

## Inactivation of Poliovirus 1 and Coxsackievirus B-2 in Broiled Hamburgers

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### ABSTRACT

Thermal stability of two enteroviruses, poliovirus 1 and coxsackievirus B-2, inoculated into ground beef patties was investigated using household broiling procedures. Internal temperatures during cooking were monitored by thermocouples placed in the centers of patties. The appearance of the centers of hamburgers correlated with the temperatures reached: red-pink-rare, 60 C (140 F); pink-brown-medium, 71 C (160 F); and brown-well-done, 76.7 C (170 F). Cooked and uncooked virus-inoculated patties were assayed for viral plaque forming units produced in Vero monkey kidney cell cultures. No viruses were detected in patties cooked to 60 C (140 F) and held at room temperature for 3 min. However, virus was recovered from 8 of 24 patties cooked to 60 C (140 F) and immediately cooled to 23 C (74 F). No viruses were detected in patties heated to 71 C (160 F) or 76.7 C (170 F) internal temperatures. Results indicate that the cooking time and temperatures used to prepare rare hamburgers wherein the center meat remains red may not be sufficient to inactivate viruses that might be present in the sample especially if the hamburger is consumed or cooled within 3 min of cooking. When frozen or partially defrosted patties are cooked, extensive external cooking can occur with little or no visible change in the coloration of the center meat.

Over  $1 \times 10^{10}$  kg (22 billion lb) of beef were consumed in the United States in 1973. Approximately half of this beef was consumed in the form of ground beef or hamburger—an average of about 454 g (1 lb) per week for each resident in the United States. There are no Federal microbiological standards for ground beef, and State and local regulations vary from being relatively strict to nonexistent. The quality of the market product basically depends on the sanitary control used by the processor and the market outlets, and on the effectiveness of the local health agencies (1).

In 1969 we reported finding viruses in 3 out of 12 market samples of ground beef (8). The isolates were identified as poliovirus 1, 2, and 3, and echovirus 6; the number of viruses recovered varied from 1 to 195 viral plaque forming units (PFU)/5-g sample. Therefore, a 113 g (¼ lb) hamburger could contain from 23 to 4,400 PFU of virus.

Because of these findings, we conducted a study to determine if household cooking processes inactivated viruses. Ground beef patties were inoculated with either poliovirus 1 or coxsackievirus B-2 and were broiled in a small household cooking unit. The directions followed

were obtained from cookbooks commonly used in the home (2, 3). Processing temperatures of 60 C (140 F), 71 C (160 F), and 76.7 C (170 F) were used for rare, medium, and well-done hamburgers (7).

### MATERIALS AND METHODS

#### *Virus*

Stock cultures of poliovirus 1 (ATCC VR 59) and coxsackievirus B-2 (ATCC VR 29) were propagated in Vero cell cultures (ATCC CCL 81). Leibovitz medium (L-15) containing 2% heat-inactivated fetal bovine serum was used to propagate virus in cell monolayers (6). Virus pools were prepared and used for the standard inocula.

#### *Media*

The diluents, suspending media, and growth media for the cells and the viral plaque assay were the same as those previously published (9). Eagle minimal essential medium (MEM), with Hanks balanced salts containing high concentrations of antibiotics, was used to prepare the meat slurries in these studies (4).

#### *Methods*

The glass wool filtration method (11) was utilized for recovery of virus from inoculated raw and cooked ground beef patties. Two-hundred milliliters of fluid diluent were added to the beef sample (a 113-g patty), the pH was adjusted to 8.5, and the slurry was shaken for 15 min. The pH was readjusted to 8.5, and the slurry was poured over glass wool. Twenty milliliters of the filtrate were assayed in 1-ml portions added to 45 cm<sup>2</sup> monolayer Vero cell cultures in 177.4 ml (6-oz) bottles. The cell cultures were overlaid with agar medium and incubated at 36 C (97 F). Virus plaques were counted and marked daily for 2 weeks.

#### *Cooking procedure*

Ground beef, 25% fat content, was purchased in a frozen lot of preformed patties. Individual patties (113-g) were thawed and placed in a plastic bag, and  $3 \times 10^5$  PFU of poliovirus 1 or  $3 \times 10^4$  PFU of coxsackievirus B-2 were added. The sample was kneaded for 2 min to evenly distribute the virus, and patties were reformed using a commercial hamburger form to produce individual units of 9 cm (3.5-in.) diameter and 1.3 cm (0.5-in.) thickness. Starting at the circumference of each ground beef unit, a copper-constantan thermocouple was inserted radially to a depth of 3.8 cm (1.5 in.). After cooking, the meat shrank to a diameter of 7.6 cm (3 in.), positioning the thermocouple at the geometric center of the patty. The patties were placed on a rack in a small commercial broiler that had been preheated to a temperature of 274 C (525 F). The hamburgers were placed 7.6 cm (3 in.) from the heating element and cooked in the following manner:

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|---------|---|
| Rare:   | Broiled for 3.5 min, then turned and broiled until the internal temperature reached 60 C (140 F). (Total avg. cooking time: 7.9 min). |
| Medium: | Broiled for 4 min, then turned and broiled until the internal temperature reached 71 C (160 F). (Total avg. cooking time: 10.3 min).  |

Well-done: Broiled for 5 min, then turned and broiled until the internal temperature reached 76.7 C (170 F). (Total avg. cooking time: 11.1 min).

After removal from the broiler, the hamburgers were left at room temperature for 3 min and then analyzed for viral content. The 3-min period was the time estimated between the end of the cooking process and the actual eating of the hamburger in the home. A number of samples were immediately disrupted into 200 ml of cold [5 C (40 F)] diluent and processed to determine whether viral inactivation was complete after the broiling process or dependent on the continuing heat treatment during the 3-min holding period.

Some consumers and commercial establishments prepare individual hamburger units and freeze them until use. The frozen or partially thawed raw patty is put directly onto the grill or into the broiler and cooked. A number of hamburgers were prepared in this manner, and the internal temperature was monitored.

## RESULTS AND DISCUSSION

Several uninoculated hamburgers were cooked and examined organoleptically. The color at the center of each patty was the most useful characteristic in subjectively determining "doneness" of the meat. The rare patties were red to pink in the center, the medium were pink to brown, and the well-done were brown.

Survival of poliovirus 1 and coxsackievirus B-2 in the 81 heated samples was seen only in the rare patties [60 C (140 F)] that were cooled to 23 C (74 F) within 15 sec by disrupting them into cold [5 C (40 F)] liquid medium; 1.4% and 1.7% of the total poliovirus 1 and coxsackievirus B-2 inoculum were recovered, respectively, from 4 of 6 and 4 of 18 cooled patties. The ranges of recoveries from these patties were 0.06% to 2.9% for poliovirus 1 and 0.01% to 9.9% for coxsackievirus B-2. The percentages of recoveries were based on the number of viral PFU recovered from uncooked positive control patties and these controls yielded an average recovery of 54% of the seed virus inputs from 26 patties. The recovery is similar to that reported in a detailed paper on this method (11). No viruses were detected in 18 uncooled patties or from nine patties cooked to 71 C (160 F) and cooled. In actual practice, such a cooling procedure would not be used; however, its effect could be duplicated by consumers who start eating the hamburger immediately upon being served. The data indicate that hamburgers should reach an internal temperature higher than 60 C (140 F) if a margin of safety is desired. The most practical indicator of the desired temperature is the color; a hamburger should be brown to pinkish brown on the inside. The 39 medium and well-done patties yielded no detectable virus. All patties held for 3 min at room temperature [21 C (70 F)] after cooking essentially maintained their internal temperatures [i.e., this temperature dropped 3 C (5.4 F) or less].

Frozen [-11.1 C (12 F)] patties containing thermocouples were broiled for the same number of minutes required to bring the unfrozen [10 C (50 F)] patties to internal temperatures of 60 C (140 F), 71 C (160 F), and 76.7 C (170 F). The temperatures actually achieved were 18.3 C (65 F), 37.8 C (100 F), and 51.7 C (125 F) with subsequent temperature drops as observed in the patties

cooked from the unfrozen state. All centers of the cooked-frozen patties were red although exteriors appeared well-done. High-temperature searing of hamburgers produces a similar product. The average time required to cook the frozen patties to the rare, medium, and well-done stages was 14, 17, and 18 min, respectively.

Thermal destruction curves of other viruses in milk and milk products have been shown to be virtually asymptotic in configuration at temperatures below 60 C (140 F). Viral destruction rates at temperatures above 60 C (140 F) approached a first-order reaction (10). It is probable that similar viral destruction rates occur during the cooking of ground beef and that the viruses surviving at 60 C (140 F) are from the second portion or "tail" of such a curve. Cooking the meat to internal temperatures above 60 C (140 F) enhances the probability of viral destruction.

The amount of virus in foods is also important. Studies on heat inactivation of Sabin type 1 poliovirus at concentrations of approximately  $1 \times 10^7$  pfu/gm in ground beef showed some virus survival in meat held at 80 C (176 F) for 5 min (5).

The incidence of viral contamination in foods is only partially known, and the thermostability of viruses in foods can differ. However, the inactivation of two enteroviruses, poliovirus 1 and coxsackievirus B-2, in ground beef patties broiled to internal temperatures above 60 C (140 F) indicates that heating food in this manner could be an effective barrier to consumption of such infectious agents. Proper cooking of hamburgers should be coupled with long-established procedures of good sanitation, such as the use of healthy animals as a food source and scrupulous cleanliness in all phases of food handling.

## ACKNOWLEDGMENT

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## REFERENCES

1. Anonymous. 1971. A close look at hamburger. *Consumer Reports* 36:478-483.
2. Anonymous. 1967. Patties, p. 64-72. *In* Ground beef cookbook. Favorite Recipes Press, Inc. 1967. Montgomery, Alabama.
3. Anonymous. 1970. Beef, p. 209. *In* Better homes and gardens new cook book. 3rd ed. Meredith Press, New York, N.Y. and Des Moines, Iowa.
4. Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. *Science* 130:432-437.
5. Filppi, J. A., and G. J. Banwart. 1974. Effect of the fat content of ground beef on the heat inactivation of poliovirus. *J. Food Sci.* 39: 865-868.
6. Leibovitz, A. 1963. The growth and maintenance of tissue-cell cultures; cultures in free gas exchange with the atmosphere. *Amer. J. Hyg.* 78:173-180.
7. Levie, A. 1970. Structure, pp. 42-47. *In* The meat handbook 3rd ed. AVI Publishing Co., Inc., Westport, Conn.
8. Sullivan, R., A. C. Fassolitis, and R. B. Read, Jr. 1970. Method for isolating viruses from ground beef. *J. Food Sci.* 35:624-626.
9. Sullivan, R., and R. B. Read, Jr. 1968. Method for recovery of viruses from milk and milk products. *J. Dairy Sci.* 51:1748-1751.

10. Sullivan, R., J. T. Tierney, E. P. Larkin, R. B. Read, Jr., and J. T. Peeler. 1971. Thermal resistance of certain oncogenic viruses suspended in milk and milk products. *Appl. Microbiol.* 22:315-320.
11. Tierney, J. T., R. Sullivan, E. P. Larkin, and J. T. Peeler. 1973. Comparison of methods for the recovery of virus inoculated into ground beef. *Appl. Microbiol.* 26:497-501.

### Specialized Items Increase, Total Production Steady, Reports 1974 "Milk Facts"

These and other pertinent economic figures are reported in "Milk Facts," the annual publication of the Milk Industry Foundation. Included in the booklet is information on milk production, processing, distribution, consumption, nutrition and marketing. The Foundation is the national association of dairy processor and distributor companies.

Some additional highlights of "Milk Facts" are that:

- \* Milk production in 1973 reached 115.4 billion pounds, approximately the same as the prior year.
- \* Output per cow rose two percent upsetting the two percent drop in milk cows.
- \* Dairy farmer cash receipts were up 16% over the previous year, totaling \$9.1 billion for milk sold to processors.
- \* The "real" price of milk rose in 1974, but was still lower than in the 1950's and early 1960's.
- \* Sales of fluid milk total 26.7 billion quarts, down two percent from 1973.
- \* Lowfat and skim milk sales were up six and one-half percent and now account for thirty percent of total fluid milk sales.
- \* Whole milk sales were down five and one-half percent, cottage cheese down eleven percent but yogurt was up eight percent.
- \* Also up were cheese (five percent), ice cream (one percent), and butter (six percent).

"Milk Facts" contains several new features this year, one being a report on consumer profile and their attitude toward yogurt and cottage cheese. Reported for instance,

was that nearly two thirds of all U.S. families bought cottage cheese during the six months period surveyed; the pint is the most popular cottage cheese size and the large urban areas show the greatest interest in cottage cheese.

Yogurt is also popular in the larger cities and strawberry is its preferred flavor. Females are greater consumers of yogurt, particularly in the 17-19 years of age bracket. Yogurt, considered a particularly good food product by persons who are weight conscious, is considered good at lunch or as a snack between meals.

Another feature of the book is a pre bicentennial section on the "History of the Dairy Industry" which recounts the beginning of the dairy industry in the U.S. from 1611 through current times.

The thirty-two page booklet of the Foundation contains interesting data on how milk moves from the dairy farm to the ultimate consumer in the home, how the industry is regulated for product wholesomeness, purity, and the basic price paid to farmers, and how milk and milk products are used all over the world. Data are presented by individual states, regions and nationally, and a segment is devoted to the various nutritional elements in fluid milk.

Copies of "Milk Facts" are available from MIF for members at 8c per copy up to 1,000 or 7c per copy for orders of 1,000 or more. Nonmembers may secure copies at 11c per copy. Orders should go to the Milk Industry Foundation, 910 17th Street, N.W., Washington, D.C. 20006.