Short Communication

Analysis of packaged orange juice volatiles using headspace gas chromatography

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ABSTRACT

    It has been demonstrated that simple headspace gas chromatography is adequate for determining the change in concentration of volatile components during shelf-life testing of orange juice. α-Pinene, octanal and *d*-limonene concentrations were measured during shelf-life testing. The time required to reach equilibrium seemed to increase with increase in temperature. A 15-min sample heating time at 50°C was determined to be the optimum headspace analysis conditions for orange juice.

INTRODUCTION

    Analytical procedures that have been used in characterizing flavor quality include solvent extraction [1], the purge and trap method [2] and direct-injection gas chromatography (GC) [3]. These methods are complex and time consuming compared with equilibrium headspace analysis. A disadvantage of equilibrium headspace analysis has been the lack of sensitivity. However, it has been reported that with certain samples, equilibrium headspace analysis yielded results equal to those given by the purge and trap method [4,5]. Lum *et al.* [6] measured concentrations of volatiles in different types of fresh orange juice using headspace GC. However, no comparison was made between the results of headspace GC and other methods of determining concentrations of volatiles. Very little information is available about the effects of heating time and temperature on headspace analysis and it is difficult to select obtimum analytical conditions without information on these variables.
    This study was performed to examine the feasibility of using a simple headspace method in place of more complicated and time-consuming methods to determine volatile contents in a shelf-life investigation of packaged orange juice. A further aim was to investigate the effects of sample heating time and temperature on headspace analysis.

EXPERIMENTAL

Headspace analysis

Sample preparation. A glass vial of internal volume 38 ml was hermetically sealed by crimp-sealing an aluminum cap with a Teflon-coated silicone-rubber septum. Each vial was tested for leaks by pressurizing it with nitrogen to 30 p.s.i.a. and placing it under water. The pressure was released by using a syringe needle. After testing for leaks and flushing with nitrogen for 15 min, a 25-ml sample of orange juice was taken from a carton by syringe and injected into the vial. Each sample was heated in a water-bath at 50, 60 and 70°C to establish equilibrium. A headspace volume of 0.5 ml was withdrawn from the vial with a 0.5-ml gas-tight syringe and injected into the gas chromatograph. The gas-tight syringe was preheated to a temperature 10°C above the temperature at which the vial was equilibrated.

Gas chromatography and mass spectrometry. A Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard Model 3396A integrator was used. The column was a Hewlett-Packard HP-5 fused-silica non-polar capillary column with cross-linked 5% phenyl methyl silicone (25 m × 0.31 mm I.D. with a 1.05-μm layer thickness). Helium was used as the carrier gas at a flow-rate of 2 ml/min. The injection and detector temperatures were 150 and 200°C, respectively. The oven temperature was programmed as follows: 50°C isothermal for 3 min, then increased at 6°C/min to 185°C and held at that temperature for 5 min. Methane was used as an internal standard (0.01-ml injection). The threshold was set at 4, peak width at 0,.04, chart speed at 0.5 cm/min, attenuation at 5 and the area reject at 30 000. Peaks were compared by means of the retention times in each sample during the storage period against a set standard. Calibration graphs were prepared by adding known amounts of pure compounds, then injecting 0.5 ml of completely the vaporized compounds into the GC system. Headspace concentrations were determined using these calibration graphs.

The same column and procedures as above were used for gas chromatography–mass spectrometry (GC–MS) for identification of the peaks, with a Hewlett-Packard Model 5971A mass spectrometric detector.

Storage test

Cartons aseptically filled with orange juice were obtained directly from a manufacturing plant. The cartons were made of paper board sandwiched between two polymer layers. Orange package samples were provided by Westvaco (Laurel, MD, U.S.A.). The samples were placed in a refrigerator at 4°C on arrival.

Microbiological analysis. The standard AOAC pour-plate technique [7] was used to determine the aerobic microbial concentration in the juice. Samples were drawn from the package with sterilized syringes and dilutions of 1:10, 1:100, 1:1000 and 1:10 000 were prepared in 0.1% aqueous peptone. Potato dextrose agar and orange serum agar were prepared and sterilized in an autoclave at 121°C for 15 min. The pH of the orange serum agar was adjusted to 4.9 with 10% tartaric acid solution just before pouring. Volumes of 1 ml of diluted orange juice samples were placed in petri dishes and about 20 ml of culture media with a temperature of 45°C were poured in, shaken and left to solidify. Duplicate plates were prepared for each sample for both media and were placed in an incubator at 35°C. Negative control plates were also made for the

media in order to observe any contamination. A positive control plate was prepared by inoculating it with spoiled orange juice. Colonies were counted after 48 h of incubation and were reported as counts per milliliter of juice.

d-Limonene analysis. The recoverable oil concentration in the orange juice was measured using the Scott method (AOAC) [8]. Isopropanol and water were added to 25 ml of orange juice and the mixture was distilled. Dilute hydrochloric acid and methyl orange were added to the distillate and the mixture was titrated with 0.0247 *M* potassium bromide–bromate solution until the color disappeared. As about 90% of the recoverable oil is *d*-limonene, it was assumed that 1 ml of titrant is equivalent to 0.0010 ml (0.00084 *g*) of *d*-limonene.

RESULTS AND DISCUSSION

A representative equilibrium headspace gas chromatogram of orange juice at 50°C is shown in Fig. 1. The chromatogram shows that there is no need for solvent extraction or the purge and trap method to obtain a good chromatographic flavor profile. Three of the major peaks were identified by GC–MS as α-pinene, octanal and *d*-limonene, as listed in Table I. These are compounds that are contributors to orange juice flavor [1,9].

Headspace samples from hermetically sealed vials containing orange juice were analyzed at different temperatures and time intervals to determine the heating time at which equilibrium is reached and the optimum analysis temperature. The plots of heating time *versus* headspace concentration for α-pinene, octanal and *d*-limonene are
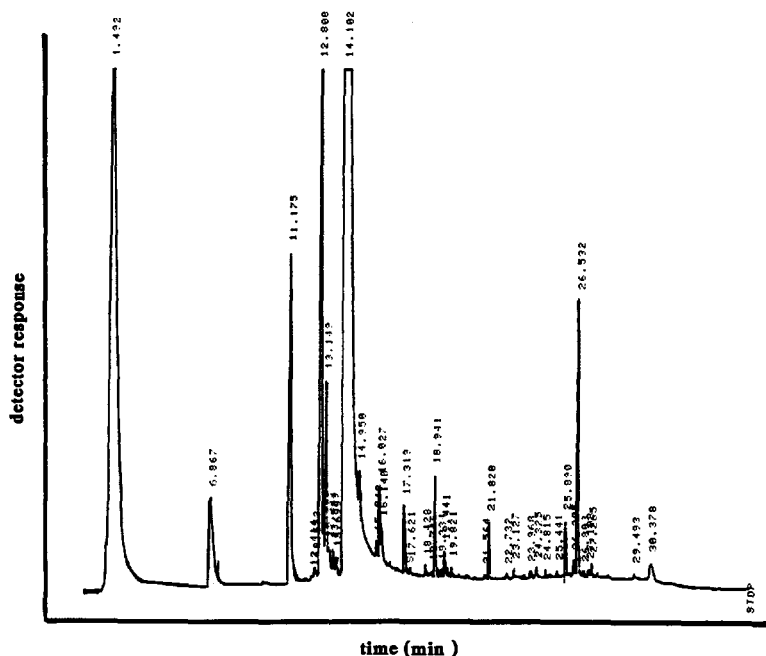

Fig.1. Typical equilibrium headspace gas chromatogram of orange juice at 50°C.

TABLE I

IDENTIFICATION OF GC PEAKS BY GC–MS

Peak No.	Retention time (min)	Compound
1	11.2	α-Pinene
2	12.8	Octanal
3	14.2	d-Limonene

shown in Fig. 2. Octanal required the longest time (15 min) to reach thermodynamic equilibrium at 50°C and both α-pinene nd d-limonene required *ca.* 10 min (Table II). It is difficult to determine the exact equilibrium time because of variatons between duplicate data (Fig. 2 and Table III). However, Fig. 2, seems to indicate that the time required to reach equilibrium increases with increase in temperature. This may be
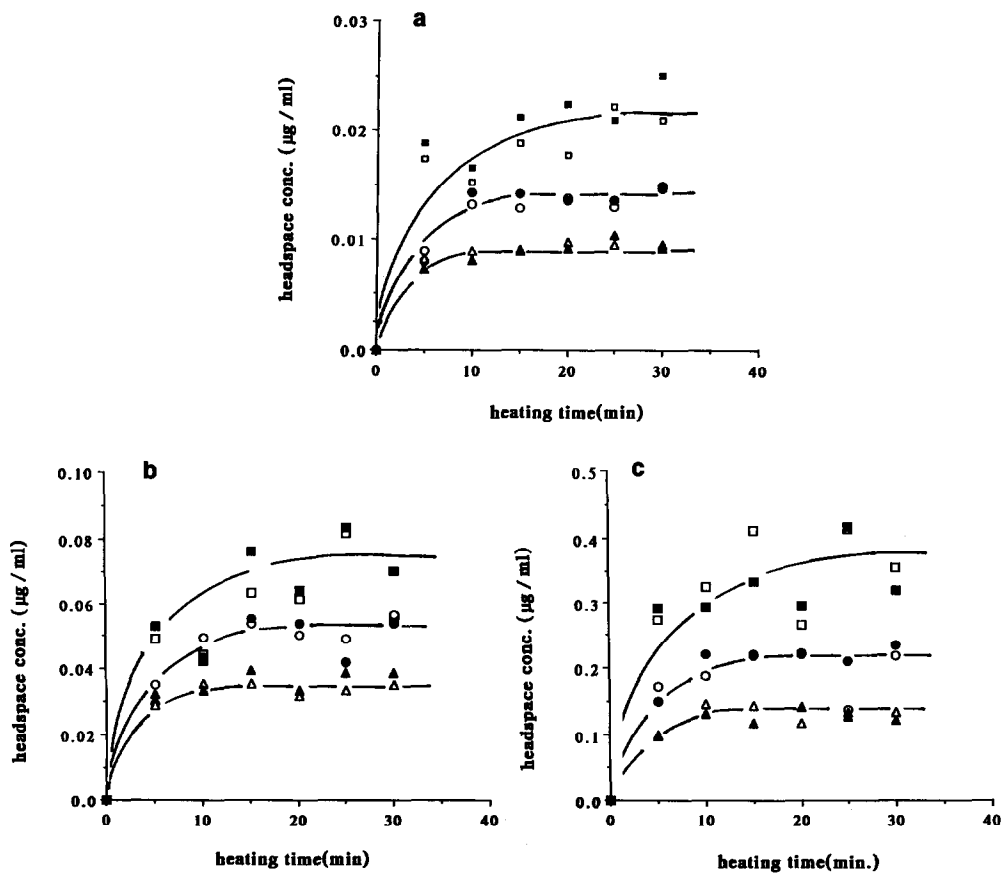

Fig. 2. Effect of heating time and temperature on headspace concentration of (a) α-pinene, (b) octanal and (c) d-limonene. □, ■ = Duplicate data at 70°C; ○, ● = duplicate data at 60°C; △, ▲ = duplicate data at 50°C.

TABLE II

TIME REQUIRED TO REACH EQUILIBRIUM CONCENTRATION IN SEALED VIALS

Compound	Approximate time required (min)		
	50°C	60°C	70°C
α-Pinene	10	15	30
Octanal	15	20	30
d-Limonene	10	15	30

TABLE III

EFFECT OF TEMPERATURE ON THE PERCENT VARIATION IN THE HEADSPACE ANAL-YSIS

Percentage variation is defined as the difference between duplicate experimental values divided by the mean of these values.

Compound	Mean variation (%)		
	70°C	60°C	50°C
α-Pinene	10.7	3.1	2.4
Octanal	7.5	5.5	3.9
d-Limonene	8.5	5.6	3.2

because the increase in the mass transfer rate is relatively smaller than the increase in equilibrium headspace concentration with increase in temperature.

As expected, the concentrations in the headspace increased with increase in temperature. Fig. 3 shows the plot of the logarithm of equilibrium headspace concentration *versus* inverse temperature. As partial pressure is linearly related to concentration, the linear plot indicates that the temperature effect on headspace
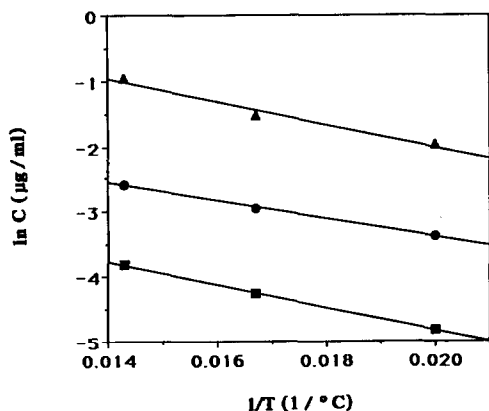

Fig. 3. Effect of temperature on equilibrium headspace concentration. ■ = α-Pinene; ● = octanal; ▲ = d-limonene.
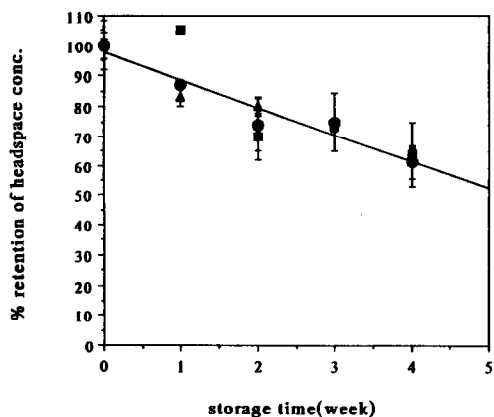
Fig. 4. Retention of orange volatile of orange juice packaged in paperboard cartons at 4°C. Symbols as in Fig. 3.

concentration follows the Clausius–Clapeyron relationship. However, the variation in headspace concentration between duplicates increased with increase in temperature (Table III). This may be due to condensation in the syringe and increased leakage in the crimp seals at higher temperature. If the sensitivity of the detector is not the limiting factor in headspace analysis, more consistent results can be obtained with this method at lower temperature. From the above results, a 15-min sample heating time at 50°C was selected as the optimum headspace analysis conditions for orange juice.

A shelf-life study of the orange juice was performed for a 4-week period to test the application of headspace analysis in monitoring volatiles. Orange juice was packaged in cartons prepared from paper board sandwiched between polymer layers and stored at 4°C. The headspace concentration was normalized and percentage retentions of all three compounds were plotted against storage time as shown in Fig. 4.
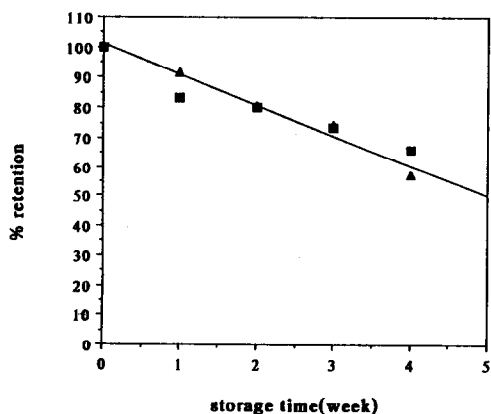
Fig. 5. Comparison of percentage retention of d-limonene in orange juice during storage at 4°C determined by (■) headspace analysis and (▲) titration.
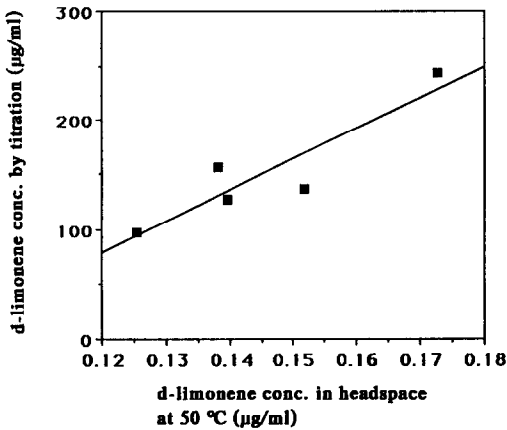
Fig. 6. Correlation of d-limonene concentration in headspace at 50°C and concentration in orange juice determined by titration. Regression equation: $y = -262.30 + 2894.9x$; $R^2 = 0.838$.

The results seem to indicate that the headspace concentrations of all three compounds decreased at about the same rate. These results are in agreement with studies performed on flavor absorption by polymeric materials during storage [2,3,10,11]. Microbial growth, which has a major effect on the orange juice quality, was not observed during the period of the shelf-life study. The positive control showed a growth of microorganisms that were too numerous to count.

The concentration in the gas phase, C_g, is related to that in the liquid phase, C_l, by the simple partition law

$$C_g = C_l/K_p$$

where K_p is the partition coefficient. Therefore, the concentration in the gas phase is representative of the concentration in the liquid phase if K_p is constant over the range of concentrations being determined. The d-limonene concentration in the liquid phase was determined for the same orange juice samples as were subjected to headspace analysis. This experiment was performed to see if the gas-phase concentration is representative of the liquid-phase concentration for d-limonene over the concentration range in the shelf-life study (150–250 μg/ml). As shown in Fig. 5, the retention of d-limonene during 4 weeks of storage was almost the same for both methods of analysis. This indicates that the gas-phase concentration of d-limonene was representative of the concentration in the orange juice and the simple method of headspace analysis is able to measure changes in the concentrations of volatiles orange juice.

Correlation of the d-limonene concentration in orange juice determined by titration and headspace analysis at 50°C is shown in Fig. 6. An R^2 value of 0.838 was obtained from the regression analysis.

CONCLUSION

About 10–15 min were required to reach thermodynamic equilibrium in the headspace analysis of orange juice. The effect of temperature on the headspace

concentration of volatiles from orange juice follows the Clausius–Clapeyron relationship. Therefore, higher headspace concentrations can be obtained at higher temperatures. However, the increase in temperature increases the experimental variation, and also increases the time required to reach equilibrium. The optimum heating time and temperature for headspace analysis were determined to be 15 min 50°C for orange juice. The results of the storage study of orange juice packages and comparison with d-limonene determination indicate that a fairly simple headspace analysis method is adequate for determining the changes in concentration of volatiles during shelf-life testing of orange juice.

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