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Research Paper:

Impacts of Carboxymethyl Cellulose Containing Propolis Extract on the Shelf Life of Trout Fillets

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ABSTRACT

Background & Aims of the Study: The present study aimed to evaluate the impact of Carboxymethyl Cellulose (CMC) coating incorporated with Propolis Extract (PE) on the shelf life properties during storage of trout fillets.

Materials and Methods: Study treatments included control, CMC, CMC-PE 1%, and CMC-PE 2%. The samples were stored in a refrigerator for 15 days, and their microbial, chemical, and sensory properties were evaluated every three days.

Results: All groups showed a significant reduction in total viable counts of Pseudomonas spp., lactic acid bacteria, Enterobacteriaceae, Psychrotrophic bacteria, and yeasts/molds population compared to the control samples. In comparison, the coated samples exhibited significantly ($P<0.05$) lower total volatile essential nitrogen, thiobarbituric acid, peroxide, and K values than the uncoated trout. Based on the sensory evaluation results, PE preserved the sensory characteristics significantly ($P<0.05$) throughout the study. The most efficient treatment in the shelf life enhancement of the studied samples belonged to CMC-PE 2%, followed by CMC-PE 1% and then CMC. Combined treatment of CMC-PE 2% increased the shelf life of the rainbow trout fillet for 12 days during refrigerated storage.

Conclusion: Eventually, incorporated PE in CMC was introduced as an alternative candidate for synthetic preservatives in refrigerated trout fillet with various health benefits.

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1. Introduction

Oncorhynchus mykiss is the scientific name of rainbow trout. It is a favorable and common edible fish in the world. Therefore, recent studies have attempted to promote its processing and production quality. Fish fillets are very sensitive to bacterial spoilage and lipid oxidation that can be related to the high moisture, pH, protein, and presence of numerous unsaturated fatty acids in it [1]. As fish products are very perishable, developing preservation approaches for increasing their shelf life is quite necessary [2]. Cold and freezing storage are major and common approaches for preserving aquaculture foods, although they cannot entirely prevent their chemical reactions.

Additionally, freezing, chilling, and super chilling for the drop marketing of these products can decrease their nutritional value by destructing their nutritious ingredients like vitamins, minerals, and proteins [1]. Other methods such as drying, canning, salting, smoking, and irradiation cannot fully protect seafood from oxidative spoilage. This failure may be related to the existence of different unsaturated fatty acids in the fish body, which become rapidly oxidized in the presence of oxygen [3, 4]. The other option is using chemical preservatives to enhance the shelf life of meat products. Consumers are now complaining about synthetic preservatives, which in their beliefs, might be detrimental to their health.

On the one hand, food industries are trying to find new methods to extend food shelf life, such as various chemical preservative applications. However, the consumers find these compounds unpleasant as they have adverse effects. Considering these problems, food researchers are searching to find novel methods to solve this problem. Natural antioxidant compounds such as different herbal extracts are the new approach of the food industries for enhancing meat foods' shelf life [5]. In other words, one of the new approaches for shelf life enhancement of perishable products such as fish is natural preservative applications instead of chemical ones. Natural food additives are used as a natural antioxidant and antimicrobial in foods, and they can elevate their nutritional value as they have high health benefit properties [6].

The *Apis mellifera* L - bees produce a resinous propolis or bee glue that protects the hive against various pests such as arthropods and microorganisms. The polyphenolic compounds of propolis are responsible for the well-known biological functions of bee glue, such as its pharmacological, anti-inflammatory, antitumor, antiviral, antifungal, antibacterial, and antioxidant properties [7, 8]. Propolis is mainly made of resin (60%) and its remaining (40%) comprised vitamins, essential oils, waxes, and microelements. Bees collect the resinous and balsamic ingredients of pollen, flours, leaves, and foliage, and after the aggregation of them in salivary secretions and enzymes, propolis is made. Because of the unique properties of propolis, it has been the subject of many studies. According to the previous studies, this product is a potent antioxidant linked to the flavonoids, cinnamic, benzoic, and caffeic acids individually or in the synergisms of these compounds [9, 10]. Recently, natural preservatives' effects have been promoted by implanting them into various edible films and coatings in foods. On the one hand, incorporating natural preservatives (antioxidants and antimicrobials) into films or coatings can enhance the films' antimicrobial and antioxidant features.

On the other hand, it delays the chemical spoilages in different ways, like oxidation of lipid and protein by oxygen or inhibition of water vapor leakage in the coated food [5, 11]. The propolis extract is a stable, potent antioxidant, antimicrobial, and odorless natural compound, which is easily found in each honey bee hive. Besides, it has beneficial effects on human health [12]. Reports have revealed the antimicrobial properties of propolis against various microorganisms, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida utilis*. These effects are mostly resulted from the polyphenolic compounds [7, 10]. Kalogeropoulos et al. assessed the antibacterial activities of Propolis extracts (Pes) from Greece and Cyprus against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* [7].

Various polysaccharides, proteins, and lipid polymers can compose different edible films and coatings with specific characteristics. Because of their special film-forming features, polysaccharides have been commonly used to build coatings and films in food backgrounds.

Carboxymethyl Cellulose (CMC) is a material with cellulose origin with many functions in the food industry, such as stabilizer, thickener, and mouth-taste enhancer. It is made of glucose units with β (1-4) links, methyl, and carboxyl groups [13, 14]. CMC, a hydrophilic polymer, can be a good choice for food coatings and films. There are also examples of biopolymer-based coating and film applications in improving the foods' shelf lives through creating water and oxygen barrier, inhibiting microbial growth, and reducing weight loss. They also protect the foods' organoleptic features by preventing and retarding protein and lipid oxidation [2, 15]. Combining hydrophobic substances such as fatty acids, vegetable oils,

resins, surfactants, and waxes in the hydrocolloid-based films is one approach to boost the moisture barrier effects [13]. Many research studies showed the antimicrobial activity of the CMC film incorporated with natural preservatives against various microorganisms [13, 14]. Shavisi et al. observed that during all storage times, fortified Polylactic Acid (PLA) film with PE could hinder oxidation reaction in the chilled minced meat [14]. The edible coating could lower the speed of the oxidation reactions and moisture retention and act as a barrier against oxygen and water permeability. In other words, various coatings such as chitosan, alginate, and CMC can increase the quality and storage life of the foods by this mechanism [4, 13, 14].

Therefore, this research planned to introduce propolis as a potent natural food preservative in fortifying CMC edible coating for elongating the shelf-life of the trout flesh under refrigeration conditions (4°C).

2. Materials and Methods

Fillet samples' preparation

The fresh trout fish (approximately 1700±100g) was purchased from a fish culture farm of Hamedan City, Iran, and was immediately taken to the laboratory in insulated boxes containing ice packs. The filleted fish were washed by sterile distilled water and sliced into 10 g pieces and covered in the coating solutions. All experiments were carried out in the Department of Food Hygiene and Quality Control, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan City, Iran, in 2019.

PE preparation

Propolis was prepared from the present beehives in the around of Kermanshah City, western Iran. The harvested propolis was air-dried at environment temperature for two weeks. Next, it was blended in a blender and mixed to ethanol with a ratio of 30:100 g/mL, agitated at 250 rpm by an Earlene shaker for 24 h. Then, the obtained solution was filtered and evaporated by a rotary evaporator (Milan, Italy) at 40ºC. Finally, the concentrated extract was dried under a vacuum at 50°C. The resulting extract was preserved at -18ºC for subsequent use [10].

The coatings and treatments preparation

The solution of coatings was made with 1% CMC (w/v) (average MW of 41 kDa, Cara gum Parsian Co. Tehran, Iran), using sterile distilled water. Glycerol at 0.5 % (v/v) was selected as a plasticizer compound into the CMC coating solution. After heating the solution at 85°C for 5 min, it was cooled at room temperature. PE (1% and 2%) was mixed with CMC coating dispersion. The prepared coating solution consisted of 1% CMC, 1.5% glycerol, alone or along with 1% and 2% PE. Four experimental groups were designated as the following groups: control (coated samples in sterile distilled water), CMC, CMC-PE 1 %, and CMC-PE 2 %. The samples were immersed in the obtained solution (sterile distilled water, CMC, CMC-PE 1%, and CMC-PE 2%) for 2 min. The coated fillets were packaged under aerobic conditions in polyethylene zip packs, and then the packs were stored in a refrigerator (4°C). Microbial, biochemical, and sensory factors of the samples were assessed at 3-day intervals for 15 days [13].

Microbiological evaluation

The 10 g sample was mixed with 90 mL of 0.1% peptone water in a special stomacher pouch and homogenized in the stomacher at 200 rpm for 60 seconds. Subsequent dilutions were prepared in tubes containing 0.1% peptone water and cultured on plates containing culture medium. Microbial tests included total viable count, psychrotrophic bacteria, lactic acid bacteria, Pseudomonas spp., Enterobacteriaceae, and yeast-molds [16].

Biochemical analysis

pH value measurement

The pH value was measured according to Brannan [17]. Five grams of the samples were homogenized in 25 mL of distilled water for 30 s. The pH values of resulting homogenates were read by a pH meter (Jenway, UK).

Peroxide value measurement

After fat extraction of the samples, their Peroxide Values (PV) were measured according to International Dairy Federation [18]. PV was expressed as mEq of O_2 per kg of fat.

Thiobarbituric Acid Reactive Substance (TBARS) measurement

This index was identified as mg of malonaldehyde equivalents per kg of the fillet. Also, 1, 1, 3, 3-Tetraethoxypropane (TEP) was considered for preparing a standard calibration curve [19].

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Total Volatile Basic Nitrogen (TVB-N) measurement

This experiment was carried out by distillation technique using a Kjeldahl apparatus (Simax, Pyrexfan, Tehran, Iran). The results were identified in mg of Nitrogen (N) per 100 g of the trout fillets [20].

K value measurement

K value (the ratio of inosine and hypoxanthine to the ATP and its decomposed products) was computed according to the modified method of Fan et al. [21]. Adenosine-Triphosphate (ATP) and its breakdown constituents were identified by HPLC (Knauer, Berlin, Germany), along with Capcell, pack ODS C18 column (4.0 × 100 mm, 3 µm). ATP and its decomposed compounds were detected and then computed by Equation 1:

Equation 1

$$\text{K value (\%)} = \frac{[(HXR)+(HX)]}{[(ATP)+(ADP)+(AMP)+(IMP)+(HXR)+(HX)]} \times 100$$

Sensory analysis

Ten Master's degree students (four females and six males between 24 and 35 years old) of the Food Hygiene and Quality Control Department performed the organoleptic analysis of the treatments. The fillet samples were cooked by steaming method for 20 min at 100±1°C after salting (1.5%) them. The sensory analysis was carried out using a 5-point hedonic scale to estimate the taste and odor scores (1: poor, 5: excellent) [4, 22].

Study analysis

A total of 52 fish were placed in four groups (each 13 fish). Microbiological and chemical analyses were repeated three times. The mean±Standard Deviations (SD) values of the analyses were demonstrated. For statistical analysis of the results, SPSS software (IBM SPSS statistics 21) was exploited. Analysis of Variance (ANOVA), Tukey test, and Independent sample t test were utilized for all data interpretation at $P<0.05$.

3. Results and Discussion

Microbiological analysis

Total viable count

Figure 1A–F displays the variations in Total Viable Counts (TVC) , Pseudomonas spp., psychrotrophic bacteria, Lactic Acid Bacteria (LAB), Enterobacteriaceae, and yeasts-molds of the fillets. Figure 1A shows the TVC during 15 days at refrigerator temperature. According to the previous report, 4-6 log CFU/g, the total bacterial counts of the fresh water fish species such as rainbow trout are very changeable [23]. In this experiment, all samples' primary level of TVC approximately was 3 log CFU/g, which agrees with prior research on the trout flesh [1, 4]. Figure 1A illustrates a significant difference among all treatments during storage days. TVC index of control samples reached 7.36 log CFU/g on the sixth day. This value is above the maximum allowable limit of 7 log CFU/g for TVC in the raw fish fillets in the cold storage time [24]. On the 12th day, the TVC amount of CMC treatment samples reached beyond the permissible limit. In the treatment sample using 1% propolis, the TVC value of the fish samples did not reach the standard limit until the 12th day; while, 2% propolis could maintain the TVC population of the fish samples in the standard range until the end of the storage period. Thus, for CMC, CMC-PE 1%, and CMC-PE 2% samples, microbiological shelf-life elongation of 9, 12, and 15 days were respectively obtained in comparison to the control sample. The shelf life increase for these groups could be induced by the antimicrobial activity of CMC and PE. According to the results, CMC-PE 2% had the highest treatment impact for the total bacterial count of the fillets, followed by CMC-PE 1% and CMC, respectively. By creating a barrier against water and oxygen, preventing microbial growth, and delaying fat oxidation, biopolymer-based films, such as CMC, can maintain the suitable quality and lengthen the shelf life of the food [2, 15]. Reports have revealed the antimicrobial properties of propolis against various microorganisms, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida utilis*. These effects are mostly resulted from the polyphenolic compounds [7, 25]. Besides, one study reported the antibacterial activity of different percentages of propolis water extract on the fresh Shubuta (*Barbus grypus*) fillets during cold storage [9].

Psychrotrophic bacteria

The primitive series of bacteria that get blamed for putrefying aerobically-stored fish meat at refrigerator

temperatures are Gram-negative psychrotrophic bacteria [26]. In this investigation, it was seen that in the studied fillets, the psychrotrophic bacteria population (day 0) was in the range of 2.63 log CFU/g to 2.82 log CFU/g. These results are consistent with previous studies [24]. As seen in Figure 1B, during the storage time, a rising trend was demonstrated in psychrotrophic bacteria numeration. Furthermore, in all days of analyses, control was the most populous group, followed by CMC, CMC-PE 1%, and CMC-PE 2% samples. On the sixth day, the psychrotrophic bacteria numeration of the control fillets reached 7.83 log CFU/g that was more than the acceptable level of 7 log CFU/g in the fresh raw fish flesh [24]. This condition continued while on the 9th and 15th days, psychrotrophic bacteria values of CMC, CMC-PE 1%, and CMC-PE 2% went beyond the allowed limit. Consistent with the results of TVC, the potent treatment in decreasing psychrotrophic bacteria population was CMC-PE 2%, followed by CMC-PE 1% and CMC, respectively. Other researchers have argued that coating can inhibit aerobic bacterial development, which acts as an effective barrier against oxygen permeability [2]. Based on Vargas-S Anchez et al., PE lowered the microbial growth (mesophilic and psychrotrophic enumerations) in the beef patties during eight storage days in a refrigerator. This finding could be linked to the polyphenolic compounds' presence, demonstrating that as a natural antimicrobial and antioxidant additive, i.e., PE has excellent potential in extending the beef patties' shelf life [27].

Enterobacteriaceae

According to the findings of Gui et al., Enterobacteriaceae were the crucial components of the spoilage microflora in the flesh, stored at 4°C [28]. Initial numeration of Enterobacteriaceae ranged from 1.75 log CFU/g to 2.005 log CFU/g in accordance with Volpe et al. on the coated trout fillets with carrageenan under refrigerator temperature [1]. After 15 storage days, Enterobacteriaceae counts reached 6.015, 6.89, 7.615, and 8.69 log CFU/g in the CMC-PE 2%, CMC-PE1%, CMC, and control fillets, respectively (Figure 1C). Also, such a steady growth in Enterobacteriaceae, according to Volpe et al., could be for the stored trout flesh in the refrigerator [1]. Such a finding was likely due to the gradual growth rate exhibited by these bacteria compared to other Gram-negative psychrotrophic spoilers. CMC and PE reduced the Enterobacteriaceae growth rate in the fillets more significantly than the control during refrigeration. Throughout the study period, the lowest Enterobacteriaceae population was found for the CMC-PE 2% containing fillets, followed by CMC-PE 1% and CMC samples. Other researchers displayed the antibacterial influence of carboxymethyl cellulose/sodium alginate coating against the major member of Enterobacteriaceae, i.e., *Escherichia coli* O157:H7, inoculated in the chilled silver carp flesh [29]. Based on the results of one study, the propolis extracts that could be considered effective natural food preservers are likely to prevent *E.coli*'s growth in vitro successfully. They reported that most propolis components, mainly flavonoids, have a phenolic origin. It is notified that polyphenols are known as strong antimicrobial substances. Similarly, Gallic acid derivatives of the propolis indicated inhibitory activities against bacteria. In response to microbial infections, plants produce flavonoids, resulting from which they are identified with antimicrobial impacts on a variety of microorganisms [30].

Pseudomonas spp

As observations suggest, Pseudomonas spp. can be a critical member of the bacterial spoilage of the meat kept in a refrigerator. Proteolysis is a determinant and effective phenomenon in meat spoilage. When the Pseudomonas spp. populations reach 10^7-10^8 CFU/g in meat products, they cause proteolysis reaction followed by slime production [31]. According to the primary count of about 3.18-3.25 log CFU/g for Pseudomonas spp. (day 0) of the trout samples (Figure 1D), similar initial numeration (day 0) related to the rainbow trout was also found by other studies [32, 33]. During the storage time, Pseudomonas spp. count raised to the final numeration of 11.12 log CFU/g (control fillets) while the counts of CMC, CMC-PE 1%, and CMC-PE 2% reached 9.98, 8.82, and 7.7 log CFU/g at the last interval, being less than the control fillets. Pseudomonas spp. count in all groups was significantly ($P<0.05$) less than the control, showing that PE-containing treatments were the strongest concerning the inhibition treatments of *P*seudomonas spp. The mentioned finding may be linked to the added antibacterial effects of CMC and PE. In their research, Raeisi et al. showed that CMC-containing coatings combined with *Zataria multiflora* Boiss essential oil and grape seed extract could lower the growth of Pseudomonas spp. in the rainbow trout flesh at refrigerated storage [13]. De Marco et al. demonstrated that as the adjuvants in treating P. aeruginosa chronic putrefaction, the usage of propolis extracts could be effective not only for their anti-biofilm features but also for their low toxicity and biological (anti-inflammatory and antioxidant) activities [25]. Some prior research also confirmed the antibacterial characteristics of several variations of propolis against *Pseudomonas aeruginosa* [8].

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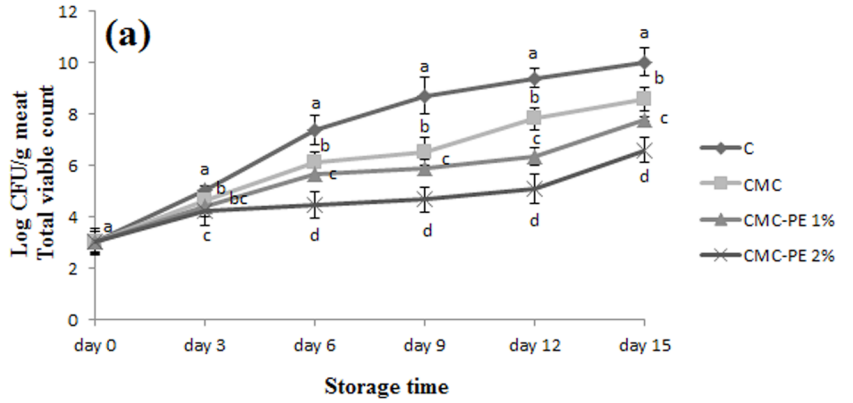

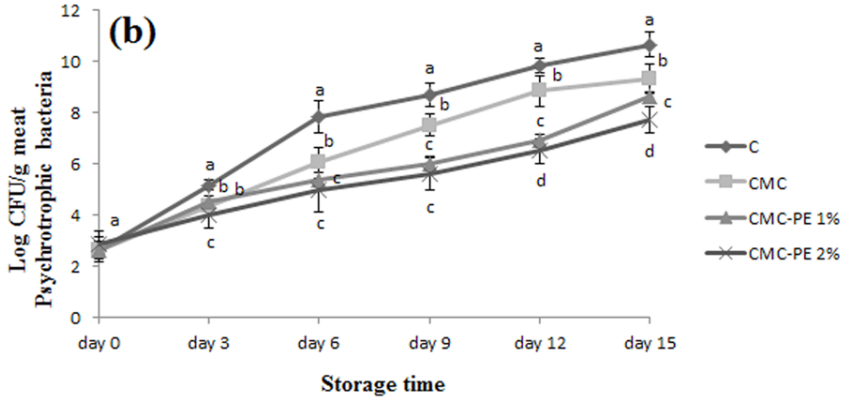

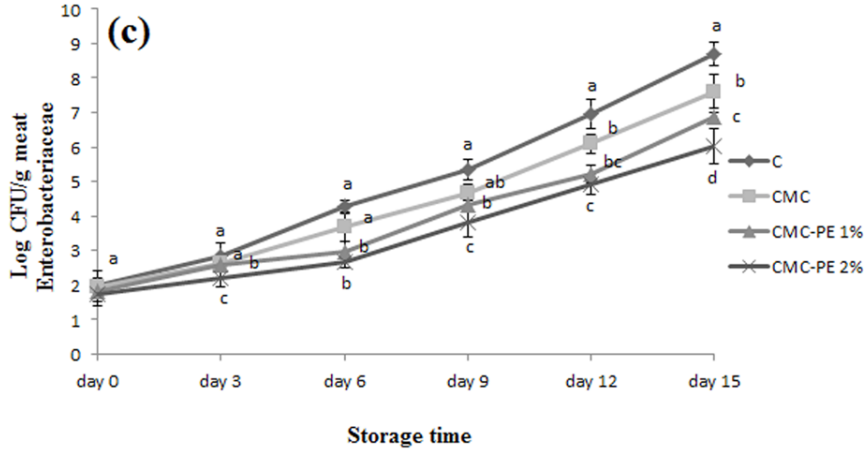

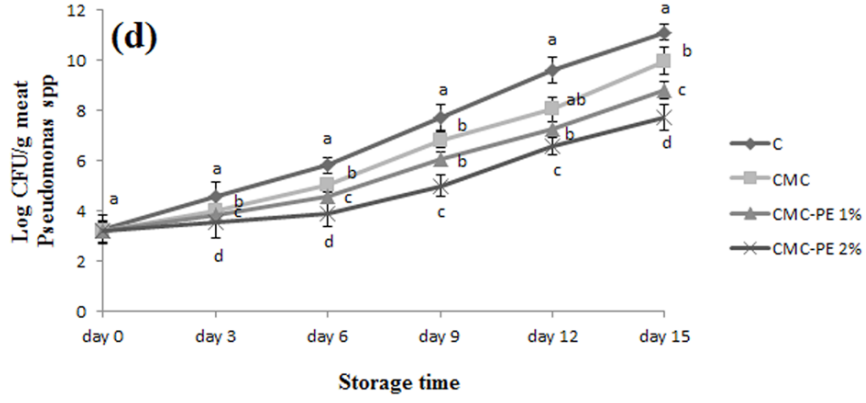

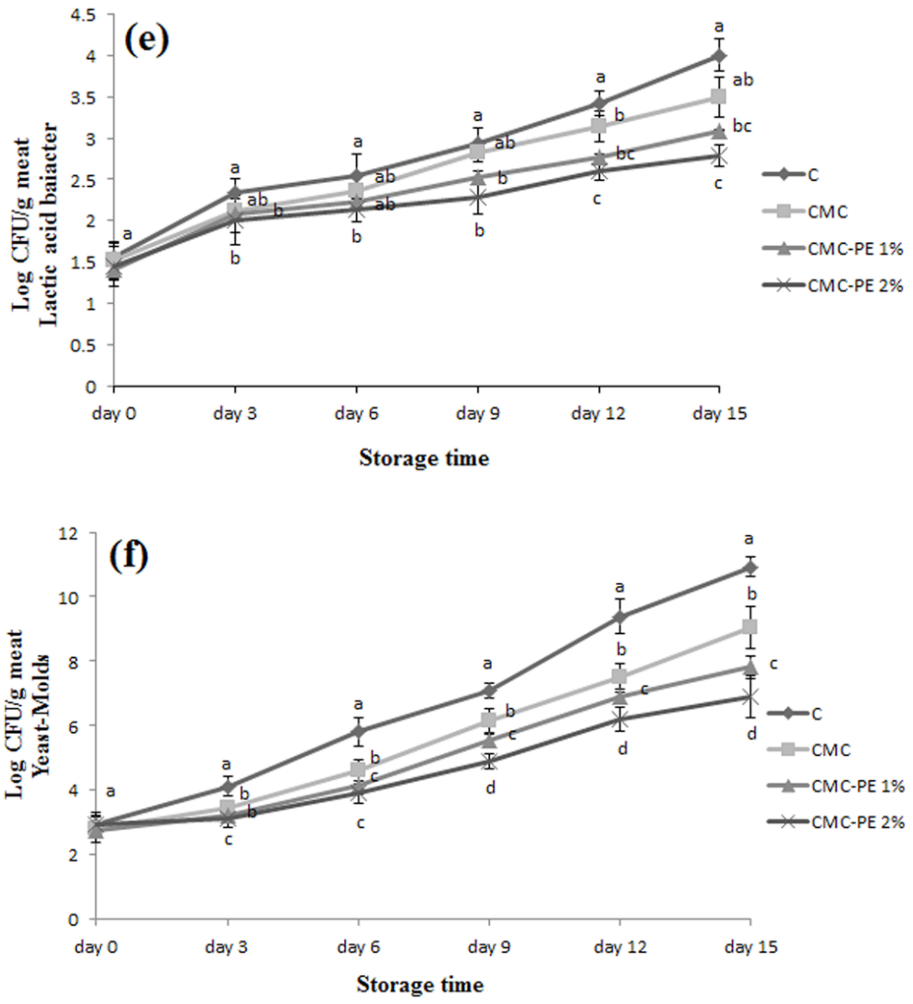

Figure 1. Variation trends of the population in TVC (a), psychrotrophic bacteria (b), Enterobacteriaceae (c), Pseudomonas spp. (d), lactic acid bacteria (LAB) (e) and yeasts–molds (f), during storage of trout fillets at 4°C

Treatments: Control (C), Carboxymethyl Cellulose (CMC); Carboxymethyl Propolis Extract 1 % (CMC-PE 1%); Carboxymethyl Cellulose containing Propolis Extract 2% (CMC-PE 2%); Different lowercases exhibit significant differences ($P<0.05$) in each interval.

Lactic acid bacteria

As a well-known facultative anaerobic bacterium, lactic acid bacteria are a part of the original microflora of the trout flesh; it can increase under aerobic and anaerobic conditions [1]. Primary Lactic Acid Bacteria (LAB) count ranged from 1.405 to 1.52 log CFU/g and did not exceed 4.01 log CFU/g in the control fillets until the 15th day of storage (Figure 1E). This condition holds while compared to the control fillets, significantly ($P<0.05$) lower LAB enumeration was found for the CMC and CMC-PE samples, which were kept during the refrigeration time. The most potent treatment was CMC-PE 2% in preventing the replication of LAB in the fillet samples among other groups of the researchbecause it induced a 1.22 log cycle reduction at the end of the refrigeration. Such a finding may be reported for the synergistic antimicrobial effect of CMC and PE. LAB is the most capable Gram-positive bacteria against antimicrobial treatments based on some research [34]. While compared to other spoilage bacteria, LAB was more resistant versus incorporated CMC with PE. In this research, the lowered LAB count by CMC-PE was less than other bacterial groups. One paper also investigated the impact of aqueous PE during storage of shibuta (*Barbus grypus*) flesh at 4°C. The conclusion was that 0.5% aqueous PE remarkably lowered the shibuta lactic acid bacteria count at all storage times compared to the control samples. As suggested, such a decrease may be related to the phenolic compounds' presence in the PE [9]. Kalogeropoulos et al. assessed the antibacterial activities of PEs from

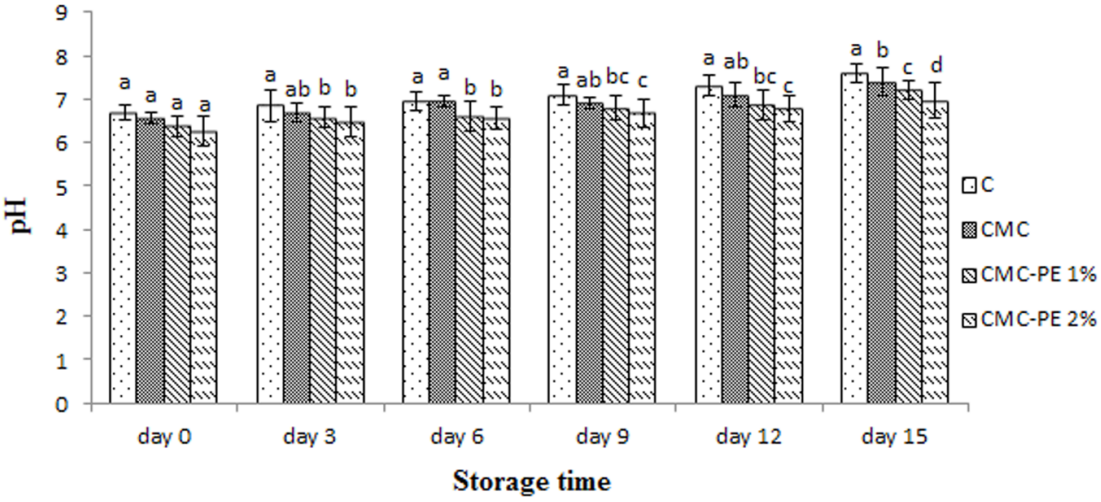

Figure 2. Variation trends of pH value during storage of trout fillets at 4°C

Treatments: Control (C); Carboxymethyl Cellulose (CMC); Carboxymethyl Cellulose containing Propolis Extract 1% (CMC-PE 1%); Carboxymethyl Cellulose containing Propolis Extract 2% (CMC-PE 2%); Different lowercases exhibit significant differences (P<0.05) in each interval.

Greece and Cyprus [7]. They concluded that compared with lactic acid bacteria, the Minimum inhibitory concentration (MIC) of all studied propolis ethanolic extracts were higher regarding the *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*.

Yeasts/molds species

The yeast and mold species are common agents of microbial spoilage in refrigerated meat. Like prior investigations, the primary count (day 0) of yeast/mold of trout fillets was 2.775-2.96 log CFU/g (Figure 1F) [26]. All used treatments in the present study induced significantly lower (P<0.05) counts of the yeasts/molds in CMC, CMC-PE 1%, and CMC-PE 2% fillet samples in comparison to the control under refrigeration condition (Figure 1F). Antifungal activity of the PE on the shibuta meat in a refrigerator was confirmed by Duman and Ozpolat [9]. Another study from Cyprus and Greece reported the antifungal feature of propolis extracts against *Candida tropicalis* and *Candida albicans* [7]. Mohammadzadeh et al. also represented in vitro antifungal effect of the Iranian propolis against *Aspergillus niger* and *Candida albicans* [8]. Noshirvani et al. showed the antifungal activity of the chitosan-CMC film incorporated with natural preservatives against *Aspergillus niger* [35].

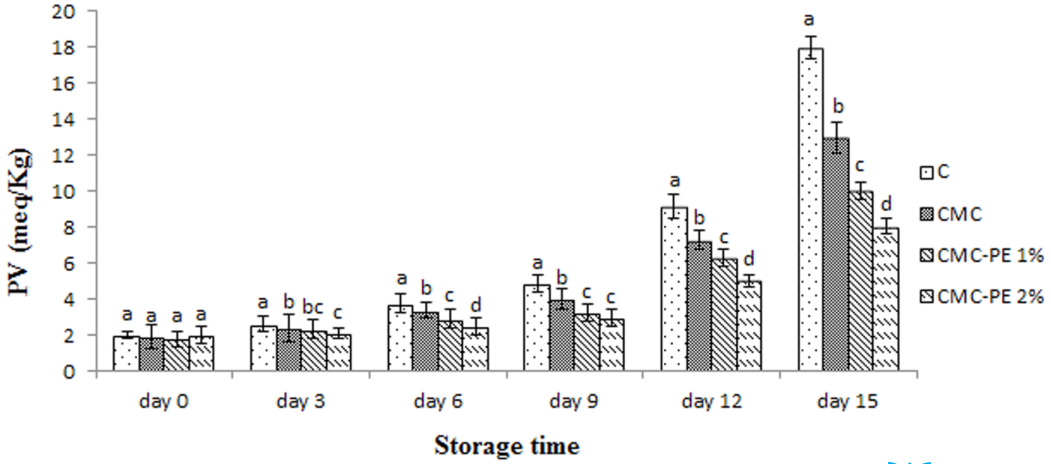

Figure 3. Variation trends of peroxide value during storage of trout fillets at 4°C

Treatments: Control (C), Carboxymethyl Cellulose (CMC), Carboxymethyl Cellulose containing Propolis Extract 1% (CMC-PE 1%), Carboxymethyl Cellulose containing Propolis Extract 2% (CMC-PE 2%). Different lowercases exhibit significant differences (P<0.05) in each interval.

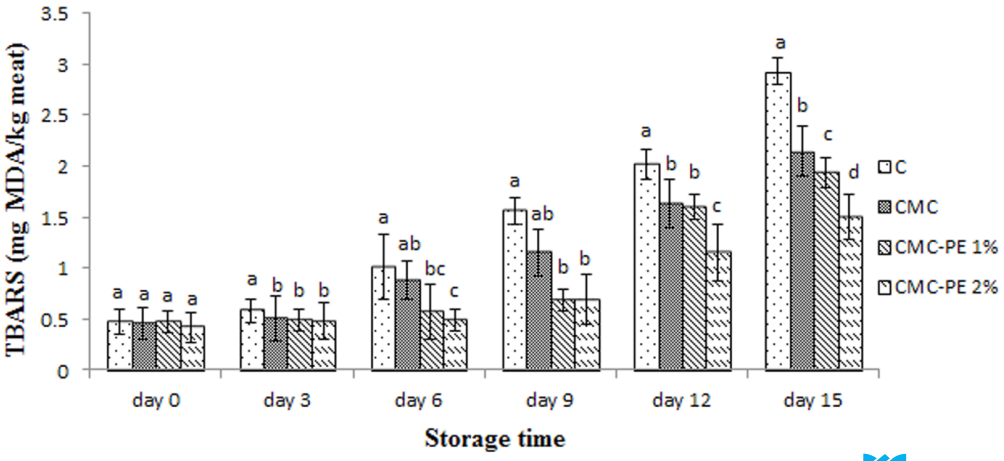

Figure 4. Variation trends of TBARS value during storage of trout fillets at 4°C

Treatments: control (C), Carboxymethyl Cellulose (CMC), Carboxymethyl Cellulose containing Propolis Extract 1% (CMC-PE 1 %), Carboxymethyl Cellulose containing Propolis Extract 2% (CMC-PE 2%). Different lowercases exhibit significant differences (P<0.05) in each interval.

Physicochemical analysis

pH value

Figure 2 illustrates pH index variations in trout fillets during 4°C storage. The primary pH of the fresh fish flesh (pH 6.27-6.69) agreed with the reports of prior scholars [1, 36], but the obtained values of the present research were slightly higher than the reported quantities by other research studies [37]. Such discrepancies in the findings may result from the dissolution and dissociation of CO_2 in the fillet samples. The pH indexes of all samples escalated in the postmortem time; this may be because of the activity of the endogenous or microbial enzymes, like lipase and protease, in the presence of oxygen, causing the enhancement of volatile bases (trimethylamine and ammonia) [21]. Degradation of nitrogenous substances led to pH enhancement, affecting the freshness of the product during storage time. Following that, the sensory features of the sample, such as taste, odor, color, texture, and acceptability, were also negatively affected [1]. In this study, after the storage time, the pH of control samples increased from 6.69 to 7.61, while the pH ranged from 7.39, 7.21 to 6.97, respectively

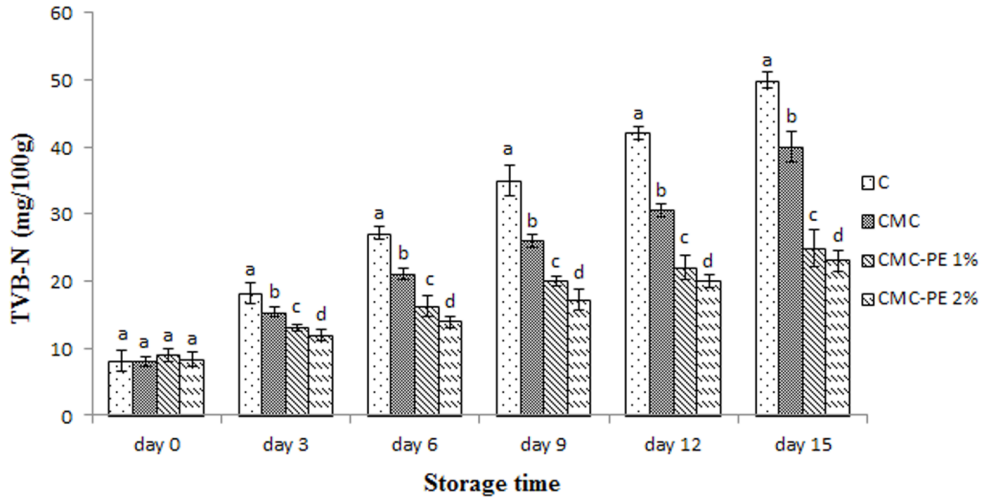

Figure 5. Variation trends of TVB-N value during storage of trout fillets at 4°C

Treatments: Control (C), Carboxymethyl Cellulose (CMC), Carboxymethyl Ccellulose containing Propolis Extract 1% (CMC-PE 1%), carboxymethyl cellulose containing Propolis Extract 2% (CMC-PE 2%). Different lowercases exhibit significant differences (P<0.05) in each interval.

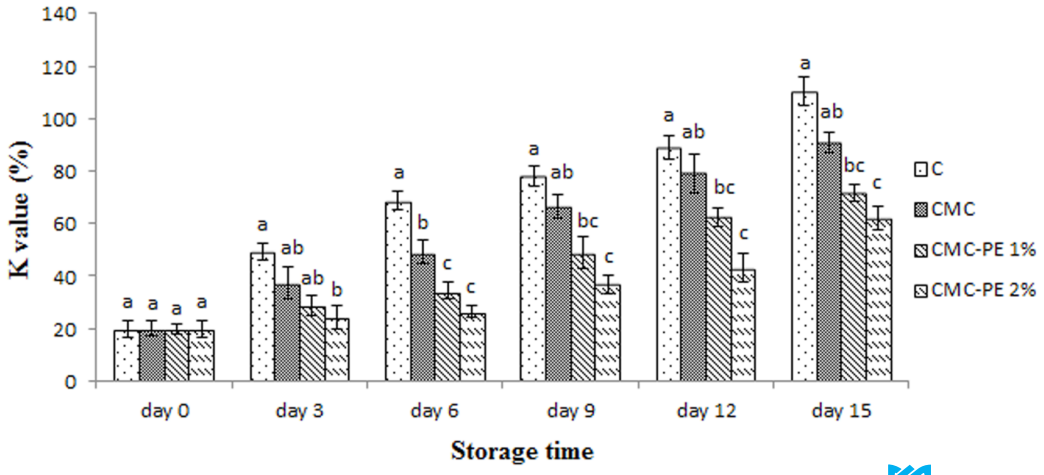

Figure 6. Variation trends of K value during storage of trout fillets at 4°C

Treatments: Control (C), Carboxymethyl Cellulose (CMC), Carboxymethyl Cellulose containing Propolis Extract 1% (CMC-PE 1 %), Carboxymethyl Cellulose containing Propolis Extract 2% (CMC-PE 2%). Different lowercases exhibit significant differences (P<0.05) in each interval.

for the CMC, CMC-PE 1%, and CMC-PE 2% samples in 15 days. These findings were exhibited for the protective action of CMC edible coating against spoiling reaction of the oxygen, significantly (P<0.05) increased by propolis, especially in the group with a higher dose. The lower pH index of other treatments (CMC, CMC-PE 1%, and CMC-PE 2%) may have occurred from preventing endogenous and exogenous (microbial) proteases' activities at various degrees of the trout fillet through the study treatment [2]. Duman and Ozpolat showed that the pH value of the fresh shibuta (*Barbus grypus*) fillets that contained propolis extract was lower compared to the control group at all storage times [9].

Peroxide value

The lipid oxidation reaction is the essential agent in the chemical spoilage of seafood. As the primary product of the auto-oxidation, the Peroxide Value (PV) estimates hydroperoxides' quantity [18]. Peroxide values of the samples are reflected in Figure 3. The PV of the control treatment raised more than other groups during the storage period. After 15 days, the PV in the control group reached from 1.99 to 17.97 mEq peroxides/kg lipid, while, during this time, the PV of CMC, CMC-PE 1 %, and CMC-PE 2 % increased from 1.89 to 12.98, 1.81 to 10.00, and 1.95 to 8.02 mEq peroxides/kg lipid, respectively. Compared to the control, all treatments demonstrated significantly reduced peroxide production during refrigeration (P<0.05). In their work, Rezaei and Shahbazi indicated that at all storage times, the composite film based on the Sodium Alginate-Carboxymethyl Cellulose (SA-CMC) could lower the peroxide index in the sauced silver carp fillet when compared to the control group [38]. The edible coating could lower the speed of the oxidation reactions and moisture retention and act as a barrier against oxygen and water permeability. In other words, various coatings such as chitosan, alginate, and CMC can increase the quality and storage life of the foods by this mechanism [4, 22, 29, 38, 39]. Utilizing CMC-PE 2% treatment, a maximal decrease in the peroxide formation was acquired, followed by CMC-PE 1% and CMC; this condition may result from the potent antioxidant activity of PE 2%. Shavisi et al. found that Polylactic Acid (PLA) film that contained propolis ethanolic extract can lower more peroxide value of the minced beef than the control samples during 11 days of storage at the refrigerator [14].

TBARS value

TBA index has widespread uses in estimating the lipid oxidation content of foods. Reactive substances of TBA developed by peroxide oxidation to ketone and aldehyde are remarkable products of auto-oxidation's terminal phase during food oxidation [19]. Findings of the TBA index of the samples under refrigeration conditions are illustrated in Figure 4. According to Figure 4, the TBARS index of all samples rose continuously during the storage period. The results of other researchers indicated the same pattern as well [12, 21, 40, 41]. Higher TBA value may have been induced from the enhanced oxidation of polyunsaturated fatty acids. As Connell suggested, a TBA index of 2 mg MDA/kg, regarded as the TBA value's maximal level, demonstrated a desirable quality of the fish fillet (chilled, frozen, or ice-stored) beyond which, the fish may reflect unfavorable sensory properties in terms of taste and odor [42]. The primary TBA index of the trout in the present research was 0.42-

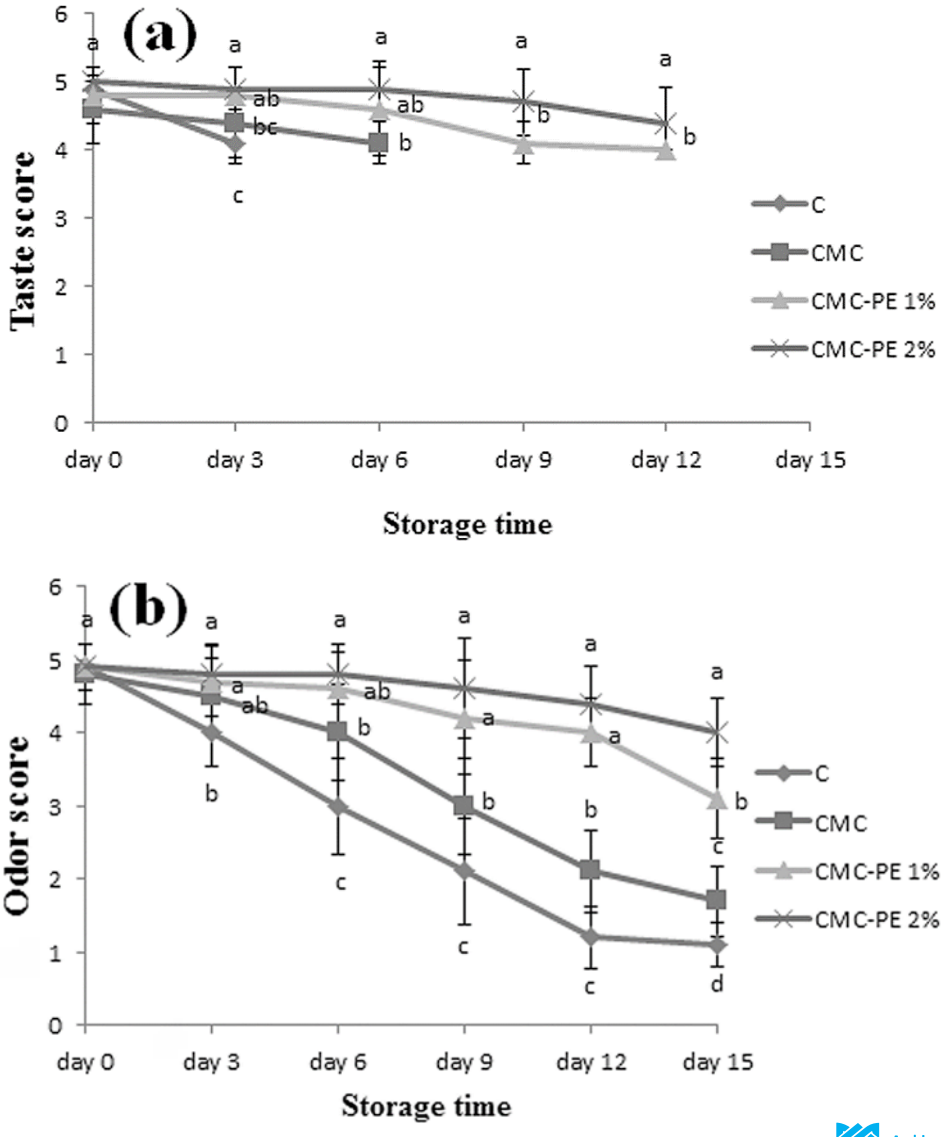

Figure 7. Variation trends of sensory characteristics (a-taste and b-odor) during storage of trout fillets at 4°C

Treatments: Control (C), Carboxymethyl Cellulose (CMC), Carboxymethyl Cellulose containing Propolis Extract 1% (CMC-PE 1%), Carboxymethyl Cellulose containing Propolis Extract 2% (CMC-PE 2%). Different lowercases exhibit significant differences ($P<0.05$) in each interval.

0.47 mg MDA/kg, which increased by 2 mg MDA/kg on the 12th day of refrigeration for the control samples. While, the TBA values of the CMC, CMC-PE 1%, and CMC-PE 2% treatments were 2.145, 1.935, and 1.495 mg MDA/kg, respectively, on the last day of the storage. All treated fillets (CMC, CMC- PE 1% and CMC-PE 2%) had significantly lower Malondialdehyde (MDA) values ($P<0.05$) than the Control (C) sample. According to Figure 4, except for day 0, significant differences ($P<0.05$) existed among designated groups during refrigeration. The TBA indexes of CMC, CMC-PE 1%, and CMC-PE 2% were significantly ($P<0.05$) lower than the control during refrigeration. This finding means that the CMC coating had the potential to prevent the lipid oxidation reaction effectively. Previous scholars acknowledged the ability of the CMC edible coating in controlling the lipid oxidation of various food models [13, 38, 43]. Lower TBA values were reflected in PE-containing treatments compared to the others, likely due to the antioxidants' presence (PE). Additionally, the best effect was reported from CMC-PE 2%. The antioxidant activity of the propolis has been reported by prior studies as well. As observed during all storage times, PLA film that contained PE could hinder oxidation reaction in the chilled minced meat [14].

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TVB-N value

Total Volatile-Based Nitrogen (TVB-N) is a standard index for the evaluation of meat spoilage. TVB-N value comprised nitrogenous substances like dimethylamine, trimethylamine, and ammonia which are probably derived from the breakdown of nitrogenous ingredients, such as nucleic acids and proteins by the exogenous (microbial) and endogenous enzymes. Increasing the TVB-N index of the meat can be a sign of spoilage. Giménez et al. reported 25 mg N/100 g of the trout fillet to be the highest allowable limit for consumers [44]. Figure 5 reflects the impacts of the studied groups on the TVB-N index of the fish flesh during refrigeration. The amount of TVB-N of all fillets continuously increased during storage. The Primary TVB-N index of the trout was 8.06-8.92 mg N/100 g that was consistent with prior findings [1, 2, 45]. Primary TVB-N indexes of large yellow croaker, northern snakehead, rainbow trout, bream, and trout were 11.502-11.893, 9.50-10.00, 9.33-12.13, 12.62, and 11.35 mg/100 g in the above-mentioned studies. From the beginning to the final day of the refrigeration period, the TVB-N indexes of C, CMC, CMC-PE 1 %, and CMC-PE 2 % were (8.15-49.91 mg N/100 g), (8.06-40.02 mg N/100 g), (8.92-24.95 mg N/100 g) and (8.38-23.07 mg N/100 g). Significant ($P<0.05$) differences were found among the considered groups in all analysis intervals. TVB-N indexes in the control and CMC samples were higher than an acceptable limit on the sixth and ninth days of refrigeration, respectively. However, until the last day of analysis, the samples that contained PE, such as CMC-PE 1% and CMC-PE 2%, remained in the allowable level of TVB-N. The TVB-N values of CMC-PE 1% and CMC-PE 2% increased by 24.95 and 23.07 mg N/100 g at the end of the refrigeration period, respectively, indicating a significant difference ($P<0.05$).

Control fillets only were fresh until the sixth day of storage based on the aforementioned standard limit, while CMC, CMC-PE 1%, and CMC-PE 2% groups retained the trout freshness for the 9th and 15th days of the refrigeration time, respectively. Through lowering gas permeability, especially oxygen, the edible coatings could raise the shelf life of the tout fillet after blocking the bacterial growth [2, 13, 29, 38]. As findings indicate, PE, an antioxidant that retains fish freshness during the refrigeration period, can rapidly reduce the bacterial ability and population for the deamination reaction of non-protein nitrogen substances [2, 4]. According to Duman and Ozpolat observations, the lowest limits of TVB-N belonged to the propolis-treated shibuta (*Barbus grypus*) fillets compared to the other treatments. The rationale for this result can be the antimicrobial effect of the propolis and the reduction ability of the bacteria to operate deamination reaction of Non-Protein Nitrogen (NPN) ingredients [9].

K value

Initiating before the bacterial spoilage, a postmortem reaction in the fish body in many respective reactions is nucleotides' decomposition in the meat until the hypoxanthine generation by the endogenous enzymes in the muscle tissues during-storage. After enhancing the bacterial growth, exogenous enzymes started linking to the endogenous ones. Estimating these resulted compounds has been concerned as an indicator of the fish freshness or K value, recognized as the ratio (×100) of the non-phosphorylated ATP's decomposed products to the total ATP's decomposed products [46]. K value variations of the fillets during the storage period are reflected in Figure 6. Based on the prior studies, trout fillet, whose K index was <20%, was relatively fresh, while the indexes <50% were known as moderately fresh, and the indexes >70% were regarded as unacceptable [28]. In the present research, the trout samples' primary K value was 15.31%-16.82%. Also, other researchers observed that their rainbow trout fillets initiated with the K values of 19.67%-19.82 % [28, 47]. Accordingly, their result is in the same line with these study's findings. As displayed in Figure 6, all samples in all groups, especially control samples, presented a rapid growth of the K value during the storage days. A similar model was found in the results of the previous studies on the bream [2], rainbow trout [28, 47], and silver carp [21]. But in other edible fish, including large yellow croaker and the black skipjack, we observed a gradually raising K value during the storage times; no variation was reported later, and the enhancement rate did not accelerate [47, 48]. Such differences may be derived from different edible fish, primary microbial load, and storage properties.

The treatments demonstrated remarkably lower K values than the control sample at all storage times ($P<0.05$). The K value of the control (77.89%), CMC (79.15%), and CMC-PE 1% (71.74%) samples raised the allowed maximum quantity (70%) on the 9th, 12th, and 15th days, respectively. During the refrigeration time, however, the K value of CMC-PE 2% did not increase by 70%. A significant difference ($P<0.05$) was found among all samples after 0 days and until the final interval. For preventing nucleotide degradation, the most potent treatments were CMC-PE 2%, followed by CMC-PE 1% and CMC. Findings confirmed the treatments with PE such as CMC-PE 1% and, specifically, CMC-PE 2% as more helpful in preventing ATP decomposition and preserving the freshness of the trout flesh.

Sensory properties

Sensory properties' differences in the steamed fillet over the storage times are represented in Figures 7A and B. Allowable fillet samples for consumption obtained the sensory grade above 4 [4, 22]. Missed grades in the taste feature, observed in Figure 7A, belong to the off-flavor and inedible samples. Taste and odor scores of studied samples decreased with increasing the storage time. According to the declaration of the panelists, the taste and odor scores of the control fillets were undesirable on the sixth day.

These observations were in line with the biochemical results of the fillets, which may have been obtained from lipid oxidation, protein degradation, and NPN decomposition compounds, including aldehyde, ketone, hypoxanthine, trimethylamine, dimethylamine, and ammonia. They produce the off odor and off flavor products that could be a rationale for these samples' poor grades. As illustrated in Figures 7 A and B, more significant differences ($P<0.05$) are seen among all groups than the control sample during the refrigeration time. Control, CMC, CMC-PE 1%, and CMC-PE 2% groups achieved admissible scores in the taste and odor after the 3rd, 6th, 12th, and 15th days of storage. Antimicrobial, antioxidant, and gas barrier effects resulting from coating minimized the spoilage reactions besides elongating the shelf life of the samples and preserving their quality. A combination of sodium alginate and CMC in another study extended the shelf life of the silver carp fillet on the eighth day of chilled condition [29].

Ojagh et al. [4] and Raeisi et al. [13] showed that the implementation of the biopolymer coatings enriched with antimicrobial substances induced a remarkable enhancement in the overall acceptability of the fish fillets. Incorporating PE into CMC coating significantly ($P<0.05$) preserved the scores of the fresh sensory features of taste and odor in the trout flesh until the last interval. The sensory analyses revealed that a combination of PE with CMC enhanced the shelf life of the samples on the 15th day; but, it did not show any additional effect on the sensory properties in terms of taste and odor, which was invisible on the surface of the rainbow trout fillet as well. As Duman and Ozpolat suggested, the simultaneous usage of the vacuum and aqueous PE increased the shelf life of the shibuta (*Barbus grypus*) fillets for about 1-2 weeks [9]. This finding can be linked to the antimicrobial effect of the PE that was derived from the phenolic components' presence in this extract. Some prior studies have also reported similar results [14].

4. Conclusion

Propolis, as a helpful product of honeybees, could lower and slow the physicochemical reactions and microbial growth. Also, its combination with CMC edible coating prolonged the shelf life of the rainbow trout fillet at the chilled storage. Concerning the point that natural food substances such as propolis are safer and more cost-effective than synthetic ones, adding these compounds into the food products may upgrade the consumers' health and the food products' preservation without any unfavorable changes in their sensory properties at low concentrations. However, to identify the effect of PE on other meat products, further studies are required. Also, the uses of other kinds of PE-containing packages or coatings in increasing the shelf life of these new food models should be tested in future works.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Behnaz Bazargani-Gilani, Mohammadreza Pajohi-Alamoti; Methodology: Mojtaba Raeisi, Parviz Hassanzadeh; Investigation, writing - original draft, and editing: All authors; Data analysis: Parviz Hassanzadeh, Mojtaba Raeisi; Funding acquisition and resources, data collection: Behnaz Bazargani-Gilani.

Conflict of interest

The authors declared no conflict of interests.

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