Journal of Food Composition and Analysis 33 (2014) 49–54

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

Chemical composition and characteristic profiles of seed oils from three Tunisian *Acacia* species

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ARTICLE INFO

Article history:
Received 30 March 2012
Received in revised form 11 October 2013
Accepted 2 November 2013

Keywords:
Acacia species
Seed oils
Food composition
Food analysis
Oil composition
Fatty acids
Phenols
NMR
Underutilized species
Biodiversity and nutrition

ABSTRACT

In this paper, physicochemical properties, fatty acid and phenolic compositions of *Acacia cyclops*, *Acacia cyanophylla* and *Acacia mollissima* oils were studied. These oils were compared in terms of physicochemical properties and fatty acid composition to soybean oil. The oil content of *Acacia* seeds is 8.85%,11.13% and 7.16%, respectively. A small difference was observed in the acid and saponification value. However, no differences were observed for refractive index, iodine value and fatty acid composition. This latter was essentially dominated by linoleic acid (56.66–60.52%), oleic acid (19.45–22.74%) and palmitic acid (9.36–12.25%). This study compares also the phenolic composition in *Acacia* seed oils. Syringic and ferulic acids were the dominant phenolic compounds observed in the studied oils. Minor amounts of phenolic acids as *o*-coumaric, *p*-coumaric and protocatechuic are also detected. The results of this preliminary study showed that *Acacia* samples are promising oilseed crops and the high level of unsaturated fatty acids makes them desirable in terms of nutrition and could be used as a potential oil in the human diet.

1. Introduction

In recent years there has been an important development of underexploited promising plant species as a source of dietary or specialty oils. Many of them contain significant quantities of oils and/or a high proportion of nutritionally, medicinally or industrial desirable fatty acid (Bowen and Clandinin, 2005). However, with a continuous increase in population, the demand for a high-quality of seed oils continues to grow up. To meet the demand, there is a need to increase the production of the major oil crops and to diversify the sources through improving and increasing the production of minor oil crops (Mulatu et al., 2011).

Vegetable oils with a high relative amount of minor lipid components are of great importance for human health (Nasri et al., 2012) and their composition is important from the nutrimental point of view. Thus, n-3 fatty acids play a very essential role in physiology, especially during fetal and infant growth (Bowen and Clandinin, 2005) and they are also important for the prevention of cardiovascular diseases as they are antithrombotic, anti-inflammatory, antiarrythmic and promote plaque stabilization (Galli and Marangoni, 2006).

Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom. They play an important role in plant metabolism, but also protect the plant against stress. Several studies have shown that plant resistance to both biotic (pathogens and predators) and abiotic (UV-radiation, drought, etc.) stresses is related to phenolic compounds. All classes of phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acid derivatives, flavonoids, polyflavans, etc.) are involved in the resistance mechanisms (Hannachi et al., 2011). Furthermore, they constitute a part of the unsaponifiable matter, also known as minor constituents of oils. These compounds have antioxidant capacities and confer a particular pungent taste to oil. It is worth noting also that phenolics play a beneficial role by preventing coronary heart diseases and cancer (colon, breast and skin) (Besbes et al., 2004).

Vegetable oils are utilized for many food and industrial purposes. Despite the wide range of vegetable oils sources, the world consumption is dominated by palm, soybean, rapeseed and sunflower oils with 38.1,35.7,17.8 and 18.2 millions tonnes consumed per year, respectively (American Soybean Association, 2007). Furthermore, worldwide interest is oriented for the recovery and exploitation of oils from natural plant resources.

Plant seeds are currently constituting new oil sources, especially from underexploited seeds such as *Acacia* genus. Little is known about the chemistry of most Acacia species, although the genus is quite large and widespread in the warm sub-arid and arid

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http://dx.doi.org/10.1016/j.jfca.2013.11.001

regions of the world. The *Acacia* genus comprises approximately 1350 species (Nasri et al., 2012). Taxonomic identification and relationships of *Acacia* species are difficult; new studies of the genus confirm that *Acacia* is an agglomeration of at least five discrete groups (Seigler, 2003). *Acacia cyclops* A. Gunn. ex G. Don is a shrubby evergreen tree (1.5–6 m high) (Kotze et al., 2010) originating in South-Western Australia, became invasive after it was introduced into South Africa in the early 1800s for dune stabilization (Impson et al., 2004). *Acacia mollissima* is a woody legume of the family Mimosaceae. It is endemic to Australia and has been introduced into many countries. The bark of this species is considered a good source of tannins. Its short fiber wood is used in pulp and fuel production (Nkuekam et al., 2008). *Acacia cyanophylla* Lindl. is a legume shrub species originally from Western Australia. It was introduced into Tunisia for range land rehabilitation, particularly in the semi arid zones. This shrub represents a potential forage resource particularly during periods of drought (Seigler, 2003). To the best of our knowledge, until now a physicochemical characterization of the oil produced from the seeds of *Acacia cyclops* grown in Tunisia has not been reported. Our investigation was undertaken to determine the physicochemical properties, fatty acid and phenolic composition of *A. cyclops*, *A. cyanophylla* and *A. mollissima* seed oils and to compare these results with those of the common soybean oil, which is the most imported and consumed oil in Tunisia.

2. Material and methods

2.1. Seed material

Mature pods of three species of *Acacia*, namely *A. cyclops*, *A. cyanophylla* and *A. mollissima*, were collected in December 2010 from three different regions in Tunisia: Beja (a city of the northwest of Tunisia with a semi-arid climate), Hammamet and Kelibia (coastal cities of the northeast of Tunisia with a Mediterranean climate), respectively. The seeds were directly isolated and then hand-picked to eliminate damaged ones. The selected seeds were sun-dried for three days, carefully cleaned and ground to powder.

2.2. Lipid extraction

Oil was extracted from *Acacia* seeds using hexane (Sigma aldrich chimie, Saint Quentin Fallavier, France), the ground dried *Acacia* seeds were placed into a cellulose paper cone and extracted with hexane using a soxhlet extraction apparatus. The solvent was evaporated under reduced pressure. The oil extracted from the seed powder was weighed and the result was expressed as the lipid percentage in the seed powder dry matter.

2.3. Physicochemical properties of seed oils

Refractive index was determined using a Sopelem series 3296 refractometer (Soplem, France).

Acid value was estimated according to the standard (ISO 660, 1996).

Iodine value was identified according to the standard (ISO 3961, 1996).

Saponification value was defined according to the standard (ISO 3657, 1996).

2.4. Fatty acid composition

2.4.1. GC analysis

The oils were converted to methyl esters using a boron trifluoride methanol complex (14% w/v) (Sigma aldrich chimie, Saint Quentin Fallavier, France). The mixture was maintained at 100 °C for one hour. The reaction was stopped with 0.5 mL of distilled water. Then, the extracted fatty acid methyl esters (FAME) were dissolved in pure hexane (Merck chimie SAS, Fontenay-sous Bois, France) for GC analyses. Individual FAMEs were separated and quantified by gas chromatography using a model 5890 Series II instrument (Hewlett-Packard, Palo-Alto, Ca, USA) equipped with a flame ionization detector, and a fused silica capillary column DB23 capillary column (60 m length, 0.32 mm i.d., and 0.25 μm film thickness; HP-Agilent Technologies, Wilmington). The oven temperature was set at 130 °C, increased to 170 °C at 6.5 °C/min, then augmented again to 215 °C at 2.8 °C/min and was held there for 12 min. Finally, it was increased to 230 °C at 40 °C/min and maintained for 20 min. Injector and detector temperatures were set at 270 °C and 280 °C, respectively. Nitrogen was used as the carrier gas at 1 ml/min and the split ratio was set at 1:5. The FAMEs were identified by comparing their retention times with respect to pure standard FAMEs purchased from Sigma and analyzed under the same conditions (Dhibi et al., 2010). *Acacia* seed FAMEs were quantified according to their percentage area, obtained by integration of the peaks. The results were expressed as a percentage of individual fatty acids in the lipid fraction.

2.4.2. ^{1}H-NMR method

In order to reinforce the chemical composition study of the three isolated *Acacia* oils, an ^{1}H-NMR spectroscopy was used.

^{1}H-NMR spectra were obtained using a Bruker AM 300 NMR spectrometer (Blue Lion Biotech, Washington-USA), at 300 *MHz* in $CDCl_3$. In a typical experiment, 10–20 mg of seed oil was dissolved in 1 mL $CDCl_3$ in an NMR tube and readings were taken between 0–14 ppm. The residual chloroform resonance was used as the internal reference. Coupling constants are given in Hertz. The chemical shifts are expressed in δ ppm (Andrade et al., 2011).

2.5. Phenolic composition

Because of the low volatility and high molecular weight of phenolic compounds, derivatization prior to gas chromatography analysis is required. The silylating reagents (Sigma Aldrich Chimie, Saint Quentin Fallavier, France) were prepared by adding 3 mL of hexamethyldisilazane and 1 mL of trimethyl chlorosilane to 9 mL of anhydrous pyridine. An aliquot of the ethanolic extracts (100 μL) and 100 μL of 0.5 mg/mL solution of 18β-glycyrrhetinic acid as internal standard were placed in 1 mL gastight vials and evaporated to dryness under a N_2 stream. The residue was dissolved in 200 μL of the silylating reagent and heated at 80 °C for two hours (Guinda *et al.*, 2010). After derivatization, an aliquot of the silylated solution was submitted to GC-FID.

Gas chromatograph: HP 5890 series II equipped with flame ionization detectors (FID), HP-5 (60 m length, 0.25 mm i.d and 0.25 μm film thickness) fused silica capillary column, carrier gas nitrogen (1.2 mL/min). The temperature oven was programmed as follow: from 90 °C (1 min) to 220 °C, then increased to 290 °C at 10 °C/min then augmented again to 300 °C at 40 °C/min. Injector and detector temperatures: 250 °C and 280 °C, respectively. Volume injected: 0.1 μL of 1% hexane solution. The identification of the phenolic compounds was performed by comparison of their retention times with those of pure authentic samples.

3. Results and discussion

3.1. Physicochemical properties of seed oils

Table 1 reports the comparison of physicochemical properties of *Acacia* seed oils with those of soybean oil. Seeds of *A. cyclops* furnished 8.85% of oil. This seed oil was comparable with that of *A.*