



## Variations in essential oil and fatty acid composition during *Myrtus communis* var. *italica* fruit maturation

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### ABSTRACT

The essential oil and fatty acid composition of *Myrtus communis* var. *italica* fruit during its ripening was determined. The effect of the harvesting time on some physical properties of *Myrtus* fruits, fruit weight and moisture content, were significant. The increase of fruit weight (from 2.54 to 8.79 g% fruits) during ripeness was correlated positively with that of moisture content (from 28% to 72%). Fruit essential oil yields varied from 0.003% to 0.01% and showed a remarkable increase at 60 days after flowering to reach a maximum of 0.11%. Forty-seven volatile compounds were identified in fruit essential oils; 1,8-cineole (7.31–40.99%), geranyl acetate (1.83–20.54%), linalool (0.74–18.92%) and  $\alpha$ -pinene (1.24–12.64%) were the main monoterpene compounds. Total fatty acid contents varied from 0.81% to 3.10% during fruit maturation and the predominant fatty acids were linoleic (12.21–71.34%), palmitic (13.58–37.07%) and oleic (6.49–21.89%) acids. The linoleic acid proportions correlated inversely with palmitic and oleic acids during all the stages of ripening.

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### 1. Introduction

The myrtle shrubs (*Myrtus communis* L.) grow wildly in the coastal regions, the internal hills and the forest areas of North Tunisia. Old local flora described the presence of two myrtle varieties called *M. communis* var. *italica* L. and *M. communis* var. *baetica* L. presenting the same vegetative characters. The morphological difference between the two varieties is the bigger size of *baetica* leaves and fruits (Pottier-Alapetite, 1979).

Myrtle is better known as a medicinal plant for its anti-hyperglycemic (Elfellah, Akhter, & Khan, 1984), antiseptic and anti-inflammatory activities (Al-Hindawi, Al-Deen, Nabi, & Ismail, 1989; Diaz & Abeger, 1987). Different parts of the plant find various uses in food and cosmetic industries (Chalchat, Garry, & Michet, 1998). Liqueurs prepared from myrtle berries became popular especially in Sardinia (Nuvoli & Spanu, 1996) while its leaves have been used as a hop substitute in beer (Buhner, 1998).

On the other hand, this species is a very aromatic plant because of the high essential oil content in its leaf, flower, and fruit glands. Different part essential oils have been employed for their antimicrobial, tonic and balsamic properties (De De Laurentis, Rosato, Gallo, Leone, & Milillo, 2005). Myrtle berries and leaves are mostly

employed for the industrial formulation of sweet liqueurs with digestive properties (Nuvoli & Spanu, 1996).

The chemical composition of the myrtle leaf essential oil belonging to the different regions and harvested at different periods has been widely studied (Bradesi, Tomi, Casanova, Costa, & Bernardini, 1997; Chalchat et al., 1998; Gardeli, Papageorgiou, Mallouchos, Theodosios, & Komaitis, 2008; Messaoud, Zaouali, Ben Salah, Khoudja, & Boussaid, 2005) and the evaluation of the fruit essential oil composition have also been reported (Mazza, 1983; Mulas, Spano, Biscaro, & Parpinello, 2000). Moreover, many phytochemical researches investigated at the same time the essential oil composition of leaves and fruits as well as the other parts of *M. communis* (Aidi Aidi Wannes, Mhamdi, & Marzouk, 2007; Boelens & Jimenez, 1992; Flamini, Cioni, Morelli, Maccioni, & Baldini, 2004; Gauthier, Gourai, & Bellakhdar, 1988; Jerkovic, Radonic, & Borcic, 2002; Tuberoso, Barra, Angioni, Sarritzu, & Pirisi, 2006) because of its great interest in various fields such as culinary, cosmetic, pharmaceutical, therapeutical and industrial. However, little has been undertaken on the fatty acid composition of myrtle fruit (Asif, Afaq, Tariq, & Masoodi, 1979; Çakir, 2004) and there is no data about the changes on essential oil and fatty acid composition of Tunisian myrtle fruit during its development.

The purpose of this work is to characterize *M. communis* var. *italica* fruit through its essential oil and fatty acid composition at different stages of ripening in order to determine the optimal

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accumulation period of desirable compounds and to try to valorize this berry fruit as source of bioactive molecules.

## 2. Materials and methods

### 2.1. Plant material

Berries of *M. communis* var. *italica* were monthly collected at different harvesting periods from plants grown in the region of Haouaria from Jbal Stara (Nabeul, North East of Tunisia). Harvesting period was stretched from 30 days after flowering (DAF) in August 2006 to 180 DAF in January 2007. Full details of fruit collection data are provided in Table 1.

### 2.2. Chemicals

All solvents used in the experiments (diethyl ether, chloroform, hexane, toluene, ethanol and methanol) were purchased from Merck (Darmstadt, Germany). Sodium methylate ( $\text{CH}_3\text{ONa}$ ), sodium chloride ( $\text{NaCl}$ ), sulphuric acid ( $\text{H}_2\text{SO}_4$ ), anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) and homologous series of  $\text{C}_8$ – $\text{C}_{22}$  *n*-alkanes used for identification were obtained from Sigma–Aldrich (Steinheim, Germany). Essential oil and fatty acid standards were purchased from Fluka (Ridel-de Haën, Switzerland) and Sigma–Aldrich (Steinheim, Germany).

### 2.3. Essential oil extraction

Essential oil was extracted by hydrodistillation over 180 min using 100 g of fruits; this time was fixed after a kinetic survey during 30, 60, 90, 120, 150, 180 and 210 min. The distillate was extracted with diethyl ether and dried over anhydrous sodium sulphate. All experiments were done in triplicates and results were expressed on the basis of dry matter weight.

### 2.4. 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 lipids was recovered and dried under a nitrogen stream. Finally, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) at –20 °C for further analyses.

### 2.5. Fatty acid transmethylation

Total fatty acids (TFA) of total lipids were transformed into their corresponding methyl esters as described by Cecchi, Biasini, and Castano (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 ( $\text{C}_{17:0}$ ) as the internal standard, 0.2 ml of 1 N  $\text{H}_2\text{SO}_4$  and 1.5 ml of 10% sodium chloride. The

hexanic phase that contains fatty acid methyl esters (FAME) was recovered and its volume reduced in a stream of nitrogen, prior to analysis.

### 2.6. Gas chromatography (GC-FID)

Essential oils were analyzed by gas chromatography (GC) using 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 ( $\text{N}_2$ , U) was 1.6 ml/min and the split ratio 60:1. Analyses were performed using the following temperature program: oven temps isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C/min, and isotherm at 205 °C over 10 min. Injector and detector temperature were held, respectively, at 250 and 300 °C.

FAMES were analyzed by GC using the same apparatus previously described. The initial oven temperature was held at 150 °C for 1 min, increased at a rate of 15 °C/min to 200 °C, and then held there for 3 min and finally ramped at 2 °C/min to 242 °C. The detector and injector temperatures were set at 275 °C and 250 °C, respectively.

### 2.7. Gas chromatography–mass spectrometry

GC–MS analyses of essential oil volatile components were carried out on a gas chromatograph HP 5890 (II) coupled to a HP 5972 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Hewlett–Packard, CA, USA) was used. The column temperature was programmed to rise from 50 °C to 240 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 ml/min; split ratio was 60:1. Scan time and mass range were 1 s and 40–300 m/z, respectively.

### 2.8. Compounds identification

Identification of essential oil volatile compounds was based on the calculation of their retention indices (RI) relative to ( $\text{C}_8$ – $\text{C}_{22}$ ) *n*-alkanes with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS data system and other published mass spectra (Adams, 2001). FAMES were identified by comparison of their retention times with those of pure reference standards. Quantitative data were obtained from the electronic integration of the FID peak areas.

### 2.9. 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 a significance level of  $p \leq 0.05$ .

## 3. Results and discussion

### 3.1. Physical characteristics of myrtle fruit

According to Traveset, Riera, and Mas (2001), the myrtle fruit's colour was adopted as a visual ripening criterion. In fact, the ellipsoidal berries turn from green (30 and 60 DAF) to pale yellow (90

**Table 1**  
*Myrtus communis* var. *italica* fruit sampling during six harvesting periods

Sampling date	03/08/ 2006	03/09/ 2006	03/10/ 2006	03/11/ 2006	03/12/ 2006	03/01/ 2007
Days after flowering	30	60	90	120	150	180
Temperature (°C)	27	24	22	18	15	14
Humidity (%)	68	76	79	79	83	82
Rainfall (mm)	27	83	123	15	133	56

**Table 2**  
Physical characteristics of *Myrtus communis* var. *italica* fruit during its ripening

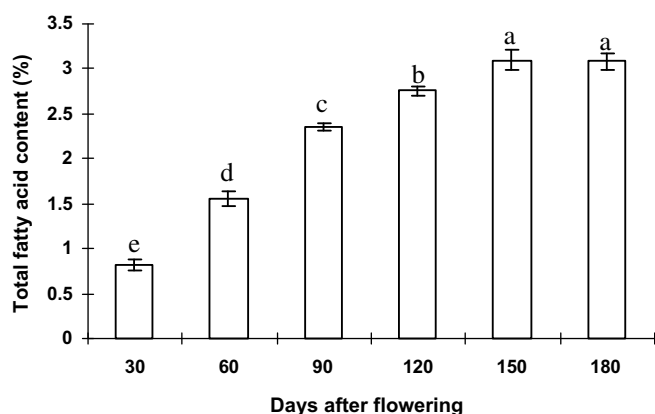
Characteristic	Days after flowering					
	30	60	90	120	150	180
Fruit colour	Green	Green	Pale yellow	Pale yellow	Dark blue	Dark blue
Fruit weight (g% fruits)	2.54 ± 0.14 <sup>d</sup>	2.80 ± 0.17 <sup>d</sup>	4.03 ± 0.23 <sup>c</sup>	5.53 ± 0.46 <sup>b</sup>	8.37 ± 0.37 <sup>a</sup>	8.79 ± 0.39 <sup>a</sup>
Moisture content (%)	28 ± 0.42 <sup>e</sup>	38 ± 0.20 <sup>d</sup>	60 ± 0.09 <sup>c</sup>	62 ± 1.69 <sup>b</sup>	71 ± 1.63 <sup>a</sup>	72 ± 2.45 <sup>a</sup>

Values followed by the same small letter did not share significant differences at 5% (Duncan test).

and 120 DAF) and finally, in the common morph, to dark blue (150 and 180 DAF) when completely ripe.

In the other hand, the effect of harvesting time on dry weight of 100 berries was significant ( $p < 0.05$ ) as shown in Table 2. The berry weight at 30 DAF (2.54 g% berries) was the lowest because of fruit immaturity. Fruits increased progressively in weight as growth progressed to obtain a maximum of 8.79 g% fruits at ripe fruit (180 DAF). The 100 fruit weight was lower than Turkish one (38.00–132.66 g% fruits) reported by Aydin and Özcan (2007). These variations could be attributed to some differences such as location, climate, environment, harvest period, berry maturity and variety type.

The increase of 100 myrtle fruit weight was strongly positively correlated ( $R^2 = 0.9415$ ) with that of moisture content from 28% to 72% during the fruit maturation. Aydin and Özcan (2007) found the same correlation between 100 fruit weight and moisture content for Turkish myrtle fruits.



**Fig. 1.** Variations in total fatty acid content (% w/w) of myrtle fruit during its ripening. TFA values with different subscript (a–e) were significantly different at  $p < 0.05$  (Duncan test).

**Table 3**  
Variations in fatty acid composition (%) of total lipids during myrtle fruit ripening

Fatty acid	Days after flowering (DAF)					
	30	60	90	120	150	180
C12:0	2.84 ± 0.12 <sup>a</sup>	0.86 ± 0.00 <sup>b</sup>	0.63 ± 0.26 <sup>b</sup>	0.64 ± 0.84 <sup>b</sup>	0.78 ± 0.69 <sup>b</sup>	0.36 ± 0.00 <sup>b</sup>
C14:0	4.21 ± 0.12 <sup>a</sup>	1.69 ± 0.00 <sup>b</sup>	0.58 ± 0.25 <sup>c</sup>	0.43 ± 0.44 <sup>c</sup>	0.55 ± 0.34 <sup>c</sup>	0.35 ± 0.01 <sup>c</sup>
C16:0	37.03 ± 0.40 <sup>a</sup>	39.81 ± 0.00 <sup>a</sup>	30.83 ± 4.77 <sup>b</sup>	14.46 ± 1.44 <sup>c</sup>	13.84 ± 1.23 <sup>c</sup>	13.58 ± 0.80 <sup>c</sup>
C16:1 (n-7)	3.50 ± 0.01 <sup>a</sup>	0.09 ± 0.00 <sup>c</sup>	0.85 ± 0.78 <sup>b</sup>	0.48 ± 0.36 <sup>bc</sup>	0.53 ± 0.34 <sup>bc</sup>	0.32 ± 0.00 <sup>bc</sup>
C18:0	9.17 ± 0.04 <sup>a</sup>	9.76 ± 0.00 <sup>a</sup>	3.96 ± 1.17 <sup>b</sup>	3.42 ± 1.16 <sup>b</sup>	2.88 ± 0.60 <sup>b</sup>	3.65 ± 0.15 <sup>b</sup>
C18:1 (n-9)	21.89 ± 0.03 <sup>a</sup>	14.70 ± 0.00 <sup>b</sup>	12.64 ± 2.43 <sup>c</sup>	7.55 ± 0.90 <sup>d</sup>	7.86 ± 1.80 <sup>d</sup>	6.49 ± 0.21 <sup>d</sup>
C18:2 (n-6)	12.21 ± 0.06 <sup>d</sup>	23.63 ± 0.00 <sup>c</sup>	32.19 ± 4.15 <sup>b</sup>	67.44 ± 3.44 <sup>a</sup>	67.25 ± 3.49 <sup>a</sup>	71.34 ± 1.04 <sup>a</sup>
C18:3 (n-3)	4.36 ± 0.03 <sup>b</sup>	1.65 ± 0.00 <sup>c</sup>	17.98 ± 2.58 <sup>a</sup>	4.03 ± 1.17 <sup>b</sup>	4.70 ± 0.61 <sup>b</sup>	3.25 ± 0.13 <sup>bc</sup>
C20:0	2.66 ± 0.02 <sup>b</sup>	4.33 ± 0.00 <sup>a</sup>	0.30 ± 0.03 <sup>c</sup>	0.74 ± 0.49 <sup>c</sup>	0.66 ± 0.46 <sup>c</sup>	0.44 ± 0.12 <sup>c</sup>
C20:1 (n-9)	2.00 ± 0.02 <sup>b</sup>	3.48 ± 0.00 <sup>a</sup>	0.03 ± 0.05 <sup>e</sup>	0.76 ± 0.69 <sup>cd</sup>	0.93 ± 0.80 <sup>c</sup>	0.21 ± 0.08 <sup>de</sup>
∑SFA	55.91 ± 0.11 <sup>a</sup>	56.44 ± 0.00 <sup>a</sup>	36.30 ± 3.50 <sup>b</sup>	19.69 ± 2.19 <sup>c</sup>	18.71 ± 2.12 <sup>c</sup>	18.38 ± 1.06 <sup>c</sup>
∑MUFA	27.39 ± 0.05 <sup>a</sup>	18.27 ± 0.00 <sup>b</sup>	13.52 ± 1.78 <sup>c</sup>	8.69 ± 0.60 <sup>de</sup>	9.32 ± 2.10 <sup>d</sup>	7.02 ± 0.12 <sup>e</sup>
∑PUFA	16.57 ± 0.06 <sup>d</sup>	25.28 ± 0.00 <sup>c</sup>	50.17 ± 5.85 <sup>b</sup>	71.47 ± 2.67 <sup>a</sup>	71.94 ± 2.89 <sup>a</sup>	74.58 ± 1.18 <sup>a</sup>
∑SFA/∑PUFA	2.94 ± 0.83 <sup>a</sup>	2.23 ± 0.00 <sup>b</sup>	0.73 ± 0.15 <sup>c</sup>	0.27 ± 0.03 <sup>c</sup>	0.26 ± 0.03 <sup>c</sup>	0.25 ± 0.01 <sup>c</sup>

Values followed by the same small letter did not share significant differences at 5% (Duncan test).

### 3.2. Total fatty acid content

The evolution of TFA content from myrtle fruit during six harvesting periods is reported in Fig. 1 which showed three phases. In fact, a rapid increase of TFA rates from 0.81% dry matter weight (DMW) at 30 DAF to 2.35% DMW at 90 DAF was observed during the first phase. During the second phase, the TFA contents raised progressively to reach a maximum of 2.75% DMW at 120 DAF. The last phase of ripe fruit (from 150 DAF to 180 DAF) was characterized by approximately stationary rates of TFA about 3.10% DMW. This latter was lower than presented by Çakir (2004) who found that Turkish myrtle ripe fruit yielded 5.25% of TFA with 0.38% for mesocarp and 4.87% for seed. No reported literature was found concerned the variation of TFA content of myrtle fruit during ripeness. However, the TFA content evolution of myrtle fruit was different to that of oleaginous fruits and seeds. For example Chahed et al. (2006) indicated that there is a slow increase of total fatty acid contents of *Pistacia vera* seeds during the first stages of maturity. Then, a rapid increase was observed to obtain a maximum of 36.79% (=367.99 mg/g DMW) at ripeness with slight decrease at final stage due to lipase activation when in the over-ripened seed.

### 3.3. Fatty acid composition

In newly formed fruit (30 DAF), saturated fatty acids (SFA) formed 55.91% of TFA with a palmitic acid (C16:0) contribution of 37.03% of TFA. This proportion decreased progressively until 13.58% of TFA at the final stage (Table 3). Other representative SFA was stearic acid (C18:0) and its proportions showed the same evolution that of palmitic acid and formed 3.65% of TFA in ripe fruit. However, lauric (C12:0), myristic (C14:0) and arachidic (C20:0) acids belong to minor fraction of SFA. Monounsaturated fatty acid (MUFA) proportions were mainly represented by oleic acid at 30 DAF with 21.89% of TFA but it decreased significantly during all the harvesting periods to obtain 6.49% of TFA at 180 DAF. The two other MUFA, palmitoleic (C16:1 n-7) and gadoleic

(C20:1 n-9) acids, were weakly represented in all the development stages. Concerning polyunsaturated fatty acids (PUFA), they were linoleic acid (C18:2 n-6) which constituted only 12.21% of TFA at 30 DAF and increased progressively to reach 71.34% of TFA in full ripe fruit (180 DAF). The second PUFA was the linolenic acid (C18:3 n-3). Its proportions varied slowly (from 1.70% of TFA to 4.65% of TFA) during the fruit ripening with the exception at 90 DAF when this fatty acid obtained 17.98% of TFA.

During maturation of the myrtle fruit, PUFA contents and mainly linoleic acid one increased despite of SFA (palmitic and stearic acids) and MUFA (oleic acid). The linoleic acid proportions correlated inversely with palmitic ( $r = -0.9728$ ), stearic ( $r = -0.8247$ ) and oleic ( $r = -0.369$ ) acids during all the stages. Hence, during the fruit ripening, there is an enhancement of enzymatic activities particularly those of desaturases, especially  $\Delta 12$ -desaturase responsible of linoleic acid biosynthesis, using 18 carbon fatty acids as substrate stimulation. This biochemical pathway has been reported in the case of fatty acid biosynthesis in cottonseed (Liu, Singh, & Green, 2002).

The ratio of saturated fatty acids to unsaturated fatty acids (SFA/PUFA) decreased during fruit maturation to reach 0.25 in fully ripe fruit. Similar results were also found in ripe coriander and niger seeds with a ratio of 0.379 and 0.370, respectively (Ramadan & Mörseel, 2006). However, the level of unsaturation/saturation (2.2%) was not different during cherry laurel fruit maturation (Ayaz & Kadioglu, 2000).

Interest in the PUFA, as health-promoting nutrients has expanded in recent years. A growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finley & Shahidi, 2001). The fatty acid composition and the high contents of PUFA at 150 and 180 DAF could be making the myrtle fruit lipids important for a variety of healthy applications.

#### 3.4. Essential oil yield

During the fruit development, essential oil yields varied from 0.003% to 0.11% DMW, reaching a maximum at 60 DAF, after which it rapidly decreased (Fig. 2). In agreement with Jerkovic et al. (2002), essential oil production of myrtle fruit has been considered as associated with early growing periods. In fact, essential oil yield in premature fruit at 60 DAF was about ten times higher than in fully ripened fruits with 0.01% at 150 DAF and 0.003% at 180 DAF as it was mentioned by Aydin and Özcan (2007) and Tuberoso

et al. (2006) in mature myrtle fruits. These results were similar to those of Flamini, Bader, Cioni, Katbeh-Bader, and Morelli (2004) who found that the essential oil yield of *Pistacia palaestina* fruits dropped from 0.16% to 0.06% in unripe and ripe fruits, respectively. However, Gauthier et al. (1988) noted that there is an obvious increase of essential oil yields from *M. communis* var. *italica* fruits during their ripening (+60%).

#### 3.5. Essential oil composition

Analysis of myrtle fruit essential oil composition showed 47 identified compounds presenting high fluctuations during its different stages (Table 4). 1,8-Cineole (7.31–40.99%), geranyl acetate (1.84–20.53%), linalool (0.74–18.92%) and  $\alpha$ -pinene (1.24–12.64%) were the main monoterpene compounds. The fruit essential oil composition was characterized by high levels of 1,8-cineole and geranyl acetate; these two compound proportions had a contrasting evolution during all the stages. When 1,8-cineole reached its highest percentages at 60 DAF (40.99%) and 150 DAF (34.86%), geranyl acetate reached the lowest ones with, respectively, 1.84% and 2.09%, but when the geranyl acetate reached a maximum percentage at 30 DAF with 20.53%, 1,8-cineole reached a minimum of 7.31%. However, linalool proportions had the same evolution that of 1,8-cineole; So, this latter showed the highest proportions at 60 and 180 DAF with, respectively, 18.92% and 9.53% and the lowest at 30 DAF forming 0.74%. The level of  $\alpha$ -pinene decreased progressively from 30 DAF to 180 DAF (from 12.64% to 1.24%). Other representative compounds were identified like  $\beta$ -caryophyllene showing its lowest values from 60 DAF to 180 DAF with an exception at 30 DAF when this compound obtained 10.83%. The highest percentage of  $\alpha$ -terpinyl acetate (8.94%) was reached at 60 DAF while at 30 and 180 DAF, it showed the lowest ones with respectively 0.92% and 0.79%. Geranyl 2-methylbutyrate proportions varied from 1.77% to 4.60% and reached at 150 DAF 8.23%. The proportions of  $\alpha$ -terpineol showed slight changes during all the stages with a maximum at 180 DAF (6.95%) and a minimum at 30 DAF (4.10%). The rest of fruit essential oil compounds represented approximately one-quarter of the total essential oil components at each sampling.

These results were different to those of Gauthier et al. (1988) who showed that there was an increase of the percentages of 1,8-cineole from 17% to 25% and myrtenyl acetate from 4% to 20% during fruit maturation of Moroccan *M. communis* var. *italica*. They also signalled the rainfall effect on mature fruit essential oil. In fact, after a rainfall period, there was a disappearance of  $\alpha$ -pinene, an increase of myrtenyl acetate percentages from 2% to 43% and a decrease of 1,8-cineole and myrtenol proportions from, respectively, 29% to 17% and 16% to 45%. Jerkovic et al. (2002) studied the changes in essential oil composition of Croatian myrtle fruit during its ripening but without variety mention. They found that the main components were myrtenyl acetate (12.20–33.30%), 1,8-cineole+limonene (10.90–21.10%),  $\alpha$ -pinene (4.00–15.30%) and linalool (4.70–7.70%). They also reported that there was a decrease of myrtenyl acetate contents during ripening period as obtained by Boelens and Jimenez (1992) for myrtle fruit essential oils from Spain. In agreement with Flamini et al. (2004) and Tuberoso et al. (2006) who studied the geographical variability of Italian fruit and leaf essential oils, the strong chemical variability in myrtle fruit essential oils could be ascribed not only to the geographical origin of the sample and its environmental conditions but also to the variety and genetic factors.

#### 4. Conclusions

The results reported on the essential oil and fatty acid composition of the myrtle fruit at different stages of ripening revealed great

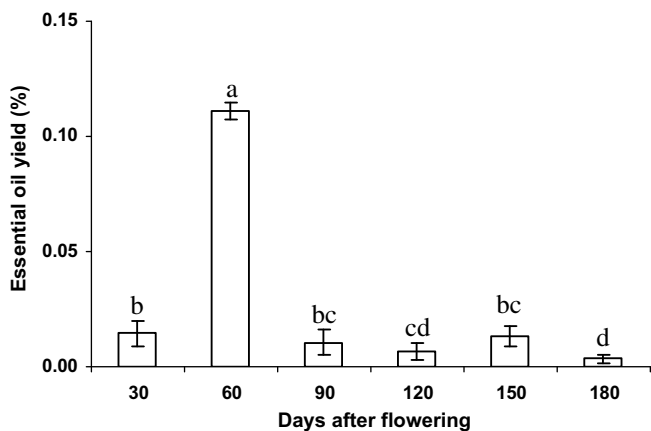


Fig. 2. Changes in essential oil yield (% w/w) during *Myrtus communis* var. *italica* fruit ripening. Essential oil yields with different subscript (a–d) were significantly different at  $p < 0.05$  (Duncan test).

**Table 4**  
Essential oil composition (% w/w) of *Myrtus communis* var. *italica* fruit during its ripening

Compound <sup>a</sup>	RI <sup>a</sup>	RI <sup>b</sup>	Days after flowering (DAF)					
			30	60	90	120	150	180
(Z)-3-Hexenol	855	1370	1.27 ± 0.39 <sup>a</sup>	0.05 ± 0.01 <sup>b</sup>	0.08 ± 0.03 <sup>b</sup>	0.09 ± 0.00 <sup>b</sup>	0.10 ± 0.05 <sup>b</sup>	0.05 ± 0.00 <sup>b</sup>
Hexanol	865	1354	–	0.02 ± 0.00 <sup>a</sup>	0.01 ± 0.00 <sup>b</sup>	–	–	0.01 ± 0.00 <sup>b</sup>
Tricyclene	924	1014	0.53 ± 0.59 <sup>b</sup>	0.44 ± 0.26 <sup>b</sup>	0.60 ± 0.17 <sup>b</sup>	0.52 ± 0.28 <sup>b</sup>	1.24 ± 0.11 <sup>a</sup>	0.58 ± 0.04 <sup>b</sup>
α-Thujene	928	1035	2.02 ± 0.04 <sup>a</sup>	0.32 ± 0.11 <sup>b</sup>	0.72 ± 0.00 <sup>b</sup>	1.08 ± 0.78 <sup>b</sup>	2.79 ± 1.10 <sup>a</sup>	0.43 ± 0.00 <sup>b</sup>
α-Pinene	939	1032	12.64 ± 0.37 <sup>a</sup>	7.20 ± 0.34 <sup>b</sup>	7.11 ± 0.20 <sup>b</sup>	7.47 ± 0.73 <sup>b</sup>	4.42 ± 0.05 <sup>c</sup>	1.24 ± 0.26 <sup>d</sup>
Camphene	954	1076	–	–	0.01 ± 0.01 <sup>a</sup>	0.03 ± 0.06 <sup>a</sup>	–	0.01 ± 0.06 <sup>a</sup>
Sabinene	975	1132	–	0.02 ± 0.02 <sup>b</sup>	–	0.18 ± 0.34 <sup>ab</sup>	0.28 ± 0.01 <sup>a</sup>	–
β-Pinene	980	1118	3.20 ± 0.32 <sup>a</sup>	2.50 ± 0.10 <sup>b</sup>	0.30 ± 0.00 <sup>d</sup>	0.41 ± 0.15 <sup>d</sup>	0.53 ± 0.14 <sup>d</sup>	1.25 ± 0.09 <sup>c</sup>
Myrcene	991	1174	–	0.02 ± 0.01 <sup>ab</sup>	0.08 ± 0.08 <sup>a</sup>	0.01 ± 0.00 <sup>b</sup>	–	0.02 ± 0.01 <sup>ab</sup>
α-Phellandrene	1006	1176	–	0.03 ± 0.03 <sup>a</sup>	–	0.04 ± 0.08 <sup>a</sup>	–	–
δ-3-Carene	1011	1159	–	0.13 ± 0.01 <sup>ab</sup>	0.32 ± 0.29 <sup>a</sup>	0.10 ± 0.19 <sup>ab</sup>	–	0.07 ± 0.02 <sup>ab</sup>
α-Terpinene	1018	1188	–	0.02 ± 0.02 <sup>ab</sup>	0.06 ± 0.07 <sup>a</sup>	–	–	–
p-Cymene	1026	1280	0.04 ± 0.04 <sup>b</sup>	0.36 ± 0.40 <sup>a</sup>	–	–	–	–
limonene	1030	1203	0.03 ± 0.03 <sup>a</sup>	0.01 ± 0.07 <sup>a</sup>	0.01 ± 0.1 <sup>a</sup>	–	–	–
1,8-Cineole	1033	1213	7.31 ± 0.89 <sup>f</sup>	40.99 ± 0.77 <sup>a</sup>	22.14 ± 0.60 <sup>d</sup>	18.38 ± 0.96 <sup>e</sup>	28.08 ± 0.51 <sup>c</sup>	34.86 ± 0.9 <sup>b</sup>
(E)-β-Ocimene	1050	1266	–	0.29 ± 0.28 <sup>cd</sup>	2.90 ± 0.45 <sup>a</sup>	0.53 ± 0.09 <sup>c</sup>	1.58 ± 0.12 <sup>b</sup>	0.43 ± 0.16 <sup>c</sup>
γ-Terpinene	1062	1255	–	0.36 ± 0.02 <sup>abc</sup>	0.97 ± 0.21 <sup>a</sup>	0.88 ± 0.90 <sup>a</sup>	0.67 ± 0.12 <sup>ab</sup>	0.09 ± 0.06 <sup>bc</sup>
cis-Linalool oxide	1074	1450	2.05 ± 2.16 <sup>a</sup>	0.04 ± 0.00 <sup>b</sup>	0.12 ± 0.03 <sup>b</sup>	0.17 ± 0.22 <sup>b</sup>	0.09 ± 0.18 <sup>b</sup>	0.30 ± 0.18 <sup>b</sup>
trans-Linalool oxide	1088	1475	0.13 ± 0.14 <sup>c</sup>	0.06 ± 0.02 <sup>c</sup>	0.07 ± 0.02 <sup>c</sup>	0.75 ± 0.76 <sup>b</sup>	0.27 ± 0.26 <sup>bc</sup>	2.03 ± 0.05 <sup>a</sup>
Terpinolene	1092	1290	0.13 ± 0.14 <sup>bc</sup>	0.13 ± 0.07 <sup>bc</sup>	0.65 ± 0.13 <sup>a</sup>	0.47 ± 0.55 <sup>ab</sup>	0.18 ± 0.17 <sup>bc</sup>	0.02 ± 0.02 <sup>c</sup>
Linalool	1098	1553	0.74 ± 0.14 <sup>f</sup>	18.92 ± 0.29 <sup>a</sup>	4.26 ± 0.33 <sup>d</sup>	3.24 ± 0.35 <sup>e</sup>	7.96 ± 0.06 <sup>c</sup>	9.53 ± 0.41 <sup>b</sup>
Borneol	1165	1719	1.13 ± 0.78 <sup>a</sup>	0.19 ± 0.01 <sup>b</sup>	0.31 ± 0.24 <sup>b</sup>	0.33 ± 0.35 <sup>b</sup>	0.72 ± 0.00 <sup>ab</sup>	0.11 ± 0.03 <sup>b</sup>
Terpinene-4-ol	1178	1611	4.26 ± 0.42 <sup>a</sup>	0.04 ± 0.02 <sup>d</sup>	0.06 ± 0.00 <sup>d</sup>	1.86 ± 0.49 <sup>b</sup>	0.77 ± 0.27 <sup>c</sup>	0.56 ± 0.01 <sup>c</sup>
p-Cymene-8-ol	1183	1864	0.04 ± 0.00 <sup>b</sup>	0.04 ± 0.00 <sup>b</sup>	0.18 ± 0.03 <sup>a</sup>	0.09 ± 0.10 <sup>b</sup>	0.11 ± 0.05 <sup>a</sup>	0.04 ± 0.10 <sup>b</sup>
α-Terpineol	1189	1709	4.10 ± 0.26 <sup>c</sup>	5.29 ± 0.52 <sup>b</sup>	6.95 ± 0.30 <sup>a</sup>	4.33 ± 0.50 <sup>c</sup>	5.07 ± 0.01 <sup>b</sup>	6.68 ± 0.10 <sup>a</sup>
Myrtenol	1194	1804	0.61 ± 0.39 <sup>a</sup>	0.07 ± 0.00 <sup>c</sup>	0.51 ± 0.32 <sup>ab</sup>	0.36 ± 0.19 <sup>abc</sup>	0.16 ± 0.00 <sup>bc</sup>	0.05 ± 0.04 <sup>c</sup>
Nerol	1228	1797	6.13 ± 0.68 <sup>a</sup>	0.14 ± 0.01 <sup>b</sup>	0.13 ± 0.10 <sup>b</sup>	0.14 ± 0.13 <sup>b</sup>	0.12 ± 0.00 <sup>b</sup>	0.04 ± 0.03 <sup>b</sup>
cis-Carveol	1247	1861	0.02 ± 0.00 <sup>c</sup>	0.02 ± 0.00 <sup>c</sup>	0.20 ± 0.10 <sup>a</sup>	0.09 ± 0.05 <sup>bc</sup>	0.24 ± 0.01 <sup>ab</sup>	0.10 ± 0.02 <sup>bc</sup>
Geraniol	1255	1857	0.68 ± 0.13 <sup>d</sup>	2.01 ± 0.11 <sup>a</sup>	1.47 ± 0.27 <sup>c</sup>	1.56 ± 0.42 <sup>bc</sup>	1.86 ± 0.00 <sup>ab</sup>	1.26 ± 0.08 <sup>c</sup>
Linalyl acetate	1257	1556	1.49 ± 0.24 <sup>b</sup>	0.25 ± 0.16 <sup>c</sup>	1.30 ± 0.29 <sup>b</sup>	1.11 ± 0.79 <sup>b</sup>	2.43 ± 0.78 <sup>a</sup>	0.15 ± 0.01 <sup>c</sup>
Bornyl acetate	1295	1597	–	0.09 ± 0.04 <sup>b</sup>	0.24 ± 0.07 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>	0.20 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>b</sup>
Tridecane	1300	1300	0.14 ± 0.16 <sup>a</sup>	0.01 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>	–	–	–
Myrtenyl acetate	1335	1707	1.43 ± 1.10 <sup>a</sup>	0.37 ± 0.01 <sup>b</sup>	0.25 ± 0.08 <sup>b</sup>	0.26 ± 0.12 <sup>b</sup>	0.23 ± 0.02 <sup>b</sup>	0.71 ± 0.05 <sup>b</sup>
α-Terpinyl acetate	1344	1706	0.92 ± 0.82 <sup>d</sup>	8.94 ± 0.27 <sup>a</sup>	2.11 ± 0.43 <sup>c</sup>	3.70 ± 0.38 <sup>b</sup>	4.17 ± 0.02 <sup>b</sup>	0.79 ± 0.07 <sup>d</sup>
Eugenol	1356	2186	0.55 ± 0.44 <sup>c</sup>	0.55 ± 0.44 <sup>c</sup>	1.69 ± 0.42 <sup>b</sup>	2.43 ± 0.93 <sup>ab</sup>	2.81 ± 0.13 <sup>a</sup>	0.45 ± 0.07 <sup>c</sup>
Geranyl acetate	1383	1765	20.54 ± 0.70 <sup>a</sup>	1.83 ± 0.04 <sup>d</sup>	7.04 ± 0.73 <sup>c</sup>	10.94 ± 0.14 <sup>b</sup>	6.48 ± 0.19 <sup>c</sup>	2.09 ± 0.30 <sup>d</sup>
Neryl acetate	1385	1733	3.76 ± 0.78 <sup>a</sup>	0.06 ± 0.02 <sup>b</sup>	0.10 ± 0.07 <sup>b</sup>	0.32 ± 0.46 <sup>b</sup>	0.10 ± 0.01 <sup>b</sup>	0.07 ± 0.04 <sup>b</sup>
β-Elementene	1391	1600	0.36 ± 0.16 <sup>a</sup>	0.10 ± 0.02 <sup>b</sup>	0.11 ± 0.01 <sup>b</sup>	0.15 ± 0.09 <sup>b</sup>	0.03 ± 0.02 <sup>b</sup>	0.04 ± 0.00 <sup>b</sup>
Methyl eugenol	1401	2030	1.14 ± 0.08 <sup>b</sup>	1.26 ± 0.00 <sup>b</sup>	3.05 ± 0.07 <sup>a</sup>	3.30 ± 0.35 <sup>a</sup>	3.05 ± 0.04 <sup>a</sup>	3.62 ± 0.76 <sup>a</sup>
β-Caryophyllene	1419	1612	10.83 ± 0.96 <sup>a</sup>	1.23 ± 0.17 <sup>c</sup>	2.16 ± 0.74 <sup>b</sup>	0.86 ± 0.13 <sup>c</sup>	0.85 ± 0.01 <sup>c</sup>	1.00 ± 0.02 <sup>c</sup>
α-Humulene	1454	1687	0.6 ± 0.41 <sup>a</sup>	0.03 ± 0.01 <sup>b</sup>	0.05 ± 0.04 <sup>b</sup>	0.08 ± 0.10 <sup>b</sup>	0.2 ± 0.29 <sup>b</sup>	0.08 ± 0.02 <sup>b</sup>
allo-Aromadendrene	1474	1661	0.33 ± 0.15 <sup>ab</sup>	0.05 ± 0.02 <sup>b</sup>	0.40 ± 0.39 <sup>a</sup>	0.08 ± 0.08 <sup>b</sup>	0.12 ± 0.00 <sup>ab</sup>	0.06 ± 0.01 <sup>b</sup>
Germacrene-D	1480	1726	0.16 ± 0.18 <sup>ab</sup>	0.23 ± 0.06 <sup>ab</sup>	0.37 ± 0.18 <sup>a</sup>	0.23 ± 0.18 <sup>ab</sup>	0.39 ± 0.13 <sup>a</sup>	0.03 ± 0.01 <sup>b</sup>
Thiophene	1501	2033	1.90 ± 0.76 <sup>ab</sup>	1.36 ± 0.42 <sup>b</sup>	1.97 ± 1.07 <sup>ab</sup>	2.92 ± 0.90 <sup>a</sup>	1.19 ± 0.09 <sup>b</sup>	0.95 ± 0.25 <sup>b</sup>
Geranyl 2-methylbutyrate	1562	1880	3.68 ± 1.00 <sup>b</sup>	1.77 ± 0.77 <sup>c</sup>	3.91 ± 0.68 <sup>b</sup>	4.41 ± 0.11 <sup>b</sup>	8.23 ± 0.26 <sup>a</sup>	4.60 ± 0.57 <sup>b</sup>
Spathulenol	1576	2144	1.06 ± 0.68 <sup>a</sup>	0.24 ± 0.17 <sup>b</sup>	0.13 ± 0.00 <sup>b</sup>	0.21 ± 0.16 <sup>b</sup>	0.13 ± 0.02 <sup>b</sup>	0.04 ± 0.04 <sup>b</sup>
Caryophyllene oxide	1581	2008	0.78 ± 0.19 <sup>b</sup>	0.78 ± 0.19 <sup>b</sup>	0.41 ± 0.30 <sup>bc</sup>	0.12 ± 0.03 <sup>c</sup>	0.54 ± 0.04 <sup>bc</sup>	1.86 ± 0.64 <sup>a</sup>
Nonadecane	1900	1900	2.70 ± 0.47 <sup>c</sup>	0.20 ± 0.12 <sup>d</sup>	3.64 ± 0.86 <sup>c</sup>	6.12 ± 0.72 <sup>a</sup>	5.18 ± 0.28 <sup>ab</sup>	4.94 ± 0.89 <sup>b</sup>
<i>Chemical classes</i>								
Monoterpenes			79.62 ± 0.19 <sup>c</sup>	94.95 ± 0.81 <sup>a</sup>	69.94 ± 1.28 <sup>d</sup>	69.61 ± 1.76 <sup>d</sup>	84.85 ± 0.48 <sup>b</sup>	71.65 ± 0.82 <sup>d</sup>
Sesquiterpenes			13.83 ± 1.71 <sup>a</sup>	2.56 ± 0.28 <sup>b</sup>	3.53 ± 1.38 <sup>b</sup>	1.57 ± 0.49 <sup>b</sup>	2.22 ± 0.45 <sup>b</sup>	3.06 ± 0.08 <sup>b</sup>
Aliphatic hydrocarbons			2.842 ± 0.64 <sup>c</sup>	0.21 ± 0.14 <sup>d</sup>	3.65 ± 0.88 <sup>c</sup>	6.12 ± 0.63 <sup>a</sup>	5.18 ± 0.25 <sup>ab</sup>	4.93 ± 0.78 <sup>b</sup>
Alcohols			1.26 ± 0.34 <sup>a</sup>	0.06 ± 0.01 <sup>b</sup>	0.08 ± 0.04 <sup>b</sup>	0.01 ± 0.00 <sup>b</sup>	0.10 ± 0.04 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>
Others			1.89 ± 0.61 <sup>ab</sup>	1.36 ± 0.41 <sup>b</sup>	1.97 ± 0.31 <sup>ab</sup>	2.92 ± 0.12 <sup>a</sup>	1.19 ± 0.96 <sup>b</sup>	1.08 ± 0.12 <sup>b</sup>
Total			99.8 ± 0.41 <sup>a</sup>	99.3 ± 0.44 <sup>a</sup>	79.3 ± 0.42 <sup>d</sup>	80.6 ± 0.83 <sup>c</sup>	93.6 ± 0.78 <sup>b</sup>	81.4 ± 0.98 <sup>c</sup>

<sup>a</sup> Components are listed in order of elution in apolar column (HP-5); RI<sup>a</sup>, RI<sup>b</sup>: retention indices calculated using respectively an apolar column (HP-5) and polar column (HP-Innowax); volatile compound proportions were calculated from the chromatograms obtained on the HP-Innowax column; values followed by the same small letter did not share significant differences at 5% (Duncan test).

differences. It may be suggested that these differences could be due to the effect of harvesting time as well as the environmental conditions. The highest TFA content (3.10% DMW) was reached in fully ripe fruit (180 DAF). Predominant fatty acid proportions varied significantly during the fruit ripening in which linoleic acid percentages had an antagonist evolution than of palmitic, stearic and oleic acids. In contrast to TFA content, essential oil yield obtained its maximum (0.11% DMW) at earlier stage of fruit ripening (60 DAF). The proportions of the main essential oil compounds (1,8-cineole, geranyl acetate, linalool and α-pinene) varied significantly

and represented approximately three quarters of the total essential oil compounds in all the stages. So, lipid and essential oil of *M. communis* var. *italica* fruit were characterized by the presence of many bioactive compounds which could have numerous applications in food, pharmaceutical, cosmetic and perfume industries.

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