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Quality characteristics of frozen horse mackerel (*Trachurus trachurus*) treated with starch–glycerol coatings containing plant extracts

Aydin Aytac Gurdal · Emre Caglak

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Abstract

Starch-based coatings are biodegradable food-contact materials, but their performance depends on plasticizer level, incorporated active compounds, and the food surface to which they are applied. This study developed starch-glycerol polysaccharide coating formulations containing aqueous extracts from persimmon fruit calyces, cherry laurel leaves, and bilberry/likapa leaves and evaluated their application to whole horse mackerel (*Trachurus trachurus*) during frozen storage at -20±2 °C for 6 months. Extract-level antioxidant activity was determined by the DPPH assay and expressed as percentage inhibition and Trolox equivalent antioxidant capacity. Comparable starch-glycerol films containing the same plant extracts had previously been characterized by Gurdal and Caglak and were used here only as formulation-characterization background. During frozen storage, TVB-N values increased but remained below 35 mg/100 g in all groups. Lipid oxidation responses were formulation-dependent: the lowest final TBA values were observed in PA and PB, whereas BA and VC showed the highest final values. pH, water activity, color, texture, and microbial results varied among treatments and storage months without supporting a uniform concentration-dependent response. The findings suggest that selected starch-glycerol formulations containing plant extracts may help modulate lipid oxidation and quality changes in frozen horse mackerel. However, the absence of a plain starch-glycerol-coated fish control, coating-uptake measurements, compound-level extract characterization, and composite-film antioxidant testing limits direct attribution of the observed effects to specific bioactive mechanisms.

Keywords Active packaging · Natural antioxidants · Horse mackerel · Frozen storage · Lipid oxidation · Quality changes · Edible coating

Introduction

Polysaccharide-based films and coatings have attracted attention as biodegradable food-contact materials because they can form continuous matrices, regulate mass transfer, and serve as carriers of functional ingredients[1,2]. Starch is one of the most widely investigated polysaccharide matrices for edible film and coating systems because of its availability, low cost, renewability, and film-forming capacity[3]. However, native starch-based films are generally

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hydrophilic and may exhibit limited mechanical and barrier performance; therefore, plasticizers and functional additives are commonly used to improve their applicability[4–7].

The incorporation of plant-derived extracts into polysaccharide matrices is a practical strategy for developing active or functional edible coating formulations. These matrices can carry phenolics and other bioactive compounds and may contribute to delayed lipid oxidation, microbial growth, or quality deterioration depending on matrix composition, extract type, food surface, and storage conditions[8–11]. Studies on fish and seafood have also shown that a plain coating control is important for distinguishing the contribution of the coating matrix from that of the incorporated active compound[12–15].

Fish and seafood are highly perishable because of high water activity, near-neutral pH, endogenous enzymes, unsaturated lipids, and microbial activity[1,2]. Freezing slows microbial and enzymatic processes but does not completely prevent lipid oxidation, protein and texture changes, moisture migration, or surface dehydration during prolonged storage. Recent reviews and application studies continue to emphasize the potential of edible coatings and active packaging systems for fish and seafood preservation, while also highlighting that coating performance is strongly dependent on matrix composition, the active ingredient, the seafood species, and the storage reg ime[1,13,14,16–19].

Coating performance on fish depends not only on the composition of a separately prepared film but also on the interaction between the coating solution and the fish surface. Surface energy, wettability, roughness, freezing state, packaging geometry, and surface moisture can affect the distribution and persistence of a coating layer. Therefore, film characterization and food-application performance should be interpreted together but not treated as identical outcomes.

Persimmon (*Diospyros kaki*) fruit calyces, cherry laurel (*Prunus laurocerasus*) leaves, and bilberry/likapa (*Vaccinium sp.*) leaves were selected as locally available plant-derived materials from the Eastern Black Sea region of Türkiye. These materials were chosen because related persimmon tissues, cherry laurel leaves, and Vaccinium leaves have been reported to contain phenolic compounds and exhibit antioxidant activity[20–24]. Nevertheless, the phenolic profile, total phenolic content, and total flavonoid content of the extracts used in the present study were not determined; therefore, the extracts are discussed as formulation components rather than as chemically defined bioactive fractions.

The aim of the present study was to prepare starch-glycerol polysaccharide coating formulations containing aqueous plant extracts, document extract-level DPPH antioxidant activity, use previously reported film-characterization information for comparable starch-glycerol films, and evaluate the quality-related responses of frozen horse mackerel during 6 months of storage. The study addresses the application of locally sourced plant materials in a simple starch-glycerol coating system for a small pelagic fish, while explicitly recognizing the limitations imposed by the absence of a blank starch-glycerol fish-control group and detailed compound-level extract characterization.

Results

Antioxidant activity of extracts and properties of separately prepared films

The DPPH radical scavenging activities of the plant extracts used in the coating formulations ranged from 69.55 ± 1.70% to 88.69 ± 1.08% (Fig. 1). The lowest inhibition value was observed for PA, whereas PB showed the highest value. Trolox equivalent antioxidant capacity ranged from 0.0322 to 0.0850 mg Trolox equivalent/mL. The response was not strictly concentration dependent. For example, BB showed lower DPPH inhibition than BA, which may reflect differences in extract composition, co-extracted non-antioxidant compounds, interactions among extract constituents, or assay-related matrix effects. Since total phenolic content, total flavonoid content, and chromatographic profiling were not performed, this pattern cannot be attributed to a specific compound group.

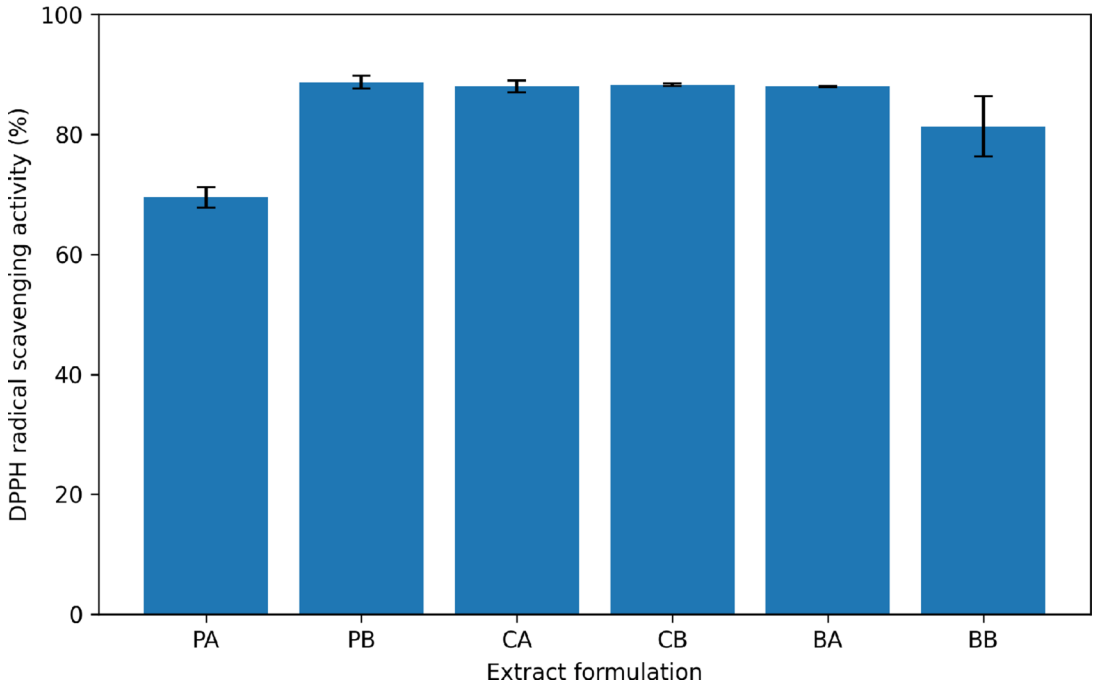


Fig. 1 DPPH radical scavenging activity of plant extracts used in coating formulations. Values are mean ± standard deviation. PA and PB: 2.5% and 5% persimmon fruit calyx extract; CA and CB: 2.5% and 5% cherry laurel leaf extract; BA and BB: 2.5% and 5% bilberry/likapa leaf extract.

Table 1 Basic technological properties of comparable starch-glycerol edible films containing plant extracts previously reported by Gurdal and Caglak[25].

Film group	Tensile strength (MPa)	Elongation at break (%)	WVP (×10–10 g H2O Pa − 1 s–1 m − 1)	Water solubility (%)	Swelling degree (%)
Plain starch-glycerol film	0.40 ± 0.03^{bc}	21.99 ± 2.17^{a}	0.42 ± 0.05^{a}	61.08 ± 0.86^{ab}	46.73 ± 0.51^{b}
PA	0.56 ± 0.06^{ab}	39.09 ± 6.81^{a}	0.57 ± 0.38^{a}	62.92 ± 0.50^{a}	40.18 ± 0.27^{c}
PB	0.61 ± 0.10^{a}	34.05 ± 10.23^{a}	0.41 ± 0.02^{a}	56.71 ± 0.79^{c}	59.62 ± 0.97^{a}
BA	0.32 ± 0.03^{c}	34.07 ± 7.15^{a}	0.34 ± 0.01^{a}	60.01 ± 0.59^{b}	36.11 ± 0.85^{de}
BB	0.46 ± 0.06^{abc}	31.97 ± 3.63^{a}	0.49 ± 0.08^{a}	63.10 ± 0.59^{a}	38.91 ± 0.83^{cd}
CA	0.52 ± 0.04^{ab}	21.43 ± 3.68^{a}	0.35 ± 0.09^{a}	58.97 ± 0.56^{bc}	35.79 ± 0.62^{e}
CB	0.47 ± 0.04^{abc}	27.54 ± 0.66^{a}	0.38 ± 0.04^{a}	61.30 ± 0.57^{ab}	36.88 ± 0.73^{de}

The technological properties of comparable starch-glycerol edible films containing persimmon, bilberry/likapa, and cherry laurel extracts were previously characterized by Gurdal and Caglak[25]. In that study, the films showed low tensile strength, moderate elongation at break, high water solubility, and measurable water vapor permeability, indicating that the starch-glycerol matrix formed handleable but hydrophilic films (Table 1). These film-property data are considered here as previously reported formulation-characterization information and are used only to support the interpretation of the coating formulations applied to frozen horse mackerel.

Values in Table 1 are mean ± standard deviation. Different lowercase letters within the same column indicate significant differences among film groups ($p < 0.05$) in the previously reported film-characterization study. These values refer to separately prepared edible films and not to the actual coating layer formed on the fish surface in the present frozen-storage trial.

Proximate composition and physicochemical changes during frozen storage

The moisture content of fresh horse mackerel muscle was 73.72%. At the end of 6 months of frozen storage, moisture values ranged from 72.72% to 76.38% among the treatment groups. Ash content was 1.35% in fresh samples and ranged from 1.12% to 1.37% at the end of storage. Crude lipid and crude protein values were determined and reported in the Supplementary Information. These proximate composition results are descriptive because natural variability among individual whole fish and analytical variation may contribute to the observed fluctuations.

The pH values remained within a relatively narrow range during frozen storage. The initial pH value was 6.69, and final pH values ranged from 6.37 to 6.97 among groups. Water activity remained high throughout storage, consistent with frozen fish muscle matrices. These results indicate that the treatments did not produce large pH or water-activity shifts during frozen storage.

TVB-N values increased during frozen storage but remained below the commonly accepted limit of 35 mg/100 g in all groups. The initial TVB-N value was 7.00 mg/100 g. At the end of 6 months, final TVB-N values ranged from 14.71 to 18.61 mg/100 g, with the highest values recorded in PA, CA, and the uncoated control. TBA values also changed during storage. Rechecking the raw dataset showed that the highest final TBA value was observed in BA (6.38 mg MDA/kg), followed by VC (6.10 mg MDA/kg), whereas the lowest final TBA values were recorded in PB (1.48 mg MDA/kg) and PA (1.89 mg MDA/kg). Therefore, the lipid oxidation response should be interpreted as formulation-dependent rather than uniformly concentration-dependent (Fig. 2).

Texture, color, and microbiological changes

Instrumental color and texture values varied among groups and storage months. Because whole small pelagic fish were coated and only skinless muscle was analyzed after thawing, variation in muscle structure, sampling location, thawing state, and individual fish composition may have contributed to the variability observed in texture and color measurements. The texture values should therefore be interpreted descriptively and not as evidence of a uniform treatment-driven structural mechanism.

Total aerobic mesophilic bacteria (TAMB), total aerobic psychrophilic bacteria (TAPB), yeast and mold, and coliform counts were below the detection limit or non-computable in several early sampling periods and became more detectable toward later storage periods. These microbial data are reported in the Supplementary Information. Because no specific spoilage organisms or foodborne pathogens were targeted, the microbiological results should be interpreted as general microbial-load indicators rather than as direct evidence of a specific antimicrobial mechanism (Fig. 3).

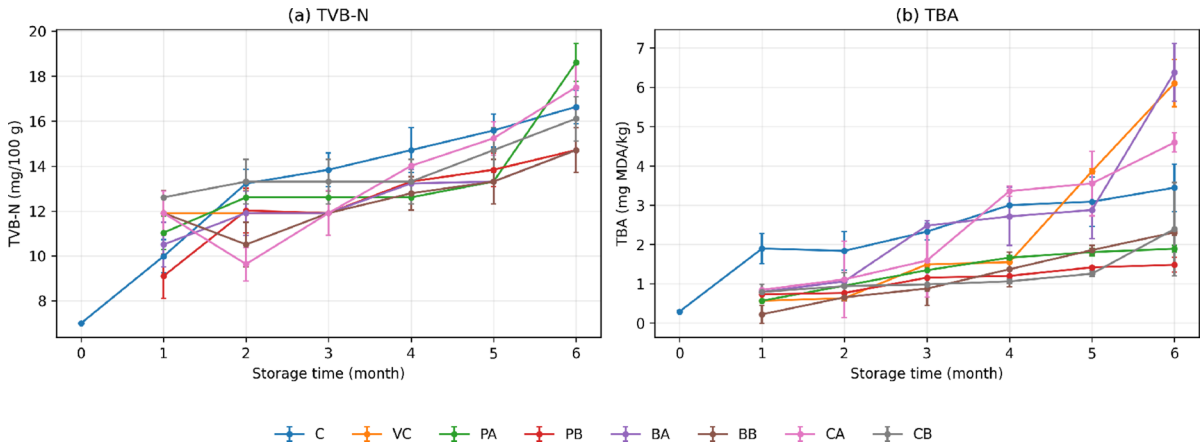


Fig. 2 Changes in (**a**) total volatile basic nitrogen (TVB-N) and (**b**) thiobarbituric acid reactive substances (TBA) during frozen storage of horse mackerel samples. Group abbreviations are defined in Table 2. Values are mean ± standard deviation.

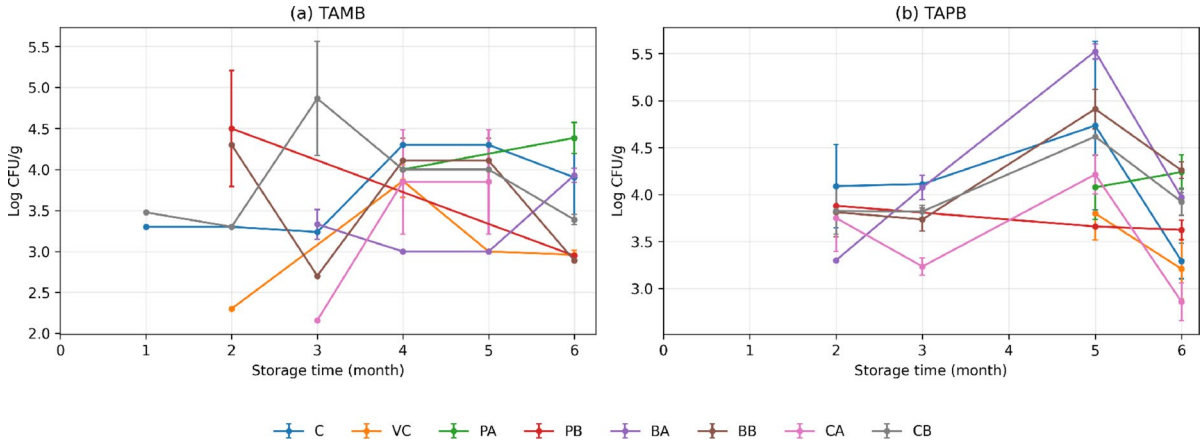


Fig. 3 Changes in (**a**) total aerobic mesophilic bacteria (TAMB) and (**b**) total aerobic psychrophilic bacteria (TAPB) during frozen storage. Group abbreviations are defined in Table 2. Values are mean ± standard deviation where replicate values were available. Values below the detection limit or non-computable logarithms were not plotted.

Discussion

The present results should be interpreted primarily as formulation-level responses of starch-glycerol polysaccharide coating systems. The previously reported film-characterization data confirmed that comparable starch-glycerol matrices containing these plant extracts could form handleable but hydrophilic films[25]. The relatively low tensile strength values are consistent with the high glycerol-to-starch ratio, because glycerol increases molecular mobility and flexibility while generally reducing the strength of starch-based films. In the present work, these film data support formulation contexts, but they do not directly quantify the thickness, mass, or continuity of the coating layer formed on frozen fish.

In the frozen fish application trial, the clearest formulation-associated response was observed for lipid oxidation. TBA values increased during storage, but the final values differed markedly among formulations. PA and PB showed the lowest final TBA values, whereas BA and VC showed the highest final values. This indicates that lipid oxidation responses were affected by the formulation but were not strictly related to extract concentration. Previous studies on frozen fish and coating or glazing systems have also reported formulation-dependent effects on lipid oxidation during frozen storage[26–30]. The relatively high final TBA value in the vitamin C treatment may reflect the complex behavior of ascorbic acid in fish matrices, where antioxidant or pro-oxidant effects can depend on oxygen exposure, transition metals, concentration, and matrix conditions. Because metal content and pro-oxidant conditions were not measured, this interpretation remains tentative.

The TVB-N, pH, water activity, microbiological, color, and texture results indicate that the coating formulations were associated with several quality-related changes during frozen storage, but the responses were not uniform across all parameters. TVB-N values remained below 35 mg/100 g in all groups, suggesting that the frozen-storage conditions limited volatile nitrogen accumulation. However, the treatments cannot be described as having a direct or specific antimicrobial effect, because the study did not include specific spoilage organisms, pathogen challenge tests, or mechanistic antimicrobial assays.

A central limitation of the experimental design is the absence of a plain starch-glycerol-coated fish group without plant extract or vitamin C. Several related studies include an uncoated control, a blank coating, and active-coating treatments to separate the physical effect of the coating matrix from the effect of the active compound. In the present study, the uncoated stretch-film group provides a practical storage control, but it does not allow the independent contribution of the starch-glycerol matrix to be separated from the contribution of the incorporated plant extracts or vitamin C. Therefore, the results should not be over-interpreted as direct proof of extract-specific bioactivity in the fish matrix.

The distinction between separately prepared films and actual food-surface coatings is also important. Characterizing edible coating formulations after application to fish is difficult because the coating becomes an inseparable part of the product[1]. In fish systems, coating performance depends on the surface properties of the product, wettability, adhesion-cohesion balance, spreading behavior, coating uptake, coating loss, thickness, and uniformity[31,32]. Since contact angle, spreading coefficient, adhesion coefficient, coating uptake/loss, and microscopic cross-sectional coating thickness were not determined on whole horse mackerel in the present study, the actual behavior of the coating layer on the fish surface could not be directly characterized.

The extract-related interpretation must also remain cautious. Previous studies have reported phenolic constituents and antioxidant activity in *D. kaki* plant tissues/leaves, cherry laurel leaves, and Vaccinium leaves[20–24]. However, the present study did not determine total phenolic content, total flavonoid content, ABTS, FRAP, HPLC, LC-MS, or other compound-level profiles of the specific aqueous extracts used. DPPH was performed only as an extract-level preliminary antioxidant screening assay and does not by itself prove antioxidant mechanisms in a frozen fish lipid matrix. In addition, the DPPH activity of the composite films was not measured.

Future studies should include a plain starch-glycerol-coated fish control, larger biological replicate numbers, factorial statistical analysis, consumer-based sensory evaluation, detailed extract profiling, composite-film antioxidant testing, coating uptake and coating-loss measurements, and specific spoilage/pathogen analyses. These additions would allow clearer separation of matrix, extract, concentration, and storage-time effects and would strengthen the mechanistic interpretation of starch-glycerol plant-extract coatings in seafood preservation.

Methods

Fish material and sample preparation

Horse mackerel (*Trachurus trachurus*) samples were obtained from a commercial fishing vessel operating in the Eastern Black Sea region of Türkiye. Immediately after capture, fish were placed in insulated boxes with ice and transported to the laboratory on the same day. The samples were whole fish and were not eviscerated or portioned before coating. The mean fish length was 13.675 cm (range: 12.0–15.2 cm), and the mean fish weight was 23.96 g (range: 15.01–35.66 g). Ten fish were used for initial fresh-sample characterization, and 10 fish were assigned to each of the eight storage groups, giving a total of 90 fish. At each sampling point, fish were thawed at room temperature, the skin was removed, and skinless muscle tissue was used for physicochemical, microbiological, color, texture, and proximate analyses.

Preparation of plant materials and aqueous extracts

Persimmon (*Diospyros kaki*) fruit calyces were collected from a private garden in Kale Neighborhood, Rize, Türkiye, with the permission of the garden owner. Cherry laurel (*Prunus laurocerasus*) leaves were collected from private gardens in Rize, Türkiye, with permission. Bilberry/likapa (*Vaccinium sp*.) leaves were collected from naturally growing plants in Handuzu Plateau, Guneysu district, Rize, Türkiye. No protected area was accessed, and no specific permit or license was required for the collection of the *Vaccinium sp.* material. Previous floristic investigations conducted in the Handuzu–Guneysu region have documented the botanical diversity of the collection area, including the occurrence of Vaccinium species[33,34]. The plant materials were identified by Emre Caglak and Aydin Aytac Gurdal based on their local/common botanical characteristics, with the Vaccinium material identified only at the genus level. No voucher specimens of the original plant materials used in the present study were deposited in a publicly accessible herbarium; therefore, no accession numbers are available. Plant materials were dried in a forced-air oven at 60 °C for 24 h, ground into powder, and used for aqueous extraction. Different extraction temperatures were selected according to the plant material and preliminary trials. Hot-water extraction gave higher antioxidant activity for persimmon fruit calyces, whereas lower-temperature aqueous extraction was

more suitable for cherry laurel and bilberry/likapa leaves. Therefore, persimmon fruit calyces were extracted at 75 °C, while cherry laurel and bilberry/likapa leaves were extracted at 40 °C. All extractions were performed for 24 h at 110 rpm, filtered through Whatman No. 1 filter paper, and stored at 4 °C until use. The possible degradation of heat-sensitive compounds during thermal extraction cannot be excluded.

DPPH radical scavenging activity of plant extracts

The DPPH radical scavenging activity of the plant extracts was determined according to Brand-Williams et al[35]. Briefly, 10 mg of DPPH (Sigma-Aldrich, Steinheim, Germany) was dissolved in 100 mL of methanol (Sigma-Aldrich, Steinheim, Germany) to prepare the stock solution. The working DPPH solution was prepared by mixing 3.5 mL of the stock solution with 6.5 mL of methanol. For the assay, 100 µL of each extract solution was mixed with 3.9 mL of the DPPH working solution in glass tubes. The mixture was vortexed and incubated at room temperature for 30 min in the dark. The absorbance was then measured at 515 nm using quartz cuvettes against a control consisting of 100 µL methanol and 3.9 mL DPPH working solution. Analyses were performed as analytical replicates, and DPPH radical scavenging activity was calculated as follows: DPPH scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$. Results were expressed both as percentage inhibition and as Trolox equivalent antioxidant capacity (mg Trolox equivalent/mL).

Preparation of starch-glycerol coating formulations

Starch-based coating formulations were prepared using starch as the film-forming polysaccharide and glycerol as plasticizer. For each formulation, starch (6.5 g) and glycerol (6.5 g) were mixed with distilled water and the respective plant extract to obtain a final liquid phase consisting of 100 mL water and 100 mL extract or vitamin C solution. The 1:1 starch: glycerol ratio was selected based on preliminary trials to obtain a stable and brushable film-forming solution. The uncoated control group did not receive a coating treatment and was packaged only with stretch film. The vitamin C group was prepared by incorporating 5% vitamin C into the starch-glycerol coating solution.

Characterization of separately prepared films

To characterize the coating formulations, comparable edible films had previously been prepared from starch-glycerol-based solutions and evaluated by Gurdal and Caglak[25]. In that study, film thickness was measured at five different points using a digital micrometer (MarCal 16 ER, Germany). Tensile strength and elongation at break were measured using a universal testing device (Zwick Roell, Germany). Water vapor permeability was determined using a modified ASTM-based gravimetric method. Briefly, films were fixed over test tubes containing silica gel, placed in a desiccator saturated with water vapor at 25 °C, and weighed at 24 h intervals for 7 days. Water solubility was determined using 2 × 2 cm film pieces dried at 105 °C for 24 h, immersed in distilled water, re-dried at 105 °C for 24 h, and calculated from the initial and final dry weights. The available experimental records included the device and general procedures, but detailed tensile-test conditions such as gauge length, crosshead speed, and conditioning conditions were not recorded. Vitamin C was included as a positive antioxidant coating treatment in the fish-storage trial, but a corresponding standalone vitamin C film was not characterized.

Experimental groups, coating application, and frozen storage

Eight fish-storage groups were prepared: C, VC, PA, PB, BA, BB, CA, and CB (Table 2). Whole horse mackerel samples were pre-frozen at −80 °C for 1 h before coating to allow rapid setting/freezing of the applied solution on the fish surface. The coating solutions were applied by brushing to both sides of the pre-frozen whole fish. Because the fish were frozen before coating, the applied solution froze rapidly on the surface and formed a

Table 2 Experimental groups and treatment descriptions.

Code	Treatment description
C	Uncoated control, packaged only with stretch film
VC	Starch-glycerol coating containing 5% vitamin C
PA	Starch-glycerol coating containing 2.5% persimmon fruit calyx extract
PB	Starch-glycerol coating containing 5% persimmon fruit calyx extract
BA	Starch-glycerol coating containing 2.5% bilberry/likapa leaf extract
BB	Starch-glycerol coating containing 5% bilberry/likapa leaf extract
CA	Starch-glycerol coating containing 2.5% cherry laurel leaf extract
CB	Starch-glycerol coating containing 5% cherry laurel leaf extract

glaze-like frozen coating layer. No additional setting or drying period was applied before packaging. The exact volume or mass of coating solution applied per fish was not measured, and coating uptake or coating loss was not determined gravimetrically. All samples were packaged with stretch film and stored at −20 ± 2 °C for 6 months.

Proximate composition

Moisture and ash contents were determined according to Gurdal and Caglak[25]. For moisture analysis, approximately 3 g of homogenized skinless muscle tissue was dried at 105 °C for 24 h until constant weight. Ash content was determined by incineration at 525 °C. Crude lipid content was determined using a Soxhlet-based method. Crude protein content was determined by the Kjeldahl method and expressed as percentage protein. Proximate composition data are reported in the Supplementary Information.

Physicochemical analyses

The pH values were measured using a calibrated pH meter according to Curran et al[36]. Water activity was determined using an Aqualab 3TE device at 25 °C. Total volatile basic nitrogen (TVB-N) was determined using the Lucke-Geidel method as described by Varlik et al[37]. and expressed as mg/100 g. Thiobarbituric acid reactive substances were determined according to Tarladgis et al[38]. Absorbance was measured at 538 nm, and results were expressed as mg malonaldehyde (MDA)/kg sample.

Microbiological analyses

Microbiological analyses were conducted according to Halkman[39]. For each analysis, 25 g of skinless horse mackerel muscle tissue was aseptically transferred into a sterile stomacher bag and homogenized with sterile diluent. Total aerobic mesophilic bacteria were enumerated on Plate Count Agar after incubation at 37 °C for 48 h. Total aerobic psychrophilic bacteria were enumerated after incubation at 7 °C for 10 days. Yeast and mold counts were determined on Potato Dextrose Agar after incubation at 25 °C for 3–5 days. Coliform bacteria were enumerated on Violet Red Bile Agar after incubation at 37 °C for 48 h. Results were expressed as log CFU/g. When plate counts generated non-computable logarithms because of zero colonies in one analytical replicate, only finite log values were used descriptively, and non-computable values were not plotted.

Color measurement

Color measurements were performed using a calibrated colorimeter (Konica Minolta CR-14, Japan). Homogenized skinless muscle samples were placed in glass Petri dishes, and measurements were taken from five points. Results were expressed using the CIE L*, a*, and b* color coordinates.

Texture profile analysis

Texture profile analysis was performed on thawed skinless muscle samples obtained from whole fish using a TA.XT Plus texture analyzer (Stable Micro Systems, UK) equipped with a cylindrical probe with a diameter of 3.5 cm. The test speed was 1 mm/s. Hardness values are reported in the Supplementary Information.

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical differences in the original dataset were evaluated using one-way analysis of variance at $p < 0.05$ in JMP 5.0.1. Because the available records were based primarily on analytical or measurement replicates and the original analysis was not designed as a full factorial biological-replicate structure, retrospective two-way ANOVA and treatment × storage-time interaction testing were not performed. Therefore, the revised manuscript presents the results descriptively and cautiously, using mean ± standard deviation and avoiding overgeneralized treatment-ranking conclusions.

Ethics statement

This study used commercially caught dead fish samples. No live-animal experimentation, experimental animal procedure, or human participant involvement was performed. Plant material collection complied with applicable local requirements, as detailed in the section "Preparation of plant materials and aqueous extracts".

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-78545-8.

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Author contributions A.A.G. carried out the investigation, collected and curated the data, performed the formal analysis, prepared the tables and figures, and wrote the original draft of the manuscript. E.C. contributed to the conceptualization and methodology of the study, supervised the research, validated the scientific content, and reviewed and edited the manuscript. Both authors interpreted the results, reviewed the final version of the manuscript, and approved it for submission.

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Data availability The data generated and analyzed during the current study are included in this published article and its Supplementary Information files. Additional information is available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval and consent to participate Not applicable. This study used commercially caught dead fish samples, and no live-animal experimentation, experimental animal procedure, or human participant involvement was performed.

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Authors and Affiliations

Aydin Aytac Gurdal[1] · Emre Caglak[2]

✉ Emre Caglak
emre.caglak@erdogan.edu.tr

[1] Yalova University, Armutlu Vocational School, Yalova, Turkey

[2] Faculty of Fisheries, Recep Tayyip Erdogan University, Rize, Turkey