Factors Affecting Growth and Survival of *Salmonella* in Fermented Salami Manufactured under Australian Conditions

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SUMMARY: Fermented salami (dry sausage), manufactured under Australian conditions has been associated with food poisoning outbreaks due to *Salmonella*. The three methods commonly used to acidify fermented salami in Australia include, natural (i.e. uncontrolled) fermentation, use of starter cultures (controlled) and inclusion of the food grade acidulant glucono-delta lactone (Gdl).

The results of experiments designed to compare and contrast the effectiveness of acidification method have demonstrated that pH reduction bought about by lactic acid production by starter cultures is far more effective for the control of *Salmonella* than the use of Gdl alone. Additionally, starter cultures capable of reducing pH rapidly (i.e. from 6.0 to 5.0 or less) within 24 hours resulted in a 3 log reduction in *Salmonella* numbers. In contrast, Gdl effected only a one log reduction under identical conditions. Depending upon the physiological state of contaminating *Salmonella*, it is possible for growth to occur when acidification is bought about by Gdl alone.

INTRODUCTION: Fermented sausages receive no heat treatment and, consequently, rely on other factors for their safety and shelf stability. Comminuted meat products such as salami can commonly be contaminated with salmonellae. Products such as these have been responsible for outbreaks of salmonellosis in both Australia and Italy (Marazza and Crespi, 1974) and an incident of staphylococcal food poisoning was traced to Genoa sausage manufactured in the United States (Genigeorgis, 1972).

Subsequent to the problem that occurred in Australia in the early 1980s, the use of Gdl became more widespread as a means of bringing about rapid pH reduction. Whilst (in Australia) salami is normally ripened under controlled conditions of temperature and relative humidity it is not uncommon to carry out the fermentation at ambient temperature and high relative humidity (approximately 90%). With this background, we have evaluated the effectiveness of Gdl in controlling the growth and survival of salmonellae in fermented salami and compared this method of acidification with that achieved by the use of starter cultures.

MATERIALS and METHODS: Salami formulation - Sausage fabrication was carried out in 15 or 20 kg batches in the Laboratory's pilot plant facility. The generic salami formulation which was common to all experiments consisted of the following: beef trims, 32%; pork trims, 32%; pork back fat, 32%; sodium nitrite, 0.0125%; sodium chloride, 3%; spices, 0.6% and glucose 0.6%. When Gdl was included the concentration was 0.5%. The tempered frozen (-5°C) or the fresh meat (0°C-2°C) was cut into cubes of approximately 3 cm prior to freezing or in

the case of experiments where salmonellae was pre-adapted to the meat environment, the m was minced through a 13 mm Hobart mincer (Model A-200 Hobart, Brisbane, Australia).

After the meat was chopped, the pork back fat (-10°C) was added and, depending upon experimental design, this was followed by either Gdl or starter culture.

The mixture was chopped in a Strommen 30 litre cutter (Model 423.13, Randers, Dema and, following inoculation with salmonellae, stuffed into 55 mm Hoechst Fibrous Casings means of a Dick, 12 litre manually operated sausage stuffer.

Sausages were weighed (about 425 g) and clipped, using a Poly-Clip System (Nieder GmbH, Frankfurt, Germany, Type 5FC). Following fabrication and stuffing, the sausa underwent a fermentation phase at 28°C and 90% R.H. for 48 hours. Upon completion fermentation, the product was placed in a drying room at 15°C for 2 days at 85% R.H. af which the R.H. was reduced to 75%. Product was held there for a further 10-12 days.

MICROBIOLOGY: Strains - The following twelve serotypes of Salmonella were used in inoculation experiments: S.adelaide, S.anatum, S.derby, S.havana, S.infant S.johannesburg, S.livingstone, S.munchen, S.newport, S.ohio, S.schwarzengrund S.typhimurium phage type 1. These organisms were chosen because these were the seroty most commonly isolated from salami responsible for the food poisoning outbreak in Austral

Inoculum - In experiments where salmonellae were not pre-adapted, cultures were gr to stationary phase by incubating aerobically overnight (16 hours) in Tryptone Soya Broth (Oxoid Code CM129). Where salmonellae were pre-adapted, the cultures were incub overnight, as previously described, then held at 5°C for 72 hours after which they w combined in a mixture, diluted in physiological saline, and mixed into the coarsely mi meat by hand. This inoculated meat was held for a further 2 days at 0°C-1°C prior to addition of Gdl or starter culture.

Enumeration of Salmonella - Salmonella was enumerated by the following method. Two grams of representative cross sectional area of the salami were removed observing aseptic technique and macerated in 80 mls of 0.1% Neutralised Bacteriological Peptone (Oxoid L34) by means of Colworth Stomacher (Model No.400). Aliquots, (0.1 ml) of appropriate serial dilutions (made in the aforementioned diluent) were spread over the surface Peptone Agar Plates (Grau, 1983) which were incubated for 72 hours under anaerobic conditions in an Oxoid Anaerobic Jar. Salmonellae appear as translucent colonies on this media and are approximately 1 mm in diameter. Because this media is nutritionally sparse the growth of the background flora (in particular lactic acid bacteria and other components of starter cultures) is kept to a minimum during this normal incubation period. A representative number of suspect colonies were purified on C.L.E.D. Agar (Oxoid Code CM 3) and colonies giving a typical reaction on this media were tested to determine that they were lysine decarboxylase positive and beta-D-galactosidase negative.

Starter cultures were enumerated on Tryptone Soya Agar (Oxoid Code CM 131) supplemented with 0.2% Yeast Extract (Oxoid Code L21) and 0.2% glucose.

pH determination - The pH of salami was determined by blending 20 grams of sausage mixture in 80 mls of distilled water and measuring with a TPS pH meter (Model LC80) fitted with a Philips C64 combined electrode.

RESULTS and DISCUSSION: The survival of salmonellae not pre-adapted to low temperature or the meat environment is exemplified by the results shown in Figure 1. In this experiment, acidification brought about by the use of Gdl resulted in only a 0.9 log reduction in the numbers of salmonellae. A starter culture which brought about a slow reduction in the pH due to lactic acid production resulted in a 1.1 log death. In this experiment the control sausages which contained neither starter culture nor Gdl but relied on the fermentative activity of the normal background flora effected about the same reduction (0.8 log) in salmonellae numbers as did those treated with Gdl. Clearly, in this situation neither Gdl nor starter culture proved effective with regard to elimination of salmonellae by the end of the drying period when the product would be released for sale. The pH profiles of the various treatments are shown on the right hand side of Figure 1.

In Figure 2 the results of a typical experiment designed to compare and contrast the effectiveness of Gdl and a starter culture capable of more rapid pH reduction are shown. A number of sausages inoculated with salmonellae but containing no Gdl or starter culture were included as controls. When a starter culture capable of more rapid pH reduction was employed a 3.0 log reduction in the numbers of viable salmonellae was achieved (Figure 2). In contrast salamis manufactured from the identical batch and acidified with Gdl showed only a 0.6 log reduction. In the sausages used as controls in this experiment a 0.3 log reduction occurred.

This experiment was repeated using a common batch of sausage mix, and inoculating with both rapid and slow acid-producing starter cultures. The results obtained confirmed those that are shown in Figures 1 and 2.

The experimental protocol was repeated but in this case the salmonellae were pre-adapted and these results are contained in Figure 3. Where product was acidified by starter culture capable of rapid pH reduction, salmonellae numbers were reduced by log 2.9. In contrast, when Gdl was used a 1.7 log increase occurred in the first three days, thereafter the viable count declined and by the end of the drying period there was a 0.9 log reduction over initial numbers. Salmonella numbers in control sausages increased by 1.3 logs after three days and by the completion of maturation (14 days) the salmonellae count was log 2.3 higher than the initial count.

These results clearly indicate that the growth and survival of salmonellae in fermented salami manufactured under these conditions is highly dependent on both the mode and the rate of pH reduction. If rapid acidification is achieved by lactic acid production by starter cultures there is no growth and a much more pronounced decline in salmonellae numbers. This occurs regardless as to whether these organisms are pre-adapted or not. When acidification is achieved by the use of Gdl or when the process relies on the acid producing

capabilities of the normal background flora, then growth and/or survival of salmonellae occur. This will depend upon the physiological status of contaminating salmonellae. Ra acidification by lactic acid production (starter cultures) in contrast to pH reduction hydrolysis of Gdl (gluconic acid release) is more effective against growth and survival salmonellae in this environment. The antimicrobial effects of lactic acid in undissociated form are well documented, particularly against Gram-negative bacteria such these (Grau, 1981; Newton and Gill, 1982; Smulders et al. 1986) and this would appear offer a partial explanation for the observed effects of rapid acid-producing star cultures as opposed to Gdl. The practical implication of these findings are manifested w dry sausage is fabricated using either frozen meat or chilled meat that has been held fo number of days at 0°C-1°C. A code of practice for the hygienic manufacture of dry and se dry sausage prepared by the National Smallgoods Council of the Meat and Allied Tra Federation of Australia recommends that the process attain a pH of 5.2 within the first hours. In addition it states that acidification can be achieved by the use of star cultures, Gdl or inoculum from a previous production of known bacterial counts and pH. results, indicate that some of these procedures may not always ensure the production fermented salami free of salmonellae.

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Figure 1 & 2: Effect of Acidification Rate and Method on *Salmonella ssp.* in Salami

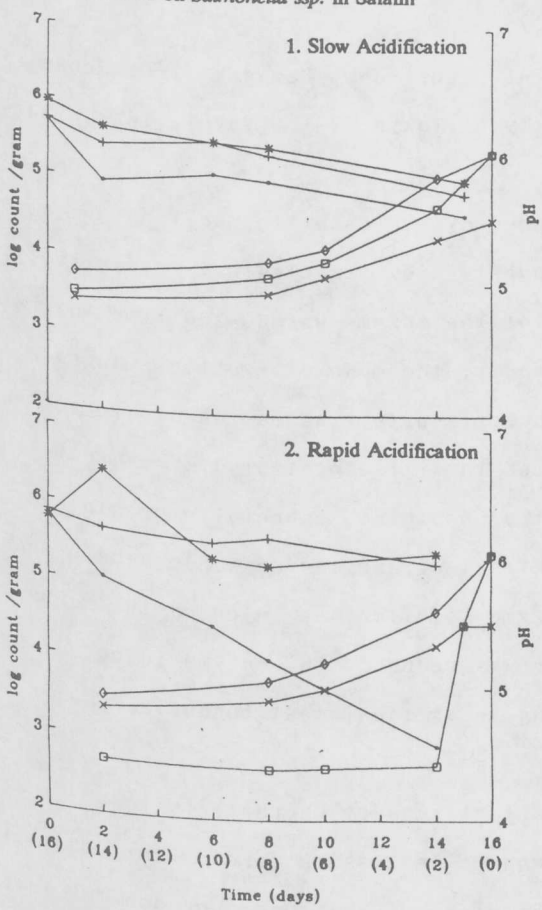

Figure 3: Effect of Rapid Acidification Rate and Method on Pre-adapted *Salmonella spp.* in Salami