Journal of Chromatography B, 972 (2014) 65–72

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Profiling of triacylglycerols in plant oils by high-performance liquid chromatography–atmosphere pressure chemical ionization mass spectrometry using a novel mixed-mode column

Na Hu[a,b], Fang Wei[a,b,*], Xin Lv[a,b], Lin Wu[a,b], Xu-Yan Dong[a,b], Hong Chen[a,b]

[a] Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, PR China
[b] Hubei Key Laboratory of Lipid Chemistry and Nutrition, PR China

ARTICLE INFO

Article history:
Received 31 May 2014
Accepted 27 September 2014
Available online 5 October 2014

Keywords:
Liquid chromatography
Mass spectrometry
Plant oils
Triacylglycerols
Barcode
Principal component analysis

ABSTRACT

In this investigation, a rapid and high-throughput method for profiling of TAGs in plant oils by liquid chromatography using a single column coupled with atmospheric pressure chemical ionization (APCI) mass spectrometry was reported. A novel mixed-mode phenyl-hexyl chromatographic column was employed in this separation system. The phenyl-hexyl column could provide hydrophobic interactions as well as π–π interactions. Compared with two traditionally columns used in TAG separation – the C18 column and silver-ion column, this column exhibited much higher selectivity for the separation of TAGs with great efficiency and rapid speed. By comparison with a novel mix-mode column (Ag-HiSep OTS column), which can also provide both hydrophobic interactions as well as π–π interactions for the separation of TAGs, phenyl-hexyl column exhibited excellent stability. LC method using phenyl-hexyl column coupled with APCI-MS was successfully applied for the profiling of TAGs in soybean oils, peanut oils, corn oils, and sesame oils. 29 TAGs in peanut oils, 22 TAGs in soybean oils, 19 TAGs in corn oils, and 19 TAGs in sesame oils were determined and quantified. The LC–MS data was analyzed by barcodes and principal component analysis (PCA). The resulting barcodes constitute a simple tool to display differences between different plant oils. Results of PCA also enabled a clear identification of different plant oils. This method provided an efficient and convenient chromatographic technology for the fast characterization and quantification of complex TAGs in plant oils at high selectivity. It has great potential as a routine analytical method for analysis of edible oil quality and authenticity control.

1. Introduction

Plant oils are an important part of human diet due to their high nutritional value. They supply energy, essential fatty acids (linoleic and linolenic acids), and fat-soluble vitamins (A, D, E and K), etc. [1]. Analysis of components in plant oils is of great importance to determine the origin and type of the oil, and to assess the quality. Triacylglycerols (TAGs), which composed of three esterified fatty acids with an attached glycerol backbone, are the main component of plant oils (95–98%) [2]. The physicochemical and nutritional properties of oils are determined by TAG molecules, thus, TAGs in edible oils are considered as good fingerprints for authenticity control. At present, TAG composition has been regarded as one of the main parameters for the official identification of olive oil by the International Olive Oil Council [3].

The profiling analysis of TAGs in plant oils is a challenging task because of the presence of numerous TAGs species with similar physicochemical properties. They are characterized by fatty acids (FAs) properties, i.e., carbon number; the number, position and configuration of double bonds (DBs) in acyl chains; and the stereospecific position of FAs on the glycerol skeleton [4]. TAGs are usually analyzed by gas chromatography (GC) and high-performance liquid chromatography (HPLC). GC is a routine method for fatty acid profiling after the transesterification of TAGs to fatty acid methyl esters (FAMEs) [5]. However, GC is strictly limited for TAG analysis because of the complexity of derivatization required before separation. High Temperature (HT) GC is employed to directly analyze TAGs, however, some unsaturated TAGs are easily degraded at high temperature. HPLC is the most widely used

* Corresponding author at: Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, PR China. Tel.: +86 27 86711669; fax: +86 27 86822291.
E-mail address: willasa@163.com (F. Wei).

http://dx.doi.org/10.1016/j.jchromb.2014.09.039
1570-0232/

separation technique for TAGs analysis with good reproducibility and high resolution. There are two main HPLC methods for the separation of TAGs species: non-aqueous reversed-phase HPLC (NARP-HPLC) and silver-ion HPLC (Ag^+-HPLC). In NARP-HPLC mode, the retention of TAGs depends on equivalent carbon numbers (ECNs), defined as the total carbon number in the acyl chains minus two times the number of DBs, i.e. ECN = CN − 2DB [6–8]. The retention of TAGs increases with increasing ECN [9]. However, NARP system has a lower selectivity for the separation of TAGs with the same ECN and TAG regioisomers, and their partial separation is feasible only with the multiple column coupling and very long retention times in the range of 100–200 min, which is not practical for the routine use [10,11]. The complementary separation mode, silver-ion chromatography, is based on the formation of weak complexes of silver ions with π-electrons of DBs in the FA residues of TAGs [12]. In Ag^+-HPLC, TAGs are separated mainly according to the number and position of DBs [13–15]. The retention of TAGs increases with increasing number of DBs. However, silver-ion HPLC suffers from a lower selectivity of TAGs differing only in the length of alkyl chains and TAG regioisomers with more than three DBs, and the reproducibility of retention times is low [16].

Compared with one-dimensional liquid chromatography, the resolution and peak capacity of two-dimensional (2D) liquid chromatography have been improved tremendously. The combination of NARP-HPLC and Ag^+-HPLC in 2D chromatography using either on-line or off-line mode with APCI-MS detection is an effective way of realizing efficient TAG analysis [17,18]. Our laboratory has successfully applied an off-line 2D system coupling NARP and Ag^+-HPLC with APCI-MS detection to the identification and quantification of TAGs in peanut oil [19]. However, off-line approaches are generally time-consuming and laborious, which requires collecting fractions from the first dimension and re-injecting them into the other column for separation in the second dimension [20]. Furthermore, it requires a large amount of organic solvent, which may subsequently lead to additional environmental pollution in the analysis. Some advantages of on-line 2D methods compared with off-line ones are the fast speed and automation; on the other hand, some drawbacks are the complexity of the system and the incompatibility of the 2D solvents [21].

In recent years, mixed-mode chromatography has received great attentions due to its great advantages over single chromatographic mode, such as great flexibility for retention and selectivity. As to TAG analysis, combinations of two or more different separation modes (mainly hydrophobic interaction and π-complexation interaction) may significantly increase the number of resolved TAGs in natural oil samples. Recently, our laboratory prepared a column with silver-ion-modified octyl and sulfonic co-bonded silica stationary phase, naming as Ag-HiSep OTS column [22,23]. This column not only could provide hydrophobic interactions by octyl groups, but also could provide π-complexation interactions with silver ions. It exhibited much higher selectivity for the separation of TAGs. However, the disadvantage of this column is that the stability of the column was affected by the water concentration in the mobile phase, for the modified silver ion might be eluted with the mobile phase from the column at water concentration above 8.0% (v/v). When the Ag^+ adsorbed on the column is exhausted, it should be regenerated with the preparation method [23].

Phenyl-hexyl column with phenyl-hexyl-bonded silica stationary phase is a commercial available column, which are commonly used for separation of aromatic analytes due to its unique selectivity for analytes containing phenyl groups [24]. The stationary phase in phenyl-hexyl column can be considered as a mixed-mode stationary phase, in which phenyl moiety could provide π–π interactions with the π electrons of DBs and the hexyl ligands could provide additional hydrophobic interactions. As to TAG analysis, combinations of two different separation modes (mainly hydrophobic interaction and π-complexation interaction) may significantly increase the number of resolved TAGs in natural oil samples. Thus, the phenyl-hexyl column would be suitable for the separation of TAGs. Furthermore, compared with Ag-HiSep OTS column, commercial available phenyl-hexyl column not only is obtained more easily, but also exhibited much better stability.

Prices of plant oils are affected by the production cost and the quality of plant oils. Higher prices of high-quality plant oils can lead to the effort of falsification by cheaper oils with a lower quality and less beneficial nutritional properties. Therefore, the authenticity of plant oils is of great importance from commercial and health aspects. The growing interest in the authenticity of oils requires reliable verification methods. Simple comparison of TAG concentrations was not sufficient for the authentication of plant oils because of the complexity of the data matrix. The statistical evaluation is a powerful tool for processing of large data sets, which enables the discrimination of different oil samples. Chemometric methods such as principal component analysis (PCA), partial least-squares analysis, linear discriminant analysis, etc., present the most successful and promising results [25,26]. Besides, barcodes contained information that often remained hidden when looking at a single chromatogram at a time and could also be extremely useful for quality control. Chambery's group used peptide fingerprint evidenced the differences between wines. The spectral traces were converted into barcodes to obtain a graphical representation of spectra [27]. The results constituted a simple tool to display differences between wine samples. LI' group also used fingerprints and barcodes of proteins to determine the geographical origin of honey [28]. Multivariate analysis for processing chromatographic data has been shown to be an efficient tool for classification and prediction in assessing authenticity of plant oils improving and searching similarities of oils samples [29].

In this investigation, a rapid and high-throughput method for profiling of TAGs in plant oils by liquid chromatography using a single column coupled with atmospheric pressure chemical ionization (APCI) mass spectrometry was reported. A novel mixed-mode phenyl-hexyl chromatographic column was employed in this separation system. This was the first attempt to apply phenyl-hexyl column for TAG profiling analysis. In comparison with the traditional C18 column and silver-ion column, which are the two main columns used for the separation of complex TAGs in natural oil samples, this novel phenyl-hexyl column, could provide hydrophobic interactions as well as complexation interactions. It exhibited much higher selectivity for the separation of TAGs, and the separation was rapid. Furthermore, compared with Ag-HiSep OTS column, commercial available phenyl-hexyl column not only is obtained more easily, but also exhibited much better stability. The retention characteristics of TAGs on this column were investigated, and separation conditions were optimized. LC method using phenyl-hexyl column coupled with APCI-MS was successfully applied for the profiling of TAGs in soybean oils, peanut oils, corn oils, and sesame oils. In addition, the TAG profiling data was converted into barcodes to obtain a graphical representation, and also analyzed by principal component analysis (PCA). This method provided an efficient and convenient chromatographic technology for the fast characterization and quantification of complex TAGs in plant oils at high selectivity. It has great potential as a routine analytical method for analysis of edible oil quality and authenticity control.

2. Experimental

2.1. Materials and chemicals

HPLC-grade methanol, acetonitrile, hexane and ammonium hydroxide solution (~10%) (NH_4OH) were purchased from CNW