Since the temperature needed for enzyme inactivation is above that used to pasteurize citrus juices, it is suggested that this rapid test for pectinesterase inactivation be used as an indication that adequate pasteurization temperatures have been used.

Summary

The new procedure has been devised and described for testing the effectiveness of the pasteurization of citrus juices. Evidence of the presence of pectinesterase is used as the index. The test is rapid in that it can be completed in four hours. A color indicator is used to detect changes in acidity in the prepared sample. The test is simple, requiring only two solutions, and can be easily performed in the control laboratory. A confirmation of the test can be obtained by setting the prepared sample in the refrigerator overnight. The test agrees well with the results of other tests.

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STORAGE CHANGES IN FROZEN CONCENTRATED CITRUS JUICES—PRELIMINARY REPORT[1] [2]

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The purpose of this investigation, started in the 1949-50 season and now in progress, is to determine what effect temperature of storage will have on the quality of frozen concentrated citrus juices. In order that high quality may be retained, frozen citrus concentrates should be stored at temperatures sufficiently low so that any chemical, microbiological, or enzymatic changes that may cause deterioration will be prevented or kept at a minimum.

Curl (3) studied the effects of degree of concentration and of temperature of storage on various characteristics of orange juices, that had been pasteurized prior to concentration and subsequently benzoated and stored at temperatures of 40° F. and above. Cotton and associates (2) made studies on frozen orange and tangerine concentrates stored at various temperatures. They reported excellent retention of aroma, taste, ascorbic acid and "cloud during storage at 0° F., but storage at 40° F. resulted in clarification, separation and flavor degradation. Rouse (4) presented data on gel formation in frozen citrus concentrates that had been thawed and stored at 40° F. His data indicated that the presence of low

[1] Cooperative research by the Florida Citrus Commission and the Florida Citrus Experiment Station.

[2] Paper presented by Richard L. Huggart at the 63rd Annual Meeting of the Florida State Horticultural Society, Winter Haven, Florida, November 1, 1950.

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methoxyl pectin, which resulted from the activity of the pectic enzyme, pectase, is the cause of gelation in frozen citrus concentrates during storage at 40° F.

At the beginning of the 1949-50 season it was evident that there was a need for more data, than were then available, in order to advise processors, transportation companies, wholesalers, retailers, and consumers concerning the changes that will take place if frozen citrus concentrates are mishandled by storage at elevated temperatures during any part of the period from the time the products are processed until they are used by the consumer. At the time this investigation was begun, it was generally believed that a temperature of 0° F. was sufficiently low to prevent deterioration, and that this low temperature should be maintained at all times during storage, transportation, and distribution of these products.

Experimental Procedure

The six packs of frozen concentrated citrus juices used in this investigation were processed in the pilot plant at the Citrus Experiment Station using an evaporator described by Atkins and associates (1), and included the following citrus varieties: Hamlin, Pineapple, and Valencia oranges, Dancy tangerines, and Duncan and Marsh grapefruit. The processing procedure used may be outlined briefly as follows: (a) fruit washed and graded, (b) juice extracted by rotary press, (c) juice for concentration passed through 0.030 inch finisher screen, (d) cut-back pulpy juice secured by passing pulp and seeds from (c) mixed with some juice through 0.125 inch finisher screen, (e) sugar added to tangerine and grapefruit juices, (f) juice concentrated to approximately 55° Brix, (g) 55° Brix concentrate cut back to approximately 42° Brix with fresh pulpy juice from (d), (h) slush frozen in Votator, (i) machine filled and vacuum closed in 6-oz. plain tin cans, and (j) frozen by storage at −8° F. This processing procedure simulated commercial methods during the 1949-50 season with the probable exception in these pilot-plant packs of lower juice yield and possible slower rate of final freezing. After the packs had been in storage at −8° F. for one day, samples for this investigation were stored at −8°, 10°, 20°, 32°, and 40° F. It was not possible to include 0° F. storage as facilities for this temperature were not available.

Periodic chemical and microbiological examinations of these packs are planned for at least a period of one year. Frequency of examination depends upon the storage temperature and other factors. Examinations include (a) Brix, (b) total acid, (c) peel oil, (d) ascorbic acid, (e) pH, (f) color, (g) pulp, (h) headspace, (i) vacuum, (j) clarification, (k) pectase activity, (l) gelation, (m) viscosity, (n) microbiological counts, and (o) flavor. Only the data summarizing the more important changes, especially gelation and clarification, that have occurred in the concentrates during storage for six months will be presented in this preliminary report.

Experimental Results and Discussion

Some of the initial analyses of the six concentrated citrus juices together with the dates at which the fruit was processed are presented in Table 1. It will be noted from this table that the tangerine concentrate had a low pectase activity as compared to the other five concentrates. All varieties of fruit were held at room temperature from the time of picking until processing.

Gelation of the Concentrates:

The data for gelation of Hamlin, Pineapple, and Valencia orange concentrates and Dancy tangerine concentrate are presented in Table 2. In this table and also in Table 3, the degree of gelation is

indicated by the numerals 0, 1, 2, 3, and 4, these referring respectively to a degree of gelation of none, very slight, slight, semi-gel, and solid-gel. The occurrence of a semi-gel or a solid-gel in a frozen citrus concentrate would cause consumer complaints. Rouse (4) shows a picture of a semi-gel and a solid-gel in frozen concentrated citrus juices.

It will be noted from Table 2 that after storage of these four concentrates at −8° F. for six months, only the Pineapple orange concentrate showed any indication of gelation and that was very slight. Very slight gelation occurred in all three orange concentrates after three months storage at 10°F., and in the tangerine concentrate after six months

TABLE 1.
INITIAL ANALYSES OF THE CONCENTRATED CITRUS JUICES.

	Hamlin Orange	Pineapple Orange	Valencia Orange	Dancy Tangerine	Duncan Grapefruit	Marsh Grapefruit
Date fruit processed	1-26-50	2-15-50	4-20-50	1-25-50	2-21-50	2-28-50
Brix, 28° C., corrected for acid	42.3	42.1	42.2	42.1	42.1	41.9
Total acid, % as anhydrous citric	2.78	2.98	3.25	2.93	4.64	4.03
Maturity ratio: Brix/acid	15.2	14.1	13.0	14.4	9.1	10.4
pH	3.6	3.7	3.6	3.6	3.1	3.3
Ascorbic acid, Mg./100 gm.	205.8	211.5	158.5	73.9	136.2	129.4
Pectase activity[1]	5.65	6.42	6.35	1.85	6.34	6.81
Microbiological count on D.A.[2]	16,500	57,200	76,200	33,300	3,700	13,200
Microbiological count on P.D.A[3]	4,480	19,100	28,300	4,200	2,020	3,860

[1] Expressed as mg. methoxyl released per hour per gm. of soluble solids.
[2] D. A. is Dextrose Agar, pH 7.0.
[3] P. D. A. is Potato Dextrose Agar, pH 3.5.

storage at this temperature. Pineapple orange concentrate showed a semi-gel after two months storage at 20°F., and after two weeks storage at 40°F. Even after six months storage at 40°F., the other two orange concentrates showed only a slight gel, and the tangerine concentrate showed only a very slight gel.

The data for gelation of Duncan and Marsh grapefruit concentrates are presented in Table 3. Duncan grapefruit concentrate showed the fastest rate of gelation of any of the six concentrates. Very slight gelation occurred in this concentrate after three months storage at −8°F., and after two months storage at 10°F. Semi-gels occurred in the Duncan grapefruit concentrate after one months storage at 20°F., and after two weeks storage at 32° or 40°F.

Marsh grapefruit concentrate showed no gelation after six months storage at −8°F., but did show very slight gelation after four months storage at 10°F., and after two weeks storage at 20°, 32°, or 40°F. Even after six months storage at 40°F., the Marsh grapefruit concentrate showed only a slight gel.

On the basis of the results presented for gelation in these six concentrate packs, it is indicated that a degree of gelation which would be definitely objectionable from the consumer standpoint occurred only in the Pineapple orange concentrate and Duncan grapefruit concentrate that were stored for sufficient periods of time at temperatures of 20°, 32°, or 40°F. This might be an indication that gelation occurs to a greater extent in concentrated juice from seedy

TABLE 2.

DEGREE OF GELATION OF CONCENTRATED ORANGE AND TANGERINE JUICES*.

Variety	Period	Time and Temperature of Storage				
		−8°F.	10°F.	20°F.	32°F.	40°F.
Hamlin Orange	Initial	0	0	0	0	0
	1 week	0	0	0	0	0
	2 weeks	0	0	0	0	0
	1 month	0	0	0	0	0
	2 months	0	0	1	2	2
	3 months	0	1	2	2	2
	4 months	0	1	2	2	2
	6 months	0	1	2	2	2
Pineapple Orange	Initial	0	0	0	0	0
	1 week	0	0	0	0	0
	2 weeks	0	0	0	2	3
	1 month	0	0	1	3	3
	2 months	0	0	3	3	3
	3 months	0	1	3	4	4
	4 months	0	1	3	4	4
	6 months	1	1	3	4	4
Valencia Orange	Initial	0	0	0	0	0
	1 week	0	0	0	0	1
	2 weeks	0	0	1	1	1
	1 month	0	0	1	1	1
	2 months	0	0	1	2	2
	3 months	0	1	2	2	2
	4 months	0	1	2	2	2
	6 months	0	1	2	2	2
Dancy Tangerine	Initial	0	0	0	0	0
	1 week	0	0	0	0	0
	2 weeks	0	0	0	0	0
	1 month	0	0	0	0	0
	2 months	0	0	1	1	1
	3 months	0	0	1	1	2
	4 months	0	0	1	1	2
	6 months	0	1	2	1	1

*Degree of gelation: 0=None; 1=Very slight; 2=Slight; 3=Semi-gel; 4=Solid gel.

TABLE 3.
DEGREE OF GELATION OF CONCENTRATED GRAPEFRUIT JUICES*

Variety	Time and Temperature of Storage.				
	Period	−8°F.	10°F.	20°F.	32°F.	40°F
Duncan Grapefruit	Initial	0	0	0	0	0
	1 week	0	0	0	0	0
	2 weeks	0	0	1	3	3
	1 month	0	0	3	3	4
	2 months	0	1	3	3	4
	3 months	1	1	3	3	4
	4 months	1	2	3	4	4
	6 months	1	2	4	4	4
Marsh Grapefruit	Initial	0	0	0	0	0
	1 week	0	0	0	0	0
	2 weeks	0	0	1	1	1
	1 month	0	0	1	1	1
	2 months	0	0	1	1	1
	3 months	0	0	2	2	2
	4 months	0	1	2	2	2
	6 months	0	1	2	2	2

*Degree of gelation: 0 = None; 1 = Very Slight; 2 = Slight; 3 = Semi-gel; 4 = Solid gel.

varieties of citrus fruit, such as Pineapple orange and Duncan grapefruit, than in the concentrated juice of varieties containing fewer seeds such as Hamlin and Valencia orange and Marsh grapefruit.

Clarification of the Concentrates

A citrus concentrate might not be acceptable to a consumer because of clarification or loss of "cloud." Loss of "cloud" is a further clarification than the usual settling of the chromatophores or color bodies of the juice. The pectic enzymes act on the pectin responsible for the "cloud," resulting in precipitation of colloidal particles, and finally leaving a clear supernatant liquid.

The turbidity or "cloud" in all concentrates was determined by centrifuging a sample of the reconstituted juice (25 gm./100 ml.) for 15 minutes at 1700 r.p.m. in an International Centrifuge, Size 1, Type SB, and reading the light transmission of the centrifugate in a Lumetron Colorimeter, Model No. 402-E, using a rectangular absorption cell (juice thickness, 10 mm.) and a filter No. 730, that was calibrated to read 100 with distilled water. With this filter a completely clarified juice will read 98 to 100 percent transmission despite its color, and the presence of turbidity or "cloud" in the centrifuged juice reduces the light transmission by dispersion and absorption.

The data for clarification of reconstituted juices of Hamlin, Pineapple, and Valencia orange concentrates and Dancy tangerine concentrate are presented in Table 4. In this table the degree of clarification is indicated by the percentage light transmission of the centrifuged juices, as follows: 50-60% = none; 60-70% = slight; 70-85% = definite; 85-100% = extreme.

The data in Table 4 indicate that a temperature of 20°F. was too high for the proper storage of the orange and tangerine concentrates on the basis that definite clarification in these concentrates, which might be noticeable to the

TABLE 4.
CLARIFICATION OF RECONSTITUTED ORANGE AND TANGERINE JUICES.*

Variety	Time and Temperature of Storage.					
	Period	−8°F.	10°F.	20°F.	32°F.	40°F
Hamlin Orange	Initial	62	62	62	62	62
	1 week	64	66	76	97	98
	2 weeks	63	66	85	98	99
	1 month	65	74	97	99	100
	2 months	64	73	97	98	98
	3 months	67	78	99	99	99
	4 months	67	81	98	97	97
	6 months	63	75	98	98	97
Pineapple Orange	Initial	58	58	58	58	58
	1 week	60	61	63	74	91
	2 weeks	62	64	72	96	98
	1 month	62	66	85	98	99
	2 months	60	64	97	99	98
	3 months	59	60	84	99	99
	4 months	61	61	97	98	98
	6 months	59	59	98	98	98
Valencia Orange	Initial	54	54	54	54	54
	1 week	57	60	61	66	81
	2 weeks	58	64	64	89	96
	1 month	55	60	65	94	95
	2 months	57	59	87	93	92
	3 months	54	56	75	89	88
	4 months	55	60	91	95	93
	6 months	56	59	91	94	91
Dancy Tangerine	Initial	55	55	55	55	55
	1 week	56	57	60	71	81
	2 weeks	57	55	58	85	98
	1 month	55	58	74	99	100
	2 months	56	59	77	99	99
	3 months	58	60	84	100	100
	4 months	57	58	84	99	99
	6 months	54	57	94	99	98

*Reconstituted 25 gm./100 ml. Clarification measured by light transmission of centrifuged juices. Degree of clarification: 50-60% = None; 60-70% = Slight; 70-85% = Definite; 85-100% = Extreme.

consumer, is indicated by a light transmission of 70 percent or higher. Definite clarification occurred after one month in the Hamlin orange concentrate stored at 10°F. At −8°F. there was no evidence of clarification in the orange concentrates or tangerine concentrate after six months storage.

The data for clarification of reconstituted juices of Duncan and Marsh grapefruit concentrates are presented in Table 5. In this table the degree of clari-

TABLE 5.
CLARIFICATION OF RECONSTITUTED GRAPEFRUIT JUICES.*

Variety	Time and Temperature of Storage.					
	Period	−8°F.	10°F.	20°F.	32°F.	40°F
Duncan Grapefruit	Initial	69	69	69	69	69
	1 week	71	68	74	95	96
	2 weeks	86	89	95	96	96
	1 month	88	94	95	97	97
	2 months	83	98	95	96	97
	3 months	90	94	96	99	99
	4 months	77	93	95	96	96
	6 months	92	95	96	96	96
Marsh Grapefruit	Initial	62	62	62	62	62
	1 week	70	68	75	92	94
	2 weeks	75	78	92	95	96
	1 month	87	89	95	97	97
	2 months	70	80	92	98	98
	3 months	72	85	94	97	97
	4 months	72	79	95	97	97
	6 months	75	88	96	96	94

*Reconstituted 25 gm./100 ml. Clarification measured by light transmission of centrifuged juices. Degree of clarification: 60-70% = None; 70-80% = Slight; 80-90% = Definite; 90-100% = Extreme.

fication is indicated by the percentage light transmission of the centrifuged juices, as follows: 60-70% = none; 70-80% = slight; 80-90% = definite; 90-100% = extreme.

The data in Table 5 indicate that a temperature of 10°F. was too high for the proper storage of the two grapefruit concentrates on the basis that definite clarification in these concentrates, which might be noticeable to the consumer, is indicated by a light transmission of 80 percent or higher. Even at −8°F., definite clarification occurred in the Duncan grapefruit concentrate and slight clarification occurred in the Marsh grapefruit concentrate. The Marsh grapefruit concentrate did not show such an objectionable degree of gelation as the Duncan grapefruit concentrate; however, it showed clarification at practically the same rate as Duncan grapefruit concentrate at storage temperatures of 20°, 32°, and 40°F.

The results on gelation and clarification indicate that the temperature of storage is an important factor and that as the temperature is increased, the rate of clarification and gelation become greater. Although both are the result of enzymatic changes, clarification took place in these citrus concentrates at a faster rate than did gelation. Slight flavor changes were noted to follow the trend of gelation and clarification.

Retention of Ascorbic Acid in the Concentrates

The average retention of ascorbic acid in all six concentrates stored at −8°F. for six months was slightly over 98 percent, decreasing gradually with an increase in storage temperature to slightly over 96 percent average retention for six months storage at 40°F. So although the concentrates in some instances may have gelled, clarified, and developed slight flavor changes, there was no appreciable decrease in the ascorbic acid content.

Microbiological Plate Counts on the Concentrates

Microbiological plate counts were made initially on the six different packs of frozen concentrate after storage at −8°F. for one day (See Table 1). The packs were examined at intervals of one month for a period of six months after

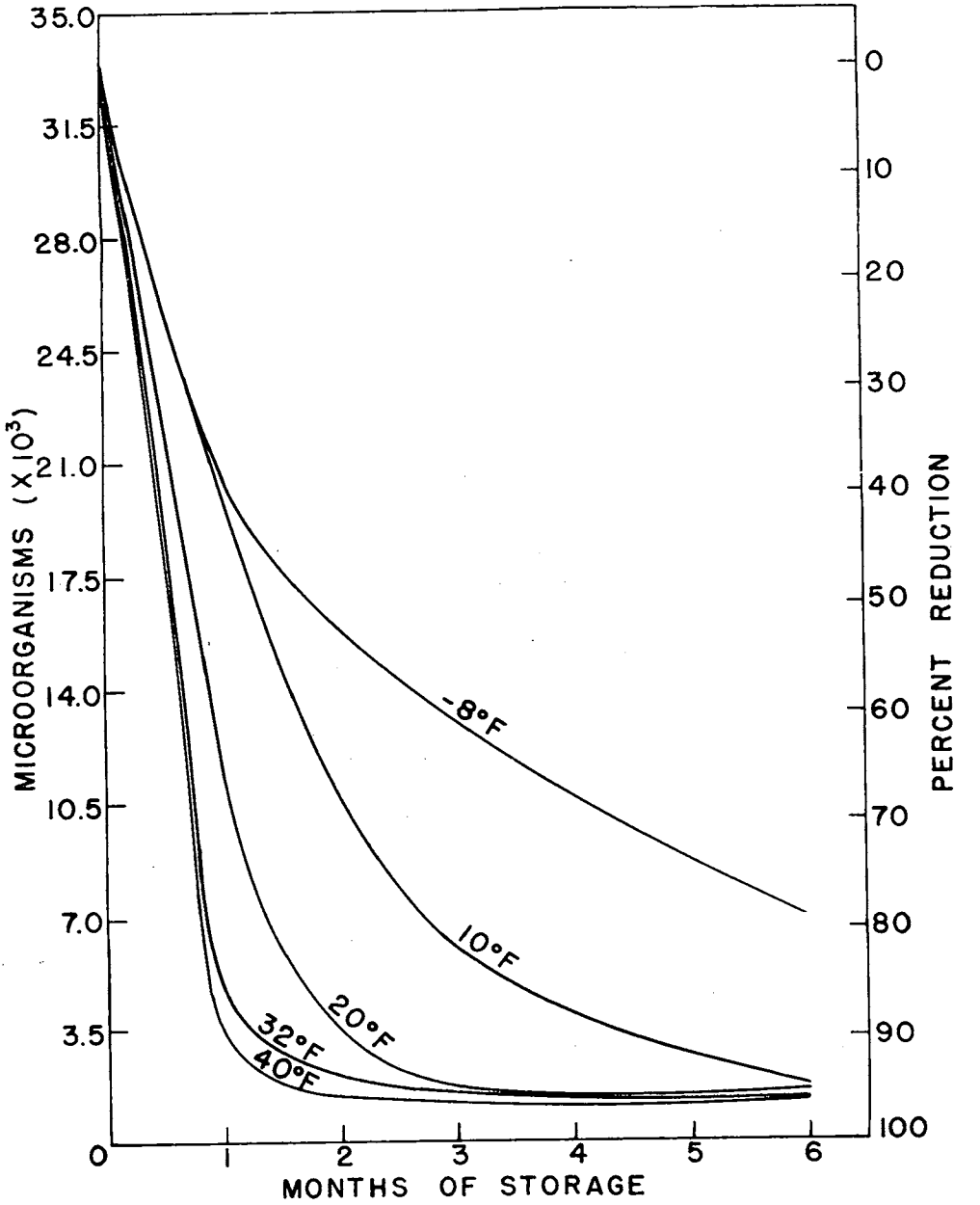

Figure 1. Trend in Reduction of Average Total Microbiological Counts of Six Packs of Citrus Concentrates Stored at Different Temperatures.

being placed at storage temperatures of −8°, 10°, 20°, 32°, and 40°F. A total count was obtained by plating with Dextrose Agar, pH 7.0, incubated for 48 hours at 30°C. (86°F.). A yeast and mold count was determined by plating on Potato Dextrose Agar, pH 3.5, incubated at room temperature for five days.

The concentrate stored at 40°F. showed an immediate sharp reduction in total count leveling off at about two months. The reduction in total count of the concentrate at −8°F. storage was much slower with a gradual decrease over the entire six months. After six months, the average reduction in total count of all packs stored at −8°F. was 80 percent while 40°F. storage packs had an average reduction in total count of 96 percent.

The packs stored at 32°F. closely paralleled the 40°F. storage packs in reduction of organisms. Packs stored at 20°F., although showing a smaller reduction in the first month, closely approached in the third month those packs stored at the two higher temperatures. Reduction of microorganism count in packs stored at 10°F. was less rapid than in packs stored at 20°F .

Average values of the total counts for all six packs stored at each temperature were calculated for each examination period in order to determine the trend in the reduction of total count for a period of six months. These trends are shown in Figure 1.

The yeast and mold count presented a somewhat different picture in that the reduction was more gradual. Initial yeast and mold counts are presented in Table 1. After the second month the yeast and mold count was very close to that of the total count in the 20°, 32°, and 40°F. packs, indicating that the total count at these later examinations was made up primarily of yeast and that few of the surviving organisms in these packs were bacteria.

Summary

Six packs consisting of Hamlin, Pineapple, and Valencia orange, Dancy tangerine, and Duncan and Marsh grapefruit concentrates were prepared during the 1949-50 season. Samples of the freshly frozen concentrates were stored at −8°, 10°. 20°, 32°, and 40°F., temperatures that approximate those to which concentrates may be subjected by processors, wholesalers, retailers, and consumers. Periodic examinations of these samples are being made over a period of one year.

Results obtained after six months indicated no appreciable decrease in ascorbic acid content in any of the samples. Gelation and clarification were more pronounced in the concentrates prepared from seedy varieties of fruit. Clarification and varying degrees of gelation occurred in all samples stored at 20°, 32°, and 40°F., and the rate of clarification was greater than that of gelation. Varying degrees of gelation and clarification occurred in some of the samples stored at 10°F. and in the Duncan grapefruit concentrate stored at −8°F. Slight flavor changes were noted to follow the trend of gelation and clarification.

The concentrate stored at 40°F. showed immediate sharp reduction in total microbiological count leveling off at about two months. The reduction in total count of the concentrate at −8°F. storage was much slower with a gradual decrease over the entire six months. After six months, the average reduction in total count of all packs stored at −8°F. was 80 percent while 40°F. storage packs had an average reduction in total count of 96 percent.

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