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Original article

Effects of laurel and myrtle extracts on the sensory, chemical and microbiological properties of vacuum-packed and refrigerated European eel (*Anguilla anguilla*) fillets

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Summary The effects of extracted natural antioxidant (laurel and myrtle) on the sensory, chemical (TVB-N, TBARS, PV, FFA and pH) and microbiological (total viable count, psychrotrophic bacteria and Enterbacteriaceae counts) properties of vacuum-packaged European eel (*Anguilla anguilla*) stored at 4 ± 1 °C were investigated. The TBARS values of myrtle were significantly lower than that of other groups. The peroxide value was low for European eel treated with myrtle and laurel extract. The FFA-free fatty acid concentration increased from 0.44 (% oleic acid) (2.03) in the eel during 24 days of storage. The values of pH showed statistically significant ($P < 0.05$) changes for all groups. The myrtle significantly reduced bacterial growth in fillets ($P < 0.05$). The microbiological limit of 7 log cfu per gram did not exceed in the treated groups. Data showed that the extracts of myrtle and laurel contain substances that inhibit oxidation of lipids and growth of bacteria in European eel, indicating the potential value of these extracts to extend the shelf life of fish.

Keywords Antimicrobial, antioxidant, European eel, laurel, myrtle.

Introduction

The presence of phenolic compounds (phenolic acids and flavonoids) in herbs and spices, along with the essential oils, has been gaining attention because of their various functions, such as antioxidant/antimicrobial capacity and flavouring properties (Lagouri & Boskou, 1995; Sacchetti *et al*., 2005; Gibis & Weiss, 2012). Consumption of food containing natural essential oils or aromatic plant extracts is expected to prevent the risk of many free radical-mediated diseases (Milan, 2006). Therefore, many scientific studies on antioxidant and antimicrobial activities of various plants have been performed (Inan *et al*., 2012).

Laurus nobilis L., belonging to the Laurel family, Lauraceae, is an aromatic plant frequently used as a spice in Mediterranean cookery and as a traditional medicine for the treatment of several infectious diseases. The major components detected in bay laurel essential oil were eucalyptol (27.2%), α-terpinyl acetate (10.2%), linalool (8.4%), methyl eugenol (5.4%), sabinene (4.0%) and carvacrol (3.2%). In addition, it exhibited strong antibacterial activity against foodborne spoilage and pathogenic bacteria (Ramos *et al*., 2012) and antifungal effects (Hassiotis & Dina, 2011; Ozcan & Al Juhaimi, 2011). Turkez & Geyikoglu (2011) also reported that laurel showed protective effects against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-mediated DNA damage. TCDD is a very toxic environmental pollutant that raises great public concern about its impact on human health.

Myrtus communis L., belonging to Myrtaceae family, is endemic in the Mediterranean area and has long been used by locals for its culinary and medicinal properties (Atzei, 2003). The main components of myrtle oil (*M. communis*) are α-pinene (36.08%), 1,8-cineole (22.63%) and limonene (15.14%) (Kiralan *et al*., 2012). Chryssavgi *et al*. (2008) researched seasonal variations in myrtle oils and found that myrtenyl acetate (23.7–39.0%), α-pinene (10.9–11.6%) and 1,8-cineol (12.7–19.6%) were the main components. It was reported that *M. communis* possessed strong antioxidant activity and high phenolic content (Chryssavgi

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et al., 2008; Tuberoso *et al*., 2010). Tumen *et al*. (2012) investigated inhibitory potential of the leaves and berries of *M. communis* L. (myrtle) against enzymes linked to neurodegenerative diseases and their antioxidant actions. They demonstrated *in vitro* neuroprotective effects of myrtle. Myrtle can be used as an affective medication in the treatment of recurrent aphthous stomatitis (RAS), one of the most common oral lesions with unknown aetiology (Mortazavi *et al*., 2012). Myrtle berries are used for food aromatisation and to prepare a typical liqueur, which has been recognised by the European Community in 2007 (Tuberoso *et al*., 2010).

In recent years, fish lipids have also assumed great nutritional significance because of their high polyunsaturated fatty acid (PUFA) levels (Ozogul & Ozogul, 2007). PUFA, particularly eicosapentaenoic acid, 20:5n3 (EPA), and docosahexaenoic acid, 22:6n3 (DHA), appear to play a key role in ontogenesis, especially in neural development and the functioning of the cardiovascular and immune systems (Lauritzen *et al*., 2001; Davis & Kris-Etherton, 2003). Lipid oxidation leads to the development of off-flavour and off-odours in edible oils and fat-containing foods, called oxidative rancidity (Hamilton, 1994; Nawar, 1996). Eel fillets are rich in PUFAs. European eel is a commercially important species because of its white flesh, flavour and high flesh yield, and the total catch was 182 tons in 2010 in Turkey (TUİK, 2013). It is also exported to European countries. Because of their high degree of unsaturation, they are less resistant to oxidation than other animal or vegetable oils (Nawar, 1996). Recently, herbs extracts have been applied to seafood products for extension of shelf life (Ozogul *et al*., 2010; Tironi *et al*., 2010; Uçak *et al*., 2011; Haghparast *et al*., 2011; Ozogul & Uçar, 2013; Li *et al*., 2012). In this research, the effects of myrtle and laurel extracts on the sensory, chemical and microbiological quality of European eel (*Anguilla anguilla*) were investigated.

Materials and methods

Plant extract preparation

Myrtle and laurel extracts were prepared from dried leaves obtained from the herbal plant (Lokman Hekim Company, Adana, Turkey). Prior to extraction with ethanol, myrtle and laurel leaves were steam-distilled for 4 h in a flask of a steam distillation unit to remove essential oils. Then, steam-distilled leaves were dried at the room temperature until completely drying. Two hundred grams of dried material was extracted with 1000 mL of 96% ethanol at 60 °C for 2 h. The extraction was repeated to recover most of the phenolic compounds. After filtration, 40 g of active carbon was added to extract. Active carbon was removed from the extract using Whatman filtration paper. Then, ethanol was evaporated in a vacuum evaporator, and the residue was stored in the dark place at −18 °C until use (Chen *et al*., 1992).

Sample preparation

Eels (*A. anguilla*) were caught in June 2010 by gill net in Mersin Bay, Turkey. They had been stored in ice for 4 h postcapture on arrival at the laboratory. The average weight and length of fish were 464.59 ± 5.85 g and 59.67 ± 4.80 cm, respectively. The fish were immediately gutted, beheaded and filleted without skin removal. After that, the fillets were washed with tap water, and then, the fillets were divided into three groups. Treated groups were immersed in a 1 L of sterile distilled water containing 10 g of sterilised myrtle or laurel extracts for 4 min. Untreated (control) and treated groups were packaged in pouches of polyamide film (Polinas, Manisa, Turkey) using a vacuum packaging machine (Reepack RV50, Seriate (BG),Via dell Artigianato, Italy). All samples were stored at 4 ± 1 °C. Sensory, chemical and microbiological analyses were performed on days 0, 4, 8, 12, 16 and 20. Data were obtained from three bags for each treatment.

Proximate composition

The proximate composition of cooked and uncooked fish fillets was determined in triplicate for protein, moisture, lipid and ash content. The crude protein content was determined by a Kjeldahl technique (AOAC, 1998). Percentage protein was calculated as% Nx6.25. Lipid content was analysed according to the procedure of Bligh & Dyer (1959). Moisture content was determined by oven-drying of 5 g of fish muscle at 105 °C until a constant weight was obtained (Method 950.46 AOAC, 1990). Ash content was determined by placing samples into a muffle furnace at 550 °C for 24 h and then weighing the remaining material (Method 938.08 AOAC, 1990).

Sensory analysis

For sensory analysis, the quality index method scheme developed by Bonilla *et al*. (2007) was used with modification. The scheme consisted of eight quality parameters (e.g. skin brightness, skin mucus, flesh texture, flesh blood, odour, colour, bright and gaping). The scheme had four simple descriptors, scoring demerit points from 0 to a maximum of 3, where 0 represented best quality and higher score, 3, indicated poorer quality (Table S1). The total sum of demerit points was 18. The panel consisted of seven regular assessors who were trained in fish quality assessment before the experiment. Triplicate samples were taken at regular intervals for sensory analysis. The panel members were

asked to state whether the fish were acceptable for the determination of shelf life of eels. Sensory evaluation for cooked fish fillets was carried out according to the method of the Paulus *et al.* (1979). Fish fillets were cooked in a microwave for 3 min (300 w) and then served to the panellists to assess. Panellists scored for colour, odour, flavour, general acceptability and texture, using a 9-point hedonic scale (1, dislike extremely; 9, like extremely).

Analytical methods

The TVB-N content of eel was determined according to the method of Antonocopoulus (1973) and expressed as mg TVB-N per 100 g of eel muscle. The value of thiobarbituric acid (TBA) reactive substances was analysed according to the method of Tarladgis *et al.* (1960) for eels' fillets to evaluate the oxidation changes during storage, and the results are expressed as TBARS value, milligrams of malondialdehyde per kg of fish flesh. Free fatty acid analysis (FFA), expressed as the percentage of oleic acid, was carried out by A.O.C.S. method (1994). Peroxide value (PV), expressed in milliequivalents of peroxide oxygen per kilogram of fat, was analysed according to A.O.C.S. method (1994). The pH of fish fillets was determined using a pH meter (315i, Germany). The sample was homogenised in distilled water in the ratio 1:10 (w/v).

Microbiological analysis

Triplicate samples were taken to estimate total viable counts (TVC) and psychrophilic viable count from each of three different groups. Fish muscle (10 g) was mixed with 90 mL of sterile Ringer solution (1/4 strength) and then stomached for 3 min. Further decimal dilutions were made, and then, 0.1 mL of each dilution was pipetted onto the surface of plate count agar (Fluka 70152, Steinheim, Switzerland) plates in triplicate. Plates were then incubated for 2 days at 30 °C and 10 days at 5 °C TVCs and psychrophilic viable count, respectively. For total Enterobacteriaceae, violet red bile agar (VRBA; Oxoid, CM0107, Hampshire, England) was used and prepared according to the manufacturer's instructions. 1-mL aliquots of the each dilution were transferred to petri dishes using the pour plate method. They were incubated for 24 h at 30 °C.

Statistical analysis

At the end of the study conducted using SPSS 13.0 package program, laurel and myrtle extract time-dependent changes between treatment groups were evaluated by Duncan's multiple comparison test (Duncan 1955). Analyses were run in triplicate for each replicate. Data were subjected to a one-way analysis of variance (ANOVA) at a 95% level of significance. Statistical significance is indicated for $P < 0.05$. The Kruskal–Wallis test and nonparametric multiple comparisons were performed to determine significant effects of storage period on sensory results.

Results and discussion

Proximate composition

Crude protein, lipid, moisture and ash contents of eels were found as 15.79%, 19.95%, 62.74% and 1.41%, respectively. Ozogul *et al.* (2005) found slightly higher protein (17.5%) and lipid (20.86%) contents for European eels. In their other study, protein and lipid contents of frozen eels were in the range of 18.08–19.64% and 19.03–24.45%, respectively. Ekanayake *et al.* (2005) reported protein (19.10%), lipid (31.50%) and moisture (56.20%) contents of fresh eels. Vishwanath *et al.* (1998) reported low level of lipid (10.74%) for fresh eel (*Monopterus albus*).

Sensory assessment

Table S2 shows the sensory scores of raw eel fillets stored at 4 ± 1 °C. Sensory score of raw eel fillets increased with an increase in storage time in all groups. This sensory assessment approach evaluates freshness by giving demerit points according to certain aspects of general appearances (i.e. skin brightness and slime, flesh texture, odour and colour). The demerit points of the control group were higher than those of treated groups. The use of natural extracts affected the shelf life of fish: 12 days for the control, 16 days for laurel and 20 days for myrtle. When the fish were rejected by the panellists, sensory scores were 10.67 for the control, 8.67 for laurel and 7.17 for myrtle. Ozogul *et al.* (2005) reported that the shelf life of iced eel and eel without ice was 12–14 days and 5–7 days, respectively. Erkan *et al.* (2011) investigated the effects of thyme and laurel essential oil treatments on the quality of bluefish during storage in ice for 13 days. According to the sensory evaluation results, the shelf life of control and treated bluefish samples stored in ice was 9 and 11 days, respectively. Kenar *et al.* (2010) also reported that the shelf life of sardine fillets was found to be 13 days for the control and 20 days for rosemary and sage groups.

Table S3 shows sensory evaluation score of cooked fish fillets. The sensory score of the cooked fillets decreased with storage time. The colour, odour, flavour and general acceptability were significantly affected by laurel and myrtle extracts. There were significant differences ($P < 0.05$) during storage period. The application of extracts to eel fillets led to an

improvement in the odour and taste of the samples. Panellists preferred samples treated with myrtle. Similar results were reported for the use of plant extracts (Kenar *et al*., 2010; Ozogul *et al*., 2010; Özyurt *et al*., 2011).

Chemical assessment

TVB-N includes the measurement of TMA, dimethylamine, ammonia and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss, 1995). Table S4 shows the changes in TVB-N content of vacuum-packed eels. TVB-N contents of all groups increased with storage time. The maximum permissible level of TVB-N in fish and fishery products is 35 mg 100 g^{-1} (EEC, 1995). The initial TVB-N value was 6.50 mg 100 g^{-1} flesh and increased to 27.36 mg TVB-N 100 g^{-1} at day 12 for the control, 20.92 mg TVB-N 100 g^{-1} at day 16 for laurel and 29.27 mg TVB-N 100 g^{-1} at day 20 for myrtle, in which all samples in vacuum pack were rejected by the sensory panellists. Ozogul *et al*. (2005) reported that the initial TVB-N value was 6.96 mg/100 g flesh for eel stored in both ice and boxes without ice. The TVB-N values were 12.4 mg TVB-N/100 g for eel stored in ice and 22.6 mg TVB-N/100 g for eel stored in boxes without ice when the eels were rejected by panellists after 15 and 8 days of storage, respectively. In this study, the TVB-N level did not exceed the maximum value for the treatment groups except the control. Significant differences ($P < 0.05$) were found in TVB-N levels after 4 days of storage among groups. The control group deteriorated more rapidly than did fish treated with natural extracts. Kenar *et al*. (2010) reported that the lowest TVB-N value was recorded for vacuum-packed sardine treated with rosemary extract and sage groups, corresponding to the value of 29.26 and 31.04 mg 100 g^{-1}, respectively. This could be due to the role of natural extracts on microbial population and the growth of bacteria as antimicrobial agent (Sacchetti *et al*., 2005; Yasin & Abou-Taleb, 2007).

The primary product of lipid oxidation is fatty acid hydroperoxide, measured as PV. Peroxides are unstable compounds, and they break down to aldehydes, ketones and alcohols that are volatile products causing off-flavour in products (Hamilton *et al*., 1997). Peroxide and TBA values are the major chemical indices to measure the degree of oxidative rancidity. In this study, the PV of oil extracted from eel fillets treated with and without antioxidants increased with storage time (Table S5). There were significant differences ($P < 0.05$) among the groups. The initial value of eel was found, 0.72 meq kg^{-1}, which was lower than 5.19 meq kg^{-1} and 6.81 meq kg^{-1} reported for iced eels by Ozogul *et al*. (2005) and for frozen eels by Ozogul *et al*. (2006), respectively. During storage period, the eels with myrtle extract showed generally low lipid oxidation compared with the control without any extract as reported for other fish species (Da Silva Afonso & Santana, 2008; Sarkardei & Howell, 2008; Kenar *et al*., 2010; Ozogul *et al*., 2010; Tironi *et al*., 2010).

Changes in TBARS values in all groups are given in Table S6. Initial TBARS values of eels were found as 0.40 mg malonaldehyde (MA) kg^{-1}. TBARS values showed fluctuation during storage time for all groups. Significant differences were observed among groups ($P < 0.05$), and the TBARS values of myrtle were significantly lower than that of other groups. This could be due to the fact that plant extracts possessed high antioxidant activity, reducing the level of oxidation. Similarly, these findings are in agreement with the antioxidative effect of natural extracts reported by Tironi *et al*. (2010), Ozogul *et al*. (2010) and Uçak *et al*. (2011). However, Kenar *et al*. (2010) reported the highest TBARS values for sage group, indicating prooxidant effect of sage tea on fish muscle.

Aryee *et al*. (2009) reported that the lipids are very prone to both lipolysis and oxidation due to high autolytic activity in fish tissue with its high PUFA content. In the present study, the release of FFA slightly increased from the initial value of 0.44 (expressed as% of oleic acid) to 2.03 for the control, 1.84 for laurel group and 1.46 for myrtle after 24 days of storage (Table S7). Significant differences were observed between groups ($P < 0.05$). FFA values of myrtle and laurel groups remained low until 12 days of storage. After that, they significantly increased until the end of storage period. FFA values of myrtle were significantly lower than that of other groups. Ozogul & Uçar (2013) reported lower FFA for chub mackerel treated with green tea, oregano and laurel groups. However, the highest FFA was found for the sage and the control groups.

The formation of lactic acid causes a decrease in the pH of the fish flesh shortly after the death of fish. During the later stages of storage, the decomposition of nitrogenous substances leads to an increase in pH value of the fish flesh (Sikorski *et al*., 1990). Postmortem pH varies from 6.0 to 7.1 depending on season, species and other factors (Simeonidou *et al*., 1998). The relatively low pH level (6.4) at the beginning of storage period reflected good state of the eels (Table S8). Ozogul *et al*. (2005) also reported initial values of 6.03 and 6.09 for iced eels and eels without ice. The values of pH showed statistically significant ($P < 0.05$) changes for all groups. There was a decrease in pH after the first day of storage, followed by an increase in pH until 24 days of storage, increasing to maximum levels of 6.43 for laurel and 6.52 for myrtle group. pH value of the control was 6.38 at the end of storage period. The increases in pH are in agreement with the findings of Erkan *et al*. (2011) for

bluefish treated with thyme and laurel essential oil and for sardine treated with rosemary extract (Ozogul *et al*., 2011).

Microbiological assessment

Figure S1 shows TVCs of all groups. The initial TVC in eels fillets was 3.92 log cfu g^{-1}, which was similar to those reported for iced ells (Ozogul *et al*., 2005). Comparison with the proposed limits (5–7 log cfu g^{-1}) for fresh fish (ICMSF, 1986) shows that ell fillets were of good quality. When fish were rejected by the sensory assessment, TVCs were 6.17 log cfu g^{-1} for the control group at day 12, 5.70 log cfu g^{-1} for laurel group at day 16 and 5.86 log cfu g^{-1} for myrtle group at day 20. Total viable counts increased with storage time for all groups (Fig. S1), and the growth of microorganisms exceeded the limit on day 20 for the control. Plant extracts have been used to preserve meat and fish products for their antioxidant and antimicrobial effects. Antimicrobial effects of nine essential oils against *Photobacterium phosphoreum*, one of the most important spoilage bacteria in fish, were investigated and found that oregano and cinnamon had strong antimicrobial effects, extending the shelf life cod fillets in modified atmosphere (Mejlholm & Dalgaard, 2002). Ozogul *et al*. (2010, 2011) reported the existence of a reduced growth of bacteria in the samples with rosemary extract in vacuum-packed sardine and frozen sardine. Similar results were found for natural extracts (Kenar *et al*., 2010; Uçak *et al*., 2011; Ozogul & Uçar, 2013). Erkan *et al*. (2011) indicated that treatment with 1% thyme and laurel essential oil was equally effective in inhibiting spoilage bacteria growth and extending the iced storage life of fish samples to 9 days compared with 13 days for treatment with essential oil.

The low initial psychrotrophic bacteria (2.87 log cfu g^{-1}) indicated very good fish quality (Fig. S2). In all groups, psychrotrophic bacteria counts did not exceed the value of 7 log cfu g^{-1}, considered as the upper acceptability limit for marine species. On the other hand, psychrotrophic bacteria counts exhibited higher growth in the control group than in other groups during storage period. When fish were unacceptable by sensory assessment, psychrotrophic bacteria were 5.83 log cfu g^{-1} for the control, 5.78 log cfu g^{-1} for laurel group and 5.47 log cfu g^{-1} for myrtle group. The use of thyme and laurel essential oil treatments reduced the growth of psychrotrophic bacteria in bluefish (Erkan *et al*., 2011). No differences were found in microbial counts as a result of the application of either rosemary or ascorbic acid during storage of gilthead sea bream (Gimènez *et al*., 2004). Natural antimicrobial compounds (thymol, lemon extract and grape fruit seed extract) to control the quality decay of a fresh fish burger were studied (Corbo *et al*., 2009). Results showed that all the active substances efficiently slowed down the growth of the spoilage microorganisms. In particular, grape fruit seed extract was the most efficient against *P. phosphoreum*, *Shewanella putrefaciens* and mesophilic bacteria, whereas thymol was the most efficient against both psychrotrophic bacteria and *Pseudomonas fluorescens*.

Figure S3 shows total *Enterbacteriaceae* counts for all groups. The initial Enterbacteriaceae count was 2.28 log cfu g^{-1}, and it increased with storage time for all groups, especially in the control group. Enterbacteriaceae counts did not exceed the limit at the end of storage period (5.93 log cfu g^{-1} for the control, 5.92 log cfu g^{-1} for laurel and 5.71 log cfu g^{-1} for myrtle). Laurel and myrtle extracts reduced the growth of bacteria in fish. Yasin & Abou-Taleb (2007) investigated the effect of marjoram and thyme on the quality of semifried mullet fish fillets during cold storage. Thyme and marjoram have strong effects against the growth of enterobacteriaceae at both concentrations (2.5 and 5%).

Conclusions

Based primarily on sensory assessment, vacuum-packed European eel reached the limits of acceptance 12 days for the control, 16 days for laurel and 20 days for myrtle. The use of natural extracts improved the sensory quality of both raw and cooked eels, most preferably eels treated with myrtle extract. Biochemical analysis showed that the use of myrtle extract in combination with vacuum pack was found to be most effective ($P < 0.05$) in controlling the rate of lipid oxidation and growth of bacteria in fish. It can be concluded that natural extracts from myrtle and laurel can be used by the food industry to extent the shelf life of seafood because they exhibited promising antioxidant and antimicrobial effects.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Total viable count in vacuum-packed eels. Data are mean values ± SD (n = 3). Vertical bars denote SD.

Figure S2. Total psychrotrophic bacteria in vacuum-packed eels. Data are mean values ± SD (n = 3). Vertical bars denote SD.

Figure S3. Total Enterbacteriaceae count in vacuum-packed eels. Data are mean values ± SD (n = 3). Vertical bars denote SD.

Table S1. Quality index method for eel fillets with skin.

Table S2. Quality index method for vacuum-packed eels.

Table S3. Sensory assessment of cooked eels during storage period.

Table S4. The changes in the level of TVB-N (mg/100 g) in vacuum-packed eels during storage.

Table S5. The changes in peroxide value of vacuum-packed eels during storage period.

Table S6. The changes in thiobarbituric acid values of vacuum-packed eels during storage period.

Table S7. The changes in free fatty acid analysis of vacuum-packed eels during storage period.

Table S8. The changes in pH value of vacuum-packed eels.