INFLUENCE OF CONTAMINATION AFTER COOKING OF ROAST BEEF ON MICROBIAL GROWTH

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INTRODUCTION

Restructuring of meat produces a uniform, reasonably priced product that is becoming popular as a restaurant menu item (Mandigo, 1975; Cross and Stanfield, 1976). The producer using a restructured meat system has almost total control of the fat content, texture, bite, shape, weight, flavor and juiciness of the finished product (Farrington, 1975).

The muscle tissue from healthy animals contains few organisms at the time of slaughter (Ockerman et al., 1964; Romans and Ziegler, 1974 and Weiser et al., 1976), but the reduction of particle size and mixing during restructuring adds and mixes microorganisms throughout the meat block (Randall and Larmond, 1977). Dennis et al., (1972), however, reported that total plate count, presumptive coliform and Clostridium perfringens in restructured beef roasts does not cause an increased public health hazard when compared with fresh beef cuts. Temperature above 60°C or below 6.7°C is important in controlling Clostridium perfringens and most other microorganisms in the meat food products (Lechowich, 1971). Brown and Twedt (1972), however, found that a 60°C holding temperature of "Beef Served to Order" might be reduced without endangering the public health from growth of Staphylococcus, Salmonella or Clostridium perfringens.

The coliform count is the traditional indicator of enteric pathogens in food products (Solberg et al., 1976; and Thatcher and Clark, 1968). The coliform group are members of the family Enterobacteriaceae which also includes the pathogenic genus Salmonella, Shigella and Yersinia (de Figueiredo and Jay, 1976). The native habitat for Escherichia coli is the enteric tract of man and warm blooded animals and range of growth is normally considered to be between 10 and 45°C (Buchanan and Gibbons, 1974).

Maxcy (1976) held hamburgers inoculated post-cooking at 50°C and 60°C with and without covers and reported that film coverings ensured surface temperature hot enough to prevent growth. Carter (1977) held restructured beef roasts at 54 and 60°C for 12 hours and found no difference in plate count due to temperature of holding and time of holding for roast in which most of the microorganisms were destroyed during cooking.

The objectives of this research was to compare total plate count and coliform count during 12 hours of holding at 54 and 60°C for fabricated beef roasts that were externally inoculated with Escherichia coli after cooking.

EXPERIMENTAL

The samples of frozen, fabricated beef (3.8±0.2 kg each) were obtained from a commercial supplier and were manufactured in one production lot. The frozen roasts were defrosted for 48 hours at 3-4°C, then covered with an aluminum foil cap. Two thermopins (Pat. No. 2835480) were inserted in the bottom one-third of the roast. The roasts were cooked in a preheated (93-100°C) forced air oven (Blodgett Model EF-111, The G. S. Blodgett Co., Inc., Burlington, Vermont) to an internal temperature of 60°C. After reaching the final internal temperature, the roasts were removed from the oven and the temperature allowed to stabilize for 30 minutes. The thermopins were removed. At this point, roasts were assigned randomly to contaminated and control treatments. The contaminated roasts were surface inoculated with a suspension of Escherichia coli (O.S.U. strain, #455, The Ohio State University Microbiology Culture Laboratory, Columbus, Ohio) at a level of approximately 10^6 cells/cm^2 (1.6×10^9 cells/roast). After inoculation, the surface contaminated roasts and the control roasts were placed in preheated warming ovens set at 54 and 60°C. The roasts were sampled after 1, 2, 6 and 12 hours of holding at each of the holding temperatures. Sampling was accomplished by taking cross-sectional slices from the center portion of the roast with an alcohol sterilized knife. The slices (40-60 g) were transferred to sterile Stomacher bags and homogenized with 1% peptone diluent. Serial dilutions were made and plated with Violet Red Bile Agar and Tryptone Glucose Extract Agar for coliform and total plate counts, respectively. Coliform plates were incubated at 37°C for 24 hours. Plates for total counts were incubated at 25°C for 4 days.

RESULTS AND DISCUSSION

Effect of Inoculation on Total Plate Counts. Escherichia coli was inoculated onto the surface of cooked roasts which were held for 12 hours at either 54 or 60°C. Samples were removed from both internal and surface regions (entire slices) of the cooked roasts after 1, 2, 6 and 12 hours of holding time. Total plate counts were initially higher at 1 hour in the inoculated than the uninoculated samples; however, there was a significant ($P<.01$) interaction between inoculation and storage time (Table 1). Figure 1 presents the comparison of total plate counts of the inoculated and uninoculated samples for the four holding times. The significant interaction (inoculation x holding time) occurred because the total plate count of the inoculated samples decreased for the first 6 hours of holding and then increased during the last 6 hours; whereas, the total plate count of the uninoculated samples increased, in general, during the total 12 hours of holding.

Effect of Holding Temperature on Total Plate Counts. There was also a highly significant ($P<.01$) interaction for total plate count between holding time and holding temperature (Table 1). Figure 2 helps to illustrate the effect of holding time and temperature on the total plate counts. The 1 hour counts for both holding temperatures were very similar. At the 60°C holding temperature, there was a steady decline in counts until 6 hours, then a

slight (less than 1 log cycle) increase at the 12 hours holding time. However, at 54°C holding temperature, there was little change in total plate count for the first 6 hours but after 12 hours, there had been an increase in this count of almost 2 log cycles. This large difference in growth rate during the last 6 hours suggest that long term holding at 54°C may not be desirable.

Table 1. Analysis of Variance of Total Plate Counts of Restructured Beef Roasts

Source	df	F	Probability
Inoculation	1	10.16	.0018**
Temperature	1	14.82	.0002**
Time	3	9.21	.0001**
Linear	(1)	4.26	.0409*
Quadratic	(1)	15.13	.0002**
Cubic	(1)	0.85	.3583
Inoc. x Temp.	1	1.27	.2624
Temp. x Time	3	5.20	.0022**
Inoc. x Time	3	12.49	.0001**
Time x Temp. x Inoc.	3	0.16	.9212

*P<.05
**P<.01

Effect of Holding Time on Total Plate Counts. Time effects were highly significant (Table 1) and proved to be quadratic (P<.01) in nature. However, both inoculation by time and temperature by time interactions were highly significant (P<.01). Figure 3 helps to show the interaction effects on holding time. The inoculated samples began with higher counts than the uninoculated for both holding temperatures. At the higher temperature, the final counts attained are lower than at the lower holding temperature. This is an indication of perhaps lethal effects of the higher holding temperature on the microbial population or more favorable growth conditions at 54°C than at 60°C for the thermoduric organisms present. This may also reflect destruction of surface microorganisms accompanied by increases in the internal population. These interactions show the presence of at least two types of microorganisms. The increase from 6 to 12 hours suggests the logarithmic growth phase of a population. This type of organism would necessarily be thermoduric, evidenced by the high growth temperature (54°C). Of course in the inoculated samples, E. coli is present. The inoculated samples show a decrease in counts from 1 to 6 hours and an increase from 6 to 12 hours only at 54°C. At the 60°C holding temperature, the counts remained constant after 6 hours. There is some suggestion in the data of a competitive effect of the inoculation of E. coli on the natural flora. For both holding temperatures, the final total plate count obtained was less for the inoculated than the uninoculated samples. The differences are quite small and not significant, but may suggest a competitive effect.

Effect of Inoculation on Coliform Counts. No coliforms were detected in the uninoculated samples. As would be expected, the analysis of variance table (Table 2) showed that inoculation had a highly significant (P<.01) effect on coliform numbers. There was also a highly significant (P<.01) interaction between inoculation and storage time, but coliforms were only detected in the inoculated samples. Figure 4 shows the effect of holding time on the coliform counts of the inoculated and uninoculated samples. No coliforms were detected at any of the holding times in the uninoculated samples. The coliform counts showed a steady decrease over the 12 hours holding time for the samples inoculated with E. coli.

Effect of Holding Temperature on Coliform Counts. Holding temperature did not significantly affect the coliform counts (Table 2). The inoculated E. coli was destroyed at both holding temperatures. The rate of destruction appears (Figure 5) more rapid at the higher temperature, but the difference is not significant.

Effect of Holding Time on Coliform Counts. The time by temperature interaction was not significant since either temperature appears to be lethal for a surface inoculation of E. coli. Holding time had a significant effect on coliforms which can be

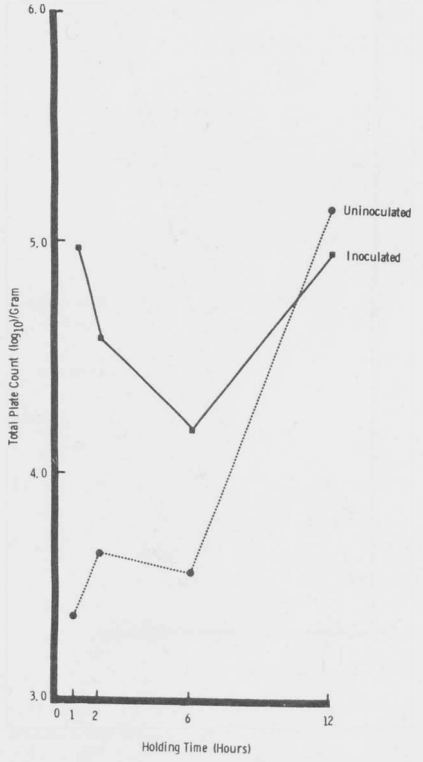

Figure 1. Total plate counts (effect of holding temperature absorbed) of restructured beef roasts uninoculated or surface inoculated (E. coli) and held 1, 2, 6 or 12 hours.

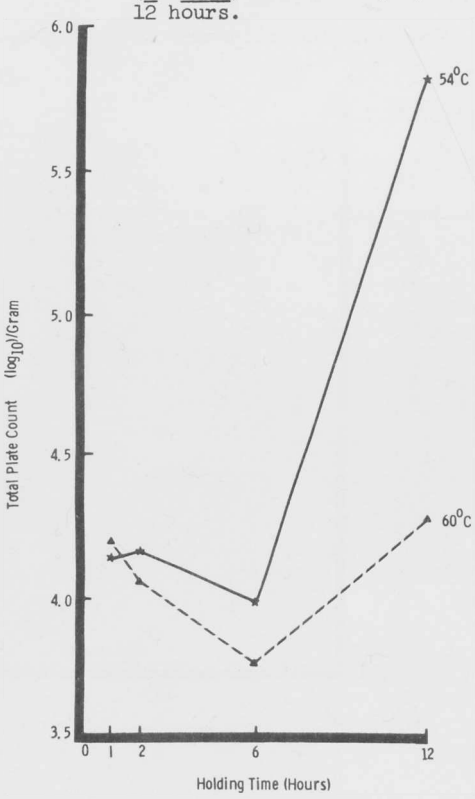

Figure 2. Total plate count (effect of inoculation absorbed) of restructured beef roasts held at 54°C or 60°C for 1, 2, 6 or 12 hours.

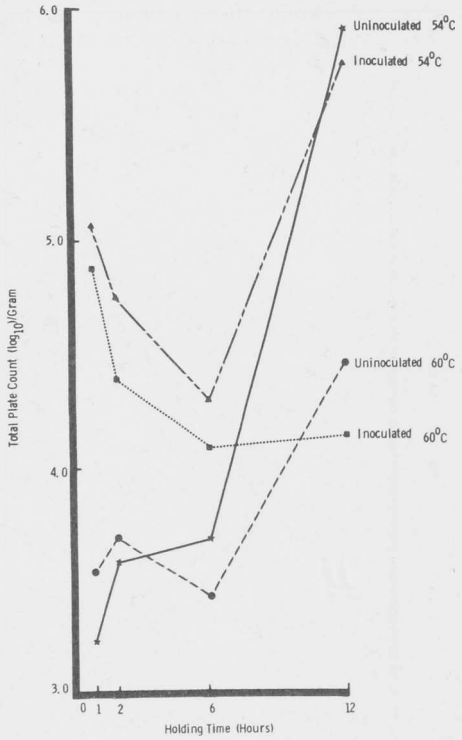

Figure 3. Total plate counts of restructured beef roasts uninoculated or surface inoculated (E. coli) and held at 54°C or 60°C for 1, 2, 6 or 12 hours.

Table 2. Analysis of Variance of Coliform Counts of Restructured Beef Roasts

Source	df	F	Probability
Inoculation	1	47.666	.0000**
Temperature	1	2.182	.1420
Time	3	28.509	.0000**
Linear	(1)	41.595	.0000**
Quadratic	(1)	23.373	.0000**
Cubic	(1)	20.558	.0000**
Inoc. x Temp.	1	2.182	.1420
Temp. x Time	3	0.684	.5632
Inoc. x Time	3	28.509	.0000**
Time x Temp. x Inoc.	3	0.684	.5698

*P<.05)
**P<.01)

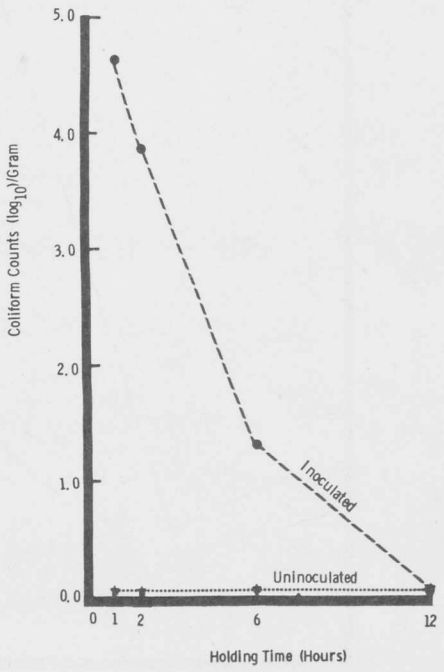

Figure 4. Coliform counts (effect of holding temperature absorbed) of restructured beef roasts either surface inoculated or uninoculated and held for 1, 2, 6 or 12 hours.

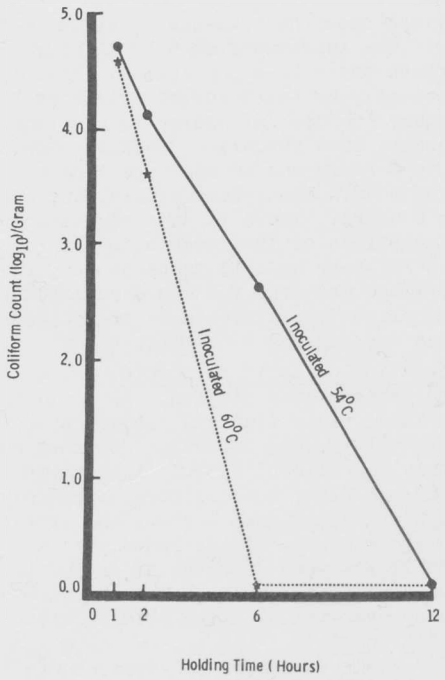

Figure 5. Coliform counts of restructured beef roasts inoculated and held at 54°C or 60°C for 1, 2, 6 or 12 hours.

seen in both Figures 4 and 5. No coliforms were detected (Figure 5) after 6 hours holding at 60°C or after 12 hours at 54°C. The decrease appeared to be more rapid at the higher holding temperature.

Combined Interpretation of Total Plate Count and Coliform Counts. Figure 3 also shows the effect of time on the destruction of the E. coli inoculum. If the decrease in total plate counts and the decrease in coliform counts are considered simultaneously, the evidence of the thermoduric population is clearly shown. The inoculated samples at both holding temperatures show decreases in total plate counts up to 6 hours, then at 60°C holding temperature the total plate count levels off while there is a dramatic increase in the total plate count of the 54°C samples. These decreases are mirrored in the coliform counts in Figure 5.

CONCLUSIONS

The holding times and temperatures employed in this study do not allow for the growth of surface contamination of the strain of E. coli inoculated. The destruction of this strain of E. coli is quite rapid (less than 6 hours) at the 60°C holding temperature. The holding temperatures do allow the growth of thermoduric organisms naturally present in the product. The higher holding temperature did not allow as rapid a rate of multiplication and resulted in lower microbial counts for the sampling times used.

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