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Effect of Oven Roasting at 200 and 300 ° F. on the Microbiology of Beef Roasts

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To the Graduate Council:

I am submitting herewith a thesis written by Marilyn Sue Williams entitled "Effect of Oven Roasting at 200 and 300 ° F. on the Microbiology of Beef Roasts." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Bernadine Meyer, Major Professor

We have read this thesis and recommend its acceptance:

J. Orvin Mundt, Frances A. Schofield

Accepted for the Council:
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(Original signatures are on file with official student records.)

February 15, 1967

To the Graduate Council:

I am submitting herewith a thesis written by Marilyn Sue Williams entitled "Effect of Oven Roasting at 200 and 300° F. on the Microbiology of Beef Roasts." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

Bernadine Meyer
Major Professor

We have read this thesis and
recommend its acceptance:

J. Orvin Mundt

Frances A. Schofield

Accepted for the Council:

Vice President for
Graduate Studies and Research

EFFECT OF OVEN ROASTING AT 200 AND 300° F. ON THE MICROBIOLOGY OF BEEF ROASTS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Marilyn Sue Williams

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CHAPTER I

INTRODUCTION

Tenderness is an important criterion of beef quality to consumers. Previous studies have indicated that tenderness is associated with cooking temperatures, lower temperatures yielding more tender meat (Cover, 1943; Bramblett et al., 1959; Bramblett and Vail, 1964; Nielsen and Hall, 1965; Simmers, 1965). It also has been established (Cover, 1943; Bramblett and Vail, 1964; Nielsen and Hall, 1965; Simmers, 1965) that the lower the oven temperature the slower the rate of heat penetration. The present study was designed to determine the effect of oven roasting at 200 and 300° F. on the microbiology of rib and top-round beef roasts.

Twenty pairs of roasts were obtained from ten steers that were finished and processed by The University of Tennessee Animal Husbandry-Veterinary Science Department. Five of the steers were of low finish, and five were of high finish. Rib roasts were bone-in 7-10 rib and averaged 10.2 and 6.7 pounds for high and low finish respectively. Boneless top-round roasts averaged 6.3 and 5.5 pounds for high and low finish respectively. One roast of each pair was cooked at 200° F. and the other at 300° F. There were five replications of each variable. Roasts cooked at 200° F. were heated to an end point of 152° F. and roasts cooked at 300° F. to 158° F., so that all roasts would be approximately medium-done. Additional information about the roasts is included in Chapter III. Internal temperature and oven temperature were

recorded automatically every three minutes to provide heat penetration data.

The data obtained for this study included rate of heat penetration and microbiological analysis of each roast. After cooking, samples from the center of each roast were removed aseptically for microbiological analysis. Sub-samples were cultured for the isolation of aerobic microorganisms and clostridia.

It was hypothesized that there would be sufficient lethal heat in the roasts under the time-temperature conditions of the study to kill all nonsporeforming microorganisms.

CHAPTER II

REVIEW OF LITERATURE

Roasting at low temperatures as a means of increasing tenderness has been investigated for several years. The high protein, vitamin, and water content of a food such as beef make it a fertile substrate for microbiological growth. In order for a food such as beef to be safe to eat, the uncooked beef must either be safe to eat or be made safe by the cooking process and properly stored to maintain that safety. A few of the studies concerning the effect of slow heat penetration on tenderness of beef, the occurrence of microorganisms in raw beef, and food-borne illnesses associated with cooked beef will be considered in this review.

I. EFFECT OF SLOW HEAT PENETRATION ON TENDERNESS OF BEEF

Cover (1943) studied the effect of extremely low rates of heat penetration on the tenderization of beef rib and chuck roasts. She attributed increased tenderness of beef roasts cooked at 176° F. over those cooked at 257° F. to a slow rate of heat penetration, and a release of the water of hydration that was slow enough to convert collagen to gelatin. Cover suggested that the length of the cooking period rather than cooking temperature was the determining factor affecting tenderness.

Bramblett et al. (1959) studied qualities of beef as affected by cooking at very low temperatures for long periods of time. They

cooked six pairs of U. S. Standard grade beef rounds at oven temperatures of 145° F. for thirty hours and 155° F. for eighteen hours. The five muscles studied were adductor, biceps femoris, gracilis, semimembranosus, and semitendinosus. Both Warner-Bratzler shear values and sensory panel scores indicated that the meat cooked at 145° F. was more tender than paired cuts cooked at 155° F. The length of time the meat was held at an internal temperature of 135 to 140° F. appeared to be a decisive factor and closely related to an increase in tenderness. The cuts cooked at 145 and 155° F. averaged eighteen and ten hours respectively between 135 and 140° F.

In further studies, Bramblett and Vail (1964) cooked paired muscles of beef rounds of U. S. Good and U. S. Standard grades to an internal temperature of 149° F. at 155 and 200° F. Tenderness scores, number of chews, Warner-Bratzler shear values, and L. E. E.-Kramer shear values all indicated that the meat cooked at 155° F. was more tender.

Nielsen and Hall (1965) used "choice grade" blade roasts to compare roasting at 225 and 325° F. and braising in an electric skillet. Also, rump cuts were roasted at 225 and 325° F. Roasted cuts were cooked to an internal temperature of 160° F. The braised blades were cooked until fork-tender. Blades roasted at 225° F. were more tender than those roasted at 325° F. and equally as tender as the braised blades. Sensory evaluations indicated that the roasted blades were juicier than braised blades. The rumps roasted at 225° F. were not superior in any way to those roasted at 325° F.

Simmers (1965) studied tenderness of beef semimembranosus muscle roasted at 200 and 300° F. in relation to cooking losses, cooking time, power consumption, and sensory properties. End point temperatures of 154 and 158° F. were used for roasts cooked at 200 and 300° F. respectively. Shear values and panel scores for tenderness indicated that the roasts cooked at 200° F. were more tender than those cooked at 300° F.

II. OCCURRENCE OF MICROORGANISMS IN RAW BEEF

Lepovetsky et al. (1953) did a microbiological study of the lymph nodes, bone marrow, and muscle tissue obtained from slaughtered cattle. The study included eleven chucks and twelve rounds representing twenty-three animals. Most of the bacteria in the deep tissues were in the lymph nodes. The predominant flora of the lymph were found to be gram negative rods and gram positive cocci. No gram positive aerobic spore-forming rods were isolated. The large numbers of organisms found in the lymph nodes suggested that these tissues might be the point from which deep spoilage in beef arises. The bone marrow and muscle tissues of the cattle were relatively free of bacteria.

Weiser et al. (1953) analyzed fresh beef that had been treated by the infusion of antibiotics. They infused twenty-three paired rounds and seven whole animals that then were tested as paired sides. One of each pair was maintained at room temperature for forty-eight hours, and the other was held under refrigeration. The greatest number of bacteria

were present in the sides held at room temperature for forty-eight hours. Organisms that were recovered from the infused animals were Bacillus megaterium, Escherichia, Streptococcus liquifaciens, and Proteus. But it was not indicated whether the bacteria were present prior to antibiotic infusion or possibly were introduced during infusion. In another report, Weiser et al. (1954) stated that the meat of an animal is never cleaner, from a microbiological standpoint, than immediately following slaughter.

Greenberg et al. (1966) examined raw pork, beef, and chicken in processing plants in the United States and Canada to determine the frequency and magnitude of the occurrence of putrefactive anaerobic sporeformers such as Clostridium botulinum. The most heavily contaminated sample had 115 putrefactive anaerobic spores per gram, but it was not stated whether this sample came from pork, beef, or chicken. The 624 samples of bloody neck area and trimmings of beef had a mean of three putrefactive anaerobic spores per gram. They concluded that the level of putrefactive anaerobic spore contamination in raw meat at the plant level is low.

Frazier (1958) pointed out that meat is contaminated during slaughter, dressing, and cutting. Microorganisms may be introduced from the exterior of the animal, its intestinal tract, knives, clothes, air, workers, and carts.

III. FOOD-BORNE ILLNESSES ASSOCIATED WITH COOKED BEEF

A food-borne illness is any disease caused by eating food (Frazier, 1958). Food poisoning is caused by a poison present in food when it is consumed (Frazier, 1958). Approximately a third of the reported cases of food poisoning in the United States are attributed to Staphylococcus aureus, and if the cause of all outbreaks were known possibly half would be attributed to this organism (Moore, 1966). Dauer (1961) stated that the actual number of food-borne and water-borne disease outbreaks might be estimated conservatively as ten to twenty times larger than the reported number. Staphylococcus aureus is nonsporeforming and it is easily killed by heat. It hs been isolated from the noses of approximately 50 per cent of normal, healthy individuals (American Meat Institute Foundation, 1960). Some strains of Staphylococcus aureus can form a toxin that is very heat resistant and is not inactivated by boiling. Angelotti et al. (1961) found the growth range for three different strains of Staphylococcus aureus to be between 44 and 114° F. in a chicken à la king. They stated that maintaining the internal temperature of foods within the mid-range of this "incubation danger zone" for more than four hours is a dangerous practice.

Food infection is a food-borne illness produced by invasion, growth, and damage to the tissue of the host by pathogenic microorganisms carried by food (Frazier, 1958). The second most common type of food-borne illness in this country, and the most common type in England, is

salmonellosis (AMIF, 1960). Salmonellae are nonsporeforming bacteria that inhabit the intestinal tract of man and many animals (Moore, 1966). Animals are carriers of Salmonellae without showing symptoms of disease. There are approximately 1,000 serotypes of Salmonellae. Angelotti et al. (1961) found the growth range to be 44 to 114° F. for three different strains of Salmonellae and recommended that holding foods between these temperatures should be avoided.

Streptococcus faecalis is a relatively heat tolerant nonsporeforming bacterium (Ott et al., 1961). The habitat of Streptococcus faecalis is the human intestine and the intestine of horses, cattle, and other domestic animals (Breed et al., 1957). Ott et al. (1961) studied the effects of heat on several precooked food products that had been inoculated with Streptococcus faecalis. The times required for 90 per cent destruction of an inoculum of 2 to 3 x 10^7 cells per milliliter in chicken à la king were 128 minutes at 140° F.; 18 minutes at 150° F.; 2.42 minutes at 160° F.; and 0.95 minutes at 165° F.

Dack (1966) criticized the Public Health Laboratories in that little or no effort is made to determine other causative agents of food-borne disease outbreaks than Clostridium botulinum, Salmonella, and staphylococci. Other agents that should be investigated in cases of food-borne disease outbreaks are Clostridium perfringens, Bacillus cereus, Vibrio parahaemolyticus, enterococci, and viruses.

Clostridium perfringens is an anaerobic sporeforming microorganism that often has been overlooked in food poisoning outbreaks due to the

difficulty of recovering it. Angelotti et al. (1962) developed a procedure for simplified identification and enumeration of Clostridium perfringens in foods that have been involved in food poisoning outbreaks. Strong et al. (1963) recovered Clostridium perfringens from two of the twenty-six beef cuts that they purchased from retail markets.

Bacillus cereus has been cited by Dauer (1961) as the probable cause of one outbreak of food-borne illness. Of all the foods eaten by those affected, only roast beef yielded Bacillus cereus.

CHAPTER III

PROCEDURE

The conventional oven temperature for roasting beef is 300-325° F. The purpose of this study was to determine the effect of oven roasting at 200 and 300° F. on the microbiology of high and low finish rib and top-round beef roasts.

I. DESIGN OF THE STUDY

Twenty pairs of beef roasts for this study were obtained from ten steer carcasses that were finished and processed by The University of Tennessee Animal Husbandry-Veterinary Science Department. Five of the steers were fed a low energy ration for 185 days before slaughter and the other five received a high energy ration for the same period of time to yield low finish and high finish animals respectively. Cuts used were rib and top-round. Rib roasts were bone-in 7-10 rib and averaged 10.2 and 6.7 pounds for high and low finish respectively. Top-round roasts were removed distal to the ilium and were boneless. They averaged 6.3 and 5.5 pounds for high and low finish respectively. The low finish steers were low U. S. Good with fat thickness at the twelfth rib ranging from two to five millimeters. The high finish steers were high U. S. Good to average U. S. Choice with a fat thickness at the twelfth rib ranging from nine to thirteen millimeters. One roast

of each pair was cooked at 200° F. and the other at 300° F. Variables included location of cut, degree of finish, and oven temperature. There were five replications of each variable.

Roasts were cooked in lots of four. For sixteen of the eighteen tests, two large roasts for this study and two smaller roasts for another study were cooked at the same time. For two of the tests all four roasts were large roasts for this study. The entire experiment included forty small family-size roasts and forty large roasts from ten steers tested in a balanced-lattice design. Only the forty large roasts were analyzed microbiologically for the present study.

II. COOKING METHOD

Roasts were kept frozen at -15 to -20° F. until one or two days before cooking. They were thawed at room temperature to an internal temperature of 32° F. at the center of the roast as measured by a sabre-couple connected to a Minneapolis-Honeywell temperature recorder. The sabrecouple was sterilized with a 1:200 dilution of 5.25 per cent sodium hypochlorite prior to insertion into the center of each roast. Defrosted roasts then were refrigerated overnight to equalize the internal temperature. Roasts were cooked in household electric ovens set at 200 or 300° F. as required by the cooking plan. The sabrecouples were again sterilized with fresh hypochlorite prior to insertion for cooking. The roasts were put into the ovens at times calculated to allow all roasts to be done at about the same time since they were involved in sensory

testing for another study. Internal temperature at the mid-point of each roast and oven temperature were recorded automatically every three minutes.

Roasts cooked at 200° F. were cooked to an end point of 152° F. and roasts cooked at 300° F. to an end point of 158° F., so that all roasts would be approximately medium-done. Thirty minutes after removal from the oven or after the internal temperature stopped rising, whichever was longer, the roasts were weighed.

Significance of the difference in cooking time per pound was calculated using the "t" test (Steel and Torrie, 1960).

III. MICROBIOLOGICAL ANALYSIS OF THE BEEF ROASTS

A slice from the center of each roast was removed aseptically onto a sterilized tray covered with sterilized aluminum foil. Four samples weighing approximately eleven grams were removed aseptically from the center of each slice and placed into weighed, sterilized jars. Two of the samples were analyzed immediately for the detection of nonsporeforming bacteria and clostridia. Sterile Butterfield buffer solution was added to each jar by sterile pipets to give a 1:5 dilution. Each sample was Osterized for two minutes with a sterile cutting head. Serial dilutions of 1:5, 1:50, and 1:500 of the beef roast suspension were inoculated into sterilized tryptic soy broth for the detection of nonsporeforming bacteria and into sterilized cooked meat medium, that had been heated to lower the oxidation-reduction potential, for the

detection of clostridia. Inoculations were done in triplicate. The other two samples from each roast were kept in sterilized jars in the refrigerator for forty-eight hours before analysis to determine if any bacteria present had undergone thermal shock rather than kill.

After two cooking tests, an additional procedure was added to help detect clostridia. Four additional samples from each roast, weighing approximately two grams each, were placed into a sterilized jar. Each sample then was submerged into fluid thioglycollate medium that had been steamed for five minutes to lower the oxidation-reduction potential. The fluid thioglycollate medium (BBL 01-136C) was modified to include dextrose, yeast extract, and gelatin. The composition of the media is shown in Table I.

Prior to each cooking test, the materials necessary to obtain the samples aseptically were sterilized at fifteen pounds pressure for fifteen minutes. This included the culture media, buffer, and pint jars with lids. Carving knives, cutting heads, caps, rubber rings, tongs, spatulas, trays, tweezers, and volumetric pipets were wrapped in paper before being sterilized. Small pipets were kept in sterilized cans. Aluminum foil was folded to keep it sterile until used.

The inoculated tubes were examined for microbiological growth after two to three days incubation at 99° F. for tryptic soy broth and five to seven days for cooked meat medium and thioglycollate. (See sample form for recording data in the Appendix, page 38.) Indication of bacterial growth in the tubes included: (1) a change of indicator from

TABLE I

COMPOSITION OF CULTURE MEDIA

Type	Ingredients	Amounts
		Per Liter
Tryptic Soy Broth	DIFCO Tryptic Soy Broth (dehydrated)	30 g.
	Brom Thymol Blue (1.6% in Alcohol)	1 ml.
	Tap Water (8 ml. per tube)	1000 ml.
		Per Tube
Cooked Meat Medium	DIFCO Cooked Meat Medium (dehydrated)	1 g.
	Distilled Water	9 ml.
		Per Liter
Thioglycollate Medium	Fluid Thioglycollate Medium (BBL 01-136C)	25.7 g.
	DIFCO Dextrose	5.0 g.
	DIFCO Yeast Extract	5.0 g.
	DIFCO Gelatin	20.0 g.
	Distilled Water (25 ml. per tube)	1000.0 ml.

blue-green to yellow, turbidity, or a pellicle in the tryptic soy broth tubes; (2) turbidity, a pellicle, odor, or gas entrapped in the meat particles in the cooked meat tubes; and (3) liquefaction of the gelatin, turbidity, or odor in the thioglycollate tubes. Burke's modification of the gram stain (Society of American Bacteriologists, 1957) was used for microscopic examination of the contents of tubes with growth.

Isolated cultures were maintained in tryptic soy broth. One drop was transferred to a fresh tube of tryptic soy broth and incubated at 99° F. for twenty-four hours before use as an inoculum for tests. When not in use, cultures were stored at 40° F. To determine lethal heat for the organisms recovered, heat tolerance studies were done in a 140° F. water bath. One-half milliliter of a twenty-four hour culture of the isolated organism in tryptic soy broth was inoculated into each of six tubes containing six milliliters of sterilized tryptic soy broth. Timing was begun when a thermometer placed in a control tube reached 140° F. Two tubes were removed after 60, 90, and 120 minutes of heating. The tubes were immediately placed in cool water to stop the heating then incubated for forty-eight hours at 99° F. Change in the indicator from blue-green to yellow and development of turbidity determined whether the organism could tolerate the heating time at 140° F. or not. That is, an organism that grew and produced enough acid to change the indicator in the tubes that were heated for sixty minutes but which did not change the indicator when heated for ninety minutes was considered to tolerate 140° F. for sixty minutes, but less than ninety minutes. Microorganisms

which could not tolerate 140° F. for as long as the roast was above 140° F. were discarded as contaminants.

Bacilli were recognized by their strong pellicle. They were identified only as to the group within the genus according to the morphology of the cell, the spore, and the sporange. Streptococci were identified by gram stain and in accordance with the outline of classification as found in Bergey's Manual (Breed et al., 1957). Clostridia were identified by gram stain, digestion of gelatin, and production of gas.

CHAPTER IV

RESULTS AND DISCUSSION

I. EFFECT OF TWO OVEN TEMPERATURES ON COOKING TIME AND HEAT PENETRATION

Cooking time. Roasts were cooked at 200° F. to an end point of 152° F. and at 300° F. to 158° F. Average weights and cooking times are shown in Table II. Roasts cooked at 200° F. required significantly longer cooking time per pound than their pair-mates cooked at 300° F. ($P < .001$ for high finish rib and top-round and $P < .01$ for low finish rib and top-round). As indicated in Table II, the average weight of high finish rib roasts was more than three pounds higher than the average weight of the low finish rib roasts. However, there was less than a pound difference between the average weights of the high and low finish top-round roasts. The cooking time per pound differed appreciably only for the rib roasts cooked at 200° F. averaging forty-six minutes per pound for the high finish and sixty minutes per pound for the low finish. It is not clear whether this difference was a function of size of roast, degree of finish, or both. Weights and cooking times for individual roasts are shown in Table VI in the Appendix, page 36.

Heat penetration. Average heat penetration curves for the rib roasts are shown in Figure 1 and for the top-round roasts in Figure 2.

TABLE II

AVERAGE WEIGHTS AND COOKING TIMES FOR RIB AND TOP-ROUND BEEF ROASTED AT 200 AND 300° F.

Cut	Finish	200° F.		300° F.		Difference in Cooking Time (min./lb.)
		Weight (lb.)	Cooking Time (min./lb.)	Weight (lb.)	Cooking Time (min./lb.)	
Rib	High	9.9 ± 1.1[a]	46	10.5 ± 1.4	27	18[b,c]
	Low	6.5 ± 1.1	60	6.8 ± 1.6	32	28[d]
Top-Round	High	6.3 ± 0.6	62	6.3 ± 0.5	29	33[c]
	Low	5.5 ± 0.5	66	5.5 ± 0.3	29	38[b,d]

[a]Standard deviation.

[b]Apparent discrepancy due to rounding.

[c]Significant ($P < .001$).

[d]Significant ($P < .01$).

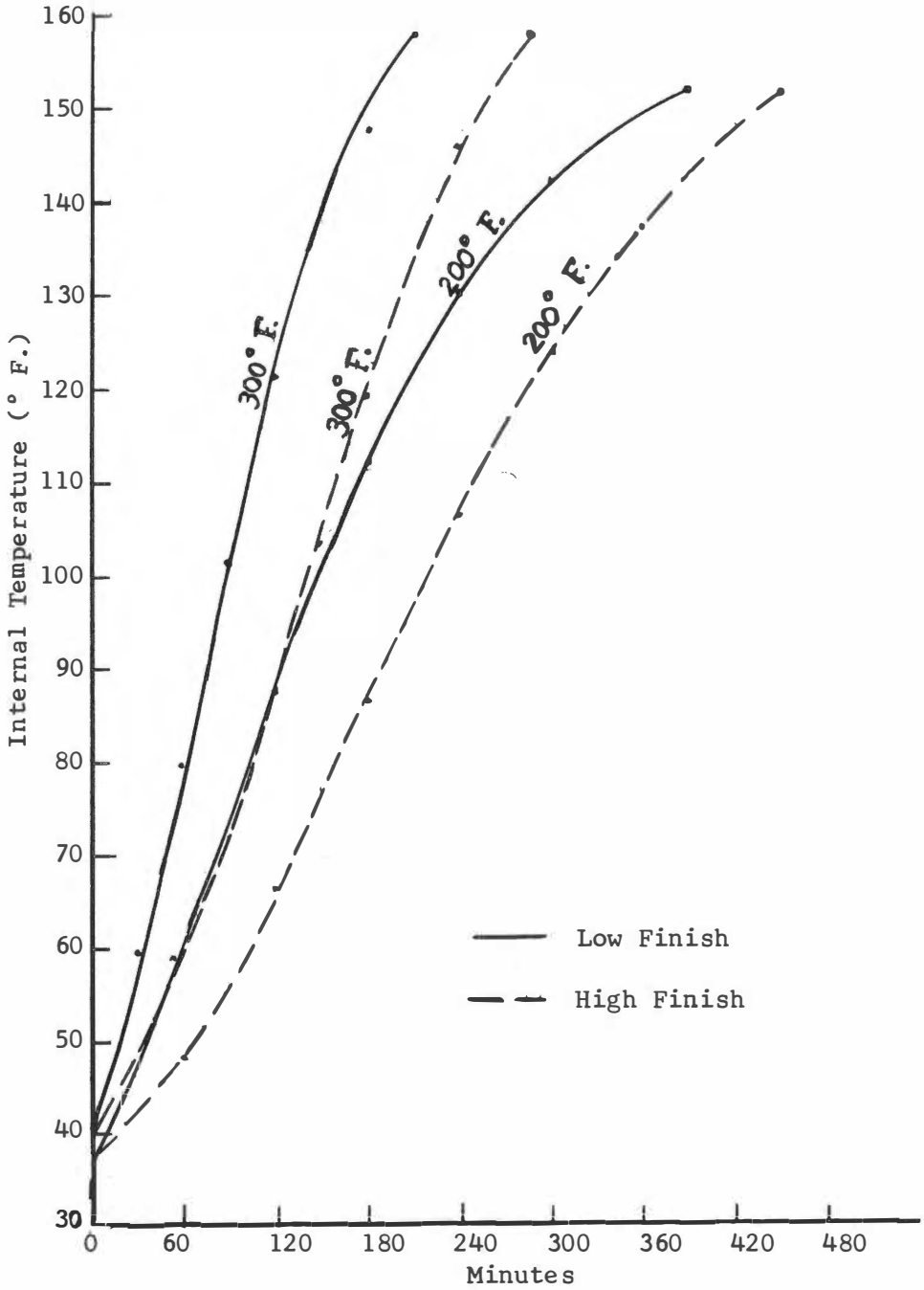

Figure 1. Average heat penetration curves for beef rib roasts of two degrees of finish cooked at two oven temperatures.

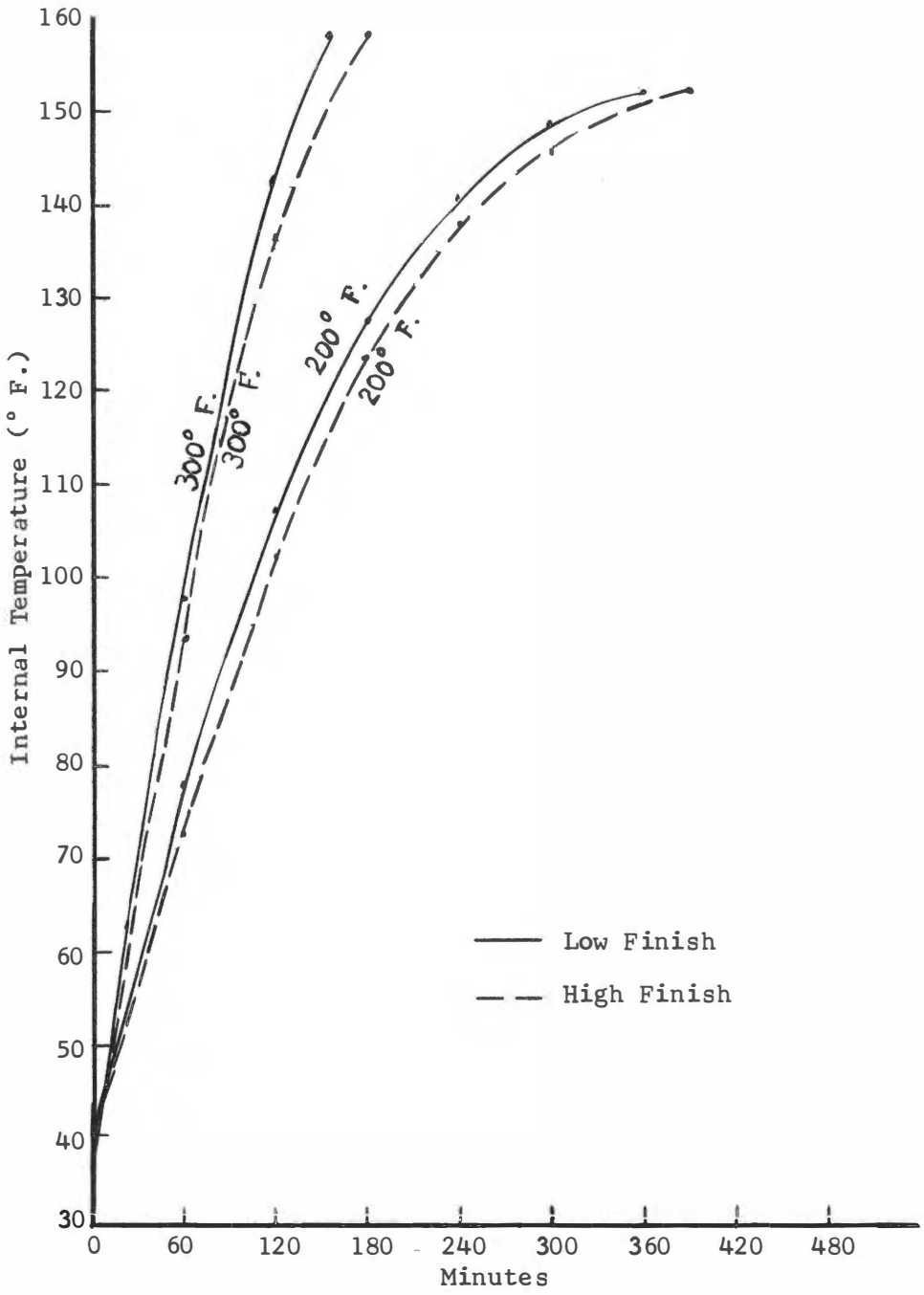

Figure 2. Average heat penetration curves for top-round beef roasts of two degrees of finish cooked at two oven temperatures.

For both rib and top-round roasts heat penetration at 300° F. was more rapid than at 200° F.

The extent to which the internal temperature of the roasts increased when removed from the oven seemed to be associated with both the oven temperature and the cut. After removal from the oven there was little or no further rise in internal temperature in any of the roasts cooked at 200° F. or in the top-round roasts cooked at 300° F. as indicated in Tables III and IV. The temperature of the high finish ribs roasted at 300° F. increased an average of 9° F. and the temperature of the low finish ribs increased an average of 5° F. Since the high finish rib roasts had a thicker cover of external fat than the low finish rib roasts, it seemed possible that this fat served as an insulator and permitted additional conduction of heat into the interior of the ribs roasted at 300° F. None of the top-round roasts had an external fat cover.

II. BACTERIA OBTAINED UPON CULTURE OF BEEF ROASTS COOKED AT 200 AND 300° F.

Most of the literature quotes thermal destruction times for non-sporeforming bacteria in terms of the number of minutes at 140° F. required for a 90 per cent destruction of the inoculum. Therefore, the minimum time that the meat was above 140° F. was determined from the heat penetration data including a thirty minute cooling period upon removal from the oven or until the internal temperature of the roast reached its peak and started to decline, whichever was longer. The low finish rib roasts

TABLE III

MICROORGANISMS ISOLATED FROM HIGH FINISH ROASTS COOKED AT 200 AND 300° F.

		200° F.				300° F.			
				Microorganisms Isolated				Microorganisms Isolated	
Animal Number	Cut	Minimum Time Above 140° F. (min.)	Highest Temperature Attained (° F.)	Cultured Fresh	Cultured After 48 Hours	Minimum Time Above 140° F. (min.)	Highest Temperature Attained (° F.)	Cultured Fresh	Cultured After 48 Hours
242	Rib	101	153	+[a,b]	+[c]	90	165	0	+[d]
223		63	149	0	+[c]	120	166	0	0
260		126	153	0	0	103	166	+[e]	+[f]
226		123	154	0	0	156	170	+[f]	0
262		123	154	0	0	192	168	+[e,f]	+[f]
Average		107	153			132	167		
242	Top-Round	141	150	0	0	97	158	0	0
223		249	152	0	0	85	158	0	+[d]
260		136	152	0	0	81	158	+[d,f]	+[f]
226		119	152	+[d]	+[f]	106	159	0	0
262		170	152	+[c]	+[f]	100	160	0	+[f]
Average		163	152			94	159		

[a] *Streptococcus faecalis*.

[b] *Bacillus sphaericus* group.

[c] *Bacillus*, unidentified.

[d] *Bacillus cereus* group.

[e] *Clostridia*, not *Clostridium perfringens*.

[f] *Bacillus subtilis* group.

TABLE IV

MICROORGANISMS ISOLATED FROM LOW FINISH ROASTS COOKED AT 200 AND 300° F.

Animal Number	Cut	200° F. Minimum Time Above 140° F. (min.)	200° F. Highest Temperature Attained (° F.)	200° F. Microorganisms Isolated: Cultured Fresh	200° F. Microorganisms Isolated: Cultured After 48 Hours	300° F. Minimum Time Above 140° F. (min.)	300° F. Highest Temperature Attained (° F.)	300° F. Microorganisms Isolated: Cultured Fresh	300° F. Microorganisms Isolated: Cultured After 48 Hours
213	Rib	115	152	+[a]	0	114	163	0	+[b]
211		124	153	0	0	75	161	0	0
258		157	152	0	0	100	162	0	0
243		141	152	0	0	111	163	0	+[a]
265		132	152	0	0	141	166	+[c]	+[c,d]
Average		134	152			108	163		
213	Top-Round	228	150	0	0	105	158	0	0
211		119	152	+[b]	0	86	158	+[e]	0
258		124	152	+[c]	+[c]	75	158	0	0
243		150	152	+[c]	0	76	159	0	0
265		177	152	0	+[c]	102	160	0	+[c,f]
Average		160	152			89	159		

[a] *Bacillus*, unidentified.

[b] *Bacillus sphaericus* group.

[c] *Bacillus subtilis* group.

[d] *Bacillus cereus* group.

[e] *Streptococcus faecalis* var. *liquefaciens*.

[f] *Clostridia*, not *Clostridium perfringens*.

and the top-round roasts of both low and high finish cooked at 200° F. were maintained above 140° F. for longer periods than the pair-mates cooked at 300° F. as indicated by data in Tables III and IV. The high finish rib roasts cooked at 300° F. cooled longer than thirty minutes due to continued rise in temperature after removal from the oven and were above 140° F. for longer than the pair-mate cooked at 200° F. Roasts were not always cooled until the internal temperature decreased to 140° F., therefore the times above 140° F. shown in Tables III and IV are minimum times above 140° F. rather than total times above 140° F. (See sample form for the calculation of cooking time in the Appendix, page 39.)

Bacilli. Of 160 meat samples from forty beef roasts, thirty samples yielded aerobic, sporeforming bacteria. They were isolated from both cuts, both finishes, and both oven temperatures. Seven of the thirty samples yielded Bacillus spp. in the second or third dilution without yielding it in the first dilution. Only three times out of eighty did duplicate samples cultured on the same day yield bacilli. This sporadic occurrence would indicate some contamination during analysis, rather than presence in the raw meat.

After five cooking tests an Andersen air sample (Andersen, 1958) was taken in the Food Science Laboratory near the ranges where the roasts were cooled and in the Bacteriology Laboratory where the analyses were performed. Most of the colonies obtained were molds. There were several

colonies of sporeforming bacteria and possibly one colony of Streptococcus. Spores may have been introduced into the center of the roast when the sabrecouple was inserted prior to cooking.

Assuming that all bacilli recovered were present in the meat prior to roasting and survived the cooking treatment, the highest recovery of bacilli, using the most probable numbers technique (American Public Health Association, 1960), was estimated as twenty-three Bacillus subtilis per gram in a low finish top-round cooked at 200° F. Estimated levels of bacilli recovered are shown in Table V.

Lepovetsky et al. (1953) did not recover any gram positive aerobic sporeforming rods from their analysis of lymph nodes, bone marrow, and muscle tissue of twenty-three beef cattle. Weiser et al. (1953) recovered Bacilli in beef cattle that had been infused with antibiotics. It is impossible to say how many recoveries of Bacilli in the present study were attributable to contamination during analysis and how many were attributable to their presence in the meat.

Clostridia. In the present study isolations of clostridia were made from three roasts, two high finish rib roasts cooked at 300° F. and one low finish top-round cooked at 300° F. (Tables III and IV, pages 22 and 23). Two isolations were in cooked meat medium and one was in fluid thioglycollate medium. None of the three isolates was Clostridium perfringens.

Other workers have reported a low incidence of clostridia in beef. Greenberg et al. (1966) noted only an average of three putrefactive

TABLE V

INCIDENCE OF BACILLI IN DUPLICATE SAMPLES OF BEEF ROASTS COOKED AT TWO OVEN TEMPERATURES

Estimated Count Per Gram	Cut	200° F.				300° F.			
		High Finish		Low Finish		High Finish		Low Finish	
		F[a]	R[b]	F	R	F	R	F	R
5.0<	Rib	0	0	0	0	0	0	0	0
1.5< 5.0		0	0	0	0	0	0	1	1
0.5< 1.5		0	0	0	0	0	1	0	0
0.1< 0.5		1	2	1	0	2	2	0	2
0		9	8	9	10	8	7	9	7
Total		10	10	10	10	10	10	10	10
5.0<	Top-Round	0	0	0	1[c]	0	0	0	0
1.5< 5.0		0	0	0	0	0	0	0	0
0.5< 1.5		0	0	1	0	0	1	0	0
0.1< 0.5		2	3	3	1	1	3	0	1
0		8	7	6	8	9	6	10	9
Total		10	10	10	10	10	10	10	10

[a]Cultured fresh.

[b]Cultured after forty-eight hours refrigeration.

[c]Estimated at twenty-three Bacilli per gram of meat.

anaerobic spores such as clostridia per gram in samples of bloody neck area and trimmings of beef. Lepovetsky et al. (1953) isolated clostridia three times from the lymph nodes of twenty-three beef cattle. The popliteal lymph node is found in the intracellular fat adjacent to the semimembranosus muscle. Since samples for the present study were obtained from the semimembranosus muscle it seems possible that the lymph node could have been the source of the organisms. However, the extent that microorganisms can move into the depths of the tissue is not known.

Pork contains clostridia more frequently than beef. Barbe et al. (1966) estimated anaerobic spore populations of rapid processed and conventionally processed ham. Only four of the thirty-eight hams tested contained anaerobic spores. They agreed with Frank (1963) that the application of heat above the growth range of some sporebearing bacteria can stimulate, or "activate," germination. Barbe et al. (1966) attributed the isolation of low levels of clostridia to spores that did not germinate during processing. In this study, there was no recovery of clostridia from roasts cooked at 200° F. possibly indicating favorable conditions for germination of spores, if present, followed by subsequent thermal destruction of the vegetative cell.

Streptococci. Heat tolerant streptococci were isolated from two roasts in the analysis performed on the cooking day. Streptococcus faecalis was isolated from one high finish rib cooked at 200° F.; Streptococcus faecalis var. liquefaciens was isolated from one low

finish top-round cooked at 300° F. (Tables III and IV, pages 22 and 23). Both the Streptococcus faecalis and the Streptococcus faecalis var. liquefaciens were found to tolerate 140° F. in tryptic soy broth for 120 minutes. Minimum time above 140° F. was 101 minutes for the high finish rib cooked at 200° F. and eighty-six minutes for the low finish round cooked at 300° F. Streptococci are found in the atmosphere, especially in a laboratory that deals intensively with their cultivation.

Lepovetsky et al. (1953) recovered streptococci thirteen times from the lymph nodes and twice from muscle tissue in their analysis of twenty-three beef cattle. Weiser et al. (1953) recovered Streptococcus liquefaciens from beef that had been infused with antibiotics.

Ott et al. (1961) found that it took eighteen minutes at 150° F. for 90 per cent destruction of an inoculum of 2 to 3 x 10^7 cells per milliliter of Streptococcus faecalis in chicken à la king. In the present study the high finish rib cooked at 200° F. was above 150° F. for forty-seven minutes; the low finish round cooked at 300° F. was above 150° F. for seventy-three minutes. Destruction of the low levels of Streptococcus faecalis reported here, estimated at 0.18 organisms per gram of meat, would be expected when these heat applications are compared to the results of Ott et al. (1961). It is impossible to say whether these two strains of Streptococcus faecalis were contaminants or survived cooking. A study in which roasts are inoculated with Streptococcus faecalis prior to cooking would help provide the answer.

Staphylococci. The staphylococci isolated from the roasts tested in this study were probably contaminants. Thomas et al. (1966) determined the thermal resistance of two strains of Staphylococcus aureus in green pea soup, beef bouillon, skim milk, and 0.5 per cent salt solution. Green pea soup, with the lowest specific heat, required 7.9 minutes for 90 per cent destruction of an inoculum of $1 \times 10^7 - 1 \times 10^8$ cells per milliliter at 140° F. It could be assumed that meat, because of its low specific heat, would require a longer time at 140° F. for 90 per cent destruction of the same size inoculum of Staphylococcus aureus. All roasts in this study were above 140° F. for at least sixty-three minutes (Tables III and IV, pages 22 and 23). These heating times would seem to provide an ample safety factor to assume that staphylococci, if present, could not have survived roasting.

Salmonellae. No Salmonellae were isolated from the roasts tested in this study. Thomas et al. (1966) found that it required 10.0 minutes for 90 per cent destruction of an inoculum of $1 \times 10^7 - 1 \times 10^8$ cells per milliliter of heat resistant Salmonella senftenberg in green pea soup. The heat treatments in this study seemed to provide an ample safety factor to assume that Salmonellae, if present, could not have survived roasting.

CHAPTER V

SUMMARY

I. SCOPE OF THE STUDY

The purpose of this study was to determine the effect of oven roasting at 200 and 300° F. on the microbiology of high and low finish rib and top-round beef roasts. Rib roasts averaged 10.2 and 6.7 pounds and top-round roasts averaged 6.3 and 5.5 pounds for high and low finish respectively. Roasts cooked at 200° F. were heated to an end point of 152° F. and roasts cooked at 300° F. to 158° F. Samples taken aseptically from the middle of a center slice of each of the forty roasts were analyzed microbiologically for the presence of aerobic microorganisms and clostridia.

II. PRINCIPAL FINDINGS

Roasts cooked at 200° F. required significantly longer cooking time per pound than their pair-mates cooked at 300° F. ($P < .001$ for high finish rib and top-round and $P < .01$ for low finish rib and top-round).

With the exception of two strains of Streptococcus faecalis that were recovered from a high finish rib cooked at 200° F. and from a low finish top-round cooked at 300° F., the hypothesis, that there would be sufficient lethal heat under the conditions of this study to kill all nonsporeforming microorganisms, appears to be acceptable. Cooking times

at both 200 and 300° F. provided large margins of safety for the destruction of staphylococci and Salmonellae, which are often causative agents of food poisoning.

Low levels of Bacilli were isolated from some roasts of each cut and each finish cooked at each oven temperature. The isolates could not definitely be attributed either to their presence in the meat before cooking or to atmospheric contamination during analysis.

Clostridia were isolated from two high finish ribs cooked at 300° F. and one low finish top-round cooked at 300° F. None of the three isolates was Clostridium perfringens.

All microorganisms were recovered at low levels. This study indicated that roasting at 200° F. as a means of increasing tenderness of beef cuts proposes no additional microbiological hazards over roasting at 300° F., a conventionally accepted temperature for roasting beef.

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APPENDIX

TABLE VI

TIME REQUIRED TO COOK RIB AND TOP-ROUND BEEF ROASTS AT 200 AND 300° F.

Cut	Finish	200° F. Weight (lb.)	200° F. Cooking Time (min./lb.)	300° F. Weight (lb.)	300° F. Cooking Time (min./lb.)	Difference in Cooking Time (min./lb.)
Rib	High	9.5	47	10.8	27	20
		8.8	53	8.9	30	23
		11.7	41	10.0	28	13
		9.4	45	10.2	28	17
		10.2	43	12.6	24	19
	Average	9.9 ± 1.1[a]	46	10.5 ± 1.4	27	18[b,c]
	Low	7.8	45	6.5	30	15
		7.2	56	4.1	42	14
		5.5	71	7.2	31	40
		6.8	59	8.0	31	28
		5.4	70	8.1	28	42
	Average	6.5 ± 1.1	60	6.8 ± 1.6	32	28[d]
Top-Round	High	6.2	69	6.1	31	38
		7.3	64	6.0	27	37
		5.6	61	6.0	28	33
		6.2	51	6.1	30	21
		6.4	63	7.2	27	36
	Average	6.3 ± 0.6	62	6.3 ± 0.5	29	33[c]

TABLE VI (continued)

Cut	Finish	200° F. Weight (lb.)	200° F. Cooking Time (min./lb.)	300° F. Weight (lb.)	300° F. Cooking Time (min./lb.)	Difference in Cooking Time (min./lb.)
Top-Round	Low	5.2	88	5.2	27	61
		6.3	50	5.7	28	22
		5.1	58	5.3	30	28
		5.3	65	5.4	30	35
		5.6	71	6.0	28	43
	Average	5.5 ± 0.5	66	5.5 ± 0.3	29	38[b,d]

[a]Standard deviation.

[b]Apparent discrepancy due to rounding.

[c]Significant ($P < .001$).

[d]Significant ($P < .01$).

H-244 BACTERIOLOGICAL ANALYSES

Cut:				
Degree of Finish:				
Cooking Temperature:				
Animal Husbandry No.:				
Raw Weight:				
Incubation Temperature:	37° C.(99° F.)			
	Cultured Fresh		Cultured After 2 days	
Date Cultured:				
Date Results Read:				
Sample Number:				
Weight of jar, cover, and core (g.):				
Weight of jar and cover (g.):				
Weight of core (g.):				
Add buffer (4 times weight of core):	x 4	x 4	x 4	x 4
Milliliters of buffer to add:				

Medium	Dilution	Number of positive tubes (Prepared in triplicate)			
Tryptic Soy	1 : 5				
	1 : 50				
	1 : 500				
Cooked Meat	1 : 5				
	1 : 50				
	1 : 500				

Comments:

H-244 HEAT PENETRATION CALCULATIONS

Cut:	
Degree of Finish:	
Cooking Temperature:	
End Point Temperature:	
Animal Husbandry No.:	
Raw Weight:	
Date Cooked:	

	Minutes
A-1. Total cooking time.	
2. Time from beginning till 150° F. (65.5° C.)	
3. Minutes of cooking time at or above an internal temperature of 150° F.	
B-1. Total cooking time.	
2. Time from beginning till 140° F. (60.0° C.)	
3. Minutes of cooking time at or above an internal temperature of 140° F.	
C-1. Total cooking time.	
2. Time from beginning till 130° F. (54.5° C.)	
3. Minutes of cooking time at or above an internal temperature of 130° F.	
D-1. Cooking time at or above 150° F. (65.5° C.) (A-3)	
2. Plus cooling time at or above 150° F.	
3. Total time at or above 150° F.	

E-1. Cooking time at or above 140° F. (60.0° C.) (B-3)	
2. Plus cooling time at or above 140° F.	
3. Total time at or above 140° F.	
F-1. Cooking time at or above 130° F. (54.5° C.) (C-3)	
2. Plus cooling time at or above 130° F.	
3. Total time at or above 130° F.	