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Rapid Transmethylation and Stable Isotope Labeling for Comparative Analysis of Fatty Acids by Mass Spectrometry

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Fatty acids covalently bonded with other molecules have been implicated in many important biological processes. We describe here a rapid approach termed isotope-coded fatty acid transmethylation (iFAT) that integrates extraction, transmethylation, and isotopic labeling into a single step with the aid of ultrasonic irradiation for comparative analysis of fatty acids by mass spectrometry. In this approach, samples without any prefractionation were mixed with a methanol solution of 0.5 M NaOH and an *n*-hexane solution. The intense wave shocks and cavitations generated by ultrasonic irradiation not only speed the alkaline-catalyzed transmethylation reaction but also facilitate the simultaneous mass transfer of fatty acid methyl esters into the top *n*-hexane extraction phase that was injected into a GC/MS system. By using commercially available d_3-methanol, we were able to compare the intensity of labeled and unlabeled methyl esters and their corresponding fragment ions. The detection limit can be down to the picogram level. Major advantages of the iFAT strategy are summarized in the following: (1) Efficient heterogeneous reactions. Solid samples such as dried cell lysates or detergent-resistant fractions can be readily transformed and analyzed with the aid of ultrasound irradiation. (2) Accurate quantification of fatty acids. Evaluation of the completeness or losses of transformation reactions across lipid classes has been hampered due to a lack of suitable methods. Isotope labeling can be used as an internal standard for accurate comparison of the fatty acid composition in different cell states. (3) Reduced interferences from complex biological context. The iFAT strategy not only differentially labels fatty acids in different samples, but also volatilizes those molecules, and thus, they are isolated from the bulk background and analyzed by GC/MS. This proposed approach has been applied to quantitatively determine the fatty acid composition in plant oil and in budding yeast cell lysates and detergent-resistant fractions. It should provide a widely applicable means for quantitative comparison of the fatty acid composition in cells and tissues.

Analysis of the fatty acid composition is important from a variety of commercial and biological perspectives. Fatty acid analysis is a useful method for identification of the authenticity of different oils[1,2] and for microbial identification.[3−5] In biomedical research, fatty acid profiles in tissues, cells, and blood have been implicated in many important biological processes. Fatty acids, covalently bonded with other molecules including glycerol, saccharides, and proteins, not only play important metabolic roles in cells but also are important signaling molecules involved in regulating the expression of a lot of genes.[6−9] They are also an integral part of cell membrane influencing membrane fluidity and channel functions.[10,11] Determination of the fatty acid composition in cells or tissues is a useful approach to monitor the physiological status and quantify biological responses to different environmental stressors.[12,13] Moreover, the clinical and basic studies of the fatty acid composition in tissues and blood have increasingly attracted attention for biomarker discovery related to cardiovascular diseases,[14,15] cancer,[16,17] and other chronic diseases.[18,19] Thus, it is necessary to develop techniques that can perform large

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numbers of samples for qualitative and quantitative analysis quickly and efficiently.

Although there are several methods for fatty acid analysis such as liquid chromatography (HPLC)[20−22] and capillary electrophoresis (CE),[23,24] the preparation of fatty acid methyl esters (FAMEs) followed by gas chromatographic analysis is routinely performed. The primary challenges associated with this technique are the throughput, detection sensitivity, and quantification. Several steps are usually involved in this technique including lipid extraction, saponification, methylation, and extraction of fatty acid methyl esters.[25] After lipids are extracted from cell or tissue homogenates by the Soxhlet procedure or with an organic solvent such as chloroform−methanol, FAMEs are generated from the lipids by either saponification and then methylation of the free fatty acids or direct transesterification of lipids catalyzed by acids or bases. The whole process is tedious and potentially causes sample loss, especially for samples with a small size.

The combination of extraction and derivatization of fatty acids has been developed.[26−28] FAMEs were generated by transesterification of lipids in a heterogeneous reaction system. This approach significantly simplifies the process of sample preparation and increases the recovery. However, due to the low solubility of fatty acids in the aqueous phase, the methylation reaction occurs on the interface between the oil and aqueous phases, which results in low efficiency of esterification and detection. Several techniques have been developed to homogenize the two-phase reaction system. Elevated temperature[29] can aid the mass transfer and increase the solubility between phases, but it takes hours to complete the reaction. Alternatively, addition of a cosolvent such as THF (tetrahydrofuran) can enhance the miscibility of the phases and create a homogeneous mixture.[30] The formation of a single-phase medium can increase the reaction rate. However, an additional procedure is needed for the separation of the mixed medium before downstream analysis. In particular, none of these techniques can avoid the side reactions caused by hydrolysis due to the presence of water in samples.

Additionally, absolute quantification of fatty acids by gas chromatographic analysis is difficult.[31,32] So far, it has not been experimentally demonstrated whether the transmethylation is quantitative across lipid classes, and no internal standards have been used to evaluate the extent of the transmethylation reactions and side reactions.[33] On the basis of these considerations, this work is aimed to develop a new method that can achieve sensitive analysis of FAMEs rapidly and quantitatively.

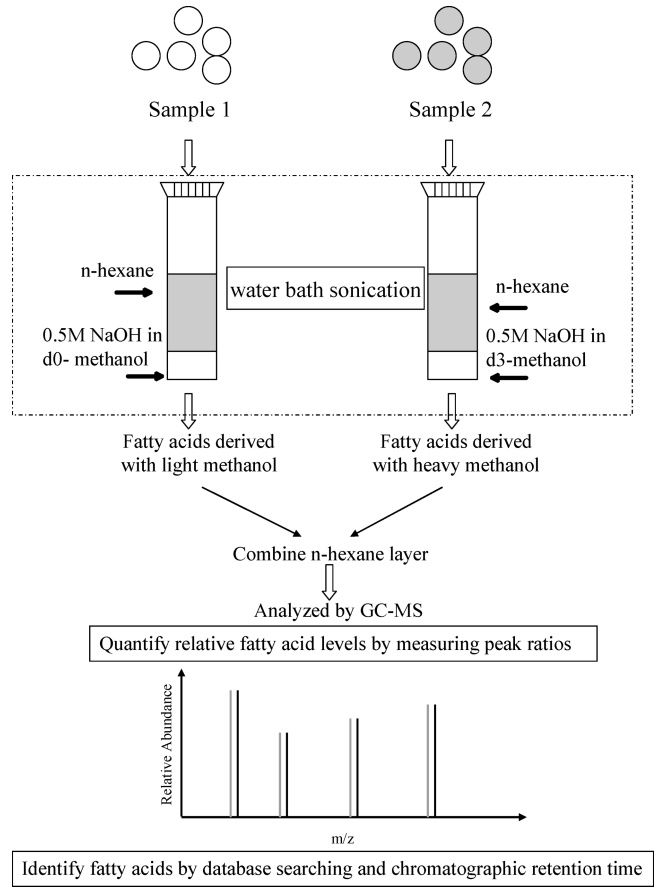

Figure 1. iFAT strategy for quantitative analysis of the fatty acid composition in different samples. Two samples have been derived with isotopically light and heavy methanol, and the resultant fatty acid methyl esters were simultaneously transferred into an *n*-hexane layer with the aid of ultrasonication. The *n*-hexane layers were combined and separated by capillary gas-phase chromatography. A pair of light and heavy methanol-derived fatty acid methyl esters are chemically identical and easily detected because they essentially coelute out and there is a 3 Da mass difference measured in a mass spectrometer. The ratio of the original amounts of the samples have been recovered in the fragment ions and molecular ions of fatty acid methyl esters. The relative quantification was determined by the ratio of peak pairs. The identification of fatty acid methyl esters was achieved by the combination of database searching and the comparison of the chromatographic retention time of the samples and standard fatty acid methyl esters.

In the past few years, ultrasonic irradiation of liquids has been reported to speed reactions markedly through the generation of transient cavitations and intense shock waves.[34] The reaction rate of heterogeneous phases can be dramatically increased due to high-velocity collisions. In this work, we describe a new method called isotope-coded fatty acid transmethylation (iFAT) as shown in Figure 1. This method integrates lipid extraction, transmethylation, stable isotope labeling, and extraction of methyl esters into one step with the aid of ultrasonication. Dried cell lysates and SDS-resistant pellets or raw plant oils without any prefractionation were directly mixed with a methanol solution of 0.5 M NaOH and an *n*-hexane solution. Transmethylation and extraction were completed within minutes under ultrasonic irradiation. This ultrasound-assisted reaction system of heterogeneous phases

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significantly improves the efficiency of FAME generation and simplifies the sample preparation. By using d_3-methanol, it provides a method to carry out comparative analysis of fatty acids from different samples via the comparison of the intensity of labeled and unlabeled fatty acid methyl esters and fragment ions. Hydrolysis caused by the presence of water can be eliminated because solid dried cell lysates can be readily transmethylated and isotopically labeled within minutes. Because of the capacity of iFAT to handle solid samples, it is thus possible to explore the most hydrophobic fraction of cells that are even resistant to the SDS solubilization. In addition, this work has also experimentally demonstrated that lipids can be quantitatively transmethylated and continuously extracted into the top *n*-hexane phase within 1 min under ultrasound irradiation. The iFAT approach has been successfully applied to quantitatively analyze fatty acid compositions not only in plant oils but also in whole cells of the budding yeast.

EXPERIMENTAL SECTION

Reagents and Apparatus. *n*-Hexane (HPLC grade) was purchased from Kermel (Tianjin, China). NaOH and Na_2SO_4 are of analytical reagent quality and were purchased from Guoyao Co. Ltd., China. Water was obtained from a Milli-Q purification system (Millipore, MA). A standard mixture of methyl esters of 37 fatty acids was purchased from Supelco (Bellefonte, PA). Anhydrous methanol and d_3-methanol are from Sigma-Aldrich (St. Louis, MO). Yeast cell lysis buffer was purchased from Shenergy Biocolor BioScience & Technology, Shanghai, China. Tryptone and yeast extract are from Oxoid (Hampshire, U.K.). Rapeseed oil and cottonseed oil are from local refineries.

Preparation of the Budding Yeast *Saccharomyces cerevisiae*. The budding yeast *S. cerevisiae* (strain YEF473A, a his3, leu2, lys2, trp1, ura3) was grown in either YPD medium or DDT containing (24 ng/μL) YPD medium, and logarithmically growing cells were harvested. The cells were washed three times with PBS buffer before lyses. Then the cells were mixed with glass beads (G8772, Sigma) and cell lyses buffer (Shenergy Biocolor BioScience & Technology, Shanghai, China) containing 1% SDS and vortexed for 15 min. They were then centrifuged at 16000*g* for 1/2 h at 4 °C. The supernatant was pipetted into an Eppendorf vial, and the insoluble pellets were transferred to another Eppendorf vial. The samples were stored at −20 °C for transmethylation and GC/MS analysis.

Isotope-Coded Transmethylation of Lipids in Raw Plant Oils and Budding Yeast Cells. Stock solutions of rapeseed oil and cottonseed oil were prepared by weighing 0.009 g of raw rapeseed oil or cottonseed oil into glass vials and dissolving them in 900 μL of *n*-hexane solution. The samples of plant oil without any prefractionation were put into glass vials and then mixed with 100 μL of either anhydrous d_0-methanol or a d_3-methanol solution of 0.5 M NaOH. Cell lysates and SDS-resistant pellets of the budding yeast cells were dried under nitrogen gas before being mixed with 100 μL of regular methanol or a d_3-methanol solution of 0.5 M NaOH. Then an *n*-hexane solution was added on the top, and the final volume of hexane was 200 μL. Each vial was treated by 1 min of ultrasonication (100 W, 40 kHz). Then the vials were allowed to stand for 5 min before the supernatant hexane parts were transferred. Equal amounts of hexane layers of d_0-methanol-derived fatty acid methyl esters were combined with those derived by d_3-methanol. The combined *n*-hexane layers were then washed with pure water three times to remove trace methanol and NaOH. Anhydrous Na_2SO_4 was used to absorb trace water remaining in the hexane solution.

GC/MS Analysis and Identification of Fatty Acid Methyl Esters. A 1 μL volume of a hexane solution of fatty acid methyl esters prepared by the previously described procedure was separated by capillary gas chromatography (Rtx-5MS, 0.25 μm in thickness, 30 m × 0.25 mm i.d.). The column oven temperature was programmed to reach 250 °C through the following steps: increased from 60 to 150 °C at a rate of 50 °C/min (held at 150 °C for 5 min), and then elevated to 250 °C at a rate of 10 °C/min. The column was then maintained at 250 °C for 10 min before completion of the whole program. During the whole process of analysis, the injector temperature was kept at 220 °C. Splitless injection was performed with a 1 min sampling time for the budding yeast cell analysis. The split ratio was 100 for the analysis of plant oil samples. The capillary gas chromatographic column was directly coupled with a quadruple mass spectrometer (GC/MS QP2010PLUS, Shimadzu, Japan). The interface temperature was set at 270 °C, and the ion source was maintained at 200 °C. A 70 eV ionization energy of impact ionization (EI) was used to fragment the eluent from capillary GC. Mass spectra were recorded in full scan mode with a mass-to-charge ratio (m/z) ranging from 50 to 600 units.

Fatty acid methyl esters derived with d_0-methanol were identified by searching against the NIST database first. Due to the structural diversity of fatty acids, we have ensured the identification by comparing the chromatographic retention time of standard fatty acid methyl esters with the experimental results. Fatty acids derived with heavy methanol were identified through the chromatographic retention time and manual interpretation according to mass spectral characteristics including molecular weight, isotopic patterns, and fragment ions compared with those derived with light methanol.

RESULTS AND DISCUSSION

Ultrasonic iFAT Strategy. Gas chromatographic analysis of fatty acids conventionally involves either acid- or alkaline-catalyzed methylation that needs several tedious steps to complete extraction, saponification, methylation, and so on. A new one-step method called iFAT is shown in Figure 1. Samples such as plant oils or budding yeast cell lysates and detergent-resistant pellets without any prefractionation were mixed with a 0.5 M NaOH solution of methanol, and *n*-hexane solvent was added on the top. After 1 min of ultrasonic irradiation, the top *n*-hexane layer can be used for GC/MS analysis. Ultrasonic irradiation speeds the transmethylation reaction and simultaneously transfers methyl esters into the *n*-hexane layer. The mixtures of fatty acid methyl esters were then separated by capillary gas chromatography and identified by mass spectrometry. By using d_0-methanol and d_3-methanol, it should be able to perform comparative analysis of fatty acids in two different samples.

The new approach iFAT is a useful tool for global comparison of the fatty acid composition in different samples. It is based on the following principles. First, ultrasonic irradiation generates strong wave shock and facilitates mass transfer among heterogeneous phases. It is thus able to handle solid samples. Second,

FAMEs can be generated from lipids by base-catalyzed transesterification, and lipids in the samples are quantitatively transmethylated and extracted. Although there are several methods for acid-catalyzed transesterification, serious side reactions have been reported.[35,36] Therefore, alkaline-catalyzed transmethylation was studied in this work. Third, the resultant FAMEs can be readily extracted by the top *n*-hexane solvent. Moreover, pairs of molecular ions of methyl esters and fragment ions tagged with light and heavy methyl esters are chemically identical and can serve as internal standards for accurate quantification. The ratios between the intensities of the light and heavy peaks provide an accurate measurement of the relative abundance of the fatty acid methyl esters. The experimental results also provide a tool to investigate whether the base-catalyzed transformation reactions across different types of lipids under ultrasound irradiation are quantitative. The isotopically labeled FAMEs can be used as internal standards for evaluating the extent of transformation reactions.

Analysis of Standard Plant Oils and Identification of the Authenticity of Plant Oils by the iFAT Strategy. A stock solution of 9 mg of rapeseed oil was prepared in 900 μL of *n*-hexane solvent. To illustrate the method, a series of standard rapeseed oil solutions containing six major fatty acids were prepared at known but different concentrations and analyzed. The fatty acid mixtures were labeled, combined, and analyzed as schematically illustrated in Figure 1. Fatty acid methyl esters were separated by capillary gas-phase chromatography and then identified and quantified by a single-quadrupole mass spectrometer directly coupled to the gas chromatograph. During the course of the 27 min of chromatographic elution, more than 2400 scans were acquired. All six fatty acids were unambiguously identified not only by database searching but also by the chromatographic retention times compared with those of standard FAMEs. Quantification of each fatty acid methyl ester was achieved by the average peak ratios of labeled and unlabeled molecular ions and fragment ions. The mean differences between the observed and expected quantities for six fatty acids ranged from 0% to 16% as shown in Table 1. All data of three repeated measurements are listed in Supporting Information Table 1. The precision of this method can be demonstrated by the standard deviations of six fatty acid methyl esters that ranged from 0.01 to 0.10 for three repeated experiments.

The detailed process is further illustrated for palmitic acid methyl ester (C16:0) as shown in Figure 2. Figure 2A is the zoom-in total ion chromatogram (TIC) of the mixture of labeled and unlabeled C16:0 methyl esters. The mixture eluted out of the column within 0.15 min (from scan 331 to scan 349). To minimize the isotope effect on the elution, spectra generated from scan 331 to scan 349 were selected and averaged. Figure 2B is the averaged mass spectrum from scan 331 to scan 349. In total, there are several pairs of fragment ions and molecular ions. Relative quantities were determined by averaging the peak ratios of fragment ions and molecular ions. The observed average peak ratio (light:heavy) is 1.02 ± 0.06 (the expected ratio is 1.00). Parts A−D of Figure 3 show the mass spectra of labeled and unlabeled C16:0 methyl esters at the expected ratios of 0.50, 0.33, 0.25, and 0.20 (light:heavy), respectively. Table 1 summarizes the identification and quantification of the six fatty acid methyl esters mixed at different known quantities. It was demonstrated that the iFAT strategy is able to obtain accurate measurements of small changes in relative fatty acid levels. The accuracy of this method can be demonstrated from the relative ratios of different amounts of rapeseed oil for which multiple fatty acid methyl esters and fragment ions have been quantified. For example, the observed mean ratios for C16:0, which is one of the six major fatty acid methyl esters, are 1.02, 0.49, 0.30, 0.25, and 0.21 while the expected ratios are 1.00, 0.50, 0.33, 0.25, and 0.20 (d_0:d_3), and the standard deviations are 0.06, 0.04, 0.03, 0.02, and 0.03. The observed mean ratios for long-chain unsaturated fatty acid methyl ester C22:1 are 1.12, 0.46, 0.32, 0.27, and 0.22 corresponding to the expected ratios of 1.00, 0.50, 0.33, 0.25, and 0.20 (d_0:d_3), and the standard deviations are 0.10, 0.03, 0.01, 0.02, and 0.01. It has been experimentally verified that the transmethylations by using the iFAT strategy are quantitative across different types of lipids. Representative mass spectra are present in Supporting Information Figure 1.

Table 1. Identification and Quantification of Fatty Acid Methyl Esters of Rapeseed Oil

FAME[a]	obsd mean ratio[b] (d_0:d_3)	expected ratio[c] (d_0:d_3)	error (%)	FAME	obsd mean ratio (d_0:d_3)	expected ratio (d_0:d_3)	error (%)
C16:0	1.02 ± 0.06	1.00	2.0	C18:0	1.00 ± 0.06	1.00	0.0
	0.49 ± 0.04	0.50	2.0		0.45 ± 0.05	0.50	10.0
	0.30 ± 0.03	0.33	9.1		0.31 ± 0.03	0.33	6.1
	0.25 ± 0.02	0.25	0.0		0.25 ± 0.02	0.25	0.0
	0.21 ± 0.03	0.20	5.0		0.21 ± 0.04	0.20	5.0
C18:2	0.96 ± 0.07	1.00	4.0	C20:1	0.95 ± 0.05	1.00	5.0
	0.42 ± 0.07	0.50	16.0		0.47 ± 0.03	0.50	6.0
	0.28 ± 0.01	0.33	15.2		0.30 ± 0.03	0.33	9.1
	0.21 ± 0.01	0.25	16.0		0.29 ± 0.04	0.25	16.0
	0.17 ± 0.01	0.20	15.0		0.22 ± 0.05	0.20	10.0
C18:1	0.97 ± 0.08	1.00	3.0	C22:1	1.12 ± 0.10	1.00	12.0
	0.47 ± 0.05	0.50	6.0		0.46 ± 0.03	0.50	8.0
	0.34 ± 0.01	0.33	3.0		0.32 ± 0.01	0.33	3.0
	0.27 ± 0.02	0.25	8.0		0.27 ± 0.02	0.25	8.0
	0.23 ± 0.02	0.20	15.0		0.22 ± 0.01	0.20	10.0

[a] Fatty acid methyl ester. [b] Ratios were calculated for each fatty acid methyl ester by averaging the peak ratios of fragment ion pairs. [c] Expected ratios were calculated from the known amounts of plant oils.

The iFAT method was applied to study the differences of the fatty acid composition between standard rapeseed oil and cottonseed oil. The same amounts of rapeseed oil and cottonseed oil were labeled, combined, and analyzed as shown in Figure 1. Table 2 presents a collection of the identified fatty acid methyl esters. The fatty acid composition has been a widely used approach to identify the authenticity of plant oil. Development of rapid, accurate, and sensitive approaches is important for public health and commerce reasons. By using the iFAT strategy, differences in six major fatty acids have been quantitatively determined as shown in Table 2. Standard deviations of the observed ratios (rapeseed:cottonseed) ranged from 0.01 to 0.16. All mass spectra are presented in Supporting Information Figure 2.

Analysis of Budding Yeast Cell Lysates and Detergent SDS-Resistant Pellets by the iFAT Strategy. To illustrate that the iFAT strategy is also useful for the global analysis of the fatty acid composition in cells, a series of a known amount of budding

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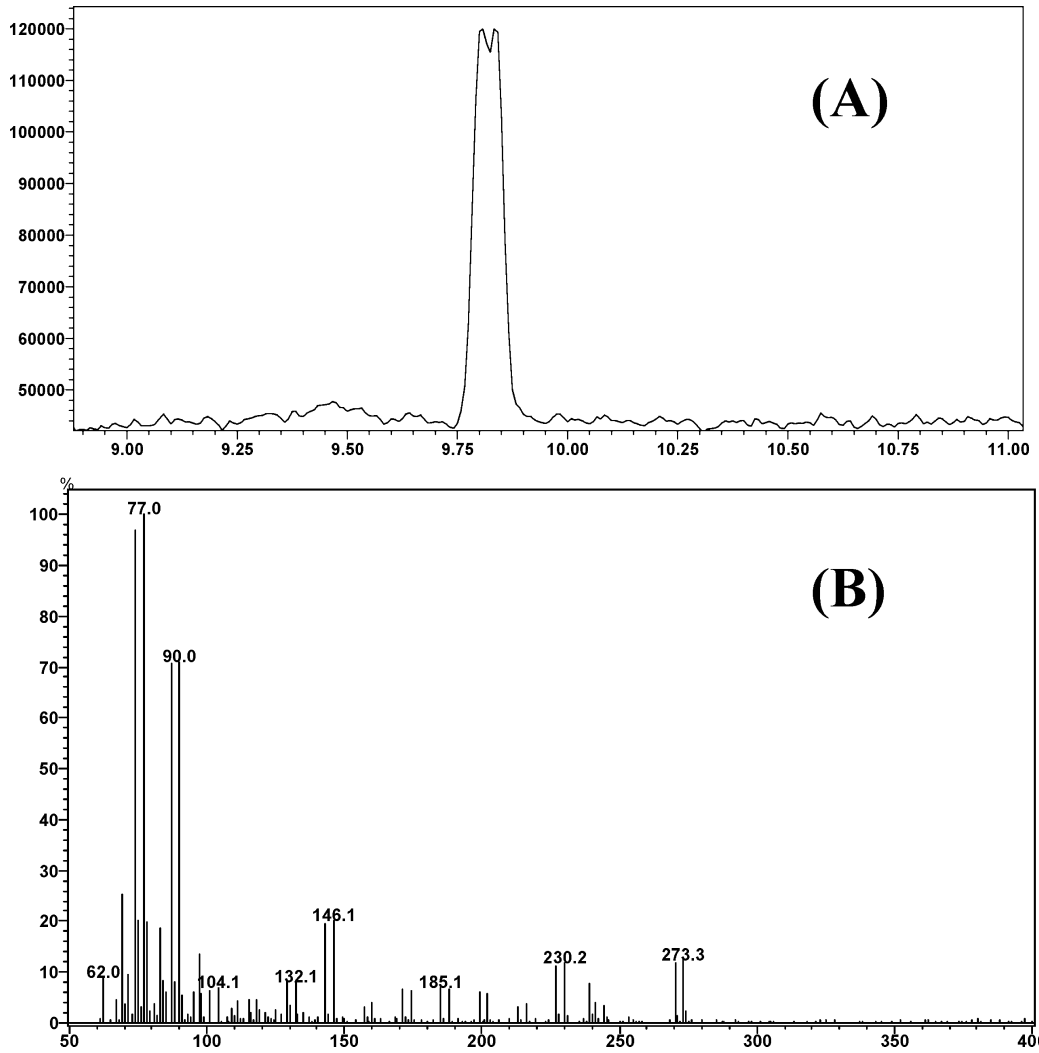

Figure 2. Isotope-coded fatty acid transmethylation and quantitative analysis of C16:0 methyl ester in rapeseed oil at the expected ratio of 1.00 (light:heavy) as shown in Table 1. (A) Zoom-in TIC of C16:0 methyl ester from scan 331 to scan 349. It eluted out within 0.15 min. Although light and heavy fatty acid methyl esters essentially coelute, there are still tiny differences in retention times due to the high separative capacity of the 30 m long capillary gas-phase chromatographic column. (B) Average mass spectrum generated from scan 331 to scan 349. There are several pairs of fragment ions, and the average ratio was used to determine the relative fatty acid methyl ester concentrations.

yeast cell lysates were analyzed. Cell lysates were dried under nitrogen gas. Without any prefractionation, the cell lysate mixtures were labeled, combined, and analyzed as schematically illustrated in Figure 1. Six major fatty acids were unambiguously identified by database searching and comparison of the chromatographic retention times with those of standard FAMEs. Quantification of each fatty acid methyl ester was calculated from the average peak ratios of labeled and unlabeled ions. Table 3 is the collection of all the identifications. The precision and accuracy of the iFAT strategy can be examined from the peak ratios of multiple fatty acid methyl esters. The mean differences between the observed and expected quantities for six fatty acids ranged from 0% to 20%, and standard deviations ranged from 0.01 to 0.17. Data of three repeated measurements are listed in Supporting Information Table 2. Representative mass spectra are presented in Supporting Information Figure 3.

The iFAT strategy was applied to study differences in stationary-phase fatty acid levels in the yeast *S. cerevisiae*, which were grown in YPD medium and DDT containing (24 ng/μL) YPD medium. Logarithmically growing cells were harvested and lysated. Cell lysates from each cell state containing 20 μg of proteins were dried under nitrogen gas, differentially labeled, combined, and analyzed as illustrated in Figure 1. It has been found that DDT can cause changes in the microbial phospholipid composition[37] and changes in the fatty acid composition of rat liver lipids.[38] Table 4 presents a collection of identified DDT-induced changes in the cellular fatty acid level of *S. cerevisiae*. It was apparent that cells of *S. cerevisiae* contained an increased level of C12:0, C14:0, C16:0, C18:0, and C18:1 but no significant changes in C16:1 when grown in DDT-containing media. The observed mean peak ratios (control:DDT-treated) were 0.65, 0.79, 0.89, 0.88, 0.90, and 0.98, respectively. Standard deviations ranged from 0.01 to 0.07. The growth of *S. cerevisiae* in the presence of DDT was retarded as shown in Figure 4A. There was a longer lag phase. These observed changes are similar to those reported in the literature.[37] Changes in the fatty acid composition could affect a number of proteins involved in signal transduction,[39] protein−lipid

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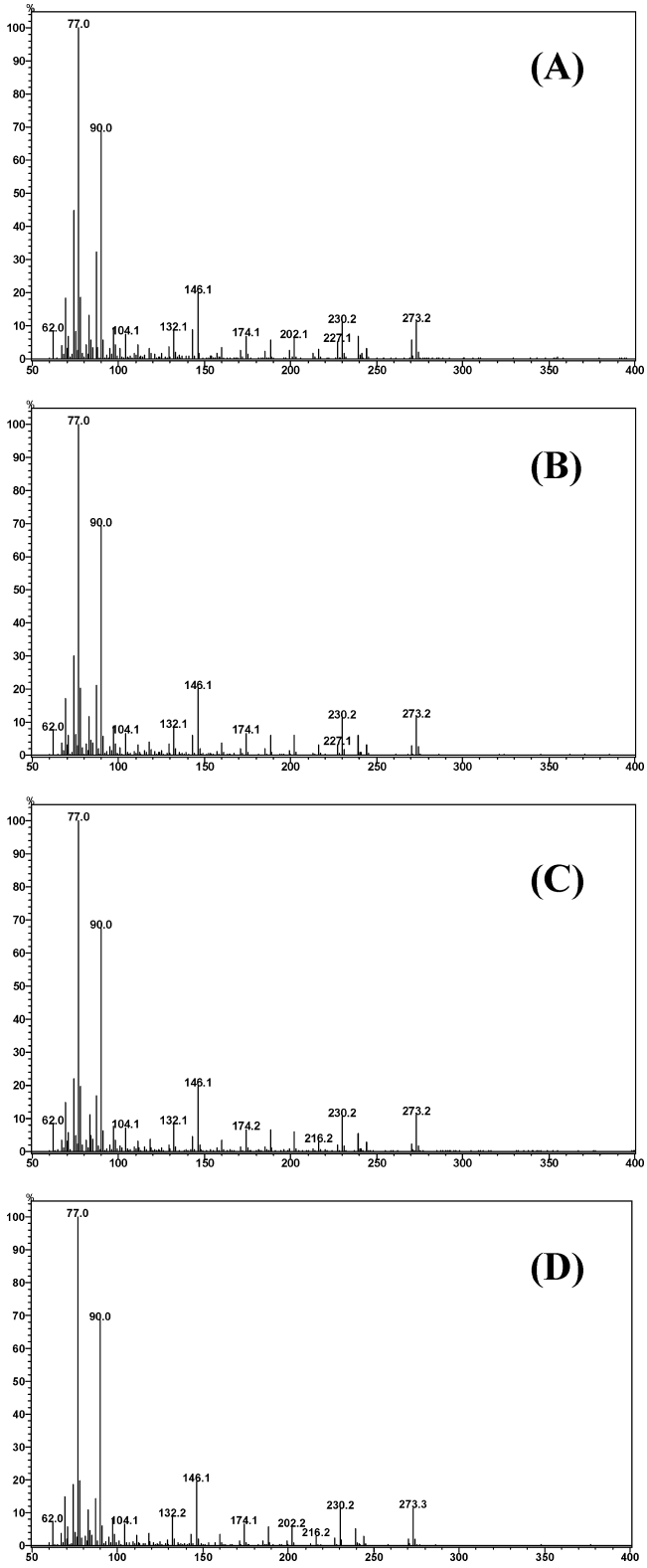

Figure 3. Isotope-coded fatty acid transmethylation and quantitative analysis of C16:0 methyl ester in rapeseed oil at different expected ratios (light:heavy) as shown in Table 1. (A−D) Average mass spectra of C16:0 methyl esters at the expected ratio of 0.50, 0.33, 0.25, and 0.20, respectively.

interactions,[40] and enzymatic activities.[41] Using the iFAT strategy, we have successfully measured the changes in fatty acid composition. Parts B and C of Figure 4 are the mass spectra of C12:0 and C16:0 methyl esters of control and DDT-treated cells, respectively.

Table 2. Fatty Acid Profiles of Rapeseed Oil and Cottonseed Oil

FAME[a]	obsd ratio[b] (rapeseed:cottonseed)	mean ± SD
C16:0	0.18	0.19 ± 0.01
	0.18	
	0.20	
C18:2	0.32	0.32 ± 0.02
	0.31	
	0.34	
C18:1	2.54	2.50 ± 0.04
	2.50	
	2.47	
C18:0	0.91	0.82 ± 0.16
	0.64	
	0.92	
C20:1	only detected in rapeseed oil	
C22:1	only detected in rapeseed oil	

[a] Fatty acid methyl ester. [b] Observed ratios were calculated by averaging the peak ratios of fragment ion pairs.

Table 3. Identification and Quantification of Fatty Acid Methyl Esters of *S. cerevisiae* Lysates

FAME[a]	obsd mean ratio[b] (d_0:d_3)	expected rratio[c] (d_0:d_3)	error (%)	FAME	obsd mean ratio (d_0:d_3)	expected ratio r(d_0:d_3)	error (%)
C12:0	1.06 ± 0.12	1.00	6.0	C14:0	1.08 ± 0.17	1.00	8.0
	0.49 ± 0.03	0.50	2.0		0.49 ± 0.05	0.50	2.0
	0.33 ± 0.01	0.33	0.0		0.39 ± 0.09	0.33	18.2
	0.25 ± 0.02	0.25	0.0		0.30 ± 0.01	0.25	20.0
	0.20 ± 0.02	0.20	0.0		0.23 ± 0.02	0.20	15.0
C16:1	1.09 ± 0.10	1.00	9.0	C16:0	1.07 ± 0.04	1.00	7.0
	0.51 ± 0.01	0.50	2.0		0.51 ± 0.03	0.50	2.0
	0.34 ± 0.01	0.33	3.0		0.37 ± 0.04	0.33	12.1
	0.25 ± 0.03	0.25	0.0		0.29 ± 0.03	0.25	16.0
	0.22 ± 0.02	0.20	10.0		0.23 ± 0.02	0.20	15.0
C18:1	1.07 ± 0.03	1.00	7.0	C18:0	0.89 ± 0.09	1.00	11.0
	0.50 ± 0.03	0.50	0.0		0.53 ± 0.03	0.50	6.0
	0.35 ± 0.03	0.33	6.1		0.35 ± 0.04	0.33	6.1
	0.28 ± 0.02	0.25	12.0		0.26 ± 0.02	0.25	4.0
	0.22 ± 0.03	0.20	10.0		0.23 ± 0.03	0.20	15.0

[a] Fatty acid methyl ester. [b] Ratios were calculated for each fatty acid methyl ester by averaging the peak ratios of fragment ion pairs. [c] Expected ratios were calculated from the known amounts of cell lysates.

Table 4. DDT-Induced Changes in the Fatty Acid Level of *S. cerevisiae* Cells

FAME[a]	mean ± SD,[b] control:DDT-treated (in soluble cell lysates)	mean ± SD, control:DDT-treated (in SDS-resistant pellets)
C12:0	0.65 ± 0.01	
C14:0	0.79 ± 0.04	1.12 ± 0.12
C16:1	0.98 ± 0.04	1.08 ± 0.25
C16:0	0.89 ± 0.03	1.14 ± 0.21
C18:1	0.90 ± 0.07	1.01 ± 0.23
C18:0	0.88 ± 0.03	1.09 ± 0.25

[a] Fatty acid methyl ester. [b] Observed ratios were calculated by averaging the peak ratios of fragment ion pairs.

All other mass spectra are listed in Supporting Information Figure 4.

In particular, due to the problem of solubility, the detergent SDS-resistant hydrophobic pellets of cells have been under-

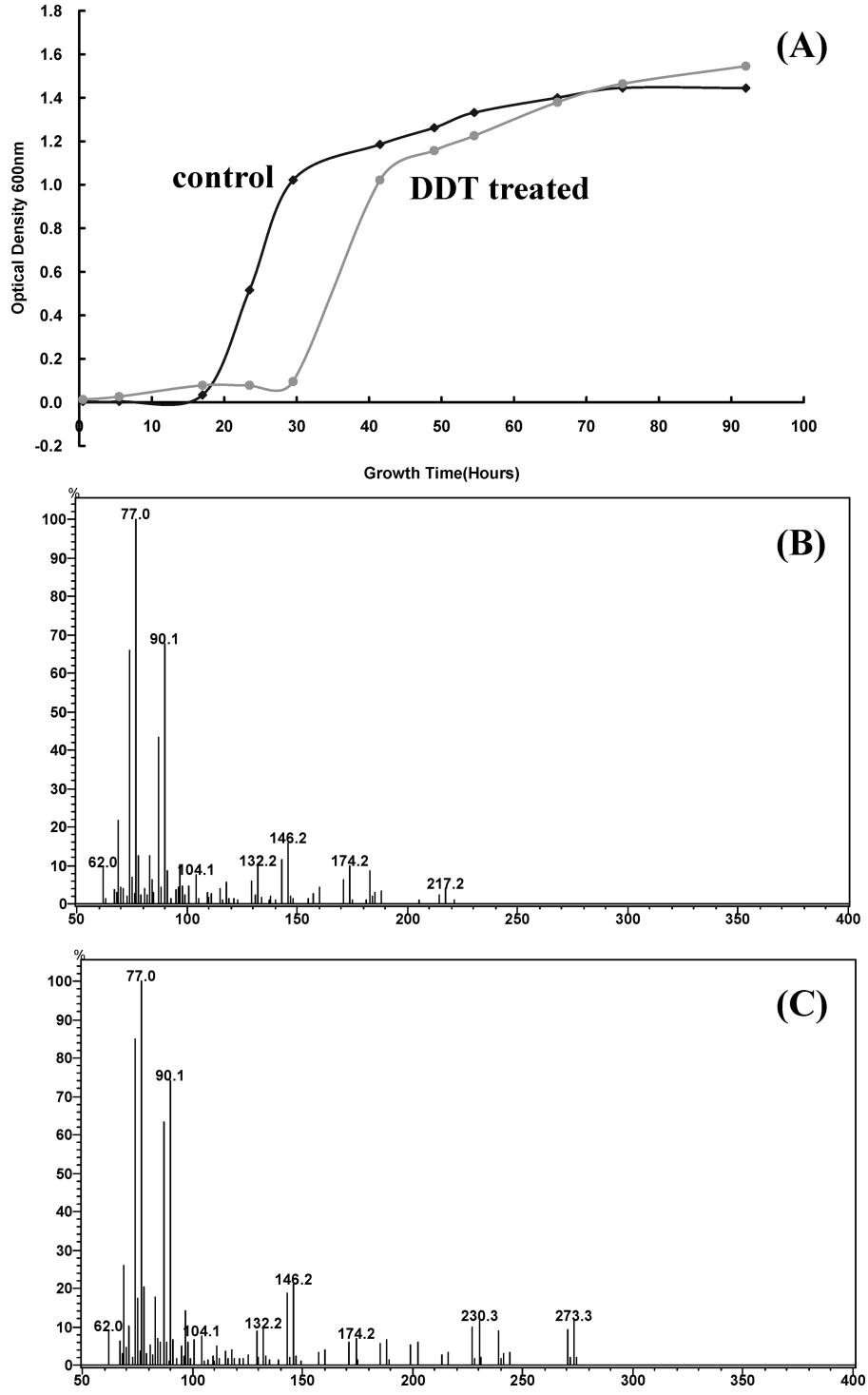

Figure 4. Isotope-coded fatty acid transmethylation and quantitative analysis of the fatty acid composition of *S. cerevisiae* cell lysates grown in normal YDD medium and DDT-containing (24 ng/μL) YPD medium. (A) Effects of DDT on the growth of *S. cerevisiae*. The blue trace represents the growth in normal YPD medium, and the red trace represents the growth in DDT-containing (24 ng/μL) YPD buffer. (B) Mass spectrum of d_0- and d_3-methanol-derived C12:0 methyl esters. (C) Mass spectrum of d_0- and d_3-derived C16:0 methyl esters.

represented.[42] Using the iFAT strategy, we have identified the changes of the fatty acid composition not only in SDS-soluble cell lysates of *S. cerevisiae* but also in the SDS-resistant hydrophobic pellets of *S. cerevisiae* grown in normal YPD medium and DDT-containing YPD medium. Fatty acids carried by lipids that are enriched in SDS-resistant pellets implicate a broad range of interest. However, identification of the composition has been hampered by lack of suitable methods. Identified fatty acids are summarized in Table 4.

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The experimental results demonstrated there are several advantages of the iFAT strategy over conventional methods: (1) It is simple and fast, and the reagents are readily available. The complex biological samples can be directly subjected to isotope-coded transmethylation and extraction without any prefractionation with the aid of ultrasonic irradiation. (2) The efficiency of the heterogeneous reaction of transesterification was greatly improved by ultrasound irradiation. The iFAT strategy can thus handle not only soluble samples but also solid pellets. The side reaction of hydrolysis caused by the presence of water in samples can be avoided because the iFAT strategy can efficiently transform dried cell lysates. In particular, the iFAT strategy provides a new avenue to explore the composition of extremely hydrophobic pellets that are resistant to SDS solubilization. The whole process can be completed within minutes. (3) There is less interference from the biological context in the GC/MS-based iFAT method compared with other LC/MS-based approaches. Isotope-coded transmethylation not only differentially labels the fatty acid methyl esters but also volatilizes the fatty acids so that they can be directly analyzed by capillary GC/MS. Compared with other quantitative methods that are based on affinity chromatography and nanoLC/MS, this strategy can efficiently avoid the interferences from the complex biological context because only volatilized samples can be extracted and analyzed by GC/M, and thus, the dimensions of separation can be greatly reduced. It significantly reduces the complexity of the mixtures. (4) At least two pairs of fragment ions and molecular ions were detected from each fatty acid methyl ester. Therefore, the identification and quantification can be redundant. (5) The derived fatty acid methyl esters provide additional information for each fragment ion and thus enhance the unambiguous identification of structurally versatile small molecules. Finally, this strategy has experimentally demonstrated that the ultrasonication-assisted one-step approach can quantitatively achieve transmethylation and extraction within minutes. It should be a useful tool for rapid analysis of fatty acid profiles in tissues, cells, or blood that has increasingly attracted attention for biomarker discovery in clinical trials and biomedical research.

CONCLUSIONS AND PERSPECTIVES

These results illustrate the potential ability of the iFAT method for quantitative analysis of the fatty acid composition not only for standard plant oil samples but also for a variety of applications to tissues, cells, and other biological fluids. The iFAT method has experimentally demonstrated that ultrasound-assisted one-step transmethylation and extraction can quantitatively derive and analyze fatty acids. It significantly reduces the sample preparation procedure. The conventional method needs several hours for lipid extraction, saponification, transmethylation, and analysis. The ultrasonication-assisted approach integrates all of these procedures into a single step. In particular, biological samples without any prefractionation can be directly subjected to isotope-coded transmethylation, and the resultant labeled and unlabeled fatty acid methyl esters are analyzed by GC/MS. It greatly avoids the interferences from the complex biological context that are confronted by LC-based methods. For the GC-based method, only volatilized components are extracted and analyzed. Therefore, other coexisting hydrophilic molecules in the samples have fewer possibilities to suppress the analytes, and the complexity of the mixture is reduced. In addition, the iFAT strategy provides a new method for the discovery of SDS-resistant pellets that has been implicated in many important biological processes. The dynamic range associated with the ability of this iFAT method to quantify the differences of fatty acid levels depends on the relative intensity of peak pairs. Differences of more than 5-fold or even larger can be determined for more abundant fatty acid methyl esters. Finally, this iFAT can be extended to quantitatively analyze protein post-translational modifications such as palmitoylation that are under-represented so far.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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