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Physical and chemical transformations of cereal food during oral digestion in human subjects

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Chemical and physical transformations of solid food begin in the mouth, but the oral phase of digestion has rarely been studied. In the present study, twelve healthy volunteers masticated mouthfuls of either bread or spaghetti for a physiologically-determined time, and the levels of particle degradation and starch digestion before swallowing were compared for each food. The amounts of saliva moistening bread and spaghetti before swallowing were, respectively, 220 (SEM 12) v. 39 (SEM 6) g/kg fresh matter. Particle size reduction also differed since bread particles were highly degraded, showing a loss of structure, whereas spaghetti retained its physical structure, with rough and incomplete reduction of particle size. Starch hydrolysis was twice as high for bread as for spaghetti, mainly because of the release of high-molecular-mass α -glucans. The production of oligosaccharides was similar after mastication of the two foods, respectively 125 (SEM 8) and 92 (SEM 7) g/kg total starch. Starch hydrolysis, which clearly began in the mouth, depended on the initial structure of the food, as in the breakdown of solid food. These significant physical and chemical degradations of solid foods during oral digestion may influence the entire digestive process.

Mastication: Oral digestion: Starch: Salivary amylase

The digestion of food in man depends on both the chemical and physical characteristics of the food and their changes during the different steps of digestion (Björck *et al.* 1994). The mouth phase provides the first step in this process since food degradation results from two simultaneous actions: mechanical grinding and enzymic hydrolysis. During mastication, food is, to a greater or lesser extent, triturated and broken down into small particles. This mechanical grinding occurs with lubrication of food by saliva, allowing the salivary amylase (EC 3.2.1.1) to reach available starch.

Although oral digestion in man involves considerable degradation of solid foods, it has been seldom studied. Enzymic and mechanical aspects of *in vivo* mouth digestion have always been investigated separately for various purposes: the mechanism of action of salivary amylase on complex carbohydrate (Kaczmarek & Rosenmund, 1977; Evans *et al.* 1986), salivary response to various stimulations (Morse *et al.* 1989; Mackie & Pangborn, 1990), the efficiency of mastication (Jiffry, 1983) and flavour release during mastication (Wilson & Brown, 1997).

In the digestive process, enzymic degradation of starch in the mouth has been considered insignificant (Dechezleprêtre & Guilbot, 1967; D'Emden *et al.* 1987) because of the short

time spent by food in the mouth. Although the salivary and the pancreatic amylases have similar enzymic properties (Kaczmarek & Rosenmund, 1977), the contribution of salivary amylase is thought to be minor compared with that of pancreatic amylase (Bernier *et al.* 1988; Leclère *et al.* 1993). However, digestion in the mouth has been found to have an impact on the overall process of digestion by increasing the blood glucose response as compared with direct swallowing of food (Read *et al.* 1986). So it needs to be established whether the amyolytic hydrolysis of starch in the mouth, occurring simultaneously with food disruption really is insignificant.

The physical degradation of solid foods has only been evaluated on model foods such as carrots and nuts in order to study chewing performance and the mechanism of mastication (Jiffry, 1983; Lucas & Luke, 1986; Garcia *et al.* 1989). Now, food structure is considered to be of general importance, affecting the metabolic response to starchy food (Granfeldt *et al.* 1992). Moreover, the disruption of the physical structure, increasing the available surface to amylase, has been shown to influence carbohydrate digestion and absorption (Liljeberg *et al.* 1992). Thus, the breakdown of foods in the mouth ought to have an impact on

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total digestion. For this reason, some *in vitro* studies of starchy food digestion have integrated the oral step, either by simulating mouth grinding (Englyst *et al.* 1992) or by practising *in vivo* mastication (Granfeldt *et al.* 1992).

To date, the simultaneous study of the chemical and physical degradations of solid foods during oral digestion has never been carried out in healthy human subjects. The aim of the present study was to evaluate the impact of the *in vivo* oral digestion on both the chemical and physical degradation of solid cereal products. We chose two foods (bread and pasta) differing in their physical structure, in order to assess the influence of characteristics of complex heterogeneous food systems on oral digestion. So we investigated the transformations of physical (particle size) and chemical (starch hydrolysis) characteristics in the mouth.

Subjects and methods

Test foods

Commercial durum wheat spaghetti and white wheat bread were supplied by Barilla (Parma, Italy). The test foods had similar chemical compositions on a DM basis (Table 1) but differed in their physical texture. The bread was stored in slices in a freezer at -20° , and thawed before use. Spaghetti samples (100 g) were cooked in 3 litres of boiling water (Evian[®], Danone, France) containing 7 g NaCl/l (NF ISO 7304); the cooking time (10 min) was determined to be optimal when complete gelatinization of the starch had occurred according to French standards (NF ISO 7304). Experimental samples of bread pieces of 3 g on a DM basis were prepared for each subject. Freshly cooked spaghetti (equivalent to 3 g DM) was weighed in order to prepare individual experimental pasta samples for each subject.

Subjects

Twelve volunteers (nine women and three men), 20–55 years of age (mean 30 years), participated in the study. All had normal dentition and were in good health. The subjects gave their informed, written consent for the study, which was approved by the local Ethics Committee (Comité Consultatif de Protection des Personnes dans le Recherche Biomédicale, Région Pays de Loire, Nantes, France). All experiments were carried out in the morning, and subjects were told to eat a standard breakfast (one cup of coffee with two rusks). The twelve volunteers were divided into three groups of four, all submitted to the same experimental procedure.

Table 1. Chemical composition of the cereal products (g/kg DM)

Products...	Bread	Cooked spaghetti
DM (g/kg fresh matter)	630	368
Crude protein (N \times 5.7)	130	157
Starch	718	719
Lipid	14	24

Experimental procedure

The entire experiment was adapted from a standardized procedure (Froehlich *et al.* 1987; Mackie & Pangborn, 1990) (Fig. 1) and was conducted in two phases. The first phase determined the physiological chewing time of bread and pasta, and the second phase investigated the results of the mastication of bread or spaghetti by the volunteers during the previously determined chewing time. Saliva and masticated bread or spaghetti were collected on separate days.

Determination of the chewing time. The volunteers were asked to chew ten samples of bread or spaghetti normally. The samples, each consisting of 3 g DM, were given in random order. The time between food intake and swallowing was recorded. Average chewing time was determined separately for each food tested and then adopted in the second phase of the experiment.

In vivo study of food degradation during the oral step. The study lasted 50 min for each volunteer (Fig. 1). During the first 10 min, basal saliva was collected (5 min collection and 5 min rest); during the last 40 min, activated saliva or mouth contents after mastication were collected during four 10 min cycles.

Collection of basal and activated saliva. Saliva was collected by forced spitting (Blum & Makhlof, 1971), before food stimulation (basal saliva) and after mastication of food (activated saliva). Basal saliva, collected during a 5 min period while volunteers performed mastication movements, was ejected four times per min into a pre-weighed container kept on ice. After food stimulation, the volunteers rinsed out their mouths with Evian[®] spring water. Activated saliva was then collected during 1 min (four times per min, as previously described) after each mastication (Fig. 1) into a pre-weighed container kept on

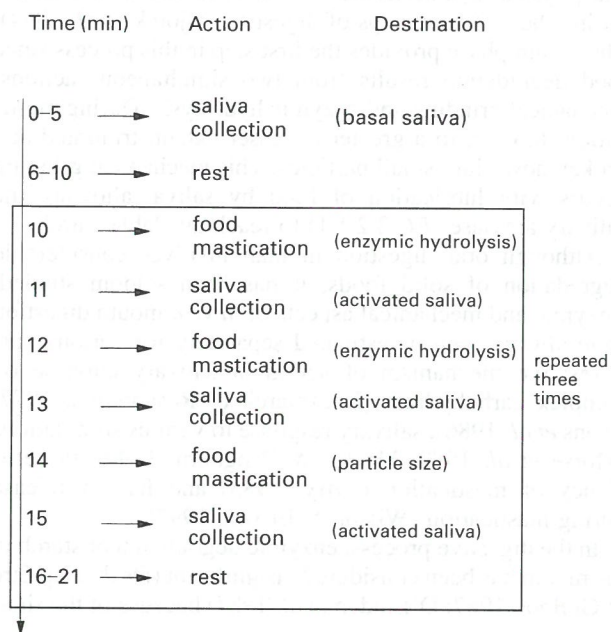


Fig. 1. Description of the experimental period of oral mastication of bread and spaghetti by healthy volunteers.

ice. Collected saliva was weighed and the mean salivary flow calculated as the difference between the weights of activated and basal saliva. After pH measurements, the samples were stored at -20° for further analyses.

The collection of masticated food samples during four 10 min cycles. Food samples were masticated during the predetermined chewing time and spat out into a container kept on ice. During each 10 min cycle, two mouth contents were pooled and kept for chemical analysis. After measurement of the DM content, the samples were freeze-dried. One mouth content of each cycle was kept individually for particle size analysis. Samples were stored at -20° and thawed just before use.

Chemical and physical analysis

Saliva analysis. The salivary impregnation was determined as the difference between the DM contents of food before and after mastication. Salivary amylase activity was measured in basal and activated saliva by a standard procedure (Phadebas test; Pharmacia AB, Uppsala, Sweden). The osmolarity of saliva samples was measured using a 13/13 DR automatic micro-osmometer (Hermann Roebling, Berlin, Germany). Protein content was determined by the Kjeldahl method using 5.7 as corrector coefficient.

Total α -glucans analysis. The total starch content of the samples was measured as described by Faisant *et al.* (1992). Oligosaccharides (degree of polymerization 1 to approximately 10) resulting from starch hydrolysis by salivary amylase were extracted by ethanol (800 ml/l). The samples (500 mg) were first mixed with 10 ml boiling ethanol (800 ml/l) for 15 min under stirring. After centrifugation (10 min at 2000 g), supernatant fractions were recovered, and two extractions were performed on the residues at room temperature. The three supernatant fractions were then pooled, and a portion was evaporated, and re-solubilized in distilled water. Oligosaccharides determination was performed using an HPLC (Waters, Milford, MA, USA) fitted with a pump (Waters 590) and a refractive index detector (Waters 410, Millipore). Samples had been previously filtered through a $0.45 \mu\text{m}$ membrane. Oligosaccharide samples (20 μl) were injected on to a C18 column (240–40 mm, DI $4 \mu\text{m}$ packed with Supersphere 250–4; precolumn: Licosphere 100, RP-18; E. Merck, Darmstadt, Germany) and eluted with water at a flow rate of 0.8 ml/min. Higher-molecular-mass α -glucans were extracted by boiling ethanol (400 ml/l) followed by two more extractions by ethanol (400 ml/l) at room temperature performed on the centrifugation residues (10 min at 2000 g). Total neutral sugars concentrations were determined on the three pooled supernatant fractions by the sulfuric-orscinol method (Tollier & Robin, 1979).

Particle size determination. Particles of masticated food were analysed by two different methods according to the particle size. After mastication, bread particles exhibited heterogeneous shape which made their characterization difficult. We chose to characterize the bread particle size by laser diffraction because the chewed bread particles were mainly less than $2000 \mu\text{m}$ in diameter. This method allowed us to obtain a quantitative approach to the physical structure

of chewed bread, representative of the size and the shape of particles. Bread particles were dispersed in isopropanol and analysed by laser granulometry using a Mastersizer IP MALVERN (Malvern Instruments Limited, Malvern, Hereford & Worcester, UK), with a 1000 lens (range of particle diameters from $4 \mu\text{m}$ to $2000 \mu\text{m}$). The analysis by laser light diffraction was performed twice for each sample. Results are presented in the form of histograms of the particle sizes. The histograms indicate the volumetric frequency of the particles as a percentage of the total volume which they occupied.

After mastication pasta was reduced to non-spherical particles more than 1 mm in length, allowing evaluation by image analysis (Bertrand *et al.* 1992). Pasta particles were individualized on a glass plate placed on a black surface and lighted by four 100 W lamps positioned on each side of the sample 320 mm from the work surface (with an angle of 60°). A CCD IAC500 matrix camera (I2S, Bordeaux, France) was fitted with a 16 mm objective lens (Nikon Corporation, Tokyo, Japan) and positioned approximately 260 mm from the sample. The monochrome images acquired were digitized in the form of matrices with 512×512 columns using a Trydin digitizing board (Info'rop, Toulouse, France). Four images were acquired and analysed together for each sample (see Fig. 4). The images were then processed according to a previously reported method (Devaux *et al.* 1992). The area of each particle was measured. The results were analysed in the form of histograms showing the percentage of the total area occupied by the particles for each class of area.

Statistical analysis

The values are expressed as means with their standard errors. One-way ANOVA was used to compare the saliva and digestion variables. All calculations were performed using Statview SE + Graphics software (Abacus Concepts, Inc., Calabasas, CA, USA) on a Power Macintosh computer.

Results

Preliminary study: determination of the chewing time

The twelve healthy subjects chewed bread significantly longer than pasta: mastication lasted 27.0 (SEM 2.0) and 20.3 (SEM 0.5) s respectively ($P < 0.05$). Chewing times of 27 and 20 s respectively were adopted in the *in vivo* study of oral digestion of bread and pasta.

Saliva secretion during oral digestion

Before food mastication, osmolarity, pH and outflow, and amylase activity of basal saliva were similar for the two foods (Table 2). No changes of salivary pH and amylase activity were found after food chewing. Food stimulation increased salivary outflow by 50 and 37% respectively for bread and pasta ($P < 0.05$). The osmolarity of saliva increased (21%) only after mastication of bread.

The DM of cooked pasta was half that of bread (650 v. 368 g/kg fresh matter) (Table 1). After mastication of pasta,

Table 2. Characteristics of saliva before (basal) and after (activated) chewing of bread and spaghetti

(Mean values with their standard errors for twelve subjects)

	Saliva	Bread		Cooked spaghetti	
		Mean	SEM	Mean	SEM
Outflow (g/min)	basal	0.9	0.1	0.8	0.1
	activated	1.3*	0.1	1.1*	0.1
pH	basal	7.1	0.1	7.1	0.1
	activated	7.4	0.1	7.4	0.1
Osmolarity (mosmol/kg)	basal	66.3	4.5	61.4	3.0
	activated	80.8*	5.7	59.3	2.7
Amylase activity (μ kat/l)	basal	3122	514	4587	737
	activated	3500	823	3983	647

Mean values were significantly different from basal saliva, * $P < 0.05$.**Table 3.** DM content of bread and spaghetti and salivary impregnation of the food bolus after mastication (calculated as the difference between the DM of food before and after mastication)

(Mean values with their standard errors for twelve subjects)

Products ...	Bread		Cooked spaghetti	
	Mean	SEM	Mean	SEM
DM (g/kg fresh matter)	430	12	329*	13
Salivary impregnation (g/kg fresh matter)	220	12	39*	6

Mean values were significantly different from those for bread, * $P < 0.05$.

the DM of the bolus did not change, whereas the DM of the bread mouthfuls was about 30% lower than the DM of bread. The moistening rates of the mouthfuls after ingestion of bread and spaghetti were in the same range, although they were significantly different (Table 3). Consequently, the salivary impregnation of bread mouthfuls was 5-fold greater than that of pasta mouthfuls (respectively 220 and 39 g/kg fresh matter, $P < 0.05$).

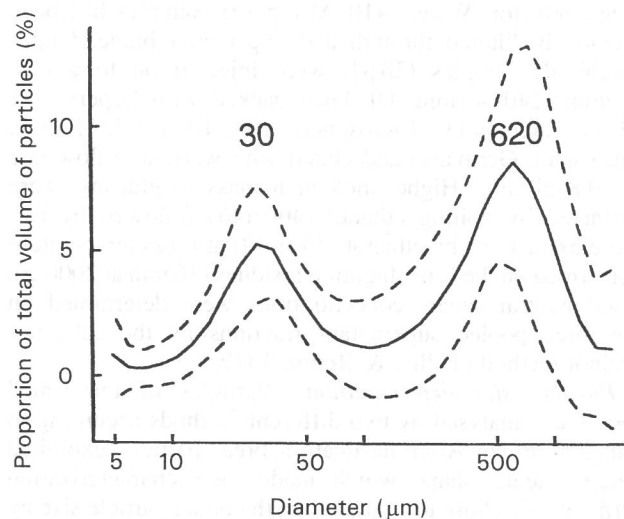
Physical degradation of food during oral digestion

The particle size of food after mastication depended on the food tested.

Bread. After mastication, bread lost its cohesive structure and was transformed into small particles. The average histogram of the bread particle size after mastication is presented in Fig. 2. The diameter of the masticated particles was between 5 and 1500 μ m. After mastication, bread particle diameters related to two maxima, respectively 30 (SD 3) μ m and 620 (SD 192) μ m. The small bread particles were isolated by liquid sieving (80 μ m screen) and characterized by staining with Lugol. This fraction of small particles represented 31% of the total DM and corresponded to starch granules (Fig. 3). Mastication of bread led to the destruction of bread and the release of starch granules from the protein network. These starch granules accounted for the small particle population.

Spaghetti. During mastication the shape of the pasta was maintained, but the length of the strands was reduced

(Fig. 4). Masticated spaghetti particles could be assimilated to small pieces of spaghetti. The particles were swallowed as cylinders ranging in length from 2.5 to 30 mm, corresponding to areas between 5 and 65 mm² (Fig. 5).

**Fig. 2.** Frequency distribution of bread particle size after mastication (3 g DM) by twelve subjects for 27 s. (—), Mean value; (---), standard deviation.

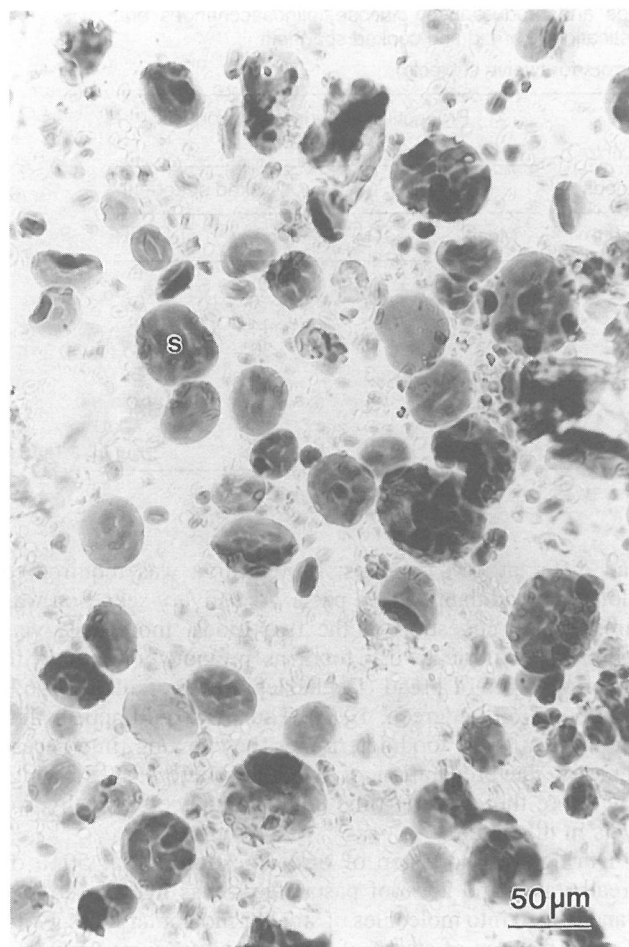


Fig. 3. Small particles of chewed bread isolated by liquid sieving; starch was visualized by microscopy after staining with Lugol solution. S, starch granule.

Chemical degradation of food during mastication

The amounts of simple sugars and oligosaccharides were higher in bread (75 g/kg total starch) than in pasta (5 g/kg total starch). The sugars found in bread were mainly glucose (21 g/kg total starch) and maltose (54 g/kg total starch) (Table 4). The amounts of α -glucans of high molecular mass found in bread and spaghetti were relatively low (respectively 70 and 34 g/kg total starch).

During the oral digestion of bread, starch hydrolysis produced mainly α -glucans (380 (SEM 34) g/kg total starch) and oligosaccharides and glucose (125 (SEM 8) g/kg total starch) (Table 4). After spaghetti mastication, the amounts of oligosaccharides and glucose (92 (SEM 7) g/kg total starch) were not significantly different from those obtained after bread mastication. They were mainly maltose and maltotriose from both ingested foods (Table 4). The significantly (two-fold) higher global starch digestion for bread (505 (SEM 28) g/kg total starch) than pasta (258 (SEM 12) g/kg total starch) resulted from a greater production of α -glucans (respectively 380 (SEM 34) g/kg total starch for bread and 166 (SEM 14) g/kg total starch for spaghetti). For both foods tested, the rates of

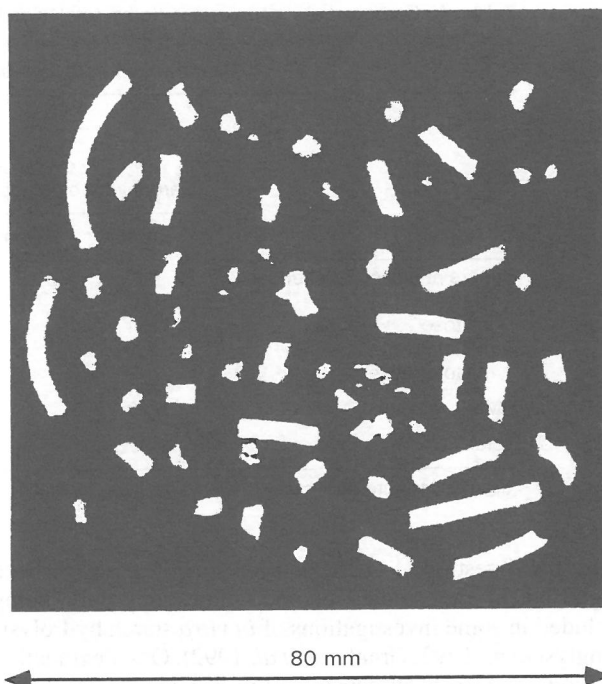


Fig. 4. Example of particles of chewed spaghetti.

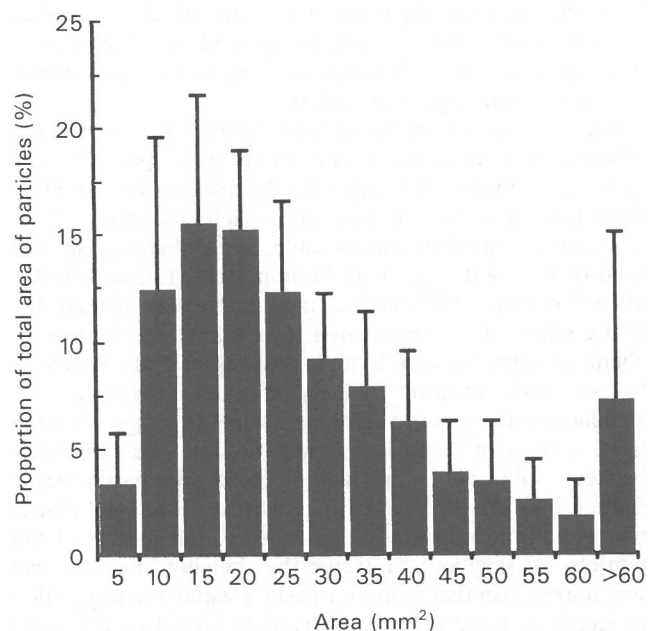


Fig. 5. Frequency distribution of spaghetti particle size after mastication of cooked spaghetti (3g DM) by twelve subjects. Values are means with standard deviations indicated by vertical bars.

starch hydrolysis were high, considering the short time that food remained in the mouth (less than 30 s).

Discussion

Oral digestion is a short step in the overall digestive process (about 20–30 s) as compared with the period of gastric and

Table 4. Carbohydrate composition (g/kg total starch) of foods and production of glucose, oligosaccharides and high-molecular-mass (HMM) α -glucans, after mastication of bread and cooked spaghetti
(Mean values with their standard errors for twelve subjects)

	Carbohydrate composition of foods		Production of hydrolysis products from starch			
			Bread		Cooked spaghetti	
	Bread	Cooked spaghetti	Mean	SEM	Mean	SEM
Sugars and oligosaccharides						
glucose	21	2	0	0	0	0
maltose	54	2	52	5	44	4
maltotriose	2	1	62	5	36*	3
maltotetraose	0	0	11	3	12	2
HMM α -glucans	70	34	380	34	166*	14
Total	147	39	505	28	258*	12

Mean values were significantly different from those for bread, * $P < 0.05$.

intestinal digestion (1–10 h) and has, therefore, often been neglected in the studies of food digestion, only being included in some investigations of *in vitro* starch hydrolysis (Englyst *et al.* 1992; Granfeldt *et al.* 1992). Oral degradation of food has also been considered in accounting for differences in starch digestibility and blood glucose response (Wolever *et al.* 1986; D'Emden *et al.* 1987). For the first time the simultaneous physical and chemical changes of food during oral digestion were investigated simultaneously. In the present study we proved the efficiency of oral digestion which should cause more interest and should be integrated in digestion studies.

Food is submitted to several actions in the mouth: lubrication with saliva, reduction to small particles and starch hydrolysis. Chewing time determines the extent of these three actions, although little is actually known about the factors controlling this variable. Some characteristics of food or the food bolus may be important (Lucas & Luke, 1986; Thexton, 1992) such as the particle size of food, the DM content of the masticated food bolus and the rate of chemical degradation of food. In the present study, we chose to test foods differing in their physical structure and to standardize the experimental procedure (average chewing time, weight of sample) in order to limit the variability between subjects. In the mouth, bread was easily transformed into small particles since its texture was not dense, whereas the mastication of pasta led to the release of big particles of spaghetti. However the chewing time of bread was longer than that found for pasta. Despite the suggestion of Lucas & Luke (1986), the particle size does not seem to determine the chewing time of food. The chewing of bread produced heterogeneous particles, composed of starch granules and fragments of initial network. The particle size of these bread fragments was very different from one distribution to the other (SEM 192 μ m), which could be explained both by the heterogeneous shape of particles and the variability of chewing between subjects. During mastication, pieces of spaghetti were cut off and reduced in size to lengths of 5–12.5 mm; the proportions of particles of different sizes varied according the chewing technique of the subjects. After mastication, the DM contents of the masticated foods were close, whereas the initial samples

had different DM contents. More saliva was required to moisten bread than cooked pasta. As salivary secretion was similar after ingestion of the two foods, more time was required to lubricate dry food, as previously found with different types of bread (Dechezleprêtre & Guilbot, 1967; Pangborn & Lundgreen, 1977). Thus, it would appear that the lubrication of food determines the chewing time necessary for the preparation of the bolus before swallowing. Therefore the chewing time could influence starch hydrolysis in the mouth.

During the short step of oral digestion, about 50 % of bread starch and 25 % of pasta starch was hydrolysed and transformed into molecules of smaller molecular mass. Only a small fraction of starch of both cereal products (approximately 10 %), produced oligosaccharides (mainly maltose and maltotriose), as could be expected (Kaczmarek & Rosenmund, 1977). The degradation of starch to α -glucans of high molecular mass was three-fold higher compared with the degradation of starch to oligosaccharides. The efficiency of this enzymic hydrolysis of starch in bread was favoured by several factors. During mastication, bread crumbs were disrupted into small particles, and about 31 % of starch granules were released from the gluten network, so they were more easily accessible to amylase. The lubrication of bread required greater volumes of saliva to coat particles, so that greater amounts of salivary amylase were likely to hydrolyse food starch. For spaghetti the hydrolysis conditions were not as favourable since the pasta particles were relatively large and the salivary impregnation was relatively low. In these conditions, starch hydrolysis was not significant as it only occurred at the surface of pasta particles. As suggested previously (Björck *et al.* 1994) there is a close interaction between physical characteristics of foods and their digestion. Thus, physical and chemical degradations occurring in the mouth during mastication might have an impact on the subsequent steps in digestion.

Although oral digestion is the shortest phase of digestion, the chemical and physical degradations of foods may play a significant role in their total digestion. In the case of rapidly disrupted starchy food such as bread, half of the starch is hydrolysed and solubilized in the digestive medium, and consequently more easily available for further

enzymic degradation. Furthermore, it has been shown previously that the breakdown of some glycosidic bonds by the endo-splitting salivary amylase leads to a loss of viscosity of gelatinized starch (Evans *et al.* 1986), which could partly explain why the viscosity of starch meals and gastric emptying were found to have no effect on glucose and insulin response in human subjects (Bornet *et al.* 1990). The action of amylase can continue, to a certain extent, during the gastric step, because amylase is partly protected from denaturation by acid pH in the stomach (Rosenblum *et al.* 1988). Thus, it would appear that oral enzymic digestion prepares food chiefly for further degradation in the gut.

In conclusion, our results demonstrate the importance of the simultaneous process of oral digestion involving mastication, saliva lubrication, mixing, and hydrolysis by salivary amylase. Particles are degraded and partially destructured and starch hydrolysis is significantly initiated. The DM content of the food bolus influences the chewing time but is not the only variable to take into account. Food palatability (Hill, 1974), satiety (Bellisle *et al.* 1984) and other more subjective factors may influence both chewing time and mastication efficiency. The size reduction of food, its de-structuring and the rate of starch hydrolysis depends on the chewing time as well as the physical characteristics of ingested food. Therefore, it is important to know to what extent oral digestion contributes to the global digestion, taking into account the different aspects of oral digestion: i.e. lubrication, grinding and salivary hydrolysis of starch. Such knowledge would help to improve *in vitro* techniques and to develop an *in vitro* method predictive of the metabolic response to starchy foods.

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