

## Microbiological status of fish products on retail markets in the Republic of Bulgaria

<sup>1</sup>\*Deyan Stratev, <sup>1</sup>Ivan Vashin and <sup>2</sup>Hristo Daskalov

<sup>1</sup>Department of Food Hygiene and Control, Veterinary Legislation and Management, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

<sup>2</sup>National Diagnostic Science and Research Veterinary Medical Institute, Bulgarian Food Safety Agency, 1000 Sofia, Bulgaria

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### Abstract

The purpose of the study was to determine the microbiological status of 161 samples cooled and frozen fish products collected from retail markets in the Republic of Bulgaria. The highest total viable counts (TVCs) in cooled fish products were established for silver carp (7.30 log cfu/g), Black sea roach (7.28 log cfu/g) and trout (7.11 log cfu/g), followed by common carp (6.81 log cfu/g), vacuum packed trout fillets (6.39 log cfu/g) and horse mackerel (5.90 log cfu/g). The highest *Aeromonas* spp. load was established in cooled trout (5.89 log cfu/g) and vacuum packed trout fillets (5.63 log cfu/g), followed by cooled silver carp (4.95 log cfu/g), common carp (4.09 log cfu/g) and horse mackerel (2.68 log cfu/g). Among frozen fish products, the highest microbial contamination was detected in frozen sprats (4.43 log cfu/g), followed by trout (4.27 log cfu/g) and mackerel (4.10 log cfu/g). *Aeromonas* spp. were not found out in Black sea roach and frozen fish samples. The relative proportion of *Aeromonas* spp. out of total microbial counts was the highest for vacuum-packed trout fillets (88.1%), followed by trout (82.8%), silver carp (67.8%), common carp (60.1%) and horse mackerel (45.4%). Cooled silver carp, Black sea roach and trout were highly contaminated with microorganisms. The significant number of contaminated cooled fish products and the high load of *Aeromonas* spp. pose a risk for the health of consumers.

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### Introduction

Fish is among the foods with shortest shelf life. Fish spoilage is caused by microbial enzymes. Bacteria colonising the skin, gills and intestines are usually harmless for live fish, and began to replicate rapidly after its death (Shamsuzzaman *et al.*, 2011). Microbial load on the skin, gills and intestines of fish living in clear waters is usually high, and muscles are assumed to be sterile (Pamuk *et al.*, 2011). The fish could be contaminated after being caught or during transportation to retail markets. After contamination and replication of microorganisms, decay occurs and the consumption becomes dangerous (Mol and Tosun, 2011; Alparslan *et al.*, 2014). Fish quality is influenced by many factors as the source, cooling methods, processing, packaging, storage conditions. The quality and freshness of fish are rapidly deteriorated through microbial and biochemical mechanisms (Al-Jasser and Al-Jasass, 2014). Bacterial activity results in unpleasant odour due to conversion of amino acids into biogenic amines, sulfides, organic acids etc. (Velu *et al.*, 2013).

The International Commission on Microbiological

Specifications for Foods poses a limit for total aerobic plate counts in fresh and frozen fish of  $10^7$  cfu/g (7 log cfu/g) (ICMSF, 1986). According to Broekaert *et al.* (2011) loads of  $10^7$ - $10^8$  cfu/g make spoilage organoleptically detectable. Fish and fish products are a source of bacteria, pathogenic for men. Pathogens could be transmitted to fish in basins or during processing under bad hygienic conditions (Uddin *et al.*, 2013). *Aeromonas* spp. are normal inhabitants of the aquatic medium. During the last years, the interest to them extended beyond the boundaries of fish pathology due to the increased incidence of human disease caused by *Aeromonas* after consumption of contaminated foods. The spoilage of foods and the pathogenicity of aeromonads is due to their ability to produce extracellular haemolysin, enterotoxins, cytotoxins, lipases and proteases (Frag, 2006). Motile aeromonads are emerging food pathogens as some isolates could produce virulence factors not only at optimum temperatures, but also under cold storage conditions (Neyts *et al.*, 2000).

Cooling and freezing are widely used methods for preservation of fish. Cooling could not prevent spoilage but shelf-life could be prolonged through

\*Corresponding author.

Email: [deyan.stratev@trakia-uni.bg](mailto:deyan.stratev@trakia-uni.bg)

decrease of body temperature. During cold or freezer storage, biochemical changes in lipids and proteins occur (Latip *et al.*, 2013). The activity of bacterial enzymes could be minimised by observing hygiene rules, proper processing, conservation and low temperatures. Maintenance of adequate hygiene in fish retail stores is a prerequisite for prevention of contamination. Fish quality is mainly assessed through the total aerobic plate counts and counts of bacteria with public health relevance (Mol and Tosun, 2011; Shamsuzzaman *et al.*, 2011). The purpose of this survey was to evaluate the microbiological status of cooled and frozen fish products on Bulgarian retail markets in order to predict the hazard for consumers' health from the presence of *Aeromonas* spp.

## Material and methods

### Sample collection

The study was conducted with 161 samples cooled and frozen fish products. Cooled fish products consisted of horse mackerel (20 samples), rainbow trout (11 samples), Black sea roach (12 samples), common carp (20 samples), silver carp (20 samples) vacuum-packed rainbow trout fillets (20 samples). Frozen fish products included rainbow trouts (19 samples), mackerel (19 samples) and sprats (20 samples). Samples were collected from special retail stores for fish and fish products, then transported to the lab in a cooler bag for analysis.

### Sample analysis

Ten grammes muscle tissue with the skin were collected aseptically and weighed in a Stomacher® 400 Circulator (Seward, England) bag. Ninety ml sample diluent (MRD, Merck) were added, and samples were homogenised at 256 rpm for 1 min. Tenfold dilutions were performed in tubes with 9 ml sample diluent (MRD, Merck). For determination of total aerobic plate counts, 0.1 ml of each dilution were inoculated on two plates with Plate count agar (Merck, Germany). Plates were incubated at 35°C for 24 hours as per Mol

and Tosun (2011). The *Aeromonas* spp. counts were determined according to Evangelista-Barreto *et al.* (2006) after inoculation of 0.1 ml of each dilution on two GSP agar plates (Merck, Germany). Plates were incubated at 28°C over 24 h. The colony counts were evaluated on a counter (Colony Counter 570, Suntext, Taiwan). All dilutions and inoculations were done in a microbiology box (Nuve MN 090, Turkey). The results are presented as log cfu/g.

### Statistical analysis

The results were statistically processed with GraphPad InStat® 3 software.

## Results

Total viable counts (TVCs) and *Aeromonas* spp. in cooled and frozen fish products are presented in Tables 1 and 2. The highest microbial loads in cooled fish products were established in silver carp (7.30 log cfu/g), Black sea roach (7.28 log cfu/g) and trout (7.11 log cfu/g), followed by common carp (6.81 log cfu/g), vacuum-packed trout fillets (6.39 log cfu/g) and horse mackerel (5.90 log cfu/g). Most contaminated with *Aeromonas* spp. were cooled trouts (5.89 log cfu/g) and vacuum-packed trout fillets (5.63 log cfu/g), followed by cooled silver carp (4.95 log cfu/g), common carp (4.09 log cfu/g) and horse mackerel (2.68 log cfu/g). Frozen fish samples exhibited lower TVCs compared to cooled fish samples. Highest microbial counts were observed in frozen sprats (4.43 log cfu/g), followed by trout (4.27 log cfu/g) and mackerel (4.10 log cfu/g). *Aeromonas* spp. were not established in frozen fish samples.

Table 3 presents the positive for *Aeromonas* spp. cooled fish samples. All samples from cooled rainbow trout and vacuum-packed trout fillets were contaminated with *Aeromonas* spp. Less positive samples were established in silver carp (85%), horse mackerel (65%) and common carp (60%). Cooled Black sea roach samples were not polluted with *Aeromonas* spp.

Table 1. Total viable counts and *Aeromonas* spp. in cooled fish products ( $\bar{x} \pm S_x$ )

Sample type	Number of samples	Total viable counts (log cfu/g)	<i>Aeromonas</i> spp. (log cfu/g)
Horse mackerel	20	5.90 ± 1.07	2.68 ± 2.12
Trout	11	7.11 ± 1.22	5.89 ± 1.96
Black sea roach	12	7.28 ± 0.33	0
Common carp	20	6.81 ± 1.17	4.09 ± 3.44
Silver carp	20	7.30 ± 0.38	4.95 ± 2.16
Vaccum packed rainbow trout fillets	20	6.39 ± 0.55	5.63 ± 0.85

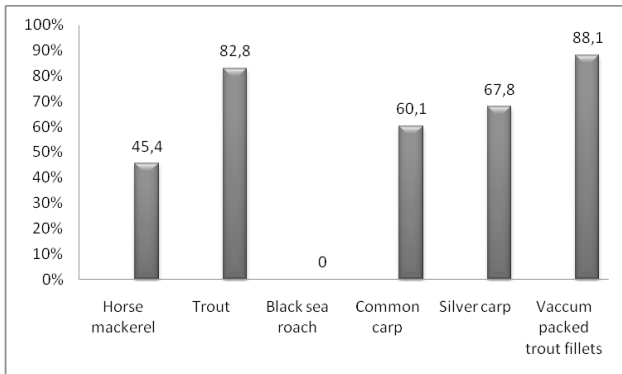


Figure 1. Relative proportion of *Aeromonas* spp. from total viable counts in cooled fish products

Figure 1 presents the relative share of *Aeromonas* spp. from the total bacterial load of cooled fish samples. *Aeromonads* relative proportion was the highest in vacuum-packed trout fillets (88.1%), followed by trout (82.8%), silver carp (67.8%), common carp (60.1%) and horse mackerel (45.4%).

**Discussion**

The complex concept of fish quality consists of safety, nutritional value, availability, integrity, freshness, eating quality, product size and type (Abbas *et al.*, 2008). The most serious problem related to fish product safety is the contamination with microbial pathogens. Furthermore, fish products are highly sensitive to spoilage because of their high water content, neutral pH, high amount of amino acids and naturally present autolytic enzymes (Jeyasekaran *et al.*, 2006). Cooling and freezing are the usual methods for conservation of fish; anyway, the quality of stored fish inevitably deteriorates with shelf-life expiration (Jeon *et al.*, 2002).

Mol and Tosun (2011) investigated the quality of fish purchased from retail markets in Istanbul. Studied horse mackerel samples exhibited high total viable microbial counts, up to 7.04 log cfu/g. We have found out in our study that horse mackerel samples were the least contaminated with bacteria compared to other cooled fish samples (5.90 log cfu/g). Duyar *et al.* (2013) outlined that studied horse mackerel samples from three different markets were of good quality, with average total mesophilic bacterial counts of 3.91, 4.22 and 4.26 log cfu/g.

In their experiments Diler *et al.* (2000) demonstrated that mesophilic bacterial counts on trout skin varied between 2 and 7 log cfu/g. Our studies showed higher average total viable microbial counts in cooled trout (7.11 log cfu/g). In contrast, Gonzales *et al.* (1999) established TVCs of 2.90 log cfu/g in rainbow trout. The TVCs in rainbow trout was reported to increase from a baseline of 4.0 log

Table 2. Total viable counts in frozen fish products ( $x \pm Sx$ ).

Sample type	Number of samples	Total viable counts (log cfu/g)
Trout	19	4.27 ± 0.89
Mackerel	19	4.10 ± 0.57
Sprat	20	4.43 ± 0.51

Table 3. Samples of cooled fish products, positive for *Aeromonas* spp.

Sample type	Number of samples	<i>Aeromonas</i> spp. positive samples (%)
Horse mackerel	20	65
Trout	11	100
Black sea roach	12	0
Common carp	20	60
Silver carp	20	85
Vacuum-packed trout fillets	0	100

cfu/g up to 7.04 log cfu/g during storage (Rezaei and Hosseini, 2008). According to Ozogul *et al.* (2013) a TVC load of 3.59 log cfu/g is a parameter for high quality of trouts. Gonzalez-Rodriguez *et al.* (2001) established total aerobic plate counts of 5.27 log cfu/g at 30°C and 4.87 log cfu/g at 25°C in packed trout fillets. However, Angis and Oguzhan (2013) outlined TVCs of 3.03 log cfu/g in rainbow trout fillets. The average value in the present study for vacuum-packed trout fillets was 6.39 log cfu/g.

According to Wenjiao *et al.* (2013), the silver carp is one of the economically important freshwater fish species for eastern countries due to its extensive growth, easy culturing, good feed conversion and high nutritional value. Anyway, the silver carp is an easily spoiled food due to high water activity, autolytic enzymes content and relatively high amounts of volatile basic nitrogen and free amino acids. The total microbial counts in silver carp muscle was 3.0 log cfu/g, indicating a good quality. In our study, the average value was 7.30 log cfu/g, i.e. exceeding the ICMSF limits (ICMSF, 1986).

Hasani and Hasani (2014) demonstrated that the total viable counts in carp fillets increased significantly during storage to 6 log cfu/g by the 6th day. Pamuk *et al.* (2011) established TVCs between 4 and 9 log cfu/g in studied common carp samples. Can (2011) established initial contamination of carp fillets with 3.88 log cfu/g and increase in microbial counts during storage. In our study, the contamination level of 6.81 log cfu/g in cooled common carp was evaluated as acceptable.

The contamination level of frozen fish was lower compared to cooled fish. The spoilage of mackerel

begins within 12 h after it is caught if not stored at suitable temperature, as the chemical composition of fish meat is an excellent medium for microbial growth (Jay *et al.*, 2005). Our study of frozen mackerel showed TVCs of 4.10 log cfu/g. Adebayo-Tayo *et al.* (2012) demonstrated higher levels in frozen mackerel varying between  $3 \times 10^5$  and  $6.3 \times 10^5$ . Popovic *et al.* (2010) have studied 30 frozen fish samples from sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), sprat (*Sprattus sprattus*), pilchard (*Sardina pilchardus*), red scorpionfish (*Scorpaena scrofa*), bluefin tuna (*Thunnus thynnus*), hake (*Merluccius merluccius*), striped red mullet (*Mullus surmuletus*). They reported that 20 samples had TVCs between 3 and 5 log cfu/g. Our results were comparable (4.43 log cfu/g in frozen sprats).

Available research showed that fish is most commonly and most extensively contaminated with bacteria from the genus *Aeromonas*. *Aeromonas* spp. positive fish samples varied from 37.3% (Thayumanavan *et al.*, 2003) to 93% (Hanninen *et al.*, 1997). Our study showed that 100% of cooled trout and vacuum-packed trout samples were contaminated with *Aeromonas* spp. Lower number of positive samples was observed in cooled silver carp (85%), horse mackerel (65%) and carp (60%). Of all cooled fish products, only Black sea roach samples were negative for *Aeromonas* spp. All frozen fish samples did not contain *Aeromonas* spp. Some researchers have isolated aeromonads from frozen fish, but after enrichment (Castro-Escarpulli *et al.*, 2003). On the other hand, the share of *Aeromonas* spp. among other microorganisms was considerable. It was the highest in vacuum-packed trout fillets (88.1%), followed by trout (82.8%), silver carp (67.8%), common carp (60.1%) and horse mackerel (45.4%). According to Palumbo (1996) *Aeromonas* spp. counts in foods depended on the initial contamination level, the type of processing, packaging and conservation methods.

The amount of motile mesophilic *Aeromonas* spp. varies from  $< 10^2$  cfu/g to  $10^5$  cfu/g (Neyts *et al.*, 2000). Herrera *et al.* (2006) reported *Aeromonas* spp. counts between 2.29 and 7.20 log cfu/g in sea fish fillet, and Salah El-Dien *et al.* (2009) - 4.91 log cfu/g in fresh mullet. Gonzalez-Rodriguez *et al.* (2002) established *Aeromonas* spp. loads between  $< 1$  and 3.37 log cfu/g in vacuum-packed cold-smoked trout fillets. In our survey, the highest *Aeromonas* spp. counts were established in cooled trout (5.89 log cfu/g) and vacuum-packed trout fillet samples (5.63 log cfu/g), followed by silver carp (4.95 log cfu/g), common carp (4.09 log cfu/g) and horse mackerel (2.68 log cfu/g).

Microbial contamination depends on the water,

fishing, processing, transportation and storage conditions. The cross contamination between raw and cooked fishes, undercooked and raw fish consumption results in food intoxications due to hydrobiont consumption (Fletcher *et al.*, 1998). The efficient bacteriological control of hygiene is important to ensure acceptable levels of contamination and prevention of food intoxications (Moyo and Baudi, 2004; Ajao and Atere, 2009). Intoxications due to fish products could be prevented by providing suitable conditions during all stages of fish processing and controlled storage temperature (Craven *et al.*, 2001). Fish product safety is influenced by many factors as origin of the fish, product characteristics, processing mode and cooking. The risk incurred by fresh fish consumption is small after proper cooking, but increased if fish is consumed raw, inadequately cooked or slightly cooked. Contaminated fish could be dangerous, especially for sensitive populations as children, elderly and immunocompromised people (Herrera *et al.*, 2006).

## Conclusion

Taking into consideration the obtained results and the recommendations of the International Commission on Microbiological Specifications for Foods, it could be concluded that microbiological load of cooled silver carp, Black sea roach and trout was unacceptable. On the other hand, the level of contamination was within the allowed range for cooled common carp, vacuum-packed trout fillets, horse mackerel and frozen fish products. The highest contamination level with *Aeromonas* spp. was observed in cooled trout and vacuum-packed trout fillets, followed by silver carp, common carp and horse mackerel. The share of *Aeromonas* spp. from the total microbial load was considerable. The high number of contaminated cooled fish products and the high *Aeromonas* spp. counts could pose a risk for human health after consumption of undercooked fish.

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