

Controlled Vapor Oven Cooking and Holding Procedures Used for the Reduction of Salmonella and Prevention of Growth of Clostridium perfringens in Boneless Beef Rib Roasts

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ABSTRACT

The FDA 2005 Food Code whole meat roast cooking guidelines were used to evaluate controlled vapor oven cooking and holding of boneless beef rib roasts weighing 13.0 to 14.0 lb (5.89 to 6.35 kg) at $130^{\circ}F$ (54.4°C). The efficacy of the controlled vapor oven cooking, holding, cooling, cold holding, and reheating procedures was evaluated on the basis of the ability of the procedures to produce a 5-log reduction of a Salmonella inoculum on the surface and in the center of the beef roasts and to control the growth of Clostridium perfringens in the center of the roasts. This study showed that 6-hour cooking of beef roasts at 130° F (54.4°C) Wet Bulb Temperature, with oven relative humidity of 30 to 90%, met regulatory requirements for the destruction of Salmonella, even though the roast temperatures reached only 120 to 128°F (48.9 to 53.3°C). There was a slight increase in C. perfringens counts during cooking, indicating spore germination and vegetative cell growth. However, when the roasts were held at 130°F (54.4°C) during post cook-hold, C. perfringens was reduced to an undetectable level. During post-cook-chill-hold and retherm (reheating), a small population of C. perfringens that had been reduced to undetectable levels (< 10 CFU/g) was detected. This observation indicates that a safer way to hold cooked roast beef is to hold the roasts at a controlled vapor oven temperature of $130^{\circ}F$ (54.4°C) until consumed rather than to cool and reheat leftovers.

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INTRODUCTION

The purpose of this study was to determine the effectiveness of controlled vapor oven processing procedures for the reduction of pathogenic bacteria such as Salmonella and Clostridium perfringens in large cuts of meat, using FDA 2005 Food Code (8) §3-401.11 (B) (1) whole meat roast cooking guidelines as a reference. The safety of controlled vapor oven cooking procedures was evaluated on the basis of the ability of the procedures to produce a reduction of a Salmonella inoculum on roast surfaces and in minced beef contained within dialysis tubes placed in the center of beef roasts, as well as the effect of cooking, holding, cooling, storage, and retherm (reheating) procedures on the outgrowth of spores and destruction of vegetative cells of C. perfringens in minced beef contained in dialysis tubes placed in the center of the roasts.

MATERIALS AND METHODS

Equipment and product

A CVap oven (Model CAC507) (Winston Industries, LLC, Louisville, KY) was used for this experiment. Operator accessible controls of this equipment are the Wet Bulb Temperature (T_{wb}) and Dry Bulb Temperature (T_{db}) (11). T_{wb} is controlled by a temperature-controlled water evaporator at the bottom of the food chamber. T_{db} is controlled by temperature-controlled air heaters in the upper food chamber.

Seven boneless beef rib roasts (USDA Choice) weighing 13.0 to 14.0 lb (5.89 to 6.35 kg) were procured from a local distributor. The roasts were stored at 32 to $34^{\circ}F$ (0 to $1.1^{\circ}C$) and used within 3 to 7 days of procurement.

Test microorganisms

Three serotypes of *Salmonella* were used: Enteritidis (ATCC 13076), Typhimurium (ATCC 14028), and Montevideo (ATCC 8387). Each *Salmonella* serotype was grown separately at 95°F (35°C) for 24 hours in Tryptic Soy Broth (TSB) and underwent at least two serial transfers. Bacterial cells for each culture were harvested by centrifugation at 10,000 × g for 10 min. and washed with Butterfield's Phosphate Buffer, pH 7.2 (BPB). Each strain was then resuspended, concentrated, and combined in BPB to obtain a final cocktail suspension containing equal concentrations of each strain (i.e., ca. 10¹⁰ CFU/ml), which was used to inoculate the product.

The three strains of C. perfringens used in this study were NCTC 8238 (ATCC 12916), NCTC 8239 (ATCC 12917), and NCTC 10240 (ATCC 14810). Each strain was individually cultured in fresh Fluid Thioglycollate Broth (FTB) and then transferred to, and individually cultured in, a medium designed to promote the maximum formation of spores (2). Spores from each culture were harvested by centrifugation at $10,000 \times g$ for 10 min. and the pellets resuspended in sterile distilled water and stored at 35 to 38°F (1.7 to 3.3°C). Aliquots of the spore suspensions were combined to prepare a final working (cocktail) suspension containing approximately equal numbers of spores of each strain (i.e., ca. 10,000 CFU/ml), which was used to inoculate the product. The final working suspension was heat treated (i.e., 20 min. at 167°F (75°C) and immediately cooled to 39°F (4°C)) just prior to inoculation to ensure that only spores were present. The final working suspension was enumerated on Tryptose Sulfite Cycloserine (TSC) agar without egg yolk.

Sample inoculation and preparation

The boneless beef rib roasts contained insertions of the Salmonella cocktail and C. perfringens spore cocktail, using the dialysis tubing technique for containment of microbial populations (1, 9). This was accomplished by mincing, inoculating, and mixing approximately 300 g of the raw product (beef roast) with ca. 30 ml of C. perfringens spore cocktail. Another ca. 300-g portion of raw product was minced, inoculated, and mixed with 3.0 ml of the Salmonella cocktail inoculum. For each pathogen, three 10-to-15-g aliquots of the inoculated minced product were analyzed microbiologically to establish initial counts. The respective inoculated product portions had ca. 5 × 10^3 CFU/g of *C. perfringens* and ca. 2 × 10⁸ CFU/g of Salmonella.

For *C. perfringens*, the remaining inoculated, minced product was portioned into 18 sample aliquots (ca. 10 g each) that were placed into moistened 20.4 mm diameter dialysis tube segments. For Salmonella, the remaining inoculated minced product was portioned into 9 sample aliquots (ca. 10 g each) that were placed into moistened 20.4 mm diameter dialysis tube segments. The dialysis segments were closed at each end with thread, resulting in a small tube of inoculated product about 3 cm long. The dialysis sample units were color coded to differentiate the Salmonella from the C. perfringens inoculum and were kept refrigerated until insertion into the beef roasts, which was performed within 24 hours.

Incisions were made in the geometric center of three roasts. Three dialysis sample units of both *Salmonella* and *C. perfringens* were carefully inserted through each incision and were placed at the approximate geometric center of each roast. The incision was then closed.

A 10 cm² surface site on each of the 3 roasts containing the dialysis sample units of Salmonella and C. perfringens was inoculated with 0.1 ml of the Salmonella suspension in 0.01 ml droplets. The inoculated surface sites were marked to identify the sites for subsequent microbial analysis. This inoculum technique delivered about 109 CFU/cm2 to each designated surface site. After surface inoculation, the roasts were held refrigerated for at least 30 minutes to allow bacterial attachment and consistent temperature equilibration prior to control sampling (time-zero) or cooking.

Experimental cooling cycle development

Procedures were tested for cooling and holding cooked roasts in a refrigerator with an air temperature of $38 \pm 2^{\circ}$ F $(3.3 \pm 1^{\circ}$ C), from 130 to 41°F (54.4 to 5°C) in a time that would allow germination and multiplication of *C. perfringens*, over approximately 24 h. This cooling time was chosen to be representative of abusive cooling of roasts that occurs in retail food establishment refrigeration units and is sufficient for the growth of *C. perfringens*.

For the roast cooling experiment, 2 roasts were fitted with thermocouples at their geometric center and heated in the controlled vapor oven set at 130°F (54.4°C) T_{wb} . When the roasts reached a temperature of 130°F (54.4°C), they were removed from the oven and placed in a refrigeration unit set at 38°F (3.3°C) and with a restricted airflow to allow slow cooling. One roast was uncovered, and one was covered with aluminum foil. This trial indicated that the roasts without a foil cover cooled from 130 to 41°F (54.4 to 5°C) in about 24 hours, thus assuring the germination and multiplication of surviving *C. perfringens*.

Processing and physical data recording

Each of the 6 roasts that contained the dialysis sample units was fitted with 2 thermocouples (one internal thermocouple at the geometric center adjacent to the inserted dialysis units and a second thermocouple at 1.6 mm below the surface), for a total of 12 thermocouples. Internal and near-surface temperatures of the designated roasts were recorded at 5-min. intervals throughout the cooking, holding, cooling, and reheating procedures.

Oven temperature was also monito red. The T_{db} temperature and relative humidity in the controlled vapor oven were electronically recorded (5- or 10-min. intervals) during all processing steps, using type K thermocouples, an Omega OMB-DAQ-55 USB data acquisition system with a OMB-PDQ1 expansion module (Omega Engineering, Stamford, CT) and a humidity probe (Global Sensors' Humidity Logger DW-HS-B-16, Mt. Holly, NC - Accuracy of 3% to 60°C and 5% to 77°C). The temperature in the refrigerator was also electronically monitored and recorded during the cooling cycle and subsequent refrigerated holding. The temperature and relative humidity display readings on the controlled vapor oven were manually recorded from the oven display panel at approximately 1-hour intervals and whenever samples were collected during processing (i.e., initial cook-hold and retherm (reheating)). Additionally, 1 roast was fitted with an internal temperature probe that was integral with the controlled vapor oven for manual recordings. The processing and sampling steps for the beef roasts were as follows:

- At time-zero, 1 roast was surface inoculated with only Salmonella; 3 roasts were surface inoculated with Salmonella and contained 3 inserted dialysis sample units of both Salmonella and C. perfringens; 3 roasts contained only inserted dialysis sample units of C. perfringens. The first inoculated roast, the control, was immediately surface sampled for Salmonella and center sampled for C. perfringens.
- 2. The controlled vapor oven was set at 130°F (54.4°C) T_{wb} and 170°F (76.7°C) T_{db} and preheated.
- 3. Three roasts were placed on a 18×26 inch sheet pan on the bottom rack of the controlled vapor oven, and 3 roasts were placed on a pan on the middle rack in the oven.
- 4. The roasts were set to cook for 6 hours to achieve a center temperature of 130°F (54.4°C). At the end of the 6 hours, 1 roast was removed from the oven. The 3 internal *Salmonella* samples in dialysis tubes were recovered and removed, along with the 3 *C. perfringens* samples in dialysis tubes. The presence of *Salmonella* on the roast surface was determined by excising the 10 cm² surface sample for *Salmonella* recovery and enumeration.
- 5. After the 6-hour cook, the T_{wh} of the oven was left at 130°F (54.4°C), and the T_{db} was set at 131°F (55°C). These settings were used to hold the beef at a center temperature of 130°F (54.4°C) and a surface temperature of 131°F (55°C) at 90% relative humidity for 121 minutes, to meet the FDA Salmonella reduction requirement (8). At the completion of this 121-minute holding step #1 (i.e., 8 hours and 1 minute after start of cook), one inoculated roast was removed from the oven for dialysis sample tube recovery and determination of Salmonella and C. perfringens.

- 6. Holding continued at 130° F (54.4°C) T_{wb} and 131° F (55°C) T_{db} for 28 hours to simulate restaurant hot holding. At the completion of this step, about 36 hours after start of cooking, one inoculated roast was removed, and *Salmonella* was recovered and enumerated from the surface of the roast. *Salmonella* and *C. perfringens* were recovered and enumerated from the dialysis tube samples within the center of the roast.
- 7. The remaining 3 roasts (which contained only *C. perfringens* dialysis tube samples) were placed in a refrigerator and cooled from 130°F (54.4°C) center temperature to 41°F (5°C) in about 24 h. After cooling to 41°F (5°C) center temperature, 1 roast was removed, and the 3 *C. perfringens* dialysis tubes from the center of the roast were removed for *C. perfringens* recovery and enumeration.
- 8. The remaining 2 roasts were held refrigerated at 38 to 41°F (3.3 to 5°C) for about 24 h. After this refrigerated hold, 1 roast was removed, and the 3 dialysis tubes were extracted for *C. perfringens* recovery and enumeration.
- 9. For the final step, the remaining refrigerated roast was reheated for 6 hours in the controlled vapor oven set at 130°F (54.4°C) T_{wb} and at 170°F (76.7°C) T_{db} . At 6 hours, the T_{db} was changed to 131°F (55°C) and the roast was held for an additional 4 hours. The roast was then removed from the oven, the dialysis tube samples were removed from the roast, and *C. perfringens* was recovered and enumerated.

Recovery

For recovery of inoculated samples, the 10-g contents of a dialysis tube were placed in a Stomacher bag with BPB to obtain a 1:10 dilution. Samples were stomached for 1 minute and the homogenate serially diluted in BPB. For the surface *Salmonella* inoculated samples, the designated inoculation sites (10

		Internal Salmonella counts		Surface Salmonella counts	
Sample Variable		CFU/g	Log CFU/g	CFU/cm ²	Log CFU/cm ²
Pre-cook	rep. l	140,000,000	8.15	1,700,000,000	9.23
	rep. 2	290,000,000	8.46	I,400,000,000	9.15
	rep. 3	280,000,000	8.45	I,500,000,000	9.18
	Mean		8.35		9.18
	Std. Dev.		0.18		0.04
Post-cook	rep. l	6,200	3.79	< 0ª	< 1.00
[6 h to 130°F (54.4°C) CT ^b]	rep. 2	5	0.70	< 10	< 1.00
	rep. 3	I,800	3.26	< 10	< 1.00
	Mean		2.58		< 1.00
	Std. Dev.		1.65		0.00
L	og Reduction	c	5.77		> 8.18
Post-cook/FDA hold	rep. l	22,000	4.34	< 10	< 1.00
[6 h to 130°F (54.4°C) CT	rep. 2	8,200	3.91	< 10	< 1.00
plus 121 min @ 130°F	rep. 3	490	2.69	< 10	< 1.00
(54.4°C)]	Mean		3.65		< 1.00
	Std. Dev.		0.86		0.00
L	og Reduction	I	4.70		> 8.18
Post-cook/Sell hold	rep. l	< 5	< 0.70	< 10	<1.00
[6 h to 130°F (54.4°C) CT	rep. 2	< 5	< 0.70	< 10	< 1.00
plus 121 min @ 130°F	rep. 3	< 5	< 0.70	< 10	< 1.00
(54.4°C) plus 28 h	Mean		< 0.70		< 1.00
@130°F (54.4°C)]	Std. Dev.		0.00		0.00
L	og Reduction	I	> 7.65		> 8.18

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^aA "less than" (<) sign indicates no surviving Salmonella were detected in the subject sample

^bCT = center temperature

^cLog Reduction = (pre-cook mean log/g) - (subject post-cook/hold mean log/g)

cm²) were sampled by use of a surface excision technique. The designated surface site was aseptically excised to a depth of 5 mm; the sample was placed in a Stomacher bag and diluted with sterile BPB diluent to obtain a 1:10 weight dilution. Excised samples were stomached for 1 min. and the homogenates serially diluted in sterile BPB diluent as required. Salmonella was enumerated by Surface Plating on XLT agar, using the Thin Agar Layer method (TAL) with Trypticase Soy Agar (TSA) to enhance recovery of sublethally injured bacterial cells (4). Clostridium perfringens was enumerated in designated samples using Tryptose Sulfite Cycloserine (TSC) agar without egg yolk and standard microbiological methods (6).

RESULTS

Results were expressed as colony forming units (CFU) per gram for internal samples and CFU per cm² for surface samples. Counts of each pathogen were converted to log values for calculation of means and standard deviations. Mean *Salmonella* log reductions (as compared to time zero) were calculated for each sample set at each process step.

Process temperatures, cooking, hold, and sell hold

Electronic temperature results indicated that the center temperatures of the roasts ranged from 120 to 125°F (48.9 to 51.7°C) at the end of cook and 123 to 128°F (50.6 to 53.3°C) at the end of the FDA 2-hour hold (8). The internal temperature of some roasts did reach 129°F (53.9°C) during the sell hold. Electronic relative humidity readings showed that the oven took about 3 hours after start of cooking to reach the range of 82 to 91% relative humidity, after which there was little variability.

Roast cooling after controlled vapor oven cook, FDA hold, and sell hold temperature data

During cooling, the internal temperature of the roasts decreased from about 130 to 41°F (54.4 to 5°C) in about 24 hours. The refrigerator temperature was steady at about 41°F (5°C) during the 24-hour hold. The electronic temperature results indicated that the internal temperature of the test roast was 126°F (52.2°C) during retherm (reheat) and sell hold.

Salmonella analysis: Pre-cook and post-cook

As shown in Table 1, the initial mean internal and surface inoculum counts were 8.35 log and 9.18 log, respectively. At the end of cook (heating), the center temperature of the beef roasts was at 120 to 126°F (48.9 to 52.2°C), while the $T_{\mu\nu}$ oven temperature was 130°F (54.4°C). At 120°F (48.9°C), the time for a 1-log reduction of Salmonella is 173 minutes, and at 125°F (51.7°C) it is 54.5 minutes (1). Some survival would be expected. Temperature at the surface was adequate for an 8.18-log reduction of Salmonella, as shown in Table 1. No Salmonella were detected on the surface of the beef roasts at the end of the 6-hour cook, and there was a 5.77-log Salmonella reduction in the center of the roast beef. A 6.5-log Salmonella reduction is required by the FDA food code (8). Although it did not meet FDA whole meat cooking guidelines exactly, the center had as much Salmonella reduction as required by the FDA code for ground meat (greater than 5 logs) at the end of the 6-hour cook. It is expected that the roast would be safe from vegetative bacterial pathogens such as *Salmonella*.

Salmonella analysis: Post-cook / FDA hold

The center temperature of the roasts continued to rise about 1.0°F (0.55°C) during the next 2 hours, until the center temperature ranged from 123 to 128°F (50.6 to 53.3°C), and the oven completed the 2-hour FDA hold mode. The thermocouple probe within the roast indicated a temperature of 127 to 128°F (52.8 to 53.3°C) when the oven registered 130°F (54.4°C). The Salmonella count was not markedly different from the count at 6 hours. As mentioned previously, if the center temperature had reached 130°F (54.4°C), it is expected that the small number of surviving Salmonella in the center would have been killed.

Salmonella analysis: Post-cook-sell-hold+28 hours at I30°F (54.4°C)

As expected, there was no Salmonella survival internally or on the surface of the roasts. This shows that the controlled vapor oven has the capability of safely holding food at 130°F (54.4°C) and preventing the growth of Salmonella. This temperature would also kill Salmonella if there is a slight amount of cross-contamination, as might occur when the meat is being taken in and out of the oven for slicing and then put back. Since vegetative bacterial pathogens such as Salmonella are killed at these temperatures, this indicates that roasts, such as the beef roasts used in this study, will meet the FDA requirement for control of Salmonella if the temperature in all parts of large cuts of meat / poultry cooked in a controlled vapor oven is greater than 130°F (54.4°C).

Clostridium perfringens: microbiological results

The microbiological results for *C. perfringens* inoculated internally into the boneless beef rib roasts subjected to

the controlled vapor oven cook, FDA hold, and sell hold process steps, as well as a designated cooling procedure, a cold-hold procedure and a CVap oven retherm (reheat) process, are presented in Table 2. Note that the pre-cook *C. perfringens* counts were comprised of spores (due to the inoculum preparation methods), whereas the counts for subsequent sampling times could be comprised of both spores and/ or vegetative cells of *C. perfringens*.

Clostridium perfringens: Post-cook / FDA hold

These results indicate that there was apparent germination and outgrowth of spores of C. perfringens in some samples during the cook process and during the combination cook / FDA hold process. As a result, C. perfringens counts were reduced, which will not occur if C. perfringens is in its heat-resistant spore state. The research studies of Willardsen et al. (10) and Shigahisa et al. (7) indicate that this is to be expected in meat when come-up time to hot holding is as long as 6 hours, as it was in this study. A temperature of 203°F (95°C) for 52 minutes, a temperature much higher than the temperatures achieved within the roasts in this study, is required for spore inactivation (3). Conversely, the vegetative cells of C. perfringens are easily inactivated at temperatures of 130°F (54.4°C) and higher (5). Some sample-to-sample variation was observed for each of these sample sets (i.e., post-cook and postcook / FDA hold), but such variation is not unusual for this kind of study. There was no C. perfringens recovered (i.e., mean count of < 10 CFU/g) after the sell hold process at 130°F (54.4°C) for 28 hours. The post-sell hold results indicate that the C. perfringens spore outgrowth as vegetative cells during the previous process steps are significantly reduced during the sell hold process.

Clostridium perfringens: Post-chill / hold

The mean *C. perfringens* count was 1.16 log CFU/g following the designated 24-hour chill procedure and 2.21 log CFU/g following the designated cold hold procedure at 38° F (3.3°C). These

TABLE 2. Clostridium perfringens results in beef roasts during controlled vapor oven processing

		Internal C. perfringens count	
Sample Variable		CFU/g	Log CFU/g
Pre-cook	rep. l	5,300	3.72
	rep. 2	5,200	3.72
	rep. 3	4,800	3.68
	Mean		3.71
	Std. Dev.		0.02
Post-cook	rep. l	< 0ª	< 1.00
[6 h to 130°F (54.4°C) CT⁵]	rep. 2	700	2.85
	rep. 3	97,000	4.99
	Mean		2.94
	Std. Dev.		2.00
Post-cook/FDA hold	rep. l	2,600	3.41
[6 h to 130°F (54.4°C) CT plus 121 min	rep. 2	120,000	5.08
@ I30°F (54.4°C)]	rep. 3	160,000	5.20
	Mean		4.57
	Std. Dev.		1.00
Post-cook/Sell hold	rep. l	< 10	< 1.00
[6 h to 130°F (54.4°C) CT plus 121 min	rep. 2	< 10	< 1.00
@ 130°F (54.4°C) plus 28 h @ 130°F	rep. 3	< 10	< 1.00
(54.4°C)]	Mean		< 1.00
	Std. Dev.		0.00
Post-chill	rep. l	10	1.00
[130 to 41°F (54.4 to 5°C) in 24 h	rep. 2	< 10	< 1.00
	rep. 3	30	I.48
	Mean		1.16
	Std. Dev.		0.28
Post-chill/Hold	rep. l	390	2.59
[130 to 41°F (54.4 to 5°C) in 24 h	rep. 2	160	2.20
plus 24 h hold @ 38°F (3.3°C)]	rep. 3	70	1.85
	Mean		2.21
	Std. Dev.		0.37
Post-retherm cook/Hold	rep. l	980	2.99
[130 to 41°F (54.4 to 5°C) in 24 h	rep. 2	8,100	3.91
plus 24 h hold @ 38°F (3.3°C)	rep. 3	13,000	4.11
plus 10 h Retherm cook]	Mean		3.67
	Std. Dev.		0.60

^aA "less than" (<) sign indicates that no surviving C. perfringens were detected in the subject sample

^bCT = center temperature

results indicate that, while undetectable at the end of sell hold, there were some low levels of spores, and there was some outgrowth of *C. perfringens* spores during the designated chill procedure and during the designated cold hold procedure. *Clostridium perfringens* does not multiply at temperatures below 50°F (10°C) (3); therefore, this apparent growth may be a result of spore outgrowth and increase in vegetative cells during slow cooling.

Clostridium perfringens: Post retherm (reheat) and hold

The mean *C. perfringens* count was 3.67 log CFU/g following the controlled vapor oven retherm process, indicating that the retherm process allowed some multiplication of *C. perfringens*.

DISCUSSION

This 6-hour cooking process of beef roasts in a controlled vapor oven meets regulatory requirements for a safe roast product. The oven relative humidity was in the range of 20 to 40% for approximately the first two hours of cooking and then increased to 82 to 91% for the remaining cooking and post-cook hold. The experiment shows that this is sufficient to assure the destruction of Salmonella on the surface of the meat. The meat surface had a greater than 6.5-log Salmonella reduction at the end of 6 hours. The center temperature of the meat was always lower than the temperature of the oven by a few degrees, and destruction of Salmonella in the center of the roast was not as rapid. If roasts are cooked to a center temperature of 130°F (54.4°C) there is a greater than 5 log reduction in the center of the roasts at the end of the 6 hour cook. Note that the log reduction after

121 minutes of hold was slightly lower (4.7 log), but within sampling variation. *Salmonella* is not a food safety concern in meat that is cooked in a controlled vapor oven. The oven used in this study effectively reduced *Salmonella* 5 logs and more.

When the meat was cooked, held for 28 hours, and cooled, C. perfringens was undetectable. However, during hot hold after cooling and holding, a small number of vegetative cells of C. perfringens were detected (probably due to spore germination and outgrowth during cooling). This means that the safest use of a slow-cook oven is not to remove food from the oven to cool it and then later retherm (reheat) it. The safest procedure is to maintain roasts in the oven at a temperature of 130°F (54.4°C) or slightly above until the entire roast is served. The controlled vapor oven is capable of stable operation at 130°F (54.4°C), and at this temperature, Salmonella and C. perfringens in cooked roasts will not multiply.

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