

Supporting document 1

Guidance on the application of microbiological criteria for
Listeria monocytogenes in RTE food (at Approval)

Background

Food Standards in Australia and New Zealand

The Australian and New Zealand food standards system is governed by legislation in the states, territories, New Zealand, and the Commonwealth of Australia; including the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act).

The FSANZ Act sets out how food regulatory measures are developed. It created FSANZ as the agency responsible for developing and maintaining the *Australia New Zealand Food Standards Code* (the Code).

Responsibility for enforcing the Code in Australia rests with authorities in the states and territories and the Commonwealth Department of Agriculture for imported food; and with the Ministry for Primary Industries in New Zealand.

Responsibility of food businesses

This Guidance document is not a legally binding document. It is designed to help interested parties understand provisions in the Code.

This Guidance document reflects the views of FSANZ. However, the Guidance document cannot be relied upon as stating the law. FSANZ is not responsible for enforcement of the Code or for providing advice on food compliance issues. In Australia, state or territory government agencies are responsible for enforcing and interpreting the Code. In New Zealand, this is the responsibility of the Ministry for Primary Industries, public health units or local governments. Legal requirements may also change, for example, as government regulations are made or changed and as courts determine cases on food law in Australia and New Zealand.

Food businesses should obtain legal advice to ensure they are aware of developments in the law and any implications of such developments.

As well as complying with food standards requirements, food businesses must also continue to comply with other legislation.

In Australia, this legislation includes the *Competition and Consumer Act 2010*; the *Imported Food Control Act 1992*; and state and territory fair trading Acts and food Acts.

In New Zealand, this legislation includes the *Food Act 1981* and *Fair Trading Act 1986*.

Disclaimer

FSANZ disclaims any liability for any loss or injury directly or indirectly sustained by any person as a result of any reliance upon (including reading or using) this Guidance document. Any person relying on this Guidance document should seek independent legal advice in relation to any queries they may have regarding obligations imposed under the standards in the *Australia New Zealand Food Standards Code*.

Purpose

The purpose of this Guidance document is to provide an overview to authorities and producers of ready-to-eat (RTE) food of microbiological criteria for *Listeria monocytogenes* (*L. monocytogenes*) in RTE foods as specified in Standard 1.6.1. RTE foods are further described on page 4.

Background

The ability of a food to support the growth of *L. monocytogenes* increases the risk that the food will contribute to listeriosis. Many factors need to be considered in determining whether growth is supported to any significant degree before the food is consumed. A consideration of these factors, including the physical and chemical characteristics of the product, shelf life and processing treatments allows RTE foods to be assessed on the basis of whether growth of *L. monocytogenes* can occur in the food or not. This approach is reflected in Standard 1.6.1 where two limits for *L. monocytogenes* are specified depending on whether growth can occur. The establishment of two limits in Standard 1.6.1 recognises that for certain RTE foods and processes, occasional low level contamination may be unavoidable and may not present a health risk for the general population.

The limits in Standard 1.6.1 are end point limits, applicable to food for sale or intended for sale. They should be met through a preventative approach to food safety which relies on food businesses implementing control measures throughout production and/or processing as required by Chapters 3 and 4 of the Code (Australia only) and associated state or territory regulations¹. To this end, microbiological sampling and testing of *L. monocytogenes* should be undertaken as appropriate to verify process control and include environmental monitoring, particularly for those RTE foods that support the growth of *L. monocytogenes*. Figure 1 depicts this integrated approach.

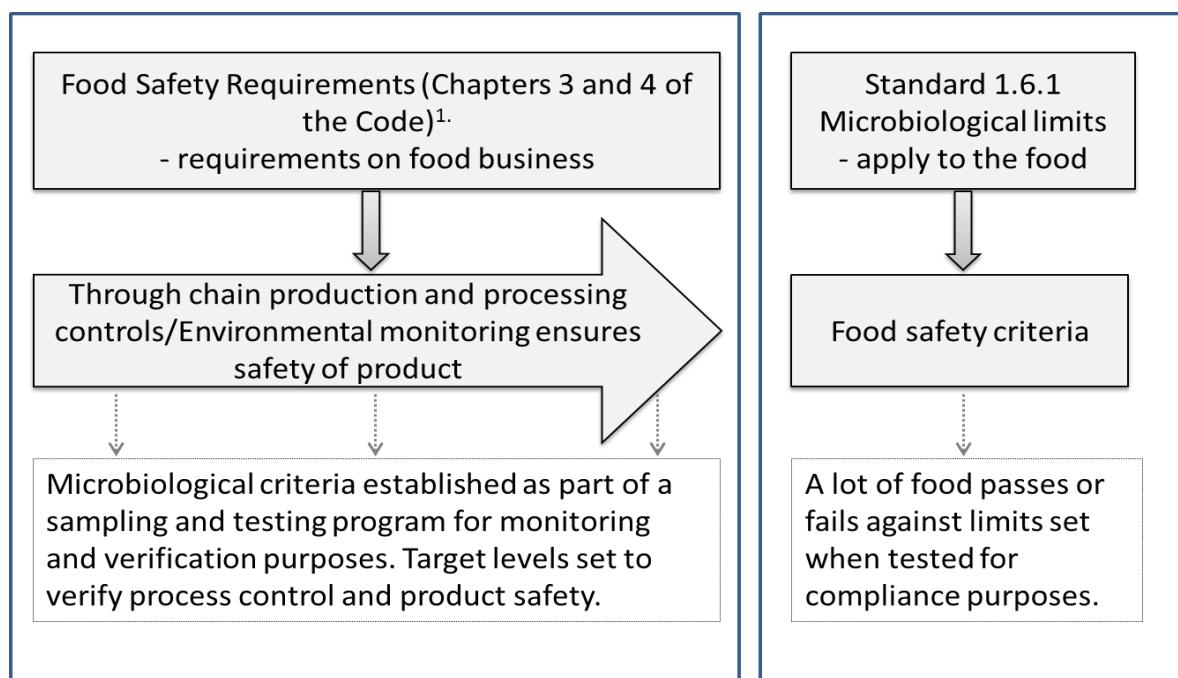


Figure 1: The role of microbiological limits in Standard 1.6.1 within a food safety system

¹ Food businesses in New Zealand manage food safety hazards via New Zealand specific food legislation (including the Food Act 1991 and the Animal Products Act (APA) 1999 - <http://www.foodsafety.govt.nz/policy-law/food-regulation/nz-food-legislation/>)

Environmental monitoring

Environmental monitoring involves testing of the processing environment (food contact and/or non-food contact surfaces) for *L. monocytogenes* or a surrogate such as *Listeria* spp. It is particularly important in facilities producing RTE foods that can support the growth of *L. monocytogenes* and should be undertaken to verify that cleaning and sanitation programs are working and there is control of niches and harbourage sites. The sampling and testing methods used should be sufficient to provide confidence that the environment is under control or identify that follow-up actions (e.g. further testing or investigation) are required.

Process control

Cross lot or between lot testing of finished product should be implemented to assess the performance of the food safety control system and verify that the production and processing controls in place are working as intended. This helps to ensure that corrective actions are implemented before microbiological criteria are exceeded. A sampling schedule (product line, number of samples to be taken, frequency etc.) should be implemented as appropriate to the operations of the food business.

Standard 1.6.1

The Schedule to Standard 1.6.1– Microbiological Limits in Food specifies end point microbiological criteria for *L. monocytogenes* in RTE foods based on whether growth of the microorganism can occur:

SCHEDULE					
Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Food	Microorganism/test	n	c	m	M
Ready-to-eat food in which the growth of <i>Listeria monocytogenes</i> will not occur	<i>Listeria monocytogenes</i>	5	0	100 cfu/g	
Ready-to-eat food in which the growth of <i>Listeria monocytogenes</i> can occur	<i>Listeria monocytogenes</i>	5	0	not detected in 25 g	

These criteria are based on the food product being produced under general principles of food hygiene to control *L. monocytogenes* in RTE foods with appropriate evaluation of the production environment and process control.

RTE foods

For some RTE foods, the application of *L. monocytogenes* limits is not relevant and testing of such foods would be unnecessary. As such, the definition for RTE foods is included in Standard 1.6.1 that specifically excludes categories of RTE foods from the limits for *L. monocytogenes* based on their nature and inherent characteristics:

ready-to-eat food means a food that –

- (a) is ordinarily consumed in the same state as that in which it is sold; and
- (b) will not be subject to a listericidal process before consumption; and
- (c) is not one of the following –
 - (i) shelf stable foods;
 - (ii) whole raw fruits;
 - (iii) whole raw vegetables;
 - (iv) nuts in the shell;
 - (v) live bivalve molluscs.

The definition for RTE food excludes shelf-stable foods, whole raw fruits, whole raw vegetables, nuts in the shell and live bivalve molluscs.

Shelf-stable foods are foods that have been processed so that they can be safely stored at ambient temperature. Such foods include canned or retorted food, bottled foods, cereals, biscuits, soft drink, sauces, confectionery, flour, sugar and dried foods.

Whole raw fruits, vegetables and nuts in the shell are generally not considered RTE foods as most are intended to undergo washing, hulling or peeling by the consumer. As these foods are raw agricultural commodities, the presence of *Listeria* species would not be unexpected and testing for *L. monocytogenes* is not useful for regulatory purposes. However raw fruits and vegetables that have been washed and either peeled, cut, sliced or shredded prior to packaging may be considered to be ready-to-eat for the purposes of Standard 1.6.1 unless they are intended to be further processed and are sold with cooking instructions on the package.

Live bivalve molluscs not intended for further processing are products that remain alive immediately prior to consumption (the shellfish will close by themselves when tapped). As for whole raw fruits and vegetables, these are raw products exposed to the environment and as such testing for *L. monocytogenes* is not useful for regulatory purposes.

RTE foods in which growth will not occur

The Schedule to the standard specifies a limit of 100 cfu /g for ready to eat foods in which the growth of *L. monocytogenes* will not occur. Clause 6 of Standard 1.6.1 sets out the criteria against which RTE foods are considered not to support growth for the purposes of applying the limits specified in the Schedule:

6 Food in which the growth of *Listeria monocytogenes* will not occur

- (1) For the purposes of the Schedule, the growth of *Listeria monocytogenes* will not occur in a ready-to-eat food if –
 - (a) the food has a pH less than 4.4 regardless of water activity; or
 - (b) the food has a water activity less than 0.92 regardless of pH; or
 - (c) the food has a pH less than 5.0 in combination with a water activity of less than 0.94; or
 - (d) the food has a refrigerated shelf life of no greater than 5 days; or
 - (e) the food is frozen (including foods consumed frozen and those intended to be thawed immediately before consumption); or
 - (f) the level of *Listeria monocytogenes* will not increase by greater than 0.5 log cfu/g for at least the expected shelf life.
- (2) A ready-to-eat food that does not receive a listericidal process during manufacture is considered as a food in which the growth of *Listeria monocytogenes* will not occur if the level of *Listeria monocytogenes* will not exceed 100 cfu/g within the expected shelf life.

Subclause 6(1) identifies a number of parameters relating to the chemical and physical characteristics of a food, as well as shelf life considerations, against which a RTE food is considered as one in which growth will not occur.

Paragraph 6(1)(f) provides for alternative processing factors to be considered and validated by providing an overall outcome that must be achieved i.e. **the level of *Listeria monocytogenes* will not increase by greater than 0.5 log cfu/g for at least the expected shelf life**. For the purposes of this guidance document, growth and shelf life can be defined as follows:

Growth – Greater than an average of 0.5 log cfu/g increase in *L. monocytogenes* levels for at least the stated shelf life of the product².

Expected shelf life – The period of time established by the food business, under intended conditions of distribution, storage, retail and use, that the food would remain safe and suitable.

Subclause 6(2) captures those RTE foods for which there is no listericidal process during production and product safety depends on the through-chain steps taken to minimise or reduce contamination. For these foods, occasional low level contamination may be unavoidable and as such a limit of 100 cfu/g should apply throughout the expected shelf life providing there is evidence that this limit will not be exceeded. Subclause 6(3) is included to clarify that such foods would normally include fresh cut horticultural produce and processed finfish.

A definition for listericidal process is included in the Standard:

Listericidal process means a process that reduces *Listeria monocytogenes* microorganisms to a safe level.

Listericidal processes may include treatments, such as a heat treatment, high-pressure processing, drying and/or acidification.

Decision framework

A decision framework (Figure 2) has been developed to assist enforcement agencies and food businesses to determine the appropriate microbiological criteria, if any, that are applicable to a RTE food product and when validation may be required to support that determination.

The initial step of the framework identifies the foods that are excluded from the definition of RTE food for the purposes of Standard 1.6.1. The definition excludes shelf stable foods, fresh whole horticultural produce and live bivalve molluscs.

The next step recognises that there may be processing factors used during manufacture that ensure *L. monocytogenes* does not present a risk and so compliance testing may be considered unnecessary. The Codex *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods* (Codex, 2007) states that testing against microbiological criteria for *L. monocytogenes* may not be useful for:

² Note that 0.5 log is two times the estimated standard deviation associated with the experimental enumeration method (viable counting/plate counts)

- a) products that receive a listericidal treatment after being sealed in final packaging that ensures prevention of recontamination until opened by the consumer or otherwise compromised,
- b) foods that are aseptically processed and packaged, and
- c) products that contain a listericidal component that ensures rapid inactivation of the pathogen if re-contaminated (e.g. products that contain >5% ethanol)

The framework then considers the chemical and physical characteristics of the food, its shelf life, and additional processing factors. RTE foods meeting the criteria defined in Standard 1.6.1 are considered as not supporting the growth of *L. monocytogenes*. Where insufficient, inadequate or no information exists to demonstrate that growth of *L. monocytogenes* will not occur in a RTE food, the food is considered to support growth and therefore a limit of 'not detected' would apply.

Food characteristics and shelf life

Knowledge of the chemical and physical characteristics of a product is needed in order to decide whether an RTE food supports the growth of *L. monocytogenes*. Criteria have been agreed internationally (Codex, 2007) for RTE foods that do not support the growth of *L. monocytogenes* where the physico-chemical characteristics fall into one of three ranges throughout the food's stated shelf life. These default criteria are:

- pH < 4.4, regardless of water activity;
- a_w < 0.92, regardless of pH; and
- combination of pH < 5.0 and water activity < 0.94.

In addition, RTE foods which are frozen are also considered to be foods in which the growth of *L. monocytogenes* cannot occur. These products include foods consumed frozen (such as ice cream) and those intended to be thawed just before consumption (to be eaten cold or reheated). Final use of the frozen product and instructions for use should be considered. If, for example, the product is intended to be thawed for retail sale it would not at that stage be a frozen product and growth may occur.

The potential for *L. monocytogenes* to grow to high levels in foods with a short refrigerated shelf life (≤ 5 days) is restricted (Health Canada, 2011; Ross 2011). Studies support the conclusion that, providing Good Manufacturing Practices (GMP)/Good Hygienic Practices (GHP) have been followed to minimise initial contamination levels, short shelf life foods pose a lower risk with respect to causing listeriosis.

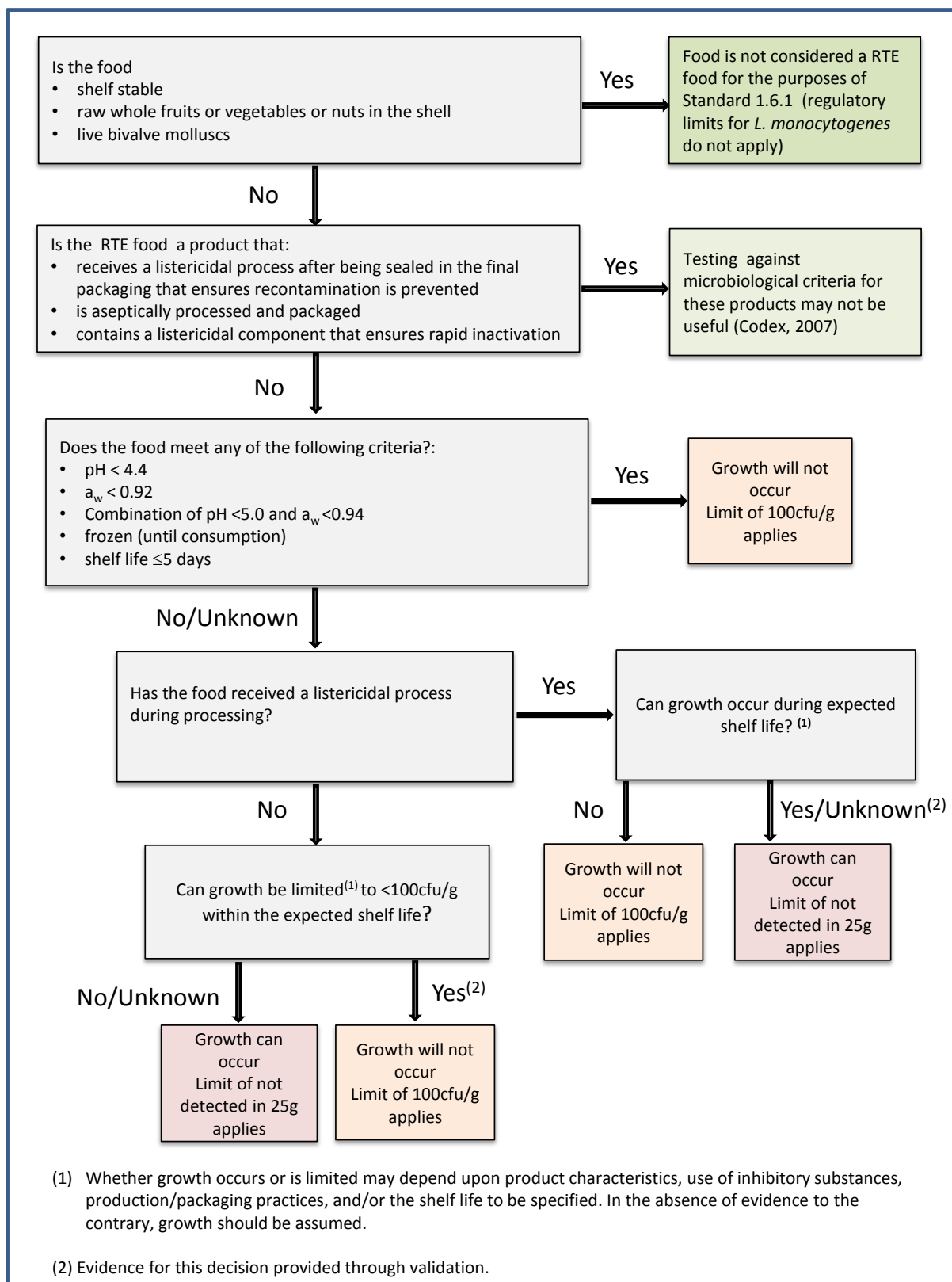


Figure 2: Recommended framework for applying limits for *L. monocytogenes* in RTE foods for the purpose of Standard 1.6.1 and where validation is required

Production processes and additional validation

1. Has the RTE food received a listericidal process?

A further consideration for determining the appropriate microbiological limit for *L. monocytogenes* is whether a RTE food receives a listericidal process during production. The presence of *L. monocytogenes* in RTE foods that have received such a treatment indicates re-contamination following the listericidal processing step. Whether growth can then occur may depend upon product characteristics, use of inhibitory substances, production and packaging practices, and/or the shelf life of the food.

In the absence of any other information, a limit of 'not detected' in 25 g should apply to these RTE foods throughout their expected shelf life. This is the default criterion should a business not have specific information available about their RTE food (e.g. unable to validate whether growth of *L. monocytogenes* does not occur during the stated shelf life).

If evidence can be provided that the RTE food will not support the growth of *L. monocytogenes*, then the limit of 100 cfu/g throughout the expected shelf life would apply.

2. RTE foods not receiving a listericidal process

Some foods do not receive a listericidal process during manufacture (e.g. smoked finfish; gravadlax finfish; fresh cuts) and ensuring product safety relies on minimising or reducing contamination during primary production, processing and distribution, and limiting growth through maintaining the cold chain and restricting shelf life. Occasional low level contamination of such products by *L. monocytogenes* may be unavoidable but may not present a risk if growth cannot occur or is limited within the stated shelf life.

If evidence can be provided that the level of *L. monocytogenes* is limited (less than 100 cfu/g) throughout the stated shelf life of these foods, then a limit of 100 cfu/g applies for the purpose of Standard 1.6.1.

Validation

Evidence that *L. monocytogenes* will not grow in a RTE food may be based upon:

- the physico-chemical characteristics of the food;
- historical information on similar or related products;
- information from the scientific literature and risk assessments;
- challenge studies;
- predictive modelling; or
- a combination of these approaches.

Validation documentation should provide the objective evidence that shows that the product does not support the growth of *L. monocytogenes* or that growth is limited (less than 100 cfu/g throughout the stated shelf life) under reasonably foreseeable conditions of distribution, storage, retail and use.

1. Key process parameters

It is recommended that process and production parameters are clearly identified and that evidence as to how these parameters are controlled and verified is provided. Information about the product that should be documented includes:

- specifications for physico-chemical characteristics of the product, such as pH, water activity (a_w), salt content, concentration of preservatives;
- the key process parameters at each stage of production
 - concentration of *L. monocytogenes* at the beginning of the shelf-life of the RTE product, pH, a_w , washing conditions, smoking or preserving conditions etc;
- the control measures in place to ensure product and process parameters are met and how these are verified;
- identification of each ingredient including its effective concentration;
 - permitted antibacterial agents, water activity depressants (sugar, salt, etc), pH reducing agents (vinegar, organic acids, etc); and
- the type of packaging system used taking into account the storage and processing conditions and the possibilities for contamination.

2. Scientific literature

A review of the scientific literature is useful to provide information on the possible level of *L. monocytogenes* contamination that could be expected and its growth profile in the food product being considered as well as similar products. Reference to published challenge studies may be useful in providing evidence on the factors that inhibit growth of *L. monocytogenes*.

3. Challenge studies

Challenge studies provide information on the response of pathogens (growth and inactivation) to changes in the intrinsic physio-chemical parameters and impact of production and processing factors. The design of a challenge study should adequately reflect the processes used to make the RTE food product.

There are a number of existing resources available to assist in the conduct of challenge and shelf life studies for *L. monocytogenes* in RTE foods, including:

- *Listeria monocytogenes Challenge Testing of Ready-to-Eat Refrigerated Foods* (Health Canada, 2012)
- Guidance document on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods, under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Commission of the European Communities, 2008)
- Shelf life of ready-to-eat food in relation to *L. monocytogenes* – Guidance for food business operators (Chilled Food Association, 2010)

In addition, the NSW Food Authority has produced a guidance document on shelf life testing with respect the establishment of 'use-by' dates that provides useful information: Shelf life testing, 'Use-by' dates for food safety (NSW Food Authority, 2010).

4. Predictive microbiological models

Predictive models that are validated, robust and built upon scientifically sound data can play an important role, along with other supporting information, in determining if a given product formulation or process will reduce the likelihood of *Listeria* spp. presence or growth.

A number of predictive microbiological models are freely available on the internet, including:

- Meljholm and Dalgaard (2009) developed a growth rate and growth boundary model for *L. monocytogenes* in RTE shrimp (Seafood Spoilage and Safety Predictor). The model includes 12 factors and their interactive effects of relevance to the prediction of the probability of growth: temperature, salt, pH, six acids (acetic acid, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid), smoke components (phenol), CO₂ in head space gas at equilibrium and nitrite. This model has been validated for meat products, seafood products, poultry products and non-fermented dairy foods (e.g. milk, cream and ice cream) (Mejlholm et al, 2010).
- Augustin et al. (2005) evaluated the performance of eight growth rate and probability of growth/no growth models using a combination of temperature, pH, the main acid present in the medium, water activity, nitrite, phenol and the proportion of CO₂ in the modified atmosphere as factors.
- ComBase (2012) is a freely available database of observed microbial responses to a variety of food-related environments and a collection of relevant predictive models. Models are also available (ComBase Predictor) to predict the response of *L. monocytogenes* to key factors such as temperature, pH and salt concentration in combination with organic acids, nitrite and carbon monoxide. The models are based on outputs from laboratory experiments observed in culture media under well controlled laboratory conditions.
- The USDA Pathogen Modelling Program (USDA, 2013) is a package of models that can be used to predict the growth and inactivation of foodborne bacteria, primarily pathogens, under various environmental conditions. The predictions are specific to certain bacterial strains and specific environments (e.g., culture media, food, etc.) that were used to generate the models.

Use of these models requires specialised technical skills and knowledge for correct utilisation and interpretation of results. Additionally, each model has inherent limitations which need to be known and understood by the user. For example, modelling programs may not extend to the impact of certain preservatives and specialised technical expertise may be required to evaluate preservative efficacy.

Predictions from models are based on observations largely made in artificial growth medium and available studies. Understanding how *L. monocytogenes* will behave in a specific product may require a challenge test.

Further information

Further information regarding validating food safety measures can be found in the Codex document *Guidelines for the Validation of Food Safety Control Measures* (Codex, 2008).

Sampling and analysis

Standard 1.6.1 stipulates two (2) class sampling plans for *L. monocytogenes* in RTE foods based on whether the food supports the growth or does not support the growth according to the methods prescribed by Australian Standard AS 5013 (or corresponding ISO Standard):

- *L. monocytogenes* not detected (5 x 25 g analytical units) with reference method AS5013.24.1-2009 Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Detection method (ISO 11290-1: 1996, MOD).

- *L. monocytogenes* not exceeding 100 cfu/g (5 x 10 g analytical units) with reference method (under the AS 5013 series) AS 5013.24.2-2009 Food Microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Enumeration method (ISO 11290-2: 1998, MOD).

When sampling for the purposes of complying with Standard 1.6.1, it is recommended that five sample units of at least 100 g or mL be taken at random and be representative of the lot or production conditions.

Prior to submitting samples for analysis, consideration needs to be given as to what limit should apply and, therefore, whether enrichment (detection) and/or a plating method is to be used; as well as the time taken for testing (which may have implications for when a lot is released to the market). It is recommended that food businesses develop procedures for sampling and analysis, including details on any hold and test procedures and corrective actions.

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