Protective effect of essential oils on the shelf life of smoked and vacuum packed rainbow trout (*Oncorhynchus mykiss* W.1792) fillets

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Abstract This study was investigated the effects of some oils on chemical, microbiological and sensory quality in vacuum packed smoked rainbow trout (*Oncorhynchus mykiss* W.1792) fillets. Acceptability scores for appearance, taste and odour of untreated and treated smoked trout decreased with storage time. The limit of sensory acceptance was reached after 56 days for the untreated samples, after 84 days for with rosemary and thyme oil-treated samples after 98 days for with sage oil-treated and after 112 days for with clove oil-treated samples. Significant differences were not found between groups as microbiological ($p>0.05$). However, significant differences were found both among groups and during the storage in term of TBA (thiobarbituric acid) and PV (peroxide value), FFA (free fatty acid) values ($p<0.05$). Essential oils as natural antioxidant can be used in conjunction with vacuum packed to enhance hot smoked fish quality.

Keywords Essential oils · Smoked fish · *Oncorhynchus mykiss* · Shelf-life · Antioxidant activity

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a fish belonging to the Salmonidae family, a species with high commercial value and much appreciated by European consumers (Cakli et al. 2006). It is sold as either whole fresh fish or in fillet form. Additionally, trout fillets in the form of smoked and vacuum packaged products are being exported to various northern European countries and consumed with no further heating

Smoking is a traditional preserving method used for both fish and meat products around the world (Doe 1998; Koral et al. 2010). Smoked fish has characteristic flavour and colour. Hot smoked fish, usually stored at refrigerator temperature, is very sensitive to deterioration and, based on sensory evaluation, has a limited shelf life often 3–4 weeks. Improvements in the shelf life of hot smoked fish product can have an important economic feedback by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets (Erkan 2011)

Essential oils has proven to be an effective preservation method for the extension of shelf life of fresh fish (Harpaz et al. 2003; Giatrakou et al. 2008; Quitral et al. 2009). Essential oils (EOs) are aromatic oily liquids obtained from plant material. Extracts from oregano, thyme, rosemary, clove, sage and mint are some of the EO that have been used to improve the sensory characteristics and extend the shelf-life of foods (Tsigarida et al. 2000; Burt 2004; Hernández-Ochoa et al. 2011; Makhal et al. 2012). Rosemary, thyme, sage and clove are considered to be the most important of the natural antioxidant and antimicrobial spice extracts (Pratt and Hudson 1990; Emir Coban and Ozpolat 2012; Karakaya et al. 2012).

The objectives of the present work were to determine the effect of the treatment with some essential oils (Rosemary,

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Thyme, Sage and Clove), as a natural preservatives, this effect on smoked rainbow trout fillets by assessing some microbiological, chemical and sensorial parameters.

Materials and methods

Raw material

Essential oils solvable in water were purchased from the Kalsec (Kalsec®, Inc, Kalamazoo). One hundred percent oil of rosemary (Herbalox® Seasoning), thyme (Aquaresin® Thyme Code: 35-06-39) sage (Aquaresin® Sage Code:07-06-39) and clove (Aquaresin® Clove Code:05-03-39) were used.

A total of 225 rainbow trout (*Oncorhynchus mykiss* W.1792), each weighting 300 ± 10 g were obtained from Keban Alabalık Co. aquaculture farm located in Keban Dam Lake, geographically located at Elazığ in the eastern Anatolia region of Turkey. Fishes were transferred within 1 h to the fish processing the laboratory of the Faculty of Fisheries of Firat University, in sealed foam boxes containing ice. They were eviscerated and washed with clean water. Fillets were separated into five groups.

Brining and addition essential oils

Essential oils were added in the brine solution. The samples were immersed in brine at a ratio of 1:1 (w/w) for 4 h at 4 °C. The brine contained 5 % NaCl.

untreated : control group

RO: 600 ppm rosemary oil was added in the brine solution

TO: 600 ppm thyme oil was added in the brine solution

SO: 600 ppm sage oil was added in the brine solution

CO: 600 ppm clove oil was added in the brine solution

Smoking and packaging process

Smoke was produced from block oak with combustion. All groups were smoked at 75 ± 3 °C. After cooling (at 10 °C for 1 h), the smoked products were vacuum packed (high barrier nylon polyethylene bags) and stored at 4 ± 1 °C until analysis on days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98 and 112.

Microbiological analyses

A sample of 25 g was taken aseptically from each samples, transferred to a stomacher bag and 225 ml of sterilized peptone water (Buffer Peptone Water, LAB M) were added, and the mixture was homogenized for

2 min with a stomacher (Stomacher 400, Lab. Blender, London, UK). Samples (0.1 ml) of serial dilutions of smoked trout 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 (Merck 1.05410), and incubated at 30 °C for 3 days. Psychrophile was determined on Plate Count Agar (PCA, Merck 1.05463) after incubation at 7 °C for 10 days. *Lactobacillus* spp. were enumerated on de Man Rogosa Sharpe agar (MRS, Oxoid, CM361) incubated at 30 °C for 5 days. Lactic *Streptococcus* spp. was determined on M17 agar (Merck 1.15108) after incubation at 37 °C for 2 days. Yeast and mold bacteria were enumerated on Potato Dextrose Agar (PDA, Merck 1.10130) incubated at 22 °C for 5 days. For lipolytic bacteria was inoculated Tributyrin agar (Merck 1.01957) incubation at 30 °C for 3 days. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g).

Chemical analyses

pH, lipid and NaCl content of fish samples were measured by standard methods, following AOAC protocols (2000). TBA was determined by a selective third-order derivative spectrophotometric method (Botsoglou et al. 1994). TBA content was expressed as mg of malondialdehyde (MDA)/kg smoked trout fillets. PV was performed using the method Wheeler (Varlik et al. 1993). The amount of FFA was calculated as oleic acid % (Yetim 2002).

Sensory analysis

Five experienced panellists (3 female and 2 male, age 28–50), who were members of academic staff and trained in sensory descriptors for smoked trout, were used to evaluate the quality of rainbow trout fillets during storage. Sensory analysis was performed using the methods of Kurtcan and Gonul (1987). Panelists were asked to evaluate sample appearance, taste and odour overall acceptability on a 5-point hedonic scale ranging from very poor (1) to very good (5).

Statistical analysis

All analytical determinations were on days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98 and 112. Experiments were replicated twice on different occasions with different fish samples. The mean each sample for each group was analysed three times. Data were subjected to analysis of variance. The tukey's honestly significant difference procedure was used to test for differences between means ($p < 0.05$) (Sümbüloğlu and Sümbüloğlu 2002).

Results and discussion

Microbiological changes during storage

The changes in the microflora (total mesophilic anaerobe bacteria, psychrophile bacteria, lactic *Streptococcus* spp., *Lactobacillus* spp., lipolytic, yeast and mold) of A, RO, TO, SO and CO groups are shown in Fig. 1. TMAB count in the fillet was detected to be $6.83 \pm 1.21 \log_{10}$ cfu/g, PB was $8.47 \pm 1.18 \log_{10}$ cfu/g, *Lactobacillus* spp. was $6.60 \pm 0.69 \log_{10}$ cfu/g, lactic *Streptococcus* spp. was $6.91 \pm 1.38 \log_{10}$ cfu/g, lipolytic was $6.60 \pm 0.69 \log_{10}$ cfu/g, and yeast-mold was $3.96 \pm 0.29 \log_{10}$ cfu/g. A decrease was observed in these microorganism levels after salting and smoking

(Fig. 1). The decrease was statistically significant ($p < 0.05$). This result can be explained by the effects of salt and smoking temperature.

Microorganism count all groups increased during storage. Increases in treated groups were less than the control group. These results proved the inhibitor effect of essential oils on microorganisms. However, the difference between the control group and treated groups were not statistically significant ($p > 0.05$). Viuda-Martos et al. (2008), reported that rosemary oil was effective on LAB; Ahmed and Abd El-Rahman (2002) reported the impact of sage on lipolytic microorganisms; whereas Rasooli and Owlia (2005) reported that thyme was effective on yeast-mold. This difference in our findings can be

Fig. 1 Changes microbial counts ($\log_{10}$ CFU/g) in production phases/smoked rainbow trout fillets during storage at 4 °C. A.Sa After salting, A.Sm After smoking

explained by the differences in application, form and concentrations of essential oils.

Chemical changes during storage

Figure 2 show the pH, lipid, salt, TBA, PV and FFA values of raw and smoked rainbow trout. The lipid and salt data for raw rainbow trout agreed with those reported by other researchers (Kolsarici and Ozkaya 1998). The percentages of total lipid, salt and pH of hot smoked rainbow trout increased due to moisture loss during processing. Similar findings were reported by Goulas and Kontominas 2005 for smoked rainbow trout.

Fig. 2 Chemical changes in production phases/smoked rainbow trout fillets during storage at 4 °C *A.Sa* After salting, *A.Sm* After smoking

have been reported by Cakli et al. (2006). TBA index value of control group increased during storage. Similar results were determined in RO, TO, SO and CO groups. Lower production of TBA index values SO and CO groups may be due to the antioxidant properties of sage oil and clove oil (Toda et al. 1994; Fasseas et al. 2007). The decrease in TBA index values may represent the breakdown of the molon-dialdehyde to tertiary degradation. After the 14th day, the differences between the control group and treated groups were found to be statistically significant ($p<0.05$). Yu et al. (2002), Quitral et al. (2009) and Erkan and Bilen (2010) observed that a rosemary, thyme and sage oil was an effective means of controlling lipid oxidation in fish meat, as reflected in thiobarbituric acid reactive substance values.

Shelf life of oil fish species is limited due to the oxidation of lipid. 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 (Ucak et al. 2011). PV value in the fillet was detected to be 1.17 ± 0.19 (millimole O_2/kg). After smoking PV for hot smoked fillets were 1.17, 1.27, 1.22, 0.90 and 1.04 millimole O_2/kg for the control, RO, TO, SO and CO respectively (Fig. 2). During storage period, increase in PV was observed for all groups. Significantly higher PV ($p<0.05$) was obtained from the control group (A). No significant differences ($p>0.05$) of PV was found for treated until 42nd day. These results proved that essential oils were effective on PV. The results of this study are similar to the findings of Quitral et al. 2009; Shahidi et al. 1995 and Perez-Mateos et al. 2006. During storage period, the lower PV was observed for Group CO.

Free fatty acids (FFA) are formed by lipolysis of triglycerides and phospholipids (Pearson et al. 1983). The increase in free fatty acid amount in the composition of foods is one of the factors that accelerate oxidation. FFA amount in the fillet was detected to be 0.63 ± 0.07 , which was approximately similar to the values reported for *Sardine pilchardus* (0.53 % oleic acid), for *Scomber japonicus* (1.64 % oleic acid) (Soyer and Şahin 1999). At the beginning of the storage period (after smoking), FFA amount of hot smoked rainbow trout were determined as 0.76, 0.68, 0.75, 0.70 and 0.76 (oleic acid %) for the control, RO, TO, SO and CO respectively. FFA increase in the control group was higher than treated groups during storage. After the 14th day, the difference between the control group and treated groups was found to be significant ($p<0.05$). At the end of the storage period of 112 days, FFA amount of CO group was found to be 3.79 (oleic acid %). The results established the antioxidant characteristics of the essential oils. Olley et al. 1969; Frega et al. 1999; Serdaroglu and Felekoglu 2005 confirmed the findings of our study. This result can be explained by increasing free fatty acids (FFA) levels due to the enzymatic hydrolysis during storage (Hwang and

Table 1 Sensory scores of smoked rainbow trout fillets during storage at 4 °C

Storage	Appearance					Taste					Odour				
	A	RO	TO	SO	CO	A	RO	TO	SO	CO	A	RO	TO	SO	CO
After smoking	4.83±0.16 ^{ax}	4.43±0.07 ^{ax}	4.57±0.10 ^{ax}	4.70±0.18 ^{ax}	4.80±0.00 ^{ax}	4.57±0.72 ^a	4.50±0.18 ^a	3.70±0.88 ^a	4.00±0.70 ^a	4.33±0.41 ^a	4.57±0.50 ^{ax}	4.80±0.41 ^{ax}	3.90±0.67 ^{ax}	4.07±0.59 ^{ax}	4.53±0.51 ^{ax}
7	4.60±0.08 ^{ax}	4.53±0.21 ^{ax}	4.67±0.00 ^{ax}	4.57±0.01 ^{ax}	4.67±0.02 ^{ax}	4.47±0.52 ^a	4.30±0.32 ^{ab}	3.60±0.90 ^a	4.17±0.74 ^a	4.17±0.28 ^{ab}	4.47±0.51 ^{ax}	4.03±0.74 ^{abx}	3.70±0.67 ^{ax}	4.36±0.63 ^{ax}	4.80±0.41 ^{ax}
14	4.50±0.18 ^{ax}	4.26±0.16 ^{ax}	4.70±0.44 ^{ax}	4.60±0.10 ^{ax}	4.50±0.46 ^{abx}	4.30±0.88 ^a	3.80±0.34 ^{ab}	3.90±0.86 ^a	4.17±0.92 ^a	4.10±0.35 ^{ab}	4.77±0.61 ^{ax}	4.47±0.61 ^{ax}	4.03±0.80 ^{ax}	4.33±0.81 ^{ax}	4.47±0.75 ^{ax}
28	4.33±0.04 ^{ax}	4.43±0.11 ^{ax}	4.57±0.18 ^{ax}	4.53±0.27 ^{ax}	4.37±0.28 ^{abx}	4.07±0.85 ^{ab}	3.60±0.30 ^{ab}	3.97±0.83 ^a	4.10±0.88 ^a	3.47±0.34 ^{ab}	4.40±0.70 ^{ax}	4.03±0.70 ^{abx}	3.83±0.75 ^{ax}	3.87±0.59 ^{ax}	4.20±0.69 ^{ax}
42	3.88±0.20 ^{abx}	4.13±0.30 ^{ax}	4.10±0.05 ^{ax}	4.07±0.10 ^{abx}	4.10±0.25 ^{abx}	3.48±0.91 ^b	3.50±0.32 ^{ab}	3.57±0.64 ^a	3.83±0.70 ^{ab}	3.90±0.30 ^{ab}	3.83±0.80 ^{ax}	4.13±0.56 ^{abx}	3.97±0.59 ^{ax}	3.53±0.63 ^{ax}	4.00±0.64 ^{ax}
56	3.13±0.33 ^{bx}	3.50±0.23 ^{abxy}	4.00±0.00 ^y	3.77±0.25 ^{aby}	3.57±0.44 ^{by}	—	3.37±0.21 ^b	3.13±0.52 ^a	3.33±0.74 ^{ab}	3.53±0.35 ^{ab}	2.27±0.96 ^{bx}	3.10±0.63 ^{bxy}	3.33±0.70 ^{abxy}	3.23±0.72 ^{abxy}	3.70±0.65 ^{aby}
70	—	2.60±0.20 ^{bx}	3.70±0.16 ^{aby}	3.10±0.16 ^{by}	3.47±0.10 ^{bxy}	—	2.60±0.20 ^b	1.93±0.70 ^b	2.90±0.68 ^b	3.26±0.22 ^b	—	2.30±0.80 ^{bxy}	2.80±0.82 ^{abx}	3.37±0.81 ^{ax}	3.43±0.64 ^{abx}
84	—	2.23±0.12 ^{bx}	2.93±0.14 ^{bx}	2.30±0.02 ^{bxy}	3.00±0.24 ^{bxy}	—	—	—	2.50±0.83 ^b	2.57±0.25 ^{bc}	—	1.90±0.51 ^{cx}	2.30±0.70 ^{bxy}	2.16±0.80 ^{bx}	3.43±0.71 ^{aby}
98	—	—	—	2.07±0.03 ^{cx}	2.50±0.60 ^{cx}	—	—	—	—	2.27±0.30 ^c	—	—	—	1.97±0.63 ^{bx}	2.77±0.49 ^{bx}
112	—	—	—	—	1.63±0.10 ^{cx}	—	—	—	—	—	—	—	—	—	2.03±0.55 ^{bx}

A: Control RO: plus rosemary oil TO: plus thyme oil SO: plus sage oil CO: plus clove oil

Values in rows followed by different superscripts are significantly different ($p<0.05$). Each value is a mean±S.D of triplicate determinations. n: 5 panelists

Regenstein 1993). The connection between lipolysis and lipid oxidation/rancidity is free (polyunsaturated) fatty acids oxidise more readily than esterified lipid.

Sensory analysis during storage

Table 1 show results of the sensory evaluation (overall taste, odour and appearance) of smoked rainbow trout. The spoilage patterns described by the panelists were as follows: softening of texture before off-odours developed and presence of bitter and rancid off-flavours. Appearance score decreased as colour discolouration increased. The limit overall appearance was reached after 56 days for the control (A), after 84 days for RO and TO, after 98 days for SO and after 112 days for CO samples. The limit of acceptability for taste and odour were reached after 42 days for the control (A), after 70 days for RO and TO, after 84 days for SO and after 98 days for CO group. Based on appearance, taste and odour scores, a shelf life of approximately 56 days for the control group, 84 days RO and TO group, 98 days SO and 112 days CO may be expected. Cakli et al. (2006) reported a shelf life for hot smoked vacuum packaged rainbow trout of 5 weeks, while Erkan (2011) reported a shelf life for hot smoked rainbow trout stored at 2 °C of 5 weeks.

The combination of vacuum and essential oils resulted in a shelf life extension of approximately 2–8 weeks attributed to the antioxidant effects of the essential oils phenolic components carvacrol, carnosic acid, thymol and eugenol known to exert antioxidant activity (Toda et al. 1994; Nychas 1995; Fasseas et al. 2007; Emir Coban and Ozpolat 2012).

Conclusion

According to chemical, microbiological and sensorial date, it was determined that the effect of essential oils on the indicators of lipid oxidation, on thiobarbituric acid (TBA) level, peroxide value (PV) and free fatty acid (FFA) levels are important; essential oils have a positive effect on the shelf life of smoked product; and especially addition of clove oil extended the products shelf life by approximately 6–7 weeks. Essential oil as natural preservative can be used in conjunction with VP to enhance smoked hot fish quality.

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