

## Shelf-Life, Sensory Attributes and Microbiological and Chemical Characteristics of Processed Mirror Carp (*Cyprinus carpio* L.) Fillets

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**Abstract:** In the present study, fresh fillets of mirror carp, a type of carp common in Keban dam lake, were subjected to 10 or 15% salt (NaCl) followed by 0% (control), 1 or 5% potassium sorbate ( $w v^{-1}$ ) treatments prior to vacuum-packaged storage at 4°C. The samples were analyzed during processing steps and on days 7, 14, 28, 42, 56, 70 and 84. Numbers of total aerobic mesophilic, psychrophilic bacteria, anaerobe bacteria, coliforms, *Staphylococcus-Micrococcus*, *Lactobacillus*, yeast and mold were found lower in the samples in 15%-salted and 5% potassium sorbate-treated group. Mold numbers reached to the highest level (4.83-7.49  $\log_{10}$  cfu  $g^{-1}$ ) on day 28 of storage followed by a consistent decrease during the remaining days and dropped below detection limit ( $<1.00 \log_{10}$  cfu  $g^{-1}$ ) on 56-84 days. The differences in mold and yeast numbers between the 10% salted and 15% salted groups were found significant ( $p<0.05$ ). pH reached to the highest level on days 14-28 of storage. Moisture level decreased to the minimum level on 56 day. Salt and  $a_w$  levels were different ( $p<0.01$ ) between salt groups. The levels of TVB-N and TBA constantly increased during storage. Sorbic acid level was determined in all groups between 102.14-593.19 mg/1000 g. Results of the present study revealed that the shelf life of the products was extended up to 2 months, overall acceptability of the products treated with potassium sorbate was higher and level of sorbic acid residues in the products did not possess a health treat.

**Key words:** Mirror carp (*Cyprinus carpio* L.), salted fish, potassium sorbate, storage, quality

### INTRODUCTION

Various processing methods are used to extend the shelf life of fish meat. Such methods provides an advantage not only for utilization of excess amount of fish that are harvested or hunted but also for offering the type of fish products to consumers as they prefer. The main fish processing methods include glazing, freezing, smoking, salting and drying (Hobbs and Hodgkiss, 1982; Horner, 1997).

Salt-preservation of fish is one of the oldest preservation methods, which is still commonly practiced in the world as well as in Turkey. Microbial activity is prevented by the bactericidal effect of salt so that fish meat is protected from microbial spoilage. The level of protection depends on the concentration of salt and as the salt level increase in the product, shelf life extends further (Horner, 1997; Del-Valle and Gonzales-Imigo, 1968).

In modern days, in addition to salt, some other antimicrobial additives are used to inhibit microbial activity, which is the major spoilage factor in foods. Among those, organic acids are of importance as indicated by the particular emphasis of preservation of fish meat by organic acids in recent studies. Among organic acids, sorbic acid and its salts such as potassium, calcium, or sodium sorbates are the most frequently used preservatives. Initially, sorbic acids and sorbates were thought to be effective only against yeast and molds. After their inhibitory effects on some bacterial species have been revealed, importance of sorbates in food preservation as well as in fish preservation has substantially increased (Saldamli, 1985; Sofos and Busta, 1981).

Sorbic acid and its salts provide important advantages in food preservation. These substances are in Generally Regarded as Safe (GRAS) status and easily metabolized by human body. The sorbates do not change

the sensory characteristics of the food when used at appropriate levels. They are highly soluble in water, have antimicrobial activity at elevated pH (i.e., pH 6.0-6.5) and inhibit formation of TMA in fish. Other advantages include low cost, ease of use and synergistic antimicrobial effect when used together with other preservative substances (Sofos and Busta, 1981; Shaw *et al.*, 1983).

Mirror carp (*Cyprinus carpio* L.) is a common carp that has been adapted in Keban dam lake. As a result, large amount of this fish have been hunted in the region. However, preservation of fresh fish is a major problem due to lack of sufficient refrigeration/freezing. Processing and preservation of fresh fish are seem to inevitable.

In the present study, changes in microbial, chemical and sensory characteristics of mirror carp fillet that are salted and treated with potassium sorbate during processing and vacuum-packaged storage were studied to support the local industry of mirror carp, a type of fish of economic and nutritional value in the region.

## MATERIALS AND METHODS

**Treatment groups:** Mirror carps weighting each between 12.6 and 13.5 kg were used in the present study. The fillets were obtained from the fishes and subjected to drying, salting and held under press for 36 h. Next, potassium sorbate ( $w v^{-1}$ ) were applied to the fillets and then fillets were dried at 25°C for 60 min using a drying oven. The resulting fillets were vacuum packaged using a vacuum packaging machine (Henkelman-TT 300/2). As a result, 6 different treatment groups were prepared. The fish products are then stored at 4°C. The samples were taken on processing steps and on days 7, 14, 28, 42, 56, 70 and 84 for microbiological, chemical analysis. Sensory evaluation was carried out on days 7, 14, 28, 42 and 56. The study was composed of 3 replicates.

**Microbiological analyses:** Pour-plating method has been used for enumeration of microorganisms. Plate Count Agar (PCA) was used for enumeration of total aerobic bacteria.

The plates were incubated at 5°C for 7 days, at 20°C for 3 days and at 35°C for 2 days. Brewer Agar was used for total anaerobic bacteria. The plates were incubated at 30°C for 5 days. Sabouraud Dextrose agar was used for enumeration of molds. The plates were incubated at 30°C for 5 days. Yeast was enumerated using Wort Agar. The plates were incubated at 30°C for 5 days. Coliform bacteria were enumerated using Violet Red Bile Agar (VRBA). The plates were incubated at 30°C for 24 h. Colonies with dark red hale were evaluated as coliforms. Mannitol Salt Agar (MSA) were used for enumeration of *Staphylococcus Micrococcus* sp. The plates were incubated at 37°C for

36-48 h. Rogosa Acetate Agar (AcA) was used for enumeration of *Lactobacillus* sp. Double layered plates were incubated at 30°C for 5 days (Harrigan and McCance, 1976; Rogosa *et al.*, 1951).

**Chemical analyses:** Water activity of the samples were determined by using water activity meter (TESTO-400). Moisture, dry substance, salt (NaCl), salt in dry substance, pH, Total Volatile Basic Nitrogen (TVB-N), Thiobarbituric Acid (TBA), sorbic acid residues of the products were analyzed (AOAC, 1984; Lang and Steinberg, 1980).

**Sensory analyses:** Sensory attributes of the products in treatment groups were evaluated by a 5-person panellist group for colour, odour, appearance, texture, flavour and saltiness. Each criteria was evaluated using a 5-point scale.

**Statistical analyses:** The data were analyzed for multiple variance analysis using SPSS software package. The differences between significant parameters were analyzed using Duncan test (Steel and Torrie, 1981).

## RESULTS AND DISCUSSION

Results of microbiological analysis, chemical analysis and sensory analysis are presented in Table 1-3, respectively.

Total aerobic bacteria were determined at different incubation temperatures (5°C for 7 days, 20°C for 3 days, 35°C for 2 days) in salt-cured, potassium sorbate treated mirror carp fillets. Psychrophilic bacteria are responsible for spoilage of fresh fish meat. It has been indicated in many studies that the psychrophilic bacteria were the predominant flora in spoiled fish. By vacuum packaging the products, growth of psychrophile bacteria are prevented so that shelf-life of the product is extended (Dalgaard *et al.*, 1993). In the present study, the average numbers of psychrophiles (5°C) in the fresh fillets were found as 4.82  $\log_{10}$  cfu  $g^{-1}$ . This level consistently increased during storage and reached to the highest level on days 42-56 of storage period. The difference in numbers of psychrophilic bacteria between 10 and 15% salted groups was significant on day 42 only and there was no significant difference between treatment groups ( $p>0.05$ ) (Table 1). These findings are in agreement with the results of previous study carried out on salt-cured mirror carp (Patir *et al.*, 2001). However, the results differed from those reported for mirror carp lakerda by Gun *et al.* (1996). Psychrophiles determined at 20°C was found as 4.95  $\log_{10}$  cfu  $g^{-1}$ , which increased until day 42. There was no significant difference in the numbers of

Table 1: The finding of microbiological analyses of mirror carp (*Cyprinus carpio* L.) fillets during production and storage ( $\log_{10}$  kob  $g^{-1}$ )

Stage of production and storage	Microorganism	Type of sample						
		10% NaCl (Control)	10% NaCl + 1% ps	10% NaCl + 5% ps	15% NaCl (Control)	15% NaCl + 1% ps	15% NaCl + 5% ps	
Fillet	T. aerob (5°C)	4.82	4.82	4.82	4.82	4.82	4.82	
	T. aerob (20°C)	4.95	4.95	4.95	4.95	4.95	4.95	
	T. mesophilic aerob (35°C)	4.97	4.97	4.97	4.97	4.97	4.97	
	T. mesophilic anaerob	4.06	4.06	4.06	4.06	4.06	4.06	
	Mold	4.05	4.05	4.05	4.05	4.05	4.05	
	Yeast	3.10	3.10	3.10	3.10	3.10	3.10	
	Coliform	3.64	3.64	3.64	3.64	3.64	3.64	
	<i>Staphylococcus-Micrococcus</i>	3.91	3.91	3.91	3.91	3.91	3.91	
	<i>Lactobacillus</i>	2.98	2.98	2.98	2.98	2.98	2.98	
	The end of salting	T. aerob (5°C)	4.59	4.59	4.59	4.39	4.39	4.39
		T. aerob (20°C)	4.85	4.85	4.85	4.71	4.71	4.71
		T. mesophilic aerob (35°C)	4.58	4.58	4.58	4.43	4.43	4.43
T. mesophilic anaerob		4.58	4.58	4.58	3.98	3.98	3.98	
Mold		3.68	3.68	3.68	3.38	3.38	3.38	
Yeast		3.37	3.37	3.37	2.80	2.80	2.80	
Coliform		2.73	2.73	2.73	2.00	2.00	2.00	
<i>Staphylococcus-Micrococcus</i>		4.31	4.31	4.31	4.18	4.18	4.18	
<i>Lactobacillus</i>		3.10	3.10	3.10	2.47	2.47	2.47	
The end of drying		T. aerob (5°C)	4.73	4.85	4.32	4.81	3.88	3.71
		T. aerob (20°C)	5.24	4.76	4.70	4.46	4.65	4.61
		T. mesophilic aerob (35°C)	5.29	5.19	4.75	4.42	4.25	4.16
	T. mesophilic anaerob	4.98 <sup>a</sup>	4.76 <sup>a</sup>	4.61 <sup>ab</sup>	4.29 <sup>bc</sup>	4.65 <sup>ab</sup>	4.18 <sup>c</sup>	
	Mold	3.82	3.31	3.48	3.18	3.24	3.01	
	Yeast	3.67	3.21	3.03	2.87	2.75	2.66	
	Coliform	2.66	3.05	2.37	1.73	1.93	1.38	
	<i>Staphylococcus-Micrococcus</i>	5.10	4.86	4.52	4.32	4.28	4.09	
	<i>Lactobacillus</i>	3.97	3.46	3.15	2.78	2.48	2.36	
	Days 7	T. aerob (5°C)	5.92	5.63	5.29	5.45	4.73	4.50
		T. aerob (20°C)	6.34	6.28	5.95	5.65	5.57	4.82
		T. mesophilic aerob (35°C)	6.38 <sup>a</sup>	6.24 <sup>a</sup>	5.98 <sup>ab</sup>	5.58 <sup>abc</sup>	5.07 <sup>bc</sup>	4.69 <sup>c</sup>
T. mesophilic anaerob		6.53 <sup>a</sup>	6.89 <sup>a</sup>	5.52 <sup>b</sup>	5.48 <sup>b</sup>	4.96 <sup>c</sup>	4.82 <sup>c</sup>	
Mold		4.89	4.43	4.26	4.42	3.72	3.95	
Yeast		4.28 <sup>a</sup>	3.77 <sup>a</sup>	3.58 <sup>a</sup>	3.19 <sup>b</sup>	2.75 <sup>b</sup>	2.60 <sup>b</sup>	
Coliform		2.51 <sup>a</sup>	2.27 <sup>a</sup>	2.10 <sup>ab</sup>	1.43 <sup>bc</sup>	1.42 <sup>bc</sup>	1.14 <sup>c</sup>	
<i>Staphylococcus-Micrococcus</i>		6.00 <sup>a</sup>	5.30 <sup>a</sup>	5.52 <sup>a</sup>	5.67 <sup>a</sup>	4.33 <sup>b</sup>	4.15 <sup>b</sup>	
<i>Lactobacillus</i>		5.17 <sup>a</sup>	4.09 <sup>a</sup>	4.74 <sup>a</sup>	3.61 <sup>a</sup>	3.46 <sup>a</sup>	2.66 <sup>b</sup>	
Days 14		T. aerob (5°C)	6.46	6.05	5.96	6.06	5.76	4.58
		T. aerob (20°C)	6.70	6.59	6.53	6.84	5.87	5.44
		T. mesophilic aerob (35°C)	7.16 <sup>a</sup>	7.06 <sup>a</sup>	6.57 <sup>a</sup>	6.78 <sup>a</sup>	5.92 <sup>ab</sup>	5.16 <sup>b</sup>
	T. mesophilic anaerob	7.86 <sup>a</sup>	7.11 <sup>b</sup>	6.70 <sup>c</sup>	6.66 <sup>c</sup>	6.90 <sup>bc</sup>	6.68 <sup>c</sup>	
	Mold	5.84 <sup>a</sup>	6.08 <sup>a</sup>	5.84 <sup>a</sup>	5.48 <sup>b</sup>	4.76 <sup>b</sup>	4.52 <sup>b</sup>	
	Yeast	5.42	5.88	5.62	5.75	4.21	3.94	
	Coliform	2.48	2.13	1.77	1.41	1.45	1.32	
	<i>Staphylococcus-Micrococcus</i>	6.20	5.56	5.68	6.28	5.31	4.48	
	<i>Lactobacillus</i>	5.64	6.08	5.90	4.99	4.12	4.15	
	Days 28	T. aerob (5°C)	7.67	7.28	7.44	7.46	6.74	6.37
		T. aerob (20°C)	8.15	8.31	8.26	7.55	6.79	7.18
		T. mesophilic aerob (35°C)	8.22	8.21	8.29	7.75	7.55	7.50
T. mesophilic anaerob		8.34 <sup>a</sup>	8.04 <sup>b</sup>	8.02 <sup>b</sup>	7.57 <sup>c</sup>	7.39 <sup>c</sup>	7.16 <sup>cd</sup>	
Mold		7.49 <sup>a</sup>	7.10 <sup>a</sup>	6.71 <sup>ab</sup>	6.98 <sup>a</sup>	5.62 <sup>bc</sup>	4.83 <sup>c</sup>	
Yeast		6.81	7.13	6.78	6.42	5.46	6.09	
Coliform		2.34	2.05	1.96	1.62	1.74	1.43	
<i>Staphylococcus-Micrococcus</i>		6.61	6.04	6.08	6.67	5.89	4.91	
<i>Lactobacillus</i>		7.69 <sup>a</sup>	7.58 <sup>a</sup>	7.22 <sup>ab</sup>	6.11 <sup>b</sup>	4.83 <sup>c</sup>	4.69 <sup>c</sup>	
Days 42		T. aerob (5°C)	9.10 <sup>a</sup>	8.13 <sup>a</sup>	8.60 <sup>a</sup>	7.67 <sup>b</sup>	7.93 <sup>b</sup>	7.52 <sup>b</sup>
		T. aerob (20°C)	9.06	8.67	8.42	8.34	8.07	7.82
		T. mesophilic aerob (35°C)	8.61	8.12	8.25	7.70	7.20	6.41
	T. mesophilic anaerob	9.53 <sup>a</sup>	7.95 <sup>b</sup>	7.82 <sup>b</sup>	7.51 <sup>b</sup>	6.61 <sup>c</sup>	6.18 <sup>c</sup>	
	Mold	6.08	5.68	5.39	5.57	5.14	4.63	
	Yeast	7.97	7.81	7.79	7.53	6.73	6.29	
	Coliform	2.29	1.76	1.84	1.55	1.55	1.42	
	<i>Staphylococcus-Micrococcus</i>	6.45	5.85	5.68	7.01	6.23	5.72	
	<i>Lactobacillus</i>	7.52	7.59	7.56	7.39	6.09	6.05	

Table 1: Continued

Stage of production and storage	Microorganism	Type of sample					
		10% NaCl (Control)	10% NaCl + 1% ps	10% NaCl + 5% ps	15% NaCl (Control)	15% NaCl + 1% ps	15% NaCl + 5% ps
Days 56	T. aerob (5°C)	8.13	8.12	8.58	8.47	8.26	8.32
	T. aerob (20°C)	7.78	7.53	7.33	7.36	7.66	6.97
	T. mesophilic aerob (35°C)	7.64	7.73	7.44	7.50	7.54	7.09
	T. mesophilic anaerob	7.43	6.80	6.45	6.93	6.69	6.86
	Mold	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
	Yeast	8.19	7.15	6.91	7.40	7.20	6.53
	Coliform	1.20	1.20	1.10	1.05	1.10	1.00
	<i>Staphylococcus-Micrococcus</i>	6.32	5.93	5.75	6.69	6.12	6.17
	<i>Lactobacillus</i>	6.60	7.11	6.74	6.92	6.01	6.37
	Days 70	T. aerob (5°C)	7.82	7.72	7.67	7.62	7.78
T. aerob (20°C)		8.32	7.64	7.82	8.64	8.22	8.09
T. mesophilic aerob (35°C)		8.77	8.25	8.17	8.61	8.34	8.11
T. mesophilic anaerob		7.60	7.48	7.70	7.30	7.47	7.60
Mold		<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Yeast		7.51	7.18	6.73	7.11	6.92	6.38
Coliform		1.55	1.32	1.10	1.10	1.23	1.04
<i>Staphylococcus-Micrococcus</i>		6.67	5.71	5.68	7.04	6.00	6.04
<i>Lactobacillus</i>		6.98	7.37	6.68	7.18	6.35	6.44
Days 84		T. aerob (5°C)	7.98	7.84	7.92	7.73	7.94
	T. aerob (20°C)	8.49	8.51	8.11	8.67	9.03	8.17
	T. mesophilic aerob (35°C)	9.33	8.78	8.40	8.23	9.18	8.22
	T. mesophilic anaerob	7.94	7.58	7.84	7.54	7.98	7.38
	Mold	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
	Yeast	7.58	7.24	7.17	7.29	6.19	6.39
	Coliform	1.84	2.19	1.38	1.43	1.58	1.78
	<i>Staphylococcus-Micrococcus</i>	6.75	6.52	6.06	7.27	6.05	6.05
	<i>Lactobacillus</i>	7.10	7.24	6.83	6.65	5.83	5.53

ps: Potassium sorbate ( $w v^{-1}$ ), a-d: Different are significant among values, which different letter in same line ( $p < 0.05$ )

Table 2: Results of chemical analyses of mirror carp (*Cyprinus carpio* L.) fillets during production and storage

Stage of production and storage	Analyses	Type of sample					
		10% NaCl (Control)	10% NaCl + 1% ps	10% NaCl + 5% ps	15% NaCl (Control)	15% NaCl + 1% ps	15% NaCl + 5% ps
Fillet	pH	6.450	6.450	6.450	6.450	6.450	6.450
	$a_w$	0.987	0.987	0.987	0.987	0.987	0.987
	Moisture (%)	78.860	78.860	78.860	78.860	78.860	78.860
	NaCl (%)	-	-	-	-	-	-
	TVB-N (mg/100 g)	5.860	5.860	5.860	5.860	5.860	5.860
	TBA (mg/1000 g)	0.310	0.310	0.310	0.310	0.310	0.310
	Sorbic acid (mg/1000 g)	-	-	-	-	-	-
The end of salting	pH	6.090	6.090	6.090	6.200	6.200	6.200
	$a_w$	0.919 <sup>a</sup>	0.919 <sup>a</sup>	0.919 <sup>a</sup>	0.871 <sup>b</sup>	0.871 <sup>b</sup>	0.871 <sup>b</sup>
	Moisture (%)	74.710 <sup>a</sup>	74.710 <sup>a</sup>	74.710 <sup>a</sup>	71.020 <sup>b</sup>	71.020 <sup>b</sup>	71.020 <sup>b</sup>
	NaCl (%)	6.820 <sup>b</sup>	6.820 <sup>b</sup>	6.820 <sup>b</sup>	10.060 <sup>a</sup>	10.060 <sup>a</sup>	10.060 <sup>a</sup>
	TVB-N (mg/100 g)	5.740	5.740	5.740	4.050	4.050	4.050
	TBA (mg/1000 g)	0.590	0.590	0.590	0.510	0.510	0.510
	Sorbic acid (mg/1000 g)	-	-	-	-	-	-
The end of drying	pH	5.960	6.080	6.070	6.170	6.180	6.190
	$a_w$	0.895 <sup>a</sup>	0.907 <sup>a</sup>	0.901 <sup>a</sup>	0.860 <sup>b</sup>	0.855 <sup>b</sup>	0.847 <sup>b</sup>
	Moisture (%)	74.100 <sup>a</sup>	74.130 <sup>a</sup>	73.550 <sup>a</sup>	69.910 <sup>b</sup>	70.600 <sup>b</sup>	70.050 <sup>b</sup>
	NaCl (%)	6.700 <sup>b</sup>	6.400 <sup>b</sup>	6.370 <sup>b</sup>	10.100 <sup>a</sup>	10.150 <sup>a</sup>	10.280 <sup>a</sup>
	TVB-N (mg/100 g)	5.140 <sup>a</sup>	5.110 <sup>a</sup>	4.830 <sup>ab</sup>	3.990 <sup>bc</sup>	3.870 <sup>c</sup>	3.830 <sup>c</sup>
	TBA (mg/1000 g)	0.860 <sup>a</sup>	0.750 <sup>ab</sup>	0.730 <sup>b</sup>	0.700 <sup>ab</sup>	0.600 <sup>c</sup>	0.590 <sup>c</sup>
	Sorbic acid (mg/1000 g)	-	131.670 <sup>b</sup>	442.470 <sup>ab</sup>	-	120.680 <sup>b</sup>	459.830 <sup>a</sup>
Days 7	pH	5.980	6.050	6.020	6.080	6.110	6.090
	$a_w$	0.884 <sup>a</sup>	0.876 <sup>ab</sup>	0.882 <sup>a</sup>	0.846 <sup>bc</sup>	0.836 <sup>c</sup>	0.839 <sup>c</sup>
	Moisture (%)	74.020 <sup>a</sup>	73.620 <sup>a</sup>	73.200 <sup>a</sup>	69.580 <sup>b</sup>	70.390 <sup>b</sup>	69.160 <sup>b</sup>
	NaCl (%)	6.850 <sup>c</sup>	6.730 <sup>c</sup>	6.680 <sup>c</sup>	10.550 <sup>b</sup>	11.010 <sup>a</sup>	11.220 <sup>a</sup>
	TVB-N (mg/100 g)	7.910	7.960	7.130	8.230	7.540	7.410
	TBA (mg/1000 g)	1.170	1.130	1.220	1.160	1.230	1.010
	Sorbic acid (mg/1000 g)	-	134.340 <sup>c</sup>	449.02 <sup>b</sup>	-	125.480 <sup>c</sup>	493.090 <sup>a</sup>

Table 2: Continued

Stage of production and storage	Analyses	Type of sample					
		10% NaCl (Control)	10% NaCl + 1% ps	10% NaCl + 5% ps	15% NaCl (Control)	15% NaCl + 1% ps	15% NaCl + 5% ps
Days 14	pH	6.000	6.080	6.070	6.110	6.130	6.100
	a <sub>w</sub>	0.879 <sup>a</sup>	0.870 <sup>a</sup>	0.865 <sup>ab</sup>	0.838 <sup>bc</sup>	0.824 <sup>bc</sup>	0.815 <sup>c</sup>
	Moisture (%)	73.490 <sup>a</sup>	73.140 <sup>a</sup>	71.470 <sup>b</sup>	69.080 <sup>f</sup>	69.950 <sup>bc</sup>	68.380 <sup>e</sup>
	NaCl (%)	6.910 <sup>e</sup>	6.860 <sup>e</sup>	6.900 <sup>e</sup>	10.860 <sup>b</sup>	11.610 <sup>a</sup>	11.350 <sup>ab</sup>
	TVB-N (mg/100 g)	9.590	9.300	9.190	9.270	9.100	8.940
	TBA (mg/1000 g)	1.540	1.430	1.310	1.520	1.580	1.370
	Sorbic acid (mg/1000 g)	-	134.870 <sup>e</sup>	477.080 <sup>b</sup>	-	136.810 <sup>e</sup>	550.210 <sup>a</sup>
Days 28	pH	5.970	6.010	6.020	6.060	6.150	6.130
	a <sub>w</sub>	0.866 <sup>a</sup>	0.860 <sup>a</sup>	0.863 <sup>a</sup>	0.806 <sup>b</sup>	0.801 <sup>b</sup>	0.794 <sup>b</sup>
	Moisture (%)	72.880 <sup>a</sup>	71.730 <sup>ab</sup>	70.780 <sup>b</sup>	68.260 <sup>f</sup>	68.640 <sup>e</sup>	67.630 <sup>e</sup>
	NaCl (%)	6.960 <sup>e</sup>	6.920 <sup>e</sup>	6.950 <sup>e</sup>	10.860 <sup>b</sup>	11.760 <sup>a</sup>	11.600 <sup>a</sup>
	TVB-N (mg/100 g)	10.270	9.410	9.210	9.320	9.150	8.970
	TBA (mg/1000 g)	2.140	2.120	1.940	2.210	2.170	1.810
	Sorbic acid (mg/1000 g)	-	139.140 <sup>e</sup>	550.010 <sup>b</sup>	-	144.010 <sup>e</sup>	593.190 <sup>a</sup>
Days 42	pH	5.860	5.830	5.980	5.940	6.100	6.070
	a <sub>w</sub>	0.854 <sup>a</sup>	0.850 <sup>a</sup>	0.857 <sup>a</sup>	0.792 <sup>b</sup>	0.787 <sup>b</sup>	0.776 <sup>b</sup>
	Moisture (%)	71.770	70.530	70.310	68.040	68.390	67.510
	NaCl (%)	6.940 <sup>e</sup>	6.910 <sup>e</sup>	6.930 <sup>e</sup>	10.790 <sup>b</sup>	11.590 <sup>a</sup>	11.300 <sup>ab</sup>
	TVB-N (mg/100 g)	10.450	9.470	9.380	9.620	9.450	9.090
	TBA (mg/1000 g)	2.640 <sup>ab</sup>	2.440 <sup>ab</sup>	2.310 <sup>bc</sup>	2.670 <sup>a</sup>	2.300 <sup>bc</sup>	2.100 <sup>c</sup>
	Sorbic acid (mg/1000 g)	-	136.270 <sup>e</sup>	528.700 <sup>b</sup>	-	142.380 <sup>e</sup>	568.140 <sup>a</sup>
Days 56	pH	5.770	5.790	5.950	5.870	6.110	6.090
	a <sub>w</sub>	0.827	0.796	0.812	0.765	0.764	0.762
	Moisture (%)	70.840 <sup>a</sup>	70.070 <sup>ab</sup>	69.470 <sup>b</sup>	67.300 <sup>f</sup>	67.640 <sup>e</sup>	67.040 <sup>e</sup>
	NaCl (%)	6.490 <sup>e</sup>	6.450 <sup>e</sup>	6.630 <sup>e</sup>	10.590 <sup>b</sup>	11.400 <sup>a</sup>	11.080 <sup>ab</sup>
	TVB-N (mg/100 g)	10.930	10.130	10.640	10.340	9.750	9.390
	TBA (mg/1000 g)	3.300 <sup>a</sup>	2.700 <sup>b</sup>	2.590 <sup>b</sup>	3.240 <sup>a</sup>	2.480 <sup>bc</sup>	2.150 <sup>c</sup>
	Sorbic acid (mg/1000 g)	-	132.970 <sup>e</sup>	499.940 <sup>b</sup>	-	135.960 <sup>e</sup>	530.880 <sup>a</sup>
Days 70	pH	5.740	5.680	5.810	5.860	6.060	6.050
	a <sub>w</sub>	0.854	0.810	0.825	0.799	0.784	0.786
	Moisture (%)	70.900 <sup>a</sup>	70.470 <sup>ab</sup>	69.700 <sup>b</sup>	67.570 <sup>f</sup>	68.580 <sup>e</sup>	68.210 <sup>e</sup>
	NaCl (%)	6.300 <sup>b</sup>	6.250 <sup>b</sup>	6.350 <sup>b</sup>	10.330 <sup>a</sup>	11.040 <sup>a</sup>	10.830 <sup>a</sup>
	TVB-N (mg/100 g)	13.19	12.76	12.090	11.400	10.270	10.370
	TBA (mg/1000 g)	3.59 <sup>a</sup>	2.97 <sup>b</sup>	2.680 <sup>b</sup>	3.400 <sup>a</sup>	2.630 <sup>b</sup>	2.280 <sup>b</sup>
	Sorbic acid (mg/1000 g)	-	121.86 <sup>b</sup>	498.770 <sup>a</sup>	-	120.680 <sup>b</sup>	499.330 <sup>a</sup>
Days 84	pH	5.63	5.64	5.670	5.730	6.000	5.990
	a <sub>w</sub>	0.877	0.849	0.851	0.822	0.831	0.841
	Moisture (%)	71.98 <sup>a</sup>	70.80 <sup>b</sup>	70.400 <sup>b</sup>	69.810 <sup>bc</sup>	69.820 <sup>bc</sup>	69.230 <sup>c</sup>
	NaCl (%)	5.99 <sup>b</sup>	6.01 <sup>b</sup>	6.10 <sup>b</sup>	10.220 <sup>a</sup>	10.900 <sup>a</sup>	10.590 <sup>a</sup>
	TVB-N (mg/100 g)	19.12 <sup>a</sup>	18.82 <sup>a</sup>	15.04 <sup>bc</sup>	15.720 <sup>b</sup>	12.910 <sup>cd</sup>	11.410 <sup>d</sup>
	TBA (mg/1000 g)	4.29 <sup>a</sup>	3.14 <sup>c</sup>	2.83 <sup>cd</sup>	3.640 <sup>b</sup>	2.710 <sup>cd</sup>	2.580 <sup>d</sup>
	Sorbic acid (mg/1000 g)	-	104.93 <sup>b</sup>	478.91 <sup>a</sup>	-	102.140 <sup>b</sup>	483.430 <sup>a</sup>

ps: Potassium sorbate (w v<sup>-1</sup>), a-d: Different are significant among values, which different letter in same line (p<0.05)

psychrophilic bacteria between the treatment groups (p>0.05) (Table 1). Likewise, in a study on Atlantic morina that were immersed to 3% potassium sorbate and stored at 10°C, no significant difference was found between treatment group and control groups (Shaw *et al.*, 1983).

The number of total aerobic mesophilic bacteria (35°C) was 4.97 log<sub>10</sub> cfu g<sup>-1</sup> in fresh fillets. The level of the bacteria increased to 7.5-8.61 log<sub>10</sub> cfu g<sup>-1</sup> on days 28-42 depending on the treatment group followed by different course of progress during the remaining period of storage. Significant differences between the treatment groups were found on days 7 and 14 (p<0.05) (Table 1). This finding is in agreement with the results of a study, in which Mirror carp were stored at 0-2°C (Gelman *et al.*, 1990). However,

results reported on *Bidyamus bidyanus*, a type of freshwater fish, morina and freshwater bass fillets and lakerda of rainbow trout are very different (Shaw *et al.*, 1983; Gun *et al.*, 1996; Gelman *et al.*, 2001; Khuntia *et al.*, 1993).

Growth of anaerobic bacteria causes spoilage of vacuum-packaged seafoods. It has been reported that numbers of anaerobe/facultative anaerobe bacteria were found higher in vacuum-packaged products compared to those packaged with aerobically-packaged foods, as explained by formation of more suitable environment for these type of bacteria as a result of vacuuming (Shalini *et al.*, 2001; Zhuang *et al.*, 1996). The significant difference between treatment groups were found at

Table 3: Results of sensory analyses of mirror carp (*Cyprinus carpio* L.) fillets during storage

Storage day	Criteria	Type of sample					
		10% NaCl (Control)	10% NaCl + 1% ps	10% NaCl + 5% ps	15% NaCl (Control)	15% NaCl + 1% ps	15% NaCl + 5% ps
Days 7	Colour	3.93	4.13	4.33	4.13	4.30	4.40
	Odour	4.13 <sup>c</sup>	4.67 <sup>b</sup>	4.67 <sup>b</sup>	4.73 <sup>ab</sup>	4.93 <sup>a</sup>	4.93 <sup>a</sup>
	Texture	4.40	4.40	4.33	4.27	4.60	4.53
	Flavour	4.13	4.27	4.33	4.27	4.40	4.53
	Saltiness	4.20	4.20	4.27	3.73	3.80	3.80
	Appearance	4.47	4.53	4.53	4.47	4.60	4.67
	Total	25.25	26.20	26.46	25.60	26.63	26.86
Days 14	Colour	3.87 <sup>c</sup>	4.13 <sup>bc</sup>	4.47 <sup>b</sup>	4.06 <sup>c</sup>	4.87 <sup>a</sup>	4.87 <sup>a</sup>
	Odour	4.40	4.60	4.73	4.53	4.80	4.87
	Texture	4.40	4.00	4.33	4.40	4.47	4.53
	Flavour	4.20	4.20	4.27	4.27	4.13	4.07
	Saltiness	3.93	4.13	4.27	3.60	3.53	3.53
	Appearance	4.07 <sup>b</sup>	4.13 <sup>b</sup>	4.13 <sup>b</sup>	4.07 <sup>b</sup>	4.67 <sup>a</sup>	4.80 <sup>a</sup>
	Total	24.87	25.19	26.20	24.93	26.47	26.67
Days 28	Colour	4.00	4.20	4.33	4.20	4.67	4.73
	Odour	4.33	4.40	4.67	4.47	4.80	4.80
	Texture	4.20	4.20	4.13	4.27	4.40	4.33
	Flavour	4.27	4.27	4.33	3.80	4.33	4.40
	Saltiness	4.13	4.00	4.13	3.53	3.60	3.80
	Appearance	3.93 <sup>b</sup>	4.07 <sup>b</sup>	4.67 <sup>a</sup>	4.60 <sup>a</sup>	4.80 <sup>a</sup>	4.73 <sup>a</sup>
	Total	24.86	25.14	26.29	24.87	26.60	26.79
Days 42	Colour	3.73 <sup>b</sup>	4.33 <sup>a</sup>	4.47 <sup>a</sup>	3.80 <sup>b</sup>	4.80 <sup>a</sup>	4.80 <sup>a</sup>
	Odour	3.93 <sup>c</sup>	4.53 <sup>ab</sup>	4.67 <sup>a</sup>	4.00 <sup>bc</sup>	4.53 <sup>ab</sup>	4.73 <sup>a</sup>
	Texture	4.00	4.20	4.20	3.87	4.13	3.80
	Flavour	3.93	4.00	4.07	3.67	3.73	3.60
	Saltiness	3.80 <sup>a</sup>	3.87 <sup>a</sup>	3.87 <sup>a</sup>	3.40 <sup>b</sup>	3.53 <sup>ab</sup>	3.40 <sup>b</sup>
	Appearance	3.73 <sup>c</sup>	4.40 <sup>ab</sup>	4.53 <sup>a</sup>	3.73 <sup>c</sup>	4.20 <sup>b</sup>	4.53 <sup>a</sup>
	Total	23.12	25.33	25.81	22.47	24.92	24.86
Days 56	Colour	2.47 <sup>b</sup>	4.40 <sup>a</sup>	4.67 <sup>a</sup>	2.67 <sup>b</sup>	4.60 <sup>a</sup>	4.33 <sup>a</sup>
	Odour	2.93 <sup>b</sup>	4.47 <sup>a</sup>	4.60 <sup>a</sup>	3.00 <sup>b</sup>	4.47 <sup>a</sup>	4.67 <sup>a</sup>
	Texture	3.33 <sup>bc</sup>	3.93 <sup>ab</sup>	4.13 <sup>a</sup>	3.00 <sup>c</sup>	4.27 <sup>a</sup>	4.33 <sup>a</sup>
	Flavour	3.00 <sup>b</sup>	4.67 <sup>a</sup>	4.27 <sup>a</sup>	2.53 <sup>b</sup>	4.07 <sup>b</sup>	4.17 <sup>b</sup>
	Saltiness	3.27 <sup>abc</sup>	3.40 <sup>ab</sup>	3.47 <sup>a</sup>	2.80 <sup>d</sup>	3.00 <sup>bcd</sup>	2.93 <sup>cd</sup>
	Appearance	2.13 <sup>b</sup>	4.67 <sup>a</sup>	4.73 <sup>a</sup>	2.20 <sup>b</sup>	4.53 <sup>a</sup>	4.53 <sup>a</sup>
	Total	17.13	25.54	25.87	16.20	24.94	24.93

ps: Potassium sorbate ( $w v^{-1}$ ), a-d: Different are significant among values, which different letter in same line ( $p < 0.05$ )

the end of drying and on days 7, 14, 28 and 42 ( $p < 0.05$ ) (Table 1). These results on anaerobic bacteria are different from what was reported for potassium sorbate treated *Lethrinus lentjan* and cat fish at storage (Shalini *et al.*, 2001; Reddy *et al.*, 1995).

Mold is not found in normal flora of fish. These microorganisms are generally of soil origin, which contaminated fish at the time of hunting/harvesting or during processing via equipment. It has been reported that muddy and moldy taste and odor is caused by the growth of *Streptomyces* sp. in fish (Goktan, 1990; Jay, 1996). In the present study, mold numbers were found in fillets as  $4.05 \log_{10} \text{ cfu g}^{-1}$ . At the end of salting, mold numbers in treatment groups were between  $3.38\text{-}3.68 \log_{10} \text{ cfu g}^{-1}$ . At the end of drying, mold numbers decreased in all groups except for control group in 10% salted products. Numbers of molds showed an increase between days 7 and 28 and then started decreasing. In all groups, mold numbers dropped below  $1.00 \log_{10} \text{ cfu g}^{-1}$  on day 56. When the salt level groups are compared, the difference

were found significant on days 14 and 28 ( $p < 0.05$ ) (Table 1). This finding is in consistence with that was reported for mirror carp fillets treated with potassium sorbate added salt cure by Patir *et al.* (2001).

Yeast number in fillets was found as  $3.10 \log_{10} \text{ cfu g}^{-1}$ . The levels of yeasts increased until days 42-56 depending on the treatment followed by different changes during the remaining period. Yeasts number were significantly ( $p < 0.05$ ) different between salt groups (10 and 15%) on day 7 whereas no significant difference was detected between treatment groups ( $p > 0.05$ ) (Table 1). This finding support the results reported by Patir *et al.* (2001).

Coliform bacteria are not found on skin or in muscles of fish, if hunted from the clean waters. Presence of these bacteria indicates presence of fecal contamination. The maximum level of coliforms has been recommended to be 2.30 or  $2.40 \log_{10} \text{ cfu g}^{-1}$  (Jay, 1996; Saunders, 1983). In the present study, initial coliform number, which was found as  $3.64 \log_{10} \text{ cfu g}^{-1}$  in the fillets consistently

decreased during processing and storage. Numbers of coliforms were lower in 15% salted samples (Table 1). This finding supports the conclusion of researchers that sorbates are more effective in the presence of higher concentration of salt, indicating a synergistic effect (Larocco and Martin, 1987).

Staphylococci are common in nature. However, they are not naturally found in seafood. If staphylococci found higher than  $2.0 \log_{10} \text{ cfu g}^{-1}$  in fish, this indicates contamination via workers or the contamination of water with waste/sludge water (Goktan, 1990). In the current study, the numbers of *Staphylococcus-Micrococcus* sp. was found as  $3.91 \log_{10} \text{ cfu g}^{-1}$  in the fillets. The numbers increased during processing and reached to the maximum level on days 28-56 depending on the treatment. After reaching to maximum level, numbers of the bacteria decreased during the remaining storage period. In general, it was seen that the samples containing potassium sorbate contained lower numbers of *Staphylococcus-Micrococcus* sp. (Table 1). This finding is in agreement with the previous studies, in which numbers of staphylococci and *Staphylococcus aureus* were found lower in potassium-sorbate treated products compared to control group (Shalini *et al.*, 2001; Tompkin *et al.*, 1974). The significant difference between treatment groups were found on day 7 ( $p < 0.05$ ).

It is known that *Lactobacillus* sp. are found in fish at lower numbers and the origin of the contamination in fillets was the processing tables and equipment. *Lactobacillus* sp. are generally more resistant to salt and low temperature (Goktan, 1990; Jay, 1996). In the present study, numbers of lactobacilli was  $2,98 \log_{10} \text{ cfu g}^{-1}$  in the fresh fillets, which then consistently increased during storage and reached to  $6,44-7.69 \log_{10} \text{ cfu g}^{-1}$  on days 28-70 depending on the treatment group (Table 1). The significant difference in lactobacilli numbers between the treatment groups were observed on day 28 ( $p < 0.01$ ). This finding is in consistence with the results published by Shalini *et al.* (2001) who reported that numbers of *Lactobacillus* sp. significantly increased in potassium sorbate-treated and vacuum-packaged *Lethrimus lentjan* fillets until the end of 21 day-storage period.

pH of the fillets increased in all groups until days 14-28 whereas, exhibited a different trend depending on the group on days 42-84. There was no significant difference in pH between treatment groups ( $p > 0.05$ ) (Table 2). The course of pH changes was similar to those reported in some studies (Gelman *et al.*, 1990; Shalini *et al.*, 2001; Doesburg *et al.*, 1969) whereas, it was different from those reported for trout lakerda by Yapar (1989). The initial increase in pH was reported to be the result of increase in volatile basic substances such as ammonia caused by bacterial activity (Reddy *et al.*, 1995). The decrease in pH toward the end of storage was the result of increases in lactic acid bacteria (Goktan, 1990).

The  $a_w$  of processed fillets was between 0.836 and 0.884 at the beginning of storage and between 0.822 and 0.877 on day 84. The significant differences in  $a_w$  of the products were found between groups at the end of salting and drying and on days 7, 14, 28 and 42 ( $p < 0.05$ ) (Table 2). This finding is contradictory to a previous study, in which  $a_w$  of fillets of freshwater bass that were processed with various preservatives (sodium benzoate, potassium sorbate and sodium dihydrogen phosphate) remained higher ( $\geq 0.80$ ) during 119 days-storage period (Khuntia *et al.*, 1993).

Moisture of the products determined in 10% salted-products was significantly ( $p < 0.05$ ) higher (Table 2). This finding is not in agreement with the results reported by Yapar (1989), who studied the effect of various salt levels on trout fillets and that by Khuntia *et al.* (1993), who reported that moisture level remained higher in freshwater bass fillets processed with preservatives during storage at  $2.5^\circ\text{C}$ .

Significant differences in salt levels was found between 10 and 15% salted groups as well as between treatment groups ( $p < 0.01$ ) (Table 2). The findings on salt level are similar to those reported by Patir *et al.* (2001) whereas, they were different from those reported by Khuntia *et al.* (1993) and Yapar (1989). The discrepancy in salt level might be explained by differences in fish species used and in salting methods such as dry-salting or brine.

In the current study, TVB-N level, which was  $5.86 \text{ mg } 100 \text{ g}$  in fresh fillets consistently increased during storage and reached to  $11.41-19.12 \text{ mg } 100 \text{ g}$  on day 84. Significant ( $p < 0.01$ ) differences in TVB-N values between treatment groups were found at the end drying and on day 84 (Table 2). This finding is different from the results of Gelman *et al.* (1990), who reported that TVB-N values started increasing on day 8 in potassium sorbate-treated fillets during storage at  $5-6^\circ\text{C}$ . The TVB-N level reported in the current study is well below the recommended maximum level ( $32-40 \text{ mg}/100 \text{ g}$ ). It can be concluded that products in groups were of good quality in terms of TVB-N level. It has been emphasized that keeping the TVB-N level lower compared to control products was the result of inhibitory effects of potassium sorbate and salt combination over microbial activity (Khuntia *et al.*, 1993).

The level of TBA ( $\text{mg malonaldehyde } 1000 \text{ g}$ ), which was  $0.31 \text{ mg}/1000 \text{ g}$  initially constantly increased during processing and storage and reached to maximum level ( $2.58-4.29 \text{ mg}/1000 \text{ g}$ ) on day 84. The difference in TBA level between 10 and 15% salted groups were significant ( $p < 0.05$ ). On the other hand, the difference between the product types were significant at the end of drying and on days 42 ( $p < 0.05$ ), 56, 70 and 84 ( $p < 0.01$ ) (Table 2). It has been reported that factors including

species of fish, fat content and season play essential role in TBA levels (Ruiz-Capillas and Moral, 2001). Our results on TBA changes in fillets are quite different from the results reported by some researchers (Gelman *et al.*, 1990; Ruiz-Capillas and Moral, 2001; Fey and Regenstein, 1982).

In the current study, the level of sorbic acid in 5% potassium sorbate-treated products were about 4 times higher than those treated with 1% (Table 2). LD<sub>50</sub> level of sorbic acid for human was reported to be 10 g kg<sup>-1</sup> of body weight (Sofos and Busta, 1981). World Health Organization (WHO) has established Acceptable Daily Intake (ADI) level for food preservatives, among which sorbates are included as 25 mg kg<sup>-1</sup> (Shaw *et al.*, 1983). Therefore, it can be said about the current study that even products with the highest sorbic acid level does not contain a health treat to consumers. The significant differences in sorbic acid levels were found at the end of drying (p<0.05) and at all sampling intervals during the storage (p<0.001).

The discrepancies between the results of the current study and in those studies mentioned so far might be probably due to differences in species of fished studied, salting method, concentration of salt and sorbate, or storage conditions.

The samples were subjected to taste-panel on days 7, 14, 28, 42 and 56 as fried. Results of sensory analysis showed that products treated with potassium sorbate received significantly (p<0.05) higher scores of acceptance compared to non-potassium sorbate treated products. All products were remained in consumable condition till day 56 of the storage. However, acceptability scores of nonsorbate treated products declined fast as of day 28 of the storage (p<0.05) (Table 3).

## CONCLUSION

As a result, numbers of microorganisms, in general, were lower in products that were subjected to 15% salting and 5% potassium sorbate. Mold numbers increased until day 28 followed by a consistent decrease and finally dropped below <1.0 log 10 cfu g<sup>-1</sup>. TVB-N, TBA and sorbic acid levels remained below the maximum recommended levels indicating that products does not posses a health risk. Our results revealed that the sensory attributes of products containing potassium sorbate was better and the products could be preserved for approximately, 2 months without spoilage.

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