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The effect of propolis application on quality properties of vacuum-packed hot smoked rainbow trout (*Oncorhynchus Mykiss*, Walbaum 1792) fillets during cold storage

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ABSTRACT

This research aimed to apply propolis extract (PE) to extend the shelf life and improve the quality of hot smoked rainbow trout while avoiding apparent changes in organoleptic properties. To this end, the effect of PE at different levels (0,25%, 0,50%, 1%, and 3%) on the physico-chemical, chemical, microbiological, and sensory parameters of vacuum-packed hot smoked rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) fillets was investigated throughout cold storage (4 ± 1 °C). The observed shelf life of smoked trout was similar in the control and 0.25% PE groups (56 days). After two weeks (on day 70), the 0.50% and 1% PE groups were spoiled. Finally, on storage day 84, 3% of the PE group was spoiled. The dose of 0.25% PE used in this study was insufficient to show the desired effects on the final product. The application of 3% PE significantly affected the microbiological and chemical properties. However, it had a negative effect on the odor and taste of the products. The 1% PE group demonstrated the most potent sensory properties in color, odor, and overall acceptability. This study shows that 1% PE is useful in hot smoked rainbow trout fillets as a natural antimicrobial and antioxidant.

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) meat has high commercial value and is widely accepted by consumers (Cakli et al., 2006). As is known, fish meat is very susceptible to microbiological spoilage and lipid oxidation during cold, refrigerated storage. Microbiological metabolites and secondary products formed as a result of lipid oxidation can lead to spoilage (Ebadi et al., 2019). While smoking was a common traditional food preservation method prior to the invention of the refrigerator, smoked products are now in demand mostly in developed countries for their sensory characteristics (Ledesma et al., 2016). The preservative effect of smoking comes from decreasing water activity (a_w) related to salting and drying and, from phenolic compounds with antimicrobial and antioxidant properties in wood smoke. Hot smoking is a process used to make cooked products sold as "*ready-to-eat*" products in the marketplace. On the other hand, hot smoked fish is generally stored in cold storage and, has a limited shelf life of mostly 3–4 weeks (Ekonomou et al., 2022; Erkan, 2012).

Propolis is an organic product that is widely distributed and traded around the world. It can be used as a natural food preservative and has antimicrobial and antioxidant effects, has numerous health benefits for consumers. In response to consumer demand for natural food preservatives perceived as safer and superior to synthetic alternatives, propolis has emerged as a popular ingredient in contemporary food manufacturing (Pobiega et al., 2019). As a food preservative, the antimicrobial and antioxidative effects of propolis come from the rich phenolic content in its chemical composition. However, in food industry, propolis is commonly extracted in solvents (water, ethanol, propylene glycol, essential oils, etc.) because of its high content of contaminants and poor water solubility. Due to its origin in plant resins, propolis has low solubility in water and is soluble in organic solvents because resins are naturally apolar. Although aqueous propolis solutions have low production costs, they have an almost tenfold lower content of phenolic compounds than ethanolic extraction. Generally, ethanol-water mixtures (frequently 70 − 80%) are used for preparing propolis extract (Bankova et al., 2021; Pobiega et al., 2019). Also, the use of propolis extract (PE) in foods is limited because of its high degree of intensive flavor and odor. Therefore, determining the concentration is

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essential to PE, for it can be applied without changing the sensory characteristics of the foods (Ebadi et al., 2019; Pobiega et al., 2019). Several researches concluded that a minimum amount of PE for sensory food product approval is 0.5% (Duman & Özpolat, 2015; Ebadi et al., 2019; Pobiega et al., 2019). Despite the fact that there have been some previous studies on propolis ingredients to develop raw fish quality parameters and add beneficial properties (Duman & Özpolat, 2015; Ebadi et al., 2019; Hassanin & El-Daly, 2013), there is no studies on the incorporation of propolis extract as a natural preservative in the smoked products to the best of our knowledge. Thus, this research focused on using propolis extract as a natural food preservative to extend the shelf life and improve the quality of hot smoked rainbow trout while avoiding apparent changes in sensory properties.

2. Materials and methods

2.1. Materials

The raw propolis was purchased from local beekeepers (Bingol, Turkey) In this research, an ethanolic extract of propolis (PE) was prepared using the maceration technique previously determined by Thamnopoulos et al. (2018) with some modifications. Briefly, 100 g of grounded raw propolis was placed into a sterile Erlenmeyer flask (covered by aluminium foil to protect it from light) which contained 1 L of ethanol with a concentration of 70%. The mixture was stirred for 15 days at room temperature in magnetic stirrer (AM4, Velp Scientifica, Usmate, Italy). After that, the extract had vacuum filtration using filter paper. And then, filtered mixture was frozen at −18 °C for 24 h and filtered again, as previously described, with the aim of eliminating waxes and other materials that exhibit lower solubility. This filtration steps were repeated an additional seven times. Finally, the yield of propolis in mixture was determined by gravimetric method, and the final concentration of propolis was set at %10 PE using by 70% ethanol solution. Approximately 2 kg of raw propolis was used to prepare the 4 L of stock 10% PE solution.

Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) (250 ± 30 g) were obtained from local fish farmer (Elazığ, Turkey). The fresh fishes were packed in a cold storage box with a cold pack and then transferred immediately to the laboratory. After that, the fresh fishes were beheaded, gutted, washed, and filleted.

2.2. Groups, brining and PE applications

In total, 74 fish were used in each replicate for microbiological, chemical, and sensory analysis, which makes 296 fish at the end of four repetitions. Before the fillets were grouped, a sample was taken at each replicate and analyzed to determine the characteristics of the raw fish meat. Fillets were separated into five groups (C, PE25, PE50, PE1, and PE3). The groups formed are shown in Table 1. The brining and PE application processes were completed concurrently. The sterile brine contained 10% NaCl, and fillets were immersed in brine at a 1:1 (w/w) ratio for 90 min at 7 ± 1 °C. Glycerol (Merck, Darmstadt, Germany) was used to increase the solubility of PE in the brine solution as E 422, which is used as a "*quantum satis*" principle food additive. The substance is miscible in water and ethanol. Also, it could be used as a "*PE carrier*" at the center of fish meat during brine salting processes. The addition ratio of glycerol was half of the amount of PE applied to each group's brine solution (Mortensen et al., 2017; Thamnopoulos et al., 2018). After the brining protocol, fillets were placed on an inclined screen to remove excess brine water from their surface for an hour.

Table 1
Groups and amount of propolis extract and glycerol addition per liter of brine solutions.

Groups	Propolis Extract	PE[a] addition in Brine solution ml/L (v/v)	Glycerol addition in brine solution ml/L (v/v)
Control (C)	0%	Untreated	
PE25	0.25%	25	12.5
PE50	0.50%	50	25
PE1	1%	100	50
PE3	3%	300	150

[a] Propolis extract as contain 10% propolis in 70% ethanolic solvent. C; Control sample without propolis extract(PE), PE25; added 0.25% v/v PE in brine, PE50; added 0.50% v/v PE in brine, PE1; added 1% v/v PE in brine, PE3; added 3% v/v PE in brine.

2.3. Smoking and packing process

All groups smoked in a smoke chamber that contained a friction smoke generator. Smoke was produced from a block of oak (*Quercus* sp., confirmed by an expert) with 1.66 bar of pressure. Also, the ventilator velocity in the smoking chamber was 3000 rpm. Fillet smoking conditions were 65 ± 5 °C, 70% relative humidity, and 50 min. After cooling in a smoke chamber (at 10 °C for 3 h), all groups were vacuum packed (VP) with polyethylene/polyamide bags (Fig. 1). Bag dimensions is 180 × 230 mm, and thickness 0.11 mm. After that, ultimate products were stored 4 ± 1 °C until analysis on days 7, 14, 21, 28, 42, 56, 70, and 84.

2.4. Physicochemical characteristics and chemical analyses

2.4.1. Basic nutrient analyses, water activity and pH

The moisture, crude ash, fat composition, and salt content of the samples were measured using methods following AOAC (2005). The water activity (a_w) values of the samples were measured with a water activity meter (650, TESTO, Germany). The pH values of samples were measured by a digital pH meter (HI, 11310, Hanna Instruments, USA). After the microbiological analyses, the stomacher bags were used for pH determination.

2.4.2. Total volatile base-nitrogen (TVB-N) analyses

TVB-N values of smoked rainbow trout fillets were determined according to the method described by Goulas and Kontominas (2005). Briefly, 10 g of sample were added in 100 mL distilled water, and then homogenized in a stomacher. This homogenate was transferred into the Kjeldahl tube. A two g of MgO and a few drops of antifoaming agents were added to tubes. Then, 200 mL distillate was collected in Erlen flask containing 25 mL of 3% boric acid solution and a few drops of Tashiro indicator. After the distillation, titration was performed with 0.1 mol equi/L HCl solution until the neutralization was completed. Results of the TVB-N quantity in fillet samples was calculated by using the following formula and presented as mg/100 g

$$\mathrm{TVB-N} = [(1.4 \times \mathrm{A}) / \ \mathrm{B}] \times 100 \ \mathrm{A} : 0.1 \ \mathrm{mol\ equi} / \mathrm{L\ HCl\ volume\ (mL)}, \mathrm{B} : \mathrm{Sample\ weight\ (g)}$$

2.4.3. Free fatty acids (FFA) analyses

The amount of free fatty acids (FFA) was calculated as gram oleic acid % fat according to the method proposed by Kurt and Kılıççeker

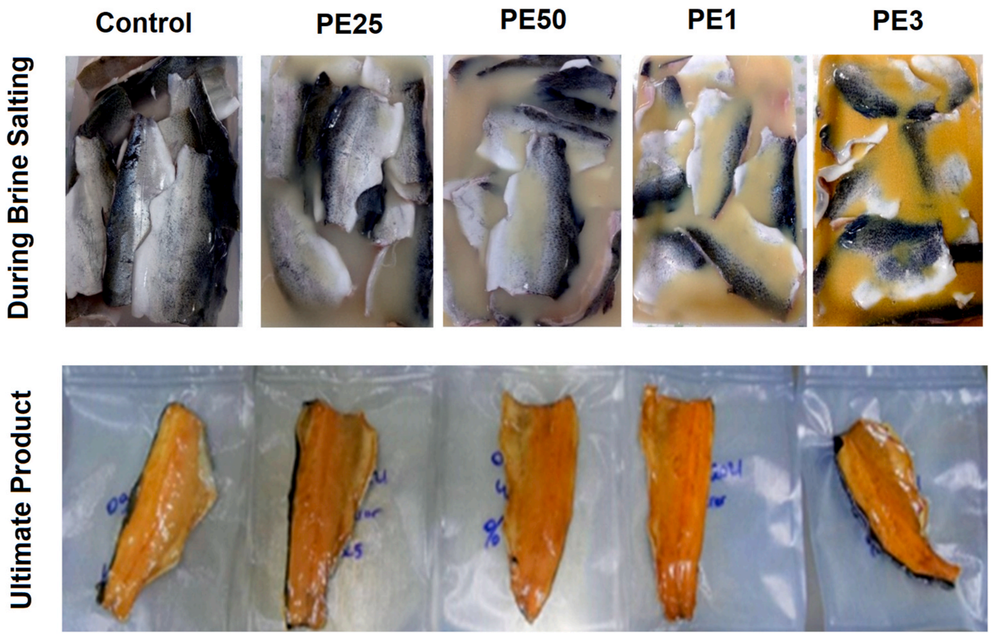

Fig. 1. The experimental groups appearance is presented at the end of the brine salting processes and after the completed protocol. (C; Control sample without propolis extract (PE), PE25; added 0.25% v/v PE in brine, PE50; added 0.50% v/v PE in brine, PE1; added 1% v/v PE in brine, PE3; added 3% v/v PE in brine).

(2011). Ten grams of ground sample was homogenized with 30 mL of chloroform containing 0.5 g of sodium sulfate, and was allowed to settle at room temperature (20 °C) for 5 min, and then filtered through filter paper. The 25 mL of filtrate was mixed with 25 mL of neutral alcohol. The FFAs in 50 mL of the solution were titrated with a 0.1 mol equi/L NaOH solution.

$$\text{FFA }(\%) = [(\text{V} \times \text{N} \times \text{F} \times 28.2)\ /\ \text{W}]\text{V} : \text{volume of titration (mL)}, \text{N} : \text{Normality of NaOH solution}, \text{F} : \text{Factor of the NaOH solution}, \text{W} : \text{Sample fat weight (g)}.$$

2.4.4. Thiobarbituric acid (TBA) analyses

Tarladgis et al. (1960) described a method for determining the Thiobarbituric acid (TBA) values of samples. Ten grams of homogenized fillet sample was mixed with 50 mL distilled water and transferred to the Kjeldahl tube. Subsequently, 47.5 mL of distilled water and 2.5 mL of HCl solution to adjust the pH to 1.5 were added. Then, distillation was performed until the 50 mL distillate collected. Five mL of the distillate was added to 5 mL of TBA reagent, and boiled for 35 min. The absorbances were measured at 532 nm (UV-VIS Mini 1240 Spectrophotometer, Shimadzu, Tokyo, Japan) against a blank reagent. The absorbance values were multiplied by a factor of 7.8 to express TBA values in mg malonaldehyde (MDA)/kg.

2.4.5. Color analyses

A colorimeter CS-10 (CHNSpec, Hangzhou, China) was used to analyse the color properties of the hot smoked rainbow trout fillet samples. The results were given in terms of the factors L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness). At room temperature, the measures of color were taken from at least four different spots on inner face of each sample (İncili et al., 2022).

2.5. Microbiological analyses

Each sampling day, a portion of 25 g of each sample package was taken aseptically and transferred to a sterile sampling bag containing 225 mL of 0.1% sterilized peptone water. The mixture was homogenized for 120 s using a stomacher (Bag Mixer 400, Interscience, France). Plate Count Agar (PCA) (Merck, Darmstadt, Germany) was used for aerobic plate counts (APC) (35 ± 1 °C, 24–48 h) and psychrotrophic bacteria (5–7 °C, 7–10 days) (USDA/FSIS, 2011). de Man Rogosa Sharpe Agar (MRS) (Biokar, France) was used (30 ± 1 °C, 72 h) for the determination of mesophilic lactic acid bacteria (International Organization for Standardization, 1998) Tributyrin agar (Liofilchem, Italy) was used (30 ± 1 °C, 48 h) for the enumeration of lipolytic bacteria (Emir Çoban et al., 2014), and Dichloran Rose Bengal Chloramphenicol (DRBC) (Merck, Darmstadt, Germany) Agar (25 ± 1 °C, 5 days) was used for yeast and mold enumeration (International Organization for Standardization, 2008).

2.6. Sensory analyses

The analysis was performed using the methods of Kurtcan and Gönül (1987). Ten qualified panellists, who were experienced sensory evaluation but received no specific training relevant to smoked foods, were evaluated the sensory quality of fillets during cold storage. In each sampling day, four fillets in each group were served to panellists in plastic plates labelled with 3-digit codes. The experiments were carried out in a room that was adequately illuminated and had proper ventilation. A cup of tap water was provided to the panellists for rinsing mouth between different samples. Scores were given by panel members based on color, odor, taste, appearance, texture, and overall acceptability on a 5-point hedonic scale ranging from 5.0 (very good) to 1.0 (poor). Samples with a score of less than 3.0 point were considered unacceptable due

Table 2
Water activity, content of salt, moisture, ash and fat (g/100 g) in the fresh fillet and stages of hot smoking of experimental groups.

	C	PE25	PE50	PE1	PE3
Water Activity (a_w)					
Fillet	0.991 ± 0.004^{a}	0.991 ± 0.004^{a}	0.991 ± 0.004^{a}	0.991 ± 0.004^{a}	0.991 ± 0.004^{a}
After Brining	0.924 ± 0.012 ^{b}	0.924 ± 0.012^{b}	0.933 ± 0.020^{b}	0.932 ± 0.014 ^{b}	0.931 ± 0.014 ^{b}
After Smoking	0.937 ± 0.010 ^{b}	0.929 ± 0.011^{b}	0.938 ± 0.018^{b}	0.939 ± 0.005 ^{b}	0.944 ± 0.018 ^{b}
Fat (g/100g)					
Fillet	2.98 ± 0.31^{c}	2.98 ± 0.31^{c}	2.98 ± 0.31^{c}	2.98 ± 0.31^{c}	2.98 ± 0.31^{c}
After Brining	5.44 ± 0.39^{b}	5.03 ± 0.58^{b}	5.26 ± 0.63^{b}	5.55 ± 1.09^{b}	5.24 ± 0.96^{b}
After Smoking	7.63 ± 0.63^{a}	7.06 ± 1.85^{a}	7.48 ± 0.61^{a}	8.11 ± 0.88^{a}	7.50 ± 0.22^{a}
Moisture (g/100g)					
Fillet	72.54 ± 1.23^{a}	72.54 ± 1.23^{a}	72.54 ± 1.23^{a}	72.54 ± 1.23^{a}	72.54 ± 1.23^{a}
After Brining	70.41 ± 3.09^{a}	70.40 ± 2.82^{a}	71.29 ± 3.05^{a}	68.53 ± 3.68^{a}	68.57 ± 3.28^{a}
After Smoking	56.99 ± 6.25^{b}	57.00 ± 8.03^{b}	59.72 ± 4.70^{b}	57.55 ± 6.44^{b}	59.65 ± 3.92^{b}
Ash(g/100g)					
Fillet	2.05 ± 0.81^{b}	2.05 ± 0.81^{b}	2.05 ± 0.81^{b}	2.05 ± 0.81^{b}	2.05 ± 0.81^{b}
After Brining	2.92 ± 0.87^{b}	2.99 ± 0.72^{b}	3.01 ± 0.74^{b}	2.77 ± 0.84^{b}	2.74 ± 0.70^{b}
After Smoking	4.25 ± 0.52^{a}	4.43 ± 0.77^{a}	5.30 ± 1.16^{a}	4.61 ± 1.31^{a}	4.35 ± 1.25^{a}
Salt (g/100g)					
Fillet	0.28 ± 0.06^{b}	0.28 ± 0.06^{b}	0.28 ± 0.06^{c}	0.28 ± 0.06^{c}	0.28 ± 0.06^{c}
After Brining	2.46 ± 0.25^{a}	2.40 ± 0.31^{a}	2.28 ± 0.24^{b}	2.22 ± 0.25^{b}	2.22 ± 0.37^{b}
After Smoking	2.72 ± 0.50^{a}	3.16 ± 0.57^{a}	3.54 ± 0.35^{a}	3.04 ± 0.58^{a}	3.10 ± 0.40^{a}

ab: Values in the same column having different superscripts for mean water activity, fat, moisture, ash and salt content for fillet, after brining phase and after smoking phase differ significantly ($P < 0.05$). Results are representing mean ± standard deviation, (n:4). C; Control sample without propolis extract(PE), PE25; added 0.25% v/v PE in brine, PE50; added 0.50% v/v PE in brine, PE1; added 1% v/v PE in brine, PE3; added 3% v/v PE in brine.

to overall unacceptability.

2.7. Statistical analyses

Normality test was done with Kolmogorov-Smirnov test and homogeneity with Levene test in all data. Results are reported as mean values ± standard deviation (m±sd). Duncan multiple comparison test ($P < 0.05$) was used to determine the significance level between groups after all data were compared with variance (ANOVA) analysis. Statistical analyses were performed by using SPSS package program version 21.0. In current research, four replications were performed.

3. Results and discussion

3.1. Physicochemical and chemical properties

As expected, there was no difference between control and propolis groups in fat, moisture, ash, salt content, and water activity (a_w) value of samples ($P > 0.05$). Table 2 shows the fat, moisture, ash, salt content, and water activity (a_w) value of the raw and production stages of hot smoked rainbow trout fillets.

Water activity (a_w) is defined as the vapor pressure of water in the hydrated substance versus to the saturated vapor pressure of pure water (Fitri et al., 2022). The a_w value was 0.991 ± 0.004 for raw fillets. After brining and smoking, a_w values were determined to be between 0.924-0.933 and 0.929–0.944, respectively. Ekonomou et al. (2022) found the a_w levels 0,965 and 0,955 in VP hot smoked rainbow trout. The variability in product ingredients, smoking conditions and production stages contribute to a_w levels of the hot smoked product, with different values reported in other works (Cakli et al., 2006; Ekonomou et al., 2022). Although there is a correlation between a_w and moisture content, there may not always be a linear relationship. A study demonstrated that the a_w values at the end of the brining and drying procedures were comparable, despite the fact that the drying process resulted in greater water loss (Andrés et al., 2005). Otherwise, glycerol is hygroscopic and absorbs water (Mortensen et al., 2017). The presence of glycerol may have explained differences in a_w values.

The fat, ash, and salt percentages increased with or without propolis due to moisture loss during hot smoking ($P < 0.05$). Various researchers have reported similar results (Bienkiewicz et al., 2019; Emir Çoban et al., 2014; Fitri et al., 2022; Goulas & Kontominas, 2005).

The changes in the pH, TVB-N, amount of free fatty acids (FFA), and TBA value of PE25, PE50, PE1, PE3, and control groups are shown in Fig. 2. The initial pH value of fresh rainbow trout was 6.48 ± 0.22. After smoking, the pH values for the C, PE25, PE50, PE1, and PE3 groups were 6.33 ± 0.14, 6.35 ± 0.15, 6.31 ± 0.08, 6.29 ± 0.16, 6.38 ± 0.22, respectively. The pH values in control and treated groups increased and decreased during storage. The observed decrease in pH might result from decomposition of muscle glycogen, chemical formation of carbonic acid, or microbiological growth in acid-producing bacteria (Ekonomou et al., 2022; Erkan et al., 2011; Fıcıcılar & Genccelep, 2017). The subsequent pH increase during the storage period might result from increased total volatile basic nitrogen compounds due to proteolytic activities by microorganisms or endogenous enzymes (Duman & Özpolat, 2015).

Total volatile base-nitrogen (TVB-N) is one of the most powerful indices of fish meat. Furthermore, it is associated with the decarboxylase activity of microorganisms during the storage period. The recommended upper limit of acceptability for rainbow trout meat is 35 mg/100 g TVB-N (Doğan & İzci, 2017; Fadıloğlu & Emir Çoban, 2018). In this study, treated groups receiving rising doses of propolis had lower TVB-N levels than the control group after 28 days of storage ($P < 0.05$). The presence of bioactive phenolics in propolis extract might inhibit the capacity of oxidative deamination in microorganisms (Ebadi et al., 2019). TVB-N values for the C, PE25, PE50, PE1, and PE3 groups were 39.4 ± 4.8, 33.1 ± 9.99, 26.3 ± 1.34, 27.4 ± 1.60, 23.1 ± 4.78 on 56th day of storage, respectively. Similar results have been reported by other researchers (Duman & Özpolat, 2015; Erkan, 2012; Fıcıcılar & Genccelep, 2017).

Free fatty acids (FFA) is the consequence of the enzymatic hydrolysis of esterified lipids. One of the reasons that accelerating oxidation is an increase in the quantity of free fatty acids in the food composition (Fıçıcılar & Genccelep, 2020). This study's results show that an amount of FFA increased during the smoking process (Fig. 2, C). Smoke drying could cause increased lipolytic enzymatic processes, which release more FFA (Fitri et al., 2022). Bienkiewicz et al. (2019) and Tenyang et al. (2018) observed same fact. The recommended acceptable limit for FFA is 1.5% (as g oleic acid) (Emir Çoban et al., 2014). However, all groups' FFA results remained below the limit throughout the storage period. According to our results on 56th day, FFA value in the samples treated by 1% and 3% PE was significantly lower than in control group ($P < 0.05$). Treated samples FFA value might be attributed to the antioxidant and antibacterial properties of PE (phenolic and flavonoid constituents of propolis), which could lead to reduce secondary lipid oxidation products like TBARS (Ebadi et al., 2019).

The TBA value is a lipid oxidation index that measures malondialdehyde (MDA) concentration. MDA is produced by hydroperoxides, which are the first results of the interaction of polyunsaturated fatty acids with oxygen (Erkan, 2012). The recommended upper acceptable limit for MDA value is 7–8 mg/kg in smoked rainbow trout (Doğan & İzci, 2017; Oğuzhan Yildiz, 2015). Initially, the PE3 group had the

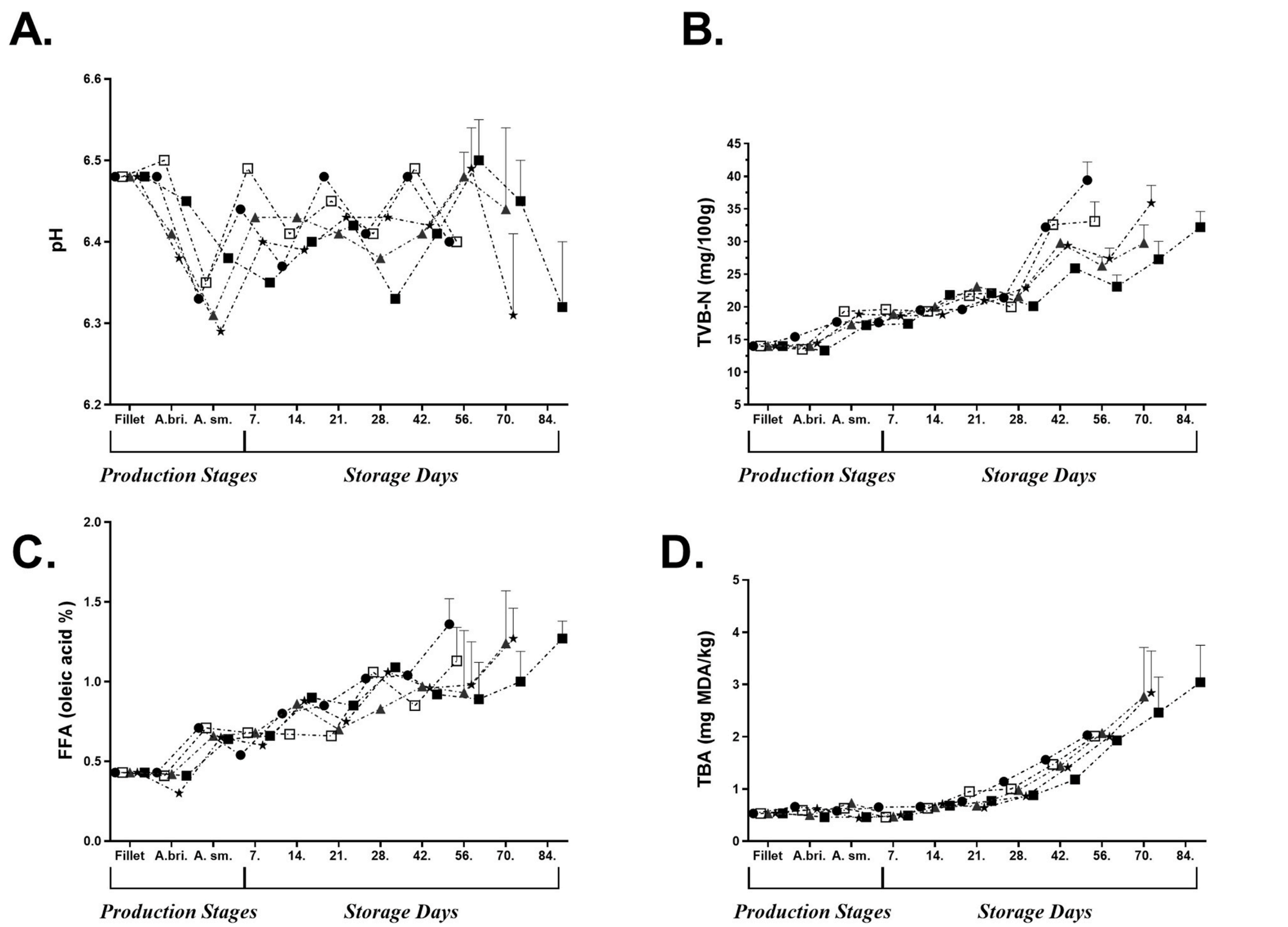

Fig. 2. Changes in pH (A), TVB-N (B), FFA (C) and TBA (D) values of vacuum packed hot smoked rainbow trout fillets in production stage and during cold storage. Results are representing mean ± standard deviation (n: 4), statistical differences $P < 0.05$. C(··●··)Control sample without propolis extract (PE), PE25(··□··); added 0.25% v/v PE in brine, PE50(··▲··); added 0.50% v/v PE in brine, PE1(··★··); added 1% v/v PE in brine, PE3(··■··); added 3% v/v PE in brine. (A.Bri.: After Brining, A.Sm.: After Smoking).

Table 3
Changes in the color properties of fresh fillet and stages of hot smoking of experimental groups.

		Control	PE25	PE50	PE1	PE3
L*	Fillet	41.82 ± 5.62	41.82 ± 5.62^{a}	41.82 ± 5.62^{a}	41.82 ± 5.62	41.82 ± 5.62^{a}
	After Brining	51.04 ± 6.79	46.38 ± 5.23^{ab}	49.87 ± 2.70^{b}	44.79 ± 3.09	51.61 ± 6.24^{b}
	After Smoking	50.60 ± 4.16^{B}	52.83 ± 3.76^{bB}	51.58 ± 4.15^{bB}	47.46 ± 5.97^{AB}	41.40 ± 4.02^{aA}
a*	Fillet	−2.43 ± 1.02^{a}	−2.43 ± 1.02^{a}	−2.43 ± 1.02^{a}	−2.43 ± 1.02^{a}	−2.43 ± 1.02^{a}
	After Brining	−5.71 ± 3.40^{aB}	−5.41 ± 3.36^{aB}	−3.97 ± 2.90^{aAB}	−2.65 ± 2.68^{aAB}	0.20 ± 0.52^{aA}
	After Smoking	6.28 ± 2.36^{b}	5.43 ± 3.13^{b}	4.90 ± 2.49^{b}	7.28 ± 0.44^{b}	5.29 ± 3.06^{b}
b*	Fillet	9.85 ± 1.03^{b}	9.85 ± 1.03^{b}	9.85 ± 1.03^{b}	9.85 ± 1.03^{b}	9.85 ± 1.03^{b}
	After Brining	1.05 ± 2.80^{aC}	0.27 ± 2.15^{aC}	4.28 ± 2.10^{aC}	14.22 ± 1.88^{aB}	19.37 ± 5.22^{aA}
	After Smoking	23.29 ± 2.00^{cB}	24.75 ± 4.83^{cB}	28.43 ± 1.32^{cAB}	28.28 ± 3.66^{cAB}	31.49 ± 2.94^{cA}

^{ABC}Different letters in the same row are significantly different ($P < 0.05$). ^{abc} The mean values with different letters in the same column are significantly different ($P < 0.05$). Results are representing mean ± standard deviation, (n: 4). C; Control sample without propolis extract(PE), PE25; added 0.25% v/v PE in brine, PE50; added 0.50% v/v PE in brine, PE1; added 1% v/v PE in brine, PE3; added 3% v/v PE in brine.

lowest MDA concentration (0.46 mg/kg), while the control group had the highest (0.58 mg/kg). The initial MDA values for VP hot smoked fillets are in agreement with Erkan (2012), and Yanar (2007). Throughout the shelf life, the MDA content increased in all groups ($P < 0.05$). However, none of the samples were above the maximum limit. A lot of research mentions that propolis can positively affect lipid oxidation (Duman & Özpolat, 2015; Pobiega et al., 2019; Yazgan et al., 2020), and propolis shows antimicrobial activity against lipolytic bacteria ($P < 0.05$). Nevertheless, no notable differences were observed between treated groups ($P > 0.05$). This can be explained by TBA reagent interacting with other molecules as well as MDA, leading to the development of secondary oxidation metabolites that do not interact with the reagent, which reacts with proteins or other compounds (Reitznerová et al., 2017; Tavares et al., 2021). In addition, glycerol shows susceptibility to oxidation (Mortensen et al., 2017). It's possible that adding more glycerol with PE helped keep the rate of oxidation similar within the control and treatment groups.

One of the first characteristics that promotes product acceptability is the color of the smoked fish, which is a strong visual indicator of quality (Fitri et al., 2022). Table 3 shows the L*(lightness), a*(redness/greenness), b*(yellowness/blueness) parameters of the raw and production stages of samples. In current study, increased doses of PE promoted greenness (a* value) in fish meat color ($P < 0.05$). Previous literature mentions that significant changes are usually detected in the b* values after drying and hot smoking. High temperatures trigger non-enzymatic browning reactions like the Maillard and protein-lipid

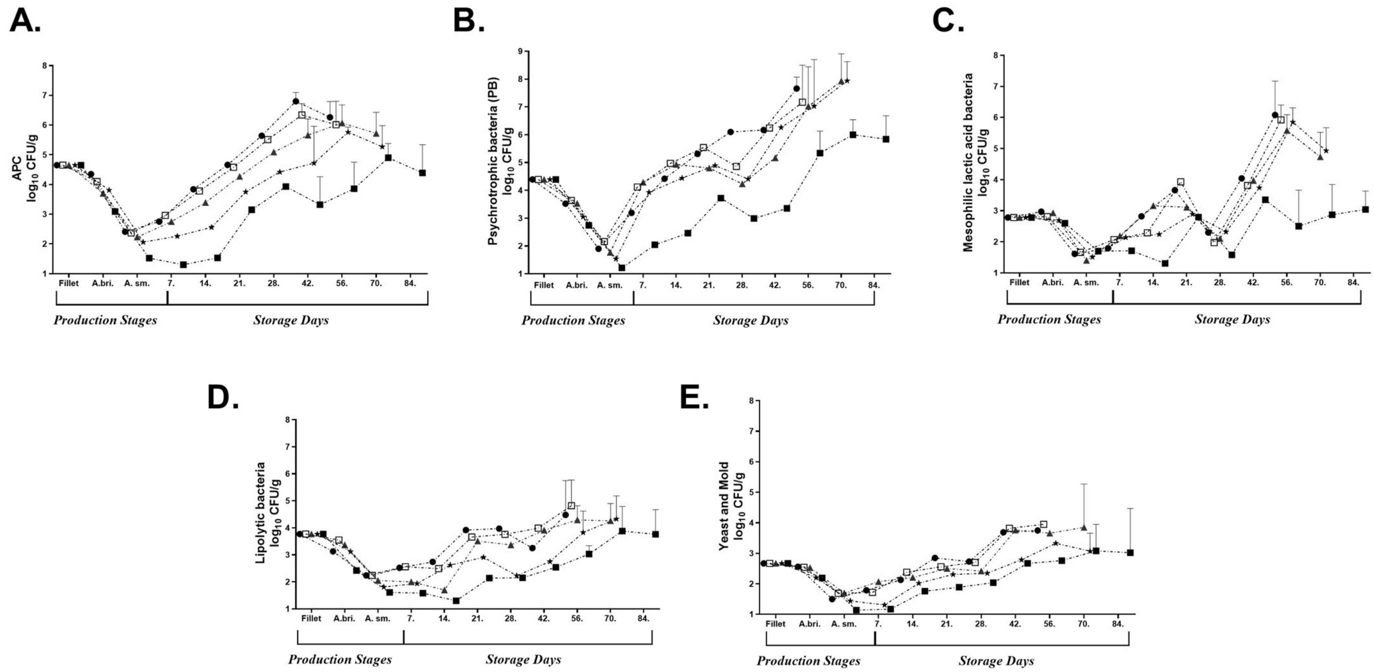

Fig. 3. Changes in APC (A), Psychotropic bacteria (PB) (B), mesophilic lactic acid bacteria (LAB) (C), Lipolytic bacteria (D) and yeast-mold (E) counts ($\log_{10}$ cfu/g) of vacuum packed hot smoked rainbow trout fillets in production stage and during cold storage. Results are representing mean ± standard deviation (n:4), statistical differences $P < 0.05$. C(··●··)Control sample without propolis extract (PE), PE25(··□··); added 0.25% v/v PE in brine, PE50(··▲··); added 0.50% v/v PE in brine, PE1(··★··); added 1% v/v PE in brine, PE3(··■··); added 3% v/v PE in brine. (A.Bri.: After Brining, A.Sm.: After Smoking).

oxidation reactions, promoting high b* value (Assogba et al., 2022; Fitri et al., 2022). In this study, the high b* value was not only contributed by the Maillard reaction but also via the surface deposition of phenolic compounds during the smoking or PE brining process (Table 3). This study shows that an increased dose of PE promoted yellowness (b* value) in smoked rainbow trout ($P < 0.05$).

3.2. Microbiological properties

Changes in Aerobic plate counts (APC), psychrotrophic bacteria (PB), mesophilic lactic acid bacteria (LAB), lipolytic bacteria, and yeast-mold of experimental groups during storage are shown in Fig. 3. Generally, microorganisms in smoked fish fillets grow during handling and filleting, reduce after smoking, and then increase again during packing, storage, and distribution.

Raw fillet APC and PB were determined to be 4.65 ± 0.26, 4.39 ± 0.70 $\log_{10}$ CFU/g, respectively, as acceptable and indicating good fish quality in reference to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986). The APC after smoking was 2.41 ± 0.58, 2.37 ± 0.48, 2.23 ± 0.20, 2.06 ± 0.21, 1.52 ± 0.47 $\log_{10}$ CFU/g while the PB was 1.90 ± 1.46, 2.16 ± 1.68, 1.77 ± 1.24, 1.54 ± 0.98, 1.21 ± 0.68 $\log_{10}$ CFU/g for the C, PE25, PE50, PE1 and PE3 groups, respectively (Fig. 3, A - Fig. 3, B). The current study agrees with those of other researchers who are investigating the shelf life of VP hot smoked rainbow trout (Cakli et al., 2006; Ekonomou et al., 2022; Emir Çoban et al., 2014; Erkan, 2012; Oğuzhan Yildiz, 2015). A dramatic reduction was observed in APC and PB levels after smoking procedure in groups because of salting, smoking, and PE application ($P < 0.05$). This result can be explained by effects of brine salt, smoke, smoking temperature and antimicrobial activity of PE (Emir Çoban et al., 2014; Yazgan et al., 2020). At the end of the storage (on 56th for C and PE25, on 70th day for PE50 and PE1, on 84th day PE3), the APC reached 6.26 ± 0.53, 6.01 ± 0.79, 6.07 ± 0.61, 5.27 ± 1.71, 4.39 ± 0.95 $\log_{10}$ CFU/g and PB reached 7.66 ± 0.41, 7.17 ± 1.33, 7.93 ± 0.68, 7.94 ± 0.69, 5.84 ± 0.84 $\log_{10}$ CFU/g respectively. PE shows antimicrobial activity against a wide spectrum of microorganisms (Pobiega et al., 2019). When examining the APC and PB results of current study, there was no significant difference between the PE50 and PE1 groups ($P > 0.05$). However, the PE3 group demonstrated significantly higher antimicrobial activity than the other groups in APC and PB ($P < 0.05$). Interestingly, the PE25 group did not affect microbiological counts in current study. This could be attributed to the dose of biologically active components in PE. According to several studies, the antibacterial activity of propolis increases with higher concentrations due to the presence of more biologically active compounds (Duman & Özpolat, 2015; Payandan et al., 2017; Yazgan et al., 2020).

Mesophilic Lactic acid bacteria (LAB) are the most common bacterial group linked with the spoilage of vacuum packed fish products. LAB were a concern as they produced characteristic acidic odors and flavors (Duman & Özpolat, 2015; Ekonomou et al., 2022). The initial LAB count in raw fillets was determined 2.78 ± 0.28 $\log_{10}$ CFU/g and, LAB counts increased in all groups throughout the shelf life ($P < 0.05$). At the end of cold storage, the LAB counts of the samples reached maximum levels of 6.08 ± 2.09, 5.92 ± 1.48, 4.73 ± 2.79, 4.93 ± 1.74 and 3.04 ± 1.59 $\log_{10}$ CFU/g for C, PE25, PE50, PE1 and PE3 samples. Duman and Özpolat (2015) applied a water extract of propolis to Shibuta fillets and found that the group that received 0.5% PE at the end of the storage had 1.26 $\log_{10}$ CFU/g less LAB than the control group. Increasing effective PE doses had an antimicrobial effect on LAB in this study ($P < 0.05$). These results are consistent with previous research that has helped explain the effects of propolis extract on LAB.

Food spoilage is associated with the proteolytic and lipolytic effects of microorganisms. Studies have indicated that the ethanolic extract of propolis inhibits the growth of proteolytic and lipolytic microorganisms (Ha El-Mossalami & Abdel-Hakeim, 2013; Pobiega et al., 2019). In this research, on the 56th day, the number of lipolytic microorganisms decreased by 0.65 $\log_{10}$ CFU/g in a 1% PE application and by 1.45 $\log_{10}$ CFU/g in a 3% PE application.

As is known, yeast and mold are common in the environment and contribute to the regular food flora. The initial yeast and mold counts of VP hot smoked rainbow trout fillets were determined 1.50 ± 0.85, 1.69 ± 0.89, 1.70 ± 0.72, 1.44 ± 0.91 and 1.14 ± 0.89 $\log_{10}$ CFU/g for C,

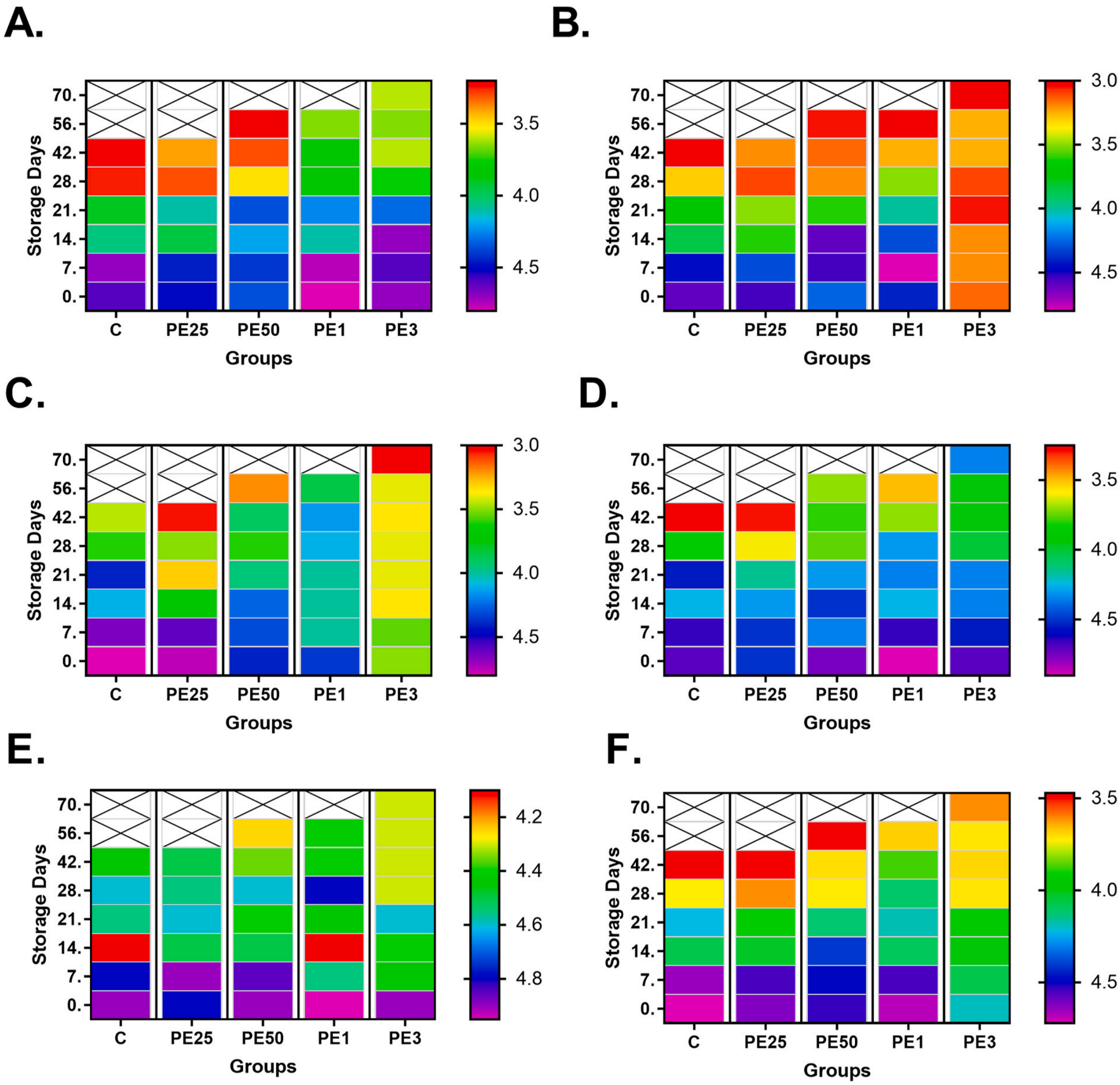

Fig. 4. Changes in score of color (A), odor (B), taste (C), appearance (D), texture (E) and overall acceptability (F) of vacuum packed hot smoked rainbow trout fillets during cold storage. Results are representing in graphically heatmap, (n:4). C; Control sample without propolis extract(PE), PE25; added 0.25% v/v PE in brine, PE50; added 0.50% v/v PE in brine, PE1; added 1% v/v PE in brine, PE3; added 3% v/v PE in brine. The cross (x) in the figure means that it has exceeded the acceptance limit. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PE25, PE50, PE1 and PE3 samples, respectively. After the 56th day, the groups reached 3.75 ± 0.79, 3.95 ± 0.50, 3.66 ± 0.53, 3.33 ± 0.32 and 2.76 ± 1.11 $\log_{10}$ CFU/g for C, PE25, PE50, PE1 and PE3 (Fig. 3, E). These results show that increasing effective doses of PE application remained desired to the antimicrobial effect on yeast and mold ($P < 0.05$). These results are consistent with most of the studies on bio-protective effects of propolis extraction (Duman & Özpolat, 2015; Ha El-Mossalami & Abdel-Hakeim, 2013).

In the current study, 0.50%, 1%, and 3% doses of PE were shown to have an effect on spoilage microorganisms ($P < 0.05$). Besides, some researchers reported that PE was shown to be effective as an antibacterial agent in preventing the growth of spoilage bacteria in fisheries products (Ebadi et al., 2019; Hassanin & El-Daly, 2013; Payandan et al., 2017). Apart from these, Ekonomou et al. (2022) reported that ın cold stored VP hot smoked rainbow trout fillets, halotolerant yeasts and LAB were the dominant microflora due to packing method and high salt content of product. Although the use of PE as an antibacterial agent prolongs the shelf life of hot-smoked vacuum-packed rainbow trout, it may not change the dominant flora responsible for spoilage. The LAB and yeast-mold data of the present study support this hypothesis. However, it still needs to be supported by another research.

3.3. Sensory properties

Fig. 4 shows the changes in, color, odor, taste, appearance, texture, and overall acceptability of VP hot smoked rainbow trout fillets during the storage period. After process, there were no samples rejected by the panellists. The highest score for overall acceptability of all groups was obtained on the first analyses day (day 0, after smoking), but the scores decreased significantly with extended shelf life in all groups ($P < 0.05$).

Firstly, there was no difference in texture between control and groups during storage ($P > 0.05$). Changes in fillet color, odor, and taste, as well as overall acceptability, occur after treatments or throughout storage. Fish with a score of less than 3.0 point were considered unacceptable due to a nasty odor, a lack of color, and overall unacceptability. The sensory analyses show that, PE application in doses over 1% had a negative effect on the odor and taste of the products ($P < 0.05$). However, it has been observed that PE1 or PE3 has a positive effect on color ($P < 0.05$). As with its microbiological and chemical properties, PE25 was not different from the control group in sensory analysis ($P > 0.05$). The difference between the PE1 group and PE50 was clearly in favor of PE1 in sensory analyses ($P < 0.05$). In addition, the lag phase of APC microorganisms in the PE1 and PE3 groups after the smoking process lasted approximately 3 weeks longer than the control group (Fig. 4, A).

In present study, the group treated with 1% and 3% PE got the highest color score throughout the storage time (Fig. 4, A). This can be explained by the distinctive golden yellow color of propolis and oak (*Quercus* sp.) wood smoke seen on fillet surfaces (Belichovska et al., 2019; Pobiega et al., 2019). It coincides with the b* values previously specified in Table 3. Also the current study, group of PE1 was the highest score in taste, odor and overall acceptability during storage time ($P < 0.05$). There are many studies indicating that high amount of PE changes the final sensory properties of the product both because of the powerful and nasty odor and taste (Ebadi et al., 2019; Irigoiti et al., 2021; Pobiega et al., 2019). This phenomenon explains why the PE3 group has lower odor and taste scores throughout storage ($P < 0.05$).

4. Conclusions

The present study demonstrates that PE application of VP hot smoked rainbow trout fillet has a significant effect on microbial growth, chemical quality, and sensory quality. In addition, 0.50% and 1% PE applications extended the shelf life of the product by about 2 weeks when compared with the control group. In particular, the 1% PE group demonstrated the best sensory properties (color, odor, and overall acceptability). The most interesting result of this study was that propolis promoted a golden yellow color in smoked fish. The 3% PE addition extends the product's shelf life by approximately 4–5 weeks. However, 3% PE may not be accepted by many consumers due to nasty odor and taste. Therefore, 1% PE application as a natural antioxidant and antibacterial may be a promising method of increasing storage quality and extending the shelf life of VP hot smoked rainbow trout.

Incorporating propolis into a food product presents an important technological hurdle due to its low solubility in water and its profound alteration of sensory properties. Therefore, propolis use demands technologically creative solutions to overcome these problems. In this study, examined the effect of propolis on hot smoked fish fillets, which have a intense aroma. The intense incense aroma can hide the undesirable effects of propolis and increase the appeal of the final product.

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Ethics approval

This research was exemption from Bingol University local ethics committee (E.3701, 03/02/2020).

Availability of data and material

Data is part of first authors Ph.D. thesis work and, is available upon request.

CRediT authorship contribution statement

Alper Güngören: Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Bahri Patir:** Supervision, Writing - review & editing. **Emine Özpolat:** Formal analysis, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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