

# Maple Sirup. XVII.

## Prevention of Mold and Yeast Growth in Maple

### Sirup by Chemical Inhibitors

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SUMMARY

Growth inhibition of mold and yeast inocula in maple sirup was studied. Low levels of esters of para-hydroxybenzoic acid (PHBA) were found to be more effective than conventional mold inhibitors (propionate, sorbate, benzoate). Sodium propyl PHBA at a concentration of less than 0.02% was inhibitory to the growth of four yeast and eight mold strains inocula in maple sirup.

IN THE PROCESS of sirup making, sap is converted to sirup by lengthy heat evaporation to remove the required amount of water. The temperature of the resulting sirup is about 217–220° F during the terminal stage of heating. All organisms initially present in the sap are destroyed during this heating process. However, when sirup is allowed to cool excessively before being packaged, contamination may enter from the air or from equipment. "Hot canning," that is, packaging maple sirup while its temperature is near 200° F, has been recommended for preventing microbial growth (6, 7, 19).

Microbial contaminants are introduced into sirup during packaging in the sugar house or during home use of small, commercial-size containers. Spoilage of maple sirup during storage of bulk stock or during consumer use of smaller containers is caused primarily by mold growth and, less frequently, by yeasts (5, 7, 8). The high osmotic pressure of standard density maple sirup prevents bacterial spoilage. However, a few molds and yeasts can survive but will not grow except where the sirup becomes diluted.

Sirups in bulk containers or in small packages usually have a free head space above the sirup. Stored sirup is usually subjected to slight changes in temperatures. During the warm cycle, moisture from the sirup distills into the head space, and this moisture condenses out as free droplets of water during the cold cycle. These droplets eventually collect on the sirup surface and dilute it.

Use of chemical inhibitors for the preservation of foods is a practice of long standing. In the United States those commonly used are: sorbate, propionate and benzoate. Recently, the esters of para-hydroxy benzoate (PHBA) have been recommended (2, 13). These esters have been employed for a number of years in cosmetics and pharmaceutical preparations (4, 11, 12, 16) and in foods in several European countries (9, 11, 16).

PHBA esters are quite soluble in aqueous and organic media (2) making them applicable to a wide variety of foods. Sokol (18) has reported that effectiveness of PHBA esters decreases as the length of the chain of the alkyl ester increases. Several investigators (2, 14, 15, 16) have reported that mixtures of the esters enhance their preservative effect, which has been termed by Littlejohn and Husa (11) a potentizing action. Because of their effective inhibition of molds, yeasts and bacteria, at low concentrations, their low toxicity (4, 10, 13, 18), and their lack of flavor, odor and color (2, 4) the esters of PHBA have been called the ideal preservative (18).

The purpose of this study was to investigate the effectiveness of chemical inhibitors, especially the PHBA esters, for preventing mold and yeast growth in pure maple sirup.

### MATERIALS AND METHODS

**Organisms.** The mold and yeast strains used in this study are shown in Table 1. Stock cultures were maintained on the modified dextrose agar medium of Sheneman and Costilow (17).

**Inocula.** Plates of dextrose agar medium were seeded with suspensions of the test organisms and incubated at 80° F. Yeast cells were harvested after one week of incubation; the mold strains were harvested after two weeks. Surface growth was removed by gently flushing with distilled water and scraping lightly with a stainless steel rod. Cell concentrations in stock

TABLE 1  
Organisms employed in study of maple sirup spoilage

Organism	Strain	Source and/or donor
<b>Molds:</b>		
<i>Aspergillus flavus</i>	ALCA-13	T. C. Cordon, Hide and Leather culture collection, EURDD
<i>Aspergillus niger</i>	2270	Maple sap, Maple Investigations culture collection, EURDD
<i>Fusarium oxysporum</i>	R-56	T. C. Cordon
<i>Fusarium</i> sp.	FBM-16	Maple sap, R. N. Costilow, Michigan State University
<i>Mucor</i> sp.	NG-12	T. C. Cordon
<i>Mucor</i> sp.	CZ-48	T. C. Cordon
<i>Penicillium oxalicum</i>	Pen. ox.	T. C. Cordon
<i>Penicillium</i> sp.	NG-1	T. C. Cordon
<i>Penicillium</i> sp.	NG-2	T. C. Cordon
<i>Rhizopus oryzae</i>	NRRL-395	T. C. Cordon
<i>Rhizopus oryzae</i>	NRRL-2004	T. C. Cordon
<i>Rhizopus</i> sp.	MX-82	T. C. Cordon
<b>Yeasts:</b>		
<i>Cryptococcus albidus</i>	496	Maple sirup, Maple Investigations culture collection, EURDD
<i>Cryptococcus</i> sp.	FBY-62	Maple sap, R. N. Costilow
<i>Rhodotorula glutinis</i>	FBY-56	Maple sap, R. N. Costilow
Unclassified	5Y	Maple sirup, Maple Investigations culture collection, EURDD
Unclassified	TY-2	Maple sirup, Maple Investigations culture collection, EURDD
Unclassified	Y-1000	Maple sirup, Maple Investigations culture collection, EURDD
Unclassified	Y-1049	Maple sirup, Maple Investigations culture collection, EURDD

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suspensions were estimated by conventional dilution and plating methods, using dextrose agar. Inocula containing desired numbers of cells were obtained by diluting the stock suspensions.

**Inoculation of maple sirup.** Appropriate amounts of the inhibitors were added, either directly or as small aliquots (1 ml or less) from stock solutions, to sterile four-ounce screw-cap bottles. U. S. Fancy grade pure maple sirup was heated to about 200° F and immediately added, in 100 ml aliquots, to the bottles containing the inhibitors. The caps were tightened, and the bottles allowed to remain undisturbed at room temperature for about one week to accomplish surface dilution (19). Bottles were then inoculated with small aliquots (1 ml or less) of appropriate dilutions of stock suspensions of the yeast and mold strains. By running the liquid slowly down the inner neck of the bottle, inocula were run on the surface "layer" of the sirup. Caps were screwed tightly in place and all bottles incubated at 80° F for at least 2 months. The viability of each dilution of the suspensions was tested by inoculating untreated sirups and checking for growth.

**Inhibitors.**<sup>b</sup> Sodium benzoate,<sup>c</sup> sodium propionate,<sup>d</sup> potassium sorbate,<sup>e</sup> methyl para-hydroxybenzoate (PHBA),<sup>f</sup> sodium methyl PHBA,<sup>f</sup> propyl PHBA,<sup>f</sup> and sodium propyl PHBA.<sup>f</sup>

**Estimation of inhibitory effect.** Inhibition was evaluated by comparing the growth (or lack of it) on treated sirups with growth of the same organism on untreated (control) sirups. Where no growth was observed on the control sirup, no evaluation could be made of the inhibitor against the strain tested. Mold growth in the inoculated sirups was examined macroscopically. The presence of discrete colonies or of a surface mat by the end of the incubation period denoted a lack of inhibition. Yeast growth in the inoculated sirups was evaluated by streaking a small aliquot (about 0.2 ml) on surfaces of dextrose agar in petri plates. The appearance of at least 3 typical yeast colonies after one week of incubation was taken as the criterion for lack of inhibition. Inhibition is expressed (Tables 2, 3, and 4) as the lowest concentration of inhibitor preventing growth in 100 ml of inoculated maple sirup.

**Estimation of flavor change in inhibitor-treated sirups.** Flavors of inhibitor-treated sirups were compared with untreated (control) sirups and evaluated by a trained, five-member taste panel.

## RESULTS

Preliminary screening of common mold inhibitors was made using potassium sorbate, sodium benzoate, and sodium propionate. Inocula of 130 cells of *Aspergillus niger*, strain 2270, were placed in bottles with 100 ml of sterile maple sirup containing 10, 20, 40, 60, 80, 100, 125, 150, 175, and 200 mg of inhibitor, respectively. Since growth was observed, after 2 weeks of incubation, in all inhibitor-treated sirups, a smaller inoculum containing only 13 cells was used in a second determination. Again, no inhibition was observed, even in sirup containing 200 mg of inhibitor per 100 ml.

The methyl and propyl esters of PHBA, as well as the sodium salts of these esters, were then tested as inhibitors of *A. niger*, strain 2270. The results observed after incubation for 69 days are shown in Table 2. While methyl PHBA inhibited growth of *A. niger*, propyl PHBA was inhibitory at the lowest level tested, 3.33 mg per 100 ml of sirup.

Since propyl PHBA was more effective than methyl PHBA as a mold inhibitor, further studies were carried out against 4 concentrations of *A. niger* using concentrations of 0.33, 1.67, 3.33, and 16.7 mg of propyl PHBA and sodium propyl PHBA per 100 ml of sirup. The results of this study, observed after

<sup>b</sup> Mention of the names of companies supplying the inhibitors mentioned herein does not imply U. S. Department of Agriculture endorsement of their products over those of companies not named.

<sup>c</sup> Eastman Organic Chemicals, Rochester, New York.

<sup>d</sup> Nutritional Biochemicals Corporation, Cleveland, Ohio.

<sup>e</sup> Union Carbide Chemicals Company, New York, New York.

<sup>f</sup> Chemo Puro Manufacturing Corporation, Newark, New Jersey.

incubation for 61 days, are shown in Table 3. The antifungal effectiveness of propyl PHBA and sodium propyl PHBA is apparent from the inhibition by low concentrations against heavy inocula. The sodium salt is slightly less effective than the acid form.

TABLE 2

Inhibition of growth of *Aspergillus niger*, strain 2270, in 100 ml of maple sirup containing esters of para-hydroxybenzoic acid (PHBA)

Inoculum Inhibitor	Lowest concentration of ester inhibiting growth, mg	
	20 cells	200 cells
Methyl PHBA.....	16.7	25
Sodium methyl PHBA.....	25	66
Propyl PHBA.....	3.33	3.33
Sodium propyl PHBA.....	3.33	3.33

Because of the ease of solubility of sodium propyl PHBA (2, 4), it was used in subsequent experiments with other mold and yeast strains. Three levels of inocula (20, 200, and 2000 cells per bottle) were tested against four concentrations (0.33, 1.67, 3.33, and 16.7 mg) of sodium propyl PHBA per 100 ml of sirup. The test organisms included a variety of mold and yeast strains, several of which had been isolated from maple sap and sirup (Table 1). The effectiveness of sodium propyl PHBA as a growth inhibitor can be seen from the results (Table 4).

TABLE 3

Inhibition of growth of *Aspergillus niger*, strain 2270, in 100 ml of maple sirup containing propyl para-hydroxybenzoic acid (PHPA) and sodium propyl PHBA

Inoculum, cells	Lowest concentration of ester inhibiting growth, mg	
	Propyl PHBA	Sodium propyl PHBA
2000.....	3.33	16.7
200.....	3.33	3.33
20.....	1.67	3.33
2.....	1.67	1.67

Since 4 of 11 mold strains tested did not grow in untreated (control) sirups, they are not included in Table 4. These strains are R-56, FBM-16, CZ-48, and NG-2. Likewise, the following yeast strains did not grow in untreated (control) maple sirup and therefore are not shown in Table 4: FBY-62, FBY-56, and Y-1049.

The taste panel was unable to detect any flavor differences among samples of maple sirup either devoid of or containing the levels of sodium propyl PHBA employed in this investigation.

## DISCUSSION

The yeasts and molds selected for this study consisted of isolates from maple sap and sirup, including some known to tolerate the osmotic pressure of standard density maple sirup. Also, other strains whose growth characteristics in maple sirup were not known were included to give a broader spectrum of representatives from these two groups of organisms. Data in Table 4 include only those strains which showed growth in the control (untreated) sirup since it is impossible to evaluate the effect of the inhibitor on those strains which did not grow. Several concentrations of inocula and inhibitors were tested to compare the effectiveness of the different inhibitors under various conditions.

Preliminary experiments showed that the PHBA esters, commonly called "parasepts" or "parabens"

TABLE 4

Inhibition of mold and yeast growth in 100 ml of maple sirup containing sodium propyl para-hydroxybenzoic acid

Inoculum Organism	Lowest concentration inhibiting growth, mg		
	20 cells	200 cells	2000 cells
<b>Molds:</b>			
<i>Aspergillus flavus</i> , ALCA-13.....	3.33	16.7	16.7
<i>Mucor</i> sp., NG-12.....	3.33	16.7	16.7
<i>Penicillium oxalicum</i> , Pen. ox.....	1.67	1.67	3.33
<i>Penicillium</i> sp., NG-1.....	3.33	16.7	16.7
<i>Rhizopus oryzae</i> , NRRL-395.....	16.7	16.7	16.7
<i>Rhizopus oryzae</i> , NRRL-2004.....	3.33	16.7	16.7
<i>Rhizopus</i> sp., MX-82.....	16.7	16.7	16.7
<b>Yeasts:</b>			
<i>Cryptococcus albidus</i> , 496.....	0.33	0.33	1.67
Unclassified, 5Y.....	3.33	16.7	16.7
Unclassified, TY-2.....	0.33	0.33	1.67
Unclassified, Y-1000.....	16.7	16.7	16.7

(13), were superior to salts of sorbate, benzoate, and propionate as mold inhibitors in maple sirup. Therefore, the latter more commonly-used food preservatives were not included in subsequent experiments.

Propyl PHBA was found to be a more effective mold inhibitor than methyl PHBA (Table 2), substantiating the findings of Mathews *et al* (13) who reported that the necessary effective concentration of PHBA esters decreased with chain length of the alkyl ester.

The acid form of propyl and methyl PHBA (Tables 2 and 3) is effective at lower concentrations than the respective sodium salts of these esters. Nevertheless, in subsequent studies involving a variety of mold and yeast strains, the sodium salt of propyl PHBA was employed since its solubility in maple sirup was greater than the acid form.

The effectiveness of sodium propyl PHBA against several concentrations of the various mold and yeast strains is shown in Tables 3 and 4. In most cases, as the size of the inoculum was increased the inhibitory concentration required also was increased. None of the inocula, even at a level of 2000 cells per 100 ml, was able to grow in the presence of 16.7 mg (less than 0.02%) of sodium propyl PHBA. Since a contamination of 2000 cells per 100 ml of maple sirup is quite unlikely, it would appear that a concentration of 0.02% sodium propyl PHBA would be effective in controlling mold and yeast growth in maple sirup during ordinary handling (packaging, storage, and consumption). The lack of taste at this concentration would not interfere with the delicate maple flavor.

#### Acknowledgments

We wish to thank Mr. R. A. Bell for his technical assistance and Drs. T. C. Cordon and R. N. Costilow for supplying many of the cultures used in this study.

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