Food Hydrocolloids 25 (2011) 659–667

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Relationships between saliva and food bolus properties from model dairy products

S.R. Drago [a], M. Panouillé [b,c,*], A. Saint-Eve [b,c], E. Neyraud [d], G. Feron [d], I. Souchon [b,c]

[a] Instituto de Tecnología de Alimentos, Universidad Nacional del Litoral, Santa Fe, Argentina
[b] AgroParisTech, UMR 782 GMPA, F-78850 Thiverval-Grignon, France
[c] INRA, UMR 782 GMPA, F-78850 Thiverval-Grignon, France
[d] INRA, UMR 1324 CSGA, 17 Rue Sully, 21065 Dijon, France

ARTICLE INFO

Article history:
Received 20 April 2010
Accepted 27 July 2010

Keywords:
Saliva
Food
Bolus
Dairy product
Oral processing

ABSTRACT

During food consumption, complex oral processing occurs to transform the food into a bolus, ready to be swallowed. The objective of this study was to relate food, saliva and bolus properties, by using model dairy products, to better understand the role of saliva in bolus formation. Un-stimulated and stimulated saliva was collected from 5 subjects and biochemical and enzymatic properties were measured. Food bolus was then obtained from 8 different dairy products, varying in composition and ranging from liquid to gelled samples. The rate of saliva incorporation, pH, spreading ability and bolus rheological properties were determined. Some correlations seemed to exist between lysozyme activity and bolus properties. Subject and food product had a significant effect on almost all bolus properties. The rheology of bolus was highly correlated with food product texture. Even though preliminary, this approach could be used to better understand stimulus release and perception during food consumption.

1. Introduction

Food consumption implies a lot of complex oral manipulations, in order to transform a food product into a food bolus ready to be swallowed. In the mouth, food is diluted and mixed with saliva and can be broken down into small pieces by mastication, depending on its initial structure (Chen, 2008). The treatment of food in the mouth has two major functions: the reduction of the particle size by mastication and the lubrication of these particles by saliva and by juices released from the food (Prinz & Lucas, 1995). During mastication, the food product mixes with the saliva to form a bolus, which is a smooth and lubricated portion of mechanically broken down food (Pedersen, Bardow, Beier Jensen, & Nauntofte, 2002). During food consumption, salivary glands are stimulated, leading to the production of stimulated saliva. Saliva, by interacting with food product, could influence not only bolus characteristics, but also flavour release and perception. Saliva is composed of a variety of electrolytes (including sodium, potassium, calcium, magnesium, bicarbonate and phosphates) and proteins (enzymes, mucines, proline rich proteins…). Among salivary enzymes, amylase is the dominating enzyme. α-Amylase is known to decrease the viscosity of starch product, such as custards (Engelen, de Wijk, Prinz, van der Bilt, & Bosman, 2003; Engelen et al., 2007) or induce breakdown of mixed protein/starch gels (Janssen, van de Pijpekampa, & Labiausse, 2009) and therefore affect mouthfeel perception. Recently it has also been shown that this activity could influence volatile release (Ferry, Hort, & Mitchell, 2004) and salty perception in viscous systems (Ferry et al., 2006). These effects have been discussed in terms of a degradation of food polymers such as starch, inducing, thereby, a release of odorants from inclusion complexes (Taylor, 1996). Other enzymatic activities have been measured in saliva, such as esterasic (Buettner, 2002), lipolytic (Voho, Chen, Kumar, Rao, & Wetmur, 2006) or proteolytic (Helmerhorst, Sun, Salih, & Oppenheim, 2008) activities but so far, their influence on bolus formation has not been studied yet.

Recently, some studies tried to better understand and to explain food destruction in the mouth and to relate it with sensorial and nutritional properties of food (Chen, 2008; De Wijk, Engelen, & Prinz, 2003). However, few studies are related to food bolus properties. For solid foods, the fragmentation pattern in the course of mastication was studied (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Peyron, Mishellany, & Woda, 2004), showing a weak interindividual effect on bolus particle size distribution before swallowing. The rheological properties of food bolus obtained from cereal products were also determined (Loret, Hartmann, & Martin, 2009; Peyron et al., 2009) and these studies highlighted the importance of the bolus water content and fluidity. To our knowledge, there are no

* Corresponding author. AgroParisTech, UMR 782 GMPA, F-78850 Thiverval-Grignon, France.
E-mail address: Maud.Panouille@agroparistech.fr (M. Panouillé).

0268-005X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2010.07.024

study dealing with the bolus formation from dairy product and the influence of saliva on bolus properties.

The aim of this work was to study saliva composition and bolus formation from model dairy products taking into account physicochemical characteristics of stimulated saliva and physical characteristics of food bolus.

2. Materials and methods

2.1. Subjects and saliva samples

Whole saliva was collected from a group of 5 volunteers from 29 to 40 years old in two times, from 9:00 to 11:30 a.m. and from 3:00 to 5:00 p.m. at 2 occasions. After brushing their teeth, donors refrained from eating and drinking, with the exception of water, for 1 h before donation. To collect un-stimulated saliva the volunteers were asked to swallow the saliva in the mouth before starting and then spit each 30 s during 5 min into ice-chilled vessels. For stimulated saliva, after rinsing their mouth with water, the volunteers chewed a piece of parafilm of 5 × 5 cm for 4 min. During this time, saliva was expectorated into ice-chilled vessels every 30 s. The first spit of saliva was discarded. During collection and handling, the samples were constantly kept on ice. Flow rate was calculated as g/min. Whole saliva samples were centrifuged at 13,400×g for 5 min at 4 °C to remove cellular debris (Eppendorf, model 5415 R, Germany). The supernatants were frozen and stored at −80 °C and used within 3 weeks.

2.2. Analysis of saliva

2.2.1. Buffer capacity and pH measurements

Buffer capacity was measured by a modified version used by Engelen et al. (2007). Two hundred microliters of saliva were mixed with 1.6 ml of 1.875 mM HCl (so 0.003 mmol of acid were used) and the pH was measured using an electrode Mettler Toledo, Intralab Expert. To determine pH, the same electrode was used and the measurement was done in a ¼ dilution of saliva in water Milli Q (Water Purification System), in order to obtain a sufficient volume to submerge pH electrode. In order to limit CO_2 formation and bicarbonate instability, buffer capacity and pH were measured immediately after sampling.

2.2.2. Conductivity

Conductivity was measured immediately after sampling in a dilution 1/10 of saliva (0.5 ml saliva + 4.5 ml Milli Q water) using a conductimeter Heitolab MPC 350, Heito Paris, conductimeter. As saliva volume was low, dilution was necessary to obtain a sufficient volume to totally submerge the electrode.

2.2.3. Protein content

Protein concentration was determined using the method of Lowry, Rosenbrough, Farr, and Randall (1951) with bovine serum albumin as a standard.

2.2.4. Enzymatic assays

Proteolytic activity was determined using Pierce fluorescent protease Assay kit, USA. This kit included fluorescein-labeled casein (FTC-casein) for use as a substrate for assessing protease activity. Fluorescence properties of FTC-casein (intact protein substrate) change dramatically upon digestion by proteases, resulting in a measurable indication of proteolysis. The measurements were performed using a fluorometer Multilabel Plate Reader "Victor 3-V", Perkin Elmer, Waltham (MA), with excitation/emission filters (485/538 nm) and using trypsin as standard provided with the kit. The results were expressed as μg of trypsin equivalent/ml of saliva.

Lysozymal activity was measured using EnzChek Lysozyme Assay Kit (E-22013), USA. The assay measured lysozyme activity on *Micrococcus lysodeikticus* cell walls, which were labeled with fluorescein. Lysozyme action relieved the fluorescence quenching, yielding a dramatic increase in fluorescence which was proportional to lysozyme activity. The standard, provided with the kit, was lysozyme from egg white, 1000 U. One unit was defined as the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25 °C. The result was expressed as U/ml saliva.

Lipolytic activity was measured following the method described by Robert (1985) which used methylumbelliferone acyl esters as non fluorescent substrate. The lipase catalyzed the cleavage of this substrate producing a fatty acid and a fluorescent molecule of 4-methylumbelliferone. After 40 min of incubation at 37 °C, the fluorescence was measured using the same fluorometer, with excitation/emission filters (350/460 nm). The standard used was umbelliferone (Sigma 93979). The result was expressed in pkat/ml saliva (or pmol/s/ml).

2.3. Food samples

A model system made of skim milk retentate powder, fat and salt was used, following the method described by Saint Eve (Saint-Eve, Lauverjat, Magnan, Déléris, & Souchon, 2009). The products, varying in ultrafiltrated skim milk retentate powder (Triballat, France) content (250 or 150 g/kg) or varying in anhydrous milk fat (Corman, Belgium) content (0–166 g/kg), were manufactured using a defined protocol. The salt (NaCl, Prolabo, France) content (10 g/kg) was constant. These model dairy products were chosen for their good repeatability between preparations and the absence of syneresis in the matrices. Two kinds of samples were produced, the samples without rennet (150/0/NG, 150/40/NG, 250/0/NG, 250/40/NG) and the samples with rennet (150/0; 150/40; 250/0; 250/40). All the samples were evaluated respect to their dry matter content. Also, the samples with rennet were evaluated in relation to textural properties using a Texture Profile Analysis (TPA) on a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 10-mm-diameter cylindrical probe made of ebonite. After storage at 4 °C, slice samples (5 cm in diameter and 1 cm in height) of model cheeses were equilibrated at room temperature (20 ± 2 °C) for 30 min before measurements were made. A double-bite compression cycle was carried out, with a rest period of 0.09 s between bites. Samples were compressed at a distance of 5 mm with a test speed of 2 mm/s during the first bite and at a distance of 5 mm with a test speed of 2 mm/s during the second one. Model cheeses were characterized in terms of firmness, adhesiveness and cohesiveness. These parameters were defined according to the study of Szczesniak (1963) and Pons and Fiszman (1996). Three replicates for each product were performed. These values are shown in Table 1, together with product compositions. Dynamic oscillation tests were also performed with a controlled-stress rheometer (RS1, Thermo Scientific, Germany), equipped with a cone–plate geometry (60 mm diameter, 2° angle) or a plate–plate geometry (35 mm diameter), depending on products (Panouillé, Saint-Eve, de Loubens, Déléris, & Souchon, in press). Table 1 describes values obtained at 1 Hz within the linear viscoelastic domain for the storage modulus (G'_{1Hz}), loss modulus (G''_{1Hz}) and complex viscosity (η^*_{1Hz}).

2.4. Food bolus sampling

The same group of volunteers was asked to produce bolus from the four rennet model products and from 250/40/NG which had also a gel-like behaviour ($G' > G''$, see Table 1). Samples of 7 g of food

Table 1
Model dairy products' composition and textural measurements.

Sample	Composition				Rheological properties					
					Texture profile analysis			Small amplitude oscillation test		
	Milk retentate powder (g/kg)	Fat (g/kg)	Fat (g/100 g DM)	Rennet addition	Firmness (N)	Adhesivity (mN mm)	Cohesion (−)	G'_{1Hz} (Pa)	G''_{1Hz} (Pa)	η^*_{1Hz} (Pa s)
150/0/NG	150	0	0	−	ND	ND	ND	6.7×10^{-2}	1.4×10^{-1}	2.6×10^{-1}
150/40/NG	150	100	40	−	ND	ND	ND	2.9×10^{-1}	8.1×10^{-1}	1.4×10^{0}
250/0/NG	250	0	0	−	ND	ND	ND	9.5×10^{-1}	4.2×10^{0}	6.8×10^{0}
250/40/NG	250	166	40	−	ND	ND	ND	3.5×10^{3}	4.3×10^{2}	5.6×10^{3}
150/0	150	0	0	+	1.2	15	0.55	1.3×10^{3}	2.6×10^{2}	2.0×10^{2}
150/40	150	100	40	+	1.0	155	0.36	3.6×10^{3}	7.4×10^{2}	5.8×10^{2}
250/0	250	0	0	+	3.7	139	0.53	1.0×10^{4}	2.1×10^{3}	1.6×10^{3}
250/40	250	166	40	+	4.2	503	0.40	2.3×10^{4}	4.7×10^{3}	3.7×10^{3}

DM: dry matter.
ND: non determined (liquid samples).

product were given to the subjects. They were instructed to eat a biscuit in order to stimulated saliva, rinse their mouth three times with water, and then put the complete sample into the mouth, taste and before swallowing, spit once into a previously weighed container. The experiment was done twice for each product.

2.5. Food bolus evaluation

The weight of food introduced in the mouth (M_{food}) and the weight of spit bolus (M_{spit_bolus}) were first determined and used to calculate the following parameters (Fig. 1).

2.5.1. Food and spit bolus dry matter content

Dry matter content of food (DM_{food}) and spit bolus (DM_{spit_bolus}) were determined after dehydration in an oven at 103 °C during 24 h. The dry weights of food and spit were determined ($M_{(d)food}$ and $M_{(d)spit\_bolus}$ respectively) and dry matter content was calculated as followed:

$$DM_{food} = \frac{M_{(d)food}}{M_{food}} \quad (1)$$

$$DM_{spit\_bolus} = \frac{M_{(d)spit\_bolus}}{M_{spit\_bolus}} \quad (2)$$

As we assumed that all dry matter in bolus came only from consumed food sample (and not from saliva), we also had:

$$M_{(d)food\_in\_spit} = M_{(d)spit\_bolus} \quad (3)$$

2.5.2. Amount of food remaining in the mouth after the spit

When a panelist was asked to produce a bolus, he put a known weight of food (M_{food}) in his mouth, chewed it and finally spit before swallowing. However, some food sometimes remained in the mouth, so M_{food} can be divided into a first part incorporated into the spit bolus ($M_{food\_in\_spit}$) and a second part remaining in the mouth ($M_{food\_in\_mouth}$) (see Fig. 1).

$$M_{food} = M_{food\_in\_spit} + M_{food\_in\_mouth} \quad (4)$$

$$M_{(d)food} = M_{(d)food\_in\_spit} + M_{(d)food\_in\_mouth} \quad (5)$$

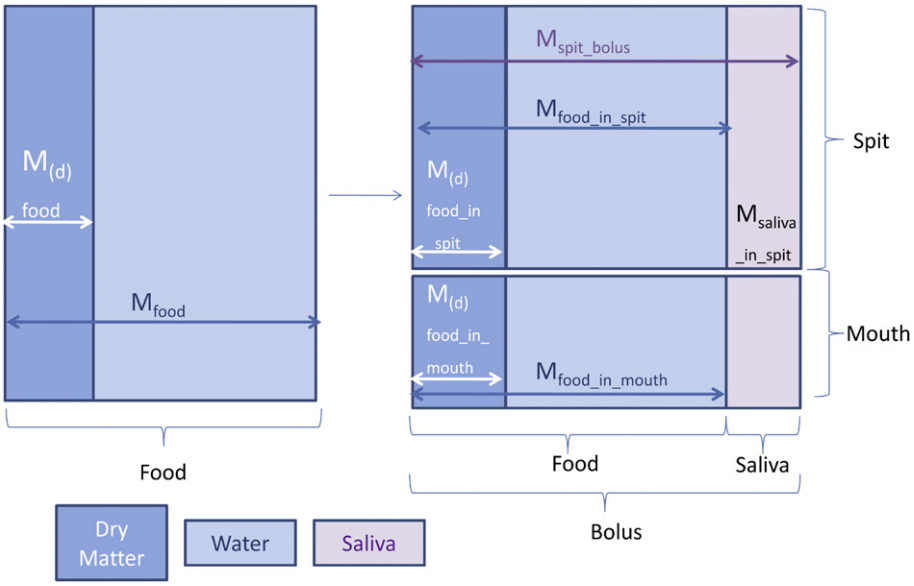

Fig. 1. Notations and scheme explaining calculations used to determine the weight (M) of food remaining in the mouth and bolus hydration by saliva.

We can calculate the weight of food solids remaining in the mouth:

$$M_{(d)food\_in\_mouth} = M_{(d)food} - M_{(d)food\_in\_spit} = M_{food} \times DM_{food} - M_{spit} \times DM_{spit} \quad (6)$$

Finally, the weight of wet food remaining in the mouth can be calculated from the dry matter food content:

$$M_{food\_in\_mouth} = \frac{M_{(d)food\_in\_mouth}}{DM_{food}} \quad (7)$$

2.5.3. Amount of saliva incorporated into the bolus

The quantity of saliva ($M_{saliva\_in\_spit}$) incorporated into the bolus was determined from bolus weight:

$$M_{saliva\_in\_spit} = M_{spit\_bolus} - M_{food\_in\_spit} \quad (8)$$

$M_{food\_in\_spit}$ was calculated from equations (2) and (3) as followed:

$$M_{food\_in\_spit} = \frac{M_{(d)food\_in\_spit}}{DM_{food}} = \frac{M_{(d)spit\_bolus}}{DM_{food}} = \frac{M_{spit\_bolus} \times DM_{spit\_bolus}}{DM_{food}} \quad (9)$$

The ratio of saliva added in the bolus was finally determined:

- respect to wet food sample (h_w):

$$h_w = \frac{M_{saliva\_in\_spit}}{M_{food\_in\_spit}} \quad (10)$$

- respect to food solid content (h_s):

$$h_s = \frac{M_{saliva\_in\_spit}}{M_{(d)food\_in\_spit}} \quad (11)$$

2.5.4. pH of bolus

The pH of bolus (pH_{bolus}) was measured and the change on pH due to saliva incorporation (ΔpH) was calculated taking into account the pH of the bolus and the pH of initial food (pH_{food}).

$$\Delta pH = pH_{bolus} - pH_{food} \quad (12)$$

2.5.5. Bolus spreading and rheological behaviour

Spreading and rheological properties of bolus were studied using a texturometer (TAXT2i Texture Analyzer, Stable Micro Systems, UK).

2.5.5.1. Initial spreading area and height. A given weight of fresh bolus was taken in a 20 ml and 20 mm diameter syringe cut at its end and carefully deposited on a plate. Depending on its consistency, the bolus spread more or less under the effect of gravity. The initial surface (S_i) of bolus was measured just after its deposit on the plate, using a photography and image analysis (Optimas 6, Media Cybernetics, USA). Height of bolus (H) before spreading was also measured with a ruler.

2.5.5.2. Spreading after compression. Bolus was then compressed between two plates. The upper plate moved down with a constant velocity of 5 mm/s until a compression force of 1 N was reached, maintained this position for 0.3 s and finally moved up. This protocol was used in order to mimic the displacement of the tongue and the compression of bolus between tongue and palate (Mossaz et al., 2010). After compression, the final bolus surface (S_f) was measured and the surface increase (ΔS) due to compression was calculated:

$$\Delta S = \frac{S_f - S_i}{S_i} \times 100$$

2.5.5.3. Force–displacement curve analysis. The force–displacement curve obtained during compression was used to obtain information on bolus rheological properties. A typical example is presented in Fig. 2. Rigidity was calculated from the initial slope of the force/distance curve and is therefore comparable to a Young modulus. Work of spreading corresponded to the area under the compression curve and work of adhesion to the negative area due to the adhesion of bolus during plate detachment.

2.6. Liquid samples evaluation

Whole stimulated saliva was collected as described above, but sample was not centrifuged. It was kept at 4 °C in an ice container until it was used within the next hour. Saliva sample was incorporated at 10% in each liquid model food product (150/0/NG, 150/40/NG, 250/0/NG). After mixing liquid food with saliva 10 s with a vortex, the apparent viscosity was measured at 37 °C during 5 min at a constant shear rate (10 s^{-1}), using a controlled-stress rheometer (MCR301, Anton Paar) equipped with concentric cylinders (outer diameter 27 mm).

2.7. Data processing and analysis

The influences of subject and stimulation on the different variables from saliva measurements were assessed by analysis of variance (ANOVA). The same test was performed to analyze the effect of subject and product on bolus variables. When significant differences were observed between products ($p < 0.05$), the mean values were compared using the Student–Newman–Keuls multiple comparison test. Correlation matrices were used to analyze the correlations among the different variables into each group, for saliva or bolus parameters. In order to relate product, saliva and bolus characteristics, correlation coefficients were also

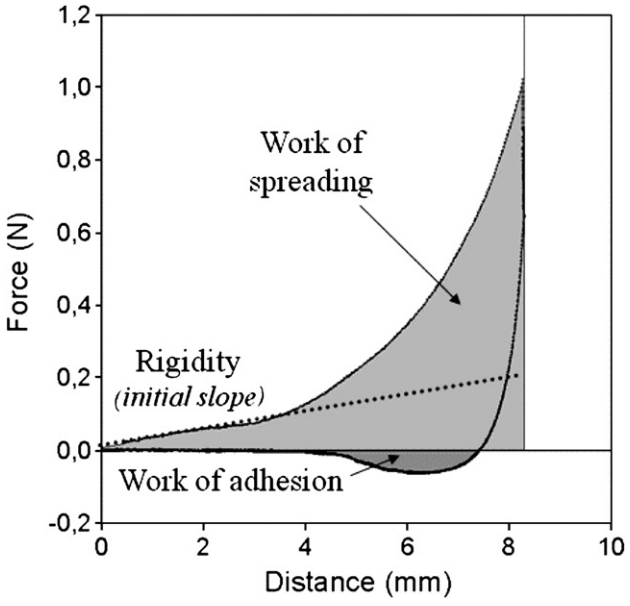

Fig. 2. Typical force/distance curve obtained during bolus compression.

calculated, first for all the data, and then product by product and subject by subject. XLStat software was used for all statistical analysis.

3. Results and discussion

3.1. Characteristics of stimulated and un-stimulated saliva

The influences of subject and stimulation on saliva parameters, analyzed by ANOVA, are presented in Table 2. Significant subject and stimulation effects ($p < 0.05$) were observed for almost all saliva parameters. The medium values of the different measurements for stimulated and un-stimulated saliva are also presented in Table 2.

The values of pH (from 7.45 to 7.84) and flow (from 0.91 to 2.58 ml/min) for stimulated saliva and pH (from 6.13 to 7.65) and flow (from 0.38 to 0.97 ml/min) for un-stimulated saliva were similar to that observed previously (Davidson, Linforth, & Taylor, 1998; Engelen et al., 2003; Humphrey & Williamson, 2001). Both total protein content for stimulated saliva (1.01–2.20 mg/ml) and for un-stimulated saliva (0.84–2.65 mg/ml), resulted in values in the same range as those described elsewhere in whole saliva (Rayment, Liu, Offner, Oppenheim, & Troxler, 2000) or parotid saliva (Neyraud, Heinzerling, Bult, Mesmin, & Dransfield, 2009). Buffer capacity was highly correlated with conductivity, i.e. ion concentrations in saliva, both in stimulated and un-stimulated saliva ($r^2 = 0.884$).

The enzymatic activities were consistent with data found in literature and confirmed the presence of proteolytic (Helmerhorst & Oppenheim, 2007), lipolytic (Stewart et al., 2010) and lysozymal activities (Chauncey, Lionetti, Winer, & Lisanti, 1954) in stimulated and un-stimulated saliva.

3.2. Bolus analysis

The effects of subject and product were analyzed using ANOVA. The statistical results are shown in Table 3, where the significant p values are presented. The medium values of the different characteristics of bolus, obtained for each sample are also shown in Table 3 and examples of photos of bolus before and after compression are shown in Fig. 3.

3.2.1. Influence of product in bolus formation

The type of food sample affected all measured parameters, except for the quantity of food remaining in the mouth and the variation of pH. It was observed that the presence of fat decreased the amount of saliva incorporated into the bolus (water content, saliva incorporation and hydration ratio h_s), but also increased the work of adhesion during compression test. The amount of protein decreased the water content of bolus and increased the self-standing properties of bolus (initial height).

3.2.2. Influence of subject in bolus formation

The subject had a significant effect on almost all the bolus variables. Statistical analyses were also performed subject by subject. Despite the little number of subjects, it was possible to distinguish between two different trends of behaviors. First of all, some subjects added a higher quantity of saliva into the bolus, which led to higher water content and hydration ratio, but also a higher variation in pH. As a result, the quantity of food remaining in mouth after spiting was lower. In terms of bolus rheology, these persons produced a more spreadable bolus (the initial height was lower and the work of spreading was lower), but also a more adhesive bolus. The second group had an opposite behaviour, adding a lower quantity of saliva, spiting a less complete bolus and producing a more self-standing bolus.

3.2.3. Incorporation of saliva into the bolus

The water content of bolus followed the same order than the water content of initial food products: the higher dry matter content of food was, the higher dry matter content of bolus was. However, 150/40 and 250/0 had the same dry matter content, but 150/40 led to a significantly less hydrated bolus, which can also be seen regarding the amount of saliva incorporated. The non renneted sample (250/40/NG) induced less incorporation of saliva into the bolus, probably because it did not require any mastication and stayed a shorter time in the mouth. When related to dry matter sample content (h_s), more saliva was incorporated into the bolus for non fat samples than for fat samples. This could also be seen in Fig. 3, where bolus from sample 150/0 before compression had saliva around the solids and was heterogeneous in relation to the others. It was also possible to observe that after compression both samples with 150 g/kg of skim milk retentate powder were less homogeneous, suggesting they had more saliva incorporated or that saliva was less mixed with the sample.

3.2.4. Relationships among the different bolus parameters

The correlation matrix obtained for bolus characteristics is presented in Table 4. Significant correlations were observed in each group of measurements (only significant correlations, evidenced by bold values in Table 4, will be described in this part). All parameters related to addition of saliva into the bolus (weight of saliva incorporated, h_w and h_s) were positively correlated. Bolus water content was positively correlated with saliva incorporation ($r^2 = 0.429$) and hydration ratios (h_s: $r^2 = 0.656$, h_w: $r^2 = 0.439$). Bolus pH and ΔpH were positively correlated ($r^2 = 0.746$). Concerning rheological properties, as expected the initial bolus height was inversely correlated with the initial surface ($r^2 = 0.741$), but positively correlated with the work of spreading ($r^2 = 0.833$) and bolus rigidity ($r^2 = 0.681$). Work of spreading was correlated to bolus rigidity ($r^2 = 0.881$). Finally, the work of adhesion was negatively correlated with bolus water content ($r^2 = 0.578$): higher bolus dry matter content led to a higher adhesivity.

Table 2
Panelist and stimulation effects on saliva characteristics obtained by ANOVA and mean values of physicochemical characteristics of un-stimulated saliva and stimulated saliva.

	Statistical analyses		Saliva measurements	
	Subject effect (p value)	Stimulation effect (p value)	Un-stimulated saliva (mean value ± SD)	Stimulated saliva (mean value ± SD)
Flow (mg/min)	$p < 0.0001$	$p < 0.0001$	0.57 ± 0.18	1.76 ± 0.49
pH	$p < 0.0001$	$p < 0.0001$	7.20 ± 0.42	7.60 ± 0.13
Buffer capacity (pH unit)	$p = 0.004$	$p < 0.0001$	3.44 ± 0.40	4.46 ± 0.92
Conductivity (μS/cm)	$p = 0.02$	$p = 0.02$	3452 ± 496	3898 ± 591
Protein (mg/ml)	$p = 0.007$	NS	1.76 ± 0.58	1.60 ± 0.33
Lysozymal activity (U/ml)	NS	$p < 0.0001$	268 ± 67	180 ± 53
Proteolytic activity (μg/ml of trypsin eq)	$p < 0.0001$	$p = 0.000$	2.22 ± 0.85	2.87 ± 0.63
Lipolytic activity (pkat/ml)	$p = 0.01$	$p = 0.08$	0.28 ± 0.25	0.53 ± 0.63

Table 3
Panelist and product effects on saliva characteristics obtained by ANOVA and mean values of physicochemical characteristics of bolus from the different products.

Measurements			Statistical analyses		Products				
		Unit	Product effect	Subject effect	150/0	150/40	250/0	250/40	250/40/NG
Food remaining in the mouth	$M_{(d)food\_in\_mouth}$	g	$p = 0.03$	$p = 0.09$	0.18a	0.23a	0.28a	0.54a	0.64a
	$M_{food\_in\_mouth}$	g	NS	$p = 0.05$	0.96a	0.92a	1.15a	1.35a	1.58a
Bolus hydration by saliva	Bolus water content	–	$p < 0.0001$	$p < 0.0001$	0.85a	0.77c	0.79b	0.66d	0.62e
	Saliva incorporation	g	$p < 0.0001$	$p < 0.0001$	1.16a	0.73b	1.19a	1.19a	0.46c
	h_w	–	$p = 0.01$	$p = 0.003$	0.19a	0.11ab	0.18a	0.20a	0.07b
	h_s	–	$p = 0.003$	$p = 0.05$	1.07a	0.46bc	0.75ab	0.48bc	0.16c
pH	Bolus pH	–	$p = 0.003$	$p = 0.01$	6.44a	6.47a	6.36ab	6.45a	6.30b
	ΔpH	–	NS	$p = 0.01$	0.03a	0.05a	0.08a	0.07a	0.07a
Spreading ability	Initial height	cm	$p < 0.0001$	$p = 0.003$	0.40c	0.47c	0.74b	1.07a	0.26d
	Initial surface	cm^2	$p < 0.0001$	$p = 0.04$	3.70b	4.06b	3.35b	2.40c	6.77a
	Final surface	cm^2	$p < 0.0001$	$p = 0.002$	5.92c	7.64b	5.21c	4.54c	9.66a
	Surface increase	–	$p = 0.02$	NS	0.64ab	0.90a	0.60ab	0.93a	0.43b
Rheological properties	Work of spreading	A.U.	$p < 0.0001$	$p < 0.0001$	0.62c	0.55d	0.69b	1.1a	0.49d
	Work of adhesion	A.U.	$p < 0.0001$	$p = 0.01$	0.05*	0.17*	0.07*	0.13*	0.29*
	Rigidity	A.U.	$p < 0.0001$	$p = 0.007$	0.11b	0.09b	0.10b	0.44a	0.12b

Different letters in the same row indicate significant difference ($p < 0.05$), using ANOVA.
* In that case, a product × subject interaction was observed.

3.2.5. Influence of food and saliva variables on bolus characteristics

In order to relate food, saliva and bolus properties, correlation matrices were calculated using the whole database, but also product by product to evidence saliva influence and subject by subject to evidence product influence on bolus properties.

The food textural characteristics influenced many of bolus rheological parameters: there was a direct relationship between food storage modulus (G′), firmness and adhesivity and:

- bolus self-standing (initial height) ($r^2 = 0.891$ for G′, $r^2 = 0.797$ for firmness and $r^2 = 0.795$ for adhesivity)
- work of spreading ($r^2 = 0.835$ for G′, $r^2 = 0.693$ for firmness and $r^2 = 0.807$ for adhesivity)
- bolus rigidity ($r^2 = 0.830$ for G′, $r^2 = 0.666$ for firmness and $r^2 = 0.930$ for adhesivity).

pH_{bolus} was correlated with pH_{food} ($r^2 = 0.659$). The bolus water content was highly correlated with food composition, especially water content ($r^2 = 0.962$) but also inversely correlated with food complex viscosity ($r^2 = 0.937$) and adhesivity ($r^2 = 0.777$). For 3 subjects, the hydration ratio h_s was correlated with sample cohesion: this suggested that when the sample was more cohesive,

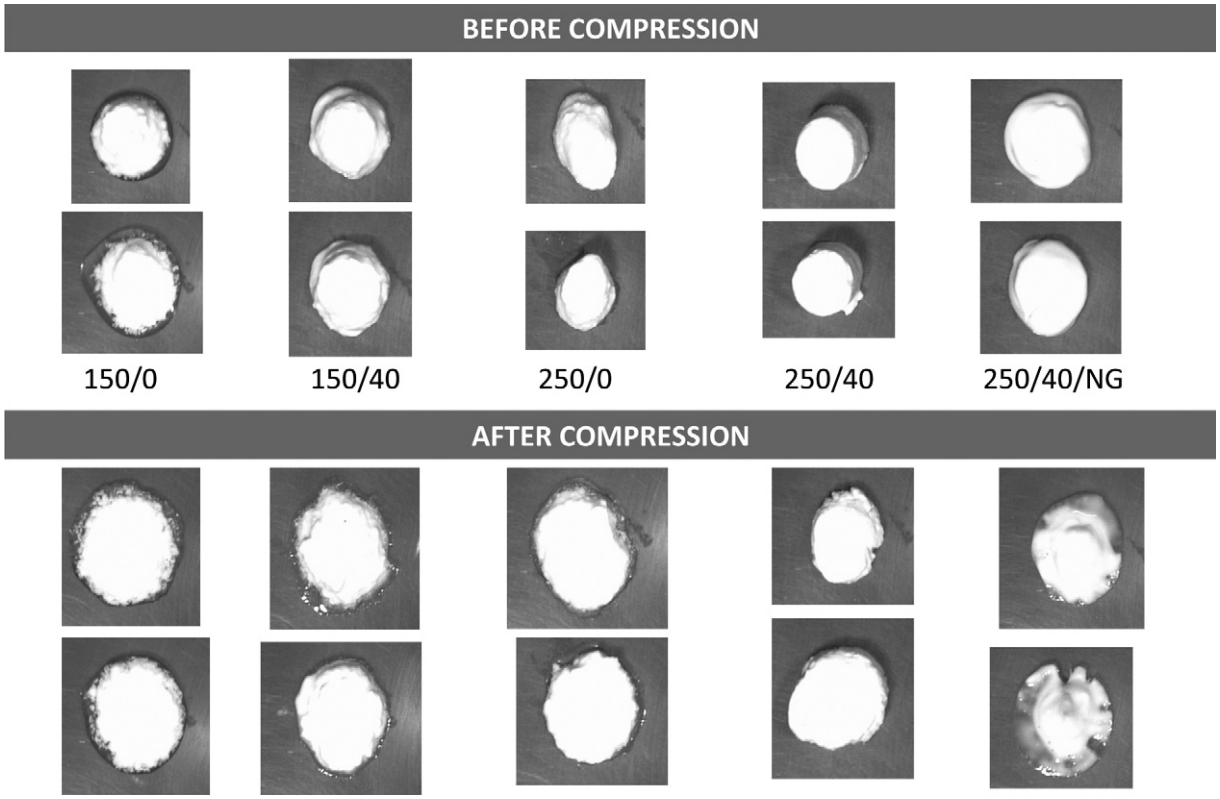

Fig. 3. Photos showing bolus spreading before (bolus was sampled with a syringe cut at its end and deposited on a plate) and after compression. 2 Different boluses produced by 2 subjects are presented as examples.

Table 4
Correlation matrix between bolus characteristics.

Variables	$M_{(d)food\_in\_mouth}$	$M_{food\_in\_mouth}$	Bolus water content	Saliva incorporation	h_w	h_s	pH	ΔpH	Initial height	Initial surface	Final surface	Surface increase	Work of spreading	Work of adhesion	Rigidity
$M_{(d)food\_in\_mouth}$	**1**														
$M_{food\_in\_mouth}$	**0.948**	**1**													
Bolus water content	**−0.540**	−0.290	**1**												
Saliva incorporation	−0.231	−0.194	**0.429**	**1**											
h_w	−0.173	−0.088	**0.439**	**0.882**	**1**										
h_s	−0.262	−0.093	**0.656**	**0.717**	**0.902**	**1**									
pH	−0.294	−0.304	0.310	**0.364**	0.300	0.254	**1**								
ΔpH	−0.071	−0.153	−0.044	0.274	0.175	0.075	**0.746**	**1**							
Initial height	0.282	0.249	−0.151	0.201	0.143	−0.075	0.126	−0.013	**1**						
Initial surface	0.092	0.022	**−0.324**	−0.304	−0.280	−0.220	**−0.414**	−0.072	**−0.741**	**1**					
Final surface	0.041	−0.043	−0.256	−0.201	−0.206	−0.177	−0.168	0.070	**−0.656**	**0.872**	**1**				
Surface increase	−0.142	−0.184	0.111	**0.364**	0.286	0.150	**0.507**	0.281	**0.364**	**−0.476**	−0.016	**1**			
Work of spreading	0.229	0.167	−0.290	−0.043	−0.027	−0.174	0.104	−0.066	**0.833**	**−0.591**	**−0.640**	0.086	**1**		
Work of adhesion	0.290	0.125	**−0.578**	−0.160	−0.176	**−0.320**	−0.100	0.200	−0.302	**0.411**	**0.454**	0.060	**−0.371**	**1**	
Rigidity	0.235	0.102	**−0.499**	−0.077	−0.049	−0.210	0.025	−0.102	**0.681**	**−0.351**	**−0.384**	0.105	**0.881**	−0.199	**1**

Significant correlations are described by bold values ($p < 0.05$).

more saliva was necessary to transform food product into a bolus ready to be swallowed. Finally, $M_{(d)food\_in\_mouth}$ was positively correlated with food dry matter content ($r^2 = 0.681$) and complex viscosity ($r^2 = 0.698$).

If food texture properties explained some differences observed in bolus properties, it was nevertheless difficult to relate them to saliva properties. However, some correlations appeared between lysozyme activity and several bolus measurements: a higher lysozyme activity seemed to be inversely correlated with the amount of saliva incorporated in the bolus ($r^2 = 0.656$), the variation of bolus pH ($r^2 = 0.658$), the bolus water content ($r^2 > 0.9$ for 3 products), hydration by saliva ($r^2 > 0.9$ for 3 products) and higher rheological properties (initial height before spreading ($r^2 > 0.8$ for 3 products), work of spreading ($r^2 > 0.9$ for 3 products) and rigidity ($r^2 > 0.8$ for 3 products)). For 3 products (150/40, 250/0 and 250/40), the water content and hydration ratios were correlated with the salivary flow ($r^2 > 0.7$). As these products were the firmest gels, they required longer mastication time and in that case the amount of saliva incorporated in the bolus was related with individual salivary flow.

3.3. Effect of saliva on bolus viscosity for liquid samples

The liquid products were diluted and mixed with water or saliva and the resulting apparent viscosity was measured at 10 s^{-1} during 5 min, in order to study the effect of saliva on food sample. The results obtained for 2 different subjects are presented in Fig. 4 as an example. For non-diluted products, a slight decrease of viscosity in time was observed for all samples, because of the thixotropic behaviour of samples. As expected, the apparent viscosity decreased after dilution with water. Concerning dilution with saliva, two different cases were observed, depending on subjects. For the first subject (J1), the apparent viscosity of the mix sample/saliva had an intermediate value between sample and sample/water viscosity. Silletti, Vingerhoeds, van Aken, and Norde (2008) found that parotid saliva had a shear-rate independent viscosity slightly higher than water, with a mean value of 2 mPa s. Although parotid saliva does not contain mucins, whole stimulated saliva contains mucins and has a shear-thinning behaviour, with a high elasticity (Stokes & Davies, 2007). The difference in water and saliva viscosity explained why dilution with water always led to a mixture with a lower viscosity. For the second subject (J2), mixing sample with saliva produced a bolus with a higher viscosity than initial sample, probably because of a higher mucin concentration in saliva. A peak in viscosity was observed in that case, because of the elastic properties of saliva (Stokes & Davies, 2007). The mix viscosity was dominated here by saliva rheology and not by product rheology. Apparent viscosity values were measured at 10 s^{-1} and the steady state value was not reached for all mixtures after 300 s, which can probably explain these high viscosity values.

When compared with dilution by water, no decrease of viscosity with time was observed when saliva was added to liquid sample, indicating that in these conditions no potential effect of enzymatic activity was observed in our experimental conditions. The most likely sources of proteolytic enzymes in whole saliva are derived from oral microbiota, gingival fluid and epithelial cells (Helmerhorst, Alagl, Siqueira, & Oppenheim, 2006). Although the proteolytic activity is important for saliva protein hydrolysis, like histatins with high content of lysine and arginine residues which makes these proteins extremely susceptible to tryptic-like digestion (Helmerhorst et al., 2006), this enzymatic activity seemed to have no visible effect on these dairy liquid products in a very short time.

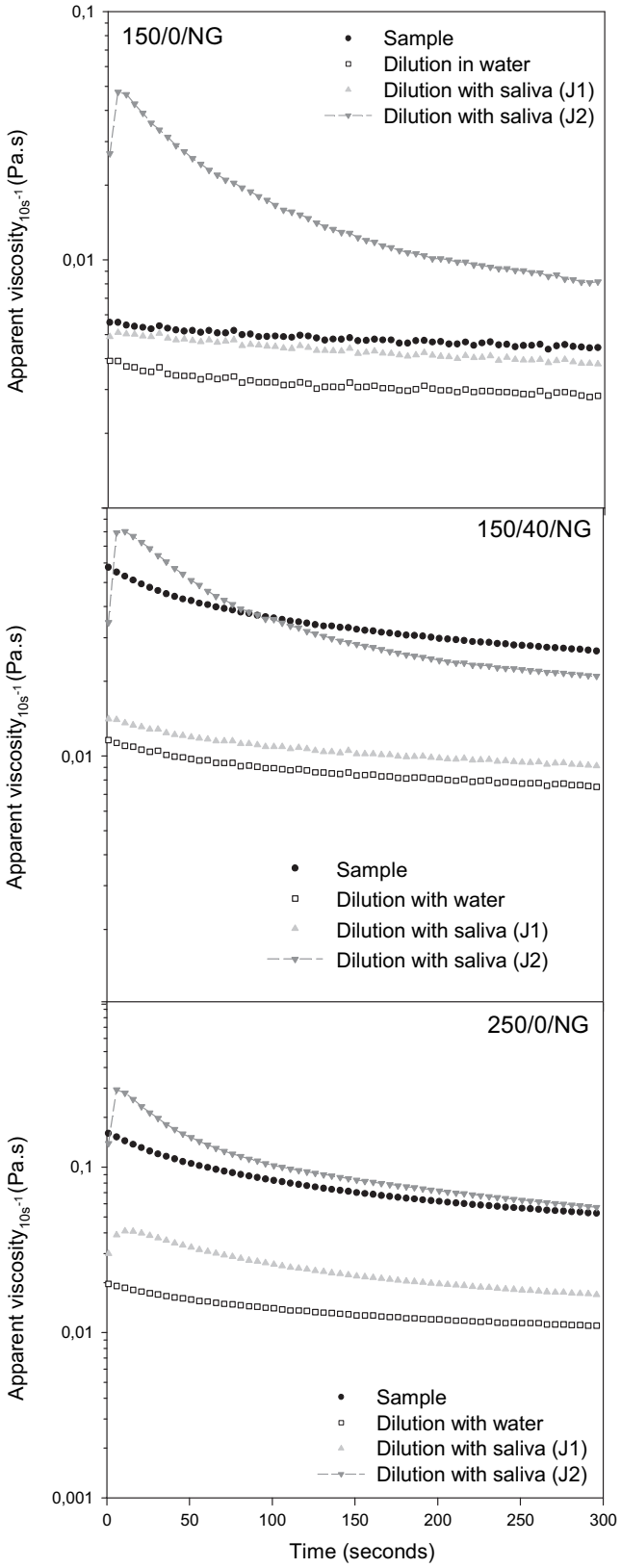

Fig. 4. Apparent viscosity of liquid products and their mixtures with water or saliva.

3.4. To go further with these results: relation with salty perception

This work was a preliminary study, carried out on a small number of subjects, to highlight interindividual differences in bolus formation related to some saliva characteristics. These results could also be used to explain some results related to stimuli perception in this type of dairy products. It was shown in another paper (Panouillé et al., in press) that product composition and texture had an influence on salty perception. Samples 250/40/NG and 150/40 were perceived as significantly more salty than the other gels (150/0, 250/0 and 250/40). If we considered bolus properties evidenced here, the 2 samples 250/40/NG and 150/40 were those which were less hydrated by saliva, but also more spreadable. As less saliva was added, the relative salt concentration in saliva was probably higher, which could lead to a higher perception of salt. Moreover, the high spreadability could lead to a higher surface of mucous membrane covered by product, i.e. a higher exchange area between salt and receptors in the tongue.

4. Conclusion

This work showed that differences in saliva properties, such as those emerging from individual differences and differences between products, relate to differences in food bolus properties. These differences in food bolus properties may well be related to differences in perceived sensory properties and may affect mastication processes. Our results pointed out the necessity to take into account saliva in modeling approaches on bolus formation or stimuli release and perception. A first trial was done to relate product, saliva and bolus properties in model dairy products. Although it was not possible to show that saliva enzymatic activities were involved in structural changes influencing bolus rheological properties, they could possibly influence stimuli release and perception. Studies implying a larger number of subjects are now required to go further in the understanding of saliva role in bolus formation and perception.

References

Buettner, A. (2002). Influence of human salivary enzymes on odorant concentration changes occurring *in vivo*. 1. Esters and thiols. *Journal of Agricultural and Food Chemistry, 50*, 3283–3289.

Chauncey, H. H., Lionetti, F., Winer, R. A., & Lisanti, V. F. (1954). Enzymes of human saliva: I. The determination, distribution, and origin of whole saliva enzymes. *Journal of Dental Research, 33*, 321–334.

Chen, J. (2008). Food oral processing - a review. *Food Hydrocolloids, 23*, 1–25.

Davidson, J. M., Linforth, R. S. T., & Taylor, A. J. (1998). In-mouth measurement of pH and conductivity during eating. *Journal of Agricultural and Food Chemistry, 46*, 5210–5214.

De Wijk, R. A., Engelen, L., & Prinz, J. F. (2003). The role of intra-oral manipulation in the perception of sensory attributes. *Appetite, 40*, 1–7.

Engelen, L., de Wijk, R. A., Prinz, J. F., van der Bilt, A., & Bosman, F. (2003). The relation between saliva flow after different stimulations and the perception of flavor and texture attributes in custard desserts. *Physiology & Behavior, 78*, 165–169.

Engelen, L., van den Keybus, P. A. M., de Wijk, R. A., Veerman, E. C. I., Amerongen, A. V. N., Bosman, F., et al. (2007). The effect of saliva composition on texture perception of semi-solids. *Archives of Oral Biology, 52*, 518–525.

Ferry, A. L., Hort, J., & Mitchell, J. R. (2004). Effect of amylase activity on starch paste viscosity and its implications for flavor perception. *Journal of Texture Studies, 35*, 511–524.

Ferry, A. L. S., Mitchell, J. R., Hort, J., Hill, S. E., Taylor, A. J., Lagarrigue, S., et al. (2006). In-mouth amylase activity can reduce perception of saltiness in starch-thickened foods. *Journal of Agricultural and Food Chemistry, 54*, 8869–8873.

Helmerhorst, E. J., Alagl, A. S., Siqueira, W. L., & Oppenheim, F. G. (2006). Oral fluid proteolytic effects on histatin 5 structure and function. *Archives of Oral Biology, 51*, 1061–1070.

Helmerhorst, E. J., & Oppenheim, F. G. (2007). Saliva: a dynamic proteome. *Journal of Dental Research, 86*, 680–693.

Helmerhorst, E. J., Sun, X., Salih, E., & Oppenheim, F. G. (2008). Identification of Lys-Pro-Gln as a novel cleavage site specificity of saliva-associated proteases. *Journal of Biological Chemistry, 238*, 19957–19966.

Humphrey, S. P., & Williamson, R. T. (2001). A review of saliva: normal composition, flow, and function. *The Journal of Prosthetic Dentistry, 85*, 162–169.

Jalabert-Malbos, M. L., Mishellany-Dutour, A., Woda, A., & Peyron, M.-A. (2007). Particle size distribution in the food bolus after mastication of natural foods. *Food Quality and Preference, 18*, 803–812.

Janssen, A. M., van de Pijpekampa, A. M., & Labiausse, D. (2009). Differential saliva-induced breakdown of starch filled protein gels in relation to sensory perception. *Food Hydrocolloids, 23*, 795–805.

Loret, C., Hartmann, C., & Martin, N. (2009). Rheological and sensory characterization of a swallowed food bolus. In *International symposium on food rheology and structure*, Zürich.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry, 193*, 265.

Mossaz, S., Jay, P., Magnin, A., Panouillé, M., Saint-Eve, A., Déléris, I., et al. (2010). Measuring and predicting the spreading of dairy products in the mouth: sensory, instrumental and modelling approaches. *Food Hydrocolloids, 24*, 681–688.

Neyraud, E., Heinzerling, C. I., Bult, J. H. F., Mesmin, C., & Dransfield, E. (2009). Effects of different tastants on parotid saliva flow and composition. *Chemosensory Perception, 2*, 108–116.

Panouillé, M., Saint-Eve, A., de Loubens, C., Déléris, I., & Souchon, I. Understanding of the influence of composition, structure and texture on salty perception in model dairy products, in press.

Pedersen, A. M., Bardow, A., Beier Jensen, S., & Nauntofte, B. (2002). Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. *Oral Diseases, 8*, 117–129.

Peyron, M.-A., Gierczinski, I., Woda, A., Loret, C., Hartmann, C., & Martin, N. (2009). Use of the texture profile analysis (TPA) test for a rheological characterization of food bolus collected in the course of mastication. In *International symposium on food rheology and structure*, Zürich.

Peyron, M.-A., Mishellany, A., & Woda, A. (2004). Particle size distribution of food boluses after mastication of six natural foods. *Journal of Dental Research, 83*, 578–582.

Pons, M., & Fiszman, S. M. (1996). Instrumental texture profile analysis with particular reference to gelled systems. *Journal of Texture Studies, 27*, 597–624.

Prinz, J. F., & Lucas, P. W. (1995). Swallow thresholds in human mastication. *Archives of Oral Biology, 40*, 401–403.

Rayment, S. A., Liu, B., Offner, G. F., Oppenheim, F. G., & Troxler, R. F. (2000). Immunoquantification of human salivary mucins MG1 and MG2 in stimulated whole saliva: factors influencing mucin levels. *Journal of Dental Research, 79*, 1765–1772.

Robert, I. M. (1985). Hydrolysis of 4-methylumbelliferyl butyrate: a convenient and sensitive fluorescent assay for lipase activity. *Lipids, 20*, 243–247.

Saint-Eve, A., Lauverjat, C., Magnan, C., Déléris, I., & Souchon, I. (2009). Reducing salt and fat content: impact of composition, texture and cognitive interactions on the perception of flavoured model cheeses. *Food Chemistry, 116*, 167–175.

Silletti, E., Vingerhoeds, M. H., van Aken, G. A., & Norde, W. (2008). Rheological behavior of food emulsions mixed with saliva: effect of oil content, salivary protein content, and saliva type. *Food Biophysics, 3*, 318–328.

Stewart, J. E., Feinle-Bisset, C., Golding, M., Delahunty, C., Clifton, P. M., & Keast, R. S. J. (2010). Oral sensitivity to fatty acids, food consumption and BMI in human subjects. *British Journal of Nutrition*. doi:10.1017/S0007114510000267.

Stokes, J. R., & Davies, G. A. (2007). Viscoelasticity of human whole saliva collected after acid and mechanical stimulation. *Biorheology, 44*, 141–160.

Szczesniak, A. S. (1963). Classification of textural characteristics. *Journal of Food Science, 28*, 385–389.

Taylor, A. J. (1996). Volatile flavor release from foods during eating. *Critical Reviews in Food Science and Nutrition, 36*, 765–784.

Voho, A., Chen, J., Kumar, M., Rao, M., & Wetmur, J. (2006). Lipase expression and activity in saliva in a healthy population. *Epidemiology, 17*, S336.