Chapter I: Article I

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

# ABSTRACT

The aim of this study was to investigate food oral processing and bolus formation in the elderly population, and their relationship with the perception of oral comfort, for two soft cereal products of different composition: sponge-cake and brioche. Twenty subjects aged 65 and over participated in the study. They were classified in two groups according to dental status (poor vs. satisfactory) and presented various stimulated salivary flow rate (SSF) in each group. Food bolus properties (hydration ratio and apparent viscosity) were characterized after three chewing stages for both groups. Results showed that chewing duration did not depend on food product but rather on physiology: subjects with a poor dental status had a shorter chewing duration. For each chewing stage, sponge-cake boli showed a higher hydration ratio than brioche boli, which showed higher apparent viscosity. For sponge-cake, perception of oral comfort was primarily driven by SSF rate, irrespective of the dental status. In the case of brioche, oral comfort was also partially explained by SSF in the case of subjects with poor dental status. This result suggests that perception of oral comfort in brioche could be driven by product related attributes rather than oral health. For both foods, a phenomenological model of bolus viscosity as a function of stimulated salivary flow and chewing duration was proposed.

# Introduction

The proportion of elderly people worldwide is growing rapidly. Over the first half of the current century, the global population aged 60 and over is projected to expand by more than three times, to reach nearly 2.1 billion in 2050 (United Nations, 2002; United Nations, 2015). Aging is often associated with a degradation of the oral health status, where tooth loss, decreased muscle strength and reduced salivary flow are among the main factors responsible for eating difficulties and loss of eating pleasure in the elderly (Laguna et al. 2016a, Ship 1999, Vandenberghe-Descamps et al. 2016, Wang & Chen 2017, Xu 2016). Moreover, olfactory and gustatory capacities are also reduced (Boyce & Shone 2006, Methven et al. 2012), increasing the risk of food intake reduction, leading to malnutrition and other diseases (Henshaw & Calabrese 2001, Maitre et al. 2014, Rolls 1999, Schwartz et al. 2017). In this context, it has therefore become crucial to develop age-friendly food products with an improved nutritional value and enhanced enjoyment of eating in order to ameliorate the quality of life of the forthcoming senior population (Giacalone et al. 2016, Schwartz et al. 2017). Food Oral Processing (FOP) has been shown to be a crucial stage for texture, taste and aroma perception, as well as for sensory pleasure (Chen 2009, Salles et al. 2011, Varela et al. 2009). A better understanding of the mechanisms involved in FOP is thus necessary (Chen 2015, Laguna & Chen 2016). Research has shown that the elderly use strategies to compensate for oral impairments such as extending chewing duration, increasing the number of chewing cycles and swallowing larger particles of food (Mioche, Bourdiol, Monier, Martin, & Cormier, 2004; Peyron, Blanc, Lund, & Woda, 2004; Peyron, Woda, Bourdiol, & Hennequin, 2017). More recently, research focusing into establishing the concept of ‘eating capability’ in this population through physiology, showed that biting force and dental status influenced the oral processing duration, the number of chewing cycles, as well as liking and difficulty perception (Laguna & Sarkar 2016; Laguna et al. 2015a,b, 2016b). Other studies have shown that food bolus properties can be related to the perception of texture and aroma (Devezeaux de Lavergne et al. 2015; Jourdren et al. 2016b, 2017). Transforming food into a bolus that is ready to swallow is the main purpose of FOP (Prinz & Lucas 1997). Therefore, studying its degree of transformation in the mouth by quantitatively characterizing its properties, such as hydration ratio, rheological behavior and particle size, is fundamental for the understanding of the underlying mechanisms.

It is common knowledge that, beyond the physiological and nutritional functions, eating is an enjoyable sensory experience that can be source of satisfaction and pleasure (Bourne 2002). However, literature regarding the food enjoyment and comfortability, especially of the elderly, is quite scarce. Recently, Vandenberghe-Descamps, Labouré, Septier, Feron, & Sulmont-Rossé (2017) developed a questionnaire to assess oral comfort for a wide variety of foods. They also investigated the impact of dental status and salivary flow on the oral comfort perception (Vandenberghe-Descamps et al. 2017b). Xu (2016) highlighted the importance of taking into account pleasure and enjoyment when designing specialized foods for the elderly, so that optimum masticatory pleasure can be achieved. However, present foods targeted for the elderly are mainly focused on the nutritional needs, without considering enjoyment. They are often found as dietary supplements that produce taste-fatigue on the long-term and have low compliance (Gosney 2003). Cereal products, besides from being staple foods in many countries, are affordable, nutritious and can be consumed regardless of culture and beliefs. They are widely consumed among the elderly population, who tends to orient towards a more ‘traditional’ dietary pattern (Andreeva et al. 2016). To this extent, the products selected for this study, sponge-cake and brioche, have been little studied and are good candidates for development since they have pleasant sensory properties. They have also a relative flexibility regarding formulation, opening the possibility for modifications including the enrichment with fibers or proteins to increase their nutritional value. Numerous studies have been carried out regarding FOP of cereal products such as bread, biscuits and breakfast flakes (Gao, Wong, Lim, Henry, & Zhou, 2015; Jourdren, Panouillé, et al., 2016; Le Bleis, Chaunier, Montigaud, & Della Valle, 2016; Peyron et al., 2011; Tournier, Grass, Septier, Bertrand, & Salles, 2014; Young et al., 2016). All of the precedent studies were conducted on middle-age population. As far as we know, there is a lack of similar investigations on elderly population.

Given this context, the aim of this study was to determine the relationships between bolus properties, oral health status and perceived oral comfort in elderly for two cereal foods: sponge-cake and brioche. Two physiology criteria were selected to assess the oral health status of participants: dental status and salivary flow rate. The tongue pressure was not included since it has been shown that solid foods require teeth action rather than tongue to be processed (Funami 2016, Ishihara et al. 2013). In this purpose, the impact of dental status and salivary flow rate on food bolus hydration ratio and rheological properties was investigated for both products. Secondly their relationships with the perception of oral comfort were assessed.

## Materials and Methods

### Subjects

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

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

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

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|  | **Poor Dental Status (PFU ≤ 4)**  **n=10** | **Satisfactory Dental Status (PFU ≥ 7)**  **n=10** | **Whole group**  **n=20** |
| **Age (years)** | 75 ± 4 | 69 ± 5 | 72 ± 5 |
| **Sex**  Number of Male  Number of Female | 5  5 | 4  6 | 9  11 |
| **Unstimulated Salivary Flow Rate (mL/min)** | 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 |
| **Stimulated Salivary Flow Rate (mL/ min)** | 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 1‑1. Subjects characteristics.

### Product samples

Brioche and sponge-cake were provided by CERELAB® (Dijon, France). Their composition and density values are shown in Table 2. Products were offered to the participants as cylinders of 20 cm3. Portions of each product were cut just before the beginning of the experimentation with a knife and a circular steel cutter of diameter (Ø)=3 cm for sponge-cake (h=2.8 cm) and Ø=5 cm for brioche (h=1 cm) and given to the subjects as mouthfuls for consumption and bolus generation during the experimental procedure.

|  |  |  |
| --- | --- | --- |
|  | Sponge-cake | Brioche |
| Proteins (g/100 g)\* | 11 | 7 |
| Fat (g/100 g)\* | 6 | 17 |
| Carbohydrates (g/100 g)\* | 55 | 46 |
| **Sucrose (g/100 g)** | 27 | 14 |
| **Starch (g/100 g)** | 18 | 30 |
| **Others (g/100 g)** | 10 | 2 |
| Density (g/cm3) | 0.21 ± 0.02 | 0.33 ± 0.02 |
| Water content (g/100 g) | 28 ± 2 | 30 ± 2 |

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

Table 1‑2. Product properties and composition (wet basis).

### Experimental procedure

Every subject participated in one collective and six individual sessions, for a total of seven sessions of approximately 1 h. The collective session aimed at determining the individual swallowing point of both products. Participants were asked to consume the product mouthful (20 cm3)in a natural manner and were recorded on video while doing it. They were asked to point out the swallowing moment by raising their hand. Total chewing duration was calculated as the time elapsed from the placement of the food inside the mouth and the swallowing point, which was defined right after the first swallow. The number of chewing cycles was determined from this recording as well and one chewing cycle was defined after a complete sequence of opening-occlusion. Chewing frequency was calculated from this data by dividing the number of chewing cycles by the chewing duration. Water (Evian, France) was offered freely after each mouthful. The procedure was repeated twice for each product. During the individual sessions, participants were asked to chew the product mouthful and to expectorate the food bolus into a Petri dish at three mastication stages according to their individual total chewing duration: 1/3 of total chewing duration (C1), 2/3 of total chewing duration (C2) and just before the swallowing point (total chewing duration, SP). They repeated the procedure once for each product. Food boli were collected at the three stages (C1, C2 and SP) for further characterization. At the end of a randomly selected individual session, participants were also asked to respond the oral comfort assessment questionnaire. They repeated the questionnaire once for each product on a different session. In both collective and individual sessions, products were randomly distributed.

### Oral comfort assessment

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

### Bolus characterization

#### Capillary rheometry

The rheological properties of products and boli were determined by capillary rheometry as previously described by Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre (2013). A mechanical texture analyzer (TA.XTplus, Stable Micro Systems, UK) equipped with a cylindrical piston with flat head and a capillary die fixed at the bottom to a cylindrical barrel, were used as a capillary rheometer. Boli were loaded into the capillary die immediately after collection. Each product was tested at three values of apparent shear rate (𝛾̇ = 10, 42 and 333 s-1) according to different combinations of the piston speed (50 or 200 mm/min) and capillary die diameter (Ø=2 or 4 mm). From these shear rate values and pressure measurements, the apparent viscosity *ƞ* was calculated. Variations of *ƞ* (𝛾̇*)* were shown to follow a power law, as reported by Le Bleis et al., 2016 in the case of bread boli, with little variation of the flow index, close to 0.3. The value of *ƞ(*𝛾̇=120s*-1)* for each subject and each chewing stage was selected to characterize bolus viscosity from a typical shear rate value of the oropharynx at the beginning of swallowing (Zhu et al. 2014). Two boli were required to repeat the measurement for each of the three chewing stages for all of the three apparent shear rate values, leading to a total of 2x3x3=18 boli per subject for each product.

#### Bolus hydration ratio

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



Where:

bolus WC =bolus water content

bolus DM =bolus dry matter

product WC =product’s water content

product DM =product’s dry matter

### Statistical analysis

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

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

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

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

## Results and discussion

### Chewing parameters and bolus properties

Average chewing parameters and bolus properties regardless of dental status and salivary flow are shown in Table 3. For the chewing duration and the number of cycles, the ANOVA performed on the last chewing stage (SP) showed a significant subject effect (p<0.0001), but no product effect. This means that despite the differences of composition and properties between the products, subjects do not modify their duration of chewing from one product to another. Comparable results were reported by Le Révérend, Saucy, Moser, & Loret (2016) in the case of healthy adults, where little variation was observed in the chewing duration and number of chewing cycles of brittle cereal products. Chewing duration and number of cycles were strongly correlated as shown by Pearson coefficients (rSponge-cake= 0.91; rBrioche= 0.94, p<0.001). Therefore, chewing frequency remained relatively constant across subjects and was the only chewing parameter where the subject effect was not significant (p>0.05), meaning there is little inter-individual variability. This result is in accordance with those previously reported for other type of foods (Devezeaux de Lavergne, Derks, Ketel, de Wijk, & Stieger, 2015; Yven et al., 2012). Indeed, chewing frequency is reported to be a distinctive feature of the human species, with values close to 1.3 Hz (Lucas 2004), and it does not seem to be affected by age (Peyron et al., 2004).

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| --- | --- | --- | --- | --- | --- | --- |
| **Product** | **Sponge-cake** | **Brioche** | **Sponge-cake** | **Brioche** | **Sponge-cake** | **Brioche** |
| *Chewing stage* | *C1* | *C1* | *C2* | *C2* | *SP* | *SP* |
| ***Chewing parameters*** |  |  |  |  |  |  |
| **Chewing duration (s)** | 11 ± 4\* | 11 ± 3\* | 23 ± 8\* | 21 ± 7\* | 34 ± 11a | 33 ± 9a |
| **Chewing cycles** | 13 ± 5\* | 13 ± 4\* | 27 ± 9\* | 27 ± 8\* | 41 ± 13a | 41 ± 11a |
| **Chewing frequency (Hz)** | 1.2 ± 0.3\* | 1.3 ± 0.2\* | 1.2 ± 0.3\* | 1.3 ± 0,2\* | 1.2 ± 0.3a | 1.3 ± 0.2a |
| ***Bolus Properties*** |  |  |  |  |  |  |
| **Hydration ratio** Added saliva (%) | 31 ± 12a | 23 ± 7b | 55 ± 21a | 35 ± 9b | 79 ± 25a | 45 ± 11b |
| **Apparent viscosity** *ƞ* (𝛾̇=120s*-1)* (Pa.s) | 464 ± 216a | 505±159a | 227 ± 83a | 358 ± 120b | 145 ± 44a | 284 ± 79b |

*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) dvalue (C1=1/3SP or C2=2/3SP).*

Table 1‑3. Chewing parameters and bolus properties for all subjects by product and chewing stage (C1, C2 and SP).

Conversely, bolus properties showed significant differences between products (p<0.0001), besides subject (p<0.0001) and chewing stage (p<0.0001) effects. Generally, the hydration ratio increased with time, and therefore with each chewing stage; while the apparent viscosity decreased. For bolus hydration ratio, products differed at every chewing stage, while for bolus apparent viscosity they only did at the last two chewing stages (C2 and SP). For both products, initial viscosity before chewing (C0) was significantly (p<0.05) higher for sponge-cake (2164±12 Pa.s) than for brioche (1561±21 Pa.s), likely because of lower fat content. This difference decreases after the first chewing stage (C1), where both products apparent viscosity becomes close to each other (i.e. 500 Pa.s). Interestingly, during the last two sequences (C2 and SP), the apparent viscosity of brioche exceeds that of sponge-cake, contrasting with their initial values. Regarding the bolus hydration ratio, even if both products have close water content values (Table 2), the amount of added saliva was significantly (p<0.001) higher for sponge-cake than for brioche. This particular feature may be explained by the higher porosity of the sponge-cake, reflected by its lower density value, although its lower fat and higher sucrose contents could also have an influence. Hence, sponge cake would absorb more efficiently the water present in saliva. This result is in agreement with those reported by Mathieu, Monnet, Jourdren & Panouillé (2016), who compared the hydration kinetics of different bread structures and found higher hydration rates for more porous structures.

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

The influence of the oral health status on the chewing parameters and bolus properties was determined by ANCOVA model, and the results are presented in Table 4. As previously mentioned, given its high correlation with the number of chewing cycles, only the chewing duration (SP) was included to represent chewing parameters. The dental status (DS) influenced the SP for both products and it was longer for participants with a satisfactory DS (positive β coefficient). However, the interaction between DS and stimulated salivary flow rate (SSF), close to significance for sponge-cake and significant for brioche, suggests that a high SSF can counterbalance the observed DS effect. As for the bolus hydration ratio, it is clear that SSF is the main factor of influence for both products, which means higher flow rates lead to more hydrated boli (positive β coefficient). The bolus apparent viscosity was also highly impacted by the SSF, although in the opposite sense (negative β coefficient). DS, on the other hand, showed little impact on bolus properties. Only in the case of brioche, individuals with a satisfactory DS produced a bolus with a higher apparent viscosity. Nevertheless, likewise the chewing duration, there was a significant interaction with SSF. A hypothesis to explain the two previous interactions could reside in the well-known theory of the ‘swallowing threshold’ (Hutchings & Lillford 1988), which stipulates that all individuals swallow at determined time, degree of structure and degree of lubrication that depends on food product. Since in this case, the SSF has shown to be the main variable involved in the reduction of bolus apparent viscosity, it could be hypothesized that individuals with a high SSF will achieve the degree of bolus viscosity needed to trigger swallowing in a shorter chewing duration, than their counterparts with a lower SSF. This could explain why individuals with a satisfactory DS, but a low SSF, need a longer chewing duration to produce a bolus with a similar degree of viscosity than those with high SSF. From another perspective, the composition of saliva, mucins in particular, adds lubricating properties to saliva (Wu et al. 1994) and therefore could also be partially responsible for the ‘faster swallowing’ of individuals with high SSF. However, in the present study the composition of saliva was not investigated, which is one of its limitations. Overall, the stimulated flow rate appears to be the key parameter that determines bolus properties just before swallowing, whatever the dental status. A similar conclusion was obtained by Yven, Bonnet, Cormier, Monier, & Mioche (2006) who worked on meat products and subjects with impaired mastication. These authors hypothesized that the level of moisture is more important in triggering the swallow event than is the level of comminution of the product.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Chewing duration (SP)** | | **Hydration ratio** | | **𝜂120** | | **Overall Comfort** | |
|  |  |  | *SC* | *B* | *SC* | *B* | *SC* | *B* | *SC* | *B* |
| **Dental Status (DS)** | F |  | **4.87** | **8.25** | 0.15 | 1.54 | 0.41 | **7.76** | 2.11 | 2.34 |
| p |  | **0.03** | **0.006** | 0.70 | 0.22 | 0.53 | **0.007** | 0.17 | 0.15 |
| β | S | **0.67** | **0.86** | -0.08 | -0.28 | 0.03 | **0.62** | 0.67 | 0.82 |
| **Stimulated Salivary Flow (SSF)** | F |  | 3.33 | 1.56 | **21.17** | **17.75** | **12.10** | **23.35** | **7.89** | 0.47 |
| p |  | 0.07 | 0.22 | **0.0001** | **0.0001** | **0.001** | **0.0001** | **0.01** | 0.51 |
| β |  | 0.01 | 0.20 | **0.37** | **0.34** | **-0.25** | **-0.20** | **0.80** | 0.41 |
| **DS\*SSF** | F |  | 3.70 | **7.60** | 0.17 | 1.76 | 0.95 | **7.10** | 1.54 | 0.30 |
| p |  | 0.06 | **0.008** | 0.69 | 0.19 | 0.33 | **0.01** | 0.23 | 1.13 |
| β | S | -0.72 | **-1.02** | 0.09 | 0.34 | -0.26 | **-0.70** | -0.67 | -0.66 |

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

Table 1‑4. 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)**.

### Phenomenological model of apparent viscosity from stimulated flow rate

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



Where:

ɸ stim =Stimulated salivary flow rate )

t CX = Chewing duration at a given sequence

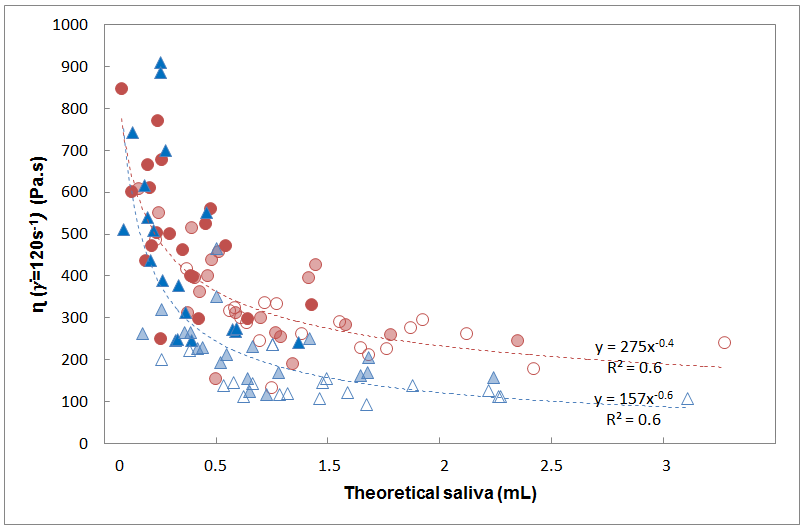
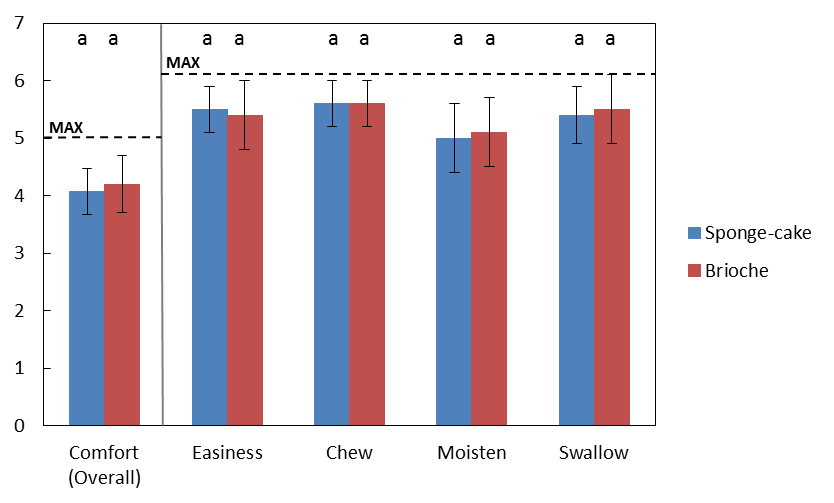


Figure 1‑1. Bolus apparent viscosity ƞ (𝛾̇=120s-1) as a function of the theoretical amount of saliva in mouth. ▲=Sponge-cake, ●=Brioche, Full symbols= C1, Mid-filled symbols=C2, Empty Symbols=SP.

From Figure 1, the decrease of the apparent viscosity over time can be fitted through a power law model and modelled from a single physiology parameter with an acceptable R2 coefficient, close to 0.6. Likely the data scattering, and lack of fit, may be due to inter-individual variability that is not explained by SSF, such as saliva composition and food fragmentation. Le Bleis et al. (2016) have taken into account the effect of fragmentation by dividing flow rate by the particle size in order to consider the increase of contact surface and absorption capacity of the food. This opens prospect for a more complete model by including other factors such as the particle size and the degree of fragmentation of food in future studies.

### Perception of oral comfort and impact of oral health status

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

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

Figure 1‑2. Mean scores for general comfort and bolus formation sections of the comfort questionnaire for all subjects and both products.

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

### Multivariate analysis and overall discussion

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



Figure 1‑3. 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; **𝜂120=**Bolus apparent viscosity at 120s-1; **SSF=**Stimulated salivary flow rate; **SP=**Swallowing point or total chewing duration.

PC 2 separated subjects according to chewing duration (R= 0.91, data not shown), but no other variable correlated to this PC, meaning that chewing time did not contribute to explain the other variables and in particular OC. This observation was confirmed by Pearson correlation analysis (r= -0.18, p>0.05). Hence, the individuals who took a longer time to swallow did not necessarily find the product uncomfortable and are probably ‘slow chewers’ in a general manner. This supports the theory that there are different chewing strategies according to the consumer preferences (Brown & Braxton 2000, Jeltema et al. 2015).

For brioche (Fig. 3, right) PC’s 1 and 2 (61 % of variability) were selected: PC 1 discriminated the subjects in terms of SSF and overall comfort, as shown by their correlations to this PC (R=0.53 and R=0.70, respectively), although they were not depicted close to each other. In fact, unlike sponge-cake, the perception of OC does not seem to be related to physiological variables but rather to sensory attributes describing OC (easy to chew, to swallow and to moisten). Interestingly, the bolus properties appeared to be orthogonal to the OC variables. Although the sticky and pasty attributes were opposed to overall comfort, and thus perceived as uncomfortable, they were poorly explained by the bolus properties or the physiology of the subjects. Previous studies in bread boli have shown a negative correlation between the perceived stickiness and the bolus hydration ratio (Jourdren et al. 2016b,a). To explain this, the authors hypothesized that a highly hydrated bolus could increase its cohesiveness and thus prevent its adhesion to the palate and teeth. Also, the perception of stickiness seemed to be influenced by the bread density: a higher density led to an increased perception of this attribute (Panouillé et al. 2014). However in our case, even if the brioche was denser and boli less hydrated, the perception of stickiness did not correlate to any of the studied bolus properties, as confirmed by Pearson correlation analysis (rBHR= -0.17 r𝜂120= 0.2, p>0.05). Also, even if the OC questionnaire does not feature a hedonic dimension, it is interesting to notice that some of the sensory attributes that were related to the perception of OC have shown to influence the liking of similar products in precedent studies (Tarrega et al. 2017). In this work, participants rated higher the products that were identified as sweet and easy to chew, and lower those that were found to be pasty and dry. This leads to think such sensory attributes could play an important role in driving consumer preferences, and thus deserve a better understanding and instrumental characterization during oral processing.’

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

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

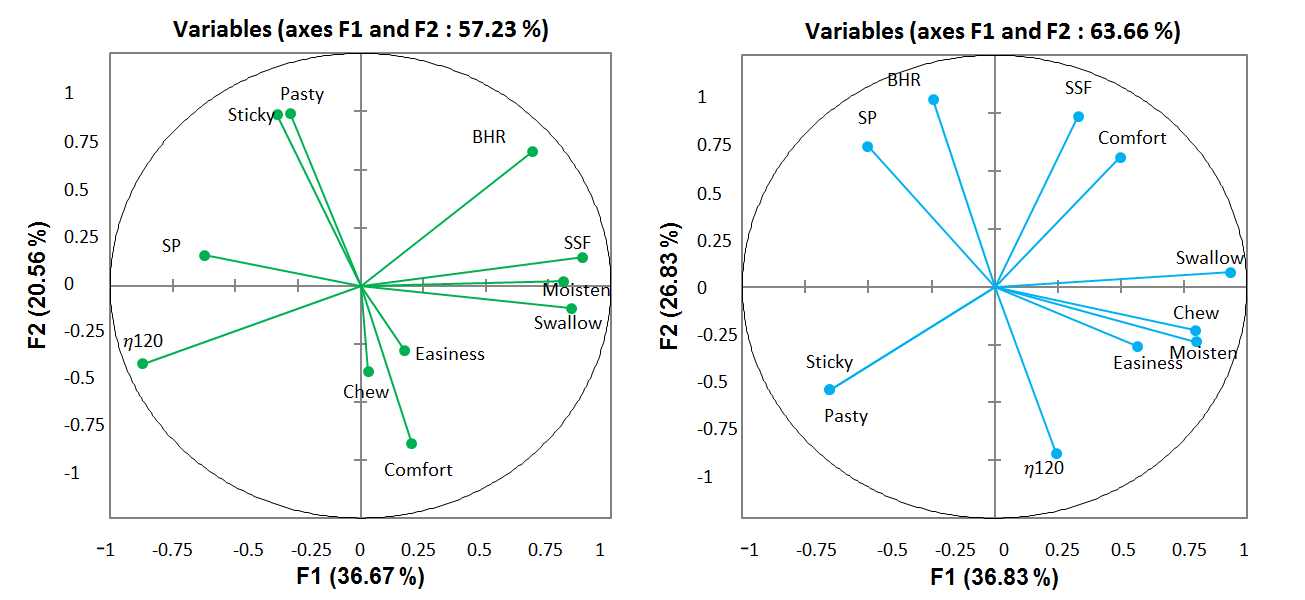


Figure 1‑4. PCA correlation circle for oral health status, bolus properties, chewing duration and in-mouth comfort variables by dental status group for Brioche. Left= Satisfactory DS; Right= Poor DS. **Comfort=**Overall oral comfort perception score; **Chew=**Perceived as easy to chew; **Moisten=**Perceived 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; **𝜂120=**Bolus apparent viscosity at 120s-1; **SSF=**Stimulated salivary flow rate; **SP=**Swallowing point or total chewing duration.

Finally, the perception of OC was not the same across the two groups. While for the satisfactory DS, the interpretation remains unchanged from the all-subject results (Fig.3, right), for the poor DS group, overall comfort was projected close to the SSF and opposed to the bolus viscosity. Moreover, all of the three variables correlated to PC2 (RComfort= 0.56; RSSF= 0.74; R𝜂120= -0.72) even though there was no direct correlation within this group between the bolus apparent viscosity and overall comfort scores, as confirmed by Pearson coefficient (r= -0.17, p>0.05). However, there was a significant one between overall comfort and SSF (r= 0.77, p<0.01). This result suggests that dental status can actually influence the perception of OC, but paradoxically highlights the importance of SSF over DS. These results also suggest that the perception of OC in a product like brioche is more complex and depends on other factors that remain to be studied such as oral lubrication mechanisms (i.e. oral tribology), physiological variables (i.e. in-mouth shear forces), bolus properties (i.e. particle size) or product characteristics (i.e. the amount of fat).

# CONCLUSIONS

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

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

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Chapter I. Article 2.

**Fragmentation of two soft cereal products during oral processing in the elderly: impact of product properties and oral health status**

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# Abstract

This study investigated the mechanisms of fragmentation leading to bolus formation during chewing in the elderly population for two cereal foods of different compositions and cellular structure: sponge-cake (SC) and brioche (B). For both products, mechanical properties were characterized by uniaxial compression and 3D cellular structure was determined using x-ray micro-tomography. Stress-strain curves showed two distinct ductile-like behaviors: product B underwent plastic deformation, whereas product SC displayed a hyper-elastic behavior. Twenty subjects aged 65 years and over with two different oral health conditions (poor *vs* satisfactory dental status, variable stimulated salivary flow rate) were asked to consume both products. Bolus particle size was determined at three different chewing stages through image analysis, and the resulting particle size distribution (PSD) curves were fitted by Gompertz model. The model parameterswere related to bolus particle heterogeneity and fragmentation, thanks to their correlations with median particle size diameter D50 and interquartile ratio (D75/D25), directly extracted from PSD curves. The use of model parameters allowed discriminating between chewing sequences for both products and revealed different fragmentation patterns: while SC boli exhibited a continuous particle size reduction during chewing, B displayed a combination of fragmentation and agglomeration. In addition, results showed that subjects with a satisfactory dental status produced significantly more degraded boli than those with a poor dental status. These results highlight distinct fragmentation mechanisms for these two soft products that were interpreted in relation to their differences in composition, structure and mechanical behavior.

# Nomenclature

a Gompertz fitting parameter, maximum size value achieved

ANSM Acronym for the French ‘National Agency of Drugs and Safety’

B Brioche

b Gompertz fitting parameter, slope at the inflexion point

c Gompertz fitting parameter, size value at the inflexion point

C1 1/3 of chewing duration, first chewing sequence

C2 2/3 of chewing duration, second chewing sequence

D25 Particle diameter of first the quartile of the distribution

D50 Median particle diameter of the distribution

D75 Particle diameter of the third quartile of the distribution

D75/25 Interquartile ratio of the particle size distribution

DS Dental status

E Young’s modulus (kPa)

FOP Food Oral Processing

P Poor (Dental status)

PSD Particle Size Distribution

PFU Posterior Functional Unit

S Satisfactory (Dental status)

SC Sponge-cake

SP Swallowing Point, total chewing duration, third chewing sequence,

SSF Stimulated Salivary Flow rate (mL∙min-1)

XR-µCT X-Ray Micro-Computed Tomography

σc Critical stress (kPa)

# Introduction

The physiological deterioration that accompanies ageing, together with the fact that the population aged 60 and over is expected to nearly triple by 2050 (United Nations, 2002), have increased the demand for foods with optimum texture design that are nutritious, safe and enjoyable (Chen, 2016; Schwartz, Vandenberghe-Descamps, Sulmont-Rossé, Tournier, & Feron, 2017).

Peleg early pointed out the need for understanding the relationship between the mechanical and geometrical properties of a food and its perceived texture in order to provide guidelines to develop specific products targeted for the elderly (Peleg, 1993). Since then, advances in the understanding of food oral processing (FOP) have been extensively reviewed (Chen, 2009, 2014, 2015) and the importance of structure and mechanical properties of foods in the bolus formation mechanisms has been highlighted (Gao, Wang, Dong, & Zhou, 2017; Pascua, Koç, & Foegeding, 2013; Witt & Stokes, 2015), as well as in the perception of flavor (Panouillé, Saint-Eve, Déléris, Le Bleis, & Souchon, 2014) and texture (Devezeaux de Lavergne, Derks, Ketel, de Wijk, & Stieger, 2015; Gao, Ong, Henry, & Zhou, 2017). These works have improved the understanding of texture by combining the studies of bolus formation mechanisms with the structural and mechanical properties of foods. The perception of texture is recognized as a dynamic process and does not depend only on the initial food properties, which govern the early stages of mastication (Kim et al., 2012; Young, Cheong, Hedderley, Morgenstern, & James, 2013), but also on bolus properties towards the middle and the end of oral processing (Devezeaux de Lavergne, van de Velde, & Stieger, 2017; Jourdren, Saint-Eve, et al., 2016). The characterization of bolus properties has thus become crucial to the understanding of FOP and perception mechanisms. This approach has been poorly addressed in the elderly, despite that such knowledge could bring new opportunities to develop food products specifically targeted for this population. Recently, we studied the relationships between sensory perception, food oral processing and bolus properties for two cereals products, namely sponge-cake and brioche, in elderly subjects varying in dental status and salivary flow rate (Assad-Bustillos, Tournier, Septier, Della Valle, & Feron, 2017). We developed a phenomenological model predicting the evolution of bolus apparent viscosity during oral processing. Viscosity was found to decrease with the theoretical amount of saliva absorbed, expressed as the product of chewing time by the stimulated salivary flow rate, irrespectively of the dental status of the subjects (Assad-Bustillos et al., 2017). However, the model displayed some dispersion, likely because the contribution of the particle size distribution of bolus fragments (PSD) was not taken into account.

The PSD of foods during oral processing has been early recognized as a crucial factor in bolus formation (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000; Olthoff, Van Der Bilt, Bosman, & Kleizen, 1984; Peyron, Mishellany, & Woda, 2004), and has been identified as a key parameter in the triggering of swallowing (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Peyron et al., 2011). Many studies have attempted to describe the comminution process of food materials after chewing by using mathematical models that consider the probability of a particle of being selected and its degree of fragmentation, which in turn depend on other factors such as its shape and mechanical properties (Lucas & Luke, 1983; van der Bilt, Olthoff, van der Glas, van der Weelen, & Bosman, 1987; van der Glas, Kim, Mustapa, & Elmanaseer, 2018; van der Glas, van der Bilt, & Bosman, 1992). To this extent, there have been attempts to relate the degree of fragmentation of several foods to their mechanical properties (Agrawal, Lucas, Prinz, & Bruce, 1997; Chen, Khandelwal, Liu, & Funami, 2013; Lucas, Prinz, Agrawal, & Bruce, 2002). From these studies, it appears that the median particle size (D50) of the bolus before swallowing is inversely related to the food hardness obtained from instrumental measurements performed by uniaxial compression. However, these observations seem to be limited to foods that exhibit brittle facture, meaning that they break in their elastic domain. As pointed out by Gao, Wang, et al. (2017), there is a lack of similar studies concerning fracture in ductile (also referred as *soft*) food materials, which are able to resist high levels of plastic deformation before breaking (e.g. bread or cakes).

As far as we know, the only cereal food exhibiting ductile behavior for which PSD after chewing has been studied and modelled is bread. Different methods have been used to characterize the PSD, such as drying, sieving and weighing the recovered fractions. Image acquisition - based on optical scanning, camera and/or laser diffraction for small particles (≤ 1mm) (Jourdren, Panouillé, et al., 2016; Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre, 2013; Pentikäinen et al., 2014) (Gao, Wong, Lim, Henry, & Zhou, 2015; Hoebler et al., 1998, 2000) - have been used to provide a more accurate quantitative analysis. The diversity of methods used has made it difficult to compare results between studies. Yet, all of them concluded that there is a general decrease of the median particle size (D50) over time, and Jourdren, Panouillé, et al., 2016 also reported an increase in bolus heterogeneity, which they chose to assess by the interquartile ratio (D75/D25). In contrast, the influence of the initial bread structure in the PSD has not been extensively studied, and so far the reported results lack of consensus. For instance, Pentikäinen et al. (2014) showed that rye wholegrain breads, which featured denser structures and thicker cell walls than traditional wheat bread, led to boli that contained smaller particles. Yet, in a similar study, Le Bleis, Chaunier, Montigaud, & Della Valle (2016) found no significant effect of structure in the D50 of boli from fiber-rich bread with different densities. In general, inter-individual variability is considered to have a large influence on oral processing and bolus properties (Panouillé, Saint-Eve, & Souchon, 2016). However, when it comes to particle size, the impact of physiology has rarely been taken into account (Fontijn-Tekamp, van der Bilt, Abbink, & Bosman, 2004; Hoebler et al., 1998; Peyron et al., 2004). Furthermore, there is a lack of focus on the elderly population, whose oral health is frequently deteriorated due to tooth loss and decreased salivary flow rate (Laguna, Aktar, Ettelaie, Holmes, & Chen, 2016; Ship, 1999; Vandenberghe-Descamps et al., 2016).

Hence, considering the various aspects involved in food fragmentation and bolus formation, the objectives of this study were, in the first place, to accurately describe and assess the fragmentation process during the chewing of two soft cereal foods with different composition and structure in an elderly panel; and secondly, to assess the impact of the oral health status of the participants in the said foods’ fragmentation process. In this purpose, we have fully characterized the PSD of sponge-cake (SC) and brioche (B) boli collected after three chewing stages from a group of elderly subjects. Additionally, the data was fitted with a mathematical model in order to be able to extract as much information as possible and avoid single parameter comparisons. With this information, the influence of the dental status (DS) and salivary flow rate (SSF) of the elderly on the PSD of the boli was evaluated.

## Materials and Methods

### Product composition, structural and mechanical properties

The sponge-cake and brioche used in this study were provided by CERELAB®, France. Their composition is detailed in Table A (Appendix).

Their instrumental texture was defined by their density, 3D cellular structure and mechanical behavior. The product density was measured by the rapeseed displacement method.

The three-dimensional cellular structure was determined by X-ray micro-computed tomography (XR-µCT), using a compact table-top system Skyscan 1174 (Bruker microCT, Belgium). A cylindrical sample of each product with a diameter of 2 cm and a height of 3 cm was prepared with a steel cutter and placed on a rotating plate while the X-ray beam passed through. A CCD camera with a resolution of 1304×1304 pixels was used to acquire the 2D radiographic images. The exposure time was 2000 ms, and the pixel size was adjusted to 22 μm. Two images were taken per rotational step (every 0.5°, until 360°) and were averaged. The projections were then reconstructed to obtain cross-sectional images using the NRecon reconstruction software (Bruker microCT, Belgium). Reconstructions were based on the Feldkamp cone-beam algorithm (Feldkamp, Davis, & Kress, 1984). After reconstruction, a stack of 1000 images in TIFF format was obtained for each sample. 3D images were therefore composed of 1304×1304×1000 voxels, coded on an 8-bit grey-scale. One replication was made for each product, for a total of four independent 3D images generated. From the images, the granulometric curves, that lead to cell wall size and cell wall thickness values, were calculated by using mathematical morphology operations (Serra, 1982). A series of openings of increasing size (image sieving) was performed on the features of interest and the sum of the volume occupied by the sieved particles, either cells or walls, was computed at each step. The results were expressed as the plot of the cumulative volume (%) of the particle *vs* the particle diameter (µm). In addition, the relative density (D) was calculated by dividing the volume occupied by the cell walls by the total volume of the sample, and the void fraction (VF), or porosity (P), was calculated as the complementary fraction D (1)

(1)

The mechanical properties were determined by uniaxial compression test. A circular steel cutter was used to prepare cylindrical samples with a diameter of 40 mm and a height of 30 mm. Both products were subjected to uniaxial compression using a universal testing machine (Adamel Lomarghy, France) equipped with a 1 kN load cell. The testing was performed with a cross head speed of 50 mm/min until 66% in height reduction between parallel plates. Five replicates were performed for each food sample. Results were expressed as the stress versus strain plot, from which Young’s modulus (E), and the critical stress (σc), when applicable, were measured. E was calculated from the initial slope within the linear elastic domain, while σc was defined as the stress value at the end of the linear domain.

### Panel composition

Twenty subjects (9 men and 11 women, aged 65–82 years, average 72 ± 5 years) participated in the study. Their dental status (DS) was assessed by determining the number of Posterior Functional Units (PFU's), allowing their classification within two groups: poor (≤ 4PFU’s) and satisfactory (≥7 PFU’s) DS. Additionally, their salivary flow rate in mL∙min-1 under mechanical stimulation (SSF), was determined for each subject. The chewing duration up to the swallowing point (SP) was determined for each subject and product through video recording. These techniques were previously used and detailed by Assad-Bustillos et al. (2017). The results obtained for the average SSF and the SP of all participants, including their standard deviation, are recalled in Table B (See Appendix). All subjects agreed on the content of the study and signed informed consent. This study was approved by the local ethical committee (CPP Est-I) and the French National Agency of Drugs and Safety (ANSM) (ID RCB n°2016-A00916-45).

### FOP assessment and bolus collection

Mouthfuls of 20 cm3 of each product were cut right before the experimentation. Each member of the panel was asked to eat a mouthful and to expectorate the generated bolus at three different chewing sequences that were defined according to each individual's swallowing point, as described in detail by Assad-Bustillos et al. (2017). The chewing stages were defined as follows: 1/3 of the total chewing duration (C1), 2/3 of total chewing duration (C2) and just before the swallowing point (SP, total chewing duration). At each chewing sequence, one bolus was generated. The bolus was suspended immediately after collection in 150 mL of glycerol (VWR International, USA) inside a plastic container with a resealable screw-lid and was agitated at room temperature for 1h using a magnetic stirrer at 170 rpm to allow particle dispersion without damaging bolus structure, according to the procedure set up by Le Bleis et al. (2013). The boli were stored at 4°C until the moment of analysis.

### Bolus particle size analysis

Before analysis, the boli suspended in glycerol were re-agitated at a rotation speed of 170 rpm during 80 min at 20°C in a water bath (Julabo SW23, Germany) to ensure homogenous particle dispersion for all samples. Bolus particles were carefully placed in a Petri dish (diameter=5.5 cm) that was placed over a matte dark background and was backlighted through an optical fiber ring (Schott DCR IV, USA) placed underneath, as described by Le Bleis et al. (2013). The images were acquired in gray level with a monochrome CMOS video camera (EXO SVS-250MGE Vistek, Germany). For each bolus, at least 90% of the total volume was characterized, with a minimum of 10 images per bolus, for a total of 1200 images. Images were saved in TIFF format as matrices of 2448×2048 pixels, with a pixel size of 15 µm. Image analysis was performed with Matlab software (Mathworks 2016b, USA). Particle size distribution (PSD) was obtained using operations of mathematical morphology by performing a series of openings of increasing size (image sieving) as described above for the 3D images. The results were expressed as a plot of the cumulative area (%) of the particle vs the particle diameter in mm, also named PSD curve.

* 1. *Data treatment and Statistical analysis*

For each subject and each chewing sequence (C1, C2, SP), the median equivalent diameter (D50) and the interquartile ratio (D75/D25) were derived from the PSD curve. The ratio (D75/D25) characterizes the heterogeneity of the bolus (Jourdren, Panouillé, et al., 2016). Moreover, to ascertain their description, all PSD (n=120) were fitted with a three-parameter Gompertz model (2). Gompertz model has been previously used to model the PSD of soils (Botula, Cornelis, Baert, Mafuka, & Van Ranst, 2013; Esmaeelnejad, Siavashi, Seyedmohammadi, & Shabanpour, 2016), *in vitro* degradability of rumen from cereal meals (Gallo, Giuberti, & Masoero, 2016) and to model the porosity kinetics of bread dough during proofing (Kansou et al., 2013). In this study, it is used to model the PSD of food particles after chewing:

(2)

Where A is the fraction of cumulated particles area (% of total particle area), p is the particle size (mm), “a”, “b” and “c” are parameters obtained by fitting. Parameter “a” is an approximation of the maximum cumulated area, “b” is the slope of the size distribution curve at the inflection point, and parameter “c” is the particle size at the inflection point. Curve fittings were performed using the modules “NumPy” and “SciPy” from Python v.3.2.5.1 software (Python Software Foundation).

A one-way ANOVA was performed to determine the differences of structural and mechanical properties between the two products. In order to investigate differences between products at each chewing stage, a repeated measures ANOVA (product + subject + chewing sequence) was carried out for the median particle size D50, interquartile ratio D75/D25 and Gompertz parameters (“a”, “b”, “c,”), with the chewing sequence as repeated factor. Additionally, a one-way ANOVA was carried out for each product to investigate differences between chewing sequences. Furthermore, to investigate the impact of oral health status, a three-way ANCOVA (Analysis of covariance) model with level 2 interactions was applied for each product (chewing duration + dental status + stimulated salivary flow + dental status×stimulated salivary flow + dental status×chewing duration + stimulated salivary flow×chewing duration). For every statistical procedure, a significance level of α=0.05 was used and results reported according to Type III sum of squares. The Student-Newman-Keuls test was used for post-hoc comparison tests. All statistical analyses were performed with XLSTAT software (v.2016 18.06, Addinsoft, USA).

## Results and discussion

### Structure and mechanical properties of the two cereal foods

The values of structural and mechanical properties of both products are reported in Table 3, together with their standard deviation. Not surprisingly, both foods show distinct structural features due to their different composition and process. The first indicator of these differences is density, where sponge-cake (SC) showed a lower value (\*= 0.21 g.cm-3) than brioche (B) (\*= 0.33 g.cm-3). This may be the reason why the cellular structure of SC displayed larger bubbles, or gas cells, while B displayed smaller cells (Fig. 1 a, b). From 3D image analysis, the relative density (D) values (D= 0.21 for SC and 0.31 for B) agree with those determined using the rapeseed displacement method (Table 1).

|  |  |
| --- | --- |
| (a) |  |
| (b) |  |
| c) | |

Figure 2‑1 (a) Crumb cross-sections and (b) 2D (top) , 3D (bottom) images of sponge cake and brioche (diameter = 20mm) obtained by micro-computed tomography (XR-µCT) and (c) the resulting cumulated size distribution of walls (dotted line) and cells (continuous line) for sponge-cake (blue) and brioche (red).

From the granulometric curves (Fig. 1 c), it can be seen that cell wall size distributions of both foods are close to each other with a median size (D50) value of ≈100 µm and ≈120 µm for SC and B, respectively (Table 1). Regarding the voxel size, i.e. 22 µm, these two values can be considered not significantly different. Conversely, the cells were found significantly larger for SC than B, with a median size of ≈300 µm and ≈200 µm respectively (Table 1). Hence, in line with the difference of density, the main difference in cellular structure between products comes from the cell size.

|  |  |  |
| --- | --- | --- |
|  | Sponge-cake | Brioche |
| Direct Measures\* |  |  |
| **Density (g/cm3)** | 0.21 (±0.02)A | 0.33 (±0.02)B |
| **Young’s modulus E (kPa)** | 5 (±1)A | 20 (±3)B |
| **Critical stress σc (kPa)** | N/A | 3 (±1) |
| 3D Image Analysis\*\* |  |  |
| **Porosity** | 0.79 (±0.01)B | 0.69 (±0.04)A |
| **Relative density (D)** | 0.21 (±0.01)A | 0.31 (±0.04)B |
| **Wall Size** |  |  |
| **D25** | 41 (±1)A | 45 (±1)B |
| **D50** | 99 (±1)A | 118 (±5)A |
| **D75** | 176 (±1)A | 200 (±12)A |
| **Cell size** |  |  |
| **D25** | 95 (±1)B | 73 (±10)A |
| **D50** | 296 (±2)B | 197 (±26)A |
| **D75** | 785 (±81)B | 403 (±40)A |

Table 2‑1. Structural and mechanical product properties.

*\*Values are average of n=5 measures (±Std. deviation).*

*\*\* Values are average of n=2 measures (±Std. deviation).*

*Different letters (A, B), indicate means that significantly (p<0.05) differ between products (Student-Newman-Keuls test).*

Differences between products with regards to their mechanical behavior can also be observed from the stress-strain curves obtained by compression tests (Fig. 2). B behaves like an elasto-plastic material, i.e. that displays inelastic permanent deformation after unloading. Its behavior features a linear elastic part, followed by a plateau-like stage where stress is kept constant due to cell wall buckling and yielding, then followed by a continuous increase of stress reflecting material densification. Conversely, SC behaves like a hyper-elastic material, i.e. it deforms elastically over a large range of loading levels, and its behavior is marked by a continuous increase of the stress until densification. The former behavior has been widely reported in baked products including different types of bread and sponge-cake (Attenburrow, Goodband, Taylor, & Lillford, 1989; Hibberd & Parker, 1985; Scanlon & Zghal, 2001; Wang, Austin, & Bell, 2011). Contrarily, the latter has been rarely observed in starch based food materials (Guessasma & Nouri, 2015; Mohammed, Tarleton, Charalambides, & Williams, 2013). Both behaviors may be assigned to ductile foams, i.e. products that have a large porosity and a cellular structure with cell wall material in the rubbery state, as described by Gibson & Ashby (1997).

|  |
| --- |
|  |

Figure 2‑2 .Average stress-strain curves obtained by uniaxial compression of the two cereal foods: sponge-cake (blue) and brioche (red). Error bars reflect the standard deviation obtained from 5 replicates.

The values of Young’s moduli (E), for both products, and critical stress (σc) for B are reported in Table 1. B had a higher value of E (20 kPa) than SC (5 kPa). This difference may be attributed mainly to the density differences, in line with Gibson & Ashby’s (1997) scaling law for solid foams.

Finally, these values of structural and mechanical properties are in the range of those found for other baked products like breads (Besbes, Jury, Monteau, & Le Bail, 2013; Gao et al., 2015; Pentikäinen et al., 2014; Van Dyck et al., 2014) and cakes (Bousquières, Michon, & Bonazzi, 2017; Dewaest et al., 2017; Lassoued, Babin, Della Valle, Devaux, & Réguerre, 2007; Sozer, Dogan, & Kokini, 2011). Median cell size (D50), however, was on the lower edge of the interval [300, 1600µm] encountered in these studies. This could be explained by the high levels of fat of both products, which, according to Brooker (1996), lead to finer crumb grains.

### Particle size distribution (PSD) of the cereal food boli: analysis and curve fitting

Cumulative particle size distributions of food boli (PSD) were determined by quantitative image analysis for each subject, each chewing sequence and each product (Fig. 3). The average values for all subjects of the median diameter (D50) and the interquartile ratio (D75/D25), an indicator of bolus heterogeneity (Jourdren, Panouillé, et al., 2016), were extracted from the PSD curves and are shown in Table 2 for both products. Firstly, B boli had significantly higher D50 values than SC at all chewing stages. Secondly, for SC, D50 was significantly reduced over the chewing sequences. B boli, on the other hand, did not show any significant variation of D50 throughout the chewing stages. Also, D50 of B boli showed a higher inter-individual variability than SC, as reflected by the higher standard deviation. In addition, D75/D25,decreased significantly for SC, meaning these boli tend to reduce particle size towards the same value as mastication progresses. Conversely, this valueincreased significantly for B boli, meaning particle heterogeneity becomes higher over the chewing sequences. The variations over time of D50 and D75/D25 for all subjects and both products are shown in Fig. 4. This figure confirms the previous analysis and clearly depicts the scattered variations of D50 for B and illustrates the complexity of chewing mechanisms in this product, likely combining fragmentation and agglomeration of food particles.

|  |  |
| --- | --- |
| (a) | (b) |
| (c) | (d) |

Figure 2‑3. Examples of cumulative size distribution curves of bolus particles for the three chewing time values C1 (blue), C2 (red) and SP (green), and their corresponding fitting by Gompertz model (dotted lines), for sponge cake (a) and, in the case of brioche, for the three patterns of fragmentation / agglomeration, I, II and III respectively (b, c, d).

|  |
| --- |
| (a) |
| (b) |

Figure 2‑4. Variations of median particle size (D50) and interquartile ratio (D75/D25) with chewing time for sponge-cake (a, blue) and brioche (b, red). Empty symbols: satisfactory dental status, filled symbols: poor dental status.

These results also show that using a single parameter from the PSD, such as D50, is not always sufficient to understand the complex variations of particle size during mastication. Therefore, PSD curves were fitted with the Gompertz three-parameter model described in 2.5 (Fig. 3), in order to integrate the whole information brought by these curves and determine if D50 and D75/D25 conveniently describe those. The average values of the fitting parameters obtained for both products and each chewing sequence are shown in Table 2. Out of 120 fitted PSD curves, 112 of them had a satisfactory fitting (R2 ≥0.9), 2 had a low quality fitting (0.6≥R2≥0.8), and 6 had an unsuccessful fitting (R2 ≤0.5) (cf. Appendix).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Product** | **Parameter** | **Chewing sequence** | | |
| *C1* | *C2* | *SP* |
| **Sponge-cake** | D50 | 1.1 (±1.3)a A | 0.5 (±0.7)b A | 0.3 (±0.1)c A |
| D75/D25 | 13.3 (±6.1)a A | 8.9 (±5.7)b A | 5.0 (±2.1)c A |
| a | 100.8 (±12.6)a A | 100.4 (±7.6)a A | 99.3 (±0.5)a A |
| b | 0.7 (±0.5)a A | 1.4 (±1.2)b A | 2.7 (±1.6) c A |
| c | 0.7 (±1.5)a A | 0.3 (±0.9)b A | 0.2 (±0.1)c A |
| **Brioche** | D50 | 2.5 (±1.5)a B | 2.5 (±2.3)a B | 2.9 (±4.0)a B |
| D75/D25 | 8.3 (±3.4)a B | 15.2 (±14.4)a B | 25.6 (±24.2)b B |
| a | 108.7 (±39.4)a A | 106.7 (±28.3)a A | 102.2 (±10.1)a A |
| b | 0.4 (±0.1)a B | 0.4 (±0.2)a B | 0.5 (±0.3)a B |
| c | 2.4 (±3.7)a B | 1.9 (±3.0)a B | -0.3 (±3.4)b A |

Table 2‑2. Gompertz model fitting parameters for particle size distributions of products per chewing sequence.

*Note: All values are means (±Std. deviation) of n=20 subjects. The negative mean value of c for brioche bolus at SP means that many small particles have a size value below image resolution.*

*Different letters (a,b,c) indicate means that significantly (p<0.05) differ between chewing sequences (Student-Newman-Keuls test).*

*Different letters (A, B), indicate means that significantly (p<0.05) differ between products (Student-Newman-Keuls test).*

As expected from cumulative curves (Fig. 3), “a” coefficient values remain unchanged, close to 100 for all products and chewing sequences, suggesting that the 112 PSD curves of food boli can be described by only the two coefficients “b” and “c”, whose values differ significantly between products for almost every chewing sequence. Coefficient “b” varies significantly between chewing sequences for SC, and coefficient “c” does it for both products. Furthermore, it was found that “c” is positively correlated to D50, (RSC=0.94, RB=0.95 p < 0.0001), and the regression line is closed to the bisector. Conversely, “b” is negatively correlated to D75/D25, (RSC=-0.65, RB=-0.49 p < 0.0001) (Fig. 5). The correlation is particularly satisfactory for both factors in the case of SC. These results confirm that the two coefficients describe completely the variations of particle size boli during chewing. Furthermore, they suggest that the variations of “c” reflect the mean size of bolus particles, and hence their degradation degree: the smaller the “c” value, the more degraded the bolus. Conversely, “b” can be considered as an index of homogeneity of the particle size distribution, at least for SC. These two parameters of the PSD model will be used in the following section to analyze the effect of the oral health status on bolus fragmentation.

|  |
| --- |
| (a) |
| (b) |

Figure 2‑5. Variations of c and b values derived from Gompertz model with respectively (a) particle median size (D50) and (b) interquartile ratio (D75/D25) for sponge cake (blue) and brioche (red).

The remaining 8 “misfit” PSD curves came from boli that featured a high percentage of large size particles, which introduced jaggedness to the distributions, hence making them difficult to fit (see Fig. 3 c,d). Interestingly, all of these boli came from B and belonged to either the second chewing sequence (C2) or the swallowing point (SP). This means the large particles were present by the end of mastication, therefore suggesting agglomeration. Indeed, a closer examination of the PSD curves and bolus images revealed the presence of three fragmentation patterns (cases I, II and III). Case I consists of an overall decrease of particle size over the chewing sequences and an increase in the number of small particles. It is represented by a curve translation towards smaller sizes (Fig 3 a,b). All of the sponge-cake (SC) boli followed case I pattern, with more than 90% of overall particles with a size lower than 6 mm (Fig. 6a). This trend was followed for brioche (B) boli for 10 out of 20 individuals (Fig. 6b). Out of the remaining 10, 2 showed a clear pattern of agglomeration (case II), which is represented by a translation of the curve towards larger size is with a jagged appearance due to large size particles (>14mm) (Fig. 3c), and is depicted by an increase in particle size during chewing until bolus becomes a single paste-like particle (size ≈20mm) (Fig. 6c). For 8 cases, a non-monotonous variation was found, with two possibilities: either an increment in particle size during C2 followed by an immediate decrease of particle size at the SP (Fig. 6d), or a decrease in particle size in C2, followed by an increase of particle size in SP (not shown), suggesting a pattern combining agglomeration and fragmentation (case III).

Actually, there was no particular relationship between the individual physiology and the agglomeration patterns, for these 10 specific cases as illustrated by Table D (Appendix).

|  |  |  |
| --- | --- | --- |
| a) |  |  |
| (b) |  |  |
| (c) |  |  |
| (d) |  |  |

Figure 2‑6. Typical examples of boli images after chewing at C1 (left), C2 (center) and at swallowing point (right) for sponge-cake (a), and for brioche, decreasing size (case I) (b), increasing size (case II) (c), combination of both (case III) (d). These images correspond to the size distributions plotted in Fig.3.

### Influence of oral health status on bolus fragmentation / agglomeration patterns

The influence of the oral health status on particle size distributions and model parameters was investigated through ANCOVA model and the results are shown in the present section. In spite of large variations of SSF, from 0.3 to 3.84 mL/min overall (see Table B in Appendix), no significant effect of salivary flow rate (SSF) on D50 or PSD model parameters was found for any of the products. For sponge-cake (SC), a significant relationship between dental status (DS) and median particle diameter (D50) was identified (p<0.05). The normalized coefficient of the model for the satisfactory DS group (**s) was -0.8. This result means that individuals with a satisfactory DS produced boli with lower D50 values than those with a poor DS. The same result was obtained when performing the analysis with “c” Gompertz coefficient instead of D50 (p<0.001, **s =-1.0). However, in this model, a significant interaction between chewing duration and DS was found (p<0.01), where **s =0.6. This positive value may reflect the limited size reduction (D50≥0.15mm), illustrated in Fig. 4a, for longer chewing duration and satisfactory DS. Conversely, for brioche (B), no significant effect of DS was found for D50.  A different result was obtained, nonetheless, with “c”, where DS had a significant effect (p < 0.01, **s =-0.3), meaning this parameter is lower for subjects with a satisfactory DS. This also means that, contrary to D50, “c” coefficient allows differentiating B boli based on the DS of subjects, and it confirms that Gompertz model parameters more completely account for PSD variations than directly extracted characteristics such as D50. Neither D75/D25 nor “b” showed significant relationships with DS or SSF, suggesting that, in the case of these soft cereal foods, bolus particle heterogeneity is independent of the oral health status. Moreover, no particular trend was found with regards to the number of agglomeration cases (n=10) and their distribution according to DS or SSF. More importantly, since no relationship with SSF was found for any of the studied parameters, it is clear that fragmentation does not depend on salivary flow.

### Overall discussion

Our results demonstrate that the Gompertz model accounts for the variability the particle size distribution (PSD) of food particles, and that the two parameters, “b” and “c” that result from it, are sufficient to discriminate between products and chewing sequences. Therefore, they are worth to be related to bolus and chewing characteristics. Also, the analysis of the quality of fit resulted in a quick way to detect atypical data, allowing the identification of different fragmentation patterns in the two studied foods, as discussed in section 3.2. While Sponge-cake (SC) boli featured a monotonous and continuous fragmentation pattern (case I), Brioche (B) boli displayed three different fragmentation patterns (cases I, II and III), including agglomeration in 50% of cases. Moreover, as observed in our previous study (Assad-Bustillos et al., 2017), B boli were perceived as sticky and pasty, which is in agreement with the observed agglomeration patterns observed in the present work. Case I type of behavior has already been observed in other ductile cereal products, like bread (Jourdren, Panouillé, et al., 2016; Le Bleis et al., 2016). However, patterns combining fragmentation and agglomeration during bolus formation, such as cases II and III, have only been reported for brittle cereal products (Rodrigues, Young, James, & Morgenstern, 2014; Young et al., 2013; Yven, Guessasma, Chaunier, Della Valle, & Salles, 2010). Yven et al. (2010) suggested that the transition from fragmentation to agglomeration during chewing is linked to a transition of the material from brittle to ductile. Such shift also seems to depend on the initial structural and mechanical properties of the food, as it occurred faster and was more abrupt for the densest and hardest foods (Young et al., 2013; Yven et al., 2010). Therefore, agglomerative patterns are somehow associated to ductile behavior, and in our case, the structural and mechanical differences between the studied foods are probably responsible for the observed fragmentation mechanisms. Among the two products, B featured a denser structure and higher values for mechanical properties; it also displayed an elasto-plastic behavior, which is known for its low energy dissipation. This means the material can undergo high levels of strain with a relatively small increase in stress. As a result, more energy and effort are needed to break down this type of materials, as much as shearing to allow cell wall breakage. A higher masticatory effort could translate in a longer chewing duration, but also in a bolus formed of larger particles (Gao, Tay, Koh, & Zhou, 2018). In our case, the chewing duration of the two products was similar, yet, the combined effect of a denser structure and elasto-plastic nature could partially account for the higher bolus particle size and agglomerative behavior of B.

Conversely, the mechanical behavior of SC was best described by a hyper-elastic constitutive law. Like previously mentioned, this behavior is characterized by a continuous non-linear increase of stress that results from reversible structural modification during compressive loading. However, SC cannot be considered as a true hyper-elastic material since it is neither isotropic nor incompressible (Mihai & Goriely, 2015). From a microstructural point of view, this behavior can be explained by the rearrangement of cells and their modification when loading is applied. In SC, it is clear that failure mechanisms are dominated by irreversible non-plastic deformation. Further experiments using high-resolution 3D image acquisition under compression and shearing would be useful to better understand these mechanisms. Still, it is possible to state that the generated cell wall damage of SC is higher than B at the early stages of compression, thus leading to an increase of stress at a faster rate. This hypothesis would explain why SC was broken down into smaller particles without increasing the chewing duration. Therefore, at product level, differences in fragmentation patterns can be partially explained by the mechanical behavior of the two foods.

At the individual level, part of the variability observed in the bolus particle size was explained by the physiology and particularly the dental status (DS) of the elderly subjects. As discussed in section 3.3, a significant relationship between a satisfactory DS and a lower bolus particle size was evidenced for both products. It was also seen that in spite of large variations of stimulated salivary flow rate (SSF), this variable is not involved in the fragmentation process, unlike other bolus properties like hydration or viscosity (Assad-Bustillos et al., 2017). Additionally, no correlation between agglomeration and DS or SSF was found. Still, it is likely that other physiology variables are involved in this mechanism, since agglomeration only occurred in 50% of the cases. According to Prinz & Lucas (1997), the tongue is highly involved in the packing and pressing of bolus particles against the palate. In the elderly, the tongue and cheek muscles that are associated with this function may be altered inducing changes in tongue activity and bite force (Laguna, Sarkar, & Chen, 2015; Laguna et al., 2016; Laguna, Sarkar, Artigas, & Chen, 2015; Peyron, Woda, Bourdiol, & Hennequin, 2017). Hence, physiological variables such as tongue pressure, tongue muscular activity and bite force may are worth to be taken into account in future studies in order to better understand these mechanisms in the elderly.

Finally, from the ANCOVA analysis performed with Gompertz model parameters, we found that DS has a significant impact on fragmentation. This result suggests that Gompertz parameters provide more information about the fragmentation properties of the food bolus than the parameters extracted directly from the distribution curves. Moreover, modelling the PSD should facilitate the implementation of numerical models based on discrete elements in similar conditions to chewing, like the one proposed by Hedjazi, Martin, Guessasma, Della Valle, & Dendievel (2014).

# Conclusion

By using quantitative image analysis of food boli taken at different steps of oral processing, we demonstrated that particle size distribution could be usefully fitted by Gompertz model. This model allows interpreting the food particle size evolution the chewing process in terms of bolus particle heterogeneity and fragmentation. We identified and described different fragmentation mechanisms for two soft cereal products differing in their initial structure and mechanical properties during oral processing in the elderly: sponge-cake was regularly fragmented, whereas brioche agglomerated. These mechanisms were explained the compressive mechanical behavior and intrinsic cell wall properties of the food products. Finally, we put into evidence the importance of the elderly dental status in the fragmentation of both foods, while salivary flow rate was not found to be involved in this process. This study also highlights the need to understand the chewing process of cereal products as a combination of fragmentation and agglomeration mechanisms, and spurs the use of mathematical models to describe the evolution of particle size in order to be able to take this complexity into account.

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Chapter II. Article 3.

**Rheological and microstructural characterization of sponge batters and cakes fortified with pea proteins**

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Keywords**:** liquid foams, stability, free-drainage, CLSM, volume air fraction.

# Abstract

The effect of pea protein fortification on the rheological properties of sponge-cake batters and their continuous phases, as well as their relationship with the final product properties were studied. Foams made out of whole egg and sugar were prepared in a planetary mixer; wheat flour (WF) was added to form a typical sponge-cake batter. Pea protein isolates (PP) were added in substitution to WF to form five batters with various PP concentrations expressed as the percentage of WF substitution: 0, 10, 20, 30 and 40%. The batter air volume fraction decreased when increasing PP concentration; this lead to an increase in the cake density, as well as the apparent Young modulus. All batters and their respective continuous phases showed shear-thinning behavior, which was modeled by a power law. The viscoelastic properties showed a predominant elastic behavior at intermediate frequencies, and a cross-over point at high frequencies. All of the rheological properties increased by a factor of ≈10 when the PP concentration increased from 0 to 40%. Free-drainage experiments showed that batter stability increased with increasing PP concentration. PP had larger particles than WF, and showed a higher water binding capacity than WF, but no significant difference in solubility. Observations of the batter and cake microstructure revealed PP formed a network of interconnected “bridged” particles in the continuous phase. These results suggest that PP act as fillers that swell and connect to each other in the continuous phase, being the main driver for the increase of rheological properties.

# Nomenclature

Apparent Young modulus (E\*)

Confocal scanning laser microscopy (CLSM)

Consistency index (K)

Crumb density (ρ\*)

Diameter ()

Flow index (n)

Loss modulus (G’’)

Mean volume diameter (d4,3)

Particle size distribution (PSD)

Pea protein isolates (PP)

Phase shift factor (tan )

Solubility (S)

Storage modulus (G’)

Student-Newman-Keuls test (SNK)

Viscoelastic linear domain (VEL)

Volume air fraction (Φa)

Water holding capacity (WHC)

Wheat flour (WF)

1. **Introduction**

The fortification of cereal products with pulse proteins, such as pea protein, is a good way to improve their nutritional properties by equilibrating the essential amino acid profile (Young & Pellett, 1994), but may have a negative effect on their texture properties (Noorfarahzilah, Lee, Sharifudin, Mohd Fadzelly, & Hasmadi, 2014).

Due to its high versatility and airy texture, sponge-cake is a very popular food among the consumers. Its simple composition and processing makes it easily available. Its porous structure allows it to absorb liquids such as syrups and jams; for those reasons it is apt for a large variety of applications and constitutes the base of many bakery specialties worldwide (Díaz-Ramírez et al., 2013).

The structure of sponge-cake is originated during the mixing of cake batter, before it becomes a soft solid foam after thermal setting during baking. Briefly, the batter process consists in forming a liquid foam by introducing air into a continuous liquid phase made of egg and sugar; to which wheat flour and other minor solid components are folded in to form the batter. In the industry, this process is known as two-stage mixing (Wilderjans, Luyts, Brijs, & Delcour, 2013). Like other egg based cakes, the final airy structure of sponge-cake depends heavily on the air trapped within the continuous phase (Conforti, 2014; Wilderjans et al., 2013). For that reason, the rheological properties, and particularly the viscosity of the batter are key factors that determine its capacity to retain air bubbles during processing (Sahi & Alava, 2003). Indeed, the air volume fraction (Φa) is a critical factor that is strongly linked to the rheological behavior of the batter (Allais, Edoura-Gaena, Gros, & Trystram, 2006), and allows its classification into two categories: bubbly liquids (Φa<0.64) and aqueous (or wet) foams (0.64<Φa<0.95) (Cantat et al., 2013). In sponge-cake and other egg based foams, Φa values commonly situate them in the aqueous foam domain; for instance Φa =0.68 for a typical foam made of whole egg; and Φa =0.74 if only egg white (Spencer, Scanlon, & Page, 2008).

Aqueous foams are made by highly packed air bubbles that form an interconnected structure, composed of films, Plateau borders and nodes (Cantat et al., 2013). This unique structure is responsible for their viscoelastic behavior at low strains (Cohen-Addad, Hoballah, & Höhler, 1998) and their shear-thinning behavior at large strains, sometimes presenting a yield stress if the bubble packing is “jammed” (Gopal & Durian, 1999). These rheological properties are intimately related to Φa and bubble size distribution (Kraynik, Reinelt, & van Swol, 2004). Moreover, since foams are not thermodynamically stable systems, they are ubiquitous and their rheology is time-dependent (Cipelletti & Ramos, 2002; Marze, Guillermic, & Saint-Jalmes, 2009); therefore, the destabilization phenomena that occur during the time scale of batter processing are to be taken into account, as they can have an influence on the structure and final properties of the cake crumb (Foegeding, Luck, & Davis, 2006). The main mechanisms that lead to foam destabilization are drainage- driven by gravity-; coarsening -due to pressure differences between bubbles of different sizes-; and coalescence, which is the bursting of liquid films separating neighboring bubbles (Cantat et al., 2013; Murray, 2007). In aqueous foams, the extent to which a particular aging mechanism dominates foam destabilization also depends considerably on Φa (Saint-Jalmes, 2006). For instance, Spencer et al., (2008) showed that destabilization of sponge-cake batters was dominated by drainage when the systems were classified as bubbly liquids, whereas coalescence was dominant in foams. In any case, the study of the stability of cake batters gives important information about their internal structure and their macroscopic behavior during processing; moreover, it can be assessed in a relatively simply manner, for instance *via* free-drainage experiences (Saint-Jalmes & Langevin, 2002).

Even though all of the above mentioned phenomena have been relatively well described in physical chemistry and soft matter science, the cake making industry could benefit from basic insights taken from said disciplines in order to improve its processing for applications such as protein and fiber fortification, sugar or fat reduction (Mezzenga, Schurtenberger, Burbidge, & Michel, 2005). In the particular case of protein fortification, the technological consequences and impact on sensory properties of adding pulse flours or isolates have been recently reviewed (Foschia, Horstmann, Arendt, & Zannini, 2017; Noorfarahzilah et al., 2014). However, there is still a lack of understanding of the mechanisms leading to those changes.

In this context, the objectives of this study were: i) to characterize the changes induced by the addition of pea protein isolates, at different levels of wheat flour substitution, on the rheological, stability and air incorporation properties of sponge-cake batters and their continuous liquid phase; ii) to relate them to the structural and mechanical properties of the corresponding baked cakes; iii) to provide insight on the possible mechanisms at the origin of these changes; iv) to discuss the possible corrective actions that could be implemented to avoid them.

## Materials and methods

### Batter and cake formulation

Five batters were prepared according to the formulae detailed in Table 1. Pasteurized liquid eggs (HORECA, France) and white sugar (Daddy, France) were bought one week before the experiment at a local store; eggs were conserved at 4°C. Wheat flour (T55, Decollogne, France), and pea protein isolates (NUTRALYS BF, Roquette, France) were bought 2 months before the experiment and stored in closed containers at 4°C. Ingredients were weighed separately in sufficient quantity to prepare 1 kg of batter. To prepare the foams, first the eggs are mixed with the sugar in a planetary mixer with rotating whisk (N50, HOBART, USA) at intermediate speed (281 rpm arm + 124 rpm whisk) during 10 minutes, plus 5 minutes at high speed (580 rpm arm + 255 rpm whisk). Then the wheat flour (WF) and pea protein isolates (PP) are added progressively to the egg-sugar foam to form the batter while mixing manually and gently to avoid bubble coalescence. For each formula, batter properties were characterized right after being prepared. Additionally, a small amount (≈15 mL) of batter was poured in a plastic test tube that was placed inside a vacuum desiccator system (without any silica particles) to remove the air bubbles during 2 hours. The properties of the airless batters, considered as their continuous phase, were characterized as for regular batters. The properties of the egg-sugar foam previous to the addition of flour and isolates were also characterized.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Batter composition**  **(% w/w)** | **Sample identification** | | | |  |
| **S0** | **S10** | **S20** | **S30** | **S40** |
| Wheat flour (WF) | 25 | 22.5 | 20 | 17.5 | 15 |
| Sugar (sucrose) | 25 | 25 | 25 | 25 | 25 |
| Egg | 50 | 50 | 50 | 50 | 50 |
| Pea protein isolate (PP) | 0 | 2.5 | 5 | 7.5 | 10 |
| **% of WF substituted by PP** | **0** | **10** | **20** | **30** | **40** |

Table 3‑1.Detailed formulae of the studied sponge-cake samples.

Since the batter properties characterizations are destructive, cakes were prepared from new batter batches by following the same formulae shown in Table 1; additionally 1% of a commercial chemical leavening agent was added (Na2CO3/Na2H2P2O7 powder mix, DGF, France) to each formula, modifying minimally their composition. For each formula, 4 cakes were prepared. Circular aluminum molds with a diameter () of 25 cm were sprayed with oil to avoid sticking, then 250 g of batter was weighed on each mold; the latter were placed on a tray and baked in a pre-heated electric deck oven at 200°C during 20 minutes. The cakes were un-molded and cooled at room temperature for 3 hours, then wrapped in plastic foil and left to rest for 24 h before the characterization of their properties.

### Batter density and air volume fraction

A 20 mL plastic container was filled with freshly prepared batter and weighed. The measurement was performed thrice. From this data, the batter density (ρ\*), in g∙cm-3, was calculated as detailed in (1):

(1)

Where:

, is the mass of the empty container, in g;

, is the mass of the container filled with the batter, in g;

, is the mass of the container filled with water, in g, and water density ≈ 1g.cm-3).

The batter air volume fraction (Φa) was calculated from the relation expressed in (2):

(2)

Where:

is the batter density, in g∙cm-3 , calculated as detailed above;

is the density of the continuous phase, in g∙cm-3, calculated from the material density of each individual component in relation to their proportion in the continuous phase:

(3)

Where

are the mass fractions of the individual components *(i, j, k…)* of the continuous phase, and;

is the material density of each individual component *(i, j, k…),* in g∙cm-3.

### Batter rheological properties

The rheological properties of the batters and their continuous phases were measured with a controlled strain rheometer (ARES, TA Instruments, USA) equipped with a parallel plate geometry (discs = 40 mm) and a 1 mm gap at 25 °C. Approximately 2 g of the sample were carefully placed with a spatula in the bottom plate, and the gap was narrowed at the minimal loading speed to avoid damaging its structure. Paraffin oil was used to cover the geometry in order prevent sample drying during the test. The measurements performed thrice.

#### Viscoelastic properties

The mechanical spectra of the samples were determined by frequency sweeps (0.01 to 100 rad∙s-1) within the viscoelastic linear domain (VEL) at a strain of 0.6%; this strain level was previously determined on a different sample by performing a strain sweep from 0.1 to 1% at a frequency of 1 rad∙s-1. From the mechanical spectra, the values of the storage modulus G’ (Pa) and the phase shift factor (tan δ) at 1 rad∙s-1 were extracted and used to characterize the structure properties of the sample at “rest”.

#### Flow properties

Following the determination of viscoelastic properties, the shear viscosity (ƞ), in Pa∙s, of the samples was measured in the range of 0.01 - 600 s-1 and was fitted with the Ostwald-de Waele power law model. The consistency index (K), in Pa∙sn, and the flow index (n) of each sample was used to characterize flow properties.

### Batter stability: free-drainage

The destabilization kinetics of the batters was measured by following the apparition of liquid at the bottom of a transparent non-graduated test tube (= 2.5 cm, h=21 cm) during a free drainage experience. The tube containing the freshly prepared sample was placed between a white halogen light source (KL 2500 LCD, Schott, Germany), and a monochrome CMOS camera (EXO SVS-250MGE, D-Vistek, Germany) equipped with a 35 mm f/1.4 lens (Myutron, Japan). The camera was set to acquire images of the entire tube at t=0 and then automatically every 10 min during 12h. The images were processed with the ImageJ freeware (<https://imagej.nih.gov>). A threshold was applied to distinguish the liquid in the image from the remaining dry foam. This allowed the quantification of the height of liquid (Hliq) in every image, which was normalized by the initial height of the foam (Hf). The results were expressed as the evolution of the ratio of Hliq / Hf over time and were used to characterize the stability properties of the samples. The measurement was performed twice.

### Measurement of pH

The pH of the continuous phases was measured using a pH meter (905 Titrando, Metrohm, Switzerland). The measurement was performed thrice.

### Cake density, water content and mechanical properties

Following cooling and rest during 24 h, the cakes were weighed and their volume was measured by the rapeseed displacement method (AACC, 2009a). Their density (ρ\*), in g∙cm-3, was calculated as their mass to volume ratio. The water content of the cakes (WC), expressed as a wet basis percentage, was determined by placing 2 g of crumb taken from the center of the cake inside an oven during 2 h at 135 °C as recommended by (AACC, 2009b). The apparent Young modulus (E\*), in kPa, of the cake crumbs were characterized from the slope of the linear part of the stress –strain curve obtained by performing a uniaxial compression test with an universal testing machine (Adamel Lhomarghy, France) on cylindrical crumb samples (=40 mm, h=30 mm) taken from the center of the cake, and using the same test conditions described by Assad-Bustillos et al. (2019). All measurements were performed thrice.

### Pea protein isolates and wheat flour characterization

#### Particle size distribution

The particle size distribution (PSD) of pea protein isolates (PP) and wheat flour (WF) in dry dispersion was determined with a light scattering instrument (Mastersizer 2000, Malvern Instruments®, UK). The PSD in wet dispersion of PP and WF in distilled water (5% w/v) was also determined within a time scale relevant to product processing (t=15 min). The refractive index was set at 1.45 as estimated for proteins, and water was set at 1.33. The mean volume diameter (d4,3) was used to characterize the particle size of the samples. Measurements were performed thrice.

#### Solubility and water holding capacity

The solubility (S) and water holding capacity (WHC) of the samples were measured for 10% (w/v) dispersions of PP and WF, based on the methods described by Peters, Vergeldt, Boom, & van der Goot (2017). Briefly, this method consisted in preparing the dispersions in Eppendorf tubes that were mixed with a vortex during 15 minutes (3 intervals of 5 minutes) to be representative of processing conditions. Subsequently, the tubes were centrifuged at 3000 rpm (845 g) for 20 min. The supernatant was separated and placed in an oven at 130°C to determine its dry mass; the pellet was weighed and afterwards placed in the oven to determine its dry mass as well. The measurements were performed five times. This data allowed the calculation of S, expressed as the percentage of solids retained in the supernatant on a dry basis, and WHC, expressed as the ratio of absorbed water to dry matter of the samples, as shown in relations (4) and (5):

(4)

(5)

Where:

is the mass of the pellet (g) ;

is the mass of the pellet after desiccation (g) ;

is the mass of the supernatant after desiccation (g).

### Pea protein isolates localization in batters and cakes

Observations of the batter and crumb microstructure were made by confocal scanning laser microscopy (CSLM), using a confocal laser scanning microscope (A1, Nikon, Japan); the excitation wavelength was set to 488 nm and the emission was recorded between 520 and 600 nm in order to identify the auto-fluorescence of PP as reported by (Nunes, Raymundo, & Sousa, 2006); no fluorophore or dying agent was added to the samples. To prepare the samples, a small amount of freshly prepared batter was carefully placed on a microscope slide within a spacer frame of 250 µm in thickness (Thermo Fisher Scientific, USA) that was sealed with a plastic cover right after to preserve the structure of the foam without crushing it and to prevent the air from escaping. The observations were made immediately after sealing, no fluorophore was added. For cakes, a cubic crumb sample taken at the center of the cake (5 cm3) was used to obtain slices of 120 µm in thickness with a cryo-microtome (HM 500 OM, Microm, France). The slices were placed on microscope slides and were left to rest for 3 days before the observations. To make sure the observed fluorescent particles correspond to PP, isolates were prepared in dry and wet 5% (w/v) dispersion, placed on a microscope slide, and covered with a classic cover slip. All samples were observed using a x20 lens (Plan APO with numerical aperture of 0.5) and a 5x digital zoom, when needed. For each sample, 3 to 5 images were taken for illustrative purposes.

### Statistical treatment

One-way analyses of variance (ANOVA) were performed in order to investigate the differences in properties between the different cakes, batters and continuous phases of different composition. Student t-tests were performed to compare the solubility and the water holding capacity of the wheat flour and pea protein isolates. For all statistical tests, a significance level of α=0.05 was used. When significant effects were found, Student-Newman-Keuls test was used for post-hoc treatment. All statistical analyses were performed with XLSTAT software (v.2016 18.06, Addinsoft, USA).

## Results and discussion

### Air volume fraction and impact on cake properties

The air volume fraction (Φa) of the egg-sugar foam is significantly (p<0.01) reduced after wheat flour (WF) is added, from 0.75 to 0.71 (Fig. 1 A). This is caused by the rupture of the air / water interfaces leading to bubble coalescence during the mixing in the presence of starch, as previously reported by Bousquières, Michon, & Bonazzi, (2017), who observed an augmentation of sponge-cake batter density when the mixing time after starch addition was increased. Interestingly, when pea protein isolates (PP) are added in substitution of WF, Φa is furtherly reduced, reaching a value of 0.61 for the highest concentration of PP. Strictly speaking, such a value of Φa is low enough (Φa<0.64) for the system to be considered a bubbly liquid, rather than a foam (Cantat et al., 2013). These results are in line with those reported by Gómez, Doyagüe, & de la Hera, (2012), where the addition of pea flour in sponge-cakes caused an increase in batter density, reflecting less air incorporation. This decrease in aeration could be responsible for the increase in the cake density reported by the same authors. Indeed, such relationship had already been reported by Bousquières et al. (2017) in regular sponge-cake batters, which suggests the dependence of the cake density on the air volume fraction is not exclusive of protein fortified systems. In our case, substituting WF by PP led to a significant (p<0.001) increase in the cake density (ρ\*), going from 0.21 ± 0.01 to 0.35 ± 0.02 g∙cm-3; these values were directly related to the batter’s air volume fraction(Φa, Fig 1 B), which confirmed the importance of the initial aeration in determining the final cake properties. Moreover, the apparent Young modulus (E\*) of the cakes also increased, going from 5 ± 1 to 17 ± 2 kPa (Fig. 1 B). Also, the values of E\* are highly correlated to the square of the crumb density (ρ\*)2 (Fig. 1 B) in accordance with Gibson & Ashby's scaling law for solid foams (1988), suggesting there is little influence of the intrinsic cell wall in the final cake properties. In baked products, high values of E\* are undesirable since they are associated with the perceived firmness of the crumb (Lassoued, Delarue, Launay, & Michon, 2008), and the latter may impact negatively the consumer’s acceptability (Angioloni & Collar, 2009; Martin, Chiron, & Issanchou, 2013). Finally, the water content (WC) of the cake crumb (not shown) increased slightly with PP addition, but not significantly (p>0.05), from 28 ± 2% in the reference cake (S0), to 30 ± 2 % for the highest level of fortification (S40).

|  |  |
| --- | --- |
| **A** | **B** |

Figure 3‑1. (A) Variation of the air volume fraction (Φa) of batter as a function of PP concentration. The red square (■) represents the Φa of the egg-sugar (e+s) foam; (B) Batter density (ρ\*,●) and apparent Young modulus (E\*, ■) as a function of cake density.

### Rheological properties of the batter and their continuous phases.

#### Viscoelastic properties

The mechanical spectra of the batters show a high frequency dependence with a predominance of the storage over the loss modulus (G’>G’’), which denotes an elastic dominant behavior; two cross-over points can be observed at low and high frequencies (Fig. 2 A). This behavior reflects the non-covalent bonding of the molecules present in the sample (Steffe, 1996). In the particular case of foams, it is likely the second cross-over point, also known as relaxation time, is a consequence of the foam aging mechanisms (mainly coalescence), that cause changes in the size of the bubbles (Cohen-Addad et al., 1998). According to Gopal & Durian (2003), the relaxation time of a foam corresponds to its “unjamming” point, where elasticity completely vanishes and the bubbles are no more closely packed. The G’ and G’’ values across the whole spectra are augmented by a factor of 3 when wheat flour (WF) is added to the egg-sugar foam, and continue to increase when the pea protein isolate (PP) are added, being multiplied by 15 at the highest PP level (S40) (Table 2). Moreover, the tan value (=G’’/G’) at 1 rad∙s-1 decreases significantly from 0.6 in the reference formula (S0), to 0.3 in the highest PP formula (S40), reflecting the enhancement of the elastic character of the batters with the addition of PP.

A similar behavior is observed for the continuous phases (Fig. 2 B). The only difference is the egg-sugar continuous phase, which spectra (not shown) reflect a dominant viscous behavior (G’’>G’) (Table 2). Overall the G’ and G’’ values are lower for the continuous phases than for batters, but they still rise by a factor of 5 when the maximum PP concentration is reached (S40). Also, no significant differences were observed in the tan δ values at 1 rad∙s-1 between the different formulae, which varied between 0.8 and 0.9, meaning the continuous phases show a more viscous character than the batters, which is coherent with the shift of the second cross-over point in the spectra, towards lower frequencies, close to 1 rad∙s-1.

|  |  |
| --- | --- |
| **A** | **B** |

Figure 3‑2. Mechanical spectra of batters with different PP concentration (A) and their respective continuous phase (B) at 0.6% strain. Legend: red=egg-sugar foam; light gray=S0; medium gray=S20; black=S40. For the sake of visibility, not all samples are shown.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **G’ at 1 rad∙s-1 (Pa)** | | **tan δ at 1 rad∙s-1** | | **K (Pa∙sn)** | | **n** | |
| **B** | **CP** | **B** | **CP** | **B** | **CP** | **B** | **CP** |
| **egg-sugar** | 7 ± 1f | 0.005 ± 0.002a | 0.7 ± 0.1a | 4.7± 0.1a | 2 ± 1d | 0.1 ± 0.05f | 0.6 ± 0.1a | 0.8 ± 0.1a |
| **S0** | 27 ± 4e | 11 ± 2e | 0.6 ± 0.1a | 0.8 ± 0.1b | 12 ± 2c | 6 ± 1e | 0.4 ± 0.1b | 0.6 ± 0.1b |
| **S10** | 39 ± 5d | 18 ± 3d | 0.4 ± 0.2ab | 0.9 ± 0.1b | 12 ± 2c | 18 ± 3d | 0.3 ± 0.1b | 0.5 ± 0.1b |
| **S20** | 67 ± 6c | 36 ± 5c | 0.3 ± 0.1b | 0.7 ± 0.1b | 22 ± 3b | 29 ± 4c | 0.5 ± 0.2ab | 0.5 ± 0.1b |
| **S30** | 141 ± 8b | 64 ± 4b | 0.4 ± 0.1ab | 0.7 ± 0.1b | 19 ± 3b | 38 ± 5b | 0.3 ± 0.1b | 0.5 ± 0.1b |
| **S40** | 410 ± 11a | 61 ± 6b | 0.3 ± 0.2b | 0.8 ± 0.1b | 97 ± 9a | 50 ± 5a | 0.4 ± 0.2b | 0.6 ± 0.2b |

*Means within the same column labelled with the same letter are not significantly different (p<0.01) (Student Newman-Keuls test).*

Table 3‑2. Summary of the rheological properties of the studied sponge-cake batters (B) and their continuous phases (CP).

#### Flow properties

Regarding the shear flow properties, both batters and continuous phases showed a non-Newtonian shear-thinning behavior (Fig. 3), as previously encountered by several authors in cake batters of similar composition (Bousquières et al., 2017; Chesterton, de Abreu, Moggridge, Sadd, & Wilson, 2013; Edoura-Gaena, Allais, Trystram, & Gros, 2007; Meza et al., 2011; Sanz, Salvador, Vélez, Muñoz, & Fiszman, 2005).

The shear viscosity (ƞ) of both, batters and continuous phases, increases significantly with the addition of WF and PP, as reflected by their consistency index (K) values (Table 2). For batters (Fig. 3 A), K increases by a factor of 6 after the addition of WF (S0); and by a factor of 8 for the highest concentration of PP (S40). Oppositely, the flow index (n) decreases after the addition of WF (S0), meaning the shear-thinning character is accentuated, but remains constant for the increasing PP formulae. For the continuous phases (Fig. 3 B), the initial increase of K after the WF addition (S0) is much more drastic, multiplying it by a factor of 60. Indeed, the K and n values of the egg-sugar continuous phase are very close to those reported for liquid whole egg (K=0.16 and n=0.84 at 20°C), which is known for its low shear viscosity and for a high flow index that makes it nearly Newtonian (Gosset, Rizvi, & Baker, 1983). In fact, adding sugar and flour to a liquid egg solution increases its viscosity in order to augment its ability to retain air bubbles (Wilderjans et al., 2013). Otherwise, the value of K of the continuous phases was multiplied by 8 when the highest concentration of PP was reached (S40); this order of magnitude is similar to the one observed in batters. Surprisingly, the K values of the continuous phases were higher than those of batters, excepting for the reference formula (S0) and the highest PP concentration (S40). These results are in contradiction to what has been previously reported by other authors that have compared foams to their corresponding “slurries”, analogous to the continuous phases in this study, where the K values always were higher for foams, i.e. Kfoam/Kslurry > 1 (Chesterton et al., 2013; Meza et al., 2011). However, these studies were carried out on batters with traditional ingredients, which did not include proteins from any other source than wheat. In our case, the presence of pea proteins could be responsible for the observed irregularities in flow properties, as reflected by the slight slope changes at larger shear rates (≈102 s-1), which could be attributed to the breakdown of the foam structure, in the case of batters, and to the breakdown of a protein network in the case of the continuous phases. *An in-depth discussion on this effect is provided in section 3.6.*

|  |  |
| --- | --- |
| **A** | **B** |

Figure 3‑3. Shear viscosity of batters of different PP concentrations (A) and their respective continuous phase (B). Legend: red dotted line ●●=egg-sugar foam; ● =S0; ●=S20; ●=S40. For the sake of visibility, curves are not shown for all samples, but were all similar.

### Batter stability

As seen from the kinetics of liquid apparition (Fig.4 A), the stability of the egg-sugar foam increases significantly when WF is added to the egg-sugar foam. This is reflected by both, the increase of the time of liquid apparition during the free drainage experience, and by the more progressive and less abrupt destabilization curve of S0, as compared to egg-sugar. Also, the stability of the batters containing PP is significantly augmented with their increasing concentration, being multiplied by 3 in the S40 sample. Similar to what occurs in S0, destabilization of samples S10 and S20 occurs progressively. In contrast, despite their larger values of liquid apparition time, the destabilization curve becomes abrupt again for the two highest PP concentrations (S30 and S40). To explain this behavior, we hypothesize that the higher viscosity of their continuous phases promotes the local accumulation of drained liquid within the Plateau borders; this type of local drainage is not macroscopically visible and does not immediately induce liquid apparition at the bottom of the tube: the liquid will continuously accumulate until its volume is high enough to cause a disruption in the internal equilibrium forces, resulting in the abrupt liberation of the accumulated liquid. Illustrations of both progressive and abrupt liquid apparition during free drainage are shown in Figure 4 (B,C).

A possible explanation for the increase of stability could rely on the migration of pea proteins and their adsorption at the water/air interface, like previously encountered by Turbin-Orger et al. (2015). However, adsorption at the interface requires proteins to be soluble in the continuous phase (Raikos, Neacsu, Russell, & Duthie, 2014). In fact, vicilins and convicilins, which are the proteins found in pulses, are highly insoluble around their isoelectric point (4<pH<6), thus showing optimum solubility at acidic and alkaline pH values (Boye, Zare, & Pletch, 2010; Gueguen, 1983). In our case, the pH of the continuous phases was found to be 7.5 ± 0.3 and did not differ significantly (p>0.05) between samples of different composition. Since this value remains close to the proteins isoelectric point, solubility may not be optimal, and thus the adsorption hypothesis seems less likely.

On the other hand, the low solubility of PP could be responsible for the viscosity increase of the continuous phase of the batters; acting as “filler” particles. Therefore, we hypothesize the viscosity increase of the continuous phase is the main mechanism that drives the stabilization of batters. However, in order to validate this hypothesis, the characterization of the solubility, particle size and water holding capacity of both WF and PP is needed.

|  |  |
| --- | --- |
| **A**  ■ e+s ■ S0 ■ S10 ■ S20 ■ S30 ■S40 | **B** |
| **C** |

Figure 3‑4. (A) Destabilization kinetics of the samples represented as the evolution of the normalized liquid fraction over time. Example of progressive S0 (B) vs. abrupt S40 (C) liquid apparition. Local liquid accumulation is indicated with red circles.

### Pea protein isolates characterization

From Table 3 it can be seen that the solubility (S) of wheat flour (WF) and pea protein isolates PP does not differ significantly (p>0.05), being very low in both cases. In WF, this is not surprising, since its main components are starch and gluten, both which are insoluble in water at pH=7 (Buleon, Colonna, Planchot, & Ball, 1998; Shewry, Tatham, Forde, Kreis, & Miflin, 1986). In PP the low solubility was also as expected from the pH value of the continuous phase (*see discussion in section 3.3)*. Conversely, in terms of water holding capacity (WHC) the difference between PP and WF is significant (p<0.01): PP absorbs ≈2 times more water than WF. In terms of particle size distribution (PSD) in dry dispersion, WF showed a bimodal distribution (*see Appendix*), with a coarse (1st mode ≈105 µm) and fine fractions (2nd mode ≈24 µm), where the second fraction seems to correspond with starch granules, which diameter is found within the range of 25-29 µm (Buleon et al., 1998); whereas for PP the distribution was monomodal (*see Appendix*). The bimodal distribution of WF illustrates its wide particle heterogeneity as compared to PP, which is not well captured by its mean volume diameter (d4,3). In any case, the differences of particle diameter between WF and PP were highly significant (p<0.001), independently on the dispersion medium used. However, the wet dispersion method seems more appropriate and relevant to our processing. Following that reasoning, it is possible to conclude the particle diameter of PP determined by wet dispersion, is significantly larger than WF.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **WHC**  **(g water/ g dry matter)** | **Solubility (%) pH=7** | **d4,3 (µm)** | |
| dry | wet  5% (w/v) |
| PP | 2.6 ± 0.1a | 5.3 ± 0.5a | 79 ± 1a | 77 ± 3 a |
| WF | 1.1 ± 0.1b | 6.1 ± 0.3a | 64 ± 1b | 45 ± 6 b |

Table 3‑3. Comparison between wheat flour (WF) and pea protein isolate (PP) properties.

The auto-fluorescence of commercial pea protein isolates at an excitation wavelength of 488 nm has been reported previously by Nunes et al. (2006). However, it is unclear which components in the isolates are responsible for the fluorescence. According to Monici, (2005), many endogenous fluorophores are present in plant cells such as proteins containing aromatic amino-acids, NADPH, flavins, lipo-pigments, chlorophylls, flavonoids and other cell wall components, such as lignin, cutin, etc. In Figure 5, we show confocal laser scanning microscopy (CLSM) images that captured the fluorescence (=488 nm) of PP in dry and wet dispersions. Clearly, PP show fluorescence emission that is bright enough to allow their localization within the batters and the cakes without the need to perform any staining that could damage or perturb the structure of the sample.

|  |  |
| --- | --- |
| **A** | **B** |

Figure 3‑5. CLSM images (  488 nm) showing the autofluorescence of PP (colored in red) in dry (A) and 5% w/v wet (B) dispersions.

### Localization of pea protein isolates in batters and cakes

Bubbles in the egg-sugar foam have a round shape and seem to be loosely packed (Fig.6 A). However after WF is added, large spherical bubbles (≈50µm) can still be seen, but there are less small bubbles, which decrease the area of water / air interface. These observations are in agreement with the decrease of air volume fraction (Φa) from 0.75 to 0.71, reported in 3.1. After adding WF, small starch granules appear finely dispersed in the continuous phase (Fig. 6 B). When PP is added, they are preferably localized near the air/water interface of the bubbles, and, to a lesser extent, in the continuous phase (Fig. 6 C). When the PP concentration is increased, their organization of the continuous liquid phase changes and PP appear to form a more or less continuous network of particles, in the liquid phase (Fig.6 D). PP particles may be linked by non-covalent interactions as previously used and described in the case of colloidal systems that stabilize emulsions (Horozov & Binks, 2006; Stancik, Kouhkan, & Fuller, 2004). Clearly, the formation of this type of network may be linked to the observed changes in the rheological properties of the batters and their continuous phases, which were particularly significant at the highest level of fortification (S40).

The images of cake microstructure (Fig. 7) are coherent with the batter observations. They help to interpret the influence of batter structure on final cake properties. During baking, starch gelatinizes, granules swell, and as water evaporates air cells expand. As a result the continuous solid phase dries and concentrates, and the swollen starch granules become the building bricks of a “brick and mortar” type structure, which is held together by strands of coagulated egg protein (Bousquières et al., 2017; Wilderjans et al., 2013). This type of structure is visible in the reference cake (S0) (Fig. 7 A).

|  |  |
| --- | --- |
| **A** | **B** |
| **C** | **D** |

Figure 3‑6. CLSM images ( 𝜆=488 nm) showing the following batter samples: (A) egg-sugar foam; (B) reference batter (S0); (C) S20; and (D) S40. Particles colored in red represent PP isolates.

However, when PP are added this structure seems less continuous, or at least two co-continuous phases of swollen starch granules and PP particles may coexist (Fig. 7 B,C,D). Like starch in WF, PP swell and this generates a competition for the water in the continuous phase. Therefore, starch granules may only achieve partial gelatinization, as previously encountered by (Hesso et al., 2015), which could partially explain the disrupted appearance of the crumb microstructure (Fig. 7 B). Additionally, when the PP concentration increases, and as previously observed in the batter, the PP particle network, is still present, and may have become covalently linked after baking, as observed for heated pea proteins (Mession, Sok, Assifaoui, & Saurel, 2013). This PP network (Fig. 7 C) might limit the air expansion process, since the air cells surrounded by the PP network appear smaller than those who are not. Many of these hypotheses should be confirmed by complementary experiments. Overall, this qualitative approach allowed us to formulate a coherent hypothesis about the organization of PP in batter cake systems, to be discussed in the following section (3.6).

|  |  |
| --- | --- |
| **A** | **B** |
| **C** | **D** |

Figure 3‑7. CLSM images ( 𝜆= 488 nm) showing the following cake samples: (A) S0; (B) S20; (C,D) S40.

### Overall discussion

By integrating all the obtained results, it is possible to plot the diagram presented in Figure 8. This representation shows the evolution of the rheological properties of batters (B) normalized by those of their continuous phases (CP), e.g. the ratios KB/KCP. and G’B/G’CP. From this diagram, it can be seen that both, viscoelastic and flow properties, evolve in a coherent manner, although KB/KCP is lower than 1 for intermediate levels of PP concentration. This relation allows defining three regions that describe rheological behavior as a function of PP concentration. The first region (KB/KCP >1, PP<10%)indicates that, at low levels of fortification, PP has not a major impact on batter structure and will behave like WF. To interpret the second (KB/KCP <1, 10 < PP substitution ≤30%) and third regions (KB/KCP >1, PP substitution>30%), the existence of a non-covalent network formed by PP during batter formation may be inferred, as suggested in the precedent section (3.5). Indeed, since PP have a larger particle diameter and higher water holding capacity than W, they are likely responsible for the viscosity increase in the continuous liquid phase. When the concentration is high enough (third region), PP may form a non-covalent network, even in the presence of bubbles (Fig. 6 D), so both KB and KCP increase, which explains KB/KCP >1. Conversely, in the second region, the non-covalent network is not formed, because of steric hindrance caused by the presence of bubbles. For this reason, KB does not increase as much as in the third region. On the other hand, the network is more strongly bound in the continuous liquid phase, which explains the more pronounced increase of KCP. This would explain the higher viscosity of the continuous phase and therefore KB/KCP <1.

Figure 3‑8. Schematic representation of the evolution of the normalized rheological and microstructural properties of batters and sponge-cakes as a function of pea protein isolate concentration.

Finally,to increase the Φa,of batters to which PP have been incorporated, it could be suggested to modify the mixing time or use more powerful mixers in order to facilitate air incorporation as shown by, Chesterton et al. (2013), or to lower the pH of the batter by adding citric acid in order to increase PP solubility (Zhang et al., 2012).

# CONCLUSIONS AND PERSPECTIVES

We have confirmed that sponge cake properties are directly impacted by their density, or by the air volume fraction of the batter (Φa). The addition of pea protein isolates (PP) in substitution of wheat flour decreased Φa and increased the viscosity of the continuous phase of the batter, as it became harder to incorporate air into the continuous phase. However, once PP incorporated, the high viscosity of the batters conferred them a large stability, far beyond the time scales that are relevant for processing. Indeed, depending on the concentration of PP, the rheological properties of the batters suggest two different behaviors It is first hypothesized that the viscosity increase mechanism by PP is due to their “filler” effect, caused by a combination of their larger particle diameter and higher water holding capacity as compared to wheat flour. Then, at higher levels of substitution, PP showed the capacity to form a non-covalent network that could be also responsible for the observed rheological behavior, and final sponge cake structure. Overall, our results suggest that the fortification, by pea proteins, of sponge-cakes and other egg foam foods can be carried with limited negative consequences on the cake properties, and some processing strategies can be envisioned to prevent or correct those changes.

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Chapter 2. Article 4

Oral processing and comfort perception of soft cereal foods fortified with pulse proteins in the elderly with different oral health status

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Keywords: food bolus, chewing, dental status, viscosity, hydration, particle size.

# Abstract

This study investigated the oral processing and bolus formation mechanisms of two soft cereal products fortified with pulse proteins, sponge-cake (FSC) and brioche (FB), in the elderly population, and their relationship with the perception of oral comfort. 20 subjects aged 65 and over participated in the study. They were classified in two groups according to dental status (poor vs. satisfactory) and presented varying stimulated salivary flow rate (SSF). Bolus properties (hydration ratio, apparent viscosity and particle size) were characterized after three chewing stages. Chewing duration was significantly impacted by the product and was longer for FB than FSC; moreover subjects with a poor dental status (DS) had a longer chewing duration for FB, while individuals with a higher SSF had a shorter duration for FSC. Compared to FSC, more saliva was added to the FB boli, and the viscosity and particle size were also higher. Since both products had similar densities, these differences were attributed to their processing and composition that led to different mechanical behavior and properties. Based on the bolus particle size and fragmentation patterns, FB was considered to be more difficult to fragment than FSC, since the latter showed a dramatic particle size reduction since the beginning of chewing. For both products, the perception of comfort depended more on the DS than on SSF. The bolus apparent viscosity was related to the perception of oral comfort in FSC, while the chewing duration and the bolus particle size at the beginning of chewing contributed to explain oral comfort in FB.

# Nomenclature

ANSM Acronym for the French ‘National Agency of Drugs and Safety’;

B Brioche;

b Gompertz fitting parameter, slope at the inflexion point

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

c Gompertz fitting parameter, size value at the inflexion point

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

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

Chew Perceived as easy to chew;

D50 Median particle diameter of the distribution

D75/25 Interquartile ratio of the particle size distribution

Dry Dryness perception;

DS Dental status;

Easiness Perceived as easy to eat;

FB Fortified brioche;

FOP Food Oral Processing;

FSC Fortified sponge-cake;

Moisten Perceived as easy to moisten;

OC Overall comfort perception;

P Poor (dental status);

Pasty Perceived pastiness;

PC Principal Component

PFU Posterior functional unit;

PSD Particle size distribution;

r Pearson correlation coefficient;

S Satisfactory (dental status);

Salty Saltiness perception;

SC Sponge-cake;

SI Structure index ;

SP Swallowing point or total chewing duration or third chewing stage;

SSF Stimulated salivary flow rate (mL/min);

Sticky Perceived stickiness;

Swallow Easy to swallow;

WC Water content (g of water/100 g of product);

Ƞ120 Bolus apparent viscosity at 120s-1 (Pa.s);

𝛾̇ Apparent shear rate (s-1);

# Introduction

It has been established that ageing causes a natural degradation of the oral health status, which could be responsible for eating difficulties and loss of eating pleasure in the elderly (Laguna, Aktar, Ettelaie, Holmes, & Chen, 2016; Peyron, Woda, Bourdiol, & Hennequin, 2017; Vandenberghe-Descamps et al., 2016; Xu, 2016). Moreover, the nutritional needs of this population differ from those of younger adults. In particular protein needs are higher in seniors (Bauer et al., 2013; Deutz et al., 2014). As a result, the demand for foods high in proteins, with optimum texture design has increased (Chen, 2016; Schwartz, Vandenberghe-Descamps, Sulmont-Rossé, Tournier, & Feron, 2017). In the past years, the study of Food Oral Processing (FOP) has proved to be highly relevant in the identification and characterization of the above mentioned eating deficiencies, and has the potential to be used as a driver to design appropriate foods for the elderly (Chen, 2016). Indeed, FOP has been identified as a key stage for the perception of texture, taste and aroma and sensory pleasure (Chen, 2009; Salles et al., 2011). Recent works have highlighted the importance of studying chewing behavior, but also the evolution of the structure and the mechanical properties of foods during bolus formation in order to improve the understanding of sensory perception (Chen, 2009, 2014, 2015; Devezeaux De Lavergne, Velde, Boekel, & Stieger, 2015). Therefore, the characterization of bolus properties is critical to the understanding of FOP and perception mechanisms. Recently, the concept of oral comfort was specially developed to assess the perception of foods by the elderly in meat, cheese and cereal products (Assad-Bustillos, Tournier, Septier, Della Valle, & Feron, 2019; Lorieau et al., 2018; Vandenberghe-Descamps et al., 2018; Vandenberghe-Descamps, Labouré, Septier, Feron, & Sulmont-Rossé, 2017; Vandenberghe-Descamps, Sulmont-Rossé, Septier, Feron, & Labouré, 2017). It takes into account all of the aspects that make a food ‘easy to eat’, in closely relation with bolus formation mechanisms.

Today, most foods targeted for the elderly are mainly focused on the nutritional aspects, without considering enjoyment. Some of them are found as dietary supplements that produce taste fatigue on the long-term and have low compliance (Gosney, 2003). Indeed, an important dimension that should be taken into account when designing specialized foods for the elderly is enjoyment (Xu, 2016). To this extent, cereal foods fortified with pulse proteins, have an enormous potential to fulfill the nutritional needs of the elderly while preserving palatability. In a previous work, we studied the relationships between oral comfort perception, food oral processing and bolus properties for two types of soft cereal foods, sponge-cake and brioche, in elderly subjects varying in dental status and salivary flow rate (Assad-Bustillos, Tournier, Septier, et al., 2019). We developed a phenomenological model predicting the evolution of bolus apparent viscosity during oral processing; furthermore, the perception of oral comfort was found to be closely related to bolus apparent viscosity. The particle size distribution of bolus fragments (PSD) was also studied, and different fragmentation patterns were observed that depended on the type of product and were explained by their mechanical properties (Assad-Bustillos, Tournier, Feron, et al., 2019). The present work focuses on the “high in protein” versions of the previously studied sponge-cake and brioche, which were developed based on the results of the first study. They are fortified with pulse proteins, from pea and faba, and the perceived oral comfort in elderly for these foods. In this purpose, we determine the impact of dental status and salivary flow rate on the food bolus hydration ratio, apparent viscosity and particle size distribution during oral processing; and we investigate their relationships with the perception of oral comfort for these cereal foods fortified with pulse proteins. Finally, we examine the impact of fortification on bolus properties, oral health status and perceived oral comfort.

## Materials and Methods

### Panel composition

Twenty French subjects (9 men and 11 women, aged 65-84 years, mean 75 ± 5 years) participated in the study. With the exception of two replacements, the panel is identical to the one reported in Assad-Bustillos, Tournier, Septier, et al. (2019). Their dental status and the salivary flow rates, with and without mechanical stimulation, were assessed as described in said study. Depending on the number of Posterior Functional Units (PFU), participants were classified within two different groups: A satisfactory (S) dental status was considered to be ≥7 PFU; while a poor (P) dental status was considered to be ≤4 PFU (Leake, Hawkins, & Locker, 1994). The mean stimulated and unstimulated, salivary flow rates along with a general description of the 20 subjects of the panel are shown in Table 1. The observed salivary flow values were not significantly different from the values reported in the previous study (p>0.05), and were not dependent on dental status (p>0.05). All subjects agreed on the content of the study and signed informed consent.This study was approved by the local ethical committee and the French National Agency of Drugs and Safety (ANSM) (ID RCB n°2016-A00916-45).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Poor Dental Status (PFU\* ≤ 4)**  **n=11** | **Satisfactory Dental Status (PFU\* ≥ 7)**  **n=9** | **Whole group**  **n=20** |
| **Age (years)** | 77 ± 4a | 72 ± 5b | 75 ± 5 |
| **Sex**  Number of Male  Number of Female | 5  6 | 4  5 | 9  11 |
| **Unstimulated Salivary Flow Rate (mL/min)** | 0.47 ± 0.29a  Min 0.12 Max 0.95 | 0.38 ± 0.17a  Min 0.13 Max 0.77 | 0.42 ± 0.24  Min 0.12 Max 0.95 |
| **Stimulated Salivary Flow Rate (mL/ min)** | 1.87 ± 0.80a  Min 0.87 Max 3.05 | 2.14 ± 0.66a  Min 1.03 Max 3.19 | 2.01 ± 0.75  Min 0.87 Max 3.19 |

*\*PFU= Posterior functional unit. Different letters (a,b) indicate values that significantly differ with p<0.05 (Student-Newman-Keuls test).*

Table 4‑1. Subjects characteristics.

## Food products

The fortified sponge-cake (FSC) and brioche (FB) holding the claim “high in protein” were provided by CERELAB® (Dijon, France). FSC was fortified with pea protein, while FB contained faba protein. Their density was measured by the rapeseed displacement method, and their mechanical properties were determined by uniaxial compression test, both methods are detailed in Assad-Bustillos, Tournier, Feron, et al. (2019). These values, along with their composition are presented in Table 2. Products were offered to the participants as cylinders of 20 cm3, which were cut before the beginning of the experimentation as described in (Assad-Bustillos, Tournier, Septier, et al., 2019) and given to the subjects as mouthfuls for consumption and bolus generation during the experimental procedure.

|  |  |  |
| --- | --- | --- |
|  | Fortified sponge-cake (FSC) | Fortified brioche (FB) |
| Energy (kcal/100 g)\* | **257** | **347** |
| Proteins (g/100 g)\* | **13** | **21** |
| Fat (g/100 g)\* | **5** | **14** |
| Carbohydrates (g/100 g)\* | **40** | **33** |
| Sucrose (g/100 g) | 26 | 7 |
| Starch (g/100 g) | 13 | 24 |
| Others (g/100 g) | 0.5 | 1.5 |
| Density (g/cm3)\*\* | 0.23 ± 0.02a | 0.21± 0.02a |
| Water content (g/100 g)\*\* | 30 ± 2b | 34 ± 2a |
| Apparent Young’s modulus (kPa)\*\* | 7±0.6 | 3 ± 0.5 |
| Critical stress (Pa)\*\* | - | 0.4 ± 0.1 |

*\*Values were determined by a certified laboratory (Eurofins, France). \*\*Values reported are the mean (n=5) ± standard deviation of measures performed in our laboratory.* *Different letters (a,b) indicate values that significantly differ with p<0.05 (Student-Newman-Keuls test).*

Table 4‑2. Product properties and composition (wet basis).

### Experimental procedure

Every subject participated in six individual sessions of approximately 1 h. At the beginning of the first session participants were asked to consume the product mouthful (20 cm3)in a natural manner and were recorded on video while doing it. From this recording, the total chewing duration, the number of chewing cycles, and the chewing frequency were determined as described by Assad-Bustillos, Tournier, Septier, et al. (2019). During the rest of the session, as well as in the other five sessions, participants were asked to chew the product mouthful and to expectorate the food bolus into a Petri dish for further characterization at three mastication stages: 1/3 of total chewing duration (C1), 2/3 of total chewing duration (C2) and just before the swallowing point (total chewing duration, SP) as described by Assad-Bustillos, Tournier, Septier, et al. (2019).

### Oral comfort assessment

Perception of oral comfort was assessed at the end of a randomly selected session by using the questionnaire developed by Vandenberghe-Descamps, Labouré, et al. (2017). The same procedure described in Assad-Bustillos, Tournier, Septier, et al. (2019) was followed.

### Bolus characterization

#### Capillary rheometry

The rheological properties of products and boli were determined by capillary rheometry by using a mechanical texture analyzer (TA.XTplus, Stable Micro Systems, UK) equipped with a cylindrical piston with flat head and a capillary die fixed at the bottom to a cylindrical barrel as previously described by Assad-Bustillos, Tournier, Septier, et al. (2019). Variations of the apparent viscosity (ƞ) as a function of shear rate (𝛾̇) showed a shear-thinning behavior and followed a power law, with flow index values comprised between 0.2-0.3. The value of ƞ at 𝛾̇=120 s-1, noted ƞ120, for each subject and each chewing stage was selected to characterize bolus viscosity from a typical shear rate value of the oropharynx at the beginning of swallowing (Zhu, Mizunuma, & Michiwaki, 2014). Finally the formerly developed phenomenological model predicting the evolution of bolus apparent viscosity during oral processing is applied to these results.

### Bolus hydration ratio

Bolus hydration ratio, defined as the percentage of added saliva, was determined as described in Assad-Bustillos, Tournier, Septier, et al. (2019), based on the equations proposed by Drago et al. (2011) and Repoux et al. (2012).

#### Bolus particle size

The particle size distribution (PSD) of boli was obtained by image analysis with a modification from the protocol presented in Assad-Bustillos, Tournier, Feron, et al. (2019). After being suspended in glycerol, and agitated with a magnetic stirrer for 1h at 170 rpm as in our previous work; bolus particles were placed over a rectangular transparent glass surface of 210 x 297 mm that was placed over a flat-bed scanner (Perfection V850 Pro, EPSON, Japan). Images were acquired in color (16-bits per pixel) with a 800 dpi resolution, using the professional mode of the scanner and without any color correction. For each bolus, 2 to 4 images were needed to characterize 100% of the total volume. Images were saved in TIFF format as matrices of 4694×7644 pixels, with a pixel size of 30 µm. Only the blue channel of the images was used for image analysis, since it offered more contrast between the particles and the background. Image analysis was carried out with Matlab software (Mathworks 2016b, USA) by performing a series of openings of increasing size. The results were expressed as the particle size distribution (PSD) curve. The diameter (D50) and the interquartile ratio (D75/D25), which characterizes the heterogeneity of the bolus, were derived from this curve. Moreover, to ascertain their description, all PSD curves (n=120) were fitted with a three-parameter Gompertz model, as previously described by Assad-Bustillos, Tournier, Feron, et al. (2019).

### Statistical analysis

Differences between products for chewing parameters and oral comfort variables were investigated using a two-way Analysis of Variance (ANOVA) model (product + subject). In bolus properties, these differences were investigated for each chewing stage using a repeated measures ANOVA model (product + subject + chewing stage), where the chewing stage was the repeated factor. When significant differences were found, the Student-Newman-Keuls test was used for a post-hoc multiple comparison test.

To investigate the impact of oral health status in the chewing duration, a two-way Analysis of Covariance (ANCOVA) model was carried out (dental status + stimulated salivary flow). For bolus properties, a three-way ANCOVA model was applied (chewing duration + stimulated salivary flow + dental status). The total chewing duration is included in the model to take into account the variability of bolus properties over time, however only the results of the oral health status effects are presented. For oral comfort variables, a three-way ANCOVA model (product + dental status + stimulated salivary flow) was carried out. Models without interaction were preferred to those with interactions, since the latter did not bring any further information. For every statistical procedure, a significance level of α=0.05 was used. Pearson correlation coefficients (r) were calculated when needed between bolus properties, oral comfort variables and chewing parameters. Finally, Principal Component Analysis (PCA) was used to study the relationship between all of the variables cited above. All statistical analyses were performed with XLSTAT software (v.2016 18.06, Addinsoft, USA). The fitting and the calculation of the coefficients of the phenomenological model of bolus apparent viscosity were performed with Matlab software (Mathworks 2016b, USA).

## Results and discussion

### Chewing parameters and bolus properties

The chewing parameters of fortified sponge-cake (FSC) and fortified brioche (FB) are in the same order of magnitude as the previously reported values concerning the standard sponge-cake (SC) and brioche (B) (Assad-Bustillos, Tournier, Septier, et al., 2019). However, significant differences (p<0.05) in all three parameters are observed between the two products, which are higher for FB. According to Gao, Tay, Koh, & Zhou (2018), a longer chewing duration can be associated with a higher masticatory effort. Paradoxically, and despite their close density values (p>0.05), FB showed a significantly (p<0.05) lower apparent Young’s modulus (Table 2). Actually, the differences in the oral processing behavior between the two foods may rely on the product’s composition, rather than on mechanical properties. Gao, Wong, Lim, Henry, & Zhou (2015) attributed the differences found in the chewing duration and the masticatory effort of breads to their different levels of gluten development, rather than their structural and mechanical features alone. In our case, FB processing involves dough mixing and kneading, which favors the development of a gluten covalent network (Stauffer, 1998); whereas in FSC, wheat proteins do not form any covalent links during the batter mixing, but thermally aggregate instead during baking (Wilderjans, Luyts, Brijs, & Delcour, 2013).

*Different letters (a,b) indicate values that significantly differ with p<0.05 (Student-Newman-Keuls test).*

Figure 4‑1. Chewing parameters boxplots data from all subjects at the swallowing point (SP) for fortified sponge-cake (FSC) and fortified brioche (FB).

Regarding bolus properties, a continuous increase of the bolus hydration ratio (BHR) and a decrease of the bolus apparent viscosity at 120 s-1 (ƞ120) are observed during chewing for the two foods (Table 3). These concomitant trends reflect the overall breakdown of food during oral processing towards a bolus ready for swallowing.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Product** | **FSC** | **FB** | **FSC** | **FB** | **FSC** | **FB** |
| *Chewing stage* | *C1* | *C1* | *C2* | *C2* | *SP* | *SP* |
| **Bolus hydration ratio** Added saliva (%) | 20 ± 9a | 25 ± 9a | 34 ± 15b | 45 ± 17a | 52 ± 22b | 61 ± 20a |
| **Bolus app. viscosity**  (𝛾̇=120s-1) (Pa.s) | 437 ± 196a | 509 ± 145a | 231 ± 65b | 304 ± 139a | 167 ± 50a | 193 ± 50a |
| **Bolus particle size**  D50 (mm) | 0.4 ± 0.2b | 9.6 ± 6.0a | 0.3 ± 0.1b | 1.7 ± 2.8a | 0.3 ± 0.1b | 0.8 ± 0.6a |
| c (mm) | 0.3 ± 0.2b | 8.3 ± 3.5a | 0.2 ± 0.1b | 3.4 ± 2.7a | 0.2 ± 0.3a | 0.4 ± 1.4a |
| D75/D25 | 6.5 ± 3.3b | 24.6 ± 33.2a | 5.3 ± 0.8b | 19.6 ± 20.5a | 5.1 ± 0.7b | 13.2 ± 5.6a |
| b (mm-1) | 1.7 ± 1.1a | 0.3 ± 0.2b | 3.1 ± 1.7a | 0.4 ± 0.3b | 2.7 ± 1.3a | 0.7 ± 0.3b |

*Different letters (a,b) indicate values that significantly differ between products for each chewing stage with p<0.05 (Student-Newman-Keuls test).*

Table 4‑3. Bolus properties for all subjects by product (FSC= Fortified sponge-cake, FB=fortified brioche) and chewing stage (C1, C2 and SP).

However, the correlation between BHR and ƞ120 is not as good in FSC as in FB (rFSC= -0.64, rFB= -0.73 p<0.0001), which suggests that factors other than water absorption contribute to explain the decrease of bolus viscosity. For the three stages, bolus viscosity was significantly larger for FB than FSC (p<0.05).

For both foods, a general decrease of bolus particle size was observed during chewing as shown by the decrease of the median particle diameter (D50) (Fig. 3) and Gompertz parameter c, both being positively correlated (r= 0.78, p<0.001). The decrease became notorious in FB during the second and third chewing stages (C2 and C3), whereas particle size of FSC was drastically reduced since the beginning of chewing (C1), barely changing over the next chewing stages. For the three chewing stages, particle size was also higher for FB than FSC. These results are in line with those observed for the standard products (Assad-Bustillos et al., 2019a,b), where bolus viscosity and particle size were also higher for B than SC, although these differences were attributed to the higher density of B. This is no more the case for the fortified products, and this difference likely relies on the deformation mechanisms of the cell wall of these soft solid foams. Indeed, the compressive mechanical behavior of the products can be described by an elastoplastic law, for FB; and by a hyperelastic law, for FSC (curves not shown), as previously encountered for the standard products in Assad-Bustillos et al., (2019), where it was also suggested that the dissipative nature of the elastoplastic behavior was responsible for the higher difficulty to be fragmented by the elderly subjects. The slight decrease of D75/D25 values, and increase of b Gompertz parameter, both being negatively correlated (r= -0.44, p<0.01), suggested that the bolus of FB became less heterogeneous by the end of chewing, whereas for FSC these changes were not significant. In general, the inter-individual variability is higher for FB than FSC, especially in bolus particle size variables, as reflected by the higher standard deviations, sometimes being higher than the mean values. Again, this is coherent with our previous results, where more scattered variations of particle size during chewing were found for B as compared to SC (Assad-Bustillos, Tournier, Feron, et al., 2019). Inter-individual variations within a same product are likely due to the physiological characteristics of individuals, for that reason, the influence of oral health status on the studied oral processing variables will be analyzed and discussed in the following section.

Figure 4‑2. Variations of median particle size (D50) with chewing time for fortified sponge-cake (▲) and fortified brioche (●).

### Impact of oral health status on chewing parameters and bolus properties

As shown by the results of the ANCOVA models (Table 4), the oral health status of the participants had a significant (p<0.05) influence in all the chewing parameters and bolus properties. Regarding the chewing duration (SP), individuals with a satisfactory dental status (DS) had a shorter SP for FB (negative 𝛽 coefficient); while for FSC, it was longer for subjects with a low SSF (negative 𝛽 coefficient). These results differ from those obtained for standard products (Assad-Bustillos, Tournier, Septier, et al., 2019), where the individuals with a satisfactory DS had shown a longer SP, but it was compensated by a high SSF. As expected, the SSF had a significant (p<0.01) influence on the bolus hydration ratio (BHR) for both products; but, interestingly, subjects with a satisfactory DS were also able to incorporate more saliva to the bolus (p<0.05, positive 𝛽 coefficient). Finally, regarding the median particle diameter (D50), individuals with satisfactory dental status produced boli with a smaller D50 (negative 𝛽 coefficient) in both products, although the effect was more significant in FB than in FSC, as reflected from their F and p-values. The same results were obtained when the analysis was performed with c Gompertz coefficient. Neither dental status nor stimulated salivary flow showed a significant effect (p>0.05, results not shown) on bolus heterogeneity (D75/D25 or b Gompertz coefficient), meaning this variable is independent of the oral health status of the individuals. Overall, these results suggest that teeth play a more important role in the oral breakdown of FB, than FSC, which, again, is coherent with the idea that brioche type foods are more difficult to break down by the elderly. In FB, 8 subjects out of the 20 followed the agglomerative-fragmentation patterns that were previously reported in Assad-Bustillos, Tournier, Feron, et al. (2019) for the standard brioche (B), where said patterns were observed in 10 out of 20 subjects. Pattern I corresponds to a decrease in particle size; pattern II correspond to an increase; and pattern III to a non-monotonous variation combining increase and decrease or *vice versa*. Like for B, no particular trend was observed regarding the DS or SFF that could explain this behavior, as summarized in the *Appendix* of this document. Conversely, no agglomerative patterns were observed for FSC, as previously encountered in SC. These results confirm that the existence of different fragmentation/agglomeration patterns depend on the product type, rather than on the individual’s physiology.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Chewing duration (SP)** | | **Bolus hydration ratio (BHR)** | | | **Apparent viscosity at 120 s-1 (𝜂120)** | | | **Median particle diameter (D50)** | |
| *Product* |  |  | *FSC* | *FB* | | *FSC* | *FB* | | *FSC* | *FB* | *FSC* | *FB* |
| **Dental Status**  **(DS)** | F |  | 0.33 | **5.49** | | 0.55 | **6.15** | | 2.2 | 1.32 | **4.34** | **9.63** |
| p |  | 0.57 | **0.02** | | 0.46 | **0.02** | | 0.14 | 0.25 | **0.04** | **0.002** |
| β | S | 0.08 | **-0.30** | | -0.07 | **0.26** | | -0.15 | -0.13 | **-0.27** | **-0.40** |
| **Stimulated Salivary Flow (SSF)** | F |  | **4.36** | 0.89 | | **17.44** | **10.32** | | **10.33** | **5.87** | 0.01 | 0.47 |
| p |  | **0.04** | 0.35 | | **0.0001** | **0.002** | | **0.002** | **0.02** | 0.93 | 0.50 |
| β |  | **-0.28** | -0.12 | | **0.37** | **0.32** | | **-0.33** | **-0.26** | 0.01 | -0.08 |

*Significant values (p<0.05) highlighted in bold.*

Table 4‑4. ANCOVA model coefficients (Type III sum of squares) for chewing duration and bolus properties for fortified sponge-cake (FSC) and fortified brioche (FB).F= Fisher ratio; p= p-value; β =normalized regression coefficients, for dental status only the Satisfactory coefficient is given (S).

### Phenomenological model of destructuration from stimulated flow rate

In our previous work (Assad-Bustillos, Tournier, Septier, et al., 2019), we presented a phenomenological model that predicts the apparent viscosity of the bolus at shear rate = 120 s-1 (ƞ120) from the stimulated salivary flow of the subjects (SSF) and the chewing time. Because of the dispersion encountered in this first model, in the present study, we propose to improve it as follows (1):

(1) *=*

Where the structure index (SI) corresponds to the ƞ120 value normalized by its initial apparent viscosity value at 120 s-1 (ƞ120 C0) measured in the same conditions. The lower the value of SI the higher is the degree of breakdown of the bolus; and the theoretical saliva in the mouth results from the multiplication of the SSF by the chewing duration time (2):

(2)

Where:

ɸ stim =Stimulated salivary flow rate )

t CX = Chewing duration at a given sequence

This model states that the viscosity reduction, expressed as the structure index (SI) as a function of the amount of theoretical saliva in the mouth, is best described by an exponential law with two coefficients: α and n. The values of α vary between 6 and 17 across the different products, including the standard matrices, with data taken from Assad-Bustillos, Tournier, Septier, et al. (2019) (Table 5). Coefficient α may reflect the interaction of food with saliva, and could be compared to the plasticization coefficient of starch by water, as previously suggested by Le Bleis, Chaunier, Montigaud, & Della Valle (2016). These authors found the α coefficient was dependent on the subject, with values ranging from 12 to 29; however, they studied only one type of matrix (fiber fortified bread). In our case, given the good correlation coefficients (R2 ranges from 0.7 to 0.9) obtained with data from all subjects and in function of the matrix, we hypothesize α is almost not dependent on the individual and that it can be used to compare the different food matrices. The coefficient n, on the other hand, remains relatively constant and only small variations are observed between product types (close to 0.4 for sponge-cakes and 0.3 for brioches). The variations of SI as a function of the theoretical amount of saliva in the mouth are shown in Figure 3. The breakdown behavior between the two sponge-cakes is similar, but the standard sponge-cake (SC) has a higher α coefficient, than the fortified sponge-cake (FSC) which has a much lower α coefficient and denotes saliva interacts less with this product. This effect may be due to the fact that there is less starch in FSC, since wheat flour was partially replaced with pea protein isolates. Additionally, pea proteins form highly cross-linked aggregates when heated (Mession, Sok, Assifaoui, & Saurel, 2013), which reduces the protein solubility and thus explain the lower interaction with water in saliva (Beck, Knoerzer, Sellahewa, Emin, & Arcot, 2017). Both, fortified (FB) and standard brioche (B) also show similar breakdown behaviors, B has a higher SI than FB, meaning that for an equivalent amount of saliva in the mouth, the bolus of B has a less breakdown degree. Clearly, from their α coefficient values, the brioche-type matrices interact less with saliva given their high fat content, almost independently of the protein fortification.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **R2** | **Ƞ120 C0 (Pa)** | **α** | **n** |
| **Sponge-cake (SC)\*** | 0.9 | 2164 | 17 | 0.4 |
| **Fortified sponge-cake (FSC)** | 0.8 | 2122 | 11 | 0.4 |
| **Brioche (B)\*** | 0.8 | 1561 | 6 | 0.3 |
| **Fortified brioche (FB)** | 0.7 | 1772 | 7 | 0.3 |

*\*Data from Assad-Bustillos et al. (2017).*

Table 4‑5. Coefficients of the proposed phenomenological destructuration model for the fortified and the standard studied matrices.

Figure 0‑3. Destructuration index (SI) as a function of the amount of theoretical saliva in the mouth for sponge-cakes fortified (⯅, FSC, R2=0.8) and standard (△, SC, R2=0.9); brioches fortified (●, FB, R2=0.7) and standard (⭘, B, R2=0.8) brioches. Data for standard matrices is from Assad-Bustillos, Tournier, Septier, et al. (2019).

### Perception of oral comfort and impact of oral health status

Significant (p<0.001) differences were found in the two principal variables of oral comfort (overall comfort and easiness) between the two fortified matrices (Figure 4). These results are in contrast with what was observed for the standard matrices (Assad-Bustillos, Tournier, Septier, et al., 2019), where no significant difference was found for any of the oral comfort variables. They show that the fortified brioche (FB) was perceived as less comfortable and more difficult to eat than the fortified sponge-cake (FSC). In order to find out if these differences are only due to the product, or are also explained by the oral health status of the subjects, an ANCOVA model was carried out, including the product effect. The results are summarized in Table 6.

Figure 4‑4. Mean scores ± S.D. of principal oral comfort variables from all subjects for both products (FSC= fortified sponge-cake; FB= fortified brioche). Easiness = Perceived as easy to eat; Chew = Perceived as easy to chew; Moisten = Perceived as easy to moisten; Swallow = Perceived as easy to swallow.

Concerning the perception of overall comfort (OC), the product effect is accountable for most of the variability (p<0.001), with FB being perceived as less comfortable (negative 𝛽); nevertheless, a trend (p=0.06) can be observed for the dental status (DS), where the individuals with a satisfactory DS dental status tend to perceive the products as more comfortable (positive 𝛽 coefficient). The FB was significantly perceived as less easy to eat (p<0.05, negative 𝛽), but the individuals with satisfactory DS perceived the products as easier to eat (p<0.05, positive 𝛽). Conversely, the perception of easiness to swallow and dryness were unaffected by the product (p>0.05), but were affected by the (p<0.05). Indeed, the values of 𝛽 coefficients denote again the subjects with a satisfactory DS perceive the products as easier to swallow and less dry than those with a poor DS. Finally, the perception of saltiness was dependent on the product (p<0.05), where FB was perceived as saltier (positive 𝛽). Saltiness was the only variable were the stimulated salivary flow (SSF) had a significant (p=0.05) effect, showing a positive value of 𝛽 coefficient, which means the higher the SSF, the higher the perception of saltiness. Again, these results are in clear contrast to the previously reported results for standard matrices, where, the SSF was found as the main driver of the OC perception, mainly in sponge-cake (Assad-Bustillos, Tournier, Septier, et al., 2019). The lower interaction of the fortified matrices with saliva, discussed in the previous section especially in sponge-cake, could explain the absence of effect from SSF. This explanation is supported by the lower bolus hydration ratio of FSC compared to SC, which was ≈50% vs. ≈80% at the swallowing point (SP), respectively. In contrast, FB absorbed more saliva than B (≈60% vs. ≈45%), but this could be attributed to the combined effect of the lower density and lower fat content of FB. In the case of brioche, it is more difficult to conclude on the sole effect of protein fortification, since adding pulse protein was not the only change that was made to its composition which also impacted its final structure. In any case, it is clear that compared to standard matrices, fortified products rely much more on the dental status to explain the perception of comfort, in particularly brioche.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Overall comfort** | **Easiness** | **Easy to swallow** | **Dry** | **Salty** |
| **Product** | F |  | **14.00** | **7.58** | 2.26 | 0.28 | **8.67** |
| p |  | **0.0001** | **0.01** | 0.14 | 0.60 | **0.01** |
| β | FB | **-0.49** | **-0.38** | -0.22 | -0.08 | **0.42** |
| **Dental Status (DS)** | F |  | *3.83* | **6.01** | **5.91** | **6.14** | 0.06 |
| p |  | *0.06* | **0.02** | **0.02** | **0.02** | 0.81 |
| β | S | *0.26* | **0.35** | **0.38** | **-0.38** | 0.04 |
| **Stimulated Salivary Flow (SSF)** | F |  | 2.88 | 1.51 | 0.03 | 1.14 | **4.05** |
| p |  | 0.10 | 0.23 | 0.87 | 0.29 | **0.05** |
| β |  | 0.23 | 0.17 | 0.03 | 0.16 | **0.30** |

*Significant values (p<0.05) highlighted in bold; trends appear in italics.*

Table 4‑6. ANCOVA model coefficients (Type III sum of squares) of oral comfort variables. F= Fisher ratio; p= p-value; β =normalized regression coefficients, for dental status and product effects, only the Satisfactory (S) and the fortified brioche (FB) coefficients are given.

### Multivariate analysis and overall discussion

In order to integrate all of the previous results, a principal component analysis (PCA) was performed including the chewing parameters, bolus properties, physiology and oral comfort variables. For the fortified sponge-cake (FSC, Fig. 5 A), it can be seen that the overall comfort (OC) is negatively correlated to principal component 3 (PC3) (R= -0.82), and it is opposed to the bolus apparent viscosity at 120 s-1 (ƞ120) at the swallowing point, which is correlated positively to PC3 (R= 0.63). Principal component 1 (PC1) is correlated negatively to the perception of easiness (R= -0.89), positively to the stickiness (R= 0.90), the chewing duration (SP) (R= 0.69) and the c Gompertz parameter (R= 0.49); while negatively correlated to the stimulated salivary flow (SSF) (R= -0.66). From this analysis, we can conclude the perception of OC in FSC is still closely related to ƞ120, as previously seen for the standard sponge-cake (SC) (Assad-Bustillos, Tournier, Septier, et al., 2019): the more viscous the bolus, the less comfortable to eat. A higher number of Posterior Functional Units (PFU) was moderately correlated to the PC3 (R=0.47), and depicted inversely to the OC, underlined the contribution of dental status to oral comfort perception, appeared as a trend in the ANCOVA model. The perception of easiness, was associated to a high SSF, and inversely related to the perception of stickiness, and to the c Gompertz parameter. This means that boli with higher particle size were perceived as more sticky and less easy to eat.

For the fortified brioche (FB, Fig. 5 B), OC is correlated to principal component 1 (PC1) (R= 0.81), as well as the easiness, easiness to chew, to moisten and to swallow (all R≈0.7). the number of PFU (R= 0.70) is also positively correlated to PC1) and negatively to the perception of stickiness (R= -0.75) and pastiness (R= -0.56). Principal component 2 (PC2) is positively correlated to the perception of dryness (R=0.64) and the amount of added saliva (BHR) (R= 0.83), and negatively to the bolus viscosity (R= -0.78). These results confirm that the dental status is paramount for the perception of comfort in FB. Moreover, as previously seen in the standard brioche (B) (Assad-Bustillos, Tournier, Septier, et al., 2019), the perception of comfort and easiness are inversely related to the pastiness and stickiness. However, none of the studied bolus properties explained these perception variables. Furthermore, as seen from the ANCOVA model, the perception of saltiness seems to be enhanced by the high SSF, and appears opposed to the c Gompertz parameter, meaning boli with smaller particle size are also perceived as saltier. Finally, neither ƞ120 nor BHR explained the perception of comfort, which is in accordance with the previous findings for B (Assad-Bustillos, Tournier, Septier, et al., 2019), even if FB absorbed more saliva. Instead, since the dental status was identified as an important factor in the perception of comfort in FB, it was not surprising the bolus particle size, represented by the c Gompertz parameter, at the beginning and the middle of chewing (C1 and C2) were opposed to OC. Interestingly, this was not the case with the c Gompertz parameter near the swallowing point. Nevertheless, since the chewing duration (SP) was negatively correlated to PC1 (R= -0.57), and opposed to OC, one possible interpretation could be that all subjects are able to fragment the particles of FB to form a bolus that is ready to be swallowed, but it takes longer for those with a poor dental status, as shown in the results from the ANCOVA model (Table 4).

In summary, the comfort perception mechanisms for the two fortified cereal foods are dependent on the physiology of the individuals, principally dental status, and secondarily on the stimulated salivary flow. Bolus viscosity contributes to explain the perception of comfort in FSC; while the bolus particle size at the beginning of chewing and the chewing duration do it in FB.

|  |  |
| --- | --- |
| **A** | **B** |
| *Comfort = Overall comfort perception; Chew = Perceived as easy to chew; Moisten = Perceived as easy to moisten; Swallow = Perceived as easy to swallow; Easiness = Perceived as easy to eat; Pasty = Perceived pastiness; Sticky = Perceived stickiness; BHR = Bolus hydration ratio or added saliva; η120 = Bolus apparent viscosity at 120 s−1; SSF = Stimulated salivary flow rate; SP = Swallowing point or total chewing duration; c= c Gompertz parameter.* | |

Figure 4‑5. PCA correlation circle for oral health status, bolus properties, chewing duration and oral comfort variables for (A) fortified sponge-cake and (B) fortified brioche.

# Conclusions

In this study we have shown that, the oral comfort perception of the protein fortified matrices, compared to their standard versions, rely more on the dental status than the stimulated salivary flow. In the case of sponge-cake, this is due to its lower interaction with the saliva, which might be due to the combined effect of less starch content and the formation of protein aggregates during baking. Also, the bolus apparent viscosity, and to a lesser extent the particle size, were related to the perception of oral comfort in this product, confirming our previous findings. As for fortified brioche, mainly the dental status, but also the stimulated flow are important in the perception of comfort; on one hand its composition and processing, probably related to the level of gluten development, grant the product more plastic mechanical properties that make it more difficult to fragment; on the other, this matrix absorbs more saliva than its standard version, probably due to its lower fat content and density. These changes had consequences on the chewing duration, which became longer, and led to a decreased perception of oral comfort. These aspects are to be taken into account in the formulation of protein fortified cereal foods, especially since adding proteins often demands other major formulation changes such as fat reduction, than can have a major impact on the product final structure and oral processing mechanisms. In this regard, these results give useful information about the different mechanisms that drive the perception of oral comfort in realistic soft cereal foods fortified with pulse protein and open prospect for the tailoring of foods for the elderly based on food oral processing.

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