should be put into force by the Inspection Service and the recovery tolerance should be eliminated with the processor allowed to recover whatever amount of juice is good and usable under strict inspection supervision. Juice once labeled substandard should not be permitted to be ever reintroduced into a retail product, but should *be forced* into beverage base products.

6. Increased automation and objective recording of data in test houses should be carried forward as rapidly as practically possible.

FURTHER STUDIES OF ETHANOL AND ACETALDEHYDE IN JUICE OF CITRUS FRUITS DURING THE GROWING SEASON AND DURING STORAGE

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Abstract

Further studies of ethanol content of citrus juice during the season, as an additional measure of maturity, showed marked varietal differences. As the season progressed, ethanol concentration in juice increased in all varieties, but at maturity Robinson tangerines and Temple oranges had lowest ethanol contents, Marsh grapefruit intermediate, and Valencia and Pineapple oranges had highest ethanol contents.

During storage in controlled atmospheres with carbon dioxide (CO_2) concentrations ranging from 10% to 30%, the ethanol content of juice of Marsh grapefruit increased with CO_2 concentration, with length of time of exposure to CO_2, and with total time of storage. Acetaldehyde content of juice, although much lower than ethanol content, increased in a similar manner.

Introduction

The ethanol and acetaldehyde contents of juice of Hamlin and Valencia oranges increase during the growing season, affording a measure of maturity in addition to the solids-acid ratio (4). These studies were extended to include other kinds of citrus fruits—Robinson tangerines, Temple and Pineapple oranges, and Marsh grapefruit.

Citrus fruits do not ripen or improve in flavor after harvest (1) and should, therefore, be stored under conditions that preserve the characteristics of freshly harvested fruit. The effects of certain storage conditions are reflected in changes in ethanol concentration in juice (4), so the determination of this component may provide a means of assessing the metabolic activity of the fruits during storage.

In a recent review by Wilkinson (8), several instances of CO_2 injury to stored products, including apples, were cited. Other fruits such as blackberries, cherries, and strawberries are apparently not as susceptible as apples to CO_2 injury, and the beneficial use of CO_2 in the transport and storage of cherries, plums, and the soft fruits has been reviewed by Smith (6). Ethanol and acetaldehyde accumulate in apple tissue damaged by CO_2, and Thomas (7) attributed the damage largely to acetaldehyde, although Smith (6) considered its presence only symptomatic of a metabolic imbalance. Brooks and McColloch (2) found that short-term exposure of grapefruit to CO_2 atmospheres avoided some of the physiological disorders associated with chilling injury.

Materials and Methods

For the study of seasonal changes, Temple and Pineapple oranges and Robinson tangerines were harvested at weekly intervals from groves in the Central Florida area; biweekly harvests were made of Valencia oranges and Marsh grapefruit from the Indian River area near Wabasso, Florida. Harvests were begun before the usual commercial shipping period for all fruits except Valencia oranges. Data for this variety were previously reported (4); the present study included a period extending 2 months beyond April, the midpoint of normal commercial shipping (1). All fruits were from commercial groves. Samples of the composite juice of 10 fruits were analyzed within 1 day of harvest for ethanol, acetaldehyde, solids, acid, and pH.

Table 1. Monthly averages of ethanol, acetaldehyde, solids, acid, and pH.*

Variety and Month**	Ethanol	Acetaldehyde	Solids	Acid	Solids/acid	pH
	(mg/100 ml)	(mg/100 ml)	%	%		
Robinson tangerine						
September‡	0.2	---	10.4	1.35	7.7	---
October	1.9	---	11.0	0.91	12.3	---
Temple orange						
October	0.5	0.10	9.9	2.50	3.9	2.85
November	1.7	0.10	10.6	2.15	4.9	2.86
December	3.9	0.18	11.8	1.78	6.6	2.96
January‡	6.7	0.17	12.1	1.50	8.1	3.06
February	12.5	0.24	13.0	1.44	9.0	3.12
March	12.0	0.23	14.0	1.17	11.9	3.17
April	12.1	0.23	14.3	1.14	12.5	3.36
Marsh grapefruit						
October‡	1.1	0.12	11.7	1.61	7.3	3.17
November	4.7	0.12	11.5	1.43	8.0	3.06
December	12.0	0.16	12.5	1.57	8.0	3.02
January	15.5	0.21	12.4	1.51	8.2	3.03
February	15.6	0.19	12.4	1.44	8.6	3.07
March	15.4	0.17	12.9	1.31	9.8	3.12
April	22.8	0.24	12.2	1.11	11.0	3.19
May	25.5	0.27	11.9	1.04	11.4	3.27
Pineapple orange						
October	4.7	0.10	10.3	1.66	6.2	3.22
November	15.7	0.20	10.4	1.47	7.1	3.26
December	29.8	0.33	11.9	1.40	8.5	3.34
January‡	45.3	0.63	13.1	1.28	10.2	3.46
February	68.1	0.63	13.4	1.12	12.0	3.68
Valencia orange						
March	68.9	0.42	12.9	1.62	8.0	3.10
April‡	69.9	0.44	13.4	1.38	9.7	3.31
May	64.0	0.58	13.5	1.24	10.9	3.41
June	66.7	0.53	13.8	1.17	11.8	3.53

*Figures represent averages of weekly or biweekly samplings of 10 fruit each.

**Approximate commercial shipping periods: Robinson tangerines, October; Temple oranges, December-March; Marsh grapefruit, November-May; Pineapple oranges, December-March; Valencia oranges, February-June.

‡Legally mature.

Table 2. Ethanol and acetaldehyde contents of juice of Marsh grapefruit, stored under CO_2, then in air, at 4.4° C.*

Date stored	Days in CO_2	Percent CO_2	1 month		2 months		3 months	
			Ethanol (mg/100 ml)	Acetaldehyde (mg/100 ml)	Ethanol (mg/100 ml)	Acetaldehyde (mg/100 ml)	Ethanol (mg/100 ml)	Acetaldehyde (mg/100 ml)
2/18/71	Initial:	Ethanol 20, Acetaldehyde 0.18 mg/100 ml						
	0	---	41	0.38	115	0.86	195	1.19
	1	10	37	0.27	95	0.61	164	0.99
		20	25	0.25	108	0.80	195	1.19
		30	34	0.34	76	0.55	172	1.19
	7	10	41	0.38	106	0.62	159	1.19
		20	87	0.57	172	0.73	244	1.39
		30	134	0.70	282	1.07	292	1.48
	14	10	74	0.50	167	0.78	270	1.39
		20	86	0.60	221	0.78	249	1.35
		30	175	1.20	289	1.78	248	1.48
4/21/71	Initial:	Ethanol 36, Acetaldehyde 0.23 mg/100 ml						
	0	---	58	0.35	65	0.48	76	0.33
	14	15	186	0.60	170	0.65	159	0.66
		20	178	1.77	231	1.33	231	0.99
		25	293	1.20	331	1.78	285	1.35
	21	15	203	0.81	161	0.85	214	0.96
		20	217	1.07	144	1.10	297	1.21
		25	260	1.57	309	1.19	348	1.35
	28	15	230	2.20	252	1.33	238	1.21
		20	190	2.61	292	2.07	331	1.71
		25	314	5.68	257	2.60	390	11.19**

*Figures represent composite juice of 5-fruit samples from 30-fruit lots.

**Severe rind damage.

In storage studies, Marsh grapefruit harvested February 18, 1971, were subjected to 10%, 20%, and 30% CO_2 for 1, 7 and 14 days at 4.4°C. Fruits from each CO_2 level and duration were then held in air for a total storage period of 1, 2, and 3 months at this same temperature. In a second test at 4.4°C, grapefruit harvested April 21 were subjected to 15%, 20%, and 25% CO_2 for 14, 21, and 28 days and then held in air at 4.4° for a total storage period of 1, 2, and 3 months.

For storage studies at 1.1°C, grapefruit harvested March 31, 1971, were exposed for 7 and 14 days to 10% and 30% CO_2 and then held in air at 1.1° for a total storage period of 1 and 2 months followed by a 2-week holding period at 21.1°.

For exposure to CO_2, fruits were placed in gas-tight containers designed for controlled-atmosphere studies (3). After an initial flushing with CO_2 to establish the desired concentration, the atmospheres were monitored continuously, and either air, oxygen (O_2), or CO_2 was added to maintain proper CO_2 levels. No attempt was made to control the O_2 concentrations, but these were fairly steady, ranging at 4.4°C from 17%-18.5% at 10% CO_2 to 11%-13% at 30% CO_2 levels. At 1.1°, the O_2 levels were 1%-2% lower at corresponding CO_2 levels.

All fruits were treated with 1,000 ppm of the fungicide 2-(4-thiazolyl) benzimidazole (TBZ) for decay control and waxed with commercial solvent-type wax before storage. Analyses were made on the composite juice of 5-fruit samples from 30-fruit lots before and after storage. Ethanol and acetaldehyde in juice were determined by gas chromatographing volatiles in headspace over the juice (5).

Table 3. Ethanol and acetaldehyde contents of juice of Marsh grapefruit, harvested March 31, 1971, stored under CO_2, then in air at 1.1° C.*

Storage conditions	On removal from 1.1° C		After 2 weeks at 21.1° C	
	Ethanol	Acet-aldehyde	Ethanol	Acet-aldehyde
	(mg/100 ml)	(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
Air storage				
1 week	36	0.24	---	---
4 weeks	49	0.30	44	0.46
8 weeks	71	0.37	91	0.75
10% CO_2				
1 week	34	0.16	---	---
1 week + 3 weeks in air	52	0.29	90	0.64
1 week + 7 weeks in air	114	0.54	144	0.88
2 weeks	76	0.29	---	---
2 weeks + 2 weeks in air	71	0.57	118	0.73
2 weeks + 6 weeks in air	142	0.97	126	0.83
30% CO_2				
1 week	94	0.56	---	---
1 week + 3 weeks in air	184	1.60	178	0.96
1 week + 7 weeks in air	223	1.60	161	1.13
2 weeks	235	1.52	---	---
2 weeks + 2 weeks in air	277	3.92	296	1.23
2 weeks + 6 weeks in air	314	5.11	---	---

*Figures represent composite juice of 5-fruit samples from 30-fruit lots.

Results and Discussion

Seasonal Changes

Marked differences in ethanol content of juice were noted among the kinds of citrus fruits tested (Table 1). Robinson tangerines, for example, averaged less than 2 mg/100 ml for the month of October, although the solids-acid ratio was 12.3. Temple oranges averaged about 12 mg/100 ml during the February-April period. Fruit of this variety is normally harvested in January and February. Marsh grapefruit was intermediate, reaching a high of 25 mg/100 ml in May. Pineapple and Valencia oranges were highest in ethanol content, reaching nearly 70 mg/100 ml. The Valencia oranges were harvested until well after the normal picking times in order to study the ethanol content during this period, and there was no perceptible change during the March-June period. The values previously reported for Valencia oranges from the Central Florida area (4) were lower than those for fruit of the same variety from the Indian River area in present tests; this difference is probably due to grove locations, or

Table 4. Chemical analyses of grapefruit stored February 18, 1971, at 4.4° C in air or under 30% CO_2 for 14 days.*

Storage conditions	Solids	Acid	Solids/acid	pH
	%	%		
Initial	12.3	1.6	7.7	2.9
1 month, total				
Air check	12.1	1.8	6.8	2.9
Under CO_2	12.1	1.3	9.4	3.0
2 months, total				
Air check	12.5	1.6	8.0	3.0
Under CO_2	11.6	1.3	8.9	3.1
3 months, total				
Air check	12.0	1.5	8.0	3.0
Under CO_2	11.6	1.2	9.5	3.2

*Figures represent composite juice from 5-fruit samples from 30-fruit lots.

possibly a difference due to year. Acetaldehyde content of juice also increased during the season with Pineapple and Valencia oranges having highest values.

Effects of Exposure to CO_2

Exposure of grapefruit for 1 day to CO_2, even up to 30%, had no apparent effect on either ethanol or acetaldehyde content of juice (Table 2). This fruit was harvested in February, treated and stored at 4.4°C. Both compounds increased during the 3-month storage at about the same rate for all exposures. Exposure of the fruit to 20% and 30% CO_2 for 7 days increased ethanol. After 1 month, for example, ethanol in fruits from 20% CO_2 was 2-fold that in air-check fruit or in fruits from 10% CO_2; and in fruits from 30% CO_2 ethanol increased about 3-fold. Acetaldehyde content of juice increased also with CO_2 concentrations but to a lesser extent. The 14-day exposure to only 10% CO_2 increased ethanol.

The patterns of increase in ethanol and acetaldehyde were similar in fruits stored in April at 4.4°C. Ethanol in the air-check fruit reached 76 mg/100 ml after 3 months, while that in CO_2-treated fruit ranged from 159 mg to 390 mg/100 ml. Fruits subjected to 25% CO_2 for 28 days and stored for a total of 3 months had 11 mg/100 ml acetaldehyde and showed severe rind damage.

In grapefruit harvested March 31, subjected to CO_2, and stored at 1.1°C, both ethanol and acetaldehyde concentrations were higher in fruit receiving 30% CO_2 than in fruit receiving 10% CO_2 exposure (Table 3). After 1 month, for example, fruit receiving 10% CO_2 for 1 and 2 weeks had 52 mg and 71 mg/100 ml ethanol in juice, while fruit receiving 30% CO_2 for 1 and 2 weeks had 184 mg and 277 mg/100 ml, respectively. Changes during the 2 weeks at 21.1° were not consistent. Ethanol and acetaldehyde concentrations increased with length of storage, the highest being 314 mg and 5.11 mg/100 ml, respectively, in fruits treated with 30% CO_2 for 2 weeks and held in air for a total storage period of 2 months.

Although much remains to be done to relate storage conditions to resulting fruit quality, it is apparent that composition of atmospheres, length of subjection to controlled atmospheres, and total length of storage period contribute to the physiological state of stored fruits as indicated by changes in ethanol and acetaldehyde. Beneficial effects of atmospheres, such as reduction in rind damage at low temperatures, must be weighed against possible internal damage.

All samples were also analyzed for total solids, total acid, and pH. Table 4 shows the changes in these constituents in fruits which were held for 14 days under 30% CO_2 and then stored for a total of 3 months at 4.4° C. There was a tendency toward decrease in total acid and increase in pH, but this trend was not entirely consistent in fruits subjected to lower CO_2 atmospheres. Thus, as far as ascertainment of physiological changes occurring in citrus fruits during storage, the measurement of ethanol and acetaldehyde may be a far more sensitive guide than measurements of solids, acid, or pH.

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