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Biological and Physico-Chemical Properties of *Lobosphaera* sp. Packed in Metallized Polyethylene Terephthalate/Polyethylene (PETmet/PE)

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Abstract

This study evaluated the effects of different storage conditions, varying in light exposure, relative humidity (RH), and packaging materials, on the physicochemical stability of *Lobosphaera* sp. biomass, the retention of bioactive compounds, and the bioactivity of its extracts. Under light and 75% RH, the biomass absorbed moisture over time, reaching 0.779 ± 0.003 g/g dry weight (DW) after three months. This was accompanied by a decline in luminosity, chroma, and hue values. In contrast, samples stored under other conditions showed minimal changes, indicating that high humidity, combined with light exposure, compromises biomass stability. Packaging in metalized polyethylene terephthalate (PETmet/PE) effectively preserved the water content, color, and carotenoid levels during a two-month storage period. Bioactive compounds extracted via hydroethanolic ultrasound-assisted extraction yielded 15.48 ± 1.35% DW. Total phenolic content (TPC) of the extracts declined over time in both PETmet/PE and low-density polyethylene (LDPE) packaging, though the decrease was less pronounced in PETmet/PE. Antioxidant activity, assessed via the ABTS assay, remained stable, regardless of storage duration or packaging. Antimicrobial activity of the extract decreased over time but remained more effective against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*), with PETmet/PE packaging better preserving antimicrobial efficacy than LDPE. These findings underscore the importance of optimized storage conditions and packaging for maintaining the quality and bioactivity of *Lobosphaera* sp. biomass and its extracts.

Keywords: *Lobosphaera*; microalgae; packaging; metallized polyethylene terephthalate; polyethylene; storage; antioxidant activity; antimicrobial activity; color; carotenoids

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1. Introduction

Lobosphaera sp. is an oleoginous green microalga well known for its high content of bioactive compounds, such as carotenoids and polyunsaturated fatty acids (PUFAs). More specifically, its ability to produce and accumulate substantial amounts of fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has attracted increasing attention in recent years as a sustainable alternative to fish and plant-based oils in food applications [1]. These compounds have shown cardioprotective effects [2]. In dietary studies, feeding *Lobosphaera incisa* to zebrafish (*Danio rerio*) has been shown to increase fatty acid

content in the gut, particularly arachidonic acid (ARA), thereby enhancing immunomodulatory functions, such as improved resistance to streptococcal infection [3]. *Lobosphaera* sp. is also rich in protein with 39.2 ± 0.7 g/100 g DW [1], which is nutritionally valuable.

Microalgae preservation faces several challenges, as the preservation of all quality attributes requires optimized storage conditions suitable for the type of film packaging used. Packaging with conventional materials (e.g., polyethylene, polypropylene) primarily focuses on barrier properties (oxygen, water vapor, and light), whereas other types of packaging, such as functional packaging, incorporate mechanisms like antioxidant, antimicrobial, anti-enzymatic, anti-browning, or pH control effects, and may include the addition of bioactive compounds to enhance packaging functionality. In conventional packaging, permeability to water vapor and oxygen can lead to lipid oxidation, resulting in oxidative rancidity caused by reactions between oxygen and unsaturated fatty acids in fats and oils or hydrolytic rancidity, which involves the breakdown of triglycerides into free fatty acids, leading to undesirable flavors [4]. Light exposure can also cause photodegradation, including the degradation of micronutrients (e.g., light-sensitive vitamins), photo-oxidation of lipids, pigments discoloration (as chlorophylls and carotenoids degrade under UV exposure), and deterioration of sensory qualities (e.g., degradation of flavor and aromatic compounds, negatively impacting taste and smell) [5].

Although studies have been performed on the applications of microalgae in food packaging [6], there is little research on packaging for microalgae in powder [7]. Petroleum is a raw material that can be manufactured into fibers, films, and objects with simple or more complex shapes [8]. Films can be produced to obtain plastic packaging for food products, including microalgae biomass in powder (freeze-dried). Polyethylene (PE) is an aliphatic hydrocarbon polymer classified as a polyolefin. Polyethylene terephthalate (PET) is classified as a partly aromatic polyester [8]. Metallized polyethylene terephthalate (PETmet) offers significant advantages over standard PET for packaging powders, particularly in terms of barrier properties. The addition of a thin metal layer (typically aluminum) on PET films enhances their oxygen and moisture barrier capabilities, which is critical for preserving sensitive components like pigments, antioxidants, and fatty acids in microalgae powders. Additionally, PETmet provides light shielding, which is especially important for preventing photo-oxidation [9,10].

The biodegradability of PET has been explored from an environmental perspective. Aboeingna et al. [11] investigated the enzymatic degradation of PET using hydrolases, identifying it as a promising strategy for recycling. In particular, enzymes from the PETase and cutinase families have demonstrated significant PET-degrading activity. Gao et al. [12] conducted a review focused on the characterization and enzymatic mechanisms of PET hydrolases. They also discussed future directions in PET biodegradation and its subsequent biotransformation into value-added compounds.

The present study focuses on the effects of using PETmet/PE packaging on the preservation of freeze-dried *Lobosphaera* sp. microalga, with a focus on the antioxidant and antimicrobial activities of ultrasound-assisted ethanolic extracts from this biomass. It also examines changes in total carotenoid and chlorophyll contents, as well as moisture contents. Additionally, the effects of the storage conditions, including light exposure (presence or absence) and relative humidity (50% or 75%) at 23 °C, on the water content and color of *Lobosphaera* sp. were evaluated.

2. Materials and Methods

2.1. Microalga Biomass

Lobosphaera sp. biomass was supplied frozen by A4F—Algae for Future, Portugal. It was stored at −20 °C until it was necessary for the packaging studies and extraction assays of bioactive compounds and then it was freeze-dried.

2.2. Storage Studies at Different Conditions of Light and Relative Humidity

Biomass (20 g) was accurately weighed and placed in a Petri dish, which was then positioned inside a designated container corresponding to each experimental condition. Containers A and B were maintained at 50% relative humidity (RH), achieved using a saturated magnesium nitrate solution. Containers C and D were exposed to 75% RH, maintained with a saturated sodium chloride solution. Each RH condition was further subdivided based on the light exposure, with containers either wrapped in aluminum foil (dark conditions) or left uncovered (light conditions). All containers were stored in a temperature-controlled room set at 23 °C. After one month of storage, three replicate samples of microalgal biomass (2 g each) were collected for color and water content analysis. This sampling procedure was repeated monthly for a period of three months, with biomass color assessed visually at each interval.

2.3. Packaging Studies

2.3.1. Packaging Materials

Two packaging materials were used: low-density polyethylene (LDPE) and metalized polyethylene terephthalate with polyethylene (PETmet/PE), with thicknesses of 0.043 ± 0.002 mm and 0.045 ± 0.001 mm, respectively (measured with a micrometer, TMI-MI20, New Castle, DE, USA). The water vapor permeability of each material was determined at 23 °C and 50% RH, by monitoring the weight change of calcium chloride (initially 5 g) enclosed in bags with an effective surface of 48 cm^2 area and stored for two months. Five replicates were performed for each material.

2.3.2. Packaging of Biomass and Quality Analysis During Storage

Previous research [7] demonstrated that packaging *Porphyridium cruentum* biomass in PETmet/PE resulted in better quality preservation compared to LDPE. Consequently, the quality assessments of *Lobosphaera* biomass during storage were conducted exclusively using PETmet/PE packaging. Biomass (3 g) was placed in bags made of PETmet/PE with a usable surface area of 48 cm^2. The bags were sealed and stored in an air-conditioned room at 23 °C and 50% RH. Four replicates were performed for each sampling time. Each sample was analyzed for color, water content, total carotenoids and chlorophylls.

Subsequently, the same procedure was followed with 4 g of biomass for the extraction of bioactive compounds. Both PETmet/PE and LDPE bags were used for packaging, and analyses included total phenolic content, as well as antioxidant and antimicrobial activities. Four replicates were performed for each packaging material and sampling time.

2.4. Physico-Chemical Characterization of Lobosphaera sp. *Biomass*

2.4.1. Color Analysis

The color analyses were performed on approximately 2 g of biomass of each replicate, by using a Minolta CR-300 colorimeter (Konica-Minolta, Osaka, Japan) in the CIE L*a*b* mode color [13]. For each sample, ten measurements were taken on each of the three replicates. The evaluated color parameters included L* (lightness), a* (green–red coordinate)

and b* (blue–yellow coordinate). The hue angle and the saturation index (chroma), and total color difference (ΔE) were calculated using the following equations:

$$\text{Hue} = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad (3)$$

L_0*, a_0*, and b_0* are the values of the control (samples not packed) at time = 0 day.

2.4.2. Moisture Content Determination

Moisture content was determined using 2 g of biomass previously analyzed for color. The sample was placed in a Petri dish and dried in an oven (FP115, Binder, Tuttlingen, Germany) at 105 °C until constant weight was achieved, following a method adapted from AOAC [14]. The determinations were performed in triplicate.

2.4.3. Carotenoid and Chlorophyll Content Determination

Total carotenoid and chlorophyll contents were determined using 0.5 g of biomass from each sampling bag, transferred to pre-weighed 15 mL Falcon tubes. To each tube, 5 mL of acetone was added. The mixtures were subjected to extraction using an Ultra-Turrax probe (IKA T18 Digital Ultra Turrax, Staufen, Germany). Following extraction, a centrifugation was carried out at 5000 rpm for 10 min. The supernatant was collected and appropriately diluted for absorbance measurements at 661, 644, and 470 nm in a UV-Vis spectrophotometer (Shimadzu, 1240, Kyoto, Japan). Chlorophyll a, chlorophyll b, and total carotenoids contents were determined using the following equations, respectively [15]:

$$\text{Chlorophyll a } (\mu g\ mL^{-1}) = Ca = 11.24A_{661} - 2.04A_{644} \quad (4)$$

$$\text{Chlorophyll b } (\mu g\ mL^{-1}) = Cb = 20.13A_{644} - 4.19A_{661} \quad (5)$$

$$\text{Carotenoids } (\mu g\ mL^{-1}) = (1000 \times A_{470} - 1.90Ca - 63.14Cb)/214 \quad (6)$$

2.5. Extraction of Bioactive Compounds from Lobosphaera sp.

The extraction of bioactive compounds from *Lobosphaera* sp. was performed according to the method described by Martins et al. [1], employing a conventional solvent extraction using a hydroalcoholic solution composed of water and ethanol in a 1:9 (*v*/*v*) ratio. One gram of *Lobosphaera* sp. powder was suspended in 30 mL of the extraction solvent and incubated at 50 °C with constant agitation at 120 rpm in an orbital shaker (MaxQ6000, Thermo Scientific, Waltham, MA, USA) for 120 min. This extraction step was repeated twice to maximize yield. Following incubation, the mixture was subjected to ultrasonic homogenization using a probe sonicator (Vibra-Cell, Sonics, Newtown, CT, USA) operated at 20 kHz, with 30-s pulses applied over a total duration of 10 min. The homogenized solution was then filtered, and ethanol was removed via rotary evaporation (Rotavapor R-210, Büchi Labortechnik AG, Flawil, Switzerland). The resulting aqueous extract was subsequently lyophilized to obtain a dry extract powder [1].

2.6. Characterization of the Bioactivity of Lobosphaera sp. *Extracts*

2.6.1. Determination of Total Phenolic Content (TPC)

The extract obtained as described in Section 2.4 was used for total phenolic content (TPC) analysis, with a total of 40 mg of the dried extract being reconstituted in 2 mL of distilled water (20 mg/mL). All experiments were conducted in triplicate.

TPC was quantified using the Folin–Ciocalteu colorimetric assay, following Martins et al.'s methodology [1]. Briefly, 100 µL of diluted Folin–Ciocalteu reagent (20% *v*/*v*) was mixed with 30 µL of extract solution, followed by the addition of 100 µL sodium carbonate (7.4% *w*/*v*). The reaction mixture was incubated for 30 min at room temperature in the dark. Absorbance was then measured at 765 nm using a microplate reader (Synergy H1, Biotek, Winooski, VT, USA). Gallic acid was used for calibration, and results were expressed as mg gallic acid equivalents per 100 mg of dry extract (mg GAE/100 mg DW).

2.6.2. Antioxidant Activity Assays

The antioxidant capacity of the extracts (20 mg/mL) was evaluated using ABTS. Each assay was performed in triplicate across three independent extractions.

The ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) assay was performed according to Martins et al.′s method [1]. The ABTS$\bullet^+$ radical was generated by reacting ABTS (0.0384 g in 10 mL water) with potassium persulfate (0.0066 g in 10 mL water) and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm. An aliquot of extract (20 µL) was reacted with 180 µL of the ABTS solution in the dark at room temperature for 5 min. Absorbance was read at 734 nm. Trolox was used as the standard, and results were expressed as µmol Trolox equivalents per 100 mg of dry extract (µmol TE/100 mg DW). The percentage of inhibition was calculated using the following equation:

$$\text{I (\%)} = [(\text{Abs A}_0 - \text{Abs sample}) \div \text{Abs A}_0] \times 100 \quad (7)$$

2.6.3. Antimicrobial Activity Assays

The antimicrobial activity assays were conducted following the methodology described by Martins et al. [16]. The extract obtained in Section 2.4. was used to prepare solutions at concentrations of 1%, 2%, and 3% (*w*/*v*) in distilled water.

An overnight bacterial culture was grown in Mueller–Hinton broth (MHB) and adjusted to an optical density of 0.2 at 610 nm (approximately 10^8 CFU/mL), then diluted to an inoculum of 10^5–10^6 CFU/mL. The bacterial suspension and extract solutions were loaded into a 96-well plate and incubated at 37 °C. Optical density (OD) readings at 600 nm were taken hourly over 24 h using a microplate reader (Multiskan GO, Thermo Scientific, Vantaa, Finland). MHB with no extract served as a positive control; sterile MHB was used as a negative control.

Bacterial growth inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = ((\text{OD bacteria control} - \text{OD bacteria})/\text{OD bacteria control}) \times 100 \quad (8)$$

Tested bacterial strains included Gram-negative species *Escherichia coli* ATCC 25922, *Yersinia enterocolitica* NCTC 10406, and *Salmonella enterica serovar* Enteritidis ATCC 13076, as well as Gram-positive species *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NCTC 2599, and *Listeria monocytogenes* NCTC 10357.

2.7. Statistical Analysis

All data are expressed as mean ± standard deviation (n = 3), with calculations and interpretations based on standard principles of statistical inference. Statistical analysis was performed using one-way repeated measures ANOVA with three conditions for the ABTS

assay and four conditions for the antimicrobial activity assay, considering a significance level of $p < 0.05$. Analyses were conducted using IBM SPSS Statistics v22. Assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests, respectively. Sphericity was assessed with Mauchly's test. If the sphericity assumption was violated, the Greenhouse–Geisser correction was applied; otherwise, the standard F-test was used. Post hoc comparisons were conducted using Fisher's LSD test for within-subject comparisons and the Bonferroni correction for between-subject comparisons.

3. Results and Discussion

3.1. Effects of the Storage of Lobosphaera sp. Biomass Under Different Conditions of Light and Relative Humidity

The water content of *Lobosphaera* biomass remained approximately constant during storage under all conditions, except under light exposure and 75% relative humidity (RH). Under these conditions, the water content increased over time (Figure 1), reaching 0.779 ± 0.003 g/g dry weight (DW) after three months of storage. This is a considerably high value, corresponding to nearly 44% of the total weight. The RH level seems to be the determining factor in this observation, as storage under light and 50% RH did not produce the same effect. Similar results have been reported for *Porphyridium cruentum* [7].

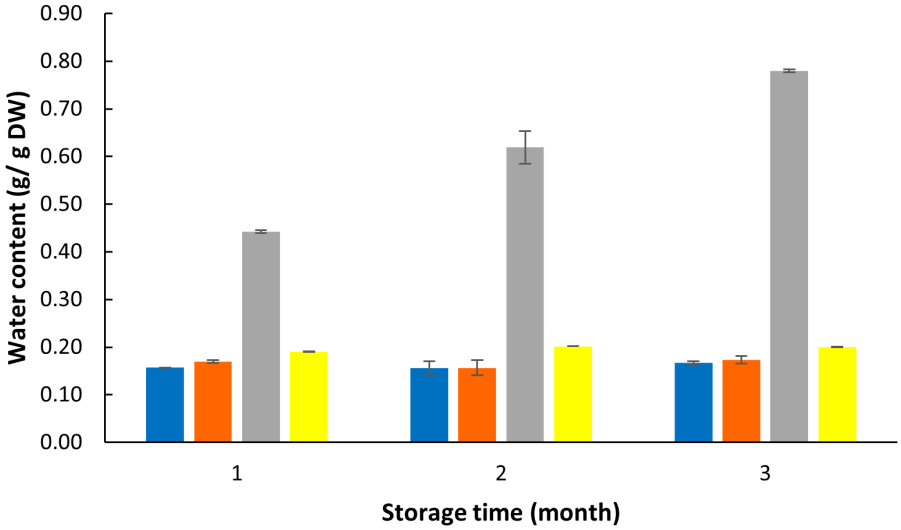

Figure 1. Water content of *Lobosphaera* exposed to light and 50% HR (blue); no light and 50% HR (orange); light and 75% HR (grey); no light and 75% HR (yellow).

The biomass luminosity was relatively low (below 26.00) and was most affected by the condition of light exposure and 75% RH, exhibiting reduced L* values compared to the other conditions, with a decrease observed during the first two months of storage (Figure 2a), indicating that the samples became darker. Hue values were also more negative under this condition than under others, although all samples showed negative hue values throughout storage (Figure 2b). Samples stored under light and 75% RH presented lower chroma values than those stored under other conditions, and these values decreased progressively during storage (Figure 2c). Nevertheless, the observed changes in color parameters were not visually perceptible. Similar results were reported by Morais et al. [7] for *P. cruentum*.

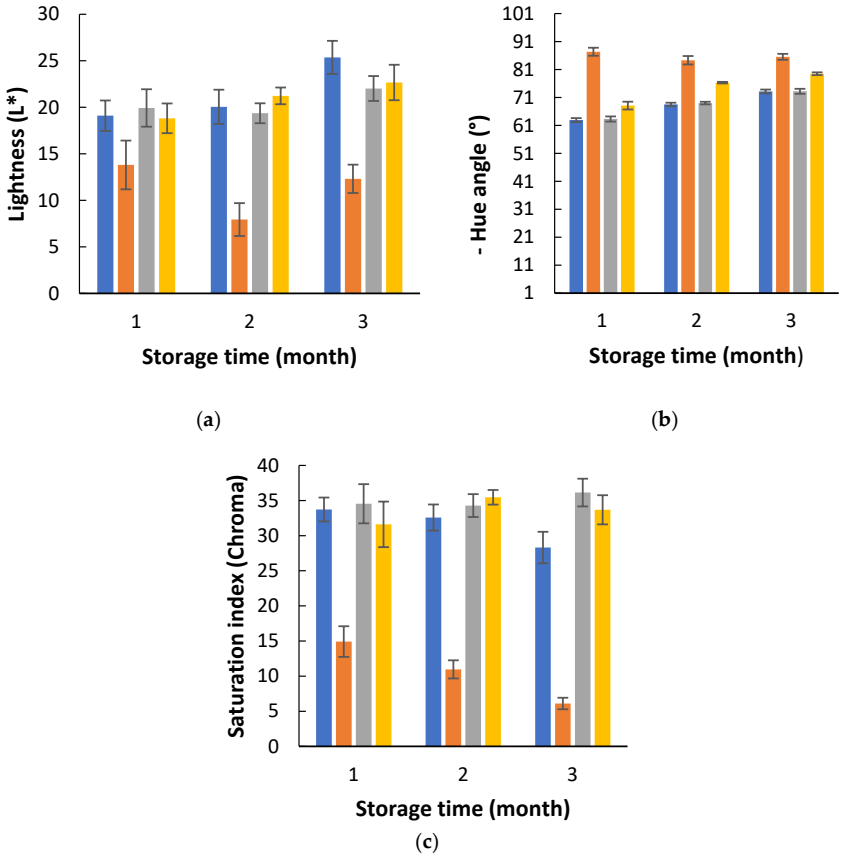

Figure 2. Color of *Lobosphaera* exposed to light and 50% HR (blue); light and 75% HR (orange); no light and 50% HR (grey); no light and 75% HR (yellow). Lightness, L* (**a**); Hue angle (**b**); Saturation index, Chroma (**c**).

Therefore, the condition of light exposure and 75% RH does not seem adequate for the preservation of *Lobosphaera* during storage.

3.2. Effects of the PETmet/PE Packaging on Biomass Quality

Figure 3 shows that the water content in biomass packed in PETmet/PE remained approximately constant throughout the storage period. These results are consistent with those reported in previous studies on *Porphyridium cruentum* [7] and suggest PETmet/PE is an effective material to protect *Lobosphaera* powder from water absorption and light, thereby allowing long-term storage.

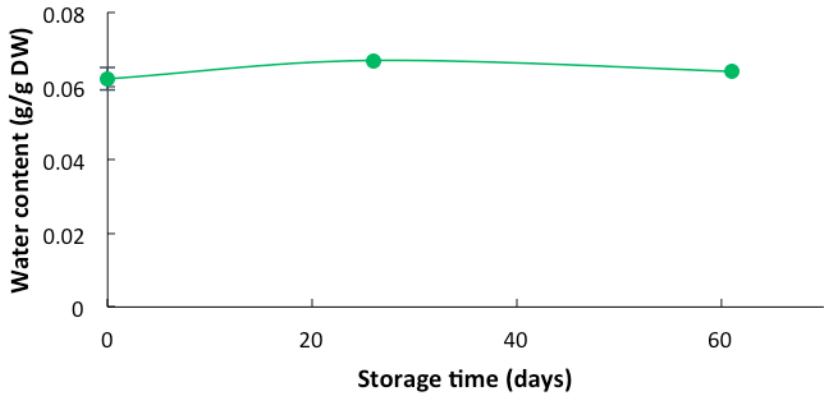

Figure 3. Water content of *Lobosphaera* packed in PETmet/PE and stored for two months.

Concerning color, it was observed that the color parameters remained relatively stable during two months of storage. There was no significant difference in the values of all color parameters between T0 (0 day) and T1 (26 days) (Figure 4). The chroma values were similar to the b* values, with the a* values being notably low (Figure 4).

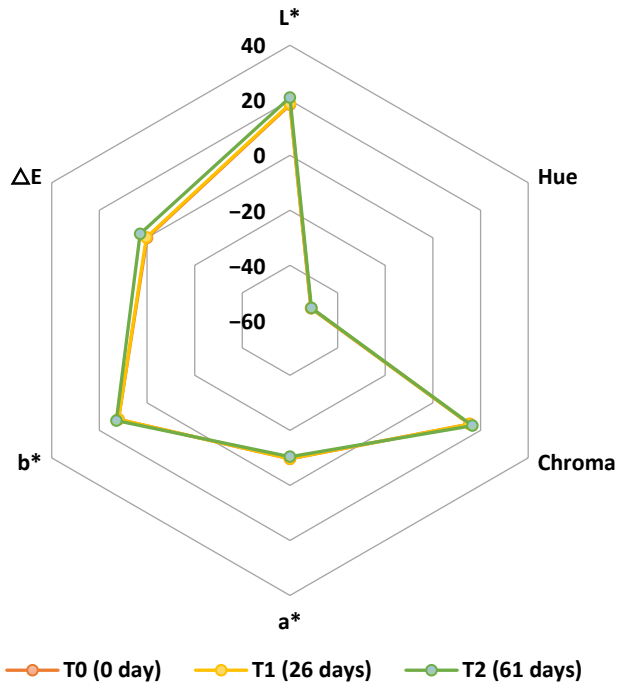

Figure 4. Color parameters of *Lobosphaera* packed in PETmet/PE and stored for two months.

A slight decrease in carotenoid content was observed between one and two months of storage (Figure 5). This finding is favorable, as carotenoids are bioactive compounds that contribute to the antioxidant activity of the *Lobosphaera* biomass. Preserving carotenoids is crucial for maintaining the quality of this microalga powder, as these compounds have significant nutritional and health-related applications. Carotenoids are important antioxidants and play a vital role in human health by contributing to the prevention of various diseases, including certain cancers and eye disorders [17].

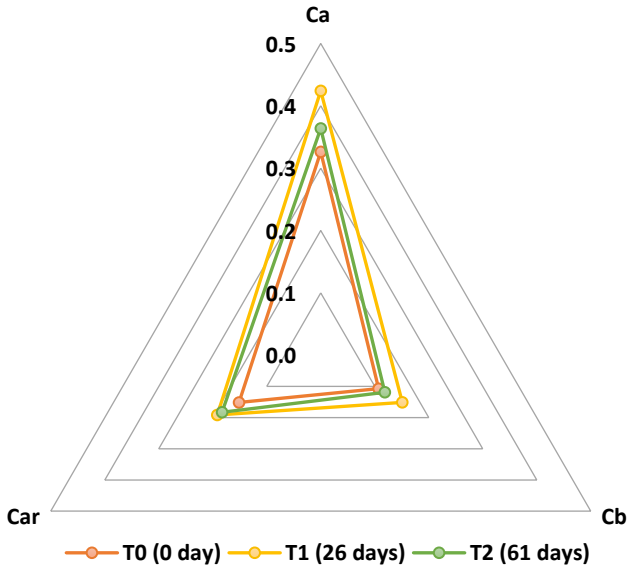

Figure 5. Carotenoids (Car), chlorophyll *a* (Ca), and chlorophyll *b* (Cb) contents of *Lobosphaera* packed in PETmet/PE and stored for two months.

3.3. Yield of Extraction of Bioactive Compounds from Lobosphaera sp.

The hydroethanolic ultrasound-assisted extraction of bioactive compounds from *Lobosphaera* sp. yielded 15.48 ± 1.35% dry weight (DW). Similarly, Martins et al. [18] reported lower extraction efficiencies for *Arthrospira* sp., 12.99 ± 0.90% DW. Monteiro et al. [19] applied ultrasound-assisted extraction using hydroalcoholic solutions at 50% and 80% ethanol to *Nannochloropsis* sp., obtaining yields of 17.33 ± 1.00% and 24.44 ± 1.88%, respectively. The same method applied to *Chlorella* sp. resulted in higher yields of 26.72 ± 1.74% and 28.05 ± 2.09%, respectively.

3.4. Effects of PETmet/PE Packaging on the Bioactivity of Lobosphaera sp. Bioactive-Rich Extracts

3.4.1. Total Phenolic Content (TPC) and Antioxidant Activity (ABTS)

Total phenolic content (TPC) reflects the total amount of phenolic compounds present, which are well known for their antioxidant properties. Bioactive compounds from *Lobosphaera* sp. have scarcely been studied in literature, although some studies are available [1,20]. The variability of TPC in microalgae is largely influenced by several factors, including the extraction methods employed as well as environmental conditions during cultivation and storage [21].

In the present study, the TPC of the *Lobosphaera* sp. hydroethanolic extract was initially 2.83 ± 0.09 mg GAE/100 mg DW and decreased after one and two months of storage for both packaging materials (Table 1). A significant effect of time was observed, indicating that TPC changed across the three time points (0, 1, and 2 months). Pairwise comparisons showed significant differences between all time points, except between 1 and 2 months, which were statistically similar. Additionally, a significant effect of packaging material was also found, confirming that the type of packaging influenced the phenolic content. A significant interaction between time and packaging material was also observed, indicating that the change in TPC over time depended on the type of packaging used.

Table 1. Total phenolic content (TPC) and antioxidant activity (ABTS) of *Lobosphaera* sp. extract after storage in PETmet/PE and LDPE for two months.

Packaging Material	Storage Time (Month)	TPC (mg GAE/100 mg Sample DW)	ABTS (µmol of TE/100 mg Sample DW)
-	0	2.83 ± 0.09	3.61 ± 0.39
PETmet/PE	1	2.53 ± 0.33	3.65 ± 0.32
LDPE		2.52 ± 0.08	3.45 ± 0.47
PETmet/PE	2	2.45 ± 0.14	3.47 ± 0.26
LDPE		2.19 ± 0.16	3.28 ± 0.46

Morais et al. [7] observed no significant changes in TPC of *Porphyridium cruentum* hydroethanolic extract during two months of storage using the same packaging materials. Notably, after two months of storage, *Lobosphaera* sp. extract presented higher TPC values (2.45 ± 0.14 mg GAE/100 mg DW for PETmet/PE and 2.19 ± 0.16 mg GAE/100 mg DW for LDPE) compared to *P. cruentum* extracts, which were around 0.6 mg GAE/100 mg DW. Martins et al. [1] reported a TPC of 1.07 ± 0.05 mg GAE/100 mg DW for *Lobosphaera* sp., lower than the initial value found here, possibly due to biomass variability. The same authors reported TPC values ranging between 1.07 ± 0.05 and 3.18 ± 0.53 mg GAE/100 mg DW across seven microalgae extracts obtained via the same methodology. Cuong et al. [22] reported a TPC of 0.894 ± 0.20 mg GAE/g DW for a 70% hydroethanolic extract of *Odontella* sp., a lower value likely reflecting a less efficient extraction method.

Results for antioxidant activity (ABTS) are presented in Table 1. No significant changes in ABTS activity were observed during storage, nor were there significant differences between packaging materials.

Similarly, Morais et al. [7] found no significant differences in antioxidant activity (measured by ABTS, DPPH, and ORAC) in *P. cruentrum* ethanolic extracts stored for two months in the same packaging materials, although ABTS and ORAC values decreased considerably. Martins et al. [1] reported ABTS values between 1.35 ± 0.27 and 2.85 ± 0.45 µmol TE/100 mg DW for seven microalgae extracts obtained by the same methodology, the highest value corresponding to *Limnospira* sp.

3.4.2. Antimicrobial Activity

The hydroethanolic extract of *Lobosphaera* sp., tested at concentrations of 1%, 2%, and 3%, exhibited antimicrobial activity against several bacteria. Bacterial growth inhibition decreased over the two-month storage period for all bacteria tested. Comparisons among the three extract concentrations showed statistically significant differences for most bacteria, except for *Listeria monocytogenes*, which showed no significant difference between 1 and 2% concentrations (Table 2). Overall, growth inhibition increased with extract concentration. Additionally, results were generally significantly different between packaging materials for all bacteria, with better inhibition observed in samples stored in PETmet/PE packaging.

Table 2. Bacterial growth inhibition of *Lobosphaera* sp. extract at 1%, 2%, and 3% concentration after storage of the microalga in PETmet/PE and LDPE for two months.

Packaging Material	Storage Time (Month)	Bacteria	Inhibition (%) 1% Extract	2% Extract	3% Extract
-	0	*Escherichia coli*	34.18 ± 4.05	39.02 ± 4.49	56.44 ± 2.85
PETmet/PE	1		24.56 ± 5.99	30.03 ± 7.01	52.60 ± 4.45
LDPE	1		6.06 ± 1.50	29.69 ± 5.58	48.93 ± 2.27
PETmet/PE	2		14.86 ± 5.25	13.80 ± 2.74	31.73 ± 2.91
LDPE	2		0.28 ± 1.11	0.72 ± 2.26	5.66 ± 1.82
-	0	*Yersinia enterocolitica*	41.25 ± 1.06	55.13 ± 1.26	71.15 ± 2.51
PETmet/PE	1		33.32 ± 1.06	37.43 ± 1.63	58.54 ± 1.96
LDPE	1		10.13 ± 3.17	19.14 ± 1.61	52.08 ± 2.02
PETmet/PE	2		14.69 ± 2.12	28.16 ± 1.92	52.45 ± 1.18
LDPE	2		1.28 ±0.72	4.16 ± 1.93	12.57 ± 1.39
-	0	*Salmonella enterica serovar* Enteritidis	41.37 ± 1.17	59.57 ± 1.04	69.35 ± 2.84
PETmet/PE	1		30.16 ± 1.99	39.65 ± 1.27	61.61 ± 1.61
LDPE	1		21.23 ± 2.39	24.41 ± 1.38	56.73 ± 1.36
PETmet/PE	2		27.33 ± 1.57	36.80 ± 1.53	46.78 ± 1.43
LDPE	2		8.03 ± 4.74	18.10 ± 0.98	23.99 ± 1.04
-	0	*Staphylococus aureus*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
PETmet/PE	1		85.92 ± 2.74	91.94 ± 1.92	98.33 ± 2.23
LDPE	1		18.13 ± 2.76	23.66 ± 2.72	51.29 ± 3.98
PETmet/PE	2		25.14 ± 1.97	31.45 ± 1.51	44.94 ± 1.37
LDPE	2		1.68 ± 1.05	2.04 ± 1.57	16.94 ± 1.60
-	0	*Bacillus cereus*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
PETmet/PE	1		91.04 ± 1.70	94.65 ± 1.44	100.00 ± 0.00
LDPE	1		16.85 ± 2.82	19.68 ± 2.72	42.33 ± 2.89
PETmet/PE	2		36.42 ± 1.66	40.81 ± 1.50	50.37 ± 1.02
LDPE	2		9.60 ± 0.98	13.41 ± 2.69	16.43 ± 1.55
-	0	*Listeria monocytogenes*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
PETmet/PE	1		96.44 ± 3.09	99.62 ± 2.43	100.00 ± 0.00
LDPE	1		5.95 ± 2.95	8.00 ± 1.89	53.99 ± 1.31
PETmet/PE	2		7.52 ± 1.72	12.84 ± 3.02	33.76 ± 1.83
LDPE	2		1.59 ± 3.80	3.32 ± 1.30	9.81 ± 1.47

The strongest inhibition (100%) was achieved against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*) at the start of storage (0 months) across all extract concentrations. Similarly, *Listeria monocytogenes* showed 100% inhibition after 1 month of storage and 3% extract concentration in PETmet/PE packaging. Lower inhibition levels were observed for Gram-negative bacteria, likely due to their lipopolysaccharide-rich outer membranes, which restrict the penetration of foreign molecules [23]. After two months of storage, the highest inhibition was seen for

Yersinia enterocolitica and *Bacillus cereus* with 3% extract concentration and PETmet/PE packaging, with values of 52.45 ± 1.18% and 50.37 ± 1.02%, respectively (Table 2). Inhibition of the other bacteria remained below 50% for both packaging materials.

Martins et al. [24] also reported notable antimicrobial properties for *Lobosphaera* sp. extract obtained via hydroethanolic ultrasound-assisted extraction. The minimal inhibitory concentrations (MICs) were 2.5 mg/100 µL for *Escherichia coli* and *Yersinia enterocolitica*, and 1.25 mg/100 µL for *Salmonella enterica serovar* Enteritidis (Gram-negative bacteria). MICs for Gram-positive bacteria were 5 mg/100 µL for *Staphylococcus aureus* and *Bacillus cereus*, and 1.25 mg/100 µL for *Listeria monocytogenes*. Minimal bactericidal concentrations (MBCs) exceeded 5 mg/100 µL for all tested bacteria.

4. Conclusions

The quality of *Lobosphaera sp.* biomass was significantly influenced by storage conditions, particularly relative humidity and light exposure. Storage under light and 75% RH led to increased moisture uptake and darker coloration, rendering it unsuitable for biomass preservation. In contrast, PETmet/PE packaging effectively maintained moisture stability, pigment retention, and the integrity of bioactive compounds over two months. The hydroethanolic ultrasound-assisted extraction method yielded a substantial quantity of bioactive compounds, with initially high total phenolic content (TPC) and strong antimicrobial activity, particularly against Gram-positive bacteria. Although both TPC and antimicrobial efficacy declined during storage, PETmet/PE packaging offered better preservation compared to LDPE. These findings highlight the critical role of controlled storage environments and advanced packaging materials in preserving the functional and commercial value of freeze-dried *Lobosphaera* sp. powder.

The environmental footprint and biodegradability of PETmet/PE warrant evaluation in future studies. Microstructural or cell integrity assessments could also help link visual and physical traits to chemical stability. In addition, expanding antimicrobial tests to include foodborne or aquaculture-relevant pathogens would enhance the applicability of the results.

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References

1. Martins, V.F.R.; Lopes, A.I.; Machado, M.; Costa, E.M.; Ribeiro, T.B.; Poças, F.; Pintado, M.E.; Morais, R.M.S.C.; Morais, A.M.M.B. Biodegradable Films with Polysaccharides, Proteins, and Bioactive Compounds from *Lobosphaera* sp.: Antioxidant and Antimicrobial Activities. *Foods* **2025**, *14*, 1327. [CrossRef] [PubMed]
2. Komprda, T. Eicosapentanoic and docosahexaenoicacids as inflammation-modulating and lipid homeostasis influencing nutraceuticals: A review. *J. Funct. Foods* **2012**, *4*, 25–38. [CrossRef]
3. Lazado, C.C.; Nayak, S.; Khozin-Goldberg, I.; Zilberg, D. The Gut Mucosal Barrier of Zebrafish (*Danio rerio*) Responds to the Time-Restricted Delivery of *Lobosphaera incisa*-Enriched Diets. *Fish Shellfish Immunol.* **2019**, *89*, 368–377. [CrossRef] [PubMed]
4. Maté, J.I.; Saltveit, M.E.; Krochta, J.M. Peanut and Walnut Rancidity: Effects of Oxygen Concentration and Relative Humidity. *J. Food Sci.* **1996**, *61*, 465–469. [CrossRef]
5. Wang, A.; Catherine, H.D.; Rachel, M.H.; Sean, F.O.; J'Nai, B.P.; Kemia, A.A.; Susan, E.D. Efficacy of light-protective additive packaging in protecting milk freshness in a retail dairy case with LED lighting at different light intensities. *Food Res. Int.* **2018**, *114*, 1–9. [CrossRef] [PubMed]
6. Dakhili, S.; Hashami, Z.; Rostami, N.; Moradi, S.; Moslehishad, M.; Shojaee-Aliabadi, S. Innovative applications of microalgae in biodegradable food packaging: A review. *J. Agric. Food Res.* **2025**, *19*, 101723. [CrossRef]
7. Morais, A.M.M.B.; Martins, V.F.; Alves, A.J.; Poças, F.; Morais, R.M.S.C. Packaging and storage of *Porphyridium cruentum*: Metallised polyethylene terephthalate/polyethylene (PETmet/PE) versus polyethylene (PE). *Acta Aliment.* **2024**, *53*, 419–431. [CrossRef]
8. Benedicte, L.; Philippe, R. Poly (ethylene terephthalate). In *Handbook of Engineering and Speciality Thermoplastics, Polyethers and Polyesters*; Thomas, S., Visakh, P.M., Eds.; Scrivener LLC: Beverly, MA, USA, 2011; Volume 3, p. 97.
9. Garnier, G.; Yrieix, B.; Brechet, Y.; Flandin, L. Influence of structural feature of aluminum coatings on mechanical and water barrier properties of metallized PET films. *J. App. Polym. Sci.* **2010**, *115*, 3110–3119. [CrossRef]
10. Anonymous. Metallized PET Film: What Can PET Met be Used for? *AlFiPa.* Available online: https://alfipa.com/applications/metallized-pet-met-foil/?utm_source=chatgpt.com (accessed on 10 July 2025).
11. Aboeinga, M.M.; Kalyaanamoorthy, S. QM/MM investigation to identify the Hallmarks of superior PET biodegradation activity of PETases over cutinase. *ACS Sustain. Chem. Eng.* **2022**, *10*, 15618–16069. [CrossRef]
12. Gao, R.; Pan, H.; Lian, J. Recent advances in the discovery, characterization, and engineering of poly(ethylene terephthalate) (PET) hydrolases. *Enzym. Microb. Technol.* **2021**, *150*, 109868. [CrossRef] [PubMed]
13. Commission Internationale de L'Eclairage (C.I.E.). Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms. Supplement No. 2 to C.I.E. Publication No. 15, (E.-1.3. 1) 1971; C.I.E.: Paris, France, 1978; Volume TC1.
14. A.O.A.C. Method, A.O.A.C. 935.29. In *Official Methods of Analysis*, 18th ed.; Association of the Official Analytical Chemists: Arlington, VA, USA, 2005.
15. Lichtenthaler, H.K.; Abuslima, E.; Nick, P. Strong increase of photosynthetic pigments and leaf size in a pruned Ginkgo biloba tree. *Photosynthetica* **2023**, *61*, 297–307. [CrossRef] [PubMed]
16. Martins, V.F.R.; Ribeiro, T.B.; Lopes, A.I.; Pintado, M.E.; Morais, R.M.S.C.; Morais, A.M.M.B. Comparison among Different Green Extraction Methods of Polyphenolic Compounds from Exhausted Olive Oil Pomace and the Bioactivity of the Extracts. *Molecules* **2024**, *29*, 1935. [CrossRef]
17. Mostafa, H.S.; Hashem, M.M. Microalgae as a source of carotenoids in foods, obstacles and solutions. *Phytochem. Rev.* **2024**, 1–43. [CrossRef]
18. Martins, V.F.R.; Poças, F.; Pintado, M.; Morais, R.M.S.C.; Morais, A.M.M.B. Edible Films with Protein and Bioactive Compounds from *Arthrospira* sp. *Biol. Life Sci. Forum* **2024**, *40*, 6.
19. Monteiro, M.; Santos, R.A.; Iglesias, P.; Couto, A.; Serra, C.R.; Gouvinhas, I.; Barros, A.; Oliva-Teles, A.; Enes, P.; Díaz-Rosales, P. Effect of Extraction Method and Solvent System on the Phenolic Content and Antioxidant Activity of Selected Macro- and Microalgae Extracts. *J. Appl. Phycol.* **2020**, *32*, 349–362. [CrossRef]
20. Corrêa, P.S.; Coimbra, R.S.T.; Caetano, N.S. Exploring the Antioxidant Potential of *Lobosphaera* Sp. and *Odontella* Sp. Biomasses under Different Extraction and Preservation Conditions. In Proceedings of the Seagriculture EU 2023 12th International Seaweed Conference, Trondheim, Norway, 21–22 June 2023.
21. Torres, P.; Osaki, S.; Silveira, E.; dos Santos, D.Y.A.C.; Chow, F. Comprehensive evaluation of Folin-Ciocalteu Assay for Total Phenolic Quantification in Algae (Chlorophyta, Phaeophyceae and Rhodophyta). *Algal Res.* **2024**, *80*, 103503. [CrossRef]
22. Cuong, D.M.; Yang, S.H.; Kim, J.S.; Moon, J.Y.; Choi, J.; Go, G.M.; Cho, S.K. Evaluation of Antioxidant and Anti-Inflammatory Activity and Identification of Bioactive Compound from the Marine Diatom, *Odontella aurita* Extract. *Appl. Biol. Chem.* **2024**, *67*, 46. [CrossRef]

23. Gómez-Cruz, I.; Cara, C.; Romero, I.; Castro, E.; Gullón, B. Valorisation of Exhausted Olive Pomace by an Ecofriendly Solvent Extraction Process of Natural Antioxidants. *Antioxidants* **2020**, *9*, 1010. [CrossRef] [PubMed]
24. Martins, V.F.R.; Lopes, A.I.; Gomes, D.; Parreira, C.; Badenes, S.M.; Costa, L.; Pintado, M.; Morais, A.M.M.B.; Morais, R.M.S.C. Unravelling the Potential of Seven Microalgae Species: Nutritional, Antioxidant, and Antimicrobial Properties and Application. *Appl. Sci.* **2025**, *15*, 6691. [CrossRef]