Production of Ethanol, Acetaldehyde, and Methanol by Intact Oranges During and After Nitrogen Storage[1]

S. M. Norman[2], and C. C. Craft[3]
U. S. Department of Agriculture, Pomona, California

Abstract. Endogenous volatiles emanating from intact navel oranges were measured by gas chromatography during and after N_2 storage. The longer the oranges were held in N_2 the greater was the rate of volatile production, both in N_2 and after the fruit were returned to air. Ethanol was the predominant volatile emanating from oranges during N_2 storage. The rate of ethanol production progressively increased in N_2 storage and decreased when the oranges were returned to air. The rate of acetaldehyde production increased slightly in N_2 storage and increased markedly to a maximum 2 to 4 hr after the oranges were returned to air, and then rapidly declined. The rate of methanol production increased in N_2 storage and declined slightly when the oranges were returned to air. Oranges held 2 hr in N_2 returned to pre-treatment volatile production rates after several days in air but those held for longer periods in N_2 did not return to pre-treatment rates. All oranges held in N_2 20 hr at 38° C or 3 or more days at 20° developed rind injury after the fruits were returned to air.

Interest in the commercial application of controlled-atmosphere storage has stimulated the study of the respiratory activity of citrus fruits under different atmospheres (2, 4, 6, 11). Knowledge of normal endogenous volatiles, other than ethylene, emanating from intact citrus fruit under different conditions is limited. Biale and Shepherd (2) showed that oranges and lemons emitted acetaldehyde when transferred from N_2 to air. Attaway and Oberbacher (1) collected vapors from 'Hamlin' oranges held in air and identified 7 major components which included ethanol and ethyl acetate. Norman et al. (10) and Norman (9) utilized direct-vapor analyses and identified ethanol, acetaldehyde, methanol, and ethyl acetate (tentative) as normal volatiles emitted from intact oranges.

Studies were undertaken to determine whether the production of ethanol, acetaldehyde, methanol, and ethyl acetate by oranges differed under aerobic and anaerobic conditions. These endogenous volatiles emanating from individual oranges were investigated with direct-vapor sampling techniques and a flame-ionization gas chromatograph to obtain production rates during and after exposure to N_2.

Materials and Methods[4]

Preparation of samples. Early-, middle-, and late-season navel oranges were obtained without packinghouse treatment and washed by hand. Single-fruit samples were used because erroneous interpretations of data were possible with multiple-fruit samples if decaying fruit were unknowingly included in the sample.

Each experimental fruit was weighed and placed in a calibrated 1-pt mason jar equipped with a lid containing 2 silicone rubber sampling ports. Controlled flows of air or N_2 (50 ml/min) passed through the jars through needles entering the sampling ports. Air and N_2 were from commercial sources. No special precautions were taken to eliminate traces of O_2 from the N_2. Analyses of the air space of empty jars did not reveal any contaminants from the silicone rubber sampling ports or rubber sealing rings. Analyses of the gas flowing over the oranges did not reveal any buildup of volatiles in the jars during purging.

Treatments. Individual oranges were held at 20° C for 2 and 20 hr and for 3, 4, and 5 days in N_2. The fruits were then transferred to air and held 12 to 14 days. Air controls were held 15 days at 20°. Other oranges were held in N_2 at 38° for 20 hr and then transferred to air at 20° and held 9 days. Air controls were held 20 hr at 38° and 9 days at 20°. Vapor samples were analyzed before the fruits were placed in N_2 and at hourly or daily intervals during the test. Triplicate single oranges were analyzed for each treatment for early-, middle-, and late-season navel oranges.

Sampling. The gas flowing through the jars was stopped and the closed jars were held for 1 hr. After 1 hr, 5-ml vapor samples were withdrawn for gas chromatographic analyses.

Gas chromatography. Direct vapor-injection analyses were carried out on a Loenco Model 150-15A flame ionization gas chromatograph equipped with a Cary Model 31 electrometer. A Honeywell Model 16 25-mv recorder equipped with a Disc integrator was employed. Chromatograms were obtained at 135° C on a 1/4-in., 6-ft column packed with 80 to 100 mesh Chromosorb 101. Gas chromatographic conditions are described in more detail elsewhere (9).

Standards. Sealed capillary tubes containing weighed amounts of ethanol, acetaldehyde, methanol, and ethyl acetate were placed in a calibrated 5-gal bottle previously flushed with N_2. The bottle was sealed with a rubber stopper equipped with 2 silicone rubber sampling ports. The glass capillary tubes were broken and the liquids allowed to vaporize and equilibrate. Vapor samples were withdrawn by syringe for gas chromatographic analyses. Quantitative estimations were made by comparing the peak areas in Disc units of the standards with those produced by the unknowns. Production rates were calculated in $\mu g/100\ g/hr$ for each fruit. All data represent the mean of 3 individual fruits, replicated 3 times.

Identification of volatiles. Identifications of ethanol, acetaldehyde, methanol, and ethyl acetate were supported by gas co-chromatography with known compounds on Apiezon L, Carbowax 20 M, Porapak Q, Chromosorb 101, and Chromosorb 104. In addition, syringe reactions using qalitative organic classification tests to indicate various functional groups were used together with gas chromatography as described by Hoff and Feit (7). Methanol and ethanol were further identified by subtractive techniques using a boric acid column as described by Ikeda et al. (8). The 2,4-dinitrophenylhydrazine derivative of the compound corresponding to acetaldehyde had the same Rf value as the known acetaldehyde 2,4-dinitrophenylhydrazine derivative on silica gel and aluminum oxide with 3 different solvent systems. Ethyl acetate was not present in sufficient quantities for positive identification and is tentatively identified on gas chromatographic retention time only.

[1]Received for publication October 12, 1970.

[2]Food Technologist, Agricultural Research Service.
[3]Plant Physiologist, Agricultural Research Service.

[4]Mention of a company name does not constitute a guarantee or warranty of the company's product by the U. S. Department of Agriculture and does not imply its approval by the Department to the exclusion of other products that may also be suitable.

Norman

Results

Fruit variability. Generally, the rates of volatile production among early-, middle-, and late-season navels did not vary more than the volatile production among single fruits. Single fruits from all 3 lots of oranges followed the same patterns of response when transferred from air to N_2 and back to air. All data presented represent the means of the seasons' fruit.

Rind injury. Early-, middle-, and late-season navels did differ in susceptibility to rind injury. Prolonged anaerobic conditions caused pitting and brown spots which developed on the surface of the rind a few hr or days after the fruit were returned to air. The rind was not injured on oranges held 20 hr or less in N_2 at 20° C but was severely injured on all fruits held 20 hr at 38° in N_2. The rind was injured on all oranges held 3 or more days in N_2 at 20°. The most severe rind injury appeared on the middle-season oranges and the least on the late-season oranges. The middle-season navels were also slightly injured when held 20 hr at 38° in air.

This rind injury had no apparent effect on the measured volatiles emitted from the oranges. Citrus oil constituents such as limonene and other terpenes released by rind infury were not eluted from Chromosorb 101 at the column temperature used for measurements.

Ethanol production. Pre-treatment rates of ethanol production by intact oranges held in air at 20° C ranged from .01 to .12 µg/100 g/hr. Rates of ethanol production by oranges held in N_2 at 20° for 2 and 20 hr and for 3, 4, and 5 days with subsequent transfer to air are shown in Fig. 1. Oranges exhibited a progressive increase in ethanol production when held in N_2, reaching nearly 450 µg/100 g/hr after 5 days. The rate of

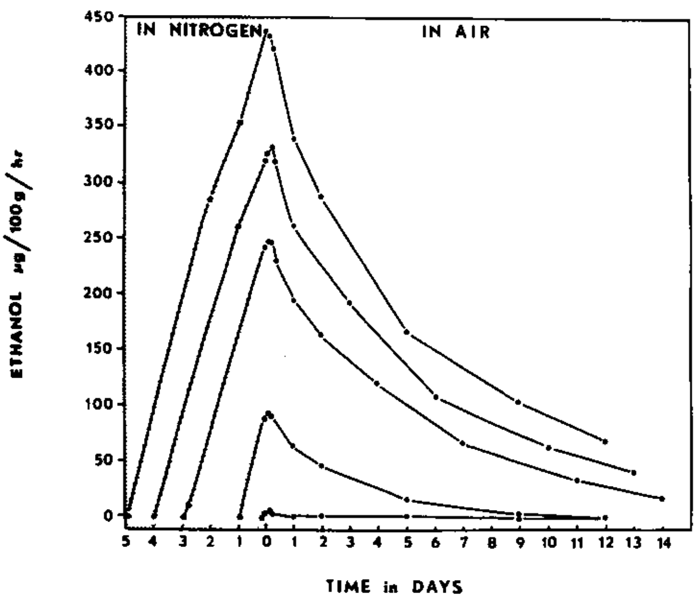

Fig. 1. Ethanol production by intact navel oranges held in N_2 at 20° C for 2 and 20 hr and for 3, 4, and 5 days with subsequent transfer to air. All values are averages of 3 replications of 3 individual fruits.

ethanol production began to decrease 4 to 6 hr after the fruit were transferred to air and continued to decrease for the remainder of the test. Five days in air were required for oranges held for 2 hr in N_2 to return to near the pre-treatment rate of ethanol production. Oranges held for 20 hr in N_2 still produced 25 times and those held for 3 to 5 days in N_2 still produced 300 to 700 times the pre-treatment rate of ethanol after 12 to 14 days in air.

Rates of ethanol production by intact oranges held in air or N_2 for 20 hr at 38°C with subsequent transfer to air at 20° are given in Table 1. The [illegible] of ethanol production increased in N_2 and decreased when the fruits were returned to air. The rate of ethanol production was still 10 times that produced by the air control at the end of the test.

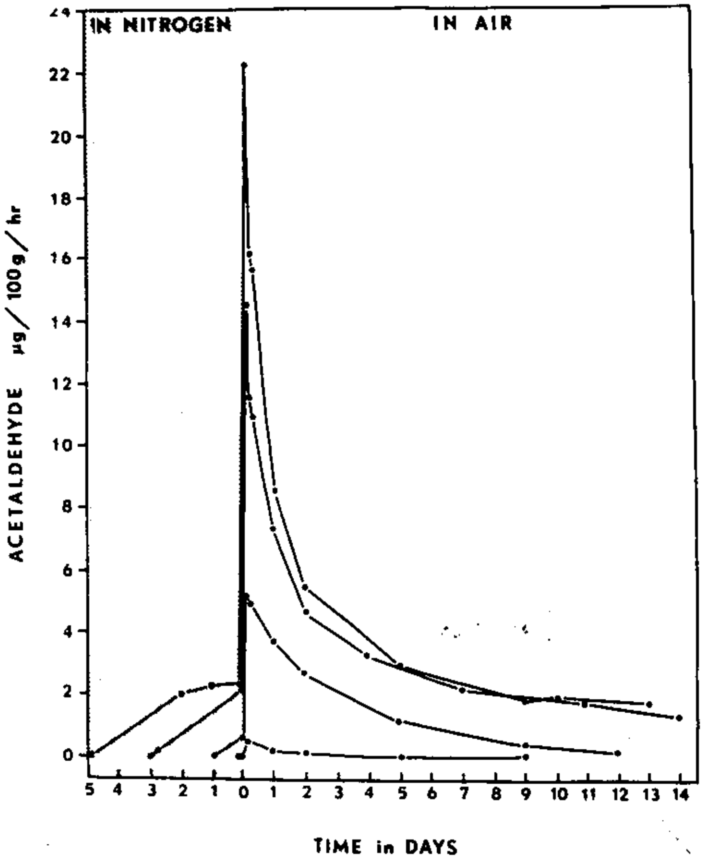

Fig. 2. Acetaldehyde production by intact navel oranges held in N_2 at 20° C for 2 and 20 hr and for 3 and 5 days with subsequent transfer to air. All values are averages of 3 replications of 3 individual fruits.

Acetaldehyde production. Pre-treatment rates of acetaldehyde production by intact oranges held in air at 20° C ranged from .04 to .08 µg/100 g/hr. Rates of acetaldehyde production by oranges held in N_2 at 20° for 2 and 20 hr and for 3 to 5 days with subsequent transfer to air are shown in Fig. 2. Oranges in N_2 showed only a slight increase in acetaldehyde production. The rate of acetaldehyde production increased 5- to 10-fold when the oranges were transferred from N_2 to air (Fig. 2). Following this peak production rate in air, acetaldehyde production declined rapidly. Four days in air were required for oranges held 2 hr in N_2 to return to the pre-treatment rate of acetaldehyde production. Oranges held for 20 hr in N_2 and then in air still produced more then twice and those held 3 to 5 days in N_2 and then in air still produced more than twice and those held 3 to 5 days in N_2 and then in air still produced some 30 times the pre-treatment rate of acetaldehyde production after 12 to 14 days in air.

The rates of acetaldehyde production by oranges held in air or N_2 at 38° C with subsequent transfer to air at 20° are given in Table 1. The rate of acetaldehyde production increased in N_2 and then increased markedly with subsequent gradual decline when the oranges were transferred to air. The rate of acetaldehyde production was 7 times that produced by air control at the end of the test.

Methanol and ethyl acetate production. Pre-treatment rates of methanol and ethyl acetate production by intact oranges held in air at 20° C ranged from .01 to .05 µg/100 g/hr. Rates of methanol and ethyl acetate production by oranges held in N_2 at 20° for 2 and 20 hr and for 3, 4, and 5 days with subsequent transfer to air are shown in Fig. 3. Oranges in N_2 showed a progressive increase in methanol production and a slight increase in ethyl acetate production. When oranges were transferred to air, methanol production decreased somewhat and then increased slightly and ethyl acetate production increased reaching a maximum 1 to 2 days after transfer to air and then declined. Oranges held 2 hr in N_2 and then in air returned to the pre-treatment rate of methanol production after 1 day in air and to the pre-treatment rate of ethyl acetate production after 9 days. Oranges held for 3 to 5 days in N_2 still produced some 20 times the pre-treatment rate of methanol production and 26 to 78 times the pre-treatment rate of ethyl acetate production after 12 to 14 days in air.

Rates of methanol and ethyl acetate production by intact oranges held in air or N_2 for 20 hr at 38° C with subsequent

Table 1. Rates of production of ethanol, acetaldehyde, methanol, and ethyl acetate by intact navel oranges held in air or N_2 20 hr at 38° C with subsequent transfer to air at 20°.

Component	Treatment 20 hr at 38° in air	in N_2	Time in air at 20° after treatment 2 hr	4 hr	1 day	5 days	9 days
Ethanol	2.4[a]		—	—	5.6	9.3	10.1
		714	319	276	243	156	107
Acetaldehyde	2.6		—	—	0.8	0.7	0.7
		27.9	38.0	27.1	12.9	5.8	5.0
Methanol	0.3		—	—	0.2	0.3	0.4
		23.5	14.8	13.5	13.6	5.7	6.9
Ethyl acetate	0.08		—	—	0.01	0.03	0.06
		0.34	0.38	0.18	0.52	0.77	0.31

[a]µg/100 g fresh weight/hr. All values are averages of 3 replications of 3 individual fruits.

transfer to air at 20° are given in Table 1. The rate of methanol production increased in N_2 and decreased somewhat when the oranges were returned to air. The rate of ethyl acetate production increased somewhat in N_2, dropped slightly when the oranges were transferred to air at 20°, and then increased to a maximum 5 days after transfer to air. The rate of methanol production was still 17 times and the rate of ethyl acetate production was still 5 times that produced by the air control at the end of the test.

Discussion

Only traces of ethanol, acetaldehyde, methanol, and ethyl acetate were emitted from oranges hed in air at 20° C. Volatile production by oranges increased 7- to 65-fold when the temperature was raised to 38°. Ethanol and acetaldehyde were the predominant volatiles emitted at both temperatures.

When oranges were held in N_2 at 20° C the volatile patterns and rates of production changed considerably. Ethanol was the dominant volatile emanating from oranges in N_2 and production increased 30-fold during 2 hr in N_2 up to several thousand-fold during 5 days in N_2. These oranges, when exposed to N_2 for 2 hr to 5 days, produced from 50 to more than 150 times as much ethanol as acetaldehyde (compare Figs. 1 and 2). Fidler (5) stated that in anaerobiosis the production of ethanol by plant tissue is accompanied by an increase in the concentration of aldehyde and that the ratio of ethanol to aldehyde content is about 100:1. Acetaldehyde production by oranges increased 10- to 50-fold during N_2 storage and after the oranges were returned to air production increased several hundred fold over the initial rate. Biale and Shepherd (2) found that oranges and lemons emitted only small amounts of acetaldehyde, even after 3 weeks' exposure to anaerobic conditions, but when transferred to air acetaldehyde production increased markedly.

Production of methanol by oranges increased during N_2 exposure at 20° C, but at a slower rate than of ethanol. Methanol production increased about 75-fold during 5 days in N_2. Production of ethanol was about 600 times that of methanol after 5 days in N_2 (compare Figs. 1 and 3).

Production of ethyl acetate (tentative) paralleled that of

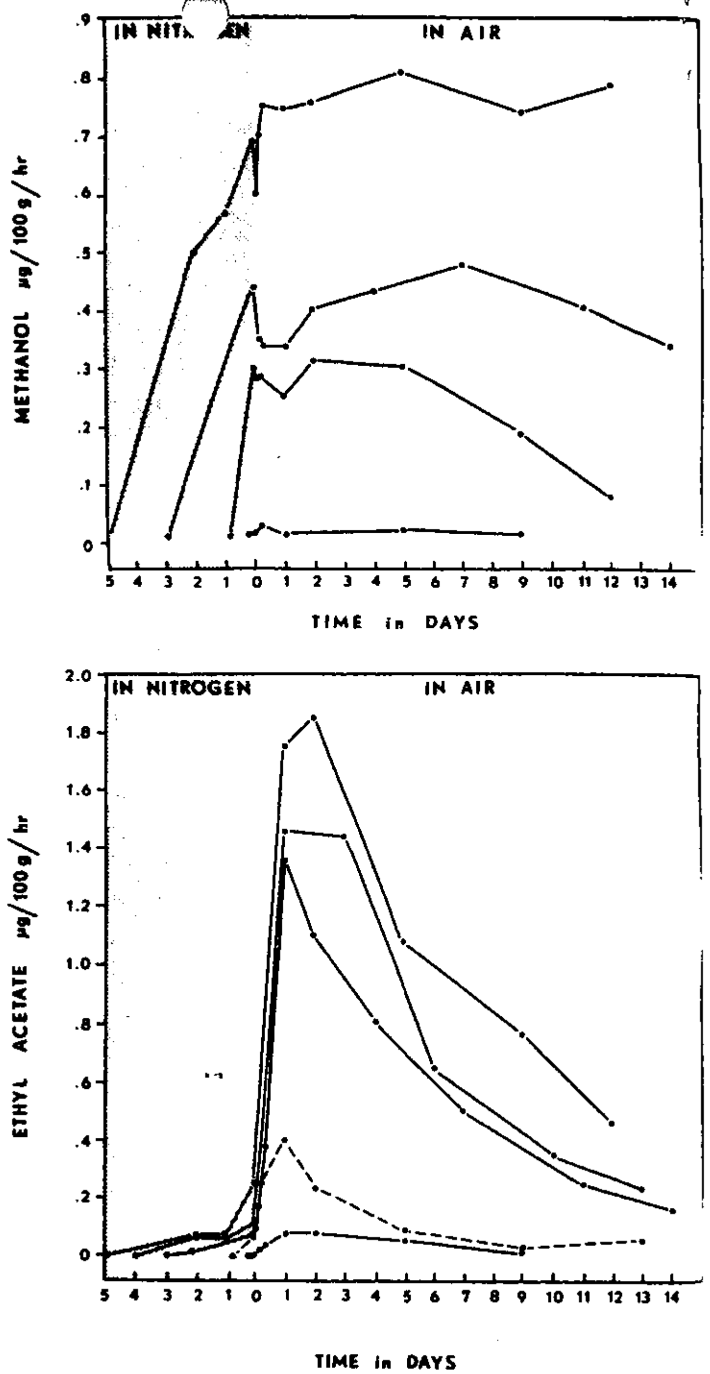

Fig. 3. Methanol (above) and ethyl acetate (below) production by intact navel oranges held in N_2 at 20° C for 2 and 20 hr and for 3 and 5 days with subsequent transfer to air. All values are averages of 3 replications of 3 individual fruits.

acetaldehyde but peaked a day or so later when the fruit were returned to air. The quantity of ethyl acetate produced was about 1/10 the amount of acetaldehyde produced (compare Figs. 2 and 3). Additional studies are required to rigorously identify this volatile as ethyl acetate.

Navel oranges held 20 hr in N_2 at 38° C emitted nearly 300 times as much ethanol, 75 times as much methanol, 10 times as much acetaldehyde, and 4 times as much ethyl acetate as the air control. Severe rind injury resulted on all the oranges when the fruit were exposed to N_2 20 hr at 38° and some rind injury developed on the air control with the middle-season navels. These results indicate that the exposure of navel oranges to anaerobic conditions at 38° is physiologically detrimental to the

Table 1. Rates of production of ethanol, acetaldehyde, methanol, and ethyl acetate by intact navel oranges held in air or N_2 20 hr at 38° C with subsequent transfer to air at 20°.

	Treatment 20 hr at 38°		Time in air at 20° after treatment				
Component	in air	in N_2	2 hr	4 hr	1 day	5 days	9 days
Ethanol	2.4[a]		---	---	5.6	9.3	10.1
		714	319	276	243	156	107
Acetaldehyde	2.6		---	---	0.8	0.7	0.7
		27.9	38.0	27.1	12.9	5.8	5.0
Methanol	0.3		---	---	0.2	0.3	0.4
		23.5	14.8	13.5	13.6	5.7	6.9
Ethyl acetate	0.08		---	---	0.01	0.03	0.06
		0.34	0.38	0.18	0.52	0.77	0.31

[a] μg/100 g fresh weight/hr. All values are averages of 3 replications of 3 individual fruits.

fruit. None of the oranges returned to the pre-treatment rate of volatile production when transferred to air at 20°. Bruemmer and Roe (3) suggest treating citrus fruits with N_2 or CO_2 at 32° to 43° for 20 hr to reduce juice acidity for product improvement before processing. Compared with an air control, Bruemmer and Roe reported a 10-fold increase in ethanol content of 'Valencia' orange juice after 20 hr in N_2 at 32°. Perhaps navel oranges are more sensitive to this type of treatment thatn 'Valencia' oranges.

Emanation of endogenous volatiles by citrus fruits is a complex process. Observations that ethanol, acetaldehyde, methanol, and ethyl acetate production rates differ in air and N_2 should be important in understanding the respiratory metabolism of citrus fruits. Bruemmer and Roe (3) suggest that increased ethanol production in anaerobiosis indicates that pyruvate is diverted from acetyl coenzyme A and citrate synthesis to acetaldehyde and reduction to ethanol. Certain metabolic systems that are O_2 dependent must be responsible for the large increase in acetaldehyde and ethyl acetate production which occurs after the oranges are transferred from N_2 to air. Until more information is available, it is difficult to draw conslusions as to the significance of these emanations.

With regard to storage of citrus fruits in controlled atmospheres, the primary interest is the relation of anaerobic production of endogenous volatiles to the physiological condition of the fruit. The present studies indicate that anaerobiosis is deterimental since all the fruits held longer than 20 hr in N_2 at 20° C exhibited rind injury and the production of volatiles did not return to the pre-treatment rate, even after several days in air. Measurement of endogenous volatiles emanating from the fruit during storage may be of considerable value in evaluating proper storage atmospheres and conditions.

Literature Cited

1. Attaway, J. A., and M. F. Oberbacher. 1968. Studies on the aroma of intact Hamlin oranges. *J. Food Sci.* 33:287-289.
2. Biale, J. B., and A. D. Shepherd. 1939. Identification of acetaldehyde among the volatile products of citrus fruits. *Proc. Amer. Soc. Hort. Sci.* 37:543-547.
3. Bruemmer, J. H., and B. Roe. 1969. Post-harvest treatment of citrus fruit to increase brix acid ratio. *Proc. Fla. State Hort. Soc.* 82:212-215.
4. Craft, C. C., G. Duncan, and D. Fouse. 1968. Respiratory activity of small lemons and lemon tissue as influence by modified atmospheres. *Proc. Amer. Soc. Hort. Sci.* 93:173-185.
5. Fidler, J. C. 1968. The metabolism of acetaldehyde by plant tissue. *J. Expt. Bot.* 58:41-51.
6. Grierson, W., H. M. Vines, M. F. Oberbacher, S. V. Ting, and G. J. Edwards. 1966. Controlled atmosphere storage of Florida and California lemons. *Proc. Amer. Soc. Hort. Sci.* 88:311-318.
7. Hoff, J. E., and E. D. Feit. 1964. New technique for functional group analysis in gas chromatography. *Anal. Chem.* 36:1002-1008.
8. Ikeda, R. M., D. E. Simmons, and J. D. Grossman. 1964. Removal of alcohols from complex mixtures during gas chromatography. *Anal. Chem.* 36:2188-2189.
9. Norman, S. 1970. Comparison of two porous polymer columns for gas chromatographic analysis of acetaldehyde, methanol, ethanol, and other volatiles emanating from intact 'Valencia' oranges. *J. Amer. Soc. Hort. Sci.* 95:777-780.
10. ———, C. C. Craft, and P. L. Davis. 1968. Volatiles from injured and uninjured Valencia oranges at different temperaturs. *J. Food Sci.* 32:656-659.
11. Vines, H. M., G. J. Edwards, and W. Grierson. 1965. Citrus fruit respiration. *Proc. Fla. State Hort. Soc.* 78:198-202.