JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Rapid Magnetic Solid-Phase Extraction Based on Monodisperse Magnetic Single-Crystal Ferrite Nanoparticles for the Determination of Free Fatty Acid Content in Edible Oils

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ABSTRACT: This study proposes a rapid magnetic solid-phase extraction (MSPE) based on monodisperse magnetic single-crystal ferrite (Fe_3O_4) nanoparticles (NPs) for determining the quantities of eight free fatty acids (FFAs), including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), and behenic acid (C22:0) in oil. The amine-functionalized mesoporous Fe_3O_4 magnetic NPs were applied as a sorbent for MSPE of FFAs from oil samples in a process that is based on hydrophilic interaction. The extraction can be completed rapidly in a dispersive mode with the aid of vigorous vortex. Additional tedious processing steps such as centrifugation and evaporation of organic solvent were not necessary with this procedure. Furthermore, esterification of FFAs can be accomplished during the desorption procedure by using methanol/sulfuric acid (99:1, v/v) as the desorption solvent. Several parameters affecting the extraction efficiency were investigated, including the matrix solvent for extraction, the desorption solvent and desorption time, and the amount of sorbent and extraction time. The pretreatment process was rapid under optimal conditions, being accomplished within 15 min. When coupled with gas chromatography−flame ionization detection (GC-FID), a rapid, simple, and convenient MSPE-GC-FID method for the determination of FFAs in oil samples was established with a total analysis time within 25 min. The limits of detection for the target FFAs were found to be 7.22−26.26 ng/mL. Recoveries in oil samples were in the range of 81.33−117.75%, with RSDs of <6.4% (intraday) and <6.9% (interday). This method was applied successfully to the analysis of dynamic FFA formation in four types of edible oils subjected to an accelerated storage test. The simple, rapid, and cost-effective method developed in the current study offers a potential application for the extraction and preconcentration of FFAs from hydrophobic sample matrices, including edible fats and oils, fatty foods, and biological samples with high amounts of lipid.

KEYWORDS: *magnetic solid-phase extraction, monodisperse magnetic single-crystal ferrite nanoparticles, free fatty acids, edible oils, GC-FID*

INTRODUCTION

Fats and oils from a wide variety of sources are important to the food industry and other industrial sectors. The analysis of many components in fats and oils is of great importance to determine the origin and type of the oil, to assess the quality, and to guide the industrial processing of the oils. Free fatty acids (FFAs) are common triacylglycerol (TAG) hydrolysis products in crude oils and are formed to some extent in refined oils as a result of oxidation or TAG degradation, impairing oil quality and functionality. Chemically, FFAs are less stable than TAGs and therefore more likely to oxidize and cause rancidity.[1] FFA content in crude oils is used to characterize high-quality pressed oils and to evaluate oil damage.[2] In edible oil refining, the FFA content is used by processors to optimize alkali and physical refining. The FFA content is also a parameter that may be used to control the oil degradation produced by storage under different conditions of moisture, temperature, oxygen, and light and to follow the thermal degradation of the oils used to cook or fry, which is very useful in specific applications, such as determining the need to replace frying fats.[3]

The determination of FFAs by using the standard method of the American Oil Chemists' Society (AOCS) involves titrating oil dissolved in alcohol with a strong base to a phenolphthalein end point,[4] which is simple, but tedious and problematic, especially when dealing with dark oils. In addition, this method requires large amounts of solvents that could pose potential environmental threats. Fourier transform infrared (FTIR) spectroscopy is a simple, rapid, and nondestructive technique, which plays a critical role in the rapid determination of various parameters including FFA contents of fats and oils.[5] However, FTIR methods are limited by their expensive instruments and are unsuitable for in-plant quality control or centralized commercial laboratories. The sensitivity of these methods is insufficient for catering to the wide range of edible oil quality. Furthermore, only total FFAs in fats and oils can be determined by both titrating method and FTIR method, but the

Received: September 5, 2012
Revised: December 11, 2012
Accepted: December 11, 2012
Published: December 11, 2012

Table 1. Fatty Acids Composition (Fatty Acid Esters as Area Percent of Total Fatty Acid Esters) of Four Different Oils

	fatty acid composition[a] (%)							
sample	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
rapeseed oil	4.73	2.00	61.99	21.62	8.36	0.45	0.84	nd[b]
soybean oil	10.95	3.29	26.28	52.39	6.40	0.32	nd	0.36
sunflower oil	6.42	4.24	26.71	60.77	0.55	0.30	0.30	0.71
corn oil	13.44	1.25	31.35	52.44	0.69	0.39	0.43	nd

[a]Values are the average of three individual samples each analyzed in duplicate with relative standard deviation (RSD) of <3.1%. [b]nd, not detected.

component of FFA and its content, which may provide valuable information for the study and control of oil degradation process, have been ignored. Therefore, an effective method for the analysis of not only total FFAs but also individual FFAs in edible oils is desirable.

Alternative chromatographic techniques, such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), provide useful quantitative and qualitative information on the fatty acid composition and its content in edible oils.[6,7] Compared with HPLC, GC technology is more appropriate in the field of fatty acid analysis, because the resolution ability of GC is much higher than that of LC. However, GC is strictly limited by sample volatility; thus, derivatization is crucial for fatty acid analysis by GC. Usually, fatty acid methyl esters (FAMEs) were prepared,[8] which complicates the analysis of FFAs in edible oils because during the methyl esterification process, TAGs (the main constituents of oils) can also be derived to FAMEs, interfering significantly with the detection of trace FFAs in edible oils. Recently, a method involving the use of dimethylamine/Deoxo-Fluor to derivatize free fatty acids to their dimethylamides was developed and successfully used for the determination of FFAs in human plasma and lipids from microalgae.[9,10] However, for the determination of trace FFAs from edible oils, even with the advent of advanced hyphenated techniques, these complex fatty matrices usually require extensive sample extraction and purification. Thus, it is necessary to develop a simple, rapid, and effective method for the enrichment of FFAs before GC analysis.

Due to the inherent complexity of oil, extraction of residue compounds at low concentration is challenging. Gilbert-López reviewed the main sample treatment methodologies for pesticide residue analysis in edible oils and fatty vegetables, which involve the use of one or the combination of some of the following techniques for both the sample extraction and cleanup steps: liquid−liquid partitioning, solid-phase extraction (SPE), gel permeation chromatography (GPC), matrix solid-phase dispersion (MSPD), etc.[11] Besides, SPE has also been used for the extraction of benzo[*a*]pyrene in edible oils with humic acid-bonded silica as a novel sorbent,[12] but most of these methods are time-consuming and not economical.

In recent years, magnetic solid-phase extraction (MSPE) has attracted much interest.[13−18] MSPE is a simple and convenient extraction technique based on the use of magnetic or magnetizable adsorbents that can be isolated readily from sample matrices with an external magnet. MSPE is also favorable to achieve high extraction efficiency in a short time, making it desirable for high-throughput sample preparations because the adsorbents can be dispersed uniformly into a sample solution by vortexing, making the contact area between the adsorbents and the analytes large enough to ensure a fast mass transfer.[19,20] Very recently, MSPE has also been used successfully for the microextraction and determination of polycyclic aromatic hydrocarbons (PAHs) and 3-monochloropropane-1,2-diol (3-MCPD) from edible oils.[21,22] Generally, the adsorbents used in MSPE are Fe_3O_4-based materials with different functional groups, which are suitable for various analytes.

In the current study, we proposed a rapid magnetic solid-phase extraction based on monodisperse magnetic single-crystal ferrite (Fe_3O_4) nanoparticles (NPs) for the determination of FFA content in edible oils. The extraction can be completed rapidly in a dispersive mode with the aid of vigorous vortex. Furthermore, the derivatization of FFAs can be accomplished during a desorption procedure by using methanol/sulfuric acid (99:1, v/v) as the desorption solvent. By coupling with gas chromatography−flame ionization detection (GC-FID) under optimal conditions, a rapid, simple, and convenient MSPE-GC-FID method for the determination of FFAs in oil samples was established, and this method was successfully applied to the analysis of dynamics of FFA formation in four kinds of edible oil accelerated storage tests.

MATERIALS AND METHODS

Reagents and Chemicals. Ethylene glycol (EG), 1,2-ethylenediamine (ETH), ferric trichloride hexahydrate ($FeCl_3·6H_2O$), sodium acetate (NaAc), sodium chloride (NaCl), and sulfuric acid (98%, w/w) were purchased from Sinopharm Chemical Reagent (Shanghai, China). Octadecyl trimethoxysilane (OTMS) was purchased from the Chemical Plant of Wuhan University (Wuhan, China). Ethanol (HPLC grade), methanol (HPLC grade), acetone (HPLC grade), and *n*-hexane (HPLC grade) were purchased from CNW Technologies GmbH (Dusseldorf, Germany). Purified water was obtained with a Millipore Milli-Q apparatus (Bedford, MA, USA). All of the chemicals were used directly without further purification.

Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), and behenic acid (C22:0) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Margaric acid (C17:0) standard, used as an internal standard (I.S.), was also purchased from Sigma-Aldrich. The individual fatty acid stock solutions and the I.S. stock solution were prepared in *n*-hexane (HPLC grade) at a concentration of 5 mg/mL. Mixed fatty acids stock solution containing 1 mg/mL of each fatty acid was prepared by mixing the individual fatty acid stock solutions. All stock solutions were kept at 4 °C in the dark. The stock solutions were diluted to the desired concentration for the following experiments.

Oil Samples. Rapeseed oil (RS), soybean oil (SB), sunflower oil (SF), and corn oil (CO) were used for this study. All were purchased from local markets in Wuhan (China) and stored at room temperature. Table 1 shows the content (% w/w) of the total fatty acids for all oils. Fatty acid contents of these oils were determined on the basis of our previous research.[8] Generally, approximately 20 mg of oil was diluted with petroleum ether. After that, 2 mL of KOH/methanol solution (0.4 mol/L) was added to the oil sample for FAME preparation, and the oil samples were esterified with a vortex mixer for 5 min. After the addition of 2 mL of distilled water, the samples were shaken for 1 min and then centrifuged at 1400*g*. FAMEs in the upper phase were separated by GC-FID.

The developed MSPE method was applied for determining the dynamics of FFA formation in an oil accelerated storage test (60 °C). Two hundred milliliters of each oil was placed into a 1000 mL flat-bottom flask closed with a stopper and kept for up to 10 days at 60 °C in the dark. Four flasks were prepared, and at each sampling day (days 0, 3, 5, and 10), one flask was taken from the incubation chamber. Flasks were stored at −20 °C after the incubation until their contents were analyzed. Triplicate analyses of each sample were performed, and average values were used for quantization.

Synthesis of Monodisperse Fe_3O_4 Magnetic Nanoparticles. Monodisperse Fe_3O_4 magnetite nanoparticles with mesoporous structure were synthesized via a solvothermal process according to a previously reported method.[23] Briefly, $FeCl_3{\cdot}6H_2O$ (5.0 g) was dissolved in EG (100 mL) to form a clear solution, followed by the addition of NaAc (15.0 g) and ETH (50 mL). The mixture was stirred vigorously for 30 min and then sealed in a Teflon-lined stainless steel autoclave (200 mL). The autoclave was heated to 200 °C and maintained there for 8 h and then allowed to cool to room temperature. The product was collected magnetically and washed with 50 mL of water/ethanol (1:1, v/v) five times and vacuum-dried at 60 °C for 6 h.

As indicated in a previous paper,[24] there exists free $-NH_2$ group on the Fe_3O_4 NPs, which may be derived from ETH. Actually, the amine-functionalized mesoporous Fe_3O_4 magnetic NPs could be easily applied as a sorbent for MSPE of FFAs in a process that was based on hydrophilic interaction.

Hydrophobic Modification of Glass Vial Inner Surface. When an untreated glass vial was used as the container, the majority of Fe_3O_4 magnetic NPs were readily adsorbed on the glass wall of the vial, which was not easily gathered by a magnet due to the hydrophilic interactions (there are some silicon hydroxyl groups on the surface of the glass). Actually, the plastic centrifuge tube was first employed as the container. However, when contacting organic solvents such as acetone and hexane for a period of time, some plastic compounds can easily perspire from the tube, which would interfere with the following analysis.[22] As a result, the glass vial was pretreated by hydrophobic modification.[22] The pretreatment of the glass vial inner surface involved two steps. In the first step, the glass vials were cleaned in an ultrasonication bath of acetone for 15 min followed by rinsing with purified water to remove the surface contamination and then dried with a stream of nitrogen at room temperature. Second, the acidic OTMS and ethanol mixture (1:19, v/v, pH 5−5.5) was added into the clean glass vials and heated to 45 °C for 24 h. The resultant vials were washed with ethanol and purified water several times and dried with a stream of nitrogen.

Magnetic Solid-Phase Extraction Procedure. A mixed fatty acids standard solution including eight fatty acids (C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, and C22:0), with the concentration of each fatty acid of 5 μg/mL, has been used for investigating the parameters that affected the extraction efficiency and desorption efficiency. Prior to extraction, Fe_3O_4 magnetic NPs (20 mg) were added into acetone (5 mL) in a 15 mL vial and ultrasonicated for 5 min to eliminate the adsorbed impurities on the surface of the Fe_3O_4 magnetic NPs. Then acetone was discarded; meanwhile, the sorbent was gathered to the vial bottom by placing a strong magnet on the outer wall of the vial. After that, 10 mL of mixed fatty acids standard solution was added. The mixture was vortexed vigorously for 8 min, and in the process, FFAs were adsorbed onto Fe_3O_4 magnetic NPs through hydrophilic interaction. Subsequently, a magnet was applied to the bottom of the vial to attract and isolate the Fe_3O_4 magnetic NPs, and the supernatant was discarded. Then, the FFA-adsorbed Fe_3O_4 magnetic NPs were washed with 2 mL of hexane/acetone (4:1, v/v) by vortexing for 1 min. After the washing solution had been discarded, the FFA-adsorbed Fe_3O_4 magnetic NPs were collected for derivation and desorption of FFAs.

For analysis of oil samples, the procedure of magnetic solid-phase extraction was similar to that described above, except that 10 mL of 0.1 g/mL oil sample with margaric acid (C17:0) internal standard (5 μg/mL) added was used for extraction.

Derivatization and Desorption. Derivatization and desorption of FFAs can be accomplished simultaneously, avoiding multiple tedious processing steps. Generally, 2 mL of methanol/sulfuric acid (99:1, v/v) was added into a vial containing the FFA-adsorbed Fe_3O_4 magnetic NPs. Then the mixture was vortexed vigorously for 5 min to convert FFAs to FAMEs and desorb them from the Fe_3O_4 magnetic NPs. To obtain samples suitable for GC analysis, 1 mL of hexane was added to extract the FAMEs from the methanol/sulfuric acid solution, along with 1 mL of sodium chloride solution (0.9%, w/w) to facilitate extraction and phase separation. The mixture was vortexed vigorously for 1 min and allowed to stand, and the supernatant was collected for GC-FID analysis.

GC-FID Analysis. The GC-FID analysis was performed on a GC (Agilent 7890N, Palo Alto, CA, USA) equipped with flame ionization detection. The GC separation was achieved on a capillary column (HP-FFAP, 30 m × 0.25 mm × 0.25 μm) purchased from Agilent (Agilent J&W GC Columns). Nitrogen (purity ≥ 99.999%) was used as carrier gas at an inlet pressure of 1.7×10^5 Pa. The temperatures of the injection port and detector (FID) were maintained at 250 and 260 °C, respectively. The oven temperature was held at 210 °C for 1.0 min, then increased to 230 °C at a rate of 10 °C/min, and held for 7.0 min. The injection volume was 1.0 μL in splitless mode. The peaks were identified on the basis of their retention times using authentic standard FAMEs, and all samples were run in duplicate. The relative peak areas (analyte area/I.S. area) were used for quantification of the fatty acids, with considered response factors.

RESULTS AND DISCUSSION

Optimization of Conditions for MSPE. To evaluate the feasibility of Fe_3O_4 magnetic NPs for the extraction of FFAs from oil samples, the parameters that might affect the performance of MSPE needed to be optimized. In this study, several major factors, namely, the type of solvent using as the matrix for extraction, the type of desorption solvent and desorption time, the amount of sorbent, and extraction time, were investigated by using a mixed fatty acids standard solution (each fatty acid 5 μg/mL), and all of the optimization experiments were conducted three times. When one parameter was changed, the others were fixed at their optimized values.

Type of Solvent Used as the Matrix for Extraction. MSPE is a new mode of extraction technique based on the use of magnetic or magnetizable sorbent, which can be uniformly dispersed into sample solution; therefore, large contact area between the sample and the extractant phase can be obtained to get fast mass transfer. Thus, the solution used as the matrix for extraction should be carefully selected. Acetone and hexane, which has the property of dissolving hydrophobic fatty samples, with different volume ratios (100% acetone; 80% acetone, 20% hexane; 60% acetone, 40% hexane; 40% acetone, 60% hexane; 20% acetone, 80% hexane; and 100% hexane) have been investigated as the matrix solution. The results showed that the Fe_3O_4 magnetic NPs can be dispersed well in 100% acetone to form a black solution and remain suspended in this solution for >30 min, which is in accordance with previous paper.[23] As hexane concentration increases, the dispersion property of the Fe_3O_4 magnetic NPs decreased, and the aggregate phenomenon of Fe_3O_4 magnetic NPs was observed, which may be ascribed to the existing hydrophilic $-NH_2$ group on the Fe_3O_4 NPs. The extraction efficiency of FFAs declined after an initial ascent with increasing hexane concentration. This phenomenon can be ascribed to the fact that the extraction efficiency of MSPE is governed by both the dispersion property of the Fe_3O_4 magnetic NPs in solvent and the influence of solvent on extraction of FFAs by Fe_3O_4 magnetic NPs. The Fe_3O_4 magnetic NPs are dispersed well in a polar solvent such as

acetone, but such a solvent may not be optimal for extraction of FFAs by Fe_3O_4 magnetic NPs through hydrophilic interaction. However, a nonpolar solvent such as hexane may be more suited to extract FFAs by Fe_3O_4 magnetic NPs through hydrophilic interaction, but the aggregate phenomenon of Fe_3O_4 magnetic NPs will occur, discouraging the extraction procedure. Therefore, 20% acetone and 80% hexane (v/v) was chosen as an optimum solvent, providing good dispersion of the Fe_3O_4 magnetic NPs and high extraction efficiency of FFAs.

Desorption Conditions. To accomplish derivatization and desorption of FFAs simultaneously, methanol/sulfuric acid (99:1, v/v) was used as the derivatization and desorption reagent. Two milliliters of methanol/sulfuric acid (99:1, v/v) was sufficient to derivatize and desorb extracted FFAs from Fe_3O_4 magnetic NPs. Because the eluent is not compatible with GC analysis, 1 mL of hexane and 1 mL of sodium chloride solution (0.9%, w/w) were added to extract the FAMEs from the methanol/sulfuric acid solution.

The derivatization and desorption time was optimized by increasing the vortex duration from 1 to 8 min (Figure 1).

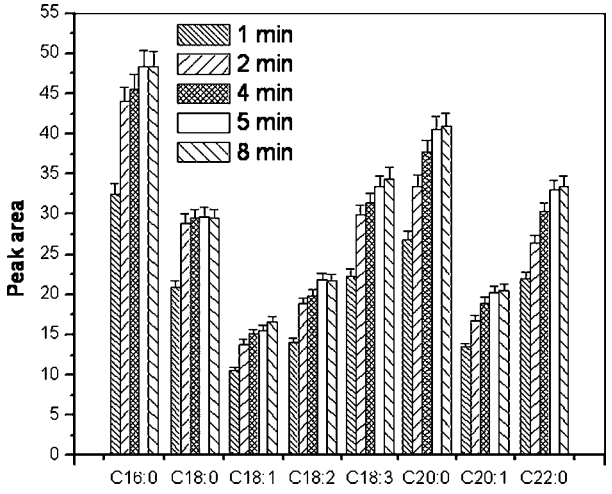

Figure 1. Effect of desorption/derivatization time on extraction efficiency of 5 μg/mL of each FFA.

Extraction efficiency increased from 1 to 5 min vortexing and then flattened out. The significant difference of the influence of derivatization and desorption time was checked by analysis of variance (ANOVA), with $P < 0.02$, and the results also showed that there is a significant difference when the derivatization and desorption time was 1−4 min, and after that no significant differences occurred. Therefore, 5 min of vortexing was selected as derivatization and desorption time, which might be enough for both effective derivatization and desorption.

In addition, the carry-over was investigated by derivatization and desorption in sequence with methanol/sulfuric acid (99:1, v/v) several times. The result suggested that >95% of the analyte adsorbed on the Fe_3O_4 could be derivatized and desorbed by 2 mL of methanol/sulfuric acid (99:1, v/v) one time (5 min). Consequently, one-time desorption (5 min) with 2 mL of methanol/sulfuric acid (99:1, v/v) was adopted in the following experiments.

Amount of Fe_3O_4 Magnetic NPs. To achieve good recovery, different amounts of Fe_3O_4 magnetic NPs ranging from 5 to 25 mg were applied to extract FFAs (as shown in Figure 2). The results show that the recovery achieved by 20 mg of magnetic sorbent is clearly higher than that achieved by

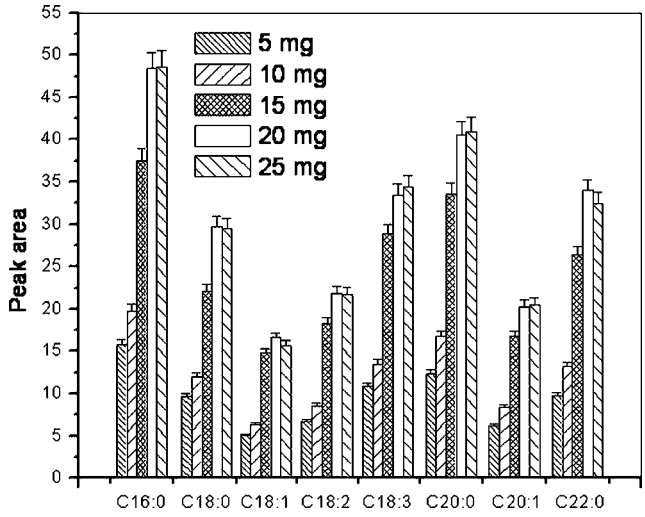

Figure 2. Influence of Fe_3O_4 magnetic NPs amount on extraction efficiency of 5 μg/mL of each FFA.

5, 10, and 15 mg of sorbent but almost the same as that achieved by 25 mg. The significant difference of the influence of the amount of Fe_3O_4 magnetic NPs was checked by ANOVA, with $P < 0.01$, and the results also showed that there is a significant difference when the amount of Fe_3O_4 magnetic NPs was between 5 and 20 mg, and after that no significant differences occurred. Thus, 20 mg was employed in the following experiment.

Extraction Time. The extraction time profiles were conducted by increasing the vortex time from 1 to 10 min. It can be seen from Figure 3 that all of the FFAs reach extraction

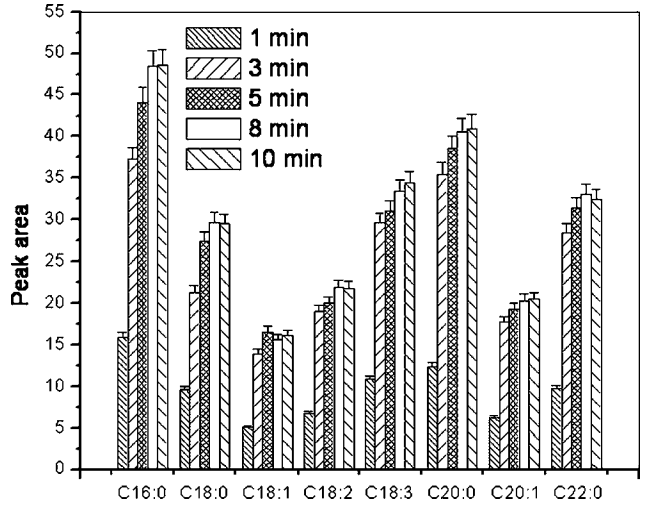

Figure 3. Effect of extraction time on extraction efficiency of 5 μg/mL of each FFA.

platforms when the vortex time is 8 min. The adsorption reached equilibrium rapidly because the adsorbents can be uniformly dispersed into sample solution by vortex, making the contact area between the adsorbents and the analytes large enough to ensure a fast mass transfer. The significant difference of the influence of extraction time was checked by ANOVA, with $P < 0.01$, and the results also showed that there is a significant difference when the extraction time was 1−8 min;

dx.doi.org/10.1021/jf303840q | J. Agric. Food Chem. 2013, 61, 76−83

Table 2. Calibration Curves and LOD and LOQ Data of Eight Fatty Acids

	linearity and sensitivity characteristics				
		regression line			
analyte	linear dynamic range (μg/mL)	linear equation	R^2 value	LOD (ng/mL)	LOQ (ng/mL)
C16:0	0.05−50	$Y = 0.2159X - 0.2175$	0.9998	7.22	24.07
C18:0	0.1−50	$Y = 0.1078X + 0.1152$	0.9997	14.89	49.64
C18:1	0.1−50	$Y = 0.0609X + 0.2718$	0.9982	26.26	87.52
C18:2	0.1−50	$Y = 0.08109X + 0.1579$	0.9977	19.80	65.98
C18:3	0.05−50	$Y = 0.19586X - 0.1547$	0.9998	7.40	24.68
C20:0	0.05−50	$Y = 0.2022X + 0.1321$	0.9998	7.33	24.44
C20:1	0.1−50	$Y = 0.07834X + 0.2847$	0.9981	20.50	68.32
C22:0	0.05−50	$Y = 0.1920X - 0.09444$	0.9998	7.50	24.99

after that, no significant differences occurred. In this study, the extraction time was set at 8 min.

Analytical Performance. Under the optimal conditions mentioned above, FFAs were quantitatively analyzed using mixed fatty acids standard solution including eight fatty acids (C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, and C22:0) with margaric acid (C17:0) added as an internal standard. The linearity was studied using the 8 mixed fatty acids standard solution at 11 different concentrations ranging from 0.05 to 50 μg/mL with margaric acid (C17:0) added as an internal standard at a constant concentration of 5 μg/mL. In the construction of the calibration curve, triplicate measurements of each concentration level of the calibration samples were performed, and the calibrations were obtained by plotting peak area ratios (analyte area/I.S. area) versus concentrations.

As shown in Table 2, satisfactory correlation coefficients for the eight compounds were obtained ranging from 0.9977 to 0.9998. The sensitivity of the method was established by examining the limits of detection (LOD) and limits of quantitation (LOQ). The LOD was defined as the lowest detectable concentration with a signal-to-noise ratio of at least 3, and the LOQ was defined as the lowest quantifiable concentration with a signal-to-noise ratio of at least 10. LOD and LOQ data were in the ranges of 7.22−26.26 and 24.07−87.52 ng/mL, respectively. The relative differences in LOD and LOQ of the different fatty acids may be attributed to the different interactions between the different fatty acids and the Fe_3O_4 magnetic NPs and the different responses of the different fatty acids with GC-FID.

Recoveries were obtained using soybean oil samples spiked with eight fatty acids at three different concentrations, ranging from 0.5 to 50 μg/mL. First, the free fatty acid concentrations in the original soybean oil sample were calculated from the calibration curves. Second, the soybean oil samples spiked with eight fatty acids at three different concentrations were also calculated by the same method. Then the amount of the spiked fatty acids was calculated by subtracting the concentration of each fatty acid in the original soybean oil from the total amount of each fatty acid of the spiked soybean oils. Finally, the recoveries were obtained by comparing the concentration of the calculated spiking fatty acids with the corresponding spiked value. Recoveries and standard deviations are summarized in Table 3. Mean recoveries were in the range of 81.33−117.75%, demonstrating that the accuracy of the present method is acceptable.

The reproducibility of the method was determined by the intra- and interday precision measurements of soybean oil spiked with eight fatty acids at three different concentrations. Intraday precision was determined using three series of six replicates each at three concentration levels. Interday precision was calculated with three replicates at the three fortification levels on three continuous days. The reproducibility of the method is measured by the relative standard deviation (RSD) of triplicate measurements. Satisfactory precisions were obtained with RSD values of <6.4% (intraday) and <6.9% (interday), as shown in Table 4, which is also in accordance with refs 21 and 22, in which MSPE has been used for the extraction of other harmful residuals in edible oil, illustrating the good reproducibility achieved by the method.

Table 3. Recoveries of Eight Fatty Acids Spiked into Oil Samples at Three Different Concentrations

	recovery[a] (%, $n = 6$)		
analyte	low (0.5 μg/mL)	medium (5 μg/mL)	high (50 μg/mL)
C16:0	113.5 ± 3.8	94.7 ± 4.3	108.2 ± 3.2
C18:0	99.1 ± 5.1	91.5 ± 3.9	117.7 ± 5.1
C18:1	87.3 ± 4.2	117.8 ± 5.2	110.2 ± 6.3
C18:2	83.4 ± 3.6	91.8 ± 4.7	96.6 ± 5.3
C18:3	112.0 ± 6.1	116.2 ± 5.3	104.7 ± 4.6
C20:0	85.2 ± 4.3	90.6 ± 5.5	100.5 ± 4.6
C20:1	88.3 ± 4.6	82.8 ± 6.1	97.0 ± 3.5
C22:0	81.3 ± 5.3	108.5 ± 4.4	106.6 ± 4.3

[a]Recoveries are given as the average value ± standard deviation of sextuple analyses.

Applications in Real Samples. To demonstrate the applicability of the method, the developed MSPE method was applied for the determination of dynamics of FFA formation in an oil accelerated storage test (60 °C). Four kinds of edible oil samples (rapeseed oil (RS), soybean oil (SB), sunflower oil (SF), and corn oil (CO)) from retail markets located in Wuhan (China) were analyzed. In Table 5, free fatty acids identified by the developed MSPE method in four various plant oils before and after 10 days of storage at 60 °C are shown. It was found that palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidic acid (C20:0) were detected in all kinds of investigated oil samples, whereas eicosenoic acid (C20:1) and behehic acid (C22:0) were detected only in RS, SF, and CO samples and SB and SF samples, respectively. It was found that the total concentration of the FFAs in all kinds of investigated fresh oils did not exceed 55 μg/mL. The highest concentration of total FFAs was found in SF oil, which was up to 53.57 μg/mL, and the lowest concentration of total FFAs was found in CO oil, which was only 16.67 μg/mL. It was also found that fresh oils were characterized by a low content of FFAs, whereas during the

Table 4. Method Precision for Extraction of Fatty Acids Spiked at Three Different Concentrations in Oil Samples

	precision (RSD %)					
	intraday (n = 6)			interday (n = 3)		
analyte	low (0.5 μg/mL)	medium (5 μg/mL)	high (50 μg/mL)	low (0.5 μg/mL)	medium (5 μg/mL)	high (50 μg/mL)
C16:0	3.2	3.8	2.9	2.5	3.5	6.6
C18:0	3.9	3.1	3.6	5.1	6.3	5.1
C18:1	5.2	2.9	5.3	6.4	6.7	6.9
C18:2	2.7	4.5	4.7	2.7	2.6	3.3
C18:3	5.3	6.2	4.6	5.2	6.2	6.4
C20:0	5.4	4.3	5.3	4.8	5.5	5.0
C20:1	6.4	5.1	4.3	5.3	5.4	4.8
C22:0	4.9	3.3	3.5	4.7	5.1	2.9

Table 5. Free Fatty Acids Identified in Four Various Plant Oils[a] before and after 10 Days of Storage at 60 °C Using the Developed MSPE Method

	fresh oils (μg/mL)				oils stored for 3 days (μg/mL)				oils stored for 5 days (μg/mL)				oils stored for 10 days (μg/mL)			
analyte	RS	SB	SF	CO	RS	SB	SF	CO	RS	SB	SF	CO	RS	SB	SF	CO
C16:0	3.14	4.77	3.14	2.57	3.35	4.83	3.68	3.15	3.45	4.88	3.87	3.27	4.23	5.16	4.17	3.32
C18:0	2.22	3.72	3.15	0.50	2.39	3.53	4.05	1.38	2.51	3.54	4.07	1.68	3.75	4.67	4.49	1.80
C18:1	37.94	16.43	16.07	5.73	38.46	16.44	17.72	5.84	38.72	18.08	18.38	6.08	43.23	20.64	21.98	7.65
C18:2	7.89	22.14	27.42	7.13	8.13	24.12	29.19	7.76	8.18	25.74	31.59	8.34	9.71	29.36	35.94	10.61
C18:3	1.62	1.53	0.81	0.27	1.70	1.71	1.07	0.69	1.70	1.73	1.08	0.95	1.83	1.94	1.19	0.95
C20:0	0.23	0.47	0.65	0.23	0.24	0.48	0.75	0.35	0.26	0.53	0.75	0.47	0.26	0.57	0.77	0.48
C20:1	0.53	nd[b]	0.72	0.24	0.54	nd	0.77	0.47	0.54	nd	0.81	0.63	0.54	nd	0.84	0.72
C22:0	nd	0.68	1.19	nd	nd	0.69	1.22	nd	nd	0.71	1.31	nd	nd	0.77	1.43	nd
total FFAs	53.15	49.74	53.57	16.67	54.81	51.80	58.45	19.64	55.36	55.21	61.86	21.42	63.55	63.11	70.81	25.53

[a]RS, rapeseed oil; SB, soybean oil; SF, sunflower oil; CO, corn oil. [b]nd, not detected.

oxidation process, the longer the storage, the higher the content of FFAs produced, which may be attributed to the increased degree of lipid peroxidation due to the high-temperature storage.[25] Figure 4 shows the chromatogram of free fatty acids detected by the developed MSPE method in soybean oils during 10 days of storage at 60 °C; it can be seen that oleic acid (C18:1) and linoleic acid (C18:2) dominated in these detected FFAs associated with stored oils.

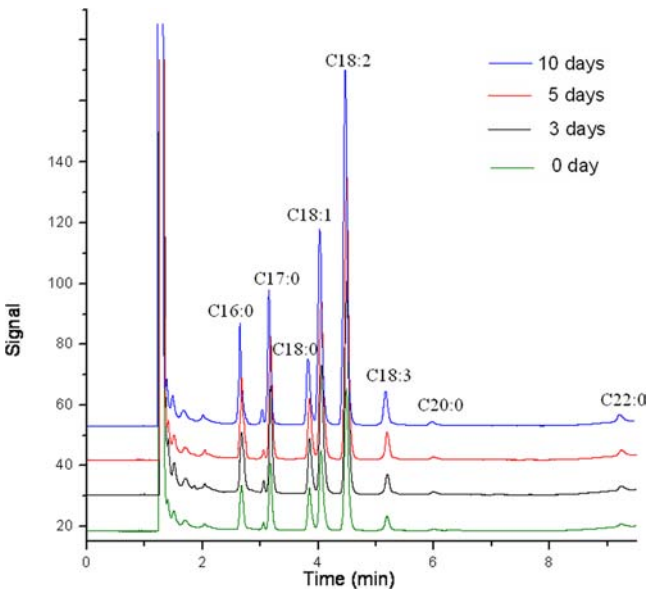

Figure 4. GC-FID chromatogram of free fatty acids detected from soybean oils during 10 days of storage at 60 °C, spiked with I.S. (C17:0) with the concentration of 5 μg/mL before extraction by the developed MSPE method.

A comparative study of our developed method to the standard method of the American Oil Chemists' Society (AOCS) involving titrating oil dissolved in alcohol with a strong base to a phenolphthalein end point was performed, and the results are presented in Figure 5. Triplicate analyses of each sample were performed, and average values were used for quantization. It can be seen that both the content of the FFAs in oils and the changing trends of the FFAs in oils during the storage examinations were roughly accordant as detected by the two methods. The significance differences between the values obtained by the two methods have also been checked by ANOVA, with $P > 0.05$, showing that there is no significant difference between the values obtained by the two methods. The results confirmed the feasibility of the proposed method for the determination of FFAs in edible oils. However, the developed method was convenient and rapid; the whole procedure of our proposed MSPE-GC method could be completed within 25 min. Many oil samples could be pretreated and analyzed simultaneously, and not only total FFAs in oils can be determined, but each FFA as well.

In conclusion, coupled with GC analysis, utilization of monodisperse magnetic single-crystal ferrite (Fe_3O_4) nanoparticles for extraction of FFAs from edible oils by the mode of MSPE was proven to be a simple, rapid, and effective method. The extraction and desorption/esterification were carried out quickly, and the whole pretreatment process could be accomplished by simple vortex and ultrasonic agitation within 15 min. The LODs and LOQs of the target FFAs by this method were in the ranges of 7.22−26.26 and 24.07−87.52 ng/mL, respectively. The recoveries in oil sample were in the range

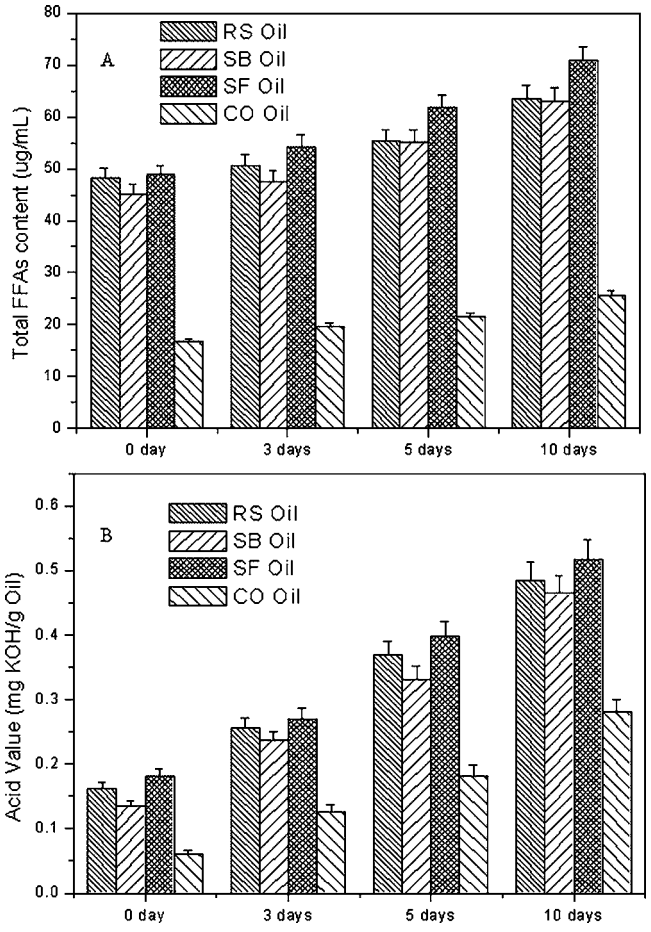

Figure 5. Comparison of the total FFA content detected by the developed MSPE method (A) and acid values detected by the American Oil Chemists' Society (AOCS) titration method (B) of the four kinds of edible oils during 10 days of storage at 60 °C. RS, rapeseed oil; SB, soybean oil; SF, sunflower oil; CO, corn oil.

of 81.33−117.75% with RSDs of <6.4% (intraday) and <6.9% (interday). This method was successfully applied to the analysis of the dynamics of FFA formation in four kinds of edible oil accelerated storage test. Our results demonstrate that the proposed method is suitable for routine analysis. Taken together, the simple, rapid, and cost-effective method developed in the current study offers a potential application for the extraction and preconcentration of FFAs from a hydrophobic sample matrix, including edible fats and oils, fatty foods, and biological samples with high amounts of lipid, and it might open up a new field in pretreatment technique for oil and lipid samples.

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Funding

This work was supported by the National Key Technologies R&D Program (2012BAK08B03), the National Natural Science Foundation of China (Grants 31271879, 31171661, and 21105119), and the National High Technology Research and Development Program of China (863 Program: 2011AA100904). We gratefully acknowledge the support of Hubei Natural Science Foundation (Grant 2011CDB357) and the Research Foundation of the Director General of Oil Crops Research Institute, Chinese Academy of Agricultural Sciences.

Notes

The authors declare no competing financial interest.

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