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## Relationships of oral comfort perception and bolus properties in the elderly with salivary flow rate and oral health status for two soft cereal foods

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30 **Keywords:** food bolus, chewing, dental status, viscosity, hydration, cereals

31

32 **Nomenclature**

33 **ANSM**= Acronym for the French 'National Agency of Drugs and Safety';

34 **B**= Brioche;

35 **BHR**=Bolus hydration ratio (g of water/100g of product);

36 **C1**= 1/3 of total chewing duration, first chewing stage;

37 **C2**= 2/3 of total chewing duration, second chewing stage;

38 **Chew**=Perceived as easy to chew;

39 **Comfort**=Overall oral comfort perception score;

40 **DM** =Dry matter (g of dry matter/100g of product or bolus);

41 **DS**= Dental status;

42 **Easiness**=Perceived as easy to eat;

43 **FOP** = Food Oral Processing;

44 **OC**= Oral comfort;

45 **Moisten**=Perceived as easy to moisten;

46 **Ø**= Diameter of capillary die or mouthful size (mm or cm);

47 **P**= Poor (dental status);

48 **PC**= Principal Component

49 **Pasty**=Perceived pastiness;

50 **PFU** = Posterior functional unit;

51 **S**= Satisfactory (dental status);

52 **SC**= Sponge-cake;

53 **SP**= Swallowing point or total chewing duration or third chewing stage;

54 **SSF or  $\Phi_{stim}$** = Stimulated salivary flow rate (mL/min or mL/s);

55 **Sticky**=Perceived stickiness;

56 **Swallow**=Easy to swallow;

57 **t<sub>cx</sub>** = Chewing duration at a given sequence (s);

58 **WC**= Water content (g of water/100 g of product or bolus);

59  **$\eta(\dot{\gamma}=120s^{-1})$** = Bolus apparent viscosity at  $120s^{-1}$  (Pa.s);

60  **$\dot{\gamma}$** = Apparent shear rate ( $s^{-1}$ );

61

## 62        **1. Introduction**

63        The proportion of elderly people worldwide is growing rapidly. Over the first half of the  
64        current century, the global population aged 60 and over is projected to expand by more than  
65        three times, to reach nearly 2.1 billion in 2050 (United Nations, 2002; United Nations, 2015).  
66        Ageing is often associated with a degradation of the oral health status, where tooth loss,  
67        decreased muscle strength and tongue pressure, and reduced salivary flow are among the  
68        main factors responsible for eating difficulties and loss of eating pleasure in the elderly  
69        (Laura Laguna, Aktar, Ettelaie, Holmes, & Chen, 2016; Ship, 1999; Vandenberghe-  
70        Descamps et al., 2016; Wang & Chen, 2017; Xu, 2016). Moreover, olfactory and gustatory  
71        capacities are also reduced (Boyce & Shone, 2006; Methven, Allen, Withers, & Gosney,  
72        2012), increasing the risk of food intake reduction, leading to malnutrition and other diseases  
73        (Henshaw & Calabrese, 2001; Maitre et al., 2014; Rolls, 1999; Schwartz, Vandenberghe-  
74        Descamps, Sulmont-Rossé, Tournier, & Feron, 2017). In this context, it has therefore  
75        become crucial to develop age-friendly food products with an improved nutritional value and  
76        enhanced enjoyment of eating in order to ameliorate the quality of life of the forthcoming  
77        senior population (Giacalone et al., 2016; Schwartz et al., 2017). Food Oral Processing  
78        (FOP) has been shown to be a crucial stage for texture, taste and aroma perception, as well  
79        as for sensory pleasure (Chen, 2009; Salles et al., 2011; Varela, Salvador, & Fiszman,  
80        2009). A better understanding of the mechanisms involved in FOP is thus necessary (Chen,  
81        2015; Laura Laguna & Chen, 2016). Research has shown that the elderly use strategies to  
82        compensate for oral impairments such as extending chewing duration, increasing the number  
83        of chewing cycles and swallowing larger particles of food (Mioche, Bourdiol, Monier, Martin,  
84        & Cormier, 2004; Peyron, Blanc, Lund, & Woda, 2004; Peyron, Woda, Bourdiol, &  
85        Hennequin, 2017). More recently, research focusing into establishing the concept of 'eating  
86        capability' in this population through physiology, showed that biting force and dental status  
87        influenced the oral processing duration, the number of chewing cycles, as well as liking and  
88        difficulty perception (L. Laguna & Sarkar, 2016; L. Laguna, Sarkar, & Chen, 2015; Laura  
89        Laguna, Hetherington, Chen, Artigas, & Sarkar, 2016; Laura Laguna, Sarkar, Artigas, &

90 Chen, 2015). Other studies have shown that food bolus properties can be related to the  
91 perception of texture and aroma (Devezeaux de Lavergne, Derks, Ketel, de Wijk, & Stieger,  
92 2015; Jourdren, Saint-Eve, et al., 2016; Jourdren et al., 2017). Transforming food into a  
93 bolus that is ready to swallow is the main purpose of FOP (Prinz & Lucas, 1997). Therefore,  
94 studying its degree of transformation in the mouth by quantitatively characterizing its  
95 properties, such as hydration ratio, rheological behavior and particle size, is fundamental for  
96 the understanding of the underlying mechanisms.

97 It is common knowledge that, beyond the physiological and nutritional functions, eating is an  
98 enjoyable sensory experience that can be source of satisfaction and pleasure (Bourne,  
99 2002). However, literature regarding the food enjoyment and comfortability, especially of the  
100 elderly, is quite scarce. Recently, Vandenberghe-Descamps, Labouré, Septier, Feron, &  
101 Sulmont-Rossé (2017) developed a questionnaire to assess oral comfort for a wide variety of  
102 foods. They also investigated the impact of dental status and salivary flow on the oral comfort  
103 perception (Vandenberghe-Descamps, Sulmont-Rossé, Septier, Feron, & Labouré, 2017).  
104 Xu (2016) highlighted the importance of taking into account pleasure and enjoyment when  
105 designing specialized foods for the elderly, so that optimum masticatory pleasure can be  
106 achieved. However, present foods targeted for the elderly are mainly focused on the  
107 nutritional needs, without considering enjoyment. They are often found as dietary  
108 supplements that produce taste-fatigue on the long-term and have low compliance (Gosney,  
109 2003). Cereal products, besides from being staple foods in many countries, are affordable,  
110 nutritious and can be consumed regardless of culture and beliefs. They are widely consumed  
111 among the elderly population, who tends to orient towards a more 'traditional' dietary pattern  
112 (Andreeva et al., 2016). To this extent, the products selected for this study, sponge-cake and  
113 brioche, have been little studied and are good candidates for development since they have  
114 pleasant sensory properties. They have also a relative flexibility regarding formulation,  
115 opening the possibility for modifications including the enrichment with fibers or proteins to  
116 increase their nutritional value. Numerous studies have been carried out regarding FOP of  
117 cereal products such as bread, biscuits and breakfast flakes (Gao, Wong, Lim, Henry, &

118 Zhou, 2015; Jourden, Panouillé, et al., 2016; Le Bleis, Chaunier, Montigaud, & Della Valle,  
119 2016; Peyron et al., 2011; Tournier, Grass, Septier, Bertrand, & Salles, 2014; Young et al.,  
120 2016). All of the precedent studies were conducted on middle-age population. As far as we  
121 know, there is a lack of similar investigations on elderly population.

122 Given this context, the aim of this study was to determine the relationships between bolus  
123 properties, oral health status and perceived oral comfort in elderly for two cereal foods:  
124 sponge-cake and brioche. Two physiology criteria were selected to assess the oral health  
125 status of participants: dental status and salivary flow rate. Other oral physiological  
126 parameters such as tongue pressure have been described also as makers of oral health in  
127 elderly (Tamine et al., 2010). However, they reflect principally dysphagia symptoms (Yoshida  
128 et al., 2006) that are not considered in this study. Moreover, it has been shown that solid  
129 foods require teeth action rather than tongue to be processed (Funami, 2016; Ishihara et al.,  
130 2013). In this purpose, the impact of dental status and salivary flow rate on food bolus  
131 hydration ratio and rheological properties was investigated for both products. Secondly their  
132 relationships with the perception of oral comfort were assessed.

## 133 **2. Materials and Methods**

### 134 *2.1 Subjects*

135 Twenty French subjects (9 men and 11 women, aged 65-82 years, mean  $72 \pm 5$  years)  
136 participated in the study. Their dental status was assessed by determining the number of  
137 Posterior Functional Units (PFU's), defined as pairs of opposing posterior teeth (premolars  
138 and molars). Depending on the number of PFU's, participants were classified within two  
139 different groups. Since the maximum number of PFU's for a complete dentition (third molars  
140 excluded) is 8, a satisfactory dental status was considered to be of at least 7 PFU's.  
141 Conversely, a poor dental status was considered to be inferior or equal to 4 PFU's (Leake,  
142 Hawkins, & Locker, 1994). Only individuals entering in these two categories were included in  
143 the study. The number of PFU's was evaluated visually by a dentist at bare eye and also by  
144 asking participants to chew a 200 $\mu$ m thick articulating paper according to the procedure  
145 described by Vandenberghe-Descamps et al. (2016).

146 The salivary flow rates (mL/min) of participants were determined on the day of the  
147 experimentation with and without mechanical stimulation as described by Neyraud et al.  
148 (2012). The mean, stimulated and unstimulated, salivary flow rates along with a general  
149 description of the 20 subjects are shown in Table 1. The observed salivary flow values are  
150 within the range usually encountered in literature for healthy adults, including elderly (Chen,  
151 2009; Vandenberghe-Descamps et al., 2016). Additionally, the salivary flow rates, both  
152 stimulated and unstimulated, were not dependent on dental status ( $p>0.05$ ) as already  
153 observed by Vandenberghe-Descamps et al. (2016).

154 All subjects agreed on the content of the study and signed informed consent. This study was  
155 approved by the local ethical committee and the French National Agency of Drugs and  
156 Safety (ANSM) (ID RCB n°2016-A00916-45).

## 157 *2.2 Product samples*

158 Brioche and sponge-cake were provided by CERELAB® (Dijon, France). Their composition  
159 and density values are shown in Table 2. Products were offered to the participants as  
160 cylinders of 20 cm<sup>3</sup>. Portions of each product were cut just before the beginning of the  
161 experimentation with a knife and a circular steel cutter of diameter ( $\emptyset$ )=3 cm for sponge-cake  
162 (h=2.8 cm) and  $\emptyset$ =5 cm for brioche (h=1 cm) and given to the subjects as mouthfuls for  
163 consumption and bolus generation during the experimental procedure.

## 164 *2.3 Experimental procedure*

165 Every subject participated in one collective and six individual sessions, for a total of seven  
166 sessions of approximately 1 h. The collective session aimed at determining the individual  
167 swallowing point of both products. Participants were asked to consume the product mouthful  
168 (20 cm<sup>3</sup>) in a natural manner and were recorded on video while doing it. They were asked to  
169 point out the swallowing moment by raising their hand. Total chewing duration was  
170 calculated as the time elapsed from the placement of the food inside the mouth and the  
171 swallowing point, which was defined right after the first swallow. The number of chewing  
172 cycles were determined from this recording as well and one chewing cycle was defined after

173 a complete sequence of opening-occlusion. Chewing frequency was calculated from this  
174 data by dividing the number of chewing cycles by the chewing duration. Water (Evian,  
175 France) was offered freely after each mouthful. The procedure was repeated twice for each  
176 product. During the individual sessions, participants were asked to chew the product  
177 mouthful and to expectorate the food bolus into a Petri dish at three mastication stages  
178 according to their individual total chewing duration: 1/3 of total chewing duration (C1), 2/3 of  
179 total chewing duration (C2) and just before the swallowing point (total chewing duration, SP).  
180 They repeated the procedure once for each product. Food boli were collected at the three  
181 stages (C1, C2 and SP) for further characterization. At the end of a randomly selected  
182 individual session, participants were also asked to respond the oral comfort assessment  
183 questionnaire. They repeated the questionnaire once for each product on a different session.  
184 In both collective and individual sessions, products were randomly distributed.

#### 185 *2.4 Oral comfort assessment*

186 Perception of oral comfort (OC) was assessed using a questionnaire recently developed  
187 (Vandenberghe-Descamps, Labouré, et al., 2017). This questionnaire is composed of 5  
188 multi-variate sections with structured scales. Each section of the questionnaire refers to a  
189 different dimension of OC: general comfort, easiness of bolus formation, pain feeling, texture  
190 and flavor of the product. Further detail of the sections and subsections is given in the  
191 appendix (Table A.1). Questions were answered by participants while consuming the  
192 products. They were asked to consume one mouthful of product for each section of the  
193 questionnaire. Water (Evian, France) was offered to rinse the mouth at the beginning and the  
194 end of the questionnaire but not in-between.

#### 195 *2.5 Bolus characterization*

##### 196 *2.5.1 Capillary rheometry*

197 The rheological properties of products and boli were determined by capillary rheometry as  
198 previously described by Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre (2013). A  
199 mechanical texture analyzer (TA.XTplus, Stable Micro Systems, UK) equipped with a



200 cylindrical piston with flat head and a capillary die fixed at the bottom to a cylindrical barrel,  
 201 were used as a capillary rheometer. Boli were loaded into the capillary die immediately after  
 202 collection. Each product was tested at three values of apparent shear rate ( $\dot{\gamma} = 10, 42$  and  
 203  $333 \text{ s}^{-1}$ ) according to different combinations of the piston speed (50 or 200 mm/min) and  
 204 capillary die diameter ( $\varnothing=2$  or 4 mm). From these shear rate values and pressure  
 205 measurements, the apparent viscosity  $\eta$  was calculated. Variations of  $\eta(\dot{\gamma})$  were shown to  
 206 follow a power law, as reported by Le Bleis et al., 2016 in the case of bread boli, with little  
 207 variation of the flow index, close to 0.3. The value of  $\eta(\dot{\gamma}=120\text{s}^{-1})$  for each subject and each  
 208 chewing stage was selected to characterize bolus viscosity from a typical shear rate value of  
 209 the oropharynx at the beginning of swallowing (Zhu, Mizunuma, & Michiwaki, 2014). Two boli  
 210 were required to repeat the measurement for each of the three chewing stages for all of the  
 211 three apparent shear rate values, leading to a total of  $2 \times 3 \times 3 = 18$  boli per subject for each  
 212 product.

### 213 2.5.2 Bolus hydration ratio

214 Bolus hydration ratio was determined on part of the bolus used for rheological  
 215 characterization, in order to reduce the number of collected boli and avoid subject  
 216 exhaustion. After capillary rheometry, part of the extruded bolus was weighed before and  
 217 after staying in an oven during 24 h at 130°C. The bolus hydration ratio was expressed as  
 218 the amount of saliva incorporated to the food product and was calculated according to the  
 219 procedure reported by Repoux et al., 2012 for cheese boli (1). All reported values are on a  
 220 wet basis.

$$221 \quad (1) \quad \text{Added saliva (\%)} = \left[ \frac{\text{bolus}_{\text{WC}}}{\text{bolus}_{\text{DM}}} \times \text{product}_{\text{DM}} \right] - \text{product}_{\text{WC}}$$

222 Where:

$$223 \quad \text{bolus}_{\text{WC}} = \text{bolus water content} \left( \frac{\text{g of water}}{100 \text{ g of bolus}} \right)$$

$$224 \quad \text{bolus}_{\text{DM}} = \text{bolus dry matter} \left( \frac{\text{g of dry matter}}{100 \text{ g of bolus}} \right)$$

225 product<sub>WC</sub> = product's water content  $\left(\frac{g \text{ of water}}{100 g \text{ of product}}\right)$

226 product<sub>DM</sub> = product's dry matter  $\left(\frac{g \text{ of dry matter}}{100 g \text{ of product}}\right)$

## 227 *2.6 Statistical analysis*

228 Differences between products and subjects for chewing parameters were investigated using  
229 a two-way Analysis of Variance (ANOVA) model (product + subject) on the last chewing  
230 stage. As for bolus properties, these differences were investigated for each chewing stage  
231 using a repeated measures ANOVA model (product + subject + chewing stage), where the  
232 chewing stage was the repeated factor. For oral comfort scores, a two-way ANOVA model  
233 (product + subject) was used. The Student-Newman-Keuls test was used for a post-hoc  
234 multiple comparison test.

235 To investigate the impact of oral health status in chewing parameters and oral comfort  
236 scores, a two-way Analysis of Covariance (ANCOVA) model with interaction (dental status +  
237 stimulated salivary flow + dental status\*stimulated salivary flow) was carried out. Regarding  
238 bolus properties, in order to take account for variability over time, a three-way ANCOVA  
239 model was applied by adding total chewing time as explanatory variable (chewing time +  
240 stimulated salivary flow + dental status\*stimulated salivary flow). For every statistical  
241 procedure, a significance level of  $\alpha=0.05$  was used.

242 Pearson correlation coefficients were calculated when needed between bolus properties, oral  
243 comfort scores and chewing parameters. Finally, Principal Component Analysis (PCA) was  
244 used to study the relationship between all of the variables cited above.

245 All statistical analyses were performed with XLSTAT software (v.2016 18.06, Addinsoft,  
246 USA).

## 247 **3 Results and discussion**

### 248 *3.1 Chewing parameters and bolus properties*

249 Average chewing parameters and bolus properties regardless of dental status and salivary  
250 flow are shown in Table 3. For the chewing duration and the number of cycles, the ANOVA  
251 performed on the last chewing stage (SP) showed a significant subject effect ( $p<0.0001$ ), but

252 no product effect. This means that despite the differences of composition and properties  
253 between the products, subjects do not modify their duration of chewing from one product to  
254 another. Comparable results were reported by Le Révérend, Saucy, Moser, & Loret (2016) in  
255 the case of healthy adults, where little variation was observed in the chewing duration and  
256 number of chewing cycles of brittle cereal products. Chewing duration and number of cycles  
257 were strongly correlated as shown by Pearson coefficients ( $r_{\text{Sponge-cake}} = 0.91$ ;  $r_{\text{Brioche}} = 0.94$ ,  
258  $p < 0.001$ ). Therefore, chewing frequency remained relatively constant across subjects and  
259 was the only chewing parameter where the subject effect was not significant ( $p > 0.05$ ),  
260 meaning there is little inter-individual variability. This result is in accordance with those  
261 previously reported for other type of foods (Devezeaux de Lavergne, Derks, Ketel, de Wijk, &  
262 Stieger, 2015; Yven et al., 2012). Indeed, chewing frequency is reported to be a distinctive  
263 feature of the human species, with values close to 1.3 Hz (Lucas, 2004), and it does not  
264 seem to be affected by age (Peyron et al., 2004).

265 Conversely, bolus properties showed significant differences between products ( $p < 0.0001$ ),  
266 besides subject ( $p < 0.0001$ ) and chewing stage ( $p < 0.0001$ ) effects. Generally, the hydration  
267 ratio increased with time, and therefore with each chewing stage; while the apparent  
268 viscosity decreased. For bolus hydration ratio, products differed at every chewing stage,  
269 while for bolus apparent viscosity they only did at the last two chewing stages (C2 and SP).  
270 For both products, initial viscosity before chewing (C0) was significantly ( $p < 0.05$ ) higher for  
271 sponge-cake ( $2164 \pm 12$  Pa.s) than for brioche ( $1561 \pm 21$  Pa.s), likely because of lower fat  
272 content. This difference decreases after the first chewing stage (C1), where both products  
273 apparent viscosity becomes close to each other (i.e. 500 Pa.s). Interestingly, during the last  
274 two sequences (C2 and SP), the apparent viscosity of brioche exceeds that of sponge-cake,  
275 contrasting with their initial values. Regarding the bolus hydration ratio, even if both products  
276 have close water content values (Table 2), the amount of added saliva was significantly  
277 ( $p < 0.001$ ) higher for sponge-cake than for brioche. This particular feature may be explained  
278 by the higher porosity of the sponge-cake, reflected by its lower density value, although its  
279 lower fat and higher sucrose contents could also have an influence. Hence, sponge cake

280 would absorb more efficiently the water present in saliva. This result is in agreement with  
281 those reported by Mathieu, Monnet, Jourden & Panouillé (2016), who compared the  
282 hydration kinetics of different bread structures and found higher hydration rates for more  
283 porous structures.

### 284 *3.2 Impact of oral health status on chewing parameters and bolus properties:*

285 The influence of the oral health status on the chewing parameters and bolus properties was  
286 determined by ANCOVA model, and the results are presented in Table 4. As previously  
287 mentioned, given its high correlation with the number of chewing cycles, only the chewing  
288 duration (SP) was included to represent chewing parameters. The dental status (DS)  
289 influenced the SP for both products and it was longer for participants with a satisfactory DS  
290 (positive  $\beta$  coefficient). However, the interaction between DS and stimulated salivary flow  
291 rate (SSF), close to significance for sponge-cake and significant for brioche, suggests that a  
292 high SSF can counterbalance the observed DS effect. As for the bolus hydration ratio, it is  
293 clear that SSF is the main factor of influence for both products, which means higher flow  
294 rates lead to more hydrated boli (positive  $\beta$  coefficient). The bolus apparent viscosity was  
295 also highly impacted by the SSF, although in the opposite sense (negative  $\beta$  coefficient). DS,  
296 on the other hand, showed little impact on bolus properties. Only in the case of brioche,  
297 individuals with a satisfactory DS produced a bolus with a higher apparent viscosity.  
298 Nevertheless, likewise the chewing duration, there was a significant interaction with SSF. A  
299 hypothesis to explain the two previous interactions could reside in the well-known theory of  
300 the 'swallowing threshold' (Hutchings & Lillford, 1988), which stipulates that all individuals  
301 swallow at determined time, degree of structure and degree of lubrication that depends on  
302 food product. Since in this case, the SSF has shown to be the main variable involved in the  
303 reduction of bolus apparent viscosity, it could be hypothesized that individuals with a high  
304 SSF will achieve the degree of bolus viscosity needed to trigger swallowing in a shorter  
305 chewing duration, than their counterparts with a lower SSF. This could explain why  
306 individuals with a satisfactory DS, but a low SSF, need a longer chewing duration to produce  
307 a bolus with a similar degree of viscosity than those with high SSF. From another

308 perspective, the composition of saliva, mucins in particular, adds lubricating properties to  
309 saliva (Wu, Csako, & Herp, 1994) and therefore could also be partially responsible for the  
310 'faster swallowing' of individuals with high SSF. However, in the present study the  
311 composition of saliva was not investigated. Overall, the stimulated flow rate appears to be  
312 the key parameter that determines bolus properties just before swallowing, whatever the  
313 dental status. A similar conclusion was obtained by Yven, Bonnet, Cormier, Monier, &  
314 Mioche (2006) who worked on meat products and subjects with impaired mastication. These  
315 authors hypothesized that the level of moisture is more important in triggering the swallow  
316 event than is the level of comminution of the product.

### 317 *3.3 Phenomenological model of apparent viscosity from stimulated flow rate*

318 Hydration ratio and bolus apparent viscosity were found to be correlated ( $r_{\text{Sponge-cake}} = -0.72$ ;  
319  $r_{\text{Brioche}} = -0.81$ ,  $p < 0.0001$ ), as previously observed by (Le Bleis et al., 2013) in the case of  
320 bread boli. Therefore, the decrease of bolus viscosity over time depends on the hydration  
321 ratio and more interestingly on the SSF of the subject. Similar results were obtained by Loret  
322 et al. (2011), who observed that rheological properties of boli from breakfast flakes were  
323 related to the bolus water content, which was concurrently correlated to the saliva flow of the  
324 subject. As a consequence, the variations of bolus apparent viscosity can be represented as  
325 a function of the theoretical amount of saliva in the mouth (Fig.1), expressed by the product  
326 of SSF by the chewing duration (Le Bleis et al., 2016).

$$327 \quad (2) \quad \textit{Theoretical Saliva (mL)} = \Phi_{stim} \times t_{CX}$$

328 Where:

329  $\phi_{stim}$  = Stimulated salivary flow rate ( $\frac{mL}{s}$ )

330  $t_{CX}$  = Chewing duration at a given sequence (s)

331 From Figure 1, the decrease of the apparent viscosity over time can be fitted through a  
332 power law model and modelled from a single physiology parameter with an acceptable  $R^2$   
333 coefficient, close to 0.6. Likely the data scattering, and lack of fit, may be due to inter-  
334 individual variability that is not explained by SSF, such as saliva composition and food

335 fragmentation. Le Bleis et al. (2016) have taken into account the effect of fragmentation by  
336 dividing flow rate by the particle size in order to consider the increase of contact surface and  
337 absorption capacity of the food. This opens prospect for a more complete model by including  
338 other factors such as the particle size and the degree of fragmentation of food in future  
339 studies.

#### 340 *3.4 Perception of oral comfort and impact of oral health status*

341 Unlike bolus properties, the perception of oral comfort (OC) was not significantly different  
342 between products, as reflected by the two-way ANOVA performed on the scores obtained for  
343 every of the 26 questions included in the questionnaire (see Appendix, Table A.2). Moreover,  
344 both products were considered to be very comfortable. The scores of the sensory attributes  
345 related to OC and bolus formation of the questionnaire are presented in Figure 2, which  
346 shows that both products were highly rated and close to the maximum.

347 In order to investigate the impact of oral health status in the perception of OC, an ANCOVA  
348 model was applied (Table 4). The results show that the stimulated salivary flow rate (SSF)  
349 had an influence in the overall comfort score of the sponge-cake, and the participants with a  
350 high SSF perceived the product as more comfortable (positive  $\beta$ ). Conversely, for brioche  
351 neither the dental status (DS), nor the SSF had an influence in the overall comfort score. In  
352 this case, the OC seems to be independent from the physiology of the subjects and could be  
353 rather explained by the product itself. Indeed, the higher level of fat contained in the brioche  
354 may have a lubricating effect which may be responsible for OC perception. Engelen, Fontijn-  
355 Tekamp, & Bilt (2005) found that adding butter to Melba toast (approximately 20 g of  
356 butter/100 g of Melba toast) reduced significantly the number of chewing cycles and the  
357 chewing duration in healthy adults. The same conclusions were obtained by Gavião,  
358 Engelen, & Van Der Bilt (2004). Therefore, the addition of fat in some dry foods may  
359 compensate for the low moisture content and facilitate the swallowing, leading to high OC  
360 scores.

#### 361 *3.5 Multivariate analysis and overall discussion*

362 The preceding study of physiological parameters highlighted their impact in the oral  
363 processing and the bolus formation process of the elderly. So, in order to consider the  
364 simultaneous action of all these variables (physiology, chewing parameters and bolus  
365 properties) and their inner relationship with oral comfort (OC) attributes, a PCA was  
366 performed. Regarding OC, only the attributes with a significant subject effect were included.  
367 For sponge-cake, principal components (PC's) 1 and 3, which together explained 57% of the  
368 total variability, were selected for a graphical projection (Fig. 3, left). PC 1 separated the  
369 subjects according to their physiology, particularly their stimulated salivary flow rate (SSF)  
370 showing a high correlation to the component ( $R= 0.81$ ). The overall comfort score was also  
371 positively correlated to this PC ( $R= 0.68$ ), as well as easiness ( $R =0.64$ ). Conversely, PC 3  
372 was driven by the differences in bolus properties, particularly the apparent viscosity, as  
373 shown by its correlation to this PC ( $R= 0.46$ ). This PC was also negatively correlated to the  
374 overall comfort score ( $R= -0.49$ ). From the correlation circle (Fig. 3, left) it can be seen that  
375 the overall comfort score was clearly opposed to the apparent viscosity of the bolus, and  
376 close to the added saliva. Moreover, the attributes easiness and easy to moisten were  
377 depicted together and in the same direction as bolus hydration. These relationships were  
378 confirmed by Pearson correlation coefficients, where the overall comfort score positively  
379 correlated to the hydration ratio, ( $r= -0.47$ ,  $p<0.05$ ) and negatively correlated to the apparent  
380 viscosity ( $r= -0.52$ ,  $p<0.05$ ). Thus, a sponge-cake bolus with a low hydration ratio and, as a  
381 consequence, a high apparent viscosity, will be perceived as uncomfortable. This result  
382 confirms an important relationship between physiology and sensory perception that could be  
383 quantified and modelled through bolus properties (See section 3.3). These results are in  
384 agreement with those of Jourden, Saint-Eve, et al. (2016), who showed by Multi-bloc Partial  
385 Least Squares (MB-PLS) regression that bread bolus properties, and hydration in particular,  
386 allow to explain better the perception of texture attributes than the characteristics of the  
387 breads themselves.

388 PC 2 separated subjects according to chewing duration ( $R= 0.91$ , data not shown), but no  
389 other variable correlated to this PC, meaning that chewing time did not contribute to explain

390 the other variables and in particular OC. This observation was confirmed by Pearson  
391 correlation analysis ( $r = -0.18$ ,  $p > 0.05$ ). Hence, the individuals who took a longer time to  
392 swallow did not necessarily find the product uncomfortable and are probably 'slow chewers'  
393 in a general manner. This supports the theory that there are different chewing strategies  
394 according to the consumer preferences (Brown & Braxton, 2000; Jeltema, Beckley, &  
395 Vahalik, 2015).

396 For brioche (Fig. 3, right) PC's 1 and 2 (61 % of variability) were selected: PC 1 discriminated  
397 the subjects in terms of SSF and overall comfort, as shown by their correlations to this PC  
398 ( $R = 0.53$  and  $R = 0.70$ , respectively), although they were not depicted close to each other. In  
399 fact, unlike sponge-cake, the perception of OC does not seem to be related to physiological  
400 variables but rather to sensory attributes describing OC (easy to chew, to swallow and to  
401 moisten). Interestingly, the bolus properties appeared to be orthogonal to the OC variables.  
402 Although the sticky and pasty attributes were opposed to overall comfort, and thus perceived  
403 as uncomfortable, they were poorly explained by the bolus properties or the physiology of the  
404 subjects. Previous studies in bread boli have shown a negative correlation between the  
405 perceived stickiness and the bolus hydration ratio (Jourdren, Panouillé, et al., 2016;  
406 Jourdren, Saint-Eve, et al., 2016). To explain this, the authors hypothesized that a highly  
407 hydrated bolus could increase its cohesiveness and thus prevent its adhesion to the palate  
408 and teeth. Also, the perception of stickiness seemed to be influenced by the bread density: a  
409 higher density led to an increased perception of this attribute (Panouillé, Saint-Eve, Déléris,  
410 Le Bleis, & Souchon, 2014). However in our case, even if the brioche was denser and boli  
411 less hydrated, the perception of stickiness did not correlate to any of the studied bolus  
412 properties, as confirmed by Pearson correlation analysis ( $r_{BHR} = -0.17$ ,  $r_{120} = 0.2$ ,  $p > 0.05$ ).  
413 Also, even if the OC questionnaire does not feature a hedonic dimension, it is interesting to  
414 notice that some of the sensory attributes that were related to the perception of OC have  
415 shown to influence the liking of similar products in precedent studies (Tarrega, Quiles, Morell,  
416 Fiszman, & Hernando, 2017). In this work, participants rated higher the products that were  
417 identified as sweet and easy to chew, and lower those that were found to be pasty and dry.



418 This leads to think such sensory attributes could play an important role in driving consumer  
419 preferences, and thus deserve a better understanding and instrumental characterization  
420 during oral processing.'

421 In all, as already seen from section 3.4, neither bolus properties nor physiological variables  
422 were related to the overall comfort perception of brioche. PC 3 correlated to chewing duration  
423 ( $R=0.94$ , data not shown) and did not correlate with other variables.

424 Additionally, two PCA were performed in order to consider poor and satisfactory dental status  
425 (DS) separately. For sponge cake, the obtained projections were similar to those described  
426 above on Fig. 3 (left) and led to identical conclusions (data not shown). In contrast, for  
427 brioche, PCA performed by DS group highlighted further relationships between physiological  
428 variables, oral processing and bolus properties that were not identified previously. For both  
429 groups, satisfactory and poor DS, the first two PC's were selected, representing 57 and 63%  
430 of the total variability, respectively. A correlation circle including said PC's was depicted for  
431 each group (Fig. 4). Some parallels and contrasts between the two groups can be outlined.  
432 For instance, in both cases, the overall comfort perception continued to be depicted in  
433 opposition to the perceived stickiness and pastiness, as seen in the all-subject results (Fig. 3,  
434 right). Also, in both cases, the perceived easiness to swallow and moisten are depicted in the  
435 same direction and contribute to discriminate subjects in PC 1, suggesting these attributes  
436 are not dependent on the DS. On the other hand, attributes such as easiness and easiness  
437 to chew appear to be distinctive between groups. In the case of the poor DS group, they  
438 appear correlated positively to PC 1 ( $R_{\text{chew}}=0.8$ ,  $R_{\text{easiness}}=0.57$ ), meaning they contribute  
439 importantly to discriminate subjects in this dimension, and differences in perception could be  
440 higher within this group. It is not the case for the satisfactory DS, where the mentioned  
441 variables did not correlate to either of the PC 1 nor PC 2. This result suggests that the  
442 perception of these variables is affected by the DS of the subjects and was probably more  
443 consensual for the satisfactory DS group. Another important difference resides in the  
444 chewing duration, which is depicted contradictorily for each DS group. These results are in  
445 agreement with the previous ones (see section 3.2, Table 4), since the bolus apparent

446 viscosity and chewing duration had already shown to be influenced positively by a  
447 satisfactory DS.

448 Finally, the perception of OC was not the same across the two groups. While for the  
449 satisfactory DS, the interpretation remains unchanged from the all-subject results (Fig.3,  
450 right), for the poor DS group, overall comfort was projected close to the SSF and opposed to  
451 the bolus viscosity. Moreover, all of the three variables correlated to PC2 ( $R_{\text{Comfort}}= 0.56$ ;  
452  $R_{\text{SSF}}= 0.74$ ;  $R_{\eta_{120}}= -0.72$ ) even though there was no direct correlation within this group  
453 between the bolus apparent viscosity and overall comfort scores, as confirmed by Pearson  
454 coefficient ( $r= -0.17$ ,  $p>0.05$ ). However, there was a significant one between overall comfort  
455 and SSF ( $r= 0.77$ ,  $p<0.01$ ). This result suggests that dental status can actually influence the  
456 perception of OC, but paradoxically highlights the importance of SSF over DS. These results  
457 also suggest that the perception of OC in a product like brioche is more complex and  
458 depends on other factors that remain to be studied such as oral lubrication mechanisms (i.e.  
459 oral tribology), physiological variables (i.e. in-mouth shear forces), bolus properties (i.e.  
460 particle size) or product characteristics (i.e. the amount of fat).

461

## 462 **CONCLUSIONS**

463 Our results have shown remarkably that for soft aerated cereal foods, stimulated salivary  
464 flow rate is the most important physiological variable that impacts the food bolus properties  
465 and the perception of oral comfort in elderly, priming over the dental status. However,  
466 increasing the amount of fat seems to lower the role of the stimulated flow rate and bolus  
467 hydration, likely by increasing lubrication. This highlights the importance of the hydration and  
468 lubrication mechanisms in the oral processing and enjoyment of eating for this type of  
469 products in the elderly. Additionally, it was seen that two products with different composition  
470 and structure show similar chewing behavior and oral comfort perception but different bolus  
471 properties and oral mechanisms. Moreover, it has been found that the evolution of bolus  
472 viscosity can be predicted through the stimulated saliva flow rate of the individual  
473 independently of the dental status. Since viscosity has been shown to influence significantly

474 the oral comfort, this relationship could be a good basis for modelling oral processing and  
475 designing foods with the desired oral comfort for the elderly.

476 In this study, salivary role has been only considered in terms of resting and stimulated flow.  
477 However, other salivary properties may influence bolus properties and sensory perception of  
478 cereal products, in particular salivary alpha-amylase and/or viscosity (Joubert et al., 2017).  
479 Knowing that salivary composition evolves significantly with age, (Nagler & Hershkovich,  
480 2005) influence of salivary composition on oral processing, food bolus properties and  
481 sensory perception of cereal products in elderly will be the subject of further investigation.

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Figure 1. Bolus apparent viscosity  $\eta$  ( $\dot{\gamma}=120s^{-1}$ ) as a function of the theoretical amount of saliva in mouth.  $\blacktriangle$ =Sponge-cake,  $\bullet$ =Brioche, Full symbols= C1, Mid-filled symbols=C2, Empty Symbols=SP.

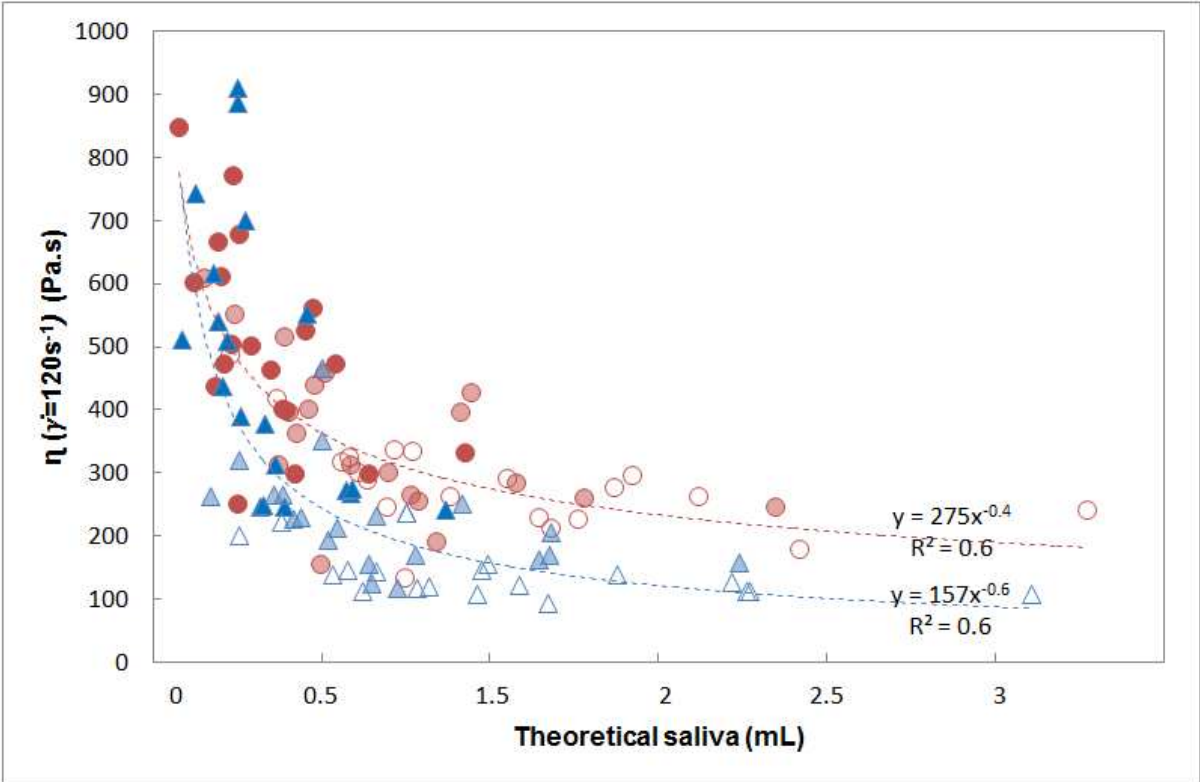
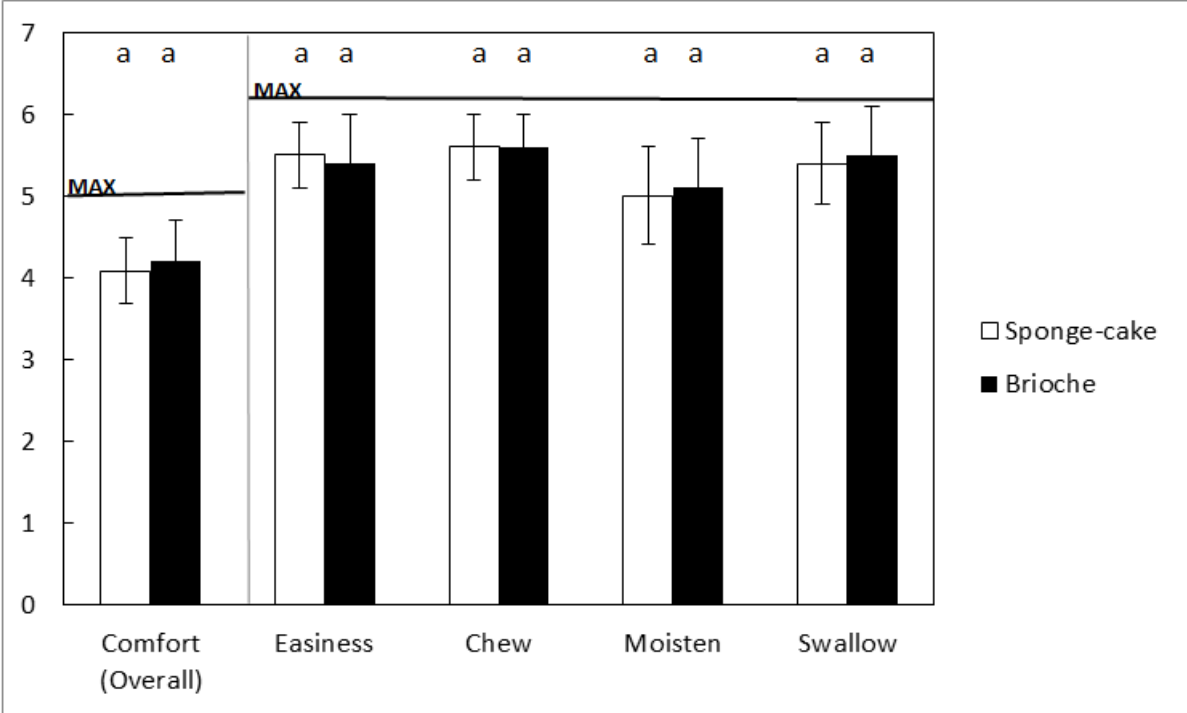


Figure 2. Mean scores for general comfort and bolus formation sections of the comfort questionnaire for all subjects and both products.



Different letters indicate means that significantly differ between products with  $p < 0.05$  (Student-Newman-Keuls test).

Figure 3. PCA correlation circle for oral health status, bolus properties, chewing duration and in-mouth comfort variables. Left= Sponge-cake, Right=Brioche.

**Comfort**=Overall oral comfort perception score; **Chew**=Perceived as easy to chew; **Moisten**=Perceived as easy to moisten; **Swallow**=Easy to swallow; **Easiness**=Perceived as easy to eat; **Pasty**=Perceived pastiness; **Sticky**=Perceived stickiness; **BHR**=Bolus hydration ratio or added saliva;  $\eta_{120}$ =Bolus apparent viscosity at 120s<sup>-1</sup>; **SSF**=Stimulated salivary flow rate; **SP**=Swallowing point or total chewing duration.

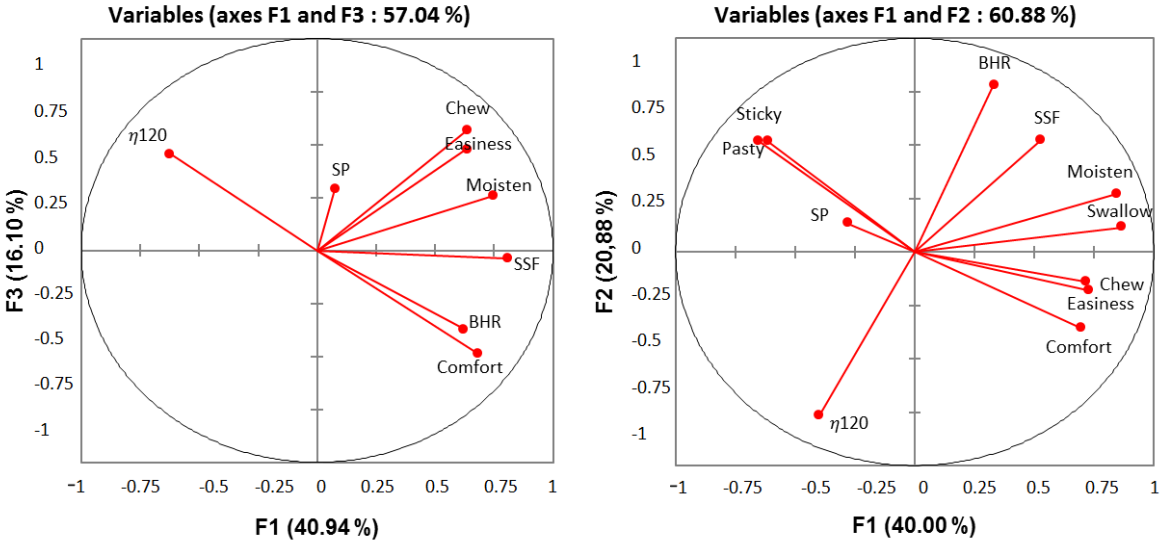


Figure 4. PCA correlation circle for oral health status, bolus properties, chewing duration and in-mouth comfort variables by dental status group for Brioche. Left= Satisfactory DS; Right= Poor DS

**Comfort**=Overall oral comfort perception score; **Chew**=Perceived as easy to chew; **Moisten**=Perceived as easy to moisten; **Swallow**=Easy to swallow; **Easiness**=Perceived as easy to eat; **Pasty**=Perceived pastiness; **Sticky**=Perceived stickiness; **BHR**=Bolus hydration ratio or added saliva;  $\eta_{120}$ =Bolus apparent viscosity at  $120s^{-1}$ ; **SSF**=Stimulated salivary flow rate; **SP**=Swallowing point or total chewing duration.

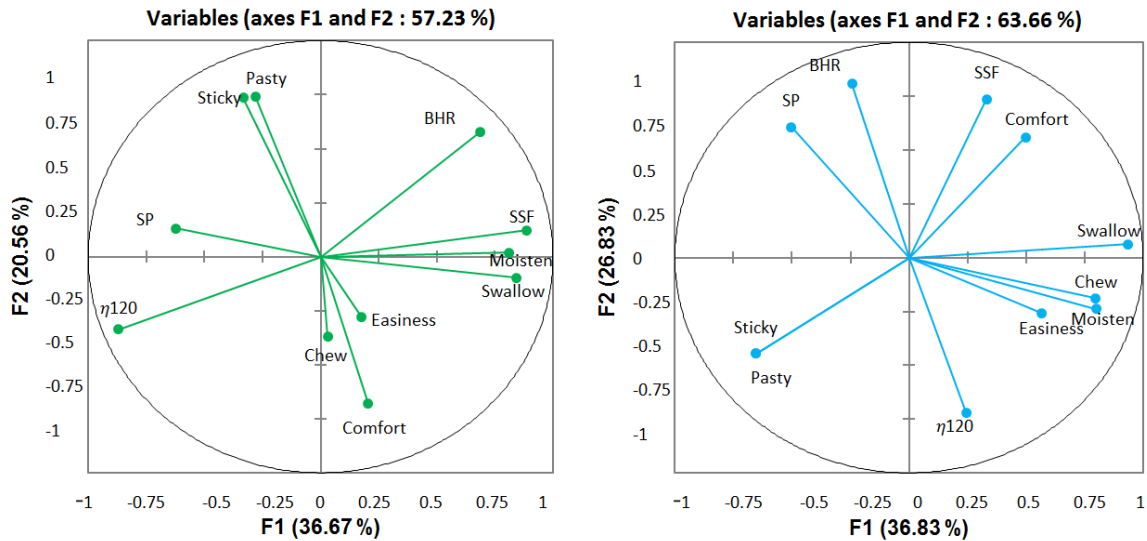


Table 1. Subjects characteristics.

	<b>Poor Dental Status (PFU ≤ 4) n=10</b>	<b>Satisfactory Dental Status (PFU ≥ 7) n=10</b>	<b>Whole group n=20</b>
<b>Age (years)</b>	75 ± 4	69 ± 5	72 ± 5
<b>Sex</b>			
Number of Male	5	4	9
Number of Female	5	6	11
<b>Unstimulated Salivary Flow Rate (mL/min)</b>	0.41 ± 0.19 Min 0.18 Max 0.75	0.33 ± 0.18 Min 0.03 Max 0.67	0.37 ± 0.18 Min 0.03 Max 0.75
<b>Stimulated Salivary Flow Rate (mL/ min)</b>	1.76 ± 0.89 Min 0.84 Max 3.70	2.05 ± 0.98 Min 0.30 Max 3.84	1.91 ± 0.92 Min 0.3 Max 3.84

Table 2. Product properties and composition (wet basis).

	<b>Sponge-cake</b>	<b>Brioche</b>
<b>Proteins (g/100 g)*</b>	11	7
<b>Fat (g/100 g)*</b>	6	17
<b>Carbohydrates (g/100 g)*</b>	55	46
Sucrose (g/100 g)	27	14
Starch (g/100 g)	18	30
Others (g/100 g)	10	2
<b>Density (g/cm<sup>3</sup>)</b>	0.21 ± 0.02	0.33 ± 0.02
<b>Water content (g/100 g)</b>	28 ± 2	30 ± 2

*\*Theoretical values based on individual ingredients composition (USDA database).*

Table 3. Chewing parameters and bolus properties for all subjects by product and chewing stage (C1, C2 and SP).

Product	Sponge-cake	Brioche	Sponge-cake	Brioche	Sponge-cake	Brioche
<i>Chewing stage</i>	<i>C1</i>	<i>C1</i>	<i>C2</i>	<i>C2</i>	<i>SP</i>	<i>SP</i>
<b>Chewing parameters</b>						
<b>Chewing duration (s)</b>	11±4*	11±3*	23±8*	21±7*	34±11 <sup>a</sup>	33±9 <sup>a</sup>
<b>Chewing cycles</b>	13±5*	13±4*	27±9*	27±8*	41±13 <sup>a</sup>	41±11 <sup>a</sup>
<b>Chewing frequency (Hz)</b>	1.2±0.3*	1.3±0.2*	1.2±0.3*	1.3±0.2*	1.2±0.3 <sup>a</sup>	1.3±0.2 <sup>a</sup>
<b>Bolus Properties</b>						
<b>Hydration ratio</b> Added saliva (%)	31±12 <sup>a</sup>	23±7 <sup>b</sup>	55±21 <sup>a</sup>	35±9 <sup>b</sup>	79±25 <sup>a</sup>	45±11 <sup>b</sup>
<b>Apparent viscosity</b> $\eta$ ( $\dot{\gamma}=120\text{s}^{-1}$ ) (Pa.s)	464±216 <sup>a</sup>	505±159 <sup>a</sup>	227±83 <sup>a</sup>	358±120 <sup>b</sup>	145±44 <sup>a</sup>	284±79 <sup>b</sup>

*Different letters indicate means that significantly differ between products for each chewing stage with  $p < 0.05$  (Student-Newman-Keuls test); Values with a \* were not measured but calculated from the SP (measured) value (C1=1/3SP or C2=2/3SP).*



Table 4. ANCOVA model coefficients (Type III sum of squares) for chewing duration, bolus properties and oral comfort by product Sponge-Cake (SC) and Brioche (B). ; **F**= Fisher ratio; **p**= p-value; **β** =normalized regression coefficients, for dental status only the Satisfactory coefficient is given (**S**).

		Chewing duration (SP)		Hydration ratio		$\eta_{120}$		Overall Comfort	
		SC	B	SC	B	SC	B	SC	B
<b>Dental Status (DS)</b>	F	<b>4.87</b>	<b>8.25</b>	0.15	1.54	0.41	<b>7.76</b>	2.11	2.34
	p	<b>0.03</b>	<b>0.006</b>	0.70	0.22	0.53	<b>0.007</b>	0.17	0.15
	β S	<b>0.67</b>	<b>0.86</b>	-0.08	-0.28	0.03	<b>0.62</b>	0.67	0.82
<b>Stimulated Salivary Flow (SSF)</b>	F	3.33	1.56	<b>21.17</b>	<b>17.75</b>	<b>12.10</b>	<b>23.35</b>	<b>7.89</b>	0.47
	p	0.07	0.22	<b>0.0001</b>	<b>0.0001</b>	<b>0.001</b>	<b>0.0001</b>	<b>0.01</b>	0.51
	β	0.01	0.20	<b>0.37</b>	<b>0.34</b>	<b>-0.25</b>	<b>-0.20</b>	<b>0.80</b>	0.41
<b>DS*SSF</b>	F	3.70	<b>7.60</b>	0.17	1.76	0.95	<b>7.10</b>	1.54	0.30
	p	0.06	<b>0.008</b>	0.69	0.19	0.33	<b>0.01</b>	0.23	1.13
	β S	-0.72	<b>-1.02</b>	0.09	0.34	-0.26	<b>-0.70</b>	-0.67	-0.66

Significant values ( $p < 0.05$ ) highlighted in bold