of California Division of Agricultural Science: Berkley, CA, 1961.
Coggins, C. W.; Hall, A. E. Effect of light, temperature, and 2′,4′-dichloro-1-cyanoethanesulfonanilide on regreening of regreened Valencia oranges. *J. Am. Soc. Hortic. Sci.* **1975**, *100*, 484–487.
Comar, C. L.; Zscheile, F. P. Analysis of plant extracts for chlorophylls a and b by a photoelectric spectrophotometric method. *Plant Physiol.* **1942**, *17*, 198–209.
Davies, B. H. Analysis of carotenoid pigments. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, New York, 1965.
Hsu, W.-J.; Yokoyama, H.; Coggins, C. W. Carotenoid biosynthesis in *Blakeslea trispora*. *Phytochemistry* **1972**, *11*, 2985–2990.
Hsu, W.-J.; Poling, S. M.; Yokoyama, H. Effect of amines on the carotenogenesis in *Blakeslea trispora*. *Phytochemistry* **1974**, *13*, 415–419.
Hsu, W.-J.; Poling, S. M.; DeBenedict, C.; Rudash, C.; Yokoyama, H. Chemical inducers of carotenogenesis. *J. Agric. Food Chem.* **1975**, *23*, 831–834.
Huff, A. Nutritional control of regreening and degreening in citrus peel segments. *Plant Physiol.* **1983**, *73*, 243–249.
Huff, A. Sugar regulation of plastid interconversions in epicarp of citrus. *Plant Physiol.* **1984**, *76*, 307–316.
Poling, S. M.; Hsu, W.-J.; Yokoyama, H. *Structure*-activity relationships of chemical inducers of carotenoid biosynthesis. *Phytochemistry* **1975**, *11*, 1933–1938.
Poling, S. M.; Hsu, W.-J.; Yokoyama, H. Synthetic regulators of carotenoid biosynthesis in *Citrus paradisi*. *Phytochemistry* **1976**, *15*, 1685–1687.
Schuetz, R. D.; Baldwin, R. A. The synthesis and properties of some substituted phenyl-w-(N,N-dialkylamine)-alkyl sulfides. *J. Am. Chem. Soc.* **1958**, *80*, 162–164.
Simpson, D. J.; Chichester, C. O.; Lee, T. H. Chemical regulation of plastid development. I. Inhibition of chlorophyll biosynthesis in detached pumpkin cotyledons by CPTA. A pigment and ultrastructural study. *Aust. Plant Physiol.* **1974**, *1*, 119–133.
Thomson, W. W.; Lewis, L. N.; Coggins, C. W. The reversion of chromoplasts to chloroplasts in Valencia Orange. *Cytologia* **1967**, *32*, 117–124.

Received for review September 14, 1987. Revised manuscript received May 9, 1988. Accepted July 18, 1988. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Reduction of Bitter Components in Grapefruit and Navel Orange Juices with β-Cyclodextrin Polymers or XAD Resins in a Fluidized Bed Process

Charles W. Wilson, III, Charles J. Wagner, Jr., and Philip E. Shaw*

β-Cyclodextrin polymer and XAD-4 and XAD-16 resins were used in a pilot-scale fluidized bed process to reduce bitterness from naringin and limonin in grapefruit juice and limonin in California navel orange juice. For β-cyclodextrin polymers cross-linked with epichlorohydrin, naringin reduction in grapefruit juice ranged from 18 to 61% and limonin reduction ranged from 28 to 67%. Limonin reduction in navel orange juice ranged from 29 to 55%. The polymer was regenerated 30 times with 2% sodium hydroxide at ambient temperature without loss in debittering capacity. Bitterness reduction in juices processed at 5 °C was similar to that found at ambient temperature. Bitterness reduction in grapefruit juice from XAD-16 resin was 55–58% for naringin and 90–97% for limonin; with navel orange juice limonin was reduced 93%. For grapefruit juice debittered with XAD-4 naringin reduction was 32–38%, and for both grapefruit and navel orange juice limonin reduction was 58%. The ability of the XAD resins to withstand repeated regeneration with 2% sodium hydroxide was not determined.

Excessive bitterness in some processed citrus juices adversely affects the flavor and marketability of products made from these juices. The bitter juices are often stored for later blending with less bitter juices. Thus, a larger portion of processed juice products is affected by these bitter juices (Shaw and Buslig, 1986). Bitterness in processed citrus juices occurs mainly in early-season grapefruit juice from Florida (processed from fruit harvested between Aug 1 and Dec 1) and in processed California navel orange juice (Wagner et al., 1988). Reducing bitterness in juices from early-season grapefruit and processed navel oranges could provide the consumer with a more acceptable juice product and the producer with a better quality product for blending.

Bitterness has been related to naringin and limonin in grapefruit juice and to limonin in processed navel orange juice. Adsorptive removal of bitter principles and titratable acid from citrus juices has been reviewed by Johnson and Chandler (1988), and some of the methods reported to remove these bitter components from citrus juices include treatment of the juice with enzymes (Kefford and Chandler, 1970), with immobilized bacteria (Hasegawa et al., 1985), and with insoluble polymers (Johnson and Chandler, 1982, 1985; Puri, 1984; Shaw and Buslig, 1986; Shaw et al., 1984; Shaw and Wilson, 1983, 1985). Recent work reported preliminary pilot scale debittering studies with grapefruit juices using insoluble β-cyclodextrin polymers in a fluidized bed process (Wagner et al., 1988).

In the current study, procedures for commercial applicability to debitter grapefruit and navel orange juices with β-cyclodextrin polymers in a pilot-scale fluidized bed column were thoroughly studied. In addition, a comparison was made between cyclodextrin polymers and neutral XAD resins used under similar fluidized bed conditions.

EXPERIMENTAL SECTION

Juices were prepared by reconstituting commercial concentrated grapefruit juice from Florida and navel orange juice from California to 10.5–11.0° Brix, except for one experimental single-strength grapefruit juice (juice E,

U.S. Citrus and Subtropical Products Laboratory, South Atlantic Area, U.S. Department of Agriculture—Agricultural Research Service, P.O. Box 1909, Winter Haven, Florida 33883-1909.

Table I. Citrus Juice Debittering with β-Cyclodextrin Polymer

juice[a]	bed residence time,[b] min	av bed ht, cm	bed vol collected[c]	% redn[d] naringin	% redn[d] limonin
			Grapefruit		
A[e]	3.3	28	2–16	36	26
	3.3	28	2–16	36	35
	4.4	25	2–16	36	53
			17–32	18	29
			2–32	27	43
	2.6	17	2–16	18	10
	2.5	19	2–16	29	23
	3.4	27	2–16	37	35
	3.5	27	2–16	37	28
B[f]	5.8	28	2–16	34	45
	3.8	38	2–16	43	37
	3.9[g] (26 °C)	40	2–16	47	50
			17–32	26	26
			33–48	17	17
			2–48	30	30
	3.6[g] (5 °C)	46	2–16	25	38
			17–32	18	35
			33–48	10	10
			2–48	18	28
C[h]	3.6	35	2–16	36	35
D[i]	3.3	32	2–16	33	39
E[i]	3.8	32	2–16	61	67
F[k]	8.5	20	3–24	40	76
			25–48	19	24
			49–72	12	3
			3–72	24	34
			Navel Orange		
G[l]	5.9	18	2–16		31
H[m]	3.5	35	2–16		55
H[n]	4.1 (5 °C)	33	2–16		45
			17–32		44
			33–48		29
			2–48		39

[a] Frozen concentrated grapefruit or California navel orange juice reconstituted to single strength, except for juice E. [b] Flow rates varied from 145 to 290 mL/min. [c] Each 2 bed volume fraction represents 1600 mL. Composite samples of eight fractions (16 bed volumes) were used for analysis. [d] Represents average percent reduction in eight, 2 bed volume composite fractions, except for bed volumes 2–32 or 2–48, which are the average total reduction for 32 and 48 bed volumes, respectively. [e] Naringin 835 ppm, limonin 5.0 ppm, 10° Brix, ratio 8.5. β-Cyclodextrin polymer A. [f] Naringin 721 ppm, limonon 9.1 ppm, 10.9° Brix, ratio 8.0. β-Cyclodextrin polymer B. Polymers A and B were different lots from same company. [g] Debittering of 48 bed volumes at 26 and 5 °C, respectively. [h] Centrifuged juice. Naringin 460 ppm, limonin 4.1 ppm, 11.0° Brix, ratio 7.5. Polymer B. [i] Naringin 475 ppm, Limonin 10 ppm, 10.8° Brix, ratio 7.9. Polymer C. [j] Single-strength juice from experimental grapefruit hybrid. Naringin 490 ppm, limonin 20 ppm. Polymer C. [k] Naringin 810 ppm, limonin 7.4 ppm, 11° Brix, ratio 7.8. Polymer D. [l] Limonin 11.7 ppm, 9.0° Brix, ratio 7.4. Polymer A. [m] Limonin 7.4 ppm, 9.5° Brix, ratio 11.6. Polymer B. [n] Debittering of 48 bed volumes at 5 °C. Polymer B.

Table I). Values for degrees Brix, Brix/acid ratio, and naringin and limonin contents for these juices are included in Tables I–III.

Four β-cyclodextrin polymers and two nonionic polystyrene divinylbenzene resins (Amberlite XAD-4 and XAD-16; Rohm and Haas, Philadelphia, PA) were evaluated. The amberlite resins were approved for food use (Johnson and Chandler, 1988), but cyclodextrin polymers have not been approved for food use. Cyclodextrin polymers A and B (the same polymer from two different lots) supplied by American Maize-Products Co., Hammond, IN, and cyclodextrin polymer C supplied by the Consortium fur Electrochemische Industry, Gmbh, Munchen, West Germany, were cross-linked with epichlorohydrin. Cyclodextrin polymer D was an experimental polymer of β-cyclodextrin cross-linked with methacrylic acid. Particle size range for cyclodextrin polymers A and B was 20–60 mesh with a dry volume of 1.37 mL/g and a hydrated volume of 4.6 mL/g. Particle size range for cyclodextrin polymer C was 40–60 mesh with a dry volume of 2.5 mL/g and hydrated volume of 5.2 mL/g. Cyclodextrin polymer D had a particle size range of 60–80 mesh with dry and hydrated volumes of 2.4 and 2.6 mL/g, respectively.

Table II. Grapefruit Juice (Juice I)[a] Debittering with Two β-Cyclodextrin Columns in Series (Polymer B)

bed residence time,[b] min 1[e]	bed residence time,[b] min 2[e]	av bed ht, cm A	av bed ht, cm B	bed vol collected[c]	% redn[d] naringin A	% redn[d] naringin B	% redn[d] limonin A	% redn[d] limonin B
3.4	3.4	33	28	2–16	45	58	44	59
				17–32	30	34	35	36
				33–48	22	32	10	23
				49–64	21	24	12	6
				2–64	30	37	25	31

[a] Frozen grapefruit concentrate reconstituted to single strength. [b] Flow rate was 280 mL/min. [c] Each 2 bed volume fraction represents 1600 mL. Composite samples of eight fractions (16 bed volumes) were used for analysis. [d] Represents average percent reduction in eight 2 bed volume composite fractions, except for 2–64, which is the average total reduction for 64 bed volumes. [e] Columns 1 and 2 were operated in a series using a single pump. [f] Naringin 760 ppm, limonin 11.3 ppm, 10.8° Brix, ratio 7.6.

The pilot-scale equipment used to fluidize the polymers or resins (Wagner et al., 1988) consisted of a glass column 76.2-cm length × 7.6-cm i.d. mounted vertically and connected at the column bottom to a metering pump (Zenith Products Co., West Newton, MA) driven by a variable-speed motor Zero-Max Company, Minneapolis, MN). Sufficient cyclodextrin polymer or XAD resin was added to give a 20.5-cm stagnant bed height after back-flushing, except for polymer D. For cyclodextrin polymer D, a 13-cm bed height was used because sufficient material of suitable mesh size was not available. The XAD resin columns were prepared by adding excess resin and fluidizing the columns at flow rates of 200 and 300 mL/min for XAD-4 and 16, respectively, to obtain a 20.5-cm bed height of uniform particle size.

The runs at 5 °C (Table I) were accomplished by moving the column into a walk-in cold room maintained at 5 °C. Juice was reconstituted and equilibrated at 5 °C prior to debittering. For the double-column run shown in Table II, a second column identical with the first was connected to it in series. Cyclodextrin polymers and XAD resins were regenerated by washing with water to remove residual juice, followed by sodium hydroxide to remove naringin and limonin (Wagner et al., 1988). Total soluble solids (corrected degrees Brix), total acid, ascorbic acid, and oil content were determined by standard methods used by the citrus industry (Praschan, 1975). Naringin values were determined by the Davis test (Praschan, 1975), which also measures other nonbitter flavanone glycosides. Limonin was determined by an HPLC method (Shaw, 1986).

RESULTS AND DISCUSSION

A pilot-scale fluidized bed column containing either β-cyclodextrin polymer or XAD resin was used to reduce the levels of the bitter components naringin and limonin in grapefruit juice and limonin in navel orange juice. The percent reduction in bitter components with cyclodextrin polymers is shown in Tables I and II and with XAD resins is shown in Table III.

For grapefruit juices debittered with cyclodextrin polymer (Table I), reduction of naringin varied in the first 16 bed volumes from 18 to 61% and limonin varied from

Table III. Citrus Juice Debittering with XAD Resins[a,b]

	residence time,[c] min	av bed ht, cm	bed vol collected[d]	% redn[e] naringin	% redn[e] limonin
			XAD 16		
			Grapefruit		
C[f]	7.2	46	2–16	82	100
J[g]	5.4	33	2–16	73	100
			17–32	42	79
			33–48	30	78
			49–64	21	63
			2–64	42	80
J	6.0	21	1–16	66	100
			17–32	44	94
			33–48	33	71
			49–64	28	66
			2–64	43	83
			63–64	22	69
			Navel Orange		
L[h]	4.7	32	2–16		96
			17–32		89
			33–48		75
			49–64		72
			65–80		65
			81–96		57
			97–112		45
			113–128		57
			129–144		54
			2–144		67
			143–144		50
			Navel Orange		
L	4.4	38	2–16		77
			17–32		68
			33–48		56
			49–64		52
			65–80		55
			2–80		62
			XAD-4		
			Grapefruit		
M[i]	5.5	35	2–16	36	64
			17–32	27	52
			33–48	27	45
			2–48	30	54
M	8.0	34	2–16	42	58
			17–32	34	57
			33–48	28	44
			2–48	35	53
			Navel Orange		
L[h]	8.1	34	2–16		63
			17–32		50
			33–48		41
			49–56		33
			2–56		47
L	5.9	35	2–16		34
			17–32		28
			33–48		26
			2–48		29

[a] XAD-16 and XAD-4 resins were used. [b] Frozen concentrated grapefruit or California navel orange juice reconstituted to single strength. [c] Flow rates varied from 139 to 283 mL/min for XAD-16 and from 114 to 168 mL/min for XAD-4. [d] Each 2 bed volume fraction represents 1600 mL. Composite samples of eight fractions (16 bed volumes) were used for analysis. [e] Represents average percent reduction in eight composite fractions (16 bed volumes). [f] See Table I, footnote *h*. [g] Naringin 695 ppm, limonin 8.2 ppm, 11° Brix, ratio 7.8. [h] Limonin 14.1 ppm, 11° Brix, ratio 13.7. [i] Naringin 710 ppm, limonin 8.5 ppm, 11° Brix, ratio 7.8.

28 to 67%. Results from juice A debittered with cyclodextrin polymer A show the polymer to be relatively durable by maintaining its ability to debitter juice thru 30 regenerations. Values were similar to those reported by Wagner et al. (1988) where only 16 bed volumes were collected. The results of debittering 32 bed volumes of juice with fractions collected in 16 bed volume increments are shown in the run with juice A at 4.4-min bed residence time. Bitterness reduction for the first 16 bed volumes was similar to values obtained for previous 16 bed volume runs. About half as much naringin and limonin was removed in the second 16 bed volumes. Total reduction in naringin and limonin for 32 bed volumes was 27 and 43%, respectively.

Some processors occasionally use hot sodium hydroxide for cleanup operations, and it would be advantageous to use this source of sodium hydroxide for column regeneration. Regeneration of the column by washing it with 2% sodium hydroxide solution at 55 °C, rather than the normal ambient temperature treatment, resulted in considerable loss in column efficiency (juice A runs at 2.6- and 2.5-min bed residence time in Table I). The physical nature of the cyclodextrin polymer was changed by this treatment, probably due to agglomeration of the polymer beads. This resulted in reduced bed heights for these two runs, even at a faster flow rate. However the runs following hot lye treatment show that subsequent regeneration with cold lye followed by washing the cyclodextrin polymer with water restored normal debittering efficiency to the cyclodextrin polymer (juice A, bed residence times 3.4 and 3.5 min).

Bed residence times and heights varied during the first two 16 bed volume runs with juice B (Table I) and afforded variable results. Longer contact time and lower bed height removed less naringin and more limonin. However, less limonin and more naringin were removed with a more expanded bed and shorter contact time. Two runs of 48 bed volumes each were conducted at ambient temperature (26 °C) and at 5 °C to determine the effect of debittering a larger volume of juice and to determine if lower temperatures, which might help preserve juice quality and prevent microbial growth, affected the debittering process (runs 3 and 4, juice B, Table I). The main difference in results from the two runs is in naringin reduction, where about half as much naringin was removed at 5 °C.

Clarified fruit juices are widely used in fruit drink beverages. Clarified grapefruit juice is a potential juice source for these beverages, but some bitterness would probably have to be removed from it prior to use. Processing bitter clarified juice (juice C, Table I) with cyclodextrin polymer debittered the juice sufficiently to consider its use in such fruit drinks.

Cyclodextrin polymer from a second commercial source (polymer C) was used to debitter grapefruit juices (juices D and E, Table I) as well. With juice D (polymer C) unusually high amounts of both bitter components were removed in the first run. However, in run E, the juice was debittered to about the same extent as for cyclodextrin polymers A and B. It has been typical of most cyclodextrin polymers that the polymer was signficantly more efficient at removing bitter components the first time it was used with juice samples than in all subsequent runs.

A polymer cross-linked with methacrylic acid (cyclodextrin polymer D) was evaluated with a juice (juice F, Table I) high in naringin content. In this run 1600 mL represents three bed volumes since the bed height was 13 cm instead of 20.5 cm used with the other cyclodextrin polymers. It reduced bitterness as effectively as the other polymers but was not as durable. Lower flow rates (8.5-min residence time, Table I) were required to establish a suitable fluidized bed. Unlike other cyclodextrin polymers studied, this polymer was very friable. Up to 50% of this polymer was lost from the fluidized bed and ended up in the juice, even at the lower flow rates.

Table IV. Percent Naringin and Limonin Reduction in Grapefruit Juice Fractions from XAD-16 Resin

	juice sample			
	C[b]		I[c]	
bed vol[a]	naringin	limonin	naringin	limonin
1–2	96		91	
3–4	88		83	
5–6	80		80	
7–8	79		70	
9–10	80		64	
11–12	78		60	
13–14	78		60	
15–16	75	100	68	100
31–32			20	65

[a] Each 2 bed volume fraction represents 1600 mL. [b] Juice C, Tables I and II. [c] Juice I, Table II. Average value from two similar runs.

Processed navel orange juice is bitter because of its limonin content and contains no naringin. Limonin reduction in navel orange juice debittered with polymers A and B (juices G and H, Table I) was 31 and 55%, respectively. Polymer A did not reduce the limonin content in 16 bed volumes of juice (juice G, Table I) as effectively as polymer B (juice H, Table I) even at lower flow rates (5.9-min bed residence, Table I). However, polymer A had been regenerated 30 times or more when the navel orange juice was debittered. Polymer B (Table I) reduced limonin as effectively at 5 °C as at ambient temperature (juice H, Table I).

Two columns were run in series (polymer B) to debitter grapefruit juice, and the results are shown in Table II. Values for 48 bed volumes of debittered juice from column A were similar to results reported in Table I (juices B and F). Reduction in bitterness for 64 bed volumes as a result of the second column was an additional 7% for naringin and an additional 6% for limonin. Juice flow from the first column, rather than a second pump, was used to create a fluidized bed in the second column.

Preparation of fluidized beds of XAD resins was difficult and required considerable back-flushing to obtain a 20.5-cm bed height of resin with sufficient density to remain in the column during fluidization. The first few runs with XAD-16 were particularly difficult because the top 4-5 cm of the bed tended to float when the bed was first fluidized. However, resin floating was eliminated after conditioning with about 5–6 column volumes of juice. Results of debittering citrus juices with XAD resins are shown in Table III. The first use of the resin with grapefruit juice (juice C) illustrates the difficulty in fluidization on start up. The bed height was considerably higher at lower flow rates than for other runs. Bitterness reduction in 16 bed volumes of juice C was 82% for naringin and 100% for limonin. In two runs with grapefruit juice of 64 bed volumes each (juice J, Table III) naringin was reduced by about 42%, with the majority of reduction occurring in the first 32 bed volumes. Limonin reduction for this juice (juice J, Table III) was about 82%, with over 50% reduction in the last 16 bed volumes. In the second run with juice J, naringin and limonin reduction in the 64th bed volume was 22 and 69%, respectively.

Naringin and limonin values for individual fractions of grapefruit juice from XAD-16 resin are shown in Table IV (juice C, Tables I and II; juice I, Table II). For the first 16 bed volumes naringin reduction varied from 96 to 75% (juice C, Tables I and II) and from 91 to 68% (juice I, Table II). For both juices 100% of the limonin was still being removed in 16th bed volume. For juice I (Table II), naringin reduction was 20% and limonin reduction was 65% in the 64th bed volume.

Navel orange juices were treated with XAD resins, also. Limonin reduction in 144 bed volumes of navel orange juice (Table III, juice L) was 67%. A reduction of 50% was still achieved in the 144th bed volume. A smaller 80 bed volume run was performed to determine the effectiveness of the resin following a longer run. Limonin reduction of individual composite fractions was not as good as in the preceding run, but 62% of the limonin was removed.

In 48 bed volumes of grapefruit juice (juice M, Table III) treated with a fluidized bed of XAD-4 resin, naringin reduction ranged from 30 to 35% and limonin reduction ranged from 53 to 54%. Average reduction in bitter components was about the same as for cyclodextrin polymer. In navel orange juice, limonin reduction varied from 29 to 47% for 48 bed volumes of juice (juice L, Table III). Values of composite samples from the first run were substantially higher than those from the second run. This indicates that either the resin lost capacity to remove bitterness because of ineffective regeneration or, as was found with cyclodextrin polymers, the initial run had greater capacity than subsequent runs.

The XAD resins effectively removed bitter components from citrus juices for a limited number of determinations, but the resins were not subjected to a sufficient number of regenerations to determine their stability in cold 2% sodium hydroxide solution. Debittered juices from the resins were not evaluated organoleptically since they were not prepared for food use according to manufacturers specifications. Therefore, the potential for development of off-flavors was not determined. There were no differences in ascorbic acid content or color score between juices from the resins and starting juice.

In conclusion, both cyclodextrin polymers and XAD resins were shown to effectively reduce bitterness levels in grapefruit and navel orange juices to acceptable levels. Cyclodextrin polymers retained their capacity for bitterness reduction for over 30 regenerations with cold 2% sodium hydroxide solution, but regeneration with hot sodium hydroxide solution caused reversible damage. The cyclodextrin polymer removed less naringin and limonin at 5 °C, but limonin was removed from navel orange juice as well at 5 °C as at ambient temperature (26 °C). Both cyclodextrin polymer and XAD-4 resin had about the same capacity for bitterness reduction, but the XAD-16 resin had more capacity for limonin reduction than either cyclodextrin or XAD-4 resin.

Registry No. XAD-4, 37380-42-0; XAD-16, 104219-63-8; naringin, 10236-47-2; limonin, 1180-71-8.

LITERATURE CITED

Hasegawa, S.; Vandercook, C. E.; Cho, G. Y.; Herman, Z.; Ou, P. Limonoid Debittering of Citrus Juice Sera by Immobilized Cells of Cornebacterium Fascians. *J. Food Sci.* **1985**, *50*, 330.

Johnson, R. L.; Chandler, B. V. Reduction of Bitterness and Acidity in Grapefruit Juice by Adsorptive Processes. *J. Sci. Food Agric.* **1982**, *33*, 287.

Johnson, R. L.; Chandler, B. V. Ion Exchange and Adsorbent Resins for Removal of Acids and Bitter Principles from Citrus Juices. *J. Sci. Food Agric.* **1985**, *36*, 480.

Johnson, R. L.; Chandler, B. V. Adsorptive Removal of Bitter Principles and Titratable Acid from Citrus Juices. *Food Technol.* **1988**, *42*, 130.

Kefford, J. F.; Chandler, B. V. *The Chemical Constituents of Citrus Fruit*; Academic Press: New York, 1970; p 140.

Praschan, V. C. *Quality Control Manual for Citrus Processing Plants*; Intercit: Safety Harbor, FL, 1975.

Puri, A. Preparation of Citrus Juices, Concentrates, and Dried Powders Which are Reduced in Bitterness. U.S. Patent 4,439,458, March 27, 1984.

Shaw, P. E. Use of a simple solvent optimization program to improve separation of limonin in citrus juices. *J. Chromatogr. Sci.* **1986**, *24*, 364.
Shaw, P. E.; Wilson, C. W., III Debittering Citrus Juices with β-Cyclodextrin Polymer. *J. Food Sci.* **1983**, *48*, 646.
Shaw, P. E.; Wilson, C. W., III Reduction in Bitterness in Grapefruit Juice with β-Cyclodextrin Polymer in a Continuous-Flow Process. *J. Food Sci.* **1985**, *50*, 1205.
Shaw, P. E.; Buslig, B. S. Selective Removal of Bitter Compounds from Grapefruit Juice and from Aqueous Solutions with Cyclodextrin Polymers and with Amberlite XAD-4. *J. Agric. Food Chem.* **1986**, *34*, 837.
Shaw, P. E.; Tatum, J. H.; Wilson, C. W., III. Improved Flavor of Navel Orange and Grapefruit Juices by Removal of Bitter Components with β-Cyclodextrin Polymer. *J. Agric. Food Chem.* **1984**, *32*, 832.
Wagner, C. J., Jr.; Wilson, C. W., III.; Shaw, P. E. Reduction of Grapefruit Bitter Components in a Fluidized β-Cyclodextrin Polymer Bed. *J. Food Sci.* **1988**, *53*, 516.

Received for review February 8, 1988. Accepted July 29, 1988. Mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture.

Influence of Various Salts on Heat-Induced ANS Fluorescence and Gel Rigidity Development of Tilapia (*Serotherodon aureus*) Myosin

Louise Wicker,* Tyre C. Lanier, James A. Knopp, and Donald D. Hamann

Changes induced by salts in thermal transition temperatures (T_r) observed in tilapia myosin with a polarity probe, 8-anilino-1-naphthalenesulfonate (ANS), and a thermal scanning rheology monitor (TSRM) followed the Hofmeister series. The T_r of myosin–ANS was easily decreased by salting-in salts, and only sodium sulfate markedly increased the T_r, indicating that ANS apparently probes for an extremely hydrophobic site on myosin. Ammonium acetate and sulfate diminished the initial (low-temperature) TSRM rigidity peak, increased the maximum rigidity attained, and increased T_r values. Salting-out salts had no effect on the second rigidity increase, indicating that nonhydrophobic interactions are primarily involved in stabilizing protein structure at higher temperatures. These studies to date suggest that, upon heating, solubilized fish myosin initially undergoes a subtle change in conformation that promotes aggregation of hydrophobic residues to form a soft but elastic gel structure. With further heating to higher temperatures, a more generalized denaturation and aggregation occurs imparting rigidity to the elastic gel.

It has been suggested that hydrophobic interactions are important in the unique setting phenomenon (gelation near 40 °C) and higher temperature gelation of fish muscle proteins (Niwa, 1975; Niwa et al., 1981a,b). In constant-rate heating studies, Wicker et al. (1986) observed a transition at 37 °C in dilute solutions of myosin–ANS and related the increase in fluorescence to a change in the hydrophobicity of myosin. Since the increase and subsequent decrease in fluorescence of myosin–ANS preceded the onset of gelation, the authors concluded that a change in conformation involving hydrophobic residues was prerequisite for gelation.

The addition of salts has proven useful in studying the role of hydrophobic interactions in the denaturation of protein (Robinson and Jencks, 1965; Schrier and Schrier, 1967; Von Hippel and Wong, 1964; Melander and Horvath, 1977; Asghar et al., 1985). Recently, Wicker and Knopp (1988) showed that the transition temperature detected by myosin–ANS fluorescence could be increased or decreased by selected salts in an order following the Hofmeister series. If a change in the effective hydrophobicity of myosin is a prerequisite for further myosin denaturation and gelation (Wicker et al., 1986), then transition temperatures of denaturation and gelation should also be affected by salts of the Hofmeister series. The objective of this study was to determine the effects of selected salts on the denaturation and gelation of fish myosin to aid in elucidating the mechanism of heat-induced gelation of fish myosin.

Department of Food Science and Technology, University of Georgia, Athens, Georgia 30602 (L.W.), and Departments of Food Science (T.C.L., D.D.H.) and Biochemistry (J.A.K.), North Carolina State University, Raleigh, North Carolina 27695-7624.

MATERIALS AND METHODS

Myosin Preparation. Myosin was prepared from tilapia (*Serotherodon aureus*) by the method of Akahane (1982) as modified by Wicker et al. (1986). Myosin was clarified by centrifugation at 100000*g* for 45 min and dialyzed overnight against 0.6 M KCl, 50 mM potassium phosphate, pH 6.5. Myosin solutions were stored under refrigeration, and experiments were completed in less than 1 week to minimize aging effects. Protein concentration was determined by micro-Kjeldahl standardized Biuret or by A_{280} ($\epsilon^{\%}_{cm}$ = 5.5; Swenson and Ritchie, 1980).

Fluorescence Thermograms. Fluorescence of myosin–ANS was measured as reported by Wicker et al. (1986). The heating rate was maintained at 1 °C/min with a Neslab programmable water bath. Fluorescence intensity (FI) was recorded on an SLM Model 8000 spectrofluorometer equipped for photon counting. The wavelengths of excitation and emission were 380 and 475 nm, respectively. The excitation and emission slits were 0.5 and 16.0 nm, respectively. The fluorescence intensity represents the number of photons counted for a period of 10 s.

Rigidity Thermograms. Changes in modulus of rigidity (G) were measured with a thermal scanning rheology monitor (TSRM) as described by Wicker et al. (1986). The myosin solution was heated at a constant rate of 1 °C/min with the same Neslab programmable water bath used in fluorescence studies.

0021-8561/89/1437-0018$01.50/0