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Understanding the relevance of in-mouth food processing. A review of *in vitro* techniques

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Abstract

Oral processing of food is the first step in the eating process. Although the food undergoes a number of changes during mastication that influence the subsequent steps, this stage has very often been neglected in studies of digestion, bioavailability, flavor release, satiety potential, glycaemic index determination, etc. The present review draws on different sources such as nutrition, medicine, phoniatry and dentistry to explain some *in vitro* oral processing methods and techniques that could be transferred to food technology studies to mimic *in vivo* comminution, insalivation, and bolus formation, describing, as a necessary reference, the respective *in vivo* physiological processes they attempt to imitate.

Developing a deeper understanding of all the aspects of in-mouth process will help food technologists to give this crucial step the necessary attention its due importance and to consider better ways to incorporate it into their studies.

Introduction

Food is a mixture of proteins, carbohydrates and lipids that interact physically and chemically in an aqueous environment to create a food-specific native or processed structure. Differences in the chemical composition of foods are therefore associated with differences in their macrostructure and texture which affect various food characteristics, including resistance to hydrolysis or to breakdown during oral food processing and simultaneous (oral) or subsequent (gastric, intestinal digestion. In-mouth actions results from a dynamic process in which the textural characteristics of food are continuously analyzed by the oral sensory systems (Pineau, *et al*., 2009). Chen (2009) reviewed the physiology as well as the rheological principles of food texture and sensory perception, since food texture is the main factor that determines the different processes for transforming food into a material that is ready to be swallowed.

In a pioneering work, Hutchings and Lillford (1988) stated that texture perception in the mouth is a dynamic sensory monitor of changes made to a food. They proposed a groundbreaking general model, defining the breakdown path of the food during oral processing through three aspects or dimensions: the mechanical and rheological behavior of the food (degree of structure), the oral experience via saliva participation (degree of lubrication), and the sequences of oral processing (time). Involving the oral experience and time in texture studies was a significant development which turned texture appreciation from a static process into a dynamic one. Several years later Prinz and Lucas (1997) proposed the optimum swallow model, in which swallowing was defined as the moment when the food bolus reaches a peak cohesive force, driven by the interaction between the food particles (degree of structure) and saliva (degree of lubrication). In this way the duality of separating thresholds for food particle size and for particle lubrication is eliminated: swallowing is initiated when it is sensed that a batch of food particles is binding together under viscous forces so as to form a bolus.

In plain words, digestion is the process of breaking food down into simpler substances that can be absorbed by the body. Food digestion in humans depends on both the chemical and physical characteristics of the food and on how it changes as it passes through the different areas of the digestive tract. Within this framework, the relevance of oral processing up to the instant of swallowing is evident.

Inside the mouth, food undergoes a number of changes. Some of them, such as comminution, are not strictly speaking digestive processes but are undoubtedly necessary before these can take place, and could be considered a “pre-digestive step”.

During in-mouth food processing, food is subjected to several major mechanical and chemical modifications. The solid food is fractured by the teeth and diluted and broken down by saliva. These joint actions induce its progressive comminution and adherence of the resulting smaller

particles through saliva impregnation, formed into a cohesive bolus and finally swallowed (Van der Bilt, Mojet, Tekamp, & Abbink, 2010). It would appear that saliva is involved at every step, not only as a digestive medium but as a lubricant, providing surface smoothness and weak inter-particle adhesive forces (Lillford, 2011). Although mastication seems a simple process, it involves many factors: the physiological characteristics of the individual performing the chewing action, such as facial anatomy, gender, age, personality type, time of day, or dentition status, as well as the properties of the food being chewed, such as hardness, moisture content, fat content, food portion size, or food structure, all have an effect on the formation of the food bolus (Bornhorst & Singh, 2012). The bolus is eventually swallowed when its structural characteristics have become suitable for safe swallowing.

Over the years, researchers from different disciplines such as nutrition, pharmacy, medicine or dentistry have been working on this subject. However, it is in the last decade that food technology research has fully approached oral processing, with enormous interest, as the bridge between food texture, microstructure and sensory perception (Stieger & Van de Velde, 2013). As it constitutes a short step (about 20–30 seconds) in the overall ingestion process compared with the length of the gastric and intestinal stages (1–10 hours), it has often been neglected in studies such as those dealing with food digestion.

In vitro studies covering bioavailability, determination of the carbohydrate glycaemic index, transportation and absorption of nutrients, flavor release, evaluation of the satiety potential of ingredients or whole food systems, etc. are only a few examples of current interests in the area of food science and technology research where the release of some food components from their physicochemical dietary matrix is necessary. This release begins in the mouth. Depending on the scope of each specific study, the selection of methods for mimicking oral actions in *in vitro* studies has to consider a number of factors.

The principal aim of the present work is to give an overview of the main strategies that could be used in Food Technology research for *in vitro* studies in which oral processing plays a role. For this purpose, it offers a review of the main equipment and techniques that have been designed to reproduce human mouth processing, emphasizing the newest of these. The physiological actions they attempt to imitate are necessary references and are also described. This paper will help Food Technology researchers to choose the proper tool for their *in vitro* studies.

Oral comminution

In vivo scenario

The oral breakdown or disruption of food during mastication is highly variable, depending on the food itself (texture, dryness, hardness, size) and on the characteristics of each person (dental health, degree of hunger, particular habits). Many authors have pointed out that the pre-swallow bolus is characterized by a specific particle size distribution that is similar across individuals for the same food (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Mishellany, Woda, Labas, & Peyron, 2006). Nevertheless, some studies have also revealed important inter-individual differences in food bolus formation and in chewing behavior (Loret, *et al*., 2011; Tárrega, Yven, Sémon, & Salles, 2011; Tournier, Grass, Zope, Salles, & Bertrand, 2012).

A recent study by Hwang *et al*. (2012), with banana, tofu, cooked rice, and biscuits eaten by healthy subjects, showed that the particle size distribution of the ready-to-swallow bolus depended essentially on food type and on mechanical properties of the food such as hardness, cohesiveness, and adhesiveness, and not on individual differences. Mishellany *et al*. (2006), working with three nuts and three vegetables, showed that the sizes of the bolus particles just before swallowing were comparable in all subjects, whereas the number of cycles and duration of sequences varied widely between individuals. They stated that fracture and fragmentation of food (ingestion involving fracture of particles by the incisors) were closely correlated with the ratio of toughness to Young's modulus in foods with approximate linear stress-strain relationships (the stress-strain gradient provides the Young's modulus value of the food and toughness is the work required to fracture it). Since the stress-strain relations of a number of food products are distinctly nonlinear, more complex fracture models have to be introduced in these cases (Lucas, Prinz, Agrawal, & Bruce, 2004). Of course, other factors such as water content, the ability to absorb saliva (Hutchings & Lillford, 1988) and the fibrous structure of the food also influence the way in which they are broken down (Mishellany, Woda, Labas, & Peyron, 2006).

In a study by Jalabert-Malbos *et al*. (2007), foods that were swallowed rapidly (14–20 masticatory cycles) were soft and had a high water content, like egg white, pickled cucumbers, mushrooms or olives. The boluses obtained from these foods contained many large particles. Harder foods such as coconuts and carrots needed more cycles and longer mastication before swallowing, probably because more time was needed to process the food and to disrupt the fibers. They also needed more complete insalivation to produce a lubricated bolus that was safe to swallow. To be swallowed easily, particles must be smaller than 2 mm, with the exception of soft particles that are not liable to injure the upper digestive mucosae. Jalabert-Malbos, Mishellany-Dutour, Woda, and Peyron (2007) showed that for a range of foods, sizes from 0.4 to 4 mm with a median of around 2 mm were found in boluses when ready for

swallowing. Mastication reduced bread to an increasing number of small particles. Le Bleis, Chaunier, Della Valle, Panouillé, and Réguerre (2013) found that mastication reduced two types of bread of different textures into an increasing number of small particles. However, the number of small particles did not always increase with the number of masticatory cycles, probably because many small particles are lost during intermediary swallows that are not generally analyzed (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007).

One important parameter that describes the bolus just before swallowing is its median particle size (d_{50}), defined as the theoretical sieve size through which 50% of its mass can pass (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Ngom, Diagne, Aïdara-Tamba, & Sene, 2007). The d_{50} value is a useful way to classify foods used in masticatory evaluation (Veyrune, Opé, Nicolas, Woda, & Hennequin, 2013) according to how easily they are processed in the mouth to form a suitable bolus.

The *in vivo* results highlight two characteristics of mastication in humans. Firstly, the intra-individual variability of food bolus particle size distribution is very narrow. Secondly, there is a contrast between the narrow inter-individual variability of the food bolus d_{50} and the much broader variability of the physiological variables among individuals, such as duration of the sequence, number of strokes, and electromyographic activity (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Mishellany, Woda, Labas, & Peyron, 2006; Peyron, Mishellany, & Woda, 2004).

Quantitative electromyography (EMG) has been used to explain the physiological process of mastication, to assess muscle function, and also to diagnose temporomandibular disorders (González, Montoya, & Cárcel, 2001). EMG emerged timidly in the late ‘80s (Boyar & Kilcast, 1986) as a new tool in texture evaluation. It is a non-invasive technique that does not interfere with the mastication process and gives a detailed account of the activity of the masticatory muscles. EMG offers the possibility of monitoring muscle activity during mastication (González, Montoya, Benedito, & Rey, 2004; González, Montoya, & Cárcel, 2001). The results obtained provide time-dependent information to characterize food texture. By monitoring the activities of the facial muscles, this technique makes it possible to correlate food physics with the physiology of oral processing and the sensory perception of food (González, Montoya, Benedito, & Rey, 2004).

Electrognathography, also known as jaw tracking (JT), is a three-dimensional method for tracking mandibular movements that provides information on mandibular velocity and direction as well as the extent of the jaw movements.

EMG and JT are the methods most commonly used to study the relationships between oral processing and food texture. Together with mechanical and sensory analyses, these two

techniques constitute a powerful combination for characterizing the complex nature of food texture (Chen, 2009). A number of EMG and JT parameters are used to understand changes in chewing behavior in relation to different textural properties. The typical measurements are number of chews, chewing time, chewing frequency, total or mean muscle activity, peak muscle activity, jaw movement amplitudes, and jaw-opening and -closing velocities, as well as opening, closing, and occlusal phase durations. These parameters can be examined over the complete chewing sequence or over different parts of it (Koç, Vinyard, Essick, & Foegeding, 2013). A new intraoral bite force recorder which would allow the study of natural mastication without an increase in the occlusal vertical dimension was recently proposed by Shimada, Yamabe, Torisu, Baad-Hansen, Murata, and Svensson (2012) for subsequent analysis of the relation between electromyographic (EMG) activity of jaw-closing muscles, jaw movements and bite force during mastication of five different types of food.

Oral physiology also exerts an important influence on chewing (Van der Bilt, Engelen, Pereira, Van der Glas, & Abbink, 2006), as do characteristics such as bite force, chewing performance and salivary flow rate. Chewing performance can be determined by quantifying the degree of fragmentation through sieving artificial (for example, silicon rubber cubes) or real food. Other methods involve evaluating the ability to mix and knead a food bolus using two-colored chewing gum or paraffin wax (Van der Bilt, Mojet, Tekamp, & Abbink, 2010).

Besides teeth, masticatory muscles, and the temporomandibular joint, the tongue plays an important role in orofacial motor behavior such as mastication and swallowing. As Kakizaki, Uchida, Yamamura, and Yamada (2002) stated, the neuronal network plays a major role in triggering and sequencing the neuromuscular events associated with movements, and the tongue and masticatory muscles have been shown to be active in a well-coordinated manner during semiautomatic movements. It is believed that the tongue senses the size and lubrication status of food particles. Chewed food particles of the right size are pushed by the elevated tongue to the back of the oral cavity (Mioche, Bourdiol, Monier, & Martin, 2002; Okada, Honma, Nomura, & Yamada, 2007), while large particles are selected for further size reduction. From a physiological point of view, it is the combined action of pushing, pulling, and twisting by the tongue that transports the food particle, either to push it back to the molar teeth for further size reduction or to pull it to the back of oral cavity for bolus formation. The structural characteristics of the tongue, which is made up of 17 muscles, allow it to perform a wide range of movements to seal the bolus content anteriorly and laterally and generate pressure for its posterior propulsion. The videofluorography technique has made it possible to track and analyze the tongue movement during mastication by gluing small lead markers to the teeth and tongue surface (Taniguchi, *et al.*, 2013).

Nevertheless, there are other food bolus characteristics that could influence the exact conditions for starting to swallow. Data involving not only granularity but also the rheological properties of the food bolus need to be collected in order to gain a better understanding of the link between physiological properties and the final d_{50} values observed just before swallowing. It could be hypothesized that the moderate correlation seen between the number of cycles and pre-swallow d_{50} reflects a need to attain certain rheological states that are partially independent of particle size. Mishellany-Dutour *et al*. (2007) reported that subjects who display long masticatory sequences, with many cycles, probably masticate less efficiently but still need to achieve certain rheological conditions in terms of the viscosity, cohesiveness or stickiness of the final bolus.

Recently, some devices have been developed to measure tongue function objectively during swallowing. Some of these methods have limitations for measuring tongue-palate contact function quantitatively. For example, dynamic palatography can be effective in showing temporary changes in tongue contact position but cannot measure the amplitude of tongue pressure (Taniguchi, Tsukada, Ootaki, Yamada, & Inoue, 2008). Developed for dysphagia rehabilitation and often used by phoniatricians, this method consists of instrumentation which records linguopalatal contacts during continuous speech and is used to evaluate areas of the palate contacted by the tongue.

A technique reported by Kieser *et al*. (2008) allowed accurate measurement of tongue pressure during swallowing, using an intraoral appliance with multichannel pressure sensors. These sensors are capable of measuring absolute pressures to a chrome-cobalt palatal appliance with a labial bow. However, the details of the movement of the tongue surface during different functions remain unclear. Sugita, Inoue, Taniguchi, Ootaki, Igarashi, and Yamada (2006) recorded tongue pressures at two sites on the palate during swallowing of model gels with different consistencies, and demonstrated that bolus consistency affected the tongue pressure of the anterior and posterior portions against the hard palate in different ways. The results suggested that a basic pattern of tongue pressure is maintained during swallowing but is modulated differently, by sensory feedback between the anterior and posterior portions of the tongue, to complete the propulsion of the bolus in the oral cavity.

In vitro scenario

A few artificial mouths that simulate mastication have been developed in recent years. One of these, called the chewing simulator (Salles, *et al*., 2007), makes it possible to set and control some of the masticatory variables, such as the number of masticatory cycles, the amplitude of the mechanical movements or the bite force. Another, the BITE Master II, can measure

variables to be replicated such as fractal force and energy to fracture, but in this case only for the first bite (Meullenet & Gandhapuneni, 2006).

Most of the existing prototypes have been developed for dental or orthodontic research and use compressive forces with teeth that have anatomical shapes. However, the complex shapes of natural teeth are operative because of the action of the central nervous system and it is very difficult to mimic this. In most machines only one functional variable (e.g. speed, deformation or piston movement) can be controlled at a time (Woda, *et al*., 2010).

Other machines are oriented towards the mechanical properties of the mouth and make no attempt to reproduce the conditions in which foods are processed within a closed mouth (Hoebler, *et al*., 2002). Conserva *et al*. (2008) developed a machine for *in vitro* study of the stress transmitted to a bone-implant in dentistry. Daumas, Xu and Bronlund (2005) developed another, called the mechatronic chewing device, to evaluate the dynamic changes in the texture of foods quantitatively, reproducing human chewing behavior. In this device, the jaw mechanism design first needs to be modelled and analyzed through simulations with the corresponding mathematical model.

Arvisenet, Billy, Poinot, Vigneau, Bertrand, and Prost (2008) also developed an artificial mouth. Their aim was not to reproduce the human mouth exactly but to determine whether mastication conditions have an effect on the release of volatile compounds.

Comprehension of the physiology of taste perception is a key to preparing some food products. Using a newly patented mastication simulator called AMADEUS (Automated Mastication for Artificial Destructuration and Extensive Understanding of Sensoriality), Guilloux, *et al*. (2013) obtained salt release kinetics and compared the results with sensory data.

Researchers from the University of Auvergne developed a mastication simulator called the Artificial Masticatory Advanced Machine (AM2) (Figure 1 and Figure 2) (Monique, *et al*., 2007). It simulates the mastication function, producing a bolus, while allowing permanent control of the process and collection of the whole bolus at any time. This simulator produces a food bolus with physical properties similar to those of the food bolus produced after natural mastication just before deglutition of the same food. In the AM2, a number of mastication variables are replicated and controlled. The experimenter can select the type of constraints exerted on the food, the number of masticatory cycles, the cycle duration and the duration of the mastication sequence, the force range applied to the food, the mastication chamber temperature and the quantity of artificial saliva. As pre-swallow food particle size distribution is a good indicator of food bolus characteristics, Mishellany-Dutour *et al*. (2011) used d_{50} to check the efficiency of AM2. They compared the d_{50} particle size values obtained in healthy human subjects with

those obtained using the AM2. The results showed that the AM2 was able to simulate the d_{50} food bolus particle size of peanuts and carrots produced by humans. Food bolus d_{50} values obtained *in vitro* and *in vivo* at different times during the mastication process were also similar.

In simulating mastication with mechanical devices, the intention has been to break down solid foods into particles of a similar average size to those achieved by chewing.

If equipment to simulate the masticatory process is not available, the sample can simply be minced. Experiments with rice, spaghetti and sweetcorn have shown that mincing is an appropriate means of mimicking mastication, giving similar starch content values to the mean values obtained by chewing. Hoebler, Devaux, Karinthi, Belleville, & Barry (2000) compared the particle sizes of food after human mastication and *in vitro* mincing. The particles obtained after human mastication were described as heterogeneous in size and shape, moist, limp, and not easily wet-sieved. The results showed that mincing gave an acceptable reproduction of the particle size distribution of bread, pasta and tortiglioni after *in vivo* mastication. The variability in size and distribution of the minced bread particles was high, but satisfactory for the purpose of *in vitro* simulation of mastication. Applied to foods of differing sizes (spaghetti and tortiglioni) and physical textures (bread and pasta), mincing allowed large amounts of food to be broken down, and thus seems to be a suitable means of mimicking chewing in a wide range of foods. This method of breaking down food is simple, suitable for routine analysis and easy to use in an *in vitro* procedure.

As discussed above, some devices have been developed to measure *in vivo* tongue function. A technique reported by Ishihara *et al*. (2013) has established an *in vitro* evaluation system for determining the deformation of both the tongue and the food, particularly tongue-palate compression, using an artificial tongue made of silicone rubber and an aluminum plate that mimics the hard palate in a conventional uniaxial compression apparatus. They used this method to determine the fracture profiles of gels prepared from different agar sources.

Consequently, existing *in vitro* models can be improved by including an *in vitro* oral phase that mimics chewing behavior. When exact imitation is not feasible, at least a particle size characterization of the sample (prior to subsequent steps) should be carried out (Van Buggenhout, *et al*., 2010).

Quantifying the bolus particle size distribution

To quantify the particle size distribution of chewed foods, the method most commonly used has been sieving. Image analysis (IA) is another frequently used method to characterize the size and shape of the bolus particles (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000). This

method has been used to determine whether the size and shape properties of a ready-to-swallow food bolus were independent of the subjects (Peyron, Mishellany, & Woda, 2004). Chen, Khandelwal, Liu and Funami (2013) used image analysis to study the correlation between the particle size distribution of food bolus and the hardness of the food. Le Bleis, Chaunier, Della Valle, Panouillé, and Réguerre (2013) also used IA to characterize the degree of fragmentation and heterogeneity of boluses from two types of bread. Mishellany, Woda, Labas and Peyron (2006) listed a number of additional methods that have been used to quantify particle size during *in vitro* digestion studies, such as laser diffraction, microscopy, sedimentation analysis and diffusion of light.

Six natural foods using sieving and laser diffraction methods were compared by Peyron, Mishellany and Woda (2004); after *in vivo* mastication, they noted that each of these two methods analyzed only one interval of the full range of particle sizes. Particles smaller than the aperture of the finest sieve were lost by sieving and laser diffraction lost large particles because of its technical limits. Therefore, food boluses of raw vegetables consisting of larger particles are better characterized by sieving but laser diffraction is the best method for measuring the granularity of dry and brittle foods such as nuts, because these contain a high percentage of small particles.

The use of IA to ascertain the particle size of food has been described as rapid, accurate and reliable, providing precise particle enumeration over a wide range of sizes with detailed two-dimensional data and obviating the unpleasant and time-consuming sieving and laser diffraction processes. However, the IA technique has the same limitation as the sieving method with respect to the range of values: the smallest particles in the food boluses are missed because they are eliminated during preparation, which involves diluting, washing and arranging the samples, so distribution curves obtained with IA are similar to those obtained by sieving. Importantly, however, the IA technique offers an additional insight, as the particle shape can be observed and quantified by the particle shape index.

Arvisenet, Billy, Poinot, Vigneau, Bertrand, and Prost (2008) studied food boluses with low levels of distinguishable particles by using an image texture analysis technique, the grey level co-occurrence matrix method (GLCM). They showed that this method can provide reliable differentiation using images of apple crunched in an artificial mouth under different compression movement frequency conditions and with different rotation speeds. Hoebler, Devaux, Karinthi, Belleville, and Barry (2000) showed that GLCM can be used to investigate food bolus formation during mastication of different breads and different types of pasta. The use of GLCM textural features for image classification enabled an average of 67% of images to be classified correctly into their respective chewing cycles. Tournier, Grass, Zope, Salles, and

Bertrand (2012) used GLCM in four different breads and identified contrast as the best marker of food degradation.

Hence, the choice of one method rather than another will depend on both the goal of the proposed study and the nature of the food. It should also be considered that whatever technique is used, not all the particles will be spat out even when the material obtained by rinsing out the oral cavity is added to the sample (Mishellany, Woda, Labas, & Peyron, 2006).

Insalivation

In vivo scenario

The oral food stage is short but it also plays another important role: hydrating and lubricating the food by mixing it with saliva. The saliva interacts with the food components, leading to structure formation or structure breakdown (Chen, 2009).

Human saliva is a complex biological fluid, consisting mainly of water (99.5% w/w), various proteins (0.3% w/w), small organic compounds and inorganic salts. It has a pH of around 6.8, rising to around 7-8 after food ingestion. Saliva is typically secreted at a rate of about 0.2 to 4 ml per minute, with a total saliva output of 500 to 1500 mL per day (McClements & Li, 2010). Resting or unstimulated salivary flow is the result of low-level autonomic stimulation by the higher brain centers. Salivary secretion is upregulated above the resting rate by taste and chewing and to a lesser degree by smell stimulation (Carpenter, 2013).

The major protein component of human saliva is mucin. Other proteins in saliva include various enzymes such as α-amylase, immunoglobulins, antibacterial proteins, proline-rich proteins (up to 45 % of the total weight of protein) and peptides such as histatins and cystatins (Sarkar, Goh, & Singh, 2009). The parotid gland contributes the greatest flow (as much as 60% of the total) to stimulated saliva but less to resting salivary flow. It secretes a serous substance that contains no mucins but is rich in amylase and in proline-rich proteins. The submandibular and sublingual glands contribute more to the resting salivary flow rate and their saliva is rich in mucins. Mucins are high-molecular-weight glycoproteins with an elongated structure that contribute significantly to the viscoelastic behavior of saliva.

Amylase is the single most abundant protein in saliva and is involved in the initial digestion of starch-containing foods. Because of this, when the food under study is rich in starch the oral digestion step has been taken into consideration, as in studies with potatoes (Parada & Aguilera, 2009), pasta (Petitot, *et al*., 2009) or a starch-based custard dessert (Engelen, *et al*., 2003). During insalivation, which is particularly important for starchy semi-fluid foods, the rapid action of salivary amylase reduces the viscosity (Hoebler, *et al*., 2002).

Since the activity of salivary amylase is greatly reduced as soon as it reaches the acidic environment of the stomach, pancreatic amylase is much more likely to be involved in the digestion of starch in foods, in the opinion of Carpenter (2013). Also, in studies on pancreatic digestion pancreatic activity has been found to overwhelm salivary amylase activity, so Woolnough, Bird, Monro, & Brennan (2010) considered that oral digestion can be neglected.

Structural variability among foods can give rise to different rates of starch hydrolysis as a consequence of their different degree of accessibility to enzymes. Hoebler, Devaux, Karinthi, Belleville, and Barry (2000) found that in cereal-based products, about 50% of bread starch and 25% of pasta starch were hydrolyzed during the short period of oral processing. Butterworth, Warre, and Ellis (2011) stated that some uncertainty still remains with regard to the physiological significance of salivary amylase. According to Nantanga, Chan, Suleman, Bertoft, and Seetharaman (2013), who worked with cooked starch treated with saliva from six participants at equal activity under conditions mimicking oral digestion, further research is needed to understand whether the hydrolyzate structure obtained, rather than the level of amylase activity, is the determinant of oral digestion of starch.

Lingual lipase is another salivary digestive enzyme. This enzyme breaks down a small fraction of dietary triglycerides in the oral cavity and stomach. However, lingual lipase is considered to be of limited significance in lipolysis for healthy individuals (Pedersen, Bardow, Jensen, & Nauntofte, 2002).

Many factors such as the flow rate, time of day, type and size of the salivary glands, duration and type of the stimulus, diet, drugs, age, sex and blood type affect the amount and composition of saliva secreted in humans (Vingerhoeds, Blijdenstein, Zoet, & Van Aken, 2005). When subjects display marked differences in their saliva composition their potential for oral interaction with food may differ, as in the subsequent release and perception of taste compounds (Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012). The role of saliva in the perception of the taste, flavor and texture of foods has been also taken into account. During consumption, food mixes with saliva, so it is not the food itself but the products of its interactions with saliva which we perceive. Consequently, the role of saliva in perception appears to be essential (Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012). For example, the action of the enzyme α-amylase, initiating the digestion of starch, can result in a drop in the perceived thickness of certain food products, as commented above. In addition, the large salivary proteins influence lubrication and hence, possibly, the perception of attributes such as smoothness and astringency (Engelen, *et al*., 2003). Saliva also plays a major role in the detection and perception of fat, as it is directly involved in the orosensory detection of triglycerides and their hydrolysis products (Feron & Poette, 2013).

411 The perception of texture attributes is strongly related to the way the food is processed during
412 food intake, mastication, and swallowing and during the cleaning of the mouth after
413 swallowing. It is also modulated by the interaction with other basic properties, such as taste
414 and aroma attributes. The most important dynamic feature of an eating process in association
415 with texture perception is the change of length scale. Understanding the in-mouth processes
416 at the colloidal scale turned out to be essential to grasping the interplay between perception,
417 oral physiology and food properties. In this regard, two aspects have to be taken into account:
418 first, food particles are chewed and reduced in size from centimeter scale initially to sub-
419 millimeter scale at the point of swallowing, and second, a thick film of food-saliva mixture
420 between oral surfaces (i.e. tongue and hard palate) is gradually reduced to a final thin film of a
421 few micrometers (Van Vliet, Van Aken, de Jongh, & Hamer, 2009). These changes have
422 important implications for the perceived texture and, more importantly, for the underpinning
423 mechanisms applied for texture perception (Chen & Stokes, 2012).
424 Saliva acts as a buffering system (De Almeida, Grégio, Machado, de Lima, & Azevedo, 2008),
425 affecting the degree to which sourness is perceived. Significant decreases in perception with
426 increasing salivary flow rates were observed for citric acid and sodium chloride. Although this
427 can partially be explained by a dilution effect, bitterness and sweetness remained unaffected
428 by the salivary flow conditions (Heinzerling, Stieger, Bult, & Smit, 2011).
429
430 *In vitro* scenario
431 The important role of saliva in the oral processing of foods makes it clear that saliva needs to
432 be used in *in vitro* studies. Exact reproduction of human saliva is especially difficult because of
433 its complexity, unstable character and inter-individual variability, as well as its dependence on
434 the type of saliva stimulation (Roger-Leroi, Mishellany-Dutour, Woda, Marchand, & Peyron,
435 2012). In addition, its complex composition varies over the day. It is thus only possible to
436 imitate an average saliva composition (Gal, Fovet, & Adib-Yadzi, 2001).
437 The compositional complexity of simulated saliva fluids (SSF) used in the literature varies
438 widely depending on the objectives of the research. Some researchers use a simple buffer
439 solution without any additional component to simulate oral conditions. Others use simulated
440 saliva fluids that contain many of the components found in human saliva, such as acids,
441 buffers, minerals, mucins and enzymes (McClements & Li, 2010). In the food technology field,
442 in studies where digestion processes are to be emulated, the SSF should be as similar as
443 possible to naturally occurring saliva. For example, Van Ruth, Grossmann, Geary, and
444 Delahunty (2001) found that significant differences in the volatility of compounds when

445 artificial saliva or water was added indicated that saliva replacement was inadequate in aroma
446 release studies.
447 Some recipes for preparing simulated saliva solutions can be found in the literature (Björklund,
448 Ouwehand, & Forssten, 2011; Gal, Fovet, & Adib-Yadzi, 2001; Leung & Darvell, 1997;
449 Mishellany-Dutour, *et al*., 2011; Sarkar, Goh, & Singh, 2009).
450 As mentioned above, during oral processing the effect of saliva on the food can lead to
451 impressive changes in rheological and other related properties. Saliva acts as a glue, holding
452 the fragmented solid particles together. The lubrication or tribological qualities of saliva are
453 central to many of its food processing roles, such as facilitating the swallowing of the food
454 bolus and its transport through the body. Surprisingly, according to Bongaerts, Rossetti and
455 Stokes (2007) there are few studies on the lubricating properties of whole human saliva in
456 terms of how it is influenced by surface roughness or surface compliance.
457 The results from the *in vitro* study carried out by Engelen *et al*. (2003) suggested that for a
458 semi-solid food like custard, breakdown by α-amylase in the mouth is limited because the time
459 it spends in the mouth (about 4-5 seconds) is too short for the saliva and custard to become
460 properly mixed, so the effects of breakdown are undoubtedly present but not extensive. In
461 contrast, during mastication of solids the mixing is more vigorous, and probably more efficient,
462 enabling the enzyme to come into contact with more starch particles rather than being
463 confined to the initial surface. Therefore, enzyme activity is more valuable for breaking down
464 solid foods that remain in the mouth for a longer time, such as bread and other cereal
465 products. Using a mixing simulator, Prinz, Janssen and de Wijk (2007) demonstrated with video
466 images of the recovered samples that saliva-induced structure breakdown exerts a dramatic
467 effect on the viscosity of starch-based custards despite the incomplete mixing of custard and
468 saliva that occurs *in vivo.* Several authors (Ferry, Hort, Mitchell, Lagarrigue, & Pamies, 2004;
469 Sorba & Sopade, 2013) used the Rapid Visco Analyser (Newport Scientific, Warriewood,
470 Australia) to measure the decrease in viscosity over time on adding amylase to starch pastes.
471 To quantify the susceptibility of starch-based semisolid foods to salivary α-amylase and the
472 rate of enzyme-induced structure breakdown, Janssen, Terpstra, de Wijk, and Prinz (2007)
473 developed a measuring system, the Structure Breakdown Cell (SBC), consisting of a helical
474 rotating vane. This system aims to achieve near-perfect mixing with saliva while monitoring
475 the resulting change in the torque required to rotate the vane through the food sample. The
476 use of complex geometries in rotational rheometry offers numerous benefits for the
477 mechanical characterization of saliva-induced breakdown, compared with the conventional
478 geometries used in rotational rheometry, as it is more effective in simulating the mixing
479 process in the mouth and tracking the evolution of the structure.

"Melting", defined by Engelen *et al*. (2003) as the rate of decrease in thickness and spreading of the product in the mouth, is a sensory attribute that could be affected considerably by the presence of salivary enzymes. Since starch is broken down by the salivary enzyme α-amylase, sensory melting could be affected more by saliva than by water. However, why does saliva affect melting more than an α-amylase solution? A possible reason is that the α-amylase in the water solution is less active than in saliva. Early work by Erickson (1992) has provided support for this explanation by showing that the presence of chloride ions is essential for α-amylase to reach full activity. The molecular basis for this effect was further studied by Qian, Ajandouz, Payan, and Nahoum (2005). Studies performed with mice have indicated that α-amylase is more active in saliva than in the gland. It can therefore be speculated that other components of saliva (e.g. hydrolyzing enzymes) or products originating in microorganisms can also influence the activity of salivary α-amylase. The choice of kinetic models for studying starch amylolysis *in vitro* is also a subject of some controversy (Butterworth, Warren, & Ellis, 2011).

As described above, several masticatory apparatuses have been employed to date to produce a food bolus with the closest possible resemblance to that resulting from *in vivo* chewing. To achieve the goal of producing the expected food bolus, Roger-Leroi, Mishellany-Dutour, Woda, Marchand, and Peyron (2012) stated that it is mandatory to develop artificial saliva with chemical and rheological characteristics that are close to those of human saliva and proposed a formulation that satisfies the major requirement of viscosity.

Bolus formation

Bolus characterization

Understanding the dynamic changes in food structure that take place during oral processing is a key factor for texture design. A knowledge of bolus rheology is one of the more important approaches to such understanding. From a rheological point of view, the bolus should behave as a weak gel for ease of mastication and swallowing. A homogeneous and cohesive state allows the mass flow of bolus through the pharyngeal phase, increasing swallowing comfort (Ishihara, Nakauma, Funami, Odake, & Nishinari, 2011).

Prinz and Lucas (1997) stated that the decisive factor for swallowing should be the combined effect of particle size and oral lubrication with the participation of saliva. According to these authors the optimum moment for swallowing is defined in terms of a peak cohesive force between food particles: a swallow should be triggered when it is sensed that a batch of food particles is binding together under viscous forces so as to form a bolus. As Chen and Lolivret (2011) commented, experimental evidence suggests that rather than maximum consistency, appropriate flow-ability is a likely trigger point for swallowing. They proved this with different

food boluses expectorated by volunteers and simulated boluses made with SSF, using a tensile method in which the boluses were stretched vertically and the force at separation was recorded as a function of stretching distance. Some other experimental evidence in the literature supports this premise. With the help of magnetic resonance imaging (MRI) and videofluorescence techniques, for example, Buettner, Beer, Hannig, and Settles (2001) observed that a food bolus became highly stretched or extensionally deformed during swallowing. This was further confirmed by Kumagai, Tashiro, Hasegawa, Kohyama, and Kumagai (2009), who observed the velocity profile of various bolus flows in the pharynx by the Ultrasonic Pulse Doppler method. Pereira, Gavião, Engelen, and van der Bilt (2007) demonstrated that the addition of fluid could significantly reduce the number of chewing cycles for some dry foods because of enhanced bolus flowability in the presence of extra fluid. The importance of bolus stretchability was also confirmed by Seo, Hwang, Han, and Kim (2007) on investigating sensory and instrumental slipperiness and compliance of foods during swallowing by human subjects using non-invasive techniques. All this experimental evidence suggests that maximum consistency is not a criterion for the point of swallowing and that the key criterion in swallowing is stretchability (Chen and Lolivret (2011).

Peyron *et al*. (2011) were also of the opinion that particle size and bolus hardness are not the only decisive factors in the swallowing threshold, since d_{50} and hardness values barely change after the middle of the masticatory sequence. Particle size (Peyron, Mishellany, & Woda, 2004), lubrication by saliva and bolus wetting (Gavião, Engelen, & Van der Bilt, 2004) are initial contributing factors to the final rheological values obtained for the swallowing threshold.

On the other hand, the several critical thresholds for swallowing may not be reached simultaneously in a bolus: the swallowing threshold is probably an integrative process that combines the perceptions of the various bolus properties enabling swallowing (Peyron, *et al*., 2011). Evidently, the swallowing threshold comprises many components. As formation of a swallowable bolus is assumed to be a key driving constraint, to avoid dangerous aspiration of small particles, each individual uses his or her physiological resources to chew a given food until a safe bolus is made and the swallowing threshold is reached.

Current techniques for studying bolus rheology

Ishihara, Nakauma, Funami, Odake, and Nishinari (2011) listed a number of techniques for inspecting the physiology of swallowing, such as videoendoscopy, the ultrasonic (ultrasound) method and acoustic analysis, not only for clinical studies but also for texture studies (Kumagai, Tashiro, Hasegawa, Kohyama, & Kumagai, 2009; Saitoh, *et al*., 2007). Other techniques such as Doppler velocimetry might allow direct information concerning bolus

velocity to be obtained without the need to track the boundaries of a bolus (e.g. in videofluoroscopy) (Engmann & Burbidge, 2013).

Videofluorography (VI) (Okada, Honma, Nomura, & Yamada, 2007; Ono, Hori, Masuda, & Hayashi, 2009) and the real-time MRI technique (Buettner, Beer, Hannig, & Settles, 2001; Kulinna-Cosentini, Schima, & Cosentini, 2007), both developed for medical applications, have been used successfully to provide insight into the visual evidence of food transformation and transportation at different stages of oral processing (Figure 3). It is foreseeable that the use of such imaging techniques, together with the classic mechanical and sensory methods, will be a powerful combination in characterizing food texture (Chen, 2009).

VI is currently one of the best ways of evaluating the swallowing function because it enables visualization of the movement of all the anatomical components related to chewing and swallowing (Ono, Hori, Masuda, & Hayashi, 2009). These components include the lips, cheeks, jaw, tongue, hyoid bone, pharynx, larynx, and esophagus. This technique also makes it possible to visualize the passage of a food or drink containing a contrast medium (typically barium sulfate powder or soluble iodine complexes) in two dimensions (sagittal and frontal). However, its application involves radiation exposure and is therefore limited to patients with severe dysfunction in chewing and swallowing.

Kulinna-Cosentini *et al*. (2007) have proved that MRI is a feasible, non-invasive method for swallowing evaluations because it has excellent potential for providing fully three-dimensional static images of the gastroesophageal junction and its anatomical structures involved in swallowing, and their degree of variation. In comparison to VI, MRI offers several advantages: it provides a better evaluation of soft tissues, the ability to acquire various series of images with excellent time resolution, and – if adequately processed, which is no trivial challenge (Engmann & Burbidge, 2013) – the possibility of resolving three-dimensional details from different angles without changing the patient's position, but its main advantage is the lack of ionizing radiation to the patient.

Currently, these physiological measurements suffer from limitations. For instance, videoendoscopy presents low quantitative performance because of the 2D projection character of the technique. The ultrasonic method is applicable preferably to females, as they lack the thyroid cartilage which could interfere with the transit of the ultrasonic pulse. Acoustic analysis is an alternative approach for recording swallowing profiles that has been utilized for diagnostic purpose as a non-invasive method in both healthy and dysphagic individuals (Lazareck & Moussavi, 2004), but has been used less in the field of food technology.

Despite the aid of the above techniques, difficulties in measuring the rheological properties of boluses still remain owing to personal physiological differences, including mastication ability

and saliva secretion, which sometimes lead to poor reproducibility of experiments. This could be one of the reasons why more research on bolus rheology has been conducted from a physiological perspective, in medical research, than by food scientists from the food technology point of view. Different stages of the swallowing mechanism, which involve different fluid mechanics regimes (from creeping flow to turbulent flow conditions) depending on the boundary conditions and bolus rheology, need to be studied (Engmann & Burbidge, 2013). It is important for food scientists to establish experimental procedures to prepare a bolus *in vitro* with high reproducibility (Ishihara, Nakauma, Funami, Odake, & Nishinari, 2011).

In silico scenario

The last few decades have been witnessing the rise of alternative research models, the so-called *in silico* approaches, using computational environments. The expression *in silico,* imitating the common biological Latin expressions *in vivo* and *in vitro*, refers to performing experiments using computers (Noori & Spanagel, 2013).

In silico models are gaining importance in the food science and technology field. The development and validation of such models require more and more in-depth knowledge of the physiological mechanisms of mastication. Mathematical models of oral processing are proposed, generally based on geometrical considerations, to emulate certain physiological features during mastication. *In vitro*, *in vivo* and *in silico* approaches have been compared when studying the dynamics of the perception of saltiness and solute release from model dairy products of varying composition and rheological behavior (Panouillé, *et al*., 2010). In another study, the mechanical human mastication of commercial breakfast cereals was modelled by using X-ray tomography data to quantify crack propagation in brittle airy products (Hedjazi, Guessasma, Martin, Della Valle, & Dendievel, 2012). Le Révérend, Loret and Hartmann (2012) studied how force is distributed along the mandibular arch and how force distribution is related to the space available to fit foods between the teeth.

In silico models have found a number of applications in characterizing mastication. Of special interest are the studies on aroma release and its particularities, some of which are more closely related to oral processing. Tréléa *et al*. (2008) described a mechanistic mathematical model for aroma release in the oropharynx reaching the nasal cavity during consumption of flavored yogurt. The model was based on the physiology of the swallowing process and was validated via mass spectrometry measurements of aroma concentration. According to the authors, this work constitutes a first step towards computer-aided product formulation. An elastohydrodynamic model of swallowing was developed by De Loubens, Magnin, Doyennette, Tréléa, and Souchon (2011) to quantify physical mechanisms that explain pharyngeal mucosa

coating. Considering complex physiological conditions, the results were applied to predicting aroma release kinetics. Using a coupled biomechanical-SPH (Smoothed Particle Hydrodynamics) model, Harrison *et al*. (2012) studied food breakdown and flavor release during mastication. SPH is a numerical method that allows complexities such as fluid free surfaces or solid fracture and interactions with complicated deforming boundaries and chemical dynamics to be modelled. De Loubens, Magnin, Doyennette, Tréléa, and Souchon (2010) developed an experimental device in order to gain insight into the biomechanics of the pharyngeal peristalsis; the results demonstrated the influence of food bolus viscosity on flavor release. Déléris *et al*. (2012) developed a mathematical model of mass transfer in the mouth during eating that made it possible to identify the parameters and properties associated with the product, or with the subject eating the product, that explain stimuli release in the mouth. To examine the effect of various oral and gastric factors, the disintegration profiles obtained by measuring the mass retention of different artificially masticated boluses were fitted to a linear-exponential model, demonstrating that the bread structure and moisture content were key features controlling the process (Bornhorst & Singh, 2012).

Model predictions have generally been in good agreement with the experimental data, so, *in silico* approaches could be a promising tool in food oral processing studies.

Conclusions

While we are eating, a whole series of transformations take place in the mouth before swallowing. Thanks to research in a number of very different disciplines we are gradually but constantly learning more about these processes, and in greater detail.

Physically, the food is broken down in the mouth into smaller particles in preparation for the following stages: gastric and intestinal digestion. Physiologically, the processes that take place in the mouth must be viewed from three different angles. The first is the beginning of starch digestion, thanks to the α-amylase in the saliva, the second is the chewing process (number of chews, chewing time, chewing frequency, bite force, fracture energy, oral – or simulation chamber – temperature, quantity and type of saliva) in relation to the food involved (size, shape, viscosity, cohesiveness, hardness, stickiness) and the third is that the particles obtained have to be formed into a cohesive, hydrated bolus that can be swallowed safely and comfortably.

While it is practically impossible to reproduce such a complicated mechanism as in-mouth processing, there are tools that can achieve similar results.

Researchers should ask themselves which steps, in relation to the food in question and the parameters to be analyzed, necessarily precede the procedures they wish to apply in their study.

The choice of one method or another will depend on the physical state of the food (liquid or solid), and its initial mechanical and structural properties. For example, a researcher who wishes to study how a food's texture affects its consumer acceptability needs to consider the in-mouth sensations aroused by all the chewing and insalivation mechanisms involved through to formation of the bolus to be swallowed, and not merely measure some single mechanical property as an indicator of texture, while the researcher who wants to know how the lipids contained in a given food could be digested by pancreatic lipases needs to consider which of the structural breakdowns the food undergoes is responsible for releasing the fat from the matrix. In addition, a cohesive, consistent bolus has many different properties to those of a food that is simply minced and diluted in water or in artificial saliva. The question is: do all these differences affect the results of my study?

The path of research related to the oral processing of food is very broad and many crossroads and shortcuts may be encountered along the way. Only a profound knowledge of the processes and a clear vision of the aims of the study will make it possible to take the right course.

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948 **Figure captions**

949 Figure 1. General view of the Artificial Masticatory Advanced Machine (AM2) masticator
950 simulator.

951 Figure 2. The AM2 masticatory chamber. It is a cylindrical cavity whose two ends are formed by
952 the stationary ‘‘maxillary disk’’ and the moving ‘‘mandibular disk’’; this can move back and
953 forth along and rotate around the central axis of the cylinder. Both AM2 disks are shown in the
954 different positions during operation.

955 Figure 3. Oral and pharyngeal segments of a subject. Dynamic sequence in the sagittal view
956 shows a normal peristaltic wave with propagation of the bolus. Upper: during rest; middle: at
957 the beginning of swallowing; bellow: complete swallowing (velopharyngeal closure prevents
958 nasal penetration). Left: videofluorography images; right: magnetic resonance images.

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