Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2000
Volume 3
Issue 2
**Series FOOD SCIENCE AND TECHNOLOGY**

Copyright © Wydawnictwo Akademii Rolniczej we Wroclawiu, ISSN 1505-0297
DĄBROWSKI W., RÓŻYCKA-KASZTELAN K., KUR J., KOTŁOWSKI R. 2000. LISTERIA MONOCYTOGENES IN SALTED HERRING **Electronic Journal of Polish Agricultural Universities**, Food Science and Technology, Volume 3, Issue 2.
Available Online http://www.ejpau.media.pl

# LISTERIA MONOCYTOGENES IN SALTED HERRING

Waldemar Dąbrowski, Kinga Różycka-Kasztelan, Jozef Kur[1], Roman Kotłowski[1]
*Department of Sea Fishery and Food Technology, Agricultural University in Szczecin, Poland*
*[1]Department of Microbiology, Institute of Technology in Gdansk, Poland*



## ABSTRACT

The following paper includes the results of research, concerning the occurrence of *Listeria* spp in salted herring and herring salads. 100 samples of traditionally and vacuum packed herring and 40 herring salads were examined. It was established that 6.6% of the samples were contaminated with these microorganisms. 4 strains of *Listeria innocua* and 5 strains of *L. monocytogenes* were isolated. *Listeria* was not found in the herring salads, which was explained by the low, <4 pH of the product. Moreover, the ability of *Listeria monocytogenes* to develop in the environment of salted herring was determined. It was established that the pathogenic microorganisms multiplies in the herring, stored at the room temperature. At 10°C the development of *Listeria* is totally inhibited. Sodium benzoate has little influence on the development of *Listeria* because it merely delays the beginning, of the logarithmic phase of the bacteria development by 2 days.

**Key words:** Listeria monocytogenes, salted herring

PRINT

## INTRODUCTION

*Listeria monocytogenes* belongs to so called facultative pathogens of the human. It is quite common in the natural environment. The pathogenic microorganism was isolated from plants, soil, sewage, sweet and sea water, bottom sediments (Watkins 1981, Colbum 1990). The following foods were most frequently reported to be the cause of Listeriosa epidemic: vegetable salad (coleslaw) (Schlech 1983), pasteurised milk (Fleming 1985), soft cheese, frankfurters, hot dogs, tongues in jelly (Jacquet et al.,1995). Apart from vegetables, dairy and meat products, *Listeria monocytogenes* also occurs in fish, crabs, shrimps and oysters (Sikorski 1996). The pathogens can be found both in fresh and frozen products (Weagant and co. 1988), and their amount does not increase when stored at 20°C (Mccarthy 1997). Barckwtt stated that frozen fish, squids and crabs were contaminated with the pathogen in 10%. It is significant that *Listeria*, present in the meat of fish, multiplies quickly at 7°C (Brackett R. E. et all 1990, Mccarthy 1997). Smoking of fish has little influence on the amount of *Listeria*, and a particularly inefficient means of the elimination of *Listeria* is cold smoking (Heinitz and others 1998). Confronting the fact of frequent occurrence of the pathogens in the sea-environment and considering their characteristic physiological features we should also expect them in salted fish because *Listeria monocytogenes* belongs to halo-tolerant bacteria. It is characterised by the ability of growth in nutrient mediums containing up to 10% NaCl. Higher concentration of salt, even up to 20% v/w , does not act bacteriacidally (Petera and co. 1986).

The ability of *L. monocytogenes* to grow in a high concentration of NaCI as well as lack of data in the literature about their occurrence in salted fish were a circumstance to do the research. It is all the more justified because currently mainly a weak salting is used, and therefore the final concentration of NaCI in the products is 7-8%.

The following paper is an introductory report presenting results of the research in which was estimated the occurrence of *L. monocytogenes* in salted herring fillets, available in retail in Szczecin. Moreover, multiplying of these micro-organisms in the process of salting and storing of herring fillets in various conditions was examined.

## MATERIALS AND METHODS

The subject matter of the research was salted herring, packed traditionally - firkins, buckets, and vacuum-packed. Also, vegetable-herring salads were examined. The occurrence of Listeria was studied in food samples weighing 25g each that were homogenised in a stomacher with 225 ml of Listeria Broth, manufactured by "Oxoid" company, with an addition of antibiotics SR. 0.1 ml of homogenate was inoculated on Listeria Agar with an addition of SR, and then incubated at 30°C. The rest of the homogenate remaining in stomacher bags was incubated at the same temperature. Inoculations onto a solid nutrient medium were repeated after 3 and 6 days. Petri dishes with Listeria Agar were incubated for 48 hours at 30°C. Characteristic colonies were subjected to an identification on API tests and haemolytic activity study. Species identity of Listeria monocytogenes was confirmed with a PCR method by determination of the HYL gene presence.

A *Listeria monocytogenes* strain, with a serotype 1, isolated from beef, and characterised by haemolytic activity and positive CAMP test, was used to estimate the *Listeria* reproduction in salted herring. Its taxonomical identity was confirmed with a PCR method. The Icelandic herring fillets were derived from frozen blocks. The salting process was conducted according to a recipe obtained from one of the producers, using brine consisting of: NaC1 - 14%, 10%

PRINT

acetic acid - 6.6%, enzymatic preparation - 0.5%, sodium benzoate - 0.2% in 1:1 ratio to the mass of the not skinned fillets. A 48-hour breed of *Listeria*, $1.5\times 10^5$/g, was added to the salted fillets. The samples were kept at 10°C and 21°C. for 21 days. The amount of Listeria as well as pH of the samples were checked every 3 days.

## RESULTS AND DISCUSSION

It is suggested that Listeria monocytogenes occurring in fish and other sea-food was the most frequent cause of Listeriosa in the humans in the USA from 1997 to 1987. It is because seafood, compared with other kinds of food, is most often contaminated with these micro-organisms (Dillon & Patel 1992). In 1992 Hertemink stated that in Iceland 56% of fresh fish on sale were contaminated with *L. monocytogenes* and other micro-organisms of this species. In Tasmania, salmon bred in fish farms contain these micro-organisms (Sikorski 1996). In 1991 Noach examined lobster tails, shrimps, fish, fresh and frozen fish fillets. He stated that 28% of the samples contained *Listeria* out of which the fresh products were contaminated in 49%, and the processed in 20%. Out of fish products, smoked fish and fish salads are contaminated with these micro-organisms most frequently. Products are infected during production processes. The bacteria are isolated from the surface of fish. They do not occur in the deeper tissues. Deep tissues are most often infected in the processes of cutting off heads, gutting, skinning, filleting. Cold smoking does not eliminate *Listeria*, and in case of their occurrence in deeper tissues there may proceed an intensive multiplying (Eklund and others 1995). Hot smoking decreases the number of positive samples but does not eliminate *Listeria* completely (Heinitz and others 1998). Also, an increase in the concentration of NaCI and an addition of sodium nitrite in smoked salmon do not inhibit the reproduction ability of *Listeria* during refrigeration (Peterson and others 1993, Pelory and others 1994). Our research, whose results we will present in a separate paper, indicates that the smoked fish available in retail in Szczecin are contaminated in a low level with *Listeria monocytogenes*, and the most often found strains are *Listeria innocua*, *Listeria welshomeri* and *Listeria seligeri.*

As it was mentioned before, *Listeria* are micro-organisms whose physiological features such as an ability of growth at low temperatures and halo-tolerance should enable the occurrence and reproduction in salted herring. Results confirmed the presumption. In total 100 samples of salted herring were examined. They were herring fillets, herring fillets a'la Matias, seagoing herring without heads, Matias herring, traditionally and vacuum-packed root Matias herring. The samples were acquired at retail sellers' in Szczecin between January 1997 and January 1998. The samples were subjected to the test of pH that ranged from 3.99 to 5.32 but more had pH higher than 4.5. The research pointed out that 6.6% of the samples contained Listeria. Data concerning the isolation are in table 1. As shown in the table, most of the isolated strains came from samples examined in January 1997. Unfortunately, the obtained results do not allow to establish whether the seasonality of the occurrence of *Listeria* is a binding rule. The samples came from 6 producers. Most often Listeria were isolated from the first producer and the third. It may have been caused by differences in the ways of defrosting of frozen fish blocks. It is interesting that one out of eight positive samples was packed traditionally and 7 vacuum-packed. The concentration of Listeria in the samples was little $<1\times 10^2$/ g. The occurrence of *Listeria* was not reported in the direct inoculation. There was a necessity of a preliminary pre-incubation of the material in the Listeria Broth at 30°C for 3 or 5 days. Having affirmed the occurrence of *Listeria* in salted herring there arises a question whether this product, belonging to the group "ready-to-eat" may constitute an endangerment to the consumers' health. Basing on large-scale research of serology, electrophoretic enzyme diversifications, and DNA structure, Boerlin suggests that strains of *Listeria monocytogenes*,

PRINT

isolated from fish and some cases of human Listeriosa, belong to two different populations (Boerlin and co. 1997). A possibility of the existence of pathogenic strains in raw fish cannot be ruled out. However, considering little concentration of *Listeria* in salted herring, <1 JKT/25 g , we can assume that the danger of illness symptoms in the consumers is little. It is confirmed by the research of Buchanan who analysed a probability of the illness occurrence depending on the quantity of *Listeria* in smoked salmon (Buchanan and co. 1997). Also, the number of yeasts in the samples was determined. There was no confirmed correlation between the number of yeasts that was very big in some of the samples and the quantity of *Listeria*. The occurrence of *Listeria* in herring-vegetable salads was examined in the research, too. In total, 40 salads containing pickled or salted herring, vegetables, and mayonnaise were tasted. Some of the salads were preserved with sodium benzoate. The presence of Listeria was not affirmed in any of the examined products. The lack of *Listeria*, despite the addition of vegetables that could be an extra source of the bacteria, can be explained by the low pH of the products that ranged from 3.8 to 4.2, and the minimum pH, needed for growth, is 4.3 - 5.2 at 30°C, and 5 - 5.7 at 4°C (Farber and others 1989).

**Table 1. Strains of Listeria ssp isolated from salted herring.**

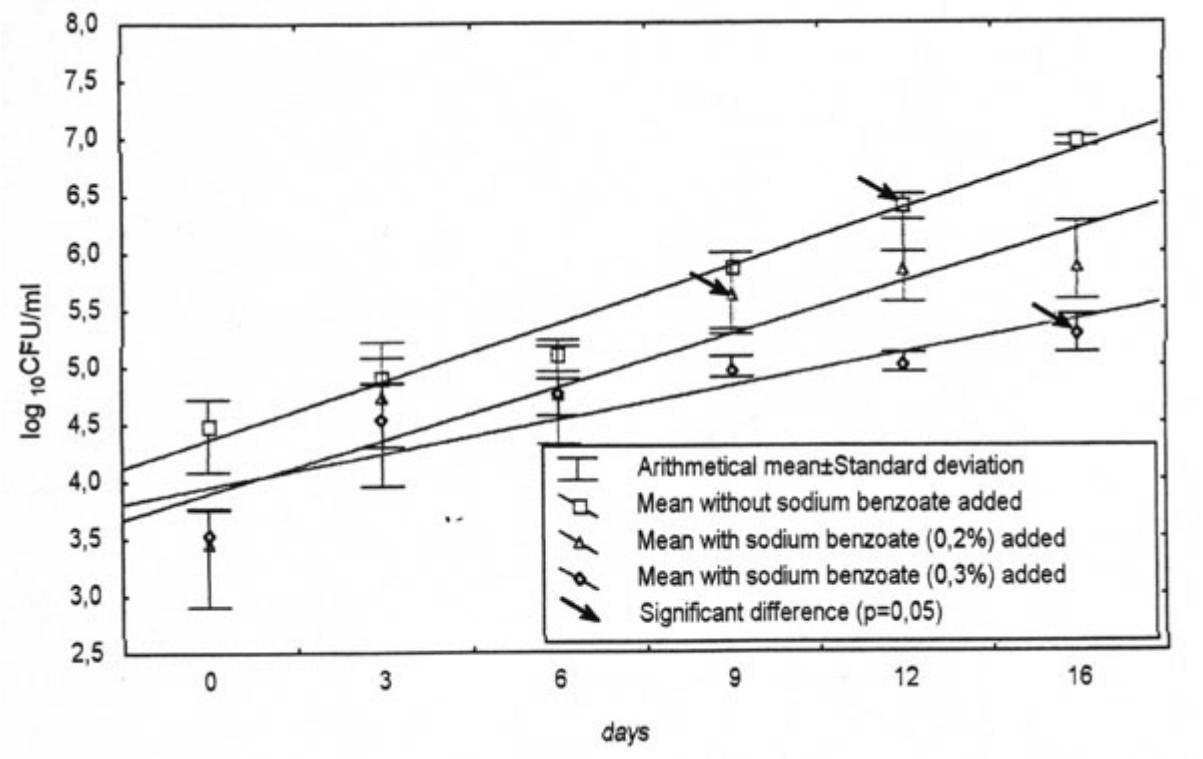
| Product | Package | Producer | Isolated species | Preincubation time [hours] |
|---|---|---|---|---|
| Herring fillets | Standard | A | *L. innocua* | 120 |
| Herring fillets | Vacuum packed | B | *L. innocua* | 120 |
| Herring fillets a'la Matias | Vacuum packed | C | *L. innocua* | 72 |
| Herring fillets a'la Matias | Vacuum packed | C | *L. innocua* | 72 |
| Herring fillets a'la Matias | Vacuum packed | C | *L. monocytogenes* | 72 |
| Herring fillets a'la Matias | Vacuum packed | C | *L. monocytogenes* | 120 |
| Herring fillets | Vacuum packed | A | *L. monocytogenes* | 120 |
| Delicate herring fillets | Vacuum packed | B | *L. monocytogenes* | 120 |
| Spicy Matias | Vacuum packed | B | *L. monocytogenes* | 120 |

The fact of the presence of *Listeria* in salted herring inclined us to determine the abilities of reproduction of *Listeria monocytogenes*. Results in fig. 1 and fig. 2 show that the micro-organisms multiply in this environment. The only barrier inhibiting the growth of *Listeria* was exclusively the temperature. Herring stored at 10°C did not develop any increase in the number of Listeria. In turn, in the product stored at room temperature there was an increase in the number of the bacteria by 3.5 logarithmic units after 7 days. Examining the ability of growth of *L. monocytogenes* in the crab meat, Bracket and Beuchat (1990) stated that the bacteria began to reproduce after 2 days at 10°C and at 5°C - after 5 days. Growth inhibition of *Listeria* in salted herring, stored at 10°C, can be explained by the influence of NaCI. As long as either the temperature of 10°C or 8% NaCI separately does not have an inhibiting

PRINT

influence on the growth of *Listeria* then both of the agents acting simultaneously gave inhibiting effects. An addition of sodium benzoate had little influence on the development of *Listeria*. Nevertheless, it was established that an addition of the preservative delayed the reproduction of *Listeria* by 3 days at room temperature in comparison to a sample without sodium benzoate. The ascertained lack of inhibitory influence of sodium benzoate on *Listeria* can be explained by an adaptation mechanism of the bacteria to preservatives due to the influence of former environmental stresses (Yuqian Lou and co. 1997). The prepared brine also contained acetic acid. When mixed with raw fish, the initial pH level was 4.5-4.66 so it was in the range tolerated by most pathogenic micro-organisms existing in food. In storage of the samples, pH gradually increased, which is depicted in the figures, to finally research 6.39 at 10°C, and 7.13 at room temperature. As it was mentioned before, Faber established that the minimum pH, required for the growth of *Listeria*, was dependent on the temperature, and the lower the temperature, the higher the pH level of the culture medium must be. Well, we cannot then rule out that the environment acidity in the first period of salting was an inhibitory factor for the growth of the bacteria at low temperatures. The above results indicate that salted herring, stored at an improper temperature, can become a dangerous to humans' health consumer product. Observations of shops point out that this product is often sold by the weight from big packages stored at room temperature. Herrings salted in a traditional way were, because of a high concentration of NaCI, regarded as bacteriologically safe products, retaining life even at room temperature. A change in the way of salting in a society carrying conviction of life and bacteriological safety of salted herring may have tragic results. It is then to the purpose to work out a herring salting recipe inhibiting the development of *Listeria monocytogenes*, regardless of storage conditions.

**Figure 1. The growth of *Listeria monocytogenes* in salted herring at 8 °C**




PRINT

**Figure 2. The growth of *Listeria monocytogenes* in salted herring at 20 °C**

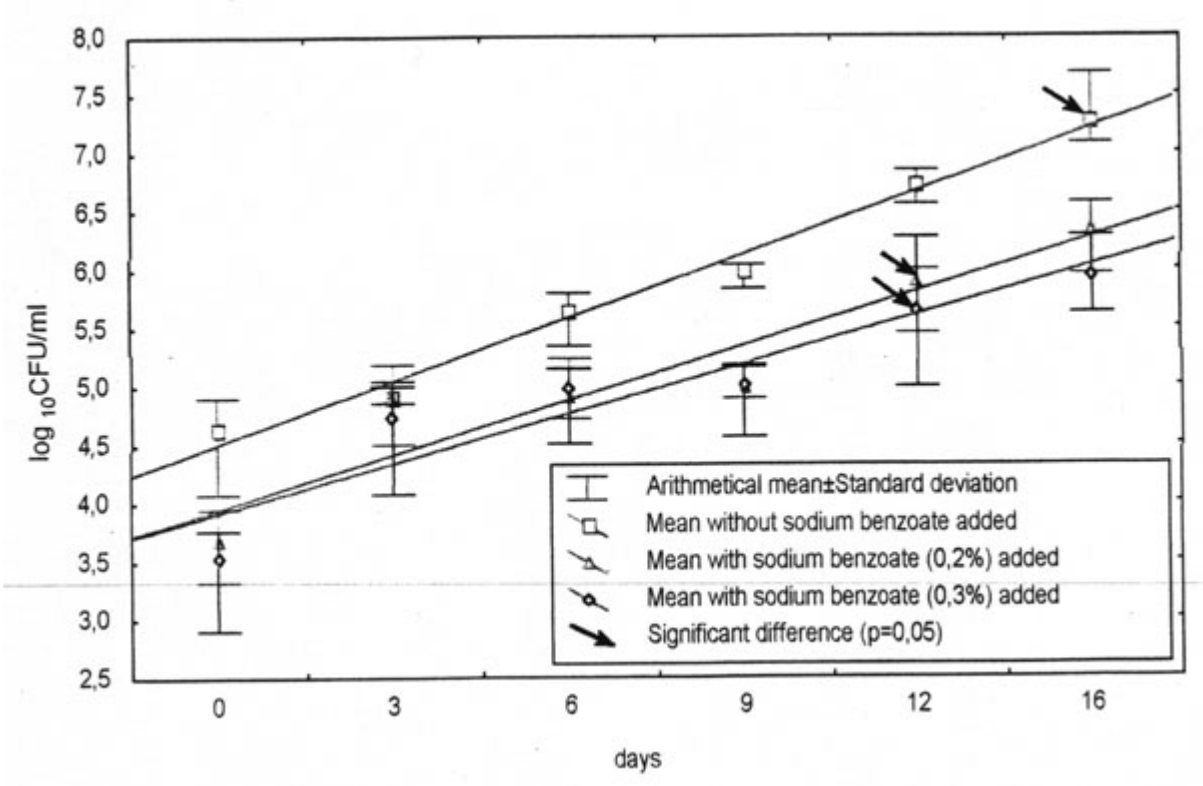
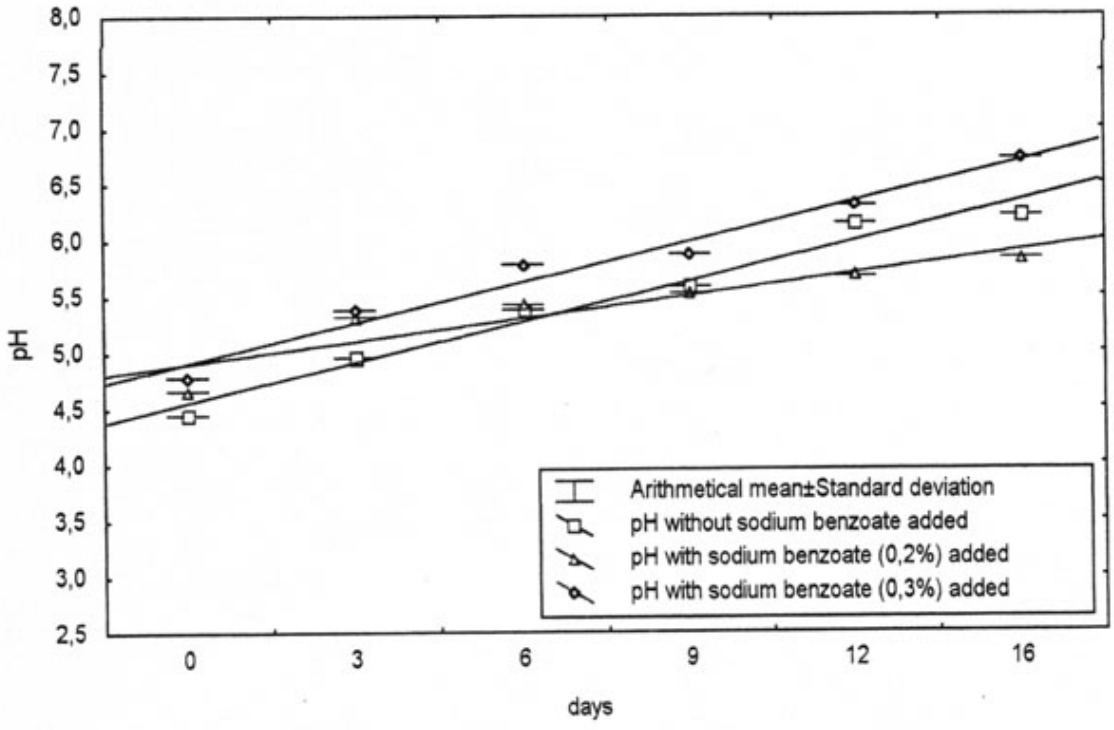
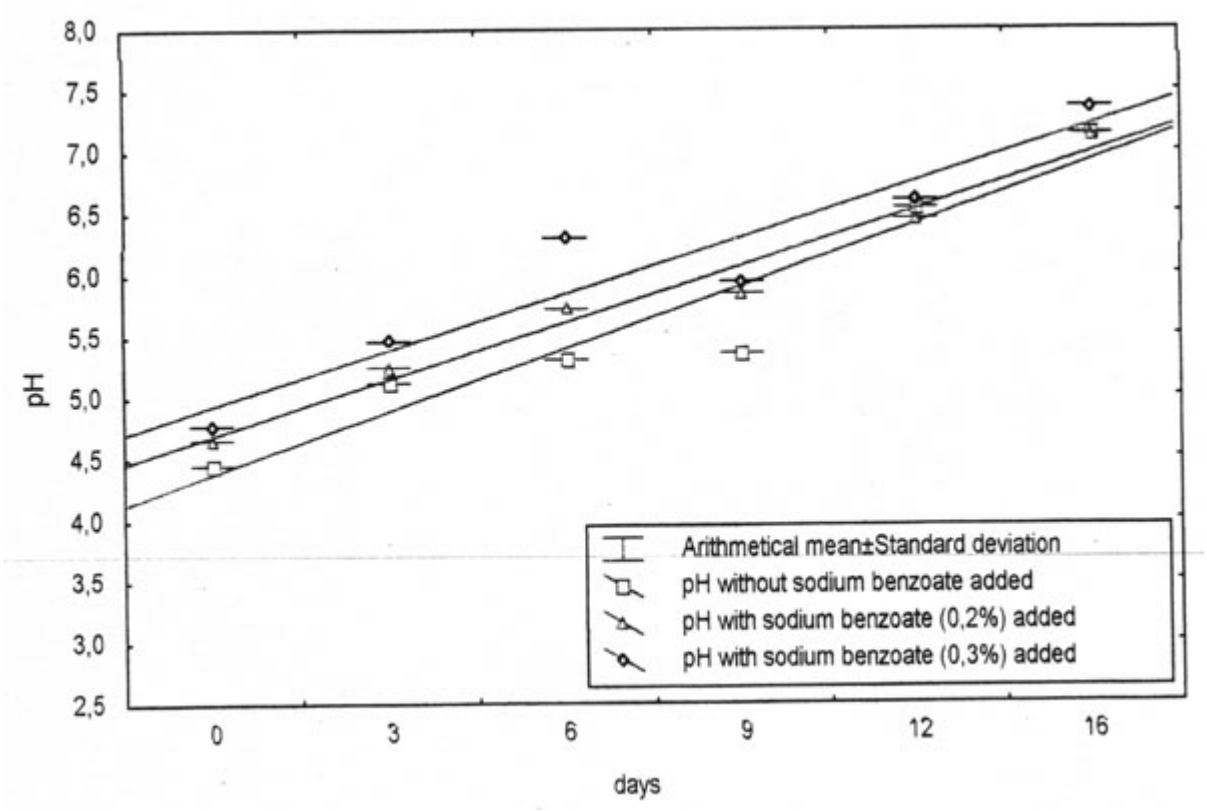



**Figure 3. pH in salted herring at 8 °C.**




PRINT

**Figure 4. pH in salted herring at 20 °C.**




## REFERENCES


1. Bergeys Mannual of Systematic Bacteriology. Vol. 2. Peter H.A., Neeat H, Williams & Wilkins 1986 Pitsburgh. s.1236.
2. Boerlin P., Boerlin-Petzold F., Bannerman E., Bille J., Jemmi T.: Typing *Listeria monocytogenes* isolates from fish products and human listeriosis cases. Appl.. Environ. Microbiol. 1997.63.12521255.
3. Brackett J. G., Beuchat L. R.: Pathogenicity of *Listeria monocytogenes* grown on crabmeat. Appl. Environ. Microbiol.1990.56.1216-1220.
4. Buchanan R. L., Damert W.G., Whiting R. C., van Schothorst M.: Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytogenes* levels and incidence of listeriosis. J.Food Prot. 1997. 60. 918-922.
5. Colbum K. G., Kaysner C. A., Abeyta C., Wekell M. M.: *Listeria* species in a California coast estuarine environmet. AppI.Environm.Microbiol.1990, 56,2007-2011.
6. Dillon R. M., Patel T. H.: *Listeria* in seaoods: A Reviev. J.Food.Prot. 1992 55.1009-1015.
7. Eklund M. W., Poyesky R. N., Lashbrook L. C., Peterson M. E., Pelory G. A.: Incidence nad sources of *Listeria monocytogenes* in cold smoked fishery products and process in plants. J. of Food Prot. 1995, 58,5, 502-508.
8. Farber J. M., Sanders G., Dunfield S., Prescot R.: 1989. The effect of various acidulent on the growth of *Listeria monocytogenes*. Lett. AppI. Microbiol. 9,181-183.
9. Fleming D. W., Cochi S. L., MacDonald K. L., Brondum J., Hayes P. S., Plikaytis B. D., Holme M. B., Audurier A., Broome C. V., Reingold A. L.: Pasteurized milk as vehicle of inection in an outbreak of listeriosis. N.EngI.J.Med. 1985 , 312. 404-407.
10. Guyer S., Jemmi T.: Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoced salmon. Appl. Environm. Microbiol.57.1523-1527.1991.
11. Heinitz M. A., Johanson J. M.: The incidence of *Listeria spp*., *Salmonella spp*., *Clostridium botulinum* in smoked fish and shellfish. J.Food Prot. 1998. 61. 318-323.
12. Hertemink R., Georgsson F.: Incidence of *Listeria* species in seafood and seafood salads. International J.of Food Microb. 1991, 12,2/3, 189-196.


PRINT

13. Jacquet C., Dehaumont P., Goulet V., Lepoutre A., Veit P., Rocourt J.: Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl. Environ. Microbiol. 1995,61,2242-2246.
14. Mccarthy S. A.: Incidence and surviwal of *Listeria monocytogenes* in ready-to-eat seaood products. J. Food Prot. 1997.60.372-376.
15. Pelory G., Peterson M., Paranjpye R., Almond J., Eklund M.: Inhibition of *Listeria monocytogenes* in cold process (smoked) salmon by sodium nitrite and packing method. J. Food Prot.1994.57.1.14-1 19.
16. Petrson M. E., Pelory G. A., Paranjpye R. N., Poysky T., Almond J. S., Eklumd M. W.: Parameters for controls of *Listeria monocytogenes* in smoked fishery products: Sodium chloride and packing method. J. Food Prot. 1993.56.938-943.
17. Schlech W., Lavigne P. M., Bortolussi R. A., Allen A. C., Haldene E. V., Wort A. J., Hightower A. W., Johnson S. E., King S. H. , Nicholls E. S., Broome C. V.: Epidemoic listeriosis - evidence for transmission by food. N. Engl. J. Med. 1983.308.203-206.
18. Sikorski Z. E.: *Listeria monocytogenes* w rybach wędzonych. Medycyna Wet.1996, 52,5,295-297.
19. Weagnt S. D., Sado P. N., Cotburn K. G., Torkelson J. D, Stanley F. A., Krane M. H., Shiefds S. C., Thayer C. F.: The incidence of Listeria species in frozen seafood products. J. Food Prot. 1988. 51.655-657.
20. Watkins J., Sleath K. P.: Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river waters. J. Appl. Bacteriol. 1981, 50.1-9.
21. Wong, Hin-Cung, Wel-Liang Chao, Shiu-Jung Lee.: Incidence and characterisation of *Listeria monocytogenes* in foods aviailable in Taiwan. AppI. Environm. Microbiol. 56.1101-.1990
22. Yoqian Lou, Yousef A. E.: Adaptation to sublethal environmental stress protects *Listeria monocytogenes* against lethal preservation factors. Appl. Environ. Microbiol. 1997.63.1252-1255.

•

---

Submited:

---

Waldemar Dąbrowski, Kinga Różycka-Kasztelan
Department of Sea Fishery and Food Technology
Agricultural University of Szczecin
4 Kazimierza Królewicza, 71-550 Szczecin, Poland
Phone (+48 91) 423 10 61 extension 222
fax (+48 91) 423 13 47

---

Responses to this article, comments are invited and should be submitted within three months of the publication of the article. If accepted for publication, they will be published in the chapter headed ‘Discussions’ in each series and hyperlinked to the article.

---

PRINT