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Effects of Blast-Freezing and Glazing on Microbiological Changes of Skinless and Skinned Tench (*Tinca tinca* L. 1758) Fillets During Frozen Storage (-18°C)

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Abstract.- In this study, we have investigated the potential changes in the number of total and psychotrophic microorganisms, coliform, *E. coli*, *S. aureus*, *Salmonella* spp. and *Listeria* spp. in the fillet of skinned and skinnless tench fish, which were treated in three different ways and cold-stored for six months. It was concluded that freezing and storing procedures could not destroy the microorganisms totally, and as the best way to inhibit the improvement of microorganisms, one should apply glaze method after treating fish skinnless.

Key word: Blast-freezing, fillet, frozen storage, glazing, tench.

INTRODUCTION

Among many currently used methods for food preservation, those based on the action of low temperatures—refrigeration and freezing—are some of the most important ones (Campanone *et al.,* 2006). At 0°C, growth of microorganisms is considerably reduced and consequently, there is a lower risk for decomposition of the principal ingredients of foodstuffs (Pavlov, 2007). Freezing, therefore is a much preferred technique to preserve food for long periods of time. Besides it does not require chemical agents or irradiation and so assures long-time shelf life (Campanone *et al.,* 2006).

Frozen fish have become an important commodity both for domestic and export markets in a number of countries of the world (Al-Harbi and Uddin, 2005). Storage time and temperature are the major factors affecting the rate of loss of quality and the shelf life of fish (Einen *et al.,* 2002; Arannilewa *et al*., 2005). Bacterial activity which depends on composition of the initial microflora related to the environment where the fish live and are caught, the seasonal period, the fishing method and the early handling, is by far the most important factor influencing fish quality (Al-Harbi and Uddin, 2005). Therefore, it is logical to use bacteria numbers as an index of quality (Suvanich *et al.,* 2000; Al-Harbi and Uddin, 2005).

A freezing process is generally used to preserve quality of products by minimizing microbiological and chemical activites. Although the process may reduce bacterial populations in products to a certain degree, it is not often used to kill bacterial cells. Bacterial content and handling of raw materials, therefore, influence the bacteriological quality of frozen fish (Antony *et al*., 2002). Consequently, fish is one of the most perishable foods and difficult to handle (Al-Harbi and Uddin, 2005). The quality of the finished product depends on the quality of the raw materials and early handling and processing method (Antony *et al.,* 2002). Therefore, products should reach the consumers as fast as possible, as fresh-chilled products to obtain maximum quality (Einen *et al.,* 2002).

The tench is a cyprinid fish species of increasing interest for european pond aquaculture (Kohlmann *et al.,* 2007), but these are commonly inhabits relatively shallow weedy lakes and slow flowing rivers (Altındag *et al*., 1998; Benzer *et al*., 2007). In Europe, tench are utilised as food and for leisure purposes such as angling (Svobodova and Kolarova, 2004). Although tench are wide-spread in Europe, these can also be found in anterior Orient and western Siberia. In Turkey, these fish live in rivers flowing into the Black Sea from Thrace and Northern Anatolia (Altındag *et al*., 1998) and they are consumed locally as skinned and skinless fillets.

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The aim of this study is to investigate the microbiological changes and influence of glazing on bacteria in skinless and skinned tench (*Tinca tinca,* L.1758) fillets stored by freezing at −18°C.

MATERIALS AND METHODS

Raw material and freezing process

Fresh tench (*Tinca tinca* L. 1758) were obtained from a local fish processing company. The tench were caught in 2006 from Beyşehir Lake (Located in Konya Closed Basin of Turkey) and transported well-iced in eight hours to the plant by frigofric vehicle. One-hundred and twenty-six fish were washed, headed, gutted and filleted by hand as two groups with skinless and skinned on the same day and treated in three different ways. At first, some of skinned and skinless fillet samples except the ones which would be used for the beginning analysis were stored at −18°C without blast freezing or applied to glaze treatment (1^{th} group). Another group of skinned and skinless fillets were layed on a tray and placed in a blast freezer (2^{nd} group). So samples were held in a blast freezer until the inner temperature reached -35°C (for six hours) (Fagan *et al.,* 2003). Microbiological analysis were performed after blast freezing. Afterwards, all the samples except the ones which would be subjected to the glaze treatment were conserved at −18°C. The last group of skinned and skinless fillets (3^{rd} group) was subjected to the glaze treatment by plunging them in water including 0.2ppm chlorine at 1±1°C. After glaze treatment, samples were held at −35°C for three hours, and then the treatment was completed. After the completion of glaze treatment, all of the samples were conserved at −18°C upon finishing the analysis the same way.

All the samples which were conserved at −18°C were stored in polyethylene nylons in cardboard packages for six months. During this time, samples were taken randomly each month to study any microbiological changes.

Before analysis, samples which were brought to the laboratory in aseptic conditions were defrozen in polyethylene bags in refrigerator conditions (+4°C for 6 h) and then broken into pieces through sterile blender. Thereafter, 10g and 25g of fish flesh were weighed aseptically and homogenized for 2 min. in stomacher bags containing 90 ml of 0.1 percent of peptone water and 225ml of buffered water and ½ strength Fraser Broth.

Microbiological analysis

Fresh and Frozen tench filletst were analyzed for total and psychrotrophic bacterial load, indicator organisms like total coliforms, *E. coli* and *S. aureus* and human pathogens such as *Salmonella* spp., *Listeria* spp.

The aerobic plate count and psychrotrophic flora count were performed on plate count agar medium incubated for 2 day at 30°C and 10 day at +4°C, respectively (Merck, 1998).

The most probable number (3-tube) method was used to determine the level of total coliforms, and *E. coli* in fish fillet samples. Coliform and *E. coli* were determined using fluorocult lauryl sulfate broth incubated for 24–48 h at 37°C. Gas positive tubes were marked as coliform and controlled through UV portable torch of 366nm long wavelength in a gloomy ambiance. Tubes with florescence beam were marked *E. coli* and final confirmation was made by applying Indole test with the addition of 1ml Kovacs Indole Reagent (Merck, 1998).

Fish homogenate was spread plated onto Baird Parkar agar and incubated at 37°C for 24 to 48 h for estimation of *S. aureus*. Typical colonies were counted, purified and subjected to further coagulase tests (using bactident coagulase) for final confirmation (Antony *et al*., 2002).

Fish sample (25g) was taken aseptically and homogenized with 225ml of buffered water and incubated at 37°C for 24 h for preenrichment. One ml of preenriched sample was transferred to 10ml of tetrathionate broth and incubated at 37°C for 24h for selective enrichment. Enriched sample was streaked onto xylose lysine deoxycholate and *Salmonella-Shigella* agar plates and incubated at 37°C for 24 to 48 h for examination of *Salmonella*. Suspicious colonies were subjected to various biochemical tests for confirmation by using triple sugar iron agar and urea broth (Halkman *et al.,* 1994).

For *Listeria* spp. analysis, 25g of sample were homogenized with ½ Fraser Broth. The homogenate was incubated for 24 hours at 30°C. 0.1ml of the

homogenate was inoculated to 10 ml of Full Fraser Broth culture. In the meantime, PALCAM and OXFORD agars were similarly smeared on the culture. PALCAM and OXFORD agars were incubated for 24 hours at 37°C, and Fraser Broth were incubated for 48 hours at 37°C. PALCAM and OXFORD agar cultures were smeared with Whole-Powered Fraser Broth culture at 24^{th} and 48^{th} hours (Merck, 1998).

After incubation, the plates were checked for typical colonies and suspected colonies were further subjected to biochemical tests for confirmation. All analysis were done in duplicate.

All chemicals were purchased from E.Merck, Germany.

Statistical analysis

To detect significant differences between batches and periods of frozen storage, one-way variance analysis (ANOVA ($P < 0.05$)) was used. This statistical test was done using SPSS 15.0 and results were given as CFU/g.

RESULTS AND DISCUSSION

The initial microbial population of fresh fish and during frozen storage period (–18°C), data of the bacterial counts of skinless and skinned tench (*Tinca tinca* L. 1758) fillets are shown in Table I.

As can be seen from Table I, some pathogen bacteria like coliform, *E. coli, S. aureus* were found in fillets processed skinned and skinless. Ideally, microorganisms such as coliforms, *E. coli*, *Staphylococcus*, should not be found on fresh fish. The presence of pathogenic bacteria should be of concern to fish processors during processing and handling of products (Liston, 1980). Thus, while processing skinned fillets to skinless fillets, though a small amount of reduction in pathogen microorganisms can still be found, existence of pathogen microorganisms caused by processing can still be found. On the other hand, the total aerobic mesophilic bacteria count is also an important indicator of hygienic quality in foods (Temelli *et al.,* 2006). Liston (1980) described that processing equipment, dips for hands and tools, and other products used in fish processing normally carried a microbial load ranging from 10^3 to 10^5 CFU/g per unit measured. Therefore, all of these factors are thought to be effective on the initial amount of microorganisms. However, 10^6–10^7 CFU/g of limit reported to be acceptable with respect to APC (El Marrakchi *et al*., 1990; Özogul *et al*., 2005) were neither at the beginning nor during storage period exceeded. In our study, we did not detect any *Salmonella* spp. and *Listeria* spp.

The microbial population of after air blast frozen and during frozen storage period (-18°C), data of the bacterial counts of both skinless and skinned tench (*Tinca tinca* L. 1758) fillets are shown in Table II.

As can be observed in Table II, microorganism population has dropped after air blast frozen in nearly all groups in comparison with the beginning amounts given in Table I. This reduction was thought to be caused by the influence of freezing application at –35°C on bacteria. Because, it was stated that the damage in cell membranes and DNA denaturation are the probably causes for death of the bacterial cells during freezing and thawing (Panoff *et al.,* 1998; Pavlov, 2007). Similar reduction of microorganisms was observed after glazing process and the results were given in Table III. In addition, disinfectant function of chlorine 0.2 ppm of which was used in glazing could also be factor in the reduction of microorganisms. With regard to freezing process, changes in microorganism population of both skinned and skinless tench fillets are given in Figures 1 and 2. The microorganism amount in both kinds of Tench fillets after the application of glazing process was determined to be less than the beginning amount and the one measured after direct quick freezing without applying glazing. In addition, microorganism amount of the samples which were subjected to the glazing process was found to be less than those which were stored for 6 months at –18°C (Table I/Group1), and which were shocked but stored without being subjected to the glazing process (Table II/ Group2).

Besides, the microorganism amount in skinless Tench fillets was determined to be less than skinned tench fillets. This situation shows that the freezing and treatment processes are effective in microorganism level.

Table I.- Initial microbial count of fresh fish an during frozen storage period (-18°C) (Group 1).

		Initial	1	2	3	4	5	6
APC (CFU/g)	Skinned	$3.1x10^3$	$2.6x10^3$	$3.3x10^3$	$2.7x10^3$	$1.4x10^3$	$3.8x10^3$	$2.1x10^3$
	Skinless	$8.8x10^2$	$7.7x10^2$	$8.6x10^2$	$9.1x10^2$	$7.5x10^2$	$8.5x10^2$	$6.4x10^2$
PFC (CFU/g)	Skinned	$2x10^2$	$2.3x10^2$	$1.6x10^2$	$2.1x10^2$	$1.9x10^2$	$3.3x10^2$	$2.6x10^3$
	Skinless	$6.6x10^1$	$4.8x10^1$	$6x10^1$	$6.7x10^1$	$7.7x10^1$	$7.2x10^1$	$8x10^1$
Coliform (MPN/g)	Skinned	$0.36x10^1$	$0.36x10^1$	$0.92x10^1$	$0.92x10^1$	nd	$0.36x10^1$	$0.92x10^1$
	Skinless	nd	nd	nd	nd	nd	nd	nd
E.coli (MPN/g)	Skinned	nd	nd	$0.36x10^1$	$0.36x10^1$	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
S.aureus (CFU/g)	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
Salmonella spp.	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
Listeria spp.	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd

APC, aerobic plate count; CFU, colony forming unit; MPN, most probable number; Nd: not detected; PCF, psychrotrophic flora count.

Table II.- Microbial count after air blast frozen (-35°C) and during frozen storage period (-18°C) (Group 2).

		After air blast frozen	1	2	3	4	5	6
APC (CFU/g)	Skinned	$3.6x10^4$	$2.8x10^4$	$7.9x10^3$	$9.8x10^3$	10^4	$8x10^3$	$1.1x10^4$
	Skinless	$8.6x10^3$	$6x10^3$	$4.6x10^3$	$2.1x10^3$	$5.1x10^3$	$1.1x10^3$	$4.4x10^3$
PFC (CFU/g)	Skinned	$4.6x10^2$	$4.2x10^2$	$3.4x10^2$	$3x10^2$	$2.8x10^2$	$2.9x10^2$	$2.5x10^2$
	Skinless	$1.8x10^2$	$1.5x10^2$	$1.7x10^2$	$2.1x10^2$	$1.9x10^2$	$2x10^2$	$1.9x10^2$
Coliform (MPN/g)	Skinned	$9.3x10^1$	$9.3x10^1$	$2.3x10^1$	$4.3x10^1$	$4.3x10^1$	$9.3x10^1$	$4.3x10^1$
	Skinless	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$1.5x10^1$	$0.92x10^1$	$0.92x10^1$
E.coli (MPN/g)	Skinned	$0.92x10^1$	$0.92x10^1$	$0.92x10^1$	$0.92x10^1$	$0.92x10^1$	$2.3x10^1$	$0.92x10^1$
	Skinless	$0.36x10^1$	$0.36x10^1$	$0.92x10^1$	$0.92x10^1$	$0.92x10^1$	$0.36x10^1$	nd
S.aureus (CFU/g)	Skinned	$5x10^1$	$6.2x10^1$	$4.4x10^1$	$3.6x10^1$	$5.2x10^1$	$3.8x10^1$	$4.1x10^1$
	Skinless	$0.6x10^1$	$0.8x10^1$	$0.7x10^1$	$0.2x10^1$	nd	$0.1x10^1$	nd
Salmonella spp.	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
Listeria spp.	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd

For abbreviations see Table I.

As can be seen in Tables I-III, neither a stable increase nor decrease in reducing microorganism level was observed during 6 months of storage period. Some degree of reduction occurred after freezing process but this trend did not continue, and the microorganism level remained almost constant in the following months. All these changes were found statistically insignificant ($p>0.05$) during the frozen period.

Al-Harbi and Uddin (2005) described the initial killing rate during freezing is rapid but it is followed by a gradual reduction of micro-organisms. In an another study, Suvanich *et al.* (2000) reported that the APC of channel catfish frame mince decreased from 10^7 to 10^5 CFU/g after 2 months frozen storage; thereafter this remained almost unchanged. The results of our study are compatible with the results of these researchers.

It is concluded that preservation by low temperature storage is highly important in the

Table III.- Microbial count after glazing process and during frozen storage period (-18°C) (Group 3).

		After glazing	1	2	3	4	5	6
APC (CFU/g)	Skinned	$3.1x10^3$	$2.6x10^3$	$3.3x10^3$	$2.7x10^3$	$1.4x10^3$	$3.8x10^3$	$2.1x10^3$
	Skinless	$8.8x10^2$	$7.7x10^2$	$8.6x10^2$	$9.1x10^2$	$7.5x10^2$	$8.5x10^2$	$6.4x10^2$
PFC (CFU/g)	Skinned	$2x10^2$	$2.3x10^2$	$1.6x10^2$	$2.1x10^2$	$1.9x10^2$	$3.3x10^2$	$2.6x10^3$
	Skinless	$6.6x10^1$	$4.8x10^1$	$6x10^1$	$6.7x10^1$	$7.7x10^1$	$7.2x10^1$	$8x10^1$
Coliform (MPN/g)	Skinned	$0.36x10^1$	$0.36x10^1$	$0.92x10^1$	$0.92x10^1$	nd	$0.36x10^1$	$0.92x10^1$
	Skinless	nd	nd	nd	nd	nd	nd	nd
E.coli (MPN/g)	Skinned	nd	nd	$0.36x10^1$	$0.36x10^1$	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
S.aureus (CFU/g)	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
Salmonella spp.	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd
Listeria spp.	Skinned	nd	nd	nd	nd	nd	nd	nd
	Skinless	nd	nd	nd	nd	nd	nd	nd

For abbreviations see Table I.

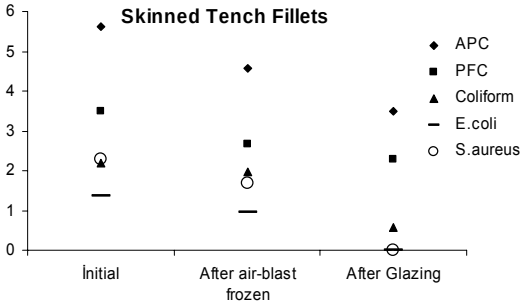

Fig. 1. Change in microorganism count in skinned tench fillet.
APC, aerobic plate count; PCF, psychrotrophic flora count.

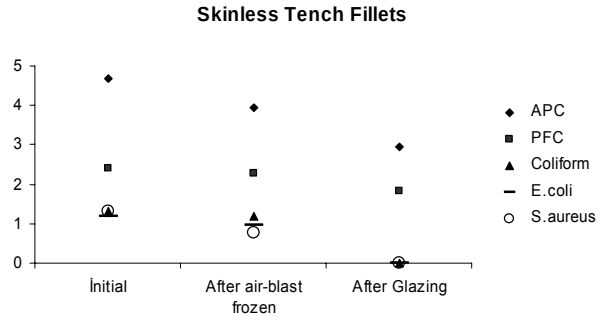

Fig. 2. Change in microorganism count in skinless tench fillet.
APC, aerobic plate count; PCF, psychrotrophic flora count.

maintenance of the quality of frozen fish. From the results of this study, it is evident that the handling method, glazing and frozen temperature are effective on microbial counts. It was determined that freezing and storage applications do not kill microorganism completely, and the method in which Tench fillet is treated skinless and then subjected to glazing was found the best way to prevent microbes.

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