

**Altering an existing recipe – Technical worked example of an approach to determining the shelf life of a ready-to-eat food in relation to *Listeria monocytogenes* (*L. monocytogenes*)**

**Brie with Garlic and Herbs**

**Background**

A small, specialist, farm based dairy has been producing a plain brie cheese for around 15 years and wishes to develop its range to include brie with additives. A market review has indicated that a garlic and herb variant has reasonable sales potential.

The dairy structure is sound and fit for purpose; process equipment was installed as a complete project by a multinational equipment supplier who provides ongoing maintenance to a defined schedule. A specialist chemical company supplies all the requisites for hygiene & cleaning and there are defined schedules and procedures for cleaning the plant and fabric. The dairy has been accredited under the SALSA scheme for around 6 months.

**1. Product characteristics and scientific literature**

The existing product is a **Ripening Brie cheese**



Milk is supplied by specified farms and delivered by a national haulier. The raw milk is pasteurised on-site at 74°C/18s, then used immediately.

The following processing aids / ingredients are supplied with specifications from a multinational specialist companies. Each company also supplies a certificate of analysis with each delivery.

- Bacterial starter culture, freeze-dried, stored frozen
- *Penicillium camemberti* ripening culture, liquid, stored chilled
- Rennet, liquid, stored chilled
- Calcium chloride, liquid, stored chilled
- Salt, solid, stored at ambient temperature (and used to prepare brine)

There is a system in place to ensure all product is stored as per the manufacturers' recommendations, durability dates are respected and the dairy keeps a record of codes used in each batch of product.

The shelf life of this product from the end of the on site maturation process was originally set at 30 days based largely upon sensory characteristics, the optimum maturity if stored at 5 to 7°C being achieved at 25 days and the product remaining acceptable to 30 days, beyond which it was considered over ripe. As the product could not be guaranteed to be free from *L. monocytogenes* it carries the warning 'not suitable for pregnant women'.

The pH and aqueous salt content of the cheese are:

Component of the cheese	Process stage	pH	Salt-on-product (%)	Moisture (%)	Aqueous salt (%)
Coat	Despatch	6.0	1.6	45	3.6
Body	Despatch	5.2	1.6	50	3.2
Coat	End of life	7.5	1.8	40	4.5
Body	End of life	7.0	1.8	45	4.0

From EC Regulation 2073/2005, products are not considered to support the growth of *L. monocytogenes* if:

- pH is no more than 4.4, or
- $a_w$  is no more than 0.92 (= 11.90% aqueous salt), or
- pH is no more than 5.0 and the  $a_w$  is no more than 0.94 (= 9.38 % aqueous salt)
- shelf life is less than 5 days

The pH and  $a_w$  values of different parts of the finished product at the start and end of shelf life suggest that *L. monocytogenes* would grow if present.

Control of *L. monocytogenes* in the cheese is achieved by:

- correct maintenance and operation of the pasteuriser.
- use of good quality, uncontaminated ingredients.
- adopting Good Manufacturing Practice and HACCP systems in all production areas to prevent cross-contamination, especially in the cheeseroom and maturation rooms.

Having said this, the following points have to be considered:

- While *L. monocytogenes* is inactivated by the standard milk pasteurisation process of 72°C/15s, *L. monocytogenes* is a ubiquitous organism that can colonise production and maturation rooms and gain access to the milk, curd or cheese as a post-pasteurisation contaminant.
- Raw milk is considered by many to be a major source of contamination of the production environment. While raw milk supplies cannot be guaranteed to be free from *L. monocytogenes* on every occasion, the incidence of *L. monocytogenes* in raw milk is surprisingly low; in a survey of UK raw milks, *L. monocytogenes* was detected in only 5.08% of samples, more than 60% of positive samples containing less than 10 cfu/ml (O'Donnell, 1995).
- Potentially 'critical' environmental sources of *L. monocytogenes* are:
  - Brine
  - Drains
  - Chiller units
  - Maturation shelves
  - Improper use of hoses
  - Moisture in the atmosphere

## 2. Historical data

The dairy has had a contract with a local accredited microbiological laboratory which processes environmental samples and product samples taken by the dairy. The microbiological sampling regime includes tests for Enterobacteriaceae, which would be primary indicators of the level of post process contamination, and *Staph. aureus* the presence of which might be considered to relate to handler hygiene practice or milk quality.

Sampling has been targeted to demonstrate the effectiveness of the hygiene controls on site and has contributed to defining and refining best practice on the cleaning procedures and schedules. Since the sampling plan was started 10 years ago the incidence of *Listeria* isolation in final product has dropped to around 25% of the original incidence.

Taking the last 2 years of data with respect to *Listeria* species, *Listeria* spp. have been found in 15% (of which one third were *Lm*) of drain samples and 2% (of which half were *Lm*) of food contact surfaces prior to cleaning. Post cleaning samples & brine samples all gave negative results.

### **Start of life testing**

200 samples were taken of product at the point of despatch and tested for presence of *Listeria* in 25g using an enrichment technique; of these 14 were positive for *Listeria* spp. and 7 were positive for *L. monocytogenes*. Enumeration of fellow samples from all of the positives gave results of less than 10/g, i.e. any contamination was below the level of detection by count.

### **End of life testing**

Over the same period 50 samples were tested 30 days after the date of despatch; these included fellow samples from all of the *Listeria* positives batches found above. The enrichment technique found 10 samples positive in 25g for *Listeria* spp. of which 6 were confirmed as *L. monocytogenes*. The enumeration technique found one single sample with a count of 400 *L. monocytogenes*/g; this was on a product which previously had been found to be absent in 25g at the start of life. All other samples were below the level of detection (i.e. less than 10/g).

It is important to note that detection of *Listeria* species from ingredients, the product or the environment, particularly food contact surfaces after cleaning, requires documented investigation and follow-up remedial hygienic action carried out and documented.

If the limit of 100 *L. monocytogenes* cfu/g is compromised during shelf life it will be necessary to withdraw or recall the product.

The pattern of *Listeria* isolation:

- **Suggests** an endemic low incidence of post process contamination with *Listeria* spp. including *L. monocytogenes*. Assuming a uniform contamination rate, the base level of contamination could be as high as 1 in 100g or as low 1 in 1,000g. That there is potential for growth of *Listeria* in the product is suggested by the increase the proportion of positive samples from start to end of shelf life and in the single detection of 400/g on one enumeration at end of life. However it is also possible that the contamination is random and sporadic and this single sample might represent an unusually high level of contamination of the sample tested rather than actual growth. The apparent increase in detection rate at 30 days may be skewed by selection of samples from batches which initially proved positive. The situation is further complicated by the variation in pH between body and coat of the cheese over the ripening period, where the ability of *L. monocytogenes* to grow is affected by the level of acidity in its immediate environment.
- **reinforces** that positive release of product on the basis of the incidence of *L. monocytogenes* at start of life would not be an effective control measure.

## Conclusions

The microbiological criterion for *L. monocytogenes* in food capable of supporting the growth of *L. monocytogenes* is absent in 25g at point of despatch, based upon n=5, c=0 in EC Regulation 2073/2005. This applies when the Food Business Operator cannot demonstrate that the criterion of 100 *L.monocytogenes*/g throughout shelf life will not be exceeded.

The data presented above suggest that the product would fail the rigour of this requirement if 5 x 25 g samples were taken on each occasion.

The historical results suggests that under normal circumstances the growth rate of *L. monocytogenes* in this product is, at best, poor, this may be due to competition effects from the cheese cultures and the chemical hurdles such as the level of salt.

Aside from the one high result at the end of life, the microbiological results suggest that the process is under control and the 30 day life given is not excessive.

Although one sample out of 50 enumerated at the target shelf life of 30 days has exhibited a count above the legal maximum for a RTE food the weight of evidence suggests that this was a rogue result and that the process is under control; however this view might change as more results are added to the data set which needs to be kept under review.

### **New Product Development: Ripening Brie cheese with garlic and herbs**

#### **1. Product characteristics and scientific literature**

The product is a **Ripening Brie cheese with garlic and herbs**. It consists of the plain ripening brie described above with the following additions.

- Garlic (peeled, boiled and puréed, stored chilled)
- Herbs (parsley and oregano), grown organically, sun-dried and finely chopped, purchased from a local farm shop and added to curd without treatment.

The physico chemical characteristics are identical to the plain brie.

- Additive ingredients such as herbs and spices may be added to the milk, curd or fresh cheese with, or without, a treatment such as boiling that would inactivate *Listeria*, yet such commodities may be grown under conditions conducive to contamination with *Listeria*; i.e. near the ground and where there may be poor hygiene standards. Such ingredients must be considered as a potential source of *L. monocytogenes* and controls implemented to minimise this potential.

#### **2. Historical data**

The historical data for the plain brie suggests that although the physical and chemical characteristics suggest that growth of *Listeria* might be supported, in practice significant growth is not detected.

The new ingredients required careful risk assessment.

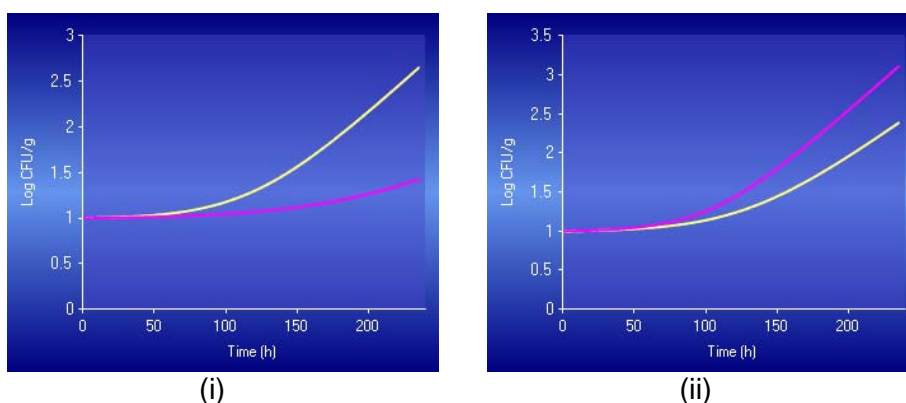
Garlic has gone through a boiling process which should eliminate *L. monocytogenes* – the product is supplied with a specification detailing the process and assures a level of absence in 25g throughout the shelf life.

The provenance of the herbs has not been tested and there is no stage in the process which would eliminate any *L. monocytogenes* (or other pathogens) naturally present. The supplier is therefore not able to guarantee that individual batches are free from contamination.

It must be assumed that *L. monocytogenes* may be present and in a vigorous condition in the herb additive and therefore if added direct into the cheese may significantly increase the loading at the start of life.

### 3. Predictive Microbiology

It may be possible to use appropriate, freely-available models such as ComBase (<http://www.combase.cc>) to predict the behaviour of *L. monocytogenes* should it be present in the maturing cheese. This software is designed to give an idea of how the pathogen might behave; however, predictive modelling may not be appropriate for some cultured foods as it does not take into account the competition that may occur between micro-organisms that can reduce the growth of *Listeria*.



**Predicted behaviour of *L. monocytogenes* in Brie cheese during storage at 5°C: (i) start of life (coat, upper curve; body, lower curve), and (ii) end of life (body; upper curve; coat; lower curve).**

These predictions suggest that if *L. monocytogenes* was present on the coat of the cheese at a level of 10 cfu/g at start of life, when stored at 5°C, growth to a level of 100 cfu/g might occur within 200 hours (8.3 days).

Utilising the data from the plain brie where the background level of *L. monocytogenes* appears to be around 1 in 100g of product, the predictive model would suggest that at 5°C a level of 100 per gram (4 log<sub>10</sub> growth) would take 4 x 8.3 days = 33.2 days.

The level of *L. monocytogenes* the herb addition is an unknown quantity and as there is no elimination step it can be assumed that there will be significant levels in certain batches. Further, the product specification provided by the supplier does not include a criterion for *L. monocytogenes*.

The addition of untreated herbs to the cheese mix at a level of 1% will increase the background level in a proportion of batches of cheese and the possibility that the storage temperatures within retail and the home may be higher would suggest that the proposed formulation is likely to exceed the legal maximum at a 30 day life.

If the herbs were subjected to a process step that would eliminate *L. monocytogenes*, such as steam treatment, then the level of *L. monocytogenes* contamination could be reduced to the point where legally it conforms to the EC Regulations for a RTE food, then the additional

loading to the cheese would not then be significant less than 1 per 100g) – this would enable a 30 day life to be applied.

#### 4. Durability studies

Durability studies are generally not applicable to determine the growth of pathogens in a foodstuff, as there is no guarantee that the pathogen will be naturally present.

Although it would appear that there is a natural background level present in this particular product there is no real evidence of an even distribution of *Listeria* in the cheese which would guarantee its presence in a 25 g sample.

If such a study was carried out, replicate samples would need to be taken from a batch of cheese over life. The storage temperature would need to replicate what would happen in reality. The samples would be tested for *L. monocytogenes* and a plot of number over time would give an indication of whether this organism could grow to a level of 100 cfu/g by the end of life of the cheese.

#### Reference

O'Donnell, E T, (1995). The incidence of *Salmonella* and *Listeria* in raw milk from farm bulk tanks in England and Wales. *Journal of the Society of Dairy Technology*, 48 (1), 25-29.