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Methanol, Ethanol, and Acetaldehyde Contents of Citrus Products

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The three major citrus volatiles methanol, ethanol, and acetaldehyde were quantitatively determined for various citrus products by gas chromatography. Methanol concentrations varied from 10 to 80 ppm, ethanol from 90 to 900 ppm, and acetaldehyde from 50 to 190 ppm (w/v). Correlations were examined between composition of volatiles and storage history or other quality factors. A positive correlation was observed between methanol content and storage time of canned grapefruit sections and between ethanol content and storage time of non-heat-treated, glass-packed grapefruit sections. Composite data for all single-strength juices (fresh and processed) showed that acetaldehyde concentration was higher and ethanol and methanol concentrations were lower in grapefruit than in orange juice. Similarly, reconstituted commericial concentrates contained less methanol and ethanol and more acetaldehyde than single-strength juices. Similarity between the profiles of volatiles for some concentrates and the profile for single-strength juice suggested that these concentrates contained essence. Volatiles in single-strength juice did not correlate with Brix, acid pulp, or storage history, but a possible relationship between ethanol and the processing date for orange juice was found. Some of these correlations might be useful in quality evaluation.

Methanol Food.

Volatiles are routinely determined when quality and orage abuse of citrus products are evaluated (Lund and namore, 1978). Diacetyl content is related to the con-tion of the fruit and the presence of microorganisms in ocessing equipment. Peel oil content is evaluated on the sis of its limonene content. Furfural in stored juice is lated to heat-induced off-flavors. For determination, all ree of these compounds are recovered by distillation and nalyzed by titration or colorimetry.

In the early work on citrus products (Kirchner et al., 53; Kirchner and Miller, 1957), volatiles were analyzed distillation and derivative formation. These studies tablished that methanol, acetaldehyde, and ethanol edominate in fresh and canned grapefruit and orange ice. Since concentrations varied widely in fresh, freshly ed, and stored canned juices, the authors implied that atile concentration might be related to processing iables and storage treatment.

More recently, a gas chromatographic (GC) headspace ocedure was employed for analysis of ethanol and ac-aldehyde in citrus fruit (Davis and Chace, 1969; Davis, 0, 1971; Davis et al., 1974; Roe and Bruemmer, 1974). anol content was found to increase considerably during growing season and was proposed as a quality indicator addition to the presently used Brix/acid ratio (Davis, 0, 1971). Acetaldehyde also increased, but not as rply. In a related study, Norman and Craft (1971) rmined ethanol, acetaldehyde, and methanol in intact nges and correlated production of these volatiles with nge of fresh fruit in air and nitrogen.

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Other GC techniques have been used to quantitate limonene and other abundant volatiles (Lund and Shaw, 1979). These various studies of citrus volatiles demonstrated that they may be analyzed by a single GC determination.

We therefore undertook to develop an improved procedure for determination of methanol, acetaldehyde, and ethanol in a variety of citrus products. These included raw, fresh juices and freshly processed single-strength (canned and glass-packed) juices, concentrated juices, and freshly processed (canned and glass-packed) grapefruit sections. Storage tests were also carried out on samples of processed single-strength juice and sections.

MATERIALS AND METHODS

General. A Hewlett-Packard Model 7620A gas chromatograph equipped with a flame ionization detector was employed. The carrier gas (helium) flow rate was 37 mL/min, and residual oxygen in the gas was removed with an Oxytrap (Altech Associates, Arlington Heights, IL). Injection port and detector block temperatures were 220 °C. The column temperature was 100 °C. The column consisted of a 1.5 m (5 ft) × 3.1 mm ($^1/_8$ in.) Teflon-lined stainless steel tube packed with 50/80 Porapak Q (Waters Associates, Milford, MA). The ends were plugged with silanized glass wool.

The injection port was modified: a removable glass liner was incorporated for easier cleaning of nonvolatile residues (Figure 1). A Teflon seat was installed at the column end, and two Teflon washers were inserted as supports for the liner, as shown in the figure (Lund and Shaw, 1979). The liner was loosely plugged at the column end with a 1-cm silanized glass wool plug and fastened at the other end with a wire loop. The stainless steel adapter between the injection port and column (6.2–3.1 mm, $^1/_2$–$^1/_8$ in.) was treated with ThetaKote (The Theta Corp., Media, PA),

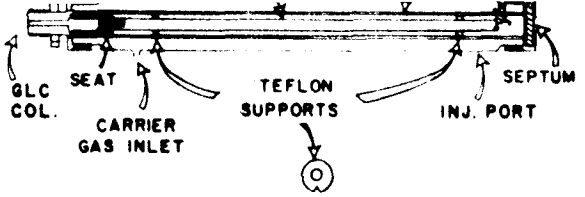


Figure 1. Modified injection port liner.

which converted the reactive steel surface to a more inert glasslike surface. A Teflon-backed, fiberglass-reinforced septum (Analabs HGC 089, Analabs, Inc., North Haven, CT) prevented contamination from the septum. These septums could be used for several hundred injections.

Products were obtained from three citrus processors throughout the season (Nov to June) and from supermarket shelves. Fresh juice samples were extracted in the laboratory or by commercial extractors. Storage studies were carried out at 28 °C for 2 months (single-strength juice and sections) and 9 months (sections).

Procedure. Since the syringe needle readily plugged when larger insoluble particles (cloud) were present, concentrated juice was reconstituted and allowed to stand at least 3 h before sampling. Canned and bottled single-strength juices and sections were also allowed to stand at least 3 h. Fresh juice was immediately filtered under pressure through a coarse sintered-glass funnel. Concentrations of the three volatiles did not change during these preliminary treatments. The juice products and liquid surrounding sections were sampled as follows: a 1-μL plug of distilled water was drawn into a 10-μL syringe followed by an air pocket (~0.3 μL) and then a 4.3-μL juice sample (includes 0.3-μL needle volume). The total volume of sample was 5.3 μL.

The sample was injected with the liner in place. For removal of less volatile components after a run, the column was purged for 5 min at 200 °C and cooled to 50–100 °C. After every second run the septum cap was unscrewed and the liner removed by grasping the wire loop with a hook. A clean liner was inserted, the septum replaced, and the column heated to 100 °C. Several minutes at 100 °C were required for equilibration of the column. The liner could usually be used twice before replacement was required; a heavy accumulation of brown deposits showed when replacement was necessary. Liners were cleaned with dichromate cleaning solution. After 1 day of operation (10–20 runs) both the adapter connecting the injection port to the column and the Teflon seat were cleaned with warm water and a pipe cleaner. The silanized glass wool plug at the upstream end of the column was also replaced. Failure to clean out the accumulated nonvolatiles caused distorted traces, an excessively long tail for the water peak, and a significant reduction in acetaldehyde peak area.

The instrument was calibrated with external standards of the three compounds in water. Peak areas were determined by planimetry. Peak height was not suitable due to distortion of peak shape. Mean values from three successive injections were used for both samples and standards.

Standards were injected in the morning, before a series of runs, and in the afternoon, after the series was completed. Although the instrument response differed somewhat from day to day, it did not vary significantly during a given day. In a few instances, the acetaldehyde peak was greatly reduced toward the end of the day. This was the result of the accumulation of nonvolatiles, since cleaning the system as described above restored the acetaldehyde peak to the normal value. Standards stored at 9 °C for 2 months in a screw-top bottle showed a reduced acetaldehyde peak, but those stored in sealed vials at −5 °C did not change during 1 year.

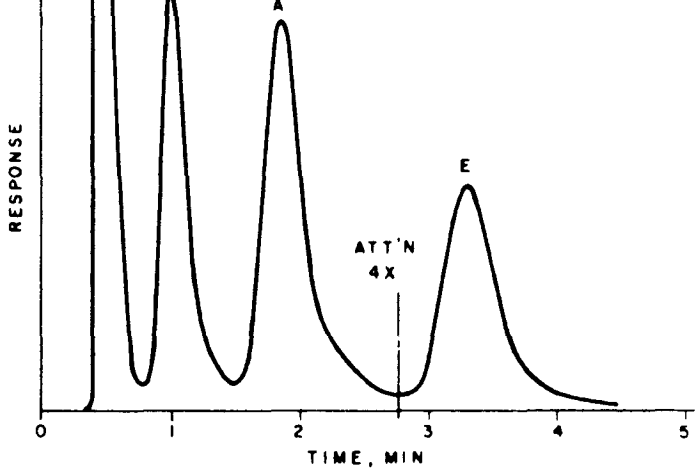


Figure 2. GC trace. M = methanol; A = acetaldehyde; E = ethanol.

Brix, acid, pulp content, and pectin methylesterase were determined by standard procedures (Hendrix et al., 1977).

RESULTS AND DISCUSSION

Method. Figure 2 is a typical GC trace and shows well-resolved peaks. The ethanol peak has a relatively long tail, which must be included in the peak area. Because of the small retention time difference between water and methanol, the detector response to methanol decreased with decreasing methanol concentration (~20% less at 0.0002% than at 0.001–0.01%).

The acetaldehyde peak was relatively variable. Peak shape and area varied among samples taken from various parts of the same container. The peak was frequently a doublet, but when it was, its total area in samples taken from a given part of the container did not change relative to the areas of single peaks. Standards rarely showed double peaks. Clean, unused liners produced sharp single peaks more often than liners that had been used once.

The accuracy of our method was estimated from the assays of two canned grapefruit juice samples and their distillates. The samples were distilled until about half the original volume had been collected (see Acknowledgment). Recovery of methanol and ethanol by distillation was 120 and 80%, respectively, but only 0.3% of the acetaldehyde was recovered. This large acetaldehyde loss must have resulted from its high volatility. It seems likely that condensation was inadequate. Although the alcohol determination appears to be relatively accurate, the large acetaldehyde losses preclude an estimate of accuracy for this compound.

Reproducibility varied from 2 to 5% (coefficient of variation). The precision for ethanol was ±2%, methanol ±4%, and acetaldehyde ±5%. Linearity of detector response was determined with standards of the three volatiles at various concentrations. It was acceptable in the 0.0002–0.2% range for methanol, 0.002–1.6% for ethanol, and 0.004–0.4% for acetaldehyde.

A similar direct injection procedure for methanol in wine was recently published (Lee et al., 1975). Recovery of added methanol in this procedure was 100.4% and the coefficient of variation was ±4.4%.

Comparison with Previous Results. Tables I and II show the range (R) and mean (M) values for the three compounds in fresh, freshly canned, and stored canned

source		concn, ppm meth-anol	acetalde-hyde	ethanol
irchner and Miller (1957)				
fresh (Valencia)		0.8	3	380
freshly canned		present	3	550
stored canned		62	0.8	480
avis and Chace (1969)				
fresh (Valencia)				400-640 (av 530)
oe and Bruemmer (1974)				
fresh (Valencia)			4.4	
avis (1970)				
fresh (Hamlin)			0.7-3	2-381
fresh (Valencia)				5-480
fresh (pineapple)			3.5	800
esent study				
fresh:	R^a	(11-80)	(70-117)	(150-159)
	M^b	0.37	91	590
freshly canned:	R	(12-60)	(50-132)	(180-700)
	M	31	83	460
stored[c]				

^{a} R, range. ^{b} M, mean. ^{c} Same as freshly canned.

le II. Single-Strength Grapefruit Juices: Comparison h Previous Values

source		concn, ppm meth-anol	acetalde-hyde	ethanol
irchner et al. (1953)				
fresh		0.2	1.45	400
freshly canned		0.2	0.33	400
stored canned		23	0.6	460
avis and Chace (1969)				
fresh (Ruby Red)				220-520 (av 400)
avis et al. (1974)				
fresh (Marsh, early)			1.5	98
fresh (Marsh, mid-season)			2.8	290
fresh (Marsh, late)			3.4	499
avis (1970)				
fresh (Marsh)				70
esent study				
fresh:[a]	M^c	43	155	220
freshly canned:	R^b	(18-40)	(70-190)	(91-500)
	M	27	150	246
stored[d]				

Single value. ^{b} R, range. ^{c} M, mean. ^{d} Same as nly canned.

es. Our results are roughly comparable with those from ier work for ethanol but are much higher for methanol acetaldehyde. We did not find the same differences ween fresh and canned juices and between stored ned and freshly canned juices observed by Kirchner Miller (1957) and by Kirchner et al. (1953). Concen-ions of the three compounds did not change in our ed canned or glass-packed juices from oranges or efruit during 2 months at 28 °C, a much longer period n required for pronounced off-flavor development. The 3 and 1957 studies, on the other hand, showed that the hanol content of stored canned juice was ~80–100 es that of freshly canned. The samples, however, had n stored for 3 or 4 years at 27 °C and were probably typical of stored canned juices. Although the storage

juice		meth-anol	acetalde-hyde	ethanol
orange				
total samples:[a]	R^b	(11-80)	(50-130)	(150-900)
	M^c	34	90	530
laboratory reamed (Valencia)				
fresh		10.8	90	150
1 h storage		15.0	80	153
20 h storage		25.2	51	155
processor extracted				
before heat treatment		37	94	530
after heat treatment (freshly canned)		25	122	470
grapefruit				
total samples:[a]	R^b	(13-40)	(40-230)	(90-500)
	M^c	27.4	154	238
processor extracted				
before heat treatment		43	155	220
after heat treatment (freshly canned)		28.4	173	179

^{a} Composite of all laboratory-reamed samples and single-strength commerical samples. ^{b} R, range. ^{c} M, mean.

period for our samples was much shorter, some increase in methanol would have been expected.

Single-Strength Juices. When juice freshly extracted in the laboratory from early Valencia oranges (Jan; Brix/acid 8.0) was held at room temperature (28 °C) for up to 20 h in a stoppered flask, methanol increased rapidly, acetaldehyde declined, and ethanol changed very little (Table III). The increase in methanol was probably the result of pectin demethylation catalyzed by pectin methylesterase. The immediate decline in acetaldehyde was not observed by Roe and Bruemmer (1974). Instead, they found that acetaldehyde increased ~30% during the first 4 h of room temperature storage and then began to decrease.

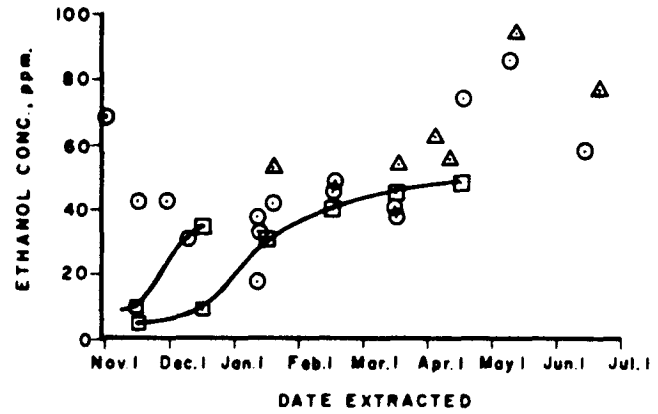
Single-strength orange and grapefruit juices extracted commercially were sampled just prior to the heat-treatment step (deoiling, degassing, and pasteurization) and directly after the canning. Analysis of these samples showed that the heat treatment caused a decline in methanol, an increase in acetaldehyde, and a slight decrease in ethanol (Table III). The alcohols could have partially volatilized, but acetaldehyde should have decreased even more rapidly than the alcohols because of the difference in volatility. The Kirchner and Miller (1957) data for oranges show acetaldehyde was unchanged and ethanol increased (Table I).

Methanol was lower, acetaldehyde higher, and ethanol lower in grapefruit than in orange juice, as shown by the values for total samples. The average ratio of acetaldehyde concentration in grapefruit juice to that in orange juice was 1.7.

Our results for the commercially canned and glass-packed juice stored at 28 °C for 2 months show that heat inactivates those enzymes that affect methanol, ethanol, or acetaldehyde concentration (note the low PEU values in Table IV). Moreover, those results indicate, surprisingly, that the concentrations of the three compounds were not significantly affected by the ongoing nonenzymatic reactions. The large methanol increase observed by Kirchner et al. (1953) and by Kirchner and Miller (1957) in stored canned orange and grapefruit juices must have re-

juice		°Brix (B)	acid (A), %	ratio of B/A	pulp, %	PEU[a] × 10[a]
orange						
single strength:	R[b]	10.7-14.6	0.58-1.00	11.4-19.7	6-20	0-7
	M[c]	12.1	0.85	14.4	12.5	0.8
concentrate:	R	44.7-46.9	2.9-3.4	13.5-16.1	10-14	0-38
	M	45.7	3.0	15.1	12	0.9
grapefruit						
single strength:	R	9.5-12.8	0.84-1.53	6.5-14.6	0.17	0-0.9
	M	10.5	1.19	8.9	8	0.3
concentrate:	R	39.4-41.0	4.1-4.7	8.6-9.9	8-12	0-4
	M	40.1	4.3	9.3	9.5	2.5

[a] Pectin methylesterase units. [b] R, range. [c] M, mean.



gure 3. Single-strength orange juice: ethanol concentration ring season. Fresh juice [(△) 6 samples]; canned and glass-cked juice [(○) 15 samples]; Davis (1970) [(□) 3 fresh samples].

lted from some unusual reaction associated with the tremely long storage period.

Table IV shows the characteristic parameters for all the ocessed juice samples analyzed in this study. Pectin ethylesterase (PEU) values were low for all heat-treated oducts; hence, enzymatic demethylation of pectin was ilikely in these products.

None of these parameters, or relatively obvious com-nations of them, could be statistically correlated to ncentrations of the three volatiles. Examination of the ta for possible correlations was centered on ethanol, ice ethanol values were much less dependent on pro-ssing variables, such as holding time for fresh juice and riable heat-treatment conditions, than the values for the her two volatiles.

The relationship between ethanol and the date processed shown for single-strength orange and grapefruit juices Figures 3 and 4. Since no significant pattern of dif-ences could be found between fresh, canned, and ss-packed orange juices, data for these juices were nsidered as a group. Figure 3 includes data from the ivis (1970) study of fresh juices prepared by standard traction procedures. With the exception of the Nov and c values, his data fall within the region of our values. ice immature fruit are not processed, his early-season lues probably represent juice from mature oranges held er from the previous season. Both our data and Davis' ggest that the ethanol content of Valencia orange juice reases as the season progresses (March to June). Our ta, however, suggest a greater increase over the season an do Davis' data.

The ethanol contents of the three grapefruit juices an-zed in Nov and Dec were higher than expected (Figure These three early samples were also unusually low in lp (5–7%); hence, pulp content may help to differentiate ces processed early in the season from those processed er. Unlike the changes in ethanol content of orange

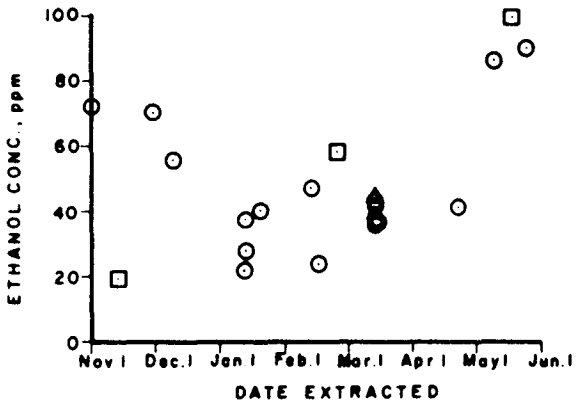


Figure 4. Single-strength grapefruit juice: ethanol concentration during season. Fresh juice [(△) 1 sample]; Davis et al. (1974) [(□) 3 fresh Marsh samples]; canned and glass-packed juices [(○) 17 samples].

Table V. Orange and Grapefruit Juice Concentrates

source	concn, ppm: methanol	acetaldehyde	ethanol
orange			
commercial evaporator			
before (fresh)	26	103	620
after (pumpout)[a]		52	
commercial samples[a]			
A		108	6.0
B	1.2	103	14.7
C	6.2	100	84
D	10.8	116	390
E	1.9	158	19
F	0.7	170	2.9
G	1.8	154	22.5
H	4.3	136	109
I	18.7	116	321
J	8.7	192	196
grapefruit			
commercial samples[a]			
A		143	4.6
B		178	0.25
C		211	0.8
D	8.7	225	0.6

[a] Reconstitute.

juice, those of grapefruit juice showed no trend; however, the May values tended to be relatively high.

Concentrates. Table V lists values for 10 commercial orange juice concentrates (reconstituted). Values for orange juice analyzed fresh and just after concentration to 69° Brix are also listed at the top of the table. Methanol and ethanol in concentrates were below detectable levels, but the acetaldehyde concentration was still 50% of that in the fresh juice. This is very surprising in view of the relatively high volatility of acetaldehyde. All three volatiles were higher in the commercial samples than in the 69° Brix

source	meth-anol	acetalde-hyde	ethanol
glass packed			
in juice, no preservative			
freshly packed	150-180	130-180	80-100
in syrup, sodium benzoate			
freshly packed			
syrup	47	65	143
inside section[a]	50-70	90-170	200-400
stored 4 days			
A (no OF)[b]	133	95	310
B (slight OF)	106	120	390
C (definite OF)	179	142	1230
freshly canned			
A: *R*[c]	(70-90)	(95-110)	(260-290)
M[d]	81	100	277
B: *R*	(50-60)	(130-170)	(110-130)
M	51	152	124

[a] Sample taken from vesicle interior. [b] OF, off-flavor or off-odor. [c] *R*, range. [d] *M*, mean.

oncentrate because of the contribution from the single-trength juice (cutback) added in the preparation of com-ercial concentrated juice. Samples D and I contained oticeably more ethanol and methanol than the other ommercial concentrates. Possibly, they had been fortified ith essence, since the ratio of the increase in methanol o the increase in ethanol was approximately the ratio of hose alcohols in commercial essence (Lund and Bryan, 977).

Four commercial samples of grapefruit juice concentrate ere also analyzed (Table V), and they were compared ith the processor-extracted, unheated grapefruit juice eported in Table III. Like the orange juice concentrates, he grapefruit juice concentrates were lower in alcohols han the fresh juice, and the three contained more acet-ldehyde than the fresh juice. On the average, grapefruit ice concentrate contained 1.4 times as much acetaldehyde s the fresh juice.

The relatively high acetaldehyde content that seems to e characteristic of concentrates may be derived from easts capable of growth in high Brix concentrates Murdock, 1977) or formed from a less volatile precursor n the GC injection port.

Sections. We examined sections that had been packed ith (a) grapefruit juice in glass bottles (no added pres-rvative), (b) with syrup and sodium benzoate as preser-ative in glass bottles, and (c) with syrup in cans (no added reservative) (Table VI). The labels for the bottled sec-ons stated that refrigerated storage was necessary. wo-months storage at 28 °C had no significant effect on e flavor or volatiles profile of the sections packed in juice ut did affect the sections bottled in syrup (data not own). In fact, changes in the latter were evident by the urth day of storage, as shown by the results for three ottles examined. In bottle A, there was no off-flavor, f-odor, or gassing, but all three volatiles had increased gnificantly. Bottle B had still more acetaldehyde and hanol, a slight off-odor, and some gassing. Bottle C owed a noticeable pressure increase, pronounced off-lor, and marked increase in the three compounds, par-ularly ethanol (9-fold). Concentrations inside a section, termined by inserting the syringe needle inside a large sicle, were much higher than in the syrup. Ethanol was rticularly concentrated inside the sections; typical values re 2–4 times the concentration in the surrounding liquid.

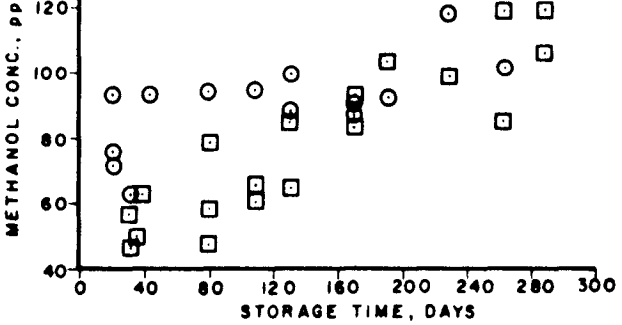


Figure 5. Canned grapefruit sections: methanol concentration during storage. Batch A (○); batch B (□).

These data show that high ethanol values correlate with spoilage of non-heat-treated glass-packed sections. However, a statistically significant test for storage history based on ethanol content would require sufficient samples for elimination of bottle-to-bottle variations.

Canned grapefruit sections were relatively stable. Huggart et al. (1955) found that the flavor of stored canned sections began changing after 6–12 months at 27 °C. We obtained canned grapefruit sections from two different processors (A and B) and analyzed them just after they had been canned (Table VI) and perodically during storage at 28 °C. The syrup in batch A was clear and that in batch B was turbid; otherwise the two appeared very similar. Acetaldehyde and ethanol did not change significantly during storage, but methanol increased. Figure 5 shows that the methanol concentration in both samples had reached 90 ppm in 180 days and in 270 days had reached 110–130 ppm. For batch B, the relationship was roughly linear; methanol in batch A, on the other hand, remained fairly constant for up to 180 days and then began to increase. We did not observe any pronounced change of color, texture, odor, or flavor in either batch. The pressure did not increase noticeably, and there was no evidence of gas production. Huggart et al. (1955) reported that although quality changes were not obvious, off-flavors in canned sections stored at 27 °C for 8 months were detectable by a flavor panel. We concluded that methanol values between 100 and 140 ppm for canned grapefruit sections can indicate an incipient flavor change resulting from extended high-temperature storage.

CONCLUSION

Certain quality-related factors, such as seasonal and varietal variations, may be correlated with ethanol content of processed single-strength juice products. Ethanol appeared to be an indicator of spoilage in non-treated, glass-packed grapefruit sections. Canned grapefruit sections showed a positive correlation between methanol and high-temperature storage time. The relative concentrations of methanol, ethanol, and acetaldehyde were characteristic of product type. Thus, acetaldehyde concentration might be used to distinguish grapefruit from orange products and single-strength juice from concentrates. Concentrates that have a volatile profile resembling that of fresh juice most likely contain essence.

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Volatile Constituents of Green Tea, Gyokuro (*Camellia sinensis* L. var Yabukita)

Kenji Yamaguchi and Takayuki Shibamoto*

The volatile constituents of Gyokuro, which had not been studied prior to this report, have been investigated by gas chromatography/mass spectrometry. Seventy-nine compounds were positively identified and ten compounds were tentatively identified in the oil obtained from a methylene chloride extract of the steam distillate of the green tea leaf. The compounds reported here include 17 hydrocarbons, 17 alcohols, 16 aldehydes, 13 ketones, 8 esters, 2 ethers, 1 acid, and 5 others. Major constituents of this oil were identified as 2,6,6-trimethyl-2-hydroxycyclohexanone, linalool, geraniol, *cis*-jasmone, β-ionone, cyclohexanone, 5,6-epoxy-β-ionone, indole, and caffeine.

Green tea was introduced to Japan from China in 1191 and quickly became one of the most popular drinks. Domestic green tea production increased steadily, reaching 98 000 tons in 1979. Recently, green tea flavor has also been used in ice cream, soft drinks, etc.

Green tea flavor has been investigated by many researchers and over 100 volatile components have been identified (Yamanishi, 1975; Kiribuchi and Yamanishi, 1963). The compounds found range from low boiling point alcohols (e.g., 2-methylpropanol) to high boiling point acids (e.g., decanoic acid).

Gyokuro, one of the highest grades of green tea (annual production in 1979 = 494 tons), gives a fresh green aroma and has a mild taste. The characteristic taste of Gyokuro is due to the use of specially treated new tea leaves. The leaves are grown in the shade under nets made by rice straw for ~20 days. Nakagawa (1973) reported that the taste of Gyokuro depends upon the relative amounts of amino acids, caffeine, and tannin present. There are, however, no reports on the volatile constituents of Gyokuro. In this study, the aroma components of Gyokuro were isolated and identified by gas chromatography/mass spectrometry (GC/MS) techniques.

EXPERIMENTAL SECTION

Tea Sample. Gyokuro (*Camellia sinensis* L. var Yabukita) was obtained from The Agricultural Institute of Fukuoka Prefecture, Tea Branch, in May 1979.

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Isolation of Volatiles. Gyokuro (220 g) was made into powder by using a blender and placed in a 2-L round-bottomed flask. Deionized water (1 L) was added, and steam distillation was performed under reduced pressure (thermometer and pressure gauge reading = 50 °C and 40 mmHg). The steam distillate (800 mL) was gathered with the condensate (50 mL) obtained from the dry ice–acetone trap. The distillate was then extracted with 300 mL of methylene chloride for 20 h by using a liquid–liquid continuous extractor. The extract was dried over anhydrous sodium sulfate for 12 h, and solvent was removed by using a rotary flash evaporator to ~10 mL in volume. Further concentration was conducted with an N_2 stream in a micro test tube. Three batches of green tea samples were treated by the above method (total green tea used: 660 g). The concentrated extracts were combined and the composite was analyzed by the GC/MS technique described by Yamaguchi and Shibamoto (1979).

RESULTS AND DISCUSSION

The volatile compounds identified in green tea (*C. sinensis* L. var Yabukita) extract are listed in Table I. Peak numbers on the left side show the elution order on the Carbowax 20M column (Figure 1); peak numbers on the right side show the elution order on the OV-101 column (Figure 2). Those peak areas (from the Carbowax 20M column) which had value of less than 0.1% are not listed. I_u designates retention indexes of unknowns. I_k represents the retention indexes of authentic samples. For some compounds, formulas were deduced from mass spectral data, but known compounds were not available. We listed those compounds as "tentatively" identified.

Several probable reaction products from β-ionone (peak 133, OV-101) were found (represented by footnote *b* in