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Physicochemical, Microbiological, and Sensory Properties of Gluten-Free Bread Fortified with *Caulerpa lentillifera* as a Natural Iodine Source

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Abstract

The rising prevalence of celiac disease and gluten-related disorders has substantially increased global demand for gluten-free bread (GFB). Such products remain nutritionally inferior to wheat-based counterparts, particularly in dietary fiber, minerals, and iodine. *Caulerpa lentillifera*, a mineral-rich green seaweed, represents a promising natural ingredient for iodine fortification of gluten-free bakery products. The present study aimed to develop iodine-fortified GFB incorporating dried *C. lentillifera* at 0% (w/w) - 3% (w/w) in jasmine rice flour-based formulations and to evaluate physicochemical properties, proximate composition, iodine content, microbiological quality, and sensory acceptability. Five formulations were prepared incorporating dried *C. lentillifera* at 0% (control), 1% (GFB-1 and GFB-2), 2% (GFB-3), and 3% (GFB-4). Salt content was 0.87% in the control and GFB-1, whereas GFB-2 through GFB-4 contained a reduced salt level of 0.435%. Specific volume, ash content, and dietary fiber increased progressively with *C. lentillifera* concentration (2.42 - 3.29 cm^3/g, 1.05% - 2.32%, and 2.24% - 3.72%, respectively). Total carbohydrate content decreased correspondingly, reflecting compositional displacement of jasmine rice flour. Iodine content increased dose-dependently from 0.36 ± 0.01 mg/kg in the control to 1.06 ± 0.01 mg/kg in GFB-4, with all %RSD values below 5% confirming high ICP-MS precision. A 50% salt reduction in GFB-2 did not significantly compromise physicochemical or sensory quality relative to GFB-1 ($p > 0.05$), confirming the technical feasibility of sodium reduction. All formulations maintained plate counts below 1×10^4 CFU/g and yeast and mold below 1×10^2 CFU/g throughout 8 days at 4 °C. Sensory acceptability did not differ significantly among formulations for any evaluated attribute ($p > 0.05$). GFB-3 was identified as the optimal formulation, achieving the highest overall acceptability score (7.47 ± 1.14) alongside notable iodine fortification (0.70 ± 0.01 mg/kg), enhanced dietary fiber and mineral content, and acceptable microbiological quality. Overall, dried *C. lentillifera* represents a multifunctional natural ingredient for developing nutritionally enhanced, iodine-fortified GFB that benefits populations at risk of iodine insufficiency.

Keywords: Gluten-free bread, *C. lentillifera*, Iodine fortification, Jasmine rice flour, Salt reduction, ICP-MS, Dietary fiber

Introduction

Consumer interest in gluten-free products has risen considerably, propelled by an increasing prevalence of gluten-related disorders and a broader shift toward gluten-free dietary patterns [1]. Despite such growing demand, commercially available GFB products consistently demonstrate nutritional inferiority relative to wheat-based counterparts, particularly with respect to dietary fiber, essential minerals, and iodine [2]. Addressing this dual challenge of technological functionality and nutritional adequacy therefore remains a critical and inadequately resolved priority in gluten-free product development. Among these disorders, celiac disease affects approximately 1% of the global population, whereas non-celiac gluten sensitivity affects up to 6% [1,3,4]. Reflecting the rising prevalence of gluten-related disorders, consumer demand for gluten-free products has grown correspondingly, with the market expanding by approximately 16% between 2018 and 2022 [1]. Such upward trends are reflected in market projections, with global consumption expected to grow from USD 6.7 billion to USD 14 billion between 2022 and 2032 [5]. The observed growth trends underscore the importance of developing high-quality, nutritionally adequate gluten-free products in this rapidly growing market.

Gluten, a storage protein complex in wheat, barley, and rye, forms a viscoelastic network critical for bread loaf volume, crumb structure, and texture, yet replicating these functional properties in gluten-free formulations remains a persistent technological challenge [4]. Rice flour is particularly favored in gluten-free bread (GFB) production due to its hypoallergenic nature, mild flavor, and widespread availability in Southeast Asia [5]. Rice-based GFB, however, is frequently associated with inferior crumb structure, lower specific volume, increased hardness, and rapid staling compared to wheat-based counterparts [5,6]. Critically, GFB products are often deficient in dietary fiber, essential minerals, and micronutrients, with only 5% of commercially available GFB fully fortified with mandatory micronutrients [2]. The identified nutritional shortcomings underscore the need for functional ingredient strategies that can simultaneously improve both the technological and nutritional profiles of GFB.

Seaweeds are recognized as functional food ingredients rich in dietary fiber, polysaccharides, minerals, and diverse bioactive compounds [7]. Among edible marine macroalgae, *C. lentillifera* (sea grape) is a green alga (Chlorophyta) widely cultivated and consumed in the Indo-Pacific and Southeast Asian regions [8,9]. *C. lentillifera* has attracted considerable scientific interest due to its rich polysaccharides, essential minerals, and bioactive compounds with demonstrated antioxidant, anti-inflammatory, and antimicrobial properties [7,8]. Of particular relevance, *C. lentillifera* is recognized as a natural iodine source, as reported iodine levels are consistent with Dietary Reference Intake recommendations [7,10]. Iodine is an essential micronutrient indispensable for thyroid hormone biosynthesis, regulating cellular metabolism, growth, and neurological development [11]. Iodine deficiency remains prevalent worldwide, particularly in inland and plant-based populations [12,13]. Seaweed incorporation into staple foods has been recognized as a sustainable dietary strategy to address iodine deficiency [14]. The incorporation of *C. lentillifera* into GFB may therefore address both the mineral and iodine deficiencies characteristic of commercially available GFB products.

From a food technology perspective, sulfated polysaccharides and dietary fiber in *C. lentillifera* may function analogously to hydrocolloids within gluten-free dough systems, increasing water-holding capacity and dough viscosity [15,16]. Such hydrocolloid-like properties may promote gas cell stabilization during fermentation, thereby improving the development of crumb structure in rice flour-based GFB [15,17]. Additionally, bioactive compounds in *C. lentillifera* exhibit antimicrobial activity against foodborne microorganisms, suggesting a potential role in extending microbiological shelf life without synthetic preservatives [18,19]. Seaweed incorporation into bread has been shown to improve nutritional value, texture, and shelf life [4,20]. However, prior studies have predominantly focused on macroalgae species other than *C. lentillifera*, or have evaluated seaweed incorporation in wheat-based rather than gluten-free bread systems [4,20]. Furthermore, iodine fortification potential has rarely been systematically quantified alongside physicochemical and sensory outcomes

within a single experimental framework [7,21]. Microalgae incorporation into GFB has nevertheless raised concerns regarding sensory acceptability and dough rheology [21]. The observed limitations underscore the importance of selecting algal species with acceptable sensory profiles, nutritional value, and technological functionality that do not compromise consumer acceptance. Bread has been widely recognized as an effective vehicle for iodine fortification at the population level [14,22,23]. Nevertheless, the potential of *C. lentillifera* as a natural iodine source in jasmine rice flour-based GFB has not yet been systematically characterized. No prior study has simultaneously characterized *C. lentillifera* as a natural iodine source alongside physicochemical, microbiological, and sensory outcomes in a jasmine rice flour-based GFB system. The combined effect of *C. lentillifera* incorporation and partial salt reduction on such parameters also remains unexamined, representing a critical gap given the growing global burden of iodine deficiency [12,13]. The novelty of the present study lies in the simultaneous characterization of dried *C. lentillifera* as a multifunctional ingredient in jasmine rice flour-based GFB. Specifically, its roles in iodine fortification, dietary fiber enhancement, mineral enrichment, and antimicrobial functionality are evaluated within a single consumer-acceptable, sodium-reduced bread system.

Therefore, the present study aimed to address these gaps through 5 specific objectives: (1) to develop jasmine rice flour-based GFB incorporating dried *C. lentillifera* at 0% (w/w) - 3% (w/w) as a natural iodine-fortifying ingredient; (2) to quantify iodine content by ICP-MS and evaluate dose-dependent iodine fortification efficiency; (3) to characterize physicochemical properties including specific volume, texture, color, and proximate composition; (4) to assess microbiological quality during refrigerated storage at 4 °C; and (5) to evaluate the feasibility of 50% salt reduction without compromising physicochemical properties or sensory acceptability.

Materials and methods

Experimental design

The overall experimental design and research workflow are illustrated in **Figure 1**. Five GFB formulations were developed using jasmine rice flour as the base ingredient, incorporating dried *C. lentillifera* at concentrations of 0% (w/w), 1% (w/w), 2% (w/w), and 3% (w/w). To independently evaluate the effect of salt reduction, GFB-1 and GFB-2 were formulated at equivalent dried *C. lentillifera* concentration (1% w/w) with differing salt contents (0.87% and 0.435%, respectively). The effect of increasing dried *C. lentillifera* concentration was evaluated across GFB-2, GFB-3, and GFB-4, all containing the reduced salt level (0.435%), thereby maintaining salt content as a controlled variable within this concentration series. The concentration range of 1% - 3% dried *C. lentillifera* was selected based on preliminary screening trials, in which concentrations below 1% produced negligible effects on bread quality, whereas concentrations exceeding 3% resulted in unacceptable sensory characteristics. Following bread preparation and baking, all formulations were subjected to 4 categories of analysis: Physical properties (specific volume, color, texture, and moisture content), chemical composition (proximate analysis and iodine content by ICP-MS), microbiological quality during refrigerated storage (Day 0 - 8), and sensory evaluation using a 9-point hedonic scale. Data were statistically analyzed by one-way ANOVA with Tukey's HSD post hoc test at $p < 0.05$, and the findings are presented in the Results and Discussion section.

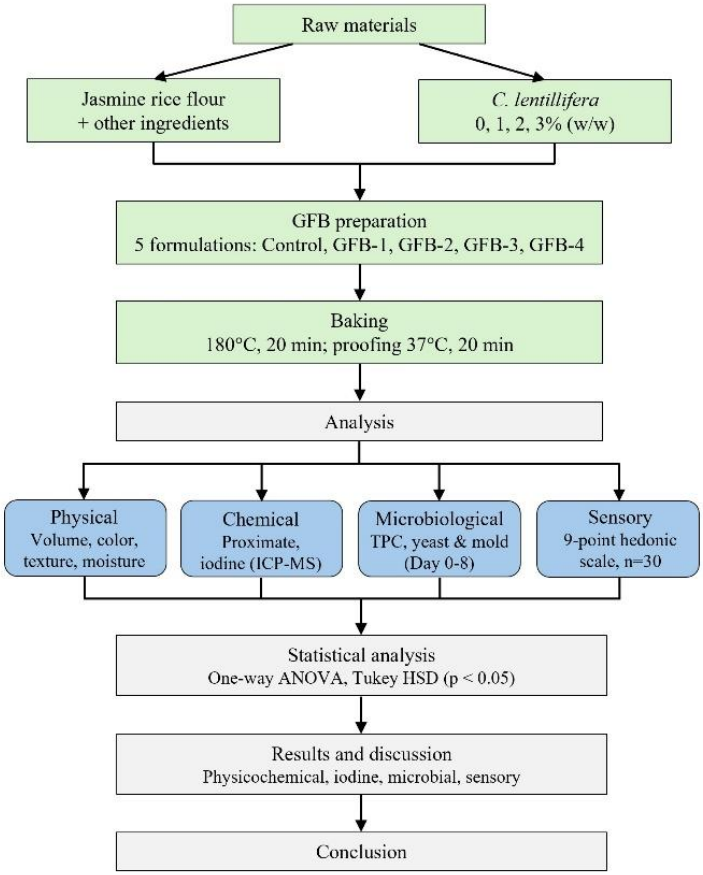


Figure 1 Experimental design and research workflow for the development of GFB fortified with dried *C. lentillifera.*

Raw materials

C. lentillifera was obtained from a local supplier in Si Kao District, Trang Province, Thailand. Jasmine rice flour (Petchpanthong, Thailand), instant dry yeast (Saf-instant, Lesaffre, France), vegetable oil, brown sugar, and salt were purchased from a local commercial market. All ingredients were of food-grade quality.

Preparation of dried *C. lentillifera*

Fresh *C. lentillifera* was cleaned, washed with potable water to remove debris, and oven-dried at 60 °C until a constant weight was achieved in a tray dryer. The dried seaweed was then ground using a laboratory blender (Waring, USA) and sieved through a 60-mesh screen to obtain a uniform particle size. The dried *C. lentillifera* was sterilized by autoclaving at 121 °C for 15 min (SX 700, TOMY, Japan) before use to eliminate microbial contamination from the raw material while minimizing heat-induced degradation of chlorophyll pigments.

Gluten-free bread formulation

Five GFB formulations were prepared with varying concentrations of dried *C. lentillifera* as shown in **Table 1**. The control formulation contained no dried *C. lentillifera*, whereas GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w). GFB-2 differed from GFB-1 only in salt content, with GFB-2 containing a reduced level (0.435%) compared to GFB-1 (0.87%). The salt level of GFB-2 (0.435% (w/w)) was set at 50% of that used in GFB-1 (0.87% (w/w)), in accordance with WHO guidelines recommending dietary sodium reduction for adults [24]. Such a reduction also aligns with current public health objectives for sodium reduction in processed bakery products. A paired formulation design at equivalent dried *C. lentillifera* concentration (1% (w/w)) enabled independent evaluation of the salt reduction effect.

Table 1 Formulation of GFB incorporating dried *C. lentillifera* (% (w/w)).

Ingredient	Control	GFB-1	GFB-2	GFB-3	GFB-4
Jasmine rice flour	52.33	51.33	51.77	50.77	49.77
Water	34.88	34.88	34.88	34.88	34.88
Vegetable oil	6.54	6.54	6.54	6.54	6.54
Salt	0.87	0.87	0.435	0.435	0.435
Brown sugar	4.36	4.36	4.36	4.36	4.36
Instant yeast	1.02	1.02	1.02	1.02	1.02
Dried *C. lentillifera*	0.00	1.00	1.00	2.00	3.00
Total	100	100	100	100	100

Bread was prepared as follows: water, brown sugar, and salt were mixed until fully dissolved. Jasmine rice flour, dried *C. lentillifera*, and instant yeast were added to a stand mixer and blended at speed level 3 until well combined. The dissolved wet ingredients were then incorporated into the dry mixture and blended until a homogeneous batter was obtained. Vegetable oil was added, and mixing continued for 20 min until a film-forming dough consistency was achieved. The dough was divided into 100 g portions, shaped, and placed into bread molds. Proofing was conducted at 37 °C for 20 min in an oven. Bread was then baked in an electric oven at 180 °C for 20 min. Following baking, loaves were carefully demolded, cooled to room temperature, and stored in airtight containers pending analysis.

Physical properties

Bread volume, specific volume, density, and baking loss

Bread volume was determined using the rapeseed displacement method in accordance with AACC International Method 10 - 05.01 [25]. Specific volume (cm^3/g) was calculated by dividing bread volume by bread weight. Bread density (g/cm^3) was calculated as the reciprocal of specific volume. Baking loss (%) was calculated using the following equation as described by Wirkijowska *et al.* [26]:

$$\text{Baking loss (\%)} = [(W_1 - W_2) / W1] \times 100$$

where W_1 is the weight of dough before baking and W_2 is the weight of bread after baking.

Color analysis

Crust and crumb color were determined colorimetrically using a ColorFlex EZ instrument (HunterLab, USA) and expressed as L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness). The total color difference (ΔE^*) relative to the control formulation was calculated using the following equation as described by Wirkijowska *et al.* [26]:

$$\Delta E^* = \sqrt{[(L^*_c - L^*_i)^2 + (a^*_c - a^*_i)^2 + (b^*_c - b^*_i)^2]}$$

where L^*_c, a^*_c, and b^*_c are the color coordinates of the control, and L^*_i, a^*_i, and b^*_i are the color coordinates of each tested formulation.

Texture profile analysis

Texture profile analysis (TPA) was performed using a texture analyzer (CT3-10KG, AMETEK Brookfield, USA) equipped with a P/36R cylindrical probe. Samples were compressed twice to 50% of their original height at a test speed of 2 mm/s. Parameters including hardness (gf), springiness, gumminess (gf), and chewiness (gf) were recorded [27].

Moisture content and water activity

Moisture content was analyzed with a halogen moisture analyzer (Mettler Toledo HE53, Switzerland) following AOAC method 925.10 [28]. Water activity (a_w) was assessed at 25 °C with a water activity meter (LabTouch-AW, NOVASINA, Switzerland).

Chemical composition

Proximate analysis

Proximate composition was determined according to standard AOAC methods [28] as follows: moisture content by oven-drying method (AOAC 925.10), ash content by dry ashing at 550 °C (AOAC 900.02A), crude protein by the Kjeldahl method using a conversion factor of 6.25 (AOAC 920.87), crude fat by the Soxhlet extraction method (AOAC 960.39), and crude fiber by the gravimetric method (AOAC 991.43). Total carbohydrate content was computed by difference as follows:

Carbohydrate (%) = 100 − (moisture + ash + protein + fat + fiber)

Energy value

Gross energy content was determined using a bomb calorimeter (Parr Instrument Company, USA).

Iodine content

Iodine content was quantified by inductively coupled plasma mass spectrometry (ICP-MS; NexION 300Q, PerkinElmer, Waltham, MA, USA) with alkaline extraction using tetramethylammonium hydroxide (TMAH), following the procedures of Photi *et al.* [29] and the USFDA Elemental Analysis Manual Section 4.13 [30]. Briefly, approximately 0.5 g of homogenized bread sample was transferred into a centrifuge tube, and 10 mL of 5% TMAH solution was introduced. The suspension underwent vortexing for 1 minute prior to incubation in a hot block at 85 °C for 150 min. Upon cooling, the extract was centrifuged at 3,000 rpm for 3 min, with the resulting supernatant collected for ICP-MS measurement at m/z = 127. The LOD and LOQ were 0.03 and 0.10 mg/kg, respectively. All determinations were conducted in duplicate.

Microbiological analysis

Bread samples were stored at 4 °C and subjected to microbiological analysis on Day 0, Day 3, Day 6, and Day 8 of storage. Total plate count (TPC) and yeast and mold count were enumerated on plate count agar (PCA) incubated at 35 °C for 48 h and potato dextrose agar (PDA) acidified with 10% tartaric acid incubated at 25 °C for 5 days, respectively. All analyses followed standard AOAC methods [28], with results expressed as colony-forming units per gram (CFU/g).

Sensory evaluation

The study protocol was approved by Rajamangala University of Technology Isan Ethics Committee (approval no. HEC-01-66-034). Sensory evaluation was conducted with 30 untrained panelists using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) [31]. Panelists were recruited from the university student and staff population, with no reported food allergies or dietary restrictions relevant to the tested products. Each panelist evaluated all 5 formulations in a single session, with samples served in randomized 3-digit coded portions of approximately 20 g at room temperature. Panelists evaluated the following attributes: Appearance, color, aroma, taste, texture, and overall acceptability. All samples were presented simultaneously under standardized lighting conditions. Water was made available for palate cleansing between samples. All panelists provided written informed consent prior to participation, in accordance with the approved study protocol. The sensory experiment was statistically arranged using a Randomized Complete Block Design (RCBD).

Statistical analysis

All experiments were conducted in triplicate using a Completely Randomized Design (CRD), with the exception of iodine content determination, which was performed in duplicate, and sensory evaluation, which used a Randomized Complete Block Design (RCBD). Data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed, and differences among means were compared using Tukey's Honestly Significant Difference (HSD) post hoc test at a 95% confidence level ($p < 0.05$). Statistical analyses were performed using JASP software version 0.18 (JASP Team, University of Amsterdam, Netherlands).

Results and discussion

Physical properties

Specific volume, density, and baking loss

The specific volume, density, and baking loss of the 5 GFB formulations are presented in **Table 2**.

Table 2 Baking loss, volume, specific volume, and density of GFB incorporating dried *C. lentillifera.*

Sample	% Loss	Volume (cm^3)	Specific volume (cm^3/g)	Density (g/cm^3)
Control	15.05 ± 0.06^{a}	1.24 ± 0.06^{d}	2.42 ± 0.04^{d}	0.41 ± 0.02^{a}
GFB-1	12.64 ± 0.14^{c}	1.28 ± 0.04^{c}	2.63 ± 0.04^{cd}	0.39 ± 0.01^{ab}
GFB-2	12.62 ± 0.33^{c}	1.29 ± 0.02^{c}	2.66 ± 0.01^{bc}	0.38 ± 0.01^{cd}
GFB-3	13.91 ± 0.20^{b}	1.32 ± 0.01^{b}	2.97 ± 0.03^{b}	0.35 ± 0.01^{c}
GFB-4	14.29 ± 0.28^{b}	1.35 ± 0.02^{a}	3.29 ± 0.02^{a}	0.31 ± 0.01^{d}

Note: Different letters ^{abcd} in the same column indicate statistically significant differences based on Tukey's HSD post hoc test at the 95% confidence level (n = 3). Significance groupings are derived from the pooled mean square error of one-way ANOVA and do not correspond to overlap of mean ± SD ranges.

Specific volume increased significantly with increasing *C. lentillifera* concentration ($p < 0.05$), ranging from 2.42 ± 0.04 cm^3/g in the control to 3.29 ± 0.02 cm^3/g in GFB-4. GFB-1 (2.63 ± 0.04 cm^3/g) and GFB-2 (2.66 ± 0.01 cm^3/g) were not significantly different from the control, whereas GFB-3 (2.97 ± 0.03 cm^3/g) and GFB-4 exhibited significantly higher values ($p < 0.05$). Correspondingly, density decreased progressively from 0.41 ± 0.02 g/cm^3 in the control to 0.31 ± 0.01 g/cm^3 in GFB-4, consistent with the inverse relationship between specific volume and density. GFB-1 (0.39 ± 0.01 g/cm^3) was not significantly different from the control, whereas GFB-2 (0.38 ± 0.01 g/cm^3) and GFB-3 (0.35 ± 0.01 g/cm^3) showed intermediate values, and GFB-4 exhibited the lowest density ($p < 0.05$). The progressive reduction in density confirms the dose-dependent effect of *C. lentillifera* incorporation on crumb expansion and the development of loaf structure. The observed increase in specific volume may be attributed to the hydrocolloid-like properties of sulfated polysaccharides and dietary fiber in *C. lentillifera*, which enhance dough viscosity and stabilize gas cells during proofing. The dose-dependent increase in specific volume was mechanistically accompanied by a corresponding decrease in density, reflecting progressive crumb expansion as a direct consequence of enhanced gas cell stabilization. At higher *C. lentillifera* concentrations, greater polysaccharide availability amplified dough viscosity, sustaining gas cell integrity throughout proofing and baking, and yielding a less dense, more open crumb matrix [4,15,17]. Such an improvement is particularly relevant, as rice-flour-based GFB typically exhibits inferior crumb structure and lower specific volume than wheat-based GFB [5].

Baking loss ranged from 12.62 ± 0.33% in GFB-2 to 15.05 ± 0.06% in the control. The control exhibited significantly higher baking loss than GFB-1 and GFB-2 ($p < 0.05$), whereas GFB-3 (13.91 ± 0.20%) and GFB-4 (14.29 ± 0.28%) were not significantly different from the control ($p > 0.05$). The lower baking loss in GFB-1 and GFB-2 may reflect the water-holding capacity of *C. lentillifera* polysaccharides, which retain moisture within the crumb matrix during baking [17]. Baking loss showed no consistent concentration-dependent trend, with GFB-3 and GFB-4 exhibiting higher values than GFB-1 and GFB-2 despite higher *C. lentillifera* concentrations. The observed trend may reflect competing effects between polysaccharide water-holding capacity and structural disruption of the starch network at elevated fiber concentrations [32]. Specifically, at lower *C. lentillifera* concentrations (1%), soluble sulfated polysaccharides effectively retained moisture within the crumb matrix, reducing baking loss. At higher concentrations (2% - 3%), insoluble fiber particles progressively disrupted starch gelatinization network continuity, reducing the structural integrity of the crumb matrix and diminishing moisture retention efficiency during baking [32,33].

GFB-1 and GFB-2 exhibited comparable specific volume and baking loss, with no statistically significant differences between the 2 formulations ($p > 0.05$). A 50% reduction in salt content in GFB-2 (0.435%) compared to GFB-1 (0.87%) did not substantially alter loaf volume or moisture retention at equivalent *C. lentillifera* concentrations. Salt reduction to 0.435% is therefore technically feasible without compromising physical bread quality.

Color and appearance

The cross-sectional appearance of the 5 GFB formulations is illustrated in **Figure 2**, and the corresponding colorimetric data for crust and crumb are presented in **Table 3**. Visual inspection revealed progressive darkening of crumb color with increasing dried *C. lentillifera* concentration. The control exhibited a white to cream appearance, whereas GFB-3 and GFB-4 developed a distinctly greenish-yellow hue. Such color changes are consistent with the natural chlorophyll and carotenoid pigments present in *C. lentillifera* [7,8].

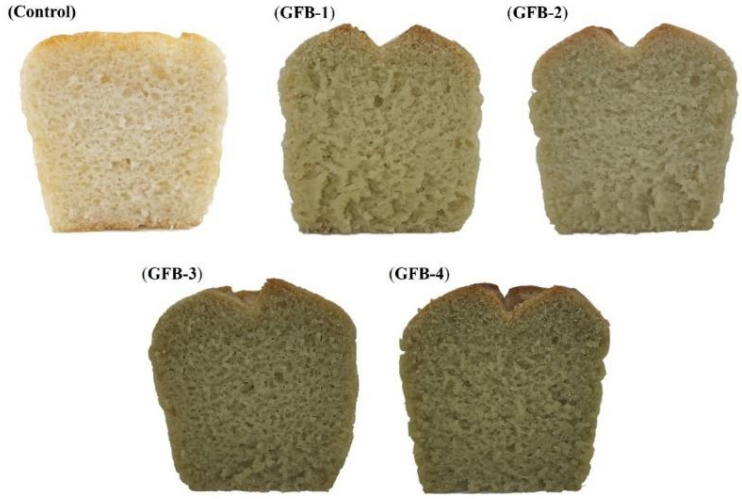


Figure 2 Cross-sectional appearance of GFB incorporating dried *C. lentillifera* at 0% (Control), 1% (GFB-1 and GFB-2), 2% (GFB-3), and 3% (GFB-4).

Table 3 Crust and Crumb color values (L^*, a^*, b^*, and ΔE^*) of GFB incorporating dried *C. lentillifera.*

Sample	Crust				Crumb			
	L^*	a^*	b^*	ΔE^*	L^*	a^*	b^*	ΔE^*
Control	64.85 ± 0.02^{a}	15.34 ± 0.06^{a}	29.54 ± 0.07^{a}	-	90.17 ± 0.93^{a}	5.19 ± 0.04^{a}	12.03 ± 0.02^{d}	-
GFB-1	50.65 ± 0.45^{d}	11.11 ± 0.16^{b}	25.06 ± 0.09^{b}	15.74 ± 0.41^{c}	68.53 ± 0.15^{b}	0.62 ± 0.03^{b}	20.16 ± 0.10^{ab}	23.20 ± 0.17^{b}
GFB-2	55.97 ± 0.17^{b}	6.69 ± 0.11^{c}	22.68 ± 0.28^{c}	14.23 ± 0.25^{d}	72.72 ± 5.61^{b}	-0.88 ± 0.09^{c}	21.74 ± 1.33^{a}	20.78 ± 3.89^{b}
GFB-3	51.78 ± 0.29^{c}	5.63 ± 0.10^{e}	18.17 ± 0.18^{e}	19.87 ± 0.13^{a}	59.03 ± 0.18^{c}	-1.91 ± 0.03^{d}	19.96 ± 0.08^{bc}	32.80 ± 0.14^{a}
GFB-4	52.17 ± 0.07^{c}	5.97 ± 0.12^{d}	20.67 ± 0.09^{d}	18.15 ± 0.05^{b}	54.97 ± 0.30^{c}	-0.57 ± 0.02^{b}	18.35 ± 0.35^{c}	35.88 ± 0.28^{a}

Note: Different letters $^{a\,b\,c\,d}$ in the same column indicates statistically significant differences at the 95% confidence level. GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

Crust L^*, a^*, and b^* values decreased significantly with increasing *C. lentillifera* concentration ($p < 0.05$). The observed darkening and reduced redness and yellowness reflected the masking effect of green chlorophyll pigments on Maillard reaction browning products [5]. The decrease in crust b^* values may further reflect spectral overlap between chlorophyll absorption bands and carotenoid-associated yellowness, whereby increasing chlorophyll concentration suppresses the perceived yellow contribution of carotenoids [7]. GFB-3 and GFB-4 were not significantly different from each other ($p > 0.05$), suggesting crust darkening reached a plateau at 2% incorporation. The plateau may reflect a balance between increasing chlorophyll input and accelerated thermal pigment degradation at higher *C. lentillifera* concentrations [34]. Crust ΔE^* ranged from 14.23 ± 0.25 in GFB-2 to 19.87 ± 0.13 in GFB-3, with all formulations exceeding the perceptibility threshold of 3.0 (the minimum ΔE^* value detectable by the human eye) [26]. GFB-2 exhibited higher L^* and lower ΔE^* than GFB-1 despite identical *C. lentillifera* concentrations (1%), attributable to the 50% salt

reduction modulating Maillard reaction intensity during baking [5]. Salt reduction in GFB-2 decreased ionic strength within the dough matrix, which may have reduced the rate of Maillard reaction between reducing sugars and amino acids during baking, resulting in less browning and higher L^* relative to GFB-1 [5,31]. The lower ΔE* in GFB-2 therefore reflects not only reduced chlorophyll-derived coloration but also attenuated Maillard browning as a direct consequence of sodium reduction.

The progressive shift in crumb color toward greenish-yellow hues is primarily driven by the chlorophyll and carotenoid content of *C. lentillifera* [7]. Crumb L^* decreased progressively from 90.17 ± 0.93 in the control to 54.97 ± 0.30 in GFB-4, reflecting concentration-dependent darkening driven by chlorophyll accumulation. Notably, crumb a^* shifted from positive values (+5.19 in the control) to negative values (−1.91 in GFB-3), quantitatively confirming the transition from reddish to greenish tones with increasing *C. lentillifera* incorporation. Baking at 180 °C may induce partial chlorophyll degradation through pheophytinization, converting green chlorophyll to olive-brown pheophytin through loss of the central magnesium ion [8,35]. At elevated *C. lentillifera* concentrations, greater chlorophyll substrate availability may accelerate pheophytin accumulation, progressively attenuating the net greenness contribution of additional seaweed incorporation [36]. Such heat-induced pigment transformation may explain the non-linear a^* trend in GFB-4, where greenness was less pronounced than in GFB-3 despite higher *C. lentillifera* content. Crust ΔE* values (14.23 - 19.87) were consistently lower than crumb ΔE* values (20.78 - 35.88), indicating more pronounced color deviation in the crumb interior than on the surface. The greater color deviation in the crumb reflects differential pigment stability under contrasting thermal and moisture conditions. The crust surface is subjected to direct dry heat at 180 °C, simultaneously accelerating both pheophytinization and Maillard browning, which partially masks chlorophyll-derived green coloration and moderates the net ΔE* magnitude. In contrast, the crumb interior retains substantially higher moisture throughout baking, moderating thermal degradation of chlorophyll and allowing greater pigment preservation, thereby producing more pronounced colorimetric deviation from the control [37].

Moisture content and water activity

Moisture content, water activity, gross energy, and iodine content of the 5 GFB formulations are presented in **Table 4**.

Table 4 Moisture content and water activity of GFB incorporating dried *C. lentillifera.*

Sample	Moisture content (%)	a_w	Kcal/100 g	Iodine content (mg/kg)
Control	32.48 ± 1.41^{a}	0.95 ± 0.00^{ns}	277.64 ± 2.21^{a}	0.36 ± 0.01^{e}
GFB-1	34.08 ± 2.08^{a}	0.95 ± 0.00^{ns}	268.68 ± 0.66^{ab}	0.59 ± 0.01^{c}
GFB-2	34.04 ± 2.40^{a}	0.96 ± 0.00^{ns}	265.04 ± 5.26^{b}	0.44 ± 0.01^{d}
GFB-3	27.38 ± 1.83^{ab}	0.96 ± 0.00^{ns}	269.39 ± 2.40^{ab}	0.70 ± 0.01^{b}
GFB-4	25.01 ± 1.97^{b}	0.95 ± 0.00^{ns}	277.75 ± 4.43^{a}	1.06 ± 0.01^{a}

Note: Different letters $^{a\,b\,c\,d}$ in the same column indicates statistically significant differences at the 95% confidence level.; Letters ^{ns} in the same column indicate non-statistically significant differences at the 95% confidence level. GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

Moisture content ranged from 25.01 ± 1.97% (GFB-4) to 34.08 ± 2.08% (GFB-1), with the control (32.48 ± 1.41%), GFB-1, and GFB-2 (34.04 ± 2.40%) showing no significant differences among each other ($p > 0.05$). GFB-3 (27.38 ± 1.83%) and GFB-4 showed significantly lower moisture content than the control, GFB-1, and GFB-2 ($p < 0.05$), reflecting a concentration-dependent decreasing trend at higher *C. lentillifera* incorporation levels. The observed decrease may reflect structural changes in the crumb matrix, where increased fiber and mineral content from *C. lentillifera* alters the starch gelatinization network and water distribution within the bread system [17,21].

Water activity remained statistically uniform across all formulations ($p > 0.05$), ranging narrowly from 0.95 ± 0.00 to 0.96 ± 0.00, indicating that dried *C.*

lentillifera incorporation at 0% - 3% did not substantially alter the thermodynamic availability of water within the bread matrix. The uniformity of a_w despite differences in total moisture content may reflect preferential water binding by dietary fiber and mineral components of *C. lentillifera* [17,21]. Such binding reduces the proportion of free water without substantially altering thermodynamic water availability at lower incorporation levels [32]. At higher *C. lentillifera* concentrations (GFB-3 and GFB-4), fiber-induced modification of the starch gelatinization network may have altered water distribution within the crumb matrix. Specifically, water redistribution from free to bound states would reduce total moisture content while maintaining a_w within a narrow range [17,21]. High a_w values (≥ 0.95) are characteristic of freshly baked high-moisture bread and are consistent with susceptibility to microbial proliferation during storage [4]. Refrigerated storage at 4 °C is therefore essential for maintaining microbiological quality throughout the shelf life of *C. lentillifera*-fortified GFB.

Texture profile analysis

Texture profile analysis (TPA) parameters including hardness, springiness, gumminess, and chewiness of the 5 GFB formulations are presented in **Table 5**. Hardness varied significantly among formulations ($p < 0.05$), ranging from 1,077.00 ± 23.26 gf in GFB-1 to 1,762.33 ± 61.23 gf in the control. GFB-1 and GFB-2 exhibited significantly lower hardness than the control ($p < 0.05$), whereas GFB-3 and GFB-4 were not significantly different from the control ($p > 0.05$). The non-linear hardness pattern reflects a concentration-dependent balance between 2 competing mechanisms. At 1% incorporation, sulfated polysaccharides in *C. lentillifera* function analogously to hydrocolloids, enhancing water-holding capacity and stabilizing gas cells during proofing, thereby producing a softer and more expanded crumb [17,20]. At higher concentrations (2% - 3%), insoluble fiber particles physically interfere with starch granule swelling and the continuity of gelatinization, progressively restricting crumb expansion and increasing rigidity [4,17,21,32,34]. Such disruption simultaneously redistributes water toward fiber-bound fractions, diminishing the plasticizing effect of free water on crumb matrix elasticity and directly contributing to the progressive increase in hardness, gumminess, and chewiness observed at 2% - 3% *C. lentillifera* incorporation [17,32,33].

GFB-1 exhibited the lowest hardness, consistent with its highest specific volume, as a more expanded crumb structure corresponds to lower crumb density and softer texture [27,38]. The inverse relationship between hardness and specific volume reflects the structural consequence of gas cell expansion within the crumb matrix. A more expanded crumb network, supported by enhanced polysaccharide-mediated gas cell stabilization, distributes compressive force across a larger cross-sectional area during TPA compression, resulting in lower measured hardness values [17,27]. A 50% reduction in salt content in GFB-2 did not substantially alter crumb hardness ($p > 0.05$), confirming that salt reduction to 0.435% is technically feasible without compromising textural quality.

Table 5 Texture profile analysis of GFB incorporating dried *C. lentillifera.*

Sample	Hardness (gf)	Springiness	Gumminess (gf)	Chewiness (gf)
Control	1762.33 ± 61.23^{a}	0.9419 ± 0.0035^{ns}	1398.67 ± 17.95^{a}	1317.33 ± 14.47^{a}
GFB-1	1077.00 ± 23.26^{c}	0.9368 ± 0.0116^{ns}	867 ± 23.64^{c}	812.00 ± 13.75^{c}
GFB-2	1206.67 ± 43.82^{c}	0.9266 ± 0.0058^{ns}	977.00 ± 53.7^{c}	913.33 ± 46.69^{c}
GFB-3	1526.33 ± 51.19^{b}	0.9439 ± 0.0060^{ns}	1230.33 ± 64.63^{b}	1161.00 ± 54.06^{b}
GFB-4	1670.33 ± 81.40^{ab}	0.9366 ± 0.0056^{ns}	1307.00 ± 90.09^{ab}	1234.00 ± 63.38^{ab}

Note: Different letters $^{a\ b\ c\ d}$ in the same column indicates statistically significant differences at the 95% confidence level. GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

Springiness was not significantly different among all formulations ($p > 0.05$), ranging from 0.9266 ± 0.0058 in GFB-2 to 0.9439 ± 0.0060 in GFB-3. In rice flour-based GFB, springiness is primarily governed by starch gelatinization during baking, as the absence of gluten renders the crumb matrix dependent on starch

network formation for its elastic recovery [4,39]. The viscoelastic nature of ulvan and other sulfated polysaccharides in *C. lentillifera* may complement the elasticity of the starch network formed during baking [17]. Such polysaccharides may undergo elastic recovery within a timescale comparable to that of gelatinized starch, without disrupting the crumb matrix structure [17]. Viscoelastic properties were therefore largely preserved across all formulations, consistent with findings reported for seaweed-incorporated GFB systems [20].

Gumminess and chewiness followed trends consistent with hardness, as both parameters are mathematically derived from hardness and cohesiveness [27]. Gumminess ranged from 867.00 ± 23.64 gf in GFB-1 to 1,398.67 ± 17.95 gf in the control, whereas chewiness ranged from 812.00 ± 13.75 gf in GFB-1 to 1,317.33 ± 14.47 gf in the control. GFB-1 and GFB-2 exhibited significantly lower gumminess and chewiness than the control ($p < 0.05$), whereas GFB-3 and GFB-4 were not significantly different from the control ($p > 0.05$). Beyond their mathematical derivation from hardness, gumminess and chewiness reflect the cohesive integrity of the crumb matrix [27]. Lower gumminess and chewiness in GFB-1 and GFB-2 indicate a crumb matrix with improved structural uniformity, attributed to enhanced starch gelatinization continuity supported by sulfated polysaccharide-mediated water retention at 1% *C. lentillifera* incorporation [17,27]. Lower gumminess and chewiness in GFB-1 and GFB-2 may be considered favorable from a consumer perspective, as excessive chewiness is frequently cited as a negative textural attribute in GFB products [20,21]. Sensory implications are further discussed in the Sensory evaluation section.

Chemical composition

Proximate analysis

The proximate composition of the 5 GFB formulations is presented in **Table 6**.

Table 6 Proximate composition of GFB incorporating dried *C. lentillifera.*

Sample	Moisture (%)	Ash (%)	Protein (%)	Fiber (%)	Fat (%)	Carbohydrates (%)
Control	41.51 ± 1.02^{ns}	1.05 ± 0.01^{d}	6.20 ± 0.02^{ns}	2.24 ± 0.11^{c}	15.09 ± 0.06^{b}	33.91 ± 0.87^{a}
GFB-1	43.03 ± 1.96^{ns}	1.25 ± 0.01^{c}	6.19 ± 0.03^{ns}	2.71 ± 0.15^{bc}	15.97 ± 0.18^{a}	30.86 ± 1.96^{ab}
GFB-2	42.65 ± 0.63^{ns}	1.29 ± 0.01^{c}	5.92 ± 0.15^{ns}	2.75 ± 0.07^{bc}	16.26 ± 0.11^{a}	31.13 ± 0.74^{ab}
GFB-3	45.79 ± 1.13^{ns}	1.68 ± 0.01^{b}	5.91 ± 0.07^{ns}	3.14 ± 0.18^{b}	14.81 ± 0.05^{b}	28.67 ± 1.28^{ab}
GFB-4	47.14 ± 1.86^{ns}	2.32 ± 0.02^{a}	5.85 ± 0.14^{ns}	3.72 ± 0.11^{a}	14.88 ± 0.17^{b}	26.08 ± 1.38^{b}

Note: Different letters $^{a\ b\ c\ d}$ in the same column indicates statistically significant differences at the 95% confidence level. Letters ^{ns} in the same column indicate non-statistically significant differences at the 95% confidence level. GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

Moisture content determined by oven-drying ranged from 41.51 ± 1.02% (control) to 47.14 ± 1.86% (GFB-4) and was not significantly different among all formulations ($p > 0.05$). Higher moisture values compared to those in Table 4 are attributable to methodological differences between the oven-drying method and the halogen moisture analyzer [28]. The oven-drying method captures total moisture loss including bound water fractions, whereas the halogen moisture analyzer may not fully volatilize tightly bound water during rapid infrared heating, yielding systematically lower values [28].

Ash content increased significantly with increasing dried *C. lentillifera* concentration ($p < 0.05$), ranging from 1.05 ± 0.01% in the control to 2.32 ± 0.02% in GFB-4. GFB-1 (1.25 ± 0.01%) and GFB-2 (1.29 ± 0.01%) were not significantly different from each other ($p > 0.05$), whereas GFB-3 (1.68 ± 0.01%) and GFB-4 (2.32 ± 0.02%) showed significantly higher ash content than all other formulations ($p < 0.05$). The progressive increase reflects the mineral-rich composition of *C. lentillifera*, which contains substantial quantities of essential minerals, including calcium, magnesium, potassium, and iodine [7,10]. Furthermore, the progressive increase in mineral content may contribute to ionic interactions within the dough matrix [40]. Divalent cations such as calcium and magnesium may influence dough rheological properties

and starch gelatinization behavior, potentially modifying water binding capacity within the crumb matrix [17,40]. Such nutritional enhancement is particularly significant given that commercially available GFB products are frequently deficient in essential minerals [2].

Crude protein content ranged from 5.85 ± 0.14% (GFB-4) to 6.20 ± 0.02% (control) and was not significantly different among formulations ($p > 0.05$), consistent with the relatively low protein contribution of *C. lentillifera* at 0% - 3% incorporation. Crude fat content did not follow a dose-dependent pattern despite the low-fat content of dried *C. lentillifera* (0.63 ± 0.31% dry weight). Variation in fat content is likely attributable to compositional displacement effects within the formulation matrix rather than a direct contribution from *C. lentillifera* [39].

Crude fiber content increased significantly with increasing *C. lentillifera* concentration ($p < 0.05$), ranging from 2.24 ± 0.11% in the control to 3.72 ± 0.11% in GFB-4. GFB-4 exhibited significantly higher fiber content than the control ($p < 0.05$), whereas GFB-1, GFB-2, and GFB-3 were not significantly different from either group ($p > 0.05$). The increase is attributable to the dietary fiber and sulfated polysaccharide content of *C. lentillifera*, including ulvan and other cell wall polysaccharides [7]. Dietary fiber in *C. lentillifera* plays a dual functional role within the bread matrix. At lower concentrations, fiber enhances crumb structure through polysaccharide-mediated water retention and gas cell stabilization [17]. At higher concentrations, fiber disrupts starch gelatinization network continuity, as evidenced by the concentration-dependent textural changes reported in the Texture Profile Analysis section [17,32]. Moreover, fiber enhancement is nutritionally significant given the previously noted scarcity of adequately fortified commercial GFB products [2]. Incorporation of dried *C. lentillifera*, therefore, represents a practical strategy for simultaneously improving the mineral and dietary fiber profiles of GFB without synthetic fortification.

Total carbohydrate content decreased progressively from 33.91 ± 0.87% in the control to 26.08 ± 1.38% in GFB-4 ($p < 0.05$), reflecting compositional displacement of jasmine rice flour by dried *C. lentillifera*. Lower carbohydrate content combined with higher dietary fiber may be nutritionally beneficial from a glycemic perspective, as dietary fiber forms complex structures that slow carbohydrate digestion and moderate postprandial glycemic response [41]. In the context of the present GFB-3 formulation, the fiber-to-carbohydrate ratio increased from 0.066 in the control to 0.110 in GFB-3, suggesting a meaningful shift toward a more fiber-dense matrix with potential glycemic benefits relevant to celiac disease patients who may have elevated metabolic risk [5,41].

Energy value and iodine content

Gross energy values and iodine content of the 5 GFB formulations are presented in **Table 4**. Gross energy ranged from 265.04 ± 5.26 kcal/100 g (GFB-2) to 277.75 ± 4.43 kcal/100 g (GFB-4). GFB-2 exhibited significantly lower energy than the control and GFB-4 ($p < 0.05$), whereas GFB-1 and GFB-3 were not significantly different from either group ($p > 0.05$). Relatively stable energy values across formulations reflect the characteristically low lipid content of dried *C. lentillifera* (0.63 ± 0.31% dry weight) and its moderate carbohydrate content, which does not substantially alter the caloric density of the final product [7,10,42]. The stability of gross energy across formulations reflects an inherent compositional balance within the bread system, whereby the simultaneous decrease in carbohydrate content and increase in ash and dietary fiber content produce a net-neutral caloric effect. Neither component individually contributes sufficient energy density to substantially shift the overall caloric value within the tested concentration range [42].

A detectable iodine level of 0.36 ± 0.01 mg/kg was observed in the control, exceeding the LOQ (0.10 mg/kg), attributable to the use of iodized salt (0.87% (w/w)). The iodine contribution from iodized salt was modest, consistent with partial volatilization during baking at 180 °C [14]. A progressive increase in iodine content was observed with increasing *C. lentillifera* concentration, ranging from 0.59 ± 0.01 mg/kg in GFB-1 to 1.06 ± 0.01 mg/kg in GFB-4. All formulations differed significantly from each other ($p < 0.05$), confirming a dose-dependent fortification effect consistent with the reported iodine content of dried *C. lentillifera* from Southeast Asian sources [7,10]. All determinations yielded %RSD values below 5%, confirming the high precision of the ICP-MS method [29]. GFB-2 was formulated with reduced salt (0.435%

(w/w)) compared to GFB-1 (0.87% (w/w)) to evaluate the effects of salt reduction at equivalent *C. lentillifera* concentrations (1%). The lower iodine content in GFB-2 (0.44 ± 0.01 mg/kg) compared with GFB-1 (0.59 ± 0.01 mg/kg) further supports the contribution of iodized salt to total iodine content.

The final iodine content of each formulation reflects the combined contribution of both sources, modulated by their relative proportions within the formulation and thermal volatilization of iodine during baking at 180°C [14]. The differential iodine content between GFB-1 (0.59 ± 0.01 mg/kg) and GFB-2 (0.44 ± 0.01 mg/kg) at equivalent *C. lentillifera* concentration (1% w/w) demonstrates that iodized salt contributes meaningfully to total iodine content beyond the seaweed-derived fraction [14]. Dried *C. lentillifera* and iodized salt therefore function as complementary rather than independent iodine sources, with their combined contribution dependent on the proportion of each within the formulation matrix [14,23]. Based on a standard serving size of 60 g (2 slices) and the recommended adult intake of 150 µg/day [43], estimated iodine intakes were 35.4 µg (24% DRI) from GFB-1, 42.0 µg (28% DRI) from GFB-3, and 63.6 µg (42% DRI) from GFB-4. GFB-2 was excluded from this intake comparison, as it served as the reduced-salt counterpart to GFB-1 at equivalent *C. lentillifera* concentration rather than as part of the concentration series. Bread has been widely recognized as an effective vehicle for population-level iodine fortification [14,22,23], and the present findings support the potential of dried *C. lentillifera* as a natural iodine-fortifying ingredient in rice flour-based GFB, particularly for populations at risk of iodine insufficiency [12,13].

Microbiological quality

The microbiological quality of GFB formulations was monitored over 8 days of refrigerated storage at 4 °C by total plate count (TPC) using PCA and yeast and mold count using PDA, evaluated on Days 0, 3, 6, and 8. Results are presented in **Tables 7** and **8**, respectively.

Table 7 Total plate count of GFB incorporating dried *C. lentillifera* during refrigerated storage at 4 °C.

Sample	PCA (CFU/g)			
	Day 0	**Day 3**	**Day 6**	**Day 8**
Dried seaweed	0	-	-	-
Control	$3.80 \pm 0.10 \times 10^{1a}$	$8.67 \pm 0.10 \times 10^{2a}$	$4.73 \pm 0.08 \times 10^{3a}$	$8.93 \pm 0.16 \times 10^{3a}$
GFB-1	$2.90 \pm 0.10 \times 10^{1b}$	$5.67 \pm 0.06 \times 10^{2b}$	$2.00 \pm 0.09 \times 10^{3d}$	$7.94 \pm 0.09 \times 10^{3b}$
GFB-2	$2.70 \pm 0.20 \times 10^{1b}$	$3.67 \pm 0.13 \times 10^{2c}$	$3.47 \pm 0.06 \times 10^{3b}$	$8.01 \pm 0.10 \times 10^{3b}$
GFB-3	$2.40 \pm 0.10 \times 10^{1c}$	$3.00 \pm 0.05 \times 10^{2d}$	$3.23 \pm 0.03 \times 10^{3c}$	$6.52 \pm 0.08 \times 10^{3c}$
GFB-4	$1.60 \pm 0.10 \times 10^{1d}$	$3.67 \pm 0.03 \times 10^{2c}$	$3.13 \pm 0.06 \times 10^{3c}$	$6.08 \pm 0.03 \times 10^{3d}$

Note: All formulas complied with microbiological standards throughout 8-day storage. Different letters $^{a\,b\,c\,d}$ in the same column indicate statistically significant differences based on one-way ANOVA with Tukey's HSD post hoc test at the 95% confidence level (n = 3). GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

Table 8 Yeast and mold count of GFB incorporating dried *C. lentillifera* during refrigerated storage at 4 °C.

Sample	PDA (CFU/g)			
	Day 0	Day 3	Day 6	Day 8
Dried seaweed	0	-	-	-
Control	$1.00 \pm 0.10 \times 10^{1a}$	$2.67 \pm 0.15 \times 10^{1a}$	$7.93 \pm 0.12 \times 10^{1a}$	$9.53 \pm 0.06 \times 10^{1a}$
GFB-1	$0.93 \pm 0.06 \times 10^{1ab}$	$1.93 \pm 0.12 \times 10^{1b}$	$5.67 \pm 0.15 \times 10^{1b}$	$8.33 \pm 0.12 \times 10^{1b}$
GFB-2	$0.80 \pm 0.00 \times 10^{1b}$	$1.97 \pm 0.06 \times 10^{1b}$	$4.90 \pm 0.10 \times 10^{1c}$	$8.07 \pm 0.15 \times 10^{1b}$
GFB-3	$0.93 \pm 0.06 \times 10^{1ab}$	$1.40 \pm 0.10 \times 10^{1c}$	$4.17 \pm 0.15 \times 10^{1d}$	$7.47 \pm 0.06 \times 10^{1c}$
GFB-4	$0.77 \pm 0.06 \times 10^{1b}$	$1.00 \pm 0.10 \times 10^{1d}$	$3.30 \pm 0.10 \times 10^{1e}$	$6.93 \pm 0.12 \times 10^{1d}$

Note: All formulas complied with microbiological standards throughout 8-day storage. Different letters $^{a\,b\,c\,d\,e}$ in the same column indicate statistically significant differences based on one-way ANOVA with Tukey's HSD post hoc test at the 95% confidence level (n = 3). GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

On Day 0, TPC ranged from $1.60 \pm 0.10 \times 10^1$ CFU/g (GFB-4) to $3.80 \pm 0.10 \times 10^1$ CFU/g (control), reflecting low initial microbial contamination consistent with adequate hygienic production conditions. Zero TPC and 0 yeast and mold count were detected in sterilized dried *C. lentillifera*, confirming that microbial counts in the final product originated from bread production conditions rather than seaweed-derived contamination. TPC increased progressively across all formulations throughout storage, consistent with typical spoilage patterns of high-moisture GFB under refrigerated conditions [4]. By Day 8, TPC of the control reached $8.93 \pm 0.16 \times 10^3$ CFU/g, whereas *C. lentillifera*-containing formulations exhibited lower counts ranging from $6.08 \pm 0.03 \times 10^3$ CFU/g (GFB-4) to $8.01 \pm 0.10 \times 10^3$ CFU/g (GFB-2). Statistical analysis revealed significant differences among formulations at each storage day ($p < 0.05$), with GFB-4 consistently exhibiting significantly lower TPC than the control from Day 0 through Day 8. GFB-2 exhibited slightly higher TPC on Day 6 ($3.47 \pm 0.06 \times 10^3$ CFU/g) than GFB-1 ($2.00 \pm 0.09 \times 10^3$ CFU/g) at equivalent *C. lentillifera* concentrations (1%). The observed difference suggests that the 50% reduction in salt content in GFB-2 may have partially reduced the preservative effect of salt during mid-storage [4]. All formulations maintained TPC below 1×10^4 CFU/g throughout 8 days of storage, remaining within internationally recognized acceptable limits for bakery products [4].

Yeast and mold counts on Day 0 ranged from $0.77 \pm 0.06 \times 10^1$ CFU/g (GFB-4) to $1.00 \pm 0.10 \times 10^1$ CFU/g (control), consistent with low initial contamination. Counts increased gradually throughout storage, with the control reaching $9.53 \pm 0.06 \times 10^1$ CFU/g by Day 8, whereas GFB-4 exhibited the lowest count at $6.93 \pm 0.12 \times 10^1$ CFU/g. Significant differences among formulations were observed at each storage day ($p < 0.05$), with GFB-3 and GFB-4 exhibiting significantly lower yeast and mold counts than the control throughout the storage period. All formulations remained below 1×10^2 CFU/g throughout storage, consistent with acceptable microbiological quality standards for bakery products [4].

GFB-3 and GFB-4 consistently demonstrated lower TPC and yeast and mold counts than the control throughout storage, suggesting a concentration-dependent antimicrobial effect attributable to bioactive compounds in *C. lentillifera* [18,19]. Beyond direct antimicrobial activity, the observed reduction in microbial counts at higher *C. lentillifera* concentrations may reflect a multifactorial outcome. Increased dietary fiber content at higher concentrations may modify crumb matrix structure, reducing water availability at the microscale and creating a less hospitable microenvironment for microbial proliferation [17,32]. The combined effect of bioactive compound activity, fiber-induced matrix modification, and refrigerated storage at 4 °C collectively contributed to maintaining microbiological quality within acceptable limits throughout the 8-day storage period [4]. The high a_w (≥ 0.95) observed across all formulations is generally conducive to microbial growth, yet refrigerated storage

at 4 °C effectively retarded both bacterial and fungal proliferation throughout the study period. A reduction in microbial counts following seaweed incorporation has been reported in other bakery products [44], supporting the role of *C. lentillifera* bioactive compounds in extending microbiological shelf life without synthetic preservatives [45].

Sensory evaluation

Sensory evaluation scores for all attributes are presented in **Table 9**. No statistically significant differences were observed among formulations for any sensory attribute evaluated ($p > 0.05$), indicating that the incorporation of dried *C. lentillifera* at concentrations of 0% - 3% did not significantly compromise consumer acceptability.

Table 9 Sensory evaluation scores of GFB incorporating dried *C. lentillifera.*

Sample	Appearance	Color	Aroma	Taste	Texture	Overall
Control	7.00 ± 1.41^{ns}	7.10 ± 1.49^{ns}	7.30 ± 1.58^{ns}	7.17 ± 1.51^{ns}	7.10 ± 1.54^{ns}	6.70 ± 1.12^{ns}
GFB-1	7.03 ± 1.45^{ns}	7.17 ± 1.44^{ns}	7.03 ± 1.40^{ns}	7.20 ± 1.37^{ns}	7.13 ± 1.33^{ns}	7.07 ± 1.17^{ns}
GFB-2	7.07 ± 1.17^{ns}	7.13 ± 1.25^{ns}	7.07 ± 1.23^{ns}	7.23 ± 1.25^{ns}	7.17 ± 1.34^{ns}	7.20 ± 1.10^{ns}
GFB-3	6.93 ± 1.36^{ns}	7.00 ± 1.31^{ns}	6.87 ± 1.43^{ns}	7.27 ± 1.41^{ns}	7.20 ± 1.42^{ns}	7.47 ± 1.14^{ns}
GFB-4	6.80 ± 1.45^{ns}	6.93 ± 1.36^{ns}	6.67 ± 1.40^{ns}	7.13 ± 1.38^{ns}	7.07 ± 1.44^{ns}	7.43 ± 1.22^{ns}

Note: Letters ^{ns} in the same column indicate non-statistically significant differences at the 95% confidence level. GFB-1 and GFB-2 contained 1%, GFB-3 contained 2%, and GFB-4 contained 3% dried *C. lentillifera* (w/w).

Color and appearance scores did not differ significantly among formulations ($p > 0.05$), ranging from 6.80 ± 1.45 to 7.07 ± 1.17 and 6.93 ± 1.36 to 7.17 ± 1.44, respectively, despite pronounced colorimetric differences observed in **Table 3**. The absence of significant differences suggests that color changes induced by *C. lentillifera* incorporation were not perceived negatively by panelists, possibly reflecting consumer familiarity with green-hued food products [21].

Aroma and taste scores were not significantly different among formulations ($p > 0.05$), ranging from 6.67 ± 1.40 to 7.30 ± 1.58 and 7.13 ± 1.38 to 7.27 ± 1.41, respectively. The mild umami flavor and aroma of *C. lentillifera* at the tested concentrations appeared to complement rather than detract from the overall sensory profile. Glutamic acid and aspartic acid, the predominant flavor amino acids in *C. lentillifera*, are well-established contributors to umami taste perception in seaweeds [7,42]. At incorporation levels of 0-3%, such amino acid-derived umami character likely enhanced rather than overwhelmed the sensory profile, contrasting with previous reports of strong algal aroma at higher microalgae incorporation levels [21].

Texture scores ranged from 7.07 ± 1.44 in GFB-4 to 7.20 ± 1.42 in GFB-3 and were not significantly different among formulations ($p > 0.05$), despite significant differences in instrumental hardness observed by TPA. The absence of significant panelist discrimination reflects that the physicochemical and compositional changes induced by *C. lentillifera* incorporation at 0% - 3% did not collectively exceed the sensory perception thresholds of the evaluated attributes. The non-linear relationship between instrumental measurements and sensory perception suggests that changes detectable by TPA did not translate to perceptible differences under normal consumption conditions [6,27].

Overall acceptability scores ranged from 6.70 ± 1.12 in the control to 7.47 ± 1.14 in GFB-3, with no statistically significant differences among formulations ($p > 0.05$). The trend toward higher overall acceptability in GFB-3 and GFB-4 suggests that *C. lentillifera* incorporation at 2% - 3% may have positively influenced the sensory experience by enhancing the mild umami flavor associated with seaweed bioactive compounds [7].

Collectively, the sensory results demonstrate that dried *C. lentillifera* can be incorporated into rice flour-based GFB at concentrations up to 3% without significantly compromising consumer acceptability across all evaluated attributes. Such findings are consistent with previous reports that seaweed incorporation at moderate concentrations does not

adversely affect sensory quality [4,20]. A 50% salt reduction in GFB-2 did not compromise sensory acceptability relative to GFB-1 ($p > 0.05$), confirming that a 0.435% salt reduction is feasible without adverse consumer impact. Considering the balance among sensory acceptability, physicochemical properties, nutritional enhancement, and microbiological safety, GFB-3 incorporating 2% dried *C. lentillifera* is identified as the optimal formulation. GFB-3 offered the most favorable combination of consumer acceptance, enhanced dietary fiber and mineral content, meaningful iodine fortification, and acceptable microbiological quality throughout 8 days of refrigerated storage. The present findings collectively support the potential of dried *C. lentillifera* as a multifunctional natural ingredient for developing nutritionally enhanced, consumer-acceptable GFB with extended microbiological shelf life.

Conclusions

The present study demonstrated that dried *C. lentillifera* at 0% (w/w) - 3% (w/w) functions as a multifunctional natural ingredient in jasmine rice flour-based GFB, simultaneously addressing iodine fortification, nutritional enhancement, microbiological safety, and sensory acceptability. Considering the overall balance among physicochemical quality, nutritional enhancement, iodine fortification potential, microbiological safety, and sensory acceptability, GFB-3 incorporating 2% dried *C. lentillifera* is identified as the optimal formulation. Specific volume, ash content, and dietary fiber increased progressively with *C. lentillifera* concentration, whereas total carbohydrate content decreased, reflecting compositional displacement of jasmine rice flour and potentially suggesting glycemic benefits. Iodine content increased dose-dependently from 0.36 to 1.06 mg/kg across formulations, supporting the potential of dried *C. lentillifera* as a natural iodine-fortifying ingredient in rice flour-based GFB. In practical production settings, dried *C. lentillifera* and iodized salt may serve as complementary iodine sources, providing additive fortification particularly beneficial for populations at risk of iodine insufficiency. A 50% salt reduction in GFB-2 did not substantially compromise physicochemical properties or sensory acceptability, confirming the technical feasibility of sodium reduction without adverse consumer impact. All formulations maintained acceptable microbiological quality throughout 8 days of refrigerated storage, with higher *C. lentillifera* concentrations exhibiting a concentration-dependent antimicrobial effect attributable to seaweed bioactive compounds. GFB-3 achieved the highest overall acceptability score, demonstrating that *C. lentillifera* can be incorporated at up to 3% without compromising consumer acceptance. Collectively, dried *C. lentillifera* functions as a multifunctional natural ingredient delivering iodine fortification, dietary fiber enhancement, mineral enrichment, and antimicrobial functionality within a single GFB system. These properties collectively support its viability as a food-based iodine fortification strategy for populations at risk of iodine insufficiency. Future studies should investigate iodine bioavailability under simulated digestion conditions, long-term stability of bioactive compounds during extended storage, and the combined iodine fortification effect under commercial production conditions.

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Declaration of Generative AI in Scientific Writing

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