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### **The Efficacy of Cecure® (CPC Antimicrobial) for Post-Harvest Decontamination of Cantaloupes and Spanish Melons**

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**Abstract:** Two trials were conducted in Costa Rica in the spring of 2007. One trial was conducted in a cantaloupe processing facility and the other in a facility that processes Spanish melons (piel de sapo). In both trials, the freshly harvested fruits were subjected to a variety of post-harvest treatments in an effort to improve the microbial condition of the fruit. In the cantaloupe trial, there were five different treatments, including fruit from the field, fruit from the field plus a 5 sec dip in 0.5% cetylpyridinium chloride (CPC), fruits that were washed commercially in 100 to 150 ppm total chlorine followed by a water rinse, washed fruits followed by a water rinse plus a 5 sec dip in 0.5% CPC and washed fruits followed by a water rinse plus a commercial fungicide. In the Spanish melon facility, there were four treatments, including fruits from the field, fruits from the field plus a 5 sec dip in 0.5% CPC, fruits that were washed in 100 to 150 ppm total chlorine (plus application of fungicide to the peduncle) and fruits that were washed in chlorine plus a 5 sec dip in 0.5% CPC. In addition, shelf-life studies at the appropriate temperatures were also conducted on both fruits; however, only the cantaloupes were subjected to a detailed sensory evaluation at the end of the shelf-life period. For field cantaloupes, the results indicate that a 5 sec dip in 0.5% CPC will allow for a 99% reduction in APC, no recovery of total coliforms and a 99.9% reduction in yeasts and molds. For Spanish melons from the field, a 5 sec dip in 0.5% CPC resulted in greater than a 90% reduction in APC and a 99% reduction in total coliforms and yeasts and molds. The commercial washing procedure in 100 to 150 ppm total chlorine followed by a water rinse (with or without the application of a commercial fungicide) was not very effective for reducing the levels of any of the groups of organisms in the cantaloupe facility. In fact, total coliforms remained unaffected by the commercial wash process. In the Spanish melon facility, the commercial wash procedure plus application of a commercial fungicide to the peduncle reduced APC by greater than 99% and total coliforms and yeasts and molds by greater than 90%. In comparison, the commercial washing process plus a 5 sec dip in 0.5% CPC resulted in the greatest reductions with almost complete elimination of APC, total coliforms and yeasts and molds from the cantaloupe and Spanish melons. The results from these trials suggest that the use of a CPC rinse solution as a treatment following commercial washing can significantly improve the overall microbial condition of fresh cantaloupe and Spanish melons. In addition, the sensory quality of cantaloupes at the end of refrigerated and retail storage was significantly improved when the fruit was subjected to a CPC-solution treatment following the commercial washing process.

**Key words:** Cantaloupe, Spanish melons, *Salmonella*, shelf-life, post-harvest, cetylpyridinium chloride, Cecure®

## INTRODUCTION

The consumption of fruits and vegetables has increased steadily in the last years in many developed countries. In an effort to meet consumer demand, the overall production of fresh fruits and vegetables has increased in many developing countries. The United States (US) and the European Community (EC) are the main importers of domestic production from these countries (USDA, 2007). As production and consumption of fresh fruits and vegetables has increased so has the importance of the microbiological safety of these products. In the last few years, food borne illness resulting from contamination of these raw agricultural products, particularly cantaloupe, has become an increasing concern (FDA, 1998).

In early 2007, Costa Rican cantaloupe producers and exporters suffered the effects of a *Salmonella*-positive result in exported product. The finding was made during a routine microbiological screening test performed by official authorities and affected fruits in commerce in the Eastern US and in Quebec, Canada. The broker decided to recall the fruits from the marketplace. The size of the recall reached more than 6100 boxes or approximately 36,600 melons (CBC News, 2007). Also in 2007, there was a report of *Salmonella*-contaminated cantaloupes on the west coast of the US, specifically in California. This time the recall amounted to 2560 boxes or approximately 15,360 melons, all coming from Costa Rica (FDA, 2007).

Fortunately, in both recalls, there were no reports of human salmonellosis due to consumption of contaminated cantaloupe. Producers and exporters in Costa Rica and in other producing countries, are now actively searching for practical solutions to eliminate pathogens of public health concern in their fruit products.

Part of the solution will arise from the understanding and implementation of Good Agricultural Practices, to reduce the incidence of *Salmonella* on melons coming from the field. *Salmonella* has been associated directly with the use of products of animal origin including organic fertilizers and contaminated irrigation water (FDA, 2006). In addition, the use of appropriate post-harvest washing and sanitizing is another key area for control of *Salmonella* on fruit (Parnell *et al.*, 2005).

Cetylpyridinium chloride (CPC) is a quaternary ammonium molecule that is highly effective for microbial destruction, including *Salmonella* (Beers *et al.*, 2006; Breen *et al.*, 1995, 1997; Kim and Slavik, 1996; Osman *et al.*, 2006). CPC is approved for use on raw poultry in the US (FDA, 2004) and certain other national jurisdictions. Currently, melon post-harvest facilities in Costa Rica are primarily using chlorine to disinfect fruit (cantaloupe, Spanish melons and other melons). However, chlorine has several technical and efficacy disadvantages against many important pathogens.

The objectives of the study were 1) to evaluate microbial reductions in the levels of total coliform (TC), Aerobic Plate Count (APC) and yeasts and molds (Y and M) on cantaloupe and Spanish melons utilizing as a post-harvest treatment the CPC-based antimicrobial solution Cecure® (Safe Foods Corporation, North Little Rock, AR, USA) 2) to visually evaluate the effects of the Cecure® treatment on the shelf life of cantaloupe stored under commercial conditions.

## MATERIALS AND METHODS

Experiments were conducted in two commercial melon production and packing plants in Costa Rica in late April, 2007. In both plants the fruit was grown less than a 10 min drive from the packing plant. One of the plants produced cantaloupe and the other plant produced a variety of melon known as the Spanish melon (piel de sapo). Figure 1 (cantaloupe) and 2 (Spanish melons) represent the process flow for each fruit within the respective plant.

Immediately after harvesting, the cantaloupes or Spanish melons were transported in open wagons to the processing facilities. Post-harvest processing of both cantaloupes (Fig. 1) and Spanish melons

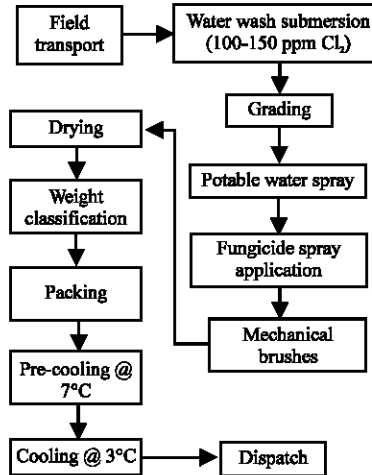


Fig. 1: Flow diagram for cantaloupe packing process

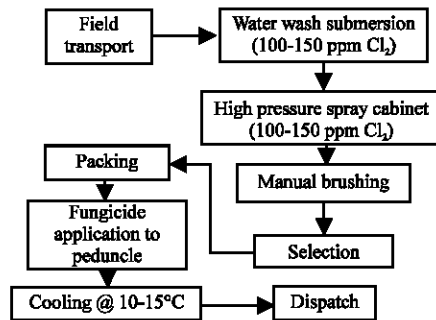


Fig. 2: Flow diagram for Spanish melon packing process

(Fig. 2) involves washing by submersion in ambient temperature water containing chlorine (100 to 150 ppm total chlorine) as a disinfectant. It should be noted that in both the cantaloupe and Spanish melon plant there is no automated overflow on the wash water tank; water is simply added when the level is too low for adequate washing or floatation of the fruit. In addition, the wash water is not drained at the end of a processing day, but is reused for as long as a week or more. In the cantaloupe plant, washing in chlorinated water is followed by grading and then low pressure spray rinsing with water. In the Spanish melon plant, the fruit is also submerged in chlorinated water (100 to 150 ppm total chlorine), but is then subjected to a high pressure chlorine (100 to 150 ppm total chlorine) spray and afterwards is manually scrubbed. To reduce fungi proliferation during storage, a commercial fungicide is directly applied to the whole fruit (cantaloupe) or to the peduncle only (Spanish melons). In the cantaloupe plant, the fungicide is continuously filtered and recycled. In the cantaloupe plant, the fruit is conveyed through a brush cabinet after the fungicide is applied. After fungicide application, the cantaloupes are conveyed to the packing area where they are boxed for subsequent cooling and transport. For the Spanish melons, the fungicide is manually applied to the peduncle only using a wand-type hand spray applicator after the melons are packed in their appropriate transport boxes.

Finally, cantaloupes are carried to a pre-cooling chamber for a period of 4-5 h at 7°C and afterwards are stored at 3°C prior to shipping. Spanish melon processing does not include a pre-cooling step, but instead the fruit is stored at 10-15°C immediately after the washing and packing steps and before shipping.

### **CPC Solution Preparation**

A 5-gallon (18.9 L capacity) plastic bucket containing 15 L of 0.5% v/v CPC was prepared by adding 187 mL of Cecure® (40% CPC concentrate in propylene glycol and water) to 14.8 L of water. To verify the concentration of active ingredient (CPC) a titration kit was used. The titration methodology utilized aryl sulfate and was developed by Global Kemical, S.A.

### **Treatments**

For cantaloupes, five different treatments were evaluated. Table 1 shows the treatments and the details for each. For Spanish melons, four treatments were evaluated. Table 2 shows the treatments and the details for each. For both the cantaloupes and Spanish melons, 0.5% CPC was applied as a 5 sec dip in the solution. Each fruit was allowed to drip for approximately 30 sec before microbiological sampling was initiated.

### **Microbiological Sampling and Procedures**

After the 5 sec CPC dip, cantaloupes or Spanish melons were individually placed in 5 L sterile plastic sampling bags. Sterile Butterfield's phosphate buffer (90 mL) was added, the bags were closed to avoid leakage and the melons were hand shaken and massaged (by the same person throughout all trials) for 1 min. Approximately 30 mL of each rinsate was poured into sterile sample cups. Aryl sulfate was added to each sample cup to neutralize any residual CPC. Sample cups were stored on ice for transport to the laboratory.

All samples were microbiologically analyzed for Aerobic Plate Count (APC), Total Coliforms (TC) and yeasts and molds (Y and M) using 3M Petrifilm™ (3M Corporation, St. Paul, MN, USA) in accordance with the manufacturer's instructions.

### **Shelf-Life Determination**

For both cantaloupes and Spanish melons, one box containing 5 melons was stored for each different treatment. The boxes were held in cooling refrigeration units (3°C for cantaloupes and 10-15°C for Spanish melons) for a period of 8 days. After the 8 day refrigeration period, boxes were held at 20°C.

On the 15th day (8 days of refrigeration + 7 days at 20°C) a very detailed visual evaluation was conducted on the outside appearance of the cantaloupes based on the level of deterioration of the fruit.

Table 1: Post-harvest treatments for cantaloupes

Treatments	Description
Field control	No wash or treatment
Field control + CPC	Not washed + 5 sec CPC dip treatment
Wash + fungicide	Commercial wash + fungicide application (whole fruit spray)
Wash + CPC	Commercial wash + 5 sec CPC dip treatment
Wash only	Commercial wash

Table 2: Post-harvest treatments for Spanish melons

Treatments	Description
Field control	No wash or treatment
Field control + CPC	Not washed + 5 sec CPC dip treatment
Wash + CPC	Commercial wash + 5 sec CPC dip treatment
Wash + fungicide	Commercial wash + Fungicide application (peduncle spray)

This evaluation was accomplished using a grading scale as follows: 1) fruit in good condition, 2) slight to moderate deterioration, 3) severe damage or deterioration. The evaluation was conducted by quality control personnel at the commercial processing and storage facility.

A similar visual evaluation was not conducted on the Spanish melons because even after 7 days at 20°C all treatments looked identical, even to the expert eye, due to the fact that none of the fruit had ripened to any significant degree.

### Statistical Analysis

All microbiological data was converted to  $\log_{10}$  cfu mL<sup>-1</sup> prior to statistical analysis. A non-parametric method called Kruskal-Wallis (one-way analysis of variance by ranks) was applied (SPSS 8.0, 2000). Comparisons between treatments were made using the Mann-Whitney test (SPSS 8.0, 2000). All tests were performed using a level of confidence of 95%.

## RESULTS

### Microbiological Results

#### Cantaloupes

Figure 3-5 shows the  $\log_{10}$  cfu mL<sup>-1</sup> reductions in the various groups of organisms on cantaloupes according to treatment in comparison to levels of microorganisms on the field control. Figure 3 specifically represents the  $\log_{10}$  reductions in APC by treatment. Reductions are based on the level of APC on the field controls which were not washed or treated in any way

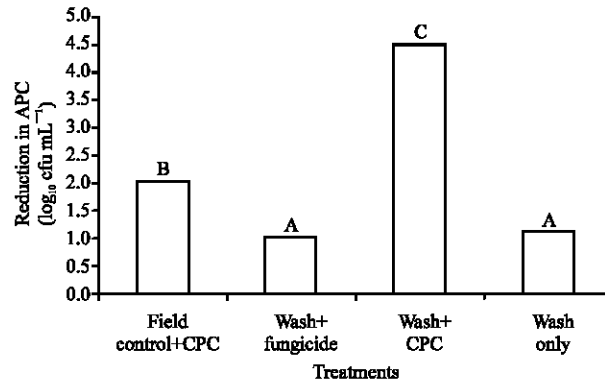


Fig. 3: Effects of various post-harvest treatments on APC on cantaloupes (n = 5)

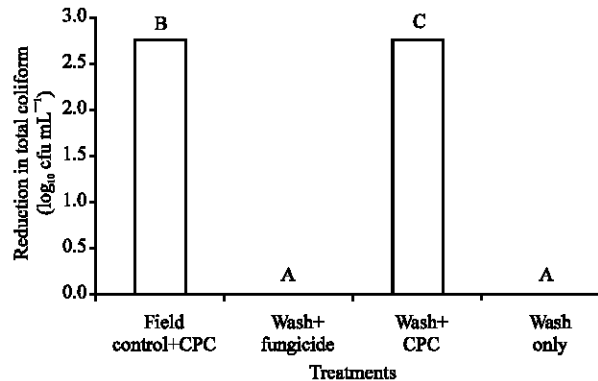


Fig. 4: Effects of various post-harvest treatments on total coliform on cantaloupes (n = 5)

(field control =  $6.7 \log_{10}$  cfu mL<sup>-1</sup>). Simply treating the unwashed field control with a 5 sec dip in 0.5% CPC reduced the APC by 2 logs; while the standard practice of washing plus fungicide only resulted in a 1 log reduction in APC. Washing only, without being followed by the fungicide spray, resulted in a similar reduction, 1.1 logs. However, commercial washing followed by a 5 sec dip in 0.5% CPC resulted in a 4.5 log reduction (> 99.99%) in APC.

The commercial wash, as well as the wash plus fungicide application, resulted in no reduction in total coliforms. However, both CPC treatments, field application or a 5 sec dip following washing, resulted in a 2.8 log reduction in total coliforms. The field control level of total coliforms was  $\log_{10}$  2.8 cfu mL<sup>-1</sup> (Fig. 4). Thus, total coliforms were completely eliminated by both of the CPC dip applications, i.e., in the field or after commercial washing.

The field control level of yeasts and molds was  $\log_{10}$  4.8 cfu mL<sup>-1</sup>. The wash only and the wash plus fungicide only resulted in a 1 log reduction in yeasts and molds. The group of cantaloupe that was treated with CPC in the field had a 3.4  $\log_{10}$  reduction in yeasts and molds; whereas the reduction was 4.2  $\log_{10}$  cfu mL<sup>-1</sup> (> 99.99%) when cantaloupes were washed and then subjected to a 5 sec dip in 0.5% CPC (Fig. 5).

**Spanish Melons**

Figure 6-8 shows the effects of various post-harvest treatments on the  $\log_{10}$  cfu mL<sup>-1</sup> reductions in microorganisms on Spanish melons in comparison to the field control. Figure 6 specifically illustrates the effects of various post-harvest treatments on the APC on Spanish melons. The level of APC on the field control melons was  $6.3 \log_{10}$  cfu mL<sup>-1</sup>. Treating the melons

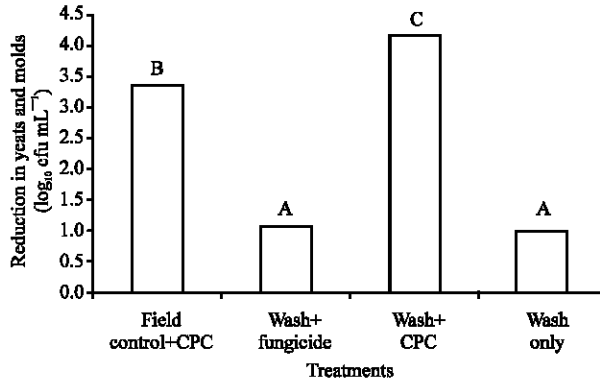


Fig. 5: Effects of various post-harvest treatments on yeasts and molds on cantaloupes (n = 5)

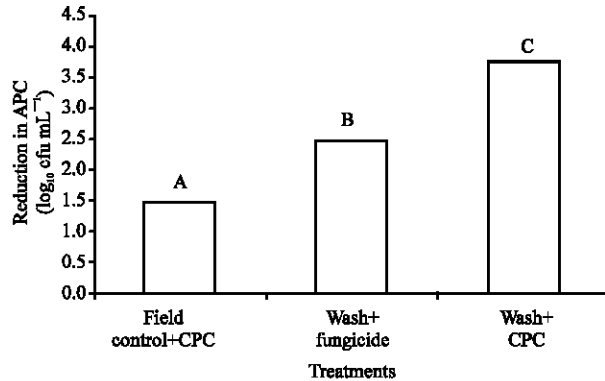


Fig. 6: Effects of various post-harvest treatments on APC on Spanish melons (n = 5)

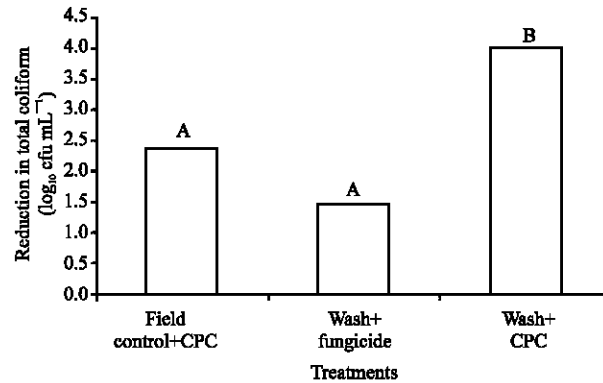


Fig. 7: Effects of various post-harvest treatments on total coliform on Spanish melons (n = 5)

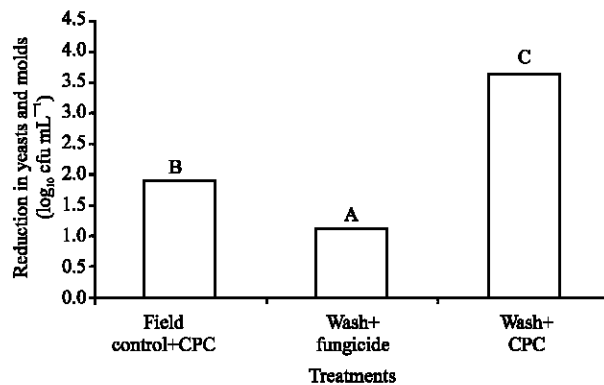


Fig. 8: Effects of various post-harvest treatments on yeasts and molds on Spanish melons (n = 5)

directly from the field with 0.5% CPC as a 5 sec dip resulted in a 1.5 log reduction in APC. The commercial process of washing plus application of a fungicide resulted in a 2.5 log reduction in APC. Washing the melons and then dipping them in a 0.5% CPC solution for 5 sec resulted in a 3.8 log reduction (> 99.9%) in APC.

The level of total coliform present on Spanish melons directly from the field was  $4.0 \log_{10}$  cfu mL $^{-1}$ . Application of 0.5% CPC directly to the melons from the field resulted in a 2.3 log reduction in total coliforms. The commercial washing procedure plus fungicide application resulted in a 1.4 log reduction in total coliforms. Application of 0.5% CPC as a 5 sec dip after washing resulted in a 4.0 log reduction (99.99%) in total coliforms, thereby eliminating this group of organisms from the melons (Fig. 7).

The level of yeasts and molds on the field control melons was  $3.8 \log_{10}$  cfu mL $^{-1}$ . The commercial process of washing plus application of the fungicide resulted in a 1.1 log reduction in total yeasts and molds (Fig. 8). Application of 0.5% CPC to the field melons resulted in a 1.9 log reduction in yeasts and molds while the application of 0.5% CPC as a 5 sec dip after commercial washing resulted in a 3.7 log reduction (> 99.9%) in yeasts and molds. Thus, application of 0.5% CPC as a 5 sec dip following washing virtually eliminated all yeasts and molds from the Spanish melons.

#### Sensory Evaluation of Cantaloupes

The results of the sensory evaluation of cantaloupes are shown in Table 3. The cantaloupes were stored at 3°C for 8 days and then at 20°C for an additional 7 days. The sensory results were



Table 3: Results from the sensory evaluation applied to cantaloupes

Samples	Field control	Field + CPC	Wash + fungicide	Wash + CPC
1	3	1	3	2
2	3	1	1	2
3	3	3	2	1
4	3	2	2	2
5	3	NA	2	3
Mode	3	1	2	2

NA: Not Available

obtained on the 7th day of storage at 20°C. Results were based on the outside appearance of the cantaloupes in terms of degree of deterioration including mold growth, indents, bruises and mushy areas. Obviously, the field control had the most deterioration at the end of the storage period. In comparison, field cantaloupes that were not washed but dipped for 5 sec in 0.5% CPC had a more acceptable overall appearance. Cantaloupes that were either washed and treated with a commercial fungicide or washed and treated with a 5 sec 0.5% CPC dip were similar in appearance.

## DISCUSSION

The results from these trials indicate that the commercial washing and disinfecting procedures presently used for cantaloupes and Spanish melons in Costa Rica could be greatly enhanced in terms of microbial efficacy by the use of cetylpyridinium chloride (Cecure®CPC Antimicrobial). The current cantaloupe washing and disinfection process is only minimally effective in reducing the APC and yeasts and molds on processed fruit. In fact, the commercial washing procedure plus application of a fungicide only reduced APC and yeasts and molds by 1 log and did not reduce total coliforms at all. With a starting level of APC at  $\log_{10} 6.7 \text{ cfu mL}^{-1}$ , a 1-log reduction leaves 5.7 logs of organisms  $\text{mL}^{-1}$  of rinsate on the fully processed fruit. Parnell *et al.* (2005) found that a 60 sec exposure period to 200 ppm total chlorine resulted in a 1.8 log reduction in nalidixic acid-resistant *Salmonella typhimurium* that were inoculated onto the surface of cantaloupe. If total coliforms are used as an indicator for incidence of potential pathogenic organisms in the current study, it is obvious that the current washing and disinfecting procedures are very ineffective since there was no reduction in total coliforms with the current commercial procedures. The difference in the current study and that conducted by Parnell *et al.* (2005) is that the current study was conducted in the field under commercial conditions, not in the laboratory where each cantaloupe was washed individually with fresh potable wash water.

The fact that the wash only (without the fungicide) provided the same microbiological results as the wash plus fungicide indicates that the current fungicide application is substantially ineffective. In comparison, the 5 sec dip of cantaloupes coming directly from the field in 0.5% CPC resulted in a 2 log reduction in APC, a 3.4 log reduction in yeasts and molds and totally eliminated total coliforms. If the 5 sec dip in CPC was applied after commercial washing, as would be suggested for commercial use, there was a 4.5 log reduction in APC and no recovery of total coliforms or yeasts and molds.

The results from the trial with Spanish melons were similar to those obtained with the cantaloupes. Again, the commercial washing and disinfecting process was only minimally effective in reducing the APC, total coliforms and yeasts and molds. Parnell *et al.* (2005) suggested that the use of brushing combined with submersion in chlorinated water could improve microbial reductions by approximately 1 log. This is consistent with the results in the current study, if the washing process for the cantaloupes (no brushing during washing) is compared to the washing process (brushing during washing) for the Spanish melons in terms of reductions in APC and total coliforms. The commercial washing procedure plus the application of a 5 sec dip in 0.5% CPC resulted in extremely effective reductions in all groups of organisms with no recovery of total coliforms.

In conclusion, the results of this study suggest that the use of a 5 sec dip in 0.5% CPC significantly improves the microbial condition and overall shelf life of cantaloupes and Spanish melons when applied either directly to field harvested melons or after the current commercial processing and washing procedures. The use of a 0.5% CPC dip could significantly reduce the risk associated with any future recall of exported fruit due to microbiological concerns.

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