Antimicrobial Activity of Butylated Hydroxyanisole and Potassium Sorbate Against Natural Microflora in Raw Turkey Meat and Salmonella typhimurium in Cooked Turkey Meat

M. M. MORAD1, A. L. BRANEN2, and C. J. BREKKE*

Department of Food Science and Technology, Washington State University, Pullman, Washington 99164-6330

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ABSTRACT

The antimicrobial activity of butylated hydroxyanisole (BHA) (100 ppm) and potassium sorbate (1000 ppm), individually and in combination, was evaluated against growth of the natural microbial flora in raw turkey meat and against Salmonella typhimurium inoculated into cooked turkey meat. Growth of the natural flora was not inhibited by using either BHA or sorbate alone; however, slight inhibition was shown using a combination of the two. BHA, sorbate and a combination were effective to the same extent in preventing growth of naturally present gram-negative organisms. Sorbate and BHA, and partially inhibited by BHA, were additive against these organisms in poultry meat (9). However, no information is available on the inhibition of the natural gram-negative flora in refrigerated meat products by BHA or a combination of BHA and sorbate at the levels of these compounds found effective in broth systems. The purpose of this study was to determine the antimicrobial effectiveness of potassium sorbate and BHA alone and in combination against the natural flora in ground, raw turkey meat and against S. typhimurium inoculated into cooked, ground turkey meat. 

Growth of several microorganisms of known importance in spoilage and food poisoning in meat products is inhibited by phenolic antioxidants and/or potassium sorbate (1,4). Butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) and potassium sorbate alone and in combination were recently shown to inhibit the growth of Staphylococcus aureus or Salmonella typhimurium in trypticase soy broth (TSB) (4). Growth of both S. typhimurium and S. aureus was totally inhibited by 150 ppm of BHA and partially inhibited by 1000 ppm potassium sorbate. A combination of 1000 ppm potassium sorbate plus 50 or 100 ppm BHA showed partial, yet synergistic, inhibition of the growth of S. typhimurium or S. aureus in TSB. It was the contention of the authors that this synergistic action could allow a reduction in the amount of potassium sorbate needed in foods when used in the presence of BHA, and that the BHA could serve a dual function as an antioxidant and an antimicrobial in food products. Although there is evidence that potassium sorbate is effective in inhibiting S. aureus and S. typhimurium in meat products (10), no information is available regarding the effect of BHA or a combination of BHA and sorbate against these organisms in meat products.

Microorganisms important in meat spoilage have also been found to be susceptible to sorbate and BHA. For example, growth of various Pseudomonas species is known to be inhibited by 0.1 and 0.2% potassium sorbate in skim milk and broth, respectively (6-8). Davidson and Branen (3) also reported that 100 ppm BHA or greater inhibited the growth of Pseudomonas fluorescens or Pseudomonas fragi in TSB. Davidson (2) tested various combinations of sorbate and BHA for antimicrobial activity against these microorganisms in TSB. He observed no synergistic activity in the combinations tested, but did find the activities were additive against P. fluorescens at 22°C and against P. fragi at 7 and 22°C. Sorbate has been found effective in inhibiting these organisms in poultry meat (9). However, no information is available on the inhibition of the natural gram-negative flora in refrigerated meat products by BHA or a combination of BHA and sorbate at the levels of these compounds found effective in broth systems. The purpose of this study was to determine the antimicrobial effectiveness of potassium sorbate and BHA alone and in combination against the natural flora in ground, raw turkey meat and against S. typhimurium inoculated into cooked, ground turkey meat.

MATERIALS AND METHODS

Potassium sorbate and BHA solutions

Potassium sorbate (Monsanto Co., St. Louis, MO) was added directly to the meat samples. Butylated hydroxyanisole (BHA) tablets were obtained from Eastman Chemical Products (Kingsport, TN). The tablets were ground, and the resulting powder was dissolved in 95% ethanol to make a 4% (w/v) stock solution. The stock solution was stored at 4°C until used.

Preparation of ground turkey meat

A frozen turkey carcass was obtained from a local supermarket, thawed at 4°C, then hand-deboned. Meat from the backs, breasts and drumsticks was cut into cubes and ground twice through a 5-mm plate.

1Information Paper, College of Agriculture Research Center, Pullman, Washington 99164-6240. Project 0252.
2Present Address: Department of Soil and Crop Sciences, Texas A & M University, College Station, Texas 77873.
3Present Address: Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68583.
Inhibition of natural flora in raw turkey meat

Four 100-g portions of ground meat were used. One portion was treated with 1 ml of 95% ethanol and served as a control. The second portion was treated with a powder of potassium sorbate calculated to give 1000 ppm based on total meat weight. The third portion was treated with a sterile (autoclaved) ethanolic solution of BHA (1% wt/vol) to give 1000 ppm BHA based on total meat weight. The fourth portion was treated with both potassium sorbate and then ethanolic BHA at the concentrations used for portions two and three. All portions were thoroughly hand-mixed with a sterile spatula for about 3 min.

Samples (11 g) of each portion were weighed and aseptically spread evenly in sterile Petri dishes (100 x 15 mm) and stored at 4°C. Samples were removed from storage at 0, 2, 4, 6, and 8 d, blended with 99 ml of phosphate buffer in a Waring Blendor, and appropriate dilutions made. Pour plates were made using plate count agar (PCA; Difco) for enumeration of total bacteria (S) and crystal violet tetrazolium agar (Difco) for enumeration of gram-negative organisms. Plates were incubated at 32°C for 48 h before counting colonies. For each treatment the data reported are means of triplicate microbial count determinations of an 11-g sample for each sampling time.

Inhibition of Salmonella typhimurium in cooked ground turkey meat

A stock culture of Salmonella typhimurium was obtained from the Washington State University Department of Food Science & Technology culture collection. The microorganisms were grown in trypticase soy broth (Difco) for 24 h before being inoculated into the meat system.

Four 100-g portions of meat were weighed and aseptically spread evenly in sterile Petri dishes (100 x 15 mm). The meat portions were autoclaved at 121°C for 10 min. After allowing the portions to cool, they were treated with 95% ethanol, BHA, sorbate or a combination of BHA and sorbate as described for the raw meat. The four portions were also inoculated with 1 ml of a 10° dilution of the stock culture of S. typhimurium. Portions were then mixed thoroughly with a sterile spatula for about 3 min. Each portion was divided into 11-g samples which were evenly spread in sterile Petri dishes (100 x 15 mm) and stored at 4°C. Samples were removed from storage at 0, 2, 4, 6, and 8 d, blended with 99 ml of phosphate buffer in a Waring Blendor, and appropriate dilutions made. Pour plates were made using PCA (Difco) and incubated at 32°C for 48 to 72 h before counting colonies (5). For each treatment the data reported are means of triplicate microbial count determinations of an 11-g sample for each sampling time.

RESULTS AND DISCUSSION

Inhibition of natural microflora in raw turkey meat

Growth of natural aerobic microflora in raw turkey meat was not reduced over 8 d of refrigerated storage with either 100 ppm BHA or 1000 ppm potassium sorbate when differences in 0-d counts are considered (Fig. 1). A combination of BHA and sorbate, at the same concentrations as used singly, resulted in a slight reduction of total aerobic plate counts compared to the control. This reduction may be due simply to increasing the total effective antimicrobial concentration. However, the results illustrate that where regulations may limit the concentration of any one additive for use in foods, additional antimicrobial benefits may be obtained by using a combination of additives.

BHA, sorbate and a combination of the two were more effective at retarding the growth of gram-negative organisms compared to the control (Fig. 2) than in retarding growth of total aerobic bacteria (Fig. 1). No appreciable difference between the antimicrobial activity of 100 ppm BHA and 1000 ppm sorbate was observed. However, unlike the results for total aerobic plate counts, a combination of BHA and sorbate did not reduce counts of gram-negative bacteria to a greater extent than when used singly (Fig. 2).

Inhibition of Salmonella typhimurium in cooked ground turkey meat

A reduction in counts of S. typhimurium occurred for all treatments and the control during 8 d of refrigerated storage (Fig. 3). Sorbate was more effective than was BHA at increasing the death rate of the pathogen. At 8 d of storage, a BHA-sorbate combination yielded a slight additional de-
crease in *S. typhimurium* counts compared to sorbate used alone, but again this may have been due to the additive effect of the two antimicrobials.

It is significant that 100 ppm of BHA was effective in inhibiting the growth of the gram-negative flora in raw turkey meat and in reducing *S. typhimurium* counts in cooked turkey meat. The partial inhibition found is consistent with that reported for 100 ppm of BHA against these organisms in broth (3,4) and is the first report of the antimicrobial effectiveness of BHA in meat products. Unfortunately, the concentration of BHA needed for inhibition exceeds the legal limit for meat by 5 to 10 times. Thus, it is unlikely that BHA could be used as an antimicrobial by itself. BHA, however, may offer some additional antimicrobial effectiveness in the presence of sorbate. Although no synergistic effect was evident in this study, a combination of 1000 ppm sorbate with 100 ppm BHA was more effective than sorbate alone in inhibiting growth of gram-negative organisms and reducing survival of *S. typhimurium*. Further studies are warranted to determine if BHA, when used at the legal concentration, also enhances the activity of sorbate.

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