was differentiated from XXI by the presence of two ring methyl absorption maxima at δ 2.3 (s) and 2.6 (s) in XXIII and the absence of the δ 2.3 (s) absorption in the spectrum of XXI. In all cases other features of the spectra also confirmed these assignments.

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Ethylene-Accelerated Limonoid Metabolism in Citrus Fruits: A Process for Reducing Juice Bitterness

Vincent P. Maier,* Linda C. Brewster, and Andrew C. Hsu

A 3-hr treatment of citrus fruits (navel oranges, lemons, grapefruit) with 20 ppm of ethylene induces accelerated limonoate A-ring lactone metabolism. Accelerated metabolism continues after ethylene exposure ceases and results in substantial loss of limonoate A-ring lactone in several days. Juice from treated fruit has a lower limonin content, is less bitter, and is more preferred by judges than juice from untreated fruit. Longer exposure to ethylene has no greater effect on limonoate A-ring lactone metabolism than the 3-hr treatment, but it can be detrimental to juice quality. The ethylene treatment has no effect on the naringin content of grapefruit juice nor on ascorbic acid content. Spraying fruit with 2-chloroethylphosphonic acid in wax is another way of achieving the ethylene effect.

The problem of delayed bitterness in citrus products is becoming more acute with the yearly increases in citrus production. As production increases, a higher percentage of the crop goes to citrus products rather than to the fresh fruit market. Citrus products from navel oranges, as well as some lemons and grapefruit, are bitter if their limonin content is over 6–9 ppm (Kefford and Chandler, 1970). Early investigators observed that juice from late-season oranges was less bitter than that from early-season fruit. Unfortunately, the low bitterness level is reached only late in the harvest season, after much of the crop has been harvested. Other investigators attempted to simulate this on-the-tree debittering by storing early-season navel oranges in warm, moist rooms (Rockland *et al.*, 1957). Although this approach had a number of serious drawbacks that prevented its commercialization, some debittering was achieved during prolonged storage.

Maier and Beverly (1968) found that delayed bitterness is caused by the conversion of the nonbitter limonoid limonoate A-ring lactone to bitter limonin by the juice acids during juice extraction. In later work, Maier and Margileth (1969) found that the metabolic debittering system of the late-season fruit acts to prevent bitterness by destroying the nonbitter precursor substance, limonoate A-ring lactone. It was reasoned that a logical approach to solving the limonin bitterness problem would be to find a way to accelerate the natural slow metabolism of limonoate A-ring lactone in the fruit.

Since limonin content had been observed to be inversely related to fruit maturity, the ripening hormone ethylene and the ethylene-generating compound 2-chloroethylphosphonic acid (CEPA) were considered likely agents to promote accelerated limonoid metabolism. Study of ethylene was also of interest because earlier investigators, working without benefit of an analytical method for limonin, found either no acceleration effect of ethylene on debittering (Samish and Ganz, 1950) or an increase in other off-flavors (Emerson, 1949). Our preliminary studies demonstrated that ethylene does, in fact, accelerate limonoid metabolism in citrus fruits (Maier and Brewster, 1971; Maier *et al.*, 1971). This paper reports the effects of ethylene and CEPA concentration, time of exposure, temperature, and length of holding time on limonin content and flavor of the juice.

Fruit and Vegetable Chemistry Laboratory, Western Region, Agricultural Research Service, U. S. Department of Agriculture, Pasadena, California 91106.

EXPERIMENTAL SECTION

Sampling. Fruit were obtained from a packinghouse before removal from field boxes or other handling had taken place. A large lot of fruit was selected from a single grove picked that morning. Fruit were of a median size and color for that grove. Fruit chosen had no bruises, as few blemishes as possible, and green, intact buttons. From the initial lot, fruit were sorted several times, first on the basis of weight and then color. A very uniform group of fruit was then chosen (average weight per fruit was 250 g), with a weight range no greater than 70 g and of approximately the same color. Samples of 15 fruit were then finally chosen, similar in color range and so close in weight that the average fruit weight per sample was within 7 g of that of the most dissimilar sample.

In each experiment, duplicate samples of 15 fruit were used for each parameter. Four samples were analyzed at the start of the treatment period to establish the zero time point. Reproducibility of the overall experimental procedure is demonstrated by the close agreement of the limonin content of the juice from replicate fruit samples, as shown in Table I. Standard deviation of the juice limonin content of the four zero-time samples is 2.7%. Unacceptable variability in limonin content among samples given similar treatment was encountered in early experiments, where less stringent sampling techniques were followed.

Fruit Preparation and Storage. Each fruit sample was washed in 0.1% sodium laurel sulfate solution, rinsed, and dried. Samples from experiments of more than 6-days' duration were also dipped in a 1% sodium borate solution, rinsed, and dried. Fruit were held no more than 2 days from picking to the start of the treatments. After treatment (or during, in the ethylene gas treatments) the samples were placed in large polyethylene bags for holding. A humidified air flow was established through each bag at a rate that replaced the bag volume in 12 to 15 min. This air flow kept fruit moisture and weight loss at a minimum.

CEPA Dip. Samples were totally immersed in a 1000-ppm aqueous solution of 2-chloroethylphosphonic acid (CEPA) (Amchem 68-240) for 1 hr unless otherwise noted. Control samples were immersed in distilled water for an equal period of time. Samples were dried at the end of the treatment period, unless otherwise noted.

CEPA in Wax. Samples were sprayed with 1000 ppm of CEPA in Flavorseal wax (FMC). Triton X-100 was added in a 50:1 ratio to the CEPA. Shaking the components resulted in a cloudy, finely dispersed emulsion which did not break during the spraying period; 100 ml of the wax solution was used to spray about 60 fruit.

Table I. Typical Experiment Showing Reproducibility of Overall Experimental Procedure Including Four Different Treatments

Treatment	Storage time, days at 70°F	Sample no., 15 fruit/sample	Juice limonin, ppm
		1	12.5
		2	11.9
Untreated	0	3	11.8
		4	12.3
Treatment A	5	1	10.4
		2	10.3
Treatment B	5	1	7.8
		2	7.7
Treatment C	5	1	7.8
		2	8.7
Treatment D	5	1	9.8
		2	8.3

Table II. Effect of CEPA (1000 ppm) and Continuous Ethylene (20 ppm) Treatments and Holding Time on Limonoid Metabolism of Navel Oranges

Days held at 70°F	Juice limonin, ppm		
	Untreated	CEPA dip	Continuous ethylene
0	12.4	12.4	12.4
5	12.4	9.9	9.9
8	10.8	8.4	9.0
15	8.8	6.4	6.8

Ethylene Gas. Ethylene gas was added to the humidified air flow system to achieve the specified ethylene concentration in the polyethylene bag. Fruit remained in this system for the entire holding period for the continuous ethylene treatment, or for a period of 3 or 5 hr for the short-term ethylene treatment. Those samples receiving the short-term treatment were then removed to another bag with no added ethylene for the duration of the holding period.

Limonin Analysis. For juice limonin, each sample of 15 fruit was juiced on a citrus reamer, which filters out all large membrane fragments and seeds. The juice from the 15 fruit was combined to form a juice sample. Two aliquots of the stirred juice sample were taken immediately after juicing. Limonin extraction and chromatographic determination were conducted on both aliquots by the method of Maier and Grant (1970), analyzing for total juice limonin.

For peel limonin, in order to obtain a representative and homogeneous sample, the entire peel from 15 fruit was ground twice through the finest plate of a food grinder and the ground peel was stirred. A 75-g aliquot of the ground peel was blended with 350 ml of distilled water in a Waring Blendor. The slurry was adjusted to pH 9 with NaOH and again blended for 5 min. The resulting slurry did not settle out in 1 hr. Two 25-g aliquots were taken of the slurry and diluted with 50 ml of water. BHT (butylated hydroxytoluene) was added (0.01% of sample weight). The mixtures were adjusted to pH 9, held for 2 hr, and stirred every 15 min. The aliquots were then filtered through a ½-in. Celite pad and combined with 4 × 50 ml washings of the Celite with water at pH 9. The filtrate was acidified to pH 2 with HCl and left standing for 1 hr, during which time it was stirred occasionally. The standard limonin extraction of Maier and Grant (1970) was followed, using the acidified filtrate as the analytical sample. Recovery of added limonin by the above method was 98%.

Moisture determinations were conducted on two aliquots of the peel slurry by drying in a vacuum oven at 60° to constant weight. Limonin determinations were reported on a dry-weight basis for all peel samples.

Gas Analyses. Four-milliliter samples of the interior gas of the fruit were withdrawn with a gas-tight syringe from the central cavity (Rasmussen, 1970). The sample was then divided to be used for both ethylene and CO_2 determinations.

The samples were analyzed for ethylene on a Beckman GC-4 with a hydrogen flame ionization detector. A 6-ft, ⅛-in. stainless steel column packed with Alumina (100–200 mesh neutral Alumina Ag7, Biorad) was used, and a 8-ft stainless steel column packed with 80–100 mesh Porapak Q (Waters Associates) was used to verify the ethylene concentration. The helium carrier gas flow rate was 25 ml/min. Temperatures of the injection port, column, and detector were 81, 73, and 180°, respectively, for both columns.

Carbon dioxide concentration was analyzed on a Microtek GC 2500R with a thermal conductivity detector. An

Table III. Effect of Time of Exposure to 20 ppm of Ethylene on Limonoid Metabolism of Navel Oranges

Treatment	Days held at 70°F[a]	Decrease in juice limonin, %
Untreated	0	0
Untreated	5	14
3-Hr ethylene	5	32
20-Hr ethylene	5	31
5-Day ethylene	5	36

[a] Total time from initiation of treatment

8-ft, ⅛-in. stainless steel column packed with Porapak Q (80–100 mesh, Waters Associates) was used with a helium carrier gas flow of 25 ml/min. Temperatures of injection port, column, and detector were 140, 65, and 190°, respectively.

Pre-Bitterness Taste Evaluation. Juice was tasted within 2 hr of juicing by a screened panel of six to eight tasters. Juice flavor was evaluated for the presence of off-flavors, as well as the taster's preference among the group tasted. In the experiment shown in Tables VI and X, sets of the two treatments plus the untreated sample were compared at each of the three holding temperatures. In the experiment shown in Table V, sets of two of the four treatments plus the untreated sample were evaluated.

Post-Bitterness Taste Evaluation. Juice to be tasted was thawed and held refrigerated overnight to allow bitterness to develop. A panel of six people screened for their ability to detect bitterness and discriminate between different flavors was asked to determine whether each sample was bitter, which sample of the pair was more bitter, and which sample was preferred, considering all taste factors. All treatments were compared, two samples at a time, with each comparison being duplicated at a later date.

Naringin Analysis. A 5-g aliquot of juice was added to 40 ml of acetone in a 50-ml centrifuge tube. The acetone was heated to boiling and then cooled in an ice bath. After it had been centrifuged in a clinical centrifuge for 5 min, the supernatant was transferred to a 100-ml boiling flask and evaporated on a rotary vacuum evaporator to approximately 5 ml. The residue was transferred to a second centrifuge tube (that contained 30 ml of acetone) with 2 × 2.5 ml washings of acetone–water (7:3). It was again heated to boiling, cooled on ice, and centrifuged. The supernatant was evaporated to 0.2 ml. This residue was then diluted to 3.0 ml with acetone–water (7:3). One microliter of the final solution was spotted on Baker Polyamide-6 tlc plates (Tatum, 1972) between naringin standards. The plate was developed twice in nitromethane–methanol (5:2), which separated naringin from naringenin 7-rutinoside. Spots were visualized by spraying the developed plates with 1% $AlCl_3$ in ethanol and viewing under uv light. Spots were quantitated by visual comparison with known naringin standards.

Using the above method, the standard deviation of replicate juice aliquots was 1.7%, and the average recovery of added naringin was 106%. The optimum naringin level for visual quantitation was 0.125–1.25 μg per spot.

Ascorbic Acid Analysis, Ascorbic acid analyses were done on filtered juice samples, using the method of Roe (1954).

RESULTS AND DISCUSSION

Ethylene Effect. *Navel Oranges.* Difficulties were initially encountered in obtaining consistent limonin values from replicate samples of fruit. However, when extreme care was taken in all details of fruit selection, subsampling, fruit treatment, and juicing, a high degree of reproducibility was achieved (Table I). To conserve space, averages rather than the individual values for replicates are given in subsequent tables, although analysis of experimental data was done using all replicate data. All conclusions were confirmed by statistical analyses of significance at at least the 95% confidence level using Fisher's F-test and Hartley's method of comparing means (Snedecor, 1956).

Table II shows the effects of a continuous ethylene treatment and a CEPA dip treatment of navel oranges on limonoid metabolism as a function of holding time at 70°F. Both treatments accelerated limonoid metabolism to a similar degree. As early as 5 days after the experiment was initiated, juice from the treated fruit had a 20% lower limonin content, while that of the untreated fruit was unchanged. Acceleration continued through the fifteenth day, when limonin content of juice from the treated fruit had decreased by 45–48%, while that from untreated fruit had only decreased 29%.

Analysis of the gas composition of the free air space inside individual oranges for ethylene and CO_2 concentration indicated that the internal ethylene concentration of the fruit being continuously gassed with 20 ppm of ethylene approached the 20-ppm level about 3 hr after exposure was initiated and remained at that level throughout the holding period. The internal ethylene concentration of the CEPA-dipped fruit also rose to a maximum in roughly 3 hr and then dropped to almost the same level as that of the untreated fruit for the remainder of the holding period. The CO_2 levels of the CEPA-treated fruit rose to a

Table IV. Internal Gas Composition of Navel Oranges Treated With 20 ppm of Ethylene

	Ethylene, ppm							
	Holding time, hr							
Treatment	0	3	5	10	24	48	72	96
Untreated	0.06	0.08	0.03	0.03	0.03	0.04	0.04	0.05
3-Hr ethylene	0.06	17	0.40	0.20	0.10	0.03	0.04	0.05
5-Day ethylene	0.06	17	17	18	18	18	17	17
	CO_2, %							
	Holding time, hr							
Treatment	0	3	5	10	24	48	72	96
Untreated	2.1	2.0	2.0	1.9	1.7	1.4	1.3	1.2
3-Hr ethylene	2.1	2.6	3.1	2.3	1.6	1.3	1.1	0.9
5-Day ethylene	2.1	2.6	3.1	3.4	4.1	5.0	5.8	5.3

Table V. Effect of Ethylene Level in a 3-Hr Treatment on Limonoid Metabolism of Navel Oranges

Treatment	Holding time, days at 60°F	Ethylene concentration, ppm	Juice limonin, ppm	Decrease in juice limonin, %
Untreated	0		5.6	0
Untreated	6	0	4.2	25
3-Hr ethylene	6	0.2	4.2	25
3-Hr ethylene	6	1.8	4.0	29
3-Hr ethylene	6	24	3.5	38
3-Hr ethylene	6	183	3.4	39

maximum that was slightly higher than that of untreated fruit in 3 to 6 hr and then dropped to about the same as that of the untreated fruit, whereas the continuous ethylene-treated fruit had CO_2 levels two or three times higher than those of the untreated fruit. These CO_2 results agree with the generally observed response of citrus fruits to ethylene, wherein respiration returns to nearly normal levels after exposure to exogenous ethylene ceases (McMurchie *et al.*, 1972).

The fact that CEPA was as effective as continuous ethylene in accelerating limonoid metabolism, even though it produced brief and much lower internal ethylene levels, suggested that very brief gassing of the fruit with ethylene might also be effective. This premise was tested in another experiment. The results show (Table III) that exposure of the fruit to 20 ppm of ethylene for only 3 hr is as effective in accelerating limonoid metabolism as is exposure to ethylene for 20 hr or continuously for 5 days. Table IV shows the concomitant internal ethylene and CO_2 levels in companion fruit. The internal ethylene levels of the fruit exposed for 3 hr rise very rapidly to about 17 ppm during exposure and then drop off rapidly when the exogenous ethylene treatment is halted. Within 48 hr, the internal ethylene concentration is similar to that of the untreated fruit. The 3-hr ethylene fruit undergo a brief period of slightly higher than normal CO_2 production, but over the main portion of the 5-day holding period, CO_2 production parallels that of the untreated fruit. In contrast, the fruit gassed continuously with ethylene during the 5-day period show high internal ethylene levels and increased (up to fourfold) levels of CO_2 during most of the holding period. Thus, the extended accelerated respiration caused by prolonged exposure to ethylene is not required for accelerated limonoid metabolism. This point is important because it shows that the 3-hr ethylene treatment specifically activates the metabolic debittering system. In practical terms, this means that an effective debittering treatment need not lead to the many changes in fruit composition (some of which may be undesirable) associated with above normal levels of respiration.

The level of ethylene needed to trigger accelerated limonoid metabolism in a 3-hr treatment was tested (Table V). Levels of ethylene above 1.8 ppm are needed to achieve maximum acceleration, although it appears that a slight acceleration is caused by the 1.8-ppm level. It is possible that longer exposure to 1.8 ppm or even lower ethylene concentrations might also accelerate limonoid metabolism. The next highest level tested in this experiment, 24-ppm, gave maximum acceleration, as did the 183-ppm level, indicating that the lowest concentration of ethylene in a 3-hr exposure that produces maximum acceleration lies between 1.8 and 24 ppm. Ethylene levels above 24 ppm cause no further increase in the acceleration effect. Hartley's comparison of means (Snedecor, 1956) supports these conclusions at the 95% confidence level.

Table VI. Effect of Temperature on Normal and Ethylene (20 ppm) Accelerated Limonoid Metabolism of Navel Oranges

Treatment	Juice limonin, ppm: Holding temperature 50°F	70°F	86°F
Untreated, zero time	24.3	24.3	24.3
Untreated[a]	22.4	19.4	14.2
3-Hr ethylene[a]	18.8	13.2	10.8
20-Hr ethylene[a]	18.3	13.6	8.2

[a] Fruit held 20 hr at 70°F, including treatment time, then held 5 days at the indicated temperatures.

Table VII. Effect of CEPA (1000 ppm) Treatment of Lemons on Limonoid Metabolism. Analysis of Juice and Peel

Days held at 72°F	Juice limonin, ppm: Untreated	CEPA dip	Peel limonin, ppm dry wt: Untreated	CEPA dip
0	6.0	6.0	374	374
6	5.5	5.0	357	285
13	4.3	2.9	238	138
20	3.0	2.4		
27	2.4	1.6		

Temperature Effect. The effect of holding temperature on ethylene-accelerated limonoid metabolism is shown in Table VI. The wide difference in the temperature effect on ethylene-accelerated and normal metabolism suggests a fundamental mechanistic difference between the two. While the metabolic pathway is probably the same in each case, the regulation of the pathway may be different. Possibly ethylene acceleration influences the synthesis of the enzymes involved in the metabolism of limonoids. The ability of ethylene to trigger selectively the synthesis of specific enzymes has been reported for peroxidase isoenzymes of sweet potato (Imaseki *et al.*, 1968).

Another possibility is that ethylene overcomes a partial or total inhibition of the synthesis of enzymes involved in limonoid metabolism or in the activities of these enzymes. It has been observed that CO_2 competitively inhibits the effect of ethylene in inhibiting growth of pea stem sections (Burg and Burg, 1967). Burg and Burg point out that many physiological effects can be explained on the basis of competition between CO_2 and ethylene. Whether such competition exists in the regulation or control of limonoid metabolism remains to be determined.

Ethylene Effect. *Lemons and Grapefruit.* The data in Tables VII and VIII show that ethylene also accelerates limonoid metabolism in lemons and grapefruit. In the lemon experiment, CEPA was used as the ethylene source, and both juice and peel were analyzed at periodic intervals. Limonoid metabolism continued, and the ethylene effect was apparent over the entire holding period of

Table VIII. Effect of Ethylene Treatment and Storage of Grapefruit on Limonoid Metabolism, and Naringin and Ascorbic Acid Content

Treatment	Limonin, ppm	Naringin, ppm	Ascorbic acid, mg/100 ml
Untreated, zero time	4.2 ± .15	433 ± 8.5	43 ± 1.5
Untreated[a]	3.6	435	43
3-Hr, 20 ppm of ethylene[a]	1.9	443	44
6-Day, 20 ppm of ethylene[a]	1.6	428	42

[a] Fruit held 6 days at 70°F, including treatment time.

Table IX. Post-Bitterness Taste Comparison of Juice From Treated and Untreated Navel Oranges[a]

Treatment	Juice limonin, ppm	Taste evaluation
Untreated, zero time	12.1	Most bitter and least preferred
5-Day ethylene[b]	7.8	
Untreated[b]	10.3	
CEPA-wax spray[b]	9.0	
3-Hr ethylene[b]	8.2	Least bitter and most preferred

[a] Paired comparisons used to arrive at evaluation order. [b] Fruit held 5 days at 70°F, including treatment time.

Table X. Relationship of Bitterness and Preference Taste Evaluation to Limonin Content of Navel Orange Juice

Treatment (holding temperature), °F	Juice limonin, ppm	Taste evaluation
Untreated, zero time	25.2	Most bitter and least preferred
Untreated (50°)[a]	23.6	
3-Hr ethylene (50°)[a]	19.1	
20-Hr ethylene (50°)[a]	18.2	
Untreated (70°)[a]	19.8	
20-Hr ethylene (70°)[a]	14.0	
20-Hr ethylene (86°)[a]	8.4	
Untreated (86°)[a]	13.6	
3-Hr ethylene (86°)[a]	10.8	
3-Hr ethylene (70°)[a]	13.0	Least bitter and most preferred

[a] Fruit held 20 hr at 70°F, including treatment time, then held 5 days at the indicated temperatures.

27 days. At all time intervals, juice and peel from treated fruit had substantially lower limonin levels than those of untreated fruit. For example, after a 13-day holding period, juice from treated fruit had a 52% lower limonin content than it did initially, while the juice from untreated fruit was only 29% lower. In like manner, the limonoid content of the peel from treated fruit dropped 63%, while that from untreated fruit dropped only 36%. Since both juice and peel undergo substantial decreases in limonoid content, it is clear that metabolism rather than translocation is responsible for the drop in limonoate A-ring lactone. Hsu *et al.* (1973) have recently isolated from citrus fruit what appears to be an initial product of limonoate A-ring lactone metabolism, 17-dehydrolimonoate A-ring lactone. Prior to that Hasegawa *et al.* (1972) reported the isolation and purification from a microorganism of limonoate dehydrogenase, an enzyme that converts limonoate A-ring lactone to 17-dehydrolimonoate A-ring lactone.

In the grapefruit experiment (Table VIII), 3-hr and continuous ethylene treatments were used, and the holding period was 6 days at 70°F. The 3-hr ethylene treatment was very effective in accelerating limonoid metabolism. The juice limonin content of the treated fruit dropped 55%, whereas that of the untreated fruit dropped only 14%. The continuous 6-day ethylene-treated fruit showed only a slightly larger ethylene effect than the fruit that received the 3-hr ethylene treatment.

At the same time, the ethylene treatments had no influence on naringin content or ascorbic acid content (Table VIII). In fact, naringin content of juice from treated fruit was unchanged from that of the untreated and zero time fruit samples, and averaged 430 ppm. Apparently naringin metabolism is so slow that no change in naringin content is detected in 6 days, even when respiration of the fruit is accelerated by continuous exposure to 20 ppm of ethylene. The same holds for ascorbic acid content. Thus, while ethylene substantially accelerates limonoid metabolism in grapefruit and thereby reduces juice bitterness caused by limonin, it has no measurable effect on naringin metabolism and the bitterness caused by naringin. The nutritional value of the juice due to its vitamin C content is unaltered by the ethylene treatment.

Ethylene Effect. *Other Agents.* Since CEPA can be used in solution it offers alternate methods of application to the use of ethylene gas as a means of accelerating limonoid metabolism. To define such alternatives better, several CEPA treatment parameters were investigated. The required fruit soaking time in a 1000-ppm aqueous CEPA solution was investigated. Similar lots of navel oranges soaked 30 sec, 15 min, and 1 hr and then held 4 days at 70°F showed juice limonin decreases of 15, 25, and 41%, respectively. While a 1-hr soak appears necessary for maximum acceleration and was suitable for experimental purposes, it would not be very feasible for commercial use. A CEPA spray treatment of the fruit, however, would be commercially feasible. The data in Table IX show that a CEPA-wax spray treatment is effective in accelerating limonoid metabolism. Other CEPA carriers such as volatile solvents could also be used in place of the wax.

Other components that decompose to release ethylene, or agents or treatments that cause the fruit to produce ethylene through an injury response, would be expected to be effective in accelerating limonoid metabolism (provided these compounds or their products have no metabolic actions that counteract the ethylene effect). One such compound, cycloheximide (Cooper and Henry, 1971), was tested by dipping navel oranges for 1 min in a 50-ppm aqueous solution. This treatment caused a typical ethylene-accelerated limonoid metabolism response. Should cycloheximide come into use as a commercial abscission agent to aid in mechanical harvesting of citrus fruits, it might have the secondary beneficial effect of promoting limonoid debittering of the fruit. In fact, any abscission agent that acts through an ethylene mechanism might serve the secondary function of promoting limonoid debittering.

Taste and Preference. In the experiments shown in Tables V and VI, the juices were tasted immediately after the fruit was juiced and before bitterness had time to develop, in order to test for off-flavors. Slight off-flavors were detected only in the 20-hr ethylene-treated fruit held at 86°F. None of the 3-hr ethylene treatments shown in Table V, including the 183-ppm ethylene-treated fruit held at 60°F for 5 days, gave evidence of off-flavor. This suggests that detrimental flavor effects from ethylene treatments tend to arise from long-term exposure to ethylene and from subsequent holding at warm temperatures.

The effect of a 3-hr ethylene treatment followed by extended storage on the pre-bitterness flavor of orange slices was also determined. Navel oranges were sliced and tasted after a holding period of 7 weeks at 60°F. The untreated, 0.2 ppm, and 1.8 ppm ethylene-treated samples had identical flavors, as determined by triangle taste tests. The 24 and 183 ppm ethylene-treated samples were distinguishable from the above, with the 24-ppm sample being preferred over all others. The 183-ppm sample was the least preferred because of off-flavors. Thus, treatment of navel oranges for 3 hr with 24 ppm of ethylene, followed by cool storage, had a beneficial effect on flavor during extended storage of the fruit.

In the experiments shown in Tables IX and X (the latter is from the experiment of Table VI), the juice was tasted after allowing sufficient time for full bitterness to develop. In general, bitterness paralleled limonin content, and preference was inversely related to limonin content. In the experiments shown in Table IX, the only exception was the 5-day ethylene-treated fruit. The low preference and high bitterness rating it received, even though it had the lowest limonin content, were apparently due to the

presence of off-flavors. Off-flavors have been noted in juice from other fruit that were treated with ethylene for several days. The presence of off-flavors tends to obscure the taster's judgment of bitterness.

In Table X, the major exception to the relationship between preference and low bitterness was the 20-hr ethylene-treated fruit that were held at 86°F. In this case off-flavor development appears to be responsible, as indicated by the previously mentioned taste tests conducted on the fresh juice before bitterness had developed. The most preferred juice was from the 3-hr ethylene-treated fruit held at 70°F.

Specificity of Ethylene Effect. The 3-hr 20-ppm ethylene treatment is a rather specific method for accelerating limonoid metabolism. It does not produce the gross metabolic and physiological effects that result from several days exposure to ethylene. Short ethylene treatment does not lead to accelerated button browning and abscission, as does longer ethylene treatment. Degreening of commercially mature fruit is only slightly affected by the short ethylene exposure, whereas longer treatment greatly accelerates degreening. Carbon dioxide production of 3-hr ethylene-treated fruit is slightly higher than that of untreated fruit for only a brief period, while longer ethylene treatment results in substantially higher carbon dioxide levels for the duration of exposure after roughly the first 10 hr of gassing.

The usual changes induced by exogenous ethylene in citrus are reversible. This is because exogenous ethylene appears not to stimulate endogenous ethylene in citrus (McMurchie *et al.*, 1972). The unique aspect of the 3-hr ethylene treatment is that it irreversibly induces accelerated limonoid metabolism. This uniqueness is fortuitous, because it permits debittering to be accelerated without greatly affecting the fruit in other ways that may be deleterious to quality.

Commercial Application. Accelerated debittering by means of the short ethylene treatment should be a simple procedure for commercial use. No expensive new facilities or equipment would be needed for the ethylene treatment. Because of the short gassing time, existing degreening gassing rooms at citrus packinghouses could be used to treat a number of charges of fruit per day. Alternatively, simple temporary enclosures or even covered-over truck trailers might be used. After ethylene treatment, the fruit could be held indoors or outdoors at either the packinghouse or processing plant, with the transit time from the packinghouse to the processing plant providing part of the holding time. The degree of debittering would increase with the holding time. A reward in the form of a higher return to the grower for fruit that yields juice of reduced bitterness might provide the incentive for appropriate holding times.

The ethylene treatment presently used on some early-season fruit to promote degreening would be expected also to accelerate debittering. However, early-season fruit have the highest limonoid content, and the juice derived is the most bitter of the season. Our findings (Table II) indicate that this situation could be improved by extending the holding period between ethylene treatment and juicing, thereby allowing metabolism of a larger amount of the limonin precursor.

As the season progresses the need for degreening ceases; however, the fruit still yield bitter juice. At this point the fruit going into products, after they are separated from the fruit going to the fresh market, should be given the short-term ethylene treatment to promote debittering. This practice should continue during the remainder of the season until the fruit reach the stage where they no longer yield bitter juice.

In areas where the ethylene degreening treatment is not used, or where the products fruit is segregated from the fresh-market fruit before the degreening treatment, only the products fruit would need to be given the short-term ethylene treatment to promote accelerated debittering.

CEPA, cycloheximide, and other compounds that decompose to yield ethylene or that promote endogenous ethylene production offer alternatives to the use of ethylene gas for achieving the fruit internal ethylene levels needed to initiate accelerated limonoid metabolism. Commercial use of such compounds would depend on prior clearance by FDA for that purpose.

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