

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

Brie with Garlic and Herbs



I wanted to make a Brie type cheese with garlic and herbs. I knew how to make the cheese as I already sold a plain Brie. However, I did not know how to work out what shelf life to put on the packets. I had heard that there was a risk of *Listeria monocytogenes* (*L. monocytogenes*) contamination. My advisor asked me a series of questions and gave me the reasons behind each one of them.

The cheese would be a “ready to eat” (RTE) food as it would not be cooked to kill off bacteria before customers ate it. I must therefore be able to demonstrate that levels of harmful bacteria were low enough to keep consumers safe.

There are strict limits on the maximum allowable level of *L. monocytogenes* at the end of shelf life.

We first looked at ingredients. They are:

- Milk supplied by specified farms and delivered by a national haulier. The raw milk is pasteurised on-site at 74°C/18s, then used immediately.
- 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)
- Garlic, peeled, boiled and puréed, stored chilled
- Herbs, (parsley and oregano) grown organically, sun-dried and finely chopped. Purchased from a local farm

Other than the herbs and milk, all the ingredients can be supplied with specifications from multinational specialist companies. The specifications should indicate that the ingredient is of suitable quality for intended use.

Each company also can supply a certificate of analysis with each delivery, which I need to keep to show I had taken the correct precautions.

The rules

The legislation requires that *L. monocytogenes* must not be present at more than 100 colony forming units/g at the end of the shelf life. So if there were any contamination to start with and the bacteria were able to grow, the shelf life may be too short for economical production.

L. monocytogenes is one of the few harmful bacteria that can grow at fridge temperatures, so storing in the fridge may not stop a small amount of contamination from becoming large by the time the cheese is eaten.

I asked my advisor if there were any other ways of stopping *L. monocytogenes* growing.

Would it grow?

If the cheese is quite acid (pH of less than 4.4) and dry (available water (a_w) less than 0.92), *L. monocytogenes* will not grow. A high salt content also slows down growth. To find out the pH and a_w we assessed both the coating and from the inside of samples of my trial cheeses, and got the following results:

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

This meant that theoretically my cheese would support the growth of *L. monocytogenes*, so we had to look at ways to limit initial contamination and then check, if there were contamination, how long it would take to reach the critical legal limit.

Limit contamination

Although it is rare for raw milk to be contaminated with *L. monocytogenes* it will be killed by proper pasteurisation (time/temperature treatment of 74°C for 18 seconds). *L. monocytogenes* is almost everywhere, so we looked carefully at the plate cooler, transfer tubes and holding tanks. Contamination often gets in from dust, the drains, chiller units, maturation shelves, improper use of hoses and moisture in the atmosphere. We looked at the additives where we were fairly confident as, apart from the herbs, they had been supplied with test results.

My test result history

Then we looked at my existing test results. Any contaminations indicate that if other bacteria could get in, so could *L. monocytogenes*. 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 other bacteria like 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 levels.

For existing cheeses, 200 samples had been 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 at the start of the shelf life.

Results from samples of the same cheese taken on the last day of the shelf life showed similar historical results, which suggested that under normal circumstances the growth rate of *L. monocytogenes* in my plain brie 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. The microbiological results suggest that the process is under control.

The occasional detection of *L. monocytogenes* may be expected in these types of product, as even the best designed and maintained factory environment will be contaminated from time to time. Positive results of this sort indicate that the sampling procedures and testing methods are working correctly.

The risk is there. What is the shelf life?

Given these results – that *L. monocytogenes* could grow and there was a possibility of contamination – my adviser recommended a mathematical prediction to suggest when levels might exceed the 100 cfu/g limit. This is called “Predictive Microbiology”. It may be possible to use appropriate, commercially-available models such as ComBase (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*.

These predictions suggested that if *L. monocytogenes* were present on the coat of the cheese at a level of 10 cfu/g at the start of life, when stored at 5°C, it could grow to a level of 100 cfu/g within 200 hours (8.3 days). This is too short to be economic.

Possible solution?

Using the data from 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 would take 4 x 8.3 days = 33.2 days. So we assumed that the addition of the herbs may account for the increase in contamination.

The level of *L. monocytogenes* in the herb addition is not known 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. 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.

So we suggested that 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 comfortably conforms to the EC Regulations for an RTE food and so the additional loading to the cheese would not be significant. In this case we could safely extend the shelf life to 30 days.

Confirmatory testing

My adviser strongly recommended shelf life tests to verify that our *L. monocytogenes* predictions on the heat treated herbs were correct. These end of life tests, he explained, were not in themselves adequate to determine the shelf life. He said that, if they did show a high level, it would be too late to do anything about it as the product would have been consumed already. As there was the possibility that the cheese could become unsafe after the 30 day life we needed to label the packets with a “Use by” date rather than a “Best before”.

He told me that there were other techniques for determining whether *L. monocytogenes* could grow in the product like the Challenge Test system. Here a known amount of contamination is deliberately inoculated into the product. Measures are taken at intervals when different storage conditions are used. The technique is not really satisfactory for solid cheeses because it may be difficult to get an even distribution of contamination. It is better for fluids.

Conclusions

Finally my adviser told me that we had established that *L. monocytogenes* could grow in my cheese if it were present in the first place. His prediction was, given the historical data that I had, a shelf life of around 8 days would be appropriate. However, if I were to sterilise the additions of herbs, the initial contaminations would be reduced and I could safely quote a shelf life of 30 days using the historical data I have for the plain brie at the end of its shelf life. All this would only be possible if I were to continue to do all my process monitoring and insist with my staff that a very high level of hygiene is maintained. I am pleased to report that I have not had a test result since we started full scale production that would indicate that our conclusions were wrong.