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 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 norvegicus), 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 the 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 UltraflexT Xtreme 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 < 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 (RTÉ) are mainly influenced by the initial status of microbial contamination and the hygienic conditions during storage. Furthermore in RTÉ and processed products, the bacterial growth dynamics are more intense, due to the facilitated availability of nutrients. It has been confirmed in processed products, in fish burgers the TVC was on the level 4.5 log10 cfu/g (3), and in the case of mackerel in two weeks of storage, the TVC increased by 2.42 log10 cfu/g and finally was 4.89 log10 cfu/g (9). It is therefore very important to achieve the
The number of staphylococcal bacteria in the tested samples differed significantly between the evaluated fish species. The staphylococcal contamination of muscle tissue of redfish on the level $2.56 \pm 0.15 \log_{10}$ cfu/g and mackerel on the level $2.13 \pm 0.15 \log_{10}$ 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 pasteurii, 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.

**References**
11. PN-EN ISO 21527-1:2009. Microbiology of Food and Animal Feeding Stuffs – Horizontal method for the enumeration of yeasts and molds – Part 1: Colony-count technique in products with water activity greater than 0.95.
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