MICROFLORA CHANGES AT HIGH (30-40°C) VERSUS LOW (15-25°C) FERMENTATION TEMPERATURES FOR BEEF SALAMI INOCULATED WITH *Pediococcus pentosaceus*

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Background

Traditional fermented sausage processes require a fermentation period ranging from 5 to 7 days in "ripening" at a general temperature range of 15-22°C. Rapid starter culture processes as used in the USA are generally conducted in time periods of 12 to 48 hr at temperatures ranging from approximately 30°C to 45°C. In the USA, the only commercially available starter culture that consists of a single bacterial specie that grows and metabolizes carbohydrate to lactic acid in the 20-37°C range is *Pediococcus pentosaceus*.

Objective

The objective of this study was to compare the effect of fermentation temperature ranges on the general microbiological profile of beef salami during each phase of manufacture with *P. pentosaceus* as the starter culture.

Experimental Methods

Beef salami was prepared with the following (per kg boneless beef): 0.15 g $NaNO_2$, 0.47 g Na erythorbate, 30.1 g NaCl, 10.0 g seasoning blend, 7.5 g dextrose, 25 g H_2O, and 5.1 ml of starter suspension (*P. pentosaceus*). Mixes were stuffed in 52-mm diameter DS fibrous casings yielding chubs of about 400 g each. After initial sampling, fermentation at 95% RH was conducted at 15, 20, and 25°C for "low" temperature range product using a chamber (Johnson and Acton, 1975) or at 30, 35 and 40°C for the "high" temperature range in a Vortron Model HL smokehouse. Low temperature range chubs were fermented for 48 hr whereas high range sausages were fermented 20 hr. Chubs were then heated with a stepwise schedule starting at 71°C for 45 min, with 5.6°C increases each 45 min until an internal product temperature of 60°C was attained. Drying was accomplished at 8°C in an environment of 80-85% RH with 15-20 air changes/hr for 12 days. Replicate sausage preparations were prepared with different lots of boneless beef.

In addition to analysis of the aged and previously frozen boneless beef used for sausage preparation, a minimum of six sausage chubs at each designated temperature were sampled at each process stage for microbiological analyses (see **Table 1**). An 11 g-center cross-section sample was aseptically removed, homogenized in a stomacher for 2 min with 99 ml of 0.1% peptone, serial diluted and duplicate pour plates were prepared for incubation. Plates were incubated at 35°C for 48 hr for coliform, micrococci and coagulase positive staphylococci and at 30°C for the others. Microbial counts were generally expressed as $\log_{10}$ colony forming units (CFU/ml) per g of sample and, in some cases, as exponential counts.

Results and Discussion

Viable microbial counts plated from boneless beef prior to sausage preparation (**Table 2**) indicate gross contamination and/or poor hygienic plant and/or storage conditions. Based on the aerobic count, indigenous lactic acid bacteria represented only 0.22% of the microflora whereas coliforms were 11.5%, much higher than the micrococci, coagulase positive staphylococci, and yeast and molds at 1.44, 0.085, and 0.03%, respectively. The majority of the aerobic count was likely pseudomonads (Hayes, 1992).

Enumerated counts for the various microbial groups at each stage of salami processing are given in **Table 3**. The lactic acid bacteria count was primarily indicative of the 10^6-10^7 inoculation with *P. pentosaceus*. Slight outgrowth during fermentation was more noticeable by the decline in pH (**Figure 1**) from 5.8-6.0 (initial) to near 4.4 at 20 hr for high temperatures and at 48 hr for low temperatures. Total time required to attain any given pH value was longer for fermentation at the lower temperatures. Coliform counts, while initially at 5.36 or 5.54 $\log_{10}$ CFU/g, were reduced during fermentation and were <1.00 $\log_{10}$ CFU/g after heating and drying. Micrococci were reduced by 0.8-1.4 $\log_{10}$ CFU/g during fermentation. Smith and Palumbo (1976) suggested micrococci decline due to lactic acid accumulation. Counts of micrococci after dehydration were <1.00 $\log_{10}$ CFU/g in low temperature fermented sausages as compared to 1.77 $\log_{10}$ CFU/g for the higher temperature fermented sausages.

For low temperature fermented sausages, coagulase positive staphylococci counts (**Table 3**) were reduced by 1.24 $\log_{10}$ CFU/g in fermentation and further reduced to <1.00 $\log_{10}$ CFU/g after heating and dehydration. However, counts for staphylococci remained at ≥ 3 $\log_{10}$ CFU/g through fermentation and heating and at 2.14 $\log_{10}$ CFU/g after dehydration for sausages fermented in the higher temperature range. Due to rapid lactic acid accumulation in fermentation and the presence of <10^5-10^6 CFU/g coagulase positive staphylococci, growth and/or maintenance of the viable staphylococci likely would not be a major concern based on the data of Niskanen et al. (1976), Daly et al. (1973) and AMI (1982). In increasingly acidic conditions and at lower population levels, staphylococci are not known to produce enterotoxin. However, enterotoxin analysis was not conducted in this study. Yeast and mold counts decreased to <1.00 log10 CFU/g after heating and dehydration, indicating no post-heating or drying phase contamination.

Conclusions

Starting with microflora of boneless beef indicating high contamination with coliforms and staphylococci, prepared salami had counts of coliforms, micrococci, and yeast and mold at or near 10^1 CFU/g after 12 days of dehydration. Low temperatures of fermentation (15-25°C) inhibited coagulase positive staphylococci during fermentation and subsequent processing steps. Higher temperatures of fermentation (30-40°C) were not effective in reducing staphylococci below 10^2-10^3/g during heating and dehydration.

References

1. AMI. 1982. Good manufacturing practices: fermented dry and semi-dry sausage. American Meat Institute, Washington, DC.
2. Niskanen, A. and Nurmi, E. 1976. Effect of starter culture on staphylococcal enterotoxin and thermonuclease production in dry sausage. Appl. Environ. Microbiol. 31:11.

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Table 1. Microbiological isolation media

Microbial Group	Isolation Media
Aerobic Plate Count	Standard Methods Agar (BBL)
Lactic Acid Bacteria[a]	MRS Broth (Difco) with Granulated Agar (BBL)
Coliforms[b]	Violet Red Bile Agar (BBL)
Micrococci[c]	Mannitol Salt Agar (BBL)
Staphylococci[d]	Baird Parker Agar Base (BBL)
Yeast and Mold[e]	Acidified Potato Dextrose Agar (BBL)

[a]Surface colonies appeared cream colored, opaque, entire and 1-2 mm in diameter; subsurface colonies were opaque, lenticular and 4-5 mm in diameter.

[b]Purplish red colonies were surrounded by a reddish zone of precipitated bile and were ≥0.5 mm in diameter.

[c]Small (2-3 mm) white colonies were surrounded by a red or purple zone.

[d]Coagulase positive staphylococci appeared as typical shiny, black, convex, 2-5 mm diameter colonies surrounded by an opaque zone.

[e]Filamentous and nonfilamentous colonies were counted.

Table 2. Microflora of boneless beef used for fermented beef salami preparation

Microbial Group	Log_{10} CFU/g	
	Average	* Range
Aerobic Plate Count	6.14	5.12 – 7.52
Lactic Acid Bacteria	2.49	<1.00 – 5.15
Coliforms	5.20	4.49 – 6.65
Micrococci	4.30	3.66 – 5.00
Staphylococci	3.07	<1.00 – 5.56
Yeast and Mold	2.68	1.65 – 4.04

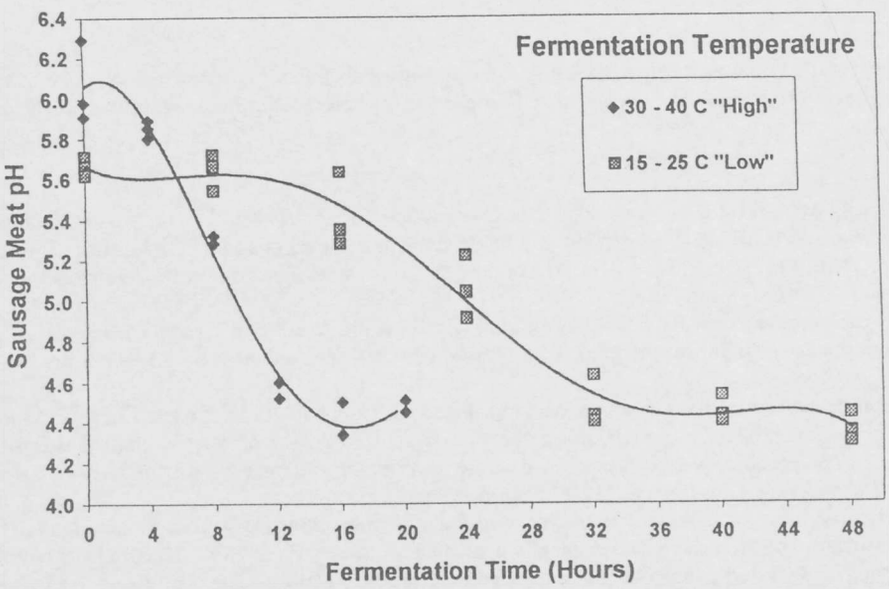

Table 3. Microbial counts (log_{10} CFU/g) of beef salami inoculated with *Pediococcus pentosaceus* at each stage of processing when fermented at "low" versus "high" temperatures

Microbial Group	Initial Mix		Fermented Mix		Heated to 60°C		Dried 12 Days	
	Low	vs. High	Low	vs. High	Low	vs. High	Low	vs. High
Lactics	7.40	6.53	7.61	7.68	6.89	6.54	7.15	6.19
Coliforms	5.36	5.54	2.50	2.69	<1.00est	<1.00est	<1.00est	<1.00est
Micrococci	4.64	4.69	3.24	3.88	1.88	2.56	<1.00est	1.77est
Staphylococci	3.33	3.84	2.09	3.69	1.23est	2.99	<1.00est	2.14
Yeast and Mold	3.66	2.44	2.48	1.78est	<1.00est	<1.00est	<1.00est	<1.00est

Est = colonies were present and counted even though <30 observed at lowest dilution