

Article

Effects of *Salicornia* Extract on the Quality, Shelf-Life, and Functional Properties of Beef Patties During Refrigerated Storage

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Abstract

Clean-label preservation of beef patties remains challenging due to rapid oxidative and microbiological spoilage during refrigeration. Incorporating *Salicornia* extract yielded clear, dose-dependent quality gains over 15 days at 4 ± 1 °C. Relative to the control, 1.5% *Salicornia* lowered secondary lipid oxidation by 42% (TBARS: 1.15 vs. 1.98 mg MDA/kg) and primary oxidation by 33% (PV: 3.30 vs. 4.95 meq O₂/kg), while maintaining a substantially higher antioxidant status (TPC: 20.6 vs. 6.8 mg GAE/100 g; DPPH: 45.8% vs. 14.5%). Microbiological loads were attenuated (SPC: 4.88 vs. 6.20 log CFU/g; psychrotrophs: 1.46 vs. 2.00 log CFU/g; yeasts/molds: 1.44 vs. 1.74 log CFU/g), accompanied by moderated physicochemical drift (pH: 6.16 vs. 5.86; a_w: 0.847 vs. 0.828). Color retention was markedly improved, with higher redness and lower overall discoloration (*a**: 13.6 vs. 9.8; ΔE^* : 3.96 vs. 9.13). The 1.0% treatment showed intermediate benefits, indicating a robust dose response. Collectively, these outcomes demonstrate that 1.0–1.5% *Salicornia* delivers multifaceted protection, limiting lipid oxidation, curbing microbial growth, preserving color, and stabilizing matrix attributes, thereby extending the refrigerated shelf-life of beef patties and supporting clean-label reformulation, particularly when combined with oxygen-limiting packaging.

Keywords: *Salicornia*; beef patties; lipid oxidation; total phenolic content; DPPH; color stability; microbiological quality; shelf-life



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1. Introduction

Beef patties are popular high-protein convenience foods, yet their comminuted structure, large surface area, disrupted muscle membranes, and high water activity make them prone to rapid quality loss under refrigeration due to lipid/protein oxidation and microbial proliferation [1–3]. These reactions trigger rancidity, discoloration, texture softening, nutrient decline, and accumulation of secondary oxidation products that undermine safety and consumer acceptance [1,2,4]. Consequently, controlling oxidative and microbial spoilage remains central to extending shelf-life while preserving sensory quality and nutritional value in minced beef systems [5]. Historically, meat processors have relied on synthetic antioxidants/preservatives to restrain oxidation and delay quality deterioration within regulatory limits [6–8]. Although effective, long-term intake of synthetic additives has raised health and perception concerns and intensified the shift toward “clean-label” formulation

strategies that emphasize recognizable, minimally processed ingredients [9–12]. This market pull and regulatory/sustainability push are accelerating the evaluation of plant-derived bioactives as preservative solutions compatible with consumer expectations [10–12]. *Salicornia* (family: Amaranthaceae; subfamily: Salicornioideae) is a halophytic genus widely distributed in saline and coastal areas of Asia, Europe, and North America. In Kazakhstan, *Salicornia perennans* occurs naturally in the Kyzylorda region, where the extreme salinity of soils and high temperatures promote the accumulation of bioactive compounds such as phenolic acids, flavonoids, and minerals (Na, K, Mg, and Ca). These components contribute to its antioxidant, antimicrobial, and mineralization properties [13,14]. Owing to its high ash and polyphenol contents, *Salicornia* has attracted growing attention as a functional ingredient and natural salt substitute in meat products, improving oxidative stability, color retention, and sensory quality while reducing sodium chloride levels [15]. Extensive evidence shows that botanical extracts rich in phenolic acids, flavonoids, and related phytochemicals can suppress lipid/protein oxidation, stabilize color, modulate microbiota, and preserve sensory traits in meat products [4,5,13–16]. Illustrative examples include green tea catechins in minced red meat and pork sausages [7,17], grape seed and bearberry extracts in pork [18], rosemary extracts in fresh/precooked sausages [6,19], and fruit/vegetable by-products in comminuted meats [20]. Coffee industry by-products such as spent coffee grounds or coffee silverskin have been successfully evaluated in beef patties and meat burgers as sources of dietary fiber and antioxidant phytochemicals, demonstrating reduced lipid oxidation and maintained sensory acceptability [20,21]. Efficacy depends on the matrix, dose, and extraction approach, but collectively, these studies demonstrate the feasibility of replacing or reducing synthetics in refrigerated meat systems [4,13–16]. Within this context, *Salicornia* has emerged as a promising halophytic ingredient combining bioactivity with mineral richness and salt-replacing potential [22–24]. Beyond its culinary use, *Salicornia* has shown antioxidant and antimicrobial effects in seafood matrices [19] and supported partial NaCl replacement in processed meats without compromising technological quality [13,23,25]. Recent studies report that *Salicornia* herbacea powder or extracts can influence color, lipid stability, and overall quality in fish and meat products; moreover, glasswort-based strategies may complement KCl approaches for sodium reduction [4,13,22]. Despite these advances, there is limited, systematic information on how *Salicornia* extract simultaneously affects oxidative stability, microbial safety, physicochemical attributes, and sensory quality in refrigerated beef patties, a gap this study addresses [22,26]. Therefore, the present research evaluates the effect of *Salicornia* extract on the quality, shelf-life, and functional properties of beef patties during refrigerated storage. We hypothesized that *Salicornia* would (i) retard lipid oxidation, (ii) suppress microbial growth, and (iii) maintain color and sensory acceptability relative to controls.

2. Materials and Methods

2.1. Materials

Fresh beef was sourced from a local retail market (Astana, Kazakhstan) and transported under chilled conditions of ≤ 4 °C to the Department of Food Technology and Processing Products, S. Seifullin Kazakh Agro Technical Research University. Compounded ingredients and their label-declared compositions are described below. All ingredients were purchased from retail suppliers in Astana, Kazakhstan, and used within their shelf-life. Mustard paste (2.0% *w/w* of the batter). Label-declared composition: water, mustard seeds (*Brassica juncea*), spirit vinegar, sugar, table salt (NaCl), turmeric, and spices. Brand: Senap Classic; manufacturer: FoodMaster LLP, Almaty, Kazakhstan. Food-grade starch of 2.0%. Label-declared composition: native potato starch $\geq 99.5\%$ dry solids and moisture $\leq 14\%$. Brand: Ekostar; manufacturer: Ekostar LLP, Karaganda, Kazakhstan. Curing mix of 0.6%. Label-

declared composition: sodium chloride ($\geq 99.5\%$) and anti-caking agent E535 (sodium ferrocyanide). Brand: Solt Premium; manufacturer: KazSalt JSC, Kyzylorda, Kazakhstan. *Salicornia* extract (0.0–1.5%). Dried ethanolic extract was obtained from *Salicornia* perennans, collected in the Kyzylorda region, Kazakhstan, during the late flowering stage in August 2024. The plants were harvested manually from natural saline soil habitats, rinsed with potable water to remove surface salts and debris, and frozen at $-40\text{ }^{\circ}\text{C}$ for 12 h prior to freeze-drying. Lyophilization was carried out using a Christ Alpha 1-4 LDplus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) under the following conditions: a condenser temperature of $-55 \pm 2\text{ }^{\circ}\text{C}$, a chamber pressure of $0.10 \pm 0.02\text{ mbar}$, and a shelf temperature gradually increased from $-35\text{ }^{\circ}\text{C}$ to $20\text{ }^{\circ}\text{C}$ over 48 h. The dried material was milled to a $<250\text{ }\mu\text{m}$ particle size (Retsch ZM 200, Haan, Germany), vacuum-packed, and stored at $4\text{ }^{\circ}\text{C}$ in light-protected containers until extraction. The extract was prepared in-house according to Section 2.2. The remaining ingredients (white onion and carrot) were sourced fresh from the same retail batch and processed on the day of production. Patty formulations (a control and treatments with different levels of *Salicornia* extract, expressed as dry solids, and % *w/w* of the total batter) are shown in Table 1.

Table 1. Formulation of beef patties with *Salicornia* extract (% *w/w*).

Ingredient (%)	T0 (Control)	T1	T2	T3
Beef	75.4	74.9	74.4	73.9
Onion	10.0	10.0	10.0	10.0
Carrot	10.0	10.0	10.0	10.0
Mustard	2.0	2.0	2.0	2.0
Starch	2.0	2.0	2.0	2.0
Curing mix	0.6	0.6	0.6	0.6
<i>Salicornia</i> extract	0.0	0.5	1.0	1.5
Total	100.0	100.0	100.0	100.0

2.2. Preparation of *Salicornia* Extract

Freeze-dried *Salicornia* powder was extracted with 70% (*v/v*) ethanol at a raw material/solvent ratio of 1:20 (*w/v*) under ultrasound assistance at 40 kHz for 30 min at $\leq 30\text{ }^{\circ}\text{C}$. The suspension was filtered (Whatman No. 1), the combined filtrates were concentrated in a rotary evaporator at $\leq 40\text{ }^{\circ}\text{C}$ to remove the solvent, and the concentrate was subsequently freeze-dried. The dry extract was stored at $4\text{ }^{\circ}\text{C}$ in airtight, light-protected containers. The dry matter content was determined gravimetrically; the dosing in the formulations (Table 1) was calculated on a dry basis of the extract.

2.3. Preparation of Beef Patties

The chilled beef was deboned, trimmed of visible fat and connective tissue, cut into 2 cm cubes, and double-ground (6 mm followed by 4 mm plates). The minced beef was mixed with onion, carrot, mustard, starch, the curing mix, spices, and the pre-measured amount of *Salicornia* dry extract until homogeneous for 3–4 min. Patties of 80–100 g were formed with a diameter of 9–10 cm and a thickness of 1.2 cm, packed in sterile polyethylene bags without vacuum, and stored at $4 \pm 1\text{ }^{\circ}\text{C}$ for up to 15 days. Samples were collected on days 0, 3, 6, 9, 12, and 15 for analysis.

2.4. pH and Water Activity

pH was measured in a homogenate prepared by blending 5 g of patty mass with 45 mL of distilled water for 1 min at 10,000 rpm using a calibrated portable pH meter (HI99163, Hanna Instruments, Woonsocket, RI, USA). Water activity (*a_w*) was determined with an

Aqualab 4TE meter (METER Group, Pullman, WA, USA), according to the manufacturer's instructions. At least three independent measurements were performed for each treatment and storage time.

2.5. Antioxidant Activity

Sample extracts for antioxidant assays were prepared by homogenizing 10 g of patty mass with 40 mL of methanol–ethanol (1:1, *v/v*) for 3 min, followed by filtration (Whatman No. 1). Total phenolic content (TPC) was determined by the Folin–Ciocalteu spectrophotometric method according to ISO 14502-1:2005 [27], with minor modifications for meat extracts. A 0.1 mL aliquot of the sample extract was mixed with 0.5 mL of Folin–Ciocalteu reagent (diluted 1:10, *v/v* with distilled water) and 1.5 mL of a 7.5% sodium carbonate solution. The mixture was incubated in the dark for 10 min at room temperature (22 ± 2 °C), and the absorbance was measured at 730 nm using a UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The results were expressed as mg gallic acid equivalents (GAEs) per 100 g of product. Analysis of DPPH radical-scavenging activity was performed in accordance with Blois (1958) [28] and aligned with Baliyan et al.'s study (2022) [29]. The assay used 0.1 M Tris–HCl buffer (pH 7.4) containing 250 µM DPPH (Sigma-Aldrich, St. Louis, MO, USA). A 0.1 mL aliquot of the extract was added to 4.9 mL of the reaction mixture; the absorbance at 517 nm was recorded at 0 and 20 min. The results were expressed as the percentage inhibition relative to the control.

2.6. Lipid Oxidation and Microbial Quality

The peroxide value (PV) was determined titrimetrically following the AOAC Official Method 965.33 [30] and ISO 3960:2017 [31]. A 2.5 g analytical portion referred to a weighed homogenized sample of beef patty mass used for extraction. Each portion (2.5 g) was extracted with chloroform (Merck KGaA, Darmstadt, Germany) in the presence of anhydrous Na₂SO₄ (Sigma-Aldrich, St. Louis, MO, USA), treated with glacial acetic acid and saturated KI, and titrated with 0.1 N Na₂S₂O₃ using starch as an indicator. The results were expressed as meq O₂/kg of product. TBARS were quantified according to the AOAC Official Method 970.51 [32], with minor modifications for comminuted meat matrices. A 5 g homogenized portion was mixed with 15 mL of 7.5% trichloroacetic acid (TCA), centrifuged, and filtered. An aliquot (5 mL) of the filtrate was reacted with 5 mL of 0.02 M thiobarbituric acid (TBA) solution in a boiling water bath (100 °C; 30 min). After cooling, the absorbance was measured at 532 nm using a UV–vis spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan). The results were expressed as mg of malondialdehyde (MDA) per kg of product. The microbiological analyses followed the APHA (2015) [33] and ISO standards for food microbiology: ISO 4833-1:2013 [34] for total aerobic mesophiles, ISO 17410:2019 [35] for psychrotrophic bacteria, and ISO 21527-1:2008 [36] for yeasts and molds. Ten grams of the sample was aseptically homogenized with 90 mL of sterile 0.85% NaCl (Biolife Italiana S.r.l., Milan, Italy) using a stomacher (BagMixer 400, Interscience, Saint-Nom-la-Bretèche, France) for 120 s. Decimal dilutions were plated and incubated as follows. SPC: Plate Count Agar (PCA, Biolife Italiana, Houston, TX, USA) at 30 ± 1 °C for 48 ± 2 h [34]; psychrotrophs: PCA at 7 ± 1 °C for 10 d [35]; and yeasts and molds: DRBC agar at 25 ± 1 °C for 5 d [36].

2.7. Color Stability (CIE L*a*b*)

Color measurements were conducted using a colorimeter (CR-400, Konica Minolta Inc., Tokyo, Japan; D65 illuminant, 2° observer, Ø8 mm aperture, SCE mode) in accordance with CIE Publication 15:2018 [37] and ISO 11664-4:2019 [38].

The instrument was zeroed and white-calibrated before each session. Patties (4 ± 1 °C) were blotted, placed on a matte black background, and bloomed for 20 min at 4 °C. For each treatment × day, *n* = 6 patties (3 batches × 2 replicates) were analyzed;

three non-overlapping spots per patty were averaged. The reported parameters were L^* , a^* , b^* , and the total color difference ΔE_{ab}^* versus day 0:

$$\Delta E_{ab} = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (1)$$

2.8. Statistical Analysis

Data are reported as means $\pm$ SDs with $n = 6$ per treatment $\times$ day 3 independent batches $\times$ 2 analytical replicates. A two-way mixed-model ANOVA was fitted with treatments T0–T3 and days 0, 3, 6, 9, 12, and 15 as fixed factors and batch as a random blocking factor; the treatment $\times$ day interaction was included. Model assumptions were checked on residuals. Variable-specific handling was as follows: microbial counts were analyzed on a log10 scale (with units reported as log CFU/g); DPPH was analyzed after arcsine–square-root transformation of the proportion; and pH, a_w , TBARS, PV, TPC, L , a , b^* , and ΔE^{***} were analyzed untransformed unless diagnostics indicated otherwise. When the interaction was significant ($p < 0.05$), simple-effects one-way ANOVAs were performed with post hoc comparisons by Duncan’s multiple-range test, where $\alpha = 0.05$, (i) among treatments within each day and (ii) among days within each treatment. When variances were unequal, the Games–Howell test was used. Exact p -values were given where feasible (otherwise, they were given as thresholds: $p < 0.05$, <0.01 , and <0.001). For microbiology, ND (“not detected”) values (observed for psychrotrophs and yeasts/molds at early times) were conservatively set to the limit of detection (LOD) for statistical testing. Analyses were conducted in SPSS v22.0 (IBM, Chicago, IL, USA). Raw replicate values and complete ANOVA output tables are provided in Supplementary Dataset S1 to ensure full transparency and reproducibility. Raw replicate values and complete ANOVA output tables underlying Tables 2–6 are provided in Supplementary Dataset S1 to ensure full transparency and reproducibility.

3. Results and Discussion

3.1. pH and Water Activity of *Salicornia* Extract-Enriched Beef Patties

The pH of comminuted meat systems is governed by raw material composition, microbial metabolism, and storage-driven chemical reactions, whereas water activity (a_w) is a primary determinant of microbial growth and shelf-life in refrigerated meats [1,4,5]. The pH and a_w values of beef patties formulated with increasing levels of *Salicornia* extract are summarized in Table 2, where T0 = control; T1 = 0.5%; T2 = 1.0%; and T3 = 1.5% extract (dry basis).

Table 2. Changes in pH and water activity (a_w) of *Salicornia* extract-enriched beef patties during refrigerated storage at 4 ± 1 °C.

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
	pH					
T0	6.22 ± 0.03 Ba	6.17 ± 0.03 Bb	6.10 ± 0.04 Cc	6.03 ± 0.03 Cd	5.95 ± 0.04 Ce	5.86 ± 0.03 Cf
T1	6.24 ± 0.03 Ba	6.22 ± 0.02 ABa	6.19 ± 0.03 Bb	6.14 ± 0.03 Bc	6.08 ± 0.03 Bd	6.02 ± 0.04 Be
T2	6.28 ± 0.03 Aa	6.27 ± 0.03 Aa	6.25 ± 0.02 Aa	6.21 ± 0.03 Ab	6.17 ± 0.03 Ac	6.12 ± 0.03 Ad
T3	6.32 ± 0.02 Aa	6.31 ± 0.02 Aa	6.29 ± 0.02 Aa	6.25 ± 0.02 Ab	6.21 ± 0.02 Ac	6.16 ± 0.02 Ad
	a_w					
T0	0.878 ± 0.003 Aa	0.867 ± 0.003 Ab	0.856 ± 0.003 Bc	0.846 ± 0.003 Cd	0.836 ± 0.004 Ce	0.828 ± 0.003 Cf
T1	0.876 ± 0.003 Aa	0.870 ± 0.003 Aab	0.860 ± 0.003 ABbc	0.850 ± 0.003 Bcd	0.842 ± 0.003 Bde	0.835 ± 0.003 Be
T2	0.878 ± 0.003 Aa	0.874 ± 0.003 Aa	0.866 ± 0.003 Ab	0.858 ± 0.003 Ac	0.850 ± 0.003 Ad	0.843 ± 0.003 Ae
T3	0.876 ± 0.002 Aa	0.876 ± 0.002 Aa	0.870 ± 0.002 Ab	0.862 ± 0.002 Ac	0.855 ± 0.002 Ad	0.847 ± 0.002 Ae

T0 = control (no *Salicornia*); T1 = 0.5% extract; T2 = 1.0% extract; T3 = 1.5% extract (all dosed on extract dry basis; % w/w of total batter). Mean values with different lowercase letters (a–f) within rows and uppercase letters (A–C) within columns differ significantly ($p < 0.05$) ($n = 6$).

All treatments showed a progressive, significant pH decrease over the storage time ($p < 0.05$), consistent with acidification driven by psychrotrophic microflora—especially lactic acid bacteria—which metabolize residual substrates to organic acids in comminuted meat systems [2,4]. The control T0 declined from 6.22 ± 0.03 on day 0 to 5.86 ± 0.03 on day 15, while *Salicornia* groups exhibited attenuated declines: T1 reached 6.02 ± 0.04 , T2 reached 6.12 ± 0.03 , and T3 reached 6.16 ± 0.02 . Between-treatment contrasts show that T2–T3 consistently retained a higher pH than T0 at corresponding time points ($p < 0.05$). These findings align with reports that plant-derived ingredients can temper storage-driven acidification in burgers/sausages by subtly influencing LAB growth and acidogenesis and/or contributing minor buffering via ionic constituents [19,24,39,40]. For *Salicornia* in particular, studies in seafood and processed meats indicate antimicrobial/quality-stabilizing effects and feasibility as a functional salty bio-ingredient [20,29,30]. *Salicornia* is rich in minerals, such as Na, K, Mg, and Ca, and phenolic compounds that can (i) modulate microbial ecology and redox balance and (ii) slightly buffer or alter the ionic strength of the meat aqueous phase [39–41]. Partial NaCl replacement with *Salicornia* herbacea in meat/seafood matrices has been shown to preserve quality and affect microbial outcomes during chill storage, supporting our observation of moderated pH decline at higher inclusion levels in T2–T3 [41–44]. While some plant extracts have neutral effects on pH depending on dose/matrix, the present data fit the commonly observed “moderation, not reversal” pattern for effective botanical levels in comminuted meats [24,39]. a_w decreased significantly in all groups across storage, a typical outcome of moisture redistribution/immobilization and solute accumulation in refrigerated minced products [1,2,6,33]. The control fell from 0.878 ± 0.003 to 0.828 ± 0.003 . In contrast, *Salicornia* groups showed smaller declines: T1 to 0.835 ± 0.003 , T2 to 0.843 ± 0.003 , and T3 to 0.847 ± 0.002 . Early differences on $\leq$ day 3 were minimal, but from mid-storage onward, T2–T3 maintained a significantly higher a_w than T0. We attribute this to the hydrophilic and ionic components of *Salicornia* that can enhance water binding/immobilization or adjust osmotic balance within the protein matrix, mechanisms consistent with water activity fundamentals and prior botanical interventions in comminuted meats [24,39,40]. Notably, glasswort-based formulations in cured pork and fish also reported quality preservation concurrent with altered mass transfer, reinforcing the plausibility of our a_w trajectory [20,29,44]. Relative to T0, 1.0–1.5% *Salicornia* in T2–T3 consistently tempered pH fall and reduced a_w decline, indicating a more stable physicochemical environment during chilled storage. Such stabilization typically co-occurs with improved oxidative and microbiological outcomes in plant-fortified meat systems, suggesting potential shelf-life benefits to be corroborated by TBARS/PV, color, microbiology, and sensory endpoints in subsequent sections [1,4,27,45].

3.2. Lipid Oxidation (TBARS and Peroxide Value) in *Salicornia*-Enriched Beef Patties

Table 3 summarizes the evolution of lipid oxidation in refrigerated beef patties at 4 ± 1 °C, formulated with graded levels of *Salicornia* extract (T0–T3).

Table 3. Changes in TBARS (mg MDA/kg) and peroxide value (meq O₂/kg) of *Salicornia* extract-enriched beef patties during refrigerated storage at 4 ± 1 °C.

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
TBARS (mg MDA/kg)						
T0	0.47 ± 0.02 ^{Ca}	0.71 ± 0.03 ^{Cb}	0.98 ± 0.04 ^{Cc}	1.27 ± 0.05 ^{Cd}	1.60 ± 0.06 ^{Ce}	1.98 ± 0.06 ^{Cf}
T1	0.46 ± 0.02 ^{Ca}	0.63 ± 0.03 ^{BCb}	0.84 ± 0.04 ^{BCc}	1.07 ± 0.04 ^{BCd}	1.31 ± 0.05 ^{BCe}	1.56 ± 0.05 ^{BCf}
T2	0.45 ± 0.02 ^{Ba}	0.57 ± 0.02 ^{Bb}	0.74 ± 0.03 ^{Bc}	0.92 ± 0.03 ^{Bd}	1.11 ± 0.04 ^{Be}	1.31 ± 0.04 ^{Bf}
T3	0.44 ± 0.01 ^{Aa}	0.52 ± 0.02 ^{Ab}	0.66 ± 0.03 ^{Ac}	0.82 ± 0.03 ^{Ad}	0.98 ± 0.03 ^{Ae}	1.15 ± 0.03 ^{Af}

Table 3. Cont.

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
			PV (meq O ₂ /kg)			
T0	1.22 ± 0.05 ^{Ba}	1.88 ± 0.06 ^{Bb}	2.58 ± 0.08 ^{Bc}	3.35 ± 0.09 ^{Bd}	4.12 ± 0.11 ^{Be}	4.95 ± 0.12 ^{Bf}
T1	1.20 ± 0.04 ^{Ba}	1.74 ± 0.05 ^{ABb}	2.34 ± 0.07 ^{ABc}	2.95 ± 0.08 ^{ABd}	3.60 ± 0.09 ^{ABe}	4.16 ± 0.10 ^{ABf}
T2	1.17 ± 0.04 ^{Aa}	1.63 ± 0.05 ^{Ab}	2.08 ± 0.06 ^{Ac}	2.64 ± 0.07 ^{Ad}	3.18 ± 0.08 ^{Ae}	3.72 ± 0.09 ^{Af}
T3	1.16 ± 0.03 ^{Aa}	1.53 ± 0.04 ^{Ab}	1.96 ± 0.06 ^{Ac}	2.43 ± 0.07 ^{Ad}	2.90 ± 0.08 ^{Ae}	3.30 ± 0.08 ^{Af}

T0 = control (no *Salicornia*); T1 = 0.5% extract; T2 = 1.0% extract; T3 = 1.5% extract (all dosed on extract dry basis; % w/w of total batter). Mean values with different lowercase letters (a–f) within rows and uppercase letters (A–C) within columns differ significantly ($p < 0.05$) (n = 6).

Across storage, TBARS increased significantly in all groups, but the rise was progressively attenuated as the *Salicornia* dose increased: T3 < T2 < T1 < T0. By day 15, TBARS reached 1.98 mg MDA/kg in T0 versus 1.15 mg MDA/kg in T3. A similar dose response appeared for PV on day 15: 4.95 vs. 3.30 meq O₂/kg for T0 vs. T3. This pattern mirrors reports where halophyte-derived matrices and other botanical antioxidants lower both primary and secondary oxidation in minced-meat models, e.g., *Lobularia maritima* essential oils/flavonoids in ground beef, and phenolic-rich plant extracts such as rosemary (carnosic and carnosol diterpenes), green tea catechins, grapeseed procyanidins, clove phenolics, and sage extract in beef patties and sausages, as well as prickly pear fruit extract and hempseed protein hydrolysates used in meatball formulations [45–51]. The concurrent reductions in PV and TBARS with *Salicornia* likely arise from (i) radical-scavenging phenolics that quench peroxy radicals and decompose hydroperoxides; (ii) metal-chelating constituents that slow Fenton-type propagation; and (iii) ionic/mineral components that may influence the micro-aqueous phase and protein–lipid interfaces. In addition, the antioxidant effect observed in this study can be attributed to the phenolic composition of *Salicornia* perennans, which is rich in hydroxycinnamic acids and flavonoids. Previous phytochemical investigations have identified caffeic acid, ferulic acid, p-coumaric acid, chlorogenic acid, gallic acid, quercetin, rutin, catechin, and kaempferol as the dominant phenolics in *Salicornia* species, all known for their high redox and metal-chelating activity [52–57]. These compounds effectively intercept lipid radicals, stabilize hydroperoxides, and inhibit the formation of secondary aldehydes, thus explaining the observed reductions in both PV and TBARS values. Such mechanisms are described for halophyte extracts and sea vegetable matrices, and they translate into lower hydroperoxide build-up and fewer secondary aldehydes in beef systems [44,58]. The steeper early-stage PV rise on days 0–9, followed by continued TBARS accumulation, is consistent with canonical lipid oxidation kinetics in beef patties: primary products form first and then decompose into secondary aldehydes under storage/processing stresses [51]. Independent studies show that process conditions accelerate both PV and TBARS in patties, and phenolic interventions blunt these trajectories, paralleling our T2–T3 curves [59–61]. Formulations with 1.0–1.5% *Salicornia* in T2–T3 delivered the lowest TBARS/PV throughout storage without eliminating oxidation entirely, an expected “moderation-not-reversal” outcome for effective botanical levels in comminuted beef. In practice, combining *Salicornia* with supportive hurdles can yield additive or synergistic suppression of both PV and TBARS in burgers [55]. Recent work has similarly shown that *Salicornia* ingredients curb primary and secondary lipid oxidation in meat products: ethanolic or powdered *S. herbacea* reduced TBARS/PV while enabling partial NaCl replacement in frankfurters and other processed meats [19]; quality improvements against oxidative deterioration were also observed in sun-dried beef jerky and semi-dried fish models fortified with *Salicornia* [13]. These effects are consistent with broader applications of glasswort as a clean-label antioxidant/salt replacer in meat systems [62]. Benchmark botanicals for raw/cooked patties include rosemary, which collectively lower hydroperoxides and

secondary aldehydes in beef systems [63]. The *Salicornia* effects observed here—concurrent PV and TBARS attenuation with retained TPC/DPPH—are comparable in pattern to these extracts, though exact magnitudes vary with matrix, dose, and packaging. Mechanistically, *Salicornia* couples phenolic radical-quenching with mineral-driven micro-environment effects, providing a complementary route to the phenolic-only mechanisms emphasized for rosemary [64].

3.3. Antioxidant Capacity (TPC and DPPH) of *Salicornia*-Enriched Beef Patties

Table 4 presents the total phenolic content (TPC (mg GAE/100 g)) and DPPH radical-scavenging activity (%) of *Salicornia* extract-enriched beef patties during refrigerated storage at 4 ± 1 °C.

Table 4. Changes in total phenolic content (TPC (mg GAE/100 g)) and DPPH radical-scavenging activity (%) of *Salicornia* extract-enriched beef patties during refrigerated storage at 4 ± 1 °C.

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
TPC (mg GAE/100 g)						
T0	11.0 ± 0.3 ^{Ca}	10.1 ± 0.3 ^{Cb}	9.2 ± 0.3 ^{Cc}	8.3 ± 0.3 ^{Cd}	7.5 ± 0.2 ^{Ce}	6.8 ± 0.2 ^{Cf}
T1	16.8 ± 0.4 ^{Ba}	15.9 ± 0.4 ^{Bb}	14.9 ± 0.4 ^{Bc}	13.8 ± 0.3 ^{Bd}	12.8 ± 0.3 ^{Be}	11.9 ± 0.3 ^{Bf}
T2	22.9 ± 0.5 ^{Aa}	21.7 ± 0.5 ^{Ab}	20.5 ± 0.4 ^{Ac}	19.1 ± 0.4 ^{Ad}	17.8 ± 0.3 ^{Ae}	16.6 ± 0.3 ^{Af}
T3	27.6 ± 0.6 ^{Aa}	26.3 ± 0.5 ^{Ab}	24.9 ± 0.5 ^{Ac}	23.4 ± 0.4 ^{Ad}	22.0 ± 0.4 ^{Ae}	20.6 ± 0.4 ^{Af}
DPPH inhibition (%)						
T0	27.5 ± 0.8 ^{Ca}	24.6 ± 0.7 ^{Cb}	21.8 ± 0.6 ^{Cc}	19.2 ± 0.5 ^{Cd}	16.7 ± 0.6 ^{Ce}	14.5 ± 0.5 ^{Cf}
T1	41.5 ± 0.9 ^{Ba}	38.4 ± 0.8 ^{Bb}	35.4 ± 0.7 ^{Bc}	32.3 ± 0.6 ^{Bd}	29.3 ± 0.7 ^{Be}	26.4 ± 0.6 ^{Bf}
T2	55.0 ± 1.0 ^{Aa}	51.5 ± 0.9 ^{Ab}	48.1 ± 0.8 ^{Ac}	44.6 ± 0.9 ^{Ad}	41.0 ± 0.8 ^{Ae}	37.6 ± 0.7 ^{Af}
T3	65.2 ± 1.1 ^{Aa}	61.6 ± 1.0 ^{Ab}	58.0 ± 0.9 ^{Ac}	54.0 ± 0.9 ^{Ad}	49.6 ± 0.8 ^{Ae}	45.8 ± 0.8 ^{Af}

T0 = control (no *Salicornia*); T1 = 0.5% extract; T2 = 1.0% extract; T3 = 1.5% extract (all dosed on extract dry basis; % w/w of total batter). Mean values with different lowercase letters (a–f) within rows and uppercase letters (A–C) within columns differ significantly ($p < 0.05$) ($n = 6$).

On day 0, the TPC rose dose-dependently with *Salicornia*—T0 < T1 < T2 < T3—and the same ordering held for DPPH. Over 15 days, both metrics significantly decreased in all groups, yet T2–T3 consistently retained higher TPC and DPPH values than T0 at each time point. This pattern reflects the phenolic-rich profile of *Salicornia* and the well-known sensitivity of extractable phenolics/antioxidant capacity to storage-driven reactions in meat matrices, which reduce assay-detectable TPC and scavenging activity over time [65–69]. The gradual loss in assayable TPC/DPPH likely arises from (i) protein–phenolic interactions that decrease extractability and quench free phenolic sites, (ii) metal-chelating and radical-quenching reactions that consume phenolics, and (iii) redistribution within the mince, all common in comminuted beef systems [70–73]. Recent studies detail how phenolics' size/structure governs their mobility and reactivity in meats and marinades, and how binding to myofibrillar proteins lowers the measured TPC despite preservation benefits in situ [6–8]. The higher TPC/DPPH retained in T2–T3 is consistent with the lower PV/TBARS profiles typically observed when phenolic inputs are sufficient to intercept primary radicals and hydroperoxides in patties. While DPPH often tracks TBARS inversely, correlations can vary with matrix and assay chemistry, so TPC/DPPH should be interpreted alongside lipid oxidation markers [74–76]. Beyond general seaweed literature, *Salicornia* spp. provide phenolic acids, flavonoids, and minerals relevant to antioxidant function. Contemporary works document their strong in vitro antioxidant capacity, feasibility as partial salt replacers in meat matrices, and technological compatibility in reduced-salt products, all supportive of the dose-responsive TPC/DPPH advantages seen here for T2–T3 [77,78]. Formulations with 1.0–1.5% *Salicornia* delivered higher initial and residual TPC/DPPH throughout storage, indicating a more resilient antioxidant “buffer” in the patties. In practice, these

levels can be paired with packaging/processing hurdles to further stabilize phenolics and sustain radical-scavenging capacity over shelf-life [79]. Multiple studies corroborate the dose-responsive increases in extractable phenolics and radical-scavenging activity when *Salicornia* is incorporated into meat matrices, with sustained advantages during storage versus controls [80]. Beyond finished products, upstream agronomic advances and biomass quality data indicate *Salicornia* species can deliver reproducible phenolic profiles suitable for food applications, supporting translational use in meat reformulation [81].

3.4. Microbiological Stability of *Salicornia*-Enriched Beef Patties

Table 5 summarizes microbial counts in *Salicornia*-enriched beef patties stored for 15 days at 4 ± 1 °C for SPC, psychrotrophs, and yeasts/molds.

Table 5. Changes in microbial counts of *Salicornia* extract-enriched beef patties during refrigerated storage (4 ± 1 °C).

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Standard plate count (SPC, log CFU/g)						
T0	2.52 ± 0.10 ^{Ba}	2.90 ± 0.11 ^{Bb}	3.60 ± 0.12 ^{Bc}	4.30 ± 0.10 ^{Bd}	5.18 ± 0.09 ^{Be}	6.20 ± 0.12 ^{Bf}
T1	2.47 ± 0.09 ^{BCa}	2.72 ± 0.12 ^{BCb}	3.18 ± 0.10 ^{Cc}	3.90 ± 0.11 ^{Cd}	4.55 ± 0.10 ^{Ce}	5.98 ± 0.11 ^{Cf}
T2	2.41 ± 0.11 ^{ABa}	2.60 ± 0.10 ^{BCb}	3.02 ± 0.09 ^{Cc}	3.68 ± 0.10 ^{Cd}	4.18 ± 0.08 ^{Ce}	5.22 ± 0.09 ^{Bf}
T3	2.40 ± 0.12 ^{Aa}	2.51 ± 0.09 ^{Ab}	2.90 ± 0.10 ^{Ac}	3.45 ± 0.09 ^{Ad}	3.86 ± 0.10 ^{Ae}	4.88 ± 0.11 ^{Af}
Psychrotrophic counts (log CFU/g)						
T0	ND	ND	ND	1.20 ± 0.08 ^{Ac}	1.52 ± 0.09 ^{Ab}	2.00 ± 0.10 ^{Aa}
T1	ND	ND	ND	1.16 ± 0.07 ^{ABc}	1.38 ± 0.08 ^{Bb}	1.86 ± 0.09 ^{Ba}
T2	ND	ND	ND	ND	1.14 ± 0.07 ^{Cb}	1.68 ± 0.08 ^{Ca}
T3	ND	ND	ND	ND	ND	1.46 ± 0.08 ^{Da}
Yeast and mold counts (log CFU/g)						
T0	ND	ND	ND	ND	1.45 ± 0.07 ^{Ab}	1.74 ± 0.09 ^{Aa}
T1	ND	ND	ND	ND	1.29 ± 0.06 ^{Bb}	1.58 ± 0.08 ^{Ba}
T2	ND	ND	ND	ND	1.19 ± 0.07 ^{BCb}	1.52 ± 0.09 ^{Ba}
T3	ND	ND	ND	ND	1.13 ± 0.06 ^{Cb}	1.44 ± 0.08 ^{Ca}

T0 = control (no *Salicornia*); T1 = 0.5% extract; T2 = 1.0% extract; T3 = 1.5% extract (all dosed on extract dry basis; % w/w of total batter). Mean values with different lowercase letters (a–f) within rows and uppercase letters (A–C) within columns differ significantly ($p < 0.05$) ($n = 6$).

All formulations showed significant SPC increases during storage, as expected for comminuted beef held under refrigeration. However, *Salicornia* produced a clear, dose-responsive attenuation: by day 15, SPC rose to 6.20 log CFU/g in the control T0 but only 4.88 log CFU/g at 1.5% in T3, with $T2 < T1 < T0$ at each time point. In fresh minced beef systems, spoilage is often associated with 7 log CFU/g aerobes and shelf-life windows of 3–5 days at 4 °C without hurdles; thus, the lower trajectories in T2–T3 indicate a meaningful microbiological slow-down relative to the control [82]. Psychrotrophs were undetectable early, appearing first in T0/T1 around days 9–12, but not until days 12–15 in T2/T3, and at lower magnitudes. Given that psychrotrophs drive off-odors/discoloration in refrigerated meat, this delay supports a protective role of the extract [65,66]. Fungi emerged late on $\geq$ day 12 across treatments, but counts remained lowest in T3. Similar reductions in yeast/mold and total aerobes have been reported when halophyte derivatives or algal phenolics are incorporated into meat/seafood matrices [83,84]. The antimicrobial effect is consistent with (i) phenolic/flavonoid constituents that disrupt membranes, scavenge radicals, and chelate pro-oxidant metals; (ii) ionic/mineral components altering osmotic balance and micro-aqueous phase; and (iii) the potential modulation of LAB/psychrotroph ecology in the comminuted matrix. Broad reviews of macroalgal antimicrobials describe phenolic acids, bromophenols, phlorotannins, and terpenoids acting against Gram-negative and Gram-positive spoilage organisms, mechanisms that plausibly underlie the lower

SPC/psychrotroph/yeast/mold counts observed here [85,86]. Direct applications of *Salicornia* in muscle foods report quality preservation and antimicrobial benefits: *Salicornia herbacea* hydrates improved functionality and process performance in reduced-salt frankfurters; powder or extract use in sausages and beef has been explored for partial NaCl replacement with technological compatibility; and studies in minced beef noted lower APC and fungal counts and longer shelf-life at 1–1.5% concentrations, comparable to our T2–T3. Our psychrotroph/yeast/mold outcomes align with these reports, while remaining within known sensory constraints for higher halophyte inclusion [87,88]. Taken together, Table 5 shows that 1.0–1.5% *Salicornia* in T2–T3 significantly slowed aerobic growth, delayed psychrotrophic onset, and reduced late-stage fungal counts relative to the control. In practice, combining *Salicornia* with packaging hurdles can further extend shelf-life by constraining oxygen and reinforcing the extract's antimicrobial action.

3.5. Color Stability (CIE $L^*a^*b^*$) of *Salicornia*-Enriched Beef Patties

Table 6 presents the color stability of *Salicornia*-enriched beef patties over 15 days at 4 ± 1 °C.

Table 6. Color stability of *Salicornia* extract-enriched beef patties during refrigerated storage at 4 ± 1 °C.

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
			L^* (lightness)			
T0	45.2 ± 0.4 Aa	46.3 ± 0.4 Bb	47.3 ± 0.5 Bc	48.3 ± 0.5 Bd	49.3 ± 0.5 Be	50.1 ± 0.5 Bf
T1	45.1 ± 0.4 Aa	45.9 ± 0.4 ABb	46.8 ± 0.4 ABc	47.6 ± 0.5 ABd	48.3 ± 0.5 ABe	49.0 ± 0.5 ABf
T2	45.0 ± 0.4 Aa	45.6 ± 0.4 Ab	46.2 ± 0.4 Ac	46.8 ± 0.4 Ad	47.3 ± 0.4 Ae	47.8 ± 0.4 Af
T3	45.0 ± 0.4 Aa	45.5 ± 0.4 Ab	46.0 ± 0.4 Ac	46.4 ± 0.4 Ad	46.9 ± 0.4 Ae	47.3 ± 0.4 Af
			a^* (redness)			
T0	16.5 ± 0.3 Ba	15.2 ± 0.3 Cb	13.8 ± 0.3 Cc	12.1 ± 0.3 Cd	10.7 ± 0.3 Ce	9.8 ± 0.3 Cf
T1	16.6 ± 0.3 ABa	15.7 ± 0.3 Bb	14.8 ± 0.3 Bc	13.6 ± 0.3 Bd	12.5 ± 0.3 Be	11.5 ± 0.3 Bf
T2	16.5 ± 0.3 ABa	16.0 ± 0.3 ABb	15.4 ± 0.3 ABc	14.6 ± 0.3 ABd	13.8 ± 0.3 ABe	13.0 ± 0.3 ABf
T3	16.4 ± 0.3 Aa	16.1 ± 0.3 Ab	15.7 ± 0.3 Ac	15.0 ± 0.3 Ad	14.2 ± 0.3 Ae	13.6 ± 0.3 Af
			b^* (yellowness)			
T0	12.2 ± 0.3 Aa	12.9 ± 0.3 Bb	13.7 ± 0.3 Bc	14.6 ± 0.3 Bd	15.3 ± 0.3 Be	16.0 ± 0.3 Bf
T1	12.1 ± 0.3 Aa	12.6 ± 0.3 ABb	13.2 ± 0.3 ABc	13.9 ± 0.3 ABd	14.5 ± 0.3 ABe	15.1 ± 0.3 ABf
T2	12.1 ± 0.3 Aa	12.4 ± 0.3 Ab	12.8 ± 0.3 Ac	13.3 ± 0.3 Ad	13.7 ± 0.3 Ae	14.1 ± 0.3 Af
T3	12.0 ± 0.3 Aa	12.3 ± 0.3 Ab	12.6 ± 0.3 Ac	12.9 ± 0.3 Ad	13.2 ± 0.3 Ae	13.6 ± 0.3 Af
			ΔE^*_{ab} (vs. day 0)			
T0	0.00 ± 0.00 Ca	1.84 ± 0.08 Cb	3.73 ± 0.10 Cc	5.89 ± 0.11 Cd	7.75 ± 0.12 Ce	9.13 ± 0.12 Cf
T1	0.00 ± 0.00 BCa	1.30 ± 0.07 BCb	2.71 ± 0.09 BCc	4.30 ± 0.10 BCd	5.73 ± 0.11 BCe	7.09 ± 0.11 BCf
T2	0.00 ± 0.00 ABa	0.84 ± 0.06 ABb	1.77 ± 0.07 ABc	2.88 ± 0.08 ABd	3.89 ± 0.09 ABe	4.91 ± 0.10 ABf
T3	0.00 ± 0.00 Aa	0.66 ± 0.05 Ab	1.36 ± 0.06 Ac	2.17 ± 0.07 Ad	3.14 ± 0.08 Ae	3.96 ± 0.09 Af

T0 = control (no *Salicornia*); T1 = 0.5% extract; T2 = 1.0% extract; T3 = 1.5% extract (all dosed on extract dry basis; % w/w of total batter). Mean values with different lowercase letters (a–f) within rows and uppercase letters (A–C) within columns differ significantly ($p < 0.05$) ($n = 6$).

Salicornia clearly improved color retention over 15 days at 4 ± 1 °C: T2–T3 limited lightness rise (L^*), slowed loss of redness (a^*), tempered yellowness increase (b^*), and yielded markedly lower cumulative color change (ΔE^*_{ab}) than T0. For example, by day 15, a^* remained at ~13.0–13.6 in T2–T3 vs. 9.8 in T0, while ΔE^* was 4.9–4.0 in T2–T3 vs. 9.1 in T0. These trends are consistent with the better preservation of oxymyoglobin and delayed metmyoglobin formation in antioxidant-fortified beef systems [89]. Meat redness reflects the redox state of myoglobin; oxidative stress and pro-oxidant catalysts accelerate oxymyoglobin → metmyoglobin, decreasing a^* and increasing ΔE^* . Polyphenolic/halophytic matrices can scavenge radicals and chelate metals, stabilizing myoglobin redox and, thus, color. The CR-400 $L^*a^*b^*$ patterns here align with this mechanism [90].

Studies using *Salicornia* herbacea in meat systems report color protection alongside sodium reduction: reduced-salt sausages with 1% *Salicornia* herbacea maintained acceptable redness/appearance relative to controls, and dry-cured pork bellies with *Salicornia herbacea* as a partial NaCl replacer showed quality preservation, including color attributes, during storage. Our findings at 1.0–1.5% mirror these beneficial effects [4,91]. Beyond *Salicornia*, seaweed ingredients in comminuted meats often darken products slightly (lower L^*) but preserve redness and limit discoloration via phlorotannins/phenolics; formulation and dose control the balance between functional protection and visual impact, consistent with the modest L^* shifts and improved a^* stability we observed for T2–T3 [92]. Incorporating 1.0–1.5% *Salicornia* yielded a meaningful delay in beef patty discoloration (lower ΔE^* ; higher a^*), congruent with myoglobin-focused color science and prior glasswort/seaweed interventions. Pairing *Salicornia* with oxygen-limiting packaging should further sustain redness during retail display [93].

4. Conclusions

In summary, adding *Salicornia* extract to beef patties produced clear, dose-dependent benefits, with 1.0–1.5% in T2–T3 performing best ($p < 0.05$): by day 15, TBARS decreased from 1.98 to 1.15 mg MDA/kg and PV from 4.95 to 3.30 meq O₂/kg in T0 vs. T3; TPC and DPPH remained higher at 20.6 vs. 6.8 mg GAE/100 g and 45.8% vs. 14.5%, respectively; SPC was lower at 4.88 vs. 6.20 log CFU/g, with reduced psychrotrophs at 1.46 vs. 2.00 log CFU/g and yeasts/molds at 1.44 vs. 1.74 log CFU/g; color was better preserved with ΔE^* at 3.96 vs. 9.13 and a^* at 13.6 vs. 9.8; and physicochemical stability improved, with the terminal pH at 6.16 vs. 5.86 and a_w at 0.847 vs. 0.828. These outcomes support *Salicornia* 1.0–1.5% as a clean-label strategy to extend the refrigerated shelf-life of beef patties, especially alongside oxygen-limiting packaging.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app152111751/s1>, Supplementary Dataset S1: Raw replicate values and full ANOVA output tables underlying Tables 2–6 of the main text. The dataset includes all physicochemical (pH and a_w), oxidative (TBARS and PV), antioxidant (TPC and DPPH), microbiological, and color (CIE L^* , a^* , b^* , and ΔE^*) parameters measured across treatments and storage days (three independent batches $\times$ two analytical replicates).

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