Proc. Fla. State Hort. Soc. 90:180-182. 1977.

TREATMENT REQUIREMENTS FOR DEBITTERING AND FORTIFYING GRAPEFRUIT AND STABLE STORAGE OF THE PRODUCT

BONGWOO ROE AND JOSEPH H. BRUEMMER
U.S. Citrus and Subtropical Products Laboratory,[1]
P.O. Box 1909, Winter Haven, Florida 33880

Additional index words. Citrus paradisi Macf., *naringin, naringinase limonin, prunin.*

Abstract. **The effects of fruit maturity and formulations with nutrients and other additives on debittering activity of naringinase in grapefruit were determined. The amount of naringinase required to debitter the albedo was 10 times as great for grapefruit harvested in fall as for grapefruit harvested in March. Glucose and fructose completely inhibited naringinase activity and sucrose and the artificial sweeteners saccharin and neohesperidin dihydrochalcone had slight effects. A vitamin-mineral mix which furnished (per fruit) 100% US RDA, ferrous sulfate at 50 mg/l and gelatin had little or no effect on activity. Grapefruit that were peeled and vacuum-infused with 35 U naringinase/liter of strawberry-flavored sucrose-gelatin solution containing 0.1% sodium benzoate and nutrients, resisted microbial contamination and retained their flavor and texture after 6 weeks of storage at 4°C. The slight initial bitterness disappeared during storage. The naringinase infusion soln was used repeatedly without loss of effectiveness when fresh enzyme soln was added to maintain constant volume.**

We reported debittering albedo of peeled grapefruit by vacuum infusing naringinase in a nutrient solution as a promising method of increasing the nutritional value of the fruit (6). We used grapefruit that were harvested in April, when the naringin content of the albedo was less than 1.5%. The levels of naringin (5), limonin (7) and citric acid (1) are higher in fruit harvested in October and November than in fruit harvested in the spring and may require higher concn of enzymes, longer treatment times, and use of sweeteners to suppress the bitterness of limonin and naringin (3) and modify the sourness of high acidity.

This report discribes treatment requirements for debittering and fortifying grapefruit and stable storage of the product.

Materials and Methods

Materials. Naringinase was obtained from Sigma Chemical Company, St. Louis, Missouri. Saccharin and neohesperidin dihydrochalcone were gifts of Dr. Paul J. Fellers, Florida Department of Citrus, Lake Alfred, Florida. The vitamin-mineral mix was supplied by Hoffman-LaRoche Company, Nutley, New Jersey. Natural and artificial strawberry flavors were supplied by Orange Products, Inc., Winter Haven, Florida. White 'Marsh' grapefruit harvested throughout the growing season were obtained from local packing plants. Strawberry-flavored Jello was purchased from a local food market. All other chemicals and materials were obtained from Fisher Scientific Company, Atlanta, Georgia.

Debittering procedure. The flavedo was removed from grapefruit with a mechanical peeler, and the fruit was vacuum-infused with naringinase in various solutions at 50°C and pH 4.5 by submerging the fruit in the soln as described (6). The fruit were then placed in clear plastic food bags and incubated at 50°C for 1, 2 or 4 hr.

We used biochemical procedures and taste tests to determine the naringinase concn and reaction times that were needed to reduce bitterness of grapefruit albedo to an acceptable level as affected by maturity of the fruit, by presence of sweeteners, flavor enhancers and nutrients, and by storage of the product.

Storage tests. We examined stability of naringinase-treated, fortified grapefruit during storage at 4°C in fruit harvested in March. Fruit were randomly assigned to 1 of 3 groups. Fruit in groups 1 and 2 were peeled and treated with naringinase for 2 and 4 hr, respectively, and stored at 4°C until scored for bitterness, flavor and texture. Fruit in group 3 were stored intact at 10°C until 1 day before the stored fruit were analyzed, when 1 fruit was peeled and treated with naringinase for 4 hr and stored at 4°C. On the day of the test, 1 fruit from each group was scored for preference by the panel of 18 tasters. Each liter of infusion solution for the storage test contained 35 U naringinase, 40 g gelatin, 150 g sucrose, 1 g sodium benzoate, 7 g vitamin-trace mineral mix (to furnish 100% US RDA of these constitutents per fruit) and strawberry extract. The soln was adjusted to pH 4.5 with 10% citric acid.

Biochemical procedure. We used the Davis Method (2) to estimate the naringin content of albedo after treating the albedo, as described previously (6).

The change in naringin concn was used to estimate naringinase activity. We added 8.8 ml of 0.05 M citrate buffer (pH 4.5) containing the sweeteners or nutrients to be tested, 1 ml 0.1% naringin, and 0.2 ml 5% naringinase to a test tube in a water bath at 30°C. After 10 min incubation, 1 ml of the reaction mixture was rapidly transferred to 5 ml of 0.1 N NaOH in 90% ethylene glycol, and the naringin content was determined by the Davis method (2). One unit of naringinase is defined as the amount of enzyme that hydrolyzes 1 μmole of naringin per min at 30°C.

Taste tests. Panels of 6, 8, and 10 tasters were selected from the Citrus and Subtropical Products Laboratory staff of 26 on the basis of sensitivity to bitterness. Samples were thin slices (2 cm^2) of grapefruit albedo and juice section. Each panelist was asked to rank samples for bitterness using 1 for least and 3 or 4 most bitter, depending on the number of samples. The sums of the rankings were analyzed for significance by the method of Kahan *et al.* (4).

The hedonic preference test was used to evaluate infused grapefruit albedo for flavor, bitterness and texture on a scale of 1 to 9 (1 = like extremely, 9 = dislike extremely). Panelists were selected from members of the Citrus and Subtropical Products Laboratory staff on the basis of analysis of variance of their scores in preliminary tests of the product. Three samples of grapefruit slices (2 cm^2 x 0.4 cm thick) containing both albedo and juice sections, were evaluated at each morning and afternoon session. Results were examined for significance by analysis of variance of test scores.

[1]One of the laboratories of the Southern Region, U. S. Department of Agriculture, Agricultural Research Service.
Mention of a brand name or trademark is for identification only and does not constitute endorsement by the U. S. Department of Agriculture over others which may also be suitable.

Results and Discussion

Debittering of early season grapefruit. Enzyme treatment decreased the naringin content of albedo of grapefruit harvested in September from 2.2 to 0.5% (Table 1). The product was liked slightly (score 3.8) by the taste panel. Adding 7.5 and 15% sucrose to the Jello infusion medium did not improve acceptability. Similar observations were made with fruit harvested in October. Jello contained about 25% soluble solids, mostly sugar. Adding more sugar apparently did not improve the product. Early season fruit contain substances that are not masked by sweeteners. The naringin contents of October and November fruit treated with enzyme in Jello were similar, but only the November fruit was liked moderately by the panel (score of 3.4).

Table 1. Effectiveness of treatment of early season grapefruit vacuum infused with 350 U naringininase/liter Jello solution with added sucrose and incubated for 4 hr at 50°C.

Date of harvested	Sucrose infused (%)	Naringin in albedo (%)	Mean hedonic score[z]
Untreated	—	2.2	—
Sept 22, 76	0	0.5	3.8
	7.5	0.5	4.6
	15.0	0.6	5.3
Oct 6, 76	0	0.5	4.2
	7.5	0.5	5.3
	15.0	0.5	5.7
Oct 21, 76	0	0.5	5.0
Nov 4, 76	0	0.5	3.4

[z]Twelve panelists scored samples on a scale of 1-9 (1 = like extremely, 9 = dislike extremely).

Debittering of midseason grapefruit. Ranking samples for bitterness, a six-member panel differentiated grapefruit infused with 18 U naringinase/l citrate buffer from those with 35 and 70 U/l (Table 2, Experiment 1A). Fruit harvested in February were not debittered satisfactorily in 4 hr with 18 U/l. The taste panel selected the 4 hr treatment with 35 U/l naringinase to be significantly superior to 1- and 2-hr treatments for reducing bitterness (Experiment 1B), but could detect no difference between 1-, 2-, and 4-hr treatments with 70 U/l naringinase (Experiment 1C). These data indicate that midseason grapefruit which had 1.7% naringin in the albedo of untreated fruit, require only about 10% as much naringinase as early season fruit, which had 2.2% naringin.

Debittering with flavor enhancement. Naringinase requirements were increased when the enzyme infusion soln contained flavored sweetener. Panelists distinguished 4-hr from 1- and 2-hr treatments at both 70 and 140 U naringinase/l of strawberry Jello (Table 2, Experiments 2B and 2C). Fruit harvested during the same month required 35 U naringinase/l of citrate buffer for the panelists to select the 4-hr treatment as superior to the 1- and 2-hr treatments (Table 2, Expt. 1B). The concn requirement for 1-hr treatment with flavor infusion appeared to be between 140 and 280 U naringinase/l of Jello (Table 2, Expt 2C and 2D). The panelists could not distinguish between 1-, 2- or 4-hr treatments with 280 U naringinase/l (Experiment 2D).

Effect of sweetener and nutrient on naringinase activity. Of the sweeteners tested, only 5% glucose and 10% fructose significantly inhibited naringinase activity (Table 3). Glucose inhibition of naringinase was shown by Thomas *et al.* (8) to occur at the second step of the hydrolysis of naringin to prunin to naringinin. Prunin accumulates in the presence of glucose and responds as naringin in the assay. Sucrose inhibited the initial rate of the naringinase reaction, but had no effect on naringin reduction in treated grapefruit (Table 1). The nutrients had only slight effects on the activity of the enzyme.

Table 2. Effectiveness of treatment of midseason (February) grapefruit vacuum-infused with various levels of naringinase in 0.005 M citrate buffer or strawberry Jello at pH 4.5 and incubated for 1, 2 or 4 hr.

Experiment	Naringinase (U/l)	Time (hr)	Sum of bitterness rankings
Citrate buffer			
1A	18	4	*17*
	35	4	10
	70	4	9
1B	35	1	*17*
	35	2	13
	35	4	*6*
1C	70	1	11
	70	2	10
	70	4	15
Strawberry Jello			
2A	35	1	13
	35	2	11
	35	4	12
2B	70	1	16
	70	2	14
	70	4	*6*
2C	140	1	16
	140	2	13
	140	4	*7*
2D	280	1	13
	280	2	14
	280	4	9

[z]Six panelists ranked 3 samples (thin slices of albedo and juice section) per experimental group using 1 for least and 3 for most bitter. Italicized sums of rankings are significantly different ($p<0.05$) from the others in their groups.

Table 3. Effect of various sweeteners and nutrients on activity of naringinase.[z]

Additive	Conc. (%)	Naringin remaining (%)
None		45
Glucose	5	97
Sucrose	5	63
Sucrose	15	75
Saccharin[y]	0.003	48
Neohesperidin dihydrochalcone	0.007	58
Fructose	10	98
Vit.-min. mix[x]	0.7	49
Fe SO_4	0.005	50
Fe SO_4[w]	0.025	57
Gelatin[v]	4	47

[z]Determined by assay of naringin remaining after reaction mixture was incubated for 10 min.
[y]Concn equivalent in sweetness to 10% sucrose.
[x]Provides 100% US RDA (adult men) per grapefruit of all vitamins and trace minerals.
[w]Provides 50% US RDA (adult men) per grapefruit.
[v]Four percent of amount in infusion soln.

Debittering as modified by sweetener. Sucrose added to the infusion medium modified the bitterness ranking of naringinase-treated grapefruit (Table 4). The taste panel ranked albedo of midseason grapefruit incubated with naringinase for 2 hr inferior to albedo of similarly treated fruit containing 15% sucrose. They also found that sucrose improved the taste of samples treated for 4 hr although the naringin content was the same with and without sweetener. Guadagni *et al.* (3) demonstrated that sweeten-

ers increased the threshold value for naringin and samples containing sucrose were less bitter than samples without sucrose.

Table 4. Effectiveness of treatment of midseason (March) grapefruit vacuum-infused with 35 U naringinase per liter of citrate buffer with added sucrose and incubated for 2 or 4 hr.

Sucrose (%)	Reaction time (hr)	Naringin in Albedo (%)	Sum of bitterness rankings
0	2	1.05	*30*
0	4	0.70	17
15	2	0.95	22
15	4	0.68	*11*

[z]Eight panelists ranked 4 samples using 1 for least and 4 for most bitter. Italicized sums of rankings are significantly different ($p<0.05$) from the others.

Reuse of naringinase infusion solution. The enzyme solution can be used repeatedly if the infusion soln is maintained at a constant volume by adding fresh enzyme soln after each infusion. The naringin content of the fifth grapefruit infused was reduced to the same level as the first grapefruit infused with naringinase (data not shown). A taste panel of 10 members ranked the samples similar in acceptability.

Storage of treated grapefruit. The debittering of naringinase-treated grapefruit continued during storage at 4°C. A panel of 18 tasters scored grapefruit stored for 33, 39 and 46 days significantly less bitter than freshly treated grapefruit (Table 5). Flavor and texture of treated grapefruit were not affected by storage at 4°C. Fruit treated for 4 hr scored slightly lower than fruit treated for 2 hr and nonstored fruit for flavor and texture. Although bitterness is an important part of grapefruit flavor, differences in bitterness did not significantly affect the total flavor scores of the samples. Average flavor scores ranged between 5 (neither liked nor disliked) and 6 (disliked slightly). Stored fruit showed no appearance of surface mold.

Conclusion

Grapefruit albedo that was treated with naringinase, sweetened, and flavored with strawberry essence was stored for 6 wk without significantly losing acceptable flavor and texture. The small amount of enzyme used in the treatment (35 U [or 0.5 g] naringinase/liter of infusion solution) its stability, which enables reuse of the infusion solution and the modification of bitterness of midseason grapefruit by sucrose are desirable features of the process. However, acceptability of product flavor was marginal (neither liked or disliked). The new challenge in research and development of this product is to find a flavor that would increase the acceptability of grapefruit albedo as a food.

Table 5. Bitterness, flavor, and texture of midseason (March) grapefruit vacuum infused with naringinase solution and stored at 4°C.

Days in storage	Average preference scores of stored fruit									
	7	11	13	18	21	25	28	33	39	46
Bitterness										
Group 1	5.2	5.4	5.8	5.9	4.8	5.1	5.0	4.3	4.5	4.0
Group 2	5.0	5.4	5.4	5.5	5.5	5.1	5.7	4.3	4.6	4.3
Group 3	5.3	6.3	6.3	6.3	6.7	6.5	6.9	*6.4*	*7.1*	*7.0*
Flavor										
Group 1	4.5	5.1	4.9	5.7	4.6	5.4	5.3	5.2	4.9	4.7
Group 2	5.0	4.8	5.1	5.2	5.5	5.4	5.4	5.1	5.2	5.3
Group 3	4.9	5.3	5.6	5.7	5.6	5.9	6.0	5.7	6.1	6.0
Texture										
Group 1	3.9	4.7	4.8	4.8	4.6	5.4	5.0	5.2	4.4	4.3
Group 2	4.3	4.7	4.9	4.6	5.2	4.5	4.8	4.4	4.6	4.5
Group 3	4.0	4.9	5.6	5.4	5.4	5.2	4.7	5.2	5.7	5.5

Fruit in groups 1 and 2 were peeled and treated with naringinase for 2 and 4 hr and stored at 4°C. Fruit in group 3 were stored intact at 10°C until 1 day before the stored fruit were analyzed, when 1 fruit was peeled and treated with naringinase for 4 hr and stored at 4°C. On the day of the test, 1 fruit from each group was scored for preference by the panel of 18 tasters. Italicized average scores are significantly different ($p<0.01$) from other groups in test.

Literature Cited

1. Collinson, S. E. 1913. Sugar and acid in oranges and grapefruit. *Fla. Agric. Expt. Sta. Bull.* 115:1-23.
2. Davis, W. B. 1947. Determination of flavanones in citrus fruits. *Anal. Chem.* 19:4760478.
3. Guadagni, D. G., V. P. Maier, and J. H. Turnbaugh. 1974. Some factors affecting sensory thresholds and relative bitterness of limonin and naringin. *J. Sci. Fd. Agric.* 25:1199-1205.
4. Kahan, G., D. Cooper, A. Paparasilion and A. Karmer. 1973. Expanded tables for determining significance of differences for ranked data. *Food Technol.* 27:64-69.
5. Maner, R. H., E. M. Burdick and C. W. Waibel. 1950. Distribution of naringin in Texas grapefruit. *Proc. Rio Grande Valley Hort. Inst.* (4th Annual) 1950:147-51.
6. Roe, B. and J. H. Bruemmer. 1976. New grapefruit product: debitterizing albedo in situ. *Proc. Fla. State Hort. Soc.* 89:191-194.
7. Scott, C. W. 1970. Limonin in Florida citrus fruit. *Proc. Fla. State Hort. Soc.* 83:270-277.
8. Thomas, D. W., C. V. Smythe and M. D. Laffec. 1958. Enzymatic hydrolysis of naringin, the bitter principle of grapefruit. *Food Res.* 23:591-598.