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Glycerolipid and fatty acid distribution in pericarp, seed and whole fruit oils of *Myrtus communis* var. *italica*

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ABSTRACT

Seed, pericarp and whole berry of *Myrtus communis* var. *italica* were compared in terms of oils, glycerolipid classes and fatty acids. The fruit is composed of pericarp and approximately 9 seeds which constituted 63.5 and 36.5% of the whole ripe fruit, respectively. The latter presented a weight of 8.8 g% fruits while seed had only 0.5 g% seeds. The moisture contents were 80.1% in pericarp, 72% in whole fruit and 39.7% in seed. The oil yield of seed (11.7%) was significantly higher than that of whole fruit (5.9%) and pericarp (2.1%). Total lipid amounts were 61.26 mg/g in seed, 28.97 mg/g in whole fruit and 4.14 mg/g in pericarp. The amounts of polar glycerolipids were lower than those of neutral glycerolipids in all samples. Triacylglycerol constituted the main neutral glycerolipid with 57.47 mg/g in seed, 25.68 mg/g in whole fruit and 1.67 mg/g in pericarp. The predominant fatty acids of total lipids and different glycerolipid classes were linoleic, palmitic, oleic and α-linolenic acids in all samples but with different proportions. Whole fruit, seed and pericarp provided low yields of oil but they were a rich source of essential fatty acids which will be important as an indication of the potentially nutraceutical and industrial utility of myrtle fruit.

1. Introduction

Myrtus communis, belonging to the Myrtaceae family, is naturally widely distributed in the Mediterranean regions (Pottier-Alapetite, 1979). It is a pleasant annual shrub with dark blue ripe fruits which have a long history of application in the perfumery, cosmetic, food and pharmaceutical industries (Nuvole and Spanu, 1996). In Sardinia (Italy), fruits are mostly employed for the industrial formulation of sweet liqueur (Mulas et al., 2000). Last decades, intensive myrtle cultivation systems have been established in various regions of the world and particularly in Sardinia in order to assure both a constant supply of good fruit material for the liquor industry and the preservation of natural myrtle populations (Mulas et al., 2002).

The myrtle fruit is mostly composed of volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids (Martiĭn Lopez et al., 1999). Until now, the majority of studies on myrtle fruit have focused on the composition of its volatile fraction (Mazza, 1983; Gauthier et al., 1988; Jerkovic et al., 2002; Flamini et al., 2004; Tuberoso et al., 2006; Aidi Wannes et al., 2007) and of phenolic compounds (Martin et al., 1990; Rosa et al., 2003; Montoro et al., 2006a, b; Tuberoso et al., 2007). However, little has been undertaken on the fatty acid composition of myrtle fruit (Asif et al., 1979; Cakir, 2004; Aidi Wannes et al., 2009).

Myrtle fruit has an appreciable oil yield about 15.40% (Asif et al., 1979) and it is rich sources of polyunsaturated linoleic acid (Asif et al., 1979; Cakir, 2004; Aidi Wannes et al., 2009). Plants which are rich in linoleic acid have been extensively cultivated and their oils widely consumed (Guil Guerrero and Rodríguez-García, 1999). However, these plants are now grown, not only for food and fodder, but also for a striking variety of products with applications in several industrial fields (Ramadan et al., 2006). In fact, oils rich in linoleic acid are used as a raw material in the manufacture of conjugated linoleic acid (Ma et al., 1999), a novel therapeutic nutrient with promising antioxidant and anti-tumor properties (Belury, 2002). This fatty acid has also important applications as a component of skin care products (Darmstadt et al., 2002). Some polyunsaturated fatty acids, such as linoleic, linolenic and arachidonic acids, known as vitamin F, are necessary for growth and protection of the skin (Cakir, 2004). A lack of these fatty acids leads to cutaneous problems such as alopecia, peeling of epidermis and eczema (Poelman, 1987). This is why vitamin F is incorporated at a concentration of about 5% into dermatological creams, especially in sun lotions and regenerating and anti-wrinkle products. As lipids of *M. communis* fruit are rich in polyunsaturated acids and as industrial interest in the lipid composition of plant oils is recently on the increase, Cakir (2004) suggested that myrtle fruit might be used in cosmetic products. Seeing that there is no investigation of the glycerolipid composition of myrtle fruit oils, the objective of this study was to evaluate the different polar and neutral glycerolipid classes as well as their fatty acid distribution in *M. communis* var. *italica* whole fruit, seed and pericarp oils. The results will be important as

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an indication of the potential utility of *M. communis* var. *italica* fruit as a raw material source for useful industrial oil components.

2. Material and methods

2.1. Plant material

Mature *M. communis* var. *italica* fruits were collected from 15 to 20 plants growing wild in Jbal Stara of Haouaria region (North East of Tunisia-Nabeul; latitude 37°03′00.13″N; longitude 11°02′43.48″E, altitude 141.43 m) in January 2007. Botanical identification of this species was carried out by Prof. A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia) according to the Tunisian flora (Pottier-Alapetite, 1979). A voucher specimen has been kept in our unit for future reference. Seeds and pericarp were isolated manually from the myrtle fruit.

2.2. Reagents and standards

All solvents used in the experiments (diethyl ether, chloroform, hexane, toluene, ethanol, acetone and methanol) were purchased from Merck (Darmstadt, Germany). Sodium methylate, sodium chloride, sulphuric acid, acetic acid and anhydrous sodium sulphate (Na_2SO_4) were purchased from Sigma–Aldrich (Steinheim, Germany). Fatty acid and Standards used for phospholipid (PL) identification, namely phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidic acid (PA), were purchased from Sigma–Aldrich (Steinheim, Germany). Standards used for neutral glycerolipid (NL) identification, including monoacylglycerol (MAG), diacylglycerol (DAG) and triacylglycerol, were procured from Fluka (Ridel-de Haën, Switzerland). Standards used for glycolipid (GL) identification, namely monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were purchased (Sigma–Aldrich, Steinheim, Germany).

2.3. Moisture content

Before analyzing oils, glycerolipids and fatty acids, an evaluation of the moisture contents of whole ripe fruit, seed and pericarp was carry out. Samples were dried in an oven at 60 °C until they reached a constant weight.

2.4. Continuous extraction of oil

Myrtle whole fruit, seed and pericarp were ground in an electric grinder. Twenty grams of each ground sample were placed in an extraction cartridge made of cellulose and placed in Soxhlet extractor. The extraction solvent used was petroleum ether. After 8 h of extraction, samples were evaporated under vacuum, weighed and their oil yield determined.

2.5. Total lipid extraction

Triplicate sub-samples of 1 g were extracted using the modified method of Bligh and Dyer (1959). Thus, fruit samples were fixed in boiling water for 5 min and then ground manually in a China mortar using a mixture of chloroform/methanol/hexane (3:2:1, v/v/v).

After washing with water of fixation and decantation during 24 h at +4 °C, the organic phase containing total glycerolipids was recovered and dried under a nitrogen stream. Finally, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) and stored at −20 °C for further analyses.

2.6. Separation of glycerolipid classes by TLC

Glycerolipid classes were separated by thin layer chromatography (TLC) using 20 cm × 20 cm × 0.25 mm silica gel plates (G60, Merck, Darmstadt, Germany). Neutral glycerolipid separation by the method described by Mangold (1964) using a development system composed of petroleum ether–diethyl ether–acetic acid (70:30:0.4; v/v/v). Polar glycerolipids were separated using as mobile phase mixture chloroform–acetone–methanol–acetic acid–water (50:20:10:10:5; v/v/v/v) as described by Lepage (1967). Glycerolipid spots were detected after a brief exposure to iodine and each spot was identified by co-chromatography of pure lipid standards.

2.7. Fatty acid methylation and analysis

Total fatty acids (TFA) and those of glycerolipid classes were transformed into their corresponding methyl esters as described by Cecchi et al. (1985). Transmethylation was made by the addition of 2 mL of hexane, 0.5 mL of 3% sodium methylate, a known amount of heptadecanoic acid methyl ester (C17:0) used as the internal standard, 0.2 mL of 1N H_2SO_4 and 1.5 mL of 10% sodium chloride. The hexanic phase that contains fatty acid methyl esters (FAME) was recovered and its volume reduced using a stream of nitrogen, prior to analysis.

FAME analysis was achieved by gas chromatography (GC) with a Hewlett-Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A HP-Innowax capillary column (polyethylene glycol: 30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used; the flow of the carrier gas (N_2, U) was 1.6 mL/min and the split ratio 60:1. Analyses were performed by using an oven temperature of 150 °C for 1 min, followed by an increase from 150 to 200 °C at a rate of 15 °C/min, and then from 200 to 225 °C at a rate of 2 °C/min and finally held there for an additional 2 min period. The detector and injector temperatures were set at 275 and 250 °C, respectively.

FAMEs were identified by comparison of their retention times with those of pure reference standards. Gas chromatograph was connected to HP Chemstation (Rev.A.0401) software for peak area and fatty acid percentage calculation.

2.8. Statistical analysis

All data were reported as means ± standard deviation of three samples. Statistical analysis was performed with STATISTICA (Statsoft, 1998). Differences were tested for significance by using the ANOVA procedure, using Duncan test with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Physical properties

The physical properties of seed, pericarp and whole fruit from *M. communis* var. *italica* are given in Table 1. The fruit is composed of pericarp and approximately 9 seeds which constituted 63.5 and 36.5% of the whole ripe fruit, respectively. The latter presented a weight of 8.8 g% fruits while seed had only 0.5 g% seeds. The moisture contents were 80.1% in pericarp, 72% in whole fruit and 39.7% in seed. The myrtle fruit presented a width of 7.4 mm and a length of 10.9 mm. The fruit length was three times higher than the seed one (3.5 mm) and this result was similar to that of Traveset et al. (2001) who also did not determine the width of myrtle seed due to its snail shape. Moreover, they determined the number of seeds