CHAPTER 9

CHEWING BEHAVIOR AND SALIVA SECRETION

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European Journal of Oral Sciences 112: 19-24, 2004

European Journal of
Oral Sciences
ISSN 0909-8836

Eur J Oral Sci 2004; 19-24

ABSTRACT

We determined the salivary flow rate in 16 healthy subjects in rest and while chewing artificial and natural foods (Parafilm, Melba toast with and without margarine, and three different volumes of breakfast cake and cheese). We also determined the duration of a chewing cycle, the number of chewing cycles until swallowing, and the time until swallowing. The physical characteristics of the foods were quantified from force-deformation experiments. The flow rates of the saliva as obtained without stimulation, with Parafilm stimulation, and with chewing on the various foods were significantly correlated. An increase in chewing cycle duration, number of chewing cycles until swallowing, and time until swallowing was observed as a function of the volume of the food. More chewing cycles were required for Melba toast than for an equal volume of cake or cheese. This may be caused by the low water and fat percentage of the Melba toast. The number of chewing cycles and the time until swallowing significantly decreased when the Melba toast was buttered, which may be caused by a facilitation in bolus formation and lubrication of the food. The number of chewing cycles until swallowing was not correlated to the salivary flow rate.

INTRODUCTION

Chewing is the first step in the process of digestion and is meant to prepare the food for swallowing and further processing in the digestive system. During chewing, the food bolus or food particles are reduced in size and saliva is secreted to moisten and lubricate the food. The urge to swallow the food could be triggered by a threshold level in both food particle size and lubrication of the food bolus (1-3).

Subjects with a reduced masticatory performance, due to an inadequate dentition, need more chewing cycles to prepare the food for swallowing than those with a good performance (4-6). Furthermore, they swallow larger food particles (6;7). Thus, subjects with an inadequate dentition compensate for their reduced chewing performance by chewing for a longer period of time and by swallowing larger food particles.

The production of sufficient saliva is indispensable for good chewing. The water in saliva moistens the food particles, whereas the salivary mucins bind masticated food into a coherent and slippery bolus that can be easily swallowed (8). It has been suggested that the swallowing process initiates when the cohesive forces that bind food particles together into a bolus are strongest (3). The important role of saliva for chewing and swallowing is demonstrated by the finding that the number of chewing strokes, hence time in the mouth, needed for swallowing significantly increases after experimentally induced oral dryness (9). Additionally, significantly more saliva is required for oral manipulation of powdered crisp bread than for pieces of crisp bread (10) as the larger surface area of the powder requires more saliva for lubrication and cohesive binding in preparation for deglutition. In a study on rabbits, it was demonstrated that greater amounts of saliva were produced for dry food than for moist food (11). The amount of saliva also plays a role in the chewing of meat, with more saliva being incorporated into a food bolus of tough meat, than into tender meat before the bolus is swallowed (12).

While saliva and chewing have been shown to be interrelated, the relationship between amount of saliva and mastication has not been studied extensively (13). During mastication it is likely that mechanoreceptors in the gingival tissues will be stimulated which may result in salivary flow (14;15). At chewing forces as low as 5% of comfortable chewing forces the masticatory-salivary reflex could already be elicited (14).

The aim of the present study was to investigate the influence of the salivary flow rate on the chewing process. We determined whole saliva flow rates under various conditions: unstimulated and stimulated by chewing artificial and various natural foods. Furthermore, we determined the number of chewing cycles and the time needed to prepare various volumes of food for swallowing. In order to relate amount of saliva not only to volumes of food, but also to physical characteristics of the foods, force-deformation experiments were performed. Widely different types of food were included in the study; dry and crisp Melba toast, sweet and moist cake and fat cheese.

MATERIALS AND METHODS

Subjects

Sixteen healthy subjects (8 males and 8 females) participated in the study. Their age ranged between 16 and 60 years (mean 35 ± 13 years). They all had a natural dentition at least up to the second molars without evident defect of dental structures, periodontal conditions or severe malocclusion. The Ethics Committee of the University Medical Center Utrecht approved the protocol. Written informed consent was obtained from each subject after a full explanation of the experiment.

Test foods

We used the following natural foods: toast (Melba toast, Buitoni, Italy, www.buitoni.com; diameter 5.0 cm, thickness 0.4 cm and volume 7.9 cm^3) with 2 g of margarine spread on one surface (Linera, Unilever, the Netherlands, www.unilever.nl), toast without margarine, three differently sized blocks of breakfast cake (Right, Peijnenburg, the Netherlands, www.right.nl; 9.2, 14.0, and 20.0 cm^3), and of aged Gouda cheese (3.0, 6.0, and 9.0 cm^3). The 3 volumes will be referred to as small, medium and large portions. Table 1 shows the characteristics of these foods. The physical properties of the food samples were tested by crushing the food in a pneumatic bite simulator. This apparatus consists of a probe attached to a pneumatic cylinder. The probe has a conical cusp with a slope of 120 degrees (16). The position of the probe during crushing was monitored by a linear variable differential transformer and the velocity was 1 mm/s. Force-deformation curves were obtained by plotting the data points of the force as a function of the percentage deformation of the food samples. From these curves, the forces and compression percentages were obtained at the yield point. Six samples of each food were measured.

Saliva collection

Saliva samples were collected in 3 different ways: unstimulated, mechanically stimulated, and food stimulated. Firstly, we collected unstimulated saliva to determine a "baseline" flow rate (17). Secondly, stimulated saliva was obtained by chewing on a piece of tasteless Parafilm (0.29 g; Parafilm "M"®, American National Can™, Chicago, IL, USA). Unstimulated and mechanically stimulated saliva were collected over a period of 5 min. Before collection, the mouth was emptied by an initial swallow. At 30-s intervals saliva was expectorated into pre-weighed containers and flow rates (ml/min) were calculated. The weight of saliva in grams was assumed to equal the millilitres of saliva secreted, because the specific density of saliva is close to 1.0 (18). Finally, saliva was obtained by chewing on the various natural foods. Before the experiments, all foods were brought to room temperature (20 ^{0}C). Margarine was stored at 4 ^{0}C. We assumed that the saliva produced equals the difference between the weight of the served food and the weight of chewed food that is collected when the subjects are ready to swallow (19). The natural test foods were given to the subjects in a predetermined sequence. The subjects were asked to chew the food in their usual manner until they wanted to swallow. Instead of swallowing they spat out the food bolus into a pre-weighed container. Prior to the experiments, it was emphasised that all chewed material needed to be recovered. Subjects were instructed to clean their mouths with tongue and cheeks while spitting into the pre-weighed containers and a probe was used to facilitate the removal of trapped particles. Tests were performed twice. In between the stimuli, subjects were allowed to sip water.

The volume of saliva was determined by subtracting the initial weight of the food from that of the food/saliva mixture. For each food, salivary flow rate was calculated as the volume of saliva secreted, divided by the time the food was in the mouth (ml/min). All samples were collected during the morning, since salivary flow rate shows a circadian rhythm (20;21). In addition, the amount of saliva secreted per gram of food was calculated (ml/g).

Chewing cycles

Masticatory mandibular movements were recorded by an optoelectronic device (Northern Digital Optotrak™; www.ndigital.com) during the chewing of natural test foods, in order to identify individual chewing cycles. The device tracks the 3-dimensional position of two small infrared light emitting diodes (LED's) that were attached to the mandible and the head. By comparing their positions we obtained the movement of the mandible with respect to the head. From the generated plots we determined the number of chewing cycles until the individuals were ready to swallow (swallowing threshold), as well as the average time of each chewing sequence.

Statistical analysis

Repeated-measures analysis of variance (ANOVA; SPSS 9.0; SPSS, Chicago, IL, USA) was applied to test the null hypothesis that there would be no statistical difference among the results obtained for the various food types and volumes. Subsequently, contrasts were determined to study the levels of the within-subjects factors (food type and volume). Furthermore, we tested whether the volume of the food caused a linear increase in saliva flow rate and chewing variables. Pearson correlations were calculated to quantify relationships among the unstimulated and parafilm stimulated saliva flow rates and the flow rates obtained for the various foods. We also tested possible relationships among the salivary flow rate obtained from chewing on a food and the number of chewing cycles needed to prepare that food for swallowing. Equal volumes of the natural foods (melba toast, small portion of cake, and large portion of cheese) were compared for cycle duration, number of chewing cycles and time until swallowing with repeated measures ANOVA.

RESULTS

The means and standard deviations of the force and deformation at the yield point of the various foods are presented in Table 1.

Table 1. Food characteristics

	Density (g cm^{-3})	Water (%)	Fat (%)	Yield point*	
				Force (N)	Deformation (%)
Melba toast	0.38	5	4.7	16.3 (1.3)	14.0 (4.5)
Breakfast cake	0.59	18	10.2	1.86 (0.24)	27.8 (6.6)
Cheese	1.08	35	31	4.90 (0.88)	20.5 (1.5)

*Means and standard deviations obtained from six measurements

Flow rates and amounts of saliva

Table 2 presents the average values for the flow rates of the saliva as obtained for the various foods. The salivary flow rates for cheese are not presented: these results were unreliable because the cheese could not be fully recovered after chewing as the cheese sticks to the teeth. This factor could even lead to calculated negative values for the salivary flow rate. Repeated-measures ANOVA on the saliva flow rates showed a significant effect for the type of saliva stimulation ($P < 0.001$). Contrast analysis showed that the flow rate obtained without stimulation was significantly lower ($P < 0.001$) than the flow rate obtained from chewing on Parafilm, whereas the flow rate obtained with Parafilm was significantly lower ($P < 0.001$) than the flow rates obtained from chewing food. No significant differences in flow rates were observed among the various foods and volumes. The flow rates of the saliva as obtained without stimulation, with Parafilm stimulation, and with chewing on the various foods were significantly correlated (Table 3). Saliva secreted per gram of food differed significantly among the foods, with toast eliciting the highest levels, followed by toast with margarine, and cake (Table 2). Less saliva per gram was observed for larger volumes of cake.

Table 2. Saliva secretion in response to different foods*

	Unstimulated	Parafilm	Toast	Toast with margarine	Cake (small)	Cake (medium)	Cake (large)
Saliva flow rate (ml min^{-1})	0.53[a] (0.28)	1.40[b] (0.67)	8.64[c] (5.06)	7.74[c] (4.97)	7.97[c] (5.02)	7.32[c] (3.97)	7.42[c] (3.61)
Saliva per gram (ml g^{-1})			1.07[d] (0.53)	0.87[c] (0.54)	0.40[b] (0.23)	0.33[a] (0.21)	0.32[a] (0.17)

* Means and standard deviations (in parenthesis) obtained from 16 subjects. Values with different superscript letters on a horizontal line are significantly different, where the letter a has the lowest value. ($p < 0.05$).

Table 3. Matrix of Pearson correlations between saliva flow rates obtained with various ways of stimulation for 16 subjects*

	1	2	3	4	5	6	7
1. Unstimulated	-						
2. Parafilm	0.74^b	-					
3. Toast	0.57^a	0.77^c	-				
4. Toast plus margarine	0.72^b	0.71^b	0.74^b	-			
5. Cake (small)	0.50^a	0.69^b	0.87^c	0.81^c	-		
6. Cake (medium)	0.71^b	0.63^b	0.66^b	0.92^c	0.81^c	-	
7. Cake (large)	0.66^b	0.66^b	0.69^b	0.89^c	0.89^c	0.94^c	-

* Superscript letters (two-sided tests): a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$

Chewing characteristics

The average duration of a chewing cycle, the number of chewing cycles and the time until swallowing for the various foods and volumes are presented in Table 4. Repeated-measures ANOVA showed that the duration of a chewing cycle linearly increased as a function of the volume of the food for both cake ($P = 0.003$) and cheese ($P = 0.002$). The cycle duration increased on average by 5 ms (cake) and 12 ms (cheese) for every additional cm^3 of food. Additionally, the number of chewing cycles and the time until swallowing linearly increased with the volume of food that was chewed for both cake ($P < 0.001$) and cheese ($P < 0.001$). On average 1.7 (cake) and 2.1 (cheese) extra chewing cycles were needed for every additional cm^3 of food, whereas the additional chewing time was 1.2 s (cake) and 1.6 s (cheese). Among the 3 foods with equal volumes (melba toast, small portion of cake, and large portion of cheese), we observed significant differences in cycle duration ($P < 0.001$). The cycle duration for cake was smaller than for toast, which was smaller than for cheese. We also observed significant larger values for the number of chewing cycles and time until swallowing for melba toast as compared to cake and cheese. No differences in number and time existed between cake and cheese. The number of chewing cycles and the time until swallowing significantly decreased when the melba toast was buttered ($P < 0.02$).

No significant correlations were observed between the salivary flow rate obtained from chewing on a food and the number of chewing cycles needed to prepare that food for swallowing.

Table 4. Average duration of a chewing cycle, number of chewing cycles, and time until swallowing for the various foods*

	Toast	Toast with margarine	Cake (small)	Cake (medium)	Cake (large)	Cheese (small)	Cheese (medium)	Cheese (large)
Cycle duration (s)	0.64[b] (0.08)	0.64[b] (0.09)	0.61[a] (0.08)	0.62[a,b] (0.07)	0.67[b] (0.10)	0.64[b] (0.07)	0.69[c] (0.10)	0.71[c] (0.11)
Number of cycles	37.6[e] (9.9)	32.4[d] (7.2)	28.4[c] (7.3)	36.9[e] (9.8)	46.4[f] (10.5)	14.4[a] (3.6)	21.8[b] (6.3)	27.0[c] (7.0)
Chewing time (s)	23.8[e] (5.8)	20.5[d] (4.5)	17.4[c] (5.2)	23.0[e] (6.5)	30.7[f] (7.2)	9.3[a] (2.6)	14.7[b] (3.9)	18.9[c,d] (4.5)

* Means and standard deviations (in parenthesis) obtained from 16 subjects. Values with different superscript letters on a horizontal line are significantly different, where the letter a has the lowest value. ($p < 0.05$).

DISCUSSION

The major functions of the oral phase in response to a meal are the breakage of food into smaller particles by chewing and the addition of saliva, so that a food bolus is produced that can be swallowed. Saliva plays a role in taste sensation, bolus formation and digestion of starch and lipids (8;22;23). We measured whole saliva rather than that of an individual gland, because whole saliva is easy to collect, causes the subjects less discomfort during collection, is readily measurable, and better represents the oral environment (13;24). Whole saliva is a combination of secretions from the submandibular, sublingual, parotid and minor glands. The composition of saliva varies and depends on the type of gland that produces it. Submandibular and sublingual saliva owes its mucous character to the relatively high levels of mucins. These mucins exhibit diverse functions in saliva, among others protection against pathogens (25), dehydration (26), and perhaps more important in this study, lubrication (27). Parotid saliva, on the other hand is practically devoid of mucins and therefore highly serous. It contains high levels of amylase, the enzyme initiating the breakdown of starch in the mouth.

The unstimulated and stimulated saliva flow rates we found (Table 2) were similar to previously reported flow rates (19;28-31). The response to chewing Parafilm was a threefold increase in the salivary flow rate compared with the unstimulated level. The salivary flow rates observed when eating (un)buttered melba toast and 3 volumes of cake ranged between 7.42 and 8.64 ml/min (Table 2). We observed no significant differences among these values. A higher salivary flow rate might have been expected for melba toast as higher bite forces are needed to fragment the toast as it has a higher yield force than the other foods (Table 1) and masticatory force has been reported to influence salivary flow (11;32). However, the higher bite forces are probably only present in the beginning of the chewing process as the toast will be softened by the saliva after a few chewing strokes. Furthermore, a harder product is also chewed for a longer time before deglutition and the salivary flow rate tends to decrease over the chewing sequence (33;34). This may counteract the effect of the higher bite forces on salivary flow rate. Due to retention of food in the mouth and inadvertent swallowing, the salivary flow rate will be slightly underestimated (18;19). However, in our study inadvertent swallowing seldom occurred as could be seen from the movement signal of the lower jaw. The flow rates elicited in response to chewing natural foods in our study concur with flow rates reported in previous studies: *e.g.* 3.4 ml/min for chewing gum (18), 6.7 ml/min for rhubarb pie (19), 4.8 ml/min for apple (29), 6.3 – 8.3 ml/min for cookies (33), and 1.8 – 6.9 ml/min for cheese (35). The relatively high salivary flow rates that we observed, may be attributed to the type of food we used. Both melba toast and cake are dry products (Table 1). These products need more saliva in order to moisten the food and form a food bolus that can be swallowed. Indeed, in a study on rabbits, higher salivary flow rates were observed for dry food (dry pellets) than for moist food (pieces of carrot) (11).

The salivary flow rates observed for the natural foods are much higher than for chewing Parafilm. Parafilm is an inert and tasteless material, so it does not cause gustatory secretory stimulation. The effect of gustatory stimulation of foods has been found to be more important than the mechanical stimulation of chewing for the saliva flow rate (19;36). Hence this may explain the much lower flow rate when chewing on parafilm than on natural foods. Furthermore, the Parafilm was chewed for a longer time (5 min) than the natural foods (30 s or less; Table 4). This may lead to lower flow rates as there is evidence of a reduced flow rate with prolonged chewing (33;34).

Significant correlations were observed between the unstimulated flow rate, the stimulated flow rate, and the flow rates elicited by the natural foods (Table 3). Thus, determining the saliva flow rate from either unstimulated chewing or chewing on Parafilm is as good a method for obtaining an indication of the salivary flow as determining the flow rate from natural foods. The Parafilm method may then be preferred, because it is the easiest and cleanest way of obtaining an adequate amount of saliva.

As stated above, we observed no significant differences among the amounts of saliva secreted per minute for the various foods and volumes. In contrast, the amount of saliva secreted per gram of product differed significantly among the different types of foods. Melba toast elicited the highest levels, followed by buttered melba toast and cake. As melba toast contains the lowest percentage of water and fat, this is evidence that a dry product needs more saliva to moisten and form a cohesive bolus suitable for swallowing which is in accordance with previous research (11). As the saliva secretion per minute was not influenced by the various foods, a dry product thus needs a longer time in the mouth to allow for enough secretion of saliva. Our results confirm a previous finding that the chewing time per weight of food is inversely related to the water content of the food (29).

We observed that the average duration of a chewing cycle increased as a function of the volume of the food. Apparently, bolus formation and size reduction of the food during a chewing cycle take more time for larger food volumes. Furthermore, the number of chewing cycles increased with volume. Equal volumes of cake and cheese were swallowed after the same number of chewing cycles on average. However, an equal volume of melba toast needed more chewing cycles to prepare for swallowing. This may be caused by the very low water and fat percentage of the toast. More saliva may be needed to obtain a food bolus that can be swallowed, and thus more chewing cycles are required. Indeed, the average number of chewing cycles needed before swallowing toast significantly decreased when the toast had 2 g of margarine on it. The margarine facilitates bolus formation and lubricates the food, which makes it easier to swallow.

No significant correlations were observed between the salivary flow rate, while chewing on a food and the number of chewing cycles needed to prepare that food for swallowing. Although large differences in flow rate among subjects are present, as can be seen from the rather large standard deviations (Table 2), these differences do not lead to corresponding differences in the number of chewing strokes. Apparently, subjects are used to their respective amounts of saliva, so that swallowing threshold is not influenced by a subject's amount of saliva. Thus, a subject with a relative large salivary flow does not necessarily swallow the food after a relative small number of chewing cycles. In a recently reported study, individual salivary flow rates did not influence sensory ratings (37). The absence of correlation between flow rate and sensory ratings was explained by the assumption that all the subjects are used to their amounts of saliva and have their own reference, probably a result of experience.

The present data shows an increase in cycle duration and number of chewing cycles until swallowing as a function of the volume of the food. Furthermore, we observed a larger number of chewing cycles until swallowing for foods with less water and fat. A dry product needs a longer time in the mouth to allow for enough secretion of saliva for the formation of a food bolus that can be swallowed, because the salivary flow rate (ml/min) was not larger for a product with less water and fat.

ACKNOWLEDGEMENTS

This work was supported by the University Medical Center Utrecht, the Netherlands Institute for Dental Sciences, the University of Campinas, and the State of São Paulo Research Foundation (FAPESP). We are grateful to Dr. F.A. Fontijn-Tekamp for her valuable comments on the manuscript, and to Dr. M. Schipper (Department of Biostatistics, Utrecht University) for her advice on the statistical analysis.

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