

## Research Article

# Oil biogenesis and antioxidant compounds from “Arauco” olive (*Olea europaea* L.) cultivar during fruit development and ripening

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“Arauco” is the only olive (*Olea europaea* L.) cultivar recognized from Argentina in the World Catalogue of Olive Varieties. Drupes from this cultivar were handpicked at different stages of fruit development and ripening—referred to as days after full flowering (DAFF)—and analyzed to assess the oil accumulation pattern, and the dynamic of the changes in fatty acid, tocopherol, squalene, and phenolic compositions. The most intense oil accumulation period occurred approximately between 80 DAFF (pit hardening) and 150 DAFF (beginning of fruit maturation). Oleic acid content increased rapidly until reaching the maximum concentration (71.4%) approximately 80 DAFF, thus indicating that the most active biosynthesis period occurred at early fruit growth stages. Later, oleic acid content decreased gradually, almost linearly, to 62% at the final sampling point (215 DAFF). The dynamic of tocopherol accumulation showed a trend characterized by high amounts of the  $\alpha$ - and  $\gamma$ -isoforms in very young drupes, a pronounced decrease during fruit development, and little or no change during the fruit maturation period. The developing drupes were found to accumulate significant amounts of squalene (up to 12 500 mg/kg of dry weight at 96 DAFF), but squalene dropped strongly after the beginning of fruit ripening. Whatever the stage of fruit development, secoiridoids were the major phenolic components. On the basis of the evolution of the analytical parameters studied, the best stage for processing “Arauco” fruits for oil production seems to be that where maturity index is higher than 1 and lower than 2.

**Practical applications:** The study focuses on biochemical aspects of oil biogenesis and minor antioxidant compounds from the cv. “Arauco” during fruit ontogeny. The results show that this cultivar has profitable characteristics for commercial production of VOO. From a practical standpoint, results also contribute to better assessment of harvest time. Findings remark on the convenience of harvesting early (at a low fruit maturity index) to get the best combination of oil quantity and quality.

**Keywords:** Antioxidant compounds / Fatty acid composition / Fruit ontogeny / Oil biogenesis / Olive (cv. “Arauco”)

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**Abbreviations:** DAFF, days after full flowering; FA, fatty acid; LA, linoleic acid; LnA, linolenic acid; MI, maturity index; OA, oleic acid; TPC, total phenol content; TT, total tocopherol; VOO, virgin olive oil

## 1 Introduction

The olive tree has been traditionally cultivated in countries from the Mediterranean Basin. In recent years, interest in olive oil production and consumption has expanded olive cultivation to countries outside the Mediterranean region, such as South America, Australia, South Africa, the USA, and China [1].

In the last two decades there has been a significant increase in olive production from Argentina. Until 1990, the

olive tree cultivation covered a total area of approximately 30 000 ha. Currently, there are about 110 000 ha under cultivation mainly in the Central-Western and North-Western regions of the country. “Arbequina,” “Manzanilla,” “Picual,” and “Frantoio” are the most extensively planted foreign cultivars. In average, 70% of the total olive production is devoted to oil production being “Arbequina” the most important cultivar for this purpose.

“Arauco” is the only cultivar recognized from Argentina in the World Catalogue of Olive Varieties [2]. Until late 1990’s, it was the most cultivated table olive cultivar in this country mainly due to its good commercial size and high flesh-to-pit ratio [3]. Nevertheless, the physical characteristics of the fruit from this cultivar may vary considerably depending upon the region in which it is cultivated. At maturity, fruits from “Arauco” have relatively high oil content. This fact and a well-balanced fatty acid composition could make cv. “Arauco” suitable for industrial oil production.

Olive fruit development and ripening last several months during which oil synthesis and accumulation take place. Olive oil synthesis comprises a combination of physiological and biochemical events that occur under strict genetic control and influence of several environmental conditions. In general, the most intense oil accumulation period occurs during the mesocarp development, after the pit hardening. During the fruit ripening period, dry matter continues to increase along with oil synthesis, although at a slower rate [4]. Several authors have studied the influence of genetic, environmental and agronomic factors on olive oil yield and quality in order to establish the optimum harvesting period [5–9]. For oil mill purposes the better harvesting time should be when the olives achieve their highest oil content and the oil quality is the best. Olive growers usually use the changes in fruit color (the maturity index, MI) as a guideline to begin the harvest period. However, this parameter is not always valid because fruit maturation and oil accumulation rate may change with the cultivar and environmental conditions.

The importance accorded to olive oil is mainly due to its particular fatty acid composition, but also to its richness in some minor components such as polyphenols, tocopherols, and squalene, which are involved in the preservation of the oil chemical quality. During the olive fruit growth and ripening, the opportunity and the magnitude of the changes in all these chemical components are mainly attributed to the cultivar. For example, in “Sayali” olive cultivar the greatest changes in fatty acid composition occur early during fruit development; only minor variations are found to occur during the fruit ripening [10]. Similarly, Baccouri *et al.* [6] and Damak *et al.* [7] have reported low variation rate in fatty acid composition during the fruit maturation process of the “Chétoui” cultivar. Differently, Baccouri *et al.* [6] found a strong drop in oleic acid content and a significant increase in both palmitic and linoleic acids from other Tunisian (“Chemlali”) cultivar evaluated throughout the entire ripening period. On the other

hand, we have observed (unpublished data) that during the fruit ripening the cv. “Manzanilla” cultivated in Argentina reached the maximum oleic acid content early at MI between 1.5 and 2.5, and thereafter it remains constant until the end of the ripening period. Regarding the cv. “Arauco,” Rondanini *et al.* [11] have found a pronounced linear decrease in oleic acid concentration when it was modeled against thermal time from flowering. Moreover, García-Inza *et al.* [12] have shown that high temperatures during the oil accumulation phase may negatively affect oil yield and quality from this cultivar growing in warm regions.

The tocopherol concentration in olive oils is mainly dependent on the cultivar, and the degree of fruit maturation. The relationship between tocopherols and fruit maturation has been analyzed in several olive cultivars [6, 13, 14]. In general, tocopherol content decreases during ripening, but that decrease takes place at different times and rates depending on the olive cultivar [8]. Squalene is the most abundant component of the hydrocarbon fraction of VOO. The importance of this triterpenic hydrocarbon arises from two main facts: it is the common biosynthetic precursor of a wide range of sterols and non-steroidal triterpenoids occurring in high amounts in the olive fruit, and it is part of a wide arrangement of natural compounds with nutritional and health properties [15–17]. In addition, squalene was reported to play a role in preventing olive oil oxidation [15]. The olive fruit is also a rich source of several phenolic compounds that contribute to oil sensory properties and protect it from oxidative degradation. Phenolic alcohols, phenolic acids, secoiridoids, lignans, and flavonoids are the most important classes of olive phenolic substances [18]. Important qualitative and quantitative differences have been observed among cultivars, fruit ripening degree and agronomic practices related to irrigation [19–22]. Oleuropein appears to be the most abundant polyphenolic compound in the olive fruit, but other secoiridoids derivatives can be also found at high concentrations. An exhaustive survey of the literature shows that no scientific information is available on tocopherol, squalene and phenolic content and composition of the cv. “Arauco.”

There is a general concern about the changes in the olive chemical composition occurring along the fruit development and ripening and their relationship with the oil quality. If fruit development and/or fruit ripening go with increasing fatty acid unsaturation level and decreasing polyphenol and tocopherol amounts, the oil becomes less stable. These changes, in turn, are of high commercial importance as they influence both the sensory characteristics and the shelf life of the oil.

On the basis of the evolution of fruit growth and ripening and oil accumulation patterns, this work primarily aimed to define the optimum harvesting period of cv. “Arauco.” In addition, the study sheds light on the dynamic of the changes in fatty acids and some minor components of olives during the lipogenesis process.

## 2 Materials and methods

### 2.1 Plant material

The field experiment was conducted at the INTA Experimental Station located at San Martín department, San Juan province (31°32' S, 68°25' W, 591 m above sea level). The region is a typical olive growing area in North-Western Argentina. It has an average annual temperature of 17.5°C and an average annual rainfall of 99.8 mm, concentrated during summer. Olive plants (*Olea europaea* L., cv. “Arauco”) used for this study are grown at planting density of 100 trees/ha (tree spacing 10 m × 10 m). They were grown under natural rainfall, plus supplemental irrigation of 800 mm/year. During both 2010/11 and 2011/12 growing seasons, drupes from five 70-year-old trees 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, 300 g of healthy drupes selected from the mid-canopy of the entire perimeter of the tree, were collected.

### 2.2 Histochemical analysis

For each sampling date and each selected tree, 10 drupes were chosen to examine structural aspects of oil accumulation in the mesocarp cells. Small, approximately 3–4 mm<sup>3</sup> cubes were cut from the near median portions of the fruit. These cubes were fixed in FAE (formalin:acetic acid:96% ethanol, 10:5:50, v/v): distilled water (65:35, v/v), cut at 30 µm thickness, rinsed with a diluted solution of NaClO in water (30:70, v/v) and then with distilled water, mounted serially and stained with Oil Red and Astra Blue for direct light microscopy observations. The stained sections were examined under a photomicroscopy (Axiophot, Carl Zeiss, Oberkochen, Germany).

### 2.3 Pit hardening and fruit maturity index

The pit hardening was determined by slicing carefully through the median portion of developing fruits with a scalpel until resistance to cutting through the seed was detected. Fruit maturity was determined using the maturity index (MI) proposed by Beltrán et al. [23]. From each fruit sample, 100-randomly selected fruits were classified into the following categories: (0) olives with intense green or dark green epidermis; (1) olives with yellow or yellowish green epidermis; (2) olives with yellowish epidermis but with reddish spots or areas over less than half of the fruit; (3) olives with reddish or light violet epidermis over more than half of the fruit; (4) olives with black epidermis and totally white pulp; (5) olives with black epidermis and less than 50% purple pulp; (6) olives with black epidermis and violet (more than 50%) or purple pulp; (7) olives with black epidermis and totally dark pulp. With *a* to *h* being the number of fruits in

each category, the MI was calculated using the following equation:

$$MI = \frac{(a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4 + f \times 5 + g \times 6 + h \times 7)}{100}$$

### 2.4 Oil content and composition

Samples (200 g) of whole drupes taken from the entire sampling period were ground (stainless steel knife mill) and lyophilized until complete dehydration. From each lyophilized sample, a 30-g aliquot was extracted with *n*-hexane using a Soxhlet apparatus following the IUPAC Standard Method [24]. The solvent was removed using a rotary vacuum evaporator at 30°C and the oil content was gravimetrically determined. Furthermore, the contribution of seed lipids to the olive oil content and composition was evaluated in drupes from the two first sampling dates. A portion of 50 g of the young drupes was subjected to manual removal of the seeds with the aid of a scalpel. Seeds were separated from the pulp and the oil content and FA composition of both seeds and pulp were determined.

For fatty acid composition, oil samples of 0.5 g were subjected to alkaline saponification (1 N KOH in methanol). Unsaponifiable matter was extracted with *n*-hexane. The FAME of total lipids were obtained using 1 N H<sub>2</sub>SO<sub>4</sub> in methanol and analyzed by GC according to Torres et al. [25]. Separations were made on a Supelcowax 10 (Supelco, Bellefonte, PA, USA) fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Peaks were identified by comparing their retention times with those of pure reference compounds [25].

Tocopherol composition was analyzed using 1-g oil aliquots obtained from lyophilized fruit samples extracted with *n*-hexane at RT in the dark. Tocopherols were identified and quantified by HPLC according to the procedure of Lazzez et al. [8] with some modifications. In brief, samples of 1 g oil were placed into 10 mL volumetric flasks. A quantity of *n*-hexane was added, swirling to dissolve the sample and making up to volume with the same solvent. An aliquot of 20 µL of this solution was injected on to a Supelcosil LC-NH<sub>2</sub>-NP column (25 cm × 4.6 mm, Supelco). The mobile phase was *n*-hexane/ethyl acetate (70/30 v/v) with a flow rate of 1 mL/min. UV detection at 295 nm was performed. Individual tocopherols were identified by comparing their retention times with those of authentic standards (ICN Biomedicals, Costa Mesa, CA). 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 twenty standard solutions with known concentrations.

Squalene determinations were done from 200-mg oil aliquots. Each oil sample was mixed with 0.5 mL *n*-hexane, 1 mL squalane solution (1 mg/mL *n*-hexane) and 1 mL KOH

solution (2N in methanol). After 1 min of vigorous shaking, the mixture was left to react for 10 min (the time required for hydrolysis of glycerides). After decanting, the upper phase (*n*-hexane) was extracted and washed twice (5 mL every time) with ethanol/water (50/50 v/v). The *n*-hexane phase was recovered and used for GC and GC–MS analyses. GC (Perkin-Elmer, Shelton, CT, USA) used a VF–5 ms (Varian, Walnut Creek, CA, USA) capillary column (30 m × 0.25 mm i.d.) coated with a 0.25 μm layer of 5% phenyl, 95% polydimethylsiloxane. The column temperature was set at 270°C, injector and detector temperatures at 290°C, carrier gas N<sub>2</sub> at 1 mL/min. GC–MS (Hewlett-Packard, Palo Alto, CA, USA) used helium (flow rate 1 mL/min) as carrier gas. The column, injector and detector temperatures were as for GC analysis. Squalene was identified by comparison of its mass spectra data with those of the Wiley mass spectra search library. Squalene concentration was calculated on the basis of the standard internal (squalane) concentration.

The total phenol content (TPC) was determined by the Folin–Ciocalteu method according to Torres et al. [25]. Individual phenolic fractions were analyzed according to the procedure previously described by Taticchi et al. [26] modified as follows: 5 g of each lyophilized fruit sample were homogenized with 50 mL of 80% methanol containing 20 mg/L of BHT (butylated hydroxytoluene); the extraction was performed in triplicate. After removal of methanol, the aqueous extract underwent phenol separation by SPE. The SPE procedure was performed by loading 1 mL of the aqueous extract into a 1000 mg Bond Elut Jr-C18 cartridge (Agilent Technologies, USA), using 50 mL of methanol as the eluting solvent. After removing the solvent under vacuum at 30°C, the phenolic extract was recovered and then dissolved in methanol (1 mL). Reverse-phase HPLC analyses of the phenolic extracts were conducted with an Agilent Technologies system Mod. 1100, which was composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, a diode array detector (DAD) and a fluorescence detector (FLD). A Spherisorb column ODS-1 250 × 4.6 mm with a particle size of 5 μm (Phase Separation, Ltd., Deeside, UK), maintained at 25°C, was employed. Sample volume was 20 μL. The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL/min. The gradient was changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, 0% A/100% B in 14 min. This composition was maintained for 10 min and then was returned to the initial conditions and equilibration in 13 min; the total running time was 73 min. Lignans were detected by using the FLD operated at an excitation wavelength of 280 nm and emission at 339 nm, whereas the other compounds were detected by using the DAD with a wavelength of 278 nm (3,4-Dihydroxyphenyl) ethanol (3,4-DHPEA) was obtained from Cayman 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 fruit following the procedure described by Montedoro et al. [27]. 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 [27, 28]. 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 <sup>1</sup>H and <sup>13</sup>C spectra using the same operating conditions reported elsewhere [27, 28].

## 2.5 Analytical indices and sensory descriptive analysis

For analytical indices and sensory analysis determinations, the maturity index of fruit samples used for oil extraction varied between 1 and 1.5. For this purpose, oil samples were obtained by using an Abencor analyzer (MC2 Ingenierías y Sistemas, Sevilla, España). This system reproduces the industrial olive oil extraction process at a laboratory scale and provides data on olive performance during crushing, malaxation and centrifugal solid–liquid separation [29]. It consists of three basic elements: hammer crusher, thermobearer, and pulp centrifuge. Immediately after harvesting, the olives were washed and milled using a stainless steel hammer crusher operated at 3000 rpm. The resulting olive paste was immediately kneaded in the thermobearer at 40 rpm for 30 min at 25°C. Centrifugation of the kneaded paste was performed at 3500 rpm. After centrifugation, the oil phase was decanted in stainless steel containers. The resulting oil samples were stored in amber glass bottles at –20°C until analyses.

Free fatty acid content, peroxide value, and UV absorption characteristics were determined following the analytical methods described in Regulations EEC/2568/91 and later modifications of the European Union Commission [30]. Chlorophyll and carotenoid contents were determined at 670 and 470 nm, respectively, in cyclohexane via specific extinction values using the method of Mínguez-Mosquera et al. [31]. The oxidative stability was measured following the Rancimat (Metrohm, Switzerland) method by using 3-g oil aliquots. Airflow rate was set at 20 L/h and temperature of the heating block was maintained at 98°C. Results corresponded to the break points in the plotted curves and were expressed as induction time in hours.

Sensory evaluation was performed by 12 selected and trained panellists from the Universidad Católica de Cuyo (Argentina) according to the method suggested by COI/T 20/Doc N° 15/Rev 1-1996 [32].

## 2.6 Statistical analyses

Statistical differences between sampling dates were estimated from ANOVA test at the 5% level ( $p \leq 0.05$ ) of significance,

for all parameters evaluated. Whenever ANOVA indicated a significant difference, a pair-wise comparison of means by least significant difference (LSD) was carried out. All statistical analyses were performed using the InfoStat program (InfoStat version 2008, National University of Córdoba, Córdoba, Argentina).

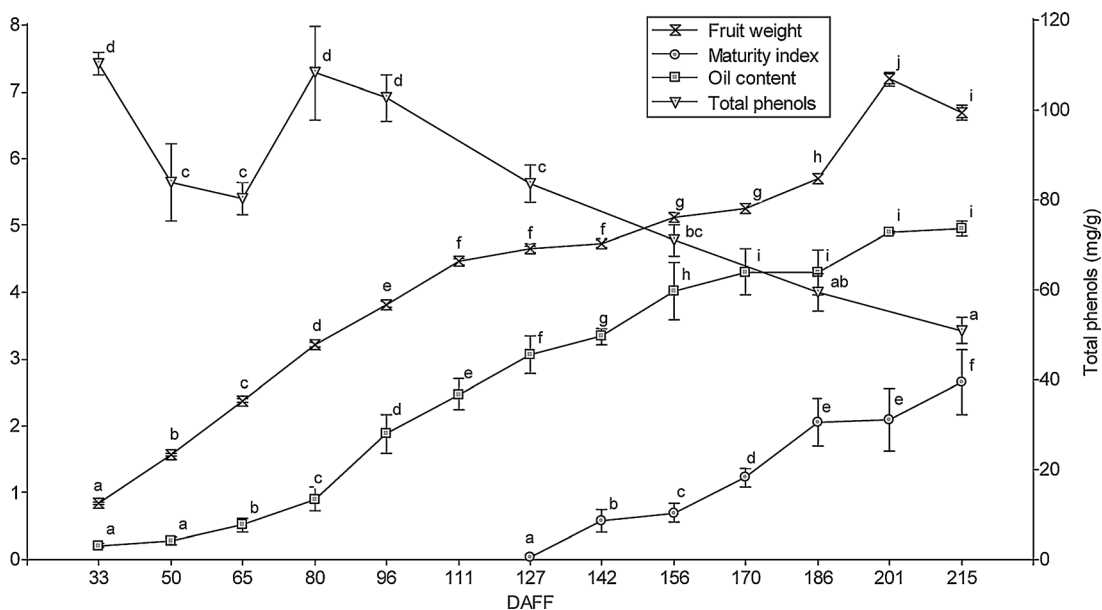
### 3 Results and discussion

Approximately 30 DAFF fruit weight began to increase quickly, almost linearly, until reaching 4.5 g/olive toward 110 DAFF (Fig. 1). After a short period (approximately 4 weeks between 110 and 140 DAFF) during which fruit weight did not vary significantly, a second phase of increment took place and a final fruit weight near 7.5 g/olive was reached at 200 DAFF. Thus, fruit weight evolution adjusted well to a sigmoid type curve, including three main phases. According to Beltrán et al. [23] these phases may be attributed dominantly, but not exclusively, to the following events: (a) the first period of rapid fruit growth due to both intense cell division and enlargement involving mainly growth and development of the endocarp, (b) the pit hardening, during which the endocarp cells stop dividing and fruit growth slows down, (c) the mesocarp enlargement due mainly to the expansion of pre-existing flesh cells.

Table 1 shows the contribution of seed and pulp tissues to both the total lipid content and the fatty acid composition in very young drupes. On a dry weight basis of whole fruit, seed

tissues had smaller oil content than pulp tissues (0.58 vs. 2.07 g/100 g fruit at 33 DAFF, and 0.21 vs. 3.90 g/100 g fruit at 50 DAFF). At the earliest fruit growth stage analyzed, seeds had greater amounts of linoleic acid but minor amounts of linolenic acid than the pulp. Palmitic and oleic acids had similar concentrations in both seeds and pulp. At the time of the second sampling date, the FA composition of whole drupes reflected that found in the pulp. This indicated that from early fruit growth stages the pulp tissues make the major contribution to oil biogenesis. Taking in mind these results, we focused on the analysis of oil accumulation during the mesocarp development. After 30 DAFF, the oil formation was identified by the presence of one, sometimes two or more, small oil bodies per cell. Oil bodies appear to form continuously during mesocarp development (Fig. 2a–c). Fusion between oil bodies was apparent after 90 DAFF (Fig. 2d). At first, small oil droplets form through coalescence of the individual oil bodies. Later, oil droplet enlargement occurs through steady fusion with other minor oil bodies. This phase of oil droplet enlargement seems to occur approximately until 150 DAFF. Finally, the oil occurs mostly as a single, spherical, large droplet (50  $\mu\text{m}$  average diameter) per cell (Fig. 2e).

In very young drupes, small oil bodies were observed in the pericarp cells before than in the mesocarp cells (Fig. 2a) thus suggesting that, at early fruit growth stages, the pericarp was more active than the mesocarp for the relative rate of lipid formation. These observations agree with those from Conde et al. [4] who found that the pericarp cells are capable of



**Figure 1.** Fruit weight (g), maturity index, oil (g/10 g fruit, dry basis), and total phenol (mg/g) contents from “Arauco” olive cultivar at distinct stages of fruit development and ripening, referred to as days after full flowering (DAFF). Each point represents the mean of two crop seasons. For each crop season five independent fruit samples were used. For each parameter, mean values with different superscript letters present significant differences ( $p \leq 0.05$ ) among DAFF.

**Table 1.** Oil content (OC, g/100 g whole fruit, dry basis) and fatty acid composition (FA, % of total fatty acids) from seeds, pulp, and whole fruits of cv. “Arauco” at early fruit development stages, referred to as days after full flowering (DAFF)

Parameter	33 DAFF			50 DAFF		
	Seed	Pulp	Whole fruit	Seed	Pulp	Whole fruit
OC	0.58 ± 0.25	2.07 ± 0.38	2.65 ± 0.51	0.21 ± 0.13	3.90 ± 0.98	4.11 ± 1.02
FA						
Palmitic	21.5 ± 0.34	24.6 ± 0.45	25.8 ± 0.39	19.2 ± 0.19	16.8 ± 0.24	17.6 ± 0.22
Palmitoleic	0.57 ± 0.10	0.31 ± 0.07	0.43 ± 0.09	2.87 ± 0.14	0.46 ± 0.08	0.51 ± 0.09
Stearic	7.78 ± 0.25	3.89 ± 0.23	3.55 ± 0.16	4.74 ± 0.19	2.71 ± 0.16	2.51 ± 0.16
Oleic	22.3 ± 0.28	24.3 ± 0.31	28.1 ± 0.36	28.6 ± 0.42	69.6 ± 1.23	67.1 ± 1.02
Linoleic	37.0 ± 0.77	23.8 ± 0.58	23.2 ± 0.54	39.2 ± 0.87	6.33 ± 0.21	7.35 ± 0.37
Linolenic	10.8 ± 0.51	23.1 ± 0.86	18.0 ± 0.53	4.77 ± 0.27	3.32 ± 0.14	4.30 ± 0.22

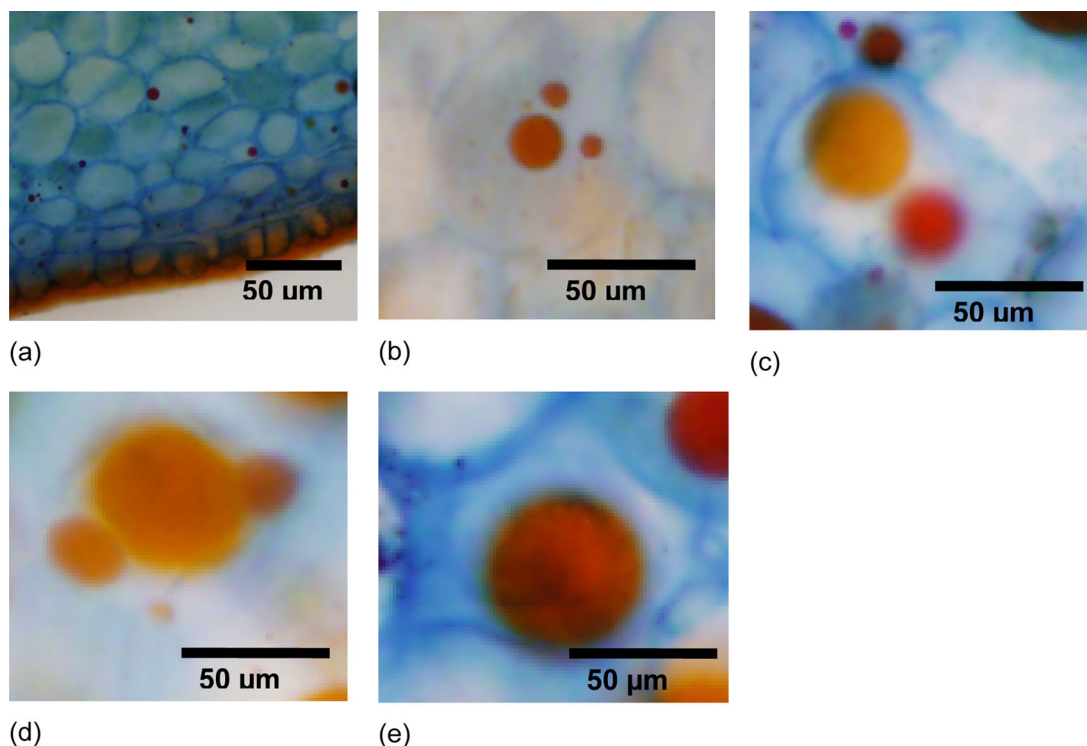
Mean values ± SD. Data are the average of two crop seasons. For each crop season five independent fruit samples were used.

synthesizing different glycerolipids by fixing atmospheric CO<sub>2</sub> in their own chloroplasts.

The formation of oil bodies and their coalescence into large oil droplets in the mesocarp cells from the cv. “Arauco” appears to follow the general pattern observed for other olive cultivars [33]. The absence of oleosin—a specific protein found in oil seeds surrounding the oil bodies—in olive mesocarp

tissues [34] results in coalescence between oil bodies through a rather large surface area (Fig. 2d). It has been suggested that the cytoplasmic coating surrounding the oil bodies becomes thinner to the point where oil to oil interfaces are bared between the oil bodies resulting in the coalescence of the oil phase [33].

In developing olives the oil content increased slowly from 0.26 g/10 g fruit (33 DAFF) until 0.90 g/10 g fruit at the



**Figure 2.** Detail of pulp cells from young drupes of the cv “Arauco”: (a) 33 DAFF, (b) 65 DAFF, (c) 80 DAFF; the oil formation was identified by the presence of one, sometimes two or more, small oil bodies per cell. (d) Formation of the oil droplet by fusion of large oil bodies in mesocarp cells (96 DAFF). (e) Detail of a mesocarp cell showing a single, spherical, large oil droplet (50 µm in diameter, in average) (156 DAFF). Micrographs correspond to light microscopy images of samples stained with Oil Red and Astra Blue.

beginning of the pit-hardening period (approximately 80 DAFF). From this fruit developmental phase, oil synthesis increased quickly and continued until the beginning of the fruit maturation period (Fig. 1). The most intense oil accumulation period occurred approximately between 80 and 150 DAFF. Although the length of this period is similar to those from other olive cultivars [4, 10], in cv. “Arauco” the oil synthesis appears to start earlier. In this cultivar the dynamic of oil accumulation follows a saturation curve, first increasing almost linearly, and then reaching a plateau toward 170 DAFF, at very low (below 1) MI value.

There is no general agreement in the literature about the developmental phase marking the beginning of oil synthesis in the olive fruit. Results from the present study indicate that in cv. “Arauco” oil biogenesis takes place concomitantly with endocarp development and continues during pit hardening, in parallel with oil body formation and coalescence.

Regarding the developmental event marking the end of oil accumulation, the present histological and chemical analyses showed that a single, very expanded oil droplet per mesocarp cell was present at the beginning of the change in color of the epicarp (approximately 150 DAF), and oil content did not increase later, indicating that oil biogenesis was complete just prior to the onset of the fruit maturation period. These findings agree with those from Sánchez and Harwood [35] who found that oil synthesis reaches a plateau at the beginning of the color change of the epicarp. In general, at this stage a single oil droplet per cell is observed [33, 36]. On the contrary, the findings mentioned previously differ from those found in some olive cultivars, such as “Picual,” “Frantoio,” and “Cornicabra” where oil accumulation continues throughout the fruit ripening period [5, 37].

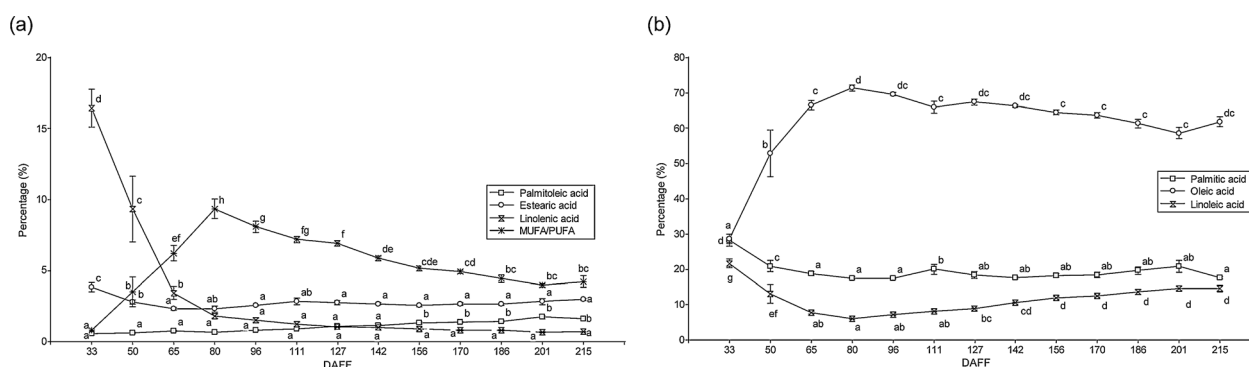
Identified fatty acids (FA) in “Arauco” olive oil were palmitic (hexadecanoic), palmitoleic (*cis*-9-hexadecenoic), margaric (heptadecanoic), stearic (octadecanoic), oleic (*cis*-9-octadecenoic), linoleic (*cis*-9-*cis*-12-octadecadienoic), linolenic (*cis*-9-*cis*-12-*cis*-15-octadecatrienoic), arachidic (eicosa-

noic), and behenic (docosanoic) acids. Palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids were measured as major FA (Fig. 3a and b).

Saturated (palmitic and stearic) FA presented similar evolution patterns: from the time of the first sampling date their concentrations decreased quickly until practically constant values were reached approximately 30 days later. Thus, palmitic and stearic acid contents dropped from mean values of 28% and 3.8% to final values of 17.6% and 2.9%, respectively. These final values were observed at early fruit growth stages. There were not significant variations in the concentrations of both saturated FA during the ripening period evaluated. The general trends mentioned previously differ partially from results described from a number of olive cultivars [6, 23, 38] in which the levels of the two major saturated FA fall during fruit maturation. Regarding the concentration of palmitoleic acid, which is formed by desaturation of palmitic acid, an increasing tendency was observed from the beginning of the fruit maturation process.

As shown in Figure 3b the young drupes (33 DAFF) exhibited a FA composition with oleic, linoleic and linolenic acids (OA, LA, and LnA, respectively) showing percentages around 28.6%, 21.6%, and 16.5%, respectively. During the course of fruit growth and ripening, the accumulation rates of OA and LA presented an opposite, well-known tendency, which has been explained by the activity of the enzyme oleate desaturase transforming OA into LA. Oleic acid content increased rapidly until reaching the maximum concentration (71.4%) approximately 80 DAFF, thus indicating that the most active biosynthesis period occurred at early fruit growth stages. Later, OA content decreased gradually, almost linearly, to 62% at the final sampling point (215 DAFF).

The highest values of both LA and LnA were found at the beginning of fruit development. Linoleic acid accumulation pattern showed a strong decrease approximately until 80 DAFF; then, its content increased linearly to final values near 10% at early ripening stages. The contribution of oleate



**Figure 3.** Evolution of fatty acid concentrations (% of total fatty acids) and monounsaturated to PUFA ratio (MUFA/PUFA) from cv. “Arauco” at distinct stages of fruit development and ripening, referred to as days after full flowering (DAFF). Each point represents the mean of two crop seasons. For each crop season five independent fruit samples were used. For each parameter, mean values with different superscript letters present significant differences ( $p \leq 0.05$ ) among DAFF.

desaturases to the increase in LA levels during the final period of fruit development has been studied by Hernández *et al.* [39], who found that LA increments correlated positively with enhanced expression of FAD2-2 oleate desaturase gene in mesocarp cells. The MUFA/PUFA ratio (Fig. 3a) increased linearly until 80 DAFF and then decreased progressively as a result of the slight decrease in OA and the greater increase in LA. The young drupes (33 DAFF) had unusually high contents of LnA (near 16%), but they dropped rapidly until 80 DAFF. Later, they declined slowly until final, constant values below 1%. No significant variations in LnA contents were found throughout the fruit ripening period evaluated.

Most of the studies on tocopherol accumulation in olive oils have focused on  $\alpha$ -tocopherol variations during fruit maturation [6, 8, 13, 40]. Studies on tocopherol biogenesis at early stages of fruit development have been of little concern. Recently, Muzzalupo *et al.* [41] showed a direct relationship between the transcript levels of geranyl-geranyl reductase and tocopherol content in olive fruits. They suggest a role of the enzyme in the synthesis of tocopherols, which were found to increase during the fruit ripening. In cv. "Arauco," high amounts of both  $\alpha$ - and  $\gamma$ -tocopherols (2622 and 2193 mg/kg oil, respectively) were observed at very early fruit developmental stages; a total tocopherol (TT) concentration near to 5000 mg/kg oil was registered in fruit samples collected 50 DAFF (Table 2). Later, a strong decrease in TT content was found to occur, mainly as a result of both  $\alpha$ - and  $\gamma$ -tocopherol decline.  $\alpha$ -Tocopherol content dropped quickly during the first 90 DAF; then, it decreased gradually, almost linearly, until early fruit ripening stages. No significant variations were found throughout the ripening period evaluated.  $\gamma$ -Tocopherol levels decreased strongly up to the beginning of the ripening period. Later, they remained practically constant until final harvest. Regarding  $\beta$ -tocopherol contents, signifi-

cantly higher values were found at the two first sampling dates. Afterwards, they decreased to practically constant values and no changes were registered along the fruit maturation period. Thus, the dynamic of tocopherol accumulation in oils from the cv. "Arauco" shows a trend characterized by high amounts of the  $\alpha$ - and  $\gamma$ -isoforms in very young drupes, a pronounced decrease during fruit development, and little or no change during the fruit maturation period.

The concentration of TT in VOO is highly variable (150–450 mg/kg) and depends strongly on the olive cultivar [14]. There is a general concern that  $\alpha$ -tocopherol is by far the most abundant isoform representing more than 90% of the TT content, followed by  $\gamma$ -tocopherol and minor amounts of  $\beta$ -tocopherol. However, tocopherol concentrations higher than 600 mg/kg oil have been found in some Italian cultivars where  $\gamma$ - and  $\beta$ -tocopherols together may account for 30% of the TT content [41]. At the time of the final sampling date analyzed, where the fruit MI was around 2.7, the oil from cv. "Arauco" had 448.4, 75.3, and 87.9 mg/kg of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherol, respectively. Hence, according to the previous statements, "Arauco" oil is at the top of olive oils with the highest concentration of both  $\alpha$ -tocopherol and TT contents.

Squalene is the major olive oil hydrocarbon. It is found to occur in concentrations ranging widely (1250–10 500 mg/kg oil). Remarkably influence of both the genotype and the fruit maturity on squalene content was reported in olive oils from several geographic origins [6, 42, 43]. At the earliest sampling date, the squalene content was the lowest (3426.5 mg/kg oil), but increased strongly in oils from drupes collected until 96 DAFF. From this time until 127 DAFF, the squalene levels remained constant. Then, they decreased progressively until final, constant values around 7000 mg/kg oil after 200 DAFF. The general trend described previously agrees partially with that reported by Sakouhi *et al.* [43] who found that squalene

**Table 2.** Concentrations of tocopherols and squalene (mg/kg oil) from drupes of cv. "Arauco" at distinct stages of fruit development and ripening, referred to as days after full flowering (DAFF)

DAFF	$\alpha$ -Tocopherol	$\beta$ -Tocopherol	$\gamma$ -Tocopherol	Total Tocopherol	Squalene
50	2622.9 ± 94.5g	427.2 ± 13.6c	2193.4 ± 95.9f	5009.3 ± 19.8g	3426.5 ± 83.5a
65	1651.9 ± 27.7f	157.7 ± 67.8b	1147.8 ± 32.4e	2957.5 ± 48.7f	8503.6 ± 268.6bcd
80	1243.5 ± 17.3e	67.5 ± 8.6a	666.6 ± 32.0d	1977.6 ± 45.7e	11149.7 ± 277.5efg
96	926.9 ± 20.3d	72.2 ± 11.8a	311.5 ± 24.0c	1338.7 ± 42.4d	12546.4 ± 364.3g
111	910.1 ± 15.3d	67.5 ± 10.1	141.6 ± 18.4	1119.3 ± 15.4cd	11673.4 ± 154.7f
127	717.6 ± 14.8cd	69.0 ± 7.3a	94.1 ± 12.7a	910.6 ± 20.4bc	11833.7 ± 238.7f
142	610.9 ± 15.3bc	77.3 ± 6.7a	87.8 ± 2.6a	805.4 ± 13.6abc	10426.3 ± 152.3de
156	502.2 ± 17.5b	68.4 ± 5.8a	85.7 ± 2.5a	656.4 ± 17.9ab	10436.5 ± 190.2de
170	436.9 ± 11.6ab	69.2 ± 8.4a	85.3 ± 2.3a	591.6 ± 11.0ab	9286.8 ± 156.0cde
186	425.5 ± 10.1ab	70.8 ± 9.7a	85.5 ± 1.8a	581.91 ± 12.3ab	8867.1 ± 84.2cd
201	329.6 ± 7.0a	68.2 ± 7.4a	82.4 ± 1.2a	468.9 ± 9.6a	7624.8 ± 40.9b
215	448.4 ± 4.7ab	75.3 ± 4.8a	87.9 ± 1.7a	631.7 ± 6.7ab	6568.8 ± 102.0b

Mean values ± SD. Data are the average of two crop seasons. For each crop season five independent fruit samples were used. Values in each column with different letters present significant differences ( $p \leq 0.05$ ) among DAFF.



concentration peaked at 21 weeks after the flowering date (WAFD), decreased strongly between 21 and 26 WAFD, and declined slowly during the fruit ripening period. In olive fruits, squalene is the common precursor of both sterols and triterpenoids compounds via the acetate/mevalonate pathway. So, the increasing squalene concentration in developing olive fruits may be the result of an increased synthesis leading to enhance sterols and triterpenoids, which are progressively accumulated at the onset of fruit development [16].

Total phenol content (TPC, as measured by the Folin–Ciocalteu reagent) dropped markedly from the first picking date until 65 DAFF (Fig. 1). Afterwards, it increased significantly reaching the highest value at the beginning of the pit hardening period. From this time, TPC decreased regularly showing the lower levels at latter sampling dates. This general trend matches well with those observed in some Spanish [44] and Italian [45] olive cultivars. Likewise, at the time of the final sampling, the TPC from “Arauco” fruits reached similar values to those obtained from fruits of both “Arbequina” and “Farga” cultivars with MI around 2 [44].

“Arauco” fruits contained different classes of phenolic compounds such as phenolic alcohols, secoiridoids, lignans, and verbascoside (Table 3). Phenolic alcohols (tyrosol or *p*-HPEA, and hydroxytyrosol or 3,4-DHPEA) presented significant changes among the sampling dates evaluated. Considering both 2010/11 and 2011/12 crop seasons, the average concentrations varied from 1.35 to 2.91 mg/g drupes (3,4-DHPEA), and from 0.44 to 1.16 mg/g drupes

(*p*-HPEA). The highest ones were observed at the beginning of the fruit ripening period, and were higher than those obtained from fruits of several olive cultivars such as “Arbequina,” “Farga,” and “Morrut” evaluated from fruit setting to harvest time [44]. In agreement with results found in drupes from many olive cultivars [6, 7, 44], secoiridoids compounds were the major phenolic components in “Arauco” olive fruits. 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 and oleuropein were the largest, ranging between 30.2–69.6 mg/g drupes and 4.42–36.6 mg/g drupes, respectively. It is remarkable the occurrence of high amounts of both 3,4-DHPEA-EDA and oleuropein at the earliest fruit developmental stage; this behavior 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. Likewise, it is noteworthy the prevalence of 3,4-DHPEA-EDA instead of oleuropein; this phenolic pattern is not common in other olive cultivars [45]. Anyway, both 3,4-DHPEA-EDA and oleuropein contents decreased significantly throughout the sampling period evaluated. Oleuropein decline is often accompanied by the accumulation of demethyloleuropein and elenolic acid glucoside [44, 45]; however, these latter were not detected at

**Table 3.** Concentrations of phenolic compounds (mg/g) from drupes (dry weight) of cv. “Arauco” at distinct stages of fruit development and ripening, referred to as days after full flowering (DAFF)

	DAFF			
	29	63	126	184
CS 2010/2011				
3,4-DHPEA	1.04 ± 0.22a	1.99 ± 0.76ab	2.84 ± 1.03b	0.89 ± 0.22a
<i>p</i> -HPEA	0.44 ± 0.11a	1.46 ± 0.77b	1.55 ± 0.25b	0.38 ± 0.08a
Verbascoside	ND	ND	4.50 ± 0.76a	5.91 ± 0.21b
3,4-DHPEA-EDA	64.4 ± 15.6c	45.1 ± 2.98b	41.3 ± 2.66b	23.8 ± 3.49a
Oleuropein	43.3 ± 13.1c	7.24 ± 1.90b	2.78 ± 0.18a	4.70 ± 0.84a
<i>p</i> -HPEA-EDA	5.81 ± 0.60c	3.43 ± 0.71b	2.25 ± 1.91a	ND
Acetoxypinoresinol	0.10 ± 0.03a	0.09 ± 0.03a	0.08 ± 0.01a	0.08 ± 0.01a
Pinoresinol	0.12 ± 0.03c	0.10 ± 0.03bc	0.05 ± 0.02a	0.05 ± 0.02a
Σ phenolic compounds	115.3 ± 28.5c	59.4 ± 6.21b	53.4 ± 6.16b	33.8 ± 7.27a
CS 2011/2012				
3,4-DHPEA	1.66 ± 0.15a	1.96 ± 0.33a	2.98 ± 1.29a	1.81 ± 0.54a
<i>p</i> -HPEA	1.28 ± 0.22b	1.45 ± 0.31b	1.56 ± 0.32b	0.50 ± 0.18a
Verbascoside	ND	ND	10.2 ± 3.54a	11.1 ± 3.55a
3,4-DHPEA-EDA	74.7 ± 9.73c	52.3 ± 9.42b	43.9 ± 9.80a	36.6 ± 4.37a
Oleuropein	29.2 ± 4.25c	7.78 ± 1.80b	4.85 ± 0.90a	4.15 ± 0.68a
<i>p</i> -HPEA-EDA	7.42 ± 1.26c	2.85 ± 0.84b	0.75 ± 0.001a	ND
Acetoxypinoresinol	0.10 ± 0.001a	0.10 ± 0.001a	0.10 ± 0.001a	0.10 ± 0.001a
Pinoresinol	0.10 ± 0.01a	0.10 ± 0.01a	0.10 ± 0.01a	0.07 ± 0.05a
Σ phenolic compounds	114.5 ± 11.7c	66.6 ± 2.40b	64.0 ± 1.10b	54.5 ± 3.68a

Mean values ± SD. For each crop season (CS) five independent fruit samples were used. Values in each row with different letters present significant differences ( $p \leq 0.05$ ) among DAFF. ND, not detected.

any fruit growth and ripening stages evaluated. Lignans included (+)-1-acetoxypinoresinol and (+)-1-pinoresinol. They were found at very low concentrations and showed minor variations during fruit physiological development. Verbascoside, a hydroxycinnamic acid derivative, was not detected at early fruit growth stages. Latter, it reached concentrations practically constant at the beginning of the fruit maturation period. *p*-HPEA-EDA showed an opposite trend with higher values in very young drupes.

The range of variation of phenolic compounds in olive fruits is really high and a marked influence of the genotype is widely reported [6, 19, 20]. Data obtained in the present study are in general agreement with those reported from some olive cultivars where phenolic content drops during fruit growth and ripening mainly as a result of a strong decrease in the concentration of secoiridoids components.

Table 4 shows the analytical parameters and chemical composition of “Arauco” oil extracted from fruits with MI varying between 1 and 1.5 by using the Abencor system. Considering the average analytical values obtained the oil

**Table 4.** Chemical and sensorial parameters<sup>a</sup> of “Arauco” olive oil extracted from whole fruits with maturity index varying between 1 and 1.5 by using the Abencor system

Free acidity (oleic acid %)	0.08 ± 0.01
Peroxide value (meq O <sub>2</sub> /kg)	5.60 ± 0.95
K <sub>232</sub>	1.90 ± 0.13
K <sub>270</sub>	0.20 ± 0.04
Fatty acids (%)	
Palmitic	18.6 ± 0.98
Palmitoleic	1.22 ± 0.21
Stearic	3.08 ± 0.15
Oleic	67.0 ± 1.11
Linoleic	9.31 ± 0.77
Linolenic	0.90 ± 0.02
MUFA/PUFA <sup>b</sup>	6.35 ± 0.57
Total tocopherols (mg/kg)	728 ± 70.5
α-Tocopherol (mg/kg)	562.0 ± 6.63
β-Tocopherol (mg/kg)	85.0 ± 4.20
γ-Tocopherol (mg/kg)	81.0 ± 1.14
Squalene (mg/kg)	1342.1 ± 76.2
Total phenols (mg/kg)	437.3 ± 17.5
<i>o</i> -Diphenols (mg/kg)	30.0 ± 6.46
Chlorophylls (mg/kg)	45.0 ± 7.74
Carotenoids (mg/kg)	22.2 ± 2.86
Oxidative stability index (h)	63.0 ± 1.70
Sensory quality	
Median of defects	0
Median of fruity	4.0 ± 0.0
Median of bitter	4.87 ± 0.25
Median of pungent	4.25 ± 0.29
Panel test classification	Extra virgin

<sup>a</sup>Mean values ± SD. Data are the average of two crop years. For each crop year five independent fruit samples were used.

<sup>b</sup>MUFA, PUFA.

was classed as “extra-virgin” because the acidity, peroxide, K<sub>232</sub>, and K<sub>270</sub> values, as well as sensory characteristics, were within the limits stated by Regulation EEC/2568/91 and latter modifications of the European Community [30] (acidity ≤ 0.8°, peroxide index ≤ 20 meq O<sub>2</sub>/kg, K<sub>232</sub> ≤ 2.5, K<sub>270</sub> ≤ 0.22). Regarding sensory quality, the median of defects was equal to 0. A good balance of fruity, bitter and pungent attributes was found with higher intensities for bitter; this latter has been mainly related to high amounts of secoiridoid phenolic compounds [46]. As regards FA composition the data differed largely from those obtained by Rondanini et al. [47]. The differences may be explained, in part, because of the effect of higher MI of the fruits they used (2.7) which gave oils with lower OA content (54.9%). In the present study, we also found that cv. “Arauco” contained higher amounts of both tocopherols and phenolic compounds as compared with several Spanish, Italian and Tunisian olive cultivars [14, 43]. All these chemical components contributed to good oil oxidative stability values, which resulted higher than those from some typical oil-producing cultivars [6, 14].

It is well-known that climate has a major influence on olive fruit growth and ripening, and hence on both the oil accumulation and the chemical composition. In Argentina, the cv. “Arauco” is cultivated mainly in the Western region of the country (La Rioja, San Juan, and Mendoza provinces). The climate at this region is considered Mediterranean with continental influence. Rainfalls occur almost exclusively in summer, and daily and annual temperature variations are greater as compared with those from the typical Mediterranean climate, such as that of the countries from the Mediterranean Basin. The cv. “Arauco” is not cultivated at this latter region. So, it is not possible to speculate on the effects of climate by comparing oils from this cultivar growing under the contrasting environmental conditions mentioned before. Nevertheless, it is clear that the oil synthesis period in olive varieties grown in Western Argentina is warmer than that from the Mediterranean countries [11, 12]. This fact could affect the oil accumulation rate and FA composition. Recently, García-Inza et al. [12] have found that high temperatures during the oil synthesis phase may negatively affect oil and oleic acid contents of “Arauco” fruits. These data and other from Rondanini et al. [11] suggest that this cultivar could reach higher oil yields and improved FA composition (higher amounts of oleic acid) if it is cultivated under milder summer temperature conditions.

## 4 Conclusions

This work shows that cv. “Arauco” has characteristic fruit growth and ripening rates, which are related to distinctive oil accumulation and composition patterns. In this olive cultivar, color changes associated with fruit ripening occur slowly whereas oil chemical changes, especially those related to fatty acid and tocopherol compositions, take place at higher rates

from early stages of fruit ontogeny. From a practical standpoint, the early OA decline, at very low MI level (<1), prior to attainment of the maximum oil potential, may be considered as a drawback in order to establish the optimum harvest time. The attainment of the highest fruit oil content is reached just prior to the onset of the turning color stage. At this stage, OA content decreases to values near 67.5%, which are approximately 4% below the highest concentration reached (71.4%) at early (80 DAFF) fruit growth stages. Regarding antioxidant components, important reductions were found to occur in both tocopherol and phenolic concentrations throughout fruit ontogeny. Whereas tocopherols decreased strongly and regularly during fruit development, total phenols showed higher values at both the first sampling date and the pit hardening period, and a progressive decrease at latter samplings. Nevertheless, oils obtained from fruits with MI between 1 and 1.5 contained high amounts of tocopherols and moderate levels of squalene and phenolic compounds. In summary, results obtained in this work demonstrate that the cv. “Arauco” has profitable characteristics for commercial production of VOO. Data contribute to better assessment of harvest time in order to maximize oil content and quality. On the basis of the evolution of the analytical parameters studied the best stage for processing “Arauco” fruits for oil production seems to be that where MI is higher than 1 and lower than 2.

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## References

- [1] Civantos, L., in: D. Barranco, R. Fernández-Escobar, L. Rallo, (Eds.), *El cultivo del olivo*, Mundi Prensa, Madrid (Spain) 2008, pp. 17–35.
- [2] IOOC: International Olive Oil Council, *World Catalogue of Olive Varieties*, Madrid (Spain) 2000.
- [3] Garrido, A., Fernández, M. J., Fernández, Díez, M. R., in: A. S. Garrido Fernández, M. J. Fernández Díez, M. R. Adams, (Eds.), *Table olives. Production and Processing*, Chapman & Hall, London, United Kingdom 1997, pp. 23–45.
- [4] Conde, C., Delrot, S., Gerós, H., Physiological, biochemical and molecular changes occurring during olive development and ripening. *J. Plant Physiol.* 2008, 165, 1545–1562.
- [5] Beltrán, G., del Río, C., Sánchez, S., Martínez, L., Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. *J. Sci. Food Agric.* 2004, 84, 1783–1790.
- [6] Baccouri, O., Guerfel, M., Baccouri, B., Cerrretani, L. et al., Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. *Food Chem.* 2008, 109, 743–754.
- [7] Damak, N., Bouaziz, M., Ayadi, M., Sayadi, S., Damak, M., Effect of the maturation process on the phenolic fractions, fatty acids, and antioxidant activity of the Chétoui olive fruit cultivar. *J. Agric. Food Chem.* 2008, 56, 1560–1566.
- [8] Lazzez, A., Perri, E., Caravita, M. A., Khlif, M., Cossentini, M., Influence of olive maturity stage and geographical origin on some minor components in virgin olive oil of the Chemlali variety. *J. Agric. Food Chem.* 2008, 53, 982–988.
- [9] Dag, A., Kerem, Z., Yogev, N., Zipori, I. et al., Influence of time of harvest and maturity index on olive oil yield and quality. *Sci. Hortic.* 2011, 127, 358–366.
- [10] Sakouhi, F., Herchi, W., Sebei, K., Absalon, C. et al., Accumulation of total lipids, fatty acids and triacylglycerols in developing fruits of *Olea europaea* L. *Sci. Hortic.* 2011, 132, 7–11.
- [11] Rondanini, D. P., Castro, D. N., Searles, P. S., Rousseaux, M. C., Contrasting patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. *Eur. J. Agron.* 2014, 52, 237–246.
- [12] García-Inza, G. P., Castro, D. N., Hall, A. J., Rousseaux, M. C., Responses to temperature of fruit dry weight, oil concentration, and fatty acid composition in olive (*Olea europaea* L. var. ‘Arauco’). *Eur. J. Agron.* 2014, 54, 107–115.
- [13] Beltrán, G., Aguilera, M. P., del Río, C., Sánchez, S., Martínez, L., Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chem.* 2005, 89, 207–215.
- [14] Uceda, M., Hermoso, M., Aguilera, M. P., in: D. Barranco, R. Fernández-Escobar, L. Rallo, (Eds.), *El cultivo del olivo*, Mundi Prensa, Madrid (Spain) 2008, pp. 699–727.
- [15] Owen, R. W., Mier, W., Giacosa, A., Hull, W. E. et al., Phenolic compounds and squalene in olive oils: The concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food. Chem. Toxicol.* 2000, 38, 647–659.
- [16] Stiti, N., Triki, S., Hartmann, M. A., Formation of triterpenoids throughout *Olea europaea* fruit ontogeny. *Lipids* 2007, 42, 55–67.
- [17] Szakiel, A., Paczkowski, C., Pensec, F., Bertsch, C., Fruit cuticular waxes as a source of biologically active triterpenoids. *Phytochem. Rev.* 2012, 11, 263–284.
- [18] Servili, M., Selvaggini, R., Esposito, S., Taticchi, A. et al., Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. *J. Chrom. A* 2004, 1054, 113–127.
- [19] Morelló, J. R., Romero, M. P., Motilva, M. J., Effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga, and Morrut cultivars. *J. Agric. Food Chem.* 2004, 52, 6002–6009.
- [20] Vinha, A. F., Ferreres, F., Silva, B. M., Valentão, P. et al., Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. *Food Chem.* 2005, 59, 561–568.
- [21] Servili, M., Esposito, S., Lodolini, E., Selvaggini, R. et al., Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils Cv. Leccino. *J. Agric. Food Chem.* 2007, 55, 6609–6618.

- [22] Pierantozzi, P., Torres, M., Lavee, S., Maestri, D., Vegetative and reproductive responses, oil yield and composition from olive trees (*Olea europaea* L.) under contrasting water availability during the dry winter–spring period in central Argentina. *An. Appl. Biol.* 2014, 164, 116–127.
- [23] Beltrán, G., Uceda, M., Hermoso, M., Frías, L., in: D. Barranco, R. Fernández-Escobar, L. Rallo, (Eds.), *El cultivo del olivo*, Mundi Prensa, Madrid (Spain) 2008, pp. 163–187.
- [24] IUPAC: Standard Methods for the Analysis of Oils, Fats and Derivates. in: C. Paquot, A. Hautffenne, (Eds.), *International Union of Pure and Applied Chemistry* Blackwell Scientific Publications Inc., Oxford United Kingdom 1992.
- [25] Torres, M. M., Pierantozzi, P., Cáceres, M. E., Labombarda, P. et al., chemical assessment of Arbequina olive cultivar grown in Córdoba province, Argentina. *J. Sci. Food Agric.* 2009, 89, 523–530.
- [26] Taticchi, A., Esposto, S., Veneziani, G., Urbani, S. et al., The influence of the malaxation temperature on the activity of polyphenoloxidase and peroxidase and on the phenolic composition of virgin olive oil. *Food Chem.* 2013, 136, 975–983.
- [27] Montedoro, G. F., Servili, M., Baldioli, M., Selvaggini, R. et al., Simple and hydrolyzable compounds in virgin olive oil 3: Spectroscopic characterization of the secoiridoids derivatives. *J. Agric. Food Chem.* 1993, 41, 2228–2234.
- [28] Servili, M., Baldioli, M., Selvaggini, R., Macchioni, A., Montedoro, G. F., Phenolic compounds of olive fruit: One- and two-dimensional Nuclear Magnetic Resonance characterization of nuzhenide and its distribution in the constitutive parts of fruit. *J. Agric. Food Chem.* 1999, 47, 12–18.
- [29] COI/OH/Doc N° 1/November 2011. *Guide for the determination of the characteristics of oil-olives*, International Olive Council, Madrid (España) 1996.
- [30] *EEC: Characteristics of olive and olive pomace oils and their analytical methods. Regulation EEC/2568/91 and latter modifications*. Official Journal of the European Community, L248. 1991.
- [31] Mínguez-Mosquera, M. I., Rejano, L., Gandul, B., Sánchez, A. H., Garrido, J., Colour pigment correlation in virgin olive oil. *J. Am. Oil Chem. Soc.* 1991, 68, 322–337.
- [32] *COI/T 20/Doc N° 15/Rev 1-1996. Reglamento CE 2568/91 Anexo II. Madrid (España)* 1996.
- [33] Rangel, B., Platt, K. A., Thomson, W. W., Ultrastructural aspects of the cytoplasmic origin and accumulation of oil in olive fruit (*Olea europaea*). *Physiol. Plant* 1997, 101, 109–114.
- [34] Ross, J. H. E., Sánchez, J., Millán, F., Murphy, D. J., Differential presence of oleosins in oleogenic seed and mesocarp tissue in olive (*Olea europaea*) and avocado (*Persea americana*). *Plant Sci.* 1993, 93, 203–210.
- [35] Sánchez, J., Harwood, J. L., Biosynthesis of triacylglycerols and volatiles in olive. *Eur. J. Lipid Sci. Technol.* 2002, 104, 564–573.
- [36] Matteucci, M., D'angeli, S., Errico, S., Lamanna, R. et al., Cold affects the transcription of fatty acid desaturases and oil quality in the fruit of *Olea europaea* L. genotypes with different cold hardiness. *J. Exp. Bot.* 2011, 62, 3403–3420.
- [37] Salvador, M. D., Aranda, F., Fregapane, G., Influence of fruit ripening on Cornicabra virgin olive oil quality. A study of four successive crop seasons. *Food Chem.* 2001, 73, 45–53.
- [38] Menz, G., Vriesekoop, F., Physical and chemical changes during the maturation of Gordal Sevillana olives (*Olea europaea* L., cv. Gordal Sevillana). *J. Agric. Food Chem.* 2010, 58, 4934–4938.
- [39] Hernández, M. L., Padilla, M. N., Mancha, M., Martínez-Rivas, J. M., Expression analysis identifies FAD 2–2 as olive oleate desaturase gene mainly responsible for the linoleic acid content in virgin olive oil. *J. Agric. Food Chem.* 2009, 57, 6199–6206.
- [40] Beltrán, G., Jiménez, A., del Río, C., Sánchez, S. et al., Variability of vitamin E in virgin olive oil by agronomical and genetic factors. *J. Food Comp. Anal.* 2010, 23, 633–639.
- [41] Muzzalupo, I., Stefanizzi, F., Perri, E., Chiappetta, A. A., Transcript levels of CHL P gene, antioxidants and chlorophylls contents in olive (*Olea europaea* L.) pericarps: A comparative study on eleven olive cultivars harvested in two ripening stages. *Plant Foods Hum. Nutr.* 2011, 66, 1–10.
- [42] Samaniego-Sánchez, C., Quesada-Granados, J. J., López-García de la Serrana, H., López-Martínez, M. C.,  $\beta$ -Carotene, squalene and waxes determined by chromatographic method in picual extra virgin olive oil obtained by a new cold extraction system. *J. Food Comp. Anal.* 2010, 23, 671–676.
- [43] Sakouhi, F., Herchi, W., Sebei, K., Absalon, C., Boukhchina, S., Characterization and accumulation of squalene and n-alkanes in developing Tunisian *Olea europaea* L. fruits. *Int. J. Food Sci. Technol.* 2011b, 46, 2281–2286.
- [44] Morelló, J. R., Romero, M. P., Ramo, T., Motilva, M. J., Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe (*Olea europaea* L.) from fruit setting period to harvesting time. *Plant Sci.* 2005, 168, 65–72.
- [45] Alagna, F., Mariotti, R., Panara, F., Caporali, S. et al., Olive phenolic compounds: Metabolic and transcriptional profiling during fruit development. *BMC Plant Biol.* 2012, 12, 162–180.
- [46] Angerosa, F., Mostallino, R., Basti, C., Vito, R., Virgin olive oil notes: Their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chem.* 2000, 68, 283–287.
- [47] Rondanini, D. P., Castro, D. N., Searles, P. S., Rousseaux, M. C., Fatty acid profiles of varietal virgin olive oils (*Olea europaea* L.) from mature orchards in warm arid valleys of Northwestern Argentina (La Rioja). *Grasas y Aceites* 2011, 62, 399–409.