

Results of 2nd Quarter National Survey 2003 (03NS2)

**European Commission Co-ordinated programme for the Official  
Control of foodstuffs for 2003**

Bacteriological Quality/Safety of Cooked Crustaceans and Molluscan  
Shellfish

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## Executive Summary

This study investigated the bacteriological quality/safety of cooked crustaceans and molluscan shellfish for the following 4 microbiological parameters: *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* and *Vibrio parahaemolyticus*. Sampling took place from April to June 2003 inclusive and included products at production level and products available on the market. The following are the main findings:

Products at production level:

- All batch samples (each batch comprised of 5 samples) were classified as satisfactory for each microbiological parameter using the criteria proposed by the EC for this survey.
- All batch samples complied with the legal criteria for *Salmonella* spp., *S. aureus* and *E. coli* as outlined in Commission Decision 93/51/EEC.

Products available on the market:

- All samples were classified as satisfactory for *S. aureus* (n=504) and *V. parahaemolyticus* (n=492); 99.8% (505/506) of samples were classified as satisfactory for *Salmonella* spp. and the same number were classified as satisfactory for *E. coli* using the criteria proposed for this survey.
- 99.8% (505/506), 97.42% (491/504), 99.2% (502/506) and 100% (n=492) of samples were classified as satisfactory for *Salmonella* spp, *S. aureus*, *E. coli* and *V. parahaemolyticus* using the national microbiological guidelines for RTE foods sampled at the point of sale.

## 1. Introduction

The fish industry plays a significant role in the Irish economy. The value of total fish production was 332.8million euro in 2002 (sea landings 63%; aquaculture 35% and inland catches 1.6%). The value of shellfish production was estimated to be worth in excess of 67 million euro in 2002 <sup>(1)</sup>.

Crustaceans and molluscs are the principle types of shellfish. Crustaceans are invertebrate animals which possess a segmented body, jointed limbs and a chitinous exoskeleton, e.g crabs, lobsters, crayfish and shrimp. Molluscs are a group of soft-bodied animals that usually have external protective shells. Bivalves (e.g. oysters, cockles, mussels and scallops) are the most important member of the molluscan group from an economic and public health point of view. They feed by filtering large volumes of water across their gills to obtain oxygen and food (i.e they are filter feeders) and by the same process they can also concentrate microorganisms from polluted waters on the mucus of their gills <sup>(2)</sup>.

The initial microflora of both live crustaceans and live bivalve molluscs (LBM) reflect the environment from which they are harvested <sup>(3)</sup>. In relation to LBM this is of public health concern as these products maybe consumed raw (crustaceae are generally cooked prior to consumption). This is addressed in Council Directive 91/492/EEC <sup>(4)</sup> where health conditions are laid down for the production and placing on the market of LBM. Under this legislation harvesting areas are assigned a classification of A, B

or C depending on the levels of *E. coli* and faecal coliforms per 100g of shellfish sampled (Table 1).

**Table 1:** Classification of harvesting areas according to Council Directive 91/492/EEC <sup>(4)</sup>

Category	Criteria	Action
<b>A</b>	<230 <i>E. coli</i> /100g or <300 faecal coliforms/100g and absence of <i>Salmonella</i> spp. in 25g	May be collected for direct human consumption
<b>B</b>	<4.6x10 <sup>3</sup> <i>E. coli</i> /100g in 90% of samples or <6x10 <sup>3</sup> faecal coliforms/100g	Must be purified or relaid to meet the requirements of category A
<b>C</b>	<6x10 <sup>4</sup> faecal coliforms/100g	Must be relaid for a long period (at least 2 months) and possibly purified to meet the requirements of category A.
<b>Prohibited</b>	>6x10 <sup>4</sup> faecal coliforms/100g	Harvesting prohibited

As outlined in Table 1 LBM harvested from areas which do not meet category A specifications must undergo a purification step. Alternatively the public health threat to consumers can be reduced by subjecting the bivalves to heat treatments (boiling/cooking) as specified in Commission Decision 2003/774/EC <sup>(5)</sup>.

This study deals specifically with the bacteriological quality/safety of cooked crustaceans and molluscan shellfish. These products are readily available on the market as a result of consumer demand for processed ready-to-eat (RTE) foods. Their microbiological status is particularly important as these products are capable of supporting the growth of a wide variety of microorganisms. In addition, certain specific features in their production make them susceptible to microbial contamination and subsequent growth e.g cooking on board fishing vessels, chilling with sea water, intensive handling and long transports <sup>(6)</sup>.

Currently, microbiological criteria for cooked crustaceans and molluscan shellfish are laid down in Commission Decision 93/51/EEC <sup>(7)</sup>. These criteria (for batch samples) include end product criteria for *S. aureus* and *Salmonella* spp., as well as process criteria for *E. coli*, thermotolerant coliforms and mesophilic aerobic bacteria (Appendix 1). In recent years the presence of *Vibrio parahaemolyticus* in cooked products has raised public health concern, however, there is currently no criterion for this pathogen in European Community legislation. It is worth noting that the European Commission is currently in the process of revising microbiological criteria for all foodstuffs and food processes. In addition, microbiological guidelines for RTE products sampled at the point of sale exist at national level <sup>(8)</sup> (Appendix 2). These guidelines are for single samples as opposed to batch samples.

This study was carried out as part of the EU Coordinated Programme for the Official Control of Foodstuffs 2003 (outlined in Commission Recommendation 2003/10/EC <sup>(6)</sup>). The aim of this study was to collate information from all member states on the prevalence of pathogenic and indicator organisms in cooked crustaceans and molluscan shellfish and to use this information to promote a high level of consumer protection (this may involve the setting of new criteria for this commodity/pathogen combination).

## 2. Specific objectives

To examine the bacteriological quality/safety (*Salmonella* spp., *S. aureus*, *E. coli*, *V. parahaemolyticus*) of cooked crustaceans and molluscan shellfish as outlined by the EU Coordinated Programme for the Official Control of Foodstuffs 2003 <sup>(6)</sup>.

## 3. Method

### 3.1 Sample source

Samples were obtained from:

- 1) Processing premises involved in the production of cooked crustaceans and molluscan shellfish and
- 2) From products available on the market, i.e.
  - Retail premises, i.e. supermarkets, shops, fishmongers etc.
  - Catering premises, i.e. restaurants and hotels
  - Wholesale and distribution premises

### 3.2 Sample description

Samples permitted for analysis included:

- Cooked crustaceans e.g. shrimp, prawn, scampi, crab, lobster, crayfish.
- Cooked molluscan shellfish, e.g. clams, oysters, mussels, scallops, cockles.
- Chilled, frozen and vacuum packed products.
- Crumbed products.
- Mussels in a butter based sauce (this is the only sauce acceptable).
- Products in brine which are packed in a plastic container (note: products in brine in tins and jars are excluded).

The following products were specifically excluded:

- Uncooked crustaceans or molluscan shellfish.
- Value-added products (e.g. chowder).
- Products in sauces, e.g. prawn cocktail.
- Crustaceans or molluscan shellfish in tins and jars.

### Sample collection and analysis

Processing samples: Batch samples (each batch comprised of 5 samples) were obtained from processing establishments. The 5 samples were taken on the same date from the same batch of finished product. If the finished product did not meet the survey criteria (e.g. if it was a value added product such as prawn cocktail); samples of the cooked shellfish (i.e. cooked crustacean or molluscan shellfish) were taken prior to inclusion in the final product.

Retail samples: Although the EU programme recommended testing samples in batches of five, it was recognised that this was not practical for products on the market. Therefore at retail/catering/wholesale/distribution level single samples were taken. In any given premises only one sample was taken from products of the same brand name. In addition, samples closest to their use-by/best before date were taken

All sampling was undertaken by Environmental Health Officers (EHOs) from the 10 health boards (Appendix 3). Sampling took place from April to June 2003 inclusive. Sufficient sample was taken to enable the microbiological analysis of 100g of meat. Samples were analysed in one of the seven Official Food Microbiology Laboratories (OFMLs – Appendix 4) using an approved/standard method.

### 3.4 Interpretation of results, reporting and subsequent actions

#### Interpretation of results

EHOs categorised samples as 'satisfactory', 'acceptable' or 'unsatisfactory', using the acceptance criteria set out in Table 1 (products at production level – batch samples) and Table 2 (products available on the market – single samples).

**Table 1:** Microbiological criteria for products at production level – Batch samples (each batch is comprised of 5 samples)<sup>‡</sup>

Parameter	Criteria (cfu/g)		
	Satisfactory	Acceptable	Unsatisfactory
<i>Salmonella</i> spp. <sup>•</sup>	Not detected in 25g in any of the 5 samples	N/A*	Detected in 25g of any of the 5 samples
<i>Staphylococcus aureus</i> <sup>•</sup>	All samples <300	All samples $\leq 10^3$ and no more than 2 samples in the range 300 - $\leq 10^3$	Any sample $> 10^3$ or 3 or more samples in the range 300 - $\leq 10^3$
<i>Escherichia coli</i> <sup>♦</sup>	All samples <300	All samples $\leq 10^3$ and no more than 1 sample in the range 300 - $\leq 10^3$	Any sample $> 10^3$ or 2 or more samples in the range 300 - $\leq 10^3$
Total <i>Vibrio parahaemolyticus</i> count <sup>*</sup>	All samples <30	All samples $\leq 10^2$ and no more than 2 samples in the range 30 - $\leq 10^2$	Any sample $> 10^2$ or 3 or more samples in the range 30 - $\leq 10^2$

<sup>‡</sup> These criteria are outlined in Commission recommendation 2003/10/EC <sup>(6)</sup>.

\*N/A -not applicable

<sup>•</sup> The criteria proposed for *Salmonella* spp. and *S. aureus* are the same as those specified in Commission Decision 93/51/EEC <sup>(7)</sup> on the microbiological criteria applicable to the production of cooked crustaceans and molluscan shellfish (Appendix 1)

<sup>♦</sup> The criterion proposed for *E. coli* is less stringent than the criterion specified in Commission Decision 93/51/EEC <sup>(7)</sup> on the microbiological criteria applicable to the production of cooked crustaceans and molluscan shellfish (Appendix 1)

<sup>\*</sup> The criterion proposed for *V. parahaemolyticus* should be used as a guideline only. There is currently no criterion for this bacterium in Community Legislation (Commission Decision 93/51/EEC) <sup>(7)</sup>.

**Table 2:** Microbiological criteria for products available on the market – Single samples<sup>♦</sup>

Parameter	Criteria (cfu/g)		
	Satisfactory	Acceptable	Unsatisfactory
<i>Salmonella</i> spp.	Absent in 25g	N/A*	Present in 25g
<i>Staphylococcus aureus</i>	<300	300 to $\leq 10^3$	$> 10^3$
<i>Escherichia coli</i>	<300	300 to $\leq 10^3$	$> 10^3$
Total <i>Vibrio parahaemolyticus</i> count	<30	30 to $\leq 10^2$	$> 10^2$

<sup>♦</sup> The EC proposed that batch samples (each batch comprising of 5 samples) should be taken from products available on the market. This was deemed inappropriate in the Irish context therefore single samples were taken and the criteria proposed in Commission Recommendation 2003/10/EC<sup>(6)</sup> were adjusted accordingly.

\*N/A -not applicable

### Reporting

The OFMLs reported the microbiological results to the FSAI and the EHOs under the normal reporting channels.

The EHOs completed a questionnaire which provided information on sample description, type of premises, best before date, sample categorisation based on microbiological results and enforcement action. All questionnaires were returned to the Food Safety Authority of Ireland. In addition, the questionnaires associated with the processing samples were forwarded by the EHOs to the relevant Sea Fishery Officer in the Department of the Communications, Marine and Natural Resources (DCMNR) for information and if necessary enforcement.

### Subsequent action

In the context of this survey no further action was required on satisfactory/acceptable samples (single/batch). For unsatisfactory single retail/catering samples the EHO decided on the enforcement action to be taken. There were no unsatisfactory processing batch samples therefore no enforcement action was required by the DCMNR.

Repeat samples were excluded from the survey results/analysis.



## 4. Results and Discussion

### 4.1 Overall results

#### 4.1.1 Products at production level (Batch samples)

##### Microbiological Data

In this study, the microbiological status of 3 batches (each batch comprised of 5 samples) of cooked crustaceans/molluscan shellfish from processing establishments were analysed for 1 or more microbiological parameter (Appendix 3).

The microbiological status of the batches was determined using the criteria proposed by the EC for this survey (Table 1). All batches were satisfactory for *Salmonella* spp., *S. aureus*, *E. coli* and *V. parahemolyticus* (Table 3).

**Table 3:** Microbiological status of production samples (batch) based on the criteria proposed\* for this EU coordinated survey

Microbiological parameter	No. of batches analysed*	Microbiological status		
		Satisfactory	Acceptable	Unsatisfactory
<i>Salmonella</i> spp.	3	3 (100)	0 (0)	0 (0)
<i>S. aureus</i>	3	3 (100)	0 (0)	0 (0)
<i>E. coli</i>	3	3 (100)	0 (0)	0 (0)
<i>V. parahaemolyticus</i>	3	3 (100)	0 (0)	0 (0)

• Criteria as specified in Commission Recommendation 2003/10/EC <sup>(6)</sup> and as outlined in Table 1

\* Additional processing samples were submitted for analysis, however, their microbiological status could not be determined using the criteria proposed by the EC (Table 1) as they did not fulfil the criteria of 5 samples per batch.

The microbiological criteria proposed for *Salmonella* spp. and *S. aureus* in this survey are the same as those specified in legislation (93/51/EEC <sup>(7)</sup> – Appendix 1). Thus all batches analysed for these 2 parameters were in compliance with the legal criteria. The criterion proposed for *E. coli* in this survey is less stringent than the criterion proposed in legislation (93/51/EEC – Appendix 1). However, even applying the more stringent legal criterion for *E. coli* to the results of this survey showed that all batches analysed were still satisfactory.

##### Questionnaire Data

Questionnaires were returned with the 3 processing batch samples. Batches were both chilled (n=1) and frozen (n=2). Information on product description (e.g. product in brine/crumbed product/vacuum packed) was not provided on the questionnaire for any of the 3 batches.

#### 4.1.2 Products available on the market (Single samples)

##### Microbiological Data

A total of 508 single samples submitted from the 10 health boards were analysed for 1 or more microbiological parameter (Appendix 3).

The microbiological status of the samples was determined using the criteria proposed for this survey (Table 2). All samples tested were categorised as satisfactory for *S. aureus* and *V. parahaemolyticus*; while 99.8% of samples were satisfactory for *Salmonella* spp. and *E. coli* (Table 4).

**Table 4:** Microbiological status of products available on the market based on the criteria proposed\* for this EU coordinated survey

Microbiological parameter	No. of samples tested	No. of samples (%)		
		Satisfactory	Acceptable	Unsatisfactory
<i>Salmonella</i> spp.	506	505 (99.8)	N/A	1 (0.2)
<i>S. aureus</i>	504	504 (100)	0 (0)	0 (0)
<i>E. coli</i>	506	505 (99.8)	1 (0.2)	0 (0)
<i>V. parahaemolyticus</i>	492	492 (100)	0 (0)	0 (0)

\* See Table 2

N/A: Not Applicable

Unfortunately, no questionnaire was returned with the sample unsatisfactory for *Salmonella* spp., therefore it is not known what enforcement action was taken.

As stated in the introduction, microbiological guidelines for ready-to-eat food (RTE) sampled at the point of sale exist at national level (Appendix 2)<sup>(8)</sup>. In the case of *S. aureus* and *E. coli* these guidelines are more stringent than the criteria proposed for this survey (Table 2). Applying these guidelines to the results of this survey; show that 99.8%, 97.42%, 99.2% and 100% of samples were satisfactory for *Salmonella* spp., *S. aureus*, *E. coli* and *V. parahemolyticus* respectively (Table 5). No sample was unsatisfactory for more than 1 microbiological parameter. A detailed breakdown of these results by health board is provided in Appendices 5-8.

**Table 5:** Microbiological status of retail samples according to the national microbiological guidelines for ready-to-eat food (RTE) sampled at the point of sale (Appendix 2) <sup>(8)</sup>.

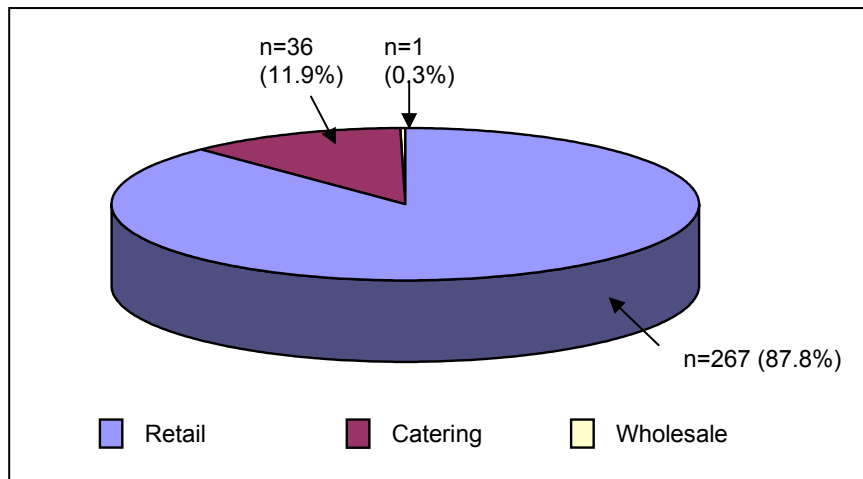
Microbiological parameter	No. of samples tested	No. of samples (%)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable/potentially hazardous
<i>Salmonella</i> spp.	506	505 (99.8)	N/A	N/A	1 (0.2)
<i>S. aureus</i>	504	491 (97.42)	5 (1)	8 (1.58)	0 (0)
<i>E. coli</i>	506	502 (99.2)	2 (0.4)	2 (0.4)	N/A
<i>V. parahaemolyticus</i>	492	492 (100)	0 (0)	0 (0)	0 (0)

N/A: Not Applicable

**Questionnaire Data**

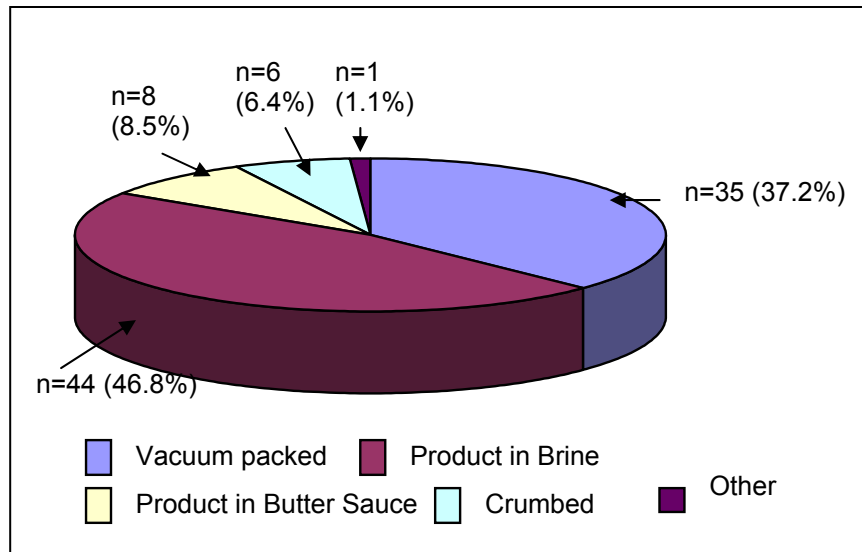
Questionnaires were returned with 304 of the 508 samples which were available on the market (i.e. the response rate was 60%, Appendix 9). The majority of samples (87.8%) were obtained from retail premises with the remaining sampled predominantly from catering establishments (11.9%) (Figure 1).

**Figure 1:** Sample distribution source (n=304)



Information on product type was only provided for 30.9% (94/304) of samples. 47% (44/94) were products in brine, while 37% (35/94) were vacuum packed (Figure 2).

**Figure 2: Product information (n=94)**



Information on storage conditions was available for 95.4% (290/304) of samples, 53% (155/290) were chilled samples while the remainder 47% (135/290) were frozen.

Although 60% of questionnaires were returned (304/508) the response rate for particular questions was very low, e.g. the question on product description was only completed on 94 out of the 304 questionnaires returned. Because of this limited information no correlation could be made between the questionnaire data and the microbiological data.

## 4.2 Results by microbiological parameter

### 4.2.1 *Salmonella* spp.

Salmonellae are bacteria which reside in the intestinal tract of infected animals and humans and which may be shed in the faeces. Their presence in raw shellfish is indicative of sewage pollution of the coastal environment (e.g. effluent run off from agricultural land). The reported prevalence of salmonellae in raw shellfish is 0.1-16% (this data is from 8 countries including Northern Ireland but not the Republic of Ireland) <sup>(9)</sup>.

Efficient cooking will quickly eliminate this pathogen, therefore its presence in cooked shellfish is indicative of poor process control and/or post process contamination. Post process contamination can occur particularly when significant hand peeling is involved <sup>(10)</sup>. The hands of workers have been identified as sources of this pathogen <sup>(2)</sup>. In addition, despite the requirements of the fishery directive with respect to the use of 'clean seawater' or drinking water for the cooling of cooked crustaceae and bivalve molluscs, it is relatively common practice in some parts of Europe for untreated seawater to be used instead. This could provide a route for the introduction of *Salmonella* spp. among other pathogens <sup>(11)</sup>.

In this study:

- *Salmonella* spp. was not detected in any batch sample obtained from a processing establishment. Therefore all batches were classified as satisfactory using the criteria proposed by the EC for this survey (Table 1) and were thus in compliance with Commission Decision 93/51/EEC <sup>(7)</sup> (Appendix 1).
- *Salmonella* spp. was detected in 0.2% (1/506) of samples available on the market (single samples). This sample was classified as unsatisfactory using the criteria proposed for this survey (Table 2) and was categorised as unacceptable/potentially hazardous using the national microbiological guidelines for RTE foods sampled at the point of sale (Appendix 2).

Very little information is available in the literature on the incidence of *Salmonella* spp. in cooked crustaceans and molluscan shellfish at either processing or retail level. However, in a study carried out in the UK <sup>(12)</sup>, *Salmonella* spp. was detected in 0.16% (4/2394) of RTE molluscs sampled from retail premises (Table 6). This finding is statistically comparable ( $p < 0.05$ ) with the finding of this Irish study.

**Table 6:** Incidence of *Salmonella* spp. in cooked crustaceans and molluscan shellfish on the market – A comparison of studies.

Origin of study	Year	Sample description	No. of samples tested	No. in which <i>Salmonella</i> spp. was detected (%)
UK <sup>(12)</sup>	1997	Cooked, RTE, out of shell molluscs	2394	4 <sup>§</sup> (0.16)
Ireland (this study)	2003	Cooked crustaceans and molluscan shellfish	506	1 <sup>∞</sup> (0.2)

<sup>§</sup> *S. Braenderup*, *S. Enteritidis* phage type 4, *S. Hadar* and *S. Ohio* were detected

<sup>∞</sup> *Salmonella* Weltevreden was detected

In the UK study it was observed that the addition of vinegar to these products did not eliminate pathogens such as *Salmonella* spp. Insufficient information on product description was obtained in this study to examine its correlation with bacteriological safety/quality.

Despite the presence of salmonellae in a proportion of marketed cooked crustaceans and molluscan shellfish, fish and shellfish have been implicated in only 1% of outbreaks of salmonellosis worldwide <sup>(13)</sup>. Based on this data the Scientific Committee for Veterinary Measures relating to Public Health (SCVMPH) in their opinion on Salmonellae in Foodstuffs <sup>(9)</sup> have implied that fish and shellfish do not pose a high risk of salmonellosis.

#### 4.2.2 *Staphylococcus aureus*

*S. aureus* is associated with the skin, nose and throat of humans and it is estimated that up to 40% of healthy individuals may be carriers of this organism. Food handlers play an important role in the transmission of this organism to food. This is particularly significant for shellfish as these products require a significant amount of handling both pre and post cooking, although the former should be eliminated by the cooking step.

Staphylococcal food poisoning is caused by ingestion of a toxin formed by *S. aureus* in the food. *S. aureus* must grow to levels of  $>10^5$  cells/g before producing sufficient quantities of the heat-stable staphylococcal toxin to cause illness. At marginal temperature abuse, staphylococci do not compete well with the normal microflora of shellfish such as crab meat <sup>(14)</sup>. However, significant growth of staphylococci can occur in temperature-abused product, this is particularly significant if the spoilage microflora has been suppressed by thermal processing <sup>(15)</sup>. This has resulted in some staphylococcal foodborne outbreaks particularly in crab meat <sup>(16)</sup>.

In this study:

- all batches were classified as satisfactory using the criteria proposed by the EC for this survey (Table 1) and were thus in compliance with Commission Decision 93/51/EEC <sup>(7)</sup> (Appendix 1).
- all products available on the market (single samples) were satisfactory for *S. aureus* using the criteria proposed for this survey (Table 2). However, applying the national microbiological guidelines for RTE foods sampled at the point of sale (Appendix 2); showed that 1.58% (8/504) of these samples were unsatisfactory for *S. aureus* (i.e.  $100 < 10^4$  cfu/g).

These findings are comparable ( $p < 0.05$ ) to the results of a UK survey (Table 7).

**Table 7:** Incidence of *S. aureus* in cooked crustaceans and molluscan shellfish on the market – A comparison of studies.

Origin of study	Year	Sample description	No. of samples tested	Count cfu/g (%)				
				ND	<20	<100	100-<10 <sup>4</sup>	≥10 <sup>4</sup>
UK <sup>(12)</sup>	1997	Cooked, RTE, out of shell molluscs	2403	2216 (92.2)*	—	157 (6.5)	29 (1.2)	1 (0.04)
Ireland (this study)	2003	Cooked crustaceans and molluscan shellfish	504	—	491 (97.4)	5 (1)	8 (1.6)	0 (0)

\* The limit of detection was not provided in this report

#### 4.2.3 *Escherichia coli*

*E. coli* is an enteric organism which is often used as an indicator organism in the determination of the microbiological status of products and/or processes.

This bacterium is used in the categorisation of shellfish harvesting areas under Council Directive 91/492/EEC <sup>(4)</sup> and as an indicator of process control for cooked crustaceans and molluscan shellfish under Commission Decision 93/51/EEC <sup>(7)</sup>. Its presence is indicative of faecal contamination and suggests that pathogens of an enteric nature may also be present. However, its applicability as an indicator of viral contamination (viral infections are now one of the most common sewage related problem for consumers of shellfish) has been questioned. This issue is being addressed at European level in the redrafting of the microbiological criteria for foodstuffs and food processes.

In this study:

- all batches were classified as satisfactory using the criteria proposed by the EC for this survey (Table 1) and they also complied with the more stringent legal criteria as laid down in Commission Decision 93/51/EEC <sup>(7)</sup> (Appendix 1).
- no sample available on the market was classified as unsatisfactory using the criteria proposed for this survey (Table 2), however, 0.4% (2/506) were classified as unsatisfactory for *E. coli* using the using the national microbiological guidelines for RTE foods sampled at the point of sale (Appendix 2).

The findings of this study are significantly different ( $p < 0.05$ ) to the findings of a UK study (Table 8). The incidence of *E. coli* at levels  $> 100$  cfu/g was lower in this Irish study.

**Table 8:** Incidence of *E. coli* in cooked crustaceans and molluscan shellfish on the market – A comparison of studies.

Origin of study	Year	Sample description	No. of samples tested	<i>E. coli</i> count cfu/g (%)	
				<100	>100
UK <sup>(12)</sup>	1997	Cooked, RTE, out of shell molluscs	2405	2304 (95.8)	101 (4.2)
Ireland (this study)	2003	Cooked crustaceans and molluscan shellfish	506	504 (99.6)	2 (0.4)

#### 4.2.4 *Vibrio parahaemolyticus*

*V. parahaemolyticus* is a halophilic<sup>‡</sup> and mesophilic<sup>§</sup> marine bacterium which is widely distributed in inshore marine waters throughout the world. It is normally isolated during the summer in temperate regions but is detected in warm water regions throughout the year <sup>(17)</sup>.

The level of *V. parahaemolyticus* in fish/shellfish is generally below  $10^3$  cfu/g; however counts may rise to  $10^6$  cfu/g in warm water <sup>(18)</sup>. The prevalence of this bacterium in shellfish/fish varies considerably between countries. In the Rapid Alert System<sup>♦</sup> this bacterium has been notified 61 times in seafood imported to Europe from countries including Bangladesh, India, Indonesia, Turkey and Vietnam <sup>(19)</sup>.

<sup>‡</sup> Halophilic organisms are those which require salt (NaCl) for growth.

<sup>§</sup> Mesophilic organisms are those organisms which grow well at ordinary temperatures (in the range of 30°C).

<sup>♦</sup> The Rapid Alert System of Food and Feed (RASFF) is a system for exchanging information between member states on food safety matters. It is managed by the European Commission



Food borne infections caused by *V. parahaemolyticus* usually present themselves as gastroenteritis and symptoms include diarrhoea, cramps, nausea, vomiting, headache, fever and chills. Onset is generally between 4 and 96 hours after ingestion and the symptoms usually resolve after 3 days <sup>(17)</sup>. Not much is known about the pathogenesis of the disease, however it is known that there is an association between the KP-positivity<sup>∅</sup> of the strain and its ability to cause gastroenteritis <sup>(20)</sup>. There is a discrepancy in the literature regarding the infective dose, however one study involving human volunteers have shown that the ingestion of  $2 \times 10^5$  to  $3 \times 10^7$  cfu/g of KP positive cells can lead to illness <sup>(19)</sup>. Disease caused by this bacterium is common in Asia and the US, however, it is rarely reported in Europe <sup>(19)</sup>.

The SCVMPH in its 'Opinion on *V. vulnificus* and *V. parahaemolyticus* in Raw and Uncooked Seafood' <sup>(19)</sup> state that the risk of infection with pathogenic *V. parahaemolyticus* is most strongly associated with the consumption of raw, undercooked or recontaminated seafood.

In this study:

- *V. parahaemolyticus* was not detected in any batch sample obtained from a processing establishment. Thus all batches were classified as satisfactory using the criteria (guidelines) proposed by the EC for this survey (Table 1).
- *V. parahaemolyticus* was not detected qualitatively (n=444) in any sample available on the market (single sample). In addition, a count of <20cfu/g (limit of detection) was recorded for all samples analysed quantitatively (n=492). Thus all samples were classified as satisfactory using the criteria (guidelines) proposed for this survey (Table 2) and using the national microbiological guidelines for RTE foods sampled at the point of sale (Appendix 2).

In a UK study<sup>(12)</sup> *V. parahaemolyticus* was detected in 2.7% (64/2375) of cooked RTE out-of-shell molluscs on retail sale and 6 of these contained *V. parahaemolyticus* at concentrations of 200 cfu/g or higher (Table 9).

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<sup>∅</sup> A thermostable direct hemolysin (TDH) is one of 4 haemolytic components in *V. parahaemolyticus*. TDH causes beta haemolysis of human erythrocytes. This phenomenon is known as the Kanagawa phenomenon (KP) <sup>(19)</sup>.

**Table 9:** Incidence of *V. parahaemolyticus* in cooked crustaceans and molluscan shellfish on the market – A comparison of qualitative studies.

Origin of study	Year	Sample description	No. of samples tested qualitatively	No. in which <i>V. parahaemolyticus</i> spp. was detected (%)
UK <sup>(12)</sup>	1997	Cooked, RTE, out of shell molluscs	2375	64 (2.7) <sup>♦</sup>
Ireland (this study)	2003	Cooked crustaceans and molluscan shellfish	444	0 (0)

<sup>♦</sup> Six samples contained *V. parahaemolyticus* at levels  $10^2$ cfu/g or higher.

## 5. Conclusions

The findings of this study are encouraging in terms of the bacteriological quality/safety of cooked crustaceans and molluscan shellfish available at both production level and on the market in Ireland. Using the criteria proposed for this survey only 1 sample was classified as unsatisfactory. This sample was unsatisfactory for *Salmonella* spp. and was available on the market. In addition,

- all processing batches complied with the legal criteria for *E. coli* (Commission Decision 93/51/EEC).
- 99.8%, 97.42%, 99.2% and 100% of samples available on the market were classified as satisfactory for *Salmonella* spp., *S. aureus*, *E. coli* and *V. parahaemolyticus* respectively using the national microbiological guidelines for RTE foods.

These microbiological results are comparable if not better than other surveys carried out on this type of product. The bacteriological quality/safety of cooked crustaceans and molluscan shellfish is related to a number of parameters including the level of process control, hygiene and handling practices. Processors, wholesalers/distributors, retailers caterers etc have an important role to play in this regard and should ensure that good hygiene practices (GHP) and good manufacturing practices (GMP) are undertaken and that a food safety management system based on the principles of HACCP is implemented.

Currently, there are no criteria for *V. parahaemolyticus* in EC legislation; however, the results of this EU Coordinated Programme may be used to develop a criterion for this product/pathogen commodity in the new microbiological regulation for foodstuffs and food processes which is currently being drafted at European level. The results may also be used to review the existing criteria.

There were a number of limitations to this survey.

- An accurate representation of the bacteriological quality/safety of products from processing environments was not obtained due to the small sample size (n=3 batches).
- Although 60% of questionnaires were returned there was a very poor response to some questions. This impacted on the analysis which could be carried out.

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**Appendix 1**  
**Microbiological criteria as specified in legislation**

Directive	Products	Micro-organism	Level (perg/ml unless otherwise specified)	Status/Action
Fish products 91/493/EEC and Commission Decision 93/51/EEC	Cooked crustaceans and molluscan shellfish	<i>Salmonella</i> spp.	Absent in 25g, n = 5, c = 0	Compulsory criteria; withhold from the market
		Other pathogens and toxins thereof	Not present in quantities such as to affect health	Compulsory criteria
	Whole products	Mesophilic aerobic bacteria (30°C)	m = 10 000, M = 100 000, n = 5, c = 2	Indicator organisms: to help manufacturers decide whether plants are operating satisfactory (Guidelines)
	Shelled or shucked products	<i>Staphylococcus aureus</i>	m = 100, M = 1000, n = 5, c = 2	Analytical criteria: organisms indicating poor hygiene
		<i>Escherichia coli</i> (on solid medium)	m = 10, M = 100, n = 5, c = 1	
		Thermotolerant coliforms (44°C on solid media)	m=10, M=100, n=5, c=2	
	Shelled or shucked products except crabmeat	Mesophilic aerobic bacteria (30°C)	m = 50 000, M = 500 000, n = 5, c = 2	Indicator organism (Guidelines)
	Crabmeat	Mesophilic aerobic bacteria (30°C)	m = 100 000, M = 1,000 000, n = 5, c = 2	

M= acceptability limit beyond which the results are considered unsatisfactory

m= limit below which all results are considered satisfactory

n= number of units comprising the sample

c= number of sampling units giving bacterial counts between m and M

**Satisfactory:** if all values observed are 3m or less;

**Acceptable :** where the maximum of c values are between 3m and 10m (M) and all values are < M

**Unsatisfactory:** if any value is above M or where more than c values are between 3m and 10m (M)

**Appendix 2**  
**National guidelines for the microbiological quality of some ready-to-eat foods**  
**sampled at the point of sale**

Criterion	Microbiological Quality (cfu/g)			
	Satisfactory	Acceptable	Unsatisfactory	Unacceptable/potentially hazardous
<b><i>E. coli</i></b>	<20	20-<100	≥100	N/A
<b><i>Salmonella</i> spp.</b>	Not detected in 25g	N/A	N/A	Detected in 25g
<b><i>S. aureus</i></b>	<20	20-<100	100-<10 <sup>4</sup>	≥10 <sup>4</sup>
<b><i>V. parahaemolyticus</i></b>	<20	20-<100	100-<10 <sup>3</sup>	≥10 <sup>3</sup>

### Appendix 3 List of Health Boards

Health board	Abbreviation	Number of samples available on the market analysed for 1 or more microbiological parameter	Number of processing batches analysed for 1 or more microbiological parameter
East-Coast Area Health Board	ECAHB	27	0
Midland Health Board	MHB	25	0
Mid-Western Health Board	MWHB	37	0
Northern Area Health Board	NAHB	58	1
North-Eastern Health Board	NEHB	23	0
North-Western Health Board	NWHB	63 <sup>∞</sup>	0
South-Eastern Health Board	SEHB	55 <sup>∞</sup>	2
Southern Health Board	SHB	98	0
South-Western Area Health Board	SWAHB	74	0
<i>Western Health Board</i>	WHB	48 <sup>∞</sup>	0
		<b>508</b>	<b>3</b>

<sup>∞</sup> An additional 8 samples were submitted from these health boards but were not analysed due to insufficient sample (WHB =1; NWHB=6; SEHB=1)



**Appendix 4**  
**List of the Official Food Microbiology Laboratories (OFMLs)**

<b>Laboratory</b>
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 5**  
**Retail samples - *Salmonella* spp. (n=506)**

Health Board	Number of Samples tested	<i>Salmonella</i> spp. not detected		<i>Salmonella</i> spp. detected	
		No.	%	No.	%
ECAHB	27	27	100	0	0
MHB	25	25	100	0	0
MWHB	35 <sup>∞</sup>	35	100	0	0
NAHB	58	58	100	0	0
NEHB	23	23	100	0	0
NWHB	63	63	100	0	0
SEHB	55	55	100	0	0
SHB	98	97	99	1 <sup>†</sup>	1
SWAHB	74	74	100	0	0
WHB	48	48	100	0	0
<b>Total</b>	<b>506</b>	505	99.8	1	0.2

<sup>∞</sup> A total of 37 samples were submitted by the MWHB, however 2 were not tested for *Salmonella* spp. due to insufficient sample

<sup>†</sup> *Salmonella weltevreden* was identified in this sample of black tiger prawns. This sample was satisfactory for *S. aureus*, *E. coli* and *V. parahaemolyticus*.

**Appendix 6**  
**Retail samples – *S. aureus* results (n=504)**

Health Board	No. of samples	<i>S. aureus</i> count (cfu/g)			
		< 20 (%)	20 - < 100 (%)	100 - < 10 <sup>4</sup> (%)	≥ 10 <sup>4</sup> (%)
ECAHB	24	21	1	2	0
MHB	25	24	1	0	0
MWHB	37	37	0	0	0
NAHB	57	54	1	2	0
NEHB	23	23	0	0	0
NWHB	63	62	0	1	0
SEHB	55	54	0	1	0
SHB	98	96	1	1	0
SWAHB	74	73	1	0	0
WHB	48	47	0	1	0
<b>Total</b>	<b>504</b>	<b>491</b>	<b>5</b>	<b>8*</b>	<b>0</b>

\* Counts of 100 cfu/g (n=1); 110 cfu/g (n=2); 120 cfu/g (n=1); 140 cfu/g (n=1); 150 cfu/g (n=1); 160 cfu/g (n=1) and 180 cfu/g (n=1) were recorded for these 8 samples.

**Appendix 7**  
**Retail samples – *E. coli* results (n=506)**

Health Board	No. of samples	<i>E. coli</i> (cfu/g)		
		< 20	20 - < 100	≥100
ECAHB	27	26	0	1*
MHB	23	23	0	0
MWHB	37	37	0	0
NAHB	58	58	0	0
NEHB	23	23	0	0
NWHB	63	63	0	0
SEHB	55	53	2	0
SHB	98	98	0	0
SWAHB	74	74	0	0
WHB	48	47	0	1 <sup>§</sup>
<b>Total</b>	<b>506</b>	<b>502 (99.2)</b>	<b>2 (0.4)</b>	<b>2 (0.4)</b>

\* A count of 100cfu/g was recorded for this sample

§ A count of 900cfu/g was recorded for this sample

**Appendix 8**  
**Retail samples – *V. parahaemolyticus* results (Qualitative and Quantitative)**

Health Board	Qualitative tests			Quantitative tests				
	No. tested	D	ND	No. quantified	<20	20-<100	100-<10 <sup>3</sup>	≥10 <sup>3</sup>
ECAHB	27	0	27	27	27	0	0	0
MHB	25	0	25	25	25	0	0	0
MWHB	37	0	37	37	37	0	0	0
NAHB	50	0	50	50	50	0	0	0
NEHB	21	0	21	21	21	0	0	0
NWHB	63	0	63	63	63	0	0	0
SEHB	55	0	55	55	55	0	0	0
SHB	98	0	98	98	98	0	0	0
SWAHB	68	0	68	68	68	0	0	0
WHB	0	0	0	48	48	0	0	0
	<b>444</b>	<b>0</b>	<b>444 (100)</b>	<b>492</b>	<b>492 (100)</b>	<b>0</b>	<b>0</b>	<b>0</b>

**Appendix 9**  
**Number of questionnaire returned by health board**

<b>Health board</b>	<b>Number of samples available on the market analysed for 1 or more microbiological parameter</b>	<b>No. of questionnaires returned</b>	<b>% Returned</b>
East-Coast Area Health Board	27	18	66.6
Midland Health Board	25	15	60
Mid-Western Health Board	37	35	94.6
Northern Area Health Board	58	41	70.7
North-Eastern Health Board	23	23	100
North-Western Health Board	63	17	27
South-Eastern Health Board	55	38	69
Southern Health Board	98	23	23.5
South-Western Area Health Board	74	51	69
<i>Western Health Board</i>	48	43	89.6
<b>Total</b>	<b>508</b>	<b>304</b>	<b>60</b>