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STORAGE STABILITY OF ORANGE SYRUPS

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Abstract. **Syrups prepared from orange juice serum were tested for stability against microbial and chemical degradation. Syrups containing 70% soluble solids (°Brix) did not support growth of osmophilic yeasts, while pure sugar syrups prepared at 70°Brix maintained cultures of yeasts for 35 days at 30°C. Therefore, orange syrups appear to contain microbial inhibitors. Nonenzymic browning of orange syrups was measured at 30°C. Browning rates were lower when syrups were prepared from serum treated with cation exchange resin to remove amino acids and lower pH. Browning rates were higher when syrups were prepared from this serum after the pH was adjusted to 4.5.**

Chilled single-strength orange juice is more convenient that frozen concentrated orange juice (FCOJ) as it can be used as purchased, without being reconstituted. Purchases of chilled single-strength juice have increased over the past several years (1). FCOJ stored at 4°C is reconstituted more easily than that stored at −15°C because it can be mixed directly (unthawed) with water. However, commercial packs of FCOJ contain about 45% soluble solids, have water activity (a_w) values of 0.90 to 0.95, and are not chemically or microbiologically stable at 4°C (7).

Orange juice concentrates at 65°Brix have a_w values of 0.80 to 0.84 and are stable against spoilage from most organisms (7). Susceptibility to nonenzymic browning, however, increases as a_w decreases (4), so that flavor and appearance of 65°Brix concentrates may change considerably. We report and discuss the microbial and chemical stability of high-Brix syrups and their potential use in citrus products.

Materials and Methods

Freshly extracted orange juice (OJ) was provided by local processing plants. Polygalacturonic acid (PGA) was obtained from Kingsley and Keith, Englewood Cliffs, New Jersey. Irgazyme, a commercial brand of pectinase enzymes, was provided by Ciba Geigy Corporation, Greensboro, North Carolina.

Saccharomyces bailii and *S. rouxii* were obtained from the Culture Collection of the USDA ARS Northern Regional Research Center. Carboxymethyl cellulose (Cellex-CM) was obtained from Bio-Rad Laboratories, Richmond, California. Constituents of culture media and other chemicals were obtained from Fisher Scientific Company, Atlanta, Georgia.

Preparation of orange juice serum. Serum was prepared from fresh unpasteurized orange juice by slight modification of the method described by Baker (3). A 3% soln of PGA adjusted to pH 4.0 with 5 N NaOH was added to the chilled orange juice to final concn of 300 ppm to aggregate suspended solids. The serum was immediately recovered by centrifugation at 8000 rpm for 2 min in a Lourdes refrigerated Betafuge Model A-2. The yield of serum averaged 86% of the juice (*14% was pulp* and cloud).

Preparation of orange syrups. We prepared 4 types of orange syrups for determining microbial and color stability (syrups 1-4) and for preparing low-pulp orange juice concentrates (syrup 1).

To prepare *orange syrup 1*, we reduced the viscosity of the serum with pectinase (Irgazyme) at final concn of 300 ppm, and storage overnight at 4°C. Then we pasteurized the treated serum at 90°C for 6 sec, cooled it rapidly to 10°C and concentrated it in a Buche Rotovapor R-20 at 30°C. Syrup Brix values were calculated from weights of serum and syrup and also from values of titratable acidity and sugars. Sugars were measured with the Abbe-3L refractometer (Bausch and Lomb, Rochester, New York). Syrups were stored at 4°C until tested; time of storage was less than one month.

Orange syrup 2 was prepared as described for syrup 1 except that after pasteurization and cooling, 6 liters of serum was passed through 1800 g Dowex 50W-8x (H^+ form, 20-50 mesh) in a 5 x 100 cm column at the rate of 20 ml/min. The effluent was monitored for amino acids with the ninhydrin spot test (5) to determine effectiveness of their removal. The holdup serum in the column was washed out with distilled water, combined with the major effluent and concentrated in the Rotovapor at 30°C bath temperature. Brix values were calculated as described for syrup 1.

Orange syrup 3 was prepared as described for orange syrup 2, except that the pH of the serum from the Dowex 50W column was adjusted to 4.5 before the effluent was concentrated to a syrup.

Orange syrup 4 was prepared as described for syrup 3 except that after the pH adjustment of the serum, it was passed through carboxymethyl cellulose in a 5 x 100 cm column at the rate of 2 ml/min to remove proteins. The effluent was tested for protein by the tetrabromophenolphthalein method (5). The holdup serum in the column was washed out with distilled water, combined with the major effluent, and concentrated to a small volume in the Rotovapor at 30°C bath temperature. Brix values were calculated as described for syrup 1.

Preparation of model syrup. We prepared model syrups with sucrose/fructose/glucose at a ratio of 2:1:1, °Brix/citric acid level at a ratio of 15.3:1, and a pH at 3.5 (adjusted with 10N NaOH). We prepared syrups at 86.5, 81, 75.7, 71.3 and 66.1°Brix. Calculations of °Brix were checked with the Abbe-3L refractometer.

Preparation of low pulp concentrates. Orange syrup 1, at 88°Brix was diluted with pasteurized single strength OJ to form concentrates at 80, 75, 70, and 65°Brix.

Osmophilic yeasts. Inoculated cultures of *Saccharomyces bailii* Y-7255, *S. bailii* Y-7262, *S. rouxii* Y-288, and *S. rouxii* Y-2547 (NRRC Collection number) were grown to population densities of about 6 x 10^8 organisms/ml in shake flasks at 100 rpm at 27°C in the following medium (pH 4.5): 0.5% tryptone, 20.1% glucose and 0.25% yeast extract in distilled water. These cultures were used as inocula (0.1 ml) for the syrups.

Microbial growth test. Orange juice concentrates were tested for contamination with pour plates made with each

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of 3 media: Difco orange serum agar (OSA), 0.5% tryptone 0.1% glucose and 0.25% yeast extract (TGY), with 1.5% agar and TGY with 20% glucose and 3% agar. The orange juice concentrates and syrups inoculated with the osmophilic yeasts were diluted 10 and 100 fold to give colony plate counts in the 30 to 300 range. Only the TGY medium containing 20% additional glucose and 3% agar was used as pour plates for growth test of the osmophilic yeasts. Plates were examined and colonies counted after 48 and 96 hr at 30°C.

Measurement of color formation. The official method for analyzing corn syrup color was modified for use with orange syrups (6). Syrups were diluted to 5 °Brix at 20°C. Absorbance at 450 nm and 600 nm were determined with a Beckman DU spectrophotometer. When 450 nm absorbance value were greater than 0.5, the syrup was diluted 1 to 10 by volume, or more if necessary, to bring absorbance below 0.5. The dilution factor includes 30 (to revert observed color to 40 Baume concentration of original) times the number of dilutions made to read on scale. Soln color = $(A_{450} - A_{600})$ x (dilution factor). The soln color was used to determine the rate of color development in the syrups.

Results and Discussion

Evaporation of OJ to 65°Brix is practiced commercially. However, pulp and juice cloud particles severely limit the efficiency of evaporation as the °Brix approaches this value. A Florida Department of Citrus Advisory Committee in proposed specifications, suggested that juice for evaporation to between 55 and 70°Brix, should have pulp content less than 9% and viscosity less than 2 centipoise at 26.7°C (2). Removing all pulp and cloud particles should increase the Brix level attainable by evaporators. We found that clarified orange juice (serum) treated with pectinase to reduce viscosity can be routinely concentrated to 85°Brix syrup. The 85°Brix syrup can be used to formulate low pulp, high Brix juice concentrates by adding high quality OJ.

Microbial growth in low pulp concentrates. Examination of the low pulp concentrates (80, 75, 70, and 65°Brix) for microbial contamination indicated that the population count was proportional to the amount of syrup in the concentrate. The 80°Brix concentrate contained the greatest proportion of syrup and had the highest initial count. (Table 1). The population of organisms declined in all concentrates during storage at 30°C. These results suggest that the concentrates do not support growth of the organisms that contaminated the products during processing.

Osmophilic yeasts are the usual contaminants of juice concentrates (7). In low pulp concentrates that were inoculated with several strains of *S. bailii* and *S. rouxii* at 6 x 10^6 organism/ml and incubated at 30°C, population increased and/or gas was produced in most of the 70 and 65°Brix samples (Table 2). The number of organisms declined in the 80 and 75°Brix samples after 12 and 20 days and no gas was formed. Our data indicated that the 70 and 65°Brix concentrates are susceptible to microbial deterioration.

Table 1. Survival of process contaminants in low-pulp orange juice concentrates at 30°C.[z]

°Brix	Microbial population (organisms/ml)					
	OSA[y]		TGYA[x]		TGY-GA[w]	
	Initial	7 Days	Initial	7 Days	Initial	7 Days
80	260	190	430	310	210	70
75	180	110	230	190	140	40
70	110	80	180	70	70	50
65	80	40	90	40	50	10

[z]88°Brix syrup was diluted with pasteurized OJ to Brix values listed. After 35 days, all concentrates contained less than 10 organisms/ml.
[y]OSA = orange serum agar.
[x]TGYA = Tryptone-glucose-yeast extract agar.
[w]TGY-GA = TGYA plus 20% glucose.

Table 2. Growth of *Saccharomyces bailii* (Y-7255 and Y-7262) and *S. rouxii* (Y-288 and Y-2547) in low-pulp orange juice concentrates incubated at 30°C.[z]

[w]Brix	Incubation (days)	Microbial population (organisms/ml)			
		Y-7255	Y-7262	Y-288	Y-2547
80	4	1 x 10^5	2 x 10^6	1 x 10^5	1 x 10^7
	12	1 x 10^3	2 x 10^6	1 x 10^3	1 x 10^3
	20	20	1 x 10^4	10	10
75	4	2 x 10^5	4 x 10^6	2 x 10^7	2 x 10^6
	12	1 x 10^3	5 x 10^5	1 x 10^3	1 x 10^3
	20	80	1 x 10^4	2 x 10^3	6 x 10^2
70	4	2 x 10^6	2 x 10^6	*1 x 10^9*	4 x 10^6
	12	6 x 10^6	5 x 10^7	*6 x 10^{11}*	1 x 10^7
	20	6 x 10^3	1 x 10^8	*8 x 10^{12}*	*8 x 10^9*
65	4	1 x 10^7	2 x 10^6	*4 x 10^{12}*	*4 x 10^9*
	12	6 x 10^6	5 x 10^7	*2 x 10^7*	*4 x 10^9*
	20	*4 x 10^4*	*2 x 10^6*	*1 x 10^{10}*	*1 x 10^6*

[z]Samples were prepared from 88°Brix syrup and pasteurized OJ and then inoculated with 6 x 10^6 organisms/ml. Italicized populations produced gas.

Microbial growth in model syrups. Model syrups inoculated with the osmophilic yeasts did not support growth and fermentation when incubated at 30°C (Table 3). The population in 86.5, 81 and 75.7°Brix syrups declined to less than 1 x 10^3 organisms/ml after 4 days' incubation (data not shown). The population of *S. bailii* (Y-7255 and Y-7262) stabilized at 1 to 6 x 10^5 organisms/ml after 35 days in the 66.1 and 71.3°Brix syrups. The population of *S. rouxii* (Y-288 and Y-2547) in those syrups declined to less than 1 x 10^3 organisms/ml after 35 days. These data show that, at 75.7°Brix and above, the model syrups inhibited growth of osmophilic yeasts. Growth of these yeasts was also inhibited in 75°Brix OJ concentrate (Table 2). Therefore the minimum Brix value that would prevent spoilage from osmophilic yeasts at 30°C is probably between 70 and 75°Brix.

Table 3. Growth of *Saccharomyces bailii* (Y-7255 and Y-7262) and *S. rouxii* (Y-288 and Y-2547) in model syrups incubated at 30°C.

°Brix	Incubation (days)	Microbial population (organisms/ml)			
		Y-7255	Y-7262	Y-288	Y-2547
71.3	4	4 x 10^5	6 x 10^5	1 x 10^3	2 x 10^3
	20	4 x 10^5	2 x 10^5	1 x 10^3	1 x 10^3
	35	1 x 10^5	6 x 10^5	1 x 10^3	1 x 10^3
66.1	4	4 x 10^5	1 x 10^6	6 x 10^3	2 x 10^4
	20	4 x 10^5	6 x 10^5	1 x 10^3	8 x 10^4
	35	2 x 10^5	4 x 10^5	1 x 10^3	1 x 10^3

[z]Inoculated to contain 6 x 10^6 organisms/ml: plate counts in 86.5, 81 and 75.7°Brix syrups were less than 1 x 10^3 cells/ml throughout the experiment.

Microbial stability of orange syrups. Orange syrups 1, 2, 3, and 4 prepared to contain 70°Brix were inoculated with osmophilic yeasts (6 x 10^6 organism/ml) and incubated at 30°C. After 4 days the cell count was less than that in the inoculum. Plate counts of each strain for each syrup were less than 1 x 10^3 organisms/ml. These results show that the syrups are microbially stable at 70°Brix and suggest that the 70°Brix juice concentrate (Table 2) was unstable

because of substances in the orange juice that was added to the syrup to prepare the concentrate. Orange syrups appear to contain microbial inhibitors, since the pure sugar model syrups at 70°Brix maintained osmophilic yeast cultures for more than 35 days at 30°C (Table 3). The pH for orange syrup 1 and the model syrups was 3.5.

Color stability of orange syrups. Orange syrup 1 darkened slightly during 1 year storage at 4°C (data not shown), and noticeably after several months at 30°C (Table 4). Comparison of unfiltered syrup 1 (pH 3.8) with Dowex-filtered syrup (pH 2.0) showed that the filtered syrup, which had its amino acids removed, had less darkening. No amino acids were detected by the spot test, at a threshold value of 1 μg/ml for arginine, in syrup 2. For syrup 3, the pH of the serum used for the concentrate was adjusted to pH 4.5, and the syrup was considerably darker than syrup 2 (pH 2.0). Carboxymethyl cellulose treatment of serum used in preparation of syrup 4 lowered protein content which resulted in a final pH of 3.7. Syrup 4 darkened less than syrup 3 (pH 4.5), but more than syrup 1 (pH 3.8), which was unfiltered. Thus, lowering the pH was probably more effective than removing amino acids for stabilizing color.

The effects of pH on nonenzymic browning in OJ and model systems were recently reviewed by Shaw *et al.* (8). The data in Table 4 support their conclusion that acidic

Table 4. Color formation in orange syrups at 30°C.[z]

	Color (A_{450}–A_{600}) x (dilution factor)			
Weeks	1	2	3	4
2	30	30	60	40
6	50	35	120	70
9	50	40	170	80
12	60	40	—	90
15	70	50	—	110

[z]Syrup 1 = unfiltered, pH 3.8; Syrup 2 = Dowex-filtered, pH 2.0; Syrup 3 = Dowex-filtered, pH adjusted to 4.5; Syrup 4 = Dowex-filtered, pH adjusted to 4.5 carboxymethylcellulose-filtered, final pH 3.7.

pH is a deterrent to nonenzymic browning. Syrups prepared from sera adjusted to pH 4.5 were less stable than syrups prepared from sera at pH 2.0.

Conclusion

Depectinized serum from clarified OJ formed a microbially stable syrup on evaporation to 70°Brix. Color stability of the syrup was improved by Dowex treatment of the serum. Stability conferred by the Dowex treatment was negated by pH adjustment to 4.5.

OJ concentrates of 75 and 80°Brix prepared from OJ syrup and high quality OJ were *microbially stable at 30°C* after inoculation with osmophilic yeasts. OJ concentrates of 65 and 70°Brix were microbially stable at 30°C as processed, but supported growth and fermentation action of inoculated osmophilic yeasts. The osmophilic yeast growth data indicate that 70°Brix OJ syrup and 75°Brix OJ concentrate are products that would be microbially stable without refrigeration.

Clarified citrus syrups can be blended with juices to form stable high Brix products. Syrups for this use should be at least 85°Brix and preferably between pH 2 and 4. Such syrups could be used as an all natural orange product to increase the sweetness of citrus juices. Certain stabilizing factors appear to be present in natural syrups but not in simple sugar syrups; these factors need further investigation.

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