Proc. Fla. State Hort. Soc. 106:258-262. 1993.

SIMULTANEOUS DISTILLATION-SOLVENT EXTRACTION OF ORANGE JUICE VOLATILES

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Abstract. **A simultaneous distillation-solvent extraction (SDE) procedure was evaluated for the recovery of volatile components from pasteurized orange juice. The Godefroot semi-micro SDE apparatus with methylene chloride solvent was used and extracts were analyzed by capillary gas chromatography. The percent recovery of ethyl butyrate, alpha pinene, octanal, 1-octanol, linalool, alpha terpineol and carvone was determined by a standard additions method. The percent recovery varied from 77% for linalool to 118% for ethyl butyrate. The coefficient of variation ranged from 1.5 to 9.9% and was less than 7.0% for the majority of the samples. The extract contained 124 detectable compounds. The SDE procedure provides a useful method for the quantitative recovery of orange juice volatile constituents.**

Methodology for the recovery of volatile constituents from orange juice for quantitative analysis has been investigated by a number of researchers. Objectives have been to recover as many volatiles as possible in detectable quantities with good reproducability and with minimum changes in the constituents caused by the recovery procedure. Different procedures evaluated include (1) direct injection of an aqueous distillate, Moshonas and Shaw (1984,1987); (2) solid phase adsorption and elution with methanol, Marsili (1986); (3) solvent extraction and evaporative concentration of the extract, Schreier et al. (1981); (4) co-distillation of juice followed by solvent extraction, Matthews and West (1988); (5) head-space analysis, Moshonas and Shaw (1992).

Several researchers utilized simultaneous-distillation extraction (SDE) for the recovery of volatiles. Schultz et al. (1977) used a modified Likens-Nickerson apparatus (1964) to evaluate recoveries of volatiles from model systems of fruit essence constituents. They simultaneously distilled 2.5 L of aqueous solution and 125 ml of solvent. Wade et al. (1992) used a modified Likens-Nickerson apparatus for vacuum SDE and compared fresh and processed orange juices. Godefroot et al. (1981) developed a small volume SDE apparatus for a heavier than water solvent. They simultaneously distilled 20 or 30 mL of aqueous solution and 1 ml of dichloromethane. Nunez (1984) used the Godefroot apparatus to isolate volatile components of grapefruit juice.

In this experiment we evaluated the Godefroot SDE apparatus for the quantitative recovery of volatiles from orange juice and orange juice plus added volatiles.

Materials and Methods

This study evaluated the recovery of seven selected volatile constituents of orange juice by SDE with the Godefroot apparatus. Known quantities (0.0, 0.25, 0.50, 0.75, or 1.00 mg/L) of selected volatiles were added to the orange juice prior to SDE. Three SDE's were performed on each juice and three gas chromatographic analyses were performed on each extract.

Materials & Equipment

(1) Methylene chloride: Fisher #D150 unstabilized; (2) Internal standard; 1-heptanol; Aldrich #H280-5; (3) Micro steam distillation apparatus for heavier-than-water solvents, Godefroot design; Alltech Associates Inc., Deerfield, IL; (4) Gas chromatograph: Perkin Elmer Auto System Model 9000, 30 meter DB-1 column, 0.32 mm I.D., film 1 um; inject 2 uL; split ratio 1:57; constant pressure 9.7 psig helium carrier gas; flame ionization detector. Temperature program: 45°C for 2 min; 3.5°C/min to 230°C; 6°C/min to 250°C; hold at 250°C for 15 min.; (5) Orange juice: commercial pasteurized not from concentrate; (6) Integrator: Perkin Elmer PE Nelson Model 1020, standard method.

Simultaneous Distillation Extraction (SDE) Procedure.

1. Measure sample into flask, 80 ml (83.7g).
2. Add three drops of Dow Corning antifoam B emulsion.
3. Add three boiling chips.
4. Add 1 ml of internal standard solution. (1-heptanol,49.3ug/g methylene chloride).
5. Pipet 1 ml methylene chloride into the solvent flask.
6. Pipet 1.5 ml methylene chloride into the separator section.
7. Pipet water into the separator section to fill.
8. Condenser: cold finger maintained at -1°C.
9. Water bath for solvent flask maintained at 78°C.
10. Oil bath for sample flask raised to 150°C initially.
11. Lower flasks into respective baths. Solvent flask will reflux for about 5 to 8 minutes while sample is heating up.
12. When the sample begins to distill, the oil bath will have dropped to about 137°C.
13. When the oil bath reaches 139°C reduce the rheostat from 70% to 64% to maintain a temperature of 140°C.
14. Continue distillation for one hour.
15. Continue solvent reflux for 5 minutes after stopping distillation.
16. Cool down apparatus then remove material in separator section.
17. Recover methylene chloride from separator section.
18. Recover methylene chloride from solvent flask.

Results and Discussion

Gas chromatographic standard curves were deter mined for seven of the volatile orange juice constituents α-pinene, linalool, ©-terpineol, ethyl butyrate, octanal, 1 octanol, and carvone (Fig 1). Serial dilutions, 100 to 2 ug/g, in 95% ethanol were quantitated. Alpha pinene ha

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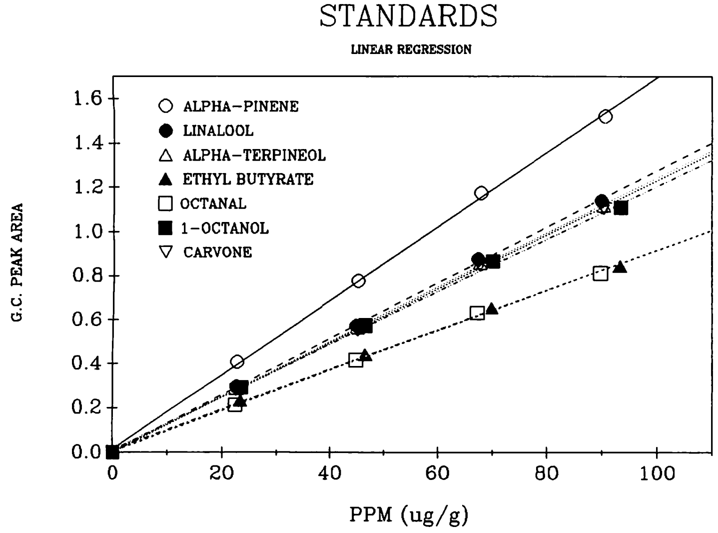


Fig. 1. Standard curve for capillary column gas chromatographic analysis of selected volatile orange juice constituents

the highest response and ethyl butyrate the lowest response of the seven compounds.

After SDE was completed, methylene chloride was recovered from the separator section and from the solvent flask. Approximately 2.5 mL was recovered from the separator section and approximately 1.0 mL from the solvent flask. Both fractions were quantitatively analyzed by gas chromatography for the seven volatiles. On the basis of gas chromatographic peak area, from 94 to 98 percent of the volatile compounds were found in the solvent flask (Table 1). Based on this data only the methylene chloride in the solvent flask was used for recovery calculations and quantitative analysis.

Table 1. Peak area for SDE volatiles recovered in the distillation separatory section and for volatiles recovered in the solvent flask, with percent in solvent flask.

Separatory Section (Peak Area)	Solvent Flask (Peak Area)	Total (Peak Area)	% in Flask
	Ethyl Butyrate		
36248	785902	8.22e+05	95.6
	alpha-Pinene		
92002	1.40e+06	1.49e+06	93.8
	Octanal		
33294	6.90e+05	7.23e+05	95.4
	1-Octanol		
13413	4.11e+05	4.24e+05	96.8
	Linalool		
22072	1.33e+06	1.35e+06	98.4
	alpha-Terpineol		
16743	6.59e+05	6.75e+05	97.5
	Carvone		
18963	2.77e+05	2.96e+05	93.6

The mean recovery for the compounds added to orange juice ranged from 77% for linalool to 118% for ethyl butyrate (Table 2). These values were determined with internal standard correction (1-heptanol) for SDE and gas chromatographic analysis. The coefficient of variation (C.V.) varied from 1.5% to 9.9%. Maignial et al. (1992), using methylene chloride solvent for SDE, had recoveries ranging from 55% for pyrazine to 118% for ethyl butyrate. Godefroot et al. (1981) had recoveries ranging from 70% for benzyl alcohol to 113% for trans-2-hexenal.

When the data are presented as a standard additions plot, the value of the compound in the unspiked orange juice is found at the intersection with the X-axis (Fig 2). In the commercial juice analyzed, these values in ug/g were: ethyl butyrate, 1.09; α-pinene, 1.11; 1-octanol, 0.38; linalool, 1.96; α-terpineol, 0.93; octanal, 1.43; and carvone, 0.29. These values are in very good agreement with the values found for the unspiked orange juice (Table 2).

When gas chromatograms of SDE's of a commercial orange juice and the same juice spiked with 1 ppm each of 10 volatile constituents are compared (Fig 3), the 1 ppm addition is easily discerned. Carvone, at 0.3 ppm in the commercial juice, is easily detected and quantified.The percent recoveries for the seven volatiles in the standard additions plot were reproducible for 0.25,0.50,0.75 and 1.0 ug/g. The percent recovery for the 0.25 ug/g addition dropped significantly for octanal, linalool and α-terpineol (Table 2).

When a chromatogram of the SDE of orange juice is compared with a chromatogram of a separatory funnel solvent extraction (Matthews and West, 1992) of the same juice (Fig 4), the greater magnitude of the peaks by SDE is very evident. With SDE, the gas chromatograph integrator quantitated 124 peaks, but with the separatory funnel extract, only 62 peaks were quantitated. Some of the additional peaks could be artifacts from the distillation process. Perhaps, thermal artifacts could be minimized using a distillation system under reduced pressure.

The SDE semi-micro procedure provides an extract of volatiles which is sufficiently concentrated to quantitatively

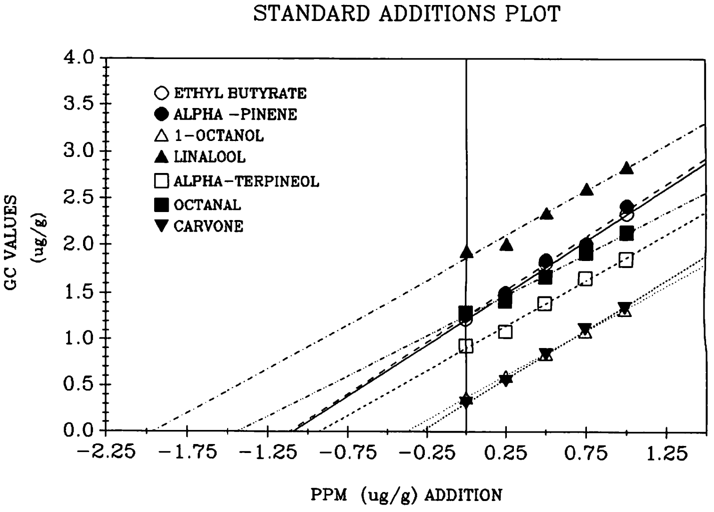


Fig. 2. Standard additions plot of SDE for selected orange juice constituents and orange juice with 0.25, 0.50, 0.75 and 1.00 ug/g addition of the constituents.

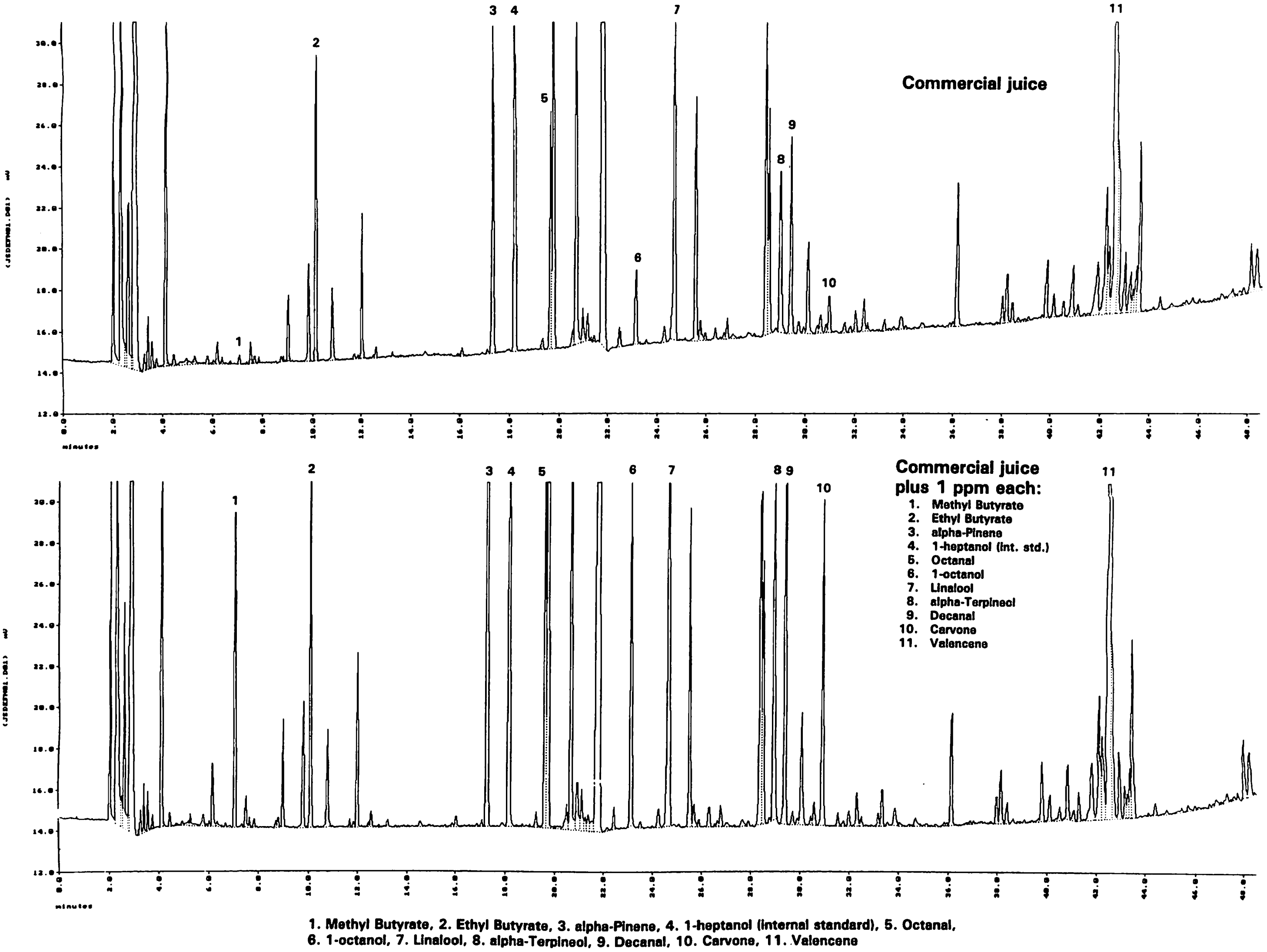


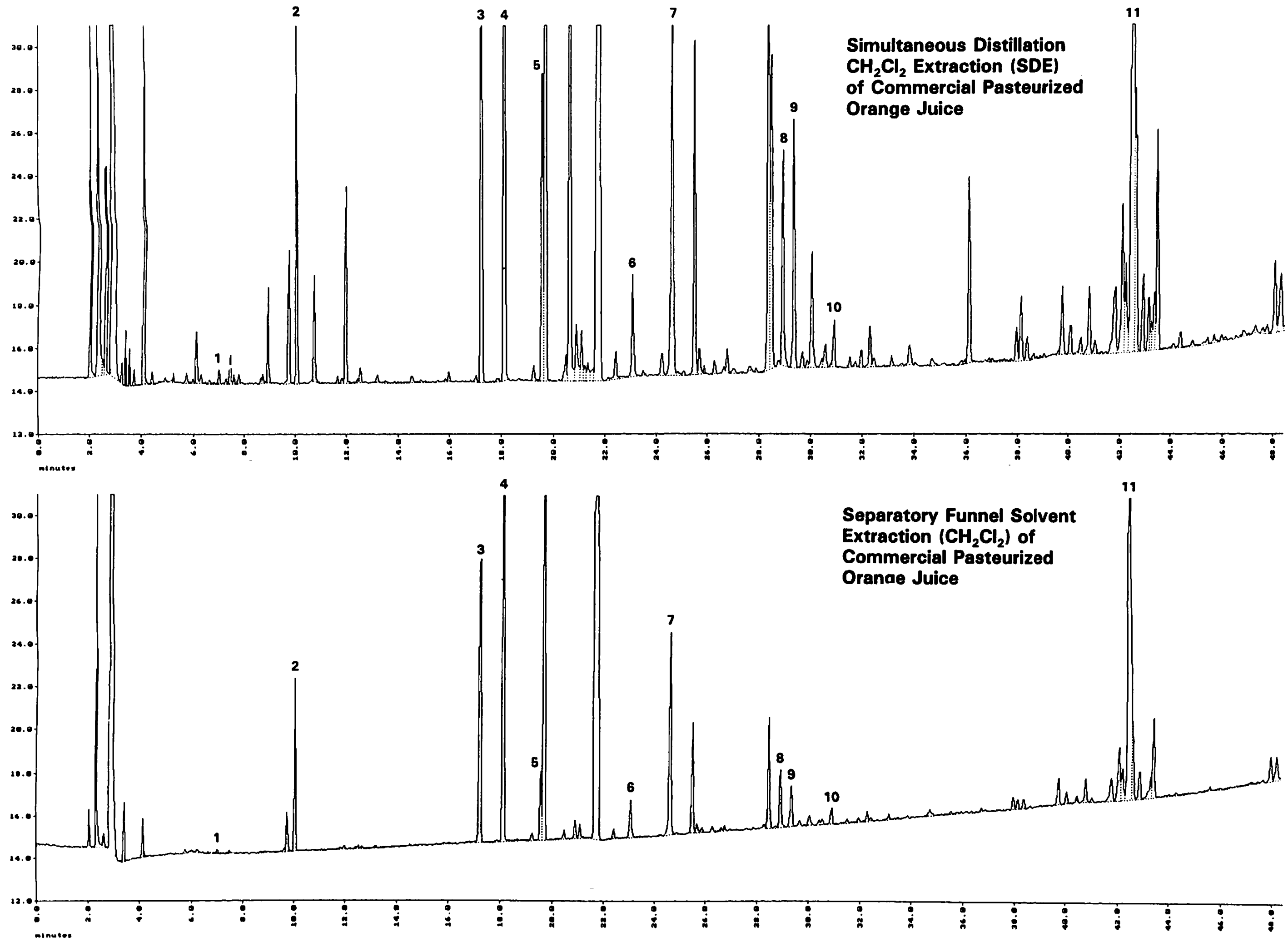
Fig. 3. Gas chromatograms of volatiles recovered by methylene chloride SDE from commercially pasteurized orange juice and juice to which 1 ppm each of methyl butyrate, ethyl butyrate, α-pinene, octanal, 1-octanol, linalool, α-terpineol, decanal, carvone and valencene had been added.

Table 2. Percent recovery and coefficient of variation of volatile constituents in orange juice with incremental addition of volatiles.

	PPM ADDED (ug/g)					
	0.00	0.25	0.50	0.75	1.00	
			Ethyl Butyrate			
MEAN/9	1.20	1.46	1.82	2.02	2.34	MEAN
INCREASE	0.00	0.26	0.62	0.82	1.13	RECOVERY
SD	0.04	0.12	0.06	0.13	0.13	
C.V.(%)	3.20	8.00	3.23	6.42	5.56	
RECOVERY(%)		106.60	132.0	114.83	120.65	118.5
			alpha-Pinene			
MEAN/9	1.27	1.51	1.85	2.02	2.42	
INCREASE	0.00	0.23	0.58	0.75	1.15	
SD	0.06	0.15	0.07	0.13	0.15	
C.V.(%)	4.61	9.90	3.66	6.62	6.38	
RECOVERY(%)		97.86	123.47	105.85	122.38	112.4
			Octanal			
MEAN/9	1.30	1.42	1.68	1.93	2.14	
INCREASE	0.00	0.13	0.39	0.63	0.84	
SD	0.07	0.08	0.04	0.07	0.08	
C.V.(&)	5.74	5.72	2.59	3.76	3.79	
RECOVERY(%)		52.66	82.38	89.13	89.25	78.4

Table 2. Continued

	PPM ADDED (ug/g)					
	0.00	0.25	0.50	0.75	1.00	MEAN RECOVERY
			1-Octanol			
MEAN/9	0.37	0.60	0.84	1.09	1.33	
INCREASE	0.00	0.23	0.47	0.72	0.96	
SD	0.03	0.02	0.01	0.03	0.04	
C.V.(%)	6.94	3.77	1.53	2.42	3.32	
RECOVERY(%)		93.91	99.51	101.28	102.00	99.2
			Linalool			
MEAN/9	1.94	2.02	2.35	2.61	2.84	
INCREASE	0.00	0.08	0.41	0.67	0.90	
SD	0.12	0.09	0.04	0.07	0.08	
C.V.(%)	6.42	4.48	1.74	2.85	2.87	
RECOVERY(%)		32.28	87.15	93.78	95.23	77.1
			alpha-Terpineol			
MEAN/9	0.93	1.09	1.40	1.66	1.86	
INCREASE	0.00	0.16	0.46	0.73	0.92	
SD	0.05	0.05	0.02	0.04	0.05	
CV(%)%	5.12	4.35	1.67	2.33	2.92	
RECOVERY(%)		65.02	98.38	102.52	98.36	91.1
			Carvone			
MEAN/9	0.31	0.55	0.84	1.12	1.35	
INCREASE	0.00	0.24	0.54	0.82	1.04	
SD	0.02	0.03	0.04	0.04	0.05	
C.V.(%)	4.95	5.13	4.41	3.64	3.45	
RECOVERY(%)		102.00	114.37	115.11	110.84	110.6



1. Methyl Butyrate, 2. Ethyl Butyrate, 3. alpha-Pinene, 4. 1-heptanol (internal standard), 5. Octanal, 6. 1-octanol, 7. Linalool, 8. alpha-Terpineol, 9. Decanal, 10. Carvone, 11. Valencene

Fig. 4. Gas chromatograms of a methylene chloride SDE and a separatory funnel methylene chloride extract of commercially pasteurized orange juice.

analyze most of the currently identified volatile constituents in orange juice. Constituents from methyl butyrate to valencene could be quantified at juice concentrations of 0.25 ug/g and above. This is attained without evaporative concentration of the approximately 1ml of methylene chloride remaining after SDE. Coefficient of variation of volatiles ranged from 1.5% to 9.9%. The procedure is useful for quantitative analysis of a wide range of orange juice volatile flavor constituents and should find increased use for recovering minor flavor constituents of fruit juices.

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Proc. Fla. State Hort. Soc. 106:262-266. 1993.

EVALUATION OF A PORTABLE SPHERE SPECTROPHOTOMETER FOR THE MEASUREMENT OF ORANGE JUICE COLOR

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Abstract. **Obsolescence of the electronics of the HunterLab Citrus Colorimeter, originally developed more than 3 decades ago, resulted in the discontinuance of this instrument in the early 1980's. Many are still in use, although the difficulty in finding repair parts for these venerable instruments is slowly forcing their replacement by more modern instruments. Dramatic advances in sensor technology and improvement in complex electronic circuitry led to the development of smaller, accurate and reproducible digital color instrumentation. A compact, hand-held, integrating sphere spectrophotometer, the Minolta Model CM-2002, employing d/0° geometry with the specular reflectance component excluded (SCE) setting, was adapted to 1 inch diameter test tubes to measure X, Y and Z color attributes for about 500 juice samples. The results were subsequently graphically compared with the Citrus Colorimeter values. The data was statistically analyzed to calculate regression equations to express color on a scale equivalent to Citrus Colorimeter color values. The expression for equivalent CN (color number) values was selected as the guide to further evaluate the performance of the Minolta CM-2002 instruments under processing plant conditions.**

Introduction

The quality grade orange juice receives includes a substantial portion derived from orange juice color. Current U. S. standards allow up to 40% of the total grade points to be allotted for color (U.S. Department of Agriculture, 1983). Originally, orange juice color was evaluated visually by comparison to a series of orange colored plastic standards under standardized lighting conditions. This method was superseded by an instrumental method relying on the HunterLab Model D45, later the Model D45D2 Citrus Colorimeter (CC), developed nearly 40 years ago (Huggart and Wenzel, 1954,1955; Hunter, 1967; Hunter and Harold, 1987). The CC permitted objective and accurate measurements of color values. The numerical scale employed by the CC was developed to coincide with the quality grade points assigned for the color part of the grading scheme (Huggart et al., 1969). Until late 1985, the CC was the only officially approved instrument for orange juice color measurement (State of Florida, Department of Citrus, 1975, et seq.; U.S. Department of Agriculture, 1983). Discontinuation of the manufacture of the widely used CC brought about testing of a wide variety of mainly reflectance mode colorimeters and spectrophotometers for their suitability for orange juice color measurement (Wagner and Buslig, 1983,1984; Berry et al., 1984; Buslig and

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