Results of 4th Quarter National Survey 2001 (NS4):

Smoked Salmon

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Summary
This study investigated the microbiological status of smoked salmon. Sampling took place during October, November and December of 2001. Samples were obtained from processing establishments and retail premises.

A total of 31 batches (each consisting of 5 samples) were tested from processing establishments. 12.9% (n=4) and 6.45% (n=2) of batches were unsatisfactory for ACC and *S.aureus* respectively. No batch was unsatisfactory for *E.coli* or *L.monocytogenes*.

A total of 321 samples from retail premises were analysed for ACC, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*. Using the criteria specified in the Commission Recommendation, 11.53% (n=37), 0.62% (n=2) and 0.62% (n=2) of retail samples were unsatisfactory for ACC, *S.aureus* and *L.monocytogenes* respectively. No retail sample was unsatisfactory for *E.coli*.

Follow up action was taken on unsatisfactory samples. This involved action on the product and/or action on the premises. This information was captured through a questionnaire.

1. Introduction
This study investigated the microbiological status of smoked salmon from processing establishments and retail premises in Ireland. This study was undertaken as part of the Official Control of Foodstuffs 2001 as outlined by the European Community (Commission Recommendation 2001/337/EC)[(1)]. Sampling took place during October, November and December of 2001. Samples were analysed for Aerobic Colony Count (ACC), *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*.

Smoking has been used as a means of food preservation for centuries. Smoking has a preservative effect because it lowers the water activity and forms a more membranous surface which acts as a physical barrier for the entry of microorganisms. Also the smoke contains a variety of compounds such as formaldehydes and phenols which are known to have bacteriostatic and bacteriocidal effects[(2)].

The process for smoked salmon production involves filleting, salting, drying, smoking, trimming and packaging. Salting is the first step in the preservation process. Salt maybe applied as dry salt, wet salt/brine (by soaking or by injection of the brine into the skin) or a combination of both. After salting the fish is air-dried and then smoked. Salmon can be smoked by either a cold or hot smoking process. Temperatures below 30^{o}C are maintained during cold smoking (this temperature prevents protein coagulation[(3)]) while temperatures between 70 ^{o}C and 80^{o}C are used in the hot smoking process (to ensure protein coagulation throughout the product[(3)]). In Ireland, the cold smoking process is most prevalent. After processing

the salmon products are generally vacuum packaged and stored at chilled temperatures. Products of this nature belong to the ready-to-eat food category.

Listeria monocytogenes is a pathogen which is ubiquitous in the environment and occurs naturally in many raw foods including fish[4]. It is of particular concern in ready-to-eat foods such as cold smoked fish which do not receive a heat treatment. It is also of concern in hot smoked fish as evidence exists that the inactivation of *L. monocytogenes* during the hot smoking process is often negated or undermined by recontamination[5]. Of further concern is the ability of this pathogen to both grow in high salt concentrations (0-10% salt) and under refrigerated conditions. In a risk assessment of *L. monocytogenes* in ready-to-eat foods carried out by the Food and Agricultural Organisation (FAO) of the United Nations, the risk of listeriosis[¥] from smoked fish was estimated as 2.1×10^{-8} cases per serving[5]. Outbreaks of listeriosis have been linked to cold smoked rainbow trout[6], smoked mussels[7] and gravad rainbow trout[8]. Listeriosis most often affects immunocompromised people, pregnant women, babies and the elderly. Symptoms of listeriosis includes infections of the central nervous system (meningitis, encephalitis), miscarriage, still births and neonatal disease.

Samples were also analysed for *Escherichia coli* and *Staphylococcus aureus*. *E.coli* is an enteric bacterium and is an indicator of faecal contamination. Its presence in smoked fish is indicative of cross contamination or inadequate controls during the production process. *S. aureus* is an ubiquitous organism but its main habitat is the skin, nose and throat of healthy people. Its presence in the final product is indicative of poor hygienic practices in food handling. The aerobic colony counts (ACC) of the smoked salmon samples were also monitored to provide information on the overall microbiological status of the product.

To date, there is no Community legislation fixing specific microbiological standards for smoked fish, however guidelines for ready-to-eat foods (including smoked fish) exist in Ireland at national level[9]. Microbiological criteria specified in Commission Recommendation 2001/337/EC[1] were used to determine the microbiological quality of the samples analysed in this survey.

2. Specific objectives

- To examine the microbiological quality (ACC, *S.aureus*, *E.coli* and *L.monocytogenes*) of smoked salmon as outlined by the EU Coordinated Programme for the Official Control of Foodstuffs 2001
- To record the action taken for 'unsatisfactory' samples by means of a questionnaire.
- To develop national survey methodology.

¥ Listeriosis is the disease caused by *Listeria monocytogenes*

3. Method

3.1 Sample source:

Samples were collected from 2 types of food business:

- Processing establishments: A batch sample consisting of 5 samples (taken on the same day from the same batch of the finished smoked salmon product) was taken from each processing establishment.
- Retail premises. On a given premises only 1 sample[¥] was taken for each brand name.
  [Food businesses are categorised in the FSAI Code of Practice No. 1[(10)]].

3.2 Sample description:

Smoked salmon which was sliced and vacuum packed and which was close to its use by date. Other fish products (e.g. gravadlax, smoked herrings etc.) were not included.

3.3 Sample collection and analysis: Environmental Health Officers (EHO's) from the 10 health boards (Appendix 1) took the samples. The samples were analysed in the 7 Official Food Microbiology Laboratories (OFML's – Appendix 2) using an approved / standard method. The microbiological quality of the samples was determined using the criteria specified in Table 1a and 1b.

Table 1a: Microbiological criteria[•] for batch samples (each batch consisted of 5 samples) from processing premises

Parameter	Criteria (cfu/g)		
	Satisfactory	**Acceptable**	**Unsatisfactory**
Aerobic colony count (30°C for 48h)	All samples ≤ 10^6	All samples <10^7 and at least three samples ≤10^6	Any sample ≥10^7or more than two samples >10^6
S. aureus[ϖ]	All samples ≤ 10	All samples < 100 and at least three samples ≤ 10	Any sample ≥ 100 or more than two samples >10
E. coli	All samples ≤ 10	All samples <100 and at least four samples ≤ 10	Any sample ≥100, or more than one sample >10
L. monocytogenes	Absent from all samples	Detected in any sample, but all samples < 100	Detected in any sample, and any ≥100

[¥] This is a derogation to Commission Recommendation 2001/337/EC where it was recommended that batch samples should be taken in both retail and processing establishments.

[•] Criteria specified in Commission Recommendation 2001/337/EC

[ϖ] Criteria for sliced, vacuum packed smoked salmon

Table 1b: Microbiological criteria for single samples from retail premises♦

Parameter	Criteria (cfu/g)		
	Satisfactory	**Acceptable**	**Unsatisfactory**
Aerobic colony count (30°C for 48h)	$\leq 10^6$	$>10^6$ - $<10^7$	$\geq 10^7$
*S. aureus*ϖ	≤10	>10 - <100	≥ 100
E. coli	≤10	>10 - <100	≥ 100
L. monocytogenes	Absent	Detected, but <100	Detected, and ≥ 100

3.4 Subtyping of *L. monocytogenes* positive samples

To obtain more information on the types of *L. monocytogenes* detected in smoked salmon, 26 positive isolates were subtyped using phenotypic and genotypic methods as outlined in Table 2.

Table 2: Methods used to subtype positive isolates *of L. monocytogenes*

Subtyping	Method	Location of study
Phenotypic	Antibody-antigen serotyping	PHL, Collindale
Genotypic	Ribotyping (Qualicon riboprinter)	Waterford Regional Hospital
	Pulse Field Gel Electrophoresis (PFGE)	Cork Institute of Technology
	Amplified Fragment Length Polymorphism (AFLP)	PHL, Collindale

3.5 Further investigation

In the context of this survey, 'satisfactory' and 'acceptable' retail samples required no further action. For 'unsatisfactory' retail samples, the EHO decided on the enforcement action to be taken with advice from FSAI or OFML, as necessary. If a follow up sample was necessary, it was not included as a survey sample. For processing samples, the EHO reported the results from batches taken at processing establishments supervised by the Department of Marine and Natural Resources (DMNR) to the Department for enforcement. Information on follow up action was captured by means of a questionnaire.

♦ The criteria for single samples presented in Table 1b are a variation of those outlined in Commission Recommendation 2001/337/EC for batch samples.

ϖ Criteria for sliced, vacuum packed smoked salmon

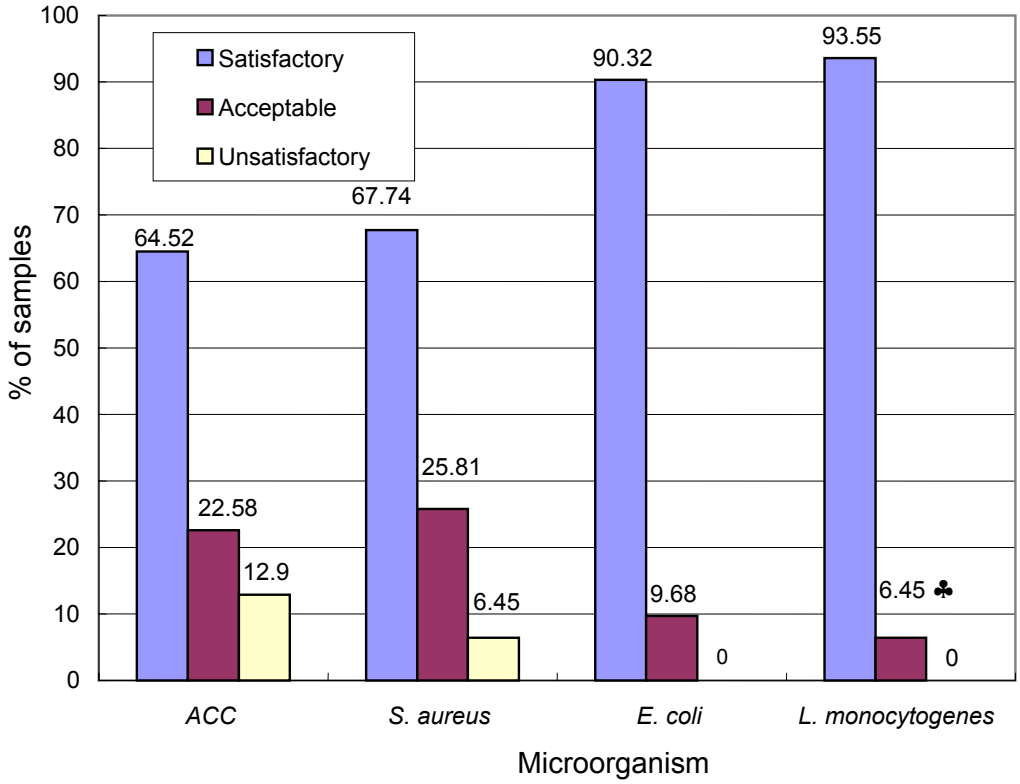
4. Results and Discussion

4.1 Overall microbiological results for processing and retail samples

4.1.1. Processing samples

A total of 31 batches (5 samples per batch) of smoked salmon were sampled from processing establishments in 8 of the 10 health boards. Figure 1 illustrates the microbiological status (ACC, *S. aureus*, *E. coli* and *L. monocytogenes*) of the 31 batches.

Figure 1: Microbiological status[Φ] of smoked salmon batches from processing establishments (n=31 batches)



♣ 6.45% (n=2) of batches were acceptable for *L. monocytogenes*. This includes 1 batch where *L. monocytogenes* was detected in 1 of the 5 samples but was not enumerated

[Φ] Microbiological status was determined using the criteria outlined in Table 1a.

Of the batches analysed, 12.9% (n=4) and 6.45% (n=2) were unsatisfactory for ACC and *S. aureus.* No batch was categorised as unsatisfactory for *E. coli* or *L. monocytogenes*. Details of the 6 unsatisfactory batches are outlined in Table 3. No batch was unsatisfactory for more than 1 microbiological parameter.

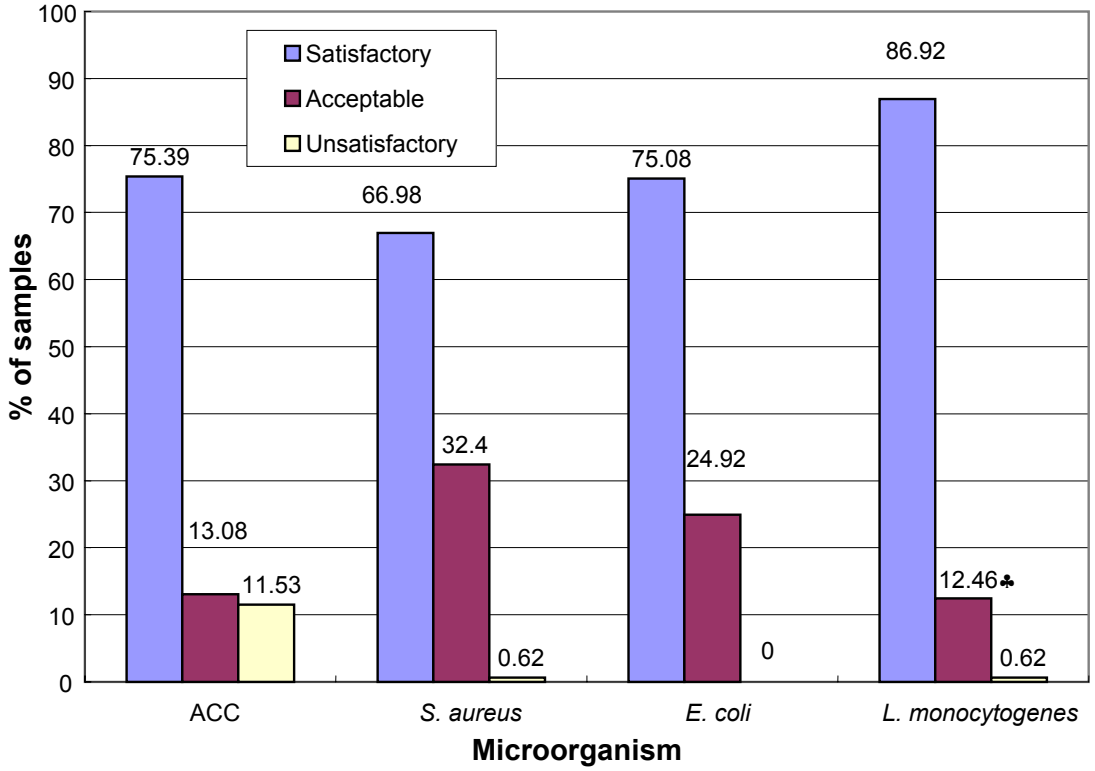
Table 3: Details of the unsatisfactory batches (n=6)

	Microbiological Status			
Batch	**ACC**	***S. aureus***	***E. coli***	***L. monocytogenes***
1	**Unsatisfactory**	Satisfactory	Satisfactory	Satisfactory
2	**Unsatisfactory**	Satisfactory	Satisfactory	Satisfactory
3	**Unsatisfactory**	Satisfactory	Satisfactory	Satisfactory
4	**Unsatisfactory**	Acceptable	Satisfactory	Satisfactory
5	Satisfactory	**Unsatisfactory**	Satisfactory	Satisfactory
6	Acceptable	**Unsatisfactory**	Satisfactory	Satisfactory

4.1.2 Retail samples

A total of 321 retail samples of smoked salmon were obtained in each of the 10 health boards. Figure 2 illustrates the microbiological status (ACC, *S. aureus*, *E. coli* & *L. monocytogenes*) of the 321 samples.

Figure 2: Microbiological status[Φ] of retail smoked salmon samples (n=321[♦])



♣ 12.46% (n=40) of samples were acceptable for *L. monocytogenes*. This includes 9 samples where *L. monocytogenes* was detected but not enumerated. These 9 samples were sampled in NAHB (1), MWHB (2), NEHB (4), SWAHB (1) and ECAHB (1).

Of the samples analysed, 11.53% (n=37), 0.62% (n=2) and 0.62% (n=2) of samples were unsatisfactory for ACC, *S. aureus* and *L. monocytogenes* respectively. No sample was unsatisfactory for *E. coli*. Of the 41 unsatisfactory samples, only 1 sample was unsatisfactory for more than 1 microbiological parameter, i.e. the

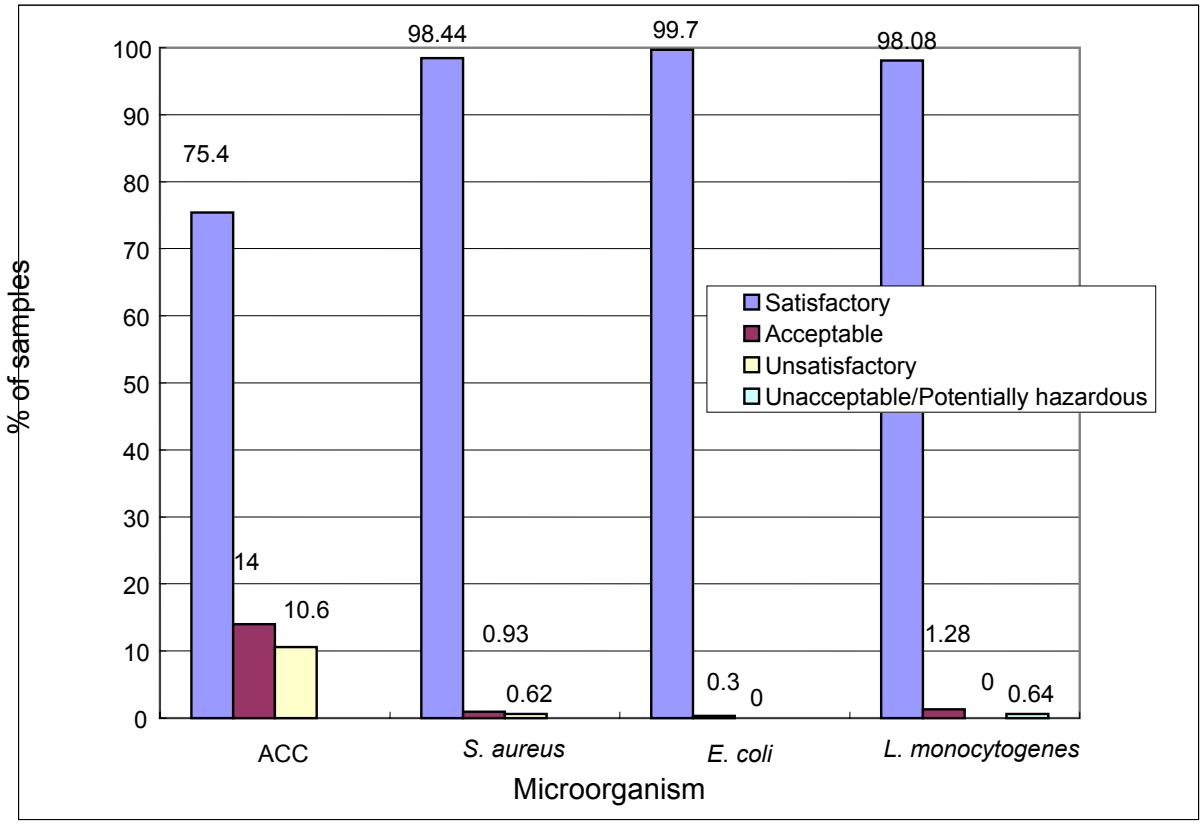
[Φ] Microbiological status was determined using the criteria outlined in Table 1b.

[♦] A total of 337 retail samples were submitted for analysis. 16 samples were excluded: 4 for missing tests (3 without *E.coli*, 1 without ACC), 9 for incorrect sample type (4 trout, 4 mackerel and 1 coley) and 3 for insufficient sample.

sample was unsatisfactory for ACC ($5.1x10^7$cfu/g) and *L. monocytogenes* ($6x10^2$cfu/g).

As stated in the introduction guidelines for the interpretation of results of microbiological analysis of some ready-to-eat foods sampled at point of sale exist at national level♦. These include guidelines for smoked salmon (Appendix 3). The following results were obtained when these guidelines were applied to the results of this survey (Fig. 3).

Figure 3: Microbiological status of retail smoked salmon samples based on the Irish microbiological guidelines♦



75.4% (n=242), 98.44% (n=316), 99.7% (n=320) and 98.08% (n=315) of all samples were satisfactory for ACC, *S. aureus*, *E. coli* and *L. monocytogenes* using the Irish national microbiological guidelines.

♦ FSAI Guidance Note No. 3: Guidelines for the Interpretation of Results of Microbiological Analysis of Some Ready-To-Eat Foods Sampled at the Point of Sale [9]

4.2 Results by microbiological parameter

4.2.1 ACC

A) Processing Samples

Of the 31 batches (5 samples per batch) of smoked salmon which were sampled form processing establishments, 64.52% (n=20 batches) were satisfactory, 22.58% (n=7) were acceptable and 12.9% (n=4) were unsatisfactory for ACC (Figure 1). These results are presented in Table 4 according to the health board where the sampling took place.

Table 4: Microbiological status$^{\Phi}$ (ACC) of smoked salmon batches from processing establishments according to health board (n=31 batches; 5 samples per batch)

Health board area$^{\varpi}$	Satisfactory i.e all $\leq 10^6$ (%)	Acceptable i.e all $<10^7$ and at least 3 samples $\leq 10^6$ (%)	Unsatisfactory i.e any sample $\geq 10^7$ or more than 2 samples $>10^6$ (%)	Total
MWHB	0	1	1	2
NAHB	1	0	0	1
NEHB	1	0	0	1
NWHB	0	0	1	1
SEHB	3	0	1	4
SHB	9	4	0	13
SWAHB	1	1	0	2
WHB	5	1	1	7
TOTAL	**20 (64.52)**	**7 (22.58)**	**4 (12.9)**	**31**

Details of the 4 unsatisfactory batches are outlined in Table 5. In 3 of the unsatisfactory batches, ACC levels of $>10^7$ cfu/g were detected in 1 or more of the 5 individual samples.

Table 5: Details of unsatisfactory ACC batches (n=4 batches, 5 samples/batch)

	Number of samples		
Batch	**$<10^6$ cfu/g**	**$>10^6$ - $<10^7$ cfu/g**	**$\geq 10^7$ cfu/g**
1	0	1	4
2	2	3	0
3	3	1	1
4	3	0	2

$^{\Phi}$ Microbiological status was determined using the criteria outlined in Table 1a.

$^{\varpi}$ See Appendix 2 for details of health boards

In a Danish study[11], variations in microbial levels were found in smoked salmon from three different processing premises (smoke houses). A number of parameters such as the quality of the raw materials, the production method, the packaging material etc. were cited as reasons for the variability in the microbiological status of samples between processing premises. In this survey it was not a requirement to monitor processes and practices in the processing establishments, therefore no correlation could be made between these and the ACC results.

B) Retail Samples

At retail level, 75.39% (n=242) of samples were satisfactory, 13.08% (n=42) acceptable and 11.53% (n=37) unsatisfactory for ACC (Figure 2). The breakdown of these results according to the health board area where the sampling took place is presented in Table 6.

Table 6: Microbiological status (ACC)$^{\Phi}$ of samples from retail premises according to health board area (n=321 samples)

Health board$^{\varpi}$	Satisfactory i.e. $\leq 10^6$ (%)	Acceptable i.e. $>10^6$ to $<10^7$ (%)	Unsatisfactory i.e. $\geq 10^7$ (%)	Total
ECAHB	11	1	4	16
MHB	20	2	1	23
MWHB	20	2	1	23
NAHB	23	3	5	31
NEHB	23	3	4	30
NWHB	29	2	6	37
SEHB	42	3	6	51
SHB	35	15	0	50
SWAHB	20	5	5	30
WHB	19	6	5	30
TOTAL	**242 (75.39)**	**42 (13.08)**	**37 (11.53)**	**321**

Of the 37 samples which were unsatisfactory for ACC, 29 samples had a count in the range 10^7-$<10^8$cfu/g, 7 samples were in the range 10^8-10^9cfu/g and 1 sample had a count $>10^9$cfu/g.

Table 7 provides information on the prevalence of ACC in retail smoked salmon in other countries (UK, Spain, Finland and Canada). The findings of this survey are statistically similar ($p<0.05$) to the findings of the 1993 UK survey (EC Coordinated Food Control Programme)[12] in which ACC levels $<10^6$ and $\geq 10^6$ were detected in 78% and 22% respectively of all samples tested.

$^{\Phi}$ Microbiological status was determined using the using the criteria outlined in Table 1b.

$^{\varpi}$ See Appendix 2 for details of health boards

Table 7: Prevalence of ACC in smoked salmon by country of study

Country	Sample	Year of study	No. of samples	ACC cfu/g (no. of samples, %)	Ref.
UK	Smoked salmon	1993¥	86	$<10^6$ (n=67, 78%) $\geq 10^6$ (n=19, 22%)	12
UK	Smoked salmon	1989-1993♦	19	$<10^6$ (n=17, 89%) $\geq 10^6$ (n=2, 11%)	
Spain	Cold smoked salmon (vacuum packed)	2002✪	30	$6.4x10^6 – 4.0x10^7$ cfu/g (n=30, 100%) (25^oc)♣	13
Finland	Hot smoked salmon – non sliced (vacuum packed)	1998✪	6	$<10^6$ (n=6, 100%)	14
Canada	Smoked fish	1988/1989	100☆ 66	$<10^6$ (n=88, 88%) $\geq 10^6$ (n=12, 12%) $<10^6$ (n=20, 30%) $\geq 10^6$ (n=46, 70%)	15
Ireland	Smoked salmon	2003	321	$<10^6$ (n=242, 75%) $>10^6$ - $<10^7$ (n=42, 13%) $\geq 10^7$ (n=37, 12%)	This study

The microflora of the ACC population was not determined in this study, however, other studies have noted that lactic acid bacteria are prevalent[11,14, 16]. Their ability to grow at low temperatures and their tolerance to CO_2 may explain their dominance in vacuum packed foods[13]. Other groups such as the *Enterobacteriaceae* and *Micrococcaceae* also account for a significant proportion of the microflora[13].

It should be noted that although the ACC level provides information on the overall microbiological status of the product and is an indicator of improper process control, post process contamination and/or poor hygienic conditions, it is not a reliable indicator of shelf life for lightly preserved fish products[13]. This is because spoilage of the product can occur due to autolytic tissue degradation when ACC levels are low.

¥ This study was undertaken as part of the EC Coordinated Food Control Programme for 1993

♦ This study was part of the routine surveillance carried out by 3 of the UK Public Health laboratories

✪ No information was provided on the survey date, therefore the publication date of the paper is presented

♣ In this Spanish study the results are presented as the range of values

☆100 samples were analysed on the day of purchase. 66 samples were stored at 4^oC and were analysed 30 days later.

4.2.2 *S. aureus*

A) Processing samples

Of the 31 batches (5 samples per batch) of smoked salmon which were sampled form processing establishments, 67.74% (n=21 batches) were satisfactory, 25.81% (n=8) were acceptable and 6.45% (n=2) were unsatisfactory for *S. aureus* (Figure 1). These results are presented in Table 8 according to the health board where the sampling took place.

Table 8: Microbiological status[Φ] (*S.aureus* [ϕ]) of smoked salmon batches from processing establishments according to health board (n=31 batches; 5 samples per batch)

Health board area[ϖ]	Satisfactory (all ≤10)	Acceptable (all <100 and at least 3 samples ≤10)	Unsatisfactory (any ≥100 or more than 2 samples >10)	Total
MWHB	0	2	0	2
NAHB	1	0	0	1
NEHB	0	1	0	1
NWHB	1	0	0	1
SEHB	3	0	1	4
SHB	9	3	1	13
SWAHB	0	2	0	2
WHB	7	0	0	7
TOTAL	**21 (67.74)**	**8 (25.81)**	**2 (6.45)**	**31**

Details of the 2 unsatisfactory batches are presented in Table 9.

Table 9: Details of batches unsatisfactory for *S. aureus* (n=2 batches, 5 samples/batch)

	No. of samples in batch		
Batch	**≤10 cfu/g**	**> 10 cfu/g**	**≥ 100 cfu/g**
1	4	0	1
2	2	3	0

[Φ] Microbiological status was determined using the using the criteria outlined in Table 1a.

[ϕ] It was assumed that all batches were sliced, vacuum packed smoked salmon

[ϖ] See Appendix 2 for details of health boards

B) Retail samples

At retail level, 66.98% (n=215) of all samples were satisfactory, 32.40% (n=104) were acceptable and 0.62% (n=2) were unsatisfactory for *S. aureus*. The breakdown of these results according to the health board area where the sampling took place is presented in Table 10.

Table 10: Microbiological status[Φ] (*S. aureus*[∞]) of samples from retail premises according to health board area (n=321 samples)

Health board area[ϖ]	Satisfactory i.e ≤10 (%)	Acceptable i.e. >10 to <100 (%)	Unsatisfactory i.e. ≥100 (%)	Total
ECAHB	8	8	0	16
MHB	18	5	0	23
MWHB	0	23	0	23
NAHB	10	21	0	31
NEHB	0	30	0	30
NWHB	37	0	0	37
SEHB	49	2	0	51
SHB	48	1	1	50
SWAHB	16	14	0	30
WHB	29	0	1	30
TOTAL	**215 (66.98)**	**104 (32.40)**	**2 (0.62)**	**321**

S. aureus counts of $2x10^3$ and $7.9x10^2$ cfu/g were recorded for the 2 unsatisfactory samples.

Very little information is available in the literature on the incidence of *S. aureus* in retail smoked salmon. In a Spanish study[13] on vacuum packed cold smoked salmon (n=30), *S. aureus* was detected at levels $<10^4$ cfu/g in 6.7% (n=2) of samples. In a Canadian study[15] on smoked fish, *S. aureus* was not detected in any sample analysed on the day of purchase (n=100) or in any sample analysed after 30 days storage at 4°C (n=66).

[Φ] Microbiological status was determined using the using the criteria outlined in Table 1b

[∞] It was assumed that all samples were sliced vacuum packed smoked salmon

[ϖ] See Appendix 2 for details of health boards

4.2.3 *E. coli*

A) Processing Samples

Of the 31 batches (5 samples per batch) of smoked salmon which were sampled form processing establishments, 90.32% (n=28 batches) were satisfactory and 9.68% (n=3) were acceptable for *E. coli.* No batch was unsatisfactory for *E. coli* (Figure 1). These results are presented in Table 11 according to the health board where the sampling took place.

Table 11: Microbiological status[Φ] (*E. coli*) of smoked salmon batches from processing establishments according to health board (n=31 batches; 5 samples per batch)

Health board area[ϖ]	Satisfactory i.e. all ≤10 (%)	Acceptable i.e. all <100 and at least 4 samples ≤10 (%)	Unsatisfactory i.e. any ≥100 or more than 1 sample >10 (%)	Total
MWHB	2	0	0	2
NAHB	1	0	0	1
NEHB	0	1	0	1
NWHB	1	0	0	1
SEHB	4	0	0	4
SHB	13	0	0	13
SWAHB	0	2	0	2
WHB	7	0	0	7
TOTAL	**28 (90.32)**	**3 (9.68)**	**0**	**31**

B) Retail Samples

At retail level, 75.08% (n=241) of all samples were satisfactory and 24.92% (n=80) were acceptable for *E. coli*. No retail sample was unsatisfactory for *E. coli*. The breakdown of these results according to the health board area where the sampling took place is presented in Table 12.

[Φ] Microbiological status was determined using the using the criteria outlined in Table 1a

[ϖ] See Appendix 2 for details of health boards

Table 12: Microbiological status[Φ] (*E. coli*) of retail samples according to health board area (n=321)

Health board area[ϖ]	Satisfactory i.e ≤10 (%)	Acceptable i.e. >10 to <100 (%)	Unsatisfactory i.e. ≥100 (%)	Total
ECAHB	8	8	0	16
MHB	18	5	0	23
MWHB	23	0	0	23
NAHB	8	23	0	31
NEHB	0	30	0	30
NWHB	37	0	0	37
SEHB	51	0	0	51
SHB	50	0	0	50
SWAHB	16	14	0	30
WHB	30	0	0	30
TOTAL	**241 (75.08)**	**80 (24.92)**	**0**	**321**

The prevalence of *E. coli* has been investigated in both a Spanish survey[(13)] (n=30) on vacuum packed cold smoked salmon and in a Canadian[(15)] (n=100) survey on smoked fish. In the Canadian survey, 66 of the 100 samples were also analysed after 30 days storage at 4°C. *E. coli* was not detected in either survey, however, in both surveys information was not provided on the limit of detection.

[Φ] Microbiological status was determined using the criteria outlined in Table 1b

[ϖ] See Appendix 2 for details of health boards

4.2.4 *L. monocytogenes*

A) Processing Samples

Of the 31 batches (5 samples per batch) of smoked salmon which were sampled form processing establishments, 93.55% (n=29 batches) were satisfactory and 6.45% (n=2 batches) were acceptable for *L. moncoytogenes.* No batch was unsatisfactory for *L. monocytogenes* (Figure 1). These results are presented in Table 13 according to the health board where the sampling took place.

Table 13: Microbiological status[Φ] (*L. monocytogenes*) of smoked salmon batches from processing establishments according to health board (n=31 batches; 5 samples per batch)

Health board area[ϖ]	Satisfactory (absent from all)	Acceptable (detected but all <100)	Unsatisfactory (Detected and any ≥100)	Total
MWHB	2	0	0	2
NAHB	0	1	0	1
NEHB	1	0	0	1
NWHB	1	0	0	1
SEHB	4	0	0	4
SHB	13	0	0	13
SWAHB	1	1	0	2
WHB	7	0	0	7
TOTAL	**29 (93.55)**	**2 (6.45)[♣]**	**0**	**31**

The raw material and the processing environment are the primary sources of *L. monocytogenes* contamination in the cold smoking process. In order to elucidate the significance of each possible contamination route, many studies have been carried out based on molecular subtyping techniques such as pulse field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) and ribotying. While there is some discrepancy in the literature, the majority of studies suggest that the processing environment is the most immediate or direct source of product contamination[(17)]. Contamination from the processing environment is also significant in the hot smoking process as it has been shown that inactivation of *L. monocytogenes* during the hot smoking process is often negated or undermined by recontamination from the processing environment.

[Φ] Microbiological status was determined using the using the criteria outlined in Table 1a

[ϖ] See Appendix 2 for details of health boards

[♣] 6.45% (n=2) of batches were acceptable for *L. monocytogenes*. This includes 1 batch where *L. monocytogenes* was detected, but not enumerated, in 1 of the 5 samples.

Regarding the processing environment, research has shown that individual processing establishments often harbour their own unique population of *L. monocytogenes*[18] and that these subtypes are capable of persisting in the environment over time [18, 19]. This may partly be explained by:

1. the ability of *L. monocytogenes* to adhere to surfaces such as stainless steel and its increased resistance to cleaning and disinfection procedures when in this adhered state[20] and
2. the formation of biofilms (in areas which may never have the opportunity to dry e.g. drains) which require more aggressive sanitation[19].

B) Retail Samples

At retail level, 86.92% (n=279) of samples were satisfactory, 12.46% (n=40) acceptable and 0.62% (n=2) unsatisfactory for *L. monocytogenes*. The breakdown of these results according to the health board where the sampling took place are presented in Table 14.

Table 14: Microbiological status$^{\Phi}$ (*L. monocytogenes*) of retail samples according to health board area (n=321)

Health board area$^{\varpi}$	Satisfactory i.e. absent (%)	Acceptable i.e. detected but <100 (%)	Unsatisfactory i.e. detected and ≥100 (%)	Total
ECAHB	14	1	1	16
MHB	18	5	0	23
MWHB	21	2	0	23
NAHB	26	4	1	31
NEHB	26	4	0	30
NWHB	32	5	0	37
SEHB	44	7	0	51
SHB	46	4	0	50
SWAHB	26	4	0	30
WHB	26	4	0	30
TOTAL	**279 (86.92)**	**40 (12.46)**$^{\blacklozenge}$	**2 (0.62)**	**321**

L. monocytogenes counts of $1.6x10^2$ and $6x10^2$ cfu/g were recorded for the 2 unsatisfactory samples.

$^{\Phi}$ Microbiological status was determined using the using the criteria outlined in Table 1b

$^{\varpi}$ See Appendix 2 for details of health boards

$^{\blacklozenge}$ This includes 9 samples where L. monocytogenes was detected but not enumerated. These 9 samples were sampled in NAHB (1), MWHB (2), NEHB (4), SWAHB (1) and ECAHB (1)).

The majority of studies which have investigated the incidence of *L. monocytogenes* in smoked salmon at retail level, report prevalence (i.e. presence/absence) rather than numerical data.

Prevalence data are presented in Table 15. These data clearly show the variability (0 to 77%) in the prevalence of *L. monocytogenes* in smoked salmon. A number of factors may be responsible for this variability including the type of smoking (hot vs. cold), the nature of the process (smoke concentration, process temperature) and the smoking environment. This type of information was not recorded in all surveys.

Numerical data are presented in Table 16 for a number of countries. In all surveys *L. monocytogenes* was detected predominantly at levels <100cfu/g. There was no significant difference ($p<0.05$) in data from the Danish and Irish surveys.

The detection of *L. monocytogenes* in retail samples is not unexpected as it is well documented that this pathogen is capable of growing in smoked salmon under typical storage conditions, i.e. refrigerated vacuum packed conditions. Research has shown that the level of contamination in the product following storage is related to the initial contamination level (i.e. the post production contamination level)[21].

Table 15: Prevalence of *L. monocytogenes* in retail smoked salmon by country of origin

Country	Sample Type	Year	Sample numbers	No. of + samples (%)	Ref.
Denmark[¥]	Cold smoked salmon	1998[♣]	190	64 (34)[☆]	22
Finland	Hot smoked salmon Non-sliced (vacuum packed)	1998[♣]	6	0 (0)	14
Japan	Smoked salmon	1999	92	5 (5.4)	23
New Zealand	Cold smoked salmon (sliced) Hot smoked salmon (fillet)	1990-1991	13 3	10 (77) 0 (0)	24
Spain	Cold smoked salmon (vacuum packed)	2002[♣]	30	0 (0)[✪]	13
UK	Smoked salmon	1993[♦] 1989-1993[◎]	86 19	2 (2) 4 (21)	12
Ireland	Smoked salmon	2001	321	42 (13.08)	This study

[¥] In this study the fish samples in retail packs were collected from the production sites rather than the retail premises.

[♣] No information was provided on the time of the survey, therefore the publication date of the paper is presented

[☆] The prevalence increased to 43% when samples were tested at the end of their commercial shelf life (i.e. 21-50 days after the production date)

[✪] *Listeria* spp. other than *L. monocytogenes* were detected (7 strains of *L. inocua*, 1 strain of *L. welshimeri* and 1 strain of *L. seeligeri* were isolated from 5 samples)

[♦] This study was undertaken as part of the EC Coordinated Food Control Programme for 1993

[◎] This study was part of the routine surveillance by 3 UK Public Health laboratories

Table 16: Numerical data for *L. monocytogenes* in retail smoked salmon by country of origin

Country	Sample Type	Year	Sample no.	Notes	No. of + samples (%)	Count (no. of samples)	Ref.
Denmark[¥]	Cold smoked salmon	1998[♦]	190	Beginning of shelf life[◎]	64 (34)	<100 (n=62) >100 (n=2)	22
			115	End of shelf life[•]	46 (40)	<100 (n=34) >100 (n=12)	
			75	End of shelf life[∅]	32 (43)	<100 (n=28) >100 (n=4)	
Japan	Smoked salmon	1999	92	No info.	5 (5.4)	<10 (n=5)	23
Ireland	Smoked salmon	2001	321	End of shelf life	42 (13.08)	<100 (n=40) ≥100 (n=2)	This study

[¥] In this study the fish samples in retail packs were collected from the production sites rather than the retail premises.

[♦] No information was provided on the time of the survey, therefore the publication date of the paper is presented

[◎] Samples analysed 4-12 days from production date

[•] Samples analysed at end of commercial shelf life, i.e. 14-20 days after production date

[∅] Samples analysed at end of commercial shelf life, i.e. 21-50 days after production date

C) Subtyping Studies

Subtyping studies were undertaken on 26 isolates of *L. monocytogenes* from retail smoked salmon samples. Subtyping studies allow for the differentiation of bacterial isolates beyond the species and subspecies level and are used not only to obtain a better understanding of the population genetics, epidemiology, and ecology of food borne pathogens but also to track sources of bacterial contamination throughout the food system.

The aim of this work was to obtain more information on the *L. monocytogenes* population in the samples examined and to compare subtyping (phenotypic and genotypic) methods. The overall results are presented in Appendix 4.

i) Phenotypic technique (Antigen-Antibody serotyping)

Using this technique, 3 main serotypes (1/2a, 1/2c and 4b) were identified in the 26 isolates positive for *L. monocytogenes* (Table 17).

Table 17: Serotypes of *L. monocytogenes* detected in smoked salmon

Serotype	No. of isolates
1/2a	21
1/2	1[¥]
1/2c	2
4b	2

According to the literature most listeriosis cases are associated with a restricted number of serotypes: 1/2a (15-25%); 1/2b (10-35%) and 4b (37-64%)[(5)]. Since serotype 1/2a was dominant in the smoked salmon isolates, it suggests that smoked salmon is a possible transmission route for this pathogen.

ii) Genotypic techniques (Ribotyping, PFGE & AFLP)

There was an increase in typing resolution when genotypic methods were used (Table 18) compared to phenotypic methods.

Table 18: Relationship between typing resolution and genotypic technique.

Genotypic technique	**No. of types**
Ribotyping	**6 Ribotypes**
PFGE	**7 PFGE types**
AFLP	**5 AFLP types**

[¥] One isolate could only be resolved to serotype 1/2.

5. Follow up Action

For the purposes of this survey, follow up action was taken on unsatisfactory samples. Follow up actions were taken on the product, the premises or on both. Details of actions taken on unsatisfactory processing and retail samples are presented in Tables 19 and 20 respectively.

Table 19: Details of follow up action taken on unsatisfactory processing batches

		Action taken©		
Microbiological parameter	**No. of unsatisfactory samples**	**None**	**Action on product**	**Action on premises**
ACC	4	0	3	4
S. aureus	2	1	1	1
E. coli	0	-	-	-
L. monocytogenes	0	-	-	-

Table 20: Details of follow up action taken on unsatisfactory retail samples

		Action taken©		
Microbiological parameter	**No. of unsatisfactory samples**	**None**	**Action on product**	**Action on premises**
ACC	37♦	10	16♣	22 ♣
S. aureus	2	1	0	1
E. coli	0	-	-	-
L. monocytogenes	2♦	0	1	2•

© Multiple actions may have been taken

♦ Includes one sample which was unsatisfactory for both ACC and *L. monocytogenes*

♣Action on both the product and premises was taken for 11 samples

• Action on both the product and premises was taken for 1 sample

6. Conclusions

The detection of ACC (12.9%) and *S. aureus* (6.45%) at unsatisfactory levels in processing samples suggests that control mechanisms were inadequate in the associated premises. Control mechanisms include the use of Good Hygiene Practices (GHPs), Good Manufacturing Practices (GMPs) and the implementation of a food safety management system based on the principles of HACCP (this is a legal requirement for all food businesses in Ireland since 1998).

Although it is very encouraging that both *E. coli* and *L. monocytogenes* were not detected at unsatisfactory levels in processing samples, it is imperative that processors do not become complacent in their control efforts. This is particularly important in relation to *L. monocytogenes* as it has been shown that this pathogen is capable of re-establishing itself in processing environments (and thus in the final product) after periods of prolonged absences. Strategies have been proposed for the control of *L. monocytogenes* in the processing environment. These strategies (based on GMP, GHP and HACCP) include the elimination of niche environments (i.e. areas which favour the establishment and proliferation of *L. monocytogenes*), environmental monitoring, raw material testing, end product monitoring etc. In recent years much research has focused on additional control strategies, these include the use of CO_2[25], the use of bacteriocins[26] and increasing the level of the indigenous microflora in the foodstuff to create a competitive environment for *L. monocytogenes*[27].

At retail level, 11.53%, 0.62% and 0.62% of samples were unsatisfactory for ACC, *S. aureus* and *L. monocytogenes* respectively. At retail level, good handling techniques, prevention of cross contamination and good temperature control are essential in maintaining the microbiological quality and safety of the foodstuff. Retailers must ensure that products are not for sale past their use-by-dates and that they are supplied with a product of high microbiological quality. This is particularly important in relation to *L. monocytogenes* as it is well documented that this pathogen is capable of growing under refrigerated conditions (temperature range 0-45^{o}C). Retailers may ensure this through supplier audits and microbiological sampling.

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8. Appendices

Appendix 1

List of Health Boards

Health board	Abbreviation
East-Coast Area Health Board	ECAHB
Midland Health Board	MHB
Mid-Western Health Board	MWHB
Northern Area Health Board	NAHB
North-Eastern Health Board	NEHB
North-Western Health Board	NWHB
South-Eastern Health Board	SEHB
Southern Health Board	SHB
South-Western Area Health Board	SWAHB
Western Health Board	WHB

Appendix 2

List of the Official Food Microbiology Laboratories (OFMLs)

Laboratory
Cherry Orchard Hospital
Mid-Western Regional Hospital
Public Analysts Laboratory, Dublin
Sligo General Hospital
St Finbarr's Hospital, Cork
University College Hospital, Galway
Waterford Regional Hospital

APPENDIX 3

Irish microbiological guidelines for retail smoked fish*

	Microbiological quality cfu/g			
Criterion	**Satisfactory**	**Acceptable**	**Unsatisfactory**	**Unacceptable/ Potentially hazardous**
ACC	$<10^6$	10^6-$<10^7$	$\geq 10^7$	N/A
S. aureus	<20	20-<100	100-$<10^4$	$\geq 10^4$
E. coli	<20	20-<100	≥100	N/A
L. monocytogenes	<20	20-<100	N/A	≥100

* In the Irish microbiological guidelines [9] smoked fish is categorised as food category D for ACC.

APPENDIX 4

Serotyping of *L. monocytogenes*

Ribotype, PFGE, AFLP and Serotype assignments of 26 *L. monocytogenes* isolates from smoked salmon

Isolate No.	Dupont. ID	Ribogroup♣	PFGE type◉	AFLP type♦	Serovar✪
1	Dup-1062	251-14-S-7	F	A	1/2a
2	Dup-1062	251-14-S-7	A	A	1/2a
3	Dup-1062	251-14-S-7	A	A	1/2a
4	Dup-1062	251-14-S-7	A	A	1/2a
5	Dup-1062	251-14-S-7	A	A	1/2a
6	Dup-1062	251-14-S-7	A	A	1/2a
7	Dup-1062	251-14-S-7	A	NT	1/2
8	Dup-1062	251-14-S-7	A	A	1/2c
9	Dup-1062	251-14-S-7	A	A	1/2a
10	Dup-1062	251-14-S-7	A	A	1/2a
11	Dup-1039	251-12-S-2	D	C	1/2a
12	Dup-1038	251-16-S-3	G	E	4b
13	Dup-1038	251-16-S-3	G	E	4b
14	Dup-1062	251-14-S-7	A	A	1/2a
15	Dup-1042	251-14-S-7	A	A	1/2a
16	Dup-1062	251-14-S-7	A	A	1/2a
17	Dup-1053	251-50-S-4	B	B	1/2a
18	Dup-1053	251-50-S-4	B	B	1/2a
19	Dup-1053	251-50-S-4	B	B	1/2a
20	Dup-1062	251-14-S-7	A	A	1/2a
21	Dup-1062	251-14-S-7	A	A	1/2a
22	Dup-1053	251-15-S-3	C	B	1/2c
23	Dup-1062	251-14-S-7	A	A	1/2a
24	Dup-1062	251-14-S-7	A	A	1/2a
25	Dup-1039	251-12-S-2	D	B	1/2a
26	Dup-1045	251-11-S-4	E	D	1/2a

♣ Ribotyping utilizes an enzyme (*Eco*R1) which cuts DNA frequently. The resulting Restriction Fragment Length Polymorphisms (RFLPS) are size separated based on their electrophoretic mobility through an agarose gel. RFLPs with complementary sequences to a labelled rRNA operon probe are visualised through chemiluminescence.

◉ Pulse Field Gel Electrophoresis (PFGE) utilises an enzyme which cuts genomic DNA infrequently e.g. *Apa*1. During electrophoresis the direction of the electric field is changed periodically. This causes continual re-orientation of DNA and therefore allows very large DNA molecules to be separated.

♦ Amplified Fragment-Length Polymorphism (AFLP) utilizes two restriction enzymes to fragment genomic DNA . Double stranded adapters are ligated to the ends of the restriction fragments. A subset of the restriction fragments are amplified using two primers complementary to the adapter and extended at their 3' ends by selective nucleotides. The amplified restriction fragments are separated by gel electrophoresis.

✪ This phenotypic method is based on the agglutination of somatic O and flagellar H antigens.