agreement between the two instruments, yielding correlation coefficients of 0.999 for both individual instruments. The combination of both sets of data also yielded a correlation coefficient of 0.999, with only a slight increase in the standard error. Of the 806 values calculated from the CR-331 data, no deviation greater than +/−0.3 CN values were found from those measured with the CC.

These results were presented to the appropriate regulatory agencies to gain approval for the use of this colorimeter as an acceptable alternative to the CC.

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SOLVENT EXTRACTION PROCEDURE FOR THE RECOVERY OF VOLATILE CONSTITUENTS FROM ORANGE JUICE

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Abstract. **A solvent extraction procedure utilizing methylene chloride for the recovery of volatile flavor components from orange juice was investigated. Extraction shake times of 10, 20 and 30 min, centrifuge times of 10, 20 and 30 min at 16,000G, and juice temperature of 1.7 and 24.0°C were evaluated. Juice temperature had a significant effect on solvent recovery volume. Recovered solvent was analyzed by capillary gas chromatography and quantitative values for forty-one compounds were determined. The coefficient of variation was less than 5 percent for most compounds in replicated trials. The procedure provides a useful quality control method for quantitatively measuring changes in orange juice constituents ranging in volatility from ethyl acetate to nootkatone.**

Different approaches have been used to recover volatile constituents from juice for quantitative analysis by gas chromatography (G.C.). Moshonas and Shaw (1984; 1987) used direct injection of an aqueous distillate from orange juice. Marsili (1986) used a solid phase adsorption-methanol elution procedure to recover volatiles from orange juice. Schreier et al. (1981) used solvent extraction of the juice followed by concentration of the extract by distillation. Matthews and West (1988) used co-distillation of juice followed by solvent extraction. This procedure resulted in a concentrated extract which provided quantitative values for a wide range of volatile compounds. However, the recovery for some compounds was as low as 43 percent. In an attempt to obtain a high recovery level for most compounds and to reduce analysis time, it was decided to eliminate the distillation step and to use direct extraction of the juice. Some researchers had indicated this was possible without excessive interference from non-volatile compounds.

This experiment evaluated the solvent extraction of orange juice and analysis of the unconcentrated extract. Methylene chloride was chosen as the solvent due to it's ability to extract a wide range of terpenes, alcohols, aldehydes and esters from orange juice (Matthews and West, 1988).

Objectives were to (1) evaluate the effect of extraction shake times, centrifuge times and juice temperatures on

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solvent recovery; (2) develop standard curves for quantitation by gas chromatography of selected volatiles; and (3) determine percent recoveries and variance for incremental additions of volatiles.

Material and Methods

This study developed a rapid quality control analytical procedure to quantitate the volatiles in orange juice. A single solvent extraction, recovery of the solvent by centrifugation and analysis of the unconcentrated extract were selected for the procedure.

Materials & Equipment.

1. Methylene chloride: Fisher #D150 unstabilized
2. Internal standard; 1-heptanol; Aldrich #H280-5
3. *Shaker:* Burrell Wrist-Action Model CC
4. Centrifuge: Sorvall RC-5 Superspeed Refrigerated Centrifuge
5. Centrifuge Rotor: GSA
6. Gas chromatograph: Perkin Elmer Auto System Model 9000, 30 meter DB-1 column, 0.32 mm I.D., film 1 μM; inject 2 μl; 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 265°C; hold at 265°C to clean column for 10 minutes; injector temp 200°C; detector temp 325°C
7. Pasteurized orange juice from concentrate
8. Integrator: Perkin Elmer PE Nelson Model 1020, Standard method

Orange Juice Solvent Extraction Procedure.

1. Transfer 200 ml sample of chilled (35°F) orange juice to a 250 ml polypropylene centrifuge bottle.
2. Add 10 ml of methylene chloride containing 100 ppm 1-heptanol as internal standard.
3. Shake vigorously by hand for 30 seconds and then place on wrist-action shaker for 10 minutes (speed level 8 in 35°F cold room).
4. Immediately place centrifuge bottles in centrifuge set at 10°C. Centrifuge at 10,000 RPM for 10 minutes.
5. Remove water phase using suction pipette.
6. Remove methylene chloride phase (beneath pulp layer) using a Pasteur pipette and transfer to a 5 ml vial and cap tightly.
7. Analyze methylene chloride extract by capillary gas chromatography.

Table 1. Effect of shake time and centrifugation time on the recovery of methylene chloride extract.[z]

CENTRIFUGE TIME	SHAKE TIME 10 MIN.	20 MIN.	30 MIN.	MEAN
	RECOVERY (ML)			
10 MIN.	3.1	3.0	2.9	3.0
20 MIN.	3.1	2.9	2.8	2.9
30 MIN.	3.2	3.0	2.8	3.0
MEAN	3.1	3.0	2.8	

[z]10 ml methylene chloride added to 200 ml OJ, all samples centrifuged at 10,000 rpm, no significant difference at $p<0.05$.

Results and Discussion

There was no significant difference in solvent recovery for centrifuge times of 10, 20 and 30 min (*Table 1*). For shake times of 10, 20, and 30 min there was a slight decrease in solvent volume recovered with increase in shake time. Temperature of the juice sample (1.7°C and 24.0°C) had a significant effect on solvent volume recovered. At 1.7°C, solvent recovery was 0.45 to 1.05 ml greater than at 24°C (Table 2). Based on the above results, the procedure outlined under the Materials and Methods section was used for evaluating percent recovery for incremental additions of volatile flavor compounds.

Standard curves were determined for each of the compounds by gas chromatographic analysis of serial dilutions in 95% ethanol. The standard curves (Fig. 1) for α-terpineol, linalool, decanal, ethyl butyrate and methyl butyrate were linear for the 5 to 100 mg/ml concentration range evaluated. α-pinene gave the greatest response per unit weight and methyl butyrate the lowest.

The percent recovery and coefficient of variation (C.V.) for six volatile flavor compounds is given in Table 3. The values were determined with internal standard correction for solvent extraction and gas chromatographic analysis. α-pinene (83.8%) and decanal (73.7%) gave the lowest recovery values. Their coefficient of variation ranged from 1.57 to 4.42 percent.

Mean recovery values for ethyl butyrate (105.5%), octanol (105.3%), and α-terpineol (99.1%) were very good. The C.V. for ethyl butyrate and α-terpineol ranged from 1.4 to 5.2%. The C.V. for octanol was much greater (2.2 to 10.5%) since problems were encountered with peak identification and quantification by the G.C. procedure used. An unknown compound did not totally separate from octanol. Linalool had a mean recovery of 133% with C.V. ranging from 2.2 to 4.7%. The high recovery was attributed to extraction of an additional component from the orange juice. This excessive recovery value, when corrected by a standard additions plot, reduces the unspiked juice value for linalool to 1.98 ppm versus an uncorrected value of 2.45 ppm (Fig. 2).

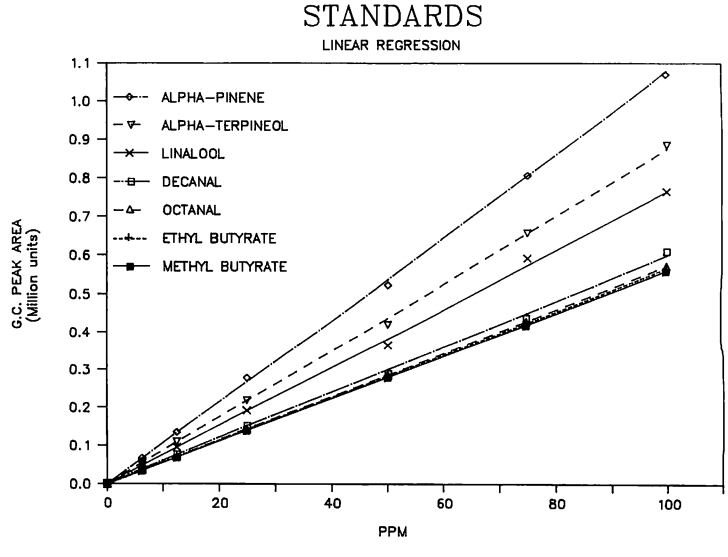

Fig. 1. Standard curve for capillary column gas chromatographic analysis of selected citrus juice volatile flavor constituents.

Table 2. Sample temperature effect on the recovery of methylene chloride extract.[z]

SAMPLE TEMPERATURE		RECOVERY (ML) SHAKE BY HAND	SHAKE BY HAND + MECHANICAL SHAKE	MEAN	+/−SD
1.7 C		4.1	3.0	3.53	0.58
		4.1	2.9		
	MEAN	4.1a	2.95a		
24 C		3.1	2.5	2.83	0.30
		3.2	2.5		
	MEAN	3.15b	2.5b		

[z]Mechanical shake, 10 min.; 10 ml solvent to 200 ml OJ, all samples 30 sec. vigorous shake by hand; centrifuged 10 min. at 10,000 rpm. Means within columns followed by the same letter are not significantly different ($p<0.05$).

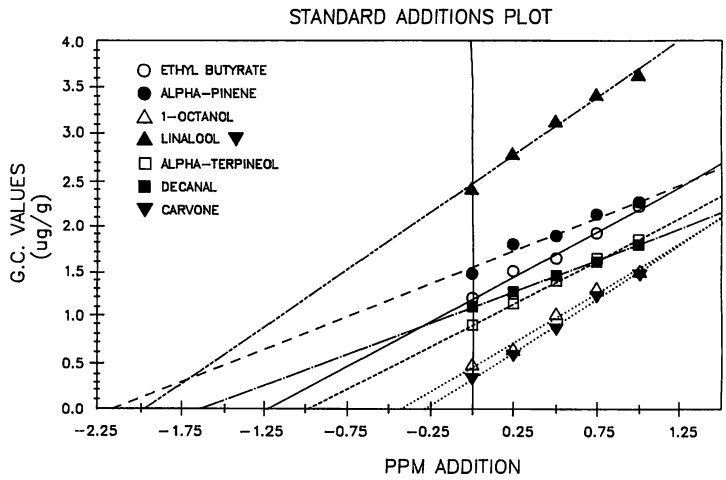

Fig. 2. Standard additions plot of solvent extract of orange juice with 0.25, 0.50, 0.75, 1.00 ppm addition of volatile flavor constituents.

The concentrations of the six compounds in the unspiked pasteurized juice were ethyl butyrate, 1.2 ppm; alpha pinene, 1.5 ppm; octanol 0.5 ppm; linalool, 2.5 ppm; alpha terpineol, 0.9 ppm; and decanal, 1.1 ppm. Fig. 3 is a plot of the values determined for unspiked juice and values for incremental additions of flavor compound. The recovery was linear for the added compounds.

Fig. 4 is a comparison of gas chromatograms for unspiked juice and juice to which 1 ppm each of ethyl butyrate, α-pinene, octanol, linalool, decanal and carvone have been added. The chromatogram provides a quantitative value for most compounds of interest from ethyl acetate to nootkatone. The 1 ppm addition is easily discernable and reproducible. There were no extraneous peaks.

Table 3. Percent recovery and coefficient of variation for volatile flavors in orange juice with incremental addition of volatiles.

	PPM ADDED V/V 0.00	0.25	0.50	0.75	1.00	MEAN RECOVERY
			Ethyl Butyrate			
MEAN/4	1.20	1.50	1.68	1.94	2.21	
INCREASE	0.00	0.31	0.48	0.75	1.02	
SD	0.02	0.05	0.01	0.10	0.07	
C.V. (%)	1.67	3.33	0.00	5.15	3.17	
RECOVERY (%)		124.00	96.00	100.00	102.00	105.5
			alpha-Pinene			
MEAN/4	1.53	1.79	1.91	2.14	2.25	
INCREASE	0.00	0.26	0.39	0.61	0.73	
SD	0.03	0.06	0.03	0.08	0.06	
C.V. (%)	1.96	3.35	1.57	3.74	2.67	
RECOVERY (%)		104.00	76.00	82.43	73.00	83.9
			1-Octanol			
MEAN/4	0.49	0.71	1.05	1.34	1.51	
INCREASE	0.00	0.22	0.57	0.86	1.03	
SD	0.07	0.04	0.11	0.03	0.10	
C.V. (%)	14.29	5.63	10.48	2.24	6.62	
RECOVERY (%)		88.00	114.00	116.22	103.00	105.3
			Linalool			
MEAN/4	2.45	2.78	3.13	3.44	3.68	
INCREASE	0.00	0.33	0.68	0.98	1.22	
SD	0.10	0.13	0.07	0.12	0.11	
C.V. (%)	4.08	4.68	2.24	3.49	2.99	
RECOVERY (%)		137.50	136.73	134.25	125.77	133.6
			alpha-Terpineol			
MEAN/4	0.92	1.18	1.40	1.66	1.86	
INCREASE	0.00	0.26	0.48	0.73	0.94	
SD	0.02	0.03	0.02	0.06	0.04	
C.V. (%)	2.17	2.54	1.43	3.61	2.15	
RECOVERY (%)		104.00	97.96	98.65	95.92	99.1
			Decanal			
MEAN/4	1.12	1.30	1.47	1.63	1.81	
INCREASE	0.00	0.18	0.35	0.51	0.69	
SD	0.03	0.04	0.04	0.05	0.08	
C.V. (%)	2.68	3.08	2.72	3.07	4.42	
RECOVERY (%)		75.00	74.47	71.83	73.40	73.7

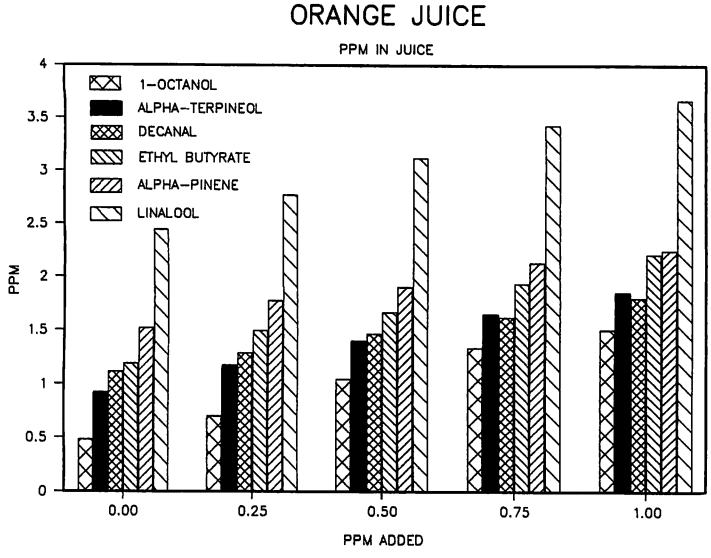

Fig. 3. Concentration of selected volatile constituents extracted from chilled orange juice and chilled orange juice with incremental addition of the volatiles.

The sample preparation procedure for G.C. analysis required less than one hour to prepare four samples. The G.C. analysis time was 52 minutes per sample. The procedure worked well, with good reproducibility and acceptable analytical variance. Some non-volatile compounds are extracted in the methylene chloride and can result in an erratic baseline and spurious peaks. Overnight bake-out of the G.C. column at 310°C restored a stable base line.

The procedure provides a relatively rapid quality control method for flavor volatiles in orange juice. The avoidance of sample distillation and extraction solvent concentration is time saving and reduces the variance potential. The procedure provides a useful quantitative measure for volatiles ranging from ethyl acetate to nootkatone.

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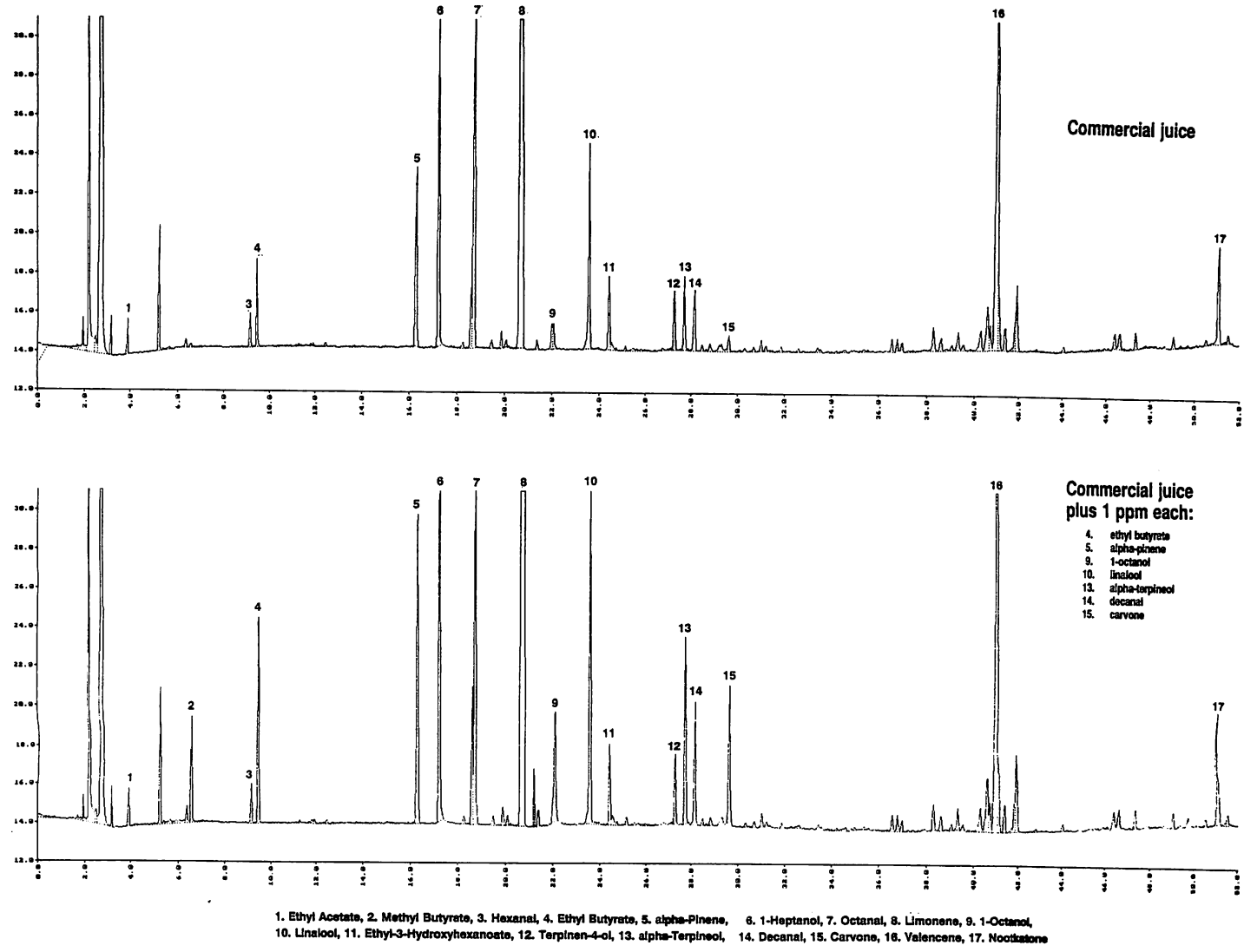

Fig. 4. Gas chromatographic trace of volatiles extracted by methylene chloride from commercial orange juice and the juice to which 1 *ppm each* of ethyl butyrate, α-pinene, octanol, linalool, decanal and carvone were added.

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COMPARISON OF OLIGOSACCHARIDES GENERATED DURING SUCROSE INVERSION AND CITRUS JUICE FERMENTATION

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Abstract. **Pure citrus juices contain few oligosaccharides (OS), which are mostly disaccharides (group I). During fermentation a second group of OS (II), predominantly trisaccharides, is generated. Concentrated juice reconstituted to 11.8° Brix with water was kept at 25°C for up to 144 hr and allowed to ferment without the introduction of extraneous microorganisms. OS were formed after 30 hr, at about the same time as the major sugars began to be utilized significantly. OS formation reached a maximum at about 50 hr, at which time they began decreasing. All saccharides were eliminated after 72 hr. During fermentation, the concentration of the preexisting Group I OS increased significantly. One of the OS belonging to group II showed a major increase while the others increased moderately or remained unaffected. Previous studies have shown that group II OS are generated during acid sucrose inversion and can be used to quantitate the addition of medium invert sugar (MIS) to juices. Fermentation generated OS interfere with those originating from added MIS. However, differences in OS composition, as well as the production of alcohol and inisitol, should allow differentiation between acid and fermentation induced OS.**

During concentration, when citrus juice is subjected to a temperature of 99°C for about 10 sec, most microorganisms are destroyed (Faville et al., 1951). However, a microbial population of up to 10000 counts per ml can still be found in juices reconstituted from concentrate (McAllister, 1980). They can eventually induce spoilage, particularly after reconstitution (Faville and Hill, 1951). During fermentation, sucrose is first hydrolyzed by invertases before being metabolized with the other sugars. These enzymes have been shown to have a transfructosidase activity that catalyses the synthesis of various OS (Hassid and Ballou, 1957). Previous HPLC studies of MIS and citrus juice OS (Cancalon, 1992a; Swallow et al., 1991; White and Cancalon, 1992) have revealed two groups of peaks. Group I eluded between 15 and 22 min, contains a mixture of di and trisaccharides, and is found in both juices and MIS. In juices, the origin of these peaks is very complex and may be due to fruit enzymes, microbial activity or acid hydrolysis within the fruit (Echeveria, 1990). The second group, mostly trisaccharides, eluded between 22 and 30 min, is largely absent from fresh juice, but contains the OS generated by keeping sugars in acidic solutions. The presence of Group II OS has been used to monitor the addition of exogenous MIS to citrus juices (Cancalon, 1992a; Swallow et al., 1991); however, OS formed during microbial activity have been shown to interfere with the analysis (Cancalon and Bryan, 1991).

In this study, OS generated during citrus juice fermentation were examined and compared with those produced during acid inversion.

Materials and Methods

Sample Preparation. Concentrated orange juice (50.8 °Brix) was diluted with HPLC grade water to 11.8°Brix and allowed to ferment at 25°C. Samples were examined at various times during fermentation. Preparation was the same as described previously (White and Cancalon, 1992). Orange juice samples were diluted to 6°Brix and centrifuged at 10000 g for 15 min. The supernatant was passed successively through Dowex AG50W-X8 (100-200 mesh) H^+-form resin, Dowex AG 1-X4 (100-200 mesh) (Bio-Rad, Richmond, CA) formate-form resin, a Sep-Pak C18 cartridge (Millipore Co., Milford, MA) and finally filtered through a 0.45 m nylon Acrodisc membrane (Gelman Sciences, Inc., Ann Arbor, MI).

HPLC Conditions. Samples were analyzed with a Waters (Milford, MA) HPLC system consisting of an Ultra WISP Model 715, a Model 625 LC pumping system, and a Model 464 metal-free electrochemical detector, set at a range of 50 A. Control of the system and data acquisition were performed with a Waters 820 Maxima 386SX work station.

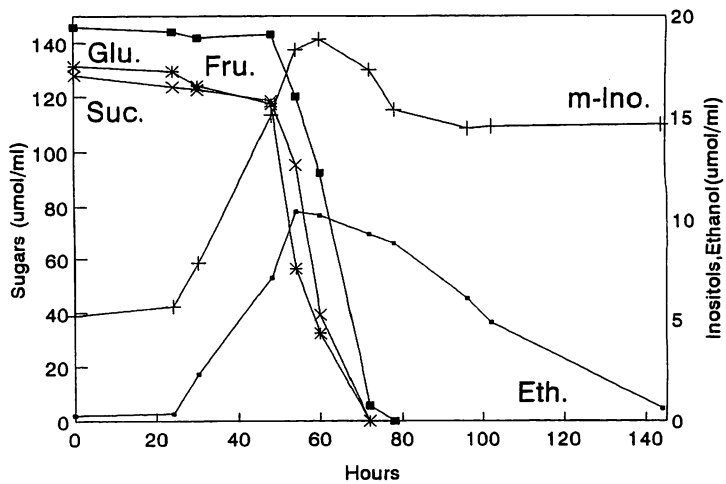

Figure 1. Changes in juice composition during fermentation. Glu: glucose, Fru: fructose, Suc: sucrose, m-Ino: meso-inositol, Eth: ethanol.