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The Effects of Salt and Storage Temperature on Microbiological Changes in Hot-Smoked Mirror Carp (*Cyprinus carpio* L.)

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Abstract: In this study, microbiological changes during processing and preservation of smoked mirror carp (*Cyprinus carpio* L.) fillets were examined. In the processing phase the brining in two different salt concentration and smoke were used. The conservation is realized in two different ambient temperatures. Starting from raw material, a_w and pH levels as well as mesophilic, psychrophilic, staphylococcus-micrococcus, coliform, the yeast and mould counts were determined at every stage. In conclusion, the period preservation of smoked mirror carp fillets was dependent on salt concentration and preservation temperature.

Key words: Mirror carp (*Cyprinus carpio*), hot smoked, microbiological quality

INTRODUCTION

Smoking is one of the oldest methods of food preservation and is still widely used in fish processing. At present, the effects of brining and smoking on the color and sensory perception of the product are at least as important as the preservation effect (Storey, 1982; Anonymous, 1982; Gogus and Kolsarici, 1992; Hall, 1999; Erkan, 2004). The growth retarding and lethal effect of smoking on the spoilage and pathogenic microflora depends on the contents of salt in the watery phase of the product, temperature, humidity and density of the smoked, duration of smoking, concentration of active components in smoke prepatations and on the time and temperature of heating (Kolodziejsks *et al*., 2002).

The preservative effect of salt has been recognized as being due to a decrease in water activity, less availability to microbial attack and enhancement of functional properties, leading to an increase of the shelf-life time (Harris and Tall, 1994).

Several studies have been reported on the effect of different smoking processes on the fish quality (Hanse *et al*., 1995; Sigurgisladottir *et al*., 2000; Goulas and Kontominas, 2005; Patir and Duman, 2006; Yanar *et al*., 2006) found that brine concentration and brining time affected the texture development of smoked fish.

In this study, it was aimed to determine the microbiological changes in mirror carp (*Cyprinus carpio*) fillets, smoked with hot smoking method using different salt concentrations (5-10%), during the processing and storing in different temperatures.

MATERIALS AND METHODS

The four sample carps used had an average weight of 16.95±3.14 were obtained from the Keban Dam Lake. The fillets were prepared from the fish within 24 h after catching.

After the initial levels of a_w and pH as well as the microbiological conditions have been determined; the samples were carved out from the fish under hygienic conditions endoavouring to obtain fillets of equal dimensions. Then the fillets were washed using tap water. Two groups of samples containing 50 pieces each were brined in two different concentrations (5 and 10%) of salted water in a 3 to 1 volumetric rate bath for 4 h. After a secondary washing, the excess salt dripping is allowed by waiting for an hour. At this point the levels of a_w, pH and the microbiological conditions of the brined samples were determined again.

Smoking process is carried out at 75°C in a semi-mechanic furnace passing the oak saw-dust smoke thorough the fish samples by a horizontal fan during 4 h. Subsequently an aeration period until the sample temperature drops to 20°C was waited. Then the samples were vacuum-packed using a HENKELMAN-T packaging machine.

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Half of the all samples were conserved in the room temperature (20°C) while the second half in a refrigerator (4°C).

This process provided 4 sample groups, namely

- A: 5% brining room temperature
- B: 5% brining refrigerator temperature
- C: 10% brining room temperature
- D: 10% brining refrigerator temperature

The conservation periods varied between 7 to 84 days with the intervals of 7 days first two weeks them 14 days eventually. After each interval the afore-mentioned analyses were repeated. All experiments mentioned above were repeated three times using a fresh but similar fish each time to provide significant average values for the results.

pH values were measured by using a digital pH meter (EDT, GP 335) at 25°C (AOAC, 1990). Water activity (a_w) of samples was determined using an electronic hygrometer (TESTO-400).

For all microbiological counts, 10 g of sample were taken and transferred into 90 mL 0.1% peptone water and homogenized. From the 10^{-1} dilution, other decimal dilutions were prepared.

Enumeration of mesophilic aerobic bacteria: Plate Count Agar (Oxoid Cm 325)) was also used for mesophilic aerobic bacteria count. In this case plates were incubated at 30±1°C for 3 days (Harrigan and McCanse, 1976; ICMSF, 1982).

Enumeration of psychrophilic bacteria: Plate Count Agar (PCA) was also used for psychrotrophic bacteria count. In this case plates were incubated at 7°C for 10 days (APHA, 1976).

Enumeration of staphylococcus-micrococcus: Mannitol salt agar medium (Oxoid) was used to count this group of microorganisms. Plates were incubated at 37±1°C for 48 h and colonies counted after incubation (ICMSF, 1982).

Enumeration of coliform group microorganisms: Violet red bile agar medium (Oxoid) was used to count this group of microorganisms. Plates were incubated at 30±1°C for 24 h. After incubation. Degrading colonies were revealed as coliform group microorganisms (ICMSF, 1982).

Enumeration of yeast-mould: Potato dextrose agar medium with pH adjusted to 3.5 with 10% tartaric acid was used. Plates were incubated at 22±1°C for 5 days and the colonies were counted (ICMSF, 1982).

Statistical evaluations of the physicochemical and microbiological analysis were made by using SPSS 10.0 programmes.

RESULTS AND DISCUSSION

A steep decrease in pH level of all groups are clearly observed after smoking process as expected. The typical values the for pH level measured in this work is 6.4 for raw material and are 5.9 and 5.4 after brining and after smoking, respectively (Table 1). But in the conservation period a readily noticed behavior does not exist; although most of the samples have increasing pH values first two weeks and an almost stable pH level around 5.6 in the remaining weeks. All four groups studied in this conservation period have not shown any significant statistical differences ($p>0.05$).

Kolodziejska et al. (2002) determined the pH values as 6.35 and 6.17 before and after hot smoking for 20% salt brining for frozen mackerel fillets. After 7 days of cold conservation in 2 and 8°C the pH values are measured as 6.10 and 6.25, respectively; on 21st day these values were found to be 6.05 and 6.22. Goulas and Kontominas (2005) reported almost stable pH values for chup mackerel stored 30 days as 6.12 in the beginning of storage then 6.10 and 5.95 for 2°C conservation temperatures. The fresh sample pH value may be considered comparable but the somewhat different values measured in the present experiments may be considered as the outcome of highly different processing technics.

All samples when fresh have approximately similar a_w level around 0.95 (Table 1). Brining drops this value to 0.9 for 10% salt concentration and to 0.93 for 5% salt concentration but all samples reach to 0.9 after smoking process. The decrease in a_w continues almost linearly until 14th day then a dispersed but generally slightly decreasing values are still observed. All samples present inconclusive variations between the values 0.77 to 0.85 after 14th day. In effect the statistical analysis is insignificant ($p>0.05$) (Table 2). It is well known that the temperature effects the vapor pressure hence the water-activity level. Dodds *et al.* (1992) have measured a long variation in the a_w levels of fish ready for consumination smoked could and hot, lowest 0.727, highest 0.997. One might consider the a_w values of this work is comparable to the ones reported ibid.

All fresh samples have almost the same initial values for total mesophilic aerobic bacteria as 5.52 $\log_{10}$ cfu g^{-1} and for total psychrotrophic bacteria as 5.48 $\log_{10}$ cfu g^{-1}. Both counts show a steep decrease after smoking process so that the total mesophilic aerobic bacteria

Table 1: Physicochemical and microbiological (Log_{10} cfu g^{-1}) results obtained in processing phase for mirror carp fillets

Processing	Salt conc. (%)	pH	a_w	*T. mesophilic aerobic*	Psychrophilic	*Staph. micr.*	Yeast-molud	Coliform
Fillets		6.41±0.08	0.958±0.02	5.52±0.08	5.48±0.34	3.63±0.30	3.74±0.08	3.49±0.50
After	5	5.93±0.58	0.927±0.06	6.09±0.63	6.00±0.78	4.07±0.15	4.03±0.16	3.33±0.35
Brining	10	6.00±0.01	0.901±0.00	5.72±0.52	5.31±0.87	3.90±0.40	3.73±0.25	2.82±0.29
After	5	5.45±0.18	0.898±0.07	2.41±1.44	1.45±0.79	1.49±0.85	1.72±0.22	1.00±0.00
Smoking	10	5.59±0.20	0.890±0.01	1.83±0.97	1.00±0.00	1.10±0.17	1.53±0.56	1.00±0.00

Table 2: Physicochemical and microbiological (Log_{10} cfu g^{-1}) results obtained in conservation period for smoked mirror carp fillets

Storage time (day)	Groups	pH	a_w	*T. mesophilic aerobic*	Psychrophilic	*Staph. micr.*	Yeast-molud	Coliform
7	A	5.57±0.25^a	0.873±0.06^a	3.43±1.93^a	1.00±0.00^a	1.82±0.78^a	1.58±0.28^a	1.00±0.00^a
	B	5.53±0.33^a	0.870±0.06^a	3.06±1.57^a	1.00±0.00^a	2.07±1.46^a	1.40±0.35^a	1.00±0.00^a
	C	5.55±0.22^a	0.877±0.01^a	2.98±1.99^a	1.00±0.00^a	3.25±2.10^a	1.33±0.58^a	1.00±0.00^a
	D	5.52±0.24^a	0.837±0.02^a	3.28±1.52^a	1.00±0.00^a	2.09±1.49^a	1.40±0.35^a	1.00±0.00^a
14	A	5.72±0.19^a	0.836±0.02^a	5.39±1.89^a	1.00±0.00^a	4.62±2.37^a	2.65±1.51^a	1.00±0.00^a
	B	5.66±0.25^a	0.854±0.09^a	3.40±2.14^a	1.00±0.00^a	2.74±1.52^a	2.29±1.22^a	1.00±0.00^a
	C	5.57±0.07^a	0.827±0.05^a	3.57±1.97^a	1.00±0.00^a	3.10±2.50^a	1.53±0.50^a	1.00±0.00^a
	D	5.69±0.09^a	0.833±0.05^a	3.12±0.91^a	1.00±0.00^a	1.78±1.35^a	1.00±0.00^a	1.00±0.00^a
28	A	5.68±0.17^a	0.834±0.17^a	4.88±2.03^b	1.00±0.00^a	3.57±2.72^a	2.80±1.48^a	1.00±0.00^a
	B	5.71±0.12^a	0.874±0.08^a	2.08±0.16^a	1.00±0.00^a	2.64±1.55^a	2.02±1.21^a	1.00±0.00^a
	C	5.64±0.02^a	0.790±0.08^a	3.63±1.34^{ab}	1.00±0.00^a	2.70±2.14^a	1.65±1.13^a	1.00±0.00^a
	D	5.52±0.15^a	0.801±0.08^a	3.13±0.82^{ab}	1.00±0.00^a	1.84±1.45^a	1.71±0.77^a	1.00±0.00^a
42	A	5.67±0.21^a	0.849±0.02^a	6.26±1.28^b	1.00±0.00^a	5.26±0.69^b	3.67±0.95^b	1.00±0.00^a
	B	5.80±0.17^a	0.844±0.04^a	2.31±0.20^a	1.00±0.00^a	1.48±0.60^a	2.65±0.90^{ab}	1.00±0.00^a
	C	5.56±0.08^a	0.809±0.05^a	3.87±0.58^a	1.00±0.00^a	2.60±1.46^a	1.62±0.56^a	1.00±0.00^a
	D	5.65±0.24^a	0.819±0.02^a	3.85±1.50^a	1.00±0.00^a	2.37±1.97^a	1.76±0.73^a	1.00±0.00^a
56	A	5.62±0.16^a	0.774±0.04^a	4.40±0.89^{ab}	1.00±0.00^a	3.85±1.56^a	3.42±1.91^a	1.00±0.00^a
	B	5.56±0.14^a	0.797±0.04^a	2.81±1.52^a	1.00±0.00^a	1.16±0.28^a	1.00±0.00^a	1.00±0.00^a
	C	5.43±0.16^a	0.772±0.07^a	5.60±1.61^b	1.00±0.00^a	4.18±2.77^a	2.37±1.59^a	1.00±0.00^a
	D	5.52±0.15^a	0.829±0.03^a	2.74±1.52^a	1.00±0.00^a	1.70±0.60^a	1.73±0.45^a	1.00±0.00^a
70	A	5.34±0.09^a	0.848±0.02^a	3.40±1.00^a	1.00±1.00^a	2.61±1.77^a	1.00±0.00^a	1.00±0.00^a
	B	5.49±0.21^a	0.835±0.07^a	3.83±2.45^a	1.00±0.00^a	2.50±1.49^a	1.99±1.71^{ab}	1.00±0.00^a
	C	5.33±0.23^a	0.812±0.06^a	7.05±0.40^b	1.00±0.00^a	6.40±0.97^b	3.26±0.13^b	1.00±0.00^a
	D	5.33±0.14^a	0.785±0.07^a	3.58±1.71^a	1.00±0.00^a	1.78±0.70^a	1.30±0.52^a	1.00±0.00^a
84	A	5.36±0.05^a	0.781±0.04^a	ND	ND	ND	ND	ND
	B	5.46±0.21^a	0.793±0.07^a	4.84±1.82	1.00±0.00	2.42±0.96	1.39±0.36	1.00±0.00
	C	5.39±0.26^a	0.843±0.02^a	ND	ND	ND	ND	ND
	D	5.48±0.26^a	0.772±0.04^a	2.98±0.98	1.00±0.00	1.65±0.60	1.00±0.00	1.00±0.00

Mean values for a particular column followed by different letter(s) different significantly ($p<0.05$). ND: Not Determined

count drops to the interval 1.83-2.41 log_{10} cfu g^{-1} and total psychrophilic bacteria drops to the interval 1-1.45 log_{10} cfu g^{-1}. In the conservation period the total mesophilic aerobic bacteria count follows a generally increasing path, probably due to the packaging faults. However the total psychrophilic bacteria count presents an almost stable value around 1. with the exception of the group A showing a sudden increase after 70th day. Similar behavior can be observed in the works reported by Colakoglu (2004), Kolsarıcı and Ozkaya (1998).

The behavior of staphylococcus-micrococcus counts, the yeast-mould counts and coliform counts display almost an identical picture. That is all fresh sample counts start from an initial constant valve (3.63, 3.74, 3.49 log_{10} cfu g^{-1}, respectively). After smoking process these valves come down to 1.49 log_{10} cfu g^{-1} for 5% 1.1 log_{10} cfu g^{-1} for 10% sample in the case of staphylococcus-micrococcus same parameters were found to be 1.72 log_{10} cfu g^{-1} for 5% sample, 1.53 log_{10} cfu g^{-1} for 10% sample for yeast and mould counts, finally 1 for coliform (Table 1-2).

In the conservation period the counts obtained for staphylococcus-micrococcus and yeast-mould cases show a generally increasing trend but the coliform counts in all cases present a stable constant value again. Those counts are comparable to the values reported by Rodriguez *et al.* (2002), Dondero *et al.* (2004) and Vishwanath *et al.* (1998).

On the other hand turbulent behavior of mesophilic aerobic bacteria, staphylococcus-micrococcus, yeast and mound night be induced because of the probable contamination imperfeding in the packaging process.

It is concluded that the mirror carp fillets gained a larger conservation period of 14 days approximately by increasing the brining concentration and decreasing the ambient temperature. The microbial quality of the products kept at 20°C was lower than that of fish stored at 4°C. However, most effective is low temperature of refrigerated storage. Thus the implementation of reliable time-temperature indicators of refrigerated fishery products would do much to establish consumer confidence in these products.

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