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Effects of microalgae incorporation on nutrient digestibility and antioxidant activity of gluten-free muffins after simulated chewing and digestion in adults and older adults

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This study aimed to evaluate the effects of microalgae incorporation, chewing conditions, and digestive models on carbohydrate and protein digestibility, total phenolic content, and antioxidant activity of gluten-free muffins, using *in vitro* boluses produced by a compression–shear–decompression process in a rheometer. To this end, gluten-free muffins, with and without microalgae (spirulina and chlorella), were formulated, and their chemical and mechanical properties were characterised. *In vitro* boluses were prepared with normal and deficient chewing, and the granulometric and mechanical properties were determined. Oro-gastrointestinal digestion was simulated *in vitro* by reproducing the conditions of adults and older adults. The results demonstrated that the rheometer was a reliable instrument for simulating *in vitro* bolus formation, closely mimicking *in vivo* conditions. The addition of microalgae increased protein, ash, and fibre content, reduced carbohydrate content, increased hardness, and decreased cohesiveness. It also improved protein digestibility, reduced starch digestibility, and increased total phenolic content and antioxidant activity. Furthermore, poor chewing reduced starch digestibility but favoured the release of phenolic compounds, whereas older adult digestion reduced both protein and starch digestibility while increasing the release of total phenolic compounds and antioxidant activity. This study highlights that both mastication and age-related digestive alterations exert a decisive impact on starch and protein digestibility, as well as phenolic compounds and antioxidant activity.

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1. Introduction

In recent years, the development of gluten-free products has experienced significant growth, driven by the increasing prevalence and diagnosis of celiac disease and other gluten-related disorders.[1,2] This trend has generated a growing demand for gluten-free food alternatives, prompting the food industry to develop products specifically tailored to this consumer segment.[3] However, many of the gluten-free products available on the market have poor nutritional profiles, characterised by high carbohydrate content and low levels of protein, fibre, and essential micronutrients.[3,4]

To enhance the nutritional profile of these products, legume and microalgae flours are being incorporated.[4,5] Legume flours, such as chickpea, lentil, and soybean flour, are rich in protein and provide significant amounts of dietary fibre, B-complex vitamins, minerals, complex carbohydrates, and bioactive compounds. Moreover, their inclusion has been shown to enhance the sensory and functional properties of gluten-free baked goods.[4] Similarly, microalgae like *Spirulina platensis* and *Chlorella vulgaris* are excellent sources of high-quality protein, dietary fibre, minerals, vitamins, polyunsaturated fatty acids, and bioactive compounds, among which phenolic compounds stand out for their antioxidant activity.[6,7] Furthermore, their inclusion could have a notable impact on texture due to their proteins,[6] which have excellent emulsifying, water-holding, and gelling properties.[5,8] These characteristics are particularly beneficial in gluten-free formulations, where the absence of gluten protein limits the formation of the structural networks typically found in traditional baked goods.

However, the nutritional quality and effectiveness of food are determined by the physicochemical and enzymatic transformations that occur during oro-gastrointestinal digestion.[9,10] Digestion models, which can be *in vivo*, *in vitro*, or *in silico*, are used to study this process.[10] In 2014, the international INFOGEST network published the first standardised *in vitro* protocol, which was further refined in 2019. This protocol

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outlines the oral, gastric, and intestinal phases of digestion, establishing a consistent ratio of food to digestive fluids in each phase based on physiological conditions. It also specifies factors such as electrolyte concentration, dilution rates, enzyme activity, bile salt concentration, pH levels, and the duration for each stage of digestion. However, the simulation of chewing in the oral phase is limited to merely grinding the sample.[11,12] In this way, there is no particle size distribution in the bolus, a characteristic that typically arises because of chewing.[13]

In recent years, several mastication simulators have been designed to clarify various aspects of the masticatory behaviour during food oral processing,[14,15] which can be used to produce the bolus *in vitro* before simulating gastrointestinal digestion. However, these simulators are not easily accessible in most laboratories; therefore, different studies have proposed the use of the rheometer as a tool for producing *in vitro* food boluses through a compression-shear-decompression cycle.[16,17] This is based on simulating the upward and downward movement of the tongue against the palate with compression and decompression movements of the upper plate, and the sliding movements of the tongue against the palate with shear movements of the upper plate. Nevertheless, it has been used so far in semi-solid foods without the use of 3M Transpore™ surgical tape to mimic the tongue surface and in many cases without the addition of saliva.

Therefore, the objective of this study was to evaluate the effects of microalgae incorporation, chewing conditions, and digestive models (adult and older adult) on the digestibility and antioxidant activity of gluten-free muffins, using *in vitro* boluses obtained through a compression–shear–decompression process in a rheometer.

2. Materials and methods

2.1 Materials and reagents

For *in vitro* oro-gastrointestinal digestion, α-amylase (A3176) and pancreatin (P7545), pepsin (P7012) and bile extract (B3883) from Sigma Aldrich, Co. (St Louis, MO, USA), as well as potassium chloride, sodium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, monopotassium phosphate, sodium bicarbonate and ammonium carbonate from Scharlau Chemie, S.A. (Sentmenat, Barcelona, Spain) were acquired.

The reagents used for subsequent analyses were Coomassie blue G-250, phosphoric acid, bovine serum albumin (BSA), L-tyrosine, L-leucine, sodium dodecyl sulphate (SDS), trinitrobenzenesulphonic acid (TNBS), sodium hydroxide, sodium carbonate, 6-hydroxy-2,5,7,8 tetramethylchromane-2-carboxylic acid (Trolox), disodium hydrogen phosphate, sodium dihydrogen phosphate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium dihydrogen phosphate anhydrous, potassium persulfate, diammonium salt of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium sodium tartrate tetrahydrate and 3,5-dinitrosalicylic acid (DNS) from Sigma-Aldrich, Co. (St Louis, MO, USA); anhydrous di-sodium hydrogen phosphate, trichloroacetic acid (TCA), hydrochloric acid 37%, gallic acid, and Folin-Ciocalteau reagent from Scharlau Chemie, S.A. (Sentmenat, Barcelona, Spain), and potassium ferricyanide and iron(III) chloride hexahydrate from MP Biomedicals, LLC. (Solon, OH, USA). All other reagents used were of analytical grade.

2.2 Preparation of the muffins

To prepare the gluten-free muffins (M), 81 g of egg, 60 g of sugar, and 50 g of milk were mixed. Next, 60 g of chickpea flour, 40 g of rice flour, and 3.7 g of baking powder were added and homogenised using an electric hand mixer (Taurus Robot 300 W, Taurus Group, S.L., Barcelona, Spain). Finally, 46 g of sunflower oil was added gradually, while the mixture was being stirred. Once the batter was prepared, the moulds were filled with 50 g of the mixture and baked at 180 °C for 24 min. To prepare the gluten-free muffins with microalgae (MM), 7.5 g of each flour was replaced by 15 g of commercial microalgae powder composed of spirulina (*Spirulina platensis*, 75%), chlorella (*Chlorella vulgaris*, 12.5%) and kale (*Brassica oleracea*, 12.5%). All ingredients were purchased from a local supermarket in Valencia.

2.3 Nutritional composition of muffins

All analyses were performed according to AOAC Official Methods.[18] Moisture content was determined by gravimetry after drying the sample at 105 °C until constant weight (AOAC 925.10). The protein content was obtained using the Dumas method (AOAC, 990.03). Total crude fat was determined by gravimetry, following acid hydrolysis using the Soxhlet extraction method (AOAC 922.06). The ash content was measured by gravimetry after incinerating the sample at 550 °C in a muffle (AOAC 942.05). Dietary fibre content was measured using an enzymatic gravimetric method (AOAC 985.29). The salt content was calculated by multiplying the sodium content determined by ICP-AES (AOAC, 984.27) by 2.5. Total sugars were determined by volumetry (AOAC 923.09), and total carbohydrates were calculated by subtracting the sum of moisture, protein, fat, ash, and dietary fibre from 100%.

2.4 Mechanical properties of muffins

The mechanical properties of muffins were determined by a Texture Profile Analysis (TPA) using a TA-TX2 texturometer (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell. For the analysis, the samples were cut into 20 mm cubes, which were doubly compressed to 50% of their original height, using a 36 mm cylindrical compression probe, a test and pre-test speed of 1 mm s^{-1}, a post-test speed of 2 mm s^{-1}, and a time between cycles of 5 s. Eight cubes per sample type were used for testing. Data were analysed with Exponent software (Stable Micro Systems Ltd), and hardness, cohesiveness, elasticity, and chewiness were determined.

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2.5 Chewing experiments

A Kinexus Pro rheometer with plate-plate geometry (Malvern Instruments Ltd, MA, USA) and a method based on a compression-shear-decompression cycle[19] was used to perform the *in vitro* chewing experiments.

The programming of the rheometer was based on *in vivo* trials to define the dynamic parameters of the method (number of cycles and gap), which were validated by superimposing the particle size distribution curves of both *in vivo* and *in vitro* gluten-free muffin boluses.[20,21]

To produce the *in vivo* boluses, four volunteers with good oral health were invited to chew 5.0 ± 0.1 g of the sample and to expectorate the bolus upon natural swallowing impulse. Each volunteer produced two boluses. All experiments were conducted in accordance with the ethical principles for human experimentation set out in the Declaration of Helsinki and were approved by the Ethics Committee of the Universitat Politècnica de València (P08_19-06-2024, 24 July 2024). Written informed consent was obtained from all participants involved in this study. Then, to produce the *in vitro* boluses, the rheometer was programmed to compress and decompress the samples, following the operational parameters whose rationale and final selection are discussed in section 3.3. The compression and decompression motions of the upper plate simulate the tongue's upward and downward movement against the palate, while the shearing motion simulates the tongue sliding against the palate.[19] Eight *in vitro* boluses of 5.0 ± 0.1 g were prepared per type of muffin, using normal chewing (NC) and deficient chewing (DC). 3M Transpore™ surgical tape was used to mimic the tongue surface,[22] and artificial saliva without α-amylase was employed to replicate conditions in the oral cavity.[20] All chewing experiments were carried out at 37 °C.

2.6 Characterisation of gluten-free muffin boluses

2.6.1 Granulometric analysis. The granulometric analyses were conducted on *in vivo* NC boluses and on *in vitro* NC and DC boluses of gluten-free muffins (8 boluses per type of muffin and chewing condition). Each recovered gluten-free muffin bolus was placed on a nylon 0.1 mm mesh, gently rinsed with tap water to disperse the particles and to eliminate residual saliva in the case of *in vivo* boluses, and dried for 35 min at 60 °C in an oven (JP Selecta, S.A., Barcelona, Spain). Then, dried particles were separated using a mechanical sieve shaker (IRIS FTL-0200, Filtra Vibración, Barcelona, Spain) operating at 1 s^{-1} vibration intervals for 3 min. Ten sieves, with apertures of 7.1, 6.3, 4.0, 2.5, 2.0, 1.4, 1.0, 0.8, 0.4, and 0.25 mm (Filtra Vibración, Barcelona, Spain), were employed. Finally, the retained particles on each sieve were weighed, and the cumulative mass distribution curve of particles passing through each specific sieve was plotted. The median particle size (d_{50}) was then determined from these curves as the aperture of a theoretical sieve through which 50% of the total particle mass would pass.[23]

2.6.2 Mechanical properties. Mechanical properties of muffin boluses were analysed by a Texture Profile Analysis (TPA) using a TA-TX2 texturometer (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell. Each bolus was placed in a container with a diameter of 28 mm and a height of 50 mm, and then compressed to 70% of its original volume, using a 20 mm cylindrical compression probe. The test velocity was set at 3 mm s^{-1}, while the pre-test and post-test velocities were both 5 mm s^{-1}. Four measurements were taken for each type of bolus and each chewing condition. Finally, hardness, adhesiveness, and cohesiveness were calculated by using Exponent software (Stable Micro Systems Ltd).

2.7 *In vitro* oro-gastrointestinal digestion tests of gluten-free muffins

For each type of muffin and chewing condition, two oro-gastrointestinal digestion scenarios were simulated: adult digestion (AD)[11,12] and older adult digestion (OD, generally referring to individuals above 65 years),[24] both performed according to the standardised INFOGEST protocols. Experimental conditions are presented in Table 1. Simulated digestion assays were conducted in duplicate at 37 °C under continuous mixing (40 rpm) using an incubation chamber (JP Selecta, S.A., Barcelona, Spain) and a rotary mixer (Intell-Mixer™ RM-2, ELMI Ltd, Riga, Latvia).

Firstly, *in vitro* NC and DC boluses were prepared as described in section 2.5 since an impairment of oral processing performance limits particle size reduction and could thereby influence both the rate and extent of digestion, as well as nutrient absorption.[20,25] However, in this case, the artificial saliva was replaced by water to simulate mouth coating and to prevent uncontrolled oral digestion by salivary α-amylase.[20] Then, the boluses were dried in an oven to remove the water added, and frozen until use.

Each digestion consisted of three phases: oral, gastric, and intestinal. For each, a simulated fluid (simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)) was prepared, as described by Minekus *et al.*

Table 1 Oro-gastrointestinal parameters to simulate the digestive conditions that could occur in normal adults and older adults

Digestion phase	Conditions	Digestion: Adult digestion	Older adult digestion
Oral	pH	7	7
	Time	2 min	2 min
	Amylase	75 U mL^{-1}	75 U mL^{-1}
Gastric	pH	3	3, 7
	Time	120 min	180 min
	Pepsin	2000 U mL^{-1}	1200 U mL^{-1}
Intestinal	pH	7	7
	Time	120 min	120 min
	Pancreatin	100 U mL^{-1}	80 U mL^{-1}
	Bile salts	10 mM	6.7 mM

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(2014).[11] After the oral phase, HCl (6 M) was added to lower the pH and simulate gastric phase conditions. After the incubation time of the gastric phase, NaOH (1 M) was added to raise the pH, stop the enzymatic reaction and simulate the conditions of the intestinal phase. Before adding the reagents of this last phase, 2 mL was extracted from each tube. After the intestinal phase, the enzymes were inactivated by placing the tubes in a 95 °C water bath for 5 min and then in an ice bath for 10 min. Finally, all samples were centrifuged at 8000*g* for 10 min at 4 °C, and the supernatants were recovered and stored at −20 °C for subsequent analysis. Blank samples, where the sample was replaced by water, were prepared in each digestion.

2.7.1 Relative protein digestibility of gluten-free muffins. To evaluate the relative protein digestibility of gluten-free muffins, total soluble protein, TCA-soluble peptides, and free amino groups were evaluated after each digestion phase. The total soluble protein content was determined using the Bradford method.[26] For this analysis, 40 µL of each sample was mixed with 2 mL of Bradford reagent and incubated at room temperature for 5 min. The absorbance was then measured at 595 nm, and the results were expressed as mg BSA per g of sample on a dry basis (d.b.). The TCA-soluble peptides were determined according to Ketnawa & Ogawa (2019).[27] Briefly, 450 µL of TCA (5%, w/v) was added to 50 µL of each sample, mixed with a vortex, and stored at 4 °C for 60 min. Then, the mixture was centrifuged at 8000*g*, 4 °C for 10 min, and the absorbance of the supernatant was measured at 280 nm. Results were expressed as mg L-tyrosine per g of sample (d.b.). Finally, free amino groups were determined following the TNBS method,[28] which specifically quantifies primary amine groups, and was used as a complementary approach together with Bradford and TCA-soluble peptide measurements to provide a comprehensive evaluation of protein hydrolysis. Thus, 40 µL of sample, 320 µL of TNBS and 320 µL of sodium phosphate buffer (pH 8.2, 0.2 M) were mixed and incubated at 50 °C for 60 min. Below, 640 µL of HCl (0.1 N) was added, and the samples were incubated at room temperature for 30 min. The absorbance was measured at 340 nm, and the results were expressed as mg L-leucine per g of sample (d.b.). All determinations were carried out in triplicate, and the absorbance was measured with a VANTAstar microplate reader (BMG Labtech, Ortenberg, Germany).

2.7.2 Relative starch digestion products of gluten-free muffins. The relative starch digestibility of the gluten-free muffins was assessed by measuring the reducing sugars' content after each digestion phase using the DNS method.[29] To this end, 500 µL of DNS solution was added to 50 µL of each sample and heated at 98 °C for 5 min. After incubation, samples were immediately cooled in an ice bath, and 4.5 mL of distilled water was added to each sample, vortexed, and the absorbance was measured at 540 nm with a VANTAstar microplate reader (BMG Labtech, Ortenberg, Germany). Samples were analysed in triplicate and results were reported as mg D-glucose per g of sample (d. b.).

2.7.3 Total phenolic content of gluten-free muffins. The determination of total phenolic content (TPC) of gluten-free muffins after each digestion phase was performed according to the method described by Teixeira-Guedes *et al.* (2019).[30] For this, 20 µL of sample was mixed with 100 µL of 10% (v/v) Folin–Ciocalteu reagent and 80 µL of sodium carbonate (7.5%, w/v) in a microplate. This was incubated at 42 °C for 30 min, and the absorbance was then measured at 765 nm using a VANTAstar microplate reader (BMG Labtech, Ortenberg, Germany). Each sample was analysed in triplicate, and the results were expressed as mg gallic acid per gram of sample (d.b.).

2.7.4 Antioxidant activity of gluten-free muffins. To evaluate the antioxidant activity of gluten-free muffins after each digestion phase, DPPH radical scavenging activity, ferric-reducing antioxidant power (FRAP) and ABTS radical scavenging capacity of the samples were determined.

The DPPH radical scavenging activity was determined according to the methodology of Bersuder *et al.* (1998).[31] Briefly, 50 µL of each sample was mixed with 250 µL of ethanol and 62.5 µL of DPPH solution (0.02%, w/v) and incubated in the dark for 60 min. Then, the absorbance of the samples was measured at 517 nm, and the results were expressed as mg Trolox per g of sample (d.b.).

The FRAP was evaluated following the methodology of Tsai *et al.* (2006),[32] with minor changes. For that, 60 µL of sample, 60 µL of phosphate buffer (200 mM, pH 6.6) and 60 µL of potassium ferricyanide (1%, w/v) were mixed and incubated at 50 °C for 20 min. Then, 60 µL of TCA (10%, w/v) was added to the samples and centrifuged at 2000*g*, 4 °C for 10 min. A total of 105 µL of the supernatant was mixed with 105 µL of distilled water and 21 µL of ferric chloride (0.1%, w/v) in a microplate, and the absorbance was measured at 700 nm. The results were expressed as mg Trolox per g of sample (d.b.).

Finally, the ABTS radical scavenging capacity was evaluated according to Re *et al.* (1999)[33] with some modifications. Firstly, 7 mM of ABTS was dissolved in 2.45 mM potassium persulfate and kept in the dark at room temperature for 12–16 h to produce $ABTS^{+}$. After this incubation period, the ABTS solution was diluted with 50 mM phosphate-buffered saline (pH 7.4) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 10 µL of the sample was mixed with 990 µL of the ABTS solution and incubated for 6 min. The absorbance was then measured at 734 nm, and the results were expressed as mg Trolox per g of sample (d.b.).

The absorbance in the three methods was measured using a VANTAstar microplate reader (BMG Labtech, Ortenberg, Germany).

2.8 Statistical analysis

Statistical analyses were performed with Statgraphics Centurion XIX software (Statgraphics Technologies, Inc., The Plains, VA, USA). Differences in particle size distribution among *in vivo* and *in vitro* NC boluses, as well as between *in vitro* NC and DC boluses, were determined by a one-way repeated measures ANOVA test, followed by Tukey–Kramer *post-hoc* test for mean comparison. Besides, a one-way ANOVA test, followed by a Tukey–Kramer *post-hoc* test for mean comparison, was applied to evaluate differences in the mechanical

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properties of M and MM. Moreover, a multifactor analysis of variance (multifactor ANOVA) was used to analyse mechanical properties and d_{50} of all NC and DC gluten-free muffin boluses, and relative protein and starch digestibility, total phenolic content and antioxidant activity of samples subjected to different digestive scenarios, with sample type (S), chewing condition (C) and digestion type (D) as factors. Data were expressed as the mean of replicates ± standard deviation (SD), and the statistical significance level was established at $p < 0.05$.

3. Results and discussion

3.1 Nutritional composition of gluten-free muffins

The nutritional composition of M and MM is summarised in Table 2. Both samples exhibited a broadly similar nutritional profile, but M had significantly ($p < 0.05$) higher carbohydrate levels, while MM presented higher contents of protein, ash, and dietary fibre. These differences can mainly be attributed to the incorporation of spirulina into the MM formulation. Spirulina, which constitutes the primary component of the microalgae powder used in this study, is characterised by its high protein content, ranging from approximately 50–70%, and its comparatively lower carbohydrate content, approximately 15–20%, of which the majority are polysaccharides.[34,35] The distinct chemical composition of spirulina directly influences the nutritional profile of the muffins, enhancing their protein and dietary fibre contents, while proportionally reducing their carbohydrate levels. Similar findings were reported by Nikolić *et al.* (2025),[2] who observed that substituting 15% of rice flour by spirulina powder in the formulation of gluten-free rice crackers resulted in an increase in protein, fibre, and ash content, along with a significant reduction in total carbohydrates. Similarly, Lucas *et al.* (2018)[36] reported an increase in protein and ash content, as well as a decrease in carbohydrates, when formulating an extruded spirulina-enriched snack. Thus, it can be stated that incorporating spirulina or other microalgae into product formulations can improve their nutritional profiles. However, a deeper evaluation of protein quality would require consideration of amino acid composition, as well as the influence of biotic and abiotic factors, such as culture systems and cultivation conditions, on their overall biochemical composition.[37]

Table 2 Nutritional composition (g per 100 g) of gluten-free muffins with and without microalgae

Component	Gluten-free muffins	Gluten-free muffins with microalgae
Moisture	24.7 ± 0.3[a]	24.85 ± 0.07[a]
Carbohydrates	43.3 ± 0.8[a]	39.9 ± 0.2[b]
Sugars	24.1 ± 0.1[a]	24.05 ± 0.07[a]
Fat	18.2 ± 0.6[a]	18.7 ± 0.2[a]
Protein	9.65 ± 0.07[b]	11.75 ± 0.07[a]
Salt	0.565 ± 0.007[a]	0.585 ± 0.007[a]
Ash	1.55 ± 0.07[b]	1.8 ± 0.0[a]
Dietary fibre	2.6 ± 0.0[b]	2.9 ± 0.0[a]

Mean (n = 2) ± standard deviation. Different letters in the same row indicate significant differences between the types of muffins ($p < 0.05$).

Table 3 Mechanical properties of gluten-free muffins with and without microalgae

Samples	Hardness (N)	Cohesiveness (%)	Elasticity (%)	Chewiness (N)
M	8.3 ± 0.8[b]	62 ± 2[a]	82 ± 3[a]	4.3 ± 0.5[b]
MM	14 ± 2[a]	54 ± 4[b]	82 ± 3[a]	7 ± 1[a]

Mean (n = 8) ± standard deviation. Different letters in the same column indicate significant ($p < 0.05$) differences between the types of muffins. M – gluten-free muffins; MM – gluten-free muffins with microalgae.

3.2 Mechanical properties of gluten-free muffins

The mechanical properties of a food product determine how it will respond to oral processing.[38] Table 3 shows mechanical parameters measured in the gluten-free muffins. MM presented the greatest hardness, probably due to its increased protein content, which can form hydrogen bonds with water[39] and promote a more compact structure. In this sense, Gün *et al.* (2022), Nurko and Nakilcioğlu (2025), and Şahin (2020)[40–42] also observed an increase in hardness as spirulina concentration increases in the formulation of biscuits, vegan cakes, and cookies, respectively. Regarding cohesiveness, MM showed significantly ($p < 0.05$) lower values compared to M, which may also be linked to its higher protein content.[43] Nejatian *et al.* (2024)[39] observed reduced cohesiveness in different products when spirulina was incorporated. Finally, it should be noted that no significant differences ($p > 0.05$) were observed in elasticity between samples, while chewiness was significantly ($p < 0.05$) higher in MM, in agreement with the results reported by Hussein *et al.* (2023).[44]

3.3 Programming of the rheometer to produce *in vitro* boluses and analysis of their granulometric properties

To produce gluten-free muffin boluses *in vitro* using a rheometer, the number of cycles and the gap between the plates had to be defined. The number of cycles was determined by *in vivo* trials, and the gap between the plates was fine-tuned

Table 4 Conditions used when programming the rheometer to mimic the *in vitro* normal and deficient chewing of gluten-free muffins with and without microalgae

Samples	Number of mastication cycles	Gap between plates[a] (mm)
M-NC	14	4–2
M-DC	14	6–4
MM-NC	17	5–3
MM-DC	17	7–5

M – gluten-free muffin; MM – gluten-free muffin with microalgae; NC – normal chewing; DC – deficient chewing. [a] Gap between plates (mm) during the decompression and compression motions of the upper plate of the rheometer.

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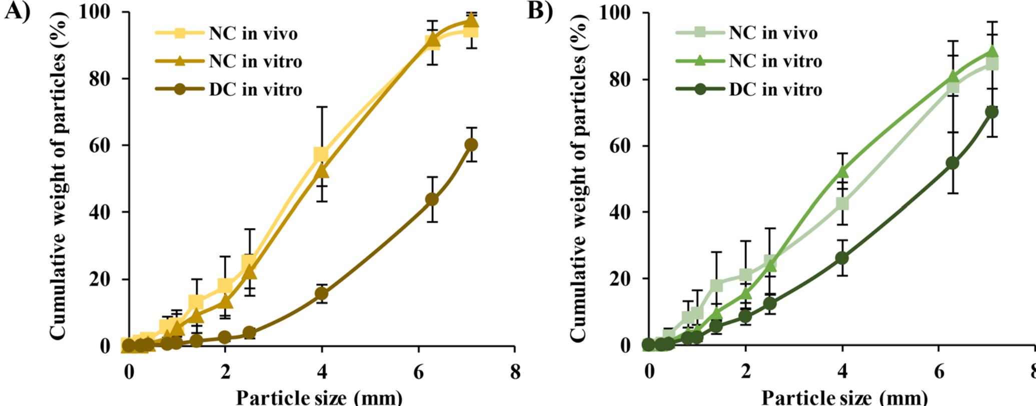

Fig. 1 Particle size distribution curves, indicated as cumulative weight of particles (%), of all boluses after *in vivo* normal chewing and *in vitro* normal and deficient chewing of gluten-free muffins (A) and gluten-free muffins with microalgae (B). Mean (n = 8) ± standard deviation. NC – normal chewing; DC – deficient chewing.

until the particle size distribution curves of the *in vivo* and *in vitro* boluses overlapped (Table 4 and Fig. 1). In the first compression cycle, the rheometer was programmed to lower the upper plate from 20 mm to the lowest gap value indicated in Table 4 at a rate of 17.8 mm s^{-1}. The sample was then decompressed (upstroke), raising the upper plate at a constant speed of 17.8 mm s^{-1} to the highest gap value (Table 4). In subsequent compression–decompression cycles, the upper plate descended to the gap value indicated in Table 4, which was different depending on the sample type. A strain rate of 10 s^{-1} was applied for 2 s per cycle.

The particle size distribution curves of *in vivo* and *in vitro* NC, and *in vitro* DC gluten-free muffin boluses of both types of muffins (M and MM) are presented in Fig. 1. No significant differences ($p > 0.05$) were observed between the particle size distribution curves of the *in vivo* and *in vitro* NC boluses, which confirms the correct programming of the rheometer. Furthermore, no significant differences ($p > 0.05$) were observed in the median particle size (d_{50}) values of the NC boluses of both types of muffins (Table 5). Nevertheless, significant differences ($p < 0.05$) were observed in the particle size distribution curves of NC and DC *in vitro* boluses for both types of muffins (Fig. 1). As expected, DC produced boluses with a higher proportion of large particles, while the cumulative weight remained below 100% due to the presence of particles larger than the maximum sieve aperture. Ribes *et al.* (2023)[45] reported a similar trend in the particle size distribution of bread boluses produced under NC and DC conditions. Finally, the d_{50} values obtained in the present study support the reduced fragmentation observed in DC boluses (Table 5).

3.4 Mechanical properties of gluten-free muffin boluses

During the oral processing of food, mastication and saliva absorption significantly alter the mechanical properties of food boluses.[46] Table 5 shows the mechanical properties of *in vitro* NC and DC boluses. The hardness of the boluses was significantly ($p < 0.05$) affected by the addition of microalgae to the muffin formulation. Regardless chewing conditions, the MM boluses were harder than the M boluses. These results may be related to the muffins' texture, as the hardness values of MM were higher than those observed in M (Table 3).

Regarding the adhesiveness of gluten-free muffin boluses, both type of muffin and type of mastication influenced this parameter. The M boluses exhibited greater adhesiveness than the MM boluses, while the DC boluses showed higher adhesiveness than the NC boluses (Table 5). Finally, regarding the cohesiveness of the boluses, significant differences ($p < 0.05$) were observed only between the formulations. The M boluses presented higher cohesiveness than the MM boluses, regardless of the chewing condition (Table 5). These findings are consistent with the texture results of the muffins, since, as pre-

Table 5 Median particle size (d_{50}) and mechanical properties of chewed gluten-free muffin boluses *in vitro*

Samples	d_{50} (mm)	Hardness (N)	Adhesiveness (N s)	Cohesiveness (%)
M-NC	3.9 ± 0.2^{Ab}	2.4 ± 0.3^{Ba}	0.0006 ± 0.0001^{Bb}	52 ± 3^{Aa}
M-DC	6.6 ± 0.3^{Aa}	2.9 ± 0.3^{Ba}	0.00066 ± 0.00003^{Ba}	57.25 ± 2.55^{Aa}
MM-NC	3.9 ± 0.4^{Ab}	5 ± 1^{Aa}	0.4 ± 0.2^{Aa}	40 ± 7^{Ba}
MM-DC	5.9 ± 0.6^{Aa}	5.3 ± 0.4^{Aa}	0.1 ± 0.1^{Ab}	39 ± 2^{Ba}

Mean (n = 5) ± standard deviation. Different capital letters in the same column indicate significant ($p < 0.05$) differences between the types of muffins, whereas different lower-case letters in the same column indicate significant ($p < 0.05$) differences between the types of mastication. M – gluten-free muffin; MM – gluten-free muffin with microalgae; NC – normal chewing; DC – deficient chewing.

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viously mentioned, M also exhibited higher cohesiveness than MM (Table 3).

3.5 Relative digestibility of gluten-free muffins

Digestibility is defined as the proportion of food components that are converted into accessible substances through various physicochemical processes that occur within the digestive system lumen.[9,10] In the present study, the relative digestibility of the protein and starch of gluten-free muffins (M and MM) was estimated by measuring the levels of total soluble protein, TCA-soluble peptides, free amino groups and reducing sugars at the end of each digestion phase. This was achieved by simulating the AD and OD (Fig. 2). A multifactor ANOVA was performed to evaluate the influence of sample type (S), chewing condition (C) and digestion type (D) on each digestion phase, and the results are presented in Table 6. Only the S and C factors were considered in the oral phase, as this stage is common to both *in vitro* digestion protocols.

Regarding relative protein digestibility, an increase in the levels of total soluble protein (Fig. 2A), TCA-soluble peptides (Fig. 2B) and free amino groups (Fig. 2C) was observed throughout oro-gastrointestinal digestion in both digestion models (AD and OD), both types of muffins (M and MM) and both chewing conditions (NC and DC). After the oral phase, the S factor was significant ($p < 0.05$) in both the soluble protein and TCA-soluble peptide content, as well as in the free amino group content. The F-ratio was significantly ($p < 0.05$) higher than other factors and interactions, indicating that the S factor had a greater influence on the observed variability (Table 6). Furthermore, the MM sample had a lower soluble protein content, but a higher content of soluble peptides and free amino groups. These results could be explained by the fact that, although MM contains a higher protein content (Table 1), protein release may be hindered by the rigid cell walls of microalgae.[5] After the gastric phase, the S factor was significant ($p < 0.05$) in all three determinations, with a high F-ratio in each case (Table 6). In general, the MM had a lower content of soluble protein and free amino groups, as well as a higher content of TCA-soluble peptides than M. These could be explained by the structural rigidity of microalgae cell walls, which makes protein release difficult. Since carbohydrase enzymes do not act during the gastric phase, the intact cell walls of microalgae could limit the protein availability.[5] Conversely, the C factor was significant ($p < 0.05$) only for soluble peptide content. In general, samples with NC had a higher content than those with DC. This trend could be attributed to particle size, as larger particles minimise protein exposure and hinder enzyme action.[47] The D factor was significant ($p < 0.05$) in the case of total soluble protein and TCA-soluble peptide content. It also showed high F-ratio values (Table 6), reflecting a clear and significant effect on the analysed variables. Samples with AD exhibited lower soluble protein content, but higher peptide content than samples with OD. This could be explained by the longer gastric phase of OD, which allows greater protein solubilisation.[48] Additionally, in OD, the higher pH and lower enzyme activity may lead to a lower degree of hydrolysis and, consequently, a lower release of peptides.[49] In the gastric phase, most of the interactions were significant ($p < 0.05$). However, the $S \times C$ interactions in TCA-soluble peptide content and free amino group content, the $S \times D$ interaction in total soluble protein content, and the $C \times D$ interaction in free amino group content presented the highest F-ratio values (Table 6). In the case of M-DC, the content of peptides and free amino groups increases, whereas in MM it decreases. The higher hardness of the muffins and their corresponding boluses (Tables 3 and 5, respectively) could explain the observed trends, as the greater hardness values of MM probably hinder the access of the enzymes to the substrate.[25] In the case of OD, an increase in soluble protein content was observed in MM and M, which could be linked to the longer gastric stage. However, this increase was more pronounced in M, which could be attributed to the rigid cell walls of the microalgae hindering protein release, as previously explained.[5] Finally, the OD simulation reduced the content of free amino groups in samples subjected to NC, whereas the samples with DC showed an increase. Although this trend was unexpected, it may be attributed to variations in bolus particle size.[20,50] The presence of smaller particles in NC boluses would facilitate enzyme access, promoting protein hydrolysis and the formation of larger peptides. In the case of DC, however, a larger particle size likely hinders enzymatic hydrolysis of protein, leading to a greater quantity of small peptides and amino acids. After the intestinal phase, the S factor was found to be statistically significant ($p < 0.05$) in all three cases with the highest F-ratio values reflecting a greater relative influence of this factor on the observed variability in the data (Table 6). The MM sample had a lower total soluble protein content, but a higher TCA-soluble peptide and free amino group values than M, suggesting that MM may be more digestible than M. These results could be linked to the hydrolysis of microalgae cell wall carbohydrates,[7] which could enhance the accessibility of proteases during this phase of digestion. The C factor was significant ($p < 0.05$) for total soluble protein and free amino groups. Regarding total soluble protein, samples with NC had a higher content than samples with DC, but a higher increase was observed in MM than in M. Therefore, the interaction $S \times C$ was also significant ($p < 0.05$). In the case of free amino groups, however, a higher content was observed in the DC samples. This unexpected trend suggests that samples with DC undergo higher protein degradation during this phase, reflected by the reduction in the total soluble protein content and increase in free amino group content. Regarding the D factor, this was only significant for total soluble protein. A higher content was observed in samples with AD compared to OD, which is consistent with the results of Ribes, Arnal, *et al.* (2023).[49] This effect could be related to the lower pancreatic activity and lower bile salt concentration used during OD, as lower pancreatic activity has been associated with poor digestion, favouring protein malabsorption and potential nutritional deficiencies.[49]

Concerning relative starch digestibility, although starch hydrolysis starts in the oral cavity, the most notable increase in

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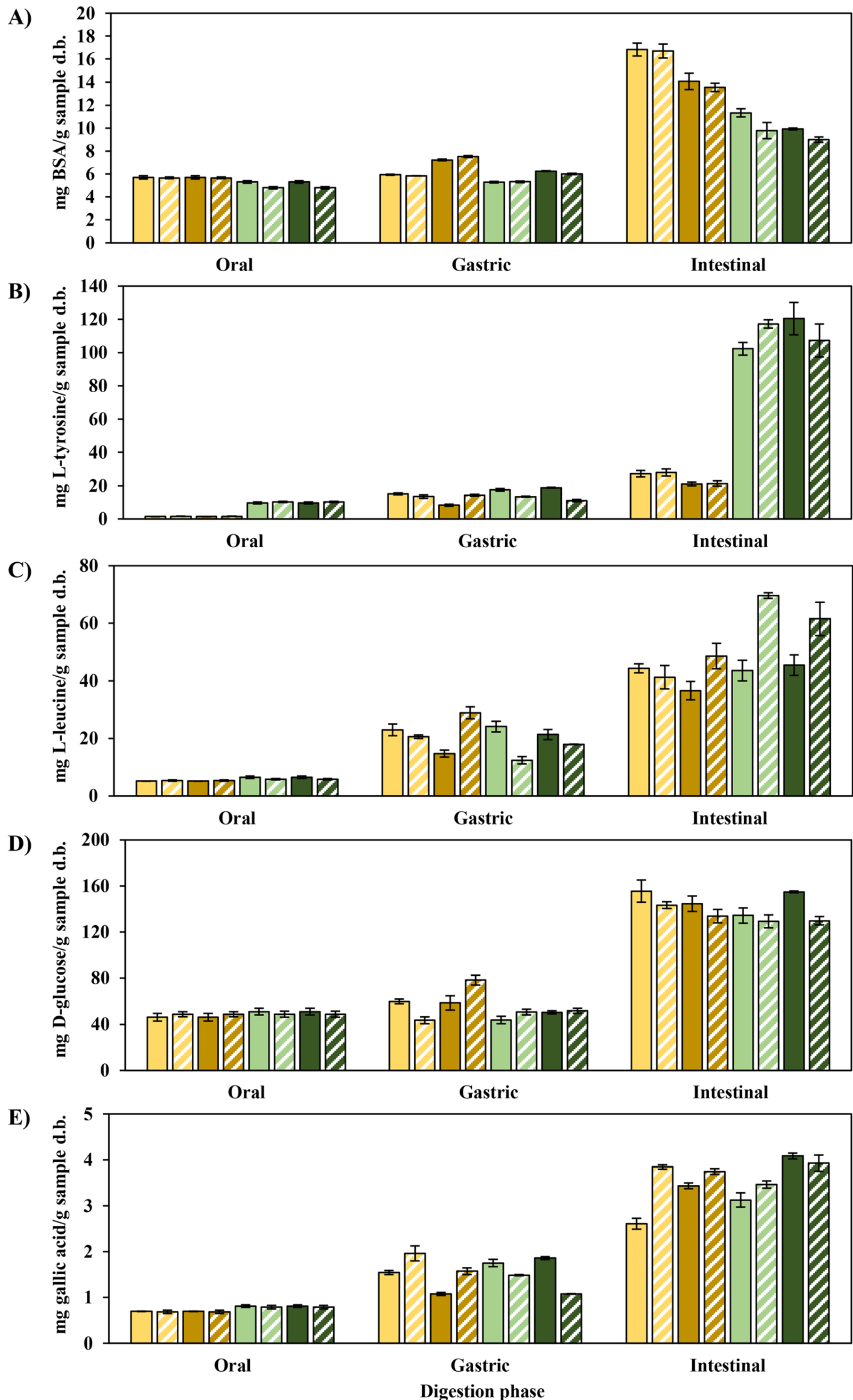

Fig. 2 Total soluble proteins (A), TCA-soluble peptides (B), free amino groups (C), reducing sugars (D), and total phenolic content (E) results of gluten-free oro-gastrointestinal digested muffins. Mean (n = 3) ± standard deviation. M – gluten-free muffin; MM – gluten-free muffin with microalgae; NC – normal chewing; DC – deficient chewing; AD – adult digestion; OD – older adult digestion.

reducing sugar content was observed after the intestinal phase (Fig. 2D), where pancreatic α-amylase is involved.[51] After the oral phase, no significant ($p > 0.05$) differences were observed in either the factors or in their interaction. Following the gastric phase, factors S and D, as well as interactions $S \times D$, $C \times D$, and $S \times C \times D$, exhibited significant differences ($p < 0.05$),

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Table 6 Results of multifactor ANOVA: F-ratio and *p*-value for factors and interactions affecting total soluble proteins, TCA-soluble peptides, free amino groups, reducing sugars, total phenolic content, and antioxidant activity of gluten-free muffins subjected to oro-gastrointestinal digestion

	Factor/interaction	Oral phase F-ratio	Oral phase *p*-Value	Gastric phase F-ratio	Gastric phase *p*-Value	Intestinal phase F-ratio	Intestinal phase *p*-Value
Total soluble proteins	*S*	98.91	0	1306.4	0	661.1	0
	C	18.89	0.0025	0.1	0.7561	14.69	0.0015
	D	—	—	2067.66	0	98.72	0
	S × *C*	13.23	0.0066	16.39	0.0009	4.84	0.0429
	S × *D*	—	—	173.49	0	21.17	0.0003
	C × *D*	—	—	1.25	0.2797	0.08	0.7753
	S × *C* × *D*	—	—	45.7	0	1.52	0.2349
TCA-soluble peptides	*S*	1346.87	0	78.94	0	1279.57	0
	C	3.18	0.1247	48.3	0	0.09	0.7744
	D	—	—	44.05	0	0.23	0.6359
	S × *C*	1.65	0.2461	220.11	0	0.01	0.9425
	S × *D*	—	—	20.59	0.0005	4.68	0.0484
	C × *D*	—	—	13.1	0.0028	8.55	0.0111
	S × *C* × *D*	—	—	105.19	0	7.92	0.0138
Free amino groups	*S*	32.44	0.0005	16.28	0.0012	48.72	0
	C	2.45	0.1562	1.4	0.257	52.23	0
	D	—		0.97	0.3409	0.89	0.3637
	S × *C*	7.78	0.0236	92.41	0	22.02	0.0004
	S × *D*	—	—	0.93	0.3517	0.65	0.4338
	C × *D*	—	—	78.67	0	0.53	0.4812
	S × *C* × *D*	—	—	8.59	0.0109	12.74	0.0034
Reducing sugars	*S*	2.31	0.1674	61.76	0	9.6	0.0069
	C	0.03	0.8678	4.31	0.0545	31.38	0
	D	—	—	54.89	0	0	0.9521
	S × *C*	2.36	0.1628	0.84	0.3732	0.62	0.4423
	S × *D*	—	—	21.32	0.0003	19.08	0.0005
	C × *D*	—	—	29.78	0.0001	3.84	0.0676
	S × *C* × *D*	—	—	55.24	0	5.07	0.0388
Total phenolic content	*S*	36.96	0.0003	0.03	0.8719	31.99	0
	C	0.85	0.3836	1.19	0.2912	101.87	0
	D	—	—	93.46	0	157.29	0
	S × *C*	0.13	0.7259	269.49	0	63.49	0
	S × *D*	—	—	21.38	0.0003	16.39	0.0009
	C × *D*	—	—	13.09	0.0023	68.87	0
	S × *C* × *D*	—	—	24.69	0.0001	6.32	0.023
DPPH	*S*	444.9	0	308.79	0	89.57	0
	C	2.32	0.1783	2.58	0.1308	2.44	0.1406
	D	—	—	0.06	0.8042	41.06	0
	S × *C*	0.67	0.4453	15.88	0.0014	18.47	0.0007
	S × *D*	—	—	1.01	0.3331	6.18	0.0262
	C × *D*	—	—	10.57	0.0058	0.76	0.3993
	S × *C* × *D*	—	—	0.38	0.5464	0.29	0.5979
FRAP	*S*	253.17	0	50.57	0	1303.02	0
	C	0	0.9575	3.09	0.0977	5.16	0.0383
	D	—	—	18.37	0.0006	4.44	0.0525
	S × *C*	8.42	0.0199	13.63	0.002	1.48	0.2427
	S × *D*	—	—	7.7	0.0135	1.51	0.2376
	C × *D*	—	—	32.31	0	9.51	0.0076
	S × *C* × *D*	—	—	0.29	0.597	2.07	0.1712
ABTS	*S*	4.88	0.0582	23.22	0.0002	5.39	0.0338
	C	1.46	0.2607	2.15	0.1621	59.84	0
	D	—	—	18.32	0.0006	498.88	0
	S × *C*	17.4	0.0031	3.31	0.0878	2.87	0.1098
	S × *D*	—	—	2.49	0.1344	12.4	0.0028
	C × *D*	—	—	6.14	0.0248	10.82	0.0046
	S × *C* × *D*	—	—	7.43	0.015	5.79	0.0286

DPPH – 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; FRAP – ferric reducing antioxidant power; ABTS – 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical scavenging capacity; *S* – sample; *C* – chewing; *D* – digestion.

with comparable F-ratio values. M samples had a higher content of reducing sugars than MM samples, which could be attributed to the muffins' chemical composition, given that M samples have a higher carbohydrate content (Table 1). Additionally, samples with OD presented a higher content of reducing sugars than samples with AD, probably due to the higher pH of the gastric phase, favouring the activity of α-amylase at the beginning of this phase,[52] and the longer dur-

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ation of this stage, which would allow a higher solubilisation of sugars.[48] Regarding the interactions observed in the statistical analysis (Table 6), the content of reducing sugars increased in both types of muffins and both chewing conditions after simulated OD, but the increase was greater in the MM and in muffins with DC. Following the intestinal phase, significant differences ($p < 0.05$) were observed for factors S and C, as well as for interactions $S \times D$ and $S \times C \times D$. Factor C had the highest F-ratio value, indicating its greater influence on the observed variability. The M samples had a higher content of reducing sugars, probably due to their higher carbohydrate content (Table 1). Additionally, NC muffins had a higher content of reducing sugars than DC muffins, which could be attributed to their smaller particle size (Table 5), making the starch bonds more accessible to pancreatic α-amylase.[20] Finally, regarding the $S \times D$ interaction, the content of reducing sugars generally decreased with OD in both types of muffins, being higher in M than in MM, possibly due to lower enzyme activity in OD.[24]

3.6 Total phenolic content (TPC) during oro-gastrointestinal digestion

Legumes and microalgae are foods rich in phenolic compounds.[7] However, the amount that can be absorbed and their capacity to exert a biological effect will be determined by the chemical and enzymatic processes that take place during oro-gastrointestinal digestion.[53] Fig. 2E shows the TPC of muffins throughout digestion, demonstrating an increase as digestion progresses. Li *et al.* (2023)[54] also observed an increase in TFC when digested spirulina and chlorella. After the oral phase, significant differences ($p < 0.05$) were only observed in the S factor, with MM showing a higher content than M. Nikolić *et al.* (2025),[2] and Nurko and Nakilcioğlu (2025)[42] also observed a higher content of TPC in spirulina-enriched crackers and vegan cakes, respectively, in comparison to the control samples. After the gastric phase, factor D, as well as all interactions, were statistically significant ($p < 0.05$), with the $S \times C$ interaction showing the highest F-ratio value. In general, samples with AD presented a higher amount of TPC, which could be attributed to the higher pepsin activity, as it is the enzyme that can hydrolyse the complexes formed between phenolic compounds and proteins, resulting in the release of phenols.[55,56] In relation to the $S \times C$ interaction, MM with DC showed lower values than those observed with NC, while the opposite trend was detected in M. The trend observed in the MM is consistent with predicted outcomes, as the larger particle size of the boluses with DC could limit the action of the enzymes.[47] However, the opposite direction was observed in M, which could be attributed to the lower hardness of muffins and boluses (Tables 3 and 5, respectively), as this lower hardness would favour particle disintegration during the gastric phase, thus facilitating enzyme action.[25] Finally, as shown in Table 6, all factors and their respective interactions were significant ($p < 0.05$) after the intestinal phase. In general, the MM presented a higher TPC than the M, and the content was also higher with DC and OD. As highlighted in the oral phase, the higher TPC of the MM could be related to the polyphenols provided by microalgae. Furthermore, the higher content of the samples with DC may be linked to the larger particle size of the boluses (Table 5), which may have conferred protection to the phenolic compounds from hydrolysis and subsequent degradation. Similarly, OD simulation implies decreased enzyme activity, thus limiting the degree of hydrolysis and degradation of the compounds. Currently, research on the bioaccessibility and bioavailability of polyphenols in older adults is limited[57] and the findings of the present work cannot be contrasted.

3.7 Antioxidant activity of muffins during oro-gastrointestinal digestion

It is essential to assess antioxidant activity using different or complementary methods, since antioxidant compounds have different associated chemical mechanisms. To measure the antioxidant activity of muffins during oro-gastrointestinal digestion, the DPPH, FRAP and ABTS methods were employed. These three methods are generally classified as electron transfer-based methods. However, the DPPH and ABTS methods can also be categorised as hydrogen atom transfer-based methods.[58,59] As can be seen in Fig. 3, antioxidant activity increases with all three methods of analysis (DPPH, FRAP and ABTS) as digestion progresses. This increase could be due to the release of antioxidant peptides or phenolic compounds complexed with protein fractions.[60]

After the oral phase, the statistical analysis revealed that the S factor was significant ($p < 0.05$) in the determinations carried out by the DPPH and FRAP methods. Similarly, the $S \times C$ interaction showed significant differences ($p < 0.05$) in determinations using the FRAP and ABTS methods (Table 6). The sample MM showed the lowest activity in the DPPH method, while it presented the highest activity in the FRAP method. The higher antioxidant activity observed in the MM is consistent with the biochemical composition of spirulina, which contains a complex mixture of antioxidant compounds, including carotenoids (such as beta-carotene and xanthophylls), chlorophyll, and phycocyanin.[34] However, this may not have been detected by the DPPH method, given that the organic reagent used could precipitate the antioxidant compounds.[61] Regarding the interaction of factors, the activity slightly increased with DC in M in both methods, whereas it decreased in MM. This trend could be related to the hardness of muffins and boluses, since M was softer than MM (Tables 3 and 5). Following the gastric phase, the S factor was significant ($p < 0.05$) in all three methods, whereas the D factor was only significant in the FRAP and ABTS methods. The MM exhibited higher antioxidant activity in the DPPH and FRAP methods. However, in the ABTS method, the M presented higher antioxidant activity, although the differences were minimal (Fig. 3C). Regarding factor D, lower antioxidant activity was observed in the FRAP and ABTS methods in samples with OD, coinciding with lower TFC release (Fig. 2E). The same trend was reported by Shang *et al.* (2022)[55] when they analysed the antioxidant activity of digested apple by the ABTS method. The interactions were all significant, but not in all methods (Table 6). The

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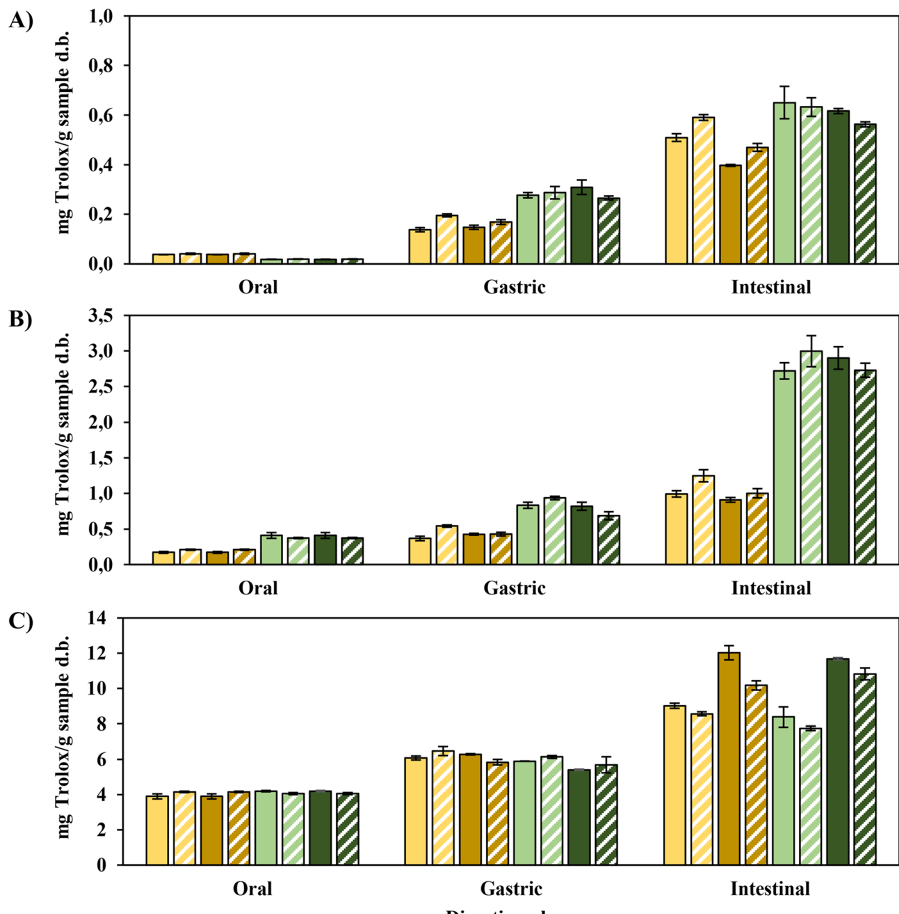

Fig. 3 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (A), ferric reducing antioxidant power (B), and ABTS (2,20-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging capacity (C) of gluten-free oro-gastrointestinal digested muffins. Mean (n = 3) ± standard deviation. M - gluten-free muffin; MM - gluten-free muffin with microalgae; NC - normal chewing; DC - deficient chewing; AD - adult digestion; OD - older adult digestion.

$S \times C$ interaction was significant ($p < 0.05$) in the FRAP and ABTS methods. In the M sample, activity increased with DC, whereas it decreased in the MM sample. As discussed in the oral phase, the reasons could be related to the hardness of the muffins. The $S \times D$ interaction was only significant ($p < 0.05$) in the FRAP method, although the F-ratio was considerably lower than that of the other factors and interactions. The $C \times D$ interaction was significant ($p < 0.05$) in all three methods. Generally, combining DC with AD was associated with increased antioxidant activity, whereas combining it with OD tended to reduce it. The trend of these results aligns with that of TFC (Fig. 2E) and may be related to the higher pepsin activity at this stage, which could hydrolyse the complexes formed between phenolic compounds and proteins,[55,56] releasing phenolic compounds with antioxidant activity. Finally, after the intestinal phase, factor S was significant ($p < 0.05$) in all three methods, factor C in the FRAP and ABTS methods, and factor D in the DPPH and ABTS methods. With the DPPH and FRAP methods, it was clearly observed that MM had higher antioxidant activity than M, which could be due to their higher TFC (Fig. 2E) and peptide content (Fig. 2B). Currently, no studies have been found that evaluate the evolution of the antioxidant activity of microalgae-enriched products during oro-gastrointestinal digestion. However, Fradinho *et al.* (2020)[6] and Nikolić *et al.* (2025)[2] observed a higher antioxidant activity in the microalgae-enriched pasta and crackers, respectively, in comparison to the control sample, although they did not evaluate its evolution during oro-gastrointestinal digestion. Depending on the method employed, muffins with NC or with DC, and with AD or OD showed higher activity. However, in relation to factor D, the ABTS method recorded the highest F-ratio value and samples with OD showed higher antioxidant activity than those with AD, which could be attributed to a higher TFC content (Fig. 2E). Similar results were observed by Shang *et al.* (2022)[55] when evaluating the antioxidant activity of apple. All interactions were significant ($p < 0.05$) in at least one of the methods analysed. However, according to the F-ratio values obtained, the $S \times C$ interactions with the DPPH method, $S \times D$ with the ABTS method, and $C \times D$ with the FRAP and ABTS methods stood out (Table 6). In samples with DC, antioxidant activity increased in M and decreased in MA. On the other hand,

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when OD was simulated, antioxidant activity increased in both types of muffins, although the increase was more pronounced in MM. Finally, when combining NC or DC with OD, it was observed that, using the FRAP method, the antioxidant activity decreased, whereas using the ABTS method, it increased. Currently, there are no studies that assess the impact of chewing and digestion on the antioxidant activity of food products. The variability in results may be attributed to several factors, such as the stability of antioxidant molecules when exposed to digestive enzymes and changes in pH, the synergistic effects produced by components within the food matrix, or the chemical reactions that can occur during the digestion process.[62]

4. Conclusions

The results of this study demonstrated that incorporating microalgae into gluten-free muffins increased protein, ash, and fibre content, reduced carbohydrate content, and modified their mechanical properties, resulting in increased hardness and decreased cohesiveness. Furthermore, it was demonstrated that the rheometer was an adequate tool for obtaining *in vitro* boluses with characteristics like those produced *in vivo*.

The addition of microalgae to gluten-free muffins improved relative protein digestibility, decreased relative starch digestibility, and enhanced total phenolic content and antioxidant activity. While chewing condition and digestion had a smaller impact overall, it is important to note that deficient chewing resulted in reduced relative starch digestibility. Additionally, older adult digestion was found to decrease both relative starch and protein digestibility. However, both inadequate chewing and the digestive processes in older adults favoured the release of phenolic compounds by the end of digestion, with older adult digestion also increasing the antioxidant activity of the gluten-free muffins.

The results of this study offer valuable insights into the physical and mechanical properties of muffin boluses and the bioaccessibility of nutrients and antioxidant activity in adults and older adults with varying oral capacities. Furthermore, this research opens new possibilities for designing gluten-free foods that incorporate microalgae, tailored for specific populations.

Author contributions

Conceptualisation: M. A., S. R., and P. T.; methodology: M. A., S. R., and P. T.; formal analysis: M. A., S. R., and N. B.; investigation: M. A., S. R., and P. T.; writing – original draft preparation: M. A.; writing – review & editing: M. A., S. R., and P. T.; visualisation: M. A., S. R., and P. T.

Conflicts of interest

There are no conflicts to declare.

Data availability

All data supporting the findings of this study are presented in the figures and tables. The raw data are available in the supplementary information (SI).

Supplementary information is available. See DOI: https://doi.org/10.1039/d6fo01526c.

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