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Factors Affecting the Growth and Survival of Salmonella in Fermented Salami Manufactured Under Australian Conditions

Fermented salamis manufactured under Australian conditions have been associated with food-poisoning outbreaks due to Salmonella. These micro-organisms are easily destroyed by heating and provided post-cooking hygiene is of a reasonable standard they should not normally pose a problem in heat-treated foods. Fermented salami receive no heat treatment and as such must rely on other environmental conditions for their microbiological safety and stability.

Funded by the Pig Research and Development Corporation, we have been examining the environmental factors controlling the growth and survival of Salmonella in salami manufactured under Australian conditions.

The two main factors contributing to the safety and stability of this product are low pH and reduced water activity (a_w), however the environmental conditions prevailing in the first 24—48 h are critical with regard to the growth and subsequent survival rate of this pathogen.

At formulation, the product has a pH of approximately 6.0 and a water activity of 0.960—0.965. At these levels these parameters neither alone or in combination are sufficient to prevent the growth of salmonellae. Reduction in water activity to control Salmonella growth is brought about by controlled moisture loss through the permeable casing. However this must be achieved slowly (about 2%/day average) otherwise uneven drying and consequent case hardening will occur.

This rate limiting a_w reduction cannot be used to control Salmonella growth in the early critical stage of manufacture, therefore pH must be reduced rapidly if Salmonella growth is to be prevented.

A number of approaches to the elimination of salmonellae from fermented salami have been examined. After formulation, the product is first fermented then it undergoes a drying process. This whole procedure usually takes a minimum of 14 days and depends to a large extent on the diameter of the product. The aim of these investigations has been to arrive at a set of recommendations that will ensure that the product is free of salmonellae by the end of the minimal ripening period and before it is released for retail sale.

The areas investigated include:

(i) the mechanism of pH reduction;

(ii) rate of pH reduction;

(iii) low temperature pasteurisation.

The pH of salami can be reduced by one of three methods. The first is the use of the food grade acidulent glucono-delta-lactone often referred to as Gdl. This is a cyclic compound which hydrolyses on contact with water to yield gluconic acid which brings about a reduction of about 0.6 of a pH unit within a few hours. The usual concentration used is 0.5%.

Alternatively the pH can be reduced by the use of starter cultures which consist of micro-organisms which utilise the added carbohydrate in the salami mix and produce lactic acid via the glycolytic pathway. The third method often referred to as back slopping consists of using ripened salami from a previous production batch in a similar way that starter cultures are used. Once again in this case, acidification occurs as a result of lactic acid production.

Apart from the mechanism of pH reduction, we have examined the effect of the rate of pH fall on the growth and survival of salmonellae. In addition to investigating the mechanism and rate of pH reduction, the effectiveness of a mild heat pasteurisation (following the ripening process) has also been examined.

Methodology

The experimental protocol consisted of manufacturing salami to a generic recipe and inoculating with a variety of Salmonella serotypes (Table 1). These particular serotypes were chosen because they were the ones most frequently isolated from salamis implicated in the outbreak of the early 1980s.

Following stuffing into moisture-permeable casing, sausages were fermented at 27°C and 90% R.H. for 48 h then dried and ripened for a further 12—14 days at 15°C and 75% R.H.

Table 1 Salmonella Serotypes – Inoculation Experiments

1. *S. anatum*	7. *S. typhimurium* phage type 1
2. *S. derby*	8. *S. infantis*
3. *S. muenchen*	9. *S. schwarzengrund*
4. *S. newport*	10. *S. ohio*
5. *S. johannesburg*	11. *S. havana*
6. *S. adelaide*	12. *S. livingstone*

In this first series of experiments (Figure 1), acidification using glucono-delta lactone was compared with use of starter culture which produced lactic acid at a relatively slow rate. In all experiments a control batch of salami made from the same mix but containing neither Gdl or starter culture was always included. In this case pH reduction had been brought about by the activity of the natural background bacteria present on the meat.

When acidification was achieved by Gdl the effect on the destruction of Salmonella was only marginally better than that brought about by the natural background flora. In the case of the slow acid producing starter culture a reduction of approx. 90% was observed, however in all cases a significant number of salmonellae still remained at the end of the ripening period. In no case did any growth of Salmonella occur.

In the next series of experiments (Figure 2), acidification of salami with Gdl was compared against the use of starter culture capable of rapid acidification. Once again a control batch was included. Elimination of Salmonella during the normal ripening period was minimal in the case of control and Gdl treatments. In the case of the control sausages, Salmonella numbers increased during the first two days. In contrast to these two treatments, when rapid acidification was achieved by a starter culture a 99.9% reduction in Salmonella numbers was observed.

In the experiments already discussed, the Salmonella cultures were added to the meat mix immediately after cutting and under these circumstances did not have an opportunity to adapt to the environment before the fermentation process commenced and the pH was reduced. Such is the case if frozen meat is used to make salami. However it is also very common to use chilled meat in the process and it has been found that chilled meat can be held for up to 5 days prior to use. Whilst at these low temperatures Salmonella cannot grow, it is possible

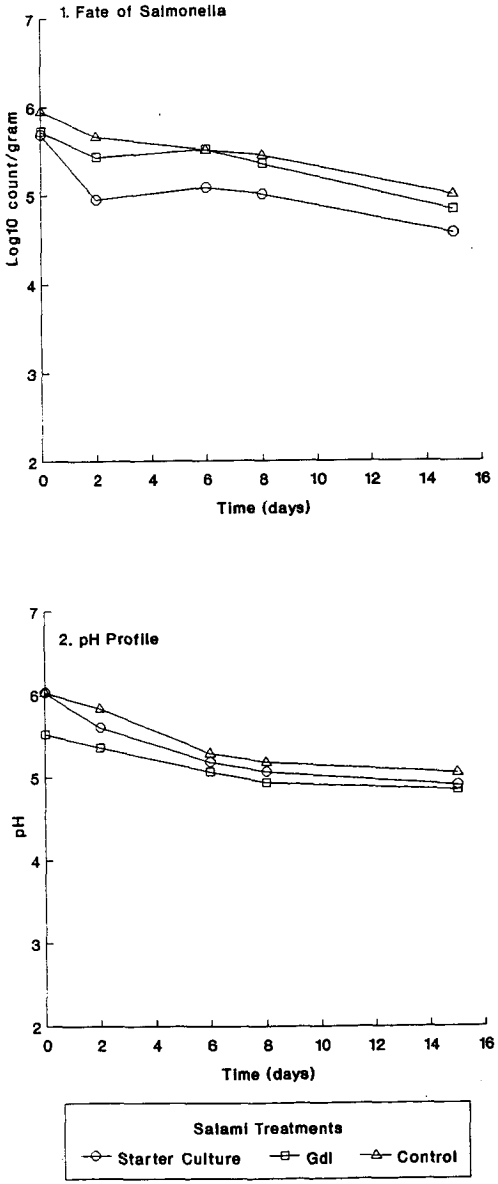

Figure 1 Effect of Slow Acidification Rate and Method on Salmonella spp. in Salami

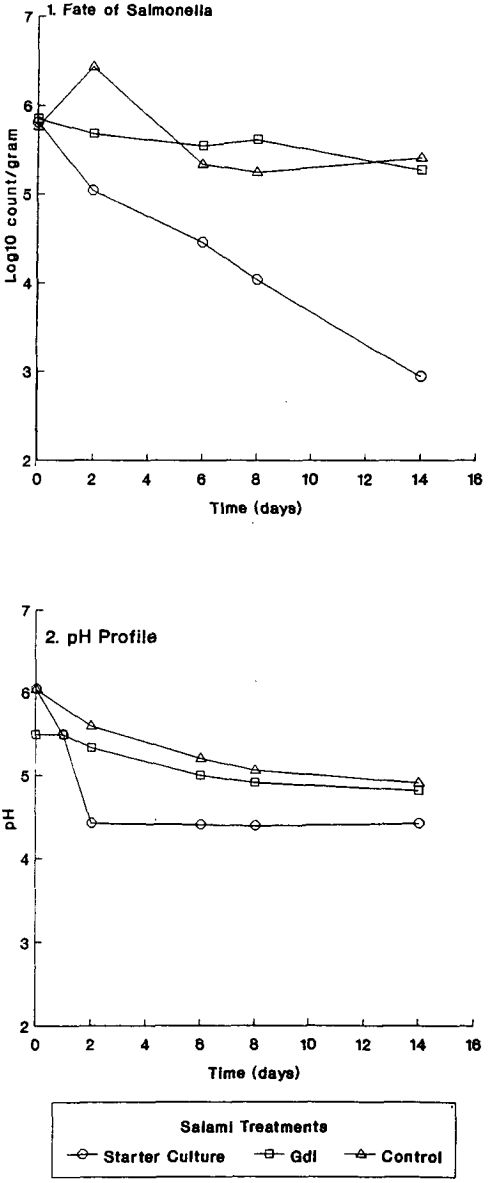

Figure 2 Effect of Rapid Acidification Rate and Method on Salmonella spp. in Salami

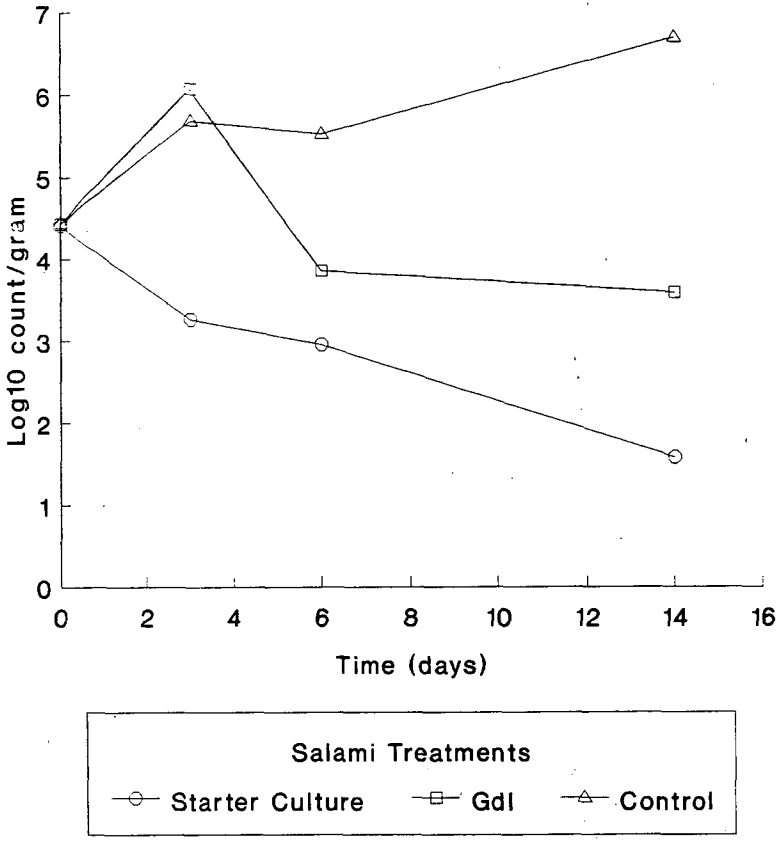

Figure 3 *Effect of Rapid Acidification Rate and Method on Pre-adapted Salmonella spp. in Salami*

for the cells to adapt to the meat environment. This situation was simulated by inoculating Salmonella onto meat and holding it at 0°C for three days. Salami was manufactured in the normal manner and held under conditions identical to those in the first series of experiments.

The results of a typical experiment designed to examine the effect of acidification rate and method on pre-adapted Salmonella are contained in Figure 3. In the case where product was acidified rapidly with starter culture, a similar effect on survival of inoculated Salmonella was observed. However when product was acidified with Gdl we observed a significant increase during the first three days. The number of viable Salmonella then declined, however by the end of

the ripening period the level of these organisms decreased only slightly over what was originally present. In the case of sausage made without either Gdl or starter culture, the Salmonella numbers had increased more than 100-fold over what were originally present.

In summarising this section of the work, rapid acidification brought about by lactic acid production by starter culture is effective in preventing the growth and reducing the survival rate of salmonellae in fermented salami, regardless as to whether chilled or frozen meat is used. In contrast, acidification brought about initially by Gdl can result in growth if the contaminating salmonellae are given an opportunity to adapt to the environment (as can be the case when chilled meat is used). Regardless as to whether chilled or frozen meat is used, acidification brought about by Gdl results in much less death of these organisms during the normal ripening period.

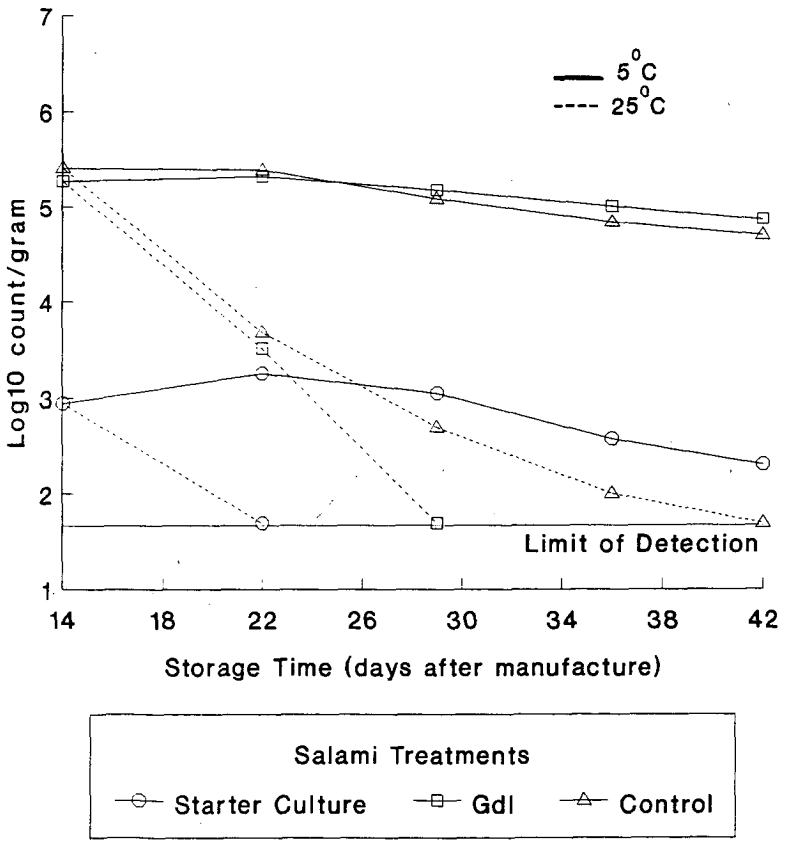

Figure 4 *Survival of Salmonella spp. Post-ripening Effect of Retail Storage Temperature*

As a corollary to this work, the death rate of Salmonella in fermented salami as a function of retail storage temperature was investigated. Salamis are often vacuum packaged after ripening to prevent further weight loss and whilst they should be shelf-stable at this point it is common to store them at the normal retail chill temperature (5°C). The results in Figure 4 demonstrate that the survival duration of Salmonella is affected by post-ripening retail storage temperature. At 5°C Salmonella remain viable in products such as these for a very much longer period than at 25°C, in fact in excess of 42 days after manufacture, whereas when product is stored at 25°C the death rate is accelerated.

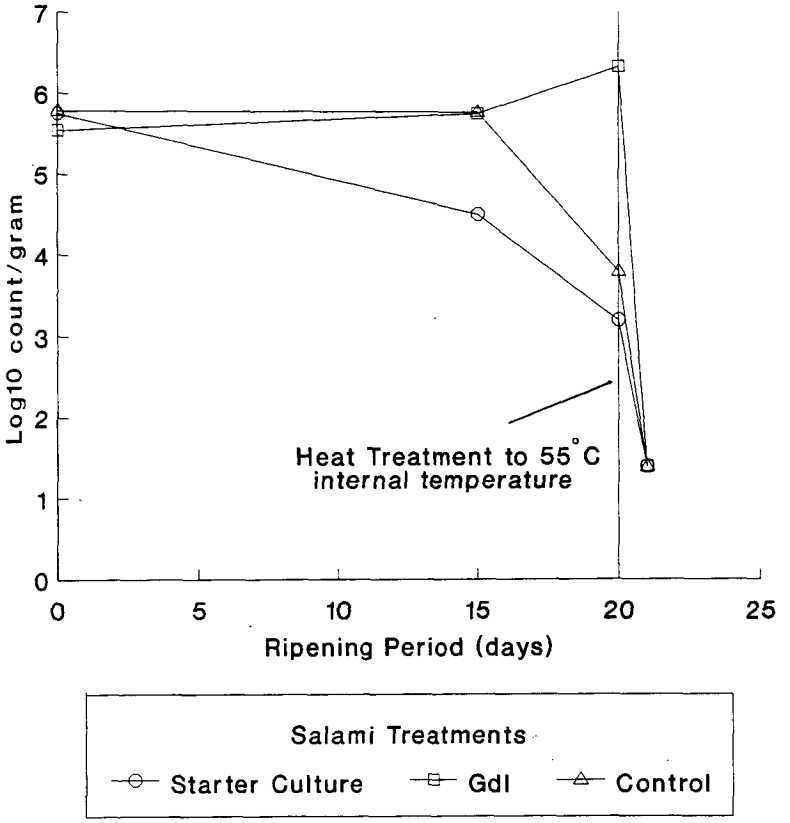

Figure 5 Reduction in Salmonella Numbers in Salami as a Result of Low Temperature Pasteurisation

Low Temperature Pasteurisation

The possibility of using low temperature heat treatment as a means of eliminating Salmonella that survive the ripening period has been investigated. The rationale for this approach was that any surviving Salmonella will be acid injured and hence more susceptible to a mild heat treatment that under normal circumstances would not be sufficient to result in significant destruction.

When heating salami there are two problems to overcome. One is the prevention of 'fat out' or fat separation as a result of heating, and the other is minimising the sensory changes that occur.

From the point of view of destruction of salmonellae, the heat treatment arrived at has to be a compromise between the extent of death of salmonellae and acceptable sensory changes.

Through experimentation it was found that the minimal effective heat treatment for Salmonella destruction under these conditions involved thermally processing the product to an internal temperature of 55°C. If this was achieved in a chamber set at 65°C and 90% R.H., it was possible to heat treat fermented salami acidified with rapid acid producing starter cultures without fat separation occurring. However the same heat treatment applied to Gdl acidified salami results in fat-out occurring. The results of the antibacterial effect of this heat treatment are shown in Figure 5. Up to a 5 log (99.999%) reduction was observed in the numbers of salmonellae following heat treatment at 55°C but it must be emphasised that this type of thermal processing is limited to salamis that have undergone rapid acidification to a pH of less than 5.0 within the first 24 h. In order to determine the sensory acceptability of the heat treatment, heated and non-heated salamis from the same batch were evaluated by a trained analytical taste panel.

Table 2 Attributes used to evaluate salami before and after heat treatment

Appearance	**Texture**
1. Colour	7. Initial Bite
2. Fat Content	8. Chewiness
3. Appearance Acceptability	9. Greasiness
	10. Texture Acceptability
Aroma	
4. Acid Aroma	**Flavour**
5. Other Aroma	11. Acid Flavour
6. Aroma Acceptability	12. Other Flavour
	13. Flavour Acceptability
	14. Overall Acceptability

The panel was asked to rate both treatments according to the attributes contained in Table 2. Where the particular attribute in this table is underlined, the panel considered the treatments to be significantly different from each other.

In summary, whilst panellists rated the colour of non-heat-treated salamis slightly higher than heat-treated product, they greatly preferred the texture of heat treated samples and rated them firmer than the non-heated counterpart. They also considered heat-treated samples to be less greasy. However it was the assessment of the flavour attribute that showed the most difference. Panellists strongly preferred the flavour of the heat-treated samples and overall considered the mild-heat-treated salami to be superior. Hence mild heating cannot only be used to reduce Salmonella numbers; according to our taste panel results it improves the sensory attributes of the product.

Subsequent to the outbreak of salmonellosis in the early 1980s, the National Smallgoods Council of the Meat and Allied Trade Federation of Australia issued a code of practice for the hygienic manufacture of dry and semi-dry sausage. Section III of this code of practice under point 8(c) contains the following:

> *pH measurements should be utilised to assure that processes attain a pH of 5.2 within 48 hours. One or more of the following means can be used to achieve this level of pH drop if these pH measurements establish that current practice is not allowing for the required rate of acid production.*
>
> *(i) Acid producing cultures which are free of contaminants and consistent in growth and acid production (starter culture);*
>
> *(ii) chemical acidulants which are thoroughly blended into the meat mixture (e.g. glucono-delta-lactone);*
>
> *(iii) inoculum from previous production of known bacterial count and pH value.*

With regard to the use of Gdl, these results have demonstrated that under certain conditions, acidification brought about by this method can result in increases in the numbers of salmonellae– a highly undesirable situation.

The American Meat Institute has issued a guideline for good manufacturing practices for fermented dry and semi-dry sausage. These guidelines recommend that pH reduction in salami be brought about through the action of lactic acid bacteria alone.

In the light of these results and in line with practices used overseas, it is perhaps timely to review our own code of practice with special regard to recommendation for pH reduction in these types of product.