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# Fermented Mechanically Deboned Poultry Meat and Survival of *Staphylococcus aureus*

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ABSTRACT

Mechanically deboned poultry meat (MDPM) was used to formulate a fermented sausage product prepared by a natural lactic acid fermentation (fermentation by indigenous lactic acid bacteria). Salted MDPM (3% NaCl) stored at 5 C promoted growth of lactobacilli to a level of $10^6$ cells/g after 12 days. During the same period the population of indigenous *Staphylococcus aureus* decreased to below a detectable level but no change was observed in the population of added *S. aureus* ($10^7$ cells/g). The MDPM attained a pH of 4.7 after 60 h of fermentation with a corresponding developed acidity of 1.6% expressed as lactic acid. The heat treatment given to the sausage (to attain an internal temperature of 60 C) brought about a reduction in the population of both the lactobacilli and *S. aureus* (4.1 and 5.6 log cycles, respectively); the latter was decreased to an undetectable level. Acid, sodium chloride and sodium nitrite in combination with a heat treatment (60 C, 60 min) gave the largest reduction of the population of *S. aureus* resulting with a D-value of 23.6 min. Succinic acid in combination with either a heat treatment (60 C, 60 min) or low temperature storage (7 C, 7 days) was the most effective treatment against *S. aureus*. Other acids active against *S. aureus* arranged in decreasing order of effectiveness were lactic, acetic and citric.

Manufacture of fermented meat products is an important and dynamic branch of the meat industry. During 1976, Federally inspected plants processed over 136 thousand metric tons of fermented (dried and semidried) sausage registering a 13% increase over the previous year's production (*1*).

Many of the older and smaller manufacturers of fermented sausage still prepare their product using a natural fermentation (fermentation by indigenous lactic acid producing bacteria in the meat) (*7*). The process includes an aging period of the salted meat to enhance growth of lactic acid producing microorganisms (*9*).

Mechanically deboned poultry meat (MDPM) from broilers' necks and backs has been used in the formulation of fermented sausage using a starter culture (*5*). However, no details are available on natural lactic acid fermentation of MDPM and the microbiological changes that occur in the various steps, including the fate of pathogenic microorganisms.

Staphylococcal food poisonings and the occurence of *Staphylococcus aureus* in fermented sausage were recorded in 1971 following outbreaks of gastroenteritis due to *S. aureus*-contaminated Genoa sausage (*2,3*). The studies of Lee et al. (*8*) and Tatini et al. (*14*) showed that *S. aureus* may grow and produce toxin in fermented and non-fermented sausage.

This work was undertaken to study the natural fermentation of MDPM and its effect on *S. aureus*. In addition, the thermal resistance of *S. aureus* in the sausage mix and the effects of different organic acids were studied.

## MATERIALS AND METHODS

### *MDPM*

The MDPM was prepared from broilers' backs and necks using a Beehive Deboner Model AUX 1272 (Beehive Machinery, Inc. Salt Lake City, Utah). After deboning the MDPM was frozen (- 25 C) until use.

### *Sausage formula*

A semidry type summer sausage was prepared using the ingredients shown in Table 1.

TABLE 1. *MDPM sausage ingredients.*

| Ingredient | Amount (g/kg MDPM) |
|---|---|
| Dextrose | 15.00 |
| NaCl | 30.00 |
| Black Pepper | 3.00 |
| Sweet Paprika | 3.00 |
| $NaNO_2$ | 0.100 |
| Sodium Isoascorbate | 0.550 |
| Coriander | 0.300 |
| Mustard Powder | 0.150 |
| Allspice | 0.300 |

### *Processing*

The MDPM was mixed with NaCl and aged for 12 days in trays at 5 C to promote growth of lactic acid-producing microorganisms. After aging, the meat was mixed with the remainder of the ingredients and stuffed into 45-mm diameter fibrous casings which were transferred into the smokehouse at 30 C. When the sausage attained a pH of 4.7 they were cooked to an internal temperature of 60 C by gradually raising the temperature of the smokehouse to 65 C.

### *pH Measurements*

The pH was measured in a slurry prepared by blending (2 min) 30 g of sausage with 270 ml of 0.1% Peptone (Difco) water. The slurry was first sampled for bacteriological examinations before pH measurements were made.

### *Titratable acidity*

The slurry prepared for pH measurements was titrated to pH 7.0 using a standard base (0.1 N NaOH) and a pH meter. The acid was expressed as percent lactic acid.

### *Bacteriological examinations*

Appropriate dilutions were prepared using 0.1% Peptone (Difco) water. Total count was estimated using APT agar (BBL) incubated at 30 C for 48 h. Lactobacilli (gram positive rods, catalase and benzidine negative) were enumerated using Rogosa SL Agar (Difco) with

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incubation for 72 h at 30 C, while coagulase-positive staphylococci were determined on Baird-Parker medium (Difco). Incubation was at 35 C for 48 h. Selected *S. aureus* colonies were confirmed by the coagulase test.

*Inoculation*

All treatments were inoculated with $1.0 \times 10^7$ cells of *S. aureus* FRI 100/g (Food Research Institute, University of Wisconsin), an enterotoxin A producing organism that had been grown in BHI broth (Difco) (35 C, 18-20 h).

*Heat treatments*

MDPM inoculated with *S. aureus* FRI 100 ($1.0 \times 10^7$ cells/g) was divided into several equal portions. Three such portions are given: (*a*) acidified to pH 4.7 using lactic acid (Mallinckrodt); (*b*) as (*a*) plus 3% NaCl; (*c*) as (*b*) plus 100 ppm $NaNO_2$. The MDPM sample of each treatment [(*a*), (*b*) and (*c*)] was divided into six 60-g subsamples which were placed each in a beaker (48-mm internal diameter) and heated in a circulating 65-C water bath. The heat treatment was timed for 60 min when the center of the meat sample attained 60 C. The coming up time was 22 to 25 min. The temperature was monitored by inserting a copper constantan thermocouple (connected to a Honeywell recorder Model 153X64 Brown Instruments Div., Philadelphia, Pennsylvania) at the center of the sample. At 10-min time intervals a beaker was removed from the water bath and chilled immediately in crushed ice (to terminate the heat treatment) and the level of *S. aureus* was monitored.

The straight line formula $\log N/N_0 = \frac{-K}{2.303}$ was used to calculate a regression line for the results of the heat treatments (where N is the number of survivor at various heating time and $N_0$ is the initial number of *S. aureus* cells). The slope of the calculated straight line, $\frac{-K}{2.303}$, is equal the reciprocal of the decimal reduction time (1/D) and K represents the death rate constant.

*Effect of organic acids*

Acetic, citric, lactic and succinic acids (Mallinckrodt) were used to acidify the MDPM inoculated with *S. aureus* FRI 100 to pH 4.7. The individual MDPM portions acidified with different acids were divided into two subportions. One subportion was subjected to a heat treatment (60 C, 60 min) while the second was stored at 7 C for 7 days. At the end of each treatment, the level of *S. aureus* was determined. Each experiment was done in triplicate.

## RESULTS AND DISCUSSION

The total count of the uninoculated sample of salted (3% NaCl) MDPM increased from about $10^5$ to approximately $10^9$ cells/g during the aging period (5 C, 12 days) (Fig. 1). Lactobacilli increased from $10^3$ to $10^6$ cells/g in the same MDPM sample. A similar increase in lactobacillus numbers was observed in MDPM inoculated with *S. aureus*. The inoculated *S. aureus* population showed little or no change but the population of the indigenous *S. aureus* decreased below a detectable level after 9 days of aging (Fig. 1). No change in the pH of the MDPM was observed during the aging period. So pH cannot be considered as a factor during this phase for reduction of the population of the indigenous *S. aureus*. It is well established that lactobacilli produce certain antibacterial agents as byproducts of their metabolism such as hydrogen peroxide antibiotics (*13*) bacteriocins (*16*) and other unidentified agents (*10*). One or more of these antimicrobial agents may have caused the reduction of the population of the indigenous *S. aureus* during aging. These results verify the finding of Raccach and Baker (*11*) and Raccach et al. (*12*) who reported that

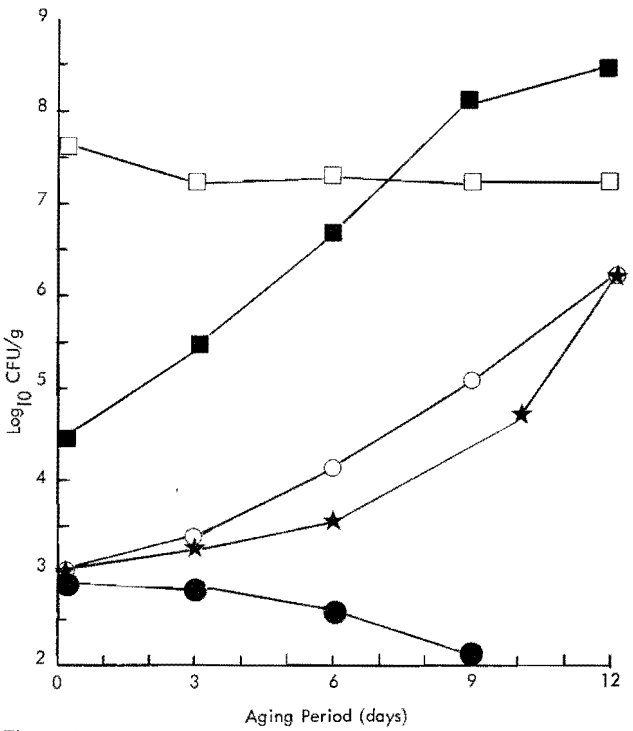


Figure 1. *The effect of aging MDPM on inoculated* S. aureus (□). *Total Count* (■); *lactobacilli in uninoculated* (○) *and inoculated* (★) *samples and indigenous* S. aureus (●).

the lactic acid-producing organisms should outnumber *S. aureus* population by a range of $10^5$ to $10^6$ to repress the pathogen. So it is not surprising that the population of the inoculated *S. aureus* ($1.0 \times 10^7$ cells/g MDPM) was not affected by the lactobacilli during this phase. The population of lactobacilli increased about 100-fold during the first 45 h of the fermentation in samples with and without added *S. aureus* (Fig. 2) but no change was observed in the total count of the uninoculated sample. The count of the population of the *Lactobacillus* on Rogosa SL agar equalled that of the total count in APT agar after 45 h of fermentation (Fig. 2). Almost all of the bacterial population isolated in APT agar consisted of gram-positive rods, catalase- and benzidine-negative. It is probable that the lactobacilli gradually became the predominant microorganisms by producing unfavorable conditions for competing flora in the sausage mix.

A pH of 4.7 was attained after 60 h of fermentation with a corresponding developed acidity of 1.6% (expressed as lactic acid) (Fig. 3). As shown in Fig. 3, more than 80% of the acidity developed before 45 h of fermentation decreasing the pH value to 5.0. The developed acidity may have suppressed the growth of many undesirable microorganisms including inoculated *S. aureus* (Fig. 2). A pH of 4.7 is the minimum for *S. aureus* growth while a pH value of 5.3 is the minimum for enterotoxin A production (*15*).

The heat treatment given to the sausage (Fig. 4) brought about a significant reduction in the different bacterial groups examined. The population of *Lactobacillus* determined with Rogosa SL agar showed a decrease of 7.7 $\log_{10}$ cycles but only 4.1 $\log_{10}$ cycles when

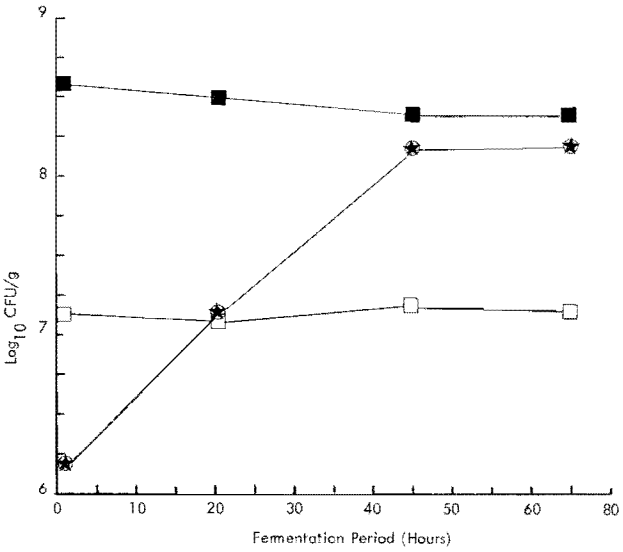


Figure 2. *The effect of MDPM-fermentation on the total count* ( ■ ); *lactobacilli in uninoculated* ( ○ ) *and inoculated* ( ✱ ) *samples with* S. aureus ( □ ).

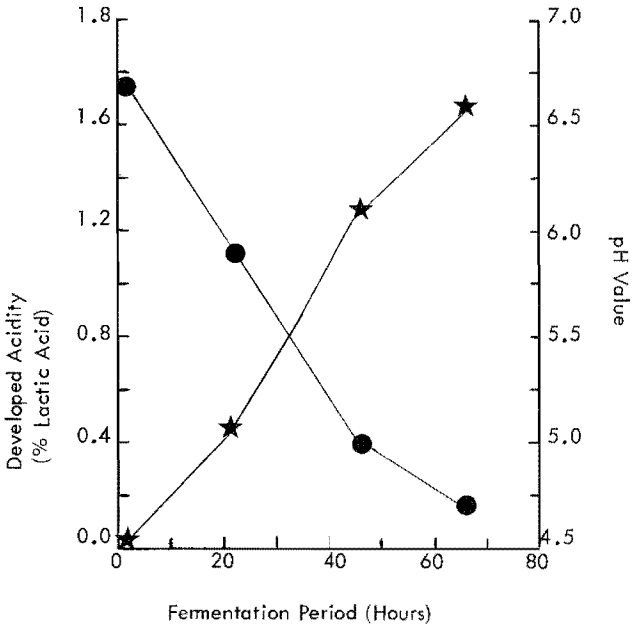


Figure 3. *Development of acid* ( ✱ ) *and changes in the pH* ( ● ) *of MDPM during the fermentation period.*

counted on APT agar. This difference may be ascribed to injured lactobacillus cells which lost their ability to form colonies on selective media. The population of inoculated *S. aureus* decreased to $< 10^2$ cells/g of sausage, a reduction of more than 5.6 $\log_{10}$ cycles. This reduction is very important especially from a public health aspect. In another study, at least $4.0 \times 10^7$ *S. aureus* cells/g were required to produce detectable enterotoxin A in sausage (*3*).

The heat treatment experiments were undertaken to examine the effect of lactic acid, sodium chloride and sodium nitrite on thermal resistance of inoculated *S. aureus* in the sausage. For comparative purposes, the D-value of *S. aureus* in acidified MDPM (44.7 min) was considered the basal value. Table 2 shows the effect of

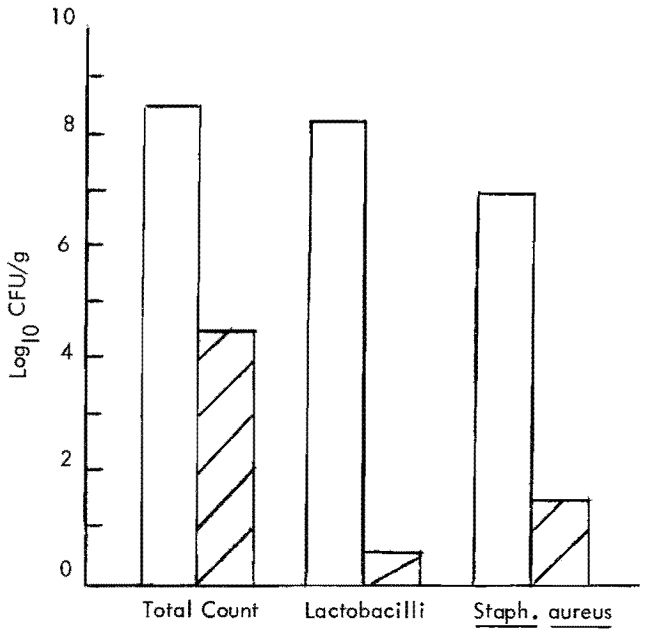


Figure 4. *The population composition of the fermented sausage before* ( □ ) *and after* ( ☑ ) *the heat treatment.*

TABLE 2. *The thermal resistance of* Staphylococcus aureus *in MDPM under different conditions.*

| Thermal resistance | Acid[a] | Acid & salt[b] | Acid, salt & nitrite[c] |
|---|---|---|---|
| Decimal reduction time (min), D | 44.7 | 32.6 | 23.6 |
| Death rate constant ($min^{-1}$),K | 0.05 | 0.07 | 0.10 |

[a]MDPM acidified with lactic acid to pH 4.7.
[b]Acidified MDPM (pH 4.7) + 3% NaCl.
[c]Salted acidified MDPM + 100 ppm $NaNO_2$.

the addition of sodium chloride alone or in combination with sodium nitrite to the acidified MDPM on the thermal resistance of *S. aureus*. Sodium chloride decreased the basal thermal resistance (D-value) by 37%. An additional 10% decrease was obtained by addition of sodium nitrite to the salted acidified MDPM. The D-value of *S. aureus* under these conditions was 50% of that in the acidified MDPM (Table 1). According to these results, addition of sodium chloride to the acidified MDPM promoted a greater reduction of the thermal resistance of the pathogens than addition of nitrite to the acidified salted MDPM. Goepfert and Chung (*6*) reported similar results. They found that a combination of acid and sodium chloride was responsible for destruction of *Salmonella* cells observed in a fermented sausage product.

Succinic acid had the greatest adverse effect on the population of *S. aureus* followed in decreasing order of effectiveness by lactic, acetic, and citric acids (Fig. 5). The heat treatment was more efficient than the low temperature storage only in combination with acetic and citric acids ($P < 0.05$). The effect of each one of these two acids in combination with the heat treatment was not significantly different ($P < 0.05$) than the control.

It is probable that heating at 60 C (a higher

temperature than the maximum growth temperature, 45 C, of *S. aureus*) of MDPM samples treated with acetic or citric acid enhanced the inactivation of staphylococci by these acids.

Citric acid treatment of the MDPM followed by low temperature storage did not inhibit *S. aureus* resulting with a survival three times greater than the control. No significant difference ($P < 0.05$) was found between the acetic acid-treated MDPM and the control at low-temperature storage. No significant difference ($P < 0.05$) was found between the heat treatment and low temperature-storage in combination with either lactic or succinic acid. Succinic acid in combination with either the heat treatment or the low temperature-storage caused about a 100-fold reduction in the population of *S. aureus*. This is comparable to the effect of lactic acid and sodium chloride in combination with the heat treatment ($D = 32.6$ min, Table 2). Lactic acid under the same conditions caused approximately a 50-fold reduction of the pathogen population. These results suggest that for direct acidification of MDPM use of lactic acid in combination with either a heat treatment or low temperature-storage is adequate even though succinic acid was superior. Since fermented sausages are acidified mainly by lactic acid and contain a minute quantity of acetic acid, it is tremendously important, especially from the practical standpoint, that these two acids were biologically active in MDPM against the population of *S. aureus*.

The results presented in Table 2 and Fig 5 showed that neither one of the acids used in combination with either a heat treatment or low temperature storage nor the lactic acid in combination with sodium chloride and sodium nitrite caused the same extent of reduction of the population of *S. aureus* observed in the sausage after it was given a heat treatment (Fig. 4). These data may suggest that the lactic acid-producing flora played a larger role than just acidifying the meat. The lactobacilli may have formed certain antibacterial agents which in combination with the heat treatment suppressed the population of *S. aureus*.

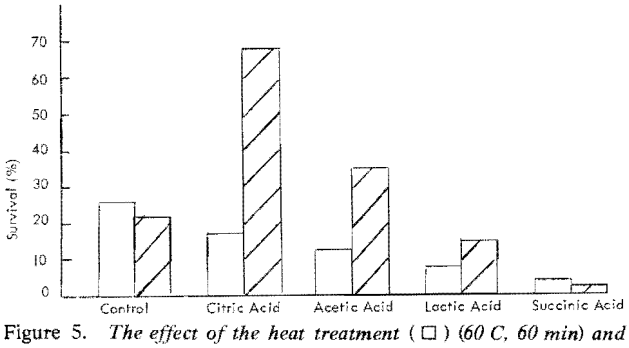


Figure 5. *The effect of the heat treatment* ( ☐ ) *(60 C, 60 min) and low temperature storage* ( ☑ ) *(7 C, 7 days) on the survival of* S. aureus *in MDPM.*

This work showed that under commercial conditions it is feasible to use MDPM for sausage production by a natural lactic acid fermentation. Even a gross contamination with *S. aureus* can be reduced below a detectable level if a proper heat treatment is given to the sausage. Nevertheless, these results do not imply that unsanitary conditions can be used along with a proper heat treatment. One should bear in mind that the heat treatment used in this work was effective against the cells of *S. aureus* but this may not be in the case against staphylococcal enterotoxin or other pathogens not examined in this study.

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