

## MICROBIOLOGY OF ORANGE JUICE

The Florida citrus industry is the largest producer of fresh and frozen juice concentrate in the USA. Citrus products are marketed as fresh or reconstituted single strength juices and as frozen concentrates. None are sterile. Microorganisms enter the processing plant on the surface of the fruit having originated from soil, untreated surface water, dust and decomposing fruit. The degree of contamination varies depending upon how the fruit was handled from the field and in the processing plant. Proper grading, washing and sanitizing the fruit contribute materially to good product quality. The low pH of fruit juices greatly limits the number and types of bacteria that can survive or grow. Lemon or lime juice is pH 2.2 to 2.6 and none of normal spoilage bacteria can grow or survive this low pH. Orange juice is pH 3.4 to 4.0 and *Lactobacillus* spp. and *Leuconostoc* spp. can survive and grow at these conditions. These bacteria can cause abnormal flavors and odors but they fail to grow at high sugar concentrations or low temperatures (45% sucrose, below 5°C) characteristic of concentrates. Acetic acid bacteria, yeasts and molds are also present and can grow when the juice is held at temperatures permitting their growth. Yeasts are primarily responsible for spoilage of chilled juice that is not sterile.

Most industrial juice concentrators use a high temperature evaporator (thermal accelerated short time evaporation) and microbes are generally killed during the process. And, many of the survivors should be killed by the freezing process even though this process will preserve the ultimate survivors. High plate counts would indicate improperly cleaned equipment or product abuse in the processing operation following the evaporator. Thus frozen concentrated orange juice should have few if any microbes.

Coliforms are rare in fruit juices. A very high occurrence of false positives result due to species of *Erwinia* and other coliform types associated with plants, these are not human or animal "fecal coliforms." Nevertheless, coliforms have been reported to retain viability in frozen concentrates but die off rapidly in fresh or reconstituted juices. Thus, coliforms are of little or no public health significance in fresh or frozen citrus products. And, even though spores of *Clostridium botulinum* can not germinate or grow, this does not rule out the importance of maintaining high sanitary standards in processing plants. Further, the rapidity at which lactic acid bacteria can grow during processing requires good sanitary practice to prevent spoilage.

This lab will investigate the differences between fresh orange juice, pasteurized orange juice and frozen concentrate as finished products and following a weeks time for spoilage using viable titers on selected media, and also measuring sugar content (Brix).

### Materials

#### FIRST WEEK

24 plates of Plate Count Agar (TGY).

24 plates of Acidified Potato Dextrose Agar.

24 plates of Orange Juice Agar.

Sterile Dilution fluid (200 ml) and Sterile 13x100 mm capped culture tubes.

Sterile 1 ml, 5 ml and 10 ml pipettes.

Spreading turntable, alcohol-beaker and spreading glass rods.  
Electronic Balance.  
4 Sterile 1 liter flasks.  
2 Sterile 100 ml flasks.  
Specific Gravity Hydrometer and 3 x 500 ml graduate cylinders.  
Samples of fresh orange juice, pasteurized orange juice and frozen orange juice concentrate.  
Alcohol and beaker of sterile Kimwipes.

#### SECOND WEEK

Glass slides, wax marking pencil, stage micrometer  
24 plates of Plate Count Agar.  
24 plates of Acidified Potato Dextrose Agar.  
24 plates of Orange Juice Agar.  
Sterile Dilution fluid (200 ml) and Sterile 13x100 mm capped culture tubes.  
Sterile 1 ml, 5 ml and 10 ml pipettes.  
Spreading turntable, alcohol-beaker and spreading glass rods.  
Specific Gravity Hydrometer.  
4 Sterile 100 ml flasks.  
Alcohol and beaker of sterile Kimwipes.

#### Procedure

##### FIRST WEEK

1. Pour about 200 ml of fresh orange juice into a 250 ml graduate cylinder, allow it to warm up to room temperature and determine the specific gravity of the orange juice. Prepare a 1X strength (>500 ml) of the frozen orange juice from the concentrate; keep chilled except for a 200 ml sample to be used in determining the specific gravity. Also measure specific gravity of the pasteurized orange juice. Make to sterilize the hydrometer after each use with alcohol.
2. Titering of orange juices: using tubes of 4.5 ml of sterile diluent prepare serial 1:10 dilutions out to  $10^{-3}$ .
3. Plate dilutions and undiluted onto duplicate plates of Plate Count Agar, Acidified Potato Dextrose Agar and Orange Juice Agar. Incubate plates at 30°C for two days.
4. Incubate samples (about >200 ml) of the fresh orange juice, pasteurized orange juice and reconstituted frozen orange juice at 25°C in 500ml graduate cylinders and cover the tops with parafilm.
5. **EACH or EVERY OTHER DAY** until the next period, a volunteer needs to come to the lab and measure the specific gravity of each juice incubating at 25°C, prepare a heat fixed slide of the juices and make observations (gas evolution, turbidity, etc.).

##### SECOND WEEK

1. Re-titer the spoiled orange juices by plating  $10^{-2}$  to  $10^{-5}$  dilutions in duplicate on to all the plates (as in the first week). Incubate the plates at 30°C for two days.

2. Determine the specific gravity of the fresh orange juice and 1X orange juice from frozen concentrate that were allowed to spoil at 25°C and in the refrigerator with a hydrometer and graduate cylinder. Calculate the approximate sugar and alcohol content of the spoiled juice.
3. Count the colonies on the plates from last week, calculate titers. Choose 5 typical colonies on each medium, Gram stain and measure their diameters (as well as describe them as you learnt in General Microbiology). Use this information to estimate the nutrient levels in orange juice.

### THIRD WEEK

1. Complete the observations. Observe all the heat-fixed slides (directly from the orange juice) after Gram Staining.
2. Count colonies on the plates from second week. Gram stain cells from 5 representative colonies. Which types of microbes are present in the spoiled juice from the organisms that grew on the plates? Which types were present in the direct stains? Do they correlate? How are the counts on acidified PDA different from Orange Juice Agar?

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#### Acidified Potato Dextrose Agar (PDA)

Prepare PDA as indicated through sterilization in the autoclave, cool to 45 to 50°C, add 17 ml of sterile 10% tartaric acid to bring the pH to 3.5 and pour plates.

Precaution: acidified media can not be re-heated, the acid conditions will destroy the solidification properties of agar.

#### Orange Juice Agar (OJA)

1. Autoclave 500 ml of Orange Juice in a 2 liter flask and in a 1 liter flask containing 500 ml distilled water and 15 grams of Bacto-Agar.
2. After autoclaving, place each in 50 to 55°C water bath...for 1 hour.
3. Aseptically pour the agar into the orange juice, mix well by gentle swirling.
4. Pour plates immediately.