High Throughput Lipidomics Analysis in Rapeseed Oil Research

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Background

Oilseed rape (Brassica napus L. and Brassica rapus L.) has been grown worldwide as one of the most important oil crops for edible uses. Oilseed rape oils nowadays are a great source of maintaining oil consumption in families. For the edible rapeseed oil, to increase oleic acid, linoleic acid contents and to decrease saturated fatty acid, linolenic acid contents in the oil is a major goal for the coming years in order to make it as the healthiest edible oil. The use of plant oils also depends on their composition and the comprehensive triacylglycerol profiling brings valuable information in this respect.

Edible oils are a complex mixture of triacylglycerols (TAGs). They are composed of a glycerol backbone esterified with saturated, unsaturated fatty acids or mixture of both. In human organism, they serve as a source of energy stored in fat tissues, thermal and mechanical protective layer surrounding important organs, source of essential fatty acids (FAs: linoleic and linolenic acids), fat-soluble vitamins and other non-polar compounds. They form an important part of human diet and their imbalances can lead to several human diseases, i.e. coronary heart disease, dyslipidaemia, obesity or inborn errors of metabolism. The deficiency of essential FAs necessary for the biosynthesis of long-chain polyunsaturated FAs important for cell membranes leads to problems in nearly every tissue in the body. The main sources of TAGs in the human diet are oil plants and especially oils prepared from them.

The structural characterization of TAG is required to know the exact chain length of the acyls group, degrees of unsaturation, and locations of double bonds. Regiospecific locations of each acyl chain are important in terms of chemical as well as physical properties like viscosity, pour point, melting point, heat of fusion, solubility, crystal structure, and polymorphism. It also affects the human absorption from different foods and is thus an important parameter for food industries.

Objectives

The objective of present study was to develop a high throughput analysis method for detailed analytical characterization of TAGs composition in a wide range of rapeseed oils important in food industry and dietetics. The mass spectrum (MS) analysis was performed by flow injection-electrospray ionization (ESI)-MS/MS with positive electrospray ionization, in the presence of ammonium ions and ammonia. The fatty acids (FAs) in each TAG and the regioisomerism of these FAs were determined from their mass spectra on the basis of specific fragmentation and relative abundances.

Methods

The separation and quantification of fatty acids were carried out by GC on Agilent 7890A instrument equipped with a split/splitless injector and an FID detector. The MS analysis was performed with a hybrid triple quadrupole/linear ion trap mass spectrometer (ABI 4000 Q-Trap, Applied Biosystems, Foster City, CA).

The analysis was performed by flow injection-electrospray ionization (ESI)-MS/MS with positive electrospray ionization, in the presence of ammonium ions and ammonia. For analysis, chloroform solution of rapeseed oil and ammonia solution was pumped using syringe pump (Cole Parmer 74900 series), at a flow rate of 5 µL/min, to the MS analysis. Neutral loss and precursor ion scanning modes were used for identification of molecular species in TAGs. The fatty acids (FAs) in each TAG and the regioisomerism of these FAs were determined from their mass spectra on the basis of specific fragmentation and relative abundances.

Results

Samples of rapeseed were harvested during the crop season (May) in 2009 at the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (CAAS). “Zhongshuang-10” was used for the evaluation of the analysis method. Fatty acids composition (%) of “Zhongshuang-10” is shown in Table 1. These compositions were determined by gas chromatography coupled with flame ionization detector based on our previous research [1].

Table 1 Fatty acids composition (%) of the “Zhongshuang-10” rapeseeds

Fatty acids	Molecular formula	m/z	Composition (%)
C14:0	$C_{14}H_{28}O_2$	228.2	0.10
C16:0	$C_{16}H_{32}O_2$	256.2	5.06
C18:0	$C_{18}H_{36}O_2$	284.2	2.70
C18:1	$C_{18}H_{34}O_2$	282.2	60.10
C18:2	$C_{18}H_{32}O_2$	280.2	20.89
C18:3	$C_{18}H_{30}O_2$	278.2	8.43
C20:0	$C_{20}H_{40}O_2$	312.3	1.33
C20:1	$C_{20}H_{38}O_2$	310.3	1.14
C22:0	$C_{22}H_{44}O_2$	340.3	0.05
C22:1	$C_{22}H_{42}O_2$	338.3	0.20

Neutral loss of the fatty acids listed above in the presence of ammonium ions and ammonia were used for identification the parent ion of each TAGs. Then (ESI)-MS/MS analysis was performed to obtain their mass spectra, the fatty acids (FAs) in each TAG and the regioisomerism of these FAs were determined from their mass spectra on the basis of specific fragmentation and relative abundances.

The regiospecific determination of the fatty acid location on the glycerol backbone plays an important role in the separation of the mixture of TAGs. Taking into account a few generally accepted considerations, TAGs positional isomers were identified. It includes, that less abundant fatty acids are often esterified on sn-3 position, and the absence of palmitic acid in the sn-2 position in plant TAGs [2]. It is also based on intensity of diacylglycerols and the fact that α and β-diacylglycerol fragments are energetically more favored. However, the sn-1 and sn-3 position are indistinguishable in MS spectrum [22, 12], also there is no official recommendation for designation of sn-1/3 position. In the ESI-MS spectrum of OLnL (Fig. 1), the m/z 855.5 and 872.6 corresponds to the protonated molecular ion, and molecular adducts of ammonium respectively. The diacylglycerols fragments at m/z 599.5, 577.5 and 575.5 correspond to LLn^+, PL^+, and PLn^+ respectively. The intensity of LLn^+ is higher than others, while PL^+ has higher intensity than PLn^+, which means that L is at sn-2 position and P and Ln bound at sn-1 and sn-3 position.

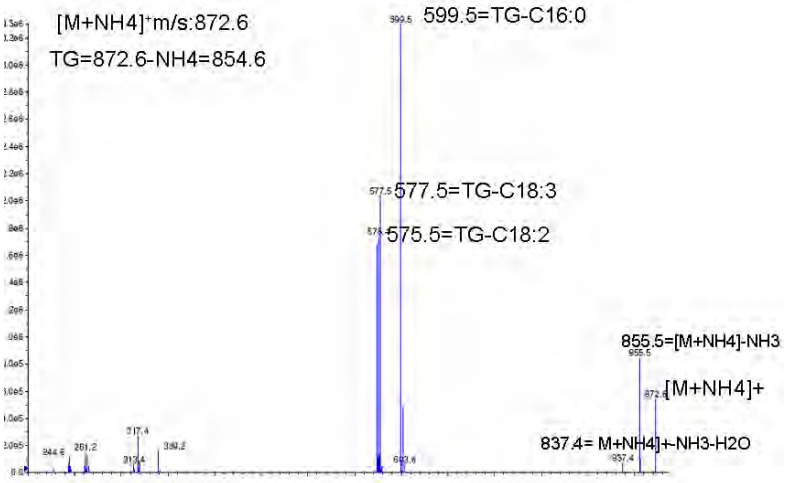

Fig. 1 The regiospecific determination of position of fatty acid in TAG using ESI-MS spectra. ESI-MS spectra of PLLn.

M[illegible] eveloped method and the results are listed in Table 2.

Table 2 Characteristic ions in mass spectra and product ion tandem mass spectra used for identification of molecular species of lipids in "Zhongshuang-10" rapeseeds oil

$[M+NH4]^+$ or other precursor ion (m/z)	Major and (minor) molecular species
850.9	TG(16:0/16:0/18:1)
871.2	TG(16:1/18:1/18:3)
873.3	TG(16:0/18:1/18:3)
875	TG(16:0/18:1/18:2)
877	TG(16:0/18:1/18:1)
879	TG(16:0/18:0/18:1)
881	TG(16:0/16:0/20:0); TG(16:0/18:0/18:0)
891	TG(18:3/18:3/18:3)
893	TG(18:2/18:3/18:3)
895	TG(18:1/18:3/18:3); TG(18:2/18:2/18:3)
897	TG(18:1/18:2/18:3); TG(18:2/18:2/18:2)
901	TG(18:1/18:1/18:2)
903	TG(18:1/18:1/18:1)
905	TG(18:0/18:1/18:1); TG(16:0/18:1/20:1)
907	TG(18:0/18:0/18:1)
909	TG(18:0/18:0/18:0); TG(16:0/18:0/20:0)
925	TG(18:2/18:3/20:1); TG(18:2/18:2/20:2)
927	TG(18:1/18:2/20:2)
929	TG(18:1/18:2/20:1)
931	TG(18:1/18:2/20:0)

Conclusion/Application to practice:

A high throughput analysis method for detailed analytical characterization of TAGs composition in rapeseed oils has been developed. In "Zhongshuang-10" rapeseeds oil, the numbers of identified TAG were about 26. The presence of intense proton ($M+H^+$), and ammonium ($M+NH4^+$), adduct ions and their respective diacylglycerols ions in the ESI-MS spectra allowed correct identification of TAG.

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