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Impact of mastication, salivation and food bolus formation on salt release during bread consumption

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Abstract

Health authorities recommend increasing fibre and decreasing salt content in bread products. However, these basic ingredients of bread composition are multifunctional, and important changes in their content influence the texture, flavour and acceptability of the product.

This study was designed to investigate the link between oral processing, bolus formation and sodium release during the consumption of four different breads that varied in composition and structure. Chewing behaviour was determined using surface electromyography, and salivation was quantified from the water content of the boluses collected. The kinetics of bread degradation during food bolus formation was characterised by measuring bolus heterogeneity using texture image analysis, and sodium release into the saliva was quantified. Mastication and salivation varied between products and between subjects, thus highlighting different bolus formation strategies. In vivo salt release was mainly explained by mastication parameters. The initial slope of sodium release increased when the chewing muscles' activity increased, and the maximum sodium concentration was reached later when more masticatory cycles were required to reach the swallowing point.

Keywords: Bread, Salt Release, Food Bolus, Mastication, Salivation

Introduction

Cereals are an important source of dietary fibres. Beside insoluble fibres, soluble fibres have beneficial effects on health through conferring a low glycaemic index to foods high in soluble fibres, in particular. The glycaemic index of bread is a specific dietary feature that may influence metabolic and cardiovascular risk factors long-term.[1-3] This index is known to depend on the bread's composition and processing. For example, the traditional baguette was found to have a lower index than that of the classic baguette,[4] and pumpernickel bread had a lower index than white bread.[5] These last years, researchers aimed to develop new cereal products with higher dietary fibre content and reduced glycaemic index.[6, 7]

After processing steps, the final bread texture, governed by the mechanical properties of the crumb and the crust, and flavour are formed. These properties mainly depend on the matrix material, such as cross-linked gluten, starch, of which the dispersion and structure are a function of both hydration and thermal history during the process, and other minor flour components. The mechanical properties of the bread depend on the density and cellular structure[8-10], and changes in the amount and nature of dietary fibres affect dough rheological behaviour and water binding, and thus significantly modify bread texture.[11, 12] The texture of food influences the oral behaviour of the consumer during eating. In particular, the mastication and saliva parameters are affected, with important consequences on the release of tastants, odourants and temporal perception during the in-mouth process.[13]

During mastication, bread is progressively hydrated by saliva and broken into particles of different sizes, increasing the interface area between the food matrix and the saliva phase and favouring the transfer of stimuli from the food bolus to the saliva phase. The saliva is a complex viscous aqueous medium containing various salts and proteins, and enzymes that are able to partially modify the initial structure and composition of the food and the availability of some flavour components during the in-mouth process. As an example, a direct relation was

found between the alpha-amylase activity level in saliva and saltiness perception of starchy matrices.[14] The breakdown of the matrix due to the action of alpha-amylase favours the release of sodium in saliva and consequently increases the perception of saltiness.

Sodium salt is another bread component linked to health issues[15]. International public health policies recommend that average population daily salt intake should be lower than 5 - 6 g per day. In most of the industrialised countries, bread, and more generally bakery products, contribute in a large part to the daily sodium intake and is the first major sodium source. As an example, in European countries, bread accounts for a range of 19-26% of the total salt intake according to the countries and the optimal salt content in white bread is between 1.29 and 1.43%.[16] However, in bakery products, salt is a common multifunctional ingredient contributing to both sensory and technological properties, such as impacting the development of gluten structures in the mixing of bread[17] and more generally fermented bakery products, inhibiting bakers' yeasts in the fermentation of bread dough and controlling the water activity in the baked products.[15, 18] In particular, the control of water activity in bakery products is critical to both product quality and safety.

In bread, many strategies have already been explored to reduce sodium content, such as partial substitution of sodium salt by potassium salt,[19, 20] potentiation of saltiness by the substitution of salt with fermented ingredients,[21] progressive reduction of salt in bread over time [22] and changes in the sodium concentration distribution.[23] These strategies only allow for partial sodium reductions. However, in-mouth salt release and saltiness perception depends on both food characteristics and oral parameters.[13] It was reported that the combined effects of food composition and chewing behaviour affected salt release in model cheeses.[24-26] In particular, fat influenced in-mouth salt release and saltiness perception differently depending on the fat level.[26] Moreover, most people develop an individual oral strategy consisting of an adaptation of oral behaviour to the food characteristics. In cheeses products, it was reported

that among the 70% of subjects who adapted their chewing behaviour, 57% adapted their behaviour via chewing time, and 40% adapted their behaviour via chewing time and muscular contraction amplitude.[27] Few studies have been reported about breads. As soon as the salt concentration is lowered in crumbs, differences in salt content can be distinguished by consumers. Moreover, salt influences not only saltiness but also aroma perception, and it masks unwanted flavour attributes, such as mustiness and flouriness.[28] Saltiness in bread is influenced by both the velocity of sodium release and the texture of the crumb.[29] Bread texture also influences saltiness perception. Among three breads of different structures and textures, the denser bread was perceived as the least salty.[30] Bread texture is also important for bolus formation and swallowing. For two breads of different textures, small particle size and an appropriate amount of saliva are both important to give to the bread bolus the adequate rheological properties to be swallowed.[31] The physical structure of bread influenced the mastication time, which lasted until the water uptake was appropriate for swallowing. In particular, the researchers reported that the plasticising effect of water on starch, which influenced the gradual decrease of viscosity of the bread bolus during chewing, is more important than particle fragmentation for the rheological behaviour of the bread bolus. However, bolus viscoelasticity did not seem to be a key parameter to trigger swallowing, and the panellists exhibited different masticatory and bolus hydration behaviour. [30]

The aim of this study is to evaluate the influence of bread characteristics and oral characteristics of individuals on in-mouth sodium release. This study, taking into account the interactions between individuals and products, should provide information on higher in-mouth sodium release for low-salt reformulated breads, thus compensating for a lower saltiness intensity.

Experimental

Breads

Four commercial breads with different textures and compositions were studied (Table1). Two breads were French baguettes. Baguettes are made from wheat flour, water, salt and yeast. The texture is characterised by a crisp eggshell crust 3 - 4 mm thick and an open and random crumb cell structure.[32] They were provided by a local supermarket (industrial manufacturing sector) and by a local bakery (artisan manufacturing sector). An industrial toast bread (white-wheat pan bread) was also studied. This type of industrial bread also contains fats and sugars. Toast bread crumb is characterised by a flexible and soft texture and small cells with thin borders. The crust is very thin and soft.[33] Finally, a German rye bread (pumpernickel) made from whole rye grains, water, salt and sourdough was selected. The texture is usually dense and composed of rye grains.

These products were characterised for their density, water content, hardness (maximum force), crust/crumb ratio and sodium content. Bread densities were measured in five replicates according to the rapeseed displacement method.[34] Water content was determined in triplicate after drying in an oven as described below. Maximum force was determined from a compression test developed to characterise the mechanical behaviour of products with different crust/crumb textures (whole breads).[35] Baguette samples were obtained by cutting the baguettes into 11-cm length slices. Samples of similar size (around 11 × 5 × 5 cm) were prepared from the toast bread and rye bread by superimposing several slices. All samples were compressed using a TAXT2 Texture Analyser (Swantech International, Gennevilliers, France) fitted with a multi-puncture probe (two series of 8 spikes). Compression was set at 60% strain of the initial height and at a speed of 0.8 mm sec^{-1}. The maximum compression force for each type of bread was determined. Eight replicates were run per type of product. The approximate crust/crumb ratio was determined by carefully separating the crust and the

crumb and weighting each portion. Sodium content was determined by atomic absorption spectrometry on the ash content (Tests carried out by Laboratoires Agrobio, Vezin le Coquet, France).

In the following experiments, breads were presented to subjects in 5 ± 0.1 g slices in a format representative of how that bread is usually consumed.[36] The breads were consumed fresh; the baguettes had been produced a maximum of 10 hours earlier, and the samples (5 g) were prepared a maximum of 15 min before the tests.

Subjects

Five subjects (two female and three male, aged 32 to 50 years, coded A to E) with good dental status participated in the study. They were selected to represent different chewing efficiencies.[36] Subjects gave their written informed consent after receiving a full explanation of the goals and schedule of the study. This study was approved by the local Ethic Committee and by ANSM (ID RCB: 2013-A01084-41).

Subjects were characterised for their salivary flow rates and amylase concentration in saliva. Salivary flow rates were determined as described elsewhere,[24] over collection periods of 10 min for saliva at rest and 1 min for mechanically stimulated saliva. Alpha-amylase levels were determined in the saliva collected at rest using a commercial kit from Biolabo (ref: 99523/99123, Maizy, France). Group averages for unstimulated and stimulated salivary flow rates were found to be 0.43 ± 0.15 and 2.14 ± 0.29 mL min^{-1}, respectively, and average amylase concentration was 10.1 (±1.4) × 10^3 IU L^{-1}. No significant differences were observed between subjects for any of these three parameters.

Experimental procedure

Mastication, salivation, bolus heterogeneity and salt release in the oral cavity were followed during bread consumption. All of these measurements were made in independent sessions. The subjects took part in eight sessions of one-hour duration each. They had eaten 2 hours before the sessions. During the experiment, they were seated comfortably in an air-conditioned room (21 ± 1 °C). Sessions 1 and 2 were devoted to the measurement of the activity of the chewing muscles. Subjects were asked to consume bread samples (5 g) in their usual manner, and chewing activity was measured throughout the chewing sequence (including swallowing) by surface electromyography (EMG). Sessions 3 through 5 were devoted to the collection of bread boluses for the study of saliva uptake and bolus heterogeneity. Subjects were asked to chew bread samples, without swallowing, in their usual manner and on a signal of the experimenter to spit out the bread boluses into a container (lid of a glass Petri dish). Boluses were collected at three different stages: 1) at 10 masticatory cycles, 2) at 20 cycles and 3) after complete mastication, when the subject felt the need to swallow (SW). For all of the experiments, the number of cycles and chewing time that induced swallowing (SW) was recorded by the researcher. Initially, boluses were also collected at 30 cycles, but as this number exceeded the number of chews for swallowing in some cases, there were some missing data for this chewing period.[36] Therefore, data collected at 30 cycles are not presented in this paper.

Subjects were trained in the procedure at the beginning of session 3 with a few samples. Boluses collection started once the subjects felt comfortable with the protocol. Boluses were collected at different stages for given bread before the next bread was offered. With a particular bread type, the subjects always started with the SW bolus, followed by other chewing durations in a random order. At the end of session 5, 180 bread boluses (5 subjects × 4 breads × 3 replicates × 3 chewing stages) were obtained.

Sessions 6 through 8 were devoted to saliva collection for further sodium analysis. The general procedure was similar to the procedure presented for bolus collection. The instructions given to subjects were to move the bolus to one side of the mouth and spit out a sample of saliva (approximately 0.5 mL) into a 1.5 mL Eppendorf® tube. At the end of session 8, 180 saliva samples were collected (5 subjects × 4 breads × 3 chewing stages × 3 replicates). A saliva sample was also collected before eating each sample (stage 0 masticatory cycle = blank). Over the eight individual sessions and the five subjects, the four breads were tested in random order. The subjects cleansed their palates with Evian® mineral water and ensured that their mouths were completely clear of any bread particles before starting the next sample. An interval of 1 minute was taken between the chewing of different samples.

Chewing activity recorded by Electromyography. The activity of masticatory muscles (superficial masseter and right and left anterior temporalis) was monitored during natural bread consumption.[37] From the electromyographic signal collected, eight variables were analysed.[38] The chewing sequence was characterised by (a) chewing time (CT in s), (b) number of bursts (i.e., masticatory cycles, BN) and (c) chewing rate (CR). Moreover, muscle activity was characterised by (d) mean and (e) maximum voltage of bursts (Vm and Vmax, respectively, in mV), (f) burst duration (BD in s), (g) total EMG activity (sum of the integrated areas of all individual masticatory cycles of the sequence; Wtot, expressed in mV s) and (h) mean EMG activity per cycle (Wc, mV s). To study chewing process dynamics, the chewing sequence was also divided into chewing periods of 10 cycles; for each one, the average EMG activity (Wc) was also calculated. Four replicates (two per session) were performed for each bread.

Heterogeneity of bread boluses. Heterogeneity was studied using image texture analysis as described in a previous study.[36] The Petri dish was closed immediately after bolus collection and four images of each bolus were acquired. These images, acquired in colour (RGB

system), were first converted into YCbCr images. Only the channel (plan) associated with Y (luminance) was processed for texture analysis. Images were analysed using the Grey level co-occurrence matrix (GLCM) method.[39] The co-occurrence matrix describes the second-order statistics in the images and allows for the calculation of textural features that are expected to represent the textural characteristics of the image studied. For a given image, we extracted 5 textural features. Among these textural features, contrast feature represents the heterogeneity of the image and was found to be a suitable feature to characterise bread degradation during the chewing sequence.[36]

Water uptake of bread bolus. The saliva taken up by the breads during oral processing was determined from the water content of the breads and of the boluses. The water content of the boluses was determined directly after image acquisition. The water content corresponded to the mass loss obtained after drying in an oven 24 h at 100 °C. For each subject, each bread and each chewing period, the percentage of saliva incorporated in the bolus was calculated as follows: Saliva (%) = ((W1 / D1) × D2) – W2 (with W1: water content of the bolus (%), D1 : dry matter of the bolus (%), D2 : dry matter of the bread (%) and W2 : water content of the bread (%)).

Sodium content in saliva

Salt release in the mouth during bread consumption was characterised from the sodium content in saliva. The saliva samples were centrifuged directly after collection at 10,000 *g* for 10 min at 4 °C (2-16K, Sigma –Aldrich, St Quentin Fallavier, France). The supernatants were collected, diluted to 1/25 in MilliQ® water and filtered (pore size: 0.45 µm, C.I.L, Sainte Foy La Grande). The amount of sodium in each sample was determined by high performance liquid chromatography using an ISC3000 Dionex system (Voisins le Bretonneux, France) whose individual components included a GP quaternary pump, an AS50 autosampler and a

detector used in conductance mode with a CSRS 300 suppressor. The loop injection was set at 20 µL (sample volume). The sodium content was analysed using a Dionex IonPac CS12A and an IonPac CG12A guard column at 25 °C. Elution was achieved with 11 mM sulphuric acid at a flow rate of 0.5 mL min^{-1}. System controls and data acquisitions were accomplished using Dionex Chromeleon 6.8 software. Quantifications were performed versus standard sodium solutions ranging from 0 to 3 mM.

Data analysis

One–way analysis of variance (ANOVA) was performed on the physical properties of the breads to assess the differences between breads. The variability in EMG parameters between subjects and products were studied using Principal Component Analysis (PCA). Variables collected for the entire chewing process, such as EMG data, number of cycles, saliva uptake of bolus at swallowing and image textural contrast of boluses collected at swallowing, were studied using two-way ANOVA with bread, random subject and the bread × subject interaction as factors. The dynamic evolution of variables collected at different stages during the chewing process (EMG data, saliva uptake and image contrast) was studied using three-way ANOVA. The factors in the model included subject (random), bread, chewing stage and interactions between these factors. Sodium release curve parameters were defined: slope (initial slope of the curve between 0 and 10 chewing cycles), Cmax (maximum concentration in sodium) corrected for initial differences in the sodium content between different breads and Tmax (number of cycles corresponding to Cmax). These parameters were analysed using 2-way ANOVA (random subjects, breads and interactions). ANOVAs were performed using the GLM procedure in SAS Software (SAS Institute Inc., Cary, NC, USA), and the LSMEANS statement was used for a post-hoc multiple comparison test. Correlation coefficients were calculated between different variables. Partial Least Squares (PLS) regression was used to

explain salt release parameters (slope, Cmax and Tmax: Block Y) in terms of oral parameters (burst duration, number of masticatory cycles observed in EMG, saliva and salt experiments, chewing rate, chewing time, mean and maximum amplitudes of muscle contraction, EMG activity per chewing cycle, total EMG activity, saliva uptake in bolus, bolus homogeneity (contrast textural feature, Block X)). As a data pre-treatment, normalisation using the 1/SDEV transform to treat all parameters as having equal potential influence was used. A full cross-validation procedure to determine the maximum number of significant dimensions was applied. The Nonlinear Iterative Partial Least Squares (NIPALS) algorithm was used. Discriminant variable selection was performed using variable importance in the projection (VIP)[40] with a threshold of 0.8.

Results

Bread characteristics

The breads significantly differed in terms of both composition and physicochemical properties (Table 1).

<Table 1>

Rye bread was significantly denser, more humid and harder than other breads. Baguettes were the least dense and the least humid products. Artisan and industrial baguettes were significantly different only in water content and hardness. Salt content was higher in baguettes. Our data were globally consistent with density values for industrial and artisan French baguettes and sodium contents in breads reported elsewhere.[41, 42]

Chewing activity and bolus properties

268 **Chewing activity.** EMG data, chewing efficiency and salivary flow rates obtained for each
269 subject and each type of bread were analysed using PCA (Fig. 1).
270
271 <Fig. 1>
272
273 The first two components accounted for 70% of the total variance. Inter-individual
274 differences were more important than differences between products. PC1 was negatively
275 correlated with the mean and maximum amplitudes of the EMG signal, chewing rate and
276 salivary flow rate and positively correlated with the chewing time, burst duration and chewing
277 efficiency on the left and discriminated between the subjects. Subjects E and C chewed breads
278 faster, and their muscle signals were stronger than those of subjects A and B. The latter
279 chewed for longer and with more chews than the former. Subjects were involved in this study
280 on the basis of their differences in chewing efficiency. It is interesting to note that no
281 correlations were observed between chewing efficiency measured by dental polymer
282 breakdown and any of the EMG parameters measured during bread consumption ($p > 0.05$).
283 PC2 was mainly correlated with EMG activities and discriminated between the products for
284 each subject. From the ANOVA results, the breads differed significantly for the mean (Vm:
285 $F_{(3, 12)} = 26.7$, $p < 0.001$) and maximum (Vmax: $F_{(3, 12)} = 18.3$, $p < 0.0001$) amplitudes, EMG
286 activity per cycle (Wc: $F_{(3, 12)} = 8.6$, $p < 0.001$) and total activity (Wtot: $F_{(3, 12)} = 18.81$, $p <$
287 0.001). Baguettes required stronger muscle contractions (work and amplitude) and longer
288 chewing times than the rye bread and toast bread. These parameters were significantly higher
289 for the baguettes than for the two other breads (data not shown). Breads varied also in
290 parameters characterising the chewing sequences. Baguettes were chewed for a significantly
291 greater number of masticatory cycles (BN: $F_{(3, 12)} = 23.9$, $p < 0.0001$) and consequently for a
292 longer period (CT: $F_{(3, 12)} = 18.9$, $p < 0.0001$) than the rye bread and the toast bread. The

293 average numbers of cycles per bread were as follows: 43.7 (average subject values ranged
294 from 36.2 to 51) for the bakery baguette (BB), 42.5 (31.2 - 50.2) for the supermarket baguette
295 (BS), 33.5 (28.2 - 44.7) for the rye bread (RB) and 32.3 (25.5 - 38.0) for the toast bread (TB).
296 No significant differences ($p > 0.05$) were observed between breads for the chewing rate (CR)
297 or for burst duration (BD).

298 Division of the EMG signal into chewing periods enabled the study of the evolution of
299 chewing activity throughout the chewing sequence. EMG activity significantly decreased
300 between chewing periods ($F_{(3, 18.5)} = 7.5$, $p < 0.001$) from 10 cycles to swallowing (Fig. 2).
301 Moreover, the chewing period × subject and chewing period × bread interactions were both
302 significant ($p < 0.001$). This suggests that the profile of chewing activity adapted depending
303 both on the subject and the type of bread, as highlighted on Fig. 2. Only subject E and Bread
304 TB are represented as an example on Fig. 2 but the same trends were observed for the others.
305
306 <Fig. 2>
307
308 **Saliva uptake in boluses.** The numbers of masticatory cycles leading to natural swallowing
309 varied between subjects ($F_{(4, 12)} = 8.93$, $p < 0.001$). On average, subjects A, B and C were
310 found to produce significantly more cycles (35.2 ± 4.8, 32.2 ± 6.9 and 38.6 ± 8.4,
311 respectively) than subjects D and E (28.4 ± 7.5 and 26.3 ± 7.5, respectively). The number of
312 cycles also varied between products. More cycles were necessary to reach swallowing for the
313 baguettes ($F_{(3, 12)} = 10.35$, $p < 0.001$) than the rye bread and the toast bread. No significant
314 subject × bread interaction was found ($p > 0.05$).

315 The amounts of saliva obtained for the different breads at three stages of the chewing
316 process are presented Fig. 3. The amount of saliva increased with the number of cycles ($F_{(2, 8)}$
317 $= 11.25$, $p < 0.01$) and depended on the type of bread ($F_{(3, 12)} = 19.90$, $p < 0.0001$). Boluses

made from rye bread had a significantly higher saliva uptake than the other breads (Fig. 3). Differences between breads were observed at 10 and 20 cycles but were more marked at swallowing, as already observed for other foods.[37, 43]

At swallowing, boluses made from the bakery baguette had higher saliva content than boluses prepared from the supermarket baguette and toast bread.

<Fig. 3>

Saliva uptake increased throughout the chewing process as the number of cycles increased. Nevertheless, we found no correlation between saliva uptake at swallowing and the number of cycles required to reach swallowing ($r < 0.2$).

Depending on the type of bread studied, the differences between subjects varied (bread × subject interaction: $F_{(12,24)} = 2.35$, $p < 0.05$). Saliva content in the swallowable bolus prepared from different subjects and different breads are presented Fig. 4. Globally, the amount of saliva taken up by swallowable boluses ranged from 13.6 to 66.6%. Our results are consistent with other studies from the literature studying salivary impregnation/uptake in bread and cereals.[44, 45]

<Fig. 4>

For the rye bread, subjects A, C and E produced significantly more saliva (up to 50% more) than subjects B and D. Moreover, subject A produced significantly more saliva than 1) subject C for the supermarket baguette and 2) all of the other subjects for the toast bread. Looking at bread differences at the individual level seems to suggest two types of salivary behaviours. Subjects A, C and E adapted their salivary production to the type of bread,

343 whereas the amount of saliva produced did not vary significantly between breads for subjects
344 B and D.
345
346 **Bolus homogeneity.** The image texture heterogeneity of each bolus collected after a fixed
347 number of cycles and at the swallowing threshold were determined using image texture
348 analysis.[36] During the chewing process, the breads were transformed into a bolus that lost its
349 heterogeneity (decrease in the contrast values) as the number of chewing cycles increased
350 ($Fcycle_{(2,8)} = 101.9$, $p < 0.0001$). In our previous study, the analysis of contrast values had
351 revealed specific patterns of bread degradation between breads and between subjects.[36] TB
352 and RB reached a homogeneity suitable for swallowing more rapidly than the baguettes.
353 Boluses prepared from different breads had different heterogeneity depending on the subject
354 considered (subject × bread interaction ($F_{(12,24)} = 6.91$, $p < 0.0001$)). Differences between
355 subjects were further studied for each individual bread and are presented Table 2.
356
357 <Table 2>
358
359 Contrast values varied greatly between subjects for the baguettes. Boluses collected from
360 subject E were significantly more heterogeneous (higher contrast values) than boluses of other
361 subjects. Inversely, Subject A (and C and D for the BS bread) produced the most
362 homogeneous boluses. These data can be partly explained by individual chewing parameters.
363 Indeed subject E had the lowest chewing efficiency and used fewer cycles to form a
364 swallowable bolus. These chewing parameters may partly explain differences observed
365 between subjects for the RB bread, too. Indeed, the most heterogeneous boluses were
366 produced by the subject (subject D) who applied the smallest number of cycles and who also
367 presented a relatively low chewing efficiency. In the case of this bread, we might also

suppose that the higher saliva uptake previously observed for subject E may have helped in preparing a more homogeneous bolus for this subject (as compared to subject D). Nevertheless, differences between subjects in chewing parameters and saliva uptake parameters did not explain all of the contrast results. Indeed, in the case of TB, no differences in contrast values were observed between boluses collected from different subjects despite the fact that subjects varied in mastication and salivation.

Sodium content in saliva

The numbers of masticatory cycles leading to natural swallowing varied between breads (Table 3) and between subjects (Table 4). As observed previously, the baguettes required more cycles to form a swallowable bolus than the rye bread and the toast bread (Table 4). The number of cycles applied to reach the swallowing stage was significantly smaller for subject E. Sodium content in saliva varied between breads (Table 3). A higher sodium concentration was observed for toast bread and rye bread than for the baguettes, and the higher concentration was also reached faster (Tmax) in those products. These effects were not subject-dependent (bread × subject interaction: $p > 0.05$).

<Table 3>

Large inter-individual differences were observed for the three parameters studied, suggesting different dynamics of the sodium release profile between subjects. Subject A released significantly more sodium than subjects B and C. Subject E had a faster release at the beginning of chewing (slope) and at the maximum concentration (Tmax).

<Table 4>

To gain deeper insight into the mechanisms influencing sodium release, a PLS regression was performed to explain the release parameters in terms of the subjects' chewing behaviour, saliva uptake in the bolus and bolus heterogeneity (Figure 5).

<Fig. 5>

On the PLS biplot associated with the two first dimensions, 40% of the variability in oral parameters explained 65% of the variability in sodium release parameters. The first axis shows Tmax on the left-hand side and Cmax on the right-hand side. These variables were well-explained by VIP. The slope variable was not explained as well by VIP and was separated on the projection along the second axis. From the beta-weight coefficients, Tmax was mainly explained by the number of masticatory cycles observed in all experiments (EMG, saliva and salt release) and the chewing time. The higher the number of cycles applied to the bread, the later the maximum salt concentration was released. Cmax was related to low cycles and high chewing rate. For the slope parameters, the EMG parameters Wtot, Wc and Vmax were the most important variables, as determined by beta-weight coefficients. The saliva uptake in bolus and bolus heterogeneity did not explain salt release parameters.

Discussion

Methodological considerations

Mastication, bolus properties (saliva uptake and image textural heterogeneity) and salt release were investigated in 3 independent studies dealing with the same subjects and the same products. We chose this set-up to avoid any potential interference between bolus collection and the natural chewing behaviour of subjects and between saliva collection and the structural

properties of collected food boluses. For all of the experiments, subjects were introduced to the protocol via a short training on a few products at the beginning of the first session. They were not intensively trained because we did not want to induce a stereotyped chewing behaviour. Unexpectedly, the number of masticatory cycles required for swallowing was significantly higher in EMG experiments than in other experiments (data not shown). This result may be related to the use of surface electrodes in EMG experiments, which unavoidably attracts the subjects' attention to mastication and may result in emphasis of their chewing behaviour. Despite this difference in chewing parameters, products were discriminated in the same way (baguettes required more cycles than the other products) in all experiments. Differences between subjects were also globally similar; subject A is always classified with the subjects producing the most cycles, and subject E systematically produced fewer cycles than subjects A, B and C. Therefore, because the numbers of cycles were different but the conclusions in term of product and subject differences were in agreement, we concluded that the data from the different experiments could be compared together.

The difficulty in exactly quantifying the salt released in saliva when chewing bread also bears mentioning. In this study, we determined salt release from the concentration in sodium measured in saliva swabs collected at different stages during the chewing process. However, saliva naturally contains sodium in low concentrations at rest but with a high inter-individual variability (i.e. 11.5 to 217.3 mmol L^{-1} according to Kallapur et al.[46]). We quantified intrinsic sodium concentrations from saliva samples collected at rest (data not shown). These concentrations were rather small compared to those obtained while chewing breads. In the literature, other authors have suggested that intrinsic sodium concentration in saliva increases when the salivary flow rate increases.[47] Sodium content in stimulated saliva by chewing parafilm on forty eight subjects has been shown to be on average four times higher than in resting saliva.[48] Because chewing real food is known to stimulate salivary flow rate, it is

443 actually difficult to quantify the exact contribution of intrinsic sodium and sodium originated
444 from the bread. Whatever the saliva sampling technique, it seems difficult at the moment to
445 be able to access to the exact sodium released from food product in a natural chewing context.
446
447 **In-mouth processing: variability between breads and between subjects**
448 **Evolution of mastication, salivation and bolus homogeneity during the chewing period.**
449 The effect of chewing cycles was observed for EMG activity, saliva uptake in boluses and
450 bolus heterogeneity. A decrease in chewing muscle activity during the chewing sequence has
451 been reported several times for different products[43, 49, 50] and is explained by an adaptation of
452 chewing behaviour to the changes in food structure during the bolus formation process. As
453 expected, saliva uptake in the bolus increased during the chewing process as a result of
454 continuous saliva production[51]. We observed different saliva uptake in boluses collected at
455 swallowing from the different breads. This result is rather in contradiction with another study
456 reporting no significant differences in bolus water content between the three bread types
457 despite their difference in structure and composition.[30] This result can be explained by the
458 greater difference between the structure of baguettes, toast breads and rye breads used in our
459 study. No correlation was found between the amount of saliva in the bolus at swallowing and
460 the number of masticatory cycles leading to swallowing. This observation is consistent with
461 other studies, according to which the number of chewing cycles until swallowing and salivary
462 flow rate were independent.[45, 52] This suggests that salivation also depends on other
463 parameters, such as product properties. In particular, the perception of texture can influence
464 salivary flow rate,[53, 54] but in the case of bread, different crumb textures were reported to have
465 no influence on salivary flow rate during chewing.[29] In parallel to the dynamic evolution of
466 mastication and salivation, breads were transformed into boluses that increase in homogeneity
467 as the number of chewing cycles increased. This result is in agreement with recent data

showing a continuous reduction of bread into many small particles throughout the chewing process.[31]

Food oral processing of different breads. Chewing behaviour was adapted to the textural properties of the food. In the literature, it has been shown that dry and hard products usually require more chewing cycles to be broken down and to capture saliva before swallowing[52, 55] and that hardness is a key parameter influencing chewing muscle activities measured by EMG.[56] In our study, toast bread was significantly less hard; it was thus quickly broken down and required less than 30 cycles to be swallowed. Toast bread also contains fat, which may have helped in-mouth breakdown. Instrumental hardness cannot account for differences observed in in-mouth processes for the other breads. The baguettes are slightly harder, less dense and less humid but need significantly more cycles, muscle activity and chewing time to be swallowed. We believe it can be explained by the presence of a thick crust, which provides resistance to biting the baguettes. The rye bread was the hardest and densest product but required similar chewing behaviour (EMG) as toast bread. We argue that it is linked to its specific structure (rye grain agglomerates), which most likely easily separate under chewing, higher humidity and higher saliva uptake. It is important to note that the higher saliva production of rye bread could also be related to the taste of the bread. Indeed, during the experiment some subjects reported that this bread had a sour taste. We can therefore hypothesise that the greater sourness might also have stimulated saliva production during eating.[57]

Food oral processing in different subjects. Variation between subjects was large in term of mastication and salivation. The subject's characterisation parameters did not predict individual mastication and salivation behaviours in a real (food product) eating context. The huge differences in chewing efficiencies (i.e., ability to break down a silicone rubber into particles in a given number of masticatory cycles[36]) between subjects was not related to

individual EMG activity or number of chewing cycles required to form a swallowable bolus from breads. Similarly, subjects did not differ in their initial salivary flow rate collected at rest and under mechanical (Parafilm®) stimulation but produced bread boluses with different saliva content. Finally, individual parameters, such as chewing efficiency or numbers of cycles required to reach swallowing, could explain the inter-individual differences in bolus homogeneity (contrast textural feature of images), but only for a few breads.

Interestingly, at the end of mastication, we did not find similar saliva content and similar homogeneity for boluses collected from different subjects. In hard and brittle products (carrots, nuts,…) a narrow inter-individual variability in particle size distribution has been observed.[58-60] Recent studies suggest that the swallowing threshold of cereal products may be more multi-components.[45, 61] A further characterisation of the physical properties of boluses, using for example the methods recently proposed,[31] seems necessary to improve our understanding of the mechanisms controlling bolus formation and swallowing in breads.

In-mouth salt release from different breads

Role of bread characteristics on salt release. More salt is released from toast bread and rye bread than from the baguettes, and two main reasons should be considered.

First, breads varied in their composition (wheat vs. rye, presence of fat and sugars, etc), which induced different chemical properties in the breads. We could suppose different interactions between salt sodium ions and bread components, such as proteins. Nevertheless, sodium-protein interactions have been reported to be sufficiently weak in breads to allow complete extraction during in-mouth processing.[28]

Second, breads also varied in their physical properties. Among the compositional factors affecting in vivo sodium release, water content has been the most frequently cited in the case of cheese products with a 50% humidity content[25, 26] and in the case of sausages.[62] Similarly,

518 we observed a higher level of release with a higher water content (Table 1) in the case of drier
519 products, such as breads. The kinetics of sodium release may be related to the mobility of
520 sodium within a food product.[63] In the case of cheeses, increasing the water content was
521 found to increase sodium mobility within the product (relaxation time measured by NMR)[64]
522 and the release of NaCl from the product to an aqueous phase, observed in vitro.[65] The lower
523 sodium release observed in baguettes could be linked to physical properties of the crust. More
524 than half of a baguette's weight is composed of a dry and crisp eggshell crust. Because of
525 higher dryness, salt may be more concentrated in the crust than in the crumb. The crust is
526 more difficult to break down and to impregnate with saliva, leading to a smaller salt
527 extraction.

528 In this study, it is actually difficult to further explain how bread factors control temporal
529 sodium release. The development of model breads with a controlled formulation and process
530 seems necessary to further understand their relative contributions.

531 **Role of food oral processing on salt release.** Salt release parameters were mainly explained
532 by chewing parameters. The maximum sodium concentration was reached later when subjects
533 applied a large number of chews and a long chewing duration, as observed in others studies.[66]
534 Rapid initial sodium release was linked to high EMG activity and signal amplitude. This is in
535 agreement with a study reporting that rapid sodium release is linked to high bite force in
536 model cheeses.[25] The effect of chewing activity on the rate of sodium release could be
537 explained by a greater breakdown of the product due to the application of stronger mechanical
538 forces between the teeth and more chews. However, this only explains the beginning of
539 release as the maximum concentration of release was related to short chewing time only. We
540 did not observe any influence of the type of bread on the sodium release velocity despite the
541 differences in bread structure and composition, even though significantly faster sodium
542 release has been reported in another study for the more coarse-pored breads.[29] These authors

543 reported that this faster release mainly occurred during the beginning of chewing that is
544 crucial for saltiness perception. According to literature, sodium release from model cheeses
545 was partly related to saliva parameters, but conflicting results were observed. High salivary
546 flow rates were linked to a high level of sodium release in one study[25] but low levels in other
547 studies.[24, 66] In our study, saliva uptake in the bolus was found to vary between 15 and 65%
548 between subjects and breads overall, but was not an important parameter for explaining salt
549 release from breads.

550 Saliva enzymatic composition needs to be mentioned as a parameter affecting food
551 breakdown and salt release. Indeed, several studies have shown that salivary alpha-amylase
552 has an impact on bread digestion.[44, 67, 68] In an in vivo study, alpha-amylase was able to
553 hydrolyse 50% of bread starch during bread mastication for chewing periods shorter or
554 similar to those observed during our study.[44] In our study, alpha-amylase concentration did
555 not explain inter-individual differences, as similar concentrations were observed between
556 subjects for saliva collected at rest. Nevertheless, it is possible that salivary alpha-amylase
557 accounted for differences observed between breads. Indeed, enzymatic degradation of starch
558 may have been different depending on the chemical and physical properties of the bread,
559 leading to variation in salt release in the saliva. This result would be in line with other studies
560 that showed that in-mouth alpha-amylase activity affected flavour release and saltiness
561 perception.[14, 69] Further experiments using more subjects and well-designed model bread
562 composition are required to better understand the link between food oral processing including
563 salivary composition, bread properties and salt release in cereal products.
564

565 Conclusion

566 Mastication and salivation are two complementary oral mechanisms that lead to food bolus
567 formation. During this study, these mechanisms were adapted to the composition and

568 structural properties of the breads. Marked inter-individual differences were observed in oral
569 parameters, leading to differences in bolus formation. Salt release in saliva was mainly
570 explained by mastication parameters. Higher chewing muscle activity induced a rapid initial
571 rate of sodium release, and longer chewing time induced later sodium release.
572

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577

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Figure captions

Fig. 1 Principal Component Analysis biplot of oral parameters obtained for each subject (A-E) and each bread (BB: bakery baguette, BS: supermarket baguette, TB: toast bread, RB: rye bread). Parameters: EMG parameters (burst duration (BD), number of bursts (i.e., masticatory cycle; BN), chewing rate (CR), chewing time (CT), mean and maximum amplitudes (Vm and Vmax, respectively), EMG activity per chewing cycle (Wc), total EMG activity (Wtot), salivary flow rate (SF), and chewing efficiency (CE).

Fig. 2 **(a)** Evolution of mean EMG activity per cycle during eating of the TB bread by five different subjects (A to E). **(b)** Evolution of mean EMG activity per cycle during eating of four breads (BB, BS, TB, RB) observed for subject E. SW: average number of chews inducing swallowing. Average value ± standard deviation (n = 4).

Fig. 3 Saliva uptake of boluses made from 4 breads (BB, BS, TB and RB) expectorated at three periods during the chewing process: at 10 (□) cycles, 20 (▨) cycles and at swallowing point (■). a, b, c, d: Mean values with different letters are significantly different ($p < 0.05$).

Fig. 4 Saliva content of boluses collected at swallowing from different subjects (A, B, C, D, E) eating different breads (BB; BS, RB, TB). a, b, c, d, e, f: Mean values with different letters are significantly different ($p < 0.05$).

Figure 5 The Partial Least Square (PLS) regression explaining salt release parameters (initial slope of release between 0 and 10 chews (Slope), maximum concentration in sodium (Cmax) and number of cycles corresponding to Cmax (Tmax); block Y) in terms of oral parameters

(EMG parameters (burst duration (BD), number of masticatory cycles (Chews-EMG), chewing rate (CR), chewing time (CT), mean and maximum amplitudes (Vm and Vmax, respectively), EMG activity per chewing cycle (Wc), total EMG activity (Wtot)), number of chews observed in saliva and salt experiments (Chews-saliva and Chews-salt, respectively), contrast textural feature from bolus images at swallowing, and saliva uptake in the bolus at swallowing (Saliva)). The method was applied to all subject × bread combinations (n = 20). **(a)** Correlation plot, **(b)** biplot of observations (subject (A-E) and bread (BB, BS, TB, RB)), plan #1-#2.

Fig. 1

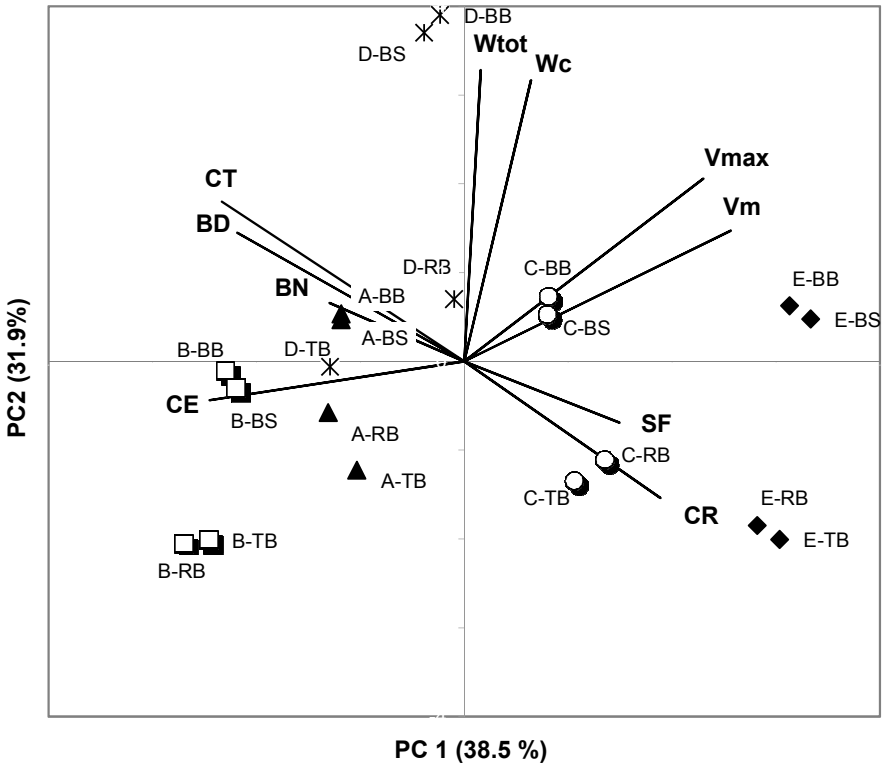
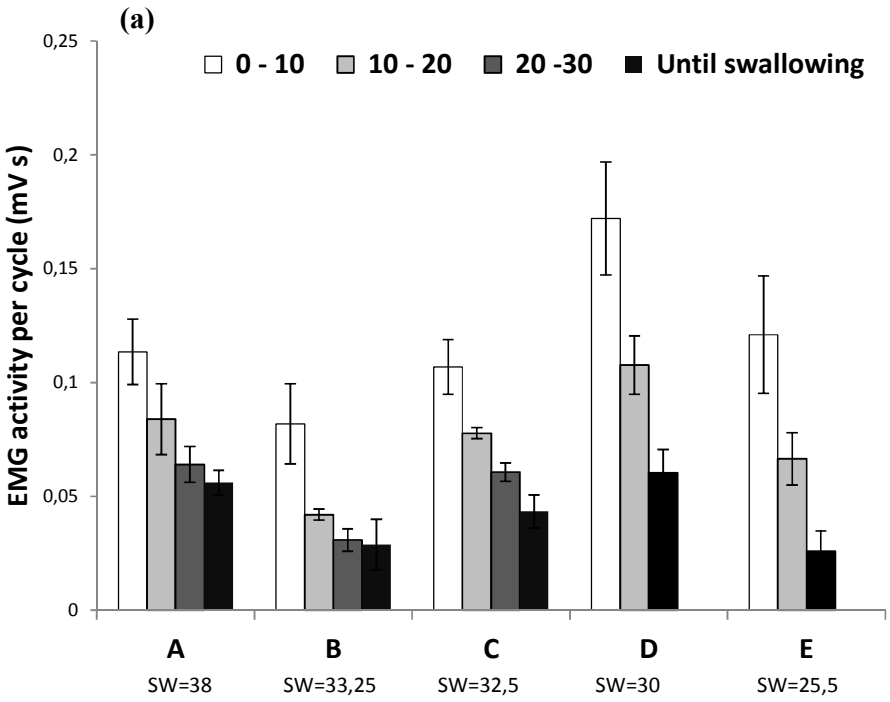

D-BB
D-BS
Wtot
Wc
Vmax
Vm
CT
BD
BN
D-RB
C-BB
E-BB
A-BB
C-BS
E-BS
A-BS
B-BB
D-TB
CE
B-BS
SF
A-RB
C-RB
A-TB
C-TB
CR
E-RB
B-TB
E-TB
B-RB
PC2 (31.9%)
PC 1 (38.5 %)

Fig. 2


(a)
0 - 10
10 - 20
20 -30
Until swallowing
EMG activity per cycle (mV s)
0,25
0,2
0,15
0,1
0,05
0
A
SW=38
B
SW=33,25
C
SW=32,5
D
SW=30
E
SW=25,5

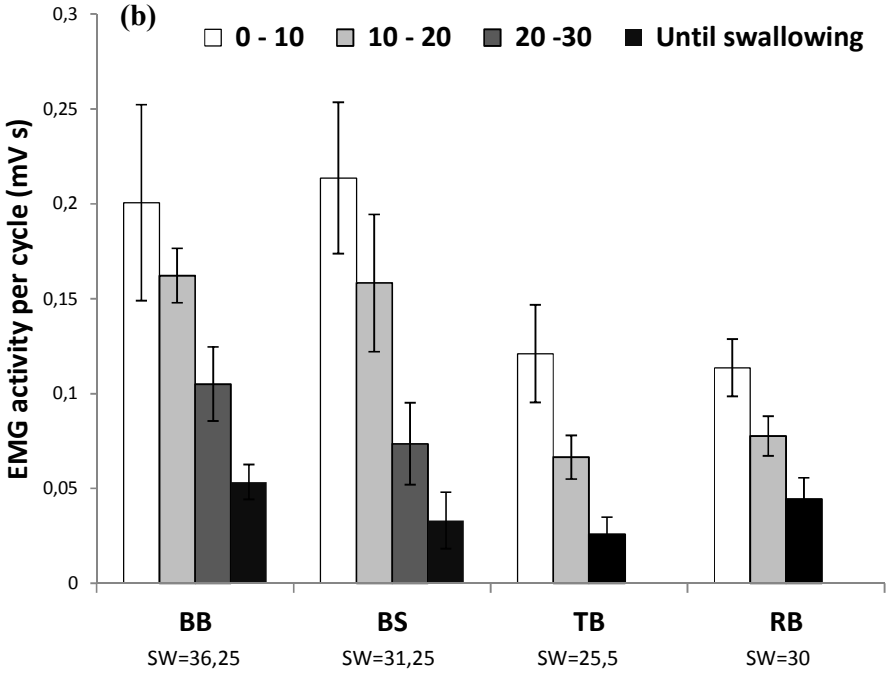

(b)
0 - 10
10 - 20
20 -30
Until swallowing
EMG activity per cycle (mV s)
0,3
0,25
0,2
0,15
0,1
0,05
0
BB
SW=36,25
BS
SW=31,25
TB
SW=25,5
RB
SW=30

Fig. 3

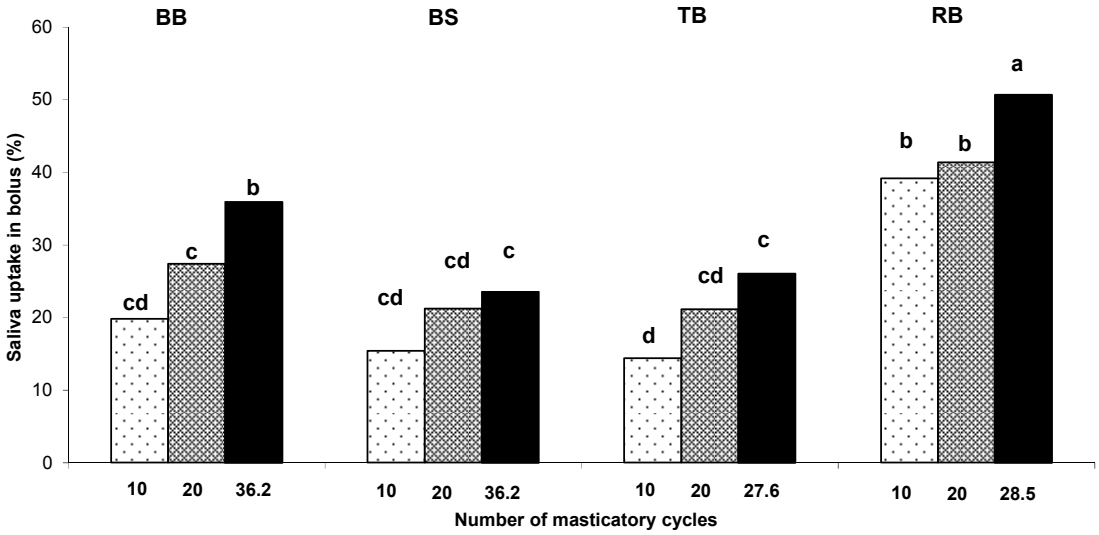

BB
BS
TB
RB
Saliva uptake in bolus (%)
60
50
40
30
20
10
0
cd
c
b
cd
cd
c
d
cd
c
b
b
a
10
20
36.2
10
20
36.2
10
20
27.6
10
20
28.5
Number of masticatory cycles

Fig. 4

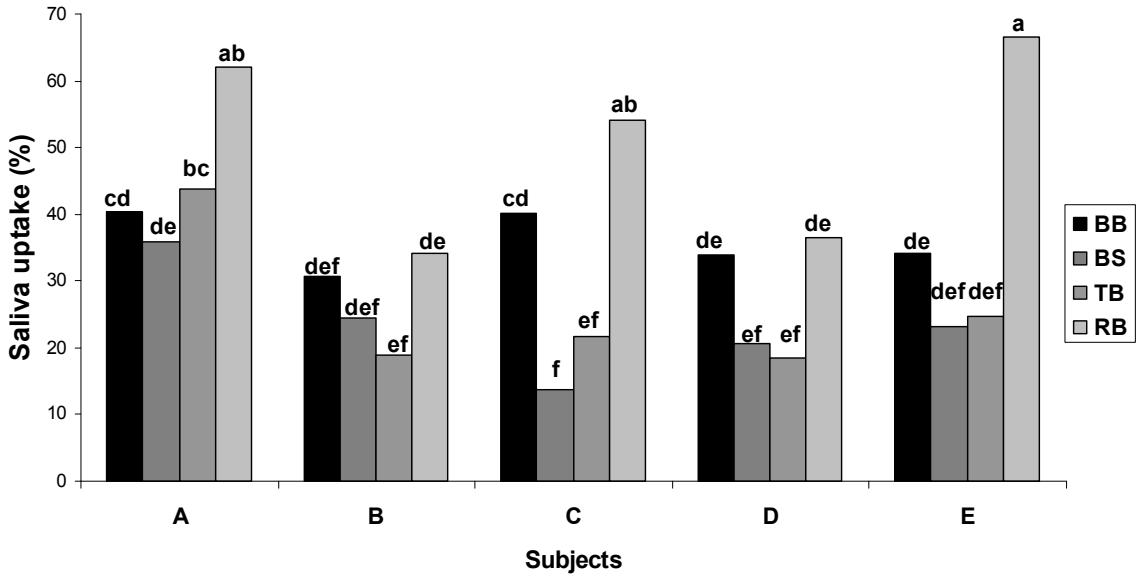
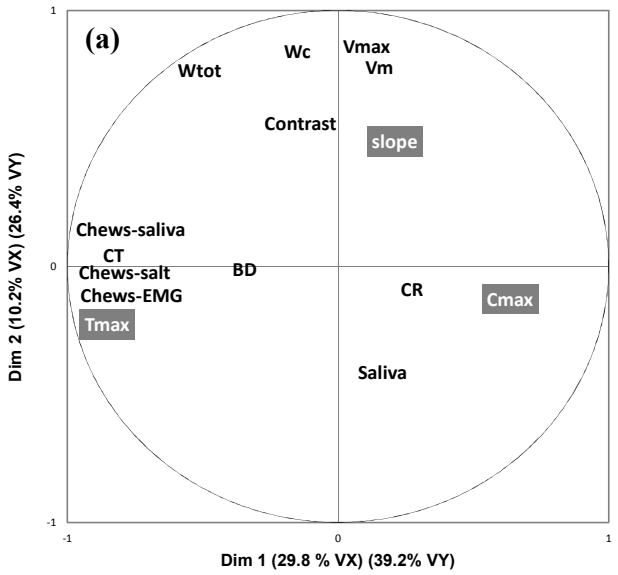

Saliva uptake (%)
70
60
50
40
30
20
10
0
cd
de
bc
ab
def
def
ef
de
cd
f
ef
ab
de
ef
ef
de
de
def
def
a
A
B
C
D
E
Subjects
BB
BS
TB
RB

Fig. 5


(a)
Wc
Vmax
Vm
Wtot
Contrast
slope
Chews-saliva
CT
Chews-salt
BD
Chews-EMG
CR
Cmax
Tmax
Saliva
Dim 2 (10.2% VX) (26.4% VY)
Dim 1 (29.8 % VX) (39.2% VY)
1
0
-1

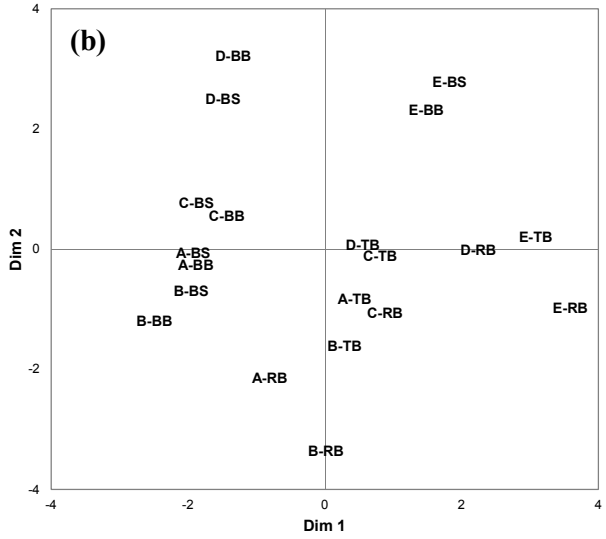

(b)
D-BB
E-BS
D-BS
E-BB
C-BS
C-BB
E-TB
D-TB
D-RB
C-TB
A-BS
A-BB
B-BS
A-TB
E-RB
C-RB
B-BB
B-TB
A-RB
B-RB
Dim 2
Dim 1
4
2
0
-2
-4

Table 1 Physical properties of the four breads studied. Different letters indicate significant differences between products ($p < 0.05$)

Code	Breads	Supplier	Brand	Density (g / mL)	Water content (g H_2O / 100 g total weight)	Approximate crust/crumb weight ratio (%)	Maximum force (N)*	Sodium content (mg / 100 g)
BB	Baguette	Artisan baker	'banette'	0.194 ± 0.01^{c}	23.2 ± 1.1^{d}	69/31	30.8 ± 6.5^{b}	575.3 ± 13.6^{a}
BS	Baguette	Supermarket	standard	0.180 ± 0.02^{c}	29.6 ± 0.9^{c}	68/32	23.7 ± 7.2^{c}	596.6 ± 10.2^{a}
TB	Toast bread	Supermarket	'Jaquet'	0.270 ± 0.03^{b}	31.3 ± 1.4^{b}	24/76	16.4 ± 1.4^{d}	438.6 ± 51.0^{b}
RB	Rye bread	Organic shop	'Pural'	0.602 ± 0.03^{a}	42.1 ± 1.9^{a}	0/100	93.4 ± 3.5^{a}	408.3 ± 41.6^{b}

*maximum force was obtained by uniaxial compression using a TAXT2 texture analyser.

Table 2 Chewing efficiency of subjects and contrast (features from textural image analysis) values of boluses collected at swallowing and the number of masticatory cycles required to form a bolus obtained from different subjects (A, B, C, D, E) eating 4 different breads (BB, BS, TB and RB). Average values ± standard deviation

		F*(p)*[1]	A	B	C	D	E
Contrast[2]	BB	13.99***	2.60 ± 0.07^{c}	2.75 ± 0.11^{b}	2.71 ± 0.11^{b}	2.79 ± 0.07^{b}	2.88 ± 0.11^{a}
	BS	28.23***	2.69 ± 0.11^{c}	2.86 ± 0.15^{b}	2.59 ± 0.08^{c}	2.63 ± 0.13^{c}	3.03 ± 0.11^{a}
	TB	2.27 NS	2.73 ± 0.05	2.84 ± 0.19	2.70 ± 0.09	2.84 ± 0.22	2.88 ± 0.25
	RB	6.51***	2.17 ± 0.06^{b}	2.19 ± 0.12^{b}	2.16 ± 0.07^{b}	2.35 ± 0.012^{a}	2.22 ± 0.14^{b}
Nb cycle required to	BB	52.91***	35.7 ± 1.8^{b}	40.3 ± 1.8^{a}	40.4 ± 0.5^{a}	34.7 ± 3.4^{b}	30.0 ± 1.7^{c}
form a bolus	BS	31.24***	38.3 ± 3.0^{b}	34.3 ± 1.3^{b}	46 ± 8.13^{a}	34.6 ± 2.1^{b}	27.7 ± 2.1^{c}
	TB	15.22***	31.3 ± 2.5^{a}	25.7 ± 3.2^{b}	32 ± 4.5^{a}	24.3 ± 3.4^{b}	24.6 ± 2.6^{b}
	RB	19.35***	35.6 ± 6.9^{a}	27.7 ± 4.0^{b}	36 ± 8.9^{a}	20 ± 4.26^{c}	23 ±1.5^{bc}
Chewing efficiency (%)		9.4***	25.4 ± 6.9^{a}	20.0 ± 5.5^{ab}	15.6 ± 4.9^{ab}	10.8 ± 0.7^{bc}	2.4 ± 0.9^{c}

[1] *F* value and associated significance effect (NS non-significant: $p > 0.05$,*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$) obtained from ANOVAs testing the subject effect,

[2] contrast: textural feature obtained from texture image analysis (a high value represents a high level of heterogeneity). a, b, c: Mean values with different letters are significantly different ($p < 0.05$).

Table 3 Effect of the type of bread (BB, BS, TB, RB) on average (n = 15) temporal sodium release parameters

	$F_{product}, p$ [a]	BB	BS	TB	RB
Number of chews	9.9**	34.2 ± 5.2 [a]	34.9 ± 6.1 [a]	28.3 ± 5.19 [b]	29.4 ± 5.9 [b]
Cmax (mg 100 g^{-1} saliva)	8.7 **	166.1 ± 51.4[b]	175.3 ± 69.1[b]	217.8 ± 59.3[a]	232.5 ± 57.7[a]
Tmax (no. of chews)	5.7 *	29.7 ± 8.9[ab]	32.8 ± 6.2[a]	26.2 ± 4.5[b]	27.7 ± 5.0[b]
Slope (mg 100 g^{-1} saliva s^{-1})	1.8 [NS]	7.0 ± 3.9	7.2 ± 4.1	6.0 ± 3.0	7.8 ± 4.4

[a] *F* and *p* values (*: $p < 0.05$, **: $p < 0.01$, NS: $p > 0.05$) obtained from the analysis of two-way ANOVA (random subjects, breads). [a, b]: average values (± standard deviation) associated with the same letters are not significantly different ($p = 0.05$)

Table 4 Effect of the subjects (A - E) on average (n = 12) temporal sodium release parameters

	$F_{subjects}$ p [a]	A	B	C	D	E
Number of chews	15.5**	35.0 ± 4.9[a]	33.0 ± 4.9[a]	33.6 ± 5.7[a]	32.0 ± 4.5[a]	23.5 ± 2.8[b]
Cmax (mg 100 g^{-1} saliva)	7.2 **	237.3 ± 62.0[a]	159.4 ± 48.1[b]	169.7 ± 68.6[b]	204.1 ± 56.1[ab]	219.2 ± 60.7[ab]
Tmax (no. of chews)	13.8 ***	30.5 ± 4.9[a]	32.3 ± 4.6[a]	29.5 ± 5.9[a]	32.0 ± 5.8[a]	20.6 ± 4.2[b]
Slope (mg 100 g^{-1} saliva s^{-1})	20.5***	7.7 ± 2.2[b]	6.2 ± 2.8[b]	3.2 ± 1.2[c]	6.7 ± 2.9[b]	11.4 ± 4.3[a]

[a] F and p values (*: $p < 0.05$, **: $p < 0.01$, NS: $p > 0.05$) obtained from the analysis of two-way ANOVA (random subjects, breads). [a, b]: average values (± standard deviation) associated with the same letters are not significantly different ($p > 0.05$)