A Simple Method to Screen Fruit Juices and Concentrates for Heat-Resistant Mold

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ABSTRACT

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 77 °C is plated. The plates and contents remaining in the bottles are incubated for periods up to 30 days at 30 °C. Several plating and liquid media for enumerating heat resistant mold were investigated.

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 *(L.)*, apples *(L)*, and strawberries *(L)*. 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 production of ascospores contained in an 8-spored 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 medium used. Usually they are characterized by buff-colored conidial structure.

*Byssochlamys* shows unusual resistance to a number of influences which are 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 *(9)* noted the spores to survive in absolute alcohol for 30 weeks. Murdock and Hatcher *(7)* found it to grow at temperatures as low as 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. Maunder *(6)* reported survival between 30 and 40 min at 86 °C in a canned grape drink. Ascospores with this degree of heat resistance are capable of surviving the normal processing temperature for fruits and fruit drinks, with subsequent germination and growth in the finished product. The pectolytic enzyme produced by this organism can destroy the texture of canned fruits *(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. 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)*. Sliepittstoesser et al. *(11)* described a method for 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 small numbers of heat resistant spores in fruit juices and/or concentrates received at a processing plant.

METHODS

Maunder and Murdock in 1968 *(5)* developed a method for 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 0.05% peptone solution. The sample was then heat shocked for 20 min at 77 °C, cooled, and then incubated at 30 °C with the bottle placed on its side, cap loosened.

This procedure was further modified in our laboratory. It has been designed specifically for checking fruit concentrates such as grape, apple, and cherry juice bases made from these products, for the presence of heat resistant mold. It consists of the following (Fig. 1): *(a)* place 50 g of product in a sterile 8-oz. medicine screw-capped bottle or a sterile 250-ml tissue culture bottle; *(b)* add 50 ml of sterile water; *(c)* spore test 30 min at 77 °C (start timing when test bottle of product containing thermometer reaches this temperature); *(d)* cool immediately; *(e)* distribute 30 to 40 ml among 4 or 5 petri plates1, add 2% plain agar and mix contents; *(f)* place bottle, containing remaining product, on its side with cap loosened, and incubate at 30 °C; *(g)* examine plates and bottles weekly for the presence of mold growth and discard after 30 days if no growth occurs; and *(h)* check outgrowth microscopically, looking for the presence of characteristic 8-spore asci as illustrated in Fig. 2.

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-g sample and do not dilute with sterile water.
RESULTS AND DISCUSSION

Comparison of media

The procedure described herein was developed to be used in those plants which have little or no microbiological equipment or selective media. One of the first items investigated was a comparison of various plating media for enumerating heat resistant mold. The media studied were Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Malt Agar (MA), and Orange Serum Agar (OSA). All of the foregoing contained 2% agar. Also included was 2% plain agar (PA). Fifty ml of 68° Brix grape concentrate was diluted with an equal volume of water in an 8-oz. screw cap bottle. The contents were sterilized for 10 min in flowing steam, cooled, then inoculated so as to contain 10 spores of B. fulva strain M-78/ml. Ten ml of the inoculated product was then distributed over five petri plates. This was repeated five times. Five plates were then poured for each test medium. The results in Table 1 show comparable counts were obtained in all media.

<table>
<thead>
<tr>
<th>Media</th>
<th>Total colonies per 5 plates</th>
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<tbody>
<tr>
<td></td>
<td>30 h</td>
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<tr>
<td>PDA</td>
<td>60</td>
</tr>
<tr>
<td>SDA</td>
<td>74</td>
</tr>
<tr>
<td>MA</td>
<td>71</td>
</tr>
<tr>
<td>OSA</td>
<td>76</td>
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<tr>
<td>PA</td>
<td>74</td>
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50 ml of 68° Brix grape concentrate diluted with an equal volume of water, 10 ml poured over 5 plates for each test medium. Plates incubated at 30 C.

The growth of heat resistant mold in a liquid medium was also investigated. In this test 50 ml of sterile 68° Brix grape concentrate in an 8-oz. screw cap bottle was diluted with an equal volume of Potato Dextrose Broth (PDB), similarly with Sabouraud Broth (SB), and another with sterile water. The bottles were inoculated to contain 10 spores per ml from the same suspension previously mentioned. They were then placed on their sides, caps loosened, and incubated at 30 C. Mold growth in all bottles appeared to be about the same (Table 2). However, colony formation took longer in the liquid media than in the plates. It appears grape concentrate supplies the necessary growth factors for mold growth and that selective media are not necessary, i.e., water can be used as a diluent for grape concentrate in the bottles and

<table>
<thead>
<tr>
<th>Media</th>
<th>Days at 30 C</th>
</tr>
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<tbody>
<tr>
<td>Grape conc. + PDB</td>
<td>2 3 5</td>
</tr>
<tr>
<td>Grape conc. + SB</td>
<td>NG + +</td>
</tr>
<tr>
<td>Grape conc. + water</td>
<td>NG + +</td>
</tr>
</tbody>
</table>

NG = No growth; + slight growth (1 or 2 colonies); ++ heavy growth (4 or more colonies).

100 × 25 mm petri dishes will prevent possible spillage of product during mixing process with 2% agar.

The test organism was from a suspension supplied by John Folinazzo of Continental Can Co., Inc. and had been heat shocked 15 min. at 80 C.

Heat shock

The heat shock of 30 min at 77 C has been designed to eliminate non-heat resistant fungi, restricting outgrowth to those organisms which may be able to survive the

1.88 × 25 mm petri dishes will prevent possible spillage of product during mixing process with 2% agar.

2. The test organism was from a suspension supplied by John Folinazzo of Continental Can Co., Inc. and had been heat shocked 15 min. at 80 C.
thermal process given the finished product. Molds of this type are able to survive several hours at this temperature. Also, Hull (2) reported optimum germination is obtained by heating the spores for 30 min, at 75°C, which is in the temperature range specified.

Screen test

Our laboratories have used this procedure since 1972 to screen incoming fruit juice concentrates for presence of heat resistant mold. Outgrowth usually occurs after 3 to 5 days of incubation, if the product contains 10 or more spores per gram. However, if concentration of spores is extremely low it may take as long as a month before colony formation appears. By incubating both plates and bottles there is a greater chance of obtaining outgrowth. 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 samples of grape base for heat resistant mold reported positive results after 5 days incubation at 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.

ACKNOWLEDGMENT

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REFERENCES