Food Chemistry 138 (2013) 1095–1100

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

An electrospray ionisation-mass spectrometry screening of triacylglycerols in developing cultivated and wild peanut kernels (*Arachis hypogaea* L.)

Aicha O. Cherif [a,*], Nathalie Leveque [b], Mhamed Ben Messaouda [c], Habib Kallel [a], Fathi Moussa [b]

[a] Laboratoire de biochimie des lipides et interactions avec les macromolécules, Faculté des Sciences de Tunis, Université Tunis-El-Manar, 2092 Tunis, Tunisia
[b] Groupe de Chimie Analytique de Paris Sud-LETIAM, Université Paris Sud, plateau de Moulon, 91400 Orsay, France
[c] Université de Carthage, Laboratoire des Matériaux, Molécules et Applications, IPEST, B.P. 51, 2075 La Marsa, Tunisia

ARTICLE INFO

Article history:
Received 30 April 2012
Received in revised form 15 November 2012
Accepted 21 November 2012
Available online 5 December 2012

Keywords:
Wild peanut
Cultivated peanut
HPLC–ESI-MS
Distribution
Triacylglycerols

ABSTRACT

The accumulation of triacylglycerols during the development of three varieties of peanuts was monitored in two Tunisian cultivated peanut (Trabelsia (AraT) and Chounfakhi (AraC)) and one wild Tunisian peanut (Arbi (AraA)). The presence of TAGs composed of rare fatty acid residues such as hexacosanoic acid ($C_{23:0}$) and heneicosanoic acid ($C_{21:0}$) among the triacylglycerols $C_{23:0}$ LL, $C_{23:0}$ OO and $C_{21:0}$ LL was noted. The major molecular species of triacylglycerol detected in the three peanut varieties were dioleoyl linoleoyl (OOL), 1,2,3-trioleyl (OOO), 1,2-dioleyl-3-palmitoyl (POO), 1,2-dilinoleoyl-3-oleyl (OLL) and 1-oleoyl-2-linoleoyl-3-linolenoyl (OLLn). The TAG composition and content were significantly different among the three peanut varieties. The three major TAGs were OOL (20.6%), OOO (15.6%) and OLLn (13.2%) in AraA; OOL (21.4%), OOO (20.1%) and POO (17.5%) in AraC and finally OLL (20.7%), OOO (19.8%) and OLL (17.7%) in AraT.

1. Introduction

The genus *Arachis*, family Fabaceae is native to South America, probably from a region including Central Brazil (Gran Pantanal) and eastern slopes of the Andes of Bolivia (Grosso, Zygadlo, Lamarque, Maestri, & Guzman, 1997). Numerous cultivated peanut and wild species have been collected in South America. Nowadays, there are 70 wild species and 17,000 cultivated species of *Arachis* in the world. Several papers suggested that the cultivated peanut, *Arachis hypogaea* L., is susceptible to a number of diseases, which increase costs and reduce yields for the farmer. For many of these diseases, good sources of resistance are lacking in cultivated germplasm, which has a very narrow genetic base (Kochert et al., 1991). Perhaps because of its limited genetic diversity, this species lacks resistance to a number of important pests, diseases and environmental constraints. In contrast, wild species of *Arachis* are genetically diverse and are rich sources of disease resistance genes (Grosso, Nepote, & Guzman, 2000).

Peanut seeds are foods with high nutritional (proteic, lipidic and carbohydrate content) content and commercial value (Rodrigues et al., 2011). In addition, the energy value of these foods is high (546 kcal/100 g) with considerably high lipid (44–56%) and protein content (22–30%) (Rodrigues et al., 2011). However, Han and Gross (2005) reported approximately 40–55% lipid content in peanuts, varying with the genotype and the seasonal conditions under which they were grown. Most plant seeds consist predominantly of triacylglycerols that generally follow a unique and typical pattern in the glycerol molecule which is characteristic to different oilseeds (Cunha & Oliveira, 2006).

Traditionally, the characterisation of fats and oils has been based on fatty acids (FA) composition. However, different TAGs blends in the right proportion could lead to similar FA profiles. Consequently, the determination of the fatty acid composition is sufficient to properly characterise a fat or an oil composition. For this reason, recent studies tend to use directly the TAG as compositional markers in order to characterise fats and oils (Bosque-Sendra, Cuadros-Rodríguez, Ruiz-Samblás, & De la Mata, 2012). The advantage of using the triacylglycerol (TAG) profile (backbone) compared to fatty acid (FA) profiles shows that the stereospecific distribution of FA on the glycerol molecule is genetically controlled and thus, the information of intact TAGs

Abbreviations: AraA, *Arachis* Arbi (wild); AraC, *Arachis* Chounfakhi (cultivar); AraT, *Arachis* Trabelsia (cultivar); DAP, days after podding; ESI-MS, electrospray-mass spectrometry; IS, immature stage; M, maturity; MIS, middle stage; MS^2, CID-MS/MS; TAG, triacylglycerols; OOP, 1,2-dioleyl-3-palmitoyl; OOO, 1,2,3-trioleyl; POO, 1,2-dioleyl-3-palmitoyl; PPO, 1,2-dipalmitoyl-3-oleyl; POP, 1,3-dipalmitoyl-2-oleyl; LLO, 1,2-dilinoleoyl-3-oleyl; OOL, 1,2-dioleyl-linoleoyl; OOO, 1,2,3-trioleyl; OLLn, 1-oleoyl-2-linoleoyl-3-linolenoyl; PLLn, 1-palmitoyl-2-linoleoyl-3-linolenoyl; PPL, Dipalmitoyl-2-linoleoyl; LgLL, 1,2-dilinoeoyl-3-lignoceroyl; LgLO, lignoceroyl-linoleoyl-oleoyl; GOO, Gadoyl-2-2-dioleoyl glycerol.

* Corresponding author. Tel.: +216 24222499; fax: +216 71288647.
E-mail address: aicha.echerif@gmail.com (A.O. Cherif).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.11.107

is usually higher (Aparicio & Aparicio-Ruiz, 2000). Therefore, TAG composition has been established as a measurement of the quality and purity of vegetable oils and is used increasingly in the food industry as a good fingerprint for adulteration detection purposes (Aparicio & Aparicio-Ruiz, 2000; Bosque-Sendra et al., 2012; Gómez-Ariza, Arias-Borrego, García-Barrera, & Beltran, 2006).

In this context, two analytical techniques have been widely employed in the analysis of TAG mixtures, silver ion normal-phase HPLC (Ag-HPLC) and non-aqueous reversed-phase HPLC (NARP-HPLC) (Lísa & Holcãpek, 2008). In this latter, TAGs are separated according to acyl chain lengths and the number of double bonds (DBs). The retention of TAGs is based on increasing partition numbers (PN). Ag-HPLC is also widely used in TAGs separations due to the formation of weak reversible complexes with silver ion (Holčapek, Lísa, Jandera, & Kàbàtova, 2005). In this case, the TAGs elution is related to an increasing total number of PN and the number of double bonds (Holcãpek, Jandera, Zderadicka, & Hruba, 2003) and the position of DBs (Lísa, Holcãpek, Rezanka, & Kàbàtova, 2007).

Different detectors have been used to determine the TAG's composition of vegetable oils. Several achievements have recently increased the interest in evaporative light-scattering detection (ELSD) (Cunha & Oliveira, 2006). It seems to be an interesting detection technique for lipids as it is sensitive. However, TAG's quantification by ELSD can be a problem as the response factor of this detector is not linear at very low or high levels of TAGs (Cunha & Oliveira, 2006).

Another tool, mass spectrometry, has facilitated a dramatic enhancement in the extremely sensitive quantification of individual molecular species of complex lipids (Hunt & Postle, 2006). Moreover, soft ionisation such as electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) not only allowed qualitative work but also static compositional measurements of TAG in lipid extract (Acheampong, Leveque, Tchapla, & Heron, 2011). The features of atmospheric pressure chemical ionisation (APCI) have also been described in the literature (Holčapek et al., 2005). Since TAGs are relatively apolar, APCI provides a better ionisation due to full compatibility with common NARP conditions, easy ionisation of non-polar TAGs as well as the presence of both protonated molecules $[M+H]^+$. APCI-MS also provides a linear calibration (Cunha & Oliveira, 2006) and information on the predominant fatty acid in the *sn*-2 position (Holčapek et al., 2005). The precise ratio of regioisomers can also be obtained by the measurement of calibration curves with both positional isomers. Electrospray ionisation mass spectrometry (ESI-MS) offers the possibility of directly analysing the total lipid extract and the characterisation of minor compounds with great success (Han & Gross, 2005). It is also compatible with the rapid and sensitive quantification of lipid species in complex matrices (Bowden, Albert, Barnaby, & Ford, 2011). Accordingly, recent studies has shown that the Ag-HPLC–ESI-MS method is sufficient to identify the TAGs present in seed oils without looking for the best optimised chromatographic conditions (Acheampong et al., 2011) and silver cationisation improved the sensitivity by a factor of 100 for TAG quantification.

Unfortunately, information related to the different changes of triacylglycerols composition and content during maturity is limited. In addition, lipid compositions of cultivated seeds have been widely studied but no reports on the triacylglycerols of wild peanuts have yet been reported. The objective of this work is to analyse oils, obtained from cultivated and wild peanuts, by Ag-HPLC–ESI-MS in an attempt to determine the distribution of various molecular species of triacylglycerols and to characterise triacylglycerol components of wild species of Arachis from Tunisia.

2. Materials and methods

2.1. Reagents and standard

Methanol and *n*-hexane, solvents for HPLC grade, were purchased from Pancreas Quimica SA (Barcelona, Spain), chloroform and petroleum ether is from Fisher Scientific SA (Loughborogh, Spain). Ethanol was purchased from Scientific Limited (Northampton, UK).

2.2. Samples

Three varieties of local peanuts were collected from plants in a private farm at Dar-Allouch in the northeastern Tunisia, in the same eco-environmental conditions: one wild variety (*Arbi* (AraA)), and 2 cultivar varieties: *Trabelsia* (AraT (Valencia type)) and *Chounfakhi* (AraC (Virginia type)). Kernels were hand-picked starting mid-May until the end of October 2008. the samples had been were collected at different intervals after the date of planting Samples had been collected at different intervals after the date of podding (DAP) until maturity. After the extractions of samples, the oils kept under nitrogen at −20 °C and independent samples extractions were analysed in triplicate.

2.3. Oil extraction

The total lipids were extracted by the method Bligh and Dyer (1959). Seeds (2.5 g) were washed with boiling water for 5 min to denature the phospholipases (Douce, 1964) and then crushed in a mortar with a mixture of chloroform–methanol (2:1, v/v). The fixing water was added and the mixture was centrifuged at 3000*g* for 15 min. The lower chloroform phase containing the total lipids was kept and dried under a stream of nitrogen.

2.4. LC–MS instrument

2.4.1. HPLC system

A quaternary HPLC system consisting of a Spectra System P 1000 XR pump (Thermo Scientific, Boston, MA, USA) was used for pumping the acetonitrile/acetone mobile phase, whereas Hewlett Packard 1050 series (Agilent Technology, Santa Clara, CA, USA) was used to pump the $AgNO_3$ on line after the column via a T connector (Interchim, Montlucon, France). A 250 × 2.0 mm × 5 μm Kromasil C18 (Eka Nobel, Bohus, Sweden) column was used throughout the experiment. The mobile phase used in isocratic mode were acetonitrile/ acetone (47:53, v/v) with a flow rate of 0.25 ml/min. $AgNO_3$ at a concentration of 100 μM was introduced into the LC effluent after the column at a flow rate of 0.05 ml/min to increase the sensitivity (Leveque, Heron, & Tchapla, 2010). Injections of 5 μl of analytical samples were made by autosampler (Thermo Separation Products, San Jose, CA, USA). The silver containing solutions were freshly prepared and protected from light during the experiments. The dead time t_0 of the column was determined by injecting pure acetonitrile.

Electrospray ionisation mass spectrometry and tandem mass spectrometry instead of MS system: the column was connected to a Thermo-Finnigan LCQ quadruple ion trap mass spectrometer (Thermo Fisher, Boston, MA, USA) equipped with an ESI ion source. The entire volume of the column effluent was directed to the mass spectrometer. Data acquisition and processing were performed using the Xcalibur data system (Woburn, MA, USA). The source voltage was 4.5 kV and the capillary temperature was 250 °C. The capillary voltage was 25 V and the lens offset 20 V. Nitrogen was used both as sheath gas and as an auxiliary gas at a flow rate of 75 and 50 (arbitrary units), respectively. The ESI spectra were