GROWTH OF PSYCHROPHILIC BACTERIA ON LAMB CARCASSES.

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INTRODUCTION

BACTERIAL build-up on meat held at refrigerated temperatures is primarily a surface phenomenon. Ayres (1960) reported that bacteria responsible for slime production on refrigerated beef held at 10°C or less were primarily pseudomonads. Ingram and Dainty (1971) found that no definite changes in meat occurred until surface bacterial counts were greater than $10^8/cm^2$. Dainty et al. (1975) reported that proteolysis was undetectable prior to spoilage of meat. Bacteria spoilage in their study occurred when surface counts of indigenous microflora reached $2 \times 10^9/cm^2$.

Hoke et al. (1976) found no differences in microbial counts from lamb carcasses of yield grades 2, 3 and 4, either at packing houses or at retail distribution points. However, there were differences among packing houses and these differences were still apparent when carcasses were sampled at retail warehouses.

This study was conducted to compare the rate of increase in numbers of psychrophilic bacteria on the surface of lamb carcasses with differing thicknesses of fat cover held at 3.3°C, 83-86% R.H. for seven days.

MATERIALS AND METHODS

TWO hundred sixty-four lambs from the University farm and the U.S. Sheep Experiment Station, Dubois, Idaho were slaughtered at the University meats laboratory. The lambs were from 6.5 to 9 months of age with an average slaughter weight of 56.2 kg. Carcasses were quality and yield graded according to USDA standards and data on carcass characteristics recorded. Weight of chilled carcasses ranged from 19.2 kg to 38.4 kg. Carcasses were chilled overnight at -2.2°C, 80% R.H. in an air blast chiller then at 3.3°C, 83 to 86% R.H. for the balance of the experiment. Bacterial samples on hot carcasses were taken immediately after inspection and prior to weighing of the carcass for hot weight data. An area of 12.9 cm^2 of each leg (biceps femoris) and flank (rectus abdominus) were swabbed using a cotton swab moistened in sterile 0.1% peptone broth. The area swabbed was controlled by sterile template. Sampling sites were the same as those reported by Sauter et al. (1979) and previously by Hoke et al. (1976). Sampling was repeated twice daily, at 7 a.m. and approximately 8 hours later each day beginning on the first day post slaughter and continuing through seven days. Swabs were placed in 9.9 ml of 0.1% peptone, mixed for 2 min. using a vortex mixer then serially diluted using 0.1% peptone broth. Plate counts were made in triplicate using Standard Methods Plate Count Agar (PCA) for both total aerobic and psychrophilic counts. Plates for aerobic counts were incubated at 35°C for 48 hrs. Those for psychrophilic counts were incubated at 7°C for 14 days. Total coliforms were enumerated using Violet Red Bile Agar (VRBA), plates were allowed to solidify then overlayed with additional VRBA. Escherichia coli and enterococci were enumerated using most probable numbers (MPN) techniques USDA (1974). Numbers of Clostridium perfringens were determined using the pour plate procedure FDA (1972) using SPS agar which was allowed to solidify and overlayed with additional SPS agar. Plates were incubated anaerobically in Gas Pak jars at 37°C for 48 hr. Nitrite positive and non motile black colonies on SPS agar were considered to be C. perfringens, Angelotti et al. (1962). An additional series of samples were taken from each hot carcass for Salmonella detection. The swab samples were incubated in Selenite Cystine broth for 22 hr at 37°C and cultures streaked on MacConkey and Salmonella-Shigella agar to determine the presence of Salmonella. Suspected colonies were then inoculated into "Roche Enterotubes" (Roche Diagnostics, Nutley, NJ).

Randomly selected plates having between 50 and 100 colonies were used to characterize the microflora of hot, chilled and 7-day lamb carcasses. All colonies were streaked onto TSA plate to obtain pure cultures. Cultures were then gram stained and inoculated into selected media for biochemical tests and ultimate identification.

Count data were converted to logarithms and analyzed by the analysis of variance (Snedecor and Cochran, 1967). Differences among means were evaluated by Duncan's 1955 Multiple Range Test.

RESULTS

AVERAGE numbers of various types of bacteria/cm^2 on the surface of hot and chilled lamb carcasses are shown in Table 1.

Table 1. Bacterial numbers/cm^2 from hot and chilled lamb carcasses.

Type of bacteria	Hot		Chilled	
	leg	flank	leg	flank
Total aerobes	2.6×10^3	5.1×10^3	1.9×10^3	4.3×10^3
psychrophils	2.1×10^3	3.3×10^3	1.8×10^3	3.2×10^3
coliforms	5.0×10^2	8.1×10^2	2.1×10^2	3.9×10^2
Enterococci	6.2×10^1	7.8×10^1	3.2×10^1	4.2×10^1
Escherichia coli	6.3×10^1	8.1×10^1	3.9×10^1	4.3×10^1
Clostridium perfringens	<10	<10	<10	<10

Generally there was a slight but insignificant reduction in bacterial numbers during over night chilling at -2.2° and 80% R.H. All lamb carcasses were negative for *Salmonella* by procedures used for detection. Numbers of *Clostridium perfringens* were below detection levels on many carcasses. *Escherichia coli* numbers ranged from 10 to 18% of the total coliform counts from hot carcasses. Relative proportions on chilled carcasses were similar.

Psychrophilic bacteria represented 81% of aerobes from leg surfaces and 65% of total aerobes from the flank area of hot lamb carcasses. Comparable percentages of psychrophils from chilled carcasses were 94% from leg and 74% from flank samples. Results indicate a greater loss of nonpsychrophilic bacteria during chilling.

Data on the build-up of psychrophilic bacteria on surfaces of lamb carcasses of various fat thicknesses during holding at 3.3°C are shown in Table 2. Since bacterial numbers from leg and flank samples were not significantly ($P < .05$) different only averages of leg and flank data are shown.

Table 2. Psychrophilic bacteria/cm^2 from lamb carcasses of differing thickness of fat cover.

Days Post slaughter	Fat Thickness		
	<.38 cm	.38 to .76 cm	>.76 cm
1 day (chilled)	$2.2 \times 10^3{}_a$	$2.3 \times 10^3{}_a$	$2.2 \times 10^3{}_a$
2	$9.8 \times 10^3{}_{ab}$	$3.0 \times 10^3{}_a$	$3.1 \times 10^3{}_a$
3	$4.2 \times 10^4{}_b$	$8.7 \times 10^3{}_a$	$8.4 \times 10^3{}_a$
4	$2.8 \times 10^5{}_c$	$5.8 \times 10^4{}_b$	$3.9 \times 10^4{}_b$
5	$1.8 \times 10^6{}_d$	$3.7 \times 10^5{}_c$	$2.9 \times 10^5{}_c$
6	$5.3 \times 10^7{}_e$	$8.9 \times 10^5{}_d$	$8.4 \times 10^5{}_d$
7	$8.2 \times 10^8{}_f$	$6.6 \times 10^6{}_e$	$6.4 \times 10^6{}_e$

Values on any line or in any column having different subscripts are significantly different ($P < .05$).

Psychrophilic bacteria on carcasses having less than .38 cm of fat cover reached much higher number during the seven-day holding period than on carcasses having more fat thickness. The increase in bacterial numbers (end of lag phase) began during the second day post slaughter on carcasses having less than .38 cm of fat, but was delayed by approximately 24 hrs on carcasses having more than .38 cm of fat cover. However, there were no significant differences in numbers of rate of build-up of psychrophilic bacteria on carcasses having from .38 to .76 cm and those carcasses having more than .76 cm of fat cover. The increased bacterial numbers with increasing holding times are shown in Figure 1. The difference in build-up of psychrophilic bacteria is apparent from the graph showing both a longer lag phase and a slower multiplication rate of bacteria on carcasses having .38 cm or more of fat cover.

A wide variety of bacteria genera were isolated and identified from hot carcasses. The more frequently isolated genera are shown in Table 3 along with approximate percentages of the total microflora.

Table 3. Surface microflora isolated from lamb carcasses.

Genera of bacteria	% of carcass microflora		
	Hot	Chilled	7 days
Acinetobacter-moraxella	14.1	14.9	7.6
Enterobacter	5.2	4.8	<1.
Escherichia	2.8	2.5	ND
Flavobacterium	12.4	12.9	<1.
Lactobacillus	14.7	14.6	<1.
Micrococcus	8.6	10.7	<1.
Leuconostoc	2.1	1.1	ND
Proteus	5.6	4.7	ND
Pseudomonas	25.6	27.5	89.8
Staphylococcus	2.7	3.2	<1.
Streptococcus	3.0	3.1	<1.
Other	3.2	ND	ND

ND - none detected.

At seven days post slaughter the percentage of the microflora represented by any one genus of bacteria had declined for all genera except Pseudomonas which greatly increases from either hot or chilled carcass percentages.

Results indicate that .38 cm of fat cover are required to restrict surface growth of psychrophilic bacteria on lamb carcasses during refrigerated storage. However, there appears to be no advantage to more than .38 cm from a microbiological standpoint.

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FIGURE 1.
GROWTH RATE OF PSYCHROPHILIC BACTERIA
ON LAMB CARCASSES HAVING
DIFFERENT THICKNESS OF FAT COVER