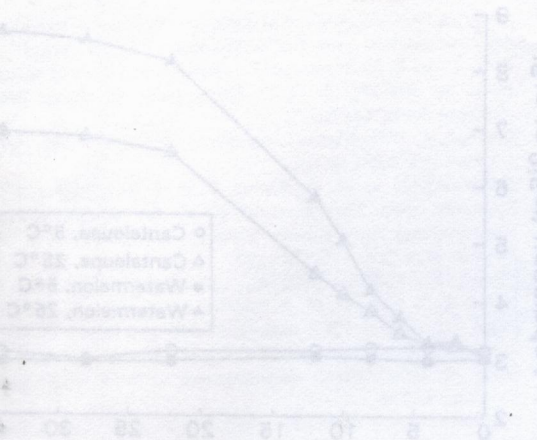

The examination of imported frozen chicken found that 32 out of 39 samples were contaminated with *Listeria*, with 18 of the 32 "positives" being confirmed as *L. monocytogenes*. This latter organism was also isolated from 12 out of 107 samples of frozen semi-processed meat products, while *L. welshimeri* was found in two samples and *L. innocua* in a further 20. Whether or not the high level of contamination of frozen chicken (82%) as against 33% for fresh product is a reflection of the contrasted sources of supply or differences in processing was not established, but clearly poultry can act as a significant carrier of *L. monocytogenes*. However, the fact that these retail items will be cooked before eating should eliminate any direct risk to consumers, for only extensive cross-contamination of other foods could lead to cell counts capable of causing disease.

The absence of contamination in any of the 197 "ready-to-eat" meals suggests that, as long as sound methods of food handling are employed in the home, "prepared foods" need not pose any risk for the consumer – at least as far as *Listeria* spp. are concerned.

A point emphasized, perhaps, by the fact that the meals included dishes based on chicken and semi-processed meats, as well as prepared salads. In other words, the range included foods where the raw materials might, on the basis of the survey, have been anticipated to be reservoirs of *Listeria*, as well as foods which, under conditions of poor hygiene, could have been subject to cross-contamination, and yet the microbiological quality of the sampled products was excellent.

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A Research Note

Survival and Growth of Enterohemorrhagic *Escherichia coli* O157:H7 in Cantaloupe and Watermelon

BRENDA A. DEL ROSARIO and LARRY R. BEUCHAT*

Center for Food Safety and Quality Enhancement and Department of Food Science and Technology, University of Georgia, Griffin, Georgia 30223-1797

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ABSTRACT

The ability of *Escherichia coli* O157:H7 to survive and grow on cubes of cantaloupe and watermelon and on the external rind surface of these fruits was investigated. Populations of the pathogen increased on cubes stored at 25°C but remained constant at 5°C over a 34-h storage period. Growth was observed on the rind of melons stored under high relative humidity at 25°C for 14 to 22 days. The pathogen rapidly died on the rind surface of melons stored at 5°C.

Key Words: *Escherichia coli* O157:H7, cantaloupe, watermelon.

Fresh fruits are seldom incriminated as vehicles of foodborne illness. Natural barriers such as skin and rind as well as a naturally acidic pH prevent or retard the growth of pathogenic bacteria. However, some fully matured fruits have pH values approaching 7 and, once cut to expose the internal flesh to environmental contaminants, can serve as substrates for the growth of bacteria. Cantaloupe and watermelon are among these fruits and are not exempt from the list of foods known to be vehicles of foodborne illness (*11*). Two outbreaks of salmonellosis have been associated epidemiologically with cantaloupes. *Salmonella chester* (*15*) and *Salmonella poona* (*5*) were the species involved. *Salmonella miami* and *Salmonella bareilly* were responsible for salmonellosis associated with the consumption of precut watermelon (*9*). In more recent outbreaks, *Salmonella oranienburg* (*6*) and *Salmonella javiana* (*3*), both originating from watermelon, were implicated as causative agents of human gastroenteritis.

Enterohemorrhagic *E. coli* O157:H7 is recognized as an important foodborne pathogen (*7,11,14*). Dairy cattle (*12*) and calves (*13*) appear to be primary reservoirs. The consumption of undercooked ground beef sandwiches (*16*), raw milk (*12*), cheese and turkey sandwiches (*17*) and ham and turkey sandwiches (*4*) has been associated with outbreaks of hemorrhagic colitis caused by *E. coli* O157:H7. In August, 1993, an outbreak of foodborne illness was linked to eating cantaloupe contaminated with *E. coli* 0157:H7 (M. Diermayer, Oregon Health Division, Portland, OR, personal communication). The study reported here was undertaken to determine survival and growth characteristics of *E. coli* O157:H7 on freshly cut cubes of cantaloupe and watermelon and on the rind surface of these fruits.

MATERIALS AND METHODS

Strains and preparation of inocula.

Four strains of *E. coli* O157:H7 (204P, 301C and 505B [obtained from Dr. Donald Conner, Auburn University] and 45753-35 [a laboratory stock culture] isolated from pork, chicken, beef and human feces, respectively) were studied. Stock cultures, maintained on tryptic soy agar (TSA; Difco Laboratories, Inc., Detroit, MI) at 5°C, were activated by transfer to tryptic soy broth (TSB) and incubation at 37°C. Two consecutive loop transfers of actively growing 24-h TSB cultures were made immediately preceding the preparation of inocula. Equal volumes (2 ml) of cultures containing 8.83 to 8.90 $\log_{10}$ CFU/ml of each strain were combined and diluted in sterile 0.1% peptone just before inoculating melons. The population of *E. coli* O157:H7 in the mixture of four strains was 8.84 $\log_{10}$ CFU/ml.

Preparation of melons and procedure for inoculation and incubation.

Cantaloupes and watermelons were purchased from the Georgia State Farmers' Market in Forest Park, GA. In experiments designed to study survival and growth characteristics of *E. coli* O157:H7 on melon cubes, the rind of whole fruits was sanitized before cutting to prepare cubes. The sanitizing process consisted of washing the melons in soapy water with the aid of a soft brush followed by rinsing in tap water, dipping in a 0.5% solution sodium hypochlorite for 1 min, rinsing again in tap water, dipping in 70% aqueous ethanol for 1 min, and finally air drying. Melons were cut with sterile knives and the flesh was cut into approximately 2-cm cubes. Cubes (50 g) were deposited in sterile stomacher™ bags and inoculated with 1.0 ml of *E. coli* O157:H7 cell suspension prepared by diluting the 4-strain mixture by 10^{-4}. After thoroughly distributing the inoculum in each sample, the bags were sealed and placed in incubators at 5° or 25°C.

Melons used in studies to determine the behavior of *E. coli* O157:H7 on the rind surface were not sanitized after purchase. Seven areas, each 2-3 cm in diameter, were delineated on the surface of each test melon. An ink pen was used to mark these areas on cantaloupes, whereas molten paraffin was used for watermelons. A diluted (10^{-2}) 4-strain mixture of *E. coli* O157:H7 cell

suspension (0.1 ml for cantaloupe and 0.2 ml for watermelon) was deposited with a pipet on each 2- to 3-cm diameter area of rind. Melons were held in covered plastic boxes at 5° or 25°C until analysis for *E. coli* O157:H7 populations.

Procedure for enumerating E. coli *O157:H7.*

Immediately after inoculating and at various intervals up to 34 h of incubation at 5° or 25°C, melon cubes were analyzed for viable populations of *E. coli* O157:H7. Samples (50 g) were combined with 50 ml of sterile 0.1% peptone and pummelled using a stomacher at medium speed for 1 min. The resulting slurry was serially diluted and surface plated (0.1 ml) in duplicate on sorbitol MacConkey agar (SMA, pH 7.1; Unipath Co., Oxoid Division, Ogdensburg, NY.). Plates were incubated at 37°C for 22-24 h before colonies were counted. Selected presumptive colonies were confirmed using an API-20E diagnostic kit (Analytab Division, Sherwood Medical, Plainview, N.Y.), the *E. coli* O157:H7 latex agglutination assay (Unipath Oxoid US), and the Bacto *E. coli* O157:H7 H antiserum assay (Difco). Confirmed populations are expressed as CFU/g of melon.

Inoculated rind surface areas (2-3 cm dia.) were analyzed for populations of *E. coli* O157:H7 immediately after inoculation. In addition, rind of cantaloupes and watermelons stored at 5° or 25°C for up to 21 days were analyzed for populations of *E. coli* O157:H7. The surface areas on which *E. coli* O157:H7 had been inoculated were excised using a sterile scalpel. Each piece of rind (2-4 mm thick) was combined with 50 ml of sterile 0.1% peptone and subjected to the same analytical procedure as described for melon cubes. Populations of *E. coli* O157:H7 recovered are expressed as CFU/cm^2 of rind surface.

Statistical analysis.

Three replicates of each experiment were done. Each sample was analyzed in duplicate. Data were subjected to the Statistical Analysis System (SAS Institute, Cary, NC) for analysis of variance and Duncan's multiple range test.

RESULTS AND DISCUSSION

Escherichia coli O157:H7 was not detected in uninoculated melons. Populations of *E. coli* O157:H7 cells detected in inoculated cantaloupe and watermelon cubes held at 5° or 25°C for up to 34 h are shown in Fig. 1. Watermelon cubes (pH 5.56) incubated at 25°C supported better growth than did cantaloupe (pH 7.01). Significant ($P<0.05$) increases in population occurred in both melons between 4 and 6 h. Populations continued to increase significantly at each analysis time, reaching 6.81 $\log_{10}$ CFU/g in cantaloupe and 8.51 $\log_{10}$ CFU/g in watermelon cubes after 28 h of incubation. The number of viable *E. coli* O157:H7 cells detected in cantaloupe and watermelon cubes held at 5°C did not change significantly throughout the 34 h test period. These observations are similar to those reported for *Salmonella* inoculated onto rind-free pieces of cantaloupe (pH 6.67), watermelon (pH 5.90) and honeydew melons (pH 5.95) (*10*). A five-strain mixture grew rapidly at 23°C on all three melons, with the highest populations occurring on watermelon (8.63 $\log_{10}$ CFU/g) within 24 h of incubation. At 5°C, populations of *Salmonella* essentially remained constant. Escartin, et al (*8*) reported that *Shigella* and *Salmonella* can survive and, in most cases, increase in population when inoculated, heat-pasteurized slices of jicama, papaya and watermelon are held at room temperature (22-27°C) for up to 6 h.

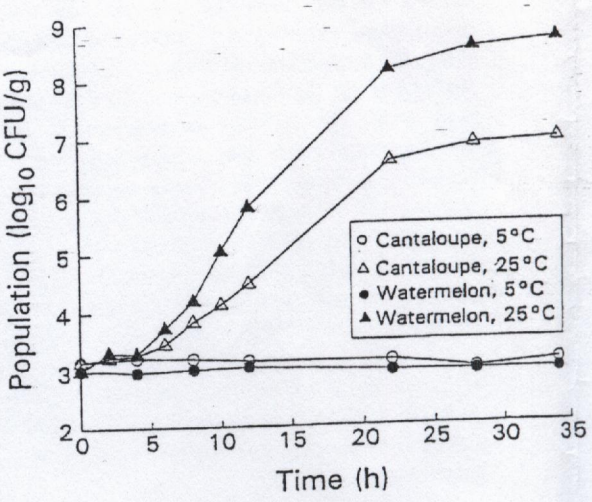

Figure 1. *Population of* E. coli *O157:H7 at 5° and 25°C on cantaloupe and watermelon cubes.*

Salmonellae are thought to have originated in the field or during transport from the field to the marketplace in outbreaks of salmonellosis associated with cantaloupe. The primary reservoir for *E. coli* O157:H7 is most likely cattle. Realizing that agronomic practices do not always preclude the application of animal manure or water contaminated with manure to melon fields and that vehicles used to transport melons from the field to the marketplace may not always be free of animal manure, it was of interest to determine the ability of *E. coli* O157:H7 to survive on the rind surface of cantaloupes and watermelons. We were surprised to observe that significant ($P<0.05$) increases in population occurred within 4 days on melons held at 25°C (Fig. 2). Populations then remained constant for the duration of the experiment. Growth was more prolific on cantaloupe than on watermelon rind. It should be pointed out that the inoculum applied to rinds contained 1% TSB and 0.1% peptone, and that the relative humidity within boxes used to store

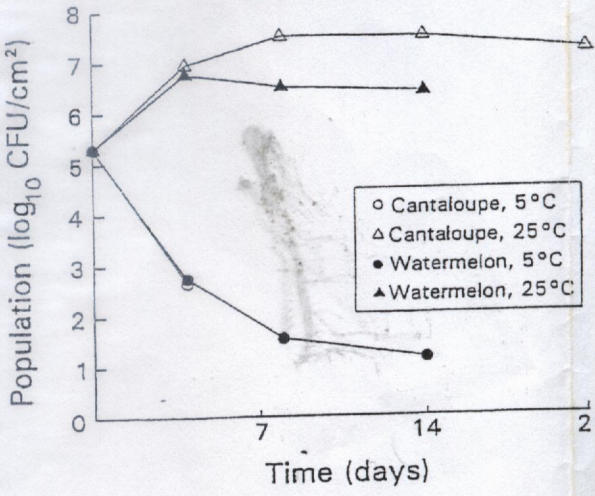

Figure 2. *Population of* E. coli *O157:H7 at 5° and 25°C on th rind surface of cantaloupe and watermelon.*