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Influence of propolis extract on microbiological and sensory quality of rainbow trout fillets

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Abstract

The aim of this study was to evaluate the effects of propolis extract (PE), on the microbiological and sensory quality of rainbow trout fillets under chilled storage for 14 days. Fish fillets were soaked into the PE and one batch was left as untreated (control). All fillets were placed into strafor plates after treatment and covered with strech film. Microbiological and sensory properties of fillets were observed during the storage. Initially total viable count, psychrotrophic viable count, and total enterobacteriaceae cells were determined as 1.48, 2.47 and 1.60 log cfu/g, respectively. During the storage period, samples treated with PE showed lower values than the control samples. Yeast and mould was observed only in control samples, while there was no growth in samples treated with PE. Acording to the sensory evaluation, PE treated group was acceptible untill at the end of the storage period, however, control group was rejected on the 6th day of the storage. The present study showed that PE can be recommended as a natural source of preservative in order to maintenance the microbial and sensory quality of trout fillets.

Keywords: Propolis extract, rainbow trout, microbiological quality, sensory quality

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INTRODUCTION

Rainbow trout *(Oncorhynchus mykiss)* is a member of the Salmonidae family and is of high commercial importance in the world. It is commercially available for consumption, either as whole fresh fish (in ice or frozen), or as frozen fillets and stored under vacuum packing conditions (Öz, 2018). However, it is highly susceptible to both microbiological and chemical deterioration due to its high water activity, neutral pH, relatively large quantities of free amino acids, high amount of polyunsaturated fatty acids, and presence of autolytic enzymes (Ghaly et al., 2010). Thus, making the extension of its shelf life is very important. Cold storage and freezing cannot prevent the spoilage of fish alone. In recent years, there has been an increasing interest in extraction of antioxidants and antimicrobials from natural sources and their effectiveness in prolonging the shelf-life of food. Different researches have been focused on the natural additives which have protective effects on the trout quality (Özoğul et al., 2017; Öz, 2018; Öz et al., 2017; Frangos et al., 2010; Berizi et al., 2018)

Propolis is a natural resinous substance collected by *Apis mellifera* from various plant sources that is used in the hive as building material and defensive agent. It has been considered a good source of natural antioxidants and antibacterials (Bankova, 2005). Propolis has been used in folk medicine all over the world. It has various biological activities, such as antibacterial, antiviral, antitumor, anti-inflammatory, anticancer, antifungal, and antitumoral properties (Falcao et al., 2010). The chemical composition of propolis is complex and varies according to its origin. In general, propolis in nature is composed of 30% wax, 50% resin and vegetable balsam, 10% essential and aromatic oils, 5% pollens and 5% various other substances, including organic compounds and minerals (Kalogeropoulos et al., 2009; Petrova et al., 2010; Tylkowskiet al., 2010). Flavonoids (flavones, flavonols and flavonones), aromatic acids and phenolic compounds are the most important active components of propolis and responsible for the biological activities of propolis (Silici and Kutluca, 2005). Many studies have been conducted in order to determine the efficiency of propolis in many ways (Duman and Özpolat, 2015; Karakaş, 2012; Kaya vd., 2012; Ucak, 2018; Rizzolo et al., 2016; Luis-Villaroya et al., 2015).

Thus, the objective of this study was to determine the effects of propolis extract on the microbiological and sensory quality of rainbow trout fillets under chilled storage for 14 days.

MATERIAL AND METHODS

Extraction procedure

Propolis was collected from Niğde Ömer Halisdemir University Animal Production Farm, Niğde Turkey. After collection, propolis was ground into powder using laboratory blender. Ultrasound-assisted extraction of propolis was conducted in an ultrasonic bath (Kudos-HP series, China) according to method of Tabaraki et al. (2012). Propolis powder and solvent (ethanol 70 %) were blended (1:10, g:ml) in conical flash and sonicated for 60 min at ambient temperature in ultrasonic bath. After extraction, the extracts were filtered through whatman no.1 filter paper and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45 °C under vacuum.

Sample preperation

Trout (*Oncorhynchus mykiss*) fillets were commercially purchased from a local fish market in Niğde, Turkey. Fish were transported to the laboratory in ice boxes in the same day. The average weight and length of fillets were 115.54 ± 4.57 g and 23.98 ± 3.26 cm, respectively. Afterward, the fillets washed with tap water and divided into two lots. One lot was used as control without extract and the other lot was soaked into the propolis extract in rate of 10% (w/v). All samples were placed in strafor plates and covered with strech film and stored at 4 ± 1 °C during 2 weeks.

Microbiological analyses

Fish samples of 10 g were taken aseptically and homogenized in a lab blender containing 90 ml pre-chilled sterile ringer solution. Further decimal serial dilutions were prepared from this homogenate. Appropriate dilutions were used for enumeration of total psychrophilic bacteria and total viable counts by plating on Plate Count Agar (PCA) and incubated at 8 ^{o}C for 7 days and 37 ^{o}C for 24-48 h, respectively. Total enterobacteriaceae were investigated by pour plating using Violet Red Bile Agar (VRBA) after an incubation period of 24-48 h at 37 ^{o}C. Total yeast and mould were enumerated by plating on Potato Dextrose Agar (PDA, with pH 3.5) and incubated at 25 ^{o}C for 5 days.

Sensory analysis

Sensory evaluation of trout fillets was performed by a panel of eight panelists aged between 25 and 35 and had experience in evaluating seafood. The evaluations were performed in separated sensory test boxes under normal daylight and ambient temperature. The panelist used water to clean their palate between samples. The panelists were not informed about the experimental approach and the samples were blind-coded with numbers. The samples were evaluated in terms of odour, texture, color, appearance and overall acceptance on a nine-point hedonic scale (Amerina et al., 1965). A score of 9-7 indicated ‘‘very good’’, a score of 6.9-4.0 ‘‘good’’, a score of 3.9-1.0 denoted as spoiled.

pH measurement

For the determination of pH value, a pH electrode was dipped into trout homogenatas prepared with distelled water (1:1). All measurements were conducted at room temperature (24 ± 1 oC) using pH-meter (Thermo Scientific Orion 2-star, Germany).

Statistical analysis

All measurements were carried out in triplicate and analysis was conducted using the SAS software (Statistical Analysis System, Cary, NC, USA). Data were evaluated using the analysis of variance (ANOVA) and differences between means of parameters were compared using the Duncan’s test at the 5% significance level.

RESULTS AND DISCUSSION

Microbiological results

The total viable counts (TVC) of trout fillets are presented in Fig. 1. The initial TVC of trout fillets was found to be 1.94 log cfu/g. During the storage period this value increased and reached 7.38 and 7.19 log cfu/g in control and propolis extract treated samples, respectively. The average TVC of fresh shibuta samples was determined as 4.2 log cfu/g by

Duman and Özpolat, 2015. After treated the samples with propolis extract they observed a microbial shelf life extension compared with the control samples. Alparslan et al. (2014) observed the initial TVC value of rainbow trout as 4 log cfu/g. Mahmoud et al. (2004) reported the initial TVC of fresh carp as 4.6 log cfu/g. In the present study, during the storage, TVC of control samples observed significantly ($P<0.05$) higher than the group treated with propolis extract. Besides control samples exceeded the limit value (IMCFS, 1986) on day 10 of storage, while the samples treated with propolis extract reached this value on day of 12. It was reported that attributed to the antimicrobial effects of the propolis extracts and especially to its phenolic components, known to exert antimicrobial activity (Ahn et al., 2007; Campos et al., 2014; Tosi et al., 2007).

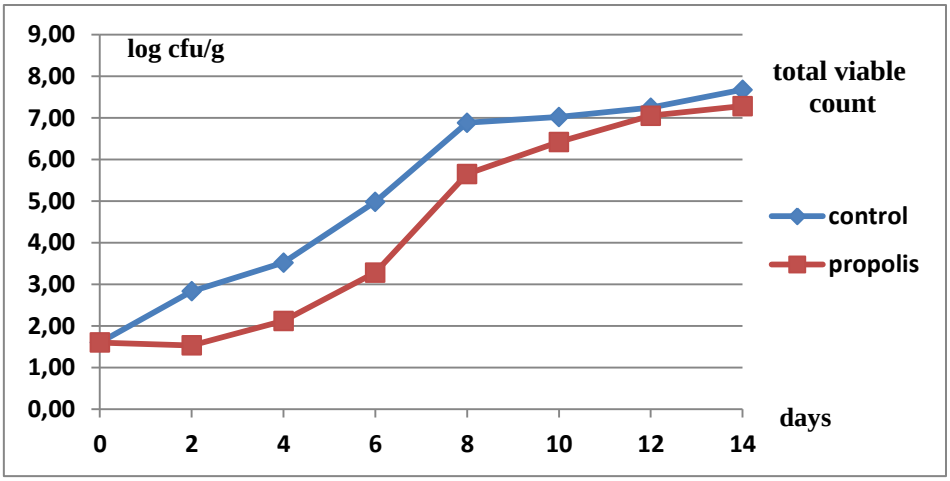

Fig. 1. Changes in total mesophilic aerobic bacteria counts of rainbow trout fillets

The psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Gram et al., 2002).Total psychrophilic bacteria counts of rainbow trout fillets are given in Fig. 2. While the initil value was determined as 2.32 log cfu/g, control group exceeded the acceptibility limit (7.0 log cfu/g, ICMSF, 1986) after 6th day of the storage period. Total psychrophilic bacteria counts of samples treated with propolis extract was still under limit value untill 12th day of the storage. At the end of the storage, total psychrophilic bacteria counts found as 7.67 and 7.28 log cfu/g in control and propolis extract treated samples, respectively ($P<0.05$). Chytiri et al. (2004) reported that bacterial spoilage in fish and fish products stored under chilled and aerobic conditions is caused by Gram--negative psychrotrophic bacteria such as *Pseudomonas, Alteromona, Shewanella* and *Flavobacterium* spp.

Duman and Özpolat (2015) determined the initial psychrotrophic bacteria counts as 3.48 log cfu/g in the fresh shibuta which is higher than the value of present study. They observed lower psychrotrophic bacteria growth in samples treated with propolis extract until at the end of the storage. In another study Özoğul et al. (2017) reported the initial psychrotrophic bacteria count of rainbow trout as 2.40 log cfu/g which is close to value determined in the present study. It has been reported that propolis possess great antimicrobial effects against Gram-positive (*Bacillus cereus, Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative (*Salmonella typhimurium, Escherichia coli* and *Pseudomonas fluorescence*) (Silici & Kutluca, 2005; Siripatrawan et al., 2013).

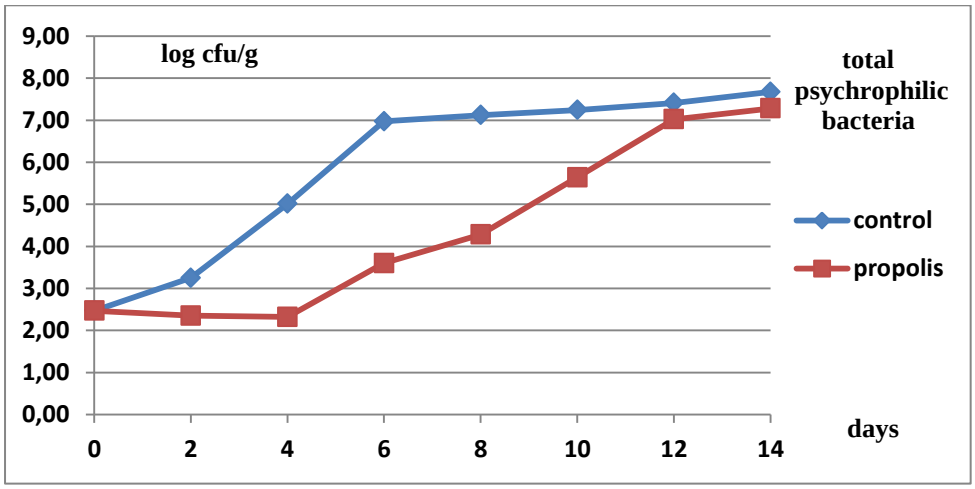

Fig. 2. Changes in total psychrophilic bacteria counts of rainbow trout fillets

Fig. 3. shows the total Enterobacteriaceae counts of rainbow trout fillets during the storage period. The initial bacteria count was determined as 1.78 log cfu/g. Enterobacteriaceae counts of the control group was significantly ($P<0.05$) higher than the samples treated with propolis extract during the storage period and detected as 5.39 log cfu/g at the end of the storage. However, this value was 3.97 log cfu/g in the samples treated with propolis extract at the end of the storage.

Enterobacteriaceae is a hygiene indicator in fish (Frangos et al., 2010; Mexis et al., 2009) and is a significant part of the spoilage microflora of trout fillets (Chytiri et al., 2004; Virta, 2009; Frangos et a., 2010). According to the results of study by Öz (2018), addition of garlic to rainbow trout diet reduced the Enterobacteriaceae counts in fish meat and kept it at a lower level during storage. In another study, Öz et al. (2017) reported the initial Enterobacteriaceae counts of rainbow trout fillets as 2.0 log cfu/g and it was observed that the addition of black cumin oil to the feed of fish reduced the number of Enterobacteriaceae. Alparslan et al. (2014) reported the total Enterobacteriaceae counts in fresh rainbow trout samples approx. 4.0 log cfu/g at the beginning the storage and 7.6 log cfu/g at the end of the storage (24 days).

Total yeast and mold was only observed in control samples. At the beginning of the storage the total yeast and mold was found as 2.56 log cfu/g and increased during the storage period. Finally reached 5.73 log cfu/g in control, while there was no growth in the samples treated with propolis extract (data not shown). Duman and Özpolat (2015) determined the initial yeast and mold counts 2.78 log cfu/g in the fresh shibuta. They reported that moulds and yeast are widely distributed in the environment and participate as the normal food flora.

According to many studies fatty acid esters, the main propolis constituents were phenolic compounds and cinnamic acid and some of them were shown to possess antibacterial activity (Greenaway et al., 1998; Kujumgiev et al., 1993)

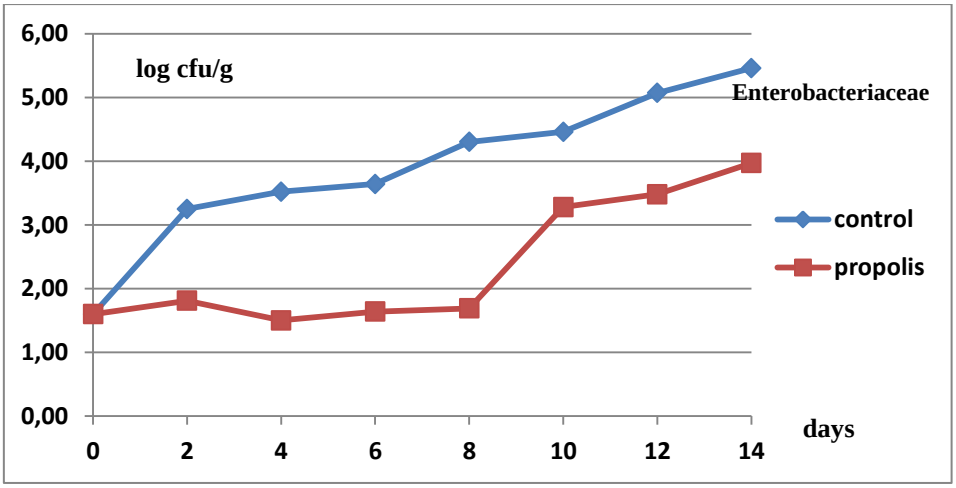

Fig. 3. Changes in total Enterobacteriaceae counts of rainbow trout fillets

Sensory results

Since the freshness is an important criterion in determining the quality of fish sensory properties of very important for the consumers (Öz et al., 2017).The results of sensory analyses of rainbow trout fillets are presented in Fig. 4-8. Throughout the storage period, sensory quality parameters of control was observed significantly ($P<0.05$) lower than the samples treated with propolis extract. The odor, texture, color and apperance properties of control sample were determined as 1.70, 1.60, 1.50 and 1.80, respectively at the end of the storage. Control group was rejected on the 6th day of the storage (3.8 overall acceptance), whereas the samples treated with propolis was still acceptable until at the end of the storage

period (5.80 overall acceptance). It was observed that the odor, texture, color and apperance properties of samples treated with propolis extract were 6.20, 5.30, 5.40 and 5.40 at the 12th day of the storage The sensory results of rainbow trout fillets were corelated with the microbiological results.

Smilar sensory results were reported by Chaillou and Nazareno (2009) who observed approximately 1-2 weeks shelf life extention with the treatment of propolis extract. In another study the shelf life of rainbow trout fillets and mince were found to be11-16 and 7-10 days at 3°C, respectively (Krizek et al., 2011). Another study reported that the shelf life of rainbow trout control group was shorter (15 days) than the samples treated with gelatine based edible films supplemented with different essential oils (20 days) (Alparslan et al. 2014).

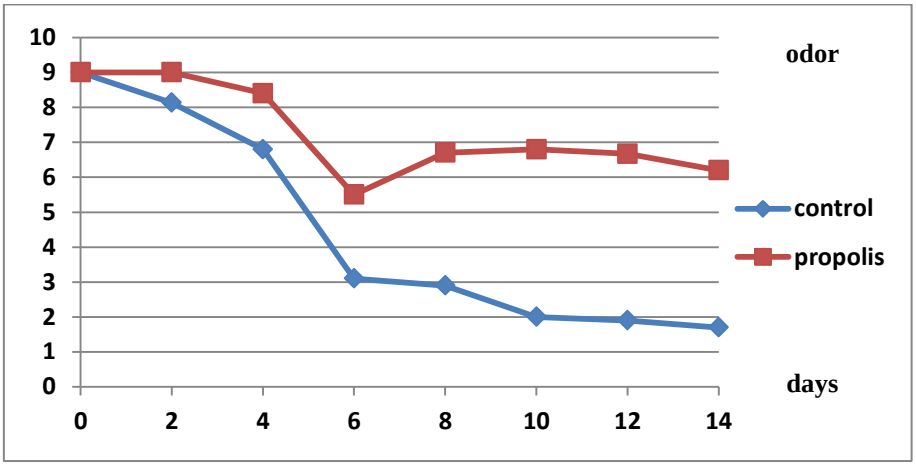

Fig. 4. Changes in odor of trout fillets

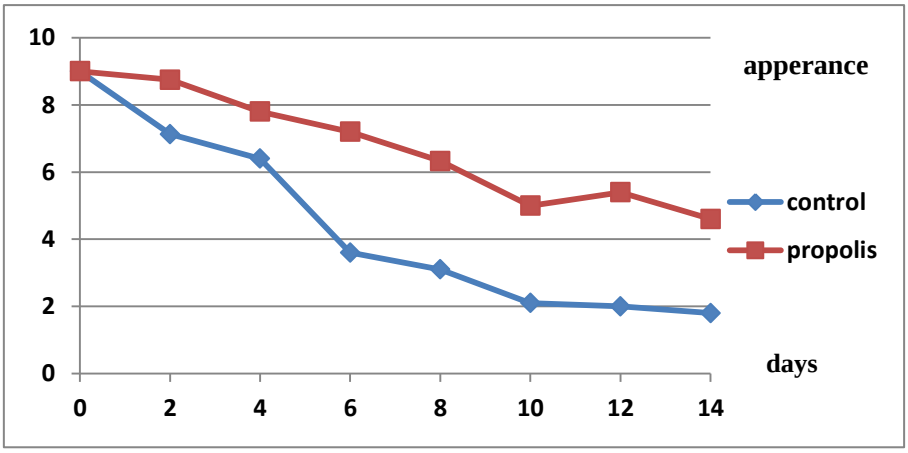

Fig. 5. Changes in apperance of trout fillets

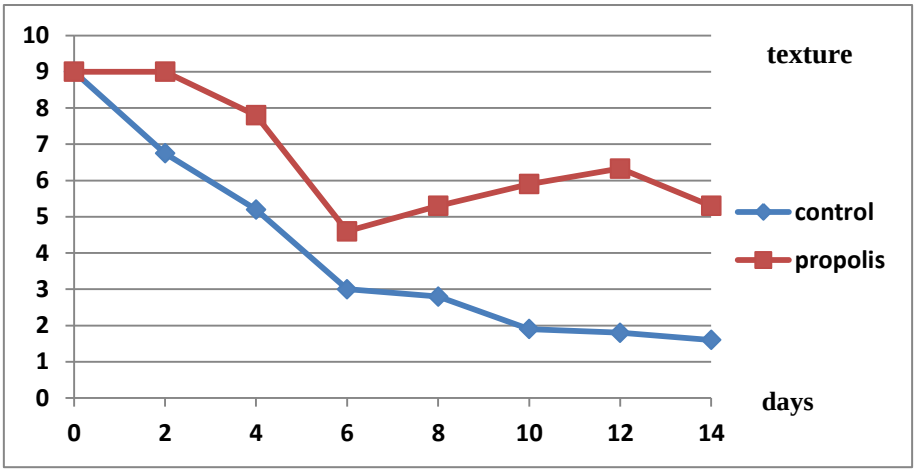

Fig. 6. Changes in texture of trout fillets

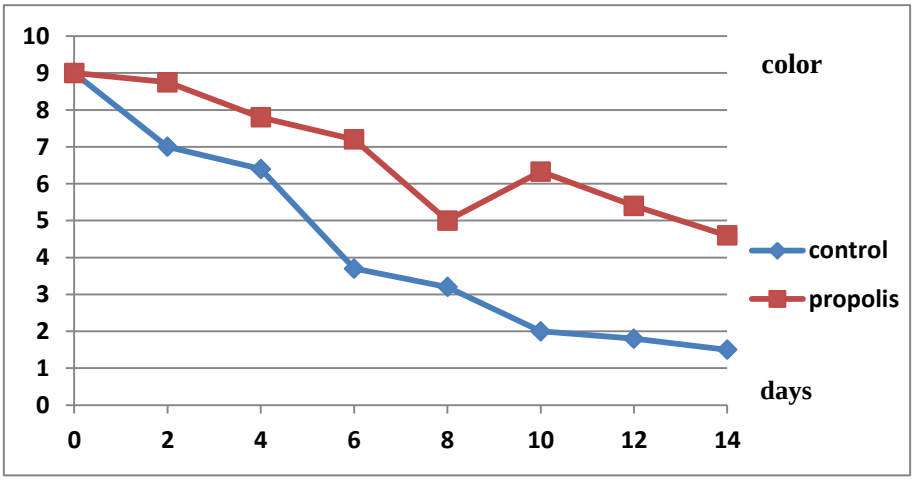

Fig. 7. Changes in color of trout fillets

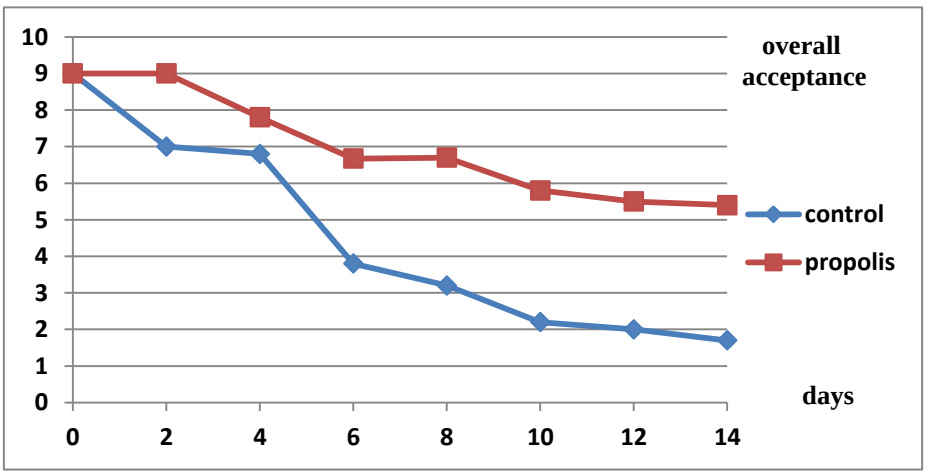

Fig. 8. Changes in overall acceptance of trout fillets

pH assesment

The effects of propolis extract on pH changes on rainbow trout fillets during storage has shown in Fig 9. At the beginning of the storage, pH value of fillets was determined as 6.57 and during the storage period this value increased in both control and propolis extract treated samples. pH value was observed higher in control group than those of samples treated with propolis extract. Finally pH values of the samples reached 6.79 and 6.67 in control and propolis extract treated samples, respectively. During the post-mortem period, pH value tends to increase because of the degradation of nitrogenous compounds (Yerlikaya et al., 2014). Baygar et al. (2012) reported that the pH value of fresh fish flesh is often between 6 and 6.5 and the upper acceptable limit for the pH of fish meat is 6.8-7.0 (Ludorf & Meyer, 1973). The initial pH value of rainbow trout samples was observed as 6.30 by Alparslan et al. (2014) and showed increase during the storage with higher values in control samples

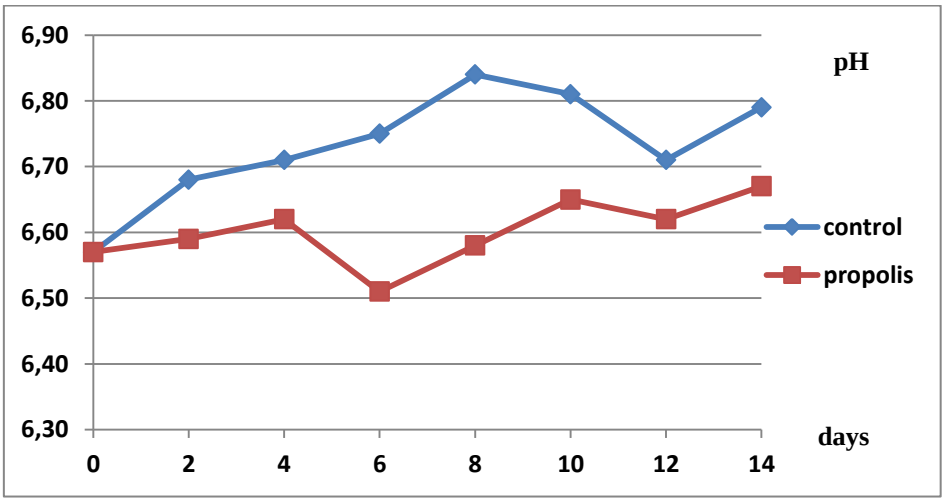

Fig. 9. Changes in pH value of trout fillets

CONCLUSIONS

According to the results of the present study, total viable counts, total psychrophilic bacteria counts and total Enterobacteriaceae counts of rainbow trout fillets treated with propolis extract were detected much more lower than the control group. Based on the sensory quality evaluation, control sample was rejected on the 6th day of the storage, while the samples treated with propolis extract still acceptable untill at the end of the storage. This study showed that propolis extract can extend the shelf life of rainbow trout fillets and can be recommended as a natural source of preservative in order to maintenance the microbial and sensory quality of rainbow trout fillets during refrigerated storage.

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