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Headspace solid-phase microextraction coupled to gas chromatography for the analysis of aldehydes in edible oils

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ABSTRACT

Oxidation has important effects on the quality of edible oils. In particular, the generation of aldehydes produced by the oxidation of oils is one of the deteriorative factors to their quality. The aim of this study was to develop a method to determine the aldehydes as lipid oxidation markers in edible oils. Seven aldehydes generated from lipid oxidation were studied using headspace solid-phase microextraction coupled to gas chromatography with a flame ionization detector. The extraction efficiency of five commercial fibers was investigated and the influence of extraction temperature, extraction time, desorption temperature, and desorption time were optimized. The best result was obtained with 85 μm carboxen/polydimethylsiloxane, extraction at 50 °C for 15 min and desorption in the gas chromatography injector at 250 °C for 2 min. Under the optimized conditions, the content of hexanal was the highest of the seven aldehydes in all edible oils. The limits of detection for hexanal in the three oils were found to range from 4.6 to 10.2 ng L^{-1}. The reproducibility of the method was evaluated and the relative standard deviations were less than 8.9%. This developed approach was successfully applied to analyze hexanal in peanut oil, soy oil, and olive oil samples, and these results were compared with those obtained using the thiobarbituric acid-reactive substances (TBARs) method.

1. Introduction

Edible oils are easily oxidized during processing, circulation, and preservation. The oxidization causes deterioration in taste, flavor, odor, color, texture and appearance, and the nutritional value decreases [1]. There have been many reports that the oxidized oils have general toxicity, cytotoxicity, genotoxicity and neurotoxicity, which can cause many diseases [2–5]. It is becoming important that a reliable method to identify the degree of oil oxidation be found. Lipids in oils are autoxidized in air and the autoxidation is a non-enzymatic autocatalytic oxidation caused by free-radical chain reactions, resulting in the formation of hydroperoxide as a primary product. Hydroperoxides are formed through the reaction of unsaturated fatty acids and triglycerides with oxygen in a free-radical process. The free-radical process involves initiation, propagation and termination stages. Hydroperoxides can also be formed from saturated or monounsaturated fatty acids or esters [6,7]. The primary products are very unstable and easily break down into many volatile and non-volatile products [8]. These products are called secondary products, including polymers, ketones and aldehydes [9]. Among the secondary products, aldehydes are the most important breakdown decomposed products of hydroperoxides from the secondary alkoxy radical cleaving [6,7]. The aldehyde content can be taken as an indicator for the oxidation level of oil products or food samples.

The solid-phase microextraction (SPME) technique is a convenient isolating and pretreatment technique, first reported in 1990 [10]. As a powerful sampling preparation technique, SPME has been proved to be a simple, fast, sensitive and solvent-free approach. It is easy to combine with gas chromatography (GC) [11] or liquid chromatography [12] and has been successfully applied in many fields, such as food [13], clinical [14], environmental [15,16], biological [17,18], and forensic analysis [19,20]. In SPME applications, three extraction modes including direct immersion (DI-SPME), headspace (HS-SPME) and membrane-SPME are available. Among them, HS-SPME exhibits the lowest background, protects the fiber from spoilage, and it is suitable for volatile analytes in most gaseous liquid and solid samples.

To date, the extent of lipid oxidation has been evaluated using peroxide value, anisidine value, conjugated diene value, conjugated triene value [21], total oxidation value [22], or thiobarbituric acid reactive substances (TBARS) value [23]. Among these methods, the easy and relatively fast TBARS has been widely

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used, however, it has been largely criticized due to its lack of specificity [24]. Compared with conventional techniques for the determination of the extent of lipid oxidation, SPME exhibits many advantages, such as easy manipulation and experimental set up, short sampling times, and high sensitivity [25–27]. In this paper, we proposed a simple and effective approach, where HS-SPME coupled to GC with a flame ionization detector were applied to the direct extraction and analysis of aldehydes generated from the oil oxidation in edible oils. Furthermore, the concentration of hexanal was related with the storage time of the edible oils. The method developed here had better accuracy and specificity than the conventional method. Our experimental results confirmed that the level of aldehydes, especially hexanal, was adequate as a parameter of oil deterioration, and could be taken as an indicator for not only edible oil oxidation, but possibly for other food matrices.

2. Experimental

2.1. Reagents and solutions

All chemicals and reagents used in the experiments were analytical grade. The aldehydes hexanal, heptanal, octanal, nonanal, decanal, 2E-decenal, and 2E, 4E-decadienal were purchased from J&K Scientific Ltd. (Beijing, China). 2-thiobarbituric acid (TBA) was supplied by Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA). Trichloroacetic acid (TCA) was obtained from Acros (Acros Organics, Morris Plains, NJ, USA). Edible oil samples were purchased from local supermarkets in Xiamen. 100 mg L^{-1} stock solutions of seven aldehydes were prepared by diluting 1000 mg L^{-1} of each compound with fresh oils (the storage time was 15 days less than that recommended by the manufacturer). The standard working solutions were prepared by diluting the mixture with oils to the required concentration. All the above solutions were sealed, and stored at 4 °C in a refrigerator. All the spiked solutions were shaken with a mini-shaker for 15 min at room temperature before it could be used.

2.2. Instrumentation

Commercial manual sampling SPME devices were from Supelco (Bellefonte, PA, USA), and the SPME fibers with 100 μm polydimethylsiloxane (PDMS), 65 μm PDMS/divinylbenzene (PDMS/DVB), 85 μm polyacrylate (PA), 85 μm carboxen/PDMS (CAR/PDMS) and 70 μm carbowax/DVB (CW/DVB) were selected and used. The analysis was carried out on a Shimadazu GC-2010 GC system coupled with a flame ionization detector (FID). All the fibers were conditioned before being used in the GC injector according to the instructions provided by the manufacturer. Separation was performed using a DB-5 capillary column (30 m × 0.25 mm I.D. and 0.25 μm, J&W Scientific, CA, USA).

The instrument parameters for the analysis were as follows: N_2 flow; 1.47 mL min^{-1}; column temperature program: held at 40 °C for 3 min, then increased from 40 to 70 °C at 15 °C min^{-1} and maintained for 1 min, and then increased to 250 °C at 30 °C min^{-1} and held for 1 min. The detector temperature was held at 280 °C. In optimized conditions, the temperature of the injector was set at 250 °C, and the desorption process was performed in splitless mode for 2 min.

Thiobarbituric acid-reactive substances (TBARs) were detected and calculated as mg of malonaldehyde (MDA), which were applied for the comparison to the SPME results. The procedures for the determination of *TBARs* followed the introduction of Deckers [23]. A UV spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) was used and the wavelength was selected at 532 nm.

2.3. Headspace solid-phase microextraction

For the HS-SPME experiments, 3 mL edible oil was placed in a 10 mL glass vial. The vial was closed with Teflon-lined septa (Supelco). The standard solutions and edible oil samples were stored at room temperature, after which a fiber was introduced into the headspace of the vial for 10 min at the same extraction temperature. After extraction, the fiber was removed from the vial, inserted into the inlet of the GC, and desorbed at 250 °C for 2 min.

3. Results and discussion

3.1. Selection of SPME mode

As is well known, the sample matrix of edible oils is very complex, containing a great amount of lipids and grease. The oils are generally very sticky, so it is unsuitable to use DI-SPME mode for the analysis of aldehydes in oil samples. Additionally, interfering effect from the matrix compounds was severe and the fiber may be damaged during the immersion. In this study, the selected aldehydes were very volatile compounds with boiling points ranging from 78 to 209 °C, which satisfied the prerequisite for the application of HS-SPME. As a result, it was expected that HS-SPME would be an ideal approach for the extraction of aldehydes in edible oils.

3.2. Extraction efficiency of different types of commercial fibers

In order to select a suitable extraction fiber, five different types of commercial fibers, 100 μm PDMS, 65 μm PDMS/DVB, 85 μm PA, 85 μm CAR/PDMS and 70 μm CW/DVB, were used. Their extraction capabilities are shown in Fig. 1. Generally, the polarity and volatility characteristics of analytes are the primary issues considered before selecting a fiber. Among the five fibers selected, it was clear that CAR/PDMS (85 μm) exhibited the highest extraction efficiency for most of the selected aldehydes. PDMS/DVB, CAR/PDMS or CW/DVB material had higher polarity than pure PDMS [28], resulting in a higher extraction capability for polar compounds such as the aldehydes involved here. In addition, CAR offered CAR/PDMS fiber a greater specific surface area, 1000–200 m^2 g^{-1} [29], which provided a larger effective extraction area

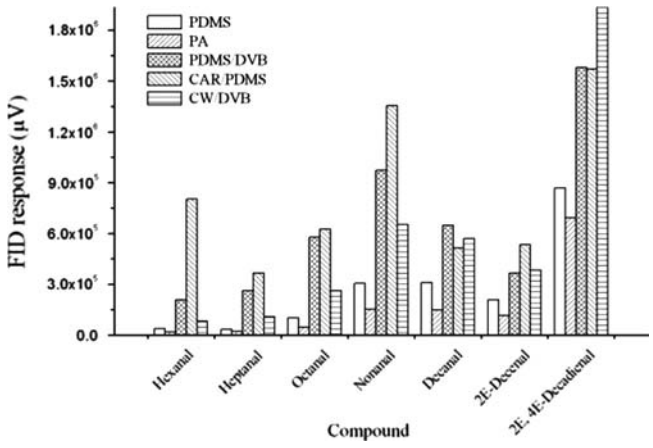

Fig. 1. Comparison of extraction amounts of commercial fibers for the spiked aldehydes from edible oils. HS-SPME conditions: sample volume, 3 mL; extraction temperature, 45 °C; extraction time, 10 min; desorption temperature, 220 °C; and desorption time, 2 min. Concentration of each aldehydes, 50 μg L^{-1}.

for the volatile organic compounds. It can be noted from Fig. 1 that the extraction efficiencies of 65 μm PDMS/DVB, 85 μm CAR/PDMS and 70 μm CW/DVB fiber were all greater than that of 100 μm PDMS or 85 μm PA fiber, and that CAR/PDMS fiber showed the best extraction efficiency for the most aldehydes. For further method optimization and all the experiments, 85 μm CAR/PDMS fiber was selected.

3.3. Optimization of the extraction process for aldehydes

3.3.1. Extraction time

The effect of extraction time on the extraction efficiency to the selected aldehydes was investigated at 1, 2, 5, 10, 15 and 20 min using the 85 μm CAR/PDMS fiber. The peak areas of the analytes increased with the extraction time from 1 to 10 min, and most of the aldehydes reached equilibrium after 10 min. No equilibrium could be found even after 15 min extraction for nonanal and hexanal (Fig.2). According to the nonequilibrium theory of HS-SPME [30], HS-SPME quantitative analysis can be utilized in a non-equilibrium situation if the extraction conditions are kept constant. In the following experiments, an extraction time of 15 min was selected as a compromise between the analysis time and the method sensitivity.

3.3.2. Extraction temperature for HS-SPME sampling

In order to investigate the effect of the extraction temperature on the extraction efficiency in the HS-SPME sampling for the selected aldehydes, different extraction temperatures of 23, 50, 70 and 90 °C were studied (Figs. 3 and 4). The temperature range was selected based on the equilibrium between the fiber coating, analytes and the sample matrix. Lower temperature favored adsorption by the fiber, but higher temperature was beneficial for the transfer of the analytes into the headspace [31]. Due to the high volatility of the aldehydes, a lower extraction temperature was enough to bring these compounds up into the headspace. For edible oil samples, although the extraction efficiency increased with temperature, too high an extraction temperature could cause by-reactions (such as oxidation) and competition adsorption from the co-existing substances. Considering the extraction efficiency and the analyte stability, an optimal extraction temperature of 50 °C was chosen.

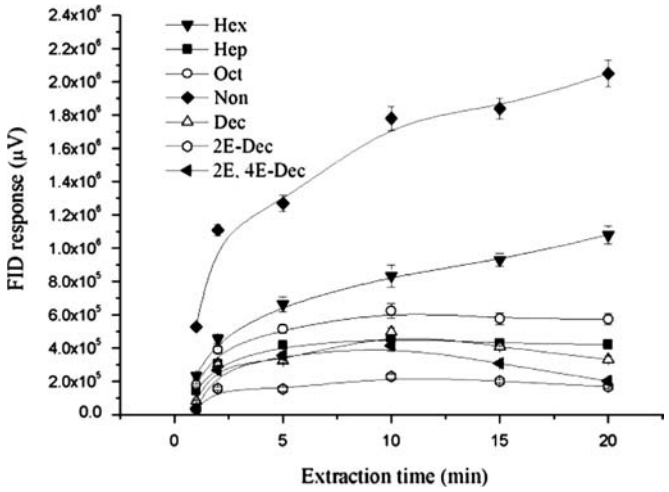

Fig. 2. Extraction time profiles for aldehydes using a CAR/PDMS fiber. HS-SPME conditions: sample volume, 3 mL; extraction temperature, 45 °C. Concentration of each aldehyde, 50 μg L^{-1}.

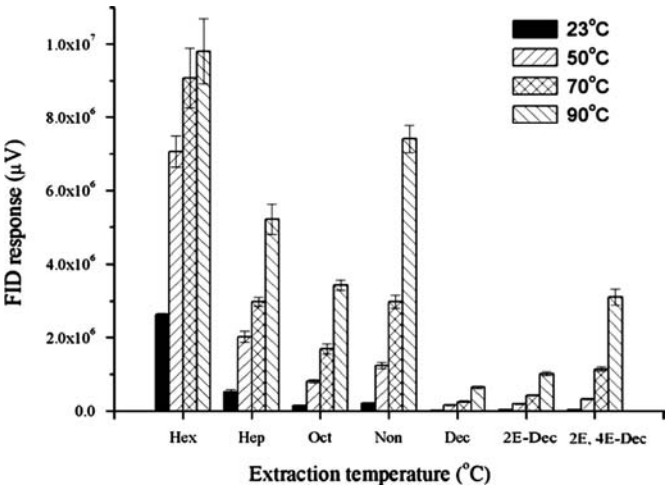

Fig. 3. Effect of extraction temperature on the extraction efficiencies of aldehydes using a CAR/PDMS fiber. HS-SPME conditions: sample volume, 3 mL; extraction time, 15 min. Concentration of each aldehyde, 50 μg L^{-1}.

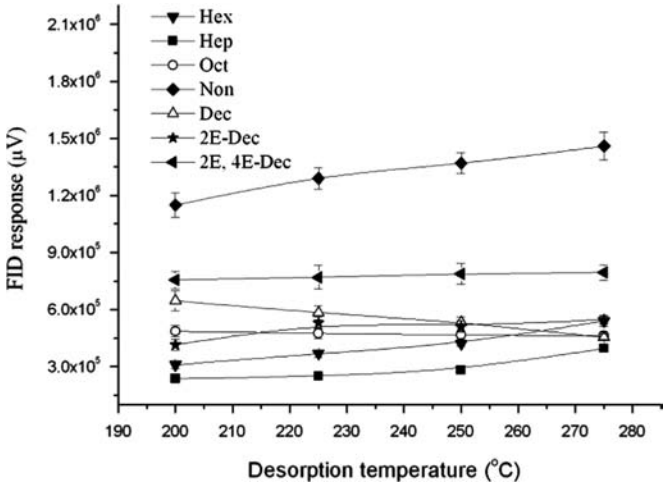

Fig. 4. Effect of desorption temperature on the extraction efficiencies of aldehydes using a CAR/PDMS fiber. HS-SPME conditions: sample volume, 3 mL; extraction temperature, 50 °C; extraction time, 15 min. Concentration of each aldehyde, 50 μg L^{-1}.

3.3.3. Desorption temperature

The desorption temperature must be high enough to effectively release analytes from the fiber. Consequently, in our experiments, desorption temperatures ranging from 200 °C to 275 °C were studied. The peak areas of all seven aldehydes remained almost unchanged above 250 °C (Fig. 4) and, although a higher desorption temperature was helpful in reducing the desorption time, the higher temperature could cause fiber coating and injector damage. Therefore, the desorption temperature of subsequent experiments was set at 250 °C.

3.3.4. Desorption time

The influence of the desorption time was checked at 1, 2, 3 and 4 min. The peak areas of the analytes increased from 1 to 2 min and reached equilibrium after 2 min. The coatings after desorption at 250 °C for 2 min were checked and no remaining analytes could be found. These results indicated that the analytes had been released completely. The rapid desorption of analytes was mainly

attributed to their volatility, and thus the desorption time was set at 2 min.

3.4. Aldehydes in edible oil samples

Under the optimized conditions, aldehydes in samples of several types of edible oil were tested (Fig. 5). Among the seven aldehydes, the hexanal content was highest in all edible oil samples. In edible oils, there are high amounts of polyunsaturated fatty acids (PUFA), and their primary oxidation products are hydroperoxides. As mentioned above, hydroperoxides are very unstable and easily degraded to volatile and non-volatile products, including polymers, ketones and aldehydes. Among these compounds, aldehydes are the most important degraded products of hydroperoxides [6]. Hexanal is a typical volatile oxidation product of n-6 PUFA, and it can be used as an indicator of lipid oxidation in foods [32]. The volatile and anti-oxidative characteristics of hexanal make headspace sampling and GC detection easy. In further studies, the hexanal content of edible oil samples was given special focus.

3.5. Evaluation of the method performance in the determination of hexanal in edible oils

3.5.1. Evaluation of method performance

The CAR/PDMS fiber was selected and used for the determination of hexanal levels in different edible oil samples. The analytical characteristics under optimized conditions are shown in Table 1. The linear ranges of the method were from 50 to 1000 μg L^{-1} and 1000 to 10,000 μg L^{-1} for hexanal in peanut, soybean and olive oil samples, and all the correlation coefficients were better than 0.99. The limits of detection, which was defined as three times the baseline noise, were found in the range 4.6 μg L^{-1} (soybean oil) to 10.2 μg L^{-1} (peanut oil). The repeatability for the method was evaluated through extracting blank edible oils spiked at 500 μg L^{-1} of hexanal (6 replicates), and the relative standard deviations (RSDs) were 5.3–8.9%.

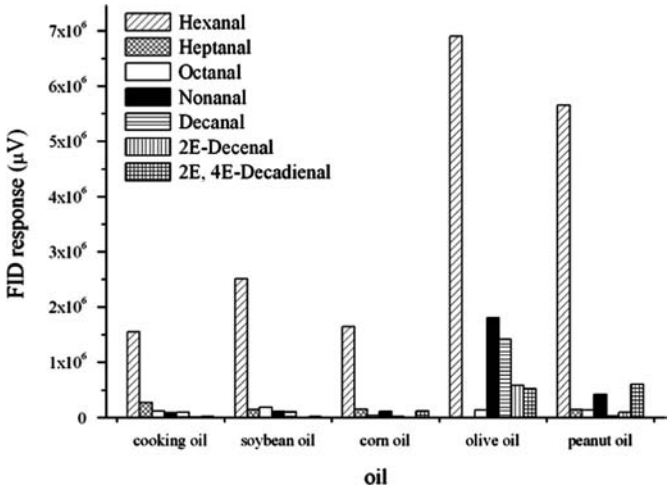

Fig. 5. Determination result of seven aldehydes in different edible oils using a CAR/PDMS fiber. HS-SPME conditions as those in Fig. 4.

3.5.2. Application to edible oil samples.

To test the method applicability, it was used to determine the hexanal in real edible oil samples. The edible oil samples were different kinds of barreled oils manufactured by the same company, and purchased from a local supermarket in Xiamen. The results for three different spiking levels are shown in Table 2. The background concentrations of the oils were 86.6 (peanut oil), 291.3 (soybean oil) and 341 μg L^{-1} (olive oil). The recovery of the hexanal spiked at 50, 100, and 500 μg L^{-1} in the edible oils samples ranged from 85.2% to 110.6%, with the RSDs ranging from 4.0% to 8.2%. In this study, we investigated the relationship between hexanal concentration and the oil storage time. When the storage time of peanut oil samples was 3, 6, 10, 15 and 21 months, respectively, the content of hexanal was found to be 232.4, 523.8, 777.7, 2310.6 and 2441.2 μg L^{-1} (Fig. 6a). The results indicated that the oil storage time presented good correlation with hexanal concentration in the oil samples, and no obvious interference effects could be found from their GC chromatograms (Fig. 6b). Jiménez et al. used a ratio between hexanal and nonanal to investigate the oil oxidation [34]. Since the nonanal peak can also be clearly observed (Fig. 6b), we calculated the ratio between hexanal and nonanal and investigated its relationship with the storage time of edible oils. We found that the ratio between hexanal and nonanal in peanut oils is decreased with the storage time from 3 months to 15 months, but increased when the storage time reached to 21 months. This result indicated that the ratio does not work perfectly as hexanal concentration in peanut oil samples for a long time. As mentioned above, TBARS is an easy and relatively fast method among the methods used to evaluate the extent of oil oxidation. This method is based on the reaction of 2-thiobarbituric acid (TBA) and malonaldehyde or malonaldehyde-type products. However, compounds other than malonaldehyde-type products may also react with TBA [33], resulting in its unsatisfactory specificity. The comparison results as shown in Fig. 6c indicate the content of hexanal regularly increased with the longer storage time from 232.4 μg L^{-1} of the primary 3 month storage to 2850 μg L^{-1} of the last 36 month storage. However, the TBARS levels are less regular as shown in Fig. 6c. The TBARS level

Table 2
Analytical results for the determination of hexanal in three kinds of edible oils.

Spiking level (μg L^{-1})	Recovery (%)		
	Peanut oil	Soybean oil	Olive oil
50	110.6 ± 7.3	89.6 ± 6.0	94.2 ± 4.0
100	109.4 ± 7.6	109.1 ± 7.2	87.6 ± 8.2
500	95.2 ± 6.9	104.4 ± 5.5	85.2 ± 5.9

Table 1
Detection limits, linear range, linearity correlation and repeatability of the proposed method.

Edible oil	LOD (μg L^{-1})	Linear rage (μg L^{-1})	Linearity correlation (r^2)	Linear rage (μg L^{-1})	Linearity correlation (r^2)	Repeatability (RSD%, $n=6$)[a]
Peanut oil	10.2	50–1000	0.9997	1000–10,000	0.9984	7.2
Soybean oil	4.6	50–1000	0.9915	1000–10,000	0.9759	5.3
Olive oil	9.0	50–1000	0.9902	1000–10,000	0.9968	8.9

[a] The concentration of the hexanal was 500 μg L^{-1} in the oil sample, and other conditions were the optimized conditions.

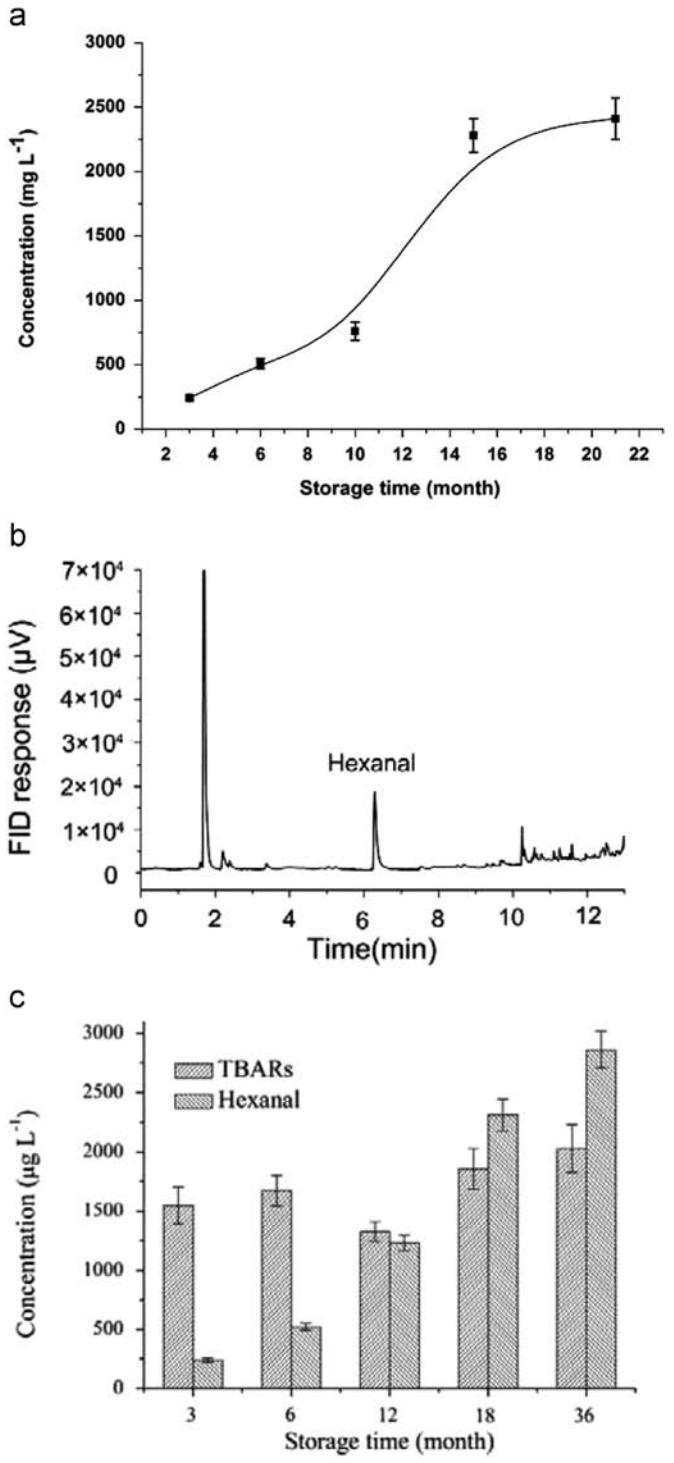

Fig. 6. (a) Concentration of hexanal in peanut oils at different storage times. (b) GC chromatogram of hexanal in peanut oils stored for 21 months. (c) Comparison of hexanal and the TBARS level of peanut oils in different storage time. HS-SPME conditions as those in Fig. 4.

increased in the primary 6 month storage, but abruptly decreased when the storage time reached at 12 month, then increased tardily. All the above results confirmed that the method we developed had better specificity than TBARS, and the hexanal concentration could be used as an indicator for oil oxidation with high stability.

4. Conclusions

For better understanding of the quality of oils, volatile aldehydes (especially hexanal), constituting key compounds from the oxidation of oils, can be taken as indicators for the oil storage situation or degree of freshness. In this paper, an HS-SPME–GC–FID method was developed to determine seven aldehydes including hexanal in different edible oil samples. The method developed was straightforward, simple, quickly specified and accurate for the analysis of aldehydes in edible oils. As volatile aldehydes may illustrate the level of oxidation and toxicity of oils or foods containing high levels of oil, more investigations must be carried out in order to improve the method. Volatile aldehydes will be good indicators to optimize processing, circulation, or preservation of oils or foods. This kind of study might be extended to other food matrices because lipid oxidation is very frequent in all industrial food processes. Additionally, the study of aldehydes in food brings more information into health research in order to better understand some unexpected phenomena.

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