had a tendency to reduce CI, but the addition of DP to Benlate-treated fruit markedly increased CI (Table 1).

Decay was generally very low at both storage temperatures. There was no statistically significant difference between temps or among treatments.

Table 1. Percentage chilling injury of 'Marsh' grapefruit picked monthly during the 1975-76 season and stored at 40°F.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 wks</th>
<th>6 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>17.5</td>
<td>33.2</td>
</tr>
<tr>
<td>EDB*</td>
<td>21.8</td>
<td>43.1</td>
</tr>
<tr>
<td>2 DP*</td>
<td>63.1</td>
<td>66.4</td>
</tr>
<tr>
<td>2 DP* + EDB*</td>
<td>57.7</td>
<td>64.9</td>
</tr>
<tr>
<td>Benlate</td>
<td>14.8</td>
<td>27.7</td>
</tr>
<tr>
<td>Benlate + EDB*</td>
<td>14.5</td>
<td>32.9</td>
</tr>
<tr>
<td>Benlate + 2 DP*</td>
<td>49.4</td>
<td>56.4</td>
</tr>
</tbody>
</table>

*Average of the 10 monthly experiments.
†Fumigated—ethylene dibromide.
*Di phenyl pads.

When DP are used for decay control of 'Marsh' grapefruit, increased CI may be expected at a storage temperature of 40°F. To avoid risk of CI, fruit of this cultivar should be stored and shipped in the temperature range of 55°F to 60°F, especially when packed with DP.

A SIMPLE METHOD FOR SCREENING FRUIT JUICES AND CONCENTRATES FOR HEAT RESISTANT MOLD

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Additional index words. Byssochlamys fulva.

Abstract. Mold growth in canned fruit juices and drinks causes a considerable amount of spoilage each year. Fungi capable of surviving the thermal process given these products are generally referred to as heat resistant molds. Organisms that fall into this classification usually belong to the genus Byssochlamys, the type species being B. fulva. These organisms are generally found in products made from grapes, although other fruits, such as cherries, apples, and strawberries, have been implicated. Spores have been known to survive one or more minutes at 200°F. A simple test is described for screening fruit juices, juice concentrates, or any suspected juice products for the presence of heat resistant mold. Product in bottles heated at 170°F (77°C) is plated. The plates and contents remaining in the bottles are incubated for periods up to 30 days at 86°F (30°C).

Mold, of the genus Byssochlamys, with heat resistant ascospores, has caused spoilage in canned fruits and in both canned and bottled fruit drinks and juices. It has been readily isolated from many fruits including grapes, cherries (13), apples (12), and strawberries (10). It was first reported in England in the 1930's (8) and in the United States in 1964 (6). Recent outbreaks have also occurred in Canada, Europe, South America, and Australia (4).

Byssochlamys is characterized by the production of ascospores contained in an 8-celled ascus. The mold has been cultured on a variety of media including Czapek Agar, Potato Agar, Potato Sucrose Agar, Potato Dextrose Agar, and Orange Serum Agar. Colony formation is dependent somewhat on the media used. Usually they are characterized by buff-colored conidial structure.

Byssochlamys shows unusual resistance to a number of influences lethal to most fungi. It can grow at low oxygen tension, hence its ability to grow in cans or bottles of processed fruit products. Olliver and Smith (8) noted the spores to survive in absolute alcohol for 30 weeks. Murdock and Hatcher found it to grow at temperatures as low as 35°F (1.7°C). Ito (3) reported 1,000 ppm chlorine solution was not sufficiently fungicidal to be effective in normal sanitizing procedures. The ascospores are also extremely heat resistant. Maundier (6) reported survival between 30 and 40 min. at 190°F (85°C) in a canned grape drink. Ascospores with this degree of heat resistance are capable of surviving the normal processing temperatures for fruits and fruit drinks, with subsequent germination and growth in the finished product. In canned fruits the pectolytic enzyme this organism produces can destroy fruit texture (9).

In recent years there has been an increase in spoilage

in thermally processed fruit juices, fruit drinks, and drink bases caused by *Byssochlamys* and other heat resistant fungi. In order to minimize this type of spoilage it is necessary to screen fruit juices and/or concentrates for the presence of heat resistant mold. A number of procedures used by various members of the food industry appear in the *Byssochlamys* Seminar Abstracts (1). Splitsstoeesser et al (11) described a method for the detection of heat resistant mold in a variety of fruit samples.

The method described herein employs a minimum of equipment. It is especially adapted for the detection of low numbers of heat resistant spores in fruit juices and/or concentrates received at a processing plant.

**Methods**

Maunder and Murdock 1968 (5) developed a method for the detection of heat resistant mold during a survey of a grape processing plant in the Midwest. A slight modification of the procedure appeared in the Proceedings of the *Byssochlamys* Seminar 1969 (1). It consisted briefly of diluting 25 ml of grape concentrate in an 8 oz. prescription bottle with an equal volume of 0.5% peptone solution. The sample was then heat shocked for 20 min. at 170°F (77°C), cooled and then incubated at 86°F (30°C) with the bottle placed on its side, cap loosened.

This procedure was further modified by our laboratory. It has been designed specifically for checking fruit concentrates such as grape, apple, and cherry and juice bases made from these products, for the presence of heat resistant mold. It consists of the following (Fig. 1):

- Place 50 grams of product in a sterile 8 oz. medicine screw-capped bottle or a sterile 250 ml tissue culture bottle.
- Add 50 ml of sterile water.
- Spore test 30 min. at 170°F (77°C) (start timing when test bottle of product containing thermometer reaches this temperature).
- Cool immediately.
- Distribute 30 to 40 ml among 4 or 5 petri plates—add 2% plain agar and mix contents.
- Place bottle, containing remaining product, on its side with cap loosened, and incubate at 86°F (30°C).
- Examine plates and bottles weekly for the presence of mold growth. Discard after 30 days if no growth occurs.

Above procedure may be used for single strength products having a Brix of 35° or less. When product of this type is being screened use 100 gram sample and do not dilute with sterile water.

**Results and Discussion**

The heat shock of 30 min. at 170°F has been designed to eliminate non-heat resistant fungi, restricting outgrowth to those organisms which may be capable of surviving the thermal process given the finished product. Molds of this type are capable of surviving several hours at this temperature. Also, Hull (2) reported optimum germination is obtained by heating the spores for 30 min. at 167°F (75°C) which is in the temperature range specified.

Our laboratories have used this procedure since 1972 to screen incoming fruit juice concentrates for the presence of heat resistant mold. Outgrowth usually occurs after 5 to 7 days incubation, if the product contains 100 or more spores per gram. However, extremely low concentrations of spores may take as long as a month before colony formation appears. If cultures are still negative after this period they should be discarded, as no further outgrowth is likely to occur. The test exhibits fairly good reproducibility. Four different laboratories checking the same sample of grape base for heat resistant mold reported positive results after 5 days incubation at 86°F (30°C). As with any other type of microbiological test, aseptic technique should be used to prevent contamination from other types of mold such as *Penicillium*.

*B. fulva* has been the species most frequently isolated. However, *B. nivea* and *Paecilomyces* have also been found. Colonies growing on the product medium may range in color from white to buff brown, with buff color usually being associated with this organism.

**Literature Cited**


**Fig. 1. Procedure for detection of heat resistant mold in fruit juice concentrates.**

1. Place 50 grams of product in a sterile 8 oz. medicine screw-capped bottle or a sterile 250 ml tissue culture bottle.
2. Add 50 ml of sterile water.
3. Spore test 30 min. at 170°F (77°C) (start timing when test bottle of product containing thermometer reaches this temperature).
4. Cool immediately.
5. Distribute 30 to 40 ml among 4 or 5 petri plates—add 2% plain agar and mix contents.
6. Place bottle, containing remaining product, on its side with cap loosened, and incubate at 86°F (30°C).
7. Examine plates and bottles weekly for the presence of mold growth. Discard after 30 days if no growth occurs.

**Fig. 2. Eight-spored asci, one of the differential characteristics of the genus *Byssochlamys* (1000X).**

8. Check outgrowth microscopically, looking for the presence of characteristic 8-spore asci as illustrated in Fig. 2.
SOFT ROT OF TOMATOES RESULTING FROM DUMP-TANK AND WASHER-WATER CONTAMINATION AND ITS CONTROL BY CHLORINATION

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Additional index words. Erwinia carotovora.

Abstract. The bacterium Erwinia carotovora Jones that causes soft rot of tomatoes is often present in tomato packinghouse dump-tank and washer waters. Incidence of fruit decay was high when large numbers of bacteria were present in dump-tank and washer waters. Chlorination (100 ppm) of the dump-tank water and washer water reduced bacterial contamination and controlled decay. However, chlorination of the dump-tank water only, or the washer water only, did not eliminate bacteria at the other site and did not effectively reduce decay. Chlorination of water effectively controlled soft rot only when water used in the packinghouse was treated. The results demonstrate that chlorine eliminated contamination in dump-tank and washer waters, but did not effectively prevent decay in tomatoes contaminated either before or after treatment.

In studies on the incidence of bacterial soft rot of tomatoes, the primary source of inoculation was postharvest exposure to the causal bacteria, Erwinia carotovora Jones, in water used for washing. When washing was performed in the field, the water was highly contaminated with E. carotovora, and decay incidence of tomatoes washed in these waters was very high (2). When fruit was washed in the packinghouse, the wash water also was contaminated, and incidence of postharvest decay was high (4). In recent years, packinghouses have installed dump-tanks containing water to receive tomatoes brought from the field in pallet bins. The water in these tanks is a source of bacterial inoculation of tomatoes.

Addition to wash water of chlorine in the form of gas, as sodium or calcium hypochlorite, effectively controlled bacterial soft rot of tomatoes (3). These chlorine compounds have little protective or eradicative effect when they are added before or after the site of contamination (1). Because of the corrosive effect of chlorine on packinghouse equipment, operators of packinghouses would prefer to apply chlorine at only one site, either the dump-tank or the washer. These studies were undertaken to determine the most effective methods for control of bacterial soft rot of tomatoes with chlorine.

Materials and Methods

Tomatoes, 'Walter' cv. at the mature-green stage were obtained from two packinghouses in the Immokalee area. These tomatoes had been hand-harvested. Three collections were made at 2-week intervals from each house during March and April 1975. The tomatoes were collected from pallet bins as they came from the field, and before they received any packinghouse treatments, and brought to the USDA Horticultural Research Lab at Orlando for post-harvest treatment. The fruit from each packinghouse was graded to eliminate culls, and divided at random into 9 lots of 50 fruit each. The tomatoes received the treatments shown in Table 1. Contamination of dump-tank water was simulated by maintaining a concentration of 1 x 10^6 bacterial cells of E. carotovora in a 50-gal tank of water. The same concn of bacterial cells was maintained in wash water by injecting these bacterial cells in water flowing from a standard high gallonage nozzle. Chlorine was maintained at 100 ppm by continuously injecting chlorine gas into the dump-tank or wash water. Following the above treatments, a petroleum-based wax, commercially used for waxing tomatoes, was brushed on the treated tomatoes in a commercial waxer. All lots of fruit were held for 2 weeks at 21°C and a relative humidity of 90%. Twice-weekly inspections were made during this holding period and decaying fruit were identified, counted, and removed to reduce secondary contamination. Data were recorded as percentage of fruit decay during the 2-week holding period.

Results and Discussion

The hand-harvested tomatoes brought from the field to the packinghouse and washed in noncontaminated water before waxing had less than 2% decay incidence after the 2-week holding period (Table 1). These tomatoes were not subjected to bacterial contamination in the simulated packinghouse test. Exposure of tomatoes to soft rot bacteria in contaminated dump-tank water only increased decay to about 15%. Exposure to the same concn of bacteria in the washer water increased the soft rot incidence to 30%. In tomatoes exposed to bacteria in both the dump-tank and washer waters, decay incidence exceeded 35%. Thus, subjecting tomatoes to contamination by soft rot bacteria resulted in a decay incidence of 15 - 85% (Table 1).