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Investigation of K-value Changes and Its Correlation with Chemical Spoilage Indicators in Rainbow Trout Fillets Coated with Gelatin Containing Polysaccharide Extracted from Loquat [Eriobotrya japonica] Seed

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ABSTRACT

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Freshness and quality of fish fillets are key indicators of safety and consumer acceptance in seafood products. This study evaluated the K-value index and its correlation with other chemical spoilage indicators, including total volatile basic nitrogen (TVB-N), trimethylamine (TMA), peroxide value (PV), thiobarbituric acid reactive substances (TBA), and free fatty acids (FFA) in rainbow trout fillets during refrigerated storage. Fillets were coated with 1% gelatin containing 0.5% or 1% loquat (*Eriobotrya japonica*) seed polysaccharide, alongside an uncoated control, and stored at 4 ± 1 °C for 12 days. Sampling was performed on days 0, 1, 4, 7, 10, and 12, and chemical analyses were conducted to determine the indices. Results showed that fillets treated with gelatin plus 1% polysaccharide had the lowest K-values and other spoilage indicators throughout storage. Correlation analysis revealed significant positive relationships between the K-value and TVB-N, TMA, PV, TBA, and FFA ($p < 0.05$). These findings indicate that the K-value is a reliable indicator for assessing the quality and spoilage of coated rainbow trout fillets during refrigerated storage. Moreover, bioactive coatings containing loquat seed polysaccharide effectively improved product quality and extended shelf life.

1- Introduction

Oxidation is an essential biological process in many living organisms for energy production; however, the generation of reactive oxygen species (ROS) during certain endogenous oxidative reactions can lead to cellular damage and has been associated with the development of various chronic diseases, including cancer, arthritis, and aging. Consequently, in recent years, increasing attention has been directed toward the development and application of natural antioxidants—particularly plant-derived polysaccharides—as effective and safe compounds for combating oxidative stress and maintaining human health [1,2]. In this context, polysaccharides extracted from plant sources have attracted considerable interest due to their ability to scavenge free radicals and inhibit oxidative reactions [3,4].

Japanese loquat (*Eriobotrya japonica*) is an evergreen tree native to southeastern China that was introduced to Japan in ancient times [5]. Each loquat fruit contains one to five brown seeds, which are the sole means of propagation for the tree. A substantial portion of the fruit, namely the seeds, is discarded as waste, despite being rich in valuable bioactive compounds such as polysaccharides. These polysaccharides can be extracted and incorporated into food packaging films, where they may play a positive role in preserving product quality and extending shelf life by inhibiting lipid oxidation and reducing the activity of spoilage-related enzymes [6]. Such applications are particularly important for highly perishable products, including meat and seafood.

Seafood products, especially fish, are highly susceptible to rapid spoilage due to their soft tissue structure, high lipid content, and intense enzymatic activity, leading to deterioration in quality, nutritional value, and consumer acceptability [7]. Among aquaculture species, rainbow trout (*Oncorhynchus mykiss*), a member of the Salmonidae family, has been identified as one of the most suitable species for farming owing to its resistance to environmental fluctuations such as changes in dissolved oxygen and carbon dioxide levels, minor pollution, and temperature variations, as well as its relatively high growth rate [8].

One of the most important approaches for evaluating fish quality during storage is the assessment of biochemical and chemical spoilage indices. The K value, which reflects nucleotide degradation in fish muscle, is recognized as a sensitive and accurate indicator of nucleotide-related spoilage and has been widely used to evaluate the quality of seafood products [2]. In addition to the K value, chemical parameters such as total volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid (TBA), and free fatty acids (FFA) are commonly employed as indicators of chemical spoilage in fish, as they reflect oxidative and hydrolytic reactions occurring in the tissue. Simultaneous evaluation of these indices alongside the K value enables a more comprehensive analysis of spoilage processes and product quality [7].

The K value is considered one of the most precise indicators for assessing freshness and chemical quality of aquatic products, as it is based on the measurement of changes in muscle nucleotide compounds, including adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine

monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx). A major advantage of this index is its ability to directly reflect the biochemical degradation of high-energy compounds following fish death, exhibiting higher sensitivity—particularly during the early stages of spoilage—compared with conventional indicators such as TVB-N or TBA [9].

The distinctive feature of the present study, compared with previous research, is the simultaneous evaluation of the effects of edible coatings containing polysaccharides extracted from Japanese loquat seeds on changes in the K value, along with their correlation with classical chemical spoilage indices. These polysaccharides, owing to their natural antioxidant and bioactive properties, have the potential to reduce oxidative processes and enhance the stability of lipids and nucleotide compounds in fish muscle [10]. This integrated assessment facilitates the use of the K value as either an alternative or complementary indicator for evaluating the quality of coated fish products.

Therefore, the objective of this study was to investigate changes in the K value and its relationship with chemical spoilage indices in rainbow trout fillets during different storage periods under refrigerated conditions. The findings of this research are expected to contribute to a better understanding of spoilage mechanisms, the identification of reliable quality indicators, and the development of effective natural strategies for improving shelf life, quality, and food safety in the seafood industry.

2- Materials and Methods

2.1. Raw materials and sample preparation

Fresh rainbow trout fillets (*Oncorhynchus mykiss*) were purchased from a local market and immediately transported to the laboratory. After washing and primary preparation, the fillets were cut into uniform dimensions. To prepare the edible coating solution, food-grade gelatin (1%, w/v) was dissolved in distilled water and heated at 50 °C for 30 min until a homogeneous solution was obtained.

Polysaccharides were extracted from Japanese loquat (*Eriobotrya japonica*) seeds using a microwave-assisted extraction method. The coating solutions were prepared with gelatin-to-polysaccharide ratios of 1:0 (G), 1:0.5 (GP0.5), and 1:1 (GP1). Uncoated fillets were used as the control sample (C). Polysaccharide concentrations of 0.5% and 1% were selected based on previous studies, as these levels provide effective antioxidant and antimicrobial activity without adversely affecting the texture or appearance of the fillets [9].

2.2. Determination of K value

The K value was determined according to the method described by Choi et al. (2007), with minor modifications [11]. Briefly, 5 g of fish sample was homogenized with 25 mL of 0.6 mmol L⁻¹ perchloric acid and centrifuged at 1940 × g for 10 min. The pH of the supernatant was adjusted to 6.5–6.8 using 1 mmol L⁻¹ sodium hydroxide. Subsequently, 20 mL of distilled water was added, and the solution was filtered through a 0.45 μm membrane filter.

ATP degradation-related compounds, including ATP, ADP, AMP, IMP, inosine

(HxR), and hypoxanthine (Hx), were quantified using high-performance liquid chromatography (HPLC). Separation was performed on an Eclipse Plus ODS C18 column (250 mm × 4.6 mm, 5 μm). A sample volume of 10 μL was injected at a flow rate of 1 mL min⁻¹, and detection was carried out at 260 nm. The mobile phase consisted of 0.04 mmol L⁻¹ potassium dihydrogen phosphate and 0.06 mmol L⁻¹ dipotassium hydrogen phosphate. The concentration of each compound was determined using external standards, and the K value was calculated as follows:

$$\text{K-value (\%)} = (\text{HxR} + \text{Hx}) / (\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}) \times 100$$

2.3. Peroxide value (PV)

Approximately 3 g of fish fillet was mixed with 30 mL of an acetic acid–chloroform solution (60% acetic acid and 40% chloroform), followed by the addition of 0.5 mL of saturated potassium iodide. The mixture was stirred for 1 min, and then 0.5 mL of 1% starch solution was added. In the presence of peroxides, a purple ring formed at the top of the flask. The solution was titrated with 0.01 N sodium thiosulfate until colorless. The peroxide value was expressed as milliequivalents of active oxygen per kilogram of sample using the following equation [12]:

$$\text{PV} = (\text{Volume of Na}_2\text{S}_2\text{O}_3 \times \text{Normality} \times 1000) / \text{Sample weight}$$

2.4. Thiobarbituric acid (TBA) value

The TBA value was determined using butanol as the solvent in the presence of thiobarbituric acid reagent, according to AOCS standards [13]. Briefly, 200 mg of fish fillet paste was weighed into a 25 mL volumetric flask and brought to volume with butanol, followed by thorough homogenization. Then, 5 mL of the sample solution was mixed with 5 mL of TBA reagent and heated in a boiling water bath at 95 °C for 2 h. After heating, the tubes were cooled under running water for 10 min. Absorbance was measured at 530 nm using a spectrophotometer.

The TBA value was expressed as mg malondialdehyde (MDA) per kg of sample and calculated as:

$$\text{TBA (mg MDA/kg)} = (A \times 50) / m$$

where *A* is absorbance at 530 nm and *m* is sample weight (g).

2.5. Free fatty acids (FFA)

Twenty grams of fish sample were homogenized with an adequate amount of chloroform using a mechanical mixer. The mixture was filtered, and the filtrate was passed through filter paper containing anhydrous sodium sulfate. A known volume of the filtrate was transferred to a pre-weighed dry flask, and after evaporation of chloroform, the lipid content was determined.

Subsequently, 25 mL of the filtrate was transferred to a 250 mL Erlenmeyer flask, and 25 mL of neutralized ethanol was added. Free fatty acids were titrated with 0.1 N sodium hydroxide using phenolphthalein as an indicator. FFA content was expressed as oleic

acid. One milliliter of 0.1 N NaOH is equivalent to 0.272 g of oleic acid [14].

2.6. Total volatile basic nitrogen (TVB-N)

TVB-N was determined using the macro-Kjeldahl method. Ten grams of minced fish sample were placed in a 500 mL Kjeldahl flask, followed by the addition of 2 g magnesium oxide as a catalyst and 300 mL distilled water for distillation.

Then, 25 mL of 2% boric acid solution was placed in the receiving flask of the Kjeldahl apparatus. Distillation was carried out for 45 min until the solution turned yellow. After distillation, the collected solution was titrated with 0.01 N sulfuric acid until the original purple color was restored [7]. TVB-N was calculated using the following equation:

$$\text{TVB-N} = (\text{Acid volume} \times 1.4 \times 100) / \text{Sample weight}$$

2.7. Trimethylamine (TMA)

Trimethylamine content was determined according to the AOAC (2016) method. Ten grams of fish muscle were homogenized with 30 mL of 7.5% trichloroacetic acid for 2 min using a homogenizer to obtain a milky solution. The homogenate was centrifuged at 2500 rpm for 10 min, and the clear supernatant was used as the extract.

Standard solutions were prepared by diluting 1, 2, and 3 mL of TMA standard solution to 4 mL with distilled water, and a calibration curve was constructed. Absorbance of the samples was measured, and TMA content was calculated using the standard curve [15]:

$$\text{TMA (mg/100 g)} = (V \times F \times (A_{\text{blank}} - A_{\text{sample}})) / m$$

where V is the final extract volume (mL), F is the standard curve factor (mg mL⁻¹), and m is sample weight (g).

2.8. Statistical analysis

To evaluate changes in the K value and its relationship with chemical spoilage indices, data were analyzed using a completely randomized design with three replicates for each treatment and storage time. Pearson correlation coefficients were calculated to assess the relationships between the K value and the evaluated indices. Simple and multiple linear regression analyses were performed to predict the K value based on independent variables using SPSS software (version 26). Data normality and model validity were assessed through normality tests and residual analysis. All statistical analyses were conducted at a significance level of $P < 0.05$. Results were expressed as mean \pm standard deviation, and graphs and regression equations were generated using Excel and SPSS software.

3- Results and Discussion

3.1. Changes in K value and nucleotide compounds

Adenosine triphosphate (ATP) and its degradation products are among the most important biochemical indicators for assessing the freshness of aquatic products [12]. In the

present study, changes in nucleotide compounds (ATP, ADP, AMP, IMP, HxR, and Hx) in rainbow trout fillets during 12 days of refrigerated storage were evaluated (Fig. 1). The results showed that ATP exhibited the highest concentration at the initial storage period in all treatments and gradually decreased over time, whereas Hx and HxR levels increased.

In the control samples, ATP degradation occurred more rapidly, and by day 12, ATP and ADP levels were almost depleted. In contrast, coated treatments—particularly those containing 1% polysaccharide—showed

a slower decrease in ATP and a more moderate increase in Hx. This effect can be attributed to the coating's ability to limit oxygen penetration and reduce the activity of nucleotide-degrading enzymes. Moreover, the antioxidant compounds present in Japanese loquat seed polysaccharides inhibit oxidative reactions and microbial activity, thereby enhancing nucleotide stability and delaying chemical spoilage [20,25]. These findings are consistent with previous studies [16,17], which demonstrated that bioactive polysaccharide-based coatings effectively preserve nucleotide integrity and reduce chemical deterioration in fish products.

Table 1. Changes in the concentration of nucleotide compounds (ATP, ADP, AMP, IMP, HxR, Hx) in mmol/kg sample and K-value (%) in rainbow trout fillets coated with different treatments during cold storage (4 °C) at different days. (C: control, G: gelatin, GP0.5: gelatin + 0.5% polysaccharide, GP1: gelatin + 1% polysaccharide)

| Index | Treatment / Day | 0 | 1 | 4 | 7 | 10 | 12 |
|-------|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|
| K (%) | C | 10.2 ± 0.4 a | 16.8 ± 0.7 a | 28.5 ± 1.2 a | 39.4 ± 1.6 a | 46.7 ± 1.8 a | 50.3 ± 2.0 a |
| | G | 10.2 ± 0.4 a | 14.5 ± 0.6 ab | 22.7 ± 1.0 b | 30.4 ± 1.3 b | 36.0 ± 1.5 b | 38.1 ± 1.6 b |
| | GP0/5 | 10.2 ± 0.4 a | 13.3 ± 0.6 b | 19.7 ± 0.9 bc | 25.8 ± 1.3 c | 30.1 ± 1.3 c | 32.6 ± 1.4 c |
| | GP1 | 10.2 ± 0.4 a | 12.1 ± 0.5 b | 17.5 ± 0.8 c | 22.4 ± 1.0 b | 25.6 ± 1.1 c | 28.4 ± 1.3 d |
| HxR | C | 0.34 ± 0.02 a | 0.56 ± 0.3 a | 0.95 ± 0.05 a | 1.31 ± 0.06 a | 1.5 ± 0.07 a | 2.82 ± 0.11 a |
| | G | 0.34 ± 0.02 a | 0.48 ± 0.3 a | 0.75 ± 0.04 b | 1.01 ± 0.05 b | 1.27 ± 0.06 b | 2.4 ± 0.1 b |
| | GP0/5 | 0.34 ± 0.02 a | 0.44 ± 0.3 b | 0.66 ± 0.04 b | 0.86 ± 0.04 c | 1.08 ± 0.05 c | 2 ± 0.09 c |
| | GP1 | 0.34 ± 0.02 a | 0.36 ± 0.2 b | 0.58 ± 0.03 c | 0.74 ± 0.04 c | 0.96 ± 0.04 d | 1.7 ± 0.07 d |
| Hx | C | 0.68 ± 0.02 a | 1.12 ± 0.05 a | 1.9 ± 0.08 a | 2.62 ± 0.1 a | 2.82 ± 0.11 a | 3.0 ± 0.12 a |
| | G | 0.68 ± 0.02 a | 0.96 ± 0.04 b | 1.5 ± 0.07 b | 2.02 ± 0.09 b | 2.4 ± 0.1 b | 2.54 ± 0.1 b |
| | GP0/5 | 0.68 ± 0.02 a | 0.88 ± 0.04 b | 1.32 ± 0.06 b | 1.72 ± 0.07 c | 2.0 ± 0.09 c | 2.16 ± 0.09 c |
| | GP1 | 0.68 ± 0.02 a | 0.72 ± 0.03 c | 1.16 ± 0.05 c | 1.48 ± 0.06 c | 1.7 ± 0.07 d | 1.92 ± 0.08 d |
| IMP | C | 5 ± 0.3 a | 4.2 ± 0.2 a | 3.3 ± 0.2 a | 2.0 ± 0.2 b | 1.3 ± 0.2 b | 1.0 ± 0.1 b |
| | G | 5 ± 0.3 a | 4.6 ± 0.3 a | 3.7 ± 0.3 a | 2.9 ± 0.2 a | 2.4 ± 0.2 a | 2.2 ± 0.2 a |
| | GP0/5 | 5 ± 0.3 a | 4.7 ± 0.3 a | 4.0 ± 0.2 a | 3.3 ± 0.2 a | 2.8 ± 0.2 a | 2.5 ± 0.2 a |
| | GP1 | 5 ± 0.3 a | 4.9 ± 0.2 a | 4.3 ± 0.2 a | 3.6 ± 0.2 a | 3.2 ± 0.2 a | 2.8 ± 0.2 a |
| AMP | C | 1 ± 0.1 a | 0.8 ± 0.1 a | 0.6 ± 0.1 a | 0.5 ± 0.1 a | 0.4 ± 0.1 a | 0.3 ± 0.1 a |
| | G | 1 ± 0.1 a | 0.9 ± 0.1 a | 0.8 ± 0.1 a | 0.7 ± 0.03 a | 0.6 ± 0.07 a | 0.5 ± 0.09 a |
| | GP0/5 | 1 ± 0.1 a | 0.9 ± 0.1 a | 0.8 ± 0.1 a | 0.7 ± 0.05 a | 0.7 ± 0.08 a | 0.6 ± 0.08 a |
| | GP1 | 1 ± 0.1 a | 0.9 ± 0.1 a | 0.8 ± 0.1 a | 0.7 ± 0.03 a | 0.6 ± 0.05 a | 0.6 ± 0.1 a |
| ADP | C | 1.2 ± 0.1 a | 1.0 ± 0.1 a | 0.7 ± 0.1 b | 0.5 ± 0.1 b | 0.4 ± 0.1 b | 0.3 ± 0.01 b |
| | G | 1.2 ± 0.1 a | 1.1 ± 0.1 a | 0.9 ± 0.05 a | 0.7 ± 0.07 a | 0.6 ± 0.07 a | 0.5 ± 0.07 a |
| | GP0/5 | 1.2 ± 0.1 a | 1.1 ± 0.1 a | 0.9 ± 0.05 a | 0.8 ± 0.05 a | 0.7 ± 0.08 a | 0.6 ± 0.05 a |
| | GP1 | 1.2 ± 0.1 a | 1.1 ± 0.1 a | 0.9 ± 0.1 a | 0.7 ± 0.1 a | 0.6 ± 0.1 a | 0.6 ± 0.08 a |

| | | | | | | | |
|-----|-------|-------------|-------------|-------------|--------------|--------------|--------------|
| ATP | C | 1.8 ± 0.2 a | 1.3 ± 0.1 a | 1.3 ± 0.1 a | 1.1 ± 0.1 a | 1.2 ± 0.07 a | 0.9 ± 0.08 a |
| | G | 1.8 ± 0.2 a | 1.6 ± 0.2 a | 1.3 ± 0.1 a | 1.3 ± 0.03 a | 1.2 ± 0.08 a | 1.0 ± 0.07 a |
| | GP0.5 | 1.8 ± 0.2 a | 1.6 ± 0.2 a | 1.3 ± 0.1 a | 1.4 ± 0.1 a | 1.3 ± 0.08 a | 1.1 ± 0.07 a |
| | GP1 | 1.8 ± 0.2 a | 1.9 ± 0.2 a | 1.4 ± 0.1 a | 1.4 ± 0.03 a | 1.4 ± 0.07 a | 1.2 ± 0.1 a |

.Statistical significance was considered at $p < 0.05$

Changes in K value during refrigerated storage

The K value of rainbow trout fillets was calculated and evaluated during a 12-day refrigerated storage period for four different treatments, including the control (C), gelatin coating (G), gelatin + 0.5% polysaccharide (GP0.5), and gelatin + 1% polysaccharide (GP1). The obtained results are presented in Fig. 1.

At the beginning of storage (day 0), the K values of all treatments were approximately at the same level, and no significant differences were observed among them (around 10%). As storage time progressed, the K value increased in all treatments, indicating the continuous degradation of ATP-related nucleotides into terminal compounds such as hypoxanthine (Hx), which reflects a gradual loss of freshness and quality. In other words, the increase in K value represents the progression of biochemical spoilage and deterioration of fish muscle quality during storage. However, the rate of increase varied among treatments.

In the control group (C), the K value increased from 10.2% on day 0 to 50.3% on day 12, showing the highest increase among all treatments. This change was statistically significant ($P < 0.05$), indicating extensive nucleotide degradation and a pronounced decline in freshness during the storage period. In the gelatin-coated samples (G), the K value reached 38.1% on day 12, which was

significantly lower than that of the control. In the GP0.5 treatment, the K value on day 12 was 32.6%. The lowest K value was observed in the GP1 treatment, where the K value was limited to 28.4% on day 12.

Based on these results, the incorporation of polysaccharides extracted from Japanese loquat seeds into the gelatin coating significantly reduced the rate of increase in the K value of rainbow trout fillets during refrigerated storage. This inhibitory effect was dose-dependent, with the GP1 treatment exhibiting the strongest suppression of K value elevation. From day 4 onward, the differences among treatments were statistically significant ($P < 0.05$).

The overall increase in K value in all treatments during storage indicates progressive nucleotide degradation and a gradual decline in freshness under refrigerated conditions. Nevertheless, the increase was markedly faster in the uncoated control samples, highlighting the protective role of edible coatings in reducing ATP degradation and its conversion into end products such as hypoxanthine [18]. In coated samples—particularly GP1—the increase in K value was significantly delayed. This effect can be attributed to the presence of bioactive compounds in the polysaccharides extracted from Japanese loquat seeds, which are likely to possess antioxidant and antimicrobial properties. These compounds, in combination with the barrier properties of gelatin, may limit oxygen penetration and inhibit microbial growth, thereby suppressing enzymatic and

oxidative reactions associated with nucleotide degradation [19].

Overall, the results demonstrate that the application of a composite gelatin-polysaccharide coating can effectively preserve the quality and freshness of aquatic products by reducing nucleotide degradation. The findings further suggest that the K value can be used not only as a sensitive indicator of freshness but also as a reliable tool for evaluating the effectiveness of protective treatments. The presence of bioactive polysaccharides, together with the film-forming properties of gelatin, creates an efficient physical and biochemical barrier against spoilage factors.

These findings are consistent with previous studies. For instance, Zarei et al. (2020) reported that the use of plant polysaccharide-based edible coatings reduced the rate of K value increase in tilapia fillets [20]. Similarly, Ramazani et al. (2018) demonstrated that gelatin-based coatings combined with plant essential oils effectively delayed nucleotide degradation in fish products [21]. Collectively, these studies confirm the effectiveness of bioactive edible coatings in controlling chemical spoilage and maintaining the freshness of aquatic foods.

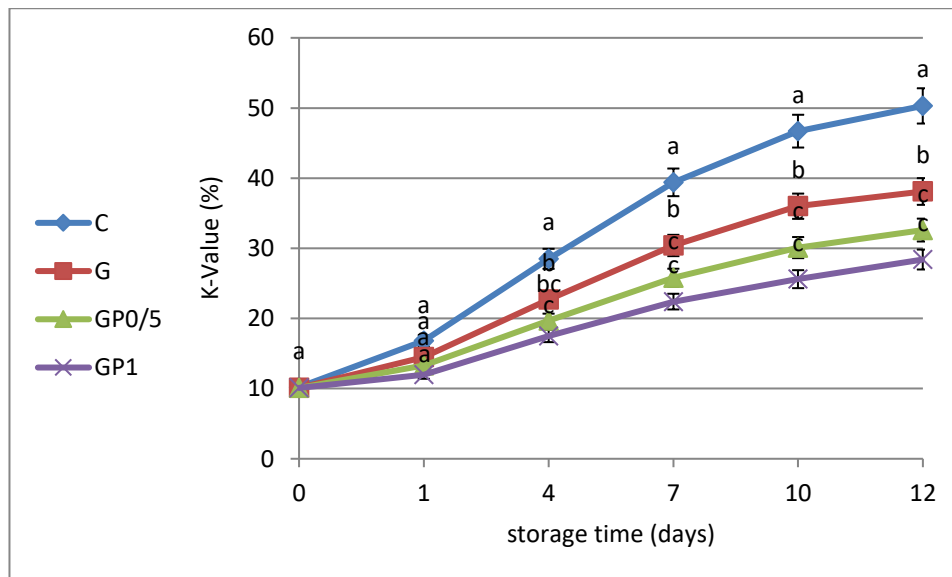


Figure 1. Changes in K-value [%] in rainbow trout fillets coated with different treatments including (C: control, G: gelatin, GP0.5: gelatin + 0.5% polysaccharide, GP1: gelatin + 1% polysaccharide) during 12 days of storage at refrigerated temperature (4 ± 1 °C). Statistical significance was considered at $p < 0.05$.

3.2. Changes in other spoilage indices

The results of chemical spoilage indices, including TVB-N, PV, TBA, FFA, and TMA, presented in Table 2, indicated that all indices increased significantly in all treatments during

the storage period, reflecting the progression of protein degradation and lipid oxidation in rainbow trout fillets. However, the coated treatments—particularly GP1—exhibited consistently lower values for all indices compared to the control (C). This finding demonstrates that the composite gelatin–polysaccharide coating effectively reduced microbial and oxidative enzyme activities and inhibited the formation of chemical spoilage compounds.

The greatest differences among treatments were observed for TVB-N and TMA, highlighting the role of the coating in suppressing the activity of enzymes responsible for the production of volatile nitrogenous compounds. In addition, the significantly lower PV and TBA values observed in coated samples indicate the effectiveness of the coatings in inhibiting both primary and secondary lipid oxidation processes [22]. The FFA content also increased at a slower rate in coated treatments, particularly GP1, suggesting reduced lipid hydrolysis and a lower release of free fatty acids.

Analysis of the overall trends revealed that TMA and TVB-N exhibited the strongest agreement with changes in the K value. Similar to the K value, both indices showed the highest increase in the control samples and remained at the lowest levels in the GP1

treatment. This consistent trend suggests that nitrogenous compounds produced during protein degradation are closely associated with nucleotide breakdown and can be used as complementary indicators for assessing fish freshness.

Previous studies support these findings. For example, Özogul et al. (2010) reported a strong correlation between the K value and both TVB-N and TMA during storage of horse mackerel, suggesting that these indices can be used complementarily to evaluate fish freshness [23]. Similarly, Ocağ et al. (2011) demonstrated in tilapia fillets that the correlation between the K value and TVB-N was stronger than its correlation with oxidative indices such as TBA and PV [24]. In another study, Fan et al. (2009) reported that increases in TMA—particularly in control samples—showed the closest agreement with increases in the K value, and that the use of antioxidant and antimicrobial coatings could simultaneously suppress the rise of both indices [25].

Overall, the simultaneous reduction of chemical spoilage indices and the K value in coated treatments indicates a potential correlation between these parameters, which will be further examined through statistical analysis in the subsequent section.

Table 2. Changes in chemical spoilage indicators in rainbow trout fillets subjected to different treatments during 12 days of storage at refrigerated temperature (4 ± 1 °C). (C: control, G: gelatin, GP0.5: gelatin + 0.5% polysaccharide, GP1: gelatin + 1% polysaccharide)

| test | Treatment / Day | 0 | 1 | 4 | 7 | 10 | 12 |
|-------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| TVB-N | C | 10.13 ± 1.12 a | 11.58 ± 1/03 a | 13.71 ± 1.55 a | 15.83 ± 1.41 a | 18.25 ± 1.95 a | 23.5 ± 2.29 a |
| | G | 10.13 ± 0.98 a | 11.47 ± 1/30 a | 12.42 ± 1/18 b | 14.42 ± 1.62 b | 16.71 ± 1.53 b | 19.42 ± 2.04 b |

| | | | | | | | |
|-----|-------|----------------|----------------|----------------|----------------|----------------|----------------|
| | GP0/5 | 10.13 ± 1.07 a | 11.40 ± 1/06 a | 11.85 ± 1.43 c | 13.59 ± 1.28 c | 15.97 ± 1.75 c | 18.15 ± 1.80 c |
| | GP1 | 10.13 ± 1.04 a | 11.23 ± 1/12 a | 11.44 ± 1/29 c | 12.11 ± 1.15 d | 13.34 ± 1.22 d | 14.73 ± 1.40 d |
| | C | 0.85 ± 0.11 a | 0.96 ± 0/08 a | 1.85 ± 0.20 a | 3.72 ± 0.42 a | 4.98 ± 0.56 a | 4.14 ± 0.43 a |
| | G | 0.85 ± 0.10 a | 0.95 ± 0/13 a | 1.61 ± 0.19 a | 3.25 ± 0.29 a | 4.38 ± 0.41 a | 3.65 ± 0.35 a |
| PV | GP0/5 | 0.85 ± 0.09 a | 0.92 ± 0/08 a | 1.41 ± 0.15 a | 2.83 ± 0.30 b | 3.63 ± 0.34 b | 3.15 ± 0.30 b |
| | GP1 | 0.85 ± 0.12 a | 0.90 ± 0/1 a | 1.23 ± 0.13 b | 2.69 ± 0.24 b | 3.22 ± 0.31 b | 3.05 ± 0.32 b |
| | C | 0.33 ± 0.03 a | 0.36 ± 0.05 a | 0.55 ± 0.07 a | 0.65 ± 0.06 a | 0.9 ± 0.08 a | 1.53 ± 0.18 a |
| | G | 0.33 ± 0.04 a | 0.37 ± 0.03 a | 0.45 ± 0.06 a | 0.5 ± 0.05 a | 0.63 ± 0.05 a | 1.1 ± 0.10 b |
| TBA | GP0/5 | 0.33 ± 0.04 a | 0.34 ± 0.04 a | 0.42 ± 0.03 a | 0.45 ± 0.06 a | 0.55 ± 0.07 a | 0.93 ± 0.10 b |
| | GP1 | 0.33 ± 0.03 a | 0.33 ± 0.03 a | 0.39 ± 0.05 a | 0.39 ± 0.04 a | 0.44 ± 0.04 a | 0.8 ± 0.09 b |
| | C | 0.42 ± 0.05 a | 0.67 ± 0.09 a | 1.7 ± 0.15 a | 2.03 ± 0.21 a | 3.25 ± 0.28 a | 4.24 ± 0.38 b |
| | G | 0.42 ± 0.03 a | 0.66 ± 0.07 a | 1.57 ± 0.18 a | 1.96 ± 0.23 a | 2.87 ± 0.32 a | 4.11 ± 0.43 a |
| FFA | GP0/5 | 0.42 ± 0.06 a | 0.65 ± 0.07 a | 1.23 ± 0.14 b | 1.65 ± 0.16 b | 2.42 ± 0.27 b | 3.44 ± 0.37 a |
| | GP1 | 0.42 ± 0.04 a | 0.65 ± 0.05 a | 1.0 ± 0.11 b | 1.47 ± 0.14 b | 2.13 ± 0.22 b | 3.4 ± 0.31 b |
| | C | 2.45 ± 0.33 a | 2.81 ± 0.27 a | 3.65 ± 0.52 a | 4.82 ± 0.42 a | 8.12 ± 0.91 a | 13.54 ± 1.25 a |
| | G | 2.45 ± 0.28 a | 2.75 ± 0.26 a | 3.44 ± 0.33 a | 4.36 ± 0.31 a | 7.32 ± 0.76 a | 11.85 ± 1.13 a |
| TMA | GP0/5 | 2.45 ± 0.30 a | 2.66 ± 0.27 a | 3.15 ± 0.36 a | 3.85 ± 0.35 b | 5.85 ± 0.62 b | 9.13 ± 0.93 b |
| | GP1 | 2.45 ± 0.31 a | 2.53 ± 0/25 a | 2.86 ± 0.30 b | 3.61 ± 0.33 b | 5.03 ± 0.47 b | 6.87 ± 0.68 c |

Statistical significance was considered at $p < 0.05$

3.3. Correlation Analysis between K-Value and Other Chemical Spoilage Indices

3.3.1. Results of the Normality Test (Shapiro–Wilk Test)

Prior to conducting correlation analysis between the K-value and other chemical spoilage indices, it is essential to assess the normality of data distribution, as many parametric statistical tests—such as linear regression and Pearson’s correlation coefficient—are based on the assumption of normality. Accordingly, the Shapiro–Wilk test, which is recognized as one of the most powerful and reliable methods for evaluating data normality, was employed in this study.

In the Shapiro–Wilk test, the W statistic reflects the degree of conformity between the data distribution and a normal distribution, with values closer to 1 indicating a higher

degree of normality. However, the primary criterion for interpretation is the p -value: when $p > 0.05$, the data are considered to be normally distributed, whereas $p < 0.05$ indicates a deviation from normality.

The results obtained from the Shapiro–Wilk normality test (Table 3) demonstrated that most of the investigated variables, including K-value, IMP, ATP, Hx, and other nucleotide-related spoilage indices, followed a normal distribution, as the corresponding p -values were greater than 0.05 in the majority of cases. These findings indicate that the null hypothesis of normality was not rejected, thereby confirming the validity and reliability of applying parametric statistical tests such as linear regression analysis and Pearson’s correlation coefficient [26,27].

The presence of normally distributed data allows for more accurate statistical inference using linear models and ensures that the results obtained in evaluating the relationships

between the K-value and other chemical spoilage parameters are supported by a robust statistical foundation.

.Table 3. Correlation analysis of normally distributed chemical spoilage indicators

| Chemical Index | W Statistic | p-value | Distribution Status |
|----------------|-------------|---------|----------------------|
| TVB-N | 0.911 | 0.298 | Normal |
| PV | 0.817 | 0.077 | Approximately normal |
| TBA | 0.889 | 0.166 | Normal |
| FFA | 0.875 | 0.219 | Normal |
| TMA | 0.829 | 0.096 | Approximately normal |
| Storage Time | 1.000 | 1.000 | Normal |

Note: A p-value greater than 0.05 indicates a normal distribution

3.3.2. Results of Spearman Correlation Analysis

Given that slight deviations from normality were observed in some variables, both Pearson and Spearman correlation tests were applied concurrently to ensure the robustness and reliability of the statistical analysis. Pearson's correlation coefficient was used for normally or approximately normally distributed data, while Spearman's rank correlation coefficient was employed for variables with uncertain or non-normal distributions. This combined analytical approach enables a more comprehensive evaluation of the relationships between the K-value and other chemical spoilage indices.

Pearson's correlation analysis is a widely used parametric method for assessing the strength and direction of a linear relationship between two quantitative variables [26]. The Pearson

correlation coefficient (r) ranges from -1 to $+1$, where values close to $+1$ indicate a strong positive correlation, values close to -1 represent a strong negative correlation, and a value of zero indicates the absence of a linear relationship. In contrast, Spearman's correlation test is a non-parametric method that evaluates the monotonic association between two variables based on ranked data and does not require the assumption of normal data distribution. This test is particularly suitable for data exhibiting non-normal distributions or potential non-linear relationships.

The results of the Spearman correlation analysis are presented in Table 4. The findings revealed significant and strong positive correlations ($p < 0.05$) between storage time and all evaluated chemical spoilage indices. These results indicate a significant increase in chemical spoilage parameters with prolonged refrigerated storage of fish fillets. Consequently, storage time can be considered a critical variable in determining fish fillet quality and the extent of chemical spoilage. Moreover, the investigated chemical indices

can be regarded as reliable indicators for monitoring quality changes in fish fillets during storage [28,29].

Table 4. Spearman correlation results between storage days and other chemical spoilage indicators.

| Chemical Index | Correlation Coefficient (r) | p-value | Correlation Description |
|----------------|-----------------------------|---------|--------------------------------------|
| TVB-N | 0.971 | 0.003 | Positive and significant correlation |
| PV | 0.886 | 0.018 | Positive and significant correlation |
| TBA | 0.971 | 0.003 | Positive and significant correlation |
| FFA | 0.886 | 0.018 | Positive and significant correlation |
| TMA | 0.829 | 0.042 | Positive and significant correlation |

3.3.3. Pearson Correlation Results between K-Value and Chemical Spoilage Indices

In the present study, the K-value was investigated as one of the key indicators of chemical spoilage in rainbow trout (*Oncorhynchus mykiss*) fillets. To evaluate the relationship between the K-value and various chemical spoilage indices, including TVB-N, PV, TBA, FFA, and TMA, Pearson correlation analysis was performed. The results (Table 5) revealed very strong and statistically significant positive correlations between the K-value and all investigated spoilage indices, with correlation coefficients (r) ranging from 0.957 to 0.997 and p -values lower than 0.01.

These findings indicate that increases in the K-value are accompanied by concurrent increases in chemical spoilage parameters, highlighting the K-value as a sensitive and

Table 5. Pearson correlation results between the K-value and chemical spoilage indicators

reliable indicator for quality monitoring and spoilage prediction in fish fillets during refrigerated storage. Similar observations have been reported in previous studies, which identified the K-value as a prominent and well-established index for evaluating fish freshness and spoilage, widely applied in seafood quality assessment [23,24].

Moreover, other studies have demonstrated that the K-value reflects post-mortem nucleotide degradation in fish muscle and serves as a practical criterion for distinguishing early freshness stages and determining the extent of spoilage. Recent research has also shown that the K-value is significantly influenced by storage temperature and preservation conditions [30]. The results of the present study are consistent with these findings and further emphasize the importance of incorporating the K-value as a reliable parameter in quality control and shelf-life evaluation of seafood products.

| Chemical Index | Pearson Correlation Coefficient (r) | p-value | Correlation Description |
|----------------|-------------------------------------|---------|---------------------------------|
| TVB-N | 0.997 | 0.00013 | Strong and significant positive |
| PV | 0.978 | 0.0003 | Strong and significant positive |
| TBA | 0.961 | 0.0012 | Strong and significant positive |
| FFA | 0.978 | 0.0003 | Strong and significant positive |
| TMA | 0.957 | 0.0017 | Strong and significant positive |

3.3.4. Scatter Plots and Linear Regression between K-Value and TMA/TVB-N

Given that TMA and TVB-N exhibited the highest conformity with changes in the K-value, scatter plots illustrating the relationships between K-value and TMA and TVB-N in rainbow trout fillets during refrigerated storage under four different treatments are presented in Figures 2 and 3. For each treatment, separate linear regression lines were plotted.

In Figure 2, a significant positive correlation between K-value and TMA was observed across all treatments. However, the slopes of the regression lines in treatments containing polysaccharide coatings, particularly GP1,

were lower than that of the control. This indicates a reduced rate of TMA increase relative to K-value in coated samples, reflecting the protective effect of the gelatin-polysaccharide coating in slowing chemical spoilage.

For instance, the regression equations for the control and GP1 treatments were as follows:

$$(C): TMA = 0.21 \times K + 8.08 (R^2 = 0.99)$$

$$(GP1): TMA = 0.14 \times K + 7.35 [R^2 = 0.96]$$

These results confirm that the addition of polysaccharide to the gelatin coating significantly reduced the rate of chemical spoilage in rainbow trout fillets [20,25].

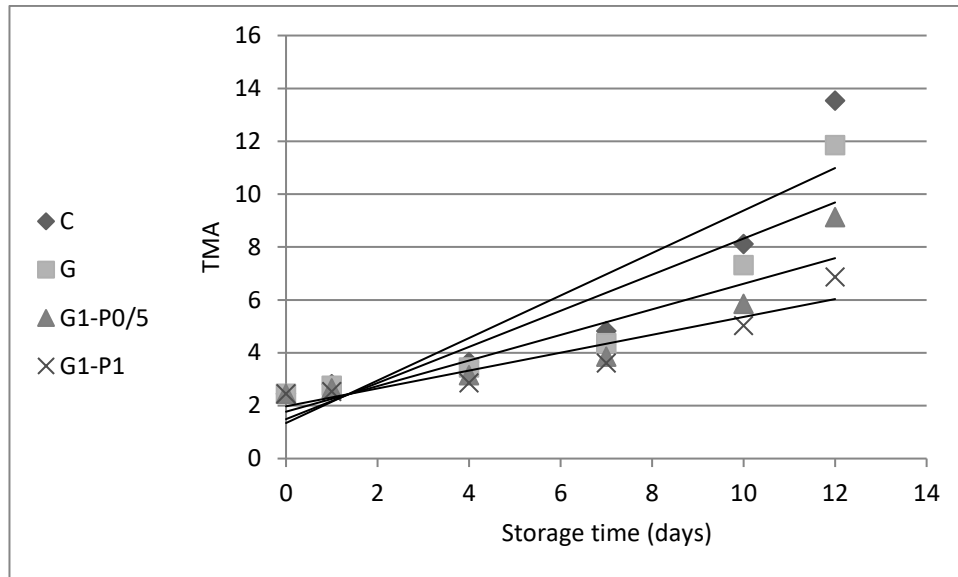


Figure 2. Scatter plot showing the relationship between the K-value and TMA index in rainbow trout fillets during cold storage under different treatments. Linear regression lines are plotted separately for each treatment. (C: control, G: gelatin, GP0.5: gelatin + 0.5% polysaccharide, GP1: gelatin + 1% polysaccharide).

3.3.5. Scatter Plot and Linear Regression between K-Value and TVB-N

In Figure 3, the scatter plot illustrating the relationship between K-value and TVB-N shows a significant positive correlation across all treatments. However, the slopes of the regression lines in treatments containing polysaccharide coatings, particularly GP1, were lower compared to the control. This indicates a reduced rate of TVB-N increase relative to K-value in coated samples, reflecting the protective effect of the gelatin-polysaccharide coating in slowing chemical spoilage.

For example, the regression equations for the control and GP1 treatments are as follows:

$$(C): TVB-N = 0.21 \times K + 8.08 \quad (R^2 = 0.99)$$

$$TVB-N = 0.14 \times K + 7.35 \quad (R^2 = 0.96)$$

(GP1):

These results confirm that the addition of polysaccharide to the gelatin coating has a significant impact on reducing the rate of chemical spoilage in rainbow trout fillets.

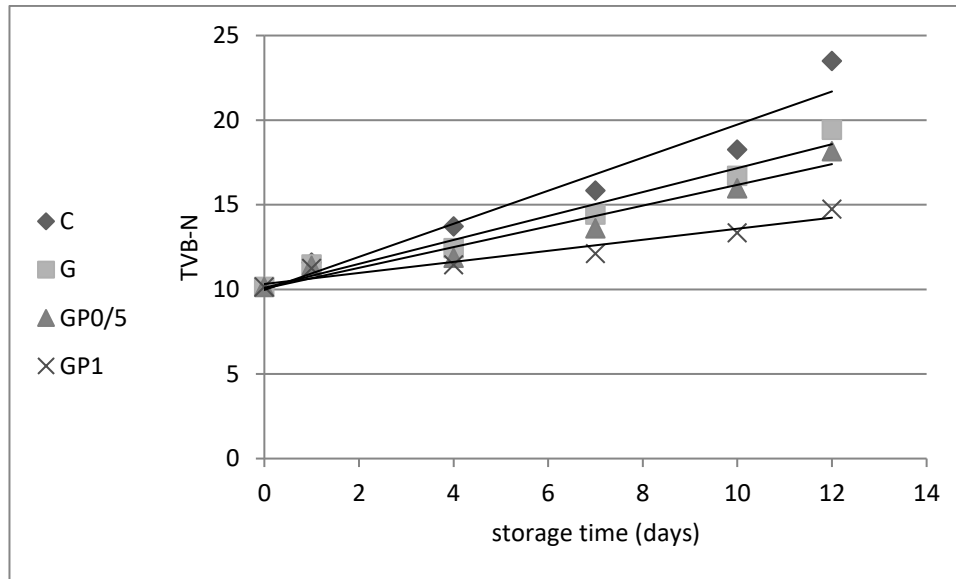


Figure 3. Scatter plot showing the relationship between the K-value and TVB-N index in rainbow trout fillets during cold storage under different treatments. Linear regression lines are plotted separately for each treatment: (C: control, G: gelatin, GP0.5: gelatin + 0.5% polysaccharide, GP1: gelatin + 1% polysaccharide).

4- Conclusion

The results of this study demonstrated that the K-value is a reliable chemical index, showing a significant positive correlation with common chemical spoilage indicators, including TVB-N, PV, TBA, FFA, and TMA. Additionally, coating rainbow trout fillets with gelatin containing polysaccharides significantly reduced the rate of increase of these spoilage indicators. Notably, treatments containing polysaccharides (GP0.5 and GP1) exhibited a stronger protective effect against chemical spoilage compared to the control and pure gelatin treatments.

This protective effect was also evident in the scatter plots of K-value versus spoilage indices, where the slopes of the regression lines for polysaccharide treatments were

lower, indicating a slower rate of spoilage. Therefore, incorporating polysaccharides into gelatin coatings can serve as an effective and natural strategy for extending the shelf life and maintaining the quality of rainbow trout fillets under refrigerated storage.

Furthermore, the K-value, due to its high sensitivity, ability to detect early stages of spoilage, and suitability for instrumental measurement, can be considered a reliable index and tool for evaluating fish quality and assessing the effectiveness of preservation treatments. These findings could contribute to the development of innovative technologies in the seafood processing industry. Considering limitations such as the investigation of a single species and specific polysaccharide concentrations, future studies are recommended to explore different species, concentrations, and additional evaluations (sensory and microbial) to provide more

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Author Contributions

All activities were carried out by the author.

Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study.

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مقاله علمی-پژوهشی

بررسی تغییرات شاخص K و همبستگی آن با شاخص‌های فساد شیمیایی در فیله ماهی قزل‌آلای رنگین‌کمان پوشش‌یافته با ژلاتین حاوی پلی‌ساکارید استخراج‌شده از هسته ازگیل ژاپنی

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تازگی و کیفیت فیله ماهی به‌عنوان یکی از شاخص‌های کلیدی در ایمنی و پذیرش مصرف‌کننده در محصولات شیلاتی مطرح است. در این مطالعه، بررسی شاخص K و همبستگی آن با دیگر شاخص‌های شیمیایی فساد شامل بازهای نیتروژنی فرار کل [TVB-N]، تری‌متیل‌آمین [TMA]، عدد پراکسید [PV]، تیوباربیتوریک اسید [TBA] و اسیدهای چرب آزاد [FFA] در فیله ماهی قزل‌آلای رنگین‌کمان طی نگهداری در شرایط سرد انجام شد. بدین منظور، فیله‌های ماهی با ژلاتین [۱٪] و پلی‌ساکارید استخراج‌شده از هسته ازگیل ژاپنی [۰/۵٪ و ۱٪] پوشش داده شدند و به همراه تیمار شاهد در دمای یخچال [±۴] درجه سانتی‌گراد به مدت ۱۲ روز نگهداری شدند. نمونه‌برداری در روزهای ۰، ۱، ۴، ۷، ۱۰ و ۱۲ انجام و آزمایش‌های شیمیایی به‌منظور تعیین شاخص‌های مذکور صورت گرفت. نتایج نشان داد تیمار حاوی ژلاتین + پلی‌ساکارید ۱٪ پایین‌ترین میزان شاخص K و سایر شاخص‌های فساد شیمیایی را طی دوره نگهداری داشت. همچنین، تحلیل آماری همبستگی‌ها نشان داد که بین شاخص K و شاخص‌های TVB-N، TMA، PV، TBA و FFA همبستگی مثبت و معناداری وجود دارد [p<0.05]. بر اساس نتایج، شاخص K می‌تواند به‌عنوان یک شاخص قابل اعتماد برای ارزیابی کیفیت و فساد فیله ماهی قزل‌آلای پوشش‌یافته طی نگهداری سرد مورد استفاده قرار گیرد. همچنین، استفاده از پوشش‌های زیست‌فعال حاوی پلی‌ساکارید ازگیل ژاپنی در بهبود کیفیت و افزایش ماندگاری محصول مؤثر بوده است.