Effect of Low-Temperature Long-Time Thermal Processing of Beef-Cuts on the Survival of Foot-and-Mouth Disease Virus

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ABSTRACT

We determined the virucidal effectiveness against foot-and-mouth disease virus of the low-temperature long-time cooking of virus-contaminated semitendinosus muscle (ST). Of the 11 time and temperature combinations examined, over a range of 63°C to 75°C for extended periods, the respective processing conditions of 71°C for 10.66 h and 75°C for 5.75 h were virucidal. Samples cooked under these temperature-time combinations were more tender (P<0.01) and had better overall acceptability (P<0.05) than beef cuts cooked by conventional commercial processes currently used in Argentina for meat to be exported. Product yields were increased from 60% for the commercial process to 67.8% or 68.6%, respectively, for the two virucidal thermal processes.

Key words: FMDV inactivation, beef cooking

Transmission of viral diseases to livestock through contaminated animal products is a serious concern in the international trade of foods and animal feeds. Animal products contaminated with foot-and-mouth disease (FMD) virus can become a source of FMD virus dissemination. Products derived from infected animals have been implicated in outbreaks that caused serious economic losses (3,13).

FMD virus is routinely inactivated in skeletal muscle as the pH decreases below 5.8 within 48 h during the onset of rigor mortis (8). However, FMD virus can persist for up to 6 months at 4°C in infected lymph nodes, clotted blood, and bone marrow of carcasses from infected cattle (8). Such tissues have been the vehicle for the introduction of FMD into susceptible livestock. Therefore, meat products originating in countries affected with FMD should be processed in order to inactivate any contaminating FMD virus present.

Several thermal processes that effectively inactivate FMD virus in specific meat products have been described by Blackwell et al. (5,6) and García Vidal et al. (9). Also, a combined treatment of heat and irradiation has been determined to be virucidal for FMD virus in meat by Lasta et al. (12). Different approaches have been tried to estimate the lethality of thermal processes on FMD virus by Blackwell et al. (4). Nonetheless, for any specific meat product to be regarded as safe, the processing conditions must be tested to demonstrate the inactivation of the FMD virus.

Temperatures over a range of 80°C to 100°C are currently applied to achieve FMD virus inactivation in commercial thermal processing of beef products. These high temperatures greatly damage the sensory quality of meat products. In Argentina, reported weight losses during commercial cooking of meat cuts in nylon tubes are about 40% (15). Reduction of processing temperatures below 80°C could result in products with better sensory qualities and higher yields.

Markets for prepared and ready-to-eat foods in developed countries demand high sensory qualities in thermally processed meat products. The importance of improving the sensory quality of cooked meat products is illustrated by the increasing interest in alternative processing technologies, such as low-temperature long-time (LTLT) cooking in water (7) and sous-vide cooking (2).

This study, therefore, examined thermal processes combining lower temperatures with longer cooking times for their ability to inactivate FMD virus, improve sensory quality, and increase product yield in meat products originating from FMD-affected countries.

MATERIALS AND METHODS

Virus

The FMD virus O, serotype (strain Campos) was obtained from the Centro de Investigaciones en Ciencias Veterinarias, Instituto Nacional de Tecnología Agropecuaria (CICV, INTA), Argentina. The virus was isolated in a field outbreak and subsequently passaged 15 times in cattle.

Cattle

Cattle obtained from an area free of FMD virus in southern Argentina were serologically tested to ensure that there had been no previous exposure to FMD virus. Steers weighing 250-350 kg, 18 months of age, were housed in animal isolation rooms at the CICV, INTA facilities.

Preparation of virus infected tissue

Cattle were inoculated intradermally into the tongue with 2.0 ml of log 10 5.7 tissue-culture 50% infectious dose (TCID 50 )/ml.
viral suspension. Bovines were slaughtered after the appearance of clinical signs of FMD, 48–50 h after inoculation. Lymph nodes from the head and body were collected, trimmed of fat, wrapped in plastic bags and immediately frozen and stored at -70°C until use.

Preparation of contaminated samples of semitendinosus muscle

Beef ST cuts weighing approximately 1.5 kg were trimmed of fat and cut into pieces of standardized dimensions (1 kg, 8 cm diameter, 20 cm length). A canal, 10–12 cm in length, was made in the major axis of each cylinder of meat using a butcher’s steel. Virus monitors were prepared by placing 10 g slices of lymph node, 2–3 mm in thickness, within the geometrical center of the canal and closing it with suture material. Samples were then placed within cook-in plastic bags (CN-510, Grace) for thermal processing.

Thermal-processing procedure

Three heating trials per each LTLT process tested were performed, except for processes G and J, where only one replication was carried out. Three contaminated ST samples were used per heating trial, making a total of 87 LTLT processed samples. Temperature and time combinations tested for thermal processes are shown in Table I. The general procedure was as follows. A stainless steel type J thermocouple probe (Termoquar S.A., Argentina) was positioned at the center of the ST sample and secured to the top of the plastic bag. Plastic bags containing samples were then immersed in a 50 L stainless-steel water bath (27 cm wide, 24 cm high, 77 cm long). The target bath temperature was controlled within 0.1°C by using an immersion circulator (Lauda, type MS, Germany). Time and temperature data for each contaminated ST piece was recorded by a data logger (Hydra 2625 A, John Fluke Mfg. Co., Inc., WA). Once the FMD virus monitor had reached the processing temperature, ST samples were maintained at this temperature for different periods of time. Immediately after completing the processing time, lymph node monitors were removed by making a lengthwise cut through the meat using a sterile surgical knife and forceps. Lymph node tissue was removed and then frozen and stored at -70°C, or alternatively kept at 4°C for no more than 48 h, until analysis.

Detection of virus

Preparation of samples for inoculation. A 20% suspension in minimum essential medium (MEM, Gibco) with antibiotics (penicillin 1,000 IU/ml, streptomycin 10 mg/ml, and gentamicin 5 μg/ml) was prepared from lymph nodes corresponding to each trial. Homogenates were centrifuged at 6,000 rpm for 30 min at 4°C in an R3RC centrifuge (Sorvall). The supernatants were immediately assayed for infectivity.

Cell culture. Confluent cell-culture monolayers of primary fetal bovine thyroid cells (FBT) were grown in 13 × 123 mm pyrex tubes containing MEM supplemented with 5% bovine serum. Cultures were grown and maintained at 37°C in a humidified 5% CO2 chamber. Serial tenfold dilutions, beginning with the clarified 20% undiluted suspensions, were prepared in chilled MEM, and 0.1 ml per tube was adsorbed on the cell monolayer for 1 h at 37°C. Three replicates per dilution were inoculated. Monolayers were examined microscopically for cytopathic effects during 7 days. Results were recorded as TCID50 per gram of product.

Cattle inoculation. If cell cultures showed no cytopathic effect, cattle were inoculated intradurally at 20 sites in the tongue, 0.1 ml per site, with the undiluted processed samples. Two steers were inoculated per sample, and were daily observed for development of vesicular lesions. Heparinized blood samples were obtained from day 0 through day 5 post-inoculation and examined for the presence of FMD virus. At 14 days post-inoculation, sera from clinically negative animals were examined for neutralizing antibodies and antibodies to virus-infection-associated (VIA) antigen. If vesicular lesions were observed, epithelium was collected and FMD virus was typed.

Sensory evaluation of thermally processed semitendinosus muscle

Twelve pairs of ST were obtained from either Aberdeen Angus or Hereford cross-breed animals (2-3 years of age). Each ST weighed an average of 1.9±0.4 kg and was thermally processed as follows. One ST from each pair was conventionally processed (processes C1, C2) by cooking in nylon tubes totally immersed in boiling water (100°C) to reach a core temperature of 85°C at the coldspot. The remaining ST from each pair was cooked by the LTLT processes E and K (6 ST each) as described in the thermal-processing procedure.

The sensory qualities of ST cuts cooked at the two separate LTLT inactivating conditions, 71°C for 10.66 h (process E) or 75°C for 5.75 h (process K), were evaluated. A comparison between LTLT cooked ST cuts and the conventionally cooked ST (processes C1 and C2) was performed simultaneously. Paired ST cuts were used to compare process E with C1 and process K with C2. A 16-member trained tasting panel evaluated ST samples for tenderness, juiciness and flavor, using an eight-point scale, with 8 representing extremely tender, extremely juicy, or having pronounced cooked-beef flavor, and 1 for extremely tough, extremely dry, or with a very weak cooked-beef flavor. LTLT samples (E and K) and the commercially cooked samples (C1 and C2) were ranked from 1 (best) to 4 (worst), according to tenderness, juiciness, flavor and overall acceptability.

Cooked samples were also qualitatively evaluated by trained panelists for their overall appearance: color, brightness and color homogeneity, and compared with conventionally cooked samples.

Product yield

The percent product yield of ST samples was determined by measuring the weight difference between raw and cooked samples, dividing by the raw weight and multiplying by 100.

Statistical analysis

Juiciness, tenderness and flavor data were analyzed using an analysis of variance for a four-factor model and a posterior simple-effect test (17). Rank preference data for sensory characteristics and overall acceptability were analyzed by Friedman's test and multiple comparison procedures appropriate for rank data (14).
RESULTS

Survival of FMD virus in contaminated meat samples

Typical curves for LTLT thermal processing showed that the rate of change in product temperature was reduced as the product temperature approached the water bath temperature (Fig. 1). As shown in Table 2, FMD virus survival was dependent upon the duration of LTLT processing. All 87 LTLT samples, processed under 11 different time-temperature combinations, were negative in FBT cell culture for FMD virus detection (Table 2). Eight of 11 thermal processes negative by cell culture were positive by cattle inoculation.

The time-temperature conditions required to inactivate FMD virus, as determined by cattle inoculation, were 10.66 h at 71°C (process E) and 5.75 h at 75°C (process K). Six cattle inoculated each with lymph node tissue from processes E and K had no clinical signs of FMD for the 14-day post-inoculation observation period. In addition serum samples were negative for the neutralizing antibodies and antibodies to the VIA antigen.

Product yield

Product yield for the LTLT thermal processes E and K were respectively 67.8% ± 3.3% and 68.6% ± 3.4%.

Sensory evaluation

The overall appearance was enhanced by the LTLT processing when compared to that of the conventionally processed samples. Mean panel score for tenderness qualified LTLT samples as very tender, while juiciness can be described as slightly dry to juicy (Table 3). Tenderness (processes K and E) and juiciness (process K) of ST were significantly greater than those of the conventionally processed samples (P<0.01) (Table 3). Process E resulted in more tender samples than process K (P<0.01) while ST cuts from process K were more juicy than process E samples (P<0.01). Flavor did not show significant differences among any of the thermal processes tested. C1 and C2 samples did not show significant differences (P>0.01) for any of the sensory characteristics evaluated.

Rank analysis (Table 4) showed that LTLT samples were better than conventionally processed samples in overall acceptability and tenderness (P<0.05). Samples from process K were ranked as more juicy than E samples (P<0.05). There were no differences (P>0.05) in flavor preference for any of the samples. The conventionally processed samples (C1, C2) were equally preferred in all characteristics examined.

DISCUSSION

The effectiveness of a range of LTLT processes for inactivating FMD virus in contaminated ST samples was studied. Data regarding the decimal reduction times of FMD virus in the meat product processed under the LTLT conditions studied have not been developed. Therefore, the LTLT processes tested were intended to cover a wide range of time-temperature combinations as a screening procedure, while temperatures selected were those commonly used in the sous-vide processes (2). The temperature-time combinations tested should be complemented in future trials with longer times at the lowest temperatures investigated.

Previous reports by House and House (10) quoting the comparable sensitivity of FBT cells with respect to the bovine infectivity assay were not confirmed in this study. We found that inoculation of the bovine tongue was a more sensitive assay system for the detection of small quantities of FMD virus, as evidenced by the development of clinical FMD in susceptible cattle after inoculation with 66 processed samples that were negative in cell culture (Table 2). These findings are in agreement with the reports from Blackwell et al. (5,6) on the survival of FMD virus in thermally processed samples. Similarly, after a virus suspension in MEM was heated at 71°C for a few minutes, FMD virus was not detected by FBT cell culture (data not shown). The FMD virus detected by bovine tongue inoculation in this study in all likelihood represents a heat-resistant fraction of the virus population, as described previously by Bachrach et al. (1).

The survival of FMD virus in virus monitor lymph-node tissue observed in most of the time-temperature combinations assayed shows that FMD virus resistance to heat must not be underestimated. Heat resistance data for FMD virus in meat products are scarce. In addition, differences in product composition and physical properties of meat products and monitor tissues make it difficult to compare data. In spite of this, it must be mentioned that the processing of 2.5 kg samples of FMD-virus-contaminated ground beef in flexible plastic cooking bags to a core temperature of 71°C failed to inactivate FMD virus (6). However, the virus was inactivated in nonformulated and formulated ground beef products when processed at a core temperature of 79.4°C (5,6). Nevertheless, inactivation of type O, FMD virus in ground lymph nodes was reported to require a heating time of more than 1 h at 82°C to achieve negative results by cattle inoculation (6).

In commercial processes using boiling water baths, when the temperature of the coldspot reaches the end temperature, the rest of the cut is exposed to higher temperatures. In contrast, during the LTLT thermal processing,
TABLE 2. Survival of FMD virus in thermally processed 1 kg ST beef muscle.

<table>
<thead>
<tr>
<th>Process Condition (°C/h)</th>
<th>Mean Virus Recovery ( \log_{10} TCID_{50}/g )</th>
<th>FBT Positive ST/ST processed*</th>
<th>Response in Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Lymph Nodes</td>
<td>Heated Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63/ 1.50</td>
<td>4.50</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>67/ 1.00</td>
<td>4.50</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>71/ 0.75</td>
<td>4.50</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>71/ 1.00</td>
<td>4.50</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>71/10.66</td>
<td>4.35</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>75/ 0.33</td>
<td>4.50</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>75/ 1.00</td>
<td>4.60</td>
<td>&lt;0.4*</td>
<td>0/3</td>
</tr>
<tr>
<td>75/ 1.50</td>
<td>4.10</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>75/ 2.00</td>
<td>4.10</td>
<td>&lt;0.4*</td>
<td>0/3</td>
</tr>
<tr>
<td>75/ 4.00</td>
<td>4.60</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
<tr>
<td>75/ 5.75</td>
<td>4.35</td>
<td>&lt;0.4*</td>
<td>0/9</td>
</tr>
</tbody>
</table>

* Number of positive ST samples in FBT cell tissue-culture test per total number of ST processed.

** Plaques not observed.

*** Negative cattle showed no visible lesions and were negative for serum-neutralizing activity and VIA antigen antibody.

TABLE 3. Mean and standard error of sensory panel scores for thermally processed ST cuts (n = 6).

<table>
<thead>
<tr>
<th>Process</th>
<th>Characteristic</th>
<th>Tenderness**</th>
<th>Flavor***</th>
<th>Juiciness****</th>
</tr>
</thead>
<tbody>
<tr>
<td>71°C \times 10.66 h (E)</td>
<td>5.51 ± 0.07c</td>
<td>4.88 ± 0.07*</td>
<td>4.41 ± 0.07b</td>
<td></td>
</tr>
<tr>
<td>75°C \times 5.75 h (K)</td>
<td>6.23 ± 0.05b</td>
<td>4.94 ± 0.07a</td>
<td>3.84 ± 0.06a</td>
<td></td>
</tr>
<tr>
<td>Commercial* (C₁, C₂)</td>
<td>5.18 ± 0.07d</td>
<td>4.68 ± 0.07c</td>
<td>4.61 ± 0.07c</td>
<td></td>
</tr>
</tbody>
</table>

*abc Means for a characteristic bearing different letters in a column showed significant differences \((P<0.01)\).

* Commercially processed samples C₁ and C₂ were not different \((P>0.01)\). Values reported are an average of samples C₁ and C₂.

** Eight-point descriptive scale \((8=\text{extremely tender and } 1=\text{extremely tough})\).

*** Eight-point descriptive scale \((8=\text{pronounced cooked-beef flavor and } 1=\text{very weak cooked-beef flavor})\).

**** Eight-point descriptive scale \((8=\text{extremely juicy and } 1=\text{extremely dry})\).

(200 g) than the ST muscle pieces in this study, and had a different consistency (ground meat, cubes or slices), with lymph nodes cut similarly to the meat. Also, only three animals were tested per product and treatment instead of the six animals used in our assays.

In the present work the possibility of improving the sensory quality of meat cuts cooked by the meat industry in Argentina, while inactivating FMD virus, was tested with promising results. There was found to be an improvement in tenderness and overall acceptability for both LTLT processed samples. Water-bath cooking at low temperatures for long times was reported earlier to result in more uniform and tender products by Buck, Hickey and Rosenau (7). The increased tenderness observed in the LTLT versus the commercial processing may be related to an increment in the solubilization of collagen during extended cooking times. Increments in collagen solubilization and tenderness have been reported to occur during long cooking times and to be favored by slow cooking rates (16). Another important product performance for this kind of noninjected ther-
nally processed meat product, where high cooking losses are otherwise found, is that the product yield was also improved. Cooking weight losses (32.2% or 31.4%) for the LTLT processes were lower than the 40% ordinarily found with commercially cooked meat in Argentina. Product yield improvements are related to lower cooking temperatures and have been reported by Laakkonen, Wellington and Sherbon (11) and Buck, Hickey and Rosenau (7) in cooked meats. This decrease in weight loss represents an obvious economic advantage.

Finally, this work will allow further studies to improve processing conditions at low cooking temperatures. The processing times estimated as safe in these assays might be decreased. Heating times also could be eventually reduced by combining thermal processing with other virucidal food preservation processes such as irradiation (12).

CONCLUSIONS

(1) Two extended LTLT processes inactivate FMD virus while increasing yield and sensory qualities such as tenderness and overall acceptability, when compared to the conventional cooking procedure used in Argentina for meat to be exported. (2) Length of cooking time is important for inactivation of FMD virus. (3) Intradermolingual cattle inoculation is a more sensitive assay system than cell culture for FMD virus detection.

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REFERENCES


