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Microbiological Changes Occurring in Trout Fillets (*Oncorhynchus mykiss* W. 1792) Salted and Treated with Potassium Sorbate During Production and Storage

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Abstract: In this study, microbiological changes during processing and storage of salted-cured trout fillets treated with potassium sorbate were investigated. For this purpose, 10 and 15% (w/w) NaCI and 1, 5 and 10% (w/v) potassium sorbate were applied to the fillets. The processed fillets were vacuum-packed and storage at 4°C. The samples were analyzed in some periods of production and in the storage days of 7, 14, 28, 42, 56, 70 and 84 for numbers of total mesophilic aerob, psycrophylic, yeast and mould. In conclusion, the microbiological quality of all samples treated with 15% NaCl and potassium sorbate were found better. Consequently, it can be concluded that the usage of potassium sorbate may be useful and a synergistic effect between salt and potassium sorbate determined.

Key words: Rainbow trout (*Oncorhyncus mykiss* W.), salted fish, potassium sorbate, microbiological, quality

INTRODUCTION

Preservation of fish is performed by methods such as freezing, smoking, salting and drying (Yetim, 1996; Horner, 1997). Salting process is one of the oldest methods applied and is performed throughout the world and in Turkey. Various antimicrobial agents are used in addition to salt, in order to prevent or delay fish spoilage. Sodium, potassium and calcium salts of sorbic acid (sorbates) are the most frequently used antimicrobial agents. The most important sorbate is potassium sorbate, which is an efficient inhibitory substance when used together with sodium chloride. It slightly delays growth when used alone and lag phase is prolonged 1 or 2 days at 25°C. The effect of potassium sorbate increases as temperature decreases (Sofos and Busta, 1981; Shaw *et al*., 1983; Gram, 1991).

After specification of the fact that sorbates are more effective against mould, yeast and many groups of bacteria, these compounds have gained increased significance in preservation (Sofos and Busta, 1981; Saldamlı, 1985; Ockerman, 1991; Yetim, 1996; Patır *et al*., 2001). In a study by Hussain *et al*. (1977), the shelf-life of the vacuum-packaged rainbow trout in 0.075 mm thick polyethylene packs was determined to be 2 weeks at 0°C. Statham and Bremner (1983) have reported that sorbates inhibited growth of bacteria significantly in vacuum-packaged fish. Villemure *et al*. (1986), on the other hand, have observed that vacuum-packaged and modified atmosphere packaged codfish fillets had acceptable sensory qualities even after 3, 6 and 14 days of preservation. Patir *et al*. (2001) specified that the number of total mesophilic aerobes, *Staphylococcus-micrococcus*, *Lactobacillus*, psychrophil, yeasts, moulds and choliform bacteria has decreased at production stages and increased at the subsequent stages in potassium sorbate treated salt processed carp fillet samples. Again in this investigation, it is reported that samples treated with 10% salt and with potassium sorbate during the preparation of the product and during preservation included a lower number of microorganisms. Gurel Inanli and Patir (2004) have reported that the preservation period of salted trout fillets was prolonged according to the amount of salt (NaCl) used and the concentration of potassium sorbate applied and that these products could be preserved under appropriate conditions at least for 70 days without being spoiled.

The aim of this study was to investigate the changes in the total number of mesophilic aerobic microorganisms, psychrophils, yeasts and moulds during production and preservation of salted and potassium sorbate applied vacuum packaged rainbow trout fillets.

MATERIALS AND METHODS

Oncorhynchus mykiss fish species (rainbow trout) from Salmonidae family (Salmons) was used in this investigation. Fish were acquired from Firat University

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Faculty of Fisheries, Trout Rearing Units. Freshly acquired fish weighing approximately 1 kg were kept at cold enviromental conditions until fillets were collected. First the skins of the fish were removed. Then fish were decapitated and inner organs were removed. Fillets were collected after strings and bones were taken out. Fillets were washed out with large amounts of clean water and made ready for the salting process. Dry salting process was performed with 10 and 15% (w/w) rock salt in types. For this purpose, salt was placed at the bottom of a suitable container and one layer of fillets were placed on top. Another layer of fillets were put over the underlying layer of salted fillets. A weight of approximately 4 times that of the fillet was placed on the stock for compression. The compressed stock was held for 36 h this way and drained at certain intervals during the process.

At the end of the compression period, fillets treated with 10 and 15% salt were separated into 4 groups. The first included the control group (A and E), the second group included the types dipped into a 1% potassium sorbate solution at 25°C and held for one minute (B and F), the third group included those kept at 5% potassium sorbate solution for one minute (C and G) and the fourth group included those kept at 10% potassium sorbate solution for one minute (D and H). Eight different types of samples were prepared this way.

After the potassium sorbate application, all the samples were put into drying process. They were dried in the fanned drying oven at 30°C for 60 min. The samples placed in polyethylene bags were packaged in the vacuum machine (HENKELMANN-TT 300/2) and preserved in fridge at 4±1°C. Three replicates were made in this study. The samples were investigated at the production stage (fillet, end of salting and end of drying) and were investigated microbiologically at certain days of the preservation (days 7, 14, 28, 42, 56, 70 and 84). One package from each type of sample was opened under aseptic conditions on the mentioned days of analysis.

Five gram of sample was weighed in a special bag of a homogenizer (Stomacher 400). Forty five milliliter of sterile 0.1% peptoned water was added on this and it was homogenized in the homogenizer. The dilutions of the samples (10^{-6}) were prepared with standard methods (Harrigan, 1998; Varlİk *et al*., 1993).

Plate Counting Agar (PCA) medium (30±1°C for 72 h) was used for counting the total amount of psychrophil and mesophilic aerob microorganisms (5±1°C for 7 days) in the samples. Wort agar medium was used for counting of yeasts (25±1°C for 5 days). Sabouraud dextrose agar medium was used for counting of moulds (3 days at 25±1°C) (Oxoid, 1982; ICMSF, 1986; Harrigan, 1998).

SPSS 10.0 computer package statistics program was used for the analysis of the microbiological changes occurring during the production and preservation of the salt processed trout fillets prepared experimentally. Independent t test was used for the comparison of groups of two, Kruskal-Wallis test was used to determine any significant difference between groups of more than two components and Duncan Test was applied to determine between which groups the differences existed (Akgül, 1997; Özdamar, 2001).

RESULTS AND DISCUSSION

Fish spoilage starts right after catching, with the effect of inner and outer factors. Natural microflora in the skin, gills and intestines of the fish and contamination with microorganisms after the fish is caught are the main causes of spoilage in fish (Table 1). Consequently, the microbial quality of the product is reduced and thus human health is risked by infections and toxication. For this reason, information about the microorganisms (number and type of microorganisms) in the muscles of the fish are quite significant in terms of human health and preservation of the product (Gram and Huss, 2000).

A total number of 5.41 $\log_{10}$ cfu g^{-1} mesophilic aerobic microorganisms detected in the salt treated trout fillets prepared experimentally in 8 different types (A, B, C, D, E, F, G and H) decreased in all types after salting and drying. It increased at day 7 in types A and B which were treated with 10% salt and at day 14 in types C and D. After a relative decrease at day 14 in type A, it reached to its highest level at day 84 in all types, increasing continuously until the end of the preservation. In the group which was treated with 15% salt, the total number of mesophilic aerobic microorganisms which started to increase on from day 7 in types E and F relatively decreased in type G at day 7 and then increased again. In type H, the decrease continued until day 14 and then an increase was recorded in the subsequent days. On the 84th day of the preservation, the number of microorganisms which reached the peak level was recorded as 5.05-6.15 $\log_{10}$ cfu g^{-1} in all types (E, F, G and H). Upon statistical evaluation of the data acquired, the changes in the total number of mesophilic aerobic microorganisms throughout the preservation was found to be statistically significant in terms of the salt rates applied (10 and 15%) at days 0, 7, 14 and 28 ($p<0.01$). Furthermore, difference was detected between different types in the group treated with 10% salt at days 56, 70 and 84 ($p<0.05$). In the group treated with 15% salt, on the other hand, difference between different types in terms of

Table 1: Microbiological analyses results during production and storage of trout fillet samples (log_{10} cfu g^{-1})

		Production stage			Storage period (day)						
Microorganisms	Sample type	Fillet	End of salted	End of dried	7	14	28	42	56	70	84
Total mesophilic aerobe	A	5.41	4.94	4.85	5.75	5.69	5.95	6.44	6.81^{b}	7.42^{b}	8.14^{c}
	B	5.41	4.94	4.71	5.27	5.39	5.75	6.36	6.77^{b}	7.08^{b}	7.31b^{c}
	C	5.41	4.94	4.72	4.34	5.17	5.27	5.56	6.07^{ab}	6.30^{a}	6.54^{ab}
	D	5.41	4.94	4.40	4.20	4.63	4.68	4.81	5.38^{a}	5.79^{a}	6.12^{a}
	E	5.41	4.54	4.29	4.37	4.86	5.16	5.77b	6.53	6.62	7.31
	F	5.41	4.54	3.99	4.04	4.17	4.62	5.90b	6.00	6.65	6.67
	G	5.41	4.54	3.72	3.52	3.61	3.82	4.26^{a}	5.31	6.03	6.16
	H	5.41	4.54	3.17	2.99	2.56	2.95	3.61^{a}	4.64	5.06	5.72
Psicrophile	A	3.80	3.35	3.44	4.38	4.85	3.98	3.39	5.05	5.17	4.53
	B	3.80	3.35	3.45	4.34	4.05	3.72	4.89	4.77	6.15	4.73
	C	3.80	3.35	3.36	4.62	4.28	3.84	3.65	4.29	4.31	3.56
	D	3.80	3.35	3.37	4.46	4.33	3.38	3.25	4.21	5.03	3.95
	E	3.80	3.72	3.38	4.90	4.69	4.11	5.44	4.43	6.10	5.51
	F	3.80	3.72	3.88	4.87	4.40	3.93	4.02	5.20	6.11	6.15
	G	3.80	3.72	3.71	4.25	4.44	3.51	5.68	5.75	5.65	5.35
	H	3.80	3.72	3.59	4.75	4.47	3.79	4.78	5.46	5.74	5.05
Yeast	A	1.93	3.56	3.47	4.28	3.82	4.09	6.08	5.28	5.05	5.63
	B	1.93	3.56	4.14	3.60	2.68	4.36	6.13	6.28	4.52	6.36
	C	1.93	3.56	3.35	4.48	3.17	3.52	4.43	6.22	5.51	5.32
	D	1.93	3.56	2.89	3.45	2.67	3.53	5.76	6.47	5.32	5.19
	E	1.93	2.81	2.58	3.06	2.67	2.84	3.01^{b}	4.48^{c}	3.34	3.38
	F	1.93	2.81	2.56	2.79	1.93	2.77	4.52^{c}	3.11^{b}	4.00	4.33
	G	1.93	2.81	1.89	2.96	2.31	2.86	3.93^{bc}	3.57^{bc}	3.07	2.61
	H	1.93	2.81	2.09	2.07	2.48	1.90	1.93^{a}	1.67^{a}	1.76	1.74
Mould	A	4.58	4.31	3.79	4.93	4.02	4.27	5.30	5.20	5.03	5.91
	B	4.58	4.31	4.46	4.31	3.53	5.18	5.61	6.22	5.60	6.62
	C	4.58	4.31	4.40	4.61	3.59	4.28	5.30	5.40	6.09	5.48
	D	4.58	4.31	4.48	4.94	3.07	4.28	4.60	5.11	5.85	6.07
	E	4.58	4.71	3.60	4.03	3.47	3.79	2.96	3.93	3.43	3.21
	F	4.58	4.71	3.82	4.11	3.66	2.62	4.36	3.42	3.67	4.91
	G	4.58	4.71	3.57	4.48	2.89	2.58	2.69	2.91	3.13	2.92
	H	4.58	4.71	3.87	4.49	3.02	1.97	1.90	1.67	3.40	2.67

A: 10% NaCl (Control), B: 10% NaCl + 1% potassium sorbate, C: 10% NaCl + 5% potassium sorbate, D: 10% NaCl + 10% potassium sorbate, E: 15% NaCl (Control), F: 15% NaCl + 1% potassium sorbate, G: 15% NaCl + 5% potassium sorbate, H: 15% NaCl + 10% potassium sorbate, a, b, c: Mean values in same rows with different superscrips are significantlry different ($p<0.05$)

number of microorganisms was detected only on day 42 ($p<0.05$) (Table 1). This decrease observed in the number of microorganisms at the end of the salting and drying processes of the fillets is due to the bacteriostatic or bactericidic effects of salt (Voskresensky, 1965; Filsinger, 1987). Furthermore, the fact that potassium sorbate treated groups (B, C, D, F, G and H) were detected to contain a lower number of microorganisms compared to the control groups (A and E) throughout the production and preservation is related to the antimicrobial effect of potassium sorbate (Sofos and Busta, 1981; Yetim, 1996; Patir *et al*., 2001; Gurel Inanli and Patir, 2004). The results acquired throughout the production and preservation are in accordance with those of some investigators (Patir *et al*., 2001; Gurel Inanli and Patir, 2004), while being in contrast with those of some others (Gelman *et al*., 2001; Shalini *et al*., 2001).

Psychrophils are microorganisms responsible for the spoilage of fresh fish. Psychrophil microorganisms were detected in various studies as the dominant microflora in spoiled fillets. Vacuuming of the products inhibits the growth of psychrophil aerobes. The resistance of the product is prolonged this way (Clingman and Hooper, 1986; Dalgaard *et al*., 1993). The number of psychrophil microorganisms appearing in average values of 3.80 log_{10} cfu g^{-1} in the fillets decreased at the end of the salting process. Number of psychrophils which increased in the beginning of the preservation presented different changes later during the preservation process, depending on different types. At day 84 of the preservation, number of psychrophils was detected to be lower in the group treated with 15% NaCl. In the statistical analysis performed, the difference detected between the groups which were treated with 10 and 15% salt was observed to be significant with $p<0.05$ at days 42, 56 and 70 and to be significant with $p<0.01$ at day 84. No difference was observed between types within groups ($p>0.05$) (Table 1). The number and growth of psychrophil microorganisms throughout production and preservation is similar to that in the study of Patir *et al*. (2001). However, it is quite different from the results of Gün *et al*. (1996), who detected that the number of psychrophils in

pickled trout samples was 2.7×10^3 cfu g^{-1} at day 0 and that it did not displayed any increase until the end of the 4th week of the storage. Discrepancy in the results is probably due to different materials and different processing technology used in the mentioned investigation. The number of yeasts in the fillet, after increasing in all types at the end of the salting process, went down in all types except for type A. At day 7 of preservation, after a relative decrease in types B and H, it increased at days 42-56 of the preservation. The number of yeasts displayed fluctuation until day 84 of the preservation in the types in both groups (10 and 15%). The number of yeasts in the types treated with 15% salt and with potassium sorbate (F, G and H) was found to be lower compared to that in the types treated with 10% salt and with potassium sorbate (B, C and D). In terms of number of yeast and salting rates (10 and 15%), the differences detected were observed to be significant with $p<0.05$ at day 70 of preservation and to be significant with $p<0.01$ in other days. However, no difference was observed in types within groups ($p>0.05$) (Table 1). These results are in accordance with those investigators (Patir *et al*., 2001) who could not detect any difference between the types during the production and preservation of the salt treated carp fillets.

Moulds are not a part of the normal flora in fish. These microorganisms are usually soil originate and fish are known to be contaminated with these from water during catching, or via the equipments and materials used after catching. The muddy, mouldy scent and taste in fish is known to occur due to growth of *Streptomcyes* species (Jay, 1996). Number of moulds was found to be 4.58 $\log_{10}$ cfu g^{-1} on average in the fillets used in the preparation of the experimental samples. In the samples treated with 10% salt, number of moulds decreased at the end of the salting process. The number of moulds, which started to increase on from day 7 of the preservation decreased again until day 14 of the preservation. In later days of the preservation, the number of moulds increased in different fluctuations. On the other hand, it relatively increased in the samples treated with 15% salt in all types (E, F, G and H) at the end of the salting process. Furthermore, it decreased rapidly at the end of the drying process. The number of moulds in the types treated with 15% salt and with potassium sorbate (F, G and H) was detected to be lower compared to that in the types treated with 10% salt and with potassium sorbate (B, C and D), throughout the production and preservation. In the statistical analysis, in terms of salting rates (10 and 15% NaCl), results related to moulds in this study was found to be significant with $p<0.05$ at the end of the salting process and at day 7 and with of $p<0.01$ at days 28, 42, 56, 70 and 84. No difference was observed between the types within groups ($p>0.05$) (Table 1). This situation observed related to the number of moulds is in accordance with the reports of the investigators stating that the effect of sorbates on microorganisms was greater compared to high salt concentrations (Sheneman and Costilow, 1955; Patir *et al*., 2001; Gurel Inanli and Patir, 2004).

Consequently, it was concluded that the rainbow trout fillets treated with 15% salt had better microbiological qualities compared to those treated with 10% salt, that the antimicrobial effect of potassium sorbate increased according to the amount of salt applied and thus there existed a synergistic association between salt and potassium sorbate.

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