JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Cultivar Differences on Nonesterified Polyunsaturated Fatty Acid as a Limiting Factor for the Biogenesis of Virgin Olive Oil Aroma

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The relationship between the content of nonesterified polyunsaturated fatty acids and the contents of oil aroma compounds that arise during the process to obtain virgin olive oil (VOO) was studied in two olive cultivars, Picual and Arbequina, producing oils with distinct aroma profiles and fatty acid compositions. Results suggest that the biosynthesis of VOO aroma compounds depends mainly on the availability of nonesterified polyunsaturated fatty acids, especially linolenic acid, during the process and then on the enzymatic activity load of the lipoxygenase/hydroperoxide lyase system. Both availability of substrates and enzymatic activity load seem to be cultivar-dependent.

KEYWORDS: Lipoxygenase pathway; polyunsaturated fatty acid; olive oil; aroma

INTRODUCTION

The benefits of virgin olive oil (VOO) consumption are related to protection against cancer and cardiovascular diseases due to its fatty acid profile and the presence of minor constituents such as phenolic compounds (*1, 2*). However, the increase in the demand for high-quality VOO can be attributed not only to its potential health benefits but also to its excellent organoleptic properties. The aim of increasing the quality standards for VOO is continuously stimulating the study of biochemical pathways related to organoleptic properties and the development of technological procedures to improve those organoleptic properties. In this sense, our group established a decade ago the participation of the lipoxygenase (LOX) pathway in the biosynthesis of compounds of six straight-chain carbons (C6 compounds) in olive oil aroma (*3*). C6 aldehydes and alcohols and the corresponding esters are the most important compounds in the VOO aroma, from either a quantitative or a qualitative point of view (*4, 5*). These compounds are synthesized from polyunsaturated fatty acids containing a (*Z*,*Z*)-1,4-pentadiene structure such as linoleic (LA) and linolenic (LnA) acids. In a first step of this pathway, LOX produces the corresponding 13-hydroperoxide derivatives that are subsequently cleaved heterolytically by hydroperoxide lyase (HPL) to C6 aldehydes (*3, 6, 7*). C6 aldehydes can then undergo reduction by alcohol dehydrogenases (ADH) to form C6 alcohols (*3, 8*) and can finally be transformed into the corresponding esters by means of an alcohol acyltransferase (*3, 9*). Moreover, Angerosa et al. (*5*) also demonstrated the relevance of compounds of five straight-chain carbons (C5 compounds) in the aroma of olive oil. C5 compounds would be generated through an additional branch of the LOX pathway that would involve the production of a 13-alcoxyl radical by LOX as demonstrated in soybean seeds (*10*). This radical would undergo subsequent nonenzymatic β-scission in a homolytic way to form a 1,3-pentene allylic radical that could be chemically dimerized to form pentene dimers or react with an hydroxyl radical to form C5 alcohols. The latter would be the origin of C5 carbonyl compounds present in the aroma of olive oil through an enzymatic oxidation by ADH as suggested to occur in soybean leaves (*11*). The lack of HPL activity gives rise to an accumulation of hydroperoxides and a subsequent increase of the homolytic LOX branch activity, producing higher contents of C5 compounds as demonstrated in antisense-mediated HPL-depleted tomato plants (*12*).

There are quite a number of studies describing the way technological procedures affect VOO aroma compound profile or the biosynthetic pathway determining this profile (*13–18*). However, as far as we know, there is no study devoted to identifying limiting factors for the biosynthesis of VOO aroma compounds. Taking into account that nonesterified polyunsaturated fatty acids seem to be the main substrates for olive LOX activity (*6*), the aim of the present work was to establish the limiting factors affecting the biosynthesis of VOO aroma compounds through the LOX pathway by studying the relationship between the content of polyunsaturated fatty acids during the process to obtain VOO and the oil aroma compound profile.

MATERIALS AND METHODS

Plant Material. Olive fruits (*Olea europaea* L.) cultivars Picual and Arbequina were harvested in CIFA Cabra-Priego orchards (Cabra, Córdoba, Spain) at ripe stage, maturity index (MI) 5, according to Uceda and Frias (*19*).

Olive Oil Extraction. Olive oil extraction was performed using an Abencor analyzer (Comercial Abengoa, S.A., Seville, Spain) that simulates at laboratory scale the industrial process of VOO production. Milling of olive fruits (1 kg) was performed using a stainless steel

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10.1021/jf071202i CCC: $37.00
Published on Web 08/21/2007

Table 1. Composition (%) of the TG and FFA Fractions of the Oils Obtained by Addition of Different Amounts of LA (mg/kg fruits) during the Oil Extraction Process

	Picual					Arbequina				
		FFA					FFA			
fatty acid	TG	0 mg	50 mg	100 mg	200 mg	TG	0 mg	50 mg	100 mg	200 mg
16:0	11.21	11.95	11.33	9.52	8.02	14.47	26.96	17.64	16.47	10.79
16:1	1.39	1.35	1.29	1.17	1.23	2.38	3.67	2.32	2.75	1.30
18:0	2.98	5.68	5.19	4.46	3.49	1.46	8.16	5.69	3.32	2.84
18:1	76.82	69.30	61.01	54.18	45.93	69.98	54.24	40.31	31.77	21.71
18:2	6.13	9.01	19.03	28.55	39.25	10.34	2.91	31.88	44.34	62.61
18:3	0.68	1.99	1.64	1.46	1.42	0.67	2.12	1.43	0.88	0.15
20:0	0.43	0.37	0.19	0.39	0.36	0.32	0.64	0.36	0.23	0.28
20:1	0.25	0.19	0.11	0.15	0.17	0.28	0.85	0.13	0.11	0.10
22:0	0.11	0.17	0.20	0.13	0.11	0.10	0.43	0.25	0.13	0.21

hammer mill operating at 3000 rpm provided with a 5 mm sieve. The resulting olive pastes were immediately kneaded in a mixer at 50 rpm for 30 min at 30 °C. Centrifugation of the kneaded olive pastes was performed in a basket centrifuge at 3500 rpm for 1 min. After centrifugation, oils were decanted and paper-filtered. Samples for volatile and fatty acid analyses (0.5 g each) were stored under nitrogen at −18 °C until analysis.

To increase the proportion of polyunsaturated fatty acids during the process to obtain the oil, different amounts of either LA or LnA in the range of 0–200 mg/kg fruit were added as sodium salts (*20*) to the olive fruits during the milling step. Duplicate experiments were carried out for each cultivar.

Fatty Acid Composition and Free Acidity. Triaglycerols (TG) and polar compounds containing free fatty acids (FFA) from the oils were fractionated by silica column chromatography according to Waltking and Wessels (*21*). The fatty acid composition of the different fractions was analyzed in triplicate by gas chromatography (GC) after derivatization to fatty acid methyl esters with 2 N KOH in methanol for the TG fraction according to the IUPAC standard method (*22*) and with diazomethane in diethylether-saturated N_2 for the FFA-containing fraction (*23*). A HP-6890 chromatograph equipped with a HP Innowax capillary column (polyethylene glycol, 30 m × 0.25 mm i.d., film thickness 0.25 μm; Hewlett Packard, United States) was used for the analysis of the methyl esters under the following temperature programme: 180 °C (4 min), 4 °C min^{-1} to 230 °C (15 min). Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1}. The temperature of both the split injector and the flame ionization detector was 250 °C. Free acidity in control oils was determined according to Annex II in EC Regulation EEC/2568/91 (*24*).

Analysis of Volatile Compounds. Olive oil samples were conditioned to room temperature and then placed in a vial heater at 40 °C. After 10 min of equilibrium time, volatile compounds from headspace were adsorbed on a SPME fibre DVB/Carboxen/PDMS 50/30 μm (Supelco Co., Bellefonte, PA). The sampling time was 50 min at 40 °C. Desorption of volatile compounds trapped in the SPME fibre was done directly into the GC injector. Volatiles were analyzed in duplicate using a HP-6890 gas chromatograph equipped with a DB-Wax capillary column (60 m × 0.25 mm i.d., film thickness 0.25 μm; J&W, Scientific, Folsom, CA). Operating conditions were as follows: N_2 as the carrier gas; injector and detector temperatures at 250 °C; and the column was held for 6 min at 40 °C and then programmed at 2 °C min^{-1} to 128 °C. Quantification was performed using individual calibration curves for each identified compound by adding known amounts of different compounds to redeodorize high oleic sunflower oil. Compound identification was carried out on a HRGC-MS Fisons series 8000 equipped with a similar stationary phase column and two different lengths, 30 and 60 m, matching against the Wiley/NBS Library and by GC retention time against standards. Volatile compounds were clustered into different classes according to the polyunsaturated fatty acid and the LOX pathway branch origin (see **Table 3**), and data were statistically evaluated using the Microsoft Excel 2002 software program.

Chemicals and Reagents. LA, LnA, and reference compounds used for volatile identification were supplied by Sigma-Aldrich (St. Louis, MO) except for (*Z*)-hex-3-enyl acetate, which was purchased from Givaudan Co. (Clifton, NJ), and (*Z*)-hex-3-enal, which was generously supplied by S.A. Perlarom (Louvaine-La-Neuve, Belgium).

RESULTS AND DISCUSSION

Two olive cultivars, Picual and Arbequina, whose oils are quite different in terms of aroma profile and fatty acid composition (*25*, *26*), were selected to study the limitation of substrate for the biosynthesis of VOO aroma compounds through the LOX pathway. For this purpose, the relationship between the content of nonesterified LA and LnA during the oil extraction process and the VOO aroma compound profile was studied. It is generally accepted that nonesterified fatty acids are the physiological substrates of plant LOX, and particularly olive LOX was reported to oxidize esterified fatty acid at a much lower velocity (<1%) than nonesterified fatty acids (*6*).

The fatty acid composition of the FFA and TG fractions was analyzed after fractionation of the oil lipids as described in the Materials and Methods. The fatty acid composition of the TG fraction did not change as a consequence of polyunsaturated fatty acid additions during the process to obtain VOO (data not shown), having the average composition displayed in **Tables 1** and **2** (TG). Data are in good agreement with the general fatty acid pattern reported for the cultivars under study (*26*), although it was observed that the LA proportion was slightly above and below the usual levels reported for Picual and Arbequina, respectively.

The composition of the FFA fraction of the oils (**Tables 1** and **2**, FFA/0 mg) showed some differences from that of TG fraction, characterized by a lower level of oleic acid (18:1) and higher levels of palmitic acid (16:0), especially in Arbequina oils, stearic acid (18:0), and the main substrate for VOO aroma compound biosynthesis, LnA (18:3). The LA proportion was different depending on the cultivar, higher than the TG fraction in Picual oils (9.01%), and significantly lower in Arbequina oils (2.91%). There is not much data reported in the literature concerning the FFA composition of olive oil. The FFA composition found in Picual and Arbequina oils is compatible with the acylhydrolase specificity against TG and phospholipids that we have previously reported in olive fruit (*3*). Those results suggest that acylhydrolase specificity from olive fruit would be the main factor responsible for the free acidity of the oils obtained from sound fruits and that this specificity could vary somewhat depending on the cultivar. Thus, differences in acylhydrolase specificity might be responsible for the slight differences found in the fatty acid composition of the FFA in Picual and Arbequina oils.

The addition of nonesterified LA and LnA (**Tables 1** and **2**, respectively) during the process to obtain VOO in both cultivars

Table 2. Composition (%) of the TG and FFA Fractions of the Oils Obtained by Addition of Different Amounts of LnA (mg/kg fruits) during the Oil Extraction Process

	Picual					Arbequina				
		FFA					FFA			
fatty acid	TG	0 mg	50 mg	100 mg	200 mg	TG	0 mg	50 mg	100 mg	200 mg
16:0	11.27	12.00	10.91	9.70	7.43	14.57	26.65	21.66	16.69	12.43
16:1	1.31	1.23	1.36	1.20	1.00	2.63	3.56	3.02	2.36	1.51
18:0	2.15	5.70	4.35	3.99	2.96	1.47	8.55	5.75	4.22	2.70
18:1	77.79	69.55	63.57	56.30	43.82	69.42	53.63	45.20	30.94	24.20
18:2	6.09	9.00	6.83	6.01	5.06	10.44	3.24	3.59	2.41	3.02
18:3	0.67	1.96	12.40	22.28	39.34	0.72	2.37	19.57	42.58	55.76
20:0	0.41	0.32	0.31	0.29	0.26	0.34	0.66	0.57	0.30	0.17
20:1	0.21	0.16	0.11	0.09	0.05	0.30	0.93	0.19	0.09	0.05
22:0	0.10	0.08	0.14	0.13	0.08	0.12	0.42	0.46	0.40	0.16

Table 3. Contents of Volatile Compounds (ng/g Oil) in Olive Oils from Picual and Arbequina Fruits[a]

volatile class	volatile compound	Picual	Arbequina
C6/LnA aldehydes	(*E*)-hex-3-enal	126.64 ± 1.83	159.04 ± 5.17
	(*Z*)-hex-3-enal	988.88 ± 93.02	88.30 ± 9.86
	(*Z*)-hex-2-enal	129.78 ± 4.42	142.20 ± 10.51
	(*E*)-hex-2-enal	2831.50 ± 74.35	13226.53 ± 216.91
C6/LnA alcohols	(*E*)-hex-3-enol	5.37 ± 1.77	3.79 ± 0.38
	(*Z*)-hex-3-enol	117.58 ± 2.55	156.89 ± 5.92
	(*E*)-hex-2-enol	7.73 ± 4.16	224.90 ± 5.89
C6/LA aldehyde	hexanal	256.97 ± 3.90	710.02 ± 26.96
C6/LA alcohol	hexan-1-ol	81.96 ± 0.24	113.30 ± 2.26
C5/LnA carbonyls	pent-1-en-3-one	221.32 ± 10.68	269.34 ± 19.81
	(*Z*)-pent-2-enal	20.74 ± 0.68	18.19 ± 0.80
	(*E*)-pent-2-enal	38.54 ± 2.05	33.26 ± 0.82
C5/LnA alcohols	pent-1-en-3-ol	82.55 ± 3.66	229.58 ± 32.16
	(*Z*)-pent-2-en-1-ol	414.43 ± 6.41	209.56 ± 7.39
	(*E*)-pent-2-en-1-ol	69.76 ± 3.27	53.58 ± 1.06
PD	pentene dimers	2726.67 ± 113.67	4216.59 ± 78.97
C5/LA carbonyls	pentan-3-ona+pentan-2-ona	58.75 ± 2.23	91.35 ± 6.36
	pentanal	6.21 ± 0.43	13.43 ± 1.07
C5/LA alcohol	pentan-1-ol	0.94 ± 0.24	21.48 ± 1.04
LOX esters	hexyl acetate	241.13 ± 7.85	48.62 ± 3.07
	(*E*)-hex-2-en-1-yl acetate	9.65 ± 2.23	39.72 ± 4.25
non-LOX esters	methyl acetate	10.72 ± 0.90	44.70 ± 3.35
	ethyl acetate	51.53 ± 2.80	20.70 ± 2.59
	methyl hexanoate	11.31 ± 2.48	18.84 ± 3.30
	ethyl hexanoate	20.91 ± 6.99	96.52 ± 8.34

[a] Compounds are clustered into different volatile classes according to the polyunsaturated fatty acid and the LOX pathway branch origin.

gave rise to an increase in the proportion of these polyunsaturated acids in the FFA fraction. Thus, additions of LA produced an increase of this acid percentage, reaching 39% in Picual oils and 62% in Arbequina oils for the higher doses (**Table 1**). Similarly, additions of LnA to olive fruits increased this acid proportion to 39 and 56% in Picual and Arbequina oils, respectively. Differences of the fatty acid percentages reached are mainly due to the different acidity of the oils from the two cultivars, higher in Picual oils (0.40%) than in Arbequina oils (0.22%).

Analysis of volatile compounds of the oils resulting after addition of different fatty acid doses was carried out. **Table 3** displays the composition of the oil volatile fraction of the two cultivars under study. Arbequina oils exhibit in general higher volatile contents than Picual oils except for the ester fraction. Increasing the level of nonesterified LA during the process to obtain VOO gave rise to an increment in the content of C6/LA compounds only in oils from cultivar Arbequina, reaching an increase of 60% for the higher LA doses (**Table 4**). This increase was mainly due to the higher levels of the C6/LA aldehyde. However, C6/LA alcohol contents remained practically unaffected. Because of the low quantitative contribution of C6/LA compounds to VOO aroma, the total volatile content remained almost unaffected. The higher proportion of LA in the FFA oil fraction and the higher level of this fraction (higher free acidity) in Picual as compared to Arbequina oils (**Tables 1** and **2**) might well explain that Picual C6/LA compound contents were not affected by LA additions. However, a modification of the content of volatile compounds coming from LnA in both cultivars as a consequence of LA additions was observed. Thus, the contents of C5/LnA compounds decreased when increasing the amount of LA, affecting mainly C5/LnA carbonyls and PD compounds, while the contents of C6/LnA compounds remained unaffected. This decrease was higher in Picual (43%) than in Arbequina oils (28%), pointing out a competitive substrate effect by LA on LOX homolytic activity that would weaken the production of C5 compounds coming from LnA. This fact and a hypothetical lower activity load of the LOX/HPL system in Picual fruits, as compared to Arbequina fruits, might explain the decrease in total volatiles observed in Picual oil aroma when increasing the LA content during the process to obtain the oil.

The level of extracted enzymatic activities from the fruit would not be representative of what is occurring during the extraction process since in the milling and malaxation steps other fruit components could interact with the proteins modifying their activity level. In this sense, higher levels of polyphenols were

Table 4. Volatile Contents (ng/g Olive Oil)[a] of the Oils Obtained by Addition of Different Amounts of LA (mg/kg Fruits) during the Oil Extraction Process

	Picual				Arbequina			
volatile[b]	0 mg	50 mg	100 mg	200 mg	0 mg	50 mg	100 mg	200 mg
Σ C6/LnA aldehydes	4076.80	4209.04	3572.35	3362.91	13616.07	13000.98	13667.92	13673.27
Σ C6/LnA alcohols	130.68	155.03	183.15	250.33	385.58	370.62	450.69	395.64
Σ C6/LnA	4207.48	4364.07	3755.50	3613.23	14001.65	13371.60	14118.61	14068.91
Σ C6/LA aldehyde	256.97	337.80	236.83	294.63	710.02	700.96	786.35	1208.17
Σ C6/LA alcohol	81.96	69.64	67.25	78.51	113.30	115.06	112.90	118.24
Σ C6/LA	338.94	407.43	304.08	373.14	823.32	816.02	899.25	1326.42
Σ C5/LnA carbonyls	280.60	272.10	248.21	171.30	320.79	257.63	226.14	179.76
Σ C5/LnA alcohols	566.74	572.76	497.55	456.11	492.72	452.77	544.00	409.04
Σ PD	2726.67	2750.98	1873.32	1396.95	4216.59	3542.24	3103.09	3049.60
Σ C5/LnA	3574.01	3595.84	2619.09	2024.35	5030.09	4252.65	3873.23	3638.39
Σ C5/LA carbonyls	64.96	63.82	74.85	60.34	104.78	103.92	109.85	101.64
Σ C5/LA alcohol	0.94	1.22	0.97	1.21	21.48	20.54	19.29	17.80
Σ C5/LA	65.91	65.04	75.82	61.55	126.26	124.46	129.14	119.44
Σ LOX esters	250.77	210.11	192.90	196.62	88.34	118.35	100.99	85.17
Σ Non-LOX esters	94.47	96.89	85.75	125.43	180.76	205.92	174.07	164.79
Σ esters	345.25	307.00	278.65	322.05	269.10	324.27	275.05	249.96
total volatiles	8531.58	8739.37	7033.14	6394.32	20250.42	18889.01	19295.29	19403.12

[a] Mean value from three determinations. The average coefficient of variance was 6.2 and 7.8% for Picual and Arbequina oils, respectively. [b] Each class of volatile compound comprises compounds listed in **Table 3**.

Table 5. Volatile Contents (ng/g Olive Oil)[a] of the Oils Obtained by Addition of Different Amounts of LnA (mg/kg Fruits) during the Oil Extraction Process

	Picual				Arbequina			
volatile[b]	0 mg	50 mg	100 mg	200 mg	0 mg	50 mg	100 mg	200 mg
Σ C6/LnA aldehydes	4241.58	4354.02	5632.39	6228.47	13382.48	22679.54	28354.33	36732.99
Σ C6/LnA alcohols	134.06	187.71	232.59	171.85	374.44	420.59	534.40	781.98
Σ C6/LnA	4375.64	4541.74	5864.98	6400.31	13756.92	23100.13	28888.73	37514.97
Σ C6/LA aldehyde	260.87	259.97	292.06	304.93	736.98	807.12	789.14	824.99
Σ C6/LA alcohol	82.21	85.01	82.94	71.31	115.56	127.87	154.63	168.29
Σ C6/LA	343.08	344.97	375.00	376.24	852.54	935.00	943.77	993.28
Σ C5/LnA carbonyls	289.78	218.08	315.29	239.14	299.91	308.11	321.72	305.06
Σ C5/LnA alcohols	580.08	512.12	667.41	583.62	452.95	557.08	573.61	557.80
Σ PD	2812.19	3134.88	5800.83	6660.72	4176.76	7486.27	10538.24	13167.69
Σ C5/LnA	3682.06	3865.08	6783.53	7483.48	4929.62	8351.46	11433.56	14030.55
Σ C5/LA carbonyls	66.76	56.55	57.64	47.38	100.79	109.59	66.88	58.54
Σ C5/LA alcohol	3.11	3.17	4.77	4.23	20.44	25.85	28.44	31.25
Σ C5/LA	69.87	59.72	62.41	51.61	121.23	135.44	95.33	89.78
Σ LOX esters	256.39	217.35	220.62	229.75	81.65	87.90	132.98	124.82
Σ Non-LOX esters	100.24	112.59	113.72	104.52	169.28	204.12	238.02	242.55
Σ esters	356.63	329.94	334.34	334.27	250.93	292.03	371.00	367.37
total volatiles	8827.28	9241.45	13420.27	14645.91	19911.24	32814.05	41732.39	52995.95

[a] Mean value from three determinations. The average coefficient of variance was 8.1 and 9.6% for Picual and Arbequina oils, respectively. [b] Each class of volatile compound comprises compounds listed in **Table 3**.

found in Picual than in Arbequina oils (*27*) and the inhibitory role of oxidized polyphenols on enzymatic activity is well-established (*28*). However, a direct relationship between LOX activity level in the fruit and the amount of volatiles in the oils was found by Ridolfi et al. (*29*) in three different cultivars.

Contrary to what was found when adding LA during the process to obtain VOO, additions of nonesterified LnA gave rise to a high modification level of the oil volatile pattern (**Table 5**). The contents of C6/LnA compounds increased when increasing LnA doses in Picual oils, reaching an average of 46% increase for the higher doses. Similarly, an increase of the C5/LnA compounds was found when increasing the amount of LnA, but it was proportionally higher than for the C6/LnA compounds (103%) and due mainly to a higher level of PD compounds synthesized. These results suggest a limitation of the HPL activity load in Picual fruits as pointed out before, being unable to sufficiently metabolize the hydroperoxides produced by LOX through a heterolytic way. A limited load of HPL activity during the process to obtain VOO would increase proportionally the level of C5/LnA, as we have previously demonstrated to occur in HPL-depleted transgenic potato plants (*12*). On the contrary, a low load of LOX activity would negatively affect both C6 and C5 compounds coming from both LA and LnA as observed in LOX-silenced transgenic potato plants (*30*).

The analysis of the aroma compound profile of oils obtained increasing the content of LnA during the oil extraction process suggest that LnA is the main limiting factor for the biosynthesis of VOO aroma compounds in cultivar Arbequina. Additions of LnA produced a high increment of the contents of the main class of VOO aroma compounds, C6/LnA compounds, for both the C6/LnA aldehydes and the alcohols, displaying a three-fold increase for the higher LnA doses (**Table 5**). Increases of the C5/LnA compounds contents were also observed to be quite in the same range than C6/LnA compounds and, as in Picual, due almost exclusively to a high increase in the content of PD compounds. Therefore, data suggest that Arbequina fruits contain not only a high LOX activity load, able to metabolize higher levels of nonesterified LnA than those currently found during the oil extraction process, but also a high level of HPL activity. This is inferred from the constant proportion found between the levels of C6 and C5 compounds coming from LnA (C6/LnA and C5/LnA) in the oils obtained after LnA additions.

In summary, results obtained from the two cultivars under study, which present significant quantitative aroma differences,

suggest that the biosynthesis of VOO aroma compounds depends mainly on the availability of nonesterified polyunsaturated fatty acid, especially LnA, to be metabolized through the LOX pathway during the process to obtain this oil. This availability seems to be cultivar-dependent. Thus, data suggest that substrate availability is comparatively lower in Arbequina than in Picual fruits. Data also indicate that the VOO aroma compound content depends on the enzymatic activity load of the LOX/HPL system as well, so that the lower aroma volatile contents found in Picual oils seem to be the result of a lower enzymatic activity load during the process to obtain the oil, despite the apparently higher availability of nonesterified polyunsaturated acids as compared to Arbequina fruits.

Therefore, breeding programs, assisted through either biochemical or molecular approaches, should take into account the combination of a high load of the LOX/HPL system activity and a high availability of nonesterified polyunsaturated fatty acids for olive cultivars producing flavorful oils.

ACKNOWLEDGMENT

We are grateful to Brígida Giménez (CIFA Cabra-Priego) for providing the fruits and to Mar Pascual for her excellent technical assistance.

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Received for review April 24, 2007. Revised manuscript received July 5, 2007. Accepted July 7, 2007. This work was supported by research project AGL2005-03959 from Programa Nacional de Recursos y Tecnologías Alimentarias funded by the Spanish Government.

JF071202I