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Quality improvement of refrigerated sardine fillets using shrimp waste-based edible coatings

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ABSTRACT

The development of sustainable packaging solutions is becoming increasingly important for preserving food quality, minimizing waste and promoting the valorisation of food industry waste/by-products. The aim of this study was to develop an astaxanthin-rich extract from red shrimp (*Aristaeomorpha foliacea*) waste for incorporation into edible chitosan-based coatings. The effectiveness of coatings with and without astaxanthin in maintaining the quality and stability of fresh sardine fillets (*Sardina pilchardus*) during cold storage was evaluated to support circular economy strategies in the seafood sector. The fillets were coated with two formulations: chitosan alone (Ch) and chitosan with 2.5 % (v/v) astaxanthin extract (Ch+Asx). A control sample without coating was also included. All samples were packed in air in polypropylene trays and stored at 4°C for 13 days and periodically analysed for headspace-gas composition, pH, water activity, colour, texture, lipid oxidation and microbial growth. The shrimp shell extract had a carotenoid content of 443.5 μg astaxanthin/g and showed strong antioxidant capacity (DPPH=77.8 %; TEAC=576.3 μmol TE/mL). Both edible coatings were able to slow down sardine deterioration compared to the control, with the Ch+Asx one showing the greatest effect in reducing microbial load (*Enterobacteriaceae:* 2.9 vs 6.3 log CFU/g; *coliforms*: 1.5 vs 5.9 log CFU/g after 13-days) and maintaining colour and texture. Lipid oxidation was significantly lower in both coated samples. These findings highlight the potential of using chitosan-based coatings enriched with natural antioxidants from shrimp waste as a promising solution for extending the shelf life of fresh seafood products while supporting waste valorisation and sustainable processing.

1. Introduction

Fish and seafood are highly nutritious foods, but they are also among the most perishable products. Their rapid deterioration is mainly due to intrinsic properties such as high-water activity, near-neutral pH and high polyunsaturated fatty acid content, which make them particularly susceptible to microbial spoilage, enzymatic degradation and lipid oxidation (Kurek et al., 2024; Mozzon et al., 2024).

This perishability limits their marketability in fresh or minimally processed form and contributes significantly to food waste along the supply chain, especially for small pelagic species such as sardines. The European sardine (*Sardina pilchardus*) is one of the most abundant small pelagic fish species in the Mediterranean and Black Sea, with average annual landings of about 141,400 tonnes in 2020–2021 (FAO, 2023). Sardines have been a staple food in the Mediterranean diet since ancient times and still play a central role in the culinary traditions of coastal regions today (Mozzon et al., 2024). Despite their cultural importance and high nutritional quality, sardines are still often considered an undervalued species (Mozzon et al., 2024). They are often landed in large quantities and marketed at lower prices compared to other fish species (Pincinato & Asche, 2018). The lack of advanced preservation and packaging techniques hinders their commercialisation in formats that meet modern consumer demands for convenient, minimally processed and sustainable seafood.

Although traditional methods such as canning, air-drying and dry salting are still widely used to preserve sardines (Mozzon et al., 2024),

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there is a growing demand for innovative strategies that maintain freshness and nutritional quality while minimising environmental impact. In this context, attention is increasingly focused on edible coatings that can be applied directly to food surfaces as primary packaging in combination with conventional secondary packaging to extend shelf life and reduce food waste (Mussagy et al., 2023). These combined systems have shown promising results in extending shelf life, particularly for perishable products such as fresh or minimally processed fish, by reducing microbial growth and oxidative degradation (Kurek et al., 2024). While edible coatings are not new to the food industry, their large-scale application remains limited by technical and economic challenges (Kurek et al., 2024). Nevertheless, they continue to generate interest as multifunctional tools that improve food safety, quality and presentation while meeting consumer preferences for natural and environmentally friendly solutions (Kurek et al., 2024).

This preservation approach has already been tested on fresh sardines in some research studies with encouraging results (Bouhanna et al., 2021; Gogus et al., 2006; Gómez-Estaca et al., 2007; Homayonpour et al., 2021; Homayounpour et al., 2020; Kanelaki et al., 2022; Mohan et al., 2012). Early research has shown the benefits of natural wax coatings in combination with lactic acid and nisin in controlling spoilage bacteria in sardines (Gogus et al., 2006), followed by the successful use of chitosan-based coatings on Indian sardines (Mohan et al., 2012). More recent work using nanochitosan-based coatings and cumin extract showed improved antioxidant and antimicrobial activity (Homayonpour et al., 2021; Homayounpour et al., 2020). Other innovations include hybrid gels and gelatin films enriched with plant extracts such as rosemary and *Arbutus unedo*, which were able to slow down the spoilage of sardines (Bouhanna et al., 2021; Kanelaki et al., 2022). Despite these advances, research specifically targeting fresh sardines is relatively scarce compared to studies on other fish species (Cetinkaya & Wijaya, 2024; Eranda et al., 2024; Kurek et al., 2024).

From the above studies, it appears that a promising strategy to improve the functional performance of edible coatings is to activate them with bioactive compounds, particularly natural antioxidants derived from food processing waste and by-products (Kurek et al., 2024). Among these, astaxanthin stands out as a potent carotenoid with strong antioxidant properties that can be extracted from crustaceans or produced by plants, algae, bacteria and fungi. Astaxanthin is a keto-carotenoid derived from β-carotene and is widely appreciated in food applications due to its natural origin, non-toxicity, high versatility and both hydrophilic and lipophilic characteristics (Sila et al., 2015). In crustaceans, it represents between 74 % and 98 % of total pigments in the shell, although this proportion can vary depending on the species and environmental conditions (Sowmya & Sachindra, 2012; Rossi et al., 2024).

Globally, shrimp and shrimp-derived products are among the most consumed seafood. Shrimp production reached 5.03 million tonnes in 2020 and is expected to increase to 7.28 million tonnes by 2025, driven by high consumer demand and the nutritional value of this product. Although shrimp are among the most common food allergens, mainly due to proteins such as tropomyosin and arginine kinase (Chen et al., 2025), their production and consumption continue to grow worldwide, leading to large amounts of side streams: processing residues such as heads and body shells can account for up to 50 % of the total shrimp biomass (Nirmal et al., 2020). These by-products are increasingly studied for their valorisation through sustainable and innovative extraction of high-value compounds such as chitin, proteins and especially astaxanthin, thus contributing to circular economy initiatives and reducing the environmental impact (de Aguiar Saldanha Pinheiro et al., 2023; Gómez-Estaca et al., 2015, 2017; Kaya et al., 2024; Rossi et al., 2024; Sachindra et al., 2006; Tran et al., 2025). *In vitro* and *in vivo* studies have shown that astaxanthin exhibits antioxidant activity up to ten times greater than other molecules such as polyphenols, cantha-xanthin and β-carotene, by quenching free radicals and reactive oxygen species (Sila et al., 2015). Moreover, its antioxidant activity has been studied in membrane systems such as liposomes, where it has shown strong potential (Sowmya & Sachindra, 2012). Beyond food preservation, astaxanthin is also being studied for its potential role in the prevention of chronic diseases including cardiovascular disorders, certain cancers and diabetes (Sila et al., 2015).

The incorporation of astaxanthin into food packaging materials has also shown the potential to increase antioxidant activity and extend the shelf life of various food products (Chandra Roy et al., 2021; Colín-Chávez et al., 2013; Hajji et al., 2018; Inthamat et al., 2024; Mussagy et al., 2023; Samsudin et al., 2014; Sanches-Silva et al., 2013; Xu et al., 2020; Yao et al., 2025). For instance, food packaging film based on chitosan and enriched with astaxanthin-containing natural deep eutectic solvents (NaDES) showed stronger free radical scavenging activity and better mechanical stability (Chandra Roy et al., 2021). In another study, astaxanthin nanoemulsions were incorporated into pullulan or gellan gum films to preserve strawberries (Yao et al., 2025). Inthamat et al. (2024) used chitosan-astaxanthin films cross-linked with genipin to improve the water resistance and oxygen barrier properties of Kraft paper. Bioactive films based on microbial astaxanthin and eutectic solvents have also been shown effective for highly perishable fruits, offering a more sustainable alternative to synthetic plastics (Mussagy et al., 2023). These findings support the idea of combining chitosan and astaxanthin to create active coatings with enhanced antioxidant and antimicrobial effects and reduced environmental impact.

Therefore, the aim of the present study was to obtain a concentrated carotenoid extract rich in astaxanthin from red shrimp (*Aristaeomorpha foliacea*) waste to be used to enrich a chitosan-based edible coating. Two types of edible coatings, one with and one without astaxanthin, were developed to evaluate their effectiveness in preserving the quality characteristics and stability of fresh sardine fillets (*S. pilchardus*) during cold storage. This approach represents a good strategy to promote the principles of circular economy, waste valorisation and food waste reduction in the seafood sector.

2. Materials and methods

2.1. Extraction of carotenoids from red shrimp waste and analytical characterization

2.1.1. Preparation of red shrimp waste

Approximately 3 kg of red shrimps (*Aristaeomorpha foliacea*), caught in the Mediterranean Sea, were obtained from a local fish processing plant Ecopesce S.R.L. SB (Cesenatico, FC, Italy) and transported to the laboratory under frozen conditions (–4 °C). After thawing, the shrimps were processed manually by removing the heads and peeling the body shells from the legs to the tail. The shrimp waste (shells and heads) was rinsed under running water, frozen at –40 °C for 48 h and then freeze-dried using an LIO2000P unit (5 Pascal, Italy). The freeze-dried material was finely ground using a food processor (Bimby TM31, Vorwerk, Italy) and stored at –20 °C for a maximum of one week until further extraction.

2.1.2. Carotenoid extraction

Carotenoid extraction was performed according to the solvent-based method described by de Aguiar Saldanha Pinheiro et al. (2023) using 40 g of freeze-dried red shrimp waste suspended in 400 mL of ethanol (1:10 w/v ratio; Carlo Erba Reagents, Italy). The mixture was magnetically stirred for 30 min at room temperature and then allowed to settle for 10 min. The supernatant was carefully collected to avoid the sediment, centrifuged at 4000 × g for 10 min at 4 °C (AvantiTM J-25, Beckman Coulter, USA) and subsequently filtered through filter paper (Whatman No. 1).

The resulting ethanolic extract was concentrated under a stream of nitrogen at room temperature for 4 h and then reconstituted in a quarter of the original ethanol volume to increase the carotenoid concentration. The remaining extract was stored at –40 °C until analysis and further

use.

The extraction procedure was carried out in triplicate.

2.1.3. Total carotenoids expressed as astaxanthin

The determination of the concentration of total carotenoids, reported as astaxanthin, was carried out by spectrophotometric analysis using a spectrophotometer UV-1601 (SHIMADZU, Japan) set at a wavelength of 470 nm by measuring the absorbance of the diluted shrimp waste extract. The concentration of total carotenoids expressed as astaxanthin was calculated using the following equation (Hajji et al., 2018; Radzali et al., 2014; Sachindra et al., 2006):

$$\text{Carotenoid yield (μg astaxanthin/g shrimp waste)} = (A_{470} \times V_{extract} \times \text{dilution}) / (W_{sample} \times 0.2) \quad (1)$$

where A_{470} is the maximum absorbance of the extract, $V_{extract}$ is volume of extract in mL (i.e., 400 mL), W_{sample} is the weight of dried and powdered shrimp waste in g (i.e., 40 g) and *0.2* is the absorbance at 470 nm of 1 μg/mL standard astaxanthin. The analysis was performed in triplicate.

2.1.4. Antioxidant activity assays

The antioxidant activity of the extract was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl, Glentham Life Sciences, UK) and ABTS·⁺ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Sigma-Aldrich Chemical Co., USA) radical scavenging assays, according to the methods described by Liu et al. (2008) and Re et al. (1999), respectively, with slight modifications.

For the DPPH assay, 100 μL of sample were mixed with 2900 μL of DPPH solution (0.5 mM in ethanol). The mixture was incubated in the dark for 30 min and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (UV-1601, SHIMADZU, Japan). The control consisted of 100 μL ethanol and 2900 μL DPPH solution. The *DPPH radical inhibition* (%) was calculated using the equation:

$$DPPH\ radical\ inhibition\ (\%) = (ABS_{control} - ABS_{sample})/ABS_{control}) \times 100 \quad (2)$$

where $ABS_{control}$ and ABS_{sample} are the absorbance of the control and sample solutions, respectively.

For the ABTS·⁺ assay, the radical cation was generated by mixing ABTS stock solution (7 mM) with potassium persulfate (2.45 mM) and incubating in the dark for 12–16 h. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.01 at 734 nm. Then, 20 μL of sample were added to 2 mL of ABTS·⁺ solution. After 6 min, the absorbance was recorded at 734 nm using a UV-Vis spectrophotometer. The control consisted of 20 μL of ethanol and 2 mL of ABTS·⁺ solution. The results were expressed as μmol Trolox equivalents (TE)/mL of extract, based on a standard calibration curve (50–400 μM Trolox in ethanol).

Both measurements were performed in triplicate.

2.2. Development of active edible coating solutions

Edible coating solutions were prepared under sterile conditions in a laminar flow hood using sterile materials to prevent microbial contamination. Two formulations were prepared: a chitosan-based coating (Ch) and an astaxanthin-enriched coating (Ch+Asx). The Ch formulation consisted of 1 % (w/v) commercial chitosan (derived from shrimp shells, ≥75 % deacetylated, Sigma-Aldrich, USA), 1 % (v/v) acetic acid, 0.3 % (w/v) Tween 80 as emulsifier and 0.5 % (w/v) glycerol as plasticiser. The Ch+Asx formulation contained the same components, with the addition of 2.5 % (v/v) concentrated carotenoid extract rich in astaxanthin. The chitosan-based formulation was adapted from Mohan et al. (2012), with modifications based on preliminary trials to optimise the coating application on raw sardine fillets.

To prepare the coating solutions, 1 g of chitosan was dispersed in 100 mL of sterile distilled water in a water bath at 40 °C for 5 min under magnetic stirring. Subsequently, 1 mL of acetic acid (Sigma-Aldrich Chemical Co., USA) was added, and the solution was stirred for an additional 30 min at the same temperature. The resulting mixture was then sonicated in an ultrasonic bath (DU-100S, ARGOLAB, Italy) at 30 °C for 5 min. After cooling to room temperature, 0.5 g of glycerol and 0.3 g of Tween 80 (Sigma-Aldrich Chemical Co., USA) were added and homogenized using magnetic stirring. In the case of the Ch+Asx formulation, 2.5 mL of concentrated carotenoid extract was incorporated into the final solution.

Each formulation was prepared in a total volume of 1 L and stored in sterile glass bottles at 4 °C for a maximum of 2 h before use.

2.3. Fresh sardine sample preparation

A batch of 4 kg of fresh sardines (*Sardina pilchardus*), mechanically filleted and refrigerated, was provided by Ecopesce S.R.L. SB (Cesenatico, FC, Italy). The fish were caught in the Adriatic Sea by trawling and filleted on the same day. The resulting fillets had an average length of 8.5 ± 0.5 cm and an average weight of 10 ± 2 g. A portion of the fillets was coated with the two edible chitosan-based coatings (samples Ch and Ch+Asx), while the remaining uncoated fillets served as a control group.

The coatings were applied to the fresh sardine fillets by dipping method. Each fillet was fully immersed in 200 mL of the respective coating solution for 30 s, followed by 15 s of draining. To maintain consistent application and avoid cross-contamination, the coating solution was replaced with a fresh one after every 20 sardine fillets. The coated fillets were then placed on sterile grids and dried in a refrigerated chamber at 4 ± 0.5 °C for 30 min. Dipping conditions were selected on the basis of preliminary tests.

All samples (control, Ch and Ch+Asx) were packed in polypropylene (PP) trays (15 × 10 cm with a height of 4 cm) sealed in air using a PP high-barrier film and a heat sealer (VGP 25, Orved, Italy). Each tray contained four fillets of a total weight of 37.50 ± 4.10 g and arranged in a single layer (Figure S1 in Supplementary Material). For each sample, 25 trays were prepared for the shelf life study, resulting in a total of 75 trays. On day 0, the sardine fillets were analysed immediately after application of the coating without packaging. For all subsequent time points (days 1, 2, 6, 9 and 13), five trays per sample group were randomly selected and used for analysis. All samples were stored at 4 °C for 13 days.

2.4. Analytical determinations during shelf life

2.4.1. Headspace gas composition

The percentage content of oxygen (O_2) and carbon dioxide (CO_2) in the package headspace was determined using a gas analyser (MFA III S/L, Witt-Gasetechnik, Germany). For each analysis, a gas sample was withdrawn from the headspace by puncturing the package with a needle, immediately before the opening.

Five measurements were performed for each sample and at each sampling time point.

2.4.2. Water activity (a_w)

Water activity (a_w) of the samples was measured at room temperature (21 ± 2 °C) using a thermoelectric dew point hygrometer (Aqualab Water Activity Meter, SERIES 3TE, Decagon Devices Inc., USA). The instrument was calibrated with two standard solutions of known a_w values: 0.760 and 0.900, with an accuracy of ± 0.003.

Three measurements were performed for each sample and at each sampling time point.

2.4.3. pH

pH measurement of the samples was performed using a pH-meter (FiveEasy Plus FP20, Mettler Toledo, Germany), calibrated with two buffer solutions at pH 4.01 and 7.00. Each sample (2 g) was diluted with 2 g of distilled water prior to measurement.

Three measurements were performed for each sample and at each sampling time point.

2.4.4. Colour analysis and image acquisition

The colour of the coating solutions and fish samples was determined with a spectrophotometer (ColourFlex EZ, HunterLab, USA) using D65 illuminant and 10° standard observer. The CIEL*a*b* standard scale was used to express the samples colour. Hue angle (h°) was calculated from the a* (greenness/redness (−/+)) and b* (blueness/yellowness (−/+)) chromatic values using the equation reported by McGuire (1992). Ten measurements were performed on the muscle side of different fillets for each sample and sampling time point.

A computer vision system (CVS) was used to acquire the images of the coating solutions and fish samples. One sardine fillet was placed inside a dark chamber against a black background, while a portion of coating solution, being transparent, was placed in a white container against a white background. All pictures were acquired under controlled lighting conditions using four fluorescent lamps (Natural Daylight, 18 W/965, 6500 K, TL-D Deluxe, Philips, the Netherlands). RGB (Red, Green, Blue) images were captured using a vertically oriented digital camera (D7000, Nikon, Japan) equipped with a 105 mm lens (AF-S Micro Nikkor, Nikon, Japan) and set to manual mode, with an aperture of f/16, shutter speed of 1/2 s, ISO 100, no zoom and no flash. Image resolution was set to 1024 × 768 pixels and images were saved in JPEG format.

2.4.5. Texture analysis

Texture evaluation of the sardine samples was carried out using a Texture Analyzer (TA.HDi500, Stable Micro Systems, UK) equipped with a 25 kg load cell. From the central region of each fillet, samples measuring 2 × 2 cm were prepared and subjected to a double compression–decompression cycle using a 5 × 5 cm square probe (TPA test), following the procedure described by Mohan et al., (2012) with slight modifications. The test was performed with a pre-test and post-test speed of 4 mm/s, a test speed of 0.25 mm/s and 45.0 % deformation. The interval between the two compressions was set at 2 s and a trigger force of 0.10 N was applied. From the force-time curves obtained, the following parameters were derived: hardness (kg), defined as the peak force recorded during the first compression, and adhesiveness (kg·s), expressed as the negative area between the first and second compression.

Five measurements were performed for each sample and sampling time point.

2.4.6. Lipid extraction and peroxide value

Lipid extraction from sardine fillet samples was performed according to the method of Bligh & Dyer (1959). The sample was homogenised, weighed (25 g) and mixed with 25 mL chloroform and 50 mL methanol. The mixture was homogenised with a blender for 30 s and then sonicated in an ultrasonic bath (DU-100S, ARGOLAB, Italy) at 21 °C for 5 min. Subsequently, 25 mL of a 0.889 % KCl solution was added to precipitate the proteins. The mixture was first filtered through a mesh sieve and then through filter paper (grade 1300/80, FilterLAB, Steroglass, Italy). The organic phase was separated using a 250 mL separatory funnel and collected in a round bottom flask. The solvents were removed by rotavapor (STRIKE 300, Steroglass, Italy) and the total lipid content was determined gravimetrically. The extracted oils were stored at –20 °C until analysis (max 1 week).

The peroxide value (PV) was determined spectrophotometrically using the ferrothiocyanate method according to Shantha & Decker (1994). Three solutions were prepared: a barium chloride solution (0.4 g $BaCl_2$ in 100 mL distilled water), a ferrous sulphate solution (0.5 g $FeSO_4 \cdot H_2O$ in 50 mL distilled water) and a 10 N HCl solution in distilled water. The Fe(II) reagent was obtained by mixing the first two solutions with 2 mL of 10 N HCl, allowing the mixture to stand for 30 min and collecting the clear supernatant. An ammonium thiocyanate solution (30 g NH_4SCN in 100 mL distilled water) was also prepared. For the assay, 50 mg of sardine oil was weighed into a 10 mL dark glass flask. Then 50 µL Fe(II) reagent and 50 µL ammonium thiocyanate solution were added. The volume was brought to 10 mL with a chloroform: methanol solution (7:3, v/v). After vortexing, the mixture was kept in the dark for 5–10 min. A blank sample was prepared with the same reagents without the lipid sample. The absorbance of the sample and blank was measured at 500 nm. Using Fe(III) standards (0–40 µg/mL), a calibration curve was prepared, which yielded an equation and $R^2 = 0.996$. The PV value was calculated using the following equation:

$$PV\ (mEq\ active\ O_2/kg\ oil) = ((As - Ab) \times m) / (55.84 \times m_0 \times 2) \quad (3)$$

where *As* is the absorbance of the sample, *Ab* is the absorbance of the blank, *m* is the slope of the calibration curve (38.098), *55.84* is the atomic weight of Fe^{3+} (g/mol), m_0 is the mass of the lipid sample (g) and *2* is the stoichiometric factor accounting for the oxidation of 2 mol Fe^{2+} per 1 mol of lipid peroxide.

Lipid extraction was performed in duplicate for each sample and sampling time point and PV determinations were carried out in triplicate.

2.4.7. Microbiological analyses

At each sampling point, 10 g of each sample was aseptically transferred to sterile stomacher bags containing 90 mL sterile saline solution (0.9 % w/v NaCl) and homogenised for 2 min using a stomacher (LAB-Blender 400, BA6021, Seward Medical, London, UK). Serial decimal dilutions were prepared with sterile saline solution. Aliquots (0.1 mL) of the appropriate dilutions were spread on the following media: Plate Count Agar (PCA) (Oxoid, UK) for total aerobic plate counts (30 °C, 48 h) and psychrotrophic bacteria (10 °C, 5 days); de Man, Rogosa and Sharpe (MRS) Agar (Oxoid, UK) for lactic acid bacteria (37 °C, 48 h); Pseudomonas Agar Base (PAB) supplemented with CFC Supplement (Oxoid, UK) for enumeration of *Pseudomonas spp.* (30 °C, 48 h); and Brilliance™ E. coli/Coliform Selective Agar (BRIEC) (Oxoid, UK) for total coliforms (37 °C, 24 h). *Enterobacteriaceae* were counted using the pour plate method with Violet Red Bile Glucose Agar (VRBGA) (Oxoid, UK). More specifically, 1 mL of the sample dilution was mixed with 10 mL of VRBGA melted medium, poured into a sterile Petri dish and covered with another 10 mL of the same agar after solidification. The plates were incubated at 37 °C for 24 h. To detect spore-forming microorganisms such as *Bacillus* and *Clostridium*, the samples were heat-treated at 80 °C for 20 min. After dilution, a surface plating on PCA (30 °C, 24 h) was performed to detect aerobic spores, while the inclusion method on Reinforced Clostridial Agar (RCA) (Oxoid, UK) (37 °C, 48 h, anaerobic conditions) was used for anaerobic spores. All microbiological determinations were performed in triplicate on independent biological replicates and results were expressed as log CFU/g.

2.5. Statistical analysis

All data are presented as means ± standard deviation (SD). Statistical analyses were performed using the XLSTAT Premium software (Lumivero, USA). Differences among samples were assessed by one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A significance level of $p \leq 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Edible and waste yields from manually processed shrimp

The yield of edible portion (muscle tissue) and processing waste of red shrimp (*Aristaeomorpha foliacea*) was found to be 1.15 kg (38.3 %) and 1.85 kg (61.7 %), respectively. Similarly, Soultani and Strati (2016) reported edible yields for manually processed *A. foliacea* and *Parapenaeus longirostris* (pink shrimp) of 48.46 % and 40.03 %, respectively,

with corresponding waste fractions of 51.54 % and 59.97 %. Kaya et al. (2024) also reported yields of 48.46 % for edible parts and 51.54 % for waste in manually processed giant red shrimp (*A. foliacea*). These findings suggest that the specimens used in the present study had a slightly higher proportion of inedible biomass, compared to previous studies. This further underscores the need to develop strategies for the valorisation of shrimp processing waste, given its abundance and potential as a source of valuable bioactive compounds such as carotenoids.

3.2. Characteristics of shrimp waste extract

Pigments, such as carotenoids, are known to play crucial physiological roles in crustacean and are primarily responsible for their characteristics pink to red coloration (Nirmal et al., 2020; Sila et al., 2015). In this study, the carotenoid content of the *A. foliacea* (red shrimp) waste extract, expressed as astaxanthin, was found to be 443.5 ± 18.0 μg/g dry weight (dw). Astaxanthin is widely recognized as the dominant carotenoid in shrimp tissues and by-products, often accounting for 75–95 % of the total carotenoid fraction (Gómez-Estaca et al., 2017; Rossi et al., 2024). It is naturally synthesized by microalgae such as *Haematococcus pluvialis* under environmental stressors, including nutrient deprivation, high salinity, or intense light, and accumulates in crustaceans through their diet (Rossi et al., 2024; Yu et al., 2022).

Astaxanthin concentrations reported in the literature for shrimp waste vary considerably, typically ranging from 30 to 600 μg/g dw, depending on multiple factors such as species, anatomical part processed (e.g., heads, shells, tails), environmental origin and extraction procedures, including the solvent system and the use of enhancing techniques (de Aguiar Saldanha Pinheiro et al., 2023; Rossi et al., 2024; Sachindra et al., 2006). For example, Sachindra et al. (2006) reported a carotenoid content expressed as astaxanthin ranging from 35 to 153 μg/g dw for Indian shrimp species depending on extraction conditions and anatomical part. Another study by de Aguiar Saldanha Pinheiro et al. (2023) found values of 47.3 μg/g dw in *Melicertus kerathurus* shrimp waste and 169.6 μg/g dw in *Aristeus antennatus* shrimp waste using ethanol solvent extraction. Additionally, Kaya et al. (2024) reported 198.8 μg/g dw in ethanol extracts from *A. foliacea* waste, while Tran et al. (2025) extracted 31.5 μg/g dw of astaxanthin using pure ethanol from *Penaeus monodon* shell powder. The value obtained in the present work (443.5 ± 18.0 μg/g dw) is notably higher than those previously reported for waste from the same species and other shrimp species under similar extraction conditions, highlighting both the high carotenoid content of *A. foliacea* shrimp waste and the efficiency of the ethanol-based extraction method.

The high carotenoid content of the shrimp waste extract, particularly astaxanthin, was associated with a marked antioxidant capacity. Indeed, the extract exhibited a DPPH radical scavenging activity of 77.8 ± 0.5 % and a Trolox equivalent antioxidant capacity (TEAC) of 576.3 ± 15.1 μmol TE/mL. These values indicate a strong antioxidant potential, consistent with the well-established bioactivity of astaxanthin and related compounds. Although astaxanthin is likely the principal contributor to this effect, other antioxidant constituents naturally present in shrimp waste extract, such as phenolic compounds, tocopherols and ubiquinols, may also play a synergistic role (Rossi et al., 2024). Comparable results have been reported in the literature; Sowmya & Sachindra (2012) confirmed strong DPPH scavenging activity in carotenoprotein isolates rich in astaxanthin from shrimp heads, with potential influenced by additional antioxidants such as tocopherols. Moreover, the study by de Aguiar Saldanha Pinheiro et al. (2023) observed antioxidant activity in ethanol extracts of *M. kerathurus* and *A. antennatus* shrimp waste, with TEAC values of 437.9 ± 27.1 μmol TE/g dw and 375.8 ± 12.4 μmol TE/g dw, respectively, using the ABTS·⁺ assay. Tran et al. (2025) reported EC_{50} values of 29.45 μg/mL and 25.11 μg/mL by ABTS·⁺ and DPPH assays, respectively, in ethanol extracts of *P. monodon*.

The potent antioxidant capacity of the carotenoid astaxanthin is primarily attributed to its unique molecular structure, which includes a polyene chain with conjugated double bonds and hydroxyl (–OH) and keto (C═O) groups on each terminal ionone ring. This structure enables efficient free radical scavenging through hydrogen hydrogen-donating capability (Nirmal et al., 2020; Tran et al., 2025).

3.3. Quality of coated raw sardine fillets during refrigerated storage

3.3.1. Physicochemical characteristics

To assess the influence of the developed edible coating on the physicochemical stability of sardine fillets during refrigerated storage, the evolution of key quality indicators was monitored. Among these, changes in headspace gas composition serve as early and sensitive markers of product deterioration. Fig. 1 shows the effect of the coatings on the evolution of headspace gas composition, specifically the percentages of oxygen (O_2) and carbon dioxide (CO_2), over time in packaged fresh sardine fillet samples stored at 4 ± 0.5 °C. Changes in headspace gas composition are indicative of alterations in the fish matrix, primarily driven by microbial growth and other related phenomena (Mohan et al., 2008, 2012).

During the storage, all samples exhibited a decrease in O_2 (Fig. 1**a**) and a concurrent increase in CO_2 (Fig. 1**b**) concentrations, with notable differences between coated and control (uncoated) samples. Specifically, after 9 and 13 days of storage, the O_2 concentration in the control sample decreased from 21.00 ± 0.05 % to 2.57 ± 1.67 % and 0.95 ± 1.16 %, respectively, whereas in the coated samples (Ch and Ch+Asx) it decreased only to 17.28 ± 0.42 % and 10.36 ± 0.06 %, suggesting a slowdown in deteriorative phenomena such as microbial respiration and oxidative processes. In the uncoated control sample, the drop in O_2 and the associated rise in CO_2 began as early as day 2, with a sharp shift observed from day 6 onward. These findings are consistent with the results of Mendes et al. (2008), who studied air-packed raw sardines fillets stored at 2 ± 1 °C during 12 days. In their work, the headspace O_2 concentration decreased from 21 % to below 10 % within just 5 days, while CO_2 increased from 0.03 % to 13.9 % by day 7, highlighting the rapid metabolic activity and microbial proliferation in raw sardine fillets. Similarly, de Aguiar Saldanha Pinheiro et al. (2019) reported that sardines packed in air showed a rapid decline in O_2 from 20.6 % at day 0 to 11.2 % after just 4 days, with a corresponding rise in CO_2 to 9.5 %. Oxygen plays a central role in promoting several quality-degrading pathways, including chemical and enzymatic oxidation, pigment and aroma deterioration, aerobic respiration, and the proliferation of aerobic microorganisms such as bacteria, molds and yeasts (de Aguiar Saldanha Pinheiro et al., 2019; Mendes et al., 2008). Therefore, the observed reduction in O_2 and increase in CO_2 in the package headspace are strong indicators of deterioration, particularly evident in uncoated control samples.

To further evaluate the effects of the edible coatings on product quality, additional analyses were carried out throughout the storage period. These measurements allowed a more comprehensive understanding of the changes in freshness, structural integrity and visual appearance of the fillets. Table 1 reports the changes in pH, water activity (a_w), colour parameters (L*, a*, h°) and texture (hardness and adhesiveness) of fresh sardine fillet samples uncoated (control) and coated with a chitosan-based film (Ch) and a chitosan-based film enriched with carotenoids (Ch+Asx) during 13 days of storage at 4 °C.

Regarding the pH, at day 0, the control fillets exhibited a higher pH value (6.17 ± 0.03) compared to samples Ch and Ch+Asx, which presented lower values of approximately 5.8. This reduction in pH can be attributed to the acid nature of the coating formulations, in particular the presence of chitosan dissolved in acetic acid, which contributes to a slight acidification of the sardine fillets. Notably, both coating solutions had a pH of 3.85 ± 0.09. These results are consistent with findings reported by Homayonpour et al. (2021), who observed similar acidifying effects in sardine fillets treated with chitosan+acetic acid-based coatings. During storage, the pH of the control sample increased significantly

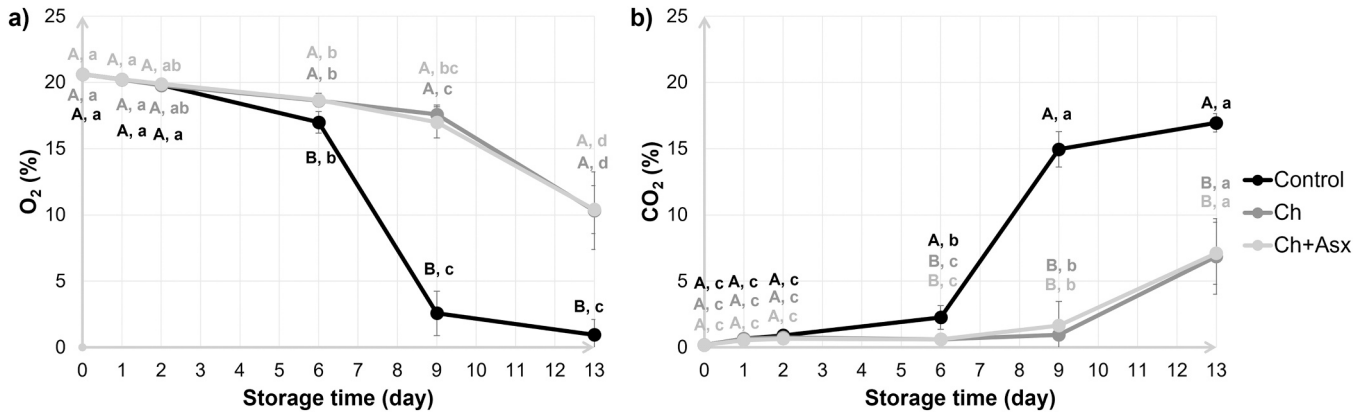

Fig. 1. Changes in (**a**) O_2 (%) and (**b**) CO_2 (%) in the headspace of the packages containing sardine fillet samples during 13 days of storage at 4 ± 0.5 °C: uncoated (control), coated with chitosan-based coating (Ch) and coated with chitosan-based coating enriched with carotenoids (Ch+Asx). Different uppercase and lowercase letters indicate statistically significant differences ($p < 0.05$) among samples at the same storage time and among sampling days within the same sample, respectively.

Table 1
Changes in pH, water activity (a_w), colour (L*, a*, h°) and texture (hardness and adhesiveness) parameters of raw sardine fillet samples during 13 days of storage at 4 ± 0.5 °C: uncoated (control), coated with chitosan-based coating (Ch) and coated with chitosan-based coating enriched with astaxanthin extract (Ch+Asx).

	Storage day					
Sample	**0**	**1**	**2**	**6**	**9**	**13**
pH						
Control	6.17 ± 0.03 $^{A, b}$	6.20 ± 0.10 $^{A, b}$	6.07 ± 0.03 $^{A, b}$	6.16 ± 0.14 $^{A, b}$	6.84 ± 0.07 $^{A, a}$	6.77 ± 0.24 $^{A, a}$
Ch	5.80 ± 0.02$^{B, a}$	5.88 ± 0.08$^{B, a}$	5.83 ± 0.02$^{B, a}$	5.83 ± 0.04$^{B, a}$	5.83 ± 0.03$^{B, a}$	5.93 ± 0.18$^{B, a}$
Ch+Asx	5.82 ± 0.06$^{B, b}$	5.86 ± 0.08$^{B, b}$	5.89 ± 0.04$^{B, b}$	5.89 ± 0.03$^{B, b}$	5.94 ± 0.10$^{B, ab}$	6.02 ± 0.11$^{B, a}$
Water activity (a_w)						
Control	0.991 ± 0.001 $^{A, a}$	0.996 ± 0.001 $^{A, a}$	0.997 ± 0.001 $^{A, a}$	0.998 ± 0.001 $^{A, a}$	0.977 ± 0.001 $^{A, b}$	0.975 ± 0.001 $^{A, b}$
Ch	0.990 ± 0.001 $^{A, a}$	0.997 ± 0.004 $^{A, a}$	0.996 ± 0.001 $^{A, a}$	0.996 ± 0.003 $^{A, a}$	0.974 ± 0.001 $^{A, b}$	0.974 ± 0.001 $^{A, b}$
Ch+Asx	0.989 ± 0.001 $^{A, a}$	0.998 ± 0.002 $^{A, a}$	0.997 ± 0.002 $^{A, a}$	0.998 ± 0.002 $^{A, a}$	0.974 ± 0.001 $^{A, b}$	0.977 ± 0.001 $^{A, b}$
L* (lightness)						
Control	51.13 ± 0.73$^{B, b}$	51.16 ± 2.74$^{B, b}$	50.95 ± 1.43$^{B, b}$	53.12 ± 3.62$^{B, a}$	52.28 ± 3.43$^{B, a}$	54.26 ± 2.23$^{B, a}$
Ch	56.69 ± 0.31 $^{A, c}$	56.73 ± 3.40 $^{A, c}$	57.51 ± 5.10 $^{A, bc}$	58.77 ± 3.09 $^{A, b}$	60.96 ± 1.04 $^{A, ab}$	61.86 ± 2.59 $^{A, a}$
Ch+Asx	55.15 ± 1.42 $^{A, c}$	54.99 ± 2.73 $^{A, c}$	56.42 ± 0.70 $^{A, b}$	57.38 ± 1.33 $^{A, ab}$	58.54 ± 1.08 $^{A, a}$	59.53 ± 1.57 $^{A, a}$
a* (redness)						
Control	1.02 ± 0.56$^{B, c}$	1.05 ± 0.43$^{B, c}$	1.00 ± 0.45 $^{A, c}$	0.47 ± 0.42 $^{A, c}$	1.35 ± 0.57 $^{A, b}$	6.60 ± 1.19 $^{A, a}$
Ch	−0.21 ± 0.14 $^{C, b}$	−0.02 ± 0.94 $^{C, b}$	−0.11 ± 0.71$^{B, b}$	−0.31 ± 0.42$^{B, b}$	−0.18 ± 0.60$^{B, b}$	0.52 ± 0.53 $^{C, a}$
Ch+Asx	2.36 ± 0.29 $^{A, a}$	1.97 ± 0.59 $^{A, a}$	1.15 ± 0.60 $^{A, b}$	0.64 ± 0.15 $^{A, b}$	1.02 ± 0.80 $^{A, ab}$	1.63 ± 1.18$^{B, a}$
h° (hue angle)						
Control	82.86 ± 2.93$^{B, b}$	83.30 ± 2.32$^{B, b}$	83.79 ± 2.31$^{B, b}$	87.95 ± 1.83$^{B, a}$	84.51 ± 2.54$^{B, ab}$	73.52 ± 1.60$^{B, c}$
Ch	91.53 ± 1.08 $^{A, a}$	90.41 ± 4.86 $^{A, a}$	90.73 ± 3.91 $^{A, a}$	91.31 ± 1.89 $^{A, a}$	90.82 ± 2.33 $^{A, a}$	88.27 ± 1.66 $^{A, a}$
Ch + Asx	76.59 ± 0.79 $^{C, b}$	79.41 ± 2.94$^{B, b}$	83.45 ± 4.13$^{B, ab}$	87.35 ± 0.53$^{B, a}$	86.17 ± 2.96$^{B, a}$	85.23 ± 3.11 $^{A, a}$
Hardness (kg)						
Control	4.27 ± 0.56 $^{A, a}$	1.78 ± 0.17 $^{A, b}$	1.69 ± 0.25 $^{A, b}$	1.39 ± 0.11$^{B, bc}$	1.33 ± 0.15$^{B, c}$	1.12 ± 0.22$^{B, c}$
Ch	3.48 ± 0.56 $^{A, a}$	1.97 ± 0.80 $^{A, b}$	1.79 ± 0.32 $^{A, b}$	1.90 ± 0.36 $^{A, b}$	2.18 ± 0.34 $^{A, b}$	2.04 ± 0.27 $^{A, b}$
Ch+Asx	3.59 ± 0.81 $^{A, a}$	1.45 ± 0.05 $^{A, b}$	1.97 ± 0.28 $^{A, b}$	2.08 ± 0.59 $^{A, b}$	2.17 ± 0.24 $^{A, b}$	1.95 ± 0.35 $^{A, b}$
Adhesiveness (kg·s)						
Control	−0.08 ± 0.04 $^{A, a}$	−0.06 ± 0.02 $^{A, a}$	−0.05 ± 0.01 $^{A, a}$	−0.06 ± 0.01 $^{A, a}$	−0.15 ± 0.08$^{B, a}$	−1.68 ± 0.31 $^{C, b}$
Ch	−0.01 ± 0.11 $^{A, a}$	−0.06 ± 0.01 $^{A, a}$	−0.05 ± 0.01 $^{A, a}$	0.03 ± 0.20 $^{A, a}$	−0.05 ± 0.01 $^{A, a}$	−0.56 ± 0.07$^{B, b}$
Ch+Asx	−0.05 ± 0.09 $^{A, a}$	−0.06 ± 0.01 $^{A, a}$	−0.05 ± 0.00 $^{A, a}$	−0.05 ± 0.02 $^{A, a}$	−0.09 ± 0.06 $^{A, a}$	−0.17 ± 0.22 $^{A, a}$

Different uppercase and lowercase letters indicate statistically significant differences ($p < 0.05$) among samples at the same storage time and among sampling days within the same sample, respectively.

from day 6 onward, reaching a value of 6.77 ± 0.24 (+9.73 %) by day 13. In contrast, the coated samples exhibited only slight increases: sample Ch reached a final pH of 5.93 ± 0.18 (+2.24 %) and sample Ch+Asx reached 6.02 ± 0.11 (+3.79 %). This difference can be attributed to the antimicrobial activity of chitosan (Homayonpour et al., 2021). The pH changes can be utilized as an indicator of spoilage in seafood products (Baek et al., 2021). An increase in pH is typically associated with the accumulation of alkaline compounds such as ammonia, trimethylamine nitrogen and other biogenic amines resulting from bacterial spoilage of fish tissue (Ayala et al., 2011; Goulas & Kontominas, 2007; Stamatis & Arkoudelos, 2007). Similar to our findings, Bouhanna et al. (2021) also reported stable pH values in sardine fillets coated with gelatin-films containing *Arbutus unedo* extract, whose polyphenolic composition was effective in slowing spoilage-related alkalinisation (Bouhanna et al., 2021).

As for water activity (a_w), all samples exhibited high values ranging from 0.998 ± 0.001–0.974 ± 0.001, which are comparable to those reported by Aoua et al. (2024) for sardine fillets (average $a_w = 0.950$) and Cakli et al. (2007) for European sea bass and gilthead seabreams (average $a_w = 0.998$). No statistically significant differences in a_w were observed among the sardine fillets samples during refrigerated storage. A slight decrease in a_w values was noted in all samples after 6 days of storage, although values remained above 0.970 throughout the entire period.

Application of the edible coatings and subsequent refrigerated storage led to significant changes in the surface colour of fresh sardine

fillets, particularly in terms of lightness (L*), redness (a*) and hue angle (h°) (Table 1). On day 0, both coated samples (Ch and Ch+Asx) showed significantly higher L* values compared to the control, a difference that persisted throughout the storage period. This increased lightness of the coated fillets is likely due to the low pH of the chitosan–acetic acid coating solution, which may have favoured the partial leaching of endogenous muscle pigments during immersion, increasing surface reflectance and resulting in a visually brighter appearance (Mohan et al., 2012). These results are consistent with previous observations by Mohan et al. (2012), who reported similar effects in sardine fillets treated with 1–2 % chitosan coatings plus 1 % acetic acid. In addition, a slight but consistent increase in L* value over time was observed in all samples, possibly due to moisture loss from the surface, which can lead changes in the light reflectance properties of the fillets.

In terms of redness (a*), the Ch+Asx sample had significantly higher initial values than the Ch and control ones. This can be directly associated to the intense reddish colour of the Ch+Asx coating solution, which had $L^* = 29.57 \pm 0.03$, $a^* = 21.02 \pm 0.22$ and $b^* = 29.04 \pm 0.29$ (Fig. 2). In contrast, the Ch coating solution had a much uncoloured and transparent appearance, with values of $L^* = 5.30 \pm 0.08$, $a^* = -1.05 \pm 0.10$ and $b^* = 0.54 \pm 0.24$. Over the course of storage, the a* value in the Ch group remained consistently low, probably as a result of pigment loss caused by the acidic environment during coating application (Mohan et al., 2012). In contrast, in the astaxanthin-enriched Ch+Asx sample, there was an initial decrease in redness by day 6, possibly due to diffusion of the pigments into the muscle tissue.

The hue angle (h°), which combines a* and b* to describe the apparent hue, provided additional support for these results. Hue values approaching 0° indicate red hues, while values close to 90° correspond to yellow hues. The control sample maintained relatively stable h° values until day 9, followed by a shift towards lower angles on day 13, indicating a perceptible increase in red pigmentation, an observation that was further confirmed by RGB image analysis (Fig. 2). Sample Ch exhibited consistently higher h° values, reflecting its lower redness, while Ch+Asx maintained a stable and intense reddish colour throughout storage.

Contrary to the present findings, Mohan et al. (2012) found no significant differences in a* values between control and chitosan-coated sardine fillets, possibly due to the use of oxygen-permeable packaging, which could have promoted similar oxidative conditions across all studied samples, potentially masking the effect of the coating. Similarly, López-Caballero et al. (2005) observed no significant colour changes in cod patties coated with a chitosan-gelatin solution.

These colour trends were paralleled by differences in physical structure, particularly texture parameters such as hardness and adhesiveness, which were selected as the most representative for discriminating between samples. Initial hardness values (Table 1) were comparable among the three groups, Control, Ch and Ch+Asx, with measures of 4.27 ± 0.56 kg, 3.48 ± 0.56 kg and 3.59 ± 0.81 kg, respectively. These are in agreement with values previously reported for fresh sardine fillets (Mohan et al., 2012). By day 1, a general decline in hardness was observed in all samples, with a slightly greater reduction in the control group, although differences between coatings were not statistically significant. The loss of tissue firmness, particularly after the first day, can be linked to post-mortem moisture loss and structural degradation of muscle fibres, which also contributes to visual changes in surface texture and reflectance (Sun et al., 2018). Notably, sardines coated with chitosan-based solutions (especially Ch+Asx) tended to retain greater structural integrity over time, in agreement with findings by Mohan et al. (2012), who observed improved textural stability in chitosan-coated fish. Adhesiveness remained relatively stable in all samples up to day 9 but decreased significantly in the control group by day 13, potentially due to enzymatic weakening of intercellular adhesion within the myofibrillar matrix. In line with this, Kanelaki et al. (2022) also demonstrated that sardines treated with structured edible coatings exhibited reduced texture degradation, highlighting the protective role of antioxidant-rich barrier layers.

3.3.2. Lipid oxidation

The sardine fillets (*Sardina pilchardus*) used in this study had an average fat content of 4.57 ± 0.71 %. This value aligns with previously reported data. Mohan et al. (2012) reported a fat content of 6.8 % in *Sardinella longiceps* harvested from the Arabian Sea off the southwest coast of India. In addition, Küçükgülmez (2012) analysed *S. pilchardus* from the eastern Mediterranean Sea (İskenderun Bay, Turkey) and found lipid contents between 4.19 % and 6.75 %, depending on the season of sampling. Similarly, Mozzon et al. (2024) found an average fat content of 4.30 % in *S. pilchardus* from the central Adriatic Sea sampled in spring and early summer. Sardines and other pelagic species are generally categorised as high-fat fish whose lipid content can exceed 20 % or fall below 4 % depending on the season (Caponio et al., 2004; Okada & Morrissey, 2007). This variability is influenced by multiple factors, including seasonality, metabolic activity, diet composition, water temperature, size, reproductive stage and geographical origin (Aidos et al., 2002; De Leonardis & Macciola, 2004; Gogus et al., 2006; Pacheco-Aguilar et al., 2000). Gogus et al. (2006) reported significant seasonal variations in lipid composition and different susceptibility to oxidation in fresh sardine fillets (*S. pilchardus*) from the Black Sea and found that sardines caught in spring and summer had a higher polyunsaturated fatty acid (PUFA) content, which made them more susceptible to oxidation.

The highly unsaturated lipids of fish are particularly susceptible to

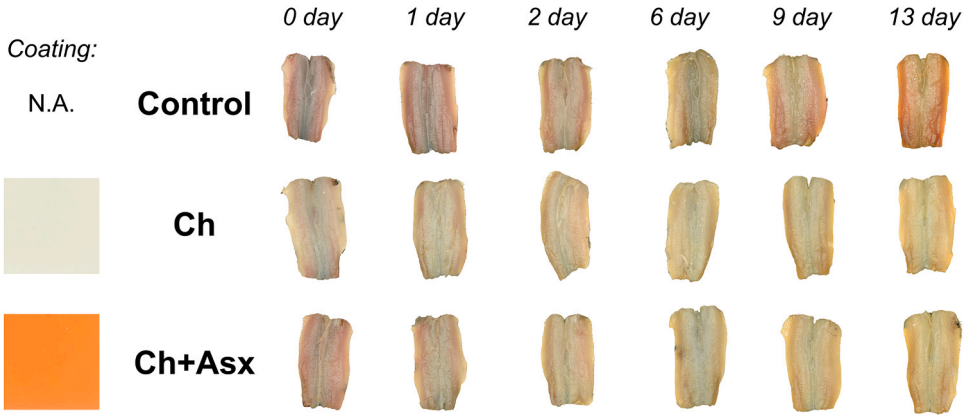

Fig. 2. RGB images acquired using a computer vision system (CVS), showing the appearance of both coating solutions (Ch and Ch+Asx) and sardine fillet samples during 13 days of storage at 4 ± 0.5 °C: uncoated (control), coated with chitosan-based coating (Ch) and coated with chitosan-based coating enriched with astaxanthin extract (Ch+Asx). N.A. = Not Applicable.

oxidation, which is one of the main causes of deterioration in the quality of oily fish and related products. Peroxides formed in the initial stages of lipid oxidation are not only unstable intermediates but are also directly responsible for the development of rancid odour and taste. Even before they are degraded to secondary volatile compounds such as aldehydes and ketones, these primary oxidation products can noticeably impair the sensory quality of fish (Barros et al., 2023; Uçak et al., 2011).

In this study, the progression of primary lipid oxidation in fresh sardine fillets stored under refrigeration was assessed by monitoring the peroxide value (PV) over 13 days (Fig. 3). At time zero (day 0), all samples had similar PVs, with an average value of 4.03 ± 0.70 mEq O_2/kg oil. This result falls within the range reported in the literature for fresh raw sardines, which is between 1.5 and 5.7 mEq O_2/kg oil depending on the season and sampling location (de Aguiar Saldanha Pinheiro et al., 2021; Homayonpour et al., 2021; Küçükgülmez, 2012; Mohellebi et al., 2024).

During storage, all samples showed a progressive increase in PV, with the uncoated control sample showing a significantly faster and more pronounced increase compared to the coated ones (Ch and Ch+Asx). Oxidation appeared to start between day 0 and day 1 in all samples, but accelerated significantly after day 6 in the control, while a more gradual increase was observed in both coated groups, especially after day 9. On day 13, the control reached a PV of 12.5 mEq O_2/kg fat, while in the Ch and Ch+Asx samples the PV values were significantly lower, 8.8 and 9.7 mEq O_2/kg fat, respectively. These results clearly indicate that the chitosan-based coatings delayed the onset and progression of lipid oxidation and remained below the PVs range of 10 and 20 mEq O_2/kg fat, which is generally considered the upper limit of food acceptability according to Raeisi et al. (2016) and Ghomi et al. (2011).

The protective effect of the coatings is consistent with the observations of Mohan et al. (2012), who found that sardine fillets treated with 1 % chitosan showed less peroxide formation after 10 days of refrigerated storage. Similarly, Homayonpour et al. (2021) demonstrated that nanochitosan coatings, both with and without cumin essential oil, effectively suppressed early oxidative processes in sardine fillets. Bouhanna et al. (2021) also reported that sardines coated with gelatin films enriched with *Arbutus unedo* extract had significantly lower PVs, highlighting the potential of antioxidant-enriched edible films to mitigate lipid oxidation during refrigerated storage. Comparable protective effects of different edible coatings have also been documented for different fish species (Cetinkaya & Wijaya, 2024; Kurek et al., 2024).

The significantly lower PVs in the chitosan-coated samples (Ch and Ch+Asx) are likely due to the antioxidant properties of chitosan. These could result from the ability of the remaining amino groups to interact with lipid oxidation products such as malondialdehyde, combined with the physical barrier effect of the coating, which limits oxygen diffusion to the fish surface and thereby slows oxidative degradation (Kanatt et al., 2013; Kurek et al., 2024). Nevertheless, contrary to expectations, the addition of carotenoid rich in astaxanthin extract to the chitosan-based coating did not lead to a measurable improvement in oxidative protection compared to chitosan alone.

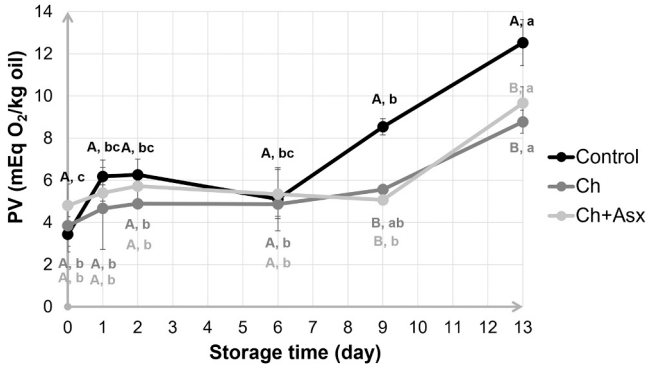

Fig. 3. Peroxide value (PV) of sardine fillet samples during 13 days of storage at 4 ± 0.5 °C: uncoated (control), coated with chitosan-based coating (Ch) and coated with chitosan-based coating enriched with carotenoids (Ch+Asx). Different uppercase and lowercase letters indicate statistically significant differences ($p < 0.05$) among samples at the same storage time and among sampling days within the same sample, respectively.

This lack of additional benefit may be attributed to the limited migration or release of astaxanthin and other possible antioxidants from the chitosan matrix into muscle tissue. As noted by Gómez-Estaca et al. (2007), the efficacy of antioxidant-enriched edible coatings is highly dependent on the polarity of the compounds contained, their physicochemical compatibility with the film-forming agents and the release dynamics into the food matrix. These factors can significantly influence the bioavailability and efficacy of the active ingredients in complex food systems such as fish fillets.

3.3.3. *Microbiological quality*

Given the complexity of spoilage mechanisms, the assessment of fish quality requires a comprehensive evaluation of the microbiological profile. In this regard, microbiological criteria for food, including fresh fish products, are established at European level by Regulation (EC) No 2073/2005. Accordingly, and in line with recent scientific literature, the present study evaluated various microbiological parameters in the sardine fillet samples during refrigerated storage, including aerobic plate count, psychrotrophic bacteria, *Pseudomonas* spp., total coliforms, *Enterobacteriaceae*, lactic acid bacteria and aerobic and anaerobic spore-forming bacteria (Table 2).

Aerobic bacterial counts increased progressively throughout refrigerated storage. According to Wu et al. (2019), Esteves & Aníbal (2021) and Duarte et al. (2020), aerobic plate counts are widely used to assess fish freshness. Values between 2 and 6 log CFU/g are generally considered acceptable for whole and filleted fish, while values above 7 log CFU/g are usually associated with sensory rejection. Furthermore, Regulation (EC) No 2073/2005Regulation (EC) No 2073/2005 sets a microbiological limit of 5 log CFU/g for fresh fish. In this study, the control sample exceeded the acceptable limit before day 6, while both the Ch and Ch+Asx coatings demonstrated a preservative effect. In particular, the Ch-coated samples maintained acceptable levels until day 6 (approx. ~4.6 log CFU/g), while the Ch+Asx-coated ones remained below the threshold until day 9 (approx. ~4.9 log CFU/g).

The number of psychrotrophic bacteria followed a similar trend. As described in the literature (Kanekar & Kanekar, 2022; Ólafsdóttir et al., 1997), monitoring psychrotrophic microorganisms is essential for assessing the quality of fish stored at low temperatures. Although the differences between the coated samples were less marked during the first 6 days of storage, a clear effect of the coatings was seen from day 9 onwards. On day 9, the microbial count in the control sample reached about 7.6 log CFU/g, compared to 4.9 and 4.2 log CFU/g in the Ch and Ch+Asx samples, respectively. By day 13, the microbial load had increased to approximately 10.4 log CFU/g in the control, 9.1 log CFU/g in Ch and 7.9 log CFU/g in Ch+Asx. These results indicate that the application of coatings effectively retarded psychrotrophic bacterial growth during cold storage, with the effect becoming more pronounced after day 6.

Pseudomonas spp., known as the dominant spoilage organisms in aerobically stored chilled fish (Duarte et al., 2020), also showed differential growth across coated samples. In the control sample (uncoated), *Pseudomonas* counts exceeded 7 log CFU/g by day 6, a threshold commonly linked to the beginning of spoilage and perception of off-odours (Gil & Barbosa, 2011). Conversely, the Ch+Asx coated samples remained below this limit until day 9, reaching about 6.6 log CFU/g, while the Ch sample had intermediate values (approx. ~7.4 log CFU/g on day 9 vs control with 9.36 log CFU/g). These findings also support the efficacy of both coatings in delaying *Pseudomonas*-associated spoilage, which is consistent with the observations of Homayonpour et al. (2021) who reported similar protective effects of nano-chitosan

Table 2
Changes in microbiological counts (log CFU/g) of raw sardine fillet samples during 13 days of storage at 4 ± 0.5 °C: uncoated (control), coated with chitosan-based coating (Ch) and coated with chitosan-based coating enriched with astaxanthin extract (Ch+Asx). Microbial parameters include aerobic plate count, psychrotrophic bacteria, *Pseudomonas* spp., coliforms, *Enterobacteriaceae*, lactic acid bacteria, aerobic and anaerobic spore-forming bacteria.

	Storage day					
Sample	**0**	**1**	**2**	**6**	**9**	**13**
***Aerobic plate count* (log CFU/g)**						
Control	$4.30 \pm 0.12^{B, a}$	$4.72 \pm 0.18^{C, a}$	$4.93 \pm 0.08^{C, a}$	$7.80 \pm 0.07^{C, b}$	$9.09 \pm 0.12^{C, c}$	$9.43 \pm 0.15^{C, d}$
Ch	$4.02 \pm 0.10^{A, a}$	$4.04 \pm 0.15^{B, a}$	$4.16 \pm 0.15^{B, a}$	$4.63 \pm 0.08^{B, b}$	$7.69 \pm 0.25^{B, c}$	$8.79 \pm 0.25^{B, d}$
Ch+Asx	$3.75 \pm 0.05^{A, a}$	$3.80 \pm 0.12^{A, a}$	$3.86 \pm 0.05^{A, a}$	$4.40 \pm 0.05^{A, b}$	$4.96 \pm 0.15^{A, c}$	$7.19 \pm 0.17^{A, d}$
***Psychrotrophic bacteria* (log CFU/g)**						
Control	$3.62 \pm 0.13^{B, a}$	$4.24 \pm 0.11^{C, b}$	$4.64 \pm 0.13^{B, c}$	$5.14 \pm 0.03^{C, d}$	$7.63 \pm 0.05^{C, e}$	$10.40 \pm 0.06^{A, f}$
Ch	$3.11 \pm 0.18^{A, a}$	$3.42 \pm 0.16^{A, b}$	$3.87 \pm 0.28^{A, c}$	$4.37 \pm 0.05^{B, d}$	$4.95 \pm 0.06^{B, e}$	$9.11 \pm 0.01^{B, f}$
Ch+Asx	$3.84 \pm 0.14^{C, a}$	$3.66 \pm 0.12^{B, b}$	$3.78 \pm 0.14^{A, c}$	$4.01 \pm 0.19^{A, d}$	$4.25 \pm 0.15^{B, e}$	$7.99 \pm 0.03^{C, f}$
***Pseudomonas* spp. (log CFU/g)**						
Control	$4.47 \pm 0.01^{C, a}$	$4.65 \pm 0.07^{C, b}$	$5.02 \pm 0.07^{C, c}$	$7.79 \pm 0.02^{C, d}$	$9.36 \pm 0.01^{C, e}$	$9.62 \pm 0.05^{C, f}$
Ch	$3.66 \pm 0.02^{B, a}$	$3.88 \pm 0.16^{B, b}$	$4.17 \pm 0.16^{B, c}$	$4.65 \pm 0.07^{B, d}$	$7.44 \pm 0.06^{B, e}$	$8.87 \pm 0.05^{B, f}$
Ch+Asx	$3.35 \pm 0.14^{A, a}$	$3.46 \pm 0.17^{A, b}$	$3.76 \pm 0.17^{A, c}$	$4.09 \pm 0.06^{A, d}$	$6.66 \pm 0.05^{A, e}$	$8.12 \pm 0.02^{A, f}$
***Total coliforms* (log CFU/g)**						
Control	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$1.87 \pm 0.25^{B, b}$	$3.87 \pm 0.15^{B, c}$	$5.52 \pm 0.27^{B, d}$	$5.93 \pm 0.12^{C, d}$
Ch	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$4.06 \pm 0.18^{B, b}$
Ch+Asx	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$1.49 \pm 0.43^{A, b}$
***Enterobacteriaceae* (log CFU/g)**						
Control	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$1.91 \pm 0.15^{B, b}$	$1.95 \pm 0.15^{B, b}$	$4.38 \pm 0.25^{C, c}$	$6.34 \pm 0.12^{C, d}$
Ch	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$1.96 \pm 0.10^{B, b}$	$4.07 \pm 0.05^{B, c}$
Ch + Asx	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$2.93 \pm 0.12^{A, b}$
***Lactic acid bacteria* (log CFU/g)**						
Control	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Ch	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Ch+Asx	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Aerobic spore-forming bacteria (log CFU/g)						
Control	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Ch	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Ch+Asx	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Anaerobic spore-forming bacteria (log CFU/g)						
Control	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Ch	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$
Ch+Asx	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$	$< \text{d.l.}^{A, a}$

Different uppercase and lowercase letters indicate statistically significant differences ($p < 0.05$) among samples at the same storage time and among sampling days within the same sample, respectively. nb: < d.l. indicates values below the detection limit, set at 1 log CFU/g.
Figure captions

coatings enriched with encapsulated essential oils on the quality of sardine fillets.

With regard to total coliforms, a noticeable inhibitory effect of the coatings was observed from day 2 onwards. In the Ch and Ch+Asx samples, the number of coliforms remained below the detection limit (<1 log CFU/g) until day 9, in contrast to the control, which had values of around 5.5 log CFU/g on day 9 and 5.9 log CFU/g on day 13. According to Regulation (EC) No. 2073/2005, the total coliform count in fresh fish should not exceed 3 log CFU/g. The control samples therefore exceeded this threshold after day 6, while the Ch and Ch+Asx samples remained compliant until day 9. By day 13, only the Ch+Asx samples remained within the legal limits.

Regarding the *Enterobacteriaceae* group (Table 2), the values of the Ch+Asx and Ch samples were significantly below those of the control sample throughout the storage period. On day 13, the control reached about 6.3 log CFU/g, compared to 4.1 log CFU/g in Ch and 2.9 log CFU/g in Ch+Asx, indicating a clear protective effect of the coatings against *Enterobacteriaceae* proliferation. Lactic acid bacteria were not detected in any of the samples during the 13 days of refrigerated storage, with all values remaining below the detection limit (<1 log CFU/g). Similarly, both aerobic and anaerobic spore-forming bacteria (*Bacillus* and *Clostridium*) remained below the detection threshold in all samples and during all sampling times.

Overall, the microbiological results confirm the antimicrobial potential of chitosan when used in active packaging systems (Sarfraz et al., 2024). Moreover, the addition of astaxanthin-rich extract was found to further enhance this functionality. Indeed, the antimicrobial activity of astaxanthin (Karpiński et al., 2022) incorporated into active packaging is consistent with previous findings (Xu et al., 2020; Mussagy et al. 2023; Yao et al., 2025). For instance, Xu et al. (2020) formulated chitosan/gelatin films enriched with ε-polylysine and astaxanthin extract and tested their antimicrobial activity *in vitro* with bacterial strains isolated from shrimps (*Litopenaeus vannamei*) and reported a significant reduction in bacterial counts. Mussagy et al. (2023) investigated the antimicrobial efficacy of alginate-based edible films containing astaxanthin extract on fresh strawberries stored at 4 °C, reporting a significant reduction in total microbial load (2.00 log CFU/mL) compared to the control sample (6.33 log CFU/mL). Recently, Yao et al. (2025) demonstrated the antimicrobial efficacy of astaxanthin nanoemulsions through *in vitro* assays against *Escherichia coli* and *Staphylococcus aureus*.

4. Conclusions

The red shrimp (*Aristaeomorpha foliacea*) waste used in the experiments proved to be a valuable source of bioactive compounds, in particular astaxanthin, a carotenoid known for its strong antioxidant activity. The ethanolic extraction process enabled the recovery of an astaxanthin-rich extract, suggesting that these by-products can be efficiently valorised as a natural antioxidant source for potential applications in the food industry, including active edible packaging. These results emphasise the potential of shrimp processing wastes as a sustainable resource for the development of functional ingredients.

Regarding the effect of edible coatings on the preservation of fresh sardine fillets (*Sardina pilchardus*), both chitosan-based formulations contributed to modulate the internal atmosphere of the packaging, lower the pH change, maintain a good colour and texture of the fish

fillets and significantly slow down lipid oxidation. In addition, the coated sardine samples showed a slower microbial growth rate compared to the untreated control. In particular, the sample coated with the astaxanthin-enriched chitosan formulation showed better overall microbiological quality than the sample coated with chitosan alone.

Apart from their demonstrated preservative effect, the use of studied coatings represents a practical strategy to improve the quality and shelf life of minimally processed and local fish species such as sardine fillets, which are often undervalued and characterised by rapid quality deterioration. The development of ready-to-use fillets coated with bioactive formulations might help to valorise this fish and support more sustainable seafood chains. Further studies could examine coated fillets after cooking, using the main culinary methods typically applied to sardines, to evaluate the impact of these coatings on the sensory and nutritional properties of the product.

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CRediT authorship contribution statement

Santina Romani: Writing – review & editing, Supervision, Conceptualization. **Francesca Patrignani:** Writing – review & editing, Supervision. **Davide Gottardi:** Writing – review & editing, Investigation. **Urszula Tylewicz:** Writing – review & editing, Supervision. **Silvia Tappi:** Writing – review & editing, Supervision. **Pietro Rocculi:** Writing – review & editing, Supervision. **Ana Cristina de Aguiar Saldanha Pinheiro:** Writing – review & editing, Investigation. **D'Alessandro Margherita:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Maria Alessia Schouten:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.fpsl.2025.101659.

Data Availability

Data will be made available on request.

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