Could squalene be an added value to use olive by-products?

Running title: squalene and olive oil

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Abstract

Background: squalene is a hydrocarbon intermediate in the biosynthesis of phytosterols and terpenes in plants. It is widely used for applications such as skin moisturizers, vaccines or carrier for active lipophilic molecules. Commonly obtained from sharks, their capture restrictions point to the need of finding alternative sources. Herein we present a scooping review of studies concerning squalene in olive grove in order to characterize its content and to provide new aspects that may increase the circular economy of this tree.

Results: there is large variation in squalene content in virgin olive oil due to cultivars and agronomical issues such as region, climate, types of soil, crop practices, harvest date. Cultivars with the highest squalene contents in virgin olive oil were Nocellara de Belice, Drobnica, Souri and Oblica. Frequently, an interaction between cultivar and aspects such as irrigation practices or agricultural season is observed. Likewise, the production of high squalene content needs an exquisite control of fruit maturation. Leaves represent an interesting source provided its extraction and yield compensate for the expenses of their disposal. Supercritical carbon dioxide extraction from olive oil deodorizer distillates offers an opportunity of obtaining high purity squalene from this derivative.

Conclusion: exploiting the obtaining of squalene from olive grove for pharmaceutical or cosmetic industries poses new challenges and opportunities to add value and recycle by-products.

Keywords: squalene; olive maturation; olive oil; olive by-products

1. Introduction

Squalene (SQ) is a hydrocarbon, $C_{30}H_{50}$, with six double bounds in its structure and an exact molecular weight of 410.3913 atomic mass units also considered an acyclic triterpenoid[1] whose formula is depicted in Figure 1. Squalene is present in several animal sources, mainly in the shark liver oil which represents its main source of obtaining and where it was originally discovered [2].

Squalene is widely used as skin moisturizer, adjuvant to boost antigenicity of vaccines and carrier for active lipophilic molecules. It has shown to have *in vivo* and *in vitro* antioxidant, anti-inflammatory, anti-atherosclerotic and anti-neoplastic properties, be able to neutralize xenobiotics and be involved in skin aging.[3, 4] These raising interests in its biomedical and industrial uses together with the recent restrictions of shark captures point to the need of finding alternative sources, being plants[4] or yeasts good candidates.[5, 6] Squalene represents an intermediate in the biosynthesis of phytosterols and terpenes in plants[7], and its levels are a balance of squalene synthase and squalene epoxidase activities (Figure 1). Despite the fact of being an intermediate metabolite, some plants accumulate to some extent.[4] Thus, vegetal products containing squalene can be a good choice to obtaining it, and manipulations of those enzymes represent future endeavours to increase its content.

In this regard, virgin olive oil (VOO) directly obtained by milling olive fruit contains a great amount of squalene (1.5 to 10.1 g kg^{-1}).[8-10] Virgin olive oil, as an expensive commodity, is one the most prone to food fraud[11]. Squalene content has also been used to identify frauds in the type of olive oil, falsifications of blended oils marketed as olive oil, and even determine the geographical origin by using its content or combined with other chemical compounds.[12-17] Unfortunately, no recommended analytical method exists to uniformly assess this compound.[18] Few studies have compared the current methods of extraction, excepting the work of Grigoriadou et al. (2007) who evaluated pretreatment of lipid extracts and found no significant differences for the compared procedures- crystallization, saponification and solid-phase extraction.[19]

However, a large variation in squalene content has been reported in these virgin olive oils (1.5 to 10.1 g kg^{-1}).[8-10] To better understand this variability, to know which cultivar is particularly rich in squalene and whether or not other olive tree by-products could also be used for squalene isolation, the present systematic review has been carried out. In order to achieve these goals, two databases, Pubmed and Scopus, have been searched using keywords olive and squalene. 471 hits were identified until July first, 2019, and the search was subsequently refined by eliminating duplicate documents. The resulting 344 papers were critically reviewed according to exclusion criteria reflected in Figure 2. This literature review covers studies related to squalene in olive groove covering aspects such as biological variation regarding cultivars, agronomical practices, presence in olive products, influence of olive milling and preparation (a total of 78 papers).

2. Variability of squalene content in olive oils

The olive tree (*Olea europaea* L.) is one of the most ancient cultivation in the Mediterranean area and its most important product, the olive oil, the main source of fat in the Mediterranean diet. The physicochemical quality and the composition of VOO, in particular of squalene, can be influenced by several factors such as the genotype or plant variety, agronomical and environmental issues -region, climate, types of soil, crop practices, harvest date, etc.-, extraction systems and refining procedures.[20, 21]

Table 1 summarizes the distribution of SQ content in different olive oils according to their cultivars. Figure 3, panels A and B, respectively displays statistical analyses, represented as boxplot and histogram distribution, of 308 individual data from 33 references. The boxplot study excluded three values (outliers) higher than 9.88 g SQ kg^{-1} VOO, and the mean value observed was 4.554 ± 2.095 g kg^{-1} VOO with a wide variation (46% as biological coefficient of variation and a median: 4.340 g SQ kg^{-1} VOO). The minimum and maximum-registered values within two standard deviations were 0.364 and 10.838 g kg^{-1} VOO. If SQ represents 80-90% of unsaponifiable fraction of VOO and the current standard applied to VOO by International Olive Council states that unsaponifiable fraction should be ≤1.5%[22], values of 12.7 g kg^{-1} would be the maximum SQ measurable. Some authors[23, 24] have found values close to theoretical figures in some cultivars.

2.1. The olive tree and its cultivars

Numerous cultivars with certain degree of genotypic heterogeneity exist. Indeed, cultivated varieties of O. *europaea* are mostly diploid (2n = 46), but some triploid, tetraploid and polysomatic (2n = 55) have also been found [25]. Thus, around 2600 cultivars can be counted in the world[25, 26], but their precise identification is hindered by more than 3000 synonyms and homonyms commonly used in areas of traditional cultivation. Likewise, several varieties can be grown for table olives, oil production and dual-purpose. Therefore, a meticulous DNA testing is required to unambiguously establish the nature of different varieties once that its genome has been completely sequenced.[27, 28]

The more than 2600 above-mentioned identified cultivars[25, 26] represent an important source of genetic variation. In addition, its genome sequence has unveiled 20,082 long non-coding RNAs[27] and 498 conserved-miRNA families[28], so an epigenetic regulation is warranted. As consequence, a wide difference in squalene content in their VOO has been reported (Table 1 illustrates this aspect). As mentioned, its levels is a balance of activities of squalene synthase and squalene epoxidase (Figure 1), genes coding for both enzymes have found to be present in olive genome, and squalene synthase mRNA levels to increase during fruit ripening.[28] To analyse the contribution of the genetic variation, only studies carried out using different cultivars in the same agronomical conditions have been taken into consideration in this review in order to minimize epigenetic influences within the same cultivar.

In 18 uncommon Spanish varieties, Beltran et al. observed a range comprised between 1.1 and 8.39 g kg^{-1} of olive oil.[29] Large differences between Arbequina and Picual cultivars were detected (2.866 vs 5.285 g kg^{-1} fruit, respectively).[30] In Italian varieties, the squalene content varied from 0.4 to 11.3 g kg^{-1}.[31-33] In Tunisia or Turkey, this difference among varieties was also observed ranging from 2 to 8.7 g kg^{-1}.[34-37] These data evidence a great difference among cultivars. Interestingly, the olive oils with the highest squalene contents as reflected in Table 2, proceeded from the cultivars Nocellara de Belice, Drobnica, Souri and Oblica. In this way, they represent the most interesting cultivars to produce olive oils rich in this particular compound.

Differences within the same cultivar were also observed[32, 37-42] which could be explained by variables such as different regions with particular environmental-climatic effects, time and year of crop and degree of maturity. All of them were also influencing the outcome in these studies.

Organic farming could represent an advantage for the consumers due to its reduced presence of pesticides and chemical fertilizers in contrast to conventional agriculture that would be in agreement with the reduction of authorized pesticides as proposed by the European Directive 91/414/EEC.[43] However, to date, no significant differences in squalene content were observed analysing olive oils from Koroneiki cultivar in organic and conventional regimens.[38] Nonetheless, further studies are required to establish the real impact of this issue on squalene content.

2.2. Olive fruit and ripening

With an oval shape, measuring from one to four cm in length, their weights vary from two to six grams. In fully developed fruits, the pulp (mesocarp) represents 70-90% of the surface, the pit 9-27% and the seed 2-3%. However, these percentages may considerably vary depending on the variety, state of maturity, tree loads, etc. In the composition of the pulp, the water content represents 500-600 g kg^{-1}, while the oil content varies between 200 and 300 g kg^{-1}.[44]

The growth of the olive fruit is a slow and long process lasting about 200 days after full flowering (DAFF)[23] or 29 weeks after the flowering date (WAFD).[45, 46] During the maturation process, the non-climacteric olive fruit experiments several changes related to compactness, colour, organic acid contents and sensory attributes[44], which contribute to overall quality of the extracted oil. In fact, the change in colour from green, spotty brown, purple and black[40] has been used as a maturity index. Basically, this method classifies 100 randomly taken fruits out of a sample of 1 kg olives according to a colour scale. The latter ranges from 0 (intense green skin) to 7 (black skin and complete purple flesh up to its inner part bordering the pit).[47] Important differences in the degree of maturity exist depending on cultivars in order to obtain high yield oil.[23, 32, 35, 38] Other aspects such as water availability, growing area, climatic and cultivation conditions, temperature and harvest time may condition the maturity stage of fruit as well.[29, 31]

In contrast, far less attention has been paid to squalene content of fruits during ripening. An attempt of comparing the different studies is troublesome considering the above-mentioned variables, that is to say, use of different varieties, different geographical locations, agricultural conditions and harvest period. Although not analysing squalene specifically, Lazzez et al. using Chemlali Tunisian varietal found that maturity stage was the main factor affecting the variability of the unsaponifiable fraction rather the studied geographical areas, harvest seasons and crop years.[48] Considering the average values of the two main Tunisian cultivars at two different indices of maturity, Ben Mansour et al. also observed changes in squalene content in the fruit comparing different months of collection in Chemlali and Oueslati cultivars.[49] With regard to squalene content during fruit maturation, an extensive work was carried out by Bodoira et al. with Arauco cultivar under rainfall and supplemented irrigation conditions in Argentine to analyse its concentration at 13 distinct stages of fruit development from 5 to 31 WAFD.[23] As reflected in Figure 4, the squalene content was the lowest (3.427 g kg^{-1} oil) at the first sampling date (7 WAFD). Later, it sharply increased to peak (12.546 g kg^{-1} oil) at 14 WAFD, around two weeks after pit hardening, then showed a progressive decrease (10.4 g kg^{-1} oil at 21 WAFD, beginning of fruit maturation) to reach 7.0 g kg^{-1} oil at last analysed time-point. Sakouhi et al. studying Meski cultivars in Tunisia observed similar results. Nevertheless, unlike the prior study, these authors began the sampling at the 21st WAFD. At that moment, they found the highest SQ content (12.8 g kg^{-1} oil) which remarkably decreased later on. In fact, the levels were 3.5 g kg^{-1} oil at 26th WAFD and 1.26 g kg^{-1} oil at complete maturity (38th WAFD).[45] Using Italian cultivar, Gentile, Gelmini et al. analysed the squalene content at 3 periods of fruit maturation: 22, 25 and 32 WAFD.[46] They reported values of 1.0, 2.2 and 1.38 g kg^{-1} oil, respectively, which shows a peak at the 25 WAFD. However, their reported amounts seem too low compared to the other studies in Arauco y Meski varieties, what it could be suggestive of lower amount in this cultivar. Recently, a study in Brač Island (Croatia) in cultivars Buhavica, Drobnica, Lastovka and Oblica has reassured the influence of time of collection.[50] Overall, these studies indicate that between weeks 20-25 after flowering (Figure 4) the SQ content falls significantly, which is crucial to obtain the greatest amount of SQ present in olives and their derivate oils. Using 98 data from the different references addressing maturity and squalene, a regression between these two aspects indicates a significant reduction of SQ concentrations (Y g $kg^{-1} = 6.919 - 0.53 * X$; $R^2 = 0.103$; $P<0.0013$) when maturity level increase. Consequently, the production of high squalene content needs an exquisite control of maturation indices for each individual cultivar.

2.3. Irrigation

The results of influence of this aspect on the squalene content in VOO are conflicting. When comparing rain-fed versus irrigated regimens using Chétoui

Tunisia cultivar, Baccouri et al. demonstrated that the squalene content was 34% less in oils from irrigated fields.[32, 39] On the other hand, using Leccino cultivar, Martinelli et al. reported the opposite.[51] Using Picual and Nevadillo negro cultivars, Romero et al. observed that the increase in squalene induced by irrigation vs rainfall was dependent on cultivar.[52] Similar results were found when comparing Leccino and Frantoio cultivars.[53] The latter was insensitive to the effect of irrigation. Using Barnea and Souri cultivars, Ben-Gal et al. also reported a different behaviour between these cultivars, being Barnea more dependent on irrigation than Souri cultivar.[54] Overall, these studies suggest an existence of interaction between genotype and irrigation, although further research is required to clarify the influence of irrigation on the squalene content and to delineate sensitive cultivars.

2.4. Agricultural season

The olive tree is perfectly adapted to the Mediterranean zone, and it is strongly rooted in this geographical location for thousands of years. The olive tree is extremely sensitive to freeze but in contrast much resistant to very high temperatures and lack of rain in non-irrigated fields. However, differences between agricultural campaigns represent an important aspect in dry land olive crops, considering the weather changes involved such as rainfall, temperature, intensity of sun radiation and cold exposure. Using Koroneiki cultivar in the region of Messina, Anastasopoulos et al.[55] analysed squalene content between years 2000 and 2004, and observed a significance difference between results obtained in the year 2000 (3.48-4.06 g kg^{-1}) compared to the 2004 (4.22-4.50 g kg^{-1}). Likewise, Samaniego-Sánchez et al. reported significant changes in squalene concentration in Picual cultivar between years 2001 and 2003 (5.01 vs 6.50 g squalene kg^{-1} oil, respectively).[42] These relevant differences were not observed in Tunisian varieties (Fakhari Douirat, Zarrazi Douirat, Chemlali Tataouine) cultivated in the arid zone of Tataouine.[34] Once again, an interaction between cultivar and agricultural season could exist.

2.5. Types of soil

Since the soil provides all nutrients and minerals to the tree, it may have an influence on squalene content as well. In this regard, analysing Kilis Yaglik cultivar, with similar age trees, Certinkaya et al. showed that soil characteristics namely, total salt content, pH, organic matter, phosphorus and potassium influenced the squalene content of the oil.[56] Further studies are required to support this contribution and to substantiate the influence of all potential components.

3. Table olives

The olive fruit is an appreciated product with great acceptation for elaboration of several dishes, particularly salads, and represents a healthy choice as snack.[57, 58] Not all fruit cultivars are adequate to be used as table olives. As reflected in Table 3, few studies have addressed squalene content in table olives from different sources. In addition, the required debittering process modifies their content. In fact, Sagratini et al. reported dramatic differences in squalene concentrations of table olives of Tenera Ascolana cultivar processed by an industrial purveyor (0.63-1.49 g kg^{-1}) or local farmer (0.54-0.76 g kg^{-1}).[59] When Ambra et al. compared different debittering processes in Nocellara de la Belice cultivar, they observed that the squalene loss was 1% using a solution of 90 g kg^{-1} NaCl in contrast to the 40% loss observed with an equal mix of NaCl and KCl at the same concentration.[60] A lower concentration of 70 g kg^{-1} NaCl also resulted in a 35% squalene loss. These studies point out to a clear influence of the type of salt used to retain their squalene content.

4. Source of squalene in olive products and derivatives

Although virgin olive oil is the foremost reason of cultivation of olive tree, exploring the squalene content in other parts or by-products may revalorize them and contribute to expand the economic value of this plant and reduce generated residues. In this section, leaves, pomace oil or derivatives generated in the process of refining low-quality VOO will be addressed.

4.1. Leaves

Accompanying olive fruits, their amount depends on the used harvest procedure. Easily separated through an inclined plane conveyor belt, they could represent a simple source of squalene or other components. With this purpose, Guinda et al. quantified the main compounds (squalene, β-carotene, α-tocopherol and β-sitosterol) in leaves from five different cultivars. They reported squalene values of 0.152, 0.111, 0.049, 0.046 and 0.038 g kg^{-1} dry matter for Picual, Hojiblanca, Arbequina, Cornicabra and Empeltre cultivars, respectively.[61] Although the amount of squalene was modest, it was obtained from a voluminous residue whose elimination requires olive mills to invest resources. Leaves as source of squalene is an interesting approach provided its extraction and yield compensate for the expenses of this by-product disposal.[61]

4.2. Pomace olive oil

Residual pomaces, dry or wet depending of the VOO extraction procedure, are processed in refineries to extract the remaining pomace olive oil. Due to the high acidity present in these extracts, they should be refined and deodorized.[62] In the traditional procedure, the recovery of the squalene was poor.[63] In order to increase this yield, ultrasounds and molecular distillation have been studied.[64-66] Using the molecular refining, it can be increased up to 740 g kg^{-1} when working with appropriate temperature conditions.[62] By physical refining, Gutiérrez et al. obtained squalene concentrations of 0.13 g kg^{-1} from olive oil deodorizer distillates.[24] Bondioli et al. analysed 10 different types of olive oil deodorizer distillates, and obtained yields of squalene between 224 and 452 g kg^{-1}.[67] This distillate was probably one of the most concentrated squalene preparations, and can be an important squalene source. Further improvements can be obtained by the use of supercritical fluids. In this sense, Catchpole et al. first reported squalene extraction with supercritical carbon dioxide from olive oil deodorizer distillates.[68] Refining the conditions, Fornari et al. obtained a purity 89.4 % with a 642 g kg^{-1} yield [69]. Later trials have further improved the yield up to 750 g kg^{-1} or even 900 g kg^{-1}.[70, 71] Thus, this technique represents an opportunity of obtaining high purity squalene from this derivative. Used for pharmaceutical or cosmetic industries [72, 73], it represents an added value to this by-product.

5. Conclusions

Squalene concentration in virgin olive oil is characteristic of the cultivar and is influenced by fruit ripening, type of soil, irrigation and others parameters (Figure 5). In this sense, cultivars such as Nocellara de Belice, Drobnica, Souri and Oblica should be used to obtain high squalene content olive oils. Although limited information exists centred on Koroneiki cultivar, olive oil squalene content seems not to be influenced by organic compared to conventional agricultural regimens.

The production of high olive oil squalene content needs an exquisite control of maturation indices in order to optimally harvest for each cultivar. Thus, new research regarding this aspect in different cultivars is required to widen our knowledge in order to produce olive fruits with high squalene content either to be used as table olives or olive oil. Further work is also required to characterize the interaction between cultivar and agricultural seasons.

New food processing technologies are required to preserve squalene content in table olives without increasing NaCl concentrations to compromise recommended requirements of dietary salts in humans.

Furthermore, by-products such as leaves and deodorizer distillates extracted with supercritical carbon dioxide may be used to obtain squalene. An optimization of the process to make it profitable would contribute to enhance the circular economy of olive grove.

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Figure 1. Structure and reactions involved in squalene metabolism in olive plant. The proposed pathways are based on information of triterpenoid analyses [7] and genome sequencing data. [28]

Figure 2. Flow chart displaying the stages used to select the references considered. EndNote X7.8 (Bld 11583 Clarivate: New York, NY, USA, 2018). * Some references may appear in more than one section of the review.

Figure 3. Variability of squalene content in virgin olive oils reported in the bibliography. A: Boxplot and whiskers showing three outlier values (n=308), and B: frequency distribution of SQ content in VOO (n=305) without outliers.

Figure 4. Influence of fruit maturation on squalene content. Studies carried out by three different research groups [23, 45, 46] are shown.

Figure 5. Overview of different aspects influencing squalene content in virgin olive oil.

Table 1. Squalene content in olive oil from different cultivars

Cultivar	Sample	Squalene (g kg^{-1})	Location	References
Aberkane	Virgin olive oil	3.750	Algeria	74
Abou Satl Mohazam	Virgin olive oil	2.720 ± 0.190	WOGC*, Córdoba, Spain	29
Adremittion	Virgin olive oil	2.550 ± 0.112	Balikesir, Turkey	75
Agello	Virgin olive oil	2.581	Umbria, Italy	76
Aguenaou	Virgin olive oil	6.770	Algeria	74
Aharoun	Virgin olive oil	3.830	Algeria	74
Aimel	Virgin olive oil	9.720	Algeria	74
Arauco	Olive oil	3.426 – 12.546^{A}	San Martin, Argentina	23
Arbequina	Virgin olive oil	1.526 – 3.495^{C}	Izmir, Turkey	37
Arbequina	Virgin olive oil	1.046	Minas Gerais, Brazil	77
Arbequina	Virgin olive oil	4.046 ± 0.060	Navarra, Spain	75
Arbequina	Virgin olive oil	1.968 – 2.374	Spain	78
Argudell	Virgin olive oil	5.330 ± 0.040	WOGC*, Córdoba, Spain	29
Arroniz	Virgin olive oil	6.420 ± 0.080	WOGC*, Córdoba, Spain	29
Barnea	Virgin olive oil	4.554 ± 0.314	Galilee, Israel	75
Bianca di Villacidro	Virgin olive oil	5.566	Sardinia, Italy	79
Biancolilla	Virgin olive oil	5.180 – 8.950^{A}	Sicily, Italy	32
Blanqueta	Virgin olive oil	4.320	Spain	78
Bosana	Olive oil	13.000 – 16.600^{A}	Sardinia, Italy	20
Bosana	Virgin olive oil	5.940 ± 0.310	WOGC*, Córdoba, Spain	29
Bosana	Virgin olive oil	6.469 ± 0.822	Sardinia, Italy	79
Bouchouk Guergour	Virgin olive oil	7.310	Algeria	74
Buhavica	Olive oil	5.659 – 9.879^{A}	Brač Island, Croatia	50
Bouichret	Virgin olive oil	2.450	Algeria	74
Buza	Virgin olive oil	7.696 ± 0.503	Croatia	80
Calega Vulgar	Virgin olive oil	4.094 ± 0.061	Alentejo, Portugal	75
Carolea	Virgin olive oil	5.730	Italy	78
Carpinetana	Olive oil	5.820 ± 0.670	Abruzzo, Italy	33
Carrasqueño de Porcuna	Virgin olive oil	4.900 ± 0.010	WOGC*, Córdoba, Spain	29

Castellana	Virgin olive oil	8.390 ± 0.150	WOGC*, Córdoba, Spain	29
Cerasoula	Virgin olive oil	1.040 – 4.010[A]	Sicily, Italy	32
Chemlali	Virgin olive oil	3.580	Algeria	74
Chemlali	Virgin olive oil	2.000 – 10.480[A]	Borj-Cédria, Tunisia	32
Chemlali	Virgin olive oil	1.185- 2.232	Kairouan, Tunisia	49, 75, 78
Chemlali-Tataouin	Virgin olive oil	2.521 – 2.896	Tataouin, Tunisia	34, 36
Chétoui	Virgin olive oil	3.580 – 8.270[A]	Tunisia	32
Chiugiana	Virgin olive oil	1.224 ± 0.124	Umbria, Italy	76
Confetto	Virgin olive oil	9.385	Sardinia, Italy	79
Coratina	Virgin olive oil	3.450 ± 0.200	Italy	81
Coratina	Virgin olive oil	4.504 – 4.649	Molise, Italy	31
Coratina	Virgin olive oil	4.659	Sardinia, Italy	79
Coratina	Virgin olive oil	3.472 ± 0.036	Giza, Egypt	82
Cordovés de la Aliseda	Virgin olive oil	7.200 ± 0.050	WOGC*, Córdoba, Spain	29
Cornicabra de Jerez Caballeros	Virgin olive oil	6.900 ± 0.230	WOGC*, Córdoba, Spain	29
Cornicabra Murciana	Virgin olive oil	4.500 ± 0.170	WOGC*, Córdoba, Spain	29, 78
Corsicana da Mensa	Virgin olive oil	5.402	Sardinia, Italy	79
Corsicana da Olio	Virgin olive oil	3.682	Sardinia, Italy	79
Curivelll	Virgin olive oil	2.770 ± 0.090	WOGC*, Córdoba, Spain	29
Dhokar Douirat	Virgin olive oil	2.363	Tataouine, Tunisia	34
Doce Agogia	Virgin olive oil	2.760 ± 0.700	WOGC*, Córdoba, Spain	29
Domat	Virgin olive oil	2.625 ± 0.054	Izmir, Turkey	75
Dritta	Olive oil	3.100 – 11.900[A]	Abruzzo , Italy	20
Dritta	Olive oil	4.840 ± 1.280	Abruzzo , Italy	33
Drobnica	Olive oil	8.748 – 12.860[A]	Korčula Island, Croatia	50, 80
Edremit	Virgin olive oil	3.070- 5.080	Burhaniye, Turkey	63
Fakhari Douirat	Virgin olive oil	4.887- 6.048	Tataouine, Tunisia	34
Figueretes	Virgin olive oil	6.290 ± 1.380	WOGC*, Córdoba, Spain	29
Frantoio	Olive oil	4.600 – 45.100[A]	Lazio-Toscana, Italy	20
Frantoio	Virgin olive oil	4.300	Marche Region, Italy	83
Frantoio	Virgin olive oil	3.620	Sardinia, Italy	79
Gentile	Virgin olive oil	3.429 – 5.099	Molise, Italy	31

Gentile di Chieti	Olive oil	6.180 ± 2.550	Abruzzo, Italy	33
Grignano	Olive oil	8.260 ± 0.750	Veneto, Italy	33
Hojiblanca	Virgin olive oil	4.100 – 4.627	Spain	75, 78
Intosso	Olive oil	7.070 ± 0.730	Abruzzo, Italy	33
Itrana	Virgin olive oil	5.705	Sardinia, Italy	79
Jemri-Bouchouka	Virgin olive oil	2.317 ± 0.010	Tataouin, Tunisia	36
Kalinjot	Virgin olive oil	1.543 ± 0.034	Vlore, Albania	75
Kilis Yaglik	Olive oil	6.900 – 10.400[D]	-	56
Kolovi	Virgin olive oil	3.288 ± 0.004	Lesvos, Greece	75
Koroneiki	Olive oil	3.760 – 4.691[A B]	Messinia, Greece	38, 81
Koroneiki	Virgin olive oil	3.078 ± 0.115	Messinia, Greece	75, 78, 84
Kotruvsi	Virgin olive oil	3.260 ± 0.010	WOGC* of Córdoba, Spain	29
Lastovka	Olive oil	5.196 – 6.029[A]	Korčula Island, Croatia	50
Leccino	Olive oil	5.600 – 58.000[A]	Italy	20
Leccino	Olive oil	5.200 ± 0.360	Abruzzo, Italy	33
Leccino	Virgin olive oil	2.746 – 3.114	Molise, Italy	31
Leccino	Olive oil	4.369	Sardinia, Italy	79
Leccino	Virgin olive oil	3.500 – 5.200	Marche Region, Italy	83
Maelia	Virgin olive oil	2.520 ± 0.250	WOGC*, Córdoba, Spain	29
Majhol	Virgin olive oil	2.830 ± 0.490	WOGC*, Córdoba, Spain	29
Maiorca	Virgin olive oil	5.726	Sardinia, Italy	79
Manzanilla	Virgin olive oil	6.511	Spain	78
Manzanilla- Cacereña	Virgin olive oil	4.445 ± 0.025	Extremadura, Spain	75
Massa Martana	Virgin olive oil	4.786	Umbria, Italy	76
Mavrolia	Virgin olive oil	3.650 ± 0.547	Messinia, Greece	84
Menara	Virgin olive oil	2.990 ± 0.370	WOGC*, Córdoba, Spain	29
Meski	Olive oil	1.265 – 12.801[A]	Bizerte, Tunisia	45
Mixani	Virgin olive oil	6.600 ± 0.100	WOGC*, Córdoba, Spain	29
Morisca de Mancor	Virgin olive oil	6.740 ± 0.100	WOGC*, Córdoba, Spain	29
Nabali Baladi	Olive oil	6.136 ± 0.100	Beni kenaneh, Jordan	85
Nabali Muhassan	Olive oil	8.800 ± 0.080	Beni kenaneh, Jordan	85
Neb Jmel	Virgin olive oil	3.221 – 3.578	Gabes & Kairouan, Tunisia	41

Nocellara de Belice	Virgin olive oil	6.270 – 9.670^{A}	Sicily, Italy	32
Nera di Gonnos	Virgin olive oil	5.831	Sardinia, Italy	79
Nera di Oliena	Virgin olive oil	6.824	Sardinia, Italy	79
Oblica	Olive oil	8.785 – 9.846^{A}	Kaštela, Croatia	50
Oliva di Tirana	Virgin olive oil	1.675 ± 0.309	Tirana, Albania	75
Olivastro	Virgin olive oil	5.885	Molise, Italy	31
Oueslati	Virgin olive oil	2.688 – 4.994	Kairouan, Tunisia	49
Paschixedda	Virgin olive oil	5.112	Sardinia, Italy	79
Passignano	Virgin olive oil	5.447 ± 0.557	Umbria, Italy	76
Peranzana	Virgin olive oil	3.066 – 3.256	Molise, Italy	31
Perafort	Virgin olive oil	2.410 ± 0.170	WOGC*, Córdoba, Spain	29
Piantone di Mogliano, Orbetana	Virgin olive oil	8.100 – 10.200	Marche Region, Italy	83
Picholine Marrocaine	Virgin olive oil	0.539	Morocco	78
Picual	Virgin olive oil	6.970 ± 0.350	WOGC*,Córdoba, Spain	29
Picual	Virgin olive oil	5.013 – 6.503^{B}	Montes de Granada, Spain	42, 81
Picual	Virgin olive oil	4.207 – 4.918	Jaen, Spain	75
Piñonera	Virgin olive oil	1.100 ± 0.010	WOGC*, Córdoba, Spain	29
Pizz 'e Carroga	Virgin olive oil	4.951	Sardinia, Italy	79
Polvese	Virgin olive oil	1.059 - 4.116	Umbria, Italy	76
Reixonenca	Virgin olive oil	3.410 ± 0.210	WOGC*, Córdoba, Spain	29
Rosciola	Virgin olive oil	3.945	Molise, Italy	31
Rosciola	Virgin olive oil	2.585	WOGC*, Córdoba, Spain	31
Royal de Calatayud	Virgin olive oil	6.520 ± 0.420	WOGC*, Córdoba, Spain	29
Rustica	Olive oil	6.830 ± 1.130	Abruzzo, Italy	33
San Mariano	Virgin olive oil	1.954 – 2.751	Umbria, Italy	76
Sayfi	Virgin olive oil	5.640 ± 0.060	WOGC*, Córdoba, Spain	29
Semidana	Virgin olive oil	6.241 ± 0.439	Sardinia, Italy	79
Shami	Olive oil	5.556 ± 0.110	Beni kenaneh, Jordan	85
Sigoise	Virgin olive oil	9.070	Algeria	74
Siviglìana da Mensa	Virgin olive oil	6.898	Sardinia, Italy	79

Sivigliana da Olio	Virgin olive oil	4.062	Sardinia, Italy	79
Sollana	Virgin olive oil	4.800 ± 0.06	WOGC*, Córdoba, Spain	29
Souri	Virgin olive oil	8.676 ± 0.007	Judea, Israel	54
Terza Grande	Virgin olive oil	5.107	Sardinia, Italy	79
Terza Piccola	Virgin olive oil	5.397	Sardinia, Italy	79
Tonda di Cagliari	Virgin olive oil	9.221	Sardinia, Italy	79
Tonda di Villacidro	Virgin olive oil	5.336	Sardinia, Italy	79
Tonda Iblea	Virgin olive oil	7.500	Italy	86
Tonda Iblea	Virgin olive oil	6.548 – 7.474	Sicily, Italy	75
Ulliri i Bardhe Berat	Virgin olive oil	7.210 ± 0.420	WOGC*, Córdoba, Spain	29
Vera	Virgin olive oil	5.680 ± 0.210	WOGC*, Córdoba, Spain	29
Verdial de Badajoz	Virgin olive oil	7.790 ± 0.390	WOGC*, Córdoba, Spain	29
Zarrazi Douirat	Virgin olive oil	2.593 – 5.015	Tataouine, Tunisia	34

WOGC*: World olive germplasm collection.

In this review, the term virgin olive oil (VOO) is used to describe the olive oil extracted and treated only by mechanical and physical processes without distinguishing extra- or virgin categories. Range of values according to [A] maturation level, [B] agricultural campaign, [C] cultivation area (location) and [D] others factors

Table 2. Ranking of squalene content in different cultivars

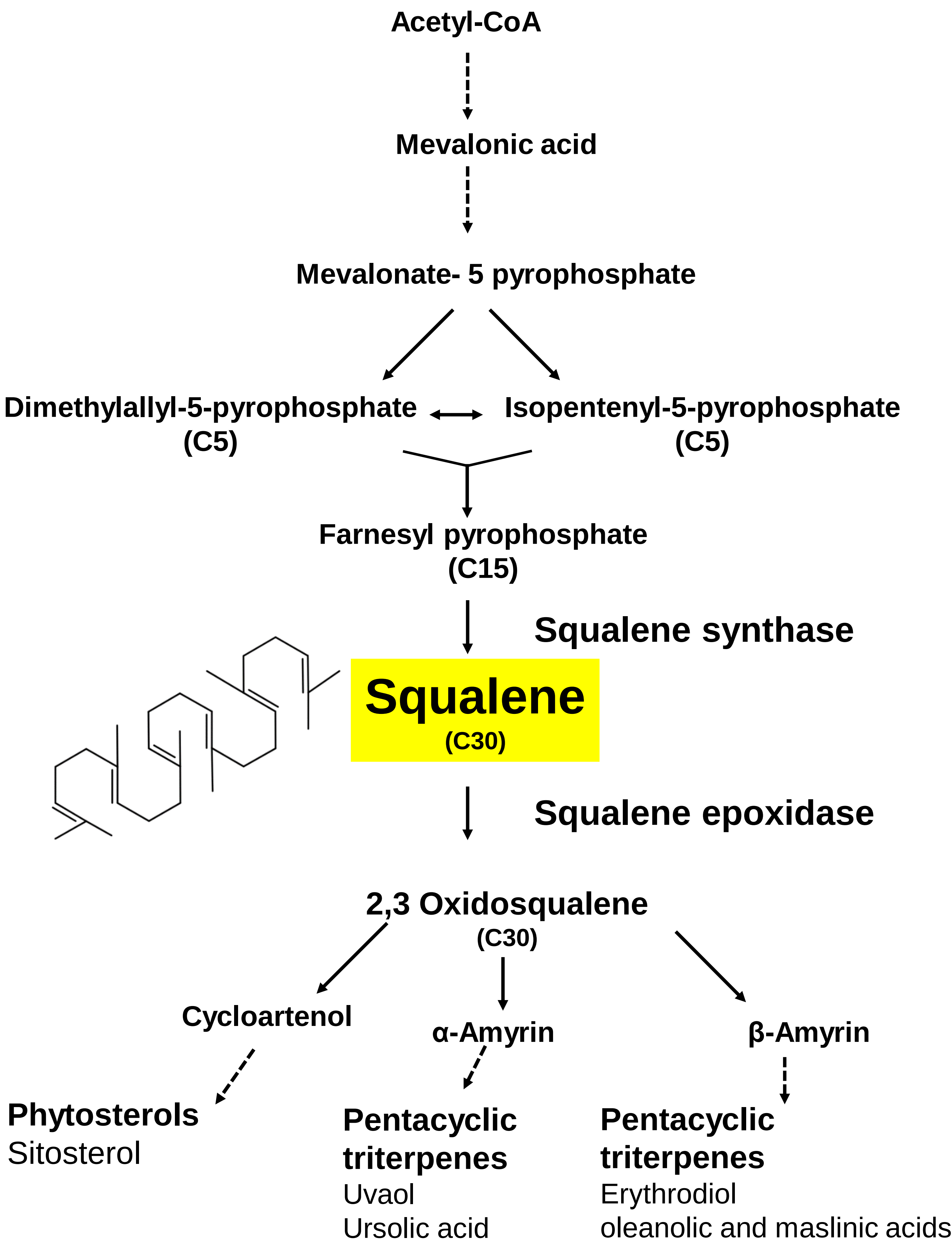
Cultivar	Count	Mean (g kg^{-1})	References
San Mariano	2	2.353 ± 0.564	76
Cerasoula	3	2.377 ± 1.507	32
Arbequina	6	2.409 ± 1.157	37, 75, 77, 78
Chemlali-Tataouine	3	2.553 ± 0.046	34, 36
Polvese	4	2.827 ± 1.326	76
Chemlali	15	3.512 ± 2.544	32, 49, 74, 75, 78
Barnea	2	3.596 ± 0.527	54
Frantoio	2	3.658 ± 0.053	79, 83
Koroneiki	25	3.781 ± 0.664	38, 75, 78, 81, 84
Oueslati	3	3.954 ± 1.169	49
Zarrazi-Douirat	4	4.077 ± 1.092	34
Edremit	4	4.145 ± 0.835	63
Gentile	6	4.171 ± 0.597	31, 33
Hojiblanca	2	4.336 ± 0.412	75, 78
Coratina	5	4.343 ± 0.507	31, 79, 81, 82
Chétoui-irrigated	5	4.688 ± 1.064	32
Leccino	4	4.795 ± 0.381	20, 31, 33, 79, 83
Fakhari-Douirat	4	5.500 ± 0.476	34
Picual	8	5.574 ± 0.889	29, 42, 75, 81
Lastovka	2	5.613 ± 0.589	50
Peranzana	2	5.803 ± 0.044	31
Biancolilla	4	5.879 ± 2.933	32
Buza	3	6.240 ± 1.267	80
Semidana	4	6.241 ± 0.439	79
Bosana	3	6.293 ± 0.657	29, 79
Tonda Iblea	3	6.798 ± 0.592	75, 86
Chétoui-rain-fed	5	7.110 ± 1.287	32
Buhavica	2	7.769 ± 2.984	50

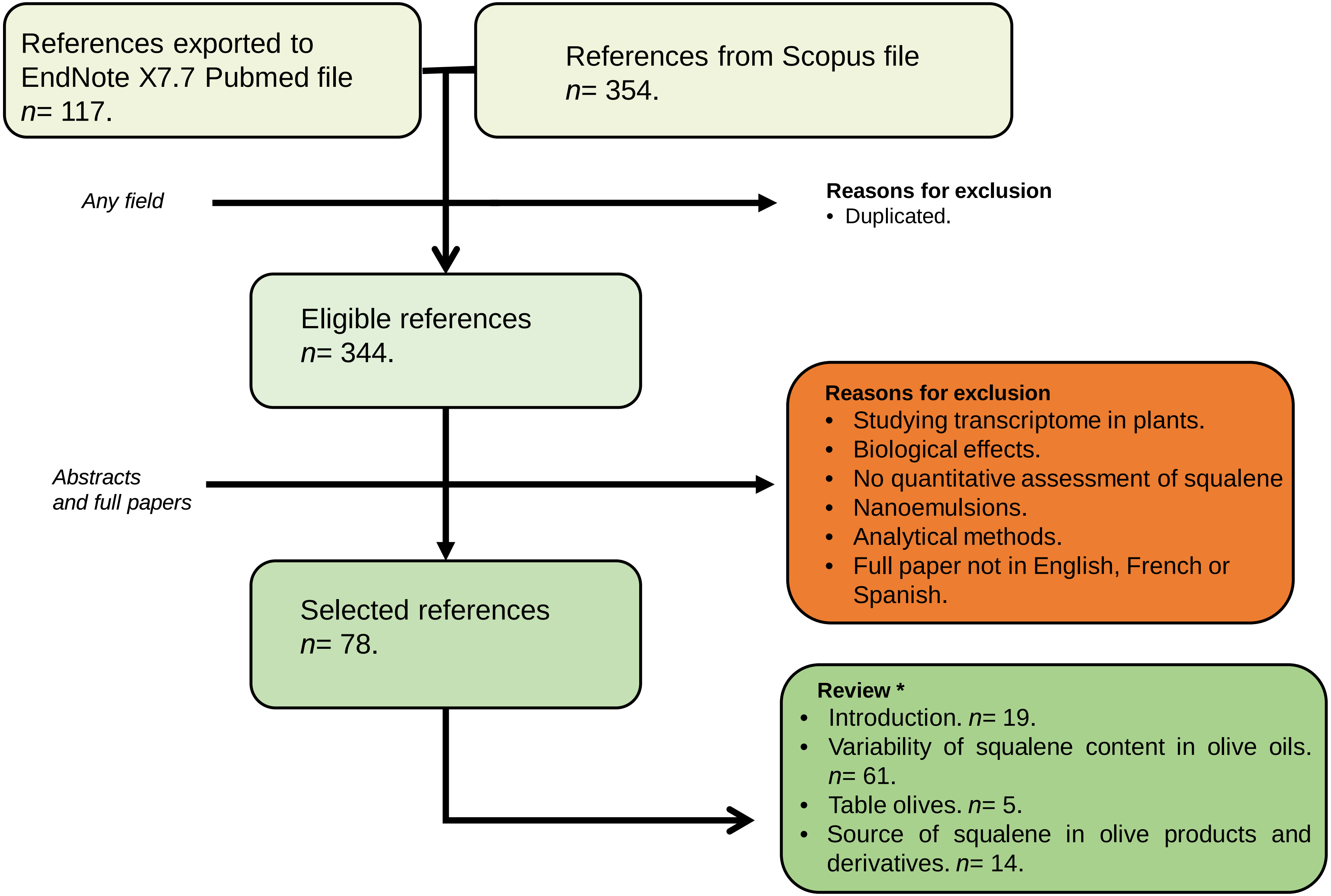
Nocellara de Belice	3	8.087 ± 1.712	[32]
Drobnica	5	8.662 ± 2.939	[50, 80]
Souri	2	8.676 ± 0.007	[54]
Oblica	2	9.316 ± 0.750	[50]

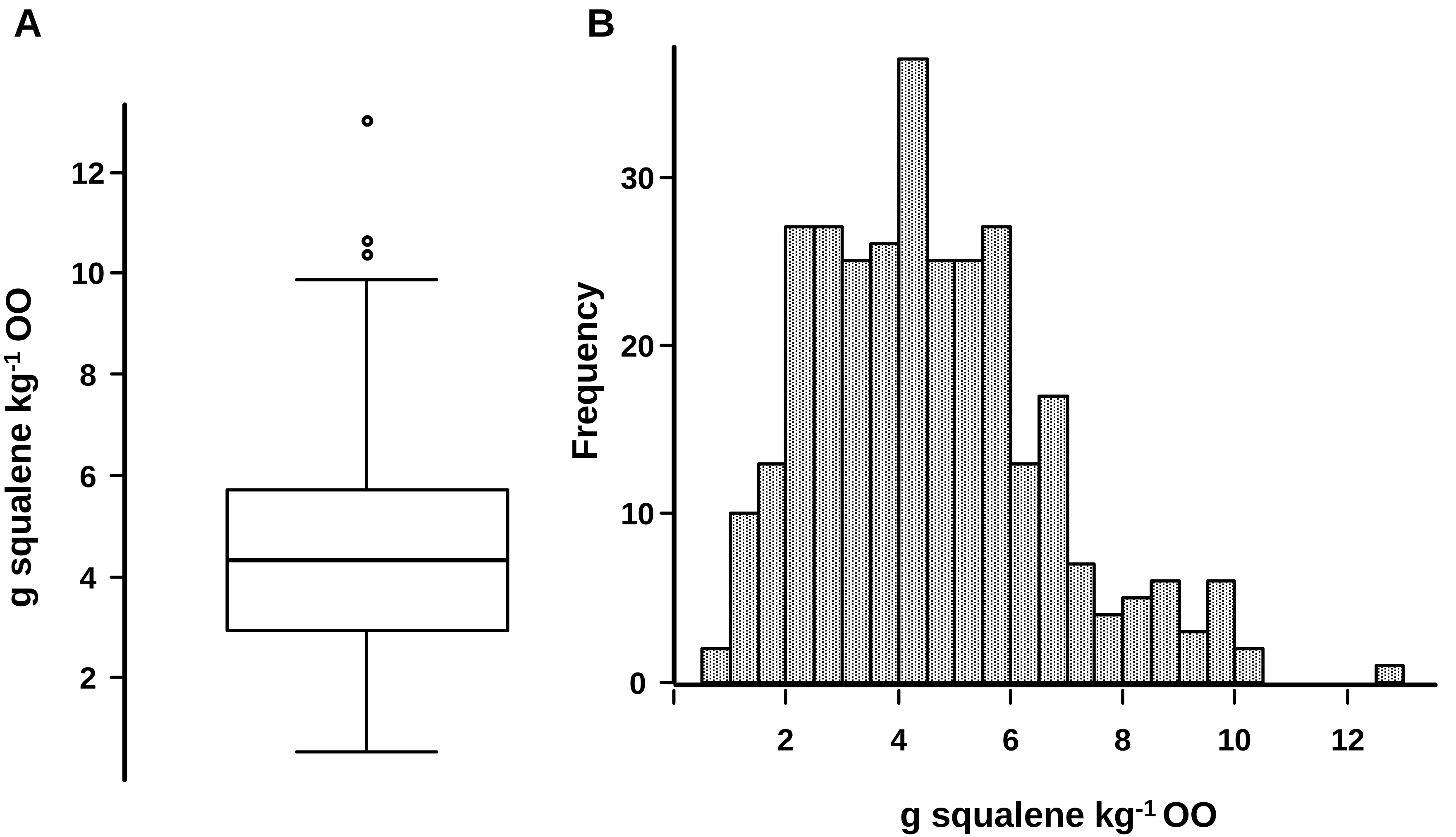
Results are shown means and standard deviations of those cultivars with at least two assays.

Table 3. Squalene content in table olives from different sources

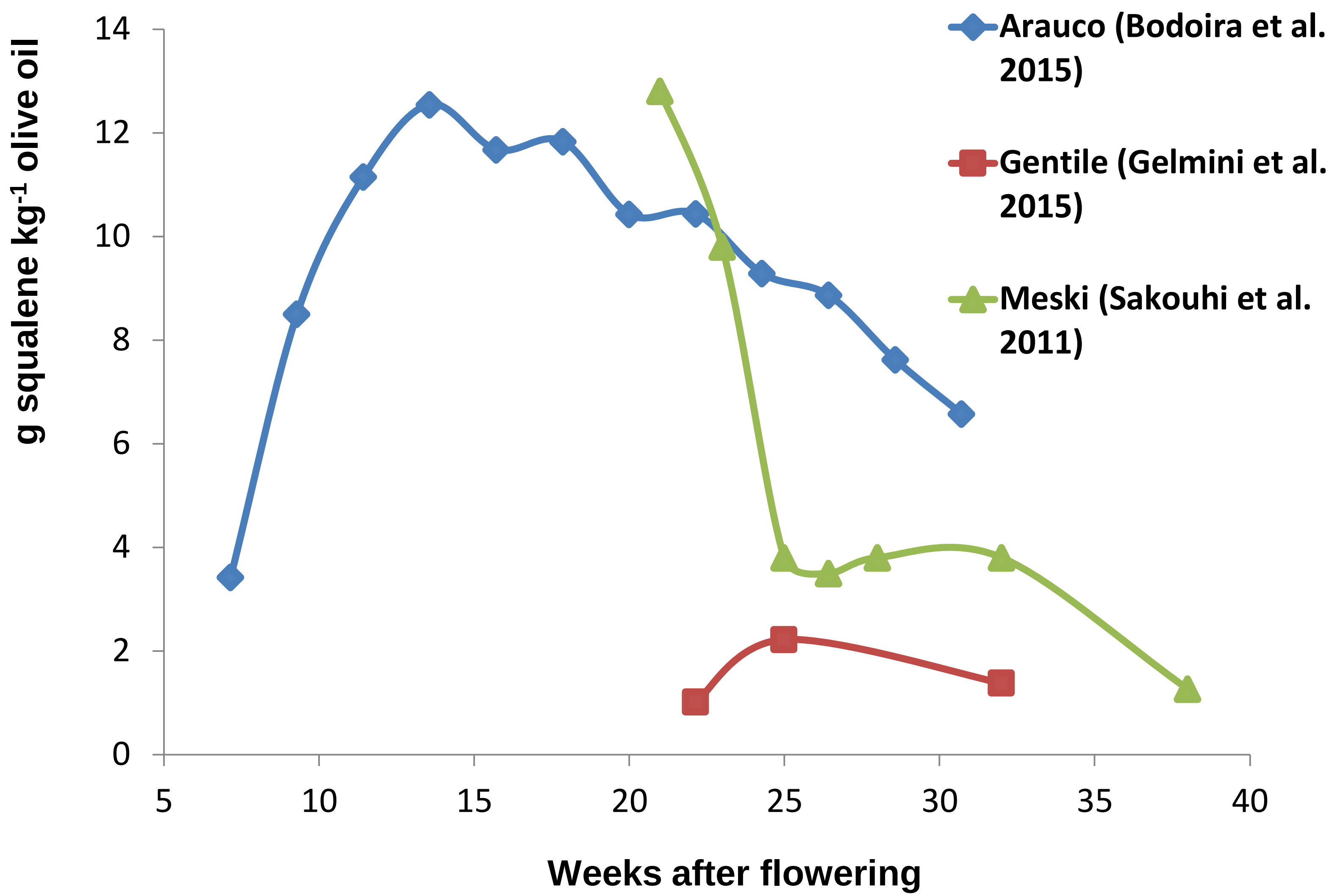
Cultivar	Squalene (g kg^{-1})	Location	References
Commercial samples	0.626 – 1.494	Italy	[59]
Tenera Ascolana	0.537 – 1.583	Italy	[59]
Stuffed from a local farmer	0.539 – 0.763	Italy	[59]
Coratina	1.056– 1.067	Molise, Italy	[31]
Edremit	2.500– 5.020	Burhaniye, Turkey	[63]
Gentile	0.831– 1.063	Molise, Italy	[31]
Hondrolia Chalkidikis	1.961– 3.423	Chalkidiki, Greece	[58]


Acetyl-CoA
Mevalonic acid
Mevalonate- 5 pyrophosphate
Dimethylallyl-5-pyrophosphate
(C5)
Isopentenyl-5-pyrophosphate
(C5)
Farnesyl pyrophosphate
(C15)
Squalene synthase
Squalene
(C30)
Squalene epoxidase
2,3 Oxidosqualene
(C30)
Cycloartenol
α-Amyrin
β-Amyrin
Phytosterols
Sitosterol
Pentacyclic
triterpenes
Uvaol
Ursolic acid
Pentacyclic
triterpenes
Erythrodiol
oleanolic and maslinic acids

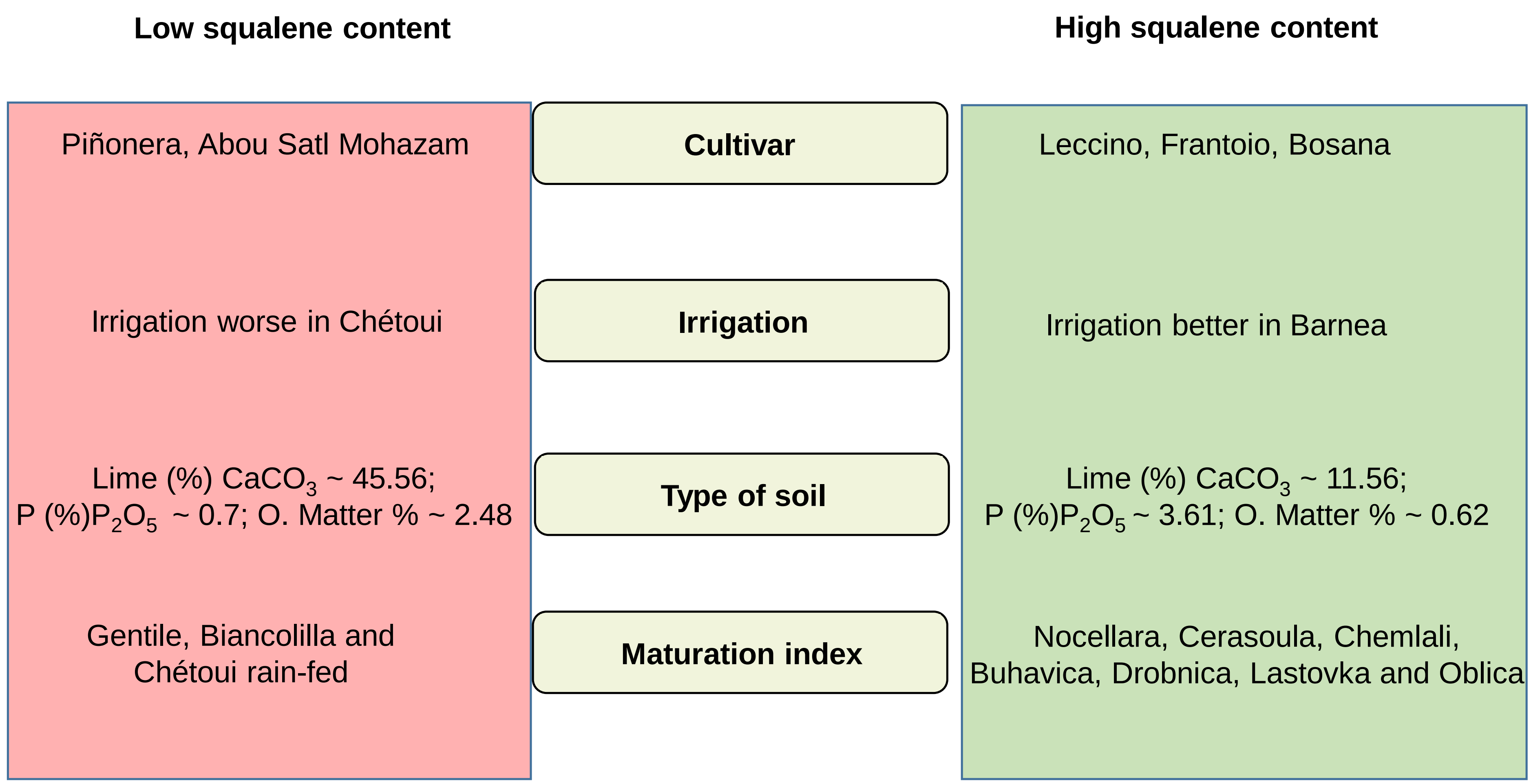

References exported to EndNote X7.7 Pubmed file *n*= 117.
References from Scopus file *n*= 354.
Any field
Reasons for exclusion
• Duplicated.
Eligible references *n*= 344.
Abstracts and full papers
Reasons for exclusion
• Studying transcriptome in plants.
• Biological effects.
• No quantitative assessment of squalene
• Nanoemulsions.
• Analytical methods.
• Full paper not in English, French or Spanish.
Selected references *n*= 78.
Review *
• Introduction. *n*= 19.
• Variability of squalene content in olive oils. *n*= 61.
• Table olives. *n*= 5.
• Source of squalene in olive products and derivatives. *n*= 14.



A
B
12
10
8
6
4
2
g squalene kg-1 OO
30
20
10
0
Frequency
2
4
6
8
10
12
g squalene kg-1 OO



g squalene kg-1 olive oil
14
12
10
8
6
4
2
0
5
10
15
20
25
30
35
40
Weeks after flowering
Arauco (Bodoira et al. 2015)
Gentile (Gelmini et al. 2015)
Meski (Sakouhi et al. 2011)


Low squalene content
High squalene content
Piñonera, Abou Satl Mohazam
Cultivar
Leccino, Frantoio, Bosana
Irrigation worse in Chétoui
Irrigation
Irrigation better in Barnea
Lime (%) $CaCO_3$ ~ 45.56;
P (%)P_2O_5 ~ 0.7; O. Matter % ~ 2.48
Type of soil
Lime (%) $CaCO_3$ ~ 11.56;
P (%)P_2O_5 ~ 3.61; O. Matter % ~ 0.62
Gentile, Biancolilla and
Chétoui rain-fed
Maturation index
Nocellara, Cerasoula, Chemlali,
Buhavica, Drobnica, Lastovka and Oblica