MICROBIOLOGICAL QUALITY OF RAW FISH AT RETAIL MARKET IN LATVIA

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Abstract. *The aim of the study was to evaluate the microbiological quality of raw fish obtained from retail market. Overall, 20 raw fish samples, including roach (Rutilus rutilus), crucian carp (Carassius carassius), European perch (Perca fluviatilis), silver bream (Blicca bjoerkna) and round gobby (Neogobius melanostomus) were collected during October, 2014. Samples were analyzed for total bacterial count, Enterobacteriaceae count, Escherichia coli, Listeria monocytogenes and Salmonella. Total bacterial count in raw fish ranged from 5.58 to 7.84 log_{10} CFU cm^{-2}. Total bacterial count in silver bream (7.57 log_{10} CFU cm^{-2}) were significantly higher ($p<0.05$) than in round gobby (6.31 log_{10} CFU cm^{-2}). Enterobacteriaceae counts ranged from 1.91 to 5.28 log_{10} CFU cm^{-2}, and there were no significant differences ($p<0.05$) between fish species. E. coli was detected in three of 20 fish samples analyzed and E. coli count ranged from 1.11 to 1.72 log_{10} CFU cm^{-2}. Overall 11 from 20 fish samples tested were positive for L. monocytogenes. However, all the samples were negative for Salmonella. This study demonstrates that high total bacterial count and Enterobacteriaceae numbers were determined in raw fish samples, as well as raw fish at retail level can serve as the potential reservoirs of L. monocytogenes.*

Key words: *raw fish, microbiological quality, Listeria monocytogenes, Salmonella.*

INTRODUCTION

Fish are a source of high quality animal protein, containing considerable quantities of valuable lipids, minerals and vitamins. It is assumed that freshwater fish products are healthy food for human nutrition [1]. On the contrary, fish nutrient composition and high moisture content allow the growth of a large range of microorganisms, which affect fish quality and safety rendering fish unacceptable for human consumption [2]. Microbiological quality of raw fish results from microbiological load of aquatic habitat, methods of capture, transportation, chilling and storage conditions [3],[4].

Total bacterial count (TBC), *Enterobacteriaceae* and *Escherichia coli* are frequently used as criteria to assess the quality and safety of foods. TBC is used to assess the general microbiological quality of fish and can be a useful indicator to predict the shelf life of raw fish [5]. *Enterobacteriaceae* and *E. coli* are used as indicators of the potential presence of pathogens and indicating hygienic status of fish [6].

Several pathogenic bacteria may either be present in the environment or contaminate fish during handling [7]. *Salmonella* and *Listeria monocytogenes* are reported to cause foodborne infection in humans by the ingestion of raw or inadequately processed contaminated fish or fish products [8]-[11]. Pathogenic bacteria can be transmitted to consumer by raw products at retail level, which develop the threat of food-borne infections [12],[13]. Therefore detection of microbiological quality and pathogens in raw products is crucial for the identification and prevention of issues related to public health and safety.

Several studies of microbiological safety and quality of raw fish in Europe have been performed [10],[14]-[16]. However, limited data on hygiene indicators and the prevalence of pathogens in raw fish at retail level in Latvia have been available. The objective of this study was to assess raw fish microbiological quality by detection of total bacterial count, *Enterobacteriaceae* and *E. coli* counts and the prevalence of *Salmonella* and *L. monocytogenes* in raw fish from retail market in Latvia.

MATERIALS AND METHODS

Fish samples

Raw, whole fish were purchased from one fish market in Riga, Latvia during October 2014. Overall, 20 samples including four roach (*Rutilus rutilus*), five crucian carp (*Carassius carassius*), five European

perch (*Perca fluviatilis*), three silver bream (*Blicca bjoerkna*) and three round gobby (*Neogobius melanostomus*) were taken. Samples were collected in plastic bags, chilled below 4°C, transported to the laboratory and tested within one hour.

Sample preparation

For detection of TBC, *Enterobacteriaceae* and *E. coli* surface samples of fish skin were collected with abrasive sponge moisturized with 0.1% peptone water by covering a 25 cm^2 or 100 cm^2 area of fish skin depending on fish size. Samples for detection of *Salmonella* and *L. monocytogenes* were consisted of pooled sample of skin, musculature and intestinal tract of each fish. In total, 25 g of material was used for detection of each pathogen.

Bacteriological analysis

For TBC and *Enterobacteriaceae* 10-fold dilutions of initial surface samples of fish skin were prepared and 1 ml of each dilution transferred into duplicate plates of Plate Count Agar (PCA, Biolife Italiana S.r.l, Milan, Italy) for TBC and violet red bile glucose agar (VRBG, Biolife) for *Enterobacteriaceae*. Inoculated PCA plates were incubated at 30°C for 72 h, according to the ISO 4332 [17] and all colonies were enumerated after incubation. Inoculated VRBG plates were incubated at 37°C for 24 h according to the ISO 21528-2 [18], using duplicate plates of and incubated at 37°C for 24 h, followed by manual counting of typical colonies.

The detection of *E. coli* was performed according to ISO 7251 [19]. An amount of 1 ml from each decimal dilution (from 10^{-1} to 10^{-5}) of the sample inoculated into 10 ml lauryl sulfate broth (Biolife). The tubes were incubated at 37 °C and after 24 h examined for gas production. Gas-negative tubes were re-incubated for an additional 24 h and reactions examined again. One 10-µl loopful of material from each tube with positive gas formation was inoculated into a tube containing 10 ml of *Escherichia coli* (EC) broth (Biolife), incubated at 44°C for 24-48 h and examined for the presence of gas production. After evalution, gas positive EC broth tubes were transferred in tripton water (TW, Biolife) and further incubated at 44°C for 48 h. Finally, the indole test was performed for the presence of *E. coli.*

The detection of *Salmonella* was done according to ISO 6579 [20]. Briefly, 25 g from each of fish samples were homogenized with 225 ml of buffered peptone water (Biolife) in stomacher for 60 s and incubated at 37°C for 18 h. An inoculum from pre-enrichment broth was inoculated into each of Rappaport Vassilliadis broth (RV, Biolife) and Muller-Kaufmann tetrathionate novobiocin broth (MKTTn, Biolife). The RV broth was incubated at 41.5°C for 24 h, while MKTTn broth at 37°C for 24 h. From both cultures, 0.1 ml was plated onto two selective solid media: xylose-lysine-desoxycholate agar (XLD, Biolife) and brilliant green agar (BGA, Biolife) and further incubated at 37°C for 24 h and examined for the presence of presumptive colonies.

The standard ISO 11290-1 [21] method was used for *L. monocytogenes* detection. An amount of 25 g of sample was added to 225 ml Half-Fraser broth (HF, Biolife) followed by homogenization for 60 seconds in a stomacher and incubated for 24 h at 30°C. Thereafter 0.1 ml aliquots of the HF broth were transferred to 10 ml of Fraser broth (Biolife) and incubated at 37°C for 48 h. An amount of 0.1 ml of both, Half-Fraser and Fraser enrichments were streaked onto Agar *Listeria* according to Ottaviani and Agosti (ALOA, Biolife) and Oxford agar (Biolife). After an incubation period of 24-48 h at 37°C, the selective agar plates were examined for the presence of the characteristic colonies that are presumed to be *L. monocytogenes*. Typical colonies of *L. monocytogenes* on ALOA agar are green-blue surrounded by an opaque halo. Suspicious colonies on ALOA agar were Gram stained, tested for hemolysis, motility and catalase activity followed by biochemical identification with the API *Listeria* system (BioMérieux, Mancy l'Etoile, France).

Statistical analysis

Statistical analyses were performed on log-10 transformed data. The probability level at which statistical analyses were accepted as significant was < 0.05. Data were analyzed (means, standard deviations, Student's *t* test) using the software Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

In silver bream the level of TBC and *Enterobacteriaceae* was the highest (7.57 and 4.91 $\log_{10}$ CFU cm^{-2}, respectively), while the lowest TBC was detected in round gobby (6.31 $\log_{10}$ CFU cm^{-2}), but the lowest *Enterobacteriaceae* in roach (3.68 $\log_{10}$ CFU cm^{-2}). While all of the freshwater fish samples were below the limit of *E. coli* enumeration (< 1 $\log_{10}$ CFU cm^{-2})**,** *E. coli* was found to be present in all round gobby samples. The number of *E. coli* count in round gobby ranged from 1.11 to 1.72 $\log_{10}$ CFU cm^{-2}. The mean values for TBC, *Enterobacteriaceae* and *E. coli* count for fish samples are given in Table 1.

Table 1

Total bacterial count, *Enterobacteriaceae* and *E. coli* in fish at retail market in Latvia

Sample	Number of samples	Total bacterial count log_{10} CFU cm^{-2}		*Enterobacteriaceae* log_{10} CFU cm^{-2}		*E. coli* log_{10} CFU cm^{-2}	
		mean ± SD	range	mean ± SD	range	mean ± SD	range
Roach (*Rutilus rutilus*)	4	6.86 ± 0.85	5.58-7.30	3.68 ± 1.03	2.15-4.36	< 1	< 1
Crucian carp (*Carassius carassius*)	5	6.64 ± 1.20	4.58-7.46	4.05 ± 1.20	1.91-4.78	< 1	< 1
European perch (*Perca fluviatilis*)	5	7.13 ± 0.39	6.64-7.52	3.97 ± 0.38	3.51-4.28	< 1	< 1
Silver bream (*Blicca bjoerkna*)	3	7.57* ± 0.37	7.15-7.84	4.91 ± 0.29	4.57-5.11	< 1	< 1
Round gobby (*Neogobius melanostomus*)	3	6.31* ± 0.46	5.84-6.76	4.86 ± 0.56	4.23-5.28	1.44 ± 0.31	1.11-1.72

* Differences of total bacterial count between silver bream and round gobby were significant ($p<0.05$)

Total bacterial count in silver bream (7.57 log_{10} CFU cm^{-2}) were considerably higher ($p<0.05$) than in round gobby (6.31 log_{10} CFU cm^{-2}). Counts of *Enterobacteriaceae* were variable, but no significantly different ($p>0.05$) between fish species. In the present study TBC and *Enterobacteriaceae* count of all raw fish samples was higher than the results of other authors. Increased numbers of *Enterobacteriaceae* and TBC can be linked to unsatisfactory storage and handing conditions at retail market. Total bacterial count was reported lower in pond raised fresh fish in Gelman et al. [22] study on silver perch (*Bidyanus bidyanus*, 1.70 log_{10} CFU cm^{-2}) and in Acuff *et al.* [23] study on tilapia (*Tilapia aurea*, 2.86 log_{10} CFU cm^{-2}). *Enterobacteriaceae* count was found to be lower (1.16 log_{10} CFU g^{-1}) on sea bream stored in ice [24]. *E. coli* possibly indicates to fecal contamination and the presence of enteric pathogens. The occurrence of *E. coli* in raw fish is related to water contamination or poor hygienic conditions during the fish handling.

Table 2

Occurrence of *Listeria monocytogenes* and *Salmonella* in raw fish from retail market in Riga, Latvia

Sample	Number of samples	Number of positive samples (%)	
		Listeria monocytogenes	*Salmonella*
Roach (*Rutilus rutilus*)	4	2	0
Crucian carp (*Carassius carassius*)	5	3	0
European perch (*Perca fluviatilis*)	5	4	0
Silver bream (*Blicca bjoerkna*)	3	2	0
Round gobby (*Neogobius melanostomus*)	3	0	0
Total	20	11	0

During study 11 of 20 raw fish samples collected from retail market were found to be contaminated with *L. monocytogenes*. Among raw freshwater fish samples, European perch had the highest occurrence of *L. monocytogenes* (4 out of 5 samples), while the roach had the lowest contamination (2 out of 4 samples).

However, no *L. monocytogenes* was found in round gobby. None of the examined fish samples contained *Salmonella*. Results for fish species and pathogens are presented in Table 2.

Our findings regarding *L. monocytogenes* are notably higher than that found in other studies in European region. The reported prevalence of *L. monocytogenes* was 0% in herring fillets of market in Belgium [25], less than 1% in raw fish of market in Northern Greece [26], 13.5% in raw fish from seafood plant in the Nordic countries [15] and 14.6% in rainbow trout (*Onchorynchus mykiss*) from fish farms around Finland [14]. *Listeria* spp. are naturally present in aquatic environment [27], therefore their presence in raw fish may be expected. Results on prevalence of *Salmonella* in our study are similar with Davies *et al.* [16] study on *Salmonella* presence in fresh fish from commercial outlets from Europe. It has been suggested that *Salmonella* is not an indigenous bacterial flora of fish [28], however occasional cases on *Salmonella* presence in fish have been reported [29]. *Salmonella* can be introduced in the aquatic environment through animal and human fecal shedding or sewage pollution [30]. *Listeria* spp. and *Salmonella* may contaminate fish in aquatic environment, during transportation to fish markets and storage.

CONCLUSIONS

Raw fish at retail market in Latvia contain high counts of TBC and *Enterobacteriaceae* among all fish species. *E. coli* was found in all round gobby samples. Although this study suggests the absence of *Salmonella*, however raw fish at retail level was found to be highly contaminated with *L. monocytogenes*, therefore consumers should exclude cross-contamination of food during fish preparation and avoid the consumption of raw fish to prevent foodborne infections. In order to assess the shortcomings of fish microbiological quality, there is a need of further studies among entire fish supply chain, including aquatic environment, transportation and storage of fish.

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