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UTILISATION OF ANTIMICROBIAL AGENTS AT PRE- AND POST-SMOKING ON THE MICROBIAL QUALITY OF HOT-SMOKED RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* FILLETS

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Utilisation of antimicrobial agents at pre- or post-smoking processes was considered for the microbial quality of hot-smoked trout fillets prepared at plant scale. Potassium sorbate (PS) and sodium lactate (SL) were used separately or in combinations at 3% (w/v) in brine for pre-smoking or with spraying at post-smoking. Pre-smoking usage could reduce the total aerobic mesophilic bacteria (TAMB) values better than the post-smoking usage during storage. However, post-smoking application was more effective at preventing the moulds and yeasts (MY) growth. PS in brine at pre-smoking was most efficient, which could keep trout fillets for even four weeks at 6±1°C storage within the range of consumable limits. Additionally, PS usage reduced the contaminated bacterial diversity and especially eliminated the outgrowth of *Serratia liquefaciens*. As a conclusion, pre-smoking application repressed the contaminated microbial growth during the storage of smoked trout fillets, which may eliminate the microbial risks.

Keywords: trout, pre-smoking, post-smoking, potassium sorbate, sodium lactate, *Serratia liquefaciens*

Rainbow trout *(Oncorhynchus mykiss)* is one of the most aquacultured fish species around the world. To increase the consumption of rainbow trout and to provide better availability, smoking is one of the trends with respect to processing and preservation. Two common methods, cold and hot, are mainly applied at smoking. Although hot smoking inhibits the resident microorganisms substantially, these products are certainly not sterile due to the small residual microbial contamination after cooking that could propagate rapidly during storage (Cakli et al., 2006; da Silva et al., 2008; Erkan, 2012). This may eventually cause spoilage and present various microbiological risks.

Generally, 1–2 log CFU g^{-1} bacterial cells can be detected after hot-smoking. The particularly heat-resistant *Bacillus coagulans* and some species of *Listeria monocytogenes, Pseudomonas, Acinetobacter, Aeromonas, Citrobacter,* and *Serratia* have been detected during storage depending on the quality of fish and production conditions (Kolsarici & Özkaya, 1998; Ghaly et al., 2010). In order to diminish safety risks and prevent economic losses, using safe preservatives in fishery products has been recommended. In the preparation of smoked catfish, carp, and salmon, different concentrations of PS and SL have been used as preservatives (Efiuvwevwere & Ajiboye, 1996; Nykänen et al., 2000; Öksüztepe & Gürel-Inanli, 2007; da Silva et al., 2008; Omojowo et al., 2009). In most preservative-based strategies, the efficient inhibition of microorganisms has been achieved at 3% concentrations of PS, SL, or sodium metabisulfite (da Silva et al., 2008; Omojowo et al., 2009), which has been also accepted as GRAS status by Food and Drug Administration (FDA).

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There is no study on preservative usage for the elimination of contaminating microflora in the production of hot-smoked trout fillets on plant scale. In this study, the aim was to determine the effect of pre- or post-smoking utilisation of antimicrobial agents to repress the growth of the contaminating microflora during storage in packaged hot smoked trout fillets, which were produced on a plant scale. In this respect, the relevant questions, which preservative and at which step it can be applied at the production of hot-smoked trout fillets to enhance the microbial safety, has been clarified in this study.

1. Materials and methods

1.1. Materials

The rainbow trout *(Oncorhynchus mykiss)* used in this study was aquacultured and supplied by a local company. The following media were used in the microbiological analyses: Plate Count Agar (PCA, Merck, Germany) for Total Aerobic Mesophilic Bacterial count, Dichloran Rose Bengal Chloramphenicol agar (DRCB, Merck) for mould-yeast (MY) count, and Violet Red Bile agar (VRB, Merck) for coliform count. Food grade sodium lactate (SL) (E325) and potassium sorbate (PS) (E202) were used for preservation and table salt was used for brining.

1.2. Fillet preparations and preservative applications

The fillet preparations were carried out in the plant processing system using standard operating conditions. All harvested trout fish were cleaned and washed immediately at chilling. The cleaned raw trout fish were divided into seven groups of twelve trout fish of equal weights (~200 g). To compare the effectiveness of preservative usage at pre- or post-smoking processes, PS and SL were applied separately to the trout with three simultaneously independent replications.

Preservatives were included in the brine at 3% and subsequently the cleaned trout were immersed in pre-smoking applications. Control samples were prepared in brine without using any preservatives. All the trout were kept in each type of experimental brine for 12 h before the smoking process was applied.

For post-smoking preservation the fillets were prepared by brining without preservatives then smoking and skinning, followed by spraying with ~2 ml preservative solution on each side.

Accordingly, the treatments were applied to both the raw and smoked trout at pre- and post-smoking, separately. Therefore in the experimental design two trouts were used for each treatment (7) and time points (6) with three replicates (2×7×6×3).

1.3. Smoking, packaging, and storage

The samples treated with or without preservatives were smoked in a smoking unit of the company using their processing parameters. Accordingly, all samples were pre-dried before smoking and smoked at 80 °C for 15 min using oak wood. The samples were then cooled to 5±1 °C and stored overnight. Then skinning was completed and these prepared fillets were packaged in high-density polyethylene bags. The antimicrobials were applied for the post-smoking samples before packaging. All samples were then stored at 6±1 °C for five weeks for microbiological and chemical analysis.

1.4. Microbiological analysis and strain identification

The TAMB, yeast-mould, and coliform counts were determined for all samples and controls as well as the raw trout and brine with the standard pour plate method (Harrigan & McCance, 1976). Accordingly, two packages of treated or untreated trout fillets were sampled at each time point (week) for each treatment. Petri dishes prepared for TAMB and MY analysis were incubated at 25 °C for 48 h and for colifom counts at 37 °C for 24 h. Also, 10 different colonies were selected from each PCA Petri dish after incubation for Gram-staining and cell morphology differentiation for further identification. The selected colonies were kept in vials containing 25% glycerol in water and stored at –20 °C. The identification of these isolates was done using the VITEK 2 ID system (bioMerieux, France) in which the obtained data was evaluated with the comprehensive database of this system.

1.5. Chemical analysis

The pH changes in the fillets were measured using a pH-meter (Hanna, UK) after homogenizing 10 g of fillet with pure water (1:1). The thiobarbituric acid (TBA) value of the samples was determined according to the method given by Tarladgis and co-workers (1960) and the values were expressed as mg malonaldehyde per kilogram of meat.

1.6. Statistical analysis

The analysis of variance (ANOVA) was performed to compare the differences in microbial (log CFU g^{-1}) and physico-chemical attributes among the treated and control hot smoked trout fillet samples using the SAS for Windows software (V9.1; SAS Institute Inc., Cary, NC). The General Linear Model (GLM) procedure was used. Tukey's studentized range test was performed for post-hoc multiple comparisons. Differences between the mean values were considered significant when $P<0.05$.

2. Results and discussion

The microbiological counts of the raw trout and four used brines are shown in Table 1. Accordingly, with the exception of raw trout and the 8% NaCI brine supplemented with 1.5% SL/1.5% PS, all microbial counts were <2 log CFU g^{-1} of fish and <2 log CFU ml^{-1} of brine. All the raw trout samples that had been cleaned previously were determined to have 3.78 log CFU g^{-1} TAMB, 1.15 log CFU g^{-1} MY, and <1 log CFU g^{-1} coliform bacteria counts.

The TAMB and MY changes during five weeks of storage for trout fillets prepared with pre- and post-smoking preservative treatments are shown in Fig. 1A. On the first day of sample preparation, the TAMB counts of all fillets were an average of 1.26 log CFU g^{-1} and were not significantly different ($P>0.05$). However, these counts increased up to 7 log CFU g^{-1} at five weeks of storage; except for the sample N2 in which 3% PS was applied at pre-smoking. The TAMB count of the control samples increased rapidly over the critical TAMB limit of 6 log CFU g^{-1} by the second week of 6±1 °C storage. According to the International Commission on Microbiological Specifications of Foods, fish and its products are accepted as high quality and consumable if the TAMB count is <5.7 and <7 log CFU g^{-1}, respectively (ICMSF, 1986). Therefore only N2 (3% PS usage at pre-smoking) preserved smoked trout fillets were of high quality for up to four weeks of 6±1 °C storage.

Table 1. Microbiological content and pH of raw trout fillets and four brines. Data are the average of the replicates and standard deviations are lower than 5%.

	TAMB	MY	Coliform	pH
	(log CFU ml^{-1})			
Raw trout fillets*	3.78	1.15	< 1	6.67
8% Brine	1.56	1.93	< 1	7.60
8% Brine + 3% SL	< 1	1.23	< 1	7.12
8% Brine + 3% PS	1.16	1.41	< 1	7.54
8% Brine + 1.5% SL + 1.5% PS	< 1	2.31	< 1	7.30

*: The unit microbial count for raw trout is log CFU g^{-1}

Among the applied preservatives, 3% PS included in brine was the most effective ($P<0.05$) at preventing TAMB increase. However, spraying the same concentration of PS on smoked fillets was not enough to extend the storage further ($P>0.05$), although the TAMB count was lower ($P<0.05$) than the untreated control. The TAMB counts in samples that had been brined with 3% PS reached 5.8 log CFU g^{-1} at week five, while the other samples were >6 log CFU g^{-1}. The TAMB counts of samples in which SL and PS were applied together were lower than when SL was applied alone. This result demonstrated that PS was more effective against the propagating microflora during storage. PS provides the best bacterial inhibition of microbial growth in smoked trout fillets during refrigerated storage, as observed by others (Efiuvwevwere & Ajiboye, 1996; Omojowo et al., 2009). The most important feature that emphasizes the use of sorbate as a preservative is the continuous antimicrobial effect of this agent at neutral pH values (Lück, 1990). The microbial growth preventing effect of PS in higher ratios compared to SL could be a result of this dissociation relationship. Also, it has been emphasized that the usage of PS could inhibit the growth of many species belonging to the *Aeromonas, Pseudomonas, Salmonella, Vibrio, Flavobacterium, Acinetobacter, Staphylococcus,* and *Micrococcus* species (Efiuvwevwere & Ajiboye, 1996; Omojowo et al., 2009).

When the preservatives used at pre- and post-smoking were compared, the TAMB counts in all samples were similar ($P>0.05$) during the 1st week. However, all preservative-treated samples except N2 had similar TAMB counts at the end of the 5th week. Nevertheless, the TAMB counts in samples treated at pre- and post-smoking with preservatives were lower ($P<0.05$) than in the control sample. These results showed that using preservatives at pre-smoking is more effective than using them at post-smoking. This is probably due to the synergistic effect occurring when salt and preservatives were used in combination. Omojowo and co-workers (2009) expressed that PS application with salt was more effective on the inhibition of microorganisms, possibly due to reduced water activity. Brining with PS before smoking for one night may enable the diffusion of the preservative through the fillets and contribute to the preservation of the product during storage.

During the storage of the smoked trout fillets, the MY counts of the control samples were higher than in the samples treated with preservatives every week (Fig. 1B). In addition, the MY counts increased in all samples until the 3rd week of storage and then there was no

further significant increase (P>0.05) observed until the end of storage. When the MY results are evaluated for the used preservatives, all of them showed a similar (P>0.05) level of antimicrobial effects. However, applying preservatives at post-smoking was more effective (P<0.05) than using them at pre-smoking during the first two weeks of storage but the difference diminished over the extended weeks. This result indicated that spraying could be effective especially against surface contamination (Fig. 1B).

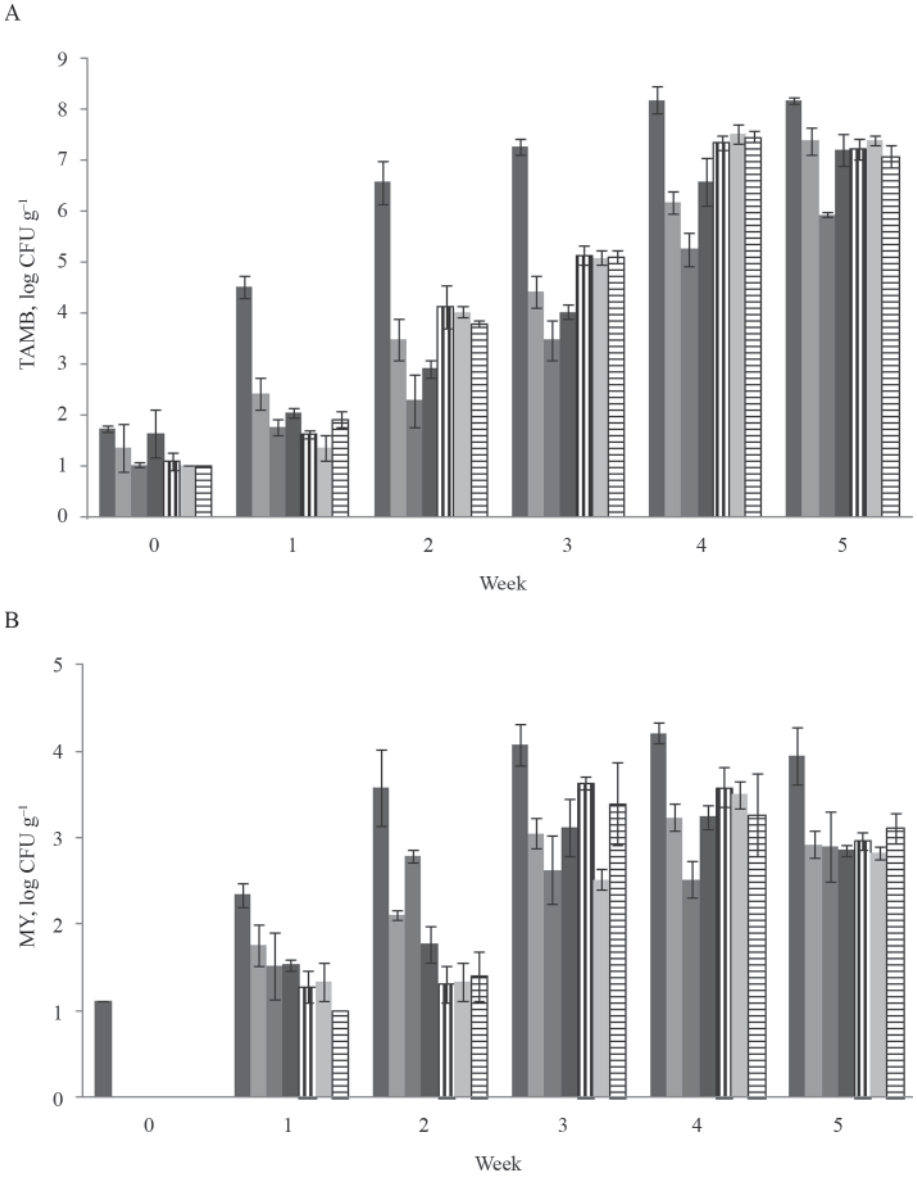

Fig. 1. Changes in A) TAMB and B) MY counts during 6±1 °C storage of smoked trout fillets. Control brine (■: C) was including 8% table salt. Pre-smoking treatments were brine plus 3% SL (■: N1); 3% PS (■: N2), 1.5% SL and 1.5% PS (■: N3). Post-smoking treatments were brine plus 3% SL (▥: N4); 3% PS (■: N5); 1.5% SL and 1.5% PS (▤: N6)

To understand which species existed in the microflora at the end of storage, ten colonies were taken from the PCA in the fifth week of product storage. All selected colonies were identified as Gram-negative long and short rods. The isolates were identified subsequently using the VITEK 2ID system. The diversity of bacteria in the samples of treated or untreated smoked trout fillets until the end of 5th week of product storage and their distribution according to treatment is shown in Fig. 2. Accordingly, seven different bacterial species (*Serratia liquefaciens, Enterobacter intermedius, Kluyvera cryocrescens, Aeromonas sobria, Citrobacter sedlakii, Citrobacter braakii, Burkholderia mallei*) were found in the smoked fish samples. The most abundant species in the samples was *S. liquefaciens*. However, the fillets preserved with brine containing 3% PS at pre-smoking consisted of low amounts of bacterial diversity without *S. liquefaciens*, and *K. cryocrescens* dominated in these samples (Fig. 2). *S. liquefaciens* is known as the cause of fish infection and is regarded as one of the main pathogens and spoilage bacteria of smoked fish products (Joffraud et al., 2006). Therefore, it is understood that *S. liquefaciens* can maintain its viability in usage at low temperatures and can proliferate at storage (Aydin et al., 2001). However, the lack of presence of this strain in samples N2 indicated that PS was effective at eliminating this species. Indeed, this antibacterial agent provided considerably lower TAMB counts.

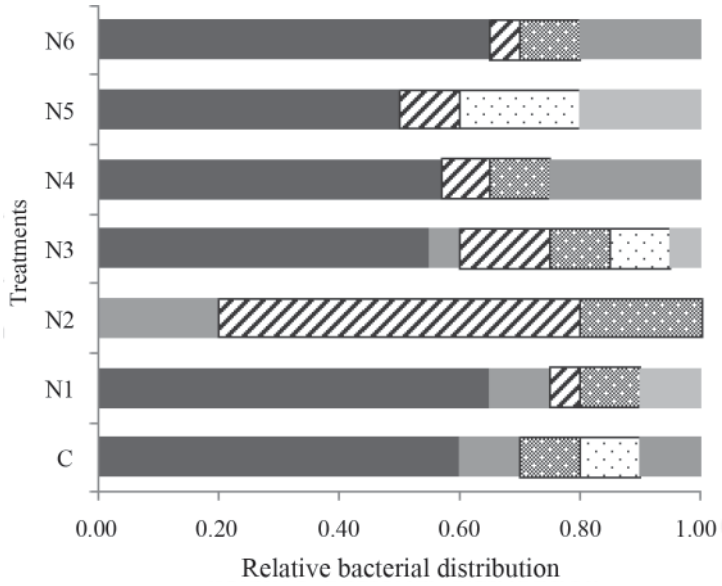

Fig. 2. Bacterial species distribution at smoked trout fillets stored for five weeks at 6±1 °C. Control brine (C) was 8% table salt. Pre-smoking treatments were brine plus %3 SL (N1), 3% PS (N2), 1.5% SL and 1.5% PS (N3). Post-smoking treatments were brine plus 3% SL (N4), 3% PS (N5), 1.5% SL and 1.5% PS (N6). : *Serratia liquefaciens;* : *Enterobacter intermedius;* : *Kluyvera cryocrescens;* : *Aeromonas sobria;* : *Citrobacter sedlakii;* : *Citrobacter braakii;* : *Burkholderia mallei*

The pH of the trout fillets decreased slightly after smoking, possibly occurring as a result of acids existing in smoke. There was no significant ($P>0.05$) difference in the pH of the trout fillets during storage for preservatives and treatment methods. The pH of the treated and untreated samples at the beginning of storage was between 6.50–6.65 and this pH had slightly decreased to 6.18–6.35 at the end of storage (Fig. 3A).

As expected, the thiobarbituric acid (TBA) values of all samples increased with storage. There were no significant differences (P>0.05) for preservatives and their applications. After five weeks of storage, the TBA value of untreated sample reached 8 mg malondialdehyde per kg. However, the TBA values of the preservative treated samples were 6.18–6.73 mg malonaldehyde per kg, meaning that the preservative treatments enabled the samples to be within the limits (8.00 mg malonaldehyde per kg) for consumption (Fig. 3B).

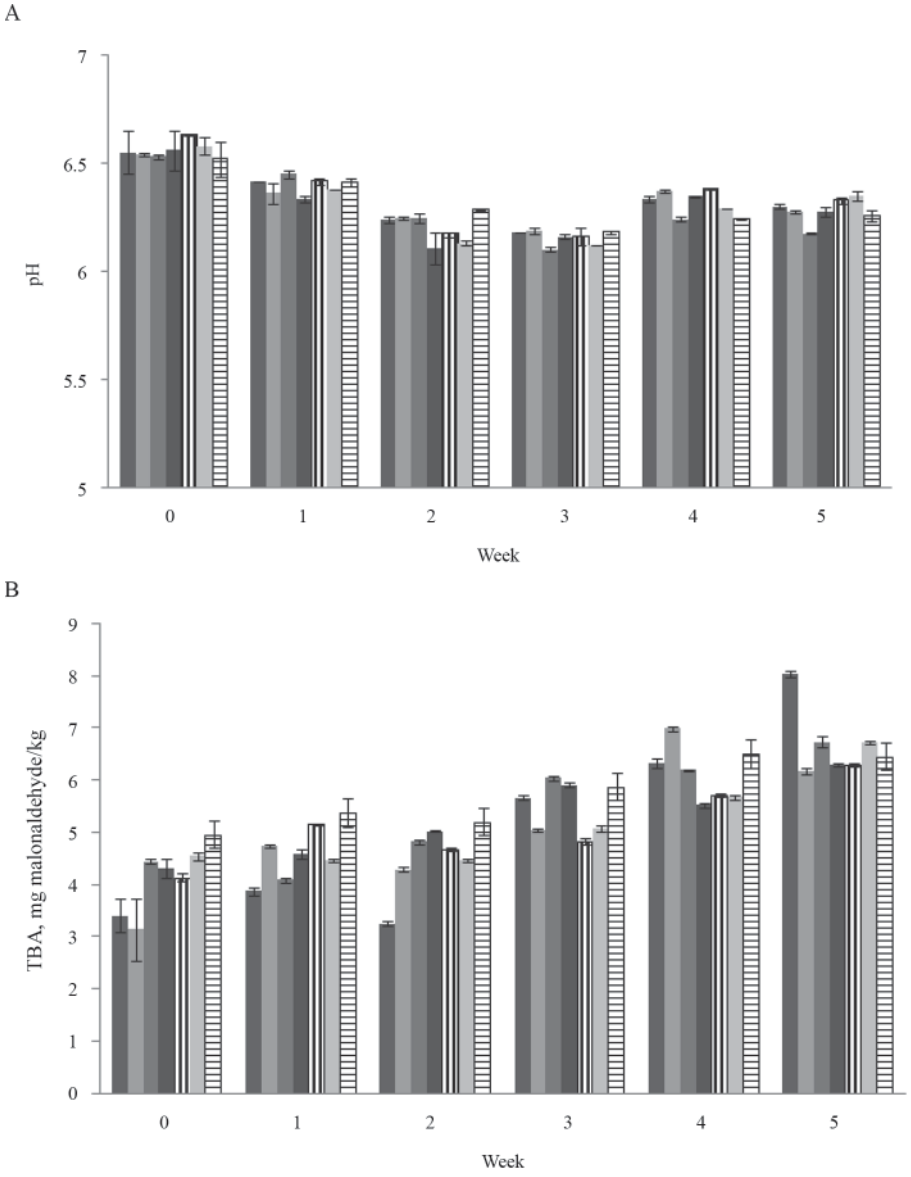

Fig. 3. Changes in A) pH and B) TBA values of smoked trout fillets stored five weeks at 6±1 °C. Control brine (■: C) was including 8% table salt. Pre-smoking treatments were brine plus 3% SL (■: N1); 3% PS (■: N2); 1.5% SL and 1.5% PS (■: N3). Post-smoking treatments were brine plus 3% SL (■: N4); 3% PS (■: N5); 1.5% SL and 1.5% PS (■: N6)

3. Conclusions

The microbial flora could be repressed in packaged smoked trout fillets when PS and SL were included separately at pre-smoking. Three percent PS in brine kept the TAMB counts and TBA values within the allowed limits for consumption (ICMSF, 1986) for up to four weeks at 6±1 °C by reducing the numbers and growth of bacterial species in the flora, notably the growth of *S. liquefaciens.*

References

Aydin, S., Erman, Z. & Bilgin, Ö.C. (2001): Investigation of *Serratia liquafaciens* infection in rainbow trout (*Oncorhynchus mykiss*) Walbaum. *Tr. J. Vet. Anim. Sci., 25*, 643–650.

Cakli, S., Kilinc, B., Dincer, T. & Tolasa, S. (2006): Comparison of the shelf lives of map and vacuum packaged hot smoked rainbow trout (*Oncorhynchus mykiss*). *Eur. Food Res. Technol., 224*, 19–26.

da Silva, A.L.V., Prinyawiwatkul, W., King, J.M., No, H.K., Bankston, J.D. Jr & Ge, B. (2008): Effect of preservatives on microbial safety and quality of smoked blue catfish (*Ictalurus furcatus*) steaks during room-temperature storage. *Food Microbiol.*, *25*, 958–963.

Efiuvwevwere, B.J. & Ajiboye, M.O. (1996): Control of microbiological quality and shelf-life of catfish (*Clarias gariepinus*) by chemical preservatives and smoking. *J. Appl. Bacteriol.*, *80*, 465–470.

Erkan, N. (2012): The effect of thyme and garlic oil on the preservation of vacuum-packaged hot smoked rainbow trout (*Oncorhynchus mykiss*). *Food Bioprocess Tech.,* 5, 1246–1254.

Ghaly, A.E., Dave, D., Budge, S. & Brooks, M.S. (2010): Fish spoilage mechanisms and preservation techniques. *Am. J. Appl. Sci.*, *7*, 859–877.

Harrigan, W.F. & McCance, M.E. (1976): *Laboratory methods in food and dairy microbiology*. Academic Press, New York, pp. 452–460.

ICMSF (1986): International commission on microbiological specifications for foods. Sampling plans for fish and shellfish. -in: *Microorganisms in foods. Sampling for microbiological analysis: Principles and scientific applications,* vol 2, 2nd ed. Blackwell Scientific Publications, Toronto, Canada, pp. 181–193.

Joffraud, J.J., Cardinal, M., Cornet, J., Chasles, J.S., Léon, S., Gigout, F. & Leroi, F. (2006): Effect of bacterial interactions on the spoilage of cold-smoked salmon. *Int. J. Food Microbiol.*, *112*, 51–61.

Kolsarici, N. & Özkaya, O. (1998): Effect of smoking methods on shelf-life of rainbow trout (*Salmo gairdneri*). *Tr. J. Vet. Anim. Sci., 22*, 273–284.

Lück, E. (1990): Food applications of sorbic acid and its salts. *Food Add. Cont., 7,* 711–715.

Nykänen, A., Weckman, K. & Lapveteläinen, A. (2000): Synergistic inhibition of *Listeria monocytogenes* on cold-smoked rainbow trout by nisin and sodium lactate. *Int. J. Food Microbiol.*, *61*, 63–72.

Öksüztepe, G. & Gürel-Inanli, A. (2007): Microbiological changes occurring in trout fillets (*Oncorhynchus mykiss* W. 1792) salted and treated with potassium sorbate during production and storage. *Pak. J. Biol. Sci., 10*, 2694–2698.

Omojowo, F.S., Idris, G.L. & Ilhuahi, J.A. (2009): Comparative assessment of potassium sorbate and sodium metabisulfite on the safety and shelf life of smoked catfish. *Nat. Sci., 7*, 10–17.

Tarladgis, B.G., Watts, B.M., Younathan, N.S. & Dugan, L. Jr. (1960): A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.*, *37*, 44–48.