Preservation of Mabí by Chemical and Physical Means[1]

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ABSTRACT

Mabí, a fermented drink made from the bark of the mabí tree (*Colubrina elliptica*), spoils if it is not refrigerated. Attempts were made to preserve mabí by adding to it 0.05 to 0.1% (w/v) of sodium benzoate and of potassium sorbate. Preservative effect was gauged by the growth of coliforms and yeasts, which are the organisms responsible for mabí fermentation. Also, mabí was pasteurized at 65° C for different periods of time. Mabí to which sodium benzoate at 0.07 and 0.09% levels was added and mabí pasteurized at 65.6° C for 15 min, kept well at room temperature (28 ± 2° C) for up to 16 weeks. These samples were rated acceptable to excellent by members of a taste panel. Potassium sorbate at up to 0.1%, as well as a mixture of 0.05% potassium sorbate plus 0.05% sodium benzoate, was less effective than sodium benzoate alone at 0.07–0.1%, in preservating mabí.

INTRODUCTION

Although mabí (maví) has been consumed by Puerto Ricans for many years, it is still produced on a day-to-day, small scale level. One reason for this is that, it has to be refrigerated immediately after preparation, or it will spoil. Even under refrigeration, deterioration occurs after about 5 days. Spoilage is due to post-fermentation, since not enough acid is produced to completely inhibit microbial activity.

Many fruit juices, fruit drinks, bottled soft drinks and syrups are preserved by the addition of sodium benzoate (2, 7, 10, 19, 23), and in some cases by sorbic acid and its salts (1, 7, 8, 22). Of the physical methods, heat is the one most commonly used to pasteurize liquid foods and drinks. The above-mentioned methods of food preservation are universally approved by the regulatory agencies and are easy and relatively cheap to apply (2, 9, 11).

Preservation of mabí by any approved chemical or physical means which does not decrease its acceptance would foster its production and distribution, so it might even compete successfully with other well-known refreshing drinks.

Previous studies by Montoya and Graham (14), have demonstrated that mabí fermentation is carried out by the joint action of bacteria of the *Enterobacter* and *Citrobacter* groups (5) and a yeast, *Saccharomyces cerevisiae*. These microorganisms occur in the unrefined sugar used and in the inoculum or starter, known locally as "pie" (12, 14, 20). The

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possibility of preparing mabí under conditions of controlled fermentation is very good. If mabí can be economically and properly preserved for storage at ambient tropical temperatures, this controlled fermentation would be a worthwhile contribution towards the commercial production of this well-liked local drink.

This paper summarizes efforts to preserve mabí by the addition of sodium benzoate or potassium sorbate and by pasteurization. It reports on the acceptance of the preserved product by a panel of consumers.

MATERIALS AND METHODS

The chemicals used were sodium benzoate, U.S.P. grade, Fisher Scientific Co.;[3] a 5% stock solution was prepared by dissolving 50 g in sterile distilled water, making up to a volume of 1 liter and storing it in the refrigerator until needed; a 5% solution of potassium sorbate, Sentry, Union Carbide Chemical Co. was prepared in the same manner as for sodium benzoate.

The mabí extract was prepared by boiling 100 g of mabí bark in 1 liter of water for 30 min, cooling the mixture and then straining it through four layers of cheesecloth. The filtrate was collected, the volume adjusted to 1 liter and then stored in the refrigerator until needed.

A 15% (w/v) solution of unrefined sugar was prepared by dissolving 15 g in tap water and making the volume up to 100 ml. Larger quantities can be made up as needed.

The "pie" or inoculum was an aliquot of a previous batch of (fermented) mabí. A 5% (v/v) inoculum was used.

The fermentation mixture was prepared by adding 15 ml of the extract [15%, (v/v)], to 935 ml of the sugar solution, mixing them well and then inoculating the sugary mixture with 50 ml of the starter or pie [5% v/v]. The total volume was 1000 ml, but larger quantities can be prepared as desired. The inoculated mixture was allowed to ferment at room temperature (28 ± 2° C) for approximately 48 h.

At the end of the fermentation period, a portion of the mabí was used for the preservation experiments, while another portion stored in the refrigerator served as control.

After each fermentation an aliquot was separated for use as "pie" or inoculum for the next batch of mabí. In this way, the production of mabí was continuous and a fresh inoculum or starter was always used.

To assess the effect of sodium benzoate or potassium sorbate, aliquots

[3] Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

of the stock solutions of the preservatives were added to freshly prepared mabí to give final concentrations of sodium benzoate of 0–0.1% and of potassium sorbate of 0.0, 0.05 and 0.1%. Table 1 shows the protocol.

Ten sterile dilution bottles (99 ml) were filled aseptically from each batch of mabí containing a specific concentration of the preservative and stored at room temperature (28 ± 2° C). The bottles containing no preservative (zero levels) served as controls and were stored in the refrigerator. At selected time intervals between the end of the fermentation period and up to 15 days thereafter, microbiological counts were made for coliforms and for yeasts. Day zero was taken as the end of the fermentation period. Counting was done by plating 1 ml of appropriate dilutions in physiological saline on violet red bile agar for coliforms, and potato dextrose agar acidified with tartaric acid to pH 3.5, for yeasts (6, 15).

TABLE 1.—*Protocol for preparing mabí with varying concentrations of sodium benzoate and potassium sorbate*

Preservative	Mabi	Preservative (5%) added	Final concentration of preservative
	ml	*ml*	%
Sodium benzoate	1000	0	0.00
	990	10	0.05
	988	12	0.06
	986	14	0.07
	982	18	0.09
	980	20	0.10
Potassium sorbate	990	10	0.05
	980	20	0.10

The pH of each preparation of mabí was measured at the varying periods with a Beckman Zeromatic pH meter.

Preservation by heat was done by aseptically placing the mabí in sterile dilution bottles, stoppering them tightly and then heating the bottled mixture in a constant temperature water bath at 65.6° C (150° F) for 0, 5, 10, 15, 20 and 30 min. An equilibrium time of 15 min was allowed for the contents of the bottle to attain the temperature of the water bath. Timing started, therefore, at the end of the equilibrium period. Equilibrium time was determined as follows: A thermometer was placed in each of the three dilution bottles containing mabí. The thermometer was fitted tightly through the bottle stopper. The bottles were placed in the agitated water bath and the time necessary for the mabí to reach 65.6° C was noted. One bottle was placed at each end of the bath and the third bottle placed in the middle of the bath.

RESULTS AND DISCUSSION

Figure 1 shows the effect of sodium benzoate and of potassium sorbate on the number of coliforms. In all cases, the number of coliforms diminished when the preservatives were added to the drink. The number of coliforms decreased slowly in the control because of the inhibitory property of the acids formed during the fermentation and because of the low temperature.

Even at a concentration of 0.05%, the number decreased to zero 5 days after the addition of sodium benzoate. As the concentration of sodium benzoate increased, the decrease in the number of coliforms was more rapid, and at a concentration of 0.9% there was a total inhibition 1 day after the addition of the preservative.

In mabí containing 0.05% sodium benzoate, turbidity and gas formation were observed, indicating that active fermentation was still taking place.

The yeast showed greater resistance to sodium benzoate (fig. 2). The number remained relatively high for up to 8 days at up to 0.06% sodium benzoate. At higher concentrations a rapid initial decrease occurred 1–3 days after the addition of the benzoate, but the destruction or inhibition was not complete until after 7 days, even at the maximum concentration of 0.1%. The control of yeasts with preservatives has been reviewed recently by Sauer (17) and Walker (21); their role in spoilage by Miller (13) and Peppler (16), and their significance in foods by Anderson (3).

Potassium sorbate at a concentration of 0.05% was much more effective against the coliforms than was sodium benzoate at the same concentration resulting in a zero count after 2 days.

Even at a concentration of 0.1% potassium sorbate, only 70% of the yeasts were destroyed after 1 day; hence this preservative had a greater effect against the coliforms than against the yeast.

Table 2 shows the percentage inhibition of coliforms and yeast after treatment with sodium benzoate, potassium sorbate and with heating at 65.6° C. Even during refrigeration, the coliform count declined rapidly, and after 7 days none was detected. At the same time, the yeast count declined very slowly, and even after 7 days was still high. At all concentrations of sodium benzoate, the coliforms were inhibited more severely than the yeasts. At 0.09% and 0.1% of sodium benzoate, none of the coliforms and less than 8% of the yeasts were detectable 1 day after addition of the preservative.

Heating mabí at 65.6° C for 15 min after the come-up time destroyed all the organisms therein.

Samples of mabí preserved with sodium benzoate at 0.07 and 0.09%, as well as freshly prepared mabí containing no preservatives, were tasted by students and faculty on three different occasions; that is, three different

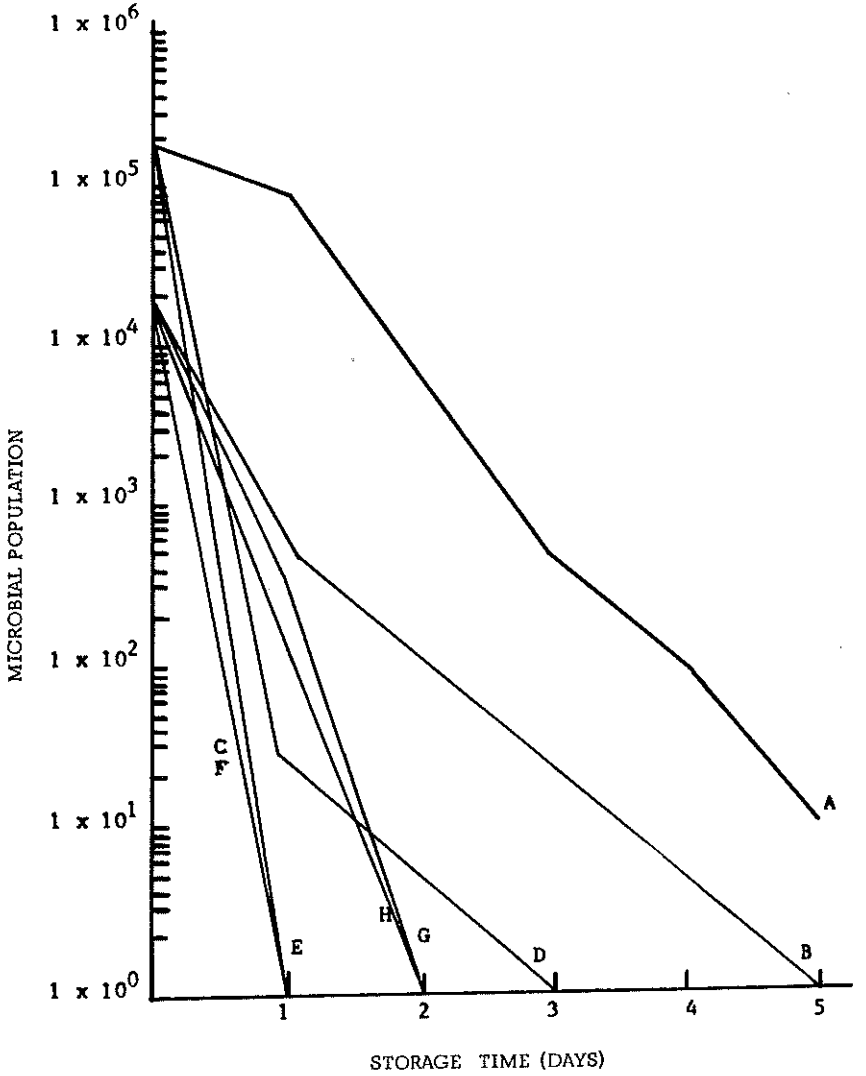

FIG. 1.—Changes in the number of coliforms due to treatment of mabi with sodium benzoate and potassium sorbate.

A. No preservative added (refrigerated);
B. Sodium benzoate, 0.05 %;
C. Sodium benzoate, 0.05 %;
D. Sodium benzoate, 0.07 %;
E. Sodium benzoate, 0.09 %;
F. Sodium benzoate, 0.1 %;
G. Potassium sorbate, 0.05 %;
H. Potassium sorbate, 0.1 %.

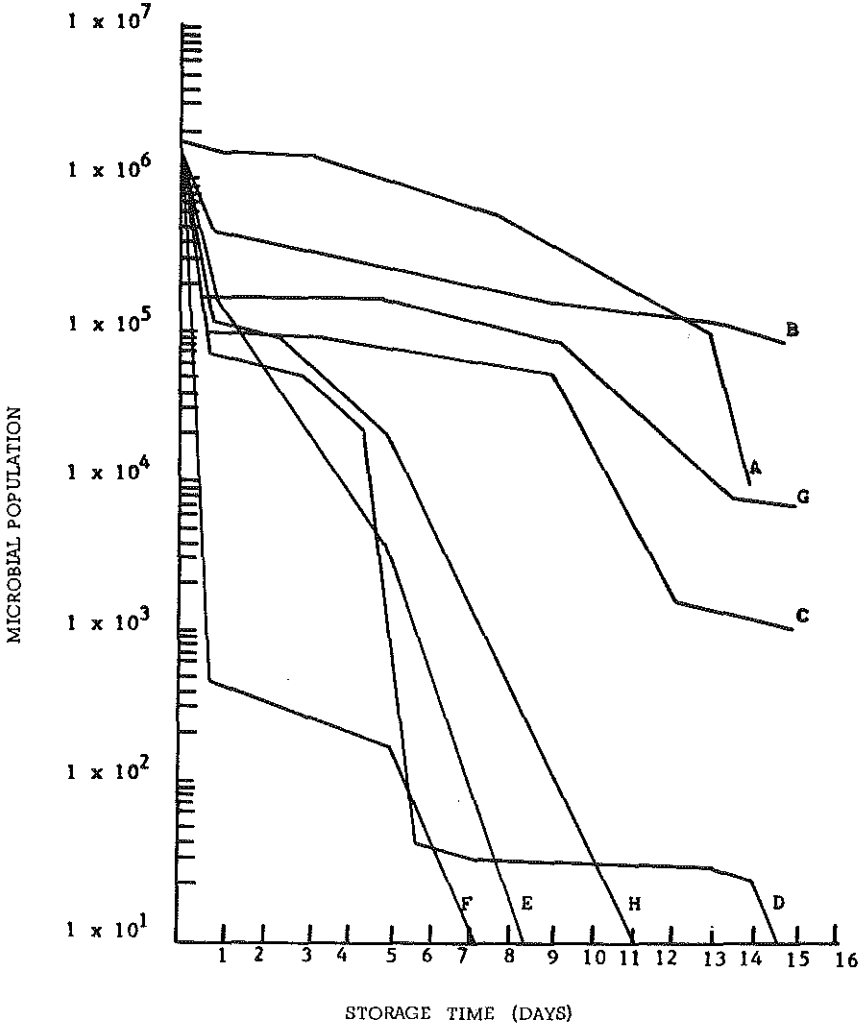

FIG. 2.—Changes in the number of yeasts due to treatment of mabi with sodium benzoate and potassium sorbate.

A. No preservative added (refrigerated);
B. Sodium benzoate, 0.05 %;
C. Sodium benzoate, 0.06 %;
D. Sodium benzoate, 0.07 %;
E. Sodium benzoate, 0.09 %;
F. Sodium benzoate, 0.1 %;
G. Potassium sorbate, 0.05 %;
H. Potassium sorbate, 0.1 %.

sets of preparations were tested. On the average, and as compared to the fresh mabí, 80% of the consumers rated mabí containing 0.07% sodium benzoate as acceptable or excellent (i.e., as having the same taste and flavor as or better than the control or unpreserved sample). Sixty percent of the tasters gave an acceptable to excellent rating for the mabí containing 0.09% sodium benzoate.

Mabí preserved with 0.1% sodium benzoate had a slightly bitter taste and received a lower rating than that preserved with 0.07 or 0.09% sodium benzoate. This bitter taste of sodium benzoate has been encountered in apple juice by Dryden and Hills (7).

TABLE 2.—*Inhibition of microorganisms in mabi by sodium benzoate, potassium sorbate and pasteurization*

Treatment	Micro-organism[1]	Days of storage				
		1	3	5	7	9
		Percent of inhibition				
No perservative added	C	52.9	99.7	99.9	100	100.0
(refrigerated)	Y	5.9	5.9	17.6	57.6	69.0
Sodium benzoate	C	97.1	99.8	100	100.0	100
(0.05%)	Y	71.1	80.7	84.0	87.0	89.1
Sodium benzoate	C	99.9	100	100	100.0	100
(0.06%)	Y	93.8	94.0	95.1		96.1
Sodium benzoate	C	100	100	100.0	100.0	100.0
(0.07%)	Y	95.2	96.4	91.1	100.0	100.0
Sodium benzoate	C	100	100	100	100.0	100.0
(0.09%)	Y	96.3	99.1	99.9	100.0	100.0
Sodium benzoate	C	100	100.0	100	100.0	100
(0.1%)	Y	97.4	100.0	100	100.0	100
Potassium sorbate	C	97.8	100.0	100	100.0	100
(0.05%)	Y	66.0	67.0	66.0	72.4	82.6
Potassium sorbate	C	99.1	100.0	100	100.0	100.0
(0.1%)	Y	70.0	89.0	94.0	96.0	100.0
Pasteurization	C	100	100.0	100	100.0	100.0
(15, 20, 25, 30, 45 min-utes)	Y	100	100.0	100	100.0	100.0

[1] C = coliforms; Y = yeasts.

Mabí without preservative and refrigerated for 5 days was less acceptable to the consumers than freshly prepared mabí. This fact indicates that even refrigeration does not maintain the original quality.

Mabí heated at 65.6° C for 15 min (after come-up time) was rated as equal in taste to freshly prepared mabí. However, samples heated for longer periods lost their flavor and tasted more like an aqueous sugar solution. This was due, most likely, to the loss of volatile flavoring substances.

Addition of preservatives caused a shift in the pH of the mabí. In all

cases, immediately after addition of sodium benzoate or potassium sorbate at concentrations of 0.05–0.1%, the pH increased. After 24 h the pH declined and kept on falling for up to 15 days or longer (table 3).

Pasteurization for 15-45 min at 65.6° C led to no change in pH, even if the samples were stored at room temperature (28 ± 2° C) for up to 5 days.

Chemical preservatives differ in their efficacy and specificity (18). Sorbic acid, a diene, is an established antifungal agent, which at low concentrations is effective in the control of mold and yeasts in fruit juices,

TABLE 3.—*Changes in pH of mabi after addition of sodium benzoate, potassium sorbate, pasteurization and storage for varying periods*

Treatment	pH[1]	pH[2]	Days of storage				
			1	3	5	7	9
No preservative added (refrigerated)[3]	3.70		3.65	3.65	3.56	3.53	
Sodium benzoate[4] (0.05%)	3.63	3.92	3.70	3.48	3.31	3.25	3.21
Sodium benzoate (0.06%)	3.63	4.02	3.81	3.62	3.47	3.44	3.42
Sodium benzoate[4] (0.07%)	3.70	4.10	4.00	3.80	3.70	3.60	3.60
Sodium benzoate (0.09%)	3.70	4.10	4.00	3.80	3.73	3.70	3.60
Sodium benzoate (0.1%)	3.63	4.10	3.97	3.72	3.60	3.59	3.57
Potassium sorbate[4] (0.05%)	3.75	4.10	3.90	3.64	3.50	3.46	3.43
Potassium sorbate[4] (0.1%)	3.75	4.20	4.15	3.81	3.70	3.64	3.60
Pasteurization (15, 20, 25, 30, 45 minutes)	3.59	3.59	3.59	3.59	3.59	3.59	3.59

[1] pH of mabi at the end of the fermentation period (48 hours).
[2] pH of mabi immediately after addition of the preservative.
[3] Storage at 9°C.
[4] Storage at 26°C.

soft drinks, wines, cheese, sauerkraut and certain meat and fish products. Its potassium salt, which is very soluble in water is used preferably in foods, especially liquid foods. Both the acid and its salts have broad spectrum activity against yeast and molds, but are less active against bacteria.

Sorbic acid is effective in most foods at a concentration range of 0.05 to 0.3%, by weight, and reportedly (1), even at the upper limit of use, no taste is detectable. Generally, the higher the level or sorbate, the more extended the period of inhibition.

Sorbates have been shown to be effective in the preservation of fruit juices, but in these experiments they were not as effective as sodium benzoate in bringing about an overall preservative effect. The presence of a mixed culture and the pH effects probably explain the relatively lower activity against the bacteria.

Sodium benzoate, which is quite soluble in water, has been used for a long time as an antimicrobial agent in foods (2, 4, 10). Generally, it is thought to be most active against yeasts and bacteria and less active against molds. It is most suitable for beverages and foods which have a natural pH range below 4.5 or 4.0 or which can be brought to such pH by acidification. Its activity is highly influenced by pH. At pH levels of 3.5–4.0, the pH range of most fruit juices, a concentration of 0.06–0.1% has been reported to be necessary to prevent the growth of most fermentation organisms. A similar trend has been observed here in that preservation of mabí was best at concentrations of 0.07–0.1% of benzoate; and mabí has a natural pH between 3.51–4.1 (14).

Sodium benzoate, therefore, seems to be a logical choice for the chemical preservation of mabí, especially in view of its long established and widespread use as an antimicrobial agent in carbonated and non-carbonated beverages, syrups and other food products and also for its legal status as a food additive permitted for use in most countries.

A mixture of 0.05% sodium benzoate and 0.05% potassium sorbate was not as effective as sodium benzoate alone at concentrations of 0.07–0.1%.

The results reported here suggest that sodium benzoate at 0.07–0.09% and pasteurization at 65.6° C for 15 min (after 15 min come up time) show promise for the preservation of mabí, thus eliminating the necessity for refrigeration of the product after fermentation.

RESUMEN

El mabí se sometió a diferentes tratamientos de preservación utilizando concentraciones de 0.05 a 0.1% de benzoato de sodio y de sorbato de potasio. También, se pasteurizó a 65.6° C a distintos intervalos de tiempo. Se realizaron catas para inquirir la aceptación del público al mabí sometido a tratamiento de preservación. Al mabí que contenía 0.07 y 0.09% de benzoato de sodio y al tratado con calor por 15 minutos los catadores los juzgaron entre aceptables y excelentes.

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