14. Models of the Oral Cavity for the Investigation of Olfaction

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In this chapter, we will briefly describe the complexity of the main mechanical, biochemical and physicochemical phenomena that occur in the mouth during food consumption using examples. To better understand the reactions occurring in the mouth during food consumption, in vitro systems called model mouths were developed to simulate food consumption and thus answer some of the more fundamental questions regarding olfactory perception. This chapter provides examples of the applications of the model mouth in performing oral functions, such as mastication, saliva production and airflow, as well as swallowing, while the released volatile compounds are measured. The recent model mouth designs represent the actual occurrence of food consumption under oral conditions in a more accurate way. We believe that this type of methodology will be even more commonly applied in the future to improve the knowledge in this field.

14.1 Oral Food Processing and the Effect on Olfaction

During food consumption, various oral processing steps are followed. Initially, the solid food is ingested, then masticated, and mixed with saliva to form a bolus. The food particles are transferred into different locations in the mouth, until they are ready to be swallowed [14.1, 2]. During the complex process of oral food consumption, the physical properties of food are modified and, as a consequence of these diverse steps as a whole, the perception of flavor and texture are affected. Therefore, the release of flavor compounds from the food and their delivery to receptors are key factors leading to flavor perception.

In the last twenty years, several devices have been proposed to simulate the various steps and parameters involved in this process [14.3, 4]. These simulators were developed to study the breakdown of food and/or the release of volatile compounds from food products of various textures. Due to the vast complexity of the mouth's functionalities, the development of mouth model had to be focused on a limited number of factors to simulate a specific process for the study under consideration.

14.1.1 Main Oral Functions

The major in-mouth phenomena that occur after the ingestion of solid food are mastication, salivation, the formation of the bolus, the transport of particles into different locations in the mouth and swallowing [14.1, 2]. Mastication is the action of breaking down food and preparing a food bolus for swallowing. During this step of oral food processing, the physical properties of the food are modified and the perception of flavor and texture are affected. The release of aroma and taste compounds from the food and their delivery to receptors are key factors leading to flavor perception. The breakdown of food into particles, associated with the effect of temperature, increases the surface area of the food that is exposed to saliva and air. This facilitates the dissolution of taste compounds within saliva and the release of volatile compounds into the gas phase of the *mouth space*, leading to flavor sensations. During this process, variations in the texture of the food and the bolus are constantly perceived and the chewing pattern is consequently adjusted according to the peripheral feedback. In addition, the masticatory pattern and the characteristics of saliva may vary considerably between individuals [14.2].

Food oral processing is divided into the three following phases: I) ingestion, II) rhythmic sequences of mastication and, III) swallowing and clearance. The properties of food (flavor and texture) are perceived during these three consecutive stages. The temporal perception of flavor and texture are important determinants in the acceptability and choice of food by the consumer, with direct consequences for his nutritional status. The breakdown patterns are specific to the food under consideration. Whereas solids must be fragmented by the teeth and be softened by and mixed with saliva to form a cohesive bolus [14.5], liquids are nearly ready to be swallowed [14.6]. Furthermore, the type and extent of oral forces change throughout the process according to the physical state of the bolus that is perceived by mechanoreceptors. Subsequent motor actions are then adapted through feedback-control mechanisms. In the case of brittle solids, the first transformation that occurs in the mouth consists of the fragmentation of the food into smaller particles. The breakage function depends mainly on the anatomical characteristics of the subjects [14.7]. The breakage function also depends on the resistance of the food to be broken [14.8]. Saliva also plays a role in the formation of a bolus from brittle food because it allows the particles to cohere due to its viscosity. Cohesive foods, such as meat and some cheeses, do not break into separate particles; instead, they are softened by chewing [14.9]. In particular, cheeses with a low lipid-to-protein ratio result in harder boli than those with a high ratio. For this type of food, the salivary intake into the food matrix is an important factor in determining the texture of the bolus: the higher the salivary intake, the softer the bolus. In the case of semisolids, the main change in food properties that occurs is the reduction of viscosity due to shearing forces, the temperature change, dilution, or chemical degradation induced by saliva.

Texture, which is dependent on the physical properties of food, is perceived through mechanoreceptors that are distributed in the oral cavity. The masticatory process can be considered as a combination of compressive and shearing activities. Studies of the mechanical properties of food have indicated that chewing and the mandibular movements occurring during the breakdown of solid food are strongly affected by the food's texture [14.10]. Thus, the hardness of the food has an effect on the number of chewing strokes necessary to trigger a swallow. The harder the food, the more chewing strokes are needed. Increasing the hardness of the food also increases the extent of the masticatory motions, thereby increasing the forces applied to the food matrix [14.11]. Compression has been shown to be dominant during the early stages of biscuit breakdown, and shearing was found to be dominant during the subsequent stages of mastication [14.12]. Food texture also affects the salivary flow rate because the chewing forces developed during mastication and the salivary flow rate, particularly the parotid gland secretion, are linked. The hardness of solid foods is therefore positively correlated with the parotid gland flow rate.

In addition to the initial hardness of the food, the progressive comminution and softening of food during mastication also affects oral physiological parameters [14.13]. Generally, the force generated by jaw muscles decreases during bolus formation due to sensory feedback between the intraoral mechanoreceptors and the muscles involved in mastication. Oral physiology and textural perception are tightly linked due to the sensory feedback between the chewing behavior and the food texture during food consumption. Moreover, tongue movements, temperature and the salivary composition are also important for textural perception because these oral parameters can modify the matrix structure of the food during in-mouth processing and consequently the sensory evaluation of texture.

In the case of brittle food (biscuits and chips), its texture is determined by the ability of the matrix to form particles, the rate of particle size reduction and the resulting particle size distribution. The level of lubrication of the particles, provided by salivation, is of obvious importance in forming a cohesive bolus prior to the swallowing step [14.5]. For nonbrittle foods, such as meat and meat products, different correlations between the oral physiological parameters and texture perception were found [14.14]. For example, the work and efficiency of chewing were found to be relevant parameters in predicting textural perception. Subjects with low efficiencies presented shorter chewing time and reached the maximal intensity of tenderness earlier. However, other works have shown that subjects presenting a lower chewing efficiency required a longer chewing time to form a bolus [14.15].

14.1.2 Release and Perception of Flavor in Oral Processing

Flavor perception during food consumption is determined by the nature and amount of volatile and nonvolatile compounds and the availability of these compounds to the sensory system as a function of time. The extent and rate of the release of flavor compounds and their subsequent transport to the receptors depend on the process of breaking down the food matrix through mastication. The progressive breakdown of the food matrix, the salivary volume and the swallowing process are important factors in the release of both nonvolatile and volatile compounds, but differences between the two compound categories can be observed.

In general, the quantity of nonvolatile compounds in saliva increases at the beginning of the chewing process, reaches a maximum, and then decreases more or less rapidly until the end of mastication. For example, the salivary concentration of nonvolatile compounds that were released from a piece of chewing gum peaked within the first minute of mastication [14.16]. The kinetics of the release of nonvolatile compounds from a model cheese were related to various chewing parameters, such as the rate, duration, and efficiency of mastication, which were closely linked to each other and to the salivary flow rate [14.17–19]. The release of sodium from the salt in model cheeses was highly affected by textural and compositional factors, and the oral physiology of individuals. For example, rapid sodium release was linked to increased bite force and slower sodium release was linked to a prolonged chewing sequence, whereas a faster perception of saltiness was related to the masticatory performance and bite force. As the area of surface contact increases during chewing, more taste papillae are stimulated and the perceived intensity increases. The compositional factors of the model cheese matrices, particularly the fat and water contents, had strong impacts on both the release of sodium in the mouth and the perception of saltiness. Increasing the fat content and reducing the water content were both found to be related to a global decrease in the release of sodium and an increase in the perceived saltiness. This result suggests that the water content affects the initial release of sodium, whereas the effect of the fat content is more pronounced in the perception of saltiness during the chewing process. However, the effect of each compositional parameter on the release of sodium differs according to the duration of chewing, and globally some of the oral parameters that affect the release of sodium are different from the parameters that affect the perception of saltiness. Thus, the significant distinction between the time of sodium release and the time of the perception of saltiness can be explained by the differential effects of both the oral and matrix parameters.

Mastication also plays a key role in the temporal pattern of the release of volatile organic compounds (VOCs), whereas processes such as swallowing and the flow of nasal air determine their subsequent delivery to receptors located in the nose. The process of mastication involves the gas-phase transfer of VOCs from the mouth to the pharynx, where they are swept through the upper airways to the nose by the air that is expired from the lungs. As is the case for nonvolatile compounds, the release of VOCs in the mouth depends on both the food characteristics and the oral parameters. For example, for cheese products, an increase in the masticatory rate increases the overall aroma release. The level of the effect of the masticatory rate differs for compounds depending on their varying mass-transfer coefficients. Most of the chewing parameters are positively correlated with a high concentration of volatiles in the nose, mainly due to the increase in the surface area due to the sample breakdown [14.20, 21]. Volatile compound release was also shown to depend on the interaction between the food matrix composition and the chewing behavior. The seal between the mouth and the pharynx opens intermittently during mastication according to the air movement in and out of the mouth [14.22]. During jaw closure, the volume of the mouth decreases, which may push some air out of the mouth into the pharynx [14.23]. However, oral behaviors vary highly according to the subject, leading to interindividual differences in the patterns of volatile compound release. For example, during the consumption of a sweet mint tablet, swallowing events were the main contributors to menthone release. The tongue and jaw movements did not induce menthone release in all of the subjects, indicating that the interindividual differences in terms of the quantity of menthone released arose from the percentage of degradation of the sweet mint tablet [14.24].

In the case of chewable products such as cheeses, it was shown that subjects differentially adapted their chewing behaviors in forming a swallowable bolus [14.25] and that this phenomenon was an important source of interindividual variability. Food texture affects both oral behavior and flavor release. An increase in firmness induced an increase in the chewing duration and the amount of saliva that was incorporated into the food bolus, which led to an increase in the total amount of released VOCs. The release rate of the volatile compounds differed according to their physiochemical properties. A higher fat content led to a larger amount of product remaining in the mouth after swallowing, resulting in the release of a smaller amount of volatile compounds and a prolonged persistence of aroma in the breath. The polarity of the volatile compound affects its release rate. A larger amount of a more polar compound, ethyl propanoate, was released during the masticatory step, whereas more nonan-2-one was released during the post-swallowing step and it was more persistent in the mouth, due to its higher hydrophobicity [14.26].

The release and perception of flavor are also affected by the composition of the food and the quantity of saliva, which vary greatly among individuals. Saliva is a complex viscous aqueous medium containing minerals and a wide variety of organic molecules, including proteins with various properties, such as enzymatic conversion, binding, and transport. Saliva plays an important role in the in-mouth breakdown process, mainly due to its hydrating, lubricating and hydrolytic capacities. Moreover, saliva dilutes the food in the mouth, thus decreasing the amount of volatile compounds that are released to the air. Saliva actively contributes to the formation of a bolus that can be swallowed and to the release of active compounds, including taste and aroma compounds, during chewing and swallowing [14.2]. Thus, saliva plays a major role in flavor perception [14.27]. Volatile compounds can interact differentially with the components of saliva [14.28]. Three types of behavior were reported based on the physicochemical properties of the volatile components: The partition coefficient of a group of compounds is not affected by mucin; mucin decreases the partition coefficient of the second group, mainly through hydrophobic interactions between saliva proteins and VOCs; and mucin decreases the partition coefficient of the last group but that process is affected by the presence of salt and sugar. Different salivary enzymatic activities are suspected to have a nonnegligible effect on taste and aroma perception. Regarding volatile compounds, their flavor can be altered by the salivary enzymatic activities [14.29, 30]. For example, ester hydrolysis by salivary extracts was found to alter flavor in model systems. The addition of human saliva to white wine led to significant changes in the volatile compound profiles. In particular, the levels of esters and fused alcohols were reduced by 32% and 80%, respectively, whereas in contrast, the levels of 2-phenyl ethanol and furfural were increased by 27% and 155%, respectively [14.31]. Regarding nonvolatile compounds, the in-mouth amylase activity, which hydrolyses bonds within amylose and amylopectin, can quickly lower the viscosity of starch in starch-thickened foods [14.32]. This enzymatic activity can reduce the perception of saltiness in starch-thickened foods as the structure of the product is changed during the enzymatic process. Thus, a direct relation was found between the level of α-amylase activity in saliva and the perception of saltiness in starchy matrices [14.33]. As another example, human salivary lipase is suspected to affect the perception of fat but this conclusion is subject to controversy. However, recent studies showed that human salivary lipase plays a significant role in the perception of fat [14.34, 35].

14.2 Simulation of Oral Processing

As described previously, human olfaction is a complex process that involves a wide array of factors that interact to affect the final perception of flavor. The main factors include mastication and the mixing of the bolus by the teeth and tongue, temperature and hydration, salivary enzymatic reactions, and airflow. Simulating in vivo oral functions using model mouth devices poses a challenge and in some cases, the actual behavior cannot be fully reproduced. However, the large deviations in the masticatory and swallowing patterns among individuals and the differences in the flow pattern and composition of saliva created the need to apply model mouths in investigations. The main advantage of these models is the ability to isolate a single parameter and study its effect on food breakdown and volatile compound release. This chapter describes the development of oral processing models in the field of volatile compound release, from the early simple devices to the recent ones. Moreover, the model-simulated oral functions and the research applications of the model mouth in food science are mentioned.

14.2.1 History of Model Mouth Designs

More than twenty years have passed since the development of the early models to simulate oral food processing and the release of VOCs. The models incorporated the following functions:

- Mastication and food breakdown
- Salivary mixing
- Airflow
- Body temperature
- VOC sampling.

The aim of each model is to determine the complexity of the model's features. The earliest models focused on sampling the VOCs that had been released into the headspace at certain intervals. A 2 l flask containing a 50 ml sample and 10 ml of artificial saliva was used

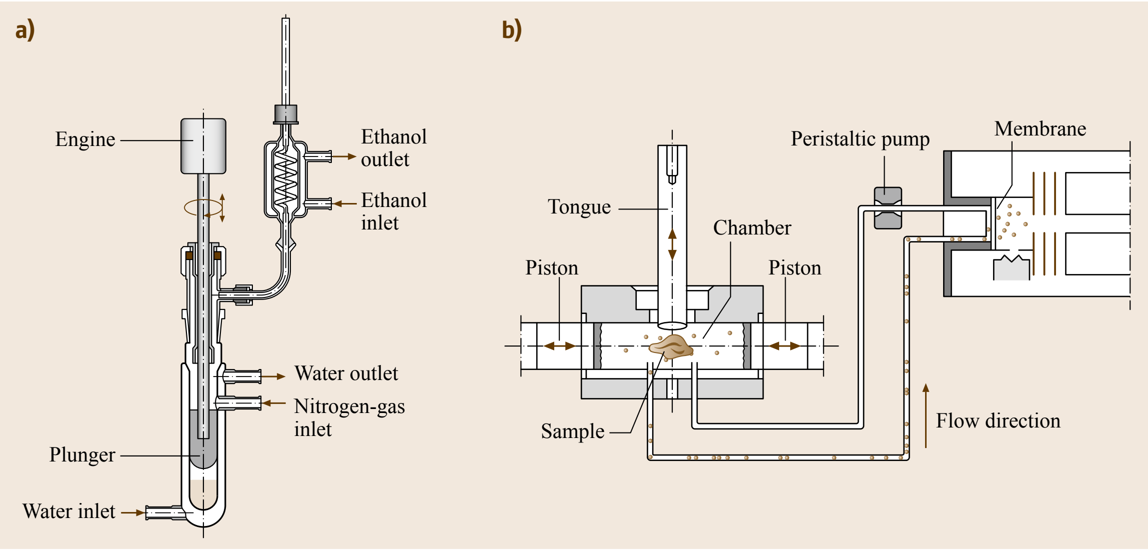

Fig. 14.1 Examples of model mouth designs to simulate oral food processing and VOC release **(a)** after [14.36], **(b)** after [14.37]

to study the release of VOCs from alcoholic beverages (old malt whiskey) [14.38]. Oral mastication in this model was mimicked by shaking a flask containing glass beads. The headspace was sampled after equilibrium was reached, at 45 min. Another device was developed [14.39] and was later used in the investigation of flavor release from dressings [14.40]; this device consisted of a temperature-controlled sample flask (70 ml) and an oscillating plunger moving vertically (4 cycles/min) to evaluate the effect of mastication on the release of VOCs from food samples (Fig. 14.1a). The samples were mixed with artificial saliva that was prepared using the major minerals and proteins that exist in human saliva. Certain VOCs were inserted into solid food (rehydrated diced bell peppers) or liquid food matrices (cream dressings or sunflower oil). The VOCs released from the food samples were trapped in a Tenax trap with a nitrogen gas flush and were later analyzed using gas chromatography with flame-ionization detection (GC-FID).

The first model mouth used to perform dynamic headspace analysis of VOC release was the so-called *retronasal aroma simulator* (RAS) [14.41]. Its design was based on a modified blender; nitrogen was purged over the sample in this blender while the temperature was held at 37 °C. The headspace was sampled at certain time points, which allowed the authors to plot the temporal release curves of several VOCs and to calculate the volatility rate constants ($k \times 10^{-5}\,\mathrm{min}^{-1}$). However, this model mouth lacked the proper dimensions and chewing geometry of the human mouth. Artificial saliva was incorporated into the device immediately before the sample was introduced, unlike in the more advanced apparatus [14.42] in which artificial saliva was pumped continuously into the glass reactor. Inside was a stirrer made of a six-star impeller, which mixed the liquid food. This model was the first to use a computer-controlled system to regulate the flow rates of the air and salivary inlets and the sample outlet. This system yielded VOC release data with a highly satisfactory level of reproducibility. The problem with this model was the nonproportionality of the conditions relative to those of a human mouth. For example, the stirrer speed of 450 rpm and the salivary flow rate of 175 ml/min deviated strongly from the real-life parameters. Later, the accuracy of the masticatory compressive and the shearing strengths was increased by using two horizontal pistons with wavy surfaces to simulate the irregularity of teeth shape and by applying one vertical piston to mimic the tongue's actions [14.37]. Together, the pistons chewed solid food (chewing gum) under the control and direction of the user via compressed air. Another unique feature of this model was the ability to measure the released VOCs online using a membrane-inlet mass spectrometer (MIMS) to simulate monitoring of their release during eating.

Despite the progress made in the development of model mouth systems, there were challenges to overcome to obtain a better resemblance to a real human mouth. The main problems with the models described above were the limited number of oral functions that could be represented simultaneously and the fact that the dimensions used were quite different from those of an actual oral environment.

Table 14.1 Comparison of functionalities between model mouth devices

Model mouth device	*Van Ruth* and *Roozen* [14.36]	*Roberts* and *Acree* [14.41]	*Woda* et al. [14.43]	*Arvisenet* et al. [14.44]	*Salles* et al. [14.45]	*Benjamin* et al. [14.46]	*Ishihara* et al. [14.47]
Airflow	Nitrogen 20 ml/min	Air or nitrogen 1200 ml/min	–	Helium not indicated	Air 30–50 ml/min	Air 1000 ml/min	–
Salivation	Constant volume	Constant volume	Small spurts	Constant volume	Constant flow rate	Constant flow rate	Constant flow rate
Mastication	Plunger screw (Compression and shearing)	Blender (Shearing)	Disks	Plunger (Compression and shearing)	Upper and lower jaws (Compression and shearing)	–	Flat plunger (Compression and shearing)
Heat	Water jacket	Water coils	Internal heater	Water jacket	Water jacket (initially) Thermofilm (now)	Water jacket	External regulation
Teeth	–	–	Shaped surfaces	Sharp shape	Human molar reproduction	–	–
Tongue	–	–	–	–	Hard cylinder (conically ended)	Soft ball	–
Measure of volatile compounds	Chemical traps	Chemical traps SPME	–	Chemical traps SPME	Connection with APCI or PTR-MS	Connection with PTR-MS	–
Measure of nonvolatile compounds	–	–	–	–	Possible by saliva sampling or introduction of sensors at different times	–	–
Food texture range	Soft–medium	All	Medium–hard	Medium–hard	All	Soft	All

14.2.2 Recent Developments in Model Mouth Devices

To address the previously mentioned limitations of the model mouth, more sophisticated and functional devices have recently been developed. This section describes several models that were designed to simulate the mastication of solid food through teeth and jaw movements and the mixing of liquid food through the pre-swallowing tongue pressure while monitoring the release of volatile compounds. A comparison of the functionalities of some model mouth devices is presented in Table 14.1.

The ability of the model to perform proper masticatory actions is well correlated with the exposure of the surface area of the bolus particles and their proper mixing with saliva. Both processes have a significant impact on VOC release [14.48]. The masticatory function of the model can be evaluated by comparing the sizes of the food particles generated with those generated by the human mouth using image analysis. The artificial mouth presented in [14.44] was developed to study the VOC release from apples crushed at different frequencies of compressive movements, speeds of rotational movement and periods of mastication. The apparatus was composed of a sample container (600 ml) and a notched plunger that rotated vertically and horizontally according to controlled motors (Fig. 14.2a). The apple samples were mixed with artificial saliva and the VOCs were extracted using solid-phase microextraction (SPME) fibers before analysis using gas chromatography (GC). The authors found that both the duration of mastication and the speed of rotational movement affected the intensity of the released aromas due to the different sizes of tissue obtained. Hence, any oxidative and enzymatic reactions that occurred accordingly affected VOC release. The same device was used to compare the aroma extract obtained from bread mastication to the aroma perceived in the human mouth [14.19]. It was found that saliva and water differentially affected the way the bread was commuted into small particles. Due to the presence of salivary proteins, saliva played a significant role in the release of VOCs according to their physicochemical properties.

A novel chewing simulator that integrated most of the main functions of the human mouth has recently been developed (Fig. 14.2b) [14.45]. This device includes a fixed upper jaw and a mobile lower jaw with teeth that reproduce the molar motif and can generate shearing forces of up to 250 N. The compressive and shearing movements are controlled in angular and vertical planes by a computer. Another unique feature of this model is that an inert material called polyetheretherketone (PEEK) was used to build the apparatus to avoid

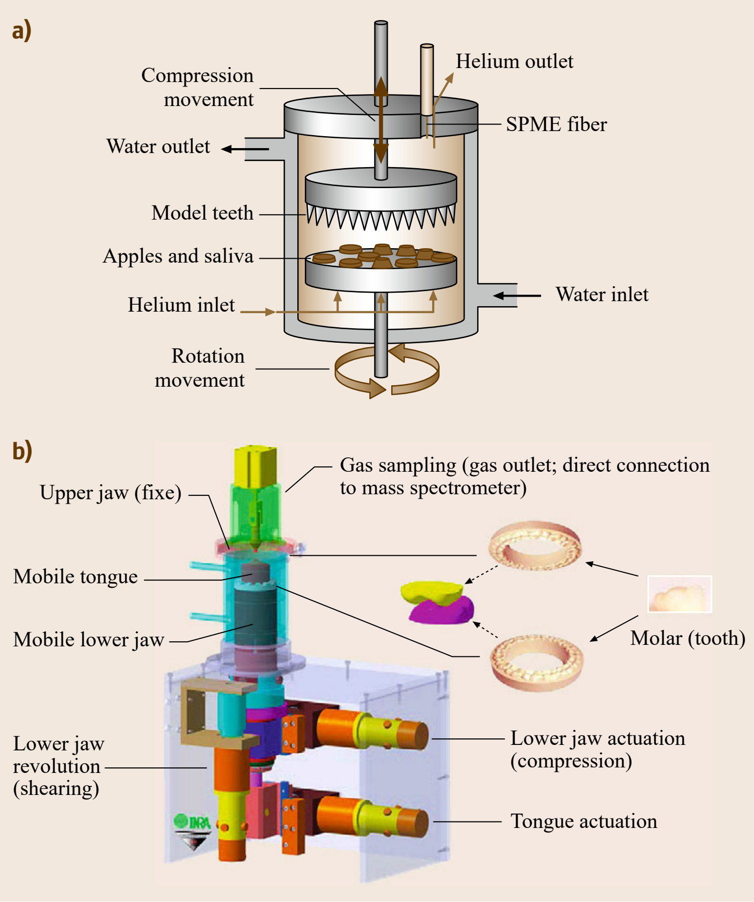

Fig. 14.2 Examples of recent model mouth designs to simulate solid food mastication and VOC release **(a)** after [14.44], **(b)** after [14.45]

the loss of VOCs through adsorption to the material. The operational volume resembles that of the oral cavity (100 ml), and artificial saliva is pumped into the chewing cell using a controlled flow system to mimic the salivary flow rate in the mouth under nonstimulated and stimulated conditions (0–5 ml/min). The masticatory capability of the simulator was tested using peanuts under oral environmental conditions. The results indicated that the course of natural food breakdown could be reproduced if the appropriate shearing forces and angles were applied as a function of the mechanical properties of the food sample. This chewing simulator was used to study the brittle behavior of cereal food products under masticatory conditions. The study reported that food fragmentation is followed by significant agglomeration after less than ten chewing cycles. Both phenomena were correlated with the magnitude of force applied and the evolution of the force. Moreover, through artificial mastication of the products, the chewing simulator was also able to discriminate products under dry masticatory conditions. The results showed a qualitative agreement with human mastication and textural properties of naturally chewed food [14.49].

The release of VOCs into the headspace is monitored in real time through a direct connection to an atmospheric pressure-ionization mass spectrometry device (API-MS) [14.50]. In particular, the temporal pattern of volatile compound release was found to change according to the oral parameters and the physico-chemical properties of the compounds.

Other devices were more dedicated to the study of food-bolus formation and the changes that occur during the chewing process. The artificial masticatory advanced machine (AM2) was developed to mimic the process of bolus formation in the mouth [14.43]. The main aim was to simulate the preparation of a food bolus in the model that had properties similar to those produced by natural mastication. The masticatory chamber is a cylindrical cavity. The two ends of the chamber are formed by the stationary *maxillary disk* and the moving *mandibular disk*. The mandibular disk can move back and forth along and rotate around the central axis of the cylinder. The authors obtained a good level of consistency between the in vivo and in vitro results for the breakdown of peanuts and carrots using different chewing cycles, with good repeatability. The in vitro and in vivo boluses displayed the same median particle size distributions for each food. The in vitro and in vivo boluses obtained at different times during the chewing process were also similar when the in vitro mechanical parameters were adjusted [14.51].

The simulation of liquid and semisolid food processing in the mouth has not progressed much, nor has the simulation of the tongue's role in volatile compound release. A mouth simulator has been specifically developed for semisolid foods, for which the oral processing is dominated by the effects of tongue movements [14.52]. The system, which is equipped with an electric motor to rotate the sample using a mixing vane, as well as a video camera, laser, optical sensor and temperature probe, is able to measure changes in viscosity due to temperature, shear, dilution and structural breakdown and to mimic the mixing pattern of semisolids and saliva in the mouth. Changes due to mixing were analyzed using a reflectance sensor (online) and image processing (offline). This study showed that enzyme-induced structural breakdown has a dramatic effect on the viscosity of starch-based semisolid products in time scales that are relevant to those of in-mouth processing.

A simpler mouth model was developed to quantify the salt release from food structures, such as biopolymer gels, after they were compressed [14.53]. The model consists of a jacketed vessel fitted with an impeller and a conductivity probe. To measure the results of diffusion, the sample is caged in the liquid phase and subjected to low shear, whereas to measure the results of compression, the sample was subjected to cyclic compressions using a texture-analyzer probe. The authors observed that salt release was affected by both the type of gelling agent used and the temperature. In particular, the compression of the gel only affected salt release when fractures occurred, which was interpreted as being a consequence of the increased surface area. A mechanical simulator was developed to mimic the action of the human jaw in the presence or absence of artificial saliva for both soft and harder foods [14.47]. The simulator consists of a cylindrical chamber composed of acrylic resin and a flat plunger with a 50 mm diameter for simulating compression and shearing simultaneously. This device was used to prepare a model bolus from various gel samples and to subject them to dynamic viscoelasticity measurements to investigate the rheological properties regarding the gel composition and the level of added saliva. As an example of its application, a model bolus prepared from a binary gel (mixture of gellan gum and psyllium seed gum) using this device showed weak-gel rheological behavior and had greater structural homogeneity than that derived from gellan gum gel. Moreover, the dynamic viscoelasticity parameters of the binary gel were less dependent on the level of saliva. The authors also reported that the greater structural homogeneity of the model bolus formed from composite gels with various physical properties were related to their greater miscibility with saliva [14.54].

Another artificial mouth has been developed to study the in-mouth processing of soft foods [14.55]. The system is composed of a 150 ml closed double-jacketed vessel with a usable volume of 100 ml. The shear rate applied to the studied mixture can be modulated using a marine propeller driven by a viscosimeter. The temperature is controlled at 35 °C and the redox potential and sodium concentration of the saliva are continuously recorded during processing by the artificial mouth. For example, one application was studying the effect of the saliva/cheese ratio and the cheese composition on salt release using pooled raw human saliva previously collected from different people, and following the changes in the composition of the saliva using different types of sensors. Regarding salt release during cheese digestion in the artificial mouth, good correlations with the sodium concentration were observed using a single sodium sensor and an array system that combined chloride and sodium detectors. By comparing domestically prepared soft cheese samples treated with deionised water and pooled human saliva in the artificial mouth system, it was found that a larger amount of sodium was released from the water-treated samples, whereas in general, a smaller amount of sodium was released from the saliva-treated cheeses. This slower release was attributed to the partial absorption of sodium by the saliva during the initial stages of digestion.

Recently, an innovative and dynamic model mouth has been developed to investigate whether the intraoral pressures produced by the tongue affect the release of VOCs [14.46]. The tongue is known to play an important role in manipulating and transporting the bolus within the mouth and lubricating it with saliva while applying pressure against the hard palate [14.2]. This model mouth incorporates some of the main human oral features to allow a better understanding of the pattern of VOC release in the mouth.

The model includes several parts that function simultaneously. The volume of the main chamber replicates that of the oral and nasal cavities in which the VOCs are released from a food bolus. Within the chamber, an artificial tongue composed of glass and silicone rubber masticates the sample. Various materials for constructing the tongue were considered to find materials with the viscoelastic properties of human muscle and inertness against the absorption of VOCs. Finally, glass material was chosen for the experiments on the release of volatile compounds, because it responds well to the pressures exerted by the elastic silicone rubber. The forces and pressures generated by the tongue are measured using two sensors: a compression load cell to assess force and a pressure transducer. The tongue movements are controlled by a computer-driven actuator and follow different mathematical movement patterns (sine wave, pulse and ramp). Figure 14.3 illustrates the pressure patterns generated by the artificial tongue made of glass and silicone rubber compared to the human tongue. The patterns for both materials follow similar curves with an average maximal pressure and duration of 20–30 kPa and 0.4–0.8 s, respectively. These values correspond to real values measured in participants while they swallowed liquids [14.56, 57]. The chamber has temperature-control circulation using a jacketed cylinder and the artificial saliva flows into the bottom and is mixed with the food. At the same time, a flow of air carries the VOCs from the headspace to the PTR-MS instrument for online detection. Thus, this model mouth can simulate a very rapid process, such as the consumption of liquid food. In the next sections, several applications of the model will be discussed.

The oral food processing is not complete without considering the swallowing phase and the intense VOC release that appears after. It was found that the majority of the VOCs are present in the thin layer coating the throat after swallowing and are carried into the nasal cavity by the exhaled air [14.58, 59]. To simulate the dynamic conditions of VOC release from liquid foods after swallowing, a model artificial throat was designed [14.60]. The system consists of vertical glass tubing that splits into the two following parts: the upper part into which the sample, artificial saliva and the cleaning solution are poured; and the bottom part from which the liquids are drained (Fig. 14.4). In the middle, there is a piece of Viton rubber that can be opened or closed using a clamp to mimic the swallowing behavior and the closure of the pharynx by the velum. A thin layer of liquid remains on the rubber surface and is responsible for the continuous release of volatile compounds. The VOCs are monitored online using an atmospheric pressure chemical ionization gas-phase analyzer (APCI-GPA). Testing the hypothesis that the release of the majority of VOCs is enhanced after swallowing yielded comparable results in the artificial throat and in humans. Compared with the results obtained using static headspace analysis or a model mouth, the relative amounts of VOCs released were much lower for the artificial throat due to the short measurement time, and closer to human values. However, the model system cannot fully simulate the events that occur in the human throat. One of the main differences between the two systems is the force that drives the swallowing, which is gravity in the artificial throat and pharyngeal peristalsis in the human throat. Therefore, the intensity of VOC release cannot be expected to be the same. In the future, the artificial throat model will undergo some modification, such as the addition of tidal airflow to simulate breathing patterns, control of the temperature and humidity of the air and the choice of material for the tube.

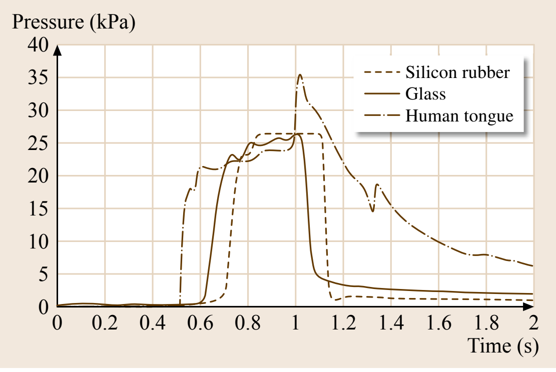

Fig. 14.3 Pressure pattern comparisons between the model mouth and human swallowing (after [14.46])

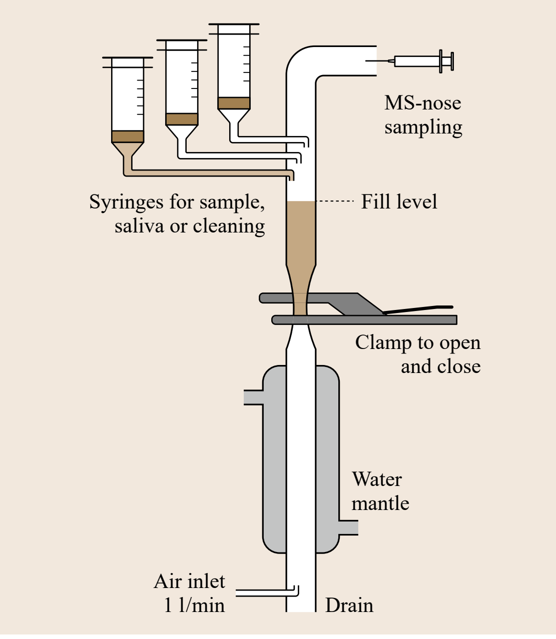

Fig. 14.4 Schematic overview of the artificial throat (after [14.60])

14.2.3 Simulation of the Oral Conditions and Oral Processing

In the section concerning the main oral functions, the high level of complexity within the mouth during oral food processing was explained. Understanding the role of each factor within the mouth and its effect on the release of flavor compounds is nearly impossible. However, a model mouth system has the clear advantage of allowing the analysis of a single parameter. The following oral functions and conditions are discussed in this section: mastication, mixing with saliva, temperature and airflow.

The mastication of solid and liquid food using the teeth and tongue has been investigated using several model mouths. One of the earlier models of *van Ruth* and *Buhr* [14.61] was applied to masticating rehydrated diced bell peppers using a plunger to make up and down screw-like movements. The intensity of the VOCs in the headspace was higher after mastication compared to that of nonmasticated material, which emphasizes the effect of the exposure of new surface areas and the efficacy of mixing food with saliva. The increased release of VOCs after mastication was also strongly perceived by assessors in terms of the odor intensity. The role of mastication for different types of foods was demonstrated using a more advanced mouth model [14.62]. The chewing efficiency of this device greatly resembled that of the panelists according to the size distribution of peanut particles that were produced at different force intensities and masticatory frequencies. The combined effect of similar mandibular, tongue and teeth shearing forces, shear angles and oral dimensions were found to be the key elements for a suitable model to simulate solid-food mastication. The structure and composition of the food affect the pattern of VOC release. This effect was clearly observed using the model mouth system. Using this device, significant differences in the release of VOCs from chewing gum compared to olives were reported.

The effect of mastication on liquid foods was studied using different mouth models [14.61, 63]. Similar to the case with solid foods, mastication generally enhances the release of volatile compounds from liquids. The turbulence from mixing and the changes in the extent of the interfacial surface area facilitate the diffusion of the VOCs into the headspace. The gas–liquid interface was increased two-fold by increasing the stirring rate from 100 to 400 rpm, which corresponded to a three-fold increase in the released VOCs [14.42]. The effects of tongue pressure and mastication on the release of VOCs in the mouth are very difficult to evaluate. However, a recently developed model mouth with an artificial tongue provides useful knowledge on the possible effects of the human tongue [14.46]. The authors applied a range of actual tongue intraoral pressures for various periods while monitoring the released VOCs online using a proton transfer reaction mass spectrometer. The findings validated the results regarding the enhanced VOC release after the mastication of solid and liquid foods. The tongue was found to create more turbulence in the liquid and more changes to the interfacial surface area when it remained in the liquid longer. The effect of the tongue on VOC release was observed as a clear peak after each masticatory cycle, following a different pattern according to the physicochemical properties of the VOC (Fig. 14.5). The location of the tongue in relation to the liquid and its direction of movement also affected the release of volatile compounds due to possible changes in the diffusion of VOCs from the liquid to the interface and then into the headspace (Fig. 14.6). The tongue is covered by a thin layer of liquid after mastication, which supports the release of more VOCs.

Finding that the composition and flow of saliva affected the release of volatiles in the mouth raised questions as to the extent of the impacts and the underlying mechanisms. Once again, the model mouth can be a suitable system in which to isolate the saliva parameter in food processing. Most of the model mouth systems use artificial saliva containing the major minerals (sodium, chloride, calcium, potassium and phosphate), proteins (mucin) and enzymes (e.g., amylase and lipase). The final composition should be close to that of human saliva and should have similar physical properties, such as the viscosity, pH and ionic strength.

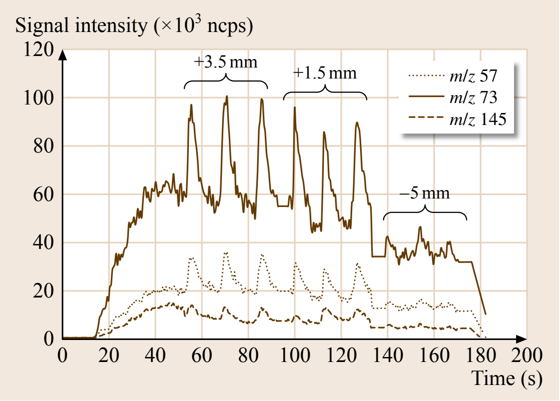

Fig. 14.5 Release curves for three volatile organic compounds (1-butanol (m/z 57), ethyl butyrate (m/z 89) and ethyl hexanoate (m/z 145)) masticated by the tongue in downward direction and different initial tongue positions from the aqueous solution surface (plus position relates to above the surface and minus position to below the surface) (after [14.64])

Among the effects of saliva on VOC release that are found in the model mouth are the dilution effect, interactions with salivary proteins and enzymes and to some extent, the possibility of *salting-out* [14.40, 65, 66]. For example, the effect of the amylase in saliva on VOC release is demonstrated by the higher odor intensity when the food contains starch. The degradation of starch by amylase leads to the release of volatile compounds that were trapped in the food complex, regardless of whether the amylase is human or porcine. VOCs are not equally affected by the presence of saliva; the effect depends on the polarity of the compound. Hydrophobic VOCs can be retained by interactions with proteins and hydrophilic VOCs can be retained by being diluted in a liquid system. An interesting finding was obtained when artificial saliva was compared to human saliva using the dynamic model mouth system with a flavored liquid sample [14.64]. These two types of saliva showed similar VOC release patterns, which supported the usage of artificial saliva as a proper alternative to human saliva. However, when saliva was replaced with water or artificial saliva lacking mucin, the extent of VOC release was higher. The authors attributed the increase in the extent of VOC release to the lower viscosities of the mixtures lacking the mucin that was present in the other types of saliva. The more viscous samples tended to adhere better to the tongue and the glass of the chamber, forming new surfaces that were exposed to the headspace. The salivary flow rate into the model chamber can be controlled at various rates to simulate a large variety of flows due to individual differences and the types of food. Consequently, the change in the flow rate corresponds mainly to the dilution effect of the sample.

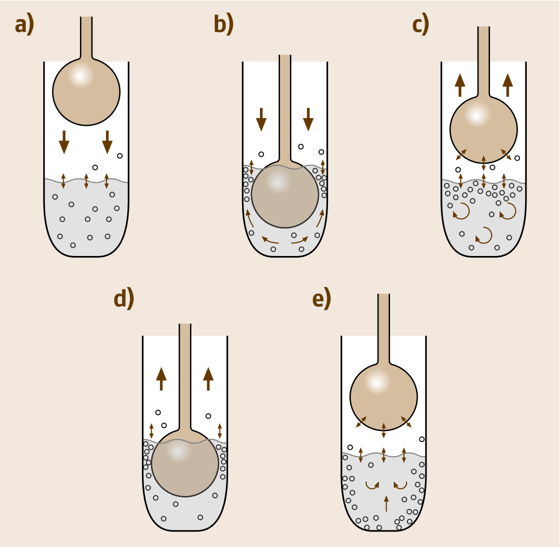

Fig. 14.6 Schematic illustrations of the artificial tongue masticating the sample at forward **(a–c)** and backward **(d–e)** movement directions (after [14.64])

The transfer of VOCs from the food to the oral and nasal cavities also depends on the respiratory rate of the lungs and the timing of the opening of the velum. To mimic the real situation of airflow, the model mouth design should consider the displacement of the volume of air in the headspace with a new supplement of air after each swallowing event, or in the case of solid food, with small volumes of air during the masticatory process. Most models used a continuous flow rate to simplify the experiment while ignoring the high level of complexity in oral physiology concerning the airflow. The model mouth designed by *Rabe* and collaborators [14.42] is most likely the one that most closely simulated the normal airflow patterns. In this model, the airflow is controlled by valves that introduce air at certain time points in correlation with the stirring and saliva-flow activities. Increasing the rate of airflow resulted in a higher flavor intensity due to the enrichment of the VOC content in the headspace. The effect of the airflow is highly dependent on the headspace volume. In the case of a large headspace volume, the enrichment process is slow when the air exchange occurs too rapidly relative to the rate of the mass transfer of VOCs.

The oral temperature is another important parameter that affects the release of VOCs. The rapid change in the temperature of a food sample once it was introduced into the mouth was measured in a model mouth system using a thermocouple sensor that was within the chamber [14.64]. In less than 70 and 100 s, oil- and water-based samples, respectively, reached body temperature (Fig. 14.7). The relationship between the temperature (T) and the partition coefficient (K) of VOCs can be described by the following equation

$$\frac{\mathrm{d}\ln K}{\mathrm{d}T} = \frac{\Delta H^\circ}{RT^2}\,, \qquad (14.1)$$

where ΔH° (kJ/mol) is the enthalpy of vaporisation of the VOCs and R is the universal gas constant. The higher the temperature becomes, the more volatile compounds are released into the headspace due to the lower ΔH°. The change in the temperature of the sample that occurs during oral processing also affects the solubility of the VOCs. Large hydrophobic VOCs were found to be less soluble at cool temperatures than were hydrophilic compounds that interacted with water through hydrogen bonds [14.64]. Moreover, the viscosity of the medium is temperature-dependent, which is more pronounced in oily systems. The viscosity of oil decreases five-fold from 60 to 4 °C, which affects the molecular and eddy diffusion of the VOCs.

Another oral phenomenon that is difficult to mimic in in vitro devices is the mucosa covering the oral surface. The artificial devices reported to date are composed of metal, glass or chemical polymers that do not confer the same mechanical, biochemical and physicochemical properties as the human mucosa. The dissimilarities have many consequences at different levels. The structure of the oral mucosa in different regions of the mouth varies considerably and consequently, the water-absorptive capacity varies according to the mouth region. The thickness of the salivary film varies in different regions of the mouth, depending on the proximity of the minor and major salivary glands. The salivary pellicle is a film that coats the oral surfaces and functions as a moisture retainer, a protective barrier, a lubricant and a determinant for microbial colonization. The pellicle is a multilayered film that is initially formed by the selective adsorption of salivary molecules to oral surfaces, followed by homo- or heterotypic complexing of these molecules with other molecules in the ambient saliva. The salivary components that adsorb to the oral mucosal epithelial cells comprise the mucosal pellicle. The forces that mediate the interactions between the salivary molecules and the epithelial cell surface most likely include noncovalent interactions involving electrostatic and hydrophobic forces. The oral mucosal pellicle that is formed by the selective adsorption of saliva to the epithelial cell plasma membrane cannot be closely reproduced in the in vitro surfaces but has many effects on the partitioning of the volatile and nonvolatile stimulatory compounds within different phases of the residence within the oral cavity and on the food-breakdown mechanisms involved in oral processing, such as tribological factors, resistance to breakdown and the deformation of the bolus. Therefore, the artificial saliva formulation is important in properly reproducing the oral phenomena through in vitro processes.

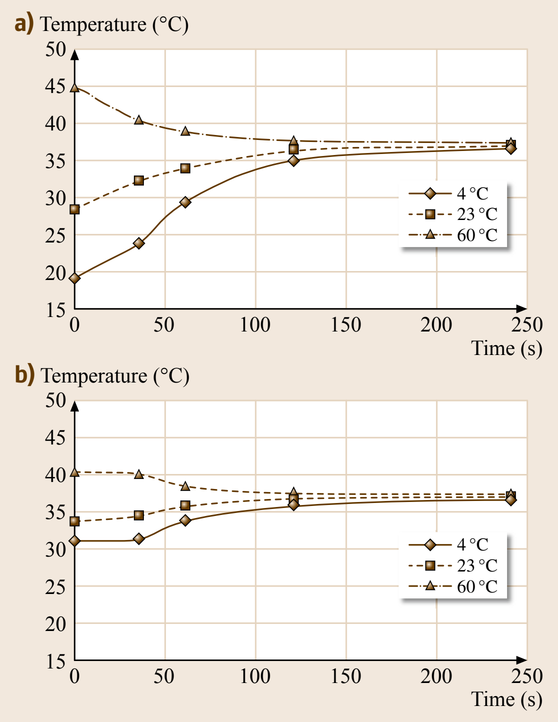

Fig. 14.7 Temperature curves in the model mouth of **(a)** aqueous solution and **(b)** oil samples versus mastication time period. The sample temperature was 4, 23 and 60 °C (after [14.64])

However, exactly reproducing human saliva is particularly difficult because of the high level of complexity of this biological fluid, its unstable character, its interindividual variability and the high cost of human salivary ingredients. The previous observations showed the importance of the physical properties of saliva in oral processes. The properties and composition of saliva are subject to significant subject variability, which is difficult to mimic using artificial solutions. Artificial saliva formulations that satisfy the viscosity requirement for the use in a masticator apparatus designed to prepare food boluses have been proposed [14.67]. The properties and composition of saliva also affect the in-mouth release of volatile compounds, which is another reason that a relevant formulation of artificial saliva is important. This statement is supported by various examples. The significant differences in the volatility of compounds that occurred when artificial saliva or water was added indicated that the saliva replacement was inadequate for studies of volatile compound release [14.68]. The salivary components differentially interact with the volatile compounds according to their physicochemical properties, leading to changes in their volatility [14.28]. The enzymatic activities of saliva can modify the composition of the released volatile compounds [14.29–31].

The human sensory system can be mimicked by electronic systems that are coupled to the mouth simulator for the detection and quantification of the released compounds. A connection to an API-MS or PTR-MS device allows the online recording of the real-time release of volatile compounds during the artificial chewing of food [14.61, 69]. However, the limitation of such detection systems is the level of sensitivity because the human olfactory system is much more sensitive and detects active odorants at concentrations at which no electronic system can detect them. There are very few reports concerning the in vitro detection of the release of nonvolatile taste compounds during oral processing. Simple sensor systems, such as pH, conductivity and sodium probes, or more complex sensor systems, such as electronic tongues, can be implemented in artificial mouth devices. Electronic tongues [14.70], which are considered artificial gustatory sensors, consist of sensor arrays and pattern-recognition systems. These systems generally aim to discriminate and analyze food and beverages [14.71, 72], but they generally need a large volume of liquid sample, are limited in sensitivity and the obtained results are poorly correlated with the taste attributes described and rated by a sensory panel because they consist only of the simultaneous measurements of chemical components by sensor-array systems. More recently, cell-based sensors have been developed, which have some advantages, such as fast response, excellent selectivity, high sensitivity [14.48], and the ability to respond specifically to compounds of a given basic taste. The use of such sensors coupled with a mouth-simulator device appears to be a promising way to mimic taste perception.

14.2.4 Model Mouth Applications

Model mouth systems have a wide range of applications in the fields of food science, nutrition, pharmacology and medicine. The following are a few examples of their applications in completed studies and future possibilities for their use:

1. Characterizing the release of VOCs from food samples according to composition. For example, the strong retention of hydrophobic VOCs in emulsions and oil-based systems was easily discerned using the model mouth [14.64].
2. Differentiating between food products as a function of the pattern of flavor release in the mouth. The release of aromas from red and white wines was compared under oral conditions using artificial saliva and a model mouth system [14.31].
3. Performing release-pattern assessments of different VOCs from food matrices during oral processing using a model mouth system.
4. Investigating oral food-processing behavior of food systems such as emulsions. Its ability to apply several oral parameters simultaneously while monitoring the release of flavor makes the model mouth a powerful research tool. For example, the model mouth can be used to gain more knowledge on the food-structure/flavor-release relationship [14.73]. This relationship was examined in two types of emulsion systems: primary and multilayered emulsions. The stability of the primary emulsion was more affected by changes in pH and saliva composition (e.g., the content of mucin) than was that of the multilayered emulsion, which also exhibited enhanced VOC release (Fig. 14.8). A multilayered emulsion consisting of two layers of pectin and β-lactoglobulin tended to better resist oil-droplet flocculation during consumption.
5. Comparing flavor release in in vitro experiments using the model mouth and in in vivo trials using participants. The results of such research can be used to evaluate the accuracy of the model mouth and to improve the elements that differ significantly [14.74].
6. The model mouth can be utilised in applications other than flavor release, such as taste analysis, salt-diffusion measurements and food-texture optimisation.
7. The model mouth can be used as a predictive tool. The oral parameters can be decoupled because each parameter is individually controlled. The model mouth could also be useful in testing mathematical models based on in vivo-acquired data.

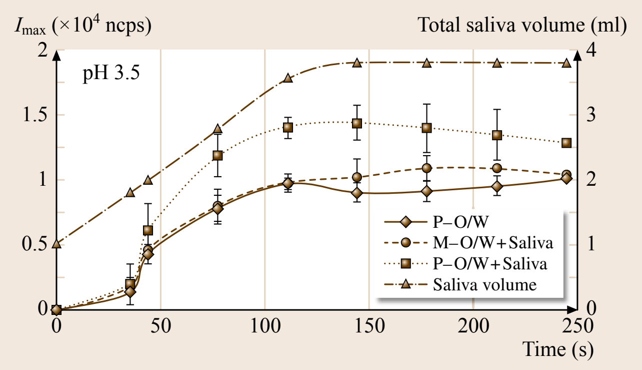

Fig. 14.8 The maximum signal intensity after mastication (I_{max}) of ethyl hexanoate from multilayer oil-in-water (M-O/W) emulsions at pH 3.5 as a function of saliva addition (after [14.73])

14.3 Conclusions

This chapter described an important tool for investigations of the oral processing of food. Using a model mouth system provides the researcher with control over different parameters that affect this complex process while investigating their impact on olfactory perception. The chapter includes a short background on the main oral functions and their influence on the release of VOCs. The history of model mouth devices and the recent improvements are considered in the chapter, together with details on certain oral functions that can be simulated by these models. Lastly, the applications of the model mouth in the field of food science are mentioned. Without doubt, research will be conducted in the future using such systems to improve the fundamental understanding of olfactory perception, the development of food products and quality assurance.

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