

Behavior of Chlorogenic Acids in Strongly Acidic Solution. Jean and Reid (8) have reported that treatment of chlorogenic acid with 5*N* sulfuric acid for 15 minutes at 20° C. produced two isomers, one of which they believe to be identical with neochlorogenic acid. They detected these isomers by paper chromatography. Since the present authors have been separating the chlorogenic acids on silicic acid columns containing 0.5*N* sulfuric acid, it was considered necessary to determine whether any isomerization occurred under these isolation conditions. Chlorogenic acid isolated from coffee as the caffeine complex and recrystallized from water was therefore chromatographed on a silicic acid column with 0.5*N* sulfuric acid as the stationary phase. No isomers were detected on scanning at 330 m μ , under conditions known to permit detection of 2 to 3% of transformation products when 1 mg. or more of an isomer is applied to the column. (Since the chlorogenic acid isomers have high absorption

coefficients, the $E_{1\text{cm}}^{1\%}$ at 330 m μ in butyl alcohol-chloroform being above 450, they can be detected at these levels by ultraviolet scanning.) Even when the chlorogenic acid in 5*N* sulfuric acid was held for 15 minutes at 20° C., the conditions used by Jean and Reid, and then chromatographed on the silicic acid column, no significant changes in the elution pattern were observed. Similar results were obtained with neochlorogenic acid. Thus, the claim for acid-catalyzed isomerization of chlorogenic acid has not been confirmed. Possibly the discrepancy between the results reported in this article and those of Jean and Reid is due to differences in the purity of the chlorogenic acid samples.

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BEVERAGE PRESERVATION

Pasteurization of Palm Sap (Neera)

Palm sap (Neera) tapped from coconut palm or date palm can be pasteurized and bottled at 170° to 175° F. without affecting its natural flavor. Pasteurization above 185° or 190° F. for 4 to 5 minutes imparts a "cooked" flavor. Yeasts are killed by holding pasteurization for 25 to 30 minutes. The presence of air increases the rate of fermentation. Addition of sodium benzoate makes pasteurization more effective. The effectiveness of benzoate is increased by a small amount of citric or malic acid, but these acids alone (pH 3.75) have no significant effect. The addition of malic acid gives the sap an off-flavor resembling that of apple juice.

THE PRESERVATION OF PALM SAP (Neera) has attracted considerable attention in India. Neera has a flavor which appeals to the palate of many Indian people, and, probably because of its vitamin content, a legend has developed regarding its value as a nutritious drink. As it contains considerable sugar, the natives have used it as the source of a fermented beverage, a use prohibited in certain states in India. The governments of these states have agencies which collect the sap and distribute it in large containers to selected stores in the cities, where it is consumed on the premises. Certain governmental officials have been interested in ascertaining whether the unfermented sap could be bottled and distributed as is done with soft drinks.

Various methods have been suggested for the preservation of Neera in bottles, but none has been found practical from either a pilot-plant or commercial point of view (1, 4). Pasteurization has often been mentioned, but its practical application has not been systematically studied.

Pasteurization, more particularly flash pasteurization (5), has been widely used in the preservation of fruit juices in the United States. Pasteurization should be preferable for Neera, as prolonged heating may cause off-flavors.

The present studies were conducted to find the optimum conditions under which Neera could be pasteurized with and without the addition of chemical preservatives, the sap being deaerated to check oxidative deterioration.

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Methods

Palm sap used in these studies was packed in dry ice and shipped by air to Chicago from Bombay, India. The sample was received within 48 hours and was fresh and clear. No fermentation had taken place. During experimentation palm sap was stored at 3° C.

To ascertain the approximate temperature and rate at which the microorganisms would be killed, a number of samples of palm sap were pasteurized by "holding" pasteurization. Temperatures were taken and plate counts were made on special agar as described by Pederson, Beavens, and Goersline (2). The hot pasteurized sap was introduced into sterile soft-drink bottles, which were immediately crowned. Later the

Table I. Composition of Neera

Fresh Date Neera	
Specific gravity	1.07
pH	6.75
Nitrogen, %	0.059
Protein, %	0.369
Total sugars, %	11.01
Reducing sugars, %	0.97
Ash (mineral), %	0.54
Calcium	Traces
Phosphorus, %	0.16
Iron, mg. %	0.0369
Riboflavin, mg./100 ml.	0.01
Vitamin B ₁ , mg./100 ml.	13.30
Vitamin C, mg./100 ml.	9.46
Total acidity, cc. 0.1N alkali/100 ml.	8.70
Coconut Palm Neera	
Specific gravity	1.07
Total solids, %	17.50
Sucrose, %	17.00
Invert sugar	Traces
Acidity	Traces
Ash, %	0.85
Undetermined nitrogen compounds, %	0.95

samples were opened and plated. Three more sets of samples were pasteurized after pretreatment with sodium benzoate, sodium benzoate and malic acid, or sodium benzoate and citric acid. After determination of the minimum time and temperature for holding pasteurization, several samples of palm sap were treated as just indicated, then pasteurized, and immediately introduced into sterile bottles, which were immediately crowned. The apparatus used in this work was fabricated as described by Pederson and Tressler (3).

Samples were withdrawn during pasteurization for plating and counting the microorganisms, and were analyzed for sugar, proteins, total acid, and hydrogen ion concentration. The specific gravity, total acidity, alcohol content, protein, carbohydrates, mineral matter, and vitamins B and C of the fresh Neera samples were determined. The holding pasteurization had to provide minimum destruction of the nutrients with maximum preservative effectiveness.

Results

Holding Pasteurization. The composition of Neera is shown in Table I. The palm sap as received was highly contaminated with yeast and bacteria. During pasteurization a temperature of 170° to 175° F. was reached in 25 to 30 minutes after the sap was placed in the pasteurizer (Table II). The plate counts showed a reduction in microorganisms from 12 to 15 minutes after pasteurization was started, when the sap temperature was between 125° and 130° F. Between 155° and 158° F. or on holding for 25 to 30 minutes most of the yeasts

Table II. Rate of Destruction of Microorganisms during Pasteurization

Time, Min.	Date Palm Neera		Coconut Palm Neera	
	Temp., ° F.	Plate count/cc.	Temp., ° F.	Plate count/cc.
0	67.0	Over 1,000,000	60	Over 1,000,000
2	85.0	500,000	94.0	175,000
4	98.0	500,000	99.0	200,000
6	107.5	125,000	105.0	120,000
8	115.0	5,400	115.0	150,000
10	122.0	3,000	120.0	100,000
12	127.0	500	125.5	45,000
14	131.0	500	131.0	5,500
16	136.0	18	135.5	1,000
18	140.0	22	140.0	26
20	144.5	...	144.0	40
22	148.0	51	148.0	18
24	152.0	16	153.5	12
26	158.0	15	159.0	7
28	164.5	17	166.0	0
30	172.0	9	175.0	0
32	180.0	6	184.0	0
34	189.5	4	190.0	0

Table III. Effect of Pasteurization on Nutrients in Neera

Nutrient, Mg./100 ml.	Pasteurization for 30 Minutes				
	Initial	160° F.	170° F.	175° F.	185° F.
Vitamin B ₁	13.30	13.26	13.25	13.20	13.00
Riboflavin	0.01	0.01	0.01	0.008	...
Vitamin C	9.46	9.22	9.05	9.05	8.55
Phosphorus, g./100 ml.	0.16	0.16	0.15	0.15	0.15
Iron	0.037	0.035	0.035	0.035	0.035

Table IV. Effect of Sodium Benzoate, Citric Acid, and Malic Acid on Pasteurization

Time, Min.	0.05% Sodium Benzoate (pH 6.35)		0.05% Sodium Benzoate 0.15% Citric Acid (pH 3.75)		0.05% Sodium Benzoate 0.1% Malic Acid (pH 3.95)	
	Temp., ° F.	Plate count	Temp., ° F.	Plate count	Temp., ° F.	Plate count
Date Palm Neera						
0	65.0	800,000	65.0	800,000	65.0	
2	90.0	425,000	89.0	420,000	90.0	420,000
4	98.0	100,000	95.0	80,000	96.0	75,000
6	105.0	45,000	102.0	6,000	104.0	7,500
8	113.5	5,600	110.0	1,200	111.0	1,000
10	122.0	1,000	129.0	190	120.0	150
12	127.0	56	125.0	75	126.0	120
14	132.0	84	131.0	85	131.0	70
16	137.0	49	137.0	75	137.0	80
18	141.5	40	142.0	45	143.0	50
20	144.5	18	145.0	20	146.0	10
22	148.0	25	148.5	7	149.5	8
24	153.0	14	154.0	7	155.0	0
26	158.0	9	159.0	0	159.5	0
28	166.0	9	166.0	0	167.0	0
30	174.5	0	175.0	0	175.0	0
32	184.0	0	183.5	0	184.5	0
34	190.0	0	187.0	0	190.0	0
Coconut Palm Neera						
0	66.0	800,000	64.0	800,000	66.0	800,000
2	92.0	500,000	90.0	450,000	91.0	450,000
4	99.0	200,000	97.0	20,000	99.0	25,000
6	107.0	50,000	103.0	4,500	104.0	3,800
8	115.0	5,000	112.0	1,500	114.0	1,000
10	124.0	1,300	120.0	500	121.0	150
12	128.5	70	127.0	60	128.0	50
14	135.0	90	132.0	80	133.0	60
16	138.0	90	139.0	50	140.0	25
18	144.0	40	143.0	50	144.0	25
20	147.0	25	147.0	35	147.0	10
22	150.0	10	149.5	10	150.5	10
24	154.0	5	156.0	7	156.5	0
26	158.0	5	160.0	7	161.0	0
28	167.0	0	168.0	0	168.0	0
30	176.0	0	176.0	0	176.0	0
32	185.0	0	184.0	0	184.0	0
34	191.0	0	189.5	0	191.0	0

Table V. Effect of Deaeration and Pasteurization on Shelf Life during First Week

Treatment	Past. Temp., ° F.	Plate Count per Cc. after Days				
		2	3	4	5	6
Coconut Palm Neera						
Without deaeration	160	175	1500	5600	5900	8600 ^a
After deaeration	160	50	125	175	690	1950
Full	160	25	75	130	250	1100
0.5 inch from top	160	125	1800	6500	7900	12000
Without deaeration	175	160	1200	4800	5000	5900 ^a
After deaeration	175	75	120	175	700	1750
Full	175	50	110	125	590	1350
0.5 inch from top	175	100	2500	3800	4900	6900
Without deaeration	185	275	590	1200	4500	6900 ^a
After deaeration	185	85	125	280	490	1800
Full	185	70	110	190	380	2000
0.5 inch from top	185	70	710	1500	5100	7500
Date Palm Neera						
Without deaeration	160	160	1150	2000	2900	4200
After deaeration	160	20	95	230	550	1000
Full	160	20	120	300	480	975
0.5 inch from top	160	130	490	850	1160	2100
Without deaeration	175	140	750	960	1300	2700
After deaeration	175	30	150	280	580	860
Full	175	25	135	280	620	1200
0.5 inch from top	175	40	590	980	1600	2200
Without deaeration	185	195	650	1400	5200	8000
After deaeration	185	70	130	195	450	950
Full	185	65	95	175	420	1100
0.5 inch from top	185	165	520	2100	4900	7800

^a Compare with over 1,000,000 count in Table II and 800,000 count in Table IV.

Table VI. Comparative Shelf Life of Pasteurized Neera with and without Preservatives

Past. Temp., ° F.	Plate Count per Cc. after Days						
	1	2	3	4	5	6	7
With Preservatives ^a							
160	20	25	75	130	250	1,100	2,500
170	25	35	95	140	390	1,200	3,000
175	20	50	110	125	590	1,350	2,500
185	20	70	110	190	380	2,000	3,000
Without Preservatives ^b							
160	25	2500	10,900	25,000	56,000
170	20	1750	9,500	29,500	60,000
175	20	2000	9,200	22,000	45,000	72,000	...
185	25	1500	3,900	11,500	35,000	60,000	90,000

^a 0.05% sodium benzoate and 0.15% citric acid (pH 3.75).

^b Molds visible in all samples without preservatives after second day (pH 6.7).

were killed in both the natural and pretreated sap. Occasionally, some mold spores were noted at 150° F., but above 165° F. the total plate count was negligible.

The pasteurization process had no significant effect on the concentration of

thiamine, riboflavin, and ascorbic acid (Table III).

The effect of the addition of benzoate, malic acid, and citric acid to the sap on the rate of killing of microorganisms by pasteurization is shown in Table IV. The addition of 0.05% sodium benzoate

increased the rate at which pasteurization destroyed the microorganisms; the rate was further enhanced by the addition of either 0.15% citric acid or 0.1% malic acid. Malic acid gave the Neera an off-flavor resembling that of apple juice. However, citric acid or malic acid alone (pH 3.75) did not appreciably change the rate of killing of microorganisms.

Pasteurization and Deaeration. The data in Table V show that 160° F. is the lowest pasteurization temperature that can be used effectively.

Some samples of the sap were deaerated before pasteurization. Some bottles were filled full, and others to within 1 inch of the top. The sap which was not deaerated and was low in acid content either fermented or showed a growth of mold.

Precidification increased the effectiveness of deaeration and pasteurization (Table VI).

Pasteurization, deaeration, and filling the bottle full preserved the sap for 4 to 5 days under refrigeration temperature (5° C.). The addition of 0.05% sodium benzoate and 0.15% citric acid provided additional shelf life without a material change in the natural flavor, when pasteurization was performed at 170° to 175° F. Pasteurization above 185° or 190° F. for 4 to 5 minutes imparted a "cooked" flavor.

Work is in progress to replace sodium benzoate by a more effective substance, to find a more effective method of deaeration, and to prolong shelf life without the addition of quantities of citric acid which affect the natural flavor.

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