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PECTINASE TREATMENT OF RAW ORANGE JUICE AND SUBSEQUENT QUALITY CHANGES IN 60°BRIX CONCENTRATE[1]

ROBERT J. BRADDOCK
University of Florida, IFAS,
Agricultural Research and Education Center,
700 Experiment Station Road,
Lake Alfred, FL 33850

Abstract. **Comparisons of pectinase-treated raw juices and 60°Brix concentrates with untreated controls indicated little or no changes in standard citrus juice quality tests, i.e., °Brix, acid, ratio, pH, pulp, color, pectinesterase activity, % oil, vitamin C, cloud and hesperidin. A significant decrease in concentrate viscosity resulted from a 1 hr/25°C treatment of raw juice with 500 ppm of the soluble solids as pectinase. The enzyme treated-concentrate contained 33% more free galacturonic acid than the control; however, this acid was only 1.2% of the total acid in the juice. Concentrations of fructose, glucose and sucrose in the concentrates and raw juices were similar, regardless of treatment. Pasteurized juice, subsequently treated with enzyme had lower viscosity and a very slight loss of cloud with increasing enzyme reaction time. Enzyme treatment improved juice pulp settling characteristics as measured by 20% less sediment in juice allowed to stand undisturbed for 72 hr.**

Modifying the nature of food products by enzymatic reactions has exciting potential. Besides traditional uses of enzymes (cheese production, fruit juice clarification, alcohol production, meat tenderization, etc.), newer uses such as corn syrup processing, vegetable protein hydrolysis, flavor modification and fruit juice processing are finding wide application (6).

Pectinases are useful during juice recovery from berries and fruits, hydrolyzing pectin to lower molecular weight (MW) fractions. This results in viscosity reduction and less gel formation via a mechanism which has been described in detail (9). The obvious advantage of viscosity reduction is that it allows a high degree of concentration for juices containing significant quantities of pectin.

Pectinase treatment of citrus juice liquids such as pulp wash is common, and concentrates over 70°Brix (°B) have been produced (3). Most quality changes resulting from pectinase treatment of pulp wash liquids are minimal, the large decreases in viscosity excepted (4). Besides viscosity reduction in pulp wash liquids, use of pectinases has been shown to improve cloud stability of orange juice (2).

Although pectinase use for pulp wash liquid is well established (5), there are concerns that such treatment of raw juice to facilitate concentration and handling at high °B might result in quality deterioration. This study was designed to compare standard citrus industry quality factors in untreated and pectinase treated orange juice and 60 °B concentrate.

Experimental

Juice. Approximately 30 boxes (1,235 kg) of mature Valencia oranges (harvested May 14, 1981) were washed and the juice extracted using an FMC Model 391 (Lakeland, FL) extractor. Finished juice yield was 49.8% (615 kg). The juice was thoroughly mixed and divided into 2 equal portions, one tank serving as a control, the other for enzyme treatment. While the control juice was being concentrated in the evaporator (pilot T.A.S.T.E. Gulf Machinery, Safety Harbor, Fla.), enzyme reaction was commenced in the other sample. Upon concentration of juice in the first tank, the evaporator was rinsed briefly with water and without interruption, the enzyme-treated juice was concentrated. Evaporation rate was approximately 182 kg H_2O/hr.

Enzyme (Biopectinase 700, Biocon, Lexington, Ky.) was added at a level of 500 ppm of the total juice soluble solids in the tank (approx. 18 g enz/tank juice). Average enzyme reaction time (1 hr) was based on evaporation rate and was calculated to include emptying the feed tank. Juice temperature was approximately 25°C. General enzyme temperature and pH optima are described in the manufacturer's technical data. For the enzyme (Biopectinase 700) used in this study, the polygalacturonase activity as supplied was approximately 3000 units (1 unit = 1 μmole galacturonic acid/g enzyme/min).

In other experiments, the above mentioned evaporator heat-stabilized control concentrate was used to study effects of enzyme addition on juice viscosity, pulp sedimentation, vitamin C, and cloud. For these studies, experiments were performed using 5 separate 500 ml samples and controls for each analysis. Enzyme (500 ppm as above) was added to the juice diluted to 12.5 °B (the original corrected °B of the raw finished juice). The enzyme was allowed to react for 0 (no enzyme control), 0.5, 1.0, 1.5, and 2.0 hr. After reaction, added pectinase was inactivated by heating the samples in a stainless steel pan with lid to 74°C, then cooled to room temperature in an ice bath. This process took about 8 minutes.

Analyses. Routine citrus juice quality tests of °B, acid, ratio, pH, sinking pulp, color score, pectinesterase activity, Scott oil analyses, cloud (% T), and hesperidin (Davis test) were performed as described in Praschan (8). Nitrogen content of 60°B concentrates was determined by micro-Kjeldahl analyses (1). Viscosities were determined with a viscometer (Brookfield Model LVF) equipped with a UL adaptor for single strength juices and spindle 2 for the 60°B concentrate (300 ml sample at 25°C in a 500 ml Berzelius beaker, 6, 12, 30, 60 rpm). Pulp sedimentation was determined after 72 hr by measuring the sediment height of juice in a 100 ml graduated cylinder (7).

Sugars and organic acids were determined by gas-liquid chromatography (GLC) of the silylated derivatives (12). The 60°B concentrates were diluted to single strength (12.49°B), 1 ml brought to 10 ml volume with 95% ethanol, mixed, 0.1 ml sat'd lead acetate added and centrifuged 1500 x g to obtain ppt. The supernatant fluid was saved. The ppt was washed 2 X 95% ethanol, 1 X acetone, 1 X ether and dried to constant weight at 75°C in an oven. The silylating reagent (1 ml TriSil, Pierce Chemical Co.) was added to the dry ppt, reacted for 30 minutes and centrifuged to obtain a clear sample for GLC injection to measure organic acids.

The supernatant fluid (1 ml) from the lead acetate ppt was evaporated to dryness under vacuum. The silylating reagent was added as before to the dry residue. This procedure prepared the sugar fractions for GLC analyses.

GLC (Hewlett Packard 5730 A with 3385 A automation, Avondale, PA) conditions were as follows: acids—2.5 μl injection; 1.8 m x 0.5 cm glass SE 30 column; column oven at

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140°C, 4 minutes, then 8°C/minute to 260°C, hold 4 minutes; duplicate samples, duplicate injections. Sugars—same as for acids, except column oven, 90°C, 4 minutes, 8°C/minute to 220°C; triplicate samples, duplicate injections. Injections were automatically performed, peak areas were integrated by computer and compared with 0.001 mg/ml standarde solutions of acids and sugars.

Results and Discussion

The cost and inconvenience of treating fruit juices with pectinases can be weighed against advantages like viscosity reduction. For orange juice to be concentrated to greater than 60°B, the cost and inconvenience of using pectinase can be estimated or calculated, after effects on quality have been defined. The following results define some of these quality effects and should assist a processor to decide whether using pectolytic enzyme treatment as a juice processing aid is justified.

Treatment of raw juice. As stated before, the major action of pectolytic enzymes in orange juice is to reduce viscosity (by fragmenting pectin molecules—see ref 9). The 50% viscosity reduction illustrated in the 60°B concentrate of Figure 1 and Table 1 was achieved by pre-determining enzyme reaction conditions in small scale laboratory experiments. Other levels of viscosity reduction could be achieved by similarly adjusting enzyme concentrations and/or reaction times. These procedures involve applying levels of enzyme to the raw juice and concentrating to about 3-fold using a lab-size vacuum evaporator. Viscosity is then measured on the obtained concentrate. The low viscosity of the control concentrate (Table 1) could be accounted for by the fact that the fruit used in the study were of good quality (Author's opinion).

Closer examination of Table 1 indicates some other differences between treatments. There was a slight increase in titratable acidity and a corresponding decrease in the ratio.

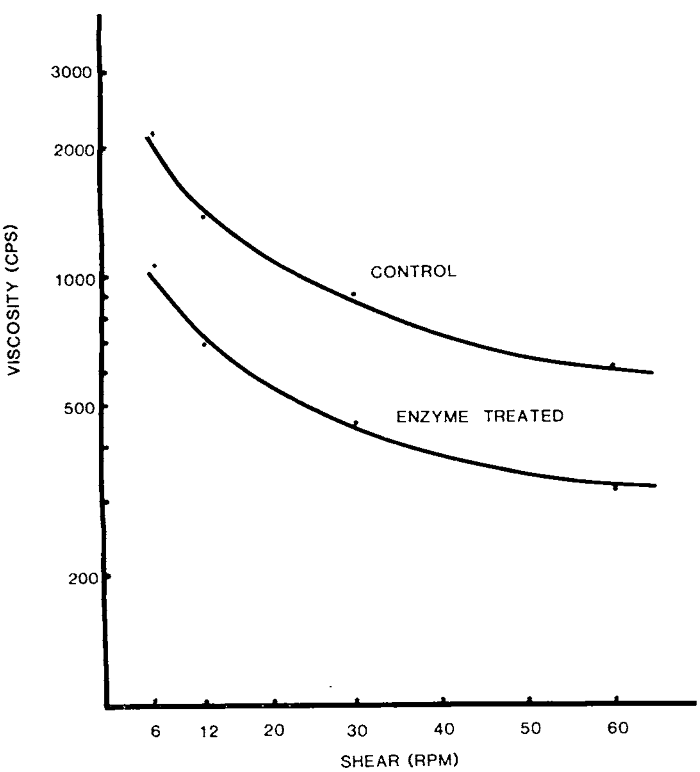

Fig. 1. Viscosity curves of 60°B orange concentrate from control and pectinase-treated raw juices. Enzyme was 500 ppm of soluble solids, 1 hr at 25°C.

Table 1. Quality factors of pectinase treated and control Valencia juices and concentrates.[z]

Factor	Control	Treated
Concentrate:		
°Brix	60	60
acid (%)	4.10	4.17
ratio (°B/a)	14.6	14.4
nitrogen (%)	0.6	0.6
viscosity (cps at 60 RPM)	600	322
Reconstituted conc:		
°Brix	12.5	12.5
acid (%)	0.86	0.87
serum viscosity (cps at 60 RPM)	1.58	1.38
pH	3.79	3.79
pulp (%)	7.4	5.6
color Cr	38.2	37.6
Cy	82.5	82.5
N	38.0	37.9
pectinesterase (P.E.U.)[y]	.035	.030
vitamin C (mg/100 ml)	36.8	36.0
hesperidin (ppm)	540	457
cloud (% T at 650 nm)[x]	13.1	13.3

[z]Values are the means of triplicate analyses.
[y]See Praschan (8).
[x]See text for raw juice cloud.

Because of the importance of ratio to flavor quality evaluation, major organic acids in the juices were analyzed more closely. Results in Table 2 show increases in free galacturonic acid in the enzyme-treated juice compared with the control. However, as can be seen in Figure 2, the galacturonic acid fraction is small (1.2% of total acid) compared with the other 2 major juice acids.

The centrifuge pulp content of the enzyme-treated sample (Table 1) was less than the control. This is probably a result of the viscosity decrease, since destruction of the pectin would allow larger sized suspended matter in the juice to precipitate more easily and form a more compact pellet in the centrifuge tube. The pulp content of these juices was lower than the 12% allowed for commercial Florida concentrate. The reconstituted juice cloud did not seem to be affected by enzyme treatment (Table 1). However, the cloud in the raw juice serum (prior to heat stablization in the evaporator) was considerably reduced, 14.5% T for control vs 24% T in the treated sample. This result could be explained by the clarifying effect of the native pectinesterase in the raw juice over the 1 hr holding time (2.5 units control vs 2.4 units treatment).

When the reconstituted juices were allowed to stand for a couple of hours, a 20 to 25% increase in pectate-type flocculation occurred in the enzyme-treated sample. Again, this was probably a function of native juice pectinesterase actions in the raw juice during holding prior to heat stabilization, as well as reduced viscosity.

Color was not significantly affected in this study by enzyme treatment, but caution should be exercised at this point. One should recognize that pectinase treatment will allow easier concentration to very high brix. Above 60°B, it could be assumed that the additional concentration of juice browning constituents, as well as a requirement for more processing (higher heat, longer evaporator residence time, etc.), could easily result in more browning in the evaporator pump-out. Increasing the free galacturonic acid content by enzymatic hydrolysis of the pectin in the juice has potential to promote browning during manufacture of concentrate. For example, Seaver and Kertesz (10) showed that rates of colored polymer formation (during heating in the presence

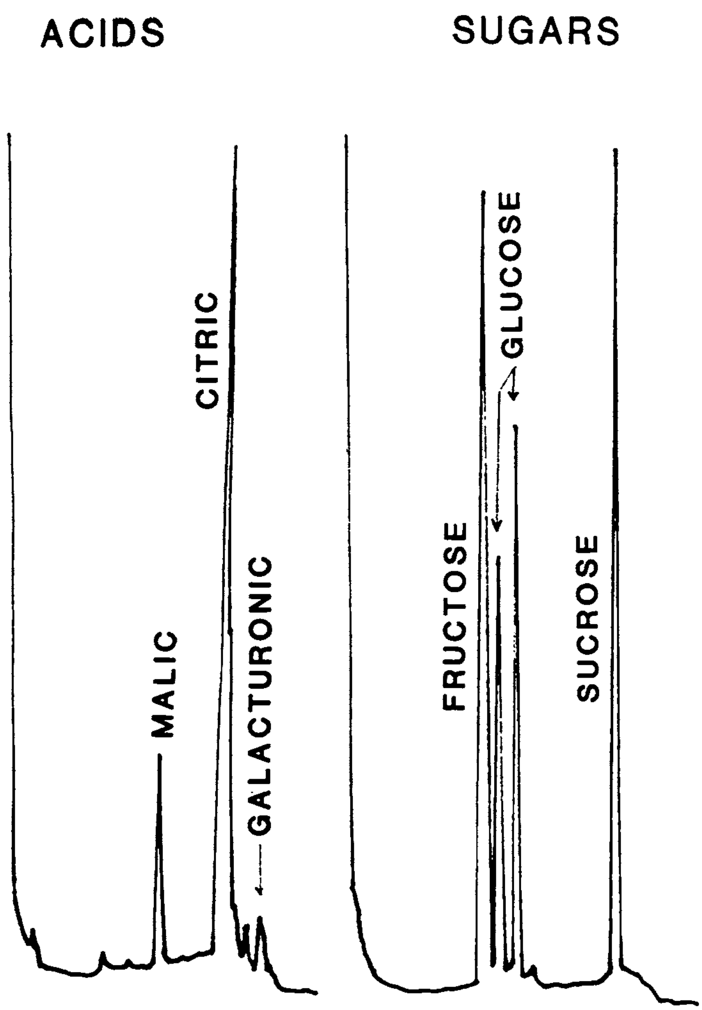

Fig. 2. Representative GLC peaks of acids and sugars from reconstituted 60°B orange concentrate prepared from either control or enzyme-treated raw juice samples.

Table 2. Quantitative GLC analyses of major acids and sugars in orange juices reconstituted from 60°B concentrates.[z]

	Control	Treated
Acids (% of total peak area)		
galacturonic	0.9,0.1	1.2,0.1
malic	7.4,0.2	7.0,0.2
citric	91.3,0.3	91.8,0.3
Sugars (% w/w)		
fructose	2.68,0.4	2.83,0.5
glucose	1.93,0.2	2.22,0.5
sucrose	3.92,0.7	4.08,1.1

[z]Values are for N = 2 experiments, duplicate analyses reported as $\bar{x}$, standard deviation.

of 0.1 M glycine) were highest for pectinic acid degraded by H_2O_2, decreasing in the order galacturonic acid, glucuronic acid, ascorbic acid and common monosaccharides.

Nitrogen content (Table 1) was not affected by enzyme treatment, the quantity being similar to previously reported values (11). There was also a slight decrease observed in vitamin C content of the enzyme-treated juice when compared to the control.

A non-trained taste panel of 15 persons was presented the samples in a multiple comparison (R = control, sample 1 = control, sample 2 = treated) difference analysis on 2 consecutive days. Ten panelists one day and 2 panelists the second day were able to identify the pectinase-treated reconstituted concentrate. From an analysis of variance, the mean square F ratio was significant for $\alpha = 0.05$. Panelists were asked to compare 2 samples coded with random numbers to the "R" sample. The major reasons given for the difference were comments like "watery," "thin," "beverage-like," "different mouth-feel." These comments indicate that mouth-feel or texture was a major reason the enzyme-treated samples could be identified.

Treatment of heat-stabilized juice. Additional data was needed comparing pectinase treatment to an enzyme-stabilized control, particularly concerning the previous discussion about cloud loss and pulp flocculation. The question to be considered is whether pulp and cloud differences are related to action of native juice pectinesterase or to commercial pectinase. Enzyme treatment of raw, unpasteurized juice was performed because of time limitations involved with using the pilot evaporator. This necessitated eliminating a 1 hr control (no enzyme) juice.

To compare cloud loss and pulp sedimentation using samples of heat-stabilized orange juice, control concentrate was diluted to 12.5°B and treated with commercial pectinase. Values in Table 3 indicate that cloud loss (measured by serum turbidity in centrifuge pulp test) was not significant during the 2.0 hr treatment of pasteurized juice with pectinase. Results in Table 3 showed that pectinase treatment also reduced the amount of pulp sedimentation, another measure of cloud loss.

Table 3. Quality factors of 12.5°B pasteurized orange juice treated with 500 ppm commercial pectinase for various time periods.

Parameter Measured[z]	Enzyme Reaction Time (hr) 0[x]	0.5	1.0	1.5	2.0
Viscosity (cps)	1.7,0.1	1.6,0.1	1.5,0.1	1.5,0.1	1.5,0.1
Sediment (%)[y]	24.6,2.9	21.0,1.1	20.2,1.8	19.8,1.6	19.5,1.0
Cloud (% T)	11.6,0.2	12.7,0.6	12.9,0.8	12.9,0.8	12.9,0.8
Vitamin C (mg/100 ml)	40.6,1.8	39.7,1.9	40.5,1.7	41.1,1.9	40.3,1.4

[z]Values are for N = 5 experiments reported as x, standard deviation.
[y]Sediment is % height of sediment from sample in 100 ml graduated cylinder after standing undisturbed for 72 hr.
[x]0 hr is a no enzyme control.

Krop and Pilnik (7) explain the beneficial effects of pectinase in stabilizing orange juice cloud as resulting from lowering the MW of pectate fractions below that MW which can be precipitated by calcium ions in the juice. They (7) also treated juice with oxalate (to bind calcium), showing significantly less pulp sedimentation than juice containing active pectinesterase but no oxalate.

Results of Tables 1 & 2 for °B, sugars and acids would imply that flavor changes might be slight. Also, the GLC data (Figure 2, Table 2) for acids (discussed above) and for individual sugars showed no significant differences. It was suggested from the data that a reduction in viscosity could be detected by sensory evaluation. Other results indicate the importance of inactivating the native juice enzymes, prior to any treatment with commercial pectinases. It is hoped that the above results and discussion will be useful, when considering the potential application of pectinases as processing aids for treating orange juices. As shown, there are some changes resulting from pectinase treatment of the juice, the most significant of which is reduction of viscosity. It should also be mentioned that standards of identity for concentrated orange juice do not include pectinase treatment as proposed.

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TECHNOLOGIC AND ECONOMIC CONSIDERATIONS OF CITRUS CONCENTRATE STORAGE[1]

F. E. STEGELIN
University of Florida, IFAS,
Food and Resource Economics Department,
Gainesville, Florida 32611

P. G. CRANDALL
University of Florida, IFAS,
Agricultural Research and Education Center,
700 Experiment Station Road,
Lake Alfred, Florida 33850

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Abstract. **Industry shifts to bulk tank storage have been partially triggered by the increased economic and operating efficiencies of tank farms over drum storage. The citrus industry has also observed a 92% increase in the level of bulk utilization since the 1977-78 season, an increased amount of concentrate reconstituted outside Florida, an increase in new and reset commercial acreage planned or planted in Florida, and increased competition from imported concentrate to meet a growing consumer demand for citrus products, especially single-strength juices. These issues give cause for managerial involvement in evaluating concentrate storage needs and techniques. The technical analyses in quality control and product standardization for concentrate storage, including alternative Brix concentrate levels and temperatures above 20°F are available as is the equipment needed for the manufacture of citrus concentrate.**

Technologic and economic considerations for storing citrus concentrate by drum, tank, and bag-in-box are presented to aid managements of small and/or potential processors evaluate their future in citrus concentrate storage.

Every gallon of 60-65° Brix citrus concentrate produced by Florida processors may be stored for extended periods of time prior to consumption. Additional storage may be used for bulk concentrate imported to supplement the concentrated orange juice produced from Florida fruit and used to manufacture frozen concentrated orange juice (FCOJ). Several factors are inherently evaluated by management in deciding how and in what form to store citrus juices.

Major considerations in these decisions, according to industry participants and published comments, include: 1. the number and volume of product categories to be inventoried; 2. product handling and transport characteristics; 3. product standardization and quality protection during storage, including uniformity of the concentrate, stratification of product, concentrate loss from blending, sanitation concerns, and microbiological risks; 4. storage space utilization; 5. types and amounts of existing storage; and 6. the costs of storage and warehousing, including investment and fixed costs as well as variable costs (1, 2, 10, 13). The focus of this paper is on describing drum, tank, and bag-in-box storage of citrus concentrate by various technical and economic considerations to aid managements of small processors and/or firms considering entrance into the processing subsector evaluate their citrus concentrate storage decisions.

Drum Storage and Tank Farms

There are several bulk storage techniques and containers used for storing citrus concentrate; however, the most prevalent bulk storage types are drums and tank farms. A comparison of these two types is presented for one million gallons of 65° Brix concentrated orange juice. The comparison includes a relative economic investment evaluation as well as a general discussion of relative advantages and disadvantages of each type of bulk storage. The composite cost approximations were supplied by representatives of various citrus concentrate storage facility manufacturers as well as citrus processors who either have recently installed additional concentrate storage facilities or have made inquires into the costs of storage expansion, but who prefer to remain anonymous.

Drums

Storage in 55-gallon drums has the advantages of handling, storing, and subsequently shipping relatively small amounts of blended concentrate—as little as one drum—thus tailoring one's merchandising program to smaller receivers. Drum storage is versatile because a number of products can be blended and inventoried separately. Drum storage also has an advantage of low initial capital requirements, pri-

[1]Florida Agricultural Experiment Stations Journal Series No. 3581. The cost figures and comparative statistics for the drum, tank, and bag-in-box concentrate storage methods were supplied by various Florida citrus processors, manufacturers representatives and IFAS personnel. The authors appreciate the interest expressed by these individuals in providing this information for this paper.