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Research Article

Evolution of Fixed Oil, Phytosterols and Polyphenols Contents during Maturation of Tunisian Wild Laurel Fruits (*Laurus nobilis L.*)

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ABSTRACT

The laurel fruits showed an increase in oil content reaching a maximum value at 140 days after flowering (23, 76%) but the later maturation stages showed a decrease in lipid accumulation. In laurus nobulis, the main fatty acid was oleic acid (C18:1) which attained a maximum value at the 140th DAF (47, 22%), before slowly decreasing at 150 DAF (46, 67%). Seven phytosterols were identified: campesterol, stigmasterol, β-sitosterol, Δ5-avenasterol, chlerosterol, Δ-5 Stigmastenol, Δ5-24Stigmastadienol. The β-sitosterol represented the main component of the phytosterols (84, 02%). During the first period of laurel fruit ripening (from 90 to 110 DAF) the amount of all sterols increased to a maximum. After the 110th DAF, the total sterols content decreased gradually to reach its lowest level at complete maturity of the fruit. The amount of peroxide index and all natural antioxydant contents increased to a maximum during the first period of fruit ripening.

Key words: Laurus nobulis; Fruit maturation; Oil; Fatty acids; Sterols; Polyphenols

INTRODUCTION

The Lauraceae has 32 genera and about 2000-2500 species. A member of the family, *Laurus nobilis L.*, mythologically named Apollo's Laurel, is a plant native to the Southern Mediterranean region and is widely cultivated mainly in Europe and the USA as an ornamental and medicinal plant [1,2]. *Laurus nobilis L.* commonly known as bay belonging to the family Lauraceae is one of the most useful essential oil and is an industrial plant used in foods, drugs and cosmetics [3].The leaves of *L. nobilis* L. are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence [4,5]. Anticonvulsive and antiepileptic activities of the leaf extract have been confirmed. The antioxidative activity of methanolic extracts of leaves, bark and fruits were tested [6]. Although several isolation and biological activity studies have been carried out on the leaves of *L. nobilis*, there has been very little work on its fruits [7]. Laurel berries are one-seeded ovoid fruits with a dark purple, thin, brittle, wrinkled pericarp, which when broken discloses the seed kernel, the seed-coats adhering to the inner surface of the pericarp. The fruits contain both fixed and volatile oils, which are mainly used in soap making, reported that the fruits of bay laurel plants contain 24-30% of fixed oil [8-10]. The poor quality fixed oil obtained from fruits by traditional method can cause loss of market value. But in Europe, the fixed oil of bay laurel is obtained by pressing technique from dried and powdered fruits. In spite of this easy method for getting oil, it remains 5 to 10% oil in oil Cake [11].

The oil extracted from berries contain fatty acids (lauric, 54%; palmitic, 5%; oleic, 15%; and linoleic, 17%) [12]. Marzouki et al., reported that quali-quantitative information on the individual fatty acids that compose the lipid classes of *L. nobilis* berries fixed oil was obtained by GC analysis. The analyzed oil showed a concentration of approximately 48% of saturated fatty acids, 29% of monounsaturated, and 23% of polyunsaturated (saturated/unsaturated fatty acids ratio of 0.9). The most represented fatty acids of fixed oil from whole berry were 12:0 (27.6%), 18:1 n-9

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(27.1%), 18:2 n-6 (21.4%), and 16:0 (17.1%) [7]. Castilho et al., [13] revealed that the fixed oil extracted from Laurus spp. fruit from Madeira Island, Portugal, is used in local traditional medicine for a wide variety of health complaints. Physical properties, density and refractive index, as well as the TAG FA composition, sterols, and waxes were determined. Oleic (30%) and linoleic (20%) acids were the main unsaturated FA, whereas lauric (18%) and palmitic (up to 22.5%) acids were the main saturated FA in the neutral lipid fraction. The oil had a sterol content on the same order as olive oil, with β-sitosterol (84%) predominating. Madeira laurel oil is not currently used as edible oil because of its very strong flavor.

The aim of this study was to determine the change in (i) pigments and polyphenols composition and (ii) the accumulation of total lipids, fatty acids and phytosterols during Tunisian wild Laurel (*Laurus nobilis*) fruit development.

MATERIALS AND METHODS

Samples

Laurel fruits were hand-harvested from the same tree at intervals of one week from the formation of the fruits (90st day after the flowering date (DAF) of laurel fruits) until there complete maturity (150th DAF). Only healthy fruits, without any sign of infection or physical damage, were selected. During this study, seven samples (one sample per 10 days for each variety) were achieved. The wild trees were grown under natural conditions in Tunis (Tunisia).

Determination of oil content

Oil content was determined by extracting dry material of *Laurus nobilis* fruits with petroleum ether using a Soxhlet apparatus [14]. 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 peanut sample before and after the extraction [15].

Peroxide index determination

The peroxide index was determined according to AOCS method (American Oil Chemist's Society: Cd 8–53 (92).

Determination of chlorophylls and carotenoids contents

The chlorophylls and carotenoids contents were determined according to the method of Minguez-Mosquera et al., [16] and they were calculated according to Ceballos et al., [17].

Gas Chromatography–Flame Ionisation Detection (GC–FID)

Fatty acids were methylated using the method of Metcalfe et al. [18] modified by Lechevallier [19]. Methyl esters were analysed by Gas Chromatography (GC) using a HP 4890 chromatograph equipped with a Flame Ionisation Detector (FID) on a capillary column coated with supelcowax TM 10 (30 m length, 0.25 id, 0.2 mm film thickness). Temperatures (°C) of column, detector and injector were: 200°, 250° and 260°, respectively.

Saponification and TLC analysis

Unsaponifiable fraction of lipids was determined by saponifying 5 g of oil mixed with both 200 µl β-cholestanol and an ethanolic KOH 12% solution; the mixture was heated at 60°C for 1.30 h. The unsaponifiable matter was extracted, washed, dried over anhydrous Na_2SO_4 and evaporated to dryness using N2. 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. After development, the plate was sprayed with 2,7-dichlorofluorescein and viewed under UV light.

Silylation of sterols

An amount of 2 mg of methylsterols residue were mixed with 125 µl of BSTFA (with 1% TMCS), 125 µl of pyridine and 450 µl of acetone, the mixture vortexed for about 10 s and heated at 70 °C for 20 min. After silylation reaction, 1.5 ml of chloroform was added to the mixture and 1 µl of the solution were directly injected to gas chromatograph.

Polyphenols analysis

Colorimetric quantification of total phenolics was determined, as described by Dewanto et al., [20]. Briefly, 125 µL of suitable diluted sample extract was dissolved in 500 µL of distilled water and 125 µL of the Folin–Ciocalteu reagent. The mixture was shaken, before adding 1250 µL Na_2CO_3 (70 g/L), adjusting with distilled water to a final volume of 3 ml, and mixed thoroughly. After incubation for 90 min at 23°C in darkness, the absorbance versus a prepared blank was read at 760 nm. A standard curve of gallic acid was used. Total phenolic content of plant parts was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. The calibration curve range was 0-400 µg/mL (R2=0.99). All samples were analyzed in three replications.2.9. Statistical analysis

The data (three replicates) were statistically evaluated using the JMP SAS version 12.6 software (Statistical Analysis System). (SAS, Institute INC, Box 8000, Cary, North Carolina 27511, USA).

RESULTS AND DISCUSSION

Evolution of oil content during maturation

The oil content of fruits (expressed as % of dry weight) was low during the first 10 days after the onset fruits of *Laurus nobilis* (8, 09 and 11,96% respectively for 90 and 100 DAF). This may be explained by the fact that the lipids synthesized by immature fruits were used for the development of new fruit tissues. The second period (between 110 and 140 DAF) was characterized by an increase in oil content, reaching a maximum value at 140 DAF (23, 76%). In this period, the laurel fruits were practically formed which favours the formation of storage lipids. The later maturation stages showed a decrease in lipid accumulation, especially at last ten days of fruit development (Figure 1). The oil biosynthesis was stopped which explains the regression of the total lipid at complete maturity of laurel fruits which the highest amount of lipid was observed at end of maturation (140 DAF).

Evolution of fatty acid (FA) content during laurel fruits maturation

Fatty acids composition at the end of maturation: Fatty acids composition of laurel fixed oil is given in Table 1 which shows that lauric (C12), palmitic (C16), oleic (C18:1) and linoleic (C18:2) acids account for more than 94,6% of the total fatty acids. We showed that the oil of laurel contained oleic and linoleic acids at high levels: 46,67% and 20,79% respectively, Saturated Fatty Acids (SFA) accounted for 30,36% and Unsaturated Fatty Acids (UFA) accounted for 69,56%. The ratio of unsaturated to saturated fatty acids (U/S) was 2, 29 and The ratio of C18:2 to C18:3 was 23, 8. Castilho et al., (2005) revealed that, in Laurus spp. fruit from Madeira Island Portugal, oleic (30%) and linoleic (20%) acids were the main unsaturated FA, whereas lauric (18%) and palmitic (up to 22.5%) acids were the main SFA in the neutral lipid fraction.

Evolution of major FA contents

In laurus nobulis, the main fatty acid was oleic acid (C18:1). The oleic acid (C18:1) content increased slowly during the first thirty days of maturation (90 to 110 DAF), after that, they showed slowly decreasing the following twenty days (110 to 130 DAF), and attained a maximum value at the 140 DAF (47, 22%), before slowly decreasing at 150 DAF (46,67%) (Table 2).

The C18:2 percentages slightly decreased from ca. 30% of the total FA to ca. 20% at 150 DAF. The C16 percentage decreased from the beginning to the ten days before fruit maturation, after that, the C16 content increased at the end of maturation. The C12 percentage, however, showed some fluctuations during the Laurel fruit maturation. The results indicate that the synthesis of some fatty acid is most intense during the second half of fruit maturation (C16 and C18:1), and it is most intense during the first half for another fatty acids (C12, C18:2).

This result can be explained by the fact that the unsaturated fatty acids such as C18: 1 was used in the composition of membrane phospholipids of newly formed cells. UFA missing mass is offset by saturated fatty acids (C16) and this is due to the fact that the reactions of elongation and desaturation are very slow at this stage (Table 1).

Evolution of total sterols content

Sterols composition at the end of maturation: Very little information is available on sterols composition of *Laurus nobilis* oil fruits. Table 3 summarized the sterol compositions of laurel oil during fruit development. In this study, we found that β-sitosterol, campesterol, stigmasterol and chlerosterol were among the major components constituting about 94, 8% of total sterols. The β-sitosterol represented the main component of the phytosterols (84, 02%), followed by campesterol (6,11%), stigmasterol (1,57%) and chlerosterol (1,49%). Δ5-Avenasterol, Δ5-24 stigmastadienol and β-5 Stigmastenol were present at lower levels. Castilho et al., (2005) revealed that the fixed oil extracted from Laurus spp. fruit had a sterol content on the same order as olive oil, with β-sitosterol (84%) predominating. Madeira laurel oil is not currently used as edible oil because of its very strong

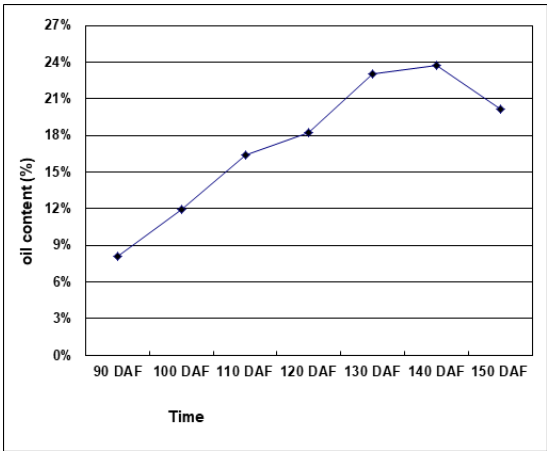

Figure 1: Evolution of oil content during maturation of Laurel fruits (Mean values, n=3). Note: (Day after the flowering date DAF).

Table 1: Evolution of SFA, MUFA ans UFA compositions.

FA	90 DAF	100 DAF	110 DAF	120 DAF	130 DAF	140 DAF	150 DAF
SFA	33,02	35,87	30,5	33,33	37,63	29,3	30,36
MUFA	32,59	35,92	42,83	41,28	41,5	48,56	47,9
UFA	65,21	63,18	69,2	66,59	63,21	70,65	69,56

Note: During maturation of Laurel fruits. **Abbreviations:** SFA: Saturated Fatty Acids; MUFA: Mono Unsaturated Fatty Acids; PUFA: Poly Unsaturated Fatty Acids.

Table 2: Evolution of fatty acids compositions (% of total fatty acid) during the development of Laurel fruits.

Fatty acids (%)	90 DAF	100 DAF	110 DAF	120 DAF	130 DAF	140 DAF	150 DAF
C6	1,78 ± 0,03	0,75 ± 0,01	0,41 ± 0,01	0,42 ± 0,01	0,59 ± 0,02	0,32 ± 0,01	0,08 ± 0,01
C8	2,41 ± 0,11	1,22 ± 0,1	0,53 ± 0,02	0,47 ± 0,01	0,52 ± 0,02	0,22 ± 0,01	0,17 ± 0,02
C10	2,03 ± 0,1	0,98 ± 0,02	0,67 ± 0,03	0,51 ± 0,01	0,64 ± 0,02	0,36 ± 0,01	0,02 ± 0,001
C12	10,49 ± 0,51	17,85 ± 0,78	12,49 ± 0,57	14,09 ± 0,61	16,59 ± 0,53	10,03 ± 0,4	9,36 ± 0,35
C14	0,62 ± 0,01	0,89 ± 0,02	0,76 ± 0,02	0,89 ± 0,03	1,05 ± 0,04	0,74 ± 0,02	0,68 ± 0,02
C16	15,57 ± 0,45	12,48 ± 0,5	13,48 ± 0,1	14,79 ± 0,8	14,63 ± 0,5	14,74 ± 0,4	17,78 ± 0,7
C16:1	0,25 ± 0,01	0,27 ± 0,03	0,19 ± 0,01	0,25 ± 0,02	0,22 ± 0,01	0,23 ± 0,01	0,41 ± 0,02
C17	0,05 ± 0,01	0,04 ± 0,01	0,03 ± 0,01	0,05 ± 0,02	0,05 ± 0,01	0,04 ± 0,01	0,03 ± 0,01
C17:1	0,11 ± 0,02	0,05 ± 0,01	0,02 ± 0,01	0,02 ± 0,001	0,01 ± 0,01	0,03 ± 0,01	0,03 ± 0,01
C18	1,48 ± 0,08	1,39 ± 0,04	2,09 ± 0,01	1,84 ± 0,02	2,25 ± 0,03	2,51 ± 0,02	2 ± 0,02
C18:1	31,47 ± 1,1	34,55 ± 0,95	41,63 ± 1,81	40,12 ± 1,52	40,37 ± 1,09	47,22 ± 1,7	46,67 ± 1,09
C18:2	30,38 ± 1,2	25,79 ± 1,2	25,05 ± 1,01	24,01 ± 1,3	20,9 ± 0,98	21,25 ± 1,1	20,79 ± 1,01
C18:3	2,24 ± 0,91	1,47 ± 0,05	1,32 ± 0,02	1,30 ± 0,01	0,81 ± 0,02	0,84 ± 0,01	0,87 ± 0,02
C20	0,34 ± 0,03	0,27 ± 0,02	0,27 ± 0,01	0,27 ± 0,02	0,31 ± 0,01	0,34 ± 0,02	0,24 ± 0,01
C20:1	0,76 ± 0,02	0,92 ± 0,03	0,99 ± 0,01	0,89 ± 0,02	0,90 ± 0,01	1,08 ± 0,04	0,79 ± 0,02

Note: All values given are means of three determinations.

Table 3: Evolution of peroxide index and antioxydant contents of Laurel fruits.

	90 DAF	100 DAF	110 DAF	120 DAF	130 DAF	140 DAF	150 DAF
Peroxid index	9,47 ± 0,1	8,73 ± 0,3	8,11 ± 0,4	6,96 ± 0,1	6,39 ± 0,2	7,02 ± 0,3	4,7 ± 0,1
Total phenolic contents (mg GAE/g DW)	11,02 ± 0,4	10,32 ± 0,19	10 ± 0,3	9,55 ± 0,1	9,2 ± 0,3	10,55 ± 0,25	8,5 ± 0,11
Chlorophylls ppm	52,44 ± 1,2	51,14 ± 0,9	45,64 ± 1,1	44,66 ± 1,5	43,37 ± 1	42,98 ± 0,8	31,94 ± 1,3
β carotene	20 ± 1,15	18,06 ± 0,9	20 ± 1,1	18,06 ± 0,81	18,06 ± 1,2	19,56 ± 0,9	17,18 ± 1,1

Note: (Mean values, n=3).

flavor.

Accumulation of total phytosterols during laurel fruit development: During the first period of laurel fruit ripening (from 90 to 110 DAF) the amount of all sterols increased to a maximum (Table 4). After the 110th DAF, the total sterols content decreased gradually to reach its lowest level at complete maturity of the fruit. The decrease in total sterol fraction may be explained by the fact that during the final days of laurel fruit 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. Sakouhi et al., [21] reported that the accumulation of total sterols during the maturity of Meski olives presented oscillations alternated with maximums and minimums of sterols levels. Herchi et al., [22] revealed that during the early stages of linseed development, phytosterols content were higher and then decreased during maturation. The seed in first stages of development had the highest levels of phytosterols; this may be explained by the fact that the early period of formation of seed is a period of intense cell divisions which required the biosynthesis of molecules essential for the construction of membranes such as phytosterols. The decrease in phytosterols could be explained by their conversion into other lipids compounds. Phytosterols could

Table 4: Evolution of sterol contents of *Laurus nobilis* fruits.

Sterols	90 DAF	100 DAF	110 DAF	120 DAF	130 DAF	140 DAF	150 DAF
Campesterol	6,92 ± 0,18	7,39 ± 0,3	8,71 ± 0,35	6,77 ± 0,2	6,75 ± 0,1	6,58 ± 0,15	6,11 ± 0,17
Stigmasterol	1,11 ± 0,02	1,96 ± 0,09	2,06 ± 0,2	1,87 ± 0,04	1,84 ± 0,05	1,7 ± 0,04	1,57 ± 0,02
Chlerosterol	1,19 ± 0,04	1,41 ± 0,01	1,96 ± 0,02	1,84 ± 0,01	1,76 ± 0,04	1,56 ± 0,03	1,49 ± 0,01
β-sitosterol	83,85 ± 1,3	88,02 ± 2,4	88,88 ± 1,9	86,42 ± 1,4	85,79 ± 2,1	84,62 ± 0,9	84,02 ± 1
Δ-5 Stigmastenol	0,89 ± 0,04	1,81 ± 0,01	2,81 ± 0,02	1,91 ± 0,04	0,7 ± 0,03	0,58 ± 0,01	0,48 ± 0,01
Δ-5 Avenasterol	0,23 ± 0,01	0,68 ± 0,02	0,73 ± 0,04	0,7 ± 0,01	0,26 ± 0,01	0,22 ± 0,01	0,2 ± 0,02
Δ5-24 Stigmastadienol	0,16 ± 0,03	0,32 ± 0,04	0,56 ± 0,02	0,54 ± 0,01	0,47 ± 0,02	0,38 ± 0,02	0,21 ± 0,01

Note: (Mean values, n=3).

be converted to steroidal hormones and vitamins that regulate the growth and the development of immature tissues [23-26].

Evolution of peroxide index and antioxydant contents

During the first period of laurel fruit ripening, the amount of peroxide index and all natural antioxydant contents (polyphenols, chlorophylls and β carotene) increased to a maximum (from 90 to 110 DAF). After the 110th DAF, these compounds contents decreased gradually to reach their lowest level at complete maturity of the fruit. Increasing antioxidant levels is related to the presence of high levels of unsaturated fatty acids on the one hand, on the other hand when fatty acids are integrated in phospholipids or TAG, levels of antioxidants decrease during laurel fruit ripening (Table 3). This study showed that the peroxide levels do not exceed 10 milli-equivalents of active oxygen per kilogram oil, these values indicate a good oil we can confirm that all the identified compounds found naturally in rapeseed oil and are not a products of oil degradation [27].

CONCLUSION

The rate of oxidation of fats and oil is affected by many factors such as light, exposure to oxygen, the presence of antioxidants and the degree of unsaturation of these fatty acids. In fact, Polyunsaturated Fatty Acids (PUFAs) with a long chain are especially sought after. Some amount of linolenic acid is required for good-flavour compounds. This is due to the formation of oxidation products, which are important flavour compounds. The great stability of vegetable oils, in conditions of oxidation, is due to the presence of an elevated rate of natural antioxidants, most of which important are tocopherols. These components are essential for protection of PUFAs in plants and animals deterioration.

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