02/03
25 September 2002

DRAFT ASSESSMENT REPORT

PROPOSAL P263

SAFETY ASSESSMENT OF RAW MILK VERY HARD COOKED-CURD CHEESES

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter: **23 October 2002** (See "Invitation for Public Submissions" for details)

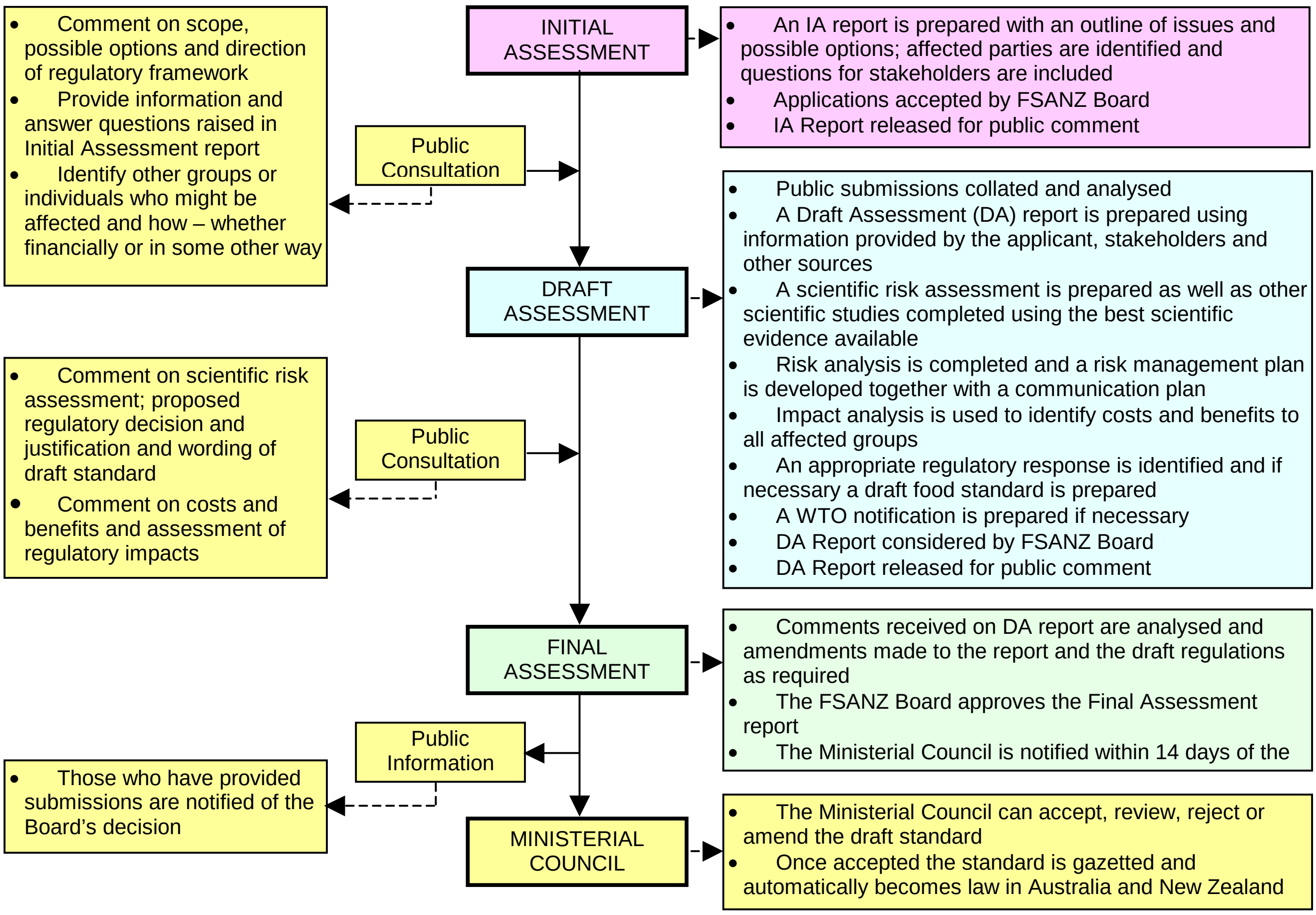
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten governments: the Federal, State and Territory governments of Australia and the New Zealand Government. It is a statutory authority under Australian Commonwealth law and an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards for food available in Australia and New Zealand including primary production and processing standards and for a range of other functions including coordinating national food surveillance and recall systems, conducting research, assessing policies about imported food and developing codes of conduct with industry.

The FSANZ Board approves new standards or variations to food standards which are then accepted by the Australia and New Zealand Food Regulation Ministerial Council (ANZFRMC), a Ministerial Council made up of Commonwealth, State and Territory and New Zealand Health Ministers. If the Council accepts the changes made by FSANZ, the food standards are automatically adopted by reference under the food laws of Australian States and Territories and New Zealand.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act* 1991 (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

The Authority has prepared a Draft Assessment Report of Proposal P263; and prepared a draft variation to Volume 2 of the *Food Standards Code.*

Under section 36 of the FSANZ Act, the Authority opted to omit one round of public consultation as it was satisfied that omitting to invite public submissions prior to making a draft assessment, would not significantly adversely affect the interests of any person or body. Subject to the *Administrative Appeals Tribunal Act 1975*, application may be made to the Administrative Appeals Tribunal, for review of the decision (under section 36) by a person whose interests are affected by the decision.

The Authority will conduct a single round of public consultation and now invites submissions on this Draft Assessment Report based on regulation impact principles and the draft variation to Volume 2 of the *Food Standards Code* for the purpose of preparing an amendment to the *Food Standards Code* for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist the Authority in preparing the Draft Assessment for this Proposal. Submissions should, where possible, address the objectives of the Authority as set out in Section 10 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). Information providing details of potential costs and benefits of the proposed change to the *Food Standards Code* (Code) from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires the Authority to treat in confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word "Submission" and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions should be received by the Authority by **23 OCTOBER 2002**. Submissions received after this date may not be considered unless the Project Manager has given prior agreement for an extension. Submissions may also be sent electronically through the FSANZ website using the Food Standards tab and then through Documents for Public Consideration. Assessment reports are available for viewing and downloading from the FSANZ website or alternatively paper copies of reports can be requested from the Authority's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au including other general enquiries and requests for information.

Questions relating to making submissions or the application process can be directed to the Standards Liaison Officer at the above address or by emailing slo@foodstandards.gov.au.

CONTENTS

FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ) ii

INVITATION FOR PUBLIC SUBMISSIONS iii

EXECUTIVE SUMMARY AND STATEMENT OF REASONS 1

Statement of Reasons 1

1.Problem 2

1.1 Background 2

1.2 Current Regulations 3

Milk and milk products 4

Legislation or documentation 4

New Zealand 5

Standard 1.6.1 – Microbiological Limits For Food 5

Escherichia coli 5

2.Objective 6

2.1 General 6

2.2 Processing requirements 6

3.Relevant Issues 7

3.1 Heat treatment of milk 7

3.1.1 Pasteurisation 7

3.1.2 Thermisation 7

3.2 Raw milk quality 8

Salmonella 9

Listeria monocytogenes 9

Staphylococcus aureus 10

Campylobacter 10

3.4 Cheese making process for very hard cheeses 10

3.5 Factors affecting the growth or survival of pathogens during cheese making 12

Temperature 12

Salt 12

Moisture content 13

Storage/ripening period 13

3.6 Survival of pathogens during the manufacture of very hard cheeses 13

Campylobacter 13

Salmonella 14

Staphylococcus aureus 14

Listeria monocytogenes 14

3.7 Quarantine requirements 15

4.Regulatory Options 17

5.Impact Analysis **18**

5.1 Option 1 .... *18*

5.2 Option 2 .... *19*

5.3 Cost benefit comparison *21*

7. Conclusion and Recommendation **22**

8. Implementation and Review **23**

ATTACHMENT 1 DRAFT VARIATION TO THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE **28**

ATTACHMENT 2 SCIENTIFIC EVALUATION OF THE SAFETY OF VERY HARD CHEESES PRODUCED FROM RAW MILK **29**

EXECUTIVE SUMMARY AND STATEMENT OF REASONS

The processing requirements for cheese and cheese products in Volume 1 and Volume 2 of the *Food Standards Code* (the Code) specify that milk for cheese manufacture must be heat treated by a pasteurisation or thermisation process. In Volume 1 of the Code, a provision exists whereby the adequate heat treatment of milk can be measured in terms of the destruction of the enzyme alkaline phosphatase. Certain very hard cheeses made from raw milk (not heat treated) have continued to be imported into Australia because, in the past, they were considered to comply with the alkaline phosphatase test. This alkaline phosphatase provision, however, has not been included in Volume 2 of the Code because it was not considered a reliable method for measuring the adequate heat treatment of milk and milk products. When Volume 1 is repealed at the end of 2002, raw milk very hard cheeses will not comply with the processing requirements of the Code (Volume 2).

Proposal P263 has been raised in order to assess the safety of very hard cheeses (<36% moisture) made from raw milk and to determine whether an amendment to the Code should be made in order to permit the continued sale of these cheeses. It is being progressed under section 36 of the *Food Standards Australia New Zealand Act 1991*, omitting the first round of public comment before draft assessment. Two options are considered by this proposal – to amend Volume 2 of the Code to permit the sale of very hard cheeses from raw milk (Option 1) or to abandon the proposal (Option 2).

The scientific evaluation of the safety of very hard cheeses produced from raw milk (Attachment 2) undertaken for this proposal indicates that these cheeses can be produced safely from raw milk given good hygienic and manufacturing practices. This is because of the high temperatures used during the curd cooking process and the long maturation/ripening periods involved in producing these low moisture cheeses.

An amendment to Volume 2 of the Code (Option 1) which allows the sale of very hard cheeses (<36% moisture) made from raw milk essentially maintains the status quo with respect to allowing the continued importation of raw milk very hard cheeses. Additionally, Option 1 allows for the domestic production of such cheeses. As the scientific evaluation indicates that these cheeses can be produced safely, the impact assessment shows that an overall benefit to all parties will be provided by an amendment to the Code and therefore this Draft Assessment report supports Option 1.

Statement of Reasons
An amendment to Standard 1.6.2 of the *Australian New Zealand Food Standards Code* is recommended for the following reasons:

- The amendment is based on scientific evidence which supports that raw milk very hard cheeses can be manufactured to achieve a safe product and as such do not pose any significant public health and safety risk.

- The amendment supports the continued importation of raw milk very hard cheeses such as Grana Padano and Parmigiano Reggiano which have been imported into Australia for many years. The amendment will also permit the production of this type of cheese domestically which could benefit Australian industry.

- The impact analysis indicates that the amendment will provide an overall benefit to key stakeholders including importers, consumers, the food service sector, specialty cheesemakers and relevant government agencies.

1. Problem

1.1 Background

The *Food Standards Code* (the Code) requires that milk and milk products for cheese manufacture must be heat treated by either pasteurisation or thermisation (see section 1.2 Current Regulations). There is, however, a provision, which allows for the use of raw milk (not pasteurised or thermised) in cheese manufacture providing that an equivalent level of safety protection is achieved to that using heat treated milk. Currently there are only three raw milk cheeses specifically permitted under this provision - Gruyère, Sbrinz, and Emmental cheeses manufactured in Switzerland. These cheeses were approved for sale in 1999 following an application from the Swiss Government. A risk assessment showed that these cheeses could be produced to an equivalent level of safety as cheese made from pasteurised milk and there was evidence that the necessary industry and regulatory control measures were in place to ensure that the requirements were met.

Raw milk very hard cooked-curd cheeses (parmesan style) such as Grana Padano and Parmigiano Reggiano manufactured in Italy are also currently permitted to be imported[1] and sold in Australia because they are considered to comply with the processing requirements of Volume 1 of the *Food Standards Code.* Volume 1 of the Code contains a provision whereby adequate heat treatment of the milk can be tested in terms of the destruction of the enzyme alkaline phosphatase (Standard H9(1)(e)). Historically, imported parmesan style Italian cheeses were considered to comply with the Code if testing of the cheese complied with the phosphatase requirement. Testing of the cheeses, and in some cases the curds, did yield phosphatase results which complied and importation of these cheeses has continued.

The alkaline phosphatase test was not included as a method for measuring the effectiveness of pasteurisation or equivalent processes in the *Australia New Zealand Food Standards Code* (Volume 2) for a number of reasons. Phosphatase may reform after heat processing of milk and milk products and the level of alkaline phosphatase may vary for different mammalian species. Goat milk, for example, has a low alkaline phosphatase activity and so a test using phosphatase activity as an indicator of effective pasteurisation would not be satisfactory for goat milk. Testing for alkaline phosphatase is also inappropriate for alternative processes to pasteurisation such as micro-filtration or heat treatment processes with lower temperatures than required for pasteurisation. Therefore the alkaline phosphatase test was considered to be an inappropriate test for this purpose.

At the end of 2002 (20 December 2002), Volume 1 of the Code will be repealed and the Joint Code (Volume 2) will come into effect. At that time, as there will no longer be any phosphatase provision to measure the adequacy of the heat treatment of milk used in cheese manufacture, raw milk hard cheeses such as Grana Padano and Parmigiano Reggiano will no longer comply with the processing requirements in the Code and these cheeses will not be able to be imported into Australia. Raw milk parmesan style cheeses from Italy have a long

[1] Around 900 to 1000 tonnes of raw milk parmesan style cheese (Grana Padano and Parmigiano Reggiano) are imported to Australia from Italy annually.

history of import into Australia and New Zealand. Comprehensive records of food-borne illness for these cheeses are not available, however, such cheeses have been extensively used over a long period without adverse reports.

ANZFA has therefore raised proposal P263 – *Assessing the safety of raw milk very hard cooked-curd cheeses (parmesan style)*. The proposal is being progressed under section 36 of the *Australia New Zealand Food Authority Act 1991* resulting in the omission of the first round of public comment. The decision to omit one round of comment was made on the basis that it would not have a significant adverse effect on the interests of stakeholders and would allow for a timely amendment to the Code (Volume 2) to be made, if appropriate.

1.2 Current Regulations

Processing requirements for cheese manufacture are specified in Standard H9 - Cheese and Cheese Products in Volume 1 of the Code and Standards 1.6.2 - Processing Requirements and 2.5.4 – Cheese in Volume 2 of the Code. In New Zealand, processing requirements for cheese and cheese products are regulated under the *Dairy Industry Act 1952* and the *Food Act 1981*. Microbiological limits for cheese, including raw milk cheese, are included in Standard 1.6.1 – Microbiological Limits for Food of Volume 2 of the Code.

Volume 1

Standard H9(1)(d) – (f)

(d) Milk and milk products used for cheese production shall -

i) be heat treated by being held at a temperature of not less than 72ºC for a period of not less than 15 seconds, or at a temperature and for a period equivalent thereto in phosphatase destruction;

ii) be subjected to a minimum heat treatment at a temperature of 62 ºC for a period of not less than 15 seconds (and the cheese may not be sold unless it has been stored at a temperature of not less than 2ºC for a period of 90 days from the date of manufacture of the cheese); or

iii) if they are specified in Column 1 of the Table to this subclause, be produced and processed using a method that:

a. ensures that the cheese produced achieves an equivalent level of safety protection as cheese prepared from milk or milk products that have been heat treated in accordance with (1)(d)(i); and
b. is set out in legislation or documentation listed in Column 2 of the Table to this subclause.

TABLE TO SUBCLAUSE (1)(d)

Column 1 Milk and milk products	Column 2 Legislation or documentation
Milk and milk products used to produce Gruyère, Sbrinz or Emmental cheese only	The Ordinance on Quality Assurance in the Dairy Industry of the Swiss Federal Council of 18 October 1995

(e) Milk and milk products used for cheese production shall be taken to have been adequately heat treated in accordance with paragraph (d)(i) of this clause if they do not exhibit a phosphatase activity in excess of that required to give a reading of 10 µg/ml of ρ-nitrophenol when tested by the current standard method in AS 2300, *Methods of Chemical and Physical Testing for the Dairying Industry*.

Volume 2

Standard 1.6.2 clause 2:

Milk and milk products used to manufacture cheese or cheese products must -

(a) be heat treated by being held at a temperature of no less than 72 ºC for a period of no less than 15 seconds, or by using a time and temperature combination providing an equivalent level of bacteria reduction; or

(b) be heat treated by being held at a temperature of no less than 62 ºC, for a period of no less than 15 seconds, and the final product stored at a temperature of no less than 2 ºC for a period of 90 days from the date of manufacture of the cheese or cheese product.

Standard 2.5.4 clause 3

Milk and milk products used to manufacture cheese or cheese products specified in Column 1 of the Table to this clause must be produced and processed using a method that –

(a) ensures that the cheese produced achieves an equivalent level of safety protection as cheese prepared from milk or milk products that have been heat treated in accordance with paragraph (2)(a) in Standard 1.6.2; and
(b) is set out in the legislation or documentation listed in Column 2 of the Table to this paragraph.

Table to clause 3

Column 1 **Milk and milk products**	Column 2 **Legislation or documentation**
Milk and milk products used to produce Gruyère, Sbrinz or Emmental cheese only	The Ordinance on Quality Assurance in the Dairy Industry of the Swiss Federal Council of 18 October 1995

NB. Section 3(a) of this standard was not adopted by New Zealand.

New Zealand

The processing requirements for cheese and cheese products specified in Standards H9 (Volume 1) and 1.6.2 (Volume 2) of the Code do not apply to New Zealand. For New Zealand purposes, processing requirements, other than for the raw milk Swiss cheeses, are currently regulated under the *Dairy Industry Act 1952* and the *Food Act 1981*. The heat treatment provisions of the *New Zealand Food Regulations 1984* for cheese are as follows:

Regulation 113:

(2) The milk or cream or mixture of milk and cream that is used in the manufacture of cheese-

- (a) Shall be subjected to pasteurisation or an equivalent heat treatment; or
- (b) Shall be subjected to heat treatment at a temperature of not less than 62 ºC for a period of not less than 15 seconds; and
  - (i) The cheese shall be labelled with the date of commencement of manufacture; and
  - (ii) The cheese shall be stored prior to sale at a temperature of not less than 2 ºC for a period of not less than 90 days from the date of commencement of manufacture; and
  - (iii) The cheese shall contain not more than:
    (aa) 100 Escherichia coli per gram; and
    (bb) 100 Staphylococcus aureus (coagulase producing) per gram; and
  - (iv) A 50 g sample of the cheese shall be free from *Salmonella*.

Standard 1.6.1 – Microbiological Limits For Food

Standard 1.6.1 – Microbiological Limits for Food includes several microbiological standards for cheese. Of relevance to this Proposal is the limit for *Escherichia coli* for all cheeses and the standards for *Listeria monocytogenes* and *Salmonella* in all raw milk cheese. The sampling plans specified in Standard 1.6.1 are provided below.

Food	**Microorganism**	**n**	**c**	**m**	**M**
All cheese	*Escherichia coli*	5	1	10	10^2
All raw milk cheese (cheese made from milk not pasteurised or thermised)	*Listeria monocytogenes*/25g *Salmonella*/25g	5 5	0 0	0 0	

Where:
n = the minimum number of sample units which must be examined from a lot of food

c = the maximum allowable number of defective sample units (the number of samples they may exceed 'm')

m = the acceptable microbiological level in a sample unit.

M = the level which, when exceeded in one or more samples, would cause the lot to be rejected.

These microbiological limits mean that raw milk very hard cheeses must have no detectable levels of *Listeria monocytogenes* and *Salmonella*. Additionally, the level of *E. coli* should not exceed 10 per gram though a maximum level of 100 per gram may be allowed for 1 in 5 samples.

2. Objective

2.1 General

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in Section 10 of the *Food Standards Australia New Zealand Act 1991*. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

The main objective in this assessment of P263 will be to protect public health and safety while minimising the barriers/restrictions on the trade of raw milk very hard cheeses.

2.2 Processing requirements

The requirement that milk for cheese manufacture be pasteurised or thermised is a public health measure. Pasteurisation destroys pathogenic bacteria such as *Salmonella*, *Campylobacter* and pathogenic *Escherichia coli* which may be present in raw (unpasteurised) milk and so then be present in the cheese. The Code does allow, however, for an alternative process to be used (e.g. the use of raw milk under Standard 2.5.4 or different heat treatments of milk under Standard 1.6.2) where it can be demonstrated that this process will achieve an equivalent level of safety protection as cheese prepared from milk that has been heat treated. This means that the process must be able to consistently produce a microbiologically safe product.

3. Relevant Issues

3.1 Heat treatment of milk

3.1.1 Pasteurisation

Pasteurisation is a heat treatment process applied to a food which is, primarily, designed to destroy pathogens. Spoilage organisms may also be eliminated during the process, increasing the stability and shelf life of the food. Vegetative bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present (the greater the number of bacteria present, the greater the rate of destruction required). This rate of destruction is dependent both on the temperature and time of exposure and the heat resistance of the organism itself. The pasteurisation parameters of time and temperature are generally determined by the most heat resistant vegetative microorganism likely to be present in the food.

The pasteurisation of milk is historically based on the destruction of *Mycobacterium bovis*, the causative agent of bovine tuberculosis which, in the past, was also responsible for causing tuberculosis infections in humans. The temperature used for milk pasteurisation was then increased to ensure the destruction of *Coxiella burnetii*, the causative agent of Q fever in humans and the most heat resistant vegetative pathogen found in milk. High temperature short time pasteurisation (HTST) of milk at 72 ºC for 15 seconds has been shown to be effective in eliminating this organism and is accepted internationally as the standard process for milk pasteurisation. Additionally, batch pasteurisation using the lower temperature of 63ºC for 30 minutes will give an equivalent measure of bacterial destruction. Other time and temperature conditions of equivalent effect can be calculated graphically by using a log time versus temperature graph. This is obtained by passing a line through the points 72º/15 seconds and 63 ºC/ 30 minutes. Equivalent time temperature conditions should not include temperatures below 63 ºC[2].

While the presence of *Mycobacterium bovis* and *Coxiella burnetii* in milk is now largely controlled by improvements in animal health and farm sanitation, pasteurisation destroys other potential milk-borne pathogens such as *Salmonella spp*, *Staphylococcus aureus*, pathogenic *Escherichia coli*, *Listeria monocytogenes* and *Campylobacter spp*. The temperature-time parameters specified for pasteurisation are generally accepted as being able to achieve at least a 5 log reduction of these pathogens and this level of reduction has been used as the benchmark measure in evaluating the effectiveness of the manufacturing processes of very hard cheeses to produce a microbiologically safe product.

3.1.2 Thermisation

The *Food Standards Code* permits a time-temperature process of milk for cheese production that is less rigorous than pasteurisation (62ºC for 15 seconds), providing that the cheese is stored for at least 90 days from the date of manufacture. This heat treatment is generally referred to as thermisation. The purpose of thermisation is to kill spoilage microorganisms (such as *Pseudomonas* and other psychrotrophs) in the milk which may cause flavour and textural defects in the cheese.[3] Thermisation results in less inactivation of enzymes and

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[2] Codex draft Code of Hygienic Practice for Milk and Milk Products, 1998.

[3] Fox P.E., Guinee T. P., Cogan T. M., McSweeney P. L. H. *Fundamentals of Cheese Science*. Aspen Publishers Inc. 2000.

nonstarter lactic acid bacteria which may be important during ripening for the development of cheese flavour.

While thermisation kills psychrotrophs (microorganisms active at lower temperatures), it may not destroy all pathogenic microorganisms that may be present. A further safeguard is therefore required and the cheese produced must be stored for at least 90 days at a temperature greater than 2 ºC. During this time, depending on the physical and chemical characteristic of the cheese such as pH, water activity and salt content, any pathogenic bacteria present die off.

3.2 Raw milk quality

The microbiological quality of raw milk for cheese manufacture is an essential component in producing good quality raw milk cheese which is microbiologically safe. Raw milk should have total bacterial counts below 20 000 cfu/ml and somatic cell counts below 400 000/ml[4]. Hygienic milk production systems should be in place, preferably as part of an overall HACCP (Hazard Analysis Critical Control Points) program. In recent years, Codex has initiated work on a *Draft Code of Hygienic Practice for Milk and Milk Products* (at step 3) which includes the hygienic production of milk as an essential element. This includes requirements relating to the areas and premises for milk production, animal health, general hygienic practice on farm and hygienic milking.

Within the European Community, Council Directive 92/46/EEC lays down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. It sets out requirements for the hygienic production of milk and provides microbiological criteria for raw milk for direct consumption and for use in the manufacture of products not involving any heat treatments. Under Council Directive 92/46/EEC, raw cow's milk must meet the following standards:

Total plate count (at 30 ºC)	must not exceed 100 000/ml
Somatic cell count	must not exceed 400 000/ml
Staphylococcus aureus (per ml)	n=5, c=2, m=500, M=2 000,

Raw goat or sheep milk intended for the manufacture of products "made with raw milk" must meet the following standards:

Total plate count (at 30 ºC)	must not exceed 500 000/ml
Staphylococcus aureus (per ml)	n=5, c=2, m=500, M=2 000

In Australia, dairy regulations covering the safe production and supply of milk are administered on a State and Territory basis. Such regulations are administered through State Dairy Authorities or food safety agencies (such as SafeFood NSW). Requirements covering the safe production and supply of milk are generally imposed as a condition of licensing. Additionally, all food produced must meet the requirements of the *Food Standards Code*.

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[4]Fox et al, 2000. *Fundamental of Cheese Science*. Aspen Publishers, Maryland.

3.3 Pathogens in milk and cheese[5]

Raw milk can be contaminated with a variety of pathogens originating from the animal, equipment, handlers and the environment. Zoonoses (organisms that can cause disease in animals and humans) such as *Mycobacterium bovis* and *Brucella* spp are controlled primarily through good animal health practices and jurisdictional requirements that milk be collected from healthy animals only. Other pathogens associated with raw milk and which have been implicated in foodborne illness due to the consumption of contaminated cheeses include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and pathogenic *Escheria coli*. Reported cases of foodborne illness internationally have not, however, been associated with the consumption of very hard cheeses. *Campylobacter jejuni/coli* has been frequently associated with human infections due to the consumption of raw milk. FSANZ has undertaken a scientific evaluation of the safety of very hard cheeses produced from raw milk (Attachment 2) which evaluates these five foodborne pathogens against the manufacturing processes for very hard cheeses.

Salmonella

Salmonella has been isolated frequently from raw milk but rarely from commercially made cheese. Outbreaks of salmonellosis due to the consumption of contaminated cheese have been ascribed to faulty control of the production process or to the use of contaminated raw milk. The source of raw milk contamination is primarily via the udder and teats. Milk can also be contaminated post-pasteurisation via the factory environment and food handlers during processing.

Depending on the production process, *Salmonella* can grow during cheese manufacture and may survive in cheese for more than 60 days. The use of thermal processes during cheese manufacture using temperatures >50 ºC will inactivate *Salmonella*. Its survival during maturation will be influenced by the pH and water activity characteristics of the cheese and the conditions of maturation.

Listeria monocytogenes

Listeria monocytogenes is ubiquitous in the environment. It can also be carried by milk producing animals and can cause disease in these hosts. While the incidence of Listeriosis is low, it can have serious health consequences for vulnerable groups (pregnant women, the elderly and the immunocompromised). Because *L. monocytogenes* is commonly found in the processing environment and may contaminate cheese throughout the various stages of the manufacturing process, it is a hazard for all cheese manufacturing processes whether using raw or pasteurised milk.

The incidence of *L. monocytogenes* in soft and mould ripened cheeses is higher than in any other cheese type. Mould-ripening has the effect of raising pH and moisture content resulting in more favourable conditions for bacterial growth than would be present in other manufacturing processes. The ability of *L. monocytogenes* to grow at refrigeration temperatures means its presence on soft and mould ripened cheeses is a particular concern. Survival of *L. monocytogenes* is dependent on the pH of the cheese and at levels of ~pH 5.5

[5] Information provided in this section has been sourced primarily from the Scientific evaluation of the safety of very hard cheeses produced from raw milk (Attachment 2). Additional reference was made to: ICMSF. 1996 *Microorganisms in foods 6, Microbial ecology of food commodities*. Blackie Academic & Professional, London.

no growth should occur (the final pH of the curd for most very hard varieties is in the range 5.0 to 5.3). Thermal processes using temperatures >50 ºC will inactivate the organism.

Staphylococcus aureus

Staphylococcus aureus can contaminate milk directly via the animal or by food handlers during milking and processing. *S. aureus* may be shed into milk in large numbers (up to 10^5 colony forming units per ml) by animals having mastitis before any clinical symptoms are shown. When conditions allow this organism to grow in the food during manufacture, enterotoxins are produced which have been shown to persist in cheese for several years. Outbreaks of foodborne staphylococcal intoxication attributed to cheese have resulted largely from poor process control and contaminated starter cultures. Proper raw milk handling and storage, and rapid acid production during acidification of cheese are important controls over this organism during cheese manufacture.

Pathogenic *Escherichia coli*

Pathogenic *E. coli* are grouped into specific pathotypes: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), diffuse-adhering (DAEC), enteroaggregative (EAEC) and enterohaemorrhagic (EHEC). Foodborne illness caused by pathogenic *E. coli* where cheese has been implicated have included EIEC, ETEC and EHEC. The dose of EHEC required to cause human illness is very low (a few cells in high-risk individuals).

Raw milk may become contaminated by *E. coli* during milk collection from the animal or from the milking parlour environment. Post-pasteurisation contamination of milk may also occur from the food processing environment. *E. coli* are sensitive to heat and are inhibited by low pH and salt. The inactivation of this organism during cheese manufacture, as for most other pathogens, depends on the thermal processes used during curd cooking, the pH and water activity characteristic of the cheese and the conditions of maturation.

Campylobacter

Campylobacter jejuni and *C. coli* are the most common *Campylobacter* spp causing foodborne illness. *C. jejuni* is more likely to be a contaminant of sheep and cows milk than *C. coli*. Milk may become contaminated with this organism from faecal material or it may be shed in the milk itself. Raw or inadequately pasteurised milk has been frequently identified as a vehicle of foodborne infection with *C. jejuni* however contaminated cheese has not been reported as a cause of Campylobacteriosis.

Campylobacters are sensitive to heat and survive poorly in mildly acidic environments and in the presence of 2% or more salt. This organism does not tolerate low moisture conditions and fails to grow at water activities of less than 0.987 (the water activity of very hard cheeses is quite low with parmesan, for example, having a typical a_w of about 0.92).

3.4 Cheese making process for very hard cheeses

Cheese can be categorised in a number of ways but more traditional characterisation schemes are based principally on moisture content such as very hard, hard, semi-hard/soft or soft. Very hard cheeses have the lowest moisture content, generally less than 35%. Because of their hard texture and strong flavour, they are generally used in small quantities as grated cheese and so are often referred to as hard grating cheeses. Codex[6] has a standard for "extra

[6] Codex International Standard for Extra Hard Grating Cheese, *CODEX STAN C-35-1978*.

hard grating cheese" which includes some principal characteristics for this cheese. The Codex standard specifies a maximum moisture content for extra hard grating cheese of 36% and a minimum period of maturation/curing of not less than 6 months. The cheese varieties typical of the very hard cheese category include Parmesan, Romano, Asiago and Montasio and are generally made from cow or sheep milk.

The majority of extra hard cheese varieties originated in Italy. Cheeses such as Parmigiano Reggiano and Grana Padano (parmesan style cheeses) are designated 'DOC' (Denominazione d'origine controllata)[7] and continue to be manufactured in Italy according to traditional methods using raw milk. However, whether using traditional methods or modern manufacturing techniques, there are characteristic steps in the manufacturing protocol of very hard cheeses which determine the nature of these cheeses. In particular the use of high curd cooking temperatures to dispel moisture and very long maturation periods are typical of these cheeses.

The main steps involved in the manufacture of very hard cheeses are outlined below.

<u>Generalised manufacturing process for very hard cheeses</u>

Raw milk

⇩

Standardised (e.g. for cream/fat content) — milk heat treated as required

⇩

Addition of starter culture/whey culture (acidification)

⇩

Coagulation (addition of rennet)

⇩

Curd cutting

⇩

Curd cooking *

⇩

Curds drained/moulded

⇩

Salting – in saturated brine (dry salting may be used for some varieties such as Romano)

⇩

Maturation/ripening (typically for periods of 12 months and longer at temperatures >10 ºC)

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[7] The DOC or Protected Denomination of Origin is a regulated and controlled qualification used within Europe for a number of products including olive oils, wines and cheeses. The Denomination of Origin regulates the area of production and the production system which makes the product unique.

*High curd cooking temperatures of up to 55-56ºC are typically used in traditional parmesan cheese manufacture. Lower temperatures (less than 50ºC) may be used in more “modern” large scale operations but are used in combination with the heat treatment of milk (pasteurisation or thermisation). A summary of the processing steps used for the manufacture of several very hard cheese varieties is provided in Table 5 of Attachment 2.

3.5 Factors affecting the growth or survival of pathogens during cheese making[8]

Several factors are involved in controlling the growth of bacteria in cheese including pH, temperature processes, the level of salt and the water activity or moisture content of the cheese. While each of these has an effect, it is their combined effect which has greatest control over the growth or survival of pathogens and other bacteria in cheese. The scientific evaluation of the safety of very hard cheeses (Attachment 2) examines the effect of the temperature processes used in the manufacture of certain very hard cheeses on bacterial pathogens, as well as the effects of pH, water activity and salt.

pH

The production of cheese curd is dependent firstly on an acidification operation. In the manufacture of very hard cheeses a starter culture or natural whey culture containing lactic acid bacteria is added to the milk and lactic acid is produced. Acid production and the resultant decrease in pH affects the growth of many non starter bacteria, including pathogens that may be present. The rate and duration of acidification is reasonably characteristic of the cheese variety. For hard cheeses, the final pH of the curd for most varieties is in the range 5.0 to 5.3.

Temperature

The time-temperature profile used during cheese making can play a major role in promoting the growth of pathogens during manufacture or may kill them. For example, if curd cooking temperatures of 24 – 40 ºC are used, this is conducive to the growth of pathogens if they are present. (In this case, rapid acid production and a quick drop in pH would be important in the process for controlling the growth of any bacteria present.) The curd-cooking temperatures used in the manufacture of very hard cheeses can be quite high (up to 55-56 ºC) as is the length of time the curd is held at these temperatures (up to 1 hour). This time/temperature process will have a lethal effect on pathogens present, the extent of destruction depending on the numbers present and the thermal resistance of the particular organism. Thermal resistance of a microorganism is expressed as a D-value (the decimal reduction time or time required for the destruction of 90% of the bacterial population, at a particular temperature).

Salt

Salting has a number of important effects on cheese quality and safety. In terms of safety, salt inhibits the growth of microorganisms, including pathogenic bacteria, which may be present. The addition of salt increases the aqueous phase of foods, causing dehydration of bacterial cells and resulting in the death or inhibition of growth, depending on the level added and the characteristics of the particular organisms present. Salt also aids in the removal of whey from the curd, thus reducing the moisture of the cheese which also influences the activity of

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[8] References used include Fox *et al*, 2000 (*Fundamental of Cheese Science*) and Fox, P.F. (ed), *Cheese: Chemistry, Physics and Microbiology.* Volume 1, General Aspects. Chapman & Hall, London.1993.

microorganisms and enzymes. Salt levels in very hard cheeses can be quite high, up to 5% for varieties such as Pecorino romano.

Moisture content
The ability of bacteria to grow or survive is largely dependent on available moisture (in combination with other factors such as pH and temperature). Cheeses with a relatively high moisture content, for example, may readily support the growth of pathogens, if present, compared to a low moisture cheese. The available moisture for bacterial growth is defined in terms of the water activity (a_w) of the food. Very hard cheese generally have quite low water activities (a typical a_w for parmesan is 0.92) which do not support the growth of pathogenic micoorganisms.

Storage/ripening period
The combined effects of pH, salt, moisture and storage temperature come into play during ripening and promote the die off of pathogens. The decline of pathogens present during this time will be influenced by the characteristics of the cheese and the temperature of storage. Very hard cheeses, which are generally ripened at temperatures above 10ºC represent quite an inhospitable environment for most bacteria such that pathogens die off during storage.

3.6 Survival of pathogens during the manufacture of very hard cheeses

The scientific evaluation of the safety of very hard cheeses produced from raw milk (Attachment 2) assessed the production process for five very hard cheeses – Parmigiano Reggiano (parmesan), Grana Padano (parmesan), Pecorino Romano, Asiago and Montasio. These cheeses are considered representative of the very hard cheese category. The manufacturing process provided for Parmigiano Reggiano and Grana Padano involved no thermal treatment of the milk (raw milk used) and involved higher curd cooking temperatures of 55 – 56°C. The specific manufacturing processes supplied for Pecorino Romano, Asiago and Montasio cheeses did included thermal treatment of the milk (pasteurisation and thermisation). While the scientific evaluation notes the effect that these thermal processes would have on the pathogens of concern, it also assesses the effect of the curd cooking processes used and the effects of ripening and storage on bacterial reduction. This allows for an evaluation of the production processes for these cheeses if raw milk is used. The manufacturing processes assessed for Pecorino Romano, Asiago and Montasio use lower curd cooking temperatures than the parmesan cheeses (42 - 48°C).

The scientific evaluation of the safety of very hard cheeses produced from raw milk provided the following conclusions for each pathogen assessed:

Campylobacter
It is concluded that *C. jejuni/coli* are unlikely to be a hazard in these very hard cheeses as thermal processing applied will inactivate bacteria present in the raw milk and the conditions in the cheeses after fermentation and ripening will further inactivate survivors. (This assumes that these processes are carried out under control programs such as HACCP and its pre-requisites.)

Pathogenic *Escherichia coli*
Hard cheeses manufactured with a curd cooking temperature of >55 ºC (30 min) and matured for a minimum of 3 months will achieve the required performance criteria of greater than a 5

log reduction in *E. coli*. Cheeses receiving a lower cooking temperature should, where possible, be manufactured from thermised or pasteurised milk. Raw milk cheeses using lower cooking temperatures should be matured for a minimum of 6 months unless supporting data on the inactivation of *E. coli* are provided.

Salmonella
Cheeses produced with curd cooking temperatures above 55 ºC are unlikely to contain viable Salmonellae at the end of maturation. Cheeses produced with curd cooking temperatures below 51ºC may still be contaminated with low levels of Salmonellae. Such cheeses should be manufactured from pasteurised or thermised milk or stored for more than 3 months at approximately 15 ºC.

Staphylococcus aureus
S. aureus should be inactivated in hard cheeses manufactured with a curd cooking temperature of > 55 ºC (30 min) and matured for a minimum of 3 months. The presence of toxin appears to be determined by the number of organisms present in the milk used for cheese making. Some growth (1 to 2 logs) of *S. aureus* would be expected in cheeses receiving a lower cooking temperature, therefore S. aureus numbers in milk used in the manufacture of these cheeses should be less than 10^3 CFU/ml. Storage of these cheeses for 3 months should ensure that they are free of viable *S. aureus*.

Listeria monocytogenes
Cooking of the curd to temperatures of >55 ºC followed by maturation at >15 ºC for more than 3 months will result in reductions in *L. monocytogenes* of more than 5 logs. Cooking at lower temperatures may result in a slight increase in *L. monocytogenes* during the early stages of production followed by a rapid decrease in numbers during storage reaching >5 logs after approximately 6 months.

The general conclusions drawn from the report are as follows:

- All very hard cheeses made, according to the process criteria provided (including acidification, curd cooking, salting and ripening conditions), will achieve a 5-log reduction in the numbers of the pathogens specified eg. *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, pathogenic *Escherichia coli* and *Salmonella*. (Note is made of the significance of staphylococcal enterotoxin production in raw milk and during manufacture.)

- The production process resulting in the greatest control/destruction of pathogens include the curd cooking together with the long maturation.

- The presence of staphylococcal enterotoxin will be dependent on the initial quality/pathogen load of the raw milk, which needs to be controlled for the production process to achieve a safe product. This is the case whether using raw milk or pasteurised/thermised milk.

Pasteurisation is generally accepted as being able to achieve at least a 5 log reduction of pathogens. This level of reduction has been used as the benchmark measure in evaluating the effectiveness of the manufacturing processes used for very hard cheeses in achieving a microbiologically safe product. For the production processes assessed, the combination of the curd cooking temperatures and maturation times given will result in this 5 log reduction. For traditional manufacturing processes using high curd cooking temperatures (55 ºC and above) this will result in the inactivation of any pathogens present (measured as a 5 log reduction) within a minimal maturation time (3 months). Where lower curd cooking temperatures are used in the manufacture of very hard cheeses (42 – 48ºC), any pathogens present should be inactivated after at least 6 months maturation. However, if there has been abuse of the raw milk or a processing problem which has enabled the growth of *S. aureus* and the production of enterotoxin, toxin will remain in the matured cheese. This is the case whether using raw milk or pasteurisation during manufacture but highlights the need for good manufacturing processes and controls for the hygienic production of milk.

3.7 Quarantine requirements

The Australian Quarantine Inspection Service (AQIS)/Biosecurity Australia maintain import requirements for dairy products into Australia. A quarantine permit must be obtained in order to import cheeses into Australia. The conditions for import depend on whether the country exporting is free from Foot and Mouth Disease. Import from these countries must be accompanied by an import permit for each consignment and each consignment must be accompanied by a specific sanitary certificate signed by an Official Government Veterinarian of the exporting country. While these requirements are mainly concerned with the transfer of Foot and Mouth Disease, they effectively require that dairy products are sourced from healthy animals and that there are appropriate controls in place within the country of origin to ensure this. The import requirements are as follows[9]:

1 The milk or the milk from which the cheese is made must originate from a country/zone recognised by the Office International des Epizooties (OIE) as foot and mouth disease-free, with or without vaccination.

2 The country of origin must have controls in place to ensure only healthy animals are used for milk production.

3 The products must be processed in a foot and mouth disease-free country/zone.

4 EITHER:

(a) The milk or the milk from which the cheese or butter was made must be subjected to one of the following heat treatments:

pasteurisation at 72°C for a minimum of 15 seconds or equivalent treatment, in terms of phosphatase destruction or

a UHT treatment of 135°C for a minimum of 1 second.

OR

(b) The milk from which the cheese was made was not heat treated as above and the milk or the milk from which the cheese or butter was made must originate from a country/zone which meets the OIE requirements for freedom from rinderpest in accordance with Code Article 2.1.4.2.

5 The packaging or immediate container must be stamped with the date of manufacture of the products.

[9] AQIS quarantine requirements for the importation of dairy products from approved countries as at 27 September 2000.

6 Cheese or butter not heat treated in accordance with requirement 4.4(a) will not be released from quarantine until the conclusion of a period of 30 days from the date of manufacture*.
*[Note: For cheese the date of manufacture is the date the curd was set.]

Conditions for the importation of cheese from countries not free from foot and mouth disease include additional requirements:

1 The milk or the milk from which the cheese is made must originate from a country/zone approved by AQIS for the export of dairy products to Australia.

2 The country of origin must have controls in place to ensure only healthy animals are used for milk production.

3 EITHER

(a) the milk from which the cheese was made was

pasteurised at a minimum of 72°C for 15 seconds or equivalent treatment, in terms of phosphatase destruction and
the cheese has attained a pH of less than 6 and the cheese has aged for 30 days or more.

OR

(b) the cheese has attained a pH of less than 6 and has aged for 120 days or more at a temperature not less than 2°C.

4 The packaging or immediate container must be stamped with the date of manufacture of the products.

5 Cheese made according to requirement 5.3(a) above will not be released from quarantine until a minimum of 30 days after the date of manufacture. Sampling of cheeses prior to release from quarantine to ensure the pH is not above 6 may be required by the Director of Quarantine.

6 Cheese made according to requirement 2.5.3(b) above shall not be released from quarantine until a minimum period of 120 days storage at a temperature not less than 2°C after the date of manufacture. Sampling of cheeses prior to release from quarantine to ensure the pH is not above 6 may be required by the Director of Quarantine.

*[Note: For cheese the date of manufacture is the date the curd was set.]

When considering the approval of countries to export dairy products into Australia, AQIS takes into account the following criteria:

- the animal health status of the country;
- the effectiveness of veterinary services and other relevant certifying authorities;
- legislative controls over animal health, including quarantine policies and practices;
- the standard or reporting to the Office International des Epizooties (OIE) of major contagious disease outbreaks;
- effectiveness of veterinary laboratory services, including compliance with relevant international standards;

- effectiveness of systems for control over certification/documentation of products intended for export to Australia.

In effect, the AQIS import requirements for dairy products provide an additional control over the source and microbiological quality of raw milk used in the manufacture of dairy products imported into Australia. Similar quarantine measures are applied in New Zealand.

4. Regulatory Options

There are two regulatory options posed by this proposal, to amend Volume 2 of the Code to allow for the sale of very hard cheeses produced from raw milk or to abandon the proposal.

- Option 1 – amend Volume 2 of the *Australia New Zealand Food Standards Code* to allow for the sale of very hard cheeses produced from raw milk

Option 1 would permit the sale of cheese from raw milk providing that the cheese has a low moisture content (no more than 36%) and has been ripened for a minimum of 6 months from the date of manufacture at a temperature no less than 10ºC. These parameters are based on the conclusions from the scientific evaluation of the safety of very hard cheeses produced from raw milk and are consistent with the Codex standard for very hard grating cheeses. In practice, very hard cheeses are generally ripened for much greater periods, up to 2 years for some parmesans such as Parmigiano Reggiano. Under Option 1, an amendment to volume 2 of the Code could be made to Standard 1.6.2 – Processing Requirements or Standard 2.5.4 – Cheese.

An amendment to Standard 1.6.2 would maintain the status quo for Australia with respect to the importation of raw milk very hard cheeses and would, additionally, allow for domestic production of these cheeses. New Zealand, however, is not covered by this standard and would need to continue with its current administrative arrangements that allow for the import of such cheeses. An amendment to Standard 2.5.4 would be applicable to both Australia and New Zealand and potentially create a more level playing field for domestic producers in both countries in that it could allow for domestic production of raw milk very hard cheeses in both countries as well as their importation. However, an amendment to Volume 2 of the Code would be more logically placed in Standard 1.6.2 as it is directly concerned with the processing requirements. As New Zealand currently has its own measures in place to deal with the importation of raw milk hard cheeses and has its own processing requirements for domestic production, an amendment to Standard 1.6.2 would not discount their interests. An amendment to Standard 1.6.2 – Processing Requirements is therefore proposed under Option 1.

- Option 2 – abandon Proposal P263

Option 2 means there would be no amendment to Volume 2 of the *Food Standards Code*.

5. Impact Analysis

The options posed by this proposal are concerned with Clause 2 of Standard 1.6.2 – Processing Requirements, which are not applicable to New Zealand. The impact analysis, therefore, focuses on the impact on Australian parties but would also be relevant to New Zealand as discussed above.

5.1 Option 1

Option 1 essentially maintains the status quo with respect to allowing the continued importation of raw milk very hard (parmesan style) cheeses into Australia. Additionally, this amendment would allow for the domestic production of very hard cheeses (no greater than 36% moisture) using milk that has not been heat treated prior to cheese manufacture, providing the cheese is ripened for at least 6 months. It is unlikely that this would result in any changes to the manufacturing protocols currently used in large scale commercial operations where milk is routinely pasteurised prior to cheese making, but could allow smaller, specialty cheese makers further scope in the production of very hard cheeses. This would, however, be subject to State and Territory requirements under dairy and health legislation.

The impact of option 1 on the parties most likely to be affected by this application is outlined below.

- Importers

The are several major importers of raw milk parmesan style cheeses. Around 900 to 1000 tonnes of raw milk parmesan style cheese (Grana Padano and Parmigiano Reggiano) are imported to Australia from Italy annually[10]. Option 1 enables importers to continue to import these cheeses as they have done for many years.

- Consumers

Consumers of Parmigiano Reggiano and Grana Padano, in particular, and of specialty cheeses in general would wish to have access to raw milk very hard cheeses. The scientific assessment has shown that these cheeses can be manufactured to achieve a safe product and should pose no additional public health and safety concern compared to a very hard cheese produced with pasteurised milk. Option 1 provides consumers with continued access to these cheeses and, potentially, further increases consumer choice through domestic production of raw milk very hard cheeses.

- Industry

The dairy industry would not support a measure that could erode domestic and export market confidence in the safety of dairy products. Pasteurisation has been viewed as a key processing requirement for maintaining the safety of milk and milk products in Australia and New Zealand. However, raw milk parmesan style cheeses have been imported for many years without any reported adverse public health consequences and the scientific assessment concludes that, because of the heat treatment of the curd and long maturation periods

[10] Import figures provided by ISTAT (Italy's National Statistical Institute) via the Embassy of Italy, Canberra.

involved in manufacture, a safe product can be produced. Quarantine requirements can provide an additional control over the milk quality used for imported products. State and Territory dairy regulations would further ensure that such products, if produced domestically, are manufactured to achieve a safe product.

The majority of parmesan style cheese consumed in Australia is produced domestically and from pasteurised milk. Australia produced around 374 261 tonnes of cheese in 2001, approximately 13 920 tonnes of this being hard grating cheese[11]. Compared to domestic production, imported raw milk parmesan style cheeses account for only a small percentage of the market. Australian specialty cheese manufacturers (in particular), however, may wish to increase the development and production of very hard cheeses using raw milk within Australia. Additionally, with the development of such products, export options may be opened up for Australian producers.

Many restaurateurs and caterers serve and use imported raw milk hard cheeses in food preparation. The food service sector would, therefore, support the ongoing supply of these cheeses into Australia.

Option 1 should result in minimal impact on the domestic dairy industry and would support the interests of the food service sector and specialty cheese manufacturers.

- Government

As with industry, State and Territory departments of health and dairy regulators would not support a measure that could compromise the safety of dairy products and impact adversely on human health. As discussed, Option 1 is supported by the scientific evaluation which demonstrates these cheeses can be manufactured to achieve a safe product using good hygienic and manufacturing practices.

For the Imported Food Program of AQIS, enforcing processing standards at the border is difficult. Option 1 does not provide an additional processing requirement but essentially provides an exemption from the heat treatment provisions for low moisture cheeses ripened for at least 6 months. Information as to the types of cheeses covered by this provision can be supplied to AQIS to support testing against the requirements of Standard 1.6.1 – Microbiological limits for food. Option 1 should not impose any additional enforcement requirements.

5.2 Option 2

Abandoning Proposal P263 would result in no amendment to Standard 1.6.2 of the *Australia New Zealand Food Standards Code* (Volume 2 of the Code). This will mean that all cheeses will be required to be manufactured from pasteurised or thermised milk as specified in this standard unless there is specific permission for an alternative process such as for Gruyere, Sbrinz and Emmental cheese produced in Switzerland. As a consequence, raw milk very hard cheeses such as Parmigiano Reggiano and Grana Padano would not be able to be imported into Australia.

[11]Australian Dairy Corporation, *Australian Dairy Industry in Focus 2001*.

- Importers

Import companies which currently have permits to import raw milk very hard cheeses into Australia would no longer be permitted to bring these products into the country for sale. Option 2 would disadvantage cheese importers.

- Consumers

Consumers who currently enjoy raw milk parmesan style cheeses imported from Italy would no longer be able to purchase these products in Australia nor enjoy them in restaurants. The Italian community, in particular, would not be able to have continued access to these traditional cheeses. As raw milk very hard cheeses pose no additional public health and safety risk to that of pasteurised milk very hard cheeses, Option 2 does not benefit consumers.

- Industry

The domestic cheese manufacturing industry would continue to supply the vast majority of very hard parmesan style cheeses consumed in Australia. There would unlikely be any significant increase in demand for Australian cheeses to compensate for the niche market that imported Grana Padano and Parmigiano Reggiano, for example, currently have. Option 2 would also maintain the status quo for specialty cheese makers, requiring all cheese to be made from pasteurised or thermised milk.

Restaurateurs and caterers would be unable to continue serving and using these cheeses. This may be considered a negative impact on the quality and range of food products served by the food service sector and would not be supported by them.

Option 2 is unlikely to provide any benefit to industry.

- Government

Stopping the trade in raw milk very hard cheeses would have no real impact on State and Territory governments. The Commonwealth government, however, may be petitioned by consumers, the food service sector and importers to allow cheeses such as Grana Padano and Parmigiano Reggiano to continue to be allowed into Australia. Italian cheese manufacturers and trade officials may also be concerned by such a barrier to trade which is not supported by the scientific evidence and petition the Commonwealth to address the issue with possible WTO implications. Option 2 may have a negative impact on government at the Commonwealth level.

For the Imported Food Program of AQIS, enforcing the requirement that raw milk cheeses are not permitted into Australia for sale (except for those specifically permitted) would not be significantly affected by Option 2.

5.3 Cost benefit comparison

A comparison of the impact of Options 1 and 2 on the parties identified is summarised in the table below.

Affected Party	Option 1	Option 2	Comments
Industry • Dairy industry (domestic)	**Costs**. No cost **Benefits.** No benefit to large manufacturers. Specialty cheese makers may benefit through increased flexibility in regulation for very hard grating cheeses.	**Costs.** No cost to large manufacturers. **Benefits.** No real benefit, though large dairy producers may feel that Option 2 best protects domestic cheese production.	The dairy industry should not be significantly affected by Option 1 or 2, though large dairy manufacturers may be more supportive of Option 2. The food service sector benefited by Option 1.
• Food service sector	**Costs.** No cost **Benefits.** The food service sector would benefit by being able to continue serving and using raw milk very hard cheeses	**Costs.** The food service sector unable to continue using raw milk very hard cheeses. **Benefits**. No benefit.	
Importers	**Costs.** No costs. **Benefits.** Maintains the status quo, enabling importers to continue the trade of raw milk parmesan style cheeses.	**Costs.** Impacts on the trade of raw milk parmesan style cheese. **Benefits.** None	Only Option 1 provides a benefit to importers – Option 2 will impart a cost through restricting trade.
Government	**Costs.** No real costs. **Benefits.** The trade in imported raw milk very hard cheeses is maintained.	**Costs.** Negative impact at the Commonwealth level through adverse reactions by importers, consumers, the food service sector and Italian trade representatives. Possible WTO implications due to restriction in trade. **Benefits**. None	The continued trade of raw milk very hard cheeses (Option 1) provides the only benefit to Government.

Outcome: Option 1 provides an overall benefit to most parties compared with Option 2.

6. Consultation

Proposal P263 has been progressed under section 36 of the *Food Standards Australia New Zealand Act, 1991*, omitting the first round of public comment. The decision to omit one round of comment was made on the basis that it would not have a significant adverse effect on the interests of stakeholders. Draft Assessment will be the first opportunity for public consultation. Comment on the recommendations of P263 will be specifically sought from relevant industry representatives and associations (such as the Australian Dairy Products Federation); State and Territory departments of health and dairy regulators; Importers and their representative bodies; AQIS, and other parties who have demonstrated an interest in raw milk cheese production and regulation in the past.

It will be recommended to the agencies responsible that the WTO be notified under the TBT and SPS agreements in accordance with Australia and New Zealand's obligations as members of the WTO, in order to enable other member countries to comment on proposed changes to standards which may have a significant impact on them.

7. Conclusion and Recommendation

The *scientific evaluation of very hard cheeses made from raw milk* (Attachment 2) concluded that the manucturing processes assessed for the very hard cheeses Grana Padano, Parmigiano Reggiano, Romano, Asiago and Montasio can achieve a 5 log reduction of the bacterial pathogens of concern when using raw milk. This benchmark level of bacterial reduction is considered as achieving a microbiologically safe product. The low moisture content of these very hard cheeses (<36%), the temperatures used during cooking of the curd and the long maturation/ripening periods involved are integral in determining the survival of any pathogens that may be present. The draft assessment of Proposal P263, therefore, proposes an amendment to Volume 2 of the *Australia New Zealand Food Standards Code* to permit the sale of very hard cheeses (< 36% moisture) from raw milk provided that they are ripened/matured for a minimum of 6 months at a temperature of at least 10ºC.

An amendment to Standard 1.6.2 of the *Australian New Zealand Food Standards Code* (Volume 2) is recommended for the following reasons:

- The amendment is based on scientific evidence which supports that raw milk very hard cheeses can be manufactured to achieve a safe product and as such do not pose any significant public health and safety risk.

- The amendment supports the continued importation of raw milk very hard cheeses such as Grana Padano and Parmigiano Reggiano which have been imported into Australia for many years. The amendment will also permit the production of this type of cheese domestically which could benefit Australian industry.

- The impact analysis indicates that the amendment will provide an overall benefit to key stakeholders including importers, consumers, the food service sector, specialty cheesemakers and relevant government agencies.

8. Implementation and Review

After the draft assessment public consultation process, it is anticipated that a final assessment of P263 will be completed before the end of October. Following approval by the Board, the amendment to Standard 1.6.2 – Processing Requirements will be notified to the Australia and New Zealand Food Regulation Ministerial Council (ANZFRMC) for their consideration. At the conclusion of the Council process, an amendment to Standard 1.6.2 may be gazetted. It is anticipated that such an amendment would be made before 20 December 2002.

ATTACHMENTS

Attachment 1. Draft variation to the Australia New Zealand Food Standards Code

Attachment 2. Scientific evaluation of the safety of very hard cheeses produced from raw milk.

ATTACHMENT 1

DRAFT VARIATION TO THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE

To commence: **on gazettal**

[1] ***Standard 1.6.2*** *of Volume 2 of the Food Standards Code is varied by omitting clause 2 and the editorial notes immediately following clause 2, substituting –*

2 Processing of cheese and cheese products

(1) Cheese and cheese products must be manufactured –

(a) from milk and milk products that have been heat treated –
(i) by being held at a temperature of no less than 72°C for a period of no less than 15 seconds, or by using a time and temperature combination providing an equivalent level of bacteria reduction; or
(ii) by being held at a temperature of no less than 62°C for a period of no less than 15 seconds, and the cheese or cheese product stored at a temperature of no less than 2°C for a period of 90 days from the date of manufacture; or
(b) such that the cheese or cheese product has a moisture content of less than 36%, after being stored at a temperature of no less than 10°C for a period of no less than 6 months from the date of manufacture; or
(c) in accordance with clause 3 of Standard 2.5.4.

Editorial note: For New Zealand, the processing of cheese and cheese products, other than those manufactured in accordance with clause 3 of Standard 2.5.4, is regulated under the *Dairy Industry Act 1952* and the *Food Act 1981*.

ATTACHMENT 2

SCIENTIFIC EVALUATION OF THE SAFETY OF VERY HARD CHEESES PRODUCED FROM RAW MILK

A report prepared for Food Standards Australia New Zealand
by
Food Science Australia

August 2002

Report prepared by:
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Summary
This report is a scientific evaluation of the safety of very hard cheeses produced from raw or heat-treated milk. Very hard cheeses considered in this report include Parmigiano Reggiano and Grana Padano, Romano, Asiago and Montasio. All of these cheeses are characterised by a low moisture content ie <35%, relatively high curd-cooking temperatures (>42ºC) and long storage/maturation times (3 to 24 months). The performance of the production process was evaluated for common foodborne pathogens eg *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, pathogenic *Escherichia coli*, and *Salmonella*.

The major source of contamination of cheese with foodborne pathogens is via the raw milk. Properly controlled processes used in the manufacture of cheese are able to inactive these bacterial pathogens. Thermal treatment of milk eg pasteurisation and thermisation, are established means for the reduction of these pathogens in raw milk. The reduction of bacterial numbers achieved with thermal processing varies depending on the combination of temperature and duration of heating employed. The fermentation and maturation processes used will also aid in the reduction of pathogen numbers depending on the level of pH and water activity, a_W.

C. jejuni/coli maybe present in the raw milk used for cheese making. *Campylobacter* spp. are unlikely to grow in the raw milk and will be eliminated by thermisation (65-68 ^{0}C) or pasteurisation (73-75 ^{0}C). During the manufacture of raw milk cheese heating the curd to > 55^{0}C for more than 5 min will result in a 5-log reduction in *Campylobacter* numbers. At low curd cooking temperatures ie < 48 ^{0}C, a 64 min cooking time would be required before a 5-log reduction in thermophilic *Campylobacter* spp. would be reached. However, after brining, ripening and storage, the conditions in the cheese are expected to be lethal to *C. jejuni*. It is concluded that *C. jejuni/coli* are unlikely to be a hazard in very hard cheeses.

Pathogenic *E. coli* may be present in raw milk and can grow in the milk if the temperature during storage and transport is greater than 7^{0}C. In some of the cheese processes milk is held at temperatures above 7^{0}C for a short time to aid in processing, at the temperatures used it is unlikely that significant growth will occur. *E. coli* are sensitive to heat and readily inactivated by pasteurisation and thermisation. Cooking of the curd will impact on any *E. coli* that have survived the processing to that stage, although at low cooking temperatures little inactivation will occur. When the curd is cooked at high temperatures eg 55^{0}C, reductions in *E. coli* numbers of between 2.5 and 6-logs would be expected. After cooking it is possible there is an increase (up to 2-logs) in numbers due to growth and the concentration of cells in the curd (syneresis). During ripening and storage the numbers of *E. coli* present in the cheese will decrease.

Salmonella has been isolated frequently from raw milk although the level of contamination should be very low. The behaviour of *Salmonella* during cheese making will be similar to that observed for pathogenic *E. coli*, although some heat resistant strains exist (ie *Salmonella* Senftenberg). Pasteurisation or thermisation of the raw milk will destroy most salmonellae. *Salmonella* will not survive high curd cooking temperatures although some increase can occur at low temperatures, possibly as a result of syneresis. The relatively high maturation temperatures and longer

storage times used in the manufacture of very hard cheeses will eliminate *Salmonella* ie >5-log reduction.

Coagulase positive *S. aureus* are the cause of foodborne intoxications. Milk usually becomes infected via the animal host or food handlers during processing. High numbers of staphylococci are required for the production of heat stable enterotoxins (10^6 to 10^7 CFU/mL). Raw milk that is not cooled rapidly or stored correctly will support growth and possible toxin production. Toxin production appears more likely associated with growth in the milk prior to cheese making rather than as a result of temperatures during curd formation. Pasteurisation and thermisation (65-68^{0}C for 63s) will inactivate *S. aureus*. However, staphylococcal enterotoxins are thermally stable and if toxin is present in the raw milk active toxin will remain in the cheese after processing. Cooking the curd at 55-56ºC will produce considerable reductions of *S. aureus*. There is little effect of cooking at lower temperatures (42-48^{0}C) on the numbers of *S. aureus*. Increases of between 1 to 2-logs can occur after cooking at low temperatures and a rapid pH fall is essential for controlling growth. *S. aureus* is inactivated in very hard cheeses during maturation and storage. The presence of toxin will be determined mainly by the number of organisms present in the milk used for cheese making.

L. monocytogenes is a zoonosis and is ubiquitous in the environment. The bacterium may be present in raw milk and is inactivated in pasteurised milk. Numbers of *L. monocytogenes* decrease during curd cooking at high temperatures (ie 55 ^{0}C). Cooking at lower temperatures will have little effect on the numbers of *Listeria*. Survival of *Listeria* during the maturation is highly variable between products and within the same product. *Listeria* has been shown to survive and even grow on the outside surface of cheese during maturation. Survival is dependent on the pH of the cheese and at levels of ~pH 5.5 no growth should occur. *Listeria* does not appear to survive in very hard cheese during maturation and storage.

According to the criteria specified by FSANZ, the following conclusions were made:

1. All very hard cheeses made, according to the process criteria provided, will achieve a 5-log reduction in the numbers of the pathogens specified eg *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, pathogenic *Escherichia coli*, and *Salmonella*. Note is made of the significance of staphylococcal enterotoxin production in raw milk and during manufacture.

2. The production process resulting in the greatest control/destruction of pathogens include the curd cooking together with the long maturation.

3. The presence of staphylococcal enterotoxin will be dependent on the initial quality/pathogen load of the raw milk, which needs to be controlled for the production process to achieve a safe product. This is the case whether using raw milk or pasteurised/thermised milk.

Terms of reference and definitions

This report is a scientific evaluation of the safety of very hard cheeses produced from raw milk prepared by Food Science Australia for Food Standards Australia New Zealand (FSANZ) according to the Schedule Item A, Agreement Services, of Food Science Australia Contract S*cientific evaluation of the safety of very hard cheeses produced from raw milk.*

The Agreement Services are to make a scientific assessment of whether very hard cheeses produced with raw milk are a safe product. This involves the following:

a. determining whether the production process provides a safe product, as demonstrated by the absence of pathogens in the final product, by achieving at least a 5 log reduction of each pathogen;
b. determining where in the production process the greatest control/destruction of pathogens is achieved (e.g. during curd cooking; during maturation/storage);
c. determining whether the production process can achieve a safe product irrespective of the initial raw milk quality/pathogen load (i.e. in the absence of any controls over raw milk quality, could a safe product still be made).

For the purposes of the Agreement between FSANZ and Food Science Australia and thus this report, the following definitions and criteria apply:

i. Raw milk is milk that has not been treated by pasteurisation or thermisation.
ii. The pathogens are *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, pathogenic *Escherichia coli*, and *Salmonella*.
iii. Very hard cheeses are those characterised by a low moisture content (<35% moisture), the use of relatively high curd-cooking temperatures (>45ºC) and long storage/maturation times (8 – 24 months). Theses cheeses include parmesan style cheeses such as Parmigiano Reggiano and Grana Padano, Romano, Asiago, Montasio.

Information was supplied to Food Science Australia by FSANZ as follows. These documents are attached as an Annex to this report.

1. "Declaration of manufacture" from Zanetti sent to AQIS, Canberra for the following cheeses:

   a. Parmigiano reggiano cheese (Zanetti Brand)
   b. Grano padano cheese (Zanetti Brand)
   c. Asiago cheese (Zanetti Brand)
   d. Montasio cheese Zanetti (Tre Canpane Brand, Three Bells Brand)

2. Documents from Istituto Sperimentale Lattiero – Caseario for parmiagiano reggiano, grana padano and pecorino romano cheeses including:
   a. statement and flow diagram for manufacture from Ministry of Agriculture,
   b. chemical composition
   c. Council Directive 92/46/EEC (Annex C) Chapter II. Microbiological criteria for milk-based products and drinking milk.

There are a variety of cheeses that fall within the group “very hard cheeses”. As information has been provided for those listed in (1) above, these are the only cheeses included in this evaluation. The information provided in the above documents have been summarised in Table 5 and Table 6. It should be noted that this evaluation was prepared using the process criteria provided in the above documents.

The format of this report is a summary of the evaluation of each pathogen under the various processing conditions outlined in the above documentation provided for the manufacture of hard cheeses. As there are both similarities and differences between the cheeses, this was considered the most efficient approach.

Introduction

The major source of contamination of cheese with foodborne pathogens is via the ingredients, from environmental sources during processing and from milkers and food handlers. Milk, both ovine and bovine, is the major ingredient in these cheeses. Raw milk may be contaminated with a variety of pathogens including all of those to be addressed in this evaluation eg *C. jejuni/coli, S. aureus, L. monocytogenes, Salmonella spp.* and pathogenic *E. coli* (Johnson et al., 1990). Other zoonotic pathogens may also be transmitted by raw milk eg. *Mycobacterium tuberculosis* and *M. paratuberculosis*, *Brucella* spp. and *Coxiella burnetii*; however, these do not fall within the terms of reference of this report. These bacteria may be both carried by healthy cattle and sheep and may cause disease in these animals. The extent of contamination of raw milk with any of these pathogens may be limited but not prevented by following good agricultural and sanitary practices on farm eg maintaining herd health, safe animal fodder, hygiene during milk collection, hygienic handling, storage, transport and delivery of milk. Growth of any of these contaminants can be limited by control of temperature during storage with rapid chilling to 4^0C or less during storage and transport. High milk quality is essential in cheese making to ensure no inhibitory substances or microorganisms are present that will interfere with the fermentation process and that pathogens and their enterotoxins are not present.

Salmonella spp. and *E. coli* have been found to contaminate dairy factory environments. In addition, *L. monocytogenes* is an environmental bacterium as well as a zoonosis and factory surfaces, equipment and the general environment are a common source of *L. monocytogenes* contamination. Food handlers may also carry or suffer illness from these pathogens and are a source of contamination if poor personal hygiene is practiced. Good hygiene and manufacturing practices and standard operating procedures that are pre-requisites for HACCP programs, if effectively employed, will limit this means of contamination.

Properly controlled processes used in the manufacture of cheese are able to inactive these bacterial pathogens although not all bacterial enterotoxins. Thermal treatment of milk eg pasteurisation and thermisation, are established means for the reduction of these pathogens in raw milk. The reduction of bacterial numbers achieved with thermal processing varies depending on the combination of temperature and duration of heating employed. Thermal processing eg cooking of curd will also inactivate vegetative cells. The fermentation and maturation processes used in cheese manufacture may further influence the populations of these pathogens and this depends largely on the level of pH and water activity, a_w.

The maximum concentration of the pathogen in the food that is considered tolerable for human protection, or the food safety objective, determines the performance required of the production process for that food (ICMSF, 2002). While the relationship between the concentration of a pathogen and the human response is generally recognised as being continuous, the probability of infection increases as the dose or concentration increases. For some food-poisoning bacteria (eg *Salmonella* and enterohaemorrhagic *E. coli*) the dose can be low, perhaps as low a one or two viable cells, particularly in foods with a high fat content such as cheese. For these bacteria it is important not only to limit contamination of the primary ingredient, raw milk, but also, for the cheese processing to perform so that the destruction or a theoretical D-value (decimal or log-cycle reduction) is sufficient to ensure a final product containing not more than the maximum tolerable level. The extent of this performance criterion necessary to provide sufficient consumer protection varies with the microorganism and the type of food.

In this evaluation the product is considered safe if the production process achieves at least a 5-log reduction in the pathogen eg the performance criterion is a 5D reduction. Therefore combinations of heating, pH and a_w through the cheese making, early ripening and late ripening must be able to reduce contaminating pathogens by 5 log-cycles. Allowing for a concentration of pathogens during syneresis (separation of the curd from the whey) a total reduction may have to be as high as 7 log-cycles from raw milk to final product. The pH and temperature limits for growth of the pathogens of concern, beyond which the bacteria can survive but not multiply are given in Table 1. Minimum a_W values are also listed in Table 1. Typical a_w value for hard cheeses such as parmesan is 0.917, which would allow growth of some of the pathogens considered here.

Table 1: Temperature and pH for growth of selected foodborne pathogens.

Organism	Temp. Range[1]	pH Range[1]	Minimum a_w values [2]
C. jejuni	25 – 43	5.5 – 8.0	0.987[3]
C. coli	30.5-45[4]		
L. monocytogenes	1 – 44	4.4 – 9.0	0.92
S. aureus	7 – 48	4.0 – 9.8	0.86
Salmonella spp.	7 – 47	4.7 – (9.0 - 11.0)	0.95
E. coli	7- 46	4.6 – 9.6	0.932

[1]Johnson *et al.*, 1990. [2] Spahr and Url, 1994. [3]ICMSF, 1996a-e [4] Benjaman et al, 1983

Foodborne pathogens in hard cheeses

Campylobacter jejuni/coli

C. jejuni and *C. coli* are the most common *Campylobacter* spp. causing human diarrhoeal disease. The clinical disease of both is indistinguishable and most laboratories do not differentiate the species so that the ratio of illness due to each species is not clear. In the USA it is estimated that 1-3% human cases are due to *C. coli* (Oberhelman and Taylor, 2000) and in a study in Denmark 6% of campylobacteriosis cases over 12 months were caused by *C. coli* (Nielsen et al, 1997). Due to the predominance in human infection of *C. jejuni* most information in foods relates to this species.

Birds and animals are the main reservoir of *C. jejuni/coli* and they are found in the intestinal tract of a wide range of healthy domesticated animals. *C. jejuni* is found in cattle and sheep, while *C. coli* is more often found in pigs and birds and less likely to be a contaminant of sheep or cow's milk than *C. jejuni*. In a nationwide study of faeces of cattle at slaughterhouses in Denmark in 1995-6, thermophilic campylobacters were isolated from 47% of 94 cattle and 46% of 316 swine (Nielsen et al, 1997). 90.9% of the thermophilic campylobacters from cattle were *C. jejuni* and 94.5% form pigs were *C. coli.* The organisms are found in the faeces of the animals and in cattle it can cause low-grade or subclinical mastitis although infrequently. Milk may be contaminated from faecal material or *Campylobacter* may be shed in the milk itself. Campylobacters have been isolated from 1-6% raw milk samples (Wallace, 1997). Raw or inadequately pasteurised milk is the most frequently identified vehicle of foodborne human infection with *C jejuni* (ICMSF, 1996a). The authors are unaware of campylobacteriosis having been caused by consumption of contaminated cheese.

C. jejuni/coli that are present in the raw milks used for the production of these very hard cheeses will not grow unless the temperature during storage and transport are at greater than 30^{0}C and the microaerophilic conditions of reduced oxygen and increased CO_2 are provided (Park, 2002). The temperatures at which the raw milks are held before processing are provided for two cheeses. The sheep's milk used in Pecorino romano cheese and cow's milk used in Grana padano cheese is held at 4^{0}C and 16-17^{0}C, respectively, and growth will not occur at these temperatures. Even for the other cheeses, the microaerophilic conditions will not prevail and growth is unlikely. The bacteria however will survive these storage temperatures.

These campylobacters are thermophilic in their growth; however, they are sensitive to heat and readily inactivated by pasteurisation and cooking temperatures for food (Park, 2002). *C. jejuni* has a D-value of 0.7-1.0 min at 55^{0}C in skim milk (Doyle and Roman, 1981). Thermisation at 65-68 ^{0}C for sufficient time or pasteurisation (73-75 ^{0}C for 15-30 sec) will result in more than a 5-log reduction in thermophilic *Campylobacter* spp. (D'Aoust et al, 1988). These thermal processes if controlled through effective HACCP programs will inactivate campylobacters present in the raw milks.

For each of the cheeses considered, raw milk is acidified and clotted by the addition of a starter culture and rennet. The starter for all except Pecorino romano is aspirated from the whey fermentation of the previous day and is composed mainly of thermophilic lactobacilli; the starter for Pecorino romano is a thermophilic lactobacilli and streptococci culture. The temperature for all is brought up to 33-40^{0}C for between 10-30 min. While these temperatures may be suitable for growth of thermophilic *Campylobacter* spp. other strict conditions required for growth are not met such as elevated CO_2 (10%) and reduced oxygen (5-6%; ICMSF, 1996a).

FSANZ has defined “very hard cheeses” for this evaluation as those that have the curd cooked at 45-55^{0}C. When pasteurised milk is used in the manufacture of cheeses (ie Asiago and Montasio, a lower curd cooking temperature is used, 42-46^{0}C for 20-30 min and 48^{0}C for 30-40 min respectively. The curd made from thermised sheep milk (Pecorino romano), is cooked at 45-48^{0}C for 10 min and is held at this temperature for up to 30 min while the curd is pressed under the whey. Raw milk used for production of Parmigiano reggiano and Grana pandano is not thermally treated and the curd is cooked at a higher temperature eg 55-56^{0}C for 15-20 min.
The curd is held for longer than this time as it is allowed to rest in contact with the whey in the cooking vat before pressing and hooping so that the time at 55-56^{0}C is a minimum of 40 min. In skim milk *C. jejuni* has a D-value of 7.2-12.8 min at 48^{0}C, 0.7 – 1 min at 55ºC (Doyle and Roman, 1981) and at 40ºC with optimum gas atmosphere death occurred (ICMSF, 1996a). *C. jejuni* would not survive the curd cooking temperatures used in the manufacture of these hard cheeses. At low curd cooking temperatures ie < 48 ^{0}C, a 64 min cooking time would be required before a 5-log reduction in thermophilic *Campylobacter* spp. would be reached.

Campylobacter survive poorly in mildly acidic environments and in the presence of 2% or more salt and fails to grow at water activities of less than 0.987 (ICMSF, 1996a). The salt concentration of these cheeses is 1.4g/100g (Parmigiano reggiano), 1.5g/100g (Grana padano), and 5g/100g (Pecorino romano). The final pHs of cheeses considered in this report are generally less than 5.5 (Grano padano, 5.4-4.6, Asiago, 5.35 and Montasio, 5.4). After brining, ripening and storage, the conditions in the cheese are expected to be lethal to *C. jejuni*.

It is concluded that *C. jejuni/coli* are unlikely to be a hazard in these very hard cheeses as thermal processing applied during pasteurisation, thermisation and/or curd cooking will inactivate bacteria present in the raw milks and the conditions in the cheeses after fermentation and ripening will further inactivate survivors. This assumes that these processes are carried out under control programs such as HACCP and its pre-requisites.

Pathogenic *Escherichia coli*

Pathogenic *E. coli* associated with foodborne disease are grouped into specific pathotypes based mainly on their virulence characteristics, mechanisms of pathogenicity and clinical syndromes: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), diffuse-adhering (DAEC), enteroaggregative (EAEC) and enterohaemorrhagic (EHEC) (Desmarchelier and Grau, 1997). The epidemiology is not clear for all of these pathotypes. Human carriers are believed to be a principal reservoir and source of EPEC, EIEC and ETEC strains involved in human illness. The intestinal tract of ruminants including cattle and sheep is an important reservoir of EHEC.

Of the pathotypes of *E. coli*, the EHEC have become the most important foodborne type, in particular those belonging to the serotype O157. This is because the incidence of EHEC foodborne disease has increased significantly in recent years and because

EHEC infection can have serious clinical outcomes such as haemolytic uraemic syndrome (HUS) and deaths occur (for a summary see Meng et al, 2001). There is insufficient data of each pathotypes' behaviour in foods and data for non-pathogenic strains is used unless a pathotype is known to behave differently. EHEC in particular are distinguished from the other *E. coli* pathotypes, as some EHEC strains are able to tolerate mildly acidic conditions in foods. The dose of EHEC required to cause human illness is also considered to be very low with a few cells required in high-risk individuals.

Pathogenic *E. coli* have been the cause of foodborne illness where cheeses have been implicated as the source of infection. These have included EIEC isolated from Brie and Camembert, ETEC associated with consumption of Brie, EHEC implicated directly or indirectly with consumption of a variety of cheeses including semi-soft cheese, cheese curds, goat cheese, Lancashire cheese (a semi-hard cheese), (MacDonald, 1995; Deschenes et al., 1996; Desenclos et al., 1996; Desmarchelier and Grau, 1997). The source of the pathogens may have been the raw milk used in the cheese manufacture (EHEC), food handlers (EIEC, ETEC, EHEC) or water used in food manufacture (EIEC).

Shiga toxin-producing *E. coli* (STEC) of which EHEC is a sub-group are found in the faeces of healthy cattle and sheep (Reviewed in Desmarchelier and Grau, 1997). Milk can become contaminated at collection or from the milking parlour environment and O157 EHEC have been isolated from raw cow's milk on farm and from bulk raw milk tankers (summarised in Meng et al, 2001; Desmarchelier and Grau, 1997). The number of bacteria present in raw milk is expected to be very low, particularly with the co-mingling of milk in bulk containers. EHEC infection has been reported following the consumption of raw cow's milk or milk contaminated post-pasteurisation (summarised in Meng et al, 2001; Desmarchelier and Grau, 1997).

E. coli grow over a temperature range of 7^0C to 46^0C and the optimum is $35\text{-}40^0$C (ICMSF, 1996b). Any contaminants present in the raw milks will grow if the temperature during storage and transport are within this range. The extent of growth depends on the temperature and duration of storage. The raw cow's milk used in Grana padano cheese is separated at $16\text{-}17^0$C for 6-8 h and a small amount of growth may occur although it is likely that the bacteria remain in the lag phase during this time. The storage temperature of sheep's milk for Pecorino romano is 4^0C and this will inhibit growth, although *E. coli* will survive. There are no temperature details provided for Montasio and Asiago cheeses or Parmigiano reggiano for this evaluation although this milk is stored for 10-12 h. Growth may occur in the latter milk depending on the temperature. It is assumed that no more than 1-log of growth is possible during the transportation and storage of milk prior to cheese making.

E. coli are sensitive to heat and readily inactivated by pasteurisation of milk (ICMSF, 1998). During thermisation at 64-68°C, significant destruction in *E. coli* will occur if the milk is heated for approximately 1 min (D-value at 64°C = 3-9.6 sec). Thermisation at 63-65°C for 15-20 sec (IDF definition) is likely to give a 2 to 5-log reduction (D'Aoust et al, 1988; Morgan et al, 1988). Pasteurisation at $73\text{-}75^0$C for 15-30 sec will eliminate pathogenic *E. coli*.

The optimum growth temperature for *E. coli* is in the range 35-40ºC. Milk used for the manufacture of these hard cheeses is heated to 33-40^{0}C for curd formation. *E. coli* has been shown to multiply in milk with starter culture added (ICMSF, 1996b). At a pH of 5.1-6.6 at temperatures of 24-36ºC from 1-2.5-log growth occurred over 5-7 h. At 32.8-40^{0}C and pH of 6.1-6.6, 3-log growth occurred over 3.5 h. While the temperature and the pH of the milk may allow growth, the durations of holding at these conditions would appear not likely to result in significant growth although *E. coli* will survive.

Cooking of the curd will impact on any *E. coli* that have survived the processing at this stage or been introduced into the product. The temperature limit for growth of *E. coli* is 46^{0}C with some strains of O157 EHEC unable to grow above 42.5^{0}C in selective media (Desmarchelier and Grau, 1997). Pasteurised milk cheeses (ie Asiago and Montasio) have relatively low curd cooking temperatures of 42-46^{0}C for 20-30 min and 48^{0}C for 30-40 min respectively. Although growth could occur, the time is short and as these milks are pasteurised there should be no survivors at this step if the pasteurisation process is adequately controlled.

The curd made from thermised sheep milk (ie Pecorino romano) is cooked at 45-48^{0}C for 10 min and is held at this temperature up to 30 min while the curd is pressed under the whey. Growth will be inhibited at this temperature. The D_{50} value for buffalo milk is 31min (ICMSF, 1996b). Thermisation of the milk will have resulted in a 2- to 5-log reduction of *E. coli*. Low temperature cooking of the curd will provide very limited further inactivation.

Raw milk used for production of some hard cheeses (ie Parmigiano reggiano and Grana pandano) is not thermally treated; however, the curd is generally cooked at high temperatures (eg 55-56^{0}C for 15-20 min) plus resting in the whey for a further 45-65 min. giving a minimum of 40 min. at this temperature. Curd heating of Swiss Emmentaler cheese is carried out at 53ºC for 45 min, including time under whey. Bachmann and Spahr (1995) indicate that a 3.5-log reduction in *E. coli* can be achieved in that process. In skim milk *E. coli* has a D_{55} value of 5.5 min and in whole milk a D_{55} value of 6.6 min (ICMSF, 1996b). Using these values *E. coli* numbers would be reduced by approximately 6-logs during this processing step. When curd used for the manufacture of cottage cheese was heated to 56 ^{0}C, *E. coli* O157:H7 numbers were reduced by ~2.5-logs (Hudson et al, 1997). No reduction was noted for the same strain at the lower cooking temperature (46 ^{0}C) used for the manufacture of Romano cheese.

At this stage if processing and hygiene is properly controlled through the application of verified HACCP programs and good hygiene practices, then there should not be *E. coli* in the Asiago, Montasio, Parmigiano reggiano and Grano padano cheeses or Pecorino romano if the thermisation of raw milk is carried out at 64 ^{0}C for at least 60 sec.

During the first 24 h of cheese manufacture any *E. coli* surviving in the milk will be concentrated in the curd during syneresis and if not destroyed during the curd cooking may also multiply during salting and ripening until the pH and a_w combinations reach the limits of no-growth. Table 2 lists the increase in *E. coli* numbers found in some cheese types during cheese making and early ripening. The pH of these cheeses at salting ranges from 4.95-5.2 and the NaCl concentration of the final cheese is 1.3-1.6g/100g with the exception of Pecorino romano that has a pH of 5.85 and a salt concentration of 5g/100g. Hudson et al, 1997, reported an increase of 1.7-logs during curd formation of Romano cheese, after 65 h in 22% brine (Table 5). For hard cheeses the amount of growth of *E. coli* during the initial stages of ripening is likely to be less than 2-logs.

Table 2: Increase in *E. coli* numbers during cheese making and early ripening.

Cheese	Strain	Log increase	Stage after:	Time from start (days)	pH (24h)	Reference
Colby	Non-O157	0.2-4.0	Press	1	4.91-5.34	Kornacki & Marth, 1982
Colby	O157	1.3	Salt	<1		Hudson et al, 1997
Cheddar	3 xO157	0.7-1.4	Press	1		Reitsma and Henning, 1996
Romano	O157	1.7	Brine	4		Hudson et al, 1997
Brick	Non-O157	2.2-2.5	Brine	2		Frank et al, 1978
Tilsiter	NCTC 9001	ca 1.5	Brine	3		Bachmann and Spahr, 1995

Romano - after 65h in 22% brine; Brick - after 24h in 22% brine; Tilsiter - after 24 h in 20% brine and 1 day ripening at 11-13°C.

During ripening and storage the numbers of *E. coli* present in the cheese will decrease. The rate of decrease will be primarily dependent on the storage temperature although further pH and a_W will contribute to the rate of inactivation (Table 3). The results of some studies are presented in Table 5 and it can be seen that the results are very variable.

Table 3: Decrease in E. coli numbers during late ripening and maturation of cheese.

Cheese	*E. coli* Strain	% Salt in Water	pH	Log decrease	Ageing Conditions	Reference
Colby	ETEC-a	3.7-4.9	4.9-5.3	>3	6.5 wk 10°C	Kornacki & Marth, 1982
Colby	ETEC-b	3.9-4.0	5-5.6	1.5-4	11 wk 10°C	Kornacki & Marth, 1982
Colby	EIEC-a	5.4-5.9	5.3-5.5	>5	3.5 wk 10°C	Kornacki & Marth, 1982
Colby	O157 EHEC		4.6	4	4 wk 13°C	Hudson et al, 1997
Cheddar	3 strains O157	3.15	5-5.2	2.8-5.8	22.5 wk 6-7° C	Reitsma & Henning, 1996
Cheddar	3 strains O157	3.34	5-5.2	ca 2.1	18.5 wk 6-7°C	Reitsma & Henning, 1996
Brick	ETEC-b		5.1-5.3	0.64 – 2.4	2 wk,15.5°C + 5 wk,7°C	Frank et al, 1978
Tilsiter	NCTC 9001	3.13	5.2-5.4	6.5	30 d 11-13°C	Bachmann and Spahr, 1995
Romano	O157 EHEC	-	5.2-5.7	>4.5	2 d,10°C + 30 d,13°C	Hudson et al, 1997

Time is from start of ageing-maturation except for Colby cheese (R Hudson et al 1997) where time and extent of decrease is from salting; Romano - after 65 h in 22% brine; Brick - after 24 h in 22% brine; Tilsiter - after 24 h in 20% brine and 1 day ripening at 11-13°C

The hard cheeses in this evaluation are matured for a minimum 3 months and up to 24 months and at temperatures of 15-22^{0}C. While there is considerable variability in survival, reductions of greater than 4.5-logs have been noted for romano matured for 30 d at 11 to 13 ^{0}C (Table 3). The higher cooking and setting temperatures (55-56ºC for 40 min) used in the production of Parmigiano Reggiano and Grana Padano will result in a greater reduction of *E. coli*, certainly more than the 5-logs required. Cheeses produced using lower cooking temperatures ie Romano type cheeses (45-48ºC), Asiago (42-46ºC) and Montasio (48^{0}C), will not produce a significant reduction in *E. coli* during cooking. These cheeses however, are usually produced with thermised or pasteurised milk and are ripened for 3 or more months. Maturation of Romano cheese at 13 ^{0}C resulted in an overall reduction in *E. coli* numbers of more than 3-logs (Hudson et al, 1997) after 21 d. While the effect of maturation for longer periods has not been reported for *E. coli*, it would appear that a 5-log reduction is possible.

Hard cheeses manufactured with a curd cooking temperature of >55 ^{0}C (30 min) and matured for a minimum of 3 months will achieve the required performance criteria of greater than a 5D reduction in *E. coli*. Cheeses receiving a lower cooking temperature should where possible be manufactured from thermised or pasteurised milk. Raw milk cheeses using lower cooking temperatures should be matured for a minimum of 6 months unless supporting data on the inactivation of *E. coli* are provided.

Salmonella

Salmonellae reside in the intestinal tract of warm and cold blooded animals. In cattle and sheep the bacterium are carried by both healthy and diseased animals and are transmitted in the faeces and hence can contaminate raw milk. Food handlers may also excrete the organisms during infection and convalescence and a small percentage become carriers. *Salmonella* has been isolated frequently from raw milk (Johnson, 1990). In the US, 4.7% milk in 678 tankers were positive. In a study of raw milk in bulk tanks in the UK in 1995, 0.36% of the tanks sampled were contaminated (O'Donnell, 1995). Both milk and milk products such as cheddar cheeses and Vacherin cheese, have been implicated in outbreaks of salmonellosis (Johnson, 1990). The source of contamination is primarily the raw milk contaminated via the udder and teats and maybe via systemic infection and milkers. Milk can also be contaminated post-pasteurisation. Product may be further contaminated via the factory environment and food handlers during processing.

Using human volunteers for infectious dose studies it has been found that 10^7 Salmonellae were required to have a significant likelihood of causing disease (ICMSF, 1996c). Outbreaks involving water, which has a minimal retention time in the stomach, and fatty or buffered foods, which protect organisms from the action of stomach acids, have been shown to result from ingestion of far fewer numbers of salmonellae (ICMSF, 1996c). Cheese implicated in Salmonellosis outbreaks has been found to contain low numbers, 0.36-9.3 cells/100 grams (D'Aoust et al, 1985) and 0.36-4.3 cells/100 grams (Hedberg et al, 1992).

The level of contamination of co-mingled raw milk would be very low. The temperature range for growth is 7-47^{0}C and the rate of growth at the extremes eg 7^{0}C is substantially reduced (ICMSF 1996c). Growth should not occur is properly chilled and stored raw milk. Holding the milk at 16-17^{0}C for 6-8h some limited growth may occur. If these milks are stored at temperature above 10^{0}C the time has to be carefully controlled.

Thermal treatment can be used to inactivate any salmonellae that may be present in raw milk. Some species of *Salmonella* (ie *Salmonella* Senftenberg) are unusually heat resistant although these species are rare. The D-value for most salmonellae in milk is 3.6 to 5.7 sec at 62.8°C. The D-value for *S. Senftenberg* is 34sec at 65.6^{0}C (ICMSF, 1996c). For pasteurised milk cheese eg Asiago and Montasio, salmonellae will be inactivated during the heat treatment of the raw milk. The thermisation of the milk eg in Pecorino romano cheese, at 65-68°C will also destroy most salmonellae if held for more than 10sec.

While concentration may occur during syneresis, decreases will occur at high curd cooking temperatures. The D-value for *S.* Typhimurium at 51.4 and 55.2ºC in laboratory media containing 10% milk solids is 49.0 min and 4.7 min, respectively (ICMSF, 1996c). A >5-log reduction will be achieved in cheeses receiving a cooking step of >55 ^{0}C for a minimum of 30 min (ie Parmigiano reggiano and Grana pandano). At lower curd cooking temperatures as found in some Romano cheeses Asiago and Montasio cheeses (45-48ºC), little if any inactivation may occur. Bachmann and Spahr (1995), demonstrated a reduction of ~2-logs in *Salmonella* after cooking at 53 ^{0}C for 45 min and an increase of ~1-log after cooking at 42 ^{0}C for 15 min. As the cheeses are ripened and matured the numbers of viable Salmonellae will decline (Table 4).

Table 4: Decrease in Salmonellae during Ageing and Maturation of Cheese

Cheese	% Salt	pH	Log decrease	Ageing Conditions	Reference
Cheddar		5.4-5.65	2.5	26 weeks at 4.5°C	Hargrove et al, 1969
		5.2-5.3	5.3	26 wk at 4.5°C	
		5-5.05	5	13 wk at 4.5°C	
		5.2-5.4	4	13 wk at 10°C	
Cheddar			4	14-16 wk at 7.5°C	Goepfert et al, 1968
			4	10-12 wk at 13°C	
Cheddar	2.1-2.3	5.2	4.8-5.2	20 wk at 7°C	Mehta and Tatini, 1994
Samsoe		5.2	4	5-6 wk at 16-20°C + ca 3 wk at 10-12°C	Goepfert et al, 1968
Montasio		5.4-5.6	ca 4.5	12-13 wk at 12°C	Stecchini et al, 1991
Manchego	2.5-3	4.9-5.0	ca 7	8 wk at 10°C	Medina et al, 1982
Manchego	2.5-3	4.9-5.0	4.6-ca 6.5	6 wk at 10°C	
Tilsiter	1.23	5.2-5.4	6.3	4 wk at 11-13°C	Bachmann and Spahr, 1995.

Time and extent of decrease in salmonellae in Cheddar is from 1 day after production; in the semi-hard cheeses, Montasio from day 3 (after brine-salting) and Tilsiter from day 3 (after brine-salting and 1 day ripening); and for Manchego, from day 2 (after brine-salting). Cheddar used by Mehta and Tatini, 1994 had an a_W of 0.95-0.97. Internal salt content of Manchego after 60 d, and for Tilsiter after 90 d.

High maturation temperatures and longer storage times will result in greater reductions in viable numbers of *Salmonella*. Maturation of semi-hard cheese at ~12 ^{0}C for 30 d (pH 5.2-5.8, 39% moisture, 1.2% w/w salt) results in a ~5-log reduction (Bachmann and Spahr, 1995). The rate of inactivation appears to be linear implying that storage for 60d would result in a ~7-log reduction, in the same experiment *Salmonella* numbers in Swiss hard cheese decreased by >6-logs after cooking.
The hard cheeses considered in this report have similar pH and moisture levels to the Swiss hard cheeses discussed above. Given this, maturing at >15 ^{0}C for more than 3 months will result in reductions of greater than 5-logs.

Cheeses produced with curd cooking temperatures above 55ºC are unlikely to contain viable Salmonellae at the end of maturation. Cheeses produced with curd cooking temperature below 51ºC may still be contaminated with low levels of Salmonellae. Such cheeses should be manufactured from pasteurised or thermised milk or stored for more then 3 months at approximately 15 ^{0}C.

Staphylococcus aureus

Coagulase positive *S. aureus* are the cause of foodborne intoxications. Illness results from the consumption of food containing staphylococcal enterotoxins produced by the staphylococci during growth in food. *S. aureus* occurs in the mucous membranes and skin of most healthy warm-blooded animals, including man and food animals (ICMSF, 1996d). This organism may be shed into milk in subclinical cases of mastitis in food animals at levels up to 10^{5} CFU/mL. The bacterium is also a common cause of wound and skin infections in personnel including food handlers and farm workers. Milk usually becomes infected via the animal host or food handlers during processing. Outbreaks of staphylococcal intoxication have been attributed to dairy products including cheeses such as Swiss style cheeses (eg Emmenthal, Gruyere and Swiss), raw milk cheddar, Colby and cheese curds (Johnson et al, 1990). These outbreaks have resulted from poor process control, contamination from infected factory workers, contaminated starter cultures and use of contaminated water. Enterotoxin production can occur in the raw milk before processing or during cheese processing. Enterotoxins have been shown to persist in cheese for several years (IDF, 1980).

High numbers of staphylococci (10^{6} to 10^{7} CFU/mL) are required for the production of heat stable enterotoxins in foods. The conditions for growth of staphylococci are listed in Table 1. *S. aureus* can grow over a temperature range of 7-48^{0}C although enterotoxin production occurs over a more restricted range. Enterotoxin production occurs between 10-48^{0}C with optimum production occurring at 35-40^{0}C and at a pH of 6.0-7.0. Production is also influenced by the salt concentration (Ash, 1987). Raw milk that is not cooled rapidly or stored correctly will support growth and possible toxin production. At 10ºC there is a long lag time (>20h) and when growth commences it is very slow (ICMSF, 1996b). *S. aureus* will grow over a wider range of a_w values than other foodborne pathogens eg 0.83-0.99, however the rate of growth is significantly slowed at values less than 0.94 (Ash, 1997).

In milk, D-values for *S. aureus* at 65, 70 and 75^{0}C have been reported to be 12, 6 and 1.2 sec respectively (ICMSF, 1996d). Johnson et al (1990) summarised heat studies of milk used for cheese making and concluded that at 70^0C a holding time of 16-18 sec was required to inactive *S. aureus*. At 65^0C, 63 sec was required for inactivation. There is evidence that *S. aureus* may become injured and is not readily recovered after heat treatment (eg 63.9-65.6 ^{0}C for 16-21 sec), although recovery may occur during cheese making (Zottola et al, 1969). Based on these studies, the thermal treatment of milk at $73\text{-}75^0$C for 15-30 sec will inactivate *S. aureus* (ie Asiago and Montasio cheeses). Treatment of milk at lower temperatures ie at $65\text{-}68^0$C will inactivate the bacterium if held for at least 63 sec, such as for the manufacture of Pecorino romano. The staphylococcal enterotoxins are thermally stable and if toxin is present in the raw milk active toxin will remain after these thermal processes (ICMSF, 1996d). For the raw milk cheeses, staphylococci could be present and management of herd health and control of hygiene in milk production are essential.

The temperatures of the milks during the initial production of these cheeses prior to curd formation are between $33\text{-}40^0$C and could allow the growth of *S. aureus*; however, the duration is not more than 30 min and provided this is controlled the extent of growth would be minimal. Growth conditions are considered less favourable in the raw milk where the lactoperoxidase system and the natural flora present inhibit staphylococcal growth (Bachmann and Spahr, 1994).

Cooking the curd at 55-56ºC for 15-20 min (ie Parmigiano reggiano and Grana padano) will produce considerable destruction of *S. aureus* as in milk the D_{50} is 10 min and D_{55} is 3min (ICMSF, 1996d). A 2 to 3-log reduction was noted after cooking at 53 ^{0}C for 45 min (Bachmann and Spahr, 1995). At lower cooking temperatures, between 42 and 48^0C, there is little effect on the numbers of *S. aureus* (Table 1). Initial increases of between 1 to 2-logs occur during the manufacture of Swiss style cheeses (cooking temperature 51 ^{0}C), due partly to the concentration of cells in the curd (Todd et al, 1981). Similar increases have been reported for semi-hard Swiss cheeses (Bachmann and Spahr, 1995). Although staphylococci can multiply up to 48ºC the lag times at these extremes of growth and the falling pH that will be occurring are likely to prevent the numbers reaching that required for enterotoxin production. If the pH does not fall rapidly growth can occur. Todd et al (1981) noted the work of Tatini et al, showing that *S. aureus* numbers can reach counts of 10^7 CFU/g in 24 h when the pH dropped to between 5.5 and 5.6. Even at pH values lower than this numbers did not reach the inoculation levels within the first 24 h. Protocols for the manufacture of Italian DOP hard cheeses state that the pH should be <5.2 within 24 h for the manufacture of Parmigiano Reggiano, Grana Padano and Pecorino Romano. Rapid pH fall is essential for preventing significant growth of *S. aureus*.

Toxin production appears to be more closely associated with growth in the milk prior to cheese making rather than as a result of temperatures allowing growth during curd formation (Todd et al, 1981). Temperature control of the raw milk used in cheese making is essential to ensure that *S. aureus* numbers remain below 10^6 CFU/ml, even if the milk is heat treated prior to use. Pre-formed enterotoxins will survive the most stringent curd heating protocols such as in traditional Canestrato Pugliese which has a curd cooking step in hot whey of 80ºC for 30 s (Albenzio et al., 2001). Because some growth of *S. aureus* can occur during manufacture, numbers in the milk should be less than 10^3 CFU/ml at the start of manufacture.

Failure of starter cultures to rapidly lower the pH or temperature abuse of the raw milk would be required to allow *S. aureus* to reach the levels required for enterotoxin production. After fermentation counts may increase during salting. Todd et al (1981) noted published work showing *S. aureus* numbers increasing to ~10^8 after 2-weeks followed by a gradual decrease in numbers until a 2-log total reduction (4-log maximum reduction) was achieved at 15 weeks. Bachmann and Spahr (1995) failed to find *S. aureus* in Swiss hard cheese immediately after cooking the curd (5-log reduction), in semi-hard cheese a 5-log reduction was observed after maturation for >30 d.

S. aureus should be inactivated in hard cheeses manufactured with a curd cooking temperature of >55 ^{0}C (30 min) and matured for a minimum of 3 months. The presence of toxin appears be determined by the number of organisms present in the milk used for cheese making. Some growth (1 to 2-logs) of *S. aureus* would be expected in cheeses receiving a lower cooking temperature, therefore *S. aureus* numbers in milk used in the manufacture of these cheeses should be less than 10^3 CFU/ml. Storage of these cheeses for 3-months should ensure that they are free of viable *S. aureus*.

Listeria monocytogenes

L. monocytogenes is carried by milk producing animals and can cause disease in these hosts. It is also ubiquitous in the environment of food production and has been linked to numerous foodborne outbreaks including coleslaw, pate, frankfurters, jellied pork tongue and raw milk and cheese (ICMSF, 1996e). *Listeria* is frequently detected in raw milk and is able to grow in properly chilled milk. Because *Listeria* is commonly found in the processing environment it is a hazard for all cheese manufacture not just that utilising unpasteurised milk.

Thermal treatment of milk will inactivate *L. monocytogenes* in milk. D-values reported for this bacterium in milk at 63.3^{0}C is 33.3 sec and at 68.9^{0}C is 7.0 and 7.2 sec (Johnson et al, 1990). Thermisation for sufficient time and pasteurisation will inactivate any organisms present in the raw milk.

Listeria numbers decrease considerably during cooking. D values in whole milk heated to 52.2 ^{0}C range between 24 and 37 mins; $D_{57.8}$ is 4.4 to 5.2 mins (ICMSF, 1996e). During the production of parmesan cheese *Listeria* numbers increased during the initial heating stages before decreasing during cooking (51^{0}C/45 min) by approximately 0.45-logs (Yousef and Marth, 1990). Cooking at a temperature of 53ºC for 45 min (pH 5.2 to 5.4) resulted in a >4.6-log reduction in *Listeria* (Bachmann and Spahr, 1995, Spahr and Url, 1994), cooking at 56ºC for 25 min gave a similar reduction (Spahr and Url, 1994). Heating whole milk at 55 ^{0}C for 40 min should result in only a ~2-log decrease in *Listeria* (from D values in whole milk), this is lower than expected from the results in experiments carried out on curd.

Cooking the curd at lower temperatures eg 42-48^{0}C will have limited if any effect on the viability of *L. monocytogenes*. In Swiss semihard cheese made from experimentally inoculated milk, after cooking the curd at 42^{0}C for 15 min a slight increase in *L. monocytogenes* was observed, probably due to concentration during syneresis.

Survival of *Listeria* during the manufacture of cheese is highly variable between products and within the same product. *Listeria* has been shown to survive and even grow on the outside surface of cheese during maturation. Survival is dependent on the pH of the cheese and at levels of ~pH 5.5 no growth should occur on the outer surface of hard cheeses (Bachmann and Spahr, 1995). Genigeorgis et al. (1991) examined the survival of *Listeria* on the surface of a variety of cheeses; non-soft cheeses made with the use of starter cultures and at pH values of ≤5.5 did not support the growth of *L. monocytogenes* at temperatures ranging between 4 and 30ºC. Contamination of the outside surface of cheese with *Listeria* should be similar for cheeses manufactured from raw or heat-treated milk.

The combination of cooking at 51 ^{0}C and low pH and high storage temperature (pH 5.1 and 12.8 ^{0}C) used in the manufacture of Parmesan cheese reduced the most persistent strain of *Listeria* by >4.5-logs after 120 d (Yousef and Marth, 1990).

Cooking of the curd to temperatures of >55 ^{0}C followed by maturation at >15 ^{0}C for more than 3-months will result in reductions in *Listeria* of more than 5-logs. Cooking at lower temperatures may result in a slight increase in *Listeria* during the early stages of production followed by a rapid decrease in numbers during storage reaching >5-logs after approximately 6 months.

Conclusions

An evaluation has been undertaken of the processing of very hard cheeses, such as Parmigiano Reggiano and Grana Padano, Romano, Asiago, Montasio under the terms of reference required by FSANZ.

The cheeses evaluated in this report fall into three broad categories:

1. cheeses made from raw milk receiving a high curd cook ie 55-56^{0}C
2. cheeses made from thermised milk and receiving a mild curd cook ie 45-48^{0}C
3. and cheeses made from pasteurised milk that receive only a mild curd cook ie 42-46^{0}C.

During the early stages of production bacteria are concentrated in the curd and at low cooking temperatures there can be a 1 to 2-log increase in pathogen numbers (due to syneresis and possibly some growth). Cooking of the curd at temperatures above 55^{0}C results in a reduction in bacteria numbers. Although the effect of cooking at this temperature is variable it is likely that a greater than 5-log reduction will occur for most of the pathogens considered. Heat resistant strains of *Salmonella* (ie *Salmonella* Senftenberg) are the exception and would survive this process although numbers may be reduced by ~2-logs as a result of cooking. Heat resistant strains of *Salmonella* are rarely encountered. Inactivation of pathogens continues throughout ripening (providing the pH is 5.5 or less) and reductions of >5-logs after 3-months should be possible for all the hard cheeses considered irrespective of the curd cooking temperature.

The presence of *S. aureus* in raw milk is a risk factor. Information on quality assurance programs for the raw milk used in cheese making was not provided as part of this assessment and this should be sought. A microbiological criterion is set for drinking milk for *L. monocytogenes* and for *Salmonella* only (Council Directive 92/46/EEC (Annex C) Chapter II). It is not known if this applies to milk used for cheese production. Growth of *S. aureus* in the raw milk can result in enterotoxins being introduced into the cheese. The cheese making process will not inactivate enterotoxin and it can persist in the cheese for several years. *S. aureus* will not grow in hard cheeses receiving a cook of >55^{0}C, therefore enterotoxins can only originate from the milk. The quality of the milk used should be monitored to ensure that *S. aureus* levels are below 10^6 CFU/ml. For cheeses receiving a milder cook it is possible that *S. aureus* can increase in numbers by ~2-logs. Therefore milk used in the manufacture of these cheeses should have <10^3 CFU/ml of *S. aureus* at the start of manufacture.

Using good manufacturing practices it is possible to produce safe raw milk hard cheese of the type specified in this report. The use of pasteurised or thermised milk for the manufacture of cheeses that have received a mild cook adds additional safety, although these cheeses can still be produced safely provided the quality of the raw material is monitored and that the maturation time is sufficiently long to ensure pathogen inactivation (ie >6-months).

According to the criteria specified by FSANZ, the following conclusions were made:

1. All very hard cheeses made, according to the process criteria provided, will achieve a 5-log reduction in the numbers of the pathogens specified eg *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, pathogenic *Escherichia coli*, and *Salmonella.* Note is made of the significance of staphylococcal enterotoxin production in raw milk and during manufacture.

2. The production process resulting in the greatest control/destruction of pathogens include the curd cooking together with the long maturation.

3. The presence of staphylococcal enterotoxin will be dependent on the initial quality/pathogen load of the raw milk, which needs to be controlled for the production process to achieve a safe product. This is the case whether using raw milk or pasteurised/thermised milk.

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Table 5 Summary of processing steps provided for very hard cheeses

Processing step	Parmigiano reggiano	Grana padano	Pecorino romano	Asiago	Montasio
Raw milk handling	Cow's milk partially skimmed No temperature provided 10-12h Whole milk added next morning	Cow's milk partially skimmed 16-17^{0}C 6-8h pH 6.4-6.7	Sheep's milk filtered Stored 4^{0}C ≤ 24h	Cow's milk partially skimmed No temperature or time provided Whole milk added next morning	Whole cow's milk No temperature and time of storage provided
Thermal treatment of milk	nil	nil	65-68^{0}C no time provided	73-75^{0}C 15-30 sec	73-75^{0}C 15-30 sec
Acidification/ Whey starter added	Natural whey starter Mainly lactobacilli	Natural whey starter Mainly lactobacilli 33^{0}C pH 6.2-6.5	Thermophilic lactobacillus & streptococcus 38-40^{0}C 30min. pH <3.5	Natural whey starter 32-36^{0}C	Natural whey starter 35^{0}C pH 6.5-6.6 before pH 6.4 after addition
Coagulation/ rennet addition	33-34^{0}C 10-12 min	33^{0}C 10-15min		35^{0}C 20-30min	35^{0}C 20min
Curd cutting/ extraction	43-44^{0}C 3-4min				42-43^{0}C
Curd cooking	55-56^{0}C 15-20min	55-56^{0}C 20min	45-48^{0}C 10min	42-46^{0}C 20-30min	48^{0}C 30-40min max
Curd resting/ hooping/ pressing	Under whey 55-56^{0}C 45-65min (minimum 40min) Pressing 3d pH 5.0-5.3	Under whey 55-56^{0}C 35-45min (minimum 40min) 8 h in wooden mould to dry 2-3d in s/s mould @ 18-20^{0}C	Under whey 45-48^{0}C 30min (minimum time)	Out of whey No temperature provided 12h	Out of whey No temperature provided Pressing 3d
Salting	 25-27d	Brine 22-26%NaCl 15-18^{0}C 25-32d	Brine 23-24% 6-10d Dry salting x3-x4 over 50-70d	Brine 20-22% 15^{0}C 5d pH 5.85	Brine 20-22% 15^{0}C 5d pH 4.95
Drying	15-18^{0}C 3d				
Ripening	 18-24month	16-22^{0}C for 14-18month	5month min.	3-12month at 15-16^{0}C (short) or at 8-9^{0}C (long)	15-18 ^{0}C 3-12month

Table 6 Summary of chemical characteristics of final product.

Property	Parmigiano reggiano	Grana padano	Pecorino romano	Asiago	Montasio
pH		5.4-5.6		5.35	5.4
Moisture	30.76	32.46	32 (5month)		
NaCl	1.3g/100g	1.4g/100g			

Annex

The following documents were provided by FSANZ and were used in this evaluation:

1. "Declaration of manufacture" from Zanetti sent to AQIS, Canberra for the following cheeses:

   d. Parmigiano reggiano cheese (Zanetti Brand)
   e. Grana padano cheese (Zanetti Brand)
   f. Asiago cheese (Zanetti Brand)
   g. Montasio cheese Zanetti (Tre Canpane Brand, Three Bells Brand)

Documents from Istituto Sperimentale Lattiero – Caseario for parmiagiano reggiano, grana padano and pecorino romano cheeses including:

   h. statement and flow diagram for manufacture from Ministry of Agriculture,
   i. chemical composition

Council Directive 92/46/EEC (Annex C) Chapter II. Microbiological criteria for milk-based products and drinking milk.