

PREVALENCE OF *STAPHYLOCOCCUS AUREUS* IN FISH PROCESSING FACTORY

Karine Grigoryan, Grigor Badalyan, Djulietta Andriasyan

ABSTRACT

The aim of the presented work was the risk assessment of distribution and prevalence of *Staphylococcus aureus* during processing of cold-smoked fish. There were totally analyzed 80 samples of fish and 50 swab samples at all stages of the processing. *Staphylococcus aureus* was detected in 74% of analyzed samples. Brining stage was the important critical control point in cold-smoked fish processing.

Keywords: *Staphylococcus aureus*, cold -smoked fish, brine

INTRODUCTION

Safety of fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of foodborne pathogens in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (FDA, 2001; Huss, 2003). The handling of fish products during the manufacturing process involves a risk of contamination by *Staphylococcus aureus*, a Gram-positive microorganism causing foodborne human intoxication (Ash, 1997; Shena et al., 2007). These bacteria are salt-tolerant and therefore can contaminate all cured preparations such as cold smoked fish, caviar and fish-based preserves (Hayes, 1995). Fish contains large amount of proteins and their breakdown into amino acids support the growth of *Staph. aureus*. *Staphylococcus spp.* may be isolated from newly caught fish, especially in warm waters (Gram and Huss, 2000). *Staphylococcus* is not found in the normal microflora of fish. This microorganism could be associated with salt (Hansen et al., 1995) or the raw fish (Ferreira et al., 2007) used in the processing. Contamination of fish products through contaminated surfaces has also been observed in many cases (Reij et al., 2003). According to Basti et al., (2003) some kinds of salt smoked fish may be considered as risk of *L. monocytogenes* and *Staph. aureus* infection and intoxication for Iranian consumers respectively. An assessment on the potential microbiological hazard associated with smoked fish fillets under refrigerated storage was made. *Staph. aureus* survived better at both storage temperatures ($T = -10^{\circ}\text{C} - 5^{\circ}\text{C}$) (Mariappan et al., 2004). According to Enclund (2004) contamination of fish by pathogens particularly such as *Staphylococcus aureus*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, and *Listeria monocytogenes*, may occur prior to harvest, during capture, processing, distribution and/or storage. Himelbloom's studies have shown, that strips handled without gloves contained 10^5 *Staphylococcus aureus/g*. Sanitary handling and air quality control will enhance seafood safety while maintaining product quality attributes. (Himelbloom et al., 1998).

In smoked and dried king salmon processed by Alaska Natives, coagulase-negative *Staphylococcus* species comprised 75% of the staphylococci isolates (Himelbloom et al., 1998). Most of the species of *staphylococci*, that have been isolated from fish are coagulase negative, namely, *Staphylococcus epidermidis*, *S. xylosus*, *S. lentus*, *S. capitis*, *S. lugdunensis*, *S. hominis*, *S. warneri*, *S. cohnii*,

S. chromogenes. High *Staph. aureus* counts (10^4 - 10^5 .g⁻¹) occurred and reached 5% of total *staphylococci* counts. Up to 10^4 *Staph. aureus* cells.g⁻¹ can be tolerated in ready-to-eat seafoods (FDA, 2004). Enterotoxin production is typically associated with coagulase-positive *Staph. aureus* when cell populations are 10^5 .g⁻¹ (Jablonski and Bohach, 1997).

The aim of the study was the assessment of prevalence of *Staph. aureus* during manufacturing of cold smoked trout.

MATERIAL AND METHODS

Sampling

There were totally analyzed 80 samples of fish and 50 swab samples.

Fish samples were taken from every step of processing: receiving raw materials, cleaning, separation of fillets, brining, cold smoking, packaging, storing. All samples of fish were collected and placed in sterile polyethylene bags, transported to laboratory and analyzed immediately upon arrival (ISO, 6887-3:2003). Swab samples were taken from all surfaces and tools by Transport swabs (MS 651, Hi Media) (APHA,1992).

Detection of *Staph. aureus*.

For determination of species of *Staph. aureus*, 10g of each samples of fish fillets were removed aseptically using a scalpel and forceps, and then transferred to sterile tubes with 90ml Baird *Staphylococcus* Enrichment Broth Salt (M-1091, Hi media) (ISO, 68881-1999). Tubes were incubated for 24 hours at 37°C. Then samples from every tube were taken with microbiological loop and spread on the surface of solid media Baird-Parker agar (M-043, Hi Media) and Mannitol salt agar (MM-118, Hi Media). All plates were examined visually for typical colony types and morphological characteristics. For confirmation of *S. aureus* strains Hicrom Aureus agar (M1468, Hi Media) was used and following biochemical tests were provided. Those results were confirmed by HiStaph TM-identification kit (Hi Media). For confirmation of DNAase reaction of *Staphylococcus aureus*, 3M Petrifilm Rapid *Staphylococcus aureus* Count plate (3MPetriFilmTM) was used.

Physical-chemical analysis

Moisture was determined by drying oven with the plates being weight until constant weight was reached AOAC 950.46B ; Novoa et al., 1994). Definition of pH was spent with pH-meter (OAKTON, USA). Determination of aw of samples was spent with AquaLab (Decagon Devices, Pullman, WA, USA). Concentration of sodium chloride was

measured by titrimetry methods accordance with the product was decreased to 6×10^2 cfu.g⁻¹. High level of

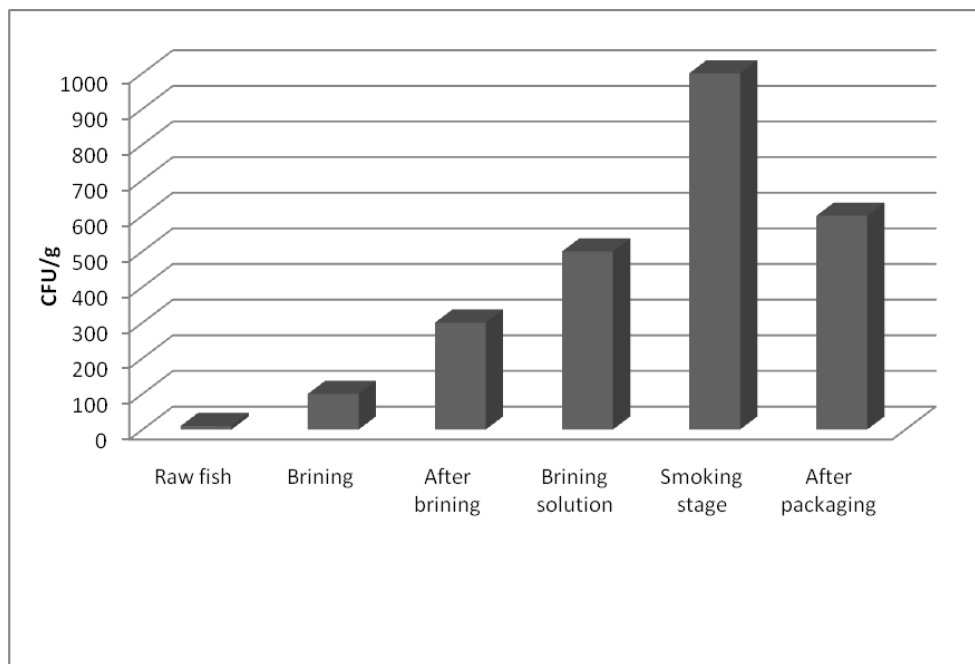


Fig. 1 Prevalence of *Staphylococcus aureus* at every technological step of manufacturing of cold smoked trout.

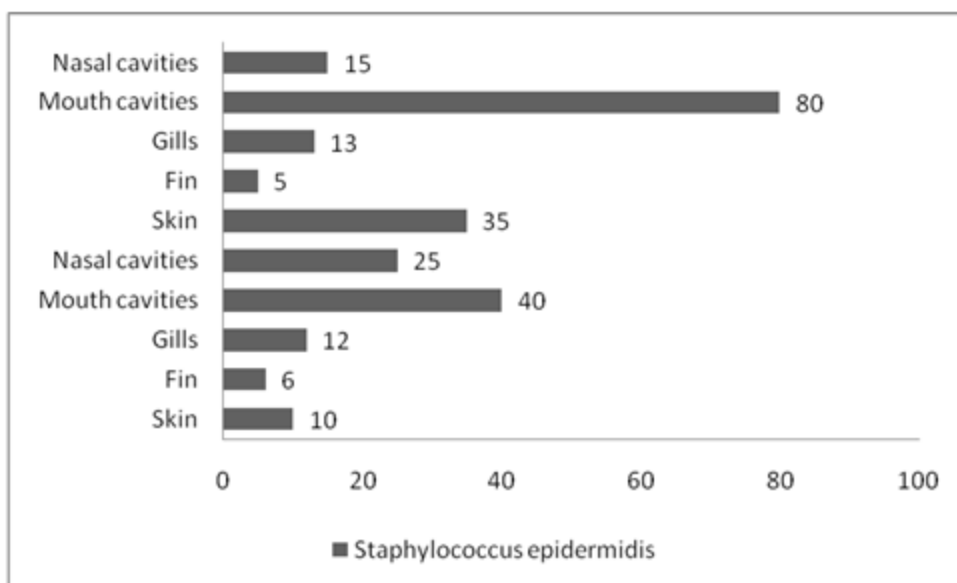


Fig. 2 Distribution of *Staphylococcus aureus* on skin, in gills, nasal and mouth cavities of live fish.

AOAC 937.09.

RESULTS AND DISCUSSION

Contamination level of raw fish by *Staphylococcus aureus* was low (10 cfu.g^{-1}). In samples of frozen fish there was no prevalence of *Staph aureus*. At the beginning of brining stage, contamination level of fish by *Staph. aureus* was 100 cfu.g^{-1} . On third day of brining the contamination level increased up to $3 \times 10^2 \text{ cfu.g}^{-1}$. In the samples of brining solution quantity of *Staphylococcus aureus* reached $5 \times 10^2 \text{ cfu.g}^{-1}$. At the smoking stage the further increase up to 10^3 cfu.g^{-1} was observed. After packaging and during storage, contamination level of the ready

contamination of ready product by *Staph. aureus* was observed in 74% of the analysed samples.

There was studied the distribution and prevalence of *Staphylococcus aureus* on skin, in gills, nasal and mouth cavities of live fish by swabbing method. Results have shown the presence of only coagulase – negative *Staphylococci*, particularly *Staphylococcus epidermidis*.

Swab samples were taken from all processing surfaces and tools (included hooks and racks).

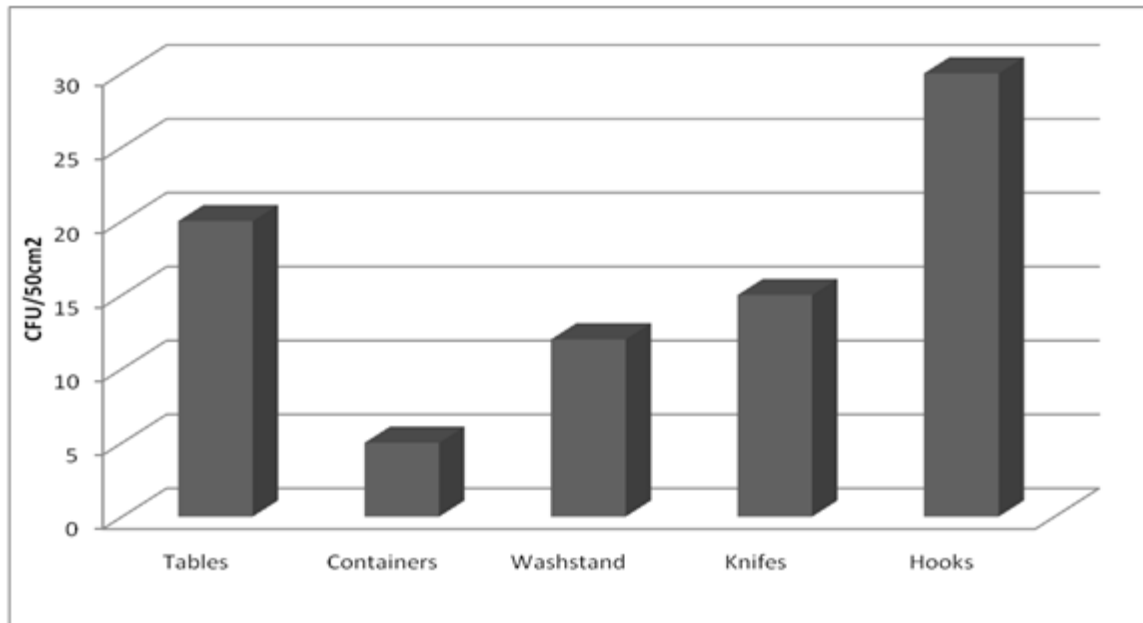


Fig. 3 Recovery count number of *Staphylococcus aureus* from contact surfaces.

High contamination level of hooks and surfaces of tables was observed. Contamination level of hooks and surfaces of tables was 28 cfu.50cm⁻² and 18 cfu.50cm⁻² respectively.

Table 1: Physical-chemical parameters of fish samples during processing

Samples	pH	a _w	NaCl %
raw material	6.9	0.986	-
cleaned fish	6.75	0.975	-
salting	5.60	0.941	5.54
after salting	5.43	0.929	6.1
smoked fish	5.39	0.927	5.2
packaged fish	5.36	0.925	4.3
storage	5.27	0.926	4.2

In all stages the physicochemical parameters were exact and at the same favorable for prevalence of *Staphylococcus spp.* In accordance with Varnam and Evans (1991), the mentioned parameters have a following ratio – pH = 4; NaCl (%) = 10-15; aw = 0.83.

Increase in contamination level was observed at the beginning of brining process. During this stage the microorganisms which are not able to grow at high concentration of salt in comparison with *Staph. aureus* are destroyed. One of the primary sources of contamination of fish by *Staphylococcus aureus* was the multiple use of the same brine solution. Our experiments have shown, that using of the dry – salting technology together with preservatives contributes to considerable decrease in the level of contamination of product after salting process.

CONCLUSION

The primary factors affecting on prevalence of *Staphylococcus aureus* and contamination level of ready to eat fish were identified in this study.

Results of our studies have shown that the brining stage is the important critical control point, during processing of cold smoked fish. Our experiments have shown, that using the dry – salting technology together with preservatives contributes to considerable decrease in the level of contamination of product after salting process. When using liquid brine solution the contamination level of fish is 4x10³ cfu/g, using the dry - salt mixture decreases in the contamination level to 10 cfu/g is occurred. Observance of hygienic requirements while preparing liquid brine solution, and also frequencies of its use accordance with (Storey et al., 1882; CAC/RCP 25-1979) are the important conditions promoting prevention of development and prevalence of *Staphylococcus aureus*.

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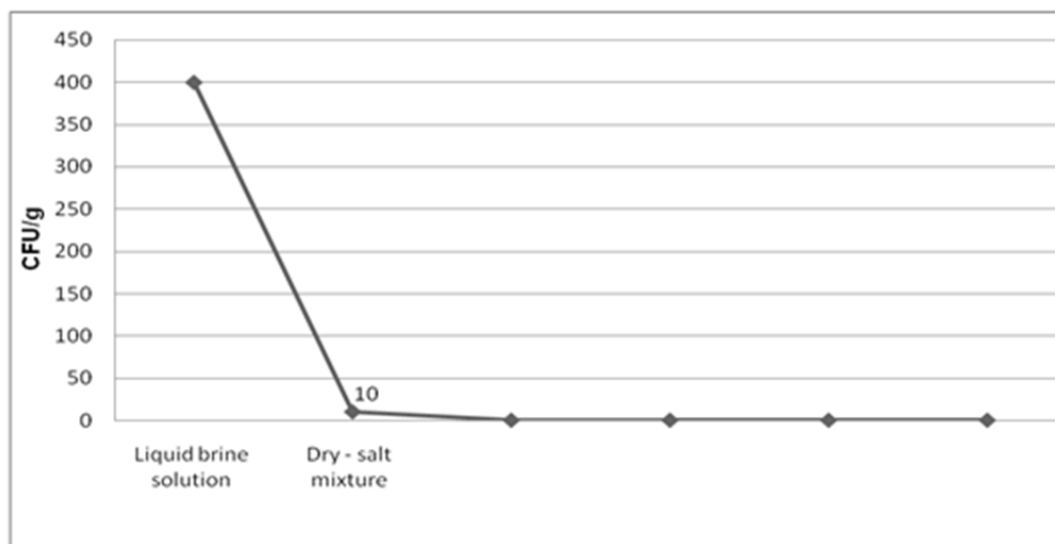


Fig. 4 Level of contamination by *Staphylococcus aureus* when using different salting technologies

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Contact address:

Dr. Karine Grigoryan, Ph.D. Yerevan State University; 1 A. Manoogyan, 0025, Yerevan, Armenia; Tel.:(37410) 55 23 52; e-mail: foodlab@inbox.ru

Grigor Badalyan, Ph.D. student., Yerevan State University; 1 A. Manoogyan, 0025, Yerevan, Armenia; Tel.:(37410) 55 23 52; t-vail: badgri@mail.ru

Djulieta Andriasyan, Ph.D. student Yerevan State University; 1 A. Manoogyan, 0025, Yerevan, Armenia; Tel.:(37410) 55 23 52; e-mail: julietta@gmail.com