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Microbiological safety and quality of salmon: health benefits and risk

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A b s t r a c t: A total of 703 samples were tested over 1-year period. Listeria monocytogenes was isolated from 12.4% and 2.3% of fish and environmental swabs, respectively. The ratio of n-6/n-3 which is between 1:1 and 4:1, as more desirable parameter of the lipid quality for nutritive benefit e.g. reducing the risk of many diseases, in the fresh salmon, cold and hot smoked salmon was close to 1:1 (fresh salmon, 1.28; cold smoked salmon, 0.98; hot smoked salmon, 1.59). The fatty acid composition of smoked salmon products was also expressed as mg 100 g^{-1} which is important from the nutritional point of view. The examined salmon products had a high content of eicosapentaenoic acid and docosahexaenoic acid (742 to 1567 mg 100g^{-1}) and fulfill requirements for their sufficient contents of recommendations in the World. On the other hand, cold smoked salmon can be naturally contaminated with low numbers of L. monocytogenes. This could represent a serious hazard for susceptible individuals or "YOPI" (young, old, pregnant or immunocompromised individuals).

Keywords: *salmon, fatty acids, Listeria monocytogenes, quality, safety.*

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 at al.,* 2016). In 2013, global per capita consumption of fish was estimated at 19.7 kg, with fish accounting for about 17 percent of the global intake of animal proteins and 6.6 percent of all proteins consumed (*FAO*, 2016). The beneficial effects to humans of consuming fish, particularly oily fish such as salmon, herring and mackerel with a high content of the *n-3* highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA; 20:5*n-3*) and docosahexaenoic acid (DHA; 22:6*n-3*), have been well documented (*British Nutrition Foundation Task Force*, 1992; *British Nutrition Foundation*, 1993; *International Society for the Study of Fatty Acids and Lipids*, 1994; *Krauss et al.,* 2000). Due to the benefits of *n-3* polyunsaturated fatty acids (PUFAs) with regard to human health, particularly the suppression of many diseases, human consumption of fish is increasing worldwide. In contrast, clinical studies indicated that high amounts of *n-6* PUFAs and high *n-6*/*n-3* ratios promote the pathogenesis of many diseases. An optimal *n-6*/*n-3* ratio, which is between 1:1 and 4:1, is more desirable for reducing the risk of many diseases (*Simopoulos*, 2002). Marine ingredients were the only sources of EPA and DHA in Norwegian salmon feed in 2012, and since fish meal and fish oil are limited resources, both the retention of EPA and DHA and the utilization of these from trimmings and by-products are important aspects (*Ytrestøyl et al*., 2015). The shortage of fish oil and the resulting increase of plant oils with a high content of *n-6* fatty acids (FAs) in salmon diets have increased the *n-6*/*n-3* ratio in salmon fillets during the last decade which raises concerns both for fish health and for the beneficial health effects of salmon for the consumer, and it is, therefore, important to optimize the retention of EPA and DHA in commercial salmon farming (*Ytrestøyl et al*., 2015). The FA composition of fish can expressed as mg 100g^{-1} wet fillets, which importantly, shows the nutritional value of fish for human consumption (*Karakatsouli,* 2012).

Salmon is also high in vitamin E and possess high antioxidant properties (*Sallam,* 2007). The product is rich in proteins, low in carbohydrates (*Chitlapilly-Dass*, 2011) and reduction of the risk of coronary heart disease (CHD) mortality and stroke are identified as the main health benefits of *n-3* PUFAs from cold smoked salmon. On the

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other hand, the high lipid content in fish may protect microorganisms from thermal treatment or freezing (*Serio et al*., 2011). Cold smoked salmon is a raw, ready to eat food and, therefore, poses a risk to human health if it is contaminated with pathogens along the food chain (*Garrido et al.*, 2008). Namely, several listeriosis outbreaks have been associated with smoked fish, including cold smoked rainbow trout (*Ericsson et al*., 1997; *Miettinen et al.*, 1999) and cold smoked salmon (*Garrido et al.*, 2008).

The objective of the present study was to examine the presence and numbers of the foodborne pathogen *Listeria monocytogenes* in salmon. The FA composition of salmon products, important from the nutritional point of view, was also determined and was expressed as mg $100g^{-1}$.

Materials and methods

Samples

A total of 703 samples of salmon were examined over 1-year period. The samples included fresh, hot and cold smoked salmon (with or without herbs) as well as samples from the salmon processing environment (surfaces and drains). The samples, each consisting of 5 sample units coming from a production batch, upon reaching the laboratory, were kept refrigerated and analyzed within 2 h. Environmental swabs were collected according to the standard reference method (*SRPS ISO*, 2010a).

Isolation, identification and enumeration of L. monocytogenes

Isolation, identification, and enumeration of *L. monocytogenes* were performed following the standard method (*SRPS ISO*, 2010b), according to the Serbian regulation on conditions of food hygiene during production, processing and transport (*Serbia*, 2010). Biochemical identification of the *Listeria* isolates was performed using the culture method in combination with commercially available API *Listeria* identification system (bioMérieux, France). API tests were performed according to the manufacturer's instructions.

Enzyme-linked fluorescent assay

The procedure of screening *L. monocytogenes* in salmon and environmental samples by compact automated mini Vidas (*L. monocytogenes* Xpress) utilised an enrichment step, as prescribed by the VIDAS LMX producer (bioMérieux). At the end of an assay, the results were analyzed automatically by the instrument, which generated a test value for each sample. This value was then compared to internal references (thresholds) and each result was interpreted as positive or negative. All positive results obtained were confirmed by the culture method (*SRPS ISO*, 2010b), or by using chromogenic agar (Agar Listeria according to Ottaviani and Agosti, Oxoid, Basingstoke).

Capillary GC analysis of FAs

Total lipids for FAs determination were extracted from six of the salmon products (3 brands, 2 batches, analyzed in duplicate) by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) as reported by *Spiric* et al. (2010). Fatty acid methyl esters (FAMEs) in the extracted lipids were prepared by transesterification using 0.25 M TMSH (trimethylsulfonium hydroxide) in methanol (*ISO*, 2000). FAMEs were determined by GC Shimadzu 2010 (Kyoto, Japan) equipped with a split/splitless injector, fused silica cyanopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA) and flame ionization detector. The injection volume was 1 μL in the split ratio of 1:50. Nitrogen was used as carrier gas at a flow rate of 1.33 mL min^{-1}. The injector and detector temperatures were 250°C and 280°C, respectively. The column oven temperature was programmed starting at 125°C to a final temperature of 230°C. Total analysis time was 50.5 min. Chromatographic peaks in the samples were identified by comparing relative retention times to FAME peaks in the Supelco 37 Component FAME mix standard. Percentages of total FAs were converted to amounts of FA per 100 g of fish products, according to *Exler* et al. (1975).

Statistical analysis

The obtained data for the FA composition of salmon are reported as mean values ± the standard deviations. Analysis of variance (ANOVA) was performed using the Tukey-Kramer HSD test to analyze the data at the 5% level of significance.

Results and Discussion

Microbiological methods and enzyme-linked fluorescent assay

The overview of analyzed samples and occurrence of *L. monocytogenes* in the selected salmon processing line is presented in Table 1.Table 1. Overview of analyzed samples and occurrence of *L. monocytogenes* in a selected fish salmon processing line.

Table 1. Overview of analyzed samples and occurrence of *L. monocytogenes* in a selected salmon processing line

Group	Sample type	Number of samples	Number (%)positive for *L. monocytogenes*
Salmon and salmon products	Fresh, hot and cold smoked salmon (with or without herbs)	218	27 (12.4%)
Environmental samples	Swabs	485	11 (2.3%)
Total		703	38 (5.4%)

Table 2. Mean total lipid content (%) and fatty acid composition (% of total FAs and mg $100g^{-1}$) of six selected salmon products

	Fresh salmon		Cold smoked salmon		Hot smoked salmon with herbs	
	(% of total FA)	(mg or g $100g^{-1}$)	(% of total FA)	(mg or g $100g^{-1}$)	(% of total FA)	(mg or g $100g^{-1}$)
Total lipid[A]		18.27±0.05^{a}		14.33±0.03^{c}		17.25±0.05^{b}
Fatty acids[B]						
C14:0	2.50±0.01^{b}	457^{y}	2.14±0.03^{c}	307^{z}	2.74±0.02^{a}	470^{x}
C15:0	0.17±0.01^{a}	31^{x}	0.16±0.01^{a}	23^{y}	0.18±0.01^{a}	30^{x}
C16:0	9.50±0.02^{c}	1733^{y}	9.93±0.05^{b}	1423^{z}	10.98±0.02^{a}	1900^{x}
C16:1*n-7*	3.60±0.03^{a}	657^{x}	2.78±0.03^{c}	398^{z}	3.41±0.02^{b}	590^{y}
C17:0	0.10±0.01	18.24	Nd	nd	0.10±0.01	20
C18:0	2.29±0.02^{a}	418^{x}	2.30±0.03^{a}	330^{y}	2.30±0.03^{a}	400^{x}
C18:1*n-9*	38.05±0.10^{c}	6941^{y}	48.57±0.04^{a}	6960^{y}	44.57±0.05^{b}	7706^{x}
C18:2*n-6*	13.67±0.77^{a}	2493^{x}	13.77±0.03^{a}	1973^{y}	12.40±0.02^{a}	2140^{y}
C18:3*n-6*	0.09±0.01^{a}	17.33^{x}	nd	nd	0.10±0.01^{a}	20^{x}
C18:3*n-3*	4.28±0.03^{b}	782^{x}	4.70±0.02^{a}	674^{z}	4.20±0.03^{b}	730^{y}
C20:0	0.20±0.01^{a}	37.39^{y}	0.27±0.02^{a}	39^{y}	0.27±0.02^{a}	50^{x}
C20:1	4.21±0.01^{b}	768^{y}	4.07±0.01^{c}	583^{z}	5.10±0.10^{a}	880^{x}
C20:2*n-6*	1.14±0.02^{a}	209^{x}	0.93±0.01^{b}	133^{y}	1.03±0.01^{ab}	180^{x}
C20:3*n-6*	0.33±0.01^{b}	61^{y}	0.55±0.01^{a}	79^{x}	0.34±0.01^{b}	60^{y}
C20:3*n-3*	3.63±0.01^{b}	662^{y}	3.51±0.02^{c}	503^{z}	4.71±0.03^{a}	810^{x}
C20:5*n-3*	3.38±0.03^{a}	617^{x}	2.13±0.02^{c}	305^{z}	2.40±0.02^{b}	410^{y}
C22:5*n-3*	2.11±0.06^{a}	386^{x}	1.21±0.02^{c}	173^{z}	1.55±0.02^{b}	270^{y}
C22:6*n-3*	5.21±0.02^{a}	950^{x}	3.05±0.02^{c}	437^{z}	3.62±0.02^{b}	630^{y}
SFA	14.78±0.04^{b}	2696^{y}	14.79±0.02^{b}	2119^{z}	16.57±0.02^{a}	2860^{x}
MUFA	51.21±0.29^{c}	9341^{x}	55.41±0.02^{a}	7940^{z}	53.08±0.03^{b}	9180^{y}
PUFA	33.23±0.01^{a}	6061^{x}	29.81±0.07^{c}	4272^{z}	30.35±0.05^{b}	5250^{y}
n-3	18.73±0.25^{a}	3416^{x}	14.58±0.02^{c}	2089^{z}	16.48±0.06^{b}	2850^{y}
n-6	14.65±0.02^{b}	2673^{x}	15.24±0.03^{a}	2190^{z}	13.87±0.03^{c}	2400^{y}
n-3/n-6	1.28±0.03^{a}		0.98±0.03^{b}		1.19±0.04^{a}	
n-6/n-3	0.78±02^{c}		1.04±0.02^{a}		0.84±0.03^{b}	

[A] Total lipids are expressed as%; [B] Fatty acids are expressed as% of total FA and mg $100g^{-1}$; All values are reported as mean±SD; nd = not detected; $^{a, b, c, x, y, z}$; Values in the same row with the same letter are not significantly different (P≥0.05).

L. monocytogenes was isolated from 12.4% and 2.3% of salmon and environmental samples, respectively. All screening-positive samples were also tested by the reference culture method (see above). The enzyme-linked fluorescent assay, mini Vidas, reported the relative fluorescent value of the sample, the relative fluorescent value of the standard (RFVs), and test value, which is a quotient of the sample value and standard value. For the positive samples, RFVs ranged from 10624 to 12243. *L. monocytogenes* was isolated from all samples which were positive according to mini VIDAS.

FA expressed as weight percent of the total FAs

The lipid composition (expressed as% and FA composition (expressed as the percentage of the total FAs and in mg $100g^{-1}$) of the salmon products are shown in Table 2.Table 2: Mean total lipid content (%) and fatty acid composition (% of total FAs and mg $100g^{-1}$) of six selected salmon products

The most predominant FAs in salmon were oleic (C18:1*n-9*), linoleic (C18:2*n-6*) and palmitic acids (C16:0). No significant differences were observed in the saturated fatty acid (SFAs) content between the fresh and cold smoked salmon ($p\geq0.05$), while the hot smoked salmon with herbs contained a significantly higher content of SFA ($p\leq0.05$). The content of monounsaturated fatty acids (MUFAs) was significantly higher in the cold smoked salmon than in the fresh salmon, but was the highest in the hot smoked salmon with herbs ($p\leq0.05$). The PUFA content was significantly higher in fresh salmon than in hot smoked or cold smoked salmon ($p\leq0.05$). Of the *n-6* series FAs among the PUFAs, higher levels were measured in fresh and cold smoked salmon, while hot smoked salmon had the lowest levels. Of the *n-3* series FAs, higher levels were detected in fresh and hot smoked salmon, with the lowest level in cold smoked salmon. The content of linolenic acid (C18:3*n-3*), which is the precursor of EPA (C20:5*n-3*) and DHA (C22:6*n-3*), was high in fresh salmon, cold smoked salmon and hot smoked salmon 4.28%, 4.70% and 4.20%, respectively. Fresh salmon contained significantly higher amounts of EPA, DPA (C22:5*n-3*) and DHA compared to cold smoked salmon and hot smoked salmon with herbs ($p\leq0.05$). No significant differences were observed in the *n-3*/*n-6* ratio in the fresh salmon (1.28), cold smoked salmon (0.98) and hot smoked salmon (1.59). However, a significantly higher n-3/n-6 ratio was observed in the salmon than in freshwater fish such as carp (*Ljubojevic et al*., 2013; *Trbovic et al*., 2013).

FAs expressed as FA amounts (mg 100 g^{-1} of product)

The FAs in the salmon were also expressed as amounts of FA per 100 g of product. These data are more useful to obtain the absolute quantities of *n-3* and *n-6* PUFA consumed per specific fish serving. Our research showed that cold smoked salmon and hot smoked salmon with herbs contained significantly lower ($p\leq0.05$) amounts of *n-6* PUFA than fresh salmon (2190 mg $100g^{-1}$, 2400 mg $100g^{-1}$ and 2673 mg $100g^{-1}$, respectively). The amounts of n-3 PUFA were the higher in the fresh salmon than in hot smoked salmon with herbs and cold smoked salmon (2673 mg $100g^{-1}$, 2400 mg $100g^{-1}$ and 2190 mg $100g^{-1}$, respectively) ($p\leq0.05$). The quantities of EPA were the highest in the fresh salmon (617 mg $100g^{-1}$) compared to the hot smoked salmon with herbs and cold smoked salmon (410 mg $100g^{-1}$ and 305 mg $100g^{-1}$, respectively). DHA content was also the highest in the fresh salmon (950 mg $100g^{-1}$) compared to hot smoked salmon with herbs and cold smoked salmon (630 mg $100g^{-1}$, 437 mg $100g^{-1}$, respectively). Thus, intake of EPA plus DHA through consumption of 100 g of fresh salmon was 1567 mg, through consumption of cold smoked salmon intake was 742 mg and through consumption of hot smoked salmon with herbs was 1040 mg.

The main factors for bacterial contamination of seafood are contamination of the raw material, from the environment and from the processing, and bacterial growth conditions (temperature, water activity, pH, microbial interactions, etc.) (*Løvdal*, 2015). Because *L. monocytogenes* is a significant food safety concern, the prevalence of *L. monocytogenes* in smoked fish products in Europe has been extensively studied. The overall rate we determined is consistent with studies (with reported incidence levels between 5% and 35%) in individual EU member states. In our study, *L. monocytogenes* was isolated from 12.4% and 2.3% of fish and environmental swabs, respectively (Table 1). All *L. monocytogenes* positive salmon samples showed a contamination level below 100 CFU g^{-1}. This is comparable with data provided by *Uyttendaele et al.* (2009). The product with the highest prevalence of *L. monocytogenes* was smoked salmon. This high prevalence could be due to the low smoking temperature involved during the cold-salmon processing, as these conditions would be ideal for the proliferation of *L. monocytogenes* if the raw salmon harbored the pathogen or acquired it from the processing environment (*Chitlapilly-Dass,* 2011). Despite considerable efforts to improve the safety

of vacuum-packed smoked fish products, listeriosis outbreaks linked to the presence of *L. monocytogenes* at infective levels in these seafood commodities have been well documented, highlighting the need for technological interventions (novel alternative technologies such as irradiation and high pressure processing) to address the food safety risk posed by the presence of this pathogen in the final products (*Tocmo et al*., 2014).

The second part of the microbiological aspect of our study was detection of *L. monocytogenes* in environmental swabs from the salmon processing line (surfaces and drains). Out of 485 environmental samples analyzed, 11 (2.3%) samples were *L. monocytogenes* positive. Environmental swabs from the surface of slicing and trimming tables, slicing machines, fish filleting and trimming knives, belt glazer, and work table were positive for *L. monocytogenes*.

Similar data for EPA and DHA to than obtained in our study was published for frozen salmon slices, cold smoked salmon and hot smoked salmon (*Lerfall et al.,* 2016). Relatively high *n-3* PUFA levels and lower *n-6* PUFA levels provided a very favorable *n*-3/*n*-6 ratio in the other studies too, e.g. the cold smoked salmon of conventional reared salmon (*Lerfall et al.,* 2016; *Ytrestøyl, et al*., 2015). The optimal n-6/n-3 ratio is between 1:1 and 4:1 (*Simopoulos*, 2002), a more desirable parameter of the lipid quality for nutritive benefit e.g. reducing the risk of many diseases, and was close to 1:1 in fresh salmon, cold and hot smoked salmon studied. The data expressed as mg $100g^{-1}$ of products are more important from the nutritional point of view and are more useful obtaining the absolute quantities of *n-3* and *n-6* PUFAs consumed per specific fish serving (*Karakatsouli,* 2012). With respect to the cardiovascular diseases, prospective epidemiological and dietary intervention studies indicate that consumption of oily fish or dietary *n-3* long-chain PUFAs (equivalent to a range of 250 to 500 mg of EPA plus DHA daily) decreases the risk of mortality from CHD and sudden cardiac death. An intake of 250 mg of EPA plus DHA per day appears to be sufficient for primary prevention in healthy subjects (*EFSA*, 2010). Among the most notable recommendations, the French Food Safety Agency have determined that for their population, EPA and DHA intakes should be at least 250 mg each (*FAO/WHO,* 2013). Therefore, the salmon products studied, having a high content of EPA and DHA (742–1567 mg $100g^{-1}$), fulfill these requirements.

Conclusion

Relatively high n-3 PUFA levels and lower n-6 PUFA levels in salmon products in the current study provided a very favorable n-6/n-3 ratio, as was shown in other published studies. In the fresh salmon, cold and hot smoked salmon, the n-6/n-3 ratio was close to 1 in our study (fresh salmon, 1.28; cold smoked salmon, 0.98; hot smoked salmon, 1.59). The FA composition of salmon, when expressed as mg $100g^{-1}$ wet provides important data, as it demonstrates the nutritional value of the consumed fish. Intake of 250 mg of EPA plus DHA per day is recommended for primary prevention of CHD and sudden cardiac death in healthy subjects. The salmon products examined had a high content of EPA and DHA (742 to 1567 mg $100g^{-1}$) and so would fulfill EFSA dietary recommendations from this point of view. Also, although the salmon studied was quite often contaminated with Listeria monocytogenes, the levels of this pathogen should not represent a hazard to healthy individuals and can be considered as natural, low level contamination.

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