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Thermal Inactivation Kinetics of Recombinant Proteins of the Lipoxygenase Pathway Related to the Synthesis of Virgin Olive Oil Volatile Compounds

María N. Padilla, José M. Martínez-Rivas, Ana G. Pérez, and Carlos Sanz*

Department of Physiology and Technology of Plant Products, Instituto de la Grasa, CSIC, Avenida Padre García Tejero 4, 41012 Seville, Spain

Supporting Information

ABSTRACT: The aim of this work was to characterize the thermal inactivation parameters of recombinant proteins related to the biosynthesis of virgin olive oil (VOO) volatile compounds through the lipoxygenase (LOX) pathway. Three purified LOX isoforms (Oep2LOX1, Oep1LOX2, and Oep2LOX2) and a hydroperoxide lyase (HPL) protein (OepHPL) were studied. According to their thermal inactivation parameters, recombinant Oep1LOX2 and Oep2LOX2 could be identified as the two LOX isoforms active in olive fruit crude preparations responsible for the synthesis of 13-hydroperoxides, the main substrates for the synthesis of VOO volatile compounds. Recombinant Oep2LOX1 displayed a low thermal stability, which suggests a weak actuation during the oil extraction process considering the current thermal conditions of this industrial process. In addition, recombinant OepHPL could be identified as the HPL activity in crude preparations. The thermal stability was the highest among the recombinant proteins studied, which suggests that HPL activity is not a limiting factor for the synthesis of VOO volatile compounds.

KEYWORDS: *Olea europaea, lipoxygenase, hydroperoxide lyase, thermal inactivation, olive oil, aroma, volatiles*

INTRODUCTION

Virgin olive oil (VOO), one of the main components of the Mediterranean diet, is related to protection against cardiovascular diseases and cancer due to its fatty acid profile and the presence of minor constituents.[1−3] A large increase in the demand for high-quality virgin olive oil during recent years can be attributed not only to its potential health benefits but also to its particular organoleptic properties. The most important compounds in the VOO volatile fraction, from either a quantitative or a qualitative point of view, are straight-chain six-carbon (C6) compounds synthesized from linoleic and linolenic acids through the lipoxygenase (LOX) pathway. In the first step of this pathway, LOX produces the 13-hydroperoxide derivatives from these polyunsaturated fatty acids[4,5] that are subsequently cleaved heterolytically by hydroperoxide lyase (HPL) to C6 aldehydes.[4,6] C6 aldehydes can then undergo reduction by alcohol dehydrogenases to form C6 alcohols[4,7] and may finally be esterified with carboxylic acids in the form of acyl-CoA derivatives, a reaction catalyzed by alcohol acyltransferases.[4,8] Additionally, Angerosa et al.[9] demonstrated the relevance of the straight-chain five-carbon (C5) compounds also present in the volatile fraction of olive oil. C5 compounds would be generated through an additional branch of the LOX pathway that would involve the production of a 13-alkoxyl radical by LOX in a homolytic way as demonstrated to occur in soybean seeds.[10,11]

The synthesis of volatile compounds responsible for VOO aroma occurs when enzymes and substrates meet as olive fruit tissues are disrupted during olive oil processing. Although most of these compounds derive from 13-hydroperoxides synthesized by 13-LOXs, crude enzymatic extracts from olive fruit mesocarp exhibit a higher 9-LOX activity, synthesizing 9-hydroperoxide derivatives,[4] indicating that 9-LOX isoforms are also present in olive mesocarp. These 9-LOX isoforms seem not to be implicated in the synthesis of VOO volatile compounds according to their regiospecificity, but they might compete for the same polyunsaturated fatty acid substrates with the 13-LOX isoforms during the milling and malaxation steps of fruit processing to obtain the oil. On the other hand, although the literature indicates the presence of LOX activity in the olive seed, there is no conclusive data about its participation in the synthesis of VOO volatile compounds. According to our previous work,[12] the seed gives rise to a slight decrease of the contents of the C6 unsaturated aldehydes in the oils. This result is compatible with the evidenced presence in the seed of enzymatic activities metabolizing 13-hydroperoxides other than HPL.

To date, four LOX genes and one HPL gene have been cloned and characterized in olive. Among the LOX genes, two 13-LOX genes were isolated from cultivar 'Picual' (*Oep1LOX2* and *Oep2LOX2*)[13] and two 9-LOX genes from cultivars 'Leccino' (*Oe1LOX1*)[14] and 'Picual' (*Oep2LOX1*),[15] respectively. Whereas *Oep1LOX2* exhibited constant expression levels in olive mesocarp during olive fruit development and ripening, *Oep2LOX2* showed a strong increase of its transcript with a maximum during turning stage that coincides with an increase in the synthesis of volatile compounds present in VOO and

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then declines in overripe fruits. These results indicate a major involvement of the *Oep2LOX2* gene in the biosynthesis of VOO volatile compounds.[13] On the other hand, Palmieri-Thiers et al.[14] suggested that the *Oe1LOX1* gene is associated with the senescence process, because it is expressed in the olive fruit mesocarp at late ripening stages, whereas the *Oep2LOX1* gene exhibits a moderate maximum transcript level at the onset of ripening in 'Picual' fruits, so it might be implicated in processes involving loss of membrane integrity occurring during ripening.[15] The *OepHPL* gene codes for a HPL protein with a strict specificity for 13-hydroperoxides, and it is expressed in fruit mesocarp displaying a slight, although significant, maximum at the onset of ripening.[16] This slight maximum preceded the fruit developmental stage, which gives rise to oils with the highest contents of C6 aldehydes.

Research is now aiming at realizing a better control of temperature during the VOO extraction process to modulate the taste, aroma, and color of this product to satisfy consumer demands. In this sense, enzymatic crude preparations from olive fruits were used as an approach for the study of the thermal stability of the LOX pathway enzymes involved in the biosynthesis of VOO volatile compounds.[17] Experimental data were compatible with the existence in olive fruit crude preparations of two LOX isoforms having different thermal stabilities, a thermolabile LOX (LOX_{lab}) and a more thermoresistant LOX (LOX_{res}), and one HPL species. Thermal characteristics of LOX and HPL enzymatic activities in crude preparations explained both the observed decrease of volatile contents in VOO and the distinct pattern of reduction in the contents of C6 and C5 compounds as a consequence of heat treatments of olive fruit. The aim of the present work was to characterize the thermal inactivation parameters of recombinant proteins related to the biosynthesis of VOO volatile compounds through the LOX pathway and try to assign them to those enzymatic activities determined in crude extracts according to their thermal inactivation kinetic parameters.

MATERIALS AND METHODS

Chemicals and Reagents. Culture media components were purchased from Pronadisa (Torrejon de Ardoz, Spain). Isopropyl-β-D-thiogalactopyranoside was purchased from Fermentas (Vilnius, Lithuania). Fatty acids, soybean LOX, antibiotics, amino acids, and reagents for enzymatic activity measurements and reference compounds used for volatile identification were supplied by Sigma-Aldrich (St. Louis, MO, USA) except for (*Z*)-hex-3-enyl acetate purchased from Givaudan Co. (Clifton, NJ, USA) and (*Z*)-hex-3-enal generously supplied by S.A. Perlarom (Louvaine-La-Neuve, Belgium).

Plant Material. Olive fruits (*Olea europaea* L.) cultivar 'Hojiblanca' were harvested in CIFA Cabra-Priego orchards (Cabra, Córdoba, Spain) at green-yellow stage, maturity index (MI) 1 (25 weeks after flowering, WAF), and at overripe stage, MI 7 (36 WAF).

Heterologous Expression and Purification of the LOX and HPL Recombinant Enzymes. Functional expression of three olive *LOX* (*Oep2LOX1, Oep1LOX2, and Oep2LOX2*) genes and a *HPL* (*OepHPL*) gene in *Escherichia coli* and purification of the corresponding recombinant enzymes were performed as described by Padilla et al.[13,15,16]

Measurement of Lipoxygenase and Hydroperoxide Lyase Activities. LOX and HPL activities were determined spectrophotometrically at 25 °C according to the method of Pérez et al.[18] For LOX activity, linolenic acid was used as substrate and the increase in absorbance at 234 nm monitored. For HPL activity, 13-hydroperoxide of linolenic acid was used as substrate and the decrease in absorbance at 234 nm determined. One unit of enzyme activity is defined as the amount of enzyme producing (LOX) or consuming (HPL) 1 μmol of hydroperoxylinolenic acid per minute at 25 °C, taking into account a molar extinction coefficient of 2.5×10^4 M^{-1} cm^{-1} for this compound.

Thermal Inactivation of Crude Enzymatic Extracts. Thermal inactivation of recombinant olive LOX and HPL enzymes was studied by isothermal heating in a thermostated water bath (20−60 °C) for up to 30 min carried out in Eppendorf PCR tubes to ensure a high heat transmission rate. At selected heating times, aliquots of the purified enzyme preparations (200 μL) were cooled immediately in ice water to avoid a further thermal inactivation, and enzymatic activities were measured. Experiments were carried out in duplicate.

Calculation of Kinetic Parameters. The possible kinetic behavior of enzymes during thermal inactivation could be explained through different mathematical models. The first-order kinetic model is described by eq 1

$$A/A_0 = e^{-kt} \quad (1)$$

where A is the activity at time t, A_0 the activity at initial time, and k the first-order inactivation rate constant.

The inactivation rate constants (k) were estimated from plots of Ln % residual activity versus time at different temperatures. The decimal reduction time (D_T) values were calculated from k according to the relationship $D_T = 2.303/k$. Activation energies (E_a) for thermal inactivation were calculated from the slopes of Arrhenius plots according to eq 2

$$\mathrm{Ln}(k) = -E_a/RT + c \quad (2)$$

where R is the gas constant and T the absolute temperature. The temperature needed to reduce the D_T value by 1 Log unit (z value) was obtained from a plot of Log D_T against temperature.

Inactivation kinetic parameters for the different enzymatic activities were estimated through linear regression analysis using the Microsoft Excel 2010 software program.

Heat Treatment of Olive Fruits and Oil Extraction. Fruits were distributed in 3.5 kg batches, dipped in a 90 L thermostatic water bath at different temperatures (20, 40, and 60 °C) for 3 min, and immediately processed. This treatment time was observed to be enough for the whole fruit (pulp and kernel) to reach the desired temperature.[19,20] Olive oil extraction was performed maintaining the same operating conditions by using an Abencor analyzer (Comercial Abengoa, S.A., Seville, Spain) that simulates on a laboratory scale the industrial process of VOO production.[21] Milling of olive fruits was performed using a stainless steel hammer mill operating at 3000 rpm provided with a 5 mm sieve. Resulting olive pastes were immediately conditioned to 30 °C before malaxation. The malaxation step was carried out always in the same experimental conditions by kneading the olive pastes 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 analysis (0.5 g) were taken in 10 mL sealed vials under nitrogen and stored at −18 °C until analysis. Treatments were carried out in duplicate.

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 fiber DVB/Carboxen/PDMS 50/30 μm (Supelco Co., Bellefonte, PA, USA). Sampling time was 50 min at 40 °C, and it was carried out in triplicate. Desorption of volatile compounds trapped in the SPME fiber was done directly into the GC injector. Volatiles were analyzed 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, USA). Operating conditions were as follows: N_2 as carrier, gas injector and detector at 250 °C, column 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 redeodorized high-oleic sunflower oil. For comparison purposes among treatments, volatiles were divided into two classes according to the method of Sánchez-Ortiz et al.:[22] C6 compounds, synthesized by the heterolytic branch of the LOX

Table 1. Rate Constants (k) and Decimal Reduction Time Values (D_T) for Thermal Inactivation of Olive Lipoxygenase and Hydroperoxide Lyase Recombinant Proteins

	$k \times 10^3$ (min^{-1})				D_T (min)			
temp (°C)	Oep2LOX1 (r^2)	Oep1LOX2 (r^2)	Oep2LOX2 (r^2)	OepHPL (r^2)	Oep2LOX1	Oep1LOX2	Oep2LOX2	OepHPL
20	18.7 (0.972)				123.16	[222.56][a]	[1239.88]	[88879.17]
25	29.8 (0.971)				77.28	[137.93]	[557.25]	[28220.84]
30	70.3 (0.991)	26.5 (0.986)			32.76	86.91	[250.45]	[8960.66]
35	195.1 (0.994)	46.5 (0.987)	20.3 (0.993)		11.80	49.53	113.45	[2845.18]
40	452.8 (0.986)	65.0 (0.995)	41.6 (0.991)		5.09	35.43	55.36	903.40
45		115.2 (0.988)	114.8 (0.996)	7.9 (0.982)	[2.39]	19.99	20.06	291.52
50		184.2 (0.976)	237.3 (0.997)	24.8 (0.982)	[1.05]	12.50	9.71	92.86
55			463.5 (0.999)	83.6 (0.996)	[0.46]	[7.81]	4.97	27.55
57				134.4 (0.995)	[0.33]	[6.45]	[3.34]	17.14
60				232.1 (0.991)	[0.20]	[4.84]	[2.06]	9.92

[a]Figures within brackets are D_T values extrapolated using the regression lines obtained from plots of the experimental log D_T against temperature for every recombinant enzyme.

pathway, comprising aldehydes and alcohols of six straight-chain carbon volatiles from linoleic and linolenic acids, and C5 compounds, grouping together five straight-chain carbon alcohols and carbonyls and pentene dimers from either linoleic or linolenic acids synthesized by the homolytic branch of the LOX pathway.

RESULTS AND DISCUSSION

As commented in the Introduction, three olive *LOX* genes and one *HPL* gene have been isolated and characterized previously by our research group. All of these genes have been overexpressed in bacteria, and the kinetics of thermal inactivation of purified recombinant Oep2LOX1, Oep1LOX2, Oep2LOX2 and OepHPL were measured in the temperature range of 20−60 °C and for incubations up to 30 min. In general, the enzymatic activity slowly decreased as the heating time increased at low heating temperatures, but a sharper decrease in activity was observed in the first minutes at higher temperatures. The semilogarithm plots of the residual activities of recombinant purified olive LOXs and HPL at different temperatures as a function of time were linear (see Figures A−D, graphs A, in the Supporting Information), which indicate that the recombinant olive LOXs and HPL enzymes are heat-inactivated in accordance with first-order kinetics. This behavior suggests that sequential inactivation pathways comprising intermediate forms with reduced activity, which has been reported in other systems,[23] are not involved. The thermal inactivation of the olive recombinant proteins comprises a simple conversion of active enzyme to denatured or inactive enzyme with a specific rate constant k (Table 1) and, consequently, it is supposed to occur also in the crude extracts or during VOO processing. Thus, the simple exponential equation from the first-order model (eq 1) is able to adequately fit the kinetic data at all temperatures with a correlation coefficient (r^2) >0.971.

As deduced from the D_T values (Table 1), both olive type 2 LOXs (recombinant Oep1LOX2 and Oep2LOX2) are around 10 times more stable than type 1 Oep2LOX1. Oep1LOX2 and Oep2LOX2 lose half of their activity in 10 min at around 40−45 °C. Oep2LOX2 is slightly more thermally stable than Oep1LOX2. By comparison of the E_a and Q_{10} values calculated for olive recombinant LOXs (Table 2) (see also Figures A and B, graphs B, in the Supporting Information) with those found for the LOX activities in olive crude extracts reported in Luaces et al.,[17] recombinant Oep1LOX2 and Oep2LOX2 could be identified with those LOX_{lab} and LOX_{res} in crude extracts,

Table 2. Activation Energies (E_a) for the Thermal Inactivation, z Values, and Q_{10} Average Values in the Assayed Temperature Intervals and in the 45−55 °C Interval of Olive Lipoxygenase and Hydroperoxide Lyase Recombinant Proteins

	E_a (kJ mol^{-1})	z (°C)	Q_{10}	Q_{10} (45−55 °C)
Oep2LOX1	125.59	13.95	5.21	5.21[a]
Oep1LOX2	77.84	24.06	2.60	2.56[a]
Oep2LOX2	134.42	14.40	4.95	4.04
OepHPL	201.88	10.04	9.92	10.58

[a]Values estimated from the slopes of the regression lines of log D_T as a function of temperature.

respectively. As shown by the inactivation constants (k) and D_T values, both LOX isoforms are quite affected by heat, comparable to those of tomato LOX2 and LOX3 and lower than that of the tomato LOX1 isoform.[24] However, they showed inactivation rate constants 1−2 orders of magnitude higher than those reported for LOX from other sources such as potato,[25] soybean,[26] or green bean.[27] Just slight differences between the D_T values of the recombinant Oep1LOX2 and the LOX_{lab} fraction were observed at different temperatures. Assuming that recombinant Oep1LOX2 and crude extract LOX_{lab} were the same, these data suggest that the medium composition did not greatly influence the rate of LOX inactivation in the case of this isoform. However, supposing that recombinant Oep2LOX2 and LOX_{res} were the same, the composition of the matrix would affect significantly the thermal stability of recombinant Oep2LOX2 as deduced from the D_T values of this enzyme (Table 1) and those of the LOX_{res} fraction reported in Luaces et al.[17] In particular, the thermal resistance of recombinant Oep2LOX2 increased around five times when present in an olive crude extract medium reinforcing its role as the main LOX isoform involved in the synthesis of VOO volatile compounds. Interaction with other constituents present within the olive fruit crude samples may contribute to the observed thermal stabilities. In this sense, pea seed contains LOX isoforms that display the same thermal characteristics in media with different compositions and isoforms that show completely different properties.[28] Moreover, the inactivation rate constants for recombinant Oep1LOX2 and Oep2LOX2 change differently as a function of temperature as occurred with the two LOX species in olive fruit crude extracts. The calculated z value for Oep1LOX2 was higher than that for

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Oep2LOX2, whereas the Q_{10} value was lower (Table 2). Actually, those E_a and Q_{10} values for Oep1LOX2 were the highest and the lowest, respectively, among the four recombinant proteins under study, suggesting that the inactivation rate constants of Oep1LOX2 are affected less by temperature among these recombinant proteins. These characteristics indicate that the thermal stability of Oep2LOX2 is higher (higher D_T values as displayed in Table 1) than that of Oep1LOX2 at temperatures below 45 °C and lower for temperatures >45 °C, the thermal stabilities of recombinant Oep1LOX2 and Oep2LOX2 being practically the same at 45 °C (similar D_T values). These differences might explain the distinct pattern of reduction of the contents of C6 and C5 volatile compounds in VOO as a consequence of increasing the temperature at milling during olive oil processing. As displayed in Figure 1, the C6/C5 compound ratio increased in oils

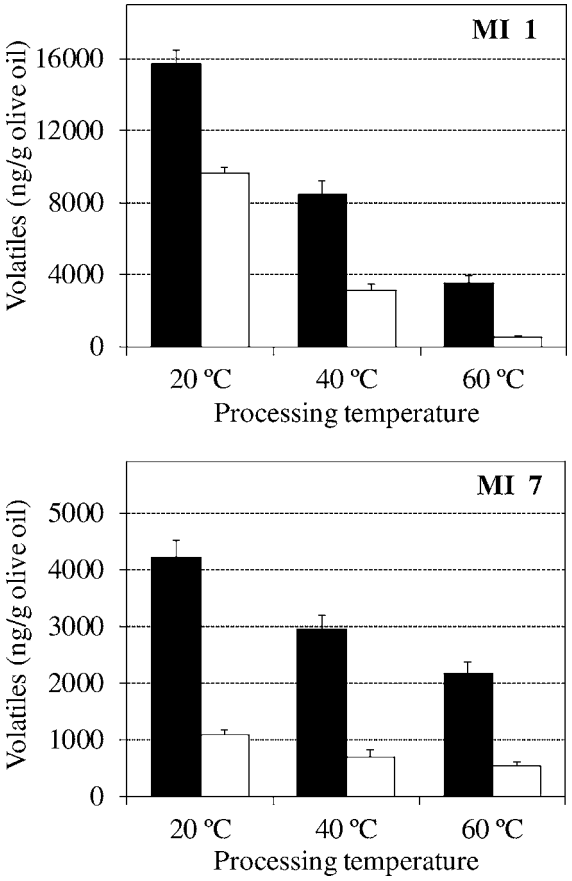

Figure 1. Contents of C6 (black bars) and C5 (white bars) compounds in olive oils extracted from olive fruits at maturity stages green-yellow (MI 1) and black over-ripe (MI 7) processed at different temperatures at the milling step.

extracted from green-matured fruits (MI 1, 25 WAF) as the processing temperature augmented, whereas this ratio was almost constant (around 4) in oils obtained from over-ripe fruits (MI 7, 36 WAF) independent of the temperature of the fruit at the milling step. Moreover, the C6/C5 compound ratio was lower in green-matured fruits than in over-ripe fruits. These results suggest that the Oep2LOX2 protein might be the main LOX isoform synthesizing C5 compounds as it is affected the most by increasing the fruit temperature at milling, in good agreement with its thermal inactivation parameters (Table 2) and that the Oep2LOX2 protein would have a higher relative contribution to the total LOX activity during the oil extraction process in green-matured fruits than in over-ripe fruits, as described in the Introduction for its putative gene transcript.

According to the decimal reduction times (D_T) calculated for each recombinant protein (Table 1), Oep2LOX1 seems to be the most thermolabile of the recombinant enzymes under study, despite the fact that it displays an intermediate inactivation dependency on temperature as deduced by the E_a, z, and Q_{10} values (Table 2) (see also Figure C, graph B, in the Supporting Information). This LOX isoform loses half of its activity at 30 °C in around 10 min. Thus, the participation of Oep2LOX1 during the oil extraction process, synthesizing 9-hydroperoxides and competing for polyunsaturated fatty acids with the 13-LOX enzymes, is questionable. The recommended temperature for the malaxation step in the VOO extraction process is around 30 °C, but it is not uncommon in the oil industry to use higher temperatures of processing. According to the experimental data of our previous work reported in Luaces et al.,[17] thermal inactivation of LOX activity in olive fruit crude preparations responded as if it contained two LOX proteins as deduced by the two separated phases found when representing the semilogarithm of LOX residual activity as a function of heating time at different temperatures. The absence of a phase corresponding to the Oep2LOX1 activity could be ascribed to the low thermal stability observed for this protein, despite the fact that most of the LOX activity in cold-extracted crude preparations is 9-LOX.[4] On the other hand, the protein encoded by the *Oe1LOX1* gene isolated by Palmieri-Thiers et al.[14] might be also contributing to the synthesis of 9-hydroperoxides. Its role as competitor of the 13-LOXs, involved in the synthesis of VOO volatile compounds, for the metabolization of polyunsaturated fatty acids, is expected to be low because the *Oe1LOX1* gene is expressed only at the end of the olive fruit ripening.

Recombinant OepHPL was found to be the most thermally resistant of the recombinant proteins under study. This observation is in agreement with the experimental data on thermal stability of HPL we have found previously in olive fruit crude extracts.[17] OepHPL loses half of its activity at 55 °C in around 10 min. With regard to the inactivation kinetic parameters, recombinant OepHPL could be identified with the HPL activity determined in olive fruit crude extracts. Calculated E_a and Q_{10} values for the olive recombinant OepHPL (Table 2) (see also Figure D, graph B, in the Supporting Information) were 201.88 kJ mol^{-1} and 9.92, whereas those calculated for olive HPL in crude extracts were 177.53 kJ mol^{-1} and 6.18, respectively. The E_a value calculated for recombinant OepHPL is quite close to that of tomato HPL (197 kJ mol^{-1}), the only one reported for this enzymatic activity from plants.[24] The D_T values found for recombinant OepHPL (Table 1) were in general double those found for HPL in olive crude extracts.[17] According to these data and assuming that recombinant OepHPL and olive crude extract HPL were the same, the composition of the crude extract seems to affect negatively the thermal stability of olive HPL.

The thermal stability of crude enzymatic extracts from olive fruit responded as if it contained only one HPL isoform.[17] In this sense, transcriptomic data have shown the presence of only one *HPL* gene expressed in olive fruit mesocarp (authors' unpublished results). However, Salas and Sanchez[6] reported the presence of two HPL isoforms in olive pulp that displayed quite similar properties in terms of optimum pH, substrate specificity,

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and thermal inactivation characteristics as suggested by the experimental data, which might be an artifact of the purification process. Unlike olive, thermal inactivation of tomato HPL displays two phases when representing the semilogarithm of HPL residual activity as a function of heating time at different temperatures: a rapid phase resulting in the loss of about 50% activity at temperatures below 50 °C and a second phase responsible for the inactivation of the rest, suggesting the presence of more than one HPL isoform in tomato with different thermal stabilities.[24]

In conclusion, the thermal inactivation kinetic parameters of recombinant enzymes from the olive LOX pathway suggest that Oep1LOX2 and Oep2LOX2 could be the LOX isoforms determined in olive fruit crude preparations, LOX_{lab} and LOX_{res}, respectively. Their roles in the biosynthesis of VOO volatile compounds are in agreement with those assigned to *Oep1LOX2* and *Oep2LOX2* genes based on their expression patterns during ripening and the catalytic properties of the proteins encoded. On the other hand, Oep2LOX1 was, on average, 10 times less thermally stable than Oep1LOX2 and Oep2LOX2, which makes uncertain its actuation during the oil extraction process as a competitor of 13-LOXs for polyunsaturated fatty acids. Similarly, according to the thermal inactivation kinetic parameters, recombinant OepHPL could be identified as the HPL activity in crude preparations. The thermal stability of recombinant OepHPL was the highest among the recombinant proteins studied, which suggests that HPL activity is not a limiting factor for the synthesis of VOO volatile compounds. Moreover, the thermal characteristics of recombinant LOX and HPL enzymatic activities would explain the observed decrease of volatile contents and changes in the C6/C5 ratio in the VOO volatile fraction as a function of the temperature of olive fruit at milling in the oil extraction process and the need to reduce the temperature of the whole process in general.

■ ASSOCIATED CONTENT

Supporting Information

Thermal inactivation curves as a function of heating time and temperature, and Arrhenius plots of enzyme inactivation rate constants for recombinant Oep1LOX2 (Figure A), recombinant Oep2LOX2 (Figure B), recombinant Oep2LOX1 (Figure C), and recombinant OepHPL (Figure D). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +34-95-4611550. Fax:+34-95-4616790. E-mail: carlos.sanz@ig.csic.es.

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Notes

The authors declare no competing financial interest.

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