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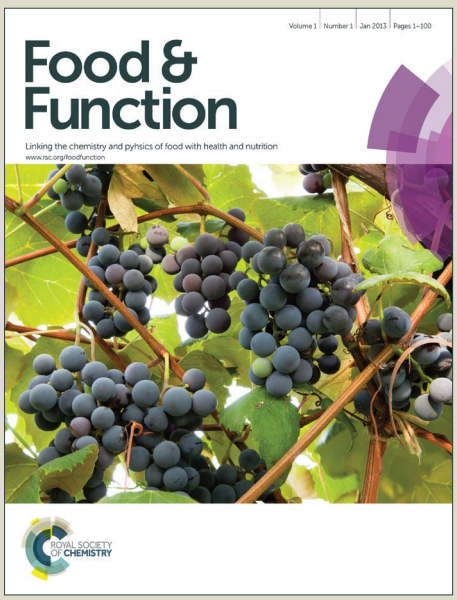

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Breakdown pathways during oral processing of different breads: impact of crumb and crust structures

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Abstract

Oral processing during bread consumption is a key process related to the dynamics of texture perceptions, sensory stimuli release and starch digestion. The aim of this study was to determine the respective contribution of bread properties (composition and structure of crumb and crust) and of the oral physiology of subjects to the breakdown pathways in the mouth. The properties of the *in vivo* bread bolus obtained from eight healthy subjects were studied at three key points in time during their oral processing. The progressive lubrication and breakdown of bread were observed, as well as the beginning of the enzymatic degradation of starch. The study showed that "time" was the factor responsible for the greatest variability in bolus properties. Breakdown pathways were established for crumbs with and without crust. The presence of crust modified the oral processing, increasing, for instance, the heterogeneity of particle size at the middle of the oral processing sequence. Moreover, the hydration capacity of crust contributed to high starch degradation at swallowing time, in comparison with crumb alone. The main subject characteristics impacting bolus properties were the in-mouth duration, the individual masticatory index and the mouth volume, while the main bread properties explaining the bolus properties were the initial composition and the water-absorbing capacity. We concluded that both crumb and crust

structures had an impact on the oral processing, affecting the capacity of hydration, the rheology and the breakdown degree of the bolus.

Keywords: Breakdown pathways, bread, oral processing, food bolus

Introduction

Carbohydrates are the main source of energy in human nutrition, supplying up to 45% of the energy requirements in developed countries, and up to 85% in developing countries.[1] Bread is a widely consumed food and thus a major source of carbohydrates in the European diet, contributing an average of 27% of the total carbohydrate intake.[2] A wide diversity of breads in terms of composition and structure exists all over the world, leading to a wide range of sensory and nutritional properties. These functional properties are known to be key factors for product acceptance by consumers and are also largely driven by food oral processing. There is a need to better understand the relationship that exists between bread characteristics, oral processing and functionalities to develop products consistent with consumer expectations.

Food oral processing is an essential step in the eating process, which aims at preparing the food for swallowing and digestion. It is also involved in the release of sensory stimuli responsible for perception,[3] essential for the palatability of foods. During oral processing, food is progressively transformed through different stages, occurring successively or simultaneously, into a bolus suitable for swallowing.[4] Bolus formation is thus a dynamic process involving at least three simultaneous phenomena: (i) the mechanical breakdown of the food product into several particles by mastication;[6,5,7] (ii) particle hydration and lubrication by saliva to form a bolus;[8,9] and (iii) enzymatic degradation, in particular, by salivary amylase in the case of starch products.[10] Both food properties and individual oral physiology have an impact on the dynamics of bolus formation and, consequently, its physicochemical properties, leading to specific "breakdown pathways".[11]

The bolus formation of starch-based products has been largely studied in the literature. For instance, in the case of starch-based custard, the role of salivary α-amylase seems to be essential to explain sweetness.[12] As illustrated with breakfast cereals, the rheological properties of the bolus such as adhesiveness, cohesiveness and springiness, and the sensory perceptions such as stickiness were identified as potential factors for swallowing initiation.[13] In the case of bread, oral processing and bolus properties have been identified as drivers for texture and salty perceptions.[14] The lubrication of a crumb bolus by saliva induces a decrease in some rheological variables (G' and G" moduli), and depends on particle size during oral processing.[15] However, it seems that bolus lubrication by saliva has a greater impact on its rheology than on its comminution. Saliva acts as a lubricant during bolus formation but is also responsible for carrying stimuli such as salt from the product to the receptors. The amount of salt released in saliva has been linked to mastication parameters: greater chewing muscle activity induced a faster release of sodium.[16] Lubrication and breakdown are not the only mechanisms that occur during the oral processing of bread. It has been shown that the digestion of starch begins in the mouth with its degradation into oligosaccharides.[10] Moreover, the density of bread had an impact on the digestibility of starch. When the bread had a higher density, the accessibility of amylase to the starch could be limited, resulting in a poor hydrolysis of the starch.[17]

Bread is a heterogeneous food composed of crumb and crust. It can be consumed in two different ways during the same meal (crumb and crust together or crumb alone). The comparison of these two types of consumption was recently performed to study their impact on food oral processing.[18] The crust had an impact on the chewing behavior of the panelist. A thick and dry crust induced the extensive breakdown of bread structure.

The mechanisms involved in bread oral processing are complex, notably due to its multiphase structure. Only one study[18] has dealt with the impact of crust on oral processing, but with only one panelist, and to our knowledge, mechanical breakdown, hydration and enzymatic degradation have not been studied simultaneously to better understand their dynamics and their respective contribution. In this context, the aims of this study are to

investigate the temporal aspect of the bolus formation of a heterogeneous food during oral processing, and to determine the respective impact of bread properties and of subject characteristics on bolus properties, including breakdown, hydration and enzymatic degradation. For this purpose, the properties of a bolus obtained *in vivo* from crumb alone or from crumb and crust were explored at three key points in time during oral processing.

Experimental

Materials

Three breads, B1, B2 and B3, were par-baked and frozen French baguettes manufactured by Lesaffre International (Marcq-en-Baroeul, France). They contained the same wheat flour but were produced through different processes to obtain different structural properties. Bread B4 was a commercial bread (Auchan, Plaisir, France) manufactured from whole wheat flour containing milled wheat grains. This bread was selected to have a density close to the one of bread B1 and a water content close to the one of bread B2 (Fig. 1). These four breads were used to study the impact of crumb structure and bread density (B1 vs. B2 vs. B3) and composition (B4 vs. B1, B2, B3) on the dynamics of bolus formation.

Breads B1, B2 and B3 were baked in a Wiesheu Minimat oven (Wiesheu GmbH, Germany) at 220 °C for 9, 7 and 8 min, respectively. A strict baking protocol was applied (control of bread positions in the oven and of baking temperature and time), and the density and Young modulus of the bread after each baking was controlled to check baking repeatability. The breads were used for tests after 2 hours of cooling and within a 2-hour interval. The commercial bread was always bought the morning of the experimentation day.

The sample size was close to the one used in natural eating behavior, as suggested by Hutchings and coworkers.[19] In order to have similar volumes for all breads for the tests, the following sampling protocol was applied (Fig. 2): crumb samples were die-cut in a cylinder (h: 2.5 cm; d: 3.0 cm) and crumb with crust samples were cut with a knife in a half-cylinder from

the side of a vertical slice (h: 2.5 cm; r: 3.0 cm). In this way, the sample contained every part of the crust (bottom, side and top).

Bread properties

Bread contents in terms of water and starch. Water content (g/100 g) was determined after drying for a minimum of 15 h and a maximum of 24 h at 110 °C in an oven for both crumb and crumb with crust samples from three baking replicates.

Total starch content of the crumb (g/100 g) was determined in triplicate according to the procedure described by Goni, Garcia-Alonso & Saura-Calixto.[20] Briefly, it consisted of a total hydrolysis of starch by amyloglucosidase. The glucose was then measured using a commercial kit from Biosentec (v10-10321, Biosentec, France).

Bread structure. Crust thickness (mm) was measured with the help of the ImageJ software (version 1.48v, National Institutes of Health, USA) on breads from three baking replicates. A horizontal slice along the complete length of the bread was scanned by an Epson GT-1500 scanner (Seiko Epson Corporation, Japan) with a resolution of 600 dpi. The crust thickness was then measured on 40 positions of the picture by visual discrimination of colors.

Crumb/crust ratio was determined in triplicate by separately weighing the crumb and the crust of a slice of bread (2.5 cm length). The crumb was separated from the crust. The crumb that could not be detached by hand was considered as part of the crust.

Bread density (no unit) was measured with the rapeseed displacement method on three baking replicates.[21] The rapeseeds had a bulk density of 0.73 g/cm^3. The measurements were performed in a rectangular box (32 cm x 22.5 cm x 9.5 cm), which was manually filled with rapeseed.

Bread properties. Crumb firmness (Young modulus, kPa) was measured by performing a compression test on crumb cylinders (h: 2.5 cm; d: 3.0 cm) from three different baking batches with a TA.XT *plus* Texture Analyzer (Stable Micro System, UK) equipped with a 3.0-cm-diameter plate and a 30-kg load cell. Compression was set at 66% of the strain of the

initial height, at a speed of 0.83 $mm.s^{-1}$. The Young modulus is the initial slope of the stress-strain curve obtained.

Water-absorbing capacity (g of absorbed water/g of dry matter) was determined in triplicate for crumb cylinders (h: 2.5 cm; d: 2.6 cm) and crumb with crust slices (length: 2.5 cm). The cylinders and the slices were weighed and submerged in a fixed volume of Milli-Q water (Merck Millipore, Merck KGaA, Germany), 35 mL and 400 mL, respectively, for 30 min. They were then drained for 20 s on a grid. The wet cylinders and the wet slices were weighed and dried in an oven for a minimum of 15 h and a maximum of 24 h at 110 °C. The water-absorbing capacity is the ratio between the amount of water that is absorbed and the dry extract of the sample.

Oral physiology of the subjects

Eight healthy subjects (four male and four female, aged from 24 to 37 years old) with good dental status were recruited for the study. Subjects gave their written informed consent to participate in this study. They were asked not to eat or drink for at least one hour before the sessions. The individual masticatory index, the salivary properties and the volume of the oral cavity were determined for every panelist in triplicate.

Oral volume. The panelists' mouth volume was measured with an acoustic rhinopharyngometer from Eccovision (Sleep Group Solutions, North Miami Beach, FL, USA). During measurement, subjects were asked to breathe through their mouths. Mouth volume (cm^3) was calculated as described by Doyennette, de Loubens, Déléris, Souchon, & Trelea.[22]

Saliva properties. Salivary flow rate (mL/min) was measured in stimulated conditions[9]. Subjects were asked to chew a piece of 0.5 g of Parafilm (American National Can Company, Menasha, WI, USA) for 6 min and to spit their saliva into a pre-weighed vessel every 30 s. Saliva collected during the first minute was not considered for calculation. The salivary flow rate was determined as the ratio between the mass of saliva that was spit out and the sampling duration, assuming that the weight of saliva is equal to its volume (density close to 1.0). A part of the saliva that was collected was immediately used after expectoration to

determine salivary viscosity and dry extract, and the other part was frozen at -80 °C for salivary amylase activity analysis.

The salivary viscosity (mPa.s) was measured with a MCR 301 rheometer (Anton Paar, Austria), fitted with a cone geometry (60 mm in diameter, 2° angle) and a plate measuring 60 mm in diameter. A quantity of 2.5 mL of saliva was spread on the plate, and a thin layer of SDS solution was applied around the rim of the plates in order to minimize protein adsorption at the air-liquid interface.[23] Measurements were performed at 35 °C and a shear-rate ramp from 2 to 450 s^{-1} was applied.

The salivary dry extract (g/100 g of saliva) was measured by drying 1 mL of saliva in an oven for a minimum of 15 h and a maximum of 24 h at 110 °C.

The salivary amylase activity (U/mL) was measured on 200 µL of saliva using a commercial kit from IBL International (ref: RE80111, IBL International GmbH, Germany). The saliva samples were defrosted two hours before the analysis at 4 °C and centrifuged at 2000 rpm for 10 min to remove all particles.[24]

Individual masticatory index. Individual masticatory index was measured for each subject by chewing standardized cylinders (3 g; h: 1.8 cm; d: 1.4 cm) of Optosil dental silicone during 20 masticatory cycles.[14] The particles obtained were dried in an oven for 1 h at 75 °C. The index was calculated as the ratio of the amount of sample that passed through a 4 mm sieve over the amount of expectorated sample.

Determination of in-mouth duration for each subject

The in-mouth duration (s) was determined per subject and per bread, for crumb with and without crust during three sensory sessions (data not shown). During these sessions, subjects performed a sensory method (Temporal Dominance of Sensations), which allowed to record the in-mouth duration on line, from the first bite to the first swallowing. The in-mouth duration that were measured here were probably longer than during natural eating due to the simultaneous achievement of a sensory evaluation task, as already shown by de Lavergne, van Delft, van de Velde, van Boekel, & Stieger.[25] Significant differences were observed

193 between breads and between samples with and without crust. However, the difference
194 between breads was too small to be controlled (especially at the beginning of oral
195 processing). The mean values of in-mouth duration were therefore calculated for each
196 subject and for crumb and crumb with crust samples. From these mean values, three
197 moments of oral processing were determined: T1 = 10% of in-mouth duration, T2 = 40% of
198 in-mouth duration and T3 = in-mouth duration.[14]
199
200 **Properties of expectorated boli**
201 **Bolus collection.** Each subject participated in eight individual sessions of 30 min for bolus
202 collection. One session was dedicated to one bread (B1, B2, B3 or B4) and one condition
203 (crumb or crumb with crust). Subjects were asked not to eat or drink for at least one hour
204 before the sessions. Samples (cylinder for crumb, half-cylinder for crumb with crust) were cut
205 for a maximum of one minute before presenting them to subjects in a cup. The first sample
206 was a warm-up product, used to stimulate salivary production. Three moments of oral
207 processing were studied (T1, T2 and T3). Samples from these three times and all replicates
208 were performed during the same session. The bolus was used immediately after
209 expectoration for every analysis except for soluble glucose content analysis, for which the
210 bolus was immediately frozen in liquid nitrogen and stored at -80 °C.
211 **Image analysis of particles on expectorated boli.** For image analysis of particles, bolus
212 samples were prepared as described by Le Bleis and coworkers.[15] After the dispersion of
213 bolus particles in glycerol, solutions were poured into Petri dishes (diameter: 140 mm). One
214 replicate was performed for each time (T1, T2 and T3) of oral processing for all breads and
215 all panelists. Particle images were acquired using a Canon EOS 700D camera (Canon Inc.,
216 Japan) and a ScanCube 308 (Altawak Technologie, France) that conferred a standardized
217 brightness. Image acquisitions were monitored with Easy ScanCube 1.9 software (Altawak
218 Technologie, France). Images were digitized as matrices of 3434 x 3434 pixels. Their
219 analysis was performed with ImageJ software (version 1.48v, National Institutes of Health,
220 USA) as follows. A black and white threshold was applied to images to provide the number of

particles and their area. After exporting these data to Excel (2007, Microsoft Corporation, USA), the median equivalent diameter D50 (mm) and the interquartile range D75/D25 (no unit) were calculated, assuming that particles could be modeled by a disc. The median equivalent diameter and the interquartile range represented the degree of degradation and the heterogeneity of the bolus, respectively. When the interquartile range D75/D25 increased, the heterogeneity of the particle size of the bolus also increased.

Water content, hydration rate and amylase activity of expectorated boli and amount of residual bolus after spitting. The water content of expectorated bolus (g/100 g of bolus) was determined in triplicate for each oral processing time by drying samples in an oven at 110 °C for a minimum of 15 h. The amount of saliva that was incorporated (hydration rate h_s, g/100 g of dry matter) and the amount of food remaining in the mouth after spitting (w/w % residues, no unit) were calculated as described by Drago and coworkers.[9] The amylase activity in bolus a_s (U/g of dry matter) was calculated as follows: $a_s = h_s \times A / 100$, where h_s is the amount of saliva incorporated into the bolus and A the salivary amylase activity of the subject.

Texture analysis on expectorated boli. Textural properties were measured by a Texture Profile Analysis (TPA) using a TA.XT *plus* Texture Analyzer (Stable Micro System, UK), fitted with a 6.0-cm-diameter plate and a 30-kg load cell. Two successive compression cycles were set at a constant speed of 0.83 $mm.s^{-1}$. A compression ratio of 65% of the strain of the initial height was selected. A resting time of 1 s between the two compressions was applied. Boli were gently shaped in a cylinder of 3.0 cm in diameter, with the help of a cut syringe. The surface was smoothed with the piston of the syringe. Three replicates were performed for each moment of oral processing (T1, T2 and T3). The parameters, hardness, adhesiveness and cohesiveness, were extracted from force-time curves. The hardness parameter (N) is defined as the maximal peak force obtained during the first compression, the adhesiveness parameter (N.s) is defined as the area under the negative curve obtained during the first decompression, and the cohesiveness parameter (no unit) is defined as the ratio between

248 the area under the curve obtained during the second compression and the area under the
249 curve obtained during the first compression.[13]

250 **Rheological analysis of expectorated boli at swallowing time.** Small-amplitude oscillatory
251 shear tests were conducted on expectorated boli at swallowing time (T3) with a rheometer
252 MCR 301 (Anton Paar, Austria), equipped with four-blade vane geometry (10 mm in
253 diameter, 8.8 mm in length, ST10-4V-8.8/88, Anton Paar, Austria). Two or three bolus
254 samples were directly expectorated into the apparatus cup (18 mm in diameter,
255 CC17/T200/SS, Anton Paar, Austria) so that the cup was full. After determining the linear
256 viscoelastic domain, a constant strain of 0.5% and a frequency of 1 Hz were chosen to
257 perform the tests. Measurements were carried out for 1 min at 35 °C in order to obtain
258 average storage (G') and loss (G") moduli (kPa). One replicate was performed for each
259 sample.

260 **Release of soluble glucose in the bolus at swallowing time.** Soluble glucose content
261 (g/100 g of bread) was measured in breads and boli at swallowing time using a commercial
262 kit from Biosentec (v10-10321, Biosentec, France). Bread samples (crumb and crumb with
263 crust) were cut into 5-mm particles with a knife and weighed. Bolus samples had previously
264 been defrosted at 4 °C and weighed. Milli-Q water (Merck Millipore, Merck KGaA, Germany)
265 was added to the samples. The amount of water that was added was equal to the bolus
266 weight or 2.5 times the bread weight for the bolus and bread analysis, respectively. After
267 manual stirring and resting for 10 min, samples were centrifuged at 11000 rpm for 5 min at 4
268 °C. The supernatant was recovered and glucose content was determined with the kit. One
269 replicate was performed on bolus samples at swallowing time (T3) for the eight subjects.
270 Three replicates were performed on bread samples. The data were corrected by the factor of
271 dilution. The release of soluble glucose (ΔGlucose) was calculated by subtracting the
272 glucose content in the bread from the glucose content in the bolus. Glucose was used as an
273 indicator of starch hydrolysis, although it is not the main reaction product.

274

275 **Statistical analyses**

All statistical analyses were carried out with XLStat software (Version 2010.4.02, Addinsoft, France).

A Kruskal-Wallis non-parametric test was performed on the bread property data. A significant level of $p < 0.05$ was chosen.

One-way ANOVA (Subject, $p < 0.05$) was performed with a multiple comparison test (Fisher's LSD test) on oral physiology parameters. A Principal Component Analysis (PCA) and a Hierarchical Clustering Analysis (HCA) were performed on reduced and centered data of oral physiology.

To obtain normally distributed data, the bolus parameters were transformed into logarithms, square roots or inverses, when necessary. Four-way ANOVA ($p < 0.05$) was performed with a multiple comparison test (Fisher's LSD test) on the transformed data of bolus properties. For crumb data and crumb with crust data, two-way ANOVA ($p < 0.05$) were performed with a multiple comparison test (Fisher's LSD test). PCA were performed on reduced and centered data of bolus properties, and were used to establish breakdown pathways.

Pearson's correlations ($p < 0.05$) between the bolus properties of crumb and crumb with crust samples were performed at swallowing time.

Partial Least Square (PLS) regressions were carried out separately for each time. The PLS were carried out to explain bolus properties (crumb with and without crust samples, Y-variables) by crumb properties, crumb with crust properties and oral physiology parameters (X-variables). The quality of the regression was judged on the R^2 value ($R^2 > 0.500$), and an X-variable was significant when the confidence interval of its normalized coefficient did not include the zero value.

Results and discussion

Bread properties

The four breads had different compositions and structural properties (Table 1). Bread B1 was characterized as a dense bread with a firm crumb and a thick crust. Its crumb and crust

contained about 10% more water than the others. Bread B2 was characterized as a bread with a low density, a soft crumb and a thick crust. Contrary to the other breads, the crust mass in a slice was higher than the crumb mass. This bread was also characterized by a high water-absorbing capacity (WAC) both for crumb and crumb with crust samples. Bread B3 had an intermediary density, a firm crumb and a thin crust. The crust of bread B3 had the capacity to absorb water (WAC of crumb with crust > WAC of crumb), which was not the case for the other breads. Bread B4 was characterized as a dense bread with a very firm crumb and a very thin crust. Its crumb contained almost 20% less starch than the others, probably because of the presence of fibers and of milled wheat grains in its composition.

Subjects characteristics

The eight subjects presented a wide range of physiologic properties. The in-mouth duration varied between 18 and 41 s for crumb samples, and between 25 and 50 s for crumb with crust samples. Their individual masticatory index ranged from 0.24 to 0.77 and their mouth volume from 27 to 74 cm^3. The stimulated saliva was characterized as a shear-thinning fluid, with a viscosity measured at 100 s^{-1} ranging between 1.07 and 2.65 mPa.s. The salivary flow of subjects varied from 1.16 to 2.59 g/min and the dry extract of saliva from 0.21 to 0.64 g/100 g of saliva. Salivary amylase activity ranged between 38 and 400 U/mL of saliva. These values were on the same order of magnitude as the ones found in the literature.[24,26] By using a HCA, two groups of subjects could be distinguished, differing in terms of the time required for swallowing and individual masticatory index: J1, J2, J4 and J5 panelists were characterized by a short in-mouth duration and a high masticatory index and were opposed to J3, J6, J7 and J8 panelists, as illustrated in Fig. 3. The individual masticatory index and the in-mouth durations were correlated ($p < 0.001$).

Bread breakdown pathways

Variation over time of the properties of expectorated boli. During the oral processing, all of the parameters significantly changed over time (Tables 2.a and 2.b.). Moreover, the effect

of time is greater than the one of bread properties (F(time) > F(bread)) for almost all of the parameters, except for heterogeneity (and adhesiveness in the case of crumb samples). This result was highlighted, in Fig. 4, by the fact that 59.1% of the variability of the PCA was contained by axis 1 that separated the three studied oral processing times. The second axis (16.0%) separated the samples of crumb and crumb with crust, and the four breads.

In the case of crumb samples (Table 2.a), a 4-fold factor can be observed on the number of particles between T1 and T3 for crumb boli ($p < 0.0001$), regardless of the bread. At the same time, the particle size decreased ($p < 0.0001$) due to the mastication process: the median equivalent diameter varied from 16.8 mm at T1 to 3.0 mm at T3. This variation had already been observed for crumb.[15] However, no variation in the D75/D25 heterogeneity over time was observed in the case of crumb samples ($p = 0.087$). The percentage of in-mouth residues increased ($p < 0.0001$) from 1.35% at T1 to 11.8% at T3, probably because of the higher number of small particles. As expected, water content increased ($p < 0.0001$) from 54.0 g/100 g at T1 to 66.3 g/100 g at T3, as more and more saliva was added (increase in hydration rate, $p < 0.0001$). The water content increased more quickly between the bread (before being put in the mouth) and the bolus at T1 than between the times T1 and T3 (Fig. 5). This is probably due to the saliva already present in the mouth before introducing food. The saliva is thus mainly incorporated at the beginning of oral processing. This result was also observed on products with a low water content like biscuits and Dutch cake.[27] The activity of salivary amylase in the bolus was multiplied by five between T1 and T3 ($p < 0.0001$), which is explained by the increase in saliva content in the bolus during oral processing. Adhesiveness generally increased between bread introduction in the mouth (T1) and T2 ($p = 0.003$). The hardness parameter was divided by six between T1 and T3 ($p < 0.0001$). Similar variations for this hardness parameter were found for cereal boli,[13] even if we did not observe an increase in the cohesiveness parameter over time in the present case but, instead, a decrease between T1 and T2 and an increase between T2 and T3 ($p < 0.0001$). In fact, cohesiveness seems to depend on the number of pieces of food that were initially taken into the mouth: cereals were composed of several petals, while the bread was

359 in one piece when it was taken into the mouth. Several petals can form a cohesive bolus as
360 their lubrication increases, whereas, in the case of bread, the initial piece of crumb probably
361 first becomes less cohesive with its destruction into particles through mastication, and could
362 then possibly be reassembled due to hydration by saliva to form a bolus ready to be
363 swallowed.
364 In the case of crumb with crust samples (Table 2.b), the same trends were observed
365 between crumb and crumb with crust samples for particle number and size, hydration rate,
366 water content, amylase activity and bolus hardness. Results from the four-way ANOVA
367 indicated a significant crust effect for these bolus properties ($p < 0.01$), except for particle
368 number ($p = 0.342$). Thus, the presence of crust in the bolus induced some changes in bolus
369 properties: larger particles, lower water content over time, lower amylase activity and a
370 harder bolus. For the other properties, the variations over time were not the same for crumb
371 and crumb with crust samples and led to different breakdown pathways (Fig. 4).
372 Heterogeneity and adhesiveness were the parameters that were the most impacted by the
373 presence of crust (main contribution to axis 2 of the PCA, separating crumb with and without
374 crust). The D75/D25 heterogeneity was maximal at T2, while there was no variation in this
375 parameter for the crumb samples. The unequal particle size probably reflected the difficulty
376 to breakdown a heterogeneous food composed of both hard and soft materials. Boli from
377 crust and crumb samples were more adhesive, especially at the end of the oral processing.
378 We observed a decrease in the cohesiveness parameter, which was probably due to
379 interactions between adhesiveness and cohesiveness, as suggested by Chen.[28] Since crumb
380 with crust boli were sticky, cohesiveness was probably interfering with adhesiveness.
381 Moreover, the differences in structure between boli at T1 (bread barely deconstructed) and
382 boli at T3 (thousands of particles pulled together with saliva) probably mean that the physical
383 origin of cohesiveness is different. On the one hand, cohesiveness is ensured by the walls of
384 air cells in the bread, while, on the other hand, cohesiveness is due to particle lubrication by
385 saliva. Contrary to crumb boli, the percentage of in-mouth residues did not change between
386 T1 and T2, but increased between T2 and T3. At T1, the amount of in-mouth residues was

higher in crumb with crust samples than in crumb samples. We can assume that the bite required in the presence of crust force was greater at the beginning of oral processing, as already shown by other authors.[5,29] This could lead to the formation of little particles at T1 that are difficult to expectorate because of their low lubrication degree. The presence of crust thus induced an adaptation of the oral processing over time.

Variations in the properties of expectorated boli between breads. Boli resulting from the four crumbs presented different characteristics (Table 3.a). The initial composition of the bread had an impact on the breakdown and hydration mechanisms, as well as on the textural properties of boli. At every studied point in time during oral processing, boli from B4 had twice as many particles as boli of B1 ($p < 0.0001$) and these particles were smaller ($p = 0.001$). This higher fragmentation could be explained by the presence of fibers and milled wheat grains in this bread. They induced a disrupted gluten network, leading to a weakening of the structure. Boli obtained from B4 crumb had a lower water content than the others at each point in time during oral processing ($p < 0.0001$), but the amount of saliva that was added was the same as in breads B1 and B3 (parallel curves in Fig. 5). Finally, bread B4 led to the hardest bolus, but only at T2 and T3 (significant interaction between bread and time, $p < 0.0001$). This was probably because its crumb was the firmest. At swallowing time, the most adhesive bolus was the one obtained from bread B4 (significant interaction between bread and time, $p = 0.022$).

The crumb structure mainly had an impact on the hydration and the texture of boli. The variation over time of the bolus water content was the same for breads B1 and B3, but the boli from the B2 crumb was hydrated faster and higher than the others (Fig. 5). This hydration capacity during oral processing could probably be explained by the water-absorbing capacity (WAC) of breads, which was also high for B2 crumb. In the case of crumb alone, boli were not very adhesive. A slight increase in the adhesiveness parameter was observed over time, except for bread B3, which explained why the pathway of this crumb was different from the others (Fig. 5).

414 The properties of boli obtained from samples with crumb and crust are also dependent on the
415 type of bread (Table 3.b). Hardness, adhesiveness and heterogeneity were the main factors
416 impacted by the presence of crust. The impact was different depending on their crust
417 structures, leading to a range of breakdown pathways (Fig. 4).
418 The crust thickness probably induced a difference in variation of bolus hardness: the
419 decrease between T1 and T3 in the hardness parameter was greater for breads B1 and B2
420 than for breads B3 and B4 (significant interaction between bread and time, $p < 0.0001$). At
421 the beginning of oral processing, the crust was probably still partially intact, strengthening the
422 bolus structure. The thicker the crust was, the more reinforcement that was provided,
423 whereas the breakdown of the crust led to a decrease in the hardness parameter. Moreover,
424 we observed that the boli from B2 were composed of more particles of a smaller size than
425 the other boli. A thick and dry crust induced a high muscular activity and led to a high
426 breakdown of the bread structure.[18] Thus, the thickness and dryness of B2 crust could
427 explain why these boli were harder at the beginning of oral processing and were quickly
428 broken down into small particles.
429 A significant interaction between bread and time was also observed for the heterogeneity of
430 particle size ($p = 0.036$). The bolus from bread B4 behaved differently than the other boli: its
431 heterogeneity was constant over time (data not shown), like that of the other crumb samples.
432 The presence of crust had no impact on the heterogeneity of the B4 bolus, probably because
433 of its very thin crust.
434 The presence of crust induced a considerable increase in the adhesiveness parameter of boli
435 from breads B1, B3 and B4, compared to the crumb sample (Fig. 4). The bolus from bread
436 B2 was less adhesive than the others, even at swallowing time.
437 **Differences between eating behavior of subjects.** The breakdown pathways varied over
438 time and between breads, but also between individuals (Fig. 6). First, regardless of the
439 subject, and especially for samples of crumb with crust, bread B2 always presented different
440 breakdown pathways, often leading to higher hydrated boli at swallowing time than the other
441 breads.

For five of the eight subjects (illustrated with breakdown pathways of J2 and J4 in Figs. 6.a. and 6.b.), the breakdown pathways of crumb samples were different from the ones of samples of crumb and crust. For the others, like subject J8 (Fig. 6.c.), few differences were observed between breakdown pathways of samples with or without crust.

Among the eight subjects that composed the panel, three subjects (J2, J4 and J8) were representative of the wide range of variability of eating behaviors. For example, for subject 2, the heterogeneity of particle size from his boli of crumb with crust was still increasing at the end of oral processing (T3) (contrary to the other subjects for whom a maximum of heterogeneity was observed at T2), and his boli were 25% less hydrated than the panel mean. Moreover, while subject J4 had distinct breakdown pathways for all of the breads, few differences were observed for subject J8. This could possibly be due to an adaptation of the oral processing of this last subject, in order to obtain similar properties of boli at swallowing.

Different eating behaviors were recently categorized in the literature.[30] “Chewer” and “cruncher” consumers prefer to process the food with their teeth, while “smoosher” and “sucker” consumers use their tongue. Following this categorization, subject J2 could be considered as a “cruncher”, leading to a more rapid breakdown of food, whereas the other subjects tend to be “chewers”.

Bolus properties at swallowing time

Rheological properties. For crumb samples, the G' modulus (tendency, $p = 0.066$) and the G" modulus (significant difference, $p = 0.010$) from bread B4 differed from the others (Table 3). The bolus obtained from B4 was more elastic and viscous than the others at swallowing. Bread density could not completely explain the difference between the G' and G" moduli of the four bread boli (in the range of density used in this study). However, when the range of density was high (from 0.25 to 0.50), it was shown that density had an impact on the G' and G" moduli by increasing in these parameters.[14] In our case, the Young modulus of bread crumb probably had an impact on the G' and G" moduli of the crumb bolus at swallowing. The same results were found with crumb with crust samples.

Enzymatic degradation. Between 2.9 and 6.3 g of glucose per 100 g of bread were released (Table 3), but no significant difference was observed between the four crumbs ($p = 0.180$). However, for crumb with crust samples, boli from bread B4 had the highest release of glucose ($p = 0.017$). The presence of milled wheat grains induced a disrupted gluten network, which probably led to a better accessibility of starch to alpha-amylase. Moreover, boli from bread B3 had a 44% increase in glucose release in the crumb and crust sample compared to the crumb sample. This large increase was not observed for the other breads. This could be explained by the high capacity of B3 crust to become hydrated (WAC of crumb with crust > WAC of crumb). This hydration capacity led to a better incorporation of saliva in the crust and, therefore, to a better degradation of starch by α-amylase.

Correlations between bolus properties. During oral processing, the action of alpha-amylase led to starch hydrolysis. The more saliva and α-amylase that were incorporated, the more glucose that was released (correlation between amylase activity in the bolus and glucose release, $r = 0.492$, $p < 0.0001$).

At swallowing time, boli were composed of large amounts of small particles lubricated with saliva. The incorporation of saliva depended on the particle size (correlation between hydration rate and D50, $r = -0.534$, $p < 0.0001$) and on the heterogeneity of particle size (correlation between hydration rate and D75/D25, $r = -0.329$, $p = 0.008$). A homogeneous distribution of small particles presented a high exchange surface, facilitating the incorporation of saliva.

The lubrication and the deconstruction of the bread had an impact on the rheology parameters of the bolus. The incorporation of saliva led to an increase in the water content of the bolus ($r = 0.876$, $p < 0.0001$), which tended to soften the bolus (correlation between water content and hardness $r = -0.856$, $p < 0.0001$). The lubrication by saliva reduced bolus adhesiveness ($r = -0.628$, $p < 0.001$). Boli composed of small particles were less hard and viscous ($r = 0.485$, $p < 0.0001$, and $r = 0.371$, $p < 0.0001$, respectively). It is important to reduce the size of food particles to allow the food to pass through the esophagus, as well as to decrease the hardness. The risk of injury during swallowing is prevented in this way.

Respective contributions of product properties and oral physiology characteristics on the variation of bolus properties during oral processing

As highlighted in the present study, bolus formation is a dynamic process that is influenced by bread composition and structure. The physiological characteristics of subjects also play an important role in the oral processing.[28] The purpose of this section is to determine the respective contribution of each parameter (bread or physiological properties) on bolus formation.

At swallowing time (Table 4), bolus hardness, adhesiveness, water content, hydration rate and heterogeneity could be explained by bread properties (mainly water content, water-absorbing capacity and the Young modulus) and subject characteristics (mainly individual masticatory index, mouth volume and in-mouth duration). Thus, a greater number of bolus properties could be explained at swallowing time than at the beginning and middle of oral processing (data not shown). It can be assumed that even if subjects have different strategies (for example, high individual masticatory index and short in-mouth duration), they all aim at producing a bolus suitable for swallowing.[7] The differences in oral processing strategies used by subjects would have different impacts on the bolus properties at times T1 and T2 than at time T3. Despite individual approaches, boli at swallowing time are similar, making it possible to establish statistical models.

The main subject characteristics that explained bolus properties at swallowing were the mouth volume, individual masticatory index and in-mouth duration. These parameters were also the ones that made it possible to discriminate between subjects in the two groups. It appears that the slow eaters produced boli that were softer, more homogeneous and more hydrated (more saliva added), and less sticky. Thus, the swallowing was safer when the eater kept the food in the mouth for a long time. Similar results were also found in the literature.[31] The in-mouth duration was also considered as a key factor to discriminate between subjects and to explain bolus properties at swallowing in a previous study.[26] At swallowing time, the bolus water content and the hydration rate were also explained by the

526 salivary flow, the mouth volume, the individual masticatory index and the in-mouth duration.
527 As expected, subjects with a high salivary flow, a big mouth and a long in-mouth duration
528 have a higher hydration of products. When the volume of the mouth is bigger, more saliva is
529 present to lubricate the food product.
530 The main bread property that determined bolus properties at swallowing was the water
531 content of the initial samples (crumb alone or crumb with crust). The water content of the
532 bolus could be explained by the water content of the initial sample at each point in time, and
533 it was also explained by the water-absorbing capacity at times T2 and T3. These results
534 were expected since more saliva is added to the dry product in these cases.[27] The present
535 results suggest that, in addition to the initial bread water content (which is responsible for the
536 absorption of saliva in the bolus), the water-absorbing capacity of the product is also a key
537 factor for understanding hydration mechanisms. This product property should be taken into
538 account when designing new products that would be easy to hydrate, notably for the elderly,
539 since the salivary flow decreases with age.[32]
540

541 ## Conclusions

542 The effects of hydration and mechanical and enzymatic breakdowns on the dynamics of
543 bolus formation were explored through the analysis of *in vivo* bolus properties for real
544 complex products.
545 The main result was that the variations over time had a greater impact on bolus properties
546 than crumb and crust structures. Therefore, in the case of a restricted category of products
547 such as bread, major differences in bolus properties are due to the time spent in the mouth.
548 Despite that, the heterogeneity of the food, studied here with the presence of crust, induced
549 a modification of the oral processing. For example, the variations over time of the
550 heterogeneity of particle size were affected. Moreover, bread properties such as water-
551 absorbing capacity or the presence of fibers contributed to a modification of hydration and
552 breakdown mechanisms.

The oral physiology of the subjects, especially their oral processing times and mouth volumes, also had a major effect on the formation of the bolus. The individual breakdown pathways revealed an adaptation of oral processing to the product for some subjects. This reinforces the fact that a panel of individuals with different oral characteristics should always be used in food oral processing studies.

Swallowing time is the moment when the greatest number of bolus properties could be explained by bread properties and subject characteristics, suggesting that this is the most relevant time to study the physical aspects of oral processing. Nevertheless, breakdown pathways could help to understand the sensory trajectories of bread. For this purpose, it is essential to study the oral processing over time.

This work helps understanding the impact of bread structure on bolus characteristics, especially the hydration phenomena and the amylase activity. This knowledge should be useful to better understand the drivers of glucose release during digestion, and also the impact of bread densities on glycemic index.

All of these results, linked to nutritional and sensory properties, should provide a solid knowledge foundation to help design new products.

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632 **Fig. 1:** Schematic representation of the product space in relation to their density and water
633 content

634 **Fig. 2:** Sampling protocol performed on a vertical slice of bread to prepare crumb or crumb
635 and crust samples. CO = Crumb Only, CC = Crumb with Crust.

636 **Fig. 3:** Principal Component Analysis (Pearson (n-1)) performed on physiological data
637 (salivary characteristics, individual masticatory index and oral volumes) for the eight
638 panelists. Two groups of panelists (colored in orange and green) were distinguished by the
639 Hierarchical Clustering Analysis on reduced centered data.

640 **Fig. 4:** Breakdown pathways of the bolus in the mouth obtained by PCA on normalized bolus
641 properties. Boli of crumb with and without crust at three key points in time during oral
642 processing (T1 = 10%, T2 = 40% and T3 = 100% of swallowing time) were plotted over
643 physical properties. CO = Crumb Only, CC = Crumb with Crust. Lines were drawn to guide
644 the reader.

645 **Fig. 5:** Bolus water contents over standardized oral processing time of the four breads for
646 crumb samples (open symbols) and crumb with crust samples (filled symbols). Error bars
647 indicate the standard error of the mean (n=24). CO = Crumb Only, CC = Crumb with Crust.

648 **Fig. 6:** Breakdown pathways of the bolus in the mouths of selected subjects (J2 (a), J4 (b)
649 and J8 (c)) obtained by PCA on normalized bolus properties. Boli of crumb with and without
650 crust, for all subjects, at three key points in time during oral processing (T1 = 10%, T2 = 40%
651 and T3 = 100% of swallowing time) were plotted over physical properties. The three graphs
652 are derived from the same PCA, but only one subject is highlighted in each graph. Lines
653 were drawn to guide the reader. CO = Crumb Only, CC = Crumb with Crust.

654

655

Table 1: Mean (M) and standard deviation (SD) of the structural and textural properties and the compositions of the four breads (three replicates). Product effect was determined by Kruskal-Wallis tests on each variable. Letters A, B and C indicate means that significantly differ between products at $p < 0.05$ (Dunn procedure).

Table 2: Mean (M) and standard error of the mean (SE) for each time of the bolus properties for (a) the crumb samples, and (b) the crumb with crust samples. F factors are derived from a two-way ANOVA (bread, time, time*bread). Probabilities are encoded as follows: NS: non-significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. Letters A, B, and C indicate means that significantly differ between times at $p < 0.05$ (Fisher (LSD)).

Table 3: Mean (M) and standard error of the mean (SE) of the properties related to bolus formation for (a) crumb samples, and (b) crumb with crust samples. The average of the three times that were studied (10%, 40% and 100% of the in-mouth duration) was calculated for each bread. F factors were derived from a two-way ANOVA (bread, time, time*bread). Probabilities are encoded as follows: NS: non-significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. Letters A, B, and C indicate means that significantly differ between products at $p < 0.05$ (Fisher (LSD)). Italics indicate that these differences are significant for the mean but not for all times (time*bread interaction significant).

Table 4: Normalized coefficients obtained by Partial Least Square (PLS) regressions between boli and bread properties (crumb samples with or without crust), and subject characteristics at the end of oral processing (T3). Blue colored coefficients are significant. Only the relevant regressions ($R^2 > 0.500$) are presented here. WAC = Water-absorbing capacity.

681 **Table 1:**

Parameter	Sample	*Unit*	B1			B2			B3			B4		
			M	*SD*		**M**	*SD*		**M**	*SD*		**M**	*SD*	
Water content	Crumb	*g/100 g of crumb*	**51.6**	*0.3*	C	**49.1**	*0.2*	AB	**50.7**	*0.2*	BC	**48.6**	*0.1*	A
	Crumb + crust	*g/100 g of bread*	**37.2**	*1.9*	B	**33.4**	*1.4*	A	**37.2**	*0.9*	B	**33.9**	*1.1*	AB
Total starch content	Crumb	*g/100 g of crumb*	**42.2**	*4.3*	AB	**43.4**	*1.3*	B	**43.6**	*2.6*	B	**34.0**	*1.1*	A
Crust thickness	Crust	*mm*	**1.07**	*0.17*	B	**1.03**	*0.28*	B	**0.82**	*0.13*	AB	**0.09**	*0.01*	A
Crumb/crust ratio (w/w)	Crumb + crust	-	**1.22**	*0.12*	B	**0.92**	*0.04*	A	**1.17**	*0.09*	AB	**1.14**	*0.14*	AB
Water-absorbing capacity	Crumb	*g of water/g of DM*	**5.9**	*0.3*	A	**12.5**	*0.5*	B	**6.1**	*0.2*	AB	**6.3**	*0.8*	A
	Crumb + crust		**4.9**	*0.2*	A	**8.2**	*0.3*	B	**6.6**	*0.1*	AB	**4.6**	*0.2*	A
Density	Crumb + crust	-	**0.25**	*0.02*	B	**0.16**	*0.01*	A	**0.22**	*0.01*	AB	**0.26**	*0.01*	B
Young modulus	Crumb	*kPa*	**7.7**	*1.7*	AB	**3.3**	*0.3*	A	**6.9**	*1.1*	AB	**9.9**	*2.3*	B

682

683

684 **Table 2:**

		F factor		Variation over time	T1			T2			T3		
Parameter	Unit	Time			**M**	*SE*		**M**	*SE*		**M**	*SE*	
a) Crumb bolus properties													
Number of particles	-	62.0	***		**629**	*75*	C	**1400**	*80*	B	**2578**	*252*	A
Hydration rate	g/100 g DM	328.5	***		**19.1**	*0.8*	C	**47.7**	*1.8*	B	**106.8**	*5.5*	A
Water content	g/100 g bolus	284.8	***	↗	**54.0**	*0.2*	C	**59.1**	*0.3*	B	**66.3**	*0.5*	A
Amylase activity	U/g DM	86.2	***		**33.3**	*2.5*	C	**82.3**	*5.7*	B	**178.7**	*12.6*	A
Quantity of residues	%	172.1	***		**1.3**	*0.3*	C	**3.9**	*0.4*	B	**11.8**	*0.8*	A
Adhesiveness	N.s	5.9	**		**1.21**	*0.06*	B	**1.39**	*0.08*	A	**1.50**	*0.08*	A
D50	mm	144.4	***	↘	**16.8**	*1.3*	A	**5.2**	*0.4*	B	**3.0**	*0.3*	C
Hardness	N	324.9	***		**11.67**	*0.61*	A	**5.01**	*0.47*	B	**1.75**	*0.11*	C
Cohesiveness	-	212.5	***	↘↗	**0.48**	*0.01*	A	**0.27**	*0.01*	C	**0.34**	*0.01*	B
D75/D25	-	2.5	NS	→	**4.2**	*0.4*		**3.9**	*0.2*		**3.2**	*0.1*	
b) Crumb with crust bolus properties													
Number of particles	-	80.6	***		**474**	*68*	C	**1339**	*91*	B	**2640**	244	A
Hydration rate	g/100 g DM	278.3	***		**17.4**	*1.4*	C	**42.8**	*2.0*	B	**87.7**	3.7	A
Water content	g/100 g bolus	307.3	***	↗	**41.6**	*0.4*	C	**49.0**	*0.5*	B	**58.0**	0.6	A
Amylase activity	U/g DM	92.5	***		**28.0**	*2.6*	C	**70.2**	*5.2*	B	**145.5**	10.3	A
Quantity of residues	%	42.4	***		**4.0**	*0.6*	B	**4.9**	*0.6*	B	**11.5**	1.0	A
Adhesiveness	N.s	189.2	***		**0.84**	*0.07*	C	**2.53**	*0.13*	B	**3.85**	0.20	A
D50	mm	214.8	***		**24.4**	*1.1*	A	**8.8**	*1.1*	B	**4.1**	0.3	C
Hardness	N	160.3	***	↘	**24.46**	*1.07*	A	**17.99**	*1.02*	B	**8.33**	0.66	C
Cohesiveness	-	388.1	***		**0.53**	*0.01*	A	**0.33**	*0.01*	B	**0.29**	0.00	C
D75/D25	-	8.3	***	↗↘	**3.9**	*0.6*	B	**5.6**	*0.5*	A	**4.0**	0.2	B

685

686

687 **Table 3:**

		F factors				B1 			B2 			B3 			B4 		
Parameter	Unit	Bread		Time*Bread		**M**	*SE*		**M**	*SE*		**M**	*SE*		**M**	*SE*	
a) Crumb bolus properties																	
Number of particles	-	10.1	***	1.0	NS	**1062**	*149*	C	**1304**	*191*	BC	**1567**	*242*	B	**2211**	*313*	A
D50	mm	6.2	***	1.0	NS	**10.3**	*1.8*	A	**7.6**	*1.6*	BC	**8.8**	*1.6*	AB	**6.7**	*1.3*	C
D75/D25	-	2.8	*	0.5	NS	**3.0**	*0.2*	B	**4.0**	*0.4*	A	**4.0**	*0.3*	A	**4.0**	*0.3*	A
Hydration rate	g/100 g DM	9.9	***	1.2	NS	**51.4**	*5.0*	B	**72.0**	*7.1*	A	**52.5**	*4.9*	B	**55.7**	*5.7*	B
Water content	g/100 g bolus	11.9	***	1.1	NS	**60.4**	*0.7*	A	**61.1**	*0.8*	A	**60.0**	*0.7*	A	**57.6**	*0.8*	B
Amylase activity	U/g DM	2.7	*	0.3	NS	**86.1**	*10.5*	B	**117.9**	*13.8*	A	**86.7**	*10.2*	B	**103.3**	*12.0*	AB
Quantity of residues	%	10.1	***	2.5	*	**5.2**	*0.8*	*B*	**8.1**	*1.1*	*A*	**4.2**	*0.6*	*B*	**5.3**	*0.6*	*B*
Adhesiveness	N.s	8.3	***	2.5	*	**1.08**	*0.06*	*C*	**1.29**	*0.07*	*B*	**1.48**	*0.09*	*AB*	**1.62**	*0.11*	*A*
Hardness	N	13.7	***	6.8	***	**5.54**	*0.62*	*BC*	**4.08**	*0.41*	*C*	**6.98**	*0.78*	*B*	**7.97**	*0.87*	*A*
Cohesiveness	-	35.1	***	10.6	***	**0.40**	*0.02*	*A*	**0.37**	*0.01*	*A*	**0.37**	*0.01*	*A*	**0.30**	*0.01*	*B*
G'	kPa	2.9	NS	-		**8.7**	*1.7*		**8.5**	*2.1*		**9.0**	*2.2*		**28.6**	*10.2*	
G''	kPa	4.5	*	-		**1.7**	*0.3*	B	**1.7**	*0.4*	B	**1.8**	*0.4*	B	**6.0**	*1.8*	A
ΔGlucose	g/100 g bread	1.7	NS	-		**3.3**	*0.4*		**4.8**	*0.3*		**2.9**	*0.5*		**6.3**	*1.6*	
b) Crumb with crust bolus properties																	
Number of particles	-	6.9	***	1.0	NS	**996**	*168*	C	**1793**	*257*	A	**1401**	*264*	BC	**1749**	293	AB
D50	mm	16.4	***	1.5	NS	**16.7**	*2.3*	A	**9.2**	*1.6*	C	**13.4**	*2.2*	B	**10.4**	1.8	C
D75/D25	-	8.8	***	2.4	*	**3.6**	*0.4*	*B*	**6.5**	*0.8*	*A*	**4.4**	*0.5*	*B*	**3.6**	0.3	*B*
Hydration rate	g/100 g DM	11.8	***	1.9	NS	**42.6**	*3.8*	B	**61.2**	*4.6*	A	**45.4**	*4.4*	B	**48.4**	5.0	B
Water content	g/100 g bolus	7.5	***	1.5	NS	**49.4**	*0.8*	B	**51.1**	*1.0*	A	**49.8**	*0.9*	AB	**47.8**	1.1	C
Amylase activity	U/g DM	3.7	*	0.5	NS	**72.8**	*8.6*	B	**101.1**	*10.5*	A	**77.2**	*10.2*	B	**74.1**	9.5	B
Quantity of residues	%	11.8	***	0.4	NS	**6.5**	*0.7*	A	**10.1**	*1.3*	A	**4.5**	*0.6*	B	**6.2**	1.1	B
Adhesiveness	N.s	13.9	***	7.6	***	**2.44**	*0.24*	*B*	**1.57**	*0.11*	*C*	**2.95**	*0.26*	*A*	**2.70**	0.21	*AB*
Hardness	N	33.7	***	16.6	***	**15.23**	*1.13*	*B*	**13.37**	*1.67*	*C*	**16.54**	*0.96*	*B*	**22.45**	1.22	*A*
Cohesiveness	-	27.2	***	17.5	***	**0.41**	*0.01*	*A*	**0.36**	*0.01*	*B*	**0.40**	*0.01*	*A*	**0.36**	0.02	*B*
G'	kPa	3.7	*	-		**25.6**	*3.3*	B	**28.8**	*9.8*	B	**27.6**	*4.6*	B	**65.4**	10.5	A
G''	kPa	4.6	*	-		**5.2**	*0.6*	B	**6.1**	*1.9*	B	**5.7**	*0.9*	B	**13.7**	2.0	A
ΔGlucose	g/100 g bread	4.0	*	-		**3.3**	*0.7*	B	**4.6**	*0.6*	AB	**4.2**	*0.6*	B	**7.8**	1.4	A

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Bolus properties at swallowing time		Hardness	Adhesiveness	Water content	Hydration rate	Heterogeneity
R^2		**0.587**	**0.546**	**0.774**	**0.627**	**0.506**
Product properties	Density	0.056	0.033	-0.023	-0.060	-0.118
	Water content	-0.434	-0.445	0.509	0.276	-0.470
	WAC	-0.193	-0.182	0.205	0.171	-0.064
	Young modulus	0.121	0.095	-0.086	-0.080	-0.055
	Total starch content	-0.111	-0.079	0.049	0.010	0.049
	Crust thickness	-0.150	-0.114	0.077	-0.006	0.000
	Crumb/Crust ratio	0.014	0.003	-0.008	-0.068	-0.107
Physiology characteristics	Salivary flow	-0.050	-0.068	0.129	0.202	-0.017
	Salivary dry extract	-0.047	-0.036	-0.002	-0.123	-0.103
	Salivary amylase activity	-0.095	-0.101	0.118	0.065	-0.118
	Viscosity of saliva	-0.005	-0.002	-0.012	-0.052	-0.033
	Individual masticatory index	0.180	0.203	-0.278	-0.254	0.196
	Mouth volume	-0.192	-0.212	0.277	0.225	-0.217
	In-mouth duration	-0.128	-0.157	0.241	0.262	-0.153

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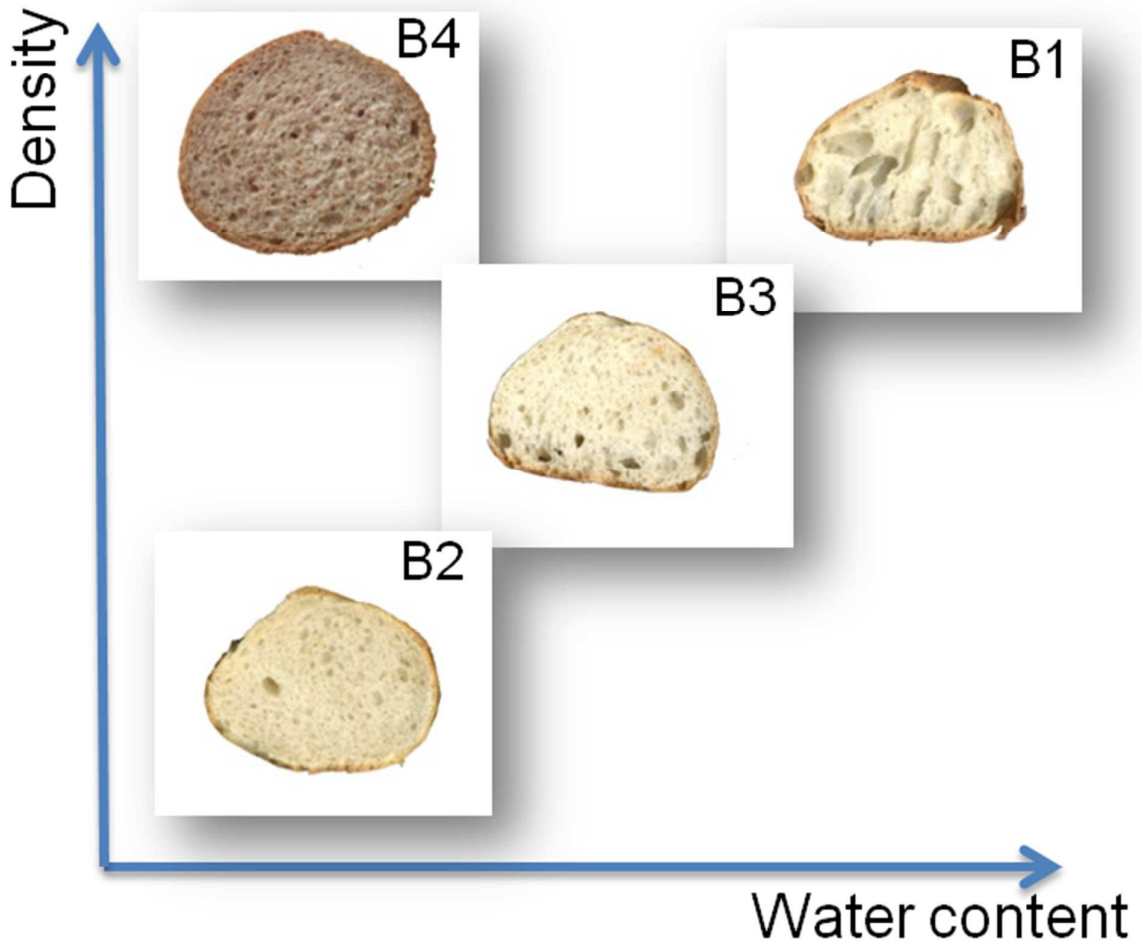

Fig. 1

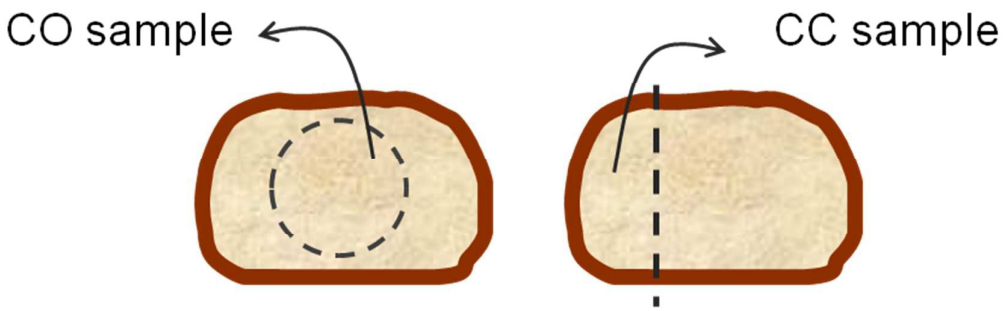

Fig. 2

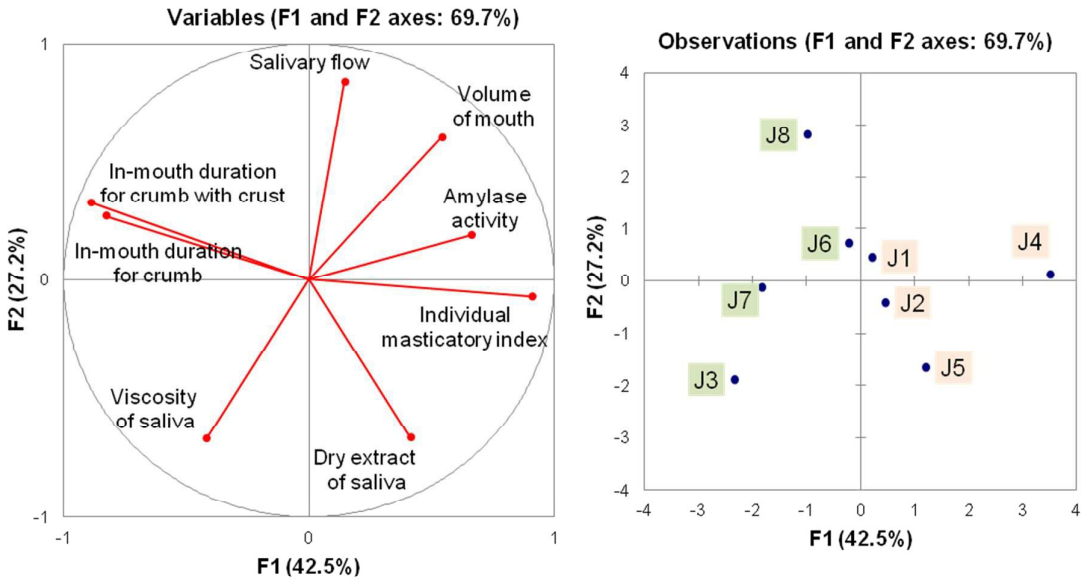

Fig. 3

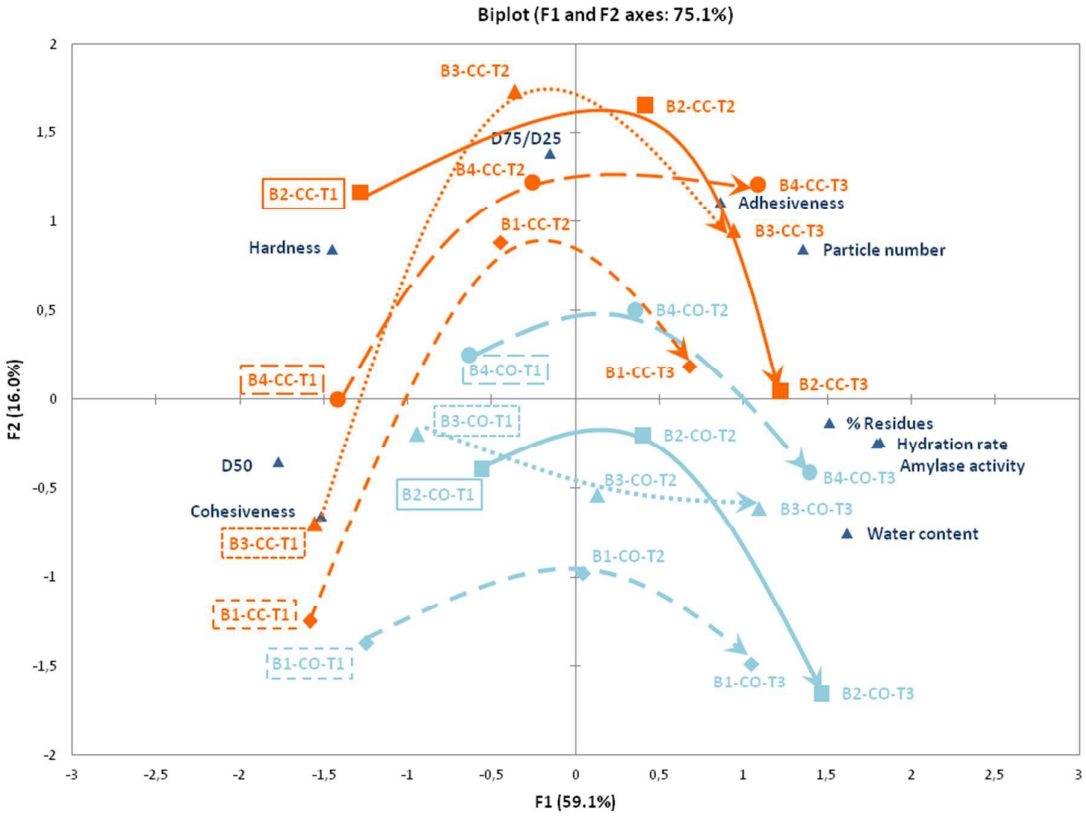

Fig. 4

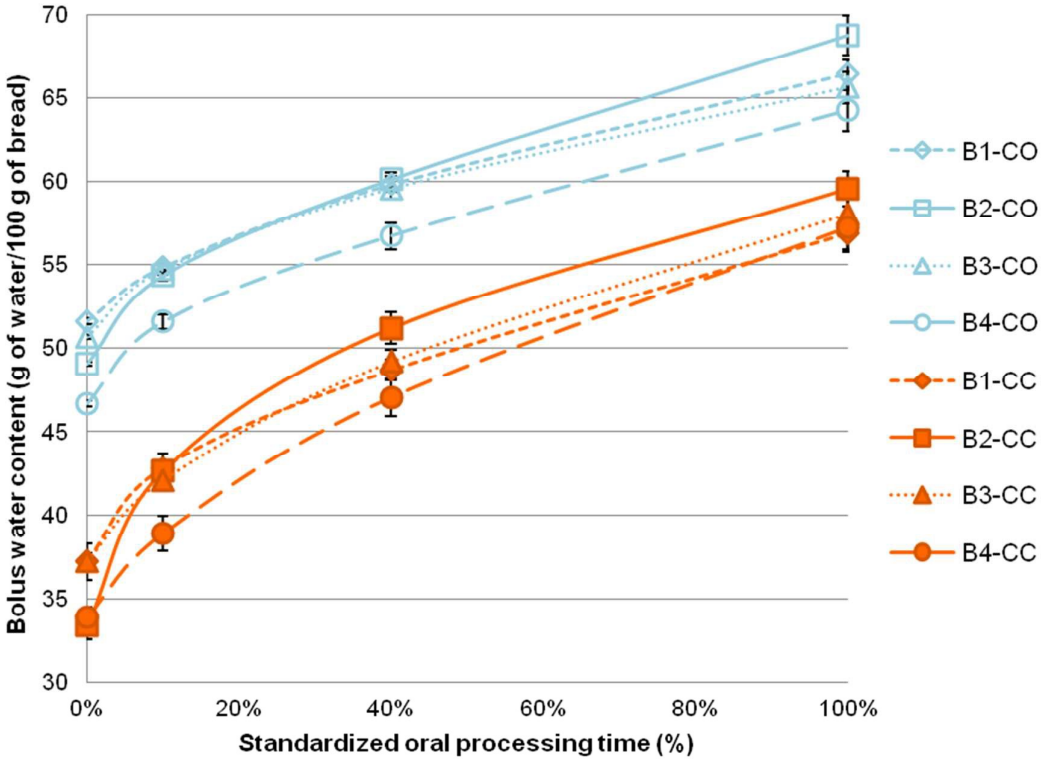

Fig. 5

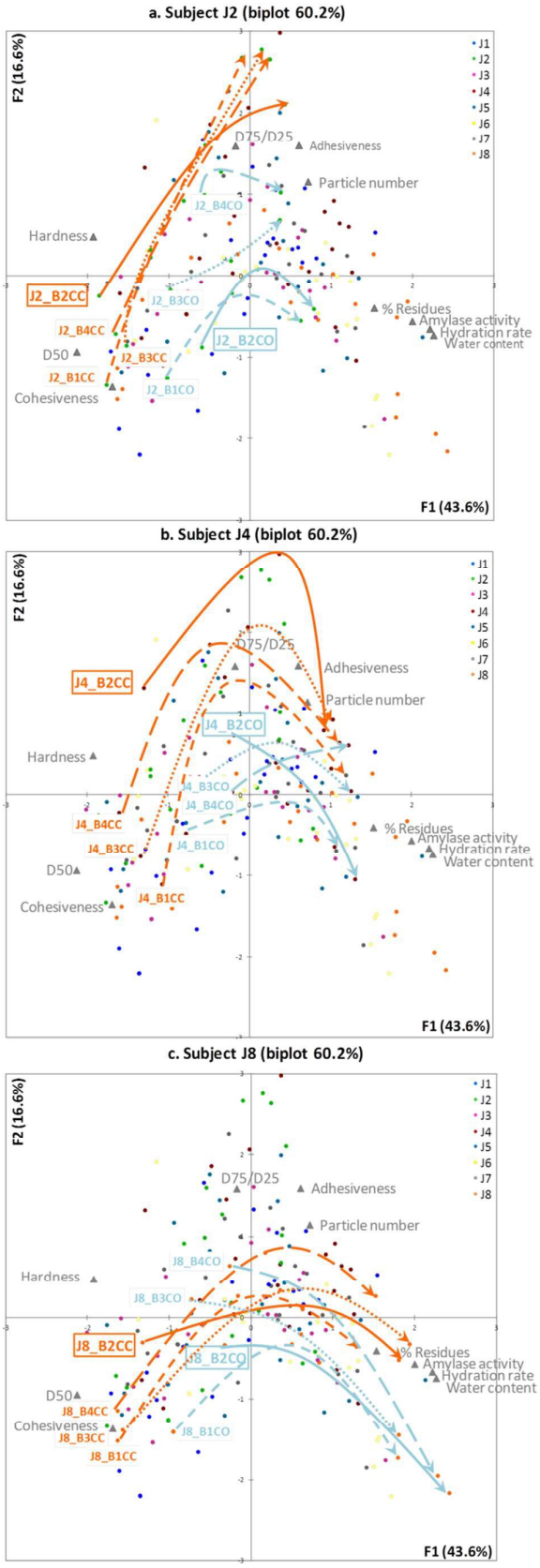

Fig. 6

Graphical abstract

An in vivo approach permitted to determine the respective contribution of bread properties and physiology characteristics to oral breakdown pathways.