JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Optimization of Solid-Phase Microextraction Analysis for Studying Change of Headspace Flavor Compounds of Banana during Ripening

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The changes of headspace flavor compounds of banana during ripening were studied by a solid-phase microextraction (SPME) method. Three temperatures, 20, 25, and 30 °C, were used to investigate the temperature effect on the changes of headspace flavor compounds of banana during ripening over a period of 8 days. Banana juice concentration, salt concentration, time, and temperature were investigated for optimizing the SPME method. The most suitable concentrations of banana juice and salt were 33.3 and 20%, respectively. The optimal temperature and time are about 50 °C and 48 min, respectively. Increasing ripening temperature could accelerate ripening rate. Ethanol developed most rapidly at 30 °C, whereas amounts of the other investigated flavor compounds stored at 25 °C were greater than those of the ones stored at 20 or 30 °C.

KEYWORDS: Solid-phase microextraction (SPME); banana; headspace flavor compounds; ripening; response surface methodology; optimization

INTRODUCTION

Flavor is one of the most important attributes of foods in determining consumer acceptance. Many methods have been used for flavor volatile analysis. The most typically utilized methods for extraction and preconcentration are headspace techniques (*1*), purge-and-trap (*2*), liquid−liquid extraction (*3*), and simultaneous distillation and extraction (*4*). The majority of these methods are time-consuming, require exhaustive concentration steps, have memory effects, and/or require dedicated headspace sampling devices (*5*).

An alternate sampling methodology, solid-phase microextraction (SPME), has the potential to reduce the time investment in sampling and should work well in combination with rapid separation and detection systems (*6*). SPME has been applied to analyze flavor in fruits (*7*), vegetable oils (*8*), and orange juice (*9*).

In the fruit-producing or -processing industry, good analytical methods are crucial to the success of any quality control during storage, processing, and/or product development studies. Therefore, a rapid, simple, and inexpensive technique for extracting and preconcentrating fruit flavor can be useful. Banana is one of the most common fruits in the tropical zones; it is nutritious, with a pleasant flavor, and consumed worldwide (*4*). As to banana flavor analysis, a simultaneous distillation and solvent extraction method was used by Shiota (*4*) to isolate volatiles of mature banana. A headspace solid-phase microextraction (HS-SPME) method has been used for analysis of volatiles of banana with a certain degree of maturity (*10*, *11*), but the SPME conditions in these studies need a more systematical study for optimization. In addition, the methods used in the studies for isolating volatiles of banana during ripening have been mainly based on purge and trap (*12*, *13*) and dynamic heaspace sampling (*1*). Banana is a climacteric fruit, so ripening conditions such as temperature and time will affect the volatile composition at each stage of ripening (*13*). Esters make the main contribution to the characteristic banana aroma (*14*). The banana-like components have been attributed to amyl esters; the fruity ones to butyl esters; and the green-note ones to pentyl and hexyl alcohols, aldehydes, and ketones (*15*). The present work aims to optimize the HS-SPME method for banana flavor analysis and to study the change of flavor compounds during ripening by this method.

MATERIALS AND METHODS

Materials. Taiwanese bananas (*Musa sapientum* L. cv. Pei-Chiao) were obtained from a banana farm in Ping-Tung, Taiwan. Isobutyl acetate, isoamyl alcohol, hexanal, isoamyl acetate, isobutyl butyrate, butyl butyrate, ethanol, and ethyl isovalerate of reagent grade were purchased from Aldrich Chemical Co. (Milwaukee, WI). An SPME fiber coated with 100 μm poly(dimethylpolysiloxane), an SPME holder, and micro stirring bars (10 × 3 mm) were purchased from Supelco Inc. (Bellefonte, PA). Teflon septa, plastic caps, and glass serum bottles (20 mL) were purchased from Tung-Kung Glass Co. (Hsinchu, Taiwan).

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10.1021/jf010891+ CCC: $22.00
Published on Web 01/11/2002

Table 1. Two-Factor Central Composite Rotatable Design

	coded value		real value	
run	X_1 time (min)	X_2 temp (°C)	X_1 time (min)	X_2 temp (°C)
1	–1	–1	20	35
2	–1	1	20	65
3	1	–1	60	35
4	1	1	60	65
5	0	–1.414	40	28.8
6	0	1.414	40	71.2
7	–1.414	0	11.7	50
8	1.414	0	68.3	50
9	0	0	40	50
10	0	0	40	50
11	0	0	40	50
12	0	0	40	50

Effect of Banana Concentration on Flavor Compound Quantity. Bananas were peeled and sliced and then homogenized with distilled water to make banana juice at three concentrations: 16.7, 33.3, and 66.7% (w/w). The banana juice was divided and placed into recloseable polypropylene bags (~15 g per bag) and stored at −20 °C. One bag only was removed and unfrozen with flowing tap water for 10 min at each analysis. An aliquot of 10 g of each concentration of banana juice was transferred into a 20 mL glass bottle and sealed with a Teflon septum and a plastic cap. All samples were prepared in duplicate.

Effect of Salt Concentration on Flavor Compound Quantity. The 33.3% banana juice was chosen to study the salt effect. Sodium chloride was added to the juice to make four levels of concentrations: 5, 10, 20, and 30% (w/w). All samples were prepared in duplicate.

Effects of Temperature and Time on Flavor Compound Quantity. A central composite rotatable design (CCRD) coupled with response surface methodology (RSM) (*16*) was employed to study the effects of time (variable x_1) and temperature (variable x_2) on the quantity of flavor compounds (response) adsorbed by the SPME fiber. The experimental design is shown in **Table 1**. The banana juice concentration used was 33.3% with 20% salt added.

Headspace Flavor Compound Analysis of Banana by SPME-GC. A 10 g aliquot of banana juice was placed into a 20 mL glass bottle containing a micro stirring bar. The sample bottle was airtightly sealed with a Teflon septum and a plastic cap. The SPME fiber coated with 100 μm of poly(dimethylpolysiloxane) was manually inserted into the headspace of the sample bottle. The SPME coating, which isolated headspace flavor compounds by adsorption, was injected into the GC injection port at 200 °C and kept for 15 min for the desorption of flavor compounds. The injection port was lined with a 0.75 mm i.d. splitless glass liner. The desorbed flavor compounds were separated by a Hewlett-Packard 6890 GC (Avondale, PA) with a capillary column (CP-Wax 52 CB, 60 m × 0.32 mm i.d., 0.50 μm film) from Chrompack (Middelburg, The Netherlands) and detected by a flame ionization detector. The temperature of the GC was programmed from 50 to 150 °C at 2 °C/min. The compounds were quantified by using their corresponding calibration lines.

Identification of Flavor Compounds. The flavor compounds of banana were identified by comparing the retention times of GC peaks with those of authentic compounds under the identical experimental conditions.

Calibration Lines of Flavor Compounds in Banana Juice. Isobutyl acetate, isoamyl alcohol, hexanal, isoamyl acetate, isobutyl butyrate, butyl butyrate, ethyl isovalerate, and ethanol were selected for analysis of banana flavor. The reason is that they are the major flavor compounds of ripening banana (*10*), and many of them have been chosen as typical flavor compounds to characterize the geographical origin of banana fruits (*17*). To determine the concentrations of these compounds in banana juice, the calibration lines of their corresponding standard compounds in 20% sodium chloride solution were prepared. The calibration lines of these compounds were obtained by plotting peak areas versus different concentrations of each standard compound in 20% sodium chloride solution.

Sample Preparation for Ripening. Freshly harvested green bananas from the same bunch were divided into three groups and immediately stored in three temperature-controlled chambers at 20, 25, and 30 °C, respectively, for 8 days. Every time, five fingers of the banana fruit in each group were removed at the scheduled time. They were peeled and evenly sampled to make a mixture of 125 g and then homogenized with distilled water to make banana juice at a concentration of 33.3% (w/w). The banana juice was divided and placed into recloseable polypropylene bags (~15 g per bag) and stored at −20 °C. One bag only was removed and unfrozen with flowing tap water for 10 min at each analysis. An aliquot of the unfrozen sample was placed into a 20 mL glass bottle and added with salt to make a final weight of 10 g with 20% salt. The sample bottle was sealed with a Teflon septum and a plastic cap and then subjected to HS-SPME analysis. All samples were prepared in duplicate.

Table 2. Effect of Banana Concentrations on the Quantity of Headspace Flavor Compounds Adsorbed by SPME

	adsorption[a] (ppm) at banana concn of		
compound	16.7%	33.3%	66.7%
isobutyl acetate	2.51 ± 0.08^{a}	2.61 ± 0.10^{ab}	3.05 ± 0.20^{b}
ethyl isovalerate	7.38 ± 0.45^{a}	9.38 ± 0.10^{b}	9.99 ± 0.90^{b}
hexanal	11.06 ± 0.86^{a}	20.94 ± 3.87^{b}	12.83 ± 0.28^{a}
isoamyl acetate	0.94 ± 0.01^{a}	0.83 ± 0.07^{a}	1.37 ± 0.42^{a}
isobutyl butyrate	0^{ab}	1.30 ± 0.30^{b}	0^{a}
butyl butyrate	1.28 ± 0.06^{a}	2.96 ± 0.19^{b}	1.58 ± 0.03^{a}
isoamyl alcohol	164.36 ± 1.17^{a}	182.59 ± 3.10^{b}	113.99 ± 5.02^{c}
total	203.54 ± 0.92^{a}	253.91 ± 6.78^{b}	209.50 ± 6.22^{a}

[a] Mean values in the same row with different letters are significantly different ($p < 0.05$). [b] Below detection limit.

Statistical Analysis. Statistical evaluations (ANOVA, response surface regression, linear regression) were conducted using Statistica for Windows (v 4.5, StatSoft, Tulsa, OK). Duncan's multiple-range test was used for comparison of mean values of obtained data at the 95% significant difference level.

RESULTS AND DISCUSSION

Effect of Banana Concentration on Flavor Compound Quantity. Three levels of concentration, 16.7, 33.3, and 66.7%, were used to investigate the effect of banana concentration on the quantity of flavor compounds adsorbed by the SPME fiber. The results showed that as the banana concentration increased from 16.7 to 33.3%, the total amount of the investigated compounds increased; however, as the concentration increased from 33.3 to 66.7%, the total amount decreased (**Table 2**). As the individual compounds were compared, the amounts of most compounds were also larger in the headspace of 33.3% banana juice than those in the headspace of the other concentrations. In HS-SPME the rate-limiting step is considered to be the diffusion of the analytes from the aqueous to the headspace. However, in a complex juice system, the matrix itself might cause the rate-limiting step affecting the transport of the analytes through the juice (*5*). Therefore, when banana concentration increased from 33.3 to 66.7%, the matrix effect seemed dominant, so that some of the headspace volatiles absorbed by the fiber did not increase but decreased. As a consequence, the 33% banana concentration gave the best adsoption result in terms of headspace volatile quantity.

Effect of Salt Concentration on Flavor Compound Quantity. Generally, the presence of electrolytes in an adsorption system can influence the adsorption in two ways: changing the properties of the phase boundary and decreasing the solubility of hydrophobic compounds in the aqueous phase (*8*). The latter is more often observed in analytical chemistry, being referred to as "salting out". The salting out effect is widely used to increase the sensitivity of an analytical method. The behavior of the selected flavor compounds in the presence of various

Table 3. Effect of Salt Concentrations on the Quantity of Headspace Flavor Compounds Adsorbed by SPME

compound	adsorption[a] (ppm) at salt concn of 0%	5%	10%	20%	30%
isobutyl acetate	2.41 ± 0.01[a]	0[bb]	2.59 ± 0.04[cd]	2.72 ± 0.12[c]	2.55 ± 0.08[ad]
ethyl isovalerate	8.62 ± 0.44[a]	7.24 ± 0.81[a]	8.27 ± 0.47[a]	10.44 ± 0.5[b]	10.80 ± 0.24[b]
hexanal	19.42 ± 2.25[a]	12.43 ± 0.28[b]	18.92 ± 0.43[a]	22.90 ± 0.58[c]	18.90 ± l.70[a]
isoamyl acetate	0.14 ± 0.08[a]	0[a]	0.07 ± 0.03[a]	0.42 ± 0.11[b]	0.19 ± 0.16[ab]
isobutyl butyrate	1.14 ± 0.30[a]	0[b]	0[b]	0[b]	0[b]
butyl butyrate	0.73 ± 0.04[a]	0.66 ± 0.05[a]	1.23 ± 0.04[b]	1.56 ± 0.24[c]	1.45 ± 0.10[bc]
isoamyl alcohol	140.87 ± 19.21[a]	113.20 ± 19.78[a]	81.98 ± 20.43[b]	113.86 ± 13.81[abc]	156.97 ± 25.77[c]

[a] Mean values in the same row with different letters are significantly different ($p < 0.05$). [b] Below detection limit.

salt concentrations in SPME adsorption was described by Yang and Peppard (*8*). In their study, depending on the compound types, the adsorption of flavor compounds could increase with salt concentration increase, then level off or decrease, and finally decrease with higher salt concentration. **Table 3** shows the salt concentration effect on the quantity of flavor compounds adsorbed by the SPME fiber. The adsorption effects of flavor compounds varied depending on the compound type. For example, the adsorption of isobutyl acetate and hexanal decreased at 5% salt but increased at 20% salt. There were no significant differences between 0, 5, and 10% salt for ethyl isovalerate and isoamyl acetate, but both compounds significantly increased at 20% salt. The addition of salt seemed to be unfavorable to isobutyl butyrate present at such low concentration because it became undetectable in the presence of salt. Except for isobutyl acetate, most compounds did not show any significant difference between 20 and 30% salt concentration. Compared with hexanal and isoamyl alcohol, the esters adsorbed by the SPME fiber were relatively low due to the banana used being still at the initial stage of ripening. However, with the addition of 20% salt, the concentrations of ester compounds (except for isobutyl butyrate) in the headspace increased as compared to those of the control sample without any salt added. This result suggested that addition of salt could improve the detection sensitivity of these ester compounds (except for isobutyl butyrate) while they were at low concentration. Similarly, addition of sodium chloride being able to lower the detection limits of esters in beer has been reported (*18*). From the results, on the whole, 20% salt was sufficient to give the best adsorption effect for most flavor compounds.

Calibration Lines of Standard Compounds. Although the matrix of a sample has a substantial influence on the volatile concentration in the headspace, actually it is impossible to duplicate a matrix free of original volatiles (*18*), especially in a complex and sticky system such as banana fruit due to its starch-based nature. Therefore, water was used to dilute the sample, and salt was added to help release volatile compounds as completely as possible, all together to minimize the matrix effect. As a result, the calibration lines were made with standard compounds in the water and salt system to estimate the volatile concentration in banana juice. The calibration linear regression lines of isobutyl acetate, isoamyl alcohol, hexanal, isoamyl acetate, isobutyl butyrate, butyl butyrate, isoamyl alcohol, and ethyl isovalerate are shown in **Table 4**. Linear relationships (denoted by R^2) between GC peak areas and the concentrations of standard compounds were >0.93 for eight standard compounds. The result also indicated that these compounds were stable and not disproportionately distributed in the water and high-salt (20%) system and thus exhibited a good linearity.

Effects of Temperature and Time on Flavor Compound Quantity. The reproducibility and sensitivity of headspace volatile compound analyses by HS-SPME are greatly influenced by the vapor pressure of flavor compounds in the bottle. Temperature and time are two of the most important factors affecting the vapor pressure and equilibrium of the flavor compounds in the bottle. Therefore, these two factors were chosen and optimized. To our knowledge, to date, the reported studies of HS-SPME on time and temperature effects have been based on one-factor-at-a-time experiments. The results of one-factor-at-a-time experiments often ignore interactions between factors, which are present simultaneously (*19*). Response surface methodology (RSM) coupled with a central composite rotatable design is an effective tool for optimizing a process (*19*). Therefore, this methodology was adopted in this study.

Table 4. Regression Equations between Flavor Compounds (ppm) and GC Peak Areas (Electronic Counts)

compound	regression equation[a]	R^2	concn range (ppm)
isobutyl acetate	$y = -117374 + 57971x$	0.930	0–65
ethyl isovalerate	$y = -251004 + 88582x$	0.953	0–65
hexanal	$y = -56231 + 85732x$	0.999	0–40
isoamyl acetate	$y = 182306 + 181753x$	0.984	0–32
isobutyl butyrate	$y = 497814 + 286300x$	0.994	0–56
butyl butyrate	$y = -285454 + 637081x$	0.971	0–8
isoamyl alcohol	$y = -8278 + 8501x$	0.996	0–1000
ethanol	$y = -303039 + 1706x$	0.987	0–4000

[a] y = electronic counts of GC peak area; x = compound in parts per million.

To obtain a large partition coefficient of flavor compounds between SPME coating and banana juice, more compounds have to pass through the banana juice, which has a low diffusion coefficient compared to the headspace gas phase, into the headspace to reach the SPME coating. To increase the diffusion of flavor compounds through banana juice, the banana juice was agitated using a magnetic bar (*9*). The effects of temperature and time on the analysis of headspace banana flavor compounds with SPME-GC are shown in **Figure 1**. From the contour plot, the temperature range of 46−60 °C and time of 38−57 min were suggested to be useful experimental condition ranges for analyzing banana flavor compounds by HS-SPME. The maximal stationary point for temperature and time can be located graphically in the contour plot, and the values are at about 50 °C and 48 min. Therefore, these values were chosen to isolate the headspace flavor compounds of banana in the subsequent ripening study. The regression equation of independent variables, time (X_1) and temperature (X_2), and response variable (Y), determined for a quadratic polynomial model for total headspace volatiles (denoted by total GC peak area in electronic counts) was $Y = -3.645e7 + 1.402e6X_1 + 5.71e5X_2 + 2.806e3X_1X_2 - 1.472e4X_1^2 - 7.489e3X_2^2$ ($R^2 = 0.976$). By error analysis, it was shown that there was no significant ($p < 0.05$) "lack of fit", which indicated the regression equation well fitted the model.

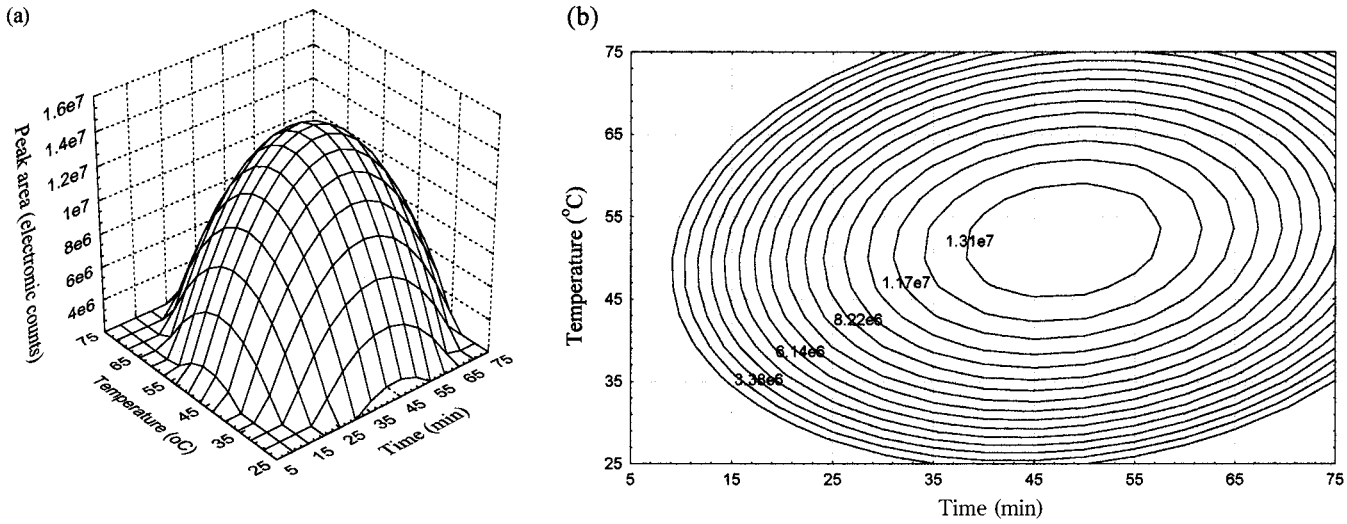

Figure 1. Response surface (a) and contour plot (b) for the effect of time and temperature on the total peak area of banana flavor compounds.

Change of Flavor Compounds during Ripening. Three temperature levels, 20, 25, and 30 °C, were used for studying the change of flavor compounds during ripening, and the results are shown in **Figures 2** and **3**. Before ethanol was emanated rapidly, isoamyl alcohol was the most abundant compound at all storage temperatures. At 20 °C, hexanal, which represents the green note, increased from day 0 to day 2 and then gradually decreased to day 8. Isoamyl acetate, which is a characteristic banana-like flavor compound, increased from day 0 to day 3 (maximum) and then gradually decreased to day 8. Isobutyl acetate also showed a trend similar to that shown by isoamyl acetate. In contrast, isobutyl butyrate and butyl butyrate, although increasing and then decreasing as well, showed only a slight change during the ripening period. The total amount of major acetate esters was found to be much larger than that of the major butyrate esters during ripening at 20 °C by Macku and Jennings (*1*), and a similar result was also observed in this study. At 25 °C, the change of hexanal was similar to that at 20 °C; on the other hand, all investigated esters, except for butyl butyrate, reached a maximum at day 3. Comparatively, the trend of changes of individual compounds stored at 25 °C was similar to that at 20 °C; however, in the former condition, the amounts of individual compounds were larger than the ones of the corresponding compounds in the latter condition. At 30 °C, hexanal, isoamyl acetate, isobutyl acetate, and ethyl isovalerate reached a maximum at day 2, 1 day earlier than in (day 3) 20 or 25 °C storage. The result showed that increasing temperature could accelerate the ripening rate. However, the amounts of these compounds (excluding ethanol and isoamyl alcohol) stored at 30 °C were similar to those at 20 °C but less than the ones at 25 °C. The reason might be due to the fact that other compounds such as ethanol increased markedly at 30 °C (**Figure 3**), resulting in relatively high vapor pressure, and were more likely absorbed onto the fiber owing to "competition effect". The general trend of changes of flavor compounds stored at 25 °C in the present study is similar to that in the study by Tressl and Jennings (*12*) under the same ripening temperature using a purge-and-trap method for flavor compound sampling. In their study the flavor compounds increased from day 0 until the day they reached a maximum and then gradually decreased. Mattei (*13*) found that the relative rates of production of volatile compounds, except for ethanol (exponential increase), increased and decreased as ripening progressed at fixed temperature, and at different temperatures, different volatiles predominated, consequently affecting the aroma composition at each stage of ripening. In the present study, at different temperatures, the volatile compositions at each ripening stage were also different;

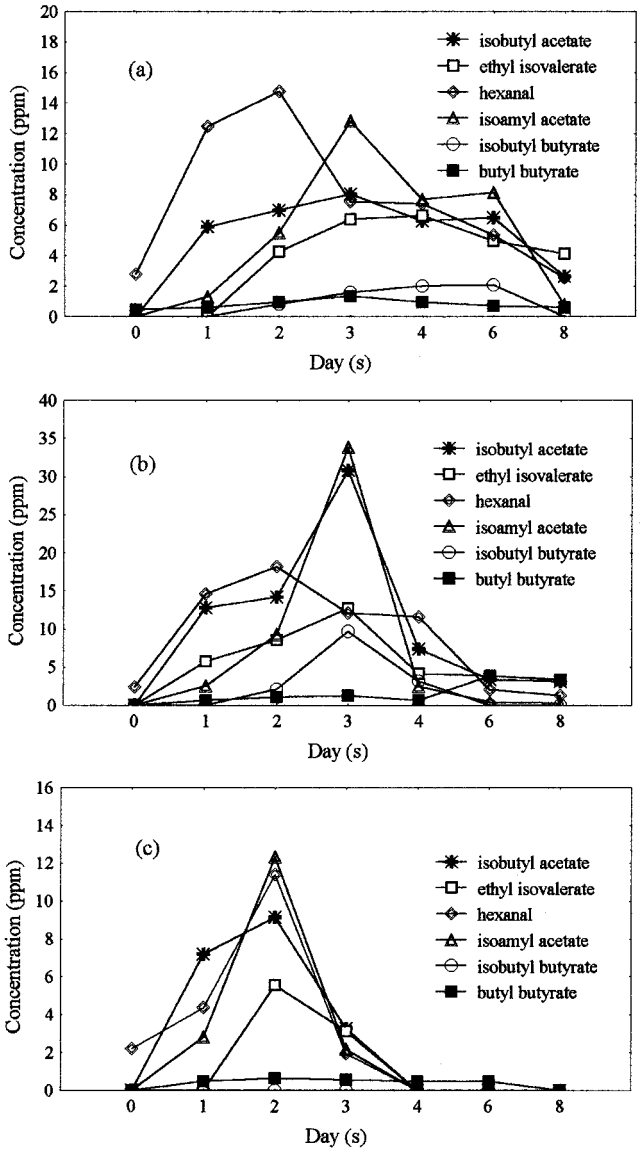

Figure 2. Changes of flavor compounds of banana during ripening at 20 °C (a), 25 °C, and 30 °C (c).

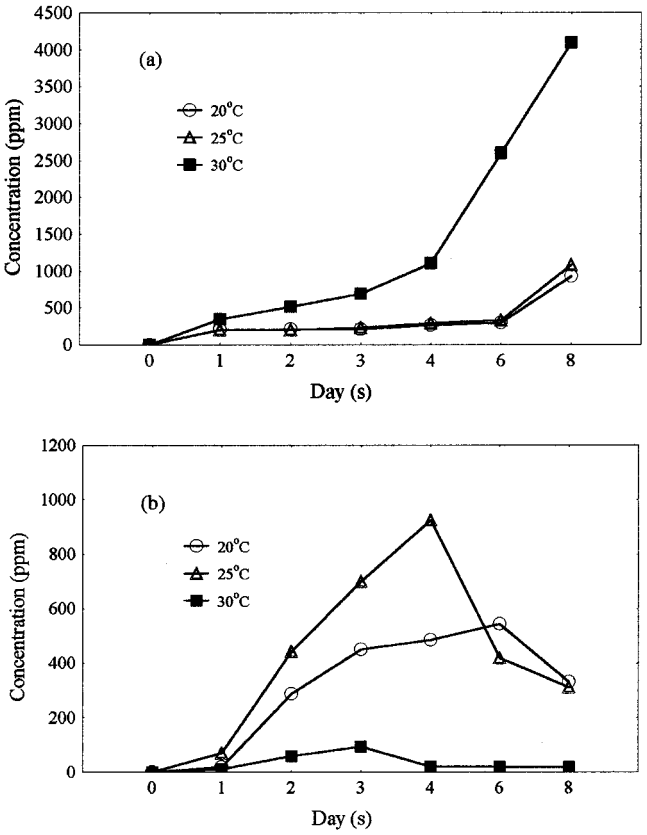

Figure 3. Changes of ethanol (a) and isoamyl alcohol (b) during 8 days of storage at 20, 25, and 30 °C.

meanwhile, ethanol increased exponentially as well at later ripening stage, which was in agreement with Mattei (*13*).

These results show that HS-SPME analysis is comparable to conventional methods. In addition, with the merits of simple operation, rapid sampling of volatiles, and relative inexpensiveness, HS-SPME is a suitable method for analysis of flavor compounds of banana during ripening.

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Received for review July 11, 2001. Revised manuscript received November 5, 2001. Accepted November 12, 2001.

JF010891+