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ORIGINAL PAPER

Dynamics of Fatty Acids, Tocopherols and Phenolic Compounds Biogenesis During Olive (*Olea europaea* L.) Fruit Ontogeny

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Abstract Fatty acids, tocopherols, and phenolic compounds biogenesis from two major Spanish olive cultivars (Arbequina and Manzanilla) were analyzed for the function of the thermal regime during the fruit ontogeny in a non-Mediterranean environment. Bilinear models characterized the dynamics of fatty acid biogenesis. Regressions between the accumulated thermal time (TT) and the levels of both oleic and linoleic acids showed different responses to temperature of each olive cultivar. After reaching similar absolute maximum oleic acid contents at similar TT, the levels of this fatty acid decreased markedly in cv. Arbequina and its final concentration was 10 % lower than that found in cv. Manzanilla. In both cultivars, concentrations of all tocopherol isoforms were negatively associated with the TT accumulated over the entire oil accumulation period. Dynamics of phenolic compounds biogenesis showed no clear tendencies with TT. Nevertheless, and whatever the stage of fruit development, secoiridoids were the major phenolic components. Results suggest greater sensitivity of fatty acid metabolism to temperature in cv. Arbequina. This fact points out the necessity of appropriate evaluation of the ambient thermal characteristics before introducing this cultivar into new growing environments.

Keywords Olive · Fatty acids · Tocopherols · Phenolic compounds · Biogenesis patterns · Fruit ontogeny · Thermal time · Growing environment

Abbreviations

DAFF	Days after full flowering
DWB	Dry weight basis
FA	Fatty acid
LA	Linoleic acid
LnA	Linolenic acid
OA	Oleic acid
PUFA	Polyunsaturated fatty acids
TPC	Total phenol content
TTC	Total tocopherol content
VOO	Virgin olive oil

Introduction

The olive (*Olea europaea* L.) is a crop well adapted to environmental conditions prevailing in countries from the Mediterranean Basin. In the two last decades, the increasing demand and consumption of olive oil and table olives have encouraged olive cultivation in several countries of the Southern hemisphere, especially in South America and Australia. These new crop environments do not have typical Mediterranean climates, and some of them are in the subtropics, where

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the response of the crop is not yet completely known. In some olive cultivation areas from central-western to Northwestern Argentina, higher spring and summer temperatures affect the timing of phenological events with respect to the olive cultivation in the Mediterranean region. So, full flowering, fruit setting, and the onset of the oil synthesis period occur earlier; the most intense oil accumulation period takes place mainly in the summer, under average temperatures warmer than those registered in Mediterranean countries, where most of the oil synthesis and accumulation occur later.

In contrast with many other edible vegetable oils, which are obtained from seeds, olive oil mostly arises from the fruit mesocarp. Olive oil biogenesis comprises a series of complex physiological and biochemical processes that take several weeks [1]. The oil synthesis is genetically regulated, but it may be modified by several factors, such as those related to climate and orchard management [2]. Thus, oil content and composition of any olive cultivar result from a complex interaction between the genotypic potential and the environmental and agronomical conditions that influence fruit growth and ripening.

Owing to the relative unresponsiveness of the olive species to photoperiodicity, studies evaluating environmental variations in FA composition of VOO have been focused on the effect of temperature. In general, it can be observed that in warm seasons and areas, olive fruits give oils with lower oleic acid contents, and higher contents of palmitic and/or linoleic acids [2–5]. Nevertheless, it has been also observed that olive cultivars may have different responses, in terms of oil fatty acid composition, to temperature regimes during the oil synthesis period [5]. Thus, according to results obtained by several authors [3, 6, 7] the seasonal or environmental variability of the olive oil composition of a given cultivar also depends on its phenotypic stability.

The importance accorded to VOO is mainly due to its particular fatty acid composition, but also to its richness in some minor components such as tocopherols and polyphenols that contribute important biological properties and protect the oil from oxidative degradation. The range of variability of these compounds at both qualitative and quantitative levels is very high and results from a complex interaction between the genotypic potential and agronomic and environmental factors [2, 8, 9].

Arbequina is a major Spanish olive cultivar, and the most used for oil production in the Southern hemisphere. Although it is considered highly adaptable to different climates and soils, FA composition of its oil may vary widely according to the geographic origin [4, 5, 10, 11]. Manzanilla is also a common Spanish olive cultivar widespread in South America. Even though a substantial part of Manzanilla olives goes to olive oil production, little scientific information is available regarding their chemical composition and, in particular, that related to tocopherol and polyphenolic components.

In this study, the biogenesis patterns of fatty acids, tocopherols, and phenolic compounds from Arbequina and Manzanilla olive cultivars were analyzed for the function of the thermal regime during the fruit ontogeny in a non-Mediterranean environment.

Materials and Methods

Plant Material and Experimental Design

The field experiment was conducted at the INTA Experimental Station (31°32′S, 68°25′W, 591 m above sea level), at San Juan province, in the central-western region of Argentina. Figure 1 shows the thermal regime at the experimental site during the period from full flowering to full fruit maturity. Table 1 reports the average seasonal maximum, minimum, and mean temperatures at the experimental site, as compared with those from typical Mediterranean regions in Spain and Italy [12].

Olive plants (*Olea europaea* L., cvs. Arbequina and Manzanilla de Sevilla) used for this study are grown in the same orchard but in different plots. They are grown at a planting density of 100 trees/ha (tree spacing 10 m × 10 m), under natural rainfall (90 mm/year, in average) plus supplemental irrigation of 800 mm water/year. During both 2010/11 and 2011/12 growing seasons, drupes were hand-picked, approximately at bi-weekly intervals, at 13 distinct stages of fruit development, referred to as days after full flowering (DAFF). For each sampling date, 200 g of healthy drupes selected from the mid-canopy of the entire perimeter of the tree, were collected. For each cultivar, five 70-year-old trees were sampled. Dynamics of fatty acids, tocopherols, and phenolic compounds biogenesis were analyzed as a function of the accumulated thermal time from full flowering. Thermal time was calculated (in °Cd units) using the single sine, horizontal cut-off method, with critical temperatures of 7 °C (lower limit) and 40 °C (upper limit) as suggested by Cherbiy-Hoffmann et al. [13]. Variations in daily ambient temperatures were registered by means of an automatic weather station (Metos, Pessl Instruments, Weiz, Austria) placed within the experimental orchard.

Oil Analyses

For each sampling date, samples (200 g) of whole drupes were ground using a stainless steel knife mill and lyophilized until complete dehydration. Aliquots of 20 g of each lyophilized sample were extracted with 100 mL *n*-hexane at room temperature in the dark. The solvent was removed using a rotary vacuum evaporator at 40 °C, and the oil obtained was used for FA and tocopherol analyses.

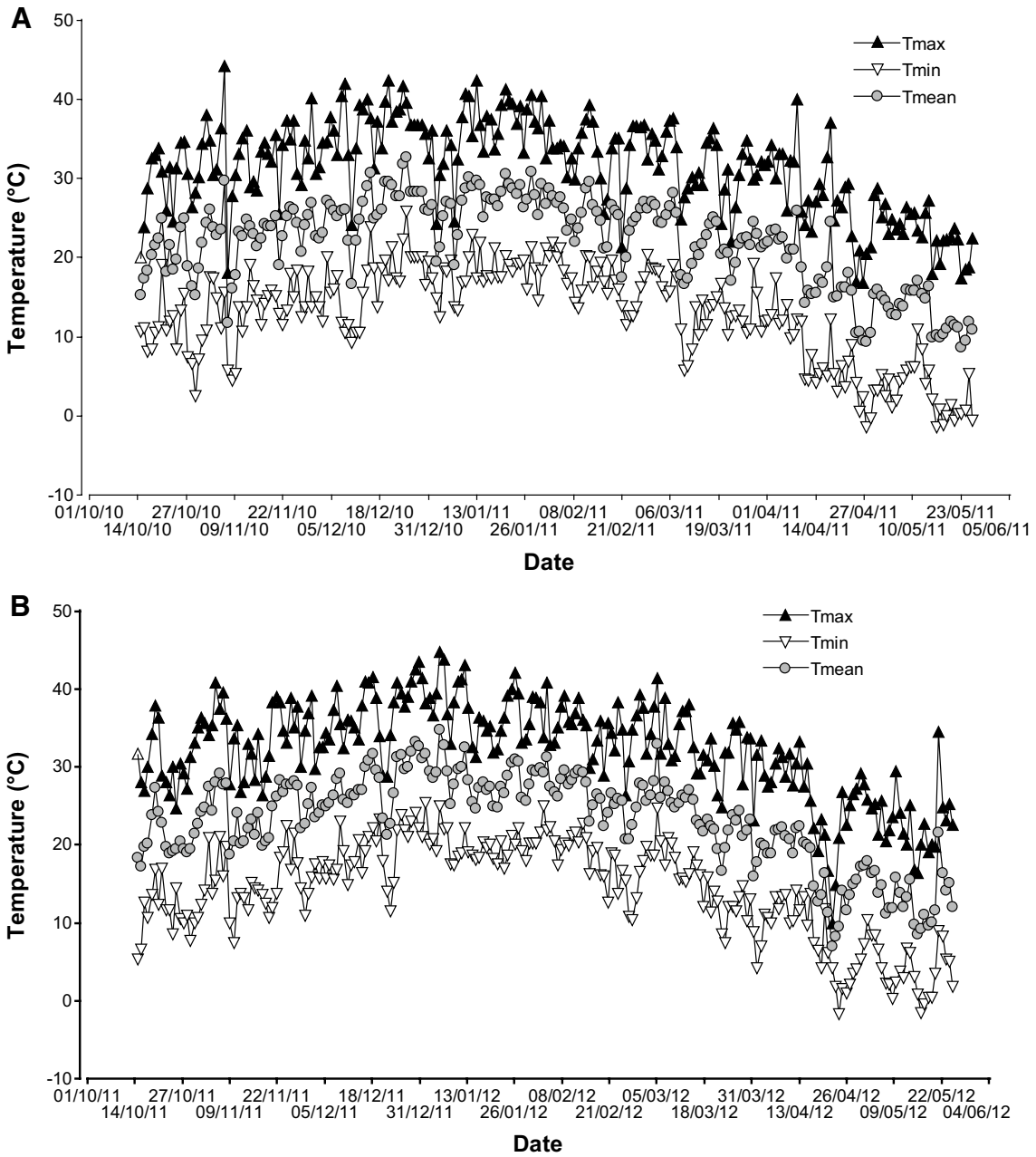

Fig. 1 Temperatures at INTA Experimental Station located in San Juan province (31°32′S, 68°25′W, 591 m above sea level), in the central-western region of Argentina, during 2010–2011 (**a**) and 2011–2012 (**b**) crop seasons. Tmax, Tmin and Tmean are the maximum, minimum, and mean temperatures (°C), respectively, during the period from full flowering to fruit maturity

Fatty acid composition was analyzed according to procedures published previously [11]. Individual FAs were identified by comparing their retention times with those of pure reference compounds (ICN Biomedicals, Costa Mesa, CA, USA), and were quantified as a percentage of total FA content.

Tocopherols were identified and quantified by HPLC (Perkin-Elemer, Shelton, CT, USA) according to the procedure of Lazzez et al. [8], with some modifications reported elsewhere [14]. Individual tocopherols were quantified by the external standard method. The linearity of the response was verified by fitting to line results of each one tocopherol individuals of 20 standard solutions with known concentrations.

Individual phenolic compounds were identified and quantified essentially according to procedures previously described by Bodoira et al. [14]. (3,4-Dihydroxyphenyl) ethanol (3,4-DHPEA) was obtained from Cayman

Table 1 Average seasonal maximum (Tmax), minimum (Tmin), and mean (Tmean) temperatures (°C) at the INTA Experimental Station (San Juan, central-western Argentina), as compared with those from typical Mediterranean olive growing regions in Spain and Italy [12]

Location	Parameter	Spring	Summer	Autumn	Winter	Annual
San Juan (Argentina)	Tmax	28.5	32.0	21.2	18.2	25.3
Latitude 31.3°S	Tmin	13.8	19.0	8.1	3.3	11.5
Altitude (masl) 591	Tmean	21.4	25.7	14.5	10.6	18.5
Jaén (Spain)	Tmax	20.8	31.1	20.8	12.3	21.3
Latitude 37.5°N	Tmin	8.8	18.3	11.9	4.1	10.8
Altitude (masl) 358	Tmean	15.1	24.7	16.2	8.2	16.0
Benevento (Italy)	Tmax	19.7	22.5	14.4	8.0	16.2
Latitude 41.1°N	Tmin	10.8	18.2	6.1	5.0	10.0
Altitude (masl) 250	Tmean	15.4	21.0	9.9	6.2	13.1

Chemicals LTD (USA) and (*p*-hydroxyphenyl) ethanol (*p*-HPEA) from Janssen Chemical Co. (Beerse, Belgium). Oleuropein glucoside was purchased from Extrasynthese (France). Verbascoside was obtained from olive fruits following the procedure described by Montedoro et al. [15]. The dialdehydic forms of elenolic acid linked to 3,4-DHPEA and *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively), (+)-1-acetoxypinoresinol and (+)-pinoresinol were obtained from VOO according to procedures reported in previous papers [15, 16]. The purity of all the substances obtained and used as standards was determined by HPLC, and their chemical structures were verified by NMR by recording ^{1}H and ^{13}C spectra using the same operating conditions reported elsewhere [15, 16].

Statistical Analyses

Dynamics of individual fatty acids and tocopherols, analyzed as a function of thermal time from full flowering, were fitted with two-segments regressions estimated by ordinary least squares according to the following equation:

$$y = \alpha + (\beta_1 x) I_{x<\gamma}(x) + [\beta_2 \gamma + \beta_2 (x - \gamma)] I_{x \geq \gamma}(x),$$

where y is the observed variable (concentration of each fatty acid or tocopherol), α the intercept, β_1 and β_2 are slopes for the first and second linear sections, respectively, x is the thermal time from flowering, I is an indicator function that equals 1 when x is a member in the defined subset or 0 otherwise, and γ the break-point, or threshold that separates both segments. Parameters on each defined segment were estimated with a linear model by restricted maximum likelihood (REML). The general model was as follows:

$$y_{ij} = \mu + C_i + Y_j + T_b + CY_{ij} + CT_{ib} + \varepsilon_{ij},$$

where μ is an intercept, C_i is the effect of the i cultivar ($i = 1, 2$),Y_j is the effect of j year ($j = 1, 2$), T_b the slope for thermal times; CY_{ij} is the interaction of i cultivar in j year, CT_{ib} the slope in thermal time for the i cultivar, and ε_{ij} is the error term for the i cultivar in the j year. Except for the error term, the stated effects were considered fixed. The error term was assumed as normally distributed with a correlation structure as a first-order continuous autoregressive function; this assumes temporal correlations of measurements within trees. The previous model was implemented as defined by Pinheiro et al. [17] in the 'nlme' package of R software. For mean comparisons, p values were adjusted by the Bonferroni criterion and compared with Fisher's least significant difference (LSD) method with a significance level fixed at 0.05. All statistical analyses were performed using InfoStat software (InfoStat, 2014, http://www.infostat.com.ar) in connection with R software.

Results and Discussion

Increasing evidence suggests that temperature, particularly from the time previous to pit hardening until fruit maturation, is probably the main environmental factor contributing to the variability in fatty acid composition of VOO [3, 5, 18]. Correlation analyses under field conditions show that olive oils arising from warm areas and seasons have consistently lower oleic acid (OA) contents which, in turn, are generally associated with higher linoleic and/or palmitic acid levels, than those from colder environments [4, 5, 19].

Monitoring the fatty acid profile from the beginning of fruit growth to maturity showed that the most active OA biosynthesis period occurred at early fruit developmental stages (Fig. 2a). In both Arbequina and Manzanilla cultivars, the dynamic of OA accumulation, modelled as a function of thermal time (Table 2), fitted well to bilinear regressions (R^2 values 0.78 and 0.80, respectively). An intense OA synthesis began after fruit setting (approximately 30 DAFF), at thermal time higher than 500 °Cd, and increased quickly, at similar rates (6.42 %/100 °Cd, Arbequina; 5.91 %/100 °Cd, Manzanilla), until reaching absolute maximum values (71.2 %, Arbequina; 72.6 %, Manzanilla) approximately at 1500 °Cd. From this time, two different patterns were observed. Whereas oils from cv. Manzanilla showed no significant changes in OA concentration, those

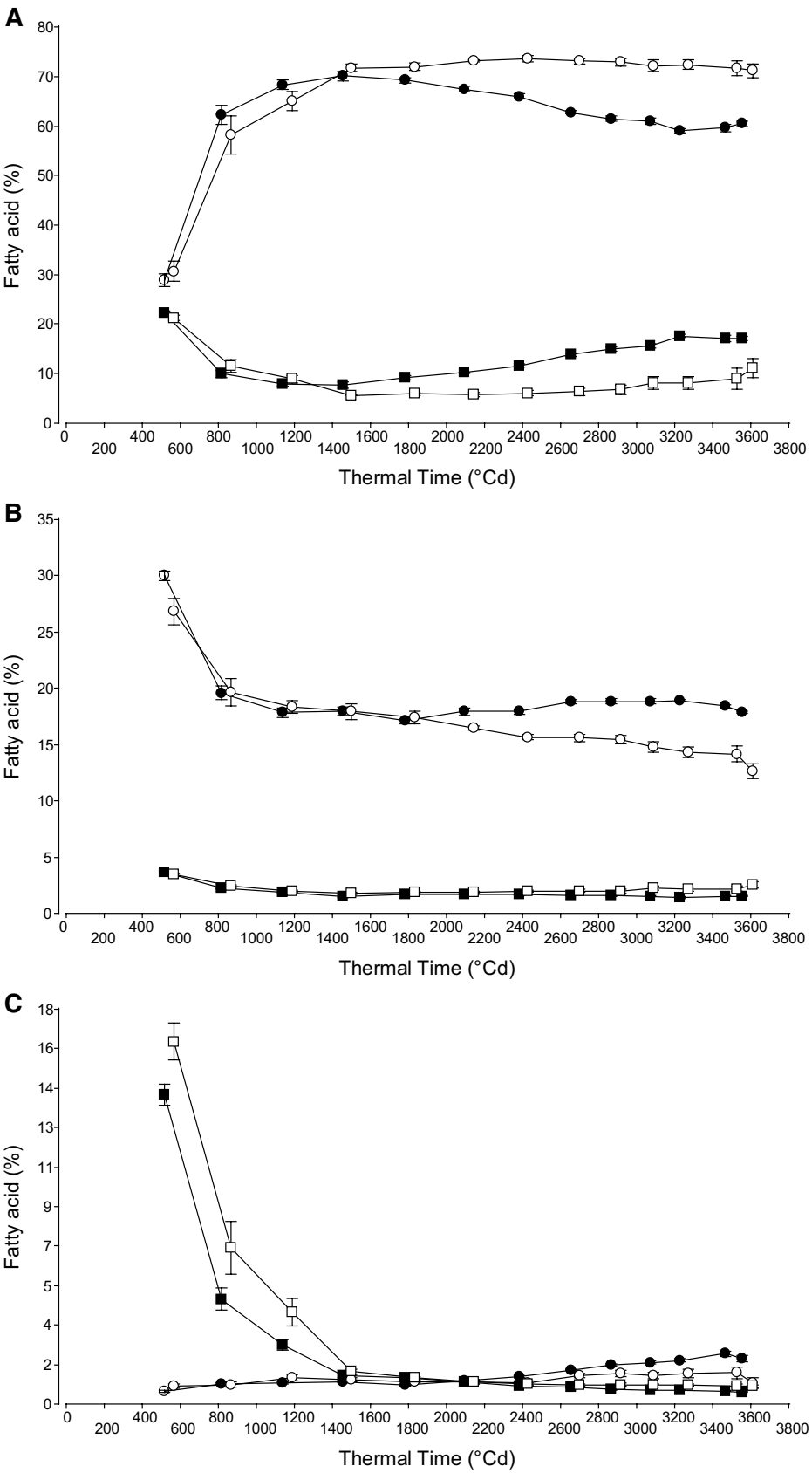

Fig. 2 Variation patterns of fatty acid (FA) composition from Arbequina (*black symbols*) and Manzanilla (*white symbols*) olive cultivars as a function of the accumulated thermal time (°Cd) from full flowering. Each point represents the mean of two crop seasons. **a** Oleic (*circles*) and linoleic (*squares*) acids; **b** palmitic (*circles*) and stearic (*squares*) acids; **c** palmitoleic (*circles*) and linolenic (*squares*) acids. Data correspond to actual FA concentrations obtained from plants at field experimental conditions stated in the "Materials and Methods" section

Table 2 Dynamics of fatty acid and total tocopherol (TT) accumulation modelled as a function of the accumulated thermal time (°Cd) from full flowering

Fatty acids	Cultivar	Slope[1] (%/100 °Cd)	Value at the break point (%)	Slope[2] (%/100 °Cd)
Palmitic	Arbequina	−3.75[a]	17.5	0.06[a]
	Manzanilla	−2.85[a]	18.2	−0.22[b]
Stearic	Arbequina	−0.28[a]	1.78	−0.01[b]
	Manzanilla	−0.25[a]	1.70	0.03[a]
Oleic	Arbequina	6.42[a]	71.2	−0.60[b]
	Manzanilla	5.91[a]	72.6	0.00001[a]
Linoleic	Arbequina	−2.32[a]	6.37	0.53[a]
	Manzanilla	−2.19[a]	4.67	0.12[b]
Linolenic	Arbequina	−1.69[a]	1.87	−0.004[a]
	Manzanilla	−1.90[a]	1.29	−0.003[a]
TT content	Cultivar	Slope[1] (mg/kg/100 °Cd)	Break point (mg/kg)	Slope[2] (mg/kg/100 °Cd)
	Arbequina	−687[b]	1272.6	−46[b]
	Manzanilla	−201[a]	883.9	−32[a]

The slope values for the first and the second sections of the fitted curves are indicated by superscript numbers, 1 and 2, respectively. For each parameter, different superscript letters indicate significant differences ($p \leq 0.05$) between cultivars in the slope values

from cv. Arbequina had a gradual decrease, at an average rate of 0.60 %/100 °Cd, reaching constant values near 60 % approximately at 3200 °Cd.

Temperature-related variations in OA concentrations have been also found in other olive cultivars such as Arauco. In this cultivar, a strong drop in OA percentage (0.86 %/100 °Cd) was observed from thermal time higher than 1000 °Cd [5]. In addition, novel experiments and findings from this cultivar [18] show that the temperature during the fruit filling period may have a direct influence on FA composition. By enclosing olive fruiting branches in controlled temperature chambers, García Inza et al. [18] found that fruits developed under temperatures 5 or 10 °C warmer than the seasonal mean ambient temperature (20.6 °C) give oils with less OA contents. Across the whole range of temperatures they explored, OA concentration decreased linearly 0.7 %/°C; on the contrary, palmitic, linoleic, and linolenic acids percentages increased with increasing temperature. Interestingly, these authors [20] also found that OA content in oils extracted from the seed and the mesocarp showed opposite responses to ambient temperature. Moreover, the response of OA concentration arising from the olive mesocarp was found to be opposite to that observed in oil-seed crops such as sunflower and soybean [21, 22].

Results from the present study show that the accumulation patterns of oleic and linoleic (LA) acids present an opposite, well-known tendency, which may be explained by the activity of the enzyme oleate desaturase transforming OA into LA [1]. From values near 20 %, obtained al thermal times between 500 and 600 °Cd, LA showed a pronounced linear decrease (both olive cultivars at similar rates, 2.19–2.32 %/100 °Cd), that reached absolute minimum values at 1100–1500 °Cd, followed by slight (0.12 %/100 °Cd, cv. Manzanilla) or moderate (0.53 %/100 °Cd, cv. Arbequina) increments. Considering all data obtained during the fruit growth and ripening periods evaluated, significant negative relationships between oleic and linoleic acids were observed ($R^2 = 0.69$ and 0.78, for cv. Arbequina and Manzanilla, respectively).

Bilinear regressions, with two negative slopes, characterized the dynamic of linolenic acid (LnA) accumulation (Table 2; Fig. 2c). High contents (14–16.5 %) were found in young drupes (30 DAFF, 500–600 °Cd) from both olive cultivars. A period of sharp decrease (between 500 and 1500 °Cd) was followed by slow decline, until final, constant values below 1 % were reached at a thermal time higher than 2500 °Cd.

From the time of the first sampling date (500–600 °Cd) until the onset of fruit ripening (1800 °Cd), the dynamics of palmitic acid (PA) accumulation showed no differences between cultivars. In this period PA concentration dropped from 27 to 30 % to values near 18 %. Later, two contrast patterns were observed: a long plateau without significant variations during fruit maturation (cv. Arbequina) and a linear decrease of moderate slope (0.22 %/100 °Cd, cv. Manzanilla) (Table 2; Fig. 2b).

Oleic acid is by far the most abundant fatty acid in VOO. As precursor of PUFA, it is also a key compound in determining the olive oil FA composition. Both Arbequina and Manzanilla olive fruits reached similar absolute maximum OA contents at similar thermal times; however, in cv. Arbequina OA decreased markedly during ripening and the final concentration was 10 % lower than that found in cv. Manzanilla. Since both cultivars were grown under identical agronomic practices and soil and climate

conditions, reasons for such differences cannot be attributed to the growing environment. Regressions between the accumulated thermal time and the levels of both OA and LA suggest different responses to temperature of each olive cultivar, which could be related to differences in the enzymatic capacities involved in OA desaturation. This hypothesis, however, needs to be examined by means of studies on regulation of genes encoding oleate desaturases.

On the other hand, OA concentration from cv. Arbequina resulted in markedly higher than the average value (51.8 %) obtained from warm, low latitudes (less than 29°S) regions in Northwestern Argentina but, on the contrary, it was lower as compared with those reported from this cultivar growing at colder, higher latitude areas in Spain [6, 23]. For cv. Manzanilla, comparisons of OA content between environments follow the same tendency, i.e. decreased concentrations in oils from warmer climates, but environmental differences are of minor importance [5]. These facts suggest greater sensitivity of fatty acid metabolism to ambient temperature in cv. Arbequina. This hypothesis is supported by findings showing this cultivar having low phenotypic stability [6, 11, 24].

Existing literature on the dynamics of tocopherol accumulation in olive fruits has been generally focused on α-tocopherol variations during fruit maturation [9, 25, 26]. There is a paucity of knowledge about the evolution of individual tocopherol concentrations from early stages of olive fruit development. The dynamics of tocopherol biogenesis in fruits of both Arbequina and Manzanilla cultivars showed similar trends (Fig. 3). The highest tocopherol concentrations were found at very early fruit growth stages; in fruit samples collected approximately at 50 DAFF (800 °Cd) the total tocopherol contents (TTC) averaged 4400 mg/kg (cv. Arbequina) and 3200 mg/kg oil (cv. Manzanilla). In both olive cultivars, the dynamics of total tocopherol accumulation, modelled as a function of thermal time, adjusted well to bilinear regressions, with two negative slopes and break points recorded at thermal times near 1250 °Cd (cv. Arbequina) and 1400 °Cd (cv. Manzanilla) (Table 2). Until these times, TTC dropped at rates of 687 (cv. Arbequina) and 201 mg/kg/100 °Cd (cv. Manzanilla) ($R^2 = 0.80$). Latter, they decreased at lesser rates (46 and 32 mg/kg/100 °Cd, respectively, $R^2 = 0.82$), until early fruit ripening stages. No significant variations were found throughout the fruit ripening period evaluated. Thus, total tocopherol concentrations were negatively associated with the accumulated thermal time over the entire oil accumulation period.

In accordance with previous studies performed in other olive cultivars [9, 14, 25–27], α-tocopherol was the most abundant isoform in all fruit developmental stages. It represented more than 80 % of the total tocopherol content, followed by γ-tocopherol and minor amounts of β-tocopherol. The general pattern of variation found in the olive cultivars analyzed here—characterized by high amounts of all α-, β- and γ-isoforms in very young drupes, pronounced decrease during fruit development, and little or no change during fruit ripening—resembled that observed in cv. Arauco growing in the same environmental conditions [14].

The concentration of tocopherols in VOO is highly variable (150–450 mg/kg) and depends strongly on the olive cultivar. Regarding oils from cv. Arbequina, studies carried out at different growing regions in Spain [23, 24] have reported a mean TTC of 250 mg/kg and α-tocopherol contents between 185 and 210 mg/kg in oils obtained from fruits at various ripening stages. On the other hand, oils from cv. Manzanilla have been found to contain from 160 to 300 mg/kg of total tocopherols, with α-tocopherol predominating largely (more than 90 % of TTC) [27]. In the present study, considering constant values (without significant differences among sampling dates) obtained from the beginning of the fruit ripening period, the average concentrations (two crop years) of α-, β- and γ-tocopherol were found to be, respectively, 385, 56, and 73 mg/kg oil (cv. Arbequina), and 264, 57, and 71 mg/kg oil (cv. Manzanilla). These values add variability to the well-known ranges of tocopherol concentration of Arbequina and Manzanilla olive oils, and suggest a possible interaction between genotype and environment that modifies the tocopherol composition. At fruit maturity, oils from these two olive cultivars growing in central-western Argentina have higher tocopherol contents than their Spanish counterparts.

The role of temperature on olive fruit phenol content is controversial, depending on the genotype and environmental conditions. In a study including many olive genotypes growing in central and Southern Italy, the higher the degree-days accumulated from fruit set to harvest, the lower the amount of total polyphenols [28]. On the contrary, total polyphenol concentration from cv. Casaliva growing at a colder region in northern Italy increased with the accumulated degree-days [29]. The effect of altitude, which is a major factor that affects thermal regime, is also unclear. Mousa et al. [30] have found that polyphenol content decreased with altitude but the opposite has been also reported [19].

In both cultivars analyzed here, the highest total phenol contents (TPC) were measured prior to the onset of the fruit ripening period. Considering the fruit growth and ripening period evaluated, no clear tendencies were found between TPC and the accumulated thermal time. TPC varied between 17,880 and 56,700 mg/kg fruit (DWB) (cv. Arbequina) and between 44,850 and 107,000 mg/kg fruit (cv. Manzanilla) (Table 3). The range of variation of phenolic compounds in olive fruits is very high, and significant effects of the growing environment, the genotype, and the fruit physiological stage at both quantitative and qualitative

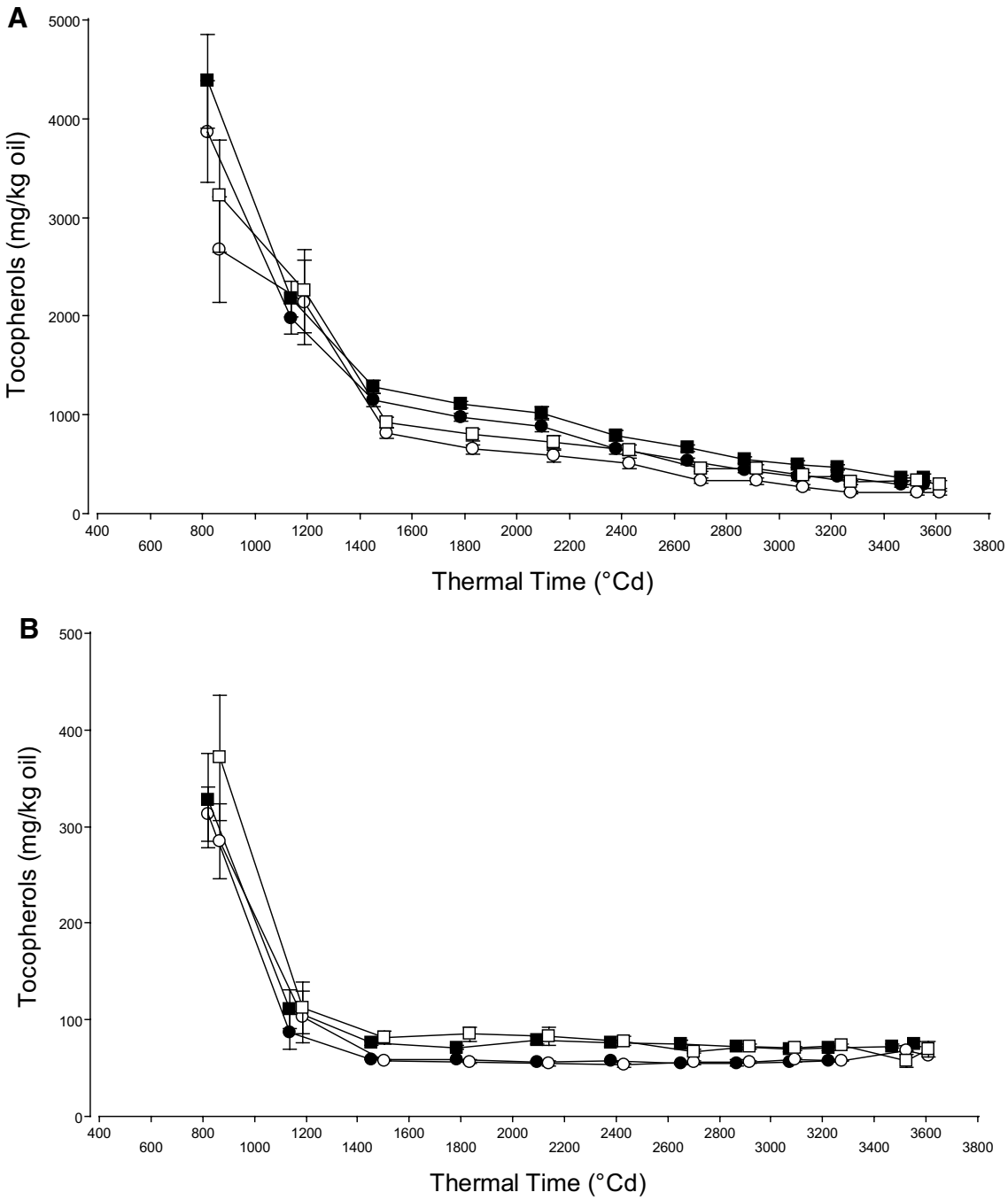

Fig. 3 Variation patterns of tocopherol concentrations from Arbequina (*black symbols*) and Manzanilla (*white symbols*) olive cultivars as a function of the accumulated thermal time (°Cd) from full flowering. Each point represents the mean of two crop seasons. **a** Total tocopherols (*squares*) and α-tocopherol (*circles*); **b** β-tocopherol (*circles*) and γ-tocopherol (*squares*). Data correspond to actual tocopherols concentrations obtained from plants at field experimental conditions stated in the "Materials and Methods" section

levels have been widely reported [14, 29, 31]. Data from the present study highlight the unusually high contents of phenolic substances obtained from Arbequina fruit samples as compared with those found by Morelló et al. [31] in drupes from this cultivar growing in the original cultivation area in Catalonia (Spain). These authors reported TPC ranging from 3899 to 18,513 mg/kg fruits (DWB), with decreasing concentrations from fruit setting to maturity.

The phenolic composition patterns from the two olive cultivars analyzed here did not show qualitative variations. Fruits from both Arbequina and Manzanilla cultivars contained different types of phenolic compounds such

Table 3 Concentrations of phenolic compounds (mg/kg) from drupes (DWB) of cvs. Arbequina and Manzanilla at distinct stages of fruit growth and ripening, referred to as days after full flowering (DAFF)

cv. Arbequina	49 DAFF	95 DAFF	140 DAFF	185 DAFF
3,4-DHPEA	1125 ± 184	1360 ± 333	1724 ± 397	1400 ± 233
p-HPEA	630 ± 242	680 ± 102	416 ± 84	270 ± 30
Demethyloleuropein	nd	nd	nd	2840 ± 755
Verbascoside	nd	20,200 ± 2323	13,200 ± 585	7500 ± 157
3,4-DHPEA-EDA	13,300 ± 1933	32,600 ± 1048	20,290 ± 649	8650 ± 117
Oleuropein	1396 ± 214	6400 ± 185	701 ± 37	nd
p-HPEA-EDA	1018 ± 34	680 ± 28	160 ± 16	nd
Quercetin-3-*o*-rutinoside	203 ± 23	240 ± 11	196 ± 7	200 ± 6
(+)-1-Acetoxypinoresinol	84 ± 7	90 ± 20	88 ± 6	160 ± 5
(+)-Pinoresinol	123 ± 19	150 ± 15	108 ± 6	70 ± 10
Total phenolic content	17,880 ± 2302	56,700 ± 1350	36,800 ± 1126	21,100 ± 1920
cv. Manzanilla	53 DAFF	98 DAFF	144 DAFF	188 DAFF
3,4-DHPEA	952 ± 37	1810 ± 87	4430 ± 59	1450 ± 27
p-HPEA	650 ± 24	860 ± 96	605 ± 22	430 ± 81
Demethyloleuropein	nd	nd	nd	nd
Verbascoside	nd	40,400 ± 4064	37,260 ± 1037	4560 ± 677
3,4-DHPEA-EDA	28,570 ± 2606	45,100 ± 2779	20,560 ± 3883	8980 ± 571
Oleuropein	11,870 ± 2709	15,900 ± 1630	4068 ± 270	nd
p-HPEA-EDA	1310 ± 247	950 ± 143	nd	nd
Quercetin-3-*o*-rutinoside	1147 ± 149	1210 ± 212	nd	nd
(+)-1-Acetoxypinoresinol	152 ± 32	270 ± 19	167 ± 40	130 ± 25
(+)-Pinoresinol	211 ± 10	150 ± 30	137 ± 13	150 ± 15
Total phenolic content	44,850 ± 4342	107,000 ± 5134	67,450 ± 7890	45,700 ± 5804

Data are the average (mean values ± standard deviation) of two crop seasons. For each cultivar five fruit samples taken from five olive plants were used

nd not detected

as phenolic alcohols, secoiridoids, lignans, one flavonoid and verbascoside (Table 3). To our knowledge, the present study is the first report on the concentration of individual phenolic compounds in fruits from cv. Manzanilla.

Phenolic alcohols comprised hydroxytyrosol (3,4-DHPEA) and tyrosol (*p*-HPEA). In cv. Arbequina, 3,4-DHPEA presented relatively stable concentrations throughout the sampling period analyzed; the average contents varied between 1125 and 1724 mg/kg and showed no trends over the fruit developmental stage. In contrast, in cv. Manzanilla the content of 3,4-DHPEA varied markedly among sampling dates and showed a peak (4430 mg/kg fruit) after the beginning of the fruit ripening period (140 DAFF). Tyrosol concentrations showed the highest values after the pit hardening and decreasing amounts during fruit ripening, in accordance with results published elsewhere [30].

In agreement with results found in drupes from many olive cultivars [31, 32] secoiridoids compounds were the major phenolic components in fruits from both Arbequina and Manzanilla cultivars. Secoiridoids included oleuropein and the aglycon derivatives of glucoside secoiridoids such as the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA, respectively). 3,4-DHPEA-EDA was the most abundant compound, ranging between 8650 and 32,600 mg/kg (Arbequina) and 8980 and 45,100 mg/kg fruit (Manzanilla). It was noteworthy there was the prevalence of 3,4-DHPEA-EDA instead of oleuropein; this phenolic pattern has been also observed in cv. Arauco from Argentina [14] but it is not common in other olive cultivars [33]. Likewise, this feature of the phenolic compositional pattern of cv. Arbequina from Argentina does not match with that observed in Spain by Morelló et al. [31] who have reported oleuropein as the main phenolic compound. It is also remarkable the occurrence of high amounts of both 3,4-DHPEA-EDA and oleuropein at the earliest fruit developmental stage; this behaviour is unusual in olive fruits since in general high amounts of 3,4-DHPEA-EDA match with the inhibition of the synthesis of oleuropein glucoside that produces an accumulation of this compound as precursor of the oleuropein synthesis. Both 3,4-DHPEA-EDA and oleuropein contents decreased strongly during fruit ripening. The decrease in oleuropein content is often accompanied by the accumulation of both demethyloleuropein and

elenolic acid glucoside [31, 33]. However, demethyloleuropein was only detected belatedly during maturity of Arbequina olive fruits, and elenolic acid glucoside was not found at any fruit growth and ripening stages analyzed. On the other hand, *p*-HPEA-EDA showed the highest amounts in very young drupes; minor or nor values were detected from the beginning of fruit ripening. This compound, also called oleocanthal, is usually present in VOOs [34] but rarely has been observed in the fruits [14].

Verbascoside, a hydroxycinnamic acid derivative, was not detected at early fruit growth stages, but before the onset of fruit ripening (95–98 DAFF) it reached unusually high contents (20,200 mg/kg in Arbequina fruits, 40,400 mg/kg in Manzanilla fruits) as compared with those found in Arbequina growing in Spain (205–540 mg/kg fruit) [31].

Quercetin-3-*o*-rutinoside was the only flavonoid identified. In cv. Arbequina it was found at all fruit development and ripening stages evaluated with concentrations varying between 196 and 240 mg/kg. In cv. Manzanilla, this flavonoid was detected at greater concentrations (1147–1210 mg/kg), but only at early fruit growth stages.

Lignans included (+)-1-acetoxypinoresinol and (+)-1-pinoresinol. In agreement with results obtained from cv. Arauco [14], they were found at very low concentrations and showed minor variations during fruit physiological development.

Conclusions

Bilinear models characterized the dynamics of FA biogenesis in both Arbequina and Manzanilla olive cultivars. Regressions between the accumulated thermal time and the levels of both oleic and linoleic acids suggest different responses to temperature of each olive cultivar, which could be related to differences in the enzymatic capacities involved in OA desaturation. Comparison of data from the present study with those obtained from regions with different thermal regimes shows Arbequina oils varying widely in OA concentrations, with values consistently lower in olives cultivated in warmer environments.

In both cultivars the dynamics of tocopherol accumulation showed very similar patterns characterized by high amounts of α-, β- and γ-isoforms in very young drupes, pronounced decrease during fruit growth, and little or no change during fruit ripening. Concentrations of all tocopherol isoforms were negatively associated with the accumulated thermal time over the entire oil accumulation period.

No clear tendencies were found between total phenol content and thermal time accumulated over the fruit growth and ripening period evaluated. Nevertheless, and whatever the stage of fruit development, secoiridoids were the major phenolic components. The prevalence of 3,4-DHPEA-EDA over oleuropein and the unusually high contents of verbascoside were the most outstanding features that characterize the polyphenolic patterns of Arbequina and Manzanilla olive fruits as compared with those from the original cultivation area in Spain.

Overall findings from this study contribute to better understanding the relationships between olive cultivars and the cultivation environment. Results suggest greater sensitivity of FA metabolism to temperature in cv. Arbequina. Although FA composition from this cultivar growing in central-western regions of Argentina and similar environments could meet current trade standards of the International Olive Oil Council for VOO, our results and others related point out the necessity of appropriate evaluation of the ambient thermal characteristics before introducing it into new growing environments.

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