Shellac Formulations To Reduce Epiphytic Survival of Coliform Bacteria on Citrus Fruit Postharvest†

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ABSTRACT

Survival of the coliform bacteria Enterobacter aerogenes and Escherichia coli was monitored in a neutral carboxymethylcellulose formulation and in shellac formulations with various pH and concentrations of ethanol and the preservative paraben; populations were subsequently measured from the surface of citrus fruit coated with these formulations. Numbers of the two bacteria increased over 24 h from $10^6$ CFU/ml to approximately $10^8$ CFU/ml in the carboxymethylcellulose solution, but over this time numbers remained little changed in the neutral solution of shellac. The Enterobacter was more tolerant of alcohol over a 3-h period; although its numbers in a shellac solution with 10% ethanol dropped from more than $10^6$ CFU/ml to just over $10^3$ CFU/ml, E. coli and a third species, Klebsiella pneumoniae, declined toward the limit of detection (5 CFU/ml) during this time. The addition of morpholine to increase the formulation pH to 9.0 caused numbers of bacteria to plummet to an undetectable level within 30 to 60 min. On Ruby Red grapefruit and Valencia oranges in storage at $13^\circ$C numbers of E. aerogenes and E. coli declined over 2 weeks from $10^5$ CFU/cm$^2$ to less than $2.5 \times 10^3$, but most of the loss in numbers occurred within 1 day. Numbers remained significantly less on shellacked fruit compared with those applied in the carboxymethylcellulose coating, and a shellac coating prepared from a pH 9 solution was more toxic to these species than one in which 12% ethanol had been added to the neutral formulation. The addition of the preservative paraben in the basic shellac was further inhibitory.

Coatings have traditionally been applied to fruit for the purpose of improving appearance and/or preventing weight loss (4). Although formulations based upon wax (such as carnauba or candelilla), cellulose, chitin, and other materials are commonly used in some applications, the coatings most often applied to citrus contain shellac, which is a purified product of the hardened resinous secretion of the scale insect Kerria lacca (Kerr), a plant parasite found in India, Burma, and Thailand (9). These coating formulations normally consist of a mixture of water, shellac, morpholine, aqueous ammonia, and alcohol with minor ingredients such as wood resin ester, carnauba wax, oleic acid, polyethylene glycol, antifoam, and fungicide, and their pH is often between 8 and 11 (4). For preserving the shellac formulation against contaminating microbes, paraben (active only at an alkaline pH) can be added.

The composition of fruit coatings has a significant influence on the numbers of microorganisms that survive the processing stage and inhabit the fruit surface during postharvest storage (10, 11, 18, 19). A liquid shellac of near-neutral pH with less than 6% alcohol is minimally toxic to many of these microbes. Such a product and neutral or mildly acidic formulations of cellulose or sucrose ester can even promote the biological control of postharvest decay afforded by certain epiphytic bacteria and yeast (12, 13).

Depending upon the fruit coating, the bacterium Pseudomonas syringae and the yeast Candida oleophila can develop populations of $10^5$ to $10^7$ CFU/cm$^2$ on the fruit surface in storage at $13^\circ$C, and such populations successfully out-compete and starve species of Penicillium that cause blue mold and green mold of citrus, as well as other fungal pathogens.

But fruit can also become contaminated at harvest and during processing with soil that may harbor human pathogens. Coliform bacteria, including fecal coliforms, are frequently associated with fresh produce, and species of Enterobacter and Klebsiella, as well as Escherichia coli, have been isolated from both fruits and vegetables (6, 14). Although each stage of processing reduces the total number of bacteria on the fruit surface, the simple addition of detergent and bleach in the wash water may fail to eliminate E. coli totally (3, 16, 17). The application of a shellac-based formulation to these washed fruit can further reduce numbers of this bacterium (16), but the combination of fruit coating and the subsequent heat of drying the fruit ($50^\circ$C or more for 2 min) provides a more significant effect (18).

Because shellac coatings can be formulated to preserve beneficial bioantagonists of fruit decay, they might also be adapted to have an opposite effect and reduce the numbers of microbes on the fruit surface. The objective of this work was to evaluate the effects of pH, alcohol content, and the preservative paraben on the survival of coliform bacteria in liquid shellac formulations and on citrus fruit to which the coatings were applied to determine whether the food safety

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of fresh fruits and vegetables might be improved by the coating process.

**MATERIALS AND METHODS**

**Fruit coating formulations.** An experimental shellac formulation was developed, based upon previous work (12, 13), that would be expected to foster growth of nonfastidious microorganisms because it had a pH near neutral and minimal quantities of morpholine and alcohol. Refined, bleached shellac (R52; Mantro-se-Hauser Co., Westport, Conn.) was formulated into a coating by addition of the polymer to a mixture of hot water, morpholine, and oleic acid. The formulation contained 20% shellac; 2.4% morpholine (Aldrich Chemical Co., Milwaukee, Wis.), and 1.0% oleic acid (Emersol 6321; Henkel Corp., Los Angeles, Calif.) and had a pH of 7.25. From this product, three additional formulations were prepared: (i) by addition of ethanol to achieve an alcohol concentration of 12%, which did not alter pH; (ii) by supplementation of the alcohol formulation with additional morpholine to achieve a concentration of 5.2% that produced a pH of 9.0; and (iii) by addition of paraben to the latter to achieve a concentration of 0.1%, which did not change the pH of 9.0.

To facilitate the adherence of bacteria to waxy fruit surfaces, an undercoating (a sticker) was also developed. Seven grams of a powder mix (71.4% gum Arabic [ Fisher Scientific Co. , Pittsburgh, Pa.], 21.4% carboxymethylcellulose [type 7LF; Aqualon, Col, Wilmington, Del.], and 7.1% whey protein isolate [Bipro; Davisco Foods International, Inc., Le Sueur, Minn.]) were added with stirring to 93 ml of warm water and 1 ml of glycerol then heated to approximately 90°C for 10 min and cooled to room temperature before use.

**Culture of coliform bacteria.** E. coli strain ATCC 25922 was obtained from VWR Scientific as a BBL QualiSwab (Becton Dickinson and Co., Cockeysville, Md.), whereas Enterobacter aerogenes strain ATCC 13048 and Klebsiella pneumoniae strain ATCC 13883, also from VWR Scientific, were from Bactrol disks manufactured by Difco Laboratories (Detroit, Mich.). Bacterial strains were routinely cultured on Bacto Pseudomonas agar F and EMB agar (both Difco Laboratories).

Preliminary tests evaluated the toxicity of ethanol to the three bacteria, comparing survival in the alcohol-free neutral shellac solution and in the neutral shellac with the addition of 2, 4, 6, 8, and 10% ethanol. To test survival, suspensions in water approximately 6 × 10^8 CFU/ml of each bacterial species were prepared from 18-h cultures on Pseudomonas agar F. From these suspensions 0.1 ml was mixed with 5.9 ml of the shellac formulations, and aliquots were then removed at 0, 0.5, 1, 2, and 3 h. The bacterial preparations were held under ambient conditions (about 24°C) and were thoroughly mixed again just prior to sampling. Samples (0.1 ml) of 10-fold serial dilutions were plated on Pseudomonas agar F, and colonies were counted after 5 days of incubation at 24°C. All tests were replicated four times. Subsequently, survival was similarly monitored over 24 h in the liquid fruit sticker and the three formulations developed from the neutral shellac containing 12% ethanol, 12% ethanol plus morpholine to pH 9.0, and the latter formulation plus 0.1% paraben.

**Survival of bacteria on the surface of citrus fruit.** Ruby Red grapefruit (Citrus paradisi Macf., diameter approximately 9 cm) and Valencia oranges (Citrus sinensis [L.] Osb., diameter approximately 7 cm) were obtained from local growers in central Florida and were thoroughly washed in water containing the label rate of Decco 241 Fruit & Vegetable Cleaner (Elf Atochem, Monrovia, Calif.). No fungicide was applied to the fruit. Twelve dozen fruit were randomized into seven groups then inoculated with either E. aerogenes or E. coli that was applied within the fruit sticker at 6 × 10^8 CFU/ml; 0.6 and 0.5 ml of the sticker was hand-rubbed over the surface of grapefruit and oranges, respectively, then fruit were allowed to dry for 1 h at 24°C. Subsequent treatments included two controls to monitor each bacterial species when no shellac was applied over the sticker, four in which E. aerogenes was overlain with the neutral shellac or the three amended shellac formulations, and one in which E. coli was overlain with the single alcohol formulation of pH 9 with paraben. For grapefruit and oranges, respectively, 0.8 and 0.6 ml of shellac was hand-applied per fruit as above. When coatings had dried for 1 h at 24°C (not at a high temperature as is the industry practice), five fruit from each treatment were individually washed in 100 ml of 0.1 M phosphate buffer (pH 7.0) containing 0.1% peptone. Fruit and buffer were placed into Ziploc freezer bags of 18 cm by 20 cm then shaken on a Lab-Line rotary shaker (model 8290; Thomas Scientific, Swedesboro, N.J.) at 200 rpm for 1 h. One-tenth milliliter from 10-fold serial dilutions of the wash was cultured on EMB agar, the selectivity of which reduced contamination by other epiphytic bacteria (recovery of the two enterics on EMB was seen to be greater than 99%). Cultures were incubated at 36°C for 2 days, at which time colonies were counted; epiphytic populations were estimated based on the surface areas of the washed fruits calculated from their diameters. The remaining fruit were stored at 13°C and 95% relative humidity for 2 weeks. For each group, five fruit were removed 1, 7, and 14 days after treatment and washed, and coliform bacteria were similarly isolated. This experiment was replicated three times with each of the two types of fruit. Also, using the Scott oil test (7), the limonene content in the buffer wash of five representative grapefruit and oranges was measured when washing was complete after 1 h; this citrus peel oil from ruptured oil glands is toxic to some microbes at 0.1% (2).

**Data analysis.** The survival of bacteria in liquid coating formulations was evaluated over a number of hours, and data (log_{10} CFU/ml) were analyzed with SAS Proc ANOVA (SAS Institute, Cary, N.C.) as a split plot over time with the hour of sampling as a secondary effect. Data (log_{10} CFU/cm^2) from population studies of fruit in cold storage were analyzed with SAS Proc ANOVA as a split plot over time with the hour of sampling as a secondary effect; bacterial species were evaluated separately. Harvest dates were treated as experimental replicates because statistical analysis determined equal variances.

**RESULTS**

**Survival of bacteria within liquid fruit coatings.** Of the three coliform bacteria, E. aerogenes was most resistant to the alcohol content in the neutral shellac (Fig. 1). This species could still be isolated from the shellac with 10% ethanol after 3 h at numbers more than 10^3 CFU/ml unlike E. coli and K. pneumoniae, which, although recovered, had decreased toward the limit of detection (5 CFU/ml).

Numbers of E. aerogenes in the liquid fruit sticker grew from 10^6 CFU/ml to more than 10^8 CFU over 24 h (Fig. 2A). In the neutral shellac this species increased to nearly 10^7 CFU/ml, but the addition of 12% ethanol reduced numbers below 10^6 CFU/ml over this time. Addition of morpholine to produce a shellac of pH 9 caused numbers to plummet below the limit measurable within 1 h. Results were similar with E. coli although populations were slightly lower (Fig. 2B).
FIGURE 1. Survival of E. aerogenes (A), E. coli (B), and K. pneumoniae (C) at 24°C in neutral shellac solutions with various amounts of ethanol; means of four tests. Least significant differences at 0.5, 1, 2, and 3 h, respectively, are 0.35, 0.36, 0.41, and 0.40 in A, 0.33, 0.35, 0.35, and 0.41 in B, and 0.51, 0.48, 0.47, and 0.48 in C.

Survival of bacteria on the fruit surface. Although applied at a rate calculated to produce $10^6$ CFU/cm² of the two bacteria on the fruit surface, numbers of E. aerogenes and E. coli were closer to $10^5$ CFU/cm² when recovered 1 h after application of the sticker coating to the fruit (Fig. 3). Limonene in the wash buffer was not detectable at a limit of 0.0002% after an hour’s agitation and, therefore, would not affect bacterial recovery. Subsequently coating fruit with the neutral shellac significantly reduced the number of E. aerogenes to $2.5 \times 10^3$ CFU/cm², but the addition of 12% ethanol had no additional effect on the numbers recovered (Fig. 3A). Increasing morpholine to make the pH of the formulation 9.0 caused numbers to significantly drop to $4.5 \times 10^2$ CFU/cm², and the addition of paraben to the latter mixture further reduced significantly the initial recovery of this species to $1.3 \times 10^2$ CFU/cm². After 1 day there was no significant difference among the shellac coatings with respect to numbers of E. aerogenes, but numbers from these treatments all were significantly less than that recovered from fruit coated with the CMC sticker. After 7 days, the pH 9 formulations supported numbers of E. aerogenes significantly less than that on the other treatments that were no longer different, and after 14 days all treatments had such low populations that no differences could be detected.

With E. coli only the sticker and the shellac formulation of pH 9 with ethanol and paraben were compared (Fig. 3B). These were significantly different over a period of 1 day, although populations were always less than those of
E. aerogenes on the same day. On days 7 and 14 no differences between the two treatments could be found; numbers of E. coli were greatly reduced and had approached the limit of detection of approximately 2 CFU/cm².

**DISCUSSION**

Particularly within the last decade, there has been an increasing concern about the contamination of foods with microorganisms that can cause disease among humans (1, 8, 14, 20). Although much of the anxiety stems from the increased importation of fruits and vegetables from regions of the hemisphere where field sanitation may be less than adequate, problems have also become apparent within our own agriculture and marketing. The risks of food contamination associated with organic gardening, as well as with the desire to reduce or eliminate the postharvest application of fungicides and other chemicals that may have compensated for surface contamination, have been increasing within the U.S.

Heat treatment or pasteurization was not a part of fresh-cut citrus or fresh-squeezed citrus juice operations prior to 1998 (18), but more fresh juice processors in Florida have adopted fruit surface heat treatment in the last 2 years (15). Nevertheless, in many cases microbial safety is greatly influenced by fruit surface microbial loads before processing. Unprocessed grapefruit can support more than 10⁴ bacteria/cm² and nearly 10³ yeasts plus 10³ other fungi (11). Similar counts were obtained by Pao and Brown (16) with oranges and tangerines. Both studies documented the reduction in microbial numbers associated with washing and the shellacking of fruit, but Pao et al. (17) also demonstrated increased efficacy when the pH of the water was 11.8. Wash water, however, could disperse contamination, and even the use of detergent and bleach does not completely kill coliform bacteria on citrus. Recycling coating materials could also disperse surface contaminants as many are not especially toxic to bacteria (11, 12).

Alcohol is added to a shellac coating formulation to improve the speed of drying, and it also acts as a preservative if the concentration is sufficient. Ethanol is toxic to many bacteria, but the time required to completely kill large numbers can be several hours, even when the alcohol concentration is 10% of the formulation. As demonstrated here, E. coli and K. pneumoniae die off more quickly in a shellac solution with 10% ethanol than E. aerogenes, but all three survive quite well during the short period a coating is applied and possibly recirculated a few times. More important in a shellac coating formulation that may be designed to kill surface microbes is the solution’s pH. Numbers of the yeast Candida oleophila in a shellac solution declined toward zero within 4 h and 1 h at a pH of 8.5 and 10.0, respectively (13). The pH is determined by addition of morpholine and/or ammonia and also contributes to the dissolution of the shellac (4), but as these volatile chemicals evaporate the pH of the drying shellac declines. Other usual constituents of shellac coatings, such as oleic acid, polyethylene glycol, and antifoaming agents, are used in amounts that minimally affect most microbes. To make a shellac more toxic to surface contaminants, little more is available than paraben, which is a registered preservative most active at an alkaline pH.

Elevating the shellac pH to 9 caused numbers of E. coli and E. aerogenes to decline below the detectable limit in a liquid shellac coating within 30 min. The addition of paraben to the liquid formulation would not be expected to affect survival during such short periods, and it was not tested in this fashion. This material did, however, reduce the recovery of E. aerogenes from fruit surfaces, relative to the pH 9 product lacking the preservative.

The CMC sticker was designed to be a nontoxic material that would improve adherence of the bacteria to the waxy citrus fruit. Commercial cellulose products normally contain a preservative such as 0.15% potassium sorbate to retard microbial growth. Declining populations of the bacteria on citrus fruit coated with the CMC sticker demonstrate, however, that in 13°C storage, these enteric bacteria do not persist well past a few days to 1 week. Survival might be expected to be greater at warmer temperatures. The drying of the coating must expose the bacteria to desiccation, and drying, whether at room temperature or around 52°C used in commercial packing lines, should be...
a significant factor that can be exploited in reducing surface contaminants. After the fruit coating has dried, those bacteria remaining would no longer be affected by the original alcohol content, however, and the surface pH could become altered by materials leaking from inside the fruit during storage. Such nutrients might sustain the enteric bacteria at warmer temperatures, but, as seen here, do not seem to sustain numbers at 13°C. As mentioned, antagonistic yeasts and bacteria compete for these nutrients with decay fungi in postharvest storage, and *P. syringae* has also been reported (5) to exclude the development of *E. coli* in wounds in apple fruit.

In conclusion, adjustment of formulation pH to 9.0 and the addition of 12% ethanol significantly reduced the numbers of coliform bacteria in liquid shellac solutions and subsequently the initial populations on citrus fruit in storage at 13°C. Numbers on fruit in cold storage continued to decline to undetectable limits within 14 days regardless of the coating formulation.

**REFERENCES**