2nd Trimester National Microbiological Survey 2005 (05NS2):

EU Coordinated programme 2005

Bacteriological Safety of Cheeses made from Pasteurised Milk

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Executive Summary

This study was carried out as part of the EU Coordinate Programme for the Official Control of Foodstuffs 2005. It investigated the bacteriological safety of cheeses (fresh, ripened and semi-hard cheeses) made from pasteurised milk for the following 4 microbiological parameters: *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*. Sampling took place from May to August 2005. Cheese samples were obtained from production premises (by Dairy Produce Inspectors) and from retail premises (by Environmental Health Officers). The following are the main findings:

Products at production level:
Batch samples (each batch sample consisted of 5 individual samples) were obtained from processing premises.

- Applying the criteria proposed by the European Commission (EC) for this survey; all batch samples (n=54) were classified as satisfactory for *Salmonella* spp., *S. aureus and L. monocytogenes*. In relation to *E. coli*, 96.3% (52/54) of batch samples were classified as satisfactory while 3.7% (2/54) were classified as unsatisfactory. The two unsatisfactory batch samples were from different production batches of the same product. Based on these results, improvements in production and process hygiene control were undertaken in the processing plant.

Products at retail level:
Single samples were obtained at retail level.

- Applying the criteria proposed for this survey; all samples were classified as satisfactory for *Salmonella* spp. (n=890). Three samples (3/884, 0.34%) were classified as unsatisfactory for *S. aureus*, one sample (1/880, 0.11%) was classified as unsatisfactory for *L. monocytogenes* and three samples (3/876, 0.34%) were classified as unsatisfactory for *E. coli*. No sample was classified as unsatisfactory for more than one microbiological parameter. The classification of one sample as unsatisfactory for *L. monocytogenes* resulted in the recall of the incriminated production batch from the market.
- Of particular concern was the finding that temperatures >5^{o}C were recorded for 26% (129/496) of samples stored under refrigerated conditions (refrigerated food should be maintained at ≤5^{o}C). This finding raises concern as maintenance of the chill chain is a critical control point in ensuring the microbiological safety of these products. It is also a legal requirement.

1. Introduction

Cheese production evolved centuries ago as a means of preserving raw milk. Over the years this process has been refined and cheese has now developed into a food of *haute cuisine* with epicurean qualities [(1)]. It is estimated that over 1400 varieties of cheese are produced worldwide [(2)]. Many attempts have been made to classify these varieties and today one of the most common classification schemes is based on moisture content (Table 1).

Table 1: A scheme for the classification of cheese [(3)]

Category	Moisture content	Examples
Hard	26-50%	• *Internally ripened, no added ripening microorganisms* e.g. Parmesan, Cheddar, Double Gloucester • *Internally ripened, added ripening bacteria* e.g. Emmental • *Internally ripened, secondary surface ripened by mould* e.g. Blue Cheshire
Semi-hard	42-52%	• *Internally ripened, no added ripening microorganisms* e.g. Lancashire, Edam • *Internally ripened, ripening mould added* e.g. Stilton, Roquefort
Semi-soft	45-55%	• *Surface ripened, ripening bacteria added* e.g. Limburger, Port du Salut
Soft	48-80%	• *Surface ripened, ripening mould added* e.g. Brie, Camembert • *Unripened* e.g. Cottage, Coulommier
Others		e.g Brined varieties, Whey cheese

The basic process for the production of cheese involves two distinct phases: 1) manufacturing and 2) ripening. The manufacturing phase is based on the lactic acid fermentation of milk. Traditionally this was achieved through the action of the indigenous microflora, nowadays it is most often achieved through the addition of specific starter cultures (e.g. *Lactococcus lactis*). When sufficient acid is produced the casein within the milk is coagulated (aided by rennet). The curd is then cut leaving a mixture of curds (solid constituents) and whey (the liquid). The liquid whey is then drained off and the curds are subjected to processes, such as shaping and salting, resulting in the production of cheese. The ripening phase determines the characteristic flavour and texture of the cheese. The period of ripening can vary from about 2 weeks (e.g. Mozzarella) to 2 years (e.g. parmigiano-reggiano or extra-mature cheddar); however, it is worth noting that some cheeses are consumed fresh. During ripening, a complex set of biochemical changes occur through the catalytic action of the coagulant, indigenous milk enzymes, starter bacteria and secondary microflora [(4,5)].

Pasteurisation of milk is one of the main critical control points (CCPs) in the cheese production process, i.e. it ensures the destruction of vegetative pathogens which may be

present in the raw milk (e.g. *Salmonella s*pp., Verocytotoxigenic *Escherichia coli* (VTEC) and *Listeria monocytogenes*). Other control steps include low pH (high acidity) and competition from starter cultures.

In general cheeses have a good record in terms of microbiological safety; however, there have been incidences where they have been implicated as vehicles in the transmission of foodborne outbreaks [6, 7]. The majority of outbreaks reported are associated with the consumption of cheese made from unpasteurised (i.e. raw/thermised) or improperly-pasteurised milk. In addition, it has been recognised that post process contamination can also occur [7]. It should be noted that the ability of pathogens to survive and grow in cheese is dictated by both intrinsic and extrinsic parameters. Intrinsic parameters of cheese include water content, pH, acidity, nutrient content, presence of antimicrobial compounds and the presence of competitive microflora. These parameters vary between cheese varieties. For example, soft cheese is a more suitable environment for the survival and growth of pathogens than hard cheese. In the latter a combination of factors including low pH, high salt content and low water activity (A_w) render the cheese microbiologically safer. Extrinsic parameters include factors such as processing steps, type of packaging and storage conditions.

At the time of this survey, microbiological standards♦ for cheese made from pasteurised milk were laid down in Council Directive 92/46/EEC [8] (this Directive was implemented in Ireland by Statutory Instrument No. 9/1996 [9]). These standards were applicable to pasteurised cheeses at the end of processing (the standards for fresh and soft cheeses made from pasteurised milk are summarised in Appendix 1). Since the 1st January 2006, Council Directive 92/46/EEC and other commodity specific Directives (17 in total) have been repealed and replaced with new legislation known as 'the hygiene package'. This package contains 5 main pieces of legislation, 3 implementing measures and 1 transitional measure. Commission Recommendation 2073/2005 on Microbiological Criteria for Foodstuffs [10] is one of the three implementing measures. It lays down microbiological standards for a variety of foodstuffs including cheese.

In addition, microbiological guidelines▲ exist in Ireland for ready-to-eat (RTE) foods sampled at the point of sale [11]. In relation to cheese, these guidelines (see Appendix 2) do not differentiate between cheese categories (e.g. soft, fresh, hard etc) or the type of milk (raw, thermised or pasteurised) used in its manufacture. Such differentitation is necessary as the microbiological risk varies between cheese categories. These guidelines are due to be reviewed by the Food Safety Authority of Ireland (FSAI) and these issues will be taken into consideration.

This study was carried out as part of the EU Coordinated Programme for the Official Control of Foodstuffs 2005 (outlined in Commission Recommendation 2005/175/EC [12]). The aim of this study was to collate information from all member states on the prevalence

♦ A microbiological standard is a microbiological criterion contained in law where compliance is mandatory.

▲ A microbiological guideline provides a benchmark against which unacceptable microbial contamination of food can be identified. It is not legally enforceable.

of pathogenic and indicator organisms in cheeses made from pasteurised milk. It follows the 2004 EU coordinated programme which collated information on the prevalence of pathogenic and indicator organisms in cheeses made from raw/thermised milk.

2. Specific Objectives

To investigate the bacteriological safety (*Salmonella* spp., *Staphylococcus aureus*, *L. moncytogenes* and *E. coli*) of cheeses made from pasteurised milk.

3. Methods

3.1 Sample Source

Samples were obtained from both processing establishments and retail premises.

3.2 Sampling Period

Sampling took place from May to August 2005 inclusive.

3.3 Sample Description

Three categories of cheese manufactured from pasteurised milk (both loose and pre-packed) were sampled:

1) Fresh cheese (Unripened soft cheese)
2) Ripened soft cheese
3) Semi-hard cheese

The following were specifically excluded from the survey:

- All hard cheeses, e.g. Cheddar
- All chesses manufactured from raw/unpasteurised milk
- All chesses manufactured from thermised milk
- All processed cheese, e.g. cheese spreads
- Cheese based products, e.g. cheese salads

3.4 Sample Collection and Analysis

Sample Collection

Batch samples from processing establishments: Sampling in processing establishments was undertaken by Dairy Produce Inspectors (DPIs) from the Department of Agriculture and Food (DAF). Sampling was carried out in accordance with the DAF Standard Operating Procedure (SOP OPS/001 – Rev. 8; 27/02/03). Each batch sample comprised of 5 individual samples. The minimum weight of each sample was 150g; thus the batch sample consisted of 5 x 150g samples. The 5 samples were obtained on the same date from the same production batch of finished product.

Retail samples: Sampling in retail premises was undertaken by Environmental Health Officers (EHOs) from the Health Service Executive (HSE) (10 HSE Areas). Single samples (150g) were obtained from retail premises (the EU suggested batch sampling but this was deemed inappropriate at retail level). EHOs were requested to obtain only one sample of each product from each premises.

Sample Analysis
Analysis was undertaken in the HSE Official Food Microbiology Laboratories (OFMLs)**.** In relation to the batch samples, each of the 5 individual samples was tested individually.

3.5 Reporting of results, Interpretation of results and Follow-up/enforcement action

Reporting of laboratory results:
The OFMLs reported the microbiological results to i) the FSAI and ii) the relevant sampling officer, i.e. the DPI or the EHO.

Interpretation of results
Upon receipt of the laboratory results, DPIs determined the microbiological safety of the batch samples from processing establishments using the criteria outlined in Table 2 and EHOs determined the microbiological safety of the single samples from retail premises using the criteria outlined in Table 3.

Table 2: Microbiological criteria* for batch samples[⊗]

Parameter	Microbiological status		
	Satisfactory	**Acceptable**	**Unsatisfactory**
***Salmonella* spp.**	Not detected in 25g in any of the 5 samples	N/A	Detected in 25g in any of the 5 samples
S. aureus	All 5 samples $<10^2$ cfu/g	No sample $>10^3$ cfu/g and no more than 2 samples in the range $10^2 – 10^3$ cfu/g	Any sample $>10^3$ cfu/g or more than 2 samples in the range $10^2 – 10^3$ cfu/g
***L. monocytogenes*♦**	Not detected in 25g of any sample	Detected in 25g and enumerated at $\leq 10^2$ cfu/g in any sample	Detected in 25g and enumerated at $>10^2$ cfu/g in any sample
E. coli	All 5 samples $<10^2$ cfu/g	No sample $>10^3$ cfu/g and no more than 2 samples in the range $10^2 – 10^3$ cfu/g	Any sample $>10^3$ cfu/g or more than 2 samples in the range $10^2 – 10^3$ cfu/g

* These criteria were proposed by the European Commission (EC) for the purpose of this survey (Commission Recommendation 2005/175/EC [12]).
[⊗]A batch sample consisted of 5 individual samples (each sample was a minimum of 150 g)
♦ Both qualitative (i.e. presence/absence) and quantitative tests were undertaken.
N/A: Not Applicable

Table 3: Microbiological criteria for products available on the market – single samples♦

Microorganism	Pathogen or Indicator	Bacteriological safety		
		Satisfactory	**Acceptable**	**Unsatisfactory**
***Salmonella* spp.**	Pathogen	Not detected in 25g	N/A	Detected in 25g
S. aureus	Pathogen	$<10^2$ cfu/g	$10^2 – 10^3$ cfu/g	$>10^3$ cfu/g
L. monocytogenes*	Pathogen	Not detected in 25g	Detected in 25g and enumerated at $\leq 10^2$ cfu/g	Detected in 25g and enumerated at $>10^2$ cfu/g
E. coli	Indicator of safety	$<10^2$ cfu/g	$10^2 – 10^3$ cfu/g	$>10^3$ cfu/g

♦The European Commission (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 outlined in Commission Recommendation 2005/175/EC [(12)] were amended accordingly.
* Both qualitative (i.e. presence/absence) and quantitative tests were undertaken.
N/A: Not Applicable

Follow-up/enforcement action:
When a sample (i.e. a batch or a single sample) was classified as unsatisfactory, follow-up (and where necessary enforcement action) was undertaken. The type of action was taken at the discretion of the sampling officer with advice as necessary from the FSAI or the OFML.

3.6 Questionnaire data
Questionnaires were distributed to all sampling officers prior to the commencement of this survey (Appendices 3 and 4). These questionnaires were completed and returned to the FSAI within one month of the survey completion date. Questionnaires were returned for 100% (54/54) of batch samples and for 66.5% (592/890) of retail samples.

4. Results and Discussion

4.1 Overall Results

4.1.1 Production samples (Batch samples)

Microbiological Data:
In this study, 54 batch samples (each batch comprised of 5 samples) were submitted to the OFMLs for analysis (Appendix 5 outlines the number of samples analysed in each laboratory). The microbiological status of the batch samples were determined using the criteria outlined in Table 2. Applying these criteria it was established that:

- All batches (n=54) were satisfactory for *Salmonella* spp., *S. aureus* and *L. monocytogenes.*
- 96.3% (52/54) batches were classified as satisfactory for *E. coli* (Table 4).

Table 4: Microbiological status of pasteurised cheese samples from processing premises

		No. of samples (%) with the following microbiological status⊗		
Microorganism	**No. of batch samples**	**Satisfactory**	**Acceptable**	**Unsatisfactory**
***Salmonella* spp.**	54	54 (100)	N/A	0
S. aureus	54	54 (100)	0	0
L. monocytogenes	54	54 (100)	0	0
E. coli	54	52 (96.3)	0	2 (3.7)*

⊗ Microbiological status was determined using the criteria outlined in Table 2
* Both batch samples were submitted to Waterford OFML for analysis

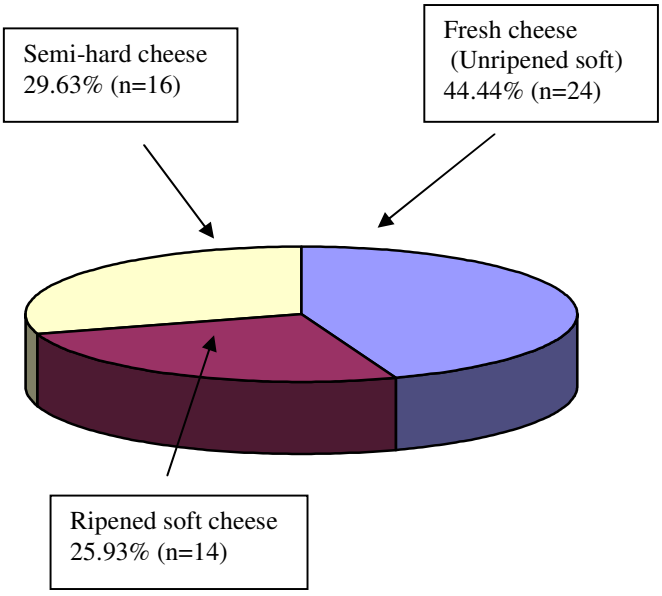
Applying the standards laid down in Council Directive 92/46/EEC [8] (Appendix 1) to the results of this survey shows that:

- 85.71% (12/14) of ripened soft cheese complied with the standard for *E. coli*
- Six batches of fresh cheese (unripened soft) complied with the standard for *S. aureus* (24 batches of fresh cheese were submitted but 18 batches could not be assessed against this standard because of the reporting mechanism used)
- All soft cheeses complied with the standard for *S. aureus*
- All fresh and soft cheese complied with the standard for *L. monocytogenes*
- All cheese and soft cheese complied with the standard for *Salmonella* spp.

Questionnaire data:
Questionnaires were returned for all batch samples (i.e. 100% response rate). Information on i) the type of cheese and ii) the follow-up action taken on account of unsatisfactory results was captured by means of questionnaire (Appendix 3). The majority (44.44%) of

batch samples were fresh cheese (unripened soft), while 25.93% were ripened soft and 29.63% were semi hard cheese (Figure 1).

Figure 1: Type of cheese sampled from processing premises (n=54 batch samples)



Details of the two batch samples which were classified as unsatisfactory for *E. coli* are outlined in Table 5. Both samples were from different production batches of the same product. Based on these results, improvements in production and process hygiene control were undertaken in the processing plant.

Table 5: Details of the batch samples (n=2) classified as unsatisfactory for *E. coli*

Batch sample	**Sample number**	***E. coli* count cfu/g**	**Type of cheese**	**Period to use by date**	**Follow-up/Enforcement action**
A	1	10	Ripened soft cheese	Information not provided	Improved in house control
	2	30			
	3	50			
	4	140			
	5	1700			
B	1	200	Ripened soft cheese	47 days	Improved in house control
	2	240			
	3	240			
	4	250			
	5	340			

4.1.2 Retail samples (single samples)

Microbiological Data:
A total of 890 samples submitted from 10 HSE areas were analysed for 1 or more microbiological parameter (Appendix 5 outlines the number of samples analysed in each laboratory).

The microbiological status of the samples was determined using the criteria outlined in Table 3. All samples were classified as satisfactory for *Salmonella* spp. (n=890); while, 99.21% (877/884), 98.86% (870/880) and 99.09% (868/876) of samples were classified as satisfactory for *S. aureus*, *L. monocytogenes* and *E. coli* respectively (Table 6). Appendix 6 outlines the microbiological status of samples submitted from each HSE area.

Table 6: Microbiological status[⊗] of pasteurised cheese samples from retail premises

		No. (%) of samples with the following microbiological status[⊗]		
Microbiological parameter	**Total no. of single samples tested**	**Satisfactory (%)**	**Acceptable[ϒ] (%)**	**Unsatisfactory[▲] (%)**
***Salmonella* spp.**	890	890 (100)	N/A	0 (0)
S. aureus	884	877 (99.21)	4 (0.45)	3 (0.34)
L. monocytogenes	880	870 (98.86)	9 (1.02)	1 (0.11)
E. coli	876	868 (99.09)	5 (0.57)	3 (0.34)

[⊗] Microbiological status was determined using the criteria outlined in Table 3.
[ϒ] No sample was acceptable for more than 1 microbiological parameter.
[▲] No sample was unsatisfactory for more than 1 microbiological parameter.

Details of the 7 unsatisfactory samples are outlined in Table 7. Of particular concern was the finding that:

- time periods in excess of 20 days remained on the shelf life of the samples unsatisfactory for *S. aureus* and *E. coli* and 6 days remained on the shelf life of the sample unsatisfactory for *L. monocytogenes*. This raises concern because if the products were subjected to temperature abuse, further microbial growth (and toxin production in the case of *S. aureus*) could occur before the end of the shelf life.
- *L. monocytogenes* was detected at an extremely high level in the unsatisfactory sample, i.e. at a level of 1.4×10^6 cfu/g (levels of *L. monocytogenes* >100 cfu/g are known to cause illness).

Table 7: Details of unsatisfactory samples from retail premises (n=7)

Micro-Organism	Bacterial count	Type of cheese	Origin of sample	Type of packaging	Period remaining on shelf life of product (days)	Storage conditions	Temp. of sample (^{o}C)	Follow-up action
S. aureus	$1.4x10^3$	Semi-hard	Irish	Pre-packed	53	Refrigerated	6	• Verbal & written warning • Improved in house control • Testing for the staphylococcal enterotoxin was undertaken and toxins g and I were found to be present ♦
	$1.1x10^3$	Semi-hard	Not Stated	Pre-packed	39	Refrigerated	4	• Written warning • Testing for the staphylococcal enterotoxin was undertaken and no toxin was detected.
	$2.2x10^3$	Ripened soft	Import	Loose	46	Refrigerated	5	• Toxin testing was negative
L. monocytogenes	$1.4x10^6$	Semi-hard	Irish	Pre-packed	6	Refrigerated	3.1	• Verbal warning which led to a product recall
E. coli	$2.0x10^4$	Ripened soft	Import	Pre-packed	36	Refrigerated	3.6	• Written warning
	$3.5x10^3$	Ripened soft	Irish	Pre-packed	21	Refrigerated	10	• Improved in-house control
	$2.8x10^5$	Not Stated	Import	Pre-packed	23	Refrigerated	4.9	• Written warning • Resample

♦ Enterotoxins G and I are not associated with human illness (staphylococcal poisoning is mainly caused by human strains of *S. aureus* producing enterotoxin A and/or D) [13].

Questionnaires were returned for 592 of the 890 samples (i.e. a response rate of 66.5%). The microbiological status of these 592 samples is outlined in Table 8. All samples were classified as satisfactory for Salmonella spp. (n=592); while, 98.81% (581/588), 99.15% (585/590) and 98.96% (573/579) of samples were classified as satisfactory for *S. aureus*, *L. monocytogenes* and *E. coli* respectively.

Table 8: Microbiological status of pasteurised cheese samples from retail premises for which questionnaires were returned (n=592)

		Microbiological status[⊗]		
Microbiological parameter	**Total no. of single samples tested**	**Satisfactory (%)**	**Acceptable (%)**	**Unsatisfactory (%)**
***Salmonella* spp.**	592	592 (100)	N/A	0 (0)
S. aureus	588	581 (98.81)	4 (0.68)	3 (0.51)
L. monocytogenes	590	585 (99.15)	4 (0.68)	1 (0.17)
E. coli	579	573 (98.96)	3 (0.52)	3 (0.52)

There is no significant difference (95% confidence interval) between the microbiological status of these samples and the microbiological status of all (n=890) samples (Table 6), indicating that samples returned with questionnaires are representative of the total sample population.

Information on 1) sample source, 2) type of sample, 3) type of packaging, 4) country of origin and 5) storage conditions were captured on the questionnaire and these data are presented in Figures 2 to 6. 86.32% of samples were sourced in supermarkets, 44.59% were ripened soft cheese, 85.81% were pre-packed, 53.72% were imported and 93.92% were stored under refrigerated conditions.

The overall microbiological status (i.e. satisfactory, acceptable or unsatisfactory) of each sample was determined and its relationship with 1) sample source, 2) type of sample, 3) type of packaging, 4) country of origin and 5) storage conditions was determined (Table 9). No parameter had a significant effect (95% confidence interval) on the overall microbiological status.

Fig 2: Sample source

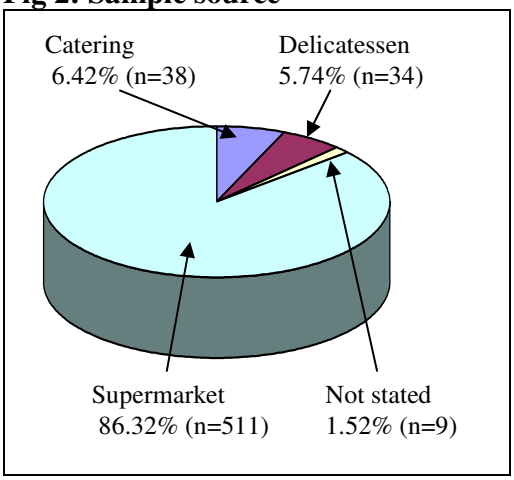


Fig 3: Type of sample

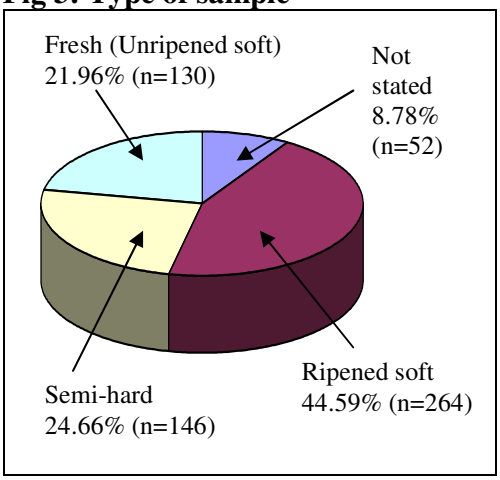


Fig 4: Type of packaging

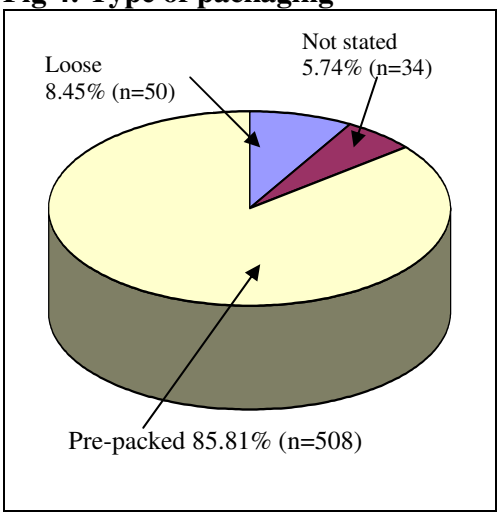


Fig 5: Country of origin

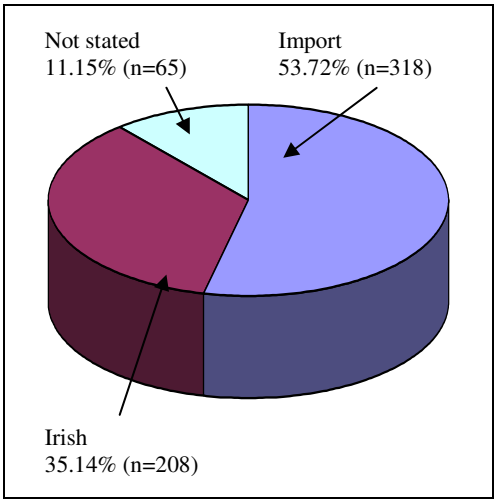


Fig 6: Storage conditions

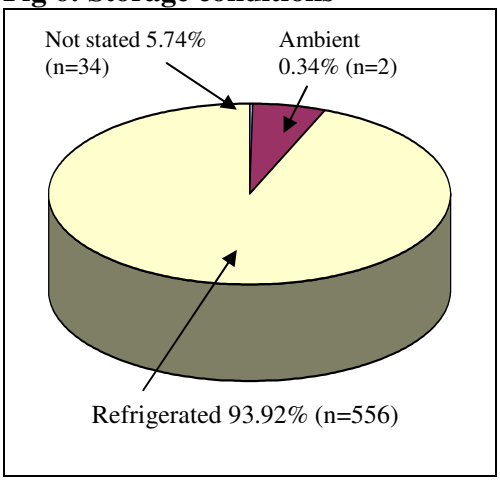


Table 9: Effect of various parameters on microbiological status of samples from retail premises (n=592)

Parameter	Parameter details	Overall microbiological status[ϒ]			Total
		S	A	U	
Sample source	Supermarket	479	9	6	494[ϒ]
	Catering	36	1	1	38
	Delicatessen	31	1	0	32[♦]
	Not stated	9	0	0	9
Type of sample	Ripened soft cheese	244	6	3	253[⊗]
	Semi-hard cheese	139	3	3	145[▲]
	Fresh cheese (Unripened soft)	123	2	0	125[Ø]
	Not Stated	49	0	1	50[*]
Type of packaging	Pre-packed	476	10	6	492[⊕]
	Loose	47	1	1	49[♣]
	Not stated	32	0	0	32[♦]
Origin of sample	Irish	195	2	3	200[℘]
	Imported	295	9	3	307[°]
	Not stated	64	0	1	65
Storage conditions	Refrigerated	523	11	7	541[♥]
	Ambient	2	0	0	2
	Not stated	30	0	0	30[♠]

[ϒ] Microbiological status determined using the criteria outlined in Table 3.

S=Satisfactory: Sample satisfactory for all 4 microbiological parameters

A=Acceptable: Sample acceptable for 1 or more microbiological parameter and satisfactory for the remaining parameters

U=Unsatisfactory: Sample unsatisfactory for 1 or more microbiological parameter and either acceptable or satisfactory for the remaining parameters

[ϒ] 511 samples submitted but 17 samples not tested for all 4 microbiological parameters
[♦] 34 samples submitted but 2 samples not tested for all 4 microbiological parameters
[⊗] 264 samples submitted but 11 samples not tested for all 4 microbiological parameters
[▲] 146 samples submitted but 1 sample not tested for all 4 microbiological parameters
[Ø] 130 samples submitted but 5 samples not tested for all 4 microbiological parameters
[*] 52 samples submitted but 2 samples not tested for all 4 microbiological parameters
[⊕] 508 samples submitted but 16 samples not tested for all 4 microbiological parameters
[♣] 50 samples submitted but 1 sample not tested for all 4 microbiological parameters
[♦] 34 samples submitted but 2 samples not tested for all 4 microbiological parameters
[℘] 208 samples submitted but 8 samples not tested for all 4 microbiological parameters
[°] 318 samples submitted but 11 samples not tested for all 4 microbiological parameters
[♥] 556 samples submitted but 15 samples not tested for all 4 microbiological parameters
[♠] 34 samples submitted but 4 samples not tested for all 4 microbiological parameters

Sample temperatures were recorded for 496 of the 556 samples which were stored under refrigerated conditions (Table 10).

Table 10: Temperatures of samples stored under refrigerated conditions (n=496)

Type of packing	No. of samples	Temperature of samples		
		Temperature range (^{o}C)	No. of samples with the following temperature	
			≤5^{o}C (% of samples)	>5^{o}C (% of samples)
Pre-packed[r]	420	-1.5 to 14.9	313 (74.5)	107 (25.5)
Loose[⊗]	44	0 to 10.8	33 (75.0)	11 (25.0)
Not stated	32	2.9 to 8.7 [r] 2.1 to 13.7 [⊗]	1 (3.1) 20 (62.5)	2 (6.2) 9 (28.1)
TOTAL	**496**		367 (74.0 %)	129 (26.0 %)

[r] Temperature measurement between packs
[⊗] Core temperature of sample

Of particular concern was the finding that temperatures >5^{o}C were recorded for 26% (129/496) of samples stored under refrigerated conditions (refrigerated food should be maintained at ≤5^{o}C). This finding raises concern as maintenance of the chill chain is a critical control point in ensuring the microbiological safety of these products. In addition, maintenance of the cold chain is now a legal requirement under Article 4 of **Regulation 852/2004 on the Hygiene of Foodstuffs** [(14)].

4.2 Results by microbiological parameter

4.2.1 *Salmonella* spp.

Salmonellae are bacterial pathogens. They reside in the intestinal tract of infected animals and humans and are shed in the faeces. They are one of the most common cause of foodborne illness (salmonellosis is the disease caused by *Salmonella* spp.). Foods including those of animal origin (e.g. dairy products, meat and eggs) and those subject to faecal contamination (e.g. fruit and vegetables) have been implicated as vehicles in the transmission of this pathogen to humans [(15)]. In Ireland, 419 clinical isolates of *Salmonella enterica* were referred to the National Salmonella Reference Laboratory in 2004 (crude incidence rate of 10.6 cases per 100,000) [(16)].

Raw milk is the principal reservoir of *Salmonella* spp. in the dairy industry. The microflora of raw milk is derived from several sources including the interior of the udder, the exterior surface of the animal, the milking equipment and the environment [(15)]. Control of *Salmonella* spp. is achieved through pasteurisation of the milk and during the cheese ripening phase.

In this study *Salmonella* spp. was not detected in any batch sample of pasteurised cheese from processing premises (n=54) or in any single sample from retail premises (n=890)). A similar finding has been reported by Norway in the *European Commissions Zoonosis Report for 2003* [(17)] (Table 11).

Table 11: Prevalence of *Salmonella* spp. in cheese made from pasteurised milk

Location	Year	No. of samples	Sample source	Type of sample	Results
Norway [(17)]	2003	278	Industry	Cheese made form heat treated milk	Salmonella not detected in any sample
Spain [(17)]	2003	1493	N/S	Ready-to-eat cheese*	Salmonella detected in 2 samples (0.13%)
Ireland (this study)	2005	54 batch samples	Processing premises	Cheese made from pasteurised milk	Salmonella not detected in any sample
		890 single samples	Retail premises	Cheese made from pasteurised milk	Salmonella not detected in any sample

* No indication is given whether the cheeses are made from raw or heat treated milk

A comparison of the salmonella data from this Irish study with data from the 2004 Irish study on C*heeses Made From Raw/Thermised Milk* [(18)] shows that *Salmonella* spp. was not detected in any cheese irrespective of milk type or sample source (Table 12).

Table 12: A comparison of Irish data: Salmonella analysis of cheese made from pasteurised milk Vs. cheese made from raw/thermised milk

Source of sample	Criteria & Results		Satisfactory	Unsatisfactory
Processing premises	***Criteria***		Not detected in 25g in any of the 5 samples	Detected in 25g in any of the 5 samples
	Results	***Cheese made from pasteurised milk***	All batch samples satisfactory (n=54)	No batch sample unsatisfactory
		Cheese made from raw/thermised milk[⊗]	All batch samples satisfactory (n=28)	No batch sample unsatisfactory
Retail premises	***Criteria***		Not detected in 25g	Detected in 25g
	Results	***Cheese made from pasteurised milk***	All single samples satisfactory (n=890)	No single sample unsatisfactory
		Cheese made from raw/thermised milk[⊗]	All single samples satisfactory (n=506)	No single sample unsatisfactory

[⊗] This Irish study was carried out as part of the EU Coordinated Programme for the Official Control of Foodstuffs 2004 [(18)].

4.2.2 *Staphylococcus aureus*

S. aureus is a pathogenic bacterium which is a common cause of food poisoning. Staphylococcal food poisoning is caused by ingestion of a heat stable toxin formed by *S. aureus* in the food (the bacterium must grow to levels $>10^5$ cells/g before producing sufficient quantities of the heat-stable staphylococcal toxin to cause illness [19]). Both the onset and the severity of the symptoms depend on the susceptibility of the person and the amount of toxin consumed. The main symptoms include abdominal cramps, vomiting and diarrhoea [20].

Staphylococcal contamination of cheese may occur from a variety of sources including raw milk produced by cows suffering from mastitis. Research has shown that *S. aureus* may be present in up to 70% of raw milk samples at levels of 10^2-10^5 cfu/ml [6]. In addition, the enterotoxin can be pre-synthesised in the udder and secreted in the milk [21]**.** Importantly, pasteurisation will kill the staphylococcal cells but it will not inactivate the heat stable enterotoxin; therefore, every effort should be made to control the pathogen in the raw milk. Staphylococcal contamination of cheese can also occur from food handlers (it is estimated that up to 50% of humans are carriers of this bacterium on their skin, nose and throat [20]) and from the environment (this pathogen is commonly found in food factories where it may become part of the flora of processing equipment and surfaces [20, 13]).

The ability of *S. aureus* to grow and produce the enterotoxin in cheese is influenced by both intrinsic and extrinsic parameters. Of particular concern is the ability of the enterotoxin to survive for months in cheese even if the viable cell counts decrease [6].

In this study:

- *Batch samples from processing premises:* Applying the criteria outlined for this survey (Table 2), all batch samples (n=54) were classified as satisfactory for *S. aureus*.
- *Single samples from retail premises:* Applying the criteria outlined for this survey (Table 3), 99.21% (877/884), 0.45% (4/884) and 0.34% (3/884) of samples were classified as satisfactory, acceptable and unsatisfactory respectively for *S. aureus*. The 3 unsatisfactory samples were different products (2 were semi hard cheeses and one was a ripened soft cheese). Staphylococcal enterotoxins G and I were detected in one unsatisfactory sample (a semi-hard cheese); however, it should be noted that these enterotoxins are not associated with human illness (staphylococcal poisoning is mainly caused by human strains of *S. aureus* producing enterotoxin A and/or D) [13].

Table 13 compares these data with data from the 2004 Irish study on C*heeses Made From Raw/Thermised Milk* [18]. Cheese type (i.e. cheese made from pasteurised milk and cheese made from raw/thermised milk) had a significant effect ($p<0.05$) on the microbiological status of cheese at both processing and retail level. The incidence of unsatisfactory samples was greater in cheese made from raw/thermised milk (Table 13).

Table 13: A comparison of Irish data: *S. aureus* counts in cheese made from pasteurised milk Vs. cheese made from raw/thermised milk

Source of sample	Type of milk	Details	Satisfactory	Acceptable	Unsatisfactory
Processing premises	***Cheese made from pasteurised milk***	*Criteria*	All 5 samples $<10^2$ cfu/g	No sample $>10^3$ cfu/g and no more than 2 samples in the range $10^2 – 10^3$ cfu/g	Any sample $>10^3$ cfu/g or more than 2 samples in the range $10^2 – 10^3$ cfu/g
		No. of batch samples (%)	54 (100)	0 (0)	0 (0)
	Cheese made from raw/thermised milk ⊗	*Criteria*	All 5 samples $<10^3$ cfu/g	No sample $>10^4$ cfu/g and no more than 2 samples in the range $10^3 – 10^4$ cfu/g	Any sample $>10^4$ cfu/g or more than 2 samples in the range $10^3 – 10^4$ cfu/g
		No. of batch samples (%)	20 (71.4)	4 (14.3)	4 (14.3)
Retail premises	***Cheese made from pasteurised milk***	*Criteria*	$<10^3$ cfu/g	$10^3 – 10^4$ cfu/g	$>10^4$ cfu/g
		No. of single samples (%)	877 (99.21)	4 (0.45)	3✦ (0.34)
	Cheese made from raw/thermised milk ⊗	*Criteria*	$<10^3$ cfu/g	$10^3 – 10^4$ cfu/g	$>10^4$ cfu/g
		No. of single samples (%)	483 (94.5)	12 (2.4)	16* (3.1)

⊗ This Irish study was carried out as part of the EU Coordinated Programme for the Official Control of Foodstuffs 2004 [18]

✦ Staphlococcal counts: 10^3-10^4 cfu/g (n=3)

* Staphlococcal counts: 10^4-$<10^5$ cfu/g (n=8), $\geq 10^5$-$<10^6$ cfu/g (n=7), $\geq 10^6$ cfu/g (n=1),

4.2.3 *Listeria monocytogenes*

Listeria monocytogenes is a bacterium which can cause a serious food borne illness called listeriosis. Although healthy people rarely contract this illness, it can be severe for certain groups of the population (e.g. newborn babies, the elderly, pregnant women and those with a weakened immune system). Symptoms include meningitis, septacemia and abortion in pregnant women.

L. monocytogenes is ubiquitous in the environment. It is present in many raw foods of animal origin including raw milk (it has been estimated that low levels of *L. monoctogenes* exist in commercial bulk-tank raw milk [22, 23]); however, it is eliminated by the pasteurisation process. *L. monocytogenes* is also a frequent contaminant of processing environments. Numerous surveys have documented the presence of listeria within the dairy plant environment including floors, freezers, processing rooms, floor mats etc. [24, 25].

Studies on the behaviour of *L. monocytogenes* during cheese manufacture and cheese ripening show that its fate varies considerably with the type of cheese. In mould surface ripened cheese *L. monocytogenes* has been shown to multiply to large numbers during the latter stages of ripening. This has been attributed to high moisture levels, high pH (due to lactate metabolism by moulds) and susceptibility to surface contamination during the ripening process [4]. Although this pathogen is rarely found in the body of the cheese, conditions under the rind create an ideal environment for its growth. In general, *L. monocytogenes* does not grow (and in most cases it decreases) during the ripening period in semi-hard and semi-soft cheese without surface ripening [6].

In this study:

- *Batch samples from processing premises:* Applying the criteria proposed for this survey (Table 2), all batch samples (n=54) were classified as satisfactory for *L. monocytogenes*.
- *Single samples from retail premises:* Applying the criteria proposed for this survey (Table 3), 98.86% (870/880), 1.02% (9/880) and 0.11% (1/880) of samples were classified as satisfactory, acceptable and unsatisfactory respectively.

The classification of one retail sample (a semi-hard, pre-packed cheese which was produced in Ireland) as unsatisfactory, resulted in the recall of the incriminated production batch from the market. *L. monocytogenes* was detected at an extremely high level in this cheese, i.e. at a level of 1.4×10^6 cfu/g (levels of *L. monocytogenes* >100 cfu/g are known to cause illness). In other studies, *L. monocytogenes* has also been detected at unsatisfactory levels (i.e. >100cfu/g, Table 14).

Table 15 compares these data with data from the 2004 Irish study on C*heeses Made From Raw/Thermised Milk* [18]. This shows show that *L. monocytogenes* was not detected in any cheese sampled from processing premises; however, it was detected in both types of cheese (i.e. cheese made from pasteurised milk and cheese made from raw/thermised milk) sampled from retail premises. Cheese type significantly affected ($p<0.05$) the microbiological status of retail samples.

Table 14: Prevalence of *L. monocytogenes* in cheeses made from pasteurised milk

Location of study	Year	No. of samples	Sample Source	Type of sample	Results	
					Qualitative (i.e. presence/absence)	Quantitative (cfu/g)
Germany [26]*	1999	163	Retail premises, wholesalers, ex-farm producers, dairies	Cheese made from pasteurised milk	13 (8%)	≤100 cfu/g (n=6) > 100cfu/g (n=7)
Sweden [27] ϒ	1989-1991	302	Supermarkets and delicatessens	Cheese made from pasteurised milk	7 (2.3%)	≤100 cfu/g (n=6) 1100 cfu/g (n=1)
Italy [28]	1987-1988	54	Dairy plants	Soft cheese made from pasteurised milk	0 (0)	N/A
Norway [17]	2003	2099	Industry	Cheese made from heat treated milk	2 (0.1%)	N/A
Ireland	2005	54 batch samples	Processing premises	Cheese made from pasteurised milk	0 (0%)	N/A
		880 single samples	Retail premises	Cheese made from pasteurised milk	10 (1.13%)	> 100cfu/g (n=1, 0.11%)

* This study investigated the incidence of *L. monocytogenes* in European smear cheese.
ϒ Cheeses were produced or imported into Sweden
N/A: Not Applicable

Table 15: A comparison of Irish data: *L. monocytogenes* in cheese made from pasteurised milk Vs. cheese made from raw/thermised milk

Source of sample	Criteria & Results		Satisfactory	Acceptable	Unsatisfactory
Processing premises	**Criteria**		Not detected in 25g of any sample	Detected in 25g and enumerated at $\leq 10^2$ cfu/g in all samples	Detected in 25g and enumerated at $>10^2$ cfu/g in any sample
	Results	***Cheese made from pasteurised milk***	54 (100)	0 (0)	0 (0)
		Cheese made from raw/thermised milk$^{\otimes}$	28 (100)	0 (0)	0 (0)
Retail premises	**Criteria**		Not detected in 25g	Detected in 25g and enumerated at $\leq 10^2$ cfu/g	Detected in 25g and enumerated at $> 10^2$ cfu/g
	Results	***Cheese made from pasteurised milk***	870 (98.86)	9 (1.02)	1$^{\blacktriangle}$ (0.11)
		Cheese made from raw/thermised milk$^{\otimes}$	492 (97.0)	14 (2.8)	1** (0.2)

$^{\otimes}$ This Irish study was carried out as part of the EU coordinated programme for the Official Control of Foodstuffs 2004 [42]

$^{\blacktriangle}$ *L. monocytogenes* count: 1.4×10^6 cfu/g

** *L. monocytogenes* count: 5.7×10^3 cfu/g

4.2.4 *Escherichia coli*

E. coli is an enteric organism. Most strains of *E. coli* are harmless; however, several are known to be pathogenic. The pathogenic strains may be categorised based on the mechanism underlying the illness. Currently four categories of pathogenic *E. coli* have been associated with foodborne illness: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC) and Enterohaemorrhagic (EHEC) *E. coli* [29].

E. coli is often used as an indicator of faecal contamination in food. Its presence in cheese suggests that other food-borne pathogens of faecal origin may also be present (e.g. *Listeria* spp., *Salmonella* spp., *Campylobacter* spp. and pathogenic *E. coli*).

In this study:
* *Batch samples from processing premises:* Applying the criteria outlined in Table 2, 96.3% (52/54) of batch samples were classified as satisfactory for *E. coli* while 3.7% (2/54) were classified as unsatisfactory. The two unsatisfactory batch samples (ripened soft cheese) were from different production batches of the same product.

* *Single samples from retail premises:* Applying the criteria outlined in Table 3; 99.09% (868/876), 0.57% (5/876) and 0.34% (3/876) of single samples were classified as satisfactory, acceptable and unsatisfactory respectively for *E. coli*. All 3 unsatisfactory single samples were pre-packed. Two were ripened soft cheese while the nature of the 3rd unsatisfactory sample was not stated on the questionnaire. All three batch samples were stored under refrigerated conditions; however, a temperature of 10°C was recorded for one of these samples.

Table 16 compares these data with data from the 2004 Irish study on C*heeses Made From Raw/Thermised Milk.* Cheese type (i.e. cheese made from pasteurised milk and cheese made from raw/thermised milk) had no significant effect (p=0.05) on the microbiological status of cheese sampled from both processing and retail level (Table 16).
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Table 16: A comparison of Irish data: *E. coli* in cheese made from pasteurised milk Vs. cheese made from raw/thermised milk

Source of sample	Type of milk	Details	Satisfactory	Acceptable	Unsatsifactory
Processing premises	***Cheese made from pasteurised milk***	*Criteria*	All 5 samples $<10^2$ cfu/g	No sample $>10^3$ cfu/g and no more than 2 samples in the range 10^2– 10^3 cfu/g	Any sample $>10^3$ cfu/g or more than 2 samples in the range 10^2 – 10^3 cfu/g
		No. of batch samples (%)	52 (96.3)	0	2 (3.7)
	Cheese made from raw/thermised milk ⊗	*Criteria*	All 5 samples $<10^4$ cfu/g	No sample $>10^5$ cfu/g and no more than 2 samples in the range 10^4– 10^5 cfu/g	Any sample $>10^5$ cfu/g or more than 2 samples in the range 10^4 – 10^5 cfu/g
		No. of batch samples (%)	28 (100)	0 (0)	0 (0)
Retail premises	***Cheese made from pasteurised milk***	*Criteria*	$<10^2$ cfu/g	10^2 – 10^3 cfu/g	$>10^3$ cfu/g
		No. of single samples (%)	868 (99.09)	5 (0.57)	3♣ (0.34)
	Cheese made from raw/thermised milk ⊗	*Criteria*	$<10^4$ cfu/g	10^4 – 10^5 cfu/g	$>10^5$ cfu/g
		No. of single samples (%)	506 (99.4)	3 (0.6)	0

⊗ This Irish study was carried out as part of the EU coordinated programme for the Official Control of Foodstuffs 2004 [18]

♣ *E. coli* counts: 10^3-10^4 cfu/g (n=1), 10^4-10^5 cfu/g (n=1), 10^5-10^6 cfu/g (n=1),

5. Conclusions

This study found that *E. coli* and two pathogens (*S. aureus* and *L. monocytogenes*) were detected at unsatisfactory levels in a small number of pasteurised cheese samples. Although the incidence of unsatisfactory results was low, the finding nonetheless raises concern as:

- epidemiological studies have shown that cheeses made from pasteurised milk have been implicated in outbreaks of food poisoning.
- the infective dose of many pathogens is quite low (e.g. *L. monocytogenes*)

Although this study did not determine where the contamination occurred, it is important to point out that control strategies must be implemented at all stages throughout the food chain. Strategies include:

- The use of active starter cultures (inactive starter cultures can lead to delayed acid formation thereby allowing acid sensitive pathogens time to grow).
- Strict plant sanitation.
- Good handling practices, good hygiene practices (GHP) and good manufacturing practices (GMP).
- Good process control including good temperature control during product storage.

All food businesses should implement a food safety management system based on the principles of HACCP. These strategies should be incorporated into this plan.

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7. Appendices

Appendix 1

Microbiological standards (Council Directive 92/46/EEC▲) for cheese made from pasteurised milk♦

Microorganism	Fresh cheese	Soft cheese
L. monocytogenes	Absent in 25g (n=5, c=0)	
***Salmonella* spp.**	Absent in 25g (n=5, c=0)	
E. coli	N/A	m=10^2cfu/g, M=10^3cfu/g, n=5, c=2ɾ
S. aureus	m=10cfu/g, M=10^2cfu/g, n=5, c=2*	m=10^2cfu/g, M=10^3cfu/g, n=5, c=2ɾ

▲ These standards were in force until 31/12/05. On 01/01/06, Council Directive 92/46/EEC and other commodity specific Directives (17 in total) were repealed and replaced with new legislation known as 'the hygiene package'. This package contains 5 main pieces of legislation, 3 implementing measures and 1 transitional measure. Commission Recommendation 2073/2005 [10] on Microbiological Criteria for Foodstuffs is one of the three implementing measures. It lays down microbiological criteria for a variety of foodstuffs.

♦ These standards were applicable to pasteurised cheeses at the end of processing.

ɾ Batch sample was classified as unsatisfactory if more than 2 of the 5 samples were in the range 10^2 to 10^3cfu/g or if any sample was > 10^3cfu/g

* Batch sample was classified as unsatisfactory if more than 2 of the 5 samples were in the range 10 to 10^2cfu/g or if any sample was > 10^2cfu/g

Appendix 2

Irish National microbiological guidelines for cheese[r] sampled at the point of sale

	Satisfactory	Acceptable	Unsatisfactory	Unacceptable/potentially hazardous
***Salmonella* spp.**	Not detected in 25g	N/A	N/A	Detected in 25g
***Campylobacter* spp.**	Not detected in 25g	N/A	N/A	Detected in 25g
S. aureus	<20	20-<100	100-<10^4	≥10^4
E. coli	<20	20-<100	≥100	N/A
L. monocytogenes	<20	20-<100	N/A	≥100

[r] No differentiation is made between cheese varieties (e.g. fresh, soft, hard etc) or between the type of milk (raw, thermised or pasteurised) used in its manufacture. These guidelines will be revised by the FSAI.

APPENDIX 3
Questionnaire – Batch Samples Sourced from Processing Premises
Bacteriological safety of Cheeses made from Pasteurised Milk

General Information:

* Sampling officer:_____
* Premises Approval No.: _____
* Laboratory Reference Numbers (upon receipt of lab report):

1)_____
2)_____
3)_____
4)_____
5)_____

Sample Information ***(to be completed at the time of sampling)*:**

* Brand name (if available): _____
* Production Batch No.: _____
* Type of sample

Unripened soft cheese (fresh) ☐
Ripened soft cheese ☐
Semi-hard cheese ☐

* Use-By Date:_____ Not Available ☐

Bacteriological Safety

(please complete for each bacteria, see section 9 of protocol):

	Satisfactory	Acceptable	Unsatisfactory
Salmonella spp.	☐	N/A	☐
S. aureus	☐	☐	☐
E. coli	☐	☐	☐
L. monocytogenes	☐	☐	☐

Enforcement Action *(This is only necessary for unsatisfactory samples; please tick as many boxes as necessary):*

None ☐
Verbal warning ☐
Written warning ☐
Improved in house control required ☐
Product recall ☐
Other (Please specify) _____

Completed by:
Area / Regional Inspector_____

APPENDIX 4
Questionnaire – Single Samples Sourced from Retail Premises
Bacteriological safety of Cheeses made from Pasteurised Milk

General Information:

* EHO Name: _____
* EHO Sample Reference Number (i.e. EHO's own personal reference number for the sample): _____
* Laboratory Reference Number (upon receipt of lab report): _____

Sample source ***(see section 3 of protocol)*****:**

Supermarket (*incl. corner shops*) □; Food stall (*e.g. country market*) □; Delicatessen shop □; Catering establishment □;
Other (*Please specify)*_____

Sample Information:

* **Brand name** (if available): _____
* **Type of sample** (*See Table 1 of protocol)*
  - Unripened soft cheese (fresh) □
  - Ripened soft cheese □
  - Semi-hard cheese □

  label):_____
* **Type of packaging:** Loose □ Pre-packed □
* **Batch Number:**_____
* **Use-By Date:** _____ Not Available □
* **Storage condition of sample in premises:**
  Ambient □ Refrigerated □
* **Sample temperature** *(See section 7 of protocol)*
  Core temperature (loose samples): _____ °C
  Between pack temperature (pre-packed samples): _____ °C
* **Plant Number** (*as recorded on*
* **Origin of sample:** Import sample □ Irish sample □
* **Information on label regarding heat treatment of milk:**
  'Made with pasteurised milk' □, None □

Bacteriological Safety *(See section 10 of protocol)*:

	Satisfactory	Acceptable	Unsatisfactory
Salmonella spp.	□	N/A	□
S. aureus	□	□	□
E. coli	□	□	□
L. monocytogenes	□	□	□

Follow up/Enforcement Action ***(see section 11 of protocol)*****:**

(This is only necessary for unsatisfactory samples; please tick as many boxes as necessary):

None □
Verbal warning □
Written warning □
Improved in house control required □
Product recall □
Other e.g. contact DAF/FSAI (Please specify)_____

Appendix 5

No. of samples analysed in each laboratory

	No. of samples analysed in each laboratory	
OFML	**Single samples**	**Batch samples**
Cherry Orchard	181	9
Cork	133	7
Galway	82	0
Limerick	60	8
Sligo	70	8
SPD	133	0
Waterford	231	22
Total	**890**	**54**

APPENDIX 6

Microbiological safety of pasteurised cheese samples from retail premises

Microbiological safety of samples✦ based on *L. monocytogenes* results

HSE Region	HSE Area	Microbiological safety			Total
		Satisfactory	Acceptable	Unsatisfactory	
HSEDMLR	East Coast Area	42	0	0	42
	Midlands Area	37	0	0	37
	South Western Area	103	2	0	105
HSEDNER	North Eastern Area	48	1	0	49
	Northern Area	71	0	0	71
HSESR	South Eastern Area	225	5	1	231
	Southern Area	132	1	0	133
HSEWR	Mid-Western Area	60	0	0	60
	North Western Area	70	0	0	70
	Western Area	82	0	0	82
Grand Total		**870 (98.86%)**	**9 (1.02%)**	**1 (0.11%)**	**880**

✦ 890 samples were submitted for analysis; however 10 samples were not analysed for *L. monocytogenes*.

Microbiological safety of samples based on *Salmonella* spp. results

HSE Region	HSE Area	Microbiological safety		Total
		Satisfactory	Unsatisfactory	
HSEDMLR	East Coast Area	42	0	42
	Midlands Area	44	0	44
	South Western Area	105	0	105
HSEDNER	North Eastern Area	49	0	49
	Northern Area	74	0	74
HSESR	South Eastern Area	231	0	231
	Southern Area	133	0	133
HSEWR	Mid-Western Area	60	0	60
	North Western Area	70	0	70
	Western Area	82	0	82
Total		**890 (100%)**	**0 (0%)**	**890**

Microbiological safety of samples⊗ based on *S. aureus* results

HSE Region	HSE Area	Microbiological safety			Total
		Satisfactory	Acceptable	Unsatisfactory	
HSEDMLR	East Coast Area	38	0	0	38
	Midlands Area	44	0	0	44
	South Western Area	105	0	0	105
HSEDNER	North Eastern Area	48	0	1	49
	Northern Area	72	0	0	72
HSESR	South Eastern Area	231	0	0	231
	Southern Area	128	3	2	133
HSEWR	Mid-Western Area	59	1	0	60
	North Western Area	70	0	0	70
	Western Area	82	0	0	82
Grand Total		**877 (99.21%)**	**4 (0.45%)**	**3 (0.34%)**	**884**

⊗ 890 samples were submitted for analysis; however 6 samples were not analysed for *S. aureus*

Microbiological safety of samples⊕ based on *E. coli* results

HSE Region	HSE Area	Microbiological safety			Total
		Satisfactory	Acceptable	Unsatisfactory	
HSEDMLR	East Coast Area	37	0	0	37
	Midlands Area	44	0	0	44
	South Western Area	105	0	0	105
HSEDNER	North Eastern Area	49	0	0	49
	Northern Area	64	1	0	65
HSESR	South Eastern Area	225	3	3	231
	Southern Area	133	0	0	133
HSEWR	Mid-Western Area	60	0	0	60
	North Western Area	70	0	0	70
	Western Area	81	1	0	82
Grand Total		**868 (99.09%)**	**5 (0.57%)**	**3 (0.34%)**	**876**

⊕ 890 samples were submitted for analysis; however 14 samples were not analysed for *E. coli.*