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EFFECT OF TEMPERATURE ON SURVIVAL OF YEAST IN 45° AND 65° BRIX ORANGE CONCENTRATE[1]

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Abstract. **A study was conducted to determine the effect of cold temperature storage on yeast survival in 45° and 65° Brix orange concentrate, under simulated conditions of bulk storage. Suspensions were prepared from each of 3 strains of yeast which had been identified as to genus and species. 45° and 65° Brix orange concentrate were inoculated to contain approximately 1,000,000 organisms per ml. Inoculated samples were then stored at 0°, 15°, 30°, 40°F (–17.8°, –9.4°, –1.10°, and 4.4°C). Samples were analyzed for total viable yeast periodically during 15 months of storage.**

No yeast growth occurred in either 45° or 65° Brix concentrate at temperatures below 40°F (4.4°C). Survival curves of one of the test organisms in both 45° and 65° Brix are presented. Yeast died faster in 45° Brix at 0°F than at 15° or 30°F, while in 65° Brix survival was greater at lower temperatures.

Mold was detected in 45° Brix at 30°F (–1°C) and below after 7 or more months. In 65° Brix, mold was observed after 12 months at all temp above 0°F (–17.8°C). Some samples showed a slight brown discoloration after extended periods of storage.

It has been the general practice in the citrus industry to store bulk product in 55 gal. drums at 0° to –10°F (–17.8° to –23.3°C). This has involved the use of thousands upon thousands of drums each season. Product in drums requires a considerable amount of handling—filling, storage, when removed from the warehouse, thawing, and again when the product is blended back into the concentrate stream. Not a very efficient operation. As the consumption of frozen orange concentrate increased, it became apparent that a more practical method was needed to handle bulk product. As a result, large stainless steel tanks have been built, some of which have a capacity of over 100,000 gals. They are housed in refrigerated warehouses, the size of the buildings depending upon the number of tanks they will accommodate. The tanks are filled with orange concentrate, usually 65°Brix, at temp which may range at some plants from 15°F (–9.4°C) to as high as 30°F (–1.1°C). Periods of storage could range from a few months to over a year. Little is known of what metabolic activity may take place under these storage conditions. Kitchel (3 investigated the survival of 4 different strains of osmophilic yeast in 60°Brix orange concentrate at 5°, 20°, and 40°F (–15°, –6.7°, and 4.4°C). All strains grew at 40°F but not at 20° or 5°F. Murdock and DuBois (4) investigated the growth of 4 strains of osmophilic yeast in 58.5° Brix orange concentrate. They reported growth at 40°F but not at 15°F and 0°F. In 70° Brix concentrate no growth occurred at 40°F. Yeast have been reported to grow in other fruit products at temperatures below 32°F (0°C). Pederson et al (6) found a *Candida sp.* to grow at 28°F (–2.2°C) in grape juice. Berry and Magoon (1) reported *Torula sp.* to grow in berries in 40% sucrose at 25°F (–4°C).

This study was conducted to determine the effect of cold temp storage on yeast survival in 45° and 65°Brix orange concentrate, under simulated conditions of bulk storage.

Experimental Procedure

Test organisms used in this investigation consisted of 3 strains of yeast identified as *Zygosaccharomyces vini* (Y-35), *Z. rouxii* (Y-36), and *Hanseniaspora melligeri* (Y-10); hereinafter referred to as strains A, B, and C respectively. Suspensions of each strain were prepared by washing growth from potato dextrose agar (PDA) slants with sterile distilled water. The concn of each suspension was determined by the Agar Plate Method using Orange Serum Agar containing 5% sucrose. These suspensions were then used to inoculate 45° and 65°Brix orange concentrate so that each contained approximately 1,000,000 organisms per ml. The appropriate amount of strain A suspension was added to the concentrate and mixed in a Waring Blender for 2 min. Inoculated concentrate was then transferred to sterile test tubes (approximately 5 ml per tube). The same procedure was repeated with suspensions of yeast strains B and C. Approximately 40 replicate tubes of each variable were then placed in cold storage for each temperature investigated (0°, 15°, 30°, and 40°F). Duplicate samples of each variable were removed at various intervals over a 15 month period and analyzed for total viable count. Each sample was plated in duplicate, using Orange Serum Agar. Plates were counted after 48-72 hrs. of incubation at 86°F (30°C).

The 65°Brix orange concentrate was prepared from 67°Brix evaporator pump out by adding sufficient sterile water to obtain the desired Brix. Commercial product was used as the source of 45°Brix concentrate. Brix-acid ratios for 45° and 65°Brix concentrate were 14:6 and 19:0.

Results and Discussion

The survival of yeast strain A in 45° and 65°Brix at 0°, 15°, 30°, and 40°F is shown in Figs. 1 and 2. The survival curves for strains B and C were quite similar to strain A; therefore they are not shown.

Yeast did not grow in 45°Brix at any of the temp investigated below 40°F. At this temp, 1 strain grew in 2 months and the other 2 strains in 3 months. Mold was detected in 7 months at 30°F and 12 months at 0° and 15°F. A slight brown discoloration was noted at 7 months at 30°F but not at 0°F and 15°F.

None of the yeast strains grew in 65°Brix, even when held for extended periods at 86°F (30°C). However, it is known that certain strains of osmophilic yeast will grow in 65°Brix. The Research Department of Continental Can Company, Inc. (2) investigated yeast spoilage in 65°Brix orange concentrate. Since our data showed no growth in 45°Brix at 30°F it is believed this would also be true in 65°Brix even if we had a yeast that would grow at this concn. Mold growth was noted at 9 months at 30°F and 12 months at 15°F, and 40°F but not a 0°F. A slight discoloration was observed at 30°F and 40°F after 15 months, but not at 0°F or 15°F.

The number of months required to reduce yeast population 90% is shown in Table 1. It is interesting to note that all 3 strains died faster in 65°Brix at 40°F than they

[1]The authors wish to thank J. F. Folinazzo of Continental Can Co., Inc., Chicago, IL for the yeast cultures used in this investigation.

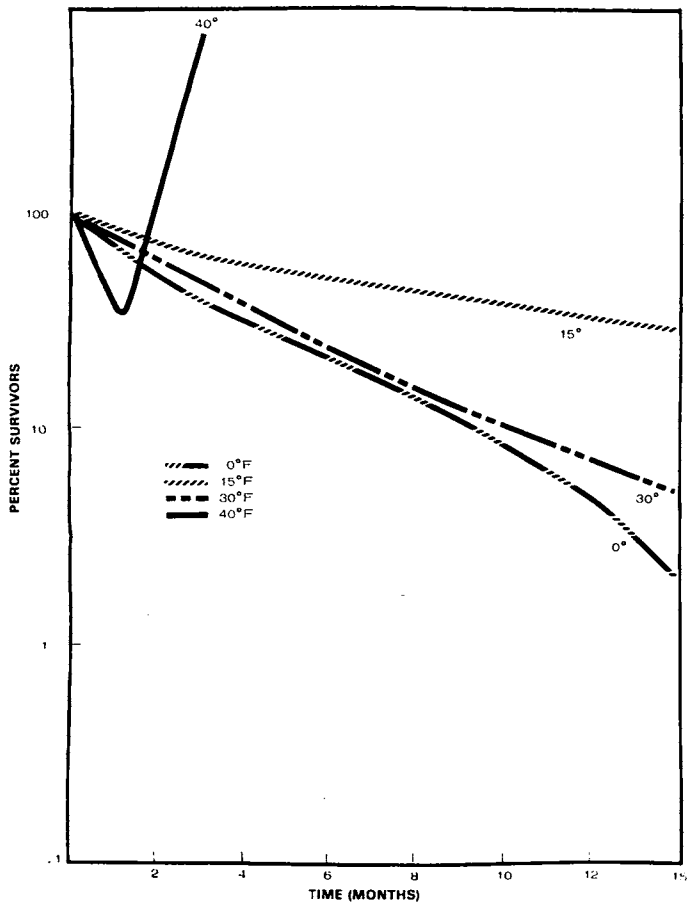

Fig. 1. Survival of yeast strain "A" in 45°Brix concentrate during bulk storage at 0°, 15°, 30°, and 40°F.

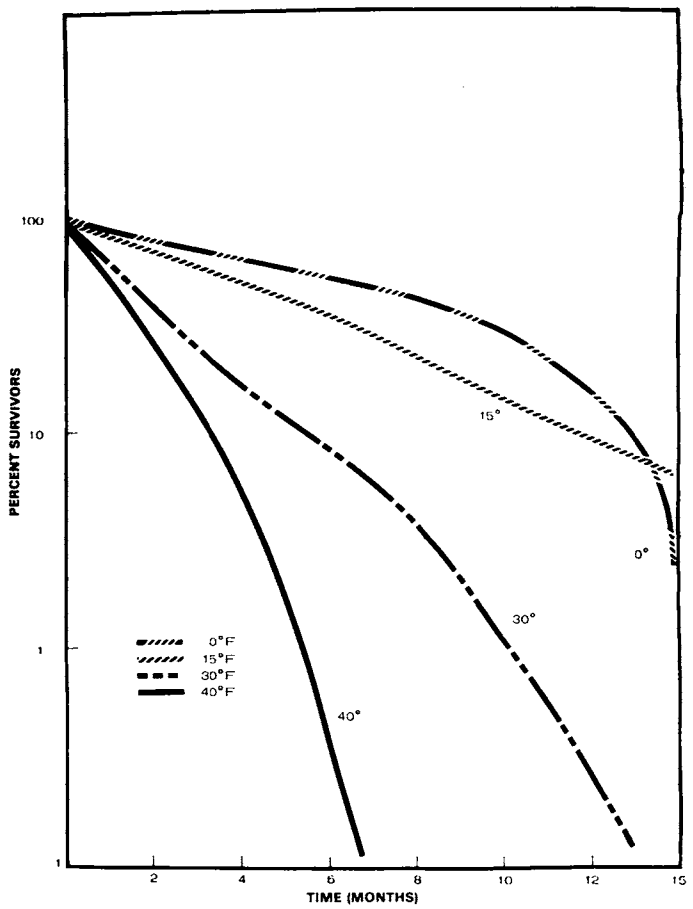

Fig. 2. Survival of yeast strain "A" in 65°Brix concentrate during bulk storage at 0°, 15°, 30°, and 40°F.

did at the other temp investigated. Except for strain B, the yeast did not die as fast at 0°F as they did at the other 3 temperatures. Generally speaking, it appears that the lower the temp the slower the death rate. However, in 45°Brix this trend was not evident. At 15°F the yeast survived in larger numbers than they did at either 0° or 30°F.

Table 1. Number of months to reduce yeast population level 90%.

	45°Brix Strain			65°BRIX Strain		
°F	A	B	C	A	B	C
0	9	3	6	13	3	9
15	>15	8	12	11	2	7
30	10	4	5	5	6	6
40	Gz	Gz	Gz	3	1	4

zGrowth

Even though our data showed no microbial growth to occur, metabolic activity still takes place in the viable cells, the end products which could possibly produce off-flavors. Murdock and Brokaw (5) noted 6 oz. cans of 42°Brix orange concentrate held at 40°F swelled and/or burst when no apparent increase in microbial population had occurred. In this particular case, the respiration of yeast apparently produced enough gas to cause the cans to swell and/or burst. It is not known whether or not this evolution of gas could possibly be a problem after long periods of storage of bulk concentrate.

Effect of Temperature Change on Yeast Population

It is the custom in the citrus industry to hold high count product in the freezer until the total viable count reaches an acceptable level. Sometimes this may occur after a few weeks, several months, or may never reach acceptable levels. Data presented herein show 2 of the 3 strains died slower in 65°Brix at 0°F than they did at the other 3 temp investigated. The effect of temp change on yeast population in 65°Brix orange concentrate was further investigated. One, designated as a laboratory study, involved inoculating 65°Brix orange concentrate with yeast strain A, placing the inoculated material into a series of test tubes, holding 1 set for 2, 4, and 6 days at 30°F and another group for the same length of time at 40°F, then placing the tubes at 0°F for 1 week, after which the product was plated for total viable yeast count. The results in Table 2 show the greatest reduction in yeast population occurred when the product was held 2 days at either 30° or 40°F. Holding it for longer periods at these temperatures did not appear to have any beneficial effect.

In another investigation, 15 — 5 gal. drums of 58°Brix orange blend were removed from 0°F storage and held 2 days at room temperature (50-60°F) and then 2 weeks at 0°F. A sizeable reduction in yeast population was obtained after this treatment (Table 3). The results represent what might occur with a mixed yeast flora, as this was commercial product. Also, no flavor degradation was noted as a result of this treatment.

Summary

In summary, our data indicated yeast did not grow in

Table 2. Effect of temp change on yeast population in 65°Brix orange concentrate.

Strain A Laboratory Study Product held 2, 4, 6 days at 30° and 40°F and 1 wk. at 0°F						
Days	Bef.	Aft.	% Dec.	Bef.	Aft.	% Dec.
		30°F			40°F	
		Org/ml x 10^3			Org/ml x 10^3	
2	640	260	59	480	280	42
4	550	290	47	550	460	16
6	450	320	29	460	310	33

Table 3. Effect of temperature change on yeast population.[z]

58°Brix orange blend	
Before treatment Original level of contamination	After treatment 2 days at RT (50-60°F) and 2 wks. at 0°F)
Yeasts per ml	Yeasts per ml
240-3000	12-825

[z]15 — 5 gal. drums of product examined before and after treatment.

45°Brix concentrate at any temp investigated below 40°F. At this temp 1 strain grew in 2 months and the other 2 in 3 months. Mold was detected in 45°Brix concentrate after 12 months at all temp investigated below 40°F. A slight brown discoloration was noted after 7 months at 30°F but was not evident in 15°F or 0°F samples. Yeast did not grow in 65°Brix concentrate at any temp over extended periods of storage. Mold growth was noted in 65°Brix concentrate after 12 months at all temperatures investigated above 0°F. A slight discoloration was observed at 30°F and 40°F after 15 months but not at 15°F or 0°F.

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ORANGE JUICE COLOR MEASUREMENT USING GENERAL PURPOSE TRISTIMULUS COLORIMETERS[1]

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Abstract. **Several general purpose colorimeters were found which under the proper sample presentation conditions could successfully be used for orange juice color measurement in place of the special purpose Hunterlab citrus colorimeter. Correlations of better than 0.98 were achieved for multiple regression equations for Agtron abridged spectrophotometer, sphere collector colorimeters, and 0-45° viewing colorimeters as compared with the citrus colorimeter color scores for 150 samples. This paper presents the methods used, regression equations, and possible reasons why certain sample presentation methods were more successful than others.**

Color has a psychological impact on the flavor of a number of food products. The effect visual color has on orange juice flavor was shown in a large scale consumer study at the New York World's Fair (3). As a result of this, the USDA grade standards allot up to forty points out of a possible one hundred for color when determining the commercial grade of orange juice (12). This is equal to the consideration accorded to the subjective flavor score for a juice.

The desire to determine juice color with an objective instrumental method, rather than a visual method led to the development by Hunter Associates Laboratories of the Citrus Colorimeter (CC) (9). The exploratory work and subsequent testing of the CC was done by Wenzel and Huggart (4, 5, 7, 13, 14, 15). They developed the multiple correlation equation (8) which has been adopted as the official color score method (11).

The major disadvantage of the Hunterlab citrus colorimeter is the calibration procedure. The State of Florida and the USDA inspection service requires all instruments used for color measurement to be annually brought to a central location, checked against a series of juices, adjusted to agree with a designated "master" instrument, and then a plastic calibration tube is given an assigned color score for routine, in-plant calibration. This type of calibration procedure is not feasible for instruments scattered over various parts of the world. Also, these plastic tubes fade from constant exposure to light and age. Transporting instruments could affect their optical alignments. Thus instruments which can be calibrated using stable, reproducible, and transportable reference standards would be preferred over the current method.

Presently, the United States standards for grades of orange juice state that "Color may be determined by any colorimeter, approved by the U. S. Department of Agriculture, which gives values equal to the USDA Orange Juice Color Standards (12)". At present, the Hunterlab D45 citrus colorimeter is the only colorimeter thus approved. The purpose of the study presented in this paper was to determine if other, commercially available colorimeters would meet the above stated criteria.

[1]Florida Agricultural Experiment Stations Journal Series No. 592.

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