# SOME OBSERVATIONS ON THE MICROBIOLOGY OF FERMENTED SAUSAGES

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# INTRODUCTION

Lebanon bologna is a highly smoked, spiced, and fermented beef sausage originally made in the Pennsylvania Dutch area around Lebanon, Pa. It is made by aging beef with salt and then smoking the sausages in special wooden smokehouses for at least 4 days at 30 to 35°C and high relative humidity. The process of Lebanon bologna manufacture appears to be similar to other fermented semi-dry sausages (1,2) though little is known about it. It has been claimed by some of the manufacturers of Lebanon bologna that the sausage cannot be made outside of the Lebanon area. It is the purpose of our study to investigate the microbiology and technology of Lebanon bologna processing.

The traditional Lebanon bologna process is presented below:

Coarse grind beef, add 3% salt

Age 10 days at 5°C in wooden barrels

Add KNO<sub>3</sub>, sugar, and spices; fine grind; stuff into cellulose casings

Smoke 4 days at 35°C and 93% RH

Mellow at least 3 days at 5°C

In the traditional process, wooden barrels are used for aging. We had assumed that the continued use of the barrels insured the buildup of the proper flora. However, we used a new wooden barrel with success and for most of our pilot studies, we simply used plastic bags to age the meat. Our work confirms the findings of others that beef contains small numbers of lactic acid bacteria as part of the normal flora (3,4). The ten day aging period seems to permit the development of a lactic acid producing flora as well as one that reduces nitrate.

### METHODS

Microbiological counts during the aging and smoking process were performed as follows: 50 g beef cubes (or 50 g material from a bologna) were aseptically removed, ground at high speed in a Waring blendor with 200 ml peptone water and appropriate dilutions were then surface plated. The types of media employed, temperature, and times of incubation are presented below:

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A. Total aerobic count on APT agar---3 days at 25°C

0

- B. Micrococcion Phenol Red Mannitol Salt agar---3 days at 32°C
- C. Lactic acid bacteria on Rogosa SL agar---3 days at 25°C
- D. Yeasts on acidified Potato Dextrose agar --- 3 days at 25°C
- E. Gram negative bacteria on Eosin Methylene Blue agar---3 days at 32°C

Gram stains of all colony types found on the various media were examined; in addition, the catalase test was performed on isolates from these colonies.

## **RESULTS & DISCUSSION**

Certain aspects of the microbiology of aging meat with and without salt are shown in Figure 1. In the absence of salt, the total aerobic count (APT) increased quite rapidly during the 10 day period. The prevailing organisms were gram negative, catalase positive rods. Very few gram negative organisms were found in the presence of 3% salt. Sodium chloride definitely stimulated the appearance of micrococci (Mannitol-salt) but inhibited the lactic acid bacteria (Rogosa). The EMB count was also inhibited by the presence of salt; the organisms present on EMB were gram negative catalase positive rods that were not typical coliforms.

The data in Figure 1 definitely established the importance of salt in the preparation of meat for Lebanon bologna processing. In another experiment, beef was aged with zero and varying amounts of salt and made into bolognas. The viable count of the aged meat in relation to the salt concentration is presented in Table 1. The microbial flora changed quite dramatically as the salt concentration in the aged meat increased from 0 to 4%. The change was especially evident with the total aerobic count (APT). With the omission of salt, the flora after ten days was predominately catalase positive, gram negative rods. The meat has the fruity smell that is associated with pseudomonas activity on fresh meat. As the salt concentration was increased, the flora shifted to gram positive, catalase positive rods and at 4% salt very few gram negative rods were found. In addition, the total aerobic count decreased as the salt level increased. The lactic acid bacteria count (Rogosa) was inhibited by increasing the salt content and the count on EMB agar was drastically reduced at high NaCl levels. The counts on mannitol salt and potato dextrose agars were the least influenced by changing the concentration of salt.

When the aged beef was made into bolognas, salt was added at the time of processing to bring the concentration to 3% (except for the 4% of course). The pH and texture of the finished bolognas are given in

(56)

Table 1. The meat had a pH of 5.6 before aging and did not change during the aging period. Good pH drops were obtained with 0 to 3% salt; 4% salt restricted the growth of lactics and the pH remained high. The poor texture of the bolognas made from meat aged with 0 and 1% salt seemed to be associated with the development of pseudomonads.

To prepare bolognas, the curing ingredients are added to the aged salted meat; the meat is fine ground, stuffed into casings and smoked at 35°C and 93% RH. The data in Figure 2 shows the progress of microbial development during smoking. The lactic acid bacteria count (Rogosa) increased and became the dominant flora. The total aerobic count (APT) was almost exclusively catalase negative, gram positive rods. The micrococci (mannitol salt count) increased during the first 24 hours of smoking and the maximum cured meat color developed during this time. Acid production increased during the first 72 hours of smoking; texture development (that is, a firm cohesive bologna) was related to acid production. Counts on EMB and potato dextrose agars decreased to levels of less than 100 microorganisms per gram.

During the period after smoking--the mellowing period--further microbial changes occurred. The most interesting change occurred on mannitol salt agar. Throughout aging and smoking, the great majority of colonies consisted of gram positive cocci but during the mellowing period the micrococci disappeared and the colonies appearing on Mannitol salt agar were catalase positive, gram positive rods. The lactic acid bacteria count showed a gradual decline during the mellowing period.

### SUMMARY

Lebanon bologna production is quite similar to that of other fermented semi-dry sausages such as thuringer and cervelat. The major points in its production are: (1) aging of beef with 3% salt to encourage the development of a flora that will produce lactic acid and reduce nitrate. The salt also inhibits the growth of gram negative rods (pseudomonads?). At least 10<sup>4</sup> lactics/gram should be present at the beginning of the smoking period in order to obtain a good pH drop. Maximum cured meat color is obtained in 24 hours of smoking and the minimum pH is obtained within 72 hours.

### REFERENCES

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1 10

	cells per gram at 10 days				Bolognas			
% NaC	1 APT	Potato dextrose	Rogosa	EMB	Mannitol-salt	pН	texture <u>a</u> /	
	Number	number	number	number	number			
0	$8.0 \times 10^9$	$4.0 \times 10^4$	5.6 x $10^5$	$1.0 \times 10^4$	$1.0 \times 10^5$	4.7	0	
1	$3.3 \times 10^{10}$	3.5 x 10 <sup>5</sup>	$2.4 \times 10^5$	$7.0 \times 10^4$	$5.0 \times 10^4$	4.8	+	
2	$2.4 \times 10^8$	$1.0 \times 10^5$	$1.0 \times 10^5$	$2.6 \times 10^3$	1.1 x 106	4.6	++	
3	$2.5 \times 10^7$	1.0 x 10 <sup>5</sup>	$2.0 \times 10^4$	2.4 x 10 <sup>3</sup>	5.3 x 10 <sup>5</sup>	4.9	+++	
4	6.0 x 10 <sup>6</sup>	$1.0 \times 10^5$	1.4 x 10 <sup>3</sup>	$0 \times 10^2$	$1.4 \times 10^{6}$	5.6	0	

Table 1. Effect of Salt Concentration of Aging Beef on the Number and Type of Microorganisms Developing.

a/The texture of the bolognas was rated on an arbitrary scale of firmness with +++ as the most firm.

# Legend for Figures

Figure 1. Influence of 0 and 3% naCl on the microbial flora during

aging of beef for Lebanon bologna manufacture.

Figure 2. Changes in microbial flora during and after smoking Lebanon bologna.



(19)



(62)