Florida Citrus Packers

a non-profit co-operative association

"Since 1960"

RICHARD KINNEY
Executive Vice President

February 12, 2002

Dockets Management Branch
HFA-305
Food and Drug Administration
5630 Fishers Lane; Room 1061
Rockville, MD 20852

Subject: Comments related to Docket Number cf0161

To Whom It May Concern:

The following comments are hereby submitted in response to the request by the Food and Drug Administration (FDA) for comments and suggestions regarding the draft document titled: "Guidance for Industry, Exemptions from the Warning Label requirement for Juice Recommendations for Effectively Achieving a 5-Log Reduction," released December 21, 2001.

Florida Citrus Packers is a non-profit trade association representing the commercial fresh citrus shippers of Florida. Our members and the industry ship approximately 50 to 60 million 4/5 bushel cartons of fresh citrus annually, grapefruit, oranges and special citrus varieties. Florida's fresh oranges and grapefruit have high juice content as well as high solids (brix) and are preferred for juice processing, especially when utilized for fresh squeezed. We have realized a significant reduction in fresh movement over the past several years due in part, we believe, to FDA's warning label rule/requirement.

While we understand and appreciate FDA's role and efforts to enhance the safety of fresh squeezed juice, we are not aware of a single, documented food safety incidence from sales of fresh squeezed juice processed at retail or facilities with central processing directly into retail containers for distributing to satellite stores. However, we supported Florida's Department of Citrus research to develop effective methods to achieve a cumulative 5-Log reduction in target pathogens, which was published June 30, 1999 (copy attached).

These methods for surface treatment of citrus fruit with sanitizers, wax and heated water and handling were published by the Florida Department of Citrus in a document titled: "Guidance Document for Retail and Roadside Fresh Citrus Juice Producers" dated June 30, 1999 (copy attached).

Fresh citrus fruit packed by commercial packinghouses are clean and wholesome. In a paper published by Pao and Brown in 1998 titled: "Reduction of Microorganisms on Citrus Fruit Surfaces during Packinghouse Operations" (J. Food Protection, vol. 61: 903-906), it was reported that fruit washing and rinsing operations in commercial citrus packinghouses

OID-0493 C 2

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resulted in significant log reduction of surface microorganisms. It was also reported that no E. coli or Salmonella were recovered from fruit packed in seven commercial citrus packinghouses. Fruit inoculated with E. coli and then washed and waxed had significantly lower surface contamination (4.8 log vs. 1.4 log CFU/cm2). Further studies using alkaline cleaners containing orthophenylphenol reduced surface microbial load by 3.5 log. Moderate grading was also found to reduce natural aciduric microflora in fresh juice by more than two logs. Washing and waxing citrus fruit was effective in achieving a 4.6 =/- 0.4 log reduction in surface inoculated E. coli. Refrigeration of fruit to 40°F can add another margin of safety by maintaining lower levels of microbial growth on the surface of fruit.

Based on these results, we believe that the use of combinations of the above mentioned procedures coupled with proper handling of fruit in transport and storage provides adequate protection against microbial contamination in fresh citrus juices. Again, fruit that is washed, sanitized in certified facilities, graded, transported in clean conveyances, refrigerated, and then squeezed into containers bottled at retail is safe. This cumulative 5-Log, steps and procedures provided in the aforementioned document, is sound science and reasonable for this category of producer. We strongly urge FDA to extend these provisions as they are currently being applied.

Regarding the issue of utilization of undamaged tree-picked fruit (USDA choice or better), Florida Citrus Packers agrees with the requirement's intent but respectfully requests flexibility in language to include "USDA choice grade or equivalent standard."

Florida Citrus Packers recognizes the importance of fresh squeezed citrus juices to consumers of this healthy, high quality product. We are concerned however, over the business viability of small and very small juice processors in having to meet unnecessary and impractical guidelines.

Respectfully submitted,

Richard J. Kinney
Executive Vice President
Florida Citrus Packers

Enclosure (5 copies of "*Guidance Document for Retail and Roadside Fresh Citrus Juice Producer)*

State of Florida - Department of Citrus

Guidance Document
for
Retail and Roadside Fresh Citrus Juice Producers

™

Prepared by the
Fresh Citrus Juice Task Force
M.A. Ismail, Chairman
June 30, 1999

STATE OF FLORIDA

DEPARTMENT OF CITRUS

SCIENTIFIC RESEARCH DEPARTMENT
700 EXPERIMENT STATION ROAD
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To: Producers of Fresh Citrus Juice at Retail and Small Roadside Outlets

From: Mohamed A. Ismail, Ph.D.
Scientific Research Director
Florida Department of Citrus
Lake Alfred, Florida

Subject: Guidance for compliance with FDA warning label rule

Date: June 30, 1999

On November 3, 1998, the Food and Drug Administration (FDA) issued a memorandum to all district office Food Team Leaders advising them that:

"Starting January 19, 1999, FDA districts should perform audit checks at citrus juice firms that have an agreement on file with the FDA District Director. The audit checks can vary in degree of complexity. They may be as simple as collecting documents from the firm on the steps it has taken to comply with the terms of the agreement, or as complex as documenting by inspection that the firm has an interim protection system in place that applies basic Hazard Analysis and Critical Control Points (HACCP) principles such as Good Manufacturing Practices, culling of damaged fruit, chemical washing, brushing and sanitizing of the fruit, and utilization of only those types of fruit with skins that are durable to be cleanable and to remain intact after cleaning; and that the firm is developing and validating procedures to achieve a 5-log reduction. CFSAN[1] will provide additional instructions concerning the audit checks as information is received and evaluated by CFSAN staff."

To assist you in providing documentation should your business location be selected for audit, the Fresh Citrus Juice Task Force was formed and operated under the aegis of the Florida Department of Citrus. It published a preliminary draft entitled "Guidance Document

[1] CFSAN: Center for Food Safety and Applied Nutrition

for Fresh Citrus Juice" on January 19, 1999. Copies were widely distributed. The document included an outline of research completed along with research in progress by the Florida Department of Citrus and the Fresh Citrus Juice Task Force. A sample check list of procedures with the corresponding log reductions for each step as supported by research was included in the preliminary draft.

The Task Force is pleased to issue its second Guidance Document for the retail and roadside producer to complement the preliminary draft and to help businesses continue production of safe, wholesome fresh squeezed citrus juice.

In preparing this document, the Task Force made every attempt to keep the material focused, clear and simple. A one-page listing of guidelines for employees in charge of preparing fresh squeezed citrus juice includes the most important steps and practices that help insure sanitary operation. This page may be copied, laminated and posted by management at the point of juice production. Individual juice businesses may wish to consult with public research agencies, private service companies or regulatory officials for additional recommendations on customized applications.

A variety of treatments and procedures are presented in this document to enable fresh citrus juice processors achieve the 5-log reduction in microbial contaminants in order to avoid use of the warning label required by the FDA. They include the use of fruit cleaners, sanitizers, wax coatings and heat treatment methods.

The latest information generated by the Florida Department of Citrus, Scientific Research staff, and members of the Fresh Citrus Juice Task Force is summarized on individual information/Data Sheets or on Fact Sheets formatted for clarity, simplicity and accuracy.

This document is not the last word on fresh juice quality, safety or sanitation. Scientific knowledge and technology are ever changing. New and more effective technological developments and information will be generated to replace less effective practices. For example, Florida Department of Citrus scientists have developed more precise methods of application of chemical sanitizers and heat treatment methods that help achieve the 5-log reduction in target pathogens mandated by the FDA. Work is under way to identify suitable surrogate microorganisms for use in verification studies at different production sites.

We have also included for your information a copy of the FDA publication titled "Guidance for Industry, Warning and Notice Statement: Labeling of Juice Products, Small Entity Compliance Guide" September 18, 1998 (*FDA,1998,* copy attached).

We hope that the information provided here will enable you to produce safe, wholesome, and good quality juice. However, the ultimate responsibility to deliver safe fresh juice products is that of the producer. We urge all fresh citrus juice producers to carefully read the following important legal statement outlining critical liability issues:

"This guidance document is for informational purposes only. It is intended to assist producers in assessing the degree to which their current procedures comply with the U.S. Food and Drug Administration mandate to take steps towards achieving a 5-log reduction in the potential for exposure to microbial contamination. The findings herein are based on the testing of specific procedures. These procedures are not a substitute for best management practices or a limitation on liability. The Fresh Juice Task Force and its individual members assume no liability arising from the use of this information. To the extent that your procedures deviate from those tested, the data may or may not be directly applicable".

Finally, I would like to acknowledge the many months of hard work by scientists and members of the Fresh Citrus Juice Task Force. Support and cooperation of the Florida Department of Agriculture and Consumer Services, Division of Food Safety is greatly appreciated.

Members of the Fresh Citrus Juice Task Force are:

Dr. Mohamed A. Ismail, Scientific Research Director, FDOC, Lake Alfred, Florida
Dr. Steven Pao, Research Scientist, FDOC, Lake Alfred, Florida
Dr. G. Eldon Brown, Research Scientist III, FDOC, Lake Alfred, Florida (Retired)
Dr. Ray Mobley (DVM), Biological Administrator for the HACCP Program, Florida Department of Agriculture and Consumer Service, Tallahassee, Florida
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Mr. Richard Kinney, Executive Vice President, Florida Citrus Packers, Lakeland, Florida
Mr. Frank Kelsey, Technical Manager, FMC Corp., Lakeland, Florida
Mr. Tom Brickweg, Sales, FMC Corp., Lakeland, Florida
Mr. Joe Gleason, Legal Counsel, Florida Citrus Mutual, Lakeland, Florida

We encourage you to refer to this document regularly and make it available to all employees engaged in fresh citrus juice production.

Additional copies of this and the preliminary document may be obtained by contacting:

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Table of Contents

Guidance Document: Section I

Guidance to Fresh Citrus Juice Producers at Retail

Florida Department of Citrus
Scientific Research Department

Citrus Fruit Factsheet

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Factsheet 1, Fresh Citrus Juice

GUIDELINES FOR EMPLOYEES CHARGED WITH RESPONSIBILITY OF PREPARING FRESH SQUEEZED JUICE AT RETAIL

The following guidelines are recommended. They must be followed, monitored and logged daily. Employees should verify completion by placing their initials in the attached monthly calendar log sheet.

1. Citrus fruit used for juicing should be procured from a commercial citrus packinghouse. Research has demonstrated a 3.4-log reduction in surface microflora can be achieved in fruit processed under citrus packinghouse practices *(Pao and Brown 1998,* copy attached). Recent research using high pH sanitizers and waxes can help achieve 4.6 ± 0.4 log reduction in surface microorganisms *(Information/Data Sheet 1).*

2. Grading of damaged and unwholesome fruit can be effective in reducing microbial load. A study on the effect of grading of late season Valencia oranges showed a significant reduction in natural juice microflora when incoming fruit was adequately graded *(Table 1, Fact Sheet 2).*

3. Fruit previously handled by consumers should not be used for production of fresh squeezed juice without a washing/sanitizing step before juice extraction.

4. Citrus fruit should be maintained in a clean, secure and sanitary environment during storage at retail location. Additional precautionary measures may include refrigeration at 40°F or below to help maintain fruit quality.

5. Employees handling fruit should maintain proper personal hygiene.

6. Extractor should be kept in a sanitary enclosed area and properly cleaned and sanitized per manufacturers guidelines. Research trials with various commercial extractors have demonstrated a 1.1 to 1.9 log reduction in test organisms (*Fact sheet 6).*

7. Containers used for packaging must be food grade and maintained in clean condition.

8. Fresh citrus juice should be properly refrigerated at 40°F or below following extraction.

9. Employee(s) are to verify daily completion of these procedures by placing their initials in the appropriate day of month.

INFORMATION/DATA SHEET 1

EFFECT OF WASHING AND SANITIZING OF CITRUS FRUIT ON MICROBIAL REDUCTION

Fruit washing can provide effective means of reducing surface microflora of citrus fruit. Research has demonstrated that surface *E.coli* counts can be reduced an average of 2.4-log cycles by washing and rinsing treatments *(Pao and Brown 1998, copy attached)*. Additional research has shown that this microbial reduction can be improved by optimizing washing treatments through the application of effective cleaners and sanitizers.

Key factors in maximizing effectiveness of fruit washing and sanitizing:

1. Prior to washing and sanitizing, all fruit should be thoroughly graded to remove damaged or unwholesome fruit (Fact Sheet 2).
2. Fruit should be washed on roller brushes using an approved fruit cleaning/washing solution to remove surface contaminants. Application of sanitizer solutions may prevent microbial buildup. Washing treatments should be followed by a potable water rinse.
3. Washing treatments must be complemented with proper hygiene equipment cleaning and sanitizing procedures to maintain optimal effectiveness.

Tested washing treatments effectiveness:

Table 1: Effect of pre-wetting and washing treatments on fruit surface-inoculated *Escherichia coli.*

Washing treatment[2]	Pre-wetting treatment[1]				
	None	Water	Chlorine	Acid cleaner	Alkaline cleaner
	E. coli reduction (log cfu/cm^2)				
Water	2.1±0.1a[3]	–	–	–	–
Acid cleaner	1.9±0.2Aa	2.4±0.3ABa	2.2±0.1ABa	2.7±0.4Ba	2.1±0.2ABa
Alkaline cleaner	2.3±0.4Aa	2.7±0.3Aa	2.7±0.2Aab	2.5±0.4Aa	2.9±0.3Aab
SOPP cleaner	3.5±0.3Ab	2.9±0.3Aa	3.5±0.5Ab	2.9±0.4Aa	3.2±0.4Ab

[1] Fruit were pre-wet on rollers by dripping various solutions for 5 seconds (5 ml/second/fruit) and followed by an additional 25 seconds of rolling. Pre-wetting solutions include 200 ppm chlorine (sodium hypochlorite bleach), 200ppm acid cleaner (a mixture of phosphoric acid, dodecylbenzene sulfonic acid, and isopropyl alcohol; pH 2.0), and alkaline cleaner (a mixture of sodium and potassium hydroxide and alkali soluble surfactants; pH 10.8).

[2] After pre-wetting, fruit were washed on a roller brush bed by spraying various solutions for 5 seconds (10 ml/second/fruit), followed by an additional 25 seconds of brushing, and a potable water rinse on the rotating brush bed for 10 seconds (20 ml/second/fruit). Washing solutions include 200ppm acid cleaner (a mixture of phosphoric acid, dodecylbenzene sulfonic acid, and isopropyl alcohol; pH 2.0), alkaline cleaner (a mixture of sodium and potassium hydroxide and alkali soluble surfactants; pH 10.8) and 2% SOPP cleaner (sodium orthophenylphenate formulated into detergent; pH 11.8).

[3] Six Hamlin oranges were treated per test and triplicated tests were conducted during this study. Means (n=3; ±S.E.) in the same row followed by the same letter (A or B) or means in the same column followed by the same letter (a or b) are not different (P>0.05).

Table 2: Effect of washing and sanitizing treatments on fruit surface-inoculated *Escherichia coli*.

Washing treatment[1]	Sanitizing treatment[2]			
	None	Chlorine	Acid sanitizer I	Acid sanitizer II
	E. coli reduction (log cfu/cm^2)			
SOPP cleaner	3.7±0.5Aa[3]	3.7±0.4Aa	3.3±0.1Aa	3.3±0.3Aa
Alkaline cleaner	3.5±0.4Aa	3.1±0.2Aa	3.3±0.2Aa	2.8±0.1Aa
NaOH solution	3.2±0.1Aa	3.0±0.3Aa	2.8±0.3Aa	2.9±0.2Aa

1 Fruit were washed on roller brush bed by spraying various solutions for 5 seconds (10 ml/second/fruit), followed by an additional 25 seconds of brushing, and a potable water rinse on the rotating brush bed for 10 seconds (20 ml/second fruit). Washing solutions were formulated to pH 11.8 and these include 2% SOPP cleaner (sodium orthophenylphenate formulated into detergent), alkaline cleaner (a mixture of sodium and potassium hydroxide and alkali soluble surfactants) and NaOH solution (a mixture of sodium hydroxide and 0.05 M sodium bicarbonate).

2 After washing, fruit were sanitized on roller brush bed by spraying various sanitizers for 10 seconds (10 ml/second/fruit), followed by an additional 20 seconds of brushing, and a potable water rinse on the rotating brush bed for 10 seconds (20 ml/second/fruit). Sanitizers include 200 ppm chlorine (sodium hypochlorite bleach), 200 ppm acid sanitizer I (a mixture of phosphoric acid, dodecylbenzene sulfonic acid, and isopropyl alcohol; pH 2.0), and 80 ppm acid sanitizer II (a mixture of peroxyacetic acid and hydrogen peroxide)

3 Six fruit were treated per test and triplicated tests were conducted during this study. Means (n=3; ±S.E.) in the same row followed by the same letter (A or B) or means in the same column followed by the same letter (a or b) are not different (P>0.05).

Table 3: Effect of application volume of selected washing treatments on fruit surface-inoculated *Escherichia coli*

Washing treatment[1]	Spray volume (ml/second/fruit)[2]		
	0	5	10
	E. coli reduction (log cfu/cm^2)		
Water	1.8±0.4a[3]	-	2.1±0.1a
SOPP cleaner	2.4±0.1ab	3.3±0.3c	3.5±0.1c
NaOH solution	2.2±0.2a	3.0±0.2bc	3.4±0.4c

1 Before spraying, the brush bed was pre-wet with washing solution. Washing solutions were formulated to pH 11.8 and these include 2% SOPP cleaner (sodium orthophenyl phenate formulated into detergent) and NaOH solution (a mixture of sodium hydroxide and 0.05 M sodium bicarbonate).

2 Washing spray was controlled to provide various volume of washing solutions (0 , 5, or 10 mil/second/fruit). Fruit were washed on roller brush bed by spraying various solutions for 5 seconds, followed by an additional 25 seconds of brushing, and a potable water rinse on the rotating brush bed for 10 seconds (20 ml/second/fruit).

3 Six Valencia oranges were treated per test and triplicated tests were conducted during this study. Means (n=3; ±S.E.) followed by the same letter (a, b, or c) are not different (P>0.05).

Prepared by: Dr. Steven Pao, Florida Department of Citrus, Lake Alfred, FL and Mr. Frank Kelsey, FMC, Lakeland, FL

INFORMATION/DATA SHEET 2

WAXING AS A MEANS OF MICROBIAL REDUCTION IN THE COURSE OF PACKING FRESH CITRUS FRUIT

The waxing process is accomplished by application of FDA approved alkaline coatings in a drip or spray over brushes. Citrus fruit is conveyed across the brush bed and additional brushing ensures uniform coverage. Fruit then enters a drying chamber where airflow and heated air are utilized to dry the coating.

Key factors to obtain microbial reduction from waxing treatments

1. Fruit must be effectively graded and washed prior to wax application (*Pao et al., 1999b).* An industry survey revealed that microbial contaminants on the surface of washed citrus were reduced more than 1 log after wax application in commercial packinghouses applying alkaline wax followed by heated air drying (*Pao and Brown, 1998*).

2. Fruit waxes are effective in reducing surface microbial contaminants. Recent study has shown that a cumulative 4.6 ± 0.4 log reduction was obtained by fruit washing with SOPP (pH 11.8) followed by waxing and air drying with alkaline wax (*Table 1*).

Table 1: Cumulative effect of washing and waxing on fruit surface-inoculated *Escherichia coli.*

Washing treatment[1]	Waxing treatment[2]			
	None	No-wax control[3]	Wax I	Wax II
	E. coli reduction (log cfu/cm^2)			
Water	2.3±0.1a[4]	1.7±0.2a	2.1±0.4a	2.9±0.1b
SOPP cleaner	3.8±0.2c	3.8±0.2c	3.4±0.0bc	4.6±0.4d

1 Fruit were washed on roller brush bed by spraying various solutions for 5 seconds (10 ml/second/fruit), followed by an additional 25 seconds of brushing, and a potable water rinse on the rotating brush bed for 10 seconds (20 ml/second/fruit). The SOPP cleaner (2% sodium orthophenylphenate formulated into detergent) was formulated at pH 11.8.

2 Waxes were applied to fruit on the roller brush bed by dripping various wax solutions for 20 seconds (0.6 ml/second/fruit). After coating, fruit were air dried with heated air at about 54 ± 1 °C (130 °F) for 2 min. Wax I and II were laboratory-prepared shellac-based wax solution formulated at pH 8.2 and pH 11.0, respectively.

3 Washed fruit (without coating) were air dried to serve as the no-wax control.

4 Six Valencia oranges were treated per test and triplicated tests were conducted during this study. Means (n=3; ±S.E.) followed by the same letter (a, b, c, or d) are not different

3. Washing and waxing remove most surface microbial contaminants. In a recent survey of seven commercial Florida citrus packinghouses, no Salmonella or E.coli were found on fruit surface following conventional washing, sanitizing, and waxing treatments (*Pao and Brown, 1998*).

4. To maintain coating application benefits, the plant should have effective hygiene and sanitation programs in place.

Prepared by: Dr. Steven Pao, Florida Department of Citrus, Lake Alfred, FL and Mr. Frank Kelsey, FMC, Lakeland, FL

Florida Department of Citrus
Scientific Research Department

Citrus Fruit Factsheet

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Factsheet 3, Fresh Citrus Juice

GUIDELINES FOR STORAGE AND TRANSPORTATION OF CITRUS FRUIT TO CONTROL MICROBIAL GROWTH

Foodborne pathogens may be introduced or allowed to grow and multiply in food items during storage or while in transit. Fruit should be protected from cross-contamination and from potential contamination by insects, chemicals, rodents, waste products, toxic material, unclean equipment, unnecessary handling, or other agents of public health significance at all times. The following guidelines for storage and transporting of fruit intended for juice products should be observed:

1. Maintain storage area in clean and sanitary manner.
2. Insure that storage areas are free of insects and rodents and constructed to prevent the entrance and harborage of insects and rodents.
3. Insure that trucks and transport cartons are clean, and free from odors, obvious dirt or debris before loading.
4. Transport washed and sanitized fruit in an enclosed vehicle.
5. Set air temperature of refrigerated conveyance at 40° F.
6. Fruit that has been sanitized, but will have a considerable delay prior to transport, may receive additional protection from microbial growth by refrigeration at 40° F.
7. Load product in a way that will minimize damage and allow for air circulation.
8. Evaluate whether prior shipments in the same trailer should preclude it from being used to transport fruit.
9. Transport animal origin products and fruit in separate conveyances.
10. All employees, including workers involved in loading and unloading of food products before and after transport, should adhere to strict personal hygiene practices.

Prepared by Dr. Ray Mobley, Florida Department of Agricultural and Consumer Services, Tallahassee, FL.

Florida Department of Citrus
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Citrus Fruit Factsheet

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Factsheet 4, Fresh Citrus Juice

CITRUS JUICE EXTRACTION TECHNOLOGY AND EXTRACTOR SANITATION

Fresh citrus juice is commonly extracted using mechanical extractors. Extraction machines are designed to separate the juice from the fruit peel, seeds, and large pieces of pulp. Small-scale extractors squeeze one fruit at a time and are often favored by retailers. They are portable, simple to clean and specially designed to attract the attention of shoppers in store or roadside operations. At juice processing plants, however, large-scale extractors are used. These machines have the capacity to extract hundreds of fruit per minute. Many of these extractors are adjustable with different kinds of cups, cutters and strainer tubes to accommodate fruit of various sizes and allow desired amounts of oil and pulp to pass into the juice. Good mechanical extraction of oranges yields roughly 45 % - 55 % juice by weight *(Carter, 1989)* and recent study indicates that the extraction process may be a significant step that affects microbial quality of fresh squeezed juice.

Key factors for juice extraction

1. Equipment must be clean and in a sanitary condition prior to juice extraction *(Schmidt et al., 1997)*. Processors must strictly adhere to manufacturer recommendations for cleaning and sanitizing extraction equipment. An individual trained in cleaning and operating the extractor should perform these duties regularly as provided in manufacturer's recommendations. The extraction process must take place in a clean and enclosed sanitary area where animals and animal products are not allowed.

2. Commercial fresh citrus juice extraction can be a contributing factor to the reduction of overall microbial contamination. Juice extraction machines reduce surface microbial test organisms by a 1.1 to 1.9 log-cycles *(Pao and Davis 1999b)*. Only a small portion of the surface microbial contaminants, either natural background microflora or inoculated test organisms, were found in the fresh juice extracted using the tested juice extractors. These results confirm recent acknowledgment by the FDA that the physical characteristics of citrus fruit may help facilitate safe and sanitary juice extraction *(FDA, 1998a)* and affirms that juice extraction can be a significant factor that contributes to accumulated microbial reduction in fresh citrus juice production.

3. Juice must be placed in new, clean containers. The containers must be stored in a clean, sanitary area where pests are excluded. Containers that have been used previously to hold juice or any other items must not be re-used to package fresh citrus juice. If juice is not transferred directly from extractor to the containers offered for sale, it is recommended that the juice be chilled or placed in a chiller-dispenser at 40° F or below.

Prepared by: Dr. Steven Pao, Florida Department of Citrus, Lake Alfred, FL and Mr. Frank Kelsey, FMC, Lakeland, FL

Guidance Document: Section II

Additional Guidance For Small Scale and Roadside Fresh Citrus Juice Producers

The following information is intended to assist small scale juice producers who may acquire fruit from various sources, including directly from the grove. It may pertain to gift fruit shippers, roadside or grove-side stands and other local independent processors. The treatments that follow supplement the guidance provided in Section I of this document.

Florida Department of Citrus
Scientific Research Department

Citrus Fruit Factsheet

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Factsheet 5, Fresh Citrus Juice

GUIDELINES FOR EMPLOYEES RESPONSIBLE FOR PREPARING FRESH SQUEEZED JUICE AT SMALL SCALE BUSINESSES

Good manufacturing practices are the foundation of any sanitation program and should be included in all training and supervision activities. The following procedures or guidelines should be followed daily, monitored and logged. Employees should verify completion by placing their initials in the appropriate calendar/day.

1. Only wholesome fresh fruit should be used for juice extraction. At minimum, fruit should be cleaned and processed in a manner consistent with commercial packinghouse processes. This cleaning process has either taken place on these premises, or at a commercial packinghouse from which the fruit was procured. Recent research using high pH sanitizers and waxes can help achieve 4.6 ± 0.4 log reduction in surface microbial contamination (Information/Data *Sheet 1 & 2*). Grading can also provide significant reduction in natural juice in microflora (*Table 1, Fact Sheet 2*).

2. Fruit previously handled by consumers should not be used for production of fresh squeezed juice without a washing/sanitizing step before juice extraction.

3. Cleaned and sanitized fruit should be maintained in a clean, secure, sanitary and enclosed environment until extraction. If a sanitary and enclosed area is not available, or if fruit is sourced directly from the field, the fruit should be treated according to one of the following methods:

   a. Repeat step one (washing on a brush bed with commercial equipment and cleaner/sanitizer immediately prior to extraction. *(Information/Data Sheets 1 & 2).*
   b. Immersion or spray (30 second contact time) with an alkaline fruit cleaner, pH 11.8 *(Information/Data Sheet 1).*
   c. Hot water immersion or spray treatment. *(Fact Sheet 6).*

4. Employees handling fruit should maintain proper personal hygiene.

5. Extractor has been properly cleaned and sanitized per manufacturers guidelines and operates in a sanitary enclosed work area. Research trials with various commercial extractors have demonstrated a 1.1 to 1.9 log reduction in test organisms.

6. Containers used for packaging must be food grade and maintained in clean condition.

7. Fresh citrus juice has been properly refrigerated at 40 F or below following extraction.

8. Employee(s) should verify daily completion of these procedures by placing their initials in the attached monthly calendar log sheet.

DAILY VERIFICATION LOG FOR FRESH CITRUS JUICE EMPLOYEES/PROCESSORS

Month _____ Year _____

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

Location: _____

Employee Name: _____

Supervisor: _____

Florida Department of Citrus
Scientific Research Department

Citrus Fruit Factsheet

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Factsheet 6, Fresh Citrus Juice

HOT WATER AND STEAM APPLICATION AS A MEANS FOR SURFACE MICROBIAL REDUCTION OF CITRUS FRUIT

Undesirable microorganisms can be effectively destroyed by thermal treatment. Since pasteurization and sanitizing treatments eliminate human pathogens and minimize spoilage organisms, they often improve product microbial safety and stability. Although thermal treatment of fresh citrus juice will render the product pasteurized, heat may be applied to sanitize fruit surfaces before juice extraction. Rapid thermal treatment of citrus fruit can reduce both fruit surface and initial juice microbial load without altering original sensory quality of fresh juice *(Pao and Davis, 1999a).*

Strategies to reduce surface carried microbes utilizing thermal processes

1. *E.coli* can be effectively reduced by hot water immersion treatments. A 5-log reduction of *E. coli* was attained by immersing inoculated fruit in hot water at 176°F for ≥ 1 minute or 158°F for ≥ 2 minutes. In general, the higher the temperature applied to the fruit, the shorter the treatment duration required to achieve a desired surface sanitizing effect.

2. Thermal treatment can also be applied as hot water spray or steam application. Initial study shows that *E. coli* was effectively reduced by hot water spray treatments. A 5-log reduction was achieved by spraying inoculated citrus fruit with hot water at 190° to 200°F for 30 to 60 seconds.

3. Thermal sanitizing treatments may be integrated into existing good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) programs to protect integrity of fresh citrus products. Applications of thermal treatment should be adapted to individual processing plants and 5-log reduction should be validated through a qualified microbiology laboratory to confirm results. Thermal treatments described above should only be applied to fruit that will be used in the production of fresh juice. These treatments are not suited for fruit destined for the fresh fruit market (such as packinghouse sourced fruit) because these treatments can cause a cosmetic degradation of the peel during transport and storage.

Prepared by Dr. Steven Pao, Florida Department of Citrus, Lake Alfred, FL and Mr. Frank Kelsey, FMC, Lakeland, FL

List of References

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Guidance for Industry

Warning and Notice Statement: Labeling of Juice Products Small Entity Compliance Guide

Additional copies are available from:

Office of Food Labeling (HFS-150)
Food and Drug Administration
200 C Street SW
Washington, DC 20204

(Tel) 202-205-5099
(Internet) http://www.cfsan.fda.gov/~dms/guidance.html

U.S. Department of Health and Human Services
Food and Drug Administration (FDA)
Center for Food Safety and Applied Nutrition (CFSAN)
September 18, 1998

WARNING AND NOTICE STATEMENT: LABELING OF JUICE PRODUCTS SMALL ENTITY COMPLIANCE GUIDE[1]

SUMMARY

There recently have been outbreaks of foodborne illness associated with the consumption of some juice products contaminated with harmful bacteria. Beginning September 8, 1998, for apple juice and apple cider and November 5, 1998, for all other juice products, FDA is requiring labeling with a warning statement those fruit and vegetable juice products (i.e., juices and beverages containing juice) that have not been pasteurized (i.e., heat treated) or treated in another way capable of preventing, reducing, or eliminating harmful bacteria by 100,000 fold. This reduction in bacteria is referred to as "a 5-log reduction."

Products required to bear the statement must be labeled with the following statement:

> **WARNING**: This product has not been pasteurized and, therefore, may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems.

Manufacturers can apply the warning statement directly on the product or, for a limited time, on signs and placards. Apple juice and apple cider manufacturers may provide the required warning statement on signs or placards in letters at least 1/4 inch in height, rather than on the labels of their products, until September 8, 1999. Manufacturers of all other juice products may provide the warning statement on signs and placards until November 5, 1999. After these dates, the warning statement must appear on the label, i.e., on the container of the products.

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[1] The Food and Drug Administration (FDA) has prepared this guide in accordance with section 212 of the Small Business Regulatory Fairness Act (P.L. 104-121). This guidance document restates in plain language the legal requirements set forth in the current regulation for the labeling of juice products that have not been processed to prevent, reduce, or eliminate harmful bacteria. Any statement in this guidance document that goes beyond merely restating the applicable legal requirements represents the agency's current thinking on this subject. The regulation is binding and has the force and effect of law; however, this guidance document does not, itself, create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. Additional copies of this guidance document are available from the Office of Food Labeling, HFS-150, 200 C Street, SW, Washington, DC 20204, (Tel) 202-205-5099, (Internet) http://www.fda.gov/~dms/guidance.html

QUESTIONS AND ANSWERS

COVERAGE

1. Question: What is the definition of "juice" for the purposes of the warning statement regulation?

   Answer: For purposes of this regulation, juice means the aqueous liquid expressed or extracted from one or more fruits or vegetables (e.g., apple juice, apple cider, orange juice, and carrot juice); a puree of the edible portions of the fruit or vegetable that is used as a beverage (e.g., banana puree or peach puree); or any concentrate of such liquids or purees (e.g., grape juice concentrate or grapefruit juice concentrate).

2. Question: What products are required to bear the warning statement?

   Answer: Any juice or beverage containing juice (i.e., any "juice product") that has not been processed in a manner capable of achieving at least a 5-log reduction in the pertinent microorganism (i.e., any "untreated juice product") for the shelf life of the product when stored under normal and moderate abuse conditions must bear the warning statement.

3. Question: If a juice product is not 100 percent juice but contains a mixture of juice and other ingredients, is the finished product required to have the warning statement?

   Answer: Juice that has not been treated must bear the warning statement. If the finished beverage is treated, the individual juice ingredients would not have to be treated. Similarly, if each individual juice ingredient has been treated the finished beverage need not be treated.

4. Question: Must untreated juice products that are to be used as ingredients bear the warning statement?

   Answer: If the juice ingredient is not for distribution to retail consumers and is used solely in the manufacture of other foods, or is to be labeled, or repackaged before sale to retail consumers, it does not have to bear the warning statement *provided* that the lack of processing to achieve the 5-log reduction is disclosed in documents (e.g. invoices, bills of lading) that accompany the ingredient.

5. Question: Are untreated juice products that are sold in retail establishments required to bear the warning statement?

Answer: If untreated juice products are sold in package form, they are required to bear the warning statement. However, untreated juice products sold in retail establishments, i.e., restaurants, delis, some grocery stores, and roadside stands, that are intended for immediate consumption and are not pre-packaged do not require warning statements.

6. Question: What juice products are not required to bear a warning statement?

Answer: Packaged juice products that have been processed in a manner to achieve, at a minimum, a 5-log reduction in the pertinent microorganism. Heat pasteurization is one process that will achieve a 5-log reduction.

7. Question: If a juice processor has strong GMP's and a strong HACCP system in place, does he have to place a warning statement on his juice products?

Answer: The warning label regulation specifies that the juice product must be processed in a manner to achieve a 5-log reduction. Therefore, only if the system in place achieves a 5-log reduction are the juice products exempted from the warning statement requirement.

8. Question: Are products other than beverages that contain juice required to bear the warning statement? For example, is a sherbet containing a fruit puree that has not been processed to achieve the 5-log reduction required to bear the statement?

Answer: No. The regulation applies only to juices and beverages containing juice. A fruit puree is included in the definition of juice because it may be used in beverages. However, if sherbet contains the puree, even if the puree is not processed to achieve a 5-log reduction, the sherbet is not required to bear the warning statement because it is not a beverage or a juice.

9. Question: Are citrus oils required to bear the warning statement?

Answer: No. Citrus oils do not fit the definition of juice because they are not aqueous liquids.

THE 5-LOG REDUCTION

10. Question: What is a 5-log reduction?

Answer: A 5-log reduction means a reduction in the number of microorganisms by 100,000-fold. For example, if a juice product contained 100,000 pertinent microorganisms, a 5-log reduction would reduce the number of pertinent microorganisms to 1.

11. Question: How does a juice manufacturer achieve a 5-log reduction without pasteurizing the product?

Answer: A manufacturer can achieve a 5-log reduction by using control measures that have been shown to be effective in reducing the number of microorganisms. A processor can use one control measure that has been shown to reduce the pertinent microorganism by at least 100,000-fold (e.g., pasteurization), or a combination of control measures that have a cumulative effect of a 100,000-fold reduction.

12. Question: What steps in the processing of juice may a manufacturer consider in determining control measures to achieve a 5-log reduction?

Answer: The control measures used to achieve a 5-log reduction may include any measure at the farming, harvesting, or processing phases over which the processor has control and which are effective in reducing the number of pertinent microorganisms.

13. Question: How can a manufacturer determine whether a process achieves a 5-log reduction?

Answer: A processor can conduct its own studies to validate the effectiveness of its process or rely upon scientific studies conducted by others (e.g., researchers, states, etc.). Validation studies may include (1) tests of the control measure with a known level of the pertinent microorganism in a controlled experimental setting which is similar to a production setting, or (2) tests with a surrogate microorganism in an experimental or process setting. Manufacturers of equipment or sanitizers that can be used to control harmful microorganisms may test the control measure they are recommending and supply the validation information to the processor.

14. Question: If I have information from validation studies done by others (e.g., researchers, states, etc.), do I have to do anything else to show that my process is validated?

Answer: Yes. A processor must show that the validated control measure is being used in the same manner as it was used in the validation study. For example, any machinery should be used in the same manner or any sanitizer at the same concentration as used in the validation study.

15. Question: What does "pertinent microorganism" mean?

Answer: The pertinent microorganism is the most resistant (i.e., most resistant to being killed by the specific treatment under consideration) foodborne pathogenic (i.e., illness-causing) microorganism that is reasonably likely to occur in a particular juice. Pathogenic microorganisms can be introduced into juice both within and outside the processing plant

environment, including before, during, and after harvesting. A pathogenic microorganism that is likely to occur in a juice is one that, based on the evidence provided by experience, illness data, scientific reports, and other information, has a reasonable possibility of occurring in the particular juice if appropriate controls to protect against its occurrence are not put in place.

16. Question: What does "surrogate microorganism" mean?

Answer: A surrogate microorganism is any non-pathogenic microorganism that has acid-tolerance, heat resistance, or other relevant characteristics similar to pertinent microorganisms. Food-grade lactic acid bacteria that have GRAS (generally recognized as safe) status are a possible option if their characteristics are similar to the pertinent microorganisms.

17. Question: What are some examples of pertinent microorganisms?

Answer: For many juice manufacturers, the most pertinent microorganism will be *E. coli* O157:H7 or *Listeria monocytogenes*. *E. coli* O157:H7 is known to be unusually acid resistant and *L. monocytogenes* is relatively heat resistant. Other microorganisms may be pertinent if they are known to be reasonably likely to occur in a particular juice product or process.

WARNING STATEMENT

18. Question: What is the required warning statement for packaged juice products that have not been pasteurized or otherwise processed to prevent, reduce, or eliminate pathogenic microorganisms that may be present?

Answer: **WARNING:** This product has not been pasteurized and, therefore, may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems.

19. Question: Where must the warning statement be placed on the label?

Answer: The statement must appear either on the information panel (the label panel immediately to the right of the principal display panel) or on the principal display panel (that part of the label most likely to be seen by the consumer at the time of purchase, generally the front of the package).

20. Question: How should the warning statement appear on a label?

Answer: The statement must appear on the label prominently, conspicuously, and must

appear in a minimum type size of one-sixteenth inch. The statement must appear in a box set off by hairlines. The word "warning" must appear in bold capital letters. For example:

WARNING: This product has not been pasteurized and, therefore, may contain harmful bacteria that may cause serious illness in children, the elderly, and persons with weakened immune systems.

21. Question: Can manufacturers use signs or placards instead of changing their labels?

Answer: Yes temporarily. Manufacturers may provide the warning statement on signs or placards, until September 8, 1999, for apple juice and apple cider and until November 5, 1999, for all other juices.

22. Question: How should the warning statement appear on signs or placards?

Answer: The statement should appear prominently and conspicuously in letters that are legible in a minimum type size of one-fourth inch.

23. Question: Where must signs or placard be placed?

Answer: The sign or placard must be placed at the point of purchase of the juice product. Point of purchase means at the place where the product is displayed, e.g, on the outside of the refrigerated case or on a shelf inside the case.

24. Question: Must the warning statement on signs be printed in professionally set type?

Answer: The regulation does not address how the sign must be printed. Therefore, the sign can be done by any means, including written by hand, as long as the statement is legible and the letters are at least one-fourth inch in height.

COMPLIANCE

25. Question: When must warning statements appear on covered products?

Answer: The warning statement must appear either on signs or placards or on the labels of covered products by September 8, 1999 for apple juice and apple cider, and by November 5, 1999, for other juice products. After these dates, the warning statement must appear directly on the packages.

26. Question: Is it the manufacturer or retailer who is responsible for providing the signs or placards?

Answer: Both share responsibility. The firm identified as the manufacturer or distributor of the product is responsible for producing the label. A firm may decide to provide signs instead of changing their labels to add the warning statement. If a firm decides not to use the label but to provide a sign, the retailer must display the sign with the product because failing to do so would constitute misbranding of the product, which is a violation of the Federal Food, Drug, and Cosmetic Act.

27. Question: How will FDA determine whether juice that is sold after the effective dates of the rule is properly labeled?

Answer: FDA may conduct inspections at juice firms that do not provide warning labels or signs and that do not pasteurize. FDA would identify the control measures that are used to reduce pathogens and review any scientific data that the firms provide to show that their process provides a 5-log reduction and, therefore, does not require the warning statement.

MISCELLANEOUS

28. Question: Can a juice product that has been heat treated to pasteurize the product be labeled "fresh?"

Answer: No. The term "fresh" implies that a food is raw and unprocessed. Juice products that have been pasteurized are processed and, therefore, can not be labeled "fresh."

29. Question: If juice products, themselves, have been treated to achieve the 5-log reduction in ways other than heat pasteurization (e.g., high pressure treatment, sodium benzoate etc.), can they be labeled "fresh?"

Answer: No. Juice products that have been preserved or otherwise processed are not unprocessed and, therefore, cannot be labeled "fresh."

30. Question: FDA encouraged voluntary warning label statement in a *Federal Register* notice in 1997. Must a manufacturer who uses a warning statement on a juice product that has different wording than the statement in the regulation have to change the labels?

Answer: A manufacturer may continue to label their products using the advice provided in FDA's August 28, 1997 notice until the label inventory is depleted. Any applicable labels printed after July 7, 1998 must use the exact warning statement as noted above in

#20.

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Research Note

Reduction of Microorganisms on Citrus Fruit Surfaces during Packinghouse Processing

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ABSTRACT

Citrus fruit surface microbial populations were evaluated following various packingline processes of seven Florida commercial packinghouses. At each packinghouse, six fruits (oranges or tangerines) were collected at each of four sampling points. The sampling was conducted in duplicate; thus, 336 fruit were evaluated during this survey. Average aerobic plate counts and yeast and mold counts on fruit surfaces before washing were about 4.0 log CFU/cm^2 and 3.3 log CFU/cm^2, respectively, and were reduced to 2.1 log CFU/cm^2 and 1.3 log CFU/cm^2, respectively, by packinghouse processing. Waxing alone reduced the average fruit surface aerobic plate counts and coliform counts from 3.7 log CFU/cm^2 and 35.2 most probable number (MPN)/cm^2, respectively, to 2.6 log CFU/cm^2 and 1.4 MPN/cm^2. No *Escherichia coli* was recovered from fruit at the end of packinghouse processing, and no salmonellae were found on fruit during the entire processing. In an inoculation study to test the effect of packinghouse processes, test organism *E. coli* was applied to fruit to achieve a high level (4.8 log CFU/cm^2) of contamination. The average *E. coli* count was reduced about 2.4 log cycles by washing and rinsing with potable water (40 psi, 25°C) for about 30 s. The combination of washing and waxing significantly reduced the inoculated level of *E. coli* from 4.8 to 1.4 log CFU/cm^2.

Fruits and vegetables are frequently in contact with soil, insects, and animals during growing and harvesting in the field. Consequently, their surfaces are not free from natural contaminants. In general, fresh produce retain populations of 10^4 to 10^6 microorganisms/g when they arrive at the packinghouse *(5)*. Coliform bacteria, including fecal coliforms, are frequently associated with fresh produce. For example, *Enterobacter* and *Klebsiella* spp. have been isolated from peas *(20)*, collards *(18)*, celery (17), and tomato *(6)*. *Escherichia coli* also has been found on both fruits *(1)* and vegetables *(18)*. These bacteria are common in soil, and their presence on raw produce do not usually represent a public health concern *(18)*. Previous researchers have studied fruit surface microbial levels to evaluate the effectiveness of various packinghouse operations *(19, 21)*.

Chun and McDonald *(8)* reported that natural microbial populations on the surfaces of mature grapefruit harvested monthly throughout two fruit seasons (from September to April 1985 to 1986 and 1986 to 1987) remained relatively stable. Temperature, total precipitation, and day length did not appear to influence the populations. Wolford *(22)* found coliforms on the surface of oranges sampled from groves, packinghouses, and juice extraction plants and concluded that they were natural contaminants of frozen concentrated orange juice. Murdock et al. *(13)* observed reduction of fruit surface microbial levels by washing and brushing at a commercial citrus juice extraction plant.

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The addition of chlorine to wash water has also been considered to be useful in preventing microbial contamination in produce processing lines *(23)*. Brown and Schubert *(7)* reported that the use of chlorine and sodium orthophenylphenate (SOPP) as surface disinfectants during washing were effective against the survival of inoculated *Xanthonomas campestris* pv. *vesicatoria* on citrus. Stapleton *(21)* studied the effects of chlorine and wax treatments on microflora of lime fruit. The author concluded that chlorine is a relatively effective biocide, and the protective wax used in this study did not increase the efficacy of chlorine treatment. The disinfection effect of chlorine treatment to surface coliforms and total microorganisms has been questioned. Albrecht et al. *(2)* reported that washing broccoli in 50 ppm chlorine for 2 min only reduced the coliform levels by approximately 1 log. Similarly, Senter et al. *(19)* found that chlorine (average 226 ppm) had little effect on the surface microflora of tomato. Brackett *(6)* concluded that chlorine compounds inactivate microbes in solutions or on equipment but only have minor effects on microorganisms on fruits and vegetables.

Fruits and their products when not handled properly can serve as vehicles for microorganisms that cause product spoilage and human disease *(6)*. Thus, the elimination of fruit surface microflora is important for the improvement of both food quality and safety. The influence of packinghouse processes on surface microflora of citrus fruit is not well documented. Updated information is needed because new treatments, such as improved detergent and wax formulations, have been introduced to packinghouses *(9, 11)*. The

objectives of this study were to determine the effects of current packinghouse operations on the surface microflora of citrus in seven commercial facilities in Florida and the effectiveness of these processes on the reduction of surface-inoculated *E. coli.*

MATERIALS AND METHODS

Packinghouse survey. Seven commercial packinghouses located at central interior and Indian River regions of Florida were investigated. At each packinghouse, six fruits (oranges or tangerines) were sampled by hand (using sterile gloves and bags) at each of four sampling points in the packingline process: after dumping, washing (following brushing, rinsing, and water elimination), waxing (following wax application and drying), and final hand packing of the waxed fruit. The sampling was conducted in duplicate at each packinghouse. Thus, 336 fruits in total were collected during this survey. The samples were chilled at 4°C before tests. One liter of 0.1% peptone (Difco, Detroit, Mich.) was added to each of the sample bags (six fruits/bag) about 24 h after sampling. Each sample bag was then placed into another plastic bag and shaken on a reciprocal shaker (120 oscillations/min; 0 to 4°C) for 2 h. The wash solutions were evaluated immediately for microbial levels.

Appropriate dilutions (in 0.1% peptone) of each wash solution were surface plated on plate count agar (PCA), orange serum agar (OSA), and acidified potato dextrose agar (APDA) (Difco). Plates were incubated for 48 h at 35 (PCA) or 30°C (OSA) or for 5 days at 25°C (APDA). After incubation, the number of colonies on PCA, OSA, and APDA represented the aerobic plate counts, aciduric microorganism counts, and yeast and mold counts *(3).* The levels of total coliforms and fecal coliforms were determined by three-tube most probable number (MPN) evaluation *(10).* The term fecal coliforms was defined as gram-negative facultative rods that ferment lactose at 44.5°C *(4).* Gram stain, oxidase and catalase reactions, and 20 biochemical tests (API20E, BioMerieux, Hazelwood, Mo.) were performed to identify fecal coliforms to species and to confirm the presence of *E. coli.* Enzyme-linked immunosorbent assay (Salmonella VIA, Tecra, Roseville, Australia) was used to detect the presence of *Salmonella.* AOAC approved methods *(10)* for sample pre-enrichment in buffered peptone water (BioPro, Redmond, Wash.) and selective enrichments in tetrathionate and selenite cystine broths (BioPro) were followed before each assay.

Inoculation study. *E. coli* ATCC 25922 (Difco) culture was maintain on tryptic soy agar (Difco) slant at 5°C and activated after three consecutive daily transfers in tryptic soy broth (Difco) at 35°C. About 58 liters of sterile cow manure slurry was prepared by blending 2.4 kg of autoclaved cow manure with water. Two liters of 1-day-old cell broth was mixed in the slurry (58 liters) to obtain an inoculum level of approximately 7.5 log CFU/ml. Surface *E. coli* inoculation that simulates a severe fecal contamination was accomplished by immersing fruit in the slurry for 5 min and draining the excess inoculum for about 1 h at ambient temperature.

Mature field-run Valencia oranges (*Citrus sinensis* L.) were purchased from a local packinghouse. Sound fruits were placed in plastic crates (100 fruits/crate) and held at ambient temperature overnight. Treatments consisted of (A) fruit inoculated with *E. coli;* (B) inoculated fruit washed with detergent on roller brushes with potable water rinse; (C) inoculated fruit washed on roller brushes with 2% of the fungicide SOPP (FMC, Lakeland, Fla.), and rinsed with water; (D) inoculated fruit washed on roller brushes with SOPP and rinsed with chlorine solution (200 ppm chlorine prepared from sodium hypochlorite bleach; James Austin Company, Mars, Penn.); and (E) inoculated fruit washed on roller brushes with SOPP, rinsed with chlorine solution (200 ppm), and coated with shellac-based water wax (590HS, FMC). Washing, brushing, and rinsing methods were performed according to Brown and Schubert *(7).* Wax was applied to fruit on six rotating (120 rpm) brushes and dried with heated air at about 52°C for 2 min.

Ten fruits were sampled by hand, using sterile latex gloves and placed in sterile plastic bags, before and after each treatment in the packingline process. The samples were held at 4°C for about 4 h before fruit surface microflora tests. Fruit wash solutions were prepared by shaking fruit in peptone water on a laboratory shaker as described above for the packinghouse survey.

Appropriate dilutions of each wash solution were pour plated with tryptic soy agar and incubated at 25°C for 2 h *(10).* The plates were then covered by an additional layer of violet red bile agar (Difco) and incubated for about 20 h at 35°C. After incubation, purple-red colonies that were ≥0.5 mm in diameter and surrounded by a zone of precipitated bile acids were counted *(10).* Coliform and *E. coli* confirmation tests were also performed on representative colonies.

Statistical analysis. The Duncan's multiple range test (Plotlt, Scientific Programming Enterprises, Haslett, Mich.) was used to determine statistical differences ($P < 0.05$) among fruit samples and between treatments.

RESULTS AND DISCUSSION

Packinghouse survey. The average surface microbial levels of unwashed fruit sampled after dumping was about 4.0 log CFU/cm² (Fig. 1A). Previous studies yielded similar microbial counts of unwashed oranges *(12, 14)* and grapefruits *(8).* The aerobic plate counts of the fruit surface were reduced ($P < 0.01$) by packinghouse procedures to 2.1 log CFU/cm² (Fig. 1A). Similarly, the processes reduced ($P < 0.01$) aciduric microorganisms from 3.9 log CFU/cm² to 2.0 log CFU/cm² and yeast and mold counts from 3.3 log CFU/cm² to 1.3 log CFU/cm². Washing reduced ($P < 0.01$) aciduric organism counts and yeast and mold counts about

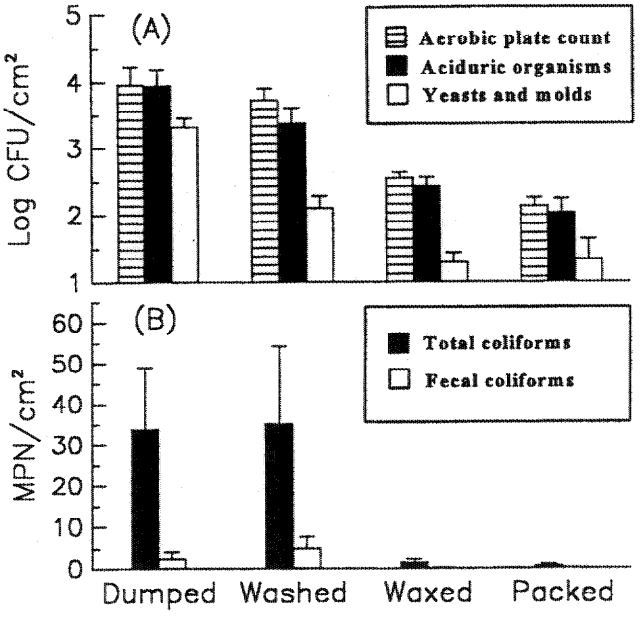

FIGURE 1. *Fruit surface (A) total microflora and (B) coliforms on citrus sampled at four stages (after dumping, washing, waxing, and packing) of a packinghouse processing line. Symbols represent the means (±SE) of seven duplicated evaluations.*

TABLE 1. *Chemical products used in fruit processing at commercial citrus packinghouses*

Packinghouse	Fruit variety	Prewashing treatment	Washing solution	Wax base	Fungicide(s) in wax[a]
A	Pineapple orange	Imazalil drenching[b]	SOPP[d]	Shellac	TBZ
B	Pineapple orange	Chlorine rinsing[c]	Detergent	Shellac	TBZ
C	Honey tangerine	None	SOPP	Shellac	TBZ
D	Valencia orange	None	SOPP	Shellac	Imazalil
E	Valencia orange	None	SOPP	Polyethylene	None
F	Valencia orange	None	SOPP	Shellac	TBZ/Imazalil
G	Valencia orange	None	Detergent	Shellac	TBZ

[a] Thiabendazole (TBZ) and imazalil were formulated into wax at 200 ppm.
[b] Imazalil concentration at 250 ppm.
[c] Chlorine concentration at 200 ppm.
[d] Sodium orthophenylphenate (2%) formulated into detergent.

0.6 and 1.2 log unit, respectively. However, washing did not significantly reduce aerobic plate counts ($P > 0.05$). Some bacteria may rapidly multiply in the fruit-washing system, particularly in soiled washer brushes, and reduce the effectiveness of washing. Waxing alone reduced ($P < 0.01$) aerobic organisms, aciduric organisms, and yeast and mold counts. This reduction in counts by waxing was generally observed in all of the packinghouses sampled (data not shown) regardless of whether fungicides were incorporated in the wax (Table 1). Final hand packing alone did not change ($P > 0.05$) any of the surface counts. Although different chemical treatments were applied before washing and during waxing (Table 1), the deviation of microbial recovery among packinghouses was minimal (Fig. 1).

Because coliforms and fecal coliforms are ubiquitous in agricultural environments, the presence of these organisms on citrus fruit surfaces was expected. The packinghouse washing process did not reduce ($P > 0.05$) populations of coliforms or fecal coliforms (Fig. 1B). However, waxing was effective in reducing coliforms. The average surface total coliforms was reduced ($P < 0.05$) from 35.2 MPN/cm^2 to 1.4 MPN/cm^2 by waxing. Fecal coliforms were found on the fruit sampled from four of seven packinghouses. Before waxing, the average fecal coliform level was about 5.0 MPN/cm^2. Fruit sampled after waxing had an average of 0.1 MPN/cm^2 fecal coliforms. Isolated fecal coliforms were identified as *Klebsiella pneumoniae, Enterobacter* spp., *E. coli,* and *Citrobacter freundii.* Because *Klebsiella* and *Enterobacter* spp. are commonly associated with agricultural environments, the presence of these fecal coliforms might not be reliable indicators of fecal contamination.

At dumping points, unwashed fruit samples of two packinghouses had detectable levels of *E. coli.* After washing and waxing, *E. coli* was recovered from fruit sampled at one of these two packinghouses. However, no *E. coli* were recovered from fruit collected at any packinghouse after packing. *C. freundii* was recovered only once in this study from washed fruit collected from one packinghouse before waxing. In addition, no salmonellae were found on any fruit samples of this study. These results suggest that current packinghouse procedures will reduce total coliforms and eliminate low levels of *E. coli* or fecal contamination on the fruit surface.

Inoculation study. The level of total coliforms on uninoculated fruit was <1 log CFU/cm^2. Cow manure slurry containing the test organism *E. coli* applied to the fruit surface left a high deposit of *E. coli* (4.8 log CFU/cm^2) contamination (Fig. 2). The average *E. coli* count was reduced ($P < 0.01$) 2.4 log cycles by washing and rinsing the fruit with potable water. Adding SOPP in the washing process and chlorine (200 ppm) in the rinsing solution did not further reduce *E. coli* populations ($P > 0.05$). The combination of washing and waxing reduced ($P < 0.01$) the level of inoculated *E. coli* from 4.8 to 1.4 log CFU/cm^2. Washing of inoculated fruit appeared to have greater impact on the reduction of coliform bacteria (Fig. 2) than that observed during the packinghouse survey (Fig. 1B). Perhaps some microbial contaminants could accumulate in commercial processing lines where much greater volumes of fruit are handled. Waxing reduced ($P < 0.05$) surface populations

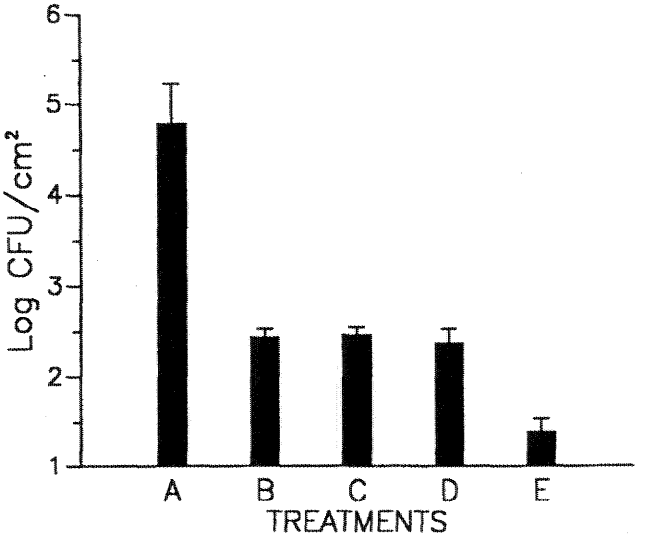

FIGURE 2. *Effect of packinghouse treatments on fruit surface-inoculated* Escherichia coli. *Packinghouse treatments were (A) fruit inoculated with* E. coli; *(B) inoculated fruit washed and rinsed with water; (C) inoculated fruit washed with SOPP and rinsed with water; (D) inoculated fruit washed with SOPP and rinsed with chlorine; and (E) inoculated fruit washed with SOPP, rinsed with chlorine, and coated with wax. Bars represent the means (±SE) of three replications.*

of the test organism, which corroborated results of the packinghouse survey. Additional research is needed to identify effective processes and components of waxing that reduce surface microorganisms.

Because heat treatment or pasteurization is not involved in fresh-cut citrus or fresh-squeezed citrus juice operations *(15, 16)*, both product microbial quality and safety are greatly influenced by fruit surface microbial loads before processing (peeling, cutting, or squeezing) and processing sanitation. Thus, it is necessary to evaluate all the handling procedures at either the packinghouse or processing plant that might influence fruit surface microflora. In this study, we concluded that commercial packinghouse procedures are generally effective in reducing fruit surface microflora. Washing is most useful in the removal of aciduric organisms and yeasts and molds. Waxing can effectively reduce surface aerobic organisms, aciduric organisms, coliforms, and yeasts and molds.

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