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

Assad-Bustillos, M.<sup>1,2,3</sup>, Tournier, C<sup>2</sup>., Feron G<sup>2</sup>., Guessasma<sup>1</sup>, S., Reguerre A.L.<sup>1</sup>, Della Valle, G<sup>1\*</sup>.

<sup>1</sup> INRA UR-1268 Biopolymères Interactions et Assemblages, 44316 Nantes, France

<sup>2</sup> Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRA, Université Bourgogne Franche-Comté, F-21000 Dijon, France

<sup>3</sup> CERELAB®, La Sucrerie 21110 Aiserey, France

\*corresponding author : [guy.della-valle@inra.fr](mailto:guy.della-valle@inra.fr)

## 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 parameters were related to bolus particle heterogeneity and fragmentation, thanks to their correlations with median particle size diameter  $D_{50}$  and interquartile ratio ( $D_{75}/D_{25}$ ), 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
D <sub>25</sub>	Particle diameter of first the quartile of the distribution
D <sub>50</sub>	Median particle diameter of the distribution
D <sub>75</sub>	Particle diameter of the third quartile of the distribution
D <sub>75/25</sub>	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 <sup>-1</sup> )
XR-μCT	X-Ray Micro-Computed Tomography

$\sigma_c$  Critical stress (kPa)

## 1. 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, Délérès, 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; Jourden, 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, Karinthe, 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 ( $D_{50}$ ) 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 fracture, 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 ( $\leq 1\text{mm}$ ) (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 ( $D_{50}$ ) over time, and Jourdren, Panouillé, et al., 2016 also reported an increase in bolus heterogeneity, which they chose to assess by the interquartile ratio ( $D_{75}/D_{25}$ ). 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 D<sub>50</sub> 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.

## **2. Materials and Methods**

### *2.1 Product composition, structural and mechanical properties*

158 The sponge-cake and brioche used in this study were provided by CERELAB®,  
159 France. Their composition is detailed in [Table A \(Appendix\)](#).  
160 Their instrumental texture was defined by their density, 3D cellular structure and  
161 mechanical behavior. The product density was measured by the rapeseed  
162 displacement method.

163 The three-dimensional cellular structure was determined by X-ray micro-computed  
164 tomography (XR- $\mu$ CT), using a compact table-top system Skyscan 1174 (Bruker  
165 microCT, Belgium). A cylindrical sample of each product with a diameter of 2 cm and  
166 a height of 3 cm was prepared with a steel cutter and placed on a rotating plate while  
167 the X-ray beam passed through. A CCD camera with a resolution of 1304×1304  
168 pixels was used to acquire the 2D radiographic images. The exposure time was 2000  
169 ms, and the pixel size was adjusted to 22  $\mu$ m. Two images were taken per rotational  
170 step (every 0.5°, until 360°) and were averaged. The projections were then  
171 reconstructed to obtain cross-sectional images using the NRecon reconstruction  
172 software (Bruker microCT, Belgium). Reconstructions were based on the Feldkamp  
173 cone-beam algorithm (Feldkamp, Davis, & Kress, 1984). After reconstruction, a stack  
174 of 1000 images in TIFF format was obtained for each sample. 3D images were  
175 therefore composed of 1304×1304×1000 voxels, coded on an 8-bit grey-scale. One  
176 replication was made for each product, for a total of four independent 3D images  
177 generated. From the images, the granulometric curves, that lead to cell wall size and  
178 cell wall thickness values, were calculated by using mathematical morphology  
179 operations (Serra, 1982). A series of openings of increasing size (image sieving)  
180 was performed on the features of [interest and the sum](#) of the volume occupied by the  
181 sieved particles, either cells or walls, was computed at each step. The results were  
182 expressed as the plot of the cumulative volume (%) of the particle vs the particle

diameter ( $\mu\text{m}$ ). 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)

$$P = VF = 1 - D \quad (1)$$

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 ( $\sigma_c$ ), when applicable, were measured.  $E$  was calculated from the initial slope within the linear elastic domain, while  $\sigma_c$  was defined as the stress value at the end of the linear domain.

## 2.2 Panel composition

Twenty subjects (9 men and 11 women, aged 65–82 years, average  $72 \pm 5$  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 ( $\leq 4$ PFU's) and satisfactory ( $\geq 7$  PFU's) DS. Additionally, their salivary flow rate in  $\text{mL} \cdot \text{min}^{-1}$  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).

### *2.3 FOP assessment and bolus collection*

Mouthfuls of 20 cm<sup>3</sup> 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.

### *2.4 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.

## 2.5 Data treatment and Statistical analysis

For each subject and each chewing sequence (C1, C2, SP), the median equivalent diameter ( $D_{50}$ ) and the interquartile ratio ( $D_{75}/D_{25}$ ) were derived from the PSD curve. The ratio ( $D_{75}/D_{25}$ ) 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:

$$A = a \times \exp(-\exp(-b \times (p - c))) \quad (2)$$

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).

258

259 A one-way ANOVA was performed to determine the differences of structural and  
260 mechanical properties between the two products. In order to investigate differences  
261 between products at each chewing stage, a repeated measures ANOVA (product +  
262 subject + chewing sequence) was carried out for the median particle size  $D_{50}$ ,  
263 interquartile ratio  $D_{75}/D_{25}$  and Gompertz parameters ("a", "b", "c,"), with the chewing  
264 sequence as repeated factor. Additionally, a one-way ANOVA was carried out for  
265 each product to investigate differences between chewing sequences. Furthermore,  
266 to investigate the impact of oral health status, a three-way ANCOVA (Analysis of  
267 covariance) model with level 2 interactions was applied for each product (chewing  
268 duration + dental status + stimulated salivary flow + dental status×stimulated salivary  
269 flow + dental status×chewing duration + stimulated salivary flow×chewing duration).  
270 For every statistical procedure, a significance level of  $\alpha=0.05$  was used and results  
271 reported according to Type III sum of squares. The Student-Newman-Keuls test was  
272 used for post-hoc comparison tests. All statistical analyses were performed with  
273 XLSTAT software (v.2016 18.06, Addinsoft, USA).

### 274 3. Results and discussion

#### 275 3.1 Structure and mechanical properties of the two cereal foods

276 The values of structural and mechanical properties of both products are reported in  
277 Table 3, together with their standard deviation. Not surprisingly, both foods show  
278 distinct structural features due to their different composition and process. The first  
279 indicator of these differences is density, where sponge-cake (SC) showed a lower  
280 value ( $\rho^* = 0.21 \text{ g.cm}^{-3}$ ) than brioche (B) ( $\rho^* = 0.33 \text{ g.cm}^{-3}$ ). This may be the reason  
281 why the cellular structure of SC displayed larger bubbles, or gas cells, while B  
282 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).

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 ( $D_{50}$ ) value of  $\approx 100 \mu\text{m}$  and  $\approx 120 \mu\text{m}$  for SC and B, respectively (Table 1). Regarding the voxel size, i.e.  $22 \mu\text{m}$ , 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  $\approx 300 \mu\text{m}$  and  $\approx 200 \mu\text{m}$  respectively (Table 1). Hence, in line with the difference of density, the main difference in cellular structure between products comes from the cell size.

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).

The values of Young's moduli ( $E$ ), for both products, and critical stress ( $\sigma_c$ ) 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 ( $D_{50}$ ), however, was on the lower edge of the interval [300, 1600 $\mu$ m] 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.

### *3.2 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 ( $D_{50}$ ) and the interquartile ratio ( $D_{75}/D_{25}$ ), 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  $D_{50}$  values than SC at all chewing stages. Secondly, for SC,  $D_{50}$  was significantly reduced over the chewing sequences. B boli, on the other hand, did not show any significant variation of  $D_{50}$  throughout the chewing stages. Also,  $D_{50}$  of B boli showed a higher inter-individual variability than SC, as reflected by the higher standard deviation. In addition,  $D_{75}/D_{25}$ , decreased significantly for SC, meaning these boli tend to reduce particle size

towards the same value as mastication progresses. Conversely, this value increased significantly for B boli, meaning particle heterogeneity becomes higher over the chewing sequences. The variations over time of  $D_{50}$  and  $D_{75}/D_{25}$  for all subjects and both products are shown in Fig. 4. This figure confirms the previous analysis and clearly depicts the scattered variations of  $D_{50}$  for B and illustrates the complexity of chewing mechanisms in this product, likely combining fragmentation and agglomeration of food particles.

These results also show that using a single parameter from the PSD, such as  $D_{50}$ , 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  $D_{50}$  and  $D_{75}/D_{25}$  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 ( $R^2 \geq 0.9$ ), 2 had a low quality fitting ( $0.6 \leq R^2 \leq 0.8$ ), and 6 had an unsuccessful fitting ( $R^2 \leq 0.5$ ) (cf. Appendix).

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  $D_{50}$ , ( $R_{SC}=0.94$ ,  $R_B=0.95$   $p < 0.0001$ ), and the regression line is closed to the bisector. Conversely, “b” is negatively correlated to  $D_{75}/D_{25}$ , ( $R_{SC}=-0.65$ ,  $R_B=-0.49$   $p < 0.0001$ ) (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.

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  $\approx$ 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).

### 3.3 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  $D_{50}$  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 ( $D_{50}$ ) was identified ( $p < 0.05$ ). The normalized coefficient of the model for the satisfactory DS group ( $\beta_s$ ) was -0.8. This result means that individuals with a satisfactory DS produced boli with lower  $D_{50}$  values than those with a poor DS. The same result was obtained when performing the analysis with “c” Gompertz coefficient instead of  $D_{50}$  ( $p < 0.001$ ,  $\beta_s = -1.0$ ). However, in this model, a significant interaction between chewing duration and DS was found ( $p < 0.01$ ), where  $\beta_s = 0.6$ . This positive value may reflect the limited size reduction ( $D_{50} \geq 0.15\text{mm}$ ), illustrated in Fig.4a, for longer chewing duration and satisfactory DS. Conversely, for brioche (B), no significant effect of DS was found for  $D_{50}$ . A different result was obtained, nonetheless, with “c”, where DS had a significant effect ( $p < 0.01$ ,  $\beta_s = -0.3$ ), meaning this parameter is lower for subjects with a satisfactory DS. This also means that,

contrary to  $D_{50}$ , “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  $D_{50}$ . Neither  $D_{75}/D_{25}$  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.

### 3.4 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 be 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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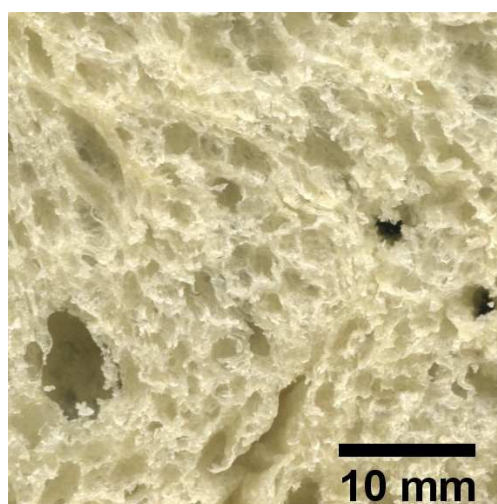
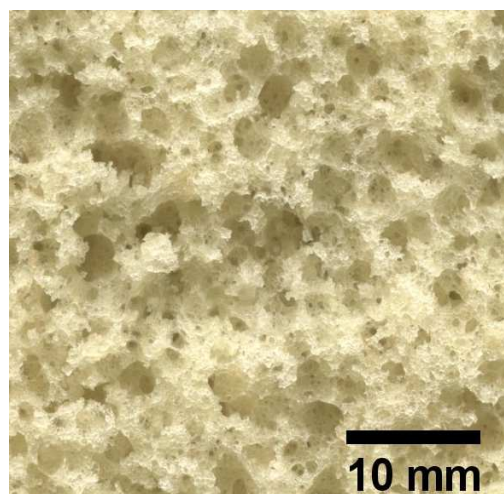
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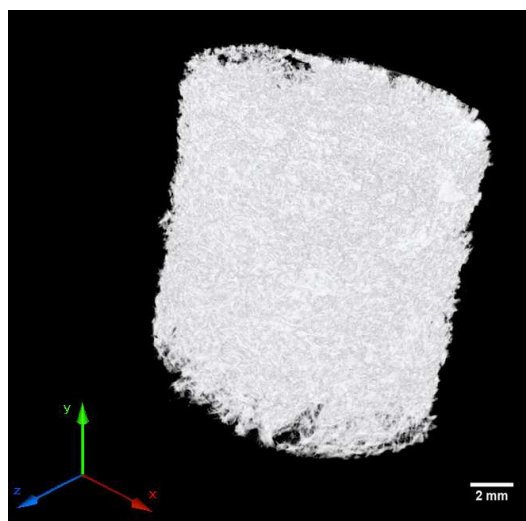
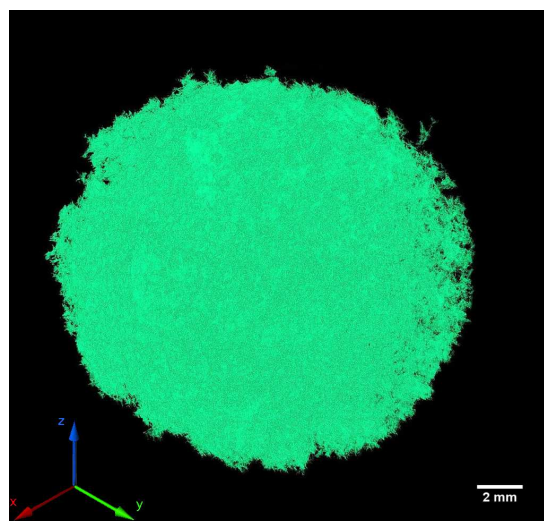
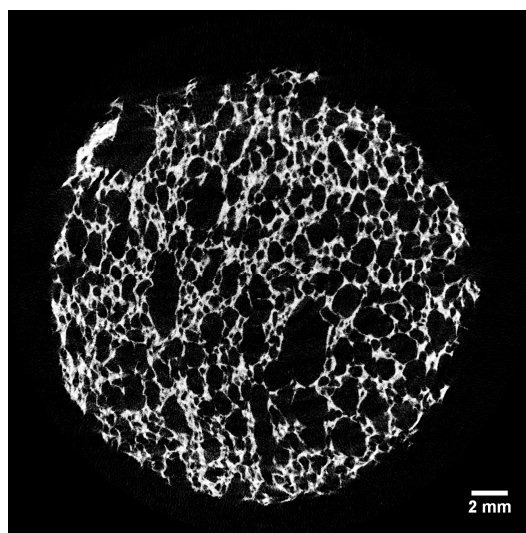
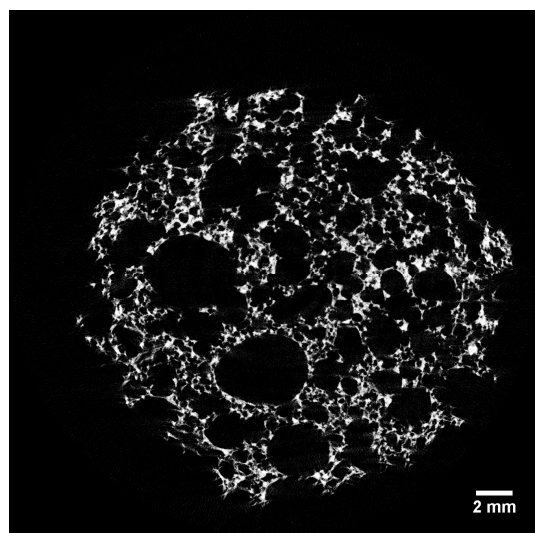
Fig. 5: Variations of c and b values derived from Gompertz model with respectively (a) particle median size ( $D_{50}$ ) and (b) interquartile ratio ( $D_{75}/D_{25}$ ) for sponge cake (blue) and brioche (red).

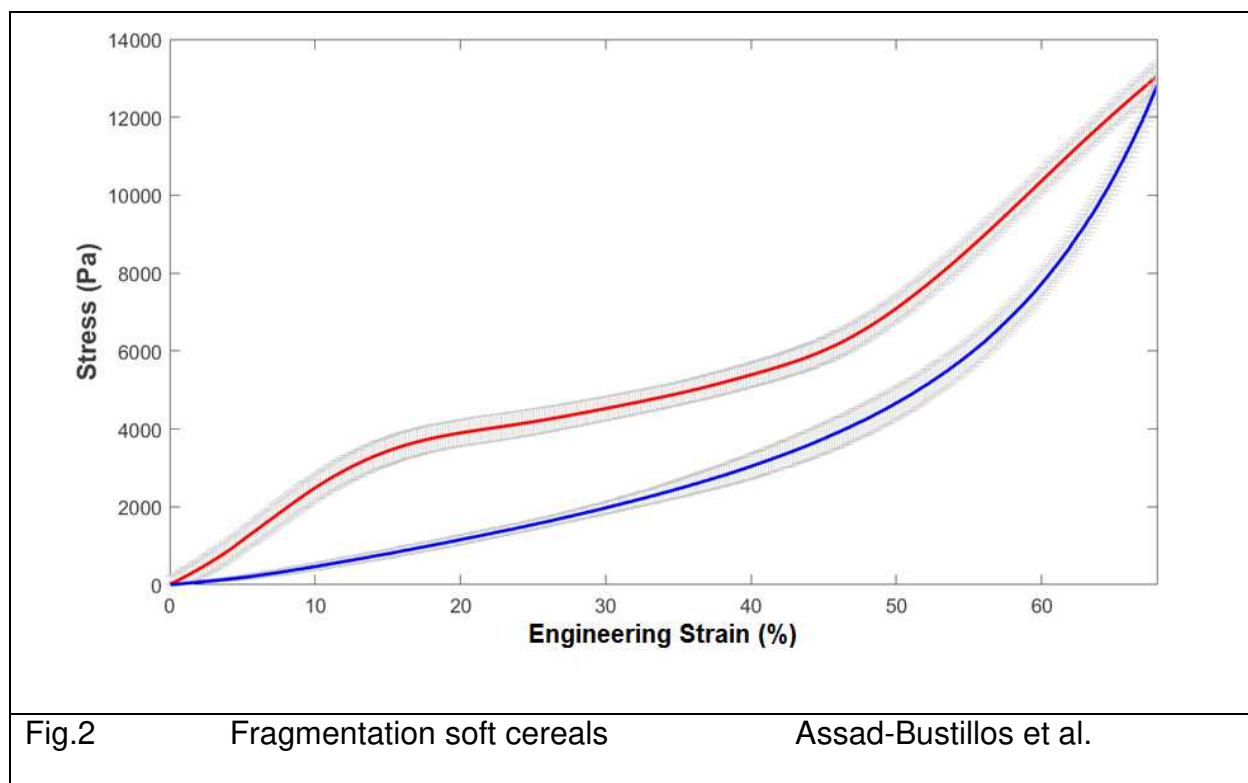
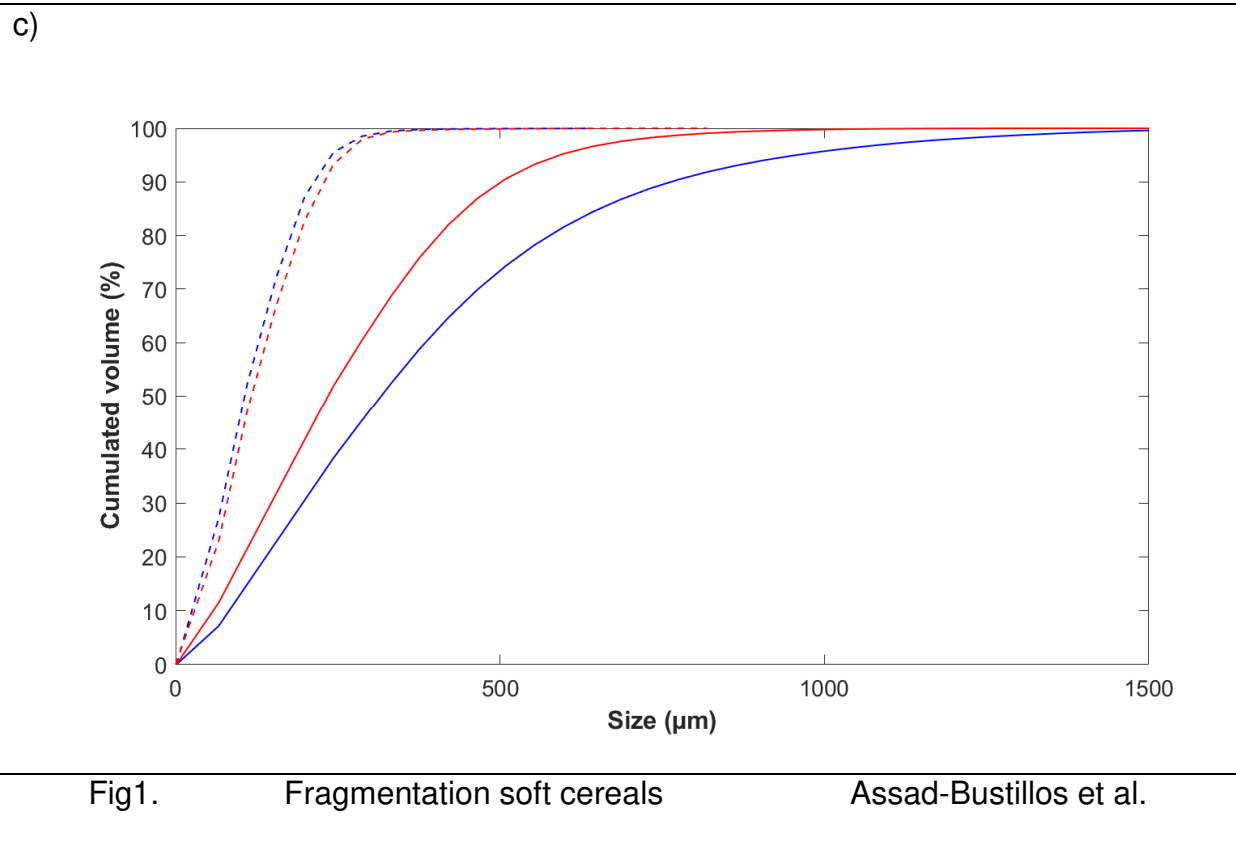
Fig.6: Typical examples of boli images after chewing at C1 (left), C2 (center) and at swallowing point (right) for sponge-cake (a), and for brioche, decreasing size (case I) (b), increasing size (case II) (c), combination of both (case III) (d). These images correspond to the size distributions plotted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

(a)



(b)





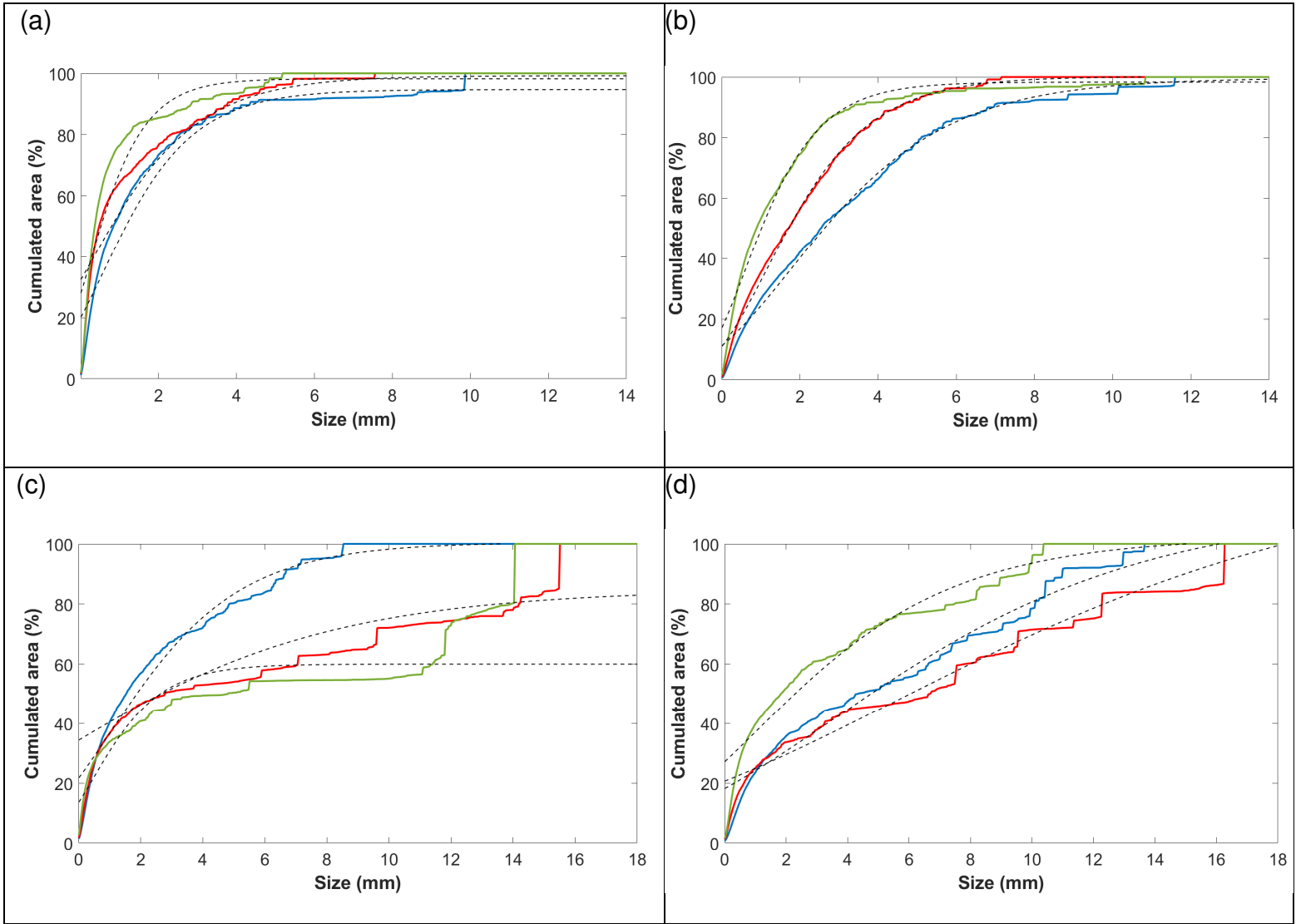
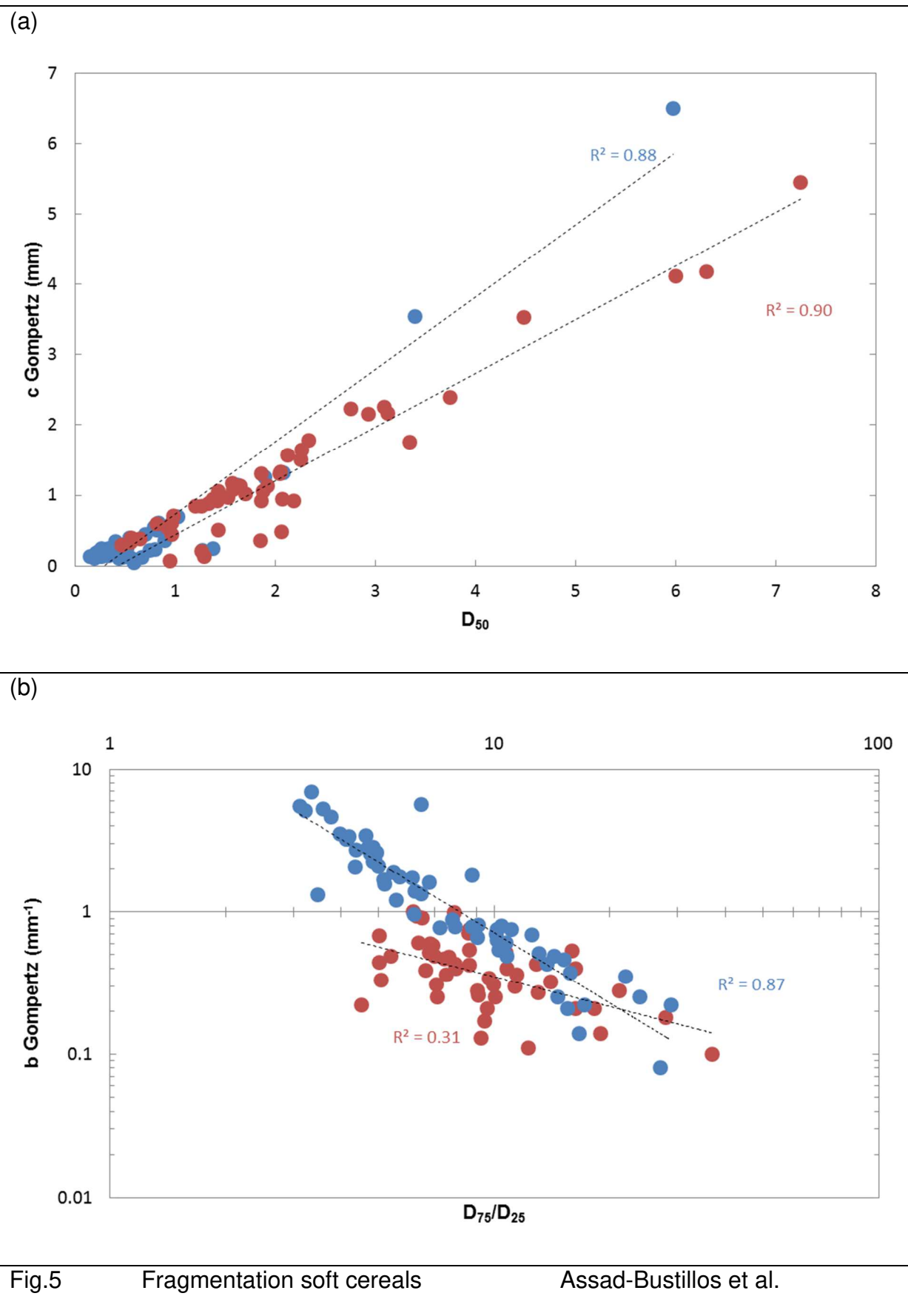


Fig.3      Fragmentation soft cereals      Assad-Bustillos et al.





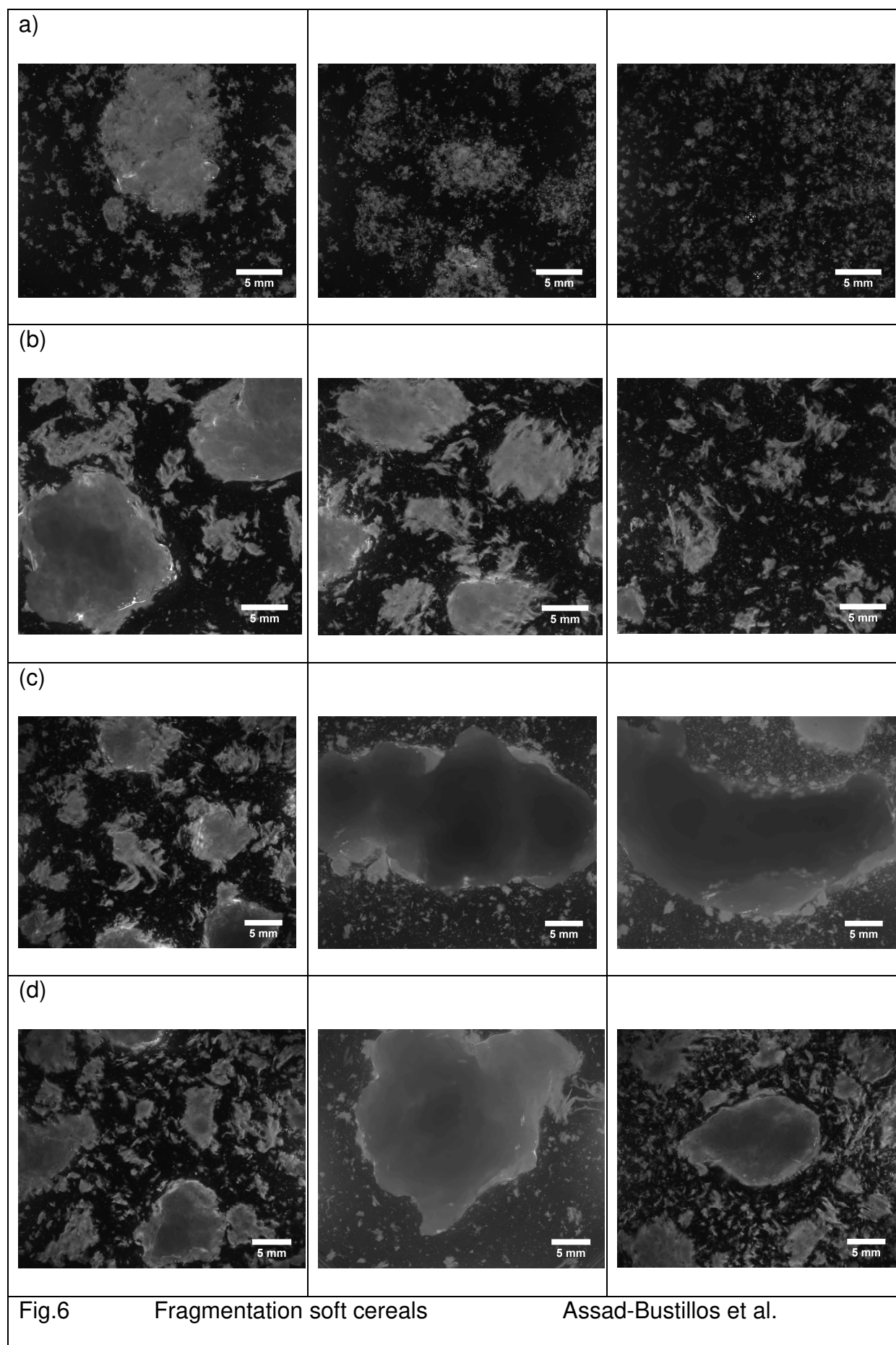


Table 1. Fragmentation soft cereals

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	Sponge-cake	Brioche
<b>Direct Measures*</b>		
Density (g/cm <sup>3</sup> )	0.21 (±0.02) <sup>A</sup>	0.33 (±0.02) <sup>B</sup>
Young's modulus E (kPa)	5 (±1) <sup>A</sup>	20 (±3) <sup>B</sup>
Critical stress $\sigma_c$ (kPa)	N/A	3 (±1)
<b>3D Image Analysis**</b>		
Porosity	0.79 (±0.01) <sup>B</sup>	0.69 (±0.04) <sup>A</sup>
Relative density (D)	0.21 (±0.01) <sup>A</sup>	0.31 (±0.04) <sup>B</sup>
Wall Size		
D <sub>25</sub>	41 (±1) <sup>A</sup>	45 (±1) <sup>B</sup>
D <sub>50</sub>	99 (±1) <sup>A</sup>	118 (±5) <sup>A</sup>
D <sub>75</sub>	176 (±1) <sup>A</sup>	200 (±12) <sup>A</sup>
Cell size		
D <sub>25</sub>	95 (±1) <sup>B</sup>	73 (±10) <sup>A</sup>
D <sub>50</sub>	296 (±2) <sup>B</sup>	197 (±26) <sup>A</sup>
D <sub>75</sub>	785 (±81) <sup>B</sup>	403 (±40) <sup>A</sup>

\*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).

Table 2. Fragmentation soft cereals

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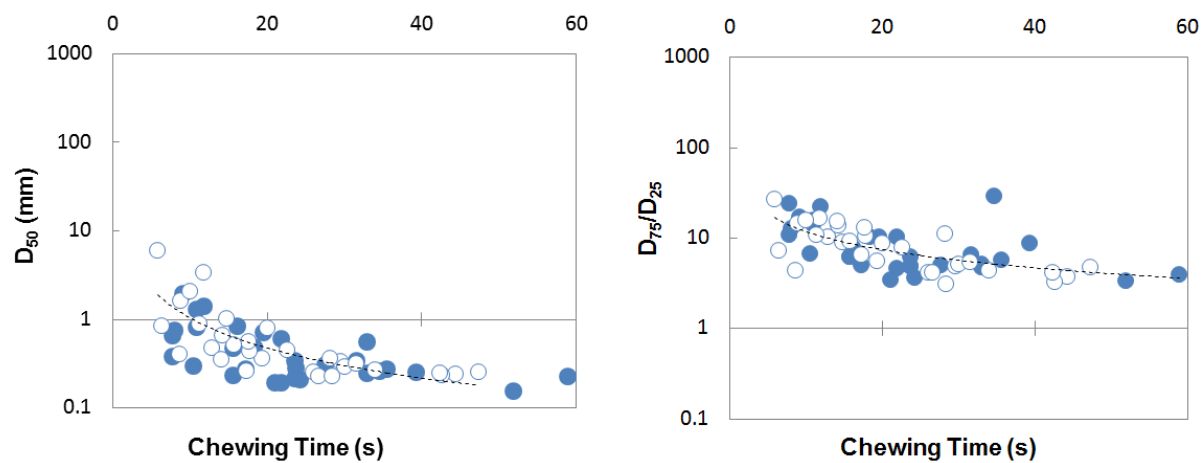
Product	Parameter	Chewing sequence		
		C1	C2	SP
Sponge-cake	D <sub>50</sub>	1.1 (±1.3) <sup>a A</sup>	0.5 (±0.7) <sup>b A</sup>	0.3 (±0.1) <sup>c A</sup>
	D <sub>75</sub> /D <sub>25</sub>	13.3 (±6.1) <sup>a A</sup>	8.9 (±5.7) <sup>b A</sup>	5.0 (±2.1) <sup>c A</sup>
	a	100.8 (±12.6) <sup>a A</sup>	100.4 (±7.6) <sup>a A</sup>	99.3 (±0.5) <sup>a A</sup>
	b	0.7 (±0.5) <sup>a A</sup>	1.4 (±1.2) <sup>b A</sup>	2.7 (±1.6) <sup>c A</sup>
	c	0.7 (±1.5) <sup>a A</sup>	0.3 (±0.9) <sup>b A</sup>	0.2 (±0.1) <sup>c A</sup>
Brioche	D <sub>50</sub>	2.5 (±1.5) <sup>a B</sup>	2.5 (±2.3) <sup>a B</sup>	2.9 (±4.0) <sup>a B</sup>
	D <sub>75</sub> /D <sub>25</sub>	8.3 (±3.4) <sup>a B</sup>	15.2 (±14.4) <sup>a B</sup>	25.6 (±24.2) <sup>b B</sup>
	a	108.7 (±39.4) <sup>a A</sup>	106.7 (±28.3) <sup>a A</sup>	102.2 (±10.1) <sup>a A</sup>
	b	0.4 (±0.1) <sup>a B</sup>	0.4 (±0.2) <sup>a B</sup>	0.5 (±0.3) <sup>a B</sup>
	c	2.4 (±3.7) <sup>a B</sup>	1.9 (±3.0) <sup>a B</sup>	-0.3 (±3.4) <sup>b A</sup>

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).

(a)



(b)

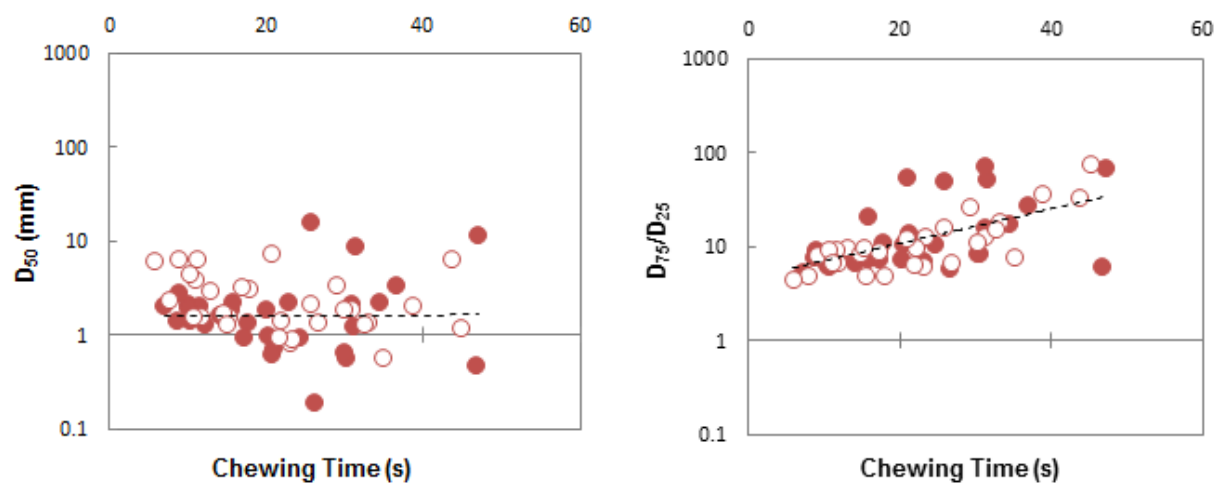
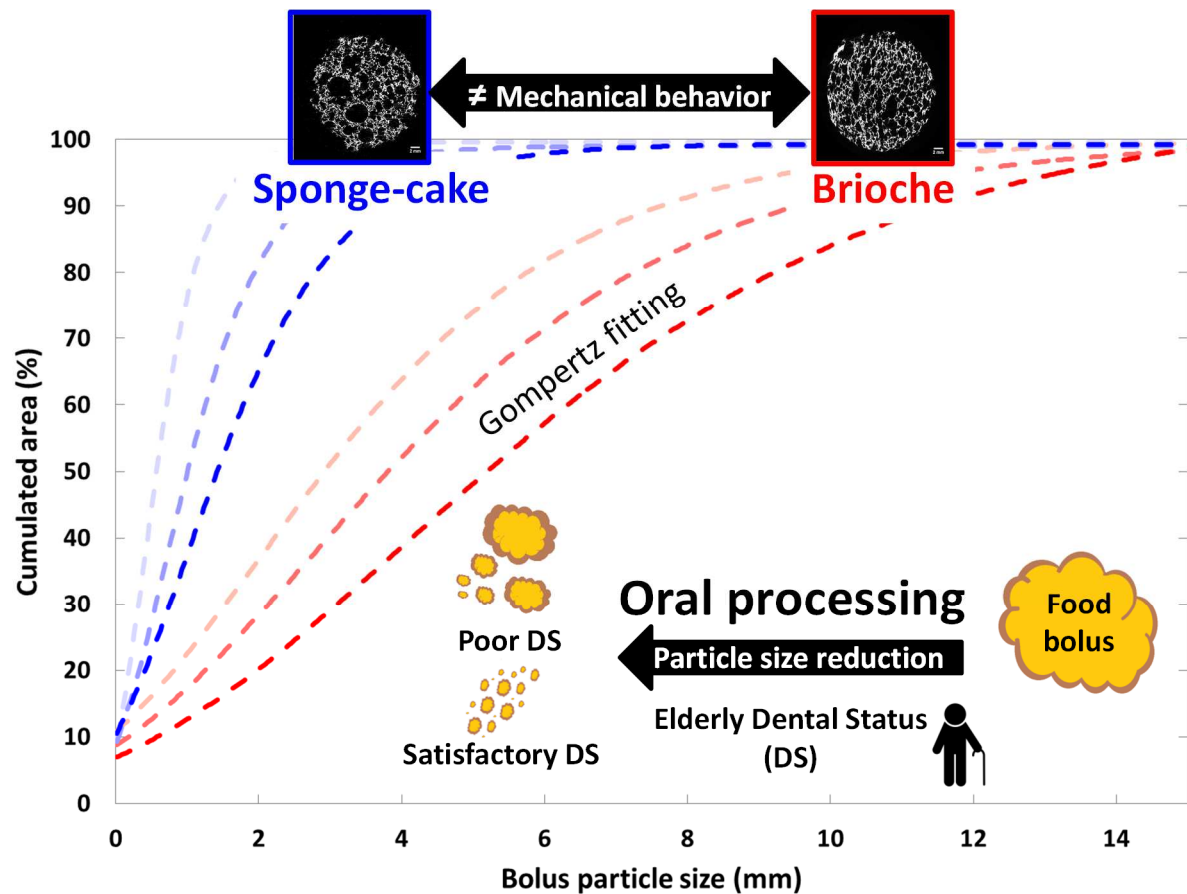


Fig.4 Fragmentation soft cereals

Assad-Bustillos et al.



**Graphical Abstract for “Cereal FOP fragmentation” by Assad-Bustillos et al.:**

The mechanisms of fragmentation of soft cereal foods during chewing are determined by image analysis and by fitting particle size distributions. This approach has allowed us to link food structure and mechanical behavior on one side, with the dental status of elderly on the other side.