Effect of Ascorbic and Isoascorbic acids on Survival of Campylobacter jejuni in Poultry Meat

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ABSTRACT

Samples of radiation-sterilized mechanically deboned turkey meat were inoculated with a strain of Campylobacter jejuni, stored at 5°C, and viable counts of the test organism determined during a 7-week period. As compared to results obtained with unsupplemented samples, addition of ascorbic acid or sodium isoascorbate (erythorbate) to the meat, at a concentration of 5 mmol/kg, caused an increase in the death rate of C. jejuni. Autooxidation of these compounds, during storage of the meat, supports the view that their toxic effect is mainly due to their oxidation products.

Ascorbic acid is added to several food products to enhance their nutritive value or improve the keeping quality (2), and to prevent generation of nitrosamines in cured meat products (3). The ascorbic acid stereoisomer, isoascorbic acid, is commonly added as the sodium salt (erythorbate) in the formulation of cured meat products to reduce the chances of nitrosamine formation (4) and as an enhancer of the antibotulinal effect of nitrite (8).

At a concentration of 5 mmol/L in nutrient broth, ascorbic and dehydroascorbic acids, but not isoascorbic acid or sodium isoascorbate, were bactericidal towards Campylobacter jejuni incubated at 42°C in a micro-aerobic atmosphere (6). The effects of ascorbic acid and sodium isoascorbate (erythorbate) on the survival of C. jejuni were studied in mechanically deboned turkey meat stored at 5°C.

MATERIALS AND METHODS

Test organism and inoculum preparation

C. jejuni C1-2, isolated from an infected human and belonging to a predominant serogroup (#2), was used in this study. It was maintained according to the procedure described in a previous paper (6). Serotyping was done at the national Center for Campylobacter, Jerusalem, using the scheme reported by Rogol et al. (7). Inocula were prepared by suspending, in 0.1% peptone, the upper portion of a 48-h-old culture, grown in 5 ml of semi-solid Brucella broth medium without FBP at 42°C in a microaerobic atmosphere. Before inoculation the suspension was standardized to approximately 10⁶ colony-forming units (CFU)/ml.

Meat samples

Mechanically deboned turkey meat (MDTM) was obtained from a local processing plant and stored at -40°C, before and after irradiation, until needed.

Irradiation

MDTM samples, packaged in sealed polyethylene bags were placed in insulated boxes containing five times their weight of dry ice and sterilized by irradiation. The samples were irradiated with a 60Co source at a dose of 1.4-1.8 KGy per h with totals of 25-30 KGy (2.5-3.0 Mrad).

Chemical supplements and their analysis

The samples of sterilized MDTM were supplemented with filter-sterilized concentrated solutions of either ascorbic acid (BDH) or sodium isoascorbate (BDH) to yield a final concentration of 5 mmol per kg. Unsupplemented meat samples were also tested. The addition of the supplements did not cause any significant changes in the pH of the meat (pH 6.0-6.1). Residual ascorbic acid was determined by titration with 2,6 dichlorophenol indophenol (1).

Meat inoculation and enumeration of C. jejuni

No viable bacteria were found in the irradiation-treated meat samples. Inocula were added to the irradiated supplemented or unsupplemented meat samples to yield a final concentration of ca. 10⁶ CFU/g. Analysis of inoculated meat sub-samples showed an even distribution of the inoculum. The inoculated samples were stored in sealed plastic containers at 5°C and C. jejuni was enumerated at selected time intervals. The levels of viable C. jejuni present in the meat samples were determined by a 3-tube MPN (most probable number) procedure. Ten- and one-g meat portions (3 of each) were mixed with 90 and 9 ml of nutrient broth (NB), respectively, the contents stirred, and decimal dilutions prepared in NB. The various dilutions were incubated for 48 h at 42°C in a microaerobic atmosphere, and those showing bacterial growth were tested for the presence of pure cultures of campylobacters. The MPN was calculated using the tables published by de Man (5). Results reported are typical of those obtained in three replicate experiments.
RESULTS AND DISCUSSION

In commercial practice, sodium isoascorbate (erythorbate) is the ascorbic acid derivative commonly used in the formulation of cured meats; it is normally added to yield a final concentration of about 500 mg per kg of meat.

Previous studies (6) have shown that, ascorbic (AsA) and dehydroascorbic acids, added to nutrient broth at a concentration of 5 mmol/L, were bactericidal towards C. jejuni grown at 42°C in a microaerobic atmosphere. The cytotoxicity of AsA may be explained by two mechanisms: (a) reactions of AsA as a reductant of metal ions, generating toxic oxygen species and especially hydroxyl radicals (9) and (b) oxidation of AsA to products such as dehydroascorbic acid, diketo-gulonic acid and others (2). The addition of known oxidants normally present in raw MDTM. As compared with sodium isoascorbate towards C. jejuni.

The addition of either ascorbic acid or sodium isoascorbate caused a significant increase in the death rate of C. jejuni in MDTM at 5°C: after 4 weeks of storage, a 2-log reduction in viable counts was found in control samples, whereas a 4 log/g reduction was found in meat samples containing either ascorbic acid or sodium isoascorbate (Fig. 1). A similar difference of ca. 2 log CFU/g was also found between supplemented and unsupplemented meat samples at the end of the fifth week. After 7 weeks, C. jejuni could not be detected in 10-g samples of supplemented MDTM (< 0.3 cells/g) whereas ca. 20 viable cells per g were found in control samples. Contrary to results obtained in vitro (6), when added to MDTM no significant differences were observed between the bactericidal effect of ascorbic acid and that of sodium isoascorbate towards C. jejuni.

Table 1 shows that within 8 d after the addition of ascorbate or isoascorbate to the meat, about 50% of the added compounds (5 mmol/kg) were autooxidized. These data support the results obtained previously in vitro indicating that the toxic effect of ascorbic acid towards C. jejuni is mostly due to the formation of oxidation products and particularly to dehydroascorbic acid (6).

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REFERENCES


CAMPYLOBACTER JEJUNI SURVIVAL IN POULTRY MEAT

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