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Microbiological status of smoked fish placed on the market in Poland*

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Summary

Fish and fishery products are considered to be one of the most nutrient-rich dietary products. The nutritional value of fish depends on its freshness. The greatest role in fish spoilage is played by microbial decomposition. Smoking is one of the methods of fish preservation based on the action of temperature and the penetration into the tissue of preservative substances produced by burning wood. Almost all microorganisms except some pathogenic bacteria are destroyed in the smoking process; nevertheless, fish can be a source of some pathogenic microorganisms, i.e. *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus* spp., which are present as the result of cross-contamination at the stage after smoking, i.e. marketing and storage (4, 5, 8). Improper and unhygienic handling practices with the ready-to-eat RTE product are also the cause of an increase of the total microbiological contamination, which can be expressed by microbiological contamination indicators, i.e. total viable count (TVC), psychrophilic bacteria count (PBC), coliform count (EBC), total yeast and mould count (TYMC) and total staphylococci count (TSC). In the performed study five smoked fish species (redfish, mackerel, codfish, sprat and herring) were tested at the marketing stage. A total number of 50 (N = 50) samples was tested according to ISO standards. The potential foodborne bacteria in the tested samples were identified using a MALDI-TOF spectrometer combined with MALDI-Biotyper 3.0 software. In the performed study, the highest microbiology contamination TVC on the level 3.63 ± 0.15 $\log_{10}$ cfu/g and PBC on the level 3.04 ± 0.24 $\log_{10}$ cfu/g has been found in redfish muscle compared to herring, codfish, sprat and mackerel muscle. A significantly lower amount of 1.26 ± 0.12 $\log_{10}$ cfu/g yeast and moulds of was found in the muscle tissue of herring, compared to the other tested fish. The highest staphylococcal contamination of muscle tissue of redfish on the level 2.56 ± 0.15 $\log_{10}$ cfu/g and mackerel on the level 2.13 ± 0.15 $\log_{10}$ cfu/g was found. MALDI-TOF identification of isolated bacteria not confirmed the presence of *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Staphylococci* and confirmed the presence of *E. coli* in tested redfish.

Keywords: smoked fish, fish meat, fish spoilage, *E. coli*, MALDI-TOF

Fish and fishery products are considered to be one of the most nutrient-rich dietary products. In particular, they are a source of high-quality protein and unsaturated fatty acids. The nutritional value of fish also depends on its freshness. The process of fish spoilage begins immediately after harvesting and killing by enzymatic digestion, oxidation of fatty acids and bacterial decomposition. The greatest role in fish spoilage by far is played by microbial decomposition. After the death

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of a fish, bacteria present in the skin and on the gills can enter the muscle tissue, causing decomposition and thus changes in the product's nutritional value. Smoking is one of the methods of fish preservation based on the action of temperature and the penetration into the tissue of preservative substances produced by burning wood. Smoking provides the product with the desired colour, taste, aroma, and longer shelf life obtained by bacteriostatic and antioxidant effects of smoke components and lowering pH. Almost all microorganisms except some pathogenic bacteria are destroyed in the smoking process (2, 7); nevertheless, fish can be a source of some pathogenic microorganisms, i.e., *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus* spp., *Clostridium botulinum* or *Vibrio* spp. (5, 8). Their sources are mainly processes associated with non-hygienic handling of the product after the smoking process (4). Incorrect handling of the finished product is also the cause of the increase in the overall microbial contamination of smoked fish, which can be expressed by microbial contamination indicators: i.e., total viable count (TVC), psychrophilic bacteria count (PBC), *Enterobacteriaceae* count (EBC), total yeast and moulds count (TYMC) and total staphylococci count (TSC).

The study aimed to indicate the initial microbiological contamination of five smoked fish species at the marketing stage and identify the potential foodborne bacteria in the tested samples.

Material and methods

The material for the study included smoked fish from a fish processing plant located in Pomeranian Voivodeship, Poland. The study included five fish species: redfish (*Sebastes norvegius*), mackerel (*Scomber scombrus*), codfish (*Gadus morhua*), sprat (*Sprattus sprattus*), and herring (*Clupea harengus*). The total number of samples tested was 50 (N = 50). From each species, ten samples (n = 10) were tested. The fish were treated with a hot smoking process, then vacuum packed, transported and stored at 4°C until the determinations were performed. Fish muscle tissue without skin from the dorsal region was collected for testing. Before sampling the skin the cutting place was disinfected with 96% ethyl alcohol (Warchem, Poland). For all microbiological counts, 10 g samples were aseptically placed into sterile lab bags with 90 mL of dilution fluid–saline peptone water (BioMaxima, Lublin, Poland) and then were homogenised for 2 min at the normal speed of 230 rpm and as required by the PN-EN ISO 6887-3:2017-05 and PN-EN ISO 7218:2008 standards (14, 16). Other decimal dilutions were prepared from the 10^{-1} dilution and were plated onto dedicated media. The samples to provide TVC and PBC were cultivated on Plate Count Agar (BioMaxima, Poland) and enumerated after incubation at 30°C for 48 h and 0-4°C for 14 days, respectively (6, 13). Bacteria belonging to the EBC group were enumerated in Violet Red Bile with Lactose Agar (BioMaxima, Poland) after incubation at 37°C for 24 h (12). The TSC was estimated using Baird-Parker agar (BioMaxima, Poland) after incubation at 37°C for 24 h (15). The TYMC was ascertained through culture on an agar medium with Dichlorate Rose Bengal and Chloramphenicol LAB-AGAR (BioMaxima, Poland). The culture was incubated at 25°C for five days (11). All bacterial populations were determined as the log of colony-forming units (log CFU g^{-1}). Isolation and determination of Salmonella *spp*. were performed according to PN-ISO 6579-1:2017-04 (10). In order to determine the presence of *Listeria* spp., an examination was performed guided by PN-EN ISO 11290-1:2017:07 (17). The potential foodborne bacteria in the tested samples were identified using a MALDI-TOF spectrometer combined with MALDI-Biotyper 3.0 software (Bruker Daltonik, Germany). The identification of bacterial isolates was preceded by preliminary extraction of proteins with ethanol and formic acid (Merck, Darmstadt, Germany). The obtained supernatant was pipetted onto a metal plate (Anchorchip 800 384, Bruker Daltonik, Bremen, Germany) and dried at room temperature, as a matrix cyano-4-hydroxycinnamic acid (Bruker Daltonik) was used. An automatic measurement of the spectrum and a comparative analysis with reference spectra of bacteria were performed using the UltrafleXtreme mass spectrometer and MALDI-Biotyper 3.0 software (Bruker Daltonik).

The identification in MALDI Biotyper 3.0 is expressed as a score index as follows: 2.300-3.000 is highly probable identification of the micro-organism to species level; 2.000-2.299 is highly probable identification of the micro-organism to genus level and likely identification to species level; 1.700-1.999 is likely identification to genus level; and 0-1.699 is unreliable identification.

The results were analyzed statistically on the basis of arithmetic means and standard deviations. The analysis included a comparison of contamination level by five groups of tested microorganisms TVC, PBC, EBC, TYMC, TSC in five species of smoked fish marketed in Poland. A normal distribution in each group was checked by the Shapiro-Wilk test. The effect of variability factors on the parameters analyzed was established on the basis of a one-way analysis of variance (ANOVA) at the 5% significance level. The post-hoc test with Tukey confidence intervals was applied to establish which species tested differed statistically significantly from each other. The significance of differences between the features under study was determined at a level of $p \leq 0.05$. The computations were made with the Statistica 13 software package (StatSoft).

Results and discussion

The most frequently used hygienic criterion providing information on the level of microbial contamination of food is the total viable count (TVC) and the psychrophilic bacteria count (PBC) for products stored under refrigerated conditions (Tab. 1). The dynamics of bacterial growth in ready-to-eat products (RTE) are mainly influenced by the initial status of microbial contamination and the hygienic conditions during storage. Furthermore in RTE and processed products, the bacterial growth dynamics are more intense, due to the facilitated availability of nutrients. It has been

Tab. 1. Microbiological contamination of smoked fish during marketing (log_{10} cfu/g)

Fish	TVC	PBC	TYMC	EBC	TSC
Herring (*Clupea harengus*)	$2.43^{A} \pm 0.13$	$1.13^{A} \pm 0.14$	$1.26^{A} \pm 0.12$	$1.92^{A} \pm 0.11$	$1.52^{A} \pm 0.20$
Redfish (*Sebastes norvegius*)	$3.63^{B} \pm 0.15$	$3.04^{C} \pm 0.24$	$2.66^{B} \pm 0.15$	$2.71^{B} \pm 0.12$	$2.56^{B} \pm 0.15$
Codfish (*Gadus morhua*)	$2.07^{A} \pm 0.21$	$2.83^{B} \pm 0.32$	$2.42^{B} \pm 0.11$	$2.56^{B} \pm 0.12$	$1.17^{A} \pm 0.11$
Mackerel (*Scomber scombrus*)	$2.47^{A} \pm 0.34$	$2.92^{B} \pm 0.34$	$2.60^{B} \pm 0.12$	$2.59^{B} \pm 0.13$	$2.13^{B} \pm 0.15$
Sprat (*Sprattus sprattus*)	$2.51^{A} \pm 0.14$	$2.85^{B} \pm 0.13$	$2.49^{B} \pm 0.17$	$2.53^{B} \pm 0.12$	$1.08^{A} \pm 0.08$

Explanation: A, B, C – means indicated by different capital letters are significantly different vertically within the bacterial group at $p \leq 0.05$

confirmed in processed products, in fish burgers the TVC was on the level 4.5 log_{10} cfu/g (3), and in the case of mackerel in two weeks of storage, the TVC increased by 2.42 log_{10} cfu/g and finally was 4.89 log_{10} cfu/g (9). It is therefore very important to achieve the highest possible microbiological quality of the product placed on the market. In the performed study, the highest microbiology contamination TVC on the level 3.63 ± 0.15 log_{10} cfu/g and PBC 3.04 ± 0.24 log_{10} cfu/g was found in redfish muscle, in comparison to herring, codfish, sprat and mackerel muscle. The determined level of TVC in the examined fish products was comparable to the TVC contamination of the catfish (*Clarias gariepinus*) (18). The level of TVC determined in the study is comparable to the level of TVC contamination found in the literature and also provides an important hygiene indicator for products that will be placed on the market or stored.

Yeasts and moulds were confirmed in all tested fish muscle samples, and their content varied between species. A significantly lower amount of yeast and moulds of 1.26 ± 0.12 log_{10} cfu/g was found in the muscle tissue of herring, compared to the other fish tested. These results were comparable to those of smoked fish obtained from the Nile River. In the case of the 'Garmout' fish, the initial amount of yeasts and moulds was 2.71 log_{10} cfu/g, and in the 'Kabarous' fish even 2.93 log_{10} cfu/g. Nevertheless, due to the inappropriate temperature and others parameters during smoking, smoked fish may have a relatively low water activity level which is a prerequisite for fungal growth, including *Aspergillus flavus* and *Aspergillus fumigatus* with mycotoxigenic potentials (1, 19). Therefore, it seems to be important to determine the level and species composition of fungi in smoked products.

Coliforms are considered as an indicator of sanitary status, and their presence indicates secondary – mostly of human origin – contamination of the final product. In the study, the lowest amount of *Enterobacteriaceae* was found in the samples of herring muscle tissue and reached 1.92 ± 0.11 log_{10} cfu/g. In the remaining examined samples, their amount was significantly lower and comparable. The presence of *E. coli* was also confirmed in the tested samples of redfish (score index 2.55). It has been confirmed that it is possible to achieve a hygienic status corresponding to 0 cfu/g in smoked fish. Furthermore, a study by Sulieman et al. on the effect of smoking on microbiological status showed the absence of coliform bacteria in the tested smoked fish (19). The presence of EBC and *E. coli* in tested samples could indicate inadequate hygienic practice during handling.

The number of staphylococci bacteria in the tested samples differed significantly between the evaluated fish species. The staphylococcal contamination of muscle tissue of redfish on the level 2.56 ± 0.15 log_{10} cfu/g and mackerel on the level 2.13 ± 0.15 log_{10} cfu/g was found to be significantly higher than in muscle tissue of herring, cod and sprat, where their numbers were comparable. In the identification of isolated bacteria the presence of pathogenic *Staphylococcus* not confirmed. In a study on the microbiological status of smoked fish obtained from Nigeria, the content of coagulase-positive staphylococci was higher, ranging from 3.95 log_{10} cfu/g in herring and 3.78 log_{10} cfu/g in catfish (2). The isolated staphylococci (*Staphylococcus pasteuri*, *Staphylococcus warneri*, *Staphylococcus epidermidis* as well as *Streptococcus intermedius*, *Staphylococcus cohnii*, *Staphylococcus xylosus*) are confirmed as commensal bacteria with increasing infectious potential.

Conclusions:

1. Commercially available smoked fish may have varying microbiological statuses depending on their species.
2. Smoked fish can be a source of *E. coli*.
3. Due to the abundant presence of staphylococci in the muscle tissue of the examined fish, it seems reasonable to undertake further studies on their species identification.

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