Elimination by Gamma Irradiation of Salmonella spp. and Strains of Staphylococcus aureus Inoculated in Bison, Ostrich, Alligator, and Caiman Meat

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Abstract

There is an expanding industry for the marketing of high- value meats from animals other than the typical domesticated species, including, but not limited to, bison, ostrich, alligator, and caiman. In this study we compared the gamma radiation resistance of a mixture of salmonellae (Salmonella dublin, S. enteritidis, S. newport, S. senftenberg, and S. typhimurium) and a mixture of Staphylococcus aureus strains (ATCC 13565, ATCC 25923, and B124) when present on ground bison, ostrich, alligator, and caiman meats at 5°C. A minimum of five doses were used to establish the D values, and the studies were replicated three times. Because the type of meat did not significantly ($P < 0.05$) alter the radiation resistance of salmonellae and of S. aureus only slightly in the case of ostrich meat, all of the results for each organism were combined to obtain radiation D values of 0.53 + 0.02 and 0.37 + 0.01 kGy for Salmonella spp and S. aureus, respectively. The authors conclude that both of these food-borne pathogens, if present, can be eliminated or greatly reduced in number, depending upon the level of contamination, from these meats by gamma radiation doses between 1.5 and 3.0 kGy at 5°C, the doses currently approved by the FDA and USDA for the irradiation of poultry. The authors also conclude that similar, if not identical, control of food-borne pathogens should be expected on edible meats in general, not just on those that are generically related.

Key words: Salmonella, staphylococcus, alligator, bison, caiman, irradiation

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Concept of "chemiclearance" has been accepted by many investigators who have studied the effects of ionizing radiation on food components (9, 18, 22, 29). Diehl and Scherz (10) and Taub et al. (30) concluded that it is possible to assess the wholesomeness, with respect to radiolytic products, of an irradiated food by extrapolating chemical data from one food to another that is generically related. The basis of the concept of chemiclearance is that the identities of the major and most of the minor constituents of foods as well as their radiation chemistries are known. If appropriate corrections are made for the differences in the amounts of these components, then it should be possible to predict the types of changes that would be produced in generically related foods when they are irradiated. Recently we tested this concept by chemical analysis of beef, lamb, pork, and turkey meats prepared and irradiated under identical conditions and found the concept to be valid (13, 15). The chemiclearance concept, however, has not generally been considered to apply to the responses of microorganisms to ionizing radiation. Many

investigators have concluded to the contrary, that the radiation resistance of an organism varies with the food substrate (2-6. 38).

The primary purpose of irradiating meat is to reduce the number of, or depending on the dose and the level of contamination, to eliminate food-born pathogens. Thus, regulatory agencies seeking to apply the concept of chemiclearance to establish wholesomeness would also, logically, wish to extrapolate data on the radiation sensitivity of a food-borne pathogen obtained with one or more meats to extend these data to include other meats not actually tested by the petitioner. Many published studies reveal significant variations in the D values for the same food-borne pathogen on different meats under apparently very similar conditions (39). Primary factors, such as oxygen tension, irradiation temperature, bacterial growth stage, amount of water, food additives, and hydrostatic pressure, and secondary factors, such as the culture medium upon which the number of survivors is estimated can greatly influence the results. Thus, results can vary with a given organism even in the same laboratory.

Significant differences between the radiation resistances of different serotypes of salmonellae tested under identical conditions on mechanically deboned chicken meat ranging from 0.38 kGy for Salmonella newport to 0.77 kay for S. enteritidis were reported by Thayer et al. (34). Radiation resistances ranging from 0.36 to 0.52 kay have been reported for Staphylococcus aureus on meat (12, 26. 33. 34, 37). Thayer et al. (34) hypothesized that differences in radiation resistance of microorganisms attributed to the food substrate might actually be mostly, if not entirely, due to variations in the intrinsic and extrinsic factors noted above. These authors compared the radiation resistances of Escherichia coli OI57:H7, Listeria monocytogenes. a mixture of Salmonella serovars, and S. aureus on beef, pork, lamb, and turkey tissues under identical conditions. They found that the radiation resistance of E. coli O157:H7 and L. monocytogenes were not different when irradiated on beef, lamb, pork, and turkey. There were small differences in the D values of S. aureus associated with the different tissues. The radiation resistance of the mixture of salmonellae was significantly less on pork than on beef, Iamb, and turkey tissue.

Bison, ostrich, alligator, and caiman meats are served in an increasing number of gourmet restaurants. Because of their limited availability, these "exotic" meats tend to be relatively high priced. The market for these products is significant in that in 1988 more than 100,000 pounds of Florida alligator meat were sold to restaurants (16). Europeans consume approximately $6 million worth of ostrich meat each year (7). In the United States the market is currently primarily in the breeder phase, with the American Ostrich Association reporting a membership of 3,800 in 1994 (24). There are 28 USDA-approved ostrich slaughter facilities and approximately 40,000 to 60,000 ostriches in the USA (11). The National Bison Association reported that 10,000 head were being slaughtered in 1995. These meats are not only exotic but tend to be leaner and lower in cholesterol than beef (17,20). Alligator or caiman meats are inspected by the Food Safety and Inspection Service only on request. Though few studies of the microbial quality of the meats from alligator, bison, caiman, and ostrich have been performed, it is not unreasonable to assume that they are subject to the same types of microbial contaminants as poultry and red meat. Staphylococcae have been identified as contaminantes of alligator meat (25).

The purpose of this study was to compare the effectiveness and predictability of the process of food irradiation on meats that are not generically related. Bison (bovine), ostrich (ratite), and alligator and caiman (reptile) were selected for this study because they represent three different classes of animals, they are available commercially, they are served in restaurants, no prior information exists on them, and two direct requests were received from processors asking for information on the potential value of food irradiation. Salmonellae and S. aureus were chosen for study because of their importance as food-borne pathogens on both poultry and other meat.

Materials and Methods

Substrates

Bison (Bison bison) top round was the gift of RC Western Meats, Inc., Rapid City, SO. Ostrich (Struthio camelus) steak was the gift of Ostrich Farms of North America, Inc., Riverview, FL. Ground ostrich meat was purchased from the Breezy Hill Meat Company, Bowie, TX. It is unknown how many birds contributed to the ground ostrich meat used in this study. Alligator (Alligator mississippiensis) and caiman (Melanosuchus crocodilas) meats were the gift of Mr. Stewart Pocock of Ocean Treats, Pompano Beach, FL. The alligator meat was from animals 8 to 10 years of age and was classified as "red," and the caiman meat was from animals approximately 2 years of age and was classified as "white" meat. The samples of bison, alligator (red meat), and caiman (white meat) were from three individual animals. All samples were fresh commercial products, vacuum packaged and shipped frozen, with the exception of the ostrich steak, which was shipped refrigerated. All meats were ground, or reground in the case of ostrich, through a 1/8-in. (ca. .32-cm)-die plate, packaged in vacuo at -0.92 bar (-92 kPa) in 100-g amounts within no.400 Stomacher bags, and overwrapped in vacuo at -0.92 bar in high-barrier pouches fabricated with 0.025-mm polycaprolactam (nylon 6) as the outside layer, O.0090-mm aluminum foil as the middle layer, and 0.051-mm polyethylene terephthalate as the inner layer (American National Can Company, Des Moines, IA) to provide better protection during storage. All samples were stored at -50°C, before and after sterilization by gamma irradiation to a dose of 42 kGy at -30°C. Prior research (31, 32, 36) demonstrated that such treatments did not significantly alter the wholesomeness and nutritional character- istics or the response of Salmonella typhimurium on chicken meat to gamma radiation.

Cultures

Salmonella dublin 15480, S. enteritidis 13076, S. newport 6962, S. senftenberg 8400, S. typhimurium 14028, Staphylococcus aureus 13565, and S. aureus 25923 were obtained from the American Type Culture Collection, Rockville, MD. S. aureus B 124 was from the culture collection of the Eastern Regional Research Center. All cultures were maintained and cloned on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI). Culture identity was confirmed by Gram stains and from reactions on gram-negative identification cards (GNI) or gram-positive identification cards (GPI), as appropriate, of the Vitek AMS Automicrobic System (bioMerieux Vitek, Inc., USA, Hazelwood, MO) (1, 19). Each isolate was cultured independently in I 00 ml of tryptic soy broth (TSB) (Difco) at 35°C. Equal amounts of the culture of each isolate of a pathogen were mixed, and then harvested by centrifugation. A 10 -fold inoculum was prepared by resuspending the cells in 0.1 volume of Butterfield's phosphate (0.25 M KH2PO4 adjusted to pH 7.2 with NaOH).

Radiation source and irradiation techniques

The self-contained 137Cs gamma-radiation source (Lockheed- Georgia, Marietta, GA) had a strength of approximately 121,220 Ci (4.48 Pbq) and a dose rate of 0.107 kGy min-1. The dose rate was established using National Physical Laboratory (Middlesex, United Kingdom) dichromate dosimeters (SD = + 1.22% at 95% confidence level). Variations in doses absorbed by experimental samples were minimized by using small samples (5.0 g), by placing them within a uniform area of the radiation field, and by using the same geometry for each study. The absorbed dose under these conditions can be calculated from the exposure time, subject to the variance in the estimate of the dose rate per minute (0.3%); the overall variance in the stated doses is estimated to be not greater than +5%. This was confirmed by the use of FWT -60 radiochromic film dosimeters (Far West Technology, Inc., Goleta, CA), which were used following the recommendations of ASTM (8). Though we could not estimate a maximum and minimum dose for such small samples there was clearly variance in the absorbed dose at least as great as that of the reference dosimeters used to determine the dose rate. Samples were maintained at 5.0 + 0.5°C during irradiation by thermostatically controlled injection of cold, gaseous nitrogen into the irradiation chamber. Sample temperature was monitored continuously during irradiation using calibrated thermocouples.

Inoculation of meat for determination of D10 values

Sterile meat was thawed rapidly (in about 5 min) by submerging the package in a 50°C water bath, inoculated with enough cells (10 ml/100g of meat) for a final population of ca. 109 stationary- phase cells per g, and stomached for 90 s in sterile no.400 polyethylene stomacher bags using a Stomacher 400 (Tekmar Co., Cincinnati, OH). Aliquots of 5.0 + 0.05g of inoculated meat were transferred aseptically to radiation-sterilized, oxygen-permeable poultry bags (E-300, Cryovac Division, W. R. Grace & Co., Duncan, SC). According to the manufacturer these bags comply with U.S. regulations for the irradiation of poultry. Inoculated meat was spread uniformly over an area of about 10 by 10 cm within the bags and heat sealed in vacuo at -0.92 bar.

Determination of DIO values

Inoculated meat samples received radiation doses of 0 to 3.0 kGy in increments of 0.5 kGy at 5 .0 + 0.5°C. Each study included one sample per dose, and each study was repeated three times.

Home |**Microbiological analysis**

Samples with serial dilutions in sterile Butterfield's phosphate were assayed for CFU by standard pour-plate procedures using TSA. Petri plates were incubated for 24 h at 35°C before counting (a 48-h incubation did not result in a significant increase in CFU). CFU on 3 petri plates with 30 to 300 colonies were counted with a New Brunswick Scientific Biotran II® automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ), and the average number was calculated for each sample.

Statistical analysis

The average number of CFU per gram was divided by the average of the three zero -dose values (No) to give a survivor value (N/No). The log survivor values (log N/No) were then used for subsequent calculations. The zero-dose values were excluded to avoid possible shoulder effects, and a minimum of five values in the linear portion of the inactivation curve were used in the calculation of each regression (35). The results from the three independent replicate studies were pooled, and the slope of the inactivation curve was determined by least squares analysis. The D values (dose in kilograys resulting in a 90% reduction of viable CFU) were the reciprocals of the slopes. Statistical calculations were performed with the general linear models procedure of the SAS statistical package (14, 28). The regressions were tested for differences by analysis of covariance.

Results and Discussion

The pooled results of the studies of the radiation resistance of Salmonella species and of S. aureus on bison, ostrich, alligator, and caiman meats are presented. Analysis of covariance revealed that D values for Salmonella spp. on each of the meats did not differ significantly ($P > 0.05$), and only the D values of S. aureus on bison and ostrich meat at the extreme ends of the distribution differed significantly ($P < 0.05$) from each other. Therefore, the individual values for the inactivation of Salmonella spp. and S. aureus on all of the meats were pooled, and average values for the slopes of the pooled inactivation curves were determined. (The regression curve for the inactivation of S. aureus on ostrich meat was not sufficiently different from that of the pooled regression for this pathogen on the other meats to warrant plotting them separately and therefore was included in the pooled values presented.)

The results of this study indicate that treating bison, ostrich, alligator, and caiman meats with ionizing radiation is an appropriate method to control contamination by Salmonella spp. and S. aureus. Based on the average D values, and the minimum dose currently approved for the irradiation of poultry (1.5 kGy), gamma irradiation would eliminate 2.8 and 4.m g units of Salmonella spp. and S. aureus, respectively.

The resistance of Salmonella spp. to gamma radiation on these "exotic" meats was less than those reported by Thayer et al. (34) for Salmonella spp. on beef, lamb, and turkey. The individual and average D values for S. aureus on bison, ostrich, alligator, and caiman were very similar to the D values obtained by Thayer et al. (34) on beef, lamb, pork, and turkey meats. The average D value for Salmonella spp. on the "exotic" meats was similar to that reported for this pathogen on pork (34). The same methods and isolates of Salmonella spp. were used in the current study as in the previous study (34); thus, the major source of difference between the studies appears to be the species and tissues from which the meats were obtained. In spite of these differences, the estimates for the D values for each of the organisms with the individual meats were remarkably similar to each other within this study and not greatly divergent from the results obtained by Thayer et al. (34).

Although these "exotic" meats were obtained from three unrelated classes of animals, their proximate analyses were, nevertheless, more similar than dissimilar. We found the amount of protein, fat, and moisture of alligator meat to be 18.4, 0.85, and 73.6%; of caiman meat to be 22.6, 0.95, and 71.4%; of bison meat to be 18.8, 1.24, and 72.6%; of ostrich steak to be 20.5, 0.26, and 73.8%; and of ground ostrich meat to be 19.5, 1.87, and 73.4%, respectively. These and other chemical results will be discussed in greater detail in a separate paper. The methodology is identical to that in the previous studies by Thayer et al. (34) and Fox et al. (13), in which beef longissimus dorsi was reported to have 19.4, 5.20, and 70.8% and turkey breast meat to have 22.4, 1.38, and 72.6% of protein, fat, and moisture, respectively. Thus, the amounts of protein in the "exotic" meats tended to be very similar to beef, but the amounts of fat were lower. The lower amount of fat in these meats would be expected to decrease the amount and possibly the types of radiolytic products (29). The amounts of total phospholipid and polyunsaturated fatty acid in bison muscles are greater than those found in the meat from Hereford or Brahman steers of similar age (21). Alligator fat contains significant amounts of unsaturated fatty acid (27). Mitchell et al. (23) discovered that the meat of two commercially farmed species of crocodile contained lower levels of stearic and oleic, higher levels of linoleic, and markedly higher levels of polyunsaturated fatty acids compared to those of chicken, beef, pork, veal, and lamb. Less saturation might indicate the potential for increased oxidative rancidity and production of radiolytic products following irradiation of the meat. These factors do not appear to have significantly altered the D values of either species of pathogen. Less saturation in the fatty acids of bison meat compared to that of beef should increase free radical scavenging by the meat, increasing, not decreasing the D value, as was found for salmonellae.

We conclude that gamma irradiation offers an excellent means for the control of food-borne pathogens on these meats and, further, that one can reasonably expect to obtain similar, if not identical, control of foodborne pathogens on edible meats and poultry products in general, not on just those that are generically related.

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