ABSTRACT

Commercial processing methods routinely used for the cooking of ground beef patties in the United States were examined for their virucidal activity against foot-and-mouth disease virus. Thermal processing of the patties in the broiler followed by 20 min oven cooking with an exit temperature of 99.7°C inactivated more than 4.0 log$_{10}$ PFU of foot-and-mouth disease virus. The electronically controlled process was not only virucidal but produced a consumer-acceptable product.

Foot-and-mouth disease (FMD) is a very serious economic disease of livestock species. The viral agent can persist for considerable periods in tissues of infected cattle and swine (5) and has survived in a number of products produced from infected animals (1,2,4). The risk of the introduction of FMD into nonaffected countries through contaminated animal products is the basis for regulations controlling the importation of animal products from countries where FMD exists.

Although limited in the resulting variety of products produced, the cooking of meat products in cans under retort conditions, cooking of cuts of beef in plastic bags in boiling water and in ovens at high temperatures for extensive periods have been effective means of producing FMD virus-free products. Recently, however, milder processing conditions of time and temperature have been used to produce a variety of products, including ground beef products, in which there was no evidence of FMD virus survival (2,4,8).

The experimental finding that FMD virus was inactivated in ground beef cooked in flexible pouches in boiling water at 75°C for 20 min (8) has stimulated interest in the examination of other relatively milder processing conditions, such as continuous belt-oven processing of beef patties, for their virucidal effect on FMD virus.

Thus, we examined the virucidal activity against FMD virus of U.S. commercial continuous belt-oven processing conditions for beef patties prepared from local sources of beef in South America where a number of countries are affected with this disease.

MATERIALS AND METHODS

Cattle

Grade Hereford steers, 12 to 18 months in age and weighing approximately 250 kg, were used in the study. Cattle were born and raised in isolation on an island in the interior of Uruguay before transport to the Direccion de Lucha Contra la Fiebre Aftosa (DILFA), Ministerio de Ganaderia, Agricultura, Y Pesca facility in Pando, Uruguay. The cattle had no previous exposure to FMD (6,8).

Preparation of virus-infected tissue

For these studies, FMD virus, O1 serotype (Strain Campos) was used. The virus was originally isolated in a field outbreak and was obtained from the DILFA repository after 16 passages in cattle. The virus was passaged once more in cattle for these studies.

Infectious FMD virus material as obtained by infecting susceptible cattle by intradermal inoculation into the tongue with 2.0 ml (0.1 ml over 20 sites) and 2.5 ml into the ventral portion of each nasal passage, respectively, with $6.3 \log_{10}$ PFU per ml of virus suspension (1,8). Cattle were slaughtered after the appearance of clinical FMD, 48 h after infection. Esophageal-pharyngeal fluid and blood for infectivity assay were collected prior to inoculation.

Because lymphatic tissue contains high concentrations of virus during the viremic stage of infection and because FMD virus persists for significant periods in these tissues, we collected lymph nodes from the head, viscera, and body at slaughter for use as monitors of viral stability in these studies.

Infectivity assay of the pooled infected lymph node tissue suspensions was performed on bovine tongue. Serial 10-fold dilutions were prepared in chilled 0.01 M phosphate buffered saline solution, pH 7.6, containing antibiotics (8).

The bovine tongue was inoculated intradermally at five discrete linear sites with 0.1 ml of the respective dilution set to determine the infectivity of the lymph node tissue samples. Vesicles...
at the site of inoculation were enumerated visually in 24 h, and the FMD virus concentration recorded as log_{10} vesicle forming unit (VFU) per g. Two susceptible bovines were used per titration.

**Preparation of beef patties**

Chilled, approximately 25 kg hind quarter batches of cuts of skeletal muscle (classified as 90-95% lean), and pieces of fat, 98% free of skeletal muscle, were received daily into the processing area of the DILFA facility from a U.S. Department of Agriculture-approved meat packing plant in Uruguay. The meat and fat were mixed at a weight-weight ratio to give an approximate range of 20-28% fat, and the mixture was ground, using a Hobart meat grinder, with a die plate of 1.6 cm. The powdered formula concentrate was reconstituted in water and mixed manually into the meat-fat mixture, then ground through a 0.3-cm die plate. Fat and moisture determinations (Standards for Meat and Meat Products, Uruguayan Institute of Technical Standards) and pH measurements (Orion Digital Research Model with Ingold 9811 probe) were made on each batch and are presented in Table 1.

The patties, 11.8 cm x 0.5 cm and weighing approximately 112 g ± 1 g, were formed in a Hollymatic former (Model Super, Hollymatic Corp., Countryside, IL). Two separate batches of ground beef were prepared for each three trials which consisted of three replications each.

Beef patties that served as FMD virus monitors were transported chilled to the Virus Laboratory and contaminated as follows: An approximate 10 g sample was removed from the center of a chilled patty, then replaced with the same quantity of FMD virus-infected lymph node tissue, previously ground in a mechanical chopper. A temperature indicator device (TID) (Telatemp Corp., Fullerton, CA) was then inserted into the infected material. The patties, 11.8 cm x 0.5 cm and weighing approximately 112 g ± 1 g, were formed in a Hollymatic former (Model Super, Hollymatic Corp., Countryside, IL). Two separate batches of ground beef were prepared for each three trials which consisted of three replications each.

**Determination of the placement of the virus monitors**

Trial processing runs were performed to determine the heating profile of the oven by arranging the patties in nine rows of five patties per row, then placing a TID in the geometric center of each row and processing at 5, 10, 15, and 20 min, respectively. The TID was then examined for the minimum core temperature achieved. Seven locations were identified as the cold zones in the oven for patties traversing the 5.9 m distance on the belt at respective belt speeds.

Thus, for each processing trial, the virus monitor patties were then placed at those locations on the belt.

Because the reciprocal exchange of heat is less with those patties in the first and last rows than those in the interior rows, the patty in Position No. 1 of the first row was selected as the temperature monitor.

The internal temperature of this virus monitor patty, as it passed through the oven, was measured by inserting the tip of a type T thermocouple (TMQ SS.05266, Omega Engineering Inc.), connected to a microprocessor digital thermometer (HH 21, Omega Engineering Inc.) by a 9 m length of OSK cable (SB-TESC-30-9), into the geometric center of the patty.

Optimum control settings were developed for the broiler and oven, respectively, that allowed for the repetitive production of hamburger patties with desirable organoleptic traits. The insertion of additional heating elements in the oven permitted the maintenance of a minimum internal temperature of the patty at 84°C and higher as it traveled through the oven and a mean exit temperature of 99.7°C. The TID present in the virus monitor patties confirmed that the minimum internal temperature had been reached.

Insertion of the thermocouple into the virus monitor patty was accomplished within 5 s, thereby assuring only a minimal transitory drop in temperature.

**Table 1. Physical characterization of raw patties.**

<table>
<thead>
<tr>
<th>Set no.</th>
<th>pH</th>
<th>% Fat</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.21</td>
<td>22.8</td>
<td>59.7</td>
</tr>
<tr>
<td>2</td>
<td>6.25</td>
<td>21.5</td>
<td>60.6</td>
</tr>
<tr>
<td>3</td>
<td>6.33</td>
<td>20.9</td>
<td>61.3</td>
</tr>
<tr>
<td>4</td>
<td>5.66</td>
<td>24.8</td>
<td>57.9</td>
</tr>
<tr>
<td>5</td>
<td>5.90</td>
<td>27.6</td>
<td>55.4</td>
</tr>
</tbody>
</table>

**Cooking process**

Processing involved the initial cooking of patties in an automatic gas broiler (NIECO, Model 824G, Burlingame, CA), followed by direct feed into a 6.7 m x 0.9 m x 0.75 m continuous belt fed oven for continued cooking in moist heat. The design of the heating system of the oven was such that desired chamber temperatures throughout the oven could be accurately controlled. The electrical system was fabricated so that additional heating elements could be placed in series to increase the chamber temperatures.

The virucidal effect of processing using the broiler plus 5, 10, 15, and 20 min cooking, respectively, was examined. Forty-five patties were cooked per replication, times three replications, per trial, times three trials per processing condition, for a total of 405 patties per test run.

**Preparation of ground beef samples for infectivity assay**

The central core was removed from each thermally processed patty contaminated with FMD virus-infected lymph node tissue pooled respectively, and homogenized as a 20% suspension in 0.01 M phosphate buffered saline solution, pH 7.6, containing 20% chloroform (vol:vol) in an Ultra Turrax sonicator. The homogenate was clarified by centrifugation at 9,000 rpm for 20 min at 4°C in a Sorval RC2B centrifuge. The supernatant fluid was aseptically removed and placed into a 3 dram (11.09 ml) vial.

**Cell culture assay**

The assay for the presence of FMD virus was performed as described previously (8). Briefly, confluent cell culture monolayers of baby hamster kidney obtained from the DILFA repository were grown at 37°C in Eagle's minimum essential medium supplemented with 5% bovine serum in 13 x 125 mm pyrex roller tubes. Serial 10-fold dilutions of 20% supernatant fluid of the heated samples were prepared in chilled Eagle's minimum essential medium after thermal processing and clarification.

An inoculum of 0.05 ml samples was adsorbed on the cell monolayer for 1 h at 37°C. Maintenance medium was added to the cells which were then incubated at 37°C for 48 h. The monolayers were examined microscopically for a period of 7 d for cytopathic effect, and when the effects of virus replication were observed, the virus concentration was recorded as tissue culture infectious dose 50’s per g of product. A minimum of three replicates per dilution was observed.

**Cattle inoculation**

Susceptible cattle were inoculated intradermally into the tongue, 2.0 ml over 20 sites (0.1 ml per site). The animals were observed daily for the development of clinical FMD. When vesicular lesions developed, samples of material were harvested and typed serologically (8). Blood samples were collected over 7 d and examined for the presence of FMD virus.

The sera of cattle that remained negative for 14 d were examined for the presence of antibody to virus infection-associated antigen (6) and of virus neutralizing activity (8).
Organoleptic evaluation
Product quality was assessed by professional taste panelists using double blind test procedures.

RESULTS

Physico-chemical characteristics of the beef patties
Product variation, including pH and percent moisture, between batches of ground beef, irrespective of collection time, was minimal (Table 1).

The fat content was within the desired 20-28% range for desired organoleptic traits.

The patty after cooking in the broiler alone had a grilled exterior but was raw on the inside. The remaining patties, processed in the broiler and cooked for 5 min and longer in the oven, appeared to be thoroughly cooked.

The product loss remained constant through 5 to 15 min oven cooking but increased by 6% at 20 min (Table 2). With an increase in overall chamber temperature, the percentage product loss at 15 and 20 min oven processing, respectively, was constant at 48 to 49% (Table 3).

The overall appearance, taste, mouth feel, and quality of the oven-cooked products were consumer acceptable.

Virucidal effect of processing
The cooking of hamburger patties over 5 to 20 min to an endpoint temperature of 90°C did not inactivate FMD virus present in the infected lymph node tissue contaminant (Table 4). The virus, however, was inactivated when such patties were processed for 20 min to an endpoint temperature of 99.7°C (Table 5).

DISCUSSION

Patties cooked in the broiler alone had pink centers permeated with a reddish colored tissue fluid. However, after 5 min cooking in the oven, the products had a thoroughly cooked appearance.

A thoroughly cooked appearance in the patty, however, was no guarantee that FMD virus had been inactivated, as evidenced by the survival of the virus in the product cooked in the broiler followed by 5 min of oven cooking.

Even under the more rigorous processing conditions, the virus persisted. For example, clinical FMD was manifested after 11 d in cattle that had been inoculated with samples prepared from pooled suspensions of patties which lost the oven at a mean temperature of 89°C, over a 20 min cooking period (Table 4). The virus was also not inactivated in the patties when cooked to an exit temperature of 99.9°C (Table 5).

The role of fat in enhancing virus survival in ground beef products during the cooking has been documented (7); however, although it may have played a role in the survival of FMD virus in the patties processed as presented in Table 4 and in those products processed for 15 min in Table 5, it did not protect FMD virus from inactivation in the product cooked for 20 min in Table 5.

<table>
<thead>
<tr>
<th>Oven cooking (min)</th>
<th>Weight of patties, g*</th>
<th>% Product loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
</tr>
<tr>
<td>Broiler alone</td>
<td>113</td>
<td>73.6</td>
</tr>
<tr>
<td>Broiler + 5 min</td>
<td>112</td>
<td>67.0</td>
</tr>
<tr>
<td>Broiler + 10 min</td>
<td>112</td>
<td>69.5</td>
</tr>
<tr>
<td>Broiler + 15 min</td>
<td>112</td>
<td>69.3</td>
</tr>
<tr>
<td>Broiler + 20 min</td>
<td>111</td>
<td>55.3</td>
</tr>
</tbody>
</table>

*Mean weight of patties.

TABLE 3. Weight loss of beef patties as a function of time of oven cooking (with extra heating elements) after broiling.

<table>
<thead>
<tr>
<th>Oven cooking (min)</th>
<th>Weight of patties, g*</th>
<th>% Percent loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
</tr>
<tr>
<td>Broiler + 15 min</td>
<td>112</td>
<td>57.9</td>
</tr>
<tr>
<td>Broiler + 20 min</td>
<td>112</td>
<td>57.4</td>
</tr>
</tbody>
</table>

*Mean weight of patties.

TABLE 4. Inactivation of FMD virus in beef patties, as a function of oven cooking after broiling.

<table>
<thead>
<tr>
<th>Oven cooking (min)</th>
<th>Mean patty temperature (°C)</th>
<th>Virus detection in cooked product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw*</td>
<td>Exit from oven</td>
</tr>
<tr>
<td>5</td>
<td>8.3</td>
<td>78.5</td>
</tr>
<tr>
<td>10</td>
<td>8.3</td>
<td>80.3</td>
</tr>
<tr>
<td>15</td>
<td>7.9</td>
<td>88.8</td>
</tr>
<tr>
<td>20</td>
<td>8.2</td>
<td>89.5</td>
</tr>
</tbody>
</table>

*Titration in the bovine tongue, the raw product samples had 4.10 log10 vesicle forming units (VFU) per g.

As reported earlier, the virucidal activity against FMD virus in a thermal process is directly related to the quantity of heat energy that the virus is exposed to (3). This fact was evident in the current study, when in order to inactivate FMD virus, it was necessary to produce those conditions in a continuous belt-fed oven that would expose the virus in
the monitor patty to a uniform quantity of heat throughout the chamber, while traversing the oven on a moving surface.

Thus, the process that we developed for the manufacture of cooked hamburger patties not only produced a consumer-acceptable product but also was virucidal for FMD virus.

ACKNOWLEDGMENTS

This study was performed under Project URU/81/TO1 of the United Nations Financing System for Science and Technology, administered by the Facultad Veterinaria, University of the Republic, Uruguay through the cooperation of DILFA, Ministerio de Ganadería, Agricultura, Y Pesca.

The studies were performed at the laboratory facilities of DILFA, Pando, Uruguay. The authors acknowledge the logistical support and encouragement for these studies by Dr. M. Cuadrado, Assistant Director, DILFA, MGAP, and the technical assistance of J. Olaso, M. Eng., Frigorifico San Jacinto, and the Staffs of DILFA and Frigorifico San Jacinto-NIREA S. A.

REFERENCES