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Enhancing and predicting the microbial and oxidative stability of ostrich meat using chia mucilage–based edible coatings enriched with *Lactococcus lactis* LBM15 supernatant and SVR model

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6 **Abstract**

7 Chia seed mucilage coatings enriched with cell-free supernatant (CFS) from *Lactococcus lactis*

8 LBM15 effectively delayed spoilage of refrigerated ostrich meat for 10 days. Compared with the

9 control, the 2% CFS coating notably limited microbial growth and oxidative spoilage: total viable

10 counts increased to 9.50 ± 0.14 log CFU/g in the control but to only 6.80 ± 0.11 log CFU/g with

11 the 2% CFS treatment. Similarly, peroxide values rose to 7.85 ± 0.13 meq O_2/kg in the control but

12 remained at 3.10 ± 0.09 meq O_2/kg with the 2% CFS. Color retention was improved, with smaller

13 declines in redness and a reduced overall color difference (ΔE). Sensory evaluations for odor,

14 color, texture, and overall acceptance were also higher in coatings enriched with CFS. Meat slices

15 were treated with a chia coating alone or with 1% or 2% CFS, along with an untreated control, and

16 assessed for microbial counts, lipid oxidation, color metrics, texture, pH, moisture, and sensory

17 attributes. Overall, combining chia mucilage with *L. lactis* CFS shows potential as a clean-label

18 method to extend the shelf life of high-value ostrich meat. This study investigates the application

19 of Support Vector Regression (SVR) to the simultaneous prediction of 12 key laboratory

20 parameters. Model performance was evaluated using Mean Absolute Percentage Error (MAPE)

21 and coefficient of determination (R^2). Results demonstrated strong predictive performance, with

22 test R^2 values ranging from 0.93 to 0.99 and MAPEs below 8% across all targets, confirming

23 SVR's effectiveness in modeling complex, nonlinear relationships in food-quality data.

24 **Keywords:** Chia seed mucilage, *L. lactis* LBM15**,** Support Vector Regression (SVR).

25
26 **1. Introduction**

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27 Ostrich meat has gained increasing attention as a premium red meat due to its low intramuscular
28 fat content, favorable fatty acid profile, and high protein value, making it appealing to health-
29 conscious consumers (Arain et al., 2025). However, its relatively high ultimate pH ($\approx$6.0–6.4) and
30 nutrient-rich matrix promote microbial growth, lipid oxidation, discoloration, and moisture loss
31 during refrigerated storage, leading to rapid spoilage and a limited shelf life (Liu et al., 2025). In
32 response to the growing demand for minimally processed foods, natural and biodegradable
33 preservation strategies have gained attention as alternatives to synthetic additives (He et al.,
34 2025a). Among these, edible coatings are an effective approach for enhancing meat stability by
35 forming thin, semi-permeable barriers that control oxygen transfer, moisture loss, and microbial
36 activity (Chen et al., 2023).

37 Polysaccharide-based edible coatings have been extensively studied due to their biodegradability,
38 safety, and strong film-forming properties, which reduce oxygen diffusion, limit moisture loss,
39 and delay microbial and oxidative spoilage (Hashemi et al., 2023). Among plant-derived
40 hydrocolloids, chia seed mucilage has gained attention because of its high viscosity, water-binding
41 capacity, and dense polysaccharide structure, enabling the formation of stable and cohesive films
42 on food surfaces (Muñoz-Tebar et al., 2021). Its application in fish, poultry, and red meat has been
43 linked to reduced microbial growth, delayed lipid oxidation, and improved surface hydration
44 during refrigerated storage (Ferraro et al., 2022). Recently, coating functionality has been further
45 enhanced through the incorporation of bioactive compounds such as postbiotics—cell-free
46 supernatants from lactic acid bacteria (LAB) containing antimicrobial and antioxidant metabolites
47 (He et al., 2025b). Among LAB species, *L. lactis* is particularly effective due to its production of
48 nisin-like compounds that are active against spoilage and pathogenic microorganisms in meat
49 systems (Ben Said et al., 2019). Accordingly, edible films enriched with LAB-derived supernatants
50 have been shown to improve microbial safety and sensory quality of refrigerated meat products,
51 supporting their use as clean-label preservation strategies (Barcenilla et al., 2022).

52 Despite these advances, significant research gaps remain. Most previous studies have focused on
53 traditional meats such as beef, poultry, and fish. In contrast, ostrich meat, despite its unique
54 physicochemical properties and increased susceptibility to oxidation and microbial spoilage, has
55 received limited research attention. Although chia seed mucilage has demonstrated effective
56 barrier and water-retention qualities in various food systems, its use as a carrier matrix for LAB-
57 derived cell-free supernatants has yet to be explored in ostrich meat. Furthermore, past studies

58 have typically assessed biopolymer coatings or microbial metabolites separately, offering limited
59 insight into their potential synergistic effects within a combined coating system. Additionally,
60 reliance on univariate statistical analyses has limited the ability to fully understand the complex
61 and interconnected changes among microbial, physicochemical, color, and sensory attributes
62 during refrigerated storage. Ensuring consistent food quality and safety remains a key challenge
63 in the modern food industry (Ghazanfari et al., 2025; Chen et al., 2022). Consequently, advanced
64 analytical approaches such as machine learning have gained increasing attention for modeling
65 complex, nonlinear relationships in food systems. Support Vector Regression (SVR), in particular,
66 has demonstrated strong predictive performance across multiple quality-related parameters,
67 particularly in datasets with multicollinearity and limited sample sizes (Jooyandeh Kar et al., 2025;
68 Namazi et al., 2025). Integrating machine learning with experimental preservation studies provides
69 a robust framework for improving quality prediction, optimizing processing conditions, and
70 supporting data-driven decision-making in food production (Rahmati-Joneidabad et al., 2025).
71 Accordingly, the novelty of the present study lies in the combined use of chia seed mucilage as a
72 biopolymer carrier and LAB-derived cell-free supernatant as a natural postbiotic system for
73 preserving ostrich meat, together with the integration of machine learning (SVR) to simultaneously
74 model multiple quality attributes during refrigerated storage.

75 This study examines the impact of chia seed mucilage–based edible coatings enriched with 1%
76 and 2% cell-free supernatant from *L. lactis* LBM15 on microbial stability, oxidative changes,
77 physicochemical properties, color, and sensory quality of ostrich meat during refrigerated storage.
78 Furthermore, the applicability of SVR modeling is evaluated for the simultaneous prediction of
79 multiple quality-related laboratory parameters, offering both a natural preservation method and a
80 predictive tool for assessing the quality of high-value meat products.
81
82 **2. Materials and Methods**
83 **2.1. Materials**
84 Chia seeds (food grade) were sourced from a local supplier. Plate Count Agar (PCA), Violet Red
85 Bile Agar (VRBA), Sabouraud Dextrose Agar (SDA), and peptone water were purchased from
86 Merck (Darmstadt, Germany). Thiobarbituric acid, trichloroacetic acid, potassium iodide, sodium
87 thiosulfate, and boric acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). All reagents
88 were of analytical grade, and distilled water was used throughout the experiments.

2.2. Preparation of Ostrich Meat

Fresh ostrich (*Struthio camelus*) meat was obtained from a certified slaughter facility and chilled at 4 °C for 24 h. The M. iliofibularis muscle was excised, transported to the laboratory under refrigerated conditions, trimmed of visible fat and connective tissue, and cut into uniform slices (2 cm thickness, 80–90 g). Surface moisture was gently removed, and samples were randomly assigned to treatments. Three independent replicates were prepared for each group (Kiakojori et al., 2024).

2.3. Extraction of Chia Seed Mucilage

Chia seed mucilage was extracted using a hydration–drying method. Seeds were mixed with distilled water (1:20 w/v) and stirred at 50 ± 2 °C for 45 min (100 rpm). The mixture was refrigerated at 4 °C for 8 h, after which the mucilage was separated, dried at 50 °C for 24 h, ground, sieved (25 mesh), and stored in sealed containers at room temperature until use (Muñoz-Tebar et al., 2021).

2.4. Preparation of Cell-Free Supernatant (CFS)

L. lactis LBM15 was cultured in MRS broth at 30 °C for 18–24 h under aerobic conditions. After incubation, cultures were centrifuged at 6,000 × g for 15 min at 4 °C to pellet cells. The supernatant was then carefully collected and passed through a sterile 0.22 µm membrane filter to remove all bacterial cells, yielding the cell-free supernatant (CFS) used in coating formulations. This method has been widely used in previous studies of LAB CFS antimicrobial activity (Manzoor et al., 2016).

2.5. Application of Coatings and Storage Conditions

For coating preparation, chia seed mucilage powder was dissolved in distilled water to obtain a final concentration of 2% (w/v). The solution was stirred until complete hydration and homogeneity before the addition of cell-free supernatant .CFS was incorporated into the chia mucilage solution at concentrations of 0%, 1%, and 2% v/v .Ostrich meat samples were coated using a dip-coating method. Briefly, meat slices were individually immersed in the prepared chia mucilage coating solutions (with or without cell-free supernatant) for 2 minutes to ensure uniform surface coverage. Excess coating solution was allowed to drain under sterile conditions, and samples were placed on sterile racks at 4 °C for 10–15 min to facilitate formation of a thin surface film. Uncoated samples were used as controls. After coating, all samples were packaged and stored

121 under refrigerated conditions for subsequent analyses. Analyses were conducted on days 1, 4, 7,
122 and 10 (Heydari et al., 2020).

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124 **2.6. Microbiological Analysis**
125 For microbiological analysis, 10 g of each meat sample was aseptically homogenized with 90 mL
126 sterile peptone water, and serial decimal dilutions were prepared. Total viable counts,
127 psychrotrophic bacteria, coliforms, and fungi were enumerated using standard plate count methods
128 on selective media after appropriate incubation. Microbial results were expressed as log CFU/g of
129 meat.(Ntzimani et al., 2022).

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131 **2.7. Lipid Oxidation (TBA and PV)**
132 Peroxide value (PV) was determined by iodometric titration. Briefly, 2 g of minced meat was
133 mixed with a chloroform–acetic acid solution, followed by the addition of potassium iodide. The
134 released iodine was titrated with 0.01 N sodium thiosulfate, and results were expressed as meq
135 O_2/kg of meat. Thiobarbituric acid (TBA) values were measured spectrophotometrically as an
136 index of secondary lipid oxidation. Meat samples were homogenized with trichloroacetic acid,
137 reacted with thiobarbituric acid reagent, heated, and absorbance was read at 532 nm. Results were
138 expressed as mg malondialdehyde (MDA)/kg of meat (Domínguez et al., 2019).

139
140 **2.8. Color Measurement**
141 Color parameters (L*, a*, b*) were measured using a CR-400 colorimeter (Konica Minolta, Japan)
142 after calibration with a white standard. Measurements were taken at three randomly selected points
143 on each sample after blooming, and the total color difference (ΔE) was calculated using the CIE
144 equation (Domínguez et al., 2019).

145
146 **2.9. pH Measurement**
147 pH was measured by homogenizing 10 g of minced meat with 90 mL of distilled water and
148 recording values using a calibrated digital pH meter (Dragon Lab, MX-S) at 25 °C (Heydari et al.,
149 2020).

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154 **2.10. Texture analysis (firmness)**

155 Firmness was evaluated using a texture analyzer with a cylindrical probe (50 mm diameter).

156 Samples were compressed to 50% of their original height at a speed of 1 mm/s and a trigger force

157 of 2 N. Results were expressed in Newtons (Zhang et al., 2021).

158
159 **2.11. Total Volatile Basic Nitrogen (TVB-N)**

160 Total volatile basic nitrogen (TVB-N) was measured by steam distillation. Minced meat (10 g)

161 was distilled with magnesium oxide, and the released volatile bases were collected in boric acid

162 and titrated with 0.01 N HCl. Results were expressed as mg nitrogen per 100 g of meat (mg/100

163 g), indicating the extent of protein degradation (Lu et al., 2025).

164
165 **2.12. Moisture Content**

166 Moisture content was measured by oven-drying the sample at 105 °C to constant weight and

167 expressed as a percentage of the initial sample mass (AOAC).

168
169 **2.13. Sensory Evaluation**

170 Sensory evaluation was performed by a trained panel of 15 laboratory staff members. Panelists

171 were trained prior to evaluation and assessed color, odor, texture, and overall acceptability using

172 a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Samples were blind-coded with

173 random three-digit numbers and evaluated under controlled lighting conditions (Civille et al.,

174 2024).

175
176 **2.14. Support Vector Regression (SVR) Framework**

177 Support Vector Regression (SVR) is a supervised learning technique rooted in statistical learning

178 theory, designed to predict continuous outcomes by leveraging the principles of structural risk

179 minimization (Akrom et al., 2025; Su et al., 2019a). Unlike conventional regression approaches

180 that minimize empirical error alone, SVR incorporates an ε-insensitive loss function, which allows

181 deviations within a predefined tolerance margin (ε) without penalization (Namazi et al., 2025; Su

182 et al., 2019b). To capture nonlinear patterns in the data, SVR employs kernel transformations that

183 implicitly project the input variables into a higher-dimensional feature space, where a linear

184 regression function is constructed. Commonly used kernels—such as the Radial Basis Function

185 (RBF)—enable flexible modeling of complex, nonlinear relationships without explicitly

186 computing high-dimensional coordinates (Megersa et al., 2025; Hu et al., 2023). The primal

187 optimization objective in SVR seeks to minimize a trade-off between model complexity and

188 empirical error, regulated by a penalty parameter C. This leads to the following constrained

189 minimization problem (Bolandnazar et al, 2023):

$$\underset{w,b,\xi,\xi^*}{Min} : \left(\frac{1}{2}\|w\|^2 + C\sum_{i=1}^{n}(\xi_i - \xi_i^*) \right) \tag{1}$$

190 Subject to:

$$y_i - \left(w.\phi(x_i) + b\right) \le \varepsilon + \xi_i \tag{2}$$

$$\left(w.\phi(x_i) + b\right) - y_i \le \varepsilon + \xi_i^* \tag{3}$$

$$\xi_i^*, \xi_i \ge 0 \tag{4}$$

191 where, w is the weight vector, C is the regularization parameter, ξ_i and ξ_i^* are slack variables,

192 $\phi(x_i)$ is the kernel function, and ε is the tolerance margin. The kernel function (e.g., radial basis

193 function) enables SVR to model nonlinear relationships effectively (Taki and Rohani, 2025).

194 Mean Absolute Percentage Error (MAPE), coefficient of determination (R^2) and Root Mean

195 Square Error (RMSE), were used to evaluate the accuracy of the models (Taki and Rohani, 2025):

$$MAPE = \frac{1}{n}\sum_{j=1}^{n}\left|\frac{d_j - p_i}{d_j}\right|.100 \tag{5}$$

$$R^2 = \frac{(\sum_{j=1}^{n}(d_j - \bar{d})(p_j - \bar{p}))^2}{\sum_{j=1}^{n}(d_j - \bar{d})^2 \sum_{j=1}^{n}(p_j - \bar{p})^2} \tag{6}$$

196 where, d_j is the jth factor of the actual output for the jth; p_j is the factor of the predicted output;

197 $\bar{d}$ and $\bar{p}$ are the mean of the actual and predicted outputs, and n is the number of output factors.

198

199 **2.15. Statistical Analysis**

200 All experiments were conducted in triplicate using a completely randomized design with a factorial

201 arrangement. Data were analyzed using Minitab software (version 16). Analysis of variance

202 (ANOVA) was performed to evaluate the effects of treatments and storage time, and mean

203 comparisons were conducted using Tukey's post hoc test at a significance level of $p < 0.05$.

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208 **3. Result and Discussion**

209 **3.1. Microbial Load Changes**

210 The microbial results clearly indicate that enrichment of chia seed gum coatings with cell-free

211 supernatant (CFS) from *L. lactis* LBM15 effectively delayed spoilage of ostrich meat during

212 refrigerated storage (Figure 1). In control samples, all microbial indices increased sharply,

213 consistent with the high-water activity and relatively elevated pH of ostrich meat. Total viable

214 counts (TVC) increased from 4.60 ± 0.12 to 9.50 ± 0.14 log CFU/g by day 10, with similar upward

215 trends observed for psychrotrophic bacteria, coliforms, and fungi, in agreement with previous

216 reports on ostrich meat deterioration (Hashemi et al., 2023). Application of chia seed gum alone

217 modestly reduced microbial growth, with counts lower than the control by day 10 (e.g., TVC: 9.10

218 $\pm$ 0.16 log CFU/g). This effect is attributed to the barrier properties of polysaccharide-based

219 coatings, which limit oxygen diffusion and surface dehydration (Hashemi et al., 2023). However,

220 the gum-only coating showed limited antifungal activity, indicating a predominantly physical

221 mode of action.

222 The most potent antimicrobial effect was observed in CFS-enriched coatings, particularly at the

223 2% level. By day 10, TVC values were reduced to 7.35 ± 0.12 and 6.80 ± 0.11 log CFU/g in the

224 1% and 2% treatments, respectively, corresponding to 28–35% reductions compared with the

225 control. Similar dose-dependent reductions were observed for psychrotrophic bacteria, coliforms,

226 and fungi. The antimicrobial effect of CFS-enriched coatings is mainly attributed to LAB-derived

227 metabolites such as organic acids and bacteriocin-like compounds, which inhibit microbial growth

228 by lowering surface pH and disrupting cell membranes. Encapsulation of these metabolites within

229 the chia mucilage matrix may further enhance their effectiveness by prolonging their retention on

230 the meat surface during storage. The enhanced efficacy of the CFS-containing coatings likely

231 reflects a synergistic interaction, in which the chia mucilage matrix acts as a controlled-release

232 system for antimicrobial metabolites, as commonly reported for active edible coatings (Ng et al.,
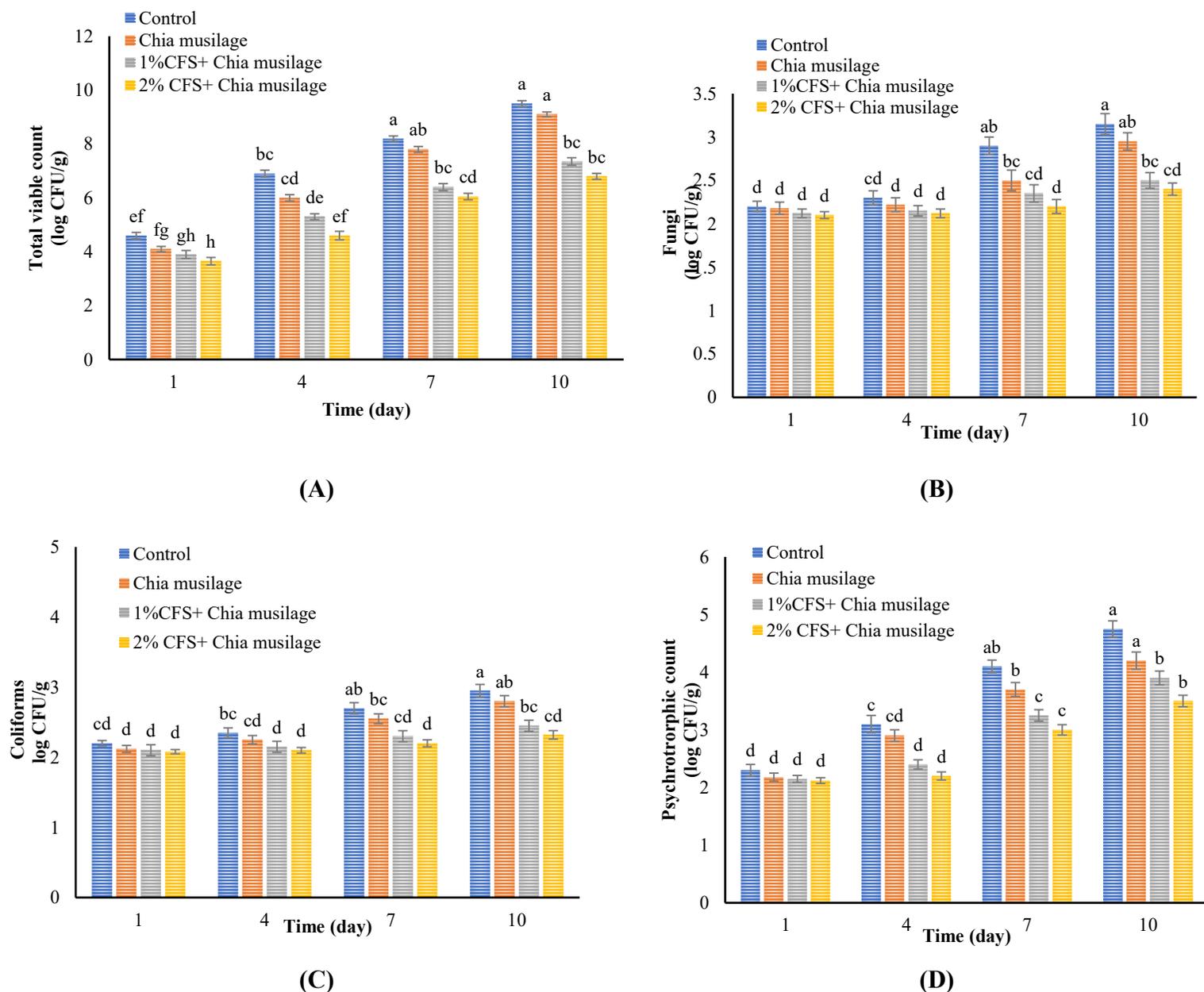
233 2020; Hashemi et al., 2023).

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(A)

(B)

(C)

(D)

Fig. 1. Changes in total viable count (A), fungi count (B), coliforms count (C), and psychrotrophic count (D) of ostrich meat samples coated with chia mucilage loaded with cell-free supernatant (CFS) of *L. lactis* LBM15 at 1% and 2% v/v levels. Samples labeled with different letters differ significantly at $p < 0.05$.

3.2. Physicochemical Changes

Lipid oxidation increased progressively across all treatments during refrigerated storage (Figure 2); however, chia seed gum coatings, particularly those enriched with *L. lactis* LBM15 supernatant, significantly delayed both primary and secondary oxidation reactions. In control samples, peroxide

248 value (PV) rose sharply from 1.15 ± 0.03 to 7.85 ± 0.13 meq O_2/kg by day 10. The gum-only

249 coating moderately reduced PV (6.70 ± 0.13 meq O_2/kg), whereas supernatant-enriched coatings

250 showed a clear dose-dependent antioxidant effect, reaching 4.80 ± 0.10 and 3.10 ± 0.09 meq O_2/kg

251 in the 1% and 2% treatments, respectively. Similar trends were observed for TBA values, which

252 increased to 1.08 ± 0.06 mg MDA/kg in the control but remained significantly lower in the 1%

253 (0.68 ± 0.06) and 2% (0.57 ± 0.06) supernatant treatments. These results are consistent with reports

254 that active edible coatings delay lipid oxidation by restricting oxygen diffusion and supplying

255 antioxidant metabolites (Moura et al., 2023). A study found that edible coatings containing

256 microbial-derived antioxidants effectively lowered both PV and TBA values in chilled meat

257 products by reducing oxygen diffusion and stopping free radical spread. Similar reduction levels

258 observed in this study indicate that the *L. lactis* cell-free supernatant exhibits potent antioxidant

259 activity when embedded in a polysaccharide matrix (Chen et al., 2023).

260 Changes in pH and moisture content further reflected the stabilizing effect of the coatings (Figures

261 2c and 2d). Although pH increased slightly in all samples during storage, the increase was more

262 controlled in supernatant-treated meat, reaching 6.08 ± 0.01 in the control compared with $5.93 \pm$

263 0.02 in the 2% treatment by day 10. This change in pH indicates reduced proteolytic activity, as

264 previously reported for LAB-based coatings (da Silva et al., 2020). Despite the antimicrobial effect

265 of CFS-containing coatings, the gradual increase in pH during storage can be attributed to residual

266 proteolytic activity and the accumulation of basic nitrogenous compounds resulting from protein

267 degradation.

268 Moisture loss was most pronounced in the control ($74.80 \pm 0.18\%$ to $64.50 \pm 0.21\%$), whereas

269 coated samples, especially the 2% supernatant treatment, retained significantly higher moisture

270 ($73.60 \pm 0.16\%$), consistent with the barrier function of polysaccharide matrices (Hashemi et al.,

271 2023). Texture analysis (Figure 2e) showed progressive softening in all treatments, with firmness

272 decreasing from 39.50 ± 1.05 to 31.10 ± 1.15 N in the control. Chia gum alone partially mitigated

273 this decline, while supernatant-enriched coatings, particularly at 2%, maintained higher firmness

274 (36.40 ± 1.13 N), likely due to improved water retention and reduced structural degradation.

275 Higher firmness values in coated samples can be explained by improved water-holding capacity

276 and reduced structural breakdown of muscle fibers, resulting from both moisture retention and

277 inhibition of proteolytic activity (Bao et al., 2025). A recent study reported that polysaccharide-

278 based edible coatings significantly improved moisture retention and texture stability in refrigerated

279 meat by reducing water vapor transmission and limiting structural protein degradation. The higher

280 firmness values observed in the supernatant-treated samples of this study further support the role

281 of active coatings in preserving meat structural integrity during storage (Rahmati-Joneidabad et

282 al., 2025). Protein degradation, assessed by TVB-N (Figure 2f), increased rapidly in control

283 samples (6.90 ± 0.18 to 34.30 ± 0.32 mg/100 g), whereas the 1% and 2% supernatant treatments

284 significantly suppressed TVB-N accumulation (21.80 ± 0.27 and 18.90 ± 0.24 mg/100 g). These

285 findings agree with previous studies showing that antimicrobial and antioxidant-enriched coatings

286 effectively limit volatile nitrogen formation during meat storage (Zhang et al., 2022). In addition,

287 recent reviews emphasize that active edible coatings have evolved beyond fundamental

288 biopolymer barriers toward multifunctional systems that regulate gas exchange, moisture transfer,

289 and the controlled release of bioactive compounds, thereby enhancing overall food quality and

290 extending shelf life in perishable products. The slower increase in pH formation in coated samples

291 indicate suppression of proteolytic spoilage microorganisms and reduced protein degradation,

292 which has been widely reported for LAB-based and biopolymer edible coatings applied to meat

293 systems (Tasnim & Islam, 2025).

294

295 **3.3. Color Measurement**

296 Color parameters (L*, a*, b*, and ΔE) exhibited clear treatment-dependent changes during

297 refrigerated storage (Figure 3). Discoloration progressed most rapidly in the control samples,

298 where lightness (L*) declined from 34.20 ± 0.55 to 28.80 ± 0.60 by day 10. The chia gum coating

299 partially mitigated this decrease (29.60 ± 0.25), while supernatant-enriched coatings preserved

300 brightness more effectively, maintaining L* values of 30.70 ± 0.37 and 30.95 ± 0.33 in the 1% and

301 2% treatments, respectively. This protective effect is attributed to reduced oxygen exposure and

302 delayed myoglobin oxidation, consistent with previous reports on mucilage-based coatings. Color

303 preservation in coated samples is associated with delayed myoglobin oxidation and reduced lipid

304 oxidation, which together limit metmyoglobin formation and surface discoloration during storage

305 (Heydari et al., 2020). Redness (a*) followed a similar trend. Control samples declined sharply

306 from 17.30 ± 0.27 to 9.10 ± 0.35, indicating rapid metmyoglobin formation, whereas gum-only

307 coatings provided partial protection (10.90 ± 0.33). In contrast, LAB-supernatant treatments

308 maintained significantly higher a* values (12.70 ± 0.40 and 13.50 ± 0.36), reflecting enhanced

309 pigment stability, in agreement with earlier studies on antioxidant- and LAB-enriched meat

310   coatings (Behbahani et al., 2024). Although yellowness (b*) decreased across all treatments,

311   coated samples—particularly the 2% supernatant formulation—retained higher values (10.85 ±

312   0.35 at day 10). Overall color difference (ΔE) clearly distinguished treatments, with the control

313   reaching 10.47, compared with lower values in the gum-only (7.39) and supernatant-enriched

314   coatings (5.67 and 5.14 for 1% and 2%, respectively), indicating superior color retention. Similar

315   ΔE reductions have been reported for active polysaccharide-based coatings containing bioactive

316   compounds (Moura-Alves et al., 2023; Pires et al., 2025). Also, previous research using isolated

317   soy protein films incorporated with peppermint essential oil demonstrated that bioactive films can

318   modify color parameters (L, a*, b*, ΔE) and exhibit antimicrobial activity, contributing to

319   extended shelf life of meat products. These results support the broader applicability of edible films

320   with functional additives for meat quality preservation (Karimian et al., 2019). Overall, chia gum

321   provided baseline protection against discoloration, while incorporation of *L. lactis* LBM15

322   supernatant markedly enhanced color stability, with the 2% formulation showing the strongest

323   effect.

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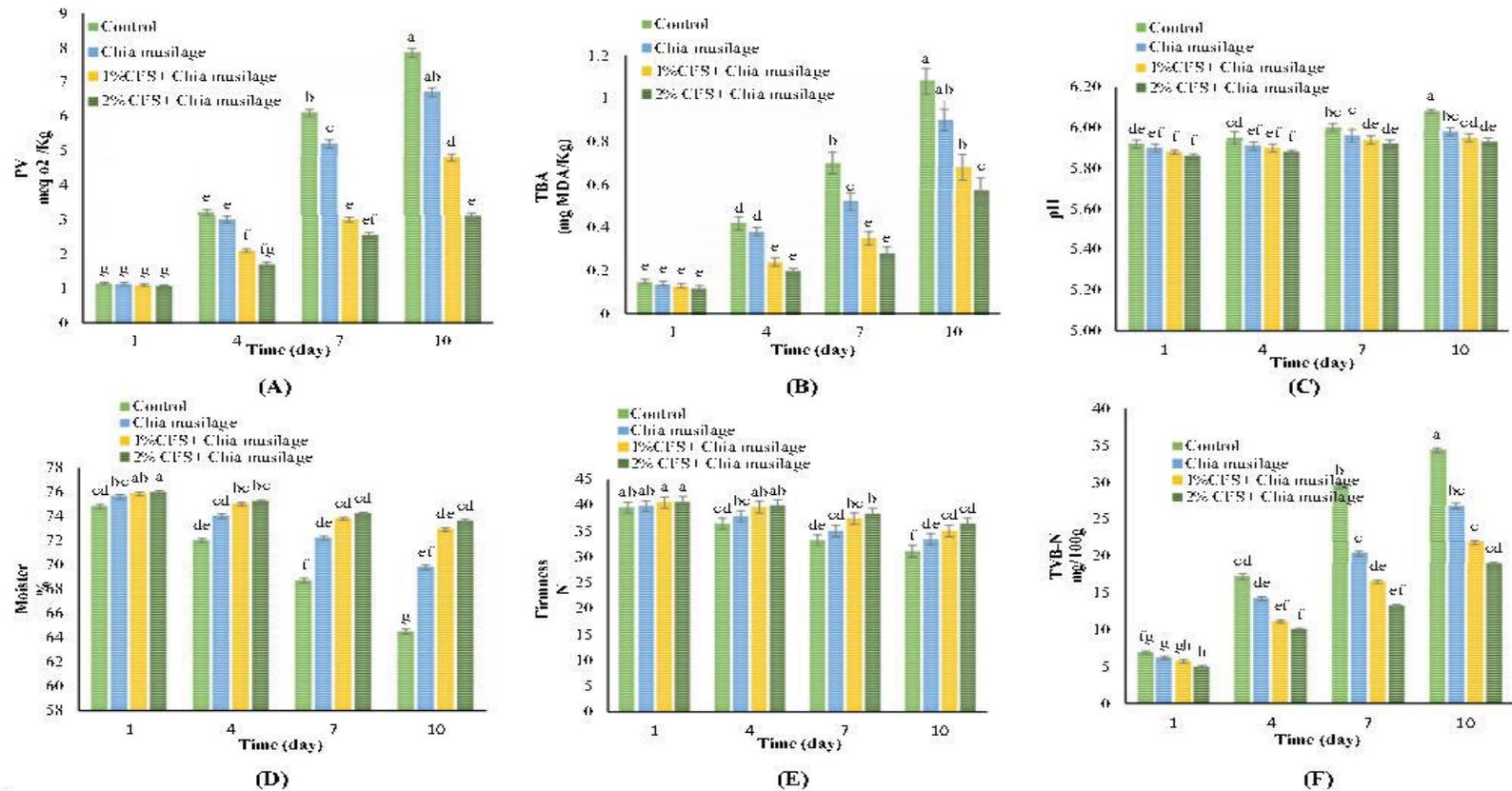
Fig. 2. Changes in PV (A), TBA (B), pH (C), moisture (D), firmness(E), and TVB-N(F) of ostrich meat samples coated with chia mucilage loaded with cell-free supernatant (CFS) of *L. lactis* LBM15 at 1% and 2% v/v levels. Samples labeled with different letters show significant differences at $p < 0.05$.
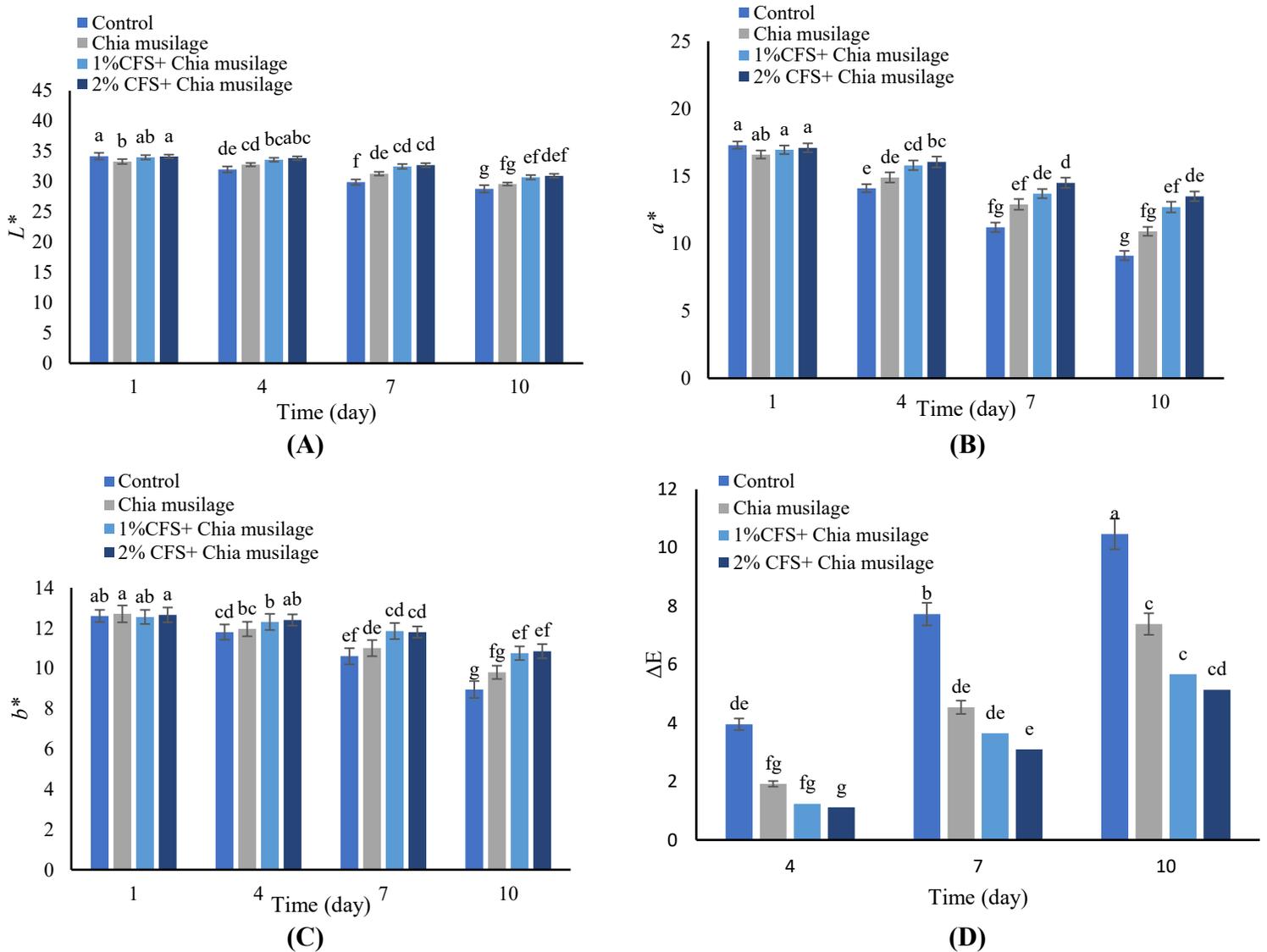
Fig. 3. Changes in L^* (A), a^* (B), b^* (C), and ΔE (D) of ostrich meat samples coated with chia mucilage loaded with cell-free supernatant (CFS) of *L. lactis* LBM15 at 1% and 2% v/v levels. Samples labeled with different letters differ significantly at $p < 0.05$.

3.4. Sensory Analysis

Sensory evaluation revealed faster deterioration of odor, color, and texture in untreated ostrich meat, whereas chia-based coatings—especially those enriched with *L. lactis* LBM15 supernatant—significantly improved sensory stability during refrigerated storage. Odor scores declined sharply in control samples from 8.20 ± 0.40 to 2.85 ± 0.45 by day 10, while gum-only coatings moderately delayed odor loss (3.65 ± 0.28). In contrast, LAB-supernatant treatments maintained higher odor scores (4.55 ± 0.42 and 4.80 ± 0.38 for 1% and 2%, respectively),

340   consistent with reduced microbial growth and lower TVB-N levels reported for LAB-derived

341   metabolites (Heydari et al., 2020; Behbahani et al., 2024).

342   Sensory color acceptability followed a similar pattern. The control declined from 8.30 ± 0.33 to

343   3.20 ± 0.37, in agreement with instrumental color deterioration, whereas LAB-enriched coatings

344   preserved higher scores (4.60 ± 0.32 and 5.00 ± 0.38). Comparable improvements in sensory color

345   have been reported for hydrocolloid-based coatings supplemented with natural antimicrobials or

346   LAB metabolites (Noshad et al., 2021). Texture scores also showed clear treatment-dependent

347   differences. Control samples decreased markedly from 8.20 ± 0.36 to 2.65 ± 0.37, reflecting

348   moisture loss and structural softening, while supernatant treatments preserved texture more

349   effectively (4.85 ± 0.33 and 5.50 ± 0.35). These improvements were reflected in overall

350   acceptance, which remained significantly higher in LAB-supernatant treatments (4.70 ± 0.39 and

351   5.10 ± 0.34) compared with the control (3.00 ± 0.30). Overall, chia gum provided baseline sensory

352   protection, whereas incorporation of *L. lactis* LBM15 supernatant offered superior preservation by

353   simultaneously limiting microbial spoilage, oxidative changes, and moisture loss (Moura-Alves et

354   al., 2023; Pires et al., 2024).

355

356   **3.5. SVR Results**

357   The SVR model was employed to simultaneously predict twelve key laboratory factors commonly

358   used to assess food quality and safety including: ΔE, TBA, PV, hardness, moisture content, TVBN,

359   pH, fungal count, PTC (psychrotrophic bacteria count), coliforms, overall acceptance and TVC.

360   Model performance was rigorously evaluated using MAPE and R^2, across training, testing and

361   total phases, ensuring a balanced assessment of predictive accuracy and generalization capability.

362   As illustrated in **Fig 5**, the SVR framework achieved consistently high predictive performance

363   across all target variables. The test R^2 values ranged from 0.93 to 0.99, indicating that the model

364   explained a substantial proportion of the variance in each laboratory factor. Notably, parameters

365   such as fungi, moisture, and coliforms exhibited the strongest fits, suggesting that their underlying

366   relationships with input features are well-captured by the SVR's nonlinear kernel mapping—likely

367   due to their more deterministic and less noisy nature compared to microbiological indicators.

368   The overall MAPE across all 12 factors remained below 8%, demonstrating the robustness and

369   practical utility of the SVR approach in this domain. The success of the SVR can be largely

370   attributed to the ε-insensitive loss function and the Radial Basis Function (RBF) kernel, which

371 together enabled the model to tolerate minor measurement noise while capturing complex,
372 nonlinear dependencies in the data. This is particularly advantageous in food science applications,
373 where sensor data, environmental conditions, and biochemical interactions often exhibit
374 nonlinearity and multicollinearity.
375 Compared with traditional regression techniques (such as multiple linear regression or artificial
376 neural networks without regularization), SVR's foundation in structural risk minimization
377 provides a principled trade-off between model complexity and empirical error, controlled by the
378 regularization parameter C and the tolerance margin ε. This balance is critical when working with
379 moderate-sized datasets, which are common in controlled food experimentation. These findings
380 underscore the potential of SVR as a reliable predictive tool for multi-response quality monitoring
381 in food processing and storage. Future work could explore hybrid models (e.g., SVR combined
382 with feature selection algorithms or ensemble methods) to further refine predictions for highly
383 variable microbiological indicators.
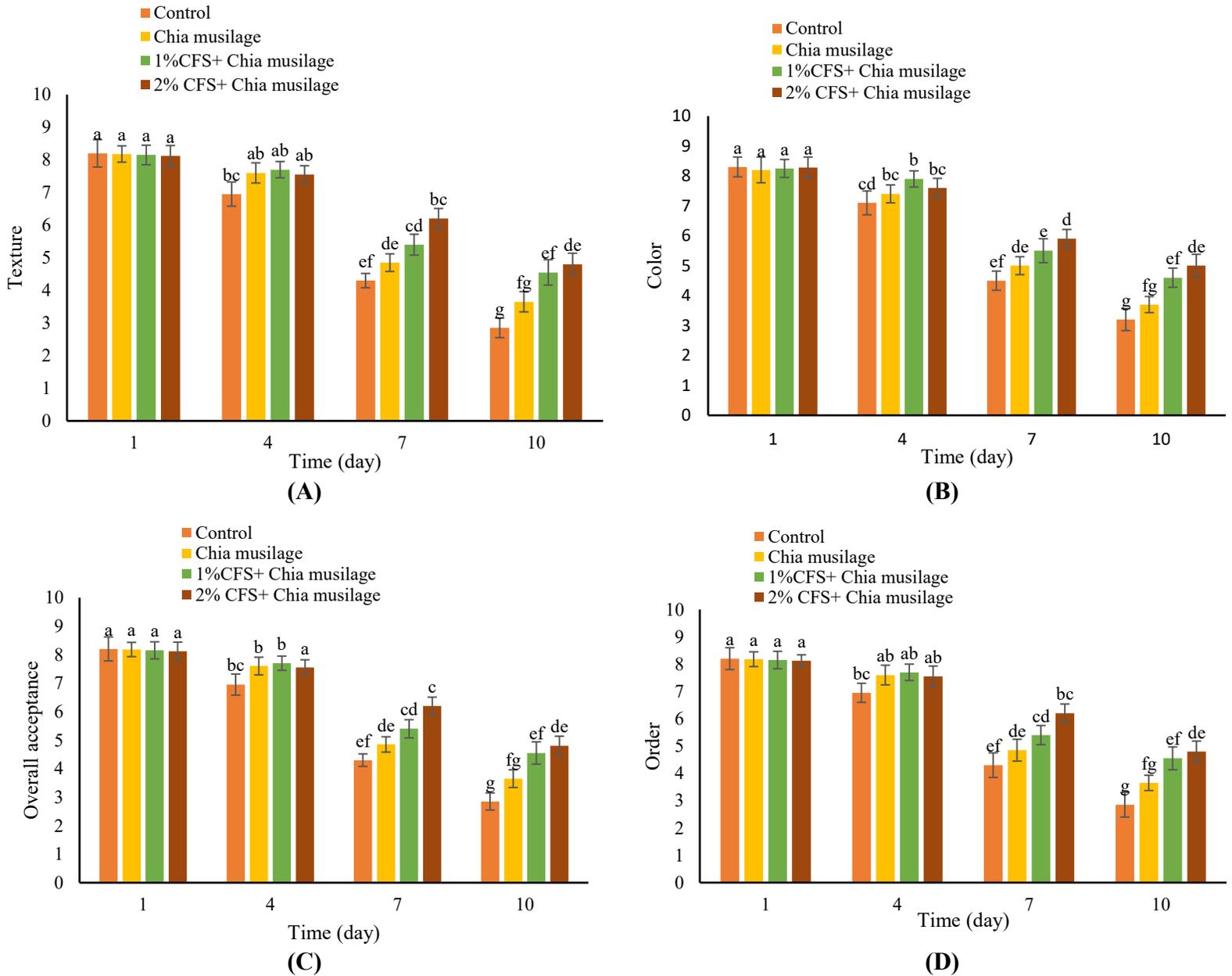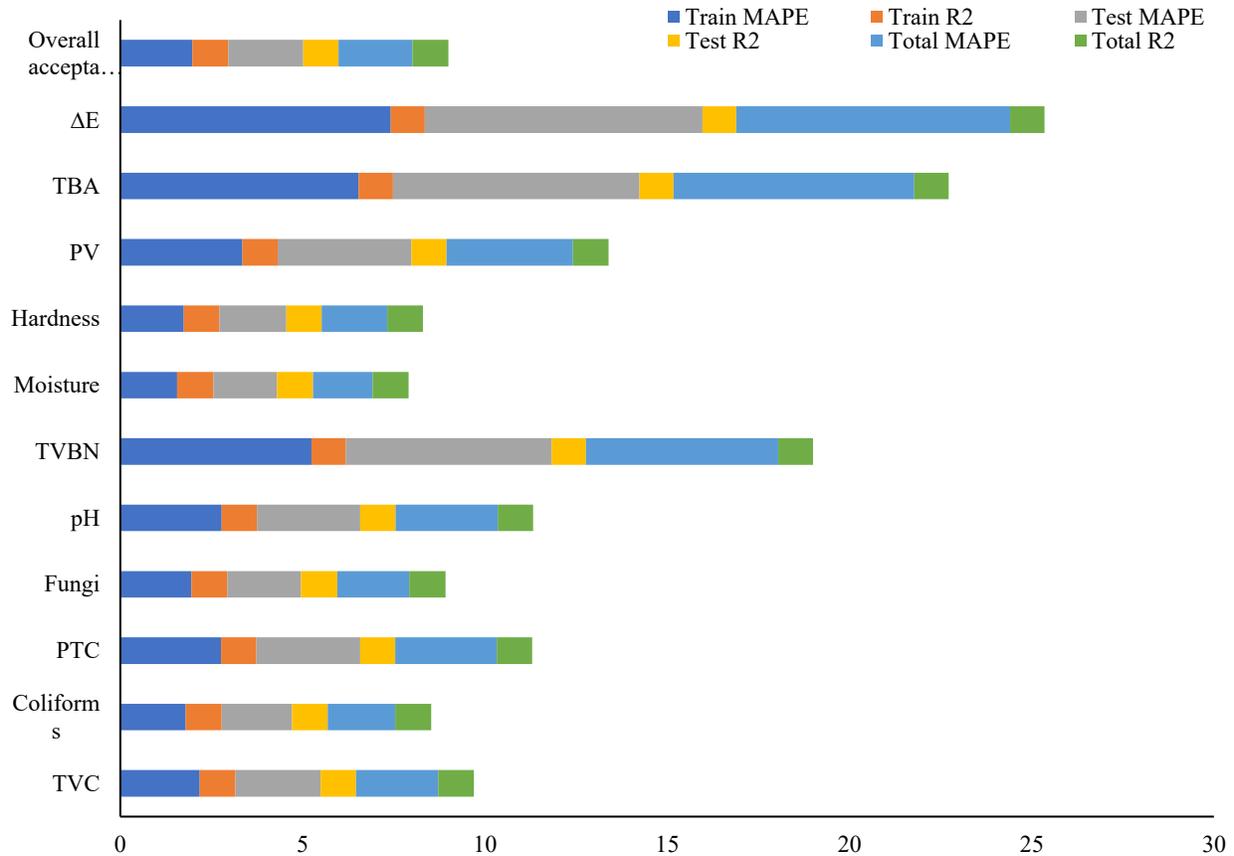
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Fig. 4. Changes in color (a), texture (b), odor (c), and overall acceptance (d) of ostrich meat samples coated with chia mucilage loaded with cell-free supernatant (CFS) of *L. lactis* LBM15 at 1% and 2% v/v levels. Samples labeled with different letters show significant differences at p < 0.05.

405

406   **Fig.5.** The results of modeling with SVR to predict all 12 laboratory factors.

407 **4. Conclusions**

408 This study demonstrates that chia seed mucilage enriched with cell-free supernatant from *L. lactis*

409 LBM15 -13, particularly at the 2% level, effectively improves the microbial, oxidative, textural,

410 color, and sensory stability of ostrich meat during refrigerated storage. These effects arise from a

411 synergistic interaction in which the hydrocolloid matrix limits gas and moisture transfer, while

412 LAB-derived metabolites inhibit spoilage-related processes. Despite these promising results, this

413 study has certain limitations. The experiments were conducted under a single storage temperature

414 and packaging condition, and the coating performance was evaluated over a fixed storage period.

415 In addition, the coating formulation was limited to two supernatant concentrations. Future studies

416 should therefore investigate the performance of this coating system under varying storage

417 temperatures, packaging atmospheres, and extended storage durations, and evaluate a broader

418 range of postbiotic concentrations and industrial-scale applicability. In parallel, the application of

419 Support Vector Regression (SVR) demonstrated strong capability in predicting 12 key laboratory

420 parameters related to food quality and safety. The model showed high generalization performance

421 (test R^2 = 0.93–0.99) with low prediction error. While physicochemical attributes were predicted
422 with exceptionally high accuracy, slightly higher errors for microbiological parameters reflect their
423 inherent biological variability. Future work may explore hybrid or ensemble machine learning
424 approaches further to improve predictive robustness for highly variable biological data.

425
426 **5. Acknowledgements**

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431 **6. References**

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588 **بهبود و پیش‌بینی پایداری میکروبی و اکسیداتیو گوشت شترمرغ با استفاده از پوشش‌های خوراکی**
589 **مبتنی بر موسیلاژ چیا غنی‌شده با سوپرناتانت *Lactococcus lactis* LBM15 و مدل رگرسیون**
590 **بردار پشتیبان(SVR)**

592 **بهاره گودرزی شمس آبادی، محمد حجتی، حسین جوینده، بهروز علیزاده بهبهانی، علیرضا وسیعی، و مرتضی تاکی**

594 **چکیده**

595 پوشش‌های خوراکی مبتنی بر موسیلاژ دانه چیا که با سوپرناتانت بدون سلول (CFS) حاصل از Lactococcus lactis LBM15 غنی شده‌اند، به‌طور مؤثری فساد گوشت شترمرغ نگهداری‌شده در شرایط سرمایش را به مدت ۱۰ روز به تأخیر انداختند. در مقایسه با نمونه شاهد، پوشش حاوی ۲٪ CFS به‌طور قابل‌توجهی رشد میکروبی و فساد اکسیداتیو را محدود کرد؛ به‌طوری‌که شمار کل باکتری‌های زنده از ۹/۵۰±۰/۱۴ log CFU/g در نمونه شاهد به ۶/۸۰± ۰/۱۱ log CFU/g در تیمار حاوی ۲٪ CFS افزایش یافت. به‌طور مشابه، مقدار پراکسید از ۰/۱۳meq O₂/kg ۷/۸۵± در نمونه شاهد به meq ۳/۱۰±۰/۰۹O₂/kg در تیمار ۲٪ CFS محدود شد. پایداری رنگ نیز بهبود یافت؛ به‌طوری‌که کاهش قرمزی و اختلاف رنگ کلی (ΔE) در نمونه‌های پوشش‌دار کمتر بود. ارزیابی‌های حسی مربوط به بو، رنگ، بافت و پذیرش کلی نیز در تیمار‌های غنی‌شده با CFS امتیاز بالاتری نشان دادند. برش‌های گوشت با پوشش چیا به‌تنهایی یا همراه با ۱٪ و ۲٪ CFS تیمار شده و در کنار نمونه شاهد بدون پوشش، از نظر شمارش‌های میکروبی، اکسیداسیون لیپیدی، شاخص‌های رنگی، بافت، pH، رطوبت و ویژگی‌های حسی مورد ارزیابی قرار گرفتند. در مجموع، ترکیب موسیلاژ چیا با CFS حاصل از L. Lactis LBM15 به‌عنوان روشی طبیعی و دارای برچسب پاک برای افزایش ماندگاری گوشت شترمرغ با ارزش اقتصادی بالا پتانسیل بالایی نشان داد. این مطالعه همچنین کاربرد رگرسیون بردار پشتیبان (SVR) را برای پیش‌بینی همزمان ۱۲ پارامتر کلیدی آزمایشگاهی بررسی کرد. عملکرد مدل با استفاده از خطای درصد مطلق میانگین (MAPE) و ضریب تعیین (R²) ارزیابی شد. نتایج نشان‌دهنده توان پیش‌بینی بالای مدل بودند، به‌طوری‌که مقادیر R² در مرحله آزمون بین ۰/۹۳ تا ۰/۹۹ و مقادیر MAPE کمتر از ۸٪ برای تمامی متغیرها به‌دست آمد که کارایی SVR را در مدل‌سازی روابط پیچیده و غیرخطی داده‌های مرتبط با کیفیت مواد غذایی تأیید می‌کند