SALIVA INCORPORATION DURING MEAT BOLI FORMATION IN RELATION TO CHEWING EFFICIENCY

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Background

Meat undergoes important structural changes during chewing (breakdown of fibers as well as saliva incorporation). During this transformation, sensory quality (texture and flavor) of meat is perceived, and determines the acceptability of the meat (Harris, 1972; Mathevon *et al*., 1995; Mathonière *et al*., 2000).

When swallowing, the properties of the boli make an important contribution to the overall acceptability of meat. It is therefore relevant to characterize these properties as well as the changes that occur in meat structure during chewing in relation to the initial properties of meat in order to get consumer-oriented clues in the understanding of meat acceptability. Cooked meat texture prior to chewing has been widely investigated and many mechanical measurements have been validated for its evaluation (Lepetit and Culioli, 1994; Culioli, 1995). Mechanical properties usually correlate with texture assessments performed during sensory analysis sessions (Bouton *et al.*, 1975; Boccard *et al.*, 1981). In contrast, properties of meat boli as affected by saliva incorporation are only sparsely documented. In a previous study (Mioche *et al*., 2002), saliva incorporation was evaluated by the variation of the weight of the samples before and after chewing. This method does not allow quantification of the meat juice lost under bite force. Therefore, methods to determine total saliva incorporation need to be developed in order to get a better estimation of net saliva incorporation along with the juice lost from the meat.

Objectives

This study aims to investigate 1) the relationship between saliva incorporation and juice release during chewing as well as 2) the relationship between saliva incorporation and the structure of the mat boli when swallowed.

Materials and methods

Subjects

Fifteen healthy elderly subjects with very different chewing efficiencies participated in the study; eight dentate (4 male, 4 female, 66.6 ± 3.1 years) and seven complete denture wearers (3 male, 4 female, 68.7 ± 5.9 years). The subjects were selected after dental examination. All dentate subjects had at least eight pairs of natural post-canines teeth. Denture-wearing subjects had been edentulous for at least five years. They wore full dentures with a correct interocclusal relationship and a satisfying prosthetic stability. Subjects felt comfortable with their dentures, which had been integrate for at least 6 months. All subjects declared to eat meat on a regular basis and all of them were able to chew the samples in the present study. All subjects were thoroughly informed and gave their consent. The protocol was approved by the Regional Ethical Committee.

Sample

Two different textures of beef meat were obtained from the muscles *Semimembranosus* of the same animal by combining different aging times and cooking temperatures ad modem (Mathevon *et al*., 1995). One muscle was aged for 3 days at 4°C and then fully cooked to 80°C (tough meat). The other was aged for 15 days at 4°C and then cooked to 65°C (tender meat). After cooking, meat was vacuum-packed, placed at -20°C (maximal storage 3 month) and then cut into cubes (5.1g ± 0.3). Just before using, the samples were thawed by immersing the packs in a 15°C water-bath for 1h.

Data acquisition:

1) Saliva collection

Subjects' saliva was collected with salivette (Sarstedt, Germany) in stimulated condition. Salivation was stimulated by chewing parafilm (American National Can, Chicago, Il., USA). Afterwards, two salivette were placed at the aperture of parotid ducts and the subjects were asked to chew a third one for 1 min. Salivette were frozen at –20°C until centrifugation at 1000g for 10 min and analysis. Saliva from three salivettes of each subject was then pooled.

2) Boli collection

Subjects were asked to chew a cold meat sample without swallowing, and to spit out the bolus when they felt that a swallow would normally be triggered. Textures were randomized among subjects and six replicates were analyzed for each texture giving twelve boli per subjects. Every four samples, the subjects were given a tender meat sample to eat in order to reset their natural chewing pattern. After collection, all boli were frozen at –20°C until analysis.

<u>Food bolus analysis</u>

Dry matter measurement

Possible losses of matter due to unexpected swallows were reported (Mishellany, Woda and Peyron, 2003). In order to evaluate the losses in meat, total dry matter of each bolus was controlled and compared to total dry matter of initial samples, calculated from cooked meat dry matter content and initial sample weight. Lower amounts of dry matter in boli than in cooked meat should reflect meat loss. For dry matter determination, samples were weighed before and after being placed in a 110°C oven for 24 hours.

Saliva incorporation measurements

Three methods were tested to evaluate saliva incorporation.

1) Weight increase: increase between the weight of meat samples and the weight of boli was determined weighing meat samples before and after chewing, which allowed saliva impregnation. To determine the losses of food matrix, boli weights were recalculated from losses evaluated by dry matter measurement.
2) Volume increase: increase in the volume of the sample was considered saliva incorporation. Volumes were measured immersing samples in water and measuring water volume increase using a graduated pipette with 0.2 ml accuracy. The volume was determined from five meat samples to obtain original meat density and from four frozen boli for each texture.
3) Marking of saliva: one bolus was collected for human secretory Immunoglobulin A (sIgA) analysis, using sIgA as a salivary marker. Boli were squeezed and the mix of meat juice and saliva was collected. The concentration of saliva in a sample was calculated using a human sIgA radial immuno-diffusion (RID) kit (The Binding Site, United Kingdom). Saliva collected with salivette was used as a reference and compared with values measured from the juice mix of saliva and meat in the boli. The quantity of sIgA in the boli was calculated from the sIgA rate in the boli obtained by RID and the quantity of the boli liquid phase obtained by dry matter measurement. From these values of sIgA in the boli and the rate of sIgA in saliva, the volume of saliva incorporated in boli was calculated.

Mechanical measurement

Samples used in this section were previously used for volume measurements. The mechanical properties of the food boli were measured by applying a shear test using a double-bladed cell with a displacement rate of 60 mm/min (Culioli, 1995). After thawing at room temperature, two boli per texture and per subject were gently placed into a U-shape mould (70 x 10 x 10 mm). Samples then had a section of 10 x 10 mm with a length depending on the bolus size. After removal from the mould, they were sheared. Three to five measurements were performed on the same bolus, 5 mm apart from each other, without any interference from one measure to another one. The maximum shear force was calculated from the force-distance curves and expressed as stress relative to the initial bolus section area. The replicates were performed to get information about structure homogeneity.

<u>Statistical Analysis</u>

Statistical analyses were carried out with SAS Analyst (SAS software, version 8.01, 1999 – SAS Institute Inc., NC, USA). To analyze correlations between variables, Pearson correlation coefficients were calculated. The SAS Mixed Model procedure was used to study the effect of dental status on saliva incorporation and on boli texture analyzed by shear tests.

Results and discussion

Total dry matter was determined in order to identify possible losses due to unexpected partial swallows. The texture of meat affected significantly the material losses, which varied from 5.6% to 12% of the initial sample weight for tender meat and tough meat, respectively. The losses were not significantly different between the two groups of subjects. This method allowed to highlight losses which were not identified in a previous study (Mioche *et al.*, 2002). In similar experiments performed with brittle foods, the losses could reach an average of 60 % of the initial sample weight (Mishellany, Woda and Peyron, 2003). Losses may be due to food thinning with saliva during mastication. These losses appeared to be out of subject control as the losses occurred for all subjects whatever the food product. Losses appeared to be related to the cohesiveness of the food, and therefore, their determination might be relevant for the acceptability of meat.

The variables describing saliva impregnation correlated with each dental status and each meat texture, except in dentate subjects chewing tough meat, where volume increase values did not correlate with the other variables. These correlations are illustrated in figure 1 showing correlations between values obtained by weight increase measurements and by the levels of sIgA on boli from tough meat. With tender meat, the Pearson coefficients of correlation were 0.796 ($p<0.05$) and 0.933 ($p<0.01$) for dentate subjects and denture wearers respectively. Variations in meat sample weight during chewing did not allow reliable separation of lost meat juice and saliva incorporation. Therefore, saliva intake during boli formation was underestimated. Volume measurements were equally underestimated, since this method did not allow quantifying the loss of meat juice, and the accuracy of the measurement was insufficient in terms of the volumes measured (from 5 to 12 ml). Using sIgA as a salivary marker permitted to determine juice losses but this method took longer and was more expensive to perform than the other two. However, the values obtained from the three methods were not significantly different.

Saliva incorporation does not vary along with food boli properties. No correlation was observed between shear stress and saliva incorporation values. Denture wearers produced boli that was less disorganized than that of the dentate subjects for each meat texture (figure 2) with the same amount of saliva. With tough meat, 5.167 ml (± 1.615ml) and 6.254 ml (± 2.832ml) of saliva was incorporated by dentate subjects and denture wearers, respectively. In this case, denture wearers characterized by reduced mastication efficiency, had their salivary flow stimulated by their denture (Veyrune and Mioche, 2000). Therefore, these values were not significantly different. However, meat texture induces large variations in the amount of saliva incorporated, tougher and dryer meat requiring more saliva than tender and juicy meat to trigger a swallow.

Conclusions

Evaluation of unexpected food matrix losses by controlling dry matter reduces the underestimation of saliva incorporation. Among the three methods tested to evaluate saliva impregnation in boli, the one using a salivary marker gave the highest estimation of saliva incorporation, but the values obtained were not significantly different from those obtained by measuring increases in weight and volume. Furthermore, it was observed that changes in chewing efficiency had a direct effect on the dynamics of meat bolus formation. With respect to the amount of saliva, the denture-wearers swallowed boli that was less disorganized to that of the dentate subjects. Therefore, saliva incorporation was not linked to the level of meat disorganization. This may suggest that a certain level of loose moisture is required to trigger a swallow.

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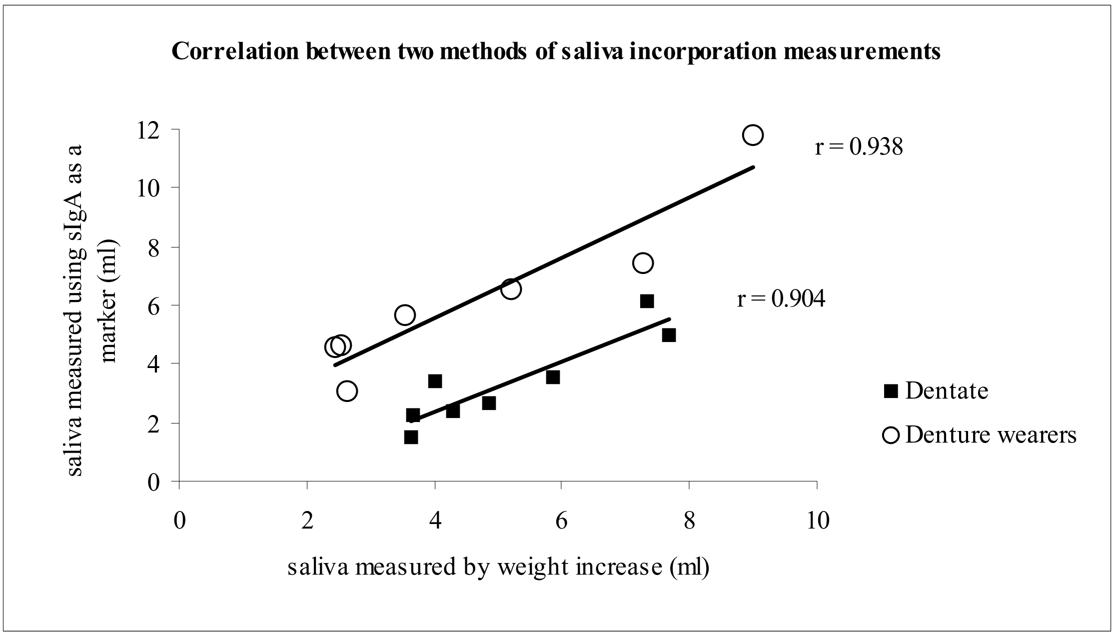

Figure 1. Correlation between the values of saliva incorporation obtained using human sIgA as a salivary marker and obtained by variations in meat sample weight corrected by food matrix losses, for each dental status, only values for tough meat are showed.

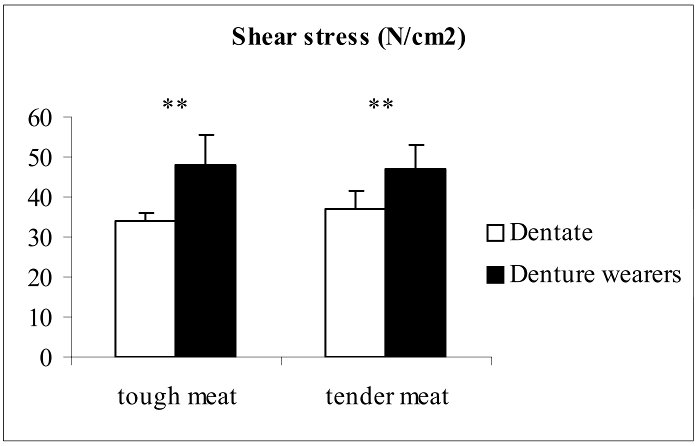

Figure 2. Variations in the shear resistance of meat boli with dental status