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Research Article

Development of fish collagen/ sodium alginate-based bio-composite loaded with green-synthesised silver nanoparticles and orange peel extract as a packaging film

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In the current investigation, an eco-friendly packaging film comprising fish skin-derived collagen (COL), Sodium Alginate (SA) functionalised with Orange Peel Extract (OPE), and silver nanoparticles (AgNPs) was fabricated. Notably, the synergistic action of nano-fillers and peel extracts in the film matrix significantly ($p < 0.05$) improves the mechanical and light barrier properties and antioxidant activity of the COL/SA bio-composite. Scanning Electron Microscopy (SEM) illustrated pronounced alterations in the microstructure of the COL/SA film. At the same time, Fourier Transform Infrared (FTIR) spectroscopy revealed intricate structural interactions within the matrix, predominantly through hydrogen bonding. Furthermore, the freshness of the fresh basa fillet attributes during 12 days of refrigerated storage was monitored, and the evaluation depicted that the film with combined treatments (COL/SA/OPE/AgNPs) retards lipid oxidation, inhibits bacterial growth, and enhances the sensory score, effectively. These findings underscore the promising potential of these films as innovative and effective packaging materials.

[**Keywords**: Eel collagen, Enhanced storage, Orange peel extract, Packaging film, Silver nanoparticles, Sodium alginate]

Introduction

Food packaging disposal is a major cause of global environmental pollution. Recently, the persistent exploitation of imperishable petroleum-based plastics has induced a profound interest in the development of biodegradable edible food packaging films[1]. Various eco-friendly biopolymers, such as proteins and polysaccharides derived from natural sources, were examined due to their abundant availability, biodegradability, biocompatibility, low toxicity, and edibility[2]. Fish collagen has received considerable scientific attention due to its evident characteristics, including greater gelling, thickening, and film-forming properties[3]. Moray eel (*Gymonothorax reticularis)*, recognised as a nutrient-rich source, supports food security through its affordability and accessibility[4]. Eel processing yields a significant quantity of fish skin with high protein content (20 – 22 %), which is often discarded due to its thickness. Converting this co-product into collagen offers a valuable opportunity to enhance eel resource utilisation and maximise its potential. Indeed, eel collagen is well documented for its better thermal stability, good imino acid content, and higher yield than other collagen sources[5,6]. However, the higher hydrophilicity and lower viscoelasticity of the collagen film limit its application in food packaging[7]. To that end, blending different biodegradable materials offer more effective way to improve the physicochemical properties of collagen-based edible films.

Sodium alginate, a brown algae-derived polysaccharide, has been extensively investigated for composites with collagen due to its superior solubility, stability, viscosity, adaptability, and safe properties[8]. For collagen/sodium alginate films, functionalisation with sodium alginate improves the barrier and thermal properties of the pure collagen film by forming a dense polyionic complex by mixing counter-charged biopolymers[9]. However, there is still a need to enhance the mechanical and antimicrobial properties to elaborate on the practical applications of collagen-sodium alginate composite films.

At present, nanomodification technologies are widely used to mechanically reinforce protein-polysaccharide blend films by integrating nanoparticles, thereby enhancing the quality of food products by reducing post-processing contamination[10]. Among these, silver nanoparticles (AgNPs) are the most notable for their thermal stability and antibacterial properties and are Generally Regarded As Safe

(GRAS) under FDA regulations (2014)[11]. Also, regarding the toxic behaviour, recent reports reveal that plant-mediated green-synthesised AgNPs with particle sizes above 20 nm exhibit non-toxic behaviour and induce less migration, distinguishing them from chemically synthesised nanoparticles[12].

Furthermore, the biological performance of the film can be improved by the addition of natural polyphenolic substances. Orange peels represent a highly promising biofilter for polymer composites, offering numerous advantages such as widespread availability, cost-effectiveness, biocompatibility, and biodegradability. Approximately 70 % of oranges are processed into juice or jam, resulting in significant quantities of orange peel that generate abundant waste material and pose substantial disposal and environmental challenges. Recent studies found that orange peel extract comprises many valuable polyphenolic compounds with anti-inflammatory, anti-atherosclerosis, and anti-carcinogenic activity, and the quinic acid, a major compound in dried orange peel extract, contains a hydroxy group, which accounts for its potential as a green and innocuous antioxidant additive[13].

Thus, the key intent of this study is to evaluate the reinforcement capability of green-synthesised silver nanoparticles on a fish collagen/sodium alginate composite film with orange peel extract as an antioxidant agent. The study also aimed to assess the impact of an eel collagen-based packaging film on the stability of basa fillets stored in refrigerated conditions.

Material and Methods

Extraction of acid-soluble collagen

The fresh skins of *Gymnothorax reticularis* (Moray eel) were obtained from a local fishing harbour, chopped into smaller pieces and then mixed with 0.1 M NaOH (1:10 w/v)[14]. The pre-treated skin was placed in 0.5 M lactic acid (1:10 w/v) at 4 °C and homogenised continuously for 36 h with a magnetic stirrer. It was filtered using a muslin cloth, and the solution thus obtained was centrifuged (10,000 g, 30 min, 4 °C). The supernatant was precipitated with 0.9 M NaCl in the presence of tris hydroxymethyl amino methane (pH 7.5) at a concentration of 0.05 M, and the precipitate was then freeze-dried to obtain the collagen powder for film preparation.

Biosynthesis of silver nanoparticles from cashew apple fruit extract

Silver nanoparticles with a spherical structure and an average size of 20 – 60 nm were synthesised from *Anacardium occidentale* (cashew apple) by adding an aqueous fruit extract to a 1 mM solution of silver nitrate (purchased from Sigma-Aldrich) at a 1:10 ratio[15]. The solution was incubated in the dark before being centrifuged for 10 min at 10,000 rpm to recover the nanoparticles. The supernatant was decanted, and the precipitate was redispersed in 15 mL milli-Q water. The progressive residue was centrifuged at 10,000 rpm for 30 min to remove the entire biomass after repeated washing with Milli-Q water. The precipitate that accumulated at the bottom of the centrifuge tube was carefully collected and then lyophilised to obtain powdered nanoparticles.

Formulation of different COL/SA films

A simple casting method was employed to formulate eel skin collagen/sodium alginate-based film using glycerol as a plasticiser[16]. Eel collagen (2 % w/v) and sodium alginate (2 % w/v) were dissolved in acetic acid and distilled water, respectively. Mixed the polymeric solution (2:3) under vigorous magnetic stirring for 24 h to obtain a collagen/sodium alginate mixture (COL/SA). Subsequently, OPE (1 wt% of the polymer) and or AgNPs (2 w% of the polymer) were added to the above-obtained polymer mixture for the preparation of COL/SA/OPE, COL/SA/AgNPs and COL/SA/OPE/AgNPs composite films. The film-forming solutions were poured onto Teflon-coated glass plates (24 cm x 30 cm) and dried for 48 h in a hot-air oven. The dried films (Fig. 1) were removed from the plates and conditioned for at least 48 h in a

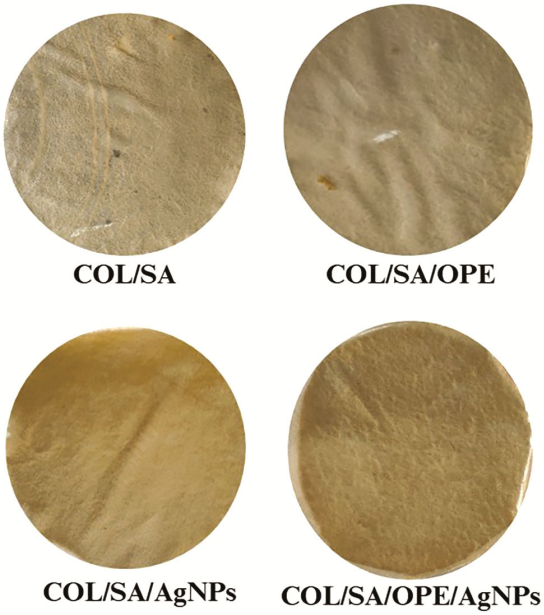

Fig. 1 — Visual images of COL/SA-based biocomposite film

humidity chamber at 25 °C and 50 % RH before characterisation.

Characterisation of different COL/SA films

Film thickness and mechanical properties

The thickness of film samples was measured using a micrometre (Yuzuki 0 – 25 mm EM02) with 0.01 mm accuracy. The average of six random measurements was taken (from the centre point to the edge) to evaluate transparency and mechanical parameters.

The ASTM D882-91 method was employed for the measurement of Tensile Strength (TS) and Elongation at Break (EB) of films using a UTM analyser (Shimadzu EZ Test EZ-LX, Trapezium Software). The rectangular sections of film specimens were made with a baseline standoff distance and a mechanical cross-head speed calibrated at 50 mm and 1 mm/s, respectively.

Water Vapour Permeability (WVP) and Water Solubility (WS)

The solubility of the samples was studied by drying the film pieces (2 × 2 cm) at 60 °C for 24 h in a hot air oven (KEMI KS.4D) and then weighing (W1). Immersed the dried film in distilled water at 25 °C, dried again, and reweighed (W2). The water solubility of the film was calculated via the following formula:

$$Soubility(\%) = \frac{W1 - W2}{W1} * 100$$

WVP of the film samples was measured gravimetrically according to the ASTM E96/E96M-16 method[17]. The test was carried out at 25 °C and 75 % RH in an air conditioning chamber.

Light transmission

The barrier property of each film sample (1 × 4 cm^2) against UV and visible light at wavelengths recorded from 200 to 800 nm was calculated using a UV-vis spectrophotometer (Hitachi UH5300).

Surface morphology and FTIR analysis

A scanning electron microscope (SEM, Joel JSM 5400) with an accelerating voltage of 12 – 15 kV was used to examine the surface morphology. Prior to SEM observation, the dry sample was sputter-coated with a thin layer of gold at 40 mA.

A Fourier Transform Infrared (FTIR) spectroscope equipped with an attenuated total reflectance system (Shimadzu IR) was used to analyse the interaction among the components in the film composition.

DPPH radical scavenging activity

The scavenging activity of the biocomposite film was measured using the DPPH method[18]. Film samples at varying concentrations (0.1 – 1.0 mg/ml) were incubated in the dark, and the absorbance was monitored at 517 nm using ultraviolet spectroscopy. Three replicates of measurements were taken.

Shelf-life evaluation of the fresh basa fillet treated with the prepared film

To evaluate the preservation effect of the developed biocomposite films, the fresh basa fillets purchased from the local market were packed with different treatments (the control, pure COL/SA film, COL/SA/AgNPs film, COL/SA/OPE film, and COL/SA/OPE/AgNPs film) and stored at 4 °C for 12 days.

Physiochemical analysis

The fish muscle was collected and ground in a mixer grinder for chemical analysis. A digital pH meter (Oakton pH 550, benchtop) was used to measure the sample pH during storage. Total Volatile Basic Nitrogen (TVB-N) values for fillet samples were assessed using the microdiffusion method[19]. Then, the samples underwent peroxide value determination according to the AOAC (1975) method[12].

Microbiological analyses

To calculate the total plate count, 25 g of the sample was weighed and added to 225 ml of 0.85 % saline solution to make a 1:10 (w/v) dilution. Then, using 0.85 per cent saline, serial dilutions of the bacterial suspensions were made, and inoculum (0.1 ml) from the respective dilution was pipetted out and spread on nutrient agar based on the spread plate method, incubated at 37 °C for 24 h, after which the colonies were counted[18].

Sensory evaluation

A 9-point hedonic scale was employed to evaluate the sensory attributes of the samples, with colour, odour, texture, flavour, and general acceptability as parameters. The samples were marked with unique three-digit numbers and presented to panel members on clean stainless-steel trays at room temperature, and a sensory score below 5 indicated the rejection of the sample in terms of shelf life.

Statistical analysis

All the experiments were conducted in triplicate, and the results were reported as mean ± standard

deviation. Statistical analysis was conducted using SPSS Version 22.0, with one-way ANOVA and Tukey's tests. Statistically significant was considered at $p < 0.05$.

Results and Discussion

Characterisation of biocomposite film

Thickness and mechanical properties

Thickness is a vital parameter for packaging films to determine physical and mechanical properties. As depicted in Table 1, COL/SA film is the thinnest (71.66±0.18) among the prepared films. The presence of OPE and AgNPs causes an increment in the thickness in a significant manner ($p < 0.05$), which could be related to the occurrence of soluble and insoluble fibres in orange peel extract and increased solid mass ratio in the respective film matrix[18]. Comparable findings were observed in the composite film of chitosan and polyvinyl alcohol incorporated with orange peel[13].

The TS and EAB reflect the packaging film's ability to maintain mechanical integrity without damage during its application. The effects of AgNPs and OPE with different contents on the film's mechanical strength were investigated, and the results are shown in Table 1. COL/SA film exhibited TS and EAB values of 20.57±0.17 MPa and 69.12±0.11 %, respectively. However, AgNP incorporation into the composite film enhances the tensile strength and reduces elongation at break. This is probably due to the uniform dispersion of nanoparticles in the polymeric matrix, which strengthens the film network[20]. Conversely, it was observed that orange peel extract-incorporated films showed a slight reduction in TS and enhancement in EAB. The presence of orange peel extract in the polymer solution causes microstructural discontinuities and weakens bonding between collagen (COL) and Sodium Alginate (SA). This results in partial ruptures of the film network and increased flexibility of the composite film[21]. For the COL/SA/OPE/AgNPs film, better mechanical characteristics could be attributed to intermolecular interactions among the COL/SA matrix, glycerol, silver nanoparticles, and OPE.

Water solubility and water vapour permeability

As presented in Table 1, the COL/SA/OPE film showed a higher water solubility of 44.22±0.68 % than the COL/SA film, which could be because the hydrophilicity of the phenolic compounds present in the OPE was stronger than that of collagen and sodium alginate molecules, which is consistent with the findings of Du *et al*.[2]. In contrast, AgNPs incorporation significantly ($p < 0.05$) enhances the water resistance of the COL/SA control film strengthening the intermolecular interactions among the polymers, OPE, and the nanoparticle, reducing the compound's hydrophilicity in the film matrix.

WVP is a key property of packaging materials, reflecting their effectiveness as barriers to water vapour transmission, and a minimal WVP value is typically crucial for ensuring the efficacy of food packaging. As shown in Table 1, COL/SA showed the highest WVP, attributed to the presence of hydrophilic groups in its structure[22]. COL/SA/AgNPs film has a moderate value because nanoparticles obstructed the water vapour pathways and altered the polymer matrix at interfacial regions[23]. Additionally, there was a significant decrease ($p < 0.05$) in the WVP after the incorporation of OPE, which could be related to the existence of aromatic rings in the phenolic structure of OPE that hinders the internal network of the film matrix and thus diminishes the affinity for water vapour[24]. Notably, in the COL/SA/AgNPs/OPE film, the pronounced interaction of AgNPs and OPE with the hydrophilic groups of polymers in the composite creates a dense network structure and minimises the availability of hydrophilic groups for water vapour sorption[25].

Microstructure and FTIR analysis

SEM analysis was conducted to analyse the microstructural alterations in the COL/SA film after blending with silver nanoparticles and orange peel extract, and the corresponding cross-sectional images

Table 1 — Thickness, tensile strength, elongation at break, water solubility, water vapour permeability of COL/SA based biocomposite films

Film	COL/SA	COL/SA/OPE	COL/SA/AgNPs	COL/SA/OPE/AgNPs
Thickness	71.66±0.18^{a}	72.33±0.17^{b}	74.66±0.31^{c}	77.66±0.18^{d}
TS (MPa)	20.57±0.17^{a}	23.76±0.03^{b}	23.97±0.08^{b}	24.57±0.07^{c}
E (%)	69.12±0.11^{a}	56.58±0.06^{c}	60.69±0.08^{b}	59.46±0.09^{c}
WS (%)	40.09±0.37^{b}	44.22±0.68^{a}	36.31±0.33^{c}	31.65±0.13^{d}
WVP (× 10−10 gm−1 s−1 Pa−1)	6.12 ± 0.11^{a}	5.16 ± 0.12^{c}	6.02 ± 0.23^{b}	5.11 ± 0.27^{c}

(Fig. 2). The COL/SA film has a compact and uniform structure with no evident phase separation, signifying the compatibility of the polymers and glycerol in the film matrix. In contrast, the functionalisation of silver nanoparticles in COL/SA-based biocomposite films resulted in white patches and changed the surface to a coarse, heterogeneous one. However, a few white spots dispersed across the surface of the films were observed after the addition of OPE, likely a result of the insoluble particles embedded in the film-forming solution. The more uneven, rough morphology in COL/SA/AgNPs and COL/SA/OPE/AgNPs could be attributed to silver nanoparticles aggregation. This is consistent with the light-transmittance analysis of the biocomposite film. The obtained results corroborate the findings of Valdé *et al.*[26].

FTIR spectroscopy was employed to examine the interactions between the polymer matrix and the additives in COL/SA-based biocomposite films (Fig. 3). The FTIR spectra of COL/SA control film showed characteristic peaks at ~ 3251 cm^{-1} (O–H stretching), 2990 cm^{-1} (CH stretching), 2318 cm^{-1} (O=C=O stretching), 1645 cm^{-1} (C=C stretching), 1482 cm^{-1} (CH bending), 1147 cm^{-1} (S=O stretching), and 1017 cm^{-1} (C–O stretching). This could be due to the strong intermolecular interaction between collagen and sodium alginate[9], which was in line with the SEM observations. Notably, when AgNPs and/or OPE were incorporated, some variations arose in the position of the peak intensity of COL/SA film. While, in COL/SA/AgNPs, the bands of O–H stretching and O=C=O shifted to 3219 and 2453 cm^{-1}, respectively. This suggested a strong electrostatic interaction between the functional group of the polymers and the nanoparticles[27]. For COL/SA/OPE, the variation in band intensity was observed at 3835 cm^{-1} and 2655 cm^{-1} relative to the control film. Finally, the O–H stretching intensity showed the highest band at of 4384.39 cm^{-1} in the COL/SA/OPE/AgNPs film,

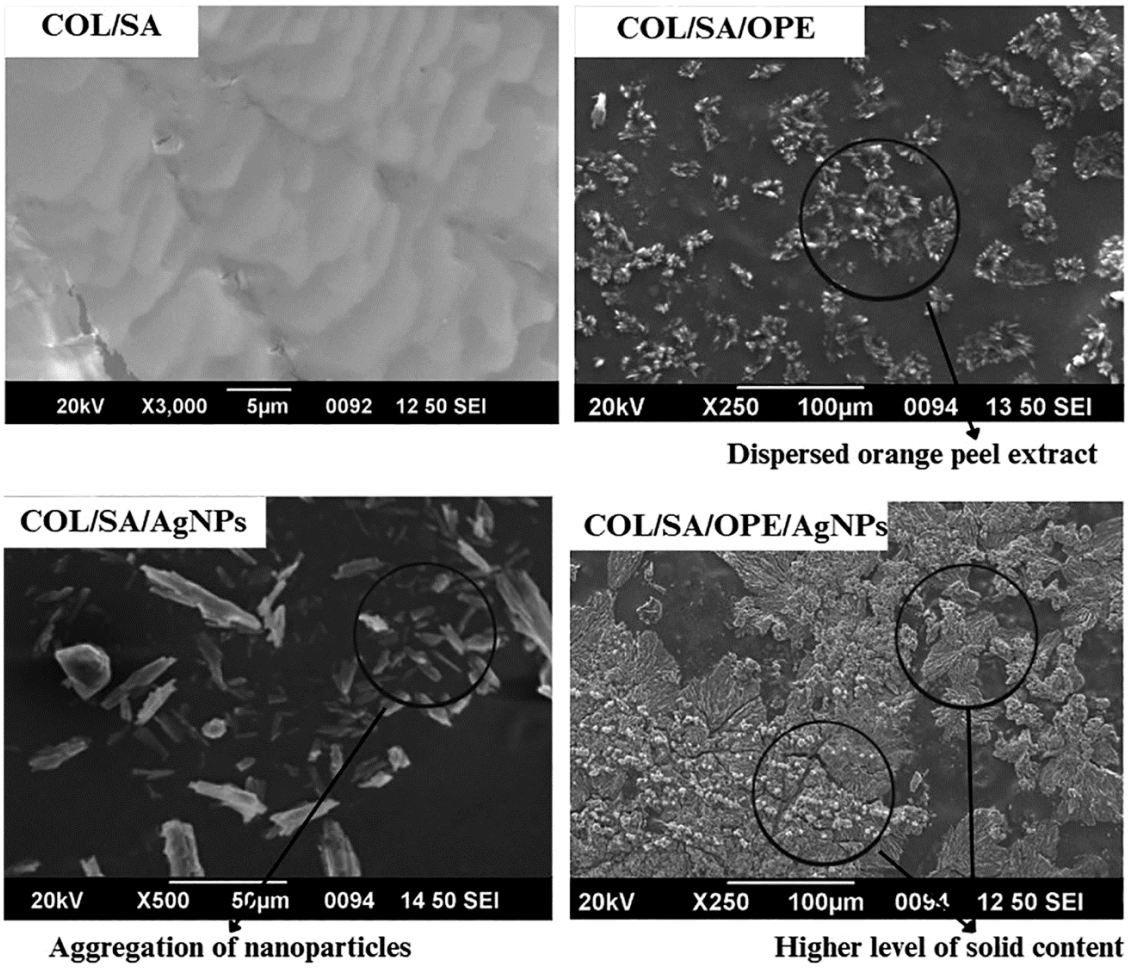

Fig. 2 — SEM images of COL/SA-based biocomposite film

indicating good miscibility between collagen, sodium alginate, silver nanoparticles, and orange peel extract.

Light transmission

Transparency is a vital characteristic of food packaging applications. It is essential to maintain an optimal balance in transparency, as overly opaque films can hinder consumers' ability to see the package's contents, while excessive transparency may compromise protection against external factors. As depicted in Figure 4, COL/SA film had the highest level of transparency (8.9 %) in a significant manner ($p < 0.05$). Adding AgNPs and OPE significantly reduced the transparency of the biocomposite film. This reduction is attributed to the light-scattering properties of the additives within the film matrix, which diminish the amount of light passing through the films. Furthermore, the increased solid content and the formation of a cohesive network among the biopolymers, nanoparticles, and peel extract contribute to this decrease in transparency. These findings are consistent with previous studies on

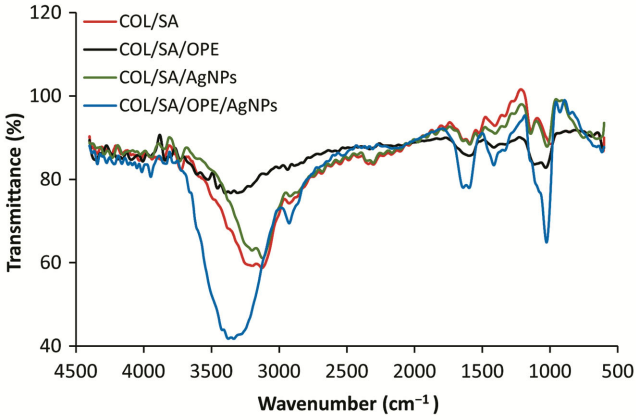

Fig. 3 — FTIR spectra of COL/SA-based biocomposite film

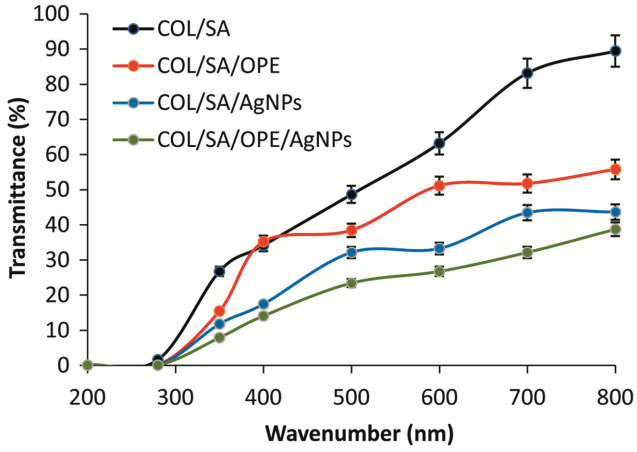

Fig. 4 — Light transmission in COL/SA-based biocomposite film

carboxymethyl cellulose and myrrh gum-based biocomposites, which incorporated TiO_2 nanoparticles and dill essential oil[16].

Antioxidant activity

Free radical scavenging activity of packaging film is significantly important to retard lipid peroxidation and delay deterioration due to oxidative processes, thereby retaining the quality of food products. As shown in Figure 5, the DPPH radical scavenging ability of COL/SA control film was calculated to be 25.49±0.13 %, indicating its deprived antioxidant property. No significant changes in the antioxidant capability of the COL/SA film were observed after incorporating AgNPs. Likewise, a similar observation was reported by Orsuwan *et al.*[28] in silver nanoparticle-reinforced agar/banana powder blend films. Conversely, COL/SA/OPE presented significantly ($p < 0.05$) higher scavenging activity because of the presence of phenolic compounds in OPE. Notably, COL/SA/OPE/AgNPs showed slightly lower antioxidant activity than COL/SA/OPE, which could be related to the antagonistic interaction among the COL, SA, AgNPs, and OPE. Previous literature has shown the presence of various polyphenols such as limonene, α-farnesene, and myrcene, contributing to the excellent scavenging ability of orange peel extract[13].

Performance of different biocomposite films on fish preservation

Biochemical analyses

Regarding the pH of the basa fillet, the initial value was 7.03. In the first days of storage, there was a

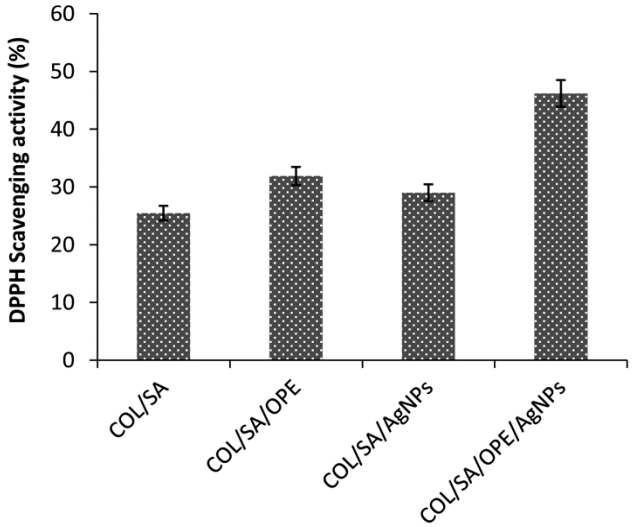

Fig. 5 — Antioxidant activity of COL/SA-based biocomposite films

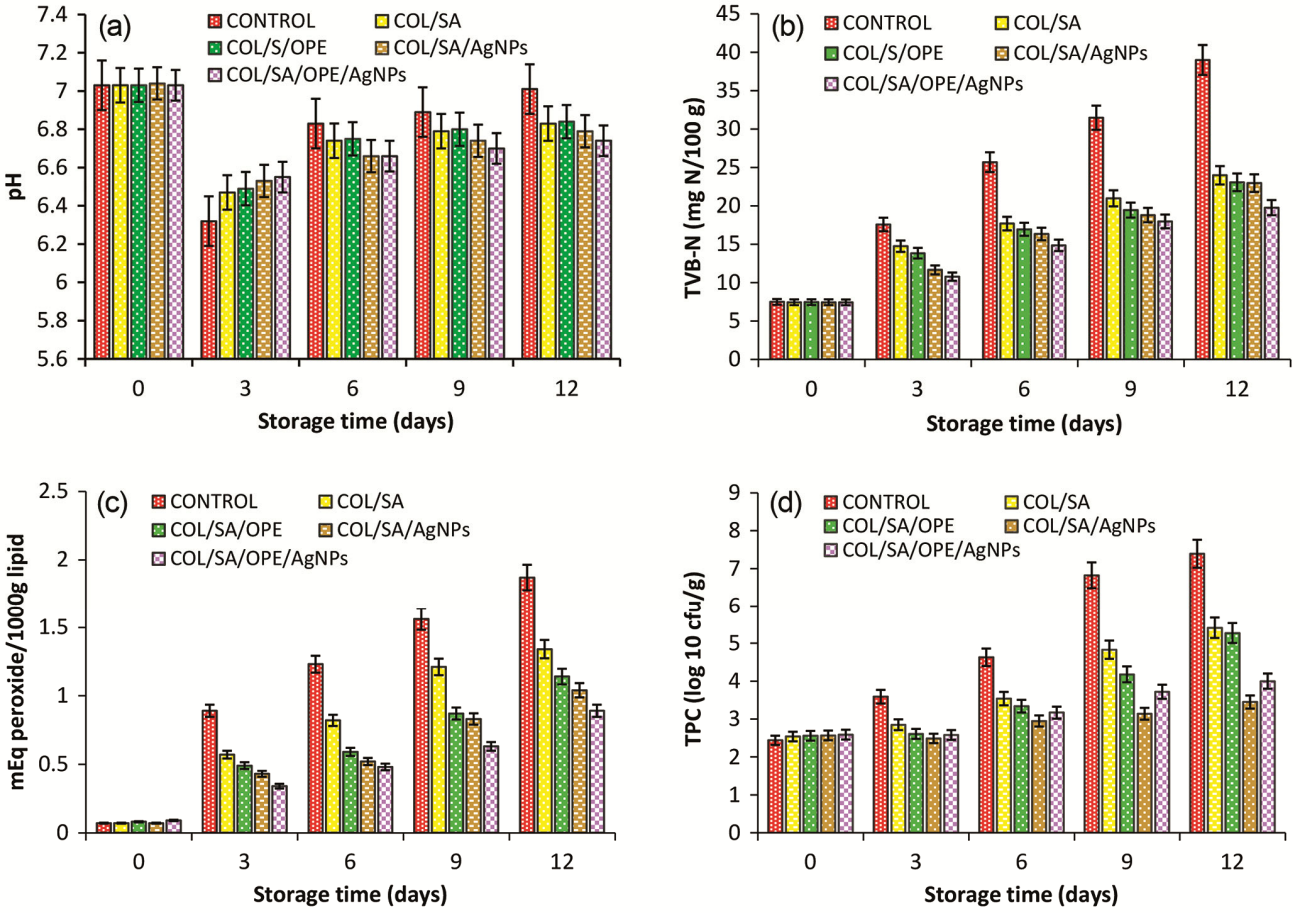

Fig. 6 — Changes in (a) pH, (b) TVB-N, (c) PV, and (d) TPC values of basa fillet during refrigeration at 4 °C

slight decrease in the pH value due to the occurrence of glycolysis, and it reached 6.33 for treated samples (Fig. 6a). In the latter stage, pH rises as a result of the accumulation of volatile compounds, which causes a negative impact on the sensory quality during storage. The combined antimicrobial and water resistance properties of silver nanoparticles and orange peel extract in COL/SA/OPE/AgNPs-treated samples help reduce pH increases and associated bacterial metabolism, thereby maintaining the quality of the fillet for longer.

Variation in TVB-N content of the samples is illustrated in Figure 6(b) in which TVB-N content of control significantly ($p < 0.05$) exceed the tolerable level (25 mg N/100 g) at 6th day of storage; while among the treated samples, COL/SA, COL/SA/OPE and COL/SA/OPE/AgNPs achieved the limit at the 9th and 12th days of storage, respectively, considered as spoilage. The results demonstrated superior antimicrobial and antioxidant activities of OPE and AgNPs, which could inhibit microbial growth by deaminating non-protein nitrogenous compounds and thus prevent enzymatic breakdown. This is consistent with the findings of bio-nanocomposite for preserving *Cyprinus carpio* meat quality[16].

The effects of different treatments on PV content in the samples throughout the storage are presented in Figure 6(c). The initial PV value was 0.07 meq peroxide/ kg of lipid, which increased ($p < 0.05$) in the control and reached a maximum on day 12 (1.87 meq peroxide/ kg of lipid). However, for the treated samples, the PV increase was slower than in the control during storage, although the values for all the samples were lower than 10 meq/ kg of lipid, regarded generally as the acceptable level[29]. It can be concluded that phenolic compounds from orange peel extract components in COL/SA/OPE and COL/SA/OPE/AgNPs could act as metal chelators, electron donors, and UV–visible light barriers to prevent lipid oxidation in fish.

Microbiological analysis

The Total Plate Count (TPC) of samples wrapped with formulated film on the 0th day was 2.54±0.02

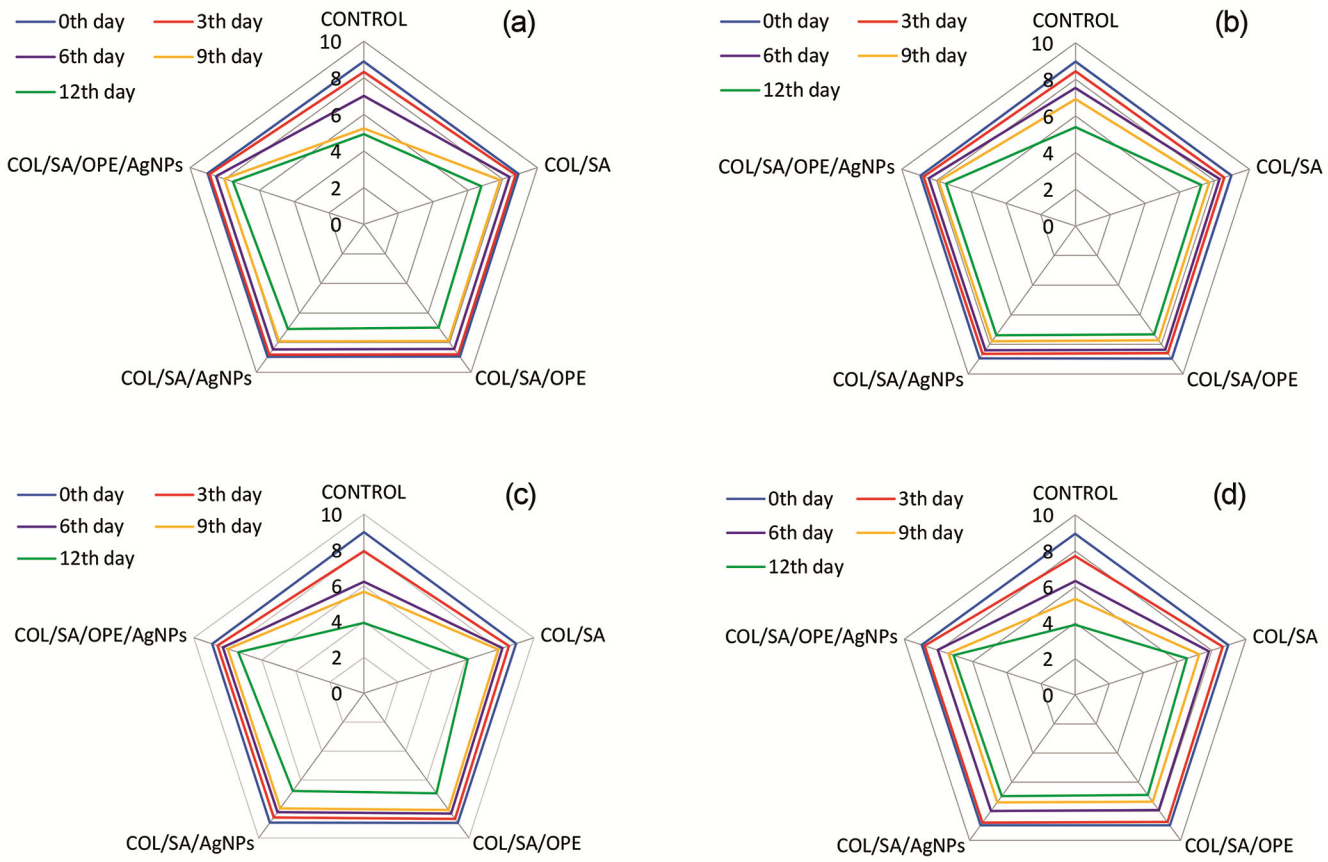

Fig. 7 — Sensory attributes score for (a) Texture, (b) Colour, (c) Odour, and (d) Overall acceptability of basa fillet during refrigeration at 4 °C

$\log_{10}$ CFU/g, indicating the fresh quality of the basa fillets (Fig. 6d). By day 6 of storage, TPC in the control sample exceeded the utmost objectionable limit of 7 $\log_{10}$ CFU/g. Moreover, the high microbial load in the COL/SA-treated sample throughout the storage period could be due to the absence of antimicrobial agents[30]. The sample treated with the COL/SA/OPE/AgNPs composite film had the lowest TPC value compared with other treatments ($p < 0.05$), indicating that the synergistic effect of OPE and AgNPs is more effective in antimicrobial action than OPE and AgNPs alone. The antioxidant characteristics of OPE are attributed to its content of many polyphenols, including anthocyanins, flavonoids, tannins, and procyanidins, which show antibacterial effects[1].

Sensory analysis

As depicted in Figure 7, OPE and AgNPs incorporated biocomposite films maintain sensory characteristics over storage. Panellists rejected the control samples on the 6th day due to putrid odour, inflexible texture, and overall unacceptability, reflected in sensory scores dropping below 5[(ref. 31)]. Underlining the positive correlation between sensory scores, TVB-N counts, and microbial spoilage, the COL/SA/OPE/AgNPs composite film achieved the highest overall liking score. This emphasises the synergistic effect of the antioxidant properties of bioactive polyphenols in the orange peel extract and the antibacterial properties of silver nanoparticles, which lowers the rate of microbial spoilage and thus preserves sensory parameters, ensuring quality maintenance.

Conclusion

In summary, the study successfully developed an eco-friendly biocomposite film consisting of fish collagen, sodium alginate, silver nanoparticles, and orange peel extract to preserve the freshness of the basa fillet. The COL/SA/AgNPs/OPE film demonstrated notable mechanical strength, excellent light transmission, and superior physical properties, as evidenced by FTIR spectra. The incorporation of OPE

and AgNPs significantly enhanced the film's antioxidant properties. These COL/SA-based biocomposite films effectively extended the preservation period of treated products by delaying lipid peroxidation and inhibiting microbial proliferation during 12 days of refrigerated storage conditions. Therefore, the potential of the fabricated biocomposite film was suggested as an effective packaging material for fresh fish preservation. The study also underscores the efficiency of converting marine disposal into valuable products to achieve sustainability and a circular economy. Further research is required to assess the biological behaviour of the biocomposite film over time in terms of cytotoxicity and migration studies, to properly optimise production parameters for scale-up at a low cost.

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Conflict of Interest

No potential conflict of interest.

Author Contributions

AA: Writing – original draft, Formal analysis, Data curation. SRRR: Writing – review & editing, Supervision, Conceptualization. AF: Writing – original draft, Formal analysis. RR: Writing – review & editing, Formal analysis.

Ethical Statement

This study did not involve endangered or protected species, and no specific ethical approval was required

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