Algal Research 50 (2020) 101998

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Microalgae biomass as an additional ingredient of gluten-free bread: Dough rheology, texture quality and nutritional properties

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ARTICLE INFO

Keywords:
Microalgae
Functional ingredients
Gluten-free bread
Physicochemical properties
Mixing properties
Sensory evaluation

ABSTRACT

Microalgae have been widely used as a source of functional ingredients such as pigments, antioxidants, vitamins, and omega-3 polyunsaturated fatty acids. They also represent a promising alternative source of protein. The objective of this study was to evaluate the impact of the addition of two green microalgae species (*Nannochloropsis gaditana* L2 and *Chlamydomonas* sp. EL5) on the techno-functional and nutritional properties of gluten-free bread. Microalgae biomass was added in the amounts of 1.0 and 3.0 g/100 g of flour. The behavior of the dough during the mixing as well as the physicochemical properties of the prepared breads were investigated. Gluten-free bread with *N. gaditana* L2 and *Chlamydomonas* sp. EL5 presented significantly higher protein and higher levels of lipids and ash, compared with the control bread. The incorporation of 3% microalgae biomass revealed a 100% increase in iron and calcium contents. The fatty acid profile of supplemented bread changed in a species-specific manner with a particular increase in linolenic acid (18:3 ω3) and a decrease in ω3/ω6 ratio. Besides, due to its original biochemical composition, mainly the highly protein content, microalgae incorporation was found to bring an overall structuring effect on the gluten-free bread texture. However, the dough mixing properties were not affected significantly by microalgae addition. A significant change in color was recorded in doughs, breads, crusts and crumbs. This was caused by the presence of pigment in microalgae biomass, which turned into more intense green-yellow tonalities. A sensory analysis revealed that the supplemented breads scored highest for nearly all the sensory parameters with the 3% *N. gaditana* L2 bread as the preferred one in terms of global appreciation. This innovative approach gives new insights of the possibility of improving gluten-free products, structurally and nutritionally, using only microalgae as a natural and a sustainable food ingredient.

1. Introduction

Gluten is a complex mixture of insoluble proteins comprising the gliadins and glutenins in wheat and equivalent proteins in barley and rye. Gluten is responsible for the viscoelastic behavior of the dough and the chewiness of foods made from wheat flour [21]. Recently, the gluten-free products market has registered a remarkable growth driven by the rapid rise of the global incidence of pathologies related to gluten intake, namely wheat allergy, celiac disease, and non-celiac gluten sensitivity, combined with the growing belief that gluten-free products are associated with a healthier life style [10,50]. In all cases, a lifelong gluten-free diet is the only treatment currently available [40]. Thus, a gluten-free product with a good sensory and nutritional quality remains the biggest wish of individuals with gluten disorders. Gluten-free bread, more than any other gluten-free product, has received a lot of attention from researchers and food technologists. Some recent studies have investigated techniques that can improve the characteristics of the final product [32]. Interesting results were reported by Clark and Aramouni [16], who used breadfruit (*Artocarpus altilis*) as a wheat flour replacement. Maize [9], vegetables [54], bee pollen [17], dietary fibers [32], and acorn flour [8] are also other functional ingredients that have been used in gluten-free bread formulations in order to increase their

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https://doi.org/10.1016/j.algal.2020.101998
Received 16 February 2020; Received in revised form 1 July 2020; Accepted 2 July 2020

nutritional profile and improve their texture and flavor.

Meanwhile, some researchers have been interested in algae biomass due to its richness in bioactive compounds [5]. Improvement of the quality of bread protein was first assessed by Arafah et al. [3], using green microalgae (*Scenedesmus obliquus*). In a more recent study, several green microalgae, such as *Isochrysis galbana* and *Nannochloropsis gaditana*, were added to bread formulations. The impact of this incorporation on both dough and bread were evaluated in relation to the mixing properties, color, and texture profile [26].

However, the examples of microalgae supplementation concern gluten-containing bread in particular [29,43]. Gluten-free bread is still under-investigated. Indeed, replicating the gluten network by adding other ingredients would affect the physico-chemical and rheological characteristics of the dough and of the resulting product. An analogous effect is expected with the microalgae supplementation, taking into consideration their biomass composition, especially the considerable amount of protein they contain.

Only a small number of microalgae are generally recognized as safe (GRAS) for food application, such as *Chlorella vulgaris*, *Arthrospira platensis,* and the diatom *Odontella aurita* (European Union [23], Novel Food catalogue). Apart from those approved species, some others that are not yet recognized as GRAS have been submitted to a toxicological evaluation and considered safe for applications in food and/or belonging to genera of approved species. In order to enlarge the nutritional application of emerging species of microalgae with the attractive advantages related to both the culturing and nutritional properties, special attention is given to the two unicellular green microalgae, *Nannochloropsis gaditana* and *Chlamydomonas* sp.

Nannochloropsis gaditana is one of the six known species of the genus *Nannochloropsis*, found mostly in marine ecosystems, but can also occur in fresh and brackish water. The *Nannochloropsis* genus has only chlorophyll-a and completely lacks chlorophyll-b and chlorophyll-c [15]. *Nannochloropsis gaditana* is considered a promising alga that can be used for industrial applications for its ability to accumulate proteins, lipids, and mainly high levels of polyunsaturated fatty acids (PUFAs) [11]. Due to these features, there is indeed a growing interest in using *Nannochloropsis* as a functional ingredient for human nutrition.

Currently, *Chlamydomonas* sp. is not approved for food applications in the European Union. Nevertheless, an oral toxicity study based on 28-day repeated-dose of dried biomass in male and female rats (ranged from 1 to 4 g/kg bw/day), revealed no mortality or treatment-related adverse [38]. Furthermore, some species of the genus *Chlamydomonas* have been studied for the production of nutrients of interest [2]. To the best of our knowledge, this work represents for the first time *Chlamydomonas* sp. as a food ingredient.

Considering the functional properties of the previously mentioned microalgae, a new healthy and structurally fortified gluten-free bread was designed with *Nannochloropsis gaditana* L2 and *Chlamydomonas* sp. EL5 biomass. Here we investigate the minimum and maximum effective dose of microalgae biomass (1% and 3% w/w) that can bring functional properties without much alteration of sensorial characteristics [5,25].

The technological, nutritional, and sensory properties of both gluten-free dough and bread, with respect to microalgae type and concentrations, were determined in order to evaluate the potential use of microalgae biomass as a natural and sustainable food ingredient in gluten-free products.

2. Materials and methods

2.1. Microalgae production

Experiments were performed with the microalgae species: *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2 which were isolated from different saline sites situated in Tunisia [31] and maintained in LIP-MB laboratory (National Institute of Applied Sciences and Technologies (INSAT)). Both strains were cultivated in artificial seawater (ASW) media. Cultures were carried out at the LNEG Lumiar campus (Lisbon, Portugal) in 20 L polycarbonate vertical column reactors in batch mode, at 25 °C, under continuous illumination, with an average light intensity of 3.2 klux (measured with a Phywe Lux-meter). The cultures were continuously supplied with filtered air with aquarium air pumps, through an air diffuser centered at the bottom.

The microalgae biomass was harvested using centrifuge Sigma 6-16KS, Germany at 13000 $\times g$ for 10 min at 4 °C and freeze-dried (Heto Power Dry LL3000, Thermo Fisher Scientific, US).

2.2. Microalgae biomass characterization

The biochemical composition of the microalgal biomass was determined in terms of proteins, carbohydrates, and fatty acids. Moisture was determined gravimetrically by drying the biomass at 105 °C.

Carbohydrate content was determined using the phenol-sulfuric acid method [19] following quantitative acid hydrolysis extraction. Protein content was estimated using the Lowry method [33] in samples previously boiled with 4 mL of NaOH 0.1M.

Fatty acid methyl esters (FAMEs) were prepared based on ISO 5509:2000 [22] (boron trifluoride method). The obtained samples were analyzed using gas chromatograph (CP-3800 GC,Varian, USA), equipped with a 30 m SUPELCOWAX 10 capillary column (0.32 mm of internal diameter and 0.25 μm film thickness). Carrier gas, He, was kept at a constant rate of 3.5 mL/min. The injector and detector (flame ionization) temperatures were kept constant at 250 and 280 °C, respectively. The split ratio was 1:50 for the first 5 min and 1:10 for the remaining time. The column temperature started at 200 °C for 8 min, then increased to 240 °C, at a rate of 4 °C/min, and kept constant at this temperature for 16 min.

Total pigments were quantified by spectrophotometry (Hitachi-2000) after extraction with 90% (*v*/v) acetone. Spectra were run between 380 and 700 nm. Calculations were performed using the Beer Lambert equation (Eq. 1) with a value of 215 L/(g·cm) for the specific optical coefficient at the wavelength of the maximum absorbance of the samples [28].

$$Total\ Pigments\ (\%) = \frac{A \times V \times f}{E_{1cm}^{1\%} \times m} \quad (1)$$

where A is the absorbance (at the wavelength of maximum absorption), V is the total volume of the pigment extract (mL), f is the dilution factor, $E_{1cm}{}^{1\%}$ is the extinction coefficient, and m the weight of the sample (g). The extinction coefficient used was based on an average of the $E_{1cm}{}^{1\%}$ of the carotenoids mainly found in microalgae, according to Gouveia & Empis [27].

2.3. Gluten-free breads preparation

The control gluten-free dough was prepared using: 31% rice flour (Espiga, Portugal), 46% buckwheat flour (Próvida, Portugal), 23% potato starch (Globo, Portugal), 4.6% hydroxypropylmethylcellulose (HPMC, WellenceTM 321, Dow, Germany), 2.8% dehydrated yeast (Fermipan®, Portugal), 2.8% sugar, 1.8% salt, and 5.5% sunflower oil. Microalgae biomass were incorporated in the mixture at 1.0 and 3.0 g/100 g of rice, buckwheat flour, and potato starch. The quantity of water (water absorption at 14% moisture basis) was adjusted using a micro-doughLAB (Perten Instruments, North Ryde, Australia), and the measurements were fixed at 69% (based on preliminary analysis). The yeast was mixed first with the sugar and warmed water for 2 min at 37 °C. The ingredients were combined all together in a bowl and mixed for 10 min with a hand blender. Gluten-free doughs (50 g) were placed in a rectangular recipient and left to ferment for 50 min at 40 °C in an electric oven (Arianna XLT133, Unox, Italy). Baking was carried in Johnson A60 oven (Johnson & Johnson, USA) at 180 °C for 30 min. After cooling at room temperature, breads were packed in a plastic and

sheltered from light. Physical analysis were done on the day of baking (color, texture, and water activity - a_w) and bread samples were crushed and frozen for further chemical characterization.

2.4. Gluten-free bread characterization

2.4.1. Analysis of mixing properties

The mixing properties of the flours were determined using a Newport micro-doughLAB mixer. The amount of water required (expressed as water absorption of the flours or the mixture of flours and microalgal biomass at 14% moisture basis) to achieve an acceptable dough consistency was previously optimized then fixed. The standard manufacturer's protocol "General Flour Testing Method" was used. The peak resistance of the optimized control-formulation was used, as a reference, to assess the optimum water absorption for each gluten-free bread formulation with microalgal addition [8]. Samples (4 g ± 0.01) were assessed at 30 °C during 20 min of mixing (63 rpm), for peak resistance (maximum torque, mNm), dough development time (DDT, the time at which the maximum torque is reached, s), softening (the difference in torque between the maximum torque and the final torque, mNm), and stability (the difference between the arrival and departure times, related to the flour tolerance to mixing, s).

2.4.2. Texture analysis

The gluten-free dough and bread texture was measured using a texture analyzer TA.XTplus (Stable MicroSystems, UK) with a cylindrical probe of 10 and 19 mm diameter (for bread and dough, respectively). Fermented doughs and bread slices of 2 cm were characterized using a Texture Profile Analysis (TPA) in penetration mode (20 mm distance, 5 s of waiting time and 1 mm/s of crosshead speed), from which texture parameters were determined: maximum resistance to penetration, considered as firmness (N), adhesiveness (−N.s), and cohesiveness. The TPA method, also known as two-bite test, was previously described by Raymundo et al. [49]. Measurements were repeated, at 20 ± 1 °C, at least four times for each formulation sample.

2.4.3. Color measurement

The color of the dough and the bread's crust and crumb were instrumentally determined using a Minolta CR-400 (Japan) colorimeter with standard illuminant D65. The results were expressed in CIELab system. The total color difference between different tested samples was measured as follow:

$$\Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2} \quad (2)$$

where L* corresponds to lightness (values increase from 0 to 100); a*, greenness to redness (−60 to 60); and b*, blueness to yellowness (−60 to 60 All measurements were conducted on the baking day, under similar light conditions, at room temperature, and replicated five times in duplicate.

2.4.4. Quality parameters: water activity, pH, and weight loss

The gluten-free bread water activity (aw) was determined through an HygroPalm HP23-AW (Rotronic AG, Switzerland), at 20 ± 1 °C. Measurements were done four times in duplicate for each formulation (crushed powder).

The dough's pH was measured using a pH-Meter Basic 20 (Crison instruments, Alella, Spain) with a penetration probe adequate for solids.

Weight loss (WL) was calculated as the difference between the weight of the dough after fermentation and the weight of the obtained bread, expressed as a percentage.

2.4.5. Chemical characterization assessment

The moisture content was measured gravimetrically through an automatic moisture analyzer PMB 202 (aeADAM, Milton Keynes, UK) at 130 °C. Total ash content was determined by incineration at 550 °C in a muffle for 24 h. Crude protein (N × 5.7) was determined by the Kjeldhal method according to the AOAC 950.36 official method [1]. Lipid content was determined by Soxhlet, according to the Portuguese standard method NP4168 [42] and as detailed by Batista et al. [5]. The carbohydrate content was calculated as the difference between the protein, lipid, ash, and moisture contents. Fatty acids were determined by gas chromatography (GC) as detailed previously. All analyses were repeated at least in duplicate.

2.4.6. Sensorial evaluation

Thirty untrained panelists, 9 males and 21 females, aged between 21 and 64, conducted sensory evaluation of the control bread and bread with 3% w/w microalgal biomass. A hedonic evaluation was performed, following the protocol previously described by Batista et al. [5] and commonly used by "LEAF- Instituto Superior de Agronomia, Portugal- Team". Bread samples were assessed for the following attributes: color, odor, taste, texture, and global appreciation (5 levels from "very pleasant" to "very unpleasant"). Panelists were also asked whether they would buy the tested bread (from "would certainly buy" to "certainly wouldn't buy"). The individuals who participated were provided with informed consent materials following in accordance with the ethical standards of the local committee responsible for human experimentation and with The Code of Ethics of the World Medical Association (Declaration of Helsinki of 1975, as revised in 2013). The objective of the analysis and the rules that should be respected during the sessions of analysis were clearly explained to the panelists through a letter of information. Samples were randomly distributed, and the panelists were invited to sufficiently cleanse their palates with water between each sample. The panelists were asked to write, in a commentary section, at the end of the sensory analysis sheet, supplementary remarks related to the product accompanied with their signature. The assays were conducted in appropriate sensory analysis room, according to the standard EN ISO 8589.

2.5. Statistical analysis

Variance analysis (one way ANOVA) of the experimental data was done using Origin Pro 8.0 software (OriginLab Corporation, MA, USA), using Tukey's test, at a significance level of 95% ($p < 0.05$).

3. Results and discussion

3.1. Effects of microalgae addition on mixing properties

In this work, the addition of *Nannochloropsis gaditana* L2 and *Chlamydomonas* sp. EL5 on the techno-functional and nutritional properties of gluten-free bread was investigated. On the first step, the biochemical composition of the used microalgae was assessed. As shown in Table 1, the two strains presented a suitable and similar

Table 1
Biochemical composition of the microalgae biomass used in the experiments (%, Dry weight).

	Moisture (%)	Protein (%)	Carbohydrate (%)	Total pigments (%)
Chlamydomonas sp. EL5	7.5[a]	21.9[a]	22.2[a]	0.8[a]
Nannochloropsis gaditana L2	7.5[a]	21.8[a]	25.9[a]	0.6[a]

Values are the average of 2–3 replications. Means followed by the same small letter in the column did not differ significantly based on Tukey's test ($p > 0.05$).

Table 2
Effect of microalgae addition on the mixing behavior parameters of gluten-free dough: Water Absorption (WA), Peak resistance in (mN·m), Dough Development Time (DDT), Softening and Stability.

Samples	WA (%)	Peak (mN·m)	DDT (s)	Softening (mN·m)	Stability (s)
Control	69	$56.33^{a} \pm 1.15$	$48.00^{a} \pm 0.00$	$9.00^{a} \pm 2.00$	$30.00^{a} \pm 0.10$
Chlamydomonas sp. EL5 1%	69	$56.00^{a} \pm 1.73$	$48.00^{a} \pm 0.00$	$9.67^{a} \pm 2.08$	$32.00^{a} \pm 0.12$
Chlamydomonas sp. EL5 3%	69	$57.00^{a} \pm 5.20$	$52.00^{a} \pm 0.06$	$11.67^{a} \pm 3.51$	$40.00^{a} \pm 0.12$
Nannochloropsis gaditana L2 1%	69	$56.33^{a} \pm 2.08$	$52.00^{a} \pm 0.06$	$8.67^{a} \pm 1.53$	$36.00^{a} \pm 0.00$
Nannochloropsis gaditana L2 3%	69	$54.33^{a} \pm 0.58$	$50.00^{a} \pm 0.06$	$7.00^{a} \pm 1.73$	$38.00^{a} \pm 0.15$

Values are means ± standard deviation (n = 3). Means followed by the same small letter in the column did not differ significantly based on Tukey's test ($p > 0.05$).

($p > 0.05$) biochemical composition in term of moisture, protein, carbohydrate, and total pigments.

The mixing behavior of the different doughs (control and with microalgal biomass) was thereafter evaluated, using the micro-doughLAB equipment. It is noteworthy that studies that provide data about gluten-free dough behaviors using a micro-doughLAB instrument are limited. Starting from this consideration and to better evaluate the effect of microalgae incorporation on the dough properties, preliminary experiments were conducted at a laboratory scale to identify the best formula that would serve as control with a sustainable dough consistency and acceptable bread texture. Similar to the control dough, water absorption was fixed at 69% for all the mixtures that were tested in the micro-doughLAB. As the dough is developed, its resistance to kneading was measured as Torque, as presented in Fig. A1 as a plot against time. From the mixing curves (Fig. A1) it is possible to obtain different rheological parameters as shown in Table 2.

As can be seen in Fig. A1, dough mixing curves were obtained with no difference in shape for the different type and amount of microalgae added and for the control. No significant variation was observed ($p > 0.05$) between the control and the doughs supplemented with microalgae, when comparing the different mixing parameters noted in Table 2. All the doughs developed an average torque of 56.3 ± 2.25 mN·m (peak torque is within 4% of the target torque-control). Therefore, it was not necessary to adjust the water absorption values for doughs enriched with microalgal biomass.

Dough development time (DDT) in different batches was found to be in the range of 48.00 to 52.00 s, being minimum for the control sample. Softening and Stability recorded a slight variation ranging between 7–9 and 30–38 mN·m, respectively, and considered insignificant at the 0.05 level ($p > 0.05$, Tukey's test). Among the three parameters, dough stability was apparently impacted ($p > 0.05$) by microalgae addition and marked the highest value with the 3% w/w *Chlamydomonas* sp. EL5 enriched dough (40.00 ± 0.12 s). It is worth mentioning that dough stability, dough development time, and softening have been significantly correlated with protein content and gluten characteristics [4,14]. This highly justified the low values of mixing parameters obtained in this study, 52 s, 11.67 mN·m, and 40 s, (the highest values obtained with 3% w/w *Chlamydomonas* sp. EL5 for DDT, softening, and stability, respectively). The values for DDT and dough stability have been reported to vary between 1.23 and 1.76 min and 1.43 to 9.13 min, respectively, in different wheat-based flours [4]. According to Mohamed et al. [37], most commercial bread wheat-flours have a stability value of up to 10 min. In the case of wheat-flours, the parameters of mixing time and stability usually reflect bread performance (specific volume and overall texture) after the baking process [56]. Assessing the correlations between dough mixing parameters and bread quality parameters lead to a better selection of mixing parameters reflecting protein quantity and quality, which in turn permits to predict the product manufacturing characteristics [46]. However, in the case of gluten-free flours, there are no standard methods and values for the optimization of mixing parameters. As previously mentioned, in this research work water absorption optimization was conducted by assessing how the bread performed during preliminary trials. As explained above, a 69% water absorption was adequate for obtaining an acceptable dough's consistency and a good bread performance. This suggests that mixing parameters herein recorded could be useful for future research when using the same type of flours. Besides, results showed that microalgae supplementation may contribute to a slight improvement ($p > 0.05$) in the dough's stability. This opens further investigation on the mixing behavior of gluten-free flours made with different microalgae strains and different level of addition.

3.2. Structural properties of dough and bread formulations

Texture behavior of gluten-free dough and bread tested formulations was evaluated on the day of baking through a texture profile analysis (TPA). The results were interpreted in terms of firmness (N), adhesiveness (-N·s), and cohesiveness, and are shown in Fig. 1 and Fig. 2, for both dough and bread. From Fig. 1, it can be observed that all microalgae enriched doughs showed values of firmness not significantly different ($p > 0.05$) from the control dough (3.9 ± 0.2 N). The two other TPA parameters did not show any noticeable variation between different formulations. Dough adhesiveness ranged between 34.2 ± 2.1 and 38.6 ± 2.0 -N·s, being the highest in 3% w/w *N. gaditana* L2 sample. All cohesiveness values were < 1 (0.9). Compared with the control sample, the effect of microalgae addition on dough texture parameters was considered insignificant ($p > 0.05$). Texture measurements of the doughs were in accordance with the microdough-LAB results. Results herein reported from the microdough-LAB and the texture analyzer can help predict the impact of microalgae supplementation (1 to 3% w/w) on the gluten-free bread as those parameters are significantly related to the consumer's judgment on bread freshness [53].

Microalgae addition was found, however, to bring an overall significant impact ($p < 0.05$) on gluten-free bread texture (Fig. 2). Due to its poor texture, gluten-free breads are known to break easily in the mouth, which generally makes them less tasty. Loaves evaluated in this study significantly increased ($p < 0.05$) in crumb firmness and adhesiveness as microalgal biomass incorporation increased. The control sample had the lowest crumb texture (3.3 ± 0.9 N; 0.7 ± 0.1 -N·s, for firmness and adhesiveness, respectively), while samples containing 3% w/w of microalgae had the highest bread texture parameters, particularly for 3% w/w *Chlamydomonas* sp. EL5 addition (26.6 ± 2.5 N; 8.1 ± 0.6 -N·s, for firmness and adhesiveness, respectively). Cohesiveness, which characterizes the extent to which the product recovers the deformation before its ruptures, was not significantly ($p > 0.05$) affected by microalgae incorporation, compared with the control bread.

As gluten-free bread crumbles easily, i.e., the increase in firmness and adhesiveness parameters, as a result of microalgae addition can be considered an encouraging result since it made the bread stronger in terms of texture. This can be confirmed by a sensory analysis, which examines the consumers' perceptions of these textural changes.

The enhancement of the texture parameters was probably caused by the presence of substantial quantity of protein in microalgal biomass (Table 1). Conte and co-workers [54] concluded in a previous study that the texture of a gluten-free bread improved as a result of incorporating different amounts of bee pollen that contained an important level of proteins (20.6%). Proteins are one of the most

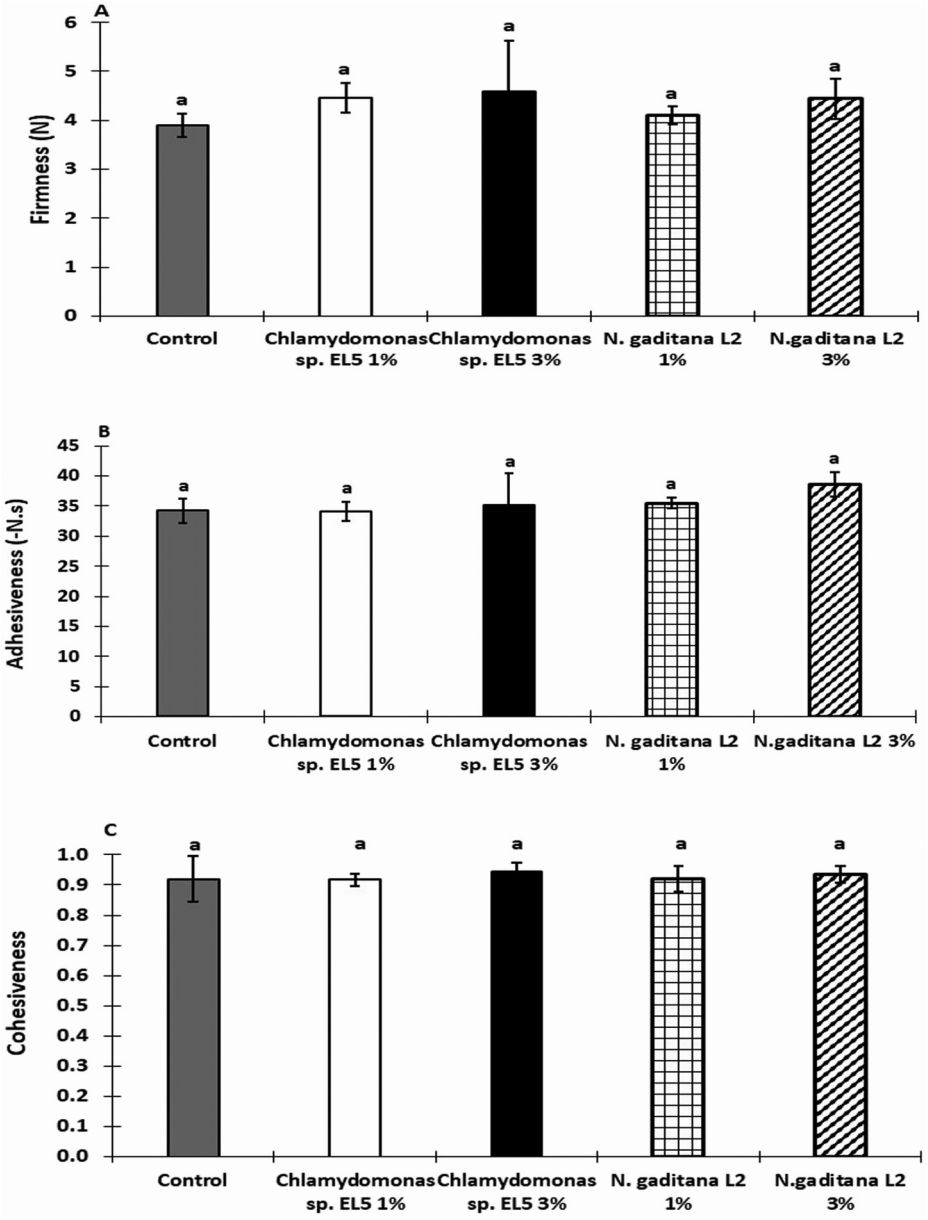

Fig. 1. Structural properties ((A) firmness, (B) adhesiveness and (C) cohesiveness) of gluten-free dough enriched with 1 and 3% w/w of *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2 biomass. Values are means ± standard deviation ($n = 4$). Means followed by the same small letter did not differ significantly based on Tukey's test ($p > 0.05$).

important components that are used to reproduce some of the gluten's properties. Thus, the use of microalgae as a source of protein (bulk protein) can be a beneficial alternative to humans, to animals and to the environment [12]. In addition, it is widely known that microalgal starch is of great importance and may play a substantial role in boosting rheological and baking properties through its filling function [41]. In a previous study, variations in firming kinetics were reported as a result of a synergic effect between proteins and gelatinized starch through the presence of hydrogen bonding between them [34]. A significant change in texture was found after 3% w/w microalgae supplementation, while a milder effect on bread texture was marked with 1% w/w microalgae addition and thus considered insignificant ($p > 0.05$). In some earlier studies, it was found that the effect of microalgal biomass incorporation to the bread texture properties was strongly related to the concentrations used for supplementation. Indeed, below 3% w/w of added biomass, no textural variation was recorded [24,29,52].

3.3. Dough and bread color

The impact of microalgae addition (1 and 3% w/w) on dough color parameters is presented in Fig. 3. Dough color was significantly affected ($p < 0.05$) by microalgae addition, and its impact was more significant for the highest level of supplementation. The lightness (L*) parameter recorded a slightly significant ($p < 0.05$) decrease with microalgae addition at 3% w/w, being *N. gaditana* L2 (3%) the darkest one ($L^* = 44.19 \pm 0.89$).

As expected, the a* parameter was considerably affected by microalgae addition, and the greenness color of dough was achieved by 3% w/w *Chlamydomonas* sp. EL5 (-18.94 ± 0.38). A similar effect was observed for the b* (yellowness) parameter. The increase in yellowness was also the result of the gradual increase of microalgae supplementation. As for the dough, the effect of microalgae addition on bread crumb and crust color parameters was evaluated. The results of this evaluation are summarized in Fig. 4 and Fig. 5, respectively.

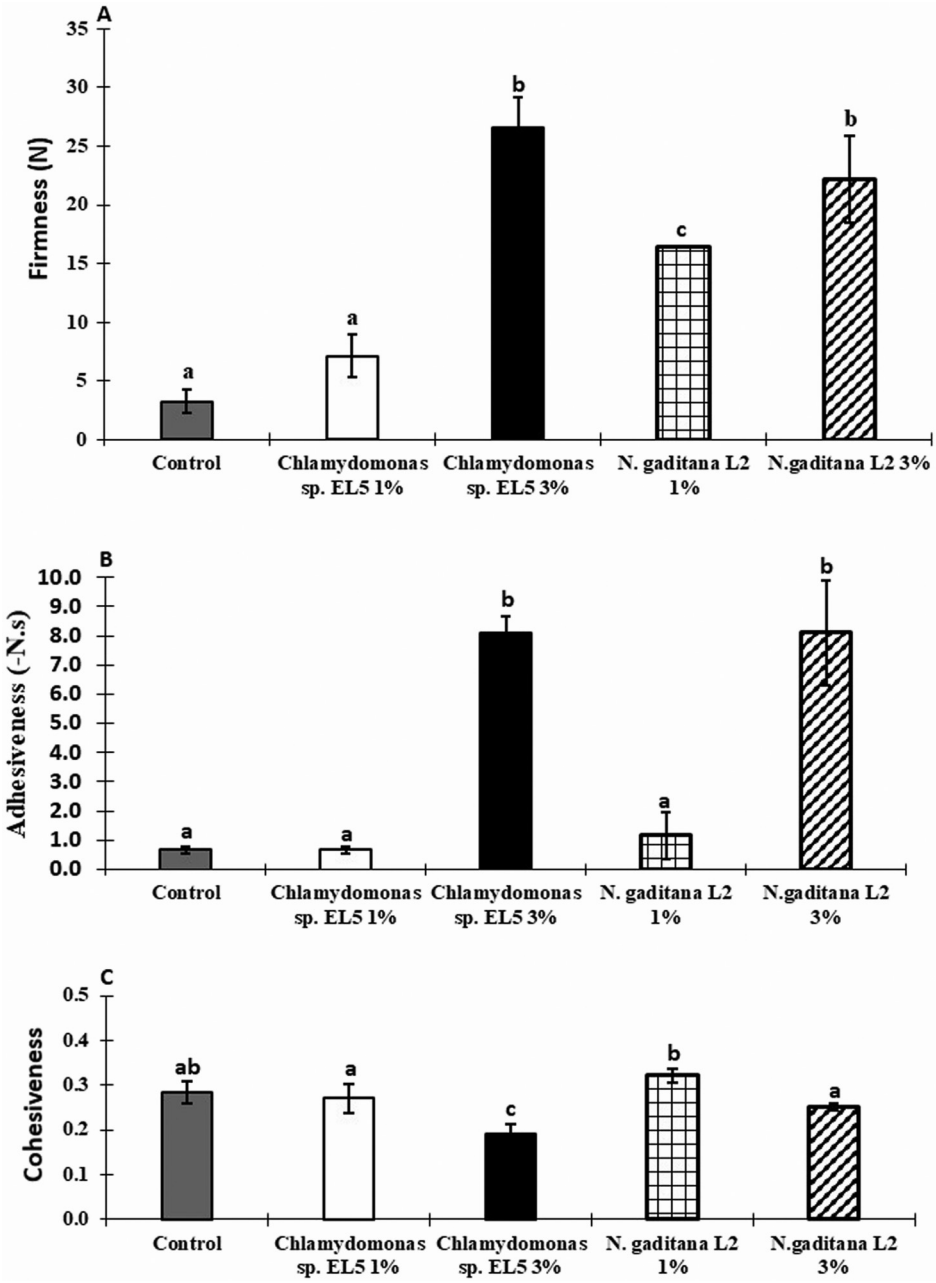

Fig. 2. Structural properties ((A) firmness; (B) adhesiveness; (C) cohesiveness of gluten-free bread enriched with 1 and 3% w/w of *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2 biomass. Values are means ± standard deviation (n = 8). Means followed by the same small letter did not differ significantly based on Tukey's test ($p > 0.05$).

Concerning the crumb, a significant reduction in lightness with more intense green (a* negative) and yellow (b* positive) color was observed as a result of microalgae incorporation, in comparison with the control (Fig. 4). In a previous study conducted by García-Segovia et al. [26], the use of four green microalgae (*Isochrysis galbana*, *Tetraselmis suecica*, *Scenedesmus almeriensis,* and *Nannochloropsis gaditana)* significantly colored the bread crumb. Różyło et al. [52] recorded a decrease in whiteness (L*) in gluten-free bread crumb color due to the use of different amounts of brown algae.

Concerning the variation in crust bread color, samples enriched with microalgae showed the lowest L* values, which was significantly associated with the level of addition. A 3% w/w incorporation of *Chlamydomonas* sp. EL5 caused a 50% decrease in L* values, compared with the control (Fig. 5). The 1% w/w incorporation of both microalgal biomass led to a significant decrease in redness values (positive a* values) which switched to green color when the amount of microalgal biomass was increased by 3% w/w.

Generally, the increase in bread and dough coloration depends on the presence of pigments in microalgal biomass, particularly chlorophyll content that characterizes green microalgae. Indeed, the darkening that was observed in both crumb and crust and that was accentuated by the degradation of microalgae pigments can be considered as a positive impact since gluten-free breads are generally characterized by a poor color compared with gluten-containing breads. Moreover, total color differences (ΔE*) were assessed in all samples (dough and bread) (Table 3), and showed a significant increase ($\Delta E^* > 5$), as a result of microalgae incorporation, which means that the gluten-free bread color differences are enough to be detected by the human eye [5].

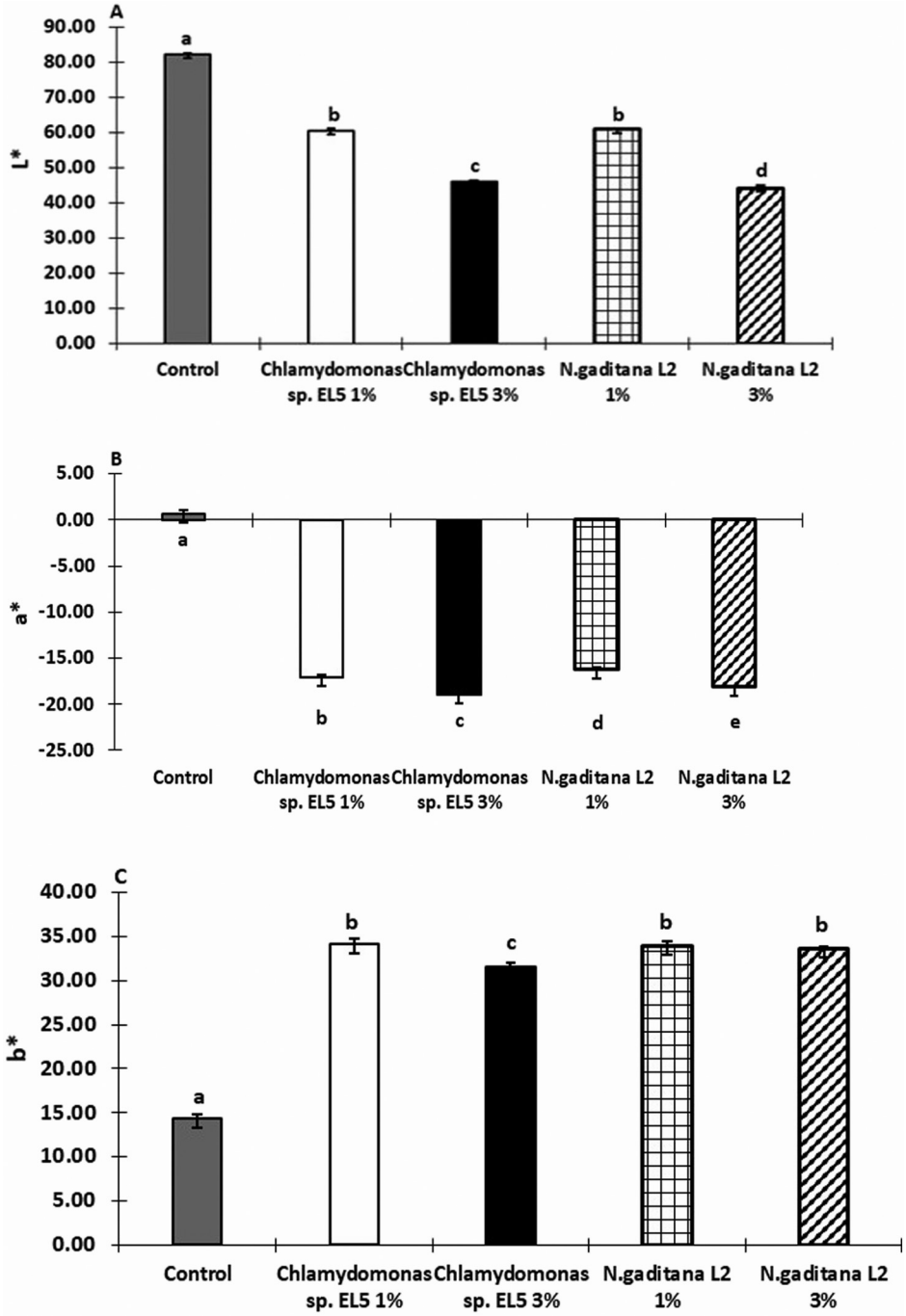

Fig. 3. Effect of microalgal biomass incorporation on color parameters ((A) Lightness (L*); (B) greenness (a*); (C) yellowness (b*)) of gluten-free dough. Values are means ± standard deviation ($n = 8$). Means followed by the same small letter did not differ significantly based on Tukey's test ($p > 0.05$).

3.4. Quality properties

Quality properties such as dough pH and bread water activity are of great importance as they determine the quality and the shelf life of the final product [52]. As can be seen in Table 4, the pH of the control dough before fermentation was 5.77 ± 0.09 and changed significantly when the amount of microalgal biomass was increased by 3% w/w (6.05 ± 0.04; 6.01 ± 0.01, for *Chlamydomonas* sp. EL5 and *N. gaditana* L2 respectively). The values of pH recorded in this study were slightly higher than those of gluten-free dough that was enriched with different amounts of brown algae [52]. It is necessary to determine the dough's pH since it influences the pH of the bread. In fact, when flour was replaced by microalgae, the source of starch was reduced. Consequently, there was less source of glucose for fermentation. For this reason, the pH reduction was less pronounced. The greater the effect was, the greater the amount of flour that would be replaced by microalgae should be (Table 4). In a previous work, neither the pH of the dough nor the pH of the bread was determined [24].

Water activity (a_w) was significantly related to the added quantity of microalgal biomass and in a species-specific manner. The control bread's water activity presented a value of 0.83 ± 0.01. The highest water activity was recorded in the bread containing 3% w/w of *N. gaditana* L2 (0.89 ± 0.02), which was lower than the previously-recorded value in the wheat bread that was enriched by 1.5% of *Nannochloropsis gaditana* biomass [26]. A slight reduction ($p < 0.05$) in moisture content was noticed in samples with microalgal biomass,

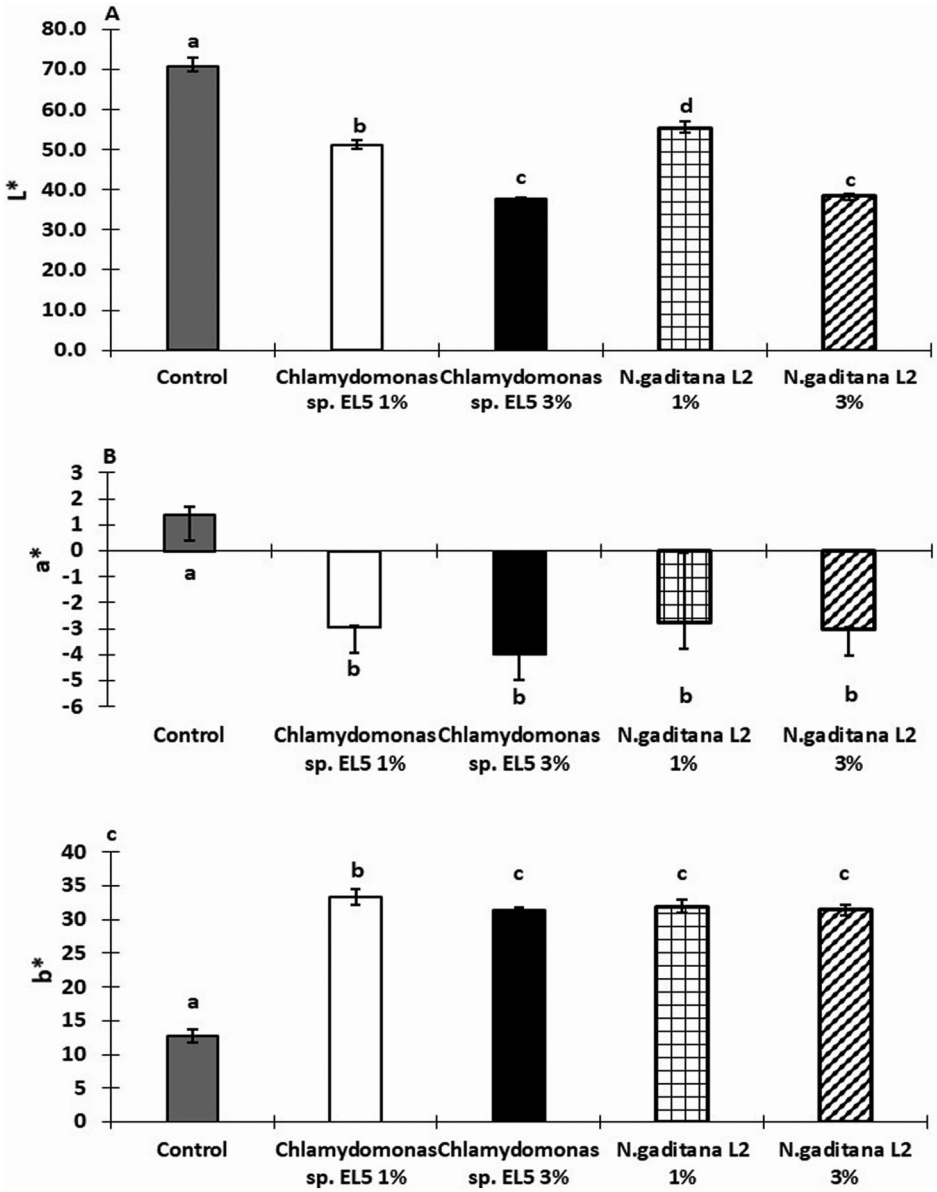

Fig. 4. Effect of microalgal biomass incorporation on color parameters ((A) Lightness (L*); (B) greenness (a*); (c) yellowness (b*)) of gluten-free breadcrumb. Values are means ± standard deviation ($n = 8$). Means followed by the same small letter did not differ significantly based on Tukey's test ($p > 0.05$).

(being highest with 3% w/w *Chlamydomonas* sp. EL5 (39.7%)) compared with the control (40.6%). The added level used in this study did not have any impact on baking weight loss, since the values were more or less the same in all the assessed samples (ranging between 15.6% to 16.6% in control bread and bread with *N. gaditana* L2 biomass, respectively).

3.5. Biochemical composition of breads

Besides their poor texture characteristics, gluten-free breads are characterized by their inadequate nutritional quality. The average composition of the control bread used in this study was 40.6% moisture, 48.7% carbohydrates, 5.6% protein, 3% fat, and 1.9% total ash (Table 5). The proximate nutritional profile of the control sample was in the range of the composition reported in Naqash et al.'s [40] study. Except for the carbohydrate content, which did not register any remarkable variation, gluten-free breads with microalgal biomass presented significant differences in all the assessed chemical parameters. Moisture values ranged from 37.0% to 40.6%, with the addition of 1% w/w *N. gaditana* L2 and *Chlamydomonas* sp. EL5 biomass, respectively, leading to a significant ($p < 0.05$) reduction in moisture content. The parameters that were mostly affected by microalgal enrichment were the ash and protein contents, even at a low addition level. Concerning the protein, microalgae breads always possessed the highest protein content (6.1 and 6.6% with 1% and 3% microalgal biomass, for both species, respectively). Figueira et al. [24] recorded an increase of 16% in protein content of gluten-free bread samples with 3% *Arthrospira platensis* (*Spirulina*) biomass, which is lower than the increase noticed in the present study (18%) with 3% w/w *Nannochloropsis gaditana* L2 and *Chlamydomonas* sp. EL5. Menezes et al. [36] have used a mixture of two-macroalgae genus (*Ulva* sp. and *Cladophora* sp.) at 7.5% in wheat bread to achieve only a 12% increase in protein content.

The ash content showed an unprecedented increase as a result of microalgae incorporation, being the highest with 3% w/w *Chlamydomonas* sp. EL5 biomass (2.4%). No significant difference was registered among the used species. Not only the total ash content but also the amounts of the essential microelements were significantly affected by microalgae addition. The most positive impact of microalgae

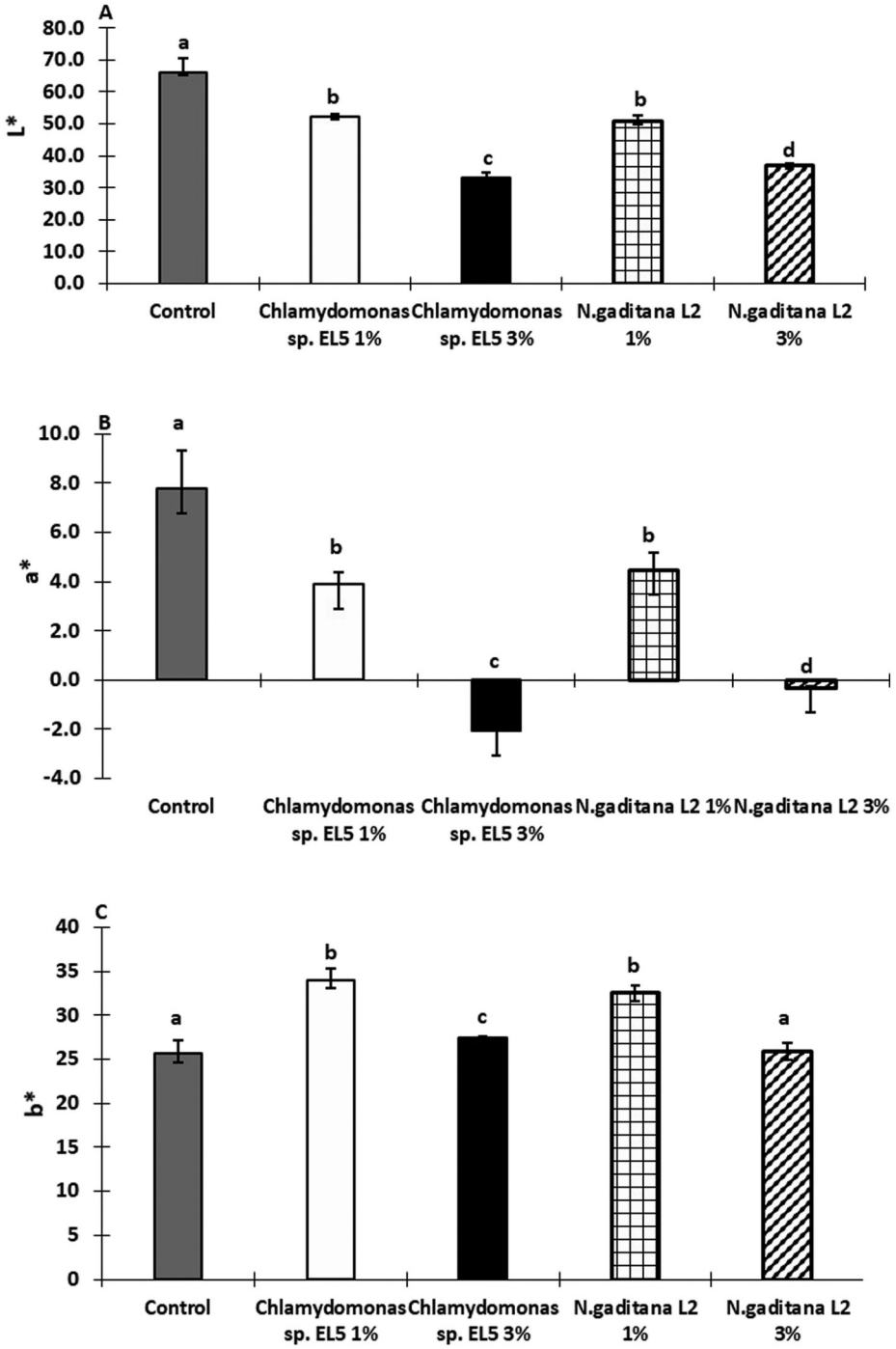

Fig. 5. Effect of microalgal biomass incorporation on color parameters ((A) Lightness; (B) greenness; (c) yellowness) of gluten-free bread crust. Values are means ± standard deviation (n = 8). Means followed by the same small letter did not differ significantly based on Tukey's test ($p > 0.05$).

Table 3
Total color difference (ΔE*), between different raw and cooked samples: Bread crust; Bread crumb; Dough (EI5- *Chlamydomonas* sp. L2- *Nannochloropsis gaditana*).

ΔE*	Bread crust				Bread crumb				Dough			
	EL5 1%	EL5 3%	L2 1%	L2 3%	EL5 1%	EL5 3%	L2 1%	L2 3%	EL5 1%	EL5 3%	L2 1%	L2 3%
Control	16.53	34.53	17.24	30.16	28.62	38.32	24.90	37.57	34.22	44.71	33.46	46.52
EL5 3%	21.23	–	–	4.62	13.69	–	–	1.23	15.05	–	–	2.79
L2 1%	2.28	–	–	15.90	4.39	–	–	16.94	0.93	–	–	16.77

Table 4
Effect of microalgae addition on basic properties of gluten-free bread: pH of dough before fermentation, moisture, water activity (a_w) and Weight Loss (WL).

Samples	pH	Moisture	a_w	WL (%)
Control	$5.77^{a} \pm 0.09$	$40.6^{a} \pm 0.1$	$0.83^{a} \pm 0.01$	15.6^{a}
Chlamydomonas sp. EL5 1%	$5.75^{a} \pm 0.01$	$37.3^{b} \pm 1.8$	$0.83^{a} \pm 0.01$	15.5^{a}
Chlamydomonas sp. EL5 3%	$6.05^{b} \pm 0.04$	$39.7^{a} \pm 0.7$	$0.86^{b} \pm 0.02$	16.1^{a}
Nannochloropsis gaditana L2 1%	$5.87^{c} \pm 0.01$	$37.3^{b} \pm 1.8$	$0.88^{c} \pm 0.02$	16.6^{a}
Nannochloropsis gaditana L2 3%	$6.01^{b} \pm 0.01$	$40.2^{a} \pm 1.6$	$0.89^{c} \pm 0.02$	16.6^{a}

Values are means ± standard deviation ($n \geq 3$). Means followed by the same small letter in the column did not differ significantly based on Tukey's test ($p > 0.05$).

addition was observed in iron and calcium (Table 6). Breads with 1% w/w microalgae samples ranged from 24 to 28 mg/100 g and 3.8 to 5.6 mg/100 g calcium and iron, respectively, while 3% w/w microalgae breads contained 51.4 to 51.6 mg calcium/100 g and 8.3 to 10.5 mg iron/100 g. Compared with the control, the incorporation of 3% w/w microalgae biomass in the tested gluten-free recipe caused more than 100% increase in iron and calcium contents (Table 6). Regardless of the strain used, 1% w/w of microalgae addition was enough to bring more than 15% of the recommended daily value in iron (Regulation (European community)), No. 1924/2006; Directive No. 90/494 (CE) (Table 6)).

More than a half of the population in the world suffers from micronutrients deficiency, particularly of Fe and Ca [35]. The presence of some essential elements is mandatory for the body's good health and functioning. Actually, it is stated that iron plays a crucial role in increasing the physical performance in all ages and boosts the mental development in children under 6 years old [35,55]. Nutritionists believe that 50% of anemia is due to insufficient dietary intake of iron [35]. An inadequate intake of iron is always associated with weakness feeling, pallor, delayed cognitive and memory deficits, some with persistent long-term effect [6,39]. On the other hand, several studies have drew the attention on the effect of adequate calcium intake on blood pressure reduction [7], the prevention of hypertensive disorders of pregnancy, osteoporosis, and colorectal adenomas [30,44,45], in addition to its well-known role on bone health [51]. Deficiency in essential elements is mostly linked to global poor nutrition and an undiversified diet. Celiac disease is one of the health conditions known to be associated with decreased minerals absorption [13]. Hence, the enhancement of gluten-free formula with microalgal biomass presents a good alternative to the issue of under-intake of essential elements.

The crude lipid content in the samples was significantly ($p < 0.05$) associated with the level of microalgal biomass added to the mixture. Using 3% w/w biomass from both used species increased the lipid level in the bread by 50% (Table 5). The main fatty acids present in the bread control – the candidates strains – and the bread samples that were enriched by 1% and 3% *w*/w microalgae are presented in Table 7.

The fatty acid profile of *Chlamydomonas* sp. EL5 and *N. gaditana* L2 was dominated by polyunsaturated fatty acids (PUFAs) (38.9% and 40.6% respectively), followed by saturated (SFA) (25.7 and 23.4%, respectively), and monounsaturated fatty acids (MUFAs) (3.2 and 6.9%, respectively). Among the PUFAs, the linoleic acid (18:2 ω6) marked the highest value (33.4 and 36% for *Chlamydomonas* sp. EL5 and *N. gaditana* L2, respectively), followed by linolenic acid (18:3 ω3) (5.5 and 4.6% for *Chlamydomonas* sp. EL5 and *N. gaditana* L2, respectively). The oleic acid (18:1ω9) is the major MUFA (2.3 and 5.9% for *Chlamydomonas* sp. EL5 and *N. gaditana* L2 respectively), and the palmitic acid (16:0) is the main SFA, representing 18.6% in *Chlamydomonas* sp. EL5 and 16.6% in *N. gaditana* L2. The results reported in the present study are in agreement with those mentioned in previous study [31].

In all the assessed bread samples (control and enriched ones), the PUFAs possessed the highest fraction (> 50% of total identified fatty acids), particularly in the 3% w/w *Chlamydomonas* sp. EL5 enriched bread where the linoleic acid (18:2ω6) is the major PUFA (53.5%). Palmitic acid (16:0) was the main SFA present in control and supplemented bread samples followed by stearic acid (18:0). The oleic acid (C18:1ω9) is the only MUFA present in the enriched breads, being the highest in the sample with 1% w/w *Chlamydomonas* sp. EL5 biomass. However, the palmitoleic acid (16:1 ω7) was present at low level in the control bread and was not detected in breads produced whether by 1% or 3% w/w *Chlamydomonas* sp. EL5 and *N. gaditana* L2 biomass.

The fatty acid profile of the breads was affected by the level of addition and the used strain. Indeed, a substitution of 1% w/w of the mixture by *N. gaditana* L2 biomass did not have any significant effect ($p > 0.05$) on the fatty acid composition compared to the control. On the contrary using 1% w/w, *Chlamydomonas* sp. EL5 was sufficient to influence the fatty acid composition of the tested mixture. A particular increase in linolenic acid (18:3 ω3) content was registered due to the microalgae incorporation, even at a low level, being the highest in gluten-free bread with 3% w/w *Chlamydomonas* sp. EL5 (2.8%). Since linolenic acid (18:3 ω3) plays an important role in preventing several diseases [18], its presence in gluten-free bread, in a considerable quantity when compared with standard formula, can be a good option for a healthy state. Furthermore, the remarkable increase in 18:3 ω3 fatty acid helped to obtain gluten-free loaves with around halved ω3/ω6 ratio (1:20; 1:19 in 1% and 3% w/w *Chlamydomas* sp. EL5 samples, respectively), compared with the control sample (1:58). It was observed in previous studies that the incorporation of *Isochrysis galbana*, *Diacronema vlkianum*, and *Undaria pinnatifida* biomass helped to bring down the ratio of ω3 to ω6 in semolina pasta [25,47] . These data evidenced the nutritional benefits of incorporating microalgae biomass in the classical recipe of gluten-free bread.

3.6. Sensorial evaluation

Gluten-free breads prepared with microalgae biomass presented an

Table 5
Main chemical composition (g/100 g) of supplemented and control gluten-free bread: moisture, carbohydrates, protein, lipid, ash, and energy value.

	Moisture (g/100 g)	Carbohydrates* (g/100 g)	Protein (g/100 g)	Lipid (g/100 g)	Ash (g/100 g)	Energy value (Kcal/100 g)
Control	$40.6^{a} \pm 0.1$	$48.7^{ac} \pm 0.3$	$5.6^{a} \pm 0.0$	$3.0^{a} \pm 0.2$	$1.9^{a} \pm 0.1$	244
Chlamydomonas sp. EL5 1%	$37.3^{b} \pm 1.8$	$50.8^{b} \pm 1.9$	$6.1^{b} \pm 0.0$	$3.4^{a} \pm 0.1$	$2.2^{bc} \pm 0.1$	258
Chlamydomonas sp. EL5 3%	$39.7^{a} \pm 0.7$	$46.8^{a} \pm 0.9$	$6.6^{c} \pm 0.0$	$4.2^{b} \pm 0.3$	$2.4^{d} \pm 0.0$	251
Nannochloropsis gaditana L2 1%	$37.3^{b} \pm 1.8$	$51.0^{bc} \pm 0.8$	$6.1^{d} \pm 0.0$	$3.4^{a} \pm 0.0$	$2.1^{b} \pm 0.1$	257
Nannochloropsis gaditana L2 3%	$40.2^{a} \pm 1.6$	$47.0^{a} \pm 1.0$	$6.6^{c} \pm 0.0$	$4.5^{b} \pm 0.1$	$2.3^{cd} \pm 0.0$	251

* Carbohydrates were calculated by difference. Values are means ± standard deviation (n = 3). Means followed by the same small letter in the column did not differ significantly based on Tukey's test ($p > 0.05$).

Table 6
Effect of microalgae addition on mineral composition (mg/100 g) of gluten-free bread.

	K	Ca	Mg	P	S	Fe	Zn
Control	$281.1^{a} \pm 26.0$	$12.0^{a} \pm 0.1$	$80.3^{a} \pm 0.4$	$176.5^{a} \pm 10.6$	$89.5^{ab} \pm 4.7$	$1.9^{a} \pm 0.4$	$1.4^{a} \pm 0.0$
Chlamydomonas sp. EL5 1%	$299.5^{ab} \pm 1.1$	$24.4^{b} \pm 0.2$	$83.0^{a} \pm 1.6$	$187.7^{ab} \pm 8.4$	$100.0^{ab} \pm 7.7$	$3.8^{b} \pm 0.3$	$1.6^{b} \pm 0.1$
Chlamydomonas sp. EL5 3%	$306.8^{ab} \pm 2.4$	$51.4^{c} \pm 0.1$	$94.1^{b} \pm 0.5$	$210.0^{b} \pm 5.9$	$104.0^{ab} \pm 8.8$	$8.3^{c} \pm 0.3$	$1.9^{c} \pm 0.1$
Nannochloropsis gaditana L2 1%	$326.0^{b} \pm 12.2$	$28.0^{d} \pm 0.9$	$93.9^{b} \pm 3.6$	$204.7^{b} \pm 8.9$	$82.7^{a} \pm 16.4$	$5.6^{d} \pm 0.4$	$1.8^{c} \pm 0.1$
Nannochloropsis gaditana L2 3%	$310.2^{ab} \pm 5.8$	$51.6^{c} \pm 0.7$	$96.2^{b} \pm 2.2$	$213.6^{b} \pm 15.5$	$110.6^{b} \pm 6.4$	$10.5^{e} \pm 0.4$	$2.1^{d} \pm 0.1$
15% RDV* (mg/100 g)	300	120	45	120	–	2.1	2.3

Values are means ± standard deviation (n = 3) Means followed by the same small letter in the column did not differ significantly based on Tukey's test ($p > 0.05$).
* According to the recommended daily values (RDV) established by Regulation (European Community), N_ 1924/2006; Directive N_ 90/494 (CE).
– Not mentioned.

Table 7
Main fatty acid profile (%) present in microalgae biomass, and in intact and supplemented gluten free bread (EL5- *Chlamydomonas* sp. L2- *Nannochloropsis gaditana*).

FAMEs	EL5	L2	GF-bread Control	GF-bread with 1% EL5	GF-bread with 3% EL5	GF-bread with 1% L2	GF-bread with 3% L2
C16:0	18.6^{A}	16.6^{B}	8.7^{a}	9.5^{b}	9.3^{b}	8.9^{a}	9.6^{b}
C16:1	0.9^{A}	1.0^{A}	0.8	n.d.	n.d.	n.d.	n.d.
C18:0	2.7^{A}	2.7^{A}	3.2^{a}	4.0^{b}	3.9^{b}	3.8^{ab}	3.9^{b}
C18:1	2.3^{A}	5.9^{B}	30.9^{ab}	31.2^{b}	30.1^{c}	30.7^{a}	30.2^{c}
C18:2	33.4^{A}	36.0^{A}	52.5^{a}	52.7^{a}	53.5^{a}	52.0^{a}	53.0^{a}
C18:3	5.5^{A}	4.6^{B}	0.9^{a}	2.6^{b}	2.8^{b}	1.2^{a}	1.7^{c}
C20:0	4.3^{A}	4.0^{A}	0.6	n.d.	n.d.	n.d.	n.d.
SFA	25.7^{A}	23.4^{B}	12.5^{a}	13.5^{a}	13.2^{a}	12.7^{a}	13.5^{a}
MUFA	3.2^{A}	6.9^{B}	31.6^{a}	31.2^{b}	30.1^{c}	30.7^{d}	30.2^{c}
PUFA	38.9^{A}	40.6^{A}	53.3^{a}	55.3^{ac}	56.3^{c}	53.1^{a}	54.7^{ac}
ω3	5.5^{A}	4.6^{B}	0.9^{a}	2.6^{b}	2.8^{b}	1.2^{a}	1.7^{c}
ω6	33.4^{A}	36.0^{A}	52.5^{a}	52.7^{a}	53.5^{a}	52.0^{a}	53.0^{a}
ω3/ω6	1:6	1:7	1:58	1: 20	1:19	1:43	1:31

Values are average of 2–3 repetitions. Means followed by the same small letter in a line did not differ significantly, between different bread samples, and means followed by the same capital letter in a line did not differ significantly between species based on Tukey's test ($p > 0.05$).
GF (gluten free); SFA (saturated Fatty Acids); MUFA (Monounsaturated Fatty Acids); PUFA (Polyunsaturated Fatty Acids); ω3 (Omega-3 fatty acids); ω6 (Omega-6 fatty acids).

attractive and innovative appearance as seen in Fig. 6. Because they presented the most interesting nutritional composition, bread samples with 3% w/w microalgal biomass were sensory evaluated in order to predict the future commercialization of the product with higher level of addition than the typical algal products (< 1% w/w). Fig. 7 presented the average answers given by the panel toward the principal sensory attributes (color, odor, flavor, texture) and the global appreciation.

Based on the scores attributed to the control bread (> 4), it can be said that the formula used was successfully chosen. The addition of both microalgae biomass had a slight but positive effect on all sensory attributes. The intense green color of the supplemented bread was greatly appreciated by the consumers. In fact, the 3% w/w *Chlamydomonas* sp. EL5 bread was the preferred one in terms of color, while for odor, taste, and texture, the panel showed a slight preference for the ones prepared with 3% w/w *N. gaditana* L2 biomass. In terms of global appreciation, the 3% w/w *N. gaditana* L2 breads scored highest followed by 3% w/w *Chlamydomonas* sp. EL5 breads, and finally by the control ones.

One of the most important issues that are related to the use of some microalgal biomass in food is the fish flavor and odor that can be unpleasant for consumers [25]. However, as mentioned earlier, the strains used in this work did not present any of these issues. Products enriched with microalgal biomass have already been sensory tested and generally appreciated. Examples include ice cream [20], bread [24,29], yoghurt [48], pasta, [25] and cookies [5]. However, in some cases the high level of addition (2%) has negatively affected the flavor parameters, leading to a general negative appreciation [25].

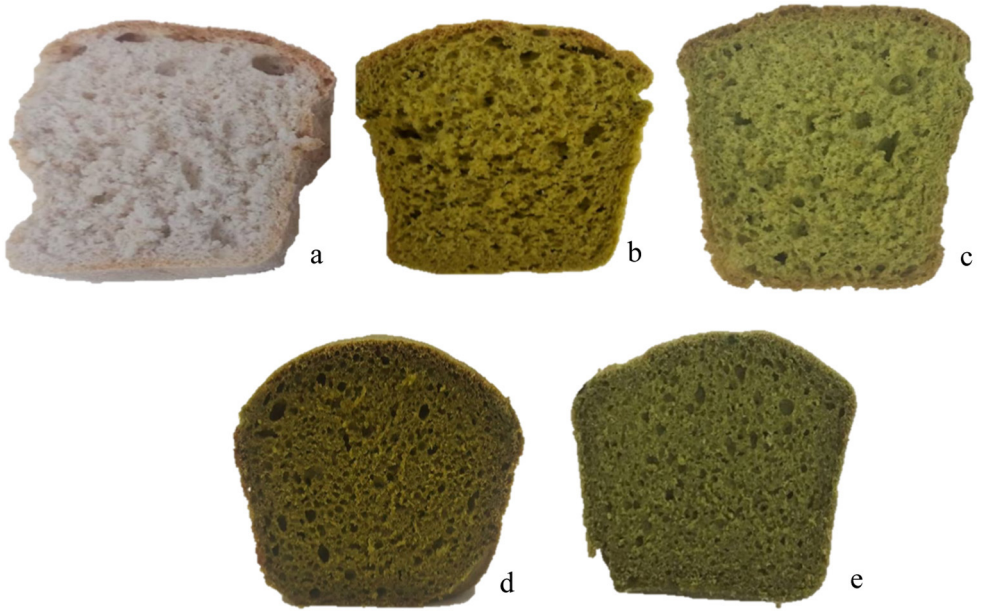

Fig. 6. Control (a), and supplemented breads with 1% w/w (b; c for *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2, respectively) and 3% w/w (d; e for *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2, respectively) microalgae biomass.

Fig. 7. Sensory evaluation results ($n = 30$) of Control and breads with 3%wt *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2 biomass.

Consumers' intention to buy the control samples and the enriched ones is mentioned in Fig. 8. Forty six per cent of the panel have chosen the proposition "would probably buy" and 20% "would certainly buy" the bread with 3% w/w *Nannochloropsis gaditana* L2 biomass. 56 and 53% "would probably buy" the control bread and the 3% w/w *Chlamydomonas* sp. EL5 bread, respectively. Actually, this simulation was an effective way to predict the acceptance of these products in the market.

4. Conclusion

Gluten-free bread, more than any other gluten-free product, is of great importance for patients of celiac disease or other pathologies related to gluten. An attractive and well-balanced functional product can be obtained by the addition of microalgae biomass, such as *Nannochloropsis gaditana* L2 and *Chlamydomonas* sp. EL5. As a whole, microalgae biomass produced gluten-free bread with a significant structuring impact in terms of firmness and adhesiveness. However, the effect of microalgae addition on dough rheological parameters was considered insignificant ($p > 0.05$). Microalgae breads always possessed the highest protein and ash content. Increasing microalgae content from 1% to 3% w/w improved significantly the iron and calcium content with a good and balanced profile of fatty acid. Attractive green tonalities (a*) with an increase of yellowness (b*) varied, according to the microalgae and the content used. A sensory evaluation revealed encouraging results. The 3% *N. gaditana* L2 bread sample had the highest score in terms of global appreciation. Overall, the current study suggests that microalgae can be considered as a suitable ingredient in gluten-free bread, enhancing its structural and nutritional profile.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2020.101998.

Statement of informed consent

No conflicts. An Informed consent was obtained from all individual participants included in the study. All sensory research procedures followed were in accordance with the ethical standards of the local committee responsible for human experimentation and with The Code of Ethics of the World Medical Association (Declaration of Helsinki of 1975, as revised in 2013).

CRediT authorship contribution statement

Sheyma Khemiri: Writing - original draft, Investigation, Formal analysis. **Nadia Khelifi:** Methodology, Formal analysis. **M. Cristiana Nunes:** Methodology, Formal analysis. **Alice Ferreira:** Investigation. **Luisa Gouveia:** Conceptualization, Writing - original draft. **Issam Smaali:** Supervision. **Anabela Raymundo:** Supervision, Validation.

Declaration of competing interest

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.

Acknowledgments

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research- University of Carthage (Tunisia) through a research grant, which permitted Sheyma Khemiri to carry out this work in the research unit UID/AGR/04129/2013 (LEAF) at Instituto Superior de Agronomia- University of Lisboa (Portugal). The authors wish to thank Graça Gomes from LNEG laboratory (Portugal)

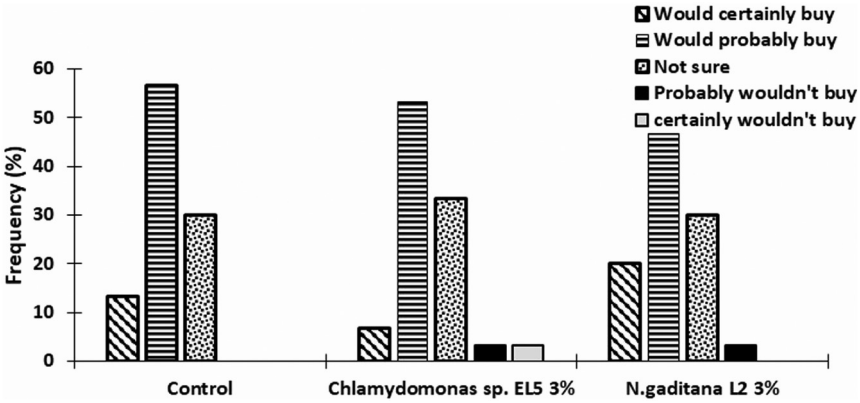

Fig. 8. The panel's buying intentions (n = 30) toward control, *Chlamydomonas* sp. EL5 and *Nannochloropsis gaditana* L2 breads.

for microalgae culture maintenance and laboratory assistance.

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