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THE EFFECTS OF DIFFERENT CONCENTRATIONS OF ROSEMARY (*ROSMARINUS OFFICINALIS*) EXTRACT ON THE SHELF LIFE OF HOT-SMOKED AND VACUUM-PACKED *LUCIOBARBUS ESOCINUS* FILLETS

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ABSTRACT

In this study, the effects of the different concentrations of rosemary (*Rosmarinus officinalis*) extract on the shelf life of hot-smoked and vacuum-packed *Luciobarbus esocinus* fillets were investigated in terms of its microbiological, chemical and sensory quality. The results showed that rosemary extract had a significant effect on lactic acid bacteria, psychrophile bacteria, yeast-mold, thiobarbituric acid and peroxide value during storage (*P* < 0.05). The shelf lives of smoked *L. esocinus* fillets to which rosemary extract was added were determined to be 42 days for 400 mL/L, 84 days for 600 mL/L and 800 mL/L brine. Also, that in the control group (without rosemary extract) was determined to be 42 days. Rosemary extract was effective in controlling the growth of bacteria and chemical indices.

PRACTICAL APPLICATIONS

Hot smoked fish stored in anaerobic conditions is very sensitive to deterioration and, based on sensory evaluation, has a limited shelf life ranging from 3 to 4 weeks at refrigerator temperature. Natural extracts have proven to be an effective preservation method for the extension of shelf life of foods. Our study has clearly shown that addition of rosemary extract in smoked fish resulted in longer shelf life, and this method could be commercially used.

INTRODUCTION

Smoking is probably the oldest known method used for preserving fish. At present, the effects of brining and smoking on color and sensory perception are at least as important as the preservative effect of modern refrigeration systems. There are three different stages of the total smoking process: brining, heating and smoking (Aminullah Bhuiyan *et al*. 1986).

Essential oils (EOs) are regarded as “natural preservatives,” as compared with chemical preservatives, and their use in foods meets the current demands of consumers for mildly processed or natural products (Nychas 1995). EOs are aromatic oily liquids obtained from plant material. Extracts from oregano, thyme, rosemary, clove, sage and mint are some of the EOs that have been used to improve sensory characteristics (taste, odor, appearance) and extend the shelf life of foods (Tsigarida *et al*. 2000). Rosemary (*Rosmarinus officinalis* L.) is considered to be one of the most important natural antioxidant and antimicrobial spice extracts (Pratt and Hudson 1990; Emir Coban and Patir 2010), and no information is yet available in the literature about the preservative effects of rosemary extract on smoked seafood.

The objective of this study was to determine the effects of the different concentrations of rosemary extract on the shelf life of smoked and vacuum-packed *Luciobarbus esocinus* fillets.

MATERIALS AND METHODS

Raw Material

*L. esocinus* (Cyprinidae) were obtained from Keban Dam Lake in Elazig located in the eastern Anatolia region of Turkey. Each *L. esocinus* was approximately 7–8 kg in weight.
Fish samples were placed in ice box and transferred within 1 h to the fish-processing laboratory of the Faculty of Fisheries of Firat University. Fish samples were washed, gutted, filleted, sliced (150 ± 10 g each) and rewashed with clean water. Sliced fish were separated into four groups: A, B, C and D.

**Rosemary Extract**

Water-soluble rosemary extract was purchased from the Kalsec (Kalsec, Inc., Kalamazoo, MI).

**Brining, Adding Rosemary Extract, Smoking and Packaging**

Group A: Control group, no extract.
Group B: 400 mL/L of rosemary extract was added in the brine solution.
Group C: 600 mL/L of rosemary extract was added in the brine solution.
Group D: 800 mL/L of rosemary extract was added in the brine solution.

All groups were smoked at 70 ± 1C by using a block of oak. The samples were immersed in brine at a ratio of 1:1 (w/w) at 2C for 10 h. The brine contained 4% NaCl. After brining and smoking, the fillets were vacuum-packed in high-barrier nylon polyethylene bags and stored at 4 ± 1C until analysis on days 0, 7, 14, 21, 28, 42, 56 and 84. Three replications were performed.

**Microbiological Analysis**

A portion of 25 g was taken aseptically from each sample and was transferred to a stomacher bag (Seward, West Sussex, U.K.) containing 225 mL of sterilized peptone water (Buffer Peptone Water, Merck KgaA, Darmstadt, Germany). The mixture was homogenized for 2 min with a stomacher. Samples (0.1 mL) of serial dilutions of smoked fish homogenates were spread on the surface of the appropriate dry media in Petri dishes for determination of the total mesophilic anaerobe on brewer anaerobe agar and then incubated at 30C for 3 days. Lactic acid bacteria (LAB) were counted on de Man Rogosa Sharpe agar and incubated at 30C for 5 days. Psychrophile was detected on plate count agar after incubation at 7C for 10 days. Yeast and mold bacteria were enumerated on potato dextrose agar incubated at 22C for 5 days. For Enterobacteriaceae, samples were inoculated with violet red bile glucose agar and incubated at 37C for 24 h.

Microbiological data were transformed into logarithms of the number of colony-forming units (cfu/g).

**Chemical Analysis**

The thiobarbituric acid (TBA) was determined by a selective third-order derivative spectrophotometric method (Bot-soglou et al. 1994). TBA content was expressed in mg of malondialdehyde (MDA)/kg smoked fillets. The peroxide value (PV) was performed using the Wheeler method (Varlik et al. 1993). The moisture, lipid composition and NaCl content of the samples were measured using standard methods, following AOAC (2002) protocols.

**Sensory Evaluation**

Five experienced panelists, academic staff who were trained in sensory descriptors for smoked fishes, were employed to evaluate the quality of *L. esocinus* fillets during storage. The panelists were asked to evaluate the overall acceptability of the appearance, taste and odor of the samples on a 5-point hedonic scale, ranging from very poor (1) to very good (5). All samples were stored at +4C until sensory analysis was performed.

**Statistical Analysis**

The experiment was replicated thrice on different occasions with different smoked fillet samples. Triplicate samples were made per attempt. Data from each replication were averaged and log transformed (cfu/g). These data were subjected to analysis of variance using sas (SAS Institute Inc., Cary, NC). Means and standard deviations were calculated, and when F values were significant at the P < 0.05 level, mean differences were separated with the least significant differences procedure.

**RESULTS AND DISCUSSION**

**Microbiological Changes during Storage**

In vacuum-packed smoked seafood, LAB is a problem as they produce typically sour odors and flavors (Leroi et al. 1998). While the LAB number in the fillets was determined to be 4.88 log10 cfu/g, a value of 2.75–3.89 log10 cfu/g was detected after smoking. It was ascertained that the statistical difference between the control group and experimental groups was significant (P < 0.05) during storage (Fig. 1a). The most effective concentration of rosemary extract was determined to be 800 mL/L brine. The LAB number in group D was 2.39 log10 cfu/g on day 84. Zaija et al. (2006) found that rosemary, marjoram, sage and thyme caused an inhibitor effect on the LAB number. Viuda-Martos et al. (2008) stated in their study, in which they sought antimicrobial effects of different EOs, that rosemary had the greatest effect on the LAB, while sage, thyme and clove, respectively, follow it. Our findings are compatible with other studies.

The number of psychrophilic bacteria detected was 4.70 log10 cfu/g per fillet. After the brining process, the number of bacteria was between 1.81 and 4.40 log10 cfu/g. After day 28 of storage, an increase in the number of psychrophilic bacteria was detected. Similar findings were reported by other
researchers (Deng et al. 1974; Schulze 1985; Emir Coban 2010). After day 21 of storage, the difference between the groups to which rosemary oil was added and the control groups was significant ($P < 0.05$; Fig. 1b).

The number of yeast-mold was determined as 2.70 log$_{10}$ cfu/g per fillet, 2.15 log$_{10}$ cfu/g after the brining process, and between 1.26 and 1.89 log$_{10}$ cfu/g after smoking (on day 0). This decline in the number of yeast and mold, following the smoking, is similar to the findings of Dondero et al. (2004). The difference between the groups with rosemary and the control group was significant during storage ($P < 0.05$; Fig. 1c).

The total number of mesophilic anaerobic bacteria, which was 0.95 log cfu/g per fillet, was 0.95 log$_{10}$ cfu/g in all groups after the smoking process (Fig. 1d). Dondero et al. (2004) argued that an increase in the total number of anaerobic bacteria in cold-smoked and vacuum-packed salmons is associated with the storage temperature. Researchers determined that the number of bacteria in each sample was 5.28 log$_{10}$ cfu/g at the beginning, 7.60 log$_{10}$ cfu/g in samples preserved at +4C or 8.77 log$_{10}$ cfu/g in samples preserved at +8C, after 26 days. These results were higher than the findings we obtained. The difference stems from the smoking temperature. The difference among groups could not be ascertained with regard to the total mesophilic anaerobe bacteria ($P > 0.05$). These results show that the addition of rosemary extract had no effect on the total mesophilic anaerobe bacteria of smoked $L$. esocinus.

Enterobacteriaceae, being psychrotolerant, are capable of growing at refrigeration temperatures; however, they cannot
compete well with other gram-negative spoilers (ICMSF 1998). The number of Enterobacteriaceae was 0.95 log cfu/g in all groups during storage (Fig. 1e). No difference was ascertained between the control group and groups to which rosemary extract was applied ($P > 0.05$).

**Chemical Changes during Storage**

The changes in the amounts of TBA, PV, lipid, salt and moisture during storage of the control group of smoked fish fillets, to which rosemary oil in different concentrations was applied, were presented in Fig. 2.

The consumability limit value of the TBA content was between 7 and 8 mg MDA/kg (Sinnuber and Yu 1958). The amount of TBA was 0.59 mg MDA/kg in the fillets. TBA values increased in all groups during storage. The difference between the control group and those to which rosemary was added was significant ($P < 0.05$). It is known that rosemary has an inhibitor effect on the TBA values (Shahidi et al. 1995; Serdaroglu and Felekoglu 2005). The TBA value was lower than consumability limits in all groups during storage. Storage at low temperature after vacuum packing, using brine method, EOs and antioxidant compounds in smoke, has been reported in many studies (Vasiliadou et al. 2005).

The PV was 1.08 meq O$_2$/kg in the fillets. Increases in PV were identified in all groups, as was the progression during storage ($P < 0.05$). The difference between the control group and those smoked with rosemary was significant ($P < 0.05$). In particular, the increase in the PV value in the group to which 800 mL/L brine rosemary extract was added was lower.
than the other groups ($P < 0.05$). PV is essential in determining the degree of lipid oxidation and rosemary extract displays a significant influence on PV (Perez-Mateos et al. 2006; Sarkardei and Howell 2008; Quitral et al. 2009). The antioxidant attributes of rosemary extract stem from the carnosol, carnosic acid and rosmarinic acid available in its structure (Richheimer et al. 1996).

Lipid content, which was 6.23% in $L. esocinus$ fillet, was between 16.01 and 16.42% after smoking. Significant increases were detected in the lipid content of smoked fishes as a result of dehydration during the brining and smoking processes (Jittinandana et al. 2002; Vasiliadou et al. 2005).

In this research, while the amount of salt in $L. esocinus$ fillets was 0.44%, this value was 2.84–3.41% after smoking ($P < 0.05$). No difference was detected among the groups during storage ($P > 0.05$). Similar findings were reported by other researchers (Kolodziejska et al. 2002).

The moisture content of raw fillets was 68.01%, and this value was ascertained to be between 45.46 and 47.20% after smoking ($P < 0.05$). Rosemary extract had no considerable effect on the moisture ratio during storage after smoking ($P > 0.05$).

**Sensory Analysis during Storage**

Figure 3 shows the changes in sensory analysis scores during storage time. Sensory scores of each sample were at “good quality” after processing. According to the statistical analysis, there were no significant differences ($P > 0.05$) in the appearance of all groups during storage. The taste of the control and group B were scored as “spoiled” by the panelists after the 42nd day, while groups C and D continued to be scored as “good quality.” The flavor and taste of rosemary extract were much stronger in group D than in groups B and C, groups B and C were mostly preferred by the panelists. The use of rosemary extract improved the sensory quality of smoked $L. esocinus$ fillets.

In this research, while the shelf life of smoked $L. esocinus$ (control group) without rosemary extract and group B were determined to be 42 days, that of smoked fillets treated with 600 and 800 mL/L rosemary extract were determined to be 84 days. These results show that rosemary extract have a positive effect on the shelf life of smoked product. Similar results have been reported in other recent studies (Emir Coban 2010; Ucak et al. 2011).

**CONCLUSION**

The results of the study showed that addition of rosemary extract in smoked fish resulted in longer shelf life compared with control. Groups containing rosemary extract were preferred by the panelists. Rosemary extract was effective in controlling the microbiological growth (LAB, psychrophile...
bacteria and yeast-mold) and chemical indices (TBA value, PV).

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