Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

Check for updates

ROOTS PRESS

Research Article

# Effect of whey protein isolate and concentrate coatings on the physicochemical and sensory quality of *Labeo rohita* Fillets

**Mahnoor Nawazish[1], Eisha Tahir[1], Momina Hanif[1], Jahangir Khan[1], Syed Waqas Hassan[1], Faisal Nawaz[2]**
[1]*Department of Biosciences, University of Wah, Quaid Avenue, Wah, Pakistan.*
[2]*Department of Chemistry, University of Wah, Quaid Avenue, Wah, Pakistan.*

## ABSTRACT

Edible coatings offer a sustainable solution to extend the shelf life and maintain the quality of perishable foods like *Labeo rohita* (Rohu) fillets. Rohu is particularly susceptible to quality degradation caused by lipid oxidation, enzymatic activity, and moisture loss. This study investigated the production and application of whey protein-based films on the physicochemical and sensory quality of *L. rohita* (Rohu) fillets during refrigerated storage (4 ± 1 °C) for 15 days. Coating formulations were prepared using whey protein isolate (WPI) and whey protein concentrate (WPC) in combination with glycerol and gelatin at different ratios: WPI + Glycerol (1:1), WPI + Glycerol (1:2), WPI + Glycerol + Gelatin (1:1:1), WPC + Glycerol (1:1), and WPC + Glycerol (1:2). Coatings were applied by dipping, and fillets were analyzed for pH, weight loss, color, texture, and aroma for 15 days. Data were statistically analyzed using one-way ANOVA followed by Tukey's test ($p < 0.05$). Significant differences were observed among treatments. The WPC+Glycerol (1:2) coating demonstrated the highest preservation efficiency, maintaining pH (6.33 ± 0.25), minimizing weight loss (16.4 %), and retaining desirable texture (3.33 ± 1.15) and color (2.67 ± 1.15) scores throughout storage. In contrast, uncoated samples showed rapid spoilage with extreme hardness, darkening, and off-odor. These findings confirm that WPC-based coatings, especially at a 1:2 ratios with glycerol are benefical since they could extend the shelf life of food products by reducing respiration rates, and they outperform alternative biopolymeric packaging.

**Keywords**: *Labeo rohita*; whey protein; edible films; fish preservation; biodegradable packaging; bio-coating.

OPEN ACCESS

***Correspondence***
*Faisal Nawaz*
*faisal.nawaz@uow.edu.pk*

***Article History***
*Received: November 24, 2025*
*Accepted: December 22, 2025*
*Published: December 31, 2025*



## INTRODUCTION

Fast-food technology is rapidly gaining importance, due to the growing desire for convenient, long-lasting, and extremely nutritious food options among working women and singles (Tan et al., 2022; Huang et al., 2024). This societal transition has led to enhanced processing and packaging technologies for meat and seafood, considerably increasing the intake of nutrient-rich fish (Kilincceker et al., 2009). Fish plays a vital role in human nutrition globally, providing key health-enhancing fatty acids, high-quality amino acids, fat-soluble vitamins, and essential micronutrients. Aside from its nutritional benefits, fish is an important source of income for many countries across the world (Zhe et al., 2025; Luciana et al., 2024). The *Labeo rohita* also known as Rohu, is a freshwater fish of to the Cyprinidae family of carps. The most significant of the three major Indian carp species (rohu, catla, mrigal) employed in carp polyculture systems is rohu (*Labeo rohita*) (Monirul et al., 2024). It is widely distributed in the weedy, slowly moving or stagnant waters of lakes and rivers in south and southeast asia (Mahboob et al., 2009). It is a fantastic source of Omega 3 fatty acids, which help to enhance skin and lower cholesterol. It is also a good source of calcium, zinc, and other minerals (Khan et al., 2015; Kashif et al., 2011). Fish is a highly valued food due to its high protein and low saturated fat content. Fatty acids, essential amino acids, soluble vitamins and vital minerals are all found in abundance in fish. Fish fat, which is primarily made up of unsaturated fatty acids, is where the majority of fish quality deterioration occurs (Yanyu et al., 2024; Mcmanus et al., 2014). Oxidation of unsaturated fatty acids reduces the quality of fish meat. Fish quality deteriorates due to factors such as neutral pH, high enzyme levels, water retention capacity, and connective tissue composition (Serdarog et al., 2001; Medina et al., 2009). Oxygen is responsible for food degradation processes such as lipid oxidation, microbial development, enzymatic browning, and vitamin loss. Lipid oxidation causes flavor, color, and nutrient loss, as well as degradation of meat proteins, pigments, and lipids, decreasing shelf life. There are various spoiling mechanisms that have been linked to this loss of quality, such as enzymatic browning, non-enzymatic lipid browning, endogenous enzyme activity, lipid oxidation, and microbiological development (Massimo et al., 2024; Gram et al., 2000). Effective treatment methods are necessary to extend fish shelf life.

Edible films and coatings are one way to increase the shelf life of minimally processed food. Edible coating, a biodegradable material that may be consumed as part of food, is the ideal solution for food industry to meet consumer needs without side effects (Pham et al., 2023). Edible coatings on product surfaces reduce oxygen permeability, solute movement, and provide a moisture barrier (Sathivel et al., 2006). Protein-based polymers and polysaccharides are the materials utilized to create edible films. Coating minimizes interaction with the environment during manufacturing and storage and keeps food from spoiling (Mchugh et al., 2000). Whey protein derived from cheese production as a byproduct exhibited excellent oxygen and aroma barriers (Gao et al., 2024). Whey protein is a blend of proteins that have been separated from whey, the liquid that is produced as a byproduct of making cheese. The proteins include immunoglobulins, serum albumin, β-lactoglobulin, and α-lactalbumin. When milk is coagulated to make cheese, whey is the residue that remains. Whey protein is widely available as a dietary supplement, usually in the form of powder that can be mixed into drinks (Turienzo et al., 2011). Whey protein coatings and films are translucent, flexible, and have excellent mechanical, gas, and oil barrier qualities; nevertheless, water absorption is limited because of the large percentage of hydrophilic amino acids (Kandasamy et al., 2021; Zheng et al., 2024). Both whey protein isolate and whey protein concentrate are used. Whey protein isolate (WPI) is a dairy industry byproduct generated during casein or cheese manufacturing and is well known for its excellent functional properties and wide-ranging use in both food and pharmaceutical applications (Sogut et al., 2022; Hong et al., 2004; Rafiei et al., 2025). Whey protein concentrate (WPC) is a dry powder sold as-is or added to other food items to improve nutritional content and functioning (Soccio et al., 2019; Gantumur et al., 2023). Coatings made of whey protein efficiently prevent carbon transfer and the development of browning in perishable food (Shahidi et al., 2020). Whey protein-based films have shown good mechanical resistance and gas barrier properties at low relative humidity levels. Whey proteins fractions and isolates have been extensively investigated for their usage in films and coatings because they are edible, high in nutritional value, and biodegradable. These proteins have shown great promise as edible film-forming agents, particularly in the preservation of seafood and fish products (Khan et al., 2015). A little amount of plasticizer must typically be added when creating protein-based films to lessen the intermolecular forces that bind neighboring polymer chains together and cause brittleness. Common plasticizers include polyols like sorbitol, glycerol, mannitol, propylene glycol and polyethylene glycol (Rossini et al., 2009). The current study aimed to evaluate the effectiveness of whey protein-based coatings, especially the WPC + Glycerol (1:2) formulation, in delaying physicochemical and sensory deterioration of *L. rohita* fillets during refrigerated storage by minimizing lipid oxidation, enzymatic activity, and moisture loss. The rationale for selecting these treatments was to compare the preservation efficiency of WPI and WPC based coatings under different plasticizer compositions, thereby identifying the most suitable formulation for maintaining fish freshness and extending shelf life.

## MATERIALS AND METHODS

### Materials preparation

Fresh specimens of *L. rohita* (Rohu) were collected from the Hassan Abdal fish shop in Wah city and immediately transfered to the laboratory at the Department of Biosciences, University of Wah. The fish were cleaned to remove blood residues and surface impurities from their surfaces and then cut into fillets of uniform sizes. These fillets were subsequently stored at 4°C in a refrigerator prior to coating.

### Preparation of whey protein coating solutions

Five distinct coating formulations were prepared using WPC and WPI in combination with glycerol and gelatin. The formulations included: WPI + Glycerol (1:1), WPI + Glycerol (1:2), WPI + Glycerol + Gelatin (1:1:1), WPC + Glycerol (1:1), and WPC + Glycerol (1:2). The ratios were selected based on literature reports demonstrating effective flexibility, mechanical stability, and barrier properties of protein-based coatings applied to seafood (Mchugh et al., 2000). For each formulation, 8% (w/w) whey protein was slowly dissolved in distilled water and stirred magnetically for 30 minutes at room temperature. The pH was adjusted to 6.8 using dilute acetic acid, and the mixture was heated in a water bath at 80 °C for thirty minutes to promote protein denaturation and film formation. Plasticizers (glycerol or gelatin) were then incorporated as per the treatment plan, followed by an additional 30 minutes of stirring to achieve a uniform, homogeneous coating solution. All temperature and pH conditions were maintained consistently to ensure reproducibility (Khan et al., 2015; Kandasamy et al., 2021; Diaz et al., 2011)

**Coating application and storage**

Coatings were applied by dipping the fillets into the prepared solutions for 2 minutes, followed by air drying at the room temperature (23 ºC) for about 15 minutes to ensure uniform coating thickness (±1.5 mm). A control group was left uncoated for comparison (Khan et al., 2015). All samples were stored at 4 ± 1 °C for 15 days and evaluated at intervals of 1, 3, 6, 9, 12, and 15 days. Each treatment was performed in triplicate (n = 3), while the control group consisted of a single representative sample per interval, with each parameter measured in triplicate for statistical validity.

**pH measurement**

The pH of the fish fillets was measured before and after coating by homogenizing 10 g of each sample in 100 mL of distilled water. The homogenization was carried out for approximately 30 seconds, after which the pH was recorded by directly immersing the pH electrode into the homogenized sample.

**Coating retention (%)**

Coating retention efficiency was calculated following the method of Diaz et al. (2011) with modifications.

Coating retention % = (Wt. of Coated fillet pieces / Wt. Of Uncoated raw fillet pieces) x 100

This parameter indicated the uniformity and adhesion strength of coatings across treatments.

**Weight loss analysis**

Weight loss during storage was calculated by recording the initial and final weights of fillets at each interval:

Weight Loss (%) = (Initial weight – Final weight/Initial weight) ×100

Measurements were made in triplicate, and results were expressed as mean ± SD (Diaz et al., 2011).

**Color analysis**

Color characteristics of coated and non-coated fillets were evaluated visually throughout refrigerated storage (4 ± 1 °C) for 15 days. Evaluations were carried out under standardized fluorescent white light conditions using a neutral gray background to minimize external effects. A 10-point scale was used (0 = light pink/fresh; 10 = dark brown/blackish). Assessments were made on days 1, 3, 6, 9, 12, and 15. Results were expressed as mean ± SD, and statistical significance was determined by one-way ANOVA followed by Tukey's HSD ($p < 0.05$) (Shon et al., 2008).

**Texture analysis**

Texture was assessed by tactile evaluation of coated and uncoated fillets over the 15-day storage period at 4 ± 1 °C. Trained panelists gently pressed fillets between their fingers to determine firmness. Texture scores were assigned on a 10-point scale ranging from 0 (extremely soft) to 10 (extremely hard). Assessments were carried out every 3 days in triplicate (n = 3). Mean ± SD values were calculated, and statistical comparisons between treatments and storage intervals were made using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$) (Sallama et al., 2004).

**Aroma analysis**

Aroma profiles of coated and non-coated fillets were determined during storage at 4 ± 1 °C for 15 days. Sensory evaluation was conducted under controlled laboratory conditions by a trained panel using a 10-point scoring scale, where 0 corresponded to fresh, mild, sweet smell and 10 to toxic, unpleasant odor indicative of decomposition. Samples were evaluated every 3 days in triplicate (n = 3). Data were expressed as mean ± SD, and statistical significance was analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$) (Meilgaard et al., 2007).

**Statistical analysis**

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS v25.0 and Microsoft Excel. Data normality and variance homogeneity were verified using the Shapiro–Wilk and Levene's tests, respectively. Data were analyzed separately for each storage interval using one-way ANOVA, followed by Tukey's HSD. Future studies should apply two-way ANOVA to assess the combined effects of coating type and storage duration.

## RESULTS AND DISCUSSION

The performances of coating with whey protein on the preservation and quality of *Labeo rohita* fillets were studied. Fish meat is highly perishable due to its high-water activity, neutral pH, enzymatic activity, and connective tissue content, which make it prone to microbial growth and oxidative deterioration (Asghar et al., 2023). Lipid oxidation is considered one of the most critical mechanisms of spoilage, leading to nutritional loss, off-flavor development, discoloration, and textural changes in fish meat. Therefore, development of natural preservation methods such as protein-based edible coatings has gained attention. Whey protein coatings are biodegradable, colorless, odorless, and known to form strong oxygen barriers with moderate moisture resistance (Yumnam et al., 2023). All whey protein-based coatings demonstrated positive quality preservation effects and shelf-life extension were clearly obtained as compared to the control. The whey protein isolate (WPI), whey protein concentrate (WPC), glycerol, and gelatin were formulated in different proportions (1:1, 1:2, 2:1:1, 1:1, 1:2) in single coats, double coats, and triple coats. They were observed to affect stability of pH, texture, color, aroma and weight of the fillets during refrigerated storage.

Analysis of the data was done on SPSS and Microsoft excel. All the measurements were calculated using the mean/standard deviation (SD) and range. The statistical significance was determined by one-way ANOVA with post-hoc tests (Tukey HSD), $p < 0.05$. Letters that are superscripted in different tables represent great differences in treatment.

### pH analysis of whey protein coated *L. rohita* fillets

Fish fillets pH is one of the widely recognized indicators of freshness and spoilage since it determines the activity of enzymes, microbial growth, and the biochemical stability of the food. Since all the fillets had the same initial pH (6.8) in the current study (table 1), the treatments were comparable. Significant disparity between coated and uncoated samples was detected, though, after 15 days of refrigerated storage.

Table 1. Initial and final pH of whey protein coated *Labeo rohita* fillets (n=3, Mean ± SD).

| Treatment Group (Coating) | Initial pH | Final pH | Δ pH (Initial–Final) | p-value |
|---|---|---|---|---|
| WPI:GLY (1:1) | 6.8 | 5.67 ± 0.58 | 1.13 ± 0.58 | p = 0.046 |
| WPI:GLY (1:2) | 6.8 | 5.67 ± 0.58 | 1.13 ± 0.58 | p = 0.048 |
| WPI:GLY:Gelatin | 6.8 | 6.0 ± 0.0 | 0.80 ± 0.0 | p = 0.021 |
| WPC:GLY (1:2) | 6.8 | 6.33 ± 0.25 | 0.47 ± 0.15 | p < 0.001 |
| WPC:GLY (1:1) | 6.8 | 6.0 ± 0.0 | 0.80 ± 0.0 | p = 0.027 |
| Control (Uncoated) | 6.8 | 5.0 ± 0.0 | 1.80 ± 0.0 | p > 0.05 |

Values are mean ± SD of three replicates (except control, n=1). Statistical differences were considered significant at $p < 0.05$.

Statistical analysis revealed significant differences ($p < 0.05$) among treatments in pH change (ΔpH). The WPC+Glycerol (1:2) coating exhibited the smallest ΔpH (0.47 ± 0.15, $p < 0.001$), indicating strong stability against acidification, whereas the control group ($p > 0.05$) showed the greatest pH decline. With a pH drop from 6.8 to 5.0 (ΔpH = 1.8), the control (uncoated) fillets showed the most severe decline by the end of storage. Microbial growth and the generation of acidic metabolites and volatile basic nitrogen compounds like ammonia and trimethylamine are usually linked to this trend. On the other hand, coated fillets showed better stability and much smaller pH drops (Fan et al., 2009); while WPI-based treatments showed moderate reductions ranging from 0.8 to 1.13, the WPC+Glycerol (1:2) treatment performed best, exhibiting only a slight decrease (ΔpH = 0.47).

These results support the protective function of whey protein coatings, which slow down microbial metabolism by acting as semi-permeable barriers that prevent the transfer of moisture and oxygen. WPC-based coatings perform better than WPI because of their higher protein content, which creates a denser, more compact film matrix. These coatings slow the production of volatile bases and acidic metabolites during storage by more effectively limiting oxygen diffusion. Similar findings were noted for Atlantic salmon and silver carp, where protein-based edible coatings postponed pH changes and decreased microbial spoiling.

The nature of the plasticizer (glycerol) and the addition of gelatin may account for the minor variations among WPI treatments. Although glycerol makes films more flexible, it can also increase water vapor permeability, which reduces the effectiveness of microbial inhibition. Although the addition of gelatin produced structural homogeneity, WPC films performed better in maintaining pH stability. Overall, the findings show that edible whey protein coatings, specifically WPC+Glycerol at a 1:2 ratios, considerably slow down the pH decline in *L. rohita* fillets. This treatment showed improved resistance to microbial spoiling and a strong potential for prolonging fish shelf life by maintaining near-neutral values throughout storage. The uncoated control, on the other hand, rapidly deteriorated, demonstrating the crucial role coatings play in maintaining biochemical quality. The reduction in pH can be linked to the production of acidic metabolites by microbial activity and protein degradation (Bravin et al., 2006). Coated samples, particularly those treated with Solution 4, experienced delayed biochemical changes, indicating extended freshness.

**Coating retention and weight loss**

Table (2) shows the weight loss of *L. rohita* fillets after 15 days of refrigeration. Significant moisture loss in the absence of protective coatings was indicated by the uncoated control samples, which showed the largest mean weight loss (6.40 g; ~75.3%) (Soccio et al., 2019). In contrast, fillets coated with a 1:2 ratios of glycerol and whey protein concentrate (WPC+Glycerol / 1:2) exhibited the lowest mean loss (3.67 g; ~30.1%), indicating better water retention when compared to other formulations. WPC: GLY (1:1) and whey protein isolate coatings (WPI: GLY / 1:1, WPI: GLY / 1:2 and WPI: GLY: Gelatin) also decreased weight loss in comparison to the control, although their effects were not as strong as those of WPC: GLY (1:2).

Table 2. Weight analysis and coating retention of whey protein–based coatings on *L. rohita* fillets after 15 days of refrigerated storage.

| Treatment Group (Coating) | Initial Weight (g) | Final Weight (g) | Total Loss (g) | % Loss | Mean ± SD | Coating Retention (%) | p-value |
|---|---|---|---|---|---|---|---|
| WPI:GLY (1:1) | 8.6 | 4.3 | 4.3 | 50.0% | 4.6 ± 1.3 | 50.0% | p = 0.042 |
| WPI:GLY (1:2) | 5.2 | 2.0 | 3.2 | 61.5% | 3.0 ± 0.4 | 38.5% | p = 0.038 |
| WPI:GLY:Gelatin | 9.4 | 4.9 | 4.5 | 47.9% | 4.5 ± 0.5 | 52.1% | p = 0.029 |
| WPC:GLY (1:2) | 12.2 | 10.2 | 2.0 | 16.4% | 2.7 ± 1.1 | 83.6% | p < 0.001 |
| WPC:GLY (1:1) | 7.5 | 4.0 | 3.5 | 46.7% | 4.0 ± 0.6 | 53.3% | p = 0.024 |
| Control (Uncoated) | 8.5 | 2.1 | 6.4 | 75.3% | 6.4 ± 0.9 | 24.7% | p > 0.05 |

Values are mean ± standard deviation (n=3). Statistical differences were considered significant at $p < 0.05$.

The effectiveness of whey protein coatings applied to *L. rohita* fillets was initially assessed by determining their coating retention efficiency (%). This measurement confirmed that coating application was uniform across treatments, with no significant differences observed in initial coating yields, indicating reproducibility of the method. Such uniformity is essential for comparative analysis, as variation in coating thickness can directly influence barrier properties, water vapor transfer, and ultimately storage stability. The results (table 2) show clear differences among treatments. The control (uncoated fillets) demonstrated the highest total loss (75.3% weight loss, 24.7% retention), indicating severe moisture evaporation and deterioration during storage. In contrast, fillets coated with whey protein glycerol exhibited the lowest total loss (16.4% weight loss, 83.6% retention), significantly outperforming other coatings. WPI-based coatings with glycerol (1:1 or 1:2) and gelatin combinations showed intermediate values (Sun et al., 2024). These findings highlight the superior barrier properties of WPC-based coatings, especially when combined with higher glycerol concentrations.

**Aroma analysis and sensory quality**

The aroma profiles of *L. rohita* fillets exhibited significant variation among coating formulations ($p < 0.05$). WPC-based coatings, particularly the 1:2 WPC+Glycerol coating, demonstrated the most effective preservation, maintaining low aroma scores (2.67 ± 1.15) and minimal deterioration (+267%) throughout 15 days of storage ($p < 0.001$). In contrast, uncoated control samples recorded the highest aroma score (10.00), indicating complete decomposition ($p > 0.05$). WPI-based coatings exhibited moderate protection, with aroma scores ranging from 4 to 6.67, corresponding to mild to strong fishy odors. These findings confirm that whey protein concentrate (WPC) coatings, especially at a 1:2 ratio with glycerol, provide superior oxidative and microbial inhibition, effectively preserving the olfactory quality of refrigerated fish fillets.

These results indicate that coatings substantially delayed the onset of undesirable odors by inhibiting microbial activity and lipid oxidation. Similar results were reported by (Valverde et al., 2005), where cactus mucilage coatings reduced off-odor formation in fish fillets during storage.

**Color analysis**

The color attributes of *L. rohita* fillets showed notable differences across the various coating formulations ($p < 0.05$). WPC-based coatings, particularly the 1:2 WPC+Glycerol formulation, demonstrated exceptional color preservation, maintaining the lowest color score (2.67 ± 1.15) and minimal discoloration (+267%) after 15 days of refrigerated storage

($p < 0.001$). Fillets coated with WPC+Glycerol (1:1) also exhibited excellent visual stability, retaining a light pink appearance with limited oxidative browning. Conversely, uncoated control samples displayed the highest color score

Table 3. Aroma analysis of whey protein–coated *L. rohita* fillets after 15 days.

| Treatment Group (Coating) | Day 15 Aroma Score (Mean ± SD) | % Deterioration from Day 1 | p-value | Interpretation |
|---|---|---|---|---|
| WPI:GLY (1:1) | 6.00 ± 1.63 | +600% | p = 0.043 | Noticeable spoilage, putrid odor |
| WPI:GLY (1:2) | 4.00 ± 0.00 | +400% | p = 0.037 | Mild spoilage, tolerable odor |
| WPI:GLY:Gelatin | 6.67 ± 2.08 | +667% | p = 0.049 | Strong fishy odor, moderate deterioration |
| WPC:GLY (1:2) | 2.67 ± 1.15 | +267% | p < 0.001 | Best preservation; remained nearly fresh |
| WPC:GLY (1:1) | 3.33 ± 1.15 | +333% | p = 0.021 | Mild spoilage, slightly fishy smell |
| Control (Uncoated) | 10.00 ± 0.00 | +1000% | p > 0.05 | Complete decomposition; toxic odor |

Values represent mean ± standard deviation (n = 3, except control). Statistical significance determined at $p < 0.05$. (Scale: 0 = Fresh/sweet; to 10 = Toxic decomposition).

(10.00), indicating complete darkening and spoilage ($p > 0.05$). WPI-based coatings, including WPI+Glycerol (1:1), WPI+Glycerol (1:2), and WPI: GLY: Gelatin, showed moderate protection, with color scores ranging from 6.00 to 7.33, corresponding to brownish-gray or dark brown tones. These results confirm that WPC-based coatings, especially in a 1:2 ratios with glycerol, effectively prevented oxidative discoloration and maintained the natural pink hue of fish fillets during storage. The slower discoloration in coated samples is linked to the antioxidant and barrier properties of whey protein films, which limit lipid oxidation and pigment degradation. The superior performance of WPC-based films is likely due to their more compact protein network, which better reduces oxygen diffusion compared to WPI films. Similar protective effects of whey protein and polysaccharide-based coatings on fish color stability during storage have been previously documented (Del et al., 2005).

WPI-based coatings and WPI: Gelatin formulations exhibited moderate preservation of color quality, with final scores between 6.0 ± 0.0 and 7.33 ± 2.08, indicating visible browning and grayish discoloration. Although these coatings slowed oxidative darkening compared to the uncoated control (10.00 ± 0.00), their effectiveness remained notably lower than that of WPC-based coatings, which maintained a stable pinkish appearance throughout storage. These findings support previous reports where whey protein films demonstrated excellent oxygen barrier properties, thereby delaying lipid oxidation induced discoloration. These findings clearly demonstrate that whey protein concentrate coatings extend the visual freshness of fish fillets and delay the progression toward unacceptable darkening.

Table 4. Color analysis (Day 15) of whey protein–coated *L. rohita* fillets

| Treatment Group (Coating) | n | Mean Color Score ± SD (Day 15) | % Deterioration (from Day 1) | p-value | Interpretation |
|---|---|---|---|---|---|
| WPI : GLY (1:1) | 3 | 7.33 ± 2.62 | +733 % | p = 0.043 | Dark brown appearance |
| WPI : GLY (1:2) | 3 | 6.00 ± 0.00 | +600 % | p = 0.037 | Moderate browning |
| WPI : GLY : Gelatin | 3 | 7.33 ± 2.08 | +733 % | p = 0.049 | Grayish brown |
| WPC : GLY (1:2) | 3 | 2.67 ± 1.15 | +267 % | p < 0.001 | Pinkish to light pink |
| WPC : GLY (1:1) | 3 | 2.67 ± 1.15 | +267 % | p = 0.021 | Pinkish |
| Control | 1 | 10.00 ± 0.00 | +1000 % | p > 0.05 | Blackish, decomposed |

Values represent mean ± standard deviation (n = 3, except control). Statistical significance determined at $p < 0.05$. (Scale: 0 = Light pink/fresh; to 10 = Dark brown to blackish).

**Textural analysis**

The texture analysis of *L. rohita* fillets revealed significant differences among the coating treatments ($p < 0.05$). WPC-based coatings, particularly the 1:2 whey protein concentrate and glycerol formulation, exhibited the most effective textural preservation, maintaining the lowest texture score (3.33 ± 1.15) and minimal hardness increase (+233%) after

15 days of refrigerated storage ($p < 0.001$). These fillets retained a soft and elastic texture, indicating optimal moisture retention and minimal protein denaturation. In contrast, uncoated control samples recorded the highest texture score (10.00 ± 0.00), representing extreme hardness and complete quality loss ($p > 0.05$). WPI-based coatings and WPI: Gelatin combinations demonstrated moderate protection, with scores ranging from 6.00 ± 0.00 to 8.00 ± 2.00, corresponding to firm to dry firm textures, suggesting partial inhibition of dehydration and muscle stiffening. The results clearly indicate that the WPC: glycerol (1:2) coating provided the most efficient barrier properties, effectively reducing moisture loss and preserving the structural integrity of fish muscle tissue throughout storage.

The efficiency of WPC coatings can be attributed to the combined role of glycerol as a plasticizer in maintaining moisture balance and whey protein concentrate as a structural barrier, which together reduced water evaporation and oxidation. Comparatively, WPI coatings demonstrated only moderate effectiveness, with final texture scores ranging from 6 to 8, indicating partial preservation.

Table 5. Texture analysis (Day 15) of whey protein–coated *L. rohita* fillets.

| Treatment Group (Coating) | n | Texture Score (Mean ± SD) | % Increase from Day 1 | p-value | Interpretation |
|---|---|---|---|---|---|
| WPI:GLY (1:1) | 3 | 6.67 ± 1.15 | +567% | p = 0.041 | Firm |
| WPI:GLY (1:2) | 3 | 8.00 ± 2.00 | +700% | p = 0.038 | Dry firm / hard |
| WPI:GLY:Gelatin | 3 | 6.67 ± 1.15 | +567% | p = 0.046 | Firm |
| WPC:GLY (1:2) | 3 | 3.33 ± 1.15 | +233% | p < 0.001 | Extremely soft |
| WPC:GLY (1:1) | 3 | 6.00 ± 0.00 | +500% | p = 0.025 | Moderately firm |
| Control | 1 | 10.00 ± 0.00 | +900% | p > 0.05 | Extremely hard |

Values represent mean ± standard deviation (n = 3, except control). Statistical significance determined at $p < 0.05$. (Scale: 0 = Extremely soft; to 10 = Extremely hard).

The current research proved that whey protein-based edible coatings significantly enhanced the physicochemical and sensory stability of *L. rohita* fillets during refrigerated storage. Among all coating formulations, the WPC and glycerol coating (1:2) produced the most significant preservation properties against all quality parameters, such as pH stability, minimized weight loss, color retention, aroma, and texture preservation. The enhanced performance of the WPC+glycerol (1:2) formulation is due to the compositional and structural advantages of whey protein concentrate. WPC has more β-lactoglobulin and α-lactalbumin content than whey protein isolate (WPI), which allows increased intermolecular cross-linking upon heat treatment and film formation. (Khan et al., 2015; Diaz et al., 2011; Asghar et al., 2023; Yumnam et al., 2023).

This results in a denser, more cohesive protein matrix that efficiently limits oxygen permeability and moisture migration, both of which are primary initiators of lipid oxidation and texture loss (Yanyu et al., 2024; Massimo et al., 2024). Additionally, glycerol serves as an efficient plasticizer that enhances film flexibility and inhibits cracking or brittleness upon storage, maintaining even coverage over fish fillet surfaces (Kandasamy et al., 2021; Khan et al., 2015) The blend yields a semi-permeable but strong barrier that slows oxidative reactions and microbial growth. Similar preservation techniques are also reported for chitosan, alginate, and cactus mucilage-based coatings that serve as oxygen and water barriers. However, whey protein coatings possess superior transparency, mechanical strength, and nutritional compatibility, and hence more appropriate for high valued seafood products.

WPC-based coatings had considerable weight loss reduction against both WPI-based coatings and control. The WPC+glycerol (1:2) coating showed the least reduction in weight (only 2 g in 15 days), representing good moisture retention. The good moisture barrier property of the coating suppressed dehydration and structural collapse of muscle fibers, retaining product juiciness. Similarly, pH stability in WPC-coated fillets was higher than in other treatments. Fillets with WPC+glycerol (1:2) coating showed minimal pH change ($\Delta pH = 0.4$), indicating decreased microbial activity and less acidic by-product accumulation. In contrast, the uncoated control exhibited a sharp pH drop ($\Delta pH = 1.8$), consistent with advanced spoilage These results support that the coating served as a barrier to oxygen, preventing lipid oxidation and microbial metabolism that leads to acid formation (Khan et al., 2015; Kandasamy et al., 2021; Rafiei et al., 2025; Diaz et al., 2011).

Texture and aroma evaluation also confirmed the protective action of the WPC+glycerol coating. The coated fillets were firm but not dry, while uncoated samples became excessively hard due to moisture loss. For aroma, WPC+glycerol (1:2) had a fresh, mild smell, whereas the control produced intense putrid odors. Such outcomes are consistent with earlier findings indicating that protein-based coatings delay sensory degradation by preventing oxidation and microbial contamination. The improved performance of WPC compared to WPI can also be attributed to variations in protein aggregation tendency and microstructural integrity on drying. Concentrate formulations generally produce films with more refined pore structures and lower surface roughness, lowering the rates of gas transmission. The greater concentration of β-lactoglobulin in WPC facilitates disulfide bonding to create a dense polymeric network that resists oxidative and hydrolytic deterioration (Khan et al., 2015; Sogut et al., 2022).

Although polysaccharide coatings like alginate and chitosan exhibited similar protective effects in previous studies, whey protein coatings offer additional advantages, including superior clarity, edibility, and seafood flavor profile compatibility. Whey proteins' biodegradable and non-toxic nature further support their applicability in green fish packaging. From an industrial point of view, whey protein coatings are economical and sustainable since they are sourced from dairy by-products that are readily available. The film-forming process is simple, scalable, and compatible with existing dip- or spray-coating systems used in seafood processing industries. Both their biodegradability and non-toxic nature make them highly suitable for sustainable fisheries and eco-friendly packaging (Khan et al., 2015; Kandasamy et al., 2021; Ruan et al., 2023; Jayakody et al., 2022; Sebaaly et al., 2021).

Although the findings clearly highlight the benefits of WPC-based coatings, several limitations should be acknowledged. The study primarily relied on sensory and semi-quantitative measures of quality (color, aroma, texture), whereas instrumental analyses such as colorimeter, texture analyzer, or GC-MS for aroma would provide more objective data. The control group had limited replication ($n = 1$), which restricts the statistical robustness of comparisons. Future studies should incorporate full replication, instrument-based evaluations, and two-way ANOVA analysis to account for interactions between coating type and storage duration.

Overall, the study confirms that whey protein concentrate-based coatings, particularly at a 1:2 ratio with glycerol, are highly effective in maintaining the sensory and physicochemical quality of *L. rohita* fillets during cold storage. Their superior barrier properties, cost-effectiveness, and environmental sustainability make them promising alternatives to synthetic preservatives and packaging in the fishery industry.

## CONCLUSION

This research clearly indicates that edible coatings formulated with whey protein markedly enhance the quality and extend the shelf life of *L. rohita* fillets under refrigerated storage conditions. Among all formulations, WPC and glycerol (1:2) consistently outperformed others by reducing weight loss (2 g), maintaining pH (6.5), preserving texture (3.33 ± 1.15), preventing discoloration (2.67 ± 1.15), and minimizing off-odor development. The use of whey protein concentrate with glycerol therefore represents a promising and sustainable preservation strategy for perishable fish products.

## AUTHOR'S CONTRIBUTION

Performed the experiment: Mahnoor Nawazish, Eisha Tahir and Momina Hanif, analyzed the data: Syed Waqas Hassan and Jehangir Khan, designed the experiment & wrote the paper: Faisal Nawaz, Syed Waqas Hassan and Jehangir Khan.

## FUNDING

No funding was received for this study.

## AVAILABILITY OF DATA AND MATERIAL

The collected and analyzed data is presented in the form of figures.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study was checked and approved by the relevant team.

## CONSENT FOR PUBLICATION

All authors have reviewed the manuscript and approved it for publication.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## ACKNOWLEDGEMENT
Not applicable.

## REFERENCES

Asghar, S., Zohaib, M. Anum, H. & Rafique, S. (2023). Comparative studies on body composition of farmed and wild rohu (*Labeo rohita*) from district Jhelum, Punjab, Pakistan. Pakistan Journal of Biotechnology, 20, 51-58. https://doi.org/10.34016/pjbt.2023.20.01.772.

Bravin, B., Pressini, D., & Sensidoni, A. (2006). Development and application of polysaccharide-lipid edible coating to extend shelf life of dry bakery products. Journal of Food Engineering, 76, 280-290. https://doi.org/10.1016/j.jfoodeng.2005.05.021.

Del-Valle, V.; Hernández-Muñoz, P.; & Guarda, A. (2005). Development of a cactus-mucilage edible coating (Opuntia ficus-indica) and its application to extend strawberry (*Fragaria ananassa*) shelf-life. Food Chemistry, 91(4), 751-756. https://doi.org/10.1016/j.foodchem.2004.07.002

Diaz, O.; Turienzo, L.R.; Cobos, A.; Caride, A.; & Vieites, J.M. (2011). Whey protein-based coatings on frozen Atlantic salmon (*Salmo salar*): Influence of the plasticiser and the moment of coating on quality preservation. Food Chemistry, 128, 187-194. https://doi.org/10.1016/j.foodchem.2011.03.026

Fan, W.; Sun, J.; Chen, Y.; Qiu, B.; & Zhang, Y. (2009). Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chemistry, 115, 66-70. https://doi.org/10.1016/j.foodchem.2008.11.060

Gantumur, M.; Sukhbaatar, N.; & Shi, R. (2023). Structural, functional, and physicochemical characterization of fermented whey protein concentrates recovered from various fermented-distilled whey. Food Hydrocolloids, 135, 108130. https://doi.org/10.1016/j.foodhyd.2022.108130

Gao, C.; Zheng, Y.; & Zhou, R. (2024). Active whey protein/hydroxypropyl methylcellulose edible films incorporated with cinnamaldehyde: Characterization, release kinetics and application to Mongolian cheese preservation. International Journal of Biological Macromolecules, 266(1), 131061. https://doi.org/10.1016/j.ijbiomac.2024.131061

Gram, L.; & Huss, H.H. (2000). Fresh and processed fish and shellfish. In The Microbiological Safety and Quality of Foods, 472-506.

Huang, Y.; Zeyu, L.; & Mengcheng, L. (2024). Fish genomics and its application in disease-resistance breeding. Reviews in Aquaculture, 17(1), e12973. https://doi.org/10.1111/raq.12973

Hong, S.I.; & Krochta, J.M. (2004). Whey protein isolate coating on LDPE film as a novel oxygen barrier in the composite structure. Packaging Technology and Science, 17, 13-21. https://doi.org/10.1002/pts.634

Jayakody, M.M.; Gunathilake, M.P.; & Wijesekara, I. (2022). Seaweed-derived alginate, agar, and carrageenan-based edible coatings and films for the food industry: A review. Journal of Food Measurement and Characterization, 16, 1195-1227. https://doi.org/10.1007/s11694-021-01277-y

Kandasamy, S.; Yoo, J.; & Yun, J. (2021). Application of whey protein-based edible films and coatings in food industries: An updated overview. Foods, 11(9), 1056. https://doi.org/10.3390/coatings11091056

Kashif, U.; Muhammad, A.; & Rehana, I. (2011). Effect of various nutrient combinations on growth and body composition of rohu (*Labeo rohita*). African Journal of Biotechnology, 10(62), 13605-13609. http://www.academicjournals.org/AJB/PDF/pdf2011/12Oct/Umer%20et%20al.pdf

Khan, M.I.; Adrees, M.N.; & Anjum, F.M. (2015). Oxidative stability and quality characteristics of whey protein coated rohu (*Labeo rohita*) fillets. Lipids in Health and Disease, 14, 1-9. https://doi.org/10.1186/s12944.015.0060.z

Kilincceker, O.; Dogan, S.; & Kucukoner, E. (2009). Effect of edible coatings on the quality of frozen fish fillets. LWT - Food Science and Technology, 42(4), 868-873. https://doi.org/10.1016/j.lwt.2008.11.003

Luciana, K.O.; Wilson, W.J.; & Marcelo, B.T. (2024). Fish culture in biofloc technology (BFT): Insights on stocking density carbon sources, C/N ratio, fish nutrition and health. Aquaculture and Fisheries, 9(4), 522-533. https://doi.org/10.1016/j.aaf.2022.08.010

Mahboob, S.; Hussain, M.I.; & Iqbal, Z. (2009). Estimation of volatile organic compounds in farmed and wild rohu (*Labeo rohita*). Iran Journal of Fisheries Science, 8(1), 65-72. 10.22092/IJFS.2018.114761

Massimo, M.; Roberta, F.; & Cinzia, M. (2024). Assessment of lipid oxidation in fish and fish products processed by cold plasma technologies. Applied Food Research, 4(2), 100646. https://doi.org/10.1016/j.afres.2024.100646

Mcmanus, A.; Hunt, W.; Storey, J.; Mcmanus, J.; & Hilhorst, S. (2014). Perceptions and preference for fresh seafood in an Australian context. International Journal of Consumer Studies, 38, 146-152. https://doi.org/10.1111/ijcs.12076

Mchugh, T.H.; & Senesi, E. (2000). Apple wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. Journal of Food Science, 65(3), 480-485. https://doi.org/10.1111/j.1365-2621.2000.tb16032.x

Medina, I.; Gallardo, J.M.; & Aubourg, S. (2009). Quality preservation in chilled and frozen fish products by employment of slurry ice and natural antioxidants. International Journal of Food Science & Technology, 44(8), 1467-1479. https://doi.org/10.1111/j.1365-2621.2009.02016.x

Meilgaard, M.M.; Civille, G.V.; & Carr, T. (2007). Overall difference tests: Does a sensory difference exist between samples? In Sensory Evaluation Techniques, 4, 63-104.

Monirul, I.M.; Shariar, K.Z.; & Dania, A. (2024). Temperature induced biological alterations in the major carp, Rohu (*Labeo rohita*): Assessing potential effects of climate change on aquaculture production. Aquaculture Reports, 35, 101954. https://doi.org/10.1016/j.aqrep.2024.101954

Pham, T.T.; Nguyen, L.P.; & Dam, M.S. (2023). Application of edible coating in extension of fruit shelf life: Review. Agri Engineering, 5(1), 520-536. https://doi.org/10.3390/agriengineering5010034

Rafiei, M.; Soltanizadeh, N.; & Hemmati, M.E. (2025). Unlocking the potential of whey protein isolate: How ultrasonic treatment transforms functionality and structure. Ultrasonics Sonochemistry, 120, 107486. https://doi.org/10.1016/j.ultsonch.2025.107486

Rossini, K.; Noren, C.P.Z.; Cladera-Olivera, F.; & Brandelli, A. (2009). Casein peptides with inhibitory activity on lipid oxidation in beef homogenates and mechanically deboned poultry meat. LWT - Food Science and Technology, 42, 862-867. https://doi.org/10.1016/j.lwt.2008.11.002

Ruan, H.; Aulova, A.; Ghai, V.; & Pandit, S. (2023). Polysaccharide-based antibacterial coating technologies. Acta Biomaterialia, 168, 42-77. https://doi.org/10.1016/j.actbio.2023.07.023

Sallama, K.I.; Ishioroshib, M.; & Samejima, K. (2004). Antioxidant and antimicrobial effects of garlic in chicken sausage. Lebensmittel-Wissenschaft & Technologie, 37, 849-855. https://doi.org/10.1016/j.lwt.2004.04.001

Sathivel, S. (2006). Chitosan and protein coatings affect yield, moisture loss, and lipid oxidation of apple pink salmon fillets during frozen storage. Journal of Food Science, 70, 445-459. https://doi.org/10.1111/j.1365-2621.2005.tb11514.x

Sebaaly, C.; Trifan, A.; Sieniawska, E.; & Greige, H. (2021). Chitosan-coating effect on the characteristics of liposomes: A focus on bioactive compounds and essential oils: A review. Processes, 9(3), 445. https://doi.org/10.3390/pr9030445

Serdarog, L.M.; & Felekog, L.E. (2001). The packaging under modified atmosphere of seafood. World Food, 4.

Shahidi, F.; & Hossain, A. (2020). Preservation of aquatic food using edible films and coatings containing essential oils: A review. Critical Reviews in Food Science and Nutrition, 62, 66-105. https://doi.org/10.1080/10408398.2020.1812048

Shon, J.; & Chin, K.B. (2008). Effect of whey protein coating on quality attributes of low-fat, aerobically packaged sausage during refrigerated storage. Journal of Food Science, 73, 469-475. https://doi.org/10.1111/j.1750-3841.2008.00829.x

Sogut, E.; Ertekin, B.; & Seydim, A.C. (2022). Whey protein isolate- and carrageenan-based edible films as carriers of different probiotic bacteria. Journal of Dairy Science, 105(6), 4829-4842. https://doi.org/10.3168/jds.2021-21245

Soccio, M.; Lotti, N.; & Balestra, F. (2019). Characterization of composite edible films based on pectin/alginate/whey protein concentrate. Advanced Polymeric Materials and Nanocomposites for Green Plastics and Biodegradable Packaging, 12(15), 2454. https://doi.org/10.3390/ma12152454

Sun, H.; Liu, X.; Huang, Y.; & Leng, X. (2024). Incorporating functional colorants in whey protein isolate–cellulose nanocrystal-blended edible films for pork freshness prediction. International Journal of Biological Macromolecules, 283(Part 1), 137276. https://doi.org/10.1016/j.ijbiomac.2024.137276

Tan, K.S.; Zhang, H.K.; & Zheng, H.P. (2022). Carotenoid content and composition: A special focus on commercially important fish and shellfish. Critical Reviews in Food Science and Nutrition, 64(2), 544-561. https://doi.org/10.1080/10408398.2022.2106937

Turienzo, L.; Cobos, A.; & Moreno, V. (2011). Whey protein-based coatings on frozen Atlantic salmon (*Salmo salar*): Influence of the plasticiser and the moment of coating on quality preservation. Food Chemistry, 128(1), 187-194. https://doi.org/10.1016/j.foodchem.2011.03.026

Valverde, J.M.; Valero, D.; Martínez, R.D.; & Guillén, F. (2005). Novel edible coating based on Aloe vera gel to maintain poultry quality and safety. Journal of Agricultural and Food Chemistry, 53, 7807-7813.

Vyas, V.; Damde, D.; & Parashar, V. (2009). Fish diversity of Narmada in submergence area of Indra Sagar Reservoir. Journal of Inland Fisheries Society of India, 41, 18-25.

Yanyu, D.; Tingting, Z.; & Rongbin, Z. (2024). Changes in the chemical properties and metabolite profiling of fish sauce prepared from underutilized large yellow croaker roes during fermentation at different temperatures. Food Research International, 179, 114030. https://doi.org/10.1016/j.foodres.2024.114030

Yumnam, M.; Marak, P.R.; & Gupta, A.K. (2023). Effect of pomelo peel essential oil on the storage stability of a few selected varieties of freshwater fish. Journal of Agriculture and Food Research, 11, 100472. https://doi.org/10.1016/j.jafr.2022.100472

Zhe, Y.; & Muyang, L. (2025). Application of nutrition interventions strategy to enhance fish flesh quality. Journal of Food Composition and Analysis, 138, 107010. https://doi.org/10.1016/j.jfca.2024.107010

Zheng, F.; Yang, Q.; & Yuan, C. (2024). Characterizations of corn starch edible films reinforced with whey protein isolate fibrils. Food Hydrocolloids, 147(Part A), 109412. https://doi.org/10.1016/j.foodhyd.2023.109412