Modifying gluten-free bread's structure using different baking conditions: impact on oral processing and texture perception

P. Puerta^{1,a}, R. Garzón^{1,a}, C.M. Rosell¹, S. Fiszman¹, L. Laguna^{1*}, and A. Tárrega¹

¹ Institute of Agrochemistry and Food Technology (IATA-CSIC). Agustín Escardino, 7, Paterna, Valencia, Spain

^a These authors have equally contributed to the work.

* Corresponding author's email: laura.laguna@iata.csic.es

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Highlights

- Gluten-free bread's structure was altered, changing water and fermentation time
- Long-fermented bread had big air cells, bolus was moister, softer and less dense
- High hydration breads were harder, chewier and less cohesive
- Oral processing was shorter for long-fermented breads
- Initial sensation of short-fermented breads was compact; long-fermented aerated.

Abstract

This study evaluated the impact of different gluten-free bread's inner structure on oral processing and texture perception. Four rice-based breads were prepared varying water hydration (H: 85 or 100 g of water/100 g of flour) and fermentation time (F: 30 or 75 minutes). Mechanical properties of breads, bolus properties (after three chews and at swallowing point), subject's chewing and swallowing activities, and perceived sensations using Temporal Dominance of Sensations were measured. Long fermentation breads (H100F75 and H85F75) had bigger air cells, less density, than short fermentation breads (H100F30 and H85F30). High hydration breads were harder, chewier, less cohesive, and resilient. Bolus from H100F75 and H85F75 breads was moister, softer, less dense, and generated smaller particles than the H100F30 and H85F30 bolus, which

was more consistent and adhesive. Before swallowing, H85F30 and H85F75 required more saliva, H100F30 and H85F30 required more time, chews and swallows. At the beginning of the mastication, short fermentation breads were perceived compact, whereas long fermentation, aerated. Independently of the formulation, all were perceived sticky. Thus, by varying breadmaking conditions like fermentation time, the structure of gluten-free breads can be modified to improve its texture sensations, elicited during consumption.

1. Introduction

The rise in coeliac disease diagnosis and its social awareness has expanded the gluten-free products available. For the food industry this represents a technical challenge, as gluten is the main structure-forming protein in baked goods (Espinosa-Ramírez, Garzón, Serna-Saldivar, & Rosell, 2018). In breads, gluten forms a network that provides cohesiveness and helps CO₂ retention, produced during fermentation, creating an open crumb structure (Deora, Deswal, & Mishra, 2014). Gluten absence drastically affects bread characteristics, commonly resulting in low volume, a crumbly texture, pale colour, and poor flavour; thus, less quality than their gluten containing counterparts (Espinosa-Ramírez et al., 2018; Houben, Höchstötter, & Becker, 2012; Matos Segura & Rosell, 2011).

Commercial gluten-free-breads are characterised by an extensive list of ingredients, including acidifiers, emulsifiers, leavening agents, preservatives, and aromas or flavourings (Matos Segura & Rosell, 2011; Puerta et al., 2020). Additionally, because of consumer demand for a clean label breadmaking (Asioli et al., 2017), breadmaking conditions have been investigated as a novel approach to improve gluten-free bread characteristics, (Vallons, Ryan, & Arendt, 2011). For example, fermentation time has shown to increase volume and produce softer texture (Cao et al., 2020), a strategy, that although not tested in gluten-free breads could change and improve the gluten-free bread structure and texture. Another approach could be to change one of the main ingredients such as water. In a previous study, this altered the bread volume and the texture of gluten-free breads (Sahin, Wiertz, & Arendt, 2020).

However, for both approaches (changing conditions or reformulation), studies are mainly based on bread's mechanical properties (Cao et al., 2020; Morreale, Garzón, & Rosell, 2018; Sahin et al., 2020; Schober, Bean, Boyle, & Park, 2008; Ziobro, Witczak, Juszczak, & Korus, 2013) and/or final sensory properties (Armstrong, Luecke, & Bell, 2009; Morais, Cruz, Faria, & Bolini, 2014). But it is still pendent to know how this alter the in-mouth process and the sensations along the different stages of the mastication.

Food oral processing studies have shown how products behave in-mouth and can help us better understand and modulate sensations perceived during consumption of products (Chen, 2014; Jourden, Saint-Eve, et al., 2016). Through food oral processing, the structure of foods is broken

down by teeth and tongue comminution, dissolving components in saliva (Devezeaux de Lavergne, Van de Velde, & Stieger, 2017) until a swallowable bolus is formed. This transition of food to a swallowable bolus, through oral processing, can be characterised at several stages with rheology (hardness, cohesiveness, viscosity, tribology) (Jourdren, Panouillé, et al., 2016; Peyron et al., 2011) or particle size characterisation (Aleixandre, Benavent-Gil, & Rosell, 2019). Particle size characterisation also provides insight about the fragmentation pattern during mastication, by using sieving (Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015; Van Der Bilt & Fontijn-Tekamp, 2004), or image analysis (Aleixandre et al., 2019; Rizo, Peña, Alarcon-Rojo, Fiszman, & Tárrega, 2018; Tournier, Grass, Zope, Salles, & Bertrand, 2012).

Additionally, to study the dynamics of perception during the full duration of the mastication process, different sensory dynamic techniques can be used, such as Time Intensity, Progressive Profiling, Temporal Check-All-That-Apply (TCATA) or Temporal Dominance of Sensations (TDS).

The TDS method allows a subject to select a dominant parameter from a list of attributes, providing the dynamics of sensations that dominate along eating time (Pineau et al., 2009). While in TCATA, each panellist can select and deselect the attributes that apply to the tested sample moment-to-moment (Castura, Antúnez, Giménez, & Ares, 2016). Both dynamic techniques have been used to further understand bolus formation and sensation in ham (Rizo et al., 2019), gels (Devezeaux de Lavergne, van de Velde, et al., 2015), and sausages (Devezeaux de Lavergne, Derks, Ketel, de Wijk, & Stieger, 2015). Furthermore, in breads, the TDS method has been used to link bolus formation and sensations along the mastication (Jourdren, Saint-Eve, et al., 2016; Panouillé, Saint-Eve, Déléris, Le Bleis, & Souchon, 2014).

Previous bread studies have investigated structural breakdown linked to perceived sensations, showing that, by only varying bread structure, the perception of salt changes, as a denser bread crumb is related to a less salty perception (Panouillé et al., 2014; Tournier, Grass, Septier, Bertrand, & Salles, 2014). Moreover, the initial bread structure is critical for gluten-free products, conditioning the textural sensations, the breakdown pattern in oral activity, and the perceived sensations (Puerta et al., 2020). Gluten-free breads with a greater number of smaller air cells in the crumb were less hard and springier than breads with few and bigger air cells. Gluten-free breads (with smaller air cells) were perceived as softer and spongier than breads with few air cells, perceived as hard breads (Puerta et al., 2020). Further, bread is usually consumed combined with other foods such as sauces (for example, gravy), or spreads (such as butter, cheese spread), which can also alter the breakdown pattern and thus, the oral behaviour, bolus properties, and sensory perception (van Eck, Fogliano, Galindo-Cuspinera, Scholten, & Stieger, 2019). Recent studies have shown how the addition of a sauce like mayonnaise facilitates the bolus formation (decreases the number of chewing cycles before swallowing), changing the perceived sensations, in special at the beginning of the oral processing (van Eck et al., 2020, 2019).

This study's hypothesis was that the modification in the structure of gluten-free breads achieved by varying breadmaking conditions can affect the texture of the bread and its breakdown pattern in mouth, eliciting the dynamics of critical sensations during consumption.

Therefore, this study aimed to study how structural changes in gluten-free breads, caused by different baking conditions (level of dough hydration and fermentation time), impact oral processing (bread breakdown and bolus formation) and sensory perception.

2. Materials and methods

2.1. Bread preparation

Gluten-free bread formulation was rice flour (Harinera La Meta, S.A., Lleida, Spain) and corn starch (EPSA Aditivos Alimentarios, Valencia, Spain) in a 70:30 (g/g) blend ratio. The remaining ingredients are expressed proportionally to the blend of flour and starch (g/100 g of flour-starch blend): compressed yeast: 3 (Lessaffre, Lille, France), salt: 1.5, seed oil: 2, hydroxipropylmethylcellulose (HPMC): 2 (Methocel K4M, Dow Chemical, Midland, MI, USA), and sugar: 1. Salt, sugar, and seed oil were purchased from a local market.

To obtain different crumb structures, preliminary tests were conducted, testing the impact of levels of hydration and fermentation times. Hydration is referred to as the amount of water added to the blend of rice flour and corn starch. Hydration levels were used because the recipe was expressed based on flour addition, as is currently used in breadmaking. Breads with two levels of hydration (H85 and H100) were obtained by adding different water amounts that corresponded to 85 and 100 g of water /100 g of the blend of flour and starch. Fermentation times were 30 or 75 min at 35 °C (F30 or F75). Combining both levels of hydration and fermentation times, four breads were obtained: H85F30, H85F75, H100F30, and H100F75.

Solids for obtaining 6 breads were blended in a kneader with a dough hook, then water was added and mixed for 8 min. Then 600 g of dough were moulded (20 × 10 × 10 cm, length, width, height) and fermented in a controlled cabinet (Salva, Guipúzcoa, Spain) at 35 °C for 30 or 75 min. After fermentation, dough was baked at 185 °C for 45 min in a convection oven (Eurofours, Gommegnies, France).

After cooling, breads H100F30, H100F75, H85F30, and H85F75 were placed in sealed plastic bags and stored at -20 °C.

2.2. Bread characterisation: grain crumb appearance, moisture content, crumb density, and texture analysis

Crumb grain appearance, moisture content, and crumb texture were evaluated after cooling for 1 h at room temperature.

2.1.1. Grain crumb appearance using imaging analysis

The bread crumb was studied using digital image analysis as previously reported (Espinosa-Ramírez, Garzón, Serna-Saldivar, & Rosell, 2018). Bread slices were scanned using a flatbed scanner (HP Scanjet 4370, Hewlett–Packard, Palo Alto, CA, USA) in the RGB (Red-Green-Blue) standard format with 1,200 dpi resolution.

Image analysis of bread slices was conducted using Fiji Image J software (Schindelin et al., 2012). For image segmentation and determination of crumb grain characteristics, image contrast was improved and then the predefined algorithm “Otsu” was applied. The crumb grain measurements included: cell density (cell/cm²), mean cell area (mm²), surface porosity (total cell area/ total slice area in percentage), and mean cell shape using the circularity factor (0-perfect circle to 1-perfect rectangle). Two slices of bread obtained in two different batches (six breads per batch) were analysed for each sample.

2.1.2. Moisture content

The moisture content of gluten-free bread samples was determined according to the International Cereal Chemistry method (ICC 110/1, 1994). Moisture content was expressed as g of water/100 g of bread crumb. Four replicates were analysed for each sample.

2.1.3. Crumb density

The crumb density was obtained from the weight and volume of a cylinder of the central part of the crumb (diameter = 3.3 cm; height = 2 cm). Three replicates were analysed for each sample.

2.1.4. Instrumental texture analysis

Crumb slices of 10 x 10 x 2 cm (length x width x thickness) were used for texture evaluation after removing the crust. Slices were double compressed to 50% of their original height using a TA-XT plus Texture Analyser (Stable Microsystems, Godalming, UK) equipped with a 5 kg load cell and a 75 mm diameter aluminium cylindrical probe. Test speed was set to 2.0 mm/s with a trigger force of 0.049 N.

Eight replicates from two different batches (n = 4 x 2) were analysed. Parameters recorded were hardness (maximum force at the first compression), cohesiveness (calculated as the ratio between the areas under the second and the first peak), springiness (calculated as the ratio between the distances at which force is maximum during the second and the first cycle), chewiness (calculated from the multiplication of cohesiveness per hardness), and resilience (calculated by dividing the upstroke area of the first compression by the downstroke area of the first compression).

2.2. Bread bolus characterisation

2.2.1. Samples and participants

Bread samples for bolus characterisation and sensory evaluation comprised a cylinder of the central part of the crumb (diameter = 3.3 cm; height = 2 cm) of the different formulated gluten-free breads (H100F30, H100F75, H85F30, and H85F75), discarding the end of loaves and crust. In all studies, samples were presented monadically following a William's design in a different order across subjects, in sealable plastic cups labelled with three-digit codes. Eleven subjects (six women and five men, aged 22-39 years old, average 30.5) with healthy complete dentition participated in the study. They gave informed consent and received compensation for their participation. Participants could rest between samples and were provided with still mineral water for rinsing their palates in all the experiments.

2.2.2. *Bolus collection procedure*

Subjects were asked to place the entire sample in the mouth, chew it as usual, and spit the bolus out into the plastic cup; either after three chewing cycles for determining particle size characterisation or after full mastication, for determining bolus moisture and mechanical properties.

2.2.2.1. Particle characterisation after three chewing cycles

Each bolus collected was spread out manually on a transparent glass surface (30 × 21 cm). Particles were manually separated and digitised in TIF format at 600 ppi on a scanner (Canon MP270 model K.10339, Canon U.S.A. Inc. Lake Success, NY., USA) using a black background. Images were analysed using Nis-Elements® BR 3.2 software (Nikon, Tokyo, Japan). Sample images were binarised using a histogram-based segmentation process, according to the predefined intensity threshold value. Particle size distribution and number of particles were obtained for each bread and subject. The median particle area (a50), which is the particle area corresponding to 50% of total area occupied, was calculated. The interquartile ratio (a75/a25) between 75 and 25% of the cumulative area occupied by particles was calculated. A cumulative curve of total area occupied by particles of assorted sizes was obtained. Two boli per sample and subject were collected over two separate sessions.

2.2.2.2. Bolus characterisation at swallowing point

Bolus moisture

Bolus moisture was determined in triplicate. Four grams of bolus were weighed, mixed with 8 g of sand, and dried to a constant weight in an oven at 105 °C for > 16 h. The difference in water content (g water / 100 g bolus) between the initial bread and bolus at swallowing point was calculated as an indicator of saliva uptake. Three boli were collected over three separate sessions from each participant.

Bolus mechanical properties

Consistency and adhesiveness of bolus obtained at swallowing point were determined using a TA.XT plus Texture Analyser (Stable Micro System, Godalming, UK), fitted with a TTC Spreadability Rig containing a male cone (90° and 40 mm diameter) that matches a glass containing the female cone fixed in an HDP/90 platform. The bolus was placed into the female cone and carefully levelled. The force when penetrating the sample with the male cone at a constant rate of 2 mm/s until a depth of 28 mm was recorded.

From the force-displacement curves, consistency values corresponded to the maximum peak force during the downstroke (N.s). Adhesiveness value (N.s) corresponded to the area under the curve while rising the probe (negative area). For each subject, three boli of each sample were collected over three different sessions. Six boli were collected over separate sessions from each participant.

2.3. Chewing and swallowing activity

Each subject was instructed to eat the bread cylinder in their usual manner while indicating the start of the test, and each of their swallows. This information was recorded using Compusense Cloud software (Compusense Inc., Guelph, Canada). From the data, the number of swallows, first swallow time, and chewing time were obtained. Participants were also recorded on video to visualise the chewing cycles count.

Three replicates per sample were performed in three separate sessions.

2.4. Sensory evaluation

Texture sensations perceived during consumption of the four bread samples were assessed by 11 subjects using the TDS method. Two previous sessions were held to establish the list of texture attributes. In the first session, each subject generated a list of attributes by comparing the different bread samples. A second session comprised a discussion and sample tasting to achieve, by consensus, a final list of attributes and their definitions. Table 1 shows the list of nine attributes and definitions (Table 1). In addition, each panellist attended one session for familiarisation with the TDS.

In the evaluation sessions, participants introduced the whole sample into the mouth and initiated the test while they pressed the “start” button and started chewing. They were asked to select the dominant sensation at each moment among the list of nine attributes while eating. They could select each attribute as often as they considered and until they stopped perceiving any sensation in the mouth.

The order of the attributes in the list was varied among participants according to a balanced design. Each panellist evaluated the four samples in each session. Six replicates were performed over six separate sessions. The serving size and sample presentation were as described in Section 2.5. The assessments took place in a temperature-controlled room under white light and in

standard sensory booths, designed in accordance with ISO 8589 (ISO, 2007). Data collection from TDS tests and analysis were conducted using Compusense Cloud (Compusense Inc., Guelph, Canada).

Experimental procedure of this work was approved by the Ethical Committee of CSIC (ethics references 018/2019).

2.5. Statistical analysis

For each instrumental parameter of breads, a one-way analysis of variance (ANOVA) was applied. Tukey's test was used to assess significant differences ($\alpha = 0.05$) among samples.

Values of bolus characterisation after three chewing cycles and at swallowing point, as well as chewing and swallowing activity parameters showed a non-normal distribution according to the Shapiro-Wilks test, and showed heteroscedasticity according to Levene's test. Therefore, they were analysed by the non-parametric Friedman's test. Nemenyi's test was used to assess significant differences ($\alpha = 0.05$) among samples.

TDS curves representing the dominance rate for each sensation at various times of the chewing period were obtained for each bread. TDS data were normalised according to individual evaluation time on a scale from 0 to 100% of consumption. Both chance and significance limits were included in the graphs. The chance level represented the dominance rate of an attribute that can be obtained by chance (chance level, $P_0 = 1/9$, as there were 9 attributes), and significance level representing the minimum value significantly higher than the chance level (significant level $= P_s = P_0 + 1.645 \sqrt{[P_0(1 - P_0) / \text{number of replicates}]}$).

XLSTAT statistical software (Version 2010.5.01, Microsoft Excel®, Addinsoft, Paris, France) was used for the statistical analysis.

3. Results

3.1. Gluten-free bread characteristics: mechanical properties, moisture content, size, and crumb grain appearance

Values of instrumental texture profile parameters for the breads are shown in Table 2. Significant variation was found among samples in hardness, cohesiveness, chewiness, and resilience ($P < 0.05$) but no significant for springiness ($P > 0.05$). Hardness values showed that longer fermentation time (F75) provided softer crumbs than short fermentation time (F30). Only in short fermentation breads, hydration influenced hardness values and were higher for higher hydration samples (H100F30). Cohesiveness and resilience (capacity to retain original height) were similar in all breads for the different fermentation times.

Gluten-free bread crumb moisture content was significantly different among samples ($P < 0.05$) according to the initial hydration level (Table 2).

As shown in Fig. 1, breads presented distinct size and crumb structure. Cell density and mean cell area values significantly varied among samples ($P < 0.05$). Main differences were related to the fermentation time. Long fermentation resulted in breads with lower cell density and higher air cell size (H85F75 and H100F75) that corresponded to the slices of greater height (Fig. 1). Accordingly, crumb density decreased with fermentation time, especially for the low hydration breads (Table 2).

3.2. *In vitro* breakdown

Breakdown degree parameter at two contrasting times is presented in Table 2. After 15 seconds of breakdown, no significant differences were observed because of fermentation time or hydration. Conversely, after 30 seconds, breads with low moisture and short fermentation (H85F30) were significantly less ($P < 0.05$) breakable than the other three breads (H85F75, H100F30, H100F75).

3.3. Bolus characterisation

3.3.1. Particle size distribution

The median particle area (a50) and interquartile ratio (a75/a25) after three chews are presented in Table 3. Significant differences ($P < 0.001$) were found among samples for both a50 and a75/a25 ratio parameters. Long fermentation breads (H85F75 and H100F75) generated more particles smaller than those obtained from short fermentation breads (H85F30 and H100F30), indicated by a50 values. Particles obtained from long fermentation breads presented lower a75/a25 ratio values, indicating that these breads fragmented into particles more homogeneous in size than short-fermented breads.

The cumulative curve of the total area occupied by particles (Fig. 3) showed that for long fermentation breads, the area is occupied mostly by smaller particles, while for short-fermented breads, particles with bigger size indicating a heterogeneous pattern of fragmentation were present.

3.3.2. Moisture content of bolus at swallowing point

Table 4 shows the moisture of bolus, at swallowing and saliva uptake, for the four gluten-free breads. Bolus moisture at the swallowing point slightly varied (64.3-66.4 g water/100g bolus) among breads ($P < 0.001$); and significant differences were found between short-fermented bread (H85F30) and long-fermented breads (H85F75 and H100F75).

Saliva uptake significantly varied among bread samples ($P < 0.001$), being higher in low hydration breads (H85F30 and H85F75) than high hydration breads (H100F30 and H100F75). Particularly, the highest significant difference was found between H85F30 and H100F75 (15.5 and 13.2 g water/100 g bolus, respectively).

3.3.3. *Mechanical properties of bolus at swallowing point*

Consistency and adhesiveness values of bolus obtained at swallowing point were significantly different among bread samples ($P < 0.05$, Table 4). Long-fermented breads (H85F75 and H100F75) resulted in a less consistent bolus at the swallowing point than shorter fermented breads (H100F30 and H85F30). This effect was higher and only significant in low hydration breads (H85). Likewise, bolus adhesiveness varied with fermentation, long-fermented breads resulted in less adhesive boli than short-fermented breads, but this effect was only significant for low hydration breads.

3.4. **Chewing and swallowing activity**

The number of chewing cycles, swallows, time to the first swallow, and total chewing time significantly varied among breads ($P < 0.005$, Table 5), because of the fermentation time. Increasing fermentation time resulted in breads (H100F75 and H85F75) that required fewer chews, swallows, time to the first swallow, and chewing time than breads with short fermentation. The effects of fermentation time were more noticeable for low hydration breads.

3.5. **Sensory evaluation**

The TDS curves in Fig. 3 show the fundamental differences among breads occurred at the beginning of the oral processing. Initial sensations described by consumers when eating short-fermented breads were compact and dry, whereas aerated was the first sensation described in long-fermented breads. Immediately after, crumbly sensation appeared as dominant for all breads, but frequency of selection was higher in long than in short fermentation breads. Bolus hard to form and grittiness were the sensations with maximum frequency in the middle of consumption for all breads. Grittiness seemed more frequent in long fermentation breads. At the end of mastication, having a sticky particles sensation was dominant in all samples, lasting longer in short fermentation breads.

Among breads with different hydration level, no differences in the TDS were found.

4. **Discussion**

In this study, breadmaking conditions (fermentation time and hydration level) of gluten-free breads were modified, affecting gluten-free breads' structure. Longer fermentation resulted in gluten-free breads with a more open structure (bigger air cells and less density) with crumbs presenting lower instrumental hardness values. This is explained by a greater gas expansion caused by long fermentation time, which increased the volume of bread (Hong & Kweon, 2020). Because of that expansion, the long-fermented gluten-free loaves were bigger than loaves using short fermentation. Hydration level also affected the structure, especially for short fermentation gluten-free breads, where low level of hydration increased the crumb density, as air cells were

smaller. Morreale et al. (2018) reported strong and moderate correlations between hydration level and instrumental parameters; with strong positive link between water quantity and crumb hardness in gluten-free breads.

Here, structural changes affected the gluten-free bread mechanical properties, and instrumental texture measurements showed that longer fermentation produced gluten-free bread with lower instrumental hardness and less chewiness.

Structural changes of gluten-free breads also affected the in mouth behaviour. During eating, chewing movements first aim to break down food. This breakage pattern was different among the gluten-free breads of this study. After three chews, breads with long fermentation broke easily into more homogeneous and smaller particles; as a lack of gluten in bread results in crumblier structures, because there is no gluten to provide a structural network (Espinosa-Ramírez et al., 2018). In our study, we showed that gluten absence and the processing can change the breakdown pattern, as longer fermentation time produced crumblier gluten-free breads. Furthermore, during eating, saliva is incorporated to foods until a safe and comfortable-to-swallow bolus is formed (Panouillé et al., 2014). At the end of the mastication, samples with lower initial hydration required more saliva incorporation, during mastication, to achieve the critical moisture level to swallow. Bolus consistency and adhesiveness were lower for long fermentation gluten-free bread bolus and higher for short fermentation and low hydration gluten-free bread bolus. Previous authors hypothesised that bolus' mechanical properties depend on the initial water content and absorbing capacity (Jourden, Panouillé, et al., 2016). In this study, the initial hydration level and fermentation time influenced the structure, because bread boli with short fermentation were more consistent and adhesive. The plasticiser role of water contributed to this behaviour, as bread de-structuration has been related to hydration and crumb fragmentation (Le Bleis, Chaunier, Montigaud, & Della Valle, 2016).

Oral activity also varied among the gluten-free breads studied. Short fermentation breads required more time and chews to form a swallowable bolus, because breads were denser, harder, less breakable. This agrees with previous studies that showed dry and hard products required more chewing cycles and time to form a swallowable bolus (Tournier et al., 2012; Engelen, Fontijn-Tekamp, & Van Der Bilt, 2005).

Structural changes also affected the sensations dynamics, different among breads at different times. These differences stand out at the beginning of the mastication, where longer fermented breads were perceived as more aerated and less dry, improving the sensory characteristics of gluten-free breads. As the mastication of the gluten-free breads progressed, all provided similar sensations. Previous studies have shown that at the beginning of the mastication the initial structure is still somewhat intact (Gao, Tay, Koh, & Zhou, 2018), having a higher impact on the sensory perception. In this study, these differences seem to be diluted as the mastication progressed, in contrast from previous studies with different commercial gluten-free breads, where

sensations differed along mastication (Puerta et al., 2020). Comparing sensations of commercial gluten-free breads with structures like regular bread, authors observed perceptual changes at the beginning (soft and spongy vs. dry and crumbly), at the middle (compact vs. sandy), and the end (pasty, sticky vs. sticky) of mastication (Puerta et al., 2020). The difference in perception between the commercial gluten-free breads and the breads of this study can be attributed to the diverse ingredients added in commercial gluten-free breads; for example, the flour; or starches blends; hydrocolloids, and fats. In this study, the recipe was kept constant and only breadmaking conditions were altered, but further investigation might be needed to improve the perceived texture sensations along the entire mastication process, not only at the beginning.

5. Conclusions

Changes in the structure of gluten-free breads were achieved by varying two conditions: water hydration and fermentation time. Both conditions influenced mechanical properties of food boli, but only fermentation time influenced oral behaviour and sensations perceived during mastication.

Oral processing was enlightened by a better understanding of the bread structure and has helped to comprehend the initial bolus formation, related with different sensations as compact or aerated. Furthermore, the dominant sensations were not discriminant, although the bolus had a different consistency before swallowing.

The implication of this study for the gluten-free bread industry is that baking conditions, such as fermentation time, can improve gluten-free bread's sensory characteristics regarding texture, creating more aerated sensations that are usually missed in gluten-free breads. However, further investigation, such as reformulation, might be needed to improve textural sensations along the entire mastication process.

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Table 1

List of texture attributes (and definitions) used in the sensory evaluation of the gluten-free breads with the TDS method.

Attributes	Definition
Compact	The bread is hard to compress when chewing because it is dense
Aerated	The bread is easy to compress, light
Roughness	The bread scratches the internal surfaces of the mouth (palate, tongue, throat)
Crumbly	The bread disaggregates and breaks down in particles easily
Bolus hard to form	Difficulty to aggregate or agglutinate the bread particles in a swallowable bolus, it is not cohesive
Hard to swallow	Bread particles remain retained through the throat
Gritty	Presence of particles in the mouth
Sticky particles	Presence of bread particles adhered to oral mucosa that need to be removed with the tongue
Dry	Bread that resulted difficult to moisturize/hydrated

Table 2

Texture parameters (n=8), crumb moisture content (n=4), crumb density (n=3) and internal crumb structure (n=2) of the gluten-free breads.

	H85F30	H85F75	H100F30	H100F75	F-ratio	P-Value
Texture parameters						
Hardness (N)	40.1 ± 3.3 b	15.2 ± 2.3 a	48.8 ± 6.8 c	16.3 ± 3.6 a	116.6	< 0.001
Springiness	0.85 ± 0.05 a	0.86 ± 0.09 a	0.88 ± 0.07 a	0.91 ± 0.05 a	1.0	0.4
Cohesiveness	0.73 ± 0.03 ab	0.76 ± 0.04 b	0.69 ± 0.03 a	0.73 ± 0.02 ab	4.9	0.01
Chewiness (N)	23.8 ± 3.7 b	9.9 ± 1.9 a	29.8 ± 4.6 c	10.9 ± 2.7 a	61.4	< 0.001
Resilience	0.40 ± 0.02 ab	0.42 ± 0.03 b	0.37 ± 0.02 a	0.40 ± 0.02 ab	5.1	0.01
Crumb moisture (g water/100g bread crumb)	48.8 ± 0 a	49.3 ± 0.2 a	52.3 ± 0.9 b	52.7 ± 0.2 b	110.8	< 0.001
Crumb density	0.54 ± 0.02 d	0.29 ± 0.01 a	0.49 ± 0.01 c	0.41 ± 0.01 b	214.8	< 0.001
Crumb structure						
Cell density (cell/cm ²)	30.0 ± 0 b	15.0 ± 1 a	26.0 ± 1 b	15.0 ± 1 a	108.9	< 0.001
Surface porosity (%)	33.6 ± 0.3 a	37.9 ± 1.5 a	32.8 ± 2.5 a	36.9 ± 2.3 a	2.5	0.20
Mean cell area (mm ²)	1.17 ± 0.17 a	2.47 ± 0.11 b	1.25 ± 0.07 a	2.50 ± 0.47 b	16.3	0.01

Different letters within rows indicate significant differences among breads according to Tukey's test ($\alpha = 0.05$).

H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75 minutes, respectively.

Table 3

Mean and standard deviation of the median particle area (a50) and interquartile ratio (a75/a25) between 75 and 25% of the cumulative area occupied by particles of bolus obtained after three chews of gluten-free breads (n=2).

Bread	a50 (mm ²)	a75/a25
H85F30	53.11 ± 4.66 b	7.41 ± 26.71 b
H85F75	24.78 ± 0.61 a	3.42 ± 8.37 a
H100F30	45.80 ± 4.06 b	6.62 ± 19.08 b
H100F75	27.93 ± 0.86 a	3.40 ± 7.32 a
Friedmans's Q	43.8	39.66
p-Value	<0.001	<0.001

Different letters within columns indicate significant differences among breads according to Nemenyi's test ($\alpha = 0.05$).

H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75 minutes, respectively.

Table 4

Mean and standard deviation of the mechanical properties and moisture content of bolus obtained from gluten-free breads at swallowing point and saliva uptake values deviation (n=6 for mechanical properties and n=3 for bolus moisture and saliva uptake values).

Bread	Bolus consistency (N)	Bolus adhesiveness (N.s)	Bolus moisture (g water / 100g bolus)	Saliva uptake (g water / 100g bolus)
H85F30	10.07 ± 6.29 c	1.62 ± 0.86 c	64.30 ± 0.04 a	15.50 ± 0.04 bc
H85F75	6.85 ± 4.49 a	1.00 ± 1.37 a	65.80 ± 0.04 b	16.50 ± 0.04 c
H100F30	8.80 ± 5.24 bc	1.32 ± 0.67 b	65.50 ± 0.05 ab	13.20 ± 0.04 a
H100F75	7.89 ± 4.79 ab	1.09 ± 0.61 ab	66.40 ± 0.03 b	13.70 ± 0.03 ab
Friedmans's Q	41.16	45.49	19.07	29.62
p-Value	<0.001	<0.001	<0.001	<0.001

Different letters within columns indicate significant differences among breads according to Nemenyi's test ($\alpha = 0.05$).

H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75 minutes, respectively.

Table 5

Mean and standard deviation values of the oral activity parameters (number of chewing cycles, number of swallows, time for first swallow and total chewing time) when consuming gluten-free breads, (n=3)

Bread	Chewing cycles	Swallows	Time first swallow (s)	Total chewing time (s)
H85F30	53.33 ± 17.58 b	5.39 ± 2.70 c	32.25 ± 7.38 b	59.54 ± 12.66 b
H85F75	38.70 ± 13.61 a	4.21 ± 2.13 a	24.04 ± 8.41 a	43.84 ± 12.04 a
H100F30	52.03 ± 16.29 b	5.06 ± 2.67 bc	32.40 ± 9.02 b	57.74 ± 14.24 b
H100F75	42.45 ± 14.23 a	4.39 ± 2.50 ab	27.44 ± 8.85 a	47.43 ± 11.66 a
Friedmans's Q	68.03	29.41	29.41	53.44
p-Value	<0.001	<0.001	<0.001	<0.001

Different letters within columns indicate significant differences among breads according to Nemenyi's test ($\alpha = 0.05$).

H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75 minutes, respectively.

Figure 1. Scanning of the sliced gluten-free breads: a) H85F30, b) H85F75, c) H100F30 and d) H100F75.

Units in the scale are expressed in cm.

H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75 minutes, respectively.

Figure 2. Cumulative curve representing the total area occupied by particles of bolus obtained after three chews of gluten-free breads H85F30 ($\square$), H85F75 ($\circ$), H100F30 ($\blacksquare$) and H100F75 ($\bullet$). H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75 minutes, respectively.

749 **Figure 3.** Dominance curves of attributes compact (—), aerated (—), roughness (—), crumbly (—), bolus hard to form (—), hard to swallow (—)
750), gritty (—), sticky particles (—) and dry (—) obtained by TDS when consuming gluten-free breads: a) H85F30, b) H85F75, c) H100F30 and
751 d) H100F75. Dotted black line indicates the chance level and solid black line, significance level.
752 H85 and H100: addition of 85 or 100g of water/ 100 of the flour and starch blend, respectively. F30 and F75: fermentation time of 30 or 75
753 minutes, respectively.

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