Oral and gastrointestinal nutrient bioaccessibility of gluten-free bread is slightly affected by deficient mastication in the elderly

Susana Ribes, Mélany Genot, Annie Vénien, Véronique Santé-Lhoutellier, Marie-Agnès Peyron

Journal Pre-proofs

Oral and gastrointestinal nutrient bioaccessibility of gluten-free bread is slightly affected by deficient mastication in the elderly

Susana Ribes, Mélany Genot, Annie Vénien, Véronique Santé-Lhoutellier, Marie-Agnès Peyron

PII: S0963-9969(23)00068-6
DOI: https://doi.org/10.1016/j.foodres.2023.112523
Reference: FRIN 112523

To appear in: *Food Research International*

Received Date: 8 October 2022
Revised Date: 13 January 2023
Accepted Date: 21 January 2023

Please cite this article as: Ribes, S., Genot, M., Vénien, A., Santé-Lhoutellier, V., Peyron, M-A., Oral and gastrointestinal nutrient bioaccessibility of gluten-free bread is slightly affected by deficient mastication in the elderly, *Food Research International* (2023), doi: https://doi.org/10.1016/j.foodres.2023.112523

Oral and gastrointestinal nutrient bioaccessibility of gluten-free bread is slightly affected by deficient mastication in the elderly

Susana Ribes[1,2]*, Mélany Genot[2], Annie Vénien[2], Véronique Santé-Lhoutellier[2], Marie-Agnès Peyron[3]

[1] *Departamento Tecnología de Alimentos. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain*

[2] *INRAE, QuaPA UR 370, F-63122 Saint Genès Champanelle, France*

[3]*Université Clermont Auvergne, INRAE, UNH, F-63000, Clermont-Ferrand, France*

***Corresponding author*: surillo@upv.es**

Abstract

The main goal of this work was to investigate the impact of impaired mastication on nutrient bioaccessibility of gluten-free bread in the elderly. *In vitro* boluses were produced with the AM^2 masticator by using two types of programming: normal mastication (NM) and deficient mastication (DM). Static *in vitro* gastrointestinal digestion was performed with the digestive physiology conditions of the elderly. Subsequently, the granulometric properties of the *in vitro* boluses produced, their starch and protein digestibility, and lipid peroxidation after *in vitro* oral and gastrointestinal digestion were evaluated. DM boluses presented higher proportions of large particles, resulting in insufficiently fragmented boluses. A delay in oral starch digestion was observed in DM boluses, probably due to the presence of larger particles that limited the bolus-saliva exchanges. Furthermore, DM boluses exhibited a lower degree of protein hydrolysis at the end of gastric digestion, whereas no differences were observed for protein hydrolysis, sugar release, and lipid peroxidation at the end of digestion (intestinal phase). The results of this study show that impaired

mastication somewhat delays the nutrient bioaccessibility of the gluten-free bread tested. Such understanding of the effect of oral decline on the nutrient bioaccessibility of foods is crucial when designing food commodities with enhanced functionalities for the elderly.

Keywords: ageing; gluten-free bread; *in vitro* mastication; starch; protein; *in vitro* digestion

1. Introduction

Increasing life expectancy and decreasing mortality have led to a society in which elderly people constitute an important segment of the worldwide population. In 2019, the number of people over 60 years old was 1 billion and this is estimated to increase to 1.4 billion by 2030 and 2.1 billion by 2050 (World Health Organization, 2022). Ageing is a natural process characterised by different changes that alter the functioning and balance of the human body, oral health being highly relevant since it can affect mastication, food bolus formation, and swallowing (Peyron et al., 2018). These deficiencies are often caused by ageing factors such as dental loss and oral function impairments, including decline in the masticatory muscle mass, consecutive bite force, jaw reflexes, and reduced salivation and xerostomia (Gaszynska et al., 2017; Müller et al., 2007; Steele et al., 2000). In elderly populations, the swallowable boluses produced are often defined by the presence of greater proportions of large particles, higher hardness, and a lower degree of structural breakdown, which could alter the oral release of nutrients and their bioaccessibility (Peyron et al., 2018; Peyron et al., 2021). Consequently, feeding this population properly is one of the most important challenges for achieving wellness in the elderly.

In recent years, commercial gluten-free products have attracted interest in both academia and the food industry due to the increasing number of gluten-related disorders detected (Henggeler et al., 2017; Sapone et al., 2012). These disorders have traditionally been recognised in children and young adults, but are often underdiagnosed in the elderly and an increasing frequency has been reported in the over-60s (Cappello et al., 2016;

Holt, 2003; Rashtak & Murray, 2009). Unfortunately, the only currently available and useful treatment for these people is a strict lifelong gluten-free diet (Ludvigsson & Murray, 2019).

Gluten is formed from glutenin and gliadin in the presence of water during kneading. It is responsible for the gas holding ability of dough during fermentation, its elasticity, resistance to extension, and extensibility (Hu et al., 2021). In baking, the removal of gluten-like proteins increases the relevance of starch in providing structure and texture to gluten-free products (Horstmann et al., 2017). These products are mainly composed of carbohydrates (38.0-58.0%), protein (1.6-8.6%), lipids (2.0-8.0%), and dietary fibre (2.0-10.4%) (Romão et al., 2020). Starch is the primary source of stored energy in plants, including cereals, legumes, tubers, and roots, and it is considered the main source of digestible carbohydrates in the human diet (Gropper & Smith, 2013). In bread and in gluten-free bread, the digestion of starch begins in the oral cavity owing to the action of the salivary α-amylase and continues to early gastric digestion (Freitas et al., 2019; Hoebler et al., 1998; Pentikäinen et al., 2014). Furthermore, pancreatic α-amylase and brush border enzymes conclude starch hydrolysis in the small intestine, where glucose is absorbed (Gropper & Smith, 2013). The digestion of proteins, such as whey protein, egg protein, and soybean protein among others, starts in the stomach and it is completed in the small intestine due to the action of proteases and peptidases (Gropper & Smith, 2013). Lipids are mostly digested in the small intestine by the emulsification of pancreatic lipase and bile (Carey et al., 1983), which is especially important since their peroxidation and production of lipid oxidation products could take place during digestion (Aksoy et al., 2022). Nevertheless, it is important to highlight that gastrointestinal functions also decline with age (Shani-Levi et al., 2017), the reduction of pepsin levels and achlorhydria and lack of gastric acid secretion in the stomach being the most typical age-related gut disorders (Wang et al., 2022). This outcome together with the presence of insufficiently fragmented food

boluses probably impacts on the oral release and bioaccessibility of nutrients (Peyron et al., 2018; Peyron et al., 2021). In this sense, some studies suggest that insufficiently fragmented food boluses, resulting from impaired mastication, alter the initiation of oral starch digestion in pasta products and affect digestive gastric events in the elderly by delaying the kinetics of lipids, protein, and peptide release in meat products (Blanquet-Diot et al., 2021; Peyron et al., 2021). Nonetheless, the effect of a deficient masticatory performance on the oral and gastrointestinal nutrient bioaccessibility of gluten-free bread has not yet been investigated. In this sense, the absence of gluten could increase starch digestibility due to the elimination of the gluten protein network, thus favouring the access of α-amylase to starch granules. Berti et al. (2004) observed higher glycaemic responses when evaluating the starch digestibility of gluten-free products; however, the fact that the oral transformation of food was not considered in the study could explain the contradictory results reported.

Therefore, the purpose of this work was to evaluate the impact of a deficient masticatory performance on the oral and gastrointestinal nutrient bioaccessibility of gluten-free bread in the elderly. To this end, the digestive physiology of the elderly was simulated to determine the impact of impaired mastication on the starch and protein digestibility and lipid peroxidation of gluten-free bread after *in vitro* oral and gastrointestinal digestion. Fourier-transform infrared (FT-IR) microspectroscopy was also employed to evaluate the effects of mastication on oral starch digestion.

2. Materials and Methods

2.1 Materials and reagents

The gluten-free bread (Dr. Schär AG/SPA, Burgstall, Italy) used in this study was bought in a local supermarket (Clermont-Ferrand, France). Table 1 presents the nutritional composition (g/100 g) and formulation of the gluten-free bread tested. The following enzymes were used to study static *in vitro* oral and gastrointestinal digestion: salivary α-amylase from human saliva (ref: A1031-5KU), pepsin from gastric porcine mucosa (ref: P6887-10g), lipase A from *Aspergillus niger* (ref: 534781-10g), pancreatin from porcine pancreas (ref: P7545-100g), and porcine bile extract (ref: B8631-100g). Analytical grade salts (potassium dihydrogen phosphate, potassium chloride, sodium chloride, sodium bicarbonate, ammonium carbonate, magnesium chloride hexahydrate, and calcium chloride dihydrate) were also employed. For analytical measurements, sodium hydroxide, boric acid, hydrochloric acid (37%), sulphuric acid (95–97%), trichloroacetic acid, phosphotungstic acid, L-methionine, DL-dithiothreitol solution 1 M, o-phthaldialdehyde, sodium tetraborate, sodium dodecyl sulphate, butylated hydroxytoluene (BHT), malondialdehyde (MDA), 1,1,3,3-tetraethoxypropane, and 2-thiobarbituric acid were employed. All these reagents were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA).

Enzymatic kits (Maltose/Sucrose/D-glucose K-MASUG and Digestible Starch and Resistant Starch K-DSTRS) were purchased from Megazyme® (Bray, Ireland).

2.2 Masticatory experiments

The AM^2 masticator was used to perform the *in vitro* masticatory experiments. This device was designed and validated to produce *in vitro* food boluses with granulometric characteristics resembling to those noted in *in vivo* food boluses (Peyron et al., 2018; Peyron et al., 2021). AM^2

masticator programming was based on prior *in vivo* trials to achieve the dynamic parameters of the masticatory sequence. This was confirmed by the superimposition of *in vivo* and *in vitro* particle size distribution curves of both gluten-free bread boluses. *In vivo* boluses were produced by 10 young subjects with good oral health, while *in vitro* boluses were prepared with the AM^2 masticator using normal mastication programming (NM) or deficient mastication programming (DM). To this end, 2.5 g of bread, 1.6 mL of tap water, 21 masticatory cycles, and a spring stiffness of 10.38 N/mm were used to programme the AM^2 masticator. The DM boluses were prepared by leaving a 4 mm space between the masticator jaws at closing, thus allowing, particles to escape compression and fragmentation. As previously indicated, tap water was added to the AM^2 masticator chamber with the aim of mimicking mouth coating and avoiding uncontrolled oral digestion by salivary α-amylase during the masticatory sequence and bolus collection; nevertheless, saliva was subsequently employed (see section 2.4). After each masticatory sequence, *in vitro* gluten-free bread boluses were recovered and analysed granulometrically. Finally, the *in vitro* boluses prepared to determine nutrient bioaccessibility after *in vitro* oral and gastrointestinal digestion were kept at - 80 °C until analysed.

2.3 Granulometric assays of *in vivo* and *in vitro* food boluses

Granulometric analyses of the *in vivo* and *in vitro* gluten-free bread boluses were conducted with a mechanical dry sieving shaker for 3 min at a vibratory amplitude of 1.7 mm (Retsch Gmbh, AS 200-digit CA, Düsseldorf, Germany). For that, the recovered gluten-free bread boluses were placed on a nylon cloth of 0.3 mm (Sefar, Switzerland) and rinsed with tap water to spread the particles out, as well as to eliminate saliva in the case of *in vivo* gluten-free bread boluses. A ventilated oven was used to dry the boluses for 30 min at 37 ºC and the dried particles were then

mechanically sieved by employing 10 sieves with apertures of 7.1, 6.3, 4.0, 2.5, 2.0, 1.4, 1.0, 0.8, 0.4, and <0.4 mm (Saulas, France). Subsequently, the particles retained on each sieve were weighed and the results were expressed as the cumulative weight curve of particles passing through each specific sieve. Lastly, the median particle size (d_{50}) from each curve was reported, which was graphically extracted from each individual curve. It is defined as the aperture of a theoretical sieve through which 50% of the gluten-free bread bolus particles' could pass (Peyron et al., 2018). The tests were conducted on each gluten-free bread bolus collected after conducting the *in vivo* and *in vitro* masticatory assays (10 *in vivo* boluses, 10 *in vitro* NM boluses, and 10 *in vitro* DM boluses).

2.4 *In vitro* oral and gastrointestinal digestion

In vitro oral and gastrointestinal digestion of gluten-free bread was performed according to the standardised static INFOGEST protocol (Brodkorb et al., 2019; Minekus et al., 2014), with minor modifications to mimic the digestive physiology of the elderly (Denis et al., 2016; Duval et al., 2020). A shaking incubator chamber (NB-205 L, Kisker Biotech GmbH & Co. KG, Steinfurt, Germany) at 180 rpm and at 37 ºC was used. To mimic the oral phase (pH 7), simulated salivary fluid and α-amylase enzyme (75 U/mL) were mixed with the *in vitro* NM and DM boluses in a ratio 1:1 (v/w) for 30 sec. In the gastric phase, simulated gastric fluid, pepsin (1200 U/mL), and gastric lipase (12 U/mL) were blended with each oral phase (1:1, v/v) for 120 min, after adjusting the pH to 3.54 with HCl (6 M). Upon completion of the gastric phase, 1 mL from each tube was withdrawn and mixed with 1 µL of NaOH (40 M) to block the enzymatic reaction. In the intestinal phase, subsequent to pH adjustment to 7 with NaOH (1 M), simulated intestinal fluid, pancreatin suspension (66.3 U/mL trypsin activity), and bile solution (6.7 mM) were mixed with the gastric

chyme (1:1, v/v) for 120 min. Ten µL of Pefabloc® (500 mM) per mL of digesta was added to inhibit the proteolysis of samples, which were immediately kept at -80 ºC until analysed. Blank samples (without gluten-free bread but with the different simulated fluids, all enzymes, and bile) and undigested samples (gluten-free bread with the different simulated fluids) were also prepared. *In vitro* oral and gastrointestinal digestion tests were performed in triplicate.

2.5 Analysis of starch digestion products

2.5.1 In vitro starch digestibility

The *in vitro* starch digestibility of the different gluten-free bread boluses, which were collected after mimicking *in vitro* oral digestion, was determined according to the manufacturer's instructions (Megazyme® K-DSTRS kit). After *in vitro* oral digestion of NM and DM boluses (section 2.4), α-amylase activity was promptly inhibited by setting the pH to 3 with HCl (6 M). Subsequently, each sample was centrifuged (4000 g, 10 min, 4 °C), the pellets were recovered, and the supernatants were stored at -80 ºC for further analysis. Then, 0.5 g of each pellet was incubated at 37 °C with amyloglucosidase (17 KU/g) and pancreatic α-amylase (40 KU/g) and different aliquots were withdrawn at 20 min, 120 min, and 240 min to determine rapidly digestible starch (RDS), slowly digestible starch (SDS), and total digestible starch (TDS), respectively. The residual starch after 240 min of digestion, described as resistant starch (RS), was also estimated. For this purpose, samples were dissolved in NaOH (1.7 M) and digested by employing amyloglucosidase (3300 U/mL). Finally, the glucose oxidase/peroxidase (GOPOD) reagent was used to estimate the release

of glucose at 510 nm with a Multiskan™ Spectrum Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). Results were expressed in percentage (%) and all measurements were run in triplicate.

2.5.2 FT-IR microspectroscopy analysis

FT-IR microspectroscopy analyses were employed to determine starch-related variations in the boluses at the end of *in vitro* oral digestion. For that, the *in vitro* gluten-free bread boluses collected after simulating *in vitro* oral digestion (pellet; section 2.5.1) were cryofixed in isopentane, which was chilled with liquid nitrogen (-196 °C), and stored at -80 ºC until analysed. Serial sections of 6 µm of the *in vitro* NM and DM boluses, which were cut with a cryomicrotome (Leica CM 1950, Leica Biosystems, Nussloch, Germany), were collected on a barium fluoride window and kept at – 20 ºC until further analysis. A FT-IR microscope (Thermo Scientific, Nicolet iN10) scanning over a wavelength range from 4000 cm^{-1} to 675 cm^{-1}, with a spectral resolution of 4 cm^{-1} and an aperture size of 30 µm x 30 µm, was employed to acquire the IR spectra. Each spectrum was the result of 64 accumulated scans and the cumulative spectra were averaged and subtracted from a background spectrum obtained at the beginning of the scan by compiling 128 scans (Renaud et al., 2022).

2.5.3 Determination of sugar content

Maltose and D-glucose analyses were performed according to the provider (Megazyme® K-MASUG kit), with minor modifications. The supernatants recovered after simulating the *in vitro* oral digestion of both *in vitro* gluten-free bread boluses (section 2.5.1), as well as the centrifuged

aliquots (4000 g, 10 min, 4 °C) obtained at the end of the gastric and intestinal digestion, were utilised to estimate the amounts of sugar released during *in vitro* oral and gastrointestinal digestion. The quantity of nicotinamide-adenine dinucleotide phosphate (NADPH) developed in the presence of the enzyme glucose-6-phosphate dehydrogenase was determined at 340 nm by using a Multiskan™ Spectrum Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). The results were expressed in g/L and determinations were performed in triplicate.

2.6 Analysis of protein digestion products

2.6.1 Determination of nitrogen fractions

Total nitrogen content of *in vitro* gluten-free bread boluses and digesta was determined with a micro-Kjeldahl (VELP Scientifica Srl, Usmate, Italy). In the case of digesta, the trichloroacetic acid-soluble nitrogen fraction was measured accounting for all peptides and free amino acids. This fraction was estimated by mixing 3.3 mL of trichloroacetic acid (48%, w/v) with 10 mL of digesta. The mixture was maintained for 30 min at 20 ºC, centrifuged (4000 g, 20 min, 20 °C), and the supernatant was recovered. Moreover, the phosphotungstic acid-soluble nitrogen fraction was analysed to account for small peptides and free amino acids. It was estimated by mixing 4.9 mL of sulphuric acid (3.95 M) and 2.1 mL of phosphotungstic acid (33.3%, w/v) with 7 mL of digesta. This mixture was kept at 4 °C overnight and then centrifuged (4000 g, 20 min, 20 °C). Aliquots of 5 mL from both centrifuged extracts were determined by using the micro-Kjeldahl. Big peptides, expressed as the difference between all peptides and free amino acid content and small peptides and free amino acid content, were also reported. Additionally, non-digested nitrogen,

which is defined as the difference between total nitrogen content and all peptides and free amino acid content, was recorded. Data were expressed in g/100 g and a nitrogen-to-protein conversion factor of 5.53 was utilised (Tkachuk, 1969). All determinations were done in triplicate.

2.6.2 Determination of the protein degree of hydrolysis

The free amino groups of the digested, undigested, and blank samples were determined by using the OPA (o-phthalaldehyde) method (Nielsen et al., 2001). The free amino groups were quantified with a L-methionine standard curve from 0 mM to 2 mM. Prior to analysis, all samples were centrifuged (4000 g, 10 min, 4 °C) and, afterwards, 50 µL of each sample was mixed with 100 µL of the OPA reagent, which was composed of sodium dodecyl sulphate (20%, w/v), DL-dithiothreitol solution (1 M), OPA solution in ethanol (10 mg/mL), and sodium tetraborate (20 mM). The blend was incubated at 20 °C for 40 min and absorbance was measured at 340 nm by means of a Multiskan™ Spectrum Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). The tests were run in triplicate.

The degree of protein hydrolysis (DH), corresponding to the proportion of cleaved peptide bonds within samples (Halabi et al., 2020), was calculated using Eq. (1).

$$\text{DH}\ (\%) = \frac{[\text{NH}_2\ \text{digested samples}] - [\text{NH}_2\ \text{undigested samples}] - [\text{NH}_2\ \text{blank}]}{[\text{NH}_2\ \text{digested samples}]} \text{ x } 100 \qquad (1)$$

where [NH_2 digested samples] represents the free amino group content in the samples upon completion of the gastric and intestinal phases (mg/L), [NH_2 undigested samples] represents the free amino group content in undigested samples, and [NH_2 blank] represents the free amino group content in the digestion blank.

2.7 Lipid oxidation analysis

The thiobarbituric acid reactive substances (TBARS) assay was used to measure lipid degradation related to oxidation, a marker of oxidative stress, of the gluten-free bread boluses at the end of *in vitro* oral and gastrointestinal digestion (gastric and intestinal phases). In the case of boluses, 1 g of sample was mixed with 10 mL of KCl-BHT buffer (0.15 M-0.1 mM), centrifuged (13000 g, 10 min, 20 ºC), and the supernatant was collected. Afterwards, 200 µL of the supernatant or centrifuged digesta samples (4000 g, 10 min, 4 °C) was mixed with 600 µL of an aqueous solution of 2-thiobarbituric acid (0.07 M), kept at 95 °C for 60 min in a water-bath, and subsequently cooled for 10 min in an ice-bath. Lipid degradation related to oxidation was quantified by using a 1,1,3,3-tetraethoxypropane standard curve from 0 µM to 120 µM. Absorbance was measured at 532 nm with a Multiskan™ Spectrum Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA) and the tests were run in triplicate. The major product stemming from the oxidation chain reaction is the toxic MDA. Thus, results were expressed in µg eq MDA/g (boluses) and µg eq MDA/mL (digesta).

2.8 Statistical analysis

Data were statistically treated by XLSTAT 2020.3.1 software (XLSTAT statistical and data analysis solution, Addinsoft, New York, USA) and presented as mean ± standard deviation (SD). Student's t-test, followed by a Fisher's F-test, was employed to evaluate the differences between samples in starch digestibility, protein digestibility, and lipid peroxidation. A one-way repeated measures ANOVA test, followed by the Tukey-Kramer *post-hoc* test, was performed to evaluate the differences between particle size distribution of *in vivo* and *in vitro* boluses, as well as NM and DM boluses. Statistical significance was considered for $p < 0.05$.

3. Results

3.1 Programming of the AM^2 masticator

The granulometric characteristics of the *in vivo* and *in vitro* gluten-free bread boluses are shown in Figure 1. Non-significant differences ($p > 0.05$) were observed between the particles size distribution curves of the *in vivo* boluses and *in vitro* NM boluses produced with the AM^2 masticator (Figure 1 A), which confirms the correct programming of the device. Additionally, non-significant differences ($p > 0.05$) were observed for the median particle size (d_{50}) values of the *in vivo* boluses (4.34 ± 0.43 mm) and *in vitro* NM boluses (4.32 ± 0.30 mm).

Regarding the deficient mastication programming, significant differences ($p < 0.001$) were noted between the particle size distribution curves of both *in vitro* NM and DM boluses (Figure 1 B). As can be seen, the DM boluses presented greater proportions of large particles and the cumulative weight did not reach 100% owing to the presence of particles larger than the largest sieve aperture. Finally, an increase in the d_{50} values

of DM boluses (5.72 ± 0.30 mm) in comparison with NM boluses (4.32 ± 0.30 mm) was observed, which can be attributed to the formation of poorly fragmented food boluses as a consequence of impaired mastication.

3.2 Analysis of starch digestion products

Table 2 summarises the starch digestibility data of both gluten-free bread boluses analysed after mimicking *in vitro* oral digestion. Concerning the starch digestibility products, non-significant ($p > 0.05$) differences were noted between *in vitro* NM and DM boluses when determined by enzymatic analysis. For instance, the rapidly digestible starch (RDS) content of *in vitro* gluten-free bread boluses ranged from 5.7 ± 0.6% to 6.9 ± 0.6%, the slowly digestible starch (SDS) values was ranged between 3.8 ± 0.4% and 4.2 ± 0.5%, and the total digestible starch (TDS) content ranged from 12.3 ± 1.2% to 12.6 ± 1.5%; whereas the resistant starch (RS) content was lower than 1.5% and the total starch content (TS) was greater than 13.0 %. In contrast, starch-related variations between *in vitro* NM and DM boluses were observed when analysed by FT-IR microspectroscopy. This technique is recognised as a non-invasive and valuable tool for the examination of bread composition and for monitoring structural changes (Kong & Yu, 2007; Ozkoc et al., 2009). Figure 2 A shows the normalised spectra of the *in vitro* gluten-free bread boluses in the carbohydrate region after mimicking *in vitro* oral digestion, as well as the FT-IR images for carbohydrates. The carbohydrate region (1200-900 cm^{-1}) was selected for more detailed analysis of the starch digestibility of gluten-free bread boluses. The FT-IR spectrum of the *in vitro* gluten-free bread boluses presented a strong tensile vibration of C–O in the saccharide structure (1023 cm^{-1}), which was greater in DM boluses (Figure 2 A). Furthermore, Figure 2B presents the FT-IR images for carbohydrates at 1023 cm^{-1}. The colour bar indicates the carbohydrate intensity in which

red represents high relative absorption intensity and blue represents low intensity. As can be seen, the greenness in the IR microspectroscopy images is more marked in DM boluses than in NM boluses after simulating *in vitro* oral digestion. This could be explained by a weaker initiation of oral starch digestion, probably due to the presence of higher particle sizes that could diminish the food-saliva exchanges and amylase penetration.

When interacting with starch, salivary α-amylase uses a multiple attack mechanism resulting in the production of a series of branched oligosaccharides, as well as sugar molecules, including maltose and D-glucose. Table 2 shows the maltose and D-glucose contents present in the liquid phase of both gluten-free bread boluses after mimicking *in vitro* oral and gastrointestinal digestion. Upon completion of *in vitro* oral digestion, non-significant differences ($p > 0.05$) were observed between *in vitro* gluten-free bread boluses regarding the maltose (> 11.0 g/L) and D-glucose (> 7.0 g/L) contents. At the end of the gastric phase, the maltose content was lower than 1.5 g/L whereas the D-glucose content was slightly higher (*ca.* 2.2 g/L). Conversely, the maltose and D-glucose contents increased at the end of the intestinal phase. In this phase, the maltose content was greater than 8.0 g/L, while lower values of D-glucose were detected (> 5.0 g/L). These data may be explained by the pancreatic α-amylase activity, which promotes the amylolytic procedure, thus favouring the hydrolysis of starch into maltose and D-glucose.

3.3 Analysis of protein digestion products

Figure 3 presents the total protein content and the nitrogen fractions of the different samples at the end of *in vitro* oral and gastrointestinal digestion (intestinal phase). Non-significant differences ($p > 0.05$) were noted between samples in total protein content and nitrogen fractions. The total protein content of the *in vitro* gluten-free bread boluses at the end of *in vitro* oral digestion was close to 2.90 g/100 g, whereas the total protein

content of NM and DM samples was close to 0.35 g/100 g upon completion of the intestinal phase (Figure 3 A-B). In addition, all peptides and free amino acid content of the NM and DM samples were higher than 0.01 g/100 g at the end of *in vitro* gastrointestinal digestion. Regarding the big peptides content, the values of both samples were close to 0.12 g/100 g, while the small peptides and free amino acid fraction was 0.02 ± 0.00 g/100 g and 0.03 ± 0.01 g/100 g for NM and DM samples, respectively (Figure 3 D-E). Finally, the non-digested nitrogen content was slightly greater than 0.20 g/100 g, regardless of the sample tested.

The degree of protein hydrolysis (DH, %) of NM and DM samples at the end of each stage of *in vitro* gastrointestinal digestion is shown in Figure 4. In relation to the gastric phase, significant differences ($p < 0.05$) were observed between the NM and DM samples, the DH being lower in the case of DM sample, probably due to its poor fragmentation (Figure 4 A). Higher particle sizes minimise protein surface exposure, thus reducing enzyme accessibility to cleavage sites. Moreover, non-significant differences ($p > 0.05$) were detected at the end of the intestinal phase between samples, with a DH higher than 91% in both cases.

3.4 Lipid oxidation analysis

Figure 5 shows the TBARS results of the different samples after simulating *in vitro* oral and gastrointestinal digestion (gastric and intestinal phases). Non-significant differences ($p > 0.05$) were noted between samples after mimicking oral digestion, with values of 3.3 ± 0.3 µg eq MDA/g and 3.7 ± 0.5 µg eq MDA/g for NM and DM boluses, respectively. Moreover, these values significantly decreased ($p < 0.05$) at the end of the

gastric stage and increased later upon completion of the intestinal phase (> 3.8 µg eq MDA/mL). The latter could be explained by oxidative processes occurring during digestion.

4. Discussion

The well-being of the elderly is a global concern that includes lifestyle and nutritional issues. Unfortunately, older people may experience various changes linked to feeding, chewing, or swallowing owing to anatomical and physiological alterations, which may impact on food intake, digestion, and nutrients assimilation. Food digestion is a complex combination of versatile and multiple-scale physicochemical processes that drive food consumption, structural breakdown, absorption of nutrients, transportation to related organs, and purging of the remaining waste (Sensoy, 2021). Digestion begins in the oral cavity and its objective is to form ready-to-swallow boluses owing to the food's mechanical breakdown and lubrication by mastication and saliva incorporation, respectively (Rémond et al., 2015; Peyron et al., 2018). The major age-related changes linked to mastication are: i) decline in motor activity of tongue and masticatory muscles; ii) lower bite force and mandibular reflexes; iii) decrease in the number of oro-sensory receptors; and iv) decline in saliva secretions (Peyron et al., 2018; Razak et al., 2014). It is important to highlight that swallowable food boluses produced by elderly people are frequently characterised by higher proportions of large particles, which can modify the bioaccessibility of nutrients (Peyron et al., 2018; Peyron et al., 2021). Additionally, ageing is frequently accompanied by modifications in gastrointestinal physiology, such as decreased gastrointestinal motility and reduced levels of gastric acid secretion and digestive enzymes (Mackie et al., 2020; Soenen et al., 2016).

The present work aimed to determine the effect of impaired mastication on the starch and protein digestibility of gluten-free bread in the elderly. To this end, a deficient masticatory performance was mimicked by employing the AM^2 masticator, which was designed to produce fractures in food matrices similar to those observed in *in vivo* food boluses (Peyron & Woda, 2016). After simulating the deficient masticatory performance, *in vitro* DM boluses exhibited higher proportions of large particles in comparison with *in vitro* NM boluses. Poorly fragmented food boluses are commonly observed in older people with impaired dental status and oral functions. In this sense, Assad-Bustillos et al. (2019) reported that elderly people with poor dental status produced significantly fewer degraded boluses than those with satisfactory dental status, when consuming two different soft cereal products. Similarly, Mishellany-Dutour et al. (2007) observed that older people using dentures presented certain difficulties in preparing carrot or groundnut boluses with small enough particles, which finally resulted in poorly fragmented food boluses.

Regarding the starch digestibility of samples, remarkable changes were observed between NM and DM boluses by means of FT-IR microspectroscopy analyses. This technique is recognised as a non-invasive and valuable tool for the examination of bread composition and for monitoring structural changes (Kong & Yu, 2007; Ozkoc et al., 2009). In this sense, a greater vibrational band corresponding to the C-O vibration in the saccharide structure (1023 cm^{-1}) of DM boluses was noted. This could be attributed to a weaker initiation of oral starch digestion as a consequence of high particle sizes, lowering the exchanges between the gluten-free bread boluses tested and saliva (Blanquet-Diot et al., 2021). This outcome was also observed in different studies where the degree of oral starch digestion increased in boluses presenting lower particle sizes (Blanquet-Diot et al., 2021; Bornhorst et al., 2013; Ranawana et al., 2010). It is noteworthy that non-significant differences were detected between samples by enzymatic tests. These could be attributed to the lower sensitivity of such tests compared to FT-IR microspectroscopy analysis given

that standard errors close to 10% are seen with samples containing > 2% w/w of digestible starch or resistant starch, whereas higher errors (*ca.* 50%) are obtained when those contents are < 2% w/w.

Following with the starch digestion products, it is important to highlight that salivary α-amylase is crucial in the starch hydrolysis of bakery products, since it attacks α-1,4 glycosidic bonds leading to a series of branched oligosaccharides and sugar molecules like dextrin, maltose, and glucose (Sharma et al., 2020; Zhang et al., 2022). Moreover, some studies report that starch can be hydrolysed during both the gastric and intestinal phases (Bustos et al., 2017; Freitas et al., 2018; Freitas & Le Feunteun, 2019). For both samples, the maltose and D-glucose contents observed at the end of the gastric phase could be ascribed to the starch hydrolysis occurring as a consequence of salivary α-amylase activity, which continues until early gastric digestion (Freitas & Le Feunteun, 2019). This outcome was also reported by Di Cairano et al. (2022) when determining the starch hydrolysis of gluten-free biscuits. Nevertheless, higher maltose and D-glucose contents were noted at the end of the intestinal phase owing to the action of pancreatic α-amylase, which favours the amylolytic process (Freitas et al., 2018). In this sense, it is well-known that glucose tolerance decreases with age due to impaired insulin secretion and action. Hence, the recommendation of gluten-free products in the elderly population should be carefully studied since these products could impact on the prevalence or development of cardiovascular diseases such as diabetes (Braun et al., 2013; Lau et al., 2015). Labelling foods with the glycaemic index, which is a value assigned to foods based on how quickly and to what extend those foods cause increases in blood glucose levels, could help consumers to select starch-based products based on their potential physiological effects (Romão et al., 2021; Venter et al., 2003). In this vein, there is evidence that food products with low glycaemic index improve

overall blood glucose, reduce body serum lipids, enhance insulin sensitivity, and decrease the risk of diabetes and other cardiovascular diseases (Bohn et al., 2018; Brand-Miller et al., 2009).

In relation to the protein digestibility of samples, the results obtained in this work suggest that poor food fragmentation resulting from a deficient masticatory performance is crucial in the kinetics of protein hydrolysis, as observed during gastric digestion. During mastication, the surface area of intracellular nutrients exposed to digestive fluids increases, augmenting their accessibility to digestive enzymes and improving overall digestion efficiency and gastrointestinal absorption of nutrients (Mandalari et al., 2008; Parada & Aguilera, 2007). Nonetheless, food boluses resulting from a deficient mastication and presenting greater proportions of large particles would hamper the access of enzymes to cleavage sites owing to their lower protein surface exposure, compared to normal masticated boluses (Paz-Yépez et al., 2019). Thus, the gastric breakdown of gluten-free bread boluses is based on the mechanical status in which they reach the stomach (Peyron et al., 2021), altering gastric digestive events by delaying protein hydrolysis. Similar results were observed by Peyron et al. (2021) who elucidated that oral impairments in elderly people significantly lower the amount of meat peptides available in the gastric compartment. Ranawana et al. (2011) also reported that small particles significantly reduce the duration of gastric emptying. Conversely, non-significant differences were observed between NM and DM samples upon completion of the intestinal phase, where hydrolysis of macronutrients is concluded. In this sense, a high degree of protein hydrolysis was obtained, which could be associated with the lower interaction between proteins and starch in gluten-free breads, making them more accessible to enzymes in the intestinal phase (Sahagún et al., 2020).

Lastly, the lipid peroxidation of gluten-free bread boluses during gastrointestinal digestion has been determined. Ageing has been linked with a decline in the ability to maintain homeostasis and triggers mitochondrial dysfunction, which favours oxidative stress and inflammatory processes extending the risk of developing diseases (López-Otín et al., 2013; Pingitore et al., 2015; Van Hecke et al., 2019). Oxidative stress happens when there is an imbalance between reactive species and antioxidant defences, resulting in cellular damage (Miranowicz-Dzierzawska, 2018; Poprac et al., 2017). The elderly population is more vulnerable to oxidative stress due to a reduction in the endogenous antioxidant system (Dani et al., 2021). In this work, lipid peroxidation significantly increased at the end of the intestinal phase probably due to the oxidative process taking place during digestion. This outcome was also observed by Ranawana et al. (2016) while investigating the effect of fortification of white breads with freeze-dried vegetables. Nevertheless, the results reported by these authors are at least 2 times lower than those observed in the present work with the gluten-free bread tested. This suggests once again the importance of being careful when recommending gluten-free breads to elderly people owing to the reduction in their endogenous antioxidant system (Dani et al., 2021), as mentioned above.

5. Conclusions

The evaluation of the oral capabilities of older people and of the physical state of swallowable boluses after mastication is essential in designing foods for specific populations, since it could affect nutrient release and bioaccessibility. The present work shows that a deficient masticatory performance has an impact, albeit slight, on the oral and gastrointestinal nutrient bioaccessibility of the studied gluten-free bread in the elderly. For the gluten-free bread tested, the level of food breakdown occurring during mastication delays oral starch digestion and gastric protein hydrolysis.

In contrast, it does not significantly affect sugar release and protein hydrolysis at the end of digestion. These results broaden knowledge of food oral processing and digestion in elderly people presenting impaired oral abilities, which is a key in formulating novel and adapted products with enhanced functionalities. However, further studies should be conducted on a huge variety of baked gluten-free products prior to recommendation to older people, since they could impact the prevalence or development of several diseases owing to the possibility of increased blood glucose levels and oxidative stress. Additionally, formulation and characteristic of gluten-free bread like texture, volume, or crumb structure should be also taken into consideration due to its relevance in starch and protein digestibility and nutrients bioaccessibility.

Acknowledgements

Authors acknowledge *Association Française des Intolérants Au Gluten (AFDIAG)* for its support.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualisation: S.R., M.G., V.S.-L., and M.A.P.; Methodology: M.G. and A.V.; Formal Analysis: S.R., M.G., V.S.-L., and M.A.P. ; Investigation: S.R., M.G., V.S.-L., and M.A.P.; Writing – Original Draft Preparation: S.R.; Writing – Review & Editing: S.R., M.G., A.V., V.S.-

L., and M.A.P.; Visualisation: S.R., M.G., V.S.-L., and M.A.P.; Supervision: S.R., V.S.-L., and M.A.P.; Project Administration: V.S.-L. and M.A.P.; Funding Acquisition: V.S.-L. and M.A.P.

Conflict of interest

None.

References

Aksoy, A. S., Arici, M., & Yaman, M. (2022). The effect of hardaliye on reducing the formation of malondialdehyde during *in vitro* gastrointestinal digestion of meat products. *Food Bioscience, 47*, 101747. https://doi.org/10.1016/j.fbio.2022.101747

Assad-Bustillos, M., Tournier, C., Feron, G., Guessasma, S., Reguerre, A. L., & Della Valle, G. (2019). Fragmentation of two soft cereal products during oral processing in the elderly: Impact of product properties and oral health status. *Food Hydrocolloids, 91*, 153-165. https://doi.org/10.1016/j.foodhyd.2019.01.009

Berti, C., Riso, P., Monti, L. D., & Porrini, M. (2004). *In vitro* starch digestibility and *in vivo* glucose response of gluten-free foods and their gluten counterparts. *European Journal of Nutrition, 43*(4), 198-204. https://doi.org/10.1007/s00394-004-0459-1

Blanquet-Diot, S., François, O., Denis, S., Hennequin, M., & Peyron, M. A. (2021). Importance of oral phase in *in vitro* starch digestibility related to wholegrain versus refined pastas and mastication impairment. *Food Hydrocolloids, 112*, 106277. https://doi.org/10.1016/j.foodhyd.2020.106277

Brand-Miller, J., Mc-Millan-Price, J., Steinbeck, K., & Caterson, I. (2009). Dietary glycemic index: health implications. *Journal of the American College of Nutrition, 28*, 446S-449S. https://doi.org/10.1080/07315724.2009.10718110

Braun, J., Bopp, M., & Faeh, D. (2013). Blood glucose may be an alternative to cholesterol in CVD risk prediction charts. *Cardiovascular Diabetology, 12*(1), 24. https://doi.org/10.1186/1475-2840-12-24

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Balance, S., et al. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nature Protocols*, 1-24. https://doi.org/10.1038/s41596-018-0119-1

Bohn, T., Carriere, F., Deglaire, A., Egger, L., Freitas, D., Golding, M., et al. (2018). Correlation between *in vitro* and *in vivo* data on food digestion. What can we predict with static *in vitro* digestion models? *Critical Reviews in Food Science and Nutrition, 58*(13), 2239-2261. https://doi.org/10.1080/10408398.2017.1315362.

Bornhorst, G. M., Kostlan, K., & Singh, R. P. (2013). Particle size distribution of brown and white rice during gastric digestion measured by image analysis. *Journal of Food Science, 78*(9), 1383–1391. https://doi.org/10.1111/1750-3841.12228

Bustos, M. C., Vignola, M. B., Pérez, G. T., & Léon, A. E. (2017). *In vitro* digestion kinetics and bioaccessibility of starch in cereal food products. *Journal of Cereal Science, 77*, 243-250. https://doi.org/10.1016/j.jcs.2017.08.018

Carey, M. C., Small, D. M., & Bliss, C. M. (1983). Lipid digestion and absorption. *Annual Review of Physiology, 45*, 651-77. https://doi.org/10.1146/annurev.ph.45.030183.003251

Cappello, M., Morrale, G. C., & Licata, A. (2016). Elderly Onset Celiac Disease: A Narrative Review. *Clinical Medicine Insights: Gastroenterology, 9*, 41–49. https://doi.org/10.4137/CGast.S38454

Dani, C., Dias, K. M., Trevizol, L., Bassôa, L., Fraga, I., Proença, I. C. T., Pochmann, D., & Elsner, V. R. (2021). The impact of red grape juice (*Vitis labrusca*) consumption associated with physical training on oxidative stress, inflammatory and epigenetic modulation in healthy elderly women. *Physical & Behaviour, 229*, 113215. https://doi.org/10.1016/j.physbeh.2020.113215.

Denis, S., Sayd, T., Georges, A., Chambon, C., Chalancon, S., Santé-Lhoutellier, V., Blanquet-& Diot, S. (2016). Digestion of cooked meat proteins is slightly affected by age as assessed using the dynamic gastrointestinal TIM model and mass spectrometry. *Food & Function, 7*, 2682–2691. https://doi.org/10.1039/C6FO00120C

Di Cairano, M., Condelli, N., Cela, N., Sportiello, L., Caruso, M. C., & Galgano, G. (2022). Formulation of gluten-free biscuits with reduced glycaemic index: Focus on *in vitro* glucose release, physical and sensory properties. *LWT, 154*, 112654. https://doi.org/10.1016/j.lwt.2021.112654

Duval, A., Sayd, T., Aubry, L., Ferreira, C., Ferraro, V., & Santé-Lhoutellier, V. (2020). Development of a Statistical Workflow for Screening Protein Extracts Based on Their Nutritional Composition and Digestibility: Application to Elderly. *Foods, 9,* 1499. https://doi.org/10.3390/foods9101499

Freitas, D., Le Feunteun, S., Panouillé, M., & Souchon, I. (2018). The important role of salivary α-amylase in the gastric digestion of wheat bread starch. *Food & Function, 9*, 200-208. https://doi.org/10.1039/c7fo01484h

Freitas, D., & Le Feunteun, S. (2019). Oro-gastro-intestinal digestion of starch in white bread, wheat-based and gluten-free pasta: Unveiling the contribution of human salivary α-amylase. *Food Chemistry, 274*, 566-573. https://doi.org/10.1016/j.foodchem.2018.09.025

Gaszynska, E., Kopacz, K., Fronczek-Wojciechowska, M., Padula, G., & Szatko, F. (2017). Electromyographic activity of masticatory muscles in elderly women – a pilot study. *Clinical Intervention in Aging, 12*, 111-116. https://doi.org/10.2147/CIA.S118338

Gropper, S. S., & Smith, J. L. (2013). Advanced nutrition and human metabolism. Cengage Learning, United States of America, 6th edn.

Halabi, A., Croguennec, T., Bouhallab, S., Dupont, D., & Deglaire, A. (2020). Modification of protein structures by altering the whey protein profile and heat treatment affects *in vitro* static digestion of model infant milk formulas. *Food & Function, 11*, 6933. https://doi.org/10.1039/D0FO01362E

Henggeler, J. C., Veríssimo, M., & Ramos, F. (2017). Non-coeliac gluten sensitivity: A review of the literature. *Trends in Food Science and Technology, 66*, 84–92. https://doi.org/10.1016/j.tifs.2017.05.018.

Hoebler, C., Karinthi, A., Devaux, M.-F., Guillon, F., Gallant, D. J. G., Bouchet, B., et al. (1998). Physical and chemical transformations of cereal food during oral digestion in human subjects. *British Journal of Nutrition, 80*(5), 429. https://doi.org/10.1017/ S0007114598001494

Holt, P. (2003). Gastrointestinal diseases in the elderly. *Current Opinion in Clinical Nutrition and Metabolic Care, 6*(1), 41-48. https://doi.org/10.1097/00075197-200301000-00007

Horstmann, S. W., Lynch, K. M., & Arendt, E. K. (2017). Starch characteristics linked to gluten-free products. *Foods, 6*(4), 29. https://doi.org/10.3390/foods6040029

Hu, X., Cheng, L., Hong, Y., Li, Z., Li, C., & Gu, Z. (2021). An extensive review: How starch and gluten impact dough machinability and resultant bread qualities. *Critical Reviews in Food Science and Nutrition, 23*, 1–12. https://doi.org/10.1080/10408398.2021.1969535

Kong, J., & Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochimica et Biophysica Sinica, 39*, 549-559. https://doi.org/10.1111/j.1745-7270.2007.00320.x

Lau, E., Soong, Y. Y., Zhou, W., & Henry, J. (2015). Can bread processing conditions alter glycaemic response? *Food Chemistry, 173*, 250-256. https://doi.org/10.1016/j.foodchem.2014.10.040

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell, 153*(6), 1194-1217. https://doi.org/10.1016/j.cell.2013.05.039

Ludvigsson, J. F., & Murray, J. A. (2019). Epidemiology of celiac disease. *Gastroenterology Clinics, 48*(1), 1-18. https://doi.org/10.1016/j.gtc.2018.09.004

Mackie, A., Mulet-Cabero, A. I., & Torcello-Gómez, A. (2020). Simulating human digestion: developing our knowledge to create healthier and more sustainable foods. *Food & Function, 11*(11), 9397-9431. https://doi.org/10.1039/D0FO01981J

Mandalari, G., Faulks, R. M., Rich, G. T., Lo Turco, V., Picout, D. R., Lo Curto, R. B., et al. (2008). Release of protein, lipid, and vitamin E from almond seeds during digestion. *Journal of Agricultural and Food Chemistry, 56*(9), 3409-3416. https://doi.org/10.1021/jf073393v

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static *in vitro* digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113-1124. https://doi.org/10.1039/C3FO60702J

Miranowicz-Dzierzawska, K. (2018). A comparative study of using free radical generators in the testing of chosen oxidative stress parameters in the different types of cells. *Toxicology and Industrial Health, 34*(4), 237-252. https://doi.org/10.1177/0748233717752090

Mishellany-Dutour, A., Renaud, J., Peyron, M. A., Rimek, F., & Woda, A. (2007). Is the goal of mastication reached in young dentates, aged dentates and aged denture wearers? *The British Journal of Nutrition, 99*, 121-128. https://doi.org/10.1017/S0007114507795284

Müller, F., Naharro, M., & Carlsson, G. E. (2007). What are the prevalence and incidence of tooth loss in the adult and elderly population in Europe? *Clinical oral implants research, 3*, 2-14. https://doi.org/10.1111/j.1600-0501.2007.01459.x

Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved Method for Determining Food Protein Degree of Hydrolysis. *Journal of Food Science, 66*(5), 642-646. https://doi.org/10.1111/j.1365-2621.2001.tb04614.x

Ozkoc, S. O., Samnu, G., Sahin, S., & Turabi, E. (2009). Investigation of physicochemical properties of breads baked in microwave and infrared-microwave combination ovens during storage. *European Food Research and Technology, 228*, 883-893. https://doi.org/10.1007/s00217-008-1001-0

Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science, 72*(2), 21-32. https://doi.org/10.1111/j.1750-3841.2007.00274.x

Paz-Yépez, C., Peinado, I., Heredia, A., & Andrés, A. (2019). Influence of particle size and intestinal conditions on *in vitro* lipid and protein digestibility of walnuts and peanuts. *Food Research International, 119*, 951–959. https://doi.org/10.1016/j.foodres.2018.11.014

Pentikäinen, S., Sozer, N., Närväinen, J., Ylätalo, S., Teppola, P., Jurvelin, J., et al. (2014). Effects of wheat and rye bread structure on mastication process and bolus properties. *Food Research International, 66*, 356–364. https://doi.org/10.1016/j.foodres.2014.09.034

Peyron, M. A., Santé-Lhoutellier, V., François, O., & Hennequin, M. (2018). Oral declines and mastication deficiencies cause alteration of food bolus properties. *Food & Function, 9*, 1112-1122. https://doi.org/10.1039/C7FO01628J

Peyron, M. A., Sayd, T., Sicard, J., Mirade, P.-S., Pinguet, J., Chambon, C., & Santé-Lhoutellier, V. (2021). Deciphering the protein digestion of meat products for the elderly by *in vitro* food oral processing and gastric dynamic digestion, peptidome analysis and modelling. *Food & Function, 12*, 7283-7297. https://doi.org/10.1039/D1FO00969A

Peyron, M. A., & Woda, A. (2016). An update about artificial mastication. *Current Opinion in Food Science, 9*, 21-28. https://doi.org/10.1016/j.cofs.2016.03.006

Pingitore, A., Lima, G. P. P., Mastorci, F., Quinones, A., Lervasi, G., & Vassalle, C. (2015). Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutrition, 31*(7–8), 916-922. https://doi.org/10.1016/j.nut.2015.02.005

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C. J., & Valko, M. (2017). Targeting free radicals in oxidative stress-related human diseases. *Trends in Pharmacological Sciences, 38*(7), 592-607. https://doi.org/10.1016/j.tips.2017.04.005

Ranawana, V., Monro, J. A., Mishra, S., & Henry, C. J. K. (2010). Degree of particle size breakdown during mastication may be a possible cause of interindividual glycemic variability. *Nutrition Research, 30*(4), 246–254. https://doi.org/10.1016/jnutres.2010.02.004

Ranawana, V., Clegg, M. E., Shafat, A., & Henry, C. J. (2011). Postmastication digestion factors influence glycemic variability in humans. *Nutrition Research, 31*, 452–459. https://doi.org/10.1016/j.nutres.2011.05.006

Ranawana, V., Raikos, V., Campbell, F., Bestwich, C., Nicol, P., Milne, L., & Duthie, G. (2016). Breads Fortified with Freeze-Dried Vegetables: Quality and Nutritional Attributes. Part 1: Breads Containing Oil as an Ingredient. *Foods, 5*(1), 19. https://doi.org/10.3390/foods5010019

Rashtak, S., & Murray, J. A. (2009). Celiac disease in the elderly. *Gastroenterology Clinics of North America, 38*(3), 433-446. https://doi.org/10.1016/j.gtc.2009.06.005

Razak, P. A., Richard, K. M., Thankachan, R. P., Hafiz, K. A., Kumar, K. N., & Sameer, K. M. (2014). Geriatric oral health: a review article. *Journal of International Oral Health, 6*,110–116.

Rémond, D., Shahar, D. R., Gille, D., Pinto, P., Kachal, J., Peyron, M. A., et al. (2015). Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. *Oncotarget, 6*(16), 13858-13898. https://doi.org/10.18632/oncotarget.4030

Renaud, C., de Lamballerie, M., Guyon, C., Astruc, T., Venien, A., & Pottier, L. (2022). Effects of high-pressure treatment on the muscle structure of salmon (*Salmo salar*). *Food Chemistry, 367*, 130721. https://doi.org/10.1016/j.foodchem.2021.130721

Romão, B., Bothlho, R. B. A., Alencar, E. A., da Silva, V. S. N., Pacheco, M. T. B, & Zandonadi, R. P. Z. (2020). Chemical Composition and Glycemic Index of Gluten-Free Bread Commercialized in Brazil. *Nutrients, 12*(8), 2234. https://doi.org/10.3390/nu12082234

Romão, B., Falcomer, A. L., Palos, G., Cavalcante, S., Assunção Botelho, R. B., Yoshio Nakano, E., & Raposo, A. (2021). Glycemic Index of Gluten-Free Bread and Their Main Ingredients: A Systematic Review and Meta-Analysis. *Foods, 10*(3), 506. https://doi.org/10.3390/foods10030506

Sahagún, M., Benavent-Gil, Y., Rosell, C. M., & Gómez, M. (2020). Modulation of *in vitro* digestibility and physical characteristics of protein enriched gluten free breads by defining hydration. *LWT, 117*, 108642. https://doi.org/10.1016/j.lwt.2019.108642

Sapone, A., Bai, J. C., Ciacci, C., Dolinsek, J., Green, P. H. R., Hadjivassiliou, et al. (2012). Spectrum of gluten-related disorders: Consensus on new nomenclature and classification. *BMC Medicine, 10*. https://doi.org/10.1186/1741-7015-10-13

Sensoy, I. (2021). A review on the food digestion in the digestive tract and the used *in vitro* models. *Current Research in Food Science, 4*, 308-319. https://doi.org/10.1016/j.crfs.2021.04.004

Shani-Levi, C., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet-Diot, S., et al. (2017). Extending *in vitro* digestion models to specific human populations: Perspectives, practical tools and bio-relevant information. *Trends in Food Science & Technology, 60*, 52-63. https://doi.org/10.1016/j.tifs.2016.10.017

Soenen, S., Rayner, C. K., Jones, K. L., & Horowitz, M. (2016). The ageing gastrointestinal tract. *Current Opinion in Clinical Nutrition and Metabolic Care, 19*(1), 12-18. https://doi.org/10.1097/MCO.0000000000000238

Steele, J. G., Treasure, E., Pitts, N. B., Morris, J., & Bradnock, G. (2000). Total tooth loss in the United Kingdom in 1998 and implications for the future. *British Dental Journal, 189*, 598-603. https://doi.org/doi: 10.1038/sj.bdj.4800840

Sharma, M., Pico, J., Martinez, M. M., & Duizer, L. (2020). The dynamics of starch hydrolysis and thickness perception during oral processing. *Food Research International, 134*, 109275. https://doi.org/10.1016/j.foodres.2020.109275

Tkachuk, R. (1969). Nitrogen-to-Protein Conversion Factors for Cereals and Oilseed Meals. *Cereal Chemistry, 46*, 419-424.

Van Hecke, T., Goethals, S., Vossen, E., & De Semet, S. (2019). Long-Chain n-3 PUFA Content and n-6/n-3 PUFA Ratio in Mammal, Poultry, and Fish Muscles Largely Explain Differential Protein and Lipid Oxidation Profiles Following *In Vitro* Gastrointestinal Digestion. *Molecular Nutrition & Food Research, 63*(22), 1900104. https://doi.org/10.1002/mnfr.201900404

Venter, C. S., Slabber, M., & Vorster, H. H. (2003). Labelling of foods for glycaemic index — advantages and problems. *South African Journal of Clinical Nutrition*, *16*(4), 118-126.

Wang, C., Zhao, F., Bai, Y., Li, C., Xu, X., Kristiansen, K., & Zhou, G. (2022). Effect of gastrointestinal alterations mimicking elderly conditions on *in vitro* digestion of meat and soy proteins. *Food Chemistry, 383*, 132465. https://doi.org/10.1016/j.foodchem.2022.132465

World Health Organization. (2022). Ageing. https://www.who.int/health-topics/ageing#tab=tab_1 Accessed July 2022.

Zhang, Y., Chen, Y., & Chen, J. (2022). The starch hydrolysis and aroma retention caused by salivary a-amylase during oral processing of food. *Current Opinion in Food Science, 43,* 237-245. https://doi.org/10.1016/j.cofs.2021.11.014

Figure captions

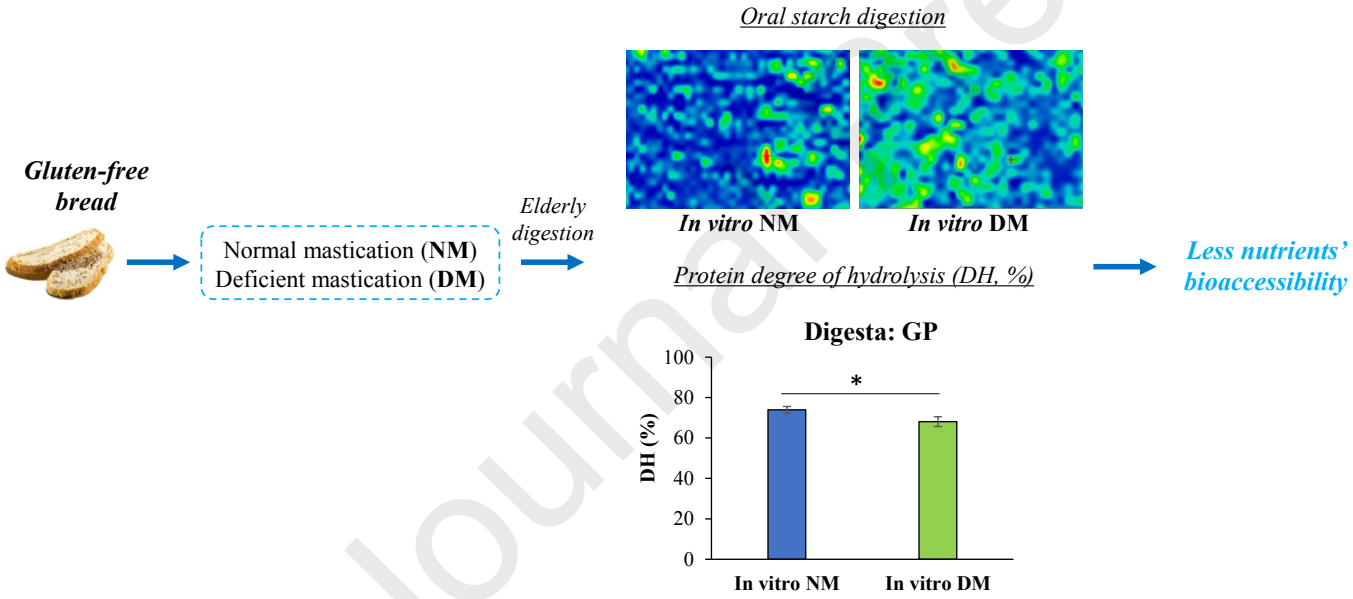
Figure 1. Granulometric properties of gluten-free bread boluses: (A) superimposed particle size distribution curves of *in vivo* and *in vitro* NM boluses that validate the programming of the AM^2 masticator, and particle size distribution curves of *in vitro* DM boluses. Curves express the mean cumulative percentages of particle weight passing through each sieve; (B) median particle size values expressed as d_{50} (mm). The d_{50} values were graphically extracted from each individual curve. Mean values (n=10) ± SD. NM: normal mastication; DM: deficient mastication. ***: $p < 0.001$.

Figure 2. FT-IR microspectroscopy analysis of starch: (A) example of normalised spectra of NM and DM *in vitro* boluses in the carbohydrate region after simulating *in vitro* oral digestion and (B) FT-IR images for carbohydrate at 1023 cm^{-1}. The colour bar shows the intensity of carbohydrates in which red represents high relative absorption intensity and blue represents low intensity. OP: oral phase; NM: normal mastication; DM: deficient mastication.

Figure 3. (A-B) Total protein content (g/100 g) of *in vitro* gluten-free bread boluses and intestinal digesta. (C-F) All peptides and free amino acids (g/100 g), big-peptides (g/100 g), small peptides and free amino acids (g/100 g), and non-digested nitrogen contents (g/100 g) of NM and DM samples at the end of the intestinal phase of *in vitro* digestion. OP: oral phase; IP: intestinal phase; FAA: free amino acids; NM: normal mastication; DM: deficient mastication. Mean values (n=3) ± SD. *p-values* > 0.05 in all cases.

Figure 4. Degree of protein hydrolysis (DH, %) of the samples at the end of the gastric (A) and intestinal (B) phases of *in vitro* digestion. GP: gastric phase; IP: intestinal phase; NM: normal mastication; DM: deficient mastication. Mean values (n=3) ± SD. **: $p < 0.05$*.

Figure 5. TBARS (µg eq MDA/g and µg eq MDA/mL) measurements of *in vitro* gluten-free bread boluses (A) and gastric and intestinal digesta (C and B). OP: oral phase; GP: gastric phase; IP: intestinal phase; NM: normal mastication; DM: deficient mastication. Mean values (n=3) ± SD. *p-values > 0.05* in all cases.



Graphical abstract

- FT-IR tests revealed *in situ* lower oral starch digestion in impaired masticated boluses.
- Gastric protein digestibility was lower in the poorly masticated gluten-free bread tested.
- Deficient mastication did not alter protein hydrolysis and sugar release at the end of digestion.
- Deficient mastication did not impact on lipid peroxidation at the end of digestion.

Conceptualisation: S.R., M.G., V.S.-L., and M.A.P.; Methodology: M.G. and A.V.; Formal Analysis: S.R., M.G., V.S.-L., and M.A.P. ; Investigation: S.R., M.G., V.S.-L., and M.A.P.; Writing – Original Draft Preparation: S.R.; Writing – Review & Editing: S.R., M.G., A.V., V.S.-L., and M.A.P.; Visualisation: S.R., M.G., V.S.-L., and M.A.P.; Supervision: S.R., V.S.-L., and M.A.P.; Project Administration: V.S.-L. and M.A.P.; Funding Acquisition: V.S.-L. and M.A.P.

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

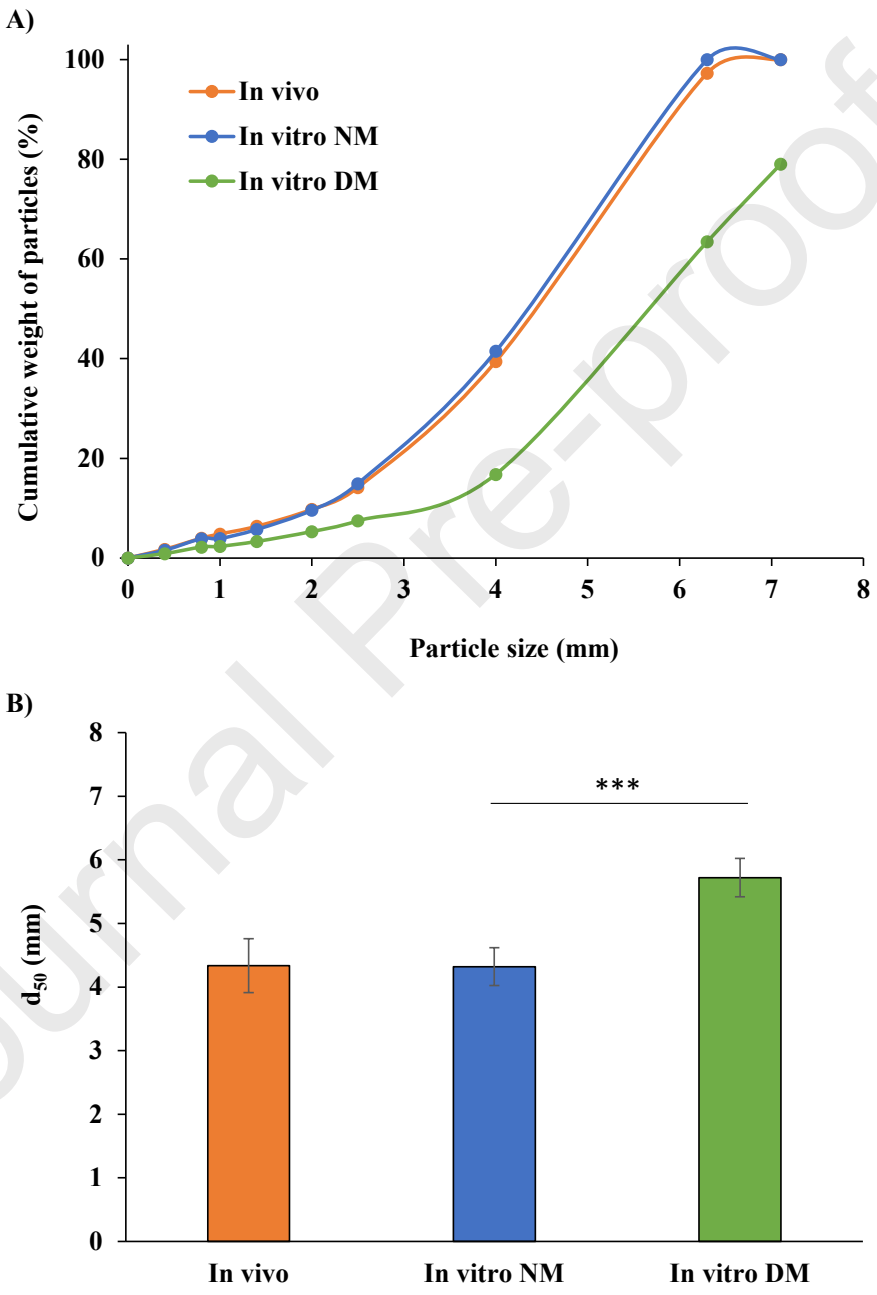
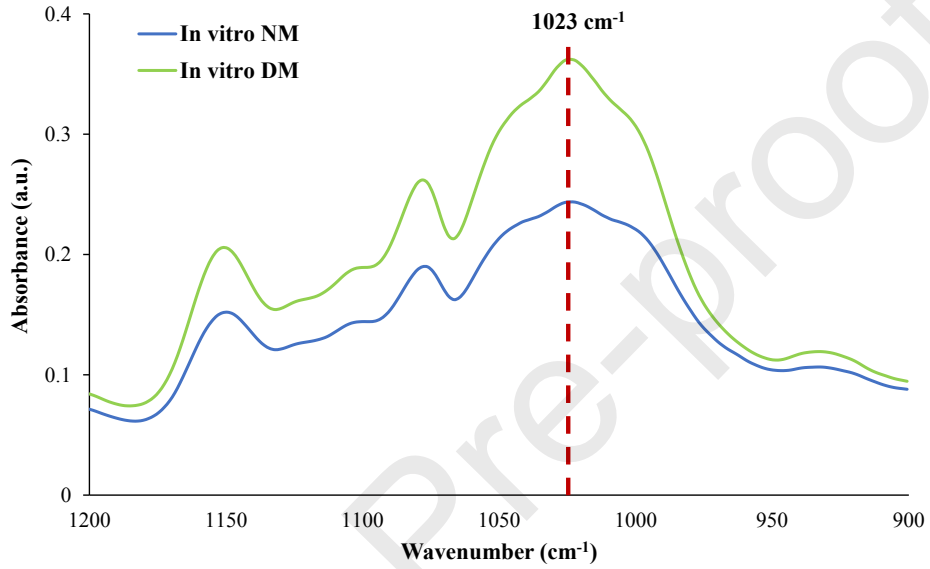


Figure 1

A)



B)

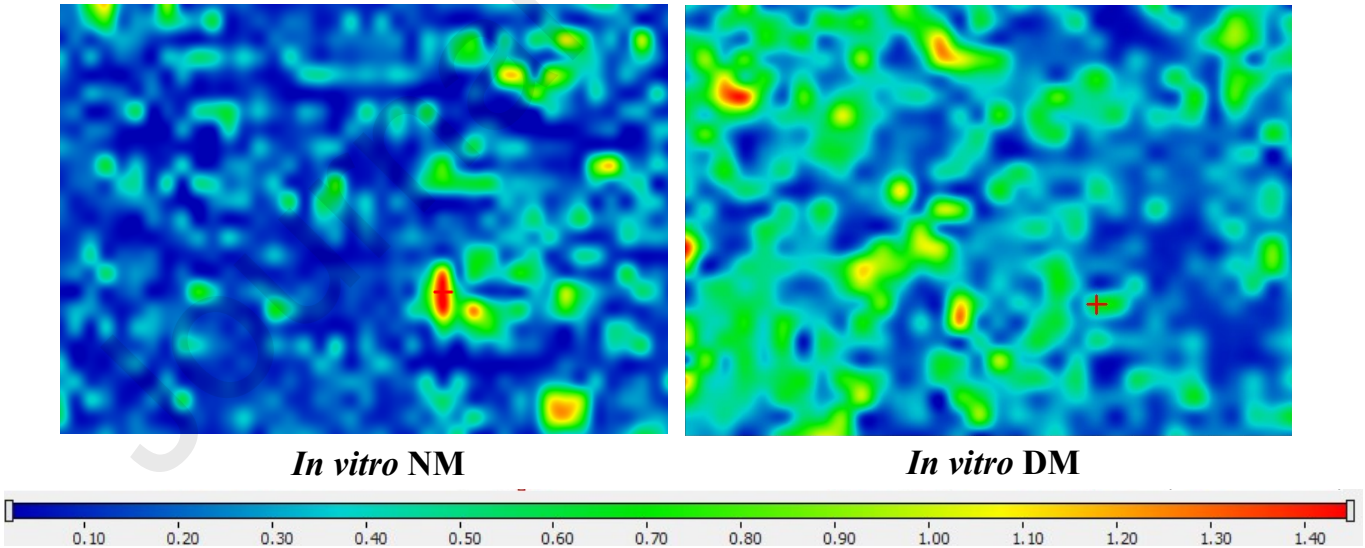
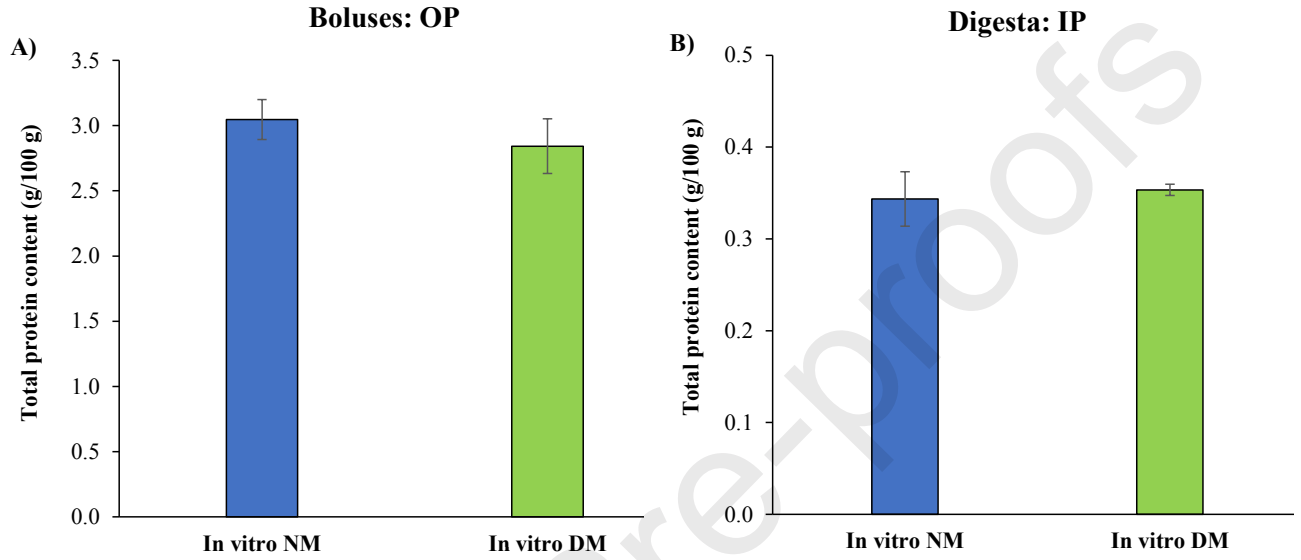
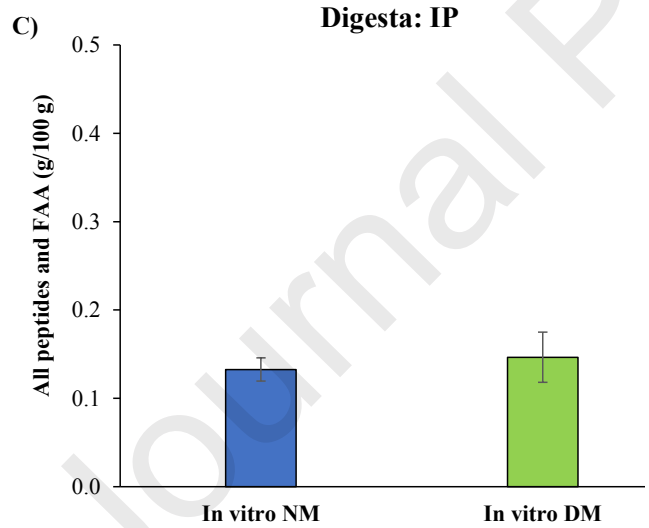
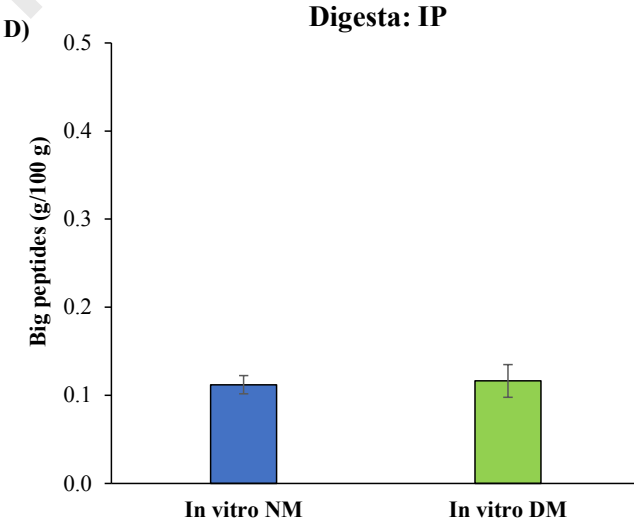
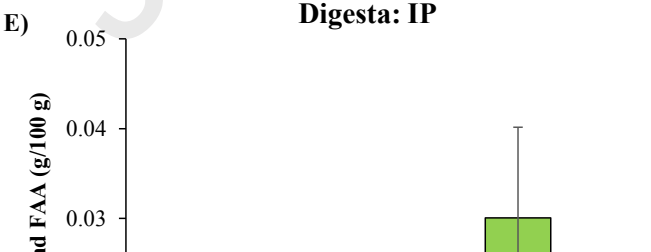


Figure 2









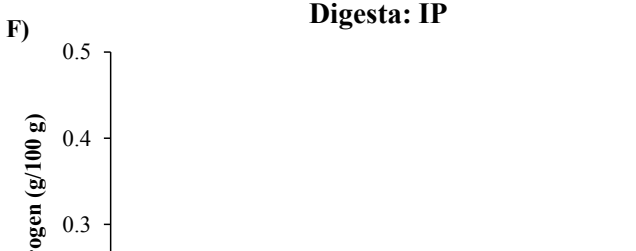


Figure 3

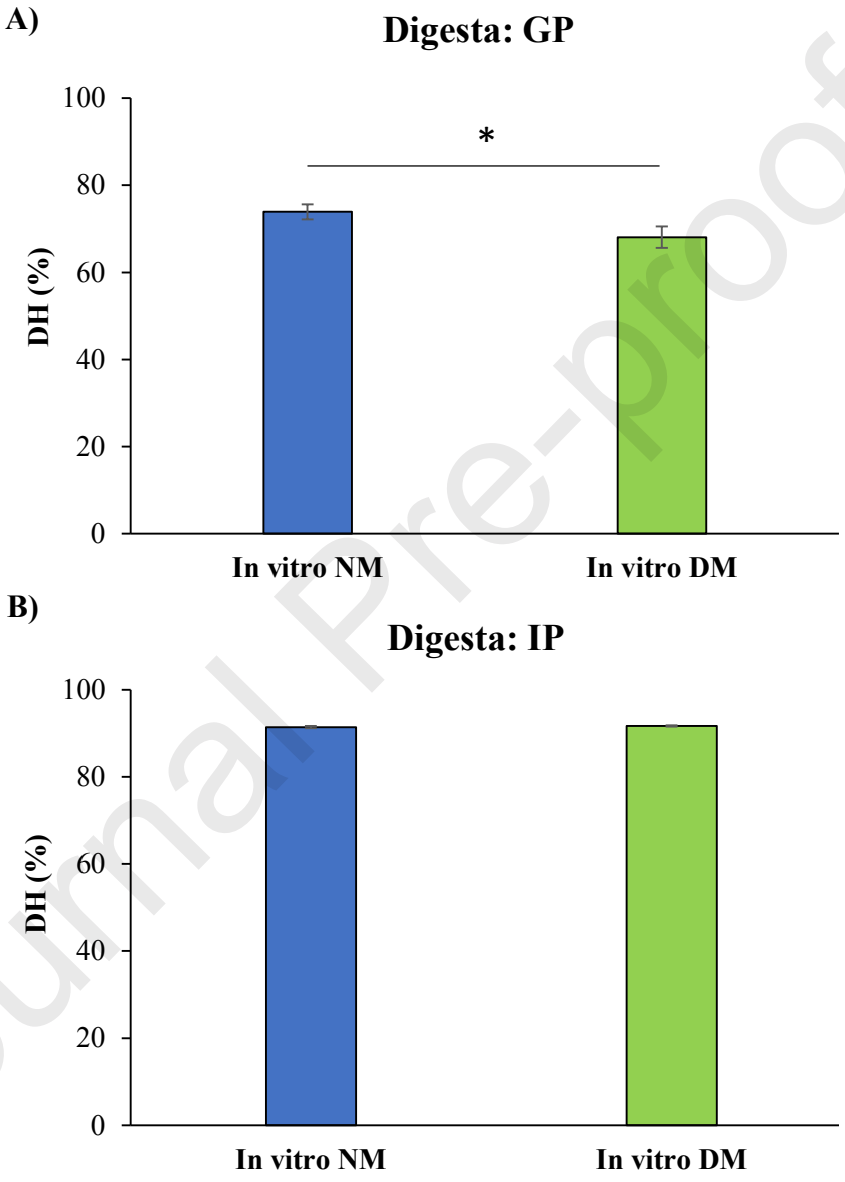


Figure 4

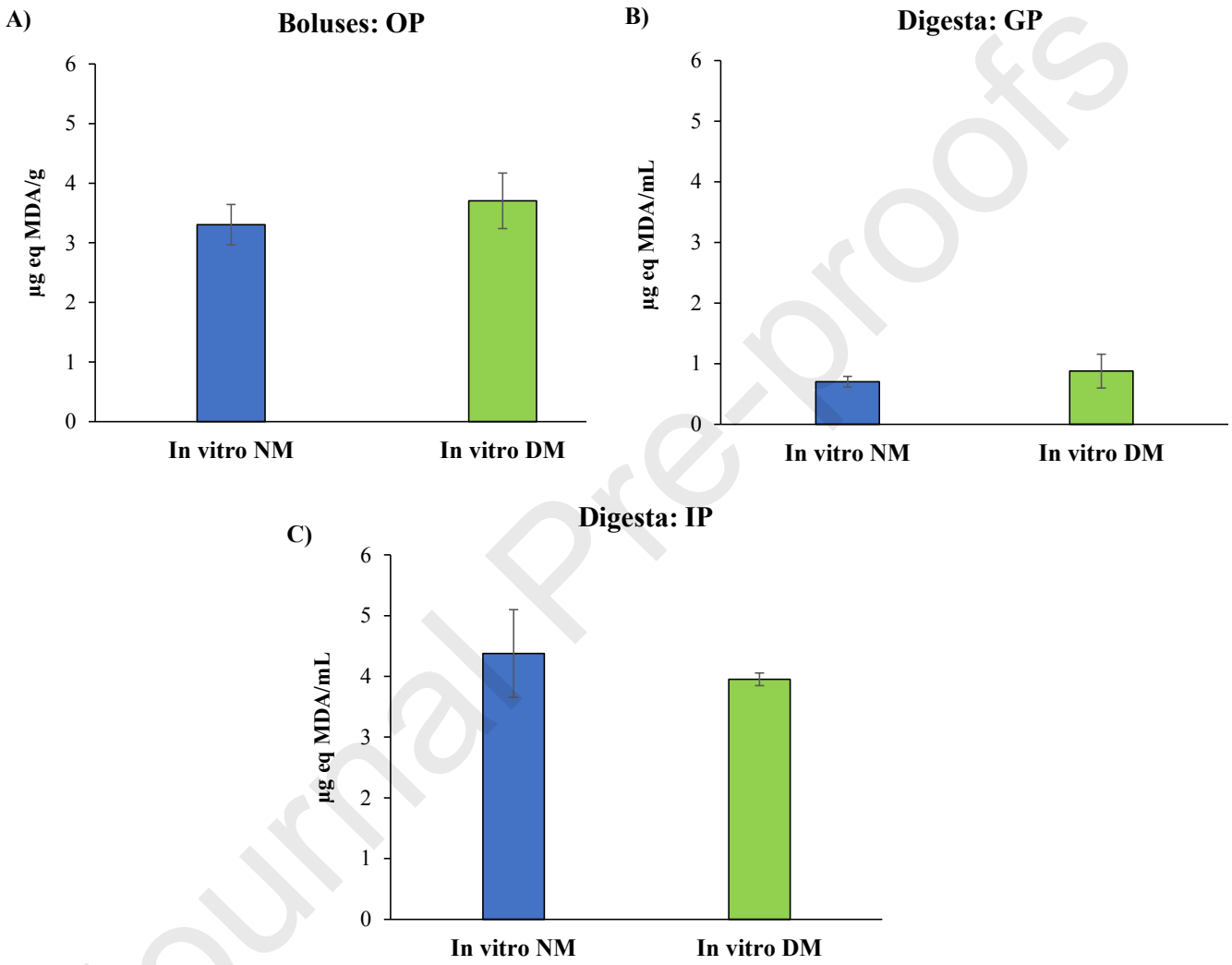


Figure 5

Table 1. Nutritional composition (g/100 g) and formulation of the gluten-free bread tested (Dr. Schär AG/SPA).

Nutrients	g/100 g
Energy (KJ/Kcal)	1108/262
Fat	3.1
Saturated fats	0.5
Carbohydrates	51
Sugars	2.6
Fibre	6.8
Proteins	4.1
Salt	1.1

Formulation: *maize starch, water, sourdough (rice flour, water) 14%, buckwheat flour 8%, rice flour, rice syrup, vegetable fibre (psyllium), rice starch, sunflower oil, soya protein, sorghum flour 2.1%, thickener: hydroxypropyl methylcellulose, yeast, salt, sugar. May contain traces of mustard. LACTOSE FREE (lactose < 0.007g/100g).*

Table 2. Percentage (%) of rapidly digestible starch (RDS), slowly digestible starch (SDS), total digestible starch (TDS), resistant starch (RS), and total starch (TS) values of the *in vitro* gluten-free bread boluses obtained after simulating the *in vitro* oral digestion, as well as maltose (g/L) and D-glucose (g/L) contents present in the liquid phase of samples after simulating the *in vitro* oral and gastrointestinal digestion. Mean values (n=3) ± SD.

		***In vitro* starch digestibility (%)**					**Sugars analysis (g/L)**	
	***In vitro* mastication**	**RDS**	**SDS**	**TDS**	**RS**	**TS**	**Maltose**	**D-glucose**
Boluses: OP	NM	5.7 ± 0.6	4.2 ± 0.5	12.3 ± 1.2	1.1 ± 0.6	13.5 ± 0.7	11.6 ± 0.5	7.0 ± 0.4
	DM	6.9 ± 0.6	3.8 ± 0.4	12.6 ± 1.5	1.4 ± 0.3	14.0 ± 1.5	11.6 ± 2.2	8.4 ± 1.0
Digesta: GP	NM	n.d.	n.d.	n.d.	n.d.	n.d.	1.2 ± 0.2	2.2 ± 0.2
	DM	n.d.	n.d.	n.d.	n.d.	n.d.	1.1 ± 0.4	2.1 ± 0.3
Digesta: IP	NM	n.d.	n.d.	n.d.	n.d.	n.d.	8.4 ± 1.4	5.1 ± 1.4
	DM	n.d.	n.d.	n.d.	n.d.	n.d.	11.1 ± 4.5	6.2 ± 1.4

NM: normal mastication; DM: deficient mastication. OP: Oral phase; GP: gastric phase; IP: intestinal phase.
n.d.: no determined.
p- value > 0.05 in all cases.