Studies of Food Stickiness in Relation to Oral Processing

Yadira Gonzalez Espinosa

THE UNIVERSITY OF LEEDS

SCHOOL OF FOOD SCIENCE AND NUTRITION

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The candidate confirms that the work that submitted is her own and the appropriate credit has been given where reference has been made to the work of others

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ABSTRACT

It is generally accepted that a food perceived as sticky would adhere easily to oral surfaces (e.g. teeth and soft tissues) and lead to many irregular movements of the jaw and the tongue. However, there are two major issues with this assumption. Firstly, there has been no supporting experimental evidence. Secondly, the stickiness measurements in the research lab are often conducted under conditions very dissimilar to those in the mouth and, therefore, their correlations to oral experience are somewhat questionable.

This project investigated food stickiness and its effects on oral experience using three approaches: instrumental characterization, sensory assessment, and oral physiological analysis. Six semi-solid confectionery foods, standardized in size and shape, were used for investigation. Their stickiness was characterized quantitatively using penetration tests performed in a Texture Analyser and evaluated sensorially through assessment by a taste panel of 14 young subjects. Oral response to food stickiness was characterised using surface Electromyography (sEMG) technique to record the activities of major oral/facial muscles.

Products were well discriminated not only according to their stickiness but also their hardness. Instrumental characterization of food stickiness was carried out with two different probes: a flat 5mm stainless steel probe and a natural tooth probe, and measured dry and wet to mimic oral conditions. It was found that food products can be categorised into two groups: those in which stickiness increased and those in which the stickiness decreased after surface wetting. The measured wet stickiness exhibited very good correlations with sensory stickiness. Measurements from the sEMG of 10 subjects showed that the activities of oral muscles during mouth closing (masseters and temporalis), mouth opening (digastric muscle), and tongue movements respond directly to food stickiness. Increases in muscle work per chewing sequence W ($\mu V \cdot s$) for both opening and clossing muscles were closely associated to the perception of food stickiness rather than with food hardness. Association of muscle activity, of closing muscles, to stickiness sensation is believed to arise from the increased difficulty and uncomfortable sensation of applying shearing action between teeth when the sticky food tends to hold them together.

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1.1 Introduction

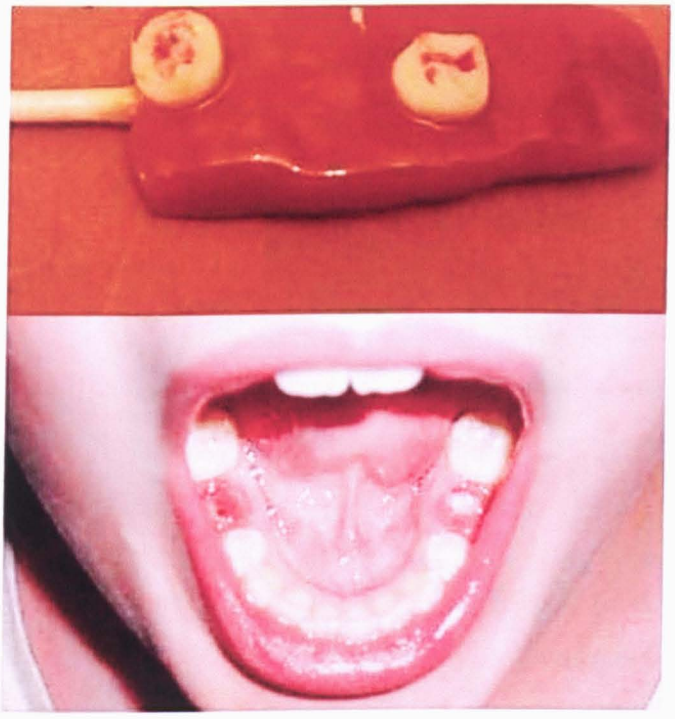


FIGURE 1.1 Sticky Foods
(KONTRABAND, 1999)

For the most part the textural properties of food go unnoticed by human beings, until they cause a bad experience. The above picture shows an extreme case of this. The little boy was probably not paying much attention to how sticky his toffee-lollipop was until it pulled out his teeth!

Of course this picture shows an event which is extremely unlikely to happen. However, it offers an excellent illustration of the negative effects which can be caused by food stickiness. A more common detrimental side effect is the pulling out of fillings, as the researcher of this study has experienced! Chewing the product found as the stickiest in this study, a toffee, could definitely lead to the pulling out of a filling. It is however important to emphasize that this would be highly dependent on the state and quality of the filling material. In this matter, to draw more concrete conclusions about the harmful effects of sticky products, more evidence would, of course, be needed.

Really it is not surprising that sticky foods can cause such damage, especially when one realises that some of the main compounds found in sticky foods have been employed extensively in the glue industry for their excellent adhesive properties. Indeed, starch and casein, two common ingredients found in foods, have been used for their characteristic adhesive properties since ancient times. The ancient Egyptians, used casein based glue in the production of murals and other artwork. Cooked starch for example has not only been reported to possess the property of sticking together but it adheres so strong in the mouth that if not degraded forms plaque biofilms. Starch granules have been recovered not only by modern-day dentists but also by archaeologists from dental calculus as evidence of the diet.

To most consumers, sticky food is unpleasant to handle and to consume. However, some highly sticky food could have more serious consequences if consumed improperly. In Japan for example, a traditional rice cake called "mochi" hits the headlines every New Year, the time when it is mostly consumed. Reports say that elderly people die by choking on this product. Stickiness in "mochi" is possibly due to the reported rheological properties of the product: It is extremely adhesive, resists large elongation, and although easy to deform it is extremely tough and hard to cut off. These properties could make the product very difficult to swallow, especially for the elderly as a consequence of physiological deterioration of their oral system which leads to the choking.

A similar effect is seen in those suffering from dysphagia. This condition leads to the weakening of the muscles involved in chewing and swallowing and a decreased capability in muscle coordination. Thus, sufferers of dysphagia are unable to breakdown sticky foods and in addition unable to swallow them safely.

It may be said that the examples presented above are extreme. However, these examples, do point to the fact that for some people sticky foods can be a severe problem. It is because of this that a focus of recent research has been on the development of special foods tailored to the needs of some vulnerable consumer groups. The textural properties of these foods, including stickiness, must be carefully controlled.

Such control of stickiness requires works which seek to deepen the understanding of food stickiness, the factors giving rise to this, and develop experimental methods which are capable of producing adequate measurements that allow accurate prediction of its perception by consumers.

1.2 Research Objectives

This project focused on the physical and oral physiological aspects of food stickiness. The main objectives of this research were:

1) To understand the governing principles of food stickiness, in particular in relation to material properties of the food, surface wetting, surface bonding, etc.

2) To reveal physiological impacts of food stickiness to our eating behaviour and sensory experience, in particular the adaptive response of orofacial muscles in consuming sticky foods.

3) To establish reliable correlations between instrumentally quantified stickiness and that sensed during oral processing.

4) It was also hoped findings from this project could provide useful knowledge to the food industry in designing and manufacturing high quality, more enjoyable food products for various consumer groups.

1.3 Thesis Outline

The work presented in this thesis is the outcome of the research carried out on the study of food stickiness and its perception during oral processing. **Chapter 2 and Chapter 3** of this manuscript present the background and the relevant literature that supports this study. In general Chapter 2 reviews the literature about texture in foods and aims to situate the term stickiness within this context. The importance not only of texture measurement in food is highlighted but also the difficulties in its achievement. The need to develop standardised methodologies, for use in the food industry, to quantify food textural properties is also discussed. The concept and theories of stickiness, in particular, are discussed. **Chapter 3** is a literature review on Surface Electromyography (sEMG), a very old technique but of relatively recent application in the evaluation of food textural properties in oral processing, and the main technique used for this project. This chapter offers an in depth review and discussion of the most current publications related to its application in eating studies.

The following chapters constitute the main body of this work, experiments performed and the results derived from them are fully discussed. **Chapter 4** Introduces the foodstuffs selected for research and other materials employed, as well as describing the corresponding methodology for their preparation. **Chapter 5** Describes the implementation of a sensory panel for the evaluation of the degree of stickiness present in confectionery products, as perceived during consumption. **Chapter 6** corresponds to the instrumental characterization of the materials using compression tests. **Chapter 7** discusses the optimization of an instrumental test to characterize the stickiness of the food materials used. **Chapter 8,** in this chapter Dynamic-stress relaxation tests were used in an attempt to measure the spreading of soft solid foods as the main mechanism involved in causing the stickiness of foods inside the oral cavity. **Chapter 9** focuses on the use of surface electromyography to characterize stickiness during oral processing and to analyse the physiological response to this textural property. **Chapter 10** analyses the effect of the sample geometry and textural properties of non-confectionery foodstuffs on masticatory response and finally **Chapter 11** presents the concluding remarks and main findings of this research. Some further work that could increase the value of, or follow, the present findings is also suggested.

Chapter 2

Literature review 1: Texture and Stickiness in Foods

2.1 Food Texture and its dynamic nature

The palatability of food depends on two factors: chemical factors comprising flavour and aroma, with the former being related to the five basic tastes (sweetness, sourness, saltiness, bitterness and unami) and physical factors, which consist mainly of texture (Nishinari, 2004). The enjoyment of food is highly influenced by its texture and in turn it is a crucial factor in determining its acceptability. This is because the way in which food behaves mechanically in the mouth influences its perception. For instance, the pleasurable experience derived from a food can be affected by the appearance of an unexpected texture. For example, if a hamburger is juicy and succulent one would tend to find it enjoyable while contrarily if in the middle of the experience one encounters a hard piece of bone or gristle the reaction of the consumer would be the immediate rejection of the product.

The term food texture has been defined in a vast number of publications, the International Organisation for Standardisation has defined food texture as "all the mechanical (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors"(ISO, 1992). Food texture is in essence a human sensory experience and as such can be perceived with all the senses, with the exception of smell and taste(Szczesniak, 2002). The perception of food texture is a dynamic process that starts even before a product is ingested (Rosenthal, 1999, De Wijk et al., 2003, Nishinari, 2004, Riqueto-Gambareli et

al., 2007). This process is illustrated in Figure 2.1 and discussed in the following paragraphs.

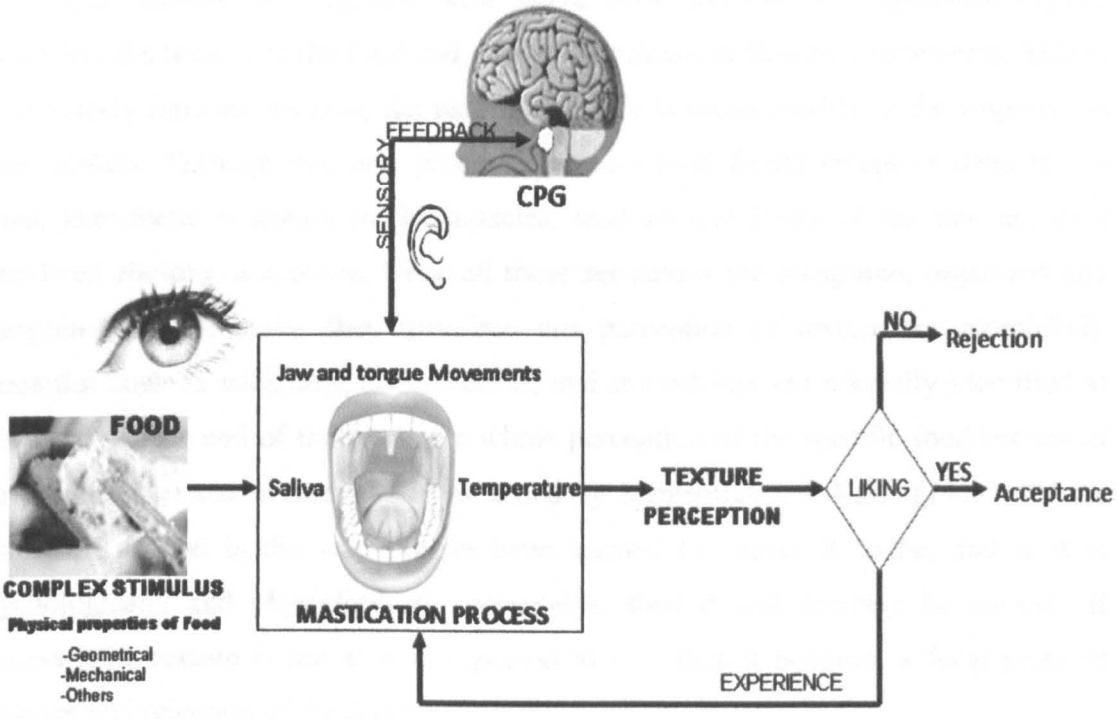


FIGURE 2.1. The dynamic process of food texture perception

Before its ingestion one may get an insight into the texture of the food by simple visual inspection, commonly referred to as appearance. For example, an impression of the roughness of the surface can be generated, although this largely depends on prior experiences. In the same way, via the sense of touch, impressions of the food's texture can be generated when it is handled. The hardness of a solid food may be judged by how it feels in the hands. The viscosity of a semi solid, or liquid, may be judged by the use of an intermediary object (such as a knife or spoon). Stimuli such as these will create expectations of how the food will feel in the mouth and thus introduce bias in a future evaluation of texture.

It is however in the oral cavity where most of sensations are evoked, while food is being subjected to varying stresses and movements, thus we sense while we eat! Right after ingestion, attributes such as crispiness, brittleness or crunchiness can be detected by interpretation of the sounds made when the food is bitten. The mechanical input (jaw and tongue movements) together with saliva flow and mouth temperature rapidly transforms the texture of the food and allows the release of flavour components. This is an extremely intricate process, the nature of which is incomparable to the response to other stimuli. Through this oral process, pressure (and force) receptors deep in the gums, kinesthetic receptors in the muscles, tendons and joints of the jaw are then stimulated eliciting sensations. Once all these sensations are integrated, organized and interpreted by the brain, they turn into our perception of texture (or mouthfeel). Properties such as stickiness, cohesiveness, and smoothness are normally identified at this stage. At the end of the process a whole perception of the specific food texture of the product ingested is formed and as stated by Szczesniak and Kahn (1971); "If the texture of a food is the way people have learned to expect it to be, and if it is psychologically and physiologically acceptable, then it will scarcely be noticed. If however, the texture is not as it is expected to be... then it becomes a focal point of criticism and rejection of the food".

Hutchings and Lillford (1988) proposed the first mouth model (Figure 2.2), which represents in a graphical way the dynamic process of texture perception inside the oral cavity. The model essentially highlights the importance of factors that are involved in the process of food and texture perception. The model includes some fundamental elements of an eating process such as: 1) The degree of structure including the rheological properties and particle size, 2) Factors that vary with time as the chewing sequence continues.(e.g. temperature of the food and the number of chews to swallow) and 3) The degree of lubrication considering the effects of saliva, moisture and fat contained in the food. The model emphasizes that in order for a food to be swallowed a certain food particle size and degree of lubrication must be satisfied. The main constraint on the usefulness of the model has been the lack of information about oral processing which is currently the challenge addressed by many research studies (Wilkinson et al., 2000, Chen, 2009).

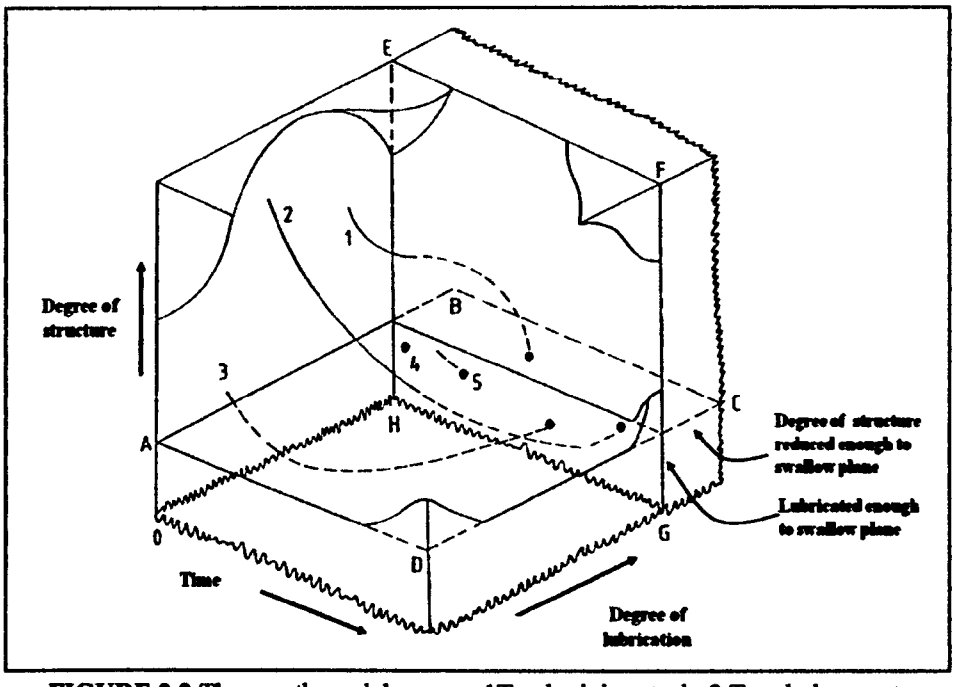


FIGURE 2.2 The mouth model process 1Tender juicy steak; 2 Tough dry meat; 3 Dry sponge cake; 4 Oyster; 5 Liquids. Below plane ABCD and across EFGH food has attained the right texture to be swallowed
(Adapted from Hutchings and Lillford, 1988)

The study of texture and rheology in foods, in recent years, has aimed to understand consumer preference. Texture preference can be affected by many factors such as: age, gender, socioeconomic class, culture and previous experiences. The most important of which is age. For example, in infants, perception of texture is largely unimportant from the point of view of personal preference; they are happy to repeatedly accept foods which have mainly a mushy, smooth, creamy and soft consistency. This may be because their physiology is not yet sufficiently developed to deal with foods having particular textures (e.g. solid foods, hard textures etc). As people grow their physiology develops and they are able to eat a diet which has a more diverse variety of textures. This allows them not only to gain more experience of different food textures but also leads to the formation of personal preferences. These preferences become an important factor in the acceptance or rejection of foods. Thus, study of consumer preference and perception allows the creation of products which will meet the textural expectations of customers. Eg: low fat yoghurt which has the same textural properties as full fat yoghurt.

With age, texture becomes not only important for food palatability but also for the safety of eating. This is the reason why the focus of recent studies has not just been on consumer preference but also on the creation of foods with textural properties tailored to the needs of specific consumer groups. One such group is the elderly (age > 60 years) which is expected to increase to over one billion, worldwide, in the coming years. With age there are many physiological changes which affect how food texture is perceived (Roininen et al., 2004, Popper and Kroll, 2003) and impair a person's ability to consume foods having certain textures. For example; the loss of teeth and their replacement with dentures reduces the sensation derived from foods and can result in food becoming trapped between the dentures and gums. Reduction in muscle activity and strength can impair a person's ability to chew and swallow food. A possible decrease in saliva production can impede the proper lubrication of food leading to problems with its processing. Thus an understanding of texture in foods and its physiological effects can make feasible its modification in order to create well designed food products targeted to protect the well being of elderly consumers.

2.1.1 Textural properties

The main problem in the study of food textures is the fact that texture is a complex stimulus embracing several textural properties. Textural properties are the group of physical characteristics that arise from the structural elements of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration, and flow of the food under a force and are measure objectively by functions of mass, time and distance (Bourne, 2002).

Studies such as those carried out by Szczesniak and Kleyn(1963) in United States, Yoshikawa *et.al.*, (1970) in Japan and Rohm, (1990) in Austria, showed not only the great variety of terms used among consumers to describe the texture of foods, but also the complexity in classifying the textural properties across different cultures and languages(Rosenthal, 1999). The diversity in the terms used to describe texture in foods, lead to the creation of texture classification systems. Classification systems were proposed by Dr Alina Szczesniack (see Table 2.1) to link rheological properties and

terms popularly used in the description of textural characteristics. Initially this was done for semi-solids and solids (Szczesniak and Kleyn, 1963) and later on for liquids (Szczesniak, 1979). The use of this approach undoubtedly came to revolutionize the study of texture in the field of foods.

TABLE 2.1 Classification systems of textural properties for solids, semi-solids and liquid foods (Adapted from Szczesniak *et. al., 1963)*

Classification of textural characteristics (Solids and semi-solid foods)	*Mechanical characteristics*		
	Primary parameters	Secondary parameters	Popular terms
	Hardness		Soft → Firm → Hard
	Cohesiveness	Brittleness	Crumbly → Crunchy → Brittle
		Chewiness	Tender → Chewy → Though
		Gumminess	Short → mealy → Pasty
			Gummy Thin → Viscous
	Viscosity		Plastic → Elastic
	Springiness		Sticky → Tacky → Gooey
	Adhesiveness		
	Geometrical characteristics		
	Class		Examples
	Particle size and shape		Gritty, Grainy, Coarse, etc.
	Particle shape and orientation		Fibrous, Cellular, Crystalline, etc.
	Other characteristics		
	Primary parameters	Secondary parameters	Popular terms
	Moisture content		Dry → Moist → Wet → Watery
	Fat content	Oiliness	Oily
		Greasiness	Greasy

From the original classification (Szczesniak and Kleyn, 1963) elasticity was replaced by the term springiness (Szczesniak, 1975) and the term firmness was added (Szczesniak and Bourne, 1969)

		Category	Typical words
Classification of mouthfeel terms (Liquid foods)	I	Viscosity-related terms	Thin, thick, viscous
	II	Feel on soft tissue surfaces	Smooth, pulpy, creamy
	III	Carbonation- related terms	Bubbly, tingly, foamy
	IV	Body-related terms	Heavy, watery, light
	V	Chemical effect	Astringent, Burning, sharp
	VI	Coating oral cavity	Mouthcoating, clinging, fatty, oily
	VII	Resistance to tongue movement	Slimy, syrupy, pasty
	VIII	Afterfeel-mouth	Sticky
	IX	Afterfeel-physiological	Clean, drying, lingering, cleansing
	X	Temperature-related	Cold, hot
	XI	Wetness-related	Wet, dry

Even when other similar systems have been proposed, such as those by Sherman (1969) and Jowitt (1974) , Szczesniack classifications of texture probably continue to be the ones most often used as a guide in food research. For solid and semi-solid foods, the usefulness of the classified terms was greatly increased by the invention of the Texture Profile Analysis technique (TPA). This is because the technique correlates the mechanical description of the terms, from instrumental tests, with sensory terms (see Table 2.2). Since its development TPA has been widely implemented and has been demonstrated to be an aid in the in the assessment of food texture (Friedman et al., 1963) and (Szczesniak and Kleyn, 1963).

2.1.2 Texture evaluation

The process of evaluating food texture started in around the 1950's, since then large efforts have been made toward the establishment of feasible physical techniques and instruments for the characterization and quantification of textural properties. Such reliable objective methods would allow food scientists: 1) to predict consumer's preference of food products; 2) to design and manufacture foods with desired textures for the consumption of different consumer groups; and 3) to have a proper quality control during manufacturing processes (Wilkinson et al., 2000). So far various reliable techniques have been made available and the Texture Analyser is probably the one which is most commonly used in research laboratories and in industry. However, a main constraint of these instruments lies in the fact that they are unable to mimic the hugely varying oral conditions occurring during chewing. This enormous variation is due to the dynamic nature of texture perception and the difference in eating styles by individuals (Riqueto-Gambareli et al., 2007, Engelen and Van Der Bilt, 2008). Such a discrepancy often leads to low correlations between instrumental prediction and the consumer's sensory perception. This is probably one of the main reasons why sensory evaluation using taste panels is still used in industry as an effective method for the evaluation of food texture and acceptability to consumers. However, disadvantages of this approach are also obvious, it is highly time-consuming and cost ineffective. The outcome from taste panel tests depends largely on the capacity of subjects to transcribe their sensations

TABLE 2.2 Definitions of mechanical parameters of texture.
(Adapted from (Szczesniak and Kleyn, 1963))

	Physical	Sensory
Primary properties		
Hardness	Force necessary to attain a given deformation.	Force required to compress a substance between the molar teeth (in the case of solids) or between the tongue and palate (in the case of semi-solids).
Cohesiveness	Extent to which a material can be deformed before it ruptures.	Degree to which a substance is compressed between the teeth before it breaks.
Viscosity	Rate of flow per unit force.	Force required to draw a liquid from a spoon over the tongue.
Springiness	Rate at which a deformed material goes back to its undeformed condition after the deforming force is removed.	Degree to which a product returns to its original shape once it has been compressed between the teeth.
Adhesiveness	Work necessary to overcome the attractive forces between the surface of the food and the surface of the other materials with which the food comes in contact.	Force required to remove the material that adheres to the mouth (generally the palate) during the normal eating process.
Secondary properties		
Fracturability*	Force with which a material fractures: a product of high degree of hardness and low degree of cohesiveness.	Force with which sample crumbles, cracks or shatters.
Chewiness	Energy required to masticate a solid food to a state ready for swallowing: a product of hardness, cohesiveness and springiness	Length of time (in sec) required to masticate the sample, at a constant rate of force application, to reduce it to a consistency suitable for swallowing.
Gumminess	Energy required to disintegrate a semi-solid food to a state ready for swallowing: a product of a low degree of hardness and a high degree of cohesiveness	Denseness that persists throughout mastication: energy required to disintegrate a semi-solid food to a state ready for swallowing.

*This term was originally named brittleness (Civille and Szczesniak, 1973)

in words and scores. This can allow for a lack of objectiveness (Boyar and Kilcast, 1986).

In order to overcome the above cited problems, physiological techniques have recently emerged as a means to bridge the gap between objective characterization and subjective perception, by underpinning the physiological responses of the human being during oral processing of foods. Electromyography (EMG) is one of the useful techniques that has been used recently to assess the physiological behaviour of oral responses in relation to varying texture of food. A fully review of this is presented in the next chapter.

Food texture is described by many factors and there is no single set of receptors responsible for its perception. Also, food texture has an extremely dynamic nature and is influenced by physiological factors. All of this makes food texture a complex stimulus which is difficult to measure accurately in a way which allows interpretation of the sensations experienced by humans.

2.1.2.1 Objective measurements (instrumental methods)

Kilcast (2004) in his publication provided a good summary of the current objective measurements available for the evaluation of food texture which is presented in table 2.3. From this table it can be clearly seen that among all measurements physiological and sound emission techniques followed by imitative tests are the ones offering the best approach to predict the consumer experience of food texture.

In general, instrumental techniques used to measure texture objectively have been classified into three main categories according to the Scott-Blair (1958) approach. A brief description of each one is given below.

TABLE 2.3 Summary of the main classes of texture measurement

(Adapted with permission from Kilcast, 2004)

Class	Food types	Initial costs	Running costs	Operating environment	Development status	Consumer relevance[6]
Sensory[1]	All	High	Moderate/High	Laboratory/QC/ production	Mature/continuing	High
Empirical[2]	All	Low	Low	QC/production	Mature/continuing	Low
Imitative[3]	Solids	Moderate	Low	Laboratory/QC	Continuing	Low/moderate
Fundamental[4]	All	High	Low	Laboratory	Mature	Low
Sound emission	Brittle solids	Moderate/High	Low/Moderate	Laboratory	Continuing	Moderate/high
Sound input	Solids	Moderate/High	Low	Laboratory	Continuing	Low/moderate
Physiological[5]	Mainly solids	High/Moderate	Moderate	Laboratory	Continuing	Moderate/high
Spectroscopic	Mainly solids	High	Moderate/High	Laboratory	Continuing	Moderate

[1]Trained profile panel [2]e.g. [2]hand-held penetrometers

[3]Intrumental test rigs, General Foods Texturometer

[4]Force-deformation devices under strictly defined operational conditions

[5]Including EMG

[6]Consumer relevance depends on ability to correlate tests with subjective textural responses

1) Empirical: These consisted of simple and rapid tests (e.g. the bloom gelometer that measures gel strength) which are most commonly used within the food industry for quality control. Parameters are not very well defined but they have shown relationships with some aspect of textural quality.

2) Imitative: These kind of tests attempt to mimic the conditions to which the food is subjected in the mouth. The tests are performed in instruments that provide stress and /or strain during the test sequence. Good correlations can be attained with sensory tests. Their main disadvantage is that they do not provided an understanding of food microstructure or force deformation and failure mechanisms at the molecular level. The texture profile method (TPA) is included within this type of test.

3) Fundamental: These tests are aimed to measure the properties of materials that determine their deformation and flow when subjected to external forces (rheological properties of materials that flow e.g. viscosity and materials that do not flow but will deform e.g Young's modulus, Poisson's ratio). These involve not only the use of well defined terms (e.g stress, strain, etc.), but also well defined conditions and methods. Since most of the rheological concepts and instruments were developed for engineering materials, their application with food materials sometimes presents experimental difficulties. This is because the tests are based on assumptions made about the characteristics of engineering materials, for which the analysis is valid, such as small strains, continuity, isotropy[1] and homogeneity, assumptions that in foods are not always valid. Fundamental tests for solids are performed on general purpose testing machines whilst for liquids rheometers are normally the choice. The physical properties obtained from fundamental measurements however have shown normally poor correlations with sensory evaluations.

[1] The term refers to the uniformity of physical properties in all directions in a material.

The importance of fundamental tests is always appreciate by food scientists because parameters obtained from such tests reflect the structural properties of the material on the molecular and microscopic levels because they are based on physical and chemical theories. Thus, when they are combined with descriptive sensory analysis, structure[2]-function relationships can be established (Barrangou et al., 2006).

These methods are regarded as limited because they are only able to measure a single property of texture which is typically deemed to be a multi-parameter attribute (Barret et al., 1998). However, as (van Vliet, 2002) argues, no one piece of equipment is able to measure, in one experiment, all the mechanical properties relevant to a sensory property. Therefore, what is most important is to determine what are the most essential properties and to which fundamental mechanical properties they are primarily linked.

In recent years food scientists have emphasized the need of measuring objectively mechanical properties in the same way as engineering materials are characterized to achieve a proper understanding of the perception of food texture (Peleg, 2006). It has been suggested that in order to gain a complete understanding of food perception studies must also include measurements of food structure, oral processing and sensory texture attributes (Foegeding, 2007).

Limitations of objective measurements

In practice instrumental tests do not always produce results which correlate well with sensory evaluations. This is because instruments measure physical properties of materials, they do not directly measure the sensory properties of food texture experienced by the consumer. In an effort to improve the correlation between objective measurements and sensory results it is important to set up the test in such a way that the

[2] The term "structure" includes how molecules are formed into assemblies that are collectively responsible for the physical properties of the foods.

conditions can mimic those found in real oral processing. Some factors that have been rarely considered in instrumental evaluations and expected to be of significance are:

- *Mechanical properties of biological tissues.* The mouth is formed from hard tissues (teeth) and soft tissues (oral mucosa). Soft tissue possesses deformability unlike the rigid probes used by the instrument. The mechanics of a system including soft deformable tissue must be considered. This is difficult to do with hard steel probes. (Peleg, 2006, Kohyama and Nishi, 1997).

- *Chewing speed.* The speed of masticatory movements is not constant throughout the stroke. In addition chewing speed varies not only between individuals, but also varies with the texture of the food consumed, e.g. tough products (requiring a high energy of mastication) are chewed more slowly than those requiring less energy of mastication. Most compression instruments, however, use a constant speed (Bourne, 2002). Rheological properties of viscoelastic food materials are strain rate dependent and thus are highly dependent on probe speed.

- *Temperature.* The temperature in the mouth is normally a few degrees below the central body temperature (37°C). The average range of for oral temperature in adult humans is ≈33.2 to ≈38.2°C. This is mainly because in the mouth radiant, conductive and convective heat exchange occurs while the mouth is open. When food is introduced, it undergoes a small change in its temperature which may lead to a change in its physical behaviour. No marked changes in temperature are assumed in the mouth within short periods of oral processing. Some foods products however are highly susceptible to mouth temperature such as: gelatine gels, chocolate, margarine and ice cream which melt in the mouth. Typical methodologies for instrumental tests however involve the introduction of the sample leaving it to reach thermal equilibrium before the execution of the test. (Ishihara et al., 2010, Rosentthal, 1999).

- *Saliva* This fluid is always present in the mouth and during eating the saliva flow increases affecting the moisture content of the food. Mixing food with saliva has not only a diluting effect but also the amylase enzyme present in saliva catalyzes the hydrolysis of starch into smaller carbohydrate molecules (e.g maltose and glucose). By hydrolysing the starch of semisolid foods into sugar molecules the starch loses its ability to bind to water resulting in a decrease of the product's viscosity (Dunnewind et al., 2004, Engelen and Van Der Bilt, 2008, Sakamoto et al., 1989). Tests are generally run under dry conditions, which means that these effects are not able to contribute to the results gained.

- Food is not a system in equilibrium therefore its properties depend on the time when it is tested(Kohyama and Nishi, 1997). Effects such as heat transfer and rates of hydration must be considered when performing measurements.

- Most of the foods are heterogeneous therefore the mechanical properties can vary significantly with the direction on which the force is being applied (van Vliet, 2002). Application of force by most equipment, used in instrumental tests, is confined to be uniaxial.

2.2 Food stickiness

From among all textural properties of a food, the measurement of hardness or firmness has been the one that has received more attention within the literature. This is probably because sensorially this property is evaluated at the beginning of oral processing when the size and shape, of the food, has not changed dramatically. The mechanical input applied to the food, the mixing of the food with saliva and the change in temperature at that stage is not enough to alter the degree of structure. Therefore, instrumental measurement of hardness is relatively easy to perform under conditions closely relevant to those found in oral processing. Thus, it is possible to establish good correlations between instrumental measurements and oral perception (Foegeding and Drake, 2007).

However, perceived adhesiveness or cohesiveness appears to be more difficult to quantify. These sensory properties are influenced by a set of contributing factors such as the mechanical input, the involvement of saliva, the heat exchange between the mouth and the food, etc. The combination and complication of all these factors makes the study of these properties more problematic. This may be the reason why up to now there is not any standard test available to measure the stickiness of foods within the food industry.

As a sensory property stickiness has a critical influence on consumer preference. The main focus of this study will be on this matter; stickiness as a sensory property.

Food stickiness as a sensory attribute can be directly perceived before ingestion, when the product comes in contact with hands or fingers or indirectly for example while pulling-off the packaging material that has been adhered to the food. It can also be experienced as a mouthfeel attribute during the chewing sequence when the food comes into contact with different parts of the mouth (teeth, cheeks, palate and tongue). In either situation an excessive stickiness is normally not desirable because of the inconvenience of handling and the unpleasant oral experience.

The term "sticky" has been one of the most used terms to describe the texture of foodstuffs as perceived in the mouth especially in products which are soft-solid or semi-solid (van Vliet et al., 2009, Kilcast and Roberts, 1998, Dunnewind et al., 2004). This indicates that it is one of the sensory attributes of food most often experienced by consumers (Szczesniak and Kahn, 1971). Stickiness can be evaluated as either a positive or a negative attribute depending on consumer preference. The preference (or liking) of sticky foods may be largely dependent on the type of product consumed, the individual experiences of the consumer, cultural factors and physiological factors. It has been reported that physiological factors can greatly influence that preference as people suffering from certain conditions seem to be extremely conscious of their ability (or lack of it) to control the food in the mouth (Szczesniak and Kahn, 1971).

The physiological changes suffered with age, such as the weakening of the chewing muscles, loss of teeth and the wearing of dentures, for instance, affect texture perception, making food textures like adhesiveness become a real problem (Kälviäinen et al., 2002, Rahn et al., 2009).

Physiological factors imposed by an illness, such as dysphagia[3], can be even more severe than those resulting from ageing as any food that may remain adhered to the mouth surfaces can cause choking and potentially lead to suffocation (Garcia and Chambers, 2010).

2.2.1 Stickiness: Adhesion and cohesion

Adhesiveness is the term that has been commonly applied in much of the literature to refer to the stickiness of materials. Adhesion has been defined as the sticking together of two materials with or without an intermediate layer. In this research however, the term stickiness will be used to allude to the physical and sensory property perceived when an appreciable force is needed to achieve the separation of two bodies immediately after they come into contact (Gay and Leibler, 1999). The term adhesion will be employed when referring specifically to the adhesion forces. The adhesion forces together with forces of cohesion partially contribute to the stickiness phenomena.

Adhesion forces are responsible for holding a sticky material to substrates or surfaces, whereas cohesion forces exist between molecules that hold the sticky material together and provide it with its bulk physical properties such as tensile strength and elongation (see Figure 2.3). Therefore the bond is not only the result of adhesion forces but is also contributed to by cohesion forces. The combined effects of these forces, among other factors, are what cause a material to be perceived as sticky.

[3] Dysphagia is characterized by difficulty in safe transfer of a liquid or food bolus from the mouth to the oesophagus, caused often by strokes or other neurologic damage.

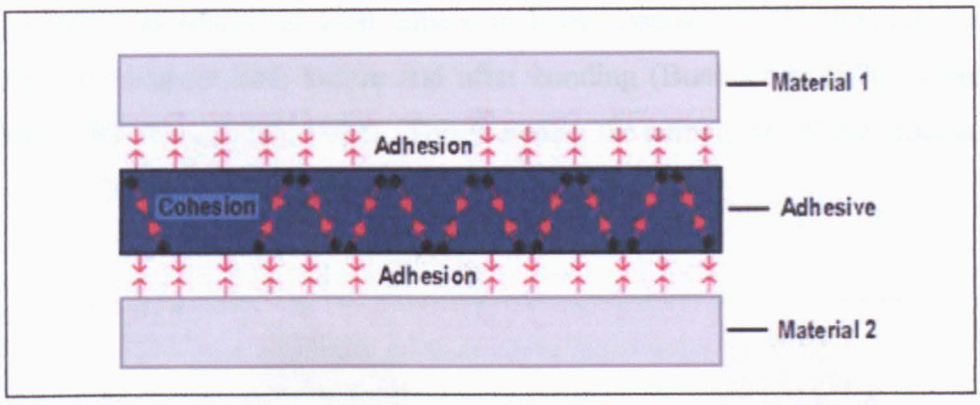


FIGURE 2.3 Forces of adhesion and cohesion involved in the process of stickiness

2.2.2 Theories of adhesion

The study of food stickiness in the mouth during oral processing can be better equated to the field of bioadhesives, especially to oral mucoadhesives rather than to the common industrial adhesives which has been the common practice. This is because for instance, bioadhesion relates to the attachment of the adhesive material to biological tissues or to the surface coating of the tissues. In the specific case of mucoadhesives, the adhesive material attaches to the mucin layer of mucosal tissue. The concept of mucoadhesives emerged within the area of pharmaceutics in response to the need to localise drugs at certain sites in the body. By improving the strength of attachment (bioadhesion) of the dosage form to the biological tissue the absorption of the drug increases as the time of the residence at the absorption site is prolonged, thus providing a more efficient delivery system. It is not surprising to find out that polysaccharides, which are widely used in food products as thickeners and stabilizers, have found major application as mucoadhesives mainly due to their non-toxicity. The stickiness of food occurs not only to the mucosa tissues, which are soft, but also to the hard tissues in the mouth (teeth). In both cases the surfaces are lubricated by salivary mucins (glycoproteins). In teeth the mucin-coating takes the form of a pellicle[4] of thickness ranging from 30 to 100nm (Lendenmann et al., 2000). Figure 2.4 shows a visual comparison between a mucoadhesive gel adhering to the mucosa and a food material adhering to the surface of a tooth.

[4] The pellicle on the tooth surface is formed when the surfaces are bathed with salivary fluid, salivary glycoproteins (positively charged) are then adsorbed to the surfaces presumably through ionic interaction, glycoproteins lose their solubility and become altered by the action of the bacterial enzymes

Furthermore, bio-adhesives must adhere to body tissues with the involvement of an aqueous environment both before and after bonding (Buonocore, 1970, Smart, 2005, Harding, 2003, Wong et al., 1999) . This increases the complexity of the process.

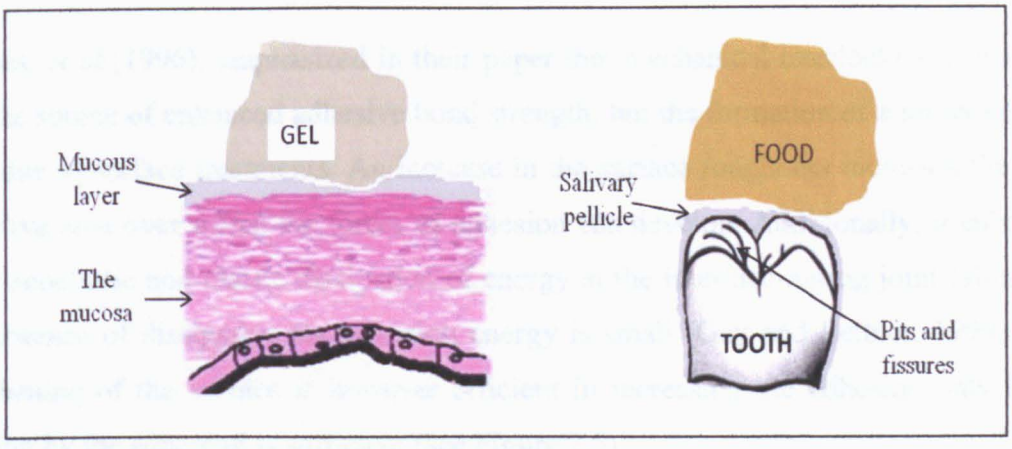


FIGURE 2.4 Bioadhesion and food stickiness. On the left, a mucoadhesive (gel) is in contact with the mucosa. On the right a food material is stuck to the surface of the tooth (hard tissue). The mucin layer is sandwiched between the adhesive and the biological substrate. In the case of the tooth, the mucin-coating is in the form of a pellicle.

There is not yet a single theory able to explain all the phenomena of mucoadhesion. However, the general theories of adhesion have been adapted to describe the mechanisms involved in the process of mucoadhesion. Of all these theories, surface energy thermodynamics theory and interface penetration, or diffusion theory, are probably the most widely accepted ones (Andrews et al., 2009). Although these theories are not able to explain fully the adhesion process, they are useful to identify parameters that are important in the process and will be discussed briefly in this section.

Mechanical interlocking theory

This theory indicates that adhesion or bonding occurs only when the adhesive material penetrates the irregularities on a rough surface, locking mechanically to the substrate. A primary condition for this form of adhesion is that the adhesive can penetrate readily into the irregularities. The adhesive must have a good wettability for the substrate and

also have the right rheological properties. For example; even though a liquid achieves excellent contact, it gives poor adhesion because it has little resistance against shear deformations. Soft solids on the contrary present some viscous dissipation, and deform to provide a good contact and resist shear.

Peppas, *et al.*(1996), emphasized in their paper that mechanical interlocking concept is not the source of enhanced adhesive bond strength, but the formation of a larger contact area due to surface treatments. An increase in the surface roughness increases the total effective area over which the forces of adhesion can develop. Additionally, it enhances the viscoelastic and plastic dissipation of energy at the interface during joint failure. In the absence of dissipation the adhesion energy is small (Gay and Leibler, 1999). The roughening of the surface is however efficient in increasing the adhesion only if the wetting by the adherend is sufficient (see Figure 2.5).

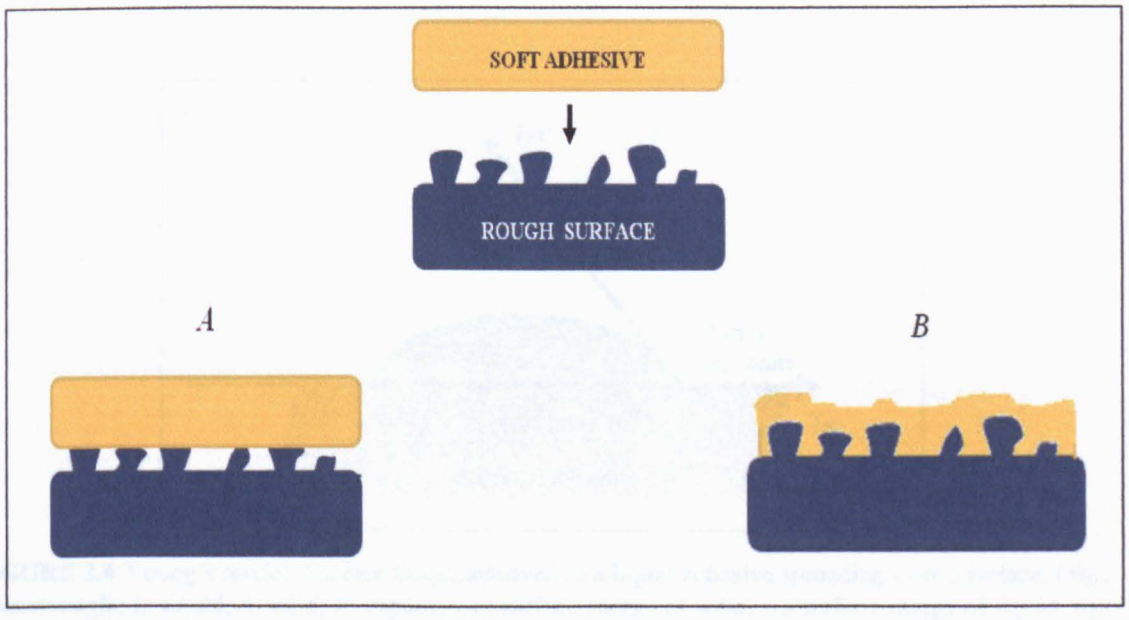


FIGURE 2.5 Degree of intimate contact achieved between a rough surface and an adhesive depending on its degree of softness. ***A.*** When the adhesive is purely elastic with a large elastic modulus, contact is restricted to the top of the hills on the surface and therefore the total contacting surface area is small. ***B.*** Softness of the adhesive is sufficient that it is able to penetrate into the valleys of the rough surface, greatly increasing the contact area. Isolated air bubbles are trapped in the valleys and generate a suction effect during debonding. In this case the roughness of the surface has contributed to an increased bond strength.

The wettability theory

This theory is based on the fact that formation of the bond usually requires a liquid-solid contact. Therefore a criterion of good adhesion is essentially good surface wettability. For this reason, this theory is mainly applicable to fluids or semi-solid materials which have, in essence, low viscosities. The wettability reflects whether the adhesive material will spread on the biological surface as a continuous film or, conversely will form several drops. Therefore, good wettability is also a good indicative of mutual affinity (Adhikari et al., 2001).

Surface wettability can be best described by Young's equation (equation 1.1), this equation represents the equilibrium state of the vector diagram shown in Figure 2.6. In this diagram, a droplet of liquid *L* (adhesive) with its vapour *V* rests on a solid surface, *S*, taking the configuration which minimizes the energy of the system.

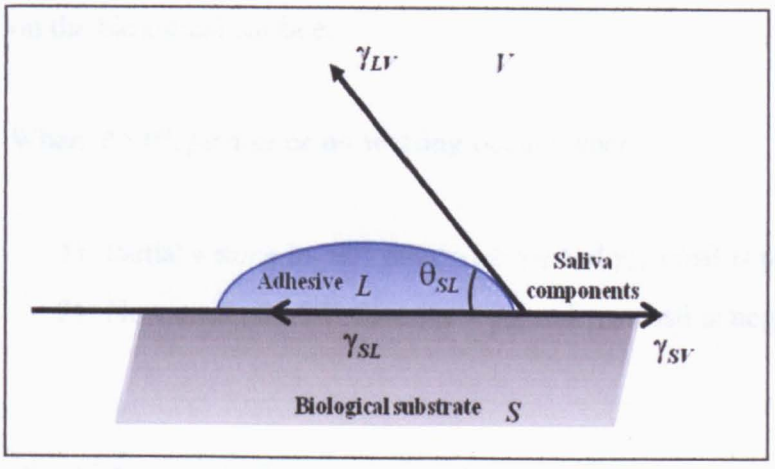


FIGURE 2.6 Young's model. Surface forces involved in a liquid adhesive spreading over a surface. (θ_{SL}: contact angle; *L*: liquid; *S*: solid; *V*: vapour; γ_{SV}: surface energy of solid; γ_{LV}: surface energy of liquid; γ_{SL}: interfacial solid–liquid energy)

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos\theta_{SL} \qquad (1.1)$$

Where,

θ_{SL}= contact angle of the liquid against the solid surface

γ_{SV}= surface energy of solid in contact with liquid vapour

$\gamma_{LV} = \gamma_L$[5] surface energy of liquid in contact with its vapour

γ_{SL}= interfacial solid-liquid energy

According to Young's equation, the contact angle is determined by the balance of the forces:

$$\cos\theta = (\gamma_{SV} - \gamma_{SL})/\gamma_{LV} \qquad (1.2)$$

- When θ=0°, ***complete wetting*** occurs, the material adheres spontaneously on the biological surface.

- When θ> 0°, ***partial*** or **no wetting** occurs where:

    1) Partial wetting θ< 90° then $\gamma_{SV} > \gamma_{SL}$ and γ_{LV} cosθ is positive
    2) No wetting θ> 90° then $\gamma_{SV} < \gamma_{SL}$ and γ_{LV} cosθ is negative

Thus, the adhesion is favoured either by large values of γ_{SV} or by very small values of the terms γ_{LV} and γ_{SL}.

[5] In the case of liquids, the surface energy is equal to the surface tension

The adsorption theory

This theory requires an intimate contact between adhesive and biological substrate, as it states that adhesion is the result of molecular-contact and forces developed between contacting molecules. The forces generated are normally secondary forces (hydrogen bonds) or van der Waals forces which require a distance separation of no more than 5 angstroms (Petrie, 2000). This process is normally known as physisorption. These interactions are more likely to be developed, causing the stickiness of food in the mouth as generated bonds are semi-permanent.

Stronger forces are generated when chemical bonds, mainly covalent, are formed by the interactions across the interface. In this case the process is known as chemisorption.

The interdiffusion theory

This theory, applied to mucoadhesion. was first introduced by Jabbari and Peppas (1994). It establishes that at an intimate contact between the polymer and the substrate, in an aqueous environment, the polymer chains have enough mobility to diffuse. Free polymer chains present, either in the mucoadhesive or in the glycoproteins, diffuse due to a chemical potential gradient. After a certain period of diffusion when enough penetration has been achieved the chains form a semi-permanent bond through effective interactions including physical entanglements and hydrogen bonds (see Figure 2.7). One indispensable factor determining the interpenetration is the chemical mutual affinity of both systems. Besides this, parameters such as time of contact between materials and the length and flexibility of the polymer chains influence the strength of adhesion. Macromolecules with high molecular weight and great number of polar groups tend to develop more intense bonds. This could be the main reason why hydrocolloids have adhesive properties (Peppas and Sahlin, 1996) and has been supported by experimental AFT-ITR spectroscopy studies (Hägerström et al., 2000).

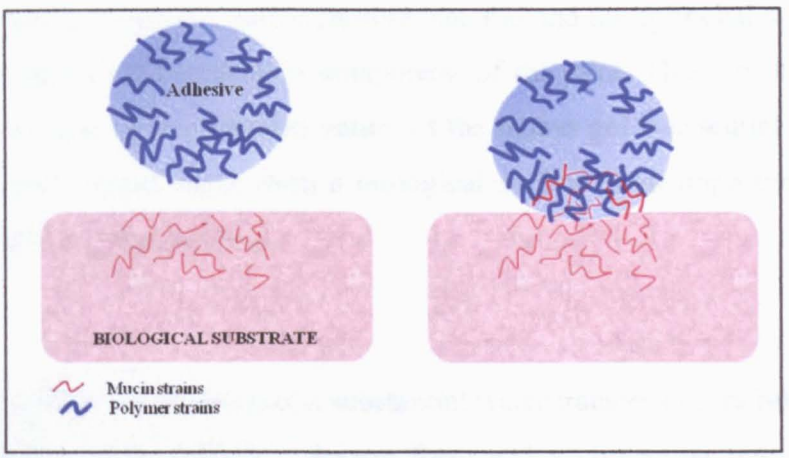


FIGURE 2.7 Molecular model of interdiffusion of polymer chains in adhesion of a polymer with the mucous layer. Left diagram shows polymer and mucous layer before the contact, whilst diagram on the right, shows the interdiffusion of chains after a certain period of contact

Degree of hydration

Chen and Cyr (1970) established that adhesion in wet conditions must be different from that occurring in dry conditions since the former is not a static state. The rate and capacity of water absorbance by the adhesive affect the amount of water present across the interface. In this respect many polymers can exhibit adhesive properties under a less hydrated condition.

Sigurdsson et al.,(2006) however indicated the need to differentiate between two different scenarios. First a "dry-on wet" adhesion, in which the sticking occurs between a dry hydrophilic polymer in contact with a wet or humid surface and second a "wet-on-wet" adhesion which refers to the adhesion of swollen adhesive polymers to mucous surfaces.

In the first case, the adhesion occurs by dehydration of the mucus gel as described by (Mortazavi and Smart, 1993). They discussed that in a mucoadhesive joint, the mucus gel is sandwiched between the mucoadhesive material and the epithelial surface, and the layer it forms would be the weakest component of the joint. Thus, in order to exhibit adhesion, an increase in the cohesive nature of the mucus gel is essential. In this sense, the capability to displace water from a biological surface is an important requirement for materials used as bioadhesives.

Mortazavi and Smart concluded that a substantial water transfer occurs between a dry or a partially hydrated mucoadhesive dosage form and a contacting mucus layer. This phenomenon seems to be more dominant than the macromolecular interdifussion, mechanism which is a much slower process. Dehydration of the mucus layer then results in a substantial increase in its cohesive and adhesive properties which in turn strength the adhesion joint. However, an excessive hydration, of the hydrocolloid, could lead to adhesion failure due to the formation of a slippery mucilage or soft paste.

On the other hand, a wet-on-wet adhesion is the case where a swollen polymer, in the presence of excess water, exhibits adhesion with the mucus layer. This can even occur when the polymer is in a fully hydrated state. In this case it is proposed that the adhesion can be better explained in terms of surface energy thermodynamics or in terms of molecular interpenetration.

The electronic theory

This theory basically states that adhesion occurs due to electron transfer upon contact of an adhesive polymer with a mucus glycoprotein network because of difference in their electronic structures (different electronegativities), resulting in a formation of an electrical double layer at the interface. The bioadhesive force is supposed to be due to attractive forces across this electrical double layer.

The fracture theory

The fracture theory relates the maximal pull-off force that the contact between the two materials can withstand, before achieving their separation, to the strength of the adhesion bond. The debonding energy (or work of adhesion[6]) which is dissipated during the debonding process provides another measurable quantity of fracture strength. Generally the greater the force or energy to break the bond the more sticky the material is. This theory is based on the fact that the way the material bonds to the substrate (biological material) is not the only important factor in contributing to the adhesion between the two. It considers that the mechanism of failure is equally important for stickiness (Adhikari et al., 2001).

Within the field of foods, the probe test has been the most common test used to measure adhesive/cohesive properties. Two main mechanisms of failure have been defined for food stickiness by Kilcast and Roberts (1998). An *adhesive failure,* that is exhibited when the cohesive strength of the food material is greater than the adhesive strength of the interface formed between the food material and the substrate to which it comes into contact with, and a *cohesive failure* present when the cohesive strength of the food material is lower than the bond strength at the food material-substrate interface Even when it has been normally established that an adhesive failure gives origin to a clean separation, (Bhandari and Howes, 2005), acknowledged the fact that an interfacial (adhesive) failure may not be common as there is not a clear-cut interface between the materials and suggested the term "adherence failure", earlier adopted by Brown (1995) They preferred the use of "adherence failure" for the apparent failure in adhesion as it is indicative of the effect of partial cohesion within the observable adhesive failure. When the material fails cohesively then two types of bond failure that could be perceived are:

[6] The work of adhesion has been considered to be a function of the reversible work of adhesion and the irreversible deformation of the substrate

Inter-phase failure: A thin layer of food material, which may be invisible to the naked eye, remaining on the probe surface.

Bulk failure: Material can be seen hanging from the probe.

Figure 2.8 shows a whole spectrum of the possible different failure modes that the debonding process of an adhesive food material can undergo. Notice that the relative magnitude of material-probe adhesion force and the cohesion strength of the sample determine the dominant mechanism of failure.

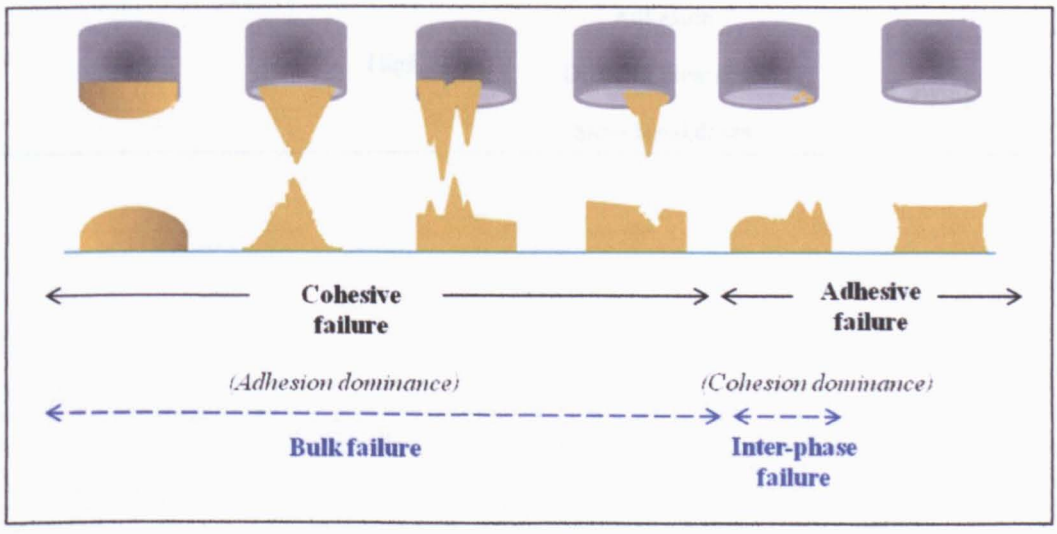


FIGURE 2.8 Spectrum of possible failure mechanisms showing adhesion or cohesion dominance

In relation to food texture perception Kilcast and Roberts (1998) associated the exhibited mode of failure to the possible outcome of stickiness perception. Their findings are displayed in table 2.4.

TABLE 2.4 Possible perceptual consequences of adhesive and cohesive failure

Adhesion	Cohesion	Behaviour	Perception
Low	Low	No adhesion Rapid clearance	Not sticky Not cloying
Low	High	No adhesion Difficult clearance	Not sticky Cloying?
High	Low	Adhesion Easily cleared (oral)	Sticky Not cloying Messy on fingers
High	High	Adhesion Difficult clearance Slow breakdown	Sticky Cloying

Chapter 3

Literature review 2: Applications of Electromyography (EMG) Technique for Eating Studies

3.1 Introduction: Mastication and electromyography

Oral mastication is a highly complicated process involving the teeth and all corresponding chewing muscles. The activities of chewing muscles drive movements of the mandible and tongue and generate biting forces to accomplish the comminution of the food and the formation of a swallowable food bolus. This neuromuscular process is influenced by individual oral physiological conditions and by the textural properties of the food. The characterisation of an eating process is extremely complicated because of physiological individuality and the continuous change of the physical and mechanical properties of the food. Such changes could affect, for example, particle size and hardness, and be influenced by saliva flow, temperature change etc. This is why often *in vitro* instrumental measurements of food texture are difficult to reproduce and often poorly correlated with sensory evaluations.

Electromyography (EMG) is a useful instrumental technique that provides access to some physiological processes by probing, evaluating and recording the electrical activities produced by skeletal muscles. The first recording of the electrical activities produced by a contracting muscle was reported more than a century ago, but it was not until the middle of 1980s when the technique was sufficiently advanced that, applications beyond clinical diagnosis became practically feasible. Pierson and Le Magnen (1970) and Boyar and Kilcast(1986) were the pioneers in the application of electromyography for the evaluation

of food textural properties by recording the activities of facial muscles during chewing. Since then, electromyographic studies have been carried out by various researchers, either to determine factors that influence human mastication or to relate masticatory parameters to the textural properties of foods. Electromyography in these studies shows the great advantages of the technique in evaluating textural properties and associated sensory perception. One unique advantage of EMG over the conventional instrumental characterization of food texture is that it follows textural changes of food throughout the whole sequence of oral processing.

The main challenge in the application of EMG technique in food texture and eating studies lies in the standardization of experimental practices and data analysis procedures to ensure that results obtained from different research groups are comparable. The main purpose of this chapter is to review experimental practices and procedures of EMG application in eating studies in relation to food texture. Surface electromyography technique (sEMG) will be the focal point. Issues such as experimental procedures, data analysis and factors causing variations will all be addressed. Much of this chapter is a literature review; however experiences gained from conducting this study form an important input into the discussion and suggested practises. This discussion is based on up-to-date studies but it must be emphasised that it will require further review, and possible modification, as more knowledge is gained in the field.

3.2 Principles of electromyography technique

3.2.1 Muscle motors and their activation

Chewing muscles are those responsible for jaw and tongue movements and mastication. Like most of the rest of the muscles in the body each chewing muscle possesses hundreds of motor units which become activated during eating. A motor unit consists of a single nerve fibre (neuron) and all of the muscle fibres it innervates. These fibres contract when the action potential (impulse) of the motor nerve reaches a depolarisation threshold as explained further below in the text. The number of fibres that are controlled by the motor

neurons correlates highly with the function of the muscle. Muscles generating large forces have a relatively larger number of muscle fibres per motor unit compared with those muscles responsible for discrete and finer movements that have relatively fewer muscle fibres per motor unit. For example, extraocular muscles have about 5-6 muscle fibres per motor unit for fine control, while large muscles of the lower limb such as the gluteus maximus and gastrocnemius have about 2000 muscle fibres per motor unit, allowing only relatively coarse control movements.

Action potentials are short-lasting events in which the electrical potential across a cell membrane rapidly rises and falls. Figure 3.1, describes the generation of action potentials which are responsible for muscle activation. At rest cell membranes are more permeable to some ions (e.g. K^+, Cl^-) than others (e.g. Na^+). Therefore sodium ions are pumped out of the cells, while potassium ions are pumped in. The outward rate of sodium ions exceeds the inward rate of potassium ions (at a ratio of about $3Na^+$ out to $2K^+$ in). Due to this difference in rates of pumping ($Na^+ > K^+$) a potential difference across the cell membrane is established. This is because the difference in pumping rates results in a difference in ion concentrations at either side of the membrane. Even though ion concentrations attempt to balance out on both sides this is not possible because of the semi permeability of the cell membrane. This leaves the interior of the cell with about a -70 mV potential difference with respect to the outside. This is known as the resting potential (Figure 3.1A). When a cell is stimulated the permeability of the cell membrane changes causing Na^+ channels to open, allowing influx of Na^+ ions into the cell (Figure 3.1B). Once the potential across the cell membrane reaches a threshold value (around -55mV) the ion channels open further allowing increased influx of Na^+ ions. The positive ion concentration is now greater inside the cell than outside and the potential becomes positive (30 mV). This process is known as depolarisation (variation in cellular potential with time) or action potential and lasts for only a few milliseconds (Figure 3.1C). After that, Na^+ channels close and K^+ channels open. As the K^+ channels remain the potential across the cell membrane is gradually restored to its rest value of -70mV (Figure 3.1D).

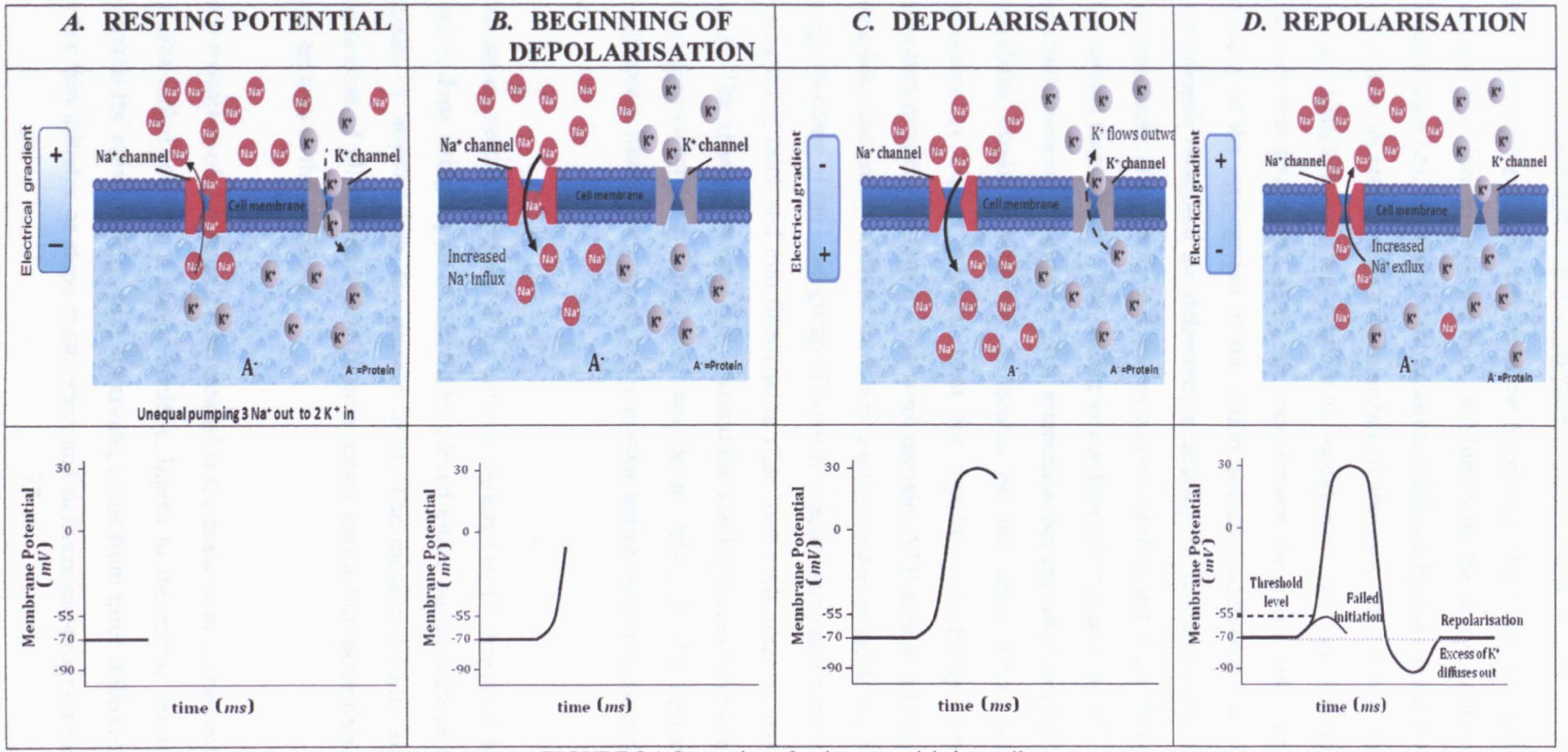


FIGURE 3.1 Generation of action potentials in a cell

When an action potential arrives to the neuromuscular junction, that is the motor end plate, it causes Ca^{2+} ions to enter the axon and bind with the acetylcholine vesicles which release acetylcholine (ACh). This neurotransmitter diffuses by exocytosis into the synapse formed at the sites where the terminal branches of the axon of a motor neuron contact a target muscle. Acetylcholine then, binds with receptors on the sarcolemma generating an action potential that propagates along the sarcolemma through a transverse system of tubules. Because of the propagation of the action potential additional Ca^{2+} ions contained in the sarcoplasmic reticulum are delivered, increasing the concentration of calcium ions around the myofibrils. Myofibrils in muscles consist of thin and thick filaments of protein; actin and myosin respectively. The sliding interaction of actin and myosin is in fact what causes the contraction of the muscle. That interaction occurs when available calcium around the myofibrils binds to troponin molecules on the actin helix, prompting tropomyosin molecules to expose binding sites for myosin cross-bridge formation. Cross-bridge formation requires binding of ATP with myosin, ATP then is split into ADP and inorganic phosphate, the energy derived from this reaction makes possible the formation of the cross-bridge (binding of the head group of myosin to actin) and entails the rotation of the globular head causing thick and thin filaments to slide over each other by shortening the sarcomere length. The neurotransmitter that initiated the whole process is broken down by the enzyme acetyilcholinesterase allowing the muscle to relax. In the abscence of calcium ions tropomyosin blocks then access to the myosin active sites on actin and the muscle returns to rest.

The action potential generated by muscle motors can be measured by EMG as a voltage. This is done using a pair of electrodes placed either on the surface of the skin above the muscle or inserted into the muscle itself. The measured EMG signal is therefore the summation of all the individual muscle motor unit action potentials (MUAPs) within the pick-up area of the electrode.

Motor neurons of jaw muscles are located in the brain stem of the central nervous system in an area called trigeminal motor nucleus. Inputs to the motor neurons which control the output to the muscle fibres, they innervate, come from three different sources 1) the motor cortex that initiates or stops mastication and delivers pre-programmed movement patterns

depending on expectations and feedback, 2) the central pattern generator (CPG) that controls rhythmic muscle activities and 3) sensory feedback from sensory receptors such as: intraoral touch receptors, muscle spindles in the jaw closing muscles, specialized mechanoreceptors in the periodontal ligament and receptors in the temporomandibular joint (Türker, 2002).

Out of all the inputs mentioned above, sensory inputs play a significant role in the mastication process by causing precise motor activities that involve the contraction of muscles. Mastication for example can occur in decerebrate animals and anencephalic patients, however the movements may be disturbed due to lack of higher control (Lavelle, 1988). See Figure 3.2 for a graphical representation of muscle activation and its measurement by sEMG.

3.2.2 Surface electromyography vs. intra-muscular electromyography

There are two different types of electromyography techniques: Surface electromyography (sEMG) and intra-muscular electromyography. Surface electromyography detects muscle activation by placing electrodes on the skin overlying the target muscles and recording action potentials. For studies of chewing muscles, surface electromyography has been the preferred technique over intra-muscular recording. The placement of the electrodes on the skin rather than the insertion of needles directly into the muscle fibres makes surface electromyography a non-invasive technique and therefore a more comfortable method without compromising accuracy.

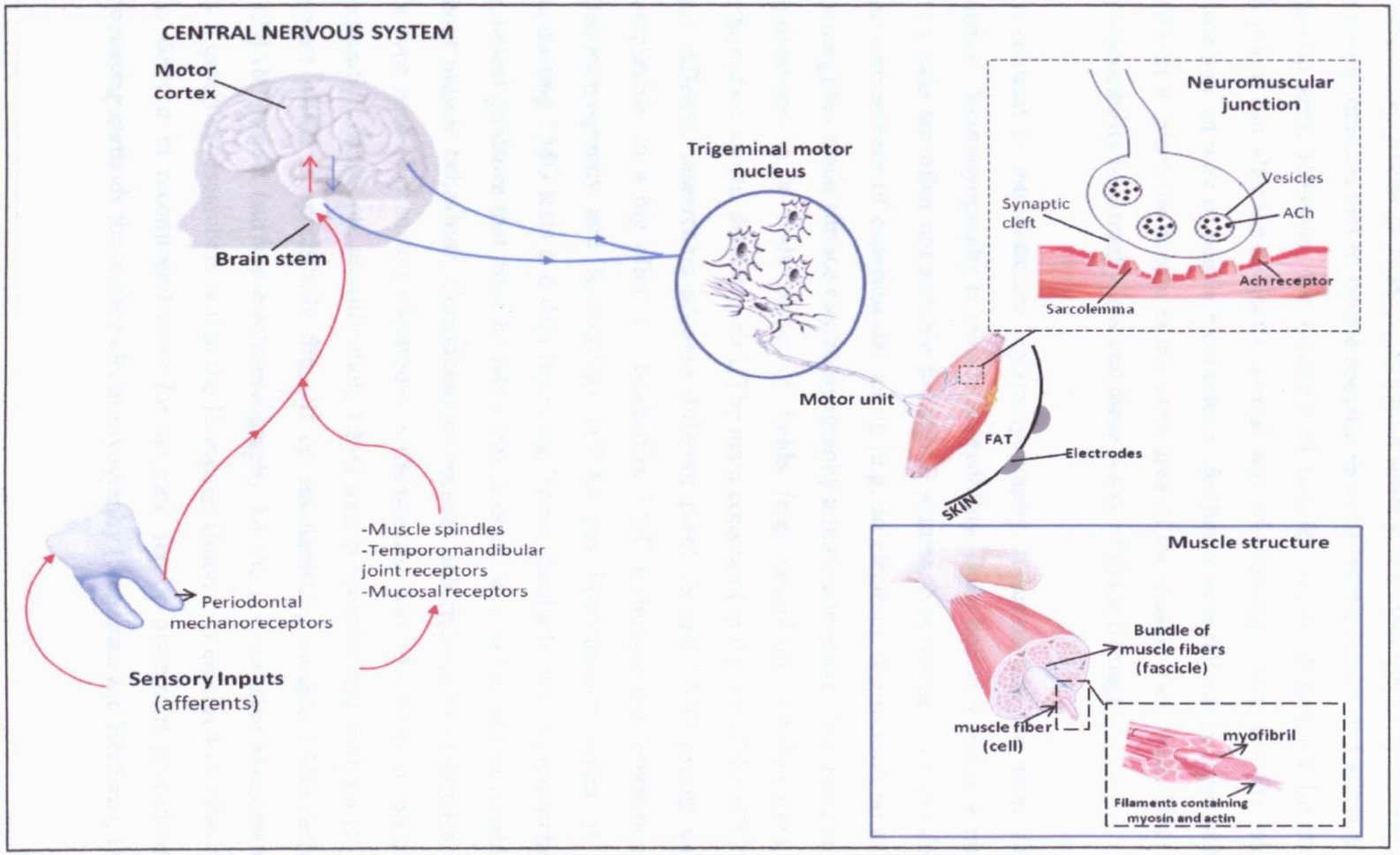


FIGURE 3.2 Process of muscle contraction

Intra-muscular electromyography has many advantages such as: the ability to test small muscles that would be impossible with a surface electrode due to cross-talk[1], the ability to test deep muscles and to isolate specific parts of large muscles and allow a more specific pick-up area. However, the insertion of needles causes discomfort for the subject and therefore can alter the subject's normal way of chewing. Moreover, the relocation of the placement of wire or needle electrodes in further tests is not easily repeatable as it is very difficult to place the needle in the same area of the muscle each time. Thus intramuscular reproducibility is normally low and there is risk of tissue damage.

In contrast to intramuscular electromyography, it seems that the main disadvantage of surface electromyography is muscle accessibility. Deep buried muscles or muscles under a thick skin are often not suitable for sEMG studies. Nevertheless, its non-invasive nature and convenience of experimental set up (e.g. no electrode sterilization no need of medical training) has made surface electromyography a more convenient technique and it has found applications in a wide range of fields (e.g. neurology, rehabilitation, ergonomics, orthopedics, sports, dentistry, etc). The main constraint in the use of the sEMG technique is that different researchers adopted different practises and EMG results were not easily comparable. In a big effort to standardize EMG technique the International Society of Electromyography and Kinesiology (ISEK) has introduced a series of standards for conducting EMG tests and data reporting. These standards provide researchers with some technical guidance that must be taken into account in order to obtain reliable information about muscle behaviour. Considerations include descriptions of electrodes depending on the type used (e.g. surface electrodes, intramuscular wire electrodes or needles), electrode preparation, detection, amplification, EMG data processing and analysis (ISEK, 1999). A project which is specifically focused on standardizing surface EMG techniques is the SENIAM project (surface electromyography for the non-invasive assessment of muscles) by a group of researchers within the European Union that emerged in 1996. Project which has derived in recommendations for sensors, sensor placement procedures, and signal processing methods for surface electromyography (Stegeman and Hermens, 1998).

[1] Cross-talk refers to the EMG signals detected by the electrode site that comes from neighbouring muscles (muscles adjacent to the muscle being recorded or deeper muscles).

3.2.3 Main mastication muscles for surface electromyography studies

A great number of muscles are involved in the mastication process. However, only some of these muscles produce strong enough electrical signals for sEMG detection. Of those muscles, the masseter lying just beneath the cheeks and the temporalis situated below the temporal fascia are the most superficial muscles that can be easily detected through palpation and their electrical activity can be easily recorded. Both muscles are of good size and belong to the group of jaw-elevator muscles (Figure 3.3), responsible for mouth closing and creating biting forces (Bourne, 2002, Ferguson, 1999). Agrawal and collaborators (1998) reported that the temporalis muscle gives a burst of activity with both a clear onset and offset during jaw closing and a clear EMG silence during mouth opening. They also reported that the recording of the masseter site gives smaller signals with less clearly defined activity and sometimes with cross-talk from other muscles such as platysma and buccinators which are active in opening. They concluded then that temporalis activity appeared unequivocally related to jaw closure.

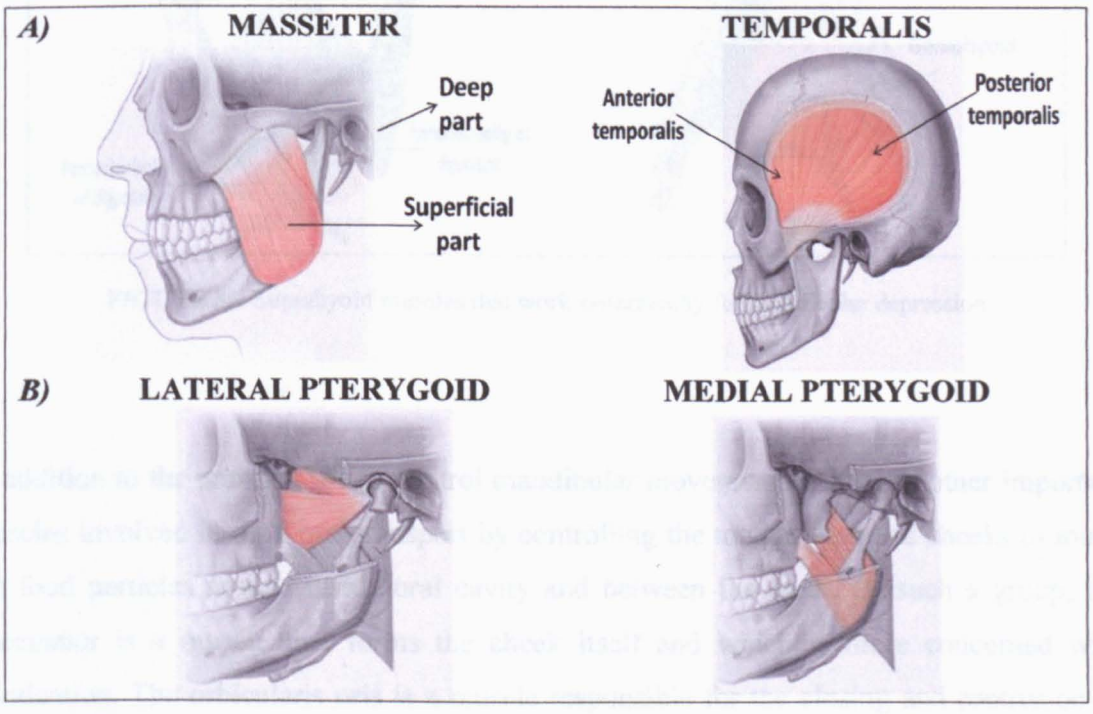


FIGURE 3.3 Jaw elevator muscles. *A)* Most superficial muscles that are easily accessed by surface electromyography *B)* Deeper closing muscles not suitable for being recorded by surface electromyography

From the group of mandibular depressor muscles, the anterior belly of the digastric muscle is often recorded. This muscle forms a part of the suprahyoid muscles (Figure 3.4) normally known as a submental muscle group, which include also the stylohyoid, the mylohyoid, and the genohyoid muscles, all covered by the platysma. All these muscles act simultaneously when the mandible depresses, therefore EMG recording will not be able to differentiate activities from each one of these muscles. However, in such cases the output of the recorded signal contributes to the same action (mandibular depression) and therefore should not represent a major problem when evaluating mandibular opening. Furthermore Winnberg and Pancherz (1983) suggested that, even though sEMG technique is not able to differentiate activities from between different suprahyoid muscles, most of the EMG activities recorded from the suprahyoid muscle group are originated from the digastric muscle.

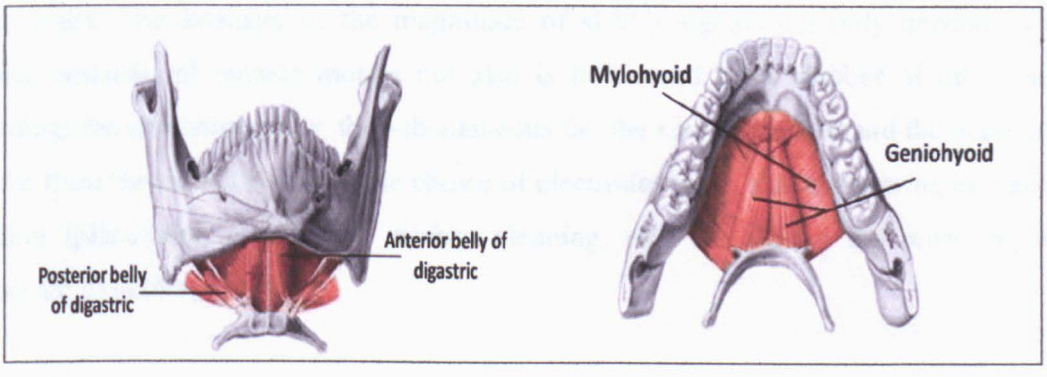


FIGURE 3.4 Suprahyoid muscles that work synergically for mandibular depression

In addition to the muscles which control mandibular movements, there are other important muscles involved in food oral transport by controlling the tongue, lips and cheeks to move the food particles around inside oral cavity and between the teeth. Of such a group, the buccinator is a muscle that forms the cheek itself and which is more concerned with mastication. The orbicularis oris is a muscle responsible for the closing and protrusion of the lips (compressing, closing, pursing or suction movements). These muscles also appear to be feasible for sEMG studies (Kohyama et al., 2010).

It has also been reported that signals of submental muscles, recorded using bipolar surface electrodes, may reflect tongue movement. Such recordings are extremely useful in investigating the role and functions of the tongue during an eating process, in particular in correlating with the changing textural properties of the food (Kohyama et al., 2010, Shiozawa et al., 1999). Another important muscle closely linked to tongue movement could be the anterior belly of the digastric muscle. Castro and collaborators (1999) observed that this muscle becomes active during all tongue movements (lateral, placement on both the hard palate and the soft palate, and on the floor of the mouth) except retraction.

3.3 Experimental design and set-up of EMG investigations

3.3.1 Electrodes, location and placement

Signals recorded from sEMG could be noisy due to temporal and spatial differences among motor units. The intensity or the magnitude of sEMG signals not only depends on the electric potential of muscle motors but also is influenced by a number of other factors including: the skin impedance, the subcutaneous fat, the size of muscle, and the depth of the muscle from the skin. Therefore, the choice of electrodes (size, shape, material, etc) and the location (placement, orientation, surface cleaning, etc) are among the most important factors for experimental set up.

Electrodes are the interface between the muscle and the recording device. Their main function is to convert the ionic current flow (biopotentials generated in muscle motors) into a flow of electrons. For that reason, their properties and their interaction with the skin must be considered carefully in order to acquire EMG signals of good quality. For instance, electrode material plays an important role in the performance of a biopotential measurement system, determining to a great extent the detection limit and the signal to noise ratio. Silver–silver chloride (*Ag–AgCl*) surface electrodes are the most commonly adopted ones in sEMG recordings due to its great stability (noise generated is less than 10 μV) and its non-polarizable behaviour (current flowing freely across the interface). Nevertheless, electrodes of other materials such as silver (*Ag*), gold (*Au*), nickel (*Ni*),

platinum (*Pt*), tin and stainless steel have also been reported for EMG recordings (Hermens et al., 2000, Cram and Kasman, 1998)

The size of the electrode detection surface, should be large enough to be able to record a reasonable pool of motor units but small enough to avoid cross-talk. The areas of the electrode recording sites of chewing muscles are relatively small because these muscles have short fibres. Therefore, it is highly recommended to use smaller electrodes for better selectivity and to avoid the pickup of signals from other muscles in close proximity. Furthermore the use of large or thick electrodes could impede the normal chewing behaviour and cause alteration in the emotional state of the subject (Lapatki et al., 2003) . In practice, circular shaped electrodes with diameters between 8 and 10 mm are commonly used for facial muscles responsible for expression and mastication. These electrodes are normally domed in order to contain an electrolyte paste or gel that provides the bridge between the electrode and the skin but also reduces the effect of electrode slippage and the resulting motion artefact[2]. The electrode is attached to the skin with the use of double sided adhesive rings to ensure that a contact pressure is always maintained between the electrode and the skin.

The configuration in which electrodes are placed is also very important in improving the quality of EMG signals. Bipolar array is typically the configuration selected especially in dynamic studies such as mastication. In this configuration two active electrodes and a ground are employed to create a differentially amplified system in which signals detected by each electrode with reference to the ground are subtracted from each other. Consequently any signal that is common to both electrodes (normally unwanted noise) will be removed. The difference between the signals therefore gives true reflection of the activities of the target muscle and will be amplified. This type of configuration can effectively minimize cross-talk.

[2] Motion artefact occurs because the electrode slips around the surface of the skin, generating an electrical potential of its own. This can be seen as direct current (DC) shifts and or massive deflections in the sEMG potentials of the raw EMG signal.

The ground electrode (also called the reference electrode) provides a common reference to the differential input of the preamplifier. Therefore, the placement of the ground is a critical factor in acquiring a clean EMG signal. It is always preferable to place the ground electrode over a bony prominence, a tissue that is electrically neutral, rather than over a muscle.

It has been widely established that the optimal location of the electrodes should be between the nearest innervation zone and the further tendon of the muscle (Rodrigues-Pedroni et al., 2005). In a bipolar arrangement it is a common practice to place one of the electrodes over the motor point of the muscle but to avoid the motor-end plate region (the terminus of the axon at the sarcolemma). The motor point is where the nerve enters the muscle and it is located on the centre of the belly of the muscle. For the second electrode, it is recommended by SENIAM that it should be situated in line with the first electrode, parallel to the muscle fibres and with a centre-to-centre distance between electrodes of 20mm (Hermens et al., 1999). This distance, known as inter-electrode distance (IED), is a parameter of considerable significance for EMG reproducibility between different subjects and between different trials of the same subject. For different trials the IED must be maintained constant to ensure that electrodes are over the same muscle fibres. For jaw-elevator muscles variations in the spectral and amplitude EMG signals have been observed even with a small electrode displacements of 2.5 mm and especially if the detection points are close to the innervation zones or tendons (Castroflorio et al., 2005). For instance, it has been reported that mean spectral frequency decreases and amplitude increases with an increasing IED (Farina et al., 2002). The use of a small IED has been suggested when single motor points are the object of the study. On the contrary, for tests to obtain an overall picture of the muscle responses, then a large inter-electrode distance is preferred (Castroflorio et al., 2008).

However, an inter electrode distance of 20mm could be sometimes difficult in practice, in particular in the case of facial muscles. Lapatki and collaborators (2003) proposed the use of 4 mm diameter electrodes with an IED of 8 mm in a bipolar array. Such an arrangement makes it possible for a simultaneous observation of multiple muscles in relatively small areas and with a much suppressed cross-talk (Lapatki et al., 2003). Large IEDs up to 30

mm are only advisable if such a distance is fixed with the use of special arrays. When two adhesive electrodes with non-fixed IED were applied in several trials significant variability was introduced in the results (Castroflorio et al., 2006).

The placement of electrodes to an optimal position on jaw elevator muscles is in practice a most challenging task. This is because the identification of a region between the innervation zone and tendon is very difficult due the fact that these muscles have short fibres and shattered innervation zones (Castroflorio et al., 2008). Therefore, the use of anatomical landmarks (dominant bone areas, prominences or other structures) is necessary to pin-point electrode sites and to ensure repeatability of electrode location.

In surface electromyography electrode placements can be designated as specific or quasi-specific. A placement is considered to be specific when the muscle to be monitored is close to the surface and is relatively easy to isolate. Temporalis and masseter muscles belong to this category. A placement is quasi-specific when an electrode is placed to record from a certain muscle but its proximity to neighbouring muscles makes it difficult to isolate. Action potentials from proximal muscles may appear in the recorded signal due to cross talk. When dealing with quasi-specific placements two strategies must be considered. One is to choose the correct size of electrode that minimizes cross-talk from neighbouring muscles. The second strategy is used when isolation of a muscle, by reducing electrode size, is impossible. In this case it is important to ensure that any proximal muscles, from which EMG signal may be recorded, contribute to or participate in the action being evaluated. The digastric muscle is a good example of this kind. Its location, small size and close proximity to other suprahyoid muscles make its recording difficult. The rest of suprahyoid muscles in close proximity to the digastric however, participate in the same action (mouth opening). Thus EMG signal recorded from this placement will correspond to the action being evaluated. In this case the inability to isolate this muscle does not represent a major problem.

Due to facial symmetry, muscle activities can be recorded unilaterally, recording electrodes of a differential amplifier are placed on one side (either right or left) of a homologous

muscle or bilaterally, each of the recording electrodes from a differential amplifier is placed on the right and left of a muscle group.

Table 3.1 summaries some good practices from recent literatures on electrode placements for studies of various facial muscles based on anatomical landmarks, function test and cross-talk. Some terms are explained below:

- ***Anatomical landmarks***. To have a precise location of a target muscle requires training and practice. One very useful guide could be the book by Lumley (2008) on clinical examination of visible and palpable anatomy. Figure 3.5 shows examples of anatomical location of closing and opening muscles.
- ***Muscle function test***. The standardization of electrode positions is in practice complicated by the unique facial anatomy of individual subjects. Among individuals there are differences in morphological characteristics such as: muscle mass, muscle length adipose tissue. It is therefore critically important that the electrodes are adjusted to fit with to the subject's anatomy. A muscle function test through visualization and palpation of the site would be essential before electrode placement.
- ***Cross-talk***. Experimenters must be aware that electrical potentials from other muscles, even those located further away from the muscle of interest, may also reach the recording site through volume conduction and contribute to the recorded EMG signal. This phenomenon is referred to as cross-talk. Cross-talk becomes a major problem when adjacent muscles are co-activated simultaneously with the target muscle. In this case the recorded signal output does not correspond to the action of investigation (motor task being performed). Strategies such as the use of proper electrode size, appropriate inter-electrode distance and where possible the exclusion of tasks that activate undesired muscles must be implemented to minimize this phenomenon.

TABLE 3.1 Electrodes location

MUSCLES	ELECTRODE SITE AND LOCATION ACCORDING TO ANATOMICAL LANDMARKS	MUSCLE FUNCTION TEST	CROSS-TALK
Anterior Temporalis	Pair of electrodes is placed over the muscle mass in a vertical direction so that active electrodes run parallel to the muscle fibers. On a straight line drawn from the outer corner of the eye to the upper attachment of the ear. The first electrode was placed 30 mm from the outer corner of the eye and 10 mm up with right angle (Akagawa and Komiyama, 1992). One electrode placed along a line from the corner of the subject's eye to the top of the ear, with the second electrode being placed approximately 1 cm superior to the first electrode in a vertical line (Takada et al., 1996). The lowest electrode is placed just above the zygomatic arch or opposite to the notch of the eye, the second is placed superior to the first and 2 cm apart (Cram and Kasman, 1998).	Palpation of the temple region while subject clenches his/her teeth, lateral deviation of the jaw, protraction and retraction of the jaw, swallowing.	Posterior temporalis, frontalis, corrugator, orbicularis oculi and masseter.
Masseter	Electrodes should be placed along an imaginary line from the corner of the jaw to the cheek bone and over the belly of the muscle (Cram and Kasman, 1998). One electrode was 10 mm below the camper's plane (a plane extending from the inferior border of the ala of the nose to the superior border of the tragus of the ear) and 20 mm back posterior from the anterior border of this muscle. The position of the second electrode is inferior to the first 20 mm apart and parallel to the longitudinal axis of the muscle fibers (Akagawa and Komiyama, 1992).	Identification of the muscle belly by palpation while asking the subject to clench his/her teeth. A forward head position may affect the resting value of the recordings.	Lateral pterygoid, Buccinator and zygomaticus.

Suprahyoid Muscles	A pair of electrodes is placed under the chin in the midline, running in the anterior-to-posterior direction (Cram and Kasman, 1998). Specific recording of anterior belly of digastric muscle has been outlined in some studies: ***Unilateral recording.*** Drawing an imaginary line which bisected the angle formed by connecting the soft tissue gonion, the soft tissue menton and the midpoint of the hyoid bone. One electrode is placed on this line 2 cm away from the soft tissue menton the second is positioned after the first on the same line with an inter-electrode distance of 2 cm. Due to the small size of digastric muscle unilateral recording in some studies has been precluded (Takada et al., 1996, Winnberg and Pancherz, 1983) ***Bi-lateral recording.*** Electrode pair is situated with one electrode over each belly (right and left portions) (Green et al., 1997)	Identification of the muscle by palpation of the area under the chin; asking the subject to swallow a few times, open the jaw or sticking the tip of the tongue to the palate towards the superior front teeth and pressing.	Platysma, sternocleidomastoid.
Buccinator	One electrode is placed just lateral to the corner of the mouth, with the second one just lateral to it (Cram and Kasman, 1998)	Ask the subject to press the cheeks against the sides of the teeth and pull corners of lips back as if to play a trumpet.	Masseter, orbicularis oris, risorius, and zygomaticus, depressor.
Inferior Orbicularis Oris	Electrodes are positioned as close as possible to the vermillon border (Lapatki et al., 2003)	Ask the subject to contract upper and lower lips (central and peripheral parts).	Adjacent facial muscles.

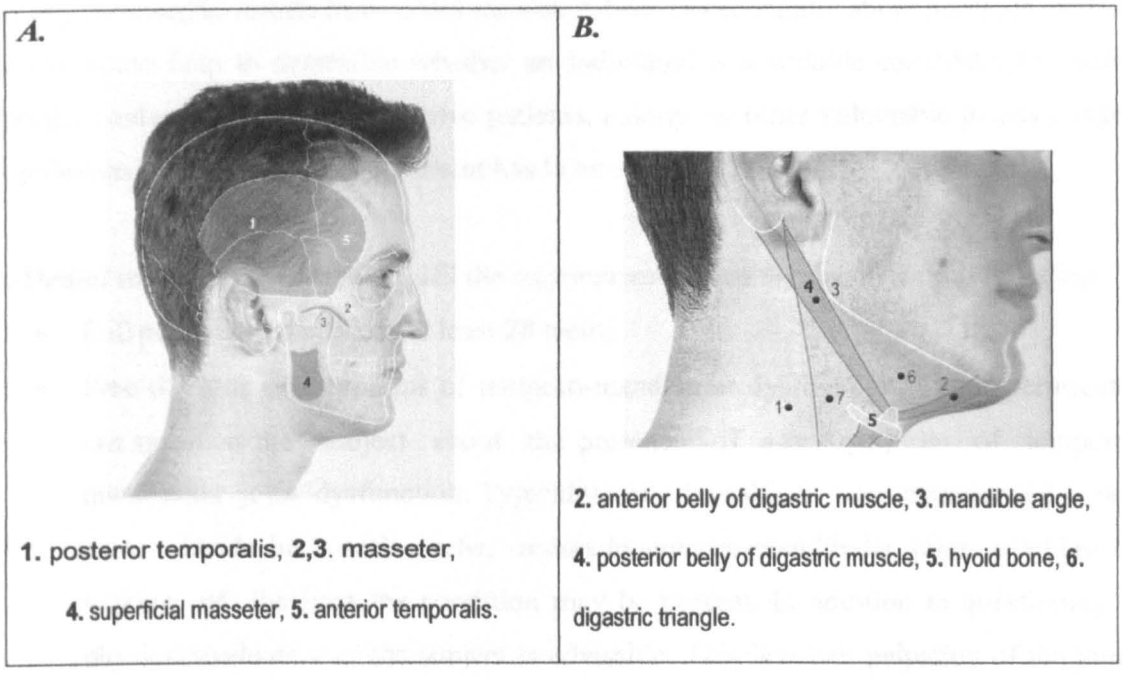


FIGURE 3.5. Recording sites for EMG tests *A.* Anatomical location of closing muscles; *B.* Anatomical location of opening muscle. (reproduced with permission from(Lumley, 2008))

3.3.2 Selection criteria for subjects for EMG studies

Experimental factors are not the only known source of variation of the bioelectrical signals generated by contraction of chewing muscles. There are other biological sources that induce variation in the recorded EMG signals. Woda and collaborators (2006) classify those sources of variation into two main categories: a) Extrinsic factors, those related to the food (size, hardness, rheological behaviour, moisture content, etc); and b) Intrinsic factors, concerning those factors which are related to the physiology of the individual (age, gender, dental health, etc). In addition to these variables González and colleagues (2004) acknowledged psychological factors (e.g. personality traits and cognitive processes) could cause significant variations in chewing patterns recorded by EMG.

It is therefore recommended that a series of criteria must be examined when selecting subject candidates for EMG studies of eating behaviour, such as:

1.-***Good general health.*** Good general health of the individual subject is critically essential for any meaningful results from EMG studies. A brief questionnaire about previous medical history could help to determine whether an individual is a suitable candidate. For some specific studies which have to involve patients, elderly, or other vulnerable disadvantaged populations, a thorough risk assessment has to be conducted.

2.-***Dental state.*** Subjects should fulfil the requirement of healthy dental status, including

- Full permanent dentition (at least 28 teeth)
- Free of signs or symptoms of temporo-mandibular dysfunction. The experimenter can question the subject about the presence of any symptom of temporo-mandibular joint dysfunction. Typically, if the subject has experienced jaw pain, headache, neck ache, noises in temporo-mandibular joint, catching or locking of the jaws the condition may be present. In addition to questioning, a physical evaluation of the subject is advisable. This involves palpation of the joint, jaw, head and neck to localize painful or tender areas. Any joint noises during mandibular movements should also be identified.
- Normal occlusion, upper teeth bite slightly ahead of the lower teeth (Angle's class I).
- No previous orthodontic treatment or orthognathic surgery.

3.-***Age.*** It is well established that chewing in humans typically emerges between 5 and 8 months old, when teeth start erupting (Sheppard and Mysak, 1984). At around 4 and 5 years the neuromuscular activity of chewing becomes well coordinated (Soboleva et al., 2005). Depending on the study it is desirable to use a selection of subjects belonging to a defined age group. Elderly people normally exhibit lower muscle activity in amplitude (either mean or maximal voltage) and longer chewing cycles. Longer chewing cycles are a product of an increase in burst and interbust durations. Such increases suggest that not only muscle activity from elevator muscles is affected but also tongue activity (Mioche, 2004, Kohyama et al., 2002). Peyron and collaborators (2004), associated age with an increase of 3 cycles per sequence per 10 years of life.

4.-*Gender*. Normally female subjects present lower muscle activities than male subjects although studies on this matter are few and not very conclusive.

5.-*Facial morphology*. Facial shape can influence chewing patterns of jaw-muscles, as it could confer a mechanical advantage to particular individuals. For instance less EMG activity is expected from an individual with greater mechanical advantage when he/she is asked to produce a given interocclusal force than from an individual with lesser mechanical advantage. Humans have a great variation in facial forms that can be roughly classified in three different types; relatively long faces (dolichofacial), average faces (mesofacial) and relatively short faces (branchyfacial). Long face subjects normally generate less activity in closing muscles and lower molar biting forces than subjects with medium and short faces. In studies this factor could be monitored by using cephalometric analysis from which several measures of facial morphology can be extracted (e.g. gonial angle, maxillary height and ramus height) (Fogle and Glaros, 1995, Vinyard et al., 2008, Grünheid et al., 2009).

6.-*Body mass index* (BMI). Muscle is a highly conductive as it contains a large amount of water (~73%) and electrolytes. Anhydrous adipose tissue lying under the skin layer is a poor conductor and can cause significant attenuation of sEMG signals. This is why obese individuals tend to have much lower amplitudes than thin individuals (Cram and Kasman, 1998). It is normally assumed that the amount of subcutaneous fat correlates well with total body fat (Mayumi et al., 2004). Therefore, it is advisable to set a BMI threshold for subject selection for specific EMG studies.

In addition to these criteria for subject selection, it is also very important to make sure that subjects are either smokers or non-smokers for particular studies. Smoking can contribute to muscle tension (Fagerström and Götestam, 1977). Subjects should also have no particular preference (like or dislike) of any test food and have no allergies to the food or food ingredients.

Ethical approval has to be obtained from an appropriate authority before EMG tests can be carried out. Even though sEMG is a non-invasive technique, the research involves human participants and data collection and, therefore, ethical approval of protocols from the

corresponding committee must be obtained. Participants who volunteer to take part in tests must be informed in detail about the objectives and procedures of the test and the potential risks involved in the experiments. It is also important to make volunteers aware that experiments could be stopped at any time, for any reason and without any negative consequences. A consent form must be filled in and signed.

3.3.3 Experimental procedures

An EMG test session consists of several stages: preparation, set-up, checking and validation, performing test, and data analysis.

PREPARATION

1. Before the session it is recommendable to ask the subject to avoid the consumption of cigarettes and caffeinated beverages, such as, coffee, cola and tea for at least two hours before the test. These foods can contribute to muscle tension

2. Ask the subject to wear appropriate clothes. The wearing of turtle neck shirts or jumpers is especially unadvisable as it can restrict the access for the proper placement of electrodes over the suprahyoid muscles region.

3. Ask male subjects cleanly shave the areas where electrodes will be placed. Recording of masseter muscles, suprahyoid muscles, and orbicularis oris muscles in men with a beard or moustache is impossible and should not be attempted.

4. Before the session, ask the subject to remove any jewellery, eyeglasses or other metal objects that may interfere with the procedure.

SET-UP

1. Locate visually the sites for placement of electrodes according to anatomical landmarks. Mark sites and orientation lines and use a flexible scale band to measure distances (see table 1).

2. Perform a muscle function test by palpation for every site to ensure the proper location of the electrode. Do this by asking the subject to perform some specific actions (see table 3.1).

3. Cleaning of the skin. Abrasion of the skin with conductive cleaning pastes or light rubbing of the skin with fine sand paper to remove dead skin cells that produce high impedance has often been reported (Lapatki et al., 2003). Fridlund and Cacioppo (1986), however, reckoned that this procedure can be annoying and painful. Since facial skin is normally very sensitive, cleaning with an alcohol swab of rough texture may be sufficient. Carefully rub the skin and let the skin dry before placement of electrodes.

4. Use double-sided adhesive rings to place the two pickup electrodes and ensure their correct adherence and good contact with the skin. Always position the electrodes oriented parallel to the muscle fibres with a set IED (see Table 3.1 for further description). It is very important that a constant IED is maintained for all the trials within a study.

5. Fill the void formed between the electrode and the skin with saline gel or paste ensuring there is no spillage out of the electrodes. Omit this step if pre-gelled electrodes are being used.

6. Attach the electrode leads to the amplifier and secure them in such way that there is enough freedom and space to perform the required actions without lifting of the electrode.

7. Attach the ground electrode away from the recording site, preferably over bony parts. Some amplifiers are designed to be placed on the top of the ground electrode.

SET-UP CHECKING and VALIDATION

1. Prior to recording allow electrodes to stabilize for a certain period of time, so that the conductive paste can adequately moisten the skin and minimise the impedance of the electrode–skin interface. Different times of stabilization have been reported, 5-6 minutes (Celebic et al., 2008), 10-15 minutes (Leung and Hägg, 2001), and 20 minutes (Marras, 1990) depending on the types of instruments and electrodes used.

2. Test electrode–skin impedance levels with an ohmmeter, ensuring these levels are less than 10 KΩ

3. Connect all the cables to the EMG equipment. All cables must be properly affixed with micropore or other suitable tape so that movements are not hindered. Sometimes cables can cause the pulling of the electrodes or the skin. This issue must be minimized as it can cause discomfort and stress to the subject and interfere with the measurement.

4. Ask the subject to sit comfortably in a straight-up position with their gaze fixed towards a target and head movement refrained. This is very important to reduce variability due to postural changes.

5. Ask the subject to relax and close their mouth in a rest position. A rest posture, or habitual mandibular position is achieved when the mandible is maintained in a reasonably constant vertical position with respect to the maxilla, with teeth remain a few millimetres apart. (Jaberzadeh et al., 2003). Although it has normally been established that under this condition, the muscle should not show any action potential, some contradictory reports suggested that muscles are active in this posture. However, EMG amplitudes at rest position are low and close to the noise level (Bérzin, 2004, Castroflorio et al., 2008)

6. Start monitoring muscle activities and check raw EMG baseline for every target muscle to be recorded while the subject is relaxed.

The EMG signal observed when the muscle is relaxed should be no higher than 10-15 μV. For a good baseline, the average amplitude could be as low as 3-5 μV. The magnitude of noise depends on the environmental noise, quality of EMG amplifier and quality of the detection of the condition being measured. It is normally recommended to record 30s of EMG signal while the muscle is relaxed to check the baseline. Apart from checking the noise level it is also advisable to ensure a zero offset before recording data and to check possible motion artefacts by visual inspection of any shifts of the baseline while recording.

7. Check validity of EMG signals. This step is very important and indispensable. This is to check that there is actually a presence of EMG activity bursts while performing a specific muscle function test. This test should be performed for every muscle being recorded to ensure that the EMG signal observed corresponds directly to the action of the muscle being evaluated.

8. Readjust electrodes if necessary and test again.

9. Start the test and perform the desired measurements.

PERFORMING A TEST

In order to produce a reliable EMG recording of chewing behaviour, it is critically essential to ensure that the subject maintains the natural way of chewing. Some influencing factors must be considered in this respect and are summarised below:

Posture, oral tasks and eye potentials. Apart from chewing, some functional and non-functional oral tasks such as: talking, drinking water, reading aloud, yawning, coughing, jaw play, lip biting, cupping of the hand on the jaw, holding the mandible protruded and laterotruded, some head movements (head yawed, flexion and extension) are normally associated with the activity of the chewing muscles (see Figure 3.6). The reflex of blinking generates some action potentials that could be included in the recording of the anterior temporal muscle. Thus in order to lessen the effect of such factors in the EMG signal, the subject should be asked to keep a comfortable upright position and their gaze fixed towards

an eye level target in the front. Speaking, drinking water or leaning must be restrained during recordings (Burdette, 1990, Farella et al., 2008)

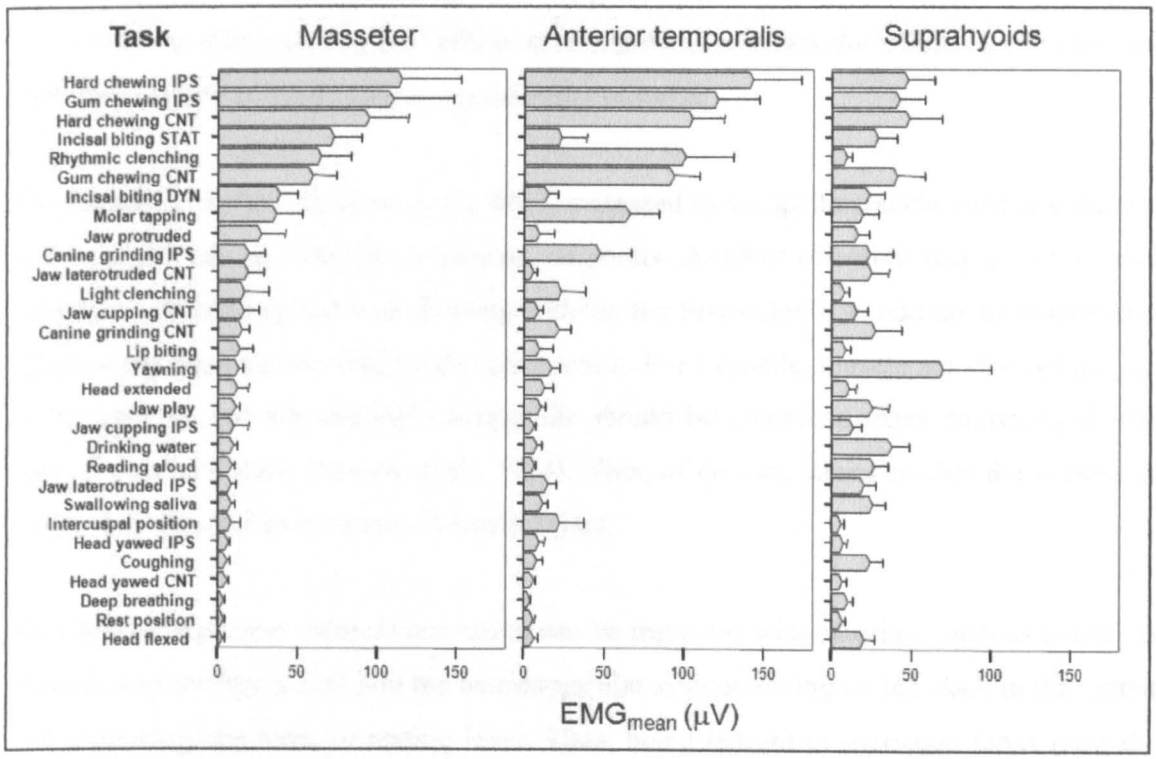


FIGURE 3.6 Mean amplitude of EMG activity obtained from the masseter, anterior temporalis and suprahyoid muscles during the individual tasks. The length of each column indicates the average (n=11), whereas the error bar indicates the upper 95% confidence limit (From (Farella et al., 2008))

Chewing style. Mastication in humans is a unilateral process in which a food is positioned between the upper and lower teeth in either the left or the right side, known as the working side. The other side is called the balancing side (Wall and Smith, 2001). However, it is commonly observed that when subjects chew the food in their habitual manner (free-style), food is continuously passed from one side to another side throughout the whole chewing sequence. In some studies subjects have been impeded to perform chewing actions in that free style manner by being asked to manipulate the food in only one side. Brown (1994) found that by doing so subjects need to concentrate on the way they chew and that the restriction is enough to alter their chewing pattern and therefore cause a change in the EMG signal. In fact Mioche and collaborators (1999) observed a lower total muscle activity of

the whole chewing sequence for closing muscles (masseter and temporalis) during free style than during side imposed mastication. They attribute this decrement to a most favourable placement of the food during the opening phase of the masticatory cycle facilitating the comminution for the following chew and therefore making the mastication process more efficient. A more efficient oral process reduces the effort of chewing and therefore muscle activity input is considerably reduced.

Carson and co-authors (Carson et al., 2003) proposed to use ipsilateral chewing in either the left or right side in order to minimize variations. Another effective way to avoid such variations when using habitual chewing without any restriction is to add up the activities of all chewing muscles involved in the same action. For example, muscle activities of left and right masseter and left and right temporalis should be combined when analyzing closing actions in mastication (Brown et al., 1994). This, of course, means double the number of electrodes and double the work of data analysis.

Psychological factors. Muscle activation can be triggered with emotions such as anxiety or fear as more energy is sent into the neuromuscular system, taking up the slack in the system and increasing the tonic or resting level. Thus, being uptight or increased tonus may also have an effect in the pattern of the movements. When subjects are exposed to stressful events the physiological response is very similar for example: speeding up their hearts, tensing their muscles or sweating (Cram and Kasman, 1998). The number of chews and the muscle activity before swallowing were found to decrease when subjects were exposed to unpleasant stimuli that modified their emotional state. It was assumed that subjects focused their attention on the stimuli rather than on the food or that the unpleasant stimuli caused a reduction in the desire of eating and therefore the behaviour of chewing (Deiss et al., 2009).

It has been suggested to use the first recording session as a means to familiarize subjects with the experimental environment. It was found that the psychological effects were the greatest in the first session as subjects are confronted with an impeding and unknown experimental context which can make them stressed and therefore alter their chewing behaviour (Foster et al., 2006, Lassauzay et al., 2000).

Fatigue effect. The fatigue of muscles is a physiological and biochemical process as a result of an intensive activity. Muscle fatigue could have two consequences in the EMG signal: 1) an increase in amplitude due to recruitment of more motor units, and 2) lowering the median frequency domain over contraction time. Chewing muscles however have been reported as being relatively resistant to fatigue. The masseter muscle may contribute to the superior fatigue resistance of jaw closing muscles, presumably due to its significant mitochondrial content. However, fatigue has been observed after subjects chew gum continuously for about 18 minutes at a rate of 80 cycles/min. But, jaw muscles of healthy subjects normally recover quickly after prolonged chewing of gum (Farella et al., 2001, Mendoça et al., 2005).

Different numbers of food samples have been used in various studies. In none of those works, muscle or intellectual fatigue experienced by the subject was reported, even when a significant number of repetitions were performed with a considerably large number of samples per session. Nevertheless it is recommendable that subjects are allowed to have sporadic breaks along the session to rest, speak or drink, and to do actions that they must refrain from doing during recording.

Food. Shape, size, textural properties and dietary variation are factors that influence jaw-muscle activity patterns.

The size of the food, normally expressed as a volume is a factor that determines the aperture of the mouth for biting and the amount of prolonged pressure when biting the food. Also, size influences the time of chewing; smaller pieces normally require less chewing while a longer time is required for chewing large pieces. The shape also affects the ease with which a food can be placed and be manipulated inside the oral cavity (Evans, 2001).

Food texture is a complex stimulus because it embraces different textural properties such as: hardness, stickiness, cohesiveness, crispiness, crunchiness, chewiness, firmness, elasticity, plasticity, etc. Textural properties are key factors influencing our chewing behaviour and the main concern of many EMG studies. During a mastication process,

sensations generated by textural attributes are elicited in the mouth and are conveyed into the brain stem through oral sensory receptors. The sensory feedback therefore generates the next corresponding inputs (physical actions) to be taken on the food. Thus, chewing behaviour has the ability to adapt to the changes in texture through mastication.

Natural foods have heterogeneous stimuli. This makes the analysis of motor response more difficult (Peyron et al., 2002). The adaptation process to food texture has been shown to be constant intra-individually when controlled settings and constant food stimuli were used but were highly variable between individuals. To overcome this problem, artificial test foods have been used in eating behaviour studies. The main advantage of artificial foods is the provision of desired and reproducible textural properties and geometry. However the disadvantage is that they cannot be swallowed, which means that only part of the eating process can be monitored (Foster et al., 2006). See Table 3.2 for a summary of some works carried out with the use of both artificial and natural foods.

The diet. Diet has been shown to influence the capacities of jaw-muscles. Studies in animals have shown that long term alteration in the pattern of muscle use can be caused by an intake of a soft diet. Jaw muscles therefore adapt morphologically and functionally to low masticatory effort for soft foods.

Such adaptation is reflected in the reductions in their muscle activity, force output, fibre cross-sectional area and percentage of slow fibres (Grünheid et al., 2009). A study on humans showed that four weeks training with a hard chewing gum seemed to influence the functional capacity of masticatory muscles and increase their strength (Kiliaridis et al., 1995).

TABLE 3.2 Studies that have applied surface electromyography to the analysis of chewing behaviours and determination of textural properties.

Publication Year	Food sample details (geometry/ amount ingested)	Number of subjects	Number of Sessions and Mastication style	Textural properties studied	Muscles and Masticatory parameters extracted	RESULTS
1991 Diaz-Tay J., Jayasinghe N., Lucas P. W., McCallum J.C. and Jones J. T.	Roasted peanuts *Unchopped* particle size median= 9.2mm range= 6.7-11.2mm *Ground* particle size median= 2.4 mm range= 2.0-2.8mm	5 females and 5 males (mean= 19.4 years)	*Sessions =1* *Style:* Habitual chewing manner	Geometrical properties (size and weight)	*Muscles:* -Left and right anterior temporalis -Left and right masseter *Parameters:* -Mean muscle activities -Peak activities -Number of chews -Duration of chews	-The masseters were more strongly affected than the temporalis. -The number of chews in a masticatory sequence increased with the increase in both size and weight, but durations of chew were weakly influence by those. -Weight of food affected muscle activity and jaw movement more that did the initial size of the food particles. Therefore total volume more important than initial particle size.
1999 Shiozawa K., Kohyama K. and Yanagisawa K.	Gumi candy (made of gelatine), Peanuts, Rice cake 5g of each gumi candy and rice cake circular in shape*	3 males and 8 females (mean of 30.6 years)	*Sessions =1* *Style:* Habitual chewing side	Hardness Adhesiveness Cohesiveness Gumminess	*Muscles:* -Masseter muscle -Anterior digastric muscle -Mylohyoid muscle *Parameters:* -Chewing time and number of cycles until first swallowing *For early stage (first five chewing cycles), middle stage (middle five chewing cycles) and late stage (final five chewing cycles):* -Chewing rhythm (average) -Cycle amplitude of EMG activity= Maximum height of integrated EMG (average)	-Decrease in the amplitude of digastric muscle is due to a reduction in particle size or volume where a wider opening of the mouth is no longer necessary. -Highly adhesive triturated food may require forceful movements of the tongue and therefore amplitude of mylohyoid muscle decreases from middle to the late stage of the masticatory sequence.

1999 Mioche L, Bourdiol P, Martin J-F and Nõel Y	Canned Frankfurters without the skin, fresh coconut, toffee and French Comtè cheese Cylindrical sample d=1.5 cm h= 1.0 cm	36 (19 males and 17 females) Mean= 20 years	*Sessions* = 1 *Style*: Free style, Right side and Left side	Viscous properties Elastic properties Firmness	*Muscles:* -Left and Right Masseter -Anterior temporalis *Parameters:* -Chewing time before the last swallow -Averaged duration of a single burst -Maximum and mean voltage of a burst -Sum of the integrated areas of all individual bursts of the sequence (burst duration x mean voltage) -Mean muscle work per chew (total muscle work/number of chews)	Total muscle work increased with hardness (stress at maximal strain) Toffee induced a shorter burst averaged duration. Increase in sequence duration of toffee could be due to its stickiness slowing down the opening phase of individual cycles Muscle work per chew could be the sensory clue used by individual to perceived stress-related variables of food texture
2000 Mathoniere, C., Mioche L., Dransfield, E. and Culioli J.	12 types of beef combining different factors such as muscle type, storage, and cooking temperature Cubes (1.5 cm) weighing ≈ 3.5 g	6 females and 5 males (25 to 50 years)	*Sessions* = 1 *Style:* Natural chewing	Elasticity, initial tenderness, juiciness and overall tenderness	*Muscles:* -Left and right temporalis -Left and right masseter *Parameters for each burst:* -Duration -Mean voltage -Maximum voltage Variables were pooled for each of the four muscles -Muscle work= duration x mean voltage *Parameters for the entire sequence=* Total muscle work= Sum of the muscle work for all chews from the four muscles	Correlation between elasticity and EMG (first two bursts) were not significant Tenderness was significantly correlated with EMG (from the third burst) Good correlation between overall tenderness and parameters from the seven middle burst of the chewing sequence Juiciness was well correlated with the first two chews variables. It was found that parameters obtained from chewing sequence better represented the sensory characteristics of the meats that any of the mechanical characteristics. Mechanical properties a low deformation were more related to juiciness while characteristics of destructive mechanical testing were more related to tenderness

2002 Peyron, M.A., Lassauzy C. and Wooda, A.	4 jellied confectionery products (gelatine) Identical size and shape[3]	15 males (22.6 ± 1.3 years)	*Sessions* = 4 (first session was discarded for data analysis) *Style:* Unilateral chewing imposed[4]	Hardness	*Muscles:* -Left and Right Masseter -Left and Right Temporalis *Parameters for the chewing sequence:* -Number of cycles -Masticatory frequency -Sequence muscular work= muscular work recorded during the whole sequence *Parameters for each cycle:* -Muscular work = sequence muscular work/number of cycles *cycles analysed individually and averaged (first five, middle three and last three cycles prior to deglutition)	-Number of chewing cycles increased with the hardness of the food. -Cycle muscular work and vertical amplitude differentiated well the products according their hardness. The second, the third, the fourth or the fifth cycle are the best choices.
2003 Carson L., Xiuzhi S., Setser C., and Peng Y.	-3 types of cakes made with: 1)Corn muffin mix 2)Corn muffin mix + polydextrose 3)White cake with all purpose flour + polydextrose 6 commercial breads (white,whole wheat, rye, pumpernickel, rye cocktail and honey cocktail) *Firmness was the same in all the cakes Cubes (20 mm x 20 mm)	9 panellists	*Sessions*=1 *Style:* Unilateral chewing (right side on the mouth)	Cohesiveness	*Muscles*: -Right Masseter *Parameters*: Total Energy, *TE* ($V \cdot s$)= Total work performed by the masseter. Muscle-area under the curve of one bite = Sum of ascending energy and descending energy Peak energy, *PE* ($V \cdot s$)= Product of the maximum voltage measurement, E_p (V) and peak width at 0.7 E_p location (s) of one bite Fourier Power, *FP* (no units)= Muscle work intensity and is a compound value of the power spectrum obtained by Fast Fourier Transform . Ascending energy *AE* ($V \cdot s$)= Area under ascending curve before the peak of one bite. Descending energy *DE* ($V \cdot s$)= Area under descending curve after the peak of one bite	-Descending energy resulted to be a suitable parameter for predicting cohesiveness. Estimation of cohesiveness as: $\frac{\Sigma DE}{\Sigma (TExPExFP)}$ Produced good correlations ($r \geq 0.80$) with sensory cohesiveness but only useful to distinguish cohesiveness among products with smaller textural differences as this attribute is thought to be masked by large differences in hardness or other textural properties.

[3] Dimensions not provided
[4] Subjects were asked to chew in their preferred side

2005 Kohyama, K., Yamaguchi M., Kabori C., Nakayama Y., Hayakawa F. and Sasaki T.	Cooked rice with different amounts of water A spoonful	10 volunteers (mean age of 32.4 years)	*Sessions* = 1 *Style:* Free style chewing	Firmness Adhesiveness Cohesiveness	*Muscles:* -Left and Right anterior temporalis -Left and Right masseter -Anterior Digastric muscles *Parameters for jaw-closing muscles:* -Number of chewing strokes -Mastication time -The amplitude or maximum voltage -Burst duration -Muscle activiy= time-integral of the EMG voltage -Cycle time for each muscle and each chewing stroke *Parameters for jaw-opening muscles:* --The amplitude or maximum voltage -Burst duration -Muscle activiy= time-integral of the EMG voltage	-Jaw closing activity reflected the firmness of the sample evaluated instrumentally -Jaw-opening activity was related to the instrumental adhesiveness of sample -Cohesiveness correlated also well with jaw-opening activity and with the adhesiveness of the rice samples tested. However muscle activity of opening muscles were not related to the cohesiveness defined by ISO 11036
2006 Foster K.D., Woda A. and Peyron M. A.	4 jellied confectionery (elastic products) 4 caramel confectionery (plastic products) Cylindrical shape (diameter=2cm and height= 1 cm)	15 males (24.1 ± 1.9 years)	*Sessions* = 1 *Style:* Unilateral chewing (preferred side)		*Muscles:* -Left and right temporalis -Left and right masseter *Parameters for the complete sequence:* -Number of chewing strokes -Masticatory frequency -EMG activity = total EMG activity for the 4 muscles during the entire masticatory sequence -Mean vertical and lateral amplitudes [5] *Parameters for each cycle:* -EMG activity= EMG activity per sequence divided by number of cycles and the 4 muscles -Opening, closing and oclusal durations -Vertical and lateral amplitudes and opening and closing velocities [6]	- Sequence duration, number of cycles, EMG activity per sequence and EMG activity per cycle increased significantly with hardness regardless the food type. -Plastic foods were chewed at lower frequencies than elastic products -The effect of hardness on masticatory frequency was only important during initial stages of a masticatory sequence and that overall frequency is better described by the rheological properties.. -Vertical amplitude is affected mostly by the rheological properties of the food.

[5] Data obtained from recording of jaw movements

2010 Kohyama K., Hanyu, T., Hayakama F. and Sasaki T.	buckwheat noodles A mouthful (15g) of: 1) standard buckwheat noodles length and 2) 3cm length	8 males and 5 females (24-43 years)	*Sessions* = 1 *Style:* Chewing or slurping Free style		*Muscles:* -Left and Right masseter -Suprahyoid musculature -Left side of the inferior orbicularis oris muscles *Parameters from masseter electromyograms:* -Number of chewing strokes -Mastication time -Mean chewing cycle time *Parameters from orbicularis oris muscles:* -Number of bursts -Cycle time *Parameters for each burst of each muscle:* -Amplitude=Maximum voltage -Burst duration -Muscle activity= time integral of the EMG voltages. Mastication effort= sum of all muscle activities of masseter	-Slurping action required a longer mastication period but a smaller EMG amplitude. -Cutting the noodles short reduced the mastication effort.

[6] Data obtained from recording of jaw movements

Training. It has been established that once an efficient chewing pattern is found, it is learned and repeated (Soboleva et al., 2005). Subject training is another important factor for EMG studies. Studies found that trained panellists in sensory assessment exhibit a more constant chewing pattern than untrained subjects across the samples and sessions. For some reasons, trained panellists were found to exert more chewing work[7] over the chewing sequence and to have longer chewing times than untrained panellists (González et al., 2002, Mioche and Martin, 1998)

3.4 Data analysis

3.4.1 Processing of raw EMG signals

The raw electrical signals obtained from the monitoring of mastication by electromyography are of the type displayed in Figure 3.7A and are normally known as raw EMG signals, plotted in a two dimensional diagram of voltage measured in microvolts (μV) against time measured in seconds (s). The raw EMG signal presents the amplified potential difference detected at the electrode recording site. The plot can be seen as a representation of the chewing pattern for the comminution of a specific food. The graph begins with a low amplitude baseline that indicates the rest position of the mandible (state at which teeth are slightly separated). When the muscle contracts to perform a chew, there is a burst of electric signals with suddenly increased amplitude. The signal quickly returns to its baseline once the activation of the muscle has ceased (occlusal stage of a chewing cycle). The EMG amplitude has a close correlation with the magnitude of the biting force, a fact suggesting that an increase in neuronal activity will result in more muscle fibres being recruited and consequently a stronger muscle contraction (Kolstra, 2002, De Luca, 2006). As a result of the rhythmic and cyclical nature of a mastication process, the EMG signal of an eating process is usually a sequence of electrical bursts with each burst correspondent to one chewing cycle. Such a pattern would normally continue until the food bolus achieves a consistency suitable for swallowing.

[7] In this study referred to as muscle activity.

The raw EMG signals are very spiky and bipolar in nature (Figure 3.7A). This makes it difficult for interpretation or numerically analysis. For instance, if the amplitude is to be quantified the average will simply be zero due to the cancellation of positive and negative values. Therefore, raw EMG signals serve more as a qualitative resource rather than being of quantitative value. Number of bursts and their corresponding onset-offset (contraction-rest states) of the muscle can be visualized and counted from this electromyogram.

In order to extract more useful information from EMG signals, a quantitative analysis of raw data is required. The most common strategies of EMG data analysis include rectification, integration and the root mean square (RMS) calculation.

Rectification Rectification is the conversion of bipolar EMG signals to unipolar signals. A rectified EMG signal will exhibit only the positive deflections of the original signal. This can be achieved in two different ways. The first, less commonly used one is called half-wave rectification, it is achieved by simply removing the EMG signals below the baseline (negative deflections). On the other hand, a full wave rectification is done by transposing the negative portion of the signal to the positive side by taking the absolute values (see Figure 3.7B). This method conserves all the energy detected and is the most preferred method for the analysis.

Integration Computing the integral value of the raw EMG signal would return a zero value due to approximately equal positive and negative excursions. Therefore, the integration must be carried out on the full rectified wave across the whole signal spectrum. A typical integration curve is shown in Figure 3.7C, where the integral expressed in microvolts seconds ($\mu V \cdot s$) raises rapidly when there are large bursts of activities and remains little changed when no muscle contraction is taking place. The value of the integral is a measure of the electrical activity within the detecting field of the electrodes. It reflects the overall muscle activity or energy produced by the muscle. Apart from full integration of a rectified EMG curve, partial integration of each individual cycle of bursts can also be done. This analysis could be of particular interest to investigate the changing behaviour of chewing

(length and muscle activity) throughout an eating process when the food changes its textural properties and more saliva gets involved.

Root Mean Square (RMS) The RMS of an EMG signal is calculated by summing the square of each sample of the EMG signal within a recording window, dividing by the number of samples within the interval of interest and finally taking the square root. The calculation of RMS does not require a full wave rectification. The general equation to compute the RMS of a given EMG signal is as follows:

$$A[n]=\left[\frac{1}{L}\sum_{n-L+1}^{i=n} S_i^2\right]^{1/2} \qquad (1)$$

Where, S_i is the EMG signal, at sample *i*, A(n) is the RMS of the EMG signal and *L* corresponds to the smoothing window length or a specific time period (Melaku et al., 2001). The RMS is calculated successively through the duration of the raw EMG signal. The time window is an important parameter when computing RMS. The smaller the time window the less smooth the resulting curve will be. However in order to get a curve that follows the trend of the underlying rectified EMG but without the spiky peaks that are evident in the signal, it is better to use a moving average in which the time windows overlap instead of covering discrete sections of EMG (Payton and Barlett, 2008). The RMS calculation is widely used as it provides graphs with waveforms that are easier to analyse than the spiky raw EMG (see Figure 3.7D). RMS is normally recommended as the preferred parameter for quantifying the EMG amplitude. The amplitude of the EMG signal is an indicator for the size of active motor units and therefore the RMS highlights the strength of the muscle contraction (Melaku et al., 2001).

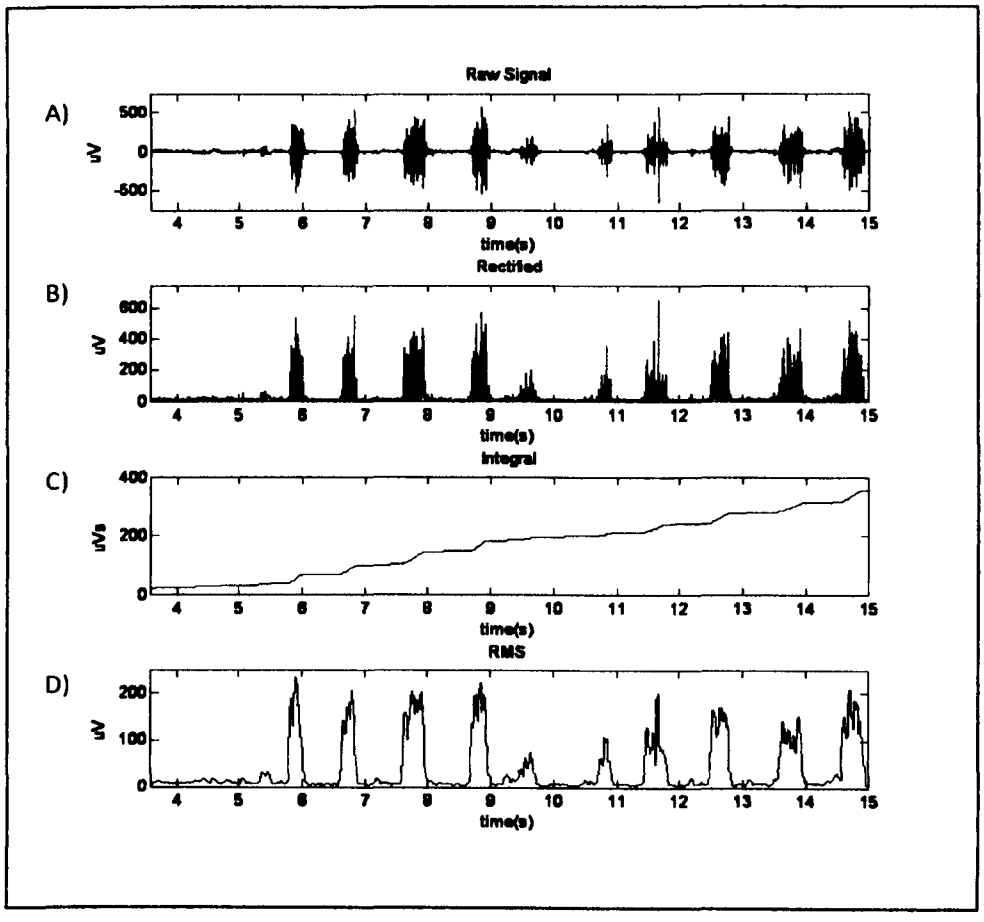


FIGURE 3.7 A typical example of the recorded EMG signal (electromyogram) and processing of the signal. *A)* Raw EMG signal (unprocessed signal), *B)* Rectified signal (full wave rectification), *C)* Integration of rectified EMG signal and *D)* Root Mean Square (RMS)

3.4.2 Masticatory parameters: analysis of chewing sequence and individual chewing cycles.

Rectified signals are often used for the analysis of chewing patterns. The analysis of the rectified results can be carried out on the whole chewing sequence, chew-by-chew or on certain sections of the chewing sequence. Various masticatory patterns could be extracted in relation to time (s) or amplitude voltage (μV), in order to characterise chewing behaviour.

3.4.2.1 Analysis of the whole chewing sequence

The masticatory sequence can be defined as the whole set of oral actions performed from the moment when the food is being ingested (opening of the mouth) until the terminal swallowing action (oral clearing) has been completed (complete removal of food from the mouth, jaw returns to stable rest position). A whole masticatory sequence can normally be divided in three phases: ingestion, main chewing sequence, and swallowing and oral clearance.

Ingestion

The ingestion phase is the transfer of food into the mouth and involves the following actions:

- The opening of the mouth;
- Incision or the biting of food by the front teeth. This action is especially performed when a suitable sized portion needs to be bitten off from a large piece of food. Soft foods can also be sheared by incisors;
- Prehension. The food is secured by lips. For example wiping off the food from a spoon when eating yoghurt or slurping when eating noodles;
- Transfer of food to between the teeth by the tongue.

Incision or prehension actions do not always occur during the chewing sequence as they depend on the food being consumed. For instance, if the food is of a size small enough for oral manoeuvring, then only mouth opening and food transportation to the molar teeth will be involved.

Main chewing sequence

This is the main part of an eating process in which food is comminuted and converted to a swallow-able bolus by rhythmic chewing. For solid foods, they undergo a series of chewing cycles and become softened with the help of saliva (Van der Bilt et al., 2006). On the other hand, low viscosity liquids are already in a swallow-able form and therefore require minimal processing other than checking. Some acidic, cold or strongly tasting liquids may

be held in the mouth for a period of time to allow buffers in saliva to raise pH, to equilibrate to body temperature, to be diluted by saliva, as well as to be fully appreciated for its taste and flavour (Prinz et al., 2006).

Many foods however, are neither perfectly solid nor perfectly liquid, existing in a state often referred to as "semi-solid" (or soft solids). Typical examples of this kind of foods are: gelatine desserts, jellies, ice creams, gum confections, peanut butter, puddings, etc. Some of these foods melt into a mobile fluid once put into the mouth (e.g. gelatine desserts, ice creams). However, some of them do not melt (e.g. agar gel which has a melting point of 98°C) and therefore need to be chewed into small lumps for swallowing (Bourne, 2002).

It was suggested that facial muscles may have a limited role when soft semi-solid foods are the focus of study. For such foods, very little muscle activity is required and there is limited mandibular movement. Therefore, tongue activity should be monitored instead(De Wijk et al., 2006). This is simply because the tongue is involved in moving and transporting food inside the oral cavity. The author observed that the suprahyoid muscles could be highly active even when the mouth is not in the opening phase, a fact that indicates close association of this muscle with tongue movement. Castro and collaborators (1999) also concluded that the anterior belly of the digastric was active in all tongue movements (lateral, placement on both the hard palate and the soft palate, and on the floor of the mouth) except in retraction. Figure 3.8 shows a recording of 60*s* of EMG activities of a female subject who was asked only to move the tongue around the mouth, simulating the removal of food from the cheeks or palate. It can be seen that masseter and temporalis muscles remain inactive and the only muscles activated during tongue movements are the suprahyoid muscles or more specifically the digastric muscle. The same tendency was also observed in twelve more subjects (9 female and 3 male). This observation shows that the recording of suprahyoid muscles could be extremely useful when soft semi-solid or fluid foods are the matter of study. For example, in an earlier study Dea *et al.* (1988) applied EMG technique for the textural evaluation of semi-fluid model foods by measuring the electrical potentials produced in the muscles located under the chin (suprahyoid muscles) to assess the perceived thickness. They found that sensory thickness correlates closely with the activity of the muscles that control tongue movement.

Clearance and swallowing

The clearance phase in an EMG spectrum is commonly characterized by the presence of some random and non-rhythmic bursts of muscle activities (Brown, 1994). Additionally, little activity in jaw-closing muscles, compared with opening muscles, is also clearly appreciated during this phase (Hiiemae et al., 1996). The duration of clearance is highly dependent on the nature of the food product. For instance, a sticky food tends to remain adhered to the teeth, tongue or palate and therefore would be expected to exhibit a prolonged period of oral clearance and increased activities of opening muscles. Visual identification of swallows within an EMG spectrum is possible and their identification could be useful for EMG analysis and for the extraction of masticatory parameters. The swallowing actions are normally classified as follows:

- *Interposed swallows.* These actions occur within the main chewing sequence, identified as short pauses within the rhythmical sequence containing usually a single burst of activity from the masticatory muscles. These swallowing actions can be preceded or succeeded by chewing cycles and therefore are normally mistakenly included as chews within the analysis. The interposed swallows can correspond to a swallow of the liquid portion and fine particles of the food, the main portion of food that is not yet ready to be swallowed is retained (Brown, 1994, Okada et al., 2007).
- *Oral clearance and terminal swallow.* This corresponds to the final swallowing action, right towards the end of an eating process. A terminal swallow is preceded by limited irregular mandibular movements, which are called pre-swallowing cycles (Okada et al., 2007)

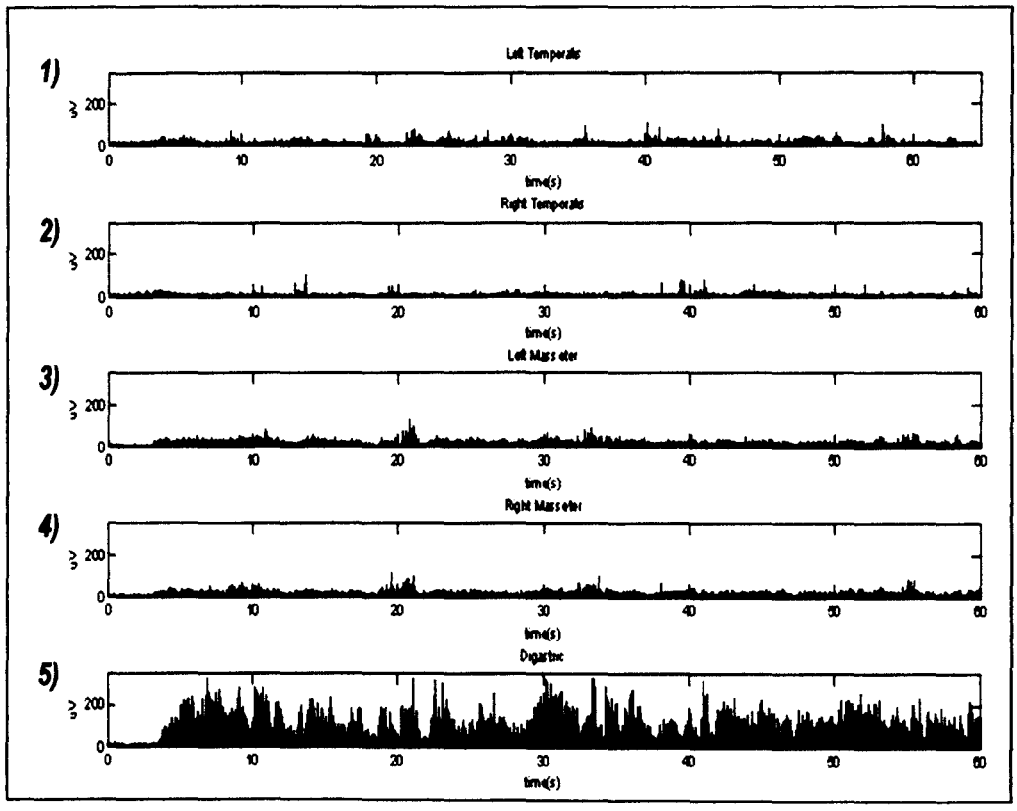


FIGURE 3.8 Rectified signals recorded during tongue movements carried out by a female subject (1: Left Temporalis; 2: Right Temporalis; 3: Left Masseter; 4: Right Masseter; 5: Bi-lateral digastric).

Figure 3.9 shows the EMG patterns of elevator and depressor muscles during eating three different products of standardised shape and size (a pectin-based jelly confection, carrot and toffee). The different phases of the eating process are clearly distinguishable. *A* represents the rest state of the muscles, *B* is the phase of ingestion, *C* is the phase of oral clearance and terminal swallow, while the main chewing sequences are seen between phases *B* and *C*.

Terminology of masticatory parameters has been diverse in the literature as shown in Table 2. Different terms have been used even when they refer to parameters of the same physical meaning. Below is a list of the EMG parameters most often used in relation to eating and food texture studies.

- **Total sequence duration (s).** This is the total time of oral processing of a food material and is considered to be the lapse between the first mouth opening (food ingestion) and the terminal swallowing.

- **Time of main chewing sequence (s).** This is the period of time that includes all the regular jaw movements (chewing cycles) excluding the period of oral clearance (pre-swallowing cycles)

- **Number of chews.** This is counted as the number of regular bursts or strokes identified within the main chewing sequence.

- **Muscle activity of a chewing sequence ($\mu V \cdot s$).** Also called the total muscle word or total energy. This is a measure of the electrical activity within the field of the electrodes. It corresponds to the area under the rectified EMG signal and can be evaluated through the integration of the full rectified signal. This parameter is a measure of the chewing or masticatory effort (Diaz-Tay et al., 1991, Kohyama et al., 2010). Dividing this parameter by the total number of chewing cycles gives the average work per chewing cycle.

- **Time of clearance (s).** This parameter is normally reported as the clearing time. It refers to the time between the end of chewing cycles within the rhythmic chewing sequence and the end of eating process when the mandibular returns to its rest position.

- **Masticatory frequency.** This is the rate of chewing and is equal to the number of total chews divided by the time of main chewing sequence.

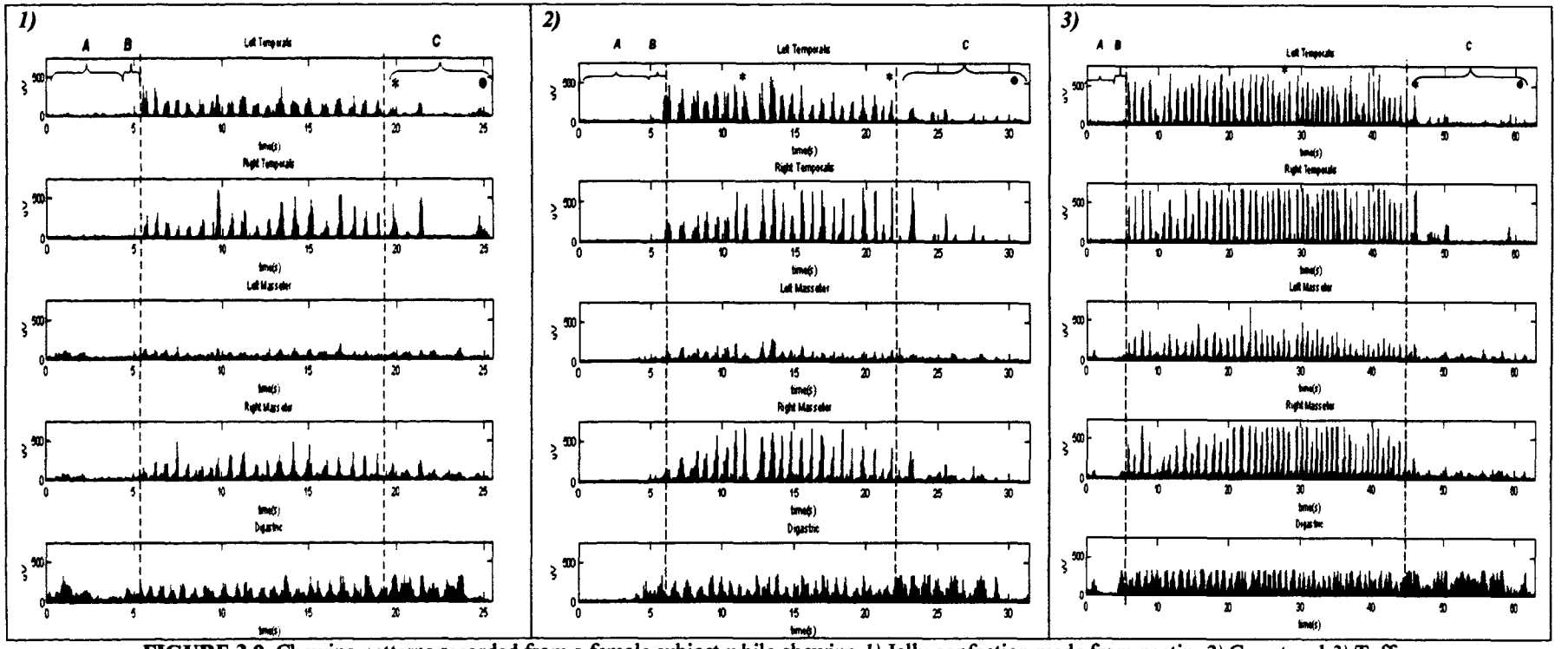


FIGURE 3.9 Chewing patterns recorded from a female subject while chewing *1)* Jelly confection made from pectin, *2)* Carrot and *3)* Toffee
EMG signals from top to the bottom: Left Tempralis, Right Temporalis, Left Masseter, Right Masseter and Digastric (signal corresponds to the suprahyoid muscles) *A)* Rest period of the muscle (mouth is closed), *B)* Ingestion phase (Opening of the mouth, activity is just observed in the digastric muscle) C) Clearance phase (irregular jaw movements or preswallowing cycles). The section between dotted lines corresponds to the main chewing sequence (rhythmic chewing movements). *Indicate possible interpose swallowing and • End of oral processing and terminal swallowing action

3.4.2.2 Analysis of Individual Chewing cycles and Muscle Bursts

A chewing cycle consists of a burst of activity, for a muscle, followed by its rest period. Different masticatory muscle signals could be out of phase, depending on their association with the oral action. For example, when closing and opening muscles are recorded simultaneously the bursts will appear alternated in time sequence. Burst activities of depressor muscles will be exhibited during the period of jaw opening while the elevator muscles remain at rest and vice versa. It should be noted that even though the elevator muscles only activate during approximation of the mandible and maxilla the digastrics, a depressor muscle, could also be activated during jaw closing. This is probably due to tongue movement or for controlling the speed and force of closing (Ferguson, 1999).

Every chewing cycle within the chewing sequence can be divided in two main phases:

1) Preparatory phase. This phase can be subdivided into two phases: *Opening Phase* and *Closing Phase*. The former consists of the jaw movement for the positioning of the food involving mainly the activation of depressor muscles while jaw elevators are relaxed. The latter refers to the period when the jaw elevates until resistance is detected between the teeth. This phase is accomplished by the contraction of the masseter, medial pterygoid and temporalis muscles.
2) Occlusal phase. Sometimes called the crushing phase, from the point when the teeth make contact with the food to until there is tooth to tooth contact or until the jaw begins to open again. During this phase there is an increasing contraction force in all elevator muscles and stimulation of periodontal receptors occurs.

Brown and collaborators (1998) associated specific jaw movements within the trajectory of a chewing cycle to their corresponding segment in EMG signal by coupling kinematic and EMG recordings. They determined that bursts of activity of closing muscles for each chew embrace two portions one correlated to a vertical closing movement followed by a clenching action which includes a degree of horizontal movement (see Figure 3.10). This differentiation is particularly important in analyzing chewing patterns of different foods. It

could provide some useful masticatory information that can be correlated with the texture changes of the food during oral processing.

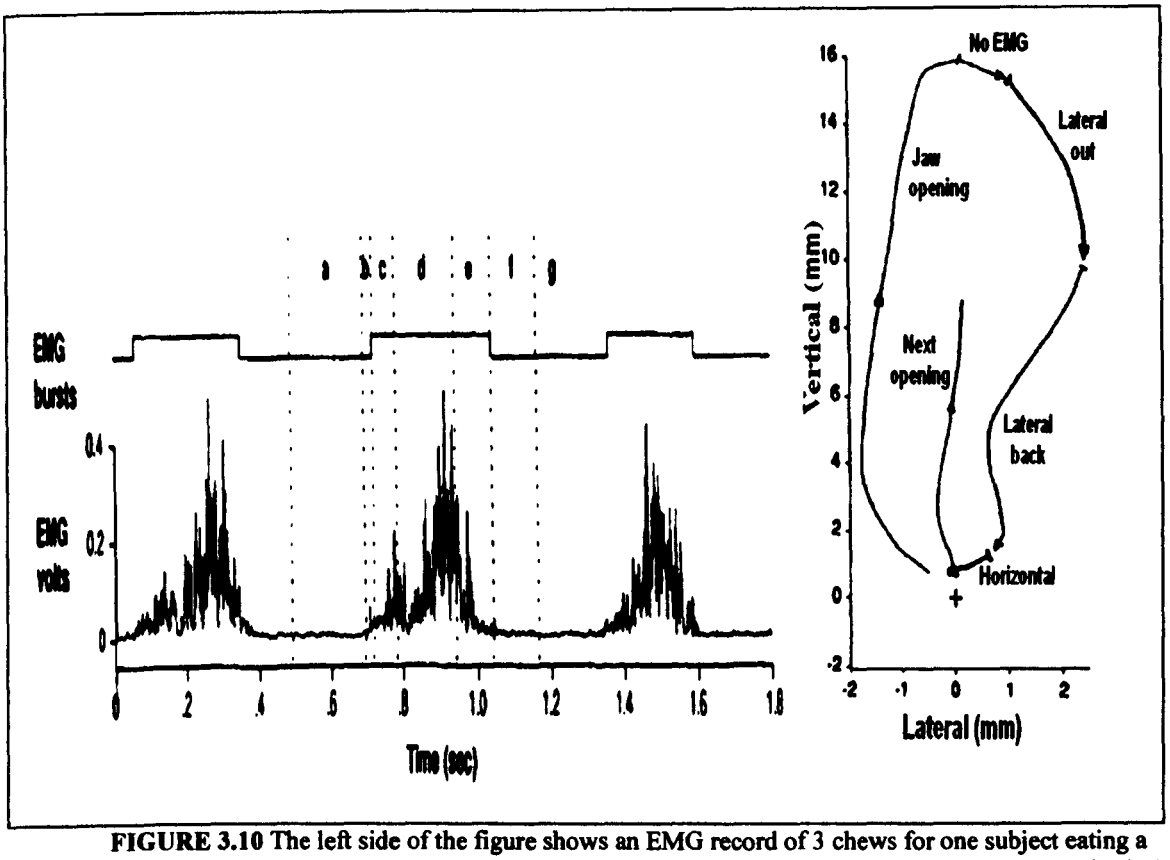


FIGURE 3.10 The left side of the figure shows an EMG record of 3 chews for one subject eating a biscuit. The pooled EMG signal is shown together with identification of the start and end of each EMG burst. a-g indicate segments of the movement pattern which are reflected in the jaw trajectory shown on the right side of the figure. a= jaw opening, b=start of jaw closing with no associated EMG signal, c= closing movement directed away from the midline (lateral out), d = closing movement directed back towards midline (lateral back), e= horizontal movement associated with EMG signal, f= movement with no associated EMG, g= next opening (modified from(Brown et al., 1998))

Due to the extensive amount of data that can be collected from each EMG test, the analysis of all oral actions is not an easy task. Most modern EMG apparatuses provide software which allows users to carry out identification of bursts manually by moving the cursor across the displayed EMG signal. However, the whole analysis could be time consuming and involve human error in determining the exact start and end point of a chewing cycle. Some studies have reported the use of a specific program or other techniques in

combination to identify bursts of activity and calculate other useful masticatory parameters. For instance Brown *et al.* (1994) reported the use of a written program Spike2 (Cambridge Electronic Design Ltd, Cambridge, UK) for the analysis of every chew but also to compute cumulative data over predefined sections of EMG data for a chewing sequence. However, scarce information was available on the details of the program. In 1997, Green *et al.* reported an algorithm for Matlab able to identify onset and offset of muscle activity, a brief explanation about the algorithm employed can be found in their paper. Abbink *et al.* (1998) published a paper about a method of automated detection of onset and offset of rhythmic muscle activity in electromyograms. Sun *et al.* (2001) developed an electronic sensing system (ESS) whose software was developed using Microsoft Visual Basic (6.0, Microsoft Corp., Redmond, WA) that allows analysis of the entire chewing sequence bite by bite, allowing observation and quantification of dynamic texture changes during mastication.

Regardless of the method used to extract them, some common masticatory parameters have been used in the analysis of individual chewing cycles. These are listed below (see Figure 3.11.

Muscle onset and offset (s): The muscle onset corresponds to the time at which the burst starts (muscle is being contracted and therefore the amplitude of the EMG signal starts increasing rapidly). On the other hand, muscle offset refers to the ending of the burst. At this point there is cessation in the activity of the muscle and therefore the EMG signal approaches the baseline (muscle returns to its rest position).

Burst duration (s): The period of time where the muscle is active. It is equal to the offset time minus the onset time.

Interbust time (s): This refers to the rest period of the muscle where no activity is present. It is the period of time between the end of the burst and the start of the following one.

Cycle Time (s): As a chewing cycle is formed by one burst of activity of the muscle and the following rest period, the cycle time is the sum of the burst duration and the interburst time.

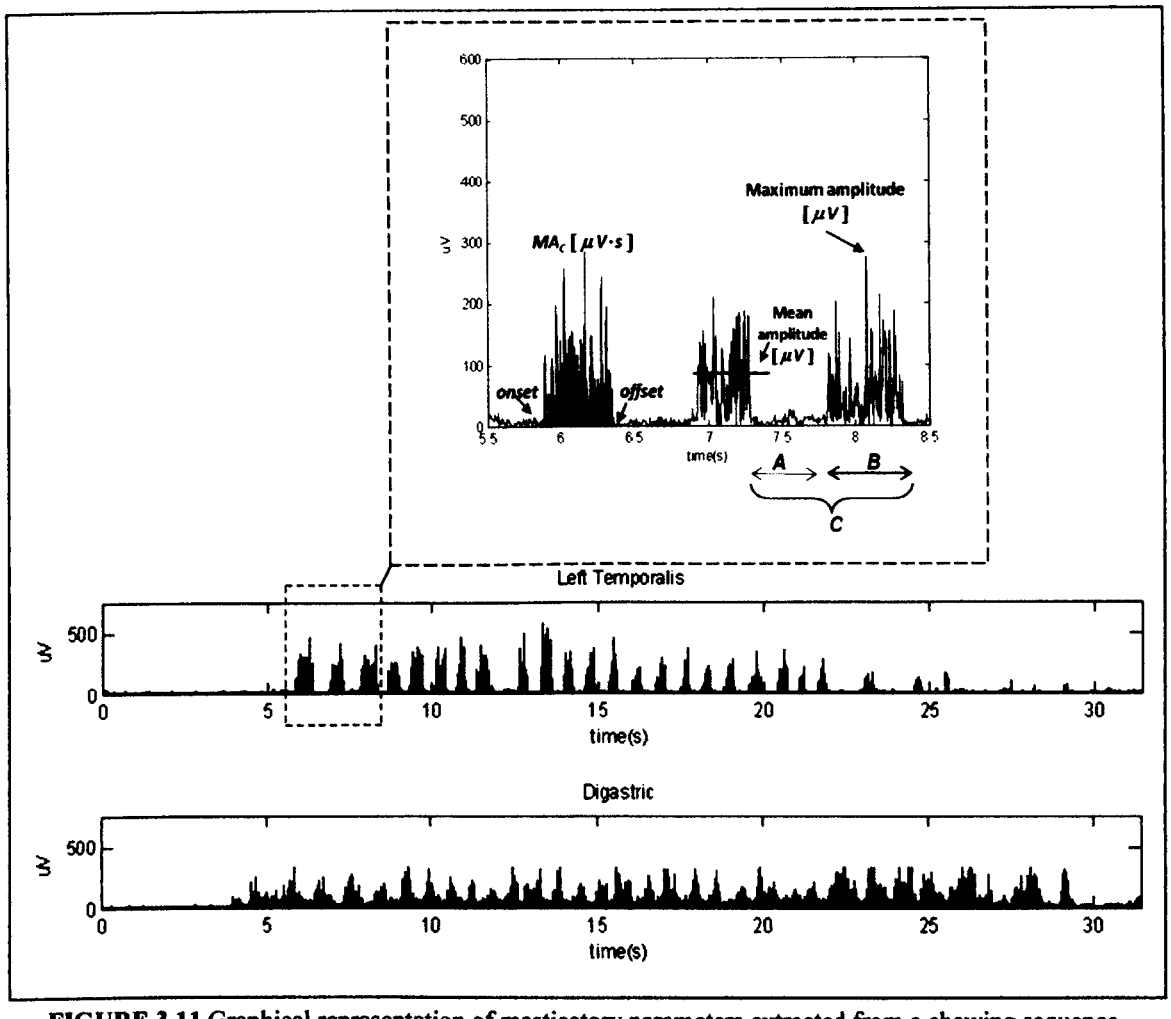


FIGURE 3.11 Graphical representation of masticatory parameters extracted from a chewing sequence. *A*: Interbust time, *B*: Burst duration, *C*: *Chewing cycle time*, MA_C: Muscle activity per chew (dark area under the curve)

Maximum amplitude (μV): This is the maximum voltage value recorded by the EMG signal within the burst (also named as peak voltage).

Mean amplitude (μV): Mean value of the voltage per burst.

Muscle activity per chewing cycle *(μV·s)*: Energy or muscle work applied in each chew to perform the corresponding muscle action (opening or closing depending on the muscle

being analysed). This value normally correlates qualitatively well with the magnitude of force applied. It corresponds to the area under the curve of each burst. It can be calculated as the integral value between the muscle onset and offset. Sometimes it can be derived as the product of the mean amplitude and the duration of the activity burst (Brown et al., 1994).

Length of chewing cycle (s): This is the time from the beginning of a burst to the beginning of the next burst.

Ascending and descending energy *(μV·s):* The ascending energy corresponds to the area under the ascending curve before the peak of the burst, while the descending energy corresponds to the area under the descending curve after the peak (Eves et al., 1988, Carson et al., 2003). Though the exact meaning of these two parameters is not yet clear, one speculation is that they could be linked respectively to what Brown and collaborators (1998) defined as the chewing work for the vertical phase and the chewing work for the horizontal phase of closing.

Peak energy *(μV·s)*: This is given as follows:

$$W_p = E_p t_p$$

Where:

W_p = Peak energy *(μV.s)*

E_p = Peak voltage (V)

t_p = Peak width at the $\frac{\sqrt{2}}{2} E_p$ locations (s)

This parameter can be larger than the total energy (Figure 3.12B) or smaller that the total energy (Figure 3.12A) of a chewing cycle. (Sun et al., 2001, Peng et al., 2002)

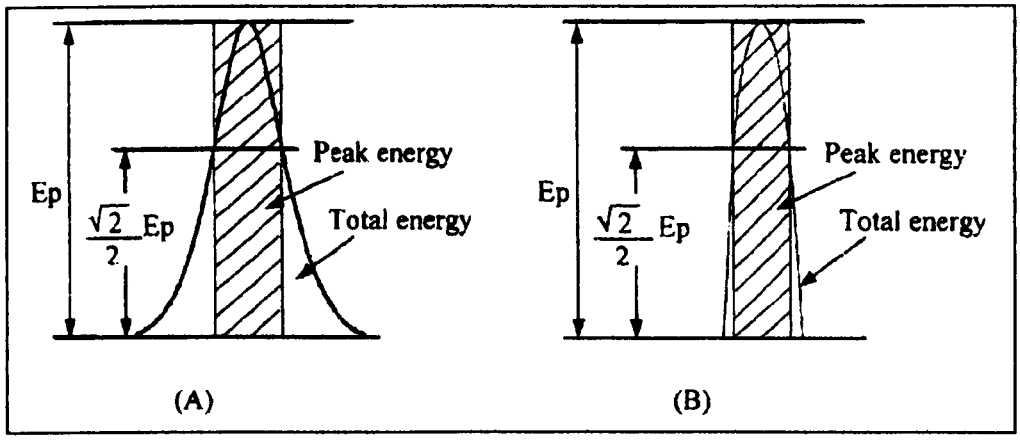


FIGURE 3.12 Definition of total energy and peak energy
The area under the curve is defined as total energy, and the shaded area is defined as peak energy
(From (Peng et al., 2002))

3.5 Summary

Since its first application in the 1980s, the EMG technique has been increasingly used for eating studies in relation to food properties, oral physiological conditions, and dental functions. The EMG technique is extremely useful to reveal oral responses to different foodstuffs (e.g. solid or fluid form, different geometries, different size or weight, or of different volume) or to understand oral physiological attributes to food texture appreciation. It is particularly useful in the study of food hardness, food toughness, food stickiness, food consistency, etc. Accounts of such research have been abundant in the literature. Table 3.2 summarised a few typical studies to highlight different experimental profiles for different applications.

The technique offers reliable real-time measurements of the activities of facial/oral muscles during a mastication process. In order to obtain good quality EMG data, experimental works have to be properly planned in detail and in advance, including the choice of electrodes, selection and location of target muscles, and the set up of the experimental environment. Selection of participating subjects must follow criteria specifically designed for the project and ethical approval must be obtained beforehand.

Data analysis and interpretation could be the most time consuming part of EMG studies and is still an area to be explored. A number of parameters can be extracted from EMG signals either for the whole eating sequence or for individual chewing cycles or even for some particular oral actions (such as mouth opening and closing, tongue movements, swallowing, oral clearing, etc). These parameters can give both qualitative and quantitative description of oral behaviour in response to specific textural properties of a food during an eating process. In general, EMG is a promising tool for food scientists and industrial researchers in studying food texture and in new product development for enhanced oral experience.

Chapter 4

Materials

4.1 Selection of samples

Foods, as the rest of other materials found in nature, do not always exhibit a tendency to adhere to a contact surface and therefore not all are regarded as sticky. In fact, Fiszman and Damásio (2000) carried out an extensive literature review of research works where the stickiness properties of several foodstuffs were the object of study in relation to food processing, handling or during consumption. They derived the classification of different foodstuffs of solid and semisolid character possessing stickiness properties into four main food categories: bakery and cereal-derived products, cheese, gelled systems, meat protein products and a miscellaneous group. The last category embraced several sticky products that did not fall in any of the former groups.

The main focus of the research was the study of food stickiness as a perceived as a sensory attribute and its association with oral behavior. Therefore based on the literature it was possible to create a classification, like the one created by Fiszman and Damásio, but which grouped food which were studied regarding their stickiness.

Such a classification is depicted in Table 4.1, it should be noticed that meat protein products group in this case has been withdrawn and dairy products have been introduced. This is because stickiness in meat has been studied merely as a factor affecting the integrity of the final product (for example the effect that certain ingredients might have on the product cohesiveness as in the filling of sausage like products) rather than its importance as a mouthfeel attribute. In that sense meat has rarely been judged as sticky product in sensory evaluations.

TABLE 4.1 Solid and semisolid foods perceived as sticky during consumption

BAKERY AND CEREAL DERIVED PRODUCTS	▪ Bread (white and whole flour)[d j] ▪ Biscuits[d j] ▪ Cake[d] ▪ Crackers[d j] ▪ Corn and flour tortillas or arepas[a] ▪ Papadum ▪ Cooked rice[i] ▪ Cooked pasta and noodles[d] ▪ Rice cake (mochi)[h] ▪ Cereal[d]
DAIRY PRODUCTS	▪ Cheese[d] ▪ Condensed milk[c] ▪ Cream[c] ▪ Yoghurt[c]
GELLED SYSTEMS	▪ Confectionery products[f, g] ▪ Jams[c] ▪ Custard[e] ▪ Tofu[k]
MISCELLANEOUS GROUP	▪ Dry fruits[j] ▪ Honey[c] ▪ Peanut butter[d] ▪ Potato chips[d j] ▪ Mayonnaise[e] ▪ Warm sauces[e] ▪ Salad dressings[c]

[a] Reyes-Vega *et al.*,1998; [c] Chen *et al.*, 2008; [d] Caldwell, 1962; [e] Dunnewind *et al.*, 2004; [f] Garcia, 2000; [g] Foegeding and Steiner, 2002; [h] Kohyama *et al.*, 2007b [i] Kohyama *et al.*, 2005; [j] Kashket *et al.*, 1991; [k] Murdia and Wadhwani, 2010.

Confectionery products were the food materials selected for this study based on several reasons that are explained next.

Working with confectionery products offered many advantages. The combination of polysaccharides and sugars, forming their structure provides a wide range of textural properties. Even when they are composed of similar ingredients, the obtained products are distinguished from one another to a large extent on the basis of their texture (DeMars and Ziegler, 2001). This allows a comparison of textural properties to be made. In such comparisons textural properties can be ranked on the basis of the intensity of the attribute present in the food material.

In many foods stickiness is normally an undesired attribute because of the unpleasant oral experience and the inconvenience of handling. In confectionery products stickiness becomes not only tolerable but many times an expected property. However, it must be

emphasized that this could largely depend on preferences and be related to the efficiencies of oral breakdown (Brown and Braxton, 2000). For example, it has been popularly recognized that stickiness is only widely accepted as a textural property in every day food by people in Japan (Tanaka, 1986). So while stickiness in cooked rice is a factor of rejection in western countries for most Asian countries that is not only pleasant but also an expected property (Kilcast and Roberts, 1998).

The fact that subjects will expected confectionery products to be sticky makes them a good candidate for inclusion especially in a sensory test which involves ranking of the products based on stickiness. It is assumed that as the subjects expect the products to be sticky their rankings will not be influenced by psychological factors.

One of the main challenges of using natural food products in texture studies has been their combination of complex structures making up complex biological compounds. The use of confections avoids to a certain extent the complexity found in biological systems due to their low water activity. Low water activity helps maintain the texture of the food and promotes bacteriological stability. This makes confections real products which are suitable for characterization of food systems.

Selection basically consisted of a preliminary evaluation of samples including the performance of a sensory test to prove, among other things, the feasibility in the preparation of samples to be tested. The sensory test was also used to find whether a difference in stickiness between the products was perceived during mastication by subjects and whether the level of stickiness present in each sample was possible to be ranked.

4.2 Description of samples

The process of sample selection resulted in the choosing of six commercially available confectionery products of semi-solid character and viscoelastic properties for investigation in this study. Products consisted of well known brands available at UK supermarkets to ensure availability throughout the whole research (see Figure 4.1)

FIGURE 4.1 Commercial brands used in the study

Table 4.2 presents a description of the main samples used in this study. Ingredients and nutrients composition are as provided by the manufacturer. The codes provided allude to the system used for identification and brevity when referring to each single sample during this study.

4.3 Description of confectionery products

In general non-chocolate confections like the ones studied can be defined as highly concentrated solutions of carbohydrates containing acid, colour, flavour, texture agents and stabilizers.

As in other food products, the texture agents in confectionery products are hydrocolloids (polysaccharides, proteins or fragments of proteins). These ingredients, due to their specific functional properties such as gelling properties, film forming, adhesion control, aeration, emulsification, binding and stabilization, are used to provide different rheological properties that give rise to the creation of a great variety of textures. The resulting texture depends on the type of gelling agent, the source and concentration as well as the physical properties of the particular system. The main biopolymers present in the confections used in this study are highlighted in Table 4.2 and described briefly next.

Gum Arabic

(Frui-tella and Milk-chews)

This polysaccharide is an exudate from trees of the Acacia Senegal species from which it receives also the name of gum acacia. It is a very soluble compound and therefore is mixed with other ingredients in order to obtain sweets. The solution is heated until achieving around 30-50% gum. In hard gums, it constitutes 50% of the total solid matter which gives a hard and short texture, whilst remaining malleable. A reduction in the content of gum with the addition of gelatine produces softer eating confections normally called pastilles. Maximum viscosity is exhibited at pH 6, falling above pH 9 and below pH 4.

Pectin

(Fruit jellies and Fry's Turkish delight)

Pectins are polysaccharides normally obtained from the cell walls of fruit. Commercially pectins are most often extracted from apple pomace and citrus peel. There are two distinctive types of pectin used in the confectionery industry according to their degree of esterification or methylation, which is represented as DM. The DM indicates the average number of methoxyl units per 100 galacturonic units. The first type of pectin is knows as high methoxyl (HM) and corresponds to those pectins which have an esterification degree greater than 50 percent. These are further subdivided, by the speed with which they set, into rapid set (DM= 68-72 %), medium rapid set (DM= 66-70%) and slow set (DM= 59-64%). The second type is the low methoxyl pectins (LM) which possess a degree of esterification of less than 50 percent.

In general HM pectins are the ingredients normally used in fruit flavoured jellies, whilst LM pectins are suitable for products with a neutral pH.

Table 4.2 General description of food samples used in this work.

Foodstuff	Manufacturer or sales agent	Sample Code	General Description	Main ingredients	Nutrition Information (where not indicated, value per 100 g)
Fruit-Tella	Perfetti Van Melle	FRUT	Flavoured chewy sweets	Glucose syrup, sugar, hydrogenated vegetable oil, fruit juices from concentrate (3%), acid (citric acid), ***gelatine****, humectant (glycerol) concentrates (eldelberry, black current), natural flavourings, ***gelling agent (gum arabic)****, dextrin, spinach and nettle extract, carrot extract, paprika extract.	NA
Werther's Original	Storck	WO	Chewy Toffees with real butter and fresh cream	Glucose syrup, ***sweetened condensed milk (21.6%)****, sugar, vegetable oil, humectant sorbitol, ***whey powder****, butter (2.4%), salt, treacle, emulsifier soya lecithin, flavouring.	Protein 3.5g, Carbohydrate 71.3g of which sugars 40.7g, Fat: 15.2g of which saturates: 9.1g, Fibre:<0.1g, Sodium 0.2g
Fruit Jellies	Thorntons	JT	Jellies made with real fruit and then lightly dusted with crisp sugar	Sugar, glucose, syrup, water, dextrose, lemon comminute, natural flavourings, orange comminute, dried apple (contains soya, lecithin), natural colors (curcumin, carbon black mixed carotenes, anthocyanins), ***gelling agent (pectin)****, citric acid, acidity regulator (sodium citrate), apple comminute, dried blackcurrants, dried strawberries, sodium polyphosphate, sulphur dioxide	Protein 0.1g, Carbohydrate: 76.8g of which sugars 76.8g, Fat trace of which saturates trace, Fibre:0.6g, Sodium 0.25g
Milk Chews	Sweetie World	MCH	Milk flavour chew candy	Glucose syrup, sugar, hydrogenated vegetable oil, ***gelatine****, humectant (glycerol), ***thickener (gum arabic)*** *, nature identical flavourings.	Protein 1g, Carbohydrate 83, 5g, Fat 7g
Fry's Turkish Delight	Cadbury	FR	Turkish Delight covered with milk chocolate	*Milk chocolate (24%):* sugar, dried whole milk, cocoa butter, cocoa mass, dried whey, vegetable fat, emulsifier (E442, E476), flavourings. Turkish delight: sugar, water, ***modified maize starch****, ***gelling agents (pectins)*** *, colour (E129), flavourings.	Protein: 1.6g, Carbohydrate 74.3g, Fat: 7g
M&S Turkish Delight	Marks & Spencer	TD	Rose and lemon flavour Turkish delight coated with icing sugar	Sugar, water, ***maize starch****, acidity regulator (E336), lemon oil, fruit and vegetable concentrates, natural flavouring.	Protein trace, Carbohydrate 85.8g of which sugars 78.6g, Fat trace of which saturates: nil, Fibre: nil, Sodium 0.10g

NA- Not Available
*Hydrocolloids- Imparters of texture properties (polysaccharides or sources of milk proteins)

Fruit jellies contain between 1.0 and 2.0 percent extra slow set HM pectin buffered with citrate or tartrate salts to control setting speed. The finished product exhibits a short tender texture with excellent flavor release. The product is normally sanded in sugar (Carr, 1996).

Pectin can also be successfully combined with modified starch. For example, in Turkish delight confection a LM pectin is combined with modified starch to yield a product of short texture and less adhesiveness than the traditional starch-based Turkish delight (Carr, 1996).

Starch

(Marks &Spencer Turkish Delight and Fry's Turkish delight)

Starch is a natural polymer, the monomeric unit of which is glucose. It exists in straight chains (amylose) and branched chains (amylopectin). Starch forms highly milk-white gels having a short texture and relatively heavy-bodied mouth-feel (Rosentthal, 1999)

Turkish delight or lokum is a sugar-based jelly-like confection. It is made from starch and sugar and it is often flavoured with rosewater or lemon. The commercial product is normally presented as a chocolate bars or dusted with sugar due to its high stickiness.

Although Turkish delight can be prepared using starch in combination with other gelling agents (e.g pectin, agar or gelatin) as in the case of Fry's Turkish delight (FR), it has been claimed that best-known brands are only starch-based as in Marks &Spencer Turkish delight (TD). Texture, surface brightness and transparency are the most important quality attributes of lokum, which are achieved by complete gelatinization, in which starch should be completely denatured. Acid modified maize starch is deemed as the best one for Turkish delight production. The use of natural starches requires more water to gelatinize, causing an opaque and amorphous structure. By using modified starches the water required for gelatinization is less. Starches which have been chemically modified reduce shear break-down and retrogradation. The texture of the finished product is normally soft and elastic (Lees and Jackson, 1973, Batu and Kirmaci, 2009).

Milk Protein

(Toffee)

Although in other countries there is a difference between the words caramel and toffee, in the UK these terms are used interchangeably and used to refer to products ranging from viscous liquids to hard sugar glasses (Edwards, 2000). All toffees contain milk solids and usually some fat (butter and vegetable fat). The milk solids are made of protein and carbohydrates (lactose). The function of milk protein apart from providing flavor and colour characteristics to the confection by undergoing Maillard reaction, is to stabilize the emulsion of fat in the sugar phase possibly by binding some of the water (Tamine, 2009). The main sources of milk solids and therefore of protein for the production of toffee is sweet condensed milk which is preferred over fresh milk. Fresh milk has a large amount of water and consequently requires longer boiling times during production. The higher the level of milk solids present, the harder the product. Casein is the major component of the milk solids and therefore contributes to the hardness of the product. Whey powder is sometimes added as a substitute for condensed milk solids.

4.4 Preparation and presentation of tested materials

The form and shape of the products employed for investigation were slightly modified from their original presentation in order to standardise them in size and geometry.

Samples in most of the cases were only cut to achieve required dimensions and dusted with icing sugar if necessary. Fry's Turkish Delight (FR) is commercially presented as a mixture of a non-chocolate confection in this case a Turkish delight (rubbery-textured sweet of sticky character) and covered with chocolate. However, for test purposes the chocolate had to be removed from the product, once done, it was cut and dusted with icing sugar to avoid the panellist generating a previous impression (visual or tactile) of the sticky nature of the product that could interfere in its sensory evaluation.

Two different geometries were obtained depending on the test to be carried out (see Figure 4.2). For sensory tests, instrumental characterization of stickiness of samples and mastication recordings cube shaped samples of size ($18 \times 17 \times 10 \pm 0.5$ mm) were

adopted. Samples used in compression and stress-relaxation tests were of size (16mm diameter × 10 mm height).

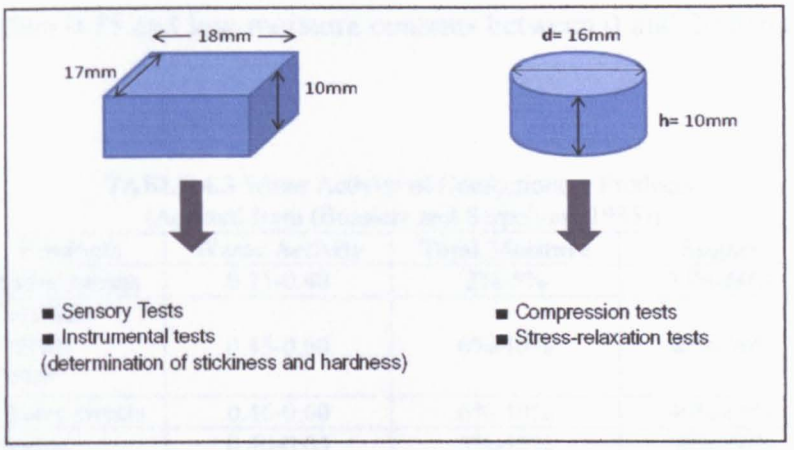


FIGURE 4.2 Sample dimensions

Size and shape have been regarded as factors that can have an influence in the oral processing of foods. The size is a factor that determines the aperture of the mouth for biting and the amount of prolonged pressure when biting the food. It could also have an effect on the time of chewing; smaller pieces normally require less chewing while a longer time is necessary to chew large pieces. On the other hand shape affects the ease with which a food can be placed between the teeth for biting (Evans, 2001).

Cutting of adequate specimens was achieved by the use of well sharpened tools. The knives used were those having flat and thin blades. Neither hollow ground nor serrated knives, or blades, were used for obtaining cube shaped samples. As the success of compression tests depends largely on the quality and accuracy of the samples sharp cork borers were employed in getting cylinder shaped specimens. It has been recommended in the literature to cut samples as slowly as possible and lubricate the tool with soapy water to avoid that the dimensions of the resulting test piece being different from the cut borer and to obtain a smooth-cut surface respectively (Gunasekaran and Mehmet, 2003). The last of these steps was however not carried out in this study as the texture of the samples was highly sensitive to moisture. Before performing every test, the dimensions of the specimen were measured with the use of a vernier scale to verify that they were within the specification established.

4.5 Storage of materials

Storage of samples played an important role in this research moisture level being the main factor to control. Confectionery products have in general low water activities (a_w) equal or less than 0.75 and low moisture contents between 0 and 20 percent (see Table 4.3).

TABLE 4.3 Water Activity of Confectionery Products
(Adapted from (Bussiere and Serpelloni, 1985))

Products	Water Activity	Total Moisture	Sugars
Boiled sweets	0.25-0.40	2%-5%	35%-60%
Caramels Toffees Fudge	0.45-0.60	6%-10%	40%-70%
Chewy sweets	0.46-0.60	6%-10%	40%-60%
Nougat	0.40-0.65	5%-10%	30%-60%
Marshmallows	0.60-0.75	12%-20%	40%-65%
Gums Jellies Licorices	0.50-0.75	8%-22%	30%-75%
Candied fruit	0.70-0.80	20%-30%	35%-100%
Jams	0.80-0.85	30%-40%	0%-70%
Fondants Creams	0.65-0.80	10%-18%	15%-30%
Chewing Gum	0.40-0.65	3%-6%	20%-35%
Soft coating	0.40-0.65	3%-6%	20%-30%
Hard coating	0.40-0.75	0%-1%	0%-20%
Lozenges Tablets	0.40-0.75	0%-1%	0%-5%

Therefore, moisture migration between the product and the atmosphere, can lead to textural changes. Moisture gain or loss induces stickiness or hardening respectively and/or crystallization of sugars giving as a result grained textures. Products with a higher equilibrium relative humidity (ERH= $a_w \times 100$) than the relative humidity of the environment (RH) will dry out during storage and vice versa; a product with a lower ERH than the RH of the environment will tend to pick up moisture (Subramaniam, 2007).

Such textural deteriorations were undesirable in the study and difficult to control. Samples such as milk chews, toffee and fruit-tella were the more sensitive to moisture migration, followed by Turkish delight (Fry's and Marks&Spencer) and fruit jellies according to Table 4.4

TABLE 4.4 Effect of humidity on various types of sugar confectionery (Adapted from (Jackson, 1995))

Type of confection	Deterioration caused	ERH
Boiled sweets	Graining and stickiness	Below 30
Toffees	Graining and stickiness	Below 30
Gum and pastilles	Stickiness, growth of moulds and yeasts	50-65
Liquorice paste goods		55-65
Turkish delight		60-70
Fruit jelly goods	Fairly stable at ambient UK conditions	60-75
Cream-paste goods		65-70
Marshmallow		65-75
Marzipan	May dry out or grow mould	70-85
Fondat cream		75-85
Jam		75-85

In an attempt to control the moisture level of the products under study, right after purchasing, these were stored in airtight plastic containers and they were kept within their original packaging until preparation for testing as the packaging is especially designed to ensure limited movement of moisture to or from the confection (Jackson, 1995).

4.6 Artificial Saliva

4.6.1 Natural saliva VS Artificial saliva

Natural saliva is the thin layer of liquid in the oral cavity that covers the surface of the cheeks, lips, teeth, tongue and the palate. It is normally called whole saliva or oral fluid to refer to the fact that it is a mixture of the secretions of the three pairs of salivary glands: the sublingual, submandibular and parotid. Composition at every gland site, that is beneath the tongue, jaw and ear respectively is different. Whole saliva is in general composed of 99.5% water, this great amount reflects its great capacity of dilution and clearance of material from the mouth. The other 0.5% corresponds to solids; 0.3% proteins and 0.2% inorganic and trace substances of which main compounds are (Schipper et al., 2007):

- ***Mucins*** Glycoproteins binding water by hydrophilic interactions which maintain the hydration of oral mucosa and thus provide lubrication. It is believed that these proteins play a special role in the stickiness according to bioadhesion studies.
- ***Amylase enzyme*** breaks down starch and glycone into dextrin and maltose.
- ***Calcium, phosphate fluoride, sodium, potassium and chloride ions*** that help in the remineralization of teeth.
- ***Lipase enzyme,*** this enzyme, because of its hydrophobic character can enter globules of fat and break down fatty acids.
- ***Lysozyme, immunoglobulin A (IgA), lactoferrin, sialoperoxidase*** Proteins with antimicrobial properties.
- ***Proline-rich proteins and statherin*** Bind calcium and reduce calculus formation.
- ***Hydrogen carbonate and urea*** Act as important buffers.

The composition of saliva varies from individual to individual and depends on type, intensity and duration of stimulation, time of the day, diet, age, sex and a variety of diseases and pharmacological agents (Darvell, 1978).

These variations in composition have been accounted for as the main hindrance not only to duplicate natural saliva but also to its use in standardized *in vitro* tests.

4.6.2 Preparation

Artificial saliva was prepared for the simulation of oral conditions in instrumental tests to determine food stickiness. The use of artificial saliva was selected for this study, because it provides a standardized procedure that would be complicated to achieve with the use of natural saliva as was mentioned previously. There are several formulations reported in the literature. However, it has been pointed out that organic compounds consisting mainly of glycoproteins (mucins) must be included within the formulation and are adequate for biological studies since they are responsible for saliva viscosity and therefore influence diffusion rates of other solutes (Gal et al., 2001).

The formulation adopted was taken from (Davis et al., 1971) and is shown in Table 4.5, together with the information of the reactants that were employed in this study. This formula is composed of substances found in appreciable quantities in whole human saliva. As can be seen from the formula, salivary mucin was substituted in this case by gastric mucin, which consists of the same general mucoproteins and mucopolysacharides.

TABLE 4.5 Formula for artificial saliva

Material	Weight/L	Reagent information
Mucin gastric	1.0 g	M1778 Mucin from porcin stomach type III (Sigma)
α-Amylase	2.0 g	10070 α-Amylase from bacillus subtilis (Fluke Analytical)
Sodium Chloride	0.117 g	Minimum 99.5% (Sigma)
Potassium Chloride	0.149 g	Reagent grade (Fisher Scientific)
Sodium Bicarbonate	2.100 g	BDH Chemicals Ltd Poole England
Distilled water	1000 ml	

The fluid was prepared by dissolving the corresponding amount of the reactants in 250 ml of distilled water. Mucin was difficult to dilute in cold therefore before diluting to volume and as an aid to the dissolution process, the solution was put in a water bath at 37±1 °C for a period of time until complete solvation. The volume was then made up to the mark and the final solution obtained was left in the water bath for an hour until thermal equilibrium of the solution was reached.

The resultant artificial saliva was opalescent and egg-shell white in colour, such as described by Davis *et al.* (1971). The pH of the solution was adjusted to 7 when necessary. Rising of the pH was sometimes observed which has been reported as a result of the loss of CO_2 when the solution is exposed to the air (Darvell, 1978). To prevent deterioration of the saliva it was prepared on the day right before the test and storage of solutions was avoided.

Chapter 5

Sensory Tests

5.1 Introduction

The sensory evaluation of the samples was conducted not only to verify that the products selected for the study could be well differentiated from one another, according to their degree of stickiness, but also to obtain a measurement of the human response to the perceived stickiness present in each one of the products. This is of significance because as Bourne (2002) clearly established in the following statement "***There is no instrument available that has the sophistication, elegance, sensitivity and range of mechanical motions as the mouth or that can promptly change the speed and mode of mastication in response to the sensations perceived during the previous chew***". Therefore the sensory differentiation of the products was essential since any data obtained from any instrument aiming to quantify textural properties as perceived by humans will be meaningless if a proper correlation between sensory data and instrumental measurements cannot be established.

The method used for sensory evaluation was the general rating scale for attribute intensity, which is the basis of the Quantitative Descriptive Analysis (QDA) most commonly employed in food evaluation (Chambers and Wolf, 1996, Schifferstein and Hekkert, 2008).

In addition to the stickiness of the food material, which was the object of study in this research, the attribute hardness was also subjected to sensory evaluation. The purpose of this was to determine whether the masticatory response to the sample was in response to the stickiness or the hardness in the product. This was because both attributes were clearly distinguishable between the samples.

Both stickiness and hardness were assessed as mouth-feel attributes. The definitions specified for the panel and used in the evaluation were as indicated in Table 5.1.

TABLE 5.1 Definitions and evaluation technique for attributes analysed

Attribute	Definition	Evaluation technique
Stickiness	It is the force required to remove the product that adheres to the mouth (especially to the teeth, cheeks tongue and palate).	Evaluation after biting the sample until termination. Panellists were asked to evaluate the attribute through the whole chewing sequence and score it after swallowing it.
Hardness	It is the force required to compress (deform) or to disintegrate the material*.	Evaluation at the beginning of the chewing sequence. Panellists were asked to compress the sample with the molars and evaluate the required force.

*Hardness was evaluated as the maximum force encountered independent of whether the material was fracturable or not.

5.2 Materials

The six confectionery products selected for this study were subject to sensory evaluation. Samples were standardised in size and shape as describe in Section 4.4. Samples at room temperature were placed on small glass boards which were wrapped with non-stick aluminium foil. A code was assigned to each sample and they were presented randomly to the panellists (see Figure 5.1). Since some of the products presented a high tactile stickiness, these samples were dusted in icing sugar to avoid panellists forming an impression before their ingestion, while handling the product. Approximated weight per sample is depicted in Table 5.2.

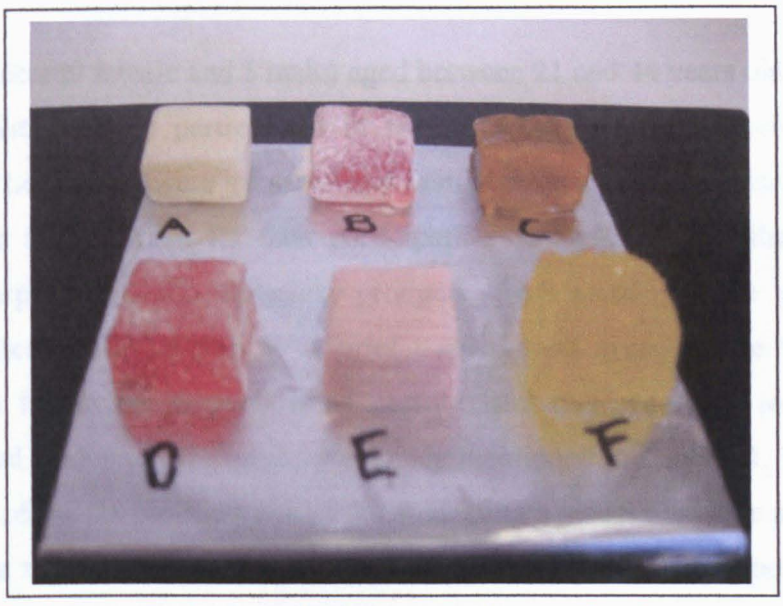


FIGURE 5.1 Six confectionery products as presented to panellists for sensory evaluation

TABLE 5.2 Sample weights

(values are presented as an average of 5 measurements ± standard deviation)

Food sample	Code*	Weight (g)
JT	**F**	4.8 ± 0.2
FR	**D**	5.8 ± 0.05
TD	**B**	5.4 ± 0.2
FRUT	**E**	4.2 ± 0.2
MCH	**A**	4.1 ± 0.2
WO	**C**	5.1 ± 0.2

*Code assigned to samples for sensory evaluation (letters correspond to samples shown in Figure 5.1)

From the weights table it can be observed that the samples had pretty similar weights ranging from 4.1g for the lightest sample (MCH) to 5.1g for the heaviest sample among the group (FR). Proportions of the samples provided nevertheless were bite sized and suitable to be easily ingested.

5.3 Method

Fourteen subjects (9 female and 5 male) aged between 21 and 44 years old (mean= 27.9, standard deviation= 5.7) participated in the test. Subjects were students from the University of Leeds who were informed and invited to take part in the analysis. None of them received any payment for their participation. It was important that none of the participants expressed dislike for sticky products which could interfere in the study as previously discussed in section #. A brief training was given before the test. Each panellist was instructed about how to perform the test according to the attribute description and evaluation technique previously mentioned in Table 5.1. The stickiness and hardness of the six products were evaluated using a visual analogue scale (VAS) of 10 cm using a non-structured line. one for each attribute, anchored at the left extremity as "non-sticky" or "soft" and "very sticky" or "hard" at the right extremity. Panellists were instructed to place a vertical mark for each sample across the line at the point which best reflected the magnitude of his or her perceived intensity of the attribute evaluated, according to the definitions and instruction provided. No information was given about the purpose of the investigation.

For sensory analysis, panellists were seated in sensory booths with appropriate ventilation and lighting. They were also instructed to chew in their habitual manner, make their response after swallowing the product, and rinse their mouth, drinking enough water to remove completely any remnants of food that could have remained in the mouth caused by the sticky nature of the samples and thus avoid any interference in the following tests. No time-limit was set for each test. Ethical permission has been granted by faculty ethics committee for carrying out the study (see Appendix A)

5.4 Results and Discussion

The marks assigned by panellists from line scales were converted to numbers by manually measuring the position of each mark on each scale, for each textural attribute being evaluated (stickiness and hardness). The scores obtained for each panellist are graphically displayed in Figure 5.2. The wide spread of sensory scores in both attributes shows that foods tested in this work have great variation of textural perception, from soft to hard and from non-sticky to very sticky.

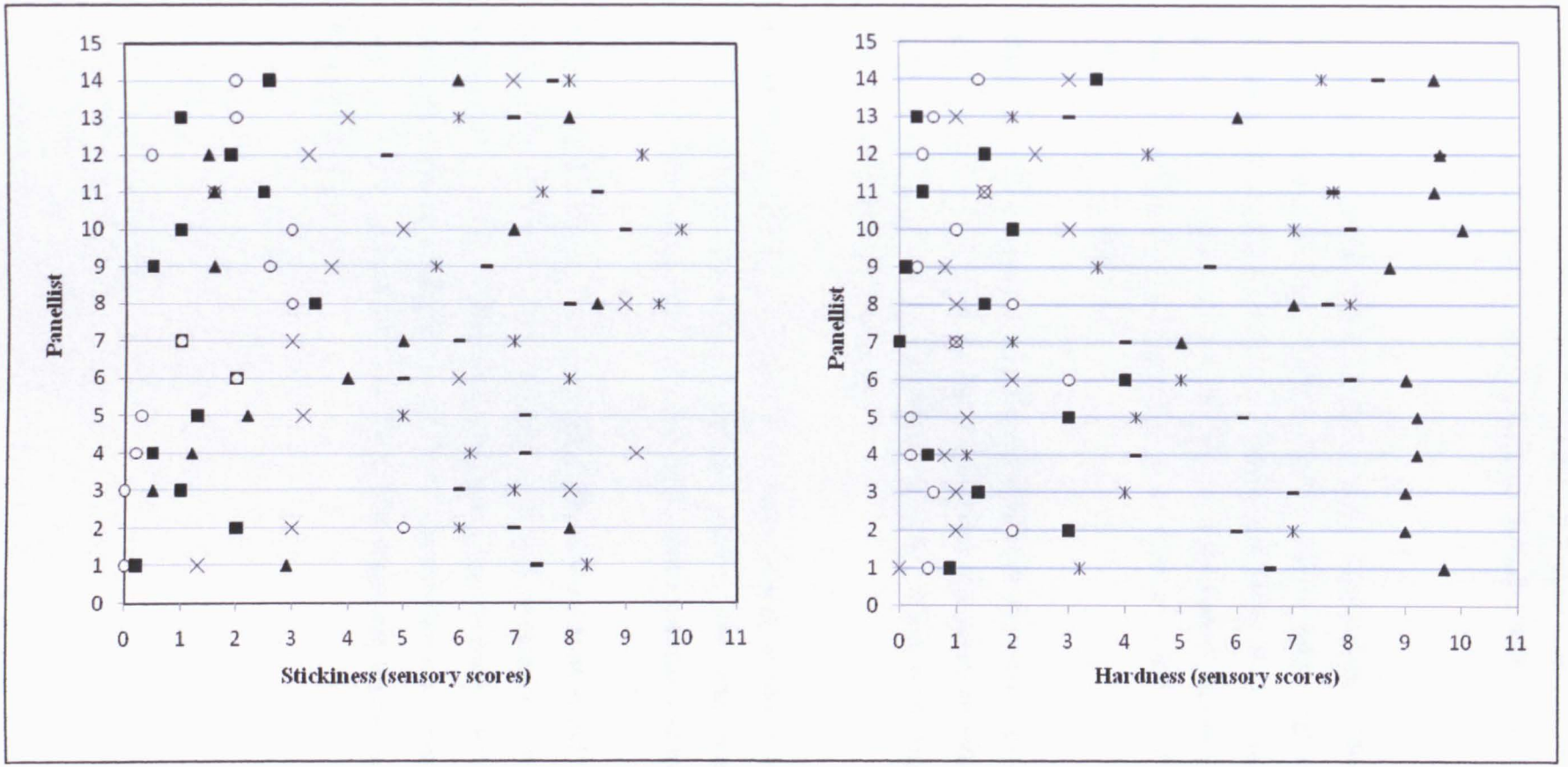


FIGURE 5.2 Sensory scores of stickiness and hardness as perceived by panellists (■: ***JT***- *Thorntons' Fruit Jelly*; ○: ***FR***- *Fry's Turkish Delight*; ×: ***TD***- *M&S Turkish Delight*; ▲: ***FRUT***- *Fruit-Tella*; –: ***MCH***- *Milk Chews*; ∗: ***WO***-*Werther's Original*).

Average scores of sensory assessment are displayed graphically in Figure 5.3. On a scale of 0 to 10, the scores for overall stickiness ranged from 1.5 (*Thorntons' Fruit Jelly*) to 7.4 (*Werther's Original*) and for hardness from 1.1 (*Fry's Turkish Delight*) to 8.6 (*Fruit-Tella*).

To determine whether the products were significantly different according to the intensity of the attribute being evaluated and to what degree difference existed, a nonparametric Friedman test was performed using R statistical computing software version 2.13.1. This test showed that products varied significantly according to their degree of stickiness and hardness $\chi^2(5) = 46.8$, $p = 6.309 \times 10^{-9}$ and $\chi^2(5) = 59.9$, $p = 1.246 \times 10^{-11}$ respectively

Therefore in both cases the null hypothesis ($Ho = \mu_{JT} = \mu_{FR} = \mu_{TD} = \mu_{FRUT} = \mu_{MCH} = \mu_{WO}$) was rejected, whilst the alternative hypothesis was accepted indicating that at least one of the products can be differentiated from the others according to the corresponding textural attribute being evaluated.

In addition multiple comparisons by ranks among products were carried out using Tukey's honestly significant difference (HSD). This was done to determine which products differed significantly according to their stickiness and hardness.

Tukey's HSD test by ranks, indicated that the products could be grouped together in three different groups; low, medium and high stickiness. The low stickiness group included JT and FR, the medium stickiness group included FRUT and TD and the high stickiness group included MCH and WO. The difference between samples in each group was not significant ($p>0.05$). However, the stickiness varied significantly between the three groups ($p<0.05$).

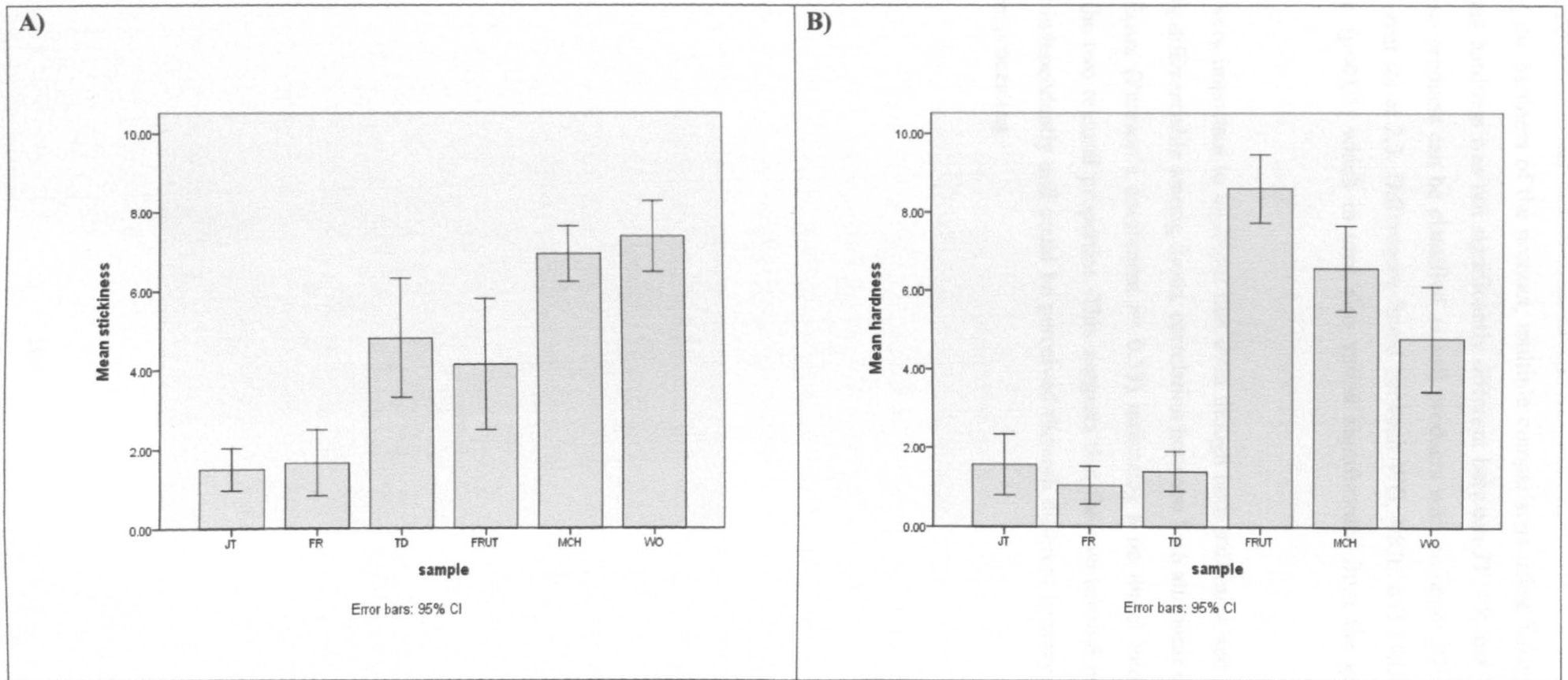


FIGURE 5.3 Average scores of stickiness and hardness obtained from sensory evaluations for each product
A) Graph for stickiness and ***B)*** Graph for hardness

Regarding the hardness of the product; multiple comparisons using Tukey's HSD test showed that hardness was not significantly different between JT, FR and TD ($p>0.05$) hence these products can be classified as soft products with an upper 95% confidence group interval set at 2.3. Differences found between WO, MCH and FRUT were very significant ($p<0.05$) which in turn also varied significantly from the group of soft products.

It is also very important to highlight that even though both stickiness and hardness are sensorially differentiable among foods, correlation between both attributes was poor and not significant (Pearson's coefficient $r= 0.53$) indicating a no direct inter-correlation between the two textural properties. This suggests that the two textural properties are exhibited independently and could be perceived through different sensory mechanisms during oral processing.

Chapter 6

Mechanical Characterization of Samples

6.1 Introduction

Uniaxial compression tests, a type of fundamental analysis was carried out to determine some important mechanical properties of food samples under study. Compressive measurement of foods is important to understand the rheological character of the food materials that is, its response or behaviour when subjected to various forces. This is relevant or meaningful as the breakdown of foods during mastication mainly occurs by mechanical actions which involve shear and compressive stresses at varying speeds. Hence as the stresses are applied to the food, it undergoes a deformation (strain). Although it has been stated that the aim of measuring fundamental rheological properties of foods is not to mimic the human sensory processes, these physical properties can lead to a better understanding of structure-function relationship by determining their relationship with the dynamic perception of food texture (Foegeding et al., 2003).

Properties such as modulus of deformability (stiffness), the fracture strain (longness), the fracture stress (firmness), the work to fracture (toughness) and the work of deformation per unit volume can be used not only to quantify the rheological character of samples but also to determine the strength of the specimens (mechanical resistance), as well as some texture properties.

For foods, the term modulus of deformability (E_D) has been preferred instead of modulus of elasticity or Young's modulus which is employed for engineering materials obeying Hooke's law. The modulus of elasticity corresponds to the ratio of stress to strain within the elastic region of the material. Its magnitude reflects an indication of the stiffness or rigidity of the material and has nothing to do with the degree of elasticity as the term could mislead (Mohsenin and Mittal, 1977). However, this is because foods are generally nonlinear viscoelastic materials in which the linear region (a region where strain is proportional to stress) from compression tests is not clearly defined. Moreover foods under compression show that elasticity as defined by Hooke's law is not obeyed as foods are unable to return to their original dimensions at even a low strain. Therefore, the use of the term modulus of deformability is considered to be more appropriate. Since stress-strain curves for most foods are often nonlinear, for convenience of comparison the modulus of deformability can be expressed as the 5% strain secant modulus (Charalambides et al., 1995)[1]

6.2 Materials and apparatus

- Texture Analyser, type TA.XT-*plus* from Stable Micro Systems, equipped with a 30 Kg load cell and linked to a computer with data recording and analysis software Texture Exponent 32 version 2.0, 4.0.
- Stainless steel probe (40 mm diameter)
- Stainless steel platform (55 mm diameter)
- The specimens employed were cylindrical shape (16mm diameter × 10 mm height) and they were prepared as discussed in section 4.4. No sugar coating of the samples was required for this test.

[1] Several other methods have been reported in literature for estimation of a representative value of the slope (Gunasekaran and Mehmet Ak, 2003)

6.3 Method

The mechanical characteristics of the six samples were measured using compression tests. The experiments were carried out on a texture analayser type TA.XT-*plus* from Stable Micro Systems equipped with parallel flat plates as shown in Figure 6.1 Cylindrical shape samples were placed on the lower platform (55 mm diameter) mounted in the texture analyser. Uniaxial force was applied with a stainless steel probe (40 mm diameter). The force was applied over cross-sectional area of the sample perpendicular to the direction of the applied force. Mineral oil was applied to upper and lower platforms and to the top and the bottom surfaces of the sample, to provide lubrication avoiding adhesion between sample and platforms and preventing barrelling of the sample caused from friction between the sample and the contacting surfaces (Casiraghi et al., 1985, Risch, 1992). Tests were performed at a constant displacement rate of 1mm/s and room temperature (22±1°C). Samples were subjected to a compression ratio of 80% of the initial sample height (distance moved by the probe= 8 mm). Large deformation was applied since while eating food is compressed over the fracture point until almost 100% strain. Thus mechanical properties obtained at higher strain and fracture properties will be more related to texture properties, than their small-deformation characteristics (Zhang et al., 2007, Kohyama et al., 2007). Tests were replicated five times per each sample.

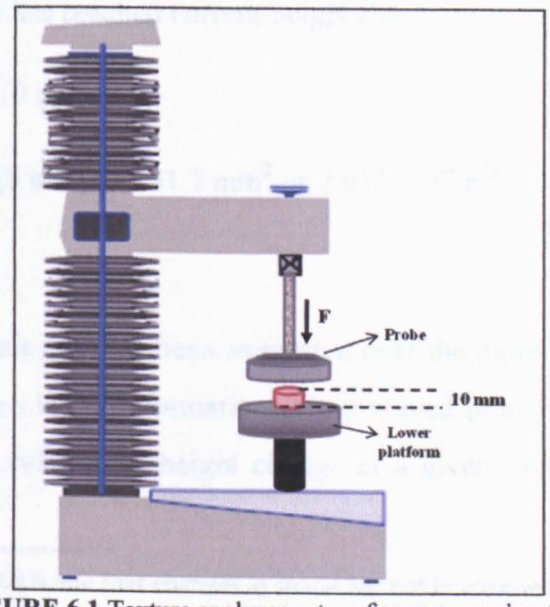


FIGURE 6.1 Texture analyser set up for compression tests.

For the analysis force-displacement data were converted to the corrected stress and Hencky strain. As the cross-sectional area of the sample changed upon deformation it was necessary to calculate an approximately corrected cross-sectional area so that true stress could be determined. Correction was done assuming that volume remained the same during compression (specimen is incompressible[2]) therefore:

$$\sigma = \frac{Fh}{A_0 H_0} = \frac{F(H_0 - \Delta H)}{A_0 H_0} = \frac{F(H_0 - vt)}{A_0 H_0} \qquad (1)$$

Where, as observed from Figure 6.2 :

σ= true or corrected stress (KPa)

F=load

h= current height = H_0 -ΔH

ΔH= distance probe has moved

v= speed at which probe moves= 1mm/s

t= time at which probe has reached current height (h)

H_0= original height = 10 mm

A_0 = original area = $\pi(8\ mm)^2 = 201.1\ mm^2$ or $2.011 \times 10^{-4}\ m^2$

The use of Hencky strain (ε_H) has been suggested over the more general used Cauchy or engineering strain when large deformations are involved (e.g. > 25%). That is because Hencky or true strain relates the height change at a given time t (ΔH) to the current

[2] An incompressible material is one that changes in shape but not in volume when compressed.

specimen height at the same time (h). Since samples in this study were subjected to 80% deformation Hencky strain was calculated as:

$$\varepsilon_H = \int_{H_0}^{h} \frac{dh}{H} = \ln h - \ln H_0 = \ln \frac{h}{H_0} = \ln \frac{H_0 - \Delta H}{H_0} = \ln \frac{H_0 - vt}{H_0} \qquad (2)$$

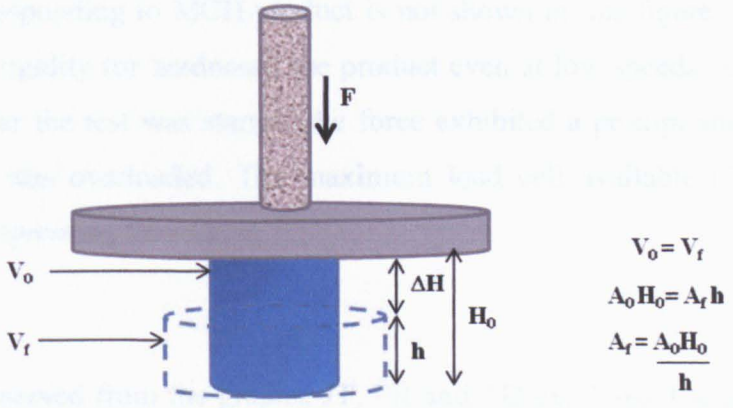


FIGURE 6.2 Diagram illustrating the change in cross-sectional area as sample undergoes deformation by uniaxial compression (A_0, A_f and V_0, V_f, initial and final area and volume respectively)

The work of deformation (W_d) was calculated as the area under the stress-Hencky strain curve from zero strain (or zero deformation) until 10, 30, 50 and 80% relative deformations (ratio of deformation to original height). Values were representative of very small, small, medium and large deformations respectively. In materials where fracture was appreciable, work up to fracture (W_f) was determined from zero loading (initial material shape) up to fracture point. The point of fracture was identified as the point where the first decrease in stress was observed. The work done per volume unit in deformation (ε_i) was estimated as follows:

$$W = \int_0^{\varepsilon_i} \sigma \, d\varepsilon \qquad (3)$$

Excel and Matlab software were employed for carrying out all the corresponding calculations.

6.4 Results and Discussion

Figure 6.3 depicts the stress-Hencky strain curves obtained for the samples under study. The curve corresponding to MCH product is not shown in this figure. This is because, due to its high rigidity (or hardness), the product even at low speeds behaves as a hard solid. Right after the test was started, the force exhibited a prompt sharp increase and the equipment was overloaded. The maximum load cell available (30 Kg) was not suitable for compressing the sample.

As it can be observed from the graphs, JT, FR and TD exhibited J-shaped strain-stress curves. This type of elastic curves has been observed in biological materials or food gels. The response is known as strain-hardening and it has been attributed generally to the extensional limitation of polymer network chains (Kendall and Fuller, 1987, Zhang et al., 2007).

At low strains (relative deformation <20%), the difference between products (JT FR and TD) were relatively small. Products start being clearly differentiated between each other approximately after 0.32 Hencky strain (over 27% relative deformation). For example stress values at 30% relative deformation from Table 6.1 are 45.12, 40.35 and 27.2 KPa respectively.

JT, FR and TD after certain amount of deformation (maximum strain) exhibited a decrease in force (stress). At this point specimen has fractured. Maximum stress values were 103.02, 73.4 and 87.15 KPa respectively. These values presented the same order that the hardness evaluated sensorially (FR > TD > JT).

In the case of WO and FRUT fracture was not appreciable instead products continued stretching radially while under compression. The force (stress) continually increased. Work of deformation per unit volume was then calculated at 80% relative deformation being equal to 234.53 and 268.22 KPa, such values agreed also with the order of hardness assigned by panellists. Although these samples showed similar patterns of deformation, higher stress values were obtained at any point for FRUT.

In general products can be classified into two main groups according to their pattern of deformation. The first group of foods corresponded to soft viscoelastic materials having mainly elastic properties as the products can partially recover their shape after being subjected to loads that are smaller than those required to cause their fracture. In the second group are found those hard and though viscoelastic materials having more plastic behaviour. These materials do not return to their original shape when stress is removed even at low strains. Once material overpasses its yield stress, it flows and is permanently deformed.

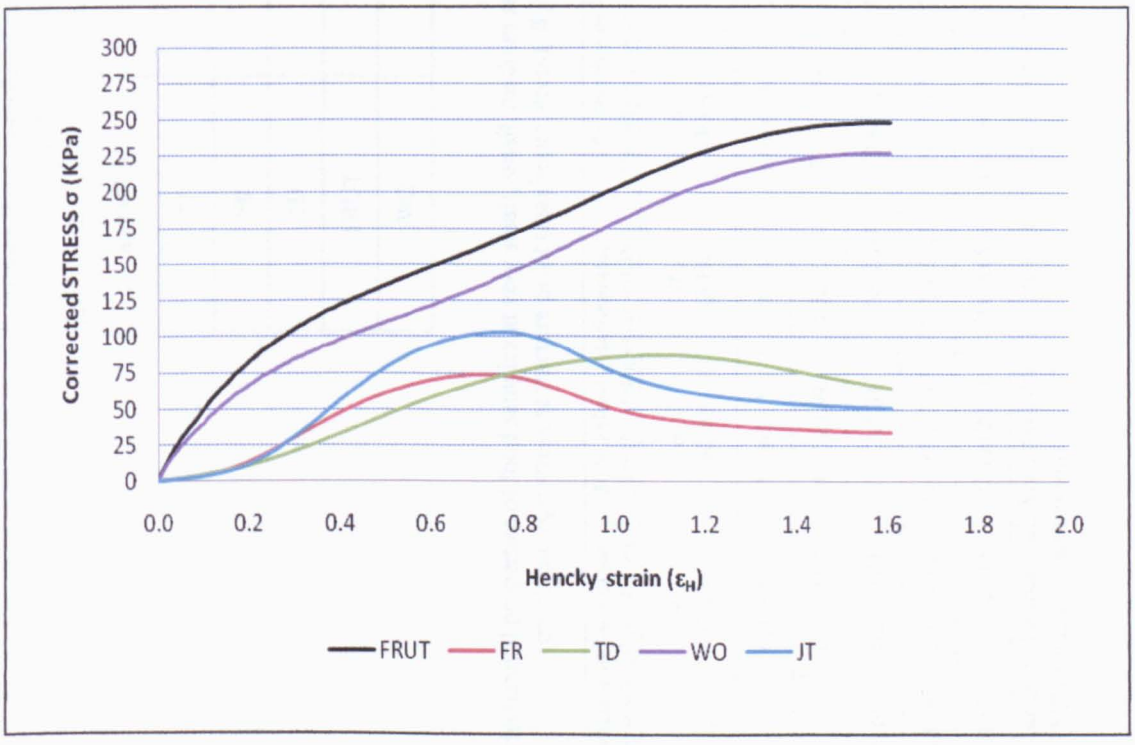


FIGURE 6.3 Stress-strain curves for materials under uniaxial compression at constant speed of 1mm/s

TABLE 6.1 Modulus of deformability for food samples
(mean values ± standard deviation)

SAMPLE	E at ε_H= 0.05 (KPa)
JT	23.4 ± 6.63
FR	31.2 ± 3.68
TD	31.0 ± 5.29
FRUT	564.5 ± 127.3
WO	466.4 ± 91.54

TABLE 6.2 Mechanical properties of food samples at very small, small, medium and large deformations. Reported values correspond to mean values ± standard deviation (σ= stress, σ_f= stress at fracture point (maximum stress), W_d= Work of deformation per volume unit, W_f= Work to fracture)

SAMPLE	10% relative deformation (Hencky strain value = 0.105)		30% relative deformation (Hencky strain value = 0.357)		50% relative deformation (Hencky strain value = 0.693)		80% relative deformation (Hencky strain value = 1.609)		At fracture		
	σ (KPa)	W_d (KPa)	σ (KPa)	W_d (KPa)	σ (KPa)	W_d (KPa)	σ (KPa)	W_d (KPa)	Hencky strain value	Maximum σ_f (KPa)	W_f (KPa)
JT	4.3±1.1	0.25±0.05	45.12±11.13	5.24±1.55	101.64±15.6	32.06±6.0	-	-	0.756±0.04	103.02±16.07	38.52±6.98
FR	5.15±0.65	0.29±0.03	40.35±4.94	5.32±0.74	73.22±5.04	25.87±2.51	-	-	0.718±0.02	73.4±4.84	27.726±1.99
TD	5.29±0.66	0.29±0.02	27.72±5.31	4.08±0.69	67.85±6.36	20.45±2.95	-	-	1.11±0.05	87.15±3.30	54.08±2.22
FRUT	52.14±8.78	3.07±0.62	114.82±12.17	25.24±3.17	159.55±17.92	71.64±8.04	247.75±33.7	268.22±34.3	-	-	-
WO	42.24±9.51	2.53±0.43	92.11±20.11	20.38±5.2	132.76±36.04	58.18±15.85	226.71±44.31	234.53±53.47	-	-	-

- Property not applicable to the material. Material either 1) fractures at relative deformations lower than 80% or 2) does not fracture in which case property is reported at maximum applied compression (80% relative deformation)

The modulus of deformability E_D and the mechanical properties of the samples are displayed in Table 6.1 and Table 6.2 respectively. The modulus of deformability was determined as the slope of the initial linear part of the stress-Hencky strain curve. Since linear regions within the initial portions of the stress-strain curves were not easily discernable, the modulus of deformability was expressed as the 5% strain secant modulus, which corresponds to the slope of a line connecting the point at zero strain (deformation) of the stress-strain curve and the point on the curve at 5% strain. This method has been also reported as to avoid the scatter produced by the end sides of the cylinders being not always exactly flat and parallel (Charalambides et al., 1995).

Linear relationship was observed between maximal stress values attained at 80% compression and sensory scores of hardness with a good correlation factor of R^2= 0.97 (Figure 6.4).

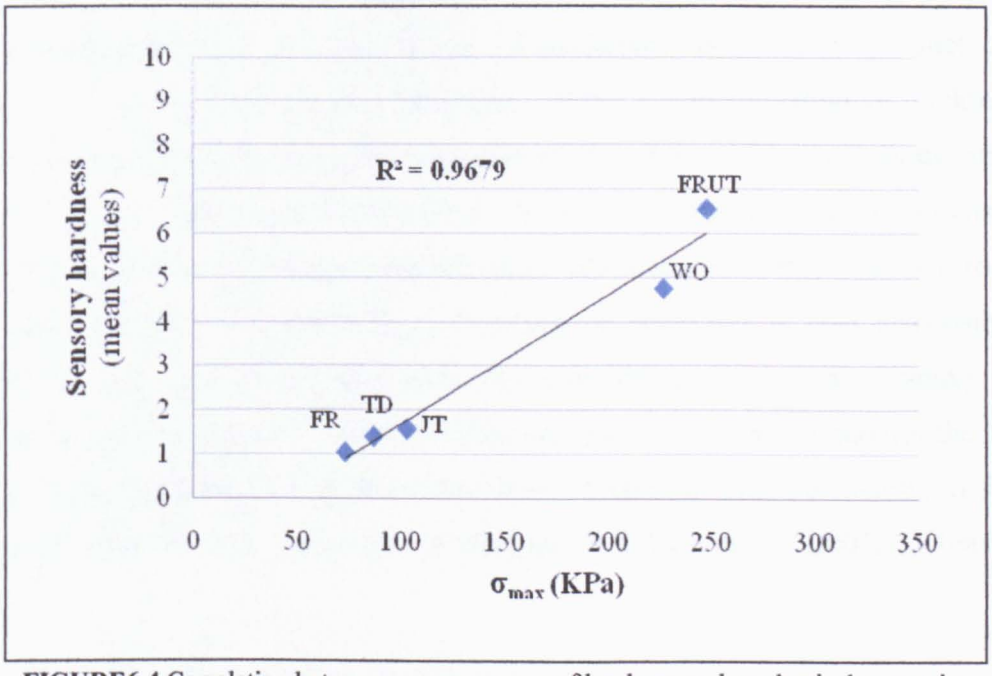


FIGURE6.4 Correlation between sensory scores of hardness and mechanical properties (maximal stress attained when samples were compressed by 80%)

Chapter 7

Instrumental Characterization of the Stickiness and Hardness of Food Samples by Penetration Tests

7.1 Introduction

As discussed before in Section 2.1.1.1 to date there is no standard method available to quantify sensory stickiness in foods. Moreover, the methods discussed within the literature have been mostly conducted towards the determination of food stickiness associated with the problems it represents in processing and packaging. For example, measurement of dough stickiness has been widely attempted as it constitutes a problem for mechanized bakeries causing halting of production and loss of product quality (Wang et al., 1996, Hoseney and Smewing, 1999). Characterization of stickiness in confectionery products, such as the evaluation of chocolate stickiness to mould surfaces (Keijbets et al., 2009) and development of test procedures to study stickiness of caramels during manufacturing process (Kilcast and Roberts, 1998), have also received great attention. This is because in such situations stickiness is also responsible for production losses and increased processing costs due to equipment cleaning. These methods have been normally based on either peel or probe tests. Although the former have been less frequently used, they have been adopted to measure sticking of food in packaging materials and stickiness of doughs (Adhikari et al., 2001, Dobraszczyk, 1997).

Probe tests, that measure the tensile strength of the bond formed between the adhesive (food material) and the substrate (probe material) have found their major application in

the measurement of stickiness of solid and semi-solid foods. The execution of most of these tests normally implies a sequence of two steps:

1) ***Bonding process.*** This stage involves the contact of the probe material with the adhesive (food material) to form a bond. The contact is achieved by any of the following strategies:

   a) By application of a particular force to the sample by compression

   b) Through the application of a certain weight; or

   c) By applying a certain degree of initial strain (or deformation)

2) ***Debonding process.*** This stage implies the destruction of the bond formed during the bonding process by removal of the probe. This normally occurs by retraction of the probe, after its contact with the adhesive material, by the application of tensile force. This action can be executed either immediately after the contact or after certain period of contact time has passed. The maximum value of the withdrawal force used to remove the probe (adhesive strength) during this stage is generally taken as a measurement of stickiness.

The above described methods in general have been shown to fail in providing good correlations with sensory evaluations of food stickiness. This is presumably due to the fact that tests have normally been performed under conditions very unlike to those occurring in the mouth. For instance, (Foegeding and Steiner, 2002) employed different probe materials, including dental enamel, to evaluate caramel stickiness and concluded that factors important to stickiness to packaging and equipment are not entirely the same as those important to stickiness as perceived in sensory analysis. The author acknowledged saliva as the factor that might have a significant contributing effect in sensory evaluations. In general probe tests involving the use of flat plates are deemed to be the most adequate for simulating adhesion to surfaces, such as food adhesion to

equipment during industrial processing or to packaging materials. Instrumental measurement of the stickiness of fluid foods by probe tensile separation using flat plates has found to correlate well with tactile sensory evaluation with the use of fingers. This is not at all surprising because finger evaluation is the most similar sensory evaluation, to instrumental testing, that involves compression and followed by separation between relative flat surfaces, with no complicating factors such as teeth shape or saliva (Chen et al., 2008).

On the other hand, probe tests involving penetration have been regarded as being the most suitable tests to mimic oral processes. (García, 2000) for example simulated biting by conducting perforation tests of gelatine confections with a 2mm plunger. This author found that the adhesive force measurements obtained from this type of tests closely correlated to the adherence of food to teeth experienced by panellists.

In past years, especially in the field of dentistry, studies attempting the measurement of adhesion of foods to teeth while using similar conditions to those found in the oral cavity, have been reported (Caldwell, 1959, Caldwell, 1962). Such studies were addressed more towards the investigation of adhesion as a factor involved in intraoral food retention and are therefore associated with dental caries. In studies carried out by Kashket *et al.*,(1991) it was determined, however, that consumer experience of food perceived as sticky correlates poorly with oral retention measurements. In a more recent paper, it has been discussed that retentiveness of foods is not the same as stickiness. A caramel or jelly bean may be sticky, but easily cleared from the oral cavity, compared to cookies or chips which are retained for longer times (Touger-Decker and Van Loren, 2003). From the works of Caldwell, described above, on adhesion, surface of biological material (hard enamel or soft oral tissues), saliva, solubility of foods, were all recognised as factors influencing the adhesiveness of foods in the mouth.

In recent years, stickiness of semi-solids has also been instrumentally evaluated using compression decompression tests, but using surfaces with similar roughness to that exhibited by the tongue (sand paper and a porcine tongue) and employing natural saliva to provide lubrication to the surfaces. Results showed that the roughness of the surface had an effect on the absolute values of stickiness obtained but not in the relative values when comparing different products analysed. The addition of saliva was shown to have an effect not only in compression movement, by decreasing the gradient of the force-distance curve, as it provided lubrication. In addition, adding saliva increased the decompression force measured at a set distance (5mm). This was attributed to the fact that adding saliva changed the extent to which necking of the sample occurred during decompression. The combination of the product plus saliva resulted in better correlations of instrumental test results with sensory evaluations of stickiness perceived in the mouth (Dunnewind et al., 2004).

This chapter describes the development of an optimized method used for the characterization of stickiness of the samples used in this study. In turn, the test served to investigate and determine potential factors critical in the perception of food stickiness. The method involved the penetration of a series of samples of constant volume to a certain set distance with two different probe geometries: a 5mm stainless steel probe and a natural tooth attached to the surface of a flat cylindrical stainless steel probe (see Figure 7.1). The method used an adaptation of the Texture Profile Analyisis (TPA) described by Friedman *et al.* (1963) and Szczesniak (1963) and later modified by Henry and Katz (1969) and Bourne, (1978) performing one single compression-decompression cycle and using oral conditions similar to those found in the mouth. A full description of the method is provided in the following sections.

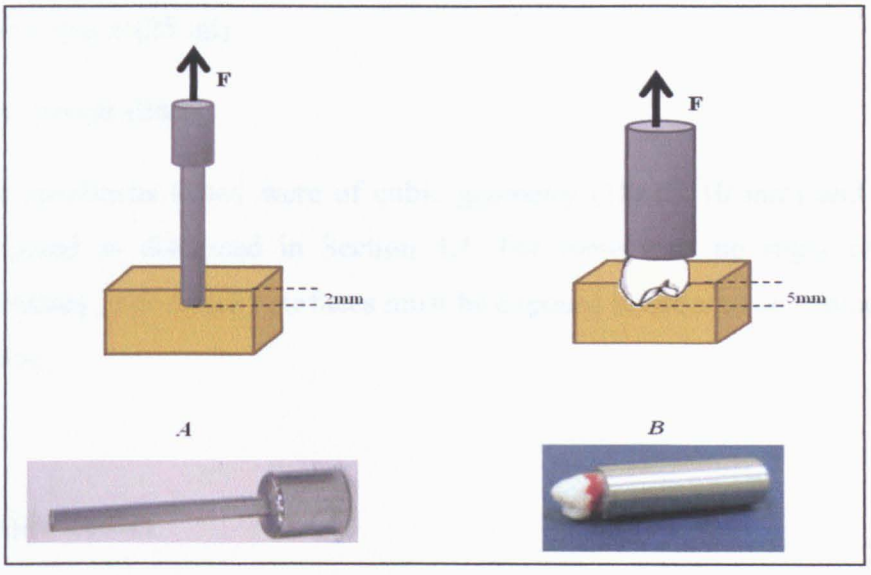


FIGURE 7.1 Probe geometries used for penetration tests ***A***. Flat cylinder stainless steel probe with 5mm diameter ***B.*** Natural tooth attached to a flat cylindrical stainless steel probe. ***F*** represents the tensile force required to break the bond formed (probe material-food material) and is taken as measurement of stickiness

7.2 Materials and Apparatus

- Texture Analyser, type TA.XT-*plus* from Stable Micro Systems, equipped with a 30 Kg load cell and software Texture Exponent 32 version 2.0, 4.0.
- A temperature controlled peltier cabinet (XT/PC) from Stable Micro Systems coupled to a Peltier Control Unit (PCU) where temperature can be easily set and displayed.
- Sample holder, customised to test requirements
- Natural tooth probe[1]
- 5mm Stainless-steel cylindrical probe

[1] The hard tissue used in this study consisted of a human premolar tooth which was provided by Leeds Dental Institute at Leeds University. After its extraction, the tooth was cleaned by removing adhering tissue and then stored in 70% ethanol until use in this study.

- Mercury thermometer
- Glass beaker (25 ml)
- Microscope slides
- The specimens tested were of cubic geometry (18x17x10 mm) and they were prepared as discussed in Section 4.4. For these tests no sugar coating was necessary, since sticky surfaces must be exposed in order to be contacted by the probe.

7.2.1 Sample holder

This sample holder was built for the purpose of clamping specimen to the base of the temperature cabinet which lies on the main platform of the texture analyser. The apparatus was especially designed to fit and be secured entirely within the temperature control unit. This fixture allowed complete penetration and withdrawal of the sample, through a square aperture, whilst avoiding sample spillage, movement or lifting. Lifting of the sample causes inaccuracies in the measurements because when the sample is stuck to the probe but lifted from the base plate, its weight then exerts a downwards force due to gravity. In this case the magnitude of the force being recorded by the texture analyser (taken as measurement of stickiness) would be an inaccurate value of the adhesion force and must be discarded. The use of glues for sticking the sample to the base plate surface has been suggested in the literature (Fiszman and Damasio, 2000). However, this option was deemed unfeasible in this study. This is because it was observed that the use of the glues introduced some problems:

-The first one is that it is difficult to find a type of glue suitable to bond different samples of different consistency to the base plate (e.g gels with wet surface, semi-solids with dry surface).

-Moreover the strength of the bond generated for each sample is not the same.

-And the last but not least, the use of glue generates a more complex system with the creation of multiple interfaces (platform glue, glue food material and food material probe). In reality, the use of a weak glue will cause uncertainty of the failure of which interface. But the use of a strong glue will cause a difficult in sample removal after the test.

As discussed above no one glue will bond all the samples to the base plate in the same way. The use of a clamp ensures that all samples are restrained in the same manner and it also simplifies the system so there is only one interface, this is the one of interest (e.g probe-food material).

7.2.1.1 Physical description of the sample holder

Figure 7.2, shows a picture of the sample holder designed for the experiments. It can be seen from the figure that, it mainly consists of two plates: 1) a base plate and 2) a clamping plate. The base plate is made from a piece of thick aluminium that is secured to the main platform of the texture analyser by four retaining bolts. Four threaded posts extend upwards from the base plate. The clamping plate is a thinner plate of aluminium which is free to slide up and down the threaded posts. There is a hole in the centre of the clamping plate to allow the probe to make contact with the sample.

The height of the clamping plate is set using four nuts. These nuts are on the threaded posts and the clamping plate is above them. Thus the plate cannot descend further than the height at which the nuts are set. Four additional nuts are tightened down on top of the clamping plate to prevent it from lifting.

During a measurement the height of the clamping plate is set such that it just rests on the top of the sample. Setting the height in this way ensures that the sample is not

compressed which could affect the measurement, whilst making sure that the sample is unable to lift during withdrawal of the probe.

The probe is then able to make contact with the sample through the hole in the clamping plate. Penetration of the sample by the probe causes deformation, especially in samples of plastic character. The probes makes contact in the centre of the sample, thus deformation occurs in the centre but does not tend to occur near the edges of the sample. The dimensions of the hole have been selected such that the clamping plate only overlaps the sample close to its edges. In this way no deformation of the sample occurs underneath of the clamping plate. This ensures that no space is created between the sample and the clamping plate. If space were created the sample would be able to lift and measurement could be affected as discussed previously (see Figure 7.3).

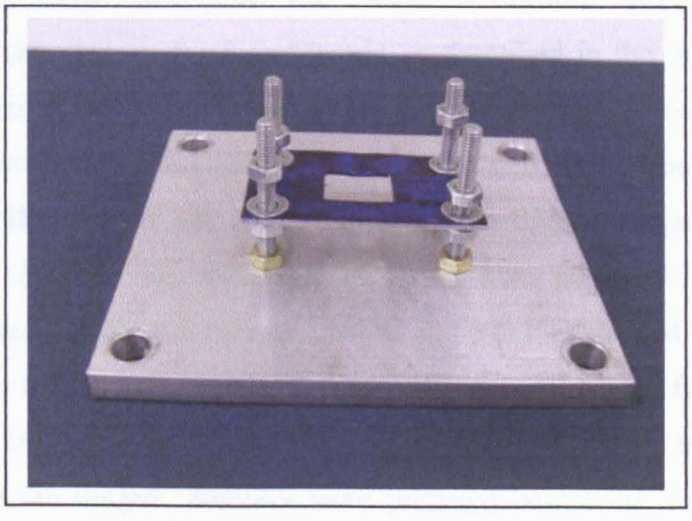


FIGURE 7.2 Sample holder

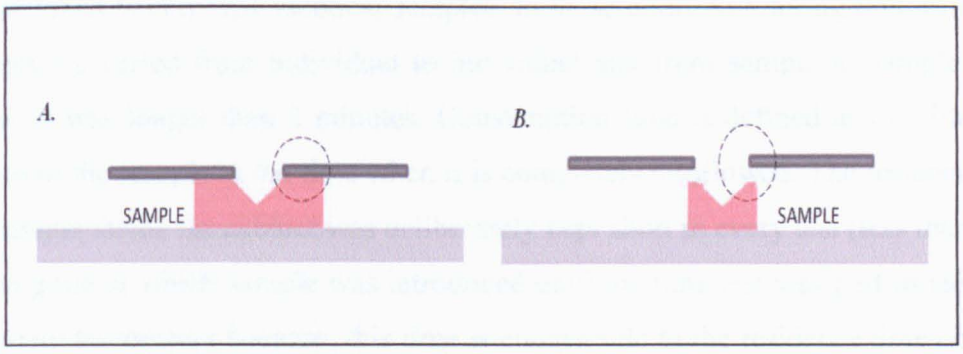


FIGURE 7.3 Diagram showing an appropriate design for a sample holder. ***A.*** Sample is bigger than the aperture in the upper plate therefore, after penetration there is no gap between the plate and the sample that could interfere with the test. ***B.*** As the sample is too small in relation to the aperture penetration creates a gap between the sample and the holder. This gap will cause the sample to lift if it is stuck to the probe when it withdraws, which will cause erroneous results in the measurement.

7.3 Method

The set-up of the equipment used to measure the stickiness and hardness by penetration of the sample is shown in Figure 7.4. Before performing any measurement the equipment was calibrated by force and height as described in the instrument manual. Calibration by height was done by setting the base of the sample holder as the initial position (distance= 0mm). The temperature was set and the cabinet was allowed to reach thermal equilibrium for 1 hour, the temperature within the cabinet was verified by the introduction of a mercury thermometer. Tests were performed at two different temperatures: a standard temperature of 25±1°C and 37±1°C, the last one of which was used to mimic the *in vivo* temperature of the mouth. A small beaker filled with distilled water was placed inside the cabinet to help keep the humidity up and to avoid premature surface drying of the sample. Samples were prepared before the test as discussed in Section 4.4 and allowed to stand at room temperature until test execution. To perform the test, the specimen was introduced into the cabinet and secured to the base of the sample holder.

The time taken to consume identical samples, to those under test during oral processing (mastication), varied from individual to individual and from sample to sample but in any case it was longer than 2 minutes. Consumption time is defined as the time from ingestion of the sample to the time when it is completely swallowed. The residence time of the sample inside the cabinet was deliberately kept short in every test (less than 3 min from the point at which sample was introduced until the time test was performed). This is important to consider because, this time is comparable to the residence time observed in real oral consumption. An increase in residence time causes alteration to the samples that affects the performance of the test (e.g samples get dry or deform loosing the original geometry).

From this it can be concluded that in the mouth the products tested did not achieve thermal equilibrium during mastication. Thus, most of the changes in texture may be the product of the mechanical input from teeth and interaction with saliva. This argument however cannot be generalised and extended to other food products. For example there are products that are very sensitive to mouth temperature (e.g products that melt at body temperature such as: chocolate, gelatine and butter). This just highlights the importance of considering an adequate temperature when measuring textural properties with the use of instruments.

The test consisted of the penetration of the sample with a probe vertically attached to the movable arm of the texture analyser (see Figure 7.4). Two probes, different in geometry and materials were evaluated; a flat cylindrical stainless steel probe with 5mm diameter and a probe having a natural tooth affixed to it.

By penetrating the sample to the set distance, deformation was applied, which imitates the chewing action of the teeth. The device was programmed to measure force in compression using the "return to start" option. Therefore, collection of data is started when the probe moves beyond the point when the trigger force is detected. Recording of data is stopped when the arm returns to the starting position. The starting position of the

probe was set at 130 mm from the base plate of the sample holder where distance is equal to 0 mm. The established distance assured complete separation of the probe from the sample after withdrawal, this is especially important in samples where cohesive failure appears to be dominant. The texture analyser settings under which tests were performed are displayed in Table7.1.

TABLE 7.1 Experimental set up of texture analyser to measure stickiness and hardness by penetration

Parameter	Value	Description
Pre-test speed	1.0 mm/s	Speed at which probe approaches the sample while searching for the trigger point
Test speed	2.0 mm/s	Speed at which probe compresses the product to approach to target distance
Post-test speed	10.0 mm/s	Speed at which probe withdraws from the sample after reaching target distance and returning to start point
Distance	5.0 mm*	Distance probe penetrates into the sample after the trigger point
Trigger Force	20.0 g	Penetration test is started after a force greater than 20g is detected. This force was suitable for all the samples and ensured full contact of the probe with the surface. Lower values caused premature triggering as forces could be detected from vibration of the moving probe or due to the fan inside the temperature cabinet.

*Penetration distance when using tooth probe.

Using the experimental procedure discussed above, tests were carried out under two different surface conditions:

1) ***Dry surface-*** Under this condition no wetting agent was added over the surface of the sample (free from sugar coating) exposed to probe contact.

2) ***Wet surface-*** The surface of the sample exposed to probe contact was wet with either distilled water or artificial saliva. Once the sample was introduced and secured to the base of the sample holder two drops (≈ 0.1g)

of the wetting agent were added over the surface and left in contact for 1 minute after which probe proceeded to penetrate the sample. Right before contact the probe was submerged into a small beaker filled with the corresponding wetting agent.

After every test, the probe was cleaned to remove any food material that could have remained attached to it. The natural tooth probe was cleaned using a tooth brush and rinsed with distilled water.

7.4 Analysis of data

Parameters were determined essentially as described by Bourne, (1978) for a typical TPA curve. However in this case the curve generated corresponded to a one only loading-unloading cycle, as a single "return to start" (or single compression) test was used. A typical example of force-time curve is shown in Figure 7.5. As can be observed from this figure the force at time 0 is 20g, which was the set trigger value indicating that the probe had made a full contact with the sample. The force then starts to rise until the probe penetrates to the set distance. It can be seen from this particular graph that force rises smoothly with time. This indicates that the material is being deformed and that the deformation is causing the material to flow. If there were an abrupt discontinuity (drop) in the force time curve this would indicate that the material had ruptured. The force achieved after reaching the set penetration distance (maximum peak) was taken as measurement of the strength or hardness (firmness) of the product. The stickiness of the product was then evaluated as the force (g) needed to withdraw the probe from the sample corresponding to the maximum negative peak in the curve. The area under the negative curve was then the work of adhesion calculated as the integral value in g·s. The stringiness, also shown in the graph, gave a measurement of the distance the product was extended during debonding before breaking off.

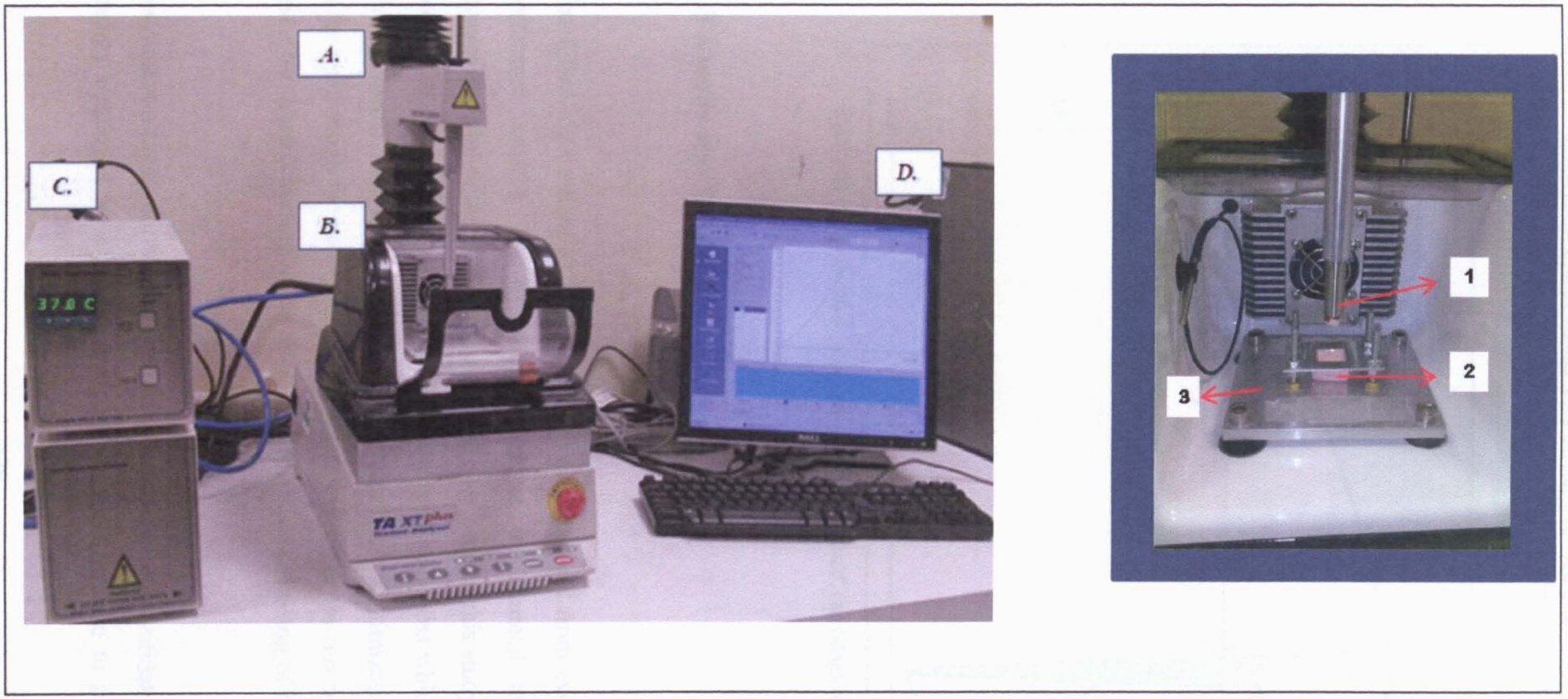


FIGURE 7.4 The picture on the left shows the instrumental set-up used for stickiness and hardness measurement by penetration tests. **A.**Texture Analyser, type TA.XT-*plus* equipped with a 30Kg load cell **B.** temperature controlled peltier cabinet (XT/PC) **C.** Peltier Control Unit (PCU) displaying the test temperature **D.** Hardware control unit displaying output from data acquisition software. The picture on the right is a close-up of the temperature cabinet. **1.** Probe (natural tooth probe) **2.** Sample position **3.** Sample holder

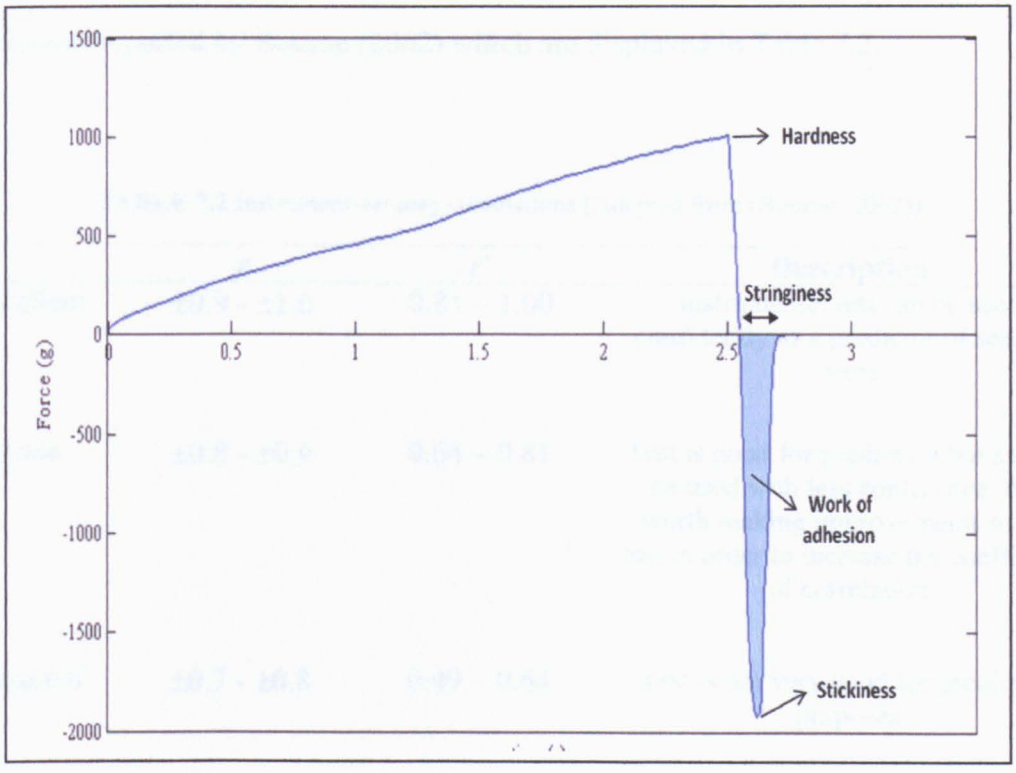


FIGURE 7.5 Force-time graph for a "return-to-start" test (single compression).

7.5 Results and Discussion

The following sections will discuss the results obtained from every set of experiments performed towards the optimization of an instrumental test that allowed the characterization of the stickiness of the samples used in this study. An optimal test was deemed to be one that produced measurements of stickiness which best correlated with measurements obtained from sensory evaluations. Optimization in this case was achieved through the incorporation of factors that made the test conditions more closely resemble those occurring in the mouth, during oral processing of food.

Instrument-sensory correlations were used as a guide to validate the suitability of the method employed as a predictor of the sensory response to the perception of food

stickiness. Pearson correlation coefficients obtained were assessed by comparison to correlations reported by Bourne (2002) which are displayed in Table 7.2.

TABLE 7.2 Instrument-sensory correlations (Adapted from (Bourne, 2002))

	r	r^2	**Description**
Excellent	±0.9 - ±1.0	0.81 – 1.00	Instrumental test can be used confidently as a predictor of sensory score
Good	±0.8 - ±0.9	0.64 – 0.81	Test is good for prediction but should be used with less confidence. It is worth making improvements to the test in order to increase the coefficient of correlation
Marginal	±0.7 - ±0.8	0.49 – 0.64	Test is not very good for predictive purposes
Poor	< ±0.7	< 0.49	Test is not useful for predictive purposes

7.5.1 Effect of probe material on the stickiness of food materials in dry and wet conditions at standard temperature (25°C)

Table 7.3 displays the measurements of stickiness obtained from experiments performed using two different probe materials (stainless steel probe and tooth probe). Two different sample surface conditions were tested in each case, a dry and wet surface where distilled water was used as the wetting agent. Experiments were run at standard temperature of 25°C. As a consequence of the difference in probe geometries, slightly different test settings had to be established for each of the probes. This prevents the making of a direct comparison between results for the two probe materials. For experiments carried out using the stainless steel probe a penetration distance of 2 mm was suitable to ensure a proper contact between the probe and the food material whilst for tests using the tooth probe the distance had to be set at 5 mm. This was because a

distance of 2 mm was not enough to achieve full contact between the tooth and the food material. At 2mm penetration only a small portion of the highest cusp of the tooth was in contact with the surface of the sample, this is why a distance of 5 mm was choose instead (see Figure 7.6). A penetration distance of 5mm ensured an adequate penetration and hence a proper contact between tooth and food material, similar to that in a real mastication process.

TABLE 7.3 Comparison of absolute value of stickiness (Maximum force value in g) of food samples for different probe materials (stainless steel vs tooth) under two different surface conditions (dry and wet surface) at 25°C (values are presented as a mean of four repetitions ± SD)

Surface Condition		SAMPLE / Probe Material	JT	FR	FRUT	TD	MCH	WO
	DRY	*Stainless steel*	127.1	119.7	25.3	133.4	6.8	158.8
		SD	46.4	20.1	18.2	17.4	13.6	53.6
		Natural tooth	141.9	147.8	43.1	226.7	0	129.5
		SD	14.3	15.0	33.5	15.9	0	95.3
	WET	*Stainless steel*	15.0	38.5	360	26.6	504.2	886.8
		SD	4.1	15.5	55.2	8.4	85.1	130.5
		Natural tooth	83.6	72.9	1366.5	107.9	2017.4	3869.7
		SD	10.0	4.0	82.3	15	351.3	441.3

NOTE: Due to differences in probe geometries penetration distance for stainless steel probe was set to 2mm whilst for natural tooth probe penetration distance was set to 5 mm to ensure sufficient contact between sample and probe.
SD= Standard Deviation

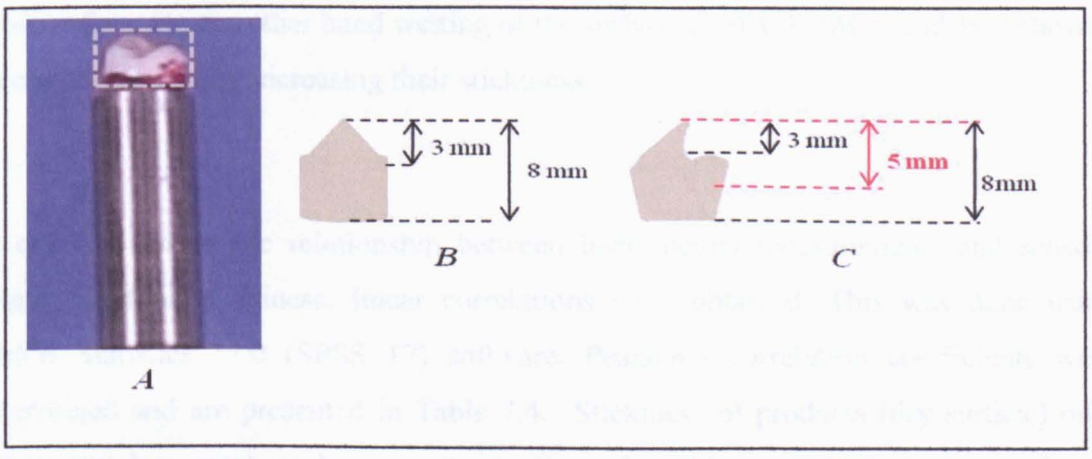


FIGURE 7.6 Degree of tooth penetration in stickiness tests. ***A.*** Tooth probe used for experiments highlighting the profile of the tooth piece. ***B.*** Tooth representation as seen from the side with the highest cusp. ***C.*** Profile of tooth piece showing the penetration distance (5mm) selected for the experiments.

In general absolute values of stickiness obtained, for the samples when using the tooth probe were higher than those observed with the use of the stainless steel probe except for the MCH and WO products. The values of stickiness for MCH and WO (dry surface) with the tooth probe were lower than those with the stainless steel probe. These differences are caused by differences in the contact areas of the materials. It is presumed in this case that the tooth has a greater contact area for adhesion due to the roughness caused by the pits and fissures present on the tooth surface. However, this argument could not be proved because estimation of the cross-sectional area of the tooth was not possible due to its complex irregular nature. Differences in stickiness for the same foods to each of these materials however, have been reported within the literature when flat surfaces with known cross-sectional areas of stainless steel and dental enamel have been used. For example (Caldwell, 1959) found smaller values for the adhesiveness of corn flakes-water mixtures to stainless steel compared to those obtained for human tooth surfaces. On the other hand; Foegeding and Steiner (2002) reported that dental enamel was the surface producing the lowest values of stickiness when evaluating caramels.

The effect of wetting of the surface using distilled water turned out to be a more important factor in the determination of the stickiness of the foods samples. As can be seen from Table 7.3, regardless of the probe material used, the values of pull-off force obtained for products such as JT, FR and TD considerably decreased after wetting of the food surface. On the other hand wetting of the surface of FRUT, MCH and WO showed an opposite effect by increasing their stickiness.

In order to assess the relationship between instrumental measurements and sensory scores for food stickiness, linear correlations were obtained. This was done using PASW statistics 17.0 (SPSS 17) software. Pearson's correlation coefficients were determined and are presented in Table 7.4. Stickiness of products (dry surface) onto either stainless steel probe or onto tooth probe did not exhibit any meaningful correlation with sensory evaluations of stickiness ($r = -0.346$, $p>0.05$ and $r = -0.203$, $p>0.05$ respectively). It was interesting to observe the effect on the measurements when the sample surfaces were wetted. The absolute values of stickiness measured varied

greatly between the two probe materials. The variation in relative values of stickiness, however, was minimal. Trends exhibited by the products according to their increase stickiness showed good correlations with sensory scores assigned in both cases ($r=0.838$, $p< 0.05$ for stainless steel probe and $r=0.834$, $p< 0.05$ for the tooth probe).

TABLE 7.4 Pearson's correlation coefficients between sensory attributes and instrumental parameters

SET CONDITIONS		Pearson's coefficient
Surface	**Probe material**	
DRY	*Stainless steel*	-
	Natural tooth	-
WET	*Stainless steel*	0.838
	Natural tooth	0.834

Note: Correlation coefficients reported were only those statistically significant (i.e $p<0.05$).

The addition of distilled water onto the surface of the sample, notably lead to an improvement in the prediction of the sensory stickiness. Measurements of stickiness in dry conditions did not show any correlation with sensory analysis, indicating that moist conditions existent in the mouth would have a notable effect on the perception of stickiness. This may be one of the main reasons why measurements of stickiness reported in the literature have hardly ever been shown as having good correlations with the sensory perception of stickiness. It seems instrumental evaluations have omitted a significant factor apparently having a crucial influence in the determination of food stickiness, as perceived in the mouth, as has been suggested in some publications (e.g (Foegeding and Steiner, 2002)).

Even though the stainless steel probe showed a slightly better relationship than the one shown by the tooth probe, the absolute values of stickiness corresponding to each product had much higher variation showing variation coefficients ranging from 15% - 40.3% whilst measurements of stickiness using the tooth probe resulted in variation

coefficients lower than 17%. Furthermore, by ordering the products according to their increasing degree of stickiness it was possible to discern that the results from using the tooth probe provided a much better trend, being closer to that obtained from sensory evaluations (see Table 7.5).

TABLE 7.5 Instrument-sensory comparisons of stickiness trends

Type of measurement	**Trend of products according to stickiness** (Least sticky product → most sticky product)
Sensory evaluations	JT < FR < FRUT < TD < MCH < WO
Instrumental test * (using stainless steel probe)	JT < TD < FR< FRUT< MCH < WO
Instrumental test * (using tooth probe)	JT < FR < TD < FRUT < MCH < WO

* Tests performed under surface wetting conditions

< stickiness less than

Looking at the trend corresponding to the tooth probe from Table 7.5 it can be seen that the FRUT product appears preceded by TD contrarily to the order in which they appeared in the sensory sequence (TD < FRUT). However, the difference between these two products according to the statistical analysis performed in Section 5.4 was not statistically significant and hence both samples were classified within the same category of stickiness as products with medium stickiness. Instrumentally however, the difference observed between these samples was considerable, where a tensile force of 1366.5 g was measured for FRUT against a force of 109.7 g exhibited by TD.

Although direct comparisons between probe materials were not possible in this study it has been well established within literature that the adhesiveness, shown by the sample, can be highly probe material specific. In order for adhesion to occur the surface energy

of the substrate, in this case the surface energy of the probe material, must be greater than that of the adhesive (food material) (Bhandari and Howes, 2005). In addition, inorganic materials have been regarded as having high surface energies compared to organic materials and polymers which have lower surface energies. Both probe materials used in this study corresponded to inorganic materials. For instance, the outermost layer of the tooth; the enamel, comprises 96% inorganic material and 4% water of its total volume, whereas stainless steel is an iron alloy with a minimum of 10.5% chromium. Surface wetting tensions for these materials have been reported as 88[2] and 71 mN/m respectively (Bhandari and Howes, 2005, Busscher et al., 1984). Even though both of the probe materials have higher surface energies, the two surface energies are notably different from each other. Moreover, the trends in sample stickiness obtained from these sets of experiments varied in relation to trends shown for sensory perception tests, as discussed above. Classification of the products, according to their increase in stickiness, obtained with the tooth probe showed the most similar trend to the trend obtained from sensory evaluations. Therefore, based on these results, the tooth probe was the material selected to carry out further experiments. In addition it has been suggested that the firm lodging of food in tooth fissures can contribute to adhesion due to the typical pressures ($1Kg/m^2$) found in mastication (Caldwell, 1962). In addition, tooth enamel is covered with a pellicle (organic mixture of substances) deposited from saliva, which has also been considered to play a role in the adhesion of foodstuffs to the oral surfaces.

7.5.2 Effect of temperature on the stickiness and hardness of food materials in wet and dry surface conditions using a tooth probe.

Figure 7.7 shows the results obtained when the samples were tested at different temperatures. It shows graphically how stickiness is affected by temperature and surface conditions.

[2] This value corresponds to ground and polished human enamel.

Values of stickiness for dry sample surfaces, for products with dominant elastic properties (JT, FR, TD) showed small variations when the temperature was raised from 25°C to 37°C. JT was the product that showed the greatest variation in this group of samples. A small decrease in the value of stickiness was measured from 141.9g to 124.4g. This change might have been caused by a slight drying out of the surface as a result of the increase temperature from 25°C to 37°C.

It should be mentioned that the temperature within the cabinet was regulated by a fan. The circulation of warm air over the surface of the sample could contribute to its drying out. It is important to stress this phenomenon because as mentioned previously the residence time of the samples inside the cabinet were short during execution of the test. If residence times were longer, such as has been common practice, in order to achieve thermal equilibrium of the samples, then samples could have experienced more drastic physical changes such as the complete drying out of the gel. This may affect the physical measurement of textural properties (hardness and stickiness in this case). This points towards the importance of simulating oral conditions when performing experimental measurements. If this is not done the parameters measured can be considerably affected.

The toffee sample (WO) when tested in dry conditions was noticed to be very sensitive to temperature even at the short residence times used in this experiment. This sample exhibited an increase in its stickiness from a value of 129.5g (25°C) to a value of 997.5 g (37°C). This variation can be explained by the material being plasticised by the effect of temperature. Plasticisation results in the softening of the sample as seen in Figure 7.9. The softening may have resulted in the stickier character developed by the product as it means that even though the sample is holding its original shape, the application of a light pressure can cause it to flow and surface spreading. This effect can be explained in terms of a change in the glass transition temperature. The glass transition temperature is the temperature at which the product changes from a glassy state to a plastic state (or rubbery state). In the plastic state, the viscosity of the product decreases allowing molecular movement. This transformation is known to have a significant impact on

product stickiness. Determination of glass transition temperature has even been recognized as an approach to describe stickiness (Adhikari et al., 2001). The effect of temperature on the FRUT and MCH samples was minor. No considerable changes were appreciated in their stickiness with the change of temperature. This may suggest that these products have higher glass transition temperatures than toffee.

The same figure also shows the effect of temperature on sample stickiness at a wet surface condition. All products showed lower values of stickiness at 37°C compared to those at 25°C

The most interesting results obtained from this set of experiments were as follows. At both temperatures, when the samples were wetted the stickiness of JT, FR and TD decreased significantly. Also, at both temperatures, when the samples were wetted the stickiness of FRUT, MCH and WO greatly increased. This suggests that two different mechanisms are involved in the stickiness of the two groups of products.

The results discussed above were the most relevant to the aim of this study because they show that the instrumental measurement of stickiness can be misleading in terms of being a predictor of sensory evaluations. As these results show, the measurements of stickiness taken under dry conditions are completely different to those taken under wet conditions. One may say that by making the test conditions more closely resemble to those found in the mouth, the measurements will be much more meaningful dramatically. Indeed, in our case, making the conditions of the test resemble those found in the mouth has increased its ability to predict the food stickiness experienced by the consumer.

Poor and not significant linear correlations ($p > 0.05$) were obtained between sensory evaluations and instrumental tests of stickiness carried out with dry surface conditions. This was even the case when using a temperature of 37°C (body temperature) (see

Figure 7.8). It is clearly observed that wetting of the samples improved notably the correlations between both measurements. Strong positive correlations showed that the best predictor of the sensory evaluation of stickiness was the instrumental test carried out under wet conditions at a temperature of 37°C ($r=0.88$, $n=6$, $p= 0.039$).

While performing experiments it was noticed that when distilled water was added to any of the products of the first group (JT, FR and TD) there was a clearly visible fast uptake of water from the surface towards the interior of the structure and gels became quickly hydrated. Water disappeared rapidly from the surface. Whereas for the second group of products (FRUT, MCH and WO), the water added tended to remain on the top of the surface. In the specific case of WO, formation of a white layer could be observed with time. This shows that WO is miscible with water and surface solubilisation could also be highly possible. It is presumed that this mixing could have played a role in the activation of the bond formed between the food material and the tooth probe.

It has been established that for hydrocolloids to exhibit adhesion, a certain amount of water is required, thus they reach a maximum adhesion at an optimum degree of hydration. Some hydrocolloids exhibit adhesiveness in the presence of very little water, where excessive water will cause the formation of slippery non adhesive mucilage (Chen and Cyr, 1970). The hydrocolloids present in JT, FR and TD were already in a hydrated state, under which they exhibited certain degree of stickiness as obtained when tested under dry surface condition. It might be possible that after wetting of the surface of the samples with distilled water, they became over hydrated. This resulted in the formation of a slippery layer over their surface. Presumably this layer causes the samples to slide easily over the teeth of panellists without adhering, resulting in low perception of stickiness.

When comparing results between stickiness and hardness (Figures 7.7 and 7.9), it is appreciable that both physical properties are quite differentiable from each other instrumentally and that there is clearly no association between both textural properties.

For instance, the stickiest product (WO) was neither the softest nor the hardest. At the same time the least sticky product (JT) was also neither the hardest nor the softest.

Regarding the hardness of the products, Figure 7.9 shows the results obtained at two different temperatures. The most appreciable effect on hardness was that exhibited when the samples were wetted with distilled water at a temperature of 37°C. In this case all the samples became softer when wet than when dry (at either temperature). Curiously the best correlations produced with sensory tests were those obtained from experiments carried out at 25°C in dry conditions. This clearly indicates that hardness is primarily an attribute that is evaluated right at the beginning of the chewing sequence when neither the saliva nor the temperature in the oral cavity exerted any great influence on the structure of the sample. It is also possible that hardness is a perceived feature of the bulk sample under compression, while stickiness is a feature closely related to sample surface.

The MCH product was not considered when plotting correlations of hardness with sensory tests (see Figure 7.10) because this product exhibited a huge variation in hardness between samples compared to the rest of the products being evaluated. This product was the least consistent batch to batch. As can be seen from the graph at 37°C the average hardness of MCH increased from its value at 25°C. The hardness would be expected to decrease or stay the same with temperature, but never increase. The only way that results like this could be obtained is if there was a significant variation in hardness between the individual MCH samples. Variation in the hardness of the MCH product was appreciated throughout the period of research. Factors contributing to this variation could include:

- Inconsistency in the product obtained from retailer, due to issues with quality control.
- Poor packaging materials which fail to prevent change in the moisture content of the product during storage. It is well known that the best way to prolong the

shelf life of commercial confectionery products is the use of good quality wrapping materials (Jackson, 1995).

- Storage conditions. These could have an effect on the samples especially as humidity was not controlled when storing the samples. However, this is less likely as no significant effect was noticed for the rest of the products. It is possible that this had an effect for MCH due to the poor quality of its wrapping materials (mentioned above).

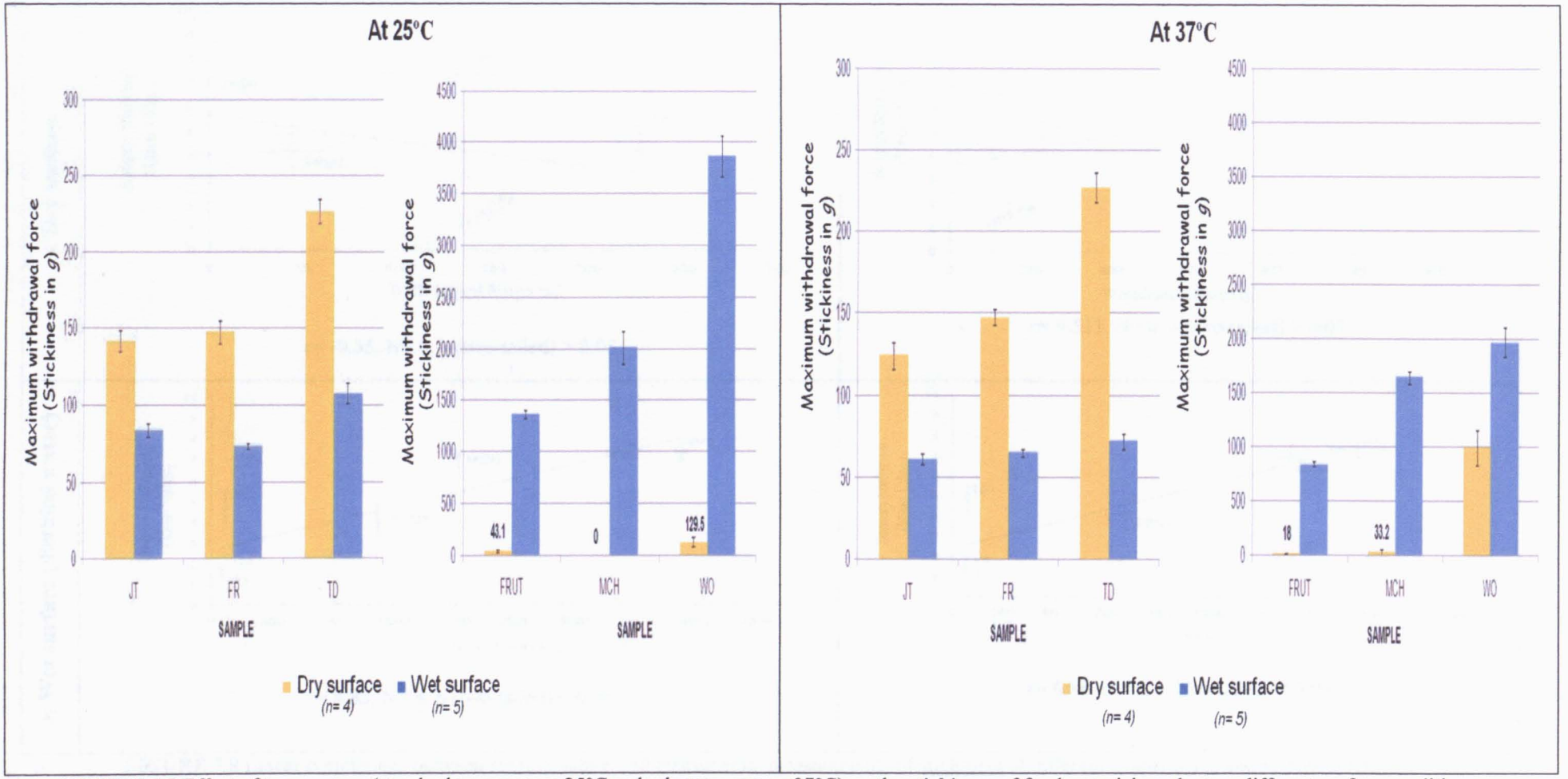


FIGURE 7.7 Effect of temperature (standard temperature 25°C vs body temperature 37°C) on the **stickiness** of food materials under two different surface conditions (dry and wet surface; use of distilled water as the wetting agent). Error bars show ±SE

SAMPLES: **JT**- *Thorntons' Fruit Jelly*; **FR**- *Fry's Turkish Delight*; **TD**- *M&S Turkish Delight*; **FRUT**- *Fruit-Tella*; **MCH**- *Milk Chews*; **WO**-*Werther's Original*

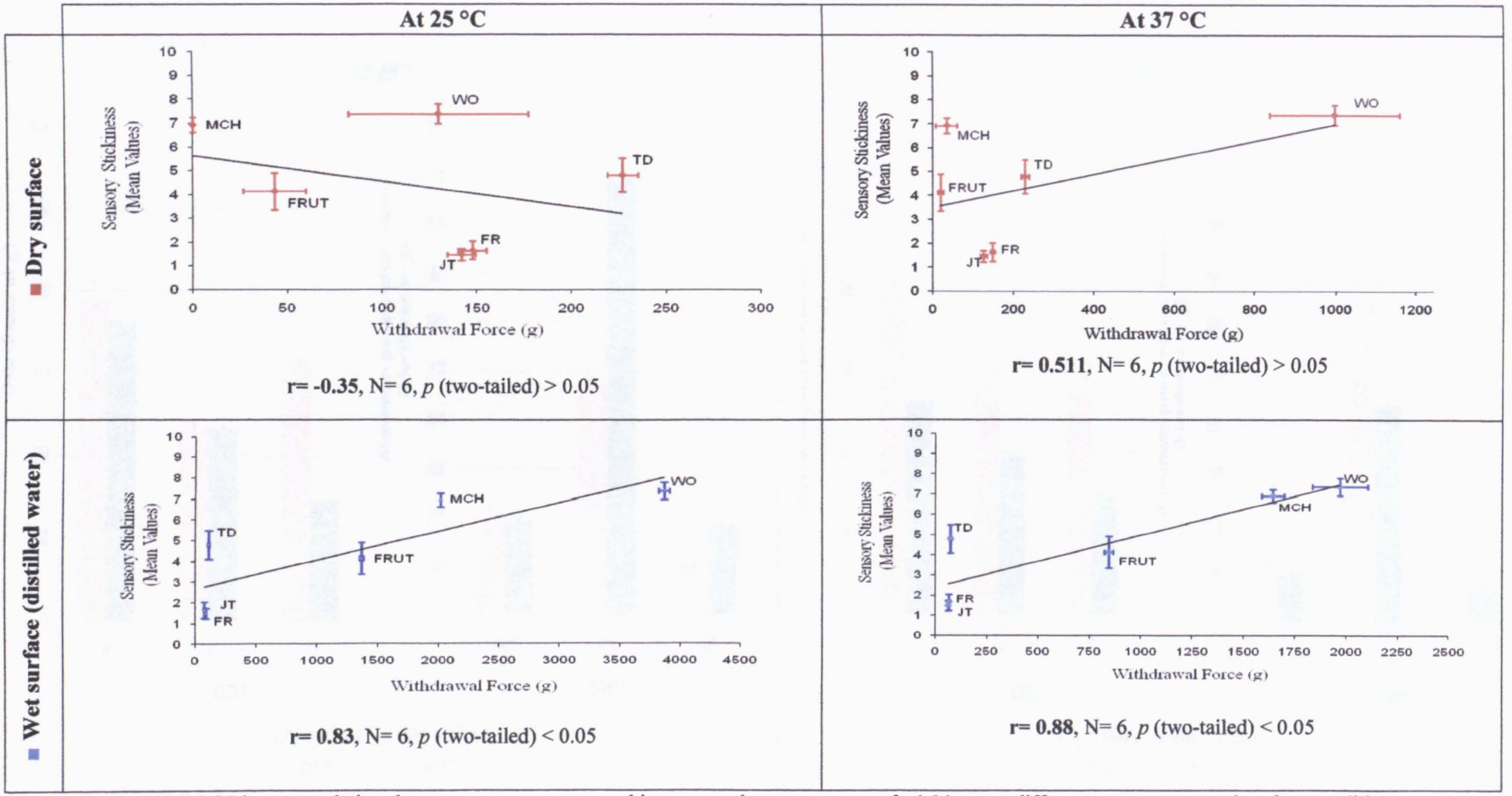


FIGURE 7.8 Linear correlations between sensory scores and instrumental measurements of **stickiness** at different temperatures and surface conditions (standard error bars are shown)

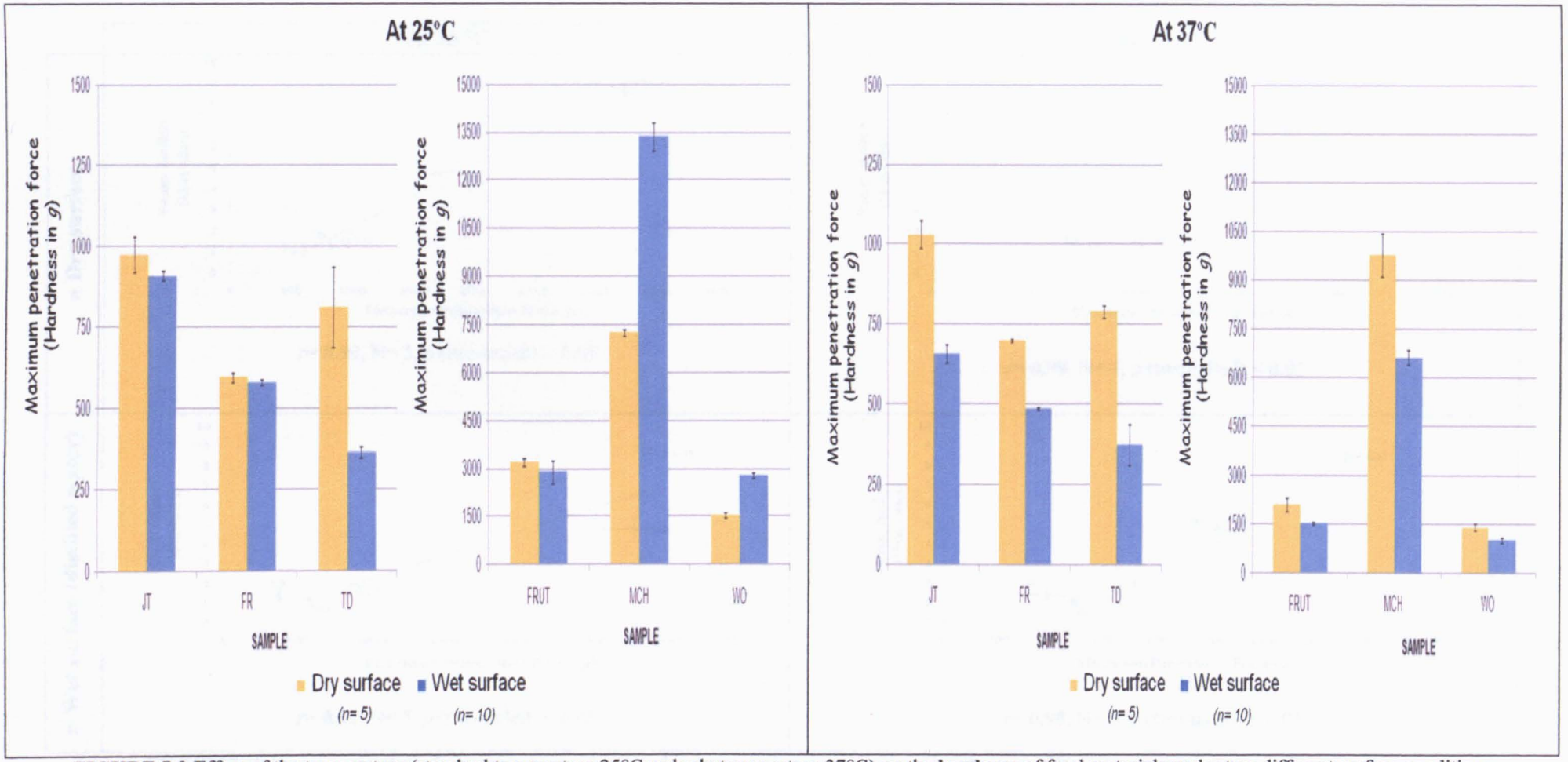


FIGURE 7.9 Effect of the temperature (standard temperature 25°C vs body temperature 37°C) on the **hardness** of food materials under two different surface conditions (dry and wet surface; use of distilled water as the wetting agent). Error bars show ±SE

SAMPLES: **JT**- *Thorntons' Fruit Jelly*; **FR**- *Fry's Turkish Delight*; **TD**- *M&S Turkish Delight*; **FRUT**- *Fruit-Tella*; **MCH**- *Milk Chews*; **WO**-*Werther's Original*

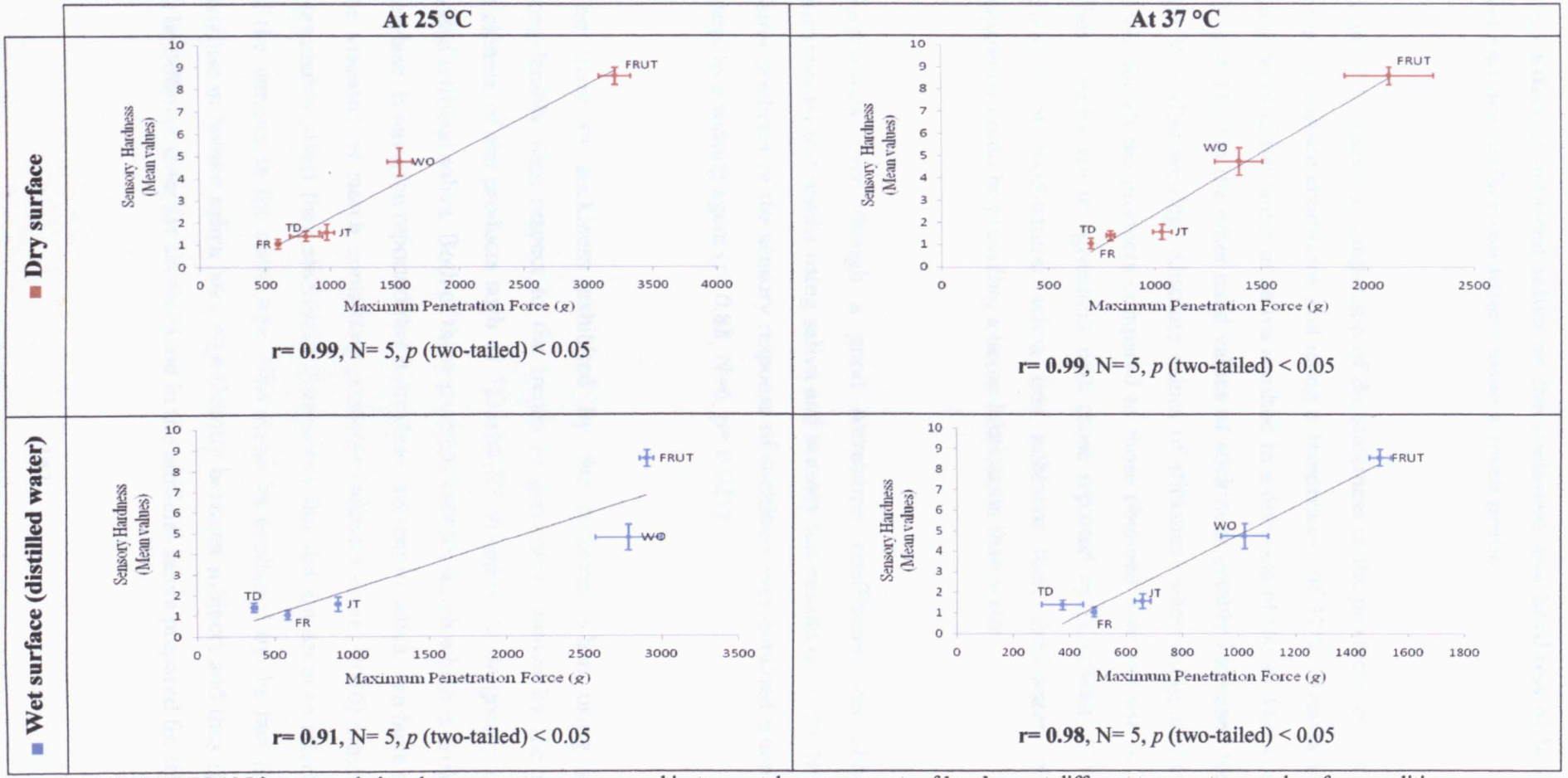


FIGURE7.10 Linear correlations between sensory scores and instrumental measurements of **hardness** at different temperatures and surface conditions (standard error bars are shown).

7.5.3 Effect of artificial saliva on the stickiness and hardness of food materials in wet and dry surface conditions using a tooth probe.

Figure 7.11 shows a comparison of the stickiness of the products, evaluated under three different surface conditions and using a temperature of 37°C. Results showed that the addition of either water or saliva resulted in a decrease of the stickiness of products JT, FR and TD. On the other hand values of stickiness notably increased for MCH, FRUT and WO after wetting. Absolute values of stickiness when using artificial saliva were lower for all the products compared to those obtained with the use of distilled water. These results are in agreement with those reported by (Caldwell, 1959), who found mixtures of food-natural saliva less adhesive than food-water mixtures. Saliva apparently could be providing a better lubrication than water.

Surprisingly even though a good correlation coefficient was obtained between instrumental test results using saliva and sensory test results ($r= 0.86$, $N=6$, $p= 0.028$) a better predictor of the sensory response of stickiness was obtained when using distilled water as a wetting agent ($r= 0.88$, $N=6$, $p= 0.021$).

The trend of stickiness exhibited by the products when using saliva differed considerably with respect to the trends in perception shown by the panellists. The stickiness of the products such as TD and FR appeared to be greatly affected by the added artificial saliva. Both of these products contain starch which is broken down by α-amylase. It has been reported that α-amylase, present in saliva, can have an influence on the viscosity of starch containing products (Mandel et al., 2010) which can, in turn, presumably affect their stickiness. Sensorially this did not seem to affect the stickiness of the samples in the same way. This could be explained by the fact that levels of α-amylase in human saliva vary significantly between subjects and thus can be expected to be different from the levels found in the artificial saliva prepared for this study.

Regarding the effect of saliva on the hardness of the products at 37°C, results showed that lower forces were required to penetrate the samples wetted with saliva than compared with a dry samples or ones which had been wetted with distilled water.

Good, significant correlation was obtained between instrumental tests performed with the addition of saliva at 37°C and sensory evaluations (r=0.853, N=5, p=0.066). This was once again lower that those obtained when tests were carried out in dry conditions.

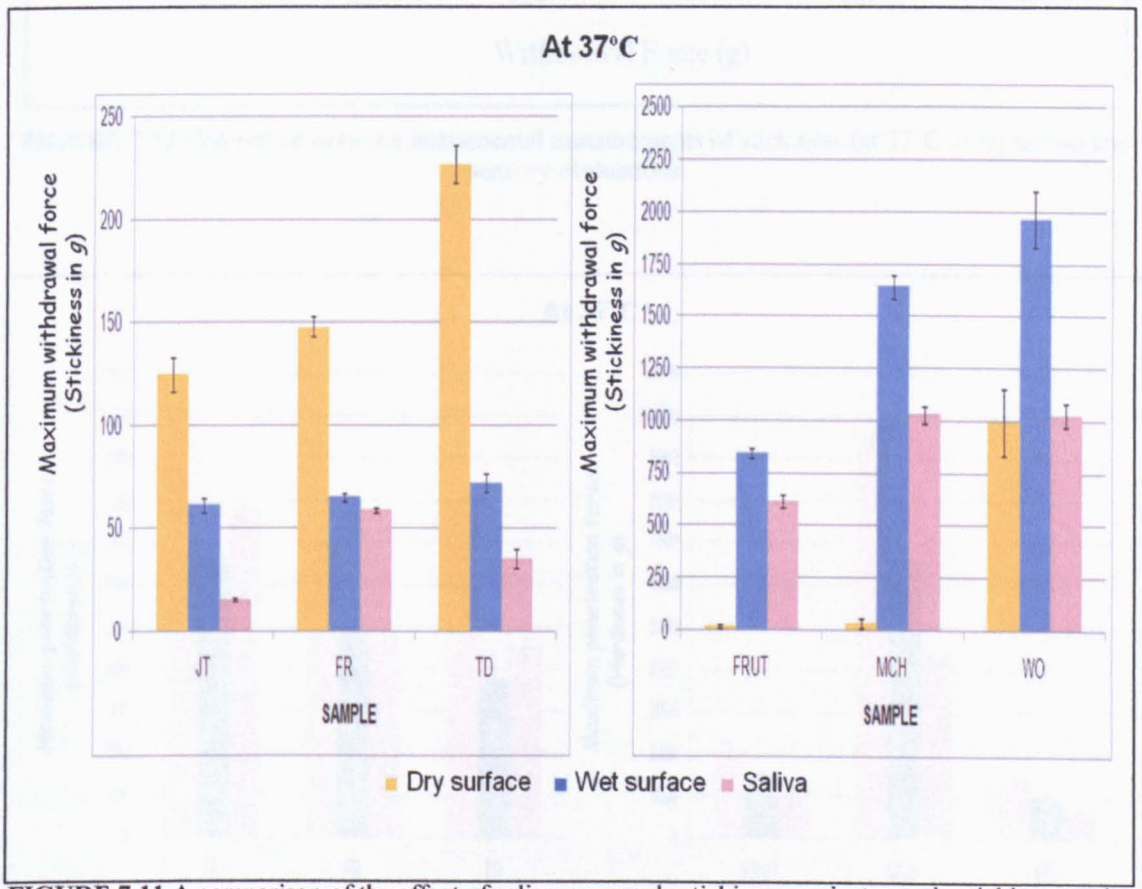


FIGURE 7.11 A comparison of the effect of saliva on sample stickiness against sample stickiness under dry and wet (distilled water) surface conditions, at 37°C. Error bars show ± SE

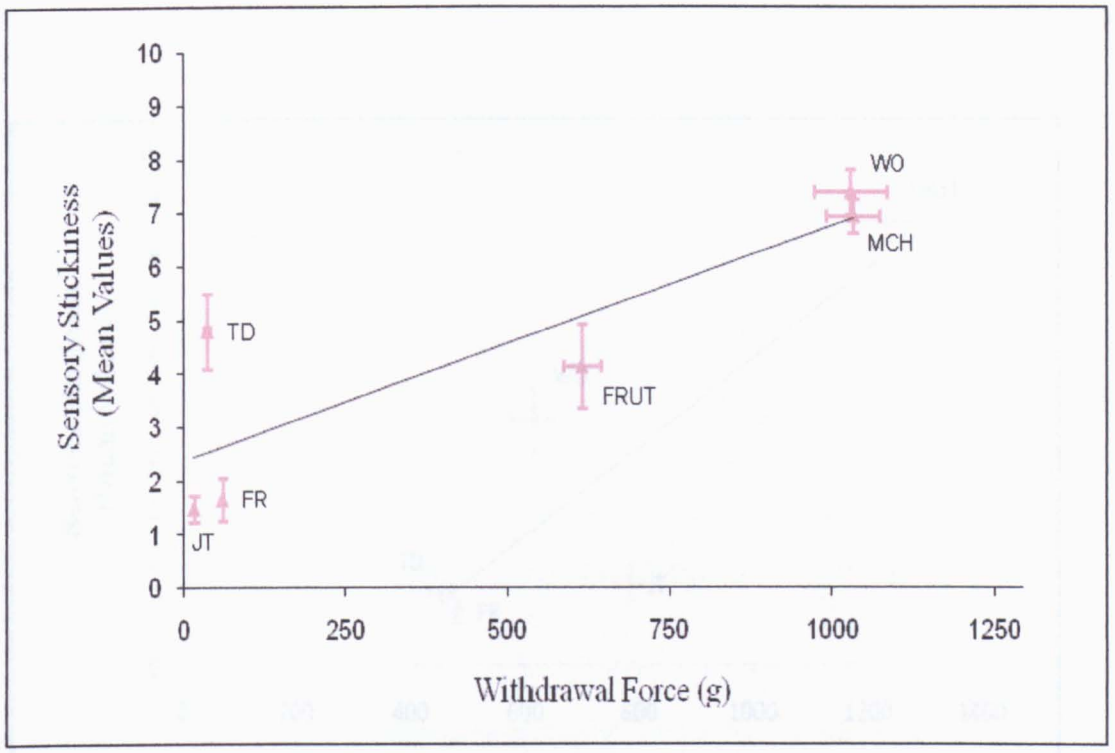


FIGURE 7.12 Correlation between instrumental measurements of stickiness (at 37°C using saliva) and sensory evaluations

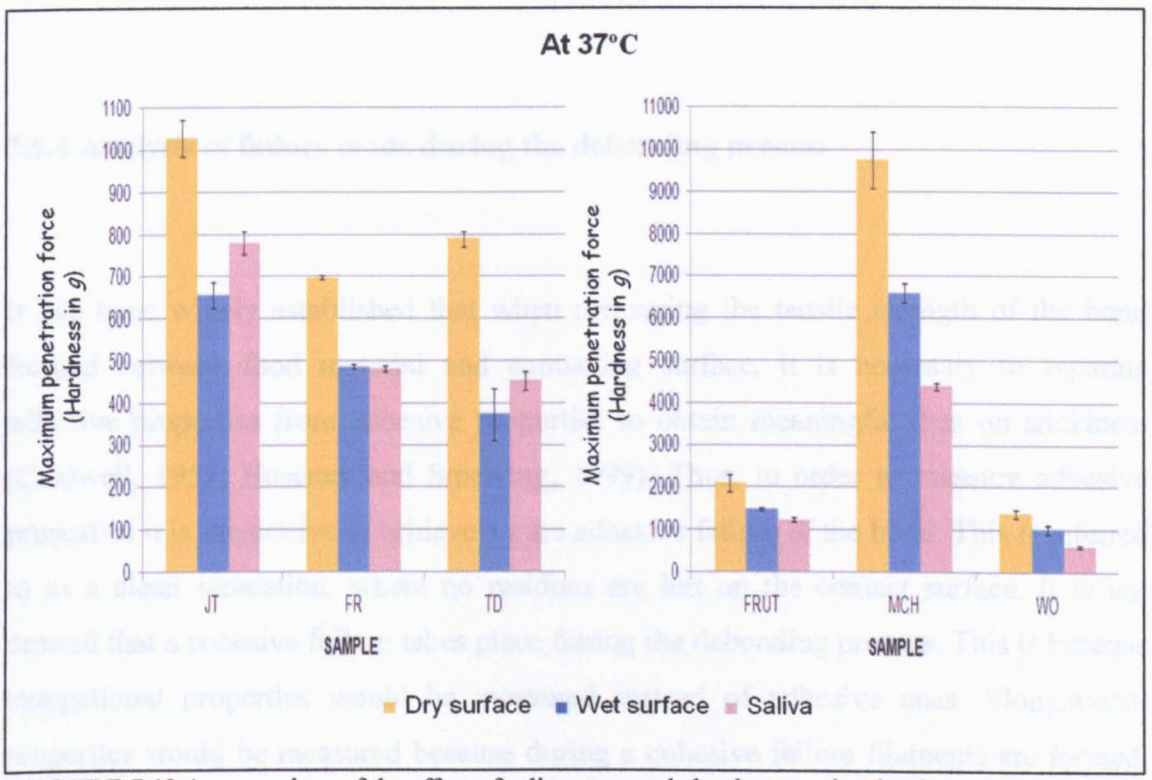


FIGURE 7.13 A comparison of the effect of saliva on sample hardness against hardness under dry and wet (distilled water) surface conditions, at 37°C.

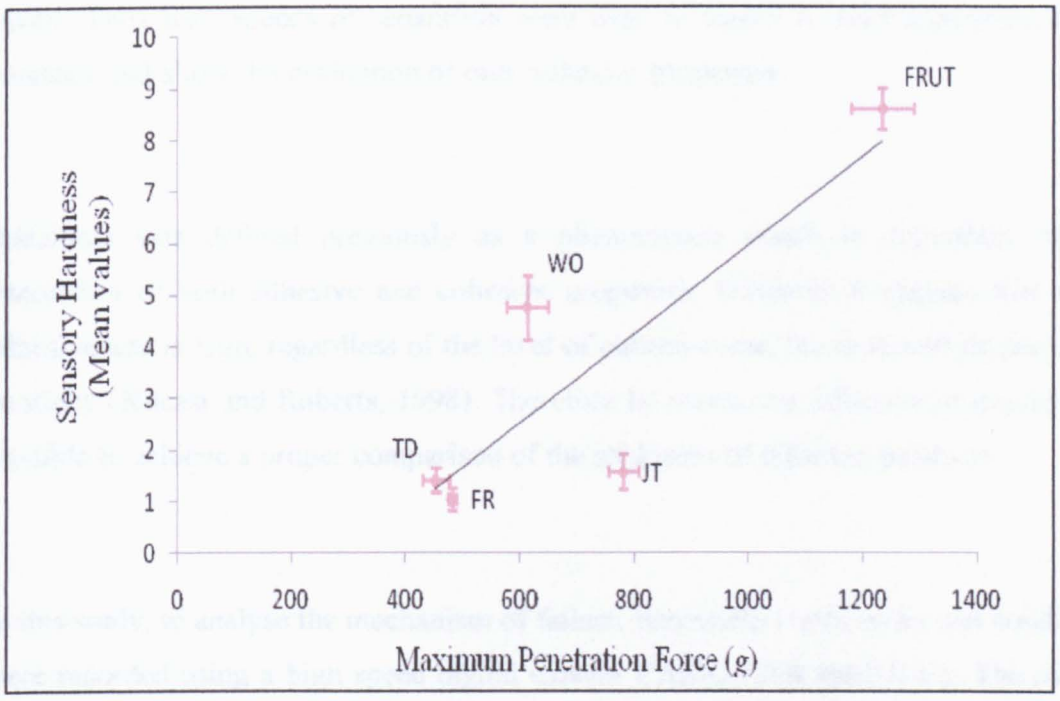


FIGURE 7.14 Correlation between instrumental measurements of hardness (at 37°C using saliva) and sensory evaluations

7.5.4 Analysis of failure mode during the debonding process

It has been widely established that when measuring the tensile strength of the bond formed between food material and contacting surface, it is necessary to separate adhesive properties from cohesive properties to obtain meaningful data on stickiness (Caldwell, 1959, Hoseney and Smewing, 1999). Thus, in order to measure adhesive properties it is imperative to achieve a pure adhesive failure of the bond. This is referred to as a clean separation, where no residues are left on the contact surface. It is not desired that a cohesive failure takes place during the debonding process. This is because elongational properties would be measured instead of adhesive ones. Elongational properties would be measured because during a cohesive failure filaments are formed. These filaments break in the middle and leave residues of the food material on the contact surface. It has been reported that both temperature and speed of separation effect

whether a cohesive failure occurs (Kilcast and Roberts, 1998). In this study temperature could not be altered, it was set at 37°C in order to mimic conditions found in the oral cavity. Thus high speeds of separation were used to ensure a clean separation at the interface and allow the evaluation of only adhesive properties.

Stickiness was defined previously as a phenomenon which is dependent on the interaction of both adhesive and cohesive properties. However it appears that when adhesiveness is high, regardless of the level of cohesiveness, the food will be perceived as sticky (Kilcast and Roberts, 1998). Therefore by measuring adhesive properties it is possible to achieve a proper comparison of the stickiness of different products.

In this study, to analyse the mechanism of failure, penetration tests under wet conditions were recorded using a high speed digital camera CASIO EXILIM EX-F1. The camera was set to a high speed movie recording mode. Even though the capacity of the camera was up to 1200 fps[3], the corresponding image size (336×96 pixels) offered a constraint to the experiment. This is because all relevant parts of the set up, both the sample and the entire range of motion of the probe could not be captured at this image size. Therefore the operating speed was set at 600 fps, giving an image size of 432×132 pixels. In order to avoid noise and motion blur in the images due to object movement or camera motion, the camera was mounted on a tripod and extra filming light was added. Video recording began right before the probe made contact with the surface of the sample and continued until the moment in which probe returned to its starting point. This high speed recorded video made it possible to shoot ultra slow-motion movies at high speeds to monitor the whole debonding process. This was done to allow visual inspection of any possible formation of filaments (or stretching of the material) that could not be seen by the naked eye. Filament formation could be an indication of the dominant type of failure mode, occurring in the products being analysed. Three repetitions per sample were recorded in order to make a comparison and detect any differences.

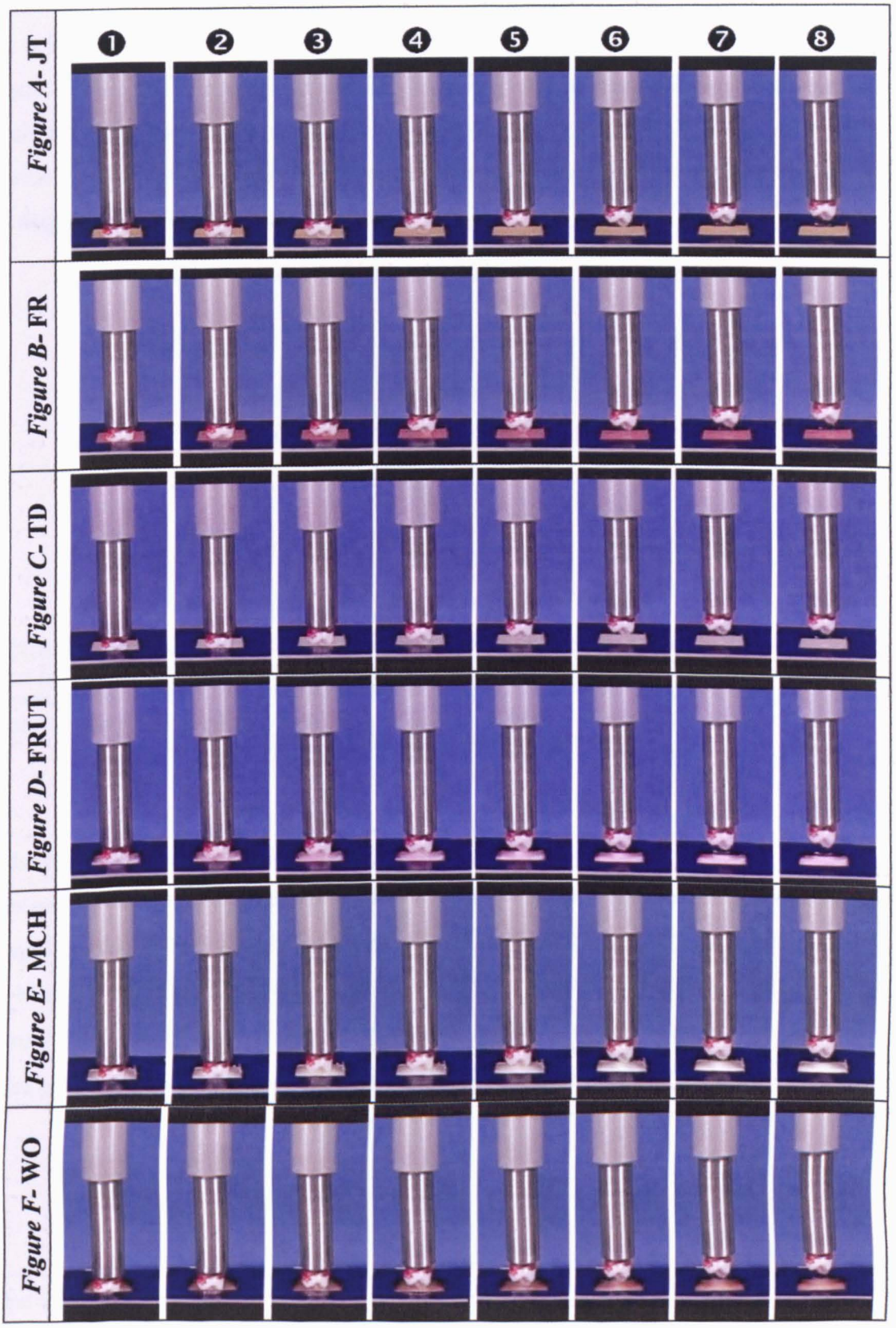
[3] fps=frames-per-second

The movie files were recorded in the ".mov" format and later converted to ".avi" format using a free-to-download converter. The files were converted to allow their processing using Microsoft® Windows® Movie Maker version 5.1. Each figure of Table 7.6 shows a set of eight snapshots extracted from each recording using Movie Maker. The snapshots correspond to a sequence of images taken throughout the separation process. Figures A to F, from the table, correspond to products JT, FR, TD, FRUT, MCH and WO. It can be well appreciated that most of the products exhibited a clear adhesive failure. The FR product, however, presented slight flow of the sample during separation, which is illustrated in frames 4, 5 and 6. After break however, no visible material was attached to the tooth probe after separation.

The experience of stickiness under the conditions occurring in the mouth is a more complex phenomenon than the stickiness that can be measured experimentally under simulated conditions. This is because in the mouth there are additional factors which are not easy to mimic under in vitro conditions. For instance; in the mouth the food is free to move around and is penetrated by teeth on both its upper and lower surfaces. This is in contrast to the instrumental test where the sample is held in place and penetrated on only one surface by a tooth probe.

In the mouth the sample contacts two surfaces. If the product is highly adhesive it is assumed that it will stick to both surfaces (upper and lower teeth). If in addition its cohesion (possibly a measure of toughness at higher compressions) is high then the product will tend to stretch as the jaw opens because is difficult to be cut off. This stretching impedes the opening motion of the jaw, which is reflected in the activity of the opening muscles and tends to increase the cycle time per chew. This will be discussed further in Chapter 10.

TABLE 7.6 Bond Failure Mechanism



Subjects are able to vary the speed of chewing, unlike in instrumental tests where the speed is constant, and texture is a regulating factor in this process. For example; one is able to open the mouth quickly when chewing a toffee but few people will attempt this, as it is highly adhesive and will probably be stuck to both the upper and lower teeth (Figure 7.15). This avoids the unpleasant "snapping" feeling caused when teeth quickly detach from the sample. A subject is much more likely to apply low speeds of jaw separation when dealing with this product. This can result in its stretching due to its high degree of cohesiveness.

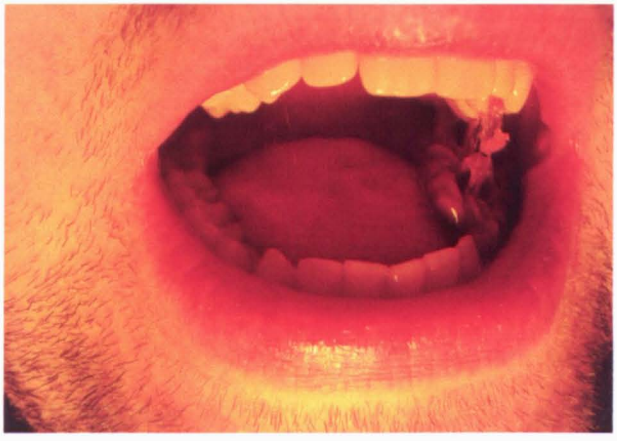


FIGURE 7.15 Picture showing a highly sticky product (WO) midway through its oral processing. The product has been fully deformed from its original shape. Observe the spreading of the food material and it fully covering the contacting teeth. Product is lodged in the pits and fissures[4] present on the surface of the teeth, because of this and due to its cohesiveness it is being stretched between upper and lower teeth. Notice also the formation of strings

On the other hand a less adhesive product such as JT is unlikely to be stuck to both the upper *and* lower teeth simultaneously. Due to its low cohesive properties (highlighted in its rapid rupture when subjected to high strains) it is unlikely that this product could ever be stretched. The fact that this product is rapidly hydrated by saliva, as observed in Figure 7.16, tends to cause it to quickly become slippery when in the mouth. All these factors contribute to the product being perceived as much less sticky.

[4] Pit and fissures correspond to the tiny hills and valleys on the top surfaces of the teeth

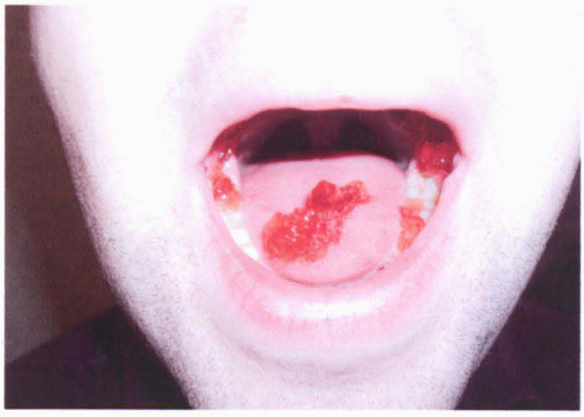


FIGURE 7.16 Picture showing a less sticky product (JT) midway through its oral processing. Food material has been deformed from its original shape. Observe that the material has suffered rupture rather than viscous flow forming a number of pieces. Notice in addition the full hydration of the product by saliva which is presumably responsible for the lower stickiness perceived.

In general it can be concluded from the information gathered during this series of tests that when the penetration tests were performed under wet conditions at 37°C mainly adhesive failures occurred for all the samples. This means that all measurements taken reflected the adhesive properties of the products. As discussed above, this allowed a full and proper comparison of the stickiness of the products under study. Results of stickiness based on tensile strength produced by these tests showed to be a good predictor of the sensory response of panellists.

Chapter 8

Dynamic Stress-Relaxation Test (Stress VS Time)

8.1 Introduction

Achieving intimate contact between the adhesive and the adherend is an essential criterion for adhesion, since the establishment of appropriate atomic and molecular distances are required for chemical interactions. Even when such chemical bonding does not occur, bringing adhesive in close proximity with the adherend increases the contact area between surfaces maximizing the forces that will keep them together. In this case wetting (or spreading); the ability of liquids to form interfaces with the solid surfaces is then maybe the most important prerequisite for the development of adhesion.

Determination of wetting is usually based on the measurement of contact angles (an angle formed by a liquid at the three phase boundary where a liquid, gas and solid intersect,θ) and surface tension. In general the smaller the contact angle and the smaller the surface tension, the greater the degree of wetting and therefore the greater adhesion exhibited, as discussed in Chapter 2. Because the food materials used in this research were not low viscosity liquids, the measurement of contact angles and surface tension properties by the usual methods was not feasible in practice. Experimental difficulties also arise from the fact that since the food samples are hydrocolloids, their surfaces could be highly active to and miscible with water. This means that the surface properties of a given hydrocolloid, as characterized in air, would be different from the same hydrocolloid in equilibrium with an aqueous environment.

The confectionery products, used in this study, behave in a very similar way to pressure-sensitive adhesives (PSAs), which despite having high viscosity are still soft enough to be deformed. Therefore they are able to achieve an intimate contact with the surface to which they are being applied, but retain some elastic memory for a time after the bonding. In such a case it is the elastic modulus which determines the resistance to flow rather than the viscosity (Dillard and Pocius, 2002).

As a result of the impracticalities in measuring contact angles, a uniaxial compression test (dynamical stress-relaxation) was adapted to compare the materials' deformation (distance measured as change in height, Δh) when subjected to a certain applied force (or stress) during a certain period of time. Test conditions were intended to approximate those occurring in the mouth during handling of food materials as will be discussed later in this chapter.

8.2 Materials and apparatus

- Texture Analyser, type TA.XT-*plus* from Stable Micro Systems, equipped with a 30 Kg load cell and software Texture Exponent 32 version 2.0, 4.0.
- A temperature controlled peltier cabinet (XT/PC) from Stable Micro Systems coupled to a Peltier Control Unit (PCU) where temperature can be easily set and displayed.
- 4 cm diameter stainless steel probe
- Stainless steel platform
- A water bath device
- Plastic petri dishes (52 mm diameter ×11 mm height)

- The specimens tested were in cylindrical shape (16 diameter × 10 mm height) and they were prepared as discussed in Section 4.4. No sugar coating of the samples was required for this test.
- Artificial saliva (pH=7.0 ± 0.2) prepared as indicated in Section 4.6.2

8.3 Method

For dynamic stress-relaxation measurements, a cylindrical sample was placed within a plastic petri dish. 24 ml of an artificial saliva solution, which had been kept in a water bath at 37 ±1°C, were then added to the sample. The plastic petri dish containing the specimen in contact with saliva was placed inside the temperature cabinet (T= 37 ±1°C) where the sample was left immersed for 2 minutes before testing. Right after the contact time was fulfilled; the petri dish was removed from the cabinet and placed directly on the lower stainless steel platform mounted on the texture analyser as shown in Figure 8.1. The test conditions were set up according to Table 8.1.

During the test the products were uniaxially compressed, using a flat stainless steel probe of 40 mm diameter, under a constant load of 4N. Stress applied, by this force, to the sample was within the range of the palatal stresses applied by humans to soft solids. This range is $4.9 \times 10^3 - 2.94\ 10^4$ N/m^2 as determined by Takahashi and Nakazawa (1991).

Even though attempts were made to run experiments applying stresses of a similar magnitude to those applied when biting, that is within the range $9.8 \times 10^4 - 1.96 \times 10^6$ N/m^2 (Takahashi and Nakazawa, 1991), in practice this was not possible. This is because various combinations of forces and speeds overloaded the equipment. For some materials, this was even the case when applying forces, to achieve stresses at the lower end of the range, at low speeds. In these instances the test was abandoned. This was due

to the viscoelastic nature of the samples tested[1] which can cause them to react as a solid in response to certain applied forces and speeds. Even when the equipment was not overloaded, for some samples (MCH in particular) the force applied initially overshot the desired 4N. This lead to the results being discarded and will be discussed later in the follow section.

The force applied to the sample (4N) was held for 10s during which the force and the distance moved by the probe were recorded. After that, the probe returned to the initial position which was set as 10 mm from the base of the petri dish. The test speed at which the probe moved, compressing the sample to reach the target force, was 0.5 mm/s. Four repetitions per sample were carried out. Force-time plots were recorded for all the samples, for further analysis.

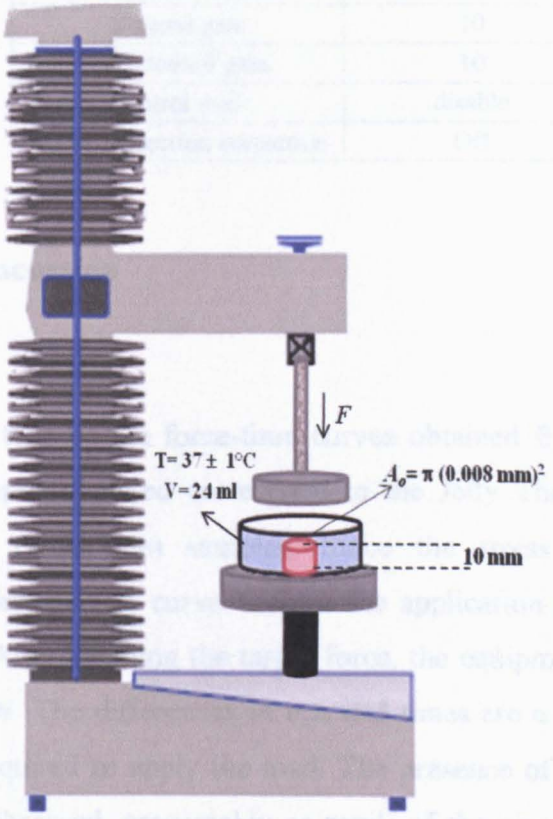


FIGURE 8.1 Texture analyser set up for dynamic stress relaxation-tests

[1] Viscoelastic materials respond more like liquids when subject to slow deformations and more like a solids when attempt to deform them rapidly.

TABLE 8.1 Texture analyser settings used to measure stress decay of the samples by uniaxial compression tests performed at constant load

Parameter	Value
Test option	Hold until time
Test Mode	Compression
Test speed	0.5 mm/s
Post-test speed	10 mm/s
Target mode	Force
Force	4 N
Hold time	10 s
Trigger type	Button
Break Mode	Off
Stop plot at	Starting position
Tare mode	Auto
Advanced options	On
Max tracking speed	5 mm/s
Proportional gain	100
Integral gain	10
Differential gain	10
Control oven	disable
Frame deflection correction	Off

8.4 Results and discussion

An example of the type of the force-time curves obtained from the test is shown in Figure 8.2. The graphs depicted correspond to the Jelly Thorntons (black line) and Werther's Original (blue line) samples. Since the stress application cannot be instantaneous, the first part of curve denotes the application of the load to reach the target force of 4N. After reaching the target force, the equipment proceeds to keep the load constant for 10s. The differences in test end times are a product of the longer or shorter rise times required to apply the load. The presence of some unloading-loading cycles can also be observed, presumably as result of the viscoelastic properties of the materials.

During deformation, in highly elastic materials, much of the stress is transmitted and stored as potential energy while it is dissipated in frictional losses for highly viscous

ones. The unloading portions of the unloading-loading cycles represent a rapid decay of the stress as a consequence of the dissipation of energy. Therefore a faster relaxation process would be an indicative of a more dominant viscous character of the material. It is important to highlight that if the material under study had been a purely elastic one, the applied force would have remained constant for the entire 10 second period, as is illustrated graphically by the solid green line plotted in Figure 8.2. In this case the material would have suffered an instantaneous elastic deformation without any further change with time and it would be expected that at the cessation of the force this would recover completely its original shape.

A comparison between the six different products was performed by using plots of stress versus time (see typical graph in Figure 8.3) which included only the first loading unloading cycle, contained within the 10 second period. This analysis was useful for the extraction of several parameters related to the viscoelastic behaviour exhibited for each product, tested under the same experimental conditions. A description of the parameters analysed is presented in Table 8.2

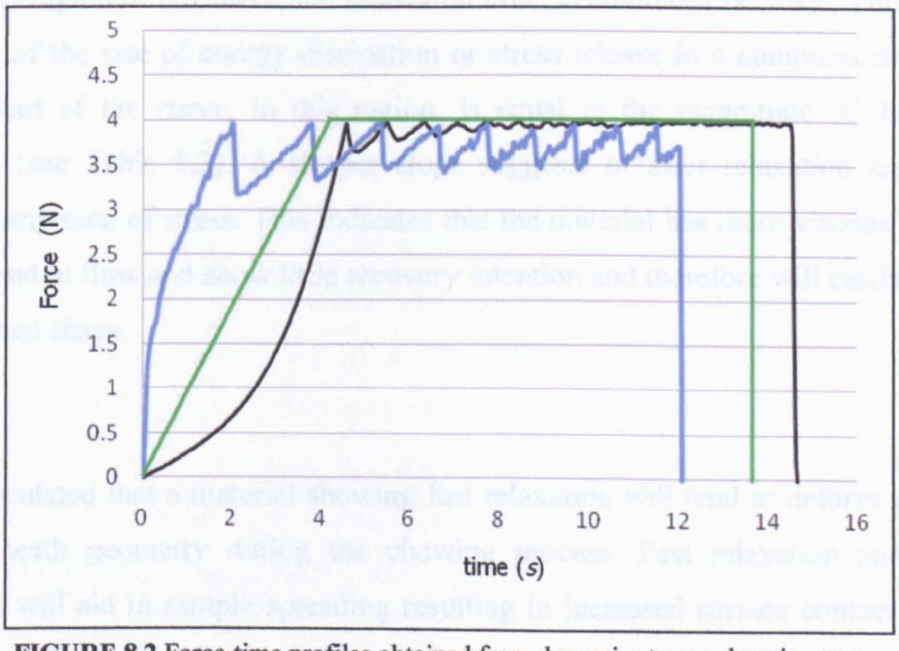


FIGURE 8.2 Force-time profiles obtained from dynamic stress-relaxation tests — WO curve — JT curve — Curve for an ideal elastic material

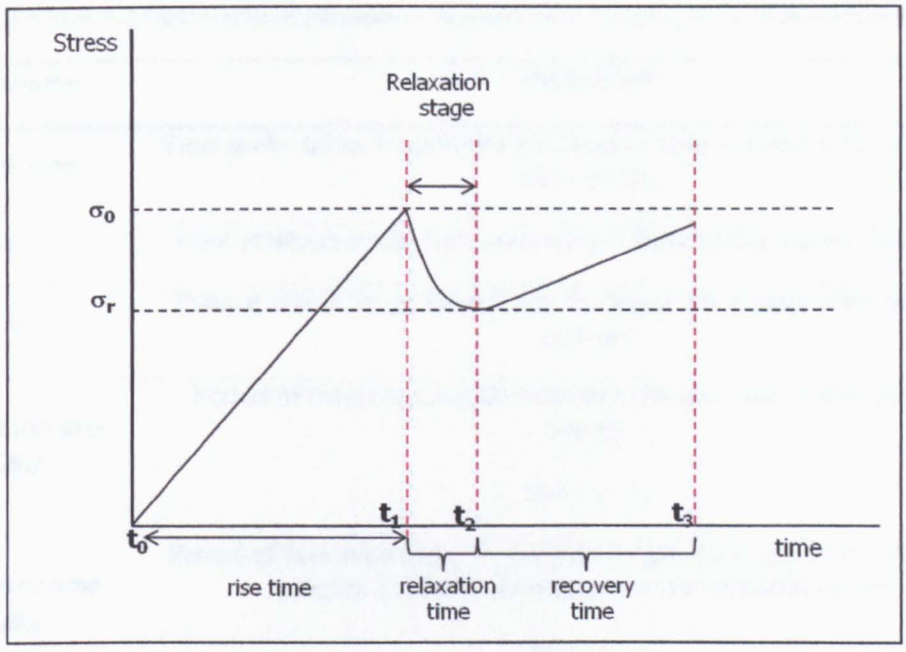


FIGURE 8.3 Typical dynamic profile of stress applied to a viscoelastic material

The first region contained between t_0 and t_1 (rise time), gives an indication of the hardness or stiffness of the product, which is related to the Young's modulus, and hence an indication of the elasticity of the material.

The second region of the curve, the relaxation time, is embraced between t_1 and t_2 is an indication of the rate of energy dissipation or stress release in a compressed material. The gradient of the curve, in this region, is equal to the magnitude of the rate of relaxation (see Table 8.2). A steeper slope suggests a faster relaxation and thus a quicker dissipation of stress. This indicates that the material has more viscous tendancy and will tend to flow and show little recovery intention and therefore will easily adapt to the deformed shape.

It was speculated that a material showing fast relaxation will tend to deform and adapt easily to teeth geometry during the chewing process. Fast relaxation and surface adaptation will aid in sample spreading resulting in increased surface contact area and thus increased bonding. It was believed that a food, having such properties, may be sensed as stickier than those having slower relaxation.

TABLE 8.2 Description of parameters obtained from dynamic stress relaxation test

Parameter	***Definition***
t_1 *(rise time)*	Time probe takes to apply the full load or time necessary to reach target force of 4N
t_2	Time at which stress has reached equilibrium after energy dissipation.
t_3	Time at which the probe reaches the target force again after relaxation process
Relaxation time (Δt_r)	Period of time required for relaxation process due to dissipation of energy $\Delta t_r = t_2 - t_1$
Recovery time (Δt_d)	Period of time necessary to reach the target force again after relaxation process in which deformation of the material occurs $\Delta t_d = t_3 - t_2$
σ_0	Initial applied stress $\sigma = \frac{F(h_0 - d)}{A_0 h_0}$ Where F= 4N, h_0-d= deformation attained at that point, A_0= Initial area of the specimen and h_0=Initial height of the specimen
σ_e	Equilibrium stress attained after relaxation process
$(\Delta\sigma_r)$	Stress decay due to dissipation of energy
Relaxation rate $(\Delta\sigma_r/\Delta t_r)$	Rate of stress decay. Where at faster relaxation rate the material will tend to exhibit a more dominant viscous character and is therefore more easily deformed

Force-time and stress-time curves showing only data for the first unloading-loading cycle and corresponding to each of the six confectionery products analysed appeared in Figures 8.4 and 8.5 respectively. It is obvious at a first glance that the products presented notably different profiles when subjected to the same load. The use of the two figures is necessary to highlight the change in stress, during the test period, as a consequence of the change in cross sectional area of the specimen. The change in area is a product of the deformation suffered when the 4N force was applied.

From Figure 8.4 it can be seen that the force applied to the MCH product increased abruptly beyond the target force, which indicates that even at very slow speeds and under small loads, the product behaved as a hard solid. Due to this overshoot and since a great variation between the four repetitions was observed, this product was not considered for further analysis. The results obtained could be adversely influenced by the fact that the equipment clearly found it difficult to maintain the desired force.

By studying the curves for the other products some general conclusions can be drawn: A shorter rise time denotes a rapid build up of stress and therefore a firmer material, under the specific tested conditions. Products which show a more elastic character, such as JT, TD and FR, tend to produce curves which have a slower relaxation rate (see Table 8.3), in the first cycle, and which stay closer to the target force. Contrarily, products whose behaviour is more viscous, such as WO and FRUT, tend to produce curves which have a much faster relaxation rate, in the first cycle, and which deviate greatly from the target force.

All the parameters describing the curves in Figures 8.4 and 8.5 are numerically presented in Table 8.3 including the average values of the rate of relaxation corresponding to each product over four repetitions.

This parameter was shown to have poor correlation with sensory stickiness (r= 0.689). This value of r is too low for the parameter to be considered confidently as good parameter in the prediction of stickiness (Bourne, 2002). This parameter gives an indication of the deformation suffered within the first loading-unloading cycle, but it does not provide any information about how many load cycles will take place before deformation of the sample stops (or stress decay has reached equilibrium). Thus it was deemed that this parameter does not reflect how viscous or elastic the material is.

Total deformation exhibited within the 10 second period (Table 8.4) turned out to be a better parameter for prediction of sensory stickiness showing a good and significant correlation (r= 0.821). Figure 8.6 shows plots of the deformation (strain) of the materials suffered in a 10 second period when a load of 4 N was applied. WO exhibited a continuously increasing deformation shown by the fact that the slope of its curve never becomes perfectly horizontal. A perfectly horizontal curve would be expected for a pure elastic material. The fact that WO does not exhibit this behaviour indicates its viscous character. This means that the product can spread well over a contacting surface (δ = 0.23). As discussed previously, this is an important prerequisite for good adhesion. This may indicate why WO was sensorially evaluated as the stickiest product, as it would easily spread over the hard and soft tissues in the oral cavity.

JT has been sensorially evaluated as the least sticky product and showed the least deformation (δ=0.04) and more tendency towards elasticity. This is indicated by an almost horizontal slope to the curve during the 10 second test period.

FRUT, which is less sticky than WO, presented a similar tendency to flow but the achieved deformation was relatively less (δ=0.14). This is in agreement with the sensorial perception stickiness which was recorded.

It can be clearly seen from the results that a more elastic material will tend to spread less, due to the application of a given load, and so achieve a less intimate contact with the contacting surface. A more viscous material, such as WO, will tend to spread more under the application of the same load. During an eating process, this will mean that the product will adapt easily to the shape of the teeth, thus allowing for a larger contact area. This effect is clearly shown in Figure 7.15. The test performed provides an effective way to characterise materials according to their viscoelastic behaviour (degree of elasticity or plasticity). Deformation measured by this method would seem to provide a good estimation of the sensory stickiness of materials.

It should be noted that the forces used in this test were similar in magnitude to palatal forces exerted in the mouth. If it had been possible to use forces representative of biting forces, some of the more elastic samples may have broken instead of just being deformed. Rupture due to the application of biting forces can be seen in Figure 7.16. In the case of rupture no spreadability is achieved and thus the product does not come into intimate contact with the oral surfaces.

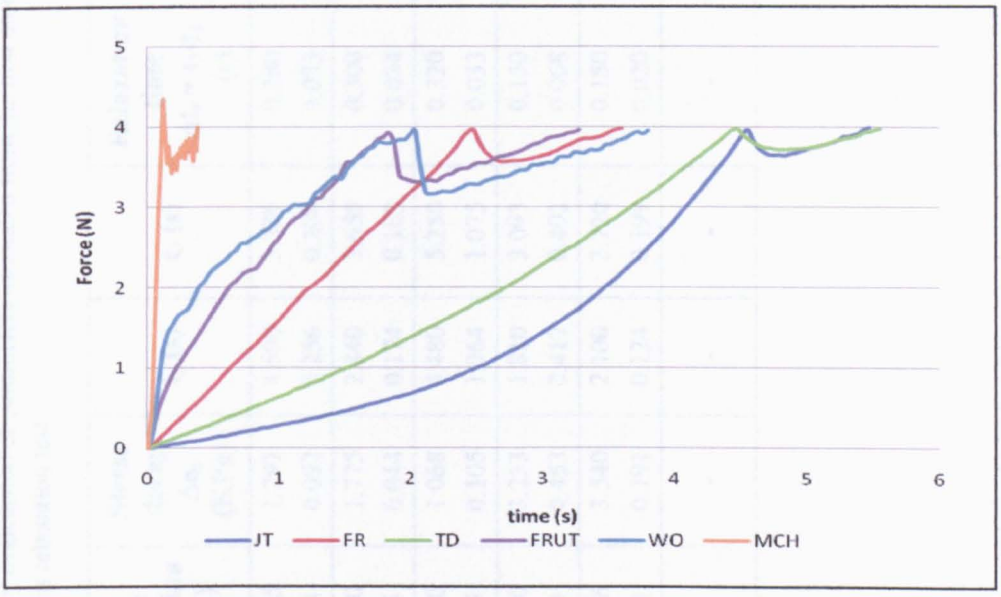


FIGURE 8.4 Force-time curves for the first unloading-loading cycle during a dynamic stress-relaxation test (sample was subjected to 4N force for a period of 10 s)

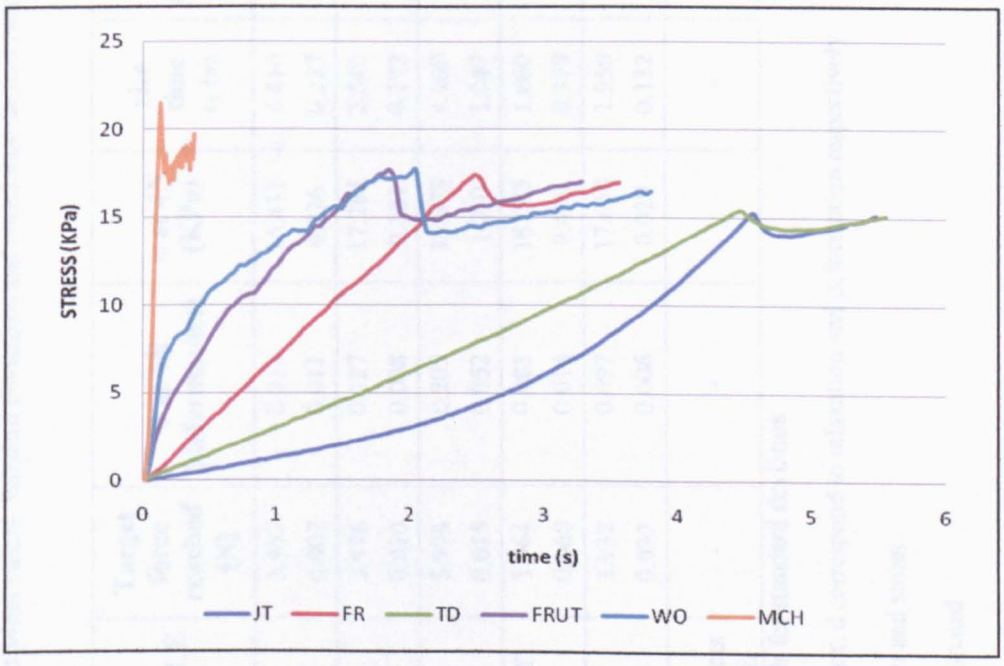


FIGURE 8.5 Stress-time curves for the first unloading-loading cycle during a dynamic stress-relaxation test (sample was subjected to 4N force for a period of 10 s).

TABLE 8.3 Mean values, standard deviations and correlation factors (with sensory evaluation) of parameters estimated from the first unloading – loading cycle of the dynamic stress relaxation test

SAMPLE	Target force reached (N)	δ at 4N (deformation)	σ at 4N (KPa)	rise time t_1 (s)	σ_e at relaxation (KPa)	Stress decay $\Delta\sigma_r$ (KPa)	t_2 (s)	t_3 (s)	Relaxation time $\Delta t_r = t_2 - t_1$ (s)	Recovery time $\Delta t_d = t_3 - t_2$ (s)	Relaxation rate $\lvert\Delta\sigma_r/\Delta t_r\rvert$ (KPa/s)
JT	3.967	0.219	15.411	4.410	14.115	1.297	4.690	5.330	0.280	0.640	4.630
SD	0.007	0.011	0.226	0.227	0.198	0.092	0.256	0.200	0.033	0.065	
FR	3.976	0.127	17.285	2.540	15.510	1.775	2.840	3.650	0.300	0.810	5.917
SD	0.010	0.008	0.183	0.172	0.146	0.044	0.174	0.163	0.034	0.050	
TD	3.974	0.205	15.678	4.160	14.610	1.068	4.480	5.250	0.320	0.770	3.336
SD	0.015	0.052	1.010	1.047	0.905	0.105	1.064	1.075	0.033	0.038	
FRUT	3.964	0.083	18.083	1.660	14.850	3.233	1.810	3.093	0.150	1.283	21.550
SD	0.060	0.018	0.450	0.379	0.370	0.453	0.417	0.402	0.068	0.113	
WO	3.932	0.097	17.658	1.950	14.118	3.540	2.100	3.720	0.150	1.620	23.600
SD	0.033	0.006	0.029	0.132	0.173	0.191	0.124	0.199	0.020	0.077	
r (stickiness scores)	-	-	-	-	-	-	-	-	-	-	0.685

SD- stands for standard deviation

Subscripts r, d correspond to relaxation and deformation respectively

δ, σ strain and stress

- Not estimated

TABLE 8.4 Sample deformation under 4N (compression force) over a period of 10s (Mean values over four repetitions ± SD)

SAMPLE	δ ± SD
JT	0.04 ± 0.007
FR	0.08 ± 0.007
TD	0.05 ± 0.003
FRUT	0.14 ± 0.000
WO	0.23 ± 0.008
r (stickiness scores)	**0.821**

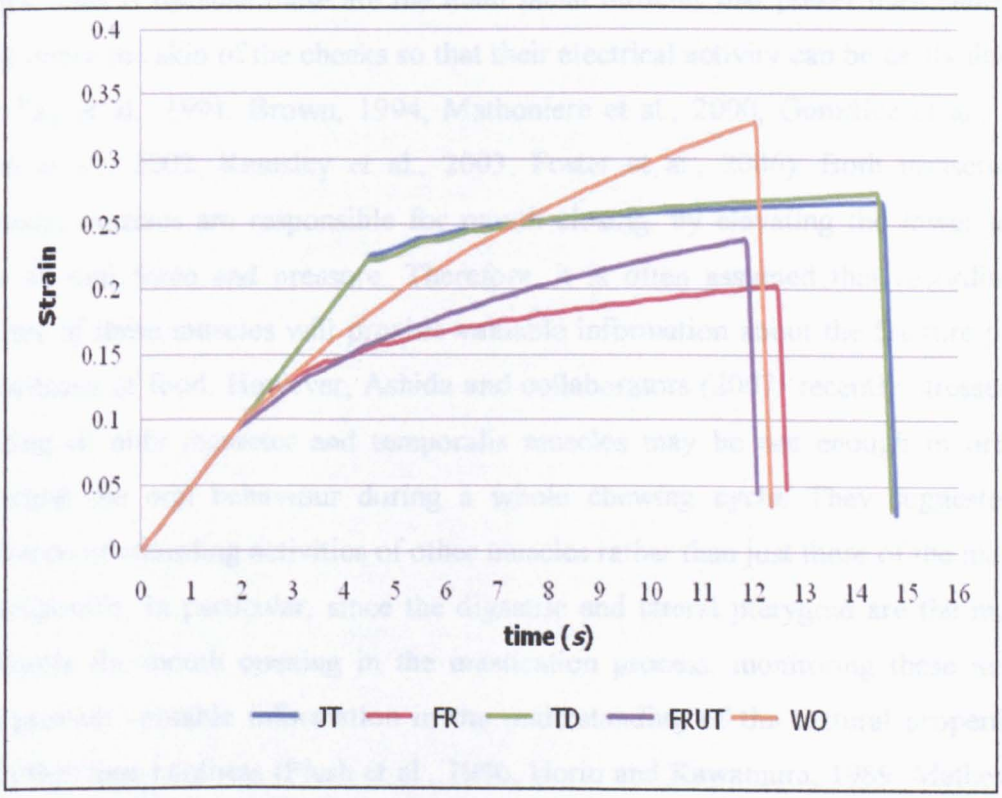


FIGURE 8.6 Strain-time curves at constant force of 4N over a period of 10s

Chapter 9

Surface Electromyography for the Study of Stickiness and Hardness of Food Samples

9.1 Introduction

Most EMG studies of mastication have monitored activities of masseter and temporalis muscles. This is because these are the main facial muscles that power mastication and lie just under the skin of the cheeks so that their electrical activity can be easily detected (Diaz-Tay et al., 1991, Brown, 1994, Mathoniere et al., 2000, González et al., 2001, Peyron et al., 2002, Kemsley et al., 2003, Foster et al., 2006). Both masseter and temporalis muscles are responsible for mouth closing, by elevating the lower jaw to create an oral force and pressure. Therefore, it is often assumed that recording the activities of these muscles will provide valuable information about the fracture pattern and hardness of food. However, Ashida and collaborators (2007) recently stressed that recording of only masseter and temporalis muscles may be not enough in order to understand the oral behaviour during a whole chewing cycle. They suggested the importance of recording activities of other muscles rather than just those of the masseter and temporalis. In particular, since the digastric and lateral pterygoid are the muscles responsible for mouth opening in the mastication process, monitoring these muscles could provide valuable information in the understanding of the textural properties of foods other than hardness (Plesh et al., 1986, Horio and Kawamura, 1989, Mathevon et al., 1995, Agrawal et al., 1998, Brown et al., 1998, Mioche et al., 1999, Kakizaki et al., 2002, Foster et al., 2006).

Stickiness is a major textural property of some food materials and is critically important in influencing consumers' oral experience as highlighted in Chapter 2. Despite this, there have been few studies on the effects of food stickiness on oral processing and oral behaviour.

In the study carried out by (Sakamoto et al., 1989), it was found that products described sensorially as very adhesive exhibited a very large chewing energy in the digastric muscle. In some other studies, adhesiveness and chewiness were related to increased chewing work and time (Brown et al., 1998). Eves and collaborators (1988) proposed that the subjective stickiness could be related to the post maximum gradient of EMG signals (i.e. the descending slope of EMG bursts). Kohyama and colleagues (2000) carried out a study addressed towards the evaluation of stickiness and found that the number of chewing strokes, masticatory time, and total duration of mastication showed a higher correlation with adhesiveness and stickiness properties than with the hardness.

In the present study; the EMG activity of both closing and opening muscles was monitored in order to study the physiological response of subjects to the stickiness of the samples they were given. It was speculated that the opening muscles could be more responsible for overcoming the effect of food sticking inside the mouth. This can be understood by considering the fact that a material which is defined as sticky requires an appreciable force to separate it from contact, as is experienced in everyday life (Gay and Leibler, 1999). It is expected that this holds true for sticky materials within the mouth. It is assumed then, that when a product is perceived as sticky, a person will use every possible oral movement to cause the separation of the material from the oral surfaces with which it is in contact. As the maxilla is fixed it will be then, the moveable mandible and tongue which will be used to achieve oral separation, thus a larger input is believed to be required from opening muscles when eating sticky foods.

Even though both the pterygoid and digasrtic muscles act in the opening of the mouth, the former was not selected for monitoring in this study. This is because the pterygoid is a smaller muscle and is located more deeply, with respect to the facial skin, meaning that needle electrodes would be required to record its activity. This is problematic as the placement of such electrodes requires clinical training and they can impede a subject from carrying out normal chewing by causing discomfort. This was discussed earlier in

Section 3.2.2. The digastric, on the other hand, was found to be much more suitable in this study as its activity is easily recorded by sEMG. Furthermore preliminary tests conducted in this research showed that the signal recorded for this muscle could embrace the activity of the tongue as presented in Section 3.4.2.1. Such activity could have a great involvement in attempting to remove the material stuck to oral surfaces during the normal mastication process.

Few studies analysing the association of the activity of opening muscles to the effect of food adhesiveness have been published. Among these is Sakamoto et al. (1989), they studied the chewing patterns of the masseter and digastric muscles of four subjects who were asked to consume forty three foods which had been sensorially evaluated. Results showed that the chewing energy of the digastric muscle increased for products which had been evaluated as very adhesive. Kohyama and collaborators (2005) also reported an increase in muscle activity in opening muscles with an increase in food adhesiveness, measured instrumentally, when evaluating cooked rice of different water content.

These previous studies have focused on the EMG response, of closing and opening muscles, to textural properties which were measured either instrumentally or sensoraliy. This chapter will present the study carried out on the effects of food stickiness on oral processing, using the results obtained from both sensory and instrumental tests in conjunction with results obtained by using the sEMG technique to analyse the oral behaviour in response to food stickiness and hardness.

9.2 Materials and apparatus

- A portable radio telemetry system (MT8 telemetry system) from MIE Medical Research Ltd. allowing the remote monitoring of EMG signals. The apparatus consists of:

  a) *A transmitter unit*. This is a small, light weight unit having eight channels to which preamplifiers and electrodes are connected (see Figure 9.1 *A*). This unit is fitted to a belt which was worn around the subject's waist.

b) A receiver unit. This unit (Figure 9.1 *B*) decodes the multiplexed signals from the transmitter. The decoded signals were seen individually on a personal computer.

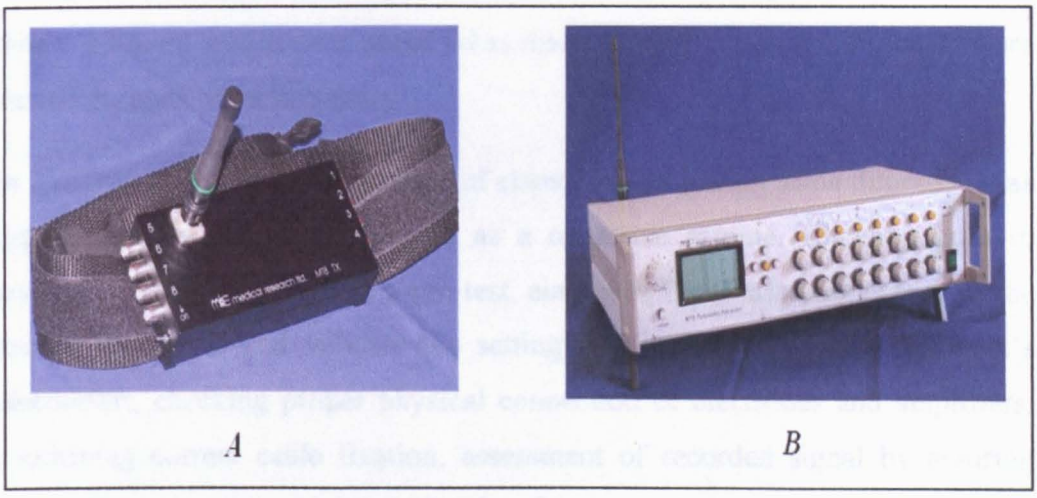


FIGURE 9.1 Radio Telemetry System for recording of sEMG signals
A. **Transmitter unit and** ***B.*** **Receiver unit**

- Trigger pulse generator, especially built for the purpose of this project. The device was designed to be plugged into one of the channels of the transmitter unit of the EMG. It served to indicate to the subject the moment that the sample must be ingested by activating a small light. At the same time the voltage pulse generated was registered on the recorded EMG signal. This served to identify the start of the chewing sequence when analysing the data.
- Reusable Ag/AgCl electrodes of 10 mm diameter
- Double-sided sticky rings for use with reusable electrodes
- Circular pre-gelled clip stud electrodes
- 4 sub miniature preamplifiers with a gain setting of ×4000
- 1 sub miniature preamplifier with a gain setting of ×8600
- 70% *v/v* alcohol swabs
- Conductive gel

- Microporus surgical tape
- Circular plastic petri dishes
- Samples used in this experiment included the 6 confections analysed previously, having the same geometry as those employed in the sensory tests (18mm × 17 mm × 10 mm), which were prepared as discussed in Section 4.4. Products were dusted in sugar when necessary.

  In addition to these samples a piece of chewing gum having same dimensions as the rest of the samples was used as a reference sample. Chewing gum in general, was used to perform a pre-test, aiming to familiarise subjects with the testing conditions and validate the setting up i.e. by evaluation of subject's discomfort, checking proper physical connection of electrodes and amplifiers, confirming correct cable fixation, assessment of recorded signal by assuring zero offset, ensuring minimum levels of noise and presence of bursts while performing specific muscle function test, and evaluation of possible motion artefacts present in the signals.

9.3 Method

Subjects

Ten subjects (2 males and 8 females) participated in the study. All subjects were students at Leeds University. Before participation they were provided with an information sheet explaining in brief the purposes of the research, safety issues and what they were required to do if they decided to take part in the study. Participants were asked to avoid cigarettes and caffeinated beverages, such as, coffee, cola and tea at least three hours before the test to preclude extra muscle tension. It was suggested that participants wear comfortable cloth excluding the wearing of turtle necks. None of them suffered any symptoms of masticatory dysfunction, pain during eating nor did they wear dental prostheses.

Approval was obtained from the faculty ethic committee of Leeds University to conduct this study (see Appendix A) and all subjects gave voluntary informed consent to take part. None of the subjects received any payment for their participation. Subjects attended one session lasting around two and a half hours, including the time used for placement of electrodes and preliminary mastication tests.

On the day of the session, subjects received a brief explanation about the test and a view of all instrumentation to be used during its execution. No deep explanation however was provided relating to the main focus of the investigation in order to avoid possible interferences with the study.

Placement of electrodes

Surface electromyography was used to record the EMG activity of the following chewing muscles:

- Left and Right Anterior Temporalis (*L. temp., R. temp.)*
- Left and Right Superficial Masseter (*L. mass., R. mass.*) and
- The Anterior belly of Digastric muscle (*Bi-lat. D*)

A pair of re-usable surface metal disk Ag/AgCl electrodes of 10 mm diameter was applied over the main belly of each muscle being monitored.

Recording of closing muscles (temporalis and masseter muscles) was done bilaterally, that is the left and right sides of each muscle were recorded with a different pair of electrodes. For each masseter muscle (left and right), the pair of electrodes was placed on an imaginary line from the gonion (back corner of the jaw bone) to the zygomatic bone (cheek bone) and parallel to the orientation of the muscle fibres with an inter-electrode distance (IED) of 10 mm. For each temporalis muscle (left and right), one electrode was placed 10 mm above an imaginary line traced from the corner of the subject's eye to the top of the ear, the second elecctrode was situated 10 mm superior to this.

Electrodes were placed after their position was confirmed by palpation of the muscle as suggested by Lapatki and collaborators (2003). In this case the subject was asked to clench their teeth together so that hard bumps of the muscle could be felt and over which the electrodes were placed and secured. The leads of the electrodes recording these four muscles were connected to sub-miniature preamplifiers with a gain of ×4000 via terminal screws.

Following the explanation given in Section 3.2.3, all opening muscles mentioned in this chapter will be mainly referred to as the "anterior belly of the digastric muscle." It was noted, that the muscle activity when recording at this site will certainly correspond to the muscle activity being emanated from all muscles localised within this region (suprahyoid muscles) as these muscles participate in the same muscle function i.e mandible depression. The anterior belly of the digastric, however, has been found to be the major source of such muscle activity (Winnberg and Pancherz, 1983). Thus, through the rest of this work the terms anterior belly of digastric muscle, digastric muscle or opening muscles will be used interchangeably to allude to the suprahyoid or submental muscles. The recording site for the digastric muscle was located immediately posterior to the mental symphysis. The small size of the muscle and the presence of substantial tissue overlying the muscles which are posterior to the chin make it impossible to have a unilateral recording, as described by Green and collaborators (Green et al., 1997). Due to difficulty in electrode placement and its small size the recording of this muscle was done bilaterally[1], with the use of a single electrode pair. One of the electrodes was placed on the left belly, of the muscle, on a line that bisects the angle formed by connecting the soft tissue gonion, the soft tissue menton and the midpoint of the hyoid bone. The second electrode was placed on the right belly situated laterally to the first 20 or 25 mm away from this, depending on the shape of the subject's mandibular body. The wideness of floor of the mouth varies greatly between individuals resulting in slightly different IEDs for different subjects. The electrode leads in this case were connected to a sub-miniature preamplifier with a gain of ×8600. As was done for the closing muscles, the electrode position for the opening muscles was verified through palpation. For this, the subject was requested to perform one or all of the following actions: opening the mouth wider, pressing the tongue against the palate or swallowing

[1] See Section 3.3.1 for a detailed explanation of the type of placement

of saliva. Performance of the last two actions in general facilitated the location of the muscle.

Schemes of surface anatomy such as those provided by Lumley (2008) and illustrated in Figure 3.5 (Chapter 3) helped in the identification of the recording sites discussed above.

Before placement of the electrodes, the facial skin of the subjects was cleaned with special 70% *v/v* alcohol swabs. This was done to remove dirt or cosmetics from the skin and so minimise the impedance to the EMG signal. Double sided adhesive rings were stuck to the facial skin of the subjects on the recording site. The electrodes were attached to the upper side of the rings.

Once the electrode was properly attached to the subject's skin, the space formed by the electrode on the skin was filled with a conductive gel. All the sub miniature amplifiers used, are designed to be placed on the top of a disposable earth electrode (pre-gelled) utilising an in-built press-stud. After placement of the recording electrodes the earth electrode was attached and placed close to a bone or to a site of low muscle activity. The preamplifiers leads were then secured to the clothes of the subject using microporus surgical tape to avoid pulling of cables that could cause discomfort for the subject and also affect the EMG signals by causing motion artifacts. Figure 9.2 presents an image showing the set up of electrodes on a subject's skin as carried out during the experiment.

Although the placement of the electrodes was time consuming, an adequate placement and assurance of electrical contact was critically important for obtaining reliable data as discussed in Chapter 3.

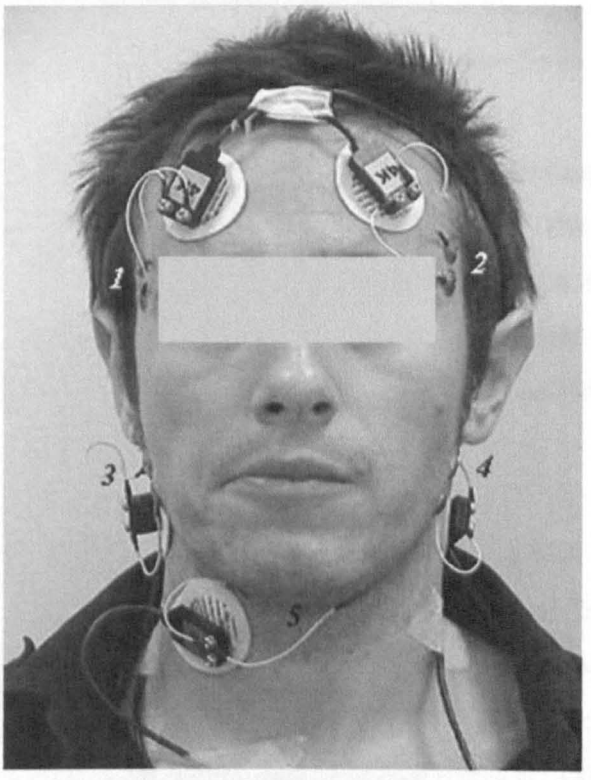


FIGURE 9.2 Set-up of electrodes on the facial skin
1, 2- Right and Left Anterior Temporalis muscle; **3, 4-** Right and Left Masseter muscle;
5- Bi-lateral Digastric muscle.

Experimental procedure

Validation of EMG signals

A series of pre-tests were required to confirm that the EMG signal was being acquired correctly. This was carried out by visual inspection of the recorded EMG signal displayed on the computer screen. The subject was asked to perform certain actions which were intended to cause the activation of the muscle being tested. The objective was to verify that the correct muscle function was being registered. The observed EMG signal in this case, had to correspond to the electrical activity of the desired action of the muscle being evaluated (e.g. for opening muscles, bursts in the signal should appear at the time when the subject opened their jaw).

During every test subjects were seated in an upright and comfortable position with their head erect and their gaze towards the front. They were asked to refrain from talking or making movements which were not requested during recordings.

Figures 9.3, 9.4 and 9.5, show examples of the EMG recordings taken from a female subject, while performing different directed actions for the pre-tests. These tests validated the EMG signals and in turn the placement of the electrodes. When EMG signals were not present or did not appear to correspond well to the expected muscle activation the setup was rechecked. When necessary the electrodes were repositioned in order to ensure the quality of the recording.

Figure 9.3A shows a recording taken with the subjects jaw at rest position. This was performed to check the stability and the baseline of the signal. For this, the subject was asked to relax and keep their mouth closed for 30s. It is clear from the figure that during the time of recording, no muscle activity was registered either for any of the closing muscles or for the opening muscles. Observe that average amplitude of the baseline along the time for any of the muscles was always $\leq 5\mu V$ which represents a good baseline as stated in Chapter 3 as noise levels are low. No motion artefact was identified through the recording which indicates good electrical contact between electrodes and facial skin.

Figure 9.3B corresponds to the EMG signal recorded, from the digastric muscle. The digastric is a depressor muscle and therefore is expected show bursts of activity when the mouth is opened. The subject was asked to open their mouth as wide as they could for 5s periods followed by a rest interval. The recorded EMG signals were checked to ensure that the bursts of activity present in the plot for the digastric muscle (Bi-lat-D) corresponded to the activation of the muscle during mouth opening. It can also be seen that the closing muscles are slightly activated together with the digastric, however their electrical activities are very low compared to that of the digastric muscle. Farella and collaborators (2008) found similar results for suprahyoid muscles when subjects performed similar actions (yawning) see Figure 3.6.

It is important to highlight that digastric muscle was also highly active with tongue movement as it was shown in Figure 3.8.

Validation of the signals from elevator muscles, which are responsible for mouth closing, was carried out in a similar way to that used for the validation of opening muscle signals. This time the subject was asked to close their mouth as tightly as possible while pressing a cotton wool ball placed between the molar teeth. The subject was instructed when to close the mouth and when to relax. Figures 9.4C and 9.4D correspond to ipsilateral chewing; left and right side respectively. In both cases it can be observed that the four elevator muscles (left and right temporalis and left and right masseter) presented clearly appreciable bursts of electrical activity during hard chewing. Low levels of activity were recorded for the digastric muscle in this case. These results confirmed that surface electromyography is able to record not only closing muscles but also capable of being used for the evaluation of opening muscles. The EMG activity recorded in both cases is quite differentiable.

Figures 9.5E and 9.5F show a comparison of opening and closing movements when no resistance was imposed (i.e. no chewing action) with opening and closing movements during chewing against resistance presented by food. When no resistance was imposed, the bursts of muscle activity for closing muscles were not well defined. The masseter muscles particularly, showed very low levels of muscle activity. The bursts of activity, for all the muscles, were significantly more defined when the subject encountered resistance, caused by the food. This in fact shows how muscle activity is mainly the response to the sensory stimulus and is targeted to overcome food resistance.

It can be clearly seen, in figure 9.5F, that there are alternate bursts in muscle activity in the opening and closing muscles, when chewing. This means that when the temporalis and masseters are active (during mouth closing), the digastric muscle remains relatively quiet and vice versa. This proves the different roles of these muscles during mouth opening and closing. However, it can also be noticed that during a burst of activity in the closing muscles the activity of the digastric does not fall to an "at rest" level. Therefore, the EMG baseline for the digastric muscle appears to be much noisier, with some continuous low intensity activity between the main bursts. This makes it rather difficult to identify the starting and ending point of single bursts for this muscle and

suggests that, even though the digastric muscle is mainly responsible for mandibular depression, it could also be active during mandibular elevation. The same findings have also been reported by Miles and Madigan (1983) and Ferguson (1999). The last author attributed such activity to a possible action by the digastric to modulate the speed and force while closing the mouth.

Test performance

After the EMG signals were completely validated ensuring a correct electrode set-up, testing was started. As for the validation trials, subjects were asked to sit in an upright and comfortable position while keeping their eyes looking to the front. Samples were presented separately in small plastic petri dishes and provided to the subject in a random order. Recording was initiated before mastication in order to record a short period (≈ 3.5 seconds) with the jaw at the rest position (mouth remained closed) to ensure a good quality of the EMG signal. This allowed checking for a good baseline by detection of any noise or extraneous activity. The Subject was instructed to ingest the food sample when a light flashed. They were told to chew in their habitual way. There was no restriction about on the method of chewing (free style). Additionally subjects were asked to indicate, by knocking on the table once, that they had completely finished the sample. A triggering pulse was generated when the light was activated which was registered in the recorded electromyogram. This was useful for posterior data analysis. Short breaks were taken between samples during which water was provided for mouth rinsing. Longer breaks were provided between test repetitions or as required by the subject. Neither muscle fatigue nor discomfort caused by cables or electrode attachment were experienced or reported by any of the participants.

After the tests the electrodes were removed carefully from the skin of the subject, to minimise skin irritation, any excess adhesive or gel remaining on the subject's skin was cleaned using moist swabs. There was no complaint of irritation after the test by any of the subjects.

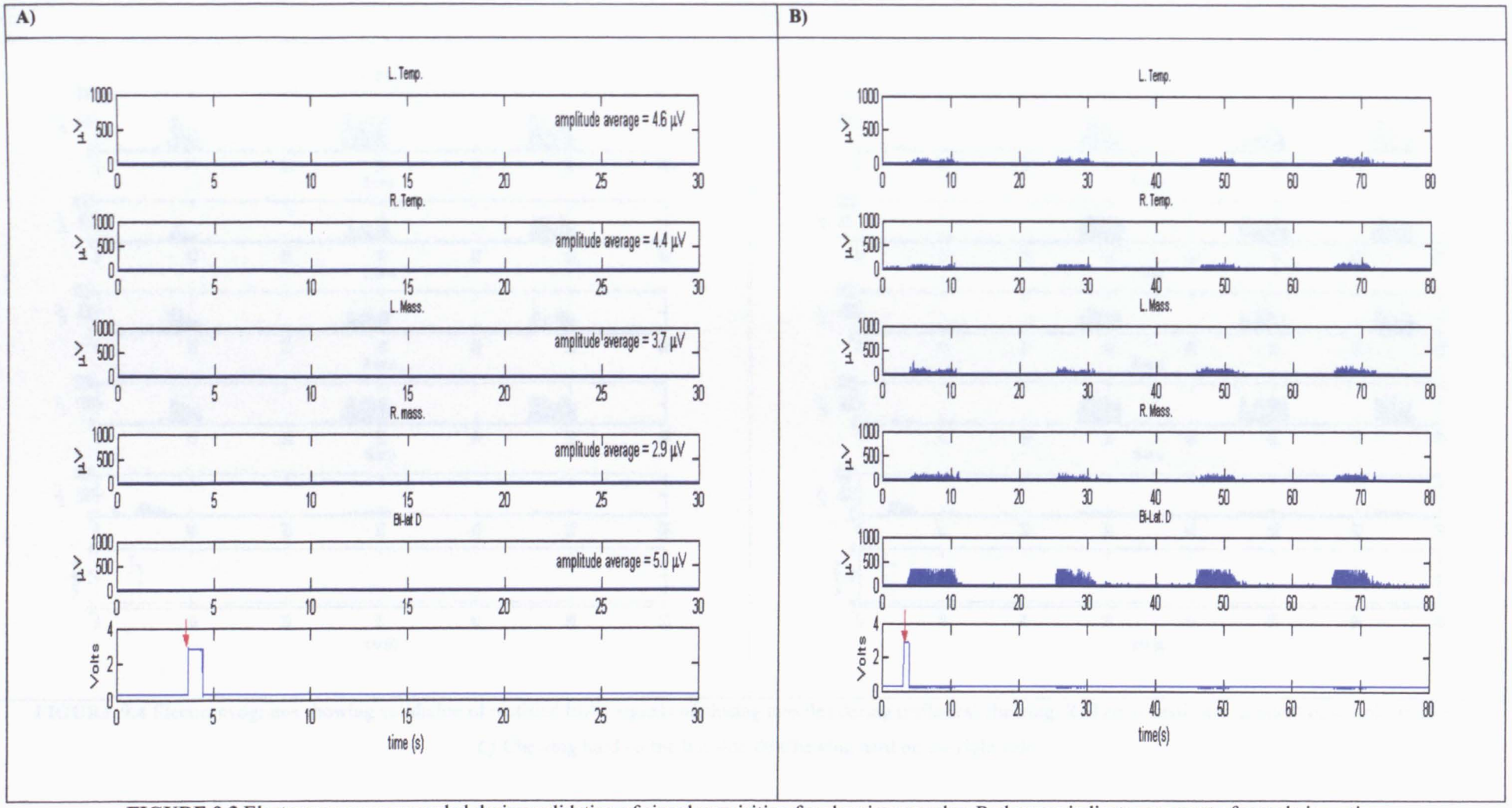


FIGURE 9.3 Electromyograms recorded during validation of signal acquisition for chewing muscles. Red arrow indicates moment of sample ingestion.

A) Signals recorded at rest position (baseline) ***B)*** Validation of digastric muscle signals during mouth opening at maximal.

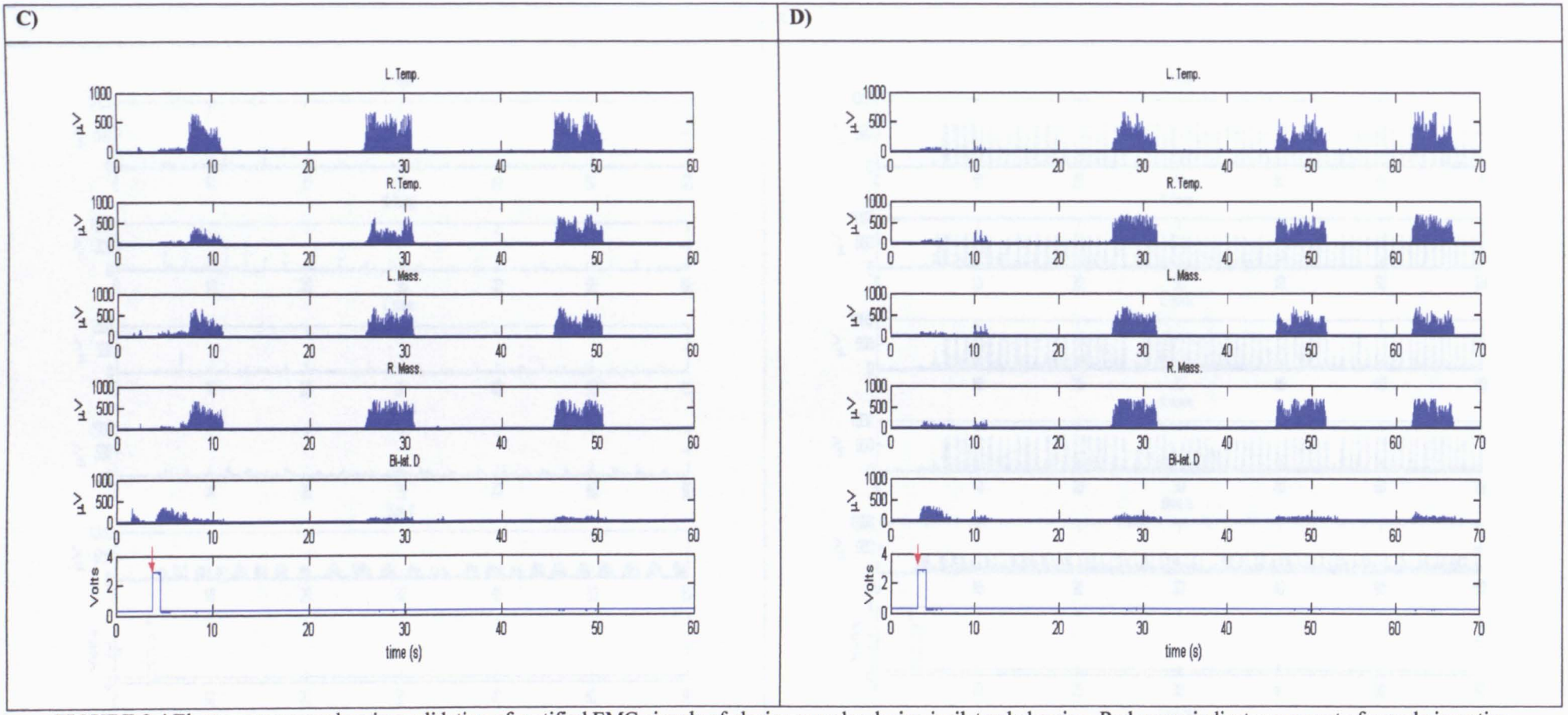


FIGURE 9.4 Electromyograms showing validation of rectified EMG signals of closing muscles during ipsilateral chewing. Red arrow indicates moment of sample ingestion.

C) Chewing hard on the left side ***D)*** Chewing hard on the right side

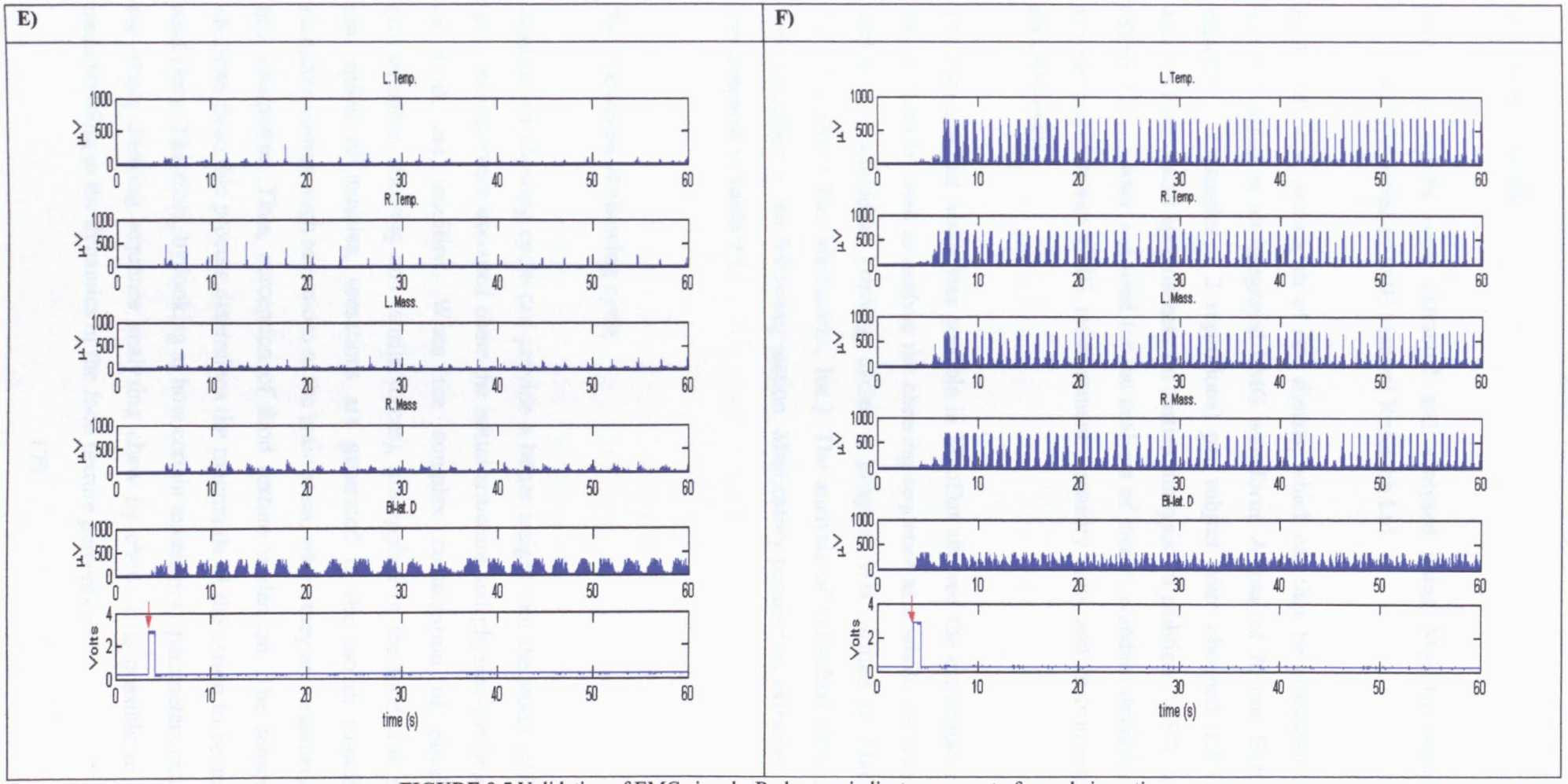


FIGURE 9.5 Validation of EMG signals. Red arrow indicates moment of sample ingestion.

E) Opening and closing jaw movements without imposed food resistance ***F)*** Opening and closing jaw movements during mastication of chewing gum

9.4 Data analysis

EMG test results were extracted and analysed using MyoDat software version 6.57.0.8130 supplied by MIE Medical Research Ltd.

Each EMG test gives a set of raw signals, which can then be processed to produce a rectified signal or an integrated EMG waveform. A total of 70 raw EMG records (5 muscles × 7 samples × 2 repetitions) per subject were obtained. EMG data were exported into excel spreadsheets for further analysis. In addition PASW statistics 17.0 (SPSS 17) software was used for the analysis of means, standard deviations and linear correlations between EMG measurements, sensory tests and instrumental stickiness measurements.

The data analysis which was possible in MyoDat allowed the extraction of parameters which could be used to analyse the chewing sequence as a whole. In order to perform analysis of individual chewing cycles a program was written in Matlab (version 7.1.0.246 (R14) The Mathworks, Inc.). The analysis of individual chewing cycles is fully described in the following section. Masticatory parameters extracted in each case are presented in Table 9.1.

9.4.1 Analysis of chewing cycle

Analysis of chewing cycle can provide a better insight into the study of food texture, since during every executed chew the initial structure and physico-chemical properties of foods are modified. When the complex combination of physical actions (compressive, shearing and tensile forces), are applied to the food with the teeth by contraction of muscles, sensations are generated in the mouth (mouthfeel). Oral receptors convey such sensations to the brain stem, where they are organized, integrated and interpreted. Thus, perception of food texture is derived. The sensory feedback obtained from this process determines the magnitude of the action to be applied in the next chew. Therefore, by looking at how certain masticatory parameters change through the whole chewing sequence analysing chew by chew, it is possible to get a better understanding of the dynamics of the food texture perception.

TABLE 9.1 Masticatory parameters extracted from EMG signals

Analysis of chewing sequence	Analysis of individual chewing cycles
Chewing sequence duration (*s*) = time of terminal swallowing – time of sample ingestion	Muscle onset (*ms*) = time of muscle activation (start of a burst)
Number of chews (chews)	Muscle offset (*ms*) = time of muscle cessation (end of a burst)
Muscle activity of closing muscles ($\mu V \cdot s$) = $MA_{\text{L. Temp.}} + MA_{\text{R. Temp.}} + MA_{\text{L. Mass.}} + MA_{\text{R. Mass.}}$	Burst duration (*ms*) = time interval from the onset to the cessation of EMG activity (jaw closing time)
Muscle activity of opening muscles ($\mu V \cdot s$) = $MA_{\text{Bi-lat. D}}$	Inter-burst duration (*ms*) = time interval from the end of a burst to the start of the next burst (jaw opening time)
	Cycle duration (*ms*) = time between the cessation of two consecutive bursts

**MA*=Muscle activity along sequence duration= Total area under the rectified EMG signal

In most of the works published on EMG in food texture studies, authors have described the development of their own method for carrying out a chew-by-chew analysis. A variety of different software has been used. However information about the algorithm employed is rarely provided and discussed. Some authors have only reported using software provided with the equipment, the equipment to perform such analysis (see Table 9.2).

The first step in any analysis regardless of the method used is the identification of single chewing cycles within the recorded electromyogram. In studies where tracking of jaw movements has been coupled with the capture of EMG signals, the turning points in vertical jaw trajectories have normally been employed to divide the chewing sequence into chewing cycles. In cases when only EMG has been performed detection of onset and termination of muscle activity is then necessary. A chewing cycle is then defined as the time from the beginning of an EMG burst to the beginning of the next recorded EMG burst.

Detection of starting and ending of muscle activity is normally done by visual inspection. This involves the identification of the earliest point at which activity has risen beyond the level of the EMG baseline (onset) and the point at which activity returns to baseline levels again (offset). Making these judgements is not easy, due to the high level of noise which is present in the EMG signal, and requires a lot of experience. This expertise is possessed, for example, by clinical experts or very highly experienced operators (Micera et al., 2001, Viera-Guerreiro and Pires-Jorge, 2008).

From the experiments carried out in this study a vast amount of recordings were gathered, resulting in a total of 700 EMG signals (7 samples × 2 repetitions × 5 muscles × 10 subjects). It is possible to manually determine the onsets and offsets of bursts in MyoDat software (version 6.57) by moving the cursor across the displayed EMG signal. Making measurements in this way would have resulted in a very time consuming task which lacked objectivity. For this reason, a program was written in Matlab (version 7.1.0.246 (R14) The Mathworks, Inc.) to automate the quantification not only of timing of muscle onsets and offsets during chewing but also other masticatory parameters that could be linked to the perception of stickiness during oral processing. Parameters such as burst area, maximum peak, ascending and descending energy per chewing cycle were also computed.

9.4.1.1 Method of Analysis

The inputs to the algorithm were the rectified EMG signal along with the RMS and integral signals. These were obtained using MyoDat and exported to spread sheets in Microsoft® Office Excel® 2007. Before proceeding to the description of the method used in this study, to carry out the analysis of chewing cycles, some of the factors which were considered will be discussed.

Figure 9.6 shows an example of a set of rectified signals recorded from a female subject while chewing the FR product. Similar[2] kinds of EMG signals were obtained from the rest of the subjects who participated in the study.

[2] Similar but not identical, since chewing patterns exhibit a lot of variation between subjects

TABLE 9.2 Different reported methods for the analysis of EMG signals

PUBLICATION	SOFTWARE	DESCRIPTION
Brown, (1994) Brown *et al.*, (1994 and 1998) Brown and Braxton (2000)	Spike2 (Cambridge Electronic Design Ltd. Cambridge,UK)	The EMG was rectified. Within selected portions of the EMG record the start and end of bursts of muscle activity were located for each muscle. The bursts were identified, from the increased frequency and amplitude of the electrical signal which occurs when the muscle contracts, using customised procedures. Six parameters were computed (duration, interbust time,cycle time, mean voltage, area and maximum voltage). The area of the EMG was derived from the product of the mean EMG voltage and duration of the activity burst.
Lassauzay *et.al*, (2000) Foster, *et.al*, (2006)*	Spike 2	The occlusal level was determined by recording the minimum vertical amplitude (subject clenched his teeth before the start of the sequence) corresponding to a baseline for occlusion. The turning points in the vertical direction identified by a minimum variation in the baseline level served to divide the cycle into opening, closing and occlusion.
Peyron *et al.*, (2002)*	Spike 2	The beginning and end of each burst were defined as an EMG signal reaching a level 10% above or below the mean area relative to the baseline 0 mV. Similarly the maximum and minimum interval durations between bursts were respectively set at 1 and 0.2s to identify individual cycles.
Mathoniere, *et al.*, (2000)	Spike 2 Software from CED	An iterative algorithm to perform burst analysis. An approximation was made of the total area (integration) under the curve during the whole chewing sequence and was divided by the total number of bursts. Then starting from the beginning of the curve, periods of 10 ms were analysed. When the area of activity of any period was more than 20% of the average area, it was recorded as a burst or part of a burst. The analysis continue along the curve in steps of 10 ms. The minimum duration between two bursts was set as 75 ms.
Kohyama, *et al.*, (2005, 2007, and 2010)	Wave analysis software (Acqknowledge®, ver 3.5.7 or ver 3.8.2, Biopac Systems)	Cycle time was read for each muscle and each chewing stroke. Data from closing muscles were averaged as signals appeared almost simultaneously when the subjects close their jaws and freely change chewing sides during mastication. From these signals for each chewing cycle: the amplitude or the maximum voltage, burst duration and muscle activity were estimated as the time- integral of the EMG voltages were read.

Carson, *et al.*, (2002 and 2003)	Microsoft Visual Basic (6.0, Microsoft Corp., Redmond, WA)	It allows analysis of the sequence bite by bite.
Diaz -Tay, *et al.*, (1991)	Not reported	Programmed calculations were used to identify the turning points in vertical jaw trajectories that serve to divide the masticatory sequences into individual chewing cycles. As a criteria for recognition of these, no defined chewing cycle was allowed a duration of less than 50ms or a vertical opening of less than 2.75 mm. For each elevator muscle, the level of its activity during jaw opening was calculated as the period from 40 ms beyond the minimal vertical movement at the start of jaw opening to 40 ms before the maximum vertical movement in each chew. This minimal level of activity was summed for all chews and a mean and SD calculated for each muscle for the chewing sequence. As the criterion for the onset of significant activity in the elevator muscles in the closing phase of each cycle, the level of electrical activity in any given record and the average of the next two successive records had to exceed the sum of the minimal level of activity plus twice its SD. Electrical activity was then accumulated without this test for at least 100ms, following which the test was repeatedly applied every 10ms. Activity was deemed to have stopped only when the test criterion ceased to be satisfied. At 2.75mm from the minimal vertical displacement, the horizontal deviation of the jaw, either to the right or the left of the midline, was used to define the working side for that chew. By making this assumption of unilateral chewing, the mean and the peak activity and the period of activity for each chewing cycle were calculated for the masseter and temporalis separately, and for both working or balancing sides.

* Paper refers to Lassauzay *et al.*, 2000
SD stands for Standard Deviation

To simplify the analysis, the four jaw elevator muscles were grouped together as a means to analyse the whole closing action. The EMG signal obtained from the recording of suprahyoid muscles (depressor muscles) was used to analyse the opening action through the chewing sequence.

EMG signals captured from the four closing muscles (left and right anterior temporalis and left and right masseter) were added together. This was possible since the signals were recorded at the same sampling rate. The rectified signals from the four closing muscles were summed at each time point obtaining a new rectified signal corresponding to the whole closing activity of the elevator muscles during mastication, as was also described in Brown *et al.* (1998).

Automatic measurement of on/off periods of EMG bursts was done based only on the signals acquired from left and right temporalis. As from the five recorded muscles, these were the ones that exhibited clearly defined bursts of activity.

EMG signals from masseter muscles were appreciably lower and with worse-signal-to-noise ratio; bursts appeared less clearly defined, possibly due to cross-talk emanated from muscles in close proximity such as buccinators and platysma, which has also been discussed in Agrawal *et al.* (1998).

The suprahyoid muscles showed large fluctuations in amplitude during mastication. As mentioned previously, the digastric muscle showed bursts of activity not only during mandibular depression, but also during mandibular elevation.

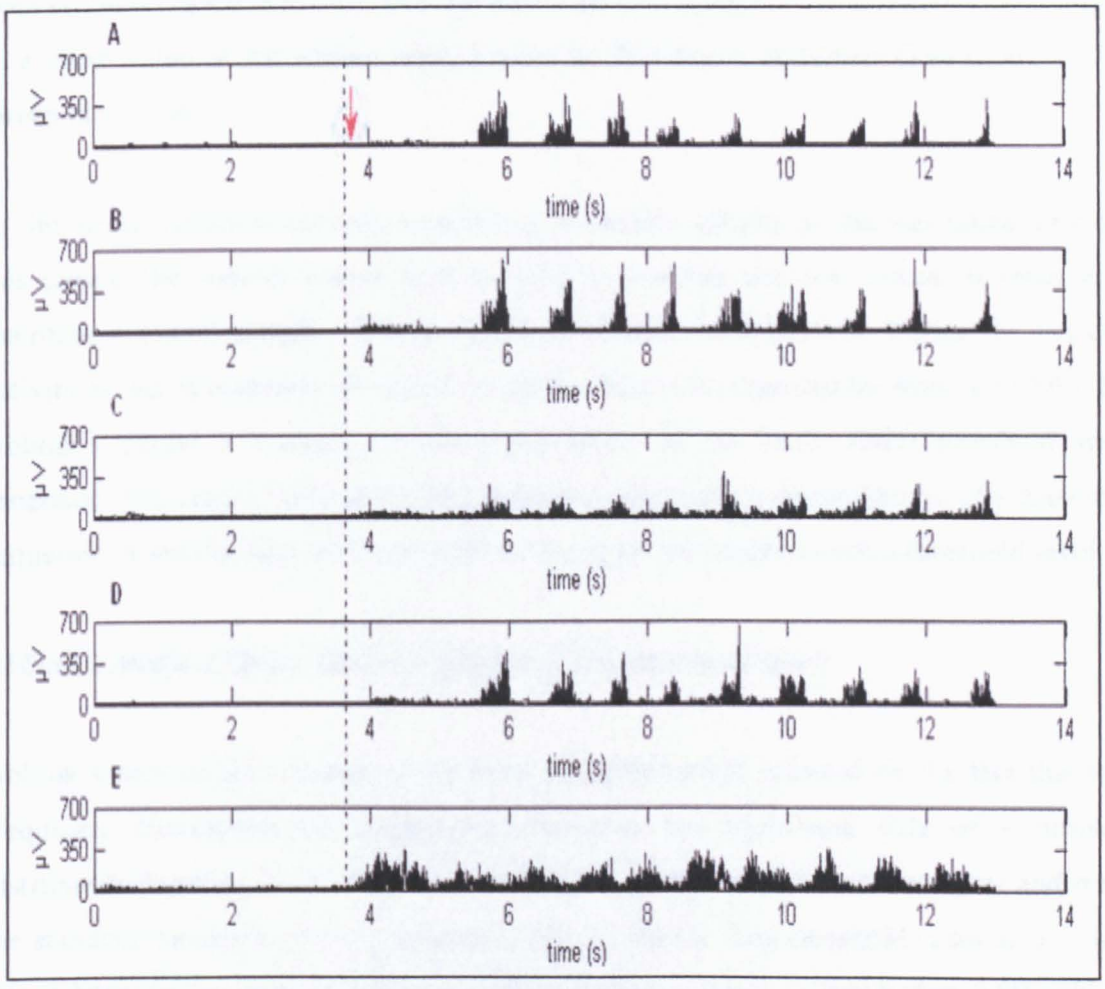


FIGURE 9.6 Rectified EMG signals of mastication muscles recorded from a female subject while chewing FR. **A.** Left temporalis **B.** Right temporalis **C.** Left masseter **D.** Right masseter **E.** Digastric. Period recorded before dotted line corresponds to muscle activity at rest. Arrow indicates the time at which sample is ingested.

Additionally, regarding the order of muscle activation, the temporalis muscle has been found to be the first muscle that contracts during mastication. The pattern most frequently observed is the simultaneous activation of the four muscles. Although in some other cases the temporalis on the working side activates first followed by temporalis on the balancing side and both masseters after a 50-100 ms delay (Lovelle, 1988, Rilo et al., 1998, Soboļeva et al., 2005). In either case using a method that obtains times from the temporalis muscles ensures that the activity from all four closing muscles is enclosed within those times.

To compute the beginning and end of muscle activity, generally algorithms involve the setting of a threshold level to find the points at which muscle activity exceeds the lowest level of muscle activity (baseline). In studies using surface electromyography it has

been common practice to calculate the threshold by adding 1, 2 or 3 standard deviations to a mean value of the resting state, known as Di Fabio's algorithm (Lee et al., 2007, Green et al., 1997).

In this study, before every test, a recording of muscle activity at rest was taken. During this period the subject seated in a straight-up position and was asked to relax and maintain a closed mouth. It was observed however that between bursts the muscle activity at rest is normally exceeded which has been also reported by Abbink (1998). In Abbink's paper, a method for the computation of the basic EMG threshold was proposed. The method is based on the frequency distribution of amplitudes of a chewing sequence. A similar approach was used in this study to compute such a threshold level.

Abbink's Method (Determination of basic EMG threshold level)

Abbink's method for computing the basic EMG threshold is based on the fact that the frequency distribution of amplitudes resembles the right-hand side of a normal distribution function N (μ,σ^2) with μ corresponding to the offset of the signal and σ to the standard deviation of the amplitudes between bursts. The threshold is set as $\mu + 3\sigma$, this is because the area of N (μ,σ^2) to the left of this values is larger than 0.99, which implies that rarely amplitudes between burst exceed that level.

The approach basically consists of building a histogram of amplitudes from the entire signal, either raw or rectified. The histogram is built by using small bin widths for good amplitude resolution, but not too small that the shape of the normal distribution gets distorted. Trial and error was used to find the best number. The threshold level is then obtained by determining the values of μ and σ from the normal distribution curve. The value of μ is estimated by looking for the bin with the largest number of entries. The value of $\mu+\sigma$ is determined by looking for the first bin with less than 0.61 times the largest number of entries, starting from maximum value. This is because the maximum of $N(\mu,\sigma^2)$ is at μ. Therefore from the normal probability density function we have that:

$$f[x] = \frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-\mu)^2}{2\sigma^2}} \qquad (9.1)$$

Where f(x) represents the probability density of x.

f= f_{max} when x= μ such that:

$$f_{max} = f[\mu] = \frac{1}{\sqrt{2\pi\sigma^2}} \qquad (9.2)$$

whereas at μ + σ

$$f[\mu+\sigma] = f_{max}\, e^{-\frac{1}{2}} \text{ or } f[\mu+\sigma] = 0.61 f_{max} \quad (9.3)$$

Equation 9.3 indicates that at μ + σ the curve has dropped to 0.61 times its maximum. Finally, knowing μ and μ+ σ, the threshold at μ + 3σ can be estimated.

Matlab Algorithm (automated extraction of masticatory parameters)

The algorithm used for extraction of masticatory parameters through analysis of chewing cycles, was written in a series of subroutines that where run sequentially. Every subroutine is discussed briefly with some additional graphic explanation.

1) Determination of onsets and offsets of muscle activation

As mentioned above the onset and off times of muscle activity were determined using only the EMG signals from both temporalis muscles. Data from rectified and RMS signals for left and right temporalis were pooled at each time point. The kind of signal obtained is displayed in Figure 9.7. When the RMS and rectified signals were overlapped, it was noticed that the RMS follows closely the trend of the underlying rectified EMG but without the variable (spikey) peaks that are evident in the rectified signal.

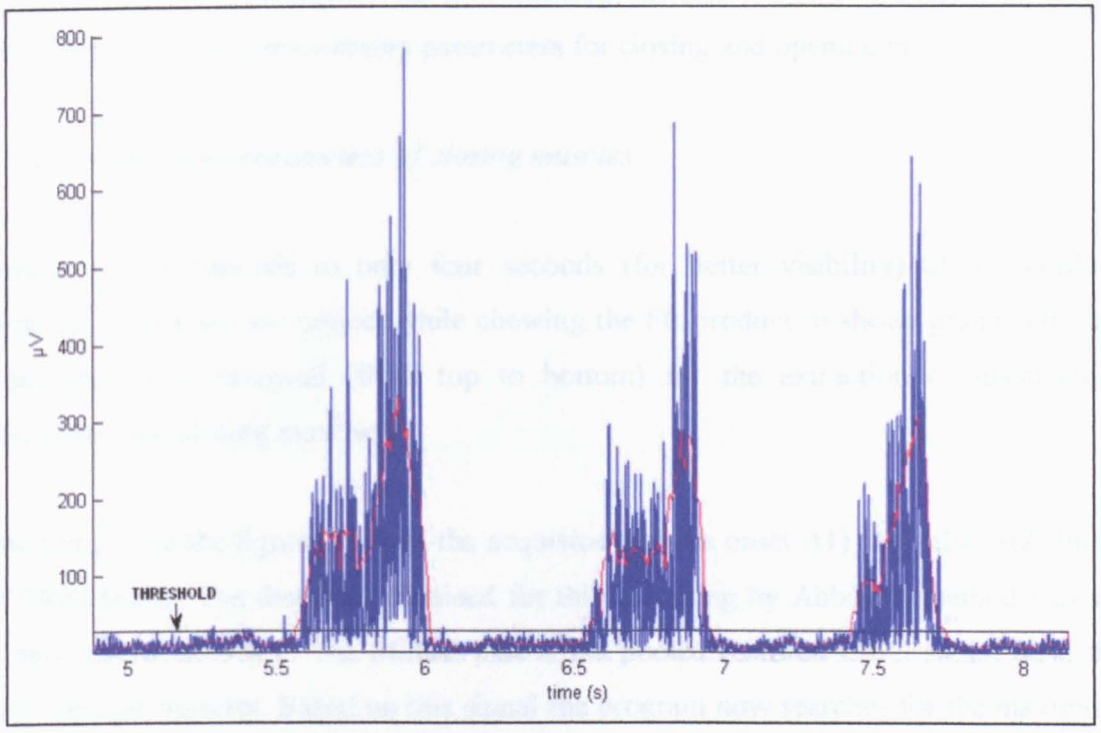


FIGURE 9.7 Rectified and RMS signals overlapped. A close up of 3 chews within a chewing sequence showing that RMS curve follows closely the trend of the rectified signal but exhibits a smoother profile.

In some of the chews there was a discrepancy between the value read manually from MyoDat software and the value obtained by the program. However the percentage error which resulted was always less than 0.5%.

The subroutine employed to find the onset and offset times basically consists of scanning through the pooled RMS signals of the temporalis muscles. The program scans through all the data samples, in time order, looking for one which has an amplitude value that exceeds the set threshold (calculated by Abbink's method). This is done by comparing every sample against the set threshold value. A burst onset then, occurs at the point when the threshold line intersects the RMS curve i.e. when a sample is found which is equal to or greater than the set threshold value. The time at which this sample occurs is recorded as t1 (onset time). After that the program continues to scan through the samples but now targeting to find one which has an amplitude equal to or less than the threshold. When a sample satisfying this criteria is found, its corresponding time is recorded as t2 (offset time). This process is repeated for every sample included in the RMS signal.

Based on the times extracted in the previous step, the following subroutines are devoted to the computation of masticatory parameters for closing and opening muscles.

2) Masticatory parameters of closing muscles

Figure 9.8 corresponds to only four seconds (for better visibility) of a recording obtained from a female subject while chewing the FR product. It shows graphically the steps that were followed (from top to bottom) for the extraction of masticatory parameters for closing muscles.

The top plot in the figure presents the acquisition of the onset (t1) and offset (t2) times of EMG bursts. The threshold obtained for this recording by Abbink's method was set in this case at 29.9 μV. The Middle plot is the pooled rectified EMG signal from the four elevator muscles. Based on this signal the program now searches for the maximum peak or maximum amplitude (P) between t1 and t2 for each burst within the chewing sequence. The value of the peak is registered together with the value of the time at which the peak occurs (tp). Finally the bottom plot corresponds to the integrated EMG for the whole chewing sequence (area under the rectified EMG signal for the four muscles). From the data of this graph the program proceeds to compute the muscle activity for each burst (area under each burst of activity). The area under the curve of each burst before the peak (ascending energy) and after the peak (descending energy) are also computed.

3) Masticatory parameters of opening muscles

For opening muscles the muscle activity in each burst was the only parameter estimated. This was done in a similar way as described for the closing muscles. This time the area under the curve was estimated between t2 and the t1 of the next burst as shown in Figure 9.9. As can be seen from the rectified signal (middle plot), the digastric muscle showed a large fluctuation in amplitude, with bursts of activity present during the closing stage as it has been discussed previously.

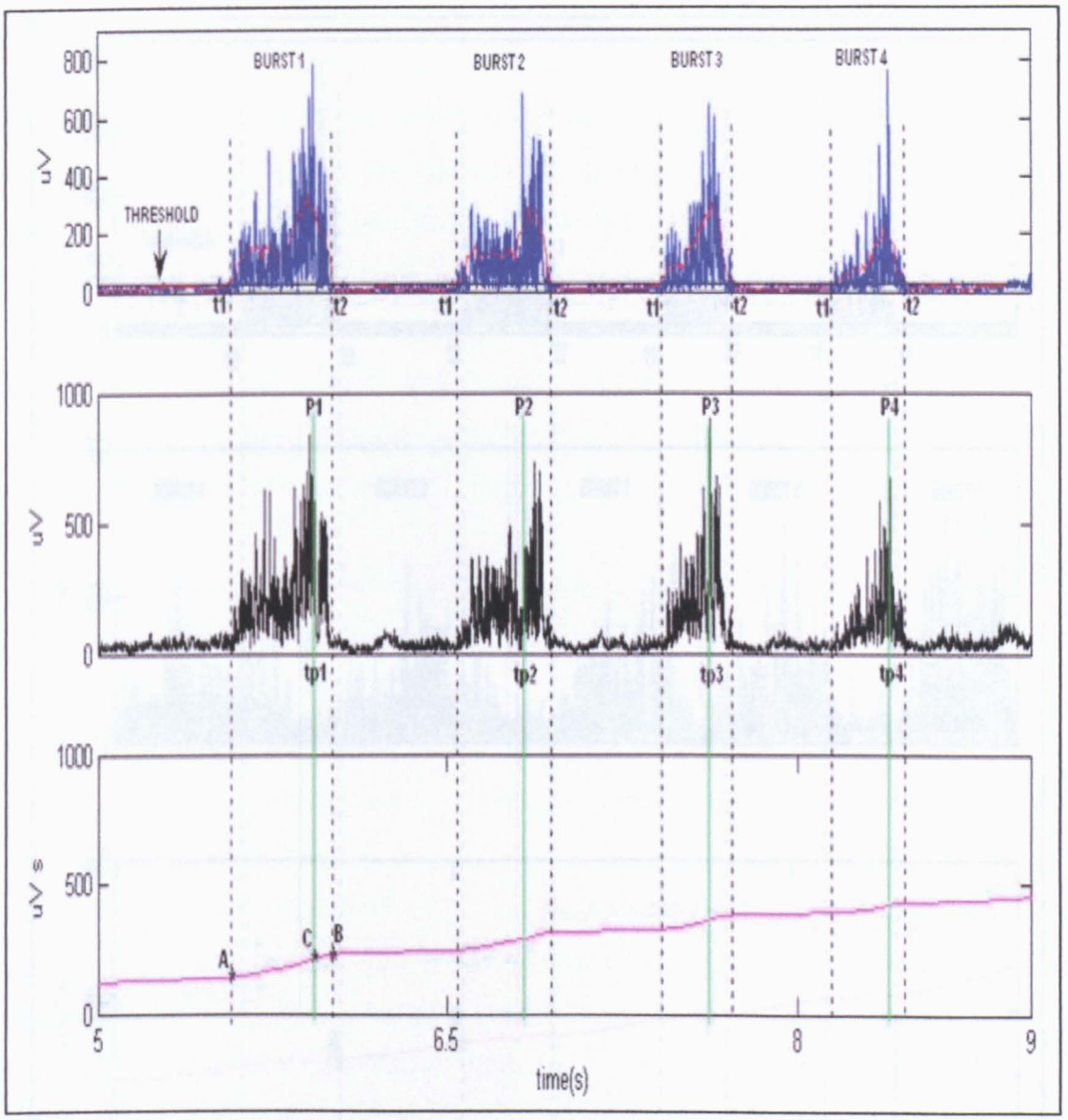


FIGURE 9.8 Analysis for closing muscles. **Upper plot**:—— Pooled rectified EMG signal from temporalis muscles (Left + Right temporalis) —— Pooled RMS signal from temporalis muscles (Left + Right temporalis) —— Calculated threshold line 29.9μV. **Middle plot:** Pooled rectified signal from 4 elevator muscles (Left temporalis + Right temporalis + Left Masseter + Right Masseter). **Lower plot**: Integrated EMG from rectified signal of 4 elevator muscles. Area before maximum peak (ascending energy) = C-A. Area after maximum peak (descending energy) = B-C. Area of burst 1= (C-A) + (B-C). Vertical dotted lines denote the onset (t1) and offset (t2) obtained from the program. Green solid lines denote the time (tp) at which maximum peak (P) occurs.

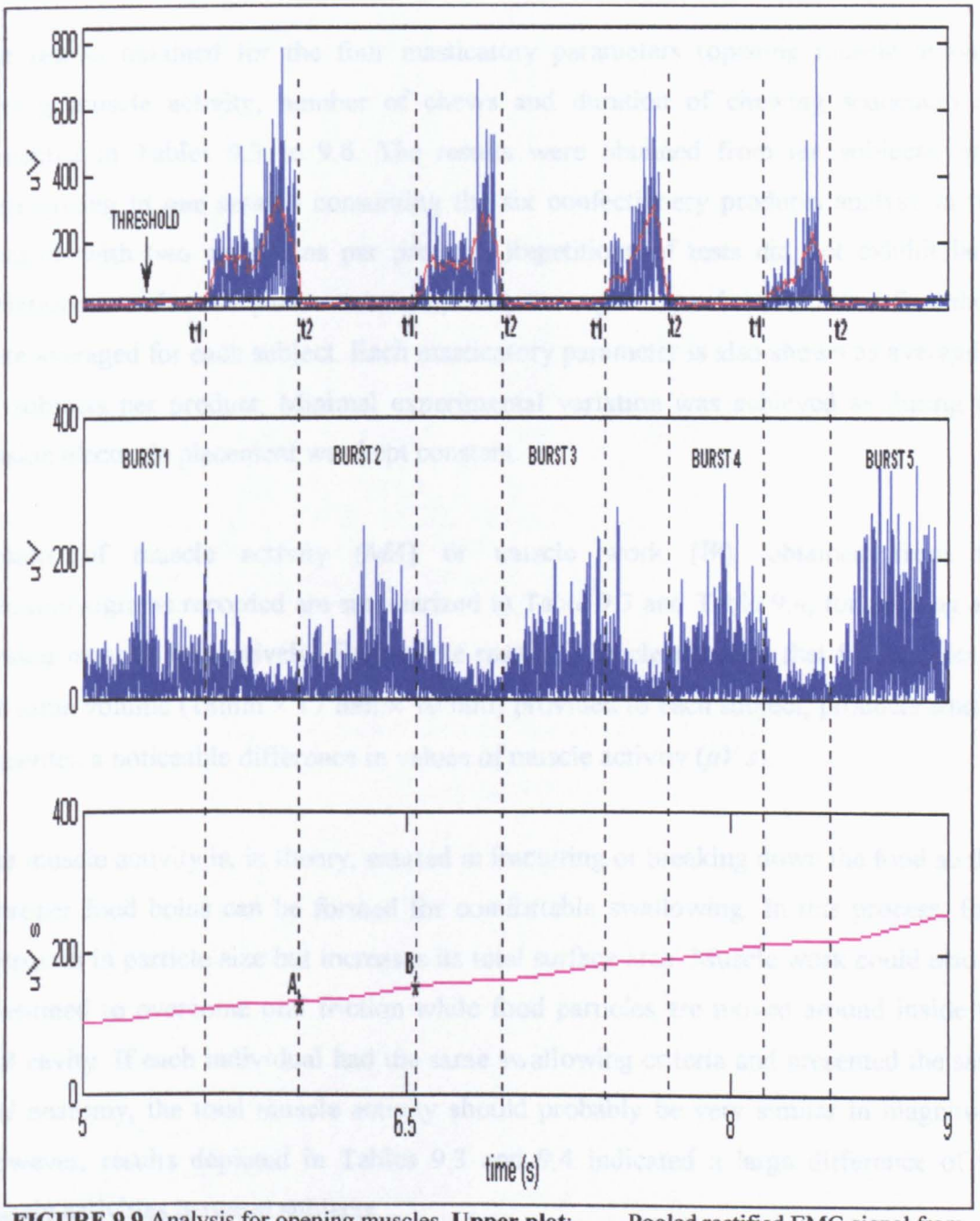


FIGURE 9.9 Analysis for opening muscles. **Upper plot:**—— Pooled rectified EMG signal from temporalis muscles (Left + Right temporalis) —— Pooled RMS signal from temporalis muscles (Left + Right temporalis) —— Calculated threshold line 29.9μV. **Middle plot:** Rectified signal from opening muscles (suprahyoid muscles). **Lower plot**: Integrated EMG from rectified signal of opening muscles. Muscle activity per burst (area under the curve between t2 and t1 of the next burst) = B-A. Vertical dotted lines denote the start and ending of muscle activation during mandibular opening

9.5 Results and discussion

9.5.1 Analysis of chewing sequence

The results obtained for the four masticatory parameters (opening muscle activity, closing muscle activity, number of chews and duration of chewing sequence) are presented in Tables 9.3 to 9.6. The results were obtained from ten subjects, each participating in one session consuming the six confectionery products analyse in this research with two repetitions per product. Repetitions of tests did not exhibit large variations in values of parameters computed, for a given product, and therefore these were averaged for each subject. Each masticatory parameter is also shown as average of 10 subjects per product. Minimal experimental variation was achieved as during the session electrode placement was kept constant.

Results of muscle activity (*MA*) or muscle work (*W*), obtained from the electromyograms recorded are summarized in Table 9.3 and Table 9.4, for opening and closing muscles respectively. From these results, it is clearly seen that for samples of the same volume (18mm × 17 mm × 10 mm) provided to each subject, products studied presented a noticeable difference in values of muscle activity ($\mu V \cdot s$).

The muscle activity is, in theory, exerted in fracturing or breaking down the food so that a proper food bolus can be formed for comfortable swallowing. In this process, food decreases in particle size but increases its total surface area. Muscle work could also be consumed to overcome oral friction while food particles are moved around inside the oral cavity. If each individual had the same swallowing criteria and presented the same oral anatomy, the total muscle activity should probably be very similar in magnitude. However, results depicted in Tables 9.3 and 9.4 indicated a large difference of the muscle activities between subjects.

Inter-subject variability is not surprising at all and has been largely reported by other studies in the literature. However, the real causes of such individual variability could vary. Lassauzay *et al.* (2000) suggested that variability could be the result of morphological differences in the masticatory system between individuals, such as, a unique anatomical relationship between the muscles, bones and teeth for each subject.

TABLE 9.3 Muscle Activity (*MA*) recorded from opening muscles (digastric muscle) for the whole chewing sequence

SUBJECT	Muscle activity of opening muscles ($\mu V \cdot s$)					
	JT	**FR**	**FRUT**	**TD**	**MCH**	**WO**
S1M	1190.2	1069.9	1652	1529.9	1724.9	1846.3
S2M	894.5	800.9	2147.6	1066.9	2033.3	2742.2
S3F	1146.4	1206.4	3270.7	1829	3660.6	4391.8
S4F	1558.3	1864.2	3695.6	2006.4	3954.5	5406.7
S5F	1324.3	2080.5	4071.5	3010.3	5459.1	8349.7
S6F	701.2	1339.4	2136.4	1488.4	3272.9	2487.4
S7F	582.9	916.5	1696.7	946.6	3229.6	2725.2
S8F	1696.1	2273.1	4063.6	2436	3783.6	4460.5
S9F	1155.9	1106.2	4674.2	1595	4467.6	4233.7
S10F	641.9	697.8	1675.9	939.1	2726.9	2181.7
MEAN* (n=10)	**1089.2**	**1335.5**	**2908.4**	**1684.8**	**3431.3**	**3882.5**
SEM	**120.5**	**173.9**	**369.6**	**210.8**	**350.6**	**620.2**

JT= Thorntons' Fruit Jelly; **FR**= Fry's Turkish Delight; **TD**= M&S Turkish Delight; **FRUT**= Fruittella; **MCH**- Milk Chews; **WO**= Werther's Original.
Letters in the subject column stand for; S= subject F= female or M= male
*Mean of 10 subjects

TABLE 9.4 Muscle Activity (*MA*) recorded from closing muscles (Left Temporalis + Right Temporalis + Left Masseter + Right Masseter) for the whole chewing sequence

SUBJECT	Muscle activity of closing muscles ($\mu V \cdot s$)					
	JT	**FR**	**FRUT**	**TD**	**MCH**	**WO**
S1M	2180.9	1778.9	5894.3	2429.6	7097.9	6644.3
S2M	1737.9	1681.1	5608.1	1992.9	5100.6	6596.1
S3F	3719.2	3811.1	10508.1	6008.3	11028.4	13387.3
S4F	4287.2	4112.7	12048.1	4210.8	11443.7	14193.4
S5F	2482.4	3797.7	10604.5	5395.7	12464.6	14499.1
S6F	1329.8	1969.9	5210	2032.7	8566.4	6654.6
S7F	1003.8	1245.7	4668.7	1362.9	11563.1	6462.5
S8F	1975.1	3042.7	9186.4	3278.3	9986.5	9397.2
S9F	2683.6	2331.5	7484.3	2933	9823.8	7826.9
S10F	2062.3	1848.3	7116.5	2571.4	16292.5	9451.2
MEAN* (n=10)	**2346.2**	**2562.0**	**7832.9**	**3221.6**	**10336.8**	**9511.3**
SEM	**320.3**	**329.3**	**821.1**	**482.9**	**969.7**	**1048.0**

JT= Thorntons' Fruit Jelly; **FR**= Fry's Turkish Delight; **TD**= M&S Turkish Delight; **FRUT**= Fruittella; **MCH**- Milk Chews; **WO**= Werther's Original
Letters in the subject column stand for; S= subject F= female or M= male
*Mean of 10 subjects

TABLE 9.5 Number of chews per chewing sequence

SUBJECT	**Number of chews**					
	JT	**FR**	**FRUT**	**TD**	**MCH**	**WO**
S1M	17	16	32	17	34	31
S2M	21	23	58	28	56	71
S3F	24	24	54	32	49	66
S4F	33	33	60	33	61	74
S5F	24	33	72	40	83	103
S6F	21	24	46	26	69	57
S7F	13	17	31	20	62	43
S8F	26	36	70	41	85	85
S9F	19	20	55	20	54	48
S10F	21	20	50	25	90	61
MEAN* (n=10)	**21.9**	**24.6**	**52.8**	**28.2**	**64.3**	**63.9**
SEM	**1.7**	**2.2**	**4.4**	**2.6**	**5.6**	**6.6**

JT= Thorntons' Fruit Jelly; **FR**= Fry's Turkish Delight; **TD**= M&S Turkish Delight; **FRUT**= Fruit-Tella; **MCH**- Milk Chews; **WO**= Werther's Original.
Letters in the subject column stand for, S= subject F= female or M= male
*Mean of 10 subjects

TABLE 9.6 Duration of chewing sequence

SUBJECT	**Sequence Duration (s)**					
	JT	**FR**	**FRUT**	**TD**	**MCH**	**WO**
S1M	34.8	29.8	42.1	44.4	39.95	41.3
S2M	23.0	22.1	53.5	26.1	48.25	67
S3F	21.8	21.75	51.2	29.9	51.3	66.9
S4F	24.2	28.05	54.5	31.5	59.4	75.7
S5F	21.9	38.5	72.9	48.3	85.15	131
S6F	24.6	33.55	54.5	37.5	82.6	60.4
S7F	15.1	22.85	35.3	21.4	73.8	52.9
S8F	34.8	47.75	69.6	46.3	82.9	91.5
S9F	20.0	17.75	55.3	25.1	56.7	54.7
S10F	16.8	20.2	41.2	21.7	77.3	56.2
MEAN* (n=10)	**23.7**	**28.2**	**53.0**	**33.2**	**65.7**	**69.7**
SEM	**2.1**	**3.0**	**3.7**	**3.2**	**5.2**	**8.1**

JT= Thorntons' Fruit Jelly; **FR**= Fry's Turkish Delight; **TD**= M&S Turkish Delight; **FRUT**= Fruit-Tella; **MCH**- Milk Chews; **WO**= Werther's Original.
Letters stand for, S= subject F= female M= male
*Mean of 10 subjects

Different swallowing criteria between individuals could be another possible contribution to the varied muscle work. Different individuals could swallow boluses composed of food particles of different sizes (Hoebler et al., 2000, Jalabert-Malbos et al., 2007). The smaller the particle size of a food bolus, the longer the chewing that will be needed and, therefore, the larger the total muscle work.

Kohyama and collaborators (2008) suggested that one method of eliminating inter-subject differences is to express the masticatory parameters as the ratio of the mean value of each parameter to the mean of all samples. However, in personal correspondence, Kohyama elaborated by saying that relative values actually have almost the same variability as absolute values, but different magnitudes. Furthermore, it was mentioned that in some cases using absolute values can actually produce better results. In this study absolute values of the masticatory parameters were used.

For the six confectionery products (JT, FR, TD, FRUT, MCH and WO), the results showed that the mean values of the four masticatory parameters (muscle activity of closing and opening muscles, number of chews and sequence duration) exhibited good and significant correlations with sensory and instrumental evaluations of stickiness. The parameters computed are presented in Table 9.7. Differences between products for each masticatory parameter were determined by means of ANOVA with repeated measures. The Results are also graphically displayed in Figure 9.10, as an average of all 10 subjects. Significant correlations were not, however, obtained between these masticatory parameters and sensory or instrumental measurements of hardness (see Table 9.8).

Figure 9.10*A* shows an increase in opening muscle activity across the range of products. The trend of increasing activity, with product, is very similar to that found by instrumental evaluation of sample stickiness (JT < FR < TD < FRUT < MCH < WO) but shows less similarity to the trend shown by sensory evaluation of product stickiness (JT < FR < FRUT < TD < MCH < WO). The Products which appeared in a different order in the two trends were TD and FRUT. Mean values of sensory scores, for stickiness, obtained for these products, however, were found not to be significantly different from each other, suggesting that the magnitude of the stickiness was possibly perceived as very similar for both products.

There was, however, a great difference in the muscular response between the products FRUT and TD, where FRUT presented considerably higher opening muscle activity than TD. This can be explained as follows:

From instrumental tests of stickiness (penetration tests discussed in Chapter 7) it was evident that the stickiness of both products was affected by wetting of the samples. In the case of TD wetting caused a decrease in stickiness whilst an increase was observed when FRUT was subjected to wetting. These different behaviours could provide an explanation for the physiological response exhibited during mastication. In this respect, it is assumed that TD is experienced as sticky at the beginning of the chewing sequence, but as oral processing continues its stickiness is reduced. It is thought that this could be due to the constant interaction between the product and saliva. The saliva acts as a solvent, by quickly dissolving the sugar in the product, and could also cause excessive hydration of the product leading to the formation of a more slippery material inside the oral cavity and possibly making it easier to be swallowed. This could be supported by the short sequence duration exhibited when chewing TD (33.2 *s*) compared to that obtained by FRUT (53 *s*).

Additionally TD contains starch. Thus, the presence of the enzyme amylase in the saliva could cause oral breakdown of the starch and lead to a dramatic viscosity reduction during the course of mastication (Prinz et al., 2007). These factors could have accounted for the lower activity of opening muscles recorded for TD compared to that corresponding to FRUT.

On the other hand, the FRUT product requires a longer period of oral processing (longer sequence duration as seen in Table 9.6) as it develops greater stickiness after it comes in contact with saliva. This leads to a longer chewing sequence, and thus the recording of a greater total muscle activity.

Apart from this discrepancy, opening muscle activity could be a good masticatory predictor of consumer experience of the stickiness of the products being studied. Table 9.8 shows the Pearson's coefficients obtained. As can be seen from this table, the coefficients indicate a good and significant correlation between opening muscle activity and stickiness evaluated sensorially and instrumentally (in all cases $r > 0.9$).

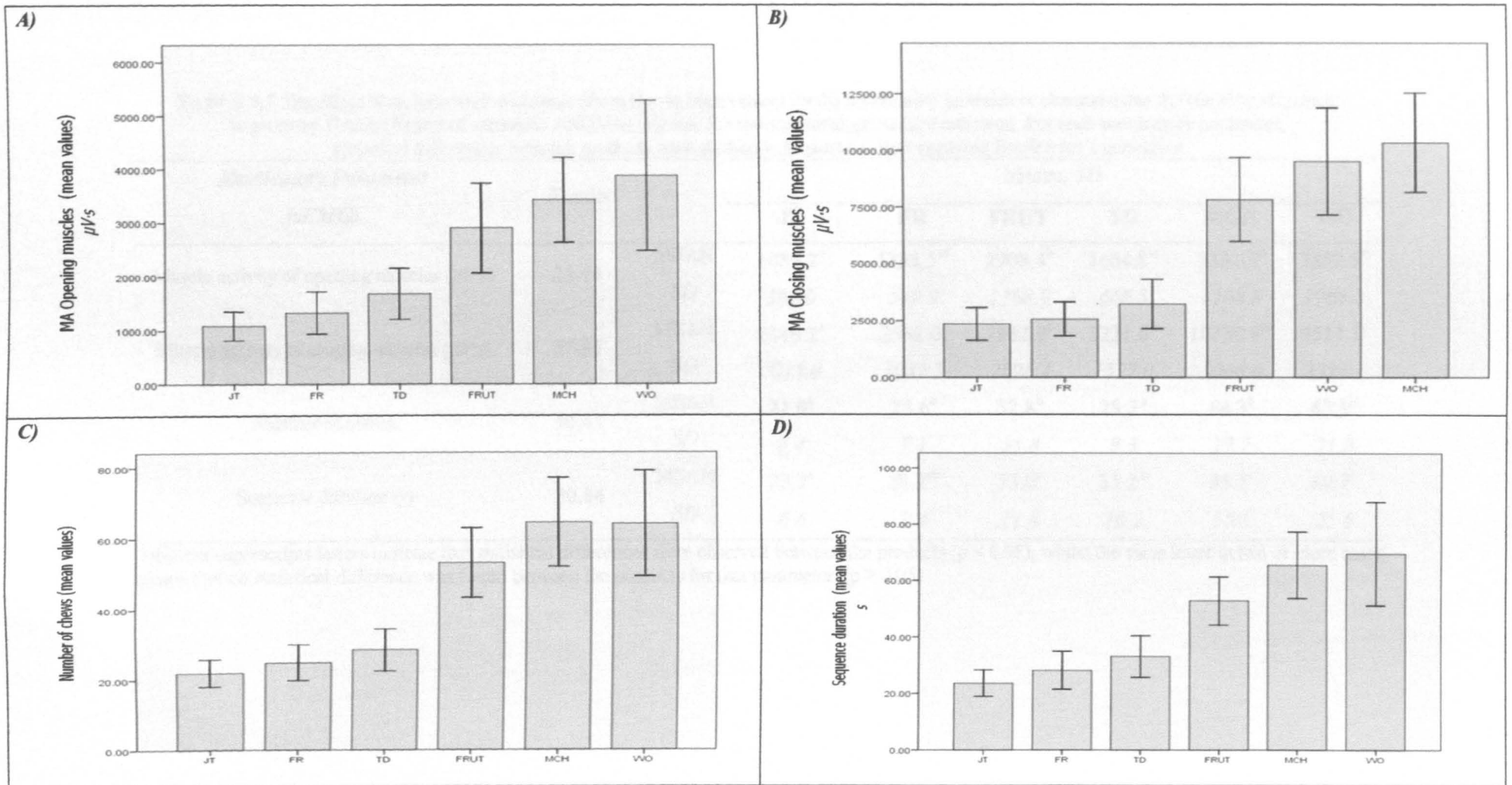


FIGURE 9.10 Masticatory parameters for analysis of the chewing sequence. Food samples correspond to six confectionery products, the main samples under study.

A) Muscle activity (*MA*) of opening muscles ($\mu V{\cdot}s$); ***B)*** Muscle activity (*MA*) of closing muscles ($\mu V{\cdot}s$); ***C)*** Number of chews; ***D)*** Sequence duration (s) Error bars: 95% CI

TABLE 9.7 The effect of an increased stickiness (from low to high values) on the masticatory parameters characterising the chewing sequence, is given by *F* ratio (Repeated measures ANOVA). Means, *SD* and statistical groups are indicated. For each masticatory parameter, statistical differences between products were studied with paired t- tests applying Bonferroni's correction

Masticatory Parameter (sEMG)	***F* ratio**		Means, SD					
			JT	**FR**	**FRUT**	**TD**	**MCH**	**WO**
Muscle activity of opening muscles ($\mu V \cdot s$)	25.74	MEAN	1089.2^{a}	1335.5^{ac}	2908.4^{b}	1684.8^{c}	3431.3^{b}	3882.5^{b}
		SD	*381.0*	*549.9*	*1168.9*	*666.5*	*1108.8*	*1961.3*
Muscle activity of closing muscles ($\mu V \cdot s$)	53.91	MEAN	2346.2^{a}	2562.0^{a}	7832.9^{b}	3221.6^{a}	10336.8^{bc}	9511.3^{c}
		SD	*1013.0*	*1041.3*	*2596.4*	*1527.0*	*3066.6*	*3314.1*
Number of chews	50.47	MEAN	21.9^{a}	24.6^{a}	52.8^{b}	28.2^{a}	64.3^{b}	63.9^{b}
		SD	*5.4*	*7.1*	*13.8*	*8.3*	*17.7*	*21.0*
Sequence duration (s)	30.84	MEAN	23.7^{a}	28.2^{ab}	53.0^{c}	33.2^{b}	65.7^{c}	69.7^{c}
		SD	*6.6*	*9.4*	*11.8*	*10.2*	*16.5*	*25.6*

Different superscripts letters indicate that statistical differences were observed between the products ($p < 0.05$), whilst the same letter in two or more cases means that no statistical difference was found between the products for that parameter ($p > 0.05$)

TABLE 9.8 Correlation coefficients (r) between Physiological measurements and Sensory evaluations and Instrumental tests of stickiness and hardness

Textural attribute	**Masticatory Parameter (sEMG)** (Mean values of 10 subjects)	**Sensory scores (mean values)**	**Instrumental measurements** (Penetration tests at 37°C using distilled water as wetting agent)	**Instrumental measurements** (Penetration tests at 37°C using saliva as wetting agent)
STICKINESS	Muscle activity of opening muscles ($\mu V \cdot s$)	0.91*	0.97**	0.98**
	Muscle activity of closing muscles ($\mu V \cdot s$)	0.87*	0.96**	0.99**
	Number of chews	0.88*	0.97**	0.99**
	Sequence duration (s)	0.91*	0.98**	0.99**
HARDNESS	Muscle activity of opening muscles ($\mu V \cdot s$)	0.73 p(2-tailed)= .165	0.70 p(2-tailed)= .187	0.31 p(2-tailed)= .618
	Muscle activity of closing muscles ($\mu V \cdot s$)	0.81 p(2-tailed)= .165	0.80 p(2-tailed)= .106	0.44 p(2-tailed)= .458
	Number of chews	0.79 p(2-tailed)= .165	0.77 p(2-tailed)= .125	0.40 p(2-tailed)= .502
	Sequence duration (s)	0.72 p(2-tailed)= .165	0.69 p(2-tailed)= .194	0.29 p(2-tailed)= .633

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)
■ Not significant correlations p(2-tailed) > 0.05 (N = 5)

The masticatory parameters analysed showed a poorer correlation with sensory or instrumental evaluations of hardness compared to those of stickiness (see Table 9.8). This result was especially interesting since closing muscle activity per sequence, number of chews and time of chewing sequence have been generally reported as increasing with food hardness.

Peyron et al., (2002) explained that a more natural product provides a more heterogeneous stimulus (i.e. the combination of several textural properties in a single food) and would be expected to produce a more complex masticatory response. Hence, they suggested that in order to gain a better understanding of the relationship between food stimulus and mastication it is necessary to use materials which present only the textural property under analysis without varying other properties. This aims to ensure that the physiological response corresponds only to that property. Another strategy would be the use of a range of real foods whose mechanical properties are well controlled and reproducible. Following such arguments, Peyron and collaborators, attempted to isolate hardness from other textural properties by employing food models (confectionery products) of predominantly elastic character and used different grades of gelatine to achieve different degrees of hardness across the foods. They reported that the best sequence descriptors of hardness were the number of chewing strokes, EMG activities of the muscles on the chewing side and the amplitude of mandibular movements.

Foster et al., (2006) arrived to similar findings regarding hardness, not only for products with elastic properties but also for those exhibiting plastic character. They concluded that mastication responds to an increased hardness by considerably increasing the number of chewing cycles and the activity of temporal and masseter muscles. With respect to the vertical amplitude of mandibular movements; Foster and colleagues found that although modified by hardness this parameter is much more affected by the elastic or plastic properties of foods.

Even though chewing sequence descriptors were good to describe the adaptation of mastication to hardness, Peyron and collaborators emphasized that to optimise the discrimination between products of different hardness the use of cycle descriptors would be more adequate. Specifically, this group of researchers suggested the use of

those descriptors related to either the first, or one of the first four, cycles rather than the use of descriptors for the whole chewing sequence. This is because the hardness of the food product changes dramatically along the chewing sequence.

In the present study the activity of closing muscles (masseter and temporalis) for an entire chewing sequence was not shown to be a good descriptor of product hardness as was found in the previous works discussed above. As can be seen from Figure 9.10 *B* the product which was sensorially and instrumentally evaluated as the hardest (FRUT) was not the one for which the highest muscle activity was recorded. Also, the softest product (JT) was not the one exhibiting the lowest closing muscle activity.

Food hardness as suggested by Peyron and collaborators (2002) is an attribute that it is rapidly lost in the first chewing cycles as saliva causes the softening of the food material. Evidence of such softening was confirmed in this study as shown by the results obtained from the penetration tests in Chapter 7 (e.g. better correlations between maximum penetration force and sensory scores of hardness were observed in dry rather than in wet conditions. Values of force were considerably reduced with wetting of the food surface) It is therefore presumed that the activity of closing muscles per chewing sequence could be modulated by the contribution of both stickiness and the toughness (similar to cohesiveness) of the product, rather than the hardness. This was highlighted by the good and significant correlation observed between stickiness and closing muscle activity in this study. This presumption could be supported by the work of Sugishita, et al.(2010), who found a significant and positive association between the feeling of thoughness and total muscle activity of closing muscles (masseter and anterior temporalis) and also with the mastication time.

It has also been widely established that fragmentation of food between teeth is largely dependent on either the food's toughness or a combination of its toughness and stiffness, which have been expressed as fragmentation indices. In this respect, muscle activity of closing muscles was correlated with the function: $(R/E)^{0.5}$, where R corresponds to the toughness of the food and E is the modulus of elasticity. The elastic modulus or modulus of deformability as discussed in Chapter 6 is related to the material's stiffness by describing its rigidity (Agrawal, et al., 1998). The use of this fragmentation function and its correlation with the EMG of closing muscles was not

possible in this study because some of the materials did not show rupture even when subjected to high deformations (80%). Toughness is the amount of energy absorbed by a material until fracture (the amount of work per unit volume), which is calculated from the total area under the stress-strain curve. Chen (2009) also suggested that the function is adequate to indicate oral performance for hard, brittle foods but is not suitable for the assessment of soft deformable foods which do not exhibit fracture.

It should be also noted that a food in the mouth is subjected to a complex combination of compressive, shearing and tensile forces during oral processing (Kilcast and Eves, 1991). In order to apply such forces during mastication, not only vertical movements are performed but also horizontal movements of the lower jaw which create a shearing action on the food. The force (and therefore the work) needed for such shearing actions may have to increase because of the increased food stickiness. For less sticky foods or foods with a slippery surface, much less muscle work will be needed for such jaw movements. Brown *et al.* (1998) suggested that food sticking to the teeth causes an uncomfortable sensation by preventing occlussion (contact between teeth). Thus that, even when teeth remain separate at the end of the stroke, there will be a greater work input to perform horizontal movement and shearing action (Brown et al., 1998).

The reason why closing muscle activity did not show an increase with food hardness as determined in previous works (Peyron et al., 2002, Foster et al., 2006) might be due to the fact that in those studies hardness was isolated from other possible textural properties by carefully preparation of the products used under laboratory conditions.

In this present study the products however consisted of more natural food products where stickiness was not isolated as the only textural property to vary. Therefore as Peyron and colleagues stated this would give a more complex response. Thus the variation in closing muscle activity is not due to hardness alone but to other textural properties present. For example stickiness has shown to have an influence onto the total closing muscle activity recorded. This suggests that in real products multiple per sequence descriptors may be required to describe any one textural property.

The total number of chewing cycles and the time of duration of the chewing sequence have been previously seen to have certain correlation with the perceived stickiness of the food (Kohyama et al., 2000). These two parameters have also been analysed from the EMG signals and results are shown in Figure 9.10 *C* and 9.11 *D*. It is clear that the total number of chewing cycles and the length of eating vary dramatically, depending on the properties of the food. The total number of chewing cycles increases with the increase of perceived stickiness, from as low as 21 for low sticky and mechanically weak foods to as high as over 63 chewing for highly sticky foods. The chewing time also increases in very similar pattern, from as low as 23.7 seconds to as high as 69.7 seconds (See Tables 9.5 and 9.6).

Figure 9.11 shows a few typical examples of rectified EMG signals for the elevator and depressor muscles being monitored during eating of three foods of different stickiness (low sticky *Thorntons' Fruit Jelly* (A), medium sticky *M&S Turkish Delight* (B), and high sticky *Werther's Original* toffee (C)), where a section of 20s EMG signals from the same subject (subject 3) was selected. While the stickiness increases for the three samples, JT was sensorially and instrumentally harder than TD sample. We can see from the figure that chewing was carried out in a regular pattern, with clearly identifiable starting and finishing for closing muscles. The muscle activity along the chewing sequence for closing and opening muscles however was found to increase significantly as the textural properties changes from low sticky to highly sticky. This highlights the dominant importance of food stickiness over the hardness in influencing muscle activities.

Even though experimental evidence from this work suggests that muscular activity has a close link with the sensory stickiness of foods and the muscular work could be used as a reliable physiological parameter to reflect the perceived sensory perception changes of food stickiness, the real situation could be much more complicated. One influencing factor as it has been highlighted in this study is the saliva contribution which modifies significantly not only the mechanical properties of foods but also their rheological properties during mastication.

From these results it can be concluded that muscular activities of closing muscles responds well with both the perceived hardness and stickiness of foods. However, the activity of the opening muscle (digastric muscle) showed very good correlation with the perceived stickiness, but relatively lower correlation with food hardness. It was also observed that digastric muscle was not only responsible for mouth opening but also highly active during tongue manipulation of food. Findings from this work confirm that human's oral physiology responses closely to the changing food properties and that physiological parameters can be used as effective objective tools to correlate oral behaviour with textural properties of food.

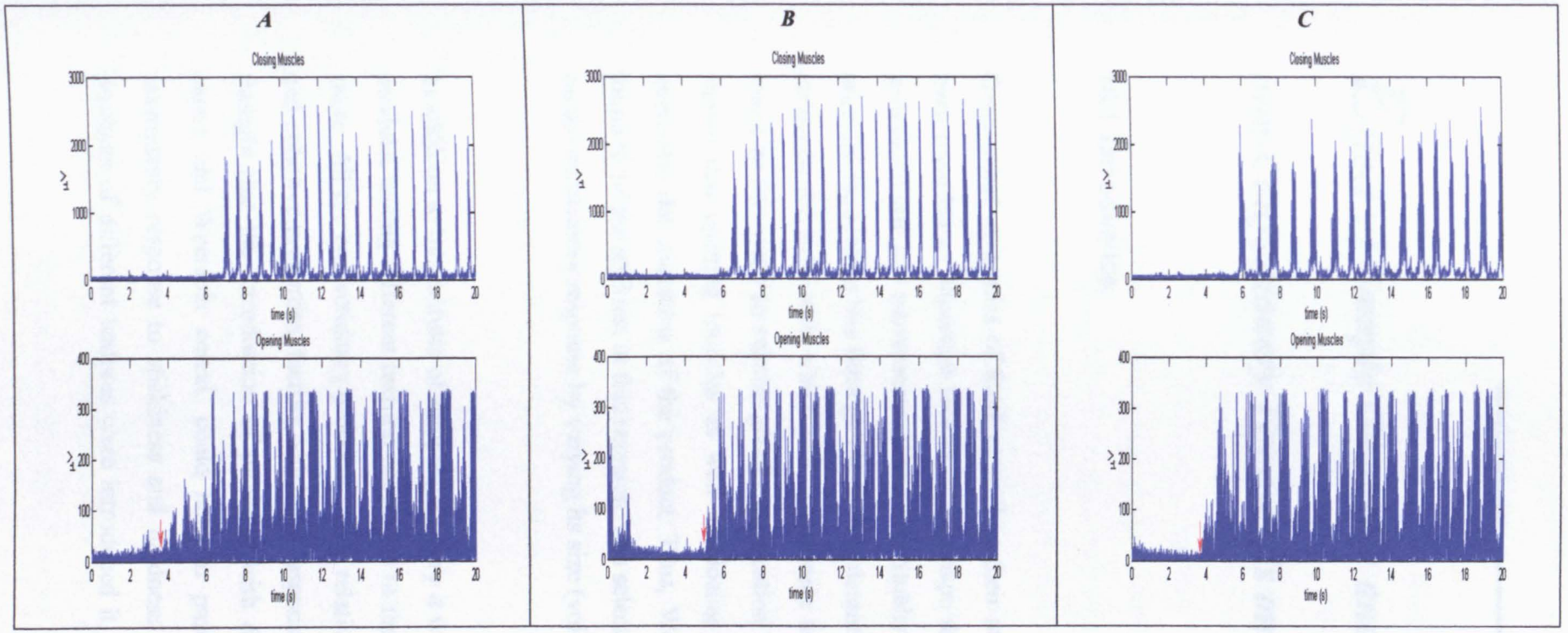


FIGURE 9.11 Individual chewing patterns of facial muscles recorded during the initial 20 second period of oral processing by subject 3. Rectified signals are from three different samples; Column ***A***: Thorntons' fruit jelly (low sticky), Column ***B***: Marks and Spencer's Turkish Delight (medium sticky) and Column ***C***: Werther's Original Toffee (high sticky). The top graph of each column corresponds to the signal from closing muscles (Left Temporalis + Right Temporalis + Left Masseter + Right Masseter) and the bottom graph corresponds to the signal recorded from opening muscles (digastric muscle). Red arrows indicate time of sample ingestion.

Chapter 10

Effect of Sample Geometry and Textural Properties of non-Confectionery Foodstuffs on Masticatory Response

10.1 Introduction

Geometrical properties of food, as well as their mechanical properties, have normally been regarded as important factors which shape the chewing pattern in terms of force generation and jaw movements (Wang and Stohler, 1991). For example, the amount of pressure required to bite through the food is determined by its size, while the shape can influence the ease with which jaw movements are performed. Sticky products were found in this study to require greater mastication effort to be orally processed. It was shown that opening muscles as well as closing muscles needed to work harder to overcome the stickiness of the product. Thus, Werther's Original toffee, the product found to be the stickiest in this research, was selected to evaluate the effect of geometry on the masticatory response by varying its size (volume) and shape.

In addition to the analysis of sample geometry it was considered beneficial to introduce products having different textural properties to the confectionery products selected for study. All the confectionary products are of relatively soft nature and present adhesive and cohesive properties, factors which are responsible for their sticky character. It was thought that the introduction of products with different textural properties, such as carrot and Weetabix cereal, could help to provide a better understanding of the masticatory response to stickiness and hardness. Specifically it was deemed that if products of different textures were introduced it may be possible to discern whether

features of the masticatory response, observed for the confectionary products, were in fact due to their stickiness.

An ideal method for determining whether masticatory response is due to stickiness would be the use of specially prepared laboratory products. These products could be made to vary only in stickiness, keeping all other textural properties constant. Thus, allowing researchers to determine the masticatory response changes in response to stickiness. Such an approach has been previously used by Peyron et al. (2002) to study the effect of hardness on masticatory response. The use of this approach, however, would be practically difficult when studying stickiness. This is because preparation of products which varied in their degree of stickiness without modification of other textural properties would be rather complicated, if not impossible, to achieve. Firstly, because of the fact that the factors causing stickiness are still not fully understood. The present research, for example, has shown that the stickiness of a product is actually dependant on other textural properties such as elasticity and plasticity. Thus creation of a product which varies only in stickiness without varying other textural properties may not be feasible. This is the reason why conventional products, having little or no sticky character, were selected for inclusion in the study.

In general fruits and vegetables are products which have not been regarded as sticky. Additionally, many raw vegetables are deemed to be hard products. Carrot a vegetable of such characteristics was introduced in the study to allow the comparison of its textural properties with those of confectionery products under study.

In addition to carrot, a cereal product (weetabix) which is dry and brittle was incorporated. Cereals have been reported as sticky after hydration, by interaction with saliva (Caldwell, 1959, Lenfant et al., 2009).

10.2 Materials, apparatus and method

EMG equipment and methodologies followed for the performance of the experiments in this chapter, have been all previously discussed in Chapter 9.

The foodstuffs used for this analysis included one of the main confectionery products studied in this research (Werther's original toffee), chewing gum and two non confectionery products (carrot and Weetabix bitesize cereal). All these products are briefly described next. See also Table 10.1 for more detail:

1) *Chewing gum.* This was used as a reference sample. Chewing gum was used to perform a pre-test, aiming to familiarise subjects with the testing conditions and validate the setting up i.e. by evaluation of subject's discomfort, checking proper physical connection of electrodes and amplifiers, confirming correct cable fixation, assessment of recorded signal by assuring zero offset, ensuring minimum levels of noise and presence of bursts while performing specific muscle function test, and evaluation of possible motion artefacts present in the signals.

2) *Toffee sample (WO).* This product was used as a representative sample of a highly sticky product according to the results obtained from the instrumental penetration tests performed in this study (Chapter 7). Two additional samples of the same product but with different geometries (cylindrical shapes with different diameters) to that previously used (cubic geometry of dimensions $18 \times 17 \times 10 \pm 0.5$ mm) were prepared. The larger of which had a similar volume ($\approx 31 cm^3$) to the cubic sample. These food samples were used to study the effect of geometry on the physiological response to textural attributes.

3) *Raw carrot.* A hard product (Szczesniak et al., 1963) generally not considered as sticky.

4) *Weetabix bitesize.* A dry piece of cereal which is moistened as the chewing sequence proceeds. Most cereals in general have been sensorially evaluated as sticky when moistened by saliva during eating.

TABLE 10.1 Samples for EMG measurement additional to the main samples under study

Food	Name/Brand	Sample Code	Sample Dimensions
Chewing gum	Hubba Bubba (Wrigley's)	CG	Cubic (18mmx17mmx10mm)
Toffee	Werther's Original (Storck)	WOb	Cylindrical large diameter (20mm diameter x 10mm height)
Toffee	Werther's Original (Storck)	WOs	Cylindrical small diameter (16mm diameter x 10mm height)
Raw carrot	N/A	CA	Cubic (18mmx17mmx10mm)
Wholegrain wheat cereal	Weetabix bitesize	WB	Cubic (18mmx17mmx10mm)

5) **N/A** Not applicable

10.3 Results and discussion

10.3.1 Effect of sample geometry on the response of masticatory parameters per chewing sequence

The four masticatory parameters: muscle activity of opening and closing muscles, the number of chews and the time per chewing sequence obtained for the three samples of toffee with different geometries are reported in Tables 10.2 to 10.5. They are identified in-column WO, corresponding to the geometry of the main sample used in this study, WOb having similar volume to WO but with cylindrical shape and WOs, being cylindrical in shape but smaller in size (smaller diameter) than WOb. Additionally, see Table 10.6 for full description of samples and Figure 10.1 for a graphical representation of the experimental design used.

TABLE 10.2 Muscle Activity (*MA*) recorded from opening muscles (digastric muscle) for the whole chewing sequence

SUBJECT	Muscle activity of opening muscles ($\mu V \cdot s$)				
	WO	WOb	WOs	CA	WB
S1M	1846.3	1831	1397.9	1615.5	1682.1
S2M	2742.2	2196.6	1775.4	815.9	943.4
S3F	4391.8	4312.3	3094.6	1751.5	1350.6
S4F	5406.7	4113.1	4134.5	1919.4	1618.6
S5F	8349.7	4644	6067.2	1719.4	1655.7
S6F	2487.4	2124.5	2376.5	825	1172.6
S7F	2725.2	2374.1	1960.5	687.3	897
S8F	4460.5	3719.6	2666.6	1011.4	1205.6
S9F	4233.7	5008.6	3958.5	1114.9	1758.5
S10F	2181.7	2051.6	1917	864.8	946.1
MEAN* (n=10)	**3882.5**	**3237.5**	**2934.9**	**1232.5**	**1323.0**
SD	**1961.3**	**1234.8**	**1429.8**	**466.6**	**336.9**

WO_b= Werther's Original (circular shape large diameter); **WO_s**= Werther's Original (circular shape small diameter); **CA**= Carrot and **WB**= Weetabix
Letters in the subject column stand for, S= subject F= female or M= male
*Mean of 10 subjects

TABLE 10.3 Muscle Activity (*MA*) recorded from closing muscles (Left Temporalis + Right Temporalis + Left Masseter + Right Masseter) for the whole chewing sequence

SUBJECT	Muscle activity of closing muscles ($\mu V \cdot s$)				
	WO	WOb	WOs	CA	WB
S1M	6644.3	6436.4	4182	4983.5	4181.6
S2M	6596.1	5550	3992.1	3808.1	2617.6
S3F	13387.3	11100	8307.4	7196.6	5922.1
S4F	14193.4	11430.4	9684.6	6207.2	5645.5
S5F	14499.1	8018	9571.9	6380.8	5421.1
S6F	6654.6	5884	4292	2040.9	3405.3
S7F	6462.5	5679.1	3726.2	1831.3	2204.9
S8F	9397.2	8849.8	6121.6	3274.2	3286.1
S9F	7826.9	9672.4	6613.9	3103.3	4163.5
S10F	9451.2	7330.2	5839.9	4526.5	4975.1
MEAN* (n=10)	**9511.3**	**7995.0**	**6233.2**	**4335.2**	**4182.3**
SD	**3314.1**	**2202.4**	**2284.2**	**1849.6**	**1295.1**

WO_b= Werther's Original (circular shape large diameter); **WO_s**= Werther's Original (circular shape small diameter); **CA**= Carrot and **WB**= Weetabix
Letters in the subject column stand for; S= subject F= female or M= male
*Mean of 10 subjects

TABLE 10.4 Number of chews per chewing sequence

SUBJECT	Number of chews				
	WO	WOb	WOs	CA	WB
S1M	31	30	24	44	28
S2M	71	60	42	37	28
S3F	66	64	42	43	32
S4F	74	61	55	53	31
S5F	103	59	78	41	33
S6F	57	53	41	24	31
S7F	43	49	26	20	22
S8F	85	75	59	33	31
S9F	48	51	41	20	23
S10F	61	51	44	40	34
MEAN* (n=10)	**63.9**	**55.3**	**45.2**	**35.5**	**29.3**
SD	**21.0**	**11.9**	**15.8**	**11.0**	**4.1**

WO_b= Werther's Original (circular shape large diameter); **WO_s**= Werther's Original (circular shape small diameter); **CA**= Carrot and **WB**= Weetabix
Letters in the subject column stand for; S= subject F= female or M= male
*Mean of 10 subjects

TABLE 10.5 Duration of chewing sequence

SUBJECT	Sequence Duration (s)				
	WO	WOb	WOs	CA	WB
S1M	41.3	50.25	37.75	51.1	47.15
S2M	67	52.6	41.55	28.5	24.2
S3F	66.9	67.3	47.65	35.0	27.3
S4F	75.7	62.4	55.65	37.0	26.2
S5F	131	72.9	98.35	36.5	29.65
S6F	60.4	54.65	60.15	24.6	32.95
S7F	52.9	46.95	38.95	16.5	21.95
S8F	91.5	73.8	61	28.8	30.65
S9F	54.7	66.95	44	15.7	19.8
S10F	56.2	48.2	47.8	26.3	25.9
MEAN* (n=10)	**69.7**	**59.6**	**53.3**	**30.0**	**28.6**
SD	**25.6**	**10.3**	**17.9**	**10.5**	**7.6**

WO_b= Werther's Original (circular shape large diameter); **WO_s**= Werther's Original (circular shape small diameter); **CA**= Carrot and **WB**= Weetabix
Letters in the subject column stand for; S= subject F= female or M= male
*Mean of 10 subjects

TABLE 10.6 Descriptors of sample geometries

SAMPLE CODE	Shape	Dimensions (mm)	Surface Area (mm^2)	Volume (mm^3)
WO	Rectangular	17 × 18 × 10	306	3060
WOb	Cylindrical	d= 20, h=10	314	3142
WOs	Cylindrical	d= 16, h=10	201	2011

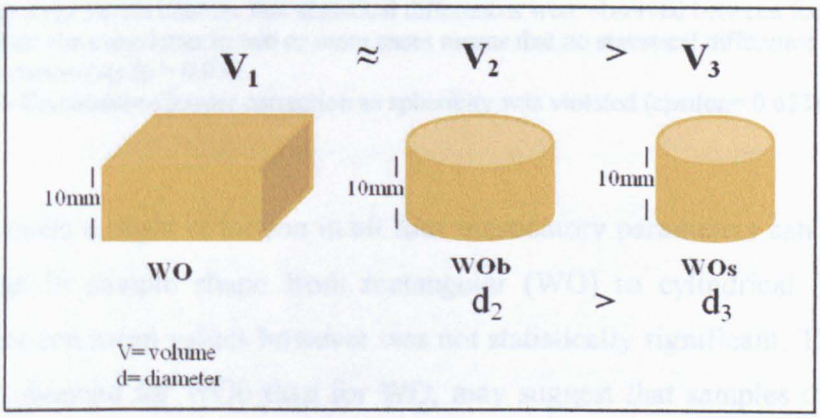


FIGURE 10.1 Geometric design of food sample (toffee) for analysis of masticatory response

Each masticatory parameter was tested by means of ANOVA with a repeated measures design. If the test was significant, a multiple comparison (paired t-test with Bonferroni's correction) was used to compare the difference between food geometries. Results of this statistical analysis are summarised in Table 10.7.

TABLE 10.7 Masticatory parameters per chewing sequence for different geometries of toffee (WO)

Masticatory Parameter (sEMG)	***F* ratio**		Means, SD		
			WO	**WOb**	**WOs**
Muscle activity of opening muscles ($\mu V \cdot s$)	5.5	MEAN	3882.5^{a}	3237.5^{ac}	2934.4^{c}
		SD	*1961.3*	*1234.8*	*1429.8*
Muscle activity of closing muscles ($\mu V \cdot s$)	20.3*	MEAN	9511.3^{a}	7995.0^{a}	6233.2^{b}
		SD	*3314.1*	*2202.4*	*2284.2*
Number of chews	13.3	MEAN	64^{a}	55^{ac}	45^{c}
		SD	*21*	*12*	*16*
Sequence duration (s)	6.0	MEAN	69.7^{a}	59.6^{ac}	53.3^{c}
		SD	*25.6*	*10.3*	*17.9*

Mean and Standard deviation (*SD*) values from 10 subjects.
Different superscript letters indicate that statistical differences were observed between food geometries ($p < 0.05$), whilst the same letter in two or more cases means that no statistical difference was found between food geometries ($p > 0.05$).
*ANOVA with Greenhouse-Geisser correction as sphericity was violated (epsilon= 0.623)

From these results a slight reduction in all four masticatory parameters can be observed with a change in sample shape from rectangular (WO) to cylindrical (WOb). The difference between mean values however was not statistically significant. The relatively lower values obtained for WOb than for WO, may suggest that samples of cylindrical shape could potentially make food consumption easier, as less mastication effort is apparently required. In circular pieces there are no corners and therefore it is presumed that this could make the manipulation of the food, inside the mouth, less difficult. Prinz and Lucas (2001), for example, found that in general, food of rectangular and square shape tends to be placed by tongue movement in such way that the longest axis is always positioned parallel to the line of the teeth. As pieces of circular shape are isometric in all surface directions (same diameter) it can be assumed that movements executed during chewing of these samples are less complex. Such tongue movements, however, are believed to occur within a short time at the beginning of the chewing sequence; before the sample is transformed and thus its shape distorted due to mechanical input, interaction with saliva and temperature inside the oral cavity. Moreover it is well known that the transformation that food undergoes during oral processing is aimed to form a bolus with geometric features which make it suitable to be swallowed. It has been observed that the food bolus acquires an oblate spherical shape

during transformation by chewing, especially in gum-type foods as in the case of toffee in this study (Liedberg and Öwall, 1995). In this sense it is presumed that cylindrical samples have a shape which requires less processing to achieve the "target bolus" than rectangular or square shaped. Square or rectangular samples may require a greater mechanical input to deform them to the point where they form a bolus which is suitable for swallowing. This could lead to a greater number of chews and tongue movements being observed for these samples. It also suggests that greater muscles activities could be recorded for square or rectangular samples.

When samples with the same shape (cylindrical) but different volume (WOb vs WOs) were compared, the only masticatory parameter which showed a statistically significant difference from one sample to the other was closing muscle activity. In this case a reduction of 36% in sample volume represented a decrease of 22% in total closing muscle activity (mean values). It can be clearly seen that ingesting a food sample of smaller size significantly reduces closing muscle activity when compared to ingesting a sample of larger size. It is interesting to note, however, that the muscle activity does not decrease by the same proportion as the size. In fact, the decrease in muscle activity is less than the decrease in sample size. This means that for a larger sample the muscle activity per unit volume is actually less than for a smaller sample. This would suggest that if a certain amount of food must be consumed; consuming it in a small number of big pieces would require less masticatory effort than consuming it in a large number of small pieces. This finding is in agreement with previous works which have investigated the effect of a reduction in sample size (weight or volume) on muscle activity (Kohyama et al., 2007a).

In this study, the sample with greater volume, in turn had a greater surface (cross-sectional) area available for contact with teeth. A larger contacting area will cause a greater resistance which opposes mandibular movement and hence requires the application of a larger force to achieve deformation. In order to exert a larger force, a larger input of muscle activity is required. Previous papers have emphasised that the force required, to achieve sample deformation, increases with thickness (Kohyama et al., 2004). In this case, as sample thickness was kept constant, the change in surface area must be responsible for the variation observed.

Results from this study showed that the mean values of the four masticatory parameters were clearly most affected when the volume and shape of the food sample were modified simultaneously. In this case the mean values of closing and opening muscle activity, number of chews and time of chewing sequence for WOs were all smaller compared to those obtained with WO. Additionally the differences between the means were statistically significant ($p<0.05$).

Previous results showed that activity of opening muscles is affected by the stickiness of the food product and therefore opening muscle activity serves as a good descriptor of that textural property. It was observed that opening muscle activity did not change significantly when either size (volume) or shape were modified separately, but differed significantly when both factors were altered simultaneously. The synergistic effect of changing both factors together must be the cause of the *significant* variation found.

Lower mean values, for the rest of the masticatory parameters analysed, were found for WOs than for WO. The differences between the means were found to be statistically significant. As the food product is the same, in all cases, it is presumed that the stickiness of the product should not vary. However the fact that the sample is smaller and shaped into a form of easy manipulation may help in its quick disappearance from the mouth which could shorten the period of sticky sensation in the mouth.

The findings of this section show that decreasing sample size is not necessarily the approach to follow to aid in the mastication process of people having eating difficulties. This is because although the masticatory effort required is reduced by reducing sample size, it is not reduced in the same proportion. i.e: If sample size is halved masticatory effort is *not* reduced by as much as half. Thus, the total effort required to process a given volume of food can actually be increased if that volume is divided in to a larger number of smaller pieces. This may be because smaller pieces require more precise jaw movements and stronger movements to place them between the teeth.

Addition, the findings also show that by preparing samples with a shape closer to that of a food bolus the mastication effort may be reduced. This is probably a reason why mushy foods are easier to process.

10.3.2 Masticatory response to non-confectionery products

Table 10.8 presents the mean values and standard deviations of all the masticatory parameters, presented in Tables 10.2 to 10.5, obtained from ten subjects when eating two non-confectionery products (carrot, CA and weetabix cereal, WB). ANOVA, with repeated measures, was used to determine how each masticatory parameter differed between the products; these differences are also highlighted in the table Results are also presented graphically in Figure 10.2 where mean values calculated for the four masticatory parameters are arranged in increasing order. The figure specifically shows where the values for CA and WB fit within the range for the six confectionery products discussed in Chapter 9.

As previously determined the muscle activity of opening muscles was shown to be significantly affected by an increase in the stickiness of the viscoelastic products used in this study. Using the opening muscle activity, the JT and FR products had been previously characterised as being low in stickiness. Although slight differences in values were observed between the mean opening muscle activities obtained from CA and those from JT and FR, these differences were not significant (as indicated in Table 10.8).Thus, CA was also put in the range of low stickiness. Curiously all these products contain pectin. Carrot for example contains about 88 % water and levels of pectin which are around 1.4%. This may suggest that pectin, as an ingredient, may provide less sticky textures. In fact it has been reported that in products like FR pectin is combined with modified starch to generate a product of short texture and less adhesive nature.

The opening muscle activities recorded for the WB product were slightly higher than those recorded for CA. Again this difference was not statistically significant and WB was also classified as in the low stickiness range.

It can be seen from Table 10.8 that although the opening muscle activities recorded for CA and WB are statistically similar to those recorded for JT and FR, placing them in the low stickiness range, they are also similar to those recorded for TD. TD is a product which has been characterized as having medium stickiness. This implies that even though CA and WB are low sticky products, they may be at the upper end of the low stickiness range.

TABLE 10.8 Results of masticatory parameters for CA and WB in comparison to confectionery products studied

Masticatory Parameter (sEMG)	***F*** ratio		Means, SD				
			JT	**FR**	**CA**	**WB**	**TD**
Muscle activity of opening muscles ($\mu V{\cdot}s$)	5.4	MEAN	1089.2^{a}	1335.5^{a}	1232.5^{ac}	1323^{ac}	2908.4^{bc}
		SD	*381.0*	*549.9*	*466.6*	*336.9*	*1168.9*
Muscle activity of closing muscles ($\mu V{\cdot}s$)	19.6	MEAN	2346.2^{a}	2562.0^{a}	4335.2^{b}	4182.3^{b}	3221.6^{a}
		SD	*1013.0*	*1041.3*	*1849.6*	*1295.1*	*1527.0*
Number of chews	9.9*	MEAN	21.9^{ab}	24.6^{ac}	35.5^{cd}	29.3^{cd}	52.8^{bd}
		SD	*5.4*	*7.1*	*11.0*	*4.1*	*13.8*
Sequence duration (s)	3.9	MEAN	23.7^{a}	28.2^{ab}	30.0^{ab}	28.6^{ab}	53.0^{c}
		SD	*6.6*	*9.4*	*10.5*	*7.6*	*11.8*

Mean and Standard deviation (*SD*) values from 10 subjects.
Different superscript letters indicate that statistical differences were observed between food samples ($p < 0.05$), whilst the same letter in two or more cases means that no statistical difference was found between food samples ($p > 0.05$).
*ANOVA with Greenhouse-Geisser correction as sphericity was violated (epsilon= 0.448)

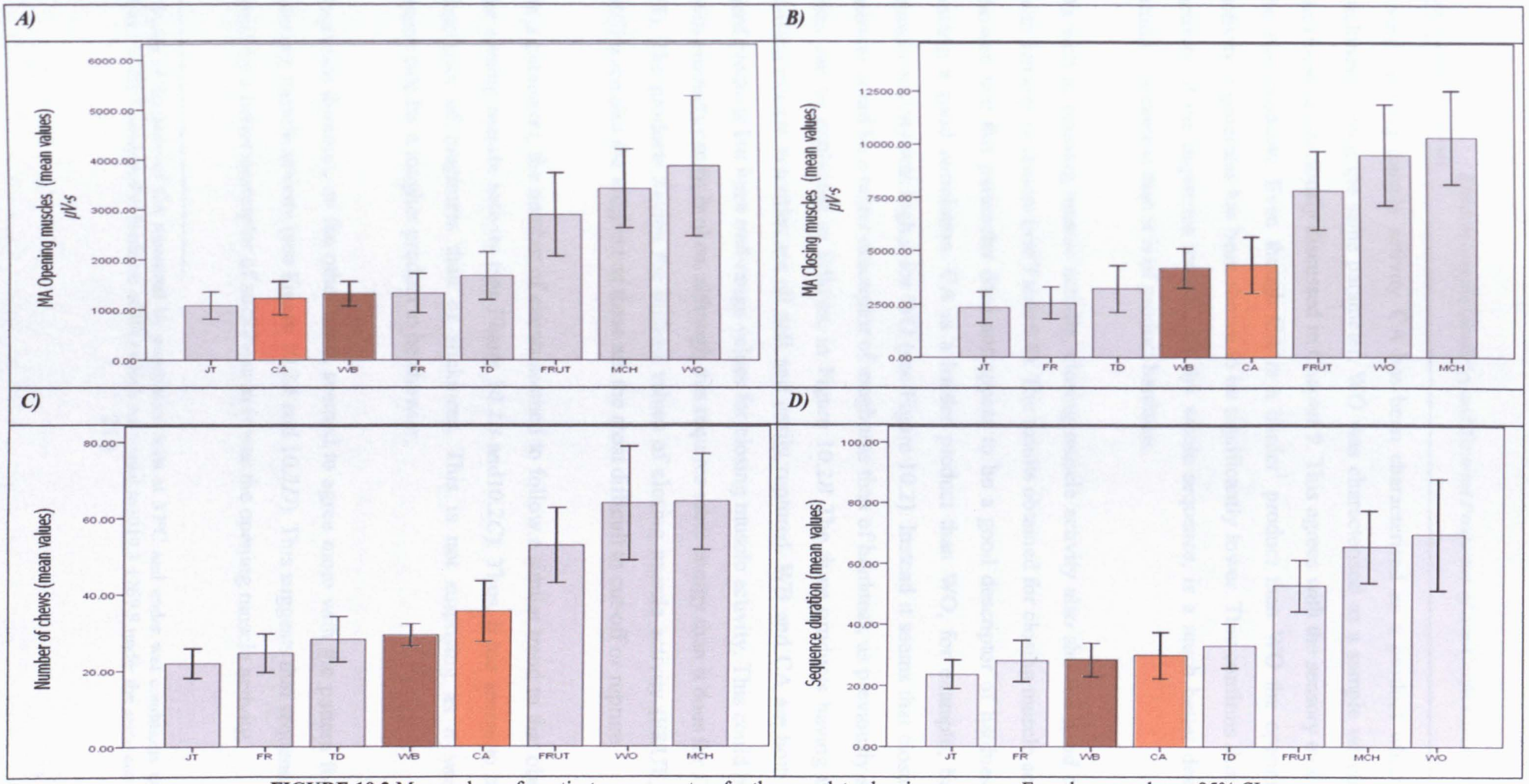


FIGURE 10.2 Mean values of masticatory parameters for the complete chewing sequence. Error bars are shown 95% CI
Samples: ■ Confectionery products (JT, FR, TD, FRUT, MCH and WO) and non-confectionery products with different textures (■ CA and ■ WB)
A) Muscle activity (*MA*) of opening muscles ($\mu V \cdot s$); ***B)*** Muscle activity (*MA*) of closing muscles ($\mu V \cdot s$); ***C)*** Number of chews; ***D)*** Sequence duration (s)

Using opening muscle activity CA has been characterised as a product which has low stickiness. Using the same parameter, WO was characterised as a sample which has high stickiness as previously discussed in Chapter 9. This agrees with the sensory evaluations of the two products. Even though CA is a harder[1] product than WO the opening muscle activity it generates has been shown to be significantly lower. This confirms that the EMG activity of the digastrics muscle, for the whole sequence, is a much better descriptor of product stickiness than it is of product hardness.

As well as opening muscle activity, closing muscle activity also showed good correlation with sensory stickiness (see Table 9.8). The results obtained for closing muscle activity also showed that this parameter does not appear to be a good descriptor of hardness, despite having a good correlation. CA is a harder product than WO, for example, but closing muscle activity was higher for WO (see Figure 10.2). Instead it seems that closing muscle activity could be a better descriptor of toughness than of hardness, as previously suggested. This can be explained as follows, in Figure 10.2*B* The three products having the lowest closing muscle activities are all soft and easily ruptured. WB and CA are both relatively hard products but have mid-range values for closing muscle activity. This could be because both products can be broken, although this requires more energy than it does for JT, FR and TD. The products having the highest values of closing muscle activity (FRUT, WO and MCH) are also the toughest as these are the most difficult to cut-off or rupture.

As a parameter, the number of chews seemed to follow a similar trend to that observed for the closing muscle activity (see Figure 10.2*B* and10.2*C*). Thus, it too seems to be a better descriptor of toughness than of stickiness. This is not surprising as it would seem reasonable for a tougher product to be chewier.

Sequence duration, on the other hand, seemed to agree more with the pattern followed by opening muscle activity (see Figure 10.2*A* and 10.2*D*). This suggests that sequence duration could be a better descriptor of stickiness as it was the opening muscle activity.

[1] Value of hardness of CA measured by penetration tests at 37°C and under wet conditions was equal to 8182.3± 705.7 compared to hardness of WO which was equal to1019.3 ±269.8 under the same conditions.

10.4 Analysis of individual chewing cycles

The program developed in Matlab for data analysis in this study allowed the extraction of multiple masticatory parameters per chewing cycle as described earlier in Chapter 9. The analysis of all those parameters would not only be extremely extensive but also not all parameters are relevant to food stickiness. Therefore, it was considered to focus the analysis on the "cycle duration" parameter as this was thought to be the parameter that could be most dependent on food stickiness.

It has been widely reported that chewing rhythm is programmed by a central pattern generator (a neural network within the central nervous system) and that this rhythm can be modified by sensory feedback obtained from sensory perception of food texture. Based on this it is reasonable to assume that a food which becomes stuck between the surfaces of the upper and lower teeth will tend to impede jaw movement and so generate sensory feedback which causes the chewing cycle time to be modified.

Additionally, the degree of attachment, of food to teeth, is not expected to be equal during every chewing cycle. This would depend considerably on the contact area and the contact force that is being applied during every chew. Therefore it was assumed that stickiness in foods could lead to the execution of irregular opening and closing movements and thus could prolong the duration of cycles.

Modulation of jaw movements in response to food stickiness was thus studied by analysing the chewing rhythm. To this end, duration of chewing burst, inter-burst duration and cycle duration were calculated. These parameters were defined in Table 9.1. The parameters were calculated using all the chews within the main chewing sequence which is defined as the portion of the electromyogram that contains the most consecutive EMG bursts, un-interrupted by swallows or long periods of tongue movement. The median value and inter-quartile range (IQR) were calculated for each parameter. This was done for each subject and every product included in the study.

The IQR was taken as an indication of the regularity in the chewing rhythm, with higher ranges indicating a great irregularity and lower ranges showing a less altered rhythmical chewing sequence from the typical value observed.

Median and IQR estimators were preferred over the more commonly used statistical estimators (mean and standard deviation) as these have the advantage of being less sensitive to extreme values. Extreme values in this case could be the product of a great number of longer cycles caused by swapping the sample from side to side, in the mouth during the mastication process. They could also be due to swallows or tongue movements carried out during mastication. A set of two repetitions were carried out for each sample.

Table 10.9 presents the values of burst and inter-burst durations for to the main confectionery products under study while Table 10.10 presents the values obtained for different geometries of Werther's Origional, along with the values for the non-confectionary products. The values correspond to the times of closing and opening of the mouth in milliseconds (median values per chewing sequence) and appear reported for each repetition carried out.

To determine whether cycle duration was modified in response to the stickiness, linear correlations between cycle duration and sensory scores of stickiness were evaluated (Table 10.11). The Pearson's correlation coefficients (r) showed that for 6 out of 10 subjects analysed the cycle durations exhibited good correlation with the sensory scores for stickiness assigned to the products in both repetitions. As seen from this table good correlations were observed for subjects 1, 3, 4, 9 and 10 with subject 7 only showing a good correlation factor for the second repetition. The rest of the subjects showed poor correlations between cycle duration values and the sensory scores of stickiness. This was greatly due to the fact that the electromyograms for products such a JT, TD and FR, for these subjects did not tend to contain long periods of consecutive EMG bursts. The chewing sequences showed lots of intermittent interruptions, taking the form of long pauses between chewing cycles. This resulted in very high median values which distorted the trend shown by other subjects. This trend was for cycle duration to

increase with stickiness. Due to this, the subjects for whom cycle duration did not show good correlations with stickiness were discarded for further analysis.

For the remaining subjects the median values of cycle duration for each repetition and product were subject to analysis (Table 10.11 and Table 10.12). A Friedman two-way ANOVA test was performed for the 10 evaluated products and 2 repetitions to find whether products were differentiable according to their cycle duration (median value) per chew. The test revealed a statistically significant difference in the cycle duration depending on the product (χ^2 (19) = 95.4, p= 3.585 × 10^{-12}). Following this, simultaneous multiple comparisons were carried out between each repetition of every product using Tukey's honestly significant difference (HSD) for ranks test. This was done to determine which pairs of products differed significantly in cycle duration per chew. The results are summarised in Table 10.11 and 10.12 and graphically represented in Figure 10.3.

Figure 10.3 shows the rank sum values for 2 repetitions of 10 products on a linear scale. The rank sums of cycle duration clearly showed an increasing trend across the products, which was similar to their increase on stickiness. For example, it can be clearly seen that products with dominant elastic character which resulted in lower values of stickiness (Chapter 7) are located to the left side of the scale, indicating short chewing cycle durations, while those with a more dominant plastic behaviour and which were found to be more sticky are located more to the right end of the scale, indicating long chewing cycle durations.

Although there was a difference between products, the Tukey HSD by ranks test indicated that this difference is only statistically significant if the distance, between two products, on the scale, is equal to or greater than the critical distance of 72.8. Therefore products embraced within one group are not significantly different from each other. Products falling in different groups show a clear statistically significant difference, at the 0.05 level, according to Tukey's test.

It can be observed that products which have been sensorially evaluated as having similar levels of stickiness (such as MCH and WO) have similar median cycle durations

and are located very close to one another on the scale. On the other hand median cycle durations are different for products which have been evaluated as having very different levels of stickiness (such as JT and WO), with the least sticky product having the shorter median cycle duration and the two products being situated in opposite extremes on the scale.

Median cycle duration for carrot (CA) a non-sticky product is significantly different from Werther's Original (WO), a highly sticky product. It can be seen on the graph that CA belongs to Group A while products with higher stickiness form Groups B, C and D.

It was interesting to note that when analysing the same product (WO) but of different geometries (WOb and WOs) the cycle duration remained similar (see Tables 10.11 and 10.12). As it is assumed that stickiness is not affected by sample geometry this indicates that cycle duration may indeed be a good descriptor of stickiness.

Another interesting finding which emerged from this analysis was that when a non-sticky product (CA) was introduced it produced the lowest cycle duration (588 *ms*). Moreover, when each subject is considered individually, it can be seen that for all of them CA produced the lowest cycle duration when compared to the other products, all of which showed some degree of stickiness. The results obtained for cycle duration for CA are in good agreement with Ahlgren (1966) who reported a cycle duration for CA of 580 *ms*, and with Karkazis and Kossioni (1997) who reported a cycle duration of 686 *ms*. This was lower than a soft gum product used in the same study. As pointed out by Karkazis and Kossioni it is believed that occasional difference can be attributed to variations in the method used and the sample selected.

It was also found that the IQR of the overall median cycle duration for CA (561.5-666.5) was the lowest out of all the products. It can be safely assumed that that as CA is a completely non-sticky product it does not impede regular jaw movements. A very sticky product, like WO, however would be assumed to present much more impedance to regular jaw movement. This is supported by the fact that the IQR for WO (774.5 – 1007.5) is greater than that for CA. These results highlight the irregularity, in the chewing cycle duration, caused by product stickiness.

Foster (2006) reported that masticatory frequency is higher for elastic products than it is for plastic products. The findings presented in this study support this. The chewing cycle frequency[1] for JT, a product with more dominant elastic character, is 1.70 *Hz*. This is higher than the frequency for WO, a product with more dominant plastic character, which is 1.17 *Hz*. A higher chewing cycle frequency indicates a higher frequency of jaw movements for elastic products. Foegeding (2007) suggested that adhesiveness of products can have an effect on jaw movement. The reason for the higher frequency of jaw movement in samples with dominant elastic character may be that plastic products, as discussed previously, tend to be stickier than elastic ones and so impede jaw movements more.

To conclude, it is clear that chewing cycle duration could be a good descriptor of stickiness in food samples. It is also clear, from the results presented, that the EMG technique is not immune to variations between subjects. This is widely reported in the literature. More study is required to understand the effect of inter-subject variation on EMG results. Standard methodologies need to be derived which minimise the effect of inter-subject variation on the results obtained. In this study great care was taken in the selection of appropriate subjects for inclusion in the study. However, it is obvious from the results obtained that the selection method needs further refinement in order to make the EMG technique more suitable for studies of food texture.

[1] Chewing cycle frequency was estimated as (1/overall median cycle duration in seconds)

TABLE 10.9 Burst duration (BD) and inter-burst duration (IBD) for main confectionery products under study

SUBJECT		JT				FR				TD				FRUT				MCH				WO			
		BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2
SM1	Median	329	311	294	243	295	311	276	291	310	325	331	248	411	462	223	201	486	485	229	230	498	545	253	229
	IQR	273	278	262	219	279	273	205	233	283	285	239	201	328	404	123	140	325	326	162	161	371	400	197	171
		350	339	395	335	329	341	421	311	358	374	353	286	517	529	325	267	521	522	335	336	615	619	345	323
SM2	Median	294	292	427	418	300	253	465	471	256	326	443	453	278	255	452	433	288	257	444	403	312	253	517	461
	IQR	228	235	363	377	250	234	368	337	209	238	321	372	259	227	395	384	247	226	399	356	237	219	444	388
		408	330	464	691	374	287	533	742	311	378	508	657	326	308	500	488	340	283	529	474	370	322	656	547
SF3	Median	247	230	407	443	250	229	469	453	259	255	509	471	285	286	503	605	294	293	595	594	278	269	559	633
	IQR	225	174	354	343	201	197	375	376	226	224	425	383	237	227	405	431	253	254	506	507	255	230	454	511
		267	263	567	536	275	272	578	492	280	263	606	552	333	308	739	729	345	346	716	715	350	333	661	741
SF4	Median	232	207	385	394	216	228	513	403	184	199	472	475	253	254	553	554	219	218	540	539	230	231	571	572
	IQR	228	188	324	357	177	193	440	323	175	164	415	406	213	214	456	457	186	185	443	444	183	184	480	481
		284	241	448	422	277	263	546	563	236	248	546	646	293	294	631	630	292	291	605	604	290	289	760	759
SF5	Median	332	314	435	399	352	341	525	453	325	305	472	439	331	295	506	435	296	319	458	465	322	309	658	583
	IQR	268	277	286	273	253	298	372	375	281	247	361	363	281	258	383	352	257	275	357	408	250	235	476	416
		389	391	491	627	452	468	720	657	371	349	749	584	388	322	728	561	345	374	627	614	396	363	940	773
SF6	Median	315	324	420	367	532	311	392	460	467	311	405	422	304	373	444	373	433	350	471	398	359	311	464	476
	IQR	271	271	406	320	466	263	366	419	363	265	371	358	233	297	422	346	559	299	382	329	305	279	403	409
		379	403	474	419	574	366	495	593	575	361	644	505	359	418	578	461	567	445	540	490	409	358	605	545

table continues

SUBJECT		JT				FR				TD				FRUT				MCH				WO			
		BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2
SF7	Median	425	313	571	477	534	340	596	534	347	315	618	590	420	499	548	536	503	497	546	541	430	400	634	592
	IQR	363	292	513	430	389	293	473	453	231	275	505	497	360	372	470	452	432	433	470	460	364	322	539	512
		462	351	670	519	588	369	677	632	381	377	712	668	509	597	736	631	679	669	758	645	564	465	857	717
SF8	Median	362	284	453	737	262	262	688	541	293	299	551	575	345	279	592	502	283	301	616	502	312	417	646	450
	IQR	294	208	411	469	229	228	478	476	243	244	482	439	267	242	487	392	233	262	531	436	250	315	563	374
		514	366	702	1291	311	317	941	675	347	329	760	991	434	343	680	593	367	358	744	639	373	487	780	673
SF9	Median	439	400	498	384	339	340	423	422	382	382	508	465	351	369	582	477	489	399	519	516	421	409	607	567
	IQR	359	366	403	339	309	310	379	380	355	355	410	406	280	315	490	417	417	347	465	431	333	353	528	466
		510	429	555	446	457	456	627	628	435	428	550	513	401	429	741	644	529	424	649	649	454	496	736	683
SF10	Median	192	230	373	365	179	208	488	358	244	225	405	407	256	255	478	449	306	275	473	464	275	265	583	521
	IQR	172	202	340	308	131	173	345	310	195	193	331	318	213	210	382	387	257	221	395	411	191	221	452	474
		221	283	427	392	222	244	652	401	271	264	493	511	283	281	595	493	367	360	545	543	320	318	707	613

SM=Subject Male, *SF*= Subject Female, ■ BD1= Burst duration (first repetition), ■BD2= Burst duration (second repetition), ■IBD1= Inter-burst duration (first repetition), ■IBD2= Inter-burst duration (second repetition).

JT= Thorntons' Fruit Jelly; **FR=** Fry's Turkish Delight; **TD=** M&S Turkish Delight; **FRUT**= Fruit- tella; **MCH-** Milk Chews; **WO**= Werther's Original

The values reported correspond to the median value of the burst and inter-burst duration per chewing sequence and inter-quartile range (IQR) corresponding to lower quartile, 25% (top value) and upper quartile, 75% (bottom value).

TABLE 10.10 Burst duration (BD) and inter-burst duration (IBD) for different geometries of a highly sticky product (Werther's Original) and non-confectionery products

SUBJECT		WOb				WOs				CA				WB			
		BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2
SM1	Median	360	560	350	193	426	465	342	239	269	286	183	186	457	391	165	228
	IQR	317	430	288	106	333	360	308	181	254	265	169	167	391	325	120	156
		527	610	468	277	495	518	415	312	290	311	240	208	493	498	251	404
SM2	Median	268	253	440	442	263	260	460	481	373	338	337	299	357	358	331	336
	IQR	249	217	374	369	225	205	401	428	323	285	280	258	324	289	257	280
		316	296	492	554	325	312	604	623	434	396	368	338	423	465	380	363
SF3	Median	273	274	691	531	290	296	581	576	271	283	350	340	261	282	368	346
	IQR	223	234	481	414	236	255	429	468	231	253	290	307	234	252	303	305
		322	306	875	695	343	332	884	787	310	299	388	434	278	293	419	379
SF4	Median	246	265	536	585	238	296	481	518	214	217	444	303	330	294	416	368
	IQR	217	205	441	513	214	222	431	431	154	183	387	258	258	234	382	337
		337	336	651	669	300	329	546	715	296	258	474	350	388	361	449	398
SF5	Median	308	297	647	540	308	261	555	535	448	410	324	250	359	327	386	304
	IQR	246	213	472	372	264	194	428	333	398	341	245	208	323	289	303	267
		353	426	109	104	367	346	672	777	512	482	355	288	531	389	453	384
SF6	Median	347	323	470	482	333	309	462	437	356	366	357	319	415	471	342	356
	IQR	317	290	357	403	279	291	416	379	294	296	319	250	358	342	277	312
		382	365	528	553	384	388	562	509	417	414	426	344	485	471	612	418

table continues

SUBJECT		WOb				WOs				CA				WB			
		BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2	BD 1	BD 2	IBD 1	IBD 2
SF7	Median	432	379	681	599	382	420	703	564	368	431	323	242	417	362	397	391
	IQR	367	326	551	516	281	399	605	511	337	354	252	119	351	332	357	340
		592	455	814	708	440	524	959	700	422	487	370	314	461	439	462	509
SF8	Median	300	311	569	512	350	288	525	594	246	222	467	421	321	291	396	437
	IQR	251	268	500	397	301	242	420	410	210	180	388	335	277	223	343	339
		363	363	726	634	408	421	661	742	311	297	545	516	380	327	476	624
SF9	Median	383	384	600	601	380	404	544	579	313	303	268	293	382	406	317	328
	IQR	356	357	533	532	274	348	510	459	297	278	240	267	351	353	278	317
		416	415	697	698	442	452	627	665	360	329	311	353	414	449	419	489
SF10	Median	258	287	541	478	269	232	644	562	300	272	294	242	305	294	295	351
	IQR	202	219	464	414	216	193	474	446	226	241	263	213	247	253	256	288
		304	344	693	600	341	293	756	701	323	295	329	272	360	328	391	377

SM=Subject Male, *SF*= Subject Female, ■ BD1= Burst duration (first repetition), ■BD2= Burst duration (second repetition), ■IBD1= Inter-burst duration (first repetition), ■IBD2= Inter-burst duration (second repetition)
WOb= Werther's Original (bigger diameter); **WOs**= Werther's Original (smaller diameter); **CA**= Carrot and **WB**= Weetabix cereal
The values reported correspond to the median value of the burst and inter-burst duration per chewing sequence and inter-quartile range (IQR) corresponding to lower quartile, 25% (top value) and upper quartile, 75% (bottom value)

TABLE 10.11 Cycle Duration (CD) for confectionery products under study

SUBJECT		JT		FR		TD		FRUT		MCH		WO		*r*	
		CD 1	CD2	CD1	CD2	CD1	CD2	CD1	CD2	CD1	CD2	CD1	CD2	1	2
SM1	Median	601	580	513	579	636	547	659	633	732	733	730	763	0.918**	0.811
	IQR	574	516	495	496	621	518	584	578	574	573	610	609		
		704	633	729	674	673	640	740	771	811	810	911	913		
SM2	Median	710	743	806	721	714	739	758	714	743	677	853	724	-	-
	IQR	668	606	654	656	594	685	667	614	682	593	727	613		
		773	921	855	998	775	951	821	810	815	782	1013	920		
SF3	Median	665	635	685	669	778	704	810	843	917	916	857	893	0.949**	0.889*
	IQR	614	598	632	651	706	642	712	730	820	821	764	834		
		811	754	820	709	855	807	1039	975	1015	1014	1005	1026		
SF4	Median	625	601	720	626	686	686	799	798	762	763	826	827	0.709	0.852*
	IQR	562	579	663	575	610	628	729	730	724	723	724	723		
		712	621	773	786	736	832	871	870	863	864	998	999		
SF5	Median	779	814	885	819	857	756	877	725	770	783	968	908	-	-
	IQR	623	592	754	678	682	628	721	633	658	693	831	740		
		877	957	1103	1025	1106	857	1075	898	935	992	1300	1138		
SF6	Median	750	731	933	815	870	719	808	787	901	764	851	783	-	-
	IQR	721	581	880	716	733	697	705	674	773	680	747	708		
		820	783	994	866	1077	854	895	846	1079	851	988	900		

table continues

SUBJECT		JT		FR		TD		FRUT		MCH		WO		*r*	
		CD 1	CD2	CD1	CD2	CD1	CD2	CD1	CD2	CD1	CD2	CD1	CD2	1	2
SF7	Median	979	795	1097	901	919	901	983	10211	1099	1114	1119	1017		
	IQR	941 1040	763 825	944 1167	793 940	866 1000	863 930	940 1091	909 1163	978 1321	949 1276	974 1293	896 1179	–	*0.813**
SF8	Median	834	1040	950	839	870	969	953	782	957	818	966	889		
	IQR	777 1021	826 1497	865 1196	782 952	789 1075	744 1190	833 1117	698 925	823 1074	730 986	875 1155	792 1053	–	–
SF9	Median	888	794	829	830	867	849	920	853	998	893	1008	974		
	IQR	828 1029	721 893	723 1039	722 1038	825 971	824 913	844 1074	774 1017	930 1151	816 1041	891 1187	860 1147	*0.864**	*0.891**
SF10	Median	575	577	648	564	624	638	727	692	774	762	836	801		
	IQR	538 617	544 659	593 814	515 602	585 722	552 777	659 787	643 752	691 897	690 929	748 951	752 885	*0.856**	*0.958***
RANK SUMS▲		**40**	**26**	**51**	**31.5**	**51.5**	**44**	**72.5**	**67.5**	**95.5**	**89**	**103**	**94.5**		
Overall median▲▲		645^{ab}	618^{abcd}	703^{abc}	648^{abcd}	732^{abcd}	695^{abcd}	805^{abcd}	821^{abcd}	846^{bcd}	828^{bcd}	847^{bcd}	860^{cd}		
IQR		594 762	589 707	648 817	613 748	664 796	635 820	721 955	730 923	772 956	770 972	756 1002	793 1013		

SM=Subject Male, *SF*= Subject Female, CD1= Cycle duration (first repetition), CD2= Cycle duration (second repetition)
JT= Thorntons' Fruit Jelly; **FR**= Fry's Turkish delight; **TD**= M&S Turkish Delight; **FRUT**= Fruit-tella; **MCH**= Milk Chew; **WO**= Werther's Original
The values reported correspond to the median value of the cycle duration per chewing sequence and Inter-quartile range (IQR) corresponding to lower quartile, 25% (top value) and upper quartile, 75% (bottom value).
r = Pearson's correlation coefficient of cycle duration with sensory scores of stickiness (*correlation significant at the 0.05 level, **correlation significant at the 0.01 level, 2-tailed).
▲Ranks adjusted for ties method. Critical distance for HSD Ranks= 72.8
▲▲ Overall median of chewing cycle duration obtained over 6 subjects showing good correlation with stickiness. Medians sharing the same letter in their superscript are not significantly different at the 0.05 level according to a Tukey HSD test by ranks; different superscript letters indicate a statistically significant difference at the 0.05 level

TABLE 10.12 Cycle Duration (CD) for different geometries of a highly sticky product (Werther's Original) and non-confectionery products

SUBJECT		WOb		WOs		CA		WB	
		CD 1	CD2	CD1	CD2	CD1	CD2	CD1	CD2
SM1	Median	777	715	805	721	462	472	635	681
	IQR	683	597	658	614	427	442	612	579
		921	857	919	835	478	507	668	776
SM2	Median	736	719	763	741	713	651	658	676
	IQR	644	640	675	659	653	583	618	610
		816	846	927	946	765	720	796	759
SF3	Median	984	819	882	872	611	642	628	630
	IQR	788	697	754	796	572	589	562	585
		1144	927	1102	1096	651	684	666	654
SF4	Median	843	877	768	836	656	535	726	654
	IQR	707	750	691	743	603	486	680	602
		939	964	849	1025	726	576	769	745
SF5	Median	1018	954	859	799	767	639	752	651
	IQR	765	647	733	588	674	562	658	576
		1401	1425	1087	1134	930	782	885	804
SF6	Median	829	837	817	779	695	619	746	774
	IQR	716	719	711	680	645	591	711	688
		897	908	927	892	759	733	1067	864

table continues

SUBJECT		WOb		WOs		CA		WB	
		CD 1	CD2	CD1	CD2	CD1	CD2	CD1	CD2
SF7	Median	1134	1057	1118	1021	704	632	799	792
	IQR	970	885	981	922	602	595	774	726
		1277	1155	1356	1178	774	661	902	887
SF8	Median	892	831	861	881	735	682	729	730
	IQR	786	719	783	791	678	621	639	688
		1051	1013	1019	1059	809	724	811	911
SF9	Median	975	974	920	990	606	597	729	773
	IQR	919	918	824	817	540	570	317	729
		1097	1096	1042	1105	633	644	819	870
SF10	Median	802	774	909	812	569	515	628	629
	IQR	726	714	815	702	541	491	572	603
		921	877	951	902	591	532	677	654
RANK SUMS[▲]		**112**	**89**	**101**	**95.5**	**15**	**11**	**36**	**35**
Overall median[▲▲]		909^{cd}	848^{d}	896^{bcd}	854^{cd}	609^{a}	566^{a}	681^{abc}	668^{abc}
IQR		757	732	785	770	557	531	592	603
		1018	946	997	1061	642	610	723	761

SM=Subject Male, *SF*= Subject Female, CD1and CD2= Cycle duration for first and second repetition
WOb and WOs= Werther's Original big and small diameter respectively, **CA**= Carrot, **WB**= Weetabix
Reported values in milliseconds are median values of cycle duration per chewing sequence and Inter-quartile Range (IQR) corresponding to lower quartile, 25% (top value) and upper quartile, 75% (bottom value)
[▲]Ranks adjusted for ties method. Critical distance for HSD Ranks= 72.8
[▲▲] Overall media of chewing cycle duration obtained over subjects *SM1*, *SF3*, *SF4*, *SF7*, *SF9* and *SF10*.
Medians sharing the same letter in their superscript are not significantly different at the 0.05 level according to a Tukey HSD test by ranks; different superscript letters indicate a statistically significant difference at the 0.05 level

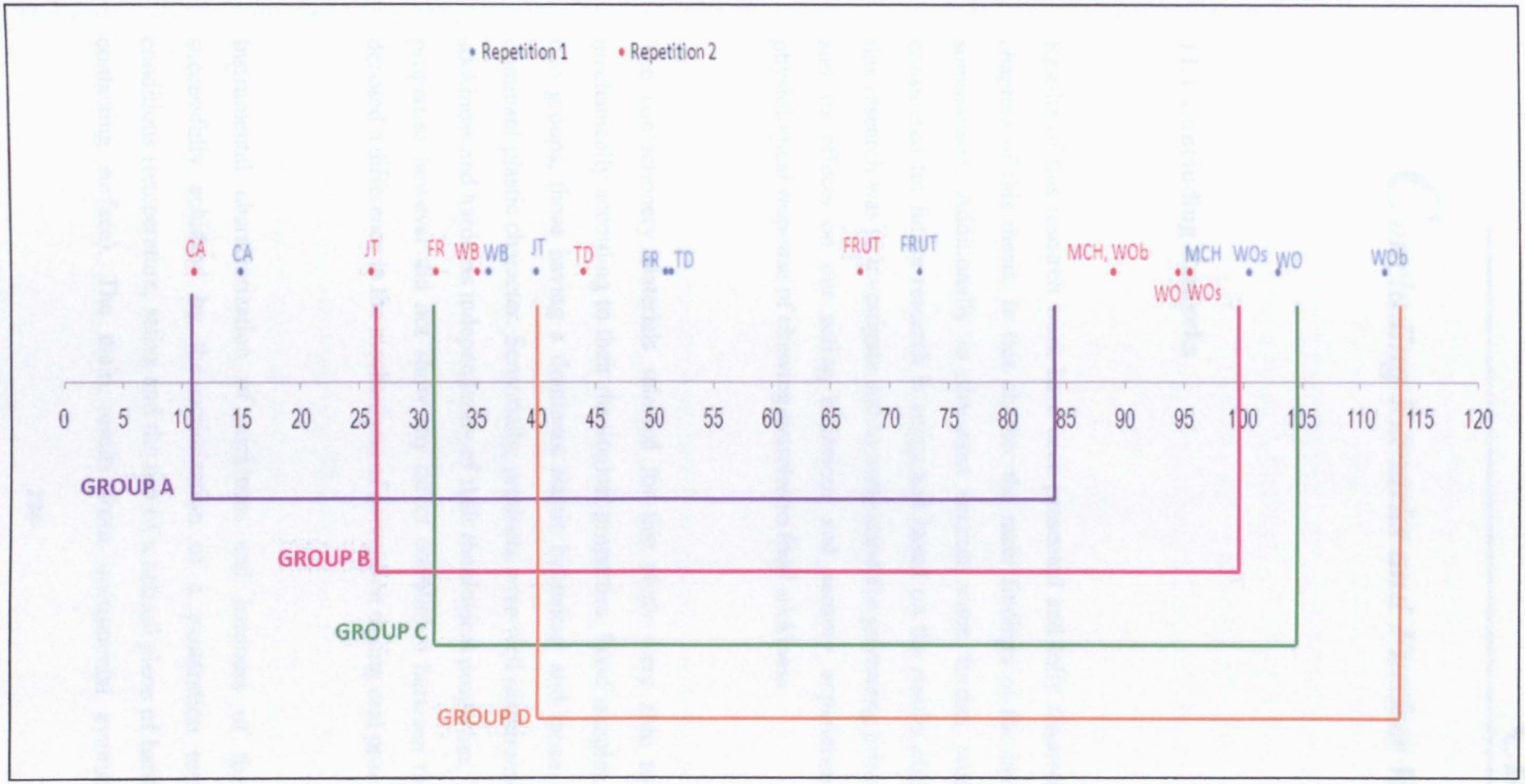


FIGURE 10.3 Multiple comparisons using Tukey's honestly significant difference (HSD) for ranks test. Products in different groups are significantly different at 0.05 level according to their cycle duration (Critical difference between ranks= 72.8) while products in the same group do not show a statistically significant difference in cycle duration.

Chapter 11

Concluding Remarks and Further Work

11.1 Concluding Remarks

Results of this research work have been presented and fully discussed in the previous chapters of this thesis. In this chapter the main findings of the investigation will be summarised. Additionally in the next section some further work that might be considered for future research is suggested based on the results obtained. The aim of this research was to investigate and to understand the governing principles of stickiness and its effects on our eating behaviour and sensory experience, particularly the physiological response of chewing muscles to food stickiness.

The confectionery materials selected for this study were able to be characterized mechanically according to their rheological properties. Food samples were divided into two groups; those having a dominant elastic behaviour and those exhibiting a more dominant plastic character. Sensorially, products were well discriminated and ranged in stickiness and hardness independently of their rheological properties. These two textural properties however did not show any direct correlation between them which clearly denoted a difference in the mechanisms of perception during oral processing.

Instrumental characterization of stickiness and hardness of food materials was successfully achieved by the optimization of a penetration test simulating oral conditions (temperature, saliva and the use of a natural piece of hard oral tissue as the contacting surface). The main results from instrumental evaluations carried out

suggested that conventional instrumental quantification of stickiness could be misleading in terms of how it relates to orosensory perception. The wet method of stickiness measurement used in this study provided a much better prediction of oral experience with good and significant correlations ($r = 0.88$ when using distilled water as wetting agent and $r = 0.86$ when using an artificial saliva solution). Two different effects on the stickiness of the samples were mainly observed 1) a decrease in stickiness by addition of the wetting agent to samples with more dominant elastic character presumably due to excessive hydration and 2) a considerable increase in stickiness for those products with dominant plastic behaviour likely as a result of the plasticization of the material.

Wetting neither improved nor made worse the characterization of material hardness. Under all tested conditions excellent linear correlations ($r > 0.9$) were obtained between measurements and sensory scores. This re-enforces the fact that hardness is an attribute evaluated right at the beginning of the chewing sequence when the sample has barely suffered any significant change in its structure or texture.

Although the use of body temperature (37°C) helped in producing good results, the time for which the samples are exposed to this temperature must be carefully controlled in tests in order to adequately mimic oral conditions of food processing. Residences times of food during normal eating processes are relatively short. Thus, care must be taken to reproduce such times if meaningful data is to be obtained for prediction of the textural properties as experienced by consumers.

It was shown that food stickiness significantly affects the chewing patterns, influencing not only muscular activities of opening muscles but also those of closing muscles. Correlations found with sensory scores of stickiness for both muscular activities were good and significant ($r \geq 0.9$). The muscular activity of the digastric muscle had much poorer and not significant correlation with food hardness. Likewise, the muscle activity of closing muscles was poorly correlated with hardness contrarily to general reports in

the literature. It is considered that hardness is a property normally lost in the first couple of chewing cycles and thus it is less likely to be described well in terms of masticatory parameters extracted from the whole chewing sequence.

In the light of the present findings it is strongly believed that muscle activity of closing muscles throughout the chewing sequence is more related to the toughness and stickiness of the food. For instance, tough materials are described as hard to cut off which emphasises their high degree of cohesiveness. Cohesiveness is also an important factor in determining material stickiness. A product that adheres to the teeth but it is in addition more cohesive would be certainly perceived as more sticky. Greater input of closing muscles, applying shearing force is necessary to attempt the detachment of the product from the contacted surface. Findings from this work confirm that human oral physiology responds closely to changing food properties and that physiological parameters can be used as an effective objective tool to correlate oral behaviour with textural properties of food. However the fact that EMG is a physiological technique means that the results obtained can be affected by variation between individuals.

Great irregularity in the chewing pattern when consuming sticky foods was confirmed in this study by looking at the variability of chewing rhythm (or cycle duration) exhibited. Longer cycle times and great inter-quartile ranges were obtained for sticky foods compared to those products of low stickiness. This outcome of the present work is only applicable and valid for the presented test conditions: healthy young adults presenting no symptoms of masticatory dysfunction, pain during eating or wearing dental prostheses. However the result suggests that such irregularity would certainly represent a great challenge for people with mastication and swallowing difficulties as greater control of muscle input is clearly required for sticky products. This could be one of the reasons why elderly people tend to dislike products of great stickiness.

Inter-individual variation for all physiological parameters was clearly observed but also expected. However for the majority of the subjects the trends observed were similar and

correlated well with sensory perception. The need to develop a refined method of subject selection was highlighted.

It could be inferred from the results of this work that pectin hydrocolloid could be suitable as a base material in designed foods for dysphagia sufferers, as it was shown to be the least sticky product. However, the reader must be remainded that the stickiness of products as found in this investigation was highly affected by the addition of saliva. Subjects taking part in this study are assumed to have standard flows of the oral fluid in the mouth. So these results would probably will not hold for people having impaired saliva production i.e. xerostomia.

It can be concluded that foods which are more plastic and tough are more likely to be perceived as sticky however interaction with saliva plays a crucial role. Faster relaxation rates and great deformations under small applied forces were obtained for such products. Materials of such characteristics are easy to spread and increase the contact area which will increase the feeling of stickiness.

In general all results gathered in this research indicate that oral physiology is an important aspect in the perception of food texture and therefore should not be neglected in research and textural measurements. All masticatory parameters analysed in this study showed clearly to have undergone certain degree of adaptation to food stickiness and hardness the most relevant have been highlighted and fully discussed.

EMG data was analysed using computerised methods. One of the main challenges in the method used was calculation of a threshold level, used to detect the onset and offset of EMG bursts. This level was calculated using Abbink's method which proved to be a good tool for proper estimation of onset and offset times. However it must be emphasised that this method was very susceptible to noise. The method offered much better results in recordings where the noise level was low.

11.2 Further work

The research that has been reported in this thesis has highlighted a number of topics that might be worthy for consideration for future research in order to improve upon present findings. Some suggestions in this respect may include:

- Application of a dynamic sensory methodology such as the use of the time intensity method, progressive profiling or the more recent Temporal Dominance of Sensation (TDS), that records dominant sensations for each product at different points of the mastication time. This would allow the tracking of the change of food texture through oral processing and will allow the analysis to be focused on the portion of the EMG record of most interest to the attribute being analysed.
- Conduction of penetration tests using probes with similar geometries to natural teeth but with quantifiable areas, using both stainless steel and synthetic dental enamel probe materials.
- Further study of chemical interactions of food material with saliva.
- Research of how to prepare a product with varying levels of stickiness whilst keeping other textural properties constant.
- Study of adhesion with the use of atomic force microscopy (AFM). This technique has been used in the study of biodhesion and has shown promising results in the study of biological surfaces. Due to the similarities between bioadhesion studies and food stickiness studies, this technique warrants further investigation.
- For further EMG studies it is strongly suggested that a methodology be developed which ensures the selection of subjects with similar dental status. More study is also required to determine the sources of variation in EMG patterns.

- Tracking jaw motion coupled with the use of the EMG technique could offer a deeper insight into the physiological response to stickiness. It has been shown in this study that jaw movements could provide a good descriptor of food stickiness.

APPENDIX A

Appendix for Chapters 5 and 9: Ethical Approval

Research Support
3 Cavendish Road
University of Leeds
Leeds LS2 9JT

Tel: 0113 343 4873
e-mail: j.m.blaikie@adm.leeds.ac.uk

MEEC Faculty Research Ethics Committee
University of Leeds

5 May 2010

Yadira Gonzalez
School of Food Science and Nutrition
University of Leeds

Dear Yadira

Title of study:	**Studies of Food stickiness in relation to oral processing**
Ethics Reference Number:	**MEEC 08-012**
Amendment Number:	**2: further clarification required**
Amendment Date:	**26/04/10**

The above amendment was reviewed by the MEEC Faculty Research Ethics Committee at its virtual meeting of 4th May 2010.

The following documentation was considered:

Document	*Version*	*Date*
Ethics Form V2.pdf	1	09/02/10
Photographic and Video Release Consent Form.doc	1	09/02/10
Consent Form (8).doc	1	09/02/10
Information sheet (2).doc	1	09/02/10
MEEC 08-012 Ethics application form.pdf	2	26/04/10
MEEC 08-012 Consent Form.doc	2	26/04/10
MEEC 08-012 Information sheet(Modified).doc	2	26/04/10

On the basis of the information provided, the Committee happy to approve this project.

Yours sincerely

J Blaikie

Jennifer Blaikie
Research Ethics Administrator, Research Support
On Behalf of Professor Richard Hall, Chair, MEEC FREC.

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