Control of *Byssochlamys* and Related Heat-resistant Fungi in Grape Products

A. DOUGLAS KING, JR., H. DAVID MICHER, AND KEITH A. ITO

*Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, California 94710 and National Canners Association, Berkeley, California 94710*

Received for publication 27 May 1969

Heat-resistant strains of *Byssochlamys fulva*, *B. nivea*, and other heat-resistant fungi were isolated from vineyard soil, grapes, grape-processing lines, and waste pomace. They are known to remain in grape juice occasionally and to grow in grape juice products. Ascospores of these fungi have a D value (decimal reduction time) of about 10 min at 190 F (88 C), but in the presence of 90 mliters of SO2 per liter (normally added to the juice) the D value was cut in half. Filtration through a commercial diatomaceous filter aid (also a common processing step) entrapped all but about 0.001 % of experimentally added spores. Thus, heat in the presence of SO2 and filtration together can reduce the population of these spores by several orders of magnitude. Growth was also prevented by benzoate or sorbate in low concentrations. Oxygen must be reduced to extremely low levels before lack of oxygen limits growth.

Heat-resistant molds have been found in processed fruit and fruit juice in England (5, 7, 10), Switzerland (12), Holland (19), Denmark (11), Australia (21), the United States (23; H. D. Michener et al., Bacteriol. Proc., p. 3, 1966), and elsewhere. Reports go back to the early 1930's. In each instance, the mold was found because it survived the heat treatment used in processing the fruit or juice. When the offending mold has been identified, it has usually been *Byssochlamys fulva*. In canned fruit juice, this forms unsightly pellets of mold growth, although these do not become large because of lack of oxygen in the cans. Growth in cans is sometimes accompanied by production of gas which causes slight bulging of the can ends. In canned fruit, its pectinolytic enzymes cause complete disintegration of solid fruit (17, 20). *B. nivea* has also been implicated in canned fruit spoilage (5, 18) and has been isolated from cucumber brine in Canada (26). A few other fungi have been reported to withstand heat processing in fruits or juices. *Penicillium* (23), *Phialophora* (11), and *Paecilomyces variotii* (13). The latter is very similar to *B. fulva* but it does not produce ascospores (2).

*Byssochlamys* is one of the ascomycetes, and the ascospores, contained in the eight-celled asci, are believed to be the heat-resistant cells (2). The conidia, produced in large numbers, lack heat resistance (10). *P. variotii*, the imperfect form of *B. fulva*, has been isolated as a heat-resistant fungus in the work described here and by other authors (13, 18). This suggests that these are strains which produce ascospores under natural conditions, but they did not do so when cultured for identification purposes or they would have been assigned to the genus *Byssochlamys*.

**MATERIALS AND METHODS**

**Preparation of ascospores**. Except as indicated, we used *B. fulva* strain no. NRRL 3493, isolated by C. W. Hesseline of the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., from canned apple-grape drink which contained diluted grape juice concentrate. Except as otherwise indicated, the suspending medium was single-strength grape juice reconstituted from grape juice concentrate.

For ascospore production, *B. fulva* was routinely grown on Potato Dextrose Agar (unacidified; Difco). Young cultures were dispersed in sterile water and spread on 5-mm layers of agar in flat-sided bottles. After incubation for 30 days at 30 C, ascii had formed in large numbers and were scraped from the agar and attached pellicle with a sterile scraper, suspended in water, and separated by decantation from mycelial fragments. This gave an almost pure suspension of intact asci. Attempts to make a suspension of free ascospores (by shaking with glass beads, by sonic treatment, and by use of a Potter-Elvehjem Teflon homogenizer) were unsuccessful. Sonic treatment gave the best results but only freed about two-thirds of the ascospores from the ascii. The experiments described here were done with the intact asci. They retained their viability for months at refrigerator temperature, at a concentration of about 10¹ ascii per ml. They settled out on the bottom of the bottle but could easily be resuspended and uniformly dispersed by shaking.
It is not known whether ascospores occurring naturally as food contaminants are solitary or in the intact ascus. We were unable to get good yields of asci from every strain. Some strains yielded asci only sparsely and one produced no asci under these cultural conditions.

RESULTS

Properties of B. fulva ascospores. Ungerminated ascospores are highly refractile under the phase microscope, but they lose this property on germination and darken before hyphae emerge (Fig. 1A–C). On emergence from the ascospore, the hypha commonly produces a basal swelling attached to the ascospore, from which one or two hyphae grow (Fig. 1C and 1D). The ascospores vary greatly in the time required for germination so that a microscope field covered with germinated ascospores and hyphae will often contain some refractile, ungerminated spores (Fig. 1E).

B. fulva has been found in grape juice and grape drink products containing grape juice concentrate that were canned or bottled in the United States. Control by heat alone is difficult because of its damaging effect on the flavor of the product. The concentrate or other ingredients were believed to contain the B. fulva spores which

---

**FIG. 1.** Germination of B. fulva ascospores. Phase microscope. X 500. (A) Asci containing refractile ascospores, before germination. (B) Ascospores darkening prior to outgrowth of hyphae. (C, D) Germinating ascospores. Note swelling of hyphae next to the ascospore from which they emerged. (E) Germinated ascospores and hyphae which they produce. Note refractile, ungerminated ascospores.
subsequently grew in the cans. The concentrate contained so few spores that they could not be detected by the usual methods of plating or culturing small samples, but when several gallons were diluted and canned, growth developed in some of the cans. Recently we have been told of the presence of *Byssochlamys* in various other food ingredients, indicating that this problem is somewhat widespread.

Ascospores germinate more readily if they are heat treated (4 min at 80 C) than if they are not heated. [See also Hull (10) and Yates et al. (27).] In this respect they resemble bacterial spores. The resemblance is probably only superficial, as the dipicolinic acid content of *B. fulva* ascospores is less than 0.02%, as compared with 5 to 15% in bacterial spores (15); furthermore, they do not have the ion-exchange properties described by Alderton and Snell (1) for bacterial spores. As a possible result, *B. fulva* ascospores differ from bacterial spores in having a high heat resistance at low pH values. In fact, it is because of this property that their presence in grape juice at a pH of about 3.8 is particularly objectionable.

The density (specific gravity) of asc and ascospores was determined by isopycnic centrifugation (14). Equilibrated spores were suspended in an aqueous potassium tartrate density gradient at room temperature. After centrifuging for 1 hr at 37,000 × g, the density at which spore bands rested was determined by density-calibrated hollow-glass beads. Ascospores and intact asc were always found together in a single band in the density gradient. The density varied somewhat in different trials but was always in the range of 1.34 to 1.37. Some dormant bacterial spores have densities within this range (14). Germinating *Byssochlamys* ascospores (with emerging hyphae) were always found in the less-dense portion of the gradient.

Specific gravities of several samples of grape juice concentrate were in the range of 1.44 to 1.46. Separation of ascospores from concentrate by centrifugation would be impractical because the spores would float rather than sediment, the concentrate is too viscous, and the difference in specific gravity is small.

**Fruit juices in which growth occurs.** It had been reported to us that *B. fulva* would not grow in certain varieties of fruit juice. We have, however, observed growth in the following canned fruit juices which had been inoculated with this organism: apple, Concord and Thompson-seedless grape, and prune juices; peach and pear nectar; and several drinks including grape-apple combinations and artificial orange drink. Ascospores germinated in commercial Concord grape juice diluted 1:1 with water as well as in the same lot of juice undiluted.

**Effects of temperature on growth.** Potato Dextrose Agar plates were inoculated in the center with *B. fulva* ascospores and incubated at several temperatures. Periodic measurements of the largest and smallest diameters of five plates were averaged (Fig. 2). Growth was optimal at 35 C and very slow at 6.5 C, the lowest temperature tested. Hull (10) reported 35 C as optimal for both growth and ascospore germination but observed no growth at 8 C. After incubation of the plates for 15 days at 53 C, we observed no growth.

**Effect of oxygen on growth.** Growth of *Byssochlamys* has been observed in cans with a limited amount of headspace. Thus, we have examined the ability of *B. fulva* to grow at low concentrations of oxygen. A gas-flow manometer control system, similar to that described by Claypool and Keefer (3), was used to test the effect of limited quantities of oxygen on growth rate. A layer of Potato Dextrose Agar in a 250-ml Erlenmeyer flask was inoculated with a loopful of ascospores in the center. The amount of oxygen in the gas was measured by a model 777 oxygen analyzer (Beckman Instruments Co., Inc., Fullerton Calif.) or a thermal conductivity gas chromatograph, both adjusted against air. Air and nitrogen were mixed to give oxygen concentrations of 21, 2.6, and 0.27% at a flow rate of 10 liters/hr. Growth at room temperature was observed in all of the flasks after 3 days. In a flask with only nitrogen (10 liters/hr) slight growth was observed after 6 days. Although the nitrogen was stated to be 99.999% pure, gas chromatog-
raphic analysis showed that it contained a trace of oxygen.

To test the ability to grow without oxygen, petri plates inoculated with ascospores were placed in a desiccator containing pyrogallic acid and Na₂CO₃ plus tubes containing reduced methylene blue as an indicator of anaerobic conditions. The desiccator was flushed several times with nitrogen and was incubated at room temperature. After 3 weeks, no growth was observed on duplicate plates of Potato Dextrose Agar, Czapek Solution Agar (Difco), or grape juice. The methylene blue remained colorless, indicating anaerobic conditions. When the desiccator was opened and the plates were incubated in air, growth began immediately. These tests show that B. fulva will not grow under strictly anaerobic conditions but that it will grow under greatly reduced quantities of oxygen.

When observed microscopically, ascospores germinated in a thin layer of grape juice or near the surface of a deep (2 cm) layer, but those that sank to the bottom of the deep layer rarely germinated within the 36-hr interval of observation. The juice used had recently been sterilized and was thus deaerated. Somewhat similarly, aeration has been shown to enhance ascospore germination in B. nivea (27).

Control of growth by chemical inhibitors. To determine whether benzoate or sorbate can inhibit growth of B. fulva, ascii were plated on acidified Potato Dextrose Agar (pH 3.5) containing 0.05% potassium sorbate or 0.1% sodium benzoate. Out of about 200 asci per plate, none grew. Several concentrations of these substances were then tested as inhibitors in reconstituted grape juice (pH 3.5). With both inhibitors, growth was prevented by concentrations of 0.025% but not by 0.01% (Table 1).

Isolation of heat-resistant molds. British workers concluded that fruit became contaminated with Byssochlamys in the field, from harvesting equipment, etc. (4, 17). We collected samples of plant tissue and soil from numerous locations in vineyards and processing plants. A total of 129 samples were collected and packed in ice until refrigerated at the laboratory.

To detect heat-resistant molds, each sample was dispersed in sterile grape juice in a blender, and a portion of the resulting slurry was heated in a 4-oz bottle for 35 min in a water bath at 80°C. The slurry was then plated on Potato Dextrose Agar and the plates were incubated at 30°C. The plates were then inspected, and as far as possible all the different morphological types were selected, purified by replating if necessary, and transferred to Potato Dextrose Agar slants. After incubation, the slants were heated with added sterile juice for 30 min at 80°C. Each of the 52 isolates surviving the second heat treatment was examined by the slide culture technique of Riddell (22). Those that we considered to be Aspergillus or Penicillium species were not further classified because these have not to our knowledge caused spoilage in grape products which have been heated. The remaining cultures were identified by C. W. Hesseltine and J. J. Ellis at the Northern Regional Research Laboratory, and have been added to their culture collection. All 52 of these isolates (Table 2) were again grown on slants and heated as before, but at 88°C for 45 min. A total of 31 cultures survived, which were again grown and heated at 88°C for 60 min, after which eight cultures survived.

Of the 25 isolates identified as Byssochlamys or Paecilomyces (Table 2), all were heat-resistant. Therefore, all must have produced ascospores if we accept the theory (2) that it is the ascospores which survive heat. Also, asci were observed

### Table 1. Effect of sorbate and benzoate on growth of Byssochlamys fulva

<table>
<thead>
<tr>
<th>Conc</th>
<th>Growth in the presence of a</th>
<th>Potassium sorbate</th>
<th>Sodium benzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

a Growth after 16 days at room temperature in a shallow layer of reconstituted grape juice inoculated with 2 × 10⁴ ascii.

### Table 2. Summary of heat-resistant cultures isolated from grape material

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates surviving successive heat treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>88°C, 30 min</td>
</tr>
<tr>
<td>Byssochlamys nivea...</td>
<td>18</td>
</tr>
<tr>
<td>Paecilomyces varioti...</td>
<td>7</td>
</tr>
<tr>
<td>Thermoascus aurantiacum...</td>
<td>4</td>
</tr>
<tr>
<td>Aspergillus sp...</td>
<td>20</td>
</tr>
<tr>
<td>Penicillium sp...</td>
<td>1</td>
</tr>
<tr>
<td>Other*</td>
<td>2</td>
</tr>
<tr>
<td>Total surviving strains...</td>
<td>52</td>
</tr>
</tbody>
</table>

* Monascus purpurea, Pullularia pullulans.
microscopically in these isolates. Nevertheless, seven isolates evidently failed to produce ascis when cultured for the purpose of identification, and as a result they were classified as Paecilomyces.

Regarding the Byssochlamys cultures, it will be noted that our reference cultures were B. fulva, whereas the new isolates were B. nivea. These species are very much alike, and from the practical standpoint it seems unimportant which species is found as a contaminant if it is able to survive processing and grow in the product. It has been stated that B. fulva is more heat-resistant than B. nivea (8); however, other authors have disagreed with this (19). Byssochlamys was isolated from grapes left in the vineyard after harvest, from leaves, vineyard soil, etc. The normal cultivation and harvest procedure is such that soil could contaminate the fruit during harvest. Byssochlamys was also found on the grapes at the crusher, on the stems, on leaving the crusher, on the skins floating on the first holding tank, in the pomace, and in a wastewater lagoon. Since the fungus was not isolated from the juice or from the concentrate, we conclude that it is usually removed with the pomace. However, recovery of spores from concentrate at the rate of one or a few per gallon has been reported to us. At this level, our plating method based on 16-ml samples would rarely detect them.

Heat resistance. Spores not removed with the pomace could be killed by heat during preparation of grape juice concentrate. In the production of concentrate, a heat exchanger is placed ahead of the vacuum pan (Fig. 3), and the juice could be heated sufficiently to kill spores if it were established that this could be done without quality loss. Subsequent cooling would be required.

For heat resistance determinations, B. fulva was grown and the ascospores were harvested and suspended in water as described above. Since almost all of the ascospores remained in intact asci, the ascus is the unit considered in the heat resistance calculations. Inoculum size was checked by plate count on Potato Dextrose Agar. Thermal death times were determined by the methods described by the National Canners Association (16).

Thermal-death-time cans containing 10 ml of juice and 10⁴ ascis were sealed under a vacuum of about 15 inches. Eight cans were heated at each of eight different intervals at three different temperatures, and were then incubated for 4 weeks. Growth and resultant pressure change within the cans were determined by measurement of the can lid with a depth gauge to detect bulging. Some negative cans were cultured to detect live spores if present. None was found. Positive cans, when opened, always showed growth. The maximum survival and minimum destruction times are listed in Table 3. From these data, the D value (decimal reduction time; 16) was determined for each temperature (Fig. 4). The z value (temperature reduction corresponding to a 10-fold increase in D value) was 11 F (6.1 C).

The thermal death time of ascis in grape juice concentrate was determined in the same way as for juice except for experimental modifications that are necessary because the spores will not grow in the concentrate. Glass thermal-death-time tubes were used because they are easier than cans to subculture. Each tube, containing 2 ml of concentrate and 3 × 10⁴ ascis, was sealed and heated (six tubes for each of eight heating times, at four temperatures). Each tube was then opened aseptically and emptied into a sterile culture tube with just enough rinse water to dilute the concentrate to a single-strength juice. The cultures were then incubated at 30 C and observed for growth. The end points found are shown in Table 3.

### Table 3. Survival and destruction times after various heat treatments in grape juice and concentrate

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temp</th>
<th>Survival</th>
<th>Destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>C</td>
<td>Cans or tubes³</td>
</tr>
<tr>
<td>Reconstituted</td>
<td>190</td>
<td>87.8</td>
<td>61.4</td>
</tr>
<tr>
<td>grape juice</td>
<td>194</td>
<td>90.0</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>198</td>
<td>92.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Grape juice</td>
<td>188</td>
<td>86.7</td>
<td>103.0</td>
</tr>
<tr>
<td>concentrate</td>
<td>193</td>
<td>89.4</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td>198</td>
<td>92.2</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>95.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Number of cans or tubes showing survival/total cans or tubes.
isolates were obtained from the NRRL culture collection; ascis were grown and harvested, and their thermal death time was determined at 190 F (87.8 C) in juice in thermal-death-time cans. None of the new strains was as heat-resistant as was the original strain. D values calculated from these data were: NRRL 1,125, < 8.5 min; NRRL 2,614, < 8.3 min; NRRL A-2,142, < 8.3 min; NRRL A-3,849, 4.8 min; NRRL 3,493 (our original strain), 11.3 min.

It has been reported that the heat sensitivity of *B. fulva* is enhanced by the presence of SO₂ (6). To investigate this, thermal-death-time tubes containing inoculated juice and known SO₂ concentrations were heated at 190 F (87.8 C) for various time intervals. The juice contained added sodium bisulfite to give calculated SO₂ concentrations of 250 and 90 μliters/liter, and subsequent analyses for total SO₂ showed these values to be substantially correct. (The original juice contained about 1.5 μliters of SO₂ per liter, which was regarded as insignificant.) After heating, the tubes were opened aseptically and rinsed into culture tubes with enough sterile juice to give a 1:10 dilution of the heated juice. The SO₂ concentration was thus reduced to at most 25 μliters/liter, which was shown in a separate experiment to be too low to prevent outgrowth of unheated *B. fulva* ascis.

Under these conditions, the D values at 190 F (87.8 C) with SO₂ concentrations of 250, 90, and 0 μliters/liter, respectively, were 1.6, 4.5, and 8.8 min. Thus the SO₂ did in fact greatly reduce the heat resistance of *B. fulva*. Gillespy (6) reported that 2 and 10 μliters of SO₂ per liter reduced the heat resistance of these spores in pH 3 citrate buffer. Using thermal-death-time cans, we found no significant reduction in D value in grape juice in the presence of 10 μliters of SO₂ per liter. Both the higher pH of the grape juice (7) and the presence of sugar which combines with part of the SO₂ (25) would reduce the effect of SO₂ in grape juice, as compared with the pH 3 buffer.

**Filtration to remove ascospores and ascis**. Since grape juice is often filtered before it is made into concentrate (Fig. 3), we investigated the possibility that diatomaceous-earth filters can entrap *B. fulva* ascospores and ascis.

Preliminary experiments were conducted with Buchner funnels to determine whether ascospores could be recovered from diatomaceous-filter aid after filtering contaminated juice. Precoat and body feed amounts for all experiments were scaled down from those suggested for commercial operation by Johns-Manville, who kindly furnished the filter aid for these experiments. The precoat amount was 10 lb/100 ft² of filter area, and the body feed was 0.8% of the inoculated

3. The D values were slightly higher than for single-strength juice made from the same concentrate (Fig. 4). The z value was 12 F (6.7 C).

Obviously, there could be *B. fulva* strains with a greater heat resistance than that measured in the above heat resistance determination. To test at least a sampling of other strains, four additional
juice. Juice containing $1.4 \times 10^4$ ascis was filtered, and portions of the Standard Super-Cel filter pad were plated. The counts on the pad indicated a recovery of $1.5 \times 10^4$ ascis. In two similar experiments, $1.3 \times 10^4$ out of $1.5 \times 10^4$ and 29 out of 30 organisms were recovered.

A pilot plant four-plate pressure filter with a total filtering area of 1.24 ft$^2$ was then used to attempt removal of ascis from grape juice. In a typical operation, a precoat pad was formed on the filter papers by pumping a suspension of filter aid or filter aid plus asbestos until it was clear. Most of the precoat liquid was pumped out of the reservoir, after which 5 gal of inoculated juice containing the body feed filter aid was added and filtered at about 3 gal/min. The filtered juice was collected in sterile carboys, filled into cans (303 by 406), sealed, and heated at 70 C (158 F) for 15 min to destroy yeasts and to heat-activate B. fulva spores. Cans containing approximately 500 ml of filtered juice were then incubated at 35 C for 3 weeks or longer, observed for swelling, and opened. The contents were observed for growth and the juice was poured through a no. 8 sieve which collected any mold pellet that grew in the can (Table 4).

Assuming that each can will swell and that mold growth will occur from one ascospore, then the reduction in count by filtering is on the order of 99.999%. The proportion of the spores passing the filter does not depend on the inoculum size and is not markedly influenced by the different filtering media (Table 4). It is concluded that a major portion of the spores were removed from the juice by diatomaceous-earth filtration.

The efficiency of filtration on a commercial scale for removal of Byssochlamys spores has not been determined. To be successful, physical arrangements should be such that the filtered juice would be removed from the filter area without recontamination by the used filter aid. It is not known whether natural populations of B. fulva exist as ascis or ascospores. Our experimental inoculum contained some separate ascospores, although intact ascis predominated. Ascospores might be more difficult to filter out but they are within the range of particle size considered to be removable by this method (24). Generally speaking, since the natural populations of B. fulva and related organisms are so small that they escape detection by conventional plating methods, a filtration system that permits passage of only one spore in $10^8$ and prevents recontamination of the filtered juice should reduce the populations to an extremely low level.

**DISCUSSION**

These data suggest measures which may be useful for control of Byssochlamys in products containing grape juice concentrate. Grape juice is commonly heated before it enters the vacuum pan. If it receives treatment the equivalent of one D value [e.g., 10 min at 190 F (87.8 C); see Fig. 4], only 10% of any ascospores will survive. Twice as much heat would permit only 1% survival. Sulfur dioxide is regularly added to the juice before it is concentrated. In a concentration of 90 µl/liter, it reduces the D value of these spores by about 50%. This would reduce the survival rates to 1 and 0.01%, respectively, after the treatments suggested above. Our data on filtration indicate removal of all but approximately 0.001% of the spores, or all but one spore in $10^6$. If the effects of heat and sulfur dioxide are added to this, our data suggest that only one spore in $10^9$ or $10^{10}$ would remain in the juice.

The population of Byssochlamys spores has, to our knowledge, never been large in grape juice; in fact, it has been so small as to escape detection by conventional plating methods. Gillespy and Thorpe (8) reported less than one spore per pound of strawberries. But if we suppose it to be one spore per milliliter (easily detectable by plating), the process suggested above should reduce this to one spore per 100,000 liters (or approximately 25,000 gallons) of single strength juice to be concentrated. But since grape juice concentrate is always diluted and reprocessed, it receives another heat treatment which can probably be the equivalent of at least one more D value, thereby effecting a further reduction of at least 90% in spore population. Thus, while we only did laboratory scale experiments, and the existence of more heat-resistant strains or higher levels of contamination is possible, our data nevertheless suggest measures which should reduce the population of Byssochlamys spores in grape juice to an extremely low level.

These measures will obviously be ineffective unless recontamination is prevented, since Byssochlamys is known to be present in the areas where grapes are processed. The usual plant sanitation procedures are important, but two particular routes of possible contamination should be mentioned. As previously stated, the filtered juice must be removed from the filter area without recontamination by the spent filter material. In the containers used to store the concentrate, any condensate which drips onto the surface of the concentrate will form "puddles." Because of the high density and viscosity of the concentrate, these may remain as pockets of dilute juice, which will be a suitable growth medium for any Byssochlamys spores that happen to be present. Thus, one or a few spores could grow into a massive contamination. This can be prevented by storing cans or drums in an area that is free from rapid temperature fluctuations, and by equipping
storage tanks with a suitable system for ventilating the headspace.

The control of B. fulva growth by elimination of oxygen is not feasible because of the requirement to maintain strict anaerobic conditions. Growth of B. fulva can be prevented, however, by addition of 0.025% sodium benzoate or potassium sorbate to fruit juices. This could easily be done commercially with fruit juice if the use of these inhibitors is permissible.

ACKNOWLEDGMENTS

We thank J. C. Lewis of this laboratory for dipicolinic acid analyses and assistance in ascopore density measurements, C. W. Hesseltine and J. J. Elia of the Northern Regional Research Laboratory for Byssochlamys cultures and for identification of the molds we isolated, and M. Seeger of the National Canners Association for assistance with the thermal death time determinations.

LITERATURE CITED