Bacterial Fermentations, Sodium Acid Pyrophosphate and Glucono Delta Lactone in Cured Sausage Production

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Food fermentations have been known and documented with the mention of bread making and wine production in the *Holy Bible*. Meat fermentations were mentioned in Homer's *Odyssey*, and the sausages were called *oryae*. Cured sausage production has fluorished since that time to each civilization and nation. The cured sausage production in the United States mirrors the population and its origins.

The Germanic population in the north central portion of Europe has given us summer sausage, cervelot, beef cervelot, pork cervelot, Thuringer, Mettwurst, and Rohwurst.

The Italian population brought with them the pepperoni, salami, mold salami, Genoa salami, Milano salami, hard salami, and Calabrese style salami.

The Scandanavian favorite, Goteborg, originated in Gothenburg, Sweden.

The French specialties *saucisson* and *sec.* (all pork) have been made here. And the Spanish favorite, chorizos, are currently produced in the same manner as in their native Iberian peninsula.

Current USDA (1980) production statistics show that 244,806,000 pounds of dry sausage and 96,390,000 pounds of semi-dry sausage were produced. Total cured sausage production was about 2.6 billion pounds for 1980 including franks and weiners, bologna, and other cured sausages.

Pederson (1971) has written an excellent review of the history of sausage uses and manufacturing. George A. Hormel Company has published an interesting and informative booklet, "The Romance of Dry Sausage," which details the dry sausages manufactured from each region of Europe and the history of each type of dry sausage.

Bacterial growth in cured sausage products can be classed as two types. The first type is the desired bacterial growth normally called fermentations; and the second type is the incidental, perhaps undesirable bacterial growth in the cured sausage.

Desirable bacterial fermentations can be achieved with the use of (1) *Micrococcus*, (2) *Lactobaccilus*, (3) *Pediococcus*, (4) *Streptococcus* (non-pathogenic), (5) *Staphylococcus* (non-pathogenic), and (6) *Leuconostoc*. Recently the use of yeast cultures in cured sausages have been introduced. These are of the *Debaryomyces* family. These bacteria and yeast are currently sold as meat starter cultures in frozen and freeze dried forms.

Undesirable or naturally-occurring microbial fermentations are usually those of (1) *Staphylococcus aureus*, (2) salmonella, (3) yeast, (4) mold, (5) clostridia, (6) bacillus. However, the use of selected types of *Penicillium* are used throughout the world for the production of unique flavors and appearance in dry sausages.

Growth Factors

The main factors which determine type of bacterial growth in cured sausages are (1) salt concentration in the sausage, (2) nutrients in the sausage, (3) temperature treatment of the sausage, (4) sodium or potassium nitrite level in the sausage, and (5) the bacterial load and the types of bacteria present in the sausage.

Sodium chloride is usually added to the sausage mixture at the rate of 2.50 to 3.75%. This would assay as about 4.0% to 6.0% initial salinity in the sausage mix. The addition of sodium chloride alone greatly influences the growth of bacteria that can grow in the sausage. The bacteria must be able to withstand the osmotic condition of at least 4.0% saline solution to grow. Leistner (1975) has postulated that each 1% of added sodium chloride depresses the water activity of meat at least .0062 units.

The carbohydrate nutrients present in meat are numerous, and meat is considered an excellent growth media. The addition of either dextrose or sucrose improves the probability of good bacterial growth in cured meat mixtures. Common ingredients which are added to cured sausages are sugar (usually corn syrup solids, dextrose, sucrose, lactose), non-fat dry milk solids, ascorbic acid, erythorbic acid, glucono-delta-lactone, and fat. All of these substances can be used by specific bacteria as a carbohydrate source.

The temperature treatment of the sausage greatly influences the bacterial growth in a cured meat product. Traditional curing procedures dictated the addition of the sodium nitrate to sausage, and the sodium nitrate would be converted to sodium nitrite by the action of Micrococci. The temperature of pan curing was usually less than 50°F (10°C). Cured sausages are normally treated with 78-156 ppm sodium nitrite to control *Cl. botulinum*. With the added use of lactic acid starter cultures, the pan curing step is eliminated from the process. Sausages can now be moved directly from stuffing to the

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smokehouse or green room for controlled fermentation. Temperatures of 70°-115°F (21°-45°C) can be used for controlled fermentation, lactic acid development, and complete curing. Lactic acid starter cultures have been developed which reduce the pH of the sausages to less than pH = 5.0 in less than 7 hours at the optimum growth temperature of the specified lactic acid starter culture. Nickerson and Sinskey (1972) noted that *Leuconostoc* and *Lactobacillus* will grow at 3.5°C (38.3°F) in cured meat products.

The types of desirable bacteria which will grow in cured sausages have a very wide temperature range, and the specific starter culture which is used will usually dictate the temperature treatment of the sausage until the pH is ≤ 5.0.

Table 1 shows the minimum growth temperature of the undesirable bacteria which will grow in cured sausages.

Table 1. Growth temperature of undesirable bacteria in cured sausages (Nickerson and Sinskey, 1972)

Type of Bacteria	*Minimum*	*Maximum*
Clostridium botulinum	3.3°C	50°C
Staphylococcus aureus	6.7°C	46.6°C
Bacillus	28°C	70.0°C
Salmonella	6.7°C	46.6°C

Therefore, the controlled fermentation of cured sausage is a manipulative mechanism inhibiting the undesirable bacteria growth and enhancing the growth of the desirable bacteria.

Staphylococcus Aureus Inhibition

Staphylococcus aureus produces an enterotoxin (types A, B, C, D, E, and F) which is a potential health hazard in cured meat sausages. Genigeorgis (1974) has published many articles concerning the growth of each type of *Staphylococcus aureus* and the production of enterotoxin. *Staphylococcus aureus* grows as low as 6.7°C, and the minimum water activity is 0.86. Fortunately, *Staphylococcus aureus* produces enterotoxin only when it grows aerobically. In cured meat sausages, the area of concern is therefore the outside 1-10 mm of the sausage.

Haymon and Gryczka (1978) showed that the intentional addition of 100,000 *Staphylococcus aureus* (ATCC 265-1, Z-88, and ATCC 265-3) produced enterotoxin in sausages fermented at 100°F. The inhibition of staphylococci in sausage is more pronounced as the ratio of lactics to staphylococci increases, and as the fermentation temperature decreases. Higher temperature (38.9°C is optimum for *Staphylococcus aureus*) and brine concentration tend to favor the growth of mesophilic, salt tolerant staphylococci (Peterson et al. 1964). The addition of *P. acidilactici* limited staphylococcal growth to less than 3 log cycles using 18 hr. old broth cultures for *S. aureus*.

Raccach (1980) has reported on the use of a low temperature lactic acid bacteria for meat fermentation, *Pediococcus pentosaceus*. The generation time of *S. aureus* inoculated into Genoa sausage in the absence of the starter culture decreased with the increase of the fermentation temperature. *S. aureus* inoculated with the starter had generations times 1.8 to 15.3 times longer (depending on the temperature) than those of the *S. aureus* growing alone, and was able to increase in population less than one log cycle.

S. aureus is not totally inhibited by sodium chloride (up to 20%), sodium nitrite, the initial pH (5.5 to 6.2) or the water activity of the sausage mix. Therefore, it appears that a short fermentation which will rapidly change the sausage internal environment to conditions unfavorable for *S. aureus* growth would prevent the *S. aureus* pathogen from attaining levels associated with enterotoxin(s) production.

The most recent data on *S. aureus* inhibition is the inoculation study with 10^5 *S. aureus* cells (mixed strains 265-1 and Z-88) (both enterotoxin A positive) in pepperoni incubated at 35°C (95°F) dry bulb and 32°C (89.6°F) wet bulb. Pepperoni inoculated with *S. aureus* alone at 10^5 viable cells per gram at the edge of the sausage (see Table 2) grew 3+ log cycles. After 18 hours, thermonuclease and enterotoxin assays were positive when the *S. aureus* population grew to 10^7 viable cells/g of meat. The pepperoni inoculated with starter culture *(Pediococcus pentosaceus)* and 10^5 *S. aureus* cells per gram of meat did not develop positive thermonuclease or positive enterotoxin. The pH of the samples showed a drop to 5.21 for the *S. aureus* alone after 52 hours, and to 4.96 after 12 hours for the starter culture, finishing at 4.68 after 24 hours.

Table 3 shows the results of another experiment where 10^4 cells *S. aureus* per gram of pepperoni grew 3+ log cycles when no starter culture was present. In the presence of starter cultures, there was only one log cycle growth; there was no indication of thermonuclease or enterotoxin production.

Metaxopoulos and Genigeorgis et al. have recently shown that three strains of *S. aureus* (S-6, 137 and 472) grow very well in Italian salami type sausages. Five levels of *S. aureus* ranging from 10^2 to 10^7 cells/g were used. The amount of growth was dependent on the inoculum size. Strains S-6 and 472 increased a mean of 2.14 log cycles at inoculum levels of 2.3×10^2 cells/g-2.5×10^3 cells/g and by a mean of 2.66 log cycles at inoculum levels of about 10^5 and 10^4 cells/g. Strain 264 increased by 1.5 logs in the presence of 5×10^5 inoculated lactobacilli/g and by 2.5 logs in the presence of 6×10^4 naturally-occurring lactic acid bacteria. They developed regression equations allowing description of the growth of inoculated *S. aureus* in the salami during manufacturing as affected by a number of variables.

Data from all of the technical literature show that lactic acid bacteria growth in association with good manufacturing practices is the most effective method for the control of the food pathogen *S. aureus*.

Antioxidant Inhibition of Lactic Acid Starter Cultures

Raccach (1980) has reported that there is an inhibition of lactic acid starter cultures by phenolic type antioxidants. Table 4 shows that *Pediococcus pentocaceus* was inhibited by BHA (Butylated hydroxy anisole), BHT (butylated hydroxy to toluene), TBHQ (tertiary-butyl hydro quinone). In combination with 3.30% sodium chloride, there was approximately 78.3% inhibition at 27°C and 83.00% inhibition at 37°C.

Table 2. Effect of lactacel® 75 on growth of *S. aureus* Z-88 and 265-1 in periphery of pepperoni inoculated with 100,000 CFU/g *S. aureus* and incubated at 35°C (80% R.H.)* (Raccach, 1980)

	Treatment					
	Pepperoni Inoculated With S. Aureus Z-88 and 265-1			*Pepperoni Inoculated With S. Aureus Z-88 and 265-1 Plus Lactacel 75*		
Time	*S. Aureus CFU/g***	*T****	*pH*	*S. Aureus CFU/g***	*T****	*pH*
0 Hrs.	1.10×10^5	Neg.	5.90	1.10×10^5	Neg.	5.90
10 Hrs.	1.73×10^6	Neg.	–	3.58×10^5	Neg.	5.38
12 Hrs.	1.02×10^7	Neg.	–	3.30×10^5	Neg.	4.96
18 Hrs.	3.57×10^7	Weak Pos.	5.55	5.00×10^5	Neg.	4.75
24 Hrs.	2.52×10^8	Pos.	5.38	3.80×10^5	Neg.	4.68
52 Hrs.	2.58×10^8	Pos.	5.21	3.10×10^5	Neg.	4.68

* = The data presented in Table 2 respresents average of two separate experiments performed on different days.
** = The *S. aureus* CFU/g numbers are averages of duplicate determinations of a total of six plates for each sausage sample dilution tested.
T*** = Thermonuclease (plates evaluated after 15 hours @ 35°C.).

Table 3. Effect of lactacel® 75 on growth of *S. aureus* in pepperoni fermented at 35°C

	Fermentation Period (Hr)			*ph Values*			*Count[A] Increase*	
Treatment	*0*	*10*	*18*	*0*	*10*	*18*	*0-10*	*10-18*
	Count/g							
S. Aureus Alone	1.1×10^4	2.0×10^5	1.7×10^7	5.95	5.60	5.62	18.2	85.0
S. Aureus and Lactacel 75	1.0×10^4	4.0×10^4	1.5×10^5	6.01	5.00	5.00	4.0	3.8

[A]Count increase = (Final count ÷ initial count).

Table 4. Inhibitory effects of phenolic type antioxidants and NaCl on acid production by lactacel® 75 (Raccach, 1980)

Chemical/Combination	*Incubation Temperature 27°C*	*37°C*
	Incubation (%)[B]	
BHA[A]	14.00	10.70
BHT[A]	53.60	57.60
Citric Acid	0.00	2.70
BHA[A], BHT[A], and Citric Acid	49.70	65.30
PG[A]	0.60	0.87
TBHQ[A]	98.00	86.90
NaCl (%)		
3.0	36.30	36.20
3.3	42.00	44.60
3.6	43.90	49.50
3.9	50.90	52.70
BHA[A], BHT[A], Citric Acid and 3.3% NaCl	78.30	83.00

[A]Added each at a concentration of 0.003% (W/V).
[B]% inhibition = (pHi ÷ pHc) 100.

TBHQ produces almost total inhibition when used at the current USDA approved level. Raccach and Henningsen (1981) recently reported that the minimum inhibitory concentration of TBHQ against *P. pentosaceus* was 15 ppm for 10^3-10^5 cells/ml and 20 ppm for 10^6 cells/ml. The inhibitory effect was bacteriostatic. TBHQ inhibited lactic acid production. TBHQ at a level of 100 ppm was bactericidal against *S. aureus* and bacteriostatic at lower concentrations.

Salmonella

In cured sausage production, the incidence of Salmonella is low compared to non-cured sausages. The effects of temperature, salt, nitrite, and lactic acid inhibit the growth of salmonella, depending upon strain, species, and the total population present in the sausage. Masters (1979) has shown that lactic acid bacteria are inhibitory to the growth of salmonellae. The mechanism of inhibition appears to be the amount of lactic acid produced and the rate of lactic acid production. Sirvio and Nurmi (1977) have shown that commercial starter cultures inhibit the growth of salmonella in dry sausages. These are all in agreement with the data of Genigeorgis' summary (1976).

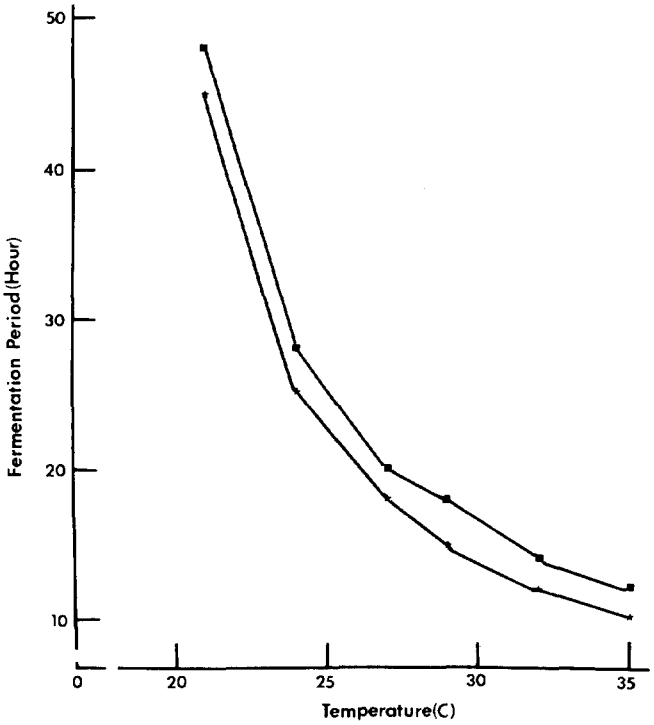

Figure 1. Fermentation time of genoa sausage prepared with lactacel® 75 (★ pH 5.3; ■ pH 5.0).

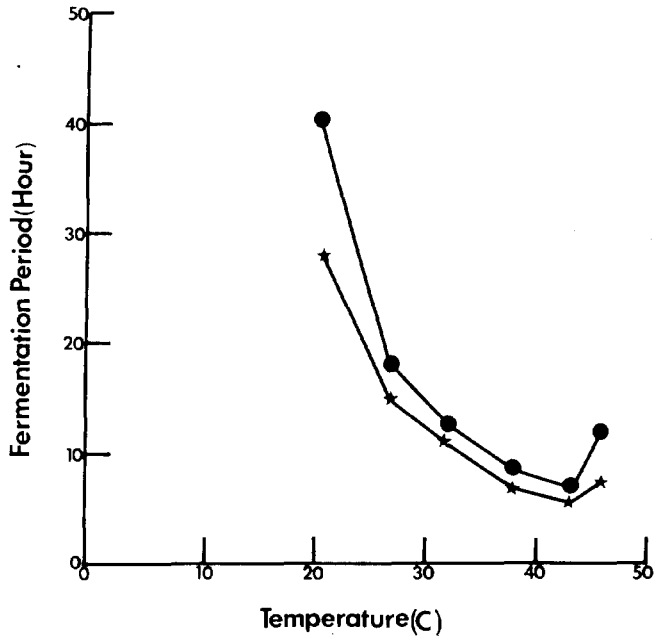

Figure 2. Fermentation time of pepperoni prepared with lactacel® 75 (★ pH 5.3; ● pH 5.0).

Sodium Acid Pyrophosphate

Sodium acid pyrophosphate (SAPP) is a USDA approved additive for frankfurters, bologna, ring bologna, knockwurst, and cooked sausages. It can be used up to 0.50% of the initial weight. At that level, this phosphate reduces the pH of the meat mixture about 0.5 units. The reduction in pH effectively increases the speed of cured pigment development, and overall curing reaction. Due to an increased total amount of cured color, the stability of the cured color is increased. The functions of other polyphosphates are also functions of SAPP. SAPP increases the firmness of the texture of the emulsion type products, and it is an excellent sequestrant and antioxidant.

Glucono Delta Lactone

The delta lactone of gluconic acid has been shown to be extremely useful as an acidulant for cured sausage production. Upon the addition of water or heating, this lactone hydrolyzes to gluconic acid and thus lowers the pH of the meat product and accelerates the cured color development. It is a USDA approved additive for cured sausage at the level of 1.0% for genoa salami, and 0.5% for all other cured sausages. At the 1% addition level, the lactone hydrolyzes sufficiently to lower the pH of the sausage one pH unit, and the 0.5% addition level will lower the pH of the sausage approximately 0.5-0.6 of a pH unit.

Evolution of Starter Cultures

Starter cultures for the meat industry have evolved from the knowledge of microbiology, and the study of naturally-occurring processes. The first starter culture was the carry over of one days' production to the next days' production, commonly called back-slop. The next type of starter culture was the in-house production of liquid cultures. Sometimes these were directly inoculated into meat, and the meat was used as the carrier for the starter organism(s). This was the state of the art, until the lyophilized starter culture was introduced by the American Meat Institute Research Foundation (Deibel, 1961).

In the late 1960's, frozen concentrated starter cultures were introduced by Microlife Technics through Merck & Co., Inc. Recently the frozen concentrated cultures have been concentrated to cell populations of 10^{12}-10^{13} cells/ml. We call these super-concentrated cultures.

The future for starter cultures appears to be very positive and optimistic. Until recently only the cured sausage industry (dry and semi-dry) could use the starter culture. The use of cultures in bacon applications are now noted in that part of the meat industry. New applications through the advances in biotechnology and genetic engineering appear on the horizon. Specifically designed cultures to perform specific tasks in the cured sausage industry may be offered to the meat industry.

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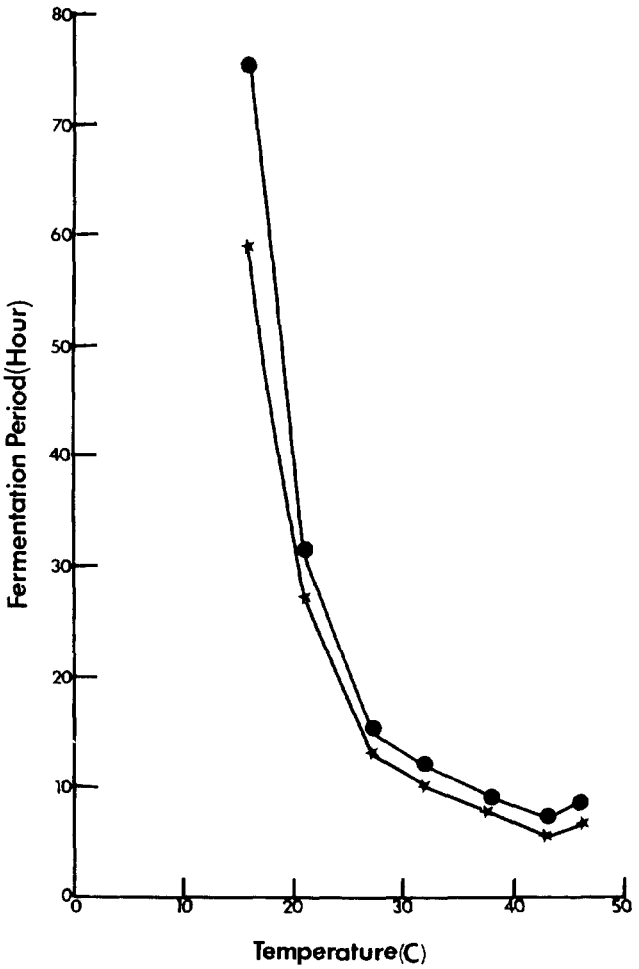

Figure 3. Fermentation time of summer sausage prepared with lactacel® 75 (★ pH 5.3; ● pH 5.0).

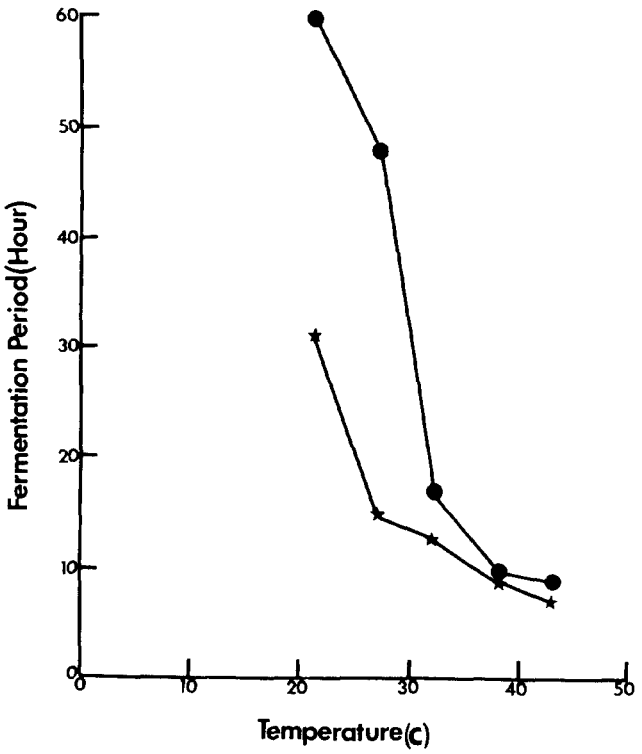

Figure 4. Fermentation time of summer sausage prepared with lactacel (●) and lactacel 75 (★).

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