DEBITTERING OF CONCENTRATED GRAPEFRUIT JUICE WITH NARINGINASE[1]

R. W. OLSEN

Florida Citrus Experiment Station

AND

E. C. HILL

Florida Citrus Commission

Lake Alfred

Over 16,500,000 boxes or 48% of the Florida grapefruit crop were used by commercial canneries during the 1961-62 season (5). Continued production and sale of processed grapefruit products in the future is necessary for the complete utilization of the Florida grapefruit crop.

Some bitterness in processed grapefruit products is acceptable to consumers but excessive bitterness is one of the major consumer objections to such products, as pointed out by Bell (1) and Birdsall (2). Increased sales of grapefruit products may result if the packing of excessively bitter products could be avoided. This could cause a greater demand for Florida grapefruit, which is desirable as recently indicated by Booz, Allen and Hamilton (3).

Naringin is the principal bitter constituent in grapefruit. Results of research in previous years have shown that the bitterness in grapefruit juice can be decreased by using enzymes, such as naringinase which hydrolyzes naringin into relatively non-bitter compounds. The purpose of this paper is to report results of an investigation to determine the effect of the concentration of naringinase and the holding temperature and time on the rate of debittering of 55° Brix frozen concentrated grapefruit juice. Such information is necessary to determine the commercial practicability of enzymatic debittering of grapefruit concentrate.

REVIEW OF LITERATURE

Considerable information is available on naringin, the principal bitter substance in grapefruit. Its intense bitterness, which is said to exceed that of quinine, is detectable in water containing as little as one part in 50,000. Results of an investigation by Kesterson and Hendrickson (11) included data on the distribution of naringin in the various component parts of different varieties of Florida grapefruit. They also listed references concerning the properties and occurrence of naringin as previously reported by other investigators. Horowitz (8) recently summarized the results of various studies to determine the chemical structure of naringin.

Ting (15) found that commercial pectic enzyme preparations, such as Pectinol 10-M and Pectinol 100-D obtained from Rohm and Haas Company, contained enzymes that hydrolyzed naringin to glucose, rhamnose and naringenin. He used Pectinol as a means of debittering grapefruit juice by the enzymatic hydrolysis of naringin in the juice. He also suggested the use of the Davis test (4) for flavanones, both before and after enzymatic hydrolysis, as a means for determining the amount of naringin in grapefruit juices. However, the degree of bitterness did not always correspond to the naringin content of the juice as indicated by this procedure. The effect of temperature, pH, enzyme concentration, concentration of substrate, citrate ions, glucose, rhamnose, and naringenin on the rate of enzymatic hydrolysis of naringin was also reported by Ting (16). The rate of hydrolysis was greatest at 60°C. but the activity was definitely inhibited at 70°C. Complete inactivation was obtained in 15 seconds at 90°C. Optimum pH range was 3.5 to 4.5. Citrate ion concentration had no effect on the enzyme activity, but glucose and naringenin decreased the rate of hydrolysis.

Thomas, Smythe and Labbee (14) prepared from microorganism cultures a partially purified enzyme, Naringinase C. Pectic enzymes were destroyed by treatment of the crude culture extracts with urea. Naringinase C was found to rapidly hydrolyze naringin in vitro in the pH range 3.5 to 5.0 and at temperatures from 20° to 50°C.; it also debittered natural grapefruit juices. It was concluded that naringinase C contained at least two glycosidases. One, a rhamnosidase, hydrolyzes the bond between rhamnose and glucose in naringin and yields prunin and rhamnose. The other, a beta-glucosidase, hydrolyzes the bond between the glucose and the aglycone, naringenin, in prunin and thereby yields naringenin and glucose. Evidence was presented that debittering of

Florida Agricultural Experiment Stations Journal Series No. 1974.

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

grapefruit juice is achieved by the conversion of naringin to rhamnose and prunin which are relatively non-bitter and further hydrolysis of prunin to naringenin is not necessary. The Davis test, generally used to measure the rate of hydrolysis of naringin, is not suitable for the measurement of debittering since prunin gives the same reaction with the Davis reagents as naringin (9). In the presence of the glucose in grapefruit juice the enzymatic hydrolysis of prunin to naringenin is comparatively slow. The use of emulsin to hydrolyze prunin to show the extent of reduction of naringin by naringinase when the Davis test was used was also described. This hydrolysis of prunin by the use of emulsin was also reported by Nystrom *et al.* (13). Previously, Hall (7) reporting the isolation of an enzyme from celery seeds, which hydrolyzed naringin to naringenin, stated that emulsin had no effect on the hydrolysis of naringin.

Griffiths and Lime (6) reported on debittering of grapefruit products with Naringinase C and discussed the conditions affecting the enzymatic debittering of grapefruit pulp and juice. They also found that neither the Davis test (4) nor the modified test proposed by Ting (15) correlated with actual bitterness and, therefore, used organoleptic procedures.

Optimum conditions for enzymatic hydrolysis of naringin in grapefruit pulp and juice to less bitter substances, prunin and naringenin, were found to be 50°C., enzyme concentrations of 0.01-0.05% and incubation periods of 1 to 4 hours. Enzyme action at 4°C. for 44 hours hydrolyzed naringin to less bitter prunin, without a corresponding decrease in the Davis test value. Use of 0.025% naringinase at 50°C. for 1½ hours reduced the bitterness of colored grapefruit pulp and made it possible to use this debittered pulp for color fortification of poorly colored, late season grapefruit juice. They considered it significant that they had not found either prunin or naringenin in bland juice from tree-ripened fruit.

Experimental Procedure

Conditions for debittering. — Concentrated grapefruit juice (55° Brix) was debittered under the following conditions. Temperatures of −8°, 60°, 80°, and 122°F. were used with Naringinase D-100 concentrations varying from .0025% to .080% by weight based on 10.5° Brix juice. Length of storage or sampling time varied since they depended upon the temperature and enzyme concentration used.

The concentrated grapefruit juice used in the series of experiments conducted at −8°, 60°, 80°, and 122°F. was prepared from seedy grapefruit picked in mid-December and processed in the Florida Citrus Experiment Station pilot plant. The juice was concentrated to 55° Brix and stored at −8°F. in polyethylene bags in metal containers holding approximately 4 gallons. As the bulk concentrate was needed, it was thawed rapidly, mixed thoroughly and divided into four equal portions. Each batch of concentrate was warmed to the temperature at which it was to be held before naringinase was added. Three different amounts of naringinase were added to three batches of concentrate while the fourth batch was used as a control. After addtion of the enzyme, each portion of concentrate was thoroughly mixed, placed in 6 oz. cans and vacuum sealed. The products were held at the temperatures selected and at intervals samples were removed and placed in boiling water for 30 minutes to inactivate the naringinase. Then the concentrates were cooled and reconstituted to 10.5° Brix.

Experiments with reconstituted juice at 40° and 80° F. were performed using grapefruit concentrate obtained from Eloise Groves Association.[2]

Determination of extent of debittering.—The Davis test, using alkaline diethylene glycol, was used for determining total flavanones in the reconstituted grapefruit juices used as controls. The Ting modification, using Pectinol to hydrolyze naringin to naringenin, was used to determine to what extent maximum debittering was possible. The Davis test was also made on the debittered juices after hydrolysis with emulsin to remove the prunin formed during the debittering process with naringinase. Prunin, as previously mentioned, is relatively non-bitter but gives a color reaction similar to naringin and thereby causes the Davis test to indicate less debittering than that actually accomplished by the naringinase. With the prunin removed by emulsin, the Davis test then reveals the amount of naringin still remaining. Therefore, the Davis test data, obtained on the emulsin hydrolyzed debittered juices, were the only results which indicated chemically the extent of debittering brought about by the naringinase. The procedure employed was to reconstitute the concentrate to 10.5° Brix ± .2°, after the naringinase had been inactivated by

[2]Name of company was recently changed to Cypress Gardens Citrus Products, Inc.

heating the can of concentrate in boiling water. A 25 ml. aliquot of juice was adjusted to pH 5.0 and transferred to a large test tube containing 0.1 gram of emulsin. The tube was vigorously shaken and placed in a 122°F. water bath. The tube was shaken again after about 1½ hr. and finally after 3 hr. the juice was cooled and filtered. The Davis test was made on this filtered juice.

Results and Discussion

In the early part of this investigation naringinase was added to reconstituted juice and held at both 40° and 80°F. Although there was a definite decrease in bitter taste, there was little indication of naringin reduction by the Davis test. The employment of the emulsin modification provided data which manifested the naringin reduction, as shown in Table 1. The naringin content of the reconstituted juice treated with 0.018% Naringinase D-100 and held at 80°F. decreased from 0.034% to 0.015% in 10 hr. At 40°F. it required 24 hr. to change the naringin content to 0.019%. Maximum reduction to 0.010% was obtained in 15 days, as determined by the Ting modification whereby reconstituted juice was treated with 0.75% Pectinol for 5 hr. at 50°C.

The effect of enzyme concentration, storage temperature and time on the removal of naringin, with a corresponding decrease in bitterness, from concentrated grapefruit juice is graphically shown in Figure 1. It is evident from this data that the naringin and, therefore, the bitterness decreased as the naringinase concentration and the storage temperature and time were increased. This is also evident from the data presented in Table 2. The reduction of naringin in 55° Brix grapefruit concentrate, containing 0.02% naringinase, and stored for 24 hr. at 60°F. and 80°F. was 21.4% and 55.2%, respectively. When this same concentrate was held for 24 hr. at 80°F. after increasing the enzyme concentration to 0.08%, the reduction of naringin was increased to 85.2% The maximum rate of reduction of naringin occurred at the optimum temperature for naringinase activity, which is approximately 122°F. (6, 16).

Naringinase activity was found to be much less in grapefruit concentrate than in reconstituted juice (Tables 1 and 2). Reconstituted grapefruit juice held at 40°F. had a 63% reduction (Table 1) in naringin after 24 hr. However, only 21.4% reduction occurred in a concentrate, debittered for 24 hr. with approximately the same amount of enzyme, but held at the higher temperature of 60°F. This inhibitory effect of concentrate on the enzyme activity was also indicated since the rate of naringin reduction at 80°F. in reconstituted grapefruit juice was virtually the same as that in the concentrate held at 122°F. containing the same amount of naringinase as the juice (Table 2). Since the concentration of glucose in grapefruit concentrate is much greater than that in reconstituted juice, this could be a major factor causing the decrease in the naringinase activity in the concentrate. Ting (16) reported that increased concentrations

Table 1. Effect of temperature and time of storage and enzyme concentration on the rate of enzymatic debittering of grapefruit juice or concentrate

Concentrated grapefruit juices						Reconstituted grapefruit juices					
Storage		Enzyme	Naringin content			Storage		Enzyme	Naringin content		
Temp. °F.	Time hr.	Conc.* %	Initial %	Final %	Reduction %	Temp. °F.	Time hr.	Conc.* %	Initial %	Final %	Reduction %
60	24	.020	.038	.032	21	40	24	.018	.034	.019	63
"	"	.040	.036	.026	38						
80	24	.020	.039	.023	55	80	10	.018	.034	.015	79
"	"	.040	.039	.017	76						
"	"	.080	.037	.014	85						
80	32	.020	.039	.021	62						
"	"	.040	.039	.016	79						
"	"	.080	.037	.013	89						
122	3 1/2	.020	.039	.023	55						
"	7	"	.039	.017	76						

* Concentration.

Table 2. Rate of enzymatic debittering of grapefruit concentrate at 122° F. and that of grapefruit juice at 80° F., using approximately the same enzyme concentration

Concentrated grapefruit juice Storage temp. = 122° F. Enzyme conc. = 0.020%				Reconstituted grapefruit juice Storage temp. = 80° F. Enzyme conc. = 0.018%			
Storage	Naringin content			Storage	Naringin content		
Time hr.	Initial %	Final %	Reduction %	Time hr.	Initial %	Final %	Reduction %
1	.039	.033	21	1	- -	- -	- -
2	"	.028	38	2	- -	- -	- -
3	"	.025	48	3	- -	- -	- -
4	"	.023	59	4	- -	- -	- -
5	"	.020	66	5	- -	- -	- -
6	"	.018	72	6	- -	- -	- -
7	"	.017	76	7	- -	- -	- -
8	- -	- -	-	8	.034	.016	75
12	- -	- -	-	10	.034	.015	79

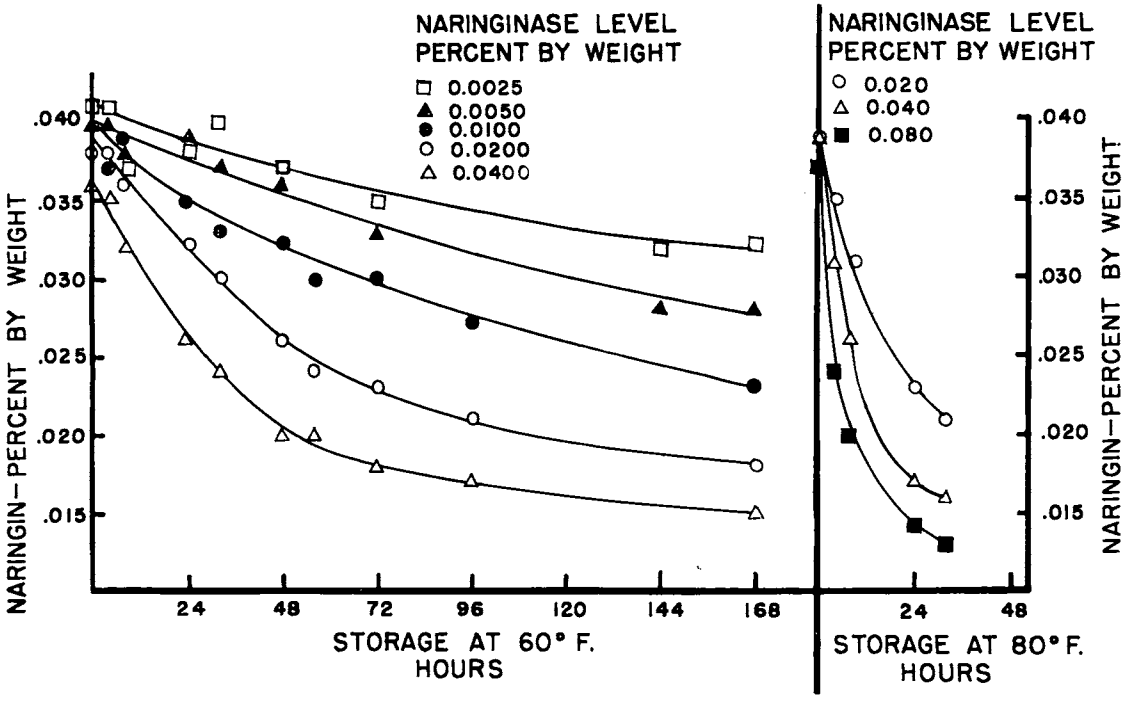

Figure 1.—Naringin content in 10.5° Brix reconstituted grapefruit juices after enzymatic debittering of 55° Brix grapefruit concentrate during storage at 60° or 80° F., using different concentrations of naringinase.

of glucose inhibited the activity of this enzyme.

The activity of naringinase in frozen concentrated grapefruit juice, stored at −8°F., was studied for one year. Concentrates with naringinase levels of .01, .02, and .04% by weight, on a 10.5° Brix juice basis, as well as a control, were canned and frozen. The concentrates were sampled at monthly intervals and Davis tests were run on the reconstituted juices, initially and after treatment with both Pectinol or emulsin. There was no indication of any naringinase activity in the concentrates throughout the twelve month period.

Organoleptic tests, using the triangular method (10, 12), were conducted to determine differences in bitterness of the reconstituted grapefruit juices. Results of these tests indicated satisfactory debittering when high concentrations of naringinase were used. However, when low enzyme concentrations were used, results were inconclusive. Therefore, additional flavor studies would be advisable and especially some to determine the degree of bitterness in grapefruit juice which would be satisfactory to most consumers.

Summary

Concentrated grapefruit juice (55° Brix) or reconstituted juice (10.5° Brix) was treated with Naringinase D-100 at levels ranging from .0025 to .08%. After being stored at temperatures of −8°, 40°, 60°, 80° and 122°F., the Davis test for flavanone glycosides was used as a chemical method for determining the reduction of the principal bitter substance, naringin, in these products and, therefore, also as an indication of the extent of enzymatic debittering. However, it was necessary at first treat the reconstituted juice with emulsin to remove the interfering and relatively non-bitter substance, prunin. The modification of Ting, whereby the Davis test was made on juice after treatment with Pectinol at 50°C. for 5 hr., was helpful as an indication of the maximum naringin reduction possible. For most juices tested this was to a level between 0.008 to 0.012% naringin, with the mode at 0.010%.

Naringin in reconstituted juice was reduced by naringinase much more rapidly than that in concentrated juice even at lower temperatures. When the enzyme concentration, the storage temperature, or the duration was increased, the rate of reduction of naringin, and consequently that of debittering, also increased.

The greatest reduction of 88.9% of naringin in grapefruit concentrate was obtained with 0.08% naringinase when the concentrate was held for 32 hr. at 80°F. Reconstituted grapefruit juice containing 0.018% naringinase and stored at 40°F. was almost completely debittered in 15 days. At −8°F. there was no apparent naringinase activity in 55° Brix grapefruit concentrate over a one year period.

Acknowledgments

The authors thank the Cypress Gardens Citrus Products, Inc., for supplying some of the concentrated grapefruit juice used in this investigation; also, Rohm and Haas Company for the Naringinase D-100 and Pectinol produced and supplied by them.

Literature Cited

1. Bell, Hugh P. 1955. Preferences for canned grapefruit juices. Proc. Florida State Hort. Soc. 68: 151-155.
2. Birdsall, Lamont. 1955. Consumer preferences in citrus juices. Proc. Florida State Hort. Soc. 68: 133-136.
3. Booz, Allen and Hamilton, Inc. 1964. Report to Florida Citrus Commission, Lakeland, Fla.
4. Davis, W. B. 1947. Determination of flavanones in citrus fruits. Anal. Chem. 19: 476-478.
5. Florida Dept. of Agriculture, Div. of Fruit and Vegetable Inspection, Winter Haven, Fla. Annual Report for 1961-62. Season.
6. Griffiths, Francis P. and Bruce J. Lime. 1959. Debittering of grapefruit products with naringinase. Food Technol. 13: 430-433.
7. Hall, H. D. 1938. A new enzyme. Chem. and Indus. 57: 473.
8. Horowitz, Robert M. 1961. Chap. 11 in "The Orange," Ed. by Walter B. Sinclair, pp. 343-345, The Regents of Univ. of California, Berkeley, Calif.
9. Horowitz, R. W. and B. Gentili. 1959. Use of the Davis method to estimate flavanones. Food Research 24: 757-759.
10. Institute of Food Technologist, Committee on Sensory Evaluation. 1964. Sensory testing guide for panel evaluation of foods and beverages. Food Technol. 18: 1135-1141.
11. Kesterson, J. W. and R. Hendrickson. 1957. Naringin, a bitter principle of grapefruit. Florida Agr. Expt. Sta. Tech. Bul. 511A.
12. Kramer, Amihud and Bernard A. Twigg. 1962. Fundamentals of quality control for the food industry. The Avi Publishing Company, Inc., Westport, Connecticut.
13. Nystrom, Charles W., Byron L. Williams and Simon H. Wender. 1954. Enzymatic hydrolysis studies on certain flavonoid gylcosides. J. Am. Chem. Soc. 76: 1950-1951.
14. Thomas, D. W., C. V. Smythe and M. D. Labbee 1958. Enzymatic hydrolysis of naringin, the bitter principle of grapefruit. Food Research 23: 591-598.
15. Ting, S. V. 1955. Application of enzymic hydrolysis in the analysis of naringin in grapefruit products. Florida Citrus Station Mimeo Report 56-9.
16. Ting, S. V. 1958. Enzymic hydrolysis of naringin in grapefruit. J. Agr. Food Chem. 6: 546-549.