Antimicrobial Activity of Selected Antioxidants

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ABSTRACT

Tripticase soy broth containing 100, 150, 200 and 400 ppm of butylated hydroxyanisole (BHA), propyl gallate, nordihydroguaiaretic acid (NDGA), or combined BHA and propyl gallate was inoculated with *Escherichia coli* or *Staphylococcus aureus* to determine the antimicrobial effect of these antioxidants. Both propyl gallate and NDGA at 400 ppm had a strong lethal effect against *E. coli*. BHA and the BHA-propyl gallate combination were more effective against *S. aureus* than against *E. coli*. NDGA had the strongest antimicrobial activity. Only 50 ppm of NDGA were highly inhibitory to *S. aureus*.

Most antioxidants in use today are phenolic compounds which are added to numerous food products to inhibit development of oxidative rancidity. Several workers have investigated the possibility that certain phenolic antioxidants might exhibit antimicrobial effect. Kaufmann and Ahmad (5) reported that 140 ppm of nordihydroguaiaretic acid inhibited growth of *Saccharomyces cerevisiae* and Epstein et al. (2) reported that the *LD*$_{50}$ for *Tetrahymena pyriformis* was only 5 ppm for NDGA. Growth of *Salmonella senftenberg* was inhibited slightly by 1.0% of butylated hydroxytoluene (BHT) (10). Recently, Chang and Branen (1) found that 250 ppm of butylated hydroxyanisole (BHA) could inhibit growth of *Aspergillus parasiticus* and 150 ppm could inactivate *Staphylococcus aureus*. In this study BHA, NDGA, and propyl gallate were tested against *Escherichia coli* and *S. aureus* to determine their antimicrobial effect. Combined BHA and propyl gallate were also tested since this combination has been widely used in fat-containing products.

**MATERIALS AND METHODS**

**Organisms**

*S. aureus*, ATCC 6538P, and *E. coli*, ATCC 9723 were used as test organisms. Twelve-hour-old cultures of each organism were prepared by transferring one loop of *S. aureus* or *E. coli* into 50 ml of Trypticase Soy Broth (TSB, BBL). Cultures were then incubated at 35°C for 12 h.

**Inhibition studies**

BHA and propyl gallate were obtained from Sigma Chemical Co. and NDGA was obtained from Aldrich Chemical Co. The above antioxidants and combined BHA-propyl gallate (50/50) each were dissolved in ethanol to achieve a 1.0% concentration of antioxidant. Ethanol was removed by evaporation in a drying oven at 120°C for 2 h. Each antioxidant was then combined with 100 ml of TSB to make stock solutions. Media containing 100, 150, 200, and 400 ppm of antioxidant were prepared by adding appropriate amounts of each antioxidant stock solution to 50 ml of TSB.

Flasks of media containing antioxidants were autoclaved. After cooling, media containing antioxidants were inoculated with 1 ml of a 12-h-old culture. After 0, 4, 8, 12 and 24 h of incubation at 35°C, 1 ml from each TSB culture was removed and appropriate dilutions were made with phosphate buffer solution. Cells were plated in Trypticase Soy Agar (BBL) and incubated for 48 h at 35°C. The antimicrobial activity was shown by the curve of log$_{10}$ cell numbers/ml vs. hours of incubation with different concentrations of antioxidants. Only one set of data is reported since it would not be valid to report mean number of cells when the initial cell numbers varied.

**RESULTS AND DISCUSSION**

Propyl gallate and NDGA had a lethal effect against *E. coli* at the 400 ppm level, (Fig. 1 and 2) but BHA and combined propyl gallate-BHA were not as effective in killing *E. coli* (Fig. 3 and 4). When tested against *S. aureus* BHA and combined propyl gallate-BHA had strong antimicrobial activity at the 400 ppm level (Fig. 5 and 6), but propyl gallate alone had little effect against *S. aureus* (Fig. 7). NDGA was the most effective inhibitor of *S. aureus* growth. Levels as low as 50 ppm had a strong lethal effect (Fig. 8).

Apparently NDGA, BHA, and the propyl gallate-BHA combination were more effective against *S. aureus* than *E. coli*. These results are in accord with the concept that phenols have more antimicrobial activity against gram-positive than against gram-negative cells (4). An exception to the above was seen when propyl gallate showed greater inhibitory effects against *E. coli* than against *S. aureus*. More study is needed to explain propyl gallate’s mechanism of lethality.

In the present study, 400 ppm of BHA were required to inhibit effectively growth of *E. coli* and *S. aureus*. Chang and Branen (1) found that 150 ppm of BHA inactivated *S. aureus* but 400 ppm were required for...
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Figure 1. Effect of propyl gallate on growth of E. coli in TSB.

Figure 2. Effect of NDGA on growth of E. coli in TSB.

Figure 3. Effect of BHA on growth of E. coli in TSB.

Figure 4. Effect of propyl gallate-BHA on growth of E. coli in TSB.

Figure 5. Effect of BHA on growth of S. aureus in TSB.

Figure 6. Effect of propyl gallate-BHA on growth of S. aureus in TSB.
NDGA was an effective growth inhibitor of both *E. coli* and *S. aureus*. Based on studies on the bactericidal power of substituted phenols and phenol derivatives by Harden and Reid (3) which showed that lethal effect against bacteria increased rapidly as the length of the intervening chain increased, it was predictable that NDGA would have strong antimicrobial activity. As little as 50 ppm of NDGA were highly toxic to *S. aureus*. It was the only antioxidant which did not disperse readily in TSB stock. Perhaps an emulsifier would have improved NDGA’s solubility and made it even more effective. Presently NDGA is used only in food packaging and no more than 50 ppm can become a part of the packaged food (9). This study tends to support Oliveto’s (6) suggestion that there is a need to reevaluate NDGA’s usage in food products because it has strong antimicrobial and antioxidant activities. Some naturally-occurring antioxidants like curcumin and chlorogenic acid with structures similar to that of NDGA may be worthy of testing for their antimicrobial activities.

**REFERENCES**