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Technical Note

A Simple Headspace–Gas Chromatographic Method for the Quantitative Determination of Organic Volatiles of Fresh Orange Juice

ABSTRACT

A fast quantitative method of determination of acetaldehyde, ethyl acetate, methanol and ethanol, in freshly squeezed Valencia orange juice using headspace–gas chromatography was developed. Filtered and unfiltered juices could be analysed using standard addition and direct calibration methods.

INTRODUCTION

Quantitative analysis of some simple volatiles in orange and other citrus fruit juices plays an important part in evaluating their freshness (Japikse *et al.*, 1986). The amounts of methanol, ethanol, acetaldehyde, ethyl acetate and other alcohols and esters are important in determining and controlling quality or checking adulterated products. A rapid quantitative method using a headspace–capillary gas chromatographic technique suitable for routine analysis of these volatiles in Valencia orange juice is now described.

MATERIALS AND METHODS

A Hewlett–Packard 5890A gas chromatograph equipped with capillary inlet system and flame ionisation detector was used. Headspace sampling was carried out using a Hewlett–Packard 19395A headspace autosampler. The chromatographic and headspace sampler conditions used are shown in Table 1.

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TABLE 1
Gas Chromatographic and Automatic Headspace Sampler Conditions

Gas chromatographic conditions:	
Carrier gas	Nitrogen
Carrier gas flow (column)	1·24 ml/min
Split ratio	1:30
Septum purge	3 ml/min
Injector temperature	250°C
FID detector temperature	300°C
Oven temperature:	
Temperature 1 (time)	40°C (0 min)
Rate 1 (40–120°) 5°/min	
Rate 2 (120–200°) 15°/min	
Temperature 2 (time)	200° (5 min)
FID hydrogen flow	28 ml/min
FID air flow	450 ml/min
Automatic headspace sampler conditions:	
Equilibration time	1–6 h
Bath temperature	60°C
Valve/loop temperature	65°C
Sampling interval	30 min
Volume of headspace vapour injected	1 ml
Volume of vial	10 ml
Carrier gas (nitrogen) flow	30 ml/min
Auxillary pressure	1·6 bar

The column used was a 30 m × 0·32 mm i.d. × 0·25 μm fused silica capillary Supelcowax-10 (Supelco, USA) polar column. A Hewlett–Packard 3393A computing integrator was used for recording chromatograms.

Analytical grade acetaldehyde, ethyl acetate, ethanol and methanol were used as standards without further purification. Double distilled water, which was distilled over $KMnO_4$, was used to prepare the standard solutions. Valencia oranges, as well as Navel and mandarin oranges from Australia, California, Portugal, Malaysia and Thailand, obtained from a local market, were used for the study.

Hand-squeezed orange juices, filtered (through cheese-cloth to remove seeds and coarse pulp) or unfiltered (5 ml) were measured into a 10 ml sampling vial and immediately capped with air-tight teflon-coated septum. Two methods of measurement were used for quantitative determination of the volatiles. In the direct calibration method, a calibration graph was constructed from a series of standards in distilled water. The concentration of the standards was then read off from the calibration graph. In the standard addition method, known amounts of a standard were added to the sample and the headspace concentration of this measured. A standard addition graph was constructed and the concentration of the standard in the sample was obtained by extrapolation. The results of the two methods were compared using the Student t-test.

All samples and standards were analysed after 1 h equilibration at 60°C in the headspace sampler. All determinations were carried out at least in duplicate, and five different batches of each type of orange were analysed.

RESULTS AND DISCUSSION

Preliminary studies showed that there was little or no fluctuation in the contents of the four volatiles determined after 1 h equilibration at 60°C in the headspace autosampler. The percentage standard deviation obtained was $< 5\%$ for all four compounds in both the samples and the standards. All determinations were, therefore, carried out after this period of equilibration.

Sampling of the volatiles in the headspace was carried out using an autosampler so as to minimise injection error. This also eliminates cumbersome and time-consuming sample preparation and hence removes introduction of artifacts as well as the uncertainty of percentage recovery. The method thus did not involve injection of large amounts of aqueous sample

TABLE 2
Acetaldehyde, Ethyl Acetate, Methanol and Ethanol in Filtered Valencia Orange Juice Determined by Standard Addition (S) and Direct Calibration (D) Methods

Origin	*Concentration* (*μl/litre*)							
	Acetaldehyde		*Ethyl acetate*		*Methanol*		*Ethanol*	
	S	*D*	*S*	*D*	*S*	*D*	*S*	*D*
Sunkist	31·0	34·3	1·4	1·7	ND		1 050	1 250
(California)	28·0	28·0	0·4	0·4	150	170	1 800	1 900
	24·3	23·5	ND		52·0	47·0	1 500	1 450
Riverland	19·0	17·5	ND		76·0	72·0	2 200	2 150
(Australia)	52·5	55·0	1·7	1·7	ND		5 200	5 300
	22·5	22·5	1·1	1·1	ND		2 100	2 050
(Thailand)	5·8	5·8	0·2	0·2	ND		43	42
Student t-test at 95% confidence level	$t = 0·756$ ($t_{tab} = 2·447$, six degrees of freedom)		$t = 1·001$ ($t_{tab} = 2·776$, four degrees of freedom)		$t = 1·137$ ($t_{tab} = 4·303$, two degrees of freedom)		$t = 1·559$ ($t_{tab} = 2·447$, six degrees of freedom)	

ND, not detected.

TABLE 3
Acetaldehyde, Ethyl Acetate, Methanol and Ethanol in Filtered (F) and Unfiltered (U) Juice Obtained by Direct Calibration Method

Origin	*Concentration* (*μl/litre*)							
	Acetaldehyde		*Ethyl acetate*		*Methanol*		*Ethanol*	
	F	*U*	*F*	*U*	*F*	*U*	*F*	*U*
Sunkist	7·8	11·0	3·6	5·1	150·5	213·0	5 050	6 700
(California)	18·0	23·5	1·3	1·1	35·0	47·0	2 270	1 450
Riverland (Australia)	17·5	19·3	ND	ND	72·0	77·0	2 150	2 350
Student t-test at 95% confidence level	t = 0·905 (t_{tab} = 4·303, two degrees of freedom)		t = 0·801 (t_{tab} = 4·303, two degrees of freedom)		t = 1·373 (t_{tab} = 2·776, four degrees of freedom)		t = 1·848 (t_{tab} = 4·303, two degrees of freedom)	

ND, not detected.

which could be detrimental to the capillary columns, thereby affecting the sensitivity of the FID.

Tables 2 and 3 show the concentration of the four volatiles of some Valencia oranges obtained by both the direct calibration and standard addition methods. Both methods gave comparable results, indicating that there was little if any matrix effect in the samples. The amounts of the volatiles varied very significantly even among oranges of the same origin. This had also previously been observed by Lund *et al.* (1981).

The standard addition method is generally considered to be more reliable as it minimises the undesirable matrix effect. However, the Student t-test

TABLE 4
Acetaldehyde, Ethyl Acetate, Methanol and Ethanol in Orange Juice Determined by Direct Calibration Method

Origin	*Concentration* (*μl/litre*)			
	Acetaldehyde	*Ethyl acetate*	*Methanol*	*Ethanol*
Navel (Malaysia)	6·0	ND	14·8	400
Mandarin (Thailand)	11·5	0·5	24·1	450
Mandarin (Australia)	30·1	8·0	138·1	3 500
Valencia (Australia)	24·5	ND	46·8	1 888
Valencia (Portugal)	15·0	0·4	ND	1 325

ND, not detected.

showed that there was insignificant difference between the two methods of quantitation (Tables 2 and 3), thus indicating little or no matrix effect in the orange juice samples. Table 3 also shows no significant differences in the concentrations of these volatiles in filtered and unfiltered juices. The concentrations of the four volatiles in other types of oranges are given in Table 4.

REFERENCES

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