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Sodium-Reduced Canned Dog Pâtés Enriched with Collagen Hydrolysate and *Salicornia perennans*: A Sustainable Strategy to Enhance Technological Quality and Oxidative Stability

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Abstract

This study evaluated the effects of enzymatically produced collagen hydrolysate and *Salicornia perennans* extract on the quality, oxidative stability, and nutritional composition of canned canine meat pâtés. Two formulations were prepared: a control 2% NaCl, no hydrolysate and an experimental sample containing 3% collagen hydrolysate sheep:camel:bovine = 1:1:1, 1% *Salicornia perennans* extract, and 1% NaCl. Physicochemical, textural, amino-acid, fatty-acid, and oxidative parameters were monitored over 10 days of storage. The treated pâtés showed similar proximate composition moisture 76.1%, protein 9.2%, metabolizable energy (ME) 102 kcal·100 g^{-1}; $p > 0.05$ but exhibited enhanced functional stability, with reduced water loss syneresis 1.8 vs. 3.1%; $p < 0.05$ and improved cohesiveness 0.46 vs. 0.41; $p < 0.05$. Amino-acid enrichment included higher aspartic acid +33%; $p < 0.05$, methionine +53%; $p < 0.05$, and tryptophan +39%; $p < 0.05$, while the lipid profile showed lower SFA 52.8 vs. 56.4%; $p < 0.05$, higher n-3 PUFA 1.5 vs. 0.8%; $p < 0.05$, and a reduced n-6:n-3 ratio 3.8 vs. 5.6; $p < 0.05$. During storage, oxidative markers decreased: TBARS −45%, carbonyls −14%, acid value −18%, and color stability improved by +2.0 pp. These findings confirm the synergistic antioxidant and structuring effects of collagen-derived peptides and Salicornia polyphenols, as evidenced by a 45% reduction in TBARS, 14% lower protein carbonyls, and 18% lower acid value relative to the control ($p < 0.05$). This synergy enabled a sodium-reduced, clean-label formulation with improved technological performance, oxidative resistance, and shelf-life stability for functional wet dog foods. In addition, it enhanced the color and visual appeal—key attributes that influence both animal palatability and the purchasing decisions of pet owners.

Keywords: collagen hydrolysate; *Salicornia perennans*; sodium reduction; oxidative stability; color stability; amino acids; fatty acids; dog food; color stability; protein oxidation

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1. Introduction

Commercial wet dog foods (pâtés and chunks-in-gravy) are a major delivery format for complete canine diets and remain central to the safety and nutritional adequacy discussions in pet nutrition [1]. The global pet food market continues to expand, with wet products accounting for approximately 35–40% of total dog food sales. According to recent market analyses, the global wet dog food sector exceeded USD 25 billion in 2024 and is projected to grow at 5–6% annually, driven by increasing pet humanization and demand for

premium, nutritionally balanced formulations [2,3]. Within this category, product quality hinges on balancing palatability, texture, nutrient density, and microbiological stability, all of which are shaped by ingredient choice and thermal processing [4]. One promising technological avenue is the use of animal-derived protein ingredients and hydrolysates that deliver functionality (gelation, water binding, emulsification) together with high protein digestibility and an amino-acid profile aligned with canine requirements [5]. Beyond generic protein sources, spray-dried animal plasma (SDAP) illustrates how animal by-products can function as high-value binders and emulsifiers in wet systems and improve product handling and structure during retort processing [6]. SDAP also brings favorable techno-functional properties and amino-acid completeness relative to alternative proteins, and has been trialed in formulations for chunks-in-gravy where it enhances the firmness, cohesiveness and juiciness of the finished pieces [7]. Importantly, product performance must be balanced with eating quality: controlled inclusion levels of such functional proteins can improve palatability in dogs, whereas excessive levels may depress preference, underscoring the need for dose–response optimization in wet foods [8]. Among the various functional proteins, collagen hydrolysates and halophyte-derived extracts such as *Salicornia perennans* have recently gained attention as dual-function ingredients—providing both structural support and natural antioxidant protection in meat and pet food matrices. Enzymatic protein hydrolysates derived from underutilized tissues are particularly attractive for sustainable innovation. Hydrolyzed collagen from sheepskins yields peptide fractions with documented antioxidant capacity and favorable physicochemical traits relevant to texturization [9]. Recent proteomic work has identified Bactrian camel hoof as a previously untapped terrestrial source of type I collagen with demonstrated emulsifying and rheological functionality—properties directly relevant to structuring wet pet foods [10]. In parallel, keratin-rich by-products (e.g., hooves, horns) represent a large global protein stream; review work highlights scalable extraction/valorization routes that can upcycle these materials into functional ingredients, aligning with circular-economy goals [11]. The use of such by-products complies with current EU feed and pet food regulations [12,13], which allow processed animal proteins derived from non-ruminant or ruminant by-products when properly hydrolyzed and sterilized to eliminate transmissible spongiform encephalopathy (TSE) risk. From a canine nutrition standpoint, diets incorporating rendered animal by-products can meet digestibility targets and support fecal quality when formulated appropriately [14]. Safety and quality assurance also require attention to biogenic amines (BAs) in canned pet foods; BA loads vary by recipe and processing but can be managed through raw-material control and thermal treatment [15]. Ingredient choices matter as well—chicken-based pet foods show BA variability across products, and hydrolyzed chicken liver–based diets have been investigated for their compositional and technological impacts in dogs [16,17]. Because wet dog foods are retorted in hermetic pouches or cans, heat-induced protein gelation and matrix setting are decisive for product texture and syneresis control [18]. Heat treatment parameters strongly influence textural properties, protein cross-linking, and oxidative stability in retorted meat systems, as reported by Benjakul et al. (2018) and Syukri et al. (2025) [19,20]. Packaging and process design therefore interact with formulation: functional/retortable packaging must preserve barrier properties while supporting efficient heat transfer and shelf stability [21–24]. Process engineering options such as variable retort temperature profiles (VRTPs) can reach lethality targets while limiting undesirable thermal markers (e.g., furan), helping maintain quality in retorted foods [22,25]. On the materials side, bio-based multilayer films for food contact are evolving quickly, opening pathways to reduce the footprint of retortable formats without compromising performance [23,26–29]. In recent years, sustainable innovation in pet food formulation has increasingly focused on valorizing animal by-products and plant-

based bioactives to improve both functionality and environmental performance. Collagen hydrolysates derived from underutilized tissues, such as the hooves of sheep, camels, and cattle, represent renewable protein sources with high techno-functional potential. These species were chosen as abundant and underutilized by-products in Kazakhstan, differing in collagen structure and composition, allowing comparative evaluation of hydrolysate functionality. These hydrolysates provide gel-forming and emulsifying capacity while contributing antioxidant peptides that can enhance product stability.

In parallel, *Salicornia perennans*, a halophytic plant naturally rich in phenolic compounds and essential minerals, offers dual benefits as a natural sodium-reduction agent and antioxidant source. Integrating these two ingredients into wet dog food formulations can address key industry challenges—namely, reducing sodium content without compromising texture, oxidative stability, or sensory quality.

Safety aspects were also considered as part of the study's design: the use of animal by-products complies with EU and national feed regulations, and biogenic amines were not directly quantified but managed through raw-material control, standardized pH, and validated sterilization conditions, discussed as part of the safety framework.

Therefore, we hypothesize that enzymatically produced collagen hydrolysate combined with *Salicornia perennans* extract can synergistically enhance the technological quality, oxidative stability, and visual characteristics of sodium-reduced canned dog pâtés.

2. Materials and Methods

2.1. Formulation and Experimental Design

Two formulations of canned meat pâtés for dogs were developed: Control, containing 2% NaCl and no hydrolysate; and experimental, containing 3% enzymatic protein hydrolysate (sheep:camel:bovine = 1:1:1), 1% *Salicornia perennans* extract powder, and 1% NaCl.

Both formulations were balanced to identical macronutrient ratios and adjusted with water to reach 100 g total mass. The ingredient composition is shown in Table 1.

Table 1. Formulations of canned canine meat pâtés (as-fed basis; g/100 g; water q.s. to 100).

Ingredient	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)	Control (No Hydrolysate, 2% NaCl)
Turkey filet	29.0	29.0
Beef liver	9.0	9.0
Beef lung	9.0	9.0
Carrot	7.0	7.0
Buckwheat groats (pre-gelatinized)	8.0	8.0
Flaxseed oil	3.7	3.7
Protein hydrolysate (sheep/camel/bovine)	3.0	—
NaCl	1.0	2.0
Salicornia extract	1.0	—
Water (q.s. to 100%)	29.3	32.3
Total	100.0	100.0

2.2. Raw Materials and Processing

Raw materials included turkey filet, beef liver, beef lung, carrot, and pre-gelatinized buckwheat groats, all obtained from a local meat-processing plant (Astana, Kazakhstan) within 24 h post-slaughter. The meat and offal were trimmed, ground through a 5 mm plate, and kept at 4 °C prior to processing. The collagen hydrolysate was produced enzymatically using Alcalase 2.4 L (1.5% E/S, 55 °C, 120 min, pH 7.5) followed by Flavourzyme (0.5% E/S,

60 min, pH 7.0), then inactivated at 90 °C for 10 min, freeze-dried, and milled. The resulting collagen hydrolysate contained crude protein 89.4 ± 0.7%. *Salicornia perennans* was harvested in the Kyzylorda region (Kazakhstan), washed, oven-dried at 45 °C, and ground to <250 µm powder. All ingredients were mixed in a vacuum mixer (Stephan UMC-5, Kreuzau, Germany) until homogeneous. The pâté batter was filled into aluminum cans (100 g portions), hermetically sealed, and sterilized in an autoclave (125 °C, F_0 = 4.2 min). The cans were cooled to 25 °C and stored at 20 ± 2 °C for up to 10 days to assess oxidative and color stability.

2.3. Physicochemical Analyses

Moisture, crude protein, fat, and ash contents were determined according to AOAC (2019) [30] methods: oven drying (950.46), Kjeldahl (981.10), Soxhlet extraction (960.39), and muffle incineration (920.153), respectively. Carbohydrates were calculated by difference. pH was measured in 1:10 homogenates using a Hanna HI 2211 pH meter. Water activity (a_p) was assessed by Novasina LabMaster-aw at 25 °C. Buffering capacity was determined titrimetrically with 0.1 mol L^{-1} HCl until pH 5.0. Centrifugal syneresis was calculated after centrifugation at 3000× *g* for 10 min at 4 °C as the ratio of expressed liquid to total sample mass.

2.4. Texture Profile Analysis

Texture parameters (hardness, cohesiveness, springiness, gumminess, chewiness, adhesiveness) were measured at 10 °C using a TA.XTplus Texture Analyzer (Stable Micro Systems, Godalming, UK) with a 50 mm compression platen (50% deformation, 1 mm/s, two-cycle test). Each value represents the mean of three replicates.

2.5. Amino Acid Analysis

Samples (2 g) were hydrolyzed with 6 mol L^{-1} HCl at 110 °C for 24 h under nitrogen. After evaporation and dissolution, amino acids were quantified using a Hitachi L-8900 amino acid analyzer (Hitachi, Zurich, Switzerland) with post-column ninhydrin derivatization (570/440 nm). Tryptophan was determined after alkaline hydrolysis (4 mol L^{-1} NaOH, 110 °C, 16 h). Results were expressed as g/100 g product.

2.6. SDS–PAGE Analysis

Protein profiles were examined by SDS–PAGE following Laemmli (1970) [31] with 12% resolving and 4% stacking gels. Samples (2 mg protein/mL) were solubilized in Tris–HCl buffer (62.5 mmol L^{-1}, pH 6.8) containing 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.01% bromophenol blue, then heated at 95 °C for 5 min. Electrophoresis was performed at 120 V. Gels were stained with 0.1% Coomassie Brilliant Blue R-250, destained, and compared with a Precision Plus Protein™ Standard (Bio-Rad, Hercules, CA, USA) covering 10–250 kDa.

2.7. Fatty Acid Analysis

Total lipids were extracted using the Folch method, methylated with 14% BF_3–methanol, and analyzed by GC-FID (Agilent 7890A, Santa Clara, CA, USA) on a DB-23 column (60 m × 0.25 mm × 0.25 µm). Fatty acid methyl esters (FAMEs) were identified by comparison with Supelco 37 Component FAME Mix standards. Data were expressed as % of total FA.

2.8. Lipid and Protein Oxidation

Peroxide value (PV) was measured iodometrically (ISO 3960:2017 [32]), acid value (AV) by titration (ISO 660:2020 [33]), and TBARS spectrophotometrically at 532 nm (mg MDA kg^{-1}). Protein carbonyls were quantified by the DNPH method (2,4-dinitrophe-

nylhydrazine, absorbance at 370 nm), expressed as nmol carbonyls mg^{-1} protein. Measurements were performed at 0, 2, 4, 6, 8, and 10 days of storage under accelerated post-opening conditions: after sterilization the cans were opened and the pâtés were held under aerobic exposure to air (simulating open storage after first use).

2.9. Color and Light Stability

Color parameters (L, a, b) were measured using a Konica Minolta CM-2300d spectrophotometer (Tokyo, Japan) (D65 illuminant, 10° observer). Each value is the mean of five readings. Light exposure tests were conducted under 1000 lx fluorescent light for 24 h at 4 °C, after which residual color intensity was expressed as % of initial (color stability).

2.10. Statistical Analysis

All analyses were conducted in triplicate (n = 3). Data are presented as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test in GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Properties

The physicochemical data are presented in Table 2.

Table 2. Composition and physicochemical properties of canned canine meat pates.

Metric	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
Moisture (%)	76.4 ± 0.3 [a]	76.1 ± 0.4 [a]
Crude protein (%)	8.8 ± 0.2 [a]	9.2 ± 0.2 [a]
Crude fat (%)	5.2 ± 0.2 [a]	5.0 ± 0.2 [a]
Ash (%)	2.10 ± 0.07 [a]	2.14 ± 0.06 [a]
Carbohydrate (%)	7.5 ± 0.4 [a]	7.6 ± 0.4 [a]
pH	6.31 ± 0.03 [a]	6.34 ± 0.03 [a]
a_w	0.983 ± 0.001 [a]	0.975 ± 0.001 [a]
Buffering capacity (mmol $H^+ \cdot kg^{-1} \cdot pH^{-1}$)	790 ± 30 [a]	510 ± 25 [b]
ME (metabolizable energy, kcal·100 g^{-1}; Atwater mod.)	101 ± 3 [a]	102 ± 3 [a]
Centrifugal syneresis (% *w*/*w*) *	3.1 ± 0.4 [a]	1.8 ± 0.3 [b]

Different superscript letters ([a,b]) in a row indicate significant differences ($p < 0.05$). * 3000× *g*, 10 min, 4 °C; % of expressed liquid relative to sample mass.

With the addition of 3% peptide hydrolysate (1:1:1 sheep–camel–bovine) and 1% *Salicornia perennans* extract with 1% NaCl, the bulk composition of canned canine pâtés remained essentially unchanged moisture 76.4 → 76.1%, crude fat 5.2 → 5.0%, ash 2.10 → 2.14%, pH 6.31 → 6.34. Metabolizable energy calculated by the modified Atwater method was similar between samples 101 ± 3 vs. 102 ± 3 kcal·100 g^{-1}; $p > 0.05$, while crude protein showed a modest increase 8.8 → 9.2%. Functionally, the treatment exhibited a slightly lower water activity (a_w) 0.983 → 0.975; $p > 0.05$ and a significant reduction in centrifugal syneresis 3.1 ± 0.4% → 1.8 ± 0.3%; $p < 0.05$, confirming enhanced water-holding capacity. This improvement likely stems from collagen-derived short peptides that strengthen water immobilization and minimize free exudate within the heat-set matrix. The modest a_w decrease reflects more effective water partitioning rather than dehydration, aligning with previous observations in high-moisture retorted meat systems [34–36]. In parallel, Salicornia extract contributed phenolic and mineral compounds that enhanced

protein–water interactions and reduced ionic strength, complementing the hydrolysate's gel-stabilizing and antioxidant effects [37–39]. Taken together, the hydrolysate + Salicornia treatment exhibited lower a_w 0.983 → 0.975; $p > 0.05$ and markedly lower centrifugal syneresis 3.1 ± 0.4% → 1.8 ± 0.3%; $p < 0.05$, indicating the improved water stability of the cooked matrix (with WHC inferred from syneresis). This metrics-based outcome is consistent with contemporary evidence on techno-functional peptides and halophyte-based sodium replacers [40].

3.2. Texture Profile

The texture parameters of the canned canine pâtés are summarized in Table 3.

Table 3. Texture profile (10 °C), mean ± SD (n = 3).

Parameter	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
Hardness (N)	32.1 ± 1.8 [a]	29.4 ± 1.5 [b]
Cohesiveness (–)	0.41 ± 0.02 [a]	0.46 ± 0.02 [b]
Springiness (mm)	4.9 ± 0.2 [a]	5.0 ± 0.2 [a]
Gumminess (N)	13.2 ± 0.9 [a]	13.6 ± 0.8 [a]
Chewiness (N·mm)	64.7 ± 4.1 [a]	68.0 ± 3.9 [a]
Adhesiveness (mJ)	0.82 ± 0.06 [a]	0.68 ± 0.05 [b]

Different superscript letters ([a,b]) in a row indicate significant differences between treatments ($p < 0.05$).

Incorporation of 3% peptide hydrolysate and 1% *Salicornia perennans* extract produced a measurable effect on several key rheological characteristics. The hardness of the experimental sample decreased from 32.1 ± 1.8 N to 29.4 ± 1.5 N ($p < 0.05$), suggesting a slightly softer and more spreadable matrix. Practically, the slightly lower hardness with higher cohesiveness may facilitate bolus formation for senior dogs and small breeds, while lower adhesiveness can improve owner handling/serving. These implications should be verified in age/size-stratified palatability tests. Although textural changes (lower hardness, lower adhesiveness, higher cohesiveness) are consistent with improved oral processing and handling, direct palatability or acceptance testing in dogs was not performed here. The present work was intentionally scoped as a technological and physico-chemical validation under sodium reduction (processability, oxidative stability, water retention), and we did not have ethical approval or animal-housing resources to run paired-preference or intake trials. Controlled feeding/acceptance tests in dogs, including age- and size-stratified panels, are therefore planned as the next step to confirm sensory relevance. This softening can be attributed to the presence of short-chain collagen peptides, which increase water retention and reduce the rigidity of the protein gel network by interfering with myofibrillar cross-linking during heat treatment [41–43]. Conversely, cohesiveness increased significantly 0.41 → 0.46; $p < 0.05$, indicating improved internal bonding strength and elasticity of the pâté matrix. The enhanced cohesiveness likely results from hydrogen bonding and electrostatic interactions between collagen-derived peptides and muscle proteins, which promote a fine and uniform gel structure [44]. Springiness, gumminess, and chewiness values remained statistically unchanged ($p > 0.05$), demonstrating that the hydrolysate did not compromise the overall viscoelastic integrity of the product. A significant decrease in adhesiveness 0.82 → 0.68 mJ; $p < 0.05$ was also observed in the hydrolysate + Salicornia formulation. Reduced adhesiveness indicates lower stickiness and better handling properties, which may reflect improved protein–water interactions and a more balanced fat–emulsion structure stabilized by the bioactive salt substitute [45,46]. The slightly lower hardness coupled with greater cohesiveness and reduced adhesiveness suggest that the combined addition of collagen hydrolysate and Salicornia enhanced the plasticity and homogeneity

of the pâté matrix. These findings align with previous studies showing that enzymatic hydrolysates from animal by-products improve moisture distribution, gel strength, and sensory texture in meat emulsions [47]. Furthermore, phenolic and mineral constituents of *Salicornia perennans* are known to stabilize emulsions and modulate ionic strength, contributing to a more cohesive and less brittle structure [48]. Overall, the synergistic use of collagen peptides and Salicornia extract improved the technological texture of dog pâtés while supporting sodium reduction and antioxidant protection.

3.3. Amino-Acid Profile and SDS–PAGE

Table 4 summarizes the amino acid composition of the control and experimental canned canine pâtés. The inclusion of 3% collagen hydrolysate and 1% *Salicornia perennans* extract resulted in modest but meaningful changes in the amino acid pattern.

Table 4. Amino acid composition of canned canine meat pâtés (as-fed basis, g/100 g product; mean ± SD, n = 3).

Amino Acid	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
Aspartic acid	2.23 ± 0.32 ^{b}	2.97 ± 0.43 ^{a}
Glutamic acid	3.86 ± 0.59 ^{a}	3.42 ± 0.51 ^{b}
Serine	0.57 ± 0.09 ^{a}	0.53 ± 0.09 ^{a}
Threonine	0.71 ± 0.11 ^{a}	0.51 ± 0.08 ^{b}
Glycine	2.39 ± 0.35 ^{a}	2.34 ± 0.35 ^{a}
Arginine	2.45 ± 0.37 ^{a}	2.25 ± 0.34 ^{a}
Alanine	2.51 ± 0.38 ^{a}	2.47 ± 0.37 ^{a}
Tyrosine	0.47 ± 0.07 ^{b}	0.55 ± 0.08 ^{a}
Cystine	0.23 ± 0.03 ^{a}	0.22 ± 0.03 ^{a}
Valine	0.76 ± 0.12 ^{a}	0.83 ± 0.13 ^{a}
Methionine	0.19 ± 0.03 ^{b}	0.29 ± 0.04 ^{a}
Isoleucine	0.56 ± 0.09 ^{a}	0.54 ± 0.08 ^{a}
Phenylalanine	0.56 ± 0.08 ^{b}	0.67 ± 0.09 ^{a}
Leucine	1.45 ± 0.22 ^{a}	1.34 ± 0.21 ^{a}
Proline	0.44 ± 0.07 ^{a}	0.40 ± 0.06 ^{a}
Lysine	1.39 ± 0.21 ^{a}	1.22 ± 0.18 ^{a}
Histidine	0.42 ± 0.06 ^{a}	0.33 ± 0.05 ^{b}
Tryptophan	0.18 ± 0.04 ^{b}	0.25 ± 0.05 ^{a}
Hydroxyproline	0.14 ± 0.02 ^{a}	0.10 ± 0.01 ^{b}

Different superscript letters (^{a,b}) in a row indicate significant differences between treatments ($p < 0.05$).

The experimental pâtés exhibited a higher content of aspartic acid +33%, methionine +53%, phenylalanine +20%, and tryptophan +39% compared with the control. The observed increases in methionine (+53%) and tryptophan (+39%) represent a nutritionally favorable trend, as these essential amino acids are often limiting factors in meat-based formulations. Their enrichment suggests improved protein quality and potential antioxidant and metabolic benefits for canine health. Elevated methionine and phenylalanine levels suggest improved nutritional balance and antioxidant potential, since both act as electron donors in radical scavenging systems [49,50]. A slight reduction in histidine −21% and lysine −12% was observed, possibly due to partial Maillard-type interactions during retort sterilization, as these residues are most reactive under heat treatment [51]. The increase in tryptophan may indicate the stabilization of aromatic amino acids by Salicornia polyphenols, which can protect indole groups against oxidative degradation [52]. The presence of *Salicornia perennans* extract likely also contributed to the preservation of sulfur-containing amino acids (methionine, cystine) via metal-chelating and antioxidant mechanisms, re-

ducing protein carbonylation observed during storage. This aligns with findings that halophyte-derived extracts enhance protein stability and nutritional amino acid retention in low-sodium meat systems [44,53]. Electrophoretic profiles (Figure 1) confirmed partial hydrolysis of high-molecular-weight myofibrillar proteins and the appearance of bands below 20 kDa in the experimental sample, indicating formation of low-molecular-weight peptides. Similar SDS–PAGE shifts have been reported in collagen hydrolysate–fortified meat emulsions, where peptide fragments increase solubility and improve digestibility without compromising structural integrity [45,54]. Overall, the enrichment of canine pâtés with collagen hydrolysate and *Salicornia perennans* resulted in a more balanced amino acid spectrum, enhanced levels of essential sulfur- and aromatic residues, and improved protein functionality, supporting both nutritional enhancement and technological stability of the product.

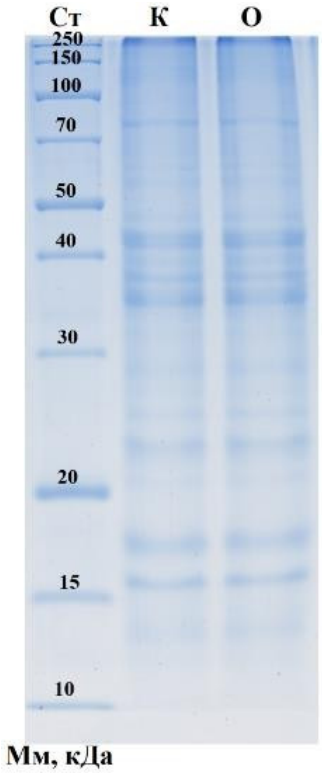


Figure 1. SDS–PAGE patterns of protein fractions in canned canine meat pâtés: C_T—molecular-weight standard 10–250 kDa, K—control sample 2% NaCl, no hydrolysate, O—experimental sample +3% collagen hydrolysate, +1% *Salicornia perennans* extract.

Figure 1 presents the SDS–PAGE protein patterns of the control (K) and experimental (O) canine pâtés enriched with 3% collagen hydrolysate and 1% *Salicornia perennans* extract. The molecular-weight marker (C_t) ranged from 10 to 250 kDa. Both samples exhibited the characteristic muscle protein bands of myosin heavy chain 200 kDa, actin 42 kDa, and tropomyosin 35 kDa; however, the experimental sample demonstrated notable structural alterations.

Specifically, the intensity of high-molecular-weight regions 50–70 kDa decreased, while several additional or more intense bands appeared between 15 and 30 kDa. These differences indicate partial proteolysis of myofibrillar proteins and the formation of soluble peptide fractions derived from the added collagen hydrolysate. The emergence of faint bands below 15 kDa further supports the presence of low-molecular-weight peptides that may possess antioxidant and metal-chelating activity, consistent with previous findings in collagen-enriched meat emulsions [43,55].

The addition of *Salicornia perennans* extract likely contributed to maintaining protein integrity through its phenolic and mineral constituents, which are capable of binding pro-oxidant metal ions and stabilizing sulfhydryl groups. This protective effect helps prevent excessive cross-linking or aggregation during thermal processing [52]. Such stabilization may also explain the lower carbonyl accumulation and reduced hardness observed in the experimental pâtés, as bioactive compounds from halophytes have been shown to attenuate oxidative modification of myofibrillar proteins [44]. In summary, the electrophoretic data confirm that the combined use of collagen hydrolysate and *Salicornia perennans* extract led to controlled proteolytic modification of the protein matrix, resulting in increased solubility, improved digestibility, and enhanced oxidative stability of the dog pâtés. These structural changes complement the improvements observed in texture and lipid oxidation indices described earlier. The suggested contribution of Maillard-type reactions to lower lysine is hypothesized; we did not quantify Maillard markers. Future studies will determine furosine, Nε-carboxymethyl-lysine (CML), HMF, and free/blocked lysine to confirm this pathway.

3.4. Fatty-Acid Profile

The fatty-acid composition of canned canine pâtés is presented in Table 5.

Table 5. Fatty-acid profile of canned canine meat pâtés (mean ± SD, n = 3).

Group/FA	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
SFA (Σ)	56.4 ± 3.1 [a]	52.8 ± 2.9 [b]
C14:0 Myristic	4.5 ± 0.4 [a]	4.1 ± 0.3 [b]
C16:0 Palmitic	27.1 ± 2.1 [a]	25.2 ± 1.9 [b]
C18:0 Stearic	21.2 ± 2.1 [a]	19.0 ± 1.7 [b]
(minor SFA C15:0, C17:0, C20:0–C23:0)	3.6 ± 0.9 [a]	4.5 ± 1.0 [a]
MUFA (Σ)	38.1 ± 2.3 [a]	39.5 ± 2.4 [a]
C18:1 cis (oleic)	30.0 ± 2.1 [a]	31.2 ± 2.2 [a]
C18:1 trans (elaidic)	3.8 ± 0.4 [a]	3.5 ± 0.3 [b]
(other MUFA C15:1, C16:1, C17:1, C20:1, C22:1, C24:1)	4.3 ± 1.0 [a]	4.8 ± 1.1 [a]
PUFA n-3 (Σ)	0.8 ± 0.4 [b]	1.5 ± 0.5 [a]
C18:3 n-3 (ALA)	0.8 ± 0.4 [b]	1.5 ± 0.5 [a]
EPA + DHA	<0.1	<0.1
PUFA n-6 (Σ)	4.5 ± 0.6 [a]	4.2 ± 0.5 [a]
C18:2 n-6 (LA)	4.0 ± 0.4 [a]	3.3 ± 0.3 [b]
(others: C18:2 trans, C20:2, C20:4)	0.5 ± 0.4 [b]	0.9 ± 0.5 [a]
PUFA total (n-3 + n-6)	5.3 ± 0.8 [b]	5.7 ± 0.9 [a]
n-6:n-3 ratio	5.6 ± 0.3 [a]	3.8 ± 0.2 [b]

Different superscript letters ([a,b]) in a row indicate significant differences between treatments (one-way ANOVA followed by Tukey's HSD; $p < 0.05$).

The experimental pâtés exhibited a reduction in total saturated fatty acids (SFAs) 56.4 → 52.8%; $p < 0.05$ primarily due to lower palmitic and stearic acids, which are often associated with oxidative instability and high melting points. Although higher n-3 PUFA increases oxidative susceptibility, mitigation was evidenced by lower PV, lower TBARS, and lower carbonyls versus control during storage. This likely reflects the combined action of peptide chelation/radical quenching and Salicornia phenolics, as well as stable emulsification that limits pro-oxidant contact with lipids. Simultaneously, the n-3 PUFA content nearly doubled 0.8 → 1.5%, leading to a more favorable n-6:n-3 ratio 5.6 → 3.8. Key *Salicornia perennans* constituents implicated in antioxidant protection include isorhamnetin glycosides, ferulic, caffeic, and chlorogenic acids, as well as minerals such as Mg and Zn,

which contribute to redox homeostasis and metal chelation [56–59]. The mild reduction in linoleic acid C18:2 n-6 and increase in α-linolenic acid C18:3 n-3 suggest that *Salicornia perennans* extract, rich in phenolics and microelements Mg, Zn, Fe, may have slowed PUFA peroxidation during sterilization. Similar protective effects have been reported for halophyte-derived extracts in low-sodium meat systems [48]. The collagen hydrolysate component likely contributed small bioactive peptides with metal-chelating and radical-scavenging properties, which suppressed lipid oxidation and preserved unsaturated fatty acids throughout processing and storage [60]. Moreover, collagen peptides may enhance emulsifying stability and lipid–protein interactions, improving lipid dispersion and reducing coalescence in the pâté matrix [61]. Future work will couple composition with microstructural imaging (CLSM/SEM) and emulsion stability indices (creaming index, droplet size by laser diffraction) to mechanistically link peptide–polyphenol interactions to lipid protection. An increase in monounsaturated fatty acids (MUFAs, mainly oleic acid) in the experimental formulation also supports improved lipid stability. Oleic acid has been recognized for its oxidative resilience and positive effect on the palatability of pet foods [62]. The reduction in trans-elaidic acid 3.8 → 3.5% of total FA; $p < 0.05$ is small in absolute terms and thus unlikely to be nutritionally impactful on its own, but it is directionally consistent with milder isomerization during processing and a modest improvement in lipid quality. Collectively, these findings confirm that the synergistic addition of collagen hydrolysate and *Salicornia perennans* extract not only mitigated lipid oxidation but also enhanced the nutritional lipid profile of dog pâtés—lowering SFAs, increasing n-3 PUFAs, and optimizing the n-6:n-3 ratio, thereby supporting both technological quality and potential health benefits for companion animals.

3.5. Dynamics of Lipid and Protein Oxidation During Storage

The oxidative stability of the canned canine pâtés was evaluated over 10 days of storage by monitoring peroxide value (PV) and protein carbonyl concentration (Table 6).

Table 6. Dynamics of lipid and protein oxidation in meat pâtés for dogs during storage (mean ± SD, n = 3).

Parameter	Day	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
Peroxide value, meq/kg	0	3.8 ± 0.4 ^{a}	3.5 ± 0.4 ^{a}
	2	4.1 ± 0.4 ^{a}	4.3 ± 0.4 ^{a}
	4	5.9 ± 0.3 ^{a}	5.5 ± 0.3 ^{b}
	6	7.1 ± 0.4 ^{a}	7.0 ± 0.4 ^{a}
	8	10.1 ± 0.5 ^{a}	9.7 ± 0.5 ^{b}
	10	14.0 ± 0.9 ^{a}	13.0 ± 0.9 ^{b}
Carbonyl compounds, nmol/mg protein	0	99.19 ± 4.1 ^{a}	95.73 ± 3.9 ^{a}
	2	112.5 ± 5.0 ^{a}	105.2 ± 4.7 ^{b}
	4	130.6 ± 5.8 ^{a}	119.0 ± 5.2 ^{b}
	6	151.2 ± 6.7 ^{a}	135.4 ± 5.9 ^{b}
	8	180.5 ± 8.1 ^{a}	158.3 ± 6.8 ^{b}
	10	215.3 ± 9.6 ^{a}	185.9 ± 8.1 ^{b}

Different superscript letters (^{a,b}) in a row indicate significant differences between treatments (one-way ANOVA followed by Tukey's HSD; $p < 0.05$).

At the end of storage, the peroxide value of the control sample increased 3.7-fold from 3.8 to 14.0 meq·kg^{-1}, while that of the hydrolysate + Salicornia sample rose only 3.4-fold from 3.5 to 13.0 meq·kg^{-1}. Similarly, the concentration of protein carbonyls—an indicator of oxidative modification of amino acid side chains—was 14% lower in the exper-

imental pâtés after 10 days 185.9 vs. 215.3 nmol·mg^{-1} protein. The improved oxidative resistance can be attributed to several mechanisms. Collagen-derived peptides act as metal chelators and hydrogen donors, scavenging lipid radicals and interrupting peroxidation chains [42]. Moreover, the phenolic constituents of *Salicornia perennans* provide additional reducing power, complementing peptide antioxidants and regenerating tocopherols or other lipid-soluble antioxidants [55]. The synergistic antioxidant action of peptides and plant polyphenols has been repeatedly demonstrated to lower both lipid and protein oxidation in low-sodium meat systems [43]. We note that the peroxide value increased rapidly within the first 10 days, reaching 14 meq·kg^{-1} in the control. This apparent 'fast' increase is consistent with early accumulation of primary lipid hydroperoxides in high-moisture, retorted meat emulsions that contain endogenous pro-oxidant metals and residual heme components. Peroxide value in meat systems is known to be highly dynamic and matrix-dependent; in some products (e.g., fermented sausages with lower water activity and ongoing reductive reactions) PV can even decrease during ripening because hydroperoxides are decomposed to secondary aldehydes and ketones rather than accumulated, as has been reported in the literature [63]. In our pâté model, the concurrent rise in TBARS (Table 7) supports this classic conversion of primary to secondary oxidation products. Importantly, despite the overall upward trend with storage time ($p < 0.05$, ANOVA with 'day' as factor), the Treatment sample consistently maintained lower PV and lower protein carbonyls than the Control at each sampling point, indicating a protective effect of the collagen hydrolysate + Salicornia system. Overall, the incorporation of collagen hydrolysate and *Salicornia perennans* extract effectively retarded lipid peroxidation and protein carbonylation, improving the shelf-life stability and functional integrity of canine pâtés. This combined approach appears promising for developing clean-label, sodium-reduced pet foods with enhanced oxidative protection.

Table 7. Dynamics of lipid oxidation in meat pâtés for dogs during storage based on thiobarbituric acid value (mean ± SD, n = 3).

Parameter	Day	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
Thiobarbituric acid value, mg MDA/kg	0	<0.039 ^{a}	<0.039 ^{a}
	2	<0.039 ^{a}	<0.039 ^{a}
	4	0.117 ± 0.012 ^{a}	0.140 ± 0.014 ^{a}
	6	1.719 ± 0.120 ^{a}	0.766 ± 0.054 ^{b}
	8	3.289 ± 0.230 ^{a}	1.910 ± 0.134 ^{b}
	10	4.865 ± 0.315 ^{a}	2.673 ± 0.189 ^{b}

Different lowercase letters (^{a,b}) in the same row indicate statistically significant differences between samples ($p < 0.05$).

3.6. Thiobarbituric Acid Reactive Substances (TBARSs)

The dynamics of secondary lipid oxidation products in canned canine pâtés were assessed by the thiobarbituric acid reactive substances (TBARS) assay (Table 7).

In both formulations, the TBARS values remained below the detection limit <0.039 mg MDA·kg^{-1} up to the second day of storage, indicating negligible oxidative degradation at the initial stage. However, after the sixth day, the control sample showed a sharp increase to 1.72 mg MDA·kg^{-1}, while the hydrolysate + Salicornia formulation exhibited a significantly lower value of 0.77 mg MDA·kg^{-1} ($p < 0.05$). By the tenth day, MDA accumulation in the control reached 4.87 mg·kg^{-1}—1.8 times higher than in the experimental pâté 2.67 mg·kg^{-1}. This substantial difference highlights the synergistic antioxidant effect of collagen-derived peptides and *Salicornia perennans* bioactives. The hydrolysate

likely contributed low-molecular-weight peptides with hydrophobic and aromatic residues capable of donating protons to lipid radicals and interrupting peroxidation chains [64]. Simultaneously, Salicornia extract, rich in phenolic compounds and minerals such as Zn and Mg, may have stabilized the lipid phase through metal chelation and radical scavenging [55]. These findings are consistent with reports that peptide–polyphenol interactions can regenerate antioxidant potential during heat processing, leading to sustained suppression of TBARS formation over storage [44]. Similar protective effects were also observed in other meat systems with reduced sodium content, where halophyte extracts decreased oxidative degradation and improved color stability [65]. In cooked meat systems, human sensory detection of rancidity is often reported around 1–2 mg MDA·kg^{-1}, depending on matrix and volatiles; dogs rely more on aroma than color, and increased TBARS is associated with stronger oxidized odors. By day 10, the control exceeded this range 4.87 mg·kg^{-1}, whereas the treatment remained lower 2.67 mg·kg^{-1}, suggesting reduced risk of rancid odor development and potentially better palatability/acceptability, though direct feeding confirmation is needed.

3.7. Dynamics of Lipid Oxidation Based on Acid Value

Acid value (AV) reflects the accumulation of free fatty acids formed during triglyceride hydrolysis and lipid oxidation. This parameter provides a sensitive indication of fat degradation processes occurring throughout product storage. The results presented in Table 8 demonstrate that acid values increased gradually in both control and experimental pâtés during storage, yet the inclusion of 3% collagen hydrolysate and 1% *Salicornia perennans* extract significantly retarded this increase.

Table 8. Dynamics of lipid oxidation in meat pâtés for dogs during storage based on acid value (mean ± SD, n = 3).

Parameter	Day	Control (No Hydrolysate, 2% NaCl)	Treatment (+3% Hydrolysate, 1% Salicornia, 1% NaCl)
Acid value, mg KOH/g	0	2.4 ± 0.2 ^{a}	2.0 ± 0.2 ^{a}
	2	3.0 ± 0.2 ^{a}	2.6 ± 0.2 ^{b}
	4	3.6 ± 0.3 ^{a}	3.1 ± 0.2 ^{b}
	6	4.2 ± 0.3 ^{a}	3.9 ± 0.3 ^{b}
	8	6.5 ± 0.5 ^{a}	5.2 ± 0.3 ^{b}
	10	8.5 ± 0.6 ^{a}	7.0 ± 0.5 ^{b}

Different lowercase letters (^{a,b}) in the same row indicate statistically significant differences between samples ($p < 0.05$).

At the beginning of storage, the acid values were comparable between treatments 2.4 vs. 2.0 mg KOH·g^{-1}, $p > 0.05$. After 10 days, the control pâté reached 8.5 mg KOH·g^{-1}, whereas the sample containing collagen hydrolysate and Salicornia extract showed a significantly lower value of 7.0 mg KOH·g^{-1} ($p < 0.05$). This reduction of approximately 18% demonstrates the capacity of the combined additives to suppress hydrolytic and oxidative lipid degradation. The protective mechanism may be linked to peptide–lipid interactions within the emulsified matrix. Collagen hydrolysate peptides—particularly those rich in glycine, proline, and hydroxyproline—have strong affinity for lipid interfaces, where they can stabilize emulsions and inhibit the catalytic activity of pro-oxidant metal ions [42]. Meanwhile, *Salicornia perennans* extract contains natural antioxidants capable of scavenging peroxyl radicals and preventing the propagation of lipid hydroperoxides [53]. A synergistic effect between collagen peptides and Salicornia polyphenols likely contributed to slower lipid cleavage and reduced formation of free fatty acids. Comparable trends were

observed in reduced-sodium meat emulsions where halophyte-based salt replacers limited lipolytic enzyme activity and improved storage stability [56]. Furthermore, the lower acid values are consistent with TBARS and peroxide results discussed above, supporting a multi-mechanistic antioxidant action involving metal chelation, radical scavenging, and emulsifying stabilization [41]. Overall, the data indicate that supplementation with collagen hydrolysate and *Salicornia perennans* extract effectively delayed lipid hydrolysis, resulting in improved oxidative stability and prolonged shelf-life of the canned canine pâtés without compromising sensory or textural properties.

3.8. Color Characteristics and Stability

Color stability is a key quality attribute of canned meat products, as oxidative discoloration directly affects consumer perception and product acceptability. Table 9 summarizes the dynamics of color parameters (L*, a*, b*) and the percentage of color retention in control and experimental dog pâtés during 10 days of storage.

Table 9. Dynamics of color characteristics and stability of meat pâtés for dogs during storage (mean ± SD, n = 3).

Day	Sample	L* (Lightness)	a* (Redness)	b* (Yellowness)	Color Stability, %
0	Control (no hydrolysate, 2% NaCl)	58.37 ± 0.44 ^{a}	7.38 ± 0.13 ^{a}	25.31 ± 0.35 ^{a}	100.0
	Treatment (+3% hydrolysate, 1% Salicornia, 1% NaCl)	51.20 ± 0.51 ^{b}	7.48 ± 0.58 ^{a}	24.35 ± 0.83 ^{a}	100.0
2	Control (no hydrolysate, 2% NaCl)	58.10 ± 0.43 ^{a}	7.25 ± 0.18 ^{a}	25.42 ± 0.37 ^{a}	99.3
	Treatment (+3% hydrolysate, 1% Salicornia, 1% NaCl)	51.15 ± 0.50 ^{b}	7.40 ± 0.54 ^{a}	24.45 ± 0.81 ^{a}	99.8
4	Control (no hydrolysate, 2% NaCl)	57.82 ± 0.46 ^{a}	7.11 ± 0.15 ^{b}	25.62 ± 0.36 ^{a}	97.2
	Treatment (+3% hydrolysate, 1% Salicornia, 1% NaCl)	51.05 ± 0.49 ^{b}	7.32 ± 0.51 ^{a}	24.61 ± 0.79 ^{a}	98.6
6	Control (no hydrolysate, 2% NaCl)	57.40 ± 0.47 ^{a}	6.97 ± 0.20 ^{b}	25.81 ± 0.39 ^{a}	96.3
	Treatment (+3% hydrolysate, 1% Salicornia, 1% NaCl)	51.00 ± 0.50 ^{b}	7.25 ± 0.50 ^{a}	24.78 ± 0.77 ^{a}	97.8
8	Control (no hydrolysate, 2% NaCl)	57.12 ± 0.48 ^{a}	6.85 ± 0.22 ^{b}	25.98 ± 0.40 ^{a}	95.4
	Treatment (+3% hydrolysate, 1% Salicornia, 1% NaCl)	50.98 ± 0.52 ^{b}	7.18 ± 0.49 ^{a}	24.90 ± 0.75 ^{a}	97.2
10	Control (no hydrolysate, 2% NaCl)	56.80 ± 0.50 ^{a}	6.73 ± 0.25 ^{b}	26.10 ± 0.42 ^{a}	94.8
	Treatment (+3% hydrolysate, 1% Salicornia, 1% NaCl)	50.95 ± 0.53 ^{b}	7.10 ± 0.49 ^{a}	24.95 ± 0.76 ^{a}	96.8

Different superscript letters (^{a,b}) in a column indicate significant differences between treatments at the same storage day ($p < 0.05$).

The experimental pâtés containing collagen hydrolysate and *Salicornia perennans* maintained significantly ($p < 0.05$) higher redness (a*) and overall color stability compared with the control throughout 10 days of storage. The a* value in the experimental sample decreased only slightly from 7.48 to 7.10 (−5.1%), whereas the control showed a more pronounced decline from 7.38 to 6.73 (−8.8%). The color stability coefficient decreased by approximately 3.2% in the experimental sample versus 5.2% in the control, indicating stronger pigment protection against oxidative bleaching. The L* (lightness) parameter remained nearly constant in the hydrolysate + Salicornia group, while a gradual lightness reduction in the control suggested pigment oxidation and myoglobin denaturation. These improvements are attributed to synergistic antioxidant mechanisms: collagen hydrolysate contributes low-molecular-weight peptides capable of scavenging free radicals and reducing metmyoglobin formation, thereby stabilizing heme pigments [66]. Concurrently, phenolic and mineral components of *Salicornia perennans* serve as natural color protectants, mitigating lipid–protein oxidative interactions responsible for discoloration [67]. Similar halophyte-based extracts have been shown to delay chromatic deterioration by suppressing

TBARS and peroxide accumulation, preserving the redness index during storage [68]. Additionally, peptides enhance water-holding capacity and microstructural uniformity, which minimizes surface oxidation and maintains the characteristic color tone of the pâté [69]. While dogs prioritize olfaction, appearance remains a purchase-driver for owners and is used as a proxy for freshness/quality. Moreover, better color retention here paralleled lower TBARS/PV, indicating genuine oxidative protection rather than purely cosmetic improvement—outcomes that can indirectly support palatability through odor preservation. Overall, the combined use of collagen hydrolysate and *Salicornia perennans* extract effectively stabilized color and delayed oxidative fading in canned canine pâtés, thereby improving their visual quality, consumer acceptability, and shelf-life stability.

4. Conclusions

The collagen-hydrolysate + Salicornia system improved process robustness and oxidative control in sodium-reduced pâtés while preserving baseline composition and feeding dosage. Practically, this supports line compatibility (no change to retort schedules), predictable yield (lower purge), and consistent shelf appearance. Texture became slightly softer yet more cohesive with less stickiness—features likely helpful for small and senior dogs; lower oxidation should better preserve aroma and, thus, palatability. For owners, stable color and a short, recognizable ingredient list are key purchase drivers. We classify the product as "clean-label" in the absence of synthetic antioxidants, nitrite, added colorants, and phosphates; use of botanical sodium reduction (Salicornia); a concise, familiar ingredient list; and the upcycling of animal by-products. These attributes mainly influence owners' decisions, while dogs' preference is driven primarily by odor/flavor—indirectly supported here via improved oxidative stability.

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