Official title of the study: Oral Processing Behavior of Gluten-free and Gluten-containing Breads

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1.1. Subjects

A total of 20 subjects were recruited for this study (9 male, 11 female, average age: 34 ± 10 years). Participants were students and staff members of the Department of Agricultural, Food, Environmental and Animal Sciences at Udine University, Italy. The inclusion criteria were: age between 18-59 years old, good general oral health (self-reported), normal smell and taste functions (self-reported), absence of mastication and/or swallowing problems, normal body mass index (BMI 18.5-24.9 kg/m^2) (based on self-reported weight and height) and absence of any food allergy or intolerance for gluten. All participants gave written informed consent. The study was approved by the ethical committee of the University of Udine (protocol number: 0002491).

1.2. Bread products

A 2x3 full factorial design was used with 2 white sandwich breads (a regular gluten-containing (GC) and a gluten-free version (GF)) and 3 spread conditions (no spread, butter and mayonnaise). The breads were commercial products kindly provided by Barilla (Parma, Italy) and Nove Alpi (Pistoia, Italy). The composition of each bread is listed in table 1.

Table 1. Ingredients and nutritional information of studied gluten-free and gluten-containing bread available on the package.

Bread	Ingredients	Energy (kcal)	Total fat (g)	Saturated fat (g)	Total carbo hydrates (g)	Sugar (g)	Fibre (g)	Protein (g)	Salt (g)
Gluten-containing	Wheat flour type "0" (70.6%), water, extra virgin olive oil (2.4%), yeast, salt, sugar, malted barley flour	272	3.9	0.6	48.4	7.3	4.5	8.5	1.25
Gluten-free	Water, corn starch, rice flour, high oleic sunflower oil, yeast, dextrose, thickeners: guar-hydroxypropylmethyl cellulose, iodized salt, soy protein, rice protein, psyllium fiber, quinoa flour, corn flour, aroma	247	5.0	0.55	44.5	3.5	6.0	3.0	1.4

Bread samples were served as two stacked cylinders resembling the consumption of a sandwich (diameter: 3.5 cm, approximate total height: 2.4 cm) (Figure 1). Due to differences in bread density, the average weight of two stacked cylinders was 5.1 ± 0.2 and 5.7 ± 0.4 for GC and GF breads, respectively.

Butter (composition in 100 g: Energy: 751 kcal, Total fat: 83 g, saturated fat: 59 g, total carbohydrates: <0.5 g, sugar: <0.5 g, protein: 0.6 g, salt: <0.01 g) and mayonnaise (composition in 100 mL: Energy: 614 kcal, total fat: 68 g, saturated fat: 8.1 g, total carbohydrates: 0.6 g, sugar: <0.5 g, protein: 0.8 g, salt: 1.1 g) were added to breads at approximately 12% w/w. These spreads were selected due to the different impact they were expected to have on assisting bolus formation. Butter, a fat based product (water in oil emulsion), was expected to increase the degree of lubrication while mayonnaise, an oil-in-water emulsion, was expected to increase lubrication and moisture content of the food bolus.

A B

Figure 1. Sample configuration: A. two stacked cylinders of bread without spread. B. two stacked cylinders of bread with spread (either butter or mayonnaise).

1.3. Test Sessions

Participants attended 2 sessions of approximately 30 min each. The first session was designed for video recording and the second session for the evaluation of texture attributes and collection of food bolus. Participants received detailed information about the test procedure but they were not informed that the objective of the study was to compare GF and GC breads. The sessions were carried out in a sensory room at 20 °C and under normal light conditions.

1.3.1. Video recording

A digital camera (Logitech C920 PRO HD webcam, resolution 1080p/30fps) was positioned approximately 50 cm from the participant's face. Participants evaluated a total of 7 samples from which the first was a test sample used to familiarize the participants with the procedure. Samples were served in randomized order. Participants were instructed to place the whole sample in the mouth (e.g. single bite) and to chew naturally until the bolus was ready for swallowing. Participants were asked to maintain the head straight to the camera, and not to block their mouth or face with their hand while eating. Between samples, participants rinsed the mouth with water.

Videos were decoded using the behavioral annotation software ELAN (version 4.9.1; Max Planck Institute for Psycholinguistics). A coding scheme was developed (Forde et al., 2013; Bolhuis et al., 2014) to record the frequency counts of chews and swallows. Measures of total eating duration (s) (i.e. starting from the moment the sample was placed in the mouth until the final swallow) were directly extracted from the videos. Coding was done by two researchers working independently.

1.3.2. Check-all-that-apply (CATA) test and bolus collection

During the second session, participants evaluated again the samples (n = 7): a test sample used for familiarization followed by the 6 evaluated samples in randomized order. Participants were instructed to

place the whole sample in the mouth and to chew naturally. After 5 chewing cycles, participants were asked to check all the attributes that describe the texture sensations perceived in the beginning of mastication. A list with attributes and their definitions were provided to guide the participants (Table 2). Participants could also indicate texture attributes not listed. Participants proceeded with chewing until the swallowing point, where they checked again all the attributes that characterize the texture sensations perceived at the end of mastication. The food bolus was then spat in a container, which was kept close to prevent water evaporation. Between samples, participants rinsed the mouth with water.

Table 2: Texture attributes and definitions used for the CATA test.

Attribute	Definition
Hard	Sensation that describes the resistance to being deformed by teeth during chewing.
Soft	The bread has a tender texture, easy to crush when chewing.
Compact/dense	The bread has a tight or little porous structure
Spongy	The bread has a light and porous structure
Crumbly	The bread is easy to disaggregate in crumbs and breaks down rapidly
Pasty	Sensation that describes the formation of a dough of the bolus
Dry	Sensation that describes the absence of moisture in mouth when chewing and the difficulty to moisturize the bread
Sticky/Adhesive	Sensation that describes the adhesion of the bolus to teeth and oral cavity during chewing
Sandy	Sensation that describes the presence of particles in oral cavity which tend to scratch the tongue and throat

1.4. Moisture and saliva content

After collection, boluses were immediately transferred to aluminum dishes, weighed and dried for 24 h at 72 °C in a vacuum oven. After drying, samples were cooled in a desiccator for 10 min and subsequently weighed. The following equation was used to calculate bolus moisture content (MC) on a wet weight basis:

$$MC\ (\%) = \frac{(w_0 - w_1)}{w_0}\ x\ 100$$

where w_0 is the weight of the sample before drying and w_1 is the weight after drying.

Bolus moisture content on a dry weight basis was calculated using:

$$MCdb = \frac{(w_0 - w_1)}{w_1}$$

The same procedure was followed to determine the moisture content of the 6 bread samples. For that, samples at the same dimensions as served to participants and containing the same proportion of butter or mayonnaise was cut in small pieces weighed and dried for 24 h at 72 °C in a vacuum oven.

Saliva content (SC) per gram dry food was determined by subtracting the moisture content on a dry weight basis of the product from the moisture content on a dry weight basis of the bolus (MCdb).

The rate of saliva incorporation (g/min) was calculated by dividing the saliva content (SC) by eating duration time. These calculations are based on the assumption that the bolus will be fully expectorated.

1.5. Data analysis

Statistical analyses will be performed using XLSTAT (Addinsoft, France). Normality of continuous variables will be checked through the Kolmogorov-Smironov test. Two-way ANOVA tests will be used to check for the effect of type of bread (2 conditions: with/without gluten) and the effect of spread (3 conditions: no spread, butter and mayonnaise) on number of chews, number of swallows, eating duration and bolus properties (saliva content). Interactions between bread and spread will be included in the analysis/model. A post-hoc test will be used for evaluating differences between values. A *p*-value lower than 0.05 will be considered statistically significant, but notable trends will also be reported and discussed. Correlation coefficients (r) will be calculated to explore the correlation between oral processing parameters (number of chews, number of swallows, eating duration), bread texture (hardness) and bolus properties (saliva content).

For the CATA test, frequency of mention for each term will be determined by counting the number of participants that used that term to describe each sample. Cochran's Q test will be carried out for each of the terms to detect differences in participants' perception of the evaluated samples.