Aroma of Virgin Olive Oil: Biogenesis of the "Green" Odor Notes

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The enzymes acylhydrolase, lipoxygenase, and fatty acid hydroperoxide lyase were found in cell-free extract of olive fruit. Triacylglycerols and phospholipids are hydrolyzed to free fatty acid, mainly polyunsaturated, by acylhydrolase. From linoleic and linolenic acids 9- and 13-hydroperoxides were formed by lipoxygenase. The lyase cleaves the 13-hydroperoxides of linoleic and linolenic acids to form the volatile aldehydes hexanal and *cis*-3-hexenal, respectively; the enzyme does not act on 9-hydroperoxides of these acids. Incubation of ripe olive fruit tissue disks resulted in biological conversion of hexanal and *trans*-2-hexenal to corresponding alcohols. Hexyl alcohols were converted to their acetate esters during the incubation with olive fruit tissue. A sequential enzymatic pathway for the formation of green odor compounds in virgin olive oil is proposed.

INTRODUCTION

Olive oil, which is one of the oldest known vegetable oils, is extracted from the fruits of the olive tree, *Olea europeae*. It is unique among vegetable oils in that it can be consumed in its crude form, called virgin olive oil. Oil properly processed from fresh mature fruits of good quality provides a delicate and unique aroma, whose composition has been reported in the past (Flath et al., 1973; Fedeli et al., 1973; Lercker et al., 1973; Olías et al., 1978). Montedoro et al. (1978), analyzing the composition of esters, alcohols, and aldehydes during maturation, found only quantitative differences. Olías et al. (1980), studying the relationship between fruit maturity and aroma components, observed that the characteristic flavor is obtained by the balance between "green" and fruity notes.

Aliphatic C_6 compounds and corresponding hexyl esters, which decisively contribute to the "unripe" component of fruit flavor, are always found in great quantities in the volatile compounds of olive oils (Montedoro et al., 1978; Olías et al., 1978; Guth and Grosch, 1991). The formation of C_6 aldehydes and alcohols in the plant is related to cell destruction. The milling of olive fruits is the first step in obtaining the oil. Milling and malaxation (continuous mixing of crushed fruit with water) prepare the paste for its extraction by pressing or centrifugation. Disruption of intact cells results in the release of lipid-degrading enzymes that degrade the membrane or stored lipids.

Among the degradation products, hexanal, *cis*-3-, *trans*-2-hexenal, and their analogous reduced products have been detected in disrupted tissues of apples, grapes (Drawert et al., 1966), and tomatoes (Kazeniak and Hall, 1970). It has long been assumed that unsaturated fatty acids are the precursors of these compounds, and a pathway involving lipoxygenase and hydroperoxide lyase has been demonstrated (Tressl and Drawert, 1973; Stone et al., 1975). Since free fatty acids do not accumulate in healthy plant tissues, the initial step in the degradation process should be the liberation of free fatty acids by lipolytic enzymes (Galliard et al., 1977). The presence of alcohols having the same chain length as the carbonyls found among the plant volatiles provides good evidence for oxidoreductase activity in tissues; finally, an alcohol acyltransferase is necessary for the formation of corresponding hexyl esters.

The work presented in this paper provides good evidence for the involvement of an enzymatic system including acylhydrolase (AH), lipoxygenase (LOX), fatty acid hydroperoxide lyase (FAHL), alcohol dehydrogenase (ADH), and alcohol acyltransferase (AAT) in the formation of green odor notes of virgin olive oils.

EXPERIMENTAL PROCEDURES

Materials. *O. europeae* cv. Arbequina in a stage of ripeness was used.

Chemicals. Ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT), polyvinylpolypyrrolidone (PVP), poly(oxyethylene ether) (Triton X-100), hexanal, *trans*-2-hexenal, and triethanolamine were from Sigma Chemical Co. (St. Louis, MO). 1,5-Diphenylcarbazide, 2,4-dinitrophenylhydrazine (DNP), and cupric nitrate were from Fluka Chemie AG, Buchs, Switzerland. Linoleic and linolenic acids were obtained from Nu-Check Prep (Elysian, MN). The other chemicals and solvents were of analytical grade. 13-Hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13LAOOH) and 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoic acid (13LNAOOH) were obtained using pure soybean lipoxygenase (L-1) (Hamberg and Samuelsson, 1967); 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid (9LAOOH) and 9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoic acid (9LNAOOH) were obtained with lipoxygenase purified from potato tubers (Galliard and Phillips, 1971).

Crude Enzyme Extract Preparation. Fresh fruits were thoroughly rinsed with water, wiped with paper tissue, and then pitted. The flesh of the fruit (10 g) was ground in 5 volumes of grinding buffer, 50 mM sodium phosphate, pH 6.8, containing 0.2 mM EDTA, 0.2% Triton X-100, 0.3 mM DTT, 10 mM sodium metabisulfite, and 20% hydrated PVPP. Grinding was done in three 20-s periods. The homogenate was filtered in vacuum to remove PVPP and centrifuged for 20 min at 20000*g*. The supernatant was clarified by passing it through four layers of gauze to exclude lipid material that separated during centrifugation.

Acylhydrolase Assay. Lipolytic activity was measured by a colorimetric method. The 1-mL reaction mixture contained 100 mM borate buffer, pH 8.5, 5 mM DTT, 2.5 mM substrate, and 0.1 mL of crude extract. The substrate, olive oil triglycerides or soybean phospholipid, was emulsified in 5% gum arabic for 1 min at high speed with a sonicator; the emulsions were prepared immediately before use. The reaction was carried out at 35 °C in a shaker water bath and stopped at 40 min by adjusting the pH to 3 with 4 N HCl. The fatty acids were converted to copper soaps and quantified using 1,5-diphenylcarbazide as the color reagent (Nixon and Chan, 1979).

Lipoxygenase Assay. Lipoxygenase activity was determined spectrophotometrically at 25 °C with linoleic acid as substrate by measuring the increase in absorbance at 234 nm arising from the conjugated double bonds formed by hydroperoxidation of linoleic acid (Axelrod et al., 1981).

0021-8561/93/1441-2368$04.00/0

Fatty Acid Hydroperoxide Lyase Assay. For assaying FAHL the method of direct analysis of volatile aldehyde in the headspace by gas chromatography was employed. The reaction mixture contained $(3 - x)$mL of 50 mM sodium phosphate, pH 5.7, 20 μM 13LAOOH, and the enzyme in a volume of x mL. The reaction was carried out in a 10-mL vial fitted with a serum stopper. After 1 min of incubation at 25 °C, the reaction was stopped by lowering the pH to 3 with 2 N HCl. The vial was then transferred into an automatic headspace sampler (Hewlett-Packard 19395A), where a 15 min equilibrium time at 80 °C was set to allow the volatiles to enter the gas phase. Gas chromatography of the headspace was performed on a column (2 mm × 2 m) containing 10% FFAP on Chromosorb W-HP (80–100 mesh), operated isothermally at 85 °C; the injector temperature was 250 °C, and the detector (FID) temperature was 275 °C. Nitrogen was used as the carrier gas (20 mL/min). Under these conditions the retention times of hexanal and *trans*-2-hexenal were 2.5 and 6.07 min, respectively.

Alcohol Dehydrogenase Assay. ADH activity was measured by analyzing in the headspace the volatile alcohol formed from aldehyde substrate by gas chromatography. The standard reaction mixture contained 2 mL of 200 mM sodium phosphate, pH 6.8, 2 mM hexanal, 1 mM NADH, and 1 g of olive tissue disks. The mixture was incubated at 30 °C for 30 min in a 10-mL sealed vial. The headspace was analyzed automatically as stated before using the same column; the only modification was in the oven temperature, which was 85 °C for 2 min and then raised to 110 °C at 3 °C/min. Under these conditions the retention times were 2.5 min for hexanal, 4.07 min for *trans*-2-hexenal, 6.52 min for hexanol, 7.63 min for *trans*-2-hexenol.

Alcohol Acetyltransferase Assay. AAT activity was assayed by analyzing the volatile ester formed in the headspace using the same procedure as stated for ADH. The reaction mixture contained 2 mL of 200 mM sodium phosphate, pH 6.8, 2 mM hexanol, 0.5 mM acetyl-CoA, and 1 g of olive tissue disks. The mixture was incubated at 30 °C for 60 min in a sealed vial. The volatiles in the headspace were determined as described in the ADH assay; the retention times of hexyl acetate and *trans*-2-hexenyl acetate were 4.6 and 6.32 min, respectively.

Identification of Products Formed by Enzymatic Action. To assess the specificity of olive acylhydrolase, 25 mg of substrate (olive triglyceride or soybean phospholipid) was emulsified with 5% gum arabic in 10 mL of 100 mM borate buffer, pH 8.5, and mixed with 10 mL of crude extract. The reaction mixture was incubated at 35 °C in a shaker water bath for a period of 45 min. The products of lipolysis were extracted, fractionated, and analyzed by GLC (Sanz and Olías, 1990).

To characterize the specificity of olive lipoxygenase, hydroperoxides were prepared by adding 2 mL of crude extract and 2 mL of 10 mM acid substrate solution (linoleic or linolenic acid) to 25 mL of oxygen saturated 100 mM sodium phosphate buffer, pH 6.1. After 20 min, the mixture was adjusted to pH 3 with 2 N HCl and passed through an octadecyl (C_{18}) extraction column from which the products were eluted with methanol. The concentrated products were esterified with diazomethane and analyzed by HPLC using a Si100 10-μm (4.6 × 200 mm) column (Olías and Valle, 1988).

To identify hydroperoxide fatty acid degradation products, the volatile aldehydes and oxo acids were determined as the 2,4-dinitrophenylhydrazone (DNPH) derivative. In a typical determination, 25 mL of sodium phosphate buffer, pH 5.7, containing 2.5 mM 13LAOOH or 13LNAOOH was incubated with 2 mL of crude extract for 10 min at 25 °C. The reaction was stopped by adjusting the pH to 3 with 2 N HCl. Next, 1 mL of 0.4% DNP in 4 N HCl was added. The mixture was shaken for 2 min, allowed to stand for 10 min, and extracted three times with 5 mL of hexane. The combined hexane was removed by evaporation and the residue dissolved in known volume of methanol. DNPHs were analyzed by HPLC (Olías et al., 1990).

Isolation and Analysis of Virgin Olive Oil Volatiles. Dynamic headspace sampling on charcoal was used to recover of volatiles from oil. In a flask (1 L) with a screw-top, containing both an inlet and an outlet tube, 500 g of virgin olive oil was placed. The flask was housed within a thermostated water bath (35 °C), and the headspace was flushed with nitrogen (99.9% pure) at a flow rate of 150 mL/min for 8 h. For sampling, a standard charcoal tube (ORBO-32, Supelco) was connected to the outlet. Extraction of the trapped headspace volatiles was carried out with carbon disulfide. The concentrated extract was fractionated at 5 °C on a column (30 × 1 cm) packed with a slurry of silica gel in pentane. The elution was performed with 75 mL of pentane, followed by 75 mL of diethyl ether (polar fraction). Identification of polar compounds was made by means of a gas chromatography–mass spectrometry system, composed of a HP-5890 gas chromatrograph directly fitted to an AEI-MS30/70 mass spectrometer and to a VG-11/250 data system (VG Analytical, Manchester, U.K.). The volatiles were separated on a 30 m × 0.25 mm i.d. fused silica capillary column OV-1. The column was held for 15 min at 45 °C and then programmed at 2 °C/min to 160 °C; the flow rate of carrier gas nitrogen was 1 mL/min. Structures were assigned by comparison of the spectra with the authentic standards (Olías et al., 1980).

RESULTS

Solubilization. Phenolic compounds, tannins, anthocyanins, and oleuropein occur in very high concentrations in olive fruits (Fernadez Diez, 1971; Vazquez et al., 1974; Amiot et al., 1986). In the isolation of enzymes from plant tissues, problems due to reaction between plant proteins and plant phenolic compounds are much more prevalent and complex than is generally recognized (Loomis and Battailes, 1966). In our case the fact that oleuropein acts as antioxidant (Vazquez, 1978) added an additional problem to the inhibition of lipoxygenase enzyme. In preliminary studies a crude extract (50 mg of protein/mL) prepared with 50 mM sodium phosphate, pH 6.8, showed low lipolytic activity and was void of lipoxygenase and hydroperoxide lyase activities. The presence of antioxidants in the extract was evident since 20 μL of solution extinguished the increase in absorbance at 234 nm (0.27 μA/min) caused by soybean lipoxygenase (2 mg/mL) at pH 9.0. Insoluble polyvinyl-*N*-pyrrolidone (PVP-P) was employed to remove phenolics from crude homogenate; although PVP-P is not sufficient to remove all phenolics, it markedly delays the loss of activity. Addition of sodium metabisulfite prevented the rapid darkening of homogenate caused by phenolic oxidation. Control experiments showed that the reducing agents did not interfere with the enzyme assays.

According to previous papers (Phillips and Galliard, 1978; Olías et al., 1990), hydroperoxide lyase is membrane-bound; in olive fruits this possibility was examined with the operational criterion that the membrane-associated activity should sediment at 150000g in 1 h. When crude extract was centrifuged under these conditions, more than 80% of lyase activity was recovered in the pellet; it was also noted that about 40% of the lipoxygenase activity was sedimented. It was therefore assumed that enzymes were adsorbed on membrane fragments or otherwise associated with particulate matter. To solubilize the enzymes, the best result in this study was obtained by adding 2 g/L Triton X-100 to the extraction buffer. EDTA and DTT were also necessary to stabilize the enzymes.

Crude extract from olive fruit pericarp prepared with this buffer, chromatographed on a Sephadex G-25 column (2.5 × 30 cm, 40 mL/min) and stored at 4 °C was found to be quite unstable. It lost about 30% of its activity in 72 h and was inactive in a week for acylhydrolase, lipoxygenase, and fatty acid hydroperoxide lyase.

Partial Characterization of Enzymes in the Crude Extract. *Crude Acylhydrolase.* The effect of pH on the relative rate of hydrolysis of olive oil triglycerides was studied using 100 mM acetate (range 4–6), phosphate (range 6–8), and borate (range 8–10) buffers. The enzyme exhibited a narrow range of pH activity with a maximum at pH 8.5; activity below pH 8 or above pH 9 was less than

Table I. Percentage of Each Fatty Acid Relative to the Total Fatty Acids Released by Olive Acylhydrolase from Triglyceride (TG) and Phospholipid (PL)

acid	TG[a]	fatty acid from TG hydrolysis	PL[a]	fatty acid from PL hydrolysis
C_{16}	8.9[b]	23.56[b]	20.50	37.9
$C_{16:1}$	1.32	1.30	0.39	
C_{18}	2.58	10.66	4.09	
$C_{18:1}$	74.58	47.13	9.41	
$C_{18:2}$	10.37	10.83	58.32	28.91
$C_{18:3}$	2.14	6.43	7.26	33.07

[a] TG (olive oil triglyceride), PL (soybean phospholipid); fatty acid percentage by transesterification with CH_3ONa and HCl–CH_3OH. [b] Average of three determinations.

Table II. Product Specificity of Olive Lipoxygenase Using Linoleic and Linolenic Acids as Substrate

fatty acid substrate	hydroperoxide isomer observed, percentage within each acid	
linoleic (LA)	9LAOOH, 65[a]	13LAOOH, 35
linolenic (LNA)	9LNAOOH, 57	13LNAOOH, 43

[a] Average of three determinations.

20%. The substrate specificity of a lipase is defined by its positional specificity, its stereospecificity, or its preference for longer or shorter saturated or unsaturated acids. In this work we were interested in checking the capacity of olive fruit AH for the release of unsaturated fatty acids from triacylglycerols and phospholipids. The results of this analysis are shown in Table I. From the pattern of hydrolysis we can assert, without going into the issue of specificity, that olive acylhydrolase releases linoleic and linolenic acids, which are specific substrates for lipoxygenase enzyme.

Crude Lipoxygenase. The effect of pH on lipoxygenase activity was studied using 100 mM sodium phosphate buffer. The enzyme showed an optimum at pH 6.1; below pH 5.2 and above pH 6.6 the activity was reduced by more than 30%. To characterize specificity for the side of oxygen insertion, linoleic and linolenic acids were used as substrates. To eliminate interference from isomerizations, the oxidation, extraction, and methylation were carried out swiftly at low concentration and temperature, and the determination of isomeric rate by HPLC was performed immediately. The 13- and 9-hydroperoxide isomers had retention times (R_t) of 8.9 and 12 min, respectively. These R_t values were identical to those of 13- and 9-hydroperoxide isomers prepared according to the methods of Hamberg and Samuelsson (1967) and Galliard and Phillips (1971). To obtain more evidence on chemical structure, the products were collected separately from HPLC and analyzed as described in Olías and Valle (1988). The ratios of isomers given by HPLC are shown in Table II. In contrast to this specificity of peroxidation, lipoxygenase from apple (Grosch et al., 1977), cucumber (Sekiya et al., 1979), and pear and strawberry (Kin and Grosch, 1978) oxidizes predominantly C-13 of the substrate, while tomato lipoxygenase (Zamora et al., 1987) produces nearly exclusively 9-isomer hydroperoxide.

Crude Fatty Acid Hydroperoxide Lyase. The activity of this enzyme in crude extract was maximum at pH 5.7, losing more than 50% of activity below pH 5.0 and above pH 7.0. To characterize the hydroperoxide degradation products, crude extract was incubated with 9LAOOH, 9LNAOOH, 13LAOOH, and 13LNAOOH; volatile aldehydes and oxo acids were determined by HPLC as the DNPH derivatives. The results are shown in Table III. With the conditions stated under Experimental Procedures

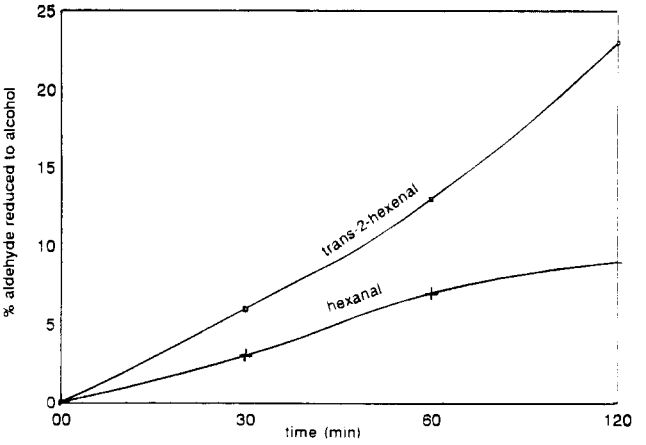


Figure 1. Time course of hexanal and *trans*-2-hexenal reduction by olive ADH. For this experiment, 10 g of olive fruit tissue was suspended in 10 mL of 200 mM sodium phosphate buffer, pH 6.8, containing 7 μmol of each carbonyl. The incubation mixture was sonicated for 2 min and held at 30 °C during the assay; at the indicated time the headspace of 2 mL of mixture was analyzed by GC.

Table III. Products Formed from Fatty Acid Hydroperoxides by Olive Hydroperoxide Lyase

hydroperoxide substrate	products analyzed	
	carbonyl	ω-oxocarboxylic acid
9LAOOH 13LAOOH	hexanal	12-oxo-*trans*-10-dodecenoic acid
9LNAOOH 13LNAOOH	*trans*-2-hexenal	12-oxo-*trans*-10-dodecenoic acid

the retention times of 12-oxo-*trans*-10-dodecenoic acid, *trans*-2-hexenal, and hexanal were 3.5, 9.5, and 10.4 min, respectively; these R_t values were identical to the DNPHs of the corresponding standards prepared as described in Olías et al. (1990). The enzyme showed lyase activity only against the 13-isomer; similar specificity has been detected in watermelon (Vick and Zimmerman, 1976), tomato (Galliard and Matthew, 1977), alfalfa (Sekiya et al., 1979), tea leaves (Hatanaka et al., 1982), and soybean (Olías et al., 1990).

It is well established that the products from 13-hydroperoxylinoleic acid are hexanal and 12-oxo-*cis*-9-dodecenoic acid (Galliard et al., 1977) and from 13-hydroperoxylinolenic acid *cis*-3-hexenal and the oxo acid stated before. Incubation of olive fruit crude extract with the 13-isomer of linolenic acid produced *trans*-2-hexenal and 12-oxo-*trans*-10-dodecenoic acid instead of *cis*-isomers. In most plants the compounds having a *cis*-3-enal structure are quickly isomerized by an isomerase enzyme to the *trans*-2-enal form (Phillips et al., 1979); we can deduce from the identity of the products in olive fruit crude extract that *cis*-3,*trans*-2-enal isomerase enzyme must exist in this extract.

Partial Characterization of Enzymes in Ripe Olive Fruit Tissue. *Alcohol Dehydrogenase.* Crude extracts prepared as stated in the previous section were ineffective in reducing the carbonyl to alcohol. Using grinding media of different compositions, including glycerol, saccharase, and β-mercaptoethanol, the extracts obtained were similarly ineffective. In assays with commercial alcohol dehydrogenase, the reduction was inhibited by adding 20 μL of any olive crude extract, which seems to show the presence of inhibitors in the extract.

The existence of ADH in olive fruits could be demonstrated working only with tissue slices. Incubation of ripe olive tissue disks with hexanal or *trans*-2-hexenal resulted in its uptake by the tissue and its conversion to alcohols

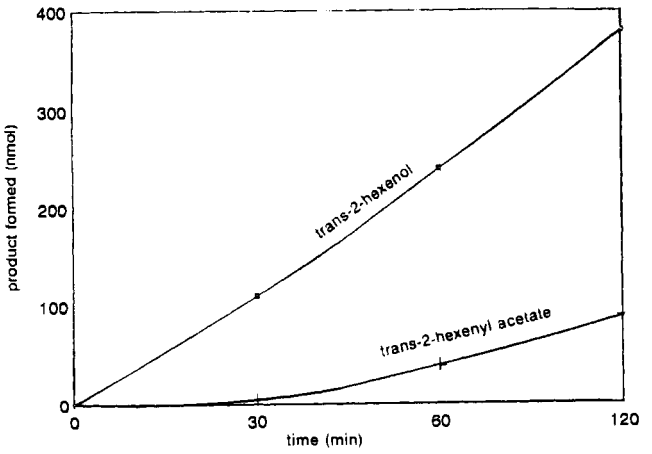


Figure 2. Time course of formation of *trans*-2-hexenol and *trans*-2-hexenyl acetate. The incubation mixture, 10 mL of 200 mM sodium phosphate, pH 6.8, contained 10 g of olive fruit tissue, 7 μmol of *trans*-2-hexenal, and 2 μmol of acetic acid; after 2 min of sonication, the mixture was held at 30 °C. At the indicated time the headspace of 2 mL was analyzed by GC.

having the same chain length as the carbonyls. Olive ADH showed optimal activity at pH 6.8 with *trans*-2-hexenal and NADH as cofactor, losing more than 40% activity below pH 5.0 and almost 50% above 8.5. Assays with tissue showed the presence of enough cofactor to carry out the reduction, although the addition of NADH accelerated the reduction. Figure 1 shows the time course of the reduction of hexanal and *trans*-2-hexenal by olive tissue. Using a partially purified ADH from pea (Erikson, 1968), it was determined that 2-alkenals are poorer substrates than the corresponding saturated compounds. In the experiments carried out with olive disks for C-6 carbonyls, there was a marked specificity for the unsaturated aldehyde, since (as can be seen in Figure 1) at whatever incubation time the production of hexenal was at least double that of hexanal.

Alcohol Acetyltransferase. Similarly to the case of alcohol dehydrogenase, the crude extracts prepared with olive pericarp did not show any esterification capacity. In contrast, incubation of ripe olive fruit tissue disks with hexanol and acetic acid resulted in its uptake by the tissue and conversion to hexyl ester. Nordström (1963) proposed that acetates are not formed through direct esterification of alcohol with acetic acid, and the participation of acetyl-CoA would be important in aroma evolution by brewers' yeast fermentation. In our laboratory, working with enzymic extracts of banana and strawberry (Pérez et al., 1993), we have been able to show that this statement is equally valid for fruits. From the incubations with olive disks, given that the presence of acetic acid was sufficient to esterify hexenol, we deduced that the tissue is able to form acetyl-CoA. Similar results have been reported in banana (Myers et al., 1970), strawberry (Yamashita et al., 1975), and apple (Berger and Drawert, 1984).

AAT showed optimal activity at pH 6.8. The best conditions of esterification are achieved by starting from the corresponding aldehyde, with the prior reduction by ADH and subsequent esterification of the alcohol formed with acetyl-CoA. Figure 2 shows the course of the reaction starting from *trans*-2-hexenal and acetic acid. Under similar conditions, only traces of hexyl acetate were formed from hexanal, which seems to indicate a greater preference for unsaturated alcohols.

DISCUSSION

Plant volatiles can be considered to be direct metabolites of intracellular biogenetic pathways. The quality and quantity of these compounds depend on genetic factors and are influenced by ripening and storage. Some of them are actually secondary products, not occurring or occurring only in traces in intact cells. They are formed very quickly during disruption of cell structure due to enzymic reactions in the presence of oxygen, e.g., by homogenization of plant material (Schreier, 1981; Tressl et al., 1983).

Aliphatic C-6 components, hexanal, *cis*-3-hexenal, *trans*-2-hexenal, hexanol, *cis*-3-hexenol, and *trans*-2-hexenol, and corresponding esters, which contribute significantly to the green notes of the aroma, fall into the category of secondary products. Figure 3 shows the chromatogram of the fraction of polar volatile compounds present in the aroma of a virgin olive oil. The compounds having green sensorial characteristics are indicated. It is obvious that the process of obtaining olive oil can be considered a good example of a system producing secondary volatiles.

It has long been assumed that unsaturated fatty acids are the precursors of these volatile compounds. The enzymes found in olive fruit and the specificities determined enable us to propose the pathway, shown in Scheme

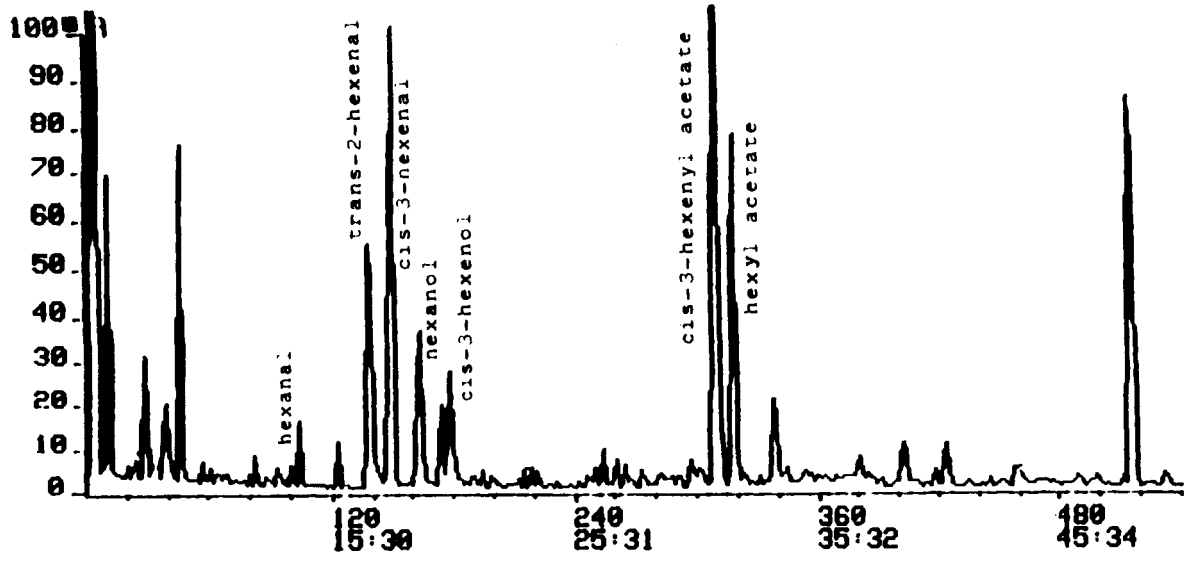
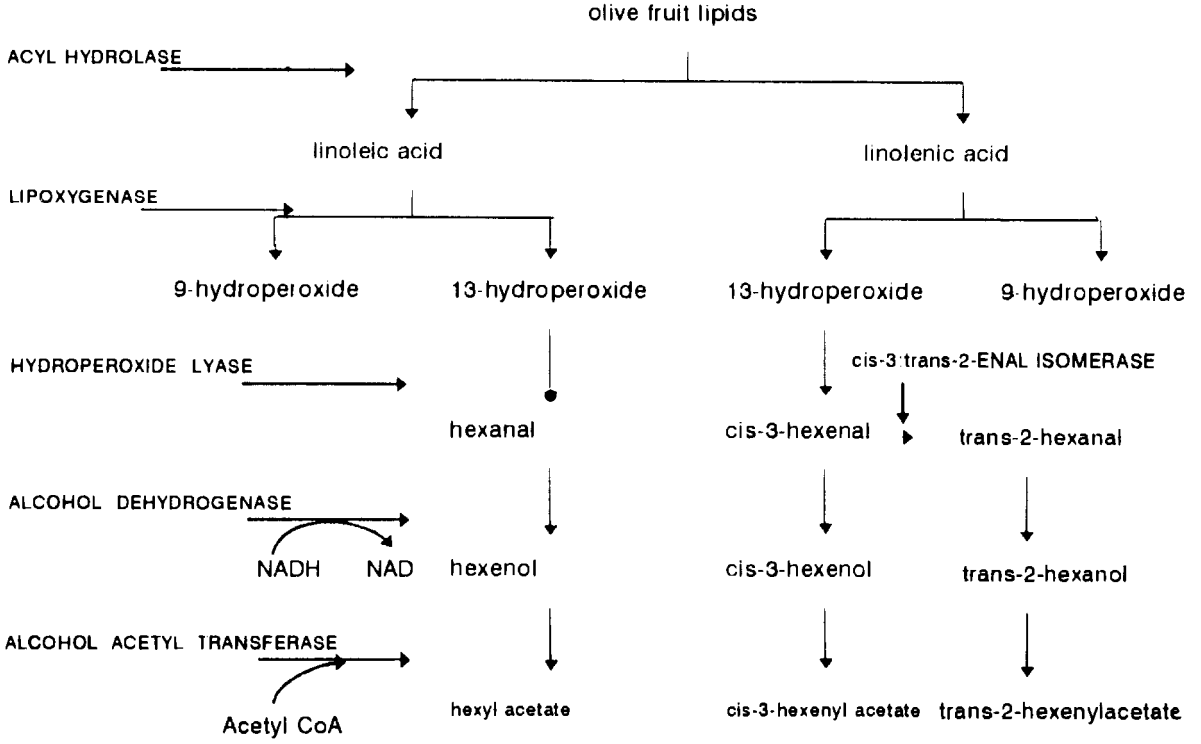


Figure 3. Ion chromatogram of polar fraction of virgin olive oil volatile compounds. Conditions were as described under Experimental Procedures. Gas chromatogram was plotted for total ion abundance vs scan number (upper line) and retention time (lower line).

Scheme I. Proposed Pathway for the Formation of C_6 Polar Volatile Compounds in Virgin Olive Oils



I , to explain the formation of the aliphatic compounds contributing to the green odor notes of olive oil aroma. Similar schemes have been proposed for the formation of volatiles in cucumber and tomato fruits (Hatanaka et al., 1975; Galliard et al., 1977).

The specificities of the fatty acid hydroperoxide lyase, alcohol dehydrogenase, and alcohol acetyltransferase enzymes are the main points of agreement between this hypothesis and the experimental data. In virgin olive oil, the major volatiles from unsaturated fatty acids are aliphatic C-6. Enzymic cleavage of 13-hydroperoxides leads to their formation, although olive lipoxygenase forms predominantly 9-hydroperoxides from $C_{18:2}$ and $C_{18:3}$. This specificity of hydroperoxide lyase for the 13-isomer is reflected in the absence of *cis*-3-nonenal and *cis*-3,*trans*-6-nonedienal in the volatile composition of virgin olive oil aroma.

The preponderance of unsaturated compounds over the saturated ones in the volatile compounds having green notes is significant. Thus, for example, the hexenal/hexanal, hexenol/hexanol, and hexenyl acetate/hexyl acetate ratio are generally greater than 1.5 (Olías et al., 1980). In in vitro assays starting from equivalent amounts of 13-hydroperoxide of linoleic and linolenic acids, hydroperoxide lyase produced approximately 4 times more hexenal than hexanal. Similarly, alcohol dehydrogenase incubated with equimolecular amounts of hexenal and hexanal produced more than double the amount of unsaturated alcohol. Similar results were obtained in the formation of hexenyl and hexyl acetate by the action of alcohol acetyltransferase.

To sum up, we can conclude that the enzymes characterized in olive fruit and their specificities verify the scheme proposed for the biogenesis of the compounds that contribute significantly to the green odor notes of virgin olive oil aroma.

ACKNOWLEDGMENT

This work was supported by Proyecto de Investigación ALI91-0421 from the Plan Nacional de Ciencia y Tecnología de Alimentos. A.G.P and L.C.S. are recipients of a grant from Junta de Andalucía.

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Received for review September 9, 1993. Accepted September 20, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, November 1, 1993.