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Professional paper

THE INFLUENCE OF HALOPHILIC BACTERIA ON THE FISH QUALITY AND SAFETY

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ABSTRACT

Salting, together with the process of drying and smoking, is one of the oldest ways of fish preserving. Salting acts on the development of microorganisms in such a way that the salt takes away the water, and consequently, growth arrest, sporulation, or death of the bacteria occurs. Salt concentration above 10% in fish meat slows down the development of most putrefactive and proteolytic microorganisms, including pathogenic species. Higher concentrations of salt have a bactericidal effect on bacteria and stop growth, while some species sporulate. Only a relatively small number of microorganisms, called halophiles, can grow on nutrient media with increased salt concentration.

Microorganisms, according to salt sensitivity, are divided into halophile microorganisms, halotolerant microorganisms, and molds. Halophiles are those microorganisms that tolerate increased salt concentrations well and are divided into facultative halophiles that do not need salt for their development, and non-facultative halophiles that need salt for growth and reproduction, in concentrations of 2%. True halophiles, which include species of the genera *Halobacter* and *Halococcus*, can reproduce at salt concentrations of 15-25%. Moderate halophiles, including species from the families Bacillaceae and Vibrionaceae, as well as species from the genus *Micrococcus*, can grow at salt concentrations of 3-15%. Halotolerant species belonging to Micrococcaceae and Corynebacterium tolerate salt concentrations up to 5%. Halotolerant bacteria are bacteria from the genera *Micrococcus, Pseudomonas, Leuconostoc, Vibrio*, and *Streptococcus*. The most sensitive genus to salt is the genus *Clostridium.*

Most halophile microorganisms are not pathogenic for humans, except for bacteria from the genera *Vibrio, Salmonella, Bacillus, Pseudomonas, Staphylococcus, Clostridium*, etc. Spoilage of food caused by halophile bacteria is manifested by a change in its organoleptic properties (color change, the appearance of mucus, and sometimes the appearance of foreign odors and gases).

This paper aims to show the importance and species of halophile bacteria in the processing of food of animal origin, as well as the influence of halophile bacteria on the fish quality and safety.

Keywords: fish spoilage, halophile microorganisms, halotolerant microorganisms, salt.

INTRODUCTION

Salting is one of the oldest ways of fish preserving fish. Salting is considered a chemical way of preserving food and belongs to the category of preserving by dehydration. It can be carried out as dry salting, wet salting, and injection of brine into the meat, as well as various combinations of these procedures (Blitz et al. 2009).

According to Karolyi (2004), in the procedures of preserving fish and fish products, salt has an antimicrobial effect, which means it prevents the development and reproduction of putrefactive bacteria. It has an inhibitory effect on the proteolytic enzymes of meat. Furthermore, it reduces the meat's ability to bind water. Adding 4 - 5% NaCl achieves the greatest increase in water-

binding capacity, and higher concentrations cause protein denaturation and a drop in water-binding capacity.
Table salt inhibits the growth of many aerobic and anaerobic bacteria that cause spoilage of fish meat. The reason for that is the fact that salt reduces the activity of water (a_w), which reduces the share of water that is needed for the growth and development of microorganisms.
Nitrites and nitrates that are added to the brine during the production of fish products also have an antimicrobial effect. Nitrites are bactericidal, but nitrates are not. Nitrates are added, because they become nitrites with reduction, thus indirectly influencing the growth and development of microorganisms. Nitrites have a particularly bactericidal effect on *Clostridium botulinum* and *Staphylococcus aureus*, as well as species of the genus *Salmonella* and *Yersinia*.
This paper aims to show the importance and species of halophile bacteria in the processing of food of animal origin, as well as the influence of halophile bacteria on fish quality and safety. The number and species of bacteria in salty food are different and depend on many factors, including the type of food, the concentration of salt, storage conditions (temperature, packaging method), etc.

The most important halophile microorganisms for the hygiene and safety of fish meat

Microorganisms, according to salt sensitivity, are divided into halophile microorganisms, halotolerant microorganisms, and molds (Bargey's Manual of Systematic Bacteriology, 2003).
Halophiles are those microorganisms that tolerate increased concentrations of salt well and are divided into facultative halophiles that do not need salt for their development, and non-facultative halophiles that need salt for growth and reproduction, in concentrations of 2 % (Beganović, 1975).
True halophiles, that include species of the genera *Halobacter* and *Halococcus*, can reproduce at salt concentrations of 15-25%. Moderate halophiles, including species from the families Bacillaceae and Vibrionaceae, as well as species from the genus *Micrococcus*, can grow at salt concentrations of 3-15%.
Halotolerant species belonging to Micrococcaceae and Corynebacterium tolerate salt concentrations up to 5%. Halotolerant bacteria are bacteria from the genera *Micrococcus, Pseudomonas, Leuconostoc, Vibrio*, and *Streptococcus*. The most sensitive genus to salt is the genus *Clostridiu*m.

Table 1. Weakly halophile microorganisms
(Bergey's Manual of Systematic Bacteriology, 2003)

Weakly halophilic microorganisms		
Genus	**Species**	**Relation to salt**
Clostridium	*C. botulinum* *C. sporogenes* *C. perfringens*	0 - 5 %
Bacillus	*B. cereus*	0 - 2.8 %
Moraxella	*M. lacunata*	1 - 4 %
Acidaminobacter	*A. hydrogenoformans*	3.20 %

Table 2. Moderately halophile microorganisms
(Bargey's Manual of Systematic Bacteriology, 2003)

Moderately halophilic microorganisms		
Genus	**Species**	**Relation to salt**
Marinobacter	*M. hydrocarbonoclasticus*	min. 0.2 - 1M NaCl
Halanaerobium	*H. praevalens*	optimal 13 % NaCl
Halobacterioides	*H. halobius*	8.4 - 14 % NaCl
Aerococcus	*A.viridans*	10 % NaCl
Enterococcus	*E. casseliflavus* *E. disper* *E. durans* *E. faecalis* *E. faecium* *E. gallinarum* *E. hirae* *E. malodoratus* *E. mundtii* *E. solitaries* *E. avium*	6.5 % NaCl
Sporohalobacter	*S. marismortui*	0.5 - 2 M NaCl
Halobacillus	*H. halophilus*	3-10 % NaCl and 0.5 % MgCl2
Methanohalophilus	*M. mahii* *M. halophilus* *M. portucalensis*	1 - 2.5 M NaCl

Table 3. Strongly halophile microorganisms
(Bargey's Manual of Systematic Bacteriology, 2003)

Strongly halophile microorganisms		
Genus	**Species**	**Relation to salt**
Staphylococcus	*S. aureus* *S. epidermidis* *S. saprophyticus*	15 % NaCl
Halococcus	*H. morrhuae*	15 - 20 % NaCl
Halobacterium	*H. salinarum*	min. 15 % NaCl (25 - 30 % NaCl)
Haloferax	*H. volcanii*	1.5 - 2.5 M NaCl
Halomonas	*H. elongata*	20 % and more
Methanohalobium	*M. evestigatus*	> 3 M NaCl
Natrinema	*N. pellirubrum* *N. pallidum*	3.4 - 4.3 M NaCl
Haloarcula	*H. vallismortis* *H. argentinensis* *H. japonica* *H. hispanica* *H. marismortui* *H. mukohataei* *H. quadrata* *H. californiae* *H. sinaiiensis* *H. aidinensis*	2 - 5 M NaCl

Halobaculum	*H. gomorrense*	1.5 - 2.5 M NaCl
Halorubrum	*H. saccharovorum*	1.5 - 5.2 M NaCl
Haloterigena	*H. turkmenica*	15 - 25 %
Natrialba	*N. asiatica* *N. magadii*	2 -5.2 M NaCl
Natronococcus	*N. occultus*	1.4 - 5.2 M NaCl
Natronomonas	*N. pharaonis*	5 M NaCl
Natronorubrum	*N. bangense* *N. tibetense*	3.4 - 3.8

Table 4. Halotolerant bacteria
(Bargey's Manual of Systematic Bacteriology, 2003)

Halotolerant bacteria		
Genus	**Species**	**Relation to salt**
Micrococcus	*M. luteus*	5 - 10 - 15 % NaC
Streptococcus	*S. termophilus*	4 - 10 % NaCl
Leuconostoc	*L. mesenterioides subsp. мesenteroides* *L. lactis*	3 - 6.5 % NaCl
Vibrio	*V. alginolyticus* *V. cincinnatiensis* *V. mimicus* *V. parahaemoliticus* *V. fluvialis* *V. cholerae* *V. damselae* *V. harveyi* *V. furnissii* *V. hollisae* *V. vulnificus*	6 - 10 % NaCl
Sarcina	*S. ventriculi* *S. maxima*	0 - 10 %
Pseudomonas	*P. aeruginosa*	1.0 % NaCl
Salmonella	*S. typhimurium*	0 - 9 % NaCl
Marinococcus	*M. halophilus*	0.5 - 20 % NaCl
Planococcus	*P. citreus*	1 - 15 % NaCl
Salinicoccus	*S. roseus*	optimal 10 % NaCl (0.9 - 25 % NaCl)
Kocuria	*K. varians* *K. rosea*	5 - 10 -15 % NaCl
Burgholderia	*B. cepacia* *B. psudomallei*	1.0 % NaCl
Weisella	*W. paramesenteroides*	3.0 - 6.5 % NaCl

Table 5. Halotolerant molds
(Bargey's Manual of Systematic Bacteriology, 2003)

Halotolerant molds		
Genus	**Species**	**Relation to salt**
Actinokineospora	*A. riparia*	< 5 % NaCl

Actinopolyspora	*A. halophila* *A. mortivallis*	20 % NaC
Amycolata	*A. autotrophica* *A. alni*	5 % NaC
Saccharopolyspora	*S. gregorii* *S. hirsuta* *S. hardei* *S. taberi* *S. rectivigula* *S. spinosa*	10 - 15 % NaC
Terrabacter	*T. tumescens*	5 % NaCl
Primicromonospora	*P. citrea*	9 % NaC

Baros & Matches (1984) stated that most of the moderate halophile bacteria involved in the spoilage of salt-preserved foods are Gram-positive species of the genera *Bacillus, Micrococcus*, and *Halodenitrificans* that have a specific requirement for NaCl. This is also true for moderately halophile *Acinetobacter/Moraxella* species isolated from salted herring, *Vibrio costicola* isolated from bacon brine, and *Vibrio alginolyticus* isolated from seafood. Salted foods that can spoil due to the presence of moderately halophilic microorganisms usually contain high numbers of halotolerant gram-positive bacteria, yeasts, and molds. Highly halophile bacteria are found in environments where the salt concentration is unusually high, as well as in sea salt obtained by evaporation in the sun. These are primarily species of the genera *Halobacterium* and *Halococcus* that produce bright red and pink pigments, grow very slowly, even under optimal conditions, and rapidly lyse when exposed to low salt concentrations (less than 10%).

Presence of halophile microorganisms in fish and fish products

The bacteriological picture of salted fish depends on many factors (fish species, storage conditions, amount of salt or brine, etc.). Most often, the amount of salt acts unfavorably for causing spoilage, but this does not mean that salting is a completely safe procedure. A salt concentration of 9-10% inhibits the growth of spoilage-causing bacteria, except for *Staphylococcus aureus*.

Spoilage of salted fish is caused by halophile bacteria and molds. The presence of halophile bacteria such as *Halobacterium salinarum* is manifested by the appearance of red (pinkish) colored colonies. Halophile bacteria can also originate from seawater.

Gray-brown colors occur because of the growth of halophile molds of the genus *Sporendonema* or *Oospora*. These phenomena can be prevented if salted fish is kept in a cold area (Blaževković - Dimovska, 2016).

During the fish salting, the ambient temperature should be constant. The growth of microorganisms is intense at temperatures higher than 10°C, which characterizes the appearance of a pinkish or gray-brown color of the fish meat. The conditions for mold growth are particularly pronounced if the salt concentration ranges from 5-13%, the air humidity is 75%, and the air temperature is 10-30°C.

Semi-canned foods that are heated to temperatures below 100°C or not subjected to heat treatment but preserved only with table salt, vinegar or other permitted preservatives also contain a certain number of micro-organisms capable of development. Halophile and halotolerant bacteria from the genera *Halobacterium, Micrococcus, Sarcina*, etc. are found in semi-canned fish meat

preserved by salting. Spoilage of canned and semi-canned fish is manifested by changes in the organoleptic properties of the contents, and sometimes by the appearance of bombage.
Moderately psychrophilic bacteria are readily found in salted and smoked fish (Onishi et al. 1980). Vilhelmsson et al. (1996) have isolated 128 strains of halophile bacteria from dried salt cod, as well as from fresh cod and brine. A total of 103 - 107 halophiles have been determined in 1 gram of dried cod. The bacteria formed smooth colonies like those of the bacterium *Halomonas salina* and grew at a salt concentration of 0.1 to 4.5 M NaCl at temperatures of 15 - 37°C.
Pediococcus halophilus is the dominant bacterium at the end of the process of salting and drying the anchovy (*Engraulis encrasicolus*). This bacterium survives in both aerobic and anaerobic conditions, shows growth at a concentration of 6.5 - 10 % NaCl, and tolerates a NaCl concentration of 15 % (Villar et al. 1985).
Šoša (1989) states that many halophile bacteria exhibit proteolytic activity by separating volatile components and producing unpleasant odors. Some of them produce mucus on the surface of salted fish, and many produce red-colored colonies. Some of them are thermophiles, with an optimal growth temperature even at 40 - 45°C. In principle, it can be said that halophile bacteria do not develop at temperatures lower than +5°C and grow at an optimal pH value of 6 - 10.
Salt has an impact on the growth prevention of many pathogenic bacteria in food. A relatively small number of bacteria can live on a substrate with an increased concentration of salt, and of all halophile microorganisms, moderately halophile bacteria that tolerate the presence of 13 - 15% NaCl in food are the most represented. The inhibitory effect of salt is greater in rod-shaped bacteria compared to cocci.
Halophile bacteria in foods of animal origin are usually introduced through sea salt that is obtained by drying it in the sun. Such salt contains 105 - 106 live bacteria per 1g of salt. That is why sea salt should be as clean as possible, without mechanical, chemical, and especially microbiological pollution. Spoilage caused by bacteria of the genus Halobacterium and Halococcus that are present in seawater can be prevented by sterilizing the salt before use.
Foods with a low concentration of NaCl will not encourage the growth of pathogenic bacteria if stored at low temperatures (optimally 4°C). If the temperature at which the food is stored is higher (4 - 12°C), the new conditions may become suitable for the development of pathogenic bacteria and the formation of toxins.
It has been observed that most human diseases caused by halophile bacteria occur in the warmer part of the year, from May to October. It is primarily caused by the consumption of thermally insufficiently processed food. Human health can be threatened by halophile bacteria from the genera *Staphylococcus, Pseudomonas, Vibrio, Clostridium, Bacillus*, as well as species from the genus *Salmonella*.

CONCLUSIONS

Salt inhibits the growth of many aerobic and anaerobic bacteria that cause fish meat to spoil. The reason for that is the fact that salt reduces the activity of water (aw), which reduces the share of water that is needed for the growth and development of microorganisms. Except for the halophile genera, most bacteria will not grow if the water activity is below 0.91. While most fresh food has a water activity of about 0.99, with the processes of salting and bringing, that activity decreases.
Microorganisms, according to salt sensitivity, are divided into halophile microorganisms, halotolerant microorganisms, and molds.

Halophiles are those microorganisms that tolerate increased concentrations of salt well and are divided into facultative halophiles that do not need salt for their development, and non-facultative halophiles that need salt for growth and reproduction, in concentrations of 2 %.
Halotolerant species belonging to Micrococcaceae and Corynebacterium tolerate salt concentrations up to 5%. Halotolerant bacteria are bacteria from the genera *Micrococcus, Pseudomonas, Leuconostoc, Vibrio,* and *Streptococcus*. The most sensitive genus to salt is the genus *Clostridium*.
Spoilage of salted fish is caused by halophile bacteria and molds. The presence of halophile bacteria such as *Halobacterium salinarum* is manifested by the appearance of red (pinkish) colored colonies. Gray-brown colors occur because of the growth of halophile molds of the genus *Sporendonema* or *Oospora*. These occurrences can be prevented if the salted fish is kept in a cool place.
The genera *Halococcus* and *Halobacterium* are responsible for the appearance of pink-colored mucus in salt, brine, and salted fish products, as well as the altered odor and taste normally associated with the spoilage process (hydrogen sulfide and indole).

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