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Nutritional Properties, Antioxidant Activity, and Consumer Acceptability of Sourdough Bread Supplemented with Marine Algae Powder Using Selected Traditional Starters

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Abstract: This study aimed at producing a sourdough bread supplemented with two marine algae powders of *Ulva lactuca* and *Gelidium corneum* at 2.5, 5, 7.5, and 10% (*w*/*w*, g/100 g DW) at the laboratory scale using mixed starters prepared with three lactic acid bacteria (LAB) combinations (LCS1, LCS2, and LCS3). The phytochemical composition, nutritional value, organoleptic properties, and acceptability of algae bread by consumers were then assessed. Good results were obtained for enriched bread with *Gelidium* at 2.5% (GB1) with a reducing sugar of 0.77 ± 0.1%, total sugar of 36.90 ± 3.15, and protein content of 8.3 ± 0.2%. While total phenolic content was 8.32 ± 1.20 mg GAE/g DW, total flavonoids was 225.00 ± 11.31 mgQE/g DW, and the antioxidant activity ranged from 71.05 ± 3.71 to 82.93 ± 3.61%. Regarding enriched bread with *Ulva* at 10% (UB12), reducing sugar was 0.48 ± 0.03%, total sugar was 45.45 ± 5.75%, and protein content was 3.7 ± 0.07%. The total phenolic content value was 6.45 ± 1.19 mg GAE/g DW, total flavonoids was 191.20 ± 12.52 mgQE/g DW, while the antioxidant activity values ranged from 52.06 ± 6.21 to 80.51 ± 1.72%. Microbiological analysis showed that all pathogenic bacteria were not detected in algae bread. The consumer acceptability test revealed that bread enriched at the level of 10% of algae powder was significant for the five selected criteria (general appearance, crumb color, odor, taste, and texture). Traditional bread supplemented with *Gelidium* powder at 2.5% and *Ulva* powder at 10%, prepared with combined sourdough (LCS1), showed good antioxidant and nutritional properties and consumer acceptance.

Keywords: marine algae; supplementation; sourdough bread; lactic acid bacteria; fermentation; nutrition; sensory testing; quality; safety

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1. Introduction

Fermentation of food raw materials is known as an ancient biotechnological process widely applied to extend food shelf life and obtain new products with particular properties [1]. The various nutrients of these raw materials are used as substrates by fermenting microorganisms to transform them into fermented food products with desirable, organoleptic, and sustainable properties. Cereals are the common agricultural raw

materials frequently used to produce fermented foods, such as beer, malt vinegar, spirits, baked goods, and sourdough bread [2,3]. Bread is usually made from wheat flour, but rye, barley, and millet bread are also common. On average, bread provides 60 to 65% of calories and proteins each day, as well as 2 to 3 g of mineral salts daily by eating bread [4]. Traditionally, Arabic flatbread is known as a local product that has been made for centuries in an artisanal way in small family bakeries over a wood fire using the natural microflora of wheat flour as a leavening agent, which consists of a natural association between lactic acid bacteria (LAB) and yeasts [5]. Because baked goods have been, and still are, a central constituent in the diet of most populations for thousands of years, their fortification with various nutritious, protective, and functional compounds is considered an effective strategy for obtaining novel or renewed high-quality products both for industry and consumers [6]. Recent studies have reported that novel bread should have a low glycemic index and fat content, and it should contain proteins with high nutritional value and balanced amounts of dietary fiber, vitamins, minerals, and phytochemicals [7].

Sourdough is an intermediate product between dough and traditional bread preparation and is considered a complex matrix originated from a mixture of flour and water fermented mainly by indigenous LAB strains and yeasts present in flour [8]. Yeasts are primarily responsible for the leavening of dough, while LAB determine the process of acidification, even though heterofermentative LAB partly contribute to the mass blowing [9]. Nowadays, more than 50 different LAB species belonging to the genera of *Lactiplantibacillus*, *Leuconostoc*, *Lactococcus*, *Enterococcus*, *Pediococcus*, or *Weissella* have been isolated from sourdoughs [10].

During sourdough fermentation, LAB produce several metabolites that have been shown to have a positive effect on bread texture and staling. Indeed, exopolysaccharides (EPS) produced by LAB have the potential to replace more expensive hydrocolloids used as bread improvers [8]. Organic acids (lactic acid, acetic acid, etc.) affect the protein and starch fractions of flour [11]. Furthermore, the decrease in pH associated with lactic acid production causes an increase in flour protease and amylase activities, which leads to a staling reduction, thus improving the textural, sensory, and nutritional qualities and bread shelf life [8]. In addition, sourdough fermented with LAB is a source of proteolytic enzymes, activated by acid production [12,13].

In an attempt to increase the nutritional value of sourdough bread, several preliminary studies have been undertaken to fortify fermented bread with natural resources, including parts of plants, algae, grains, etc. These studies have reported the importance of bread enrichment with different plant parts to produce bread with a better nutritional profile. The commonly used bread fortification micro-/macroalgae species include *Arthrospira platensis*, *Chlorella*, *Tetraselmis chuii*, *Nannochloropsis gaditana*, *Cladophora* spp., *Ulva* spp., *Sargassum subrepandum*, *Saccharina latissimi*, *Fucus vesiculosus*, and *Ascophyllum nodosum*. These marine species are known as a source of proteins, fibers, lipids, and micronutrients, which improves the nutritional value of the fortified bread, enhances its quality, and has a nutritional profile that better meets human nutritional requirements [12,14–20]. Furthermore, bread fortification with algae (e.g., *Ulva lactuca* powder) possesses considerable potential for market differentiation because of its high nutritive value, easy nutrient attainment, well-balanced amino acid profile, and its ability to drive protein enrichment in gluten-free bakery products [21,22].

Morocco holds a large bio-ecological diversity of seaweeds (Rhodophyta, Phaeophyceae, and Chlorophyta), which consists of 381 species along the Mediterranean coast and 323 species along the Atlantic coast [18], including most known species, especially *Gelidium corneum*, *Ulva lactuca,* and *Sargassum muticum* [19,20]. The choice of algae for bread fortification was based on the fact that seaweed powder may contain various po-

tentially bioactive compounds, some of which are not present in terrestrial plants. Some macroalgae species, such as *G. corneum*, are rich in proteins. On the other hand, *U. lactuca* is known to contain various bioactive compounds with potential health benefits. Moreover, polysaccharides such as fucoidan and carrageenan, significant classes of compounds found in *Gelidium* spp., exhibit various biological activities, including anticancer, antioxidant, anti-inflammatory, and immunomodulatory effects. Indeed, the bioactive compounds of algae may have health-promoting properties and play a role in modulating some chronic diseases [23,24]. Indeed, marine algae are very rich in antioxidant substances like tannins, phenols, flavonoids and saponins, which prevent oxidative stress, neutralize free radicals and protect against several pathologies [25].

To the best of our knowledge, there is sufficient published data on the enrichment of baker's yeast bread with microalgae and seaweed for increasing its nutritional value and screen health advantages, including immunomodulatory properties and chronic disease prevention. However, up until now, no scientific information is available regarding seaweed fortification of sourdough bread elaborated using selected LAB starters. Thus, the purpose of this study is therefore to investigate the nutritional properties and antioxidant activity of seaweed powder of *U. lactuca* and *G. corneum*, and those of a novel enriched sourdough bread made with algae powder on a laboratory scale using selected LAB strains, as well as its consumer acceptability.

2. Materials and Methods

2.1. Seaweed Preparation

2.1.1. Seaweed Powder Preparation

Two seaweed species (*Gelidium corneum* (Hudson) J.V.Lamouroux, 1813 and *Ulva lactuca* Linnaeus, 1753) were collected from the Atlantic coast of Sidi Bouzid at El Jadida, Morocco. Fresh samples (Figure 1) of *G. corneum* and *U. lactuca* were transported to BIOMARE Laboratory, then washed twice to remove salt and debris. Seaweed samples were dried in the shade for 3 days and ground at high speed (28,000 rpm) using a blender (Moulinex, Labbox, Rungis, France) to obtain a fine powder (<355 µm). The obtained powder was well sealed and stored in glass boxes at 4 °C until further analysis.

(**a**) (**b**)

Figure 1. Samples of marine algae collected along the Moroccan Atlantic coast of Sidi Bouzid (El Jadida, Morocco). Note: (**a**) *U. lactuca*; (**b**) *G. corneum*.

2.1.2. Seaweed Powder Characterization

Reducing Sugars

Determination of reducing sugars in seaweed powder was performed according to the method of Miller [26]. Briefly, 3 mL of seaweed extracts (1 g of algae powder added to 9 mL of distilled water) were mixed with 3 mL of 3,5-Dinitrosalicylic acid (DNS) reagent (Sigma-Aldrich, Darmstadt, Germany) and 3 mL aliquots of glucose solution in 14 mm tubes. The mixtures were heated for 5 min in a boiling water bath and then quickly cooled under running tap water adjusted to ambient temperature to stop the reaction between

DNS and sugars. The color intensities were measured using a UV-VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China) at 575 nm.

Total Sugars

Total sugars were analyzed in algae powder according to the method of Montenegro et al. with slight modifications [27]. The principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectable color. The standard procedure of this method is as follows. A 2 mL aliquot of each seaweed extract was mixed with 1 mL of a 5% aqueous solution of phenol in a test tube. Subsequently, 3 mL of concentrated sulfuric acid at 99.99% (Sigma-Aldrich, Saint-Louis, MO, USA) was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm was recorded on the UV-VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd., China). Reference solutions were prepared in an identical manner as above, except that the 2 mL algae extracts were replaced by bidistilled water. The phenol used in this procedure was redistilled, and 5% phenol in water (w/w) was prepared immediately before the measurements. The calibration curves were performed using standard solutions prepared from the following concentrations: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.9, and 1 $g \cdot L^{-1}$ of glucose.

Phenolic Compounds Content

Phenolic compound contents in algae extracts were determined and the results were expressed as gallic acid equivalents (GAE). One gram (1 g) of each seaweed powder was dissolved in 9 mL of ethanol ($\geq$99.5%, VWR International, Rosny-sous-Bois, France). The 0.5 mL extract solution was taken into a vial (which contained the 5 mL distilled water). The 0.5 mL of Folin–Ciocalteu reagent (Sigma-Aldrich, USA) and 2 mL of sodium carbonate (Sigma-Aldrich, USA) solution at 10% (w/v) in distilled water were added to the vial and mixed well by a vortex. After 10 min of incubation at room temperature, the absorbance was measured at 730 nm using a UV-VIS spectrophotometer (Shanghai Metash, China). Ethanol served as a blank solution for the experiment [28]. The calibration curve was carried out using the concentrations of 10, 25, 50, 100, 150, 250, and 500 mg/L of gallic acid standard solution, and the results were expressed as a percentage of DW.

Flavonoids Contents

Algae powder flavonoid contents were determined by using the aluminum chloride colorimetric method. 1 g of seaweed powder (*Gelidium* and *Ulva*) was extracted with 9 mL of distilled water. Then 0.5 mL extract, 0.1 mL aluminum chloride (dissolving 10 g in 100 mL of distilled water: w/v), 0.1 mL potassium acetate (1 M), and 4.3 mL of distilled water were mixed and incubated at room temperature for 30 min. The absorbance was measured at 415 nm using a UV-VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd., China). In this experiment, quercetin was used to make the calibration curve in the range of 50–450 mg/L [28].

Microbial Load of Algae Powder

Enumeration of total aerobic mesophilic flora (TAMF) was carried out using plate count agar (Oxoid Ltd., Basingstoke, UK). Results reading was performed after 24 h of incubation at 30 °C. Enumeration of coliforms was carried out on MacConkey agar (Oxoid, UK). The red colonies that ferment lactose and have a diameter of more than 0.5 mm, appearing after 24 h of incubation at 37 °C for total coliforms and at 44 °C for fecal coliforms, were counted. Enumeration of pathogenic staphylococci was carried out on

Baird–Parker agar (Merck KGaA, Darmstadt, Germany). After 48 h of incubation at 37 °C, black colonies with white margins were enumerated. Detection of *Salmonella* was carried out following the recommendations of the international standard (ISO 1990). Indeed, 25 g of each algae powder sample were diluted in 225 mL of buffered peptone water (BPPW) (Oxoid Deutschland GmbH—Wesel, Germany). After 18 h of incubation at 37 °C, 1 mL of the inoculated BPPW was transferred to a tube containing 9 mL of Rappaport Vassiliadis (RV) broth (Merck, Germany) and incubated at 42 °C for 24 h. Using a loop, a single culture was taken from RV and inoculated by spreading on the surface of Hektoen agar (Oxoid Deutschland GmbH—Wesel, Germany). The plates were incubated at 37 °C for 24 h, and suspect *Salmonella* colonies were characterized by a blue-green or green color with or without a black center. Enumeration of fungal flora (yeasts and molds) was performed on "Dichloran-Rose Bengal Chloramphenicol" (Biokar diagnostics, Allonne, France). Prepared plates were incubated at 25 °C for 72 h for yeasts and for 5–7 days for molds [29].

2.2. Development of Sourdoughs Recipe

2.2.1. LAB Strains

A total of thirteen (n = 13) LAB strains (*Lactiplantibacillus plantarum* (n = 1), *Companilactobacillus paralimentarius* (n = 1), *Bifidobacterium pseudocatenulatum* (n = 1), *Enterococcus hirae* (n = 1), *Lentilactobacillus parabuchneri* (n = 2), *Levilactobacillus brevis* (n = 2), and *Pediococcus pentosaceus* (n = 5)), previously isolated from Moroccan traditional sourdoughs, were used in this study. These LAB strains were previously isolated and selected based on their criteria, including CO_2, lactic acid, and exopolysaccharides (EPS) production [30]. LAB isolates were inoculated in MRS broth (de Man, Rogosa and Sharpe, Biokar Diagnostics, France) under sterile conditions and incubated at 30 °C for 24 h.

2.2.2. Mono-Inoculated Sourdoughs

To select the most performant ferments for bread making, a total of 13 separated sourdoughs were prepared at the laboratory scale according to the Moroccan traditional method as previously reported [30]. Indeed, the first step consisted of sourdough preparation by fermenting wheat flour with each LAB strain, as indicated in Table 1. Briefly, 50 g of wheat flour were added to 50 mL of tap water and 5 g of salt and mixed in a transparent polypropylene plastic box. The mixture was well homogenized using a sterilized spatula, and 5 mL of each LAB inoculum was added. Then, the doughs were homogenized for the second time, the boxes closed properly, and they were incubated at 30 °C for 24 h. A control assay was prepared by adding 50 g of wheat flour to 50 mL of tap water, as previously reported [31]. The performance of elaborated sourdoughs was characterized by the determination of pH, titratable acidity (TA), and dough increase (CO_2 production).

Table 1. Sourdough ferments prepared using selected LAB strains.

Sourdough Ferments	Inoculated LAB Strain
1	*Levilactobacillus brevis* S3
2	*Companilactobacillus paralimentarius* S5
3	*Enterococcus hirae* S4
4	*Pediococcus pentosaceus* S6
5	*Levilactobacillus brevis* S8
6	*Bifidobacterium pseudocatenulatum* S12
7	*Pediococcus pentosaceus* S15
8	*Lentilactobacillus parabuchneri* S17

Table 1. *Cont.*

Sourdough Ferments	Inoculated LAB Strain
9	*Lactiplantibacillus plantarum* S21
10	*Lentilactobacillus parabuchneri* S23
11	*Pediococcus pentosaceus* S24
12	*Pediococcus pentosaceus* S27
13	*Pediococcus pentosaceus* S28

2.2.3. Mixed-Culture Sourdoughs

A total of eight (n = 8) mixed-culture sourdoughs were prepared by combining the initial sourdoughs cited above and that responded to the mentioned criteria (pH, TA, and CO_2 production). The final fermentation of mixed-culture sourdoughs was performed at 30 °C. Development of bubbles and sweet-smelling odor was observed. The sourdough samples were analyzed for their pH, TA, and CO_2 production [32]. At the end of the experiment and based on the characteristics previously mentioned, three combined sourdoughs (LCS1, LCS2, and LCS3) represented in Figure 2, showing high performances, were selected to elaborate bread supplemented with algae powder.

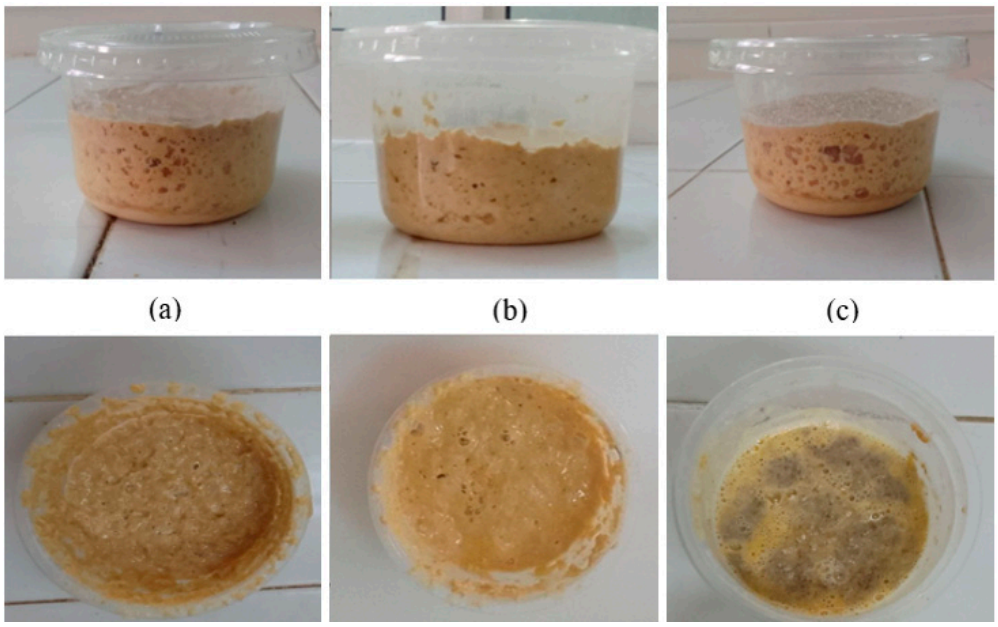

Figure 2. Three (3) selected sourdoughs showing high performance. Note: (**a**): LCS1; (**b**): LCS2; and (**c**): LCS3.

2.2.4. Analyses of Sourdoughs

pH and Titratable Acidity

The pH of dough and bread samples was determined using a pH meter AD1000 (Adwa Kft, Szeged, Hungary). The pH electrode was punched in 20 g slurries of dough disaggregated in 10 mL of distilled water [30]. For TA measurements, the previous homogenized mixture was titrated using NaOH (0.1 N), and phenolphthalein drops were added as a color indicator [33].

CO_2 Production

The one of the most important criteria used to judge the performance of the sourdoughs, since it affects the texture of the breadcrumbs after baking. To test the ability of the

selected sourdough to produce CO_2, 50 g portions of dough, prepared from commercial soft wheat flour *Alitkane* (Zine Capital Invest, Casablanca, Morocco), were inoculated with a 2 mL suspension (10^6 cfu/mL) of each selected LAB strain. Then, the portions of dough were placed in a transparent polypropylene plastic box test to fill a volume of approximately 88 cm^3. Transparent polypropylene plastic box tests were closed and incubated at 30 °C for 24 h. The variation in volume (ΔV) was measured at the end of incubation; this experiment was performed in duplicate with slight modifications [30].

2.3. Development of Algae-Enriched Bread

2.3.1. Bread Making

For making bread enriched with algae powder, three formulations were tested: a control wheat sourdough (CWS) and two enriched breads prepared with *G. corneum* (GB) and *U. lactuca* (UB) powder using the three LAB combinations (LCS1, LCS2, and LCS3) (Table 2). Then, bread was prepared at the laboratory scale according to the slightly modified protocol [34]. Briefly, wheat flour (66 g) was supplemented separately with *U. lactuca* and *G. corneum* powder at different fortification levels of 2.5, 5, 7.5, and 10%. Then, 4 g of NaCl and 66 mL of warm tap water (30 °C) were added. All ingredients were mixed slowly for 2 min and fast for 7 min manually using sterile gloves. After resting for 30 min, the dough was divided into 66 g pieces and kept for fermentation at 30 °C for 60 min. Bread pieces were prebaked in a deck oven preheated at 250 °C. The baked bread samples were kept in the freezer for further analysis. The pH and TA of enriched bread samples were measured as described above.

Table 2. Combined sourdoughs developed using selected LAB strains (ratio 1:1:1).

Combined Sourdough	Inoculated LAB Strain
1	*Lactiplantibacillus plantarum* S21 *Pediococcus pentosaceus* S6 *Lentilactobacillus parabuchneri* S17
2	*Lactiplantibacillus plantarum* S21 *Pediococcus pentosaceus* S15 *Pediococcus pentosaceus* S27
3	*Lactiplantibacillus plantarum* S21 *Levilactobacillus brevis* S3 *Levilactobacillus brevis* S8
4	*Pediococcus pentosaceus* S6 *Lentilactobacillus parabuchneri* S17 *Pediococcus pentosaceus* S15
5	*Pediococcus pentosaceus* S6 *Pediococcus pentosaceus* S27 *Levilactobacillus brevis* S3
6	*Lentilactobacillus parabuchneri* S17 *Pediococcus pentosaceus* S15 *Pediococcus pentosaceus* S27
7	*Lentilactobacillus parabuchneri* S17 *Levilactobacillus brevis* S3 *Levilactobacillus brevis* S8
8	*Pediococcus pentosaceus* S15 *Pediococcus pentosaceus* S27 *Levilactobacillus brevis* S8

2.3.2. Analyses of Algae-Enriched Bread

Nutritional Composition

Enriched algae bread samples were analyzed for reducing and total sugars, total flavonoids, and phenolic content as described above (Section 2.1.2). Protein measurement was carried out using the Lowry method [35]. Briefly, 200 µL of bread extract supernatant (1 g of baked bread in 9 mL of distilled water well mixed and centrifuged at 8000× *g* for 10 min) were added to 100 µL of 0.5% (*w*/*v*) copper sulfate solution (Sigma-Aldrich, USA), 100 µL of 1% (*w*/*v*) potassium-sodium tartrate (Sigma-Aldrich, USA) solution, and 10 mL of 2% (*w*/*v*) sodium carbonate solution (Sigma-Aldrich, USA). The resulting mixture was shaken and incubated in the dark for 15 min at room temperature. Then, 200 µL of Folin–Ciocalteu reagent (Sigma-Aldrich, USA), half diluted in 0.1 N NaOH, were added. The mixture was shaken once more and kept in the dark for 30 min for color changing. Absorbance of mixtures was measured at 660 nm with a UV-VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd., China). Bovine serum albumin (BSA, Sigma-Aldrich, USA) served as a control, and the calibration curve was prepared with the following concentrations: 2, 4, 6, 8, 10, 15, 20, and 30 µg/mL [35,36].

Antioxidant Activity (DPPH)

The DPPH (2,2-diphenyl-1-picrylhydrazul) assay is based on the scavenging of DPPH radical by antioxidants, producing an absorbance decrease at 517 nm. The antioxidant activity in bread (*G. corneum* and *U. lactuca*) samples was measured following the method of Chen et al. [37]. Briefly, 1 g of baked bread was added to 9 mL of distilled water, mixed well, and centrifuged at 8000× *g* for 10 min; then 1 mL of supernatant (FS) was added to 2.0 mL of ethanolic DPPH solution (0.05 mM) (Sigma-Aldrich, Darmstadt, Germany), mixed vigorously, and then incubated at room temperature (25 °C) in the dark for 30 min. DPPH and distilled water solution were used as controls, while ethanol mixed with the free supernatant was used as a blank. The absorbance was measured at a wavelength of 517 nm, and the scavenging ability was expressed as follows [23]:

$$\text{DPPH scavenging ability (\%)} = (\text{Blank absorbance} - \text{sample absorbance/blank absorbance}) \times 100.$$

β-Carotene–Linoleic Acid Assay

In this assay, the antioxidant capacity was determined indirectly by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (LC grade). Then, 25 µL of linoleic acid and 200 mg of Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen was added with a vigorous shaking. A total of 2.5 mL of this reaction mixture was dispersed to test tubes, and 350 µL portions of the oils prepared at 2 g/L concentrations were added, and the emulsion system was incubated up to 48 h at room temperature. The same procedure was repeated with synthetic antioxidants, butylated hydroxytoluene (BHT), curcumin, and ascorbic acid as positive controls. The absorbance of the mixture was measured at 490 nm. Values were presented as means ± SD of two parallel measurements [38].

2.4. Sensory Evaluation of Algae-Enriched Bread

To evaluate final algae bread products (GB1 to GB12 and UB1 to UB12), the sensory assessment test was realized at laboratory scale. Thus, two groups of untrained panelists (9 females and 11 males aged 20–28 years) participated in the sensory test. The first group

(n = 5 females and n = 5 males) participated in GB samples assessment, and the second group (n = 4 females and n = 6 males) in UB samples in adequate conditions (absence of disruptive elements, better light intensity, and drinking water bottles).

A total of 24 bread samples (n = 12 for GB and n = 12 for UB) were evaluated (Table 3). The untrained panelists were instructed to use water after each taste and to note parameters scores. Each panelist evaluated 12 seaweed bread samples separately (GB or UB), and data were recorded. Seaweed bread samples were tested for five characteristics including crumb color, odor, texture, taste and general appearance by giving the following scores (1 = very bad, 2 = bad, 3 = fair, 4 = good, and 5 = very good). Sensory evaluation of the algae bread was carried out using a hedonic test 24 h after baking the algae bread samples.

Table 3. Bread making using different LAB strains combination at various fortification levels (%) of algae powder.

LAB Combination	*Ulva* Bread	*Gelidium* Bread	Fortification Level (%)
LCS1	UB1	GB1	2.5
	UB2	GB2	5
	UB3	GB3	7.5
	UB4	GB4	10
LCS2	UB5	GB5	2.5
	UB6	GB6	5
	UB7	GB7	7.5
	UB8	GB8	10
LCS3	UB9	GB9	2.5
	UB10	GB10	5
	UB11	GB11	7.5
	UB12	GB12	10

Note. UB: *Ulva* bread; GB: *Gelidium* bread; LCS1 (*B. pseudocatenulatum*, *L. parabuchneri*, *Lp. plantarum*); LCS2 (*Lev. brevis*, *B. pseudocatenulatum*, *P. pentosaceus*); LCS3 (*P. pentosaceus*, *L. parabuchneri*, *P. pentosaceus*).

2.5. Statistical Evaluation

Data analysis, including analysis of variance (ANOVA), was carried out using the free Past software (Palaeontologia Electronica, Norway, 2020). The significant variations among the results of the algae bread sensory test were determined by Tukey's test ($p < 0.05$). All analyses have been performed in triplicate (n = 3) in this study.

3. Results and Discussion

3.1. Algae Powder Characterization

3.1.1. Reducing and Total Sugars

Results of the biochemical characterization of algae powders are summarized in Table 4. As shown, reducing sugars of *G. corneum* and *U. lactuca* powder were 0.7 and 0.9% DW, respectively. While total sugars were 35 and 28.8% DW for *G. corneum* and *U. lactuca* powder, respectively. These values showed that algae powder is very rich in carbohydrates. Similar data has been reported by Mohy El-Din et al., who reported that the maximum carbohydrate amount in *G. corneum* and *U. lactuca* is more than 21.98% DW [39].

Table 4. Chemical composition of the studied algae powder.

Algae Powder	RS (%)	TS (%)	Proteins (%)	PC (mg GAE/g DW)	F (mg QE/g DW)
G. corneum	0.7	35	0.76	0.47	11.4
U. lactuca	0.9	28.8	0.33	9.95	21.39

Note. RS: reducing sugar; TS: total sugar; PC: phenolic content; F: flavonoids.

3.1.2. Total Phenolic Content (TPC)

Biochemical analysis of *U. lactuca* and *G. corneum* powders showed that PC amounts in both algae powders were 9.95 and 0.47 mg GAE/g DW, respectively. These results are higher than those (3.55 mg GAE/g DW) reported for *U. lactuca* by Tolpeznikaite et al. [40]. Another study conducted on the seasonal nutritional profile of *G. corneum* from the center of Portugal reported that the PC amount varied depending on seasons and reached the maximum in summer with 6.8 mg GAE/g DW [41].

3.1.3. Flavonoids Contents

Flavonoid contents in analyzed algae powders are shown in Table 3. These compounds reached 11.4 and 21.39 mg QE/g DW in *G. corneum* and *U. lactuca* powders, respectively. These results are similar to those found in a study on bioactive and biochemical constituent evaluation of *U. lactuca*, where the amount of flavonoids was 20.79 mg QE/g [42]. A more recent study has reported lower amounts of flavonoids in Mediterranean red algae (*Gelidium* sp.) using different extraction methods ranging from 0.38 to 1.56 mg QE/g DW [43].

3.1.4. Microbial Load

Results of the microbiological profile of algae powder are shown in Table 5. As shown, TVC charges were 3.27×10^3 cfu/g and 4.18×10^3 cfu/g for *G. corneum* and *U. lactuca*, respectively. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., and yeast and molds were absent in the two analyzed algae powders. These results showed that few microorganisms can grow directly on algae powder because of the absence of simple fermentable sugars and probably the need for a specialized enzymatic arsenal for the degradation of the polysaccharides, major components of seaweed powder. Moreover, our results are in agreement with the findings of Stabili et al. [44], who reported that *Escherichia coli* and *Enterococcus faecalis* could be present in algae powder at low counts [44].

Table 5. Microbial load (cfu/g) of the studied algae powder.

Algae Powder	TVC	EC	SA	SM	YM
G. corneum	3.27×10^3	nd	nd	nd	nd
U. lactuca	4.18×10^3	nd	nd	nd	nd

Note: nd: not detected; TVC: total viable count; EC: *Escherichia coli*; SA: *Staphylococcus aureus*; SM: *Salmonella* sp.; YM: yeast and molds.

3.2. Performance of Developed Sourdough

3.2.1. pH and Titratable Acidity

Regarding the preparation of sourdoughs at the laboratory scale, the study started with screening thirteen sourdoughs containing one traditional starter each, which were then examined to distinguish the highest LAB strain parameters efficacy (dough volume, odor, pH, and TA). Then, the second step consisted of testing eight LAB combinations (three LAB strains/mixed-culture sourdoughs). The development of combined LAB strains of sourdoughs was performed based on the performance of mono-inoculated sourdough strains for testing their role in the final algae bread composition. Then, the final step was

the selection of three sourdoughs with LAB combination (LCS1, LCS2, and LCS3), which showed good baking properties in the screening and thus had the potential to have an effect on algae bread's nutritional and chemical composition (Figure 2), including a reduction in protein and sugar contents while increasing phenolics and antioxidant activity. Reduction in proteins would be beneficial and avoid certain diseases. Indeed, it was reported that protein restriction in humans has been associated with reduced cancer, diabetes, and overall mortality [45].

Regarding pH and TA, obtained results are summarized in Figure 3. As shown, initial pH values (0 h) ranged from 5.88 to 6.00. After 24 h, the pH values decreased significantly for the thirteen sourdough samples to final pH values of 3.42–4.15. When LAB strains were combined, pH values of mixed-culture sourdoughs decreased from an initial value of 5.91 to 3.62 after 24 h. It was reported that the pH decrease is particularly associated with the presence of acidifying LAB strains, since no other microorganisms other than LAB had a significant effect on the decrease in pH of sourdoughs [30].

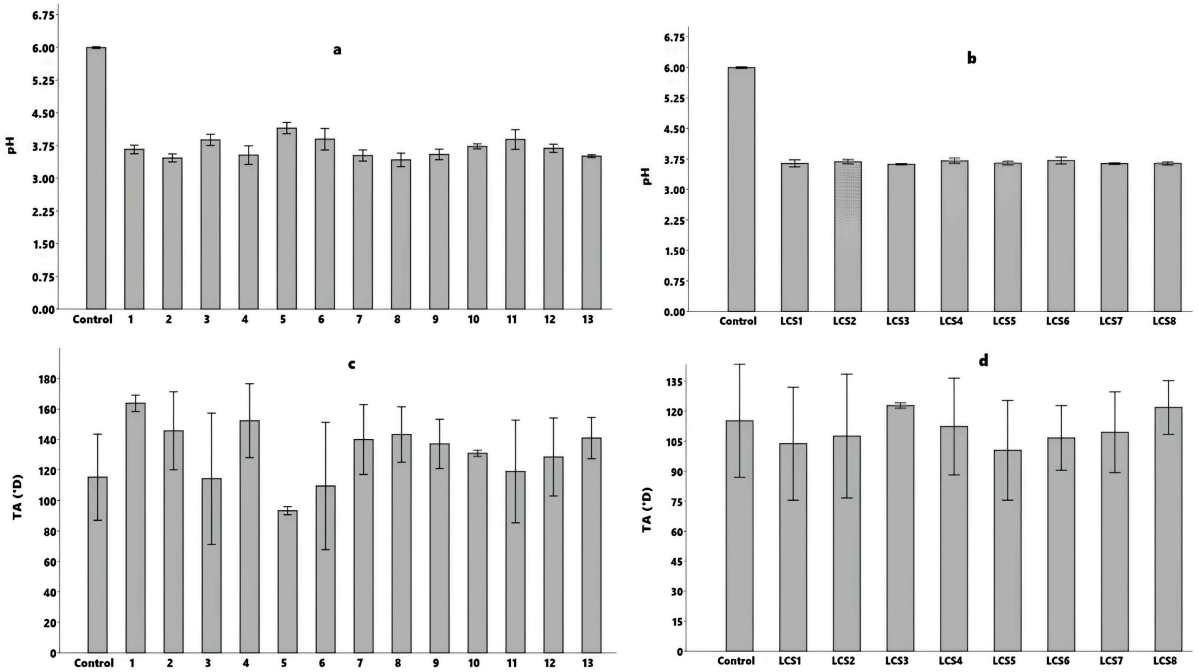

Figure 3. pH and TA of sourdoughs. Note: Mono-inoculated (**a**,**c**); Mixed-culture sourdoughs (**b**,**d**).

Concerning TA amounts during sourdough fermentation, initial values varied from 29.52 to 43.81 Dornic degrees (°D) for all prepared samples. However, a drastic increase in TA was observed after 24 h to final TA values of 93.33–163.81 °D. Concerning the combined sourdoughs, the TA values increased considerably to a maximum level of 142.86 °D. These promising findings could be explained by pH values reached in the analyzed samples and are higher than those found by Viola. et al. [46]. The acidity reaches its peak directly during the leavening process and then decreases during baking. Acidity is not only a crucial process parameter but also a significant quality indicator of the final bread because it affects the taste of fermented bread [47].

3.2.2. CO_2 Production

The fermentative activity of the selected LAB strains was determined by measuring their capacity to produce carbon dioxide (CO_2). For this, a control (non-inoculated sourdough) was used to make a comparison with inoculated sourdoughs (enriched with LAB strains). A subtraction was made between the CO_2 value of the tested strain and the control. Figure 4 shows the dough elevation according to the different tested LAB

strains. As shown, the best CO_2 production (ΔV) was noted in sourdoughs n°4, n°5, n°9, and n°12 (one LAB strain inoculation batch) with, respectively, 84 ± 9.90, 86.2 ± 6.36, 88 ± 16.97, and 79.5 ± 4.95 cm^3. Regarding the mixed-culture sourdoughs, these values reached 45.5 ± 3.41 and 43.5 ± 4.95 cm^3 for the LAB combination LCS1 and LCS5, respectively (Figure 4). According to these findings, there was no significant difference observed between the analyzed samples. On the other hand, obtained results regarding dough elevation levels were higher than those reported by El Khaider et al., who found ΔV values ranging from 2 to 25 cm^3 [30].

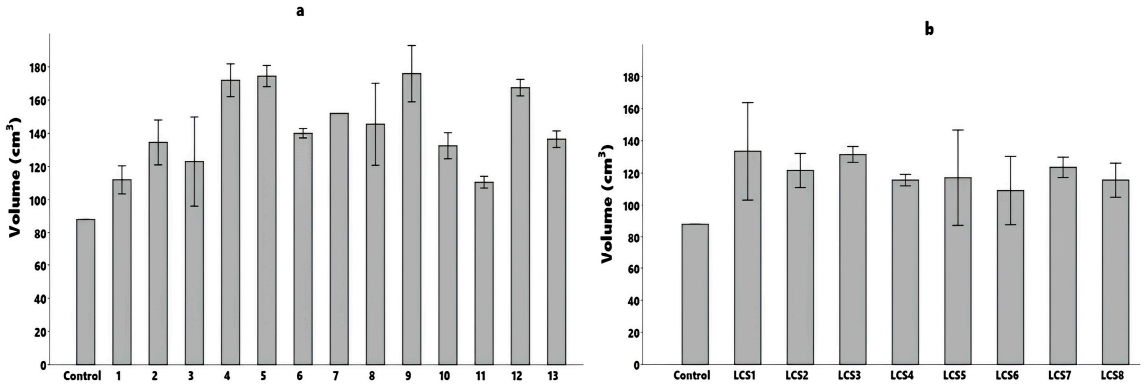

Figure 4. Volume of sourdoughs. Note: Mono-inoculated sourdoughs (**a**); Mixed-culture sourdoughs (**b**).

3.3. Nutritional and Functional Properties of Algae-Enriched Bread

3.3.1. pH and Titratable Acidity

Data of pH and TA measurement in bread samples enriched with *U. lactuca* and *G. corneum* powder are shown in Figure 5. The final pH values were found to be around 5.5 for all baked bread samples, while TA levels ranged between 17.14 and 73.33 °D. These results are similar to those reported by Garzon et al. [48], who reported that the pH of fortified bread with microalgae ranged between 5.27 and 5.83 [48]. The findings indicated that the pH was slightly acidic (around 5) and did not reach low levels due to the limited duration of bread fermentation (90 min). However, a study on four fortified breads with microalgae had reported a pH value more than 6.00 [49].

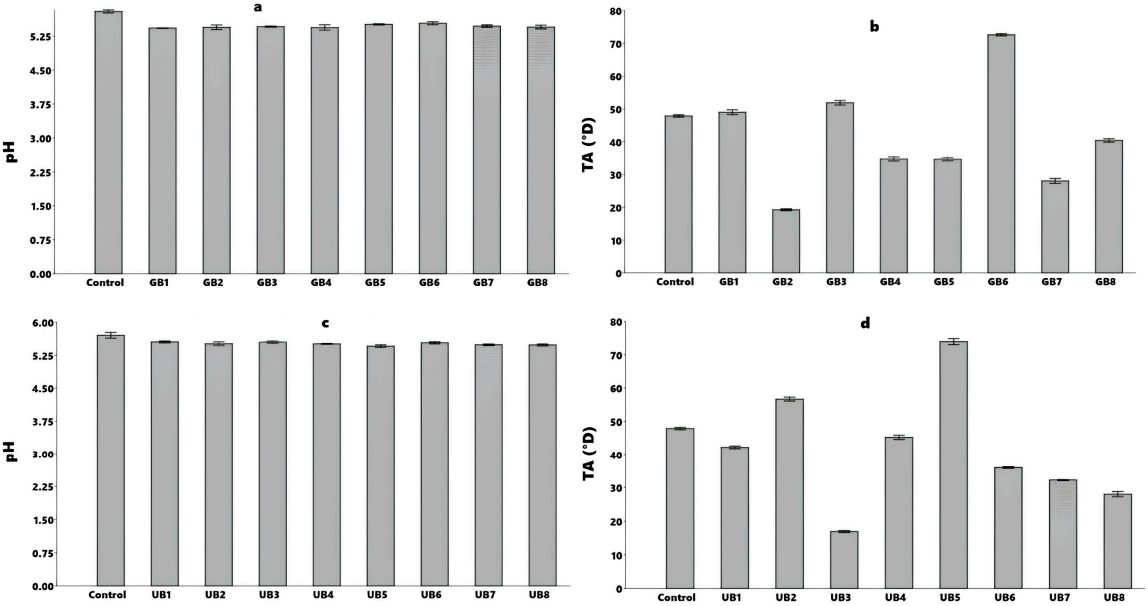

Figure 5. pH and TA of enriched bread samples. Note: *Gelidium* bread (**a**,**b**), *Ulva* bread (**c**,**d**).

Recent studies have shown that pH, which is influenced by the LAB fermentation process, significantly affects the color, taste, and aromatic parameters of the crumb of bread enriched with bran, whey protein, and plant, which are also affected by the circumstances and duration of the fermentation process [49,50]. Despite being one of the most consumed foods worldwide, bread is also one of the most thrown out because of its short shelf life (staling process) and susceptibility to mold deterioration. Thus, strategies to extend the shelf life of bread are highly desirable in order to prevent wastage. Choosing starting cultures that have both enhanced antifungal activities and strong biotechnological properties is one way to extend the shelf life of bread [51]. A variety of LAB strains are able to excrete numerous metabolites, including acetic, propionic, and caproic acids that are known for their antifungal properties. It is also recognized that bread with low pH reduces the gluten disulfide bonds (especially glutenin macropolymer GMP) by LAB acidification and glutathione reductase activity that makes possible the generation of oligopeptides and amino acids by activation of flour protease [13,52].

3.3.2. Nutritional Content

Reducing and Total Sugars

Results of total sugar contents in enriched bread are represented in Table 6. Total sugar contents ranged from 20.92 ± 5.22 to 45.22 ± 5.33% for *Gelidium* bread and from 34.42 ± 6.43 to 53.10 ± 1.97% for *Ulva* bread. Regarding reducing sugars, amounts ranged from 0.46 ± 0.09 to 0.77 ± 0.1% for *Gelidium* bread and from 0.28 ± 0.1 to 0.83 ± 0.07% for *Ulva* bread samples. Nachi et al. [53] have reported similar results, while Rico et al. found that total sugars are lower in a functional bread made with carob byproducts and seaweeds [54].

Table 6. Nutritional composition, phytochemicals and antioxidant properties of enriched algae bread samples (mean ± SD).

	Bread Samples	RS (%)	TS (%)	TP (%)	TPC (mg GAE/g)	TF (mgQE/g)	DPPH (%)	β-Carotene Assay (%)
Gelidium corneum	Control	0.74 ± 0.09	45.45 ± 7.68	12.0 ± 0.4	0.31 ± 0.01	-	52.96 ± 3.24	28.86 ± 1.05
	GB1	0.77 ± 0.1	36.90 ± 3.15	8.3 ± 0.2	8.32 ± 1.20	225.00 ± 11.31	82.93 ± 3.61	71.05 ± 3.71
	GB2	0.67 ± 0.07	34.87 ± 2.27	6.1 ± 0.7	8.32 ± 1.90	302.50 ± 20.51	64.30 ± 2.96	48.61 ± 2.54
	GB3	0.75 ± 0.1	39.82 ± 2.34	5.8 ± 0.8	7.47 ± 0.92	203.70 ± 19.81	85.27 ± 2.51	64.37 ± 4.89
	GB4	0.53 ± 0.08	32.40 ± 3.91	8.2 ± 0.6	6.11 ± 0.11	171.20 ± 12.17	74.71 ± 3.15	62.28 ± 2.88
	GB5	0.57 ± 0.09	29.47 ± 3.48	9.9 ± 0.1	5.80 ± 2.31	157.50 ± 8.91	0.00	60.03 ± 5.95
	GB6	0.52 ± 0.07	20.92 ± 5.22	9.4 ± 0.0	6.65 ± 1.18	166.20 ± 21.53	64.91 ± 2.20	54.39 ± 1.11
	GB7	0.74 ± 0.07	23.17 ± 6.12	6.6 ± 0.6	7.07 ± 2.53	320.00 ± 17.52	39.21 ± 2.86	56.82 ± 3.32
	GB8	0.50 ± 0.1	33.75 ± 5.41	7.5 ± 0.8	6.94 ± 2.82	306.20 ± 27.10	4.61 ± 1.10	45.85 ± 2.86
	GB9	0.69 ± 0.09	44.55 ± 7.17	14.4 ± 0.1	6.32 ± 1.93	136.20 ± 13.35	61.76 ± 3.35	59.01 ± 3.83
	GB10	0.58 ± 0.07	45.22 ± 5.33	4.3 ± 0.8	6.85 ± 1.14	120.00 ± 15.61	38.47 ± 0.95	42.64 ± 1.09
	GB11	0.46 ± 0.09	44.10 ± 5.10	10.3 ± 0.7	6.40 ± 2.20	140.00 ± 12.37	7.27 ± 1.14	51.99 ± 2.80
	GB12	0.71 ± 0.03	30.82 ± 4.53	5.8 ± 0.6	5.70 ± 2.71	162.50 ± 14.57	12.91 ± 0.83	59.52 ± 5.33

Table 6. *Cont.*

	Bread Samples	RS (%)	TS (%)	TP (%)	TPC (mg GAE/g)	TF (mgQE/g)	DPPH (%)	β-Carotene Assay (%)
Ulva lactuca	UB1	0.49 ± 0.09	47.25 ± 2.27	8.4 ± 0.1	6.49 ± 1.94	216.20 ± 10.92	44.21 ± 1.53	59.02 ± 4.13
	UB2	0.47 ± 0.09	40.05 ± 4.45	9.7 ± 0.5	5.92 ± 0.95	98.70 ± 9.85	85.55 ± 2.19	54.32 ± 3.63
	UB3	0.42 ± 0.01	43.42 ± 4.82	11.1 ± 0.9	4.65 ± 0.36	158.70 ± 10.31	82.73 ± 1.10	61.90 ± 7.52
	UB4	0.49 ± 0.09	34.42 ± 6.43	4.2 ± 0.1	6.78 ± 1.86	110.00 ± 20.32	77.84 ± 0.81	73.80 ± 3.61
	UB5	0.36 ± 0.08	53.10 ± 1.97	6.1 ± 0.6	5.74 ± 2.15	136.20 ± 8.64	36.84 ± 1.56	51.41 ± 2.85
	UB6	0.28 ± 0.1	51.07 ± 10.42	5.0 ± 0.8	6.65 ± 1.61	81.20 ± 7.89	76.72 ± 2.23	48.45 ± 6.13
	UB7	0.83 ± 0.07	43.87 ± 4.48	12.1 ± 0.5	6.49 ± 2.05	173.70 ± 14.53	80.42 ± 1.98	66.30 ± 5.67
	UB8	0.40 ± 0.01	38.47 ± 2.56	10.0 ± 0.7	6.94 ± 1.86	116.20 ± 17.65	85.00 ± 2.54	51.40 ± 7.15
	UB9	0.38 ± 0.07	49.95 ± 4.92	4.9 ± 0.6	6.41 ± 1.97	177.50 ± 8.88	55.46 ± 2.80	78.68 ± 4.56
	UB10	0.28 ± 0.08	43.87 ± 3.81	5.5 ± 0.9	6.07 ± 0.86	140.00 ± 11.26	83.65 ± 1.78	43.05 ± 2.81
	UB11	0.37 ± 0.05	38.25 ± 5.63	4.7 ± 0.08	6.09 ± 0.68	212.50 ± 10.13	62.05 ± 3.62	50.69 ± 5.13
	UB12	0.48 ± 0.03	45.45 ± 5.75	3.7 ± 0.07	6.45 ± 1.19	191.20 ± 12.52	80.51 ± 1.72	52.06 ± 6.21

Note: GB: *Gelidium* bread; UB: *Ulva* bread; RS: reducing sugars; TS: total sugars; TP: total proteins; TPC: total phenolic content; TF: total flavonoids.

Algae are known as a natural source of essential nutrients, including macronutrients (carbohydrates, proteins, and lipids) and micronutrients (vitamins). Carbohydrates account for up to 60% of all bioactive compounds in seaweed. Algal carbohydrates include glycosaminoglycans (GAGs), also known as mucopolysaccharides, which are composed of amino sugars and uronic sugars, as well as fucans, mannitol, sorbitol, carrageenans (natural hydrocolloids), and agar, which is used as a natural thickening and gelling agent [55].

Proteins Contents

Protein contents in *Gelidium* bread varied between 4.3 ± 0.8 and 14.4 ± 0.1% and from 3.7 ± 0.7 to 12.1 ± 0.5% for *Ulva* bread, which probably indicates the digestibility effect of supplemented LAB strains on the biodegradation of seaweed protein. The obtained values of proteins were higher than those reported by Khoozani et al. [56]. Seaweeds are a rich source of proteins and amino acids, such as glycoproteins, metalloproteins, and exogenous amino acids such as alanine, asparagine, glycine, lysine, serine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine [55].

Total Phenolic Compounds and Flavonoids

Phenolic compound amounts in *Gelidium*- and *Ulva*-enriched bread samples ranged between 5.70 ± 2.71 and 8.32 ± 1.90 mg GAE/g and from 4.65 ± 0.36 to 6.94 ± 2.05 mg GAE/g DW, respectively. Flavonoid contents in *Gelidium* and *Ulva* bread samples ranged between 120 ± 15.61 and 320 ± 17.52 mg QE/g DW and from 81.2 ± 7.89 to 216.2 ± 10.92 mg QE/g DW, respectively. Our findings showed that the PC values are higher than those reported by Özcan [57], who reported that PC in enriched bread ranged from 0.37 to 0.58 mg GAE/g DW, and flavonoid amounts varied from 0.61 to 0.99 mgQE/g DW. Moreover, PC amounts are higher when compared to those reported by Rico et al. [54], who found that PC amounts in enriched bread with algae (8%) were 24.05 µmol GAE/g DW.

Phenolic compounds and flavonoids are commonly known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. These metabolites have been reported for their antioxidant, anticancer, antibacterial, cardioprotective, and

anti-inflammatory properties promoting the protection of the skin against UV rays, and are an interesting candidate for pharmaceutical and medical applications [58].

It should be highlighted that the decrease of pH obtained during LAB fermentation facilitates phenolic compound availability for nutrition. Thus, sourdough and acidified breads theoretically should have more available phenolics in the free form when compared to the control dough. Phenolic compounds must be first released from the bread matrix during solubilization in order to express their antioxidant activity. Their chemical structure as well as the food matrix interactions (such as with carbohydrates, proteins, and lipids) are factors that impede the bioaccessibility and the bioavailability of phenolic compounds [30].

The bioavailability of phenolic compounds may substantially change based on the ability of the organism to take up the polyphenols from the food matrix and on the interaction between polyphenols and blood protein as well as cell transporters. For these reasons, the most abundant polyphenols in the diet might not necessarily be those showing the highest bioavailability and leading to the highest concentrations of active metabolites. Phenolic compounds exist in nature predominantly as O- or C-glycoside conjugates, and it has been evidenced that only slight modifications occur along the upper gastrointestinal tract. Once reached the small intestine, polyphenols undergo extensive metabolism by enzymes able to release the aglycone, i.e., cytosolic β-glucosidases [59].

Antioxidant Activity

In recent years, there has been a great demand for natural antioxidants as an alternative to synthetic ones. Indeed, in animal models, a number of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have demonstrated harmful effects or carcinogenic effects. Although most natural antioxidants currently available on the market are derived from terrestrial plants, seaweeds are being considered more often as a potential source of natural antioxidant compounds for the food industry [60].

Obtained results showed that recorded antioxidant activity in *Gelidium* bread samples was important: 85.27 ± 2.51, 82.93 ± 3.61, and 74.71 ± 3.15% for GB3, GB1, and GB4, respectively (Table 6). This percentage decreased in GB5 (undetected activity) and slightly in bread GB8, GB11, and GB12 (4.61 ± 1.10–12.91 ± 0.83%). Regarding *Ulva* bread samples, the antioxidant activity values were found to be relevant and ranging between 76.72 ± 2.23 and 85.55 ± 2.19% for UB6, UB4, UB7, UB12, UB3, UB8, and UB2, respectively. Other samples showed values less than 70.00% (Table 6). Samples of *Ulva* bread showed high antioxidant activity when compared to *Gelidium* bread samples.

The antioxidant activity of algae bread was performed with another test (β-carotene assay). Results of this assay showed that *Gelidium* bread samples reached their maximum of 71.05 ± 3.71% in GB1, while in the *Ulva* bread samples, UB9 reached 78.68 ± 4.56% compared to the control (28.86 ± 1.05%); and eight samples, both in *Gelidium* and *Ulva*, showed rates of more than 60%. Moreover, the results showed that the algae powder fortification level has an impact on the antioxidant activity, either with *Gelidium* or *Ulva* species. Thus, fortification levels of 2.5% with *Gelidium* and 7.5% with *Ulva* resulted in an increase in the antioxidant activity of enriched bread ranging from 60 to 82% for GB and from 50 to 82% for UB, respectively.

It was reported that astaxanthin and carotenoid (compound) are lipophilic scavengers that enhance the antioxidant activity of *U. lactuca*. Additionally, these compounds depended on the switching of aromatic rings and the arrangement of the hydroxyl moieties. Numerous earlier studies on different algal extracts of DPPH, ORAC, ABTS, and TAC have also been studied, which have proved their antioxidant capacity. On the other hand, probiotics and their byproducts are acknowledged as a novel source of potent antioxidants. They enhance the action of antioxidant enzymes or alter oxidative stress in the circulatory system [61].

LAB isolated from a marine source was used to ferment Sargassum sp., with significant increases in antioxidant activities. The Lactobacillus plantarum fermentation of three edible Irish brown seaweeds promoted an increase in the antioxidant activity of the fermentation broth. Although these results indicated that LAB fermentation can change free TPC and antioxidant ability, its effects on the free and bound polyphenol profiles of seaweeds have not been well studied [62].

Obtained results are similar to those of Arufe et al., who reported that the antioxidant activity of seaweed-enriched bread after baking ranged between 43.8 ± 1.7 and 76.0 ± 0.7% DW [63]. Furthermore, Rico et al. [54] reported that bread formulated partially with seaweeds showed the lowest PC content without significant differences between seaweed species. In addition, no correlation was observed by the authors between PC amounts and antioxidant activity, suggesting that the major antioxidant compounds in breads might be non-phenolics [54]. It should be noted that in our case the dough used for bread preparation plays a significant role in the DPPH activity increase of enriched breads augmented with the increase of algae enrichment level, indicating probably higher levels of hydrophilic compounds like ascorbic acid and hydrophobic compounds like pigments.

The total carbohydrates and proteins digestion showed better rates within the *Gelidium* bread samples of GB11 and GB10 (0.46 ± 0.09 and 4.3 ± 0.8%), while UB10 and UB11 showed 0.28 ± 0.08% and 4.7 ± 0.0%, respectively. Based on these results, both algae bread samples of *Gelidium* and *Ulva* showed better digestion of carbohydrates and proteins into simple sugars and peptides.

According to the obtained results, a personal daily intake of algae bread could be recommended at 100 g for *Gelidium* bread (e.g., GB3 at 7.5%), which showed high phenol compounds (TPC: 7.47 ± 0.92 mg GAE/g DW and TF: 203.70 ± 19.81 mg QE/g DW) and an antioxidant activity (64.37 ± 4.89 and 85.27 ± 2.51%). Moreover, the intake of *Ulva* bread (UB2, 5%) could also be recommended at 100 g, which corresponds to high phenolic compounds (TPC: 5.92 ± 0.95 mg GAE/g DW and TF: 98.70 ± 9.85 mgQE/g DW) and an antioxidant activity (54.32 ± 3.63 and 85.55 ± 2.19%).

3.4. Consumer Sensory Evaluation

The sensory properties of the enriched bread with the two algae powders showed some differences depending on the algae fortification level. Moreover, results showed that the fortified level of 10% gave the best scores and significances (vs. negative control) compared to the enrichment levels of 2.5, 5, and 7.5% (vs. negative control), which showed no significant score values when compared to controls. The mean data from the consumer panel evaluations (at a 10% fortification level) are presented in Table 7.

Table 7. Sensory properties of enriched bread samples with *G. corneum* and *U. lactuca* powder at 10% with different sourdoughs formulas.

LAB Combination	Samples	Crumb Color	Odor	Texture	Taste	General Appearance
-	Control	3.70 ± 1.42	2.50 ± 1.08	3.00 ± 1.56	2.90 ± 0.56	2.70 ± 1.63
LCS1	GB1 (10%)	4.00 ± 1.33	3.80 ± 1.03	3.30 ± 1.41	3.40 ± 0.84	3.90 ± 1.10
LCS2	GB2 (10%)	3.90 ± 1.45	3.50 ± 1.08	3.50 ± 1.08	3.30 ± 1.06	3.60 ± 1.43
LCS3	GB3 (10%)	3.80 ± 1.31	3.80 ± 1.03	2.60 ± 1.50	3.00 ± 0.66	3.20 ±1.26
LCS1	UB1 (10%)	3.20 ± 1.03	3.50 ± 1.08	2.10 ± 0.73	2.90 ± 0.56	2.60 ± 1.26
LCS2	UB2 (10%)	3.20 ± 1.03	3.50 ± 1.35	2.80 ± 1.03	3.20 ± 0.78	3.00 ± 1.33
LCS3	UB3 (10%)	3.70 ± 1.16	3.30 ± 1.42	2.70 ± 1.42	3.20 ± 0.63	2.70 ± 1.16

Note. GB: *Gelidium* bread; UB: *Ulva* bread; LCS: lactic acid bacteria sourdoughs.

As shown in Table 7, the taste point of bread samples increased from 2.90 (control), 3.00–3.40 for *Gelidium* bread (10%), and 2.90–3.20 for *Ulva* bread (10%) with different sour-

dough LAB combinations. While the odor point changed from 2.50 (control) to 3.50–3.80 for *Gelidium* bread and to 3.30–3.50 for *Ulva* bread samples, respectively. Moreover, crumb color values of bread samples fortified with 10% of algae powder varied from 3.70 (control) to 3.80–4.00 for *Gelidium* bread and to 3.20–3.70 for *Ulva* bread, respectively, while the texture point varied from 3.00 (control) to 2.60–3.5 for *Gelidium* bread and to 2.10–2.80 for *Ulva* bread, respectively. Finally, for the visual appearance point, the values increased from 2.70 (control) to 3.2–3.9 for *Gelidium* bread and to 2.70–3.00 for *Ulva* bread, respectively.

Regarding LAB combination sourdoughs (LCS), it was observed that LCS1 and LCS2 showed high point scores for *Gelidium* bread samples compared to *Ulva* bread, and no significant difference was observed for LCS3 between *Gelidium* bread and *Ulva* bread samples.

In general, the most acclaimed bread by the panelists was the *Gelidium* bread enriched with 10% algae powder. Regarding sensory results, and excepting the modest appreciation of bread sample texture, the other sensory parameters revealed acceptable appreciation by the untrained panelists for seaweed bread enrichment with algae powder. Indeed, bread enrichment with *Gelidium* and *Ulva* algae powders improved bread odor and taste for all bread samples prepared with different sourdough LAB combinations.

The general evaluation of sensory properties of enriched bread made from wheat flour and algae powder shows the potential application of algae flour in the bakery industry. As a result of the analysis of the fortified bread made at the laboratory scale, the most liking was taken to bread fortified with *G. corneum* and *U. lactuca* powders at a concentration of 10% that gave the best scores, and this rate could be recommended in bread making. In addition to the high consumer acceptability, the bread fortified with algae powder showed interesting nutritional properties, such as decreasing sugar amounts (both reducing and total sugars) and protein levels in fortified bread samples.

Obtained results suggest that seaweed bread production with different fortification levels and its use as a human nutritional model by producing an enriched bread with fewer carbohydrates could be suitable for people suffering from chronic diseases such as diabetes (types 1 and 2).

It has been reported that *Lactobacillus* spp., prevalent in the microbiota, are able to cleave gluten peptides, thereby reducing their immunogenicity via the indigenous protease by LAB acidification [60]. Thus, according to the results obtained, LAB sourdough strains showed the ability to reduce proteins with different decrease rates, which might suggest such consumption of seaweed bread for a healthy diet.

At the same time, results of the different assays showed an increase in enriched bread antioxidant activity, which could be directly related to the increase in PC and TF amounts. Indeed, the benefits of a high level of antioxidant activity could contribute to avoiding several types of diseases, such as cancer and gastrointestinal tract disorders, etc., via phenolic compounds. The use of different levels of seaweed powder (*G. corneum* and *U. lactuca*) in enriched bread production based on LAB sourdoughs revealed high sensory acceptability scores and gave recognized nutritional properties by decreasing protein levels and probably generating free amino acids and/or bioactive peptides necessary for human well-being and high antioxidant activity to protect the human organism from oxidative stress consequences.

With regard to the large-scale human intake of algae, environmental issues vary from one country to another. While Spanish consumers consider microalgae to be environmentally friendly, the French have the opposite perception, and the Belgians do not see this as an incentive to consumption. On the regulatory aspect, in the United States, the FDA grants algae-based products the status of products generally recognized as safe (GRAS) for consumption.

It should be noted that seaweed has been used as food for a long time, with a strong market in Asia and a growing market in Europe, driven by increasing consumer awareness of healthy and safe food. In addition, seaweeds are a sustainable source of valuable natural bioactive compounds. There is very limited data on seaweed consumption in Europe, but it is expected to be significantly lower (<50%) than in Asian countries, where the average daily consumption of seaweed by adults, for example, ranges from 4 to 8.5 g. As an example, consumption of dried seaweed in Japan is around 2 kg per capita per year. Product launches of traditional foods containing seaweed ingredients, such as bread and other products, are on the rise in the European market, holding a 1.34% share of new European food and drink launches since 2017 [64].

It should also be noted that in Morocco and neighboring North African countries (Algeria, Tunisia, etc.), traditional fermented bread is of major social and cultural importance. Its fortification with seaweed powder rich in bioactive compounds, resulting in particular organoleptic properties and nutritional and biological activities, would be a novelty in bakery products on the national and regional scale, where the demand for local and natural products has been growing constantly in recent years.

4. Conclusions

In conclusion, enrichment of sourdough bread with seaweed powder (*G. corneum* and *U. lactuca*) using indigenous LAB fermentation starters showed high antioxidant activity in the final bread. In parallel, a decrease in carbohydrate and protein amounts was observed after LAB fermentation of wheat flour supplemented with both tested seaweed powders when compared to the negative control. Regarding the general consumer evaluation, the sensory test showed a good, enriched bread acceptability (10%) with both seaweed powders, with better parameter scores for *Gelidium* bread compared to *Ulva* bread. The used fortification level (10%) gave a typical bread with good nutritional properties characterized by a high antioxidant activity due to the generated phytochemicals (phenolic compounds, flavonoids, etc.) and a considerable reduction in sugar levels. These findings suggest the use of seaweed powder as a promising ingredient in bread making to improve the nutritional quality and phytochemical properties of traditional sourdough bread, which could constitute a new opportunity for the marketing of novel bakery products with recognized added value. Nevertheless, it is necessary to assess the bioavailability and stability of phenolic compounds and flavonoids during the digestion and the storage of enriched bread. Moreover, the shelf life, the marketability, and the environmental feasibility of producing algae-enriched bread needed to be performed on a commercial scale.

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