Oral processing of heterogeneous foods

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Abstract

Food manufacturers could potentially benefit from foods designed to influence mastication and the breakdown of food into a bolus. Mastication and the properties of the food bolus have been linked to the sensory and nutritional properties of foods. This research aimed to investigate the mastication and particle size distribution of the food bolus of heterogeneous food systems, where one food component is combined with another, with a view to indentifying parameters that influence mastication and the food bolus. A range of matrices of contrasting physical properties, which were embedded with peanut pieces of contrasting physical properties, were investigated.

Trials involved serving these heterogeneous foods to subjects standardized by volume (concluded as the most suitable serving method following an investigation of natural bite size). Subjects were asked to chew and expectorate the bolus (where the number of chews and chewing time were recorded) before the matrix of the expectorated bolus was washed away to isolate the peanut particles, and the peanut particle size distributions determined using image analysis. A Rosin-Rammler function was fitted to the cumulative distribution data of each bolus to derive peanut particle size parameters (d_{50} and broadness (b)).

Results demonstrated that in heterogeneous food systems the presence of one food component (the matrices) can alter the breakdown of another food component (the peanuts) embedded inside that matrix. The properties of the matrix influenced mastication, the rate of peanut particle size reduction, and the spread of the distribution of peanut particle size inside the matrix, but did not influence the d_{50} of the peanut particle size distribution inside the bolus. Peanut properties did not influence mastication, but influenced the d_{50} of the peanut particle size distribution, the rate of particle size reduction, and the retention of peanuts in the bolus. It is postulated that the properties of the matrices largely influence the probability teeth contact peanut particles (known as the selection function), and the properties of the peanuts largely influence particle fracture per chew (known as the breakage function).

Acknowledgements

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I feel obliged in this section of my thesis to finish up with a profound and philosophical statement. The best I could come with is a quotation by social commentator Karl Pilkington, who once said “Any problem solved is a new problem made”. This thesis is typical of any piece of science where the quest for greater knowledge and understanding will always continue.

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Experiments conducted in Chapter 4 were registered as low risk with the Massey University ethics committee. Experiments conducted in Chapter 6 (Southern A Application 08/17) and Chapters 7-10 (Southern A (Application 09/24) were approved by the Massey University ethics committee.

List of publications and presentations

Publications in international journals

Hutchings, S.C., Bronlund, J.E., Lentle, R.G., Foster, K.D., Jones, J.R., & Morgenstern, M.P. (2009). Variation of bite size with different types of food bars and implications for serving methods in mastication studies. *Food Quality and Preference*, 20, 456-460 (Appendix G).

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Conference presentations (oral and poster presentations)

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R., & Morgenstern, M.P. (2010). 'The importance of chewing in food technology'. Oral presentation, NZIFST annual conference, Auckland, New Zealand.

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R., & Morgenstern, M.P. (2010). 'Selection of subjects for mastication studies'**.** Poster presentation, Food oral processing, international conference, Leeds, UK.

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R., & Morgenstern, M.P. (2010). **'**Mastication of heterogeneous foods: peanuts inside food matrices'. Poster presentation, Food oral processing, international conference, Leeds, UK.

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R., Sun, C., & Morgenstern, M.P. (2009). 'Mastication of peanuts embedded inside different food matrices'. Oral presentation, NZIFST annual conference, Christchurch, New Zealand.

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R., Sun, C., & Morgenstern, M.P. (2009). 'Mastication of peanuts embedded inside food matrices: A pilot study'. Poster presentation, Delivery of functionality in complex food systems, international conference, Wageningen, the Netherlands.

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R., & Morgenstern, M.P. (2008). 'Variation of bite size with different types of food bars and implications for serving methods in mastication studies'. Oral presentation, Biomouth symposium 2008, Rotorua, New Zealand.

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Chapter 1 : Introduction

Mastication is the process of breaking down food particles into a food bolus suitable for swallowing (Woda et al., 2006a). The food bolus is deemed safe to swallow once it reaches certain criteria in terms of particle size, lubrication, and cohesion (Hutchings & Lillford, 1988; Engelen et al., 2005b; Chen, 2009). It is an instinctive behaviour common to all *Homo sapiens,* which provides satisfaction during the consumption of food. By reducing the particle size of foods up to three orders of magnitude, mastication allows the high energy requirements of the human body to be met (Lucas, 2004).

Mastication is a complex process as food variables and human variables interact simultaneously. This process is summarised in Figure 1-1. The properties of food have been widely shown to influence mastication (Hiiemae et al., 1996; Hiiemae & Palmer, 1999) and the properties of the swallowed food bolus (Hoebler et al., 2000; Jalabert-Malbos et al., 2007). For instance the toughness (Proschel & Hoffman, 1988), hardness (Peyron et al., 2002), rheological properties (Foster et al., 2006) and overall size on ingestion (Lucas & Luke, 1984) of foods are known to influence chewing behaviour. Human factors such as dentition, age, and gender have also been widely shown to influence mastication (Peyron et al., 2004a; Mishellany-Dutour et al., 2008), and the food bolus (Jiffry, 1983; Fontijn-Tekamp et al., 2004b).

The food industry is interested in the mastication process because chewing behaviour and the particle size distribution of the food bolus has been related to the glycemic response (Read et al., 1986; Suzuki et al., 2005; Ranawana et al., 2010a), texture perception (Brown et al., 1994; Brown & Braxton, 2000), and the extent of flavour release (Taylor, 1996; Alfonso et al., 2002). Consequently, an opportunity exists for food manufacturer's to take advantage of the chewing process for improving the sensory and nutritional benefits in manufactured foods. In particular, the opportunity presents itself in heterogeneous foods, where more than one type of food is combined with another (such as a muesli bar containing peanuts, oats, and raisins). The bulk of material that is eaten is heterogeneous in composition, and most manufactured foods are heterogeneous. However, very little work has been undertaken investigating

mastication and the resulting food bolus of such foods. It is possible that the different food components of heterogeneous foods interact affecting mastication and the formation of the food bolus.

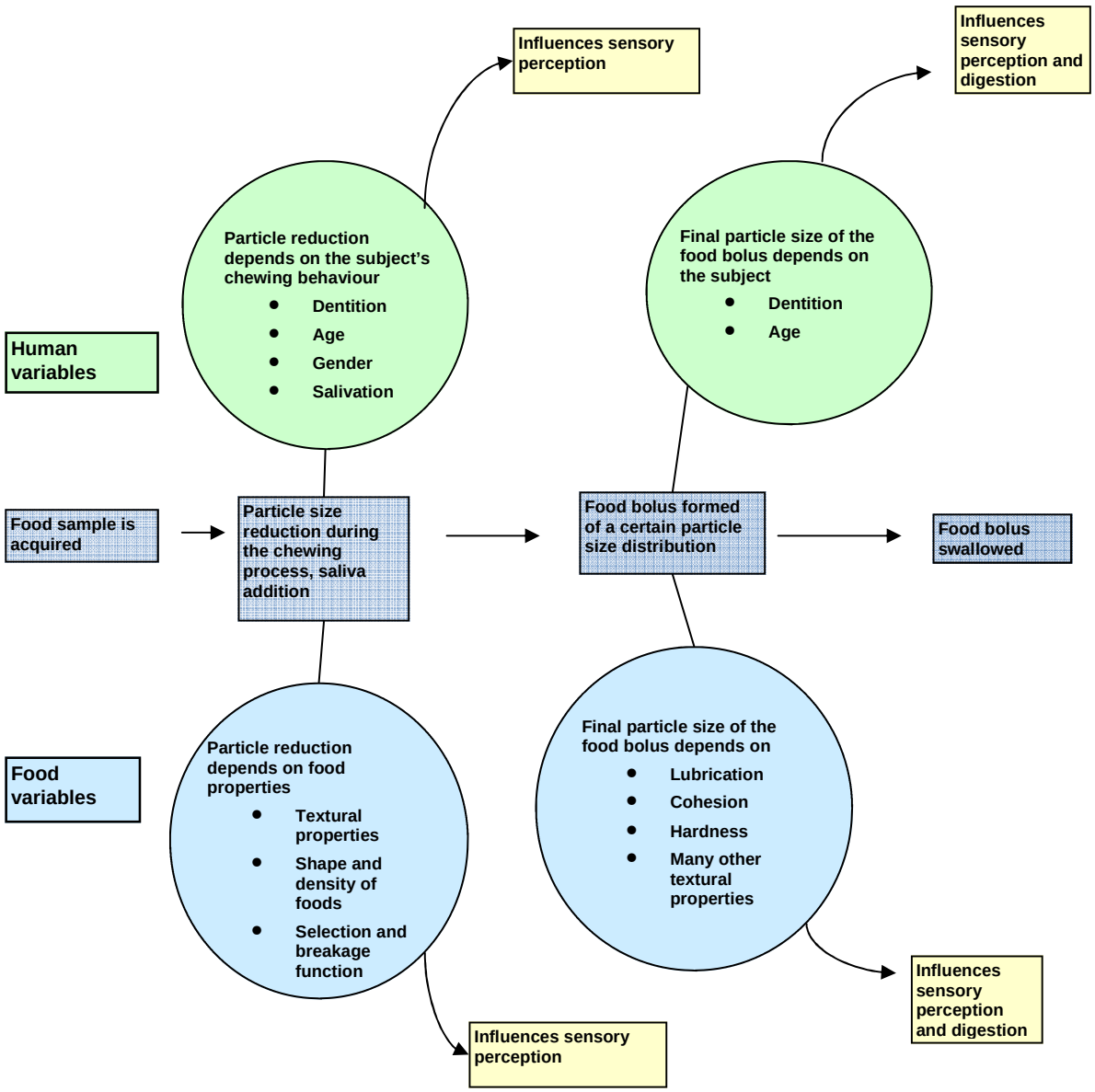

Figure 1-1: Diagrammatical representation of the chewing process and its outcomes, illustrating the influence of food and human variables.

The aim of this project was to manipulate chewing behaviour and the resulting food bolus using heterogeneous food systems. Experiments were designed using continuous food matrices (gelatine gel, chocolate, scone, and brownie) containing embedded test

pieces (peanuts), where the properties of the matrix and the properties of the internal test piece were modified.

The purpose of this work was to evaluate the effect of food structure on oral processing, rather than to explore trends in chewing behaviour among a population. Consequently, many studies in this thesis have used single subjects to function as 'chewing devices' to explore a wide range of food variables. These subjects were carefully selected to avoid participants whose oral processing characteristics were highly variable or unusual. Major findings have then been validated with a multiple subject study to complete the thesis.

The project had the following main objectives:

1. To identify the most suitable technique to standardise serving size for mastication studies.
2. To develop appropriate methods for the selection of single subjects in mastication studies.
3. To investigate the effect of matrices of contrasting physical properties on mastication, the particle breakdown process, and the particle size distribution of the bolus in heterogeneous food systems (where peanut pieces are embedded inside a continuous matrix).
4. To investigate the effect of test pieces of contrasting physical properties on mastication, the particle breakdown process, and the particle size distribution of the bolus in heterogeneous food systems (where peanut pieces are embedded inside a continuous matrix).
5. To develop a set of food design principles that can be used by food manufacturers to manipulate chewing behaviour and particle size in the food bolus. These design principles should lead to ideas for controlling digestion and the sensory appeal of food products.

Chapter 2 : Literature review

This literature review covers the basic anatomy of the mouth and the physiology of the mastication process. The influence of human variables on mastication and the food bolus is discussed, as is the influence of mastication on digestion and sensory perception. The review then focuses on the affect of food properties on mastication and the food bolus in great detail. The chapter is concluded with a summary of the current techniques used to monitor mastication and the bolus.

2.1 Purpose of mastication

Mastication is the first stage of food breakdown in the human body. Food enters the mouth, is broken down into a bolus and is swallowed (Woda et al., 2006a). Mastication is driven by a brain stem central pattern generator (CPG) which triggers a motor program that directs the actions of the entire mouth (Yamada et al., 2005). The properties of food contribute to the output of every mastication cycle (Foster et al., 2006).

It is widely known that human beings masticate to meet the high energy and nutrient requirements of the human body (Chen, 2009). In particular, chewing plays a role in the reduction of food particle size, saliva incorporation, sensory assessment, and temperature assessment, ready for transfer of the food bolus to the stomach for digestion (Health & Prinz, 1999).

According to Lucas (2004), a high energy requirement to maintain an elevated and constant body temperature is the most important reason mastication has evolved in mammals. Other vertebrates such as reptiles do not maintain high body temperatures, and do not masticate their food. By reducing food particle size mastication increases the surface area of the food and thus the rate of chemical and biochemical breakdown. Particle size is reduced by 2 to 3 orders of magnitude during chewing, and by 20 orders of magnitude in the stomach (Bourne, 2002).

Chewing is critical for breaking up food and lubricating it to be safely swallowed (Yeatman & Drake, 1973). Mastication allows food to be mixed with saliva and other fluids that exist within the food (Hutchings and Lillford, 1988). Mixing with saliva is also the first step where enzymes begin to break down foods (Yeatman & Drake, 1973).

Furthermore, the texture, taste, sound and odour of food is closely examined during chewing (Bourne, 2002). This has evolved in all mammals as a primary way to decide if a food is safe and suitable for swallowing. If food is sensed as unsuitable during mastication it will immediately be rejected (Lucas, 2004).

2.2 Anatomy and physiology of the mouth

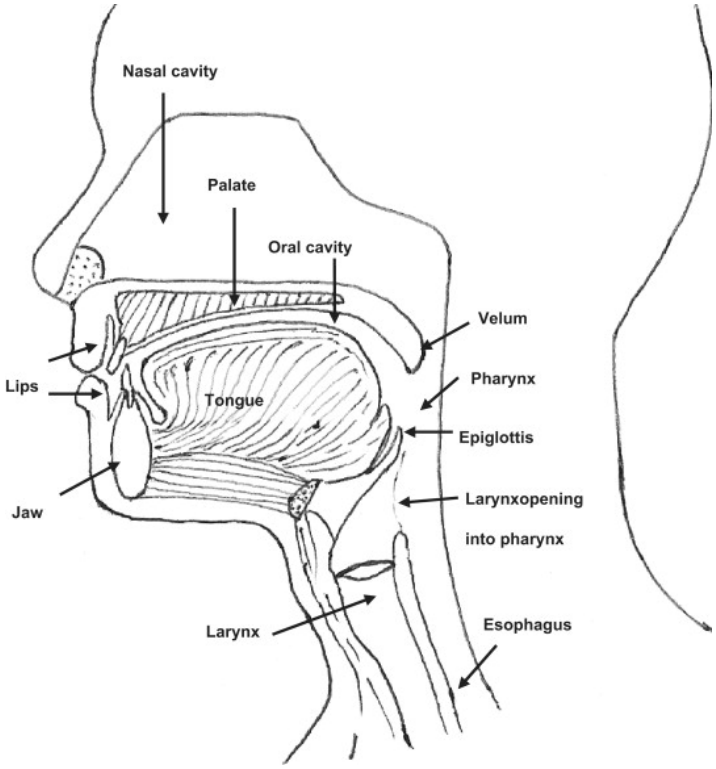

Figure 2-1: Components of the human body critical for mastication (Chen, 2007).

Mastication depends on the effective functioning of a vast number of components of the mouth, including the teeth, tongue, jaw, and lips (Figure 2-1). These are discussed in more detail as follows.

2.2.1 Teeth

Composition

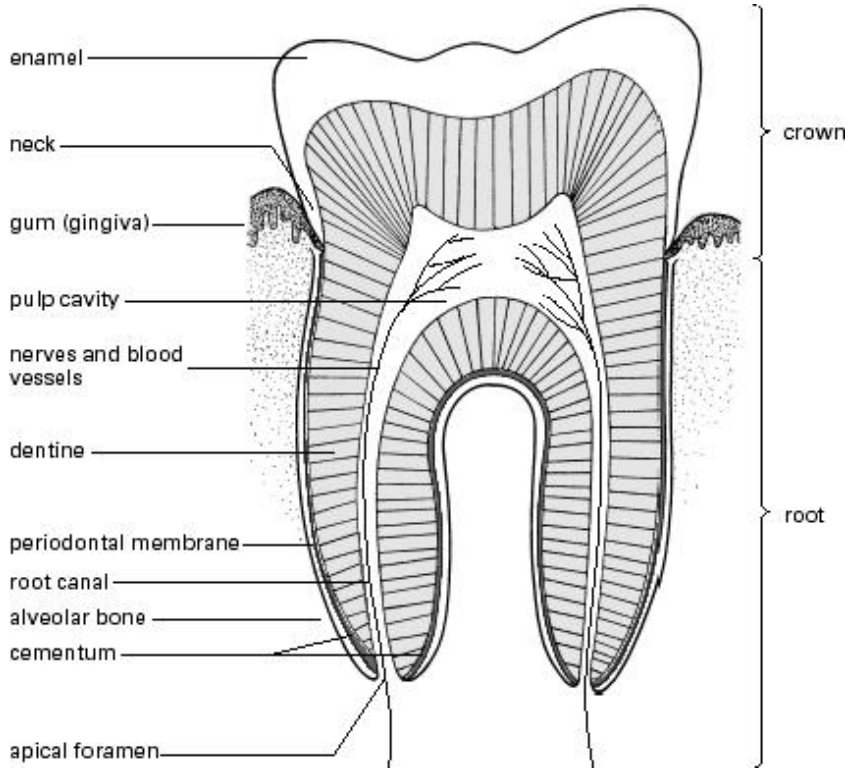

Figure 2-2: Cross section of a tooth (Oxford Reference Online, 2008).

Teeth are the main tool used during mastication for breaking down food into smaller particles for swallowing (Tortora & Grabowski, 2003). They can be divided into two sections, the crown and the root. The crown is the visible section of the tooth that sits above the gums, and the root is the section holding the teeth to the jaw bone.

Teeth are made up of contrasting materials (Figure 2-2). The upper surface of the tooth is made of enamel which makes contact with food during chewing. Enamel is the hardest material in the body. It is made up predominantly of calcium salts, and is densely packed with mineral crystals. Dentin makes up the majority of the mass of the tooth, which has similar properties to bone (Marieb, 2004).

The peridontal membrane is a layer of connective tissue between the jaw bone and cementum, which serves to support the position of the tooth. Seventy five percent of the periodontal membrane is fibrous tissue in long bundles. Within this fibrous tissue

are cells, interstitial fluid, blood vessels, lymphatics and nerves (Bourne, 2002). Periodontal receptors in the periodontal membrane provide information about the forces teeth are subjected to, and are therefore critical in mastication control and in the prevention of tooth wear (Bosman et al., 2004; Lucas, 2004).

Pulp is the soft tissue that sits in the centre of the tooth. Inside the pulp cavity are nerves, arteries, veins, and blood vessels, which are connected to the rest of the jaw vessels through the tip of the root (MacKinnon & Morris, 2005).

Types of teeth

It is well known that every human with complete dentition has several types of teeth. A fully-grown adult has 4 incisors, 2 cuspids, 4 premolars and 6 molars attached to the lower jaw (mandible), and the same attached to the upper jaw (maxilla) (Mac Kinnon & Morris, 2005).

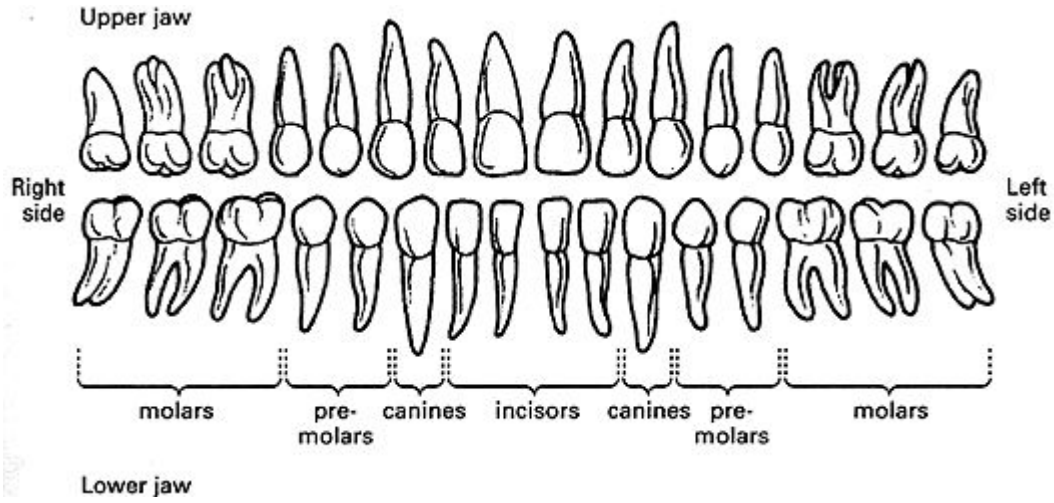

Figure 2-3: Permanent teeth of the mouth (Martin & Hine, 2008).

Figure 2-3 shows the different types of teeth in the mouth. Incisors sit at the front of the mouth, and either side of the incisors are the canines (cuspids). Further back are the premolars (bicuspids), followed by the molars.

Shape and alignment

The unique structure of each tooth is a result of their different roles during mastication and acquisition (biting). Incisors have a sharp flat edge for cutting and biting food, and canines have a pointed edge useful to tear food. The premolars are larger and have two cusps, used to crush or tear food. Molars are larger again, with several cusps to grind and crush food (Seikel et al., 2010).

The upper and lower teeth of most vertebrates apart from mammals never contact, instead only making contact with the food. However the need of mammals to reduce particle size means the molars and pre molars touch each other when the teeth are fully closed (full occlusion). It is vital for mastication that the teeth from the upper and lower jaw match up properly during full occlusion. To achieve this, teeth sit in sockets that slowly shift the teeth into the correct alignment. These sockets exist as part of the jaw bone and are connected to the teeth via the periodontal ligament (Lucas, 2004).

2.2.2 Jaw bones

The jaw comprises two jawbones that contain the teeth, the maxilla and mandible. The upper jawbone is the maxilla. It is mostly immobile acting as a hard surface that the lower jaw can crush food against (Rohrle & Pullan, 2007). The lower jaw bone is a single fused bone called the mandible and is the bone which is in motion during chewing (Tortora & Grabowski, 2003).

2.2.3 Muscles

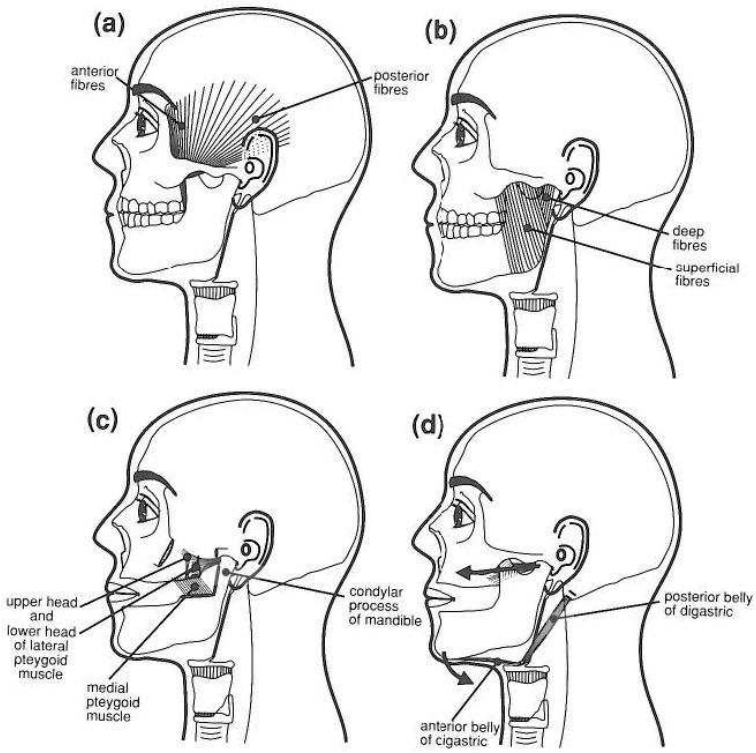

Figure 2-4: Orientation of the main muscles involved in mastication (Lucas, 2004) (a) Masseter (b) Temporalis (c) Medial and Lateral pterygoid (d) Digastric.

The action of muscles have evolved to maximise efficiency and minimise forces on the jaw joint (Crompton, 1963). Mastication depends on the activity of large muscles to move the jaw (Figure 2-4). The jaw operates in a complex muscle system where five different movements are possible. The muscles of mastication, regarded as some of the strongest in the body, operate collaboratively for effective breakdown of food. The major jaw muscles are the massetor, temporalis, and medial pterygoid (closing muscles), lateral pterygoid (for protrusion), and digatricus, mylohyoid, geniohyoid, and platysma (opening muscles) (Seikel et al., 2010).

2.2.4 Temporomandibular joint

The temporomandibular joint (TMJ) is a specialised joint that links the mandible and the temporal bone, and allows the mandible to move. The mandible and temporal bone are connected by an articular disc (cartilage) and an articular capsule (joint capsule).

The TMJ allows the jaw to open, close, and move forwards, backwards, and side to side. During normal chewing it operates as a hinge joint where mandibular movements are in a vertical direction. When the jaw is opened wide or shifted forward the mandible and articular disc shifts to a second compartment (Boyar & Kilast, 1986; Tortora & Grabowski, 2003).

2.2.5 Tongue

The tongue is a complex muscle occupying most of the oral cavity. During mastication it assists in controlling the position of food between the teeth, mixes food with saliva, and sorts out unsuitable particles (Hiiemae et al., 2002). The tongue also contains specific cells to detect the five basic tastes (sweet, salty, bitter, sour, and unami) (Chandrasheka et al., 2006), and contains receptors which can discriminate the presence of separate particles down to 2 mm apart (Ringel & Ewanoski, 1965).

Movement of the tongue is driven by various muscles. Changes in shape (such as extension, contraction, and rotation) are controlled by intrinsic muscles, whereas changes in overall position are controlled by movement of the hyoid and extrinsic muscles (Hiiemae et al., 2002).

The tongue places and maintains food between the teeth (with the assistance of the buccinator (a cheek) muscle), and sorts out particles which are ready for bolus formation from particles which require further breakdown. Particles which require further chewing head to the centre of the tongue, and those which are small enough head to the lateral areas of the tongue, before being shifted to the back of the oral cavity. During mastication the tongue also makes side to side churning movements to incorporate saliva and mucus, and the tip is raised and placed against the hard palate for swallowing to take place (Abd-El-Malek, 1955).

2.2.6 Salivary glands and saliva

Saliva is a fluid comprised of approximately 98% water, as well as electrolytes, mucins, proteins, glycoproteins, and enzymes. It has a role to play in lubrication, buffering, maintenance of tooth integrity, antibacterial protection, taste, and digestion (Humphery & Williamson, 2001; Chen, 2009). On average, humans will produce 600 mL of saliva per day. Flow rates are highest during mastication and lowest during sleep (Edgar & O'Mullane, 2006). The pH of saliva can range from 5.3 during low flow to 7.8 in peak flow (Humphery & Williamson, 2001).

Salivary glands produce saliva in the mouth whenever food is chewed. The three main pairs of salivary glands are: The sublingual gland below the tongue, the submandibular gland behind the jaw at the floor of the mouth, and the parotid gland below the ear (Edgar & O'Mullane, 1996).

When the salivary glands are unstimulated saliva production is reported to be around 7% from the sublingual, 65% from the submandibular, 20% from the parotid, and around 8% from the minor glands. However flow rates change when the salivary glands are stimulated (i.e. during chewing), where the parotid contributes more than 50% of total secretions (Humphrey & Williamson, 2001).

The excretion of saliva is predominantly achieved by the stimulation of nerve endings in the periodontal membrane as a result of movement during chewing and the presence of food against the mucosa and the teeth. Taste and olfactory signals also play a major role. Stimulated saliva is believed to contribute 80-90% of average daily production (Humphery & Williamson, 2001; Edgar & O'Mullane, 2006).

2.2.7 Other features of the mouth involved in mastication

The hard and soft palate, oral mucosa, lips, cheeks, gums, and pharynx all assist mastication. The hard and soft palate separates the upper respiratory tract from the mouth to allow simultaneous eating and breathing. The hard palate is a bony piece of the mouth covered by mucosa, whereas the soft palate is a fold of the mucous

membrane that contains muscles, blood vessels, nerves, and mucous glands (Mac Kinnon & Morris, 2005; Seikel et al., 2010).

The oral mucosa (or mucous membrane) is the outer layer that lines the mouth like a thin skin. It is made up of an outer layer of epithelium that contains glands which secrete mucus, and an inner layer of connective tissue and muscle (Mac Kinnon & Morris, 2005; Seikel et al., 2010). The oral mucosa has been proposed as the main site for detecting food particles (Prinz & Lucas, 1995).

Lips move to allow the entry of food into the mouth and their sensory receptors accurately sense food texture and temperature. Cheeks ensure that food stays between the teeth for each bite, and the gums surround and anchor the teeth. The pharynx, a space linking the oral and nasal cavities to the oesophagus and thus down to the stomach, also plays a role in mastication. It pushes the food bolus down to the oesophagus via constrictor muscles (Bourne, 2002).

2.3 The mastication process

2.3.1 The overall process

The overall mastication process can be divided into five steps from initial entry of the food until emptying of the mouth:

1. Acquisition

A unit of food, usually a ‘mouthful’, is deposited in the oral cavity on the tongue (Bourne, 2002).

2. Stage 1 transport

If the food is assessed as suitable, it is transported from the incisors to the cuspids, bicuspids, and then molars. Some foods will be punctured and torn using the incisors, canines, and premolars before transport to the molars (Thexton & Hiiemae, 1997; Hiiemae, 2004).

3. Chewing and compression against the hard palate

Reduction of particle size takes place using the molars to masticate the food. Compressing some foods against the hard palate using the tongue also achieves a smaller food particle size (Thexton & Hiiemae, 1997; Hiiemae, 2004).

4. Stage 2 transport

Masticated food is shifted from the mouth into the oropharynx, an area further back in the mouth below the junction of the hard and soft palate. In this region, the masticated food and saliva are mixed to form a food bolus. The tongue and teeth contribute to the formation of the bolus by mixing. The formation of the bolus usually takes place at the same time as particle size reduction in step 3, because in a given ‘mouthful’ that is being processed, some food particles are small enough to begin to form a bolus, while

others require further chewing. This can result in several swallows for one 'mouthful' of food (Thexton & Hiiemae, 1997; Hiiemae, 2004).

5. Swallowing

Swallowing is undertaken by transporting the bolus from the oropharynx to the oesophagus. Food that is not ready for swallowing is retained in the mouth. Chewing, mixing and swallowing will continue until the mouth is empty and ready to take in a new piece of food (Thexton & Hiiemae, 1997; Hiiemae, 2004).

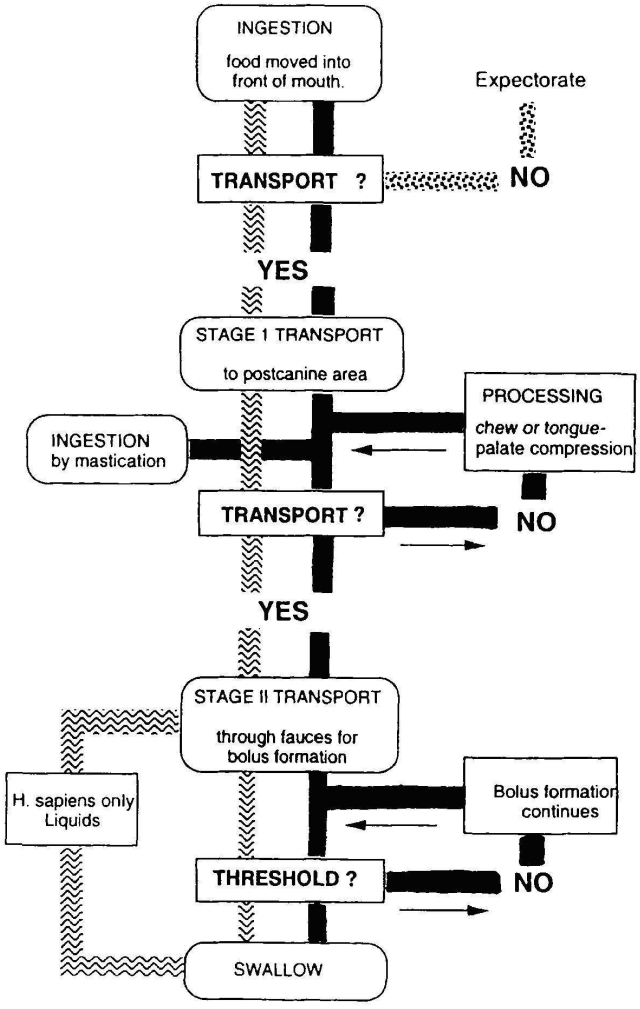

Figure 2-5: A model for the process of feeding (Hiiemae, 2004).

Hiiemae (2004) presented a model summarising the various sensorimotor gates food passes through before swallowing (Figure 2-5). Liquids follow a different path to solids (liquids follow the path with the wavy fill, solids with the black fill).

2.3.2 Acquisition

Acquisition is the process of taking a bite of food, where a unit of food is deposited on the oral cavity of the tongue using the incisors, the tongue, or the lips. Once food is acquired it is assessed by sensory organs where it is either rejected or completely ingested (Thexton & Hiiemae, 1997; Bourne, 2002; Hiiemae, 2004).

In terms of solid foods, the bite is conducted by forcible occlusion of the opposing edges of the upper and lower incisors (Okada et al., 2007). The speed of biting, in particular during late phase contact with the food, depends on food toughness (Ang et al., 2006). The force applied during biting is likely to provide an initial evaluation of the work required during subsequent mastication (Ang et al., 2006), and is useful in evaluating the hardness (Brandt et al.,1963; Boyd & Sherman, 1975; Vickers & Christensen, 1980) and the thickness of food (Peyron et al., 1997).

2.3.3 The chewing cycle

The chewing cycle involves movements of the jaw that are not simple unilateral movements. The speed and trajectory of movement is highly variable and depends on the stage of the chewing cycle, the type of food and the individual (Bourne, 2002; Lucas, 2004).

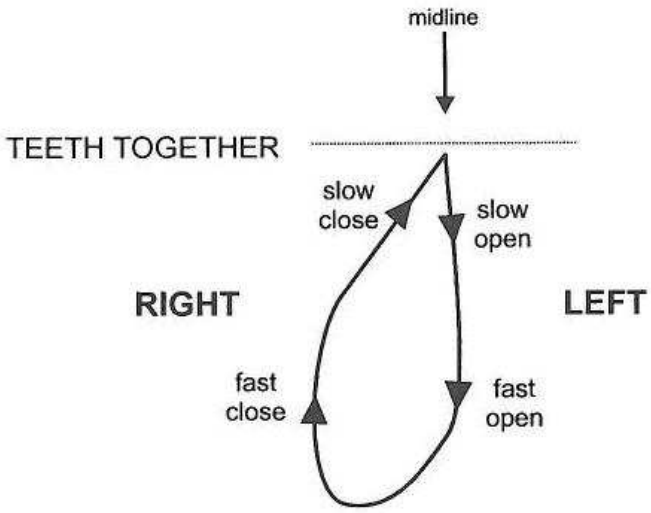

Figure 2-6: The trajectory (viewed from the front) of a point on the mandible (such as an incisor) during a typical chewing cycle (Lucas, 2004).

Figure 2-6 shows a common path that the teeth would follow during a chewing cycle. Fast and slow phases and the differing path from opening to closing are found in all cycles (Lucas, 2004).

The beginning of the closing phase is the fast close phase, where jaw movement is free until the teeth contact the food (Hiiemae, 2004). Once tooth-food-tooth (*tft*) contact occurs, the slow close phase begins. Resistance of the food slows the jaw and the masseter, medial pterygoid and temporalis muscles become active as the food is compressed and sheared. This phase may also be slow to assist texture perception (Lucas, 2004).

The jaw moves laterally to one side during the early closing period to fracture particles usually on one side of the mouth (Lucas, 2004) (in Figure 2-6 the food is on the left side of the mouth when viewed from the front). The pathway is more centred during the later closing stages. As time passes for a particular chewing sequence, the amplitude at *tft* contact decreases (Hiiemae, 2004).

At the end of closing phase is the intercuspal phase, known as full occlusion, followed by the beginning of the opening phase (Hiiemae, 2004). The most important functions of the opening phase are achieved by the tongue (Lucas, 2004). During slow jaw

opening the tongue moves forward to catch falling food, mixes food with saliva, and presses it against the hard palate for sensory analysis. Depending on the state of the food after sensory analysis, it may be returned between the teeth or undergo stage 2 transport (Figure 2-5). The cheeks also play a role in returning food between the teeth during the chewing cycle, as particles which fall too far laterally will be reflected back onto the teeth by the contraction of the buccinator (cheek muscle) (Lucas, 2004).

2.3.4 Control of mastication

Mastication is essentially a sensory-motor activity, where the central pattern generator (CPG) and the cerebral cortex of the brain (the higher part of the brain) combine to produce the chewing output (Yamada et al., 2005) which is modified by sensory input.

The CPG is located below the cerebral cortex, composed of a group of cells that excite and inhibit closer and opener motor neurons, to generate the fundamental rhythm of mastication (Dellow & Lund, 1971). This basic motor command is similar to respiration. The role of the CPG can be divided into 3 processes: the generation of chewing rhythm, the generation of a pattern of activities of the jaw, tongue, and face, and the coordination of the action of these muscles (Nakamura & Katakura, 1995).

As physical properties differ between foods, and the physical properties of the bolus also change during a mastication sequence, chewing behaviour must be able to adjust to changing conditions detected by sensory receptors inside the mouth (Bosman et al., 2004). Consequently the inputs from the cerebral cortex and peripheral sensory inputs modulate the mastication output depending on the food and food bolus properties (Yamada et al., 2005; Foster et al., 2006). This allows the action of the mastication muscles to drive the teeth to produce force for efficient breakdown of food particles.

Research is also showing that mastication output is based on pre-programmed or anticipatory behaviour, which is also altered according to sensory feedback. van der Bilt et al. (1995) discovered that 30% of the muscle force is in anticipation, and 70% is based on sensory experience after food contact for that particular chew. Foster et al. (2006) presented a dual hypothesis for mastication control, where a cortical-brain stem

pre-programmed mechanism to change jaw movements according to food rheological properties is linked to a brain stem mechanism that controls muscle force according to food hardness.

2.3.5 The sensation of texture and particles inside the oral cavity

One of the most important sensations that affect mastication output is texture. The definition of texture is complex, however it is essentially a group of properties that derive from the structure of food, primarily sensed by the feeling of touch inside the mouth (Bourne, 2002). It is sensed by the palate (on the oral mucosa), tongue, gums, periodontal membrane, and by the muscles and tendons involved in chewing. The mouth, tongue, and lips are considered to be the second most sensitive surfaces in the body in terms of touch (the finger tips are the most sensitive). The sensation of touch inside the mouth can be divided into four major sensory functions. Discriminative touch recognizes the size, shape and surface characteristics of foods, proprioception detects the static position and movement of the jaw, nociception is the recognition of pain, and temperature-sense monitors if foods are warm or cold (Guinard & Mazzucchelli, 1996; Bosman et al., 2004).

The sensory modalities for perceiving texture can be divided into three categories: Mechanoreceptors on the exterior oral structures (tongue, hard and soft palate, gums), mechanoreceptors in the periodontal ligament below the teeth, and mechanoreceptors in the muscles spindles and tendons (Guinard & Mazzucchelli, 1996).

Single particles as small as 2 mm can be detected in the oral cavity, as shown by two-point discrimination tests (Ringel & Ewanoski, 1965; Laine & Siirila, 1971 in Lucas, 2004). Furthermore, particles as small as 8-15 μm can be discriminated by the teeth (Utz 1986, in Lucas, 2004). This fine sensitivity has evolved as a means to avoid dental wear (Lucas, 2004). Owall (1970) and Owall & Vorwerk (1974) showed fine steel balls inserted into peanut samples could be detected down to 0.69 mm during mastication.

Interestingly, the size of a particle determines size detection whereas density does not. Engelen et al. (2002) investigated ball bearings (4-11 mm in diameter) made of 4

different materials (steel, nylon, PTFE, and polypropylene) of contrasting densities. Volume of the ball bearings influenced size detection, however no differences in size detection were found between different materials of the same volume.

Moreover, particle sizes as small as 2 µm can be detected by the palate to influence textural perception by vibration or friction (Engelen et al., 2005a). The size and shape of particles has also been shown to influence perception, where harder and irregular particles are detected as larger than soft and round particles of the same size (Engelen et al., 2005b).

Imai et al. (1995) trialled micro crystalline cellulose of different particle size (6-79 µm) and concentration embedded in 5 different model systems: aqueous suspensions, low and high viscous suspensions, and soft and hard gels. The perception of grittiness was shown to increase with particle size, but decreased with concentration. The perceived grittiness in cream cheese also increases with the concentration and size of added particles (0.04-850 µm) (Sainani et al., 2004).

The medium surrounding particles has also been shown to influence perception. Imai et al. (1995) found that the perception changed between different model systems which were containing the particles. Grittiness perception reduced from the aqueous suspension to the viscous suspensions to the gels. Essentially as the viscosity of the medium increased the ability of the medium to mask the presence of the particles increased. However in contrast, viscosity of a custard dessert (3 Pa.s versus 6 Pa.s) was not shown to influence size perception of silicon dioxide and polystyrene particles (Engelen et al., 2005a). The authors suggested that greater changes in viscosity than used in their study were required to affect texture perception.

2.3.6 Particle breakdown

As mentioned larger particles are broken into smaller particles during mastication. The resulting food bolus contains particles whose size distribution is nearly always skewed at the smaller end (smaller particles exist in greater mass than relatively larger

particles). Increasing the number of chews reduces the mean and median particle size of the distribution (Lucas, 2004).

The rate of particle size reduction depends significantly on the probability of a food particle being contacted with the teeth, and the extent of particle fragmentation when the food does make contact with the teeth. The former is the selection function, and the latter is the breakage function (Voon et al., 1986; Lucas et al., 2002; Lucas, 2004).

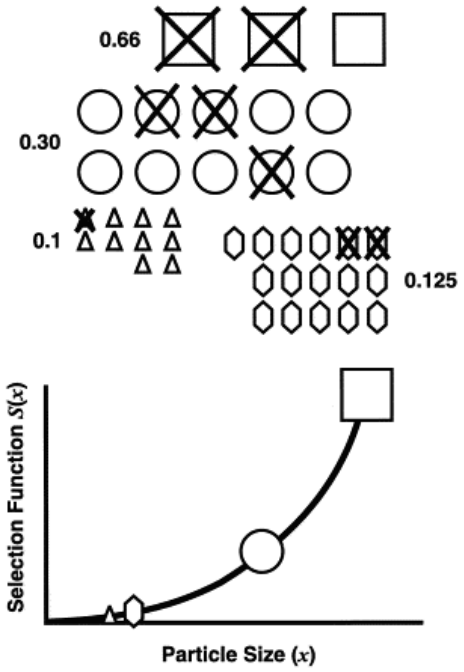

Figure 2-7: The concept of the selection function (Lucas et al., 2002).

The selection function increases exponentially with particle size (Figure 2-7) as larger particles are more likely to come into contact with teeth during a chewing stroke (i.e. they are more likely to be 'selected' for breakdown) (Lucas & Luke, 1983; van der Glas et al., 1987). The selection function also depends on the molar surface area of individuals (van der Glas et al., 1992).

Lucas & Luke (1983) calculated the selection function for different size ranges (where *x* denotes a particular size range being studied) using carrots according to Equation 2-1.

$$S(x) = 1 - (C_2 - C_1)\sqrt{\frac{P_2}{P_1}}$$ **Equation 2-1**

Particles were marked with a stain after a certain number of chews (C_1) to identify the percentage of those particles in size range x (P_1). These particles were then re-chewed, and those that remained in that size range (and hence how many have been reduced) (P_2) were determined after a certain number of chews (C_2).

van der Glas et al., (1987) also studied selection function using Opstosil particles. These particles had a specific form (cubes or half cubes), and were of seven particle colours (1.2-8.0) mm. The colour of the fragments indicated their original size.

The selection function was determined according to Equation 2-2:

$$S_{x(1)} = 1 - (1 - S_{x(N)})^{1/N}$$ **Equation 2-2**

Where $S_{x(1)}$ was the average selection chance per chew for the particle size range x that was being studied, and $S_{x(N)}$ the experimentally determined selection chance over N cycles (5 or 10 in this study). $S_{x(N)}$ was then be determined from observing the number of cubes or half cubes (of a certain size) which were fractured over 5 or 10 chewing cycles.

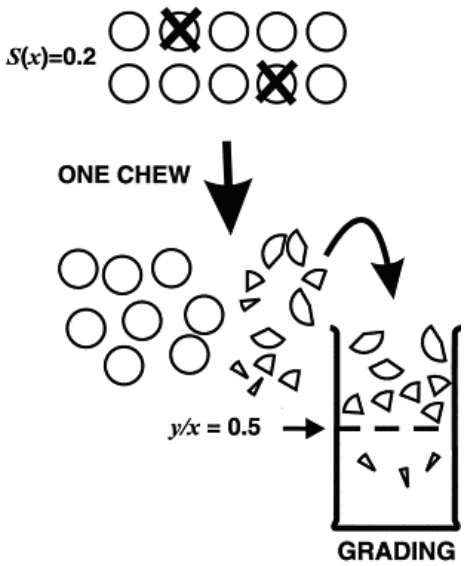

Figure 2-8: The concept of the breakage function (Lucas et al., 2002).

The breakage function quantifies the distribution of the fragments after each chew, and depends on physical properties of the food (Lucas et al., 2002). The concept is shown in Figure 2-8. A distribution is set up in reference to the mesh size *y*. As an example, if a particular set of particles are sent through a sieve with a mesh size 50% of the original fragment size then y/x=0.5. If 10% of the fragments pass through the mesh then the breakage function, $B_{(0.5)}$=0.1.

The nature of fracture during mastication is complex and is different for different foods. However Lucas et al. (2002) has described two extreme scenarios for crack initiation:

1. The first scenario involves bending a material like a 3-point bend test using the cusps. A crack forms away from the cusps as shown in Figure 2-9. In this scenario, the fragmentation is limited by the displacement that the teeth can achieve.

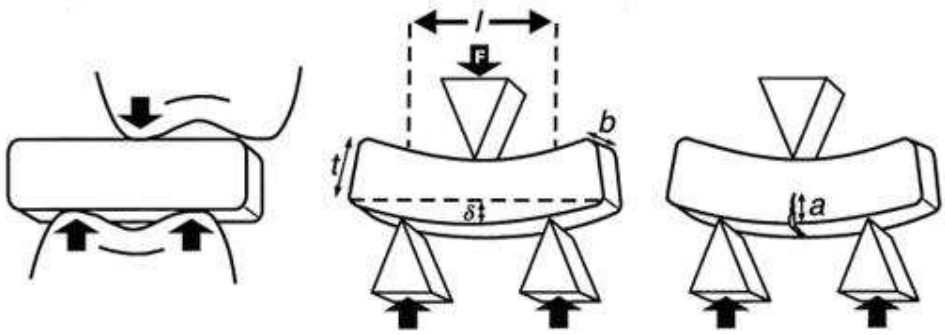

Figure 2-9: Cracking away from cusp tips (Lucas et al., 2002).

2. The second scenario is when a crack forms right next to a cusp during indentation. This is shown in Figure 2-10. In this scenario, the fragmentation is limited by the stress the cusp can apply to the food surface.

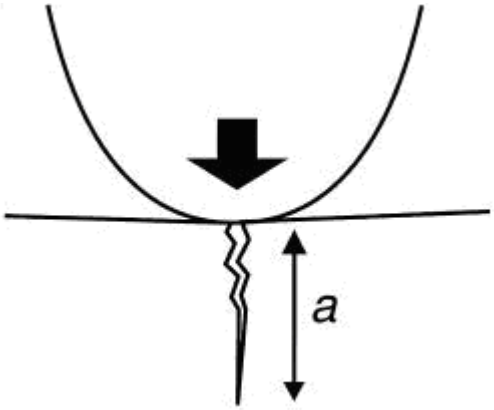

Figure 2-10: Cracking as a result of indentation (Lucas et al., 2002).

The breakage function of a particular food is usually measured by asking subjects to take one chew of the food product, and measuring the particle size distribution of the resulting fragments. This is easily achieved using image analysis (Agrawal et al., 1997).

Breakage properties are considered to be a defining property of a given food, however, the breakage properties of large particles are also different to smaller particles of the same food product (van der Glas et al., 1987). Small particles are likely to be only squeezed between tooth surfaces, medium particles cut by cusps, and when large

particles are fractured a significant portion of the particle is often not subject to breakage.

2.3.7 Dynamic changes in the food bolus during the chewing sequence

Mastication is a dynamic process, where jaw trajectories (Mioche et al. 2002b; Peyron et al., 2002; Kohyama & Mioche, 2004) (in vertical, horizontal, and lateral directions) and muscle activities (Figure 2-11) (Kohyama et al., 2002; Gonzalez et al., 2002; Peyron et al., 2002) constantly change throughout the chewing sequence in response to changes in the physical ands chemical properties of the food bolus.

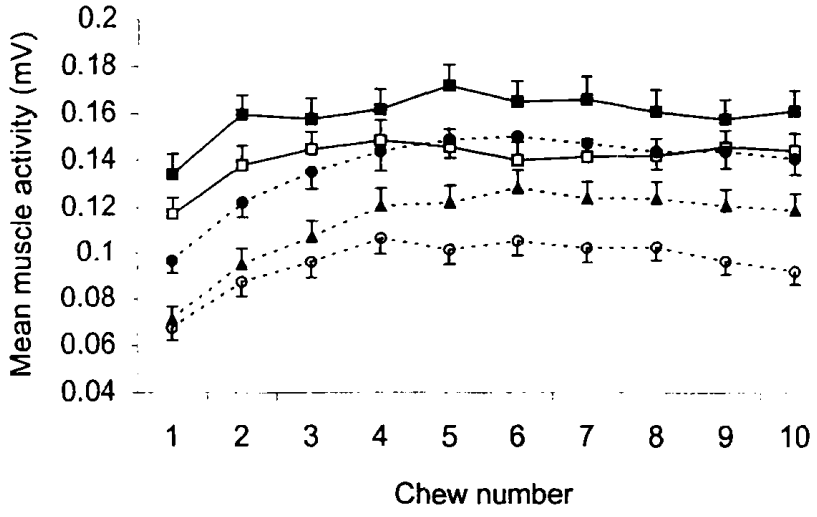

Figure 2-11: Amplitude of the mean voltage of the first 10 cycles while chewing meat and wafers (Mioche, 2004). The dotted lines are wafers while continuous lines are the meat. The tough meat is shown as black squares, and the tender meat as open squares.

The weight of the food bolus declines throughout the chewing sequence. Particle weight retention has been measured after one quarter, one half, and a full chewing sequence using peanuts, almonds, pistachio nuts, carrots, radishes, and cauliflower (Peyron et al., 2004a). The weight recovered decreased significantly between each stage. It is likely that weight decreases because particles are lost during the chewing sequence via intermediate swallows prior to the main swallow (Lucas & Luke, 1986), and also from dissolution into the saliva during the chewing sequence.

The particle size distribution changes throughout the chewing sequence. The $d_{50,}$ a measure of the sieve aperture at which 50% of the particle weight would fall between, typically decreases throughout the chewing sequence, and does so with an exponential decay. This has been shown with peanuts (Kawashima et al., 2009), brazil nuts and carrots (Lucas & Luke, 1986), and an artificial test food known as Optosil (Olhoff et al., 1984). In rare cases the d_{50} can increase later in the chewing sequence if particles agglomerate (Yven et al., 2010).

The spread of the particle size distribution, otherwise known as the broadness, is also altered during chewing. Some studies report the spread of the distribution to decrease with time (Lucas & Luke, 1986) while other studies suggest that the spread increases (Yven et al., 2010) and in some cases increases then decreases if mastication continues for a sufficient period of time (Olthoff et al., 1984). Changes in spread are likely to be dependant on the food product.

Rheological properties of the bolus are modified during mastication. The most well known example is the increase in lubrication (Hutchings & Lillford, 1988). Such increases in lubrication depend on the food, and result from free moisture in the mouth, expulsion of water and fat from inside the food during mechanical breakdown, and saliva addition.

Prinz & Lucas (1997) presented a model proposing that as mastication progresses cohesion increases and then decreases, and it is the point where cohesion is at a maximum where swallowing is initiated (See Section 2.3.8). Expectorated boluses near the swallow point are reported to cohere together, whereas boluses expectorated well after the natural swallow point are reported to fall apart as saliva floods the bolus and particles are separated due to a lack of cohesion. Mathematical models describing the changes in adhesion and cohesion can be found in Section 2.3.8.

Mioche et al. (2002a) investigated the textural properties and saliva content of meat. Throughout the chewing sequence mechanical shear force decreased, and saliva content increased. Significant differences in texture were found for samples before chewing, mid way through the chewing sequence, and when the bolus was ready for swallowing, however differences were smallest in the ready to swallow bolus.

Texture Profile Analysis (TPA) (see Section 3.2.2) of breakfast cereal boluses has shown hardness to decrease and cohesiveness, springiness, and adhesiveness to increase (Peyron et al., 2009) as the chewing sequence progresses.

2.3.8 Swallowing: The process and requirements of the food bolus to be suitable for swallowing

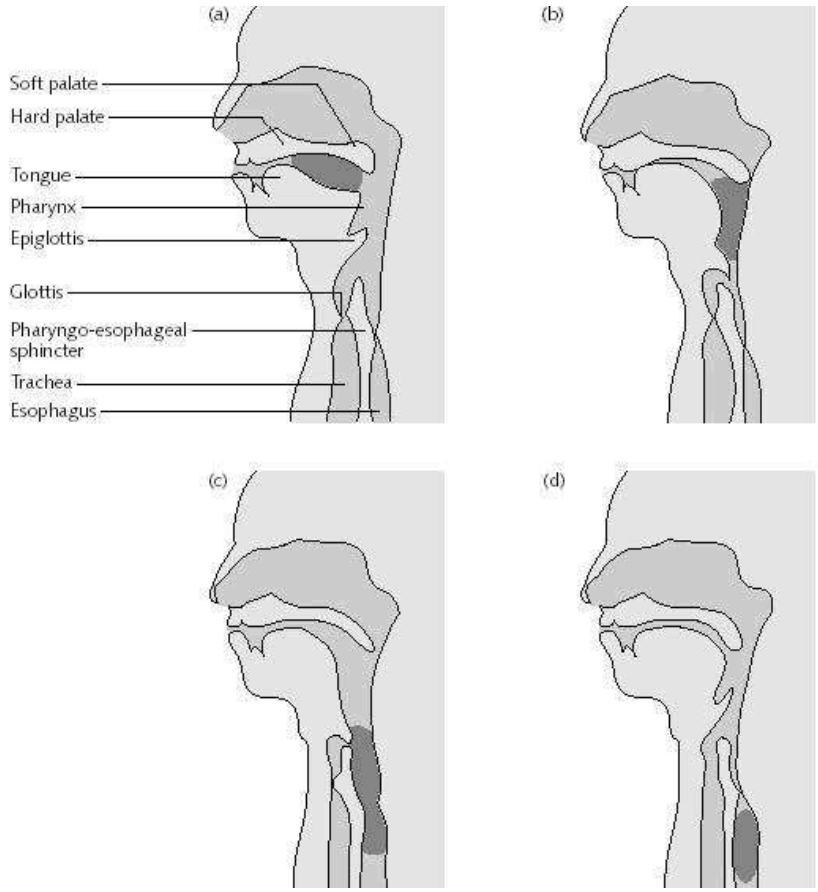

Figure 2-12: The process of swallowing (Thexton, 2001). The food bolus is dark grey.

Swallowing is the transportation of food from the mouth into the stomach, and can be divided into four stages: oral preparatory stage, oral stage, pharyngeal stage, and oesophageal stage (Gleeson, 1999).

The oral preparatory stage involves ingesting and masticating food, the addition of saliva, the formation of the food bolus, and the trapping of the bolus between the tongue and the hard palate (Figure 2-12a). The oral stage begins once the decision to swallow has been made, the tongue traps the bolus against the hard palate, the back of the tongue forms a 'chute' and the bolus moves into the oropharynx. In the pharyngeal stage the soft palate is raised to stop the bolus entering the nasal cavity and contractions along the pharyngeal wall and soft pallet allow the bolus to enter the pharynx (Figure 2-12b). The

epiglottis moves to cover the larynx and the hyoid bone and larynx also move upwards to stop the bolus entering the windpipe (Goyal & Mashimo, 2006).

The bolus then moves into the oesophagus in the oesophageal stage by the tongue pressing against the soft palate to close off the oral cavity and the lower pharynx muscles relaxing (Figure 2-12 c & d). The upper pharynx muscles also contract to force food into the oesophagus. The oesophagus relaxes to receive the bolus. Foods then move along the oesophagus via peristaltic contractions of the oesophagus muscles, although liquids can travel by gravity alone in some cases. When food reaches the end of the oesophagus, muscles guarding the entrance of the stomach relax. The same muscles contract once food has entered the stomach to prevent regurgitation (Bourne, 2002; Goyal & Mashimo, 2006).

The operation of the mouth, pharynx and oesophagus are integrated by a neuronal network, where sensory input and feedback are vital in controlling all four phases described (Miller, 1999). One aspect of this sensory control is the state of the food bolus before it is ready for swallowing. The food bolus must meet a certain physical state before it is suitable for swallowing (Hutchings & Lillford, 1988), although the exact criteria to meet this state are unknown.

Several different variables have been presented as critical parameters which initiate when swallowing will take place. Hutchings & Lillford (1988) introduced the dual-threshold model, where the bolus needed to reach a certain particle size and lubrication threshold to trigger swallowing. The model involved three dimensions: ‘Degree of structure’, ‘Degree of lubrication’, and ‘Time’ (Figure 2-13). As an example, this model explains why oyster can be swallowed with almost no chewing (at a large particle size) because of its lubricant properties, where as peanuts require extensive chewing to reduce particle size and for saliva to be incorporated. The importance of lubrication was confirmed by Prinz & Lucas (1995). Brazil nut particles were suspended in yoghurt at varying concentrations and particle size. The number of chews and chewing time increased significantly with increasing concentration of nuts (relative to yoghurt) and particle size.

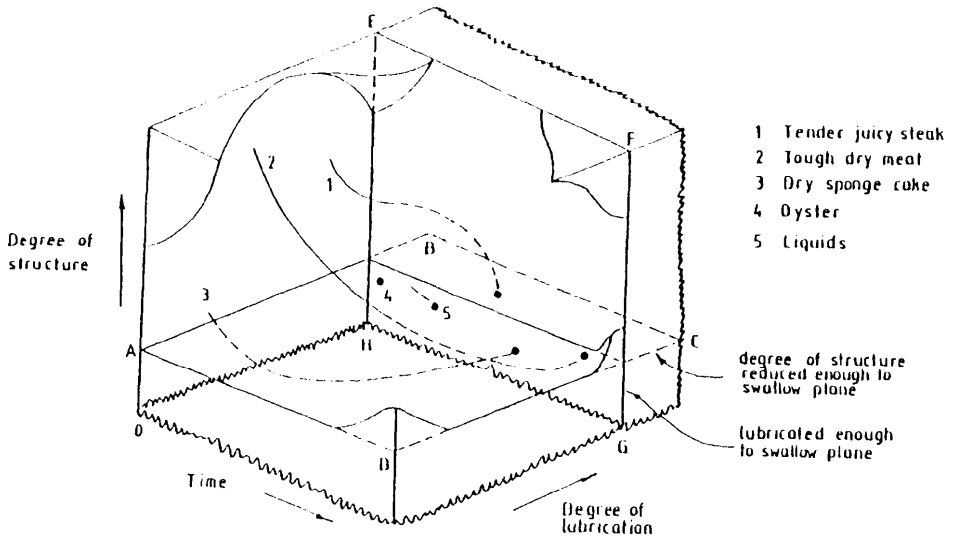

Figure 2-13: A model describing the requirements for swallowing (Hutchings & Lillford, 1988).

Another model, known as the bolus model, has been proposed by Prinz & Lucas (1997) in an attempt to explain what forces keep the food bolus together. This model is based around the trigger for swallowing being the point at which the net cohesive force of the bolus is at a maximum (Figure 2-14). The net cohesive force is defined as *Fv-Fa*, the force required to separate the mass of particles (*Fv*) subtracted by the surface tension force pulling a particle towards the oral mucosa (*Fa*). The safest time for swallowing is when *Fv-Fa* is greatest, so that minimal particles are left behind. *Fv* and *Fa* can be defined by the following parameters:

$$Fv = \frac{3\pi\eta D^4}{64d^2t} \qquad \textbf{Equation 2-3}$$

$$Fa = 4\pi r\lambda \qquad \textbf{Equation 2-4}$$

Where:

D is the size of the bolus, η is the salivary viscosity, *d* is the average separation between particles, and *t* is the time span over which separation is attempted. *r* is the size of the particles, and λ is the surface tension of saliva. Some of the superficial receptors in the tongue are suggested to detect these low attractive forces within the bolus (some times around 0.01 N) (Trulsson & Essick, 1997).

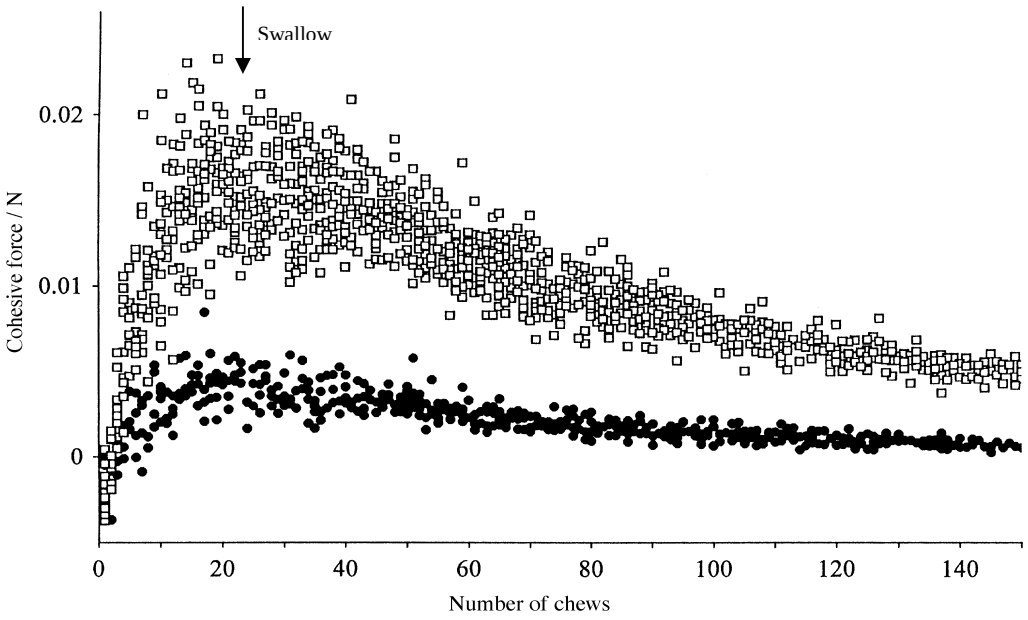

Figure 2-14: The cohesive force, F*v* - F*a*, plotted against the number of chews taken in the masticatory sequence for raw carrot (closed circles) and brazil nut (open squares) according to the model presented by Prinz & Lucas (1997). Swallowing is believed to take place at the point of maximum cohesive force (as indicated).

The model by Prinz & Lucas (1997) has been supported by research from Peyron et al. (2009) which investigated the texture of the bolus of breakfast cereals using TPA. Cohesiveness, adhesiveness, and springiness were at a maximum point when the bolus was ready to swallow.

The rheology of ready to swallow boluses of different wheat flakes has also been studied (Loret et al., 2009). Significant differences were found in the storage (G') and loss (G'') modulus, and yield stress. No significant differences were found between in the yield strain and moisture content. The authors suggested that bolus moisture content may be a trigger for swallowing.

2.4 Nutritional importance of mastication

2.4.1 Glycaemic response

The glycaemic response of foods is measured to understand how quickly carbohydrates in a food product become present as glucose molecules in the bloodstream. The most common measure is the glycaemic index (GI). Foods with a high GI are digested quickly and the blood glucose concentration increases quickly. Foods with a low GI are digested slowly and the blood glucose concentration increases slowly. It is believed that the rate of glucose entry into the blood and the period of time that blood glucose levels are high affect hormones and the body's metabolism (Brouns et al., 2005).

The first studies of GI compared 50g samples of foods and 50g of glucose, where blood samples were taken over a 2 hour period. Calculations involve taking the difference in area under a glucose-time curve between fasting and consuming the food of interest. The GI is the percentage area under a curve for a particular food compared to the area under a curve obtained for glucose. It can be measured *in vivo* by taking blood samples after food consumption, or *in vitro* in laboratories using pancreatic and brush-border enzymes (Brouns et al., 2005). Glycaemic load (GL) is an alternative way to express blood glucose response and takes into account the serving size of a meal (Mela, 2006).

2.4.2 Relationship between chewing, bolus particle size, and nutrient uptake

The in-vitro digestibility of foods is influenced by the food structure and the degree of processing. Processing which disrupts tissue or cell structure can increase the metabolic response (Bjorck et al., 1994). Snow & O'Dea (1981) found that the physical form of a wide range of cereals affects the hydrolysis rates of starch, and Fardet et al. (1998) showed the enzymatic degradation of pasta is significantly influenced by food structure.

Food structures have also been suggested to have influence *in vivo* digestion. Insulin response in the blood (a response to glucose uptake in the blood) is much smaller after

ingestion of whole apple than the apple puree and the apple juice of equal total carbohydrate content (Haber et al., 1977). Blood glucose responses in whole brown rice are significantly lower than ground rice (Collier & O'Dea, 1982), and the insulin responses in blood increased stepwise with the extent of processing (from whole grains to cracked grains to coarse flour to fine flour) in wheat, maize, and oats (Heaton et al., 1988). Correlations between *in vitro* and *in vivo* studies are often weak however (Heaton et al., 1988).

Moreover, chewing behaviour and the resulting particle size distribution has been shown to affect *in vitro* and *in vivo* digestion. Ranawana et al., (2010b) investigated the *in vitro* and *in vivo* glucose response between naturally masticated rice boluses from different subjects. The particle size distribution of masticated rice differed significantly between subjects, and the rate of *in vitro* digestion of rice decreased as particle size increased. However *in vivo* results were less clear, as particle size correlated with *in vivo* glucose response at 30 minutes post ingestion, but did not correlate with the total area under the blood glucose curve.

A similar study by the same research group involving greater within subject replicates (Ranawana et al., 2010a) investigated the glycaemic response of rice and spaghetti. The particle size distribution of the bolus differed significantly between subjects, and correlations between particle size and the glycaemic response was found in rice but not with spaghetti. The authors suggest that in the case of foods such as rice where an intact grain is masticated into smaller pieces, particle size strongly influences glycaemic response because starch resides within the storage cells of the seed. For spaghetti, which uses wheat that has already been broken into fine particles (200-400 μm), starch is more available for digestion, and hence glycaemic response is less dependant on particle size.

One study compared the difference in glycaemic response when sweetcorn, white rice, diced apple and potato was chewed naturally or swallowed whole (Read et al., 1986). When subjects did not chew the peak blood plasma concentration was significantly lower, as was the area under a 150 min glucose profile. The authors suggested chewing may increase the digestibility and absorption of carbohydrates by reducing particle size and thereby increasing the transit of food from the stomach to the small intestine,

increasing surface area for enzyme attack, and enhancing salivation on the food for breakdown by amylase enzymes in the mouth.

However, the relationship between longer chewing and increasing the rate of glucose uptake may not be linear (Suzuki et al., 2005). Subjects were asked to chew a small piece of hamburger steak for 10 seconds (termed 'natural mastication') and for 30 seconds (termed 'thorough' mastication). 'Thorough mastication' caused significantly lower glycaemic response compared to 'natural mastication', whereas the opposite trend was expected (longer chewing results in smaller particle size).

2.5 The influence of mastication on sensory perception

2.5.1 Flavour release

The extent of mastication affects the release of flavour from food particles. As food is chewed the substances which provide odour and flavour are released (Bourne, 2002). Figure 2-15 shows the pathways of flavours, beginning with breakdown in the mouth, and ending at the sensory receptors. Chemicals which are responsible for flavour perception must be released from the food matrix and transported to the flavour receptors for flavour to be experienced (Taylor, 2002).

Mastication rate and the chewing time, as well as other physiological factors such as salivary flow, will determine the concentration and rate at which flavour chemicals reach the sensory receptors in the mouth and nose. When particles are crushed due to mastication, particle surface area increases, which increases the rate that flavour diffuses into the saliva (Taylor, 1996). Volatiles will not be detected simply by being present in a food, but only by being released in the mouth (Piggott, 2000).

Alfonso et al. (2002) studied subjects chewing similar strength gelatine gels containing 0, 40, 70 or 100 µmol/L quinine. It was observed that assessors who chewed more rated higher for bitterness. Ingham et al. (1995) showed that the aroma detected from strawberries is greater the more they are broken down via homogenisation.

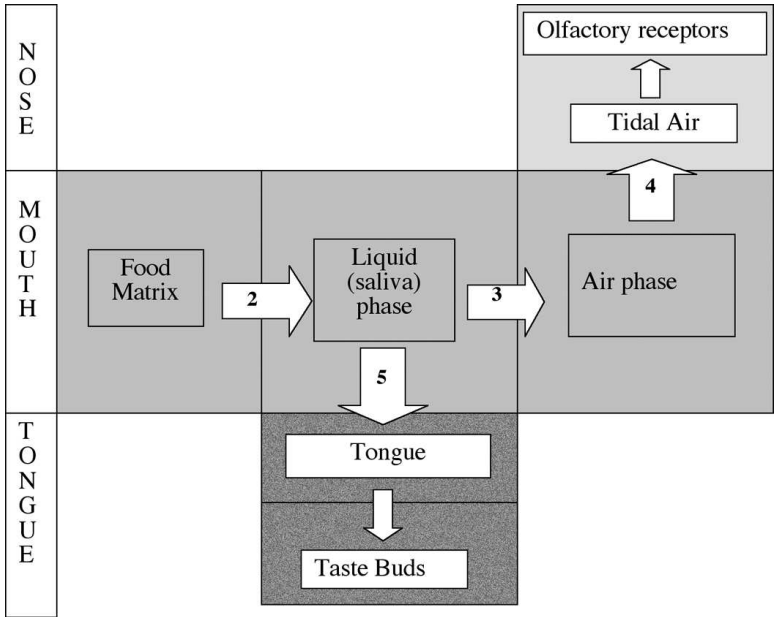

Figure 2-15: Transport of flavours from the food to the taste buds and olfactory receptors (Taylor, 2002).

2.5.2 Texture perception

It is also widely accepted that chewing behaviour and chewing strategies affect texture perception. Much work in this area has involved characterising populations into specific groups according to chewing behaviour, and assessing differences in sensory perception between these groups.

Brown et al. (1994) characterised 52 subjects into 5 sub groups according to chewing behaviour measured with EMG (Electromyography, see Section 2.8.6). Sensory ratings of carrot, apple, roast pork, salami, biscuit and toast in terms of firmness and rubberiness were significantly different between the 5 sub groups.

Subjects have also been classified according to chewing efficiency, by using weight loss from a standard chewing gum and assessing the particle size outcome of almonds. Using this classification significant differences have been found between groups in terms of the tenderness perception of meat (Braxton et al., 1996) and hardness, crunchiness, and crumbliness of biscuits (Brown & Braxton, 2000).

Differences in saliva flow and composition have also been shown to influence textural perception in semi solid foods (Engelen et al., 2007). Salivary parameters (total protein concentration, mucin concentration, buffer capacity, and alpha amylase) varied significantly between subjects, and this variation correlated with differences in sensory perception of a number of flavour, mouth feel and after feel attributes in mayonnaise and custard dessert.

2.6 The influence of physiological variables on chewing behaviour

Many physiological variables influence chewing behaviour. This section introduces several of the most well studied physiological variables.

2.6.1 Age

Age influences chewing behaviour. Mishellany-Dutour et al. (2008) showed the elderly required more chewing cycles and a longer chewing sequence to chew nuts and carrots compared to the young. Work on visco-elastic model foods found age related to an increase of 0.3 cycles per sequence per year of life, and therefore a 50% increase in the total number of chews from 25 to 75 years (Peyron et al., 2004a). It is probable that the chewing sequence becomes longer with age as elderly seem to adapt their chewing behaviour to weakening muscle activity (Kohyama & Mioche, 2004). Mastication frequency was not found to differ significantly with crispbread between young and old (Karlsson & Carlsson, 1990), whereas Peyron et al. (2004a) found cycle duration and opening phase duration fell slightly with age.

The total amount of muscle work added to a given amount of food increases with age because the number of chews increases (Peyron et al., 2004a). This strongly suggests that the jaw muscles of older people are working closer to their maximum capacity than young people (Woda et al., 2006b). Changes in muscle activity per chewing cycle is less clear. Studies by Mioche et al. (2004) on meat suggest the voltage per chew decreases with age, and work by Kohyama & Mioche (2004) on rice, beef, cheese, crispy bread, apple and peanuts suggests maximum EMG amplitudes decline with age. However, no significant difference was found in the EMG per chew between old and young (Peyron et al., 2004a) with viscoelastic model foods.

The influence of age on properties of the final bolus is unclear. The bolus produced by older subjects generally has a higher proportion of large pieces, however if missing teeth are taken into account then the impact of aging may be negligible (Woda et al. 2006a; Woda et al. 2006b). In fact Mishellany-Dutour et al. (2008) found older subjects

with complete dentition showed greater particle size reduction than younger subjects with complete dentition.

2.6.2 Gender

Various chewing parameters differ between males and females. The average number of chews subjects apply to a given food is not considered to be different between genders (Woda et al. 2006b), however parameters such as the mastication frequency (Youssef et al., 1997), vertical amplitude (Castro et al., 2002; Peyron et al., 2004a), and total EMG per sequence are higher in males (Peyron et al., 2004a).

2.6.3 Dental state

Dental status is of primary importance to mastication (Woda et al. 2006b), and has been described one of the most important factors determining food choice and nutrition in the older population (Mioche et al., 2004).

Masticatory performance is influenced by dental status (Fontijn-Tekamp et al., 2004b). van der Bilt et al. (1993c) compared subjects missing on average 5.7 post canine teeth with subjects that had normal dentition. Those with missing dentition chewed food a greater number of times, and the size of swallowed food particles were significantly larger.

Dentures also reduce masticatory performance. Full denture wearers have been shown to produce boluses with particle sizes double that of subjects with mixed dentition (Jiffry, 1983). Furthermore, particle size in the bolus is reported to be larger in denture wearers despite an increase in the number of chews, chewing time, and EMG activity per sequence (Kapur et al., 1964; Slaghter et al., 1993; Mishellany-Dutour et al., 2008). However, EMG activity per cycle and mastication frequency is similar between dentate subjects and subjects with dentures (Woda et al., 2006a).

A range of other problems can also reduce chewing performance such as dental malocclusion and temporo-mandibular disorder (Woda et al., 2006b).

2.6.4 Salivary flow

Differences in salivary flow rate are common between individuals (Gavio et al., 2004; Engelen et al., 2005b), however the influence of contrasting salivary flow on chewing behaviour is unclear. Engelen et al. (2005b) found a significant correlation between total salivary flow rate and the number of chewing cycles, where a higher flow rate resulted in less chewing cycles. In contrast, Gavio et al. (2004), found no significant correlation between the total salivary flow rate of subjects and the number of chewing cycles needed to prepare a bolus for swallowing.

2.7 The influence of food variables on chewing behaviour

2.7.1 The influence of foods on general chewing behaviour

The properties of food, particularly texture, have been shown by numerous studies to alter the way food is chewed (Hiiemae et al., 1996; Hiiemae & Palmer, 1999; Mioche et al., 2002b).

Table 2-1: The affect of different foods and textures on chewing (Koyahama et al., 2002).

Elderly subjects N=23	F_{sample}	apple	cheese	bread	meat	peanut	rice
Number of bursts	156 ***	21.1 f	25.8 e	37.4 c	52.5 a	42.8 b	34.1 d
Chewing duration (sec)	148 ***	13.4 e	20.1 d	26.7 bc	38.9 a	28.4 b	26.0 bc
Clearance duration (sec)	24.7 ***	5.0 c	7.7 b	7.8 b	7.6 b	7.7 b	11.9 a
Total muscle activity (mV·sec)	292 ***	8.6 e	10.5 d	26.3 b	41.4 a	28.1 b	17.8 c
Temporal-masseter ratio	3.0 *	0.92 ab	0.86 b	0.83 b	1.08 a	1.03 ab	0.95 ab
Duration of one burst (sec)	17.5 ***	0.35 c	0.38 a	0.38 a	0.37 b	0.35 c	0.39 a
Inter-burst duration (sec)	48.7 ***	0.29 d	0.40 a	0.34 b	0.39 a	0.32 c	0.39 a
Muscle activity per chew (mV·sec)	136 ***	0.42 d	0.43 d	0.77 b	0.82 a	0.74 b	0.56 c
Mean voltage (mV)	154 ***	0.32 c	0.29 c	0.53 a	0.56 a	0.55 a	0.37 b
Maximum voltage (mV)	142 ***	2.61 c	2.47 c	4.45 a	4.47 a	4.53 a	3.21 b
Young subjects N=14	F_{sample}	apple	cheese	bread	meat	peanut	rice
Number of bursts	170 ***	12.9 f	20.7 e	27.2 d	38.3 a	29.3 c	36.1 b
Chewing duration (sec)	163 ***	8.0 d	13.8 c	17.9 b	25.4 a	18.3 b	24.0 a
Clearance duration (sec)	13.6 ***	3.5 c	5.3 b	5.0 b	5.0 b	5.9 b	7.5 a
Total muscle activity (mV·sec)	127 ***	7.9 e	12.0 d	28.8 c	40.3 a	35.6 b	29.0 c
Temporal-masseter ratio	4.3 ***	1.05 b	1.24 a	1.13 ab	1.20 ab	1.17 ab	1.28 a
Duration of one burst (sec)	9.0 ***	0.35 ab	0.36 a	0.34 bc	0.33 c	0.32 d	0.35 ab
Inter-burst duration (sec)	11.3 ***	0.29 c	0.33 ab	0.33 ab	0.35 a	0.31 b	0.35 a
Muscle activity per chew (mV·sec)	91.9 ***	0.59 d	0.61 d	1.08 b	1.14 ab	1.18 a	0.89 c
Mean voltage (mV)	109 ***	0.43 d	0.43 d	0.82 b	0.85 b	0.94 a	0.63 c
Maximum voltage (mV)	91 ***	4.21 d	4.15 d	7.92 a	7.18 b	8.31 a	6.03 c

Mean values for each product are shown.
Effect of subject is significantly ($P < 0.001$) different for all the cases.
***; $P < 0.001$, *; $P < 0.05$.
Values in a row with different letters differ significantly ($P < 0.05$) with Student-Newman-Keuls test.

Table 2-1 summarises changes in chewing parameters across a range of foods. For every chewing parameter differences are statistically significant between foods.

A study by Hiiemae et al. (1996) is typical of research showing changing chewing patterns with food type. Results showed that food type determined the number of chewing cycles prior to swallowing and the total length of a chewing sequence. More chewing cycles and a longer chewing time was required for biscuit than apple, and for apple than banana prior to the first 'in sequence' swallow. Results also showed the chew-swallow ratio (defined as the total number of chews/total number of swallows) and duration of clearance decreased from biscuit to apple to banana. Furthermore,

stage-one transportation was significantly shorter for banana than apple and biscuit. Mioche et al. (2002b) studied the chewing of banana, tough meat, tender meat, and biscuit. Meat and biscuit were chewed for much longer than the banana, which was ready for swallowing sooner.

Moreover, chewing trajectories (in terms of vertical, lateral, and anterior-posterior movement) change from food to food (Hiiemae et al., 1996). Brown et al. (1998) studied apple, carrot, and biscuits by monitoring 3D jaw movement. Apple and carrot were chewed mainly along the vertical plane by crushing the food. Biscuits were crushed vertically during early chews, but were usually grinded laterally later in a sequence.

2.7.2 The influence of different foods on acquisition

Studies have found significant differences in mean bite weight between foods. This has been shown between banana, apple, and cookies (Medicis & Hiiemae, 1998), and between bread, sausages, rice, and apple (Yagi et al., 2006). However, little information is available on the distribution of bite size across a population, and on what food properties influence bite size.

Differences in bite volume between foods do not appear to have been studied, although natural sip size was measured by Medicis & Hiiemae (1998) and Lawless et al. (2003). Hiiemae et al. (1996) also determined the volume of solid foods using density, however the data is not reported.

2.7.3 The influence of different foods on bolus properties

The initial properties of a food influence the final state of the food bolus. The final particle size distribution, and final textural properties of the bolus, vary between food types.

Jalabert-Malbos et al. (2007) measured the particle size distribution of the ready to swallow food bolus of a wide range of foods: coconuts, carrots, peanuts, chicken, ham,

egg white, emmental, olives, mushrooms, and gherkins. The particle size among the 10 foods varied greatly, with the d_{50} (a measure of the sieve aperture 50% of the weight of particles would pass through) ranging from 0.82 mm to 3.04 mm. The particle size distributions in the food bolus of bread, spaghetti and tortiglioni have also been shown to differ significantly (Hoebler et al., 1998; Hoebler et al., 2000). Bread particles were broken down with a complete loss of structure, whereas spaghetti was only partially reduced and much of its structure retained.

Furthermore, the particle size distribution of the ready to swallow bolus has been shown to be similar within some food groups of related initial physical properties, but to be different between food groups of distinctly different physical properties. For example, the particle size distributions of peanuts, almonds, pistachio nuts have been found to not differ significantly, and the particle size distributions of cauliflower, radish and carrots have also been found to not differ significantly. However, the particle size in the bolus of this group of vegetables is reported to be significantly larger than the group of nuts (Peyron et al., 2004b; Mishellany et al., 2006).

The initial toughness of meat has also been shown to influence the toughness of the bolus (Hiiemae & Palmer, 1999; Mioche et al. 2002a; Mioche et al., 2003; Hiiemae, 2004). Mioche et al. (2003) found that tougher meat resulted in a tougher bolus.

Interestingly, variation in the particle size distribution between subjects is regularly reported to be smaller than between foods (Peyron et al., 2004b; Woda et al., 2006b; Jalabert-Malbos et al., 2007). Peyron et al. (2004b) observed no significant inter-individual variability between subjects, and Mishellany et al. (2006) and Jalabert-Malbos et al. (2007) found differences between subjects to be markedly smaller than between different foods. Similar size distributions are noteworthy considering the large variability of chewing time, chewing frequency, vertical and lateral amplitude, jaw velocity, and EMG activity for a group of subjects chewing a particular food (Mishellany et al., 2006). It is proposed by these authors the lack of significant differences is a requirement to obtain a safe bolus for swallowing, and that people with normal dentition use their masticatory apparatus in varying ways to achieve a similar bolus (Woda et al., 2006b).

2.7.4 The influence of different foods on muscle activity and force

The compressive, shear, and elastic parameters of foods, as well as sensory properties, are well known to correlate with total muscle activity (Mathevon et al., 1995). A study by Mathoniere et al. (2000) tested 12 different samples of beef by varying muscle, myofibrillar status and cooking temperature. A sensory panel evaluated tenderness of the different samples, and the muscle work required to chew the sample was evaluated using EMG. A strong relationship was found between muscle work and the perceived tenderness of beef (Figure 2-16).

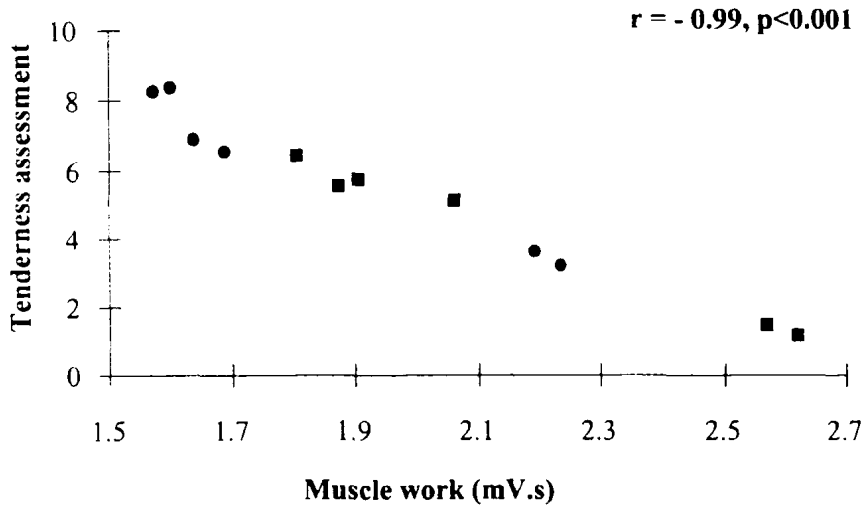

Figure 2-16: Relationship between muscle work and meat tenderness assessment during chewing (Mathoniere et al., 2000).

The activities of the main jaw closer muscles, the masseter and temporalis, are reported to be greater for foods with the highest stress at maximum strain during compression (Mioche et al., 1999). It has also been proposed that the temporalis activity is more influenced by food texture than the masseter (Mioche et al., 1999).

Forces applied by the teeth also vary between foods. Bourne (2002) presented a study by Yurkstas & Curby (1953) that measured forces experienced by dentures incorporated with a strain gauge. Forces on the teeth between varied from 0.4 kg for cabbage to 1.4 kg for tender steak.

2.7.5 The influence of different foods on saliva addition

Different foods require different quantities of saliva to be incorporated into the food bolus. Hoebler et al. (1998) found that 220 g/kg $_{(\text{fresh matter})}$ and 39 g/kg $_{(\text{fresh matter})}$ of saliva was added to bread and spaghetti respectively. Mioche et al. (2003) compared saliva addition between tough and tender meat, and found the mean weight increase of the bolus after chewing was 36% and 30% respectively.

Harder and drier products require greater amounts of saliva before swallowing. Saliva production during the oral processing of toast, toast with margarine, and cake was investigated by Gavio et al. (2004). The flow rate of saliva (ml/min) did not vary significantly between foods, however the saliva addition per gram increased from cake, to toast with margarine, to plain toast. Engelen et al. (2005b) studied toast, cake, cheese, and peanuts, and found harder and drier products required greater amounts of saliva. These products also required more chewing cycles and a longer time in the mouth before swallowing. This further indicates the need for a swallow safe bolus to be produced from chewing as explained Section 2.7.3.

2.7.6 The influence of different foods on intra-oral bolus position

The bolus position in the oral cavity differs during chewing of different foods. Depending on the product, foods will be chewed unilaterally (on one side of the mouth), bilaterally (on both sides of the mouth), or in shift cycles (the bolus is moved from one side to the other during a sequence). Different foods also require different degrees of tongue pushing and cheek pushing to keep the food bolus on the occlusal surface (Mioche et al., 2002b).

2.7.7 The influence of specific food properties on mastication

A vast range of food properties, from physical parameters such as hardness and toughness, to food dimensions such as serving size and initial particle size, all have an influence on the mastication process. This section summarises the variables which have been most widely studied.

Taste

The extent of mastication may be reduced by increasing (Bellisle et al., 2000) or decreasing (Neyraud et al., 2005) sensory acceptability of a food product. Bellisle et al. (2000) investigated five different flavoured sandwiches which were evaluated for palatability by sensory tests using visual analogue scales. Sandwiches were then consumed in a separate trial and chewing parameters analysed. As palatability increased chewing time was shorter, fewer chews were observed, and pause duration between food pieces was shorter. The difference between these parameters was more significant at the beginning of a meal than at the end.

In contrast, Neyraud et al. (2005) studied gelatine gels containing varying concentrations of a bitter compound called quinine (0 to 1446 μmol/kg). As the concentration of quinine increased, acceptability decreased and sweetness decreased, and the number of chews, chewing time, and clearance time after chewing decreased. Mastication frequency and salivation rate were unchanged with increasing quinine concentration.

Moisture content and lubrication

Studies with bread, toast, melba toast, breakfast cake, peanuts, and cheese show that dry products require more chewing cycles than moist products (Gaviao et al., 2004; Engelen et al., 2005b). It is believed that dry foods require greater time in the mouth for lubrication to take place (via saliva) to form a suitable bolus. Lubrication of the bolus is considered to be a critical parameter in determining when to bolus is ready for swallowing (Hutchings & Lillford, 1988).

Furthermore, the addition of butter to toast, melba toast, and cake has also been shown to reduce the number of chews and chewing time (Gaviao et al., 2004; Engelen et al., 2005b). The authors suggest butter acts as a lubricant, reducing the extent of breakdown required by mastication before the bolus is suitable for swallowing. However, the addition of butter to bread is not reported to effect mastication (Engelen et al., 2005b).

Initial particle size

The initial particle size in food products may (Diaz-Tay et al., 1991; Kohyama et al., 2007) or may not (Lucas et al., 1986) influence chewing behaviour.

Diaz-Tay et al. (1991) found that different initial sizes of peanut pieces (2.4 mm-9.2 mm) served to participants induced significantly different jaw movements and muscle activities. Kohyama et al. (2007) found significant differences in muscle activity between blocks and finely cut samples of carrot, cucumber, pork, and surimi gels.

However, Lucas et al. (1986) concluded jaw movements were unrelated to the initial particle size of peanuts. Vertical displacement, lateral displacement, and cycle duration were not significantly different between nuts with an initial particle size of 6.7 - 8.0 mm, 5.6 – 6.7 mm and 4.0 – 5.6 mm. It is unknown if the initial particle size affects the final particle size outcome in the food bolus.

Bite Size and Serving size

Increasing the serving size, also known as the bolus size, increases the number of chewing cycles and the chewing time. Increasing serving size has been shown to increase the number of chews and chewing time with breakfast cake (Gavio et al., 2004), peanuts (Lucas & Luke 1984; Fontijn-Tekamp et al., 2004b), carrots (Fontijn-Tekamp et al., 2004b), cheese (Fontiijn-Tekamp et al., 2004b), and gels (Daet et al., 1995; Miyawaki et al., 2000). The mastication frequency (number of chews/ chewing time) was influenced by serving size in studies with breakfast cake (Gavio et al., 2004), but was unchanged in studies with chewing gum (Bhatka et al., 2004).

A larger serving size has been shown to increase vertical jaw movement (downwards movement of the mandible during chewing) in peanuts (Lucas et al., 1986; Diaz-Tay et al., 1991), gels (Daet et al., 1995), and chewing gum (Bhatka et al., 2004). The increasing serving size has also been shown to influence lateral jaw movement with chewing gum (Bhatka et al., 2004), but not with peanuts (Lucas et al., 1986). Serving size is also known to affect jaw velocity (speed of the mandible during chewing). Miyawaki et al. (2000) and Bhatka et al. (2004) found that jaw closing and jaw opening velocities were significantly faster with larger samples of chewing gum and gels respectively. Furthermore, muscle activity has been shown to increase with serving size in a wide range of foods (Diaz-Tay et al., 1991; Miyawaki et al., 2001; Kohyama et al., 2007).

Altering the serving size changes the particle size distribution of the bolus. Buschang et al. (1997) studied the affect of changing the size of an artificial food called Cuttersil ©. The artificial food was served in 2.5, 2.0, and 1.25 g servings, and particle size measured after 20 chews. The results found that smaller boluses had a smaller and wider particle size distribution. Work by Lucas & Luke (1984) on peanuts found that increasing the bolus size increased the particle size of ready-to-swallow food boluses, and decreased the number of chews per unit of food. This is despite a recorded longer chewing time for larger peanut boluses.

Thickness and shape

Vertical jaw motion and jaw velocity has been shown to increase with sample thickness (Peyron et al., 1997). Bite force has also been shown to increase with thicker foods (Kohyama et al. 2004a; Kohyama et al., 2005). Moreover, the shape of food alters chewing behaviour. Meat samples with a rectangular face tend to be orientated longitudinally along a tooth row, where as the orientation of cubes is random (Mioche et al., 2002b).

Toughness

Toughness is generally defined as the measure of the amount of energy a material can absorb (or the work required) until it will fracture. The fundamental units of toughness

are generally stated as energy per unit volume (J/m^3) (Smith, 1993). Toughness can also be described in terms of fracture toughness. This is the resistance to fracture propagation when a crack is present in a material (see Figure 2-9). The units of fracture toughness are generally stated as the energy per unit of area created during fracture propagation (J/m^2) (Callister, 2007). Toughness can be measured instrumentally, but is also reported as a sensory attribute.

The toughness of a food influences the way it is chewed. The tougher foods become, the more likely the teeth will be used to break them down (Bourne, 2002). The toughest foods tend to be chewed with greater lateral motion than softer foods (Proschel & Hoffman, 1988). Tougher foods will also be chewed for a longer period (Togashi et al., 2000).

A tough food also requires greater muscle activity during chewing than a soft food. Mioche et al. (2003) worked on tough and tender meat cut into 2 x 2 x 1.5 cm cubes. Results showed the average muscle activity (measured in millivolts) was significantly greater for tough meat. Tough meat also required more saliva to be incorporated into the meat.

The rate of particle breakdown during chewing can be predicted by a ratio between the fracture toughness (R) and the Young's modulus of a food (E). Agrawal et al. (1997) established a relationship between fracture toughness, Young's modulus, and particle breakdown. This was discovered by measuring the change in specific surface area from the mastication of 28 different foods from three food groups (cheeses, nuts, and raw vegetables). As explained in Section 2.3.6, particle breakdown is believed to be limited by either the displacement or stress the teeth can inflict on a food particle. If the fragmentation of a particular particle is limited by stress then the rate of breakdown can be estimated by a function of $(ER)^{(0.5)}$. If fragmentation is limited by displacement, the rate of breakdown can be estimated by a function of $(R/E)^{(0.5)}$. Hence these parameters therefore provide information about the resistance to crack formation and therefore the breakage function (Agrawal et al., 1997). This work has led to other authors (Lucas et al., 2002; Lucas, 2004; Jalabert-Malbos et al., 2007) suggesting that fracture toughness and the Young's modulus are the link between breakage properties and the particle size distribution of the bolus.

Further work by Agrawal et al. (2000) identified that the relationship established for displacement limited fragmentation, (the case for most particles being chewed), is related to movement of the jaw. Significant correlations were obtained from this study between $(R/E)^{(0.5)}$ and the horizontal jaw movement and closing angle of the jaw for 15 different foods. By undertaking a study on the temporalis muscle while ten subjects chewed a range of foods, Agrawal et al. (1998) also found that the muscle activity of the jaw is highly correlated to the $(R/E)^{(0.5)}$ value of foods.

Hardness

Hardness can be defined as the resistance of a material to permanent deformation (Smith, 1993). Hardness can be evaluated with instrumental tests but is commonly reported as a sensory attribute.

The effect of hardness on mastication is widely studied. Increasing hardness has been shown to increase the number of chews and chewing time in a large variety of foods (Horio & Kawamura, 1989; Takada et al., 1994; Hiiemae et al., 1996; Peyron et al., 2002; Engelen et al., 2005). However, the hardness of a food is reported to have a minimal impact on mastication frequency (Peyron et al., 2002; Foster et al., 2006; Woda et al. 2006a).

The lateral amplitude of jaw movements increase significantly with hardness (Figure 2-17) (Takada et al., 1994; Peyron et al., 2002; Mizumori et al., 2003). Vertical movements may (Peyron et al., 2002) or may not (Takada et al., 1994) be influenced by hardness, however anterior-posterior motion is not reported to change with hardness (Takada et al., 1994).

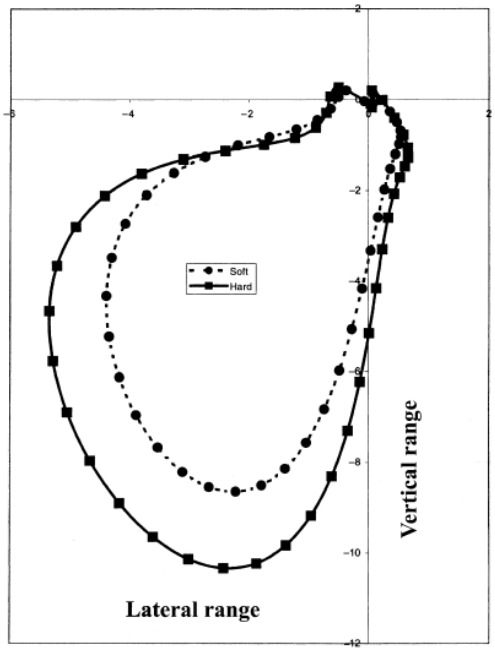

Figure 2-17: Vertical and lateral movement while chewing hard (■) and soft (●) chewing gum (units: mm) (Anderson, Throckmorton, Buschang, & Hayasaki, 2002).

Moreover, hardness increases muscle activity during chewing (Mioche et al., 1999; Woda et al., 2006a). A study on gelatine based model foods found the total muscular work (measured in mV.s, the area under an EMG wave) of the massetor and temporalis muscles (of chewing and non chewing sides) both increased significantly with hardness (Peyron et al., 2002). Foster et al. (2006) obtained similar results, where total muscular work (mV.s) of the massetor and temporalis (on both sides) increased with hardness for elastic and plastic model foods. EMG activity per cycle (average area under the EMG wave for all four muscles per sequence) also increased with hardness for elastic and plastic foods (Foster et al., 2006). Mioche et al. (1999) suggests the muscle activity of the temporalis is more dependant on hardness than the massetor. Hard foods also require a greater bite force (Mioche & Peyron, 1995; Hidaka et al., 1997; Kohyama et al., 2004b) than soft foods.

Rheology

Limited research has been undertaken assessing the influence of rheology on mastication, however Foster et al. (2006) investigated the mastication of elastic and plastic model foods of the same mechanical hardness. A change in rheology from elastic to plastic reduced the chewing frequency. Changes in the shape of masticatory

cycles were also observed between elastic and plastic foods, as can be seen in Figure 2-18. Different chewing patterns have also been observed between agar gels of varying cohesion, adhesion, and hardness by Ashida et al. (2007), however cofounding of variables mean the results are difficult to interpret.

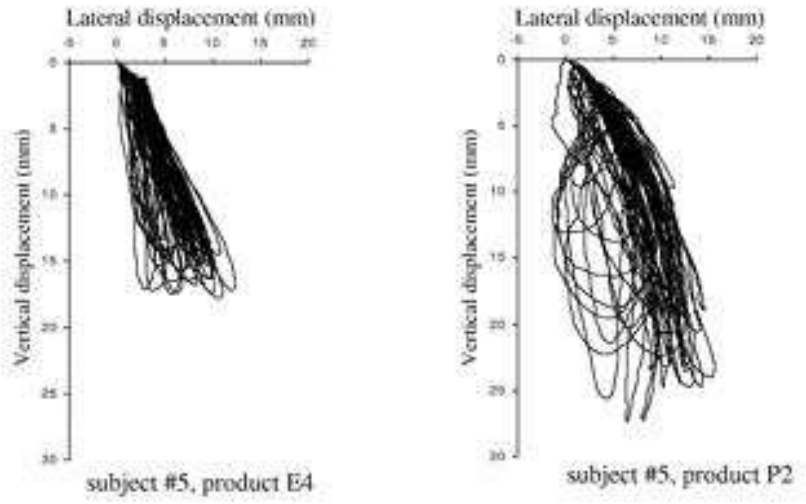

Figure 2-18: Differences in chewing paths between one elastic (left) and plastic (right) food of the same mechanical hardness (Foster et al., 2006).

2.8 Measuring and monitoring mastication

2.8.1 Serving size

In mastication studies where different foods are being compared, fixed weight or volume samples are usually used. Constant volume samples were used by Agrawal et al. (1998) (2 cm^3), Engelen et al. (2005b) (10 cm^3), and Foster et al. (2006) (3 cm^3) while Mioche et al. (2002a) (5 g), Fontijn-Tekamp et al. (2004a) (3,6,9 and 12 g), and Hiiemae (2004) (8 g) served constant weight samples. A small number of studies, such as Hiiemae et al. (1996), have allowed subjects to take natural bites of food. As suggested by Medicis & Hiiemae (1998), the weights and volumes that are commonly served tend to be lower than what appears to be a natural bite size. Furthermore, it is unclear whether constant volume or constant mass is more suitable for standardising serving size. Such standardisation is important given that the size of sample that is served has a significant influence on mastication and particle size outcome (Section 2.7.7).

2.8.2 Sample size (number of subjects)

In most mastication studies assessing foods or food properties, the number of subjects used is usually between 10 and 20. Miyawaki et al. (2000) used 16 subjects, Mioche et al. (2003) used 25 subjects, Kohyama & Moiche (2004) used 20 subjects, Peyron et al. (2004b) used 10 subjects, and Foster et al. (2006) used 16 subjects.

2.8.3 Human measurements

Even in recent years, mastication studies often use an assessor to manually count the number of chews and to time the duration of the entire chewing sequence (Peyron et al., 2004b; Mischellany et al., 2006; Jalabert-Malbos et al., 2007). The mastication frequency can then be derived. This technique is simple, cost-effective, and time-effective.

2.8.4 Jaw trajectory

Measurement of jaw trajectories are widely used in mastication studies. The most common measures of jaw trajectory involve the production of a magnetic field, the detection of light movement and infra-red emitting diodes, and videographic techniques.

Magnetic field techniques

Magnetic field based systems are the most commonly used technique to track jaw movement (Chew et al., 1988; Lassauzay et al., 2000; Kakizaki et al., 2002; Inoue et al., 2004; Kohyama & Mioche, 2004). Lucas et al. (1986) used a Sirognathograph where magnets were cemented to the front of the lower incisors. Sensors were attached to a frame fastened to the subjects head, and detected 3D movement of the magnets. A Kinesograph can also be used, where magnetic field variations are created by moving magnets attached to the teeth (Horio & Kawamura, 1989; Castro et al., 2002). Peyron et al. (1996) used a device known as an electromagnetic articulograph (AG100), which involved the subject placing their head inside a large frame creating a magnetic field. Miniature coils were attached to the middle and lower incisors.

Light based techniques

Light can be used to monitor jaw movement. Reflective markers have been employed by Haggman-Henrikson & Eriksson (2004), and van der Bilt et al. (1995), Miyawaki et al. (2000) and Bhatka et al. (2004) used light emitting diodes. Such techniques often use cameras to track jaw movement.

Infra-red techniques

Infra-red studies are also common. Peyron et al. (1997) mounted four infra-red emitting diodes (IRED) to a frame connected to the subjects head. An IRED was also attached on the subjects chin, and the subtraction between the head and chin revealed jaw movement. Infra-red light emitting diodes have also been glued to stainless steel connected to the teeth (van der Bilt et al., 1991). An opto-electronic system is usually used to track the 3D movement of the IRED's, where cameras are connected to computers.

2.8.5 Videographic techniques

Videoflourography involves xray images being recorded on a videotape, allowing movements of the tongue and food to be monitored in detail (Hiiemae & Palmer, 1999; Mioche et al., 2002b). Mioche et al. (2002b) glued radio plaque markers to the upper and lower canines, and dusted food with barium sulphate. Videotapes were recorded at 30 frames per second, and data was analysed by looking at each frame in slow motion.

2.8.6 Muscle activity

Measuring muscle activity to monitor chewing is common. Electromyography (EMG) is frequently used, and other techniques such as Vibromyography (VMG) are emerging.

Electromyography

Electromyography (EMG) operates by electrodes monitoring electrical signals created during muscular contractions to evaluate energy expended by the muscles (Brown et al., 1994). The massetor and temporalis are usually the muscles studied in mastication research. Electrodes can be applied to the surface of the muscle (surface electrodes), or inserted into the muscle. Surface electrodes are reported to be more accurate and user friendly (Armijo-Olivo et al., 2007).

Set-up involves cleansing the skin with alcohol or soap, shaving off hair, and placing electrodes in a precise position by the subject clenching their teeth. Conductive gels are often used between electrodes and the skin (Lassauzay et al., 2000). Voltage and time are the directly measured parameters, and results can be reported in terms of chew work (area under the EMG wave (mV.s)), average voltage, maximum voltage, chew work rate (muscle work within a section/time), and proportional work (% work of a section compared to entire sequence) (Gonzalez et al., 2001).

Vibromyography

Vibromyography (VMG) measures the mechanical muscle activity during muscle contractions. Contact sensors read vibrations of the muscle fibres on the skin surface

produced from lateral expansion of the muscle. As with EMG, voltage and time are the directly measured parameters (Mananas et al., 2002).

VMG is reported to discriminate absolute muscles forces between subjects better than EMG (Matheson et al., 1997), and is useful for assessing muscle fatigue (Herzog et al., 1994). However, VMG is not reported to discriminate forces within a subject as effectively as EMG (Matheson et al., 1997).

2.8.7 Food bolus analysis

Particle size distribution

Analyzing the food bolus is a critical part of understanding the result of mastication and relating it to nutrient release in the stomach. Studying bolus particle size is the most common way the bolus is evaluated. Analysis can be difficult however because up to 60% of the bolus can lost before expectoration (Jalabert-Malbos et al., 2007). Measurement of particle size is most commonly undertaken using sieving, image analysis, or laser diffraction.

A. Sieving

Analyzing particle size using wet sieving and dry sieving is a common technique (Woda et al. 2006a). The bolus can be evaluated through one sieve or multiple sieves. Single sieving evaluates chewing performance by determine the percentage weight of the bolus that passes through a sieve of a standard mesh size (van der Bilt, & Fontijn-Tekamp, 2004). This method is faster, however provides limited information about spread of particle sizes the bolus.

The multiple sieve method is more common, and is recommended over the single sieve method because the proportion of particles in a particular size range can be evaluated (van der Bilt, et al., 1993b). Results are often described with a cumulative weight distribution. The d_{50}, a measure of the sieve aperture at which 50% of the weight would pass, has been shown as an effective method of summarizing the particle size distributions (van der Bilt et al., 1993a).

Sieving analysis is simple, and results can be easily compared with previous papers given the large volume of work which has used this technique. However, sieving is a time consuming process, and results with non spherical particles can be misleading (van der Bilt et al., 1993b).

B. Image analysis

Image analysis is a technique where an image of the food bolus is analysed by computer software to evaluate the particle size distribution. Shi et al. (1990), Hoebler et al. (1998), and Hoebler et al. (2000) are examples studies that have used image analysis. A typical approach was taken by Hoebler et al. (1998) where bolus particles were spread out on a glass plate, photographed, digitised, and analysed using computer software. Results were displayed in histograms in terms of particle area.

Image analysis requires less time than sieving, and is more useful for evaluating irregular shapes where the smallest diameters do not represent the actual size (e.g. spaghetti). However illuminating particles, differentiating particles from other air bubbles, and spreading out particles, can be troublesome (van der Bilt et al., 1991; van der Bilt et al., 1993b; Hoebler et al., 2000).

C. Laser light diffraction

Laser light diffraction is another technique to evaluate the particle size distribution. Laser beams interact with the particles, and the diffraction angle depends on particle size (Woda et al. (2006a). A mastersizer is usually used for this technique. Laser light diffraction is considered more useful for measuring boluses containing particles less than 1 mm in diameter, but is ineffective with larger particles (Hoebler et al., 2000).

Rheological measurements of the food bolus

Until recently, few techniques have been developed for measuring the rheological properties of the bolus. Loret et al. (2009) used a rheometer to undertake oscillation and rotation measurements to determine storage and loss moduli, and viscosity, of the bolus. Peyron et al. (2009) undertook TPA analysis of the food bolus using an Instron set up with a flat piston head and cylindrical cup to determine hardness, adhesiveness,

cohesiveness and springiness of the bolus at various points in the chewing sequence. However, drying of the bolus after expectoration is reported to be a problem with rheological measurements of the bolus (Loret et al., 2009; Young, 2010 (personal communication)).

2.8.8 Test foods

Test foods used in mastication studies

Mastication studies have involved many different test foods, from almonds, to broccoli, to beef, to gelatine gels, to artificial test foods. The selection of a test food depends entirely on the particular objectives on the experiment. For mastication studies where particle breakdown is the focus, foods that break down into discrete particles which do not fracture or break during measurement, such as nuts (Lucas & Luke, 1984; Hiiemae, 2004), and carrots (Peyron et al. 2002; Fontijn-Tekamp et al. 2004b), are commonly studied.

Specialised artificial test foods are also commonly used (Olthoff et al., 1984, van der Bilt et al., 1993b). The most widely studied test food in mastication literature are peanuts, used in over 30 published papers such as Lucas & Luke (1984), Peyron et al. (2004b), and Engelen et al. (2005b). Peanuts are useful as their physical properties are also relatively easy to manipulate via simple processing. Oven roasting of peanuts will disrupt the cytoplasmic network, lipid bodies are burst and protein bodies are expanded, and a crunchy texture is created (Young & Schadel, 1990). Changes in moisture content reduces crispiness, crunchiness, and hardness (Lee and Resurreccion, 2006).

Test foods usually pose problems in mastication studies if they are inconsistent (often due to seasonal variation or variation in production) and thus introduce unwanted variability into results (Bronlund, 2007(personal communication)).

2.9 Conclusions from the literature

The general process of mastication and the formation of the food bolus for swallowing is well understood. Food is masticated until the bolus reaches a threshold in terms of particle size, lubrication, and cohesion. The rates food particles are broken down depend on the breakage properties of the particles and the probability of the teeth coming into contact with the particles. The process is influenced by human variables such as age, gender, and dental status, as well as food variables such as hardness, toughness, and lubrication.

This review has described in detail the influence of different foods and food properties on mastication and the food bolus with homogeneous foods. Significant differences in the mastication and food bolus exist between different natural homogenous foods such as nuts, vegetables, rice and pasta. However, an understanding of mastication and the bolus in heterogeneous foods is limited. Many modern food products involve more than one food component combined with another, and whenever a meal is consumed, more than one food is usually masticated at the same time.

Mastication studies generally serve constant mass or constant volume samples to standardise the chewing process. However, the weights and volumes that are served are often chosen without consideration of the natural bite size from the foods being studied, and are commonly too small. It is unclear whether serving constant mass or constant volume is more representative of natural biting than the other. It is also possible that other serving methods, such as allowing subjects to take natural bites, should be employed.

Moreover, mastication can be studied by making basic measurements (counting the number of chews and timing the duration of a chewing sequence), by monitoring jaw trajectories and muscle activity, or by assessing the properties of the food bolus.

In addition, research which investigates the relationships between food properties and chewing behaviour typically uses 10 to 20 subjects. This number of subjects may limit

the range of food properties which are studied because of the time involved in preparing test foods and analysing the food bolus.

Finally, mastication and the food bolus may influence sensory perception and digestion. Unfortunately, little is known about how foods can be designed to manipulate mastication and the bolus with the aim of influencing such factors. The overall goal of this thesis is to develop design principles to take advantage of the chewing process to influence the sensory perception and digestion of manufactured foods.

Chapter 3 : Methodology and method development

3.1 Method development

3.1.1 A heterogeneous food system: Food matrices with embedded test pieces

Model foods needed to be developed to satisfy the objectives of this research which was to investigate mastication in a heterogeneous food system, with a view to identifying parameters to manipulate chewing behaviour and the resulting particle size distribution in the food bolus. The concept of a food matrix containing embedded test pieces was therefore developed (Figure 3-1).

Matrices of contrasting physical properties could potentially alter mastication, the cohesion and lubrication of the bolus, the detection of internal particles inside the bolus by sensory receptors in the mouth, and the availability of internal particles for selection by the teeth for mastication. Consequently, the rate of particle breakdown of the internal test piece, and the size of the internal particles required to reach the swallowing threshold could potentially be manipulated. In addition, the physical properties of the internal test piece could be manipulated to change mastication and the resulting particle size in the bolus. This could also alter breakdown rates and the particle size required to reach the swallowing threshold. The other advantage of this type of model food is, with careful design, that the matrices can be washed away to isolate the internal particles for particle size analysis.

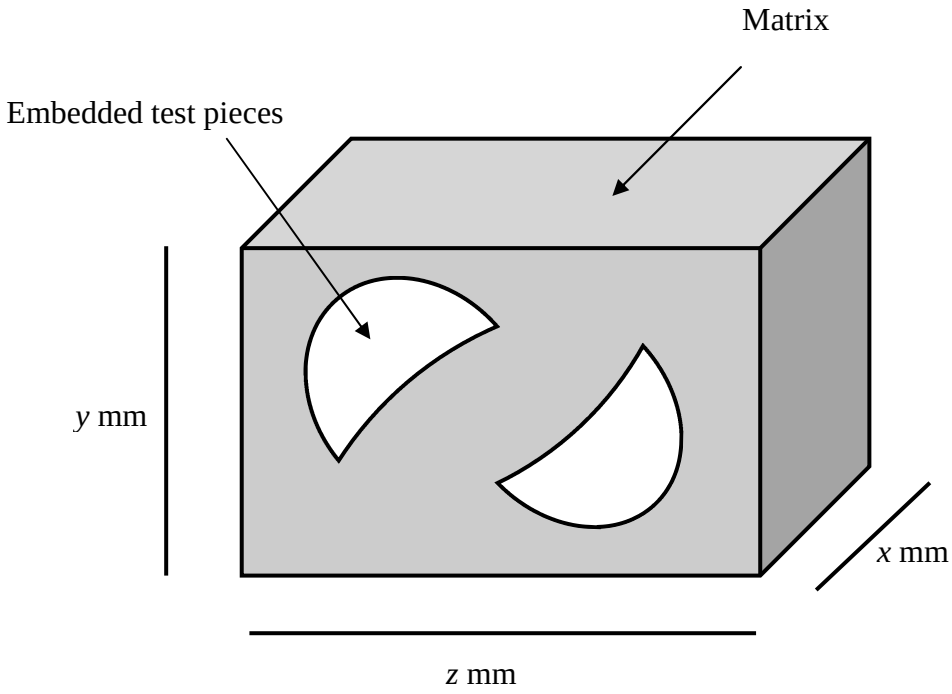

Figure 3-1: The concept test food: A continuous food matrix embedded with an internal test piece.

3.1.2 Selection of test pieces

A test piece needed to be selected for the heterogeneous system. The test piece needed to meet the following criteria:

1. Solid.
2. Fractures and reduces in size during mastication.
3. Low variability in physical properties.
4. Consistent properties during storage.
5. Palatable.
6. Suitable for particle size assessment using image analysis and sieving (cannot change in size during particle size assessment).
7. Physically suitable for embedding inside food matrices (cannot fracture or change its physical characteristics while inside the matrices).
8. Cost effective.

Peanuts (*Arachis hypogaea*) were selected as the test piece for this project. Peanuts have been used in over 30 published papers on mastication, including work by Lucas & Luke (1984), Peyron et al. (2004b), and Engelen et al. (2005b). During mastication

peanuts fracture into small particles which can be easily measured using sieving or image analysis (Mishellany et al., 2006; Jalabert-Malbos et al., 2007). They were also observed to be relatively resilient during handling in the laboratory.

Unsalted peanuts were used in every trial (Virginia cultivar, Prolife Foods, Hamilton, New Zealand). The average nutritional composition is given in (Table 3-1).

Table 3-1: Composition of Virginia cultivar peanuts used in this project supplied by Prolife Foods, Hamilton, New Zealand.

Energy (kJ)	2392
Protein (g)	24.8
Fat (g)	47.3
-saturated	5.4
Carbohydrate (g)	9.0
-sugars	5.1
Sodium (mg)	1

Roasted peanuts were predominantly used (roasted by Prolife foods, Hamilton, New Zealand). Modifications to peanut properties were made for particular trials by moisture content changes or different forms of heat treatment. Peanuts pieces were served as quarters (kernel halves) unless otherwise specified (Figure 3-2). Different treatment conditions and physical properties of the peanuts in each trial will be shown in the relevant sections.

Figure 3-2: Peanut pieces (quarters), as served in the current study.

3.1.3 Selection of food matrices

Requirements of the food matrix

A series of different matrices needed to be developed. The matrices were required to have the following properties:

1. Physically stable when peanuts were embedded inside them–cannot break apart.
2. Contrasting physical properties between each type of matrix, and each type of matrix should induce differences in chewing behaviour.
3. Repeatable between batches of the same matrix type.
4. After mastication the matrix in the bolus must be able to be washed away to isolate the peanut particles in the bolus.
5. A distinct colour difference from peanuts so that peanut particles in the bolus can be seen when washing the matrix away.
6. Palatable.
7. Able to be prepared or set in the form of constant volume bars – (20x30x200 mm).
8. Once prepared as bars must be able to be cut into small bite size portions.
9. Cost effective.
10. Consistent between batches and during storage.

The five matrices

Five different matrices were developed for use during this research: scone, gelatine gel (200 bloom), gelatine gel (250 bloom), brownie and chocolate.

The scone matrix was prepared using a proprietary premix (Fino Scone mix, Bakels, Auckland, New Zealand) in a proportion of 59% (wt/wt) in water. This mixture was blended and 5% (wt/wt) sugar added. Cocoa was also added (3% wt/wt) to colour the matrix and thus allow easier identification of the peanuts particles in the bolus during their subsequent extraction from the bolus. The mixture was then baked at 220 ºC for 12 min inside the mould.

The 200 bloom gelatine gel matrix was prepared using gelatine (200 bloom, Gelita ®, Christchurch, New Zealand) in a procedure used by previous workers in the field (Laussauzay et al., 2000) with a reduced citric acid content to 0.4% (w/w).

The 250 bloom gelatine gel matrix was prepared using gelatine (250 bloom, Gelita ®, Christchurch, New Zealand) following the identical procedure described by Lassauzay et al. (2000), with a citric acid content of 1.5% (w/w).

The brownie matrix was prepared by combining a brownie premix (75% wt/wt; Double choc brownie mix, Edmonds ®, Goodman Fielder, Auckland, New Zealand) with egg (8% wt/wt), water (7% wt/wt), and melted butter (10% wt/wt). The mixture was blended and then baked at 180 ºC for 28 min.

Samples with a chocolate matrix were prepared by melting proprietary chocolate (Dairy Milk ®, Cadbury confectionary ©, Dunedin, New Zealand) in a microwave, pouring the chocolate into the mould, and allowing it to set in a refrigerator (4 ºC).

With the exception of the scone matrix (which was frozen and defrosted for serving), all samples were stored at 4 ºC pending use. All products were wrapped in aluminium foil during storage, and discarded after a maximum of 3 days storage.

The physical properties of the matrices are provided in each chapter where the relevant matrix was used.

3.1.4 Development of an image analysis technique for determining peanut particle size

A technique for the analysis of peanut particle size

In order to measure the particle size distribution of the peanut bolus an accurate and repeatable technique needed to be developed. A time efficient method was also required, and consequently an image analysis procedure was used. The procedure is shown below.

The image analysis procedure

Each bolus sample and debri washings sample (obtained by each subject expectorating the bolus after mastication) was frozen at -18 °C following each trial (See Section 3.2.1). The bolus and washings were thawed at 20 ºC for 30 min, before being combined so that the entire mass of food could be analysed together (samples were frozen separately to minimise losses when combining the bolus). The complete bolus (original bolus and washings) was sieved across a 355 µm sieve with warm water for 4 min (Figure 3-3). This process caused the bulk of the matrix to be washed through the sieve to isolate the majority of peanut particles for analysis (particles below this sieve aperture were lost). Peanut particles were then placed on a Petri dish (140 mm diameter) (Biolab, Auckland, New Zealand) and 60 mL of ethanol (absolute) (Polychem Marketing Ltd, Auckland, New Zealand) was added to assist in particle separation and to prevent fat globules from forming (Figure 3-4). A plastic spatula was also used to separate particles for analysis to ensure an even distribution of particles.

Particles in the entire Petri dish were scanned at 800 dpi (Epson Perfection, 3490, Photo) in grayscale (Figure 3-5, Appendix A). The scan was then repeated after redispersal of the contents by shaking and respreading with the plastic spatula. Processing of the 4300 x 4300 pixel images was conducted using Image J ® (1.37a, National Institute of Health, USA). A black and white threshold was applied to obtain binary images (Figure 3-6, Appendix B). A nucleus counter in conjunction with a watershed algorithm was used to separate any abutting particles assessed particle size.

The particle size output from Image J (area in mm^2 for each counted particle) was exported to a software program in order to create cumulative particle size distributions in terms of area. An example of this output is shown in Appendix C. It was assumed the area of each particle was of a circular particle, and the diameter was derived. Each particle was then allocated to one of a series of classes based on particle diameter. Particles were categorized into 8 diameter classes of a well known power series: 0.355-0.5, 0.5-0.7, 0.7-1, 1-1.4, 1.4-2, 2-2.8, 2.8-4, ≥4 mm.

Figure 3-3: Washing the matrix away, using warm water across a 355 µm sieve, to isolate the peanut particles.

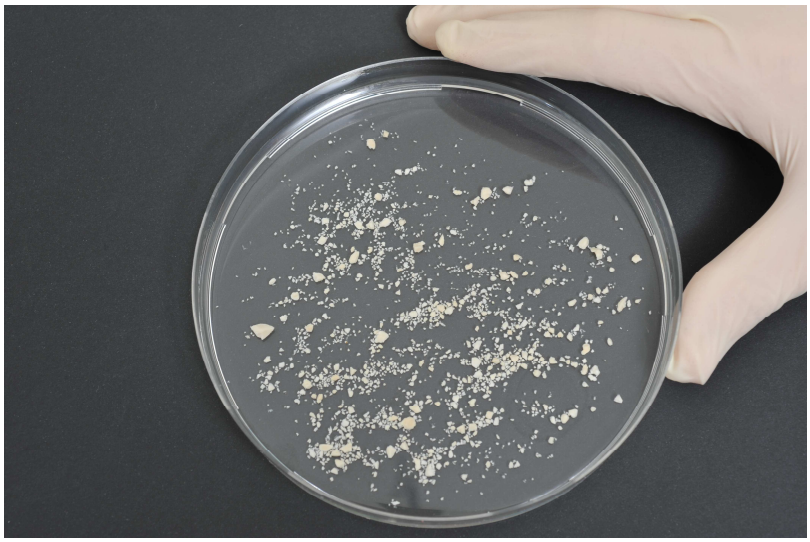

Figure 3-4: The isolated peanut particles for image analysis, sitting in ethanol inside a Petri dish.

Assessment of retention of the peanuts (percentage dry weight of peanuts remaining after mastication with respect to the initial dry weight of peanuts served) was undertaken by decanting ethanol, drying peanut particles for 24 h at 105 ºC in an air dry

oven (Labserv ®, Biolab, Auckland, New Zealand), and determining the dry weight. The initial dry weight of peanut pieces in the sample served was determined by calculating the initial moisture content of the peanuts (see Section 3.2.2).

Retention was also assessed by estimating the volume of peanuts remaining in each bolus from the photos taken during image analysis. Each particle was assumed to be spherical, and hence the area of each particle was converted to a volume, and the volume of the constituent particles for each bolus was summed.

Standardising the image analysis technique

Following development, the reliability of the image analysis technique needed to be assessed.

A. Visual assessment of particle counting

Figure 3-5, Figure 3-6, and Figure 3-7 show the conversion from a scanned photo to the counting of peanut particles in Image J. The individual particles were counted by Image J, and the watershed algorithm separated particles which were abutting. Generally the watershed algorithm correctly separated the adhering particles, however in a few cases whole particles would be divided (see particles 14 and 16 in Figure 3-7). This was considered acceptable given that each bolus would be subject to the same minor error.

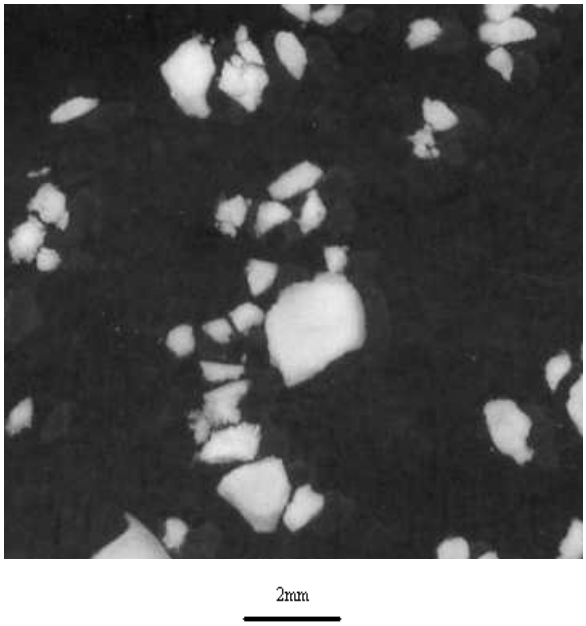

Figure 3-5: Grey scale image of peanut particles extracted from a bolus. Photo is in greyscale, 800 dpi.

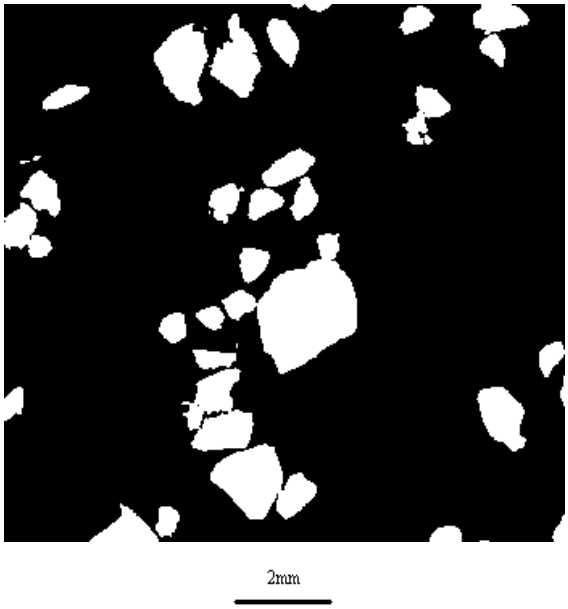

Figure 3-6: The same peanut particles after the binary threshold was applied using Image J ®.

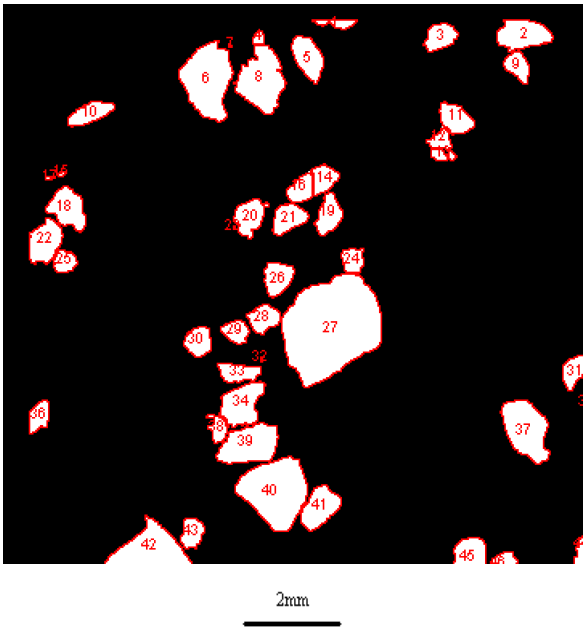

Figure 3-7: The same peanut particles after Image J has run the particle count and watershed. Numbers indicate each particle which has been counted and the lines show where the watershed algorithm has identified each particle's outline.

B. Assessing the consistency of the image analysis technique

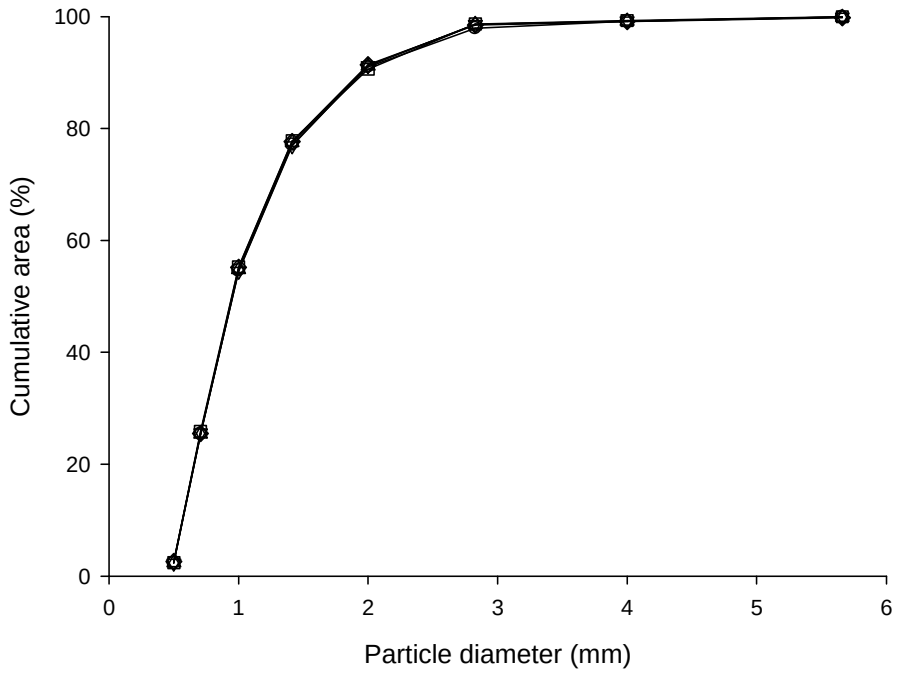

Figure 3-8: Replicate cumulative particle size distribution of the same peanut bolus. The particle size distribution of a peanut bolus was photographed, re-dispersed, and photographed again (this procedure repeated five times). Photo 1: ○, Photo 2: ·, Photo 3: □, Photo 4: ◊, and Photo 5: Δ.

The image analysis technique was performed on the same peanut bolus 5 times to assess consistency (Figure 3-8). A photograph of a peanut bolus immersed in ethanol on a Petri dish (pre sieved across a 355 μm sieve to eliminate small particles) was taken using the scanner, the bolus was re-dispersed on the Petri dish, and another photo taken (this was repeated five times). Each photograph was then processed using Image J. The particle size distribution was consistent between replicates.

C. Comparison between image analysis and sieving

The image analysis technique was compared with wet sieving, a common technique used in a vast number of mastication papers (Lucas and Luke, 1984; Olfhoff et al., 1984; Peyron et al., 2004b), to understand the extent of differences in reported particle size between the two techniques. Five peanut boluses of different particle size were prepared, and sieved across a 355 μm sieve to remove small particles. Each boluses was processed using image analysis, before undergoing wet sieving (on the same day). An estimated weight distribution curve was generated from the image data by converting the area of each counted particle to volume and assuming the peanut particles were spherical. A software program (Labview 8.5, National instruments) assisted with this procedure.

The wet sieving involved the following sieves: 0.355, 0.5, 0.7, 1, 1.4, 2, 2.8, and 4 mm, based on a sieve aperture ratio of $2^{1/2}$. The bolus was deposited at the top sieve (4 mm) and then washed with water for 30 s on each sieve. The peanut deposit on each sieve was carefully transferred to pre weighed and pre dried filter paper, and dried in an air dry oven for 24 h at 105 ºC, before being re weighed.

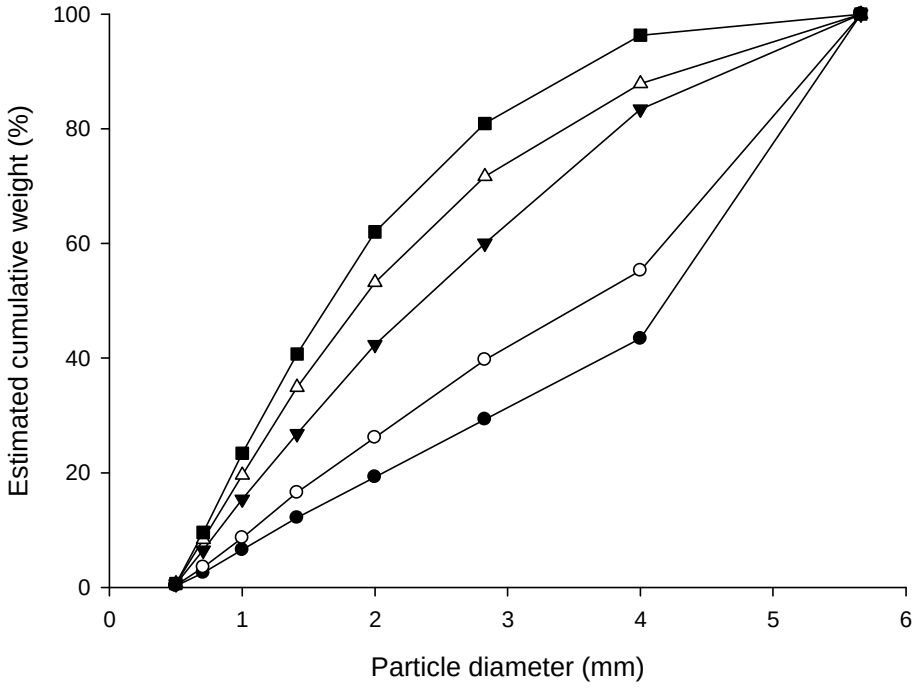

Figure 3-9: Estimated cumulative peanut particle weight distribution using image analysis. Bolus 1: ●, Bolus 2: ○, Bolus 3: ▼, Bolus 4: ∆, and Bolus 5: ■.

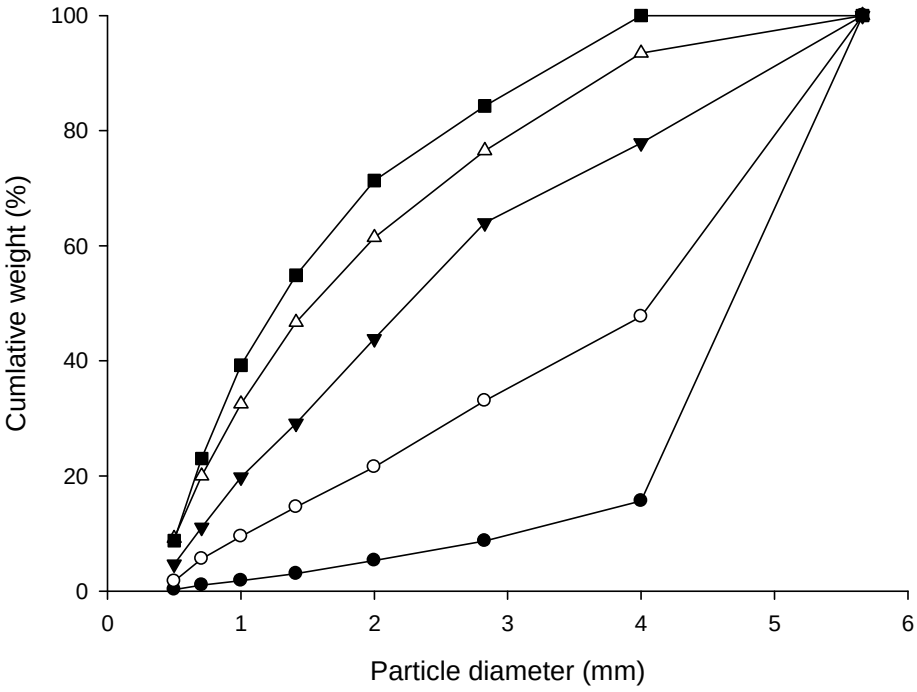

Figure 3-10: Cumulative peanut particle weight distribution using wet sieving. Bolus 1: ●, Bolus 2: ○, Bolus 3: ▼, Bolus 4: ∆, and Bolus 5: ■.

Figure 3-9 and Figure 3-10 show the particle size distribution between the two methods was similar. Importantly, the trends between the boluses are similar for image analysis and wet sieving, with Bolus 1 having the largest particles, and Bolus 5 the smallest particles. More spread in size between the five boluses can be observed for peanuts measured by the sieving technique, which is likely to be due to the watershed procedure cutting large particles into smaller pieces (Bolus 1 and 2). Boluses assessed throughout this thesis are typically closer in size to Bolus 3, 4 and 5, where distributions are comparable between the two techniques.

D. The effect of storage in ethanol of the size of peanut particles

Following sieving of the food bolus (to remove the matrices), the retained peanut particles were stored in ethanol before the bolus was photographed for image analysis. Consequently, the effect of storage in ethanol needed to be assessed to ensure the particles were not changing in size.

A typical bolus was analysed after being immediately placed in ethanol after sieving the matrix away. The particles were then stored in ethanol for 7 days and the same particles assessed again (five replicate photos were taken on day 1 and day 7).

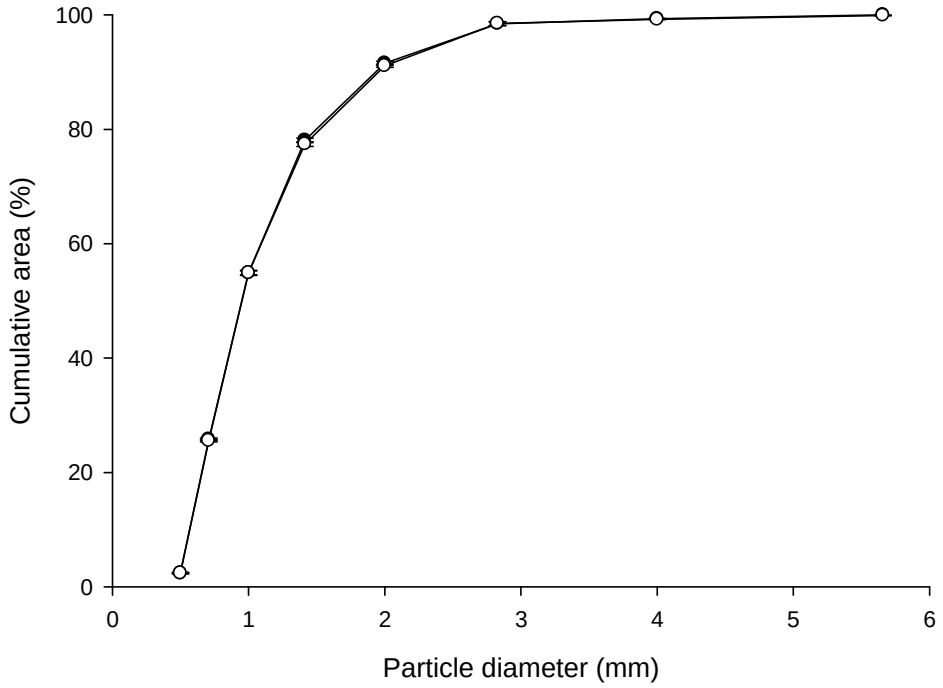

Figure 3-11: Cumulative particle size distribution of peanut particles on day 1 (●) and day 7 (○) after storage in ethanol (mean±SD).

Figure 3-11 shows that the particle size distribution from day 1 to day 7 were identical, showing that storage in the ethanol did not change particle size.

E. The effect of storage inside different matrices on the size of peanut particles

Experiments in the current study sought to analyse only differences in particle size resulting from the chewing process, and avoid confounding changes of peanut particle size afterwards. Consequently, any changes in peanut particle size during storage inside boluses of different matrices (of contrasting moisture contents and water activities) after the bolus had been expectorated also needed to be assessed. Moisture migration has been shown to result in expansion in peanuts (El-Masry et al., 2009).

An assessment of peanut particle size changes inside the gelatine gel (250 bloom) and chocolate boluses was conducted. These were the two matrices used most extensively for comparison in this thesis, and formed boluses which had large differences in moisture content and water activity (Gelatine gel: 80.3 gH_20/100g total mass, 0.919 aw; Chocolate: 37.0 gH_20/100g total mass, 0.799 aw - duplicate readings of the boluses after mastication by a single subject).

Five chocolate and 5 gelatine gel (250 bloom) (2x3x1.15 cm in size) matrices were masticated by a single subject, and the resulting boluses for each matrix were collected in individual containers. Dry roasted peanuts were crushed using a pestle and mortar, and dry sieved across sieves of the following sieve series: 0.355-0.5, 0.5-0.7, 0.7-1, 1-1.4, 1.4-2, 2-2.8, 2.8-4, ≥4 mm. Peanut particles which fell on the 1.00 mm sieve were collected, and 0.35±0.01 g samples (randomly obtained from the 1.00 mm sieve) of the particles were embedded within each bolus. To simulate the storage conditions during an experimental trial samples were kept at 20 ºC for 2 h, and then frozen for 24 h at -18 °C. Boluses were then thawed for 30 min at 20 ºC and washed for 4 min using water at 45 ºC across a 355 µm sieve to remove the matrix to obtain peanut particles for image analysis.

The particle size distribution was similar between the 5 peanut particles obtained from the gelatine gel and from the chocolate (Figure 3-12). As this was a narrow distribution in terms of particle size, the average particle area (mm^2) was also determined by using Image J for each bolus (average area=total area/particle count). Average particle area of peanuts was also similar between matrices. Average particle area inside the chocolate was determined to be 1.00±0.04 mm^2 (mean±SD), and 0.99±0.03 mm^2 (mean±SD) inside the gelatine gel. Therefore, no evidence for changes in peanut size after mastication was observed.

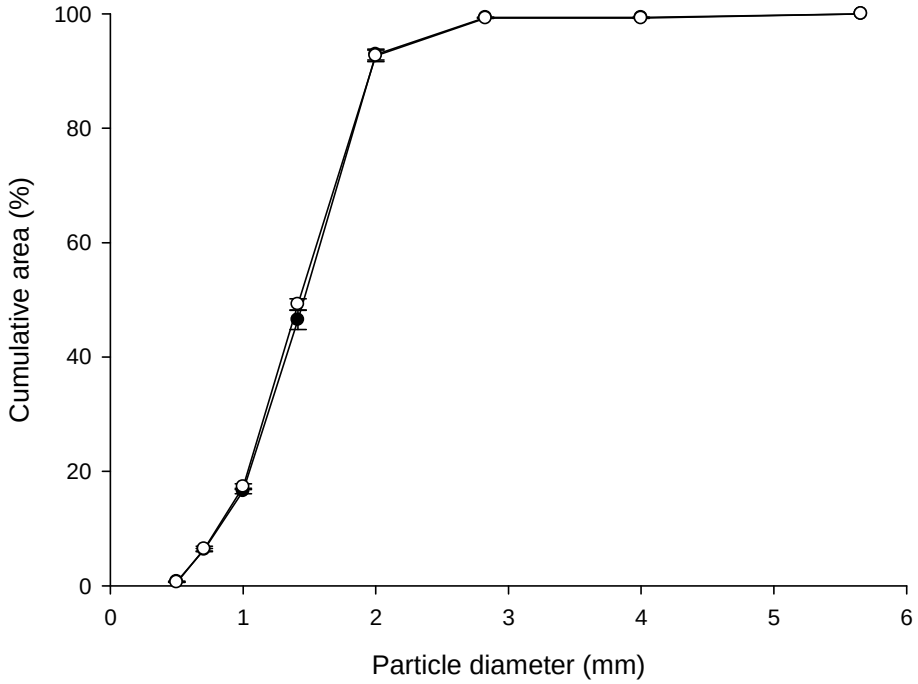

Figure 3-12: Cumulative particle size distribution of peanut particles after storage inside chocolate (●) and gelatine gel matrices (○) (mean±SD).

F. The effect of storage inside ethanol on the dry weight retained

Ethanol dissolves fat. Consequently, fat loss from peanut particles during storage in ethanol needed to be quantified, as did the influence of the particle size of peanuts on fat loss into ethanol, to assess to influence of ethanol on dry weight data.

Peanuts were crushed using a pestle and mortar, and then dry sieved across the following sieve series: 0.355-0.5, 0.5-0.7, 0.7-1, 1-1.4, 1.4-2, 2-2.8, 2.8-4, ≥4 mm. Ten samples of particles (1.00±0.01 g (mean±SD)) were obtained from the 2.8 mm sieve and from the 1.0 mm sieve. Five samples from the 2.8 mm sieve and the 1.0 mm sieve were immersed in ethanol for 24 h at 20 ºC, and five samples from the 2.8 mm sieve and the 1.0 mm sieve were stored in air tight containers as controls for 24 h at 20 ºC.

Following the 24 h storage treatments, the dry weight of each sample was determined by drying in an air oven at 105 ºC for a further 24 h. By comparison of the average dry weight between samples stored in ethanol with the control samples, the percentage loss in dry weight (likely to be predominantly fat loss) could be calculated.

Table 3-2: Estimating the loss of dry of weight from peanut particles during storage in ethanol.

Particle size	Treatment	Average Dry wt (g) (mean±SD)	Estimated % loss of dry weight during storage
1mm	Soaked in ethanol for 24 h	0.77±0.01	
1mm	Control	0.97±0.01	20.4±0.3
2.8mm	Soaked in ethanol for 24 h	0.82±0.01	
2.8mm	Control	0.97±0.01	15.6±0.2

Considerable reduction in dry weight took place over 24 h in ethanol for peanut particles obtained from the 1.0 and 2.8 mm sieves (Table 3-2). Greater losses were observed from particles obtained from the 1.0 mm sieve. This is likely to be as a result of an increased surface area to volume ratio for solids loss into the ethanol with the smaller particles.

Hence results showed considerable solids loss (likely to be fat loss) was taking place during storage in ethanol. Consequently, this needed to be taken into consideration when dry weight retention results of peanuts were presented. Retention is therefore likely to be slightly greater than data presented.

G. Evaluation of the technique

Image analysis is regularly used in mastication studies looking at the food bolus (Hoebler et al.,1998; Mishellany et al., 2006). The image analysis procedure counts individual particles and then groups them, where as sieving counts mass fractions of the bolus. Image analysis also measures the complete 2D area of each particle rather than separating particles based on the smallest diameter (as in sieving). The validation trials above show the Image J program and watershed algorithm was counting individual peanut particles, particle outcome was consistent between replicates, particle size was stable during storage in ethanol and in boluses from different matrices, and trends in particle size were similar between wet sieving and image analysis. The method was considered to be significantly faster than wet sieving, and therefore allowed for a greater number of replicates to be conducted throughout this project.

The main disadvantage of the technique was fat loss during storage in ethanol influencing the weight retention results (despite particle size being unaffected during

storage in ethanol). Therefore, estimated volume in the bolus from the images was also used as an alternative measure to assess peanut retention, given the stability of particle size (even over a one week period) in ethanol.

Describing the particle size distribution

To describe the particle size distribution of the peanut particles, a Rosin-Rammler distribution function was fitted to the cumulative area distribution determined from each bolus (Equation 3-1):

$$Q = 1 - \exp\left[-\left(\frac{x - 0.354}{d_{50} - 0.354} \right)^{b} .\ln 2 \right] \qquad \textbf{Equation 3-1}$$

Where x is the sieve class (mm), d_{50} is the theoretical sieve size through which 50% of the 2 dimensional particle area will fall (mm), b is the broadness of the cumulative area distribution (the slope of the cumulative curve, where increasing values correspond to particle size distributions that are less broad), and Q the area fraction of particles that have a smaller diameter (assuming circular particles) than x. The value of the baseline constant, 0.354 was chosen, rather than calculated from the curve fit, since all particles below 0.355 mm had been removed from the bolus. This method was based on a similar function presented by Olthoff et al. (1984).

Figure 3-13 shows a fit of the Rosin-Rammler curve to the actual bolus particle size distribution.

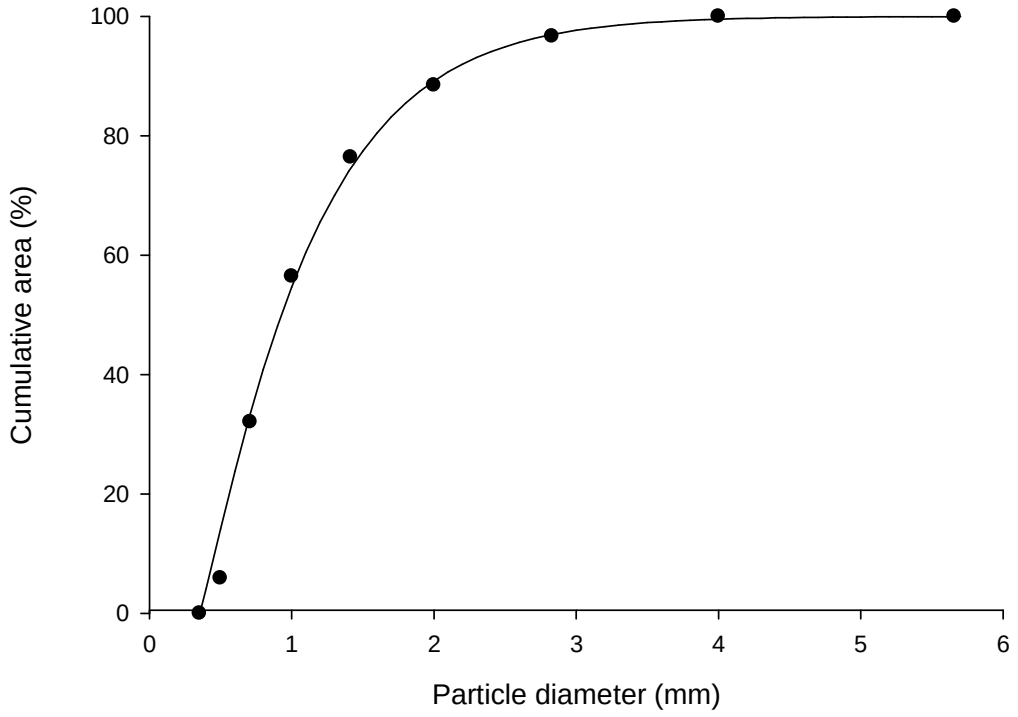

Figure 3-13: A typical cumulative area distribution of bolus peanut particles, with the Rosin-Rammler fit. Raw area data from a typical bolus: ●, Rosin-Rammler fit: ─.

3.2 General methodology

3.2.1 Experimental procedure for mastication trials

Mastication trials in the current study involved serving test food samples of a specific volume (see Chapter 4), and asking each subject to chew each sample until the point at which they felt the impulse to swallow and then to expectorate the bolus at this point. The number of chews and chewing time were recorded manually. Each experimental session was conducted in a room at 20 ºC, and test foods placed at 20 ºC at least 1 h before serving. Test foods were wrapped in foil prior to serving to eliminate moisture loss during the time between preparation in the laboratory and serving to the subjects (Figure 3-14). Subjects were asked to eat a meal within 1 to 2 h prior to each session.

Figure 3-14: Typical setup for a single test sample. Containers from left to right: water sample for rinsing the mouth (25 mL), matrix, and peanut pieces.

Following expectoration each subject rinsed their mouth with 25 mL water and expectorated this into a container. Both the bolus and the washings (debris) were weighed and frozen at -18 ºC. Boluses and debris were frozen for several days before thawing and particle analysis took place.

3.2.2 Analysis of the physical properties of the test foods

The density of the peanuts was measured by determining the volume displacement of a known mass of peanuts in toluene. Analytical grade toluene (Scharlau, Barcelona, Spain) was used as it is considered to be absorbed by peanuts to a lesser extent than water (Aydin, 2006).

The moisture content of peanuts was determined by vacuum drying of a known mass at 88 kPa at 100 ºC. The method used was based on the AOAC manual for determination of moisture contents of nuts (AOAC official method 925.40 moisture in nuts and nut products).

Water activity was measured at 25 ºC using AW SPRINT TH-500 (Novasina, Lachen, Switzerland). Samples of size 1x1x0.5 cm were used for the matrices, and peanut halves (one kernel) were used for the peanuts. Dimensions of matrices were smaller than the test samples (see Chapters 6-10) to fit matrix pieces inside the water activity meter.

Textural analysis of the matrices and peanuts were conducted using a Texture Analyser TA-XT2 (Stable Microsystems, Surrey, UK), using two successive uni-axial compression tests with a flat cylindrical probe (diameter: 50 mm) (Texture profile analysis, TPA). TPA of peanuts was conducted by compressing peanut halves to 50% strain with a test and post test speed of 1.67 mm/sec. TPA of the matrices was conducted by compression of samples (13x20x15 mm, where the 20x15 mm portion was lying flat) to 80% strain, with a test and post test speed of 0.8 mm/sec. Different conditions were used for matrices and peanuts to optimise the level of compression within the limit of the 50 kg load cell, and to minimise variability. Dimensions of the matrices were smaller than the test samples (in Chapters 6-10) to also optimise the level of compression within the load cell limit. Textural analysis of the food bars used in Chapter 4 was also undertaken, and is described in Section 4.2.2.

TPA parameters were then calculated according to standard methods outlined in Bourne (2002). These parameters were developed by General foods as a bridge between

sensory evaluation and the instrumental measurement of texture (Szczesniak, 1963), and are derived from TPA curves (Figure 3-15). They are related to the forces of attraction of matter within each food, and how the matter which makes up these foods opposes disintegration (Sczesniak, 1963). According to Szczesniak (1963), Pons & Fisman (1996), and Bourne (2002) these parameters can be defined as:

Hardness (N): The height of the force peak on the first compression cycle (Hardness 1). This instrumental measurement has been developed to represent sensory firmness (or softness) of a food.

Cohesiveness: The ratio of the positive areas of the first and second compression (Area 2/Area 1). Given constant speed during the TPA analysis, this is effectively a comparison of work required on the second compression against the work of the first compression (Work done=Force x distance). This instrumental measurement represents the strength of the internal bonds making up the food.

Springiness (m): The distance that the food recovered in height between the end of the first compression and the beginning of the second compression (originally labelled elasticity). Distance can be calculated using the constant test speed. This instrumental measurement has been developed to represent the level of plasticity or elasticity in a food.

Chewiness (J): The product of hardness × cohesiveness × springiness. Chewiness is a measurement of work required to masticate a solid product to a state ready for swallowing. This instrumental measurement represents sensory tenderness or toughness of a food.

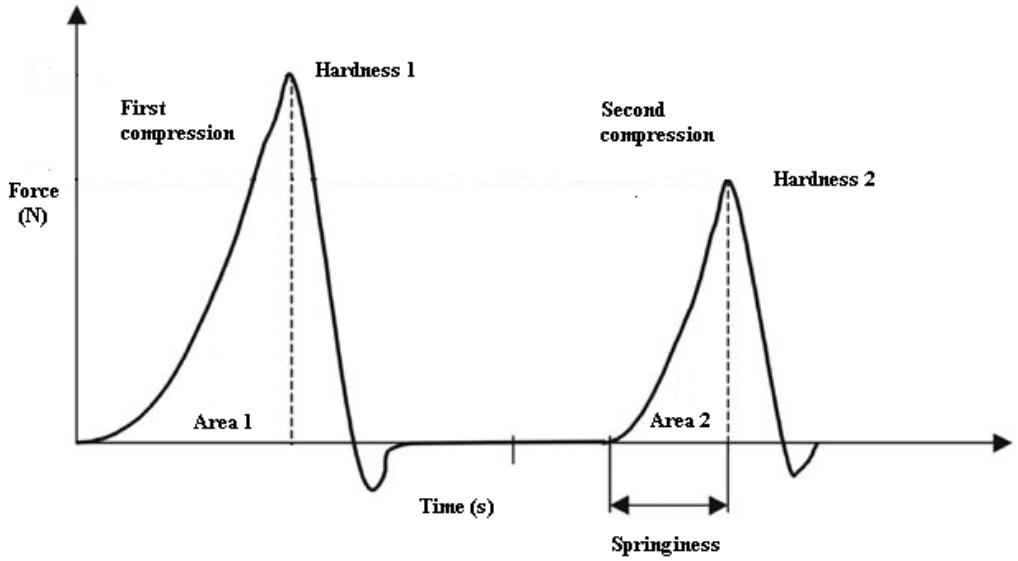

Figure 3-15: A general TPA curve obtained from the Texture Analyser (TA –XT2).

Chapter 4 : An investigation of natural bite size in a population and the development of a method to standardize serving size

4.1 Introduction

Bite size is important in the design of mastication studies when selecting and standardising serving size. It is important to ensure that the quantity of food served falls within the range of what would be naturally acquired. Serving size influences the number of chewing strokes and chewing time, (Fontijn-Tekamp et al., 2004a; Gaviao et al., 2004), and the particle size distribution of the food bolus (Lucas & Luke, 1984; Buschang et al., 1997).

A number of mastication studies have compared foods or food properties on a basis of standardised mass (Mioche et al., 2002a; Fontijn-Tekamp et al., 2004b; Hiiemae, 2004) while others have standardised volume (Agrawal et al., 1998; Engelen et al., 2005b; Foster et al., 2006b). However, as explained in Section 2.8.1, it is unclear whether serving samples that are standardised with a constant weight, constant volume, or by using alternative serving methods, provides the most accurate basis for mastication studies.

The process of biting (also known as acquisition), involves external assessment by sensory organs, occlusion of the upper and lower incisors, and the deposit of a unit of food in the oral cavity on the tongue (Section 2.3.2). During acquisition sensory organs will determine if the food product is suitable for ingestion, otherwise it will be rejected (Thexton & Hiiemae, 1997; Bourne, 2002; Hiiemae, 2004). The work required for mastication (Ang et al., 2006), as well as the hardness (Brandt et al., 1963; Boyd & Sherman 1975; Vickers & Christensen, 1980) and the thickness of the product (Peyron et al.,1997), are also assessed during acquisition.

Section 2.7.2 outlined that the natural size of the bite that people take varies between foods. Differences in bite weight have been shown between bread, rice, sausage, and

apple (Yagi et al., 2006), and between banana, apple, cookies, and peanuts (Medicis & Hiiemae, 1998). It is unclear from literature what parameters largely influence bite size, however volume (de Wijk et al., 2008) and cross sectional area (Forbes, 1987) may be important.

The aim of this study was therefore to examine variation in the bite weight, volume, and length of a variety of food bars, between subjects and between bars. Based on these findings the most useful parameter for standardisation of samples in mastication studies comparing different foods could be identified, and thus a technique for standardising serving size could be developed.

Much of this chapter, including many figures and tables, is based on work which has been published (Appendix G) (Hutchings et al., 2009).

4.2 Methodology

4.2.1 Subjects

Forty five subjects (24 females, and 21 males, aged 27.8±7.4 years) were selected on the basis of having good oral and general health with no pain during chewing, complete natural dentition, no history of recent orthodontic treatment or jaw injuries, and who were currently not on medication that could affect mastication or salivation. The questionnaire used to select subjects for this study is included in Appendix D.

The study was registered as a low risk category application with the Massey University Ethics Committee. All subjects gave informed consent following an explanation of the study. The subjects were not informed that bite size was being investigated in an effort to maintain the natural character of acquisition.

4.2.2 Experimental procedure

Six food bars commonly available in New Zealand were used for the study: Moro (chocolate and nougatine whip, Cadbury ©), Crunchie (hokey pokey and chocolate, Cadbury ©), Fruit and Nut Bar (Tasti Products Ltd.), Muesli Bar (Flemings ®), Apricot Pie (doughy bar with an apricot filling, Tasti Products Ltd.), and Pixie Caramel (hard chocolate and caramel, Nestle). The bars were chosen on the basis of their distinctive physical properties.

Each subject attended two experimental sessions, in which three types of bars were served in a randomised order. Hence each subject took bites from a given bar on only a single occasion. The subjects were asked to bite and chew in a manner which felt natural and comfortable, and instructed to take a single bite from the bar and to take a subsequent bite from a second bar of the same type once the first bite had been completely chewed and swallowed. This was designed to ensure that every subject had short term knowledge of the products sensory properties for the second bite, as during the first bite only some subjects had prior knowledge of the product.

The bars were weighed before and after the bite. The cross sectional area of the end of the bars was derived by dividing the average volume by the average length of each bar. Dimensions were taken from five replicates of each bar. The volume and length of bar bitten off were calculated from the bite weight, and the average density and average dimensions of the bars. Each sequence of acquisition and chewing was video taped on a Quick Cam 8.48 (Logitech Asia Pacific Ltd, Hong Kong). The number of chews between acquisition and swallowing of each bite was subsequently determined manually from the recordings.

The average density of each bar was determined using a dry volume displacement method. A bar of known weight was submerged in rapeseed inside a measuring cylinder. Hence the volume of the bar was calculated from the difference in the level of rapeseed in the cylinder when the bar was completely covered. Five replicates were conducted on each type of bar and the mean volume used in subsequent calculations. Density was calculated as the average bar weight divided by the average bar volume.

Textural analysis was undertaken using a Texture Analyser TA-XT2 (Stable Microsystems, Surrey, UK) using cuboid samples of each bar (1.2 cm x 1 cm x 0.7 cm). Compression and incision tests were used to evaluate hardness and work done. Data acquisition was carried out using a 50 kg load cell and a sample frequency of 40 Hz. Four replicates were conducted for each test on each bar. Compression tests were based on principles from Bourne (2002), and were conducted to 75% strain at a test speed of 2 mm/s using a cylindrical shaped probe 61 mm in diameter. Hardness was taken as the maximum force measured during a compression test. Work was taken as the area under the force-displacement curve from start to 75% compression.

An incision test was also conducted using the Texture Analyser to assess incision hardness and work using bars cut to 4 cm length and 3 cm width. An ‘axe’ shaped probe was allowed to penetrate half way down each bar at a perpendicular angle to its length, until it reached 3 mm from the base. Hardness was taken as the maximum force during incision, and the work for incision as the area under a force-displacement curve until the maximum force was reached. This test was devised to simulate the physical process of biting.

4.2.3 Statistical analysis

Statistical analyses were performed using SPSS ® (version 15.0 for Windows). To assess normality a Kolmogorov-Smirnov test (with Lillifors significance correction) was conducted on the data set for the first and the second bite. The bite size data were all normally distributed ($P>0.05$) except for the second bite of the Pixie Caramel bar ($P=0.04$ for weight, volume, and length). The number of chews were not normally distributed, however after a log transformation, all data were normally distributed, except for the number of chews from the first bite of the apricot pie bar ($P=0.02$).

Two-way repeated measures ANOVAs, with bar and bite as the within subject factors, found significant interactions between bite number and bar type for weight, volume, and length of bite. This significant interaction indicated changes in biting behaviour from bite 1 to bite 2. Consequently, all results presented and subsequent statistical analysis used only the second bite data. Statistical analysis involved one-way repeated measures ANOVAs with bar as the only within subject factor.

When repeated measures ANOVA indicated significant differences between bar quantities, post hoc Bonferroni tests were undertaken to compare individual bars. Where the assumption of specificity had been broken, degrees of freedom were adjusted using Greenhouse-Geisser estimates of sphericity.

The similarities in the various parameters of bite quantities (weight, volume and length), as well the number of chews, were compared overall by plots of raw data in cumulative form.

4.3 Results

4.3.1 The physical properties of the bars

The food properties varied across the bars (Table 4-1). The following differences were noted:

- The Crunchie bar was the least dense and required the lowest level of incision work.
- The Fruit and Nut bar had the largest cross–sectional area and was relatively high in the level of incision work.
- The Muesli bar had one of the smallest cross sectional areas, was relatively hard and required a relatively high compression and incision work.
- The Apricot Pie bar was the softest product having the lowest compression hardness and work.
- The Pixie Caramel bar was the hardest and densest bar requiring the highest levels of incision and compression work. It also had a relatively small cross-sectional area.

Table 4-1: Food properties of the bars (mean±SE).

	Moro	**Crunchie**	**Fruit and Nut**	**Muesli Bar**	**Apricot Pie**	**Pixie Caramel**
Density (g/cm^3)	0.95±0.01	0.58±0.01	0.75±0.02	0.85±0.04	0.75±0.02	1.27±0.03
Cross Sectional Area (cm^2)	5.7±0.1	5.4±0.1	6.3±0.2	3.6±0.1	5.9±0.1	3.5±0.2
Compression Hardness (N)	20±1	41±2	52±13	82±16	8±1	238±16
Incision Hardness (N)	2.7±0.1	5.9±0.3	5.4±0.1	6.8±0.3	2.1±0.1	9.8±0.6
Work for compression (mJ)	52±3	153±16	122±32	243±44	38±4	1108±68
Work for incision (mJ)	297±25	91±9	528±26	486±26	202±89	670±52

4.3.2 Interaction between bite 1 and bite 2

A significant interaction term for bite weight between bite number and bars ($F(2.8,122.95) = 5.01$, $P<0.01$) was found, indicating that the type of bar caused changes in bite size from first to second bite. A similar pattern of significance occurred with bite volume ($F(4.02, 176.79) = 3.83$, $P<0.05$) and bite length ($F(2.99, 131.53) = 4.57$, $P<0.05$).

4.3.3 Bite weight

There was a noticeable spread between the cumulative distribution curves of the bite weights for each of the bars, particularly between the Crunchie and the Moro (Figure 4-1A). Significant variation between subjects ($F(1,44) = 418.6$, $P<0.0005$) and between bars ($F(3.44,151.2) = 22.3$, $P<0.005$) was found. Post hoc Bonferroni tests found the bite weight of the Moro to be significantly different from all bars except the Apricot Pie bar (Table 4-2).

4.3.4 Bite volume

There was a noticeable spread between the cumulative distribution curves of the bite volumes for each of the bars, particularly between the Pixie Caramel and Muesli bar from the other bars (Figure 4-1B). Again significant overall variation between subjects ($F(1,44) = 436.1$, $P<0.0005$) and between bars ($F(4,178.2) = 36.32$, $P<0.005$) was seen. Post hoc Bonferroni tests identified the Pixie Caramel and the Muesli bar to be significantly different from each other and all other bars (Table 4-2).

4.3.5 Bite length

Distinctly less spread was observed between the cumulative distribution curves of bite length (Figure 4-1C). Significant overall variation between subjects ($F(1,44) = 440.4$, $P<0.0005$) and between bars ($F(4.32,190) = 9.5$, $P<0.005$) was indentified. Only the

Muesli bar was significantly different from the other bars according to Post-hoc Bonferroni results (Table 4-2).

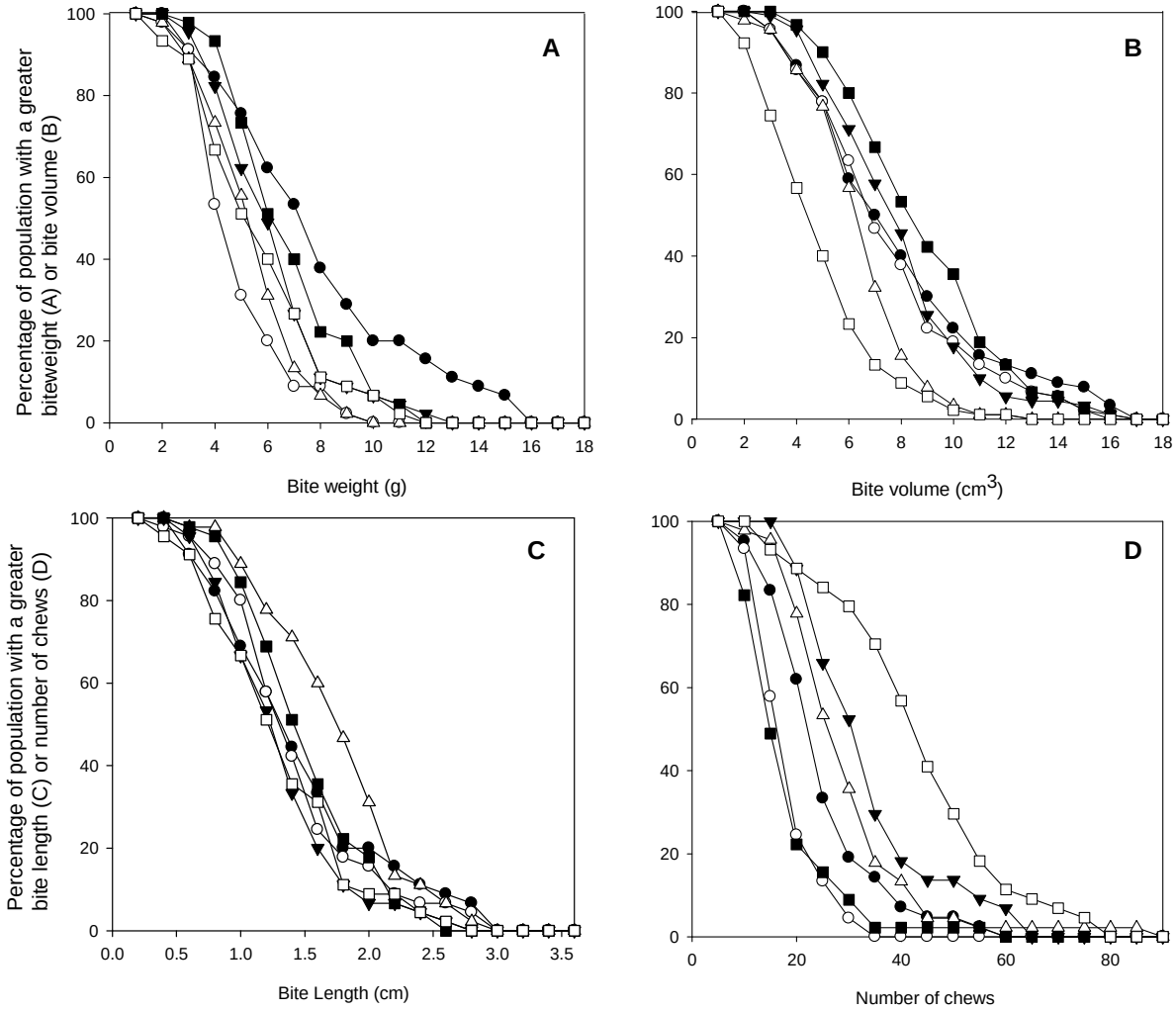

Figure 4-1: Cumulative distribution of bite weight (A), volume (B), length (C) and the number of chews (D) for the second bite. Moro: ●, Crunchie: ○, Fruit and Nut: ▼, Muesli Bar: ∆, Apricot Pie: ■, Pixie Caramel: □.

Table 4-2: Bite size (weight, volume, and length) and the corresponding number of chews from six food bars for the second bite (mean±SE).

Parameter	Moro	Crunchie	Fruit and Nut	Muesli Bar	Apricot Pie	Pixie Caramel
Weight (g)	7.66±0.54 a	4.63±0.27 b	6.02±0.32 cd	5.17±0.25 b	6.60±0.32 ac	5.48±0.36 bd
Volume (cm^3)	8.04±0.57 a	7.48±0.45 a	8.02±0.43 a	6.07±0.30 b	8.75±0.42 a	4.32±0.28 c
Length (cm)	1.41±0.10 a	1.39±0.08 a	1.28±0.07 a	1.7±0.08 b	1.46±0.07 a	1.26±0.08 a
Number of chews	24.64±1.68 a	17.49 ±0.96 b	34.8±2.41 c	28.80±2.00 d	17.80±1.49 b	44.53±2.94 e

Different letters (a,b,c,d,e) across each row indicate a significant statistical difference after a one-way repeated measures ANOVA using post-hoc Bonferroni tests ($P<0.05$).

4.3.6 The number of chews per bite

Significant differences, even greater than those observed for bite weight and bite volume, were observed in the cumulative distribution curve (Figure 4-1D). Significant overall variation between subjects ($F(1,44) = 3479.4$, $P<0.0005$) and between bars ($F(3.87,158.7) = 120$, $P<0.005$) was seen. Post hoc Bonferroni tests showed numerous significant differences in the number of chews between bars (Table 4-2).

4.4 Discussion

4.4.1 Variation in bite size

Results demonstrate that natural bite size varies greatly between subjects (Figure 4-1). Such spread in bite size within subjects has been found in every food so far examined in literature, from bananas to biscuits. Bratley & Hackett (1999) observed similar variation in carrots, and Yagi et al. (2006) in apples. It is likely that individuals develop distinctive overall biting strategies depending on their physical and behaviour characteristics.

Interestingly, the variation in bite size is likely to be greater than what would be expected due to physical dimensions alone, such as variation in jaw size. Further work is required to identify what causes this variation. Behavioural factors may be far more important than physical factors.

4.4.2 The influence of first bite v. second bite

The significant interaction between bar type and bite number showed that the bite size changed between the first and second bite according to bar type. This indicates that properties of the bars induce assessment and readjustment. Prior to the first bite many subjects had limited knowledge of the products properties, however prior to the second bite subjects could take a bite knowing what to expect. For example the high hardness and work values of the Pixie caramel (Table 4-1) may have resulted in readjustment to a smaller second bite for many subjects.

4.4.3 Variation in bite size between bars

These results show that each subject's bite size was not based on acquiring a particular mass or volume across different bar types, however bite size may be based on acquiring a particular length across different bar types. There were significant differences between the bars in terms of all measured bite size variables (weight, volume, and

length), but the difference in bite length between bars was not as great as seen for weight and volume (Figure 4-1). Post hoc analysis showed that only the Muesli bar was significantly different from the other bars in terms of length (Table 4-2). Interestingly, the bars which produced the greatest separation by weight differed from those which produced greatest separation by volume.

The regularity in natural bite length may mean the physical shape and the density of the food bar may have a stronger influence on bite size than textural properties such as hardness and work during compression.

Previous studies have found significant differences in mean bite weight between different foods (Hiiemae et al. 1996; Medicis et al. 1998; Yagi et al. 2006). Previous research has not compared natural bite volume or bite length between solid foods, although some information on sip volume for liquids is available (Medicis et al., 1998; Lawless et al., 2003; de Wijk et al., 2008).

4.4.4 Variation in the number of chews between bars

Results showed the 6 food bars differed greatly in the way they were chewed (Figure 4-1D, Table 4-2). Hiiemae et al. (1996) has also shown differences in chew number between foods acquired from natural bites.

4.5 Conclusions

The results of this study show that the bite size varies significantly between subjects but that bite size also varies with food even when different foods are presented in a similar shape (as food bars). Variation between bars was high in terms of bite weight and bite volume, but considerably lower in terms of bite length. Consequently, constant volume samples may represent natural feeding more so than constant mass, as volume differences will match length differences if the cross sectional area is constant.

These results highlight the importance of considering the manner in which foods are served to subjects in mastication studies. The administration of a chosen weight of food is likely to produce different results from a chosen volume of food.

4.6 Applications for serving methods in mastication studies

These results have applications for standardising portions in mastication studies. Despite the finding that neither bite mass nor bite volume were consistent between food bars for each subject, the regularity in bite length between bars suggests constant volume servings may be more appropriate to represent typical feeding. If bite length is consistent between many bar shaped food products (where the cross sectional area does not limit acquisition), bite volume should also be as consistent if the cross sectional area across for the different bars (area of the end of the bar) is kept constant (length x cross sectional area = volume). A study using bars of the same cross sectional area could be undertaken to confirm regularity of bite volume with regular cross sectional area.

The significant differences between products in terms of bite mass, as also shown by Hiiemae et al. (1996), Medicis et al. (1998), and Yagi et al. (2006), and significant differences in bite volume, highlights the importance of researchers carefully selecting a serving size.

An alternative option for serving is to allow subjects to take natural bites for mastication studies, which reflects typical feeding more so than serving constant sized samples. Taking natural bites from food bars of the same cross sectional area could be particularly effective, given the regularity in bite length.

4.7 The method implemented for this project

Based on conclusions drawn from the bite size study, a method for preparing constant volume samples was developed. In Chapters 6-9, a constant volume serving size was determined by asking the selected single subject to take test bites from the different test foods (used in the particular study in that Chapter) all prepared as bars with constant cross sectional area and volume, and recording the bite length from each food type (each bar). Replicate bites were taken from each bar, and a bite length (and therefore bite volume) which fitted within the subject's natural bite range for all the foods to be test in that trial was chosen. The bite length which was selected was often close to the average bite length of the subject. Thus to serve the samples in the mastication trials, the samples were prepared by cutting all the bars (with constant cross sectional area) at the selected bite length, to produce constant volume samples for all the foods to be tested in the trial.

In Chapter 10, where 8 subjects were used, an identical procedure was used for each subject as for the single subject studies. However, the selected bite length was based on an average bite length across the 8 subjects.

A multi-purpose aluminium mould was constructed for setting and baking matrices, to produce matrices (as bars) of a constant shape and volume (20 x 30 x 200 mm) (Figure 4-2). Bars were cut at the desired bite length to produce constant volume pieces. Any increase in the volume of the matrix by upward expansion during preparation was prevented by application of a flat tray on top of the mould during baking.

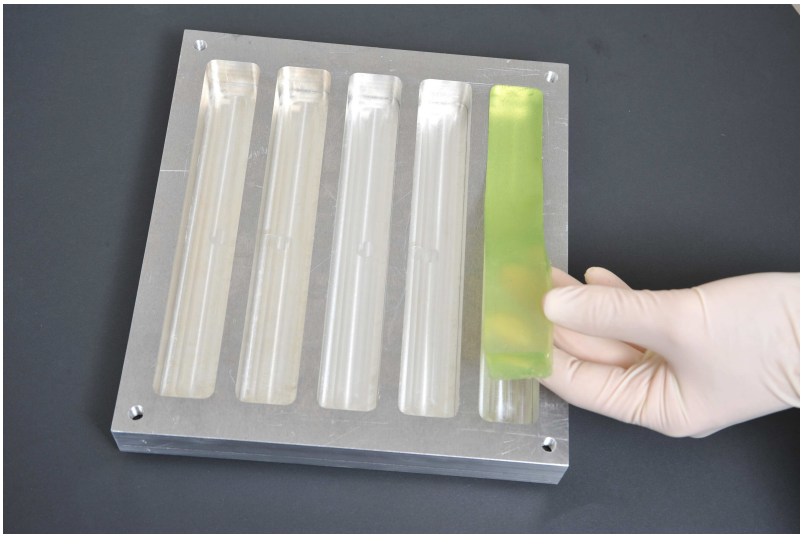

Figure 4-2: The aluminium mould used to prepare matrices.

Chapter 5 : Selection of subjects in mastication studies

5.1 Introduction

The purpose of this research was to evaluate the effect of food structure (of heterogeneous foods in particular) on oral processing and the food bolus, with the goal of establishing food design principles for food manufacturers to influence sensory and nutritional properties.

The practicalities of this work, in terms of requirements to investigate a wide number of food variables for establishing these design principles meant that multiple subject studies were not feasible throughout the project. This is because multiple subject studies report wide variability in mastication behaviour (Lassauzay et al., 2000; Peyron et al., 2002), such as the number of chews, chewing time, and mastication frequency, and also some variability in the food bolus (Mishellany et al., 2006; Jalabert-Malbos et al., 2007). Consequently large numbers of replicates are required to obtain significant results, which constrains what variables can be explored, is time consuming, and can lead to complicated statistical analysis.

Consequently, single subjects were used in Chapters 6-9 of this thesis, and a multiple subject study was used in Chapter 10 to validate the major findings of this work with a population. This approach minimized variability in the data resulting from variation between individuals to simplify analysis and enabled a greater range of food variables to be tested. Variability within subject replicates is much smaller than between subjects in terms of mastication parameters (Lassauzay et al., 2000) and particle size distribution in the food bolus (Mishellany et al., 2006).

As this work was not specifically designed to investigate the physiology of chewing or trends in chewing amongst a population, the single subjects functioned effectively as reproducible chewing devices which responded to changes in food properties. Single subject studies have been used previously in mastication literature (Howel, &

Brudevold, 1950; Yven et al., 2010), and are often used in research involving glycaemic index (Parada & Aguilera, 2009). A wide number of masticatory robots that are also being developed (Salles et al., 2007; Xu et al., 2008, Woda et al., 2010), which is testament to the effectiveness of the use of single subject studies to evaluate changes in mastication to varying food properties.

A rigorous procedure was undertaken to eliminate the selection of subjects with highly unusual or highly variable oral processing characteristics for these studies. The selection approach was based on assessing the consistency of bite and chewing behaviour of each applicant with the Fruit and Nut bar used in Chapter 4, and assessing if applicant's bite and chewing characteristics of the Fruit and Nut bar were similar the general population studied in Chapter 4. The selection approach could be applied more loosely for multiple subject studies, as undertaken in Chapter 10. Selected subjects also met strict dental and health criteria alongside the assessment of oral processing characteristics.

Applicants for selection were recruited via advertising and word of mouth. Subject 1 participated in the work presented in Chapter 6, Subject 2 participated in the work presented in Chapter 7, and Subject 3 participated in the work presented Chapter 8 and 9. All mastication trials in this thesis were approved by the Massey University ethics committee (Southern A Application 08/17 and 09/24), and all applicants gave informed consent.

It must be noted that as mastication data and particle size data were both key parameters measured in the current study, a technique to assess the particle size of applicants boluses (with a test food that could be compared to particle size data of a population) was considered as alternative for this screening out unusual subjects. However, this was deemed unnecessary given that it is mastication variables that vary more widely than particle size variables. Dental screening of subjects also reduced the likely hood of obtaining subjects who would produce boluses with unusual particle size distributions.

5.2 Assessment of the health and dental status of the applicants

The health and dental history of each applicant was assessed using methods commonly used in mastication studies (Lassuzay et al., 2000; Foster et al., 2006; Jalerbert-Malbos et al., 2007). This involved the administration of a screening questionnaire (Appendix E), and the use of a qualified dentist to assess dental status and occlusion (Appendix F).

The single subjects were required to have class 1 occlusion (correct alignment between the teeth of the maxilla and mandible when the jaw is closed (occluded)), no significant tooth crowding, no obvious tooth decay, and healthy periodontal condition. They were not allowed to have any functional disturbance to mastication such as pain or clicking during chewing, or any other known oral or general health issues that could influence oral processing.

5.3 Assessment of oral processing characteristics

Applicants who passed the health and dental screening were asked to bite, chew and swallow (as in Chapter 4) a standard Fruit and Nut bar (a bar used in Chapter 4) five times. Bite weight and the number of chews of each volunteer were recorded. Applicants who were the most consistent and representative were selected, and hence applicants with unusual oral processing characteristics could be eliminated.

Applicants with the lowest standard deviation for each parameter were considered most consistent. The degree to which each applicant's parameters were similar to the larger population was assessed by comparison of their means with those from the large bite size study in Chapter 4. Bite weight and chewing behaviour of the Fruit and Nut bar was seen as an indicator of how a potential subject would bite and chew other products, given the significant positive correlations for bite weight and the number of chews between the Fruit and Nut bar and the other bars used in Chapter 4 (among the 45 subjects) (Table 5-1, Figure 5-1). Hence a subject who took an unusually large number of chews of the Fruit and Nut bar was considered to be likely to chew other products a large number of times, and was therefore not selected.

Correlations were undertaken with data that was normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction) where $P>0.05$ (a $\log_{10}$ transformation was applied to the number of chews). All Pearson correlation coefficients were significant ($P<0.05$).

Table 5-1: Pearson correlation coefficients between Fruit and Nut and other bars in Chapter 4 among 45 subjects.

	Moro	**Crunchie**	**Flemings**	**Apricot Pie**	**Pixie Caramel**
Bite weight (g)	.647	.642	.636	.655	.638
Number of chews (after $\log_{10}$ transformation)	.819	.756	.797	.875	.649

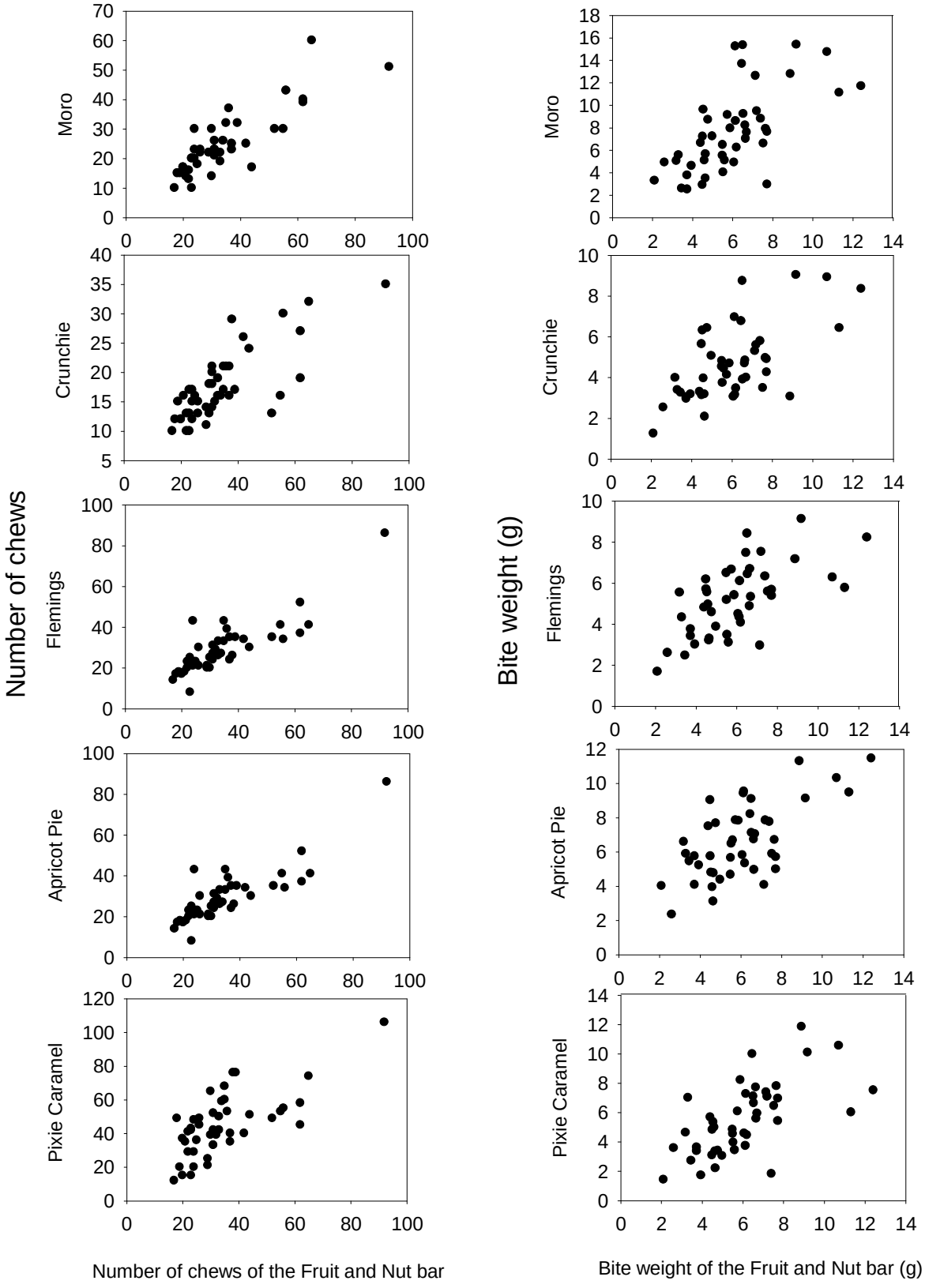

Figure 5-1: Scatter plot showing the relationship between chewing behaviour of the Fruit and Nut bar and the other bars used in Chapter 4.

5.4 Example of selecting a subject

Below is an example set of data used to select the subject in Chapter 6. This selection technique was used for choosing each single subject. Selected subjects were required to be within one standard deviation of the population mean for bite weight and the number of chews (population standard deviation of the Fruit and Nut bar), and required a standard deviation of less than 15% of the mean across five replicates for bite weight and number of chews (%SD with respect to the mean of the replicates of the Fruit and Nut bar).

Ten subjects volunteered to take part in trial 1 (Chapter 6). After an oral inspection by a qualified dentist, seven of the subjects were asked to bite and chew the Fruit and Nut bar (5 replicates).

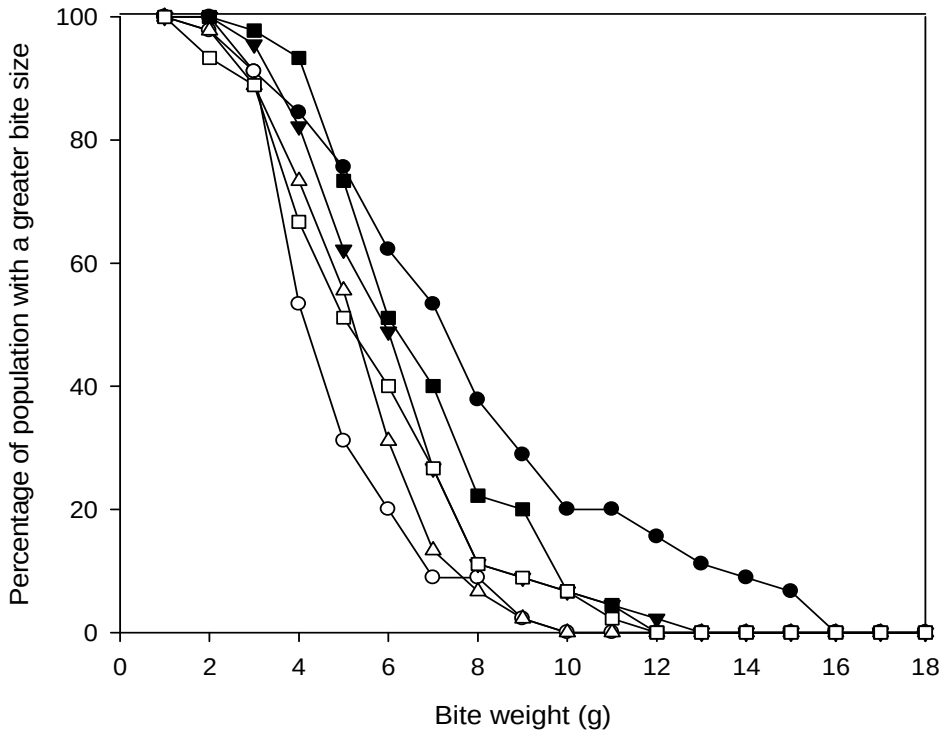

Figure 5-2: The cumulative distribution of natural bite weight of 45 subjects for Moro: ●, Crunchie: ○, Fruit and Nut: ▼, Muesli bar: △, Apricot Pie: ■, and Pixie Caramel: □.

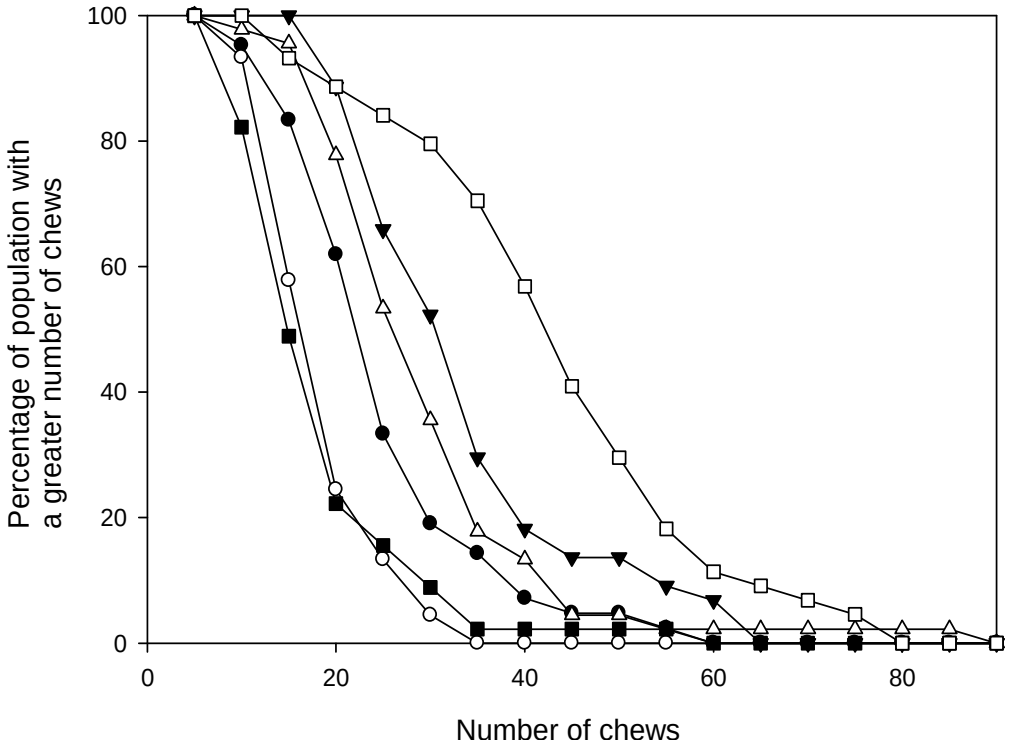

Figure 5-3: The cumulative distribution of the number of chews of 45 subjects for Moro: ●, Crunchie: ○, Fruit and Nut: ▼, Muesli bar: △, Apricot Pie: ■, and Pixie Caramel: □.

Figure 5-2 and Figure 5-3 show the cumulative distributions of bite weight and number of chews, which can be used as a benchmark for screening subjects (from Section 4.3). The Fruit and Nut bar has been used for selection (▼). The bite weight and number of chews of each applicant were compared with the cumulative distributions, to assess each applicant with reference to the population.

Selection decisions were made in terms of bite weight and number of chews (Table 5-2). Subject 6 was selected for this single subject study. This subject had a mean bite weight and number of chews well within 1 standard deviation the population mean, and had a low standard deviation among the 5 replicates for bite weight (7.8% of the mean) and the number of chews (7.2% of the mean). Subjects were rejected on the basis of bite weight or chew results which were outside the population mean or were highly variable (high standard deviation).

Table 5-2: The screening data using the Fruit and Nut bar (mean±SD) and decisions made for each applicant.

Subject	Bite weight (g)	Number of chews	Selection decision
1	6.00±0.98	25.8±2.4	High variability in bite weight
2	8.90±0.60	49.6±0.6	Large bite weight and number of chews
3	7.00±0.82	18.2±1.5	Low number of chews
4	2.68±0.44	33.6±0.4	Small bite weight
5	3.88±0.79	22.2±3.5	Small bite weight
6	5.41±0.42	29.2±2.1	Selected subject
7	4.88±0.19	18.4±1.1	Low number of chews
Population (45 subjects)	6.02±0.19	34.8±6.2	

Chapter 6 : Serving peanuts prepared inside different matrices

Four distinct single subject trials (Chapters 6-9) were undertaken to meet the remaining objectives of this research, before a multiple subject study (Chapter 10) was used to validate results with a larger population. This chapter is the first of the single subject trials investigating mastication and the food bolus of the matrices embedded with peanut particles.

6.1 Introduction

As outlined in Section 2.7, the physical properties of homogenous foods, notably texture, alter chewing behaviour (Hiiemae et al., 1996; Brown et al., 1998; Hiiemae & Palmer, 1999), and the final properties of the food bolus (Hoebler et al., 2000; Jalabert-Malbos et al., 2007). Current knowledge of mastication and the state of the ready-to-swallow food bolus in heterogeneous foods is limited. Consequently, the mechanism by which simple heterogeneous food systems are broken down, such as a system containing a single type of solid test piece embedded in a continuous matrix, is unknown. It is possible that the properties of both the test piece (peanuts in this case) and the matrix will influence chewing behaviour and the state of the food bolus. In particular, it is hypothesised that the matrix will influence mastication and the cohesion and lubrication in the bolus, and consequently alter the final particle size distribution of peanuts in the bolus.

Therefore, the aim of this study was to examine the chewing behaviour and chewing outcome of standardized heterogeneous foods, by preparing peanuts inside four different types of matrices (scone, gelatine gel (200 bloom), brownie, and chocolate), with a view to indentifying parameters that influenced chewing behaviour and the size distribution of peanut particles in the food bolus.

6.2 Methodology

6.2.1 Subject screening and selection

The subject used in this study was selected according to the procedure outlined in Chapter 5. The subject gave informed consent, and the study was approved by the Massey University ethics committee (Southern A Application 08/17).

The subject selected was a 26 year old male with class 1 occlusion, no significant tooth crowding, no obvious tooth decay, and healthy periodontal condition. He had no functional disturbance to mastication such as pain or clicking during chewing, nor did he have any other known oral or general health issues that could influence oral processing. The subject showed high levels of consistency for bite weight and the number of chews of the Fruit and Nut bar, with the means of these parameters lying within 1 standard deviation of the means of the reference population (Table 6-1).

Table 6-1: The bite weight and number of chews of the Fruit and Nut bar of a previous population studied and the selected subject (mean±SD).

	Bite weight	Number of chews	n
Previous population	6.02±2.15	34.8±16.2	45
Selected subject	5.41±0.42	29.2±2.1	1

6.2.2 Experimental procedure

Two trials were conducted:

1. Serving 4 types of matrices (scone, gelatine gel (200 bloom), brownie, and chocolate) each containing embedded peanut pieces, and serving peanut pieces on their own (no matrix) in a random order (4 matrix variants + 1 control (no matrix, peanuts only), 2 sessions, 3 replicates per session). The aim of this trial was to determine the influence of the physical properties of the matrix on the chewing behaviour and particle size distribution of peanuts after mastication.

2. Serving peanut pieces that had been removed from each of the matrices prepared as in trial one in a random order (4 peanut variants removed from matrices after preparation + 1 control (peanuts not prepared in a matrix), 1 session, 4 replicates). The aim of this trial was to quantify the effect of preparation inside the matrices on chewing behaviour and particle size outcome of peanuts on their own (i.e due to physical changes in the peanuts during baking or setting inside various matrices).

Experimental conditions and protocol followed that outlined in Section 3.2.1.

6.2.3 Assessment of natural bite size and selection of serving size

The selection of serving size was based on the findings and methods developed in Chapter 4. The natural bite length was determined by the subject taking natural bites from the four matrices (scone, gelatine gel (200 bloom), brownie and chocolate) prepared as bars of identical shape containing peanut pieces (20 mm height, 30 mm width, 100 mm length, containing 11.3% peanut quarters (v/v)). An overall mean bite length of 13±2 mm (mean±SD) was determined, and therefore a constant volume serving size of 7800 mm^3 (20x30x13 mm) was adopted for trials involving matrices containing peanuts to fit within the subjects natural bite range for the matrices.

6.2.4 Preparation of test foods

Matrices were prepared as bars containing peanut quarter pieces inside (where the kernel, a peanut half, was cut in half again), according to methods outlined in Section 3.1.3. Roasted unsalted peanuts were used in all matrices and in the control. All peanut quarters were sieved across a 4.75 mm sieve prior to preparation in the matrix to ensure no small particles were included. The bars were cut at 13mm intervals to obtain test pieces.

All servings were also weighed (Table 6-2). Based on the cut length of 13 mm, 1 g of peanuts was contained in each sample on average (equivalent to 11.6% peanuts by

volume). When peanuts were served without a matrix, 1.00±0.02 g (mean±SD) samples were used.

Analysis of the physical properties of the matrices and peanuts were undertaken according to methods described in Section 3.2.2. Textural analysis of the matrices involved 12 replicates. Four replicates were used for peanut moisture content, 12 replicates were used for peanut hardness, and 6 replicates for peanut density.

6.2.5 Analysis of the food bolus

Analysis of the particle size distribution of peanuts particles in the bolus was conducted according to Section 3.1.4 where a Rosin-Rammler function was fit to the data. Weight retention of peanuts was not assessed in this study as the exact weight of peanuts in each test piece was unknown, however estimates of the volume of peanuts in each bolus were undertaken.

6.2.6 Statistical analysis

Statistical analyses were performed using SPSS ® (version 15.0 for Windows). The Kruskal-Wallis test was used to assess significant differences in the parameters of mastication (number of chews, chewing time, and mastication frequency), and bolus parameters (d_{50}, b, and volume retention of peanut particles). These parameters were the dependant variables, and matrix was the factor. Where significant differences were found, the Kruskal-Wallis test was also run pair-wise to identify individual differences between each matrix (or each peanut group removed from each matrix). The Kruskal-Wallis test was used for statistical analysis of single subject trials in Chapters 6-8. The test was deemed to give a significant difference within factors when $P<0.05$.

6.3 Results

6.3.1 Properties of the food matrices and peanuts

The properties of the various matrices differed (Table 6-2). The following differences were noted:

- The scone matrix had the highest water activity, and was the softest matrix.
- The gelatine gel matrix was the chewiest, springiest and most cohesive matrix, and also had a high water activity.
- The chocolate was the hardest matrix, and had the lowest water activity.
- The peanut pieces absorbed moisture from the surrounding matrix and the extent of moisture absorption differed between matrices. Peanuts inside the scone matrix absorbed the largest amount of moisture (5% increase), while peanuts inside the chocolate matrix did not appear to absorb moisture.

Table 6-2: Properties of the test foods (mean±SE).

	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (mJ)	Water activity (aw) (at 25 ºC)	Serving weight (g) (Trial 1)	Peanut moisture content after preparation inside matrices* (gH_20/100g total mass)	Peanut density after preparation inside matrices* (g/cm^3)
Scone	31±2	0.32±0.01	5.46±0.27	56±5	0.903±0.011	5.67±0.18	7.2±0.35	1.07±0.01
Gelatine gel (200 bloom)	71±4	0.62±0.03	9.96±0.09	433±33	0.779±0.002	10.71±0.16	3.43±0.10	1.08±0.01
Brownie	126±9	0.23±0.01	2.07±0.14	60±6	0.634±0.009	4.36±0.13	2.69±0.32	1.08±0.01
Chocolate	389±12	0.15±0.01	1.70±0.16	99±10	0.505±0.004	9.96±0.19	1.94±0.07	1.07±0.02
Peanuts	140±13	0.25±0.02	1.12±0.05	42±7	0.442±0.003	1.02±0.02	1.99±0.10	1.07±0.01

*determined by preparing matrices with peanut pieces inside, and storing them for 24 h in the manner they were stored during the trial

6.3.2 Parameters of mastication

The number of chews ($H(3) = 15.127$, $P<0.005$), and the mean chewing time ($H(3) = 15.487$, $P<0.005$) differed significantly between matrices (Table 6-3) but the frequencies of mastication did not differ significantly ($H(3) = 5.040$, $P>0.05$). The gelatine gel (a chewy, cohesive, and springy matrix) was chewed the greatest number of times and for the longest period, and the brownie (the matrix with the lowest density) was chewed for the least number of times and for the shortest period (36.0 chews compared to 17.7 chews on average).

When peanut pieces that had been removed from the various matrices were chewed neither the number of chews ($H(4) = 5.503$, $P>0.05$), the chewing time ($H(4) = 6.729$, $P>0.05$), or the mastication frequency ($H(4) = 5.318$, $P>0.05$) differed significantly with the matrix type from which they originated (Table 6-4).

Table 6-3: Effects of matrices on parameters of mastication of test foods and particle size distribution of peanuts particles in the food bolus: Peanuts inside the matrix (mean±SE).

Matrix containing peanuts	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	d_{50} (mm)	*b*	Volume of peanuts in bolus (mm^3)
Scone	28.7±1.9 ac	17.47±0.82 ac	1.64±0.06	1.46±0.06 a	1.19±0.02 ac	1650±290 ac
Gelatine gel (200 bloom)	36.0±3.8 a	22.29±2.00 a	1.61±0.01	1.43±0.06 a	1.14±0.02 a	1950±240 a
Brownie	17.7±0.8 b	10.97±0.55 b	1.6±0.01	1.22±0.03 b	1.24±0.02 c	1260±170 bc
Chocolate	25.3±1.7 c	15.93±1.07 c	1.59±0.02	1.18±0.03 b	1.13±0.03 a	1300±160 ac
No Matrix (peanuts only)	8.00±0.4	4.97±0.30	1.61±0.04	0.98±0.03	1.14±0.03	980±40

Different letters (a & b) down each column indicate a significant statistical difference after pair-wise Kruskal-Wallis tests ($P<0.05$). Peanut pieces which were not embedded in a matrix at all were not included in the Kruskal-Wallis tests (Pair-wise analysis was only conducted when a significant overall difference was found).

Table 6-4: Effects of preparation inside different matrices on parameters of mastication of peanut pieces and resulting particle size distribution of peanut particles in the food bolus: Peanuts prepared inside the matrix and REMOVED before mastication (mean±SE) (WITHOUT matrices).

Matrix	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	d_{50} (mm)	*b*	Volume of peanuts in bolus (mm^3)
Scone	7.8±0.4	5.04±0.18	1.58±0.03	1.24±0.04 a	1.13±0.03	1640±70 a
Gelatine gel (200 bloom)	7.5±0.3	4.57±0.10	1.64±0.03	1.13±0.03 ab	1.19±0.02	1350±40 b
Brownie	7.5±0.3	4.76±0.15	1.58±0.04	1.04±0.02 b	1.13±0.03	1270±20 b
Chocolate	7.0±0.4	4.57±0.06	1.53±0.02	1.04±0.02 bc	1.14±0.01	1020±40 c
No Matrix (peanuts only)	7.8±0.2	5.00±0.16	1.55±0.05	0.97±0.01 c	1.17±0.02	980±20 c

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$) (Pair-wise analysis was only conducted when a significant overall difference was found).

6.3.3 Properties of the collected boluses

A. Peanuts served inside the matrices

The particle size distributions of the peanut particles after mastication in the various matrices were different in terms of the range of the standard error (Figure 6-1). The size distributions of peanut particles from the scone and gelatine gel were outside the range of both the brownie and the chocolate. Similarly they all differed from that of the particles of peanuts without a matrix. These differences were confirmed by Kruskal-Wallis test of the d_{50} values ($H(3) = 14.129$, $P<0.005$). By running the Kruskal-Wallis test pair-wise it was found the d_{50} of peanut particles from the scone and gelatine gel was significantly larger than the brownie and chocolate, and all were significantly larger than particles from peanuts without a matrix (Table 6-3). On average the peanut particles from the scone matrix had a d_{50} of 1.46 mm, where as peanut particles from the chocolate matrix had a d_{50} of 1.18 mm.

The broadness value (*b)* (derived from the Rosin-Rammler function, Chapter 3, Section 3.1.4), which describes the spread of the peanut particle size distribution, also differed significantly between matrices according to the Kruskal-Wallis test ($H(3) = 8.491$, $P<0.05$). Pair-wise analysis showed the broadness values of the peanut particles inside

the brownie were significantly larger (and therefore less spread in the particle size distribution) in comparison with the gelatine gel and the chocolate (Table 6-3). The estimated volume of peanut particles in the bolus (based on the 2D particle area and assuming sphericity, see Section 3.1.4) did not differ significantly ($H(3) = 5.507$, $P>0.05$) between matrices (Table 6-3).

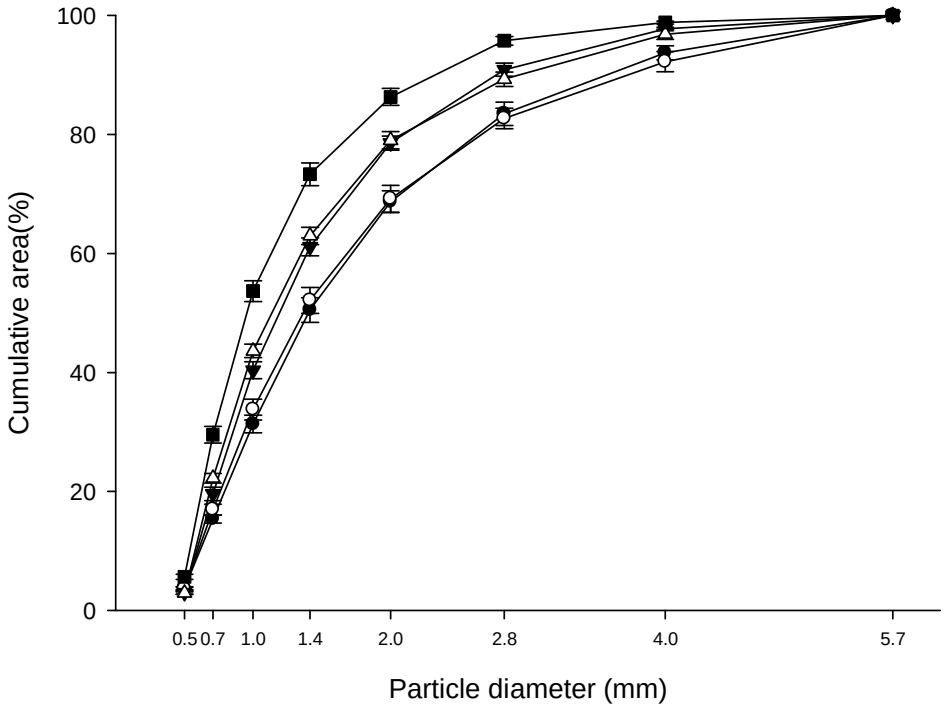

Figure 6-1: Cumulative particle size distribution of peanut particles in the food bolus where peanut pieces were prepared and served inside different food matrices. Scone: ●, Gelatine gel: ○, Brownie: ▼, Chocolate: Δ, and No matrix: ■. Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (6 replicates) (mean±SE).

B. Peanuts removed from the matrices and served alone

The particle size distributions after mastication of peanut pieces removed from the various matrices (and then masticated) were also different in terms of the range of the standard error (Figure 6-2). The ranges of all distributions differed except for particles of the peanut pieces removed from the brownie and chocolate, which were similar. The d_{50} of particles differed significantly between peanut pieces removed from various matrices according to the Kruskal-Wallis test ($H(4) = 16.294$, $P<0.005$). Pair-wise analysis found the particle size of the bolus of peanut pieces removed from the scone was significantly larger than those removed from the brownie and the chocolate, and all

were significantly larger than particles of peanut pieces that were not prepared in a matrix (Table 6-4) (the d_{50} was 1.24 mm on average for bolus particles of peanuts removed from the scone, and was 1.04 mm on average for those removed from the chocolate and brownie).

The broadness values (*b)* of the peanut particle distribution did not differ significantly between pieces removed from various matrices (H(4) = 5.496, P>0.05) according to the Kruskal-Wallis test, however there was a significant difference in the estimated volume of peanut particles in the bolus between the matrices (H(4) = 19.025, P<0.005) (Table 6-4).

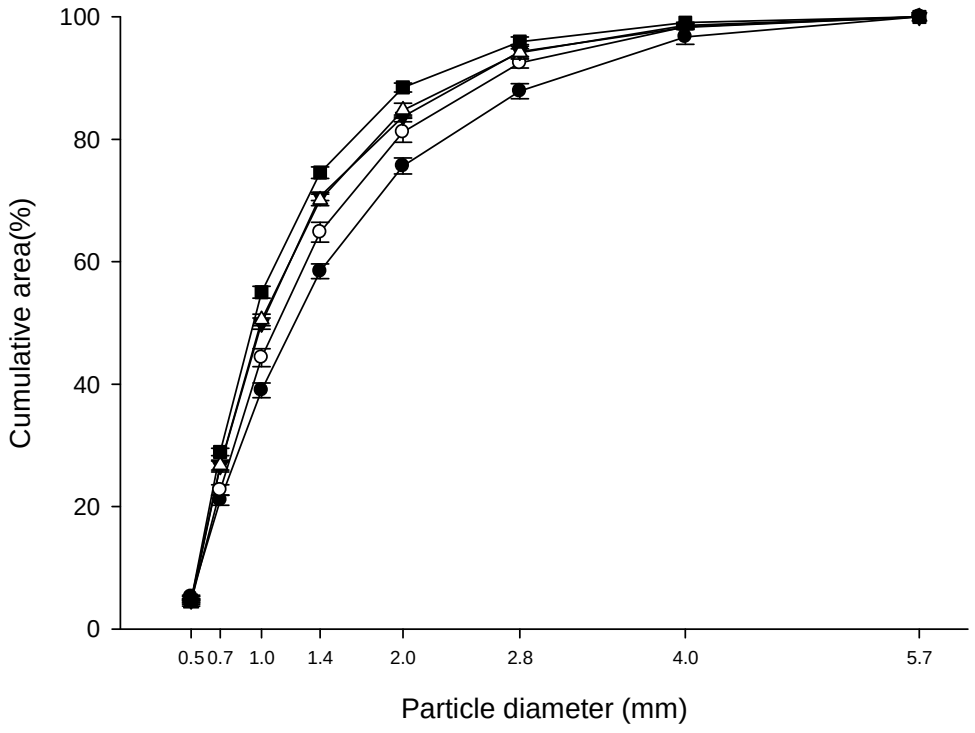

Figure 6-2: Cumulative particle size distribution of peanut particles in the food bolus where peanuts were served WITHOUT matrices (after being prepared inside different food matrices). Scone: ●, Gelatine gel: ○, Brownie: ▼, Chocolate: ∆, and No matrix: ■. Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (4 replicates) (mean±SE).

6.4 Discussion

6.4.1 Relationships between food properties, chewing behaviour, and bolus particle size

As the initial moisture content of peanut pieces increased the d_{50} of peanut particles in the bolus increased. This relationship can be seen clearly when peanuts pieces were removed from the matrices and served (Figure 6-3B) but is also evident for peanut pieces served inside the matrices (Figure 6-3A). There were no trends seen between d_{50} of peanut particles in the bolus and chewing behaviour (Figure 6-3C). As the number of chews applied to the test foods increased, the corresponding d_{50} of peanut particles in the bolus increased rather than decreased as would be expected. Previous studies in homogeneous foods have shown significant correlations between an increasing number of chewing cycles and a decreasing d_{50} (Olthoff et al., 1984; Jalabert-Malbos et al., 2007).

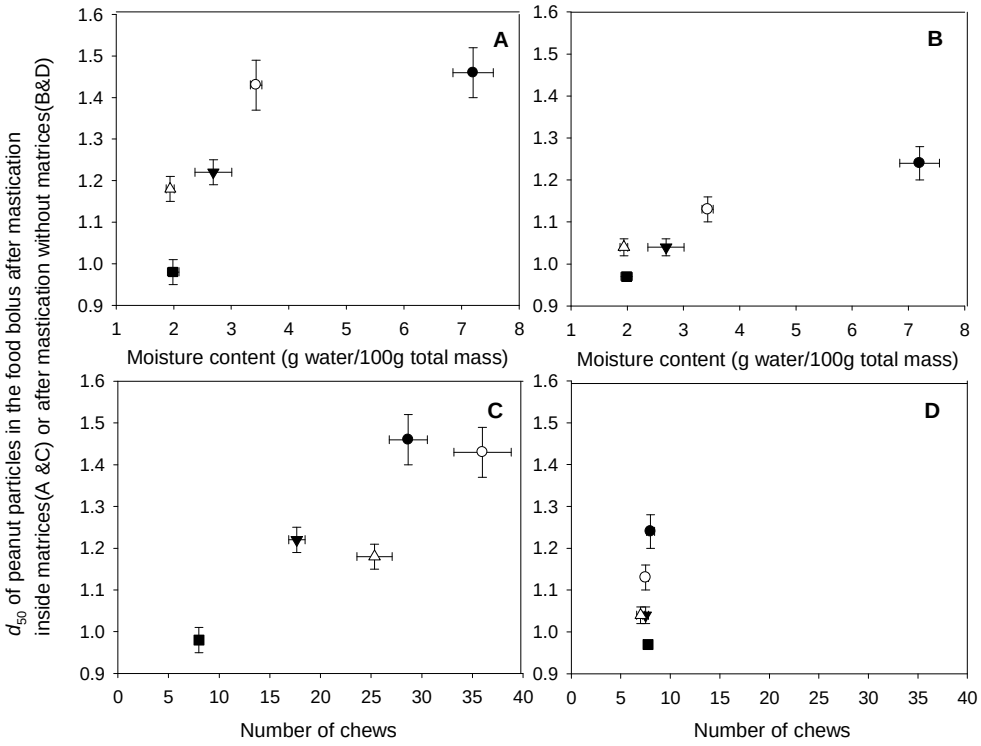

Figure 6-3: The relationship between d_{50} (estimated sieve size at which 50% of the bolus area of peanut particles would pass), moisture content, and the number of chews during mastication of peanuts served inside matrices, and served after removal from matrices. Scone: ●, Gelatine gel: ○, Brownie: ▼, Chocolate: Δ, and No matrix: ■ (mean±SE).

6.4.2 The influence of the matrix and the peanut piece on mastication

The significant differences in chewing behaviour between matrices are consistent with other published data for homogenous foods (Foster et al., 2006; Jalabert-Malbos et al., 2007). Interestingly, where peanut pieces that were removed from matrices were chewed, no significant differences in mastication parameters were observed, despite large differences in moisture content (Table 6-4, Figure 6-3D). This suggests that chewing behaviour was not affected by the embedded peanut piece but by the matrices.

6.4.3 The influence of the matrix and the peanut piece on particle size outcome in the food bolus

A. Peanuts served inside the matrices

The matrices caused significant differences in the peanut particle size distribution of the bolus in terms of the d_{50} and broadness *(b)* values (Figure 6-1, Table 6-3). As the differences in chewing behaviour between matrices did not correspond to expected trends in d_{50} of peanut particles in the bolus (Figure 6-3C), and as d_{50} trends between the two trials were similar (Figure 6-1, Figure 6-2), the influence of the matrix on the d_{50} of peanut particles in the bolus appears limited. However, the significant difference in broadness of the peanut particle size distribution between matrices (which was not found with peanuts removed from the matrices) suggested the matrices may influence the spread of the size distribution. Further work is undertaken in Chapter 7 to clarify the effect of matrix properties on peanut particle size by eliminating the confounding effect of moisture migration.

B. Peanuts removed from the matrices and served alone

Changes in the peanut properties during preparation have caused differences in the d_{50} of peanut particles in the bolus (Figure 6-2, Table 6-4). The relationship between moisture content and d_{50} (Figure 6-3B), and differences in water activity between matrices (Table 6-2), suggests moisture migration during baking or setting is causing this difference. Uptake of water has been widely reported to influence the textural

properties of foods (Roos, 1995), particularly in nuts (Visvanathan et al.,1996; Paksoy & Aydin 2004; ElMasry et al., 2009). Moist, soft peanuts may have less friction against the oral mucosa than hard, dry peanuts, and so can be therefore be swallowed at a larger particle size as a masticated bolus. Differences in the particle size distribution are known to occur when different types of foods are masticated and expectorated at the swallowing point (Hoebler et al., 2000; Mishellany et al., 2006; Jalabert-Malbos et al., 2007), and also when peanuts have been subjected to different heat processing conditions (Mac Kiernan & Mattes, 2010).

The significant difference in d_{50} of peanut particles in the bolus is present despite no overall significant difference in chewing behaviour, which suggests that the breakage function of peanuts has changed during preparation and storage inside the matrices (the extent that the particle fragments per chew) (Lucas et al., 2002; Lucas et al., 2004). The breakage function is known to be influenced by the toughness and Young's modulus of foods (Agrawal et al., 1997; Lucas et al., 2002).

6.5 Conclusion

This study found that in a heterogeneous food system where peanut pieces were prepared within various matrices, chewing behaviour and the peanut particle size outcome (in terms of d_{50} and broadness (b)) in the food bolus differed between matrices. Peanut particle size outcome (in terms of d_{50} but not broadness (b)) also differed between peanut pieces which had been removed from the matrices, despite there being no differences in chewing behaviour. Moisture content changes in the peanut pieces during preparation are likely to be the main cause of d_{50} differences in the heterogeneous system. The influence of the matrix without the confounding influence of changes in peanut properties during preparation remains unclear, and requires further investigation.

Chapter 7 : Serving peanuts manually inserted into different matrices after preparation

7.1 Introduction

Moisture migration occurs between two mediums of contrasting water activity, where a water activity gradient exists (Labuza & Hyman, 1998). The uptake of water has been shown to alter the textural properties of food products including those of nuts (Visvanathan et al., 1996; Paksoy & Aydin 2004; ElMasry et al., 2009).

Chapter 6 showed there were significant differences in the mastication of test foods (different matrices prepared with peanuts inside) and the resulting particle size distribution of peanut particles in the food bolus. However, these results had been confounded by the uptake of moisture into the peanut pieces during preparation inside the various matrices. Results suggested that the uptake of water into the peanut pieces was having a significant influence on the size distribution of peanut particles in the food bolus.

Consequently, further experiments were required to eliminate the confounding effect of moisture into the peanut pieces, to exclusively assess the influence of the matrices on mastication and the food bolus. To achieve this, matrices were prepared without peanut pieces, and the peanut pieces were subsequently inserted manually into the matrices immediately prior to being served to the subject. This prevented any effect from moisture migration during preparation inside different matrices, and also allowed a precise weight (and hence volume) of peanut pieces to be present in each test sample.

Furthermore, a comparison was made between the mastication of matrices with and without peanut pieces. It has been shown that mastication is different between food products (Brown et al., 1998; Hiiemae & Palmer, 1999), and it is known that serving size influences mastication (Daet et al., 1995; Gavio et al., 2004), however the effect of removing the peanut pieces from the matrices on mastication was unknown. It is

hypothesised that the presence of the peanuts will alter mastication (and perhaps the relative differences in mastication between matrices).

Therefore, the aim of this study was to examine the chewing behaviour and resulting particle size distribution of standardized heterogeneous foods (peanut pieces inside different types of matrices (scone, gelatine gel (200 bloom), brownie, and chocolate) with the effect of moisture migration eliminated. In addition, the study aimed to investigate the mastication of the matrices without peanut pieces present inside (a homogenous matrix), to compare the influence of matrices with and without peanuts.

7.2 Methodology

7.2.1 Subject screening and selection

The subject was selected according to the procedure outlined in Chapter 5. The subject gave informed consent, and the study was approved by the Massey University ethics committee (Southern A Application 09/24).

The subject selected was a 20 year old female with class 1 occlusion, no significant tooth crowding, no obvious tooth decay, and healthy periodontal condition. She had no functional disturbance to mastication such as pain or clicking during chewing, nor did she have any other known oral or general health issues that could influence oral processing. The subject showed high levels of consistency for bite weight and the number of chews of the Fruit and Nut bar, with the means of these parameters lying within 1 standard deviation of the means of the reference population (Table 7-1).

Table 7-1: The bite weight and number of chews of the Fruit and Nut bar of a previous population studied and the selected subject (mean±SD).

	Bite weight	Number of chews	n
Previous population	6.02±2.15	34.8±16.2	45
Selected subject	7.56±1.00	26.0±1.9	1

7.2.2 Experimental procedure

The study involved two trials:

1. Peanuts served inside 4 different matrices in a random order (scone, gelatine gel (200 bloom), brownie and chocolate) containing peanut pieces (4 matrix variants, 2 sessions, 3 replicates per session). The aim of this trial was to determine the influence of the physical properties of the matrix on the chewing behaviour and size distribution of peanut particles after mastication, without the influence of moisture migration into the peanuts.

2. Four different matrices (scone, gelatine gel (200 bloom), brownie and chocolate) were served as matrices without any peanuts in a random order (4 matrix variants, 2 sessions, 3 replicates per session). The aim of this trial was to understand how mastication of the matrices changed when no peanuts were present in the system.

Experimental conditions and protocol followed that outlined in Section 3.2.1.

7.2.3 Assessment of natural bite size and selection of serving size

The selection of serving size was based on the findings and methods developed in Chapter 4. The natural bite length was determined by the subject taking natural bites from the four matrices (scone, gelatine gel (200 bloom), brownie and chocolate) prepared as bars of identical shape containing peanuts (20 mm height, 30 mm width, 100 mm length, containing 11.3% peanut quarters (v/v)).

An overall mean bite length of 19±3 mm (mean±SD) was determined, and a constant volume serving size of 10800 mm^3 (20x30x18 mm) was adopted for both trials to fit within the subjects natural bite range for the matrices.

7.2.4 Preparation of test foods

Matrices were prepared without peanuts inside according to methods outlined in Section 3.1.3. Peanut pieces were manually inserted into the matrix test piece (cut from the matrix bar according to the subjects bite length) immediately before each sample was served to the subject to ensure no unwanted moisture migration took place (peanut moisture content: 1.99±0.20 gH_20/100g). Roasted, unsalted peanuts were used in all matrices. All peanut pieces were sieved across a 4.75 mm sieve prior to preparation in the matrix to ensure no small particles were included. All servings of matrices and peanuts were weighed (Table 7-2). Each sample in Trial 1 contained 1.34±0.04 g peanuts (mean±SD, 4-5 peanut quarter pieces), and therefore each matrix contained 11.5% (v/v) peanuts (where $\rho_{peanuts} = 1.08$ g/cm^3).

Analysis of the physical properties of the matrices and peanuts were undertaken according to methods described in Section 3.2.2. Textural analysis of the matrices involved 12 replicates, and 6 replicates were used for measuring peanut density.

7.2.5 Analysis of the food bolus

Analysis of the particle size distribution of peanuts particles in the bolus was conducted according to methods outlined in Section 3.1.4, where a Rosin-Rammler function was fit to the data.

7.2.6 Statistical analysis

Statistical analyses were performed using SPSS ® (version 16.0 for Windows). The Kruskal-Wallis test was used to assess significant differences in the parameters of mastication (number of cycles, chewing time, and mastication frequency), and bolus parameters (d_{50}, b, weight retention, and volume retention of peanut particles). These parameters were the dependant variables, and matrix was the factor. Where significant differences were found, the Kruskal-Wallis test was also run pair-wise to identify individual differences between each matrix. The Kruskal-Wallis test was deemed to give a significant difference within factors when $P<0.05$.

7.3 Results

7.3.1 Properties of the food matrices and peanuts

Table 7-2: Properties of the test foods (mean±SE).

Matrix	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (mJ)	Serving weight of matrix (Trial 1) (g)	Serving weight of matrix (Trial 2) (g)
Scone	31±2	0.320±0.01	5.46±0.27	56±5	6.39±0.10	7.88±0.13
Gelatine gel (200 bloom)	71±4	0.615±0.03	9.96±0.09	433±33	11.15±0.06	13.70±0.17
Brownie	126±9	0.230±0.01	2.07±0.14	60±6	5.04±0.03	5.77±0.09
Chocolate	389±12	0.151±0.01	1.70±0.16	99±10	12.36±0.11	14.36±0.10

The contrasting physical properties of the matrices are shown in Table 7-2 (identical to Chapter 6). The total volume of each test sample was the same in both trials of this chapter (10800 mm^3), however matrix serving weights for Trial 2 were greater than Trial 1 as no peanuts were present in Trial 2 test pieces.

7.3.2 Peanuts embedded inside four different matrices

Table 7-3 shows the scone was chewed for the greatest number of chews on average (25.8), where as the brownie was chewed for the shortest number of chews on average (20.3). The number of chews did not differ significantly between matrices according to the Kruskal-Wallis test ($H(3) = 5.180$, $P>0.05$), however the mean chewing time ($H(3) = 9.294$, $P<0.05$) and mastication frequency ($H(3) = 13.112$, $P<0.005$) differed significantly between each matrix. By running the Kruskal-Wallis test pair-wise it was found the brownie was chewed for the shortest period of time, and the chocolate at the slowest frequency (Table 7-3).

The particle size distributions of the peanut particles after mastication in the various matrices were all similar in terms of the range of the standard error (Figure 7-1). The similarity in particle size was confirmed by Kruskal-Wallis test of the d_{50} values ($H(3) =$

3.805, P>0.05), and the broadness values *(b)* (H(3) = 5.805, P>0.05), which showed a lack of significance in both cases.

Weight retention (H(3) = 6.068, P>0.05) and volume of peanuts in the bolus (H(3) = 3.246, P>0.05) did not differ significantly between matrices (Table 7-3).

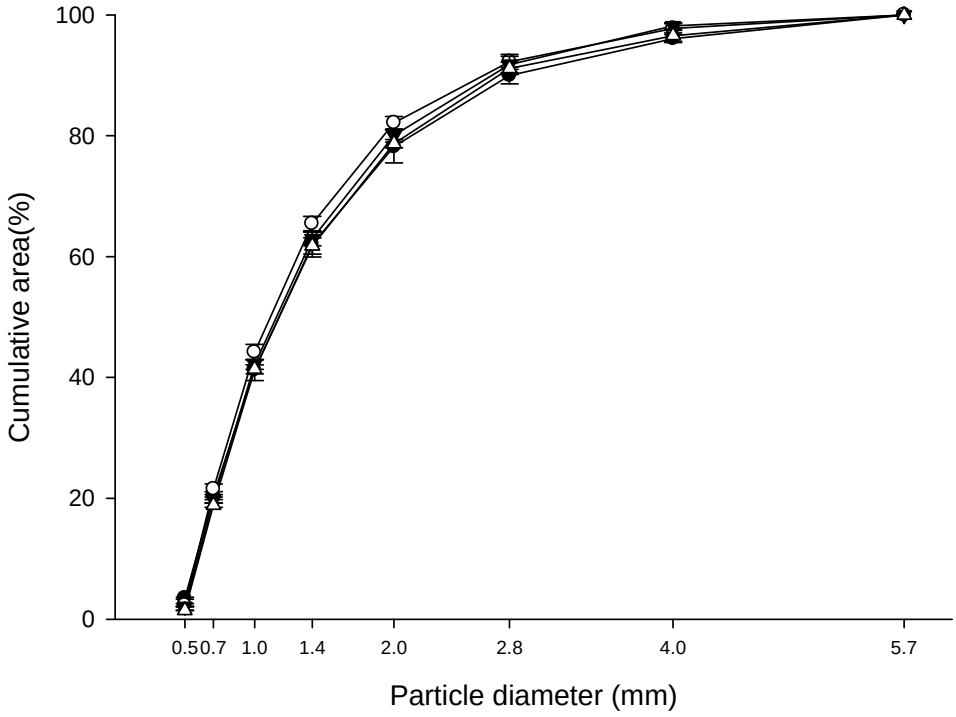

Figure 7-1: Cumulative particle size distribution of peanut particles in the food bolus where peanuts were prepared and served inside different food matrices. Scone: ●, Gelatin gel: ○, Brownie: ▼, Chocolate: Δ. Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (6 replicates, mean±SE).

Table 7-3: Mastication of peanuts embedded inside four different matrices: Parameters of mastication, particle size, and bolus retention (mean±SE).

Matrix containing peanuts	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	d_{50}	b	% Peanut weight retention (drywt/dry wt)	Volume of peanuts in bolus (mm^3)
Scone	25.8±1.8	18.27±1.42 a	1.42±0.02 a	1.22±0.04	1.17±0.02	21.47±1.29	1460±90
Gelatine gel (200 bloom)	24.3±2.0	17.40±1.35 a	1.40±0.02 a	1.18±0.03	1.22±0.05	28.23±2.04	1620±70
Brownie	20.3±1.0	14.19±0.74 b	1.43±0.02 a	1.20±0.02	1.28±0.03	25.53±1.54	1550±60
Chocolate	23.3±1.5	19.33±1.11 a	1.21±0.03 b	1.26±0.02	1.26±0.03	25.35±1.92	1480±40

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$). Peanuts which were not embedded in a matrix at all were not included in the Kruskal-Wallis tests (Pair-wise analysis was only conducted when a significant overall difference was found).

7.3.3 Four different matrices without peanuts

The number of chews ($H(3) =16.819$, $P<0.005$), the chewing time ($H(3) = 14.154$, $P<0.005$) and mastication frequency ($H(3) = 11.172$, $P<0.05$) were significantly different between matrices (Table 7-4). The gelatine gel was chewed the greatest number of times and for the longest period (15.5 chews on average), and the brownie was chewed the least number of times and the shortest period (9.5 chews on average). The number of chews and chewing time is vastly shorter when peanuts were not included inside the matrices, despite the same volumes being served.

Table 7-4: Mastication of four different matrices with peanuts removed: Parameters of mastication (mean±SE).

Matrix	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})
Scone	11.5±0.8 a	8.45±0.61 ac	1.36±0.02 a
Gelatine gel (200 bloom)	15.5±0.6 b	10.94±0.50 b	1.42±0.03 a
Brownie	9.5±0.3 c	7.15±0.33 a	1.33±0.04 ab
Chocolate	11.8±0.6 a	10.01±0.50 cb	1.19±0.05 b

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$).

7.4 Discussion

7.4.1 Peanuts embedded inside four different matrices

Results showed that different matrices were being processed differently in the mouth (chewing time and mastication frequency were significantly different, and differences were seen in the number of chews despite a lack of significance) (Table 7-3). However, as the properties of the internal peanut piece were kept constant, no differences in the properties of the bolus were found in terms of d_{50}, broadness (*b)*, volume retention, or weight retention of peanut particles.

This shows that the significant differences in d_{50} in Chapter 6 resulted from differences in properties of the peanut pieces inside the matrix (i.e moisture), rather than from differences the matrices may have on manipulating the d_{50} required to form a swallow-safe bolus. Hence these results suggest that despite differences in chewing behaviour and physical properties of the matrix, the peanut particles needed to be reduced to a similar particle size distribution no matter which matrix it is inside. The difference in chewing behaviour but similarity in particle size in the bolus indicates the matrices may be influencing the selection of peanut particles between the molars.

The influence the matrix has on the broadness (*b*) value of the peanut particle size distribution inside the bolus remains unclear. Chapter 6 suggested the matrix may cause differences in the broadness of the distribution, however the differences are not significant is this study. Further investigation is undertaken in Chapters 8-10.

The similarity in peanut particle size outcome somewhat challenges previous literature surrounding a swallow-safe bolus. Boluses are believed to be safe for swallowing once particles reach a certain size distribution and degree of lubrication (where both variables vary between foods) (Hutchings and Lillford, 1988), and the bolus reaches a maximum degree of cohesion (Prinz & Lucas, 1997). Different matrices are likely to induce different rheological properties (such as lubrication and cohesion) in the food bolus, however this has not induced differences in the size of peanut particles between matrices.

One possible explanation for the similarity in particle size outcome is that receptors inside the mouth can precisely detect the texture and size of the peanut particles within all the different matrices. Such acute detection of particles may mean that differences in cohesion or lubrication of the surrounding matrix are largely irrelevant in determining final peanut particle size. In the test foods used, the size of particles may also be the priority in preparing a swallow safe bolus, and the cohesion or lubrication of the surrounding matrix is not deemed as important. Receptors on the tongue and oral mucosa can detect single particles as small as 2 mm (Ringel & Eawonski, 1965; Lucas, 2004), and the threshold discrimination on teeth is reported to be as small as 8-15 μm by some authors (Utz 1983 in Lucas, 2004). Furthermore, particles as small as 2 μm (Engelen et al., 2005a) to 10 μm (Imai et al., 1995) can influence textural sensations.

It is also feasible that the peanut particles are treated independently from the matrix during mastication. Peanuts could be isolated or removed from the matrix early in the chewing sequence, and could be broken down and determined suitable for swallowing separately of the matrix. In this case peanut particles may be stored in a different part of the oral cavity (a different compartment) than the matrix during the chewing sequence, where the matrix and peanuts are returned separately between the molars for communition. Flynn (2010) presented a hypothesis that mastication occurs within a multi-compartment system where particles are stored in at least one compartment (i.e the tongue), but can settle in a separate compartment (i.e around the cheek or the sides of the molars).

7.4.2 Four different matrices without peanuts

Mastication of matrices without peanut pieces showed that the presence of peanuts greatly increases the time and number of chews spent chewing the same initial volume of food (Table 7-3 and Table 7-4). The presence of peanuts in a system may increase the mastication required to prepare the bolus for swallowing because peanuts are a hard and crunchy material (Young & Schadel, 1990) in comparison to the matrices.

Some differences in chewing trends can also be seen between the two trials (in terms of which matrix was chewed for the longest time and greatest number of chews). Most

notably, the scone is chewed for a longer period of time and for a greater number of chews than the gelatine gel when peanuts were present, where as the opposite was found without peanuts in the matrices. This suggests a small interaction in chewing behaviour between peanuts and matrices.

7.5 Conclusion

When peanuts of identical physical properties where manually inserted into different food matrices and masticated, differences in chewing behaviour were observed between matrices, however the particle size distribution of the peanut particles in the bolus was similar. Weight and volume retention of peanuts the bolus after mastication were not influenced by the type of matrix. When the matrices (of an identical initial volume) were masticated without peanuts, the number of chews and chewing time was greatly reduced.

Chapter 8 : Identification of parameters to manipulate particle size in the food bolus

8.1 Introduction

Knowledge of techniques to manipulate particle breakdown rates and the particle size distribution of the ready-to-swallow food bolus is limited. Much research compares the particle size distribution of different food types, but does not investigate how different particle size outcomes could be obtained in food products by careful food manufacturing and design. It is well known that the particle size of the food bolus differs between foods (Hoelber et al., 2000; Peyron et al., 2004b; Michellany et al., 2006), and has also been shown between foods subjected to differing heat treatments (McKeirnan & Matts, 2010). Furthermore, almonds subjected to different roasting conditions have different breakage properties, and these breakage properties have been linked to differences in consumer acceptability (Varela et al., 2008).

The work discussed in Chapter 7 had shown that the test matrices used were not suitable media for changing the final d_{50} of peanut particles in the food bolus, but suggested that they could potentially change the selection function of the peanuts. Furthermore, the work in Chapter 6 suggested moisture of the internal peanut piece influenced the breakage function and final particle size outcome of peanut particles in the bolus. Therefore, the aim of this study was to clarify the effect of moisture content of peanut pieces and the effect of matrices, and to explore a wider range of variables for changing mastication and the bolus particle size. A number of experiments were devised to achieve this:

1. Maximising differences in matrix and peanut properties

Chapter 6 showed increasing the moisture content of peanuts resulted in peanut particle size distributions with higher d_{50}'s, and Chapter 7 showed different matrices resulted in different chewing behaviour but not in d_{50} of peanut particles in the bolus. The magnitude of differences in the initial matrix and peanut properties could be enhanced

(by increasing differences in textural properties of the matrices and increasing differences in moisture content of peanuts) to seek larger changes in oral processing and particle size outcome. The interaction between matrices and peanut properties was also unknown. It was hypothesised that trends in peanut particle size outcome between the two matrices may be different for dry and moist peanuts, given that the type of peanut and type of matrix could interact to influence the final particle size in a swallow safe bolus.

2. The concentration of peanuts inside the matrices

As serving size has been shown to influence mastication (Lucas & Luke 1984; Fontijn-Tekamp et al., 2004b) and particle size outcome in the food bolus (Buschang et al., 1997) it was postulated that changing the initial quantity of peanut pieces inside the matrices may also influence mastication and the bolus. Reducing the concentration of peanuts inside the matrices may result in less competition between matrices and peanuts at the occlusal surfaces, or may reduce the ability of mechanoreceptors on the surfaces of tongue and oral mucosa to detect peanut particles during mastication.

3. The initial particle size of peanuts inside the matrices

Initial particle size has been shown to influence jaw movement (Diaz-Tay et al., 1991; van der Bilt et al., 1991), and jaw muscle activity (Kohyama et al., 2007). Therefore changing the initial size of peanut pieces inside the matrices may also result in differences in chewing behaviour and peanut particle size outcome.

4. Saliva stimulants (sialogogues)

Food properties influence the quantity of saliva that is added to the food bolus (Hoebler et al., 1998), where harder and dryer products required greater amounts of saliva before the bolus reaches the swallowing threshold (Gavio et al., 2004; Engelen et al., 2005b). Moreover, salivary flow rate may (Engelen et al., 2005b), or may not (Gavio et al., 2004) affect the number of chews to prepare a food for swallowing. Consequently, it was postulated that changing the quantity of saliva stimulated during the mastication of

peanuts may influence mastication and particle size outcome of peanuts in the food bolus.

5. Heat treatment of peanuts

Given the influence of moisture on bolus particle size (Chapter 6), various forms of heat treatment could be used to change moisture content and peanut structure. Heat treatment could therefore alter textural properties, and induce differences in fracture propagation and the final size of peanut particles formed in a swallow safe bolus.

8.2 Methodology

8.2.1 Subject screening and selection

The subject used in this study was selected according to the procedure outlined in Chapter 5. The subject gave informed consent, and the study was approved by the Massey University ethics committee (Southern A Application 09/24).

The subject selected (from a group of 6 subjects) was a 28 year old male with class 1 occlusion, no significant tooth crowding, no obvious tooth decay, and healthy periodontal condition. He had no functional disturbance to mastication such as pain or clicking during chewing, nor did he have any other known oral or general health issues that could influence oral processing. The subject showed high levels of consistency for bite weight and the number of chews, and his mean bite weight and number of chews from the Fruit and Nut bar was well within 1 standard deviation of the means of the reference population (Table 8-1).

Table 8-1: The bite weight and number of chews of the Fruit and Nut bar of a previous population studied and the selected subject (mean±SD).

	Bite weight	Number of chews	n
Previous population	6.02±2.15	34.8±16.2	45
Selected subject	6.73±0.88	32.0±1.9	1

8.2.2 Experimental procedure

Trial 1: Evaluated the effects of matrix type and moisture content of peanuts. The peanut pieces were manually inserted in a matrix of either gelatine gel (250 bloom) or chocolate. This gelatine was chosen to increase chewing work required by the subject in comparison with the previous gelatine. Peanuts of a high moisture content (22.21±0.18 gH_20/100g total mass) or peanuts of a regular moisture content (1.99±0.20 gH_20/100g total mass) were embedded inside both matrices (4-5 peanut quarter pieces). A constant volume of peanuts was served in both cases (11.3% peanuts (v/v)) inside

each matrix (2 matrix variants x 2 peanuts variants x 1 session x 4 replicates = 16 samples). Samples were served in a random order.

Trial 2: Evaluated the effect of matrices at lower concentrations of peanuts (3.1%(v/v) peanuts). One dry peanut quarter piece was embedded and then served in either one of three different matrices: scone, chocolate and gelatine gel (200 bloom) in a random order (3 matrix variants x 1 session x 5 replicates = 15 samples).

Trial 3: Evaluated the effect of the initial size of the dry peanut pieces embedded in the matrix. One peanut quarter piece or small peanut pieces (7-8 pieces) were embedded in a scone matrix with the total volume of peanut remaining constant (3.1 % (v/v) peanuts) (2 peanut size variants x 1 session x 4 replicates = 8 samples). Samples were served in a random order.

Trial 4: Evaluated the effect of increasing the amount of saliva secreted during oral processing by the use of icing sugar and citric acid as secretary stimulants. Peanut quarter pieces were mixed with either sherbet (10% citric acid, 90% icing sugar (wt/wt)), icing sugar, or served alone with no added material (3 stimulant variants x 1 session x 5 replicates = 15 samples). Samples were served in a random order.

Trial 5: Evaluated the effect of heat treatment on peanuts. Peanut pieces (peanut halves rather than quarters in this case to avoid shattering) were served to the subject. Raw blanched peanuts were roasted, boiled and dried, or were not subjected to any treatment (3 peanuts heat treatments x 1 session x 5 replicates = 15 samples). Samples were served in a random order.

Where food matrices were used, peanuts were randomly embedded inside each matrix immediately before serving so that moisture migration from matrices to peanuts would not take place.

Experimental conditions and protocol followed that outlined in Section 3.2.1.

8.2.3 Assessment of natural bite size and selection of serving size

The selection of serving size was based on findings and methods in Chapter 4. The subject took part in a total of 5 trials.

In Trials 1 to 3, the size of the serving was standardized by volume. The serving volume was determined by asking the subject to take bites from the four matrices used in this study (gelatine gel (250 bloom), gelatine gel (200 bloom), scone, and chocolate) prepared as constant bars of identical shape containing peanut quarters (20 mm height, 30 mm width, 100 mm length, containing 11.3% peanut quarters (v/v)). An overall mean bite length of 15±3 mm (mean±SD) was determined, and serving sizes of 9000 mm^3 (20x30x15 mm) were adopted for trials involving matrices containing peanuts to fit within the subjects natural bite range for the matrices. All final samples were also weighed.

In Trials 4 and 5, the serving size was standardized by weight. The subject was instructed to grasp (using his hands) samples of peanuts from a small bag of a size he would typically put into his mouth to chew. The mean weight obtained was 4.4±1.0 g (mean±SD), and hence a serving weight of 4.4 g was selected.

8.2.4 Preparation of test foods

All peanuts used in this study were obtained from Prolife, New Zealand (Virginia Peanuts, China). Peanuts used in Trials 1-4 were roasted (unsalted) by the supplier (unless specified these were the peanuts used). The moist peanuts used in Trial 1 were prepared by immersing the peanut pieces (quarters) in water for 120 min and subsequently removing and storing the moist mass for 48 h at 4 ºC in water tight containers. Raw blanched peanuts were used in Trial 5. These peanuts were then roasted at 180 ºC for 20 min, or boiled for 30 min before being dried at 105 ºC for 16 h in an air oven, or served untreated. All peanuts were packed in foil to prevent oxidation and moisture migration.

Matrices were prepared without peanuts inside according to methods outlined in Section 3.1.3. Peanuts were manually inserted into the matrix immediately before each sample was served to the subject to ensure no unwanted moisture migration took place. All peanut quarters were sieved across a 4.75 mm sieve prior to preparation in the matrix to ensure no small particles were included, with the exception of small particles used in Trial 3, which were sieved across the 1.00 mm sieve to remove small particles. Serving weights can be found in the results section of each trial.

8.2.5 Analysis of the physical properties of the matrices and peanuts

Analysis of the physical properties of the matrices and peanuts were undertaken using the methods described in Section 3.2.2. Four replicates were used for peanut moisture content, 10 replicates were used for peanut hardness, and 6 replicates for peanut density. Texture analysis of the matrices was performed with 12 replicates.

The breakage properties of the peanuts were investigated by a single compression test as described in Section 3.2.2 (however using a single compression rather than double compression), and determining the resultant peanut particle size by image analysis. Image dimensions of constituent peanut particles were then categorised into 10 width classes: 0.355-0.5, 0.5-0.7, 0.7-1, 1-1.4, 1.4-2, 2-2.8, 2.8-4, 4-5.7, 5.7-8, $\geq$ 8 mm (as particles obtained after a single compression were generally larger than the particles in the food bolus, larger width classes were used) (8 replicates).

8.2.6 Analysis of the food bolus

Analysis of the particle size distribution of peanut particles in the bolus was conducted according to methods in Section 3.1.4. For Trials 4 and 5 where larger quantities of peanuts were present, each bolus was placed across 2 Petri dishes.

8.2.7 Statistical analysis

Statistical analyses were performed using SPSS ® (version 15.0 for Windows). The Kruskal-Wallis test was used to assess significant differences in the parameters of mastication (number of chews, chewing time, and mastication frequency), and bolus parameters (d_{50}, b, weight, and volume retention of peanut particles). These parameters were the dependant variables, and matrix, peanut type, initial peanut size, saliva stimulant, or heat treatment, were the factors. To identify individual differences within factors the Kruskal-Wallis test was also run pair wise. The Kruskal-Wallis test was deemed to give a significant difference within factors when $P<0.05$.

8.3 Results

The contrasting physical properties of the matrices and the peanuts used in these 5 trials are shown in Tables 8-2 and 8-3.

Table 8-2: Properties of the matrices (mean±SE).

Matrix	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
Scone	31±2	0.32±0.01	5.5±0.3	56±5
Gelatine gel (200 bloom)	71±4	0.62±0.03	10.0±0.1	433±33
Gelatine gel (250 bloom)	252±8	0.89± 0.01	10.1±0.1	2270±90
Chocolate	400±8	0.17±0.01	2.0±0.2	150±20

Table 8-3: Properties of the peanuts (mean±SE).

Peanuts	Trial number	Moisture content ($gH_20/100g$ total mass)	Hardness (N)	Density (g/cm^3)
Roasted (from supplier)	1-4	1.99±0.10	140±13	1.08±0.01
Raw, unblanched	5	3.50±0.05	85±14	1.12±0.01
Roasted (20 min at 180 ºC, covered)	5	0.73±0.03	70±8	1.03±0.01
Boiled for 30 min and dried at 105 ºC for 16 h	5	0.88±0.02	41±3	1.05±0.01
Soaked in water for 120 min, 48 h to equilibrate	1	22.21±0.09	52±7	1.06±0.01

8.3.1 Mastication of different matrices containing peanuts at different moisture contents (Trial 1)

A. Parameters of mastication

The number of chews ($H(3) = 12.478$, $P<0.0005$), chewing time ($H(3) = 11.669$, $P<0.005$), and mastication frequency ($H(3) = 8.577$, $P<0.05$), differed significantly between test foods (Table 8-4). Pair-wise analysis showed the gelatine gel matrix was chewed significantly longer and for a greater number of chews than was the chocolate, but there were no significant differences is the mastication of moist and dry peanut pieces in the same type of matrix. On average the gelatine gel was chewed for 53.8 and 60.3 cycles when containing dry and moist peanuts respectively, and the chocolate for 30.8 and 32.8 cycles when containing the dry and moist peanuts respectively.

B. Properties of the collected bolus

The particle size distributions of the peanut particles in the various test foods differed after mastication (Figure 8-1). Similarly, the d_{50} ($H(3) = 12.507$, $P<0.0005$), broadness (*b)* ($H(3) = 8.559$, $P < 0.05$), weight retention ($H(3) = 13.059$, $P<0.0005$), and volume of peanuts ($H(3) = 13.059$, $P<0.0005$) also differed significantly (Table 8-4). Pair-wise analysis showed the d_{50} was always significantly larger in the moist peanut particles than the dry peanut particles for each matrix, and the d_{50} of peanut particles inside the gelatine gel (250 bloom) was significantly smaller than the chocolate for the moist peanut particles. The average d_{50}'s were 1.09 mm and 1.17 mm for dry peanut particles inside the gelatine gel and the chocolate matrices respectively, and 1.22 mm and 1.38 mm for the moist peanut particles inside the gelatine gel and chocolate matrices respectively. Pair-wise analysis showed no difference in the broadness (*b*) of the peanut particle size distribution from moist and dry peanut pieces for each matrix. However, significant differences in broadness (*b*) of the peanut particle size distribution were found between matrices for the dry peanut particles, where the gelatine gel (250 bloom) had a significantly smaller *b* value, and hence a wider particle size distribution. The average broadness (*b*) value for the dry peanut particles was 1.13 inside the gelatine gel, and 1.34 inside the chocolate. The peanut weight retention and volume of retained peanuts were significantly greater for the moist peanuts within each matrix.

There were large differences in the particle size distribution (and hence the breakage properties) of the moist and dry peanut particles after a single compression to 50% strain with respect to the standard error of the mean area at each sieve aperture (Figure 8-2). The dry peanuts were shattered into finer pieces than the moist peanuts during the compression. No particles below 8 mm in diameter were obtained in the moist peanut fragments.

Table 8-4: Mastication of different matrices containing peanuts at different moisture contents (Trial 1). Parameters of mastication (mean±SE).

Matrix	Peanut moisture content (gH_20/100 g total mass)	Weight of matrix (g)	Weight of peanuts (g)	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	$d_{50\ (mm)}$	*b*	% Peanut weight retention (drywt/drywt)	Volume of peanuts in the bolus (mm^3)
Gelatine gel (250 bloom)	2	12.48±0.07	1.16±0.01	53.8±1.7 a	40.18±2.36 a	1.34±0.04 ab	1.09±0.04 a	1.13±0.04 a	22.57±2.42 a	1100±60 a
Gelatine gel (250 bloom)	22	12.31±0.09	1.12±0.01	60.3±2.5 a	42.59±1.61 a	1.41±0.01 a	1.22±0.02 b	1.21±0.04 ab	41.68±1.33 b	1740±30 b
Chocolate	2	11.39±0.08	1.16±0.02	30.8±0.8 b	23.87±0.68 b	1.29±0.01 b	1.17±0.02 ab	1.34±0.03 b	24.80±0.69 a	1200±50 a
Chocolate	22	11.31±0.07	1.13±0.01	32.8±1.4 b	24.75±1.41 b	1.33±0.02 b	1.38±0.04 c	1.26±0.04 ab	49.64±2.01 c	1890±40 c

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$) (Pair-wise analysis was only conducted when a significant overall difference was found). The weight of dry peanut pieces in the matrices was slightly greater than the moist peanut pieces to accommodate density differences, and thus ensure the volume of peanuts in each matrix was the same.

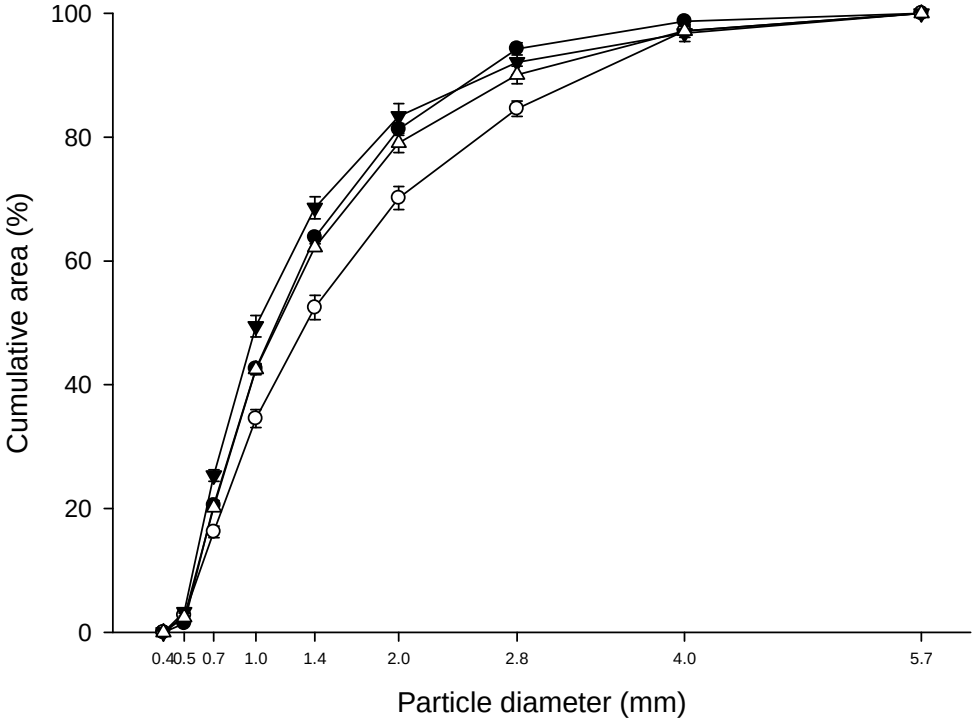

Figure 8-1: **Cumulative peanut particle size distribution of the bolus after mastication of peanuts served inside gelatine gel and chocolate matrices at moisture contents of 22.21±0.18 gH_20/100g total mass and 1.99±0.20 gH_20/100g total mass (mean±SD) (Trial 1). Dry peanuts in chocolate: ●, Moist peanuts in chocolate: ○, Dry peanuts in gelatine gel (250 bloom): ▼, Moist peanuts in gelatine gel (250 bloom): Δ (1.10±0.05 g inside a 9000 mm^3 matrix, peanut quarters). Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (4 replicates) (mean±SE).**

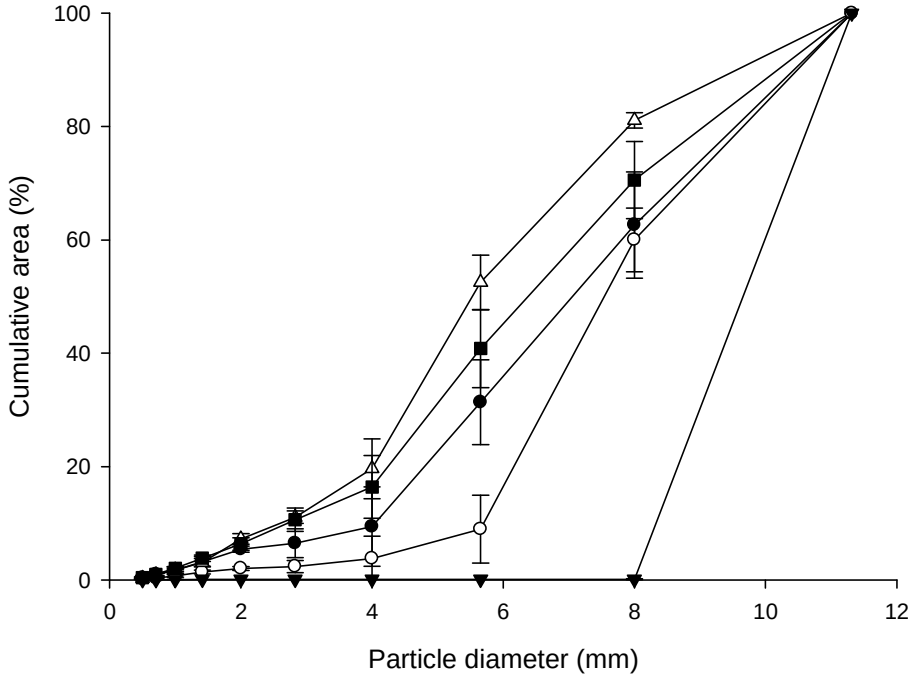

Figure 8-2: Breakage properties of peanuts: Cumulative particle size distribution of peanut fragments after a single compression to 50% strain using a flat cylindrical probe. Moist roasted (by supplier): ▼, Dry roasted (by supplier): Δ, Raw blanched: ○, Boiled and dried (in lab): ●, Roasted (in lab): ■ (8 replicates) (mean±SE).

8.3.2 Mastication of different matrices containing peanuts at lower concentrations (Trial 2)

A. Parameters of mastication

The number of chews ($H(2) = 10.049$, $P<0.05$), chewing time ($H(2) = 6.140$, $P<0.05$), and mastication frequency between matrices ($H(2) = 9.620$, $P<0.05$) differed significantly (Table 8-5). Pair-wise analysis showed the gelatine gel (200 bloom) and scone were chewed significantly longer and for a greater number of chews than the chocolate. On average the gelatine gel, scone, and chocolate were chewed 42.6, 36.2, and 28.4 times respectively.

B. Properties of the collected bolus

The particle size distributions of peanuts in the various matrices were similar after mastication, although differences can be seen at the upper end (>2.0 mm) of the

cumulative distribution (Figure 8-3). The d_{50} (H(2) = 2.220, P>0.05), weight retention (H(2) = 2.060, P>0.05), and volume of peanut particles in the bolus (H(2) = 1.520, P>0.05) did not differ significantly between matrices. However, the broadness (*b*) of peanut particle size distribution differed significantly between matrices (H(2) = 7.620, P<0.05). Pair-wise analysis showed the broadness (*b*) values of the peanut particle size distribution in the gelatine gel (200 bloom) were significantly smaller than the chocolate (Table 8-5). On average the broadness (*b*) value in the gelatine gel, scone, and chocolate was 1.14, 1.21, and 1.31 respectively.

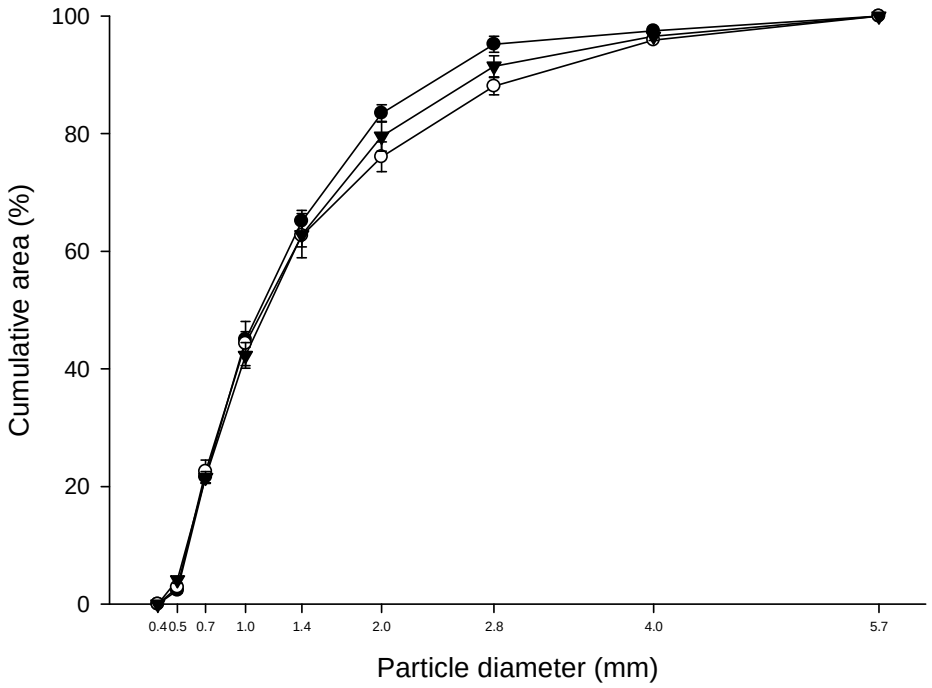

Figure 8-3: **Cumulative peanut particle size distribution in the bolus after mastication of peanuts served inside different matrices at a low concentration. Scone: ▼ Gelatine gel (200 bloom): ○ Chocolate: ● (0.28±0.02 g, one quarter, inside a 9000 mm^3 matrix, peanut moisture content was 1.99±0.10 g$H_2$0/100g total mass) (Trial 2). Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (5 replicates) (mean±SE).**

Table 8-5: Mastication of different matrices containing peanuts at lower concentrations (Trial 2). Parameters of mastication (mean±SE).

Matrix	Weight matrix (g)	Weight peanuts (g)	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	$d_{50\ (mm)}$	*b*	% Peanut weight retention (drywt/drywt)	Volume of peanuts in the bolus (mm^3)
Scone	6.28±0.07	0.30±0.01	36.2±1.8 a	28.86±1.74 ab	1.23±0.02 a	1.17±0.04	1.21±0.03 ab	16.69±1.83	340±20
Gelatine gel (200 bloom)	11.52±0.14	0.29±0.01	42.6±2.3 a	33.57±1.92 a	1.27±0.01 a	1.20±0.06	1.14±0.03 a	18.86±1.77	350±30
Chocolate	12.43±0.20	0.29±0.01	28.4±1.1 b	25.56±0.94 b	1.11±0.01 b	1.13±0.03	1.31±0.04 b	16.26±2.22	310±20

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$) (Pair-wise analysis was only conducted when a significant overall difference was found).

8.3.3 Mastication of a scone matrix containing peanuts of a different initial size (Trial 3)

A. Parameters of mastication

The number of chews ($H(1) = 0.021$, $P>0.05$), chewing time ($H(1) = 0.083$, $P>0.05$), and mastication frequency ($H(1) = 1.333$, $P>0.05$) did not differ significantly between scone matrices containing peanut pieces of different initial size (Table 8-6).

B. Properties of the collected bolus

The peanut particle size distributions after mastication of the scone matrices containing peanuts of different initial size were similar (Figure 8-4). Small differences can be seen in the centre of the distribution, however the standard error is high. The d_{50} ($H(1) = 0.750$, $P>0.05$), broadness (*b)* ($H(1) = 1.333$, $P>0.05$), weight retention ($H(1) = 0.750$, $P>0.05$), and volume in the bolus of peanut particles ($H(1) = 2.083$, $P>0.05$) did not differ significantly.

Table 8-6: Mastication of a scone matrix containing peanuts of a different initial particle size (Trial 3). Parameters of mastication (mean±SE).

Initial particle size	Weight matrix (g)	Weight peanuts (g)	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	$d_{50\ (mm)}$	b	% Peanut weight retention (drywt/drywt)	Volume of peanuts in the bolus (mm^3)
Large (one quarter piece, ~0.28g)	5.96±0.18	0.28±0.01	35.5±2.4	28.34±1.70	1.25±0.02	1.19±0.02	1.28±0.05	26.88±1.44	290±10
Small (7-8 pieces, ~0.04 g each, ~0.28g in total)	6.20±0.11	0.27±0.01	35.5±2.6	29.23±2.38	1.22±0.02	1.37±0.12	1.23±0.07	27.56±0.65	270±10

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$) (Pair-wise analysis was only conducted when a significant overall difference was found).

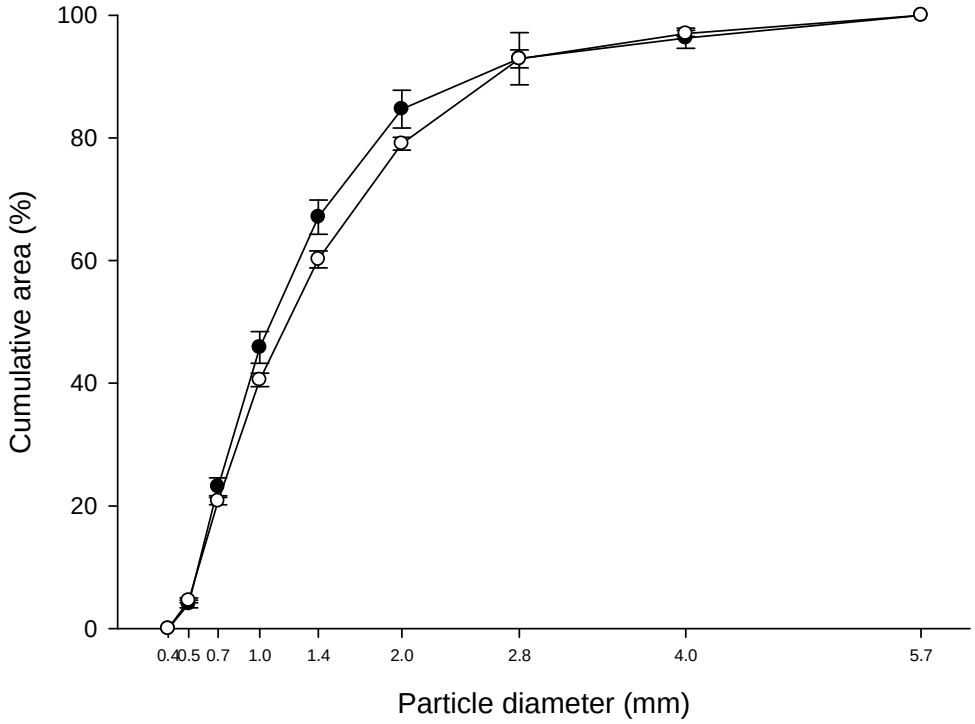

Figure 8-4: **Cumulative peanut particle size distribution after mastication of scone matrices containing large (●) (0.28±0.02 g, one quarter) or small (○) (0.04±0.01 g, 7-8 pieces) peanut pieces when served (total quantity of peanut kept constant, 0.28±0.02 g of peanut inside a 9000 mm^3 matrix, moisture content of peanuts were 1.99±0.10 gH_20/100g total mass) (Trial 3). Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (4 replicates) (mean±SE).**

8.3.4 Mastication of peanuts containing saliva stimulants (Trial 4)

A. Parameters of mastication

The number of chews (H(2) = 0.866, P>0.05), chewing time (H(2) = 1.297, P>0.05), and mastication frequency (H(2) = 1.95, P>0.05) did not differ significantly when peanuts with various saliva stimulants were chewed (Table 8-7).

B. Properties of the collected bolus

The peanut particle size outcome was similar between treatments (Figure 8-5). d_{50} (H(2) = 0.980, P>0.05) and *b* (H(2) = 3.500, P>0.05) was not significantly different, however peanut retention (H(2) = 9.980, P<0.005) and volume of peanuts the bolus (H(2) = 9.680, P<0.005) was significantly different (Table 8-7). Pair-wise analysis

showed the weight retention and volume of the peanut particles in the bolus produced when peanuts with the icing sugar stimulant were chewed were significantly less than the control (and the sherbet stimulant in the case of weight retention).

Interestingly, moisture content between the boluses were significantly different ($H(2) = 10.260$, $P<0.001$). Pair-wise analysis showed that the moisture content of the boluses with the two stimulants was significantly higher than the bolus with no stimulant.

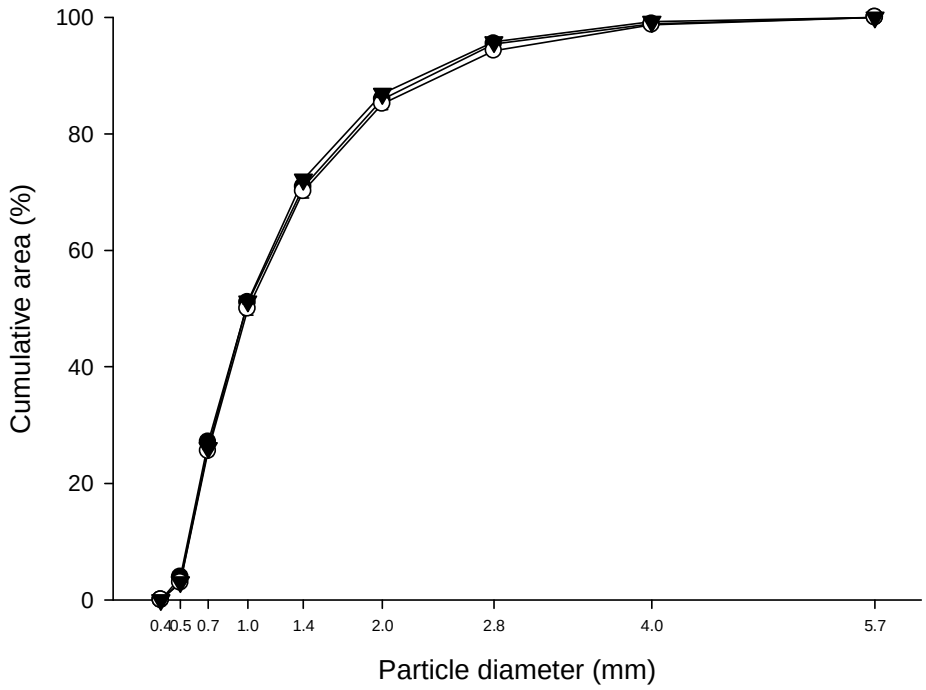

Figure 8-5: **Cumulative peanut particle size distribution after mastication of peanuts containing saliva stimulants (Trial 4). Sherbet: ▼, Icing Sugar: ●, No Saliva stimulant: ○ (4g of peanut quarters, with 0.5g stimulant or 4g with no stimulant, peanut moisture content was 1.99±0.10 gH_20/100g total mass). Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (5 replicates) (mean±SE).**

Table 8-7: Mastication of peanuts containing saliva stimulants. Parameters of mastication (mean±SE).

Saliva stimulant	Weight stimulant (g)	Weight peanuts (g)	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	$d_{50\ (mm)}$	Moisture content of bolus (gH_20/100g total mass)	*b*	% Peanut weight retention (drywt/drywt)	Volume of peanuts in the bolus (mm^3)
Sherbet	0.51±0.01	4.39±0.01	28.2±0.9	22.40±0.82	1.27±0.01	1.03±0.01	54.41±0.72 a	1.21±0.02	22.29±0.72 a	3940±100 ab
Icing sugar	0.51±0.01	4.44±0.02	29.6±2.7	23.56±2.03	1.26±0.03	1.04±0.02	52.73±0.86 a	1.16±0.02	16.29±1.03 b	3550±170 a
No stimulant	NA	4.39±0.02	31.6±2.6	25.23±2.27	1.26±0.02	1.06±0.02	40.85±0.70 b	1.17±0.02	23.81±1.19 a	4180±40 b

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$)
(Pair-wise analysis was only conducted when a significant overall difference was found).

8.3.5 Mastication of peanuts subjected to different heat treatments (Trial 5)

A. Parameters of mastication

The number of chews ($H(2) = 5.370$, $P>0.05$) and mastication frequency ($H(2) = 1.823$, $P>0.05$) when peanuts subjected to different heat treatments were chewed did not differ significantly, however the chewing time was significantly different between treatments ($H(2) = 6.416$, $P<0.05$) (Table 8-8). Pair-wise analysis shows that the roasted peanuts were chewed for a significantly shorter period of time than the raw and 'boiled and dried' peanuts.

B. Properties of the collected bolus

The mean d_{50} of the peanut particle size distribution following chewing of peanuts subjected to different heat treatments did not differ significantly ($H(2) = 2.880$, $P>0.05$) (Figure 8-6). However, a significant difference was found in broadness *b* of the peanut particle size distribution ($H(2) = 9.980$, $P<0.05$), weight retention ($H(2) = 8.000$, $P<0.05$), and volume of bolus ($H(2) = 7.280$, $P<0.05$) between treatments. Pair-wise analysis showed that the broadness values (*b)* of the raw peanut particle size distribution were significantly smaller than both heat treated peanut particle size distributions. The average broadness value *b* for the raw, roasted, and 'boiled and dried' peanut particle size distributions was 1.04, 1.18, and 1.16 respectively.

The weight retention of the raw peanuts was significantly lower than the roasted peanuts, and the volume of the raw peanut particles in the bolus was significantly lower than the 'boiled and dried' peanut particles.

Differences were found in the particle size distribution of peanut fragments between the raw blanched, and the roasted and 'boiled and dried' peanuts after a single compression of 50% strain (Figure 8-2). The heat treated peanuts shattered into finer particles than the raw blanched peanuts, which demonstrated a higher breakage function in the heat treated peanuts.

Table 8-8: Mastication of peanuts subjected to different heat treatments. Parameters of mastication (mean±SE).

Heat treatment	Weight peanuts (g)	Number of chews	Chewing time (s)	Mastication frequency (s^{-1})	$d_{50\ (mm)}$	*b*	% Peanut weight retention (drywt/drywt)	Volume of peanuts in the bolus (mm^3)
No heat treatment	4.41±0.02	35.2±1.1	27.86±0.73 a	1.26±0.01	1.04±0.01	1.04±0.03 a	16.12±0.96 a	3630±170 a
Roasted	4.41±0.02	30.8±1.0	24.21±0.69 b	1.27±0.02	1.08±0.01	1.18±0.01 b	21.63±0.62 b	4150±80 ab
Boiled and dried	4.40±0.01	33.6±1.4	27.18±1.12 ab	1.23±0.02	1.06±0.01	1.16±0.01 b	18.89±1.33 ab	4340±120 b

Different letters (a & b) down each column indicate a significant statistical difference after pair wise Kruskal-Wallis tests ($P<0.05$) (Pair-wise analysis was only conducted when a significant overall difference was found).

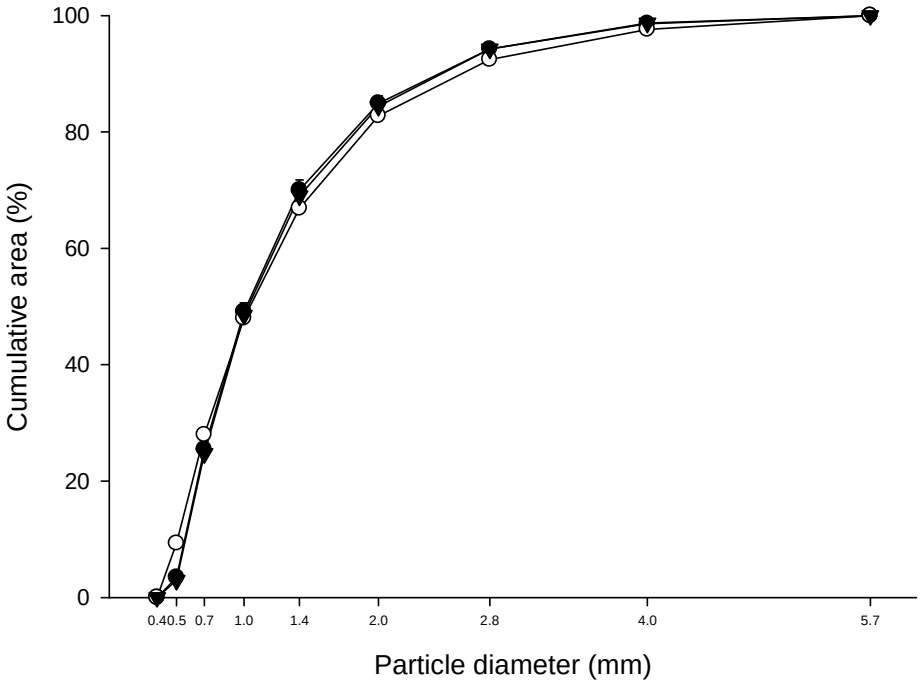

Figure 8-6: **Cumulative peanut particle size distribution after mastication of peanuts subjected to different heat treatments (Trial 5). Roasted (moisture content: 0.73±0.03 gH_20/100g total mass): ▼, Boiled and dried (moisture content: 0.88±0.02 g/100g): ●, No heat treatment (moisture content: 3.50±0.05 gH_20/100g) : ○ (4g of peanut halves). Data was obtained after a complete sequence of mastication on expectorated boluses, before swallowing (5 replicates) (mean±SE).**

8.4 Discussion

8.4.1 Mastication of different matrices containing peanuts at different moisture contents (Trial 1)

The significant differences in the subject's mastication of different matrix types indicates that the properties of the matrices appear to largely determine chewing behaviour in the test foods used in this trial. The lack of difference in the subject's mastication of the same matrix containing different peanuts suggests the properties of the peanuts have a limited affect on mastication in this case (Table 8-4).

However, the properties of the bolus seem to be influenced by the type of peanut piece and the matrix. In terms of comparisons between peanut pieces masticated within a given matrix the d_{50}, weight retention, and volume, of peanut particles in the bolus were greater when moist peanuts were chewed, while broadness (b) of the peanut particle size distribution was unchanged (Figure 8-1, Table 8-4). d_{50} results reflect what was found in Chapter 6 where moist peanut pieces were masticated to a larger d_{50} of peanut particles than the dry peanut pieces. A higher weight and volume retention for moist peanut pieces compared to dry peanut pieces may be a result of smaller particles from the dry peanuts being shifted more easily into the oro-phrarynx (as suggested by Flynn et al., (2010)) where they are not collected by rinsing the mouth with water. Alternatively, this could be due to a greater quantity of smaller particles from the dry peanuts passing through the 355 μm sieve during the washing of the bolus (see Section 9.4.3), and due to fat loss during image processing in the case of weight retention (see Section 3.1.4).

In terms of comparisons between matrices masticated with a given peanut piece, results are more complex. Generally, peanut particles in the bolus after mastication in the gelatine gel were smaller and had a greater spread (Table 8-4 & Figure 8-1). However, results are not all significant, and interactions also appear to have taken place between peanut pieces and what matrix the peanuts are embedded inside. These results require validation with a larger population to allow more powerful statistical assessment to clarify the effect of the particle type and matrices, and the interaction between them.

Results also indicate differences in breakdown rates are present. The difference in d_{50} despite no difference in chewing behaviour, and in the distribution after a single compression using the Texture Analyser (Figure 8-2), strongly indicates a difference in the breakage function between moist and dry peanut pieces. Differences in the selection function may also be present between matrices, as the d_{50} was similar between matrices despite significant differences in the chewing behaviour (in the case of the dry peanuts). The effect of matrices on the breakdown rates also requires clarification.

8.4.2 Mastication of different matrices containing peanut pieces at lower concentrations (Trial 2)

Increasing concentrations of particles inside continuous mediums have been shown to increase (from 5 - 25% (w/w)) (Sainani et al., 2004) or decrease (Imai et al., 1995) (0.5-3.0% (w/w)) grittiness perception. However, it appears any changes in perception by reducing the concentration of peanut pieces from 11.3% (v/v) (used in all trials thus far) to 3.1% (v/v) did not cause any notable change on the effect of matrices on the peanut particle size outcome in the bolus.

Differences in mastication between matrices were accompanied by significant differences in the broadness (*b*) of the peanut particle size distribution, however no significant differences were found for d_{50} or retention of peanut particles (Figure 8-3, Table 8-5). As found in Chapter 7 and Trial 1 of this chapter (with dry peanuts), the lack of significant difference in d_{50} between matrices despite differences in chewing behaviour suggests the rate of particle breakdown is different from matrix to matrix.

8.4.3 Mastication of a scone matrix containing peanut pieces of a different initial size (Trial 3)

Differences in chewing behaviour (Diaz-Tay et al., 1991; Kohyama et al., 2007) and grittiness perception (Imai et al., 1995; Sainani et al., 2004; Engelen et al., 2005a) have been reported with contrasting initial particle size. However no difference in chewing behaviour, peanut particle size outcome, or peanut retention was found in this trial (Figure 8-4, Table 8-6) by changing initial size of the peanut pieces. It likely that after several chews the peanut particles inside samples with a large initial peanut size have been reduced to a similar size of peanuts with a small initial size. By the end of the chewing sequence all fragments are well below the initial peanut size, and the identical physical properties of the peanuts and matrices mean that the size of peanut particles in the bolus is unchanged.

8.4.4 Mastication of peanuts containing saliva stimulants (Trial 4)

The addition of saliva stimulants caused significant differences in the moisture content of the bolus (due to the increased production of saliva), however this did not cause changes in chewing behaviour or particle size outcome of the peanuts (in terms of d_{50} or broadness(b)) (Figure 8-5, Table 8-7). Some authors (Loret et al., 2009) have proposed that moisture content is a trigger for swallowing, however these differences suggests that this is unlikely.

Changes in moisture content are likely to have caused differences in cohesion and lubrication of the bolus, and therefore changes would be expected to alter the particle size required to prepare a swallow-safe bolus, according to the model presented by Hutchings & Lillford (1988). However, it is possible that much greater changes in lubrication (more than a 10-15% increase in moisture content) would be required to induce significant differences in bolus particle size.

8.4.5 Mastication of peanuts subjected to different heat treatments (Trial 5)

Heat treatment of raw peanuts produced peanuts with different breakage properties (Figure 8-2). The heat treated peanuts (which shattered into finer particles than the raw peanuts after one compression with a Texture Analyser) were chewed for a significantly shorter period and smaller number of chews, but formed boluses that had a statistically similar d_{50} of peanut particles compared to the raw peanuts (Figure 8-6, Table 8-8). However, the heat treated peanut particles had a significantly narrower size distribution in the bolus (larger broadness value (*b)*) which is likely to be linked to the contrasting breakage properties. Mc Keirnan & Matts (2010) published data (after laboratory work and analysis for this study was undertaken) showing similar results, where roasted peanuts were chewed for a significantly lower number of chews and shorter chewing time than raw peanuts, and size distributions of peanut particles also varied significantly. Retention was significantly lower in the raw peanuts in this trial (Table 8-8). Again this is likely to be as a result of a higher proportion of fine particles present in the bolus, which are lost more easily during chewing, washing, and image processing.

8.5 Conclusions

The results of this study show several parameters can be used to manipulate chewing behaviour and the properties of the bolus.

The addition of saliva stimulants and changing the initial size of peanut pieces inside a food matrix did not influence mastication or peanut particle size in the bolus. Embedding peanut pieces inside different food matrices changed mastication and in most cases the broadness (*b*) of the peanut particle size distribution, but had a negligible effect on the d_{50} of peanut particles. It is likely that matrices are also changing the rate of peanut particle breakdown.

Heat treatment changed the breakage properties of peanuts as well as chewing behaviour and broadness (*b*) of the peanut particle size distribution, but did not change the d_{50} of the peanut particle size distribution. Increasing the moisture content of peanuts had the most notable effect on changing particle size outcome of the peanut particles, by increasing d_{50} substantially. Increasing the moisture content also changed the breakage properties of the peanuts.

Further work is required to quantify and understand how the matrices are changing the rate of particle breakdown. A study assessing bolus properties at specific intervals in the chewing sequence is presented in Chapter 9.

Chapter 9 : Particle breakdown dynamics of peanuts embedded inside different food matrices

9.1 Introduction

Chewing is a dynamic process where the food bolus is constantly changing until it is suitable for swallowing. During chewing, jaw movements (Mioche et al., 2002b; Peyron et al., 2002 ; Kohyama & Mioche, 2004) and muscle activity (Gonzalez et al., 2001; Kohyama et al., 2002; Peyron et al., 2002) are continually changing to accommodate changes in the food bolus. Moreover, communition of particles takes place (Lucas & Luke, 1983; Olthoff et al., 1984), and the mass of bolus inside the oral cavity decreases (Peyron et al., 2004b), until the bolus reaches a suitable state for swallowing. Textural properties such as stickiniess, springiness, adhesion, and cohesion of the bolus tend to increase, while hardness decreases (Lefant et al., 2009; Peyron et al., 2009). Throughout the chewing sequence lubrication will increase as a result of free moisture in the mouth, expulsion of water and fat from fracturing of particles, and by ongoing secretion of saliva (Hutchings & Lillford, 1988).

A study of the dynamic changes in the bolus of the heterogeneous foods used in the current study was required to further understand the mechanism of breakdown in these test foods. Therefore, a need arose to analyse the peanut particle size distribution, as well as other physical properties of the bolus (such as wet and dry bolus weight), of the heterogeneous matrices throughout the chewing sequence. All previous studies on dynamic changes in bolus properties (such as particle size and rheological properties) have all involved homogenous foods (Lucas &Luke, 1983; Lefant et al., 2009).

Previous single subject studies in this thesis have shown almost no differences in the d_{50} of peanut particles in the food bolus after mastication of different matrices containing peanut pieces (where the properties of the peanut piece were kept constant). This is despite significant differences in most cases between matrices in terms of mastication and the broadness (b) of the size distribution of the peanut particles in the bolus. It appears the rate of particle breakdown (and hence most probably the selection function) varies

between matrices. The selection function is a measure of the probability a given food particle will make contact with the teeth for fracture during a chewing stroke, where larger particles have a higher selection function than smaller particles (Lucas et al., 2002; Lucas, 2004).

The aim of this study was to compare the size distribution of peanut particles inside the gelatine gel and chocolate matrices (using only dry peanuts) at regular points in the chewing sequence until the point of swallowing. Changes in matrix retention, peanut retention, and moisture content of the bolus during the chewing sequence were also sought after to understand the dynamics of breakdown between these matrices.

9.2 Methodology

9.2.1 Subject screening and selection

Subject screening followed the methods as described in Chapter 5. The screening data for this subject is shown (Table 9-1).

Table 9-1: The bite weight and number of chews of a Fruit and Nut bar of a previous population studied and the selected subject (mean±SD)

	Bite weight	Number of chews	n
Previous population	6.02±2.15	34.8±16.2	45
Selected subject	6.73±0.88	32.0±1.9	1

The selected subject was a 29 year old male. The subject had class 1 occlusion, no significant tooth crowding, no obvious tooth decay, and healthy periodontal condition. He had no functional disturbance to mastication such as pain or clicking during chewing, nor did he have any other known oral or general health issues that could influence oral processing. This study was reviewed and approved by the Massey University Human Ethics Committee: Southern A (Application 09/24).

9.2.2 Experimental procedure

The experimental procedure involved serving two test foods: a gelatine gel (250 bloom) matrix and a chocolate matrix containing embedded peanut quarters.

The subject was asked to expectorate food boli after 5, 10, 15, 20, and 25 chews, and at the point he wanted to swallow. The subject was not told how many times he would have to chew for, but was told to stop at the predetermined point by the researcher (apart from when samples were obtained at the normal swallowing point). The order of test foods, and the order of the number of chews the subject conducted, was randomised. Six sessions were undertaken, and each session contained 12 samples (6 gelatine gel samples chewed to 5, 10, 15, 20, 25 chews or the swallowing point, and 6 chocolate samples chewed to 5, 10, 15, 20, 25 chews or the swallowing point).

Four of the sessions were used to obtain boli for particle size analysis of the peanut particles and peanut retention, and two sessions were used to obtain boli for assessment of the total dry weight of the bolus and determination of moisture content. Data on the total wet weight of the bolus was obtained from every session.

Each test food was placed between the subjects molars on his preferred chewing side (the subjects right side) before mastication began. The researcher then signalled to the subject when to begin mastication.

Experimental conditions and protocol followed that outlined in Section 3.2.1.

9.2.3 Assessment of natural bite size and selection of serving size

A constant volume serving size of 2x3x1.5 cm (9 cm^3) which had been determined for this subject in Chapter 8 (the same subject was used in this chapter) was used again in this trial. Serving weights are shown in Table 9-2. Each test piece contained 11.3% (v/v) peanut pieces, where $\rho_{(peanuts)} = 1.08 \pm 0.01$ (g/cm^3).

Table 9-2: Serving weights of the test foods (mean±SD)

Test component	Serving weight (g)
Chocolate matrix (g)	10.62±0.16
Gelatine matrix (g)	11.59±0.13
Peanuts (g)	1.13±0.02

9.2.4 Preparation of test foods

Chocolate matrices and gelatine gel (250 bloom) matrices were prepared as outlined in Section 7.2 and 8.2 respectively. Peanut pieces (quarters) were manually inserted into the matrices and served immediately to eliminate any moisture migration into the peanuts prior to mastication (peanut moisture content: 1.99±0.20 $gH_20/100g$). All peanut quarters were sieved across a 4.75 mm sieve prior to preparation in the matrix to ensure no small particles were included. Six peanut pieces (quarters) were used in every test piece, with three pierced on the front side, and 3 on the back side.

Textural properties of the matrices were assessed using identical methods to those described in Section 3.2.2 using 12 replicates.

9.2.5 Analysis of the food bolus

Boli from the first four sessions were analysed using methods described in Section 3.1.4 to measure the particle size distribution and dry weight of peanuts retained.

As described in Section 3.1.4 the Rosin-Rammler function was used to derive the broadness (*b*) of the particle size distribution of each bolus. The R-squared of fits for distributions from all boli were all above 0.98, apart from the distributions after 5 chews and 10 chews in the gelatine gel, which were above 0.91 and 0.94 respectively. As fits after 5 and 10 chews were not as strong as fits in previous chapters, the d_{50} was determined by manually to improve accuracy by plotting a cumulative distribution curve for each bolus, and determining the sieve aperture at which 50% of the area had accumulated. Specific surface area was calculated by measuring the total 2D surface area of peanut particles in each bolus, and dividing by the dry weight of peanuts retained.

Wet weight of the bolus, and wet weight of material (debris) obtained from the rinse phase (material collected by rinsing the mouth with 25 mL of water after the bolus is expectorated), was also measured for each bolus of every session. The total wet weight of the bolus was then determined. Total dry weight of the boli were analysed in sessions five and six, by placing each bolus (and rinse collection) in an air dry oven at 105 ºC for 24 h (and measuring weight before and after).

9.2.6 Statistical analysis

To quantify relationships between each dependant variable and the number of chews, simple linear regression was undertaken on the data from each matrix treatment using SPSS ® (version 16.0 for Windows) (SPSS Inc., USA). The *Y* series (dependant variable) was linearised where needed to obtain a simple linear relationship:

$$Y=a+bx \qquad \textbf{Equation 9-1}$$

Where *Y* was the dependant variable, *x* the number of chews, *a* the intercept, and *b* the slope. The intercept and slope were significant when *a* and *b* were significantly different from 0 respectively ($P<0.05$).

Multiple regression with dummy variables was also undertaken using SPSS ® (version 16.0 for Windows) (SPSS Inc., USA) to compare significant differences in the slope and intercept between gelatine gel and chocolate treatments for each dependant variable. The *Y* series (dependant variable) was linearised where needed, and the following linear model was fitted to each linear curve:

$$Y=a+bx_1+cx_2+dx_2x_1 \qquad \textbf{Equation 9-2}$$

Where *Y* was the dependant variable, x_1 was the chew number, and x_2 the variable that was 0 for gel and 1 for chocolate. Hence the linear model was fit to the gelatine gel curve (*a* was the intercept of the gelatine curve, and *b* was the slope of the gelatine curve), and fitting constants to modify this fit to the chocolate curve were determined. Where the constant *c* was significant ($P<0.05$), the intercept was significantly different between the gelatine gel and chocolate curves, and where *d* was significant ($P<0.05$), the slope was significantly different between gelatine gel and chocolate curves.

This analysis is equivalent to an ANCOVA.

9.3 Results

9.3.1 Peanut particle size distribution changes during the chewing sequence

The matrices had contrasting physical properties (Table 9-3).

Table 9-3: Properties of the matrices (mean±SE)

Matrices	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
Gelatine gel (250 bloom)	252±8	0.89±0.01	10.1±0.1	2270±90
Chocolate	400±8	0.17±0.01	2.03±0.23	150±20

Breakdown of peanuts followed contrasting pathways between matrices (Figure 9-1). At early stages (5-10 chews) and middle stages (15-20 chews) of the chewing sequence a greater proportion of large particles were present in the gelatine bolus compared to the chocolate. After 25 chews, nearing the swallowing point for the chocolate matrix but not for the gelatine gel matrix, differences are much smaller. At the swallowing point for both matrices the peanut particle size distributions appear similar. The gelatine gel required a greater number of chews to prepare the bolus for swallowing than the chocolate (Figure 9-1).

Dynamic changes in d_{50} of the peanut particle size distribution

The d_{50} of peanut particles decreased significantly with number of chews according to the following relationship for chocolate (Equation 9-3) and gelatine gel (Equation 9-4) using simple linear regression (Figure 9-2) (where x is the number of chews). The d_{50} relationship was an exponential decay (Figure 9-2) (weaker fits were observed without natural log transformation). After natural log transformation, the d_{50} of the chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where $P>0.05$.

Chocolate: $\ln(d_{50})=0.56-0.02x$, r = 0.84, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-3

Gelatine gel: $\ln(d_{50})=1.11-0.02x$, r = 0.72, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-4

There was a significant difference in the intercept (P<0.005) but not the slope (P>0.05) between the chocolate and gelatine gel for the d_{50} of peanut particles (Figure 9-2) according to the multiple regression.

With the exception of the boli expectorated at the swallow point, the d_{50} of the peanut particles was smaller in the chocolate bolus than the gelatine gel bolus at all other intervals in the chewing sequence (Figure 9-2). Once the bolus was ready for swallowing, d_{50} of peanut particles was similar between matrices.

Dynamic changes in the broadness value (b) of the peanut particle size distribution

The broadness value (*b*) of the peanut particle size distribution increased significantly with the number of chews according to the relationships below for chocolate (Equation 9-5) and gelatine gel (Equation 9-6) using simple linear regression (Figure 9-3) (where *x* is the number of chews). The broadness value (*b*) of the chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where P>0.05.

Chocolate: $b=0.901 - 0.010x$, r = 0.70, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-5

Gelatine gel: $b=0.847 -0.004x$, r = 0.64, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-6

There was no significant difference in the intercept (P>0.05) but a significant difference in the slope (P<0.05) for the broadness (*b*) (Figure 9-3) according to the multiple regression. The broadness value (*b*) of the peanut particle size distribution was higher in the chocolate matrix than the gelatine gel throughout the chewing sequence, i.e. there was a narrower spread of particles.

Dynamic changes in the specific surface area of peanut particles

The specific surface area of peanut particles increased significantly with the number of chews according to the relationships below for chocolate (Equation 9-7) and gelatine gel (Equation 9-8) using simple linear regression (Figure 9-4) (where *x* is the number of chews) (the data did not follow an exponential decay as weaker fits were observed when a natural log transformation was used on the surface area data). The specific surface area of the chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where $P>0.05$.

Chocolate: SSA=971 + 130*x*, r = 0.87, P(intercept) <0.0005, P(slope) <0.0005 **Equation 9-7**

Gelatine gel: SSA=342+81*x*, r = 0.90, P(intercept) >0.05, P(slope) <0.0005 **Equation 9-8**

Furthermore, there was no significant difference in the intercept ($P>0.05$) but a significant difference in the slope ($P<0.05$) for the specific surface area (Figure 9-4) according to the multiple regression. The specific surface area of peanut particles inside the chocolate matrices were greater than inside the gelatine gel matrices at all intervals in the chewing pathway until the bolus was ready for swallowing (Figure 9-4).

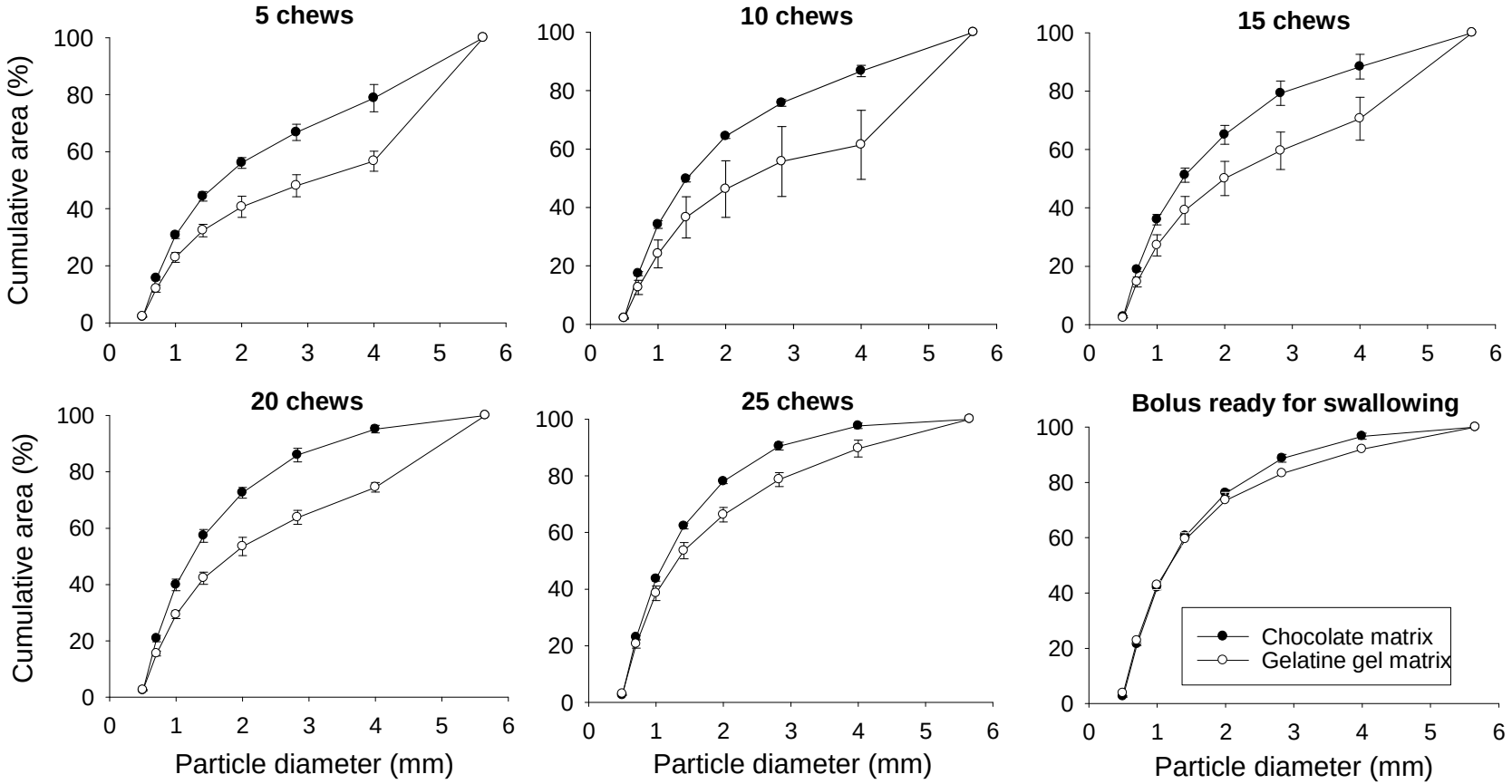

Figure 9-1: Cumulative peanut particle size distribution in the food bolus at different points in the chewing sequence. Chocolate matrix containing peanuts: ● (26±1 chews), Gelatine gel matrix containing peanuts: ○ (43±1 chews) (mean±SE).

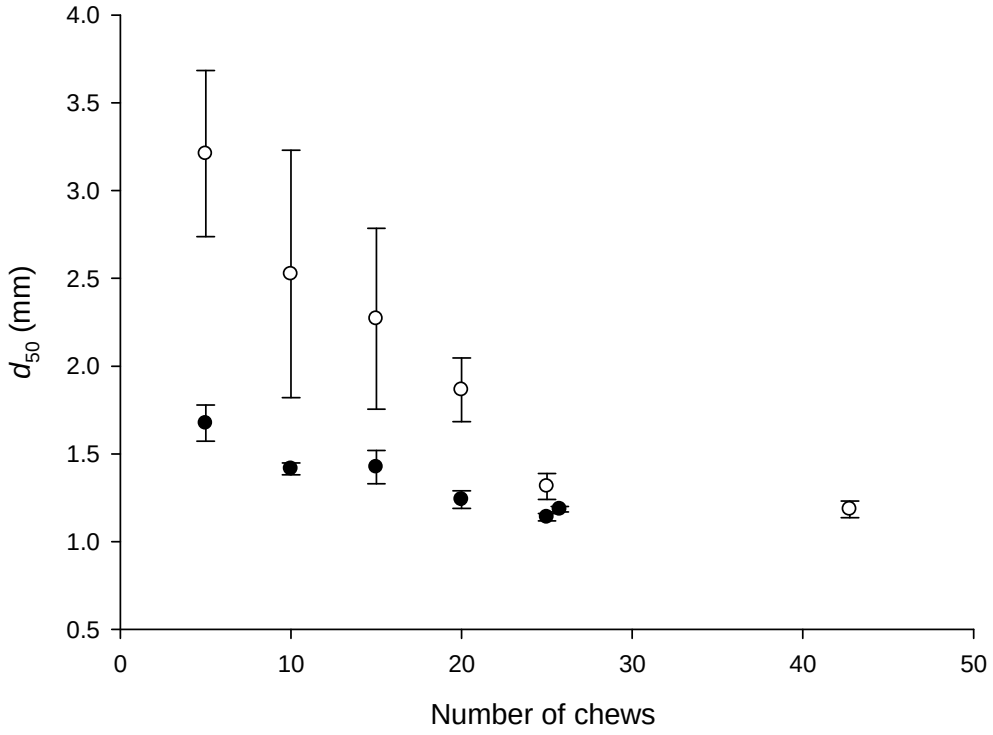

Figure 9-2: d_{50} (sieve aperture at which 50 % of the particle size area would fall below) of the peanut particles in the food bolus at different points in the chewing sequence (mean±SE). Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○. Difference between matrices: P(intercept) <0.005, P(slope) >0.05.

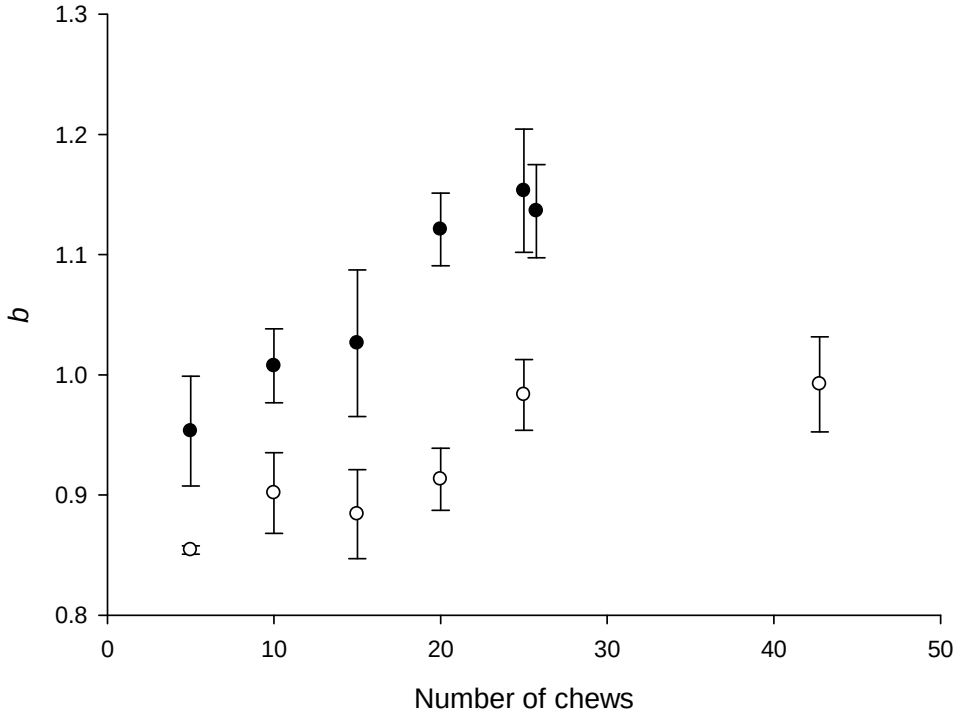

Figure 9-3: Broadness (*b*) of the peanut particle size distribution in the food bolus at different points in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○ (mean±SE). Difference between matrices: P(intercept) >0.05, P(slope) <0.05.

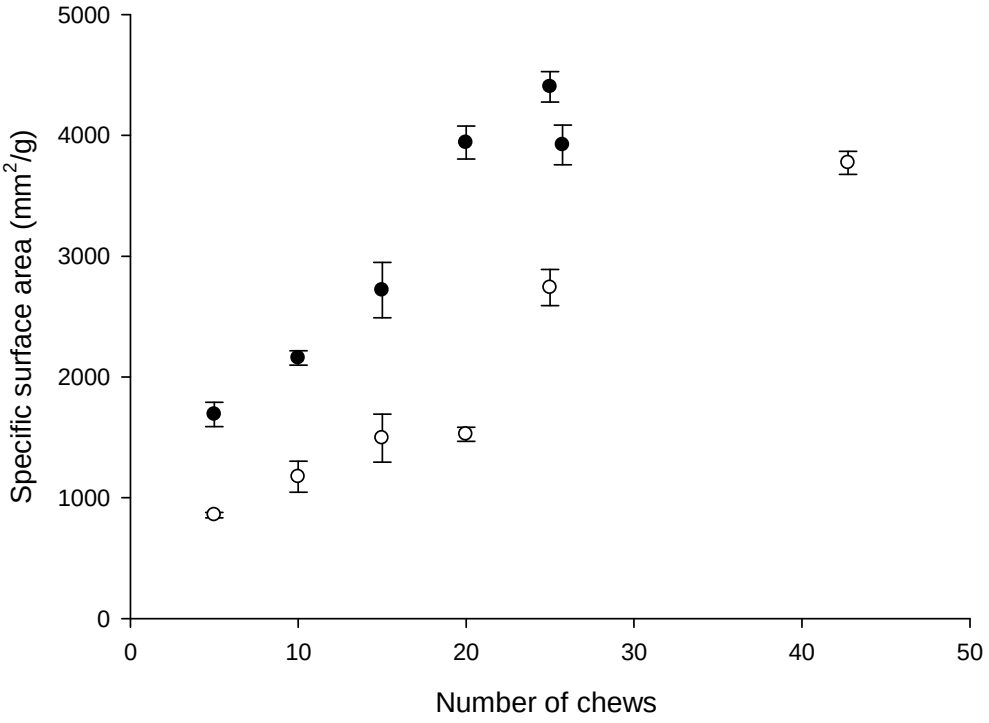

Figure 9-4: The specific surface area of the peanut particles inside the food bolus at different stages in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○ (mean±SE). Difference between matrices: P(intercept) >0.05, P(slope) <0.05.

9.3.2 Peanut retention

Retention of peanuts (on a dry mass basis) decreased significantly with the number of chews, and followed an exponential decay shape (Figure 9-5) (weaker fits were observed without the natural log transformation on the dry weight data). After natural log transformation, the chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where P>0.05. The following relationships were identified for dry weight retention of peanut particles in the chocolate (Equation 9-9) and gelatine gel (Equation 9-10) using simple linear regression (Figure 9-5) (where *x* is the number of chews):

Chocolate: ln(%Peanut dry weight retention)= 4.21-0.03*x*, r = 0.89, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-9

Gelatine gel: ln(%Peanut dry weight retention)= 4.37-0.02*x*, r = 0.87, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-10

There was no significant difference in intercept (P>0.05), but a significant difference in slope (P<0.05) according to the multiple regression. Loss from the chocolate matrices was greater than the gelatine gel throughout the chewing sequence until the bolus was ready for swallowing.

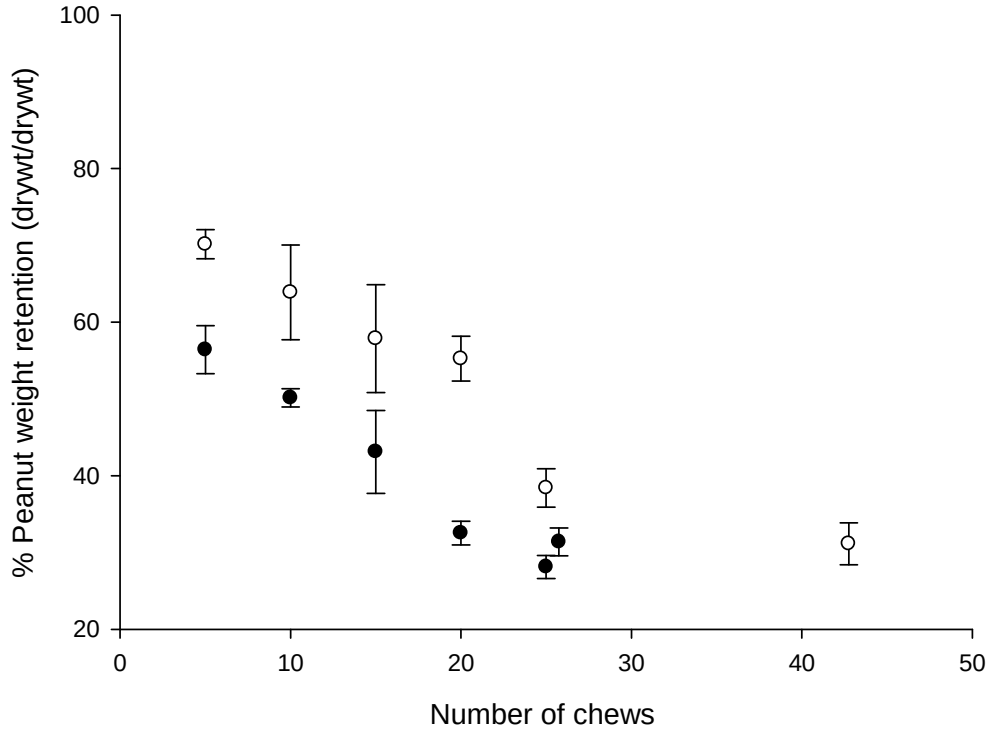

Figure 9-5: The dry weight of peanuts remaining inside the boli at different stages in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○, (mean±SE). Difference between matrices: r = 0.90, P(intercept) >0.05, P(slope) <0.05.

9.3.3 Total dry weight of bolus

Total dry weight of the bolus decreased significantly with the number of chews for the chocolate matrix, but not for the gelatine gel matrix. The chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality, where P>0.05. The magnitude of dry weight reduction was approximately 11.5 g to 10.5g for the chocolate, and appeared unchanged with the gelatine gel (Figure 9-6). The following relationships were identified for chocolate (Equation 9-11) and gelatine gel (Equation 9-12) using simple linear regression (Figure 9-5) (where *x* is the number of chews).

Chocolate: Total dry wt = 11.51 - 0.04*x*, r = 0.86, P(intercept) <0.0005, P(slope) <0.0005
Equation 9-11

Gelatine gel: Total dry wt = 9.03 - 0.01*x*, r = 0.10, P(intercept) <0.0005, P(slope) >0.05
Equation 9-12

Using multiple linear regression, a significant difference in the intercept (P<0.0005) and the slope (P<0.0005) was found between matrices. In comparison with the loss of peanut dry weight (Figure 9-5), the total dry weight loss of the bolus (matrix and peanuts) was minor (losses were approximately 70% for peanut dry weight).

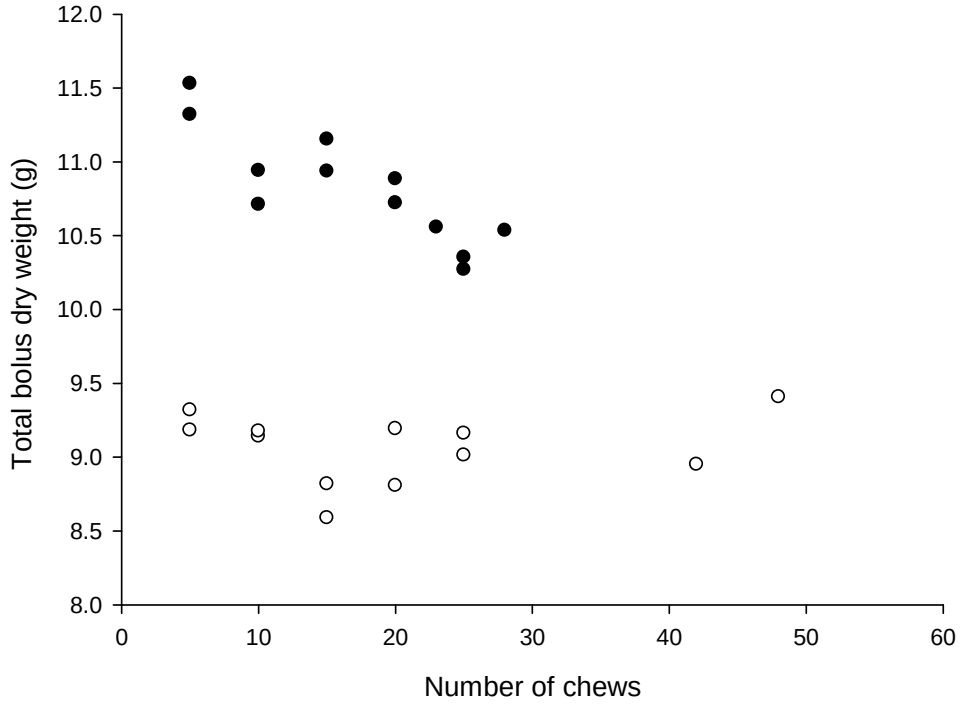

Figure 9-6: Total dry weight of boli recovered at different points in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○. Difference between matrices: P(intercept) <0.0005, and P(slope) <0.0005 (Note: The difference in initial position is due to differences in density and moisture content in the matrices served (matrices were standardised by volume)).

9.3.4 Wet weight of bolus

Dynamic changes in the total retained weight wet of the bolus

Total wet weight of the bolus + debris increased significantly for both matrices (Figure 9-7) with the number of chews. The chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where P>0.05. Using simple linear regression, the following relationships were obtained (Equation 9-13 and Equation 9-14) (where *x* is the number of chews):

Chocolate: Total wet bolus wt = 11.79 + 0.13*x*, r = 0.91, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-13

Gelatine gel: Total wet bolus wt = 12.88 + 0.12*x*, r = 0.96, P(intercept) <0.0005, P(slope) <0.0005 Equation 9-14

A significant difference in the intercept (P<0.0005), but no significant difference in the slope (P>0.05), was found according to multiple regression. Figure 9-7 shows a similar linear increase in the total dry weight of both boli.

Dynamic changes in the bolus weight

Bolus weight increased significantly for the gelatine gel but not for the chocolate (Figure 9-8) with the number of chews. The chocolate data set was normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where P>0.05, however the gelatine gel data was not normally distributed, P=0.005. Using simple linear regression, the following relationships were obtained (Equation 9-15 and Equation 9-16) (where *x* is the number of chews):

Chocolate: Bolus wt = 11.21 +0.03 *x*, r = 0.31, P(intercept) <0.0005, P(slope) >0.05 Equation 9-15

Gelatine gel: Bolus wt =12.55 + 0.09*x*, r = 0.93, P(intercept) <0.0005, P(slope) >0.05 Equation 9-16

For bolus weight the intercept (P<0.0005) and the slope (P<0.0005) were significantly different between matrices according to the multiple regression (Figure 9-8). Bolus

weight during the chewing sequence was greater for gelatine than the chocolate matrix, and increased by a greater amount per chew.

Dynamic changes in the weight of debris

The weight of debris increased significantly for both matrices with the number of chews (Figure 9-9). The chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), where P>0.05. Using simple linear regression, the following relationships were obtained (Equation 9-17 and Equation 9-18) (where *x* is the number of chews):

Chocolate: Rinse wt = 0.58 + 0.10*x*, r = 0.79, P(intercept) <0.05, P(slope) <0.0005 **Equation 9-17**

Gelatine gel: Rinse wt = 0.45 + 0.04*x*, r = 0.70, P(intercept) <0.0005, P(slope) <0.0005 **Equation 9-18**

According to the multiple regression there was no significant difference in intercept (P>0.05) between matrices, however there was for the slope (P<0.0005). The weight of debris was greater for the chocolate matrix than the gelatine gel matrix throughout the chewing sequence, and increased by a greater amount per chew (Figure 9-9).

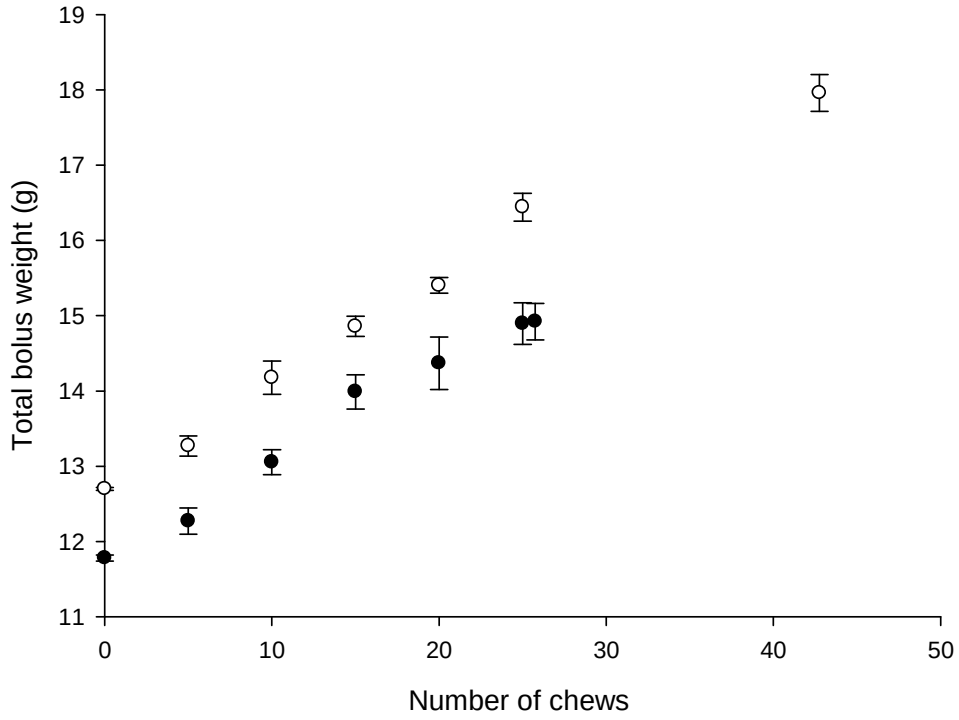

Figure 9-7: Total wet weight of the bolus recovered (bolus expectorated + debris recovered via a rinse) at different points in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○ (mean±SE). Difference between matrices: P(intercept) <0.0005, P(slope) >0.05.

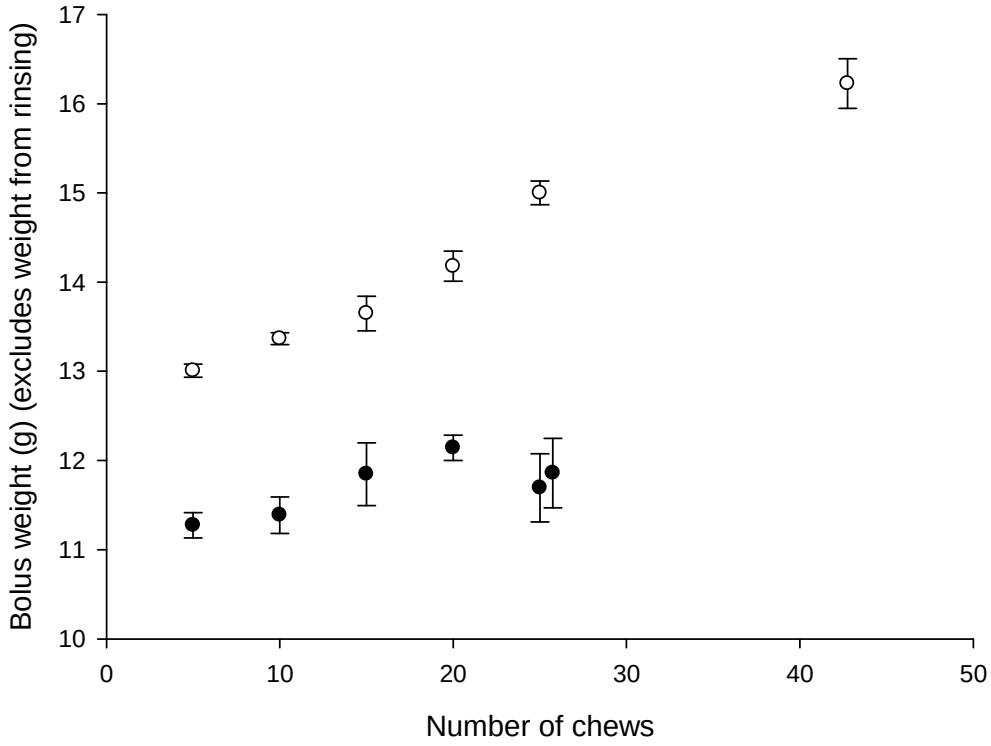

Figure 9-8: Weight of boli recovered (from expectoration) at different points in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○ (mean±SE). Difference between matrices: P(intercept) <0.0005, P(slope) <0.0005.

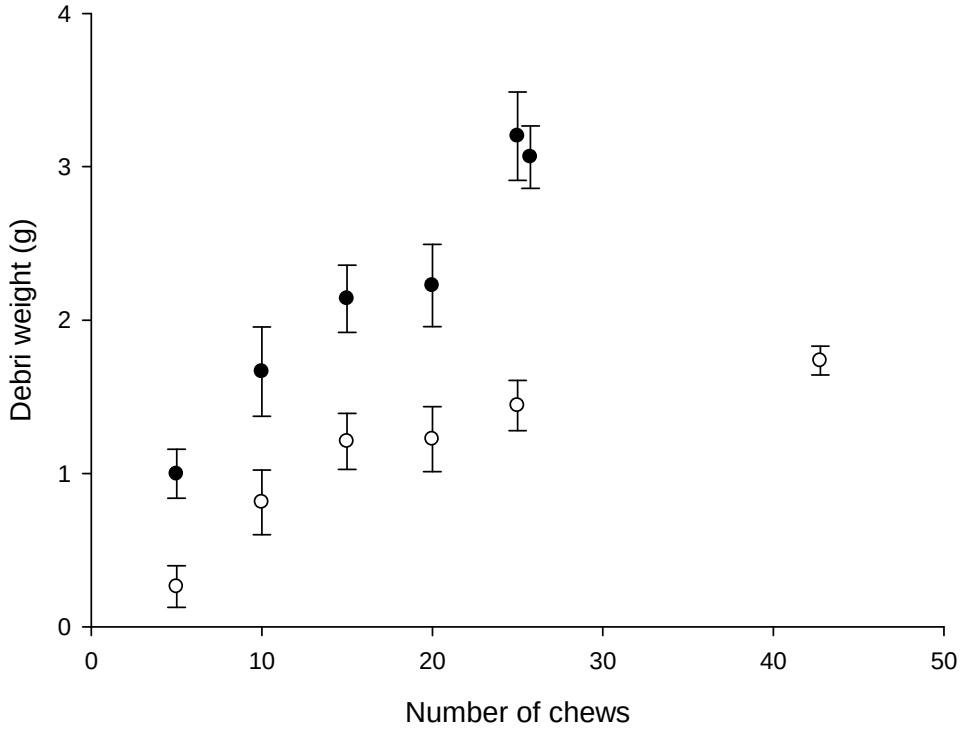

Figure 9-9: Weight of debris recovered from rinsing of the mouth after expectoration. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○ (mean±SE). Difference between matrices: P(intercept) >0.05, P(slope) <0.0005.

9.3.5 Bolus moisture content

The moisture content of the bolus increased significantly with the number of chews for both matrices (Figure 9-10). The chocolate and gelatine gel data sets were normally distributed according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction), P>0.05. Using simple linear regression, the following relationships were obtained (Equation 9-19 and Equation 9-20) (where *x* is the number of chews):

Chocolate: %moist = -3.0 + 1.6*x*, r = 0.97, P(intercept) >0.05, P(slope) <0.0005 **Equation 9-19**

Gelatine gel: %moist = 43.1 + 0.9*x*, r = 0.89, P(intercept) <0.0005, P(slope) <0.0005 **Equation 9-20**

There was a significant difference in the intercept (P<0.0005), but no significant difference in the slope (P<0.05) between matrices according to the multiple regression.

The moisture content of the gelatine gel matrix was much greater than the chocolate matrix early in the chewing sequence. This difference remained throughout the chewing sequence. The final moisture content of the gelatine bolus ready for swallowing was approximately twice the moisture content of the chocolate bolus (Figure 9-10).

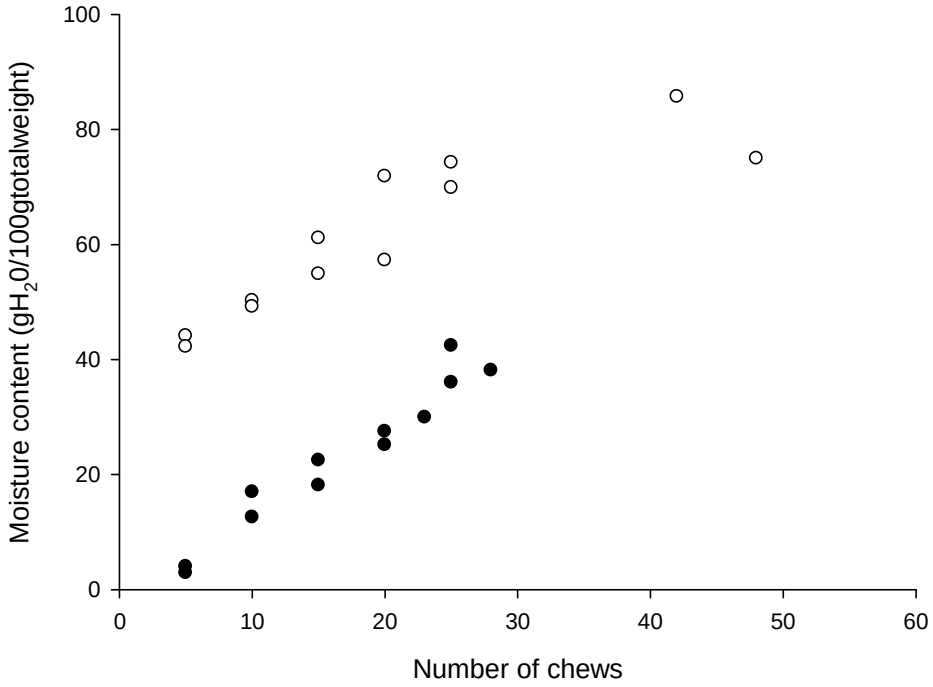

Figure 9-10: The moisture content of the boli at different points in the chewing sequence. Chocolate matrix containing peanuts: ●, Gelatine gel matrix containing peanuts: ○. Difference between matrices: P(intercept) <0.0005, and P(slope) <0.05.

9.4 Discussion

9.4.1 The pathway of peanut particle breakdown

The exponential decline in the d_{50} of the peanut particle size distribution is typical of data that has been obtained using homogenous foods. This has been shown in previous studies involving peanuts (Kawashima et al., 2009), brazil nuts, and carrots (Lucas & Luke, 1986), and an artificial test food, Optosil (Olthoff et al., 1984). An exponential decline in d_{50} is likely to occur because large particles are more easily broken into small particles than small particles are broken into fine particles (Lucas, 2004). Once particles reach a certain size the chewing process becomes less efficient in further reducing particle size. This can be seen from 20 chews to the swallow point in the chocolate matrix (26±1 chews, mean±SE) and from 25 chews to the swallow point in the gelatine gel matrix (43±1 chews, mean±SE) (Figure 9-2).

The trends in the broadness value (*b*) show that the spread of the peanut particle size distribution is reducing throughout the chewing sequence. This was seen for peanuts inside both matrices. This result is an agreement with work by Lucas & Luke (1986) where cumulative distributions of raw carrot boli were plotted after 5, 10, 15, 20, 25 and 30 chews. However, work with Optosil has found a slight decrease in the broadness value (*b*) between 10-50 chews, before an increase in later cycles between 50-200 chews (Olthoff et al., 1984). The broadness value (*b*) of cornflake particles also steadily decreased throughout the natural chewing sequence (5-30 chews) (Yven et al., 2010). Trends in the broadness are likely to be dependant on the type of food (or combination of foods in the case of this study) that is served. Lucas (2004) explains that after a few chews the long tail of the distribution is usually out to the left, and later moves right as particles get smaller with further chewing (when presented as a probability distribution). As the chewing process goes on, the fracture mechanics change because it is more difficult to break smaller particles than larger ones.

Changes in specific surface area were expected to increase exponentially as peanuts are masticated and d_{50} declines. However, the large losses in peanut weight (and volume) in

the bolus (Figure 9-5) are likely to have caused specific surface area trends to follow more of a linear shape (Figure 9-4).

9.4.2 The influence of the matrices on the rate of peanut particle size reduction

The type of matrix served has influenced the breakage pattern of the peanut particles in the bolus (Figure 9-1, Figure 9-2, Figure 9-3, & Figure 9-4). Particle size (in terms of d_{50} and broadness (b)) and specific surface area differed between matrices at each chewing interval, apart from the swallowing point (where d_{50} was similar). d_{50} of the peanut particle size distribution was greater inside the gelatine gel throughout the chewing sequence. The broadness (b) value of the distribution and specific surface area of peanut particles was greater inside the chocolate throughout the chewing sequence.

This strongly suggests that the selection function differs between matrices. The selection function is typically measured by colour marking of particles of a certain size range, and determining how many particles of that size range are fractured after a given number of chews. Large particles have been found to have a higher selection function than small particles (Lucas & Luke, 1983; van der Glas et al., 1987).

One possible explanation is that the matrix influences how easily the molar surface of the teeth can encounter the peanuts for fracture (or remove peanuts from the matrix and then encounter). The TPA parameters of cohesiveness (describing the strength of internal bonds within a food), chewiness (a measurement of the work required to masticate a food), and springiness (a description of the elastic properties of the food) were all substantially higher in the gelatine gel (Szczesniak, 1963; Pons & Fisman, 1996; Bourne, 2002). Consequently, it is likely to be more difficult for the molar teeth to break through the gelatine gel to encounter the peanut pieces in comparison with the chocolate. The chocolate is also likely to soften and melt at temperatures found inside the mouth (Do et al., 2007) to aid how easily teeth can encounter the peanut particles.

An alternative explanation is that the gelatine gel requires greater masticatory work. Peanut pieces may be isolated from both matrices early in the chewing sequence, but are

masticated intermittently while the matrix is being masticated. Given the large differences in cohesiveness, chewiness, and elasticity, it is possible that more time must be spent breaking down the gelatine gel matrix than the chocolate matrix to produce a swallow-safe bolus. As a result, less time is spent masticating the peanuts in the case of the gelatine gel, and consequently the particle size reduction rate is slower.

However, it cannot be disregarded that different matrices could change the breakage function of the peanuts rather than the selection function. The breakage function is a measure of particle fragmentation per chew (Lucas et al., 2002). As differences in textural properties exist between the matrices, it is likely that the pathway of the molars differs depending on the matrix. It is well known that chewing trajectories (in terms of vertical, lateral, and anterior-posterior movement) change from food to food (Hiiemae et al., 1996). Furthermore, tougher foods tend to be chewed with greater lateral motion than softer foods (Proschel & Hoffman, 1988) (the gelatine gel may require greater lateral motion during mastication that the chocolate). Consequently, differences in molar trajectories could influence manner by which the molars contact the peanut pieces, and influence the degree of fragmentation to take place after contact.

9.4.3 Losses in dry weight during the chewing sequence

Peyron et al. (2004b) measured particle retention after one quarter, one half, and a full chewing sequence with peanuts, almonds, pistachio nuts, carrots, radish, and cauliflower. A significant reduction from one quarter to one half of a sequence, and from one half to a full sequence was found for all six foods. In this chapter a reduction in total peanut dry weight was also found during the chewing sequence, where total peanut dry weight in the gelatine matrix was greater than in the chocolate matrix at each chewing interval, while the final total peanut dry weight was similar (Figure 9-5). Surprisingly, losses in total dry weight of the boli were small compared to losses of peanut dry weight. No significant losses of gelatine bolus total dry weight, and small but significant losses in chocolate bolus total dry weight were observed (Figure 9-6).

Similar peanut losses have been reported in boli at the end of the chewing sequence (Jalabert-Malbos et al., 2007; Flynn et al., 2010). Losses are suggested to take place due

to intermediate swallowing (Jalabert-Malbos et al., 2007), fat loss into saliva, transportation to the oropharynx (where particles will not be collected using a rinse after expectoration), and sieving (Flynn et al., 2010). It is probable that the loss of peanut dry weight in this study has been caused by movement of peanut particles into the oro-pharynx and during sieving across the 355 μm sieve to isolate peanut particles from the matrix. Loss has also been shown to take place during image analysis when the peanut particles are stored in ethanol (Section 3.1.4).

Greater peanut dry weight losses in the chocolate compared to the gelatine gel during the chewing sequence (but not at the swallow point) may be a function of a smaller particle size at each interval. Smaller particles are likely to be shifted more easily into the oro-pharynx, will exhibit greater loss through the 355 μm sieve during washing, and will release a greater quantity of fat into ethanol during image analysis (Section 3.1.4).

It is unclear why losses of total bolus dry weight were small in comparison with total peanut dry weight in this study, and in comparison with other published literature assessing peanut retention (Peyron et al., 2004b; Jalabert-Malbos et al., 2007; Flynn et al., 2010). It is possible that the mouth is able to efficiently retain the matrices more efficiently throughout the chewing sequence, whereas peanut particles are lost more readily from the matrix into the oro-pharynx. However, peanut dry weight retention is reduced by other sources of loss during washing across the 355 μm sieve and image processing.

9.4.4 Changes in bolus properties throughout the chewing sequence as particles are broken down

There was a significant linear increase in the total bolus weight and moisture content during the chewing sequence (Figure 9-7 & Figure 9-10) due to saliva addition. Saliva content has been shown to increase in several studies during the chewing sequence (Mioche et al., 2002a; Loret et al., 2009). The similar rates of increase in total bolus weight and moisture content (Figure 9-7 & Figure 9-10) between matrices suggest that saliva flow was comparably stimulated between matrices. However, given the difference

in the number of chews (and hence chewing time) more saliva would have been incorporated into the gelatine gel bolus prior to swallowing.

Significant differences between matrices in the slope of the bolus weight and debri weight plots suggest differences exist in rheological properties between matrices throughout the chewing sequence (Figure 9-8 & Figure 9-9). The amount of debris was greater and increased at a greater rate for chocolate than the gelatine gel. This is likely to be due to the chocolate adhering to the walls of the mouth more so than the gelatine gel.

9.5 Conclusion

Embedding peanuts inside two different matrices (chocolate and gelatine gel) resulted in different peanut particle breakdown pathways between matrices. Dynamic changes during the chewing sequence in the $d_{50,}$ the broadness (b), and specific surface area of peanut particles were significantly different between matrices. Contrasting matrices also induced different patterns of weight loss in terms of the peanut and total bolus dry weight, and wet bolus and debri weight. However, dynamic changes in total bolus wet weight and bolus moisture content were unaffected by the type of matrix.

Chapter 10 : The influence of peanuts and food matrices on mastication and the peanut particle size distribution of the bolus in a population (multiple subject study)

10.1 Introduction

The main results from the four separate single subject studies (Chapters 6-9), which have investigated mastication and the food bolus in heterogeneous systems, could be summarised as:

1. The properties of the matrices determine the chewing behaviour, whereas the properties of the peanuts appear to have almost no influence.
2. The properties of the peanuts (modified using moisture) influence the final d_{50} of the peanut particle size distribution and weight and volume retention in the bolus, and the breakage properties of the particles.
3. The properties of the matrices influence the final spread (broadness, *b*) of the peanut bolus particle size distribution, and the rate the peanut particles break down.
4. Interactions between peanuts and matrices may also be influencing mastication and bolus properties.

However, the single subject approach has the obvious limitation that results cannot be concluded for a population. Significant variability in chewing behaviour (Lassauzay et al., 2000; Peyron et al., 2002) and particle size outcome (Mishellany et al., 2006; Jalabert-Malbos et al., 2007) is shown throughout mastication literature. The sources of variability in mastication include age (Peyron et al. 2004a; Mishellany-Dutour et al. 2008), gender (Youssef et al.1997; Peyron et al. 2004a), dental condition (van der Bilt et al. 1993c; Fontijn-Tekamp et al. 2004a), and salivary flow (Engelen et al., 2005b). Effective screening measures can reduce the effect of these variables, but will never eliminate significant variation. Moreover, the extent of variability in heterogeneous food systems is unknown.

Hence these results required validation and clarification with a multiple subject study. This would also provide an understanding of the variability of mastication and food bolus data between subjects for foods used throughout this thesis, to give perspective to the single subject results. A multiple study was carefully designed, where 4 heterogeneous test foods (2 matrices variants and 2 peanut variants) were served to 8 subjects (4 male and 4 female).

Therefore, the aim of this study was to investigate variation among a population in mastication and particle size outcome when different heterogeneous foods are chewed. In particular, to compare the effects of two types of solid test pieces with different physical properties (moist and dry peanuts) embedded inside two types of matrices (chocolate and gelatine (250 bloom)) on mastication and the final peanut particle size distribution in the food bolus.

Much of this chapter, including many figures and tables, is based on work which has been published (Appendix H) (Hutchings et al., 2011).

10.2 Methodology

10.2.1 Subject selection

Eight subjects (4 male and 4 female, 25.6±4.3 years) were selected for this study according to guidelines outlined in Section 5.3. The process of selection was not as strict as in previous chapters given that a population was selected rather than a single subject. However, several volunteers that demonstrated unusual oral processing behaviour in terms of the bite size and the number of chews applied to the standard Fruit and Nut bar were screened out.

Potential subjects were assessed by asking them to bite, chew and expectorate the standard Fruit and Nut bar on three separate occasions. The selection procedure also assisted in familiarising subjects with the process of expectorating a food bolus for the trial. Selected subjects also met strict dental criteria. All subjects had class 1 occlusion, no significant tooth crowding, no obvious tooth decay, and a healthy periodontal condition. They had no functional disturbance of mastication evidenced by pain or clicking during chewing, and no other known oral or general health issues that could influence oral processing. This study was reviewed and approved by the Massey University Human Ethics Committee: Southern A (Application 09/24).

10.2.2 Experimental procedure

Following the screening session, each subject attended one experimental session. Two types of matrix (gelatine gel (250 bloom) or chocolate) containing one of two types of peanut (moist (22.21±0.18 gH_20/100g total mass) or dry (1.99±0.20 gH_20/100g) (mean±SD)) were served in a randomised order. Four replicates of each food type (16 samples in total) were served for each subject.

Experimental conditions and protocol followed that outlined in Section 3.2.1.

10.2.3 Assessment of natural bite size and selection of serving size

Serving size was determined by the assessment of the natural bite length of the subjects (and hence natural bite volume) as discussed in Chapter 4. Each subject was asked to take 2 natural bites from chocolate bars (containing 11.3% peanut quarters (v/v)) with a constant shape (20 mm height, 30 mm width, 100 mm length). A mean bite length of 16±6 mm (mean±SD) was determined from the 8 selected subjects, and therefore a constant volume serving size of 9600 mm^3 (20x30x16 mm) for matrices (containing 11.3% peanut pieces (v/v), 4-5 quarter pieces) was adopted, where ρ(moist peanuts) = 1.06 g/cm^3 and ρ (dry peanuts) = 1.08 g/cm^3. The weights of the servings were determined (Table 10-1). The weight of dry peanuts was slightly greater than moist peanuts to ensure the volume of peanuts served was the same (given the small density differences).

Table 10-1: Serving weights of the test foods (mean±SD).

Test component	Serving weight (g)
Chocolate matrix (g)	11.26±0.20
Gelatine matrix (g)	12.30±0.21
Dry peanuts (g)	1.14±0.03
Moist peanuts (g)	1.12±0.03

10.2.4 Preparation of the test foods

Matrices were prepared without peanuts inside according to methods outlined in Section 3.1.3. Peanuts were manually inserted into the matrix immediately before each sample was served to the subject to ensure no unwanted moisture migration took place. Roasted, unsalted peanuts were used in all matrices. All peanut pieces (quarters) were sieved across a 4.75 mm sieve prior to insertion into the matrix to ensure no small particles were included. Moist peanuts were prepared by soaking the peanut quarters in water for 2 h and equilibrating in a water tight container for 46 h in a refrigerator (4 ºC).

10.2.5 Analysis of the physical properties of the matrices and peanuts

Analysis of the physical properties of the matrices and peanuts were undertaken according to methods described in Section 3.2.2. Four replicates were used for peanut moisture content, 10 replicates were used for peanut hardness, and 6 replicates used for peanut density. Textural analysis of the matrices involved 12 replicates.

TPA and stress-strain curves from compression of the matrices and peanuts are also presented to enhance an understanding of the physical properties. The stress-strain curves were obtained from single uni-axial compression using the same test conditions outlined for the TPA procedure (Section 3.2.2) for matrices and peanuts respectively. Given that the cross sectional area of peanuts was not known, a cross sectional area of 1 cm^2 was assumed for both dry and moist peanuts to calculate stress.

10.2.6 Analysis of the food bolus

Analysis of the peanut particle size distribution in the bolus was conducted according to methods Section 3.1.4.

10.2.7 Statistical analysis

Statistical analyses were performed using SPSS ® (version 16.0 for Windows) (SPSS Inc., USA). The fit of the cumulative distribution function of particle area by the Rosin Rammler equation was determined for each bolus by the R^2 values.

The distributions of the parameters (number of chews, chewing time, mastication frequency, d_{50}, broadness (*b)*, dry weight retention, and volume retention) were considered to be normally distributed when the P value was greater than 0.05 according to the Kolmogorov-Smirnov test for normality (with Lillifors significance correction). A two-way repeated measures ANOVA, with matrix type and peanut type as within subject factors, was used to assess the significance of the differences in results between

matrices and peanuts. The following transformations were made to normalise the data (conversion from x to y):

- The number of chews and chewing time were normally distributed following $\log_{10}$ transformation, and the mastication frequency was normally distributed following exponential function transformation (e^x).
- d_{50} was normally distributed following a Johnson transformation in MINITAB (version 15, Minitab Inc.) with the following formula:

y = -2.23950 + 1.51986*Asinh(x-0.918140)/0.143034) **Equation 10-1**

- The broadness value (*b)* was normally distributed without transformation.
- Dry weight retention of peanuts was normally distributed following Johnson transformation in MINITAB (version 15, Minitab Inc.) with the following formula:

y = 0.318810 + 0.847683 * ln((x - 8.67778) / (72.5068 - x)) **Equation 10-2**

- Peanut volume retention was normally distributed following Johnson transformation in MINITAB (version 15, Minitab Inc.) with the following formula:

y = -0.215711 + 0.635957 * ln((x - 707.488)/(2035.88 - x)) **Equation 10-3**

10.3 Results

10.3.1 Physical properties of the test food components

As in previous chapters the physical parameters differed between the matrices and between the peanuts (Table 10-2). The cohesiveness, springiness (elasticity), and chewiness were all greater in the gelatine gel than the chocolate matrix. The chocolate matrix was harder than the gelatine gel matrix, and the dry peanuts were harder than the moist peanuts.

Figure 10-1 and Figure 10-2 are curves obtained from TPA and single uni-axial compression of the matrices respectively, showing distinct differences in texture between the chocolate and gelatine gel.

The gelatine gel demonstrated highly elastic properties, whereas the chocolate demonstrated plastic properties (Figure 10-1). The force-time profile of the gelatine gel is similar in the first compression compared to the second compression, showing almost complete recovery to its original state. The profile of the chocolate differs markedly between compressions, showing significant unrecoverable deformation after the first compression. This is shown most clearly by the time delay in the second compression between the chocolate and the gelatine gel, but is also evident by the differences in area under the curve from the first to second compression. Stress of the chocolate and gelatine gel matrices also differed in response to deformation during a single compression (Figure 10-2). The slope (Young's modulus) differs markedly between matrices.

A stress-strain curve of the single uni-axial compression of the peanuts (to the point of fracture) is also presented (Figure 10-3). Dry peanuts were subject to fracture at a lower strain. The slope (Young's modulus) is significantly smaller in the moist peanut. However, the area under the curve until fracture takes place appears similar between peanut types (the toughness, see Section 2.7.7).

Table 10-2: Properties of the test foods (mean±SE) (Note: The hardness of the matrices and peanuts were undertaken using different TPA conditions).

Matrices					**Peanut piece (quarter)**		
	Hardness (N)	**Cohesiveness**	**Springiness (mm)**	**Chewiness (mJ)**		**Moisture content (gH_20/100g total mass)**	**Hardness (N)**
Gelatine gel (250 bloom)	252±8	0.89±0.01	10.1±0.1	2270±90	Dry peanut	1.99±0.10	78.04±9.27
Chocolate	400±8	0.17±0.01	2.03±0.23	150±20	Moist peanut	22.21±0.09	51.63±6.93

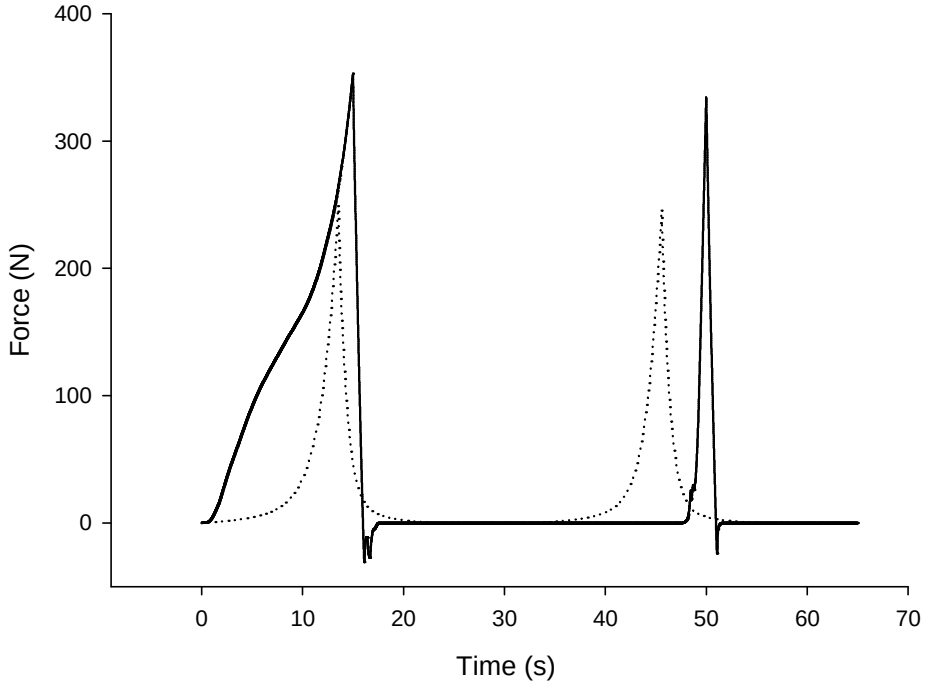

Figure 10-1: TPA curve of the chocolate matrix (solid line) and the gelatine gel matrix (250 bloom) (dotted line).

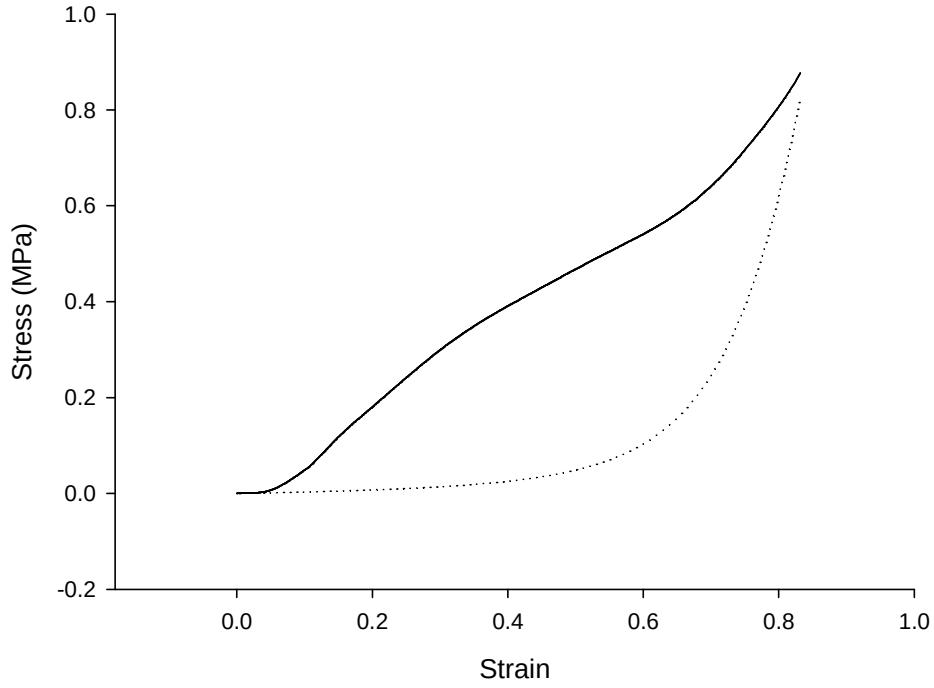

Figure 10-2: Stress-strain curve of the chocolate matrix (solid line) and gelatine gel matrix (250 bloom) (dotted line).

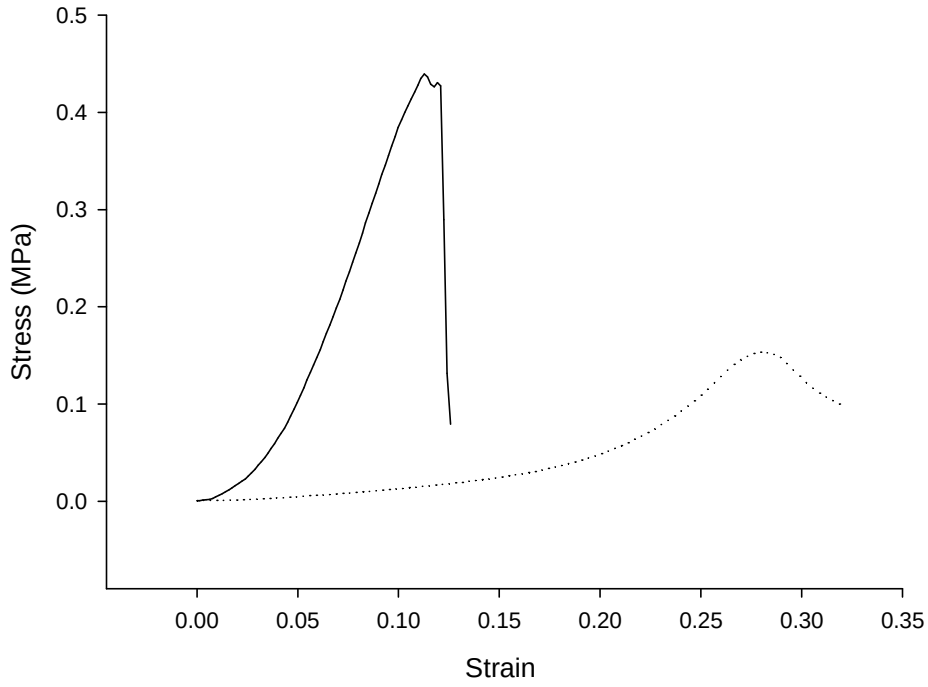

Figure 10-3: Stress-strain curve of the dry peanut (solid line) and moist peanut (dotted line) to the point of fracture (cross sectional area of peanuts was assumed to be 1 cm^2).

10.3.2 Parameters of mastication

A. Variation in mastication between subjects

Large variation in mastication parameters occurred between subjects. The number of chews ($F(1,7) = 971.3$, $P<0.0005$), chewing time ($F(1,7) = 676.0$, $P<0.0005$), and mastication frequency ($F(1,7) = 228.2$, $P<0.0005$) all differed significantly between subjects according to the repeated measures ANOVA for the effect of matrix type and the type of peanut piece inside each matrix.

B. The effect of the type of matrix and peanut type on mastication

Compared to chocolate, the gelatine gel was masticated for a significantly greater number of chews ($F(1,7) = 98.8$, $P<0.0005$) and consequently chewing time ($F(1,7) = 54.2$, $P<0.0005$), and at a significantly higher mastication frequency ($F(1,7) = 10.8$, $P<0.05$) (Table 10-3). For moist and dry peanut pieces in a given matrix, there were no significant differences either in the number of chews ($F(1,7) = 4.4$, $P>0.05$), chewing time ($F(1,7) = 2.2$, $P>0.05$), or mastication frequency ($F(1,7) = 0.8$, $P>0.05$) (Table 10-3). Interactions between matrix and the type of peanut piece were not significant for the number of chews ($F(1,7) = 3.7$, $P>0.05$), chewing time ($F(1,7) = 1.2$, $P>0.05$), or mastication frequency ($F(1,7) = 2.2$, $P>0.05$). The gelatine gel was chewed 37.8 and 40.5 times on average when containing the dry and moist peanut pieces respectively. The chocolate was chewed 23.8 and 23.9 times on average when containing the dry and moist peanut pieces respectively.

10.3.3 Food bolus particle size

A. Variation in peanut particle size in the bolus between subjects

There was large variation between subjects in the peanut particle size distribution of individual boluses (Figure 10-4). Hence d_{50} ($F(1,7) = 294.8$, $P<0.0005$), and broadness (b) ($F(1,7) = 2873.4$, $P<0.0005$) of the peanut particle size distribution differed significantly between subjects on a repeated measures ANOVA for the effect of matrix type and type of peanut piece.

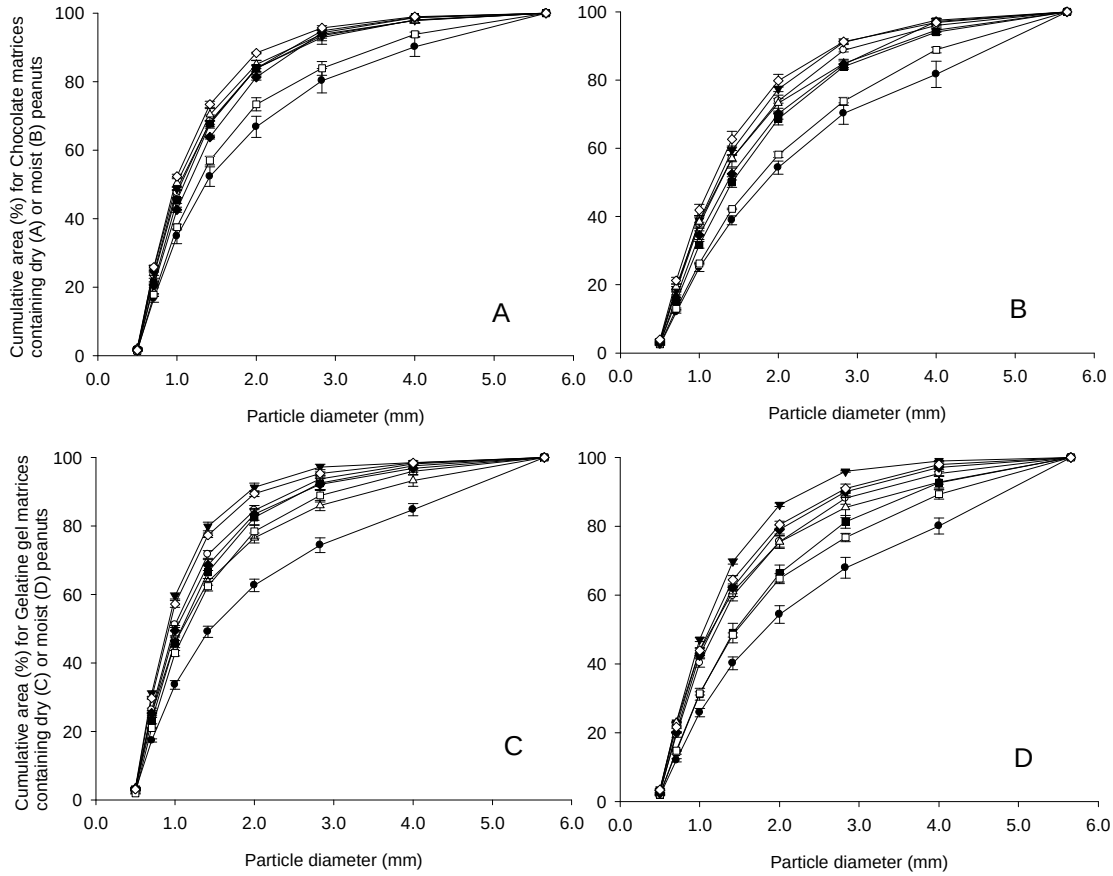

Figure 10-4: Cumulative peanut particle size distributions obtained from the boli of all subjects for chocolate containing dry peanuts (A) and moist peanuts (B), and gelatine gel containing dry peanuts (C) and moist peanuts (D). Subject 1: ●, Subject 2: ○, Subject 3: ▼, Subject 4: Δ, Subject 5: ■, Subject 6: □, Subject 7: ♦, and Subject 8: ◊ (mean±SE).

B. The effect of matrix type and peanut type on the bolus peanut particle size distribution

The mean peanut particle size distribution curves are shown in Figure 10-5. There was significant variation between matrix and between peanut types in the particle size parameters. Whilst d_{50} ($F(1,7) = 4.0$, $P>0.05$) did not vary significantly between matrices, the d_{50} value for moist peanut particles was significantly larger than that for the dry peanut particles ($F(1,7) = 147.5$, $P<0.0005$). The average d_{50} of the dry peanut particle size distribution was 1.14 mm and 1.18 mm with the gelatine gel and chocolate matrices respectively, whereas the average d_{50} of the moist peanut particle size distribution was 1.37 mm and 1.43 mm with the gelatine gel and chocolate matrices respectively. There was no significant interaction between matrix and peanut type for d_{50} ($F(1,7) = 0.251$, $P>0.05$) (Figure 10-6, Table 10-3).

The broadness (*b*) values of the peanut particle size distribution ($F(1,7) = 8.9$, $P<0.05$) were significantly higher for boli with a chocolate matrix than those with a matrix of gelatine gel, showing the spread in the size distribution of peanut particles in the gelatine gel matrix was greater than that in the chocolate matrix. Peanut type did not affect the broadness value (*b*) ($F(1,7) = 0.0$, $P>0.05$), however the interaction of the effects of the matrices and peanut type on broadness (*b*) was significant ($F(1,7) = 11.0$, $P<0.05$) (Figure 10-6, Table 10-3). The interaction can be seen in Table 10-3, where *b* values were higher in peanut particle size distributions of the moist peanuts compared to dry peanuts in the gelatine gel, and higher for dry peanuts compared to moist peanuts in the chocolate. On average, the broadness (*b*) values of the moist and dry peanut particle size distributions inside the gelatine gel were 1.13 and 1.17 respectively, and the broadness (*b*) values of the moist and dry peanut particle size distributions inside the chocolate matrix were 1.23 and 1.19 respectively.

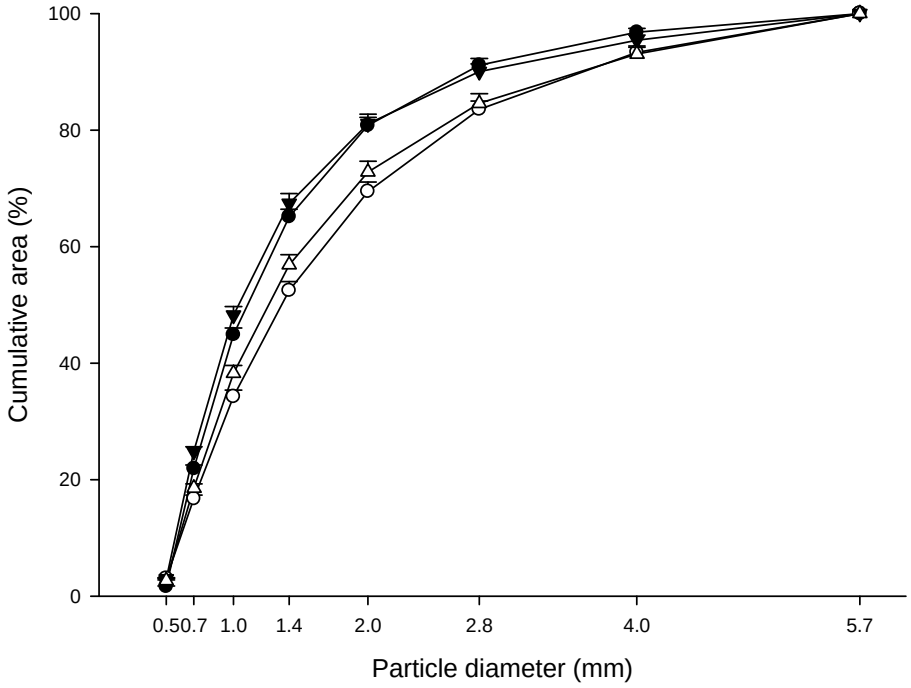

Figure 10-5: Mean cumulative peanut particle size distribution obtained from the bolus of gelatine gel containing dry (▼) or moist peanuts (∆), or chocolate containing dry (●) or moist (○) peanuts (mean±SE).

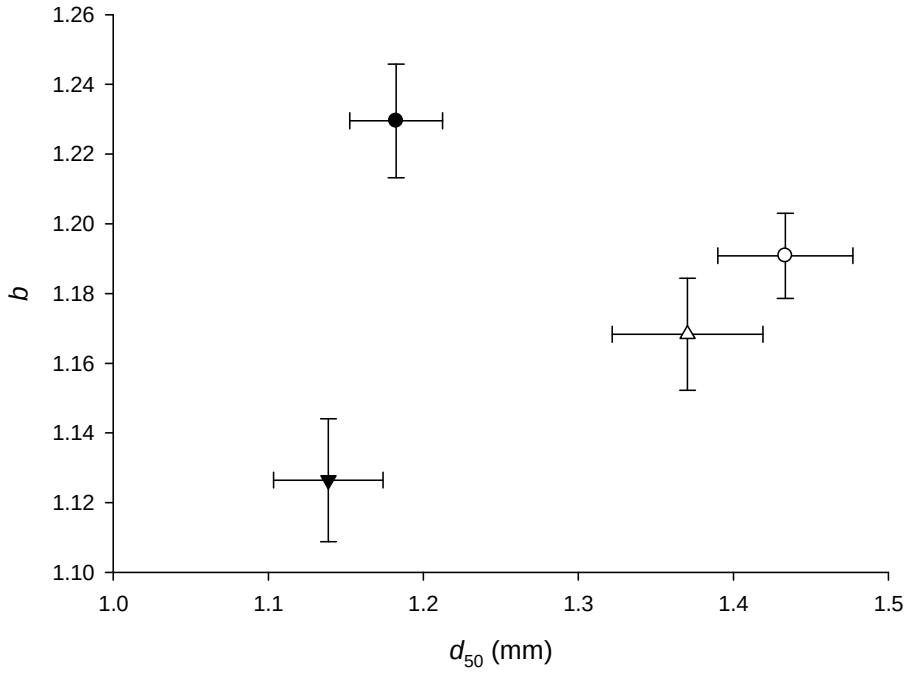

Figure 10-6: Mean broadness (*b*) against mean d_{50} of the peanut particle size distribution obtained from the bolus of gelatine gel containing dry (▼) or moist peanuts (∆), or chocolate containing dry (●) or moist (○) peanuts (mean±SE).

10.3.4 Retention of peanuts in the bolus

A. Variation in peanut retention between subjects

Dry weight retention of peanut particles ($F(1,7) = 318.7$, $P<0.0005$) and volume of peanut particles in the bolus ($F(1,7) = 789.5$, $P<0.0005$) varied significantly between subjects on a repeated measures ANOVA for the effect of matrix type and peanut type.

B. The effect of matrix type and peanut type on peanut retention

Dry weight retention of peanut particles ($F(1,7) = 2.1$, $P>0.05$) and volume of peanut particles in the bolus ($F(1,7) = 0.8$, $P>0.05$) did not differ significantly between matrices on repeated measures ANOVA (Table 10-3). However, dry weight retention of peanut particles ($F(1,7) = 40.1$, $P<0.0005$) and volume of peanut particles ($F(1,7) = 216.8$, $P<0.0005$) was significantly greater when moist peanuts were chewed than when dry peanuts were chewed (Table 10-3). There was no significant interaction between the effect of matrix and peanut type for dry weight retention ($F(1,7) = 0.0$, $P>0.05$) or volume of peanuts in the bolus ($F(1,7) = 0.0$, $P>0.05$) on repeated measures ANOVA.

Table 10-3: The effect of food matrices and peanut type on the mastication and bolus parameters (mean±SE).

Matrix	**Gelatine gel**	**Chocolate**	**Gelatine gel**	**Chocolate**
Peanut type	**Dry**	**Dry**	**Moist**	**Moist**
Number of chews	37.8±2.1	23.8±1.1	40.5±2.4	23.9±1.2
Chewing time (s)	27.66±1.53	18.58±1.12	28.77±1.61	18.56±1.06
Mastication frequency (Hz)	1.38±0.04	1.32±0.04	1.41±0.03	1.32±0.04
d_{50} (area) (mm)	1.14±0.04	1.18±0.03	1.37±0.05	1.43±0.04
b	1.13±0.02	1.23±0.02	1.17±0.02	1.19±0.01
% Peanut weight retention (drywt/drywt)	24.52±1.64	26.13±1.43	46.84±1.64	50.42±1.42
Volume of peanuts in bolus (mm^3)	1120±50	1150±40	1780±20	1800±20

10.4 Discussion

10.4.1 Variation between subjects

Variation between subjects was large in terms of the mastication parameters and particle size distributions despite efforts to select a population without unusual oral processing characteristics. Significant variation between subjects has also been reported previously in homogeneous foods in terms of the parameters of mastication (Peyron et al., 2004b; Foster et al., 2006) and particle size (Mishellany et al., 2006; Jalabert-Malbos et al., 2007). However, literature does suggest that variation in the particle size distribution of the food bolus is small in comparison with that of the parameters of mastication, and in some cases is not always found to be significant at all (Peyron et al., 2004b). Differences in the peanut particle size distribution of the bolus between subjects may be exaggerated when heterogeneous food systems such as the ones used in this study are chewed (Figure 10-4).

Interestingly, the magnitude of differences between subjects was greater than the magnitude of differences between food variables. Importantly, the results largely reflect the trends observed in Chapters 6-9. This study can therefore serve as some form of validation of the results in the single subject studies in this thesis.

10.4.2 The influence of the matrix and the peanuts on mastication

The significant differences in chewing behaviour when different matrices were chewed reflects reports of similar variation when different homogeneous foods are chewed (Hiiemae et al., 1996; Brown, et al., 1998; Mathoniere et al., 2000). This is also typical of results presented in Chapters 6-9, where gelatine gel matrices were chewed for greater periods of time and subjected to more chewing cycles than the chocolate matrices. This confirms that the matrix affected the various modalities of sensation during mastication and caused different chewing strategies to form a suitable bolus (Table 10-3). In this trial, the properties of the peanuts embedded inside the matrix did not appear to alter the chewing behaviour even though their different physical properties

did influence the peanut particle size outcome in the bolus, which was also found in Chapters 6-9.

The TPA curve (Figure 10-1) suggests that the application of stress by the molars during mastication will result in permanent deformation of the chocolate matrices (due to plastic behaviour) but significantly less permanent deformation in the gelatine gel (due to elastic behaviour). Flow of the matrices are also likely to be different in response to stress (Figure 10-2). When the differences in cohesiveness and chewiness (Table 10-2) are also considered, it is probable (as suggested in Section 9.4.2) that molar trajectories differ between matrices. Wider trajectories, particularly in terms of lateral grinding and cutting, would be expected in the gelatine gel compared to the chocolate.

10.4.3 The influence of the type of peanut on the particle size distribution of the food bolus

Matrices containing moist peanut pieces yielded higher d_{50}'s in the peanut particle size distribution than those containing dry peanut pieces, despite no significant difference in parameters of mastication (as also shown in Chapters 6 and 8). This shows the particle size that is required to prepare a safe-to-swallow bolus is larger with the moist peanuts. Uptake of water has been widely reported to influence the textural properties of foods (Roos, 1995), and nuts in particular (Visvanathan et al., 1996; Paksoy & Aydin 2004; ElMasry et al., 2009). Harder particles are also believed to be detected as larger than soft particles of the same size inside the mouth (Engelen et al., 2005c). Results indicate that changing the physical properties of the peanuts has caused differences in the rate of particle breakdown (as shown in Chapter 6 and 8), which is likely to be induced by differences in fracture propagation.

It is therefore highly likely the breakage function of the peanuts has changed by the addition of moisture. The breakage function depends on the physical properties of the food, where brittle foods that fracture into greater number of smaller particles have a higher breakage function (Agrawal et al., 1997). It appears that moist, soft peanuts

break into larger pieces during contact with the teeth, where as dry peanuts shatter into smaller pieces.

A comparison in the slope of the stress-strain curves (Figure 10-3) shows the Young's modulus (E) of the moist peanuts is markedly smaller than the dry peanuts. Assuming the toughness (R) of the material is similar between moist and dry peanuts (as suggested in Figure 10-3 where the total area under the curve is alike for both peanut types), the reduction in Young's modulus of the moist peanuts can explain the change in breakage function. According to Agrawal et al. (1997), where the breakage function was described by a highly negatively correlated relationship with $(R/E)^{(0.5)}$ (Lucas et al., 2002), a decrease in E will increase $(R/E)^{(0.5)}$, and hence decrease the breakage function. Thus when the dry peanut is shattered, more fragmentation takes place in comparison with the moist peanut. Lucas et al. (2002) presents data showing the breakage function of white bread soaked in water is smaller than dry white bread, and the breakage function of raw peanuts is smaller than roasted peanuts. Raw peanuts also shattered into larger pieces than roasted peanuts following a uni-axial compression test in Section 8.3.1, Figure 8-2.

10.4.4 The influence of the matrix on the particle size distribution of the food bolus

The two matrix types exhibited significant chewing behaviour differences but this did not affect the d_{50} for any one peanut type. This indicates the large differences in breakdown rates between matrices (likely to be due to differences in the selection function, the probability that particles of a given size present themselves to the occlusion zones (Lucas et al., 2002)) is valid for a larger population. The properties of the matrix (such as those shown in Figure 10-1 & 10-2) are likely to influence the selection function of peanuts that lie within. Differences in flow, plastic-elastic deformation, and chewiness may all be influencing how readily particles can be encountered by the teeth inside the matrices. Section 9.4.2 presented several hypothesises to describe differences in breakdown rates between matrices.

The type of matrix that was chewed also affected the spread of the resultant peanut particle size distribution (broadness (b)) (similar differences in broadness (b) were also

found between matrices in Chapters 6, 8, and 9). The higher broadness (*b*) values from boli containing chocolate (showing peanut particle size in the chocolate had a smaller spread) may reflect the differences that are seen in the rate of particle breakdown. Chocolate is renowned for melting rapidly in the mouth (Do et al., 2007), had considerably lower values of cohesiveness and chewiness (Table 10-2), and exhibited plastic deformation (Figure 10-1). Consequently, it may be that larger particles are more likely to be selected over smaller particles in the chocolate matrix, resulting in a tightening of the particle size distribution where less spread is observed. Alternatively, differences in broadness could be attributed to contrasting molar trajectories inside the matrices (see Section 9.4.2). A more lateral motion through the gelatine gel may result in fracture propagation where the particle size distribution has a greater spread.

Moreover, the significant interaction for broadness (*b*) between matrices and peanut type (Section 3.3.2) shows the ability of the molars to uniformly masticate the peanut particles depends not only on the matrix, but on how the peanuts behave inside that matrix. The manner by which the peanut surfaces interact with the matrix could contribute to adhesion to the matrix, and thus again influence how easily the molars can encounter the peanuts for fracture.

Despite differences in broadness (*b*), the observation that the d_{50} of the peanut particle size distributions are statistically similar between matrices challenges current literature. A bolus is deemed to be safe to swallow when it reaches a certain particle size, cohesion, and lubrication (Hutchings and Lillford, 1988; Prinz & Lucas, 1997). It therefore would be expected that different matrices would induce sufficient differences in cohesion and lubrication of the bolus (Section 9.3.4 strongly suggests this is the case) to change the d_{50} of the particles size distribution.

As discussed in Section 7.4.1, it is possible that receptors inside the mouth can precisely detect the texture and size of the peanut particles within all the different matrices, where particle size is considered pivotal in preparing a safe bolus. As a result, the cohesion and lubrication of the surrounding matrix may have no influence on the final d_{50}. Receptors on the tongue and oral mucosa can detect single particles as small as 2 mm (Ringel & Eawonski, 1965; Lucas, 2004), and particles as small as 2 µm (Engelen et al., 2005a) can influence textural sensations.

An alternative hypothesis, linked to a two compartment theory introduced by Flynn et al. (2010), may also offer an explanation for the similarity observed in d_{50}. In this case the peanuts could be separated from the matrix early in the chewing sequence, and would be broken down and determined suitable for swallowing independently of the matrix (by being stored in different compartments of the mouth during mastication).

10.4.6 The influence of the matrix and the peanut on retention in the food bolus

No more than 50% of the dry weight of peanuts in the initial sample was retained in the bolus for each test food. Such losses have been reported in previous studies involving peanuts (Peyron et al., 2004b; Jalabert-Malbos et al., 2007; Flynn et al., 2010), and were similar in Chapters 7-9 at the swallowing point. Losses are expected to be high given the number of steps involved in the experimental procedure. As explained in Section 9.4.3 losses may take place by particles shifting into the oropharynx, during washing through the 355 μm sieve, and from fat migration into ethanol during image analysis (Section 3.1.4).

The weight and volume retention of the moist peanut particles was significantly greater than for the dry peanut particles (Table 10-3). Losses are likely to be higher in boluses containing a greater proportion of finer particles (in the case of the dry peanuts) as finer particles may be more readily lost into the oropharynx and through the sieve, and will loose more fat into ethanol.

Despite differences between matrices in chewing behaviour, deformation in response to stress, particle breakdown rates, and broadness of the particle size outcome, peanut retention (in terms of dry weight and volume) is unaffected by the type of matrix (Table 10-3). The reasons for this are unclear, but may indicate that peanut particle loss is simply a function of average particle size (ie d_{50}). This may even suggest again that matrices and peanuts are stored in different compartments of the mouth during mastication, where peanut communition and loss is independent of the matrix.

10.5 Conclusion

This multiple subject study showed the mastication of moist and dry peanut pieces inside gelatine gel and chocolate matrices, and the resulting peanut particle size distribution of the food bolus, was significantly different between subjects. Results have clarified among a population that when heterogeneous test foods are chewed, the properties of one food component can influence the breakdown of another food component. More specifically, when matrices (prepared as gelatine gel or chocolate matrices containing either moist or dry peanut pieces) were chewed, the matrix influenced mastication (in terms of the number of chews, chewing time, and mastication frequency) and broadness (b) of the peanut particle size distribution in the bolus. However, the matrix did not influence the d_{50} of the peanut particle size distribution, or retention of the peanut particles after mastication. Furthermore, the properties of the embedded peanut piece influenced the d_{50} of the peanut particle size distribution but had no affect on chewing behaviour.

Chapter 11 : Overall discussion and conclusions

11.1 Overall discussion

This is one of the first pieces of research into the mastication of and resulting food bolus from heterogeneous food systems, where more than one food type (such as a peanut particle embedded inside a food matrix), is involved in the mastication process. Almost all current mastication literature has looked at homogeneous foods (e.g. Kohyama et al., 2004b; Peyron et al., 2004b; Foster et al., 2006). Understanding of how heterogeneous systems breakdown inside the mouth is important as the bulk of material that is eaten is heterogeneous in composition.

The project had the following main objectives:

1. To identify the most suitable technique to standardise serving size for mastication studies.
2. To develop appropriate methods for the selection of single subjects in mastication studies.
3. To investigate the effect of matrices of contrasting physical properties on mastication, the particle breakdown process, and the particle size distribution of the bolus in heterogeneous food systems (where peanut pieces are embedded inside a continuous matrix).
4. To investigate the effect of test pieces of contrasting physical properties on mastication, the particle breakdown process, and the particle size distribution of the bolus in heterogeneous food systems (where peanut pieces are embedded inside a continuous matrix).
5. To develop a set of food design principles that can be used by food manufacturers to manipulate chewing behaviour and particle size in the food bolus. These design principles should lead to ideas for controlling digestion and the sensory appeal of food products.

To meet these objectives, this research has investigated the natural bite size of manufactured food bars in order to identify a technique for standardising serving size, and has developed a methodology for the selection of single subjects in mastication studies where the assessment of food properties is of interest. A series of studies have been undertaken with heterogeneous test foods to analyse mastication and the food bolus, where peanut test pieces of contrasting properties were embedded inside various food matrices of contrasting properties. Initial studies involved carefully selected single subjects, and main findings were validated with a multiple subject study.

Standardising serving sizes in mastication studies

It is currently unclear how serving size should be standardised in mastication research. Some studies standardise by weight (Mioche et al., 2002a; Fontijn-Tekamp et al., 2004a; Hiiemae, 2004) and others by volume (Agrawal et al., 1998; Engelen et al., 2005b; Foster et al., 2006). Serving size is important because it influences the number of chews and chewing time of the mastication sequence (Fontijn-Tekamp et al., 2004a; Gaviao et al., 2004), and the particle size distribution of the food bolus (Lucas & Luke, 1984; Buschang et al., 1997).

A trial was undertaken using 45 subjects with 6 common food bars of contrasting physical properties, where variation in bite weight, bite volume, and bite length were assessed between bars (Chapter 4). It is well known that bite size varies significantly between subjects (Hiiemae et al., 1996; Brately & Hackett, 1999), and between different food products (Medicis & Hiiemae, 1998; Yagi et al., 2006). Variation in bite length was much smaller than bite weight or bite volume between bars. The process of acquisition may therefore be limited by the length of a bite, and hence the physical shape and density of a bar may be much more important than the textural properties in determining the size of a bite.

Consequently a serving method was developed where foods to be studied were served to subjects as constant volume bars (with constant dimensions and cross sectional area), and subjects' asked to take natural bites of the bars. The bite length of each product was measured. A common bite length was then determined which fitted within the subject or subjects' natural bite range for the foods in that particular trial, and hence a standard

volume sample could be implemented for each trial (based on the length that was determined as the original cross sectional area of the bar). Standardising bite size using this method is recommended for other work in the mastication field, however researchers must decide on an appropriate serving method depending on the particular objectives of each trial.

Selection of subjects in mastication studies and the use of single subject studies

Large subject studies can be expensive and time consuming, and variability between subjects is widely reported in both mastication behaviour (Lassauzay et al., 2000) and the properties of the food bolus (Jalabert-Malbos et al., 2007). Variability within subjects is smaller (Mishellany et al., 2006; Jalabert-Malbos et al., 2007). For studies investigating the effects of food variables rather than human variables, smaller studies using a single subject can overcome these problems. Major findings from a series of single subject studies can then be validated with a multiple subject study. This was the approach taken in this work.

A selection procedure was developed to select single subjects from a larger group of applicants who did not display unusual biting and chewing characteristics, and who also met strict dental criteria (Chapter 5). Potential subjects were asked to bite and chew the Fruit and Nut bar used in the bite size study. Subjects who were the most consistent in terms of the spread of bite and mastication data, and who also had bite size and mastication data nearest to the mean of the 45 subjects in the bite size study (Chapter 4), were selected.

Single subject studies offer the potential to explore a greater range of variables over a shorter period of time. Initially they are limited because results cannot be representative of the broader population, however the most important findings can then be validated with a larger population.

The influence of changing the properties of internal test pieces (peanuts) in a heterogeneous system, where peanuts are embedded inside food matrices, on mastication and peanut particle breakdown

Changing the physical properties of the peanut pieces was primarily undertaken by immersing peanuts in water (Chapter 8 and 10). It was also undertaken by using various forms of heat treatment (Chapter 8), and took place when peanuts were prepared inside matrices of different physical properties (Chapter 6). A small section of work also changed the initial size of peanuts inside the matrix (Chapter 8).

Mastication was not altered by the changes in the properties of the peanuts when the peanut pieces were embedded in the matrices (Chapters 8 and 10) or when peanuts with differing physical properties were served without matrices (Chapter 6 and 8). However, changing the properties of the peanuts had a significant effect on the resulting peanut particle size distribution in the food bolus (Chapters 6, 8, and 10). The particle size distribution of the bolus is known to vary significantly between different foods (Hoebler et al., 2000; Peyron et al., 2004b).

Most notably, the d_{50} of the peanut particles was strongly influenced by the properties of the peanuts (Chapters 6, 7, 8, and 10) irrespective of the matrices they were inside. Mastication of peanuts with a higher moisture content (which reduced the hardness, fracturability, and Young's modulus) resulted in a particle size distribution with a larger d_{50} than peanuts with a lower moisture content (Chapters 6, 8, and 10). However the broadness (b) (spread) of the peanut particle size distribution was unchanged (Chapters 8 and 10). Hence the particle size required to prepare a swallow safe bolus of lower moisture content particles (harder particles) was smaller than for the higher moisture content particles (softer particles).

Moreover, immersing the products in water, and consequent changes in physical properties of the peanuts, appears to have altered the breakage function of the peanuts (the breakage function is a measure of the extent that the particle fragments during contact with the teeth (Lucas et al., 2002)). This was shown as a significant difference in d_{50} alongside a lack of significant difference in chewing behaviour (Chapters 6, 8 and 10). In addition, single compression tests on peanut halves resulted in large differences in the size and number of particles between peanuts subjected to different treatments

(Chapter 8), where moist peanuts were broken into fewer pieces at a larger size, and dry peanuts broken into more pieces at a smaller size. The change in breakage function can be explained by the reduction in the Young's modulus (E) of the moist peanuts (Chapter 10), as the breakage function has been shown to be highly negatively correlated to $(R/E)^{(0.5)}$ (Agrawal et al., 1997).

Peanuts which were heat treated under different conditions resulted in particle size distributions with different broadness (*b*) values but similar d_{50} values after mastication. Changes in the fracture propagation (Chapter 8) are likely to be responsible for these differences. Changing the initial size of the peanut pieces did not change d_{50} or broadness (*b*) (Chapter 8).

The influence of changing the matrix in a heterogeneous food system where peanuts are embedded inside food matrices, on mastication and peanut particle breakdown

Peanut pieces were embedded inside food matrices (gelatine gel (250 bloom and 200 bloom), chocolate, scone and brownie) of contrasting physical properties (Chapter 6, 7, 8, 9, and 10). Mastication (measured in terms of the number of chews, chewing time, and mastication frequency) was significantly different between matrices, as has been found between different homogeneous foods (Hiiemae et al., 1996; Mioche et al., 2002b). Most notably, the gelatine gel (250 bloom and 200 bloom) and scone matrices were chewed for a significantly greater number of chews and a longer period of time than the chocolate and brownie matrices.

Despite differences in mastication between matrices, no significant difference in the d_{50} of peanut particles in the bolus were found between matrices which contained the same type of peanut pieces (Chapter 7, 8, 9, and 10). Receptors inside the mouth appear to deem a precise particle size of peanuts vital to produce a swallow-safe bolus, regardless of differences in cohesion or lubrication of the surrounding matrices. Receptors on the tongue and oral mucosa can detect single particles as small as 2 mm (Ringel & Eawonski, 1965; Lucas, 2004), and particles as small as 2 µm influence textural sensations (Engelen et al., 2005a). It is also possible that peanuts are separated from the matrix early in the chewing sequence, and are broken down and determined suitable for

swallowing independently of the matrix. In such a scenario they could be stored different compartments of the mouth during mastication (Flynn et al., 2010).

The lack of significant differences in d_{50} alongside a significant difference in chewing behaviour (Chapter 7, 8, and 10), and the significant difference in breakdown rates between matrices (Chapter 9), strongly suggests the matrices influenced the selection function of the peanuts. The selection function is a measure of the probability of a food particle coming into contact with the occlusal surfaces of teeth (Lucas & Luke, 1983; van der Glas et al., 1987).

The difference in particle breakdown rates was shown most clearly between the gelatine gel (250 bloom) and chocolate matrices (Chapter 8, 9, and 10). The gelatine gel required significantly greater mastication (in terms of the number of chews and chewing time) than the chocolate, and the d_{50} of peanut particles in the ready to swallow bolus was not significantly different (Chapter 8 and 10). Furthermore, differences in the breakdown rate of peanuts were shown between these two matrices at separate chewing intervals as the chewing sequence progressed (Chapter 9).

Significant differences in the broadness (*b*) (spread) of the peanut particle size distribution were also found between matrices (Chapters 6-10). This was again shown most clearly in studies comparing the gelatine gel (250 bloom) and the chocolate matrices (Chapter 8, 9, and 10). The broadness (*b*) values of the peanut particle size distributions in the chocolate were greater than in the gelatine gel, which showed a narrower spread in the size of peanut particles in the chocolate bolus.

The different breakdown rates between matrices may result from the matrices determining how easily the teeth can encounter (or remove and then access) the peanuts for particle breakdown. It is also possible that as different matrices require different amounts masticatory work, the proportion of cycles spent comminuting peanuts varies from matrix to matrix.

Differences in broadness (*b*) may also be linked to these differences in selection, where for example the large particles inside the chocolate are more easily size selected than large particles inside the gelatine gel. Consequently peanuts inside the chocolate follow

a finer distribution. Differences in broadness (*b*) could also have resulted from varying molar trajectories between matrices leading up to contact with the peanut particles, causing differences in the fracture propagation of the peanuts.

The influence on changing the matrix and type of peanut in a heterogeneous food system on peanut retention

Significant losses in peanut dry weight took place in every test food, where no more than 50% of the initial dry weight was retained (Chapters 6-10). Similar losses have been reported in studies using peanuts as a homogeneous test food (Peyron et al., 2004b; Jalabert-Malbos et al., 2007; Flynn et al., 2010). The type of peanut had a significant influence on the weight retention and volume of peanut particles the food bolus (Chapter 8 and 10), but the type of matrix did not influence weight retention and volume of peanut particles at the swallowing point (Chapter 7, 8, and 10). Interestingly, peanut dry weight retention differed significantly between matrices during the chewing sequence. Total dry weight of the bolus (matrix and peanuts) was largely unchanged throughout the chewing sequence (Chapter 9).

Peanut losses are likely to be taking place by particles shifting into the oropharynx, during washing through the 355 μm sieve, and from fat migration into ethanol during image analysis (Section 3.1.4). Smaller particles are more prone to being lost in such processes, and therefore peanut retention in boli with smaller peanut particles was generally less than in boli with larger peanut particles (ie larger d_{50}).

Food design principles

Based on these findings, the following food design principles have been developed for food manufacturers to manipulate chewing behaviour and particle size in the food bolus. These design principles are linked to literature where chewing behaviour and the particle size distribution in the food bolus is related to sensory perception (Brown et al., 1994; Alfonso et al., 2002) and digestion rates (Read et al., 1986; Ranawana et al., 2010a).

1. Manipulating natural bite size

A regular bite length suggests that natural bite size could be altered by changing the density and cross sectional area of bars. Bite size has been shown to influence chewing behaviour and the particle size outcome in the food bolus (Buschang et al. 1997; Fontijn-Tekamp et al., 2004a).

2. Changing the mechanical properties of the food particle

Where peanuts were subjected to different physical treatments such as immersion in water (which induced differences in the physical properties of the peanuts), the breakage properties and particle size distribution in the bolus were altered. Food manufactures could therefore alter the processing conditions (time, heat, moisture addition or removal) of foods to manipulate the rate of particle breakdown (by changing the breakage function) and the boli particle size distribution.

3. Embedding food particles inside different matrices

Where peanuts were embedded inside different food matrices the rate of particle breakdown changed. The physical properties of food matrices can therefore be altered to change the rate of breakdown of particles contained within them. Food manufacturers could use food matrices to develop products with contrasting rates of flavour release.

11.2 Conclusions

A series of studies involving heterogeneous test foods, where peanuts were embedded inside food matrices, showed that the presence of one food component can alter the breakdown of another food component. In particular, changing the properties of the matrix influenced mastication, the rate of peanut particle breakdown, and the broadness (spread) of the distribution of peanut particles inside the matrix. However, changing the properties of the matrix did not influence the d_{50} of the peanut particle size distribution inside the bolus. The same studies also revealed that changing the properties of the peanut particles did not influence mastication, but influenced the d_{50} of the particle size distribution, the rate of peanut particle breakdown, and the retention of peanuts in the bolus. Results show the properties of the peanuts largely influence the peanut particle size required to reach the swallowing threshold. It is postulated that the properties of the matrices influence the selection function of particles, and the properties of the peanuts influence the breakage function of particles.

11.3 Recommendations for future research

It is recommended that future research investigates the difference in rheological properties of the boli (between different matrices containing peanuts) at various points of the chewing sequence and the swallow point. Given the differences in d_{50} during the chewing sequence and similarity once the bolus is ready for swallowing, it would be valuable to determine properties such as cohesion, lubrication, adhesion, storage modulus (G'), and loss modulus (G'') during the same stages. An investigation of the changes in the surface interaction (particularly adhesion) between peanuts and the matrices during the chewing sequence would also be valuable. Such work has the potential to offer important information for the triggers of swallowing and the importance of particle size to the swallowing threshold in heterogeneous food systems. Sensory changes using Temporal Dominance of Sensation (TDS) or Time Intensity (TI) could also be monitored throughout the chewing sequence. An understanding of perception would complement information about rheological changes in the bolus leading up to the swallow point.

Greater understanding of the dynamic movement of the teeth and the bolus during the mastication of these heterogeneous foods is required. Determining the physical and fracture properties of the matrices using instrumental measurements, followed by investigations of molar trajectories through the matrices with articulography, may offer insights into the mechanisms by which the matrices are influencing the rate of particle breakdown. Tracing the movement of the bolus (and perhaps distinguishing between peanuts and the matrix) using video fluorography or x-ray techniques could also clarify how the heterogeneous bolus is prepared and deemed suitable for swallowing.

The relationship between matrix properties, particle breakdown dynamics, and the particle size distribution of the food bolus is somewhat quantitative in this study. It is therefore recommended that a range of matrices of various specific physical properties (such as toughness, elasticity, hardness, cohesiveness) are trialled with peanuts embedded inside. Correlations between the number of chews, particle breakdown rates and the particle size in the ready to swallow food bolus could be undertaken to grasp what particular physical properties are influencing the process. Furthermore,

quantifying the effect the current matrices have on the selection function should be clarified. This could be undertaken by marking coloured particles of various sizes which are inserted inside the matrices, as in van der Glas et al., (1987).

In addition, work with heterogeneous food systems would benefit greatly from the exploration of test pieces beyond peanuts. Research with test pieces such as seeds, oats, and rice grains, may show that different particles behave differently than peanuts inside the same matrices.

The relationship between changes in physical properties of the peanuts (such as moisture) causing changes in the particle size distribution is also somewhat qualitative. Using peanuts only (without matrices), a large range of specific physical properties could be induced in the peanut pieces (such as hardness, Young's modulus, lubrication, and cohesion of the peanuts, and also adhesion of the peanuts to the oral mucosa) by the addition of moisture, heat treatment or other techniques. The resulting particle size distribution of the bolus could be measured throughout the chewing sequence and at the point of swallowing. Correlations between such physical properties and particle size could be undertaken. This would provide further insight into the mechanism involved in initiating swallowing in homogenous foods.

Finally, it is recommended research investigates the direct influence of embedding test pieces in contrasting food matrices, or manipulating the physical properties of the test pieces, on sensory perception and digestion. In particular, opportunities exist using heterogeneous model foods (where test pieces are embedded inside continuous food matrices) to directly investigate how changing the properties of matrices can influence flavour release and texture perception. Opportunities also exist to investigate how changing the fracture or breakage properties of foods can influence the glycaemic response.

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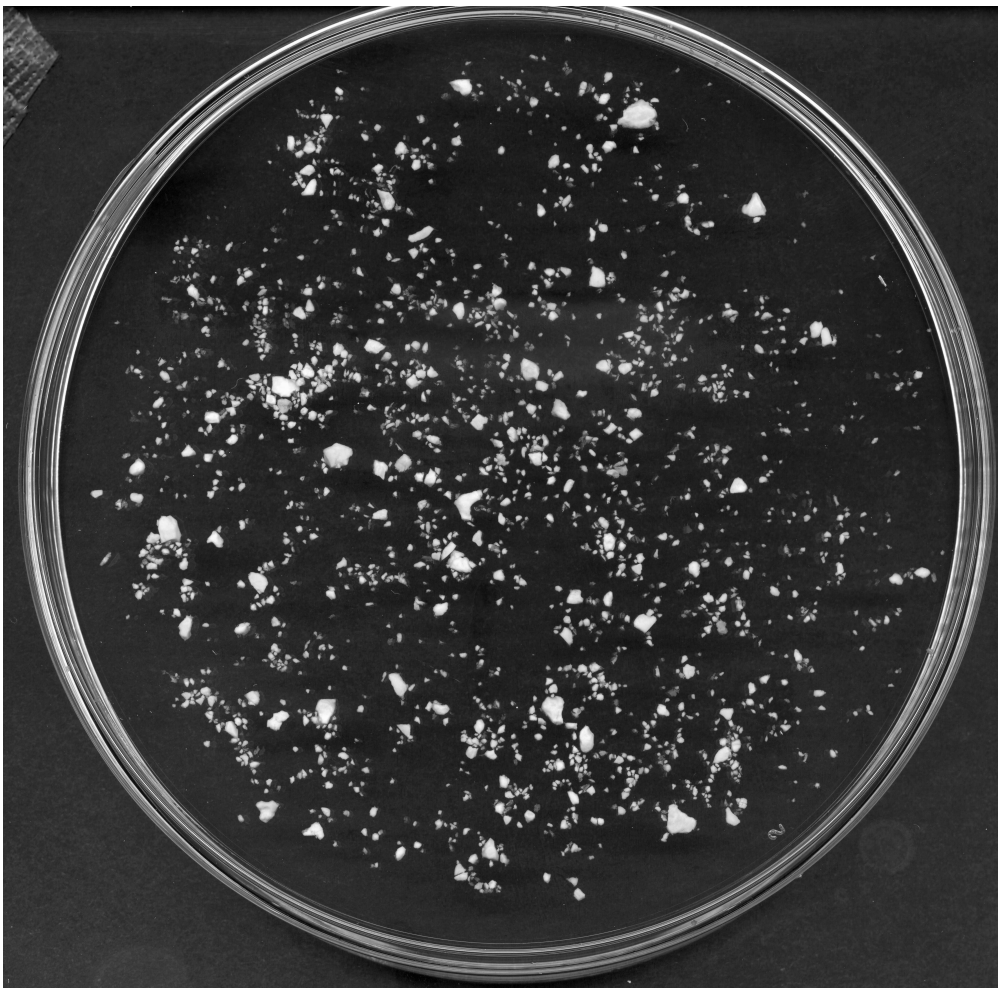
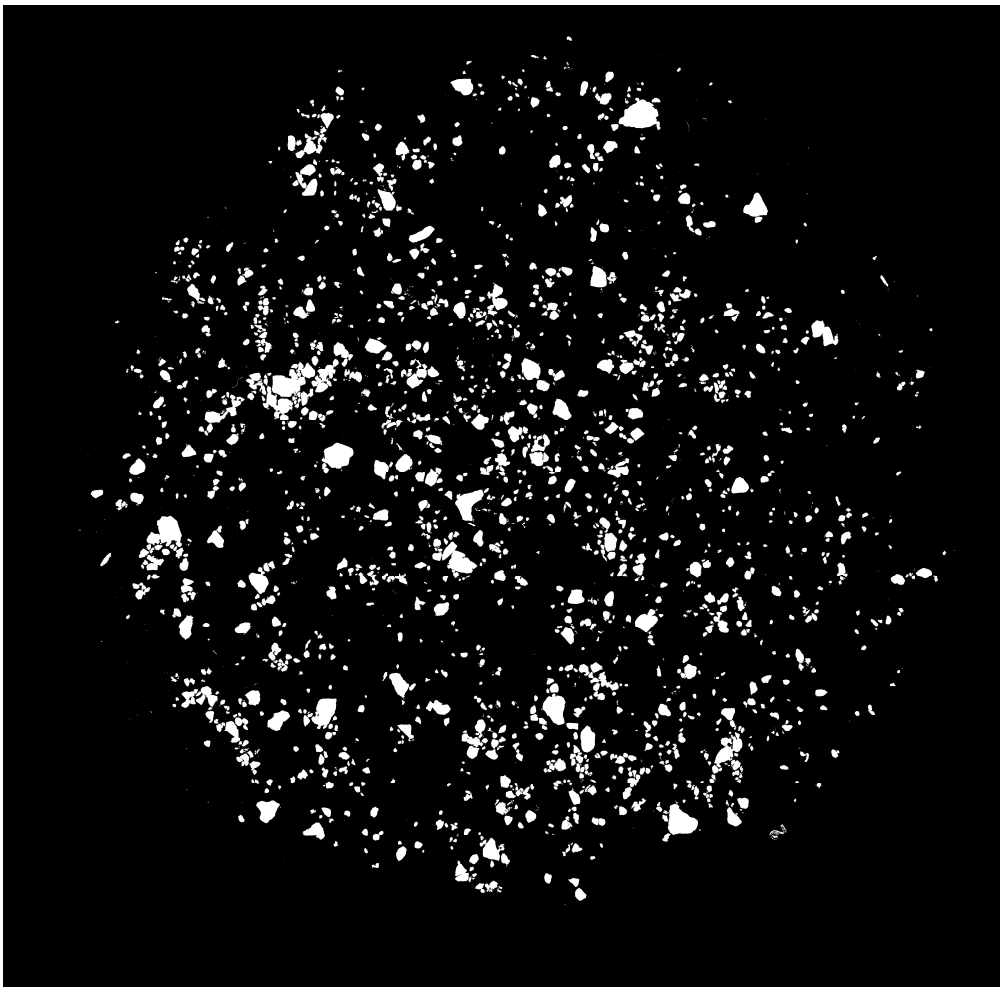
Appendix A: Typical photo of peanut bolus using Flat bed scanner

Below is a typical gray scale image taken by the flat bed scanner (Epson Perfection, 3490, Photo) of the peanut particles from the bolus on a Petri dish.



Appendix B: Typical bolus after the application of the black and white threshold

Below is the same photo of the peanut particles in the bolus from Appendix A, converted into a black and white threshold using Image J.



Appendix C: Typical output from Image analysis

Below is an example of a typical output from Image J. Thousands of particles would be counted, where 2D area (mm^2) was determined by the program for each particle. Information in the area column was sent to Matlab and Labview codes for Rosin-Rammler fitting and cumulative distribution curves respectively. Radius and volume were then calculated for estimating volume of the bolus.

Particle	Area (mm^2)	Radius (mm)	Volume (mm^3)
1	2.0	0.80	2.13
2	0.7	0.47	0.44
3	0.2	0.25	0.07
4	1.4	0.67	1.25
5	0.3	0.31	0.12
6	3.7	1.09	5.35
7	1.1	0.59	0.87
8	4.3	1.17	6.71
9	0.2	0.25	0.07
10	0.1	0.18	0.02
11	0.4	0.36	0.19
12	1.5	0.69	1.38
13	3.1	0.99	4.11
14	0.1	0.18	0.02
15	1.0	0.56	0.75
16	0.2	0.25	0.07
17	0.1	0.18	0.02
18	0.2	0.25	0.07
19	0.2	0.25	0.07
20	0.1	0.18	0.02
21	0.1	0.18	0.02
22	0.1	0.18	0.02
23	0.2	0.25	0.07
24	0.6	0.44	0.35
25	0.2	0.25	0.07
26	0.1	0.18	0.02
27	0.2	0.25	0.07
28	0.3	0.31	0.12
29	0.6	0.44	0.35
30	0.2	0.25	0.07
31	0.1	0.18	0.02
32	0.1	0.18	0.02
33	0.1	0.18	0.02
34	0.6	0.44	0.35
35	0.3	0.31	0.12
n			

Appendix D: Questionnaire for the bite size study

To select subjects for the bite size study, the following form was used to inform potential subjects about the research, and question them about their health and dental status.

QUESTIONAIRE

This study will investigate the general feeding trends of common manufactured food bars found in New Zealand supermarkets. Understanding feeding trends between people and between bars will provide useful information for mastication and digestion studies.

You will be asked to fill in a short questionnaire about your age, gender, weight, and height, prior to the commencement of the study.

During the study you will be given 3 bars on the first day and asked to take 2 separate bites from each bar. You are then required to return on the following day to repeat this procedure. You will be videotaped during biting and chewing.

We greatly appreciate your interest in taking part in this study. You are welcome to take part provided you can answer **NO** to all of the following questions:

Are you missing any teeth?
Y N

Do you experience any regular pain/discomfort while chewing?
Y N

Have you suffered any serious jaw injuries in the past?
Y N

Do you currently wear braces?
Y N

Are you on any form of a diet to loose weight?
Y N

Do you have a problem with dry mouth or salivary flow?
Y N

Are you allergic to any of the following ingredients in any of the food bars used in this study ? (an ingredients list was supplied)
Y N

(If you have any food allergies please check this list very carefully)

Appendix E: Screening questionnaire used for single subject trials and multiple subject study

QUESTIONAIRE

We greatly appreciate your interest in taking part in this study. If you can answer **NO** to all of the following questions you will be asked to undergo an inspection of your teeth, and an assessment of your biting and chewing behaviour:

Is your age less than 18 or greater than 30?
Y N

Do you have any missing teeth?
Y N

Do you experience any pain/discomfort while chewing?
Y N

Have you suffered any serious jaw injuries in the past?
Y N

Do you currently wear tooth braces?
Y N

Are you on any form of a diet to loose weight?
Y N

Do you have a problem with dry mouth or salivary flow?
Y N

Are you allergic to any of the following ingredients that will be used in this study? (This was provided on an information sheet)
Y N

Do you have any known allergies to adhesives or glues?
Y N

Do you have a cardiac pacemaker or any other biomechanical device that may be subject to interference by magnetic fields?
Y N

Are you a smoker?
Y N

Do you have a disorder of the mouth?
Y N

Do you currently have any significant problems with tooth decay or gum disease?
Y N

Have you noticed any tooth grinding or excessive tooth clenching while chewing?
Y N

Are you pregnant?
Y N

Are you aware of any other health problems that may inhibit your ability to take part in this study or put your health at risk in any way?
Y N

Appendix F: Dental examination form for the selection of single subjects

This form was used by a qualified dentist to evaluate the state of potential subjects teeth for the single subject studies. In Chapter 10 (multiple subject study) the same sheet was used to examine potential subjects teeth, however this was undertaken by the author.

Dental Examination

Personal Details

Code:
Date of birth (day/mth/yr):
Date of examination:

Part 1: Jaw and articulation examination

Does jaw movement exhibit any of the following?:
Clicking
Cracking
Restriction
Discomfort
Pain

Comments:

Part 2: Teeth and Gums Examination

Full set of natural teeth with no restoration work: YES NO

If no, which teeth are missing, and/or what is extent of the restoration work?

Class of occlusion

Please circle the appropriate class for this subject:

Class 1
Class 2
Class 3

Comments:

Crowding of the teeth

Please describe the extent of teeth crowding (if any can be seen)

Please briefly describe the presence of any dental caries

Please briefly describe the periodontal condition

Please make any other important comments about the condition of this subject's teeth

Appendix G: Journal article

Hutchings, S.C., Bronlund, J.E., Lentle, R.G., Foster, K.D., Jones, J.R., & Morgenstern, M.P. (2009). Variation of bite size with different types of food bars and implications for serving methods in mastication studies. *Food Quality and Preference*, 20, 456-460.

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Variation of bite size with different types of food bars and implications for serving methods in mastication studies

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ABSTRACT

Acquisition has a considerable influence on the process of mastication. The aim of this study was to examine variation in the natural bite weight, volume, and length of different food bars, to assess whether serving constant mass samples, constant volume samples, or alternative methods, are most appropriate for mastication studies.

Six types of manufactured food bars were assessed with 45 subjects (21 males and 24 females). Bite weight was determined and the volume and length of each bite were calculated using the density and dimensions of each bar. Natural bite weight, volume, and length varied significantly between bars. Bite length varied least. The results suggest that food bite size is not controlled by weight nor volume, but by bite length, when food bars are being consumed.

No ideal serving method exists however the relative regularity of bite length suggests constant volume servings may represent normal feeding behaviour more so than constant mass.

1. Introduction

Acquisition, commonly known as biting, involves external assessment by sensory organs, the use of the incisors, and the deposit of a unit of food in the oral cavity on the tongue. Once food is acquired it is assessed by sensory organs where it is either rejected or completely ingested (Bourne, 2002; Hiiemae, 2004; Thexton & Hiiemae, 1997). The bite is conducted by forcible occlusion of the opposing edges of the upper and lower incisors (Okada, Honma, Nomura, & Yamada, 2007). The speed of biting, in particular during late phase contact with the food, depends on food toughness (Ang, Lucas, & Tan, 2006). The force applied during biting is likely to provide an initial evaluation of the work required during subsequent mastication (Ang et al., 2006), and be useful in evaluating the hardness (Boyd & Sherman, 1975; Brandt, Skinner, & Coleman, 1963; Vickers & Christensen, 1980) and the thickness of food (Peyron, Maskawi, Woda, Tanguay, & Lund, 1997).

Literature on bite size in humans is limited, however it is clear that the natural size of the bite that people take varies between foods. Yagi, Matsuyama, Mitomi, Taguchi, and Noda (2006) found differences between subjects natural bite weights of bread, rice, sausage, and apple, and Medicis and Hiiemae (1998) found differences between banana, apple, cookies, and peanuts. Hiiemae et al. (1996) also discovered differences between banana, biscuit, and apple. The major variables which influence bite size are largely unknown, however studies of biting in herbivores suggests that cross sectional area has a large affect (Forbes, 1988), whilst work on human subjects sipping liquids suggests that volume is important (de Wijk, Zijlstra, Mars, De Graaf, & Prinz, 2008).

Bite size is important in the design of masticatory studies when selecting and standardising serving size. It is important in such studies where food is served rather than bitten, to ensure that the quantity of food served falls within the range of what would be naturally acquired. Serving size influences the number of chewing strokes and chewing time, (Fontijn-Tekamp et al., 2004a; Gaviao, Engelen, & Van Der Bilt, 2004), and the particle size distribution of the food bolus (Buschang, Throckmorton, & Travers, 1997; Lucas & Luke, 1984).

A number of mastication studies have compared foods or food properties on a basis of standardised mass (Fontijn-Tekamp, Van Der Bilt, Abbink, & Bosman, 2004b; Hiiemae, 2004; Mioche, Bourdiol, Monier, & Martin, 2002) others have standardised volume (Agrawal, Lucas, Bruce, & Prinz, 1998; Engelen, Fontijn-Tekamp, & Van Der Bilt, 2005; Foster, Woda, & Peyron, 2006). However, it is unclear whether serving samples standardised with a constant weight, constant volume, or using alternative serving methods, provides the most accurate basis for mastication studies.

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The aim of this study was to examine variation in the bite weight, volume, and length of bar acquired, between subjects and between food bars, with a view to determine which parameter is most useful as a basis of standardisation in masticatory studies comparing different foods.

2. Materials and methods

2.1. Subjects

Forty five subjects (24 females, and 21 males, aged 27.8 ± 7.4 years) were selected on the basis of having good oral and general health with no pain during chewing, a complete natural dentition, no history of recent orthodontic treatment or jaw injuries, and who were currently not on medication that could effect mastication or ensalivation.

The study was registered as a low risk category application with the Massey University Ethics Committee. All subjects gave informed consent following an explanation of the study. The subjects were not informed that bite size was being investigated in an effort to maintain the natural character of acquisition.

2.2. Experimental procedure

Six food bars commonly available in New Zealand were used for the study: Moro (chocolate and nougatine whip, Cadbury), Crunchie (hokey pokey and chocolate, Cadbury), Fruit and Nut Bar (Tasti Products Ltd.), Muesli Bar (Flemings), Apricot Pie (doughy bar with an apricot filling, Tasti Products Ltd.), and Pixie Caramel (hard chocolate and caramel, Nestle). The bars were chosen on the basis of their distinctive physical properties.

Each subject attended two experimental sessions, in which three types of bars were served in a randomised order. Hence each subject took bites from a given bar on only a single occasion. The subjects were asked to bite and chew in a manner which felt natural and comfortable, and instructed to take a single bite from the bar and to take a subsequent bite from a second bar of the same type once the first bite had been completely chewed and swallowed. This was designed to ensure that every subject had short term knowledge of the products sensory properties for the second bite, as during the first bite only some subjects had prior knowledge of the product.

The bars were weighed before and after the bite. The cross sectional area of the end of the bars was derived by dividing the average volume by the average length of each bar. Dimensions were taken from five replicates of each bar. The volume and length of bar bitten off were derived from the bite weight, and the average density and average dimensions of the bars. Each sequence of acquisition and chewing was video taped on a Quick Cam 8.48 (Logitech Asia Pacific Ltd, Hong Kong). The number of chews between acquisition and swallowing of each bite was subsequently determined manually from the recordings.

The average density of each bar was determined using a dry volume displacement method. A bar of known weight was submerged in rapeseed inside a measuring cylinder. Hence the volume of the bar was calculated from the difference in the level of rapeseed in the cylinder when the bar was completely covered. Five replicates were conducted on each type of bar and the mean volume used in subsequent calculations. Density was calculated as the average bar weight divided by the average bar volume.

Textural analysis was undertaken using a Texture Analyser TA-XT2 (Stable Microsystems, Surrey, UK) using cuboid samples of each bar (1.2 cm × 1 cm × 0.7 cm). Compression and incision tests were used to evaluate hardness and work done. Data acquisition was carried out using a 50 kg load cell and a sample frequency of 40 Hz. Four replicates were conducted for each test and each bar. Compression tests were based on principles from Bourne (2002), and were conducted to 75% strain at a test speed of 2 mm/s using a cylindrical shaped probe 61 mm in diameter. Hardness was taken as the maximum force measured during a compression test. Work was taken as the area under the force–displacement curve from start to 75% compression.

An incision test was also conducted using the Texture Analyser to assess incision hardness and work using bars cut to 4 cm length and 3 cm width. An 'axe' shaped probe was allowed to penetrate half way down each bar at a perpendicular angle to its length, until it reached 3 mm from the base. Hardness was taken as the maximum force during incision, and the work for incision as the area under a force–displacement curve until the maximum force was reached. This test was devised by the authors to simulate the physical process of biting.

2.3. Statistical analysis

Statistical analyses were performed using SPSS® (version 15.0 for Windows). To assess normality a Kolmogorov-Smirnov test (with Lillifors significance correction) was conducted on the data set for the first and the second bite. The bite size data were all normally distributed ($P > 0.05$) except for the second bite of the pixie caramel bar ($P = 0.04$ for weight, volume, and length). The number of chews were not normally distributed, however after a log transformation, all data were normally distributed, except for the number of chews from the first bite of the apricot pie bar ($P = 0.02$).

Two-way repeated measures ANOVAs, with bar and bite as the within subject factors, found significant interactions between bite number and bar type for weight, volume, and length of bite. This significant interaction indicated changes in biting behaviour from bite 1 to bite 2. Consequently, all results presented and subsequent statistical analysis used only the second bite data. Statistical analysis involved one-way repeated measures ANOVAs with bar as the only within subject factor.

When repeated measures ANOVA indicated significant differences between bar quantities, post-hoc Bonferroni tests were undertaken to compare individual bars. Where the assumption of specificity had been broken, degrees of freedom were adjusted using Greenhouse-Geisser estimates of sphericity.

The similarities in the various parameters of bite quantities (weight, volume and length), as well the number of chews, were compared overall by plots of raw data in cumulative form.

3. Results

3.1. The physical properties of the bars

The food properties varied across the bars (Table 1). The following differences were noted:

- The Crunchie bar was the least dense and required the lowest level of incision work.
- The Fruit and Nut bar had the largest cross–sectional area and was relatively high in the level of incision work.
- The Muesli bar had one of the smallest cross sectional areas, was relatively hard and required a relatively high compression and incision work.
- The Apricot Pie bar was the softest product having the lowest compression hardness and work.
- The Pixie Caramel bar was the hardest and densest bar requiring the highest levels of incision and compression work. It also had a relatively small cross sectional area.

Table 1
Food properties of the bars (mean ± SE).

	Moro	Crunchie	Fruit and nut	Muesli bar	Apricot pie	Pixie caramel
Density (g/cm^3)	0.95 ± 0.01	0.58 ± 0.01	0.75 ± 0.02	0.85 ± 0.04	0.75 ± 0.02	1.27 ± 0.03
Cross sectional area (cm^2)	5.7 ± 0.1	5.4 ± 0.1	6.3 ± 0.2	3.6 ± 0.1	5.9 ± 0.1	3.5 ± 0.2
Compression hardness (N)	20 ± 1	41 ± 2	52 ± 13	82 ± 16	8 ± 1	238 ± 16
Incision hardness (N)	2.7 ± 0.1	5.9 ± 0.3	5.4 ± 0.1	6.8 ± 0.3	2.1 ± 0.1	9.8 ± 0.6
Work for compression (mJ)	52 ± 3	153 ± 16	122 ± 32	243 ± 44	38 ± 4	1108 ± 68
Work for incision (mJ)	297 ± 25	91 ± 9	528 ± 26	486 ± 26	202 ± 89	670 ± 52

3.2. Interaction between bite 1 and bite 2

A significant interaction term for bite weight between bite number and bars ($F(2.8,122.95) = 5.01$, $P < 0.01$) was found. A similar pattern of significance occurred with bite volume ($F(4.02, 176.79) = 3.83$, $P < 0.05$) and bite length ($F(2.99, 131.53) = 4.57$, $P < 0.05$).

3.3. Bite weight

There was a noticeable spread between the cumulative distribution curves of the bite weights for each of the bars, particularly between the Crunchie and the Moro (Fig 1A). Significant variation between subjects ($F(1,44) = 418.6$, $P < 0.0005$) and between bars ($F(3.44,151.2) = 22.3$, $P < 0.005$) was found. Post-hoc Bonferroni tests found the Moro to be significantly different from all bars except the Apricot Pie bar (Table 2).

3.4. Bite volume

There was a noticeable spread between the cumulative distribution curves of the bite volumes for each of the bars, particularly between the Pixie Caramel and Muesli bar from the other bars (Fig 1B). Again significant overall variation between subjects ($F(1,44) = 436.1$, $P < 0.0005$) and between bars ($F(4,178.2) = 36.32$,

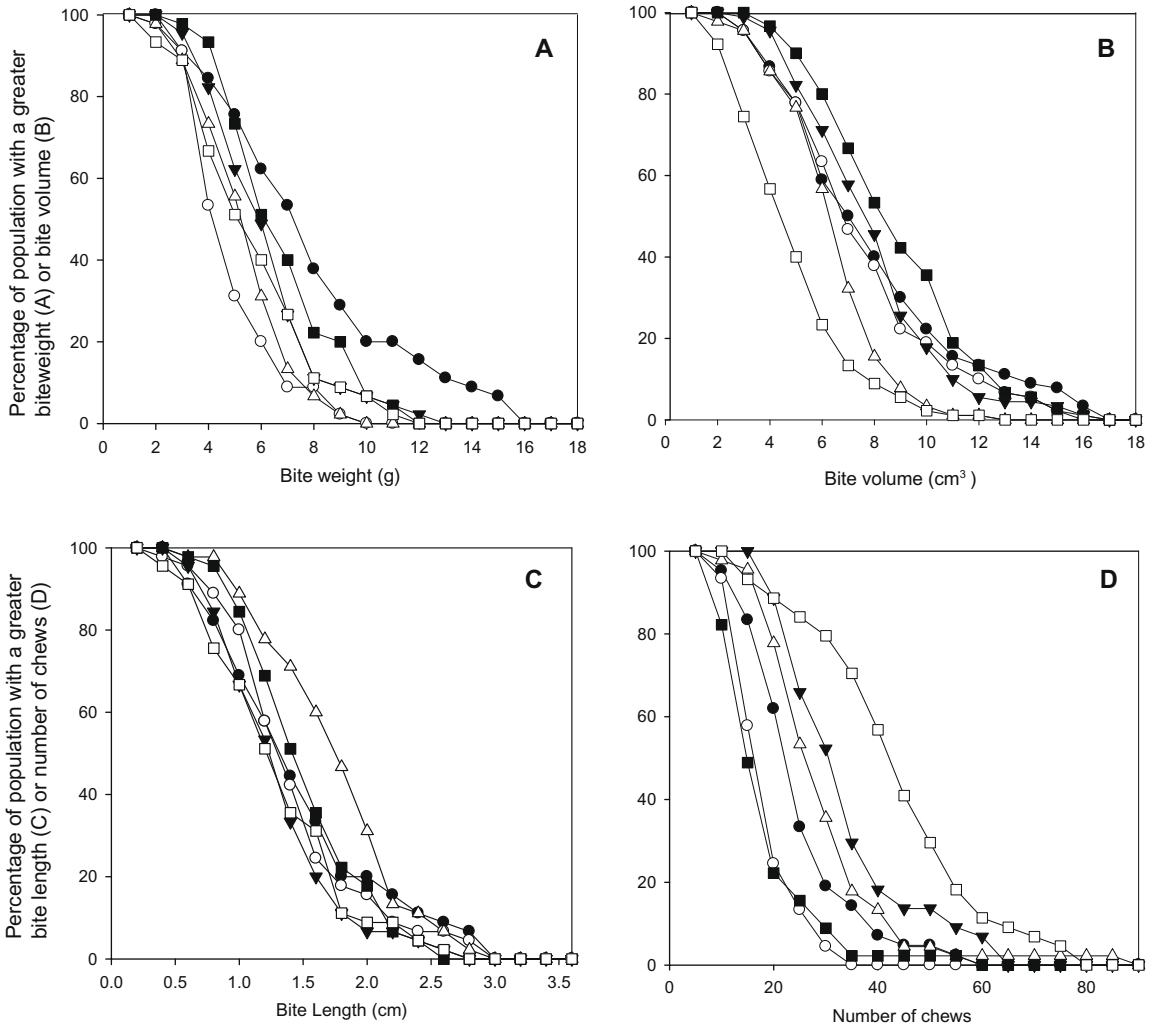

Fig. 1. Cumulative distribution of bite weight (A), volume (B), length (C) and number of chewing cycles (D) for the second bite. Moro: ●, crunchie: ○, fruit and nut: ▼, muesli bar: Δ, apricot pie: ■, pixie caramel: □.

Table 2
Bite size (weight, volume, and length) and the corresponding number of chews from six food bars for the second bite (mean ± SE).

Parameter	Moro	Crunchie	Fruit and nut	Muesli bar	Apricot pie	Pixie caramel
Weight (g)	7.66 ± 0.54[a]	4.63 ± 0.27[b]	6.02 ± 0.32[cd]	5.17 ± 0.25[b]	6.60 ± 0.32[ac]	5.48 ± 0.36[bd]
Volume (cm^3)	8.04 ± 0.57[a]	7.48 ± 0.45[a]	8.02 ± 0.43[a]	6.07 ± 0.30[b]	8.75 ± 0.42[a]	4.32 ± 0.28[c]
Length (cm)	1.41 ± 0.10 [a]	1.39 ± 0.08 [a]	1.28 ± 0.07 [a]	1.7 ± 0.08 [b]	1.46 ± 0.07 [a]	1.26 ± 0.08 [a]
Number of chews	24.64 ± 1.68[a]	17.49 ± 0.96[b]	34.8 ± 2.41[c]	28.80 ± 2.00[d]	17.80 ± 1.49[b]	44.53 ± 2.94[e]

Different letters (a,b,c,d,e) across each row indicate a significant statistical difference after a one-way repeated measures ANOVA using post-hoc Bonferroni tests ($P < 0.05$).

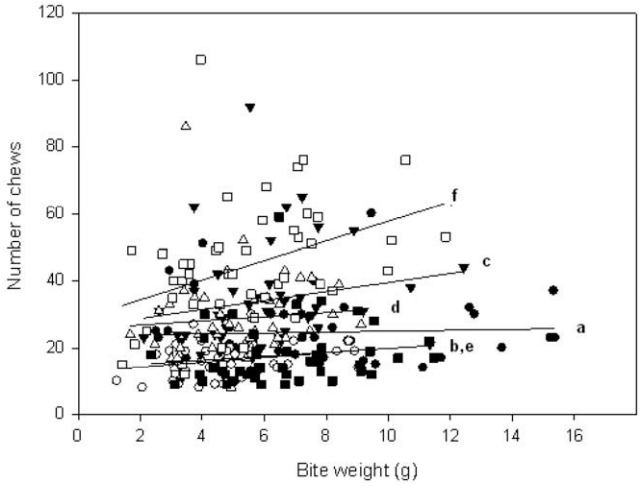

Fig. 2. The relationship between bite weight and the number of chews for the second bite. Moro: ●, crunchie: ○, fruit and nut: ▼, muesli bar: Δ, apricot pie: ■, pixie caramel: □ (moro(a): $r^2 = 0.002$, $P = 0.771$, crunchie(b): $r^2 = 0.134$, $P = 0.366$, fruit and nut(c): $r^2 = 0.038$, $P = 0.200$, muesli bar(d): $r^2 = 0.007$, $P = 0.585$, apricot pie(e): $r^2 = 0.019$, $P = 0.366$, pixie caramel(f): $r^2 = 0.148$, $P = 0.009$).

$P < 0.005$) was seen. Post-hoc Bonferroni tests identified the Pixie Caramel and the Muesli bar to be significantly different from each other and all other bars (Table 2).

3.5. Bite length

Distinctly less spread was observed between the cumulative distribution curves of bite length (Fig 1C). Significant overall variation between subjects ($F(1,44) = 440.4$, $P < 0.0005$) and between bars ($F(4.32,190) = 9.5$, $P < 0.005$) was indentified. The post-hoc Bonferroni reflected these results, as only the Muesli bar was significantly different from the other bars (Table 2).

3.6. The number of chews per bite

Significant differences, even greater than those observed for bite weight and bite volume, were observed in the cumulative distribution curve (Fig 1D). Significant overall variation between subjects ($F(1,44) = 3479.4$, $P < 0.0005$) and between bars ($F(3.87,158.7) = 120$, $P < 0.005$) was seen. Post-hoc Bonferroni tests showed a high number of significant differences between bars (Table 2).

Fig. 2 reports a plot of the number of cycles against bite weight. A weak positive correlation was seen between bite size and the number of chews. Only the correlation with the Pixie caramel bar was significant.

4. Discussion

4.1. Variation in bite size

Results demonstrate that natural bite size varies greatly between subjects (Fig. 1). Such spread in bite size within subjects has been found in every food so far examined in literature, from bananas to biscuits. Bratley and Hackett (1999) observed similar variation in carrots, and Yagi et al. (2006) in apples. It is likely that individuals develop distinctive overall biting strategies depending on their physical and behaviour characteristics.

Interestingly, the variation in bite size is likely to be greater than what would be expected due to physical dimensions alone, such as variation in jaw size. Further work is required to identify what causes this variation. Behavioural factors may be far more important than physical factors.

4.2. The influence of first bite vs. second bite

The significant interaction between bar type and bite number showed that the bite size changed between the first and second bite according to bar type. This indicates that properties of the bars induce assessment and readjustment. Prior to the first bite many subjects had limited knowledge of the products properties, however prior to the second bite subjects could take a bite knowing what to expect. For example the high hardness and work values of the Pixie caramel (Table 1) may have resulted in readjustment to a smaller second bite for many subjects.

4.3. Variation in bite size between bars

These results show that each subject's bite size was not based on acquiring a particular mass or volume across different bar types, however bite size may be based on acquiring a particular length across different bar types. There were significant differences between the bars in terms of all measured bite size variables (weight, volume, and length), but the difference in bite length between bars was not as great as seen for weight and volume (Fig. 1). Post-hoc analysis showed that only the Muesli bar was significantly different from the other bars in terms of length (Table 2). Interestingly, the bars which produced the greatest separation by weight differed from those which produced greatest separation by volume.

The regularity in natural bite length may mean the physical shape and the density of the food bar may have a stronger influence on bite size than textural properties such as hardness and work during compression. This is an area which requires further research.

Previous studies have found significant differences in mean bite weight between different foods (Hiiemae et al., 1996; Medicis & Hiiemae, 1998; Yagi et al., 2006). Previous research has not compared natural bite volume or bite length between solid foods, although some information on sip volume for liquids is available (de Wijk et al., 2008; Lawless, Bender, Oman, & Pelletier, 2003; Medicis & Hiiemae, 1998).

4.4. Variation in the number of chews between bars

Results showed the 6 food bars differed greatly in the way they were chewed (Fig. 1, Table 2). Hiiemae et al. (1996) has also shown differences in chew number between foods acquired from natural bites.

Interestingly, weak positive correlations were seen between the number of chewing cycles and the bite size of the population (Fig. 2). As highly significant differences were seen between subjects for bite size, and the raw data indicated, in general, that a particular subject makes comparable bite sizes for all the bars (e.g. small biters take small bites for all bars tested), this figure suggests that large biters do not compensate for a larger mouth fill by using a greater number of chewing cycles.

4.5. Applications for standardising serving size

These results have interesting applications for standardising portions in mastication studies. Despite the finding that neither bite mass nor bite volume were consistent between food bars for each subject, the regularity in bite length between bars suggests constant volume servings may be more appropriate to represent typical feeding. If bite length is consistent between many bar shaped food products (where the cross sectional area does not limit acquisition), bite volume should also be as consistent if the cross sectional area across for the different bars (area of the end of the bar) is kept constant (length $\times$ cross SA = volume). A study using bars of the same cross sectional area could be undertaken to confirm regularity of bite volume with regular cross sectional area.

The significant differences between products in terms of bite mass, as also shown by Hiiemae et al. (1996), Medicis and Hiiemae (1998), and Yagi et al. (2006), and significant differences in bite volume and to a lesser extent bite length, highlights the importance of researchers carefully selecting a serving size. While sample size is not chosen at random, studies rarely provide details on how it is selected. Assessing the subjects natural bite size for the foods of interest could be a simple method of choosing a serving size before undertaking a mastication study. Even brief details explaining the reasoning for the selection of a particular sample size could be useful in publications.

An alternative option is to allow subjects to take natural bites for mastication studies, which reflects typical feeding more so than serving constant sized samples. Taking natural bites from food bars of the same cross sectional area could be particularly effective, given the regularity in bite length.

5. Conclusions

The results of this study show that the bite size varies significantly between subjects but that bite size also varies with food even when different foods are presented in a similar shape (as food bars). Variation between bars was high in terms of bite weight and bite volume, but considerably lower in terms of bite length. Consequently, constant volume samples may represent natural feeding more so than constant mass, as volume differences will match length differences if the cross sectional area is constant.

These results highlight the importance of considering the manner in which foods are served to subjects in mastication studies. The administration of a chosen weight of food is likely to produce different results from a chosen volume of food. It may be useful for mastication studies to provide greater details on how sample size was chosen.

Ultimately, an ideal method for serving size in mastication studies is yet to be identified and researchers need to carefully consider what particular serving method will most effectively match the requirements of their work.

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Scott Christopher Hutchings

Name/Title of Principal Supervisor: Dr Kylie Foster

Name of Published Paper: Variation of bite size with different types of food bars and implications for serving methods in mastication studies.

In which Chapter is the Published Work: 4

What percentage of the Published Work was contributed by the candidate: 80%

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Appendix H: Journal article

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Mastication of heterogeneous foods: Peanuts inside two different food matrices

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ABSTRACT

We investigated chewing behaviour and particle size outcome in the oral processing of heterogeneous foods using peanuts embedded within two types of food matrices. Eight subjects, selected according to strict dental and mastication criteria, were served four different model foods. Each model food comprised one of two matrices of different physical properties (chocolate and gelatine gel) which were embedded with one of two physically different particles (peanuts with low moisture or high moisture content). A standard volume of each model food was chewed to the point of swallowing and expectorated and the number of chews and chewing time was recorded. The matrix of the expectorated bolus was washed away to isolate the peanut fragments, and the particle size distributions of the peanut fragments were determined using image analysis. A Rosin–Rammler function was fitted to the cumulative distribution data of each bolus to derive particle size parameters (d_{50} and broadness (b)).

Results showed the properties of one food component (the matrix) can influence the breakdown of another food component (the peanuts) in a heterogeneous system. The properties of the matrix caused differences in mastication (measured in terms of the number of chews, chewing duration, and frequency) and broadness of the peanut particle size distribution, but did not influence the final d_{50} of the peanut particle size distribution. Conversely the physical properties of the embedded peanut influenced the d_{50} of the particle size distribution but had no effect on chewing behaviour. It is likely that properties of the matrix influenced the rate at which particles of various sizes were selected for chewing (known as the selection function), whereas the physical properties of the peanuts influenced extent of breakage when the teeth made contact with the particles (known as the breakage function).

1. Introduction

Investigations into the mechanism of mastication have typically employed relatively homogenous foods to simplify the dynamics of fracture and the measurement of the food bolus particle size distribution. Hence the mastication of foods such as peanuts (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007), carrots (Lucas & Luke, 1986), and pasta (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000) are well described. Work has studied the influence of the gross physical properties of such foods on mastication (Hiiemae & Palmer, 1999; Hiiemae et al., 1996) and on the final properties of the food bolus (Hoebler et al., 2000; Jalabert-Malbos et al., 2007). However, mastication often involves the breakdown of more than one food type in the mouth. Many commercially available foods are heterogeneous, such as a muesli bar, where oats, raisins, and nuts are chewed together. The consumption of a meal also often involves the mastication of multiple foods at once.

Currently, little is known about physiological responses or the mechanical outcome when heterogeneous foods are masticated and swallowed. In particular, different particles may be interposed between the occluding surfaces of the teeth at the same time. It is not known how the simultaneous interposition of particles that differ in their physical properties will influence the duration and force applied in the chewing cycle, or the particle size distribution of the food bolus. Foods that exhibit structural heterogeneity (such as apples where the shape and size of cells and spaces vary from the surface through to the core) will fracture differently according to their orientation with respect to the dental array (Khan & Vincent, 1993; Wang, 2003).

An understanding of the dynamics and particulate outcome of the oral processing of heterogeneous foods may allow food manufacturers to develop foods for sensory and nutritional benefits. It is possible that optimising chewing behaviour and the particle size distribution of the food bolus can benefit the glycaemic response

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(Ranawana, Henry, & Pratt, 2010; Read et al., 1986; Suzuki et al., 2005), amino acid assimilation (Remond et al., 2007), texture perception (Brown & Braxton, 2000; Brown, Langley, Martin, & Mac Fie, 1994), and the extent of flavour release (Alfonso, Neyraud, Blanc, Peyron, & Dransfield, 2002; Taylor, 1996).

The aim of this study was therefore to investigate mastication and particle size outcome when a series of simple heterogeneous foods are chewed. In particular, to compare the effects of two types of solid particle with different physical properties (moist and dry peanuts) embedded inside two types of matrix (chocolate and gelatine gel) on mastication and the final particle size distribution in the food bolus.

2. Methodology

2.1. Subject selection

Eight subjects (four male and four female) were selected for this study (25.6 ± 4.3 years, mean ± SD). Subject selection involved screening subjects in terms of biting and chewing characteristics, and also dental criteria. Potential subjects were asked to bite and masticate a standard 'Fruit and Nut' bar (Tasti Products Ltd., Auckland, New Zealand) (as used by Hutchings et al. (2009) with a large population). Subjects who showed highly unusual behaviour, such as large variability in terms of bite weight and the number of chews, or large differences in the mean bite weight or the number of chews compared to the population in Hutchings et al. (2009), were screened out. The selection procedure also assisted in familiarising subjects that were selected with the process of expectorating a food bolus.

Dental screening involved selecting only subjects with class 1 occlusion, no significant tooth crowding, no obvious tooth decay, and a healthy periodontal condition. They had no functional disturbance of mastication evidenced by pain or clicking during chewing, and no other known oral or general health issues that could influence oral processing. This project was reviewed and approved by the Massey University Human Ethics Committee: Southern A (Application 09/24).

2.2. Assessment of natural bite size and selection of serving size

Serving size was determined by the assessment of the natural bite length of the subjects (and hence natural bite volume). Each subject was asked to take 2 natural bites from chocolate bars (containing 11.3% peanut quarters (v/v)) with a constant shape (20 mm height, 30 mm width, 100 mm length). A mean bite length of 16 ± 6 mm (mean ± SD) was determined from the eight selected subjects, and therefore a constant volume serving size of 9600 mm^3 (20 × 30 × 16 mm) for matrices (containing 11.3% peanut quarters (v/v)) was adopted. The weights of the servings were then determined (Table 1). The concept of standardising by a common bite length is discussed in Hutchings et al. (2009).

2.3. Experimental procedure

Four different test foods were served in a randomised sequence with four replicates of each food type (16 samples in total per subject). Two types of matrices (gelatine gel or chocolate) were used, which were embedded with moist or dry peanuts (2 matrices × 2 peanut types = 4 test foods). The subjects were allowed to chew each sample for as long as necessary until they felt the impulse to swallow, at which point they expectorated the bolus into a container. Following expectoration each subject rinsed their mouth with 25 mL distilled water and expectorated this into a separate container. Both the bolus and the washings were weighed and frozen (−18 °C), as done with meat boli by Mioche, Bourdiol, Monier, and Martin (2002). The number of chews and chewing time of each sample were recorded by the researcher. Each experimental session was conducted in a room at 20 °C, with test foods equilibrated at 20 °C at least 1 h before serving.

Table 1
Serving weights of the test foods.

	Avg. serving weight (g) (±SD)	Avg. density (g/cm^3) (±SD)
Chocolate matrix (g)	11.26 ± 0.20	1.36 ± 0.02
Gelatine matrix (g)	12.30 ± 0.21	1.49 ± 0.03
Dry peanuts (g)	1.14 ± 0.03	1.08 ± 0.01
Moist peanuts (g)	1.12 ± 0.03	1.06 ± 0.01

2.4. Preparation of the test foods

Roasted unsalted peanuts sourced from Prolife Foods Ltd. (Hamilton, New Zealand) were used in all trials. Peanuts kernels (halves) were cut into quarters, and sieved across a 4.75 mm sieve prior to inclusion into the matrices to ensure no small particles were included. Moist peanuts were prepared by soaking the peanut quarters in water for 2 h and equilibrating in a water tight container for 46 h in a refrigerator (4 °C). Peanut quarters were inserted into each matrix during the experimental session (immediately prior to serving a particular sample to the subject) to ensure no unwanted moisture migration took place. Fig. 1 illustrates the test foods and sample dimensions, with peanuts inside the matrix.

The chocolate matrix was prepared by melting chocolate (Dairy Milk®, Cadbury confectionary©, Dunedin, New Zealand) in a microwave, pouring the chocolate into an aluminium mould (20 mm height, 30 mm width, 100 mm length), and allowing it to set in a refrigerator (4 °C). The gelatine gel matrix was prepared with gelatine (250 bloom, Gelita, Christchurch, New Zealand) using the procedure described by Lassauzay, Peyron, Albuisson, Dransfield, and Woda (2000). The mixture was poured into an identical mould as used with the chocolate matrix, and left to set at 20 °C.

2.5. Analysis of the physical properties of the matrices and peanuts

The density of the peanuts was measured by volume displacement in toluene as this is reported to give more precision than water (Aydin, 2007). Analytical grade toluene was used (Scharlau, Barcelona, Spain).

The moisture content of peanuts was determined by drying for 24 h at 105 °C in an air dry oven (Labserv®, Biolab, Auckland, New Zealand) (4 replicates of ~10 g samples).

Texture analysis of the matrices (without peanuts), and of the peanuts, was conducted on a Texture Analyzer TA-XT2 (Stable Microsystems, Surrey, UK), using two successive uni-axial compression tests with a flat cylindrical probe (diameter: 50 mm) (Texture profile analysis, TPA). Data acquisition was carried out using a 50 kg load cell at a sample frequency of 40 Hz.

Analysis of the peanuts was conducted by compressing peanut halves to 50% strain (automatic trigger force of 0.1 N, test and post test speed: 1.67 mm/s, 10 replicates). Analysis of the matrices was conducted by TPA compression of samples to 80% strain (13 × 20 × 15 mm, where the 20 × 15 mm side was facing the compression probe, automatic trigger force of 0.1 N, test and post test speed: 0.8 mm/s, 24 replicates). Dimensions were smaller than the test samples so forces were within the limit of the 50 kg load cell during TPA analysis. The parameters were each calculated according to standard methods (Bourne, 2002), and all tests were

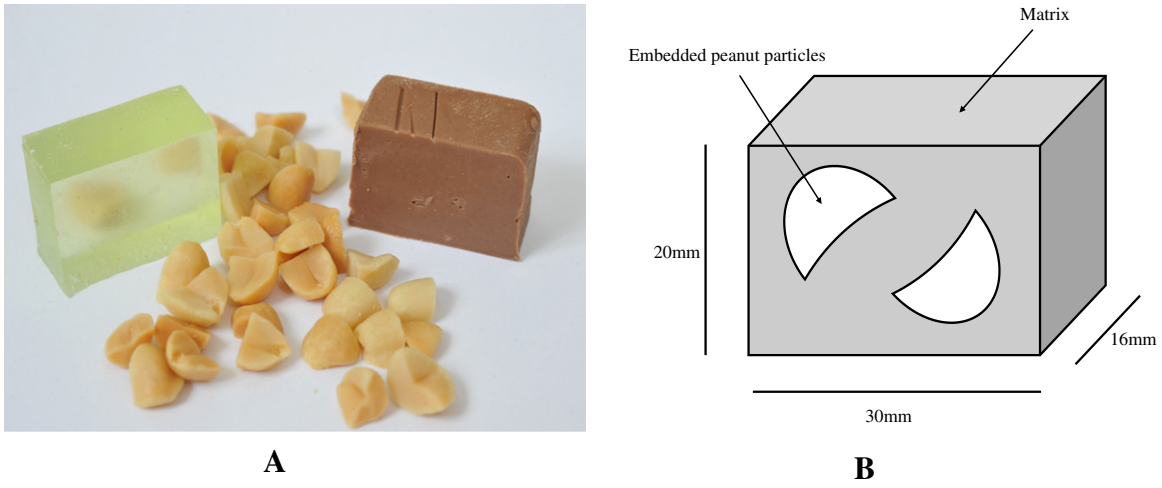

Fig. 1. (A) The gelatine gel matrix (left), the chocolate matrix (right), and peanut quarters. (B) An illustration of the test food, with peanut quarters embedded inside the food matrix.

undertaken at 20 °C. As measurements were undertaken using different test conditions for peanuts and matrices, results are only compared between matrices and between peanuts. Different conditions were used for matrices and peanuts to optimise the level of compression within the limit of the 50 kg load cell, and to minimise variability.

2.6. Analysis of the food bolus

The bolus and washings were thawed at 20 °C for 30 min, before being combined. Each complete bolus (original bolus and washings) was sieved across a 355 μm sieve with water at 45 °C for 4 min. This process caused the bulk of the matrix to pass through the sieve and the majority of peanut particles to be retained. Hence particles less than this sieve aperture in size were discarded. The retained peanut particles were then placed on a Petri dish (140 mm diameter) (Biolab, Auckland, New Zealand) and 60 ml of ethanol (absolute) (Polychem Marketing Ltd., Auckland, New Zealand) added to assist in particle separation and to prevent fat globules forming. Particles were dispersed with a plastic spatula prior to scanning.

The contents of the Petri dish were scanned at 800 dpi (Epson Perfection, 3490 Photo, Epson, China) in grayscale (Fig. 2A) and the scan repeated following re-dispersal of the contents. Processing of the 4300 × 4300 pixel images was conducted using Image J® (1.37a, National Institute of Health, USA). A black and white threshold was applied to obtain binary images (Fig. 2B). A nucleus counter in conjunction with a watershed algorithm was used to separate any abutting particles.

The particle size output from Image J (area in mm^2 for each counted particle) was exported to Labview™ (National Instruments©, USA) in order to create cumulative particle size distributions in terms of area. It was assumed the area of each particle represents a circle, and the diameter was derived. Each particle was then allocated to one of a series of classes based on particle width. Particles were categorized into 8 width classes that follow a well-known power series: 0.355–0.5, 0.5–0.7, 0.7–1, 1–1.4, 1.4–2, 2–2.8, 2.8–4, ⩾4 mm.

To ensure no changes in particle size were taking place during storage in ethanol (which was rarely longer than 24 h), and to assess repeatability of the image analysis procedure, a peanut bolus was analysed immediately after being placed in ethanol, and after 7 days of storage in ethanol. The cumulative area distributions of the two boluses did not change after this treatment (Fig. 3).

The cumulative particle area distribution of the peanut particles in each bolus was fitted to a Rosin–Rammler distribution function (Eq. (1)):

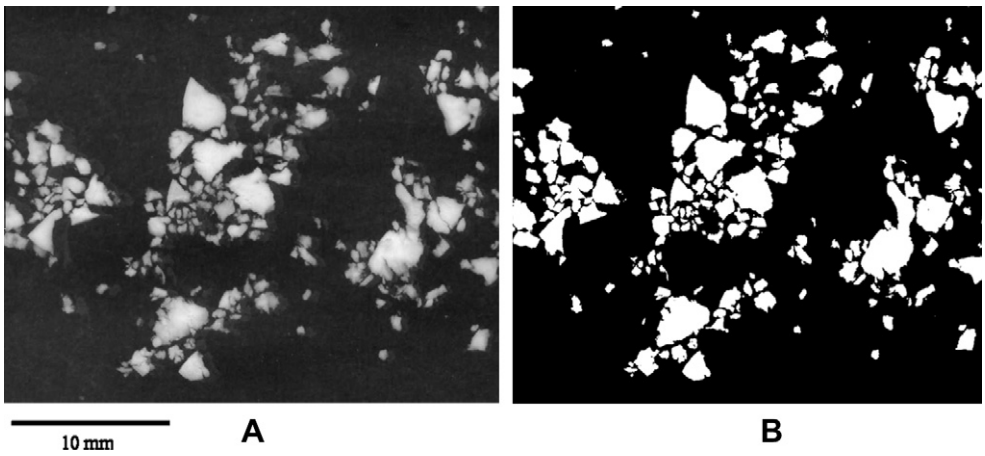

Fig. 2. The image analysis process. A gray-scale photo was taken using a flatbed scanner (A), and a black and white threshold was applied (B). (Note: This is a zoomed image showing a portion of a typical peanut bolus.)

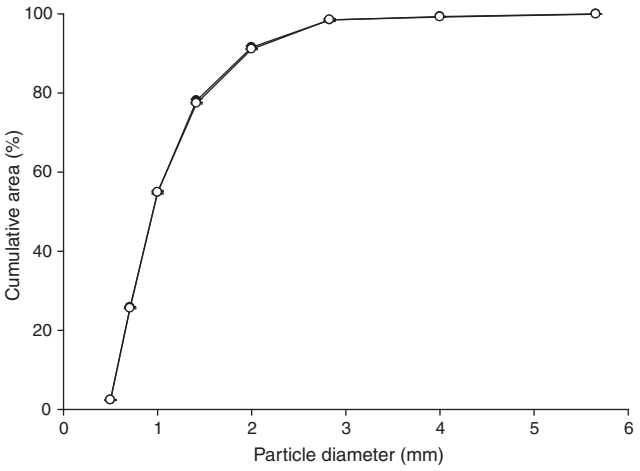

Fig. 3. Cumulative area distribution of a peanut bolus analysed on day 1 (●) and day 7 (○) during storage in ethanol. The cumulative percentage area (±SD) in each class is based on determining a particle width assuming each particle is spherical.

$$Q = 1 - \exp\left[-\left(\frac{x - 0.354}{d_{50} - 0.354}\right)^{b} . \ln 2\right] \quad (1)$$

where x is the sieve class (mm), d_{50} is the theoretical sieve size through which 50% of the 2 dimensional particle area will fall (mm), b is the broadness of the cumulative area distribution (the slope of the cumulative curve, where increasing values correspond to particle size distributions that are less broad), and Q the area fraction of particles that have a smaller diameter (assuming circular particles) than x. The value of the baseline constant, 0.354 was chosen, rather than calculated from the curve fit, since all particles below 0.355 mm had been removed from the bolus. This method was based on a similar function presented by Olthoff, van der Bilt, Bosman, and Kleizen (1984).

Following particle size analysis, the mass of particles in each bolus was determined by decanting the ethanol, and drying the peanuts for 24 h at 105 °C in an air dry oven (Labserv®, Biolab, Auckland, New Zealand) (thus determining dry weight). The percentage of the dry mass retained was calculated by estimating the dry weight of peanuts in the original food portion (as the moisture content of peanuts and total weight of each peanut serving was known). Comparative loss of material from different boli was also estimated by comparing the total volume of peanuts remaining in each bolus after sieving from the photos taken during image analysis. Each particle was assumed to be spherical and of a diameter equal to the maximum diameter of the particle. The volumes of constituent particles were summed to derive a figure for the total volume of particles in each bolus. Mass and volume retention were both determined as it was likely that loss of fat from the peanuts into the ethanol during the imaging procedure would influence the results based on proportion of mass retained.

2.7. Statistical analysis

Statistical analyses were performed using SPSS® (version 15.0 for Windows) (SPSS Inc., USA). The fit of the cumulative distribution function of particle area by the Rosin–Rammler equation was determined for each bolus by the R^2 values.

A two-way repeated measure ANOVA, with matrix type and particle type as within subject factors, was used to assess the significance of the differences in results between matrices and particle type. The distributions of the parameters (number of chews, chewing duration, frequency, d_{50}, broadness (b), dry weight retention, and volume retention) were considered to be normally distributed when the P value was greater than 0.05 according to the Kolmogorov–Smirnov test for normality (with Lillifors significance correction). The following transformations were made to normalise the data (conversion from x to y):

- The number of chews and chewing time were normally distributed following $\log_{10}$ transformation, and the frequency was normally distributed following exponential function transformation (e^x).
- d_{50} was normally distributed following a Johnson transformation in MINITAB (version 15, Minitab Inc.) with the following formula:
  $y = -2.23950 + 1.51986 * \text{Asinh}(x - 0.918140)/0.143034)$
- The broadness parameter b was normally distributed without transformation.
- Dry weight retention of peanuts was normally distributed following Johnson transformation in MINITAB (version 15, Minitab Inc.) with the following formula:
  $y = 0.318810 + 0.847683 * \ln((x - 8.67778)/(72.5068 - x))$
- Peanut volume retention was normally distributed following Johnson transformation in MINITAB (version 15, Minitab Inc.) with the following formula:
  $y = -0.215711 + 0.635957 * \ln((x - 707.488)/(2035.88 - x))$

3. Results

3.1. Physical properties of the test food components

The physical parameters differed between the matrices and between the peanuts (Table 2). The cohesiveness, springiness, gumminess, and chewiness were all greater in the gelatine gel than the chocolate matrix. The chocolate matrix was harder than the gelatine gel matrix, and the dry peanut was harder than the moist peanut.

3.2. Parameters of mastication

3.2.1. Variation in mastication between subjects

Large variation in chewing parameters occurred between subjects. The number of chews ($F(1,7) = 971.3$, $P < 0.0005$), chewing duration ($F(1,7) = 676.0$, $P < 0.0005$), and frequency (number of chews/chewing duration) ($F(1,7) = 228.2$, $P < 0.0005$) all differed significantly between subjects according to the repeated measures ANOVA for the effect of matrix type and particle type.

3.2.2. The effect of matrix type and peanut type on mastication

The gelatine gel matrices (containing either moist or dry peanuts) were masticated for a significantly greater number of chews ($F(1,7) = 98.8$, $P < 0.0005$) and consequently chewing duration

Table 2
Properties of the test foods (mean ± SE). (Note: The hardness of the matrices and peanuts were undertaken using different TPA conditions).

Matrices						Particle		
	Hardness (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)		Moisture content (gH_20/100g total weight)	Hardness (N)
Gelatine gel	252 ± 8	0.89 ± 0.01	10.1 ± 0.1	225 ± 8	2270 ± 90	Dry peanut	1.99 ± 0.10	78.0 ± 9.3
Chocolate	400 ± 8	0.17 ± 0.01	2.03 ± 0.23	70 ± 2	150 ± 20	Moist peanut	22.21 ± 0.09	51.6 ± 6.9

Table 3
The effect of food matrices and particle type on the mastication and bolus parameters (mean ± SE).

Matrix Particle type	Gelatine gel Dry	Chocolate Dry	Gelatine gel Moist	Chocolate Moist
Number of chews	37.75 ± 2.11	23.75 ± 1.14	40.50 ± 2.44	23.91 ± 1.21
Chewing duration (s)	27.66 ± 1.53	18.58 ± 1.12	28.77 ± 1.61	18.56 ± 1.06
Frequency (Hz)	1.38 ± 0.04	1.32 ± 0.04	1.41 ± 0.03	1.32 ± 0.04
d_{50} (area) (mm)	1.14 ± 0.04	1.18 ± 0.03	1.37 ± 0.05	1.43 ± 0.04
b	1.13 ± 0.02	1.23 ± 0.02	1.17 ± 0.02	1.19 ± 0.01
% Peanut weight retention (g drywt/100g initial drywt)	24.52 ± 1.64	26.13 ± 1.43	46.84 ± 1.64	50.42 ± 1.42
Estimated volume of peanuts in bolus (mm^3)	1123 ± 47	1154 ± 38	1776 ± 24	1801 ± 23

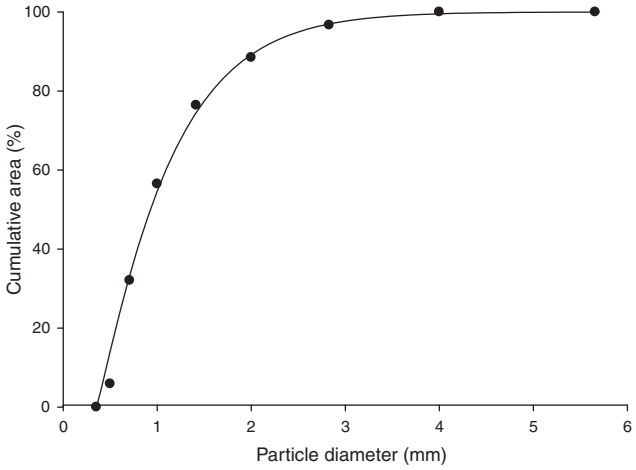

Fig. 4. A typical cumulative area distribution of bolus peanut particles, with the Rosin–Rammler fit. Raw area data from a typical bolus: ●, Rosin–Rammler fit: —.

($F(1,7) = 54.2$, $P < 0.0005$), and at a significantly higher frequency ($F(1,7) = 10.8$, $P < 0.05$), than the chocolate matrices (containing either moist or dry peanuts). There were no significant differences either in the number of chews ($F(1,7) = 4.4$, $P > 0.05$), chewing duration ($F(1,7) = 2.2$, $P > 0.05$), or frequency ($F(1,7) = 0.8$, $P > 0.05$) between the type of peanut that was embedded in the matrices. Interactions between matrix and particle type were not significant for the number of chews ($F(1,7) = 3.7$, $P > 0.05$), chewing duration ($F(1,7) = 1.2$, $P > 0.05$, or frequency ($F(1,7) = 2.2$, $P > 0.05$) (Table 3).

3.3. Food bolus particle size

Fig. 4 shows the fit of the Rosin–Rammler curve to a typical bolus particle size distribution. Values for R^2 were greater than 0.99 for all particle size distributions.

3.3.1. Variation in bolus particle size between subjects

There was large variation between subjects in the particle size distribution of individual boluses (Fig. 5). Hence d_{50} ($F(1,7) = 294.8$, $P < 0.0005$), and broadness (b) ($F(1,7) = 2873.4$, $P < 0.0005$) differed significantly between subjects on a repeated measures ANOVA for the effect of matrix type and particle type.

3.3.2. The effect of matrix type and peanut type on the bolus particle size distribution

The mean particle size distribution curves are shown in Fig. 6. There was significant variation between matrix and between particle types in the particle size parameters. Whilst d_{50} ($F(1,7) = 4.0$, $P > 0.05$) of peanut particles in the bolus did not vary significantly between matrices they were inside, the d_{50} value for moist particles was significantly larger than that for the dry particles ($F(1,7) = 147.5$, $P < 0.0005$) within each type of matrix. There was no significant interaction between matrix and particle type for d_{50} ($F(1,7) = 0.251$, $P > 0.05$) (Fig. 7, Table 3).

The broadness (b) ($F(1,7) = 8.9$, $P < 0.05$) of the peanut particles in the bolus was significantly higher for peanuts chewed inside a chocolate matrix than those in the gelatine gel, showing the spread in the distribution of peanut particles in the gelatine gel matrix was greater than that in the chocolate matrix. Peanut type did not affect the broadness (b) ($F(1,7) = 0.0$, $P > 0.05$), however the interaction between the matrices and particle type on broadness (b) was significant ($F(1,7) = 11.0$, $P < 0.05$) (Fig. 7, Table 3). While peanut particles in the chocolate bolus had greater (b) value than in the gelatine gel, the values tended to be higher for moist peanuts compared to dry peanuts inside the gelatine gel, and higher for dry peanuts compared to moist peanuts inside the chocolate.

3.4. Retention of peanuts in the bolus

3.4.1. Variation in particle retention between subjects

Dry weight retention ($F(1,7) = 318.7$, $P < 0.0005$) and volume retention ($F(1,7) = 789.5$, $P < 0.0005$) of peanuts were significantly different between subjects on a repeated measures ANOVA for the effect of matrix type and particle size.

3.4.2. The effect of matrix type and peanut type on peanut retention

Dry weight retention ($F(1,7) = 2.1$, $P > 0.05$) and volume retention ($F(1,7) = 0.8$, $P > 0.05$) of peanuts did not differ significantly between matrices which peanuts were embedded inside on repeated measures ANOVA. However, dry weight retention ($F(1,7) = 40.1$, $P < 0.0005$) and volume retention ($F(1,7) = 216.8$, $P < 0.0005$) were significantly greater in moist than in dry particles within each matrix. There was no significant interaction between the effect of matrix and particle type for weight ($F(1,7) = 0.0$, $P > 0.05$) or volume retention ($F(1,7) = 0.0$, $P > 0.05$) (Table 3).

4. Discussion

4.1. Variation between subjects

Variation between subjects was large in terms of the mastication parameters and the particle size distribution despite the selection procedures employed. Significant variation between subjects has been reported in homogeneous foods in terms of the parameters of mastication (Foster, Woda, & Peyron, 2006; Kohyama & Mioche, 2004) and particle size (Jalabert-Malbos et al., 2007; Mishellany, Woda, Labas, & Peyron, 2006). However, literature does suggest variation in the particle size distribution of the food bolus is small in comparison with parameters of mastication, and in some cases is not found to be significant at all (Peyron, Mishellany, & Woda, 2004). Differences in the particle size distribution between subjects may be exaggerated in heterogeneous food systems (Fig. 5).

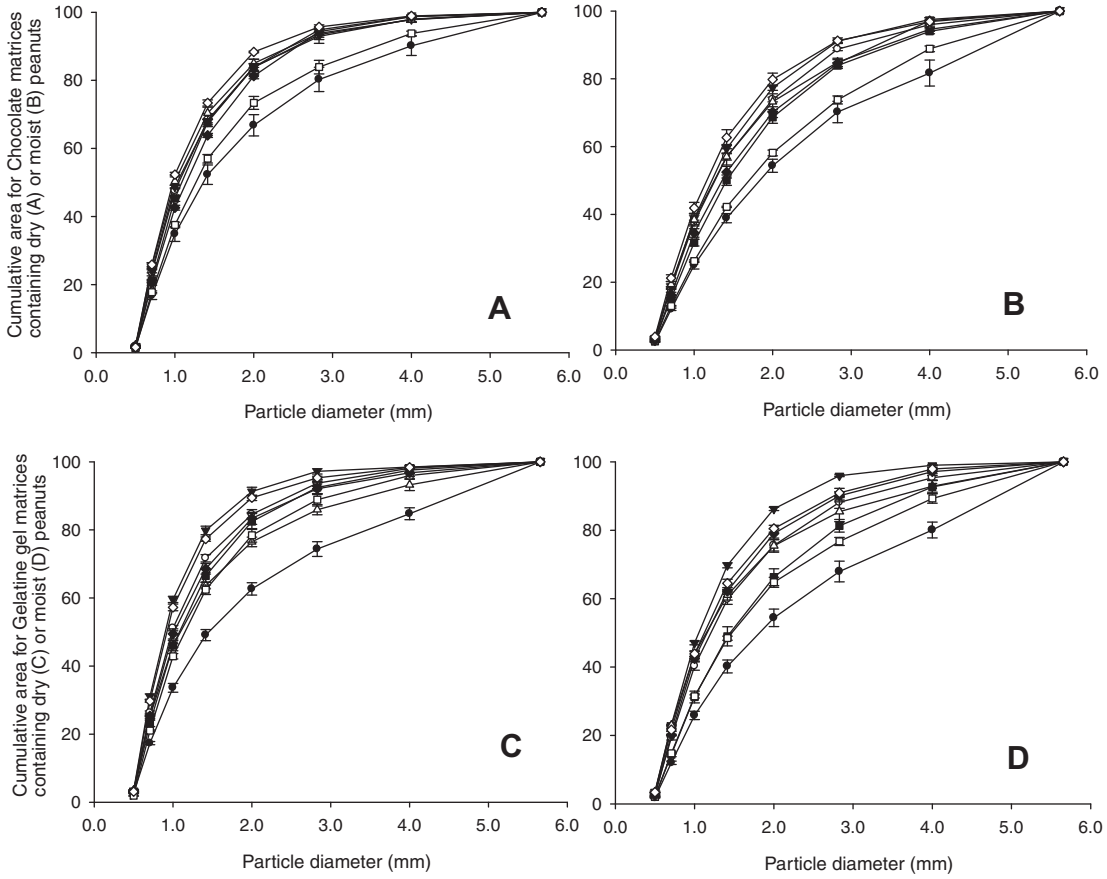

Fig. 5. Cumulative distribution of peanut particles obtained from the boli of all subjects for chocolate matrices containing dry peanuts (A) and moist peanuts (B), and gelatine gel matrices containing dry peanuts (C) and moist peanuts (D). The cumulative percentage area for each subject (mean ± SE) in each class is based on determining a particle width assuming each particle is spherical. Subject 1: ●, Subject 2: ○, Subject 3: ▼, Subject 4: Δ, Subject 5: ■, Subject 6: □, Subject 7: ♦, and Subject 8: ◇.

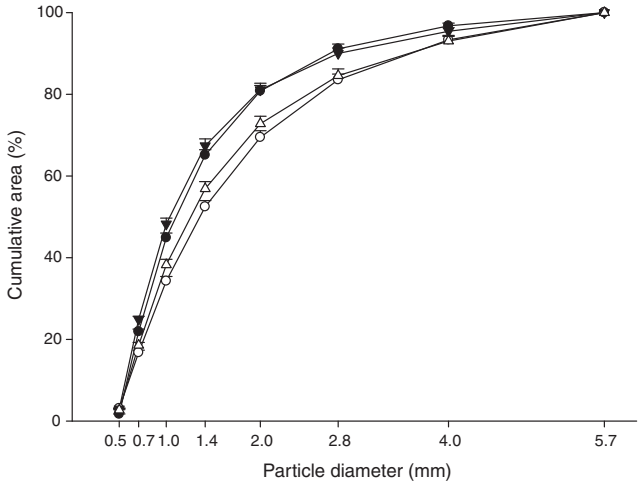

Fig. 6. Mean cumulative area distribution of the peanut particles obtained from the bolus of gelatine gel containing dry (▼) or moist peanuts (Δ), or chocolate containing dry (●) or moist (○) peanuts. The cumulative percentage area (mean ± SE) in each class is based on determining a particle width assuming each particle is spherical.

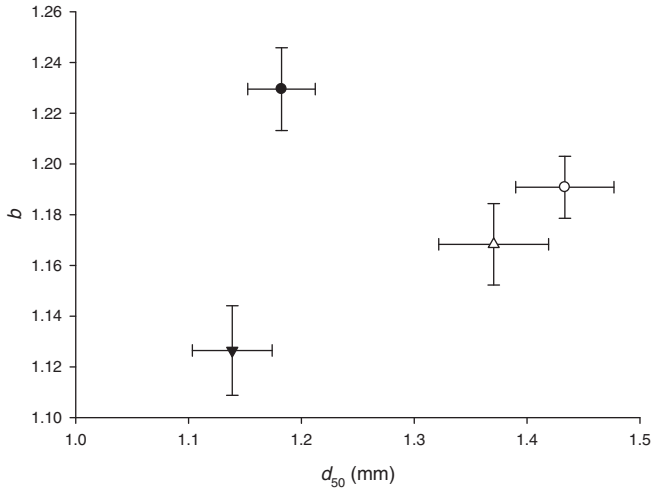

Fig. 7. Mean broadness (b) against mean d_{50} of the peanut particles obtained from the bolus of gelatine gel containing dry (▼) or moist peanuts (Δ), or chocolate containing dry (●) or moist (○) peanuts (means ± SE).

4.2. The influence of the matrices and the peanuts on mastication

The significant differences in chewing behaviour between matrices are similar to results with homogeneous foods (Brown, Eves, Ellison, & Braxton, 1998; Hiiemae et al., 1996; Mathoniere, Mioche, Dransfield, & Culioli, 2000). This indicates that the matrix caused different chewing strategies to form a suitable bolus (Table 2). Interestingly, the properties of the peanuts embedded inside the matrix did not alter the chewing behaviour even though their different physical properties did influence the peanut particle size outcome (Table 2, Fig. 7).

4.3. The influence of the matrices and the peanuts on the particle size distribution of the food bolus

Matrices containing moist peanuts yielded higher d_{50}'s than those containing dry peanuts. Uptake of water has been widely reported to influence the textural properties of foods (Roos, 1995), particularly in nuts (ElMasry, Radwan, ElAmir, & ElGamal, 2009; Paksoy & Aydin, 2004; Visvanathan, Palanisamy, Gothandapani, & Sreenarayanan, 1996). Interestingly, harder particles are believed to be detected as larger than soft particles of the same size inside the mouth (Engelen et al., 2005). As a greater d_{50} in the moist peanuts is accompanied by no significant difference in mastication between moist and dry peanuts within the matrices, the changing physical properties of the peanuts have resulted in different breakage functions, which is likely to be induced by differences in fracture propagation. The breakage function depends on the physical properties of the food, notably its resistance to fragmentation (Agrawal, Lucas, Prinz, & Bruce, 1997), where brittle foods fracture into a greater number of smaller particles.

The two matrix types exhibited significant chewing behaviour differences but did not affect the d_{50} for any one peanut type. This indicates that the properties of the matrix may influence the selection function, which is the probability that particles of a given size present themselves to the occlusion zones (Lucas, Prinz, Agrawal, & Bruce, 2002). It is possible the gelatine gel matrix limits how easily particles of different size can be removed from the matrix and moved to the occlusal plane. In comparison, peanuts are likely to be more easily removed from the chocolate matrix for the molars to access (chocolate is renowned for melting rapidly in the mouth, and had considerably lower values of gumminess, chewiness, and springiness than the gelatine gel (Table 2)).

The type of matrix also affected the spread of the particle size distribution. The higher broadness values from boli containing chocolate (showing peanut particle size in the chocolate had a smaller spread) may reflect the differences that are seen in selection function. Larger particles are more likely to be selected over smaller particles in the chocolate matrix than the gelatine matrix, resulting in a tightening of the particle size distribution (less spread).

Moreover, a significant interaction for broadness between matrices and particle type (Section 3.3.2) shows the ability of the molars to uniformly masticate the particles depends not only on the matrix, but on how the particle behaves inside that matrix. The manner by which the particle surfaces interact with the matrix could affect adhesion to the matrix, and thus influence how easily the particles can be broken down.

4.4. The influence of the matrices and the peanuts on peanut retention in the food bolus

No more than 50% of the initial dry weight of peanuts in the initial sample was retained in the bolus for each test food. Such losses have been reported in previous studies involving peanuts (Jalabert-Malbos et al., 2007; Peyron et al., 2004). Losses are expected to be high given the number of steps involved in the experimental procedure. Hence solids losses may occur during mastication, expectoration, washing to remove matrices (across a 355 μm sieve), and dissolution of fat in ethanol.

The weight and volume retention of the moist particles was significantly greater than for dry particles, however these losses were not influenced by the surrounding matrix (Table 3). Losses are likely to be higher in boluses containing a greater proportion of finer particles (in the case of the dry peanuts) as finer particles may be more readily lost during chewing and washing.

5. Conclusion

This study shows that when heterogeneous test foods are chewed, the properties of one food component can influence the breakdown of another food component. More specifically, when matrices (prepared as gelatine gel or chocolate matrices containing either moist or dry peanuts) are chewed, the matrix influences mastication (in terms of the number of chews, chewing duration, and frequency) and broadness of the peanut particle size distribution in the bolus. However, the matrix does not influence the d_{50} of the peanut particle size distribution. Furthermore, the properties of the embedded peanut influence the d_{50} of the peanut particle size distribution but have no affect on chewing behaviour. We postulate that physical properties of the ingested food matrix influence the selection function whilst the physical properties of the embedded peanut fragments influence the breakage function.

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

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Name/Title of Principal Supervisor: Dr Kylie Foster

Name of Published Paper: Mastication of heterogeneous foods: Peanuts inside two different food matrices

In which Chapter is the Published Work: 10

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