Effect of anchovy by-product protein coating incorporated with thyme essential oil on the shelf life of anchovy (*Engraulis encrasicolus* L.) fillets

Serpil Tural¹ · Sadettin Turhan²

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Abstract The effects of anchovy by-product protein coatings incorporated with thyme essential oil (TEO) on the shelf life of anchovy (*Engraulis encrasicolus* L.) fillets stored at 4 ± 1 °C were investigated. We grouped fillets in three categories: untreated fillets, fillets treated in coating solution alone, and fillets treated in coating solution containing 1.5% TEO and analyzed on 0, 3, 6, and 9 days of storage. It was observed that the total volatile basic nitrogen (TVB-N) and pH of all the fillet groups increased under cold storage conditions; however, this increase was much slow in both the coated anchovy fillets. Both coating applications slowed down the lipid oxidation but the coating containing TEO exhibited better effect than coating alone. The coating application alone or with essential oil had limited effect on microbial growth but positively affected the sensory quality. These results revealed that anchovy by-product protein coating mixed with TEO may prolong anchovy fillets' shelf life stored under cold condition.

Keywords Anchovy by-product protein coating · Thyme essential oil · Anchovy fillet · Shelf life

Introduction

Fish are highly susceptible to microbial spoilage during cold storage, mainly due to rapid growth of microorganisms naturally present in fish or from contamination. As the microorganisms grow, they utilize nutrients and produce metabolites such as sulfuric compounds, aldehydes, ketones, esters, trimethylamine, and total volatile base nitrogen. It is well known that the primary cause of the sensory rejection of fresh fish is due to the accumulation of metabolic products during storage. In addition, these metabolic products can occasionally result in either health-related or economic problems [1]. Fish are known to be very sensitive to lipid oxidation because they contain unsaturated lipids at high levels; moreover, the metal concentration in their muscles is quite high. The lipid oxidation significantly lowers fish lipids' nutritional value and leads to the development of undesirable rancidity and potentially toxic reaction products [2]. Therefore, preventing microbial growth and delaying lipid oxidation in fish and fish products are very important. Many food preservation methods are developed to delay the spoilage and growth of pathogenic microorganisms and impede lipid oxidation; however, there is still a need for novel techniques to further slowdown, reduce or eliminate them. Moreover, an increasing consumer demand for less chemical food additives and products that have less effect on the environment has been observed. Here edible coatings are one of the important and new strategies that can be used for this purpose.

Edible coatings bear the potential of extending shelf life and thus improving the fishery products' quality. They can be used to restrict oxygen absorption, reduce lipid migration and moisture loss, serve as a physical protection, enhance the properties of mechanical handling, or provide

✉ Sadettin Turhan
sturhan@omu.edu.tr

¹ Republic of Turkey Ministry of Food, Agriculture and Livestock, Veterinary Control Institute, 55200 Samsun, Turkey

² Department of Food Engineering, Engineering Faculty, Ondokuz Mayıs University, 55270 Samsun, Turkey

a substitute for commercial packaging materials in versatile food products. They can be obtained from different materials such as protein, lipid, and polysaccharide [3]. Various researchers have reported the successful use of fish by-product proteins among these materials in the development of edible coatings [4–7]. In addition, it can be noted that fish-processing by-products have been effectively assessed to prevent environmental pollution as well as achieve high added value products by increasing the variety of products [5]. Moreover, edible coatings may be used as carriers for essential oils, which may inhibit microorganism growth and delay lipid oxidation and hence extend the shelf life of food products [3, 4].

Thyme essential oil (TEO) has been evaluated as a crucial essential oil; it contains 31 different compounds, and most of them possess substantial antimicrobial and antioxidant properties [8]. Its most important active compounds are thymol (46.42%), carvacrol (12.42%), p-cymene (22.31%), and γ -terpinene (7.50%), which exhibit antimicrobial and antioxidant properties in a wide spectrum [9]. The effects of TEO on the shelf life or quality properties of fish and fish products were studied by various researchers [3, 9]. Recently, we characterized the physicochemical and mechanical properties of films made from anchovy (*Engraulis encrasicolus*) by-product proteins as a function of glycerol and protein concentration; moreover, we determined that edible film containing 4% protein and 40% glycerol (w/w of protein) was the best film [6]. TEO-added anchovy by-product protein films' properties and antioxidant capacities were investigated in our previous study [7], and we also reported that film incorporated with 1.5% TEO exhibited the highest antioxidant capacity. Depending on these results, this study aims to evaluate the effect of anchovy by-product protein coating mixed with thyme essential oil on the microbiological, sensory, and chemical properties of anchovy (*Engraulis encrasicolus* L.) fillets stored for 9 days at 4 ± 1 °C.

Materials and methods

Materials

Anchovy (*Engraulis encrasicolus* L.) processing by-products, including head, frame, and viscera (30.23% dry matter, 12.58% protein, 13.02% fat, and 3.43% ash), were procured from SASTAS A.S. (Samsun, Turkey) and used in the anchovy by-product proteins (ABP) production. First, by-products were minced using an electronic meat mincer (Mado Primus, MEW 613 model, Eilenburg, Germany) and mixed with water at 1:2 ratio (w/v) in a blender (Sinbo, SHB 3062 model, Istanbul, Turkey) for 1 min. NaOH (2 M, pH 11.2) was added to the homogenized

mixture under continuous agitation for 1 h via a magnetic stirrer (MTOPS, HS15-03P model, Yangju-City Kyunggi-do, Korea) for protein solubilization, and the mixture was centrifuged at $9000 \times g$ for 30 min in a laboratory centrifuge (Nuve, NF 800R model, Ankara, Turkey) at 4 °C. The supernatant was collected; it was acidified for protein precipitation with 2 M HCl (pH 5.35), mixed for 5 min by a magnetic stirrer (MTOPS, HS15-03P model, Yangju-City Kyunggi-do, Korea), and again centrifuged for 15 min at $9000 \times g$ in a laboratory centrifuge (Nuve, NF 800R model, Ankara, Turkey) at 4 °C. Subsequently, we dried the precipitated proteins under forced air at 35 °C in an oven (JSR, JSOF-50 model, Gongju-City, Korea) for 24 h; they were milled and then stored at +4 °C in glass jars prior to coating solution preparation [5]. The protein content of ABP powder was determined according to AOAC [10] procedure and it presented 68.58% protein.

Dried thyme (*Thymus vulgaris* L.) was purchased from a local market (Samsun, Turkey), powdered using a household coffee grinder (Sinbo, 2909 model, Istanbul, Turkey) and hydrodistilled via a Clevenger apparatus (Sesim Kimya Laboratuvar, Ankara, Turkey) using 500 mL distilled water and 50 g dry material for 3 h. We collected the extracted essential oils, then dehydrated it with anhydrous sodium sulfate, and kept at +4 °C in the dark prior to usage. Tween 80 and glycerol were purchased from Merck (Darmstadt, Germany).

In this research, we used anchovy (*Engraulis encrasicolus*, L) obtained from the Black Sea as the coating application material. We procured the fresh fish from Samsun Fish Bazaar, placed in a box full of ice, and then immediately transferred to the laboratory. Afterwards, we eviscerated the fish after beheading them and then washed using tap water; finally, they were dried for 10 min on a plastic sieve. We measured the weight of anchovy fillets to be between 5 and 6 g.

Coating solutions preparation, application to anchovy fillets, and storage

The coating solutions were prepared in accordance with Limpan et al.' method [11] after several modifications. ABP (4.0 g) was added to 100 mL distilled water; after mixing with 5 M NaOH, the solution pH was adjusted to 11.5, and then we added glycerol at 40% (w/w of ABP) as a plasticizer. We homogenized the solution for 5 min with a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany) at $11,500 \times g$, which was stirred for 60 min at 85 °C on a magnetic stirrer (MTOPS, HS15-03P model, Yangju-City Kyunggi-do, Korea). We used a laboratory centrifuge (Nuve, NF 800R model, Ankara, Turkey) to centrifuge at $3000 \times g$ for 10 min, and then applied filtration to remove unresolved materials; furthermore, the solution was cooled

it to 40 ± 2 °C. Essential oil and Tween 80 were mixed with the coating solution at a level of 0.05% (v/v) to dissolve the essential oil easily, and TEO at 1.5% (v/v) was added to the coating solution. A coating solution without TEO incorporation was also prepared. Finally, the solutions were emulsified via a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany) for 3 min at $11,500 \times g$ and applied to anchovy fillets. Before coating application onto fillets, all solutions were cooled to room temperature.

The anchovy fillets were randomly divided into three groups, and the first group was used as the control (without coating application) (CNT1), the second group was treated with coating solution without TEO (CNT2), and the third group was treated with coating solution with 1.5% TEO (TEO15). For coating applications, we immersed the anchovy fillets in the solutions for 1 min, air-dried for 1 min, and then immersed them again in the solutions for 1 min and once more air-dried for 1 min. Furthermore, fillets without or with coating were packaged in polyethylene terephthalate (PET) tray, kept at 4 ± 1 °C for 9 days and analyzed on 0, 3, 6, and 9 days of storage.

Determination of pH

We stirred 10 g sample with 100 mL distilled water using a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany) for 1 min; we measured the mixture's pH value using a digital pH meter (Cyberscan PC 510, Singapore). The same person performed all the measurements, and we used the average of the three measurements.

Determination of the total volatile basic nitrogen (TVB-N)

We stirred 10 g sample with 100 mL distilled water using a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany) for 1 min and the mixture was transferred to a Kjeldahl distillation flask. This mixture was incorporated with a few drops of antifoam solution and 1 g magnesium oxide, and then the mixture was distilled using a Buchi distillation unit (B-324 model, Switzerland). The distillate was absorbed by 10 mL aqueous solution of boric acid (4%) with a mixed indicator derived from 0.1 g methylene blue in 100 mL ethanol and 0.1 g methyl red. Finally, we titrated boric acid solution with 0.1 M HCl, and TVB-N content of the samples was measured in terms of HCl consumption [12].

Determination of conjugated diene (CD)

We determined the CD value via Soyer's method [13] with several modifications. First, using a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany), we

homogenized 0.5 g minced anchovy fillets with 5 mL distilled water for 2 min. The homogenate (0.5 mL) was then mixed with 5 mL hexane-isopropanol (3:1) mixture, homogenized for 1 min using a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany), and then centrifuged for 5 min at $500 \times g$. Supernatant's absorbance was designated at 233 nm using a UV-Vis spectrophotometer (Agilent Technologies, Cary 60 model, Victoria, Australia) and defined as the CD value.

Determination of thiobarbituric acid reactive substances (TBARS)

We determined TBARS according to the method of Witte et al. [14] by applying some modifications. We combined 10 g anchovy fillets with 20 mL distilled water and 25 mL 20% trichloroacetic acid, which was homogenized for 2 min using a homogenizer (Ultraturrax-IKA, T25 model, Staufen, Germany). Afterwards, we filtered the homogenate using a Whatman No.1 filter paper and then combined 5 mL filtrate with 5 mL 0.02 mol/L thiobarbituric acid (TBA) reagent and heated for 30 min in boiling water. We cooled down the samples under cold flowing water and read the absorbance at 538 nm against the appropriate blank. We prepared a standard curve using 1,1,3,3-tetraethoxypropane and showed the results as mg malondialdehyde (MDA) per kg sample.

Microbial analysis

We aseptically transferred 10 g anchovy fillets into individual stomacher bags filled with 90 ml sterile saline solution (NaCl 0.85%) and used a laboratory stomacher for homogenization for 1 min to prepare the initial dilution. Then, we prepared appropriate serial decimal dilutions in saline solution (NaCl 0.85%) and took 0.1 mL of each dilution Plate Count Agar (Merck) plates' surface by means of a pipette for psychotropic count (PTC) and total viable count (TVC), and for the coliform bacteria count, we took them onto the surface of Violet Red Bile Agar (Lab M). We incubated the inoculated plates for 5 days at 25 °C for TVC for 10 days at 7 °C for PTC and for 24 h at 37 °C for coliform bacteria count. We expressed all counts in log cfu/g.

Sensory analysis

We determined the sensory attributes on both raw and cooked anchovy fillets. We cooked anchovy samples separately on an electrical grill (Arcelik Mini Firin, Turkey). We served them warm to a trained panel comprising ten volunteers from the Ondokuz Mayıs University, Department of Food Engineering in Turkey. We instructed the

panelists to clean their palates with water between the samples then we performed the sensory evaluations under fluorescence lighting. For scoring sensory attributes, we utilized 9-point hedonic scale, wherein 1 represented dislike extremely and 9 represented like extremely, and the acceptability limit was 5. We calculated the overall acceptability for raw anchovy fillets considering odor, texture, and appearance (each with 33.33%), whereas we calculated the overall acceptability for cooked samples considering texture and flavor (each with 50%) [15].

Statistical analysis

We subjected all data to analysis of variance and used Duncan's multiple range test ($p < 0.05$) to compare the significant differences among the means. For data analysis, we utilized SPSS statistical program (SPSS 17.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

pH

The pH value can be an indicator of spoilage in fishery products [16]. Changes in anchovy fillets' pH value during refrigerated storage are shown in Fig. 1(A). As seen, the initial pH values belonging to uncoated (CNT1) and coated (CNT2 and TEO15) anchovy fillets were observed as 6.80, 6.72, and 6.72, respectively. The pH value of all the groups gradually increased during cold storage for 9 days ($p < 0.05$). This significant increase could be associated with the volatile basic components, e.g., trimethylamine and ammonia produced by either endogenous or microbial

enzymes [12], and the increase in TVB-N can confirm this situation [Fig. 1(B)]. In coated fish, similar results were obtained by many authors. For example, Souza et al. [17] observed that the pH value of salmon (*Salmo salar*) coated with chitosan increased during 18 days of cold storage. Similarly, Mohan et al. [16] found that pH value of Indian oil sardine (*Sardinella longiceps*) coated with chitosan increased as a function of storage period and the pH values were measured as 6.66 and 6.58 in 1 and 2% chitosan coated sardine after 9 days of cold storage, respectively.

The coating application with or without TEO to anchovy fillets slowed down the increase speed of pH, and no significant differences in pH values were observed between the coated groups throughout the study (except 6 days of storage). This demonstrated that addition of TEO had no effect on the pH of anchovy fillets. The pH values of CNT1, CNT2, and TEO15 groups were determined as 7.10, 6.98, and 6.95 on day 9 of the storage, respectively. According to the results, the acceptable upper limit value of 7.0 [18] was exceeded by uncoated anchovy fillets on day 9 of the storage, whereas coated fillets did not exceed it. The surface of the fillet with coating under atmospheric effects is preserved against oxygen's spoiling action that is noted to possibly cause an increase in the pH value due to the effects of basic amines production [19]. Moreover, Ahmad et al. [20] presented similar results in sea bass slices coated with gelatin film with or without lemongrass essential oil.

Total volatile basic nitrogen (TVB-N)

TVB-N content is known to be an important reference for evaluating the freshness of the fish, and the increase in TVB-N content could be caused by endogenous enzyme

Fig. 1 Changes in pH (A) and TVB-N (B) values of anchovy fillets during refrigerated storage (CNT1: uncoated, CNT2: coated with anchovy by-product protein solution, and TEO15: coated with

anchovy by-product protein solution containing 1.5% thyme essential oil). The values are the mean of four determinations in two experiments and the bars indicate the standard deviation

activity and spoilage bacteria activity [21]. Changes in TVB-N contents in fish samples during storage for 9 days are presented in Fig. 1(B). As seen, the initial TVB-N content of anchovy fillets ranged from 11.17 mg/100 g in TEO15 group to 12.16 mg/100 g in CNT1 group, which is within recommended ranges of TVB-N in fresh fish (5–20 mg/100 g) [18]. During cold storage for 9 days, the TVB-N content of all groups gradually increased ($p < 0.05$); however, this increase was much slower in the coated anchovy fillets. Moreover, no significant differences in TVB-N contents were observed between the CNT2 and TEO15 groups throughout the study. The increase of TVB-N content during storage may be associated with the activity of endogenous enzymes and spoilage bacteria and subsequently attributed to the accumulation of trimethylamine, dimethylamine, monoethylamine, ammonia, and other volatile bases [21, 22]. Similar to these results, it is also reported that the coating applications with or without essential oil slowed down the formation of volatile basis in bream [12], Indian oil sardine [16], salmon [17], sea bass [20], silver carp [22], and rainbow trout [18, 19, 21, 23].

The TVB-N content of CNT1 group increased to 46.11 mg/100 g during storage on the 6th day, whereas the TVB-N content of CNT2 and TEO15 groups increased to 42.15 and 41.85 mg/100 g on the 9th day of the storage, respectively. A spoilage upper limit of 30 mg/100 g was applied to the fishery products [24]. Hence, uncoated anchovy fillets were observed to exceed this limit during the storage on the 6th day, whereas coated fillets exceeded it on day 9 of the storage. However the addition of TEO into coating solution did not affect the TVB-N formation ($p > 0.05$). According to the results, the coating applications have an effect in regulating anchovy fillets' TVB-N value, delaying the formation of volatile bases. This effect could be explained by the oxygen resistance layer formed on the fish surface and prevention of microbial contamination of coating application.

CD

In the primary step of lipid oxidation, non-conjugated double bonds within unsaturated fatty acids turned into to conjugated double bonds observed at 232–234 nm [25]. UV absorbance measurements of CD values at 233 nm are shown in Fig. 2(A). As seen, the CD values of all the groups increased during cold storage for 9 days ($p < 0.05$) and the increase was calculated as 60, 43, and 29% for CNT1, CNT2 and TEO15 groups, respectively. In line with the results of our study, increases in the CD value during cold storage of chitosan-coated herring and cod fillets were also determined by Jeon et al. [26]. These authors reported that the CD values of chitosan-coated herring and cod samples increased by 4–9 and 6–12-fold at the end of the

storage of 12 days, whereas the increase in the herring and cod samples without coating were 19 and 27-fold, respectively.

The highest CD value during all storage periods was observed in the uncoated group. However, no significant differences in CD values were observed between the CNT1 and CNT2 groups on days 0, 3, and 6 of the storage. The TEO15 group had a lower CD value than that of the CNT1 and CNT2 groups on days 3 and 9 of the storage ($p < 0.05$). Riveros et al. [27] also reported that TEO added to the carboxymethyl cellulose coating was useful in protecting roasted sunflower seeds from lipid oxidation, and a lower CD value was observed in the roasted sunflower seeds with carboxymethyl cellulose coating containing TEO compared with uncoated samples. The antioxidant capacity properties of TEO were well documented and suggested as a natural food antioxidant [4, 7, 8, 27]. This property of TEO is related to the presence of carvacrol and thymol, two phenolic compounds showing antioxidant capacity [28]. Phenolic compounds do not behave as oxygen absorbers but they inhibit fatty free radical formation, which engages in a reaction with oxygen or absorbs it during autoxidation process, and therefore, delays the beginning of an autoxidative process in foods [2]. These results showed that coating application with TEO was useful in hampering the lipid oxidation of anchovy fillets stored under cold conditions.

Thiobarbituric acid reactive substances (TBARS)

We used TBARS for the measurement of secondary oxidation products that may cause color and odor changes, off-flavor, and result in the deterioration of the texture of fish products [23]. Figure 2(B) shows the changes in the TBARS values of the CNT1, CNT2, and TEO15 groups during storage for 9 days. Anchovy is a fatty fish, and its lipids are quite susceptible to oxidation [2]. As seen, early TBARS value of anchovy groups ranged from 1.07 mg MDA/kg for TEO15 group to 1.18 mg MDA/kg for CNT1 group and continuously increased ($p < 0.05$) with different rates depending on anchovy groups during cold storage. The results from numerous studies are compatible with this observation [12, 16, 17, 23]. We can associate the increase in TBARS value during storage with the higher levels of unsaturated fatty acids oxidation and partial dehydration of fish [12]. TBARS value of the CNT1, CNT2, and TEO15 groups reached 27.73, 22.84, and 12.85 mg MDA/kg on day 9 of the storage and the increase rates were 24, 20, and 12-fold, respectively. According to Sallam [29], the maximum TBARS value is 5 mg MDA/kg tissue, demonstrating the good quality of the fish (chilled, frozen, or stored with ice), although the fish may be consumed up to 8 mg MDA/kg tissue. According to the results of this study,

Fig. 2 Changes in CD (A) and TBARS (B) values of anchovy fillets during refrigerated storage (CNT1: uncoated, CNT2: coated with anchovy by-product protein solution, and TEO15: coated with

anchovy by-product protein solution containing 1.5% thyme essential oil). The values are the mean of four determinations in two experiments and the bars indicate the standard deviation

TBARS values of CNT1 and CNT2 groups (7.94 and 7.02 mg MDA/kg, respectively) transcended the limit of 5 mg MDA/kg, which is the maximum limit of good quality on the 3rd day of storage, which points to lipid oxidation at high levels in the samples and the TBARS value of TEO15 group transcended it on the 6th day of the storage. However, all of uncoated and coated anchovy fillets transcended the maximum limit for consuming on the 6th day of the storage.

The TBARS value of CNT2 group was observed to be significantly lower ($p < 0.05$) compared with the value of CNT1 group on days 3 and 9 of the storage. The lower TBARS value can be associated with oxygen resistance layer formation on the fish surface [12, 16]. Dangaran et al. [30] reported that most protein films provide excellent barriers to oxygen. Moreover, light transmission of fish protein films is generally low and such films can hinder lipid oxidation caused by UV light in a food system [31]. Ozyurt et al. [23] also reported reduced lipid oxidation in rainbow trout fillets coated with fish protein. Similar to CD value, the lowest TBARS values ($p < 0.05$) were determined in the TEO15 group for all storage periods. This result indicated an antioxidative effect of TEO in anchovy fillets. As mentioned above, this effect of TEO is related to its thymol and carvacrol content with known antioxidant capacity [28]. Similar results were also obtained when fish products were coated materials with TEO. Chanamara et al. [32] showed that coating application containing TEO to rainbow trout samples resulted in reduction in the TBARS values during storage. Jouki et al. [9] reported that the incorporation of TEO into a quince seed mucilage film improved the resulting film's antioxidant property, which

can also be observed in the lower TBARS of rainbow trout fillets coated with QSFM + TEO. These results indicated that both the coating applications could inhibit the lipid oxidation in anchovy fillets but coating containing TEO had a better effect than coating alone.

Microbial quality

The composition of fish muscle makes it favorable for microbial growth; thus, fish spoilage occurs during storage mainly as a result of microbial activity [17]. Figure 3 shows the changes in TVC, PTC, and coliform bacteria counts of CNT1, CNT2, and TEO15 groups during storage for 9 days. As seen in Fig. 3(A, B), early values of TVC and PTC of anchovy groups ranged between 3.65 and 4.21 cfu/g and 3.65 to 3.98 cfu/g, respectively, indicating that the anchovies studied were slightly contaminated. The TVC and PTC of all the groups increased during cold storage for 9 days ($p < 0.05$). The lowest TVC value was observed in the TEO15 group on day 3 of the storage, whereas no significant differences were observed between uncoated and coated groups on days 6 and 9 of the storage. The PTC of uncoated group was similar to that of the coated groups on day 0 of the storage and the lowest PTC was observed in TEO15 group on days 3, 6, and 9 of the storage. However, no significant differences in PTC were observed between the CNT2 and TEO15 groups on days 3 and 6 of the storage. The TVC and PTC of all the groups exceeded 7 cfu/g, considered as the upper recommended microbiological limit for fish [33] on the 6th day of storage. These results showed that coating application alone or with essential oil slightly delayed microbial growth during cold

Fig. 3 Changes in TVC (A), PTC (B) and coliform bacteria (C) count of anchovy fillets during refrigerated storage (CNT1: uncoated, CNT2: coated with anchovy by-product protein solution, and TEO15: coated with anchovy by-product protein solution

containing 1.5% thyme essential oil). The values are the mean of four determinations in two experiments and the bars indicate the standard deviation

storage. This low effect might be explained with the low concentration of TEO applied because the antimicrobial effect of TEO varies depending on its concentration [9]. Moreover, this low effect might be explained with the prepotency of lactic acid bacteria in the TVC and PTC because they are proponent microflora in fresh meat with vacuum package and are known to show resistance to antimicrobial treatments. Karagoz Emiroglu et al. [34] also reported similar findings in fresh ground beef patties coated with 5% TEO-added film. However, many studies showed that TEO has antimicrobial properties and could effectively inhibit most spoilage organisms; therefore, it has been widely used as a natural food preservative [9, 32]. The antimicrobial activity of TEO could be attributed to its thymol, carvacrol, p-cymene, and γ -terpinene content [9].

As seen in Fig. 3(C), the initial coliform bacteria count of anchovy groups ranged from 0.60 to 1.19 cfu/g and bacteria count increased in all the groups during cold storage for 9 days ($p < 0.05$). The coliform bacteria count in CNT1, CNT2, and TEO15 groups was determined as 5.15, 5.06, and 4.93 cfu/g on day 9 of the storage,

respectively. In all storage period, the lowest coliform bacteria count was observed in TEO15 group; however, no significant differences were observed between TEO15 and the others groups on days 3 and 9 of the storage. Similar to TVC and PTC, coating application with essential oil slightly delayed coliform bacteria growth during cold storage. This could be explained with abovementioned factors.

Sensory assessment

Changes in the overall acceptability scores of raw and cooked samples during storage are shown in Fig. 4. As seen in Fig. 4(A), the acceptability score of uncoated group was similar to that of the coated groups on days 0, 3, and 6 of the storage, whereas the uncoated group received lower score than that of the coated groups on day 9 of the storage. The overall acceptability scores of all groups decreased ($p < 0.05$) during the cold storage for 9 days and were determined as 3.10, 6.00, and 6.33 for the CNT1, CNT2, and TEO15 groups on day 9 of the storage, respectively.

Fig. 4 Changes in overall acceptability [raw (A), cooked (B)] scores of anchovy fillets during refrigerated storage (CNT1: uncoated, CNT2: coated with anchovy by-product protein solution, and TEO15:

coated with anchovy by-product protein solution containing 1.5% thyme essential oil). The values are the mean of four determinations in two experiments and the bars indicate the standard deviation

According to these results, uncoated anchovy fillets received unacceptable score on day 9 of the storage, whereas the coated fillets received acceptable scores. This could be explained with the antioxidant, antimicrobial, and barrier properties of protein coatings without or with TEO, and this result is also supported by the results of microbial and chemical analysis. Furthermore, several studies have been conducted on the positive effect of edible coatings alone or with essential oil on the sensorial properties of fish products [12, 16, 23].

As seen in Fig. 4(B), the overall acceptability score of the cooked anchovy groups was similar to each other on days 0, 3, and 6 of the storage but the CNT1 group was not analyzed on day 6 of storage due to poor microbial quality and high TVB-N and TBARS values. The overall acceptability scores of CNT2 group decreased ($p < 0.05$) during cold storage, whereas the scores of CNT1 and TEO15 groups did not change. As in the raw anchovy fillets, the cooked CNT2 and TEO15 groups were also acceptable on day 6 of the storage. This could be explained with abovementioned factors. Chamanara et al. [3] also presented similar results in cooked fish samples and these authors reported that chitosan coating alone or enriched by TEO positively affected the taste scores of rainbow trout during cold storage. Despite the positive effect of coating applications alone or with TEO, in terms of shelf life, no good relationship was established between chemical, microbiological, and sensory properties.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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