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Dynamic accumulation of 4-desmethylsterols and phytostanols during ripening of Tunisian *Meski* olives (*Olea europea* L.)

Faouzi Sakouhi [a,*], Christelle Absalon [b], Saoussem Harrabi [a], Christiane Vitry [b], Khaled Sebei [a], Sadok Boukhchina [a], Eric Fouquet [b], Habib Kallel [a]

[a] *Laboratoire de Biochimie des Lipides, Département de Biologie, Faculté des Sciences de Tunis, 2092 El Manar II, Tunisia*
[b] *Centre d'Etude Structurale et d'Analyse des Molécules Organiques, Institut des Sciences Moléculaires, Université de Bordeaux1, 351, Cours de la Libération, 33405 Bordeaux, France*

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ABSTRACT

Eleven 4-desmethylsterols and two phytostanols were identified by GC–MS during the ripening of *Meski* olive. The maximum levels of 4-desmethylsterols (1300 mg/100 g oil) and phytostanols (7.5 mg/100 g oil) were reached at the 26th week after the flowering date (WAFD) of fruit. β-Sitosterol (72–86% of total 4-desmethylsterols) was the major 4-desmethylsterols during the maturity of fruit, while sitostanol was the predominant phytostanols (75–85% of total phytostanols). Δ5-Avenasterol (2–18%) and campesterol (1.6–4%) were the second and the third 4-desmethylsterol levels detected, respectively, during the ripening of *Meski* olive. The levels of campestanol varied from 15% to 25% of phytostanols. The rate of accumulation of those compounds occurred before 30th WAFD. Some of these compounds (4-desmethylsterols and phytostanols) showed nearly the same profile during the ripening of *Meski* olive which could be linked to the relation between these compounds during their biosynthetic pathway.

1. Introduction

Phytosterols and phytostanols are a group of minor bio-molecules present in vegetable oils and are very important in the human diet. These compounds have been widely studied for their health properties. Clinical studies confirmed that phytosterols have hypocholesterolemy, anti-inflammatory and anti-carcinogenic effects (Awad, Downie, Fink, & Kim, 2000; Beveridg et al., 2002; Conchillo et al., 2005; Hicks & Moreau, 2001). Physiologically, phytosterols have essential functions on cellular membrane; they participate with phospholipids to regulate membrane fluidity and permeability (Darnet & Rachier, 2004). Phytosterols, which represent the major portion of unsaponifiable matter of vegetable oils, are divided into three main classes: 4-desmethylsterols (sterols), 4-monomethylsterols and the 4,4′-dimethylsterols (Benveniste, 2002). Methylsterols are generally considered to be intermediates in the biosynthetic pathway of 4-desmethylsterols. Phytosterols are present in three forms: free sterols (the major form), steryl esters and steryl glucosides sterols (Benveniste, 2004). Generally, in the edible oil sterols mainly occur as free sterols or steryl esters of fatty acids (Verleyen et al., 2002). According to the position of double bond at C-5 or C-7 in A ring, 4-desmethylsterols are present in two groups which are Δ5-desmethylsterols and Δ7-desmethylsterols. The most proportion of 4-desmethylsterol is Δ5-desmethylsterol which contained mainly β-sitosterol, Δ5-avenasterol and campesterol (Pardo, Cuesta, & Alvarruiz, 2007).

Phytostanols, a completed saturated phytosterols, are less abundant in nature than 4-desmethylsterols (Nair, Kanfer, & Hoogramartens 2006). These compounds are mainly sitostanol and campestanol which are the hydrogenated form of β-sitosterol and campesterol, respectively (Venkatramesh, Karunanandaa Cunter, Gunter, Boddupalli, & Kishore, 2002). Despite their minority, phytostanols are more effective than phytosterols in lowering cholesterol levels in mammals (Normén et al., 2006). This better effectiveness is related to the fact that phytostanols, which have practically no absorption, remain for a longer period in the intestinal lumen where they interfere continually and in a more efficient way with the absorption of cholesterol (Hicks & Moreau, 2001; Nguyen, 1999). Diminishing of plasmatic cholesterol levels is vital for the prevention of cardiovascular diseases, which are the main cause of death in Europe (Santos et al., 2007). Physiologically, campestanol is to a far extent linked to biosynthesis of brassinosteroids compounds (Jang, Han, & Kim, 2000) which are steroidal plant hormones that regulate plant physiologic phenomenon such as stem elongation, photomorphogenesis, senescence, sex and vascular plant organ development (Clouse & Sasse, 1998; Jang et al., 2000; Kim, Kim, & Kim, 2005). Due to their role in lowering plasmatic cholesterol levels, recently food products are enriched in soluble phytosterols and phytostanols. Thus, the objective of industry is to identify the plant matrices rich in those compounds. In addition to their healthy proprieties, phytosterols (methylsterols and

* Corresponding author. Tel.: +216 99 550297; fax: +216 71 885480.
E-mail address: Faouzi.Sakouhi@fst.rnu.tn (F. Sakouhi).

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desmethylsterols) and phytostanols have been used to detect admixture of vegetable oils. It is know that because of similarities in fatty acids and triacylglycerols composition, hazelnut oil, which is less expensive, is used to adulterate olive oil (Azadmard-Damirchi & Dutta, 2006).

Although sterols composition has been studied at ripeness stage of olives (Azadmard-Damirchi & Dutta, 2006; Casas, Bueno, García, & Cano, 2004; Cuncha, Fernandes, & Oliverira, 2006; Salvador, Aranda, & Fregapane, 2001), very little information is availale on 4-desmethylsterols and phytostanols accumulation during the maturity of olive. The aim of the present study is therefore, first to carry out a qualitative and quantitative characterisation of 4-desmethylsterols and phytostanols during the ripening of *Meski* olive (Tunisia), and then to determine the date in which the *Meski* olive accumulated to a maximum those high value-added compounds.

2. Materials and methods

2.1. Reagents and standard

Acetone, chloroform, diethyl ether and petroleum ether were purchased from Fisher Scientific SA. (Loughborough, Spain). Ethanol was from Scientific Limited (Northampton, UK). Pure molecules of sterols (4-desmethylsterols, stanols and 5-α-cholestanol (I.S)) and *N,O*-bistrimethylsilyltrifluoroacetamide (BSTFA) were acquired from Sigma (St. Louis, MO, USA). TLC silica plates (silica gel 60 G F254, 20 × 20 cm, 0.25 thickness), Potassium hydroxide pellets, pyridine and anhydrous sodium sulphate were from Merck (Darmstadt, Germany). Pyridine was purchased from Fluka (Neu-Ulm, Germany).

2.2. Sample

The variety of *Meski* olive (*Olea europea* L.) was grown on the Agronomy farm of the O.T.D.G (Office Terres Domaniales Ghzala) in the north of Tunisia. Fruits were hand-harvested from the same tree at intervals of one week from the formation of the olive (21st week after the flowering date (WAF) of *Meski* olive) until its complete maturity (38th WAF). Only healthy fruits, without any kind of infection or physical damage, were selected.

2.3. Determination of oil content

Oil content was determined by extracting dry material of olives (olives were dried at 20 °C in dry air sterilizers) with petroleum ether using a Soxhlet apparatus. This extraction takes 4 h at 42 °C and repeated three times for each sample. The extract was dried in a rotary evaporator at 32 °C. Oil was weighed and stored at −10 °C. The oil content was determined as different in weight of dried olive sample before and after the extraction (AOCS, 1989).

2.4. Saponification

Unsaponifiable fraction of lipids was determined by saponifying 5 g of oil mixed with both 200 μl 5-α-cholestanol solution (internal standard; 0.2% (w/v)) and an ethanolic KOH 12% (w/v) solution; the mixture was heated at 60 °C for 1.30 h. After cooling, 50 ml of H_2O was added. The unsaponifiable matter was extracted four times with 50 ml of petroleum ether. The combined ether extract was washed with 50 ml of ethanol–water (1:1). The extracted ether was dried over anhydrous Na_2SO_4 and evaporated to dryness using N_2. The dry was dissolved in chloroform for TLC analysis.

2.5. Thin layer chromatography

The unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates (silica gel 60G F254) using one-dimensional TLC with hexane–diethyl ether (6:4, v/v) as the developing solvent. The unsaponifiable fraction diluted in chloroform was applied on the silica gel plates. So as to correctly identify the sterols band, a reference sample of purified sterol, 5-α-cholestanol, was applied on the left and the right sides of the TLC plates. After development, the plate was sprayed with 2,7-dichlorofluorescein and viewed under UV light. The band corresponding to sterols was scraped, extracted three times with chloroform–diethyl ether (1:1, v/v), filtered to remove the residual silica, dried in a rotary evaporator and stored at −10 °C.

2.6. Silylation of sterol fraction

An amount of 2 mg of sterol residue was mixed with 125 μl of BSTFA (with 1% TMCS), 125 μl of pyridine and 450 μl of acetone, the mixture vortexed and heated at 70 °C for 20 min. After silylation reaction, 1 ml, 5 ml of chloroform was added to the mixture and 1 μl of the solution was directly injected to gas chromatograph.

2.7. Gas chromatography–mass spectrometry detection

GC–MS analyses were performed using a DB-5MS fused silica capillary column (30 m × 0.25 mm I.D., 0.25-m film thickness; J&W Scientific, Folsom, CA, USA) in an Varian SAR 3400C× gas chromatograph coupled directly to the mass detector (MS Varian SATURN). Helium was used as carrier gas, with a constant flow rate of 1 ml min^{-1}. The injector and detector temperatures were 250 °C. The oven temperature was programmed from 150 to 300 °C at 4 °C min^{-1}. The final temperature was held constant for 10 min and the transfer line temperature was 250 °C. Electron impact mass spectra were measured at acceleration energy of 70 eV. Manual injection of 1 μl of the solution of sterol was performed in the split mode at a 60:1 split ratio. The phytosterols and phytostanols compounds were identified by comparing their relative retention times and mass spectra with those of the authentic standard. The peaks were also confirmed with NIST Mass Spectral Library. The retention time and mass spectrometric data of 4-desmetylsterols and phytostanols identified by GC–MS were shown in Table 1.

2.8. Gas chromatography–flame ionisation detection (GC–FID)

Due to the higher sensibility of GC–FID detector compared to GC–MS one (Azadmard-Damirchi & Dutta, 2006; Cuncha et al., 2006), the quantification of 4-desmetylsterols and phytostanols was performed using GC–FID apparatus. The GC system used was a HP 4890A gas chromatograph (Hewlett–Packard, Avondale, PA) equipped with a split–splitless injector, a FID and a DB-5MS (30 m × 0.25mm I.D., 0.25-m film thickness; J &W Scientific, Folsom, CA, USA) column. The initial column temperature was 150 °C and programmed to increase at a rate of 4 °C min^{-1} to 300 °C and then held for 25 min. The injector and detector temperatures were 280 and 310 °C, respectively, and nitrogen was used as carrier gas at an inlet pressure of 12 psi, giving a column flow of 1 ml min^{-1}. Quantification of sterols was achieved by addition of 5-α-cholestanol as an internal standard. The level of each sterol was calculated as milligrams per 100 g of oil using the following formula; amount = $100 - (PA_s)(m_{is})/(PA_{is})(m)$, where PA_s = sterol peak area, PA_{is} = internal standard area, m_{is} = weight (mg) of the internal standard and m = weight (g) of oil taken for analysis. For each sample three determinations have been done.

Table 1
Retention time and mass spectrometric data for trimethylsilyl derivatives of sterols identified by GC–MS

Peak number	Retention time (mn)	Compounds	Molecular weight	Mains fragmentation ions (*M*/*Z*)
1	43.35	Cholesterol	458	369, 264, 255, 129, 83, 73
2	43.55	α-Cholestanol (I.S)	460	369, 305, 257, 215, 83, 75
3	44.78	24-Methylene cholesterol	470	255, 211, 173, 163, 129, 73
4	45.05	Campesterol	472	399, 382, 250, 203, 129, 73
5	45.27	Campestanol	474	394, 255, 147, 129, 83, 73
6	45.44	Stigmasterol	484	394, 255, 175, 129, 83, 73
7	45.63	Δ7-Campesterol	472	255, 187, 163, 147, 83, 73
8	46.14	Clerosterol	484	255, 173, 147, 129, 105, 73
9	46.48	β-Sitosterol	486	396, 381, 357, 213, 129, 73
10	46.63	Sitostanol	488	398, 383, 290, 230, 215, 75
11	46.68	Δ5-Avenasterol	484	395, 342, 296, 255, 129, 73
12	47.38	5.24-Stigmastadienol	484	396, 255, 218, 173, 129, 73
13	48.44	Δ7-Stigmastenol	486	377, 255, 215, 190, 83, 75
14	48.97	Δ7-Avenasterol	484	357, 310, 252, 201, 171, 55

3. Results and discussion

3.1. Evolution of total lipids content

During the ripening of *Meski* olives, the total lipids accumulation (expressed in g/100 g of dry weight of olive) increased to attain a maximum level of fat (83.73 g/100 g d.w) at the 34th WAF (Fig. 1a), then it decreased at complete maturity of fruit. From the 21st to 27th WAFD, the lipid biosynthesis was much slower. This result can be explained by the fact that in this period (21st to 26th WAF), the lipids synthesized by immature olive were used for the development of new fruit tissues. Between 27th and 34th WAFD, the lipid accumulation was very important and reached its maximum level. This increase in total lipids could be explained by the fact that, in this period the fruit was practically formed which favours the formation of reserve lipids. During the remaining WAFD, the lipids biosynthesis was stopped which explains the regression of the total lipid at complete maturity of fruit. The study of the lipids accumulation during the ripening of olives is important to decide the best moment for the harvest of olives. Indeed, in *Meski* cultivar the highest amount of lipid was observed at 34th WAFD which corresponds to the best time of *Meski* olives harvest and guarantees a significant production of oil.

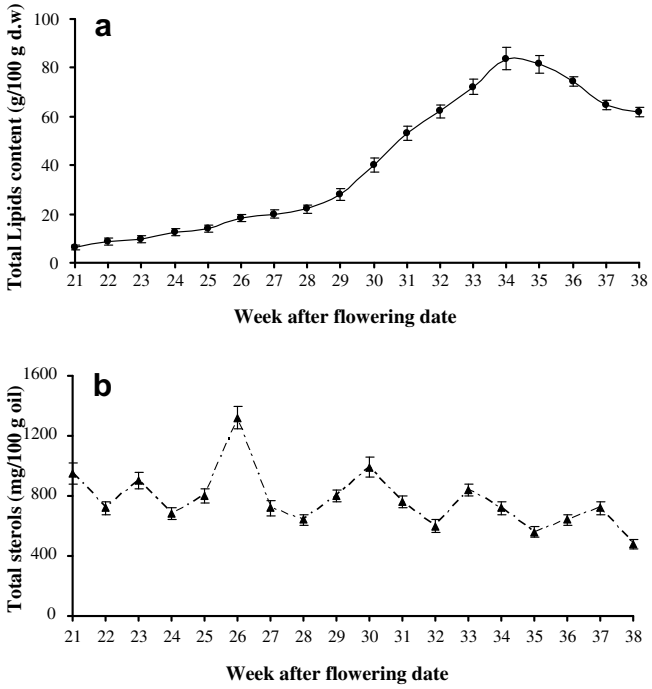

Fig. 1. Changes in total lipids and total sterols contents during ripening of *Meski* olive: (a) total lipids amount (expressed in g/100 g of fruit dry weight), (b) total sterols content (expressed in mg/100 g of oil).

3.2. Evolution of total sterols content

During the first period of *Meski* olive ripening (from 21st to 25th WAFD), the amount of total sterols (expressed in mg/100 g of oil) was about 800 mg/100 g oil. Then it increased to a maximum (1320 mg/100 g oil) at 26th WAFD. After the 30th WAFD, the total sterols content decreased gradually to reach its lowest level (480 mg/100 g oil) at complete maturity of the fruit (Fig. 1b). The decrease in total sterol fraction could probably be explained by the fact that during the final weeks of olive ripening, the enzymatic activity of sterols biosynthesis was stopped which generated the decrease of sterols accumulation. Indeed, during this period sterols were not synthesized but we assisted to the conversion of existed phytosterols to other sterols forms (stanols and steryl esters) implying some dehydratation, hydrogenation and dehydrogenation reactions (Venkatramesh et al., 2002). The accumulation of total sterols during the maturity of *Meski* olives presented oscillations alternated with maximums and minimums of sterols levels. These intermediate decreases in sterol levels may be explained by the fact that at some precise moments of olive ripening the plant led to the conversion of their existed sterols to the steroidal hormones and vitamins that regulate the growth and the development of immature tissues (Jang et al., 2000). The total sterols profile showed that the *Meski* olive cultivar accumulated the highest level of sterols (4-desmethylsterols and phytostanols) at the 26th WAFD. Thus, at this date the *Meski* olive may be a potential source of these health-enhancing compounds for functional foods and nutraceutical applications. The total sterols profile did not give an idea about the level of each 4-desmethylsterol and phytostanol compound. So, a quantitative characterisation of each sterol compound may be done.

3.3. Dynamic accumulation of phytostanols

Phytostanols were found to be minor components of the unsaponifiable fraction (Harrabi et al., 2007). The average dietary consumption of phytostanols is approximately 25 mg/day (Nair et al., 2006). The change in the phytostanol (sitostanol and campestanol) level during olive ripening has never previously been studied. Fig. 2 shows that the greatest change in the amounts

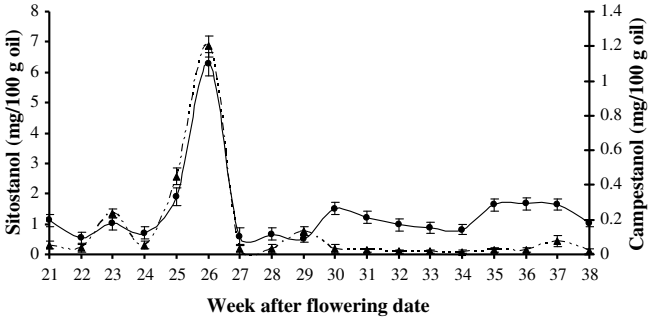

Fig. 2. Variation of phytostanols concentration (expressed in mg/100 g of oil) during ripening of *Meski* olives: sitostanol (●) and campestanol (▲).

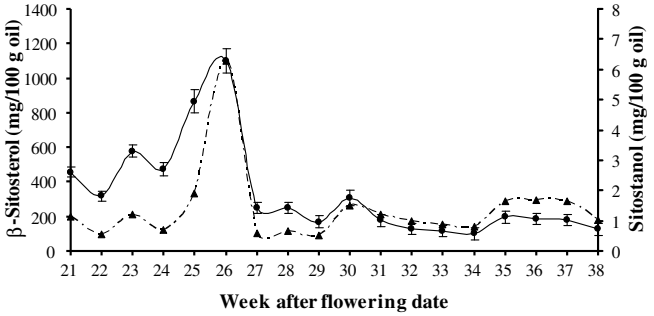

Fig. 3. Change in β-sitosterol (♦) and sitostanol (▲) contents (expressed in mg/100 g of oil) during ripening of *Meski* olives.

of these phytostanols (expressed in mg/100 g of oil) occurred during the first weeks of *Meski* olive development. The major increase in the total amount of phytostanols was observed from 24th (0.8 mg/100 g oil) to 26th WAFD (7.48 mg/100 g oil). Then, a dramatic decrease was noted from the 27th WAFD. This decrease in total phytostanols may be due to the conversion of these compounds to steroidal hormones such as brassinosteroids which regulate the growth of immature tissues. Fujioka & Acadia, 2003 reported that campestanol is the precursor of C_{28} brassinosteroids, whereas stigmastanol (or sitostanol) is the precursor of C_{29} brassinosteroids. The patterns of phytostanol showed that sitostanol (6.2 mg/100 g oil) accumulated more than campestanol (1.28 mg/100 g oil at 26th WAFD) in olive tissues. This result can be explained by the fact that β-sitosterol, precursor of sitostanol (Venkatramesh et al. 2002), which was found at higher levels (93% of total phytosterols) during ripening than campesterol precursor of campestanol. At complete maturity of *Meski* olive the amount of sitostanol and campestanol was in agreement with the results from Spanish (Casas et al., 2004), Italian (Azadmard-Damirchi & Dutta, 2006) and Portuguese (Cuncha et al., 2006) olives cultivars.

3.4. Dynamic accumulation of 4-desmethylsterols

4-Desmethylsterols are the major components of phytosterol matter in most vegetable oils (Azadmard-Damirchi & Dutta, 2006). The average dietary consumption of phytosterols is approximately 250 mg/day (Nair et al., 2006). Little information is available about the accumulation of 4-desmethylsterols. At complete maturity of *Meski* olive, β-sitosterol (125.23 mg/100 g oil), Δ5-avenasterol (21.51 mg/100 g oil), campesterol (3.24 mg/100 g oil), cholesterol (1.69 mg/100 g oil) and stigmasterol (1 mg/100 g oil) were the major 4-desmethylsterols accounting for over 92% of total sterols. 5,24-Stigmastadienol (0.28 mg/100 g oil), Δ7-avenasterol (0.27 mg/100 g oil) and Δ7-campesterol (0.03 mg/100 g oil) amounted to less than 8% of total sterols content. The qualitative characterisation of ours samples was in agreement with those listed in the literature (Azadmard-Damirchi & Dutta, 2006; Casas et al., 2004; Cuncha et al., 2006). However, the quantitative characterisation was different which can be explain by the fact that the level of 4-desmethylsterols is affected by geographical growing area and difference in olives varieties (Casas et al., 2004; Koutsaftakis, Kotsifaki, Stefanoudaki, & Cert, 2000; Stefanoudaki, Chartzoulakis, Koutsaftakis, & Kotsifaki, 2001). The previous works have been concerned with the 4-desmethylsterols characterisation only at complete maturity of olives. Fig. 3 shows that the amount of β-sitosterol increased from the 22nd (317 mg/100 g oil) to the 26th (1100 mg/100 g oil) WAFD, but a dramatic decrease of this compound was observed from 27th WAFD. Thus, at 26th WAFD *Meski* olive matrices contained the highest level of β-sitosterol.

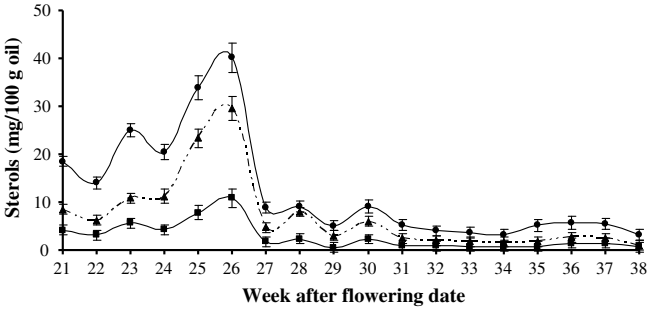

Fig. 4. Variation in campesterol (●), stigmasterol (▲) and clerosterol (■) amounts (expressed in mg/100 g of oil) during the maturity of *Meski* olives.

Among the various phytosterols, β-sitosterol has been the most intensively investigated with respect to its beneficial and physiological effects on health (Yang, Karlsson, Oksman, & Kallio, 2001). The profiles of β-sitosterol and sitostanol accumulation were similar during the repining of *Meski* olive. This similarity could be explained by the fact that during the biosynthetic pathway of phytosterols, β-sitosterol was the precursor of sitostanol (Venkatramesh et al., 2002).

Yoshida and Niki (2003) reported that campesterol, stigmasterol and clerosterol exerted antioxidant effects on the oxidation of methyl linoleate oil solution. Fig. 4 shows that the change in profiles of campesterol, stigmasterol and clerosterol was very similar during ripening of *Meski* olive. This result could probably be explained by the fact that these compounds had the same biosynthetic precursor. The highest levels of these compounds were detected at 26th WAFD and were 40.12; 29.59; and 10.79 mg/100 g oil, respectively, for campesterol, stigmasterol and clerosterol. From later results we deduced that during the fruit ripening process, the olive favours the biosynthesis of campesterol more than stigmasterol and clerosterol. The amounts of these 4-desmethylsterols at the complete maturity of *Meski* olives were in agreement with those listed in the literature (Azadmard-Damirchi & Dutta, 2006; Casas et al., 2004). Δ5,24-Stigmastadienol and cholesterol were expressed at lower levels than the other Δ5-sterols during the maturity of *Meski* olive. The highest amount of Δ5,24-stigmastadienol (15.46 mg/100 g oil) was reached at the 26th WAFD. Cholesterol which was found in higher levels in mammal tissues was presented in low concentrations in vegetable tissues. Indeed, in vegetable cell, cycloartenol which is the precursor of all sterols, was more converted to 24-methylene cycloartenol than to 24-dihydrocycloartenol which is the precursor of cholesterol.

The Δ7-sterols compounds were mainly represented by Δ7-avenasterol (5.73 mg/100 g oil), Δ7-stigmastenol (5.19 mg/100 g

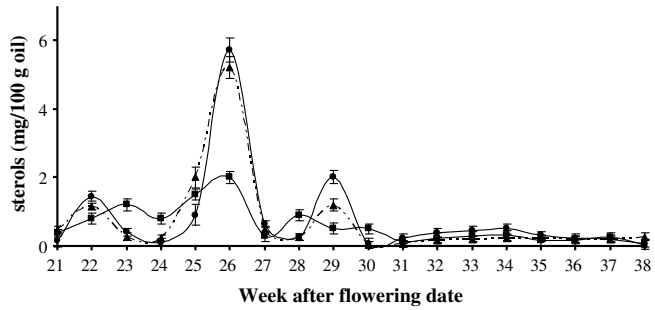

Fig. 5. Evolution of Δ7-stigmastenol (●) Δ7-avenesterol (▲) and Δ7-campesterol (■) contents (expressed in mg/100 g of oil) during maturation of *Meski* olives.

oil) and Δ7-campesterol (2.01 mg/100 g oil at 26th WAFD). During the ripening of *Meski* olive, Δ7-avenasterol and Δ7-stigmastenol had similar patterns which differed to that of Δ7-campesterol. Fig. 5 shows that Δ7-avenasterol and Δ7-stigmastenol were practically accumulated in equal proportions during the olive ripening process. The amounts of Δ7-sterols at complete maturity of *Meski* olive were in agreement with those reported previously for olive oils (Pardo et al., 2007; Rivera del Álamo, Fregapane, Aranda, Gómez-Alonso, & Salvador, 2004). The Δ5-sterols were mainly accumulated in olive tissues with respect to Δ7-sterols at complete maturity of fruit (Casas et al., 2004). During the ripening of *Meski* olive, a great change of the Δ5-sterols to Δ7-sterols ratio (Δ5-S/Δ7-S) was observed. The highest Δ5-S/Δ7-S ratio level (590) was detected at the 30th WAFD. Thus, during the ripening process, olive moderated the biosynthesis of Δ5-sterols and Δ7-sterols according to the olives need for these sterols to regulate the growth and the development of new tissues. The Δ5-sterols were mainly accumulated in the plasma membrane where they are believed to regulate membrane fluidity (Jiang & Wang, 2005). The qualitative and quantitative characterisation of 4-desmethylsterols (Δ5-sterols and Δ7-sterols) of *Meski* olive at complete maturity was different from the other vegetable oils. In fact, Harrabi et al. (2007) reported that in maize oil the percentage of 4-desmethylsterols was 65–80% of total sterols. This corresponding figure in hazelnut oil was 88–99% (Azadmard-Damirchi & Dutta, 2006). However, in our samples the percentages of the 4-desmethylsterols moved from 51% to 61%. Clerosterol was not detected in both maize and hazelnut oils. Thus, these qualitative and quantitative differences observed in the components of the 4-desmethylsterol fraction of vegetable oils suggested that this fraction may be used as a marker for the detection of oil adulteration.

4. Conclusion

In summary, the 4-desmethylsterols and phytostanols were present at all stages of olive maturity. Δ5-Sterols were present in higher amounts than Δ7-sterols during the maturity of olive. During the ripening process, the 4-desmethylsterols and phytostanols compounds were highly accumulated at the 26th WAFD of *Meski* olive. Thus, this date is the best moment to exploit the maximum level of these high value-added compounds in *Meski* olive. The qualitative and quantitative characterisation of 4-desmethylsterols and phytostanols seem to be useful for the detection of vegetable oils adulteration and the quality control of food products.

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References

AOCS (1989). *Official methods and recommended practices of the American oil chemist's society*. Champaign: American Oil Chemist's Society. Method Ce-66.

Awad, A. B., Downie, A., Fink, C. S., & Kim, U. (2000). Dietary phytosterol inhibits the growth and metastasis of MDA-MB-231 human breast cancer cells grown in SCID mice. *Anticancer Research, 20*, 821.

Azadmard-Damirchi, S., & Dutta, P. C. (2006). Novel solid-phase extraction method to separate 4-desmethyl-, 4-monomethyl-, and 4,4′-dimethylsterols in vegetable oils. *Journal of Chromatography A, 1108*, 183–187.

Benveniste, P. (2002). The Arabidopsis book. ⟨http://www;aspb.org/publications⟩.

Benveniste, P. (2004). Biosynthesis and accumulation of sterols. *Annual Review of Plant Biology, 55*, 429–457.

Beveridge, T. H. J., Li, T. S. C., & Drover, J. C. G. (2002). Phytosterol content in American ginseng seed oil. *Journal of Agriculture and food chemistry, 50*(4), 744–750.

Casas, J. S., Bueno, E. O., García, A. M. M., & Cano, M. M. (2004). Sterol and erythrodiol + uvaol content of virgin olive oils from cultivars of Extremadura (Spain). *Food Chemistry, 87*, 225–230.

Cuncha, S. S., Fernandes, J. O., & Oliverira, M. B. P. P. (2006). Quantification of free and esterified sterols in Portuguese olive oils by solid-phase extraction and gas chromatography–mass spectrometry. *Journal of Chromatography A, 1128*, 220–227.

Clouse, S. D., & Sasse, J. M. (1998). Brassinosteroids: Essentiel regulators of plant growth and development. *Annual Review of Plant Biology, 49*, 427–451.

Conchillo, A., Cercaci, L., Ansorena, D., Rodriguez-Estrada, M. T., Lercker, G., & Astiasanan, I. (2005). Levels of phytosterol oxides in enriched and nonenriched spreads: Application of a thin-layer chromatography–gas chromatography methodology. *Journal of Agriculture and Food Chemistry, 53*, 7844–7850.

Darnet, S., & Rachier, A. (2004). Plants sterol biosynthesis: Identification of two distinct families of sterol 4α-methyl oxidases. *Biochemical Journal, 378*, 889–898.

Fujioka, S., & Acadia, T. (2003). Biosynthesis and metabolism of brassinosteroids. *Annual Review of Plant Biology, 54*, 137–164.

Hicks, K. B., & Moreau, R. A. (2001). Phytosterols and phytostanols: Functional food cholesterol busters. *Food Technology, 92*(12), 478–480.

Harrabi, S., Sakouhi, F., St-Amand, A., Boukhchina, S., Kallel, H., & Mayer, P. M. (2007). Accumulation of phytosterols, triterpene alcohols and phytostanols in developing Zea mays L. Kernels. *Journal of Plant Sciences, 2*(3), 260–272.

Kim, Y. S., Kim, T. W., & Kim, S. K. (2005). Brassinosteroids are inherently biosynthesized in the primary roots of maize, Zea mays L. *Phytochemistry, 66*, 1000–1006.

Koutsaftakis, A., Kotsifaki, F., Stefanoudaki, E., & Cert, A. (2000). Estudio sobre las variaciones de determinados parámetros químicos y de los components menores de los aceites de oliva virgin obtenidos de aceitunas recolectadas en distintas fases de maduración. *Olivae, 80*, 22–27.

Jang, M. S., Han, K. S., & Kim, S. K. (2000). Identification of brassinosteroids and their biosynthetic precursors from seeds of pumpkin. *Bulletin Korean Chemists' Society, 21*, 161–164.

Jiang, Y., & Wang, T. (2005). Phytosterols in cereal by-products. *Journal of American Oil Chemist's Society, 82*, 439–444.

Nair, V. D. P., Kanfer, I., & Hoogramartens, J. (2006). Determination of stigmasterol and stigmastanol in oral dosage forms using high performance liquid chromatography with evaporative light scattering detection. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 731–737.

Nguyen, T. U. T. (1999). The cholesterol-lowering action of plant stanol esters. *Journal of nutrition, 129*, 2109–2112.

Normén, L., Ellegârd, L., Janssen, H. G., Streenbergen, H., Trautwein, E., & Andersson, H. (2006). Phytosterol and phytostanols esters are effectively hydrolised in the gut and do not affect fat digestion in ileostomy subjects. *European Journal of Nutrition, 45*, 165–170.

Pardo, J. E., Cuesta, M. A., & Alvarruiz, A. (2007). Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, Spain). *Food Chemistry, 100*, 977–984.

Rivera del Álamo, R. M., Fregapane, G., Aranda, F., Gómez-Alonso, S., & Salvador, M. D. (2004). Sterol and alcohol composition of Cornicabra virgin olive oil: the campesterol content exceeds the upper limit of 4% established by EU regulations. *Food Chemistry, 84*, 533–537.

Salvador, M. D., Aranda, F., & Fregapane, G. (2001). Influence of fruit ripening on Cornicabra virgin olive oil quality a study of four successive crop seasons. *Food Chemistry, 73*, 45–53.

Santos, R., Limas, E., Sousa, M., da Conceição Castilho, M., Ramos, F., & Noronha da Silveira, M. I. (2007). Optimization of analytical procedures for GC–MS determination of phytosterols and phytostanols in enriched milk and yoghurt. *Food Chemistry, 102*, 113–117.

Stefanoudaki, E., Chartzoulakis, K., Koutsaftakis, A., & Kotsifaki, F. (2001). Effect of drought on qualitative characteristics of olive oil of cv Koroneiki. *Grasas y Aceites, 52*, 202–206.

Venkatramesh, M., Karunanandaa Cunter, A., Gunter, B. S., Boddupalli, S., & Kishore, G. M. (2002). *Phytochemistry, 62*, 39–46.

Verleyen, T., Forcades, M., Verhe, R., Dewettink, K., Huyherbaert, A., & De Greyt, W. (2002). Analysis of free and esterified sterols in vegetable oils. *Journal of the American Oil Chemists' Society, 56*, 531–535.

Yang, B., Karlsson, R. M., Oksman, P. H., & Kallio, H. P. (2001). Phytosterols in sea buckthorn (*Hippophaë rhamnoides* L.) berries: Identification and effects of different origins and harvesting times. *Journal of Agriculture and Food Chemistry, 49*, 5620–5629.

Yoshida, Y., & Niki, E. (2003). Antioxidant effects of phytosterol and its components. *Journal of Nutritional Science and Vitaminology, 49*, 277–280.