Microbiological Quality of Fresh-Squeezed Orange Juice and Efficacy of Fruit Surface Decontamination Methods in Microbiological Quality

UFUK BAGCI AND AYHAN TEMIZ*

Department of Food Engineering, Hacettepe University, 06800 Beytepe, Ankara, Turkey

ABSTRACT

The aims of this study were to evaluate the microbiological quality of fresh-squeezed orange juice and to reduce the microbial population by using various chemical and physical fruit surface decontamination methods. In the first step of the study, polyethylene-bottled fresh-squeezed orange juice samples purchased in Ankara, Turkey, were examined. The average aerobic plate count (APC) and coliform count (CC) varied within the ranges of 3 to 5 log CFU/ml and 1 to 4 log MPN/ml, respectively. Ten of 60 samples contained various levels of Escherichia coli, while Salmonella spp. and E. coli O157:H7 were not detected in any of the samples. Comparing the efficacy of various fruit surface decontamination methods on microbial population of oranges, the best results were obtained following two applications of submersion in boiling water and 5% H2O2 solution for both the uninoculated and inoculated samples. Orange juice samples obtained from surface-inoculated and decontaminated oranges were also examined. We showed that about 17.4% of the E. coli population was transferred to orange juice after extraction, indicating the separation of microbial contaminants from fruit peel during extraction. Finally, the levels of microbial contamination occurred throughout the extraction process on the inner surfaces of a commercial juice extractor at one of the sale points investigated. Significant (P < 0.05) increases in the APC and CC were determined in surface samples of the extractor after the extraction. Surface decontamination and extraction are critical steps in fresh juice production for preventing microbial contamination. Immersion in boiling water for 0.5 min, without using any chemicals, can be offered as an effective method to reduce microbial population on orange surfaces.

Consumption of fresh-squeezed fruit juices has increased in recent years due to their high nutritional value and superior taste (2, 14). The feature of fresh-squeezed fruit juice that sets them apart from conventional fruit juice products is the lack of pasteurization. Heating processes used in pasteurization of conventional juices increases shelf life by inactivating certain enzymes and microorganisms. However, flavor losses and heat-induced chemical changes are the main drawbacks of the pasteurization process, leading to a remarkable diminishing of the quality of the natural fresh juice (14, 27).

Citrus juices are the most widely consumed fresh-squeezed fruit juices worldwide (19). Fresh-squeezed citrus juice is pure juice obtained from mature citrus fruit and has not been further pasteurized, frozen, or concentrated after extraction (15, 23). Among citrus juices, orange juice is the most appreciated and consumed because of its pleasant taste and its high content of vitamin C (20).

Fresh fruit juices have a short shelf life, even when kept under refrigeration (0 to 4°C) (7). It has been recognized that the shelf life and quality of fresh juice products are directly related to the care and sanitation steps taken in fruit handling and processing. Reported initial microbial levels of fresh citrus juice vary from 1.3 to 5.3 log CFU/ml. Extremely high initial microbial levels may result from the use of deteriorated fruit, insufficient cleaning, or poor equipment sanitation (3, 9, 15).

Acidic foods such as fruit juices were not recognized as vehicles of foodborne illness until major outbreaks involving Escherichia coli O157:H7 and Salmonella spp. in orange and apple juices occurred (6, 31). Pathogenic microorganisms do not grow in fruit juices due to their low pH but can survive and become adapted to acidic environments (12, 28). E. coli O157:H7 and Salmonella spp. can survive for extended periods of time in high-acid environments under refrigerated storage. Zhao et al. (35) reported that E. coli O157:H7 could survive up to 31 days at 8°C in unpasteurized apple cider (pH 3.6 to 4.0). Salmonella enterica serovars Hartford, Gaminara, and Typhimurium have been shown to survive up to 24, 15, and 15 days, respectively, at pH 3.5 and 4°C storage temperature (18).

In 2001, the U.S. Food and Drug Administration (FDA) established that juice processors were required to implement hazard analysis critical control point programs. The regulation requires implementation of a process capable of
reducing the pertinent pathogen by 5 log units (30). For achieving this goal, surface decontamination is one of the most critical processing steps in the fresh juice production, affecting the safety and quality of the end product. Chemical sanitizers have been widely used in food processing to reduce undesirable microorganisms (21). Chlorine has been used for the sanitation process in food processing for several decades and is perhaps the most widely used sanitizer in the food industry. Brackett (4) concluded that chlorine compounds inactivate microorganisms in solutions and on equipment but only have minor effects on microorganisms existing on fruit and vegetable surfaces. Liquid chlorine and hypochlorites are generally used in the 50- to 200-ppm concentration range with a contact time of 1 to 2 min. Hydrogen peroxide, generally used in 1 to 5% concentrations, is a highly effective antimicrobial agent against bacteria but is less active against yeasts, fungi, and viruses. The major advantages claimed for H₂O₂ are its sporocidal activity and rapid breakdown into oxygen and water upon contacting organic material, thus having no long-term residual activity (17, 23). An alternative to chemical sanitizers is hot water immersion (surface pasteurization). This has the advantage of retaining the simplicity of a purely water-based process (2, 8, 10). Hot water can also be utilized to sanitize orange surfaces without altering the original sensory quality of fresh juice (14).

The objectives of this study were to (i) determine the microbiological quality of commercial fresh-squeezed orange juice during storage; (ii) determine the microbial load on commercial-type juice extractor before and after extraction; and (iii) determine the sanitizing effects of hot water, chlorine, and H₂O₂ on the surfaces of noninoculated and E. coli ATCC 25922–inoculated oranges.

MATERIALS AND METHODS

Commercial orange juice samples. Fresh-squeezed unpasteurized orange juice samples in polyethylene bottles were collected from five different commercial producers in Ankara, Turkey. Each bottle consisted of approximately 330 ml of fresh-squeezed orange juice. In a 1-year period, two bottles of juice samples were collected from each producer at six different time points. All samples were transported to the laboratory under cold-storage conditions (0 to 5°C). Samples were stored at 4°C for 3 days, and microbial analyses were performed during the 3 days of storage.

Inoculum preparation. E. coli ATCC 25922 was used for inoculum preparation. The stock culture of E. coli was activated in tryptic soy broth (TSB; Merck, Darmstadt, Germany) at 37°C for 24 h prior to inoculum preparation. Activated E. coli was subcultured in 10 liters of TSB at 37°C for 20 h. Cells in the subculture were separated by centrifugation (3,000 × g for 10 min) at 4°C. Pellets were washed and centrifuged in sterile peptone water (1 g/liter) twice. Cells were resuspended in sterile peptone water in order to number about 10⁷ CFU/ml.

Inoculation of oranges. Oranges were purchased from a local supermarket in Ankara, Turkey. Before inoculation, oranges were washed with tap water to remove dirt on the surface and were allowed to dry. Oranges were then immersed in the inoculum for 15 min. After inoculation, oranges were drained for 1 h to remove excess inoculum before juice extraction.

Surface decontamination of oranges. Distilled water, 200 ppm of chlorine, 5% H₂O₂ solution, and boiling water were used as surface decontamination solutions. Chlorine solution (200 ppm) was prepared from a hypochlorite solution (15.8% active chlorine; Ak-kim Chemistry Co., Istanbul, Turkey), and a 5% H₂O₂ solution was prepared from a 30% H₂O₂ solution (Mercer) by using deionized water.

Both the uninoculated oranges (UO) and inoculated oranges (IO) were separately immersed into 5 liters of freshly prepared distilled water, 200 ppm of chlorine, 5% H₂O₂, and boiling water for 2, 8, 2, and 0.5 min, respectively. The oranges were stirred with gentle continuous agitation. Then, the surface decontamination solutions were drained off. Chlorine-treated samples were rinsed with the same amount of sterile deionized water for 30 s. Non-surface-decontaminated oranges were considered controls.

These trials were repeated six times.

Surface microbial load of oranges. The surface microbial loads of oranges were determined according to the method of Pao and Davis (14) with some modifications. Surface microbial loads of UO and IO were determined by washing six oranges in a sterile plastic bag containing 1 liter of peptone water (1 g/liter) for 15 min with gentle continuous agitation. This washing solution was immediately used for microbial evaluation (14).

Juice extraction. To obtain orange juice samples, 15 oranges per treatment were squeezed by a hand-type steel juice extractor. Extractors were sanitized before juice extraction. Juice samples were immediately used for microbial evaluation after extraction.

Surface microbial load of commercial extractor. A small-scale extractor (Zumex Z38, Valencia, Spain) that was used by one of the commercial fresh-squeezed orange juice producers in Ankara, Turkey, was analyzed in order to determine the surface microbial load. The swab (3M Swab-Sampler with buffered peptone water, 3M, St. Paul, MN) samples were collected from four different surface points (blade, upper pressing unit, lower pressing unit, and plastic juice filter) of the extractor. The first swab sample was taken before extraction and the second 2 h after extraction. Aerobic plate counts (APCs) and coliform counts (CCs) were determined before and after extraction.

Microbial analysis. For commercial orange juice samples and surface microbial loads of commercial extractors, APCs were determined on plate count agar (Merck) following the pour plate method by incubation at 37°C for 48 h. The CCs and E. coli counts were determined by using the three-tube most-probable-number (MPN) method. Appropriate dilutions were inoculated into Fluorocult lauryl sulfate tryptose (LST-MUG; Mercer) broth with Durham tubes and incubated at 37°C for 24 to 48 h. One loopful of culture from the tubes with gas formation at 24 and 48 h was transferred to brilliant green bile broth (Merck) and incubated at 37°C for 24 to 48 h. The brilliant green bile broth tubes with gas and turbidity formation were used for the enumeration of coliforms by using the MPN table. For the determination of E. coli numbers, gas- and turbidity-positive LST-MUG broth tubes at 24 to 48 h were examined for blue fluorescence at a wavelength of 366 nm by using a handheld UV lamp (CAMAG, Muttenz, Switzerland). As a confirmation, the indole test was performed using Kovac’s reagent. The gas-, fluorescence-, and indole-positive LST-MUG broth tubes were used for the determination of E. coli numbers by using the MPN table (29).

For the detection of E. coli O157:H7, 25 ml of orange juice was preenriched in 225 ml of modified TSB (containing 0.02 g/
liter novobiocin; Merck) at 37°C for 24 h and then plated onto cefixime tellurite sorbitol MacConkey agar (Merck) and incubated at 37°C for 24 h. After incubation, the plates were checked for the presence of sorbitol-negative, 1- to 2-mm-diameter colorless colonies. Sorbitol-negative colonies were analyzed with an O157 latex test (Oxoid, Basingstoke, UK) and modified brilliant green phenol red lactose sucrose agar (Merck), xylose lysine desoxycholate agar (Merck), and Rambach agar (Merck), and all plates were incubated at 35°C for 24 h. Then, the cultures were streaked onto Rambach agar (Merck), xylose lysine deoxycholate agar (Merck), and modified brilliant green phenol red lactose sucrose agar (Merck), and all plates were incubated at 35°C for 48 h. Pure cultures of the presumptive-positive colonies were transferred to the triple sugar iron agar (Merck), urea agar (Merck), and lysine iron agar (Merck) slants and incubated at 35°C for 24 h (29).

For the detection of E. coli from the IO surfaces and orange juice samples, wash solutions or juice samples were pour plated by using tryptic soy agar (Merck) and incubated at 25°C for 2 h. The plates were then covered with an additional layer of violet red bile agar (Merck) and incubated for 20 h at 45.5°C. Typical colonies were counted (13).

All serial decimal dilutions were prepared in sterile buffered peptone water.

**Statistical analysis.** The Duncan’s multiple range tests were performed by SPSS 12.0 software (Chicago, IL) to determine statistical differences ($P \leq 0.05$) between surface decontamination trials.

**RESULTS AND DISCUSSION**

**Microbiological quality of commercial fresh-squeezed orange juice during storage.** Microbiological analysis results of the commercial orange juice samples with respect to five distinct points of sale are given in Table 1. Based on measurements conducted within the first day of storage, the lowest count was determined as 3.33 log CFU/ml at the first point of sale and the highest count was determined as 4.85 log CFU/ml at the third point of sale. At the first day of storage, the lowest coliform bacteria count was 1.88 log MPN/ml at the first point of sale and the highest coliform bacteria count was 4.35 log MPN/ml at the third point of sale. Although there were no significant differences in average APC from all producers throughout the storage, there was a 0.25-log CFU/ml increase in average APC during the second day of the studied samples and a 0.49-log CFU/ml increase during the third day in comparison with the measurements conducted during the first day of storage. The CC decreased throughout the storage period. Initial populations of ca. 2.8 log MPN/ml were reduced to 2.15 and 1.51 at the second and third days of storage, respectively. The decrease in the CC at the end of the storage was significant in comparison with the first storage day ($P < 0.05$) (Table 2).

Only 10 of 60 orange juice samples from the second, fourth, and fifth points of sale contained E. coli. The highest count of E. coli was determined as 1.18 log MPN/ml. Neither E. coli O157:H7 nor Salmonella spp. were detected in any of the orange juice samples acquired from five distinct points of sale in Ankara, Turkey.

There is limited information regarding the microbiological quality of commercial fresh-squeezed orange juice. APCs of fresh-squeezed orange juice samples collected from street vendors in Mexico ranged between >2 and >6 log CFU/ml, and one-third of the samples were determined to have APCs of about >5 log CFU/ml. It was indicated that 75% of juice samples contained E. coli, having counts of up to 4 log CFU/ml (5). They also showed that 9% of the juice samples were Salmonella positive. According to these authors, the lack of resources such as potable water for washing oranges or hands for street vendors explains the magnitude of objectionable contamination (5). Similar to our results, de Souza et al. (7) showed that there was no

### TABLE 1. Aerobic plate count (APC), coliform count (CC), and pH values taken over a 3-day storage period at 4°C for freshly squeezed unpasteurized polyethylene-bottled orange juices purchased in Ankara, Turkeya

<table>
<thead>
<tr>
<th>Producer no.</th>
<th>Analysis day</th>
<th>APC (log CFU/ml)</th>
<th>CC (log MPN/ml)b</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3.33 ± 0.36 A</td>
<td>1.88 (1)</td>
<td>3.37 ± 0.10 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.65 ± 0.31 AB</td>
<td>0.85</td>
<td>3.36 ± 0.12 AB</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.05 ± 0.54 B</td>
<td>0</td>
<td>3.40 ± 0.12 B</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3.88 ± 0.82 A</td>
<td>2.17 ± 0.24 A</td>
<td>3.43 ± 0.18 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.07 ± 0.81 A</td>
<td>1.52 ± 0.53 A</td>
<td>3.42 ± 0.19 A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.69 ± 1.67 A</td>
<td>0.55 ± 0.67 B</td>
<td>3.45 ± 0.19 B</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>4.85 ± 0.51 A</td>
<td>4.35 ± 0.44 A</td>
<td>3.48 ± 0.17 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.00 ± 0.39 A</td>
<td>4.00 ± 0.55 A</td>
<td>3.47 ± 0.18 A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.94 ± 0.82 A</td>
<td>3.65 ± 0.02 A</td>
<td>3.52 ± 0.18 A</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>3.81 ± 1.18 A</td>
<td>2.61 ± 2.03 A</td>
<td>3.42 ± 0.16 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.99 ± 1.30 A</td>
<td>2.25 ± 1.99 A</td>
<td>3.44 ± 0.16 A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.46 ± 1.71 A</td>
<td>1.94 ± 1.55 A</td>
<td>3.45 ± 0.17 A</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>3.51 ± 1.23 A</td>
<td>3.17 ± 0.29 A</td>
<td>3.40 ± 0.10 A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.69 ± 1.60 A</td>
<td>2.13 ± 0.71 A</td>
<td>3.39 ± 0.09 A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.89 ± 1.39 A</td>
<td>1.58 ± 1.04 A</td>
<td>3.39 ± 0.09 A</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations of the population recovered ($n = 12$). Values followed by different letters in the same column are significantly different ($P < 0.05$) for each point of sale.

b Numbers in parentheses indicate the number of samples of the 12 samples taken with CC >3 MPN/ml.
Salmonella or fecal coliform in polyethylene-bottled unpasteurized orange juice samples during 72 h of storage at 4°C. Fellers (9) studied the shelf life of fresh-squeezed polyethylene-bottled orange juice (from Valencia orange, Hamlin orange, and Pineapple orange). Initial total plate counts varied from 3.91 log CFU/ml (Valencia orange juice) to 5.28 log CFU/ml (pineapple orange juice). In contrast to our results, in general, over the approximate shelf life period (10 to 16 days at 4.4°C) the numbers of viable colonies recorded for total plate counts declined from the initial counts of 0.08 and 1.93 log CFU/ml. The differences between the results may be due to the differences in storage periods.

Effects of surface decontamination methods on uninoculated and E. coli–inoculated oranges. The effect of surface decontamination treatments on UO and IO is shown in Table 3. The average values of APC of UO surfaces were 2.76 log CFU/ml. E. coli, E. coli O157:H7, and Salmonella spp. were not detected on the surfaces of UO. The effectiveness of surface decontamination processes on UO was evaluated with respect to APC. Washing with water had no significant effect on APC. Significant reductions by chlorine, H₂O₂, and hot water treatments were observed. Although no statistical differences were found between these three surface decontamination treatments, H₂O₂ was determined to be the most effective surface decontamination method, achieving a 1.70-log CFU/ml reduction with respect to the control. The effectiveness of this method was followed by washing with chlorine, submerging into boiling water, and washing with tap water treatments with 1.29–, 1.21–, and 0.19-log CFU/ml reductions, respectively (Table 3).

Since the highest initial APC found on UO was 3.03 log CFU/ml (data not shown), it was not possible to observe the 5-log reduction required by the FDA (30). Therefore, orange surfaces were inoculated with E. coli ATCC 25922 to get higher initial counts. After the inoculation, E. coli counts on the orange surfaces varied within the range of 4.80 to 5.98 log CFU/cm², with an average of 5.51 log CFU/cm². All decontamination methods reduced the E. coli counts significantly (P < 0.05). Up to 3.37-log CFU/cm² reductions were achieved by submersion into boiling water, showing the highest rate of contaminant reduction. Treatment with the 5% H₂O₂ solution and the 200-ppm chlorine solution resulted in 2.91- and 2.54-log CFU/cm² reductions, respectively, whereas washing with tap water reduced the initial load by only 0.96 log CFU/cm² (Table 3).

Compared with the other methods, water treatment did not reduce the microorganism level effectively. Gil et al. (11) mentioned that large quantities of water would be required to achieve the same level of microbial reduction without the use of sanitizers. In our study, there were 1.29- to 2.54-log reductions in the microbial load with chlorine treatment. Published data indicated that at permitted concentrations, population reductions on produce surfaces will be within the range of 1 to 2 log units. This is due in part to the rapid breakdown of chlorine in the presence of organic matter (17, 22). Sapers and Jones (24) showed that 200 ppm of Cl₂ (for 3 min, at 20°C) reduced the E. coli population by 1.70 log on tomatoes. Hydrogen peroxide treatment has been shown to be a good alternative to chlorine with its generally recognized as safe status. Hydrogen peroxide treatment was shown to reduce the population by up to 2.91 log. Similar to our results, Sapers et al. (25) reported a 2.34-log CFU/g reduction in the level of E. coli following by 5% H₂O₂ treatment for 2 min on apples. The best decontamination results were obtained by boiling water, with a reduction of 3.37 log in our study. Several studies demonstrated the efficacy of hot water treatments on reducing human pathogens and native microflora on fruit surfaces. Hot water treatment (76°C for 3 min) has been reported to be superior to chlorine (20 ppm, 5 min).

### Table 2. Average aerobic plate counts (APCs) and coliform counts (CCs) of freshly squeezed unpasteurized polyethylene-bottled orange juice samples from all producers throughout the 3-day storage period at 4°C

<table>
<thead>
<tr>
<th>Analysis day</th>
<th>APC (log CFU/ml)</th>
<th>CC (log MPN/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.87 ± 0.59 A</td>
<td>2.80 ± 1.10 A</td>
</tr>
<tr>
<td>2</td>
<td>4.12 ± 0.52 A</td>
<td>2.15 ± 1.25 A</td>
</tr>
<tr>
<td>3</td>
<td>4.36 ± 0.50 A</td>
<td>1.51 ± 1.29 B</td>
</tr>
</tbody>
</table>

* a Values are means ± standard deviations of populations recovered (n = 60). Values followed by different letters in the same column are significantly different (P < 0.05).

### Table 3. Effects of different surface decontamination treatments on uninoculated and E. coli ATCC 25922–inoculated orange surfaces and orange juice from these inoculated oranges

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Distilled water (2 min)</th>
<th>Boiling water (0.5 min)</th>
<th>Chlorine (200 ppm, 8 min)</th>
<th>H₂O₂ (5%, 2 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated orange surfaces (APC)</td>
<td>2.76 ± 0.24 A</td>
<td>2.57 ± 0.15 A</td>
<td>1.55 ± 0.80 B</td>
<td>1.47 ± 0.62 B</td>
<td>1.06 ± 0.57 B</td>
</tr>
<tr>
<td>Inoculated orange surfaces (E. coli counts)</td>
<td>5.51 ± 0.43 A</td>
<td>4.55 ± 0.54 B</td>
<td>2.14 ± 0.46 C</td>
<td>2.98 ± 0.21 D</td>
<td>2.60 ± 0.53 DC</td>
</tr>
<tr>
<td>Orange juice from inoculated oranges (E. coli counts)</td>
<td>4.75 ± 0.29 A</td>
<td>3.96 ± 0.50 B</td>
<td>1.69 ± 0.37 C</td>
<td>2.53 ± 0.34 D</td>
<td>2.04 ± 0.46 C</td>
</tr>
</tbody>
</table>

* a Values are means ± standard deviations of populations recovered (n = 6). Values followed by different letters in the same row are significantly different (P < 0.05).

b E. coli level in inoculum used for inoculation of orange surfaces, 8.98 ± 0.22 CFU/ml.

c pH of orange juice, 3.41 ± 0.06.
TABLE 4. Aerobic plate counts (APCs) and coliform counts (CCs) from four different sample points before and after 2-h extraction by commercial orange juice extractor

<table>
<thead>
<tr>
<th>Sample point</th>
<th>Before extraction</th>
<th>After extraction</th>
<th>Before extraction</th>
<th>After extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APC</td>
<td>CC</td>
<td>APC</td>
<td>CC</td>
</tr>
<tr>
<td>Upper pressing unit</td>
<td>1.78 ± 0.39 A</td>
<td>4.21 ± 0.26 b</td>
<td>ND*</td>
<td>2.50 ± 0.30 b</td>
</tr>
<tr>
<td>Lower pressing unit</td>
<td>2.09 ± 0.52 A</td>
<td>4.20 ± 0.25 b</td>
<td>ND</td>
<td>3.07 ± 0.23 b</td>
</tr>
<tr>
<td>Juice extractor</td>
<td>1.85 ± 0.37 A</td>
<td>3.04 ± 0.51 b</td>
<td>ND</td>
<td>1.40 ± 1.21 b</td>
</tr>
<tr>
<td>Blade</td>
<td>2.10 ± 0.08 A</td>
<td>3.32 ± 0.32 b</td>
<td>ND</td>
<td>1.54 ± 1.42 b</td>
</tr>
<tr>
<td>Juice filter</td>
<td>2.05 ± 0.64 A</td>
<td>3.83 ± 1.01 b</td>
<td>ND</td>
<td>2.27 ± 1.03 b</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations of populations recovered (n = 3). Values with different letters in the same row are significantly different (P < 0.05) for APC and CC.

20 min) in effectively reducing microbial populations of whole cantaloupes (8). E. coli O157:H7 was not detected on the surface of apples inoculated with E. coli O157:H7 followed by hot water treatment at 95°C for 60 s (10).

In this study, we were unable to achieve a 5-log reduction through any surface decontamination methods against the tested E. coli strain. Wisniewsky et al. (32), Pao et al. (16), Fleischman et al. (10), Pao and Davis (14), and Sapers et al. (26) were also unable to achieve a 5.0-log reduction by several surface decontamination methods that were applied on the surfaces of apple and orange samples.

In our study, the treatment of submersion in boiling water reduced E. coli counts of IO surfaces by up to 3.37 log, whereas a 1.21-log reduction in APCs of the uninoculated samples occurred (Table 3). Similarly, there were higher reductions with the remaining decontamination methods on IO than on UO. Several researchers obtained similar results between natural and inoculated microfloras of the studied fruit samples. For example, a 5-log reduction in E. coli counts was attained by immersing the IO in hot water at 80°C for 1 min or 70°C for 2 min, while these treatments reduced the APC about 3 log on natural surface microflora of the orange surfaces (14). Annous et al. (1) reported that hot water treatment at 76°C for 3 min resulted in a 5-log reduction of Salmonella-inoculated cantaloupes but was able to reduce the total plate counts by only 1 to 2 log. These results showed that the natural microflora was more resistant to decontamination treatments than the artificial microflora. Differences in biofilm formation between natural and artificial floras and the presence of inaccessible areas (the stem and the calyx regions) could limit the efficacy of sanitizing agents (22).

Effects of surface decontamination methods on orange juices obtained from IO. E. coli counts of the juice obtained from surface-decontaminated oranges are provided in Table 3. The average E. coli count was recorded as 4.75 log CFU/ml. The highest decontamination rate was recorded as 3.06 log CFU/ml, obtained by submersion in boiling water. The difference between submersion into boiling water and washing with H2O2 solution was determined to be statistically insignificant, while the statistical differences between the effectiveness of all other methods were found to be significant (P < 0.05).

For citrus products, an extraction procedure is commonly employed in which juice is extracted while largely maintaining peel integrity. Fresh juice can be selectively separated from the fruit peel and surface microbial contaminants during commercial juice extraction. In our study, we observed that about 17.4% of E. coli cells from the fruit’s surface were transferred to orange juice after extraction by a hand-type juice extractor. Similarly, Pao and Davis (15) showed that transfer of E. coli into orange juice was about 1.3% from the surface of IO after extraction by a commercial extractor. These results suggest that 1 to 2 log of the surface microbial load can be eliminated by juice extraction.

Microbial load on commercial-type extractor before and after the extraction. The APCs and CCs of the surface samples of commercial-type extractor before and after the extraction of the fruit juices are shown in Table 4. The extraction process resulted in an increase in the APC of 2.43 and 1.19 log CFU per surface at the upper pressing unit and juice extractor, respectively. An average increase of 1.75 CFU per surface was obtained from all sample points. The CC was determined as <3 MPN per surface in all surface samples taken before extraction, and it increased up to 3.07 log MPN per surface following extraction. After extraction, there was a significant (P < 0.05) increase in the APCs and CCs at all sample points. These results clearly revealed that the extractor may be an important source of contamination of the fruit juice and must be cleaned and sanitized periodically during the production day.

This study gives a general overview of microbiological quality of commercial fresh-squeezed orange juice in Ankara, Turkey, and the use of surface decontamination methods on orange surfaces. The presence of an average of 4 to 5 log CFU/ml in the APC load together with CC and E. coli within a number of orange juice samples procured from an open market indicated that certain hygiene and sanitation guidelines were not followed throughout the production of freshly squeezed orange juice in general. Significant increases in both the APC and CC were recorded on extraction spots of the commercial extractor between
preextraction and postextraction counts. These results reveal that extraction is a critical step and a potential source of microbial contamination in fresh juice production. Thus, producers should ensure proper hygiene of extractors through frequent cleaning, reconditioning, and proper sanitization throughout the production cycle day. Surface decontamination is another crucial step in fruit juice production that may contribute significantly to attain the 5-log reduction of pathogens on fruit intended for fresh unpasteurized juice production. In this study, decontamination methods applied on orange surfaces did not ensure a 5-log reduction in *E. coli* ATCC 25922. Nevertheless, these methods provided a significant reduction in *E. coli* counts both within the orange juice and on the orange surface. Hydrogen peroxide was shown to be a good alternative as a surface decontaminant since it breaks down into nontoxic compounds rapidly upon contacting organic materials.

Sanitizing with H$_2$O$_2$ exposed to the surface of oranges for a period of 2 min at room temperature can reduce up to 2.91 log CFU of the tested *E. coli* strain per cm$^2$. On the other hand, submerging oranges into boiling water resulted in the highest decontamination impact. *E. coli* reductions of up to 3.38 log CFU/cm$^2$ were achieved upon exposure to boiling water for 30 s. According to the results presented in this study, a short period of hot water immersion, without the use of chemicals, can be offered as an effective method to reduce microbial population on orange surfaces.

**ACKNOWLEDGMENTS**

The authors thank Hacettepe University Research Fund (project no 0401602001) and Gökhan Artar for financial support.

**REFERENCES**


