

# Rapid Authentication of Edible Oils by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry

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## ABSTRACT

We have developed direct analysis of edible oils using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The data acquisition can be computer-controlled and MALDI-MS spectra of high quality and high reproducibility can be obtained for edible oil samples, and a preliminary spectral database of edible oils has been set up. Since different types of edible oils have different MALDI-MS spectral patterns, the authenticity of an edible oil sample can be determined by comparing its MALDI-MS spectrum with those of its labeled oil in the established database. Our results revealed that mixed edible oils and recycled cooking oils could be screened out from the pure edible oils after the analysis, since heating and mixing of edible oils could change their MALDI-MS spectral patterns. This method is thus capable of rapidly authenticating edible oils and screening out fraudulent mislabeling of edible oils, with a speed of several minutes for one sample.

**Keywords:** edible oils, rapid authentication, direct analysis, MALDI-MS, spectral database

## 1 INTRODUCTION

Edible oils are daily used for cooking and food preparation. Different edible oils have different nutritional values and can have very different market prices. Counterfeit and adulteration of edible oils have been frequently reported [1]. Authentication of edible oils has been a long-term issue in food safety, and becomes particularly important with the widespread use of recycled cooking oils (gutter oils) in recent years. Nevertheless, there is no widely accepted scientific method for rapid identification of gutter oils. Detection of food residue markers such as capsaicinoids (marker of chili peppers) was reported for identification of gutter oils [2]. However, such markers may not necessarily present in all the oil samples, and can only be applied in some cases. Development of a reliable and general approach for rapid authentication of edible oils is thus highly desirable.

Techniques such as gas chromatography-flame ionization detection (GC-FID) are currently used for authentication of edible oils. These techniques typically

require sample extraction and chemical derivatization [3]. After that, chromatographic separation is needed to generate desirable results for fatty acid analysis. The whole process can take more than an hour. It is not efficient to analyze large amount of samples using these methods.

MALDI-MS, on the other hand, has desirable advantages over conventional techniques, e.g., short analysis time, high sensitivity, and the capability to directly analyze complex samples without chemical derivatization and chromatographic separation [4]. MALDI-MS could thus be a potential technique for analysis of edible oils, and has been attempted for such a purpose [4-7]. The reported MALDI-MS method for analysis of edible oils typically involves procedures including sample extraction with solvents, mixing with MALDI matrix, sample loading onto the MALDI plate, air drying and MALDI-MS analysis. MALDI-MS spectra acquired from edible oil samples were demonstrated to allow differentiation of the oil species [5-7].

We have developed a simplified protocol for MALDI-MS analysis of edible oils [7]. In this method, oil samples are directly loaded onto the MALDI plate pre-deposited with MALDI matrix, and then introduced into the mass spectrometer for automatic spectral acquisition. This method requires no sample extraction and solution mixing, and thus allows faster and simpler MALDI-MS analysis. Different types of edible oils can produce MALDI-MS spectra with different patterns. Adulterated and cooked edible oils could be rapidly screened out of the pure ones, since mixing and heating could change the MALDI-MS spectral patterns of edible oils. A preliminary mass spectral database of edible oils has been established, and the authenticity of an edible oil sample can be determined by comparison of the acquired spectra with the reference spectra. Using this new protocol, the time for analyzing one edible oil sample can be reduced to several minutes.

## 2 EXPERIMENTAL

### 2.1 MALDI-MS analysis

Aliquots of 0.5  $\mu\text{L}$  of 100 mg  $\text{mL}^{-1}$  2, 5-dihydroxybenzoic acid (DHB) in acetone were loaded onto spots of the MALDI plate and air-dried to form matrix layers. About 0.2  $\mu\text{L}$  of each oil sample was then transferred by pipette tip or cotton tip to form a thin oil

layer on the matrix layer. The plate was then introduced into the mass spectrometer for MALDI-MS analysis. Waters Micro-MX MALDI time-of-flight (TOF) mass spectrometer and Bruker ultrafleXtreme MALDI-TOF/TOF mass spectrometer were used for the analysis. Mass range of 500-2000 Da was acquired. The mass spectrometers were calibrated with the polyethylene glycol (PEG) mixture (PEG600/PEG1000/PEG2000/NaI = 1/2/2/5 (v/v)).

## 2.2 Principal component analysis and hierarchical clustering analysis

The background of each MALDI-MS spectrum was subtracted and the mass spectral peaks were smoothed and centered. For triacylglycerol (TAGs) signals with intensity higher than 2%, the normalized intensities (absolute intensity of TAGs observed / total absolute intensity of TAGs observed in the mass spectrum) of their monoisotopic peaks were input into the statistics software (Umetrics Simca 13.0) for principal component analysis (PCA) and hierarchical clustering analysis (HCA).

## 3 RESULTS AND DISCUSSION

### 3.1 The simplified protocol for MADLI-MS sample preparation

This protocol allows direct loading of oil samples onto MALDI plate spots pre-deposited with the DHB matrix. Our study demonstrated that the dried DHB layers were very stable and no significant differences of the resulting spectra were found even the DHB layers on the MALDI plate were stored for one week. Therefore, the matrix on the plate can be prepared before the analysis, and when new samples are received, the only procedure is to directly load the samples onto the MALDI plate. Compared to previous sample preparation methods that required sample extraction and mixing of sample and matrix [4-5], the present protocol is much simpler and easier for rapid analysis, and allows generation of spectra with high quality and high reproducibility.

### 3.2 MALDI-MS spectra of edible oils

A MALDI-MS spectrum of peanut oil is shown in Figure 1. Signals corresponding to fragments and sodium adducts of triacylglycerols (TAGs) were predominated in the range of 500-1000 Da of the mass spectrum. TAGs are the main components of edible oils which consist of three fatty acid units such as palmitic acid (P), oleic acid (O), linoleic acid (L) and stearic acid (S) (Figure 1b). The MALDI-MS spectra of different edible oils have different TAGs patterns (Figure 2), which can be used as the fingerprints for authentication of edible oils. In this study, authentication of edible oils was based on the TAGs region in the spectra.

Mixing and heating of edible oils can change the TAGs content and generate MALDI-MS spectra that are different from those of the pure edible oils. This allows rapid screening of mixed edible oils and gutter oils from the pure one. Heating of edible oils can produce oxidized products such as oxidized TAGs, TAGs complex and TAGs dimers [6]. As shown in Figure 3, the oxidized products could be observed in the spectra of the heated oil samples while no similar peaks were found in the control sample, indicating that the oxidized products could be useful for identifying recycled cooking oils.

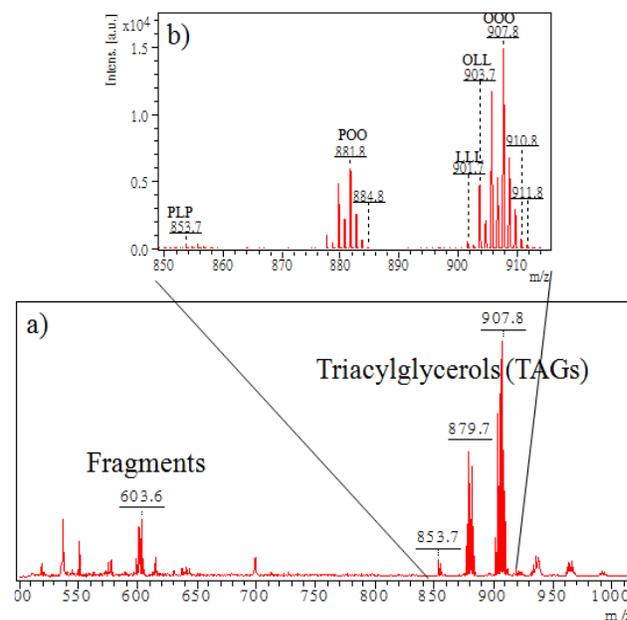


Figure 1. MALDI mass spectrum of peanut oil: (a) full spectrum ( $m/z$  range 500-1000 Da); (b) enlarged view of the TAGs region. TAGs were detected as  $\text{Na}^+$  adducts in the spectrum. P: palmitic acid; O: oleic acid; L: linoleic acid.

### 3.3 MALDI-MS spectral database and authentication of edible oils

Different oil species displayed different patterns of their MALDI spectra, allowing them to be distinguished from each other. A complete MALDI-MS mass spectral database containing a variety of edible oils can thus be established to provide reference spectra for comparison with the acquired spectra of the measured samples. The authenticity of edible oils can be determined by comparing the MALDI-MS spectra of the samples with those in the database.

A preliminary database has been established in our studies [7]. The TAGs signals of the reference edible oils in the database as well as the samples were input into statistics software for PCA and HCA processing for authentication of the samples. This method has been validated with control pure vegetable oil samples, including peanut oil, olive oil, canola oil and corn oil. The PCA plot indicated that the spot of each oil sample fell into the correct cluster, allowing clear identification of the sample. Ten recycled oil (gutter

oil) samples were also examined. The PCA results indicated that these gutter oils did not belong to any of the vegetable oils. The gutter oils could be screened out after the analysis.

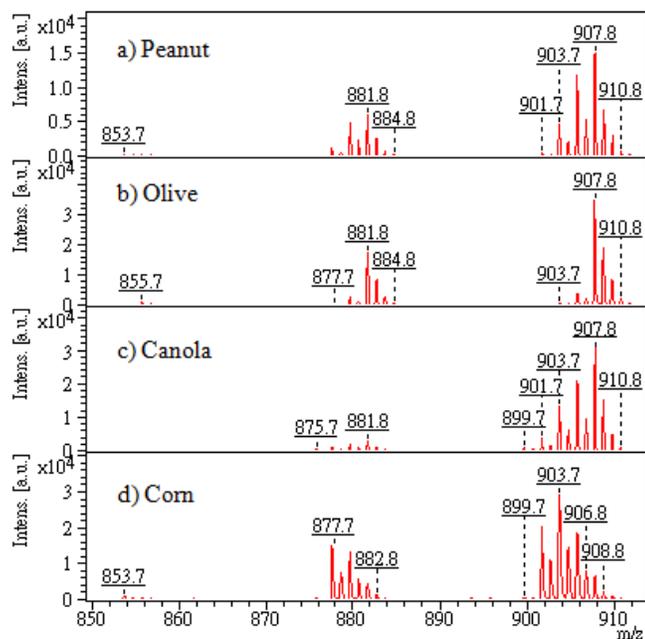


Figure 2. The TAGs regions of MALDI mass spectra of (a) peanut oil, (b) canola oil, (c) olive oil, (d) corn oil.

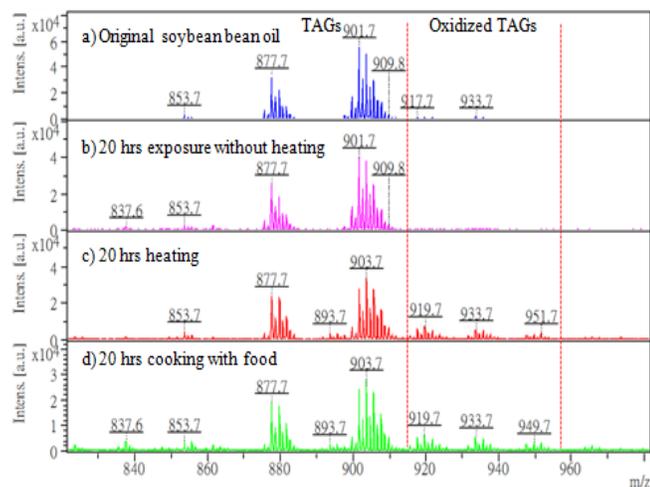


Figure 3. MALDI-MS spectra (TAGs region) obtained for (a) original soybean oil, (b) soybean oil with 20 hours exposure (control), (c) soybean oil with 20 hours heating and (d) soybean oil with 20 hours cooking with food.

A more complete MALDI-MS spectral database is being established. More than five hundred different edible oil products is being collected. PCA and HCA results of the new data suggested around thirty types of edible oils can be differentiated and authenticated based on their MALDI-MS spectral patterns.

## 4 CONCLUSION

MALDI-MS is a simple and rapid technique for direct analysis of edible oils. The analysis of one edible oil sample can be achieved within minutes using our method. The authenticity of edible oils can be determined by comparing the MALDI-MS spectra of samples with those in the database using statistical analysis, and mixed edible oils and gutter oils can thus be screened out from the pure edible oils. Those results suggested that MALDI-MS analysis with database matching is highly useful for rapid authentication of edible oils.

## ACKNOWLEDGMENT

Thanks are given to Innovation and Technology Fund, Hong Kong (Grant no. ITS/070/15) and The Hong Kong Polytechnic University (PolyU) for the financial supports and to Prof. Huiqin Wu in National Analytical Center, Guangzhou, China for providing the gutter oil samples used in this study. The Bruker ultrafleXtreme MALDI-TOF/TOF mass spectrometer is provided by The University Research Facility in Life Sciences, PolyU.

## DECLARE

Journal paper related to this study has been published by the authors (Ng et al., *Anal. Chim. Acta.*, 884, 70-76, 2015).

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