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Chewing behaviour and bolus formation during mastication of meat with different textures

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Summary During chewing, meat is mashed under compression and shear bite forces whilst saliva is incorporated. The resulting mixture is shaped into a cohesive bolus by agglomeration of small particles, and triggers a swallow. This study aimed to investigate the relationship between chewing behaviour and bolus formation of meat with different textures. Twenty-five consenting young adults participated in this study. Electromyographic activity was recorded from surface electrodes on the elevator muscles (masseter and temporalis) during mastication of cold beef. Two different textures (T_1: tough and dry; T_2: tender and juicy) were studied, and subjects were asked to chew the beef and then spit out the bolus either: (1) after a constant chewing period of 7 s or (2) when the bolus was ready to be swallowed. Meat samples were weighed before and after chewing to determine weight changes due to saliva incorporation and the release of meat juice. Cutting tests were applied to measure the maximum shear force. The mechanical shear force was maximal for meat before chewing ($T_1 = 124$ N/cm^2; $T_2 = 83$ N/cm^2) and decreased with increased chewing duration. Texture differences analysed from mechanical measurements remained significant even when the boli were ready for swallowing ($T_1 = 39$ N/cm^2; $T_2 = 32$ N/cm^2); the toughest meat gave the toughest bolus. Muscular activity adapted to the texture of the meat as soon as chewing began, and remained constant over the observed chewing period. Mean muscular activity was higher during the chewing of tough meat than during the chewing of tender meat. As a consequence, by the time a bolus was ready to be swallowed, more saliva had been incorporated into the tough meat samples (mean weight increase: 36%) than the tender meat samples (mean weight increase: 30%).

Introduction

Mastication is a complex sensory-motor activity whereby the ingested food is first transported to the post-canine teeth by the tongue and then processed into a bolus suitable for swallowing. This involves the breakdown of solid food into smaller particles, the incorporation of saliva, the agglomeration and shaping of the resulting mixture into a cohesive bolus, and finally the transport of the bolus to the pharynx. Food texture has been shown to affect various aspects of the masticatory process,[1—4] and the relationship between chewing activity and texture perception has been established.[5,6] The effect of texture on salivary flow has also been identified,[7—9] although the main effect could be due to the biting force developed during chewing.[10] Saliva participates in bolus formation by providing cohesiveness to food particles.[11,12] Models of bolus formation have been developed for brittle foods,[12,13] but the bolus properties of cohesive foods, such as

Abbreviations: EMG, electromyography; GLM, general linear model

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meat, are only documented sparsely.[14] It has been shown that food properties modify the transport duration of the bolus between the oral cavity and the oropharynx, but the duration of swallowing itself is not affected.[15]

Meat consumption represents the major source of protein intake in the western diet, and the acceptability of meat is driven by texture perception.[16] This perception is elaborated during the entire chewing sequence.[6] A better understanding of the mechanisms underlying texture perception requires identification of the relationship between the texture of meat before chewing and the properties of the food bolus. This study aimed to investigate bolus formation in relation to chewing behaviour during mastication of meat with different textures.

Materials and methods

Subjects

Twenty-five healthy human subjects (11 female, 14 male), aged 25–30 years, participated in this study. A full dental examination was performed and all subjects had at least eight pairs of natural post-canine teeth. All subjects gave informed consent and the protocol was approved by the regional ethics committee.

Preparation of meat samples

Meat samples of two different textures were obtained from the same muscle (semi-membranosus) by combining different aging times and cooking temperatures ad modem.[5] Half of the muscle was aged for 2 days at 4 °C and then cooked at 80 °C (Texture 1 (T_1)), and the other half was aged for 14 days at 4 °C and then cooked at 65 °C (Texture 2 (T_2)). After cooking, the meat was vacuum-packed, placed at −20 °C (maximum storage 3 months) and cut into cubes (ca. 2 cm × 2 cm × 1.5 cm). Just before use, the samples were thawed by immersing the packs in water at 15 °C for 1 h. The overall loss of juice corresponding with cooking and freezing was determined before chewing.

After these treatments, T_1 was tough and dry (maximum shear force = 124 N; 35% juice loss), and T_2 was tender and juicy (maximum shear force = 83 N; 25% juice loss) (see mechanical measurements below).

Food bolus analysis

Determination of saliva incorporation

The meat samples were weighed before and after chewing. After chewing, the variation in weight corresponded with loss of meat juice under bite force and saliva incorporation during chewing. Food boli were then frozen until mechanical measurements were carried out.

Determination of the mechanical properties of the boli

The mechanical properties of the boli were measured using a cutting test.[17,18] After thawing at room temperature, individual boli were gently placed into a U-shape mould (70 mm × 10 mm × 10 mm), and a 10 mm × 10 mm section was taken with length depending on the bolus size. After removal from the mould, a double-bladed shearing cell was used with a displacement rate of 60 mm/min (Fig. 1). Several measurements were performed on the same bolus 5 mm apart without interference between measurements. The maximum shear force was calculated from the force–distance curves and expressed as stress relative to the initial bolus area. Three to five replicates per bolus were performed to gain information about structure homogeneity.

Data acquisition

Subjects were asked to chew cold meat samples normally (at room temperature) and then to spit out the bolus at the point when swallowing would normally have been triggered. In addition, without

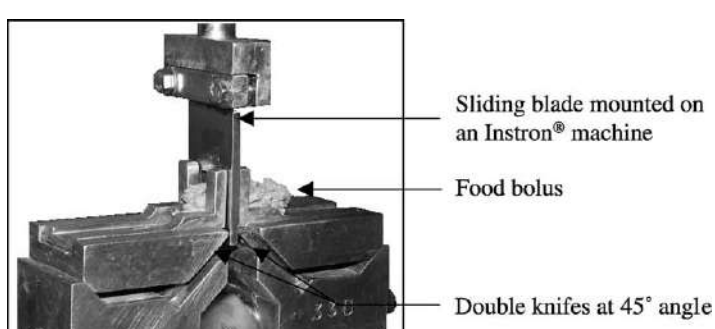

Figure 1 Cutting cell device used to measure shear stress of meat boli.

prior warning, subjects were asked to stop after a short chewing time (7 s). This allowed collection of boli formed over the same chewing period for all subjects. Texture order and chewing conditions were randomized among subjects, and two replicates were performed for each condition. In total, each subject produced eight boli.

Deglutition was monitored using a necklace strain gauge which provides a straight baseline during chewing; a variation in the baseline indicated when a swallow was triggered. In three of 200 recordings (25 subjects who each chewed eight meat samples), swallowing occurred within the chewing sequence, associated with a decrease in weight of the food bolus. These recordings were discarded.

Mastication recordings and analysis

The left and right superficial masseter and anterior temporalis muscles of each subject were located by palpation when subjects clenched their teeth. After careful cleaning of the overlying skin, two surface electrodes (Bionic, France) coated with conductive paste were fixed with adhesive, 2 cm apart, along the length of each muscle. An additional earth electrode was attached to the subject's ear lobe.

The electromyographic (EMG) signals were filtered (0—10 kHz) and amplified (500$\times$) using a digital amplifier (Grass Link 15, Grass Instruments, USA). An analogue/digital conversion was performed at 1000 Hz using a CED 1401 (Cambridge Electronic Design, Cambridge, UK). Data were collected using Spike2 software from CED.

After rectification of EMG signals, five variables were analysed for: (1) 7 s of chewing; and (2) chewing until the boli were ready to swallow:

(a) mean voltage of bursts;
(b) muscle work: sum of the integrated areas of all individual bursts of the sequence (expressed in mV/s);
(c) mean muscle work per chew (ratio between the total muscle work and the number of chews);

and, when assessing chewing sequences until the boli were ready to swallow:

(d) chewing time;
(e) number of bursts.

These values were collected from each of the four muscles and then averaged.

Statistical analysis

Statistical analysis was performed using SAS 6.07. The general linear model (GLM) procedure was used to study the effect of the different rheological factors on EMG variables. Subject effect nested to gender was used as the error term for all analyses. When the F ratio was significant, Student—Newman and Keuls tests were used to compare the differences of the means. Samples were designed to be the same weight, but they showed slight differences (4.7 ± 0.5 g). To avoid any bias due to sample weight, this was introduced as a co-variable in the GLM.

Results

Gender and subject effect

The effects of gender disparity and between-subject variability nested to gender are summarized in Table 1. Bolus properties and chewing variables were affected by gender, but mean muscle work was unaffected. Gender did not have a significant effect on salivation parameters. However, all variables expressed the same trend: females chewed for a shorter duration and developed less muscle activity (total muscle work) than males. Consequently, when ready for swallowing, the bolus texture was less comminuted (higher stress value) and less homogenous (higher standard deviation).

Subject effect (nested to gender) was significant for all chewing and salivation variables, but subject variability decreased for mechanical properties of the bolus.

Our protocol was designed to have a constant initial sample weight. However, slight variations in weight were observed due to difficulties in shaping the samples. Therefore, we introduced the initial sample weight as a co-variable in our GLM procedure. This calculation allowed the subsequent statistical analysis to be free from this effect. Interestingly, we found that the initial sample weight only had a significant effect on the mean muscular work per burst ($F = 10.4$, $P < 0.001$) and on mechanical shear force by time unit ($F = 7.48$, $P < 0.01$). The initial weight of the sample had no significant effect on salivation.

Influence of meat texture on bolus properties

Meat texture was transformed during mastication. Fibres were broken down progressively, and saliva and air were incorporated. These aspects contributed to a visible increase in volume although it was difficult to quantify (Fig. 2). The mechanical shear stress characterizes the resistance of the fibres under shear forces. These values were maximal for meat before chewing and decreased with increased chewing duration. The difference

Table 1 Results from the general linear model for the three groups of studied variables for gender effect and subject effect nested to gender (mean ± S.E.M.).

Variables	Gender effect				Subject effect	
	Male	Female	*F*	*P*	*F*	*P*
Salivation						
Weight bolus increase (g)	1.28 ± 0.11	0.93 ± 0.095	2.5	ns	14.98	***
Salivary/flux (g/s)	0.07 ± 0.004	0.07 ± 0.004	0.15	ns	19.57	***
Food bolus mechanical properties						
Shear stress (N/cm^2)	43.29 ± 2.01	52.64 ± 2.29	6.15	*	2.18	*
Shear stress per time unit ($N/cm^2/s$)	4.57 ± 0.37	5.76 ± 0.44	6.57	*	4.44	***
Number of chews	29.9 ± 2.26	20.8 ± 1.46	6.9	*	8.49	***
Chewing parameters						
Chewing duration (s)	19.63 ± 1.63	14.37 ± 1.14	4.02	*	7.45	***
Mean muscle work (mV/s)	0.18 ± 0.006	0.15 ± 0.007	1.33	ns	39.46	***
Total muscle work (mV/s)	5.24 ± 0.46	3.01 ± 0.27	6.11	*	14.64	***

* $P < 0.05$.
*** $P < 0.001$.

between the two textures was highly significant before chewing and after chewing for 7 s ($F = 22$, $P < 0.001$). When the boli were ready to swallow, the difference between the two meat textures was reduced (Fig. 3), but the shear stress remained significantly different ($F = 6.03$, $P < 0.05$). The tougher meat gave a tougher bolus even after a longer chewing period.

Influence of texture on chewing activity and salivary flow

Fig. 4 shows progression of mean voltage, averaged over the 25 subjects, during the first 11 bursts regardless of chewing duration. The average was calculated from two replicates (when the boli were spat out when ready to swallow) for all subjects. After 11 bursts, some subjects had already stopped chewing, and therefore, the average could not be further calculated from the same recordings. From the curves, a clear texture effect was visible from the first chew onwards. A higher mean voltage was elicited when biting on the tougher meat. The mean voltage increased from the first to the third chew and reached a plateau in a fairly parallel way for both textures. The difference in voltage remained fairly constant over the observed chewing period. When averaged for each cycle of the chewing sequence, this variable gave the highest texture discrimination (highest *F* value, Table 2).

Texture was also found to have a significant effect on other chewing variables and, consequently, on

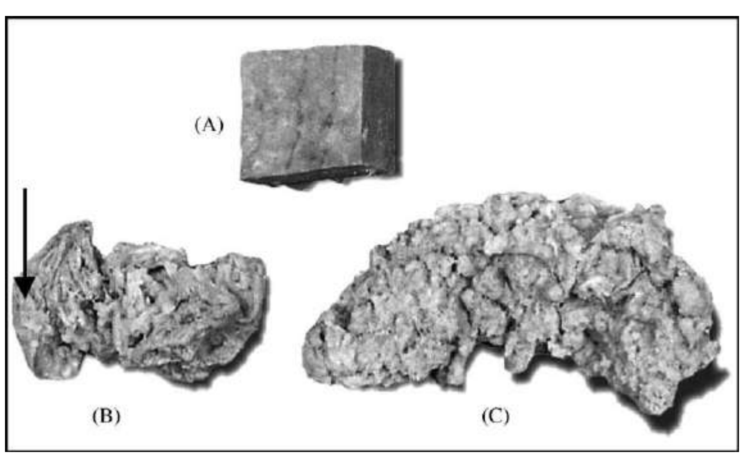

Figure 2 Meat sample and food bolus in the three stages studied: (A) a sample of 5 g before chewing; (B) a sample chewed by the same subject after 7 s; and (C) a sample when ready to be swallowed (30 s chewing). Both the shape and the volume changed during chewing with a large increase of the exposed surface. The meat was mashed and flattened, and saliva was incorporated. After 7 s of chewing, some fibres were still intact (arrow) but other parts were already well comminuted. When ready to be swallowed, the meat bolus looked like shaped minced meat.

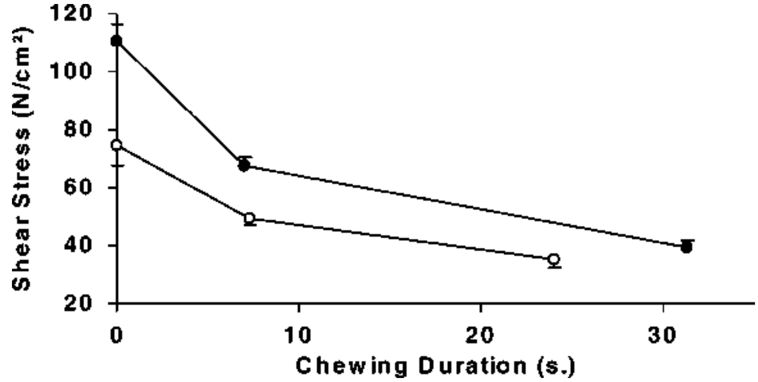

Figure 3 Mean mechanical shear stress in terms of the chewing duration. Texture 1, black dots; Texture 2, white dots. Vertical bars indicate the standard error of the mean (S.E.M.) for meat before chewing ($n = 8$), for boli gathered after 7 s of chewing ($n = 50$: two replicates × 25 subjects), and for boli that were ready to be swallowed ($n = 50$).

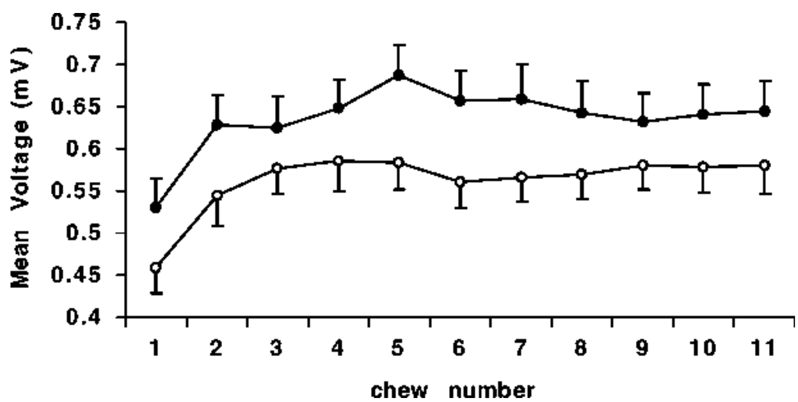

Figure 4 Mean muscle work per burst for the first 11 cycles. Texture 1, black dots; Texture 2, white dots. Vertical bars indicate S.E.M. ($n = 50$: two replicates × 25 subjects).

Table 2 Results from the general linear model for the three groups of studied variables for the texture effect tested using subject effect nested to gender as error term.

Variables	Texture effect			
	T_1	T_2	F	P
Salivation				
Weight bolus increase (g)	1.925 ± 0.18	1.467 ± 0.14	11.47	**
Salivary/flux (g/s)	0.064 ± 0.005	0.065 ± 0.006	0.02	ns
Food bolus mechanical properties				
Shear stress (N/cm^2)	39.486 ± 2.11	32.257 ± 2.28	6.03	*
Shear stress per time unit (N/cm^2/s)	5.7 ± 0.17	4.5 ± 0.25	4.67	*
Chewing parameters				
Number of chews	45.122 ± 3.07	36.020 ± 2.6	13.78	**
Chewing duration (s)	31.226 ± 2.29	23.991 ± 1.97	11.03	**
Mean muscle work (mV/s)	0.170 ± 0.008	0.144 ± 0.007	14.64	***
Total muscle work (mV/s)	7.964 ± 0.78	5.315 ± 0.53	13.41	**

Variables were calculated from chewing sequences before swallowing (mean ± S.E.M.).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

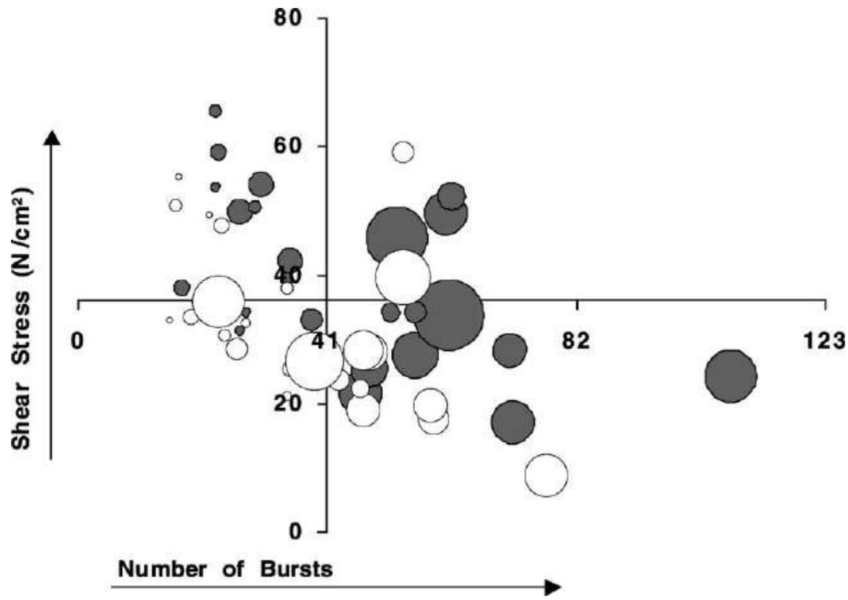

Figure 5 A three-dimensional representation of the relationship between mechanical properties (shear stress), salivation (bolus weight increase) and chewing activity (number of bursts before swallowing). The increase in bolus weight during chewing is represented by the diameter of the dot (Texture 1, gray dots; Texture 2, white dots). Axes cut on mean values and define four quadrants. The upper left quadrant shows boli that were ready to swallow after a small number of chewing cycles, with only a small quantity of saliva incorporated and with a low level of comminution (higher shear stress values). In contrast, by the time the boli grouped in the lower right quadrant were ready to be swallowed, more saliva had been incorporated and they were well comminuted (lower shear stress) after more chewing cycles than boli in the diagonally opposite quadrant.

the total amount of saliva secreted. Significantly more saliva was incorporated into the tough, dry meat (T_1) than into the tender, juicy meat (T_2). When ready for swallowing, the increase in bolus weight varied, depending on subjects, from 9.8 to 85% (mean: 36%) for the tough meat and from 3.8 to 76% (mean: 30%) for the tender meat. However, when divided by the chewing time (to express the salivary flow), no significant texture effect was observed (Table 2). In addition, good correlation was found between salivation (bolus weight increase) and total muscle work (T_1: $r = 0.73$, $P < 0.001$; T_2: $r = 0.59$, $P < 0.001$).

The relationship between the mechanical properties of a bolus (shear stress), salivation (bolus weight increase) and chewing activity (number of bursts before swallowing) is summarized in Fig. 5. The greater the degree of comminution of the meat (lower shear stress), the more saliva was incorporated into the bolus (larger dots) and more bursts were used to prepare the bolus for swallowing.

Discussion

These results provide new information to facilitate understanding of the chewing process during meat mastication and bolus formation. During 'natural' chewing, swallows are basically triggered under reflex control and, for most of the time, the chewing of solid food induces multiple swallows.[2] It is possible to delay a swallow consciously provided that the bolus remains anterior to the faucial pillars. This probably limits the tongue selection function that separates the already comminuted particles from the rest of the bolus. Nevertheless, the chewing duration obtained in this study was within the range of chewing durations obtained in a previous study with similar pieces of meat.[6]

Meat texture, specifically tenderness and juiciness, is a determinant factor of its acceptability.[16] These two parameters were controlled in this study by modifying the aging and cooking temperature. Mastication pattern and, more specifically, muscular activity from jaw elevator muscles, were found to be well adapted to the texture of the chewed material.[3,4] The present results clarify the adaptation of muscular activity within the chewing sequence. During the first bite, a clear texture effect was found from the muscle activity elicited during the closing phase. This clearly confirms Ottenhoff et al.'s[19] results showing that muscle activity developed to overcome food resistance started about 20 ms after tooth—food contact. This delay is compatible with an adaptation of the elevator muscles' activity to food texture occurring within the first stroke.

However, meat tenderness is not usually assessed from the first bite but depends on sensations experienced by the consumer while it is being transformed in the oral cavity.[6] Mechanical properties are well identified for intact meat but the relationship between the meat texture before chewing and its properties when transformed into a bolus during chewing are not documented. This study characterizes some meat texture transformations in relation to chewing activity.

One of the most marked results of this study is that texture differences, identified from intact meat, are evident to the last swallow. The toughest meat gives the toughest bolus, despite being chewed with more strokes, more work being done, and more salivary uptake.

Measuring the shear forces at several points of the bolus appeared to be a relevant method to assess chewing efficiency of a cohesive, fibrous food such as meat. The dynamics of fragmentation and bolus properties in terms of particle size and cohesiveness have been analysed with brittle products,[20,21] but these models are not suitable for a cohesive bolus such as meat. During chewing of meat, a combination of tongue and cheek activity maintain the food's position on the occlusal plane using a combination of rhythmic tongue-pushing and cheek-pushing.[22] This movement of the food ensures that different parts of the sample undergo occlusal forces in successive cycles. This progressive comminution of a meat sample explains why samples still have some intact fibres after 7 s of chewing.

Two thresholds were described to trigger a swallow: food particle size and lubrication threshold.[13] These two types of threshold may be not achieved simultaneously in the same food.[23] We found large variations in the way our subjects dealt with bolus properties. Some subjects were able to swallow a tough bolus with little saliva incorporated, and cohesiveness was due to weakly comminuted meat fibres. In contrast, other subjects swallowed well-comminuted boli with more saliva incorporated.

This study was performed with meat samples differing in toughness and juiciness. The tougher meat was also drier and our protocol did not allow separate analyses of variations in juice release and saliva incorporation. The actual secreted saliva is thus under-reported. The specific role played by juice and saliva in bolus formation is not clear. Juice is thought to interfere with viscous properties of saliva by lengthening bolus formation. This can explain the weakness of the relationship between food toughness and saliva flow found in this study. Indirect evidence suggests a stronger relationship. Indeed, during chewing, parotid saliva flow increases with EMG activity of the ipsilateral muscles,[10] itself closely related to food toughness.[3–6] In denture wearers, juiciness of meat was found to be more important than tenderness in terms of acceptability, suggesting that juicy meat promotes swallowing of poorly comminuted boli in subjects with low chewing efficiency.[24]

Increased chewing duration of a meat sample lowers its mechanical strength and increases the quantity of saliva that is incorporated. These results fit perfectly with one of the major roles of saliva, namely to provide cohesion between particles.[12] Our results also show that the more comminuted the bolus after longer chewing duration and/or higher bite force, the greater the saliva generation and incorporation prior to swallowing.

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