Microbiological Studies of Mabí Fermentation[1]

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ABSTRACT

Commercial samples of mabí, mabí bark and refined sugar were obtained from various sources and examined for the types of microorganisms in them.

The principal microorganisms found in commercial mabi were: *Enterobacter aerogenes* (1); *Enterobacter cloacae* (2); *Citrobacter intermedius* (3); and the yeast *Saccharomyces cerevisiae* (4). *Escherichia coli* (5) was isolated from one sample only.

Mabí bark contained 2 and 3, while sugar samples contained 1, 3, and 4. An *Asperigillus* species and a *Penicillium* species were found in the sugar samples.

Mabí prepared in the laboratory with pure cultures of (1+2+3+4), or (1+4) tasted like commercial mabí, according to 78% of the members of a taste panel.

It is concluded that mabí fermentation is a natural fermentation carried out mainly by bacteria 1 and 2, the yeast *Saccharomyces cerevisiae*, and possibly by *Citrobacter intermedius*. *E. coli* does not seem to be involved, unless unsanitary conditions prevail during the preparation of the drink.

INTRODUCTION

Mabí is an acidic, low alcoholic, sweet beverage which is very popular in Puerto Rico. According to Cabanillas de Rodríguez (5) mabí was consumed in Puerto Rico as a popular beverage as early as the 16th century. The drink is known also in other islands of the Caribbean, in Surinam, South America, and other places. Alvarez (1) pointed out the resemblance of the name mabí to a fermented drink in the Belgian Congo made from the bark of a tree. In many of the West Indian Islands, drinks with names pronounced similarly to mabí are found but the spelling is different. In addition, it seems that the drink is prepared differently, is not as sweet and, in some cases, is used mostly as a tonic rather than as a refreshing beverage.

In Puerto Rico, mabí is prepared through the fermentation of a mixture of unrefined sugar, an aqueous extract of the bark of the mabí tree or shrub and an inoculum or starter. The starter, known locally as "pie", is simply a portion of previously fermented mabí.

The mabí tree or shrub *Colubrina elliptica* occurs principally in the southwestern regions of Puerto Rico. It is about 10–15 feet high and less than 4 inches in diameter at the trunk. Besides Puerto Rico, its occurrence has been noted in other Caribbean islands, Surinam, South America, the Bahamas, Florida, Yucatán and Guatemala (7).

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Despite the long use and popularity of the drink in Puerto Rico, mabí fermentation has not received much scientific investigation. Soltero and Hernández-Mora (10) suggested that coliforms were involved in the fermentation, but as far as can be ascertained no other report on the fermentation is available. Therefore, in view of the paucity of information, studies were initiated to gain information on the microorganisms involved in mabí fermentation.

MATERIALS AND METHODS

Ten commercial samples of mabí were obtained from the following eight locations: Mayagüez (2), Cabo Rojo (1), Toa Baja (1), Barceloneta (1), Vega Baja (2), Río Piedras (1), Juana Díaz (1), and Bayamón (1).

Two samples of unrefined sugar were analyzed for their microbiological content. The samples were from Mayagüez and Yauco.

Dry mabí bark was obtained from commercial places in Yauco, Mayagüez and Cabo Rojo. Green bark was obtained from Cerro Vertero, Lajas.

Samples of tap water were collected in sterile bottles from the following locations: Añasco, Mayagüez, Cabo Rojo, Arecibo and Lajas. The bottles were taken to the laboratory and placed in the refrigerator.

Microbiological examinations were made on the samples as soon as possible after arrival at the laboratory. In the case of the mabí and water samples, analyses were done immediately after arrival. The pH of the mabí and of the water were measured with a Beckman pH meter.

Microorganisms in the samples of mabí, sugar and bark were isolated by the procedures outlined by the Difco Manual of Dehydrated Culture Media and Reagents (6), and the American Public Health Association (2, 3). Microorganisms in the water samples were isolated by the procedures outlined by the American Public Health Association (3).

The bacteria isolated were classified and identified according to Bergey's Manual (4). Yeasts were classified according to information outlined in Lodder (8) and fungi identified by reference to Moore-Landecker (9).

Aliquots of the mabí and the water, after appropriate dilution, were plated on the following media to facilitate propagation and isolation. The propagation media were: trypticase soy broth for aerobes and fluid thioglycollate medium for anaerobes and microerophilics, both incubated at 32°C for 24 hours; Sabouraud liquid medium for yeasts and molds, and malt extract broth for yeasts, both incubated at 28°C for 48 hours.

The isolation media were staphylococcus agar for staphylococcus, blood agar for streptococcus, and McConkey agar for coliforms, the three of which were incubated at 32°C for 24 hours; and Sabouraud agar for yeasts and molds and malt agar for yeasts both of which were incubated at 28°C for 3 days.

After incubation in the propagation media, samples were removed

aseptically for Gram staining and for inoculation of the different isolation media listed above.

For analysis of the mabí bark, a weighed amount was placed in a sterile Waring blender containing phosphate buffer at pH 6.8 and macerated for 2 minutes. From this mixture an aliquot was used to inoculate the propagation media, from which the isolation media were, in turn, inoculated.

Identification and classification of the microorganisms were based on morphological and biochemical characteristics. Morphological characteristics of cells growing in liquid media and of colonies growing on solid media were observed. Freshly cultivated cells were Gram stained.

The biochemical tests performed were as follows: fermentation of sugars in Durham tubes with bromthymol blue as indicator; utilization of sugars, employing the auxonogram technique; production of H_2S in agar medium containing lactose, sucrose and iron; production of indole in tryptone medium using the Kovacs reagent to read the test; production of acid in Voges-Proskauer methylene red medium; production of acetylmethylcarbinol (Voges-Proskauer test); utilization of urea in urea-agar medium; and hydrolysis of gelatin.

These tests were done with the purified cultures of the microorganisms isolated as described previously.

Based on their morphological characteristics and biochemical activities, the principal organisms present in the commercial samples of mabí, the water, the sugar and the bark were identified. Analysis of the data indicated the ones which were most prevalent and active in mabí fermentation. These cultures were then used to inoculate sterile solutions of bark and unrefined sugar. The pure cultures of microorganisms served, therefore, as inoculum (starter or pie) in these controlled or laboratory fermentations.

The following variables were investigated in different batches of this controlled or laboratory-fermented mabí: the relationship of initial pH to final pH in the fermented drink; relative changes in the population of the principal fermenting organisms; and consumer acceptance of the laboratory or controlled, fermented beverage as compared to the product prepared under commercial conditions. The term "commercial" is used here to indicate the everyday procedure used by mabí vendors.

PREPARATION OF MABI BY "COMMERCIAL" PROCEDURE

The basic ingredients are an aqueous extract of mabí bark, unrefined (brown or dark sugar), water, and an inoculum or starter.

For the preparation of the extract, 100 g of mabí bark were boiled in water for 30 min. The mixture was cooled and strained through a clean piece of cheesecloth (several layers, if necessary). The filtrate was made

up to 1 L with tap water and stored in the refrigerator until required for use.

The sugar solution was prepared with 15 g of unrefined sugar dissolved in tap water and the volume made up to 100 ml.

The starter or inoculum was simply a portion of a batch of mabí previously prepared. It contained the active microorganisms to carry out the fermentation. It is known locally as "pie". The amount of starter (inoculum or pie) used is, generally, equal to 5% (V/V) of the total volume of the fermenting mixture.

For each 100 ml of the fermenting mixture there were added 1.5 ml. of the aqueous mabí bark extract (1.5% V/V); inoculum or pie—5 ml (= 5% V/V); and sugar solution—93.5 ml = 93.5% (V/V). The mixture was shaken well, placed in a large-mouth flask, bottle or carboy, depending on the quantity, and allowed to ferment for approximately 48 hr at 28 ± 2° C. At the end of this period, the fermented mixture was placed in the refrigerator overnight and served cold.

PREPARATION OF MABÍ BY CONTROLLED FERMENTATION

The preparation of the mabí bark extract and of the sugar solution were the same as in the preparation of commercial mabí. However, these preparations were sterilized in the autoclave for 30 min at 15 lb/in^2 of pressure (121°C).

Ten-ml portions of sterile trypticase broth and of Sabouraud broth were inoculated with pure cultures of *Enterobacter aerogenes* and of *Saccharomyces cerevisiae,* respectively. The former (bacterial culture) was incbated at 32° C for 12 hr and the latter (yeast culture) was incubated at 28° C for 24 hr.

One hundred ml of the sterile substrate were inoculated with these cultures to provide the starter (pie or inoculum) for further studies. The substrate consisted of 1.5 ml of the mabí bark extract and 98.5 ml of the sugar solution.

The following starters or inocula were used: bacterium only—*Enterobacter aerogenes;* yeast only—*Saccharomyces cerevisiae;* and bacterium plus yeast.

For each 100 ml of the final mixture, 1.5 ml of the bark extract were added. This mixture was autoclaved for 30 min at 15 lb/in^2. The mixture was cooled and then inoculated with the starters.

For studies on the relationship of initial pH to the final pH of the fermented mixture, three separate batches were prepared from the following inocula: Bacterium only; 5 ml of the *Enterobacter aerogenes* starter was added to the sterilized substrate. Yeast only: 5 ml of the yeast starter (*Saccharomyces cerevisiae*) was added to the sterilized substrate. Bacterium plus the yeast starter: 5 ml of the starter (*Enterobacter*

aerogenes) and 5 ml of the yeast starter (*Saccharomyces cerevisiae*) were added to the sterilized substrate. In all cases, the total basic volumes were 100 ml. When necessary, the initial pH of the substrate plus the inoculum was adjusted to the desired level by the addition of sterile 10% lactic acid or 5% dibasic potassium phosphate.

Changes in the population of the bacteria and the yeast during fermentation were studied by inoculating the sterile substrate with a mixed starter of *Enterobacter aerogenes* (5% final concentration, V/V) and of *Saccharomyces cerevisiae* (5% final concentration, V/V).

Samples for tasting were as follows: 1) Control, mabí prepared according to commercial practices as previously outlined; 2) controlled or laboratory fermented mabí. Three different types were prepared using as starter or inoculum: *Enterobacter aerogenes, Saccharomyces cerevisiae,* and a mixed starter of *Enterobacter aerogenes* plus *Saccharomyces cerevisiae.*

RESULTS AND DISCUSSIONS

Table 1 shows the protocol for identifying the bacteria in the samples examined. The bacteria encountered were all gram-negative rods.

Table 2 shows the characteristics of the yeast isolated and identified as *Saccharomyces cerevisiae.*

Table 3 summarizes the bacteria and yeast present in the commercial samples of mabí, the coliform as well as the yeast populations in them and the pH of each sample. Neither fungi nor bacilli were found in any of the commercial samples examined. *Saccharomyces cerevisiae* was present in all of the samples. One sample of sugar contained a Gram-positive spore-forming bacillus.

Only one sample contained *Escherichia coli.* Pathogenic organisms were not detected in any sample. This is a tribute to a commodity which is prepared and sold so widely in small establishments and modest mobile units around school premises, factories, sports arenas and other public places.

In all cases, the yeast count was much higher than the bacterial count. Since these were commercial samples, many of the more acid-sensitive enteric bacteria could have died off because the history of the products was not known. This dying-off probably accounted, at least partly, for the complete absence of bacteria in two samples (M-3 and M-4).

Table 4 summarizes the microorganisms found on the mabí bark (B) and in the unrefined sugar samples. On the bark, *Enterobacter cloacae* was found, but fungi, yeasts and bacilli were absent. *Enterobacter aerogenes, Aspergillus* sp., *Penicillium* sp. and yeasts were found in the sugar samples. Characterization and identification of the fungi *Aspergillus* and *Penicillium* were based on growth on potato dextrose agar, mycelia, conidiophore and spore formation and other salient characteristics.

The results indicate that the principal microorganisms in mabí are derived from the ingredients used. The inoculum (starter or pie) and the sugar contribute bacteria and the yeast. The bark makes no microbial contribution to the fermentation mixture because, in practice, the bark concoction is boiled vigorously in preparing the extract and so microorganisms thereon are killed, except probably for very resistant microbial

TABLE 1.—*Characterization of bacterial colonies isolated*

Character or reaction	Cultures isolated and characterized			
	1	2	3	4
Gram stain	—	—	—	—
Size (microns)	1.0–2.0 ×0.6–0.8	1.0–2.0 ×0.5–1.0	1.0–3.0 ×0.5–0.6	1.0–30 ×0.5–0.6
Motility	+	+	+	+
EMB agar	–	–	+[1]	+[1]
H_2S production	–	–	–	–
Indole	–	–	+	–(+)[2]
Citrate	+	+	–	+
Methyl red	–	–	+	+
V. P. reaction	+	+	–	–
Catalase	+	+	+	+
Urea	+	+	–	+
Gelatin hydrolysis	–	+	–	–
	Fermentation of Sugars			
Glucose	A + G[3]	A + G	A + G	A +G
Lactose	A + G	A + G	A + G	A +G
Sucrose	A + G	A + G	A + G	A +G
Classification	*Enterobacter aerogenes*	*Enterobacter cloacae*	*Escherichia coli*	*Citrobacter intermedius*

[1] Positive reaction on Levine's EMB (Easin Methylene blue) agar. Typical *E. coli* colonies are deep blue black in color and have a metallic sheen caused by reflected light. Typical colonies of *Enterobacter aerogenes* (*Aerobacter aerogenes*) do not have a metallic sheen and are pale pink or lavender in color.

[2] Two cultures gave negative indole test while two gave positive indole test.

[3] A = acid; G = gas.

spores, should any be present. The molds noted in table 4 were most likely contaminants in these particular samples of sugar.

There were isolated several cultures whose colony form and morphological characteristics resembled those of *E. coli* and of *Enterobacter aerogenes,* but their biochemical characteristics differed in two respects: namely, in the production of indole and in the utilization of citrate. These were originally designated as so-called "intermediate" coliforms, i.e., intermediate between *Escherichia coli* and *Enterobacter aerogenes (Aerobacter aerogenes).*

From the biochemical characteristics displayed by these colonies (table 6) they seem to fit the designations for *Citrobacter intermedius,* as outlined by the 8th edition of Bergey's Manual (4) p. 297.

When sterile mabí extract substrate was inoculated with yeast only, the organism multiplied rapidly up to the eighth hour and then the population remained constant between the 8th to the 36th hour of fermentation (table 5). However, when *Enterobacter* alone was inoculated into the medium, the bacterium multiplied rapidly up to the 12th hour and then declined drastically between the 24th and 36th hour. This

TABLE 2.—*Characteristics of yeast isolated from samples*

Multilaterally budding cells, occurring in culture singly or in short chains
Cells 6-12 microns long by 4-8 microns wide, stained Gram positive
Generally 1-4 oval or spheroidally shaped spores per ascus (ascospores).
Growth on:
Potato dextrose agar slants
Moderate growth
Pseudomycelium produced after 8 days
Malt agar medium
Large, raised, yellowish white colonies
Malt broth medium
Abundant growth after 48 hours with much sediment and no film formation.
Biochemical characteristics
H_2 production ..... negative
Fermentation of sugars:

Glucose	Galactose	Sucrose	Maltose	Raffinose	Soluble Starch	Melibiose
AG[1]	AG	AG	AG	A	—	—

Auxonogram:
(Assimilation of sugars)

+	+	+	+	+		

Conclusion = *Saccharomyces cerevisiae.*

[1] A = acid produced; G = gas produced.

probably was due to the increased acidity produced over this period of the fermentation. The enteric organisms, including *Enterobacter aerogenes,* are sensitive to low pH (13).

When the medium was inoculated with a mixture of both organisms (bacterium and yeast) the yeast population increased more slowly than when growing alone, for up to 12 hr. However, between 12 to 36 hr, the population was the same as when grown pure in the medium. In association with the yeast, the bacterium likewise multiplied less rapidly than when present alone for up to the 12th hr, and then declined up to the 36th hr. However, the decline between the 24th and 36th hr was less than when the bacterium was growing alone.

When the bacterium and the yeast were inoculated into the sterile

TABLE 3.—*pH, microorganisms and microbial counts of commerical mabí (M) samples*

Sample No.	pH	*Enterobacter aerogenes*	*Enterobacter cloacae*	*Citrobacter intermedius*	*Escherichia coli*	*Saccharomyces cerevisiae*	Coliform—count/ml	Yeast—count/ml
M-1	3.60	neg.	neg.	pos.	neg.	pos.	2.0×10^3	5.0×10^6
M-2	3.65	pos.	neg.	neg.	neg.	pos.	1.5×10^5	3.8×10^7
M-3	3.30	neg.	neg.	neg.	neg.	pos.	0	3.0×10^2
M-4	3.50	neg.	neg.	neg.	neg.	pos.	0	2.9×10^6
M-5	3.55	pos.	neg.	neg.	neg.	pos.	3.0×10^2	1.5×10^7
M-6	3.60	neg.	neg.	pos.	pos.	pos.	3.0×10^2	1.5×10^7
M-7	3.63	pos.	neg.	neg.	neg.	pos.	2.3×10^4	3.0×10^7
M-8	3.60	neg.	pos.	pos.	neg.	pos.	3.0×10^2	3.5×10^7
M-9	3.50	neg.	neg.	pos.	neg.	pos.	2.7×10^5	3.0×10^7
M-10	4.00	neg.	pos.	neg.	neg.	pos.	1.0×10^4	5.0×10^6

mabí substrate, individually or together, the initial pH's were 5.6, 5.1 and 5.3, respectively, and the corresponding final pH's 4.1, 3.6 and 3.8, respectively (table 6). Since the pH of the commercial samples were from 3.3 to 4.0 (table 3), other experiments were designed adjusting the initial pH of the medium to 5.1, 5.3 and 5.6. Each medium, at each pH, was inoculated with the bacterium and the yeast, individually and together, and the pH monitored every 6 hr between 0 to 48 hr. The results are summarized in table 6.

From an initial pH of 5.1, the final pH's for sterile media inoculated with the yeast (1), bacterium (2) and a mixture of both (3), were 4.0, 4.3 and 3.7, respectively. For an initial pH 5.3, the final pHs were 4.2, 4.3 and 4.1, respectively, and for an initial pH 5.6, they were 4.1, 4.4, and 4.1, respectively, (table 7).

TABLE 4.—*Microorganisms isolated from samples of mabí bark (B) and unrefined sugar (S)*

Sample	*Enterobacter aerogenes*	*Enterobacter cloacae*	*Escherichia coli*	*Saccharomyces cerevisiae*	*Aspergillus* sp.	*Penicillium* sp.	*Bacillus* sp.
B_1	neg.	pos.	neg.	neg.	neg.	neg.	neg.
B_2	neg.	pos.	neg.	neg.	neg.	neg.	neg.
B_3	neg.	pos.	neg.	neg.	neg.	neg.	neg.
B_4	neg.	pos.	neg.	neg.	neg.	neg.	neg.
S_1	neg.	neg.	neg.	pos.	pos.	neg.	neg.
S_2	pos.	neg.	neg.	pos.	pos.	pos.	neg.

Great variation in the flavor of the final product occurred with variation in the initial pH of the fermentation mixture. The best flavor resulted when the initial pH was between 5.1 and 5.3. A higher initial pH resulted in a drink with less acceptable flavor. An initial pH lower than 5.1 resulted in a slower fermentation and a sweeter drink since less sugar was metabolized.

Table 6 shows that the decrease in pH was essentially the same when sterile medium was inoculated with the mixture of the bacterium and the yeast, although the initial pH varied between 5.1 and 5.6. The substrate of initial pH 5.6 attained a final pH of 4.1 after 48 hr of fermentation, as did the substrate of initial pH 5.3. However, the flavor of the drink prepared from substrate of initial pH 5.6 was less acceptable than that of drinks prepared from substrate with initial pHs of 5.3 and 5.1. Apparently, the production of flavoring substances occur principally during the early stages of the fermentation and an initial pH of 5.1 to 5.3 is critical for such development. Analysis of table 7 lends credence to this hypothesis. It can be seen here that at an initial pH of 5.1, the pH of the fermenting medium approached that of commercial mabí (table 3) after 30 hr. At an

initial pH of 5.3, this was attained after 42 hr. However, at an initial pH of 5.6 it took 48 hr. An initial pH of between 5.1 to 5.3 is, therefore, recommended for the development of acceptable mabí flavor within the usual fermentation time of 48 hr.

Drinks prepared by fermentations using the yeast only, (1); the bacterium only (2); and (1) and (2) together, (3) were tasted and scored by 23 persons. Seventy-eight percent (78%) indicated that No. 3 was the one which most resembled commercial mabí. Drink No. 1 tasted like beer, while No. 2 had a sweetish taste of no particular flavor. This indicated clearly that mabí fermentation entails the joint fermentative action of the coliform organism(s) and a yeast, *Saccharomyces cerevisiae*. Both types of microorganisms participate in the degradation of the substrate

TABLE 5.—*Changes in population of microorganisms growing on sterile mabí substrate*

Inoculum	Number of microorganism per ml Fermentation time (hr)					
	0	4	8	12	24	36
S. cerevisiae	4×10^6	7×10^6	3×10^7	3×10^7	3×10^7	3×10^7
Enterobacter aerogenes	8×10^6	2×10^7	5×10^7	5×10^7	5×10^6	3×10^6
S. cerevisiae * plus *Enterobacter aerogenes*[1]	4×10^6 / 8×10^6	4×10^6 / 2×10^7	1×10^7 / 3×10^7	3×10^7 / 3×10^7	3×10^7 / 7×10^6	3×10^7 / 4×10^6

[1] Inoculated together.

with the production of the flavoring substances, alcohol and acids. The extremely sweet taste of the mabí is due to the large amount of sugar which remains unfermented. Since the natives have developed a taste for this level of sweetness, it has become a characteristic of the drink, even though for the metabolic support of the organisms much lower concentrations could be used.

During fermentation, organic acids (acetic, lactic and formic) are produced. Other products are acetoin, ethanol, CO_2 and hydrogen. These are typical metabolic products formed through the fermentative action of bacteria of the enteric group. (11, 12, 14)

Since *E. coli* was absent from all but one of the commercial samples, it seems, therefore, that *Enterobacter* species and *Citrobacter intermedius* are most likely, the principal bacteria in mabí fementation. Since each pie (inoculum or starter) might differ in microbial load and microbial species, isolates from any one batch or even from several batches, will differ. The sensitivity of the coliform enteric bacteria to relatively high acidity (13) compounds this difference. The age of the pie is also another

factor which would influence the types and number of microorganisms present.

Yeast, on the other hand, is quite acid-tolerant and would survive well in the pie for longer periods. This is reflected in the isolation of yeast from all the commercial samples.

Enterobacter aerogenes was chosen as the principal bacterium in

TABLE 6.—*Initial and final pH of sterile mabí substrates inoculated with pure cultures of microorganisms*

Initial pH	Inoculum			Decrease in pH
	S. cerevisiae	*Enterobacter aerogenes*	*S. cerevisiae Enterobacter aerogenes*	
		Final pH		
5.1[1]	3.6			1.5
5.3[1]			3.8	1.5
5.6[1]		4.1		1.4
5.1[2]	4.0			1.1
5.1[2]		4.3		0.8
5.1[2]			3.7	1.4
5.3[2]	4.2			1.1
5.3[2]		4.3		1.0
5.3[2]			4.11	1.2
5.6[2]	4.1			1.5
5.6[2]		4.4		1.2
5.6[2]			4.1	1.5

[1] Unadjusted pH of sterile mabí substrate + inoculum.

[2] Sterile mabí substrate + inoculum adjusted to indicated pH with sterile 10% lactic acid or 5% dibasic potassium phosphate.

TABLE 7.—*Changes in pH of mabí substrate, adjusted to 3 different initial pH levels and inoculated with a mixture of Enterobacter aerogenes and Saccharomyces cerevisiae*

Initial pH	Fermentation time—hr							
0	6	12	18	24	30	36	42	48
5.1	4.7	4.4	4.3	4.2	4.0	3.9	3.7	3.7
5.3	4.8	4.6	4.5	4.4	4.3	4.2	4.1	4.1
5.6	4.8	4.7	4.7	4.5	4.4	4.3	4.2	4.1

studies on pH and population changes, etc., because it was isolated from the commercial mabí samples as well as from sugar and its metabolism is well documented. However, *Citrobacter intermedius,* which was isolated from both sources could also be equally important. *Enterobacter cloacae,* found also in the commercial samples could be another important participating bacterium. However, samples of mabí prepared using

all three bacterial cultures plus the yeast, tasted the same as that prepared with only *Enterobacter aerogenes* and *Saccharomyces cerevisiae*.

No role is ascribed to *E. coli* since it is considered a fecal contaminant and was isolated from only one sample. This chance isolate could have been due to the use of water of poor quality in the preparation of this particular sample or to unsanitary post-preparation procedures.

The tasting experiments indicated that the best flavor acceptance occurred when the sugar, at a concentration of 15% (W/V), and extract, equal to 1.5% (V/V) of the fermenting mixture, are used.

Isolation and identification of the microorganisms involved in mabí fermentation have thrown light on this important local drink and should allow for more detailed study under controlled conditions.

RESUMEN

El mabí es un refresco fermentado muy popular en Puerto Rico, el cual se prepara con la corteza del arbusto mabí (*Colubrina elliptica*).

Muestras de varias fuentes del mabí comercial, de la corteza y del azúcar sin refinar se examinaron para determinar los tipos de microorganismos comunes en ellas.

En el mabí comercial se encontraron *Enterobacter aerogenes (1), Enterobacter cloacae (2), Citrobacter intermedius (3),* y la levadura *Saccharomyces cerevisiae (4).* En la muestra de la corteza se encontraron 1 y 3, y en la de azúcar, 1, 3 y 4. En las muestras del azúcar también se encontraron una especie de *Aspergillus* y otra de *Penicillium.*

Para el 78% de los catadores no hubo diferencia en el sabor entre el mabí comercial y el preparado en el laboratorio con cultivos puros de los inóculos 1+2+3+4 o de 1+4.

Se concluye que la fermentación del mabí es una fermentación natural. Las bacterias 1, 2 y 3, la levadura,y posiblemente *Citrobacter intermedius* participan en la fermentación.

Escherichia coli, aislado de una muestra solamente, aparentemente no interviene, pero puede estar presente en la bebida por causas insanitarias que la puedan contaminar al prepararla.

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