Survival of *Salmonella typhimurium* and *Staphylococcus aureus* in Genoa Salami of Varying Salt Concentration

S. Messier, H.J. Smith and F. Tittiger

ABSTRACT

Genoa salami prepared using three different salt concentrations (2.0, 2.75 and 3.3%) were inoculated with 2.0 x 10^3 and 1.1 x 10^3 bacteria/g of *Salmonella typhimurium* and *Staphylococcus aureus* respectively. Over a period of 74 days samples were taken and analyzed for water activity and pH, counts of *S. aureus* and presence of *Salmonella*.

After 11 days of dry-curing *Salmonella* could no longer be detected by preenrichment followed by selective enrichment procedures. Viable *S. aureus* were still found after 74 days of dry-curing. The results of this study would suggest that water activity and pH measurements are useful in evaluating the safety of dry-cured products.

RÉSUMÉ

Cette expérience portait sur des échantillons de salami Genoa qui contenaient respectivement 2%, 2,75% et 3,3% de sel; elle consistait à leur inoculer *Salmonella typhimurium*, à raison de 2 x 10^3 salmonelles/g, et *Staphylococcus aureus*, à raison de 1,1 x 10^3 staphylocoques/g. Au cours des 74 jours qui suivirent, on en préleva des échantillons pour vérifier l'activité aqueuse et le pH, ainsi que pour y dénombrer les staphylocoques et vérifier la présence de salmonelles.

Après 11 jours de salaison à sec, on ne pouvait plus détecter de salmonelles en utilisant un enrichissement avant l'ensemencement et un milieu de culture pourvu d'un enrichissement sélectif. Au bout de 74 jours, on réussit cependant à recouvrer des staphylocoques viables. De tels résultats semblent suggérer l'utilité de la détermination de l'activité aqueuse et du pH, lors de l'évaluation de l'innocuité des produits salés à sec.

INTRODUCTION

Because Genoa salami is prepared from raw pork the curing process is relied on to destroy enteric pathogens which may be present. Salt concentration, pH, and water activity (a_w) are the main parameters affecting the safety of dry cured products (1).

The influence of a_w on bacterial growth has been reviewed by Sperber (2). Concentrations of salt have a marked effect on a_w. Measurements of pH and a_w offer a ready means for predicting survival of specific organisms and shelf life. For example, Leistner *et al* (3) proposed that canned meat products with a pH value greater than 6.3 had to have an a_w value of less than 0.94 to prevent growth of bacterial spores, especially those of *Clostridia*. Rodel and Leistner (4) have proposed a classification of meat products into highly perishable, perishable and shelf stable based on pH and a_w.

Salmonella and enterotoxin producing staphylococci surviving the dry curing process are a major concern for the safety of these products. The following study was conducted to evaluate the influence of modification in salt concentration on the survival of these pathogens in Genoa salami.

MATERIALS AND METHODS

BACTERIAL STRAINS

Staphylococcus aureus ATCC #25923 and *Salmonella typhimurium* ATCC #14028 strains were used. Bacteria were grown in 100 mL of brain heart infusion (BHI) (Difco, Detroit, Michigan) at 37°C with agitation (100 rpm). Aliquots from the logarithmic phase of each bacterial strain were collected and diluted appropriately with 0.1% peptone water (Difco, Detroit, Michigan) to inoculate the meat preparation of Genoa salami. The inoculum contained 2.0 x 10^3 and 1.1 x 10^3 bacteria/g of ground meat for *S. typhimurium* and *S. aureus* respectively, as determined by plate counts.

PREPARATION OF GENOA SALAMI

Genoa salami at three different salt concentrations (2.0, 2.75 and 3.3%) were prepared as described elsewhere (5). Bacteriological analysis of the raw meat, prior to inoculation, gave the following results: total aerobic count of 1.5 x 10^4 bacteria/g; a *S. aureus* count of 64 bacteria/g; and no *Salmonella*.

ENUMERATION OF *S. AUREUS*

On days 1, 4, 11, 18, 25, 32, 40 and 74 postpreparation, samples were tested for *S. aureus* and *S. typhimurium*. Five 25 g samples of product from each salt concentration were each put into 225 mL of nutrient broth (NB) (Difco, Detroit, Michigan) and homogenized with a Stomacher (Seward Medical, London, England). Tenfold dilutions were made in 0.1%

Agriculture Canada, Laboratoire d'hygiène vétérinaire, 3400 boul. Casavant, Saint-Hyacinthe, Québec J2S 8E3 (Messier), Agriculture Canada, Health of Animals Laboratory, P.O. Box 1410, Sackville, New Brunswick E0A 3C0 (Smith) and Agriculture Canada, Meat Hygiene Division, Halldon House, 2255 Carling Avenue, Ottawa, Ontario K1A 0Y9 (Tittiger).

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peptone water and 0.2 mL of the 10^{-1} and 10^{-2} dilutions spread on Baird-Parker plates (Gibco, Madison, Wisconsin). Suspect colonies of *S. aureus* were counted after 48 h incubation at 37°C. To confirm suspect colonies of *S. aureus*, a representative number of colonies were inoculated into rabbit plasma (Difco, Detroit, Michigan) to detect coagulase-positive colonies.

ISOLATION OF *SALMONELLA*

The NB containing the homogenized product was incubated for 20-24 h at 37°C. Following incubation, 1 mL aliquots from each sample were inoculated into selenite cystine (SC) (Gibco, Madison, Wisconsin) and tetrathionate brilliant green (TBG) broth (Gibco, Madison, Wisconsin) to increase the chances of isolating *Salmonella*. The SC tubes were incubated at 37°C and TBG tubes at 43°C for 20-24 h. Following incubation each SC and TBG tube was inoculated onto a bismuth sulfite (BS) (Gibco, Madison, Wisconsin) and a brilliant green sulfa (BGS) plate (Difco, Detroit, Michigan) and the plates incubated at 37°C for 20-24 h. Suspect colonies were then picked, identified biochemically by reactions on triple sugar iron (TSI) (Difco, Detroit, Michigan), urea (Becton Dickinson and Co., Cockeysville, Maryland) and lysine iron agar (LIA) (Difco, Detroit, Michigan), and confirmed serologically as *Salmonella* sp. using a polyvalent antiserum (Difco, Detroit, Michigan).

WATER ACTIVITY AND pH MEASUREMENTS

Water activity (a_w) and pH were measured on all sampling days. Two composite samples were made up for each salt concentration by picking at random five 10 g pieces of salami, not closer than 1 cm from the outer surface, and mixing them together. One was used for a_w and the other for pH measurement. The a_w was determined using an a_w-value analyzer model 5803 (G Lufft, Stuttgart, West Germany) according to the manufacturer's instructions. The pH values were obtained using a pH meter Accumet model 630 (Fisher Scientific, Ville St-Laurent, Québec) with a flat surface electrode model E-5 D (Fisher Scientific, Ville St-Laurent, Québec).

STATISTICAL ANALYSIS

Staphylococcus aureus counts among batches for each sampling day were analyzed by one-way ANOVA.

RESULTS

The five samples of Genoa salami from each salt concentration analyzed day 1 postpreparation were all positive for *Salmonella*. On day 4 postpreparation, all five samples from the 2.0 and 2.75% salt concentrations were positive for *Salmonella* and from the 3.3% salt concentration, four out of five samples were positive for *Salmonella*. All *Salmonella* identified were of the same serotype as the one used in the inoculum. On the 11th day postpreparation, and on all subsequent sampling days, all five samples from each of the salt concentration were negative for *Salmonella*.

The mean numbers of *S. aureus* found in Genoa salami at day 1 postpreparation were 50 to 70% of those of the inoculum (Fig. 1). Numbers of *S. aureus* at the 2.75 and 3.3% salt concentration remained low throughout the sampling schedule except on one occasion (40 days postpreparation) when the mean number of *S. aureus* was found to be 595 bacteria/g in the 3.3% salt concentration sample. For the 2.0% salt concentration, numbers of *S. aureus* were variable from one sampling day to another and

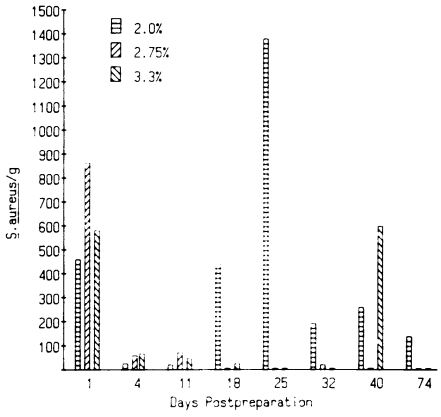

Fig. 1. Mean numbers of *Staphylococcus aureus* found in Genoa salami with salt concentration of 2.0, 2.75 and 3.3% at intervals postpreparation.

TABLE I. Water Activity and pH Measurements for Genoa Salami with Salt Concentration of 2.0, 2.75 and 3.3% on Days Postpreparation when Bacteriological Analyses Were Performed

Day Postpreparation	% Salt	Water Activity	pH
1	2.0	0.98	4.99
	2.75	0.98	5.48
	3.3	0.93	5.51
4	2.0	0.98	5.66
	2.75	0.97	5.77
	3.3	0.96	5.79
11	2.0	0.96	4.84
	2.75	0.95	4.86
	3.3	0.93	4.87
18	2.0	0.94	4.92
	2.75	0.93	4.89
	3.3	0.94	4.89
25	2.0	0.94	4.77
	2.75	0.94	4.78
	3.3	0.93	4.76
32	2.0	0.93	4.90
	2.75	0.94	4.83
	3.3	0.93	4.79
40	2.0	0.93	4.84
	2.75	0.95	4.81
	3.3	0.90	4.79
74	2.0	0.88	4.81
	2.75	0.87	4.92
	3.3	0.87	4.82

the highest mean number was found on day 25 postpreparation.

On day 25 postpreparation, numbers of *S. aureus* present in the 2.0% salt concentration samples were significantly higher ($p < 0.01$) than those in the 2.75 and 3.3% salt concentration samples. A significant difference ($p < 0.05$) was also observed on day 74 postpreparation between the 2.0% and the 2.75 and 3.3% salt concentrations.

Although fluctuations were observed, the decrease in a_w during the course of the experiment was not influenced by variation in the salt concentration (Table I). A lower pH was noted on day 1 postpreparation with the 2.0% sample compared to the two other salt concentrations (Table I), but on all other sampling days pH values were very similar in the three salt concentrations.

DISCUSSION

Even though the processing method employed by this establishment was unusual in that fermentation and drying were conducted at refrigeration temperatures the results of this study should also be valid for dry-cured products prepared in other establishments. The rapid drop of pH was unexpected but may explain the marked inhibitory effect on the pathogens observed.

In this study *Salmonella* organisms were no longer isolated from the Genoa salami after the third sampling date, day 11 postpreparation, and on all subsequent sampling days. This appears consistent with the observation that there is a rapid decline in the numbers of gram-negative bacteria after only a few days of the fermentation process (6). Bacterial death was responsible for the disappearance of *Salmonella* because bacteria injured by the dry-curing process would have recovered in the preenrichment step with the NB. On day 11 postpreparation it was noted that pH values for all three salt concentrations were below 5.0 and remained below this level throughout the remaining of the sampling schedule. On days 1 and 4 postpreparation all but one a_w values were above 0.95 which is the minimum a_w reported for growth of *Salmonella* (2). On day 11 postpreparation, the a_w readings were above the 0.95 value for the 2.0% salt level but at 0.95 and 0.93 for the 2.75% and 3.3% levels respectively. This exemplifies the concept that the a_w value of a food is only one of the factors that interacts with others (pH, temperature, chemical preservatives) to create a preservative system (7).

The findings were less clear for *S. aureus* than for *Salmonella*. At all three salt concentrations, the rapid drop followed by the low counts of staphylococci up to the third sampling date would appear to be as expected considering the fact that staphylococci do not compete well with lactic acid producing bacteria involved in the fermentation process (6), and that the dose of inoculum used was low in comparison to levels that are required to cause staphylococcal food poisoning (8). At the 2.75% and 3.3% salt concentrations, counts of *S. aureus* remained low except on day 40 postpreparation in the salami with 3.3% salt concentration. A possible explanation may be that there was an uneven distribution of the staphylococci in the meat during the inoculation. In retrospect, it appears that each of the five subsamples could have been made up of five smaller samples pooled together. This could have decreased the variations observed. After reviewing the raw data (individual counts of the five subsamples for that day) it was noted that three out of the five subsamples had counts of 0 *S. aureus*/g, one had a count of 325 *S. aureus*/g and one had a count of 2 650/g. In the salami with 2.0% salt concentration, the higher counts following the initial decrease may also be due in part to uneven distribution of the inoculum. On day 25 postpreparation, two of the five subsamples had high counts of 2 500 and 3 000 organisms/g. On the other sampling days, although mean counts were not high, they were still higher than mean counts observed in salami with 2.75% and 3.3% salt levels, except on the one occasion as mentioned. This may be due to the lower salt concentration since there are no differences between the a_w and pH values. Levels of *S. aureus* never reached critical numbers where formation of enterotoxin might be expected to occur (6,8).

The above results are in keeping with the concept of other investigators, that various parameters, such as salt, nitrite, temperature, pH and a_w interact to create the desired effect of dry-curing. Measurement of pH and a_w appear to be useful for evaluating the safety of the products.

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