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Effects of water extract of propolis on fresh shibuta (*Barbus grypus*) fillets during chilled storage

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ABSTRACT

The present study examined the effects of water extract of propolis on the chemical, microbiological and sensory quality in vacuum-packed fresh shibuta (*Barbus grypus*) fillets during storage at 2 °C. Treatments in the study included the following: control (P0) without extract of propolis, 0.1 (P1), 0.3 (P3) and 0.5 (P5) % (v/w) the water extract of propolis, respectively. After 24 days of storage, the total volatile basic nitrogen (TVB-N) values were 57.76, 44.66, 42.23 and 36.5 mg/100 g, and total viable counts (TVC) were 8.9, 8.3, 7.96 and 6.95 log cfu/g, for water extract of propolis additions of 0.1 (P1), 0.3 (P3), 0.5 (P5) and 0 (control; P0) % (v/w), respectively. The highest acceptable TVB-N value was adopted as 30 mg/100 g, corresponding to shelf lives of 9, 15, 18 and 21 days for P0, P1, P3 and P5, respectively. Addition of 0.1% water extract of propolis extended the product's shelf-life by approximately 6 days, whereas the 0.5% water extract of propolis resulted in a significant shelf-life extension of the shibuta fillets, i.e. by approximately 12 days, according to sensory data, as compared to the control sample.

1. Introduction

Fresh fish are highly perishable products due to their biological composition. Under normal refrigerated storage conditions, the shelf life of these products is limited by enzymatic and microbiological spoilage (Ashie, Smith, Simpson, & Haard, 1996; Sivertsvik, Jeksrud, & Rosnes, 2002). Fish is preserved when the basic causes of its spoilage are controlled. The methods for preserving food are varied and, depending upon their basic approach, may be effective for either short or long periods of spoilage. Preservation of high moisture-fresh food as fish may be accomplished by low temperature, but only for a short time (Ghaly, Dave, Budge, & Brooks, 2010; Tosi, Re, Ortega, & Cazzoli, 2007).

Increasingly, consumers are demanding more natural, minimally processed products. An increasing awareness of the consumers for the use of synthetic preservatives needs research for more efficient antimicrobials with fewer side effects on human health (Tosi et al., 2007). The use of various combined preserving methods and substances is under consideration. Polyphenols from various natural sources has plants, apple skin and propolis, among others, have been reported to have a variety of biological effects, including antimicrobial activities (Aliyazıcıoglu, Sahin, Erturk, Ulusoy, & Kolayli, 2013; Gülçin, Bursal, Sehitoglu, Bilsel, & Goren, 2010).

Propolis is a natural substance, produced by honeybees from the gum of various plants and trees, is thought to be used in the beehive as a protective barrier against their enemies. Propolis usually contains a variety of chemical compounds, such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids, and amino acids, volatile aldehydes and ketones (Chaillou & Nazareno, 2009; Ghisalberti, 1979; Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008). Several authors reported that some compounds in propolis extracts could have antibacterial antioxidant activity (Basim, Basim, & Özcan, 2006; Silva, Souza, Matta, Andrade, & Vidal, 2006; Uzel et al., 2005).

The freshwater fish the shibuta (*Barbus grypus* Heckel, 1843), is a cyprinid which is found along the Euphrates and Tigris Rivers in Turkey, Syria and Iraq and is abundant and commercially important (Oymak, Dogan, & Uysal, 2008). It reaches a maximum size of 2 m and 60 kg (Zivotofsky & Amar, 2006).

Development of natural preservative with high antioxidant, antibacterial activities that prolong the shelf life of fish and fish products is desirable. The objectives of the present work were to determine the antimicrobial and antioxidant effects of propolis, as a natural preservation of shibuta fresh fillets. Chemical, microbiological and sensory analyses were done to investigate the quality changes and to determine the shelf-life of shibuta fresh fillets during storage at 2 °C.

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2. Materials and methods

2.1. Sample preparation

Shibuta (*B. grypus*) with an average weight of 6633 ± 96,090 kg were purchased at local markets and were transferred to the laboratory. Fish samples were placed in ice boxes and transferred within 1 h to the laboratory. Immediately after delivery, whole fish were filleted (150 ± 10 g each) manually. Clean fish fillets were separated into four groups. P0, control sample, without added water extract of propolis and P1, P3, P5, treated samples with water extract of propolis 0.1, 0.3 and 0.5% (v/w), respectively, under vacuum packed. Water extract of propolis (Fanus Natural Company, Trabzon, Turkey) was added on the surface of fish samples in appropriate volumes by using a micropipette, followed by mild massage (directly with the fingers) of the oil for each sample. After addition of propolis extracts, samples were vacuum packed in nylon/LDPE pouches using a Henkelman packaging machine (Boxer 42, Henkelman Ind Co., Netherland). Samples were stored in a refrigerator (2 ± 1 °C) and analysed at 3 day intervals to determine the shelf life. Experiments were conducted twice and in each study three replicate samples were analysed for each treatment.

2.2. Proximate composition

The moisture content and crude ash were determined in an oven at 103 °C and 550 °C, respectively, until the weight became constant. The total crude protein was determined by Kjeldahl's method (AOAC, 1984) and the fat content was analysed according to the procedure of Bligh and Dyer (1959).

2.3. Chemical analyses

The pH values were measured by immersing a glass-calomel electrode directly into the sample by using a pH meter (Thermo Scientific Orion 3-Star Benchtop, Cambridge, UK). The total volatile basic nitrogen (TVB-N) content was determined according to the method of Antonocopoulus (1973). The value of thiobarbituric acid (TBA) was determined according to Tarladgis, Watts, and Yonathan (1960) to evaluate the oxidation stability during storage and the results expressed as mg of malondialdehyde/kg (mg MDA/kg) fish muscle.

2.4. Microbiological analysis

A sample of fish (10 g) was diluted with 90 ml sterile 0.1% peptone water and homogenised in a Stomacher (Model 400, Seward, London, UK) at regular speed for 2 min. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptone water) of fish homogenates were spread on the surface of dry media. Total viable counts (TVC) were determined using Plate Count Agar (PCA, Merck) after incubation for 48 h at 30 °C. Plate count agar was used for psychrotrophic bacteria and incubated at 7 °C for 10 days. Lactic acid bacteria (LAB) were enumerated on MRS medium (Merck, 110660) and incubated at 37 °C for 3 days. Yeast and mould bacteria were enumerated on potato dextrose agar incubated at 22 °C for 5 days (ICMSF, 1986). Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample.

2.5. Sensory evaluation

The sensory quality of cooked shibuta fillet samples was evaluated at each sampling time by a seven member trained panel. Fillets samples were cooked individually in a microwave oven at full power (1600 W), for 2 min and immediately presented to the panellists. Fish samples were assessed on the basis of appearance, odour, taste and texture characteristics using a nine point descriptive scale. A score of 7–9 indicated "very good" quality, a score of 4.0–6.9 "good" quality, a score of 1.0–3.9 denoted as spoiled (Amerina, Pangborn, & Roessler, 1965).

2.6. Statistical analysis

All statistical analyses were analysed by one-way analysis of variance (ANOVA), using the SPSS statistical package for windows version 16.0 (SPSS Inc., Chicago, IL, USA). Statistical significance level was considered to be $P < 0.05$. All data were expressed as mean ± SD in each group.

3. Results and discussion

3.1. Proximate composition

The moisture, crude protein, lipid and crude ash contents of shibuta were found to be 74.78%, 20.42%, 3.50% and 1.3%, respectively. Similar the proximate composition were reported by Gokce et al. (2011).

3.2. Chemical analysis

Results of chemical analysis are given in Fig. 1a–c. The initial pH of the fish samples was 5.98. All samples showed an increased pH value with extended storage period (Fig. 1a). Significant statistical differences were found between the samples ($P < 0.05$). At the end of storage time, the pH values of the samples in the present study reached maximum levels of 6.42, 6.33, 6.33 and 6.3 for P0, P1, P3 and P5, respectively. The increase of pH values during the storage period may be attributed to the accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, mainly derived from microbial action (Schormüller, 1969).

TVB-N is one of the most widely used indices of seafood quality and is associated with the amino acid decarboxylase activity of microorganisms during storage (Huss, 1995). TVB-N values for shibuta fillets are shown in Fig. 1b. The initial (TVBN) values in fillet were determined as 18.41 mg/100 g flesh and increased with time of storage in all groups. Its increase is related to the activity of spoilage bacteria and endogenous enzymes because enzymes are still active. Similar TVB-N values were reported by Ali, Kassem, and Atta-Alla (2010). A level of 30 mg N/100 g TVB-N has been considered the upper limit above which fishery products are considered spoiled and unfit for human consumption (El-Marrakchi, Bennour, Bouchriti, Hamama, & Tagafait, 1990; Harpaz, Glatman, Drabkin, & Gelman, 2003). Significant statistical differences were found between the samples ($P < 0.05$). At the end of storage time, the TVB-N values of the samples in the present study reached maximum levels of 57.76, 44.66, 42.23 and 36.5 for P0, P1, P3 and P5, respectively. Samples treated with P5 had the lowest levels of TVB-N compared to the other samples. This finding could be due to the antimicrobial activity of the propolis and the reduction of the capacity of the bacteria to carry out oxidative deamination of non-protein nitrogen compounds (Fan et al., 2009). On the basis of the present data (TVC, sensory evaluation), a similar TVB-N limit value of 30.0 mg N/100 g may be proposed for the initiation of fresh shibuta spoilage.

TBA (thiobarbituric acid) index is a widely used indicator for the assessment of degree of lipid oxidation (Nishimoto, Suwetja, & Miki, 1985). Determination of TBA is based on the measurement of malondialdehyde determining the secondary oxidation products related to spoilage of fish (Al-Bandak, Tsironi, Taoukis, &

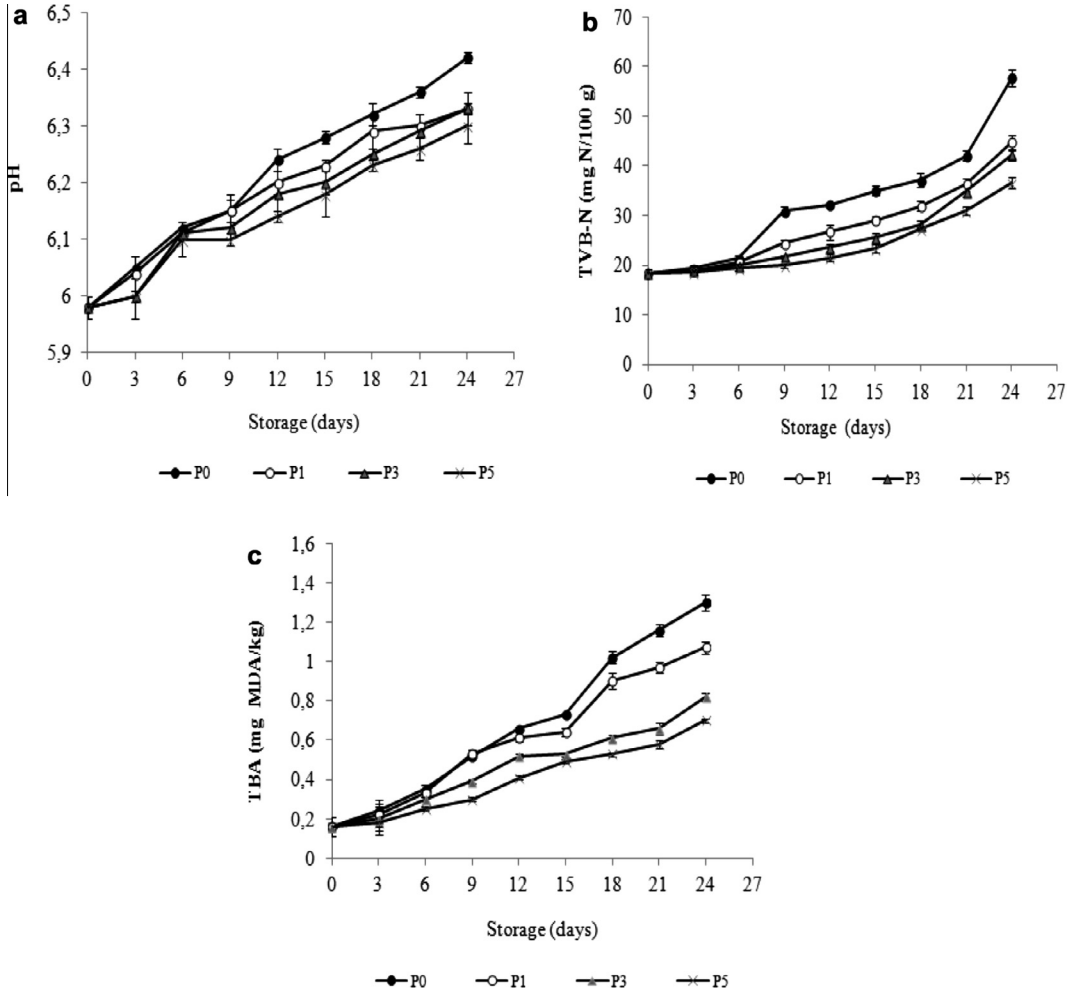

Fig. 1. Changes in pH (a), TVB-N (b) and TBA index (c) values of shibuta fillets during storage at 2 °C. P0 control, P1 plus 0.1% (v/w) water extract of propolis, P3 plus 0.03% (v/w) water extract of propolis, P5 plus 0.5% (v/w) water extract of propolis.

Oreopoulou, 2009) which are the initial products created by the reaction of polyunsaturated fatty acids with oxygen. Changes in TBA values for shibuta fillets samples are presented in Fig. 1c. In the present study, the initial TBA value of raw shibuta was 0.16 mg MDA/kg. TBA value showed a general and gradual increase with the storage time for all groups. However, the lowest TBA values were obtained from P5 samples. Significant statistical differences were found between the samples ($P < 0.05$). Similarly, these findings are in agreement with the antioxidative effect of propolis extracts reported by Hassanin and Eldaly (2013). At the end of storage time, the TBA values of the samples in the present study reached maximum levels of 1.3, 1.07, 0.82 and 0.7 for P0, P1, P3 and P5, respectively. This effect may be related to the presence of flavonoids in the propolis because flavonoids have an antioxidant effects. Generally, propolis partly inhibited hydrolytic rancidity according to control. Schormüller (1969) suggested 7 to 8 mg MDA/kg as a limit of acceptability for fish. Therefore, the parameter of TBA is not useful as a physicochemical index of quality decay or in predicting the shelf-life of shibuta samples.

3.3. Microbiological analysis

Results of microbiological analysis are given in Fig. 2a–d. Total viable counts in the fishery products are the useful tool for quality evaluation of shelf-life and post-processing contamination, while psychrotrophic bacteria are particularly the major group of microorganisms responsible for spoilage of fresh seafood (Bensid, Ucar, Bendeddouche, & Ozogul, 2014; Huss, 1995) The average TVC of fresh samples was 4.2 log cfu/g, indicates good fish quality, in agreement with results (4.6 log cfu/g) reported by Mahmoud et al. (2004) for fresh carp. P0, P1, P3 and P5 fish fillets samples exceeded the value of 7 log cfu/g for TVC, considered as the upper acceptability limit for fresh marine species (ICMSF, 1986) on days 9, 15, 18 and 24 of storage, respectively (Fig. 2a). Thus, compared with the control (P0) samples, a microbiological shelf-life extensions of 6, 9 and 15 days were achieved for P1, P3 and P5 fish samples, respectively, as determined by TVC data, 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).

The psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Gram et al., 2002). The initial the psychrotrophic bacteria of the fish samples was 3.48 log cfu/g (Fig. 2b). In control and P1 group samples psychrotrophic bacteria counts exceeded the value of 6 log cfu/g, on 15th and 18th storage day, respectively. On the other hand, in P3 and P5 group samples psychrotrophic bacteria counts exhibited a growth under the 6 log cfu/g on the 21th storage day. Statistically significant differences were found between the samples with respect to the storage

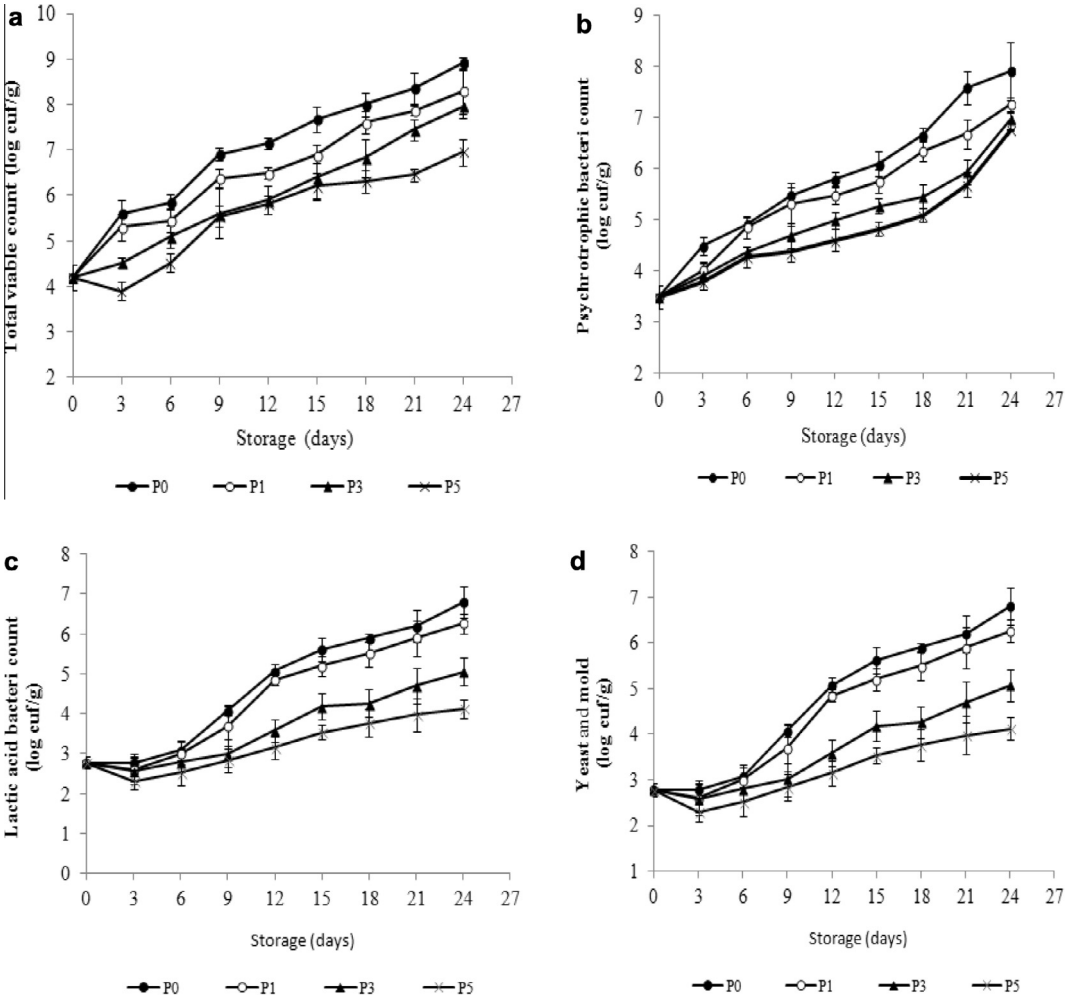

Fig. 2. Changes in total viable counts (a), psychrotrophic bacteria counts (b), lactic acid bacteria counts (c) and yeast–mould counts (d) of shibuta fillets during storage at 2 °C. P0 control, P1 plus 0.1% (v/w) water extract of propolis, P3 plus 0.03% (v/w) water extract of propolis, P5 0.5% (v/w) water extract of propolis.

duration ($P < 0.05$). The antimicrobial properties of propolis extracts have been reported in the literature (Castro & Higashi, 1995; Grange & Davey, 1990).

Lactic acid bacteria (LAB) are the major bacterial group associated with the spoilage of vacuum-packed fish products. LAB is known to produce organic acids and ethanol as typical fermentation end products (Gottschalk, 1986). The initial LAB count of the fresh samples was determined to be 3.3 log cfu/g (Fig. 2c). Lactic acid bacteria, which were in the minority at the beginning increased to 4.6, 4.51, 4.05 and 3.85 log cfu/g for P0, P1, P3 and P5, respectively, during the 9 day. At the end of storage time, the LAB counts of the samples in the present study reached maximum levels of 7.81, 7, 6.75 and 6.55 log cfu/g for P0, P1, P3 and P5 samples, respectively. Significant statistical differences were found between the samples ($P < 0.05$). These results may explain the effects of extract of propolis on LAB (Tosi et al., 2007).

Moulds and yeast are widely distributed in the environment and participate as the normal food flora. The initial yeast–mould counts of shibuta fillets were determined as 2.78 log cfu/g in all samples (Fig. 1d). After the 9 days, yeast mould counts were 4.08, 3.7, 3.01 and 2.84 log cfu/g for P0, P1, P3 and P5, respectively. Yeast–mould counts reached final counts of 6.8, 6.26, 5.06 and 4.12 log cfu/g, respectively, for P0, P1, P3 and P5 shibuta fillets samples (Fig. 1b). Interestingly, P1 and P5 fillets samples, on 24 days of storage, had significantly lower ($P < 0.05$). Similar results have been reported in other recent studies (Ali et al., 2010).

Thus, while some authors have suggested that extract of propolis have significant bacteriostatic/inhibition properties for pathogenic and spoilage micro-organisms (Burdock, 1998), the results of the present study showed that the propolis extracts used in fresh shibuta fillet leads to a reduction in microbial contamination during long storage time. This may be attributed to the presence of phenolic compounds in the extract of propolis (Gómez-Caravaca, Gómez-Romero, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2006).

3.4. Sensory analyses

Acceptability scores for odour, taste and texture of shibuta fillet samples evaluated by the panellists, decreased significantly ($P < 0.05$) with time of storage, as shown in Fig. 3a–c. A score of 4 was taken as the lower limit of acceptability equivalent to slight off odour or off taste development. The first sensory changes of fish during storage are concerned with appearance, texture and flavour of the fish.

Odour (Fig. 3a) and taste (Fig. 3b) showed a similar pattern of decreasing acceptability. The limit of acceptability of odour was reached after 12 days for the control samples, after approximately 15 (P1) 21 (P3) and 24 days (P5) for the propolis samples. The same comments hold for taste scores.

The differences observed between odour and taste scores in the control without propolis samples may be attributed to the fact that

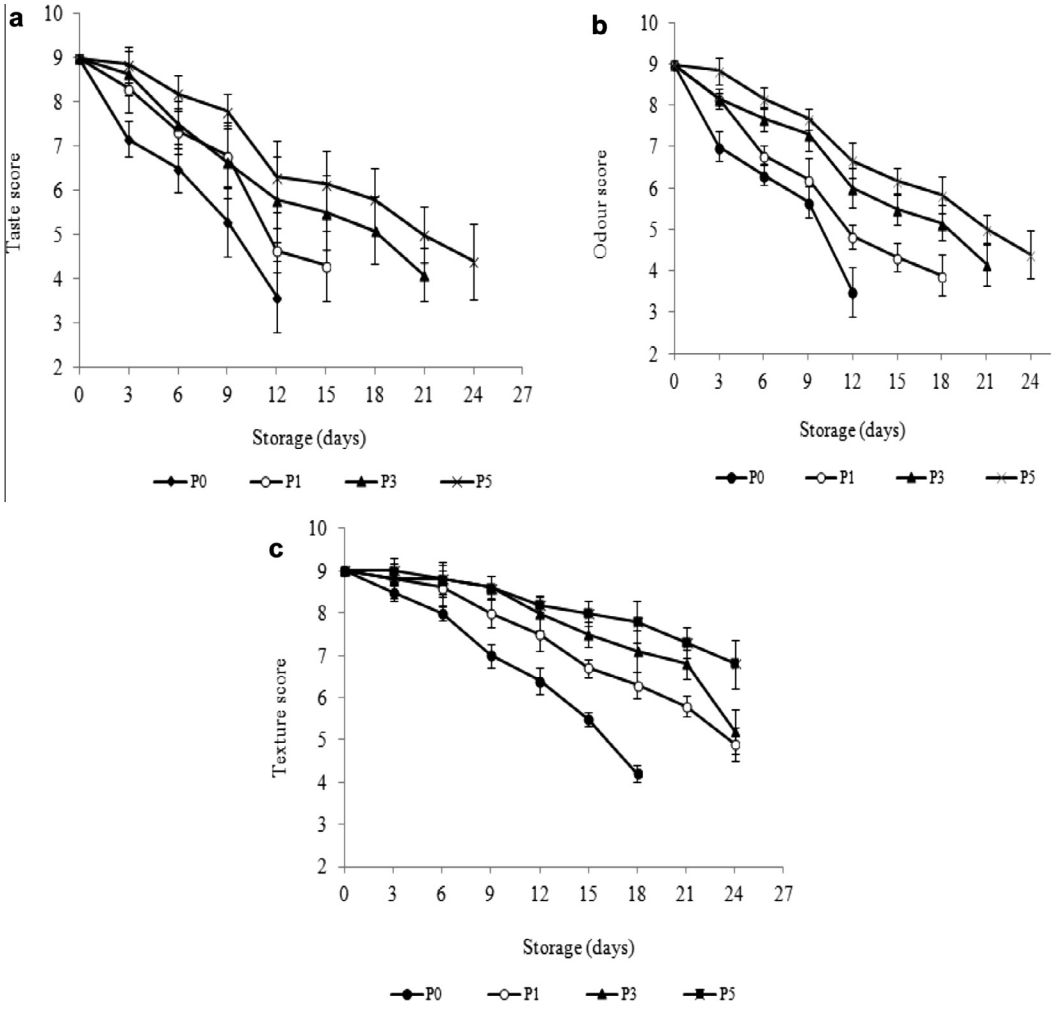

Fig. 3. Changes in taster (a), odour (b), texture (c), overall acceptance (d) of shibuta fillets during storage at 2 °C. P0 control, P1 plus 0.1% (v/w) water extract of propolis, P3 plus 0.03% (v/w) water extract of propolis, P5 plus 0.5% (v/w) water extract of propolis.

the majority of bacterial metabolic products, which contribute to the sensory deterioration, are volatile and are assessed more readily by odour. On the contrary, in the propolis extract samples volatile bacterial metabolic products were either broken down by flavonoids present in propolis. Similar results have been reported in other recent studies (Hassanin & Eldaly, 2013).

Texture scores of both control samples and extract of propolis samples decreased at slower rate than odour and taste scores. The limit of acceptability for texture was reached after 18 days for filleted shibuta samples, while this limit was never reached for control samples throughout the entire storage at 2 °C. Present organoleptic data are in good agreement with microbiological data (upper limit of total viable count of 7 log cfu/g, ICMSF, 1986). There was a significant difference ($P < 0.05$) between samples with and without extract of propolis (Fig. 3c).

These results showed that samples with high extract of propolis have acceptable overall scores, due to the limiting effect of propolis on microbiological activity and TVB-N value. P5 samples were assessed as the most acceptable products by the panellists.

The combination of vacuum and water extract of propolis resulted in a shelf life extension of approximately 1–2 weeks, attributed to the antimicrobial effects of the propolis extract phenolic components known to exert antimicrobial activity (Chaillou & Nazareno, 2009). Similar results have been reported in other recent studies (Hassanin & Eldaly, 2013).

4. Conclusion

The present experiment demonstrates that addition of water extract of propolis to vacuum-packed shibuta fillets has a profound effect on sensory quality, TVB-N value and microbiological growth. P1 and P3 samples had a shorter shelf life than P5 samples, but three had longer shelf life than the control samples without water extract of propolis. Water extract of propolis can therefore be viewed as a natural preservative for fresh fish products.

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