Research Note

Trial of Heat Inactivation of Selected Viruses Following Irradiation

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ABSTRACT

Four selected viruses were irradiated in ground pork with an electron beam at absorbed doses of 4.4 to 5.27 kGy. Irradiated and nonirradiated viruses were heated at four temperatures for four time intervals and assayed for surviving virus. Data were examined for evidence of irradiation-heat interaction to determine whether absorbed irradiation would sensitize virus so that a lesser amount of heat would be required for inactivation. It was determined that irradiation does not increase lability to heat to a level that has practical application in virus inactivation.

Key words: Irradiation, virus, pork, heat

Methods are needed for the long-term preservation of pork products which reduce possible contaminating viral agents to negligible levels. Foodborne viral infections (4) and thermal resistance (8) of the viruses involved in foodborne infections have been reported.

A preliminary study (11) was done in which ground Pork was contaminated with three viruses and irradiated at an absorbed dose of 1 kGy. There was no significant decrease in viable virus at this dose.

The American Medical Association endorses irradiation of foods (1, 2) and the U.S. Department of Agriculture has approved appropriate and safe levels of irradiation to be used in foods (3).

Certain traditional methods for processing meat products were examined for their virucidal effects on three serotypes of foot-and-mouth disease virus (FMDV) (9). Aging, curing, and heating at 78°C for 20 min did not alter the sensory characteristics of the meat when used singly or in combination. The only processing treatment that was virucidal was the combination of heat and gamma irradiation.

Eight viruses were each separately suspended in bovine serum and treated with gamma radiation at −68°C (7). The $D_{10}$ values for the eight viruses ranged from 0.25 kGy for Albana virus to 1.07 kGy for minute virus of mice, a parvovirus.

Ionizing radiation was shown to be capable of serving as an effective sanitizing treatment, improving the quality of shellfish and providing an increased margin of safety for shellfish consumers (10). A $D_{10}$ value of 2 kGy was determined for depletion of hepatitis A virus in clams and oysters. A $D_{10}$ value of 2.4 kGy was determined for depletion of rotavirus SA-11 in clams and oysters.

The recovery was studied of polio virus from a variety of foods inoculated with virus after four processing procedures: freeze drying, "pasteurization" by cobalt 60 gamma irradiation and storage at 4°C and 20°C (6). A decrease in virus recovery occurred in each of the operations. Complete loss of recoverability was observed only in highly acidic foods (below pH 2.9). The poor correlation between the rate of loss of pasteurized virus in fish fillets and suspensions suggests a significantly greater loss of recoverability of virus from the food.

Because viral inactivation conducted in water, saline, or culture medium does not reflect actual treatment practice, inactivation experiments were carried out in 500-ml containers of serum (5). Six viruses were studied. It was concluded that gamma irradiation inactivation of biological contaminants in aqueous solution depends on the strandedness of the viral nucleic acid, the target size, and the milieu in which the irradiation occurs. The difficulty in inactivating porcine parvovirus is due in large part to the extremely small size of the virus.

Decimal reduction values (D values) were determined for 30 viruses (15). The D values of viruses suspended in Eagle's minimal essential medium ranged from 0.39 to 0.53 Mrads. It was found necessary to increase the radiation dose by a factor of >3 to inactivate virus suspended in Eagle's medium, compared to inactivation of the same virus suspended in distilled water.

The question subsequently arose whether an absorbed dose of irradiation of between 4 and 5 kGy would sensitize virus to a requirement for a lesser amount of heat for inactivation. A study was thus designed to investigate this possibility.

MATERIALS AND METHODS

Viruses and cell cultures

The viruses and cell cultures are listed in Table 1. With the exception of PK-15 cells, the cells were propagated in minimal
TABLE 1. Some characteristics and irradiation exposure of viruses assayed

<table>
<thead>
<tr>
<th>Virus, TCID&lt;sub&gt;50&lt;/sub&gt;/ml</th>
<th>Type</th>
<th>Stranded</th>
<th>Host cells used</th>
<th>Enveloped</th>
<th>Irradiation (kGy) exposure dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno, 1 x 10&lt;sup&gt;8.43&lt;/sup&gt;</td>
<td>DNA</td>
<td>DS</td>
<td>MA-104</td>
<td>No</td>
<td>4.4</td>
</tr>
<tr>
<td>ECHO-7, 1 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>RNA</td>
<td>SS</td>
<td>LLCMK-2</td>
<td>No</td>
<td>4.9</td>
</tr>
<tr>
<td>Encephalomyocarditis, 1 x 10&lt;sup&gt;6.3&lt;/sup&gt;</td>
<td>RNA</td>
<td>SS</td>
<td>Vero</td>
<td>No</td>
<td>4.46</td>
</tr>
<tr>
<td>Pseudorabies, 1 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>DNA</td>
<td>DS</td>
<td>PK-15</td>
<td>Yes</td>
<td>5.27</td>
</tr>
</tbody>
</table>

essential medium supplemented with 10% fetal bovine serum and antibiotics. The PK-15 cells were propagated in minimal essential medium plus 3% bovine virus diarrhea (BVD)-free swine serum and antibiotics.

**Pork**

Eighty percent lean ground pork was obtained frozen in 1-lb (ca. 0.45 kg) sealed packages from the Iowa State University meat laboratory. The pork was thawed and weighed as needed.

**Preparation and irradiation of viruses in pork**

Ten percent suspensions of the four viruses were made by adding 1 part of stock virus to 9 parts of lean pork (vol/wt). The pork-virus mixtures were homogenized by vigorous shaking by hand and vortexing 8 to 10 times with 10-s bursts. The 50-ml plastic centrifuge tubes containing the homogenates were kept in an ice bath when not being handled. As soon as homogenates were prepared for a given virus, the appropriate number of tubes were taken to the Iowa State University Linear Accelerator Facility on ice and irradiated using an electron beam (linear accelerator). The minimum dose occurred at the surface of the product. This dose was approximately 4.2 kGy and varied slightly from run to run (±4.0%). The maximum dose occurred within the product and could only be estimated. This value was 5.2 to 5.5 kGy. The dosimeters were calibrated to a nationally or internationally recognized standard. These standards were performed at a secondary laboratory that, through collaborative studies, has ties to the standards at the National Institute of Standards and Technology.

The characteristics of the electron beam were as follows. The linear accelerator was operated at 20 Mev, 6.9 kev, and a mean beam current of approximately 692 μamp. The samples moved at a speed of 8.25 ft/min (ca. 2.51 m/min). The placement of the sample was in the center of a cart on a honeycomb pad. The beam was scanned at 60 cm. across the cart so that the product was in the center of the beam scan.

The tubes were placed horizontally during irradiation. The greatest thickness of the layer to be irradiated was 1.0 cm. Dosimeters were placed above and below the layer to be irradiated and postirradiation readings from each pair were averaged in calculating absorbed doses.

Heating of samples was done in four separate water baths as follows. Five milliliters of samples to be heated were placed into sterile 10-ml vaccine vials, closed with sterile aluminum rings and rubber stoppers. Samples heated at 40°C were equilibrated submerged for 0.75 min, those at 50°C for 0.50 min, those at 60°C for 0.25 min, and those at 70°C for 0.15 min. Heating times began after equilibration. Heated samples in vials were placed into an ice bath immediately after heating.

Irradiation exposure was determined by dosimetry (alanine dosimeters), and expressed in kGy (Table 1). Irradiated preparations were centrifuged at 1,400 × g for 25 min in a refrigerated centrifuge, similar supernatants pooled, were divided into aliquots, and frozen at −80°C until they were titrated for virus. The list of irradiated, heated, and control virus preparations is presented in Table 2.

**Titration of principal and control sample preparations**

Titrations were carried out in 96-well cell culture plates. Samples for titration were diluted from 10<sup>−1</sup> through 10<sup>−11</sup> in saline G (12). Eight replicates of 100 μl of each dilution were titrated. Titerers were calculated as 50% endpoints (13).

**RESULTS**

Assessment of reduction in titer (50% endpoints) by irradiation and by irradiation and heat was made by graphical presentation as shown in Figures 1 through 4.

![Figure 1. Heat inactivation of adenovirus: (a), in saline G; (b), in pork; (c), following irradiation.](image-url)
Irradiation at the levels applied reduced the titer of the test adenovirus by approximately 1.5 log units (Fig. 1), of ECHO-7 virus by approximately 2.0 log units (Fig. 2), and encephalomyocarditis virus approximately 1.0 log\textsubscript{10}. Pseudorabies was completely inactivated.

**Heat inactivation in pork or saline G**

Adenovirus (Figure 1a to 1c). Placing adenovirus in pork greatly increased heat resistance. At 40°C in meat, the virus was stable. In saline G the titer dropped >1.0 log unit in 10 min and further dropped 1.0 log unit at 20 to 40 min. At 50°C in pork the virus was stable. In saline G, the titer dropped >2.0 log units at 10 min and further decreased with heat in 20 to 40 min.

ECHO-7 virus (Figure 2a to 2c). Placing the virus in pork increased the heat resistance. At 40°C and 50°C heating lowered the virus titer by an average of 1.2 log units, from 10 to 40 min in saline G.

Encephalomyocarditis virus (Figure 3a to 3c). In saline G, the virus was stable at 40 and 50°C for 10 min, and then there was some increased inactivation from 20 to 40 min of heating at both 40 and 50°C. In pork, the virus was stable at 10 min of heating at both 40 and 50°C. At 40°C heating, the virus lost stability in pork at 40 min. At 50°C heating, the virus lost stability in pork at 40 min.

Pseudorabies virus (Figure 4a to 4b). Placing the virus in pork greatly increased heat resistance. At 40°C in pork the virus was stable, but was inactivated at 50°C for 20 min and above. In saline G (control), the titer dropped from 6.2 to 3.2 log at 40°C for 10 min and 3.0 to 2.2 log for 40 minutes. At 50°C for 10 minutes the titer was also 2.2 log but was completely inactivated at 20 to 40 min.

**Heat inactivation following irradiation in pork**

Adenovirus (Figure 1a to 1c). Placing the virus in pork (control) lowered the virus titer from 5.2 to 3.5 log compared to saline G. Irradiation in pork (control) lowered the virus titer from 3.5 to 1.8 log. Heating at 40 and 50°C for 10 to 40 min did not further reduce the titer. Heating at 60°C for 10 min inactivated the virus whether it was irradiated or not. DNA can be damaged by a variety of physical agents, ionizing radiation being one. There is a chance that damage sustained by DNA can be repaired because genetic information is stored in both strands of the double helix, so that genetic information lost by one strand can be retrieved from
The results of this study did not indicate that irradiation increased lability to heat to a level that has practical application in virus inactivation. This was confirmed by the fact that infectious virus was still present, with the exception of pseudorabies virus, following irradiation and heating up to the point where heating alone was sufficient to inactivate viable virus.

Perhaps other combinations, including irradiation, should be tried to inactivate virus added to ground pork, e.g., chemical sprays used before irradiation or spices added after irradiation.

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REFERENCES