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VOLATILE CONSTITUENTS OF FRESH ORANGE JUICE RECOVERED BY SIMULTANEOUS DISTILLATION EXTRACTION

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Abstract. **Volatile constituents were recovered from fresh hand squeezed unpasteurized orange juice by simultaneous distillation extraction (SDE) with methylene chloride. Ninety volatile constituents were detected in the extract. Of these, twenty-one volatiles were identified and quantified by capillary gas chromatography. The SDE procedure was compared with room temperature (RT) methylene chloride solvent extraction of the orange juice. The heat of distillation was found to produce some artifacts in the SDE juice extract. Furfural was identified as an artifact. Nootkatone was identified as a fresh orange juice constituent in the SDE samples and in the RT solvent extract.**

Several researchers utilized simultaneous distillation extraction (SDE) for the recovery of volatiles. 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 heavier than water solvents. Nunez (1984) used the Godefroot apparatus to isolate volatile components of grapefruit juice. Matthews and West (1993) evaluated the Godefroot SDE apparatus for recovery of volatiles from pasteurized orange juice.

In this experiment we compared the volatiles recovered from fresh orange juice by room temperature (RT) methylene chloride solvent extraction and by SDE with methylene chloride using the Godefroot apparatus. Volatile compounds were identified by gas chromatographic retention times and/or electron impact mass spectra. Compounds were quantified from standard curves, Matthews and West (1992). If pure standards were not available quantitation was based on gas chromatographic response data of similar compounds.

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Materials and Methods

Valencia oranges were obtained from the UF Citrus Research and Education Center on 3/25/94 and 4/12/94. Juice was obtained from the fresh fruit by hand squeezing with a Waring mighty squeeze Model 11JC21 juicer. A minimum of 12 oranges was juiced for a sample. Three extractions were made per sample and two gas chromatographic analyses per extract.

Materials & Equipment. (1) Methylene chloride: Fisher #D150 unstabilized; (2) Microsteam distillation apparatus for heavier-than-water solvents, Godefroot design; Alltech Associates Inc., Deerfield, IL; (3) Internal standards; 1-heptanol, Aldrich #H280-5; methyl anthranilate, Aldrich #23, 645-4; (4) Gas chromatograph: Perkin Elmer Auto System 9000, 30 meter DB-5 column, 0.32 mm I.D., film 1um, inject 2 uL, split ratio 1:57, Constant pressure 9.7 psig helium carrier gas, flame ionization detector. Temperature program: 45C for 2min, 3.5C/min to 230C, 6C/min to 250C, hold at 250C for 15 min.

Simultaneous Distillation Extraction (SDE) Procedure. Procedure as per Matthews and West (1993) with the following modifications: (1) 1-heptanol and methyl anthranilate internal standard solutions (400 ug/g each), 1 ml of each solution added. (2) Water bath for solvent flask maintained at 74C. Oil bath maintained at 130C.

Room Temperature (RT) Solvent Extraction Procedure. Procedure as per Matthews and West (1992).

Mass Spectrometry-Gas chromatography. Hewlett Packard 5890 Gas chromatograph interfaced to a Hewlett Packard 5989A Mass Spectrometer operating in the electron impact ionization mode. Chromatographic conditions as described above.

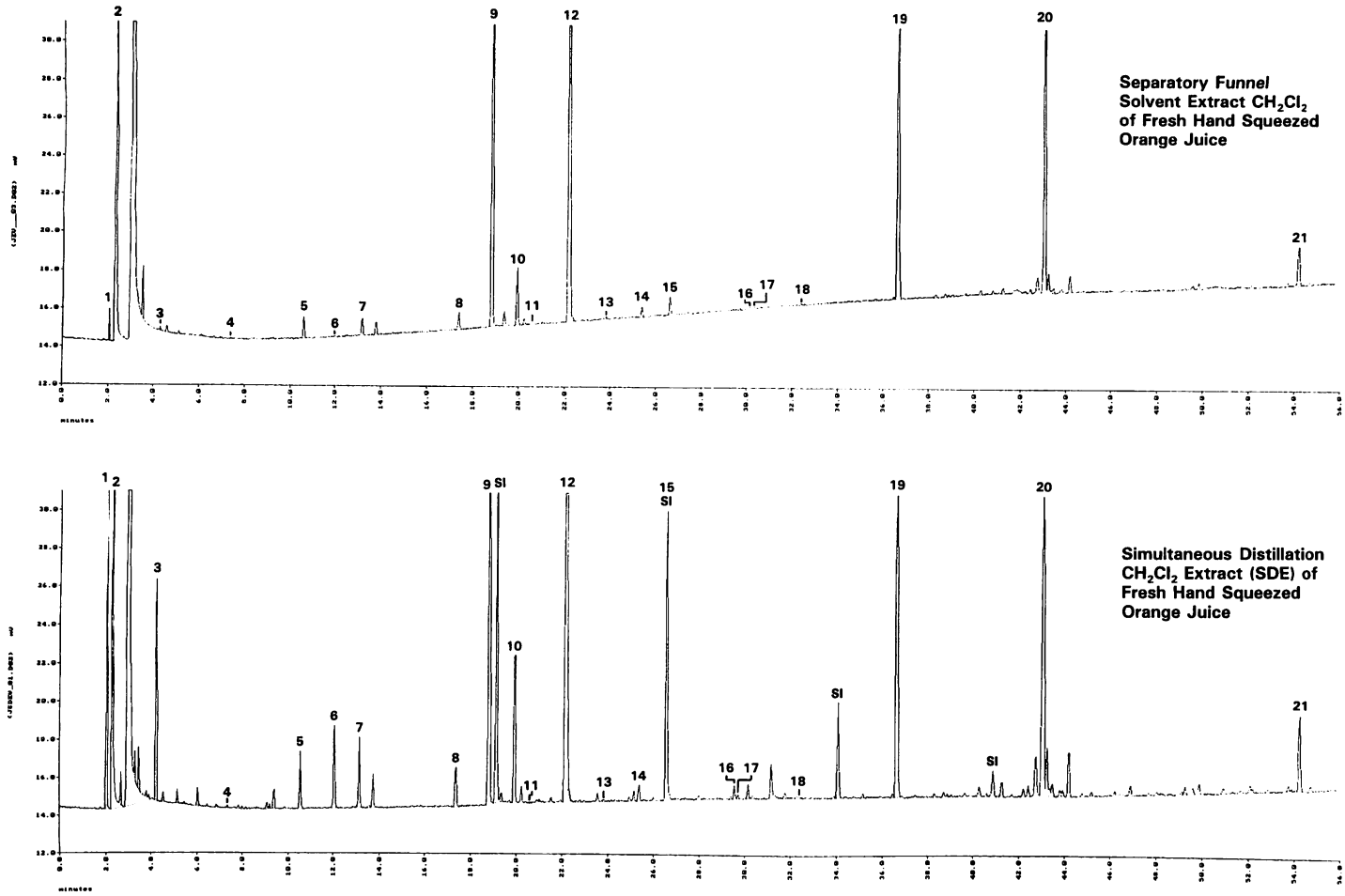


Figure 1. Gas chromatograms of volatiles recovered from fresh hand squeezed orange juice by simultaneous distillation extraction and by separatory funnel solvent extraction.

Results and Discussion

In this experiment and in previous research, (Matthews and West, 1993) the SDE with methylene chloride provided excellent recovery of orange juice volatiles. Ninety volatile compounds could be detected in the extract. Twenty-one of these volatiles were identified and quantitated (Fig. 1). Some peaks of significant magnitude were identified as artifacts from the antifoam added to the orange juice prior to distillation. The peaks were silicon (SI) compounds and one interfered with the quantitation of ethyl-3-hydroxy hexanoate. Furfural was also identified as an artifact in the SDE extract and was the only component identified that was presumed to be formed by the heat of distillation. Furfural was not detected in the methylene chloride extract from room temperature separatory funnel solvent extraction.

The amounts of identified volatiles recovered by the SDE procedure and the RT-Extraction procedure values were similar for most compounds (Table 1). There was no significant difference between the values for 14 of the 21 volatiles quantitated. The SDE value for ethyl acetate was nearly four-fold that in the RT-Extract. The SDE value for valencene was nearly three-fold that of the RT-Extract.

Table 2 lists the values for volatiles of fresh orange juice reported by Wade et al. (1992), Moshonas and Shaw (1994)

Table 1. Volatile Constituents of Fresh Orange Juice by Simultaneous Distillation Extraction (SDE) and by Separatory Funnel Extraction.

	SDE		Separatory Funnel	
	Mean (ppm)	%Rel SD[z]	Mean (ppm)	%Rel SD[z]
Methanol	1.2	7.12	0.26	4.02
Ethanol	45.9	5.47	73.7	4.75
Ethyl Acetate	1.09	3.43	0.28	1.95
Methyl Butyrate	0.33	1.11	0.27	1.24
Ethyl Butyrate	0.74	4.36	1.48	1.86
Furfural	0.66	2.13	0.00	—
cis-3-Hexanol	0.38	2.78	0.31	5.81
Hexanol	0.27	6.52	0.25	3.51
a-Pinene	0.14	3.34	0.17	3.73
Sabinene	0.09	—	0.16	2.42
Myrcene	0.29	2.49	0.39	2.71
Octanol	—	—	0.33	—
Limonene	12.4	5.18	17.2	1.72
g-Terpinene	0.09	4.03	0.08	—
Octanol	0.059	16.16	0.05	16.45
Linalool	0.24	2.04	0.25	3.02
Ethyl 3-OH hexanoate	—	—	0.48	2.17
a-Terpineol	0.42	1.90	0.31	1.76
Decanal	0.26	0.84	0.23	1.56
d-Carvone	0.46	1.40	0.38	3.46
Valencene	7.8	3.77	2.9	5.88
Nootkatone	1.39	4.66	1.06	9.54

[z]% Rel SD = Percent Relative Standard deviation

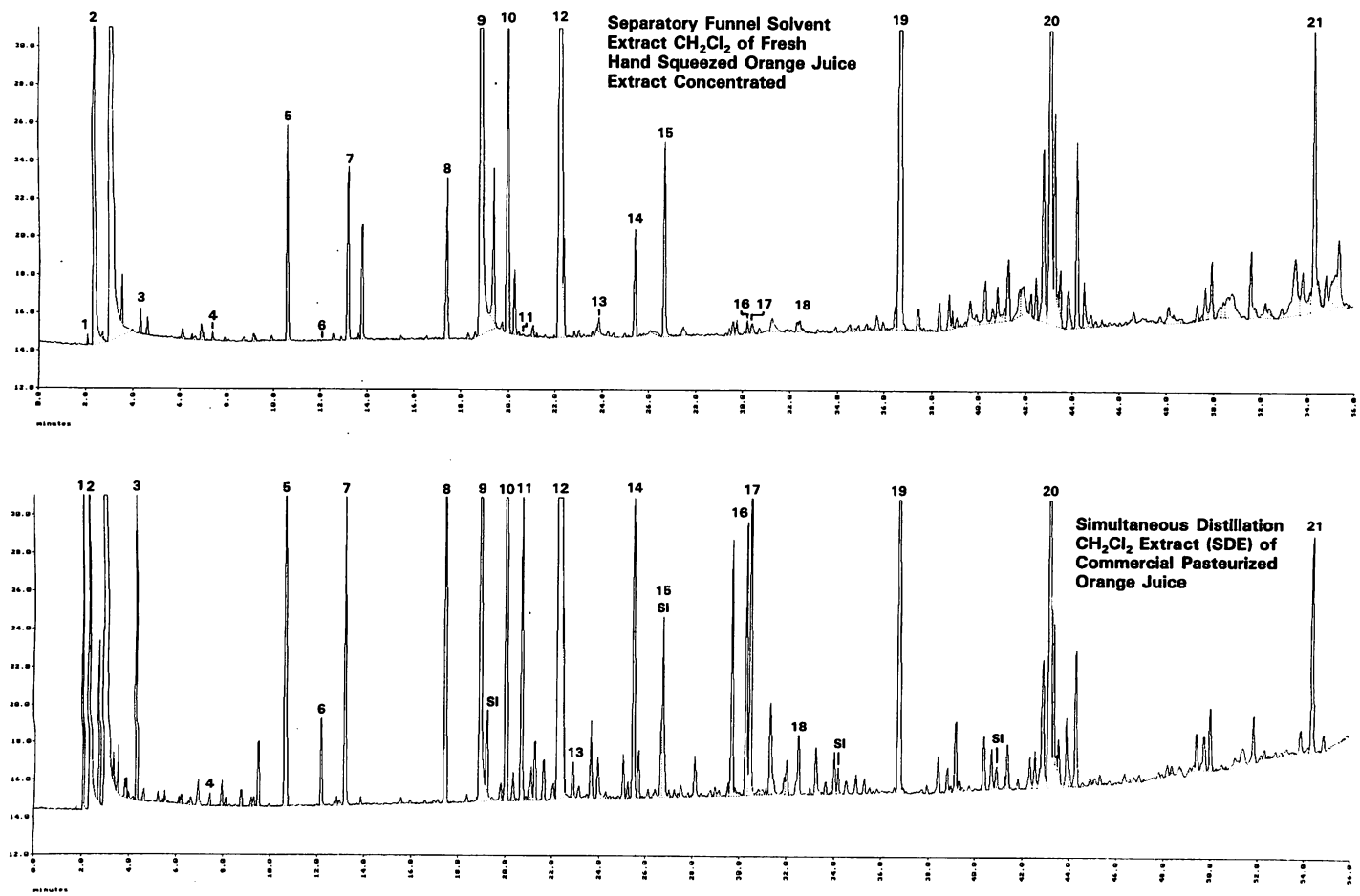
Table 2. Reviews of Volatile Constituents of Fresh Orange Juice Concentration, ppm

	Wade, et al., 1992	Moshnonas and Shaw 1994	Nisperos-Carriedo and Shaw 1990
Methanol	77.7	37	38.0
Ethanol	640.1	1150	420.0
Ethyl Acetate	—[z]	0.28	0.20
Methyl Butyrate	—	0.016	0.03
Ethyl Butyrate	1.45	0.84	0.54
Furfural	—	—	—
cis-3-Hexanol	0.028	0.017	0.29
Hexanol	0.039	0.12	0.12
a-Pinene	0.071	0.10	0.10
Sabinene	—	0.023	0.02
Myrcene	0.233	0.34	
Octanal	0.024	0.004	0.009
Limonene	14.0	18.0	40.0
g-Terpinene	0.008	0.002	0.15
Octanol	0.031	0.089	—
Linalool	0.092	0.13	t[z]
Ethyl 3-OH hexanoate	1.34	0.28	—
a-Terpineol	0.044	t[z]	t[z]
Decanal	0.088	0.016	—
d-Carvone	0.010	0.004	—
Valencene	1.12	3.30	8.0
Nootkatone	0.058	—	—

[z]t = Trace, — = not reported.

and Nisperos-Carriedo and Shaw (1990) for comparison with our SDE values. Methanol and ethanol were much less for the SDE procedure. This is expected since methylene chloride does not extract these low molecular weight alcohols to any degree (Moshonas and Shaw, 1983). The SDE procedure gave a high value for valencene (7.8 ppm). Nisperos-Carriedo and Shaw reported 8.0 ppm valencene in fresh juice. Nootkatone is seldom identified as a constituent of orange juice, although it is considered a major contributor to grapefruit juice flavor. In this research we found nootkatone at slightly more than 1.0 ppm in both the SDE sample and in the separatory funnel extract. Schreier (1981) reported nootkatone as a constituent of freeze concentrated orange juice and also orange juice from thermal concentration. Schreier et al. (1979) reported that nootkatone was formed in orange juice aroma during the course of the evaporation process. The odor threshold in water for nootkatone has been reported to be 0.8 ppm and it is found at 6ppm in grapefruit juice (Shaw, 1991). Wade et al. (1992) noted the presence of nootkatone at levels less than 0.1 ppm in fresh orange juice and in frozen concentrated orange juice. Our research also found nootkatone in commercial pasteurized orange juice (Fig. 2).

The SDE procedure provided excellent recovery of most fresh orange juice volatiles. Even though the results must be corrected for furfural formed from the heat of distillation and for silica compounds introduced by the antifoam, the



1. Methanol, 2. Ethanol, 3. Ethyl Acetate, 4. Methyl Butyrate, 5. Ethyl Butyrate, 6. Furfural, 7. cis-3-hexen-1-ol, 8. alpha-pinene, 9. 1-heptanol (internal standard), 10. Myrcene, 11. Octanal, 12. Limonene, 13. 1-octanol, 14. Linalool, 15. Ethyl-3-hydroxyhexanoate & SI, 16. alpha-terpineol, 17. Decanal, 18. Carvone, 19. Methyl anthranilate (internal standard), 20. Valencene, 21. Nootkatone. SI = Silicon

Figure 2. Gas chromatograms of a simultaneous distillation extract of commercial pasteurized orange juice and a concentrated separatory funnel extract of fresh hand squeezed orange juice.

procedure is useful for evaluating the composition of orange juice.

Acknowledgments

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BIOCONVERSION OF MUSCADINE GRAPE WASTE INTO LIVESTOCK FEED

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Abstract. **The United States and the world at large are facing monumental problems in the future when considering the issue of waste disposal. Alternatives to landfills must be found. In this work two waste products, pomace and vine clippings from muscadine grapes, are being examined as potential sources of animal feed. The conversion of these wastes involves three stages of operation: size reduction, where the raw pomace (residue after grapes are crushed for juice and/or wine) or the grapevine clippings are reduced in particle size to increase available surface area; chemical pretreatment, where the materials under study are modified (usually in crystal structure) to a more suitable form for biological action; and bioconversion, which involves exposure of treated waste materials to cellulolytic fungi in order to increase overall digestibility. The work discussed here was primarily directed at the chemical pretreatment step, but the other steps are also discussed. Sodium hydroxide was effective in modifying the structure of the grape pomace. This was shown by measuring crude fiber percentage (by AOAC methods) of pomace before and after processing. A decrease in crude fiber percentage indicates an increase in available nutrients from the waste material. Later, this modified material would go through a bioconversion stage. The final stage is testing the product by conducting feed trials on ruminants.**

Introduction

The problems of waste disposal in the United States are tremendous. Landfills are being rapidly filled to capacity, and new landfills are an unpopular solution to waste disposal problems. Recycling and incineration are being attempted, each with its own advantages and disadvantages. New solutions must be found to the problems of wastes. Some of the wastes being disposed of are agricultural in nature. This project involves one set of wastes, those from grape and wine production. In the United States, the grape industry produced 5.7 million tons of grapes in 1989 with an estimated market value of $16.6 billion in 1990 (USDA, 1991). From this, an estimated 2.5 million tons of pomace (which comprises the pulps and seeds left over after pressing grapes for juice and wine) were discarded at a cost of $25 million. Instead of disposing the pomace as wastes, it could have been converted into 930,000 tons of animal feed with an estimated market value of $279 million. The two materials being examined for conversion to animal feed in this study are muscadine grape pomace and grapevine clippings.

Grape pomace has been investigated previously as a potential feed for livestock (Grujic et al., 1992; Aguilera, 1987; Famuyiwa and Ough, 1990; Famuyiwa and Ough, 1982). Generally the digestibilities determined for various ruminants have been seen as approximately half that of grains. Little work has been performed on vine clippings as a source for animal feed, and no studies have been done on muscadine pomace or vine clippings. The goal of this research project is to achieve improvement in digestibility through chemical pretreatment and solid state fermentation (SSF) of the grape wastes, and later to develop a system that can be used on the farm. As muscadine grapes are common in Florida, this project has the potential to generate wide spread interest among grape growers.

Grujic et al. (1992) reported on their attempt of SSF on grape pomace using *Chaetomium cellulolyticum* as an inoculum. However, their work focused on presterilized non-muscadine pomace. Improvements in their process can be made through pH control and pretreatment optimization.

Three fundamental steps are involved in bioconversion of agricultural residues to livestock feed. The first step is size reduction. In this step, the waste materials must be processed in such a way as to maximize the surface area available for chem-