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Relationships between saliva and food bolus properties from model dairy products

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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

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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