Because the decline in malate and citrate ion concentrations compares so well with the decrease in acidity of the juice, the terminal product is probably a neutral compound.

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RELATION OF ETHANOL CONTENT OF CITRUS FRUITS TO MATURITY AND TO STORAGE CONDITIONS

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ABSTRACT

Ethanol content of juice of citrus fruits increased during the growing season. The range and degree of increase were greater than changes in solids-acid ratio. Ethanol content affords an additional measure of maturity. Ethanol content of juice of stored fruit increased with decreasing oxygen concentration in controlled-atmosphere storage. Waxed fruit consistently had higher ethanol content in juice than nonwaxed fruit. Acetaldehyde in juice also increased during the growing season and during storage but to a minor degree. Both ethanol and acetaldehyde increased during a 1-week holding period in air at 21°C following storage at lower temperatures.

INTRODUCTION

Ethanol concentration in juice of fresh oranges and grapefruit grown in California has been reported by Kirchner *et al.* (10, 11) to be about 40 mg/100 ml. Walford *et al.* (12) observed some indication of seasonal changes in ethanol content of juice, but interference of solvent used in extraction made quantitative evaluation difficult.

From results of controlled-atmosphere (CA) storage experiments, Chace *et al.* (5) suggested that off-flavors may be related to ethanol production by the fruits. Metabolic processes depending on respiratory oxygen (O_2) and involving alcohols and aldehydes are considered by Bruemmer *et al.* (2, 3) to be related to flavor development. Craft *et al.* (7) have cautioned that physiological effects of atmospheres should be considered before recommendations are made for CA storage.

In order to obtain more information on the ethanol content of juice, studies were made of seasonal changes and changes during storage attributable to atmospheres and to waxing.

MATERIALS AND METHODS

Tests were conducted with Hamlin oranges grown on sour orange and Cleopatra mandarin rootstocks, Pineapple and Valencia oranges and Marsh grapefruit on rough lemon rootstocks, Temple oranges on Cleopatra mandarin, and Ruby Red grapefruit on sour orange.

For a study of seasonal changes in ethanol content, fruits were harvested from the same trees at weekly intervals. The composite juice of 10-fruit samples was analyzed within 1 day of harvest. Hamlin oranges were harvested from September through December and Valencia oranges from October through April.

In CA storage studies, Pineapple oranges and Marsh grapefruit were stored for 6 weeks at 4.4°C. Temple oranges were stored for 10 weeks at 4.4°, Valencia oranges for 12 weeks at 1.1°, and Ruby Red grapefruit for 12 weeks

at 7.2°. Comparable lots of waxed and non-waxed fruits were used. Fruits were maintained in gas-tight containers, and the atmospheres of O_2 down to 2.5% and CO_2 up to 10% were monitored continuously (4, 5).

The ethanol content of juice was determined by gas chromatographing volatiles in headspace over the juice (8). Immediately after juicing, samples were placed in a water bath maintained at 35°C and analyzed after 1 hour. Ethanol standards were maintained in the same bath. A Micro-Tek[1] GC-2000R dual flame gas chromatograph was used with the following operating conditions: column, ¼" x 9' carbowax 20 M on Gas Chrom Q, oven 100°, inlet 135°, detector 200°, and N_2 carrier gas at 80 ml/min. Calculations of concentration of ethanol were made considering 95% ethanol standards to be 92.3% by weight with a density of 0.810 at 25°.

[1]Manufacturer's name is given for identification and is not intended to be an endorsement by the U. S. Department of Agriculture of this product over any other.

Acetaldehyde determinations were made concurrently with the gas chromatographic ethanol analyses.

Results and Discussion

Seasonal Changes.—Ethanol in juice from both Hamlin and Valencia oranges increased during the season. At the beginning of October the ethanol content of juice was less than 1 mg/100 ml for both varieties. By December, the midpoint of normal commercial shipping for Hamlin oranges (1), those grown on sour orange rootstock had nearly 50 mg/100 ml, and those grown on Cleopatra mandarin rootstock had more than 30 mg/100 ml. Valencia oranges reached 50-60 mg/100 ml by April, the midpoint of the normal commercial shipping season for this variety (1). Results by monthly averages are summarized in Table 1. To illustrate the more rapid change in ethanol than in usual measurements of maturity, the results of weekly

Table 1. Monthly averages of ethanol, solids, acid, and pH of juice of Hamlin and Valencia oranges[1/]

Variety	Season	Month	Ethanol (mg/100 ml)	Solids (%)	Acid (%)	Solids/acid	pH
Hamlin on Cleopatra mandarin rootstock	1968-69	October	4.0	9.3	1.05	8.9	---
Do	1968-69	November	20.0	9.5	0.89	10.6	---
Do	1968-69	December	25.4	10.4	0.87	12.0	---
Do	1969-70	September	0.2	8.9	1.46	6.3	3.044
Do	1969-70	October	1.7	9.4	1.04	9.1	3.243
Do	1969-70	November	9.3	10.1	0.94	10.7	3.371
Do	1969-70	December	24.5	10.8	0.91	12.0	3.467
Hamlin on sour orange rootstock	1969-70	September	0.2	9.2	1.53	6.1	3.057
Do	1969-70	October	0.8	9.4	1.21	7.9	3.043
Do	1969-70	November	9.1	10.0	0.97	10.3	3.333
Do	1969-70	December	38.1	10.6	0.94	11.2	3.417
Valencia on rough lemon rootstock	1968-69	October	0.5	8.5	2.83	3.0	---
Do	1968-69	November	4.7	9.1	2.28	4.0	---
Do	1968-69	December	9.5	10.6	1.88	5.8	---
Do	1968-69	January	28.9	11.3	1.67	6.7	---
Do	1968-69	February	40.0	11.7	1.46	8.0	---
Do	1968-69	March	45.7	12.4	1.29	9.7	---
Do	1968-69	April	48.0	11.8	1.04	11.5	---

1/ Figures represent an average of four weekly samplings of 10 fruits each.

pickings of Hamlin oranges grown on sour orange rootstock are plotted in Figure 1. Measurements of weekly pickings of Valencia oranges grown on rough lemon and Hamlin oranges on Cleopatra mandarin rootstocks showed essentially the same pattern as that of Hamlin on sour orange in Figure 1.

Although the legal standards for maturity of citrus fruits have been based largely on solids-to-acid ratio, a high ratio does not necessarily mean juice of superior quality nor a low ratio imply juice of inferior quality (9). A measure of ethanol content of juice might afford an additional criterion of quality. The rapid increase in ethanol concentration during the season provides a sensitive indication of maturity. Juice of Hamlin oranges grown on Cleopatra mandarin rootstock, for example, increased in ethanol content from 4 to 25 mg/100 ml during the October-December period, whereas the solids-to-acid ratio increased from 8.9 to 12.0. The establishment of reliable standards of maturity is a lengthy process, but these results indicate that further investigations of ethanol content are warranted.

Acetaldehyde, one of the precursors of ethanol, also increased during the season. In juice of Hamlin oranges grown on sour orange rootstock, for example, the concentration of acetaldehyde increased from 0.07 mg/100 ml in October to 0.3 mg/100 ml in December (data not shown). This low concentration and narrow range, however, seem to preclude this component as a useful indicator of maturity.

Effects of Storage Atmospheres and Waxing. —Ethanol content of juice of stored citrus fruits was affected by atmospheres and by waxing. Both Pineapple oranges and Marsh grapefruit stored for 6 weeks had lowest ethanol content in juice of fruits held at 21% O_2 - 0% CO_2 (Table 2). The ethanol content of juice increased with decreasing O_2 concentration and with increasing CO_2 concentration. Juice of fruit stored at 5% O_2 - 0% CO_2 had the highest ethanol content. Juice of waxed fruit consistently had higher ethanol content than that of nonwaxed fruit. During a 1-week holding period at 21.1°C following storage, ethanol content of juice increased in both waxed and nonwaxed Pineapple

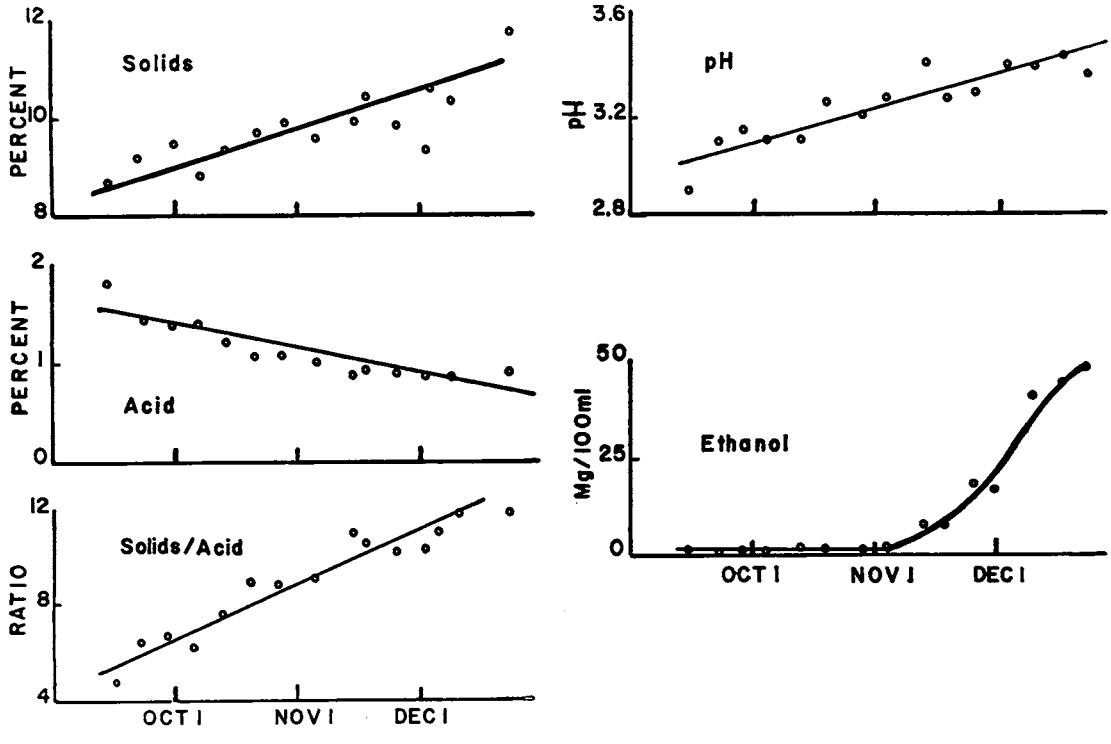

Fig. 1.—Weekly averages of solids, acid, solids/acid ratio, pH, and ethanol content of juice of Hamlin oranges grown on sour orange rootstock, 1969-70. Analyses made on comb ined juice of 10-fruit samples.

Table 2. Ethanol content of juice of Pineapple oranges and Marsh grapefruit after controlled-atmosphere storage for 6 weeks at 4.4° C.

Variety	Atmosphere		Ethanol content of juice[1]				
			Before storage	On removal		After 1 wk in air at 21° C	
	O_2	CO_2		Waxed	Nonwaxed	Waxed	Nonwaxed
	(%)	(%)	(mg/100 ml)	(mg/100 ml)	(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
Pineapple oranges	5	0	80	477	405	740	470
Do	15	0	80	275	255	---	---
Do	21	0	80	233	118	449	253
Do	21	5	80	289	134	---	---
Do	21	10	80	370	233	545	328
Marsh grapefruit	5	0	7	203	167	---	---
Do	15	0	7	99	55	---	---
Do	21	0	7	70	44	---	---
Do	21	5	7	108	70	---	---
Do	21	10	7	124	94	---	---

1/ Figures represent 10-fruit samples from 100-fruit lots.

oranges.

Acetaldehyde content of juice of Pineapple oranges increased only slightly, from 0.35 mg/100 ml before storage to an average of 0.52 mg/100 ml on removal from storage, with no apparent effect of storage atmospheres (data not shown). During 1 week at 21.1°C following storage, the acetaldehyde content of juice increased about three-fold. Acetaldehyde content of juice of grapefruit was lower than that of oranges and also not affected by storage atmospheres.

In longer-term experiments with Valencia and Temple oranges and Ruby Red grapefruit, carbon dioxide content (up to 5%) of the storage atmosphere had no apparent effect on ethanol content of juice, and the results were grouped according to O_2 concentration (Table 3). With each variety, decreasing O_2 concentration of the atmosphere resulted in increasing ethanol in juice, and waxed fruit usually had higher ethanol content than nonwaxed. Lowering the O_2 concentration to 15%, however, had less effect on ethanol buildup in nonwaxed fruit than did lower O_2 levels. This is in accord with the finding that nonwaxed Valencia oranges stored at 15% O_2 - 0% CO_2 had higher flavor ratings than those stored under other O_2 - CO_2 combinations (6). Apparently this concentration of O_2 even for a 3-month period does not induce excessive anaerobic respiration, as indicated by ethanol levels.

The ethanol content of juice is easily determined by gas chromatographic procedures and affords one measure of metabolic activity of citrus fruits in storage. One source of ethanol is by way of reduction of acetaldehyde during anaerobic respiration, and storage conditions which increase ethanol should be studied carefully before recommendations are made. It would seem that the increase of ethanol in juice is a desirable phenomenon related to maturity and that requirements for storage of citrus fruits should be chosen to maintain as nearly as possible their metabolic state at the time of harvest. Studies of the relation of ethanol to atmospheres,

Table 3. Ethanol content of juice of Temple and Valencia oranges and Ruby Red grapefruit after controlled-atmosphere storage

Variety	O_2	Temperature	Length of storage	Ethanol content of juice on removal 1/	
				Waxed	Nonwaxed
	(%)	(° C)	(Weeks)	(mg/100 ml)	(mg/100 ml)
Temple oranges	21	4.4	10	14	15
Do	15		10	64	17
Do	10		10	89	34
Do	5		10	180	152
Valencia oranges	21	1.1	12	91	66
Do	15		12	158	94
Do	10		12	179	147
Ruby Red grapefruit	21	7.2	12	17	16
Do	15		12	63	40
Do	10		12	120	100
Do	2.5		12	306	248

1/ Figures represent average of at least 10 fruit from 2-bushel lots.

length and temperature of storage period, and degree of maturity at time of storage, are currently in progress in this laboratory.

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