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An Active Linoleate 13-Lipoxygenase is Found in Virgin Olive Oil

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Introduction

Olive oil is one of the oldest known vegetable oils, and it is almost unique in that it can be consumed without any refining treatment One of its most important quality problems is oxidative rancidity due to the oxygenation of polyenoic fatty acids and formation of compounds that derive from these fatty acid hydroperoxides (Boskou, 1996) Among oxidizing enzyme activities within olive oil an activity towards polyenoic fatty acids was suggested to be a lipoxygenase (LOX) (EC 1 13 11 12), which may originate from olive endosperms and survived at least in part olive oil production (Georgalaki *et al* , 1998) For storage lipids of various oilseeds a number of oxygenated fatty acid derivatives, such as 9- and 13-hydroxy octadecadienoic acid, have been described, which might be derived from the action of a LOX form located within the developing seed (van de Loo *et al* 1993) This may implicate the occurrence of LOX forms within this tissue Plant LOXs are non-heme iron-containing dioxygenases They oxygenate polyenoic fatty acids to S configurated fatty acid hydroperoxides, and are classified into 9- and 13-LOX with respect to their positional specificity in linoleic acid oxygenation (Rosahl, 1996) In this communication we present a partial characterization of an active LOX form within commercially available olive oil which might be derived from olive endosperm

Experimental

Virgin olive oil extraction, preparation of protein extracts from olive oil, spectrophotometric determination of LOX activity with linoleic acid as substrate, size exclusion chromatography of olive oil protein extracts, and other analytic procedures were performed as previously described (Georgalaki *et al.*, 1998). Determination of product specificity of olive oil LOX and analysis of endogenous LOX-derived fatty acid derivatives were performed by HPLC-, GC-MS- and ^{1}H-NMR analysis as described before (Feussner *et al.*, 1997).

Results and Discussion

Protein extracts produced from olive oil by low speed centrifugation following size exclusion chromatography on Superose 6 HR 10/30 or Sepharose 4B columns were used for LOX analysis. In both cases nearly all LOX activity eluted at the void volume of the column. The preferential occurrence of LOX activity in fractions related to an unexpected high molecular mass suggested that the enzyme might be predominantly associated with membrane compartments of olive endosperms transferred in olive oil during its production. Light microscopic inspection of these fractions revealed the occurrence of entire lipid vesicles of a mean diameter of 0.75-1.5 µm. The calculated phosphate-, neutral lipid- and protein-content of this fraction and the finding that phosphatidylcholine was the major phospholipid identified may suggest that these vesicles are derived from oil bodies and that the LOX activity might be associated with their membrane. The characterization of olive oil LOX as a membrane-associated activity is furthermore strengthened by experiments using the Triton X-114 two-phase partitioning technique, as performed previously with cucumber LOX (Feussner and Kindl, 1994), which revealed that the olive oil LOX activity behaved like a particulate enzyme (data not shown).

TABLE 1 (A) Analysis of LOX products within olive oil. Total lipids from olive oil were extracted and fractionated into esterified fatty acids and free fatty acids. (B) Analysis of the products from the reactions of protein preparations from olive oil with linoleic acid. 900 µl of olive oil extract in 50 mM sodium phosphate buffer pH 6.0 was reacted with 300 µg of linoleic acid for 30 min at room temperature.

	Positional isomers		Optical isomers (S : R)	
	13-(*ZE*)-HODE	9-(*EZ*)-HODE	13-(*ZE*)-HODE	9-(*EZ*)-HODE
A) esterified fatty acids	70 : 30		67 : 33	50 : 50
free fatty acids	90 : 10		92 : 8	50 : 50
B) pH 6.0	91 : 9		91 : 9	75 : 25

We further analyzed olive oil for the content of LOX-derived oxygenated fatty acid derivatives, such as (9*S*,10*E*,12*Z*)-hydroxy-(10,12)-octadecadienoic acid or (13*S*,9*Z*,11*E*)-hydroxy-(9,11)-octadecadienoic acid (9-HODE or 13-HODE, respectively), indicating the specific action of one or more LOX forms (Table 1). Oxygenated fatty acids could be detected

within the esterified fatty acid- and the free fatty acid fraction. In both fractions the 13-isomer of hydroxy linoleic acid was the main product and only this positional isomer turned out to be preferentially the S enantiomer, indicating the specific formation of these substances by a LOX *in vivo*. These findings together with the observation that the oxygenated free fatty acid derivatives showed a higher amount of 13-HODE than the esterified fatty acids, suggest a preferential oxygenation of free fatty acids by this LOX form. Most interestingly, the enzyme remained active during the production process within all olive oil charges under investigation. Table 1 shows also the analysis of the products formed by the enzyme extracted from olive oil with linoleic acid at pH 6.0 indicating this LOX form as a linoleate 13-LOX. Furthermore, approximately 1.6% of all linoleic acid molecules within the olive oil samples had been converted into 13-HPODE as determined by ^{1}H-NMR analysis.

Taken together, our results may suggest that a linoleate 13-LOX presumably located at the membranes of oil bodies of olive endosperms is the LOX form detectable in samples of virgin olive oil, where it remains at least in part as an active enzyme and it may oxygenate free linoleic acid within the olive oil.

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