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Identification of triacylglycerol using automated annotation of high resolution multistage mass spectral trees

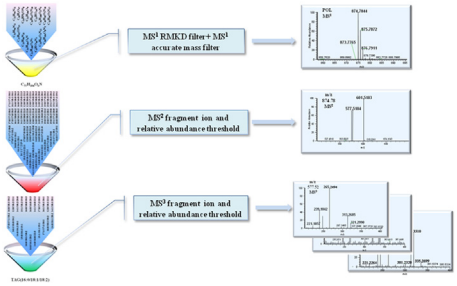
Xiupin Wang [a, b, c, d, *, 1], Qingzhi Peng [f, 1], Peiwu Li [a, b, c, d, **], Qi Zhang [a, c, d, e], Xiaoxia Ding [a, c, d, e], Wen Zhang [a, c, d, e], Liangxiao Zhang [a, c, d, e]

[a] Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China
[b] Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, China
[c] Key Laboratory of Detection for Mycotoxins, Ministry of Agriculture, Wuhan 430062, China
[d] Laboratory of Risk Assessment for Oilseeds Products (Wuhan), Ministry of Agriculture, Wuhan 430062, China
[e] Quality Inspection and Test Center for Oilseeds Products, Ministry of Agriculture, Wuhan 430062, China
[f] College of Food Science and Technology of Huazhong Agriculture University, China

HIGHLIGHTS

- TIT software can identify TAGs using a "stage-by-stage elimination" strategy.
- TIT software can discriminate unique elemental composition candidates by utilizing the MS^1 accurate mass and referenced RKMD.
- The regiospecific isomers of fatty acyl chains for TAGs will be distinguished using MS^2 and MS^3 fragment spectra.

GRAPHICAL ABSTRACT



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ABSTRACT

High complexity of identification for non-target triacylglycerols (TAGs) is a major challenge in lipidomics analysis. To identify non-target TAGs, a powerful tool named accurate MS^n spectrometry generating so-called ion trees is used. In this paper, we presented a technique for efficient structural elucidation of TAGs on MS^n spectral trees produced by LTQ Orbitrap MS^n, which was implemented as an open source software package, or TIT. The TIT software was used to support automatic annotation of non-target TAGs on MS^n ion trees from a self-built fragment ion database. This database includes 19108 simulate TAG molecules from a random combination of fatty acids and corresponding 500582 self-built multistage fragment ions ($MS \leq 3$). Our software can identify TAGs using a "stage-by-stage elimination" strategy. By utilizing the MS^1 accurate mass and referenced RKMD, the TIT software can discriminate unique elemental composition candidates. The regiospecific isomers of fatty acyl chains will be distinguished using MS^2 and MS^3 fragment spectra. We applied the algorithm to the selection of 45 TAG standards and demonstrated that the molecular ions could be 100% correctly assigned. Therefore, the TIT software could be applied to TAG identification in complex biological samples such as mouse plasma extracts.

* Corresponding author. Current address: Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Xudong 2nd Road 2#, Wuchang District, Wuhan, 430062, China.
** Corresponding author. Current address: Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Xudong 2nd Road 2#, Wuchang District, Wuhan, 430062, China.
E-mail addresses: xiupinwangyls@hotmail.com (X. Wang), lipeiwu@caas.cn (P. Li).
[1] These authors contributed equally to this work.

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1. Introduction

Glycerolipids are composed of mono-, di-, and tri-substituted glycerols, with the most well-known one being fatty acid triester of glycerol, which is called triglycerides (TAGs) [1]. TAGs were stored in the fat of animal and plant tissues as energy storage [2]. TAGs are highly complex and diverse due to various combinations of types and number of fatty acyl chains, *cis*-/*trans*-configuration of double bonds, and their positions on the glycerol skeleton (such as regioisomers and enantiomers) [3]. To discover new TAG species and to identify known TAG isomers in biological systems are both challenges for analysis of all TAGs [4]. Establishing software packages that contain reasonable TAG identification rules and comprehensive TAG databases is an approach to solve the problems.

Several software packages from either commercial or open sources have been suggested for identification of TAGs. Kurvinen [5] developed the MSPECTRA software package, which was applied to automatic processing of TAG molecular mass distribution spectra and collision induced dissociation (CID) product ion spectra based on $[M\text{-}H]^-$ ions, fragment ions $[RCO_2]^-$, and corresponding $[M\text{-}H\text{-}RCO_2H\text{-}100]^-$ ions from electron ionization (EI) sources. However, this software was not well suited for most TAGs because TAGs had a high boiling point and were difficult to gasify, which was easily detected using LC-MS/MS with atmospheric pressure ionization (API) sources [6]. Mitsui Knowledge Industry Co., Ltd and the Taguchi laboratory developed Lipid Navigator (http://lipidsearch.jp/LipidNavigator.htm), which is a high-throughput web-based tool and an automated system for identification of glycerophospholipids and sphingolipids [7]. However, this website does not support TAG search. Cvacka and his associates developed the TriglyAPCI software [8], which is used for identification of MS^2 fragment ions and molecular adduct ions from atmospheric pressure chemical ionization (APCI) mass, as well as conjecture of possible TAG structures. But this software can be used to analyze data only from low resolution APCI mass. Although a recent study showed that commercial software used to identify TAGs had been developed, such as LipidView from the AB SCIEX company, it was feasible only for MS^2 mass spectra and cannot be used to distinguish regiospecific isomers of TAG fatty acyl chains.

Recently, liquid chromatography coupled with accurate mass has been described as a powerful tool for non-target compound identification [9–11]. Compared to MS/MS generating MS^2 fragments, the multistage mass (MS^n) ion tree technique leads to deeper and more detailed fragmentation pathways, thereby enabling chemical characterization, even in the absence of data from reference compounds or NMR [12–16]. The linear ion-trap MS^n ($n \geq 3$) on the $[M+Li]^+$ ions with the approach of electrospray ionization (ESI) sources coupled with RP-HPLC could be applied in the characterization of individual TAGs, particularly the identification of the positions of fatty acid substituents and that of double bond(s) of the unsaturated fatty acyl moieties on the glycerol backbone [17]. However, the TAGs were overlapped using RP-HPLC separation except some isomeric disaturated and monounsaturated TAGs [18]. Therefore, regiospecific isomers of TAG fatty acyl chains on the overlapping TAGs cannot be discriminated using the above method. We have developed the TAG ion tree (TIT) software to support TAG identification by annotating all fragmented TAGs in LC-MS^n data sets with candidate molecules taken from self-built multistage databases. The software is based on the "stage-by-stage elimination" strategy of accurate mass MS^n ion tree and can be used to distinguish overlapped regiospecific isomers of TAG fatty acyl chains. The software can be downloaded from the TIT website (http://www.oilcrops.com.cn/ArticleView.aspx?id=1342).

Computational algorithms were used to assign elemental formulas to mass peaks based on the MS^1 accurate m/z values and Kendrick mass defect (KMD) [19–22]. The referenced kendrick mass defect (RKMD) allows identification of TAG classes without prior knowledge of the lipid class of any TAG mass in the spectrum [23]. Therefore, to combine the MS^1 accurate mass with the RKMD is a good method for assigning TAG elemental formulas. However, one elemental formulas may represent only hundreds of known TAGs, but there may be an even larger number of unknown TAGs [24]. Recently, a refined method has been described for the MS^n substructure of isomers using MS^n spectrum matching scores, which can be used to rank different candidate structures for an unknown metabolite for the purpose of its identification [25]. The "matching score" method requires perfect reproducibility of MS^n spectra and good MS^n accurate mass. However, the sample and time limitations may not allow optimization of the experimental conditions for the best accuracy of mass spectrometer in routine operations [26,27]. In addition, some approaches rely on similarity with the spectral trees of known compounds to derive structural features [27,28], even though such reference information is not always available. In this study, we described a novel strategy named "stage-by-stage elimination", which can greatly reduce mass accuracy requirements. This strategy derives the ion tree database of generated substructures to explain multistage spectral data and eliminates "false" isomers at each MS level using the ion tree database.

Herein, we presented a software package for efficient identification of non-target TAGs based on raw file input, which is implemented as open source software. The new software achieves identification of TAGs, and it is fully automated without highly specialized knowledge. Moreover, this software can accurately distinguish regiospecific isomers of fatty acyl chains based on the accurate mass and "stage-by-stage elimination" strategy. This strategy is obviously advantageous over the "matching score" method because reproducibility of MS^n spectra is less demanded, particularly across different dissociation models [29]. The qualitative limit of detection of TAGs was decreased significantly and the application range of the software was expanded, while the use of the identification method on the number of MS^3 fragment ions. This study also evaluated the reproducibility of the TAG MS^n spectral tree technique, and the obtained results were stable over a 3 month period, a 1000-fold concentration change, more than 30% collision energy (CE), and different collision models (CID and ETD). The performance of the TIT software package was verified by analysis of 45 TAG standards. The false negative rate was 0%, and there were false positives because the TIT software cannot distinguish regioisomers.

2. Experimental

2.1. Materials and reagents

MS grade acetonitrile (ACN) and isopropyl alcohol (IPA) were purchased from Fisher Scientific (Pittsburgh, PA). TAG standards were purchased from Larodan Fine Chemicals (Malmö, Sweden), and all standards had the purity of more than 98%. The detailed list of all TAG standards is given in the Supporting Information, Table S-1. Four-week-old female BALB/c mouse were purchased from Centers for Disease Control and Prevention of Hubei Province (Wuhan, China).

Each TAG standard was used as a solution in isopropanol/methanol (1/1; v/v) at the concentration of 10 μg/mL to be analyzed on an LTQ Orbitrap Elite mass spectrometer in the Z-spray geometry. These standard stock solutions were stored at −30 °C. Before use, a mixed standard solution was diluted to the desired concentration