

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

Cold smoked salmon and fresh watercress sandwich

I wanted to extend my range of sandwiches to include ones filled with smoked salmon and chopped watercress. I had heard that smoked salmon sometimes contained *Listeria* (*L. monocytogenes*) so I thought it wise to consult my professional adviser to see what sort of a shelf life I could safely put on. If he said the life would be too short, it would not be worth going ahead as we would not have time to sell them before they went out of date.



The first question he asked me was about the ingredients we were intending to use. I said I would use the highest quality ingredients available and would obtain them from reputable suppliers. These supply a 'certificate of compliance' (to my requirements) for each batch of ingredients supplied. The details of the ingredients are:

- Salmon which is supplied in 1kg packs with a 10 day chilled shelf life. The salmon is cold-smoked by a process of 30°C for 16 hours and has a salt content of 3.5%;
- Fresh shredded watercress, supplied in 500g packs with a 7 day chilled shelf life.
- Sliced wholemeal bread, supplied in 800g bags with a 7 day ambient shelf life.
- Butter, supplied in 2kg tubs with a 6 week shelf life.

The Rules

The legislation requires that *L. monocytogenes* must not be present at more than a very low level (no more than 100 colony forming units per gram) 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 must be limited.

Could there be contamination?

He then explained that there was a real risk that some of these ingredients could be contaminated with *L. monocytogenes*. He said that the following points have to be considered:

- The 'heat process' used in the cold-smoking of Salmon (30°C for 16h) is not sufficient to inactivate *L. monocytogenes*. (A process equivalent to 70°C for 2 minutes is required for this). Also, the salt concentration of 3.5% is not sufficient to control growth as *L. monocytogenes* can grow in the presence of salt at 10%, and survive in conditions of 25% salt. Some protection may be afforded by the preserving effect of

the smoking, and competitive effects of the indigenous microbiological population of the component.

Cold-smoked salmon has been shown to be contaminated by *L. monocytogenes* at frequencies of 2-21% (McLauchlin and Nichols, 1994). Levels were generally less than 100 cfu/g with the highest count between 100 and 1000 cfu/g. This is in line with findings by the Food Standards Agency, where 1344 samples of cold smoked fish were sampled at retail during 2006. Nearly 300 (282, 20.5%) samples contained *Listeria* spp, and 236 (17.4%) *L. monocytogenes* with all levels below 100 cfu/g (<http://www.food.gov.uk/multimedia/pdfs/fsis0508.pdf>).

- Since *L. monocytogenes* is a relatively common bacterium in the environment, watercress might be expected to be occasionally contaminated with it. Washing the watercress in chlorinated water will help to reduce the level of *L. monocytogenes*.

Bell & Kyriakides (2005) mention a survey of 11 samples of watercress, 2 of which were found to be contaminated with *Listeria*. (One was found to be *L. welshimeri* the other was not identified.)

- Bread has no history of being contaminated with *L. monocytogenes* as it is prevented from growing on it because appropriate nutrients are not available.
- Butter has been associated with listeriosis, but this is the exception rather than the rule, and came about as a result of incorrectly made butter. (Butter is an emulsion of water droplets in a fat matrix. *L. monocytogenes* is normally controlled by the water droplets being of insufficient size to physically allow growth.)

In an outbreak in England, testing confirmed the presence of *L. monocytogenes* at 180 cfu/g in a batch of butter although it was only detectable at low levels (less than 20 cfu/g) in other batches (ACMSF, 2003).

Would it grow?

Having established that there was a risk of *L. monocytogenes* contamination in the ingredients, my adviser then went on to show that the bacterium could grow. I knew that *L. monocytogenes* could grow at fridge temperatures or below, but he explained that unless the acidity (pH) were very low and the product is fairly dry *L. monocytogenes* would grow. He worked out for my sandwich these approximate figures.

Component	pH	a _w
Salmon	6.0	0.95 (3.5% salt)
Watercress	6.5	0.98
Bread	6.1	0.97
Butter	6.6	0.96 (aqueous phase)

NB the values used here are for illustration purposes only

From Regulation (EC) No. 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, or
- pH is no more than 5.0 and the a_w is no more than 0.94
- shelf life is less than 5 days

("Bread" is one of the foods specifically mentioned as being excluded in the Regulation).

So we concluded that the pH and a_w values of the ingredients suggest that *L. monocytogenes* would grow in them if present. Although the proposed shelf life of the completed sandwich is less than 5 days, the age of one or more of the ingredients may be older than this. Now we had to determine whether any initial contamination would exceed the legal limits by the end of my proposed shelf life. So my adviser looked at the data that I had on ingredients I already used.

My test result history

Historical data show that the results of microbiological testing of supplied ingredients; sandwich-manufacturing environment; and finished product throughout shelf life could contain *L. monocytogenes*. Although information and data on *Listeria* and *L. monocytogenes* is of prime importance, other microbiological data, for example Aerobic Plate Counts, can be used to indicate if production is generally under control.

Useful information can be obtained from suppliers, such as evidence of absence of *L. monocytogenes* in the environment and ingredients they are supplying. The level of confidence increases with the amount of data available. Ideally, this should cover eventualities of variability such as seasonality of ingredient/component supply. Data acquired from one supplier is not applicable to another or all potential suppliers of the same component.

Evidence of the absence of *L. monocytogenes* in ingredients where this microorganism can grow (such as Salmon and Watercress), is important to show that the sandwich produced is acceptable. Counts of *L. monocytogenes* at less than 100 cfu/g at end of life of the sandwich are useful, as is evidence that counts of *L. monocytogenes* are 'less than 10 cfu/g' or 'less than 20 cfu/g' at the start of life of the sandwich or its ingredients. This is however not evidence that *L. monocytogenes* will not grow to levels above 100 cfu/g by the end of life of the product and therefore necessitate being withdrawn from sale. It does however strongly suggest that the controls in place are working.

Occasional counts of *L. monocytogenes* are to be expected in this type of product, as ingredients and factory environments will be contaminated from time to time. Positive results of this sort indicate that sampling procedures and testing methods are working.

The risk is there. What should be the shelf life?

Having established that there is a real possibility that *L. monocytogenes* could be present in the sandwich at the point of sale, the task now is to be certain that the count does not exceed 100 cfu/g at the end of the proposed shelf life. My adviser explained that there are three generally accepted methods of checking this:

i. Predictive Microbiology

The behaviour of *L. monocytogenes* should it be present in the sandwich ingredients, can be predicted using appropriate freely-available models such as ComBase (<http://www.combase.cc>). This software is designed to give an idea of how the pathogen might behave, it does not take into account factors such as: the anti-microbial effects of smoking the salmon; competing microflora in the salmon or watercress; and so on.

The predictions for the ingredients discussed in this example indicate that if *L. monocytogenes* were present at a level of 10 cfu/g in the salmon or watercress at start of life of each component, even if they were kept at 5°C, the number is likely to

reach 100 cfu/g (2 logs) before the end of life of the ingredients and probably the sandwich made from them.

ii. Durability studies

Durability studies are generally not relevant to determining the growth of pathogens in a foodstuff, as there is no guarantee that they will be naturally present. If such a study were carried out, replicate samples would need to be taken of the ingredients and sandwich over life. The temperatures that these foodstuffs were held at 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 would grow to a level of 100 cfu/g by the end of life of the sandwich.

iii. Challenge test

A challenge test study may be used to determine the behaviour of a pathogen in a foodstuff over life. As for the durability study, the number of the relevant organism is determined over the life of the foodstuff.

The advantages of a challenge test over the other methods of shelf life determination mentioned here, is that a known number a particular species of microorganism can be added at the start of the study. And units initially inoculated at the start of the study, can be analysed at end of life.

To reflect reality, the sandwich would need to be made from the ingredients when the salmon was no more than 7 days old and the watercress no more than 4 days old – so that the shelf life of the sandwich could be taken into account.

Conclusion

If the results of these tests are satisfactory, then my adviser said it may be concluded that a three day shelf life as proposed is valid. In this case, he would still recommend that ingredients are used early in their life to minimise any potential growth of *L. monocytogenes* that might already be present.

He added that if the tests indicate that the 100 cfu/g were to be exceeded, then either the shelf life must be reduced or further precautions taken with the ingredients and processing (e.g. use 'hot-smoked' or 'canned' salmon instead of 'cold-smoked') to eliminate the risks during production.

The result of these discussions with my professional adviser was that I could safely put a three day shelf life on the sandwiches if I took all the precautions mentioned above. He drew my attention to several references in addition to Regulation (EC) No. 2073/2005 which defines the limits for *L. monocytogenes* at the end of shelf life assuming the sandwich was for general consumption and not for vulnerable people more susceptible or more likely to develop foodborne disease, e.g. pregnant women, the elderly, children and people with weakened immune systems...

References

Advisory Committee on the Microbiological Safety of Foods (ACMSF) (2003) Information paper. Recent Trends in Listeriosis in the UK. ACM/667. December. London, UK.

Bell, C and Kyriakides, A (2005) *Listeria: a practical approach to the organism and its control in foods*, 2nd Edition. Wiley-Blackwell.

McLauchlin, J and Nichols, G L (1994) *Listeria* and seafood. *PHLS Microbiology Digest* 11(3), 151-154.