



MICROBIOLOGICAL EVALUATION OF FISH

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ABSTRACT

Fish meat has a specific composition that positively influences human health. Thanks to this composition, it is an excellent nutritional medium for growth and reproduction of undesirable microorganisms, which may cause spoilage and they can also lead to alimentary illnesses. Microbiota of fish is dominated by Gram negative and psychrophilic bacteria. Microbial contamination causes fish deterioration and leads to the end of its shelf-life when reaches levels between 10^7 and 10^9 CFU.g⁻¹. The most appropriate temperature for storage of fish is between -1 °C and 4 °C and the ideal relative air humidity is 80 to 85%. The objective of the work was to evaluate microbiological quality of fresh fish (Rainbow Trout, Atlantic Salmon, Atlantic Cod) bought in various types of stores in the Czech Republic and to evaluate if different storage temperatures have influence on the quantity of microorganisms. The following microorganisms were monitored: the total aerobic count (TAC), coliform bacteria, *E. coli*, *Salmonella* spp. and *Vibrio parahaemolyticus*. Based on the obtained results it is possible to state that difference between individual stores ($p > 0.05$) in the total aerobic count and the quantity of *E. coli* (except for cod) was not proven. After 2 days of storage there was increase ($p < 0.05$) of the total aerobic count in case of all monitored fish species from all stores. In case of coliform bacteria and *E. coli* there was increase ($p < 0.05$) of their quantity in a majority of the analysed samples. Different storage temperature (4 °C and 8 °C) did not have influence ($p < 0.05$) on the TAC, the quantity of coliform bacteria (except for cod) and the quantity of *E. coli* (except for trout).

Keywords: fish; TAC; *E. coli*; *Salmonella* spp.; *Vibrio parahaemolyticus*; storage temperature

INTRODUCTION

Fish plays an important role in the human diet and there is an observed increase in the consumption of fish per capita in Europe (Novoslavskij et al., 2016). Consumption of fish meat in the Czech Republic is low in comparison to another EU countries (Buchtová 2001). Consumption of two portions of fish per week is recommended as prevention of cardiovascular and oncological diseases (Clonan et al., 2012). Fish is a source of many beneficial substances such as vitamins, polyunsaturated fatty acids, mineral substances and in some countries of the world they are practically the only source of animal protein (Matyáš et al., 2002). Generally, fish meat is sterile while it is alive. However, a large number of bacteria are found on the outer surface, scales, gills and intestine (Hempel et al., 2011). Microbiota of fish is dominated by Gram negative and psychrophilic bacteria (Görner, Valík, 2004). During the capture and handling of fish, the muscle is colonized by these microorganisms. Microbial contamination causes fish deterioration and leads to the end of its shelf-life when reaches levels between 10^7 and 10^9 CFU.g⁻¹ (Scheleguedaa et al., 2016). Fish meat is a very suitable substrate for growth and reproduction of microorganisms due to high water content (Pipová et al., 2006). Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue and only small amounts of lactic acid are produced post mortem. This has important consequences for the microbiology of fish (Gram, Huss, 1996).

Safety of fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of foodborn 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 (Grigoryan, Badalyan Andriasyan, 2010).

The most suitable storage temperature for fish is between -1 °C and 4 °C. The ideal relative air humidity is 80 to 85%. Chilled fish stays “fresh” approximately for 4 days (in crushed ice), if fish is sliced, the shelf-time decreases to two days at 4 °C (Buchtová, 2001).

The objective of the work was to evaluate microbiological quality of meat of Rainbow Trout, Atlantic Salmon and Atlantic Cod, and to find out if storage temperature and the type of store, where fish was bought, have impact on the quantity of monitored microorganisms.

MATERIAL AND METHODOLOGY

Samples of fresh fish bought in the regular sales network in the Czech Republic were observed. 3 representative samples of every fish species were analysed. They included samples of Rainbow Trout (*Oncorhynchus mykiss*), Atlantic Salmon (*Salmo salar*) and Atlantic Cod (*Gadus morhua*). Samples were bought in the store focusing on sale of fresh fish and fish products, it has several branches in the Czech Republic and it guarantees fast transport of fish from a place of catching and thus also

freshness of freshwater as well as saltwater fish (denoted as the store 1). The second set of samples was bought in one of the store chains that offers food as well as non-food products (denoted as the store 2). The third set of samples was bought in a small store that is specialized on sales of fish and it is owned by private persons (denoted as the store 3).

Samples were transported into the microbiological laboratory of the Institute of Food Technology at MENDELU in Brno in a cooling box, stored at the temperature of melting ice and the microbiological analysis was carried out on the day of purchasing. The subsequent microbiological analysis was carried out after 2 days of storage at different temperature conditions at 4 °C and 8 °C.

For every sample of fish the following microbiologic indicators were determined:

The total aerobic count (TAC). Culture on the growth medium Plate Count Agar (PCA, NOACK, France) according to ISO 4833 at 30 °C for 72 hours.

Quantity of Coliform bacteria. Culture on the growth medium Violet Red Agar (VRBL, NOACK, France) according to ISO 4832 at 37 °C for 24 – 48 hours.

Quantity of bacteria *E. coli*. Cultivation on Chromocult Coliform agar (Merck, France) for 24 to 48 hours at 37 °C.

***Vibrio parahaemolyticus*.** Pre-reproduction in peptone water with higher salt content at 41.5 °C for 18 hrs. Cultivation on Thiosulfate-citrate-bile salts-sucrose agar (TCBS, Biokar Diagnostics, France) for 24 hrs at 37 °C. Biochemical confirmation, oxidase test.

***Salmonella* Species.** Cultivation after pre-reproduction on Salmonella Enrichment with addition of IRIS Salmonella selective supplement (18 hrs at 41.5 °C) in IRIS Salmonella® Agar (Biokar Diagnostics, France) for 24 hrs at 37 °C. Biochemical confirmation.

Sampling and processing was carried out based on ČSN ISO 7218 and ČSN EN ISO 6887-1.

The following methods were used for statistical evaluation: the calculation of basic statistical parameters (mean, standard deviation, standard deviation of the mean) and the simple sorting method of analysis of variance (ANOVA, Turkey test). Evaluation was performed using the programme STATISTICA CZ, version 10.

RESULTS AND DISCUSSION

The total aerobic count

The total aerobic count (Figure 1) found in the samples of Rainbow Trout bought in the individual stores was comparable ($p > 0.05$). In the store 1, the total aerobic count was 6.1 log CFU.g⁻¹ (1.3×10^6 CFU.g⁻¹), in the store (2) 6.4 log CFU.g⁻¹ (2.2×10^6 CFU.g⁻¹) and in the store (3) 6.6 log CFU.g⁻¹ (3.7×10^6 CFU.g⁻¹).

Also in the samples of Atlantic Salmon the total aerobic count was comparable ($p > 0.05$). The highest values of the total aerobic count were found in the sample from the store (3), specifically 6.6 log CFU.g⁻¹ (3.6×10^6 CFU.g⁻¹), the lowest in the store (1) 6.1 log CFU.g⁻¹ (1.2×10^6 CFU.g⁻¹).

Regarding cod, the highest ($p > 0.05$) total aerobic count was recorded in the sample bought in the store (1) specifically 7.0 log CFU.g⁻¹ (10.7×10^6 CFU.g⁻¹), the lowest quantity ($p < 0.05$) was found in the sample from the store (2), specifically 6.3 log CFU.g⁻¹

(1.9×10^6 CFU.g⁻¹). As it is stated by Ingr (2010), according to currently invalid Notice no. 132/2004 Coll., fish and its parts intended for heat treatment may contain the total aerobic count 6 log CFU.g⁻¹ (10^6 CFU.g⁻¹), according to ČSN 56 9609 (2008) the quantity of 6.7 log CFU.g⁻¹ (5.10^6 CFU.g⁻¹) is permissible. The total aerobic count in the observed samples was lower in all fish samples bought in all stores, except for cod bought in the store (1). If we compare the found values of TAC and results of Kordiovská et al. (2004), then the quantities found by our research are higher. In the above mentioned experiment of Kordiovská et al. (2004), the total aerobic count fresh fish bought in the sales network was 6.2×10^4 CFU.g⁻¹ (4.8 log CFU.g⁻¹). On the contrary Nespolo et al. (2012) present in salmon TAC comparable with our study (1.1×10^3 to 3.9×10^6 CFU.g⁻¹).

During capture and storage fish are almost invariably come into contact with nets, decks, ropes, boxes, human hands and clothing. These contacts are introducing microorganisms from other sources such as humans, birds and soil (Fernandes, 2009).

During storage (Figure 2) after 2 day there was increase ($p < 0.05$) of the total aerobic count in the all observed species of fish from all stores. Considering storage of fish at different temperatures, there was no difference in TAC recorded ($p < 0.05$). TAC was comparable after two days of storage at the temperature 4 °C as well as 8 °C. The highest TAC was found in the sample of trout, specifically 9 log CFU.g⁻¹. With this high total aerobic count there already occurs spoilage and sensory changes (Miks-Krajník et al., 2016). Parlapani and Boziaris (2016) also carried out monitoring spoilage microbiota, where the total aerobic count detected at the beginning of storage was 3.5 log CFU.g⁻¹, and after storage at 5 °C then 8.1 log CFU.g⁻¹, which is lower than we found during the analyses.

The quantity of coliform bacteria

The quantity of coliform bacteria (Figure 3) in the samples of Rainbow Trout bought in the individual stores was comparable ($p > 0.05$), only in the store (1) there was higher quantity of coliforms recorded ($p > 0.05$) in comparison to the store (2), specifically 4.7 log CFU.g⁻¹ (4.8×10^4 CFU.g⁻¹); respectively 4.4 log CFU.g⁻¹ (2.3×10^4 CFU.g⁻¹) in the store (2).

In case of Atlantic Salmon higher ($p < 0.05$) quantity of coliform bacteria was recorded in the store (2) in comparison to the store (1), 4.5 log CFU.g⁻¹ (3.2×10^4 CFU.g⁻¹) and 3.5 log CFU.g⁻¹ (3.2×10^3 CFU.g⁻¹) respectively.

Regarding the store (3), 3.7 log CFU.g⁻¹ (5.10^3 CFU.g⁻¹) was found in salmon, which is comparable quantity of coliforms as in the store (1). In comparison to Kordiovská et al. (2004), higher quantities of coliform bacteria were recorded in our experiment. In the above mentioned experiment there was found the quantity 2.9 log CFU.g⁻¹ (7.6×10^2 CFU.g⁻¹) of coliform bacteria. Lower quantities were also recorded in fresh fish before storage by Miks-Krajník (2016), specifically up to 4 log CFU.g⁻¹.

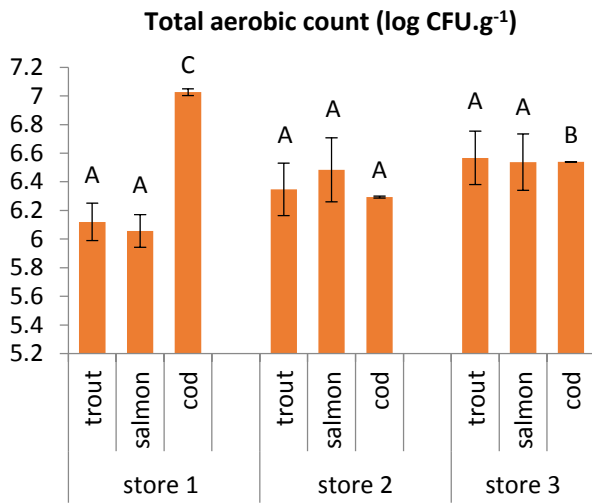


Figure 1 Comparison of the total aerobic count (log CFU g⁻¹) in the samples of fresh fish from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger's). The averages marked with different letters are statistically different ($p < 0.05$); $n = 3$ within the observed factor (store).

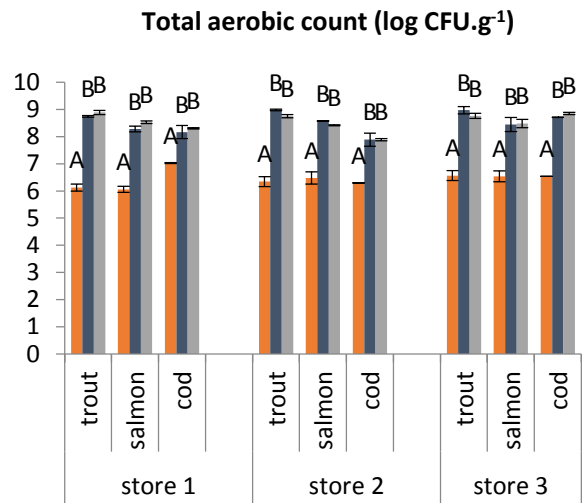


Figure 2 Comparison of the total aerobic count (log CFU g⁻¹) in the samples of fresh fish (marked red) and fish stored 2 days at the temperature 4°C (marked blue) and at the temperature 8°C (marked green) from three different stores (1=specialized wholesale of fish, 2=chain store, 3=fishmonger's). The averages denoted by different letters are statistically different ($p < 0.05$); $n = 3$ within the observed factor (storage temperature).

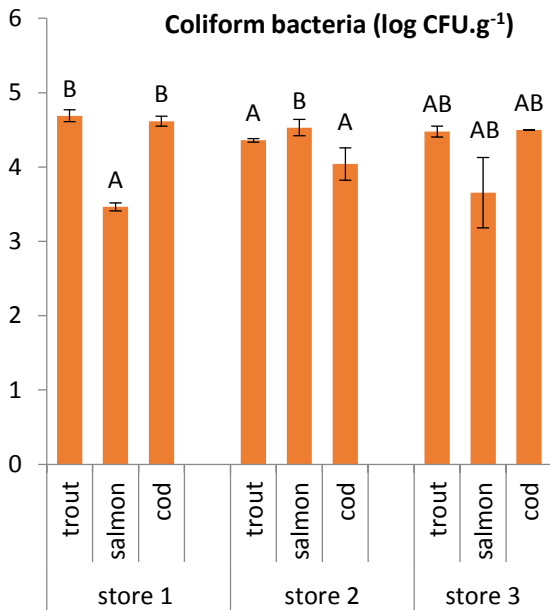


Figure 3 Comparison of the quantity of coliform bacteria (log CFU.g⁻¹) in the samples of fresh fish from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger's). The averages denoted by different letters are statistically different ($p < 0.05$); $n = 3$ within the observed factor (store).

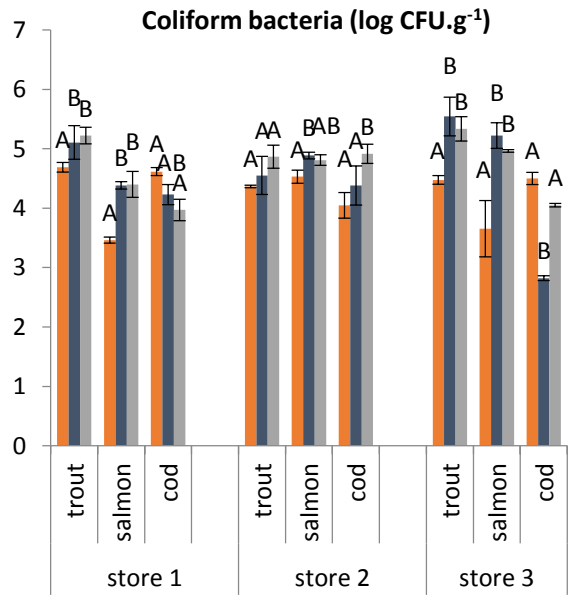


Figure 4 Comparison of the quantity of coliform bacteria (log CFU.g⁻¹) in the samples of fresh fish (marked red) and fish stored for 2 day at the temperature 4 °C (marked blue) and at the temperature 8 °C (marked green) from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger's). The averages denoted by different letters are statistically different ($p < 0.05$); $n = 3$ within the observed factor (storage temperature).

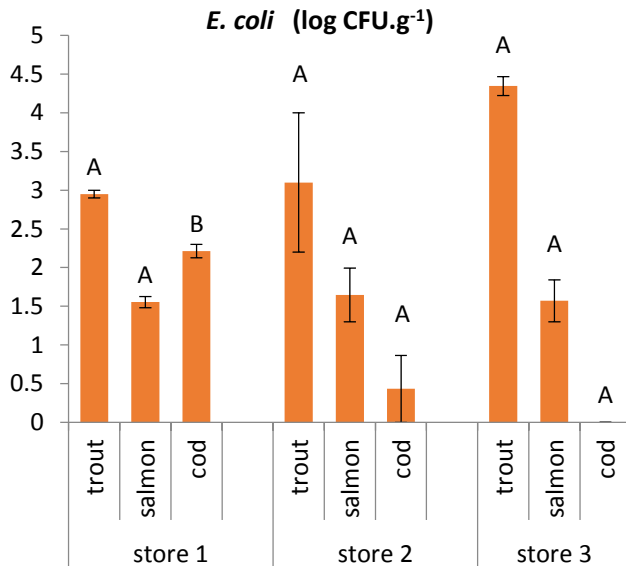


Figure 5 Comparison of the quantity of *E. coli* (log CFU.g⁻¹) in the sample of fresh fish from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger's). The averages denoted by different letters are statistically different ($p < 0.05$); $n = 3$ within the observed factor (store).

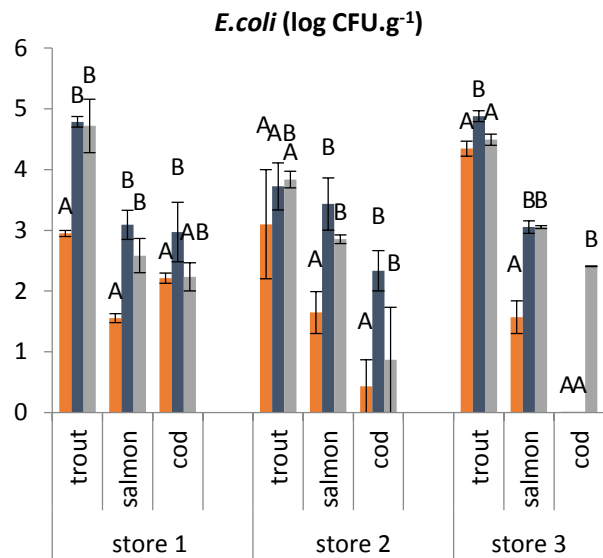


Figure 6 Comparison of the quantity of *E. coli* (log CFU.g⁻¹) in samples of fresh fish (marked red) and fish stored for 2 days at the temperature 4 °C (marked blue) and at the temperature 8 °C (marked green) from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger's). The averages denoted by different letters are statistically different ($p < 0.05$); $n = 3$ within the observed factor (storage temperature).

Coliform bacteria belongs to the family of *Enterobacteriaceae*, which can play a key role in food spoilage due to their ability to metabolize amino acids to malodorous volatile compounds (Remenanta et al., 2015). Higher quantity of coliforms in food is attributed especially to incorrect treatment of food and to not keeping of storage temperatures (Görner, Valík, 2004).

During storage (Figure 4) there was increase ($p < 0.05$) of the quantity of coliform bacteria in a majority of the observed samples. The highest value was recorded in trout bought in the store (3), specifically 5.5 log CFU.g⁻¹. Different storage temperature did not have (except for the cod samples) impact on the quantity of coliform bacteria ($p > 0.05$).

The quantity of bacteria *E. coli*

In case of Rainbow Trout and Atlantic Salmon (Figure 5) the individual stores were not different in the quantity of *E. coli* ($p > 0.05$). The highest values were recorded in the sample of trout bough in the store (3), specifically 4.4 log CFU.g⁻¹ ($2.2 \cdot 10^4$ CFU.g⁻¹). Considering salmon, the quantities of *E. coli* were comparable and they were from 1.6 log CFU.g⁻¹ (3.5×10^1 CFU.g⁻¹) to 1.7 log CFU.g⁻¹ (4.5×10^4 CFU.g⁻¹).

In the samples of Atlantic cod, higher quantity ($p < 0.05$) of *E. coli* in the sample from the store (1) was recorded: 2.2 log CFU.g⁻¹ (1.6×10^2 CFU.g⁻¹), in the samples from the store (3) bacteria *E. coli* were not detected.

According to ČSN 56 9609 (2008) the highest permitted quantity for *E. coli* in fresh fish and their parts intended for heat processing is 2 log CFU.g⁻¹, respectively 2.7 log CFU.g⁻¹ (5×10^2 CFU.g⁻¹) in two samples from five. In our experiment the maximal legislative limit was not kept only in case of the trout samples. Despite norms do not have a binding character, it is necessary to take

these values as limiting in case of missing legislative requirements. The Commission Regulation (ES) 2073 (2005) states the highest permitted values of *E. coli* only for products of boiled crustaceans and molluscs.

During storage (Figure 6) there was increase ($p < 0.05$) in the quantity of *E. coli* a majority of the observed fish samples, except for trout (the store 2). The highest quantity of *E. coli* was recorded in trout bought in the store (3), specifically 4.9 log CFU.g⁻¹. Different storage temperature did not have (except for the samples of trout from the store 3) impact on the quantity of *E. coli* ($p > 0.05$). The highest permitted quantity (2.7 log CFU.g⁻¹) was exceeded by all fish samples at the storage temperature 4 °C, except for two cod samples. At the storage temperature 8 °C it was 4 samples. If we would take 2 log CFU.g⁻¹ as the highest threshold, then only two analysed samples would meet this requirement.

Vibrio parahaemolyticus

Bacteria *Vibrio parahaemolyticus* were detected in 4 samples of fresh fish, especially in the samples of trout from the store (2) 0.9 log CFU.g⁻¹, in the samples of salmon from the stores (2) or (3) 1.8 log CFU.g⁻¹, and 0.5 log CFU.g⁻¹ respectively, and in the sample of cod from the store (1) 0.3 log CFU.g⁻¹.

Vibrio parahaemolyticus is widely distributed in the marine environments and considered as the leading cause of human gastroenteritis (Alaboudi et al., 2016). This pathogen is connected especially with fish products and sea products (Komprda, 2004). Higher quantities of the species *Vibrio* were proved in fish by Aagesen and Häse (2014), especially in case of violation of cooling chain or repeated temperature fluctuations. Major outbreaks are associated with the warmer month. Control of *Vibrio parahaemolyticus* growth in shellfish meats is temperature

depended in the first place (Fernandes, 2009). The negative criteria in 25 grams is stated in fish in case of *V. parahaemolyticus* (ČSN 56 9609, 2008).

Salmonella species

Bacteria of the *Salmonella* species were detected in 4 samples. It included two samples of trout from the store (1), one sample of salmon from the store (1) and one sample of salmon from the store (2). The Commission regulation (ES) 2073 (2005) on microbiologic criteria for food requires no presence of salmonella in 25 g of a sample. The above stated samples thus do not correspond with the legislative limit. On contrary Patil et al. (2013) during observing microbiological quality of fish stored at 4 °C did not detect salmonella in samples.

The presence of *Salmonella* in seafood may derive from contamination occurring in the natural aquatic environment, in aquaculture or cross-contamination during store, transportation and processing (Amagliani et al., 2012). *Salmonella* was not isolated also from the freshwater fish in the study of Terenjeva et al. According this study salmonella-negative results are in agreement with those of previous reports for France, Great Britain, Portugal, Czech Republic, Slovakia, and the United States for marine and freshwater fish. But, the presence of *Salmonella* in fish was detected in several countries of Asia and Africa (Terenjeva et al., 2015). Yang et al. (2015) study implied that pollution by human or animal feces and sewage may be a major reason for the high prevalence of *Salmonella* in freshwater fish samples.

CONCLUSION

Ensuring health safety of food is a basic responsibility of all producers. Controlling bacterial contamination is important all the way from catching and handling to processing. In processing and producing operation there are therefore implemented HACCP systems, which are responsible not only for analysing danger, but especially for eliminating this danger at an acceptable level and to choose effective preventive measures.

Microbial criteria and monitoring of microbial levels are important part of food inspection and determine acceptability or unacceptability of fish and seafood for the consumers.

In conclusion, it is possible to state that quality of fish bought by us was in all types of stores comparable and that a consumer does not have to be afraid to buy fish also in stores that are not exclusively specialized on sale of these products. Despite our results of the quantity of observed microorganisms in comparison to results of another studies were higher, they met legislative requirements (except for five analysed samples). Obviously, pathogens (*Salmonella* spp., *V. parahaemolyticus*) should not occur in products offered to consumers. Considering fish storage, it is not possible to recommend storage at fridge temperatures because this temperature is often higher than 8 °C and fish stored like this would reach limits of spoilage after 2 days (8 log CFU.g⁻¹). Based on the obtained results it is possible to recommend consumption of fish on the day of purchase or storage at the temperature of melting ice, specifically from -1 °C to 2 °C.

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