# THE PARTICLE SIZE DISTRIBUTION OF SOLID FOODS AFTER HUMAN MASTICATION

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# ABSTRACT

Bolus formation is a complex process for which two theories are generally accepted, both theories describe how food changes through the mastication process which results in specific properties of the bolus being detected in the mouth to initiate swallowing. This research aimed to identify how food type, portion size (2 g and 4 g) and subjects affects the fate of ingested food solids and their particle size distributions, and bolus moisture content at the swallow point. Then the dynamics of bolus formation up to and past the point of natural swallowing were investigated by the use of a single subject to identify key trends.

Trials involved up to five processed foods; subjects were asked to chew portions of food and expectorate the bolus at the point they felt ready to swallow, or to expectorate the bolus at a specific number of chewing cycles. The solids loss from the bolus and moisture content of the bolus was determined. Particle size distribution (PSD) was measured for the expectorated bolus, and the debris (solids rinsed from the mouth after the bolus).

The food type had the greatest influence on the bolus moisture content, loss of solids from the bolus and PSD of the bolus and debris fractions. Solids are lost from the bolus progressively from the first chew cycle. PSD differed significantly between the bolus and debris fractions, and the PSDs were characteristic for each food type. The rate of change in PSD appears to plateau near the swallow point for some foods, whilst moisture addition continues to increase up to and past the point of swallowing. The bolus moisture content at the swallow point was approximately 50%, despite the differences in chewing strategy between subjects. Saliva does not appear to be added at a constant rate due to no significant effect of portion size.

The results from these studies indicate that bolus does not have to meet specific particle size criteria to achieve a safe swallow, and that particles circulate in multiple compartments during mastication. Results suggest a defined moisture content is required for a safe swallow.

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# LIST OF PUBLICATIONS AND PRESENTATIONS

**Peer-reviewed publications**

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**Conference presentations**

Lawrence, C. S.*, Bronlund, J. E., Jones, J. R., Lentle, R. G., Morgenstern, M. P. & Foster, K. D. *The effect of food type on the particle size distributions of the bolus and remaining particles after human mastication.* Biomouth Symposium, Auckland, NZ, 13-14 December 2006 (*Oral presentation).

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Flynn, C. S.*, Bronlund, J. E., Foster, K. D., Jones, J. R., Lentle, R. G. & Morgenstern, M. P. *Dynamics of mastication: changes in bolus composition during mastication*. 1st Food Oral Processing Conference – Physics, Physiology and Psychology of Eating, Leeds, UK, 5 – 7 July 2010. (*Oral presentation).

Flynn, C. S.*, Bronlund, J. E., Foster, K. D., Jones, J. R., Lentle, R. G. & Morgenstern, M. P. *Mastication in a multi-compartment system: food bolus properties just before swallowing*. 1st Food Oral Processing Conference – Physics, Physiology and Psychology of Eating, Leeds, UK, 5 – 7 July 2010. (*Poster presentation).

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| | |
|---|---|
| ANOVA | Analysis of variance |
| BMI | Body Mass Index |
| CPG | Central Pattern Generators |
| d.p. | Decimal place |
| DMB | Dry Mass Basis |
| EMG | Electromyography |
| $IDM_{food}$ | Ingested dry food solids (g) |
| K-S test | Kolmogov-Smirnov test |
| LSD | Least Significant Difference |
| $M_{(d)bolus}$ | Mass dry bolus (g) |
| $M_{(d)food}$ | Mass dry food (g) |
| $M_{(d)pellet}$ | Mass dry food centrifuge pellet (g) |
| m.s. | mean square |
| $M_{bolus}$ | Mass wet bolus (g) |
| $MC_{bolus}$ | Moisture content bolus (g/100 g dry solids) |
| $MC_{food}$ | Moisture content food (g/100 g dry solids) |
| $M_{food}$ | Mass wet food (g) |
| $M_{pellet}$ | Mass wet food centrifuge pellet (g) |
| $M_{sample}$ | Mass of ingested sample (g) |
| MSDS | Material Safety Data Sheet |
| $N_{chew}$ | Chew cycle number |
| P | Probability |
| PC | Principal Components |
| PCA | Principal Component Analysis |
| PSD | Particle Size Distribution |
| SEM | Standard Error of the Mean |
| $S_{insol}$ | Insoluble solids (%) |
| $S_{loss}$ | Bolus solids loss (g/100 g dry solids) |
| $S_{sat}$ | Saturated solids (g/100 g dry solids) |
| $S_{sol}$ | Soluble solids (%) |

| | |
|---|---|
| $t_{chew}$ | Chewing time (s) |
| TMJ | Temporomandibular Joint |
| TPA | Texture Profile Analysis |
| VFG | Videofluorography |
| WHC | Water holding capacity |
| WMB | Wet Mass Basis |
| $\Delta MC_{bolus}$ | Change in moisture content of the bolus (g) |
| $\upsilon_{chew}$ | Chewing frequency (1/s) |

# CHAPTER 1: PROJECT OVERVIEW

## 1.1 INTRODUCTION

Humans enjoy eating food, and it is an essential function to meet nutritional requirements. Much thought may go into planning a meal and choosing what to consume, but little thought is required by humans to transform an ingested portion of food in the mouth into a swallowable bolus by the process of mastication.

Mastication is the simultaneous breakdown of food and lubrication by tituration of saliva of the resulting particles (Prinz & Lucas 1997). The particles are then formed into a bolus and swallowed (Hiiemae *et al.* 1996). A few prominent theories of swallowing have been put forward for certain criteria to be reached for a swallow to be initiated: particle size, lubrication and bolus cohesion. Mastication can be highly variable depending on the individual (Mioche 2004; Kohyama & Mioche 2004; Mishellany-Dutour *et al.* 2008), and food texture and composition (Mioche *et al*. 2003; Gaviao *et al*. 2004; Engelen *et al*. 2005; Foster *et al.* 2006; Loret *et al*. 2011). Key factors affecting the stages of mastication and swallowing are outlined in Figure 1.1.

Masticatory efficiency has been researched over the past sixty years, but Hutchings and Lillford (1988) were the first to discuss sensory texture perception as a dynamic process based on the mouth detecting the changes occurring to food during mastication. More recently research has focussed on the composition of the food bolus to determine how oral physiology and food properties affect intra-oral processing (Mioche *et al.* 2002; Mioche *et al.* 2003; Peyron *et al.* 2004 & 2011; Engelen *et al.* 2005; Loret *et al.* 2011). The characterisation of the bolus is of interest to researchers in many different fields as it may enable identification of the parameters that initiate swallowing. If the food bolus has the same characteristics between individuals for the same food, this would indicate that there is a defined trigger for swallowing, even though it seems individuals might use different chewing strategies to reach the same characteristics of the food bolus.

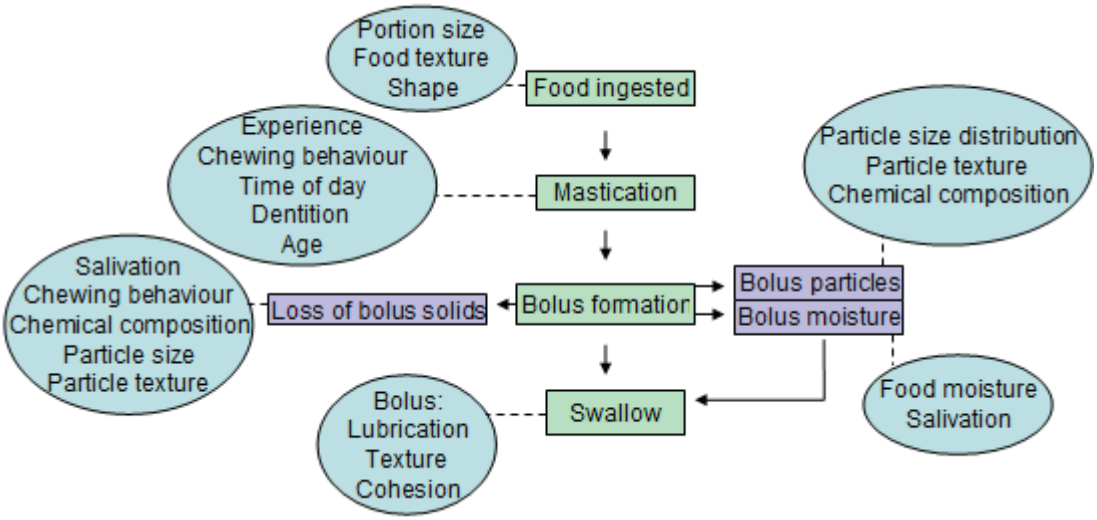



**Figure 1.1 Factors that may affect the mastication process**

To date there are limited studies that investigate the mastication of processed heterogeneous foods or a range of textures in one mouthful which is most commonly what humans ingest when consuming a varied diet. It is also known that not all ingested food solids are present in the bolus at the point of swallowing (Schneider & Senger 2001; Peyron *et al.* 2004b). Therefore in characterising food breakdown through mastication it is of interest to identify the fate of all food solids ingested and how they affect food bolus formation.

## 1.2 RESEARCH OBJECTIVES

The purpose of this work was to investigate, with a group of subjects, the fate of food solids after human mastication, for a range of processed food products that are of differing textures and composition. The bolus particle size distribution (PSD), solids content and moisture are of particular interest to try and identify critical bolus properties that may initiate swallowing. The research initially focussed on the outcome of bolus formation, and then the process of bolus formation was followed by the use of a single subject to identify trends in how bolus properties were achieved.

The specific aims of this research were to:

- Investigate the fate of ingested food particles in the mouth at the swallowing point, for five processed foods and two portion sizes.
- Investigate the PSD of recovered food particles at the swallowing point.
- Investigate the effect of food type and portion size on bolus moisture content at the swallow point.
- Investigate the dynamic process of PSD changes occurring during bolus formation and the effect of food type.
- Investigate the bolus moisture content and fate of bolus solids during bolus formation and determine whether these properties may initiate swallowing.
- Determine the factors affecting the PSD of the bolus, including variability within and between individuals.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 INTRODUCTION

Mastication is a process in which pieces of food are ground into a fine state, mixed with saliva, and brought to approximately body temperature in readiness for transfer to the stomach where most of the digestion occurs (Bourne 2004). The main purpose of mastication is to produce a food bolus suitable for swallowing (Chen 2009). Thexton & Crompton (1998) state that swallowing is a clearing process that is initiated voluntarily, but later becomes reflex once food particles have been cleared by the tongue.

The processes of mastication and swallowing are affected by oral physiology, initial food properties, the breakdown pathway of the food and food bolus formation. To gain understanding of the processes the physiology of the mouth must be explained, and then related to its functions as masticatory apparatus. It is not just the breakdown of food that is important but the intra-oral transport that is an essential requirement of the process (Thexton 1992). The effect of food properties will be explained in relation to both mastication and swallowing; how the mouth identifies differences between foods and relates these to control mastication and form a food bolus that initiates swallowing. A food bolus needs to be swallowed before further mouthfuls can be ingested, and there are a number of theories that have been proposed to explain mechanisms for swallowing initiation, which will be reviewed.

## 2.2 ORAL PHYSIOLOGY

The anatomy of the oral digestive system consists of the mouth and its structures, Figure 2.1. The mouth is divided into two parts, the vestibule, which is the space at the front between the lips, cheeks and teeth, and the oral cavity, which lies behind the teeth. The oral cavity consists of the hard and soft palates which make up the 'roof of the mouth', the entrance to the oropharynx at the back, the tongue and floor of the mouth (Ross *et al*. 2003). Considering the process of mastication these structures are important to the ingestion, taste, texture detection, quality assessment, and the particle size reduction of food materials.

The formation and function of the structures relevant to mastication will be reviewed in detail in the following sections so that they can be understood in relation to the processes of mastication, food bolus formation and swallowing.

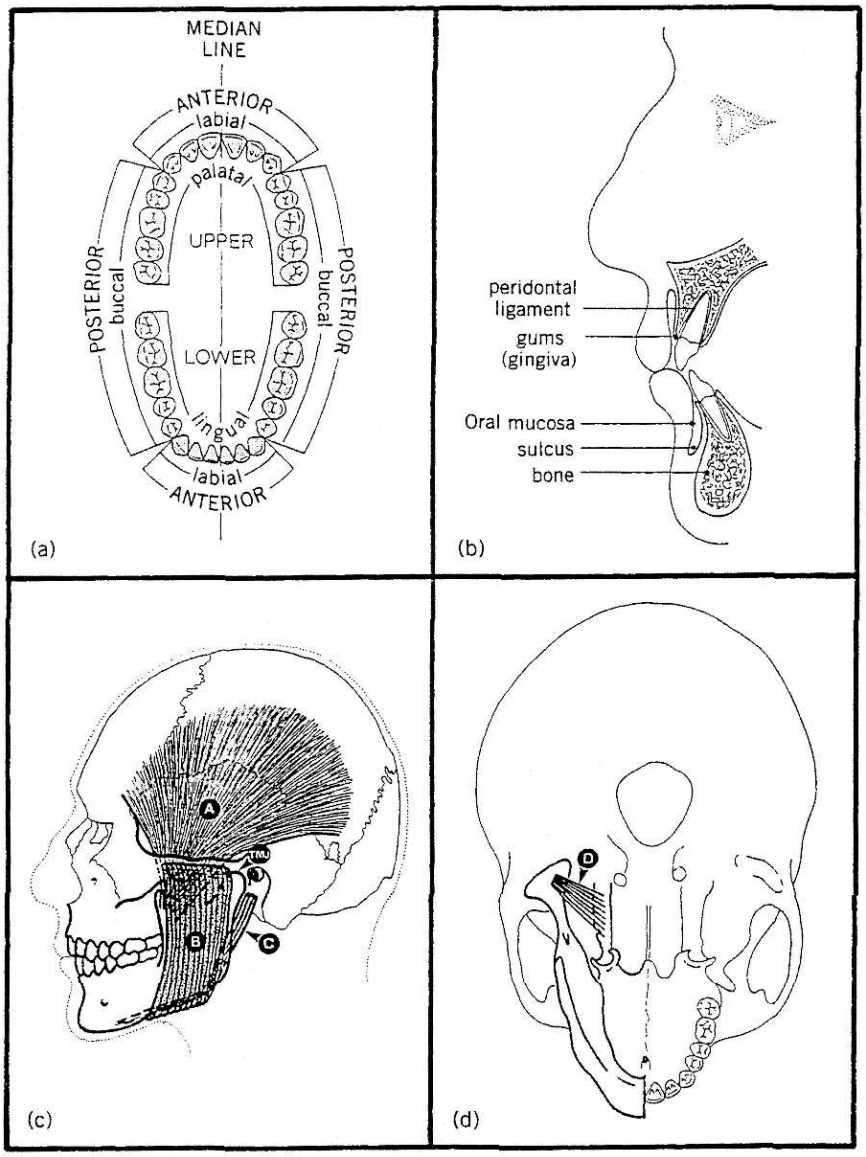
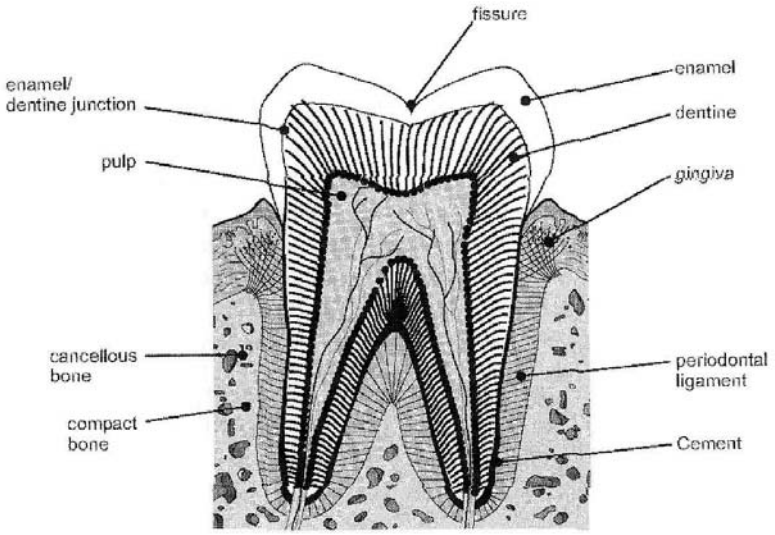



**Figure 2.1 Human masticatory apparatus (from Harker *et al.* 1997)**

(a) The surface of the teeth
(b) The maxilla (upper jaw) and mandible (lower jaw) including teeth anchorage.
(c) and (d) Muscles of mastication, temporalis (A) and masseter (B) are jaw closing muscles. Digastric (C) and lateral pterygoid (D) are jaw opening muscles. Temporomandibular joint (TMJ).

## 2.2.1 TEETH

Teeth are a major component of the mouth. In adults there are 32 teeth, with 8 on each side of each jaw consisting of two incisors, one canine, two pre-molars or bicuspids, and three molars; half of these are set into the jawbone of the maxilla (upper jaw), and half are set in the mandible (lower jaw). Within each jaw bone the incisors and canines are grouped as the anterior teeth, and the pre-molars and molars are the posterior teeth (Jenkins 1978; Lucas 2004). The posterior teeth are responsible for mastication and the anterior teeth are used to bite mouthfuls from larger pieces of food and sculpt foods, such as separation of peel from apple (Jenkins 1978; Lucas 2004).




The crown covered by enamel projects into the oral cavity. The roots are anchored into the tooth socket by the periodontal ligament (much narrower than shown) and the gingiva (gum) protects it. Blood vessels and nerves enter the through the roots. The former supply the odontoblasts (dentine forming cells), the latter supply the periodontal ligament and tooth respectively

**Figure 2.2 Structure of a human molar tooth. (from Lucas 2004)**

It is important to know the basic structure of the teeth to understand some of their functions in mastication (Figure 2.2). The visible part of the tooth in the mouth is called the crown, of which the working surface of this is called the cusp. The posterior teeth have multiple cusps and fissures that run between the bases of the cusps. The part of the tooth that anchors into the jaw bone is called the root, which is fixed in place by soft tissue, the periodontal ligament or direct to bone (Jenkins 1978). The root is lined by

cementum which is a collagenous bone-like material to protect dentine and pulp. This provides anchorage for the collagen fibres of the periodontal ligament from the tooth to the socket (Jenkins 1978; Lucas 2004). Teeth are made up of specialised tissues, enamel and dentine, which require more detail below.

Enamel is a mineralised tissue that covers the crown of the tooth. The enamel provides a tough surface used to break down food particles and protects the dentine. Dentine is a calcified mineral that forms most of the tooth substance. It contains tubules that wind down from the enamel to the pulp cavity (Jenkins 1978). Dentine is secreted by odontoblast cells that retreat in a row away from the tissue towards the pulp as dentine is produced. The odontoblasts end up lining the pulp cavity as dentine is formed continuously during the life of the tooth, therefore the pulp cavity decreases in volume with age (Jenkins 1978; Ross *et al.* 2003; Lucas 2004). The odontoblast processes serve a transducer function in transmitting stimuli, such as the occurrence of tooth-on-tooth contact, from the tooth surface to the nerves in the dental pulp. When they receive a sufficiently intense signal they produce dentine at an increased rate to strengthen the tooth (Lucas 2004).

The teeth are developed inside the jaw, and erupt through the soft lining of the tooth socket (Lucas 2004). It is important that teeth are aligned to limit damage that could occur to them during the high pressures of mastication with tooth-food-tooth contact, and tooth-on-tooth contact (Lucas 2004). The tooth-on-tooth contact can cause the most abrasive wear due to tooth hardness which is a major factor in wearing down of teeth. Alignment of teeth is termed occlusion, and the precision of this depends on the type of tooth and its ability to adjust in the jaw (Jenkins 1978; Lucas 2004).

The opposing post-canine teeth in mammals require surfaces that match well as they need to touch when the jaw is closed to be able to fragment food particles thoroughly (Lucas 2004). To manage this, mammals can subtly adjust a tooth in the jaw due to the mode of attachment of the teeth to their sockets. Collagen fibres and elastic fibres are components of the periodontal ligament which secures the tooth to the surrounding bone (Figure 2.1(b)). These fibres allow slight movements naturally, and forms the basis of

orthodontic procedures used to straighten teeth to reduce malocclusion (misalignments) of the biting and grinding surfaces of the teeth (Jenkins 1978).

Good occlusion in mammals not only protects teeth from wear by spreading the force of tooth contacts across dentition, it also increases chewing efficiency. A study by Bourdiol & Mioche (2000) has shown that an increase in the occlusal surface area, as the higher the number of pre-molar and molar pairs in the mandible and maxilla, significantly correlates with an increase in functional surface area in mastication. This increases chewing efficiency by resulting in a reduced number of chewing cycles required to prepare a hard food for swallowing. For the most effective bite force of food on the molars, the muscular force must be directed along the tooth long axis which will also increase chewing efficiency (Lucas 2004).

Evidence of tooth wear increases with time, and depends on the past functional and acquired habits of the individual (Bourdiol & Mioche 2000; Lucas 2004). Side preferences of chewing also contribute to tooth functional surface wear. In the study by Bourdiol & Mioche (2000), evaluation of the functional surface ratio between left and right sides identified the individual's preferred chewing side.

### 2.2.2 SOFT TISSUES

The soft tissues in the mouth are the tongue, gingiva (gums), hard palate, soft palate and pharynx. The oral cavity and its tissues are lined by a masticatory mucosa, a lining mucosa and specialised mucosa. The masticatory mucosa is found on the gingiva and the hard palate. The function of this mucosa is to protect tissues from friction by food particles (Lucas 2004). It is keratinised to provide toughness, resulting in the relative immobility of these tissues, which protects against subsequent wear (Jenkins 1978; Lucas 2004). The lining mucosa is found on the lips, cheeks, soft palate, and floor of the mouth and under the tongue. It protects the underlying muscles, contains minor salivary glands, taste buds and sensory receptors, but is flexible to adjust to the movement of its muscles. The specialised mucosa is restricted to the dorsal surface of the tongue, where it contains papillae and taste buds (Jenkins 1978). To avoid friction from dry foods the oral mucosa are constantly lubricated by saliva (Lucas 2004). Saliva will be reviewed in more detail later.

The gingiva are part of the supporting tissues of the teeth. They are firmly attached to teeth and underlying bone. The gingiva is composed of two parts gingival mucosa which is synonymous with the masticatory mucosa, and the junctional epithelium which adheres firmly to the tooth. In young individuals, the attachment is to the enamel; in older individuals the gingiva starts to decrease and expose the root of the tooth, the attachment is then to the cementum (Ross *et al.* 2003). This gum receding can result in disease, and or the eventual loss of teeth, which affects masticatory function (Ross *et al.* 2003).

The hard palate is important to separate the masticatory apparatus from the airway above (Lucas 2004). The hard palate contains some taste buds and mechanoreceptors, resulting in the high sensitivity to touch. Texture of food is detected mostly by this surface and a loss of 'taste' is reported by full denture wearers due to the reduced ability to judge texture, taste and temperature of food (Jenkins 1978; Hildebrandt *et al*. 1997).

The pharynx is the opening to the oesophagus and its surface is smooth to allow food particles to slip over it easily. The pharynx has a series of constrictor muscles attached to the back of the mouth and which are contracted rapidly to assist in pushing the food bolus down the oesophagus as quickly as possible to avoid choking as this is where the food passes the airway (trachea) (Lucas 2004).

#### 2.2.2.1 TONGUE

The functions of the tongue in mastication are numerous. First, it is often how food is taken into the oral cavity if a bite sized piece is ingested (Hiimae *et al.* 1996). The surface of the tongue is roughened with keratinised projections called filiform papillae which prevent the slipping of the mass of food (Jenkins 1978; Lucas 2004). Secondly, the tongue pushes the food onto the occluding surfaces of the teeth along the food's long axis to obtain maximum exposure to functional surfaces of the posterior teeth (Jenkins 1978; Prinz & Lucas 2001; Lucas 2004; Okada *et al.* 2007). Thirdly, the sensory endings of the tongue enable it to elect those parts of the food mass which are sufficiently well masticated to be ready for swallowing and to separate them from parts requiring further mastication (Jenkins 1978; Heath 2002). The tongue helps to mix

saliva into the bolus by dipping its tip into the pool of saliva which forms under the tongue and then touching the food bolus (Prinz & Lucas 1997). The tongue may also have a direct crushing effect on food by pressing it against the hard palate, and after swallowing has occurred, sweeping movements of the tongue help to remove food residues which have become trapped in the vestibule, between the gingiva and elsewhere (Jenkins 1978; Hiimae *et al*. 1996; Mioche *et al*. 2002b; Okada *et al.* 2007).

The structure of the tongue that enables it to carry out the functions mentioned above are due to the muscles, taste buds and mechanoreceptors that are specialised in this tissue (Figure 2.3). The tongue projects into the oral cavity from the floor of the mouth and anchored to the hyoid bone which makes it very responsive (Jenkins 1978; Lucas 2004). The tongue can project outside the mouth to receive foods for ingestion; this is done using the extrinsic muscles that are connected to the hyoid bone and the muscles that move the hyoid bone. These muscles produce anterior (genioglossus muscle) or posterior (styloglossus and hyoglossus muscles) movement, depression (genioglossus and hyoglossus) and elevation (styloglossus and palatoglossus), changing both the tongue shape as well as its position (Lucas 2004).

The arrangement of muscle fibres allows flexibility and precision in the movements of the tongue which is also due to intrinsic muscles which move the tongue laterally and involve unilateral contraction (Lucas 2004). The intrinsic muscles have no attachments to bone. There are three sets of muscles arranged in bundles that run in three planes within the tongue, each arranged at right angles to the other two in vertical, transverse, and longitudinal directions (Jenkins 1978; Lucas 2004). These muscles are essential to the role of the tongue in mastication, swallowing and human speech.

The tongue contains three taste bud containing regions called papillae. The fungiform contain 1 – 4 taste buds and represents approximately 20% of the total number of taste buds in the oral cavity. Foliate taste buds lie in two areas on opposite sides of the tongue and comprise about 33% of all taste buds. The circumvallate taste buds lie in the posterior part of the tongue (Ross *et al.* 2003). Taste compounds are released from foods, dissolved in saliva and then detected by the taste bud. Release of compounds is enhanced by the fracture of food particles. The taste buds provide an important

detection of compounds in the mouth so that the mouth can react (Katz *et al.* 2000). Sour is detected by low pH, which stimulates the release of saliva to neutralise the acid as this erodes enamel. Sweet may be detected as sugars have significant impact in tooth decay, it may also identify carbohydrates for the body (Katz *et al.* 2000; Lucas 2004).

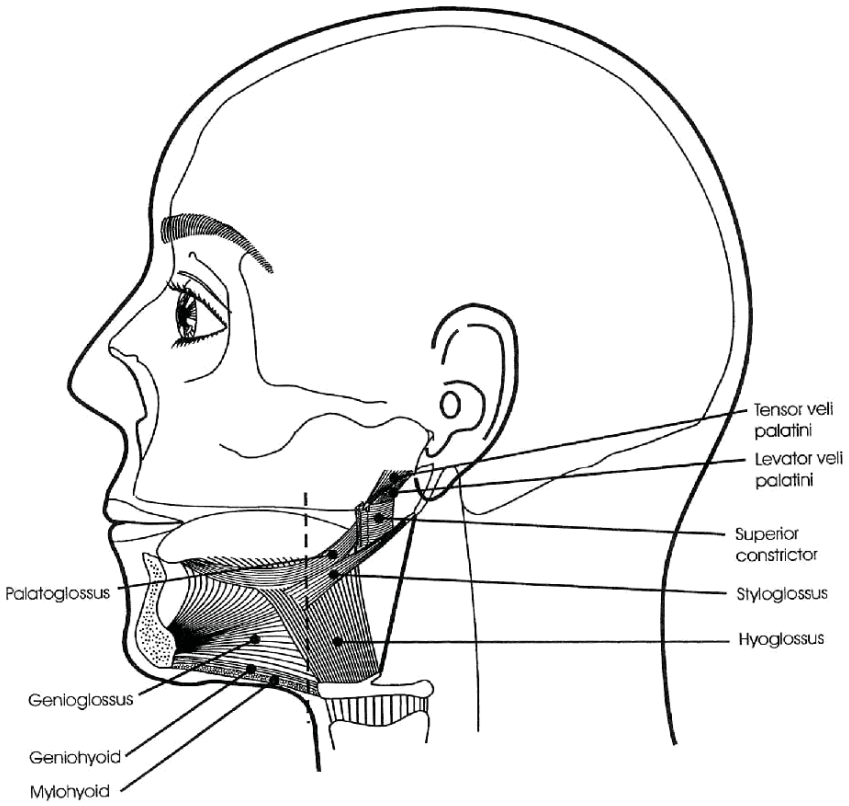



**Figure 2.3 The muscles of the tongue, soft palate and pharynx. (adapted from Lucas 2004)**

Taste receptors are also found in other areas of the oral cavity, including soft palate, epiglottis, oesophagus, pharynx and the inside of the cheeks. Of these the most important is the soft palate, where 15% of the total numbers of taste buds are found (Jenkins 1978). Taste receptors have a role in mastication by detecting where food particles are in the mouth, this aids bolus formation and clearance of the mouth.

The tongue has very sensitive touch discrimination and investigations have been carried out to identify whether finger touch or tongue touch has the best two-point discrimination. Two-point discrimination is the distance between two points that are both detected and the most sensitive is the detection of the two closest points (Ringel & Ewanowski 1965). A study using specially designed equipment to monitor force

applied to the points resulted in the tip of the tongue having a mean two-point discrimination of 1.7 mm which was more sensitive than the finger tip (Ringel & Ewanowski 1965). Another study resulted in a mean tongue tip discrimination of 1.25 mm, with a range of 1 – 2 mm, but finger touch was found to be more sensitive (Laine & Siirila 1971). This indicates how the tongue selects food particles above 1.2 mm for movement to occlusal surfaces for comminution (Jiffry 1983; Okada *et al.* 2007).

### 2.2.3 JAW BONES AND THE TEMPOROMANDIBULAR JOINT

The jaw bones house the teeth in the dental arches surrounding the anterior part of the tongue. There are two jaw bones found in the mouth; the mandible and the maxilla. The mandible (or lower jaw) has to be able to move laterally to align the upper and lower post-canine teeth properly for chewing. The jaw bone contains blood vessels which feed cells, allowing them to respond to changes in mechanical strain within the bone. When food is loaded between the teeth, the teeth and surrounding bone are deformed. It is thought that bone tissue remodels in response to strain levels that it sustains, resulting in different bone structures depending on the types of foods that are consumed; although most people eat a variety of textures in their diet (Jenkins 1978; Lucas 2004).

The upper and lower jaws are connected together by the temporomandibular joint (TMJ) which is attached to the temporal bones of the head (Figure 2.1(d)). The mandible acts as a condyle with a fibrocartilage disc allowing the joint to slide against the maxilla. The TMJ has a hinge-like movement which is used for the biting of food. A movement that may be employed in the early stages of mastication is the protrusion and withdrawal of the mandible. In most individuals, the lateral movement is approximately symmetrical, but the presence of a tender tooth, among many other causes, may lead to the establishment of habits of unilateral chewing. Such habits may persist indefinitely, long after the original cause has been removed (Jenkins 1978; Bourdiol & Mioche 2000; Lucas 2004). The condyle disc that facilitates the movement of the mandible occasionally causes trouble in humans by not moving in complete synchrony with it, this may reduce masticatory efficiency (Lucas 2004). The mandible is controlled by a number of muscles and these contribute to control of the mastication cycle and bite forces, which will be explained in the following section.

## 2.2.4 MUSCLES

The muscles of mastication can be classified into those concerned with opening or closing (Figure 2.4). Muscles responsible for opening the jaw include the lateral pterygoid, the digastric and other suprahyoid muscles (such as mylohyoid, geniohyoid and sternohyoid). The closing muscles consist of the temporalis, used in quick closure and gentle biting, and the masseter and internal pterygoid muscles which are required for more powerful crushing movements (Jenkins 1978; Lucas 2004). The muscles of the lips and cheeks also take part in mastication (Figure 2.5).

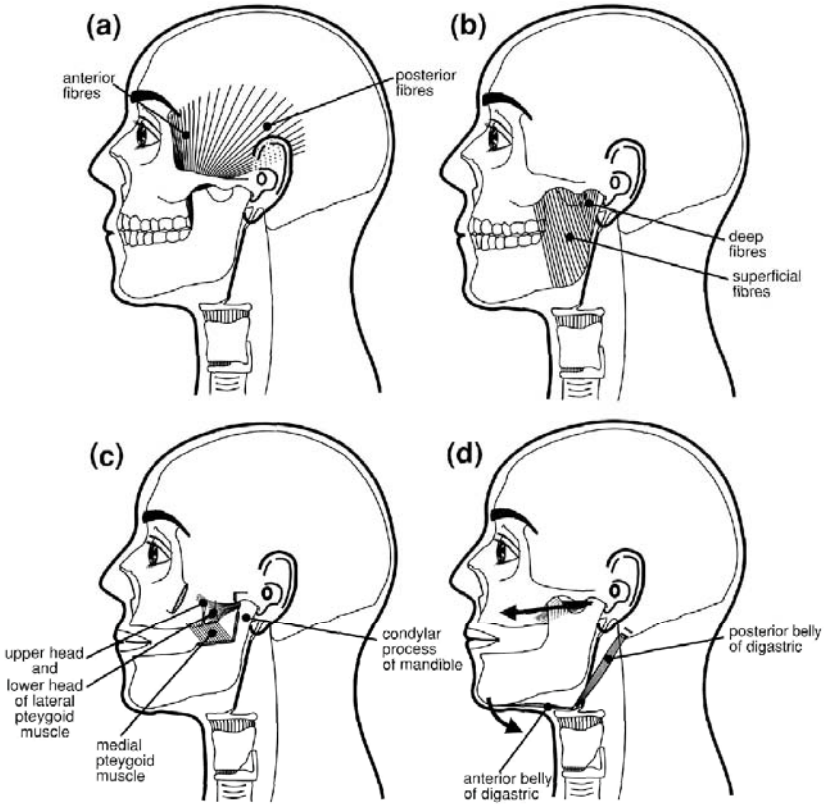



**Figure 2.4** **The muscles of mastication (from Lucas 2004)**

(a) Temporalis; (b) Masseter; (c) Medial and lateral pterygoid; (d) Lateral pterygoid and digastric

### 2.2.4.1 JAW OPENING AND CLOSING

The jaw opening muscles are also called the jaw depressors or abductors. In humans, the anterior digastric and the lateral pterygoid muscles act together to open the jaw by both rotation of the TMJ (at any angle of opening) and anterior translation of the condyle (pronounced at wider gapes). The mammalian mandible can be moved from

side-to-side, up, down, forwards and backwards (Hiiemae *et al.* 1978). The lateral pterygoid muscle in particular, acting just on one side of the jaw, drags the lower jaw towards the opposite side, but other jaw muscles are potentially capable of assisting it depending on whether they are situated so as to produce a smooth movement. There can be considerable variation in lateral movement even within a masticatory sequence, when processing one mouthful of food (Peyron *et al.* 1996; Lassauzay *et al.* 2000; Mioche *et al.* 2002b). In contrast, when jaw movements are viewed from the side, they appear stereotyped, varying only in amplitude. Movement in a anterior-posterior direction is evident in the mastication of very large mouthfuls of food and this is to enable them to be processed bilaterally (i.e. on both rows of anterior teeth at once) (Mioche *et al.* 2002b; Lucas 2004).

The jaw closing muscles (elevator or adductor muscles) provide the major work input for fracturing food particles. These are the masseter, temporalis and medial pterygoid muscles (Lucas 2004). During mastication, the good control of mandible movement by opening and closing muscles enables speeds of 75 mm/s to be achieved during the fast stages (Harker *et al.* 1997). This slows when teeth are very close or food is detected between the posterior teeth, or near maximum opening during a chew cycle (Harker *et al.* 1997; Hiiemae 2004; Lucas 2004). Bite forces are often measured and analysed in relation to masticatory efficiency (Engelen *et al.* 2005). Maximum bite force is not usually applied during mastication of foods (Atkinson & Shepherd 1967).

The mylohyoid muscle is connected to the mandible and to the hyoid bone (Figure 2.3). This muscle moves constantly during the masticatory sequence as the tongue is constantly moving (Hiiemae & Palmer 1999; Lucas 2004). As swallowing commences there is a burst of activity, measured by electromyography (EMG), from the mylohyoid muscle as the muscles in the tongue contract and propel food towards the oesophagus, this is affected by food texture (Shiozawa & Yanagisawa 1999). During a swallow the mylohyoid muscle moves vertically, and this is the mechanism for any swallow whether solid food or saliva only (Hiiemae *et al.* 2002).

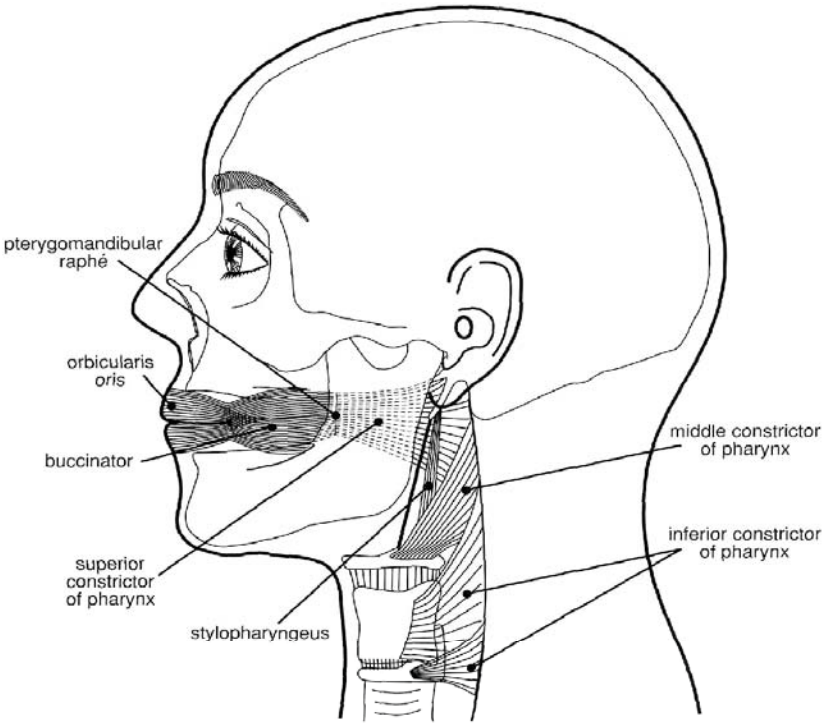



**Figure 2.5** **The cheek and lip muscles (from Lucas 2004)**
Including the muscles of the pharynx.

The buccinator is the cheek muscle that is important for its manipulation of food particles during mastication (Figure 2.5). The muscle fibres have attachments to the maxilla and mandible, and are attached to a knot of tissue by the corners of the lips (modiolus) (Lucas 2004). The buccinators are essential for ensuring that food particles are kept on the functional surface of the molars by pressing onto the lateral side of the teeth, and not letting the particles fall into the vestibule (Casas *et al.* 2003; Lucas 2004). This coordination is well controlled as cheek biting only rarely occurs (Casas *et al.* 2003).

A circular muscle called the orbicularis oris forms the lips, which surround the entrance to the mouth. This acts as a sphincter as the width of the opening is controlled through a number of muscles that converge from above and below onto the modiolus (Lucas 2004). The movement of the lips affects the modiolus position which is important for the control that the buccinator requires in mastication, as mentioned above.

### 2.2.5 SALIVATION

There are three major paired salivary glands, meaning that they exist in both sides of the mouth. They secrete fluid into the oral cavity to lubricate the mouth. The parotid glands are located just behind the lower jaw, and excrete serous (protein) saliva into the vestibule of the mouth opposite the molar teeth where the teeth are sprayed directly. The submandibular glands are located in the floor of the mouth and its main ducts open together just behind the incisors and others enter individually into the oral cavity. They are mixed glands that are mostly serous secreting. The sublingual glands and minor salivary glands produce mainly viscous mucus (mucin) secretions that cover the mucous membrane (Jenkins 1978; Lucas 2004). This thin covering is not lost at swallowing and is needed to keep friction in the mouth low (Prinz 2004).

Saliva includes the combined secretions of all the major and minor salivary glands and is composed of water, proteins, glycoproteins and electrolytes. The volume of saliva produced varies among individuals, but approximately 1200 ml can be produced per day. One of the unique features of saliva is the large volume produced which must be related to it having so many functions, only some of which are concerned with digestion (Ross *et al.* 2003).

The functions of saliva include moistening the oral mucosa to reduce friction between food and mucosa to low levels. This requires a fairly high glycoprotein concentration that results in a surface tension well below that of water (Ross *et al.* 2003). Saliva is good at wetting exposed surfaces of food produced by particle fragmentation. It encourages them to bind together to form a bolus due to the viscosity of saliva being well above water, this stickiness is also due to high levels of protein and glycoprotein. Saliva also starts carbohydrate digestion with the enzyme α-amylase that breaks 1-4 glycosidic bonds, and continues to act after reaching the stomach (Prinz & Lucas 2000; Ross *et al.* 2003; Lucas 2004).

The water component, comprising approximately 98%, of saliva provides a medium to solubilise potential taste compounds so that they can be sensed on the tongue. Saliva buffers the contents of the oral cavity so preventing acid erosion of the mineralised tissues, because of its high bicarbonate ion concentration. It is a source of calcium,

phosphate and fluoride ions which are essential for normal tooth development and maintenance. Other functions of saliva include performing immunologic functions as it contains antibodies, immuglobin A, G and M. It also controls the bacterial flora of the oral cavity by use of lysozyme (Jenkins 1978; Ross *et al.* 2003).

### 2.2.6 SENSATION AND NEURAL FEEDBACK

Food processing in the mouth requires continuous neural feedback from sensory receptors (Lund 1991). Information is required about where food is in the mouth and its condition; this enables control of the position of the jaw to optimise processing and minimise any damage structures in the oral cavity (Jenkins 1978).

Mechanoreceptors are present in the oral mucosa, periodontal ligament and temperomandibular joint. These monitor and react to mastication events and ensure the success of chewing cycles (Jenkins 1978). During the slow phase of jaw opening the food properties are being evaluated by the oral mucosa (Jenkins 1978). Throughout each chew cycle, receptors supply information about jaw displacement and regulate the vertical amplitude of a chew stroke (Jenkins 1978). During jaw closing when tooth-food-tooth contact occurs, the fracture events in the food particles are monitored in the tooth through the sensitivity of the fluid movement through tubules of dentine (Paphangkorakit & Osborn 2000) and by mechanoreceptors in the periodontal ligament (Jenkins 1978; Lucas 2004). The periodontal ligament deforms under pressure and the nerves respond by controlling mastication. The nerves can limit mastication if excessive pressure is felt or pain results (Jenkins 1978). In the late phase of jaw closing it moves slowly to avoid damage to the teeth, then stops and opens as soon as movement is obstructed, this occurs about 12 ms after tooth-on-tooth contact (Gibbs *et al.* 1981).

It would seem that teeth sensitivity is excellent, as they are able to sense particles between them of between 8 – 15 μm in thickness or diameter (Utz, 1986, cited by Lucas, 2004). The effect of abrasives on the chewing process was tested by Prinz (2004) and it was found that particles of 250 μm diameter were detected in the mouth and resulted in a reduction of food manipulation. These functions require skilled

movements of the tongue which are controlled by neural feedback mechanisms (Jenkins 1978).

## 2.3 MASTICATION AND SWALLOWING

Humans ingest food to gain their fuel for metabolism and nutrients. Whether humans need to masticate their food or not is non-conclusive in studies to date. Research has stated that many foods do not require chewing in order for absorption of nutrients in the gastrointestinal tract (Farrell 1956). However research has shown that the inability to masticate may result in the omission of some hard/tough foods from the diet which will lead to an increased risk of dietary deficiency (Heath 1972; Chauncey *et al*. 1984; Hildebrandt *et al.* 1997; Sheilam & Steele 2001). Many researchers believe that maintenance of a high degree of masticatory efficiency is important for human health and to meet the demand of their high metabolic rates (Akeel 1992; Prinz & Lucas 1997); full understanding is still to be obtained from studies in this area.

Prior to ingestion, potential foods are first analysed visually, then by hand-touch, then smell to deem its suitability as a food product (Bourne 2004). Tools, hands, teeth, or a combination of these will be utilised to obtain a bite-sized piece to put into the mouth – this is the first physical analysis of texture (Heath 2002). The mouth initially evaluates the material to determine whether it is to be further ingested; it will be assessed externally by lips and tip of the tongue, then internally by incisor bite (Okada *et al.* 2007), and mucosal receptors will assess flavour. At this point the material will either be expectorated or moved further into the mouth for processing (Heath 2002).

Food is transformed during the process of mastication (Mioche 2004). The main objective of this transformation is to reduce food size by two or three orders of magnitude, via the fracture of particles which exposes food surface area (Heath 2002; Bourne 2004; Lucas *et al.* 2004). This creation of surfaces releases tastes and aromas, and later in the gut increases the rate at which chemicals and enzymes act on the food to result in a further 20 orders of magnitude of size reduction. This provides the body with energy at a higher rate (Prinz & Lucas 1997; Heath 2002; Lucas *et al*. 2004). If food particles cannot be reduced sufficiently nutrients are not absorbed into the blood to be metabolised and are then excreted (Bourne 2004).

A mouthful of food is acted on during mastication; it is comminuted by crushing, shearing and the incorporation of saliva. The resulting mixture of small particles are agglomerated, which is shaped into a bolus and swallowed (Jiffry 1981 & 1983; Hutchings & Lillford 1988). This set of functions corresponds to a highly complex sensory-motor activity integrating various components of the masticatory system, such as teeth, jaw muscles, lips, cheeks, tongue and the production of salivary secretions (Mioche 2004). The mouth and chewing apparatus are complex, and most of the time used sub-consciously.

## 2.3.1 MASTICATORY SEQUENCE

The masticatory sequence is the oral processes of chewing and swallowing that involve controlled coordinated function of the lips, cheeks, tongue, teeth, hard palate and oropharynx. Fluids are dealt with using a different sequence to the management of solid foods, and both have been studied in depth by Hiiemae & Palmer collaborations for over ten years, summarised in Hiiemae (2004). Only the processes of solid foods that require chewing will be reviewed here (Figure 2.6).

### 2.3.1.1 STAGE 1 TRANSPORT

Regardless of initial food texture, a bite size piece will be manoeuvred by the tongue to the occlusal surfaces of the posterior teeth. This is termed stage I transport and has been investigated by Hiiemae *et al*. (1996) using videofluorography (VFG). Food is dusted or mixed with a small quantity of barium paste and the subject is instructed to chew and swallow as normal while being recorded. VFG tapes at 30 frames per second were recorded from each subject and analysed using slow motion. VFG shows the movement of jaws, teeth, hyoid, tongue and the position and state of the food (Hiiemae & Palmer 1999). The food will be held on the midline of the tongue by the filliform papillae, and then the tongue orientates the food on the molar teeth along its long axis, to enable the most efficient chewing (Prinz & Lucas 2001; Mioche *et al.* 2002b). This positioning process can take up to two seconds during which there is an initial quality assessment of the food (Hiiemae 2004). The food is then ready to be processed.

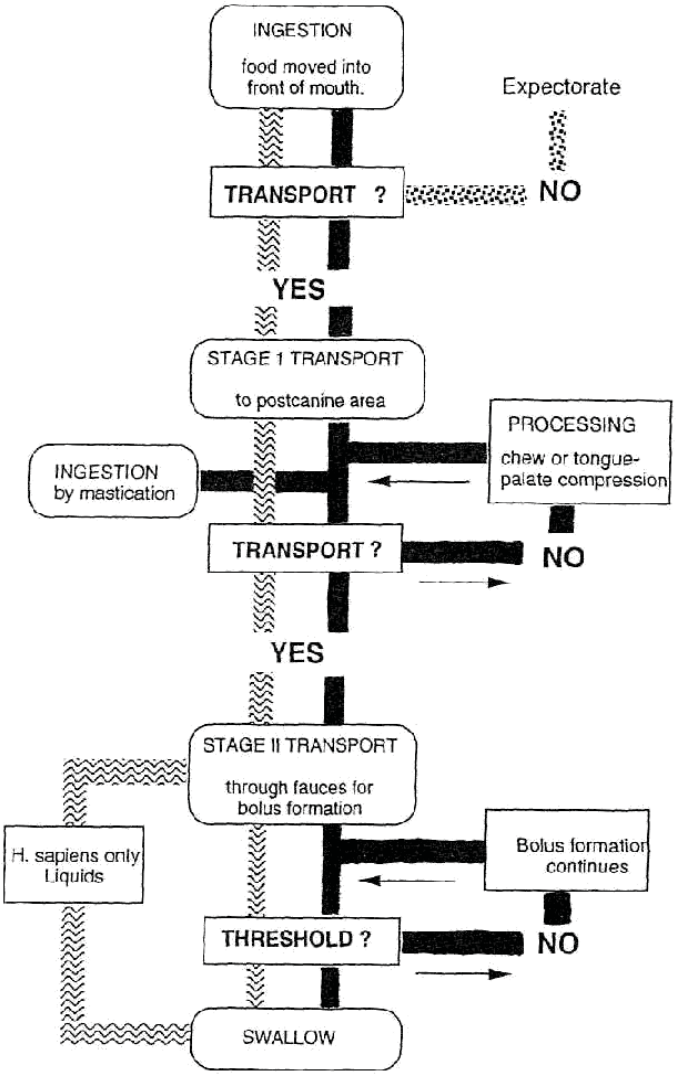



**Figure 2.6 The process model of feeding (from Hiiemae 2004)**

### 2.3.1.2 ORAL PROCESSING

The chewing forces and number of chewing cycles depends on the individual, but in healthy adults differences can be seen that correlate with food texture (Prinz & Lucas 1997; Hiimae & Palmer 1999; Lassauzay *et al*. 2000; Mioche *et al*. 2002a; Mioche 2004; Peyron *et al.* 2004a; Foster *et al.* 2006). Chewing efficiency has been of interest to dentists and studied over the past sixty years, using sieve analysis after a food material or test food has been subjected to a particular number of chews or increasing numbers of chewing cycles (Yurkstas 1951; van der Glas *et al.* 1987). Chewing forces have also been studied by inserting transducers into dentures or partial dentures and monitoring changes and maximum force during chewing (Anderson 1955), and recently mini transducers have been inserted into natural dentition (Mioche *et al.* 1994; Peyron & Mioche 1994; Mioche *et al.* 2004a).

The closing and opening strokes of the chewing cycle change in their rates depending on the food bolus properties and at what point in the chewing stage the individual is at (Hiiemae 2004). The lower jaw movement follows a particular pattern where the jaw moves in a series of orbits controlled by central pattern generators (CPG) (Lucas 2004) which most individuals tend to conform to. The CPG are areas of the brain stem that send out rhythmic impulses in response to intra-oral stimuli, which responds to the constantly changing bolus in the mouth (Lund 1991). As the gape of the mouth closes to move the mandibular molars to the maxilla molars, it goes through a fast closing stroke controlled by the masseter, temporalis and medial pterygoid muscles until they measure resistance with tooth-food-tooth contact. When tooth-food-tooth contact occurs, the slow close phase crushes the food initially then shears as the jaw moves in a lateral direction. The canines act to guide posterior teeth contact during lateral movements of the jaw (Bourdiol & Mioche 2000). The intercuspal phase is the slight separation of the jaws, when the tongue rises and rotates to reposition the food and lap into the pool of saliva under the tongue which coat the food particles. This is followed by the opening stage, which can sometimes occur at two different rates, but depends on food type (Bourdiol & Mioche 2000; Lucas 2004).

The pattern of chewing is constantly monitored by mechanoreceptors around the mouth and feedback during closing from the periodontal ligament (Guinard & Mazzucchelli 1996). The food bolus properties are analysed during the chewing cycles and muscle activity, jaw movement, saliva flow and tongue movement vary accordingly (Hiiemae *et al.* 1996; Lassauzay *et al.* 2000; Mioche 2004; Foster *et al.* 2006). During the slow close phase, if the food material is of brittle texture, particles will fracture and spill out on to the tongue and the cheek will hold larger particles onto the occlusal surface for the next chewing cycle, then the tongue pushes on more particles (Prinz & Lucas 1997; Lucas 2004). The rate of comminution depends on two factors, originally described by Epstein (1947) (cited by Prinz & Lucas 1997). The selection function, which is the chance of a particle being contacted by the teeth per chew, and the breakage function which is the degree of size reduction produced by the teeth when a particle breaks (Orchardson & Cadden 1998; Lucas *et al.* 2002). Investigations have found that large

particles are fractured more commonly than small particles (Yurkstas 1965; Lucas 1983; van der Glas *et al.* 1987; van den Braber *et al.* 2002).

#### 2.3.1.3 STAGE 2 TRANSPORT

The tongue is very important for intra-oral manipulation of food and the food bolus. During mastication, the tongue cycles to keep food on the occlusal surfaces and carries out sweeping movements to collect comminuted food from around the anterior teeth and hard palate to move to the anterior of the mouth for bolus formation (Hiiemae 2004).

Food bolus formation occurs in the oropharynx, after material has been selected to have met unknown swallow criteria (Thexton & Crompton 1998). The material is selected in the oral cavity between the tongue and hard palate, and then slips back to the oropharynx for a few seconds where more material merges together, termed stage 2 transport (Ardran & Kemp 1960; Hiiemae & Palmer 1999). It has been proven through VFG studies that the soft palate does not form a seal with the tongue, as used to be believed by other researchers, as material passes to the oropharynx continuously during chewing cycles (Hiiemae & Palmer 1999). At some point a swallow is initiated and the bolus is propelled through the pharynx into the oesophagus (Hiiemae & Palmer 1999).

#### 2.3.1.4 SWALLOW

Swallowing may be triggered by the forces of attraction between food particles and saliva. These forces can be very low, around 0.01 N (Prinz & Lucas 1997) but within the detection range of the tongue (Trulsson & Essick 1997; Engelen *et al.* 2002). This also has an important function in the sensory feedback control of mastication as the tongue touches the food bolus between each tooth-food-tooth contact chewing cycle (Mioche 2004). Section 2.4.2 reviews the theories of swallowing.

#### 2.3.1.5 CLEARANCE

Clearance is the final stage in the mastication process. It occurs after a meal has been consumed. The tongue is essential for this to search around the oral cavity and the vestibule of the mouth for stray particles of food to bring them together to form a bolus for swallowing (Hiiemae *et al.* 1996; Mioche *et al.* 2002b). This action produces irregular jaw movements as the tongue is reaching across the occlusal surfaces to reach and collect particles. The lips are closed during clearance and a negative pressure is

formed as the tongue forms a seal against the surfaces that it is clearing. The food fragments are brought onto the tongue for bolus formation using the same movement as in stage I transport (Thexton 1992; Hiiemae 2004), ready for the final swallow of the meal.

### 2.3.2 TRANSPORT MECHANISMS OF SWALLOWING

The momentum for a swallow is provided by the tongue. This action occurs to clear a swallowable bolus from the oropharynx, or all the food in the oral cavity (Hiiemae 2004). The lips will be sealed and the teeth sometimes in occlusion (Thexton 1992). The function of the soft palate is in swallowing where it moves upwards to close off the nasal passage. The hyoid bone moves upwards and forwards which opens the oesophagus; the tip of the tongue touches the hard palate just behind the incisors then contracts in a wave-like motion backwards (Thexton 1992). The bolus is then propelled into the pharynx (Hiiemae 2004). The swallowing action is patterned by CPG (Jean 2001), and once activated, continues without further sensory input (Thexton 1992).

During the involuntary part of the swallow, the bolus is detected in the pharynx as it contacts the oral mucosa. It is not clear exactly what is being detected, but the presence of taste buds in this region could contribute to monitoring the bolus progression to ensure that the bolus is moved into the oesophagus and not the trachea (Thexton, 1992). The most sensitive area in the oral cavity was found to be the pillars of fauces at the entrance to the pharynx. When this area is anaesthetised, a swallow is unable to occur, indicating that this area could function to detect a swallowable bolus and monitor the bolus (Thexton 1992). For ease of bolus transport, the bolus must be encapsulated by a thin film of saliva to ensure it travels as smoothly, and safely as possible to the stomach (Prinz & Lucas 1997). The larynx also moves upwards and becomes closed off so as not to receive the bolus, this action stretches the wall of the oesophagus which makes it easier to propel the bolus through.

### 2.3.3 FACTORS AFFECTING MASTICATION

Food texture is defined by Bourne (2002) as: “The textural properties of a food are that group of physical characteristics that arise from structural elements of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration,

and flow of the food under a force, and are measured objectively by functions of mass, time and distance". Food texture affects mastication, and during the breakdown of food the changes in food bolus properties result in changes in the mastication strategy (Hiiemae *et al.* 1996). Food texture has been found not to affect the chewing rate, although the rate is different between individuals, this is likely to be due to CPG rhythm (Kohyama *et al.* 2002; Foster *et al.* 2006). Contrary to this, some studies have found that the rate changes with texture in the first five chews (Brown *et al.* 1998a; Lassauzay *et al*. 2000; Foster *et al.* 2006).

Some physiological factors have been shown to affect mastication. Although individuals still exhibit differences in their chewing efficiency, number of chewing cycles and chewing duration, even when controlled for dentition, age, and food consumed. This means that there are many physiological differences between people that do not inversely affect mastication as they can produce the same particle size distributions in the food bolus if they consume the same food material (Peyron *et al.* 2004b).

Electromyography (EMG) has been widely used to monitor mastication (Boyar & Kilcast 1986; Brown 1994; Brown *et al*. 1994a, 1998a & b; Hiiemae *et al.* 1996; Agrawal *et al*. 1998; Hoebler *et al*. 1998 & 2000; Mioche & Martin 1998; Shiozawa & Yanagisawa 1999; Peyron *et al.* 2004a; van der Bilt & Fontijn-Tekamp 2004; Foster *et al*. 2006). EMG is the measurement of the electrical activity of muscles. It has been used to measure the activity of the masseter, anterior digastric, mylohyoid, and the anterior temporalis muscles (Brown 1994; Brown *et al*. 1998a, 1994a & b; Agrawal *et al.* 1998; Shiozawa & Yanigsawa 1999; Mioche *et al*. 2004b; Peyron *et al*. 2004a). From the EMG signals, bursts of activity can be used to calculate the chew cycles, the duration of the mastication sequence, total muscle work per chew, sum of muscle activity, maximum amplitude, activity duration and inter-activity duration (Kohyama & Mioche 2004).

#### 2.3.3.1 FOOD PROPERTIES

The food structure breakdown will differ with all foods between the initial bite and subsequent mastication with the anterior teeth (Hutchings & Lillford 1998). Between

each chewing cycle the food particles are sprayed with saliva, any moisture present/trapped in the foods will be released and the resulting bolus is pressed to different strengths against the hard palate resulting in a shearing effect that can change food properties (Heath 2002). A temperature change in the food occurring with mastication may result in a phase change of components (Heath 2002). This will change the structural properties of the foods and therefore the breakdown routes of the foods with each cycle (Lillford 1991). Information regarding mixtures of food properties in one bite has not been published, but is likely to result in different jaw movements when each chewing cycle comes into contact with a newly exposed texture in the food, with the bolus texture exhibiting its own properties (Hiiemae 2004).

**HARDNESS**

Softer foods or foods with two distinct textures such as an apple are often consumed using different jaw movements and a reduced number of chewing cycles to prepare into a swallowable bolus compared to harder foods (Jiffry 1981; Mioche *et al.* 2002b). Studies using VFG have shown that although some chewing cycles may occur, they are not always the main method of size reduction. A banana is often 'mushed' between the tongue and hard palate and then swallowed with a couple of mid-sequence swallows (Hiiemae *et al.* 1996; Mioche *et al.* 2002b). A piece of apple is often sculpted using the incisors to remove the peel, therefore the juice and pulp are swallowed mid-sequence after a couple of chewing cycles (Hiiemae 2004). Soft foods, which are not often studied, can be compressed by the tongue to incorporate saliva for bolus formation and this affects the amount of time that the bolus is in the oropharynx before swallowing (Hiiemae *et al.* 1996). Larger particles of a soft food are easier to swallow than a hard rigid food particle (Hoebler *et al.* 2000).

The elastic behaviour of food is important as it can predict the amount of work required to expose new surfaces in size reduction of foods (Lucas *et al.* 2004). The bite force and chewing force applied to foods during mastication is closely related to food hardness (Peyron & Mioche 1994). Properties of the initial food tend to carry through to the food bolus such as hardness. The duration of swallows and clearance may also be affected by these properties as they do exhibit different times with different food types (Shiozawa & Yanagisawa 1999; Mioche 2004).

Vertical jaw movements, sequence duration, number of chew cycles and total chewing work increase with an increase in food hardness (Foster *et al.* 2006; Peyron *et al.* 1996; Brown *et al*. 1998a; Lassauzay *et al.* 2000). Muscle work for the first five chews was summed and correlated significantly with sensory assessments for hardness. This is due to the main activity of mastication being particle size reduction and harder samples require more force to achieve this (Brown *et al.* 1998b).

Hard foods with high elastic modulus and a large surface area result in fewer chewing cycles (Bourdiol & Mioche 2000). Toffee behaves differently due to the temperature affect of the mouth. It is initially hard to chew then softens as it warms to mouth temperature (Bourdiol & Mioche 2000). Temperature effects many foods as some cool or warm to mouth temperature, causing their food properties and therefore bolus properties to change (Heath 2002).

**TOUGHNESS**

Toughness is the force that produces unit area of indentation in the food (Agrawal *et al.* 1998) and this can be related to bite forces that are required to deform a food. Agrawal *et al*. (1997) measured a range of cheese, nuts and raw vegetables for their values of toughness and Young's modulus, then the breakage function was calculated. Within the food groups, cheese and nuts had similar ranges of toughness, but different breakage functions, therefore the toughness value alone does not correlate with the breakage function. A correlation was found between $\sqrt{R}/\sqrt{E}$ (where R is toughness and E is Young's modulus) and the breakage function of the foods (Agrawal *et al.* 1997). Total muscle work correlates significantly with a $\sqrt{R}/\sqrt{E}$ mechanical index of foods (Agrawal *et al.* 1998). Vertical and lateral jaw movements were not found to relate to the food index, but correlated significantly with the closing angle of the jaw at tooth-food-tooth contact (Agrawal *et al.* 2000).

The particle size reduction of tough foods is quicker with a small mouthful (Lucas *et al.* 2004). Products of high toughness are first fractured into particles then absorb some saliva to form a paste-like bolus (Mioche *et al.* 2002a; Casas *et al.* 2003). These products are often subject to unilateral and bilateral chewing cycles, where food is on the occlusal surfaces on both sides of the mouth, to sufficiently comminute the product with saliva. The use of bilateral and unilateral chewing was also exhibited during

mastication of meat when observed with VFG, where manipulation of the bolus into a single mass occurred prior to swallowing (Mioche *et al.* 2002b).

During comminution of food the hardness or elastic modulus of solid foods should be reduced considerably, although harder foods produce a harder bolus (Shiozawa & Yanagisawa 1999). Carrots and brazil nuts have different values of toughness and this may be the reason for the difference in breakdown rates between the products, although other properties could be involved (Lucas & Luke 1984 & 1986).

#### ADHESIVENESS AND COHESIVENESS

Shiozawa & Yanagisawa (1999) found that the adhesiveness of a food affects the jaw movements late in the chewing sequence. Miyawaki *et al.* (2000) observed that when hard cohesive food is chewed, the chewing frequency becomes slower, even though the jaw movement is faster and a stronger shearing force is produced.

#### FLAVOUR

Whether food flavour release as saliva penetrates the bolus affects the length of chewing has not been investigated, but it is postulated that as food moves around the mouth and new surfaces of the bolus are exposed, or if the flavour is liked, it may be chewed for a longer period of time (Heath 2002).

### 2.3.3.2 PHYSIOLOGICAL FACTORS

A range of food textures in the diet is desired by most people and their mouth needs to be able to detect these differences before sufficient mastication of foods can be achieved (Heath 2002). There are essential physiological requirements for mastication to be able to occur, and these include teeth, specifically posterior teeth (natural or artificial), tongue, lips, hard palate, cheeks, and saliva production.

#### TEETH

Texture appreciation changes through life, mainly through changing dentition. Children will accept some textures and not others, and only when they have their full set of adult teeth (not including third molars) in their teenage years do they show preferences for a wide range of food textures and have efficient oral management of them (Szcesniak & Kleyn 1963: Heath 2002; Bourne 2004).

The state of dentition is of great importance to mastication, the occlusal surfaces is where size reduction of foods occurs except in very soft foods which can use the hard palate (Hiiemae *et al.* 1996). Particle size reduction is more efficient where there is broad occlusal contact area as opposed to a few cusp contact points (Wilding 1993). Bourdiol & Mioche (2000) found there to be high correlation between particle size and distance between occlusal contacts of between 0.2 and 0.45 mm. There is significant correlation between occlusal surface area and functional surface area of posterior teeth, and a tendency for fewer chewing cycles with larger functional surface area. Functional surface area can be used to identify what side of the mouth is used most for chewing (Bourdiol & Mioche 2000). Values of mean duration of tooth contact vary between 0.02 and 0.37 s, but a constant pattern can be observed in individuals (Neill 1967).

Patterns of mastication may alter to unilateral cycles for different food textures, as a result of missing teeth, joint pain or other oral health issues (Bourdiol & Mioche 2000). Unilateral chewing cycles are not normally exhibited and even if instructed for test purposes to chew on a particular side of the mouth, it automatically changes during cycles to the other side, and sometimes bilateral chewing cycles are seen (Hiiemae 2004). Good oral health is also important as periodontal disease can reduce the sensory feedback capability required for control of chewing and could result in tooth-tooth contact, which may damage teeth (Jenkins 1978; Lucas 2004). Loss of dentition is not necessarily a factor of age, but oral health issues during a persons lifetime (Mioche 2004).

### MUSCLES

Control of lips, cheeks and tongue are very important for stage I transport, maintaining food on occlusal surfaces, stage II transport and the action of swallowing and clearance (Thexton 1992; Hiiemae *et al.* 1996). Muscle activity has been shown to reduce with age and this can also result in lower bite and chewing forces which affect the particle size distribution of the food bolus (Kohyama *et al.* 2002). Reduced muscle activity results in a weaker bite force (Helkimo *et al.* 1977) and slower jaw closing in chew cycles (Kohyama & Mioche 2004). Weaker total muscle activity was partly compensated by increasing the number of chew cycles (Kohyama *et al*. 2002; Kohyama

& Mioche 2004; Peyron *et al.* 2004a & b). Peyron *et al.* (2004a) found there to be an increase of 0.3 chewing cycles per sequence per year increase in age.

The tongue is essential for efficient mastication, food bolus formation and swallowing (Hiiemae 2004). Any impairment, loss of neurological control, or reduction in muscular strength may cause issues with foods that are highly adhesive as the tongue has been shown to manipulate the foods to coat them in saliva and to manoeuvre the bolus material round the mouth (Shiozawa & Yanagisawa 1999). In Japan, a New Year tradition is to eat an adhesive, sticky cake made from glutinous rice, called Mochi, and often elderly die by choking whilst trying to consume this product due to loss of muscular control (Nishinari 2004). It is important with the aging population growth, that more is understood about how food is managed in the mouth and how food properties affect this. Foods could be designed for this age group so that they can still enjoy eating a wide range of food textures safely (Mioche 2004; Nishinari 2004).

**INGESTION QUANTITY**

When the quantity of initial food is varied, an increase in food weight results in an increase in the number of chews taken prior to swallowing, mean vertical jaw movement, and mean and peak muscle activity (Lucas *et al.* 1986; Diaztay *et al.* 1991; Miyawaki *et al.* 2001; Bhatka *et al.* 2004). The muscle activity on the chewing side of the mouth is higher than on the non-chewing side and changed proportionately in response to food size (Miyawaki *et al.* 2001). An increase in the ratio of activity in the anterior temporal muscle was similar to the increase in ratio between the heights of food ingested. Therefore the angle of jaw closing reflects the height of the bolus (Miyawaki *et al.* 2001).

Vertical jaw displacement during comminution of food was affected by the selected volume of food broken by teeth in one chew stroke and not total volume of food in the mouth (Lucas *et al.* 1986). No changes in cycle duration were observed during chewing of different ingestion quantities of peanuts (Lucas *et al.* 1986; Diaztay *et al.* 1991), but cycle duration increased with a larger food sample for gummy jellies and a synthetic test food (Miyawaki *et al.* 2001; Bhatka *et al.* 2004). When two portions of the same weight, but different particle size were consumed no change could be seen in number of chews until swallow, vertical jaw movement or cycle duration (Lucas *et al.* 1986).

Food sample weight has a greater affect on mastication than initial particle size of the food sample (Diaztay *et al.* 1991). Food length was found to be more consistent than weight and volume for a group of subjects taking a single bite from six types of manufactured food bars. This suggests that the length and shape of food samples in mastication studies be considered carefully and that investigating samples controlled by portion weight is likely to be the least representative of natural feeding behaviour (Hutchings *et al.* 2009).

## 2.4 FOOD BOLUS

The food bolus is the product of mastication. The bolus is composed of particles produced during the mastication process incorporated with saliva and any expelled moisture from the food (Hutchings & Lillford 1988; Lillford 1991). The result is a cohesive mixture of liquid coated particles that stick together (Prinz & Lucas 1997; Peyron *et al*. 2004b).

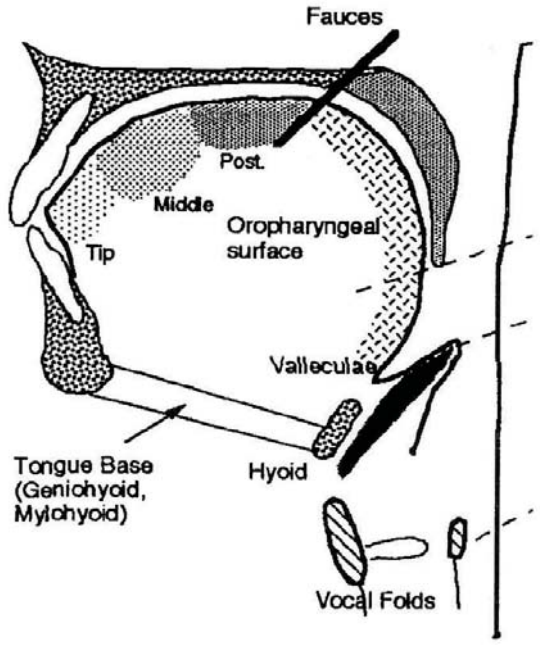



**Figure 2.7** **The features of the oral cavity used in bolus accumulation and swallowing (adapted from Hiiemae & Palmer 1999)**

The cohesive food bolus is very important to enable safe swallowing. There is a risk of fatal accidents occurring if food particles enter the trachea (Smith 1992). The food

bolus is a coherent mass which keeps food particles retained as is passes through the pharynx into the oesophagus thus reducing the risk of inhalation (Prinz & Lucas 1997). Forming a food bolus from a mouthful of ingested food is an efficient way to clear the mouth of food particles ready to ingest the next mouthful (Lillford 1991).

Swallowing also needs to be comfortable so the food particles that are produced from mastication should be small and refined enough to ensure this (Yurkstas 1951). Yurkstas (1951) and Lucas & Luke (1986) propose that deformability of the bolus could be important as hard foods will not conform easily to the shape of the pharyngeal passageway through which it is propelled. Hard foods will require smaller particles than soft foods for a comfortable swallow (Lucas & Luke 1986). Prinz & Lucas (1995) suggest that larger particles can be swallowed if they are easily deformed but will be affected by the person's tolerance of discomfort. As dentition generally depreciates through life and therefore chewing efficiency is reduced, individuals do not tend to compensate by a longer period of mastication, but adapt to swallowing larger particles of food (Yurkstas 1951; Jiffry 1983; Mioche *et al.* 2004b; Mishellany-Dutour *et al.* 2008). Lucas & Luke (1986) found that denture wearers did compensate when chewing carrots by chewing for a longer period of time but still swallowed a larger particle size than dentate individuals.

## 2.4.1 FORMATION OF THE FOOD BOLUS

The food bolus is formed from food particles resulting from mastication, fluid from saliva and expelled moisture from food. Formation occurs in the mouth and involves the hard and soft palates, tongue and oropharynx (pharyngeal surface of the tongue). The bolus formation occurs during stage II transport (Hiiemae *et al.* 1996; Hiiemae & Palmer 1999; Mioche *et al.* 2002b; Hiiemae 2004) with the tongue packing food particles together by applying pressure against the hard palate, squeezing saliva through the bolus, then the tongue coats the bolus in saliva (Prinz & Lucas 1997; Hiiemae & Palmer 1999; Shiozawa & Yanagisawa 1999; Mioche *et al.* 2003; Hiiemae 2004).

### 2.4.1.1 FOOD PARTICLES OF THE FOOD BOLUS

Food particles have an affinity with other food fragments in the mouth and the oral mucosa (Lucas *et al.* 2004). Studies by Malone *et al.* (2003) investigated food

hydrocolloid and emulsion interactions with oral mucosa and perceived sensory characteristics of the products. They suggest that the average lubrication level of particles to be perceived as "slippery" is a coating of 1.5 – 2.5 μm. The friction coefficient in emulsions was reduced as oil concentration increased, this is related to the lubrication of the tongue and hard palate as they touch during oral processing of foods (Malone *et al.* 2003). The sensation of astringency is the precipitation of salivary proteins with molecules such as polyphenols and tannins (Prinz & Lucas 2000; Malone *et al.* 2003). Interactions within the bolus depend on the cohesion of the food particles to each other, or their adhesion to the oral mucosa, and the surface tension and viscosity of oral fluid (Lucas *et al.* 2004). The tongue needs to move food particles and help coat them in saliva, where the mucins help decrease friction between oral mucosa and other food particles in order for them to start forming a cohesive bolus (Prinz & Lucas 1997; Pedersen *et al*. 2002). The movement of the tongue is important to provide shear forces that mix the bolus. Salivary protein interactions between the food and the thin film of saliva coating teeth and oral mucosa are enhanced if the food is not moved around sufficiently to lubricate the food particles to encourage them to cohere together (Heath 2002).

#### 2.4.1.2 JAW AND TONGUE MOVEMENT IN BOLUS FORMATION

Videofluorography (VFG) and EMG have been used to observe chewing cycles and jaw movement in many studies and have also been used to monitor food texture (Ardran & Kemp 1960; Boyar & Kilcast 1986; Brown *et al.* 1986, 1998a & b, 1994a & b; Hiiemae & Palmer 1999; Shiozawa & Yanagisawa 1999; Mioche *et al*. 2002b). The muscle activity of the mylohyoid in late stages of chewing reflected the adhesiveness of the food bolus and the manipulation of the bolus by the tongue as it is compressed against the hard palate (Shiozawa & Yanagisawa 1999). A W-shaped jaw movement is associated with tongue movements during processing just prior to stage II transport (Hiiemae *et al.* 1996; Heath 2002; Mioche *et al.* 2002b). These jaw and tongue movements occur to manipulate the food bolus to expose more surface area during subsequent chewing cycles, or move the bolus material to the middle of the tongue to press against the hard palate (Heath 2002; Mioche *et al.* 2002b). It is also postulated that the movements of the tongue prior to swallowing are to lubricate the outside of the

bolus to encapsulate it and aid propulsion through the oesophagus (Shiozawa & Yanagisawa 1999; Lucas *et al*. 2002).

#### 2.4.1.3 TYPES OF BOLUS FORMATION

Hutchings & Lillford (1988) proposed that at least three different types of bolus formation occur depending on the foods initial properties and breakdown properties. The subdivision model is where foods are broken down by the teeth into small particles, probably in the low millimetre range then swallowed (Lillford 1991). The rolled bolus model for meats, requires that meat fibres are weakly comminuted (Mioche *et al.* 2003), then rolled into a ball for swallowing. The shatter model requires breakage of low forces, then saliva incorporation to form a single unit (Mioche *et al.* 2002a) which is swallowed as a ball also. Optimum cohesion of the bolus is between 15 – 30 chew cycles, depending on food mechanical properties and breakdown rates (Lucas *et al.* 2002). Bolus shape is an important feature of the mastication process. If the shape is not suitable for swallowing the masticatory process is inefficient (Liedberg & Owall 1995).

#### 2.4.1.4 SWALLOWING

A mouthful of food does not always form one swallowable bolus (Hiiemae & Palmer 1999; Okada *et al.* 2007). Small quantities of food bolus may move to the anterior of the tongue as it has met the criteria to be swallowed. Bolus material accumulates on the oropharyngeal surface for up to ten seconds, while chewing continues on the rest of the food, more material will then move to the waiting swallowable bolus (Hiiemae & Palmer 1999). On the oropharyngeal surface, the consistency of the bolus becomes mouldable by the saliva that has penetrated it and starts to set. This improves the cohesiveness of the bolus for a safe swallow (Heath 2002). When the bolus is ready it is swallowed, with the trigger yet unknown (Thexton & Crompton 1998; Peyron *et al.* 2004b), although sensory studies by Jack *et al.* (1994) showed that cheddar cheese samples were always masticated to a defined texture before a swallow occurred. Multiple swallows may occur on one mouthful of food (Mioche *et al.* 2002b; Hiiemae 2004; Okada *et al.* 2007). Some parts of the bolus are kept in the cheek while harder particles are broken down on the occlusal surface then, either pushed onto the occlusal surfaces, or pushed to the tongue to be swallowed with the rest of the bolus with no

further processing (Heath 2002). If swallowing is delayed during mastication, saliva floods the bolus and separates the particles (Lillford 1991; Prinz & Lucas 1997).

Most investigations have been carried out on foods that fragment into particles, such as carrots, peanuts and dental material (Lucas & Luke 1983; Olthoff *et al*. 1984; Lucas *et al.* 1986), some of which do not form a cohesive bolus, for example, carrots (Prinz & Lucas 1997). Models that have been developed for these types of product will not apply to non-brittle products, e.g. banana, meat, sandwich paste (Mioche *et al.* 2003). In the models that have been developed, properties of the food are assumed to remain constant (Prinz & Lucas 1997), which is not a true reflection of the changes occurring during processing in the mouth (Lillford 1991). When natural foods are used in investigations, they can be very complicated to standardise but they give valuable information about how the particular food is managed in the mouth (Liedberg & Owall 1995).

## 2.4.2 THEORIES OF SWALLOWING

There have been a few prominent theories of the initiation of a swallow and they have been studied over the last sixty years (Dahlberg 1942; Yurkstas 1965) with new theories evolving in the last thirty years.

### 2.4.2.1 PARTICLE SIZE THRESHOLD

The particle size threshold model has been prominent due to the fact that size reduction is one of the main purposes of mastication (Yurkstas 1951). A particle size threshold in the low millimetre range is generally the size range of the average swallowed particles found at the end of human mastication (Yurkstas & Manly 1950; Lillford 1991). The tip of the tongue and anterior hard palate mucosa can detect particles between 1 – 2 mm (Ringel & Ewanowski 1965; Laine & Siirila 1971). Particles smaller than this would only be detected if they were sharp or rigid, therefore there is no advantage in continuing to chew such particles. As the initiation of a swallow, this theory has been disproved, as a smaller ingestion quantity of food is always swallowed at a smaller particle size distribution than a larger one (Jiffry & Molligoda 1983; Lucas & Luke 1984) and different food types are swallowed at different particle sizes, for example, nuts and vegetables (Peyron *et al.* 2004b).

### 2.4.2.2 TIME AND VOLUME TRIGGER

Hiiemae *et al.* (1978) hypothesised that a time/volume trigger occurred in the oropharynx. Either a set volume of bolus forms to trigger a swallow; or a bolus is accumulated for a set length of time in the oropharynx then a swallow is triggered. This was due to the finding that small quantities of bolus slip to the back of the tongue and sometimes stay for several seconds while more bolus material is accumulated, in nonhuman mammals (Hiiemae *et al.* 1978). This has been disproved by observing in humans that after several seconds in the oropharynx the bolus will be swallowed regardless of size, and that time varied with food type (Hiiemae 2004).

### 2.4.2.3 DUAL THRESHOLD MODEL

Hutchings & Lillford (1998) developed the particle size theory further by suggesting that dual threshold criteria are met before swallowing can be initiated. Either the food structure must have been reduced below a certain level, or lubrication must be above a certain level. In both cases there must be the factor of time to develop and detect both parameters in the mouth (Hutchings & Lillford 1988; Lillford 1991). The food breakdown pathway is individual to the consumer, the eating situation and dependent on initial food properties and how they change through comminution (Hutchings & Lillford 1998).

Prinz & Lucas (1995) set up an investigation to test the dual threshold theory by varying the particle size of brazil-nut pieces and the solid/liquid volume ratio with plain yoghurt independently, so that they could establish the mixture that can be swallowed without chewing. They found that particles of 2.0 mm or smaller, at concentrations of 20% or less, were wetted enough to be swallowed immediately. If the concentration is above 20% the mixture must be comminuted to wet the food particle surfaces (Prinz & Lucas 1995). The concentration of a swallowable bolus of carrot particles in saliva is a lot higher than the limits found in this study (Prinz & Lucas 1997). Prinz & Lucas (1995) concluded that particle size threshold is food dependent as the 2.0 mm limit found was smaller than in previous studies. The dual threshold theory has not been successfully proven but it is very important in highlighting considerations for a different view on the food breakdown path, intra-oral manipulation and texture detection.

#### 2.4.2.4 COHESION MODEL

The cohesion/adhesion model was put forward by Prinz & Lucas (1997). They suggested that the end point of mastication is marked by a peak in the cohesive force that binds food particles together into a bolus. An analytical program was developed to model the binding action of spherical particles. Adhesion is the initial force that sticks particles to the oral cavity by surface tension. The second force, known as cohesion, that acts within the bolus and holds the particles together is the "gluing" of particles as the saliva sets (Roberts 1977, cited by Prinz & Lucas 1997). The program was applied using results from previous studies on brazil-nuts and raw carrots, it was assumed that the only difference between particle size reduction rates depended on the breakage function of the different food types (Prinz & Lucas 1997).

The model shows that adhesive force is primarily acting on the particles, then cohesive force rises rapidly and peaks at about 20-25 chews for both food types. Further mastication results in a decline in cohesive force (Prinz & Lucas 1997). At peak cohesion, there is approximately a 9 – 25 fold increase in the surface area over that at ingestion (Prinz & Lucas 1997). If there was an increase in taste components of the products to increase gustatory saliva production or juice expressed from foods, this should result in the bolus becoming flooded earlier, therefore requiring fewer chewing cycles; or if stabilisers are added to the initial food matrix, this should increase the saliva viscosity and help the cohesive forces to increase and form a bolus earlier (Prinz & Lucas 1997).

Lucas and Luke (1986) suggest that the tongue and hard palate may sense the point when the bolus forms a food paste which has flowing characteristics. When cohesion between food particles in the bolus starts to reduce, the detection point between tongue and hard palate may sense this and trigger a swallow (Prinz & Lucas 1997; Peyron *et al*. 2004b). Bolus cohesion is important to hold food particles together and must be stronger than adhesion to oral mucosa, to trigger a safe swallow (Prinz & Lucas 1997).

Peyron *et al.* (2011) investigated cereal bolus properties using texture profile analysis (TPA) and particle size distribution, comparing results to those provided by a sensory panel applying the method of temporal dominance analysis. The particle d50, sensory

and TPA hardness decreased, whilst measures of sensory stickiness and TPA adhesiveness increased through the chewing sequence. These parameters changed at different rates, and seemed to reach their thresholds at different times in the chewing sequence, with changes in stickiness and adhesiveness present near the swallowing point which is indicative of the achievement of optimum cohesive forces in the bolus required for swallow.

## 2.4.3 FACTORS AFFECTING THE FOOD BOLUS AND ITS FORMATION

Certain food properties have been found to significantly affect bolus formation more than physiological factors (Engelen *et al.* 2005). Although, there are a wide range of food properties and physiological factors that can effect food bolus formation and the properties of the food bolus.

### 2.4.3.1 FOOD PROPERTIES

Food properties that have been investigated and found to effect bolus formation include initial toughness, dryness, adhesiveness, particle size, water content and hardness. Other structure components that may have an effect on bolus formation are the presence of cells with moisture, food that can absorb water or saliva, and fibrous foods (Lucas & Luke 1984; Mioche *et al.* 2003). These types of structures can also affect the food properties, for example, hardness, toughness, moisture content. The breakdown path of food is characteristic to the food type (Peyron *et al.* 2004b; Mishellany *et al.* 2006; Jalabert-Malbos *et al.* 2007; Lenfent *et al*. 2009).

#### MOISTURE CONTENT

Dry food products require more chewing cycles before a bolus suitable for swallowing is formed (Yurkstas 1965; Hiiemae *et al.* 1996; Engelen *et al.* 2005) and they stimulate an increase in saliva flow to aid cohesion of the forming bolus (Mioche 2004). Engelen *et al.* (2005) investigated the effect of modifying one initial food property in a range of foods on their bolus formation with 87 healthy adult subjects with natural dentition (aged 42 ± 12 yrs). Breakfast cake, melba toast, bread and toast were modified by the addition of 0.8g of butter, thereby enhancing lubrication. They succeeded in significantly reducing the number of chewing cycles required to form a swallowable

bolus for the breakfast cake, melba toast and toast. The bread did not reduce in chewing cycles as this has significantly higher moisture content than the other products. The results of butter addition substantiate findings that saliva flow rate is significantly and negatively correlated with the number of chew cycles required for the breakfast cake, melba toast and toast as these foods need a longer time in the mouth to enable formation of a cohesive bolus (Engelen *et al.* 2005).

Peyron *et al.* (2004b) investigated how two different groups of foods affected the particle size distribution of the boluses produced at the end of mastication. The foods investigated were dry nuts of 9% water content and raw vegetables of 87% water. Ten subjects (aged 36.7 ± 9.5yrs) with healthy dentition were selected to chew the foods until just prior to swallowing the six individual portions of the following foods: five peanuts, four pistachios, three almonds (2.5 – 4 g), and cylindrical samples (1 cm height, 2 cm diameter) of cauliflower, radish, carrot (3.5 – 4 g), and then expectorate for later analysis. The subjects were timed for the duration of the chewing sequence and the number of chew cycles counted, this enabled the calculation of masticatory frequency. The subjects were also asked to chew for half the number of cycles and then quarter the number of cycles and expectorate the sample for analysis. Particle size distributions were measured using sieve analysis and laser diffraction. Distribution of particle size across sieves was similar for all subjects, the vegetables showed similar particle size distributions with the modal size of 2.5 mm. The peanuts showed a peak in the size distribution at 1.4 mm. The moisture content of the foods was presented as being the cause of the differences in particle size distribution between the two groups of foods.

**TOUGHNESS**

The affect of toughness has been described in a few very different foods. Mioche *et al* (2002a & 2003) has studied the effect of meat toughness on bolus properties. Two meat samples from the same muscle were aged and cooked differently to ensure different values of toughness (124 N and 83 N). Twenty five subjects (aged 25 – 30 yrs, all with at least 8 pairs of natural postcanine teeth) were asked to chew the meats individually and expectorate at the point just prior to swallowing. The boluses were characterised using an Instron machine with a double blade shear cell at a displacement rate of 60 mm/min and the shear stress calculated. The significantly different results for mean

shear stress of the meat boluses were 39.486 ± 2.11 N and 32.257 ± 2.28 N respectively. If an initial property of a meat sample is high toughness this has been shown to result in a bolus being produced of high toughness (Mioche *et al.* 2002a & 2003). For tougher meat, an increase in the number of chewing cycles is required to breakdown fibres to allow enough saliva to incorporate into the bolus so that a bolus ready for swallowing is formed. Meat toughness has also been shown to affect the length of time the bolus spends in the oropharynx before swallowing (Hiiemae & Palmer 1999: Mioche *et al.* 2002a & 2003).

### HARDNESS

Hard foods such as cookie, have also been shown to affect the duration of the swallowable bolus in the oropharynx before swallowing (Hiiemae *et al.* 1996). Shiozawa & Yanagisawa (1999) tested three foods: gummy candy, peanuts and rice cake and they were measured using a creep meter double bite test to obtain a value for food hardness. 5 g of each food was given individually to each subject for them to chew and expectorate at the point when they felt ready to swallow. The food boluses were held separately in a glass ring at 37$^{o}$C then measured for hardness value. Peanuts were the hardest of the foods tested and the resulting bolus was the hardest. Although the hardness is significantly reduced with mastication, the ratio of initial food harness is also present in the resulting boluses (Shiozawa & Yanagisawa 1999).

### ADHESIVENESS AND COHESIVENESS

The adhesiveness of the initial food product results in greater forces applied by the tongue to manipulate the bolus into a swallowable bolus than for products of lower adhesiveness and the bolus also has a higher adhesiveness in relation to other food boluses as measured using a creep meter, double bite test by Shiozawa & Yanagisawa (1999). EMG recording of the mylohyoid muscle showed a burst in activity in the late stage of chewing, and it was significantly different between foods and correlated with the foods significantly different values of adhesiveness (Shiozawa & Yanagisawa 1999). The adhesiveness of the product will lead to particles adhering to the mouth and the cohesiveness of the product to other food particles which increases the amount of work that the tongue needs to do to form a swallowable bolus against high friction (Lucas *et al.* 2004).

#### FOOD PARTICLE SIZE

The particle size distribution of the initial mouthful of food has been shown to affect the particle size distribution of the bolus (Yurkstas 1951; van der Glas *et al.* 1987; van den Braber *et al.* 2002). Yurkstas (1951) changed conditions that might affect the ability to masticate food, a 9 g sample of a test food was divided into different portions: six 1.5 g pieces, three 3 g pieces, two 4.5 g pieces and one 9 g piece. Ten subjects were recruited to chew each portion for 30, 60 and 120 chew cycles and expectorate the sample for sieve analysis. Masticatory efficiency was calculated from the percentage of food passing through a particular sieve size (10 mesh for easily broken down foods, and size 5 mesh for difficult foods). In this study the portion composed of six 1.5 g pieces of test food had the highest average masticatory efficiency compared to the other portions, with the single portion of 9 g having the lowest mean masticatory efficiency. The oral cavity is more efficient when smaller particles are introduced into it. The ten subjects were then asked to chew the same portion sizes until they felt ready to swallow then expectorate the sample for particle size analysis and the swallow threshold calculated. An increase in the initial portion size resulted in a decrease in the number of chew cycles per gram of food, and resulted in larger food particles being swallowed (Yurkstas 1965). To identify whether there is any affect of abrasives on intra-oral processing, Prinz (2004) coated two coloured pieces of gum evenly in pumice of 250 μm diameter, and found that after 10 strokes it was evident that there was a reduction in mixing of the two colours of gum, and this was a significant difference compared to a non-abrasive gum. Prinz (2004) suggests that the reason for this is to reduce wear of the teeth.

### 2.4.3.2 ORAL PHYSIOLOGICAL FACTORS

There are many physiological factors that may affect bolus formation such as dentition, tongue muscle control, salivation and the ability to detect the food bolus in the mouth for swallow initiation. All these factors will be different between individuals and may result in the soft tissues of the pharynx and oesophagus adjusting to swallow a bolus regardless of the discomfort the individual may have to sustain if they want to consume a range of food products (Yurkstas 1951).

#### INGESTION QUANTITY

Food ingestion quantity is the amount of food a person chooses to consume per mouthful or per bite. In a study of a young group of four subjects aged 8 – 11 years old

with mixed dentition and an older group aged 50 – 60 yrs with full dentures, Jiffry (1983) found that older subjects who wear dentures ingested a larger quantity of hard-baked soya beans than younger subjects with mixed dentition. It was suggested that this may be due to slightly larger oral cavity volume due to maturity (Jiffry & Molligoda 1983). The subjects were asked to chew the portions until they were ready to swallow and expectorate the sample for particle size analysis. The number of chewing cycles used per mouthful of food was counted by the investigator and were found not to increase with age. Therefore the mean particle size produced at the end of mastication was a mode value of around 4 mm which was significantly larger than younger subjects with mixed dentition who resulted in a mode value of around 2 mm (Jiffry 1983; Jiffry & Molligoda 1983; Engelen *et al*. 2005). When the younger subjects ingested double their chosen quantity of food, the same quantity as the older subjects chose, they produced a swallowable bolus with a similar particle size distribution to the older subjects, with more than 60% of particles above 1.5 mm.

Lucas & Luke (1984) asked six subjects to chew different weights of peanuts for a specific number of chews then expectorate the sample for analysis of their particle size distributions. The number of chews varied from 1 to 20 with the 1 g sample size, and 10 to 100 cycles for the 12 g sample. A total number of five different chew cycles for each ingestion size (1, 2, 5, 8 and 12 g) was completed by each subject. The number of chews required to swallow each mouthful were also counted, then this was repeated with the subject expectorating the sample just prior to swallowing for analysis. The smaller mouthful exhibited a faster rate of breakdown than the larger mouthful, and the number of chews to prepare the food sample for swallowing increased with the larger mouthful. The smallest particle sizes were swallowed with the smaller ingestion quantity. Lucas & Luke (1984) also tested whether there was any correlation between freely chosen ingestion quantity and oral cavity volume and found that there was no significance.

Ingestion quantity was also investigated by Fontijn-Tekamp *et al* (2004), using three natural foods: unsalted peanuts (6, 9 and 12 g), carrots and cheese in cubes (3, 6 and 9 g). Masticatory efficiency was established with 87 healthy subjects with good natural dentition with the number of occlusal units (corresponding pairs of molars and pre-

molars) noted. 3.7 g of a silicone test food was used to measure masticatory efficiency and the subjects were asked to chew until just prior to swallowing and expectorate, and then on a second sample for 15 chews only. Particles were sieved and median particle size determined, this also quantified the swallow threshold. The subjects ate and swallowed the natural foods as normal while the researcher counted the number of chew cycles required until a swallow. The number of chew cycles increased with ingestion sample size. From the swallow threshold it was found that more cycles before a swallow resulted in finer particles being produced and subjects swallowed larger particles if their masticatory performance was poorer (Fontijn-Tekamp *et al.* 2004).

**AGE**

A direct result of aging is the reduction in muscular activity, which results in reduced bite force and chewing forces and therefore lower chewing efficiency (Mioche 2004; Engelen *et al.* 2005). Elderly use less force per chew but require more chewing cycles to form a swallowable bolus (Kohyama *et al.* 2002). In young subjects there was a difference in muscle work dependent on meat texture. This was not detected in elderly subjects and increasing the number of chewing cycles would help to compensate for this (Mioche 2004).

Mioche *et al.* (2004a) recruited 25 healthy subjects aged 25 – 30 yrs, and 20 healthy elderly aged 68 – 73 yrs. All had at least six pairs of natural postcanine teeth. Two meat samples were prepared to have different textures of toughness, and the subjects were asked to chew the samples until just prior to swallow and expectorate the bolus. Duration of sequence, number of cycles, muscle activity and total muscle work was interpreted from EMG of the masseter and anterior temporalis. Bite force was also monitored of some subjects from both groups during chewing with a mini force transducer on left and right second molars. While chewing subsequent meat samples the subject was stopped after seven seconds chewing duration. The older subjects used a significant increase in the number of chews to form a bolus regardless of meat texture, but despite this, the bolus was still less comminuted compared to the younger subjects, as measured by textural analysis. No texture effect was evident in older subjects for muscle activity or the amount of saliva present in the bolus (Mioche *et al.* 2004a).

#### SALIVATION

The incorporation of saliva into the bolus is important for bolus formation. This can be product dependent. Some people are unable to produce saliva at all due to illness, effect of medication or medical treatment; or can only produce reduced quantities of saliva. This often occurs with age. During the chewing of meat samples, it was found that elderly incorporated less saliva than the young subjects (Mioche 2004). Some people can swallow a meat bolus with only a small quantity of saliva absorbed, and others carry out more chewing cycles and incorporate more saliva (Mioche *et al.* 2003).

Yurkstas (1965) simulated a dry mouth and a moist mouth to monitor the effect this had on the mastication of peanuts. Atropine sulphate was given to the subjects to simulate a semi-dry mouth and one hour later they carried out the test on a 3 g portion of peanuts. They were asked to chew the peanuts while the researcher counted the number of chew cycles, and then expectorate the sample just prior to swallowing for sieve analysis. Masticatory performance and swallow threshold were calculated. The test was repeated with a 3 g sample of peanuts ingested under normal mouth conditions, then a moist mouth was simulated by adding 5 ml of water with each sample of peanuts. Yurkstas (1965) found that masticatory performance was best under normal conditions, but swallow threshold and number of chew cycles decreased with increased moisture content. The increase in moisture content resulted in larger food particles being swallowed.

## 2.4.4 TECHNIQUES FOR CHARACTERISING THE BOLUS

The food bolus is most commonly characterised by its particle size distribution. It has also been analysed for particular textural properties, bolus shape and extent of lubrication. The results are applied to understanding what properties of the food bolus are important for initiating a swallow.

### 2.4.4.1 PARTICLE SIZE ANALYSIS

The percentage of particles in a food bolus that pass a particular mesh size is used as a measure of masticatory efficiency (Dahlberg 1942; Yurkstas & Manly 1950; Yurkstas 1951; Jiffry 1983 & 1987; Jiffry & Molligoda 1983; Lucas & Luke 1983 & 1984 & 1986; van der Glas *et al.* 1987; Kapur *et al.* 1990; van der Bilt *et al*. 1993; van den

Braber *et al*. 2002; Fontijn-Tekamp *et al*. 2004; van der Bilt & Fontijn-Tekamp 2004). Sometimes the bolus is collected for analysis after a particular number of chew cycles (Fontijn-Tekamp *et al.* 2004; Hutchings *et al.* 2012), or a range of chew cycles, e.g. from 10 to 100 (Lucas and Luke 1984). It is also used to characterise the bolus in relation to its swallow threshold by asking the subject to chew the food until just prior to swallowing (Yurkstas 1965; Jiffry 1981; Lucas & Luke 1984; Hoebler *et al*. 2000; Peyron *et al*. 2004b; Mishellany *et al.* 2006; Jalabert-Malbos *et al.* 2007; Hutchings *et al*. 2011).

#### SINGLE SIEVE METHOD

The single sieve method has predominantly been used to calculate masticatory efficiency. Chauncey *et al.* (1984) asked subjects to chew a 3 g portion of raw carrot, habitually until just prior to swallowing then expectorate into a cup. The mouth was then rinsed twice and this expectorated into the cup also. The number of chew cycles and time of sequence was noted by the researcher. The chewed carrot and rinsings was washed through a 4 mm sieve onto a finer sieve. Particles from each sieve were then transferred to a 15 ml graduated centrifuge tube and water added to bring up to the 10 ml level. The tubes were centrifuged for 3 minutes at 1500 rpm. The swallow threshold index, a measure of masticatory efficiency, was measured by dividing the volume of test food passing through the 4 mm sieve by the total volume of recovered food, then multiplying by 100 to obtain the test index value.

At present we do not know the optimal size of food particles to be swallowed, so there are no criteria for selection of the sieve aperture size, which is critical to the value of the results (van der Bilt & Fontijn-Tekamp 2004).

A comparison of single and multiple sieve methods was carried out by van der Bilt & Fontijn-Tekamp (2004). 176 dentate subjects were asked to chew for 15 strokes on a 3 $cm^3$ portion of silicone test food and expectorate into a beaker. The particles were then washed, dried then sieved by two different methods. To determine how sieve aperture affected the results of the single sieve method, three sizes (1, 2 and 4 mm) were selected to carry out the analysis. The percentage of particles that passed through was termed the mastication performance index. Samples of the chewed test food were sieved through a stack of 12 sieves, ranging from 8 – 0.5 mm aperture. The distribution of

particle sizes by weight was used to calculate the cumulative frequency curve. The results of the single sieve method showed that for this test food only 9% of particles passed the 1 mm aperture, 24% through the 2 mm sieve and 65% through the 4 mm sieve. From the multiple sieve method it was calculated that the median particle size produced by the subjects was 3.1 mm. If the sieve size employed in the single method moves too far away from the median particle size of the chewed food the reliability of the method is reduced. The selected sieve size needs to be suitable for all subjects in the investigation to reliably calculate the mastication performance index. If more detailed information about the sample is required the multiple sieve method should be used.

**MULTIPLE SIEVE METHOD**

The multiple sieve method was employed by Jiffry (1981) for the analysis of the particle size distribution of different hard-baked pulses to identify a test food for further investigations. Subjects were asked to choose a mouthful of food sample, this was then weighed, chewed until just ready to swallow then expectorated. The number of chewing cycles and time of sequence was noted. The mouth was rinsed with 150-200 ml of water and rinsings added to the bolus. To reduce the sticky affect of the saliva, 100 ml of calcium hydroxide solution was added to the bolus material and stirred for 10 minutes, then left for half an hour for sedimentation of particles to occur. The supernatant was decanted off and the rest filtered through a Buchner funnel. The filter paper and funnel were then dried in an oven for 3 - 4 hours at 70$^{o}$C, and then passed through a stack of sieves ranging from 4750 μm to 53 μm, while shaking for 5 minutes. Particles on each sieve were then weighed and the percentage of particles passing through each sieve calculated. Occasionally a small amount of material would pass through the smallest aperture, but the whole range of sieves was required to obtain a frequency distribution of the particle sizes produced from mastication.

Lucas & Luke (1983) tested carrot as a test food with ten subjects. They were asked to chew for 5, 10, 15, 20, 25 and 30 chews then expectorate the sample into a beaker, rinse their mouth with water and add rinsings to the beaker. The bolus material was then washed through a stack of nine sieves ranging from 11.2 to 0.5 mm aperture, whilst shaking for two minutes. Each sieve was then washed individually with a wash bottle then particles from each sieve transferred to 15 ml graduated centrifuge tubes and spun

for 2 minutes at 1100 g. The apparent volume of particles in each tube was measured, and then cumulative frequency curves were plotted.

**IMAGE ANALYSIS**

Experiments to compare optical scanning against multiple sieving have been investigated (van der Bilt *et al.* 1993). The optical scanning was done with a Seescan device (256 x 256 pixels with 80 x 80 mm platform). Particles need to be scattered at least 0.3 mm apart to enable the scanner to measure them individually which is time consuming, therefore particles below 1 mm were sieved out first. The particles were measured in their longest and shortest diameters then cumulative volume distributions calculated to compare with the sieve method. The multiple sieve method by van der Bilt & Fontijn-Tekamp (2004) is described previously. In this experiment they found that both methods resulted in the same median particle size by volume and same total known volume, to obtain useful information a range of sieves is required and the scanning method is useful to detect the smaller particles (van der Bilt *et al.* 1993). Both methods are accurate when correct assumptions have been made, optical scanning can be more time consuming at the data collection stage, but then no data entry is required as with sieving.

Image analysis was used by Hoebler *et al*. (1998 & 2000) when analysing chewed pasta that was non-spherical and generally longer than 1 mm. Pasta particles were isolated on a glass plate, on a black surface, lighted by four 100W lamps on each side of the sample (320 mm distance from the work surface, $60^{o}$ angle). A CCD IAC500 matrix camera (I2S, Bordeaux, France) was fitted with a 16 mm objective lens (Nikkon Corp., Tokyo, Japan) and positioned 260 mm away from the sample. Monochrome images were acquired in the form of matrices (512 x 512 pixels) using a Trydin digitizing board (Info'rop, Toulouse, France). Four images were acquired and analysed together for each sample. The area of each particle was measured and percentage of total area occupied by the particles for 13 size classes between 0 and 60 $mm^2$ calculated. The results found that spaghetti were shortened in length from original sample and swallowed in lengths from 2.5 – 30 mm. Tortiglioni lost its initial shape and was reduced to varied particles. The mode values of area were spaghetti 12 – 20 $mm^2$ and tortiglioni 7.5 – 12.5 $mm^2$.

#### LASER DIFFRACTION

Laser light diffraction has been used by Hoebler *et al*. (1998 & 2000) to analyse chewed bread. Bread particles were heterogeneous in shape after mastication and generally below 2 mm diameter, so laser diffraction was used to quantify size and shape of particles. The bread particles were suspended in isopropanol and analysed using a Mastersizer IP (Malvern Instruments Ltd., UK) with a 1000 lens to measure a size range of 4 to 2000 µm. Results were presented in histograms of particle size volumetric frequency. The diameter of chewed bread samples was between 5 and 1500 µm, with two modes of 30 µm and 620 µm. The smaller fraction was isolated and characterised by staining with Lugol, which corresponded with starch granules (Hoebler *et al*. 1998 & 2000).

Laser diffraction was used by Peyron *et al*. (2004b) to widen the range of particle sizes that could be analysed. Particles above 2 mm were sieved out, and then the remaining particles analysed using a Mastersizer S (Malvern Instruments Ltd., UK) with a 1000 lens which measures particles between 5 and 2000 µm. The particle size distributions were expressed as percentage of total volume, then converted to mass using volumetric mass and plotted as cumulative frequency. Sieve analysis was used to characterise particles above 2 mm. Combining the use of two methods widens the range of particle size distribution to provide more information about the food bolus, especially with dry brittle foods like peanuts that contain a high percentage of particles below 400 µm, but it is difficult to combine results (Peyron *et al.* 2004b).

### 2.4.4.2 ANALYSIS OF BOLUS TEXTURE

Texture of initial food has been shown to affect mastication and the resulting texture of the food bolus prior to swallowing (Lucas & Luke 1984; Lucas *et al.* 1986; Hiiemae *et al*. 1996; Shiozawa & Yanagisawa 1999; Hoebler *et al*. 2000; Amemiya *et al*. 2002: Mioche *et al.* 2002a & 2003; Engelen *et al*. 2005; Peyron *et al.* 2011). Texture measurements have been taken on the bolus produced from a variety of different foods. Mioche *et al.* (2003) measured the texture of meat boluses of different initial known toughness using a double bladed shearing cell used on an Instron machine. Several measurements were made on each bolus, on at least three boluses produced from the same initial meat toughness and the maximum shear force was calculated (Mioche *et al.*

2003). Ameniya *et al*. (2002) developed a model of how the bolus moves under occlusal forces, and they measured the flow and elasticity of biscuit bolus to apply to the model. A texture analyser with compression and extension capability was used to measure the Young's modulus and viscosity of a bolus prepared to swallow, then 2 cycles prior to swallow, then 2 cycles more than swallow. Results showed that the biscuit bolus had viscoelastic properties that changed with mastication duration (Amemiya *et al.* 2002). A creep meter, applying the Texture Profile Analysis double bite test was used to measure hardness, cohesiveness and adhesiveness of boluses produced from three different types of food by Shiozawa & Yanagisawa (1999). Results showed that adhesiveness affected the tongue forces applied to form the bolus and swallow the food, and the food characteristic carried through to the bolus.

#### 2.4.4.3 SHAPE

A bolus of hard particles may not form easily into a shape that is required before it can easily be propelled through the pharynx and oesophagus (Yurkstas 1951; Lucas *et al.* 1986; Hutchings & Lillford 1988). Therefore the effect of bolus shape formation has been highlighted as an area of importance for swallowing by Liedberg & Owall (1995). Shape was investigated with two colours of chewing gum by monitoring bolus formation. A rating scale was developed prior to the investigation on chewing gum boluses after different numbers of chewing cycles. Two experimental groups of different dentitions were recruited to chew two colours of gum for multiples of 10 cycles and spit the sample out for rating later. The two colours of gum also enabled gum mixing to be assessed using another pre-determined scale. Ten chew cycles was found to be sufficient to differentiate between people to provide a value for masticatory efficiency (Liedberg & Owall 1995; Prinz 2004).

Prinz (1999) also investigated bolus shape and mixing of chewing gum using a modified version of Liedberg & Owall's (1995) method. A two-colour, two-flavour gum was given to 10 healthy subjects with full natural dentition in different portion weights (1.8, 3.75 and 7.5g). Subjects were asked to chew them for different specified numbers of chews between 2 and 30 cycles, then expectorate the sample. The extent of mixing was evaluated by squashing the gum between two plates 1 mm apart, and digital images (320 x 240 pixel) of both sides of the gum taken, and a computer program analysed the

images. The image analysis of mixing was comparable with subjective evaluation in accuracy, and flattening the bolus had no effect on quantifying mixing. The small size of gum was mixed, whereas the larger pieces were mixed in parts while some was held in the cheek. Chewing results in lengthening of the bolus as it is rolled between the teeth, so it must be shortened by folding it (Prinz 1999). Rolling of the bolus, rotating it about its long axis, occurred with every chew stroke and folding of the bolus along its long axis was observed, and this occurred sporadically (Prinz & Heath 2000).

#### 2.4.4.4 LUBRICATION

Lubrication is important for a bolus to be swallowable, and this will predominantly be a factor of saliva, but may also be due to expressed moisture from the food, melted fat, or consuming a beverage while eating (Jiffry 1981 & 1983; Lucas & Luke 1984; Hutchings & Lillford 1988; Lillford 1991; Prinz & Lucas 1997 & 2000; Heath 2002; Mioche *et al*. 2002a; Pedersen *et al*. 2002; Casas *et al*. 2003: Malone *et al*. 2003; Bourne 2004; Peyron *et al.* 2004b; Engelen *et al.* 2005). Saliva stimulation varies with food properties (Pangborn & Lundgren 1977; Engelen *et al.* 2005). Some investigators have attempted to monitor saliva impregnation by calculating the difference between dry matter content of food before and after mastication (Hoebler *et al.* 2000), or by weighing the bolus and calculating the difference between initial food weight and bolus weight after mastication which corresponds with saliva absorption and food moisture content loss (Mioche *et al.* 2003). Note that saliva uptake and inherent moisture of the food is non-distinguishable.

## 2.5 CONCLUSIONS

Mastication is a highly variable process affected significantly by features of oral physiology including: state of dentition, salivation, muscle activity reduction associated with healthy aging and selected food ingestion quantity. These factors not only affect oral processing, but stage 2 transport and bolus formation also. It is important when recruiting subjects to select on age group, strict dental criteria, good oral health, good general health, and to control food portion size.

The properties of the food ingested also affect the masticatory sequence. Food properties that have been found to be significant are initial particle size, toughness,

rigidity (Young's Modulus), hardness, moisture content, adhesiveness and cohesiveness. These properties also affect bolus formation and the characteristics of the expectorated bolus. Food properties have been found to affect bolus formation significantly more than physiological factors (Engelen *et al.* 2005). The foods selected for investigation should be measured for as many of the above parameters as possible to identify relationships. Variability within natural foods can be high but they provide real information about how food is managed intra-orally. A controlled test food, for example gelatine gums made to a range of hardness, provide useful information for method development and the effects of one property of the test food can be monitored on chewing and bolus formation.

A few prominent theories of swallowing have been put forward. The particle size threshold model states that a percentage of the particles must be below a particular size to trigger a swallow (Yurkstas 1951). The dual threshold model determines that the food bolus must be below a particular particle size or above a level of lubrication before a swallow will be triggered (Hutchings & Lillford 1988). The cohesion model stipulates that the particles in the bolus must have higher cohesion to each other than adhesion to the oral mucosa; the detection of these forces is the trigger for a swallow (Prinz & Lucas 1997). The trigger may also be related to the production of a bolus that optimises the transport of food for a safe swallow, which may be based on several criteria to be met, at different rates, prior to swallow (Peyron *et al.* 2011).

The particle size threshold model has been disproved by a number of different studies. It has been shown that different foods are swallowed at very different particle size distributions (Yurkstas 1965; Peyron *et al.* 2004b). Increasing the ingestion quantity increases the mean size of the particles in a bolus and larger initial food particle sizes result in larger mean particle size at swallowing (Jiffry & Molligoda 1983; Lucas & Luke 1984). The dual threshold model and the cohesion model have not been verified as the trigger for swallowing, at present.

The food bolus is most commonly characterised by its particle size distribution. Different methods have been utilised including sieve fractionation, image analysis and laser diffraction (Hoebler *et al.* 1998 & 2000; Peyron *et al.* 2004b; van der Bilt &

Fontijn-Tekamp 2004). A multiple sieve method presents good particle size distribution data for foods tested to date, but these have tended to be dry, brittle foods. Image analysis can be employed to obtain information about particle shape and surface area of particles for heterogeneous foods. Laser diffraction is particularly useful for small particle sizes down to 4 μm and gives information about particle shape. Rheological methods of analysis have been applied to the characterisation of the food bolus (Lucas & Luke 1984; Lucas *et al*. 1986; Hiiemae *et al*. 1996; Shiozawa & Yanagisawa 1999; Hoebler *et al*. 2000; Amemiya *et al*. 2002; Mioche *et al.* 2002 & 2003; Engelen *et al.* 2005) and are particularly useful for research focussing on the cohesion model of swallowing.

For the range of natural food products that has been investigated there appears to be a characteristic particle size distribution of the swallowable bolus. It is of interest to identify if this finding is observed for processed heterogeneous food products. There is little discussion in the literature regarding the fate of food particles. From a food technology perspective it is of interest to understand how the food particles breakdown, identify factors affecting this, and investigate their role in bolus formation as it is recognised that not all particles are recovered in the food bolus (Lucas 2004; Peyron *et al.* 2004b). The first researchers investigating mastication and swallowing focussed on only one or two subjects to identify key parameters, but more recently researchers have studied controlled groups of subjects to gain more relevant information for specific populations. To understand mechanisms of food breakdown and bolus formation it may be more appropriate to study single subjects to collect detailed information, which could be verified later in group studies.

# CHAPTER 3: MATERIALS AND METHODS

## 3.1 FOOD TYPES

The food types were selected to provide a range of textural properties and chemical compositions. The foods were commercially produced for human consumption, available in most supermarkets in New Zealand. Production date information on the food packaging was used to ensure that the age of the samples presented to consumers was the same across all subjects, although the product age differed between food types. The foods chosen for investigation were muesli bar (Uncle Tobys, Forest Fruits Chewy Bar), cooked pasta (San Remo, Large Instant Lasagne), cake (Ernest Adams, Madeira), cereal bar (Griffins, Fruitli Apricot), and peanuts (unsalted roasted). Refer to Appendix 1 for the allergen statements and ingredient listings.

## 3.2 SAMPLE PREPARATION AND PORTION SIZE

Portion size was standardised by weight, 2 ± 0.01 g and 4 ± 0.02 g (SEM), for all food types and presented to the subjects in individual containers (Biolab, 120 mL screw cap, autoclavable polycarbonate, 46 x 92 mm). Subjects were given small samples to avoid subsequent intra-oral division into multiple boluses (Hiiemae *et al.* 1996; Okada *et al.* 2007). All food types were cut into cubed portions detailed in Table 3.1, with the exception of peanuts which were presented as split kernels. All food samples were weighed prior to each study session.

**Table 3.1** **Cubed dimensions for each food type for the required portion weights.**

| Food Type | Cube Dimensions (mm) (h x w x d) | |
|---|---|---|
| | 2 g | 4 g |
| **Cake** | 16 x 16 x 16 | 21 x 21 x 21 |
| **Cereal bar** | 12 x 13 x 13 | 12 x 19 x 19 |
| **Muesli bar** | 12 x 12 x 12 | 12 x 17 x 17 |
| **Pasta** | 4 slices x 16 x 16 | 4 slices x 22 x 22 |

The cake sample required freezing to ensure that the same production date samples were presented to participants during the study sessions. On the day of purchase, the cake had all outer "crust" cut off with a knife, it was cut into the cube sizes (Table 3.1), and stored in labelled containers at -18°C. The samples were thawed and brought to room temperature for serving 1 h prior to the study session. The other foods were prepared on the morning of the study. The cereal bar edges were hard baked, so these were removed to ensure that each participant consumed representative samples.

Three types of pasta were initially investigated to determine the most appropriate for these studies. Large Lasagne, Wide Lasagne and Spiral (all San Remo brand) were tested for ease of sample preparation, how comfortable a subject would be in masticating the sample and expectorating, and reproducibility of results (Table 3.2). The Large Lasagne was used in the studies as the pasta sample as it was the most reproducible for bolus moisture content, number of chews to swallow point and chewing time for one test subject. Refer to Sections 3.7.3 & 3.4.9 for measurements and calculations of mastication physiological parameters in the study sessions.

**Table 3.2** **Type of pasta: food sample weight and moisture content, chewing strategy and bolus moisture content.**

| Pasta Type | Sample weight (g) | Number of chew cycles | Chewing time (s) | Moisture content bolus (g/100g DMB) | Moisture content food (g/100g DMB) |
|---|---|---|---|---|---|
| **Large Lasagne** | 5.68 ± 0.12 | 20 ± 0.0 | 15 ± 0.7 | 235 ± 10 | 183 ± 7 |
| **Wide Lasagne** | 7.81 ± 0.06 | 16 ± 0.4 | 15 ± 1.9 | 289 ± 16 | 245 ± 10 |
| **Spiral** | 6.01 ± 0.55 | 14 ± 0.7 | 12 ± 0.7 | 250 ± 67 | 176 ± 1 |

The pasta was prepared to be ready at the start of each study session. One dry lasagne sheet was cut in half with a knife, while a pot of water was brought to the boil. The lasagne was added to the water and left to simmer for 10 min. The pasta was drained then rinsed with cold water for 1 min, and the excess water removed by blotting with a paper towel. After cutting to the required size, the pasta cube was skewered with a cocktail stick for easy transfer to the mouth.

Due to the random order of sample presentation used to collect bolus samples in Chapters 4 and 5 (Table 3.9), it was noted that the pasta sample drying out over the session time may have resulted in less reproducible results. It was decided to present samples as per the previous instructions, but immersed in deionised water (16.5°C ± 0.1). The subject was instructed to gently agitate the pasta sample to remove excess water before placing in their mouth. This resulted in minimal changes in pasta food moisture content at the time it was placed in the mouth so this method was used in the studies covered in Chapters 6 and 7.

## 3.3 FOOD COMPOSITION AND TEXTURE ANALYSIS

### 3.3.1 MOISTURE CONTENT

The moisture content of the foods was determined by drying prepared samples in an oven at 105°C for 24 h until constant weight was achieved (ISO 712). For the studies in Chapters 4 and 5 samples were weighed and placed in the oven immediately after the subject had masticated the food study samples. In Chapters 6 and 7 the samples were placed in the oven half-way through the study sessions. The food moisture content on a dry mass basis was calculated as g moisture/100g dry food using Equation 3.1. This was calculated for each food type for each session in duplicate. The values were used to calculate food water holding capacity, and for each individuals study session the bolus moisture content and loss of bolus solids. Mean data across all studies is given in Table 3.3.

$$MC_{food} = \left(\frac{\left(M_{food} - M_{(d)food}\right)}{M_{(d)food}}\right)100 \qquad \textbf{Eq. 3.1}$$

Where, $MC_{food}$ is the moisture content food (g/100g dry solids), $M_{food}$ is the mass of wet food (g), and $M_{(d)food}$ is the mass of dry food (g).

**Table 3.3 Mean (±SEM) food moisture content data.**

| Food Type | Moisture content (g /100g dry food) |
|---|---|
| **Peanuts** | 2.1 ± 0.1 |
| **Cake** | 23.5 ± 0.7 |
| **Cereal bar** | 13.1 ± 0.4 |
| **Muesli bar** | 11.1 ± 0.2 |
| **Pasta** | 159 ± 3.0 |

## 3.3.2 CHEMICAL COMPOSITION

Fat content was determined as percentage crude fat by acid hydrolysis and extraction with mixed ethers according to the AACC method 30-10.01. pH was determined by measuring the hydrogen ion activity using the AACC method 02-52.01. The percentage of protein was determined by nitrogen content extracted by Dumas combustion. Total sugars data was taken from the nutrition information panel on the food packaging. Mean results for all foods are given in Table 3.4.

**Table 3.4 Mean (±SEM) food chemical composition data.**

| Food Type | pH | Total Sugars % | Protein % | Total Fat % |
|---|---|---|---|---|
| **Peanuts** | 6.62 | 4.3 | 27.2 ± 0.0 | 32.1 ± 0.2 |
| **Cake** | 6.51 | 31.8 | 5.0 ±0.03 | 17.1 ± 0.2 |
| **Cereal bar** | 5.32 | 34.8 | 7.3 ± 0.2 | 15.9 ± 0.1 |
| **Muesli bar** | 4.44 | 34.8 | 6.1 ± 0.3 | 13.8 ± 0.4 |
| **Pasta** | 6.23 | 0.3 | 6.3 ± 0.01 | 1.1 ± 0.02 |

## 3.3.3 WATER HOLDING CAPACITY

The water holding capacity (WHC) of each food type was measured by weighing 0.7 ± 0.16 g of the food into a centrifuge tube, adding 20 g of deionised water, covering and leaving to stand at room temperature for 24 h. The sample was then centrifuged for 20 min at 3000 rpm. The supernatant was decanted and the wet food pellet was weighed and then transferred to a pre-weighed moisture dish. The pellet was then dried in an oven at 105°C for 24 h to achieve a stable weight then re-weighed. The WHC, insoluble solids, saturation of solids, and soluble solids were calculated (Equations 3.2 to 3.5). Mean results for all food types are shown in Table 3.5.

$$WHC = \frac{(M_{pellet} - M_{food})}{M_{food}}$$ **Eq. 3.2**

Where, *WHC* is the water holding capacity, $M_{pellet}$ is the mass of the wet food centrifuge pellet (g), $M_{food}$ is the mass wet food (g).

$$S_{insol} = \left(\frac{M_{(d)pellet}}{IDM_{food}}\right)100$$ **Eq. 3.3**

Where, $S_{insol}$ is the insoluble solids (%), $M_{(d)pellet}$ is the mass of the dry food centrifuge pellet (g), and $IDM_{food}$ is the ingested dry food solids (g) (Eq. 3.6).

$$S_{sat} = \left(\frac{(M_{pellet} - M_{(d)pellet})}{M_{(d)pellet}}\right)100$$ **Eq. 3.4**

Where, $S_{sat}$ is the saturated solids (g/100 g dry solids).

$$S_{sol} = \left(\frac{(IDM_{food} - M_{(d)pellet})}{IDM_{food}}\right)100$$ **Eq. 3.5**

Where, $S_{sol}$ is the soluble solids (%).

**Table 3.5 Mean (±SEM) water holding capacity data**

| Food Type | Water holding capacity | Insoluble solids (%) | Saturation of solids (g/100g dry solids) | Soluble solids (%) |
|---|---|---|---|---|
| **Peanuts** | 0.84 ± 0.05 | 91.8 ± 0.7 | 106 ± 5 | 8.2 ± 0.7 |
| **Cake** | 2.93 ± 0.18 | 49.9 ± 0.2 | 872 ± 32 | 50.1 ± 0.2 |
| **Cereal bar** | 0.56 ± 0.05 | 44.3 ± 0.9 | 293 ± 5 | 55.7 ± 0.9 |
| **Muesli bar** | 1.48 ± 0.21 | 66.1 ± 1.8 | 311 ± 20 | 33.9 ± 1.8 |
| **Pasta** | 1.20 ± 0.06 | 93.3 ± 0.5 | 470 ± 14 | 6.7 ± 0.5 |

### 3.3.4 TEXTURE ANALYSIS INSTRUMENTAL

The textural properties were characterised by texture profile analysis (TPA) (Bourne, 2002) using an Instron Universal Testing machine model 4444 with a 500 N load cell. A double compression to 50% of the foods original height was performed using a 65mm diameter plate at a crosshead speed of 100 mm $min^{-1}$. The following parameters were determined: Hardness (the maximum force applied during the first compression cycle); Springiness (the food's ability to spring back to its original shape); Cohesiveness (the work done during the second compression cycle compared to the first cycle);

Fracturability (the force at the first significant break in the curve during the first compression cycle); Adhesiveness (the work done to pull the plate away from the sample after the first compression cycle) and Chewiness (cohesiveness x hardness x springiness) which reflects how readily the food is broken down under compression (Bourne 2002). Mean results for all food types in Table 3.6.

**Table 3.6 Mean (±SEM) texture profile analysis (TPA) data**

| Food Type | Texture Profile Analysis (TPA) | | | | | |
|---|---|---|---|---|---|---|
| | Hardness (N) | Cohesive – ness | Adhesiveness (N x mm) | Chewiness (N x mm) | Springi – ness (mm) | Fracturability (N) |
| **Peanuts** | 102 ± 9 | 0.13 ± 0.04 | - | 2.7 ± 0.8 | 0.22 ± 0.01 | 41.3 ± 5.1 |
| **Cake** | 9.4 ± 0.5 | 0.39 ± 0.01 | 0.09 ± 0.01 | 2.8 ± 0.2 | 0.76 ± 0.002 | - |
| **Cereal bar** | 210 ± 11 | 0.17 ± 0.01 | 0.03 ± 0.06 | 13.3 ± 1.2 | 0.36 ±0.02 | - |
| **Muesli bar** | 319 ± 21 | 0.12 ± 0.01 | 0.98 ± 0.16 | 13.8 ± 1.6 | 0.35 ± 0.03 | - |
| **Pasta** | 207 ± 12 | 0.70 ± 0.03 | 3.00 ± 0.51 | 132 ± 5.3 | 0.92 ± 0.04 | - |

## 3.4 METHODOLOGY DEVELOPMENT

There is no set recommended method for particle size distribution (PSD) analysis of the food bolus. Previous researchers who have investigated PSD of the bolus for understanding of food breakdown have tended to use a multiple sieve method, but all using different aperture sieves and a different number of sieves (Peyron *et al.* 2004b, Jalbert-Malbos *et al.* 2007). Also some researchers have applied their sieve method with a range of food types, or others have worked solely on brittle foods or food analogues (Olthoff *et al.* 1984; van der bilt & Fontijn-Tekamp 2003; Lucas 2004). Previous work has shown that most of the recovered food bolus particles are in the range of 0.1 to 5.0 mm. Peanuts are a commonly studied food type and Peyron *et al.* (2004b) showed that most particles are between 0.4 to 2.0 mm in size.

The aim of this work was to compare wet and dry multiple sieve methods and give recommendations for future work.

### 3.4.1 BOLUS COLLECTION

In this study peanuts were selected as they are commonly investigated in mastication studies. Natural peanuts were chosen (from Peanut & Raisin mix, Total RePac Ltd., Christchurch, NZ), the kernels were split into two and standardised by weight at 4 ± 0.01 g portion size.

The three subjects who participated were not controlled for dentition, gender, or age. They were instructed to chew the sample and swallow naturally then raise a hand to indicate the swallow point. The researcher timed the chewing sequence and counted the number of chew cycles taken to reach a natural swallow for each subject, this was measured in triplicate.

Subjects were told the group mean number of chew cycles (27) that they had taken for the natural swallow, and were asked to use only that number of chew cycles on the test sample, and expectorate the bolus into a container. The subject then rinsed their mouth with 75 mL water and added the debris washings to the container with the bolus. The chewing sequence time was recorded and number of chew cycles noted. Subjects did comment that sometimes the bolus did not feel ready for swallow using the chew cycle number as a control for the swallow point. The collection method was repeated four times for each subject, for each of the PSD methods. The bolus was analysed the same day it was collected.

### 3.4.2 WET SIEVE METHOD

The PSD was determined by washing through a series of 8 sieves (200 mm diameter, and 50 mm height), with the apertures: 0.125, 0.25, 0.5, 0.71, 1.0, 1.4, 2.8, 4.0 mm (Endecott, London, UK). The sieves were ordered in series from largest to smallest aperture. (Eight sieves were used due to a constraint of the number of sieves that could fit in the sieve shaker for the dry method.)

The bolus sample was poured onto the 4.0 mm sieve at the top of the sieve stack and washed using tap water for 4 min at a flow rate of 8 L/min. Each of the fractions was recovered using a brush and placed on separate pre-weighed filter papers (LabServ, 4 series, coarse retention, fast flow rate). The filter papers were then carefully transferred

using tweezers onto a foil baking dish (Confoil pie dish) which were then dried at 65°C for 1.5 h, then weighed on a balance to 3 d.p. It was noted in this study that some filter papers (with no sample) lost weight with drying (0.01 g) but as this change was intermittent and within the accuracy of the balance, it was not taken into consideration with the results.

The total mass of solids recovered was determined by simple summation. The mass of particles in each of the sieve fractions was calculated as a percentage of the total solid matter retained on the sieves.

### 3.4.3 DRY SIEVE METHOD

The bolus produced was found to be cohesive and this increases with any time delay between collection and analysis. Therefore, prior to dry sieving the bolus had to have the saliva removed by washing with tap water on the 0.125 mm sieve at a flow rate of 8 L/min for 4 min. This treatment of the bolus pre-sieving and the dry sieve method is similar to that used by Peyron *et al.* (2004b). The sample was then scraped onto a pre-weighed foil sheet and dried in the oven at 65°C for 1.5h as this dried the particle surfaces sufficiently to facilitate their separation. The dry bolus particles were poured into the 4.0 mm sieve which was stacked in series in the sieve shaker (Octagonal digital shaker, Endecott, London, UK). The sieves were then shaken for 2 min (amplitude 6). The sieves were removed individually, the particles brushed onto pre-weighed foil, and each fraction weighed.

### 3.4.4 COMPARISON OF THE WET AND DRY SIEVE METHODS

The subjects chewing sequence time was recorded (Table 3.7), which showed very little variation in the chew frequency between subjects when instructed to chew for the same number of chew cycles prior to expectorating the sample.

**Table 3.7 Mean (±SEM) chewing parameters and method comparison**

| Food Type | Chewing Time (s) | Chew Frequency (1/s) | Ingested Solids Recovered (%) | |
|---|---|---|---|---|
| | | | Wet Sieve Method | Dry Sieve Method |
| **Peanuts** | 29.63 ± 0.73 | 1.10 ± 0.03 | 51.26 ± 1.85 | 58.8 ± 2.18 |

The percentage of ingested solids recovered (Table 3.7) was slightly higher than found in previous research (Peyron *et al.* 2004b). The wet sieve method resulted in greater losses when compared to the solids recovered using the dry sieve method, but the wet sieve method showed less variation when repeated.

The comparisons of PSD is shown for each subject as they each had their own chewing strategy and were not a controlled group of subjects (Figure 3.1). For each subject, for each sieve fraction, the range of variation produced by the different sieve methods was similar. The PSD produced by each sieve method exhibited little intra-method variation, although they showed inter-method variation at certain parts of the PSD curve.

The PSD differed significantly between the two sieve methods for the particles present in the 1.0 mm and finer fractions for two of the subjects (Figures 3.1. B & C). There was no significant difference between the methods for the quantities of particles recovered in the fractions 1.4 mm and larger. The 0.25 mm fraction was significantly different between the methods with the dry method producing a peak, and the 0.125 mm fraction also resulted in a higher quantity of material when the dry sieve method was applied.

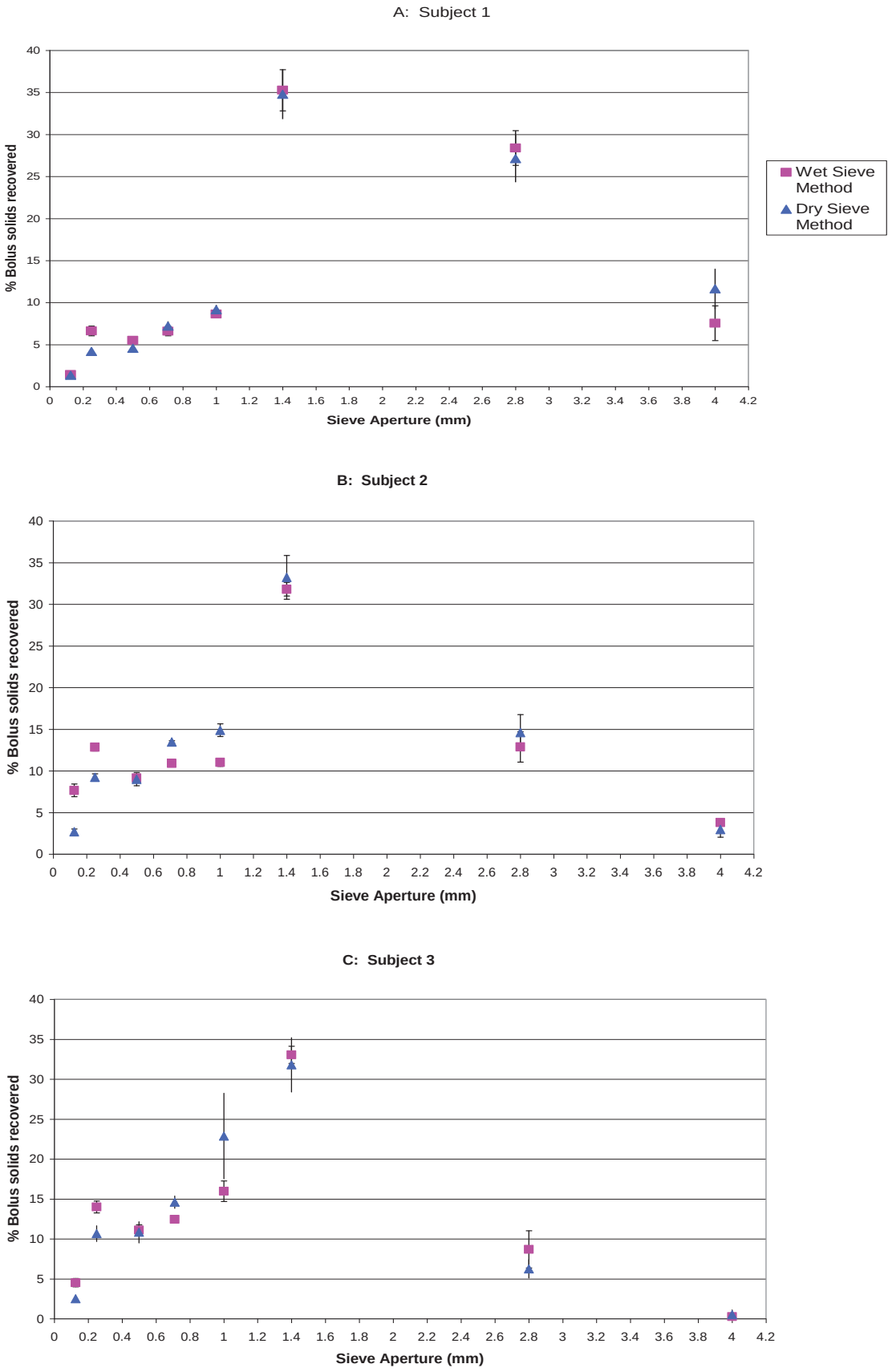



**Figure 3.1 (A-C) PSD of the recovered bolus particles, mean (±SEM).**

### 3.4.5 DISCUSSION AND CONCLUSIONS

The dry and wet sieve methods investigated were both suitable for analysing the PSD for peanuts. It was decided that the most appropriate method for a range of foods to be analysed was the wet sieve method, as it showed the best reproducibility within a subject. The dry sieve method had a couple of steps where particles could be formed and damaged: insufficient bolus washing and transfer of dried bolus to sieves.

In this study the reason that the subjects varied in PSD produced was not only that they were not a controlled group of subjects, but that they were instructed to chew to the mean number of chew cycles for the group. Therefore the percentage of solids recovered for this study may be higher than other researchers as these subjects had not masticated the food to a swallowable composition prior to expectorating.

As the debris washings were collected it was decided that it would be of interest to analyse these separately in future studies to determine if a different PSD results which will provide more information about the mastication process. Adding the debris sample to the bolus for analysis is not a true representation of the PSD of the material that the subject would normally swallow.

During method development, each bolus was collected and immediately analysed due to the observation that the bolus dried out and became set or more cohesive if left in ambient conditions or a fridge prior to analysis. For the dry method the bolus was washed on a sieve under running tap water to facilitate separation of particles (Peyron *et al.* 2004b). Further study was undertaken to find a more appropriate method especially as large numbers of samples (more than can be analysed per day) will be collected from subjects in future study sessions.

The issues identified during these method investigations were improved prior to further application of the wet sieve method.

### 3.4.6 FINALISED WET SIEVE METHOD

The recommended wet sieve method is applied in all studies, and the laboratory set up pictured in Figure 3.2.

The PSD of the bolus and debris were separately determined by a wet sieve method. A water bottle with spout containing deionised water was used to wash particles through a series of 11 sieves. The sieves used were 0.125, 0.177, 0.25, 0.344, 0.5, 0.71, 1.0, 1.4, 2.0, 2.8, and 4.0 mm, which was every fourth sieve in the ISO preferred number series (ISO 3310-1:2000).

**Figure 3.2 Laboratory set up for wet sieve analysis.**

Filter papers were prepared by placing in a numbered foil baking dish (Confoil pie dish), covered with a foil sheet lid and put into an oven to dry (105°C for 24 h). The foil dish was transferred to a desiccator for 1 h, and then each dish was weighed. A pre-weighed filter paper was taken from a dish (number noted), positioned in a Buchner funnel with a Venturi pump connected to remove excess water, as the material retained on each of the sieves was subsequently back-washed onto filter paper. This was repeated for each of the sieves in the series. These were then dried at 105°C for 24 h, then transferred to a desiccator and weighed. The sum of each of the bolus and debris fractions were calculated by combining the mass of each of the bolus and debris fractions retained on each of the sieves. These sums were used to calculate the ratio of

the total debris solids to total bolus solids, and the total dry mass of solids recovered as a percentage of the ingested food solids. The calculation of ingested food solids (g) (Equation 3.6) used the food moisture content (or dry matter) data calculated on the session day and the actual weight of the food sample provided to the subject in that session. The mass of particles on each of the sieves was calculated as a percentage of the total solid matter retained on the sieves.

$$IDM_{food} = \left(\frac{100 - M_{food}}{100}\right) M_{sample} \qquad \textbf{Eq. 3.6}$$

Where, $IDM_{food}$ is the ingested dry food solids (g), $M_{food}$ is the mass of wet food (g), and $M_{sample}$ is the mass of ingested sample (g).

In initial studies the recovery of ingested solids was incomplete, and it was of interest to identify what happens to all particulates during mastication of solid foods. Therefore, in Chapter 7 the session protocol instructed the subject to inspect their mouth with a mirror between samples and use a tooth pick to remove any left over particles. Any particulates collected were put onto a pre-weighed Petri dish, dried in an oven (105°C for 24 h), then weighed. Analysis of the pan water post-sieving was modified to a centrifuge method. The pan water was transferred to centrifuge tubes (Beckman 500 mL), and the sample was then centrifuged for 5 min at 3500 rpm. The supernatant was decanted and the wet pellet was weighed and then transferred to a pre-weighed filter paper in foil dish (as per the sieve method). This was then dried in an oven (105°C for 24 h) to achieve a stable weight then re-weighed.

### 3.4.7 BOLUS STABILISATION TRIALS

As acknowledged in Section 3.4.5, the bolus collection process had to be improved due to a minimum of 14 samples per study session being collected for PSD analysis. It was not possible to analyse 14 samples as they were produced by the subject as this would have taken weeks for collection and analysis. Also this would not have been a controlled method of collection as it is likely they would have had to be collected at different times during the day to be able to analyse them immediately. If the number of samples collected per day were reduced, this would have reduced participant

recruitment, retention, and possibly their compliance. To deal with 14 samples per session a stabilisation and storage method was developed.

Three methods were identified for trial to facilitate separation of the bolus and debris fractions into discrete particles prior to storage. The rational for choosing the final method was based on observation, minimum damage to particulates assessed by observation and the subjective decision regarding "ease of sieving" through the 4.0 mm sieve by the researcher.

The methods identified were: Peyron *et al.* (2004b), who washed the bolus under running tap water for 1 min on the 0.4 mm sieve, then dried the sample (40°C for 1h) prior to analysis by dry sieving. Jiffry (1981) added 100 mL slaked lime solution ($Ca(OH)_2$ 0.051 g in 150 mL water) to the bolus material and stirred for 10 min. This suspension was left for 30 min for sedimentation of particles to occur. The supernatant was decanted and the rest filtered through a Buchner funnel. The filter paper and funnel were then oven dried (70°C for 3 – 4 h). Jiffry (1981) noted that this reduced the sticky effect of the saliva in the bolus samples. The third method was not previously applied to food bolus samples, but to food digesta from the brown kiwi (*Apteryx mantelli*) (Potter *et al*. 2006), where samples were suspended and agitated in TRIS buffered saline (pH10, 1.21 g of trihydroxymethylamine (refer to Appendix 2 for MSDS) plus 4.4 g NaCl, made up to 500 mL with deionised water) to hydrolyse adhered mucus immediately prior to PSD analysis. All methods were modified and tested to suit the experimental conditions.

A bolus sample was collected onto a mesh (0.120 mm) positioned over a beaker (250 mL) (Figure 3.3), then it was washed with 100 mL of one of the three solutions to be tested, using a water bottle with spout. This was repeated for each of the foods to be studied (Section 3.1) with each solution.

### 3.4.8 METHOD FOR BOLUS STABILISATION AND STORAGE

The bolus and debris samples washed with 100 mL TRIS buffered saline were separated most readily into discrete particles. The samples were stored on the mesh at -80°C for

24 h and then at -20°C pending sieving. Samples were thawed for 1 h at ambient temperature then sieved. The samples washed with TRIS buffered saline were most easily sieved, and the mass of particles on the 4.0 mm sieve most similar to that of a fresh bolus washed with water and sieved immediately. The TRIS buffered saline was used in the stabilisation procedure for all bolus and debris samples for sieve analysis.

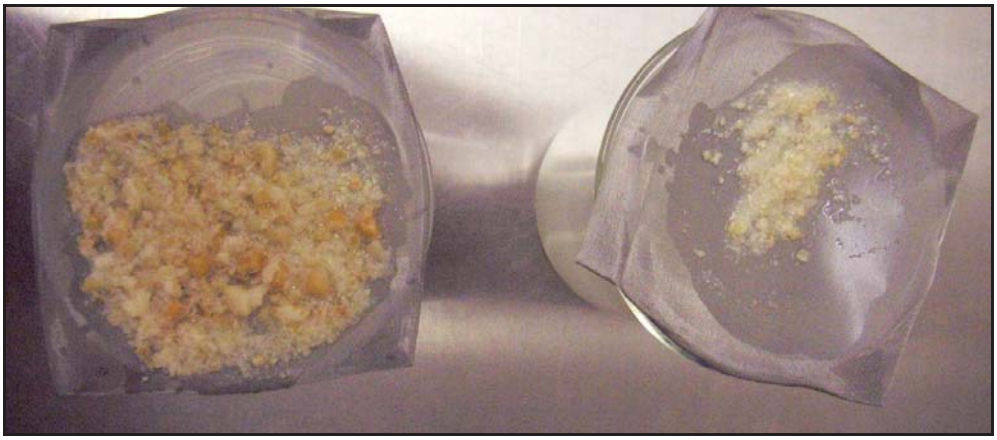


**Figure 3.3** **Bolus (left) and debris after washing with TRIS solution on the 0.12 mm mesh.**

### 3.4.9 BOLUS MOISTURE CONTENT AND SOLIDS LOSS

Food bolus moisture content was investigated at the point that the subject felt ready to swallow. Bolus samples were expectorated onto a pre-weighed glass Petri dish which was then dried in an oven (105°C for 24 h) to achieve constant weight. The moisture content of the bolus was calculated on a dry mass basis and the percentage loss of solids from the bolus were determined by Equations 3.7 and 3.8. The change in moisture content (g moisture increase/100g dry solids) from that of the ingested food to bolus moisture content was calculated by Equation 3.9.

$$MC_{bolus} = \left(\frac{\left(M_{bolus} - M_{(d)bolus}\right)}{M_{(d)bolus}}\right)100 \qquad \textbf{Eq. 3.7}$$

Where, $MC_{bolus}$ is the moisture content bolus (g/100 g dry solids), $M_{bolus}$ is the mass of the wet bolus (g), and $M_{(d)bolus}$ is the mass of the dry bolus (g).

$$S_{loss} = \left(\frac{\left(IDM_{food} - M_{(d)bolus}\right)}{IDM_{food}}\right)100 \qquad \textbf{Eq. 3.8}$$

Where, $S_{loss}$ is the bolus solids loss (g/100 g dry solids), and $IDM_{food}$ is the ingested dry food solids (g).

$$\Delta MC_{bolus} = MC_{bolus} - MC_{food} \quad \textbf{Eq. 3.9}$$

Where, $\Delta MC_{bolus}$ is the change in moisture content of the bolus (g), and $MC_{food}$ is the moisture content of the food (g/100 g dry solids).

## 3.5 DATA ANALYSIS AND STATISTICS

A Kolmogorov-Smirnov (K-S) test (with Lilliefors significance correction) for normality was conducted on all data sets for participant measures of mastication, particle size distribution sieve fractions, bolus moisture content and solids loss. Where necessary the data was transformed to achieve normality (log or square root). These statistical analyses were performed using GenStat version 12 (VSNi Ltd, UK) or SYSTAT® version 11 (Systat Software Inc., USA). A P-value of <0.05 was considered to be statistically significant.

### 3.5.1 PARTICLE SIZE DISTRIBUTION DATA ANALYSIS

The distribution of particle sizes in each of the bolus and debris compartments departs from normality. Therefore, the percentage particle size distribution from each sieve fraction was log transformed to achieve a normal distribution. This is inconsistent with the findings reported by other researchers, Peyron *et al.* (2004b) and Jalabert-Malbos *et al.* (2007) who found that the masticated bolus (with debris fraction combined) PSD for a range of food types was "normal in all cases".

Principal Component Analysis (PCA) was applied to the log transformed PSD data as each size fraction may be considered as independent variables. This methodology was applied as it describes the whole data set and reduces the number of measured variables to a few principal components (PC). The number of PC generated is equal to the number of variables, so to maintain available degrees of freedom the results were discarded from one sieve; data from the largest sieve (4.0 mm) was removed from the analysis due to having the least data across the data set. After consulting the scree plot produced, the factors were chosen that produced eigenvalues greater than 0.8, which

gave three PC that explained the highest percentage of total variance. The component loadings for each of the three PC describe the key patterns of variation for the PSD.

The factor scores produced for the PCA were saved, and checked for normal distribution by K-S test. Initially, the bolus and the debris scores were each analysed for food type and portion size effects, with subject and subject x food type x portion size as strata. To compare bolus versus debris, the data was combined, and factor scores were analysed using a three-way analysis of variance (ANOVA) model with subject x food type x portion size x replicate, and subject x food type x portion size x bolus vs debris as strata fitted, with bolus vs debris and the interactions with food type and portion size as factors. Where significant differences were identified, post hoc tests for comparison of means (Fisher's LSD) were carried out. Further, the residuals of these analyses were checked for normality graphically and by K-S test (with Lillefors significance correction).

### 3.5.2 BOLUS MOISTURE AND SOLIDS DATA ANALYSIS

Two-way ANOVAs stratified by subject, and subject x food type x portion size were applied to examine the effect of portion size, food type and subjects on the (log of) debris/bolus ratio and (log of) the mass of debris solids data. Post hoc tests for comparisons of means (Fisher's LSD) assessed significant effects and interactions. The residuals of these analyses were checked for normality by K-S test (with Lilliefors significance correction).

Two-way stratified ANOVAs (strata as above) with post hoc adjustment for multiple testing to assess interaction effects, were undertaken to examine the effect of portion size, food type and subjects on the log of: bolus moisture content, and loss of solids data. The residuals of these analyses were checked for normality graphically and by Kolmogov-Smirnov test (with Lillefors significance correction).

### 3.5.3 MASTICATION DATA ANALYSIS

Two-way stratified ANOVAs (strata as in Section 3.5.2) with post hoc adjustment for multiple testing to assess interaction effects were undertaken to examine the effect of portion size, food type and subjects on the chew frequency data and log of: chew cycles,

and chewing time data. The residuals of these analyses were checked for normality graphically and by Kolmogov-Smirnov test (with Lillefors significance correction).

### 3.5.4 VARIANCE TESTS FOR BOLUS MOISTURE AND SOLIDS LOSS DATA

Three-way ANOVAs stratified by subject, subject x food type, and subject x food type x variance test, (with food type, variance test and their interactions as factors) were applied to examine the variability when samples were repeated by subjects on a separate test day. These effects were examined for the chew frequency data, and the log of: chew cycles, chewing time, loss of solids, and bolus moisture content data. Where significant differences were identified, post hoc tests for comparison of means (Fisher's LSD) were carried out. Further, the residuals of these analyses were checked for normality graphically and by K-S test (with Lillefors significance correction).

### 3.5.5 DATA ACCURACY AND ERROR REPORTING

The balance (Sartorius CP225 D) used for all the study sessions was calibrated daily and calibration checked regularly. The balance used was accurate to 0.0001 g, and data will be presented to 3 decimal places. Where experimental errors are presented these refer to standard error of the mean ($x \pm y$, where $x$ is the mean and $y$ is the standard error of the mean), unless stated otherwise. The researcher completed sieve analysis for particle size distribution for all samples to minimise experimental error and ensure reproducibility of methodology between samples.

## 3.6 PARTICIPANTS

Human ethics approval was granted by the Massey University Human Ethics Committee (approval number ALB05/25) and all study documents and processes approved are in Appendix 3. Recruitment commenced using poster advertising and face-to-face recruitment of potential participants by the researcher around Massey University Albany campus and the local village, sports centres and shopping mall. Potential subjects were given an information sheet and if interested in the study gave their informed consent. Subjects were then required to fill out a screening questionnaire which covered familiarity of food types to be investigated, general and dental health.

They were informed that they would be reimbursed for their time with supermarket vouchers.

Participants were required to be familiar with and willing to consume the food types to be studied, deemed by their self to be of good general health, and willing to have a dental examination. Participants gave informed consent to undergo a dental examination, which was organised and paid for by the researcher. They filled out a specific dental questionnaire for the dentist to ensure that they were on no medication that would interfere with saliva flow, that they had good oral health, no history of major medical problems, no missing teeth and class I molar dentition. Dental impressions using alginate in moulds were collected by the dentist if the participant met the dental criteria. These moulds were taken to a Dental Solutions laboratory to be cast in yellow stone, which were kept in locked storage with all participant documentation by the researcher. Confidentiality agreements were signed by dentist's who had access to participant information.

Participants who met all criteria were invited to a familiarisation session to go through the study protocol, refer to Section 3.7.3 for details, and gave their consent to continue with the study, the mean physiology characteristics measured for the subjects are presented in Table 3.8. Subjects were assured they had the right to withdraw from the study at any time, but informed that this stage would not affect the investigation. Participants then booked in for their first study session for data collection. Data presented is from subjects who attended all study sessions.

**Table 3.8** **Subject physiological data mean (±range)**

| Subject physiological data | Chapters 4 & 5 | Chapters 6 & 7 |
|---|---|---|
| Age (yr) | 21.5 ± 3.5 | 27 |
| BMI (Weight (kg)/Height ($m^2$) | 24.1 ± 9.0 | 24.6 |
| Mouth volume (mL)* | 84.3 ± 28.2 | 87.5 ± 7.7 |

*Mouth volume is the maximum capacity of water that the subject could take into their mouth from a cup.

# 3.7 SAMPLE COLLECTION

## 3.7.1 STUDY DESIGN FOR CHAPTER 4 AND CHAPTER 5

The samples to be presented per session and order of presentation were randomly selected to minimise order effects with the following constraints: each subject could assess no more than 18 samples per session as sensory fatigue could affect the mastication outcomes; each subject assessed only one portion size per food type per session, so that results were not affected by the subjects awareness of the two portion sizes as they were visually apparent; each subject assessed all food types per session. Each food type and size was assessed in triplicate over 4 study sessions. Sessions were randomised between subjects, an example of study design for one subject is shown in Table 3.9.

**Table 3.9** **Study design for one subject from the group studies.**

| Session | Food | Weight (g) | Number of Replicates |
|---|---|---|---|
| 1 | A | 2 | 4 |
| | B | 4 | 3 |
| | C | 4 | 3 |
| | D | 2 | 4 |
| | E | 2 | 4 |
| 2 | C | 2 | 4 |
| | A | 2 | 3 |
| | E | 4 | 4 |
| | B | 2 | 3 |
| | D | 4 | 4 |
| 3 | E | 4 | 3 |
| | D | 4 | 3 |
| | B | 2 | 4 |
| | A | 4 | 4 |
| | C | 2 | 3 |
| 4 | B | 4 | 4 |
| | E | 2 | 3 |
| | D | 2 | 3 |
| | C | 4 | 4 |
| | A | 4 | 3 |

### 3.7.2 STUDY DESIGN FOR CHAPTER 6 AND CHAPTER 7

The samples to be presented per session and order of presentation were randomly selected with the following constraints: a subject could assess no more than 14 samples per session; only one food type was to be assessed per session. Each food type was assessed in triplicate.

### 3.7.3 SESSION PROTOCOL

The subjects attended sessions spaced at least 24 h apart, held in the morning 1 to 2 h after a meal. Health and safety questions were asked at the start of the session to ensure that the subject's health was not adversely affected by participating, and that the samples collected are comparable to other sessions. For example, if the subject had tooth pain or any cold or flu symptoms this was expected to affect their mastication strategy, such as modification of chewing behaviour, affecting changes in saliva flow, lessened sense of smell and taste which would affect the reproducibility of the data and samples collected. These subjects were asked to reschedule the session when they had returned to good health.

Instructions were given at the start of each session describing what the researcher would do and what was expected of the subject (refer to Appendix 4). At all sessions the subjects chewed and swallowed the first sample of each food type. Subjects were asked not to touch the food with their hands, but to tip the food samples directly from the container into their mouth to avoid tactile assessment. The researcher observed the subject and when they commenced chewing, the duration of the chewing sequence was timed and the number of chew cycles counted until the subject reached a swallowable bolus. The subject also indicated this by the raising of a hand. The first sample was to familiarise the subject with the sample and to obtain data for a natural swallow to confirm that samples collected for analysis were similar to the normal process for the individual, and confirm their reproducibility. Chew frequency (1/s) is calculated by Equation 3.10.

$$v_{chew} = \frac{N_{chew}}{t_{chew}} \qquad \textbf{Eq. 3.10}$$

Subsequent samples were chewed and bolus expectorated onto a sieve (0.12 mm aperture, stainless steel, Mounts Wire Industries Ltd.) positioned on a 250 mL glass beaker. The subject then rinsed the remaining debris from around their mouth with 50 mL deionised water and expectorated the debris onto a second sieve on a beaker. The specific details regarding the samples to be collected are detailed in each chapter. An inter-stimulus interval of approximately 2 min was used, unless the subjects indicated a longer break was required.

To study ensalivation and solids loss from the bolus, samples were collected for moisture content analysis. The subjects chewed the samples until they felt the urge to swallow, while the researcher recorded chewing sequence time and number of chew cycles. The bolus was then expectorated onto pre-weighed glass Petri dish for analysis.

# CHAPTER 4: IDENTIFICATION OF MULTIPLE COMPARTMENTS PRESENT DURING THE MASTICATION OF SOLID FOOD

## 4.1 INTRODUCTION

In mastication studies, a bolus is generally considered the main bulk of food which is masticated at a given point in time within a chewing sequence or the mass of food which is initially swallowed. The debris is the residual material left in the mouth after the bolus has been swallowed. After further clearance, some of the debris may also be swallowed however, we are still normally left with some material trapped in various locations within the oral cavity. It could be considered that the ingested food dissociates and becomes located in two 'compartments', i.e. within the main bolus or dispersed elsewhere within the mouth.

The assessment of masticatory function by means of comparison of particle size distributions (PSDs) (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007; van der Bilt & Fontijn-Tekamp 2004; Jiffry 1981; Lucas & Luke 1983) is straightforward if, as is generally assumed, mastication takes place in a single compartment in which the probability of fracture is related principally to its size (van den Braber *et al.* 2002; Lucas *et al.* 2002; van der Glas *et al.* 1987). However such assessment becomes more complex when particles circulate through more than one compartment and the probability of fracture differs between compartments. This could arise, for example, if a fraction of the particulate material adheres temporarily to the teeth. The existence of additional compartments is supported by videofluorographic evidence showing that a proportion of particulate material generated during mastication is not incorporated into the bolus and remains in the mouth after swallowing (Hiiemae *et al.* 1996; Hiiemae 2004; Okada *et al.* 2007).

The general assumption that mastication takes place in a single 'chewing' compartment may have resulted from early studies of fracture dynamics which used foods or food analogues that were prone to simple fracture, and generated particles that did not adhere

to each other (van den Braber *et al.* 2002; Lucas *et al.* 1987; van der Glas *et al.* 1987; Schneider & Senger 2001). Subsidiary compartments (non bolus) are postulated to be generated only during the chewing of sticky foods (Lucas 2004), although there is evidence that this 'loss' of solid material during chewing for a range of food types, with generally less than sixty percent of particulates recovered in the swallowable bolus (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007). This "loss" is evident even when insoluble foods such as coffee beans are chewed (Schneider & Senger 2001). There have been no published studies comparing the PSD of the bolus and debris, and where debris has been collected following the expectoration of the bolus, it has been added to the bolus for subsequent particle size analysis (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007; Mishellany *et al.* 2006; Ohara *et al.* 2003).

The extent to which particles are retained in subsidiary compartments is likely to be influenced by their physical properties, e.g., their size. It is known that larger particles generated during the mastication of some foods may lodge in interproximal spaces (Newell *et al.* 2002; Every *et al.* 1998), and, for example, that grain particles smaller than 0.25 mm do not become impacted between the teeth of rats (Hoppert *et al.* 1932). Particle properties such as tribology, coherence and viscoelasticity (Hiiemae *et al.* 1996; Hiiemae 2004; Mioche *et al.* 2002; Thexton 1992) are also likely to affect compartmentalisation; they may either promote adherence to dental and mucosal surfaces and lodgement between teeth (Newell *et al.* 2002; Every *et al.* 1998; Hoppert *et al.* 1932; Konig 1961; Brudevold *et al.* 1990; Kashket *et al.* 1991; Kilcast & Roberts 1998) or aid the binding of particles within a bolus (Kilcast & Roberts 1998; Dunnewind *et al.* 2004).

If the physical characteristics of particles generated from a given food do not lead to subsidiary compartmentalisation, i.e., the mouth operates as a single compartment, then the PSD of the material within the mouth will be uniform regardless of the site from which it is sampled. Conversely, if subsidiary compartmentalisation has occurred then the PSD will vary between the compartments, i.e., with site of sampling, given that the pattern of PSD changes rapidly during early chewing (Lucas 1994; Rensberger 1973).

The purpose of this work was to investigate the effect of human mastication on the breakdown of solid foods of a range of chemical compositions and homogeneity. To determine the extent to which subsidiary compartments are formed by comparing differences in PSDs between the expectorated bolus and remaining debris. If the bolus and debris PSD are significantly different, it is hypothesized that a two compartment system exists where particles are comminuted in at least one compartment within the oral cavity. The effect of food portion size on compartmentalisation was also examined. Some of the results from this chapter have been presented in Flynn *et al.* (2011).

## 4.2 MATERIALS AND METHODS

Five foods were chosen with a differing range of chemical composition and homogeneity (Sections 3.1 & 3.3): cake, muesli bar, cereal bar, pasta, peanuts. The food samples were standardised by weight, either 2 g or 4 g, and actual weight noted (Section 3.2). Food moisture content (Equation 3.1) was measured in duplicate for each food type per session and used to calculate the quantity of ingested solids (Equation 3.6).

The samples presented per session were randomly selected for each subject, and then ordered randomly for each study session following the study design (Section 3.7.1). Ten male subjects were recruited through the process outlined in Section 3.6. Overall, each sample was assessed in triplicate over four study sessions following the session protocol described in Section 3.7.3. A total of 60 bolus and debris samples were collected from each subject for analysis. The bolus and debris samples were immediately stabilised (Section 3.4.8) as collected. At the end of the session all the samples were stored pending sieve analysis.

The determination of the PSD was by the finalised wet sieve method described in Section 3.4.6. The mass of particles on each of the sieves was calculated as a percentage of the total solid matter ingested. The total mass of particles in each of the bolus and debris fractions were calculated by combining the mass of particles retained on each of the sieves. The total dry mass of solids recovered was found by summing the total mass of each of bolus and debris fractions together. The percentage of ingested solids recovered was calculated from the mass of solids recovered and the initial solids

content of the ingested sample. The results were statistically analysed following the procedures described in Section 3.5.

## 4.3 RESULTS

### 4.3.1 MASS RECOVERED IN BOLUS AND DEBRIS COMPARTMENTS

The total dry mass of solids recovered on the sieves, expressed as a percentage of the dry matter ingested was incomplete for all food types. It varied from 14 to 61% between foods, with the bulk being recovered in the bolus (Figure 4.1) (Table 4.1). The bolus and debris sieve pan washings were separately dried and calculated to be approximately 8 – 45% of the total solids recovered from the sieving process depending on the food type (Figure 4.2). The pan washings were composed of soluble solids and very small particles <0.125 mm, and these were excluded from further calculations as the focus of this study was the PSD. Also the method to retrieve these solids was significantly different from that used for the particles on the sieve fractions used for determining PSDs.

The ratio of debris to bolus solids data (Table 4.1) differed significantly between food types ($F(4,90) = 11.1$, $P<0.001$). Cake, cereal bar and peanuts had similar ratios (0.26-0.35 debris:bolus) while muesli bar produced more debris (ratio of 0.55) and pasta produced less debris (ratio of 0.15). The ratio of debris to bolus solids also shows a general trend that there was a higher proportion of debris for the 2 g portion size compared to the 4 g portion (0.36 compared to 0.30 respectively), indicating that proportionately less solids were lost to the debris compartment when a larger portion size was masticated. But overall there was no significant difference between the portion sizes ($F(1,90) = 2.86$, $P=0.094$).

The mass of debris solids recovered was significantly greater in the 4 g portion compared to the 2 g portion, except for cake (Table 4.1). This may be expected since double the quantity of food was ingested, but the mass of debris recovered was only double for muesli bar and pasta, and not for the other food types.

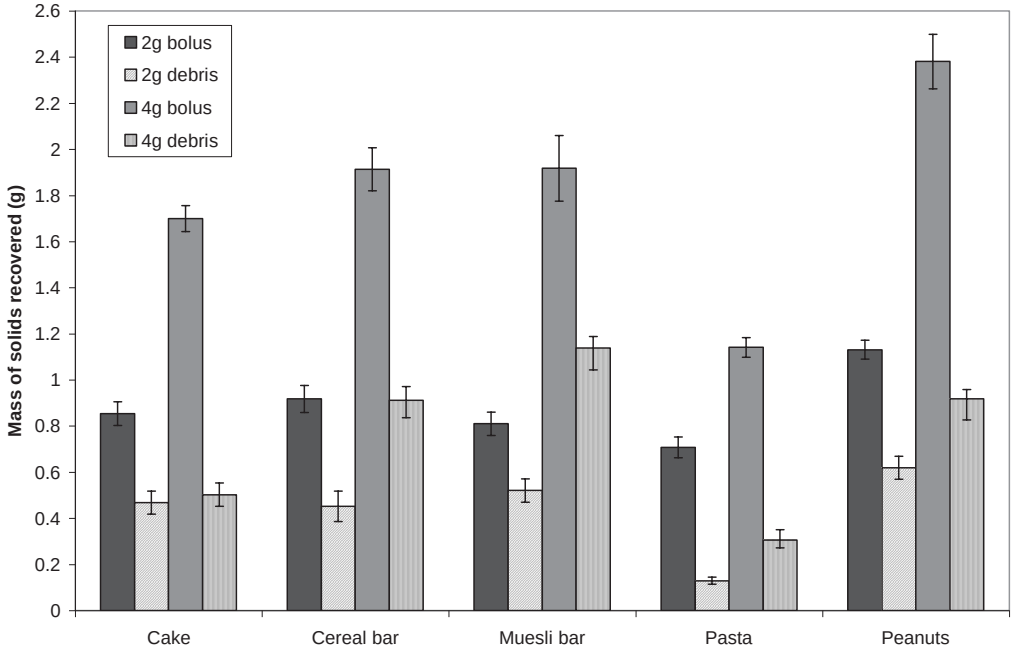



**Figure 4.1** **Quantity of solids recovered in the bolus and debris compartments for each food**

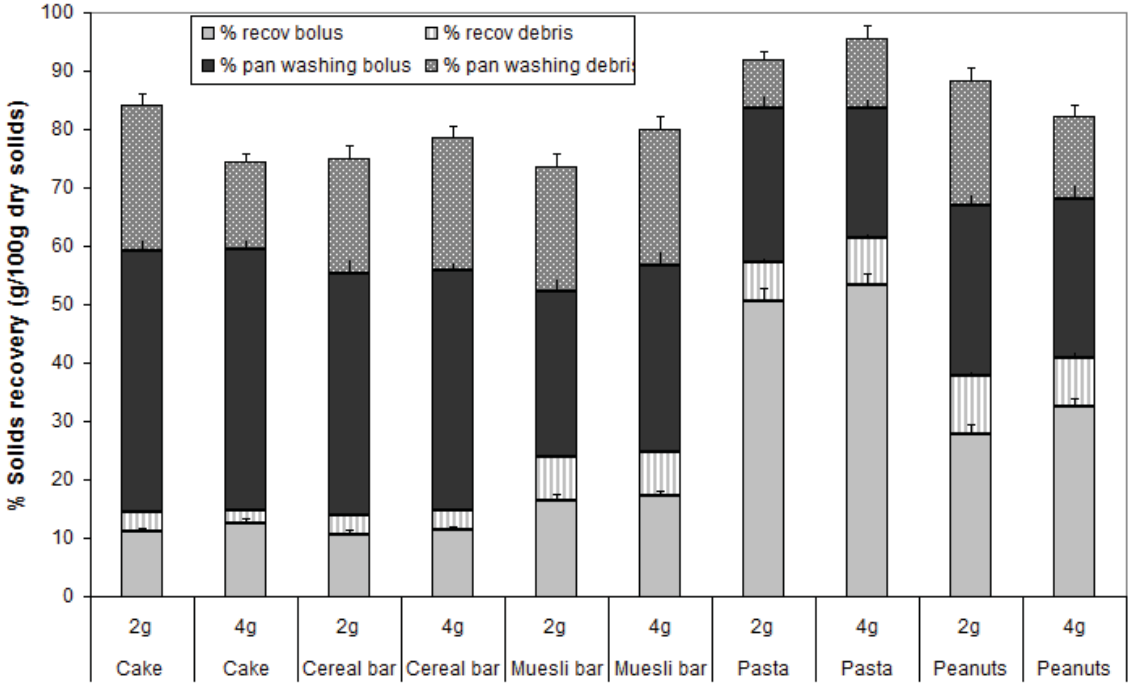



**Figure 4.2** **Sieve pan solids as a proportion of total recovered solids for bolus and debris compartments for each food**

**Table 4.1** **Recovery of solids into the bolus and debris compartments**

| | Cake | | Cereal bar | | Muesli bar | | Pasta | | Peanuts | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2g** | **4g** | **2g** | **4g** | **2g** | **4g** | **2g** | **4g** | **2g** | **4g** |
| **Solids recovery (%)** | 14.4±0.7 | 14.7±0.9 | 13.9±0.7 | 14.4±0.5 | 23.8±0.9 | 24.7±0.7 | 57.2±2.2 | 61.4±2.0 | 37.5±1.8 | 41.0±1.4 |
| **Mass of dry debris solids (g)#** | 0.05±0.004 a | 0.06±0.006 A | 0.06±0.005 a* | 0.1±0.013 AB* | 0.13±0.01 b* | 0.27±0.025 C* | 0.06±0.006 a* | 0.12±0.015 B* | 0.19±0.015 b* | 0.34±0.039 C* |
| **Ratio of debris to bolus solids #** | 0.33±0.04 b | 0.19±0.02 AB | 0.34±0.04 b | 0.30±0.05 B | 0.58±0.10 b | 0.53±0.08 C | 0.14±0.02 a | 0.16±0.02 A | 0.40±0.05 b | 0.30±0.05 BC |

Significant differences between food types within each portion size are indicated by different letters (a,b,c or A,B,C,D for 2 or 4 g portion sizes respectively).

* indicates a significant difference between portion sizes within the same food.

# ANOVA and post hoc Bonferroni testing performed on log transformed data.

### 4.3.2 PARTICLE SIZE DISTRIBUTIONS (PSDS)

The average PSDs for each food type are shown in Figure 4.3 (A–E). Differences between these distributions by food type are apparent, for example, with cake having an overall smaller particle size and pasta having an overall larger particle size. There also appears to be differences within foods between portion size and bolus and debris PSDs. Principal components analysis showed variation on three orthogonal axes (i.e. 3 components were identified: PC1, PC2, PC3) which together accounted for 83% of the total variance (50, 23 and 10% for PC1, 2 and 3 respectively). The components described the PSDs as follows (Figure 4.4 (A-C)): PC1 contrasts the proportion of particles 0.5 mm and below with those 1.4 mm and above (thus a high PC1 score indicates a larger proportion of finer and smaller proportion of larger particles); PC2 contrasts the proportion of particles >2.8 mm with those between 0.71 and 2.0 mm (thus a high PC2 score indicates a larger proportion of particles >2.8 mm than those in the fractions 0.71-2.0 mm); PC3 contrasts the proportion of particles in the 0.125 mm fraction with those in the 0.5 mm fraction (thus a high PC3 score results from a low proportion of particles at 0.5 mm compared to those in the 0.125 mm fraction).

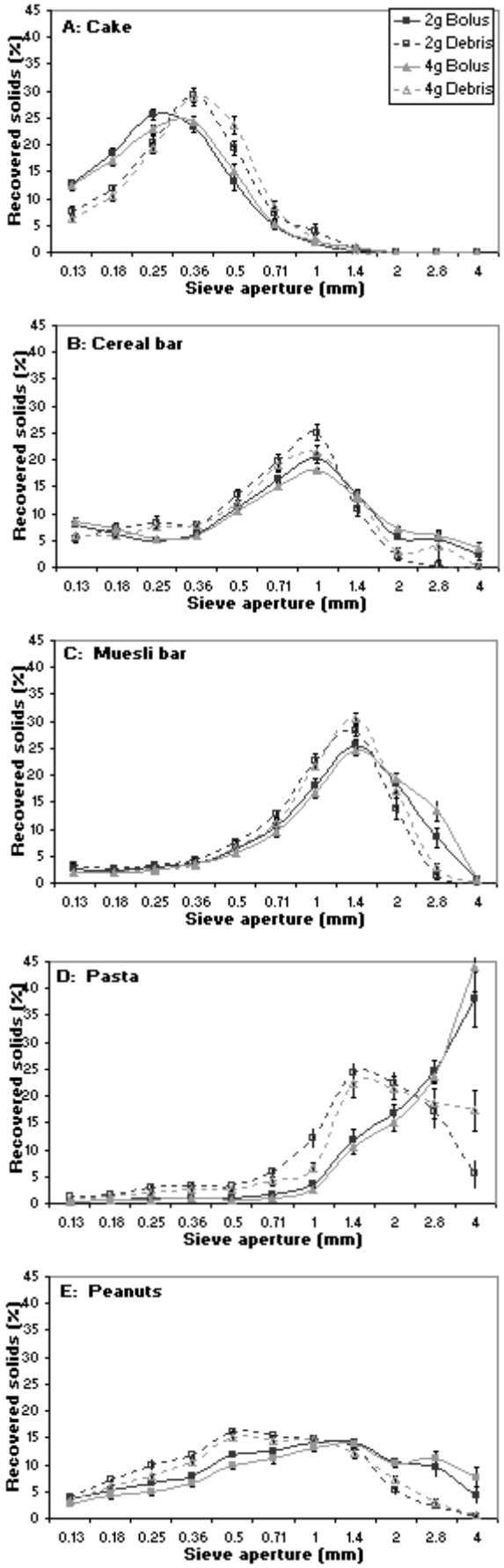
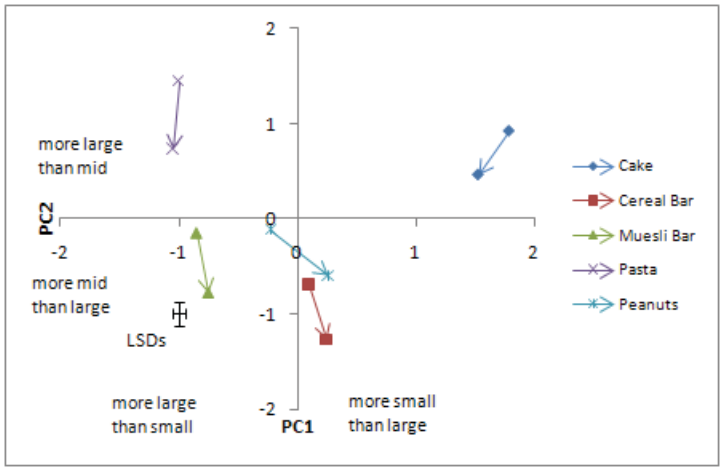
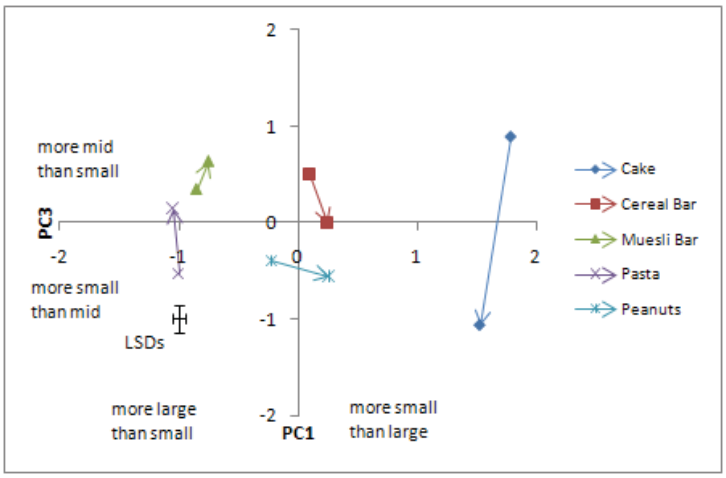



**Figure 4.3 (A-E) Comparison of mean PSD of bolus and debris compartments for each food.**

**A.**




**B.**




**C.**

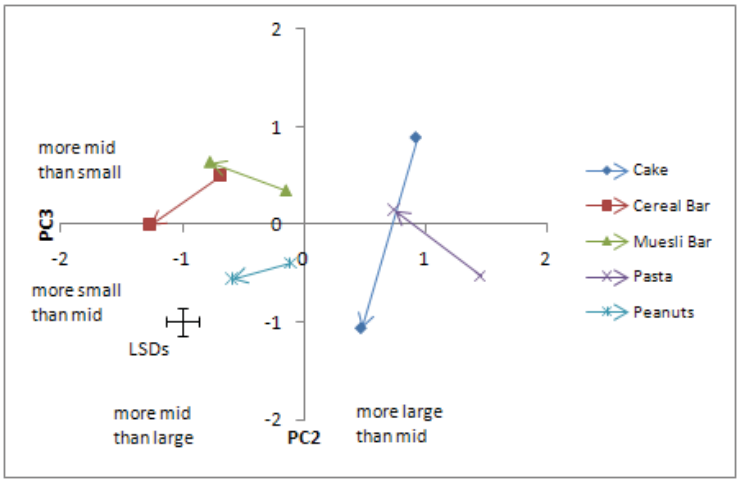



**Figure 4.4(A-C) Principal component (PC) plots of mean PC values for each food type. Arrows on the lines point from the bolus mean to the debris mean. A. PC2 vs PC1. B. PC3 vs PC1. C. PC2 vs PC2. Means that differ by more than the LSD (least significant differences) are significantly different. Comments, such as "more large than mid" refer to PC values corresponding to more large sized than mid sized particles.**

#### 4.3.2.1 EFFECT OF FOOD TYPE ON BOLUS PSDS

Overall the PSDs of the food boluses were all significantly different between the food types ($F(4,81) = 487.9$ for PC1, 92.6 for PC2 and 15.8 for PC3, $P<0.001$ for all PCs) (Figure 4.3). Cake boluses contained the largest proportion of small particles (<0.5 mm) with almost no particles above 1.4 mm. Pasta showed a reverse trend to cake with almost all particles being greater than 0.71 mm in size. Muesli bar also had a relatively larger quantity of mid range particles in the PSD compared to the other foods tested, with the majority of particles between 0.71 – 2.8 mm. Cereal bar had most particles in the range of 0.355 to 2 mm, although there were significant proportions of particles outside that range. Peanuts showed a broader distribution with most particles being above 0.5 mm in size.

#### 4.3.2.2 EFFECT OF FOOD TYPE ON DEBRIS PSDS

Overall, the debris fraction PSDs were significantly different for each food type ($F(4,81) = 398.9$ for PC1, 75.7 for PC2, 21.2 for PC3, $P<0.001$ for all PCs). The general trends observed in section 4.3.2.1 for the bolus samples were also evident in the debris samples for both 2 and 4 g portion sizes (Figure 4.3).

#### 4.3.2.3 COMPARISON OF BOLUS AND DEBRIS PSDS

Significant differences were identified between the bolus and debris fractions for all food types (Figure 4.3) ($F(1,90) = 12.6$ for PC1, 114.6 for PC2, 28.2 for PC3, $P<0.001$), although for PC1 and PC3 there were also significant food type x bolus/debris interactions ($F(4,90) = 25.4$ for PC1, 52.8 for PC3, $P<0.001$). Debris from all foods contained a significantly higher proportion of particles in the 0.5 – 1.0 mm range and a lower proportion of particles >2.8 mm compared to the bolus (Figure 4.3). For individual foods other trends were also significant, cake and cereal bar had less small particles (<0.18 mm) in contrast to a higher proportion in the mid-range (0.355 - <1.4 mm) in the debris, with the opposite trend occurring in the bolus (Figure 4.3 (D-E)).

### 4.3.3 THE EFFECT OF PORTION SIZE

There was no significant effect of food portion size on bolus particle sizes ($F(1,81) \leq 2.2$ for all PCs, $P \geq 0.144$). For the debris, the 4 g portion size contained a significantly higher proportion of large particles (>2 mm) and a lower proportion of small particles

(<0.5 mm) compared to the 2 g portion ($F(1,810) = 4.3$, $P=0.042$ on PC1)) (Figure 4.3). The portion size x food type interactions were not significant for the bolus or debris.

### 4.3.4 SUBJECTS

Variations between subjects, between PSDs for different foods and between bolus and debris, were larger than the corresponding replicate to replicate variation within subjects (variance ratios range from 6.8 to 0.9). But these variations were smaller than the difference between food types, or between bolus and debris. (All *F* ratios quoted above are relative to the subject to subject variation.)

## 4.4 DISCUSSION

### 4.4.1 LOSS OF TOTAL SOLIDS

The quantity of total solids recovered by the wet sieving technique was significantly lower than that ingested, and was of similar levels to those reported by other researchers (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007). This loss probably results from a combination of processes: the dissolution of soluble components in the foods during oral processing and wet sieving (cake and cereal bar contained the highest concentration of sugar and had the highest loss of solids); the melting and extraction of fats; the loss of material finer than the smallest sieve (<0.125 mm); transport of the bolus to the oropharynx prior to swallowing; and intermediary swallows (Hiiemae *et al.* 1996; Hiiemae 2004; Mioche *et al.* 2002; Hutchings *et al*. 2011). A high level of solids was captured in the sieve pan, this data was excluded from the PSD analysis as it was composed of soluble solids and fine particles, and not processed in the same way as the sieve fractions (Figure 4.2). This data highlights the importance of dissolution of nutrients in oral processing and the limitations of wet sieving methods which may wash out additional solids, and assess only particulate material. Also, although the quantity of solids recovered in the debris is smaller than in the bolus, it is a significant proportion of the total solids recovered.

### 4.4.2 VARIATION IN BOLUS PARTICLE SIZE DISTRIBUTIONS WITH FOOD TYPE

The significant variation in bolus PSDs produced from different food types supports previous findings (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007; Jiffry & Molligoda 1983; Lucas & Luke 1984; Hoebler *et al.* 2000; Fontijn-Tekamp *et al.* 2004) in that there is no particle size threshold for swallowing. The dynamics of dissolution and particle fracture are likely to operate in different ways that are dependent on the physical properties of the food that is being consumed. Cake and cereal bars consist of inherently small particles bound together in a soluble matrix by relatively weak bridges. The low TPA chewiness scores (Table 3.6) for these foods indicate that these matrices may be readily broken down to give distributions dominated by these small particles. Breakdown would be further enhanced by the relatively high soluble solids content.

Pasta is more cohesive, very adhesive, has a higher TPA chewiness score (Table 3.6) and a sparse soluble matrix so that the generation of finer particles requires greater comminution. The low cohesiveness and chewiness scores of peanuts suggest they are brittle and readily comminuted. The cereal and muesli bars had very similar TPA scores except for the muesli bar having greater adhesiveness. The less homogenous muesli bar matrix contains relatively large intact ingredients (grains, raisins etc.) bound together and set with a sugar syrup. This suggests an initial phase during mastication where dissolution of the sugar releases the larger particulate material followed by breakage of these particles.

### 4.4.3 BOLUS VERSUS DEBRIS

The differences in PSD between bolus and debris support the hypothesis that the resultant PSD of a food depends on the dynamics of the mouth as a multi-compartment rather than a single compartment system. Comminution occurs in at least one compartment (i.e. on the molars), but not necessarily to food present in the other compartments (e.g. interproximal spaces) although moisture adsorption and dissolution of soluble materials are likely to occur in all compartments. The general trend across all foods that debris contained greater proportions of mid-range particles than the bolus, suggests that whilst particles are retained in this compartment they are withheld from

the occluding surfaces. Particles over 4.0 mm in size were found in debris from both peanuts and pasta. The PSD of the debris may be the result of optimum bolus cohesion requiring particles of a wider range of PSD, therefore the mid-range particles may be 'ignored' for retention in the debris compartment.

One of the mechanisms for particles to move into the debris compartments, are particles sticking to the teeth. In this case the tooth area for this to occur is finite and therefore it is possible that the mass of particles that can be lost into the debris compartment is also finite. The variation between the PSD of the bolus and debris that occurs between food types indicates that either the amount of particulate material retained, or the duration of its retention in non-chewed compartments, may be influenced by the physical properties of the food. It is noteworthy that some degree of compartmentalisation between debris and bolus was observed in all foods in this investigation, despite their differing initial physical and chemical properties.

Examination of the variations in the ratio of debris to bolus solids (Table 4.1) with food properties (Tables 3.3, 3.4 & 3.6) gives some insight into the mechanisms that operate to retain particles in the non chewed compartments. In the high sugar content foods, such as muesli bar, debris particles had a greater chance of being retained in non-chewed compartments whilst debris from lower sugar content foods, such as pasta, was not so readily retained. However, debris from other low sugar content food, e.g. peanuts, were also retained in non chewed compartments to a greater extent than pasta. This may have been due to compaction i.e. elastic elements being packed into non chewed compartments such as the fissures in the molar surfaces.

It is likely that the debris compartment in the masticatory system itself consists of multiple components. Some particles are likely to stick to teeth or mucosal surfaces, other particles will become impacted into molar fissures, others will re-join the bolus in the oral cavity, and yet others will simply lodge in non mixing zones of the oral cavity. The degree to which debris particles adhere will depend on the relative force balance between adhesive (between food and surface) and cohesive (interstitial to food) forces and other forces active during mastication, for example compression, suction, and shear together with the tribological properties of the bolus. It is also possible that liquid

surface tension affects the internal and external properties of the bolus. Of the particles that adhere to the teeth, it is possible that these preferentially originated from larger particles (Newell *et al.* 2002; Every *et al.* 1998; Hoppert *et al.* 1932).

It is not known what happens to the debris if a subsequent bite of food occurs, as in eating a meal or the entire muesli bar. The debris proportion could reach a level of saturation where no more debris is added to a particular compartment, or the debris portion could circulate continuously and join the bolus portion while other material moves to the non-chewed compartments. At the end of a meal, a clearance stage (Hiiemae *et al.* 1996; Hiiemae 2004; Okada *et al*. 2007; Mioche *et al.* 2002) would be expected and this debris could be subjected to further chews prior to swallowing. In future work, it is of interest to identify what happens when subsequent portions of foods are consumed, and to determine whether these particles re-circulate, reducing further in size and aiding bolus formation. Or to find out whether, once a proportion of particles are lost to non chewed compartments, the majority of these stay until the end of the feeding sequence when they are removed with clearance.

### 4.4.4 PORTION SIZE

When portion size increased, the mass of solids in the bolus also increased. The PSDs were slightly influenced by portion size; the resulting PSD shifted to a higher proportion of larger particles with an increase in portion size, however, this was only significant in the debris fraction. This indicates that particle fracture, matrix dissolution and liberation of fine particles occurs in larger boluses with equal efficiency to smaller boluses; this would need to be assessed for portion sizes >4 g**.**

### 4.4.5 IMPLICATIONS

This work demonstrates that the debris is a significant proportion of the total mass of solids recovered and, having a different PSD to the bolus, should not be excluded from particle size analysis if the aim of research is to understand food breakdown in the mouth and the fate of food particles. To gain understanding about the swallowable bolus PSD it is not appropriate to combine the bolus and debris fractions as this will significantly modify the result, and is not representative of the bolus at the swallowing point. Although it is likely that most of the debris fraction will be swallowed at some

point during or after a meal, it is unknown what parameters are met prior to swallowing of the debris.

## 4.5 CONCLUSIONS

The significant differences found between the PSDs of the expectorated boluses and debris for all foods investigated suggests that mastication occurs within a multi-compartment system where particles are comminuted in at least one compartment. Overall, increasing mouth fill resulted in an increase in larger particles, although this was only significant for the debris fraction. The complex process of forming a swallow safe bolus involves not only particle size reduction as discussed in this chapter, but also the processes of moisture addition and loss of solid particles from the bolus. These two processes are investigated in Chapter 5 to gain knowledge about the swallowable bolus in relation to the formation of a cohesive bolus (Prinz & Lucas 1997).

# CHAPTER 5: EFFECT OF MASTICATION OF SOLID FOOD ON BOLUS MOISTURE CONTENT AND LOSS OF SOLIDS

## 5.1 INTRODUCTION

In Chapter 4, five different processed foods' bolus and debris solids were recovered at the natural swallowing point for a group of subjects, and it was noted that in the fractions only 8 – 45% of the ingested solids were recovered. These fractions were then separately analysed to determine their PSDs by wet sieving and a significant difference was found between them, suggesting that mastication occurs within a multi-compartment system. The methodology applied in Chapter 4, may have led to a decrease in the total solids recovered, so a method with less handling of the particles should provide more details on the concurrent processes of bolus solids loss and moisture addition.

Food acceptance has been linked to the matching of perceived and expected food breakdown dynamics (Guinard & Mazzucchelli 1996). Where expectations are unfulfilled, texture defects are detected and acceptance is compromised. To characterise the dynamics of food breakdown, it is first necessary to understand the bolus properties required for swallowing, the end point of mastication. With this knowledge, insight can be gained into how food properties change during chewing and how the masticatory process is adapted in response to these changes. Many theories have been proposed which speculate what properties of the bolus must be detected before swallowing is initiated, although it is generally accepted that the primary purpose of mastication is the production of a swallow-safe bolus (Coster & Schwarz 1987; Nishinari 2004).

Two accepted theories are the dual threshold model and the cohesion model. The dual threshold model determines swallowing to occur when a requisite level of particle breakdown has occurred or a particular level of lubrication has been reached (Hutchings & Lillford 1988). In contrast, the cohesion model stipulates that swallowing occurs

when the cohesion forces between particles within the bolus are higher than their adhesion to the oral mucosa. Prinz & Lucas (1997) demonstrated this for Brazil nuts and carrots using a simple numerical model. The theory has not been extended to other food types, most probably because of the complex interactions between added saliva and most food particles that occur during the agglomeration process of food bolus formation.

Cohesion within particulate agglomerates has been studied extensively in the granulation literature (Iveson *et al.* 2001; Iveson *et al.* 2002). Moisture causes liquid bridges to form which, with increasing moisture, advance through the pendular, funicular and eventually capillary states. Cohesion increases with moisture content because the internal interfacial surface area is increasing but, as the capillary state approaches, the direction of change in cohesion depends on the relative importance of inter-particle friction. Fine particles have high inter-particle friction and so a viscous binder acts as a lubricant; adding more moisture will cause cohesion to decrease. For coarse agglomerates inter-particle friction is negligible and so capillary forces dominate; adding more moisture will cause cohesion to increase. Cohesion is also greater when the void volume is lower. With further moisture addition, saturation may be reached beyond which the particles disperse with shear as typical in slurry flow.

The amount of moisture required for these different states, and the resulting cohesion strength, is dependent on the properties of the particles, their size, shape and the void volume (Iveson *et al*. 2002). Unlike granulation, during oral processing particle size is reduced and bolus solids are lost (Chapter 4), which is likely to cause subsequent changes in the packing of particles. For this reason, the cohesive state of a food bolus will depend on three rate processes: particle size reduction, loss of solids and saliva addition to form liquid bridges.

The relative rates and the extent of salivation vary with food composition and structure, and between people (Brudevold *et al.* 1990; Gaviao *et al.* 2004; Engelen *et al.* 2005; Neyraud *et al.* 2003; Mioche 2004; Watanabe & Dawes 1988b). In many foods, moisture is absorbed as particles hydrate, thereby making it unavailable for liquid bridging. Also, added moisture may dissolve soluble components from food particles

during mastication (Watanabe & Dawes 1988a; Wright & Hills 2003). Such dissolution affects the formation of liquid bridges in two ways; first, moisture is associated with the soluble solids washed out of the food, thereby reducing the amount that could be available to form liquid bridges; and second, the removal of soluble solids alters the size and structure of the particles remaining in the food bolus. The rates of both hydration and dissolution are likely to increase with decreasing particle size during mastication (Wright & Hills 2003).

In the food systems studied by Prinz & Lucas (1997), hydration or dissolution were not likely to be important. Particles such as carrots are already saturated with moisture so any moisture added will participate in liquid bridging. In nuts or foods high in oils, the rates of moisture uptake will be slower than more porous food particles. If masticated for a very long time it might be expected that all food systems will tend towards a collection of insoluble particles (washed of all soluble materials), bound together by liquid bridges. The degree to which this occurs will be a function of the net rate of each of the following processes: size reduction, bolus solids loss, moisture addition, dissolution, and particle hydration. These processes then determine the amount of moisture available for liquid bridging and as a result the cohesive state of the bolus. In this work this mechanism is explored by assessing the masticatory outcomes for a number of different foods. For each food the following was assessed: the moisture changes that occur, bolus solids loss, the extent to which the bolus particles are saturated and soluble solids are washed out, and infer the relative importance of these processes on bolus formation. Some of the results from this chapter have been prepared in a manuscript for publication (Flynn *et al.* (2012). Manuscript under review).

## 5.2 MATERIALS AND METHODS

Five foods were chosen with a differing range of chemical composition and homogeneity (Sections 3.1 & 3.3): cake, muesli bar, cereal bar, pasta, peanuts. In particular the water holding capacity of the foods was measured (Table 3.5). The food samples were standardised by weight, either 2 g or 4 g and actual weight noted (Section 3.2). Food moisture content (Equation 3.1) was measured in duplicate for each food type per session to ensure that the most accurate data was used to calculate the quantity of ingested solids (Equation 3.6).

The samples presented per session were randomly selected for each subject, and then ordered randomly for each study session following the study design (Section 3.7.1). The samples collected for this study were collected at the same time as the samples for Chapter 4. Seven male subjects were recruited through the process outlined in Section 3.6. Each sample was assessed in duplicate over four study sessions following the session protocol with the recording of chewing data for all samples, described in Section 3.7.3. A total of 20 bolus samples were collected from each subject for analysis. The samples were processed immediately after expectoration, following the procedure described in Section 3.4.9. The moisture content of the bolus was calculated on a dry mass basis of the sample (Equation 3.7) and the percentage loss of solids from the bolus was determined by Equation 3.8. The results were statistically analysed following the procedure in Sections 3.5.2 and 3.5.3., and Pearson's correlations were investigated for evidence of relationships between parameters. A proximate measure of saliva flow rate (g/s) was calculated by dividing the moisture content of the bolus by the chew time.

### 5.2.1 VARIANCE TESTS FOR BOLUS MOISTURE CONTENT

A second study was carried out with five of the subjects who participated in the main study to monitor the variance in results produced. Subjects attended an extra study session where they chewed and expectorated samples of the five food types, in triplicate, for one portion size only. A total of 15 bolus samples were collected from each subject for analysis, following the procedure described in Section 3.4.9. Bolus moisture content and solids loss were determined by Equations 3.7 and 3.8. The results were statistically analysed following the procedure in Section 3.5.4.

## 5.3 RESULTS

### 5.3.1 CHEWING BEHAVIOUR

The chew time ($F(1,54) = 131$, $P < 0.001$) and number of chew cycles ($F(1,54) = 117$, $P < 0.001$) varied significantly with portion size, with larger portion sizes requiring a greater number of chew cycles and time (Figures 5.1 & 5.2). Although not directly proportional, as may be expected, the chew time and number of cycles do increase with

an increase in the portion size of the food. This effect was also seen by Gaviao *et al.* (2004) who found that chew time and number of cycles increased with the volume of the food portion but not in direct proportion to the increase in food volume. Because there was a strong correlation (r = 0.997) between chew time and chew number to reach the swallow point for each of the food types (Figure 5.3), only chew time was analysed in any further detail. Overall there was a significant difference in the chew time with food type ($F(4,54) = 24.6$, $P < 0.001$). Cake (fastest chew time) and peanuts (slowest chew time) were both significantly different from all other food types. There were no significant differences between cereal bar, muesli bar and pasta (Table 5.1). All subjects followed these trends although there was variation between subjects ($F(6,54) = 93.4$, $P < 0.001$). Overall the chew frequency was significantly different for food type ($F(4,54) = 7.07$, $P < 0.001$), and did not vary with portion size ($F(1,54) = 3.06$, $P = 0.086$) (Table 5.1). All subjects followed these trends, although there were significant differences between subjects ($F(6,54) = 32.64$, $P < 0.001$).

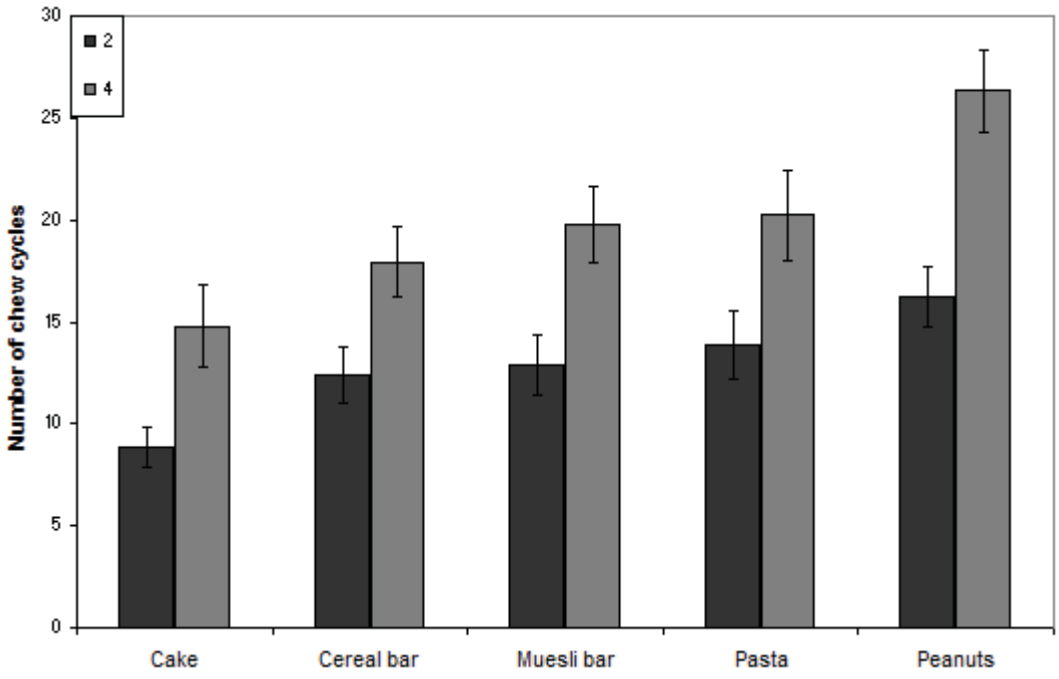



**Figure 5.1** **Food type and portion size effect on mean number of chew cycles (±SEM) to form a swallowable bolus.**

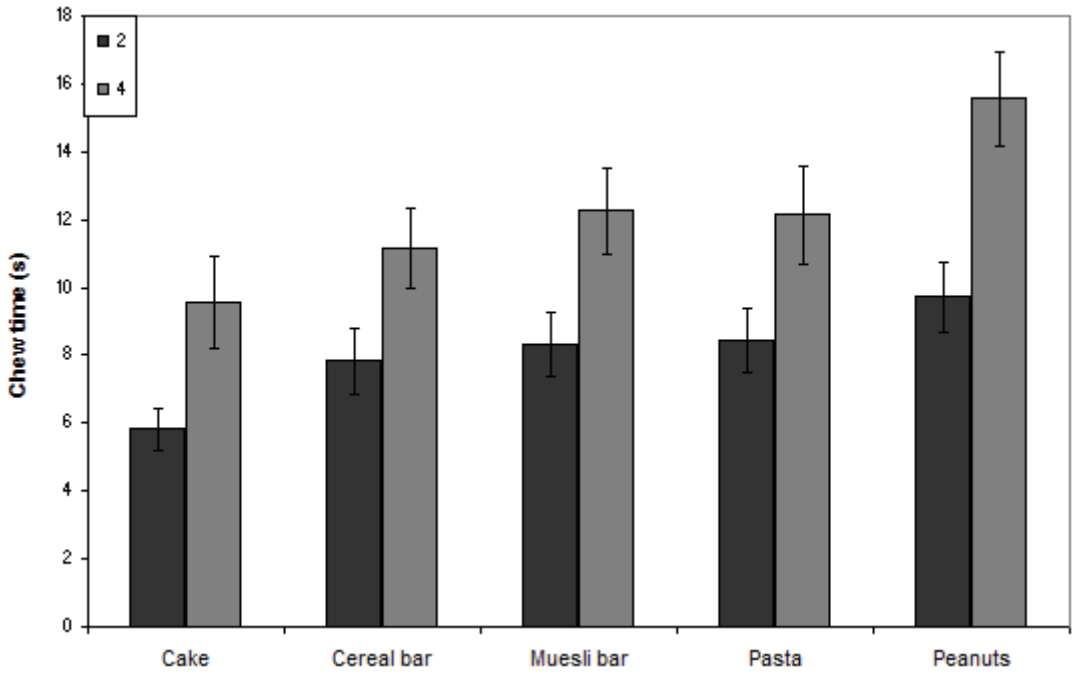



**Figure 5.2** **Food type and portion size effect on mean chew time (±SEM) to form a swallowable bolus.**

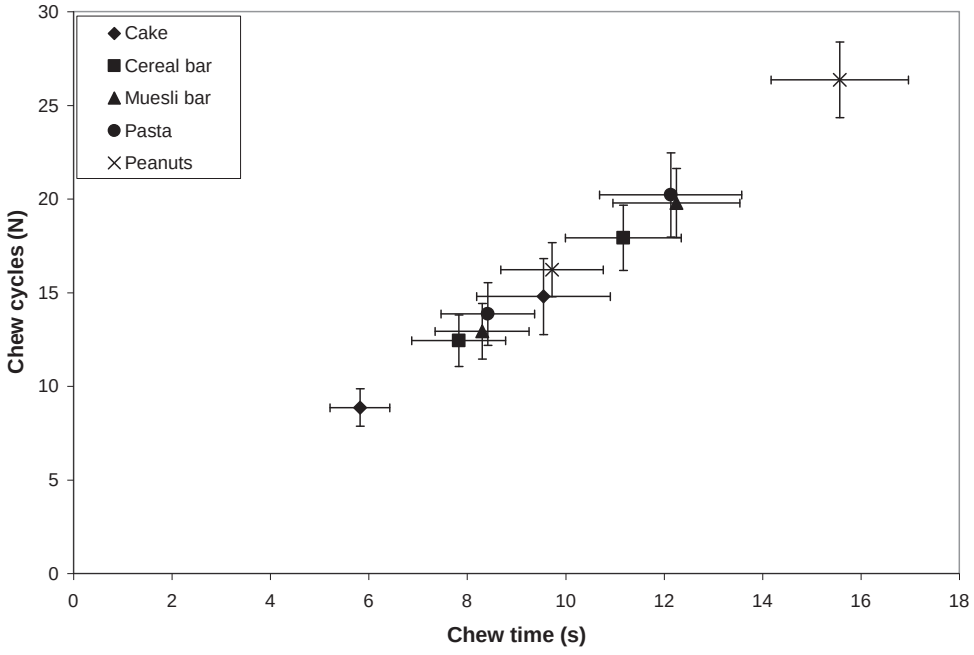



**Figure 5.3** **Correlation between chew time and number of chew cycles for each food type and portion size. Mean data (±SEM).**

**Table 5.1** **Statistical significance of the effects of food type and portion size on the mean (±SEM) number of chew cycles, chew time, chew frequency, saliva flow rate, bolus loss of solids and bolus moisture content.**

| Food type and portion size (g) | | Chew cycles | Chew time (s) | Chew frequency (1/s) | Saliva flow rate (g/s) | Loss of solids (g/100g dry solids) | Bolus moisture content (g/100g dry solids) |
|---|---|---|---|---|---|---|---|
| **Cake** | **2** | 8.86±1.00 a | 5.82±0.61 a | 1.52±0.07 a | 13.3±1.69 a | 26.83±2.23 a | 66.37±3.85 a |
| | **4** | 14.79±2.03 b | 9.55±1.36 b | 1.54±0.06 a | 8.50±1.46 b | 21.11±2.89 a | 60.28±2.80 a |
| **Cereal bar** | **2** | 12.43±1.37 c | 7.83±0.95 c | 1.61±0.07 a,b,c | 7.45±1.53 c | 28.06±4.19 a | 46.31±3.05 b |
| | **4** | 17.93±1.74 d | 11.17±1.18 d | 1.62±0.06 a,b,c | 4.49±0.42 d | 26.72±4.22 a | 45.57±3.74 b |
| **Muesli bar** | **2** | 12.93±1.48 c | 8.31±0.95 c | 1.56±0.06 a,b | 6.92±0.74 c | 42.49±5.24 b | 51.34±4.94 b |
| | **4** | 19.79±1.84 d | 12.25±1.29 d | 1.65±0.06 a,b | 4.59±0.60 d | 36.36±3.84 b | 50.22±5.47 b |
| **Pasta** | **2** | 13.86±1.67 c | 8.42±0.95 c | 1.63±0.04 b,c | 30.6±4.49 e | 15.07±2.56 c | 213.05±8.48 c |
| | **4** | 20.21±2.25 d | 12.14±1.44 d | 1.69±0.06 b,c | 21.03±2.95 f | 18.57±4.10 c | 210.76±12.72 c |
| **Peanuts** | **2** | 16.21±1.45 e | 9.72±1.04 e | 1.71±0.05 c | 4.88±0.56 d | 30.36±2.49 a,b | 42.00±3.88 b |
| | **4** | 26.36±2.02 f | 15.57±1.40 f | 1.74±0.06 c | 3.12±0.29 h | 32.47±3.14 a,b | 45.95±4.11 b |

Values with different letters (a,b,c) on a vertical line are significantly different after a two-way stratified ANOVA and post hoc Bonferroni test.

### 5.3.2 MASS OF BOLUS RECOVERED

The mean mass of the wet bolus expectorated for most of the different food types and portion sizes studied resulted in a decrease compared to the mass of the sample ingested (Table 5.2). Only the 2 g portion of pasta resulted in a mean increase in mass, with 4 g cake showing no change between the mean masses of samples. The 4 g portion of muesli bar showed the greatest losses with the mean wet bolus mass only 77% of the ingested mass, which could be due to the heterogeneous composition of the product, as it is made up of a range of ingredients of a range of sizes bound in a sugar matrix. When the sugar is dissolved this results in a loss of soluble solids, and releases the particulate ingredients of the muesli bar which may be more readily trapped in oral sulci, leading to the result of greater loss of solids from the bolus.

**Table 5.2** **Wet mass (±SEM) of sample ingested, bolus expectorated and the percentage change in mass.**

| Food Type | Portion size (g) | Mass ingested (g) | Bolus mass (g) | Change in mass (%) |
|---|---|---|---|---|
| Cake | 2 | 1.73±0.05 | 1.66±0.05 | -4.0±2.4 |
| | 4 | 3.85±0.09 | 3.85±0.14 | 0.1±2.9 |
| Cereal bar | 2 | 2.11±0.04 | 1.87±0.09 | -10.6±4.3 |
| | 4 | 4.00±0.04 | 3.73±0.19 | -6.8±4.6 |
| Muesli bar | 2 | 1.97±0.04 | 1.51±0.12 | -23.3±5.9 |
| | 4 | 4.07±0.06 | 3.49±0.24 | -13.9±6.0 |
| Pasta | 2 | 2.15±0.04 | 2.22±0.06 | 3.5±3.0 |
| | 4 | 4.06±0.08 | 3.89±0.23 | -4.8±4.8 |
| Peanuts | 2 | 2.06±0.03 | 1.98±0.06 | -3.8±2.9 |
| | 4 | 4.09±0.03 | 3.93±0.20 | -3.8±4.7 |

### 5.3.3 SOLIDS LOSS

Overall the fraction of solids lost from the bolus varied significantly with food type ($F(4,54) = 11.76$, $P < 0.001$) (Figure 5.4). Pasta differed from all other food types with the lowest loss of solids, and muesli bar differed from all food types except peanuts, exhibiting the highest loss of solids (Table 5.1). There was also significant variation between subjects ($F(6,54) = 7.95$, $P < 0.001$) which is likely to relate to different chewing strategies employed and salivation variations. The effect of portion size on the fraction of solids lost was not shown to be significantly different within each food type ($F(1,54) = 0.00$, $P = 0.981$). There was no correlation ($r = 0.12$) between bolus solids

loss and the chew time taken to reach the swallow point for the different foods and portion sizes which demonstrates the relative independence of the two processes and the variation that occurs within a group of subjects (Figure 5.5).

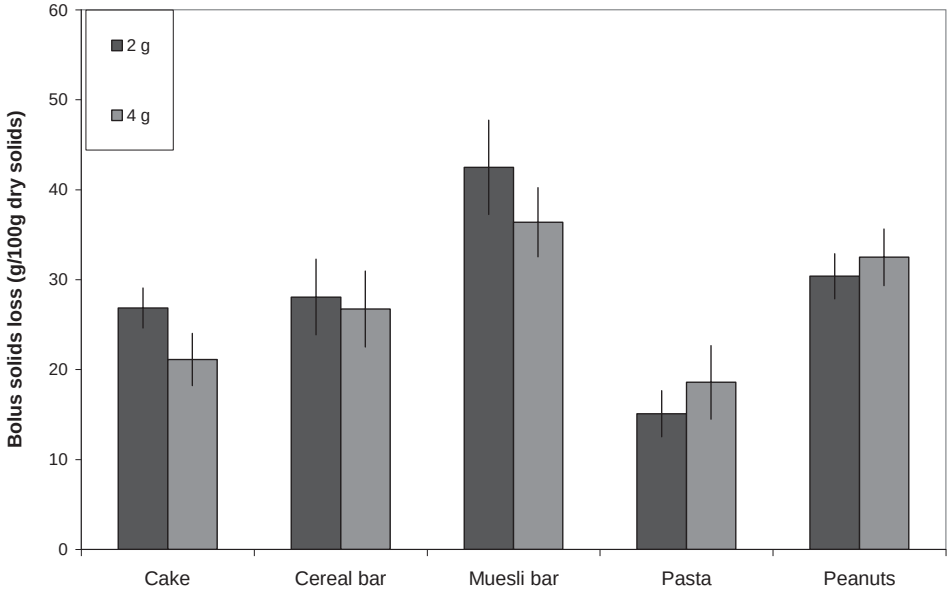



**Figure 5.4** **The effect of food type and portion size on the solids loss from the bolus.**

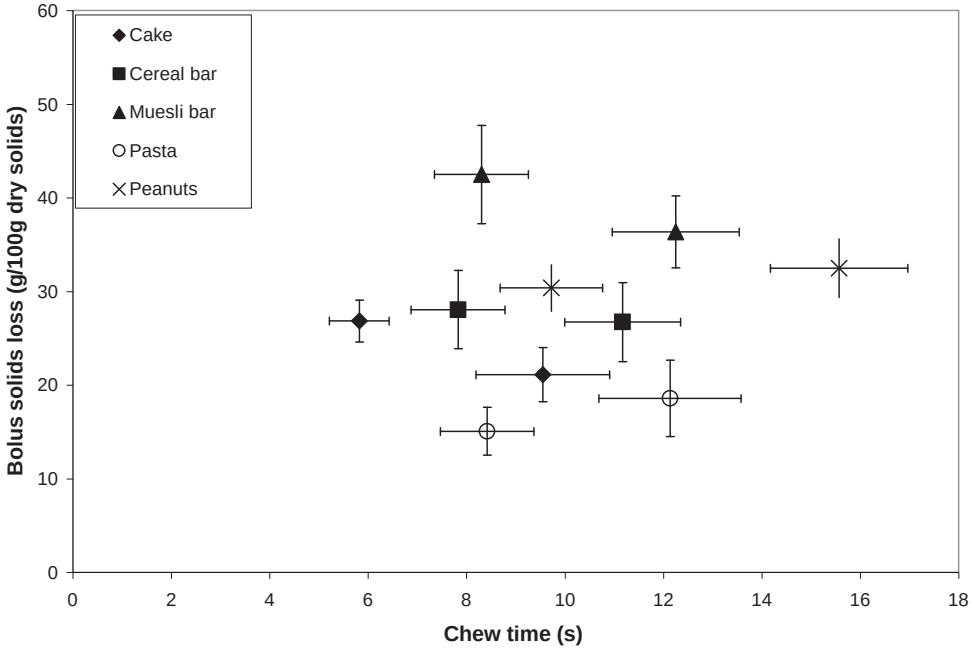



**Figure 5.5** **Relationship between chew time and bolus solids loss.**

## 5.3.4 BOLUS MOISTURE CONTENT

The moisture content of the bolus was similar across all foods except pasta, and also between portion sizes (Figure 5.6). Overall there was a significant difference between food types ($F(4,54) = 260$, $P < 0.001$). There was no significant difference between cereal bar, muesli bar and peanuts (Table 5.1), with only pasta and cake being significantly different from each other and the other food types with higher bolus moisture contents. There was no significant effect of portion size on bolus moisture content ($F(1,54) = 0.23$, $P < 0.636$). Overall the moisture content of the bolus varied significantly between subjects ($F(6,54) = 17.0$, $P < 0.001$), although there were similarities between subjects suggesting similar end-points in bolus moisture (Figure 5.6, Table 5.1). The saliva flow rate was similar for cereal bar and muesli bar (Figure 5.8), but significantly different between all other food types ($F(4,54) = 155$, $P < 0.001$). There was also a significant effect of portion size within each food type the flow rate was slower for the 4 g portion size compared to the 2 g ($F(1,53) = 66.6$, $P < 0.001$). There was no correlation ($r = -0.02$) between bolus moisture content and the chew time taken to reach the swallow point for the different foods and portion sizes which demonstrates the relative independence of the two processes with a study on a group of subjects (Figure 5.7).

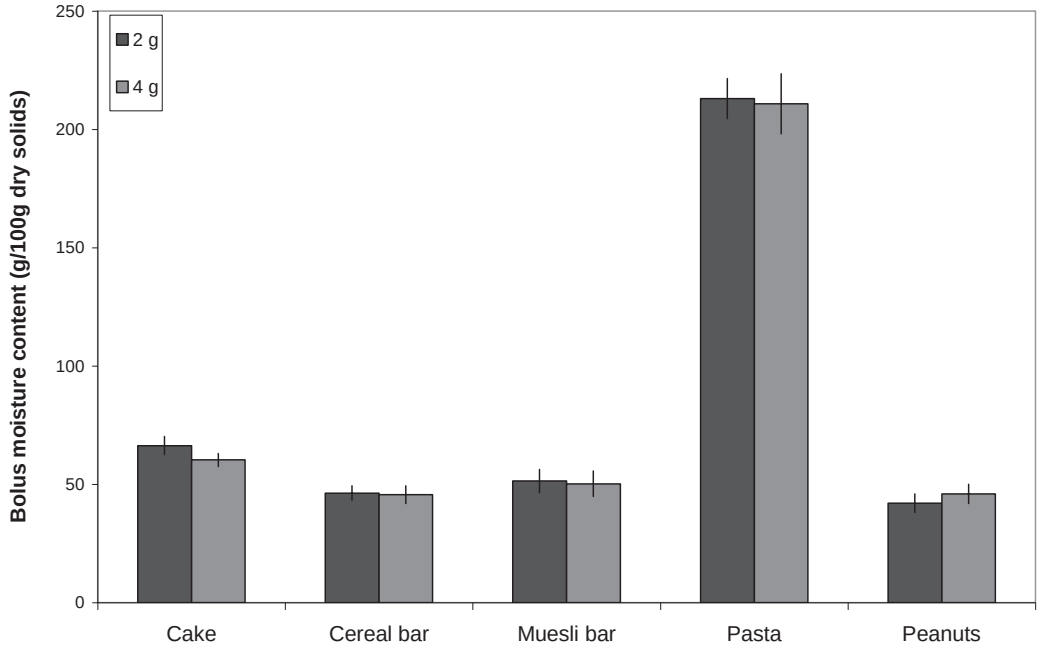



**Figure 5.6 The effect of food type and portion size on the bolus moisture content.**

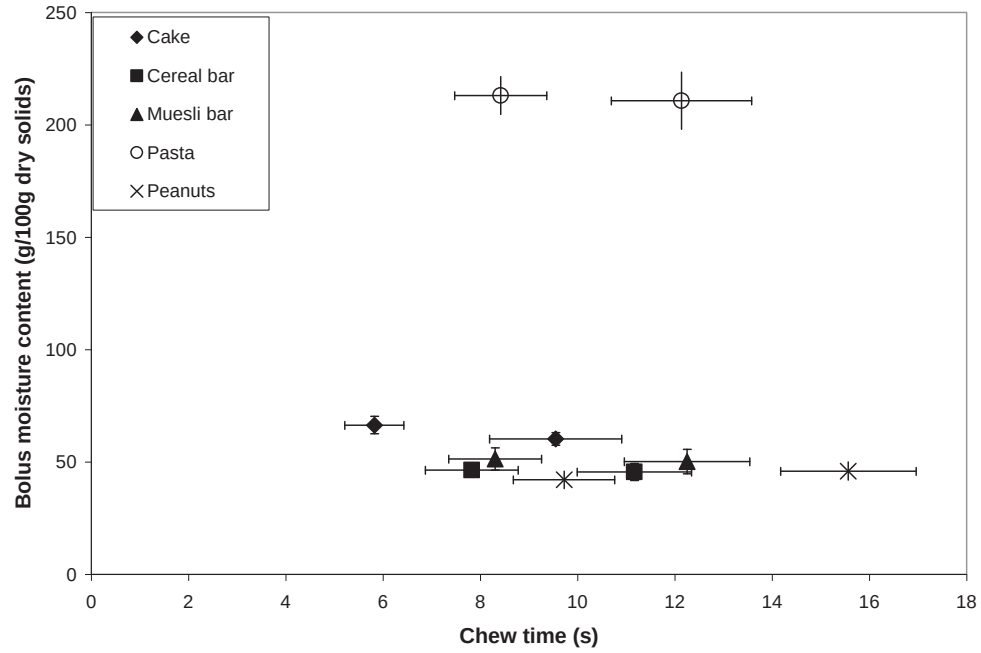



**Figure 5.7** **Relationship between chew time and bolus moisture content.**

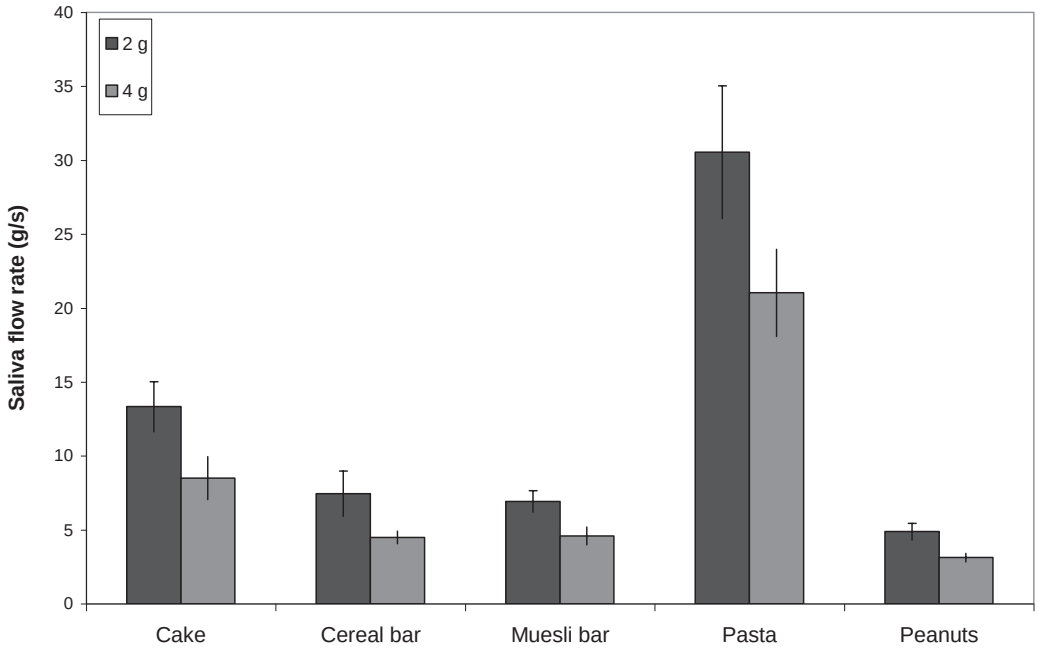



**Figure 5.8** **The effect of food type and portion size on saliva flow rate.**

There was significant correlation (r = -0.72) between bolus moisture content and the bolus solids loss at the swallow point for the different food types, regardless of portion size. This indicates that these two processes are food type dependent and although there are differences between subjects a similar end point is reached for both processes of moisture content and solids loss (Figure 5.9).

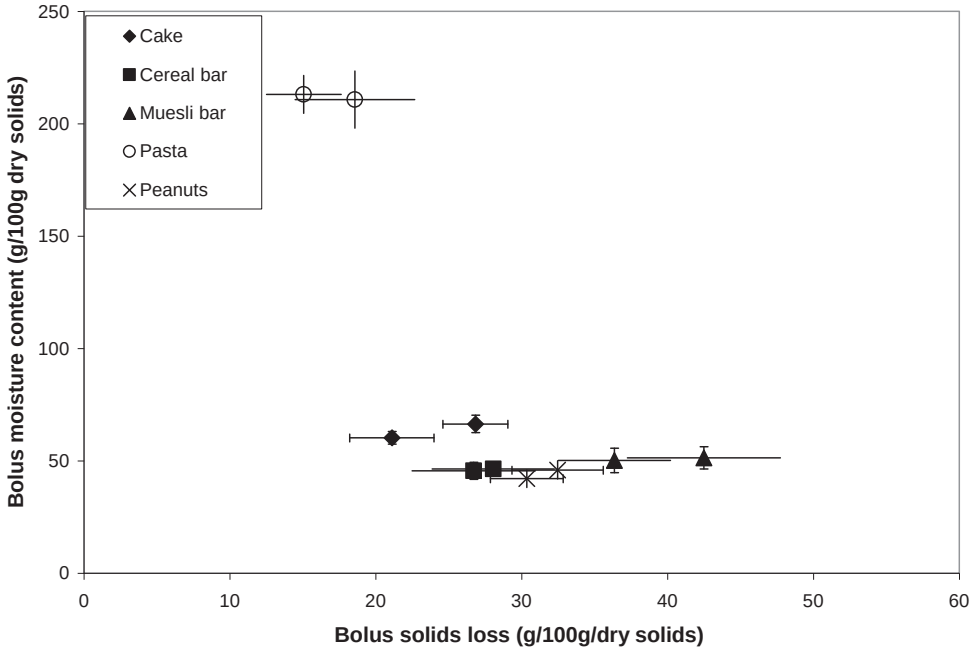



**Figure 5.9 Relationship between bolus moisture content and bolus solids loss (±SEM).**

## 5.3.5 VARIANCE TESTING AND WITHIN SUBJECTS EFFECTS

The within subjects' replicate to replicate variation (Table 5.3) were calculated from the ANOVA output for the results presented in Table 5.2. The variability ratio for within subjects' chew time is considered high which means that the within subjects variation was small, and not significantly different. The bolus moisture content within subjects' variation was small, and not significantly different between replicates. Therefore the chew time and bolus moisture content are more consistent and reproducible within subjects' than between subjects. The bolus solids loss ratio for within subjects' variation was small, but this was significantly different between replicates. Within subjects results are reproducible with minimal differences in bolus solids loss within subjects', and all parameters investigated showed variation between subjects.

**Table 5.3** **Within subjects' replicate to replicate variation.**

| | Log bolus moisture content | Log loss of solids | Log chew time |
|---|---|---|---|
| **P =** | 0.161 | 0.014 | 0.128 |
| **Variability ratio** | 1.29 | 1.75 | 1.33 |

**Table 5.4** **Variance session: effects of food type and portion size on the mean (±SEM) number of chew cycles, chew time, chew frequency, bolus loss of solids and bolus moisture content.**

| Food type & portion size (g) | | Chew cycles | Chew time (s) | Chew frequency (Hz) | Saliva flow rate (g/s) | Loss of solids (g/100g dry solids) | Bolus moisture content (g/100g dry solids) |
|---|---|---|---|---|---|---|---|
| **Cake** | **2** | 8.56±1.38 | 4.75±0.62 | 1.74±0.09 | 13.7±1.89 | 19.22±1.27 | 57.09±3.75 |
| | **4** | 9.50±1.62 | 6.63±1.37 | 1.49±0.08 | 9.78±1.79 | 25.42±2.39 | 55.11±2.79 |
| **Cereal bar** | **2** | 12.56±1.77 | 6.70±0.88 | 1.85±0.04 | 7.05±0.70 | 20.00±3.03 | 43.55±4.30 |
| | **4** | 13.83±1.84 | 9.20±1.60 | 1.55±0.07 | 4.68±0.42 | 35.24±6.51 | 40.83±4.75 |
| **Muesli bar** | **2** | 10.89±1.82 | 6.02±0.90 | 1.75±0.07 | 7.63±0.84 | 31.83±7.71 | 42.19±5.72 |
| | **4** | 14.83±1.51 | 10.57±1.45 | 1.46±0.12 | 3.85±0.43 | 53.14±7.24 | 39.05±5.15 |
| **Pasta** | **2** | 11.56±1.62 | 6.26±0.64 | 1.78±0.11 | 33.61±2.69 | 42.74±3.00 | 199.8±13.1 |
| | **4** | 14.17±3.80 | 8.91±2.74 | 1.69±0.08 | 31.39±7.28 | 47.18±6.79 | 198.4±17.5 |
| **Peanuts** | **2** | 14.00±1.98 | 7.38±0.97 | 1.88±0.05 | 5.42±0.88 | 24.16±2.06 | 34.27±2.65 |
| | **4** | 20.50±2.17 | 13.07±1.87 | 1.61±0.07 | 2.96±0.25 | 33.32±3.44 | 36.79±2.62 |

The variance session data (Table 5.4) was used to compare individual subjects' data with their test data to check for reproducibility. Overall there were small but consistent differences between the variance session and the test data. The loss of solids showed differences between the variance and test sessions ($F(1,75) = 17.77$, $P < 0.001$). There was also a significant interaction effect between food x between test sessions ($F(4,74) = 13.58$, $P < 0.001$). Post hoc tests showed that the only significant difference was for pasta samples, which was due to a change in methodology in handling the pasta sample. The moisture content of the bolus showed a significant difference between the variance and test sessions ($F(1,75) = 5.92$, $P = 0.024$) as the variance session resulted in lower mean bolus moisture contents about 9% lower than the test session. There were no significant interaction effects. There were significant differences between the variance session and the test sessions for the chew time ($F(1,75) = 12.81$, $P < 0.01$) and number of chew cycles ($F(1,75) = 10.39$, $P < 0.01$), with no significant interaction effects. The variance session had consistently shorter chew time and lower numbers of chew cycles to form a swallowable bolus (12% less time and cycles compared to test sessions). Due to the consistent effects of chew time and chew cycles, the chew frequency showed no significant differences between the variance and the test sessions ($F(1,75) = 2.43$, $P = 0.135$). There were no significant differences exhibited between the test and variance sessions for the saliva flow rate ($F(1,75) = 0.59$, $P = 0.45$).

## 5.4 DISCUSSION

### 5.4.1 CHEWING BEHAVIOUR AND ENSALIVATION

In this study, significant differences were observed in the chewing strategy used by different subjects. Chewing time varied for the different foods studied, and although there was variation between subjects they followed the same trend for increasing chew time in this order: cake<cereal bar<muesli bar<pasta<peanuts. The increase in portion size resulted in an increase in chewing time, but not in direct proportion to the increase in portion size (Table 5.1, Figure 5.2), which is in agreement with previous studies demonstrating subject, food and portion size effects on chewing time and frequency (Gaviao *et al.* 2004; Yurkstas 1965; Lucas & Luke 1984; Fontijn-Tekamp *et al.* 2004; Peyron *et al.* 2004b, Engelen *et al.* 2005). As reported Chapter 4, the particle size

distributions were also significantly different between the different foods (Figure 4.3(A-E)). Despite these large differences in chew time and the resulting particle size distributions for the different foods, the differences between the moisture content and the proportion of solids lost from the bolus during processing were small. Within subjects data shows that they were reproducible during the test sessions, although a separate one-off variance session showed less reproducibility within subjects when comparing data from the two experiments. The variance session resulted in significant reductions in chewing time taken to form a swallowable bolus, which is most likely due to changes in sample collection procedure (Section 5.2.1). The different chewing strategy in the variance session also resulted in minor but significantly lower bolus moisture contents for all food types, with no significant change to chewing frequency or solids loss from the bolus.

It is widely reported in the literature that salivary flow rates vary between individuals (Gaviao *et al.* 2004; Neyraud *et al.* 2003), can vary with the time of day (Engelen *et al.* 2005) and can be stimulated by low pH (Watanabe & Dawes 1988b), and high salt or sugar content in foods (Neyraud *et al.* 2003). As shown in Table 3.4, the foods studied in this work with high sugar content were cake (31.8%) and cereal bar (34.8%), and muesli bar had the lowest pH (4.4) and as such it might be expected that these foods would promote more extensive rates of saliva incorporation during bolus formation. Despite this, the increase in moisture content was relatively constant across the range of foods except for the pasta, suggesting a similar end-point in bolus moisture. This was regardless of the chewing strategy applied, which did vary significantly between subjects. Also there was no effect of food portion size on the bolus moisture content, which is evident from the saliva flow rate results, as saliva was added at a significantly slower rate for the larger portion size. Saliva flow rate was only a proximate measure of the saliva added for the duration of the chewing sequence, it is unknown if this flow rate is at a constant rate for the chewing sequence, or how the result is affected by the residual volume of saliva that is in the mouth before the food is introduced, or how it is stimulated initially by the subjects' anticipation of the food.

### 5.4.2 BOLUS MOISTURE CONTENT

While the ANOVA demonstrated an overall difference between bolus moisture content between the foods, post-hoc tests (Table 5.2) showed that this was due to the pasta and cake results. For each food, bolus moisture content was not affected by portion size suggesting that there is a defined amount of moisture required to form a suitable bolus for each food type to allow a safe swallow. To achieve the same moisture content for increased portion size, saliva was added at a slower rate when comparing the mass of saliva added over the duration of the chewing sequence. This was achieved by an increase in mastication time, but not in direct proportion to the increase in portion size, and is also related to the concurrent process of solids loss during mastication. Saliva was not added to the bolus at a constant rate during chewing of the foods studied here in contrast to observations by Mioche *et al.* (2002) for chewing meat. A more detailed analysis of how the moisture content of the bolus changes with time during mastication is required to develop further understanding of how saliva addition is controlled. This research is presented in Chapter 7.

The moisture content of all food boluses were well below (less than 50% of) the measured saturation moisture contents of the insoluble food particles reported in Table 3.5. This clearly shows that the bolus does not reach an equilibrium state in which only completely hydrated insoluble particles remain. This was true even in the case of pasta samples which were cooked in water and had very high initial moisture content. These findings suggest that the particle hydration is incomplete, and variations in moisture uptake rate, the rate of particle size breakdown, salivary flow and the chewing time for different foods may influence the end point bolus moisture content.

### 5.4.3 BOLUS LOSS OF SOLIDS

Another process occurring during chewing is the dissolution and subsequent loss of soluble solids in the food. This process requires addition and subsequent loss of moisture from the bolus. It is obvious that this process occurs, from the sensation of taste that occurs during oral processing (Watanabe & Dawes 1988a). This dissolution has been used to explain the differences between initial and recovered solids contents of foods during mastication trials (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007). To investigate this, the experimentally observed solids lost were compared to the amount of

soluble solids in the initial foods (Table 3.6). It was found that for cake and cereal bar samples, the solids lost could account for removal of at most approximately half of the soluble solids in the initial food samples (48 and 49% respectively). For muesli bar samples, enough solids were lost to account for all the dissolvable solids (108%).

These findings suggest the dissolution process is advanced but not necessarily complete by the time the food is ready for swallowing. The rate of dissolution will be important in the formation of a cohesive bolus for three compounding reasons: first, dissolution will reduce the solids fraction and particle composition of the bolus as soluble solids are washed out; second, this dissolution process causes loss of moisture that could have contributed to particle hydration; and third, dissolution will increase the liquid volume that contributes to liquid bridging within the bolus.

The quantities of solids loss from the expectorated boluses were slightly less than previously reported for peanuts (Chapter 4; Peyron *et al*. 2004b), probably due to the additional solids loss during particle sieving in those studies. For peanuts and pasta the amount of solids lost was 2 - 4 times greater than the amount of soluble solids originally present in the food, suggesting the loss of solids was primarily due to loss of insoluble particles during chewing.

In Chapter 4, the bolus and the debris remaining in the mouth after expectoration of each food bolus was collected. Using the data reported for 4 g portions (Table 4.1), the weight of debris retained on the sieve stacks (smallest sieve size of 0.125 mm) were recalculated to be 23, 16, 20, 35 and 13% of the total sieved particle solids recovered for peanuts, cake, cereal bar, muesli bar and pasta samples respectively. These calculations represent a conservative estimate of the amount of particles that are lost from the bolus during mastication as some soluble material would be washed out of the particles during the wet sieving procedure used. On comparison of these results with Table 5.1 it can be seen that loss of particles from the bolus into other parts of the mouth can account for most of the solids lost for the peanut samples (70-100%). This suggests that both dissolution of soluble material and the loss of particles from the bolus, are important to the bolus PSD, mass and composition at the point of swallowing.

### 5.4.4 EFFECT OF MASTICATION STRATEGY ON BOLUS FORMATION

It might have been expected that moisture content or fraction of solids lost would be greater for boluses that were chewed for longer times. This was observed to some extent (for example for muesli bar and cereal bar), but not universally across all foods (overall r is -0.02 and 0.12 respectively) (Figures 5.5 & 5.7). Some correlation was found between the fraction of solids lost and moisture content of the bolus (Figure 5.9). This suggests that moisture addition and solids lost are related to some extent. This would be expected if solids loss is partially due to dissolution and may be more prevalent where there are higher degrees of moisture addition.

The particle granulation literature report that agglomerate adhesion increases significantly when the void volume between particles is more than approximately 40% saturated with water (Rondet *et al.* 2008). When the void volume is completely saturated, increasing moisture contents result in dispersion of particles and therefore reduced cohesion (Iveson *et al.* 2001). It would be expected that a larger internal interfacial surface area within the bolus would result in increased cohesive strength. Thus, boluses made with smaller particles attain their maximum cohesive strength at lower levels of inter-particle void volume saturation (i.e. cake would form a cohesive bolus at lower degrees of void volume saturation than muesli bar or pasta). Although the particle size distributions were different between the foods (Chapter 4), in a well mixed system like mastication where the bolus is not undergoing confined compression, the particles will be loosely packed and the inter-particle void volume fraction is not likely to vary dramatically. The void volume fraction of loosely packed particles is reported to be in the range 0.3624 to 0.42 (Sherrington & Oliver 1981). Therefore, it could be expected that the uptake in moisture required to form a swallow safe bolus would be similar between foods if hydration of particles does not dramatically change. There is some evidence of this in that the moisture content of the bolus was not significantly different between peanuts, cereal bar and muesli bar samples (as shown by post-hoc Bonferroni tests), despite the large differences in particle size observed for these foods (Figure 4.3(A-E)).

Clearly, hydration and dissolution would both modify the moisture content of the particles, but both of these processes do not reach completion. From the moisture content data recorded for the pasta samples, this food behaved quite differently to the other foods in that much more moisture is required to make the bolus safe to swallow. This was also evident in the physical appearance of the expectorated boluses, where suspensions of large particles in saliva were found rather than the cohesive pastes observed for the other foods. It is likely that this is because, for pasta, the breakdown of the initial food into small particles was much more difficult than for the other more brittle or crumbly foods studied. This is evident in the high cohesiveness and chewiness scores measured for this food in the TPA tests (Table 3.6). In this way moisture addition proceeds more quickly than the particle size reduction rate and as such a relatively dilute suspension is formed that allows the particles to be swept or washed down as part of a liquid swallow. The other foods broke down rapidly to a point where bolus formation becomes possible when enough moisture is added.

## 5.5 CONCLUSIONS

In this study boluses were characterised from five different foods at the swallow point. The key finding was that a defined amount of moisture was required to achieve a swallow safe bolus for each food type. Understanding the role moisture/saliva plays in providing the required bolus functionality to allow swallowing is complicated by concurrent processes of particle hydration, dissolution and particle losses, each of which is dependent on the changing particle size distribution. It was shown that extents of particle hydration and dissolution of solutes from the food particles were incomplete by the end of chewing. Both insoluble and soluble solids losses from the bolus are important to the size and composition of the finished bolus at the swallow point. For foods with high insoluble solids content, solids loss during chewing was primarily due to particle loss from the bolus during mastication. In other food matrices, greater degrees of solids loss occurred as moisture content increased; a result consistent with washing of soluble materials from the bolus. It could be expected that the uptake of saliva/moisture required to form a swallow safe bolus would be similar if hydration of particles do not dramatically change.

In future work investigations should characterise the distribution of water in the resulting bolus and the dynamics of moisture absorption and solids loss during mastication (Chapter 7). Understanding of the role moisture/saliva on bolus formation in this way can provide a basis for food product developers to design foods with pre-defined mastication time and solute/flavour release rates. With more information the concept provided by Prinz & Lucas (1997) could then be strengthened to provide a more accurate model of bolus formation for a wider range of food products.

# CHAPTER 6: DYNAMICS OF BOLUS FORMATION: PARTICLE SIZE CHANGES IN MULTI-COMPARTMENT BREAKDOWN OF FOOD

## 6.1 INTRODUCTION

In Chapters 4 & 5, findings were presented showing that the initial food solids are not all recovered in the swallowable bolus, and that a debris compartment exists which results in a significant difference between the PSD of the bolus and debris solids recovered. Many researchers have recognised that measurements of the bolus do not identify the end point of all particulates, as not all food particles are present in the bolus at the time they are expectorated (Hiimae *et al*. 1996; Brudevold *et al*. 1990). In some studies debris particles have been collected and added to the bolus proportion for further analysis (Peyron *et al*. 2004b), whilst other studies have disregarded the losses (Schneider & Senger 2001).

In Chapter 4, the debris proportion was collected and particle size distribution analysed for a range of food types. Compared to the bolus, the debris has a significantly higher quantity of mid-range (0.7 – 1.4 mm) particles, with some particles in the debris over 4 mm in diameter, and it is not composed predominantly of the smallest particles as previously postulated (Schnieder & Senger 2001). Particulate material can be retained in multiple secondary compartments within the mouth (e.g. adhering to the teeth, retained in sulci) (Brudevold *et al.* 1990; Kashket *et al.* 1991; Newell *et al.* 2002). It is probable that a number of physical attributes of food particles (e.g. their tribology, coherence, viscoelasticity), and the changes that take place during mastication (e.g. particle size reduction, particle selection, saliva addition, etc.) may lead to particles being separated and withheld from the dental mill for periods of time during oral processing. It is not known whether the stages in bolus formation and breakdown of particles follow a similar process despite food type, especially where similar chewing strategies (number of chew cycles and chew time) are applied.

To date there have been no studies carried out to track the dynamics of loss of particles to or from these secondary compartments. This study is a preliminary study applying the use of a single subject as an "instrumental" measure to identify areas for further research and verification within a population. The purpose of this work was to determine the changes in PSD during bolus and debris formation up to and past the natural swallow point. This was to identify if there are similarities between the mastication of three processed foods, whether selection and breakage theories are supported, how the debris proportion is formed, and if these processes affect the rate of bolus formation.

## 6.2 MATERIALS AND METHODS

Three food types were chosen with a differing range of chemical composition and homogeneity: muesli bar, pasta and peanuts (Section 3.1). The food samples were prepared and standardised by weight at approximately 4 g (Section 3.2), and the actual weight recorded. Food moisture content (Equation 3.1) was measured in duplicate for each food type per session to ensure that the most accurate data was used to calculate the quantity of ingested solids (Equation 3.6). The samples for moisture content analyses were placed in an oven half-way through the study session.

The samples presented per session were randomly ordered with only one food type studied per session following the study design outlined in Section 3.7.2. One male subject (age 27 y) was recruited through the process outlined in Section 3.6. Each of the samples collected were assessed in triplicate over nine study sessions following the experimental procedure detailed in Section 3.7.3. A total of 192 bolus and debris samples were collected for analysis. The bolus and debris samples were immediately stabilised post-expectoration following the procedure described in Section 3.4.8. At the end of the session the samples were put into storage pending sieve analysis.

### 6.2.1 EXPERIMENTAL PROCEDURE

The session protocol outlined in Section 3.7.3 was followed, except that instead of the subject chewing the sample to a swallowable bolus, the researcher instructed the subject to expectorate the bolus at a specific number of chew cycles (1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35). (Only peanuts and muesli bar were chewed for 35 chew cycles). The subject

did not know the number of chew cycles being investigated with each sample until they were asked to expectorate the sample. The order of the number of chew cycles used in each trial was randomised across all sessions to minimise any order effects, and a complete set of data was collected for all chew cycle numbers in each session. The numbers of chew cycles up to, and past the point of natural swallowing were determined from observation of the subject during the familiarisation session. The bolus was not expectorated and analysed at the subjects natural swallowing point, only the chew time was recorded for a natural swallow (in triplicate for each food type) to confirm that the subject was within the range of chewing behaviours exhibited in a previous group study (Chapter 4). Chew frequency was calculated for all samples (Equation 3.10).

The subject was provided with a mirror and toothpicks to ensure that their mouth was clear of any food particles at the start of the session and after each sample. Any additional particles collected after each sample (termed "brushings") were put onto a pre-weighed dish which was then dried in an oven at 105 °C for 24 h. From this, the mass of dry solids was determined and percentage of the initial ingested solids present in the recovered brushings was calculated (g solids/100 g initial food ingested). The dry mass of the brushings particles were added to the sum of the recovered solids from the bolus and debris sieving, and pan washings. It should be noted that the dissolved solids content was not measured in this study.

The particle size distribution (PSD) was determined using the finalised wet sieve method described in Section 3.4.6. The mass of particles recovered on each sieve fraction and pan washings (<0.124 mm) for the bolus (or debris) fraction show the changes in PSD with the specific numbers of chew cycles studied. The mass of particles on each of the sieves and pan washings were also calculated as a percentage of the total bolus (or total debris) solids recovered, and results presented as the cumulative distribution. The sum of the mass on the sieve fractions was used to calculate the percentage of initial ingested solids present in the recovered total bolus (or total debris). The percentage of initial ingested solids recovered in the sum of bolus and debris proportions combined (excluding particles <0.124 mm) was calculated to confirm whether the percentage of recovered ingested solids are similar to the results presented in Chapter 4.

# 6.3 RESULTS

## 6.3.1 CHEWING BEHAVIOUR

Overall for pasta and muesli bar, the chewing frequencies exhibited low reproducibility for the initial chew cycles, and the frequencies increase up to eight cycles then dip by the tenth cycle (Figure 6.1). The peanuts show fluctuations, which reduce in magnitude for the first ten chew cycles. From ten cycles onwards, chewing behaviour for muesli bar and peanuts exhibit the same chew frequencies applied for the increasing number of chew cycles, with good reproducibility up to and past the swallow point. The pasta showed a reduction in chew frequency past ten cycles then it plateaued post swallow point. The mean chew frequency near the swallow points for each of the foods in the chew number study was similar to that of the sample that was chewed and swallowed naturally at the start of each session (Table 6.1). The chew frequency for pasta to the swallow point was not notably different from that of the group study (Chapter 5), but muesli bar and peanuts exhibited lower chew frequencies compared to the group study.

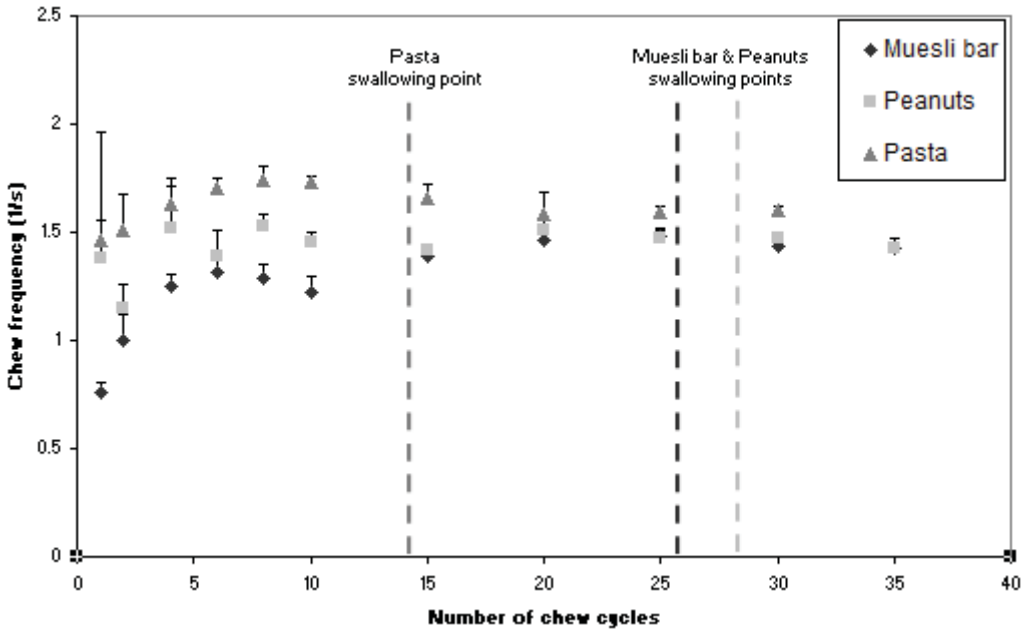



**Figure 6.1** **Chew frequency changes with chew cycle number (±SEM).**

**Table 6.1** **Effect of food type on the mean (±SEM) number of chew cycles, chew time, chew frequency and ingested solids recovered, measured at the subject's natural swallowing point.**

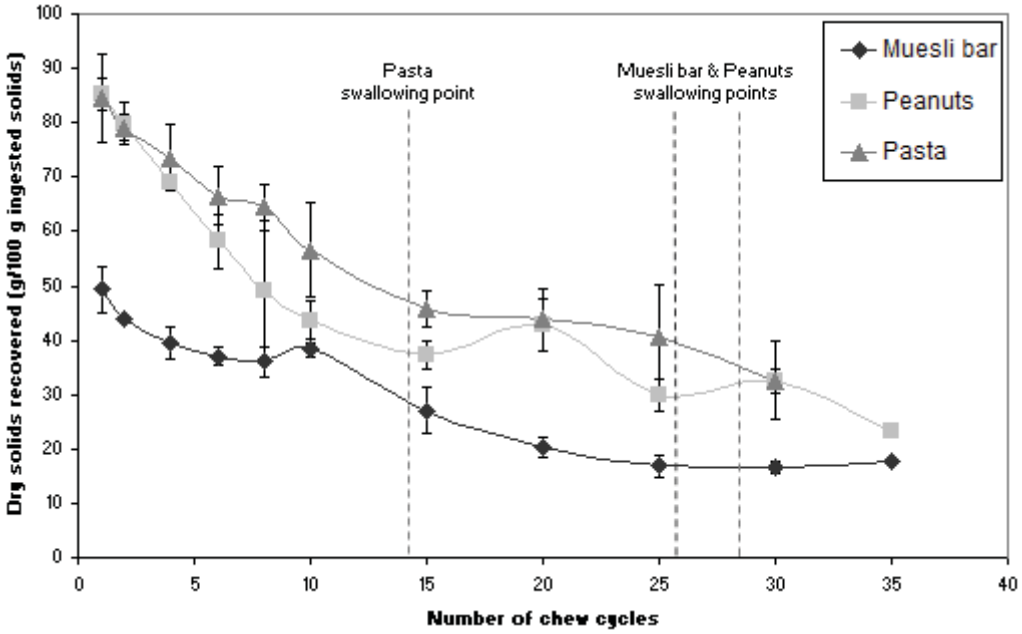
| Food type | Chew cycles | Chew time (s) | Chew frequency (1/s) | Ingested solids recovered >0.125 mm (%) |
|---|---|---|---|---|
| **Muesli bar** | 27 (±2.0) | 19.5 (±1.00) | 1.39 (±0.04) | 17.6 (±0.84) |
| **Peanuts** | 28 (±2.0) | 19.6 (±1.36) | 1.43 (±0.03) | 37.4 (±6.61) |
| **Pasta** | 14 (±1.0) | 8.69 (±0.94) | 1.66 (±0.09) | 84.9 (±7.81) |

### 6.3.2 MASS RECOVERED IN BOLUS AND DEBRIS COMPARTMENTS

One hundred percent recovery of ingested food solids was not achieved for any food type and losses were observed from the first chew cycle. Overall, the bolus for all food types showed a reduction in solids recovered with an increase in the number of chew cycles. The percentage of bolus solids recovered nearest each of the swallowing points was significantly different between the three food types (Figure 6.2A). Debris particles were collected from the first chew cycle for all food types and the percentage of solids recovered fluctuated through the chew cycles depending on food type. For the debris, no more than 11% solids were recovered for any of the chew cycles studied for the three food types (Figure 6.2B).

The bolus solids recovery for muesli bar samples for the first chew cycle (49%) was the lowest of the three food types studied. Chewed muesli bar samples gradually increased in debris mass recovered up to 30 chew cycles, then reduced by 35 chew cycles. In muesli bar samples, the percentage of total solids in the recovered bolus and debris fractions at 25 cycles (chew number nearest the subject's swallowing point, Table 6.1) was significantly lower compared to the group study mean results of 25% (± 0.7) (Table 4.1). The total recovery at the first chew cycle for peanuts bolus was 85% of the ingested solids. Masticated peanuts showed an increase in debris up to 10% by 6 chew cycles, this then reduced to less than 5% by 15 chew cycles then stayed fairly constant up to 35 chew cycles.

**A.**




**B.**

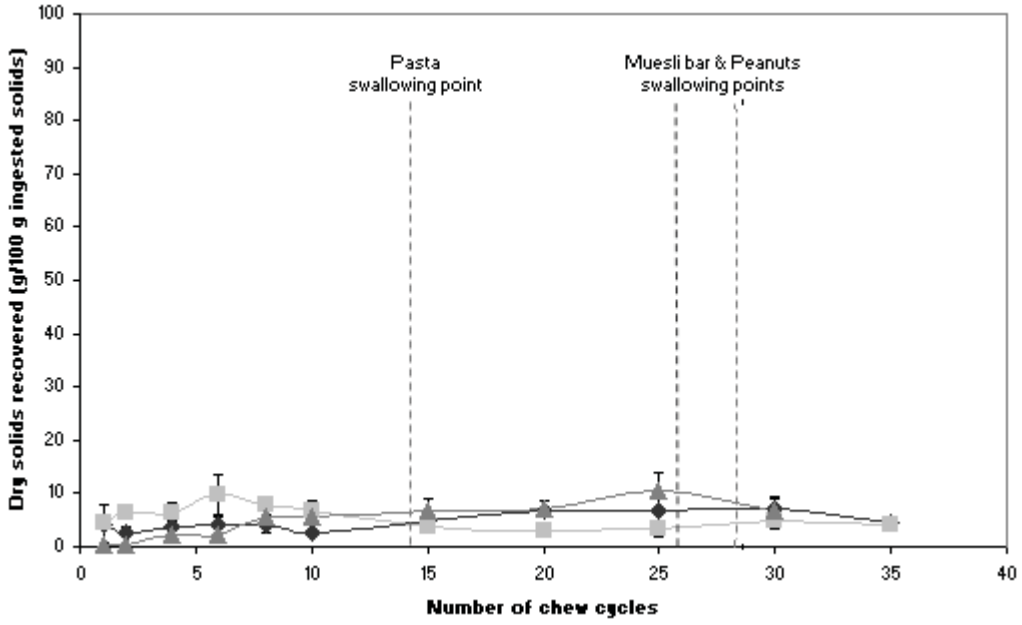



**Figure 6.2** **Percentage of the initial ingested solids recovered in, A: Bolus. B: Debris.**

For peanuts, the percentage solids recovered in bolus and debris at 30 chew cycles (chew number nearest the subject's swallowing point, Table 6.1) was significantly lower compared to the group study mean results of 41% (± 1.4) (Table 4.1). The recovery of solids for pasta was 85% after one chew cycle with almost all these solids as part of the bolus. The pasta increased in debris with increasing number of chew cycles, with a reduction in solids at 35 chew cycles, perhaps due to reaching 10.5% at 30 chew cycles. The percentage of total solids recovered at 15 chew cycles (chew number nearest the subject's swallowing point, Table 6.1) was significantly lower compared to the group study mean results of 61% (± 2.0) (Table 4.1).

Brushings samples were collected by the subject, after expectorating the bolus and rinsing debris, to ensure their mouth was clear prior to masticating the next sample. This mass was collected for peanuts and muesli bar only, and was the smallest proportion, contributing <0.5% of the ingested solids recovered. This data was not included in further particle size distribution analysis as the brushings could not be attributed to either the bolus or the debris proportion.

### 6.3.3 DEBRIS: BOLUS SOLIDS RATIO

The ratio of debris to solids differs between food types, although for the three foods the trend shows that proportionately more solids were lost to the debris compartment with an increase in the number of chew cycles (Figure 6.3). The peanuts debris to solids ratio fluctuates with a decrease in debris proportion from 10 to 20 chew cycles, then increases. The ratio of debris to bolus near the swallow point for peanuts is similar to the ratio produced after six chew cycles which is due to the reduction in recovery of bolus solids rather than a significant increase in the debris proportion.

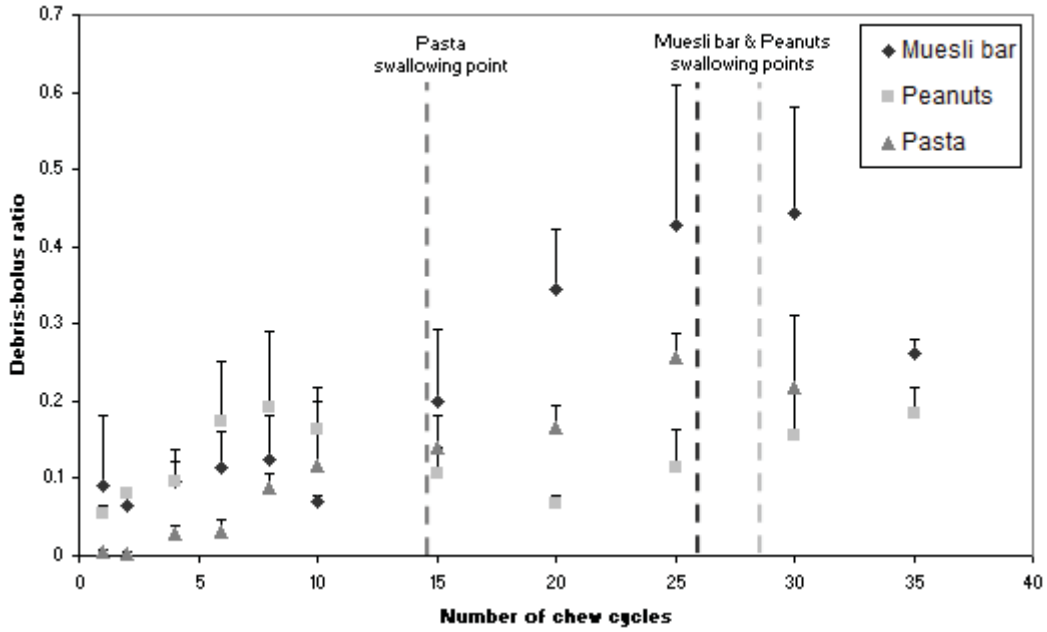


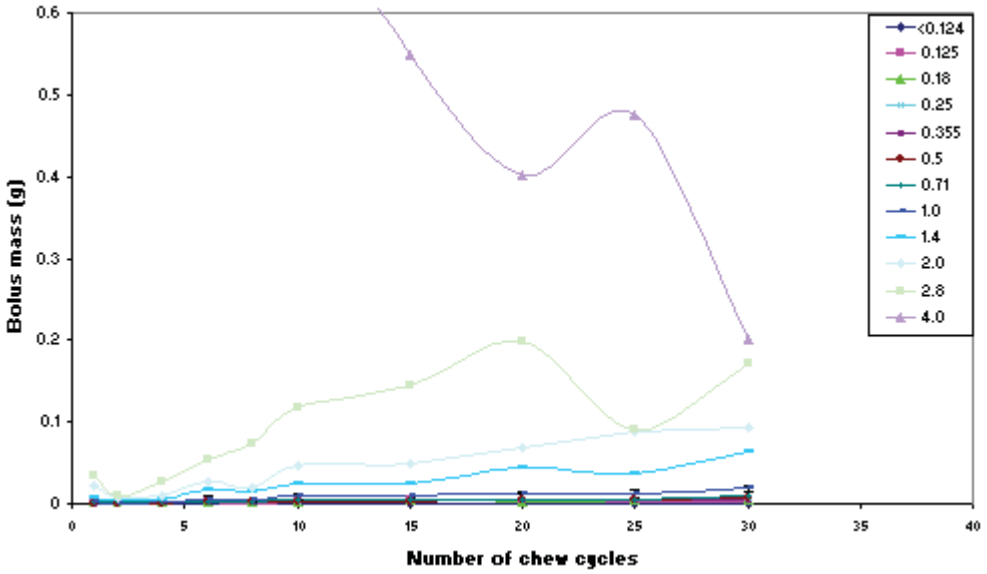

**Figure 6.3** **The changes through the chewing sequence of the ratio of debris:bolus for chewed muesli bar, peanuts and pasta.**

### 6.3.4 CHANGES IN PARTICLE SIZE DISTRIBUTION (PSD)

The PSDs differ significantly between the bolus and debris compartments and between the food types (Figures 6.4 - 6.9). It is also evident that as mastication progresses the PSD changes within each of the compartments. The subject's samples showed good reproducibility in PSD for the three replicates for each of the chew numbers studied. For clarity, the error bars are only shown on each figure for the 1.0 mm sieve fraction (Figures 6.4, 6.6 & 6.8) or the chew cycle number nearest the subject's natural swallowing point (Figures 6.5, 6.7 & 6.9). The mean data (±SEM) for the mass of solids recovered for a specific number of chewing cycles (Figures 6.4, 6.6 & 6.8) is presented in Appendix 5.

There was a reduction in bolus particles greater than 4 mm for each of the food types with increasing chewing cycles. Overall the trends for chewed peanuts and muesli bar show that there is a reduction in the larger particle size fractions (>1.0 mm) and an increase in the smaller fractions in the bolus (<0.5 mm) (Figures 6.6A & 6.8A). The debris followed similar changes, with a reduction in the larger particles and increase in the mid-range particles 0.5 to 1.0 mm as chewing progressed (Figures 6.6B & 6.8B). The pasta showed a reduction in particles in the 4.0 mm fraction in the bolus and an increase in all other size fractions, the debris fraction exhibited an increase in all size fractions with increasing number of chew cycles (Figure 6.4). At the swallowing points for muesli bar and peanuts >78% of bolus particles were below 1.4 mm, whereas only 4.0% of pasta bolus particles were below 1.4 mm (Figures 6.5, 6.7 & 6.9).

**A.**




**B.**

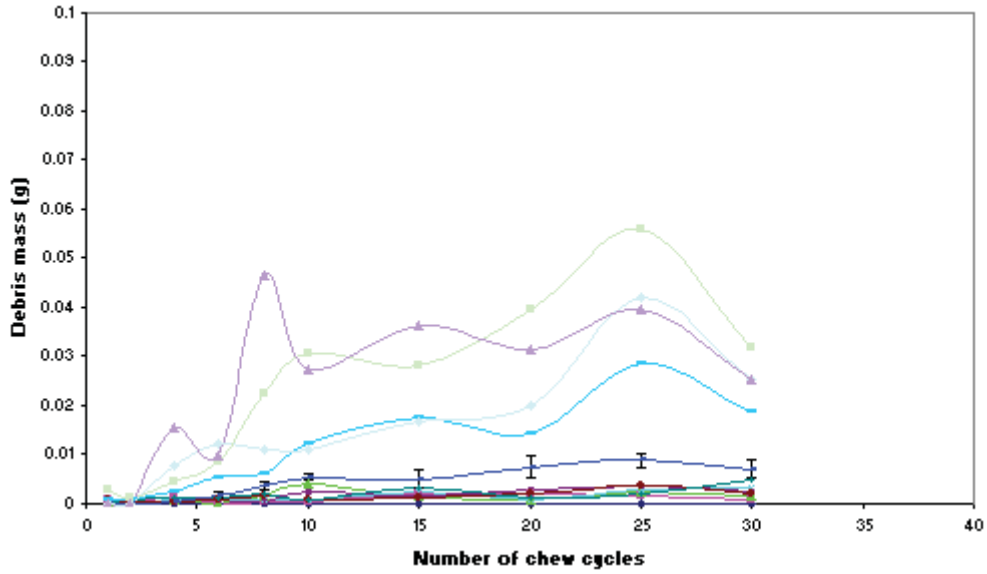
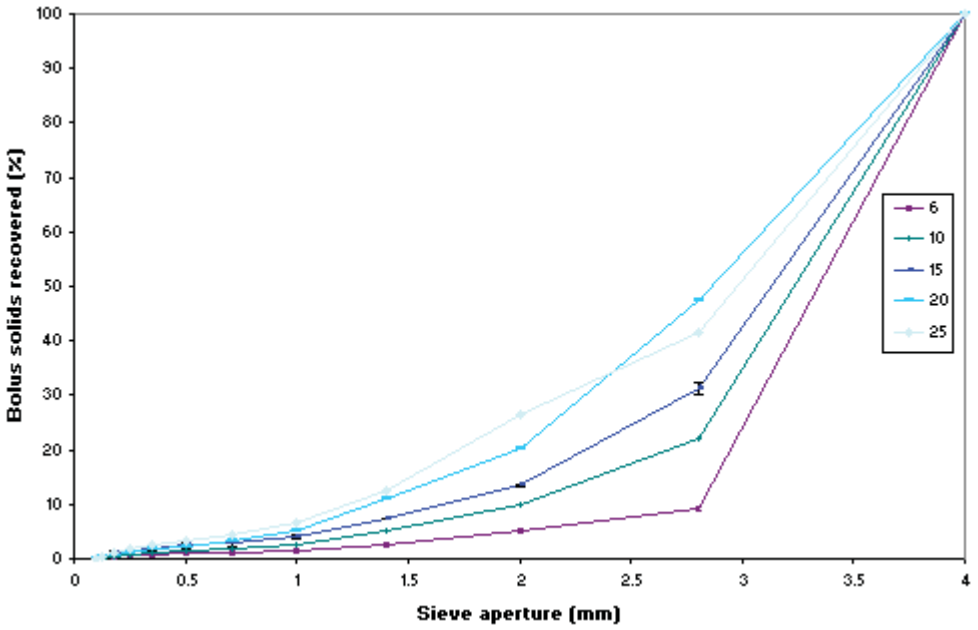



**Figure 6.4** **Masticated pasta PSD shown as the mass of solids recovered, for a specific number of chewing cycles, A. Bolus. B. Debris.**

Figure 6.4A does not show the 4.0 mm fraction changes for chew cycles 1 – 10: 1.36g, 1.30 g, 1.20 g, 1.11 g, 0.99 g, 0.79 g.

**A.**




**B.**

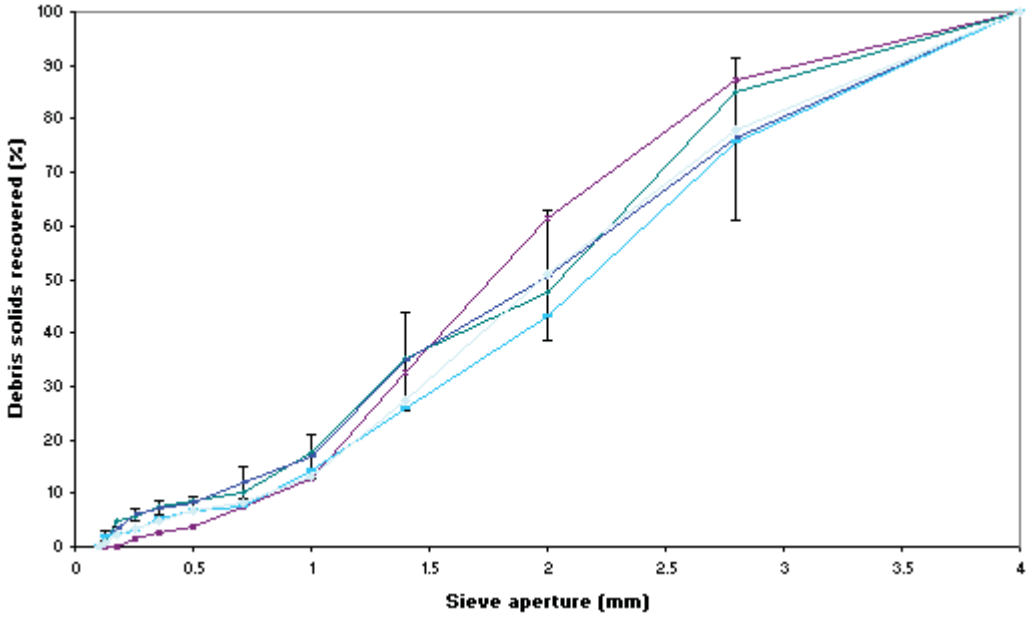



**Figure 6.5** **Cumulative distribution of masticated pasta showing changes in PSD with specific numbers of chew cycles. Mean data presented as the percentage of solids recovered in, A. Bolus. B. Debris.**

The pasta bolus formation over the first 10 chew cycles (Figures 6.4A & 6.5A) showed that with increasing number of chew cycles there was a reduction of the proportion of particles recovered on the 4.0 mm sieve, and an increase in the proportion in all the other sieve fractions. From 4 – 10 chew cycles the bolus was composed of less fines, more particles in the 1.4 – 2.8 mm fractions, and the largest proportion of particles were present on the 4.0 mm sieve. The PSD of the debris formation (Figures 6.4B) showed initially that there were no particles in the 4.0 mm sieve fraction for the first two chew cycles, the greatest proportion of particles were in the 2.8 mm fraction and from 2 – 10 chew cycles the mass of particles >1.0 mm show the largest increases. The PSD of the debris formation from chew cycles 1 – 10 is more erratic than for the bolus formation which exhibits more steady change (Figures 6.4A & B).

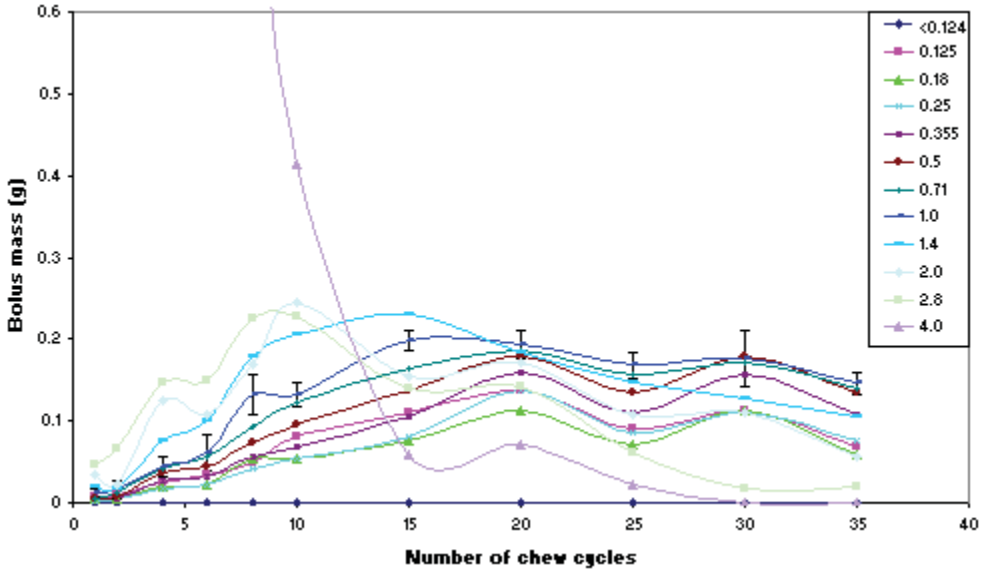
The subject's natural swallow point for pasta was 14 chew cycles. Near this, at 15 chew cycles, the majority of bolus particles (68.6%) were in the 4.0 mm fraction, and 28.5% were present in fractions 1.0 – 2.8 mm (Figure 6.5A). The debris compartment at the swallow point was composed of 23.7% particles in the 4.0 mm fraction, and 64.3% in the 1.0 – 2.8 mm fractions (Figure 6.5B). It can therefore be seen that there is a defined bolus PSD achieved at the swallow point, and the five chew cycles prior and post swallow exhibit different PSDs to the bolus at the swallow point (Figure 6.4A & 6.5A). For the debris compartment PSD the five chew cycles prior and post swallow are similar to the PSD at the swallow point, except there are a slightly higher proportion of particles in the 2.0 and 2.8 mm fractions post swallow (Figure 6.4B & 6.5B).

The pasta bolus PSD for chew cycles post the natural swallowing point showed a continual reduction in particles in the 4.0 mm fraction and an increase in the mass of particles on all other sieves (Figure 6.4A & 6.5A). At 10 chew cycles past the point of natural swallowing (25 chew cycles) there was an overall reduction in the mass of the bolus recovered resulting in a corresponding increase in the mass of particles recovered in the debris compartment (Figure 6.4). The resulting changes to PSD showed a reduction in bolus particles <4.0 mm and an increase in particles in the 4.0 mm fraction, with a corresponding increase in the debris mass of particles in the fractions <4.0 mm (Figure 6.4).

The peanuts bolus formation over the first 10 chew cycles showed that with increasing number of chew cycles there was a reduction in the proportion of particles recovered on the 4.0 mm sieve, and an increase in the proportion in all the other sieve fractions, with each chew cycle measured (Figures 6.6A & 6.7A). At 10 chew cycles the 4.0 mm fraction is still the largest proportion of particles in the bolus PSD. The PSD of the debris formation (Figures 6.6B & 6.7B) shows that the 4.0 mm fraction increased over the first 8 chew cycles then started to decrease, the particles < 4.0 mm increased in mass over the first 6 chew cycles then decreased.

From 15 chew cycles onwards, the bolus PSD showed a reduction in the 4.0 to 1.4 mm fractions, and an increase in fractions <1.4 mm (Fig 6.6A). The PSDs for chew cycles 15 to 35 exhibit similarities, which indicates a slower rate of change. The debris PSD from 15 chew cycles onwards exhibited a continual reduction in the 4.0 mm fraction, and resulted in no particles in this fraction by 25 chew cycles onwards. The masses of particles reduced in all other fractions and then increased in particles <1.0 mm near the subjects swallowing point (Figure 6.6B). Near the natural swallowing point, at 30 chew cycles the bolus was composed of the majority of particles below 2.8 mm, with almost equal proportions of particles in the 0.5 – 1.0 mm fractions (13.7% ±0.2), and nearly 40% of particles between 0.125 and 0.355 mm fractions (Figures 6.6A & 6.7A). The debris compartment near the swallow point contained only particles below 2.8 mm, with the highest amount in the 0.5 mm fraction and approximately equal proportions on the other sieves (Figures 6.6B & 6.7B). The bolus PSD achieved near the swallow point (30 cycles) is similar to the PSDs five chews pre and post swallow (Figure 6.6A). Conversely, the debris compartment changes in the five chews pre and post swallow, with a gradual increase in the total debris by 35 chew cycles with a reduction in particle fractions >1.4 mm and increase in fractions <1.0 mm (Figure 6.6B).

**A.**




**B.**

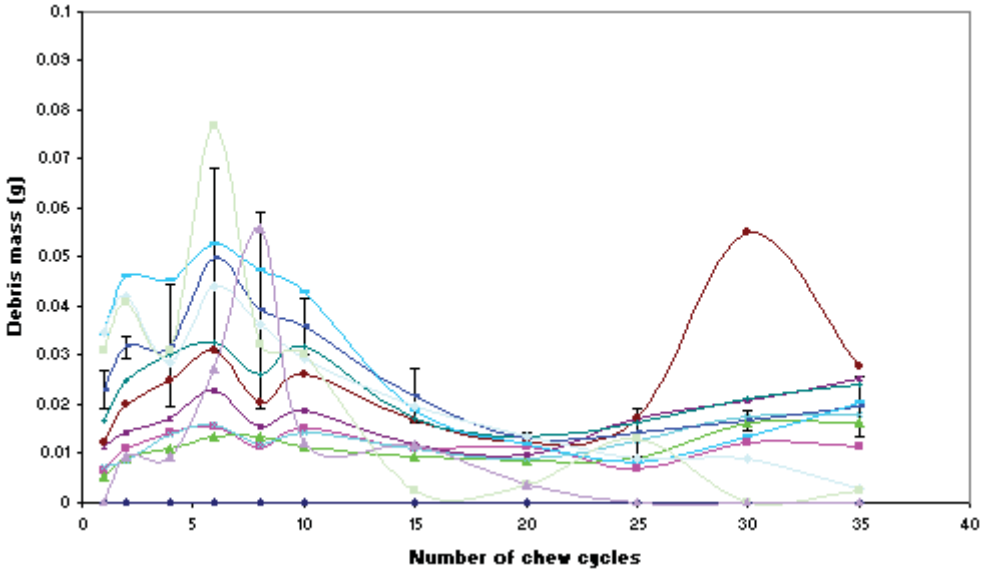
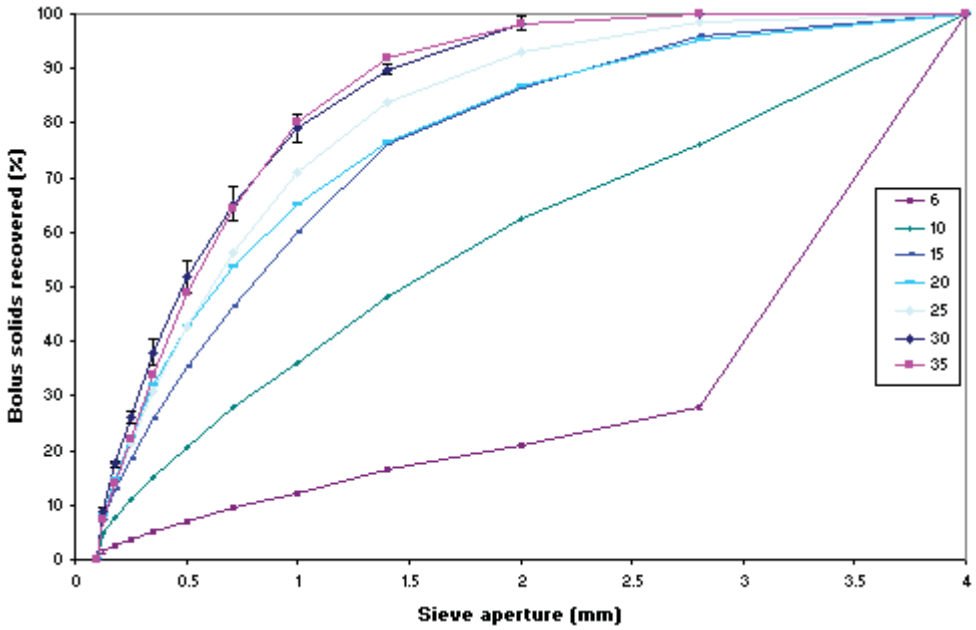



**Figure 6.6** **Masticated peanuts PSD shown as the mass of solids recovered, for a specific number of chewing cycles, A. Bolus. B. Debris.**

Figure 6.6A does not show the 4.0 mm fraction changes for chew cycles 1 – 8: 3.22g, 2.96 g, 2.15 g, 1.67 g, 0.87 g.

**A.**




**B.**

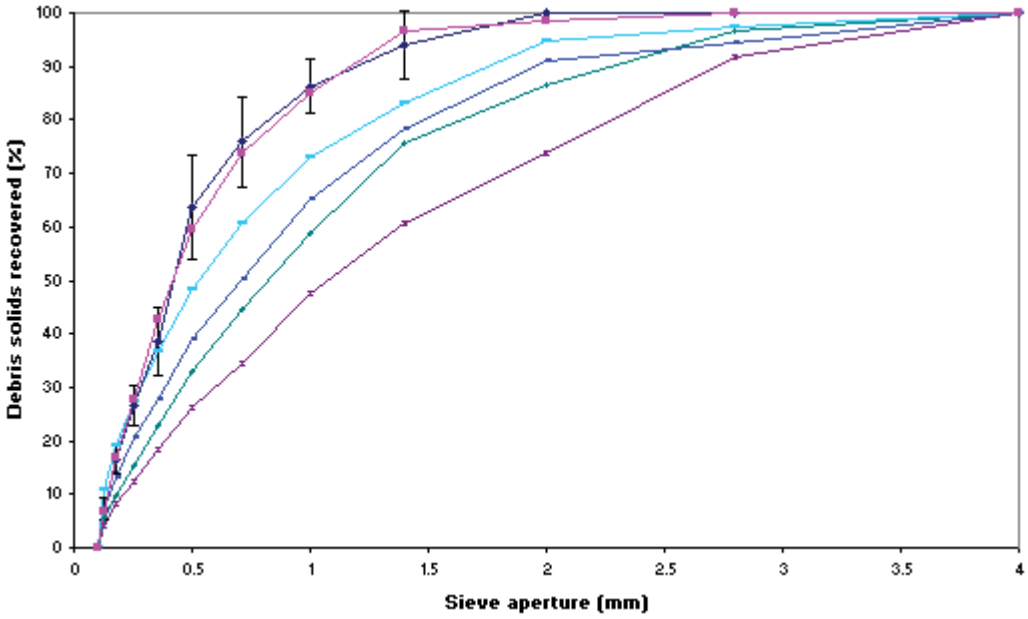
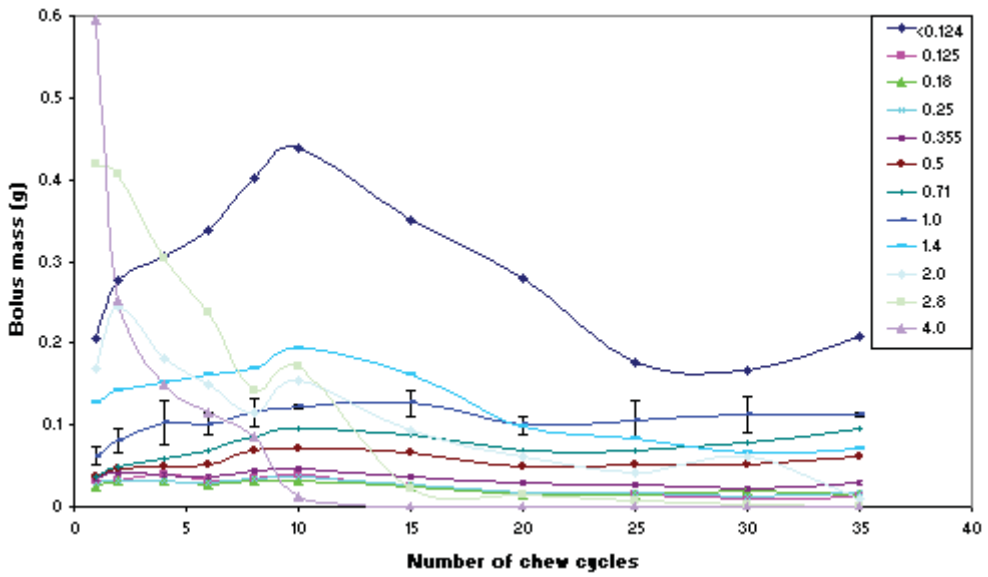



**Figure 6.7** **Cumulative distribution of masticated peanuts showing changes in PSD with specific numbers of chew cycles. Mean data presented as the percentage of solids recovered in, A. Bolus. B. Debris.**

**A.**




**B.**

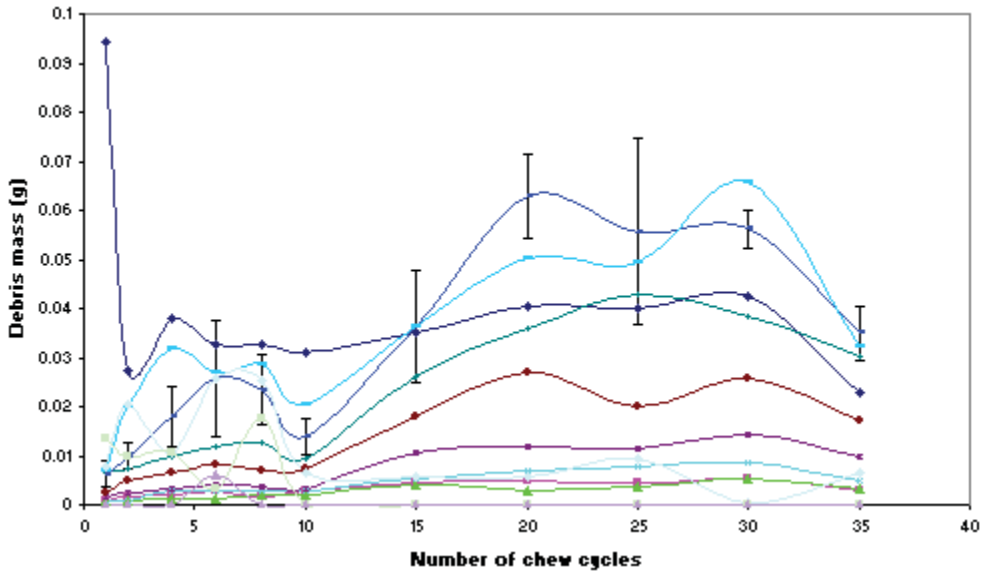
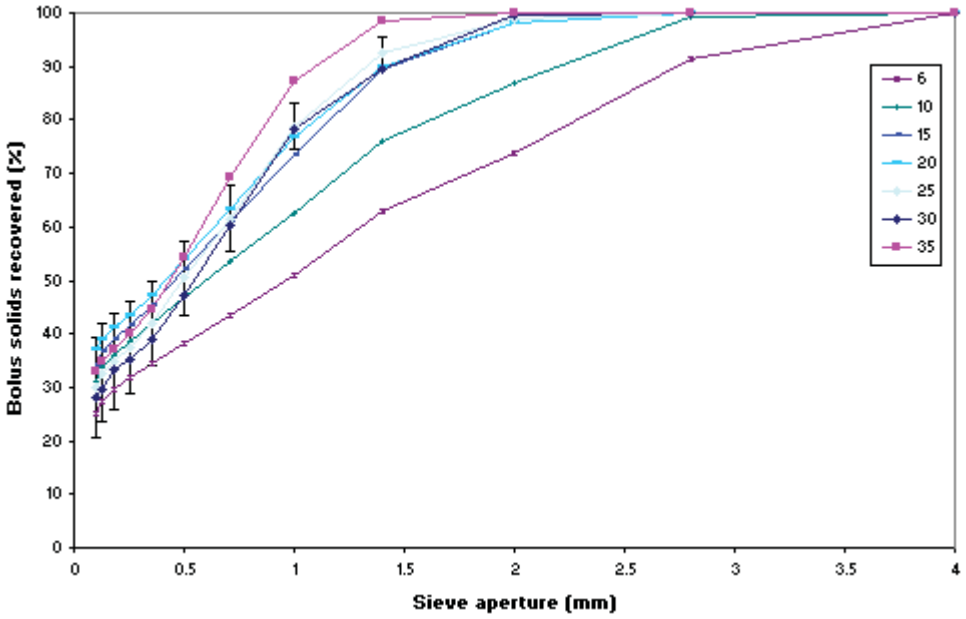



**Figure 6.8** **Masticated muesli bar PSD shown as the mass of solids recovered, for a specific number of chewing cycles, A. Bolus. B. Debris.**

**A.**




**B.**

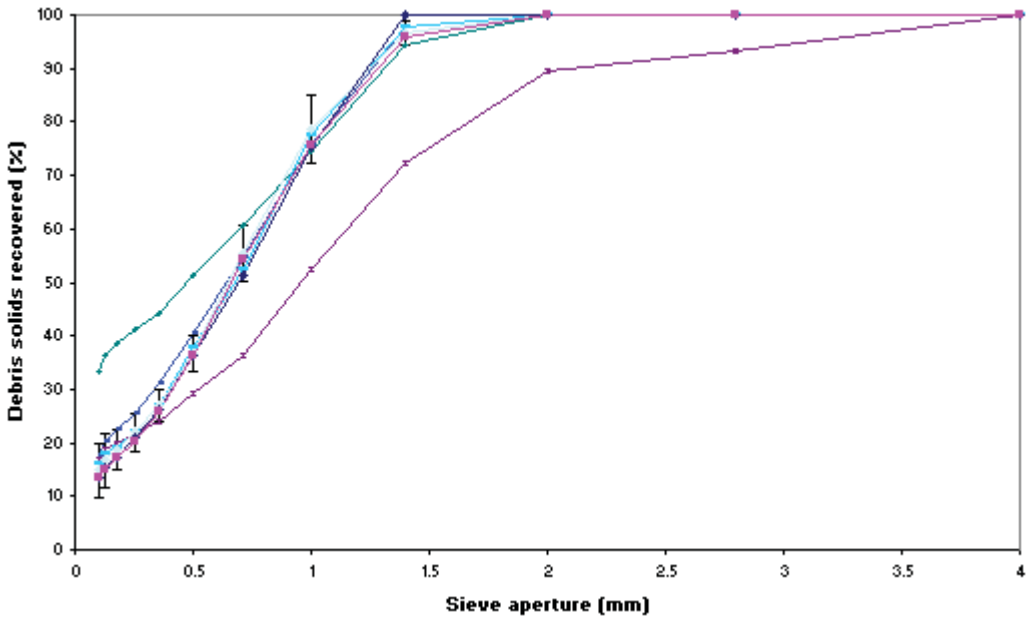



**Figure 6.9** **Cumulative distribution of masticated muesli bar showing changes in PSD with specific numbers of chew cycles. Mean data presented as the percentage of solids recovered in, A. Bolus. B. Debris.**

The muesli bar bolus formation over the first 10 chew cycles (Figures 6.8A & 6.9A) shows that with increasing chew cycles there was a reduction in the amount of particles recovered on the sieves 2.8 mm and greater, and an increase in all fractions <2.0 mm, in particular by 10 chew cycles the greatest proportion of particles are present in the fines <0.124 mm. The PSD of the debris compartment fluctuates over the first 10 chew cycles particularly in fractions >2.8 mm, a large increase in particles between the 1.0 – 2.0 mm fractions, and a steady increase in the mass of particles <0.71 mm (Figure 6.8B). The highest proportion of particles were in the fines <0.124 mm which after a large initial decrease, was fairly constant.

The PSD of the bolus from chew cycles 15 to 35 (Figures 6.8A & 6.9A) show a decrease in the proportion of particles between 1.4 and 2.8 mm, with none present in the 4.0 mm fraction, an increase in the fractions below 1.4 mm, and a reduction in the <0.124 mm fraction. The PSD of the debris from chew cycles 15 to 35 show very little change in distribution (Figure 6.9B), but gradually increase in the amounts of particles in the <0.124 to 1.4 mm fractions (Figure 6.8B). Near the subject's swallowing point (25 chew cycles) the bolus was composed of 30% of particles <0.125 mm, and almost equal proportions on fractions in the 0.71 – 1.4 mm range totalling 42% of the bolus, with a small percentage in the 2.8 mm fraction (Figures 6.8A & 6.9A). At the swallow point the debris contained 60% of the particles in the 0.71 – 1.4 mm fractions, 15% of particles were in the <0.124 mm fraction (Figures 6.8B & 6.9B). Five chew cycles pre and post swallow show that the bolus and debris PSD are fairly constant indicating a slower rate of change around the swallow point.

## 6.4 DISCUSSION

### 6.4.1 CHEWING BEHAVIOUR

The chew time taken to form a bolus suitable for swallow varied significantly with food type, and chew time increased in this order: pasta<muesli bar<peanuts. This order is different from the results presented in Chapter 4, where although there was variation between subjects, they followed the same trend for increasing chew time: muesli bar<pasta<peanuts. The reduction in chew time for pasta in this study is most likely due to the change in serving method used, as it was stored in water until the subject ingested the sample. The addition of fluid has been shown previously to result in a lower number of chew cycles prior to swallow (van der Bilt *et al.* 2007; Pereira *et al*. 2006; Shiozawa & Kohyama 2011). There was good reproducibility of chew time taken for specific numbers of chew cycles, with lower reproducibility for the sample that was chewed naturally to the swallow point. This was consistent with the within-subject affect in Chapter 4 which showed some variability in chew time taken to form a bolus for swallow. This is likely to be due to the natural variances in the food texture and composition, with food particles not fracturing identically in each chewing sequence; resulting in food particles in the oral cavity not circulating in exactly the same way during each chewing sequence, therefore requiring a variation in the number of chew cycles to form a suitable bolus for swallow.

The three food types investigated exhibited differences between their chew frequencies for the first 10 chew cycles, and then muesli bar and peanuts were similar in chew frequency up to and past the point of the natural swallow. The pasta showed a steady chew frequency from the swallow point onwards, which was higher than the other two foods studied, which may be due to the bolus texture (Hiimae *et al*. 1996; Lucas *et al*. 1986). The low reproducibility, or more erratic changes in chewing frequency for the initial ten chew cycles for all food types is likely to be due to the initial exploratory chew cycles and change in food texture from cycle to cycle (Langley & Marshall 1993; Hiimae *et al*. 1996). This variability occurs as the PSD is reduced, solids are lost to the debris compartment, the bolus is triturated with saliva, and soluble solids are dissolved (Chapter 4; Chapter 5; Lucas *et al*. 1986). Not only is the initial assessment of the food properties, such as hardness, being carried out, but the subject is ensuring that the food

is safe to consume (Heath 2002). The large initial fluctuations in chew frequency for the peanuts may also be due to the discrete particles introduced into the mouth (i.e. split peanut kernels) that fracture during breakage and, specifically in the early stages before a unified bolus is formed, particles need to be selected and positioned in the dental mill; compared with less fluctuation in chew frequency for the rectangular shaped single portions of pasta and muesli bar, neither of which exhibit fracture properties (Table 3.7). It is also likely that error is introduced in the measurement of the first chew cycle due to the researcher reaction time for recording of start/stop of the chew cycle. The regularity of the chew frequency past the natural swallow points for the three food types could be due to the attentiveness of the subject in continuing to chew when they have passed their trigger to swallow, as it is not a natural process to over chew food.

### 6.4.2 LOSS OF TOTAL SOLIDS

It was observed that the food types in this study did not result in 100% solids recovery for any of the chew cycles studied, and that debris was formed from the first chew cycle. The solids losses could be due to transport of the bolus to the oropharynx prior to swallowing and intermediary swallows (Hiiemae *et al.* 1996; Hiiemae 2004; Mioche *et al.* 2002b; Okada *et al*. 2007), although it is surprising if this transport occurs so early in the mastication sequence. Alternatively it could be due to poor retrieval of solids from the interproximal spaces (Newell *et al.* 2002) or oral sulci during rinsing. The low recovery of muesli bar solids is most likely due to the higher quantity of total sugars (Table 3.5) some of which would have been dissolved during mastication, with further dissolution during the wet sieving process applied (Hutchings *et al*. 2011). If the total sugars are added back to the muesli bar solids recovered for the first chew cycle, this would result in approximately 89% recovery solids of which is comparable to the other food types. A different methodology (with no wet sieving) is applied in Chapter 7 for calculating bolus moisture uptake and solids loss from 15 chew cycles onwards. This alternative method results in significantly lower solids loss, which gives more detail about bolus solids during mastication.

It was observed during this study that a non-cohesive bolus formed at <15 chew cycles for the food types studied as the chewing sequence was, at a maximum, two thirds complete; other researchers have also noted that food bolus samples expectorated early

in the chewing sequence are not completely masticated or cohesive (Mioche *et al.* 2002 & 2003; Lillford 1991; Hutchings & Lillford 1988). It was also observed that to expectorate the bolus at a specified number of chew cycles <15, the subject also expectorated extra saliva that was not "bound" into the food bolus particles, which seemed to assist the ease of bolus retrieval. Moisture content analysis on the 1 – 10 chew cycle samples would not be accurate, but PSD analysis by wet sieving is suitable with stabilised bolus samples as the PSD should not be changed with this additional saliva. During the study it was found that the reproducibility of bolus and debris solids recovery for 4 to 10 chew cycles replicates were good. The results for solids recovered for the first and second chew cycles studied were more erratic, and likely to be due to the initial exploratory cycles. This is consistent with the variable chew frequency that was calculated for the initial chew cycles.

### 6.4.3 DYNAMIC CHANGES IN BOLUS FORMATION

The greatest changes in mass of recovered bolus solids occurred over the first 10 chew cycles, this trend was also evident in the PSD changes of the bolus for each of the foods studied. It has been noted previously during PSD studies with carrot that the patterns for selection and breakage functions were evident by 10 chew cycles (Lucas & Luke 1983), although it is clear that larger particles are more readily selected as the 4.0 mm fraction is the first to be reduced significantly for the three food types. Particle size has been shown to influence the breakage function (Olthoff, *et al.* 1984; van der Bilt *et al.* 1987; van der Glas *et al.* 1987) with larger particles more susceptible to breakage than smaller ones which is supported by the results in this study. Also, muesli bar and peanuts showed evidence that when the largest particle fraction has been significantly reduced in proportion the next size fraction is selected. This supports the findings that selection chances have been shown to be influenced by larger particle sizes (van der Glas *et al.* 1987; Lucas & Luke 1983). The three food types in this study show evidence that the PSD of the bolus results in an increase of particles <2.0 mm with increasing number of chew cycles. This is contrary to findings by other researchers who found that increased chances of all particle sizes were found to be susceptible for breakage by enhanced competition for breakage sites; due to increased chances of smaller food particles being retained in teeth fossa if the occlusal area and the total amount of food was constant (van der Glas *et al.* 1985; van der Glas *et al.* 1992; van der

Bilt *et al.* 1992; Lucas & Luke 1983). Although it can be seen in this study that the mass of food is not kept constant at any stage during mastication, as solids loss is observed with every chew cycle which may have resulted in increased chances of larger particle selection. The formation of the debris compartment could reduce selection chances of smaller particles to be broken by the teeth by changing the mass of food competing for breakage sites. There is also evidence of this changing bolus mass resulting in increased chances of larger particle selection due to peanuts being embedded in two different matrices: gelatine and chocolate (Hutchings *et al.* 2012). There are greater losses of solids from the chocolate sample bolus to the debris fraction (and in total solids losses over a chewing sequence), and the PSD of the peanuts is not significantly different between the two matrices at the subject's swallow point; this results in a significantly shorter chewing sequence for the chocolate sample as the larger particles have increased chances of selection and more quickly reach the required PSD for a safe swallow.

Muesli bar and peanuts debris fractions were composed of the majority of particles <2.0 mm, although pasta debris contained particles in the 4.0 mm fraction from 4 chewing cycles onwards. This could be due to differences in their textural properties and their detection in the mouth, as softer particles are not as easily detected as they are perceived as smaller, compared with harder particles of the same size (Engelen *et al*. 2005; Hiiemae *et al.* 1996; Hiiemae 2004; Mioche *et al.* 2002; Thexton 1992). The debris was composed of a majority of mid-range particles (0.5 – 1.4 mm) compared to the bolus which consists of a wider range of particle sizes which could make the bolus more cohesive and suitable for swallowing (Chapter 5). The three food types studied showed a trend where the debris fraction gradually increased with increasing chew number, although the debris compartment may have a fill level as none of the foods studied had over 30% of the recovered solids in the debris, even when the bolus was chewed past the natural swallow point and there were a large number of small particles present. For example, when peanuts debris neared this 'fill level' the debris proportion reduced and seems to have re-circulated for its availability to be incorporated into the bolus as there was a corresponding increase in the mass of solids recovered in the bolus compartment. This movement of particles from bolus to debris, circulation between compartments and the chances of particle selection in the mouth is likely to be influenced by saliva

addition (Chapter 5; Chapter 7), bucco-lingual food engagement (van der Bilt *et al.* 1987), the tongue's ability to reload teeth (Voon *et al.* 1986), particle lodgement (Newell, *et al.* 2002; Every *et al.* 1998; Hoppert *et al.* 1932) and cusps of teeth deflecting particles with the direction of mandibular movement (Lucas & Luke 1983; van der Bilt *et al*. 1987). The food composition and properties, such as muesli bar's high sugar content or TPA adhesiveness, may have led to particles being held in teeth interproximal spaces or fossae. This could be one explanation for why the muesli bar and peanuts differed in bolus to debris compartment behaviour, even though they shared the tendency to form a cohesive bolus and showed similarities in the way the PSD changed with increasing chew number.

### 6.4.4 DYNAMIC PARTICLE SIZE BREAKDOWN

The three food types investigated showed characteristic PSDs for each of the chew cycles studied indicating that initial food properties have a persistent affect during bolus formation. The effect of food type has been shown previously for the swallowable bolus composition and confirms that there is no particular particle size threshold for swallowing (Chapter 4; Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007; Jiffry & Molligoda 1983; Lucas & Luke 1984; Hoebler *et al.* 2000; Fontijn-Tekamp *et al.* 2004). This study indicates that particle selection and breakage operate in different ways that are dependent on the initial physical properties of the food that is being consumed, this is also observed in the study by Hutchings *et al.* (2012).

The rate of change of the bolus PSD seemed to decline as it nears the swallow point for each of the foods studied, which has been suggested to be due to the detection of the number of median size particles in the bolus being sensed by the oral mucosa (Lucas & Luke 1983; Prinz & Lucas 1995), perhaps as a particle size threshold in bolus preparation for swallow is approached (Hutchings & Lillford 1988). With chewing 5 cycles past the natural swallow point there were similarities between that PSD and the swallow point PSD for both peanuts and muesli bar. Pasta showed continual change in PSD with the increasing number of chew cycles. This difference in processing is likely to be due to the type of bolus that is formed by the pasta as it is not as cohesive as the other food types investigated, and still contains the highest proportion of particles in the 4.0 mm fraction. Also the initial high food moisture content of pasta does not result in

the bolus reaching the same end point as muesli bar and peanuts (Chapter 5; Chapter 7). Pasta also showed a reduction in particles <4.0 mm at 10 chews past the natural swallow point; this is likely to be due to a continued increase in saliva addition which would have reduced cohesive forces between the particles which were then lost to the debris proportion (Iveson *et al*. 2002; Reynolds *et al*. 2005; Rondet *et al*. 2008).

Each of the food types investigated show that the PSD of the debris proportion is fairly constant by 15 chew cycles, but that the mass on each sieve fraction increases with increasing chew number. It seems that there may be a level of saturation within the debris proportion where no more debris is added, and this is not reached by the swallow point and is dependent on food type. Chewing past the point of natural swallow shows evidence of particulate movement between bolus and debris compartments but this is likely to be due to the relationship with bolus moisture content resulting in reduced cohesion with the concurrent saliva addition and further particle size reduction.

The bolus and debris compartments exhibited significantly different PSDs in the group study in Chapter 4 for all food types studied, but in this study there were similarities between the compartments for peanuts PSD from 15 chew cycles onwards. This could mean that the debris is continually being circulated and masticated further by the teeth to change the PSD, and this can be seen in the change of mass in the sieve fractions for the peanuts which fluctuates. This could be due to the initial food properties, rigidity of particles (Hutchings & Lillford 1988), and their dynamic changes during the mastication process with concurrent addition of saliva to efficiently achieve a swallow-safe bolus (Lillford 1991; Prinz & Lucas 1997). This relationship between bolus solids loss and moisture content is investigated further in Chapter 7.

## 6.5 CONCLUSIONS

This work provides insight into the dynamic particle size breakdown of food during bolus formation. The three food types investigated showed characteristic PSDs for each of the chew cycles studied, indicating that initial food properties have a persistent affect during bolus formation. This study on processed solid foods shows evidence supporting the theories of selection and breakage functions which have been previously studied

with artificial food, hard brittle carrot, or brittle peanut particles embedded in matrices (Lucas & Luke 1983; van der Glas *et al.* 1987; van der Bilt *et al.* 1987; Hutchings *et al.* 2012). The muesli bar and peanuts also showed evidence that when the largest particle fraction has been significantly reduced in proportion the next size fraction is then selected.

The analysis of changes in PSD with an increasing number of chew cycles for the bolus and debris compartments show that bolus formation is food type dependent. Although, for the three food types the particles predominantly selected for breakdown for the specific chew cycles studied were those >2.0 mm. This is most likely due to the loss of bolus solids as this appears to have resulted in increased chances of large particle selection. Particles are found in the debris compartment from the first chew cycle, and with increasing number of chew cycles (cycles 1 to 10) the PSD of the bolus and debris change significantly as a more unified bolus is formed. The rate of change in the PSD of the bolus slows down for the chew cycles studied around the natural swallow point which suggests specific bolus characteristics are being monitored (e.g. bolus moisture content or bolus cohesion), and greater changes in PSD are exhibited at 10 cycles post natural swallow, most likely due to excess saliva and reduced bolus cohesion (Chapter 7). Although the PSD of the bolus and debris shows fewer changes from 15 chew cycles up to 5 cycles past the natural swallow; it can be seen that the masses on the finer sieve fractions continue to increase for pasta and muesli bar as the largest particles are preferentially selected for breakage. The debris sieve fractions then reduce 10 -15 cycles beyond the swallow point most likely due to a reduction in the cohesiveness of the bolus due to excess saliva (Chapter 7) resulting in liberation of particles which results in a corresponding increase in the mass of bolus sieve fractions. Fluctuation in bolus sieve masses is seen for peanuts when the recovered debris sieve fractions reaches 20% which could indicate a food type specific 'fill level' where no more debris is added. This indicates that particles circulate between the bolus and debris compartments continually and is likely to be affected by the concurrent addition of saliva (Chapter 7) and affect the cohesiveness of the bolus.

# CHAPTER 7: DYNAMICS OF BOLUS FORMATION: FOOD SOLIDS LOSS AND MOISTURE CONTENT CHANGES

## 7.1 INTRODUCTION

In Chapter 5, for a group of controlled subjects the swallowable bolus moisture content and the fraction of solids loss from the bolus, was predominantly influenced by food type, with no significant affect of food portion size. The chewing time increased with larger food portion size, although did not increase in direct proportion to the increase in portion size. This raised questions regarding the dynamics of bolus formation, as it seems that saliva is not added to the bolus at a constant rate (Chapter 5). Bolus formation is a dynamic process where the initial food properties are changed significantly and continually until the bolus is suitable for swallowing; this has been investigated by food texture sensory analysis, as it is known that subjects can detect changes occurring throughout mastication (Guinard & Mazzucchelli 1996; Wilkinson *et al*. 2000). The challenge of defining a specific bolus trigger for swallowing by sensory (Lenfant *et al*. 2009), instrumental texture analysis (Chen & Lolivret 2011) or physical composition (Peyron *et al*. 2004b; Engelen *et al*. 2005; Jalabert-Malbos *et al*. 2007) has not yet been found due to the variability between subjects and effect of food type (Chapter 5).

In Chapter 6 findings were presented that showed solids losses from the bolus for the three food types studied were evident from the first chew cycle and that the rate of change of the particle size distribution (PSD) of the bolus slows near the natural swallowing point, which may be due to another bolus parameter being detected to trigger a safe swallow. Within subjects', the variation in bolus physical composition is low (Chapter 4; Chapter 5; Loret *et al*. 2011) which is likely to be due to each subject being familiar with their individual oral physiology and from personal experience. It also seems that, within a food category, there may be detectable similarities in bolus texture and particle composition that may be important to trigger a swallow (Chapter 4; Chapter 5; Chapter 6; Loret *et al*. 2011; Lenfant *et al*. 2009; Peyron *et al*. 2004b).

Although, across food categories when the initial food moisture content is below 50%, there seems to be a similar end point prior to swallowing (Chapter 5), even with significant differences between subjects with the chewing strategies applied.

Bolus moisture content is governed by: inherent food moisture content and how this is bound in food particles or how readily it is expressed to aid lubrication of the bolus (Hutchings & Lillford 1988); and the stimulation of saliva during the mastication process (Heath 2002). It is hard to fully differentiate where the moisture present in the bolus comes from, other than making the assumption that the dry solids of the bolus recovered contained the original food moisture content and that any additional moisture was added by saliva (Chapter 5; Drago *et al*. 2011). Some studies have analysed the bolus moisture content as the mass of expectorated bolus minus the mass of the food sample intake (Mioche *et al*. 2002a; Brudevold *et al*. 1990), which does not take into account the fact that there are usually food particles left in the mouth after expectoration (debris), and dissolution of an unknown proportion of soluble solids will have occurred (Chapter 4; Chapter 5; Brudevold *et al*. 1990; Drago *et al*. 2011; Heath 2002). The debris (and loss of soluble solids) explains why the mass of some bolus samples recovered are less than the mass of the food ingested, and often these sample results are disregarded (Mioche *et al*. 2002a). Significant correlations have been found between subjects salivation rates (stimulated (using Parafilm) or unstimulated) and bolus moisture content for natural food samples at the end of the chewing sequence (Gavaio et al. 2004; Engelen et al. 2005). Although it is not known whether the moisture content of the bolus increases at a steady rate through the chewing sequence for food, and whether there is any interaction effect with loss of solids from the bolus.

Cohesion of the bolus increases throughout the chewing process with food particle size reduction and addition of saliva (Prinz & Lucas 1997) until an optimum level is reached where, it is hypothesized, a swallow may then be triggered. In the study of granulation, it has been shown that further moisture addition may result in reduced cohesion and particles become dispersed with shear (Iveson *et al*. 2002; Reynolds *et al*. 2005; Rondet *et al*. 2008). The dynamic mastication process depends on continual particle size reduction and salivation rates. The saliva may be absorbed by food particles and dissolve soluble components from the food (Watanable & Dawes 1988a) with the rates

of these processes increasing with decreasing particle size (Wright & Hills 2003). Concurrently, particles are witheld from the bolus adhering to the teeth and oral mucosa (Chapter 4; Chapter 5; Chapter 6; Hiimae *et al*. 1996; Drago *et al*. 2011). In single food portion studies, this debris material is collected by the tongue during a clearance phase, formed into a bolus and swallowed (Hiimae *et al*. 1996). This process of solids loss from the bolus to be swallowed later may seem inefficient, but is likely to decrease the mastication time required to form a bolus of swallowable composition, such as reducing the time taken to achieve a 50% moisture content threshold (Loret *et al*. 2011).

In this study, the rates of moisture addition and loss of solids from the bolus for three food types were measured at specific numbers of chew cycles up to and past the point of natural swallowing. This is to gain understanding on how these mastication processes influence bolus formation and whether they support the cohesion theory of swallowing. This was a single subject study to eliminate between subjects variation in their individual chewing strategies as it has been shown previously that the swallowable composition of the bolus is similar between subjects (Chapter 5).

## 7.2 MATERIALS AND METHODS

Three food types were chosen with a differing range of chemical composition and homogeneity: muesli bar, pasta and peanuts (Section 3.1). The food samples were prepared and standardised by weight at 4 g (Section 3.2), and actual weight noted. Food moisture content (Equation 3.1) was measured in duplicate for each food type per session to ensure that the most accurate data was used to calculate the quantity of ingested solids (Equation 3.6). The samples for moisture content analysis were placed in an oven half-way through the study session.

The samples presented per session were randomly ordered with only one food type per session following the study design in Section 3.7.2. The samples collected for this study were collected at the same time as the samples for Chapter 6. One male subject (age 27 y) was recruited through the process outlined in Section 3.6. Each of the study samples (Section 7.2.1) was assessed in triplicate over three study sessions. A total of 45 bolus samples were collected for analysis. The samples were processed immediately as they were provided by the subject, following the procedure described in Section

3.4.9. The moisture content of the bolus was calculated on a dry mass basis of the sample, the change in moisture content from ingested food to bolus, and the percentage loss of solids from the bolus were determined by equations 3.7, 3.8, and 3.9. Relationships between changes in bolus moisture content and dynamics of bolus PSD changes were explored using d50, d75 and d90 data which is taken from the cumulative graphs in Chapter 6 (Figures 6.5A, 6.7A & 6.9A).

### 7.2.1 EXPERIMENTAL PROCEDURE

The session protocol outlined in Section 3.7.3 was followed, except that instead of the subject chewing the sample to a swallowable bolus, the researcher instructed the subject when to expectorate the bolus at a specific number of chew cycles (15, 20, 25, 30, 35). The subject did not know the number of chew cycles being investigated with each sample until they were asked to expectorate the sample. A complete set of data was collected for all chew cycle numbers in each session, but the order of chew cycles was randomised across all sessions to minimise any order effects. These numbers of cycles were up to and past the point of natural swallow (by approximately ten chew cycles). This was determined by the mean number of chew cycles from a previous group study that used these same food types (Chapter 5), and confirmation from observation of the subject during the familiarisation session. Chew time was recorded and chew frequency determined.

# 7.3 RESULTS

## 7.3.1 CHEWING BEHAVIOUR

The subject chewed and swallowed the first sample in every session to familiarise themselves with the food type, which provided data on their natural swallow. The mean number (±SEM) of chew cycles for muesli bar, pasta, and peanuts were 27 (±2.0), 14 (±1.0), and 28 (±2.0), respectively (Table 7.1). The reproducibility of the chew time for the swallow samples was lower than for expectorating samples at specific numbers of chew cycles, but this is due to the varying number of chew cycles taken to reach the swallow point dependent on when the subject felt the bolus was ready for swallowing.

The chew time increase was directly proportional with the increasing number of chew cycles, which is confirmed by the chew frequency data showing a constant rate for each number of chew cycles studied for each food type (Figure 7.1). Only the muesli bar swallow sample showed a lower mean rate than the chew number sample near the swallow point.

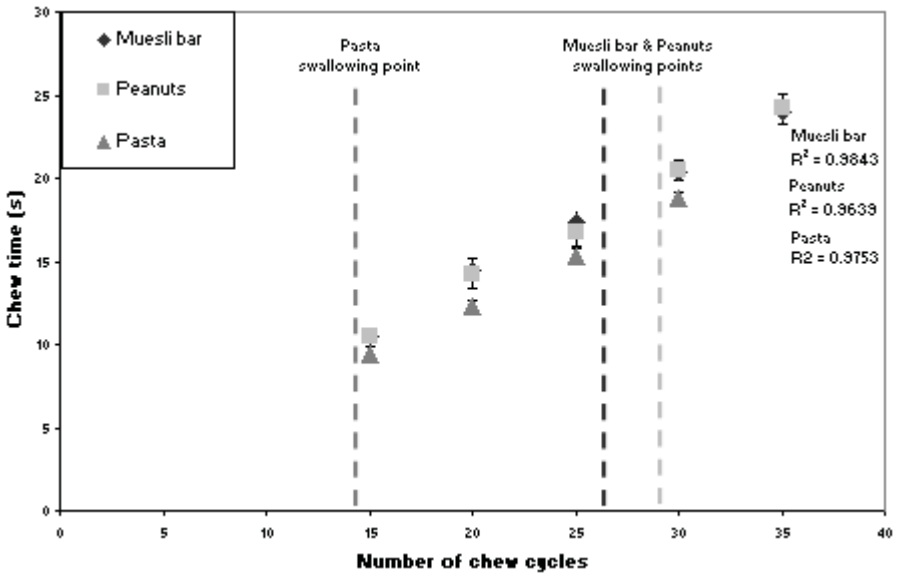



**Figure 7.1** **Chewing frequency at specific numbers of chew cycles and for the subject's natural swallowing point.**

### 7.3.2 MASS OF BOLUS RECOVERED AND SOLIDS LOSS

The mass of the wet bolus expectorated for the range of chew numbers studied for pasta and peanuts resulted in an increase compared to the mass of the sample ingested (Table 7.1). The muesli bar bolus changed in wet mass with specific numbers of chew cycles, but was generally lower in mass than the ingested samples, with the highest loss at 20 and 25 chew cycles then lower losses of bolus mass by 30 and 35 chews.

**Table 7.1 Mass (±SEM) of sample ingested and bolus expectorated**

| Food Type | Number of chew cycles to swallow point | Cycle number | Ingested wet mass (g) | Bolus wet mass (g) | Change in mass (%) |
|---|---|---|---|---|---|
| Muesli bar | 27 (±2.0) | 15 | 4.10±0.05 | 4.18±0.11 | 2.07 |
| | | 20 | 4.07±0.05 | 3.20±0.29 | -21.37 |
| | | 25 | 4.17±0.09 | 3.20±0.20 | -23.35 |
| | | 30 | 3.98±0.14 | 3.67±0.23 | -7.7 |
| | | 35 | 4.13±0.11 | 3.91±0.64 | -5.25 |
| Pasta | 14 (±1.0) | 15 | 4.08±0.20 | 4.46±0.09 | 9.25 |
| | | 20 | 4.15±0.19 | 4.66±0.36 | 12.16 |
| | | 25 | 4.14±0.16 | 4.15±0.29 | 0.06 |
| | | 30 | 4.11±0.15 | 4.36±0.53 | 5.95 |
| Peanuts | 28 (±2.0) | 15 | 4.04±0.02 | 4.22±0.15 | 4.21 |
| | | 20 | 4.10±0.06 | 4.53±0.19 | 10.53 |
| | | 25 | 4.05±0.10 | 4.48±0.19 | 10.4 |
| | | 30 | 4.16±0.03 | 4.64±0.05 | 11.52 |
| | | 35 | 3.97±0.09 | 4.21±0.22 | 5.91 |

The three foods studied showed that there were food type dependent solids losses from bolus samples when compared to the solids of the ingested food, for all the numbers of chew cycles studied (Figure 7.2). Overall there is greater solids loss with increasing numbers of chew cycles for all food types, this relationship is not linear but showed changes with the specific number of chew cycles studied. Muesli bar resulted in higher solids losses from 20 chew cycles onwards compared to the other food types. With muesli bar there was a significant increase between 15 and 20 chew cycles, and when the food was chewed past the swallow point for up to 30 and 35 chew cycles there was slightly lower solids loss and less reproducibility between replicates.

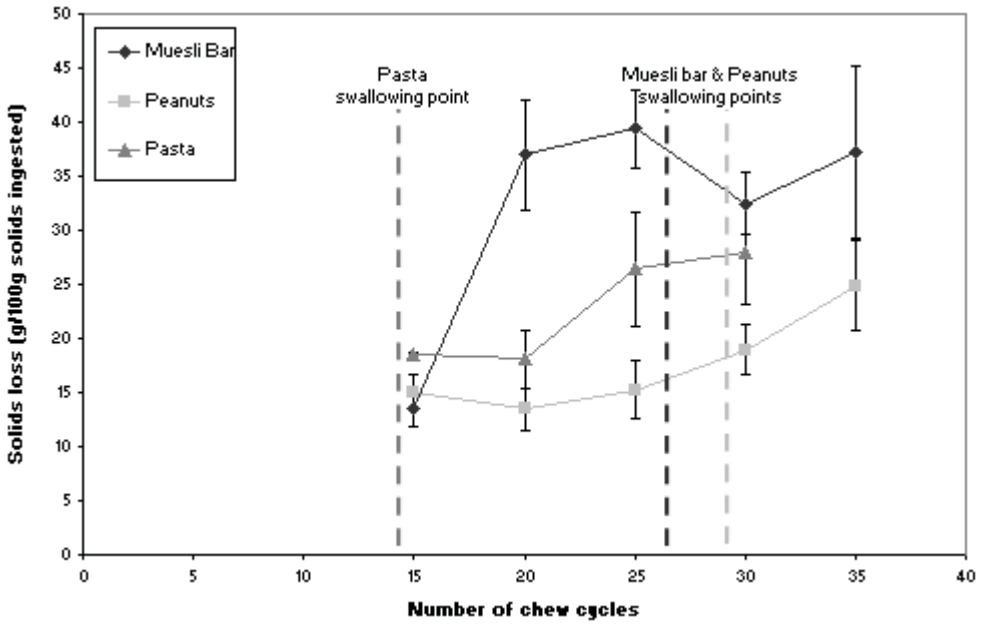



**Figure 7.2** **Mean solids loss (±SEM) from the bolus at specific numbers of chew cycles for each food type.**

The pasta solids loss near the swallow point at 15 chew cycles exhibited good reproducibility, and the solids loss stayed constant until 20 chew cycles. Increasing the number of chew cycles, well past the natural swallow point for pasta showed a significant increase in solids loss and a decrease in the reproducibility between replicates. The peanuts showed a steady increase in solids loss with increasing numbers of chew cycles, with a decrease in reproducibility between replicates when the sample was chewed past the natural swallow point. The peanuts samples exhibited a relationship between increasing chew cycle number and an increase in the quantity of solids loss from the bolus ($r = 0.87$). Pasta showed a stronger correlation between the increase in solids loss with increasing chew cycle number ($r = 0.92$). This relationship was minimised for muesli bar ($r = 0.64$), which showed fluctuations in the quantities of solids loss, possibly due to the heterogeneous matrix of ingredients that compose the muesli bar.

### 7.3.3 BOLUS MOISTURE CONTENT

The moisture content of the bolus for the three foods studied showed a steady linear increase with increasing numbers of chew cycles (Figure 7.3). The bolus moisture content is dependent on food type. Muesli bar and peanuts resulted in moisture contents just under 50g/100g dry solids at the swallow point which is consistent with previous findings for the group study of the food bolus at the swallow point (Chapter 5). The moisture content of the bolus for pasta was significantly higher than the other food types due to the high initial food moisture content. The pasta also exhibits less reproducibility between replicates compared to the other food types.

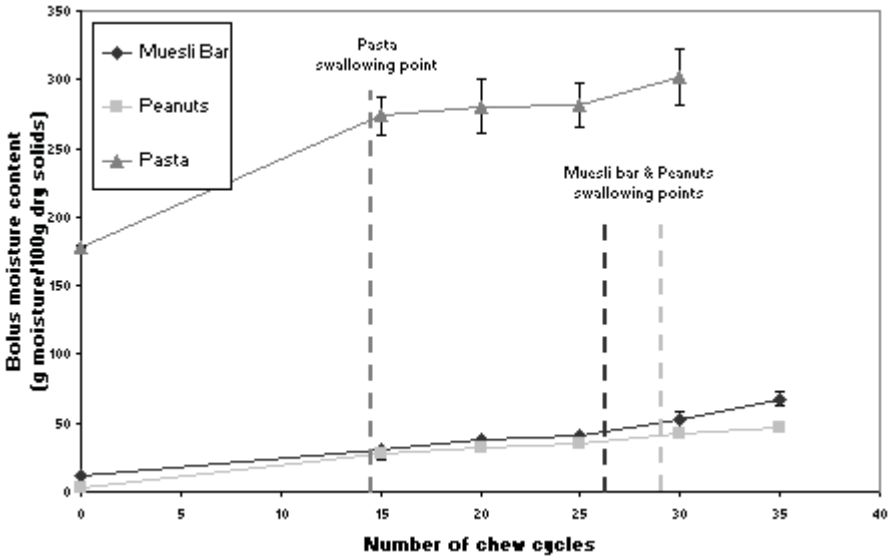



**Figure 7.3** **Mean moisture content (±SEM) of the initial food ingested and the bolus at specific numbers of chew cycles for each food type.**

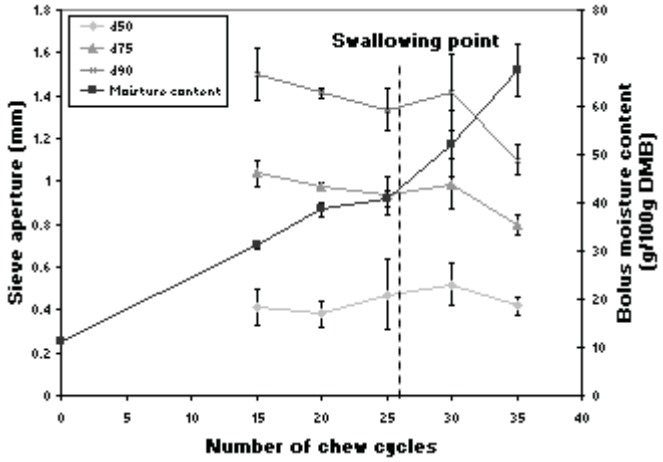
The change in moisture content from ingested food to bolus shows how much saliva was added, with the assumption that there are no moisture losses from the initial food ingested (Figure 7.3). The peanuts and muesli bar showed very similar quantities of added moisture for each of the specific number of chew cycles studied, but with muesli bar showing a significant increase when over-chewed 10 cycles past the natural swallow point. The pasta results in significantly higher quantities of added moisture with each number of chew cycle studied compared to the other food types, with high variability between replicates. Also from ingested food moisture content to the bolus moisture content at the swallow point had the largest quantity of saliva added of the three foods

studied. This clearly shows that the rate of saliva addition is more dependent on food type than chewing strategy. In this study it was found that increasing chew cycle number correlates with the increase in bolus moisture content ($r > 0.89$). This overall trend was not seen previously, as the bolus moisture content variation with chew numbers was due to between subjects' variation in a group study, and they achieved a similar end point of bolus moisture content (Chapter 5). The mean quantity of saliva added between each of the specific chew cycles studied is food type dependent, and fluctuates between the numbers of chew cycles studied within a food type (Figure 7.3).

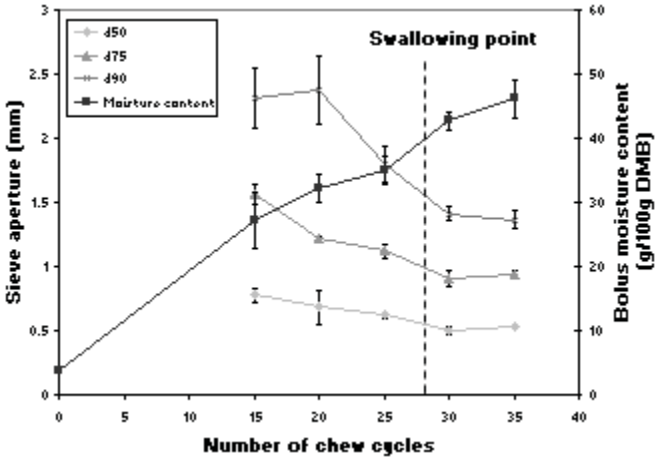
### 7.3.4 RELATIONSHIP BETWEEN BOLUS MOISTURE AND PSD

Overall for the three food types studied it appears that the rate of change in bolus PSD slows as it nears the subject's natural swallowing point, and the moisture content of the bolus continues to increase at a steady rate up to and past the point of natural swallow (Figure 7.4). The muesli bar bolus d50 increases near the swallow point due to an increase in all particles recovered <1.4 mm, but it can be seen that there is a plateau exhibited when looking at the d75 between 20 and 30 chew cycles, which indicates that the limiting step in forming a bolus suitable to swallow is the rate of saliva addition (Figure 7.4A). The peanuts bolus d50 (plus d75 and d90) plateau once the swallow point has been reached, which also indicates that reaching a specific bolus moisture content may be critical to initiate swallowing (Figure 7.4B). The pasta bolus continues to reduce in particle size after the swallowing point has been reached, so it is hard to determine whether moisture or particle reduction is most critical for swallowing (Figure 7.4C).

**A.**




**B.**




**C.**

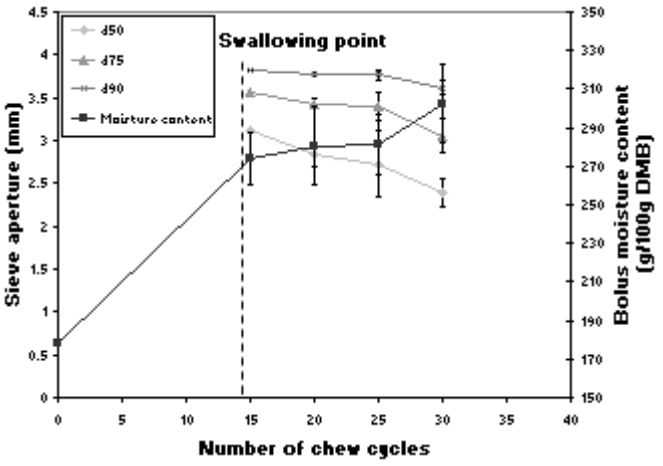



**Figure 7.4** **Relationship between bolus moisture content and particle size d50, d75 and d90 for: A. Muesli bar, B. Peanuts & C. Pasta.**

Note that different scales are used for bolus moisture content and sieve aperture for each of the foods investigated.

Comparing the pasta to muesli bar and peanuts boluses, they are swallowed at very different moisture contents (Figure 7.4). It may be that the high bolus moisture content of pasta enables larger particles to be swallowed, as the d50 of pasta is 3.11 mm (±0.02) compared to that of muesli bar 0.47 mm (±0.16) and peanuts 0.50 mm (±0.03), at their respective swallowing points. In addition, Pearson's correlations between bolus moisture content and solids loss for the numbers of chew cycles studied for muesli bar, pasta and peanuts are r = 0.52, 0.75, and 0.87, respectively.

## 7.4 DISCUSSION

Chewing strategy shows large inter-individual variability in group studies where subjects have been asked to chew solid foods to the natural swallow point (Chapter 5; Mishellany *et al*. 2006; Peyron *et al*. 2004b). Even with the variability between subjects in group studies, the effect of food type has been more influential on the chewing strategy and the subjects show minimal variation in bolus composition when the sample is expectorated at the natural end point of mastication within food type (Loret *et al*. 2011; Mishellany *et al*. 2006). The selection of a single subject to focus on the effect of chew number on the mastication of three manufactured foods has eliminated inter-individual variation. In essence the subject is selected as a 'chewing device' to reproducibly masticate food bolus samples for analysis (Hutchings *et al.* 2012). Intra-individual variability was present but not as great as the variability between food types for most of the parameters studied. When the subject chewed to specific chew numbers, the chew time variation was smaller than the subject's replicates of chewing to the natural swallow point. This is likely to be due to the small variations in texture, composition and initial portion weight of the natural food samples studied.

Overall chew time increased with increasing number of chew cycles, and the chew frequency stayed constant for the duration of each of the chew numbers studied within food type. There is correlation between cycle number and chew time ($r > 0.998$ for each of the food types), supporting evidence presented in most studies on mastication behaviour (Loret *et al.* 2010; Peyron *et al*. 2004; Mishellany *et al*. 2006). In this study as the samples were collected from a single subject, the chew frequency was steady for each food type and specific number of chew cycles studied and this relationship between chew time and chew number was directly proportional. Results from group

studies do not show this relationship as the chew frequency varies between individuals as they use different chewing strategies to reach a swallowable composition of bolus (Chapter 5).

For all foods studied, it was noted that the subject found it "strange" to chew for the numbers of chew cycles that were past the natural swallow point, and that they did not feel as though they would be able to swallow the bolus as it felt "flooded", which supports findings by Prinz & Lucas (1997). However, there was no obvious change in the chew frequency when chewing past the natural swallow point (Figure 7.1).

### 7.4.1 BOLUS MASS, MOISTURE CONTENT AND RATE OF MOISTURE ADDITION

This study aimed to gain understanding of some of the mastication parameters which affect bolus cohesion and adhesion, as a theory of swallowing (Prinz & Lucas 1997). After preliminary investigation, it was decided to focus on chew numbers that produced bolus samples where some degree of cohesion was present, also enabling the subject to expectorate the sample easily without additional saliva to facilitate. Chew numbers below 15 cycles did not result in a bolus that was cohesive enough to expectorate for the food types investigated.

The pasta and peanuts exhibited similarities when comparing the mass of ingested food to the wet bolus mass expectorated; at all specific numbers of chew cycles studied there was an increase in mass in the bolus. The muesli bar bolus mass changes with chew cycle number, and resulted in losses from 20 chew cycles onwards (Figure 7.1). The food composition is likely to have affected the changes in mass, for peanuts and pasta this could be due to the low levels of soluble solids and high levels of insoluble solids (Table 3.6). Muesli bar showed reverse trends with high soluble solids and low insoluble solids. The heterogeneous matrix of the muesli bar may also affect the loss in mass of the bolus due to the wide range of ingredient textures and distribution of particle sizes inherent in the initial food. Mioche *et al*. (2002a) presented data on increased mass of expectorated meat bolus samples (although bolus samples exhibiting weight loss were excluded from the study) and used this as a measure of saliva

incorporation, the bolus weight increased between 30 – 36% dependent on the meat toughness. This study showed fluctuations in the change in mass through the chewing cycle (Table 7.1) for each of the food types. The changes in mass from ingested food to bolus are complex as it has been shown that for the swallowable bolus there is solids loss and moisture addition occurring during the mastication process (Chapter 5).

Mioche *et al*. (2002a) found that more saliva is incorporated into a piece of meat the more it is chewed, overall this finding is consistent with this study, but if the specific chew numbers are investigated further to identify the quantity of added moisture for each increase of 5 chew cycles, it can be seen that there are fluctuations and intra-individual variations (Figure 7.3). This indicates that the rate of moisture addition is not constant during the mastication process (Brudevold et al. 1990), and is contrary to other studies that reported a reduction in saliva flow over the chewing sequence as this was not seen for the food types studied (Gaviao *et al*. 2004; Watanabe & Dawes 1988a). Also the effect of food type can be seen to affect the rate of addition which was not observed in the study by Mioche *et al*. (2002a) when meat bolus samples were expectorated after 7s chew time duration for different textured meat samples.

The bolus moisture content of the muesli bar and peanuts samples near their swallow points were approximately 50g moisture/100g dry solids, which are consistent with previous findings for group studies with food types that have initial food sample moisture contents below this level (Chapter 5; Loret *et al*. 2011). This result is independent of the initial food moisture content, unless it is initially higher than 50g/100g, such as the pasta sample. Loret *et al*. (2011) also showed that this moisture content is achieved independent of mastication strategy applied, but seems to adapt to product type. This cannot be seen here due to the similarity of chew time and cycle number between muesli bar and peanuts, but with different chew numbers it can be seen that the rate of moisture addition is product dependent and not only related to mastication strategy.

It is known that salivary flow rate can be stimulated by low pH (Watanabe & Dawes 1988b), high sugar content and salt content (Neyraud *et al*. 2003), low fat content (Gavaio, *et al*. 2004) and food texture (Agrawal *et al*. 1998; Mioche *et al*. 2003; Mackie

& Pangborn 1990). However, despite these influences, the increase in moisture content was relatively constant for muesli bar and peanuts, although in this study it is not seen how initial saliva flow is affected as food particles are exposed during the initial mastication sequence before a more cohesive bolus is formed. The bolus moisture content of the pasta is more variable which is likely due to the type of bolus formed of a high proportion of particles >4.0 mm bound in a moisture matrix to enable a safe swallow (Chapter 4; Hoebler *et al*. 2000). Although the change in moisture from ingested food to bolus is more than three times as high compared to peanuts and muesli bar, the added moisture per 5 chew cycles studied, as viewed by the slope of the curve, showed that the moisture addition level varied (Figure 7.3). This implies that the main function of mastication of pasta is moisture addition opposed to particulate breakdown resulting in lower chew cycle numbers required to form a swallowable bolus, which could be related to the cohesiveness of the initial food. It also indicates that composition of the food type is detected for the pasta to have had significantly more moisture added to the bolus compared to muesli bar and peanuts.

Moisture contents of all the food bolus samples, even when formed past the natural swallow point were well below (<64%) the measured saturation moisture content of the insoluble food particles (Table 3.5). Near the swallow point the moisture contents were less than 58% of the saturation of insoluble food particles, which is similar to findings from a previous group study (Chapter 5). These results support the hypothesis that the bolus does not reach an equilibrium state in which only hydrated particles remain (Chapter 5).

### 7.4.2 BOLUS SOLIDS LOSS

The levels of solids losses from the expectorated boluses near the swallow point for pasta and muesli bar were similar to previously reported data (Chapter 5). The solids loss for peanuts, even with over chewing at 35 chew cycles (Figure 7.2) was not as high as seen in a previous group study which was 30 – 40 g/100 g dry solids (Chapter 5). In this study the overall trend showed that an increase in the number of chew cycles resulted in increased solids loss, this was not seen previously as the variation with chew numbers was due to between subjects variation (Chapter 5). In studies that have measured food bolus solids lost in group studies for foods within a category (e.g. cereals

and model cheeses) with initial food texture and moisture content differences, there has been no effect of product or within subjects effect (Drago *et al*. 2011; Loret *et al*. 2011), but significant differences between subjects. Food type differences are found as the foods in this study are from different categories and the study by Loret *et al*. (2011) identifed that within a category differences in bolus solids lost may not be evident, particularly on an individual level. This validates that research using a single subject to study the mastication process for specific numbers of chew cycles to identify what happens during the process is most suitable to understand bolus formation.

This study shows that the rate of solids loss is not constant through the mastication process so does not seem to be related specifically to increasing chew cycle number, and is dependent on food type. There is a large change in the quantity of solids loss for muesli bar between 15 and 20 chew cycles which does not seem to be related to bolus moisture uptake (Figure 7.4) or particle size distribution of the bolus or debris (Chapter 6). It is likely these changes are related to the dissolution of soluble solids from the food as particulates are exposed during the mastication process.

The percentage of solids loss from the bolus does not distinguish between insolubles and solubles that were present in the initial food samples. The dissolution effect has been proposed to explain the differences found in mastication studies between initial and recovered solids contents of foods (Peyron *et al*. 2004b; Jalabert-Malbos *et al.* 2007). It is obvious that this process occurs, from the sensation of taste that occurs during oral processing, but the losses found are much greater than the soluble solids of the food types studied (Chapter 5). To investigate the potential effect of initial food soluble solids, the amounts were compared to the experimentally observed solids lost. The soluble solids content of peanuts and pasta were low (Table 3.5). For the chew cycle numbers studied, it was found at 15 cycles peanut had lost nearly double the quantity of insoluble solids, and for the number of chew cycles to reach the natural swallow point, nearly 2.5 times the soluble solids level in the initial food were lost. Pasta exhibited a similar trend; at 15 chew cycles (which was also the natural swallow point) approximately 2.8 times the quantity of the soluble solids was lost. Muesli bar showed that less than 50% of the soluble solids would account for the quantity of solids loss from the bolus at 15 chew cycles, and by 25 chew cycles which is near the natural

swallow point, 100% of the dissolvable solids would account for the solids loss from the bolus.

These results show that the dissolution process is possibly complete by the time the food is ready for swallowing for all food types studied, especially for muesli bar where the ingredients are bound together with a sugar solution and set. The solids loss in pasta and peanuts is likely to be due to the large particles that are recovered in the debris proportion (Chapter 4). Also, as there is a significant proportion of particles over 4.0 mm in size in both the bolus and debris proportions, these would not have been processed sufficiently to dissolve any solids.

These results suggest that both dissolution of soluble material and loss of particulates from the bolus are important to the size and make up of the bolus at the point of swallowing. Both processes will be affected by the rates of the addition and subsequent loss of moisture during bolus formation, with the concurrent particle size reduction during mastication.

### 7.4.3 INTERACTIONS BETWEEN BOLUS MOISTURE CONTENT AND SOLIDS LOSS

As mentioned in Chapter 5, it seems that 50g moisture/100g dry bolus solids may be an optimal level of bolus moisture content to initiate a swallow for the food groups studied (except pasta which has a high initial food moisture content) and that subjects are able to detect this parameter of the bolus immediately prior to swallow. The concurrent particle size reduction of particles in the bolus (Chapter 6) assisting the loss of solids from the bolus, and particle dissolution with the addition of moisture resulting in the trend for a constant increase in bolus moisture content with increasing number of chew cycles (15 cycles onwards).

There is an expected relationship between bolus solids loss and moisture content, partially due to dissolution of soluble solids content of initial food through moisture addition (Jalabert-Malbos *et al*. 2007); potential for hydration of particles and liquid bridging between particles for bolus formation to achieve a cohesive force in the bolus suitable for a safe swallow (Chapter 5; Prinz & Lucas 1997); and also a moisture phase

assisting the movement of particles from the bolus to be withheld in the debris compartment (Chapter 4; Chapter 6). This study and results from Chapter 6 indicate that initially particles in the debris compartment circulate and PSD continues to change in the bolus and debris compartments, until just after the swallow point. Post natural swallowing point, due to the reduction in bolus cohesiveness and excess saliva (Figure 7.4), changes can be seen in the bolus PSD more noticeably for pasta and muesli bar, and changes in the mass of solids recovered can be seen for the three food types.

In this study the increasing number of chew cycles served as an indicator of these process rates, it can be seen in Figure 7.3 that the rates of moisture addition with every 5 chew cycles studied varies, yet the actual bolus moisture contents were increasing steadily. This indicates that the loss of solids from the bolus kept the moisture content at a steady increase, as the rate of moisture addition is varied. There were significant correlations between bolus moisture content and solids loss for the numbers of chew cycles studied for muesli bar, pasta and peanuts. As these samples were chewed past the natural swallow point, the relationship between bolus solids loss and moisture content is possibly weaker than expected for a bolus that is chewed to the swallow point (Figure 7.4). The relationship between bolus moisture content and the dynamic changes of bolus PSD indicate that the bolus moisture content is more critical with respect to the swallow trigger. Although it is likely that the swallow trigger is the function of the two independent processes reaching an optimum cohesiveness within the bolus for a safe swallow.

The three food types investigated in this study and Chapter 6 were reported to be less suitable for swallowing when mastication continued past the natural swallowing point due to the increasing bolus solids loss and moisture content. This is likely to be due to the continuation of the processes of dissolution, hydration and liquid for bridging (Iveson *et al*. 2001) within the bolus, which disrupts the integrity of the bolus. The results support the theory of Prinz & Lucas (1997) that bolus cohesive forces reduce with over-chewing.

## 7.5 CONCLUSIONS

In this study the boluses from three different food types were characterised at specific numbers of chew cycles up to and past the natural swallowing point to give insights into the mechanisms involved in preparing the bolus, and the changes that occur past the swallowing point. During bolus formation, food type affected the rate of bolus solids loss which may be related to texture changes of the bolus through the mastication process. The rate of moisture content changes during bolus formation fluctuated with the specific numbers of chew cycles studied, and it is likely this is related to the rate of solids loss and dissolution of solids that occurs with concurrent particle size reduction. It seems that a target end point determining bolus suitability for swallow is 50g moisture per 100g dry solids for foods that have initial food moisture contents below this level, and that the subject can consistently identify when the bolus is suitable for swallow and uses the same chewing strategy for a specific food type consistently.

Past the natural point of swallowing, the moisture content of the bolus for each of the food types studied continued to increase. The changes in solids loss from the bolus were dependent on food type, the bolus cohesion reduced and for all food types the subject did not feel that the bolus was suitable for swallow. The chew time required to prepare a bolus for swallowing was directly proportional to the number of chew cycles (including chew cycles past the natural swallow point) and this (the chew frequency) correlated with the initial food cohesiveness. Direct proportionality in the relationship between chew time and chew number is only seen in this study due to investigating a single subject, therefore inter-individual variation has been removed. Chew frequency was consistent for each of the specific chew cycles studied for each of the foods studied, indicating that frequency does not change as bolus consistency changes through the chewing sequence.

It seems that the moisture content of the bolus may indicate a trigger to swallow for certain categories of foods. It would be interesting to identify if sensory or analytical textural parameters correlate with the bolus moisture content and be used to monitor transformations of the initial food into a swallowable bolus through the mastication process for a range of food types.

# CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

## 8.1 CONCLUSIONS AND ORIGINAL CONTRIBUTION TO THE LITERATURE

This research contributes to the knowledge of the mastication of solid processed foods and the formation of a bolus suitable for swallowing. Hutchings & Lillford (1988) were the first to discuss sensory texture perception as a dynamic process based on the mouth detecting the changes occurring to food during mastication. This study has been approached from a food technology perspective to investigate how processed foods breakdown in the mouth, the fate of food particles, and the extent that particles are lubricated during bolus formation to enable a safe swallow. These results are relevant for the development of food products, nutritional science and sensory perception.

It was found that during mastication solids are lost from the bolus for the five food types studied (Chapters 4, 5, 6 & 7), and that these solids are lost progressively from the first chew cycle (Chapter 6). The bolus and debris solids were collected separately for PSD analysis which determined that there were characteristic bolus and debris PSDs for each of the food types investigated. And for the five food types studied the PSD differed significantly between the bolus and debris fractions (Chapter 4). The results from these studies indicate that bolus does not have to meet specific particle size criteria to achieve a safe swallow, due to the range of PSD achieved for the different food types (Chapter 4 & 6). Although due to the characteristic PSD at swallowing, particular bolus criteria appear to be required prior to swallowing and are dependent on the initial food texture and composition (Chapter 4). There was significant variation between subjects exhibited for bolus and debris PSDs, but this was less than the effect of food type (Chapter 4). Within subject's differences were minimal, which supported the idea to study the dynamics of PSD changes during bolus formation with the application of a single subject as an instrument to identify trends (Chapter 6).

It was also found that portion size exhibited a significant effect on the PSD of the debris fraction only, with the 4 g sample containing a higher proportion of large particles (>2.0 mm) and a lower proportion of fines (<0.5 mm), compared to the 2 g sample (Chapter 4). The rates of bolus solids loss and PSD changes in the bolus must have also changed in response to the portion size for no significant affect to be noted. This is probably why the PSD of the debris was affected by portion size and contained a higher proportion of mid-range particles and fewer fines, and supports the hypothesis that the target of mastication is to most efficiently achieve a bolus suitable for safe swallowing.

The difference in PSD between the bolus and debris fractions indicates that compartmentation exists where, for a particular time, particles may be withheld in the debris compartment, unavailable for particle selection for size reduction on the dental mill (Chapter 4 & 6). The majority of debris particles at the swallowing point were found to be in the mid-range sizes (0.5 – 1.0 mm). Some particles greater than 4.0 mm were also present in the debris for some foods (Chapters 4 & 6), this is contrary to previous hypotheses that debris would only be composed of small particles (Schneider & Senger 2001; Lucas 2004). The PSD of the debris fraction for each of the foods studied was fairly consistent by 15 chew cycles. Fluctuation in bolus sieve masses is seen for peanuts and pasta when the recovered debris sieve fractions reached 20% of the recovered debris solids. This indicates that a food type specific 'fill level' may exist, where no more debris is added and that the debris particles circulate between the bolus and debris compartments and masticated further (Chapter 6). This circulation between compartments is likely to be influenced by the concurrent addition of saliva (Chapter 7) and affect the cohesiveness of the bolus.

The dynamics of mastication changes in PSD with increasing number of chew cycles support the theory of selection and breakage functions occurring during mastication, as it was identified that larger particles are more susceptible to breakage than smaller particles (Chapter 6). Also, the muesli bar and peanuts bolus samples showed evidence that when the largest particle fraction has been significantly reduced in proportion, the next size fraction is then selected. The rate of change of both the mass of bolus solids loss, and the PSDs of the bolus and debris fractions were seen to decrease as they neared the subject's swallowing point (Chapters 6 & 7).

It was found that bolus moisture content was approximately 50% moisture immediately prior to swallow, when the food ingested is initially below 50% moisture content (Chapter 5). There was no significant effect of portion size on the bolus moisture content. This suggests that for the foods studied, saliva is not added to the bolus at a constant rate during chewing, as there is a defined amount of moisture required to form a suitable bolus to achieve a safe swallow, therefore, to achieve the same moisture content for increased portion size, more saliva must have been added.

In Chapter 7 results showed that rates of moisture addition with every 5 chew cycles studied varied and were dependent on food type, yet the actual bolus moisture contents increased steadily. This indicates that the loss of solids from the bolus kept the moisture content at a steady increase, as the rate of moisture addition is varied. With continued chew cycles past the swallow point, the bolus moisture content continued to increase steadily for all food types; and for muesli bar and peanuts that form a paste-like bolus, the changes in PSD indicate a plateau is reached near the swallow point (Chapter 6). Past the natural swallowing point the bolus samples were less cohesive and deemed unsuitable for swallow by the subject. The relationship between bolus moisture content and the dynamic changes of bolus PSD indicate that the bolus moisture content is more critical with respect to the swallow trigger. Although it is likely that the swallow trigger is the function of the two independent processes reaching an optimum cohesiveness within the bolus for a safe swallow.

## 8.2 SUGGESTIONS FOR FUTURE RESEARCH

In recent years the interest in mastication has grown considerably and the inaugural international conference on Food Oral Processing – Physics, Physiology, and Psychology of Eating was held in Leeds, UK in 2010. This event initiated discussions and sparked many ideas for studies and raised questions from the research presented in this thesis, the following areas could be the focus of future research.

It is of interest to identify what happens when subsequent portions of food are consumed, and to determine whether there is a saturation ‘fill level’ for debris particles to re-circulate, reducing further in size and aiding bolus formation, or whether once a

proportion of particles are around the mouth, the majority of these stay until the end of the feeding sequence when they are removed with clearance.

In this study, the high reproducibility within and between subjects for bolus PSD and moisture content indicate that the continuous neural feedback from perireceptors and mechanoreceptors in the oral mucosa detect when the bolus is suitable for safe swallow. Loret *et al.* (2011) found that "fluidity" was a sensory parameter for cereal analysis that could indicate suitability of the bolus for swallow. It would be of interest to identify if correlations exist between the dynamic sensory analysis of foods through the mastication sequence and mastication outcomes from a cycle by cycle bolus formation study.

The moisture content of the bolus seems to be a critical parameter indicating suitability of a cohesive bolus for swallowing and it would be of interest to measure this for a wider range of food types that have initial moisture contents below 50%. Further studies on model foods could be used to understand the moisture changes occurring during bolus formation like the study designed by Drago *et al.* (2011).

As PSD does not seem to be the single trigger for swallowing it would be of interest to measure the textural characteristics and tribology of the food bolus through the breakdown process. Chen & Lolivret (2011) have measured the rheology of model boluses and noted that extensional stretch-ability was important for the ease of swallowing for liquid and semi-solid foods. It would be appropriate to further develop techniques to measure the 'cohesiveness' of the bolus produced near the swallow point to provide support to the cohesiveness theory of swallowing (Prinz & Lucas 1997).

Mastication and swallowing are controlled by the autonomic nervous system, and unless something interferes with this process, mastication and swallowing usually occur subconsciously. It would be of interest to monitor the debris compartment or solids loss from the bolus in groups of subjects who have impairments to the mastication process (e.g. xerostomia, reduced muscle function) to identify if there is a difference from healthy subjects. Current work in this area focuses on changes in masticatory function

(muscle activities, jaw movements and PSD of the bolus) without consideration of the debris compartment or loss of solids during oral processing.

Research into the effect of human physiology and psychology of eating could have an affect on the mastication outcomes. For example, subjects' hydration status was not monitored in these studies and could affect salivation rates. Can the mastication outcomes be affected by the time of the study? In this thesis the studies were all controlled to morning sessions 1 – 2 hrs after a meal and food is more commonly consumed at set times throughout the day. Is there a link between mastication and satiety or obesity? It was noted that in the small group studies that the subjects with the highest BMI recorded the shortest chew times and those with the lowest BMI recorded the longest chew times. Are the in vitro and in vivo glycaemic responses affected by the bolus composition? Can the subjects' mood, eating experience or food preferences influence the outcomes of mastication? If the participants' favourite food is studied, do they consume it differently to a similar but alternative product?

Any future research on mastication and swallowing, if approached from a food technology perspective will provide further insights to those presented in this thesis, which may be beneficial to the food industry, services in health sciences, and enable greater understanding about consumer's sensory perception.

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**APPENDIX 1: Food ingredient listings.**

**Muesli bar**

Cereals (45%) (rolled oats, whole wheat, puffed rice (rice flour, sugar, salt, malted barley extract, emulsifier (471)), wheat flakes (whole wheat, salt, malted barley extract)), glucose (contains sulphur dioxide), sugar (white and brown), fruit (5%) (raisins, dehydrated apple, apricot fruit grains, (humectant (422), acid (330, 332), stabiliser (pectin), antioxidant (300), colour (annatto)), vegetable fat (hydrogenated palm kernal oil, emulsifier (soy lecithin)), humectant (420, 422), peanuts (2.8%), vegetable oil (canola contains antioxidant 319), sunflower seeds, coconut (may contain sulphur dioxide), emulsifier (soy lecithin), salt.

**Contains wheat, oats, barley, peanuts, sesame seeds, soy and sulphites.**

**May contain traces of milk solids and other nuts.**

**Peanuts**

Peanuts, vegetable oil (contains antioxidant 319)

**Contains Peanuts**.

**Lasagne**

Durum wheat semolina

**May contain traces of egg and soy**.

**Soft cereal bar**

Fruit (25%) (raisins, apricot, apple), cereals (15%), (oats, wheat bran, cornflakes), sugar, wheat flour, vegetable fat, (antioxidant (306)), glucose syrup, milk solids, humectants (420, 422), coconut, whole egg powder, raising agents (450, 500), stabilisers (1442, 461, 440), salt, food acids (330, 296, 300), flavours, antioxidant (332), preservatives (202, 224), colour (106b).

**Contains wheat, oats, milk, egg, soy and sulphites.**

**Produced in a factory handling egg, sesame seeds, soy bean products, peanuts and other nuts.**

**Cake**

Sugar, wheat flour, egg, water, butter (milk), animal & vegetable fat/oils, glucose syrup, skim milk powder, humectants (420, 422), emulsifiers (soy lecithin, 435, 471, 481, 491), modified starch (1442), raising agents (500, 541), salt, preservatives (200, 202), flavour, antioxidant (320), acidity regulator (330), colour (160a).

**Contains gluten – containing cereal, egg, milk and soy**.

**Made on a line that also produces almonds.**

## APPENDIX 2: MATERIAL SAFETY DATA SHEET

**1. Identification of the substance/preparation and of the company/undertaking**
***Identification of the product***
Catalogue No: 10315 ID No.: 1031500
Product name: **Tris(hydroxymethyl)methylamine AnalaR (tris buffer)**
***Manufacturer/supplier identification***
Company: BDH Laboratory Supplies, Poole, Dorset, BH15 1TD, England
Telephone : + 44 (0) 1202 660444 Telefax : + 44 (0) 1202 666856
Emergency telephone No.: + 44 (0) 1202 669700
**2. Composition/information on ingredients**
***Chemical characterization***
Organic base
Product name: Tris(hydroxymethyl)methylamine
CAS number: 77-86-1 EC-No.: 201-064-4
Molecular formula: $NH_2.C(CH_2OH)_3$= 121.14 g/mol
**3. Hazards identification**
Irritating to eyes and skin.
**4. First aid measures**
- Eye contact: Irrigate thoroughly with water for at least 10 minutes. If discomfort persists, obtain medical attention.
- Inhalation: Remove from exposure, rest and keep warm. In severe cases obtain medical attention.
- Skin contact: Wash off thoroughly with soap and water. Remove contaminated clothing and wash before re-use. In severe
cases, OBTAIN MEDICAL ATTENTION.
- Ingestion: Wash out mouth thoroughly with water and give plenty of water to drink. OBTAIN MEDICAL ATTENTION.
**5. Fire-fighting measures**
***Special risks:***
Combustible. May evolve toxic fumes in fire.
***Suitable extinguishing media:***
Water spray, foam, dry powder or carbon dioxide
**6. Accidental release measures**
Wear appropriate protective clothing.
If local regulations permit, mop up with plenty of water and run to waste, diluting greatly with running water. Otherwise transfer
to container and arrange removal by disposal company. Wash site of spillage thoroughly with detergent and water.
For large spillages liquids should be contained with sand or earth and both liquids and solids transferred to salvage containers.
Any residues should be treated as for small spillages.
**7. Handling and storage**
***Handling:***
Wash hands and face thoroughly after working with material. Contaminated clothing should be removed and washed before
re-use.
***Storage:***
Store at room temperature (15 to 25°C recommended). Keep well closed and protected from direct sunlight and moisture.
**8. Exposure controls/personal protection**
***UK Exposure Limits:***
None assigned
***Personal protective equipment:***
As appropriate to the situation and the quantity handled.
- Respirator: Dust respirator
- Ventilation: Extraction hood

- Gloves: Rubber or plastic
- Eye Protection: Goggles or face-shield
- Other Precautions: Plastic apron, sleeves, boots - if handling large quantities

**9. Physical and chemical properties**

***General information:***

Form: solid
Colour: white
Odour: odourless

***Health, safety and environmental information:***

Melting temperature 170°C
Boiling temperature n/a
Density(g/ml) n/a
Solubility in water Very soluble
pH value 10.5 (6 g/l)
Flash point Not applicable
Explosion limits: lower: No data
Auto-ignition temperature No data

**10. Stability and reactivity**

Stable.
Substances to be avoided
oxidizing agents.
The possibility of reaction with other substances cannot be excluded.

**11. Toxicological information**

- After skin contact: Irritation.
- After eye contact: Irritation. Risk of corneal clouding.

Degreasing effect on the skin, possibly followed by secondary inflammation.

- After ingestion: nausea, vomiting, agitation, confusion, cyanosis, collapse, spasms, muscular symptoms, coma.

***Further data***

LD50 5900 mg/kg oral, rat.
We have no evidence of carcinogenic effects. We have no evidence of mutagenic or teratogenic effects.

**12. Ecological information**

Degradability: 89%/28d (hydrochloride)
Quantitative data on the ecological effect of this product are not available.
Do not allow to enter drinking water supplies, waste water, or soil!

**13. Disposal considerations**

Chemical residues are generally classified as special waste, and as such are covered by regulations which vary according to
location. Contact your local waste disposal authority for advice, or pass to a chemical disposal company. Rinse out empty
containers thoroughly before returning for recycling.

**14. Transport information**

Not subject to transport regulations.

**15. Regulatory information**

***Labelling according to EC directives***

Symbol: Xi Irritant.
R-phrases: R36/38
Irritating to eyes and skin.
S-phrases:
EC-No.: 201-064-4

***Local Regulations***

Within the UK, the use of this material must be assessed under the Control of Substances Hazardous to Health (COSHH)
regulations.

**16. Other information**

Revision.
Supercedes issue of 18/08/94
Changes in Section : 12
Date of issue: 13/02/01
Date of print: 21/04/04

**APPENDIX 3: Human ethics application, Participant information and procedures**

## Information Sheet for Participants

Hi! My name's Christine and I am a student at Massey University Albany studying for my PhD in Food Technology. I would like to invite you to participate in my studies investigating the particle size of foods after chewing. This is to understand whether different foods are broken down to different sizes, and to find out if everybody chews the same foods to the same state. My findings will be related to nutrition studies.

**Who can participate?**

Men, born in New Zealand, aged between 18 – 25 years old with a full set of natural teeth. You must be able to eat single portions of peanuts, muesli bar, soft cereal bar, cake and cooked pasta. You also need to be willing to chew foods in front of the researcher (me), and spit them out, just when you feel ready to swallow. You need to be available for one hour any time before 11 am to participate in the study, and attend at the same time for the duration of the study.

**What will my participation involve?**

- Complete a screening questionnaire in order to determine whether you can participate in the study (20 minutes of your time).
- If invited, be prepared to undergo a Dental Examination on 11$^{th}$ August by a qualified dentist, at which point you will be informed if you do not meet the recruitment criteria. If suitable for the study, the dentist will take a mould of your teeth to enable the calculation of chewing surface areas. (10 minutes of your time and dental exam paid for by researcher).
- You will then be invited to proceed with the study. There will be eight sessions of 30 minutes to 1 hour duration held at Massey University Albany. The first session is to familiarise yourself with chewing and spitting foods, and getting used to doing this in the interview room in front of the researcher.
- You will be observed by the researcher during the study to count the number of chews taken, and timed to measure how long it takes to chew a food until the point where you are ready to swallow.

**What are my rights as a participant?**

- You are under no obligation to accept this invitation to participate in the study.
- You may withdraw from the study at any time up until the end of the study sessions.
- Prior to each session you will be provided with a list of ingredients of all the foods you will be chewing. If you are allergic to anything contained on the list, please inform me and this product will be removed from the investigation. If you feel that you cannot chew any of the products being presented to you, you may decline from consuming them.
- You will be compensated for your time after you have attended all eight study sessions, this will be in the form of supermarket vouchers.
- You are encouraged to ask any questions you may have at any time during the study. You can decline to answer any particular question, although this may affect your participation in the study for health and safety reasons.

**Will the results of the trials be provided to me?**

At the end of the study, you may request a copy of the results. No individual data will be presented. The information collected in this study will be used in research papers for publication in scientific journals and for the writing of a PhD thesis.

**Who has approved the go ahead of this study?**

This project has been reviewed and approved by the Massey University Human Ethics Committee, ALB Application 05/25. If you have any concerns about the conduct of this research, please contact Associate Professor Kerry Chamberlain, Chair, Massey University Human Ethics Committee: Albany, telephone 09 414 0800 Ext 9078, email humanethicsalb@massey.ac.nz.

**For more information contact:**

**Christine Lawrence**

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Chewing Study
Massey University
Private Bag 102 904
North Shore Mail Centre
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**Office:**
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Oteha Rohe Campus
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**Email:**
c.s.lawrence@massey.ac.nz

**Telephone:**
09 414 0800 Extn 9827
(Office hours)

My supervisor is Dr Kylie Foster and her details are:

**Postal address:**
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Massey University
Private Bag 102 904
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Auckland

**Office:**
Building 20, IFNHH
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Auckland

**Email:**
k.foster@massey.ac.nz

**Telephone:**
09 414 0800 Extn 9845
(Office hours)

## Consent Form

I have read the information sheet and questionnaire and have had details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand I have the right to withdraw from the study at any time and to decline to answer any particular questions or consume any food that I do not wish to consume.

I agree to provide information to the researchers on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project).

I agree to participate in this study under the conditions set out in the Information Sheet.

I have read the ingredient list and, to my knowledge, I am not allergic to anything contained on the list.

Signed: ……

Name: ……

Date: ……

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COLLEGE OF SCIENCES

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AND HUMAN HEALTH
Private Bag 102 904
North Shore Mail Centre
Auckland
New Zealand
T 64 9 443 9770
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www.massey.ac.nz

**Screening Questionnaire**

All information you provide will be treated as confidential.

May I keep your details on file for future studies, if for some reason you are unable to participate in this one? YES NO

**Personal Details**

Name: …….

Date of Birth (day/mth/yr): ……

Ethnic Group: ……. Nationality: ……

Height (m): ……... Weight (Kg): ……...

**Contact Details**

Address: ……..

……..

Telephone: Home: …… Mobile: ……...

Availability: Please indicate all availability by circling times below. (You will only be required to attend one hour per day, maximum).

| DAY | TIME | | | | |
|---|---|---|---|---|---|
| Monday | 8 – 9am | 8.30-9.30am | 9 – 10am | 9.30-10.30am | 10 – 11am |
| Tuesday | 8 – 9am | 8.30-9.30am | 9 – 10am | 9.30-10.30am | 10 – 11am |
| Wednesday | 8 – 9am | 8.30-9.30am | 9 – 10am | 9.30-10.30am | 10 – 11am |
| Thursday | 8 – 9am | 8.30-9.30am | 9 – 10am | 9.30-10.30am | 10 – 11am |
| Friday | 8 – 9am | 8.30-9.30am | 9 – 10am | 9.30-10.30am | 10 – 11am |
| Saturday | 8 – 9am | 8.30-9.30am | 9 – 10am | 9.30-10.30am | 10 – 11am |

Any time when you are unavailable due to holidays/work/exams:

**Foods to be investigated**

The following foods will be presented to you at different times over the sessions. Please indicate in the boxes provided if you are willing/able to eat the above foods, and if you have any known allergies to ingredients in the foods. (A complete list of ingredients will be given to you before you undertake the study).

| Food Type | Allergies | Willing to consume |
|---|---|---|
| Peanuts | | |
| Muesli Bar | | |
| Soft Cereal Bar | | |
| Cake | | |
| Pasta (cooked) | | |

Comments:

**General Health**

Please circle the appropriate answer to the following questions, and add any details where necessary.

Please describe your overall general health.

…….....

Are you taking any medication which affects
muscle function, or saliva flow? YES NO

Do you have any allergies? YES NO

If yes, Please detail ……..

**Dental Health and Habits**

Please circle the appropriate answer to the following questions, and add any details where necessary. A qualified dentist will carry out the examination and take moulds of teeth.

Are you prepared to undergo a dental examination to determine suitability to participate in this study? YES NO

Are you willing to have moulds taken of your teeth? YES NO

- If there are any details that you would not like to give here, please discuss further with the dentist conducting the examination.

Do you wear dentures or have any prosthetic teeth? YES NO

Do you have any teeth missing or loose? YES NO

How many? …….. Tooth type? MOLAR INCISOR

Have you had any major dental work carried out in the last six months? YES NO

Do you feel any pain when eating foods? YES NO

Are there any foods you have difficulty chewing? YES NO

Do you chew chewing gum? YES NO

How often? ……

Do you generally chew on one side of the mouth? YES NO

If yes, which side? RIGHT LEFT

Are you right or left handed when writing? RIGHT LEFT

Do you grind your teeth? YES NO

Do you brush your teeth after each meal? YES NO

**THANK YOU FOR YOUR TIME.**

Please return the questionnaire in the envelope provided, either by mail or in person. If you have any questions regarding this questionnaire or the study, please contact me.

Christine Lawrence

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**Email:**
c.s.lawrence@massey.ac.nz

**Telephone:**
09 414 0800 Extn 9827
(Office hours)

My supervisor is Dr Kylie Foster and her details are:

**Postal address:**
Chewing Study
Massey University
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North Shore Mail Centre
Auckland

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Building 20, IFNHH
Massey University
Oteha Rohe Campus
Albany
Auckland

**Email:**
k.foster@massey.ac.nz

**Telephone:**
09 414 0800 Extn 9845
(Office hours)

## Dental Examination Consent Form

I have read the information sheet and have had details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand I have the right to withdraw from the study at any time until after the first study session. I also have the right to decline to answer any particular questions, but realise that this may exclude me from the study if criteria to participate is not met, so that I can not come to any harm.

I agree to provide information to the researchers on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project).

I agree to undergo a Dental Examination by a qualified dentist to be able to participate in this study under the conditions set out in the Information Sheet. I agree to allow a mould of my teeth to be taken if the dentist confirms that I have met the criteria outlined on the Information Sheet.

I understand that all Examination records and Teeth Moulds will be disposed of confidentially if I withdraw from the study or am not eligible to participate further in the study.

Signed: ……

Name: ……

Date: ……

# Confidentiality Agreement

I …… (Full Name – printed) agree to keep confidential all information concerning the project "Particle Size Distribution of Solid Foods After Human Mastication".

I will not retain or copy any information involving the project.

Signature: …….. Date: ……..

# Dental Examination

**Personal Details**

Code:

Date of birth (day/mth/yr):

Date of examination:

**Part 1: Medication and Dental Health**

| | | |
|---|---|---|
| Have you had any major dental work carried out in the last six months? | YES | NO |
| Do you feel any tooth pain when eating foods? | YES | NO |
| Do you grind/clench your teeth excessively? | YES | NO |
| Are you taking any medication which affects muscle function, or saliva flow? | YES | NO |

If yes, details ……...

**Part 2: Jaw and articulation examination**

Does jaw movement exhibit any of the following:

Clicking

Cracking

Restriction

Discomfort

Pain

**Part 3: Teeth and Gums Examination**

Full set of natural teeth with no restoration work YES NO

Dental Classification

| | | Cl II1 or II2 | Cl I – Cl II | Class I | Cl I – Cl III | Class III |
|---|---|---|---|---|---|---|
| Molar Classification | Right<br>Left | | | | | |
| Canine Classification | Right<br>Left | | | | | |

Central position of incisors

Dental Scheme

| | |
|---|---|
| 8 7 6 5 4 3 2 1 | 1 2 3 4 5 6 7 8 |
| 8 7 6 5 4 3 2 1 | 1 2 3 4 5 6 7 8 |

Dental Caries

Periodontal Condition

Good OK Bad

**Part 4: Moulds of teeth taken** YES NO


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# Confidentiality Agreement

I …… (Full Name – printed) agree to keep confidential all information concerning the project "Particle Size Distribution of Solid Foods After Human Mastication".

I will not retain or copy any information involving the project.

Signature: …….. Date: ……..

**APPENDIX 4: Study protocol (information also provided for ethics approval).**

## Study Session Procedure

**Personal Details:**

Code no: …….....

Date of Session: …….

Time: ……..

**Session 1: Familiarisation**

**Health and Safety Questions:**

| | | |
|---|---|---|
| Are you allergic to any of the ingredients on this list? | YES | NO |
| Do you feel any tooth pain when eating foods? | YES | NO |
| Do you have any loose teeth ? | YES | NO |
| How many? ……... Tooth type? | MOLAR | INCISOR |
| Are there any foods you have difficulty chewing? | YES | NO |
| Are you in good general health? | YES | NO |

**Instructions:**

You will be timed for the duration of the chewing to identify whether you take the same time on replicate samples and whether this differs between food types. I will also count the number of chews taken to reach a swallowable condition in the food. Please let me know when you have consumed each sample. If you would like a break at any time, please let me know, and we can continue with the study when you are comfortable. You are required to rinse your mouth with 25ml water and spit this into individual sample pots between each food type. Then you are welcome to consume water that is provided in between samples, I will note the amount of water consumed and when you consume it. If you decide that you are not happy to continue with the study at any point in today's session, you are under no obligation to stay and may leave the study at any time as this is the last point to pull out.

**Procedure:**

**Sample 1_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Rinse Quantity | |
| Weight of bolus | |
| L or R | |
| Time | |
| Water Consumed | |

**Sample 2_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Rinse Quantity | |
| Weight of bolus | |
| L or R | |
| Time | |
| Water Consumed | |

**Sample 3_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Rinse Quantity | |
| Weight of bolus | |
| L or R | |
| Time | |
| Water Consumed | |

**Sample 4_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Rinse Quantity | |
| Weight of bolus | |
| L or R | |
| Time | |
| Water Consumed | |

**Sample 5_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Rinse Quantity | |
| Weight of bolus | |
| L or R | |
| Time | |
| Water Consumed | |

**Post-session:**

Would you like to continue with the next six sessions? YES NO

If no, please explain (you do not have to answer this) ……

……..

Next session date and time ……..

## Study Session Procedure

**Session**

**Personal Details:**

Code no: …….....

Date of Session: …….

Time: ……..

**Health and Safety Questions:**

| | | |
|---|---|---|
| Are you allergic to any of the ingredients on this list? | YES | NO |
| Do you feel any tooth pain when eating foods? | YES | NO |
| Do you have any loose teeth ? | YES | NO |
| How many? ……... Tooth type? | MOLAR | INCISOR |
| Are there any foods you have difficulty chewing? | YES | NO |
| Are you in good general health? | YES | NO |

**Instructions:**

You will be timed for the duration of the chewing to identify whether you take the same time on replicate samples and whether this differs between food types. I will also count the number of chews taken to reach a swallowable condition in the food. When you are chewing the food, please can you remember the side of the mouth you were chewing on and let me know when you have finished with each sample. If you would like a break at any time, please let me know, and we can continue with the study when you are comfortable. You are required to rinse your mouth with 50ml water and spit this into individual sample pots between each food type. Then you are welcome to consume water that is provided in between samples, I will note the amount of water consumed and when you consume it.

**Sample 1_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** | **C** | **D** |
|---|---|---|---|
| No. of Chews | | | |
| Time to swallow | | | |
| Rinse Quantity | | | |
| Weight of bolus | | | |
| L or R | | | |
| Time | | | |
| Water Consumed | | | |

**Sample 2_____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** | **C** | **D** |
|---|---|---|---|
| No. of Chews | | | |
| Time to swallow | | | |
| Rinse Quantity | | | |
| Weight of bolus | | | |
| L or R | | | |
| Time | | | |
| Water Consumed | | | |

**Sample 3 _____**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** | **C** | **D** |
|---|---|---|---|
| No. of Chews | | | |
| Time to swallow | | | |
| Rinse Quantity | | | |
| Weight of bolus | | | |
| L or R | | | |
| Time | | | |
| Water Consumed | | | |

**Sample 4**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| **Sample** | **A** |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| **Sample** | **B** | **C** | **D** |
|---|---|---|---|
| No. of Chews | | | |
| Time to swallow | | | |
| Rinse Quantity | | | |
| Weight of bolus | | | |
| L or R | | | |
| Time | | | |
| Water Consumed | | | |

**Sample 5**

I will time you and count the number of chews you take to eat the food sample in front of you. Please place the entire sample in your mouth at once, and proceed to chew. Raise a hand as soon as you make the first swallow, so that I can make a note of the time taken. Then let me know when you feel happy that your mouth is clear of the food.

| Sample | A |
|---|---|
| No. of Chews | |
| Time to swallow | |
| Time to clear | |
| Water consumed | |

This is the same sample as you just ate, please place the entire sample in your mouth and proceed to chew. Raise a hand at the point you feel ready to swallow then spit the food out as quickly as possible, into the sample pot provided.

| Sample | B | C | D |
|---|---|---|---|
| No. of Chews | | | |
| Time to swallow | | | |
| Rinse Quantity | | | |
| Weight of bolus | | | |
| L or R | | | |
| Time | | | |
| Water Consumed | | | |

**Next Session, date and time: …….…….**

**APPENDIX 5: Mean mass (g) of recovered solids (±SEM).**

Results presented and discussed in Chapter 6.

Pasta bolus mean mass (g) of recovered solids (±SEM)

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | <0.124 mm | | 0.125 mm | | 0.18 mm | | 0.25 mm | | 0.355 mm | | 0.5 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 0.00E+00 | 0.00E+00 | 1.53E-03 | 1.18E-03 | 1.03E-03 | 8.73E-04 | 2.17E-03 | 2.18E-03 | 4.67E-04 | 3.19E-04 | 6.00E-04 | 7.35E-04 |
| 2 | 0.00E+00 | 0.00E+00 | 1.93E-03 | 1.18E-03 | 1.07E-03 | 8.64E-04 | 1.63E-03 | 8.84E-04 | 3.17E-03 | 2.19E-03 | 9.00E-04 | 6.36E-04 |
| 4 | 0.00E+00 | 0.00E+00 | 2.07E-03 | 8.20E-04 | 2.27E-03 | 9.65E-04 | 1.70E-03 | 1.24E-03 | 1.70E-03 | 1.09E-03 | 1.13E-03 | 7.79E-04 |
| 6 | 0.00E+00 | 0.00E+00 | 2.57E-03 | 1.66E-03 | 1.97E-03 | 7.88E-04 | 2.67E-03 | 1.27E-03 | 2.37E-03 | 2.20E-03 | 2.50E-03 | 9.72E-04 |
| 8 | 0.00E+00 | 0.00E+00 | 4.00E-04 | 2.83E-04 | 2.10E-03 | 1.37E-03 | 1.73E-03 | 1.46E-03 | 1.53E-03 | 1.47E-03 | 1.97E-03 | 1.28E-03 |
| 10 | 0.00E+00 | 0.00E+00 | 2.10E-03 | 1.48E-03 | 2.57E-03 | 1.27E-03 | 2.80E-03 | 1.16E-03 | 3.83E-03 | 1.03E-03 | 2.53E-03 | 1.27E-03 |
| 15 | 0.00E+00 | 0.00E+00 | 4.23E-03 | 2.24E-03 | 4.27E-03 | 1.00E-03 | 3.93E-03 | 7.79E-04 | 3.83E-03 | 1.44E-03 | 3.57E-03 | 7.08E-04 |
| 20 | 0.00E+00 | 0.00E+00 | 1.97E-03 | 1.92E-03 | 2.57E-03 | 1.23E-03 | 4.60E-03 | 1.16E-03 | 4.20E-03 | 1.06E-03 | 5.13E-03 | 2.35E-03 |
| 25 | 0.00E+00 | 0.00E+00 | 2.83E-03 | 7.79E-04 | 4.20E-03 | 1.74E-03 | 5.17E-03 | 1.54E-03 | 4.50E-03 | 2.02E-03 | 5.00E-03 | 1.21E-03 |
| 30 | 0.00E+00 | 0.00E+00 | 3.47E-03 | 1.22E-03 | 5.92E-03 | 1.28E-03 | 6.60E-03 | 5.52E-04 | 5.33E-03 | 5.31E-04 | 6.53E-03 | 9.60E-04 |

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | 0.71 mm | | 1.0 mm | | 1.4 mm | | 2.0 mm | | 2.8 mm | | 4.0 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 1.27E-03 | 1.05E-03 | 3.07E-03 | 2.44E-03 | 7.07E-03 | 6.63E-03 | 2.12E-02 | 2.00E-02 | 3.34E-02 | 4.09E-02 | 1.36E+00 | 9.17E-02 |
| 2 | 1.37E-03 | 9.91E-04 | 1.17E-03 | 5.21E-04 | 3.78E-03 | 1.81E-03 | 6.80E-03 | 9.72E-04 | 9.83E-03 | 7.04E-03 | 1.30E+00 | 4.20E-02 |
| 4 | 1.40E-03 | 9.57E-04 | 2.70E-03 | 2.02E-03 | 4.37E-03 | 2.89E-03 | 9.30E-03 | 5.81E-03 | 2.65E-02 | 2.09E-02 | 1.20E+00 | 1.42E-01 |
| 6 | 2.30E-03 | 1.63E-03 | 4.67E-03 | 4.28E-03 | 1.64E-02 | 7.15E-03 | 2.78E-02 | 6.69E-03 | 5.29E-02 | 1.20E-02 | 1.11E+00 | 8.55E-02 |
| 8 | 1.67E-03 | 1.50E-03 | 3.90E-03 | 5.10E-04 | 1.52E-02 | 2.39E-03 | 2.06E-02 | 3.34E-03 | 7.31E-02 | 1.57E-02 | 9.90E-01 | 9.32E-02 |
| 10 | 4.77E-03 | 9.34E-04 | 9.57E-03 | 3.58E-03 | 2.37E-02 | 4.35E-03 | 4.73E-02 | 4.97E-03 | 1.16E-01 | 1.79E-02 | 7.88E-01 | 1.57E-01 |
| 15 | 3.77E-03 | 1.03E-03 | 9.27E-03 | 1.08E-03 | 2.52E-02 | 1.70E-03 | 4.95E-02 | 6.98E-03 | 1.44E-01 | 1.63E-02 | 5.48E-01 | 5.42E-02 |
| 20 | 5.77E-03 | 1.12E-03 | 1.34E-02 | 6.42E-04 | 4.51E-02 | 1.16E-02 | 6.93E-02 | 5.45E-03 | 1.99E-01 | 1.77E-02 | 4.01E-01 | 9.38E-02 |
| 25 | 5.67E-03 | 2.24E-03 | 1.27E-02 | 5.54E-03 | 3.60E-02 | 1.97E-02 | 8.85E-02 | 6.47E-02 | 9.15E-02 | 4.55E-02 | 4.75E-01 | 2.84E-01 |
| 30 | 8.90E-03 | 8.86E-04 | 1.94E-02 | 3.85E-03 | 6.32E-02 | 2.04E-03 | 9.27E-02 | 5.35E-03 | 1.71E-01 | 1.83E-02 | 2.01E-01 | 6.31E-02 |

Pasta debris mean mass (g) of recovered solids (±SEM)

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | <0.124 mm | | 0.125 mm | | 0.18 mm | | 0.25 mm | | 0.355 mm | | 0.5 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 0.00E+00 | 0.00E+00 | 1.00E-03 | 6.28E-04 | 3.33E-04 | 4.08E-04 | 3.67E-04 | 2.27E-04 | 7.00E-04 | 4.64E-04 | 8.67E-04 | 1.06E-03 |
| 2 | 0.00E+00 | 0.00E+00 | 5.33E-04 | 6.53E-04 | 0.00E+00 | 0.00E+00 | 1.33E-04 | 1.63E-04 | 2.33E-04 | 1.78E-04 | 2.33E-04 | 2.86E-04 |
| 4 | 0.00E+00 | 0.00E+00 | 1.03E-03 | 9.76E-04 | 9.33E-04 | 1.08E-03 | 7.00E-04 | 8.57E-04 | 4.67E-04 | 5.72E-04 | 4.67E-04 | 2.94E-04 |
| 6 | 0.00E+00 | 0.00E+00 | 3.33E-05 | 4.08E-05 | 3.33E-05 | 4.08E-05 | 9.67E-04 | 1.18E-03 | 9.00E-04 | 1.10E-03 | 8.33E-04 | 1.02E-03 |
| 8 | 0.00E+00 | 0.00E+00 | 4.67E-04 | 5.72E-04 | 1.47E-03 | 7.08E-04 | 8.00E-04 | 6.28E-04 | 3.67E-04 | 3.89E-04 | 1.73E-03 | 7.12E-04 |
| 10 | 0.00E+00 | 0.00E+00 | 4.33E-04 | 5.31E-04 | 4.00E-03 | 3.02E-03 | 9.67E-04 | 8.44E-04 | 2.43E-03 | 2.62E-03 | 6.33E-04 | 7.76E-04 |
| 15 | 0.00E+00 | 0.00E+00 | 1.87E-03 | 5.21E-04 | 1.77E-03 | 1.08E-03 | 2.50E-03 | 5.52E-04 | 1.67E-03 | 6.68E-04 | 1.13E-03 | 4.60E-04 |
| 20 | 0.00E+00 | 0.00E+00 | 2.10E-03 | 5.61E-04 | 7.67E-04 | 8.20E-04 | 9.00E-04 | 4.30E-04 | 3.03E-03 | 2.23E-03 | 2.17E-03 | 1.46E-03 |
| 25 | 0.00E+00 | 0.00E+00 | 1.80E-03 | 6.28E-04 | 2.27E-03 | 7.36E-04 | 2.67E-03 | 1.95E-03 | 3.87E-03 | 2.66E-03 | 3.87E-03 | 1.23E-03 |
| 30 | 0.00E+00 | 0.00E+00 | 8.33E-04 | 7.22E-04 | 1.73E-03 | 1.02E-03 | 3.47E-03 | 2.00E-03 | 2.30E-03 | 6.16E-04 | 2.17E-03 | 1.64E-03 |

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | 0.71 mm | | 1.0 mm | | 1.4 mm | | 2.0 mm | | 2.8 mm | | 4.0 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 0.00E+00 | 0.00E+00 | 2.67E-04 | 3.27E-04 | 9.00E-04 | 1.10E-03 | 0.00E+00 | 0.00E+00 | 2.70E-03 | 3.31E-03 | 0.00E+00 | 0.00E+00 |
| 2 | 8.00E-04 | 5.10E-04 | 2.00E-04 | 2.45E-04 | 7.00E-04 | 8.57E-04 | 0.00E+00 | 0.00E+00 | 1.37E-03 | 1.67E-03 | 0.00E+00 | 0.00E+00 |
| 4 | 1.40E-03 | 3.08E-04 | 6.00E-04 | 3.94E-04 | 2.43E-03 | 1.51E-03 | 7.60E-03 | 1.18E-03 | 4.63E-03 | 1.56E-03 | 1.55E-02 | 1.11E-02 |
| 6 | 1.30E-03 | 8.15E-04 | 1.70E-03 | 6.12E-04 | 5.23E-03 | 4.69E-03 | 1.21E-02 | 6.57E-03 | 8.40E-03 | 2.12E-03 | 9.83E-03 | 1.20E-02 |
| 8 | 2.23E-03 | 1.40E-03 | 3.67E-03 | 7.88E-04 | 5.93E-03 | 1.83E-03 | 1.10E-02 | 3.58E-03 | 2.24E-02 | 4.20E-03 | 4.65E-02 | 1.98E-02 |
| 10 | 8.33E-04 | 6.87E-04 | 5.50E-03 | 7.38E-04 | 1.24E-02 | 1.14E-03 | 1.11E-02 | 5.14E-03 | 3.07E-02 | 9.30E-03 | 2.73E-02 | 3.35E-02 |
| 15 | 3.37E-03 | 2.04E-04 | 5.10E-03 | 1.84E-03 | 1.75E-02 | 3.22E-03 | 1.68E-02 | 4.56E-03 | 2.82E-02 | 8.78E-03 | 3.63E-02 | 2.48E-02 |
| 20 | 1.13E-03 | 2.48E-04 | 7.47E-03 | 2.35E-03 | 1.43E-02 | 3.11E-03 | 1.98E-02 | 2.79E-03 | 3.98E-02 | 1.42E-02 | 3.12E-02 | 2.12E-02 |
| 25 | 2.17E-03 | 7.88E-04 | 8.87E-03 | 1.47E-03 | 2.86E-02 | 1.22E-02 | 4.19E-02 | 9.29E-03 | 5.61E-02 | 3.27E-02 | 3.96E-02 | 2.45E-02 |
| 30 | 4.97E-03 | 2.31E-03 | 7.10E-03 | 1.84E-03 | 1.86E-02 | 5.59E-03 | 2.54E-02 | 7.98E-03 | 3.18E-02 | 1.44E-02 | 2.53E-02 | 2.53E-02 |

Peanuts bolus mean mass (g) of recovered solids (±SEM)

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | <0.124 mm | | 0.125 mm | | 0.18 mm | | 0.25 mm | | 0.355 mm | | 0.5 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 0.00E+00 | 0.00E+00 | 6.17E-03 | 3.04E-03 | 4.00E-03 | 1.21E-03 | 1.77E-03 | 3.27E-04 | 7.63E-03 | 9.91E-04 | 4.23E-03 | 1.76E-03 |
| 2 | 0.00E+00 | 0.00E+00 | 9.50E-03 | 3.26E-03 | 5.53E-03 | 2.34E-03 | 4.33E-03 | 1.42E-03 | 4.83E-03 | 3.43E-03 | 8.53E-03 | 4.62E-03 |
| 4 | 0.00E+00 | 0.00E+00 | 2.56E-02 | 2.74E-03 | 1.97E-02 | 2.31E-03 | 1.81E-02 | 1.87E-03 | 2.74E-02 | 2.21E-03 | 3.57E-02 | 4.36E-03 |
| 6 | 0.00E+00 | 0.00E+00 | 3.53E-02 | 1.86E-02 | 2.27E-02 | 1.01E-02 | 2.27E-02 | 1.13E-02 | 3.26E-02 | 1.56E-02 | 4.41E-02 | 2.18E-02 |
| 8 | 0.00E+00 | 0.00E+00 | 4.92E-02 | 1.43E-02 | 5.33E-02 | 2.41E-02 | 4.21E-02 | 4.58E-03 | 5.64E-02 | 7.67E-03 | 7.31E-02 | 6.65E-03 |
| 10 | 0.00E+00 | 0.00E+00 | 8.10E-02 | 1.62E-02 | 5.27E-02 | 8.24E-03 | 5.50E-02 | 4.08E-03 | 6.79E-02 | 1.03E-02 | 9.60E-02 | 1.49E-02 |
| 15 | 0.00E+00 | 0.00E+00 | 1.09E-01 | 1.62E-02 | 7.66E-02 | 7.83E-03 | 7.97E-02 | 3.72E-03 | 1.06E-01 | 4.88E-03 | 1.38E-01 | 5.66E-03 |
| 20 | 0.00E+00 | 0.00E+00 | 1.38E-01 | 2.56E-02 | 1.13E-01 | 3.47E-02 | 1.37E-01 | 4.16E-02 | 1.60E-01 | 4.01E-02 | 1.80E-01 | 3.26E-02 |
| 25 | 0.00E+00 | 0.00E+00 | 9.03E-02 | 1.27E-02 | 6.98E-02 | 5.80E-03 | 8.56E-02 | 1.17E-02 | 1.10E-01 | 1.00E-02 | 1.35E-01 | 9.30E-03 |
| 30 | 0.00E+00 | 0.00E+00 | 1.12E-01 | 2.57E-02 | 1.12E-01 | 2.96E-02 | 1.10E-01 | 3.17E-02 | 1.57E-01 | 5.69E-02 | 1.79E-01 | 5.39E-02 |
| 35 | 0.00E+00 | 0.00E+00 | 6.88E-02 | 1.13E-02 | 5.96E-02 | 6.06E-03 | 7.55E-02 | 3.76E-03 | 1.07E-01 | 3.06E-03 | 1.36E-01 | 2.97E-03 |

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | 0.71 mm | | 1.0 mm | | 1.4 mm | | 2.0 mm | | 2.8 mm | | 4.0 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 6.07E-03 | 4.27E-03 | 1.24E-02 | 4.88E-03 | 1.90E-02 | 5.62E-03 | 3.34E-02 | 8.63E-03 | 4.63E-02 | 2.11E-02 | 3.22E+00 | 1.52E-01 |
| 2 | 1.18E-02 | 7.26E-03 | 1.60E-02 | 1.10E-02 | 1.80E-02 | 1.52E-02 | 2.23E-02 | 1.77E-02 | 6.73E-02 | 6.68E-02 | 2.96E+00 | 3.12E-01 |
| 4 | 4.05E-02 | 1.87E-03 | 4.35E-02 | 1.17E-02 | 7.63E-02 | 1.38E-02 | 1.24E-01 | 3.06E-03 | 1.47E-01 | 1.90E-02 | 2.15E+00 | 7.32E-03 |
| 6 | 5.56E-02 | 2.63E-02 | 6.09E-02 | 2.20E-02 | 9.97E-02 | 3.60E-02 | 1.08E-01 | 4.17E-02 | 1.49E-01 | 5.28E-02 | 1.67E+00 | 3.38E-01 |
| 8 | 9.22E-02 | 7.34E-03 | 1.33E-01 | 2.41E-02 | 1.80E-01 | 4.03E-02 | 1.69E-01 | 2.63E-02 | 2.25E-01 | 1.07E-02 | 8.74E-01 | 5.36E-01 |
| 10 | 1.23E-01 | 1.40E-02 | 1.32E-01 | 1.53E-02 | 2.06E-01 | 6.49E-03 | 2.45E-01 | 4.15E-03 | 2.27E-01 | 2.07E-02 | 4.14E-01 | 1.33E-01 |
| 15 | 1.63E-01 | 4.00E-03 | 1.99E-01 | 1.15E-02 | 2.31E-01 | 1.36E-02 | 1.53E-01 | 2.37E-02 | 1.41E-01 | 3.60E-02 | 5.91E-02 | 4.50E-02 |
| 20 | 1.85E-01 | 2.57E-02 | 1.93E-01 | 1.75E-02 | 1.83E-01 | 6.12E-03 | 1.71E-01 | 8.31E-03 | 1.43E-01 | 2.85E-02 | 6.99E-02 | 4.18E-02 |
| 25 | 1.56E-01 | 1.24E-02 | 1.68E-01 | 1.59E-02 | 1.47E-01 | 9.45E-03 | 1.08E-01 | 1.46E-02 | 6.08E-02 | 1.19E-02 | 2.13E-02 | 2.61E-02 |
| 30 | 1.71E-01 | 4.04E-02 | 1.76E-01 | 3.41E-02 | 1.28E-01 | 3.61E-03 | 1.10E-01 | 3.47E-02 | 1.84E-02 | 1.27E-02 | 0.00E+00 | 0.00E+00 |
| 35 | 1.40E-01 | 9.56E-03 | 1.46E-01 | 1.37E-02 | 1.06E-01 | 3.05E-03 | 5.73E-02 | 8.34E-03 | 1.87E-02 | 1.68E-02 | 0.00E+00 | 0.00E+00 |

Peanuts debris mean mass (g) of recovered solids (±SEM)

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | <0.124 mm | | 0.125 mm | | 0.18 mm | | 0.25 mm | | 0.355 mm | | 0.5 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 0.00E+00 | 0.00E+00 | 6.67E-03 | 1.31E-03 | 5.50E-03 | 1.84E-03 | 7.47E-03 | 2.04E-03 | 1.14E-02 | 4.02E-03 | 1.24E-02 | 4.40E-03 |
| 2 | 0.00E+00 | 0.00E+00 | 1.09E-02 | 2.08E-03 | 8.90E-03 | 1.24E-03 | 8.70E-03 | 1.02E-03 | 1.41E-02 | 1.78E-03 | 1.99E-02 | 2.79E-03 |
| 4 | 0.00E+00 | 0.00E+00 | 1.41E-02 | 2.61E-03 | 1.12E-02 | 4.27E-03 | 1.40E-02 | 5.23E-03 | 1.71E-02 | 4.21E-03 | 2.48E-02 | 7.21E-03 |
| 6 | 0.00E+00 | 0.00E+00 | 1.57E-02 | 5.07E-03 | 1.37E-02 | 3.04E-03 | 1.57E-02 | 7.37E-03 | 2.27E-02 | 8.56E-03 | 3.09E-02 | 1.50E-02 |
| 8 | 0.00E+00 | 0.00E+00 | 1.14E-02 | 6.79E-03 | 1.33E-02 | 4.14E-04 | 1.16E-02 | 4.12E-03 | 1.55E-02 | 5.92E-03 | 2.05E-02 | 9.42E-03 |
| 10 | 0.00E+00 | 0.00E+00 | 1.53E-02 | 4.90E-03 | 1.15E-02 | 2.55E-03 | 1.44E-02 | 2.59E-03 | 1.88E-02 | 3.51E-03 | 2.63E-02 | 4.96E-03 |
| 15 | 0.00E+00 | 0.00E+00 | 1.11E-02 | 3.86E-03 | 9.27E-03 | 3.44E-03 | 1.11E-02 | 4.11E-03 | 1.18E-02 | 5.29E-03 | 1.68E-02 | 5.87E-03 |
| 20 | 0.00E+00 | 0.00E+00 | 1.16E-02 | 3.16E-03 | 8.53E-03 | 6.68E-04 | 8.97E-03 | 1.00E-03 | 9.97E-03 | 1.86E-03 | 1.24E-02 | 2.14E-03 |
| 25 | 0.00E+00 | 0.00E+00 | 6.97E-03 | 2.21E-03 | 8.83E-03 | 1.14E-03 | 1.27E-02 | 3.80E-03 | 1.71E-02 | 5.58E-03 | 1.72E-02 | 6.41E-03 |
| 30 | 0.00E+00 | 0.00E+00 | 1.21E-02 | 1.57E-03 | 1.65E-02 | 3.13E-03 | 1.75E-02 | 4.54E-03 | 2.09E-02 | 4.87E-03 | 5.50E-02 | 4.27E-02 |
| 35 | 0.00E+00 | 0.00E+00 | 1.15E-02 | 1.31E-03 | 1.64E-02 | 1.47E-03 | 1.81E-02 | 1.78E-03 | 2.52E-02 | 3.60E-03 | 2.76E-02 | 1.53E-03 |

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | 0.71 mm | | 1.0 mm | | 1.4 mm | | 2.0 mm | | 2.8 mm | | 4.0 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 1.66E-02 | 1.78E-03 | 2.30E-02 | 3.93E-03 | 3.43E-02 | 4.64E-03 | 3.45E-02 | 1.65E-02 | 3.11E-02 | 8.66E-03 | 0.00E+00 | 0.00E+00 |
| 2 | 2.50E-02 | 1.37E-03 | 3.17E-02 | 2.27E-03 | 4.60E-02 | 5.41E-03 | 4.19E-02 | 4.05E-03 | 4.08E-02 | 1.16E-02 | 9.23E-03 | 1.13E-02 |
| 4 | 3.04E-02 | 1.03E-02 | 3.20E-02 | 1.26E-02 | 4.54E-02 | 1.59E-02 | 2.87E-02 | 9.87E-03 | 3.11E-02 | 6.30E-03 | 9.20E-03 | 1.13E-02 |
| 6 | 3.27E-02 | 1.57E-02 | 4.96E-02 | 1.87E-02 | 5.25E-02 | 2.65E-02 | 4.39E-02 | 6.79E-03 | 7.67E-02 | 5.23E-02 | 2.74E-02 | 2.22E-02 |
| 8 | 2.62E-02 | 1.20E-02 | 3.92E-02 | 2.01E-02 | 4.75E-02 | 2.42E-02 | 3.62E-02 | 1.47E-02 | 3.21E-02 | 4.56E-03 | 5.60E-02 | 4.15E-02 |
| 10 | 3.17E-02 | 8.65E-03 | 3.60E-02 | 5.65E-03 | 4.30E-02 | 4.28E-03 | 2.92E-02 | 7.26E-03 | 3.02E-02 | 1.76E-02 | 1.21E-02 | 1.49E-02 |
| 15 | 1.73E-02 | 6.00E-03 | 2.18E-02 | 5.66E-03 | 1.86E-02 | 5.50E-03 | 1.97E-02 | 1.32E-02 | 2.53E-03 | 3.10E-03 | 1.19E-02 | 1.45E-02 |
| 20 | 1.33E-02 | 7.76E-04 | 1.32E-02 | 9.51E-04 | 1.17E-02 | 5.86E-03 | 1.37E-02 | 7.19E-03 | 3.60E-03 | 4.41E-03 | 3.67E-03 | 4.49E-03 |
| 25 | 1.64E-02 | 5.82E-03 | 1.43E-02 | 4.76E-03 | 7.97E-03 | 5.92E-03 | 9.17E-03 | 6.51E-03 | 1.30E-02 | 1.60E-02 | 0.00E+00 | 0.00E+00 |
| 30 | 2.13E-02 | 2.64E-03 | 1.68E-02 | 2.08E-03 | 1.34E-02 | 4.00E-03 | 8.90E-03 | 8.14E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| 35 | 2.39E-02 | 2.70E-03 | 1.96E-02 | 6.05E-03 | 2.04E-02 | 8.05E-03 | 3.00E-03 | 1.97E-03 | 2.30E-03 | 2.82E-03 | 0.00E+00 | 0.00E+00 |

Muesli bar bolus mean mass (g) of recovered solids (±SEM)

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | <0.124 mm | | 0.125 mm | | 0.18 mm | | 0.25 mm | | 0.355 mm | | 0.5 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 2.06E-01 | 3.38E-02 | 3.28E-02 | 2.79E-03 | 2.44E-02 | 5.40E-04 | 2.90E-02 | 2.31E-03 | 3.32E-02 | 1.95E-03 | 3.79E-02 | 1.60E-03 |
| 2 | 2.77E-01 | 2.03E-02 | 3.22E-02 | 2.38E-03 | 3.18E-02 | 2.58E-03 | 3.29E-02 | 1.62E-03 | 4.10E-02 | 3.33E-03 | 4.73E-02 | 2.59E-03 |
| 4 | 3.06E-01 | 2.73E-02 | 3.81E-02 | 2.45E-03 | 3.15E-02 | 3.04E-03 | 3.26E-02 | 5.41E-03 | 4.03E-02 | 4.53E-03 | 5.01E-02 | 9.53E-03 |
| 6 | 3.39E-01 | 2.65E-02 | 3.25E-02 | 5.21E-03 | 2.75E-02 | 1.08E-03 | 2.98E-02 | 5.38E-03 | 3.77E-02 | 5.44E-03 | 5.04E-02 | 8.70E-03 |
| 8 | 4.02E-01 | 1.02E-02 | 3.41E-02 | 4.82E-03 | 3.27E-02 | 4.14E-03 | 3.55E-02 | 2.86E-03 | 4.50E-02 | 5.07E-03 | 6.88E-02 | 7.79E-03 |
| 10 | 4.38E-01 | 4.60E-02 | 3.84E-02 | 1.99E-03 | 3.27E-02 | 5.12E-03 | 3.58E-02 | 3.46E-03 | 4.67E-02 | 4.07E-03 | 7.11E-02 | 4.17E-03 |
| 15 | 3.50E-01 | 7.09E-02 | 2.54E-02 | 2.71E-03 | 2.36E-02 | 1.97E-03 | 2.57E-02 | 1.63E-03 | 3.75E-02 | 2.99E-03 | 6.51E-02 | 4.40E-03 |
| 20 | 2.79E-01 | 4.51E-02 | 1.48E-02 | 2.14E-03 | 1.53E-02 | 3.78E-03 | 1.73E-02 | 4.49E-03 | 2.99E-02 | 4.65E-03 | 4.96E-02 | 6.43E-03 |
| 25 | 1.77E-01 | 3.91E-02 | 1.57E-02 | 2.44E-03 | 1.37E-02 | 2.59E-03 | 1.62E-02 | 3.65E-03 | 2.76E-02 | 6.41E-03 | 5.15E-02 | 1.02E-02 |
| 30 | 1.67E-01 | 2.71E-02 | 1.04E-02 | 7.08E-04 | 2.08E-02 | 1.15E-02 | 1.25E-02 | 1.57E-03 | 2.21E-02 | 5.12E-03 | 5.04E-02 | 9.65E-03 |
| 35 | 2.08E-01 | 2.61E-02 | 1.22E-02 | 5.72E-04 | 1.43E-02 | 2.03E-03 | 1.72E-02 | 3.19E-04 | 2.87E-02 | 1.20E-03 | 6.17E-02 | 3.89E-04 |

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | 0.71 mm | | 1.0 mm | | 1.4 mm | | 2.0 mm | | 2.8 mm | | 4.0 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 3.63E-02 | 2.96E-03 | 6.24E-02 | 1.06E-02 | 1.28E-01 | 2.06E-02 | 1.70E-01 | 2.83E-02 | 4.19E-01 | 3.47E-02 | 5.96E-01 | 2.32E-01 |
| 2 | 4.91E-02 | 4.14E-03 | 8.09E-02 | 1.53E-02 | 1.41E-01 | 9.93E-03 | 2.45E-01 | 3.39E-02 | 4.06E-01 | 6.99E-02 | 2.53E-01 | 4.86E-02 |
| 4 | 5.97E-02 | 8.80E-03 | 1.03E-01 | 2.75E-02 | 1.52E-01 | 2.93E-02 | 1.81E-01 | 1.61E-02 | 3.04E-01 | 5.89E-02 | 1.49E-01 | 9.73E-02 |
| 6 | 6.88E-02 | 1.37E-02 | 1.02E-01 | 1.40E-02 | 1.62E-01 | 2.42E-02 | 1.50E-01 | 3.74E-02 | 2.38E-01 | 7.23E-02 | 1.16E-01 | 2.69E-02 |
| 8 | 8.68E-02 | 1.02E-02 | 1.15E-01 | 1.77E-02 | 1.69E-01 | 2.78E-02 | 1.16E-01 | 2.93E-02 | 1.41E-01 | 3.94E-02 | 8.45E-02 | 1.03E-01 |
| 10 | 9.58E-02 | 6.24E-03 | 1.23E-01 | 2.10E-03 | 1.94E-01 | 1.86E-02 | 1.54E-01 | 1.63E-02 | 1.71E-01 | 4.84E-02 | 1.29E-02 | 9.70E-03 |
| 15 | 8.90E-02 | 5.48E-03 | 1.27E-01 | 1.63E-02 | 1.61E-01 | 3.80E-02 | 9.31E-02 | 3.22E-02 | 2.18E-02 | 1.41E-02 | 0.00E+00 | 0.00E+00 |
| 20 | 6.96E-02 | 6.26E-03 | 9.99E-02 | 1.12E-02 | 9.91E-02 | 8.14E-03 | 6.15E-02 | 1.51E-02 | 1.42E-02 | 9.08E-03 | 0.00E+00 | 0.00E+00 |
| 25 | 6.91E-02 | 1.32E-02 | 1.06E-01 | 2.32E-02 | 8.34E-02 | 1.28E-02 | 4.05E-02 | 1.58E-02 | 6.83E-03 | 4.31E-03 | 0.00E+00 | 0.00E+00 |
| 30 | 7.92E-02 | 1.76E-02 | 1.12E-01 | 2.22E-02 | 6.68E-02 | 4.62E-03 | 6.08E-02 | 3.23E-02 | 1.30E-03 | 1.59E-03 | 0.00E+00 | 0.00E+00 |
| 35 | 9.46E-02 | 6.50E-03 | 1.13E-01 | 2.77E-03 | 7.01E-02 | 1.64E-02 | 9.17E-03 | 4.71E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |

Muesli bar debris mean mass (g) of recovered solids (±SEM)

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | <0.124 mm | | 0.125 mm | | 0.18 mm | | 0.25 mm | | 0.355 mm | | 0.5 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 9.44E-02 | 1.06E-01 | 8.67E-04 | 6.79E-04 | 7.67E-04 | 6.34E-04 | 7.33E-04 | 3.56E-04 | 1.80E-03 | 1.28E-03 | 2.40E-03 | 1.17E-03 |
| 2 | 2.73E-02 | 8.92E-03 | 1.47E-03 | 5.35E-04 | 8.00E-04 | 4.64E-04 | 9.00E-04 | 9.25E-04 | 2.37E-03 | 1.53E-03 | 5.00E-03 | 1.87E-03 |
| 4 | 3.80E-02 | 2.56E-02 | 2.03E-03 | 8.04E-04 | 1.27E-03 | 5.93E-04 | 2.80E-03 | 8.57E-04 | 3.33E-03 | 1.61E-03 | 6.43E-03 | 3.43E-03 |
| 6 | 3.25E-02 | 2.69E-02 | 2.93E-03 | 1.36E-03 | 1.40E-03 | 8.83E-04 | 2.80E-03 | 1.85E-03 | 4.23E-03 | 3.40E-03 | 8.27E-03 | 4.35E-03 |
| 8 | 3.25E-02 | 1.88E-02 | 1.43E-03 | 1.08E-03 | 2.17E-03 | 1.91E-03 | 2.87E-03 | 2.02E-03 | 3.63E-03 | 3.62E-03 | 6.87E-03 | 4.04E-03 |
| 10 | 3.09E-02 | 1.08E-02 | 2.80E-03 | 3.94E-04 | 2.17E-03 | 3.49E-04 | 2.67E-03 | 1.37E-03 | 3.17E-03 | 1.68E-03 | 7.23E-03 | 1.96E-03 |
| 15 | 3.51E-02 | 1.65E-02 | 4.30E-03 | 1.60E-03 | 4.10E-03 | 1.83E-03 | 5.43E-03 | 2.13E-03 | 1.07E-02 | 4.64E-03 | 1.78E-02 | 7.39E-03 |
| 20 | 4.06E-02 | 1.23E-02 | 4.87E-03 | 1.00E-03 | 2.73E-03 | 1.29E-03 | 6.87E-03 | 2.08E-03 | 1.20E-02 | 2.07E-03 | 2.70E-02 | 4.40E-03 |
| 25 | 3.99E-02 | 1.93E-02 | 4.57E-03 | 1.87E-03 | 3.80E-03 | 1.24E-03 | 7.90E-03 | 2.07E-03 | 1.16E-02 | 2.70E-03 | 2.00E-02 | 3.75E-03 |
| 30 | 4.23E-02 | 3.26E-02 | 5.23E-03 | 2.23E-03 | 5.30E-03 | 1.28E-03 | 8.73E-03 | 1.60E-03 | 1.41E-02 | 4.28E-03 | 2.58E-02 | 6.55E-03 |
| 35 | 2.29E-02 | 1.01E-02 | 2.77E-03 | 5.76E-04 | 3.17E-03 | 6.98E-04 | 4.73E-03 | 5.76E-04 | 9.63E-03 | 2.01E-03 | 1.73E-02 | 3.10E-03 |

| | Sieve aperture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chew cycles | 0.71 mm | | 1.0 mm | | 1.4 mm | | 2.0 mm | | 2.8 mm | | 4.0 mm | |
| | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| 1 | 6.47E-03 | 1.63E-03 | 6.30E-03 | 2.65E-03 | 6.97E-03 | 3.77E-03 | 7.83E-03 | 4.72E-03 | 1.34E-02 | 9.92E-03 | 0.00E+00 | 0.00E+00 |
| 2 | 7.33E-03 | 2.55E-03 | 9.90E-03 | 2.62E-03 | 2.01E-02 | 7.94E-03 | 2.02E-02 | 3.75E-03 | 9.60E-03 | 1.18E-02 | 0.00E+00 | 0.00E+00 |
| 4 | 9.80E-03 | 4.64E-03 | 1.80E-02 | 6.16E-03 | 3.19E-02 | 1.27E-02 | 1.08E-02 | 3.20E-03 | 1.05E-02 | 6.55E-03 | 0.00E+00 | 0.00E+00 |
| 6 | 1.17E-02 | 7.02E-03 | 2.57E-02 | 1.20E-02 | 2.71E-02 | 7.51E-03 | 2.57E-02 | 1.00E-02 | 3.43E-03 | 4.20E-03 | 5.97E-03 | 7.31E-03 |
| 8 | 1.27E-02 | 6.10E-03 | 2.35E-02 | 7.19E-03 | 2.88E-02 | 4.40E-03 | 2.54E-02 | 8.80E-03 | 1.77E-02 | 1.59E-02 | 0.00E+00 | 0.00E+00 |
| 10 | 9.53E-03 | 1.85E-03 | 1.38E-02 | 3.62E-03 | 2.04E-02 | 7.46E-03 | 6.47E-03 | 6.51E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| 15 | 2.62E-02 | 9.80E-03 | 3.62E-02 | 1.14E-02 | 3.62E-02 | 8.41E-03 | 5.90E-03 | 5.13E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| 20 | 3.61E-02 | 2.57E-03 | 6.29E-02 | 8.48E-03 | 5.02E-02 | 7.01E-03 | 5.87E-03 | 4.94E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| 25 | 4.28E-02 | 8.15E-03 | 5.57E-02 | 1.89E-02 | 4.93E-02 | 2.83E-02 | 9.30E-03 | 5.84E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| 30 | 3.83E-02 | 9.11E-03 | 5.62E-02 | 3.90E-03 | 6.59E-02 | 2.48E-02 | 2.67E-04 | 3.27E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| 35 | 3.01E-02 | 6.68E-03 | 3.50E-02 | 5.47E-03 | 3.21E-02 | 7.53E-03 | 6.73E-03 | 2.76E-03 | 0.00E+00 | 0.00E+00 | 0.00E+00 | 0.00E+00 |