Polar Compound Concentrations in Virgin Oils from Stored Cultivar Picual Olive Fruits

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Olive fruits (*Olea europaea* cv. Picual) were stored for up to 60 days under five different storage conditions: three in controlled atmosphere at 5 °C (air + 3% CO_2; 5% O_2 + 3% CO_2; 5% O_2 + <1% CO_2) and two in air (one at environmental temperature (between 6 and 17 °C) and the other in cold room at 5 °C). After conservation, the oils were extracted from the fruits and nonpolar compounds (mainly nonaltered triglycerides) and polar compounds (triglyceride polymers, oxidized triglyceride monomers, diglycerides, monoglycerides, and fatty acids) were determined. Polar compounds were determined by high-performance size exclusion chromatography after nonaltered compounds were discarded by column chromatography. The results demonstrated that storage of olive fruits at 5 °C produced a lesser amount of polar compounds in the oils extracted from them, compared to classical conservation (environmental temperature). At 5 °C neither increasing CO_2 to 3% nor decreasing O_2 to 5% affords additional adventages as compared to storage in air.

INTRODUCTION

Inappropriate storage of olive fruits may give the oil extracted from them certain chemical and sensory characteristics that are unacceptable under the technical and health regulations established for its human consumption (Martínez Suárez, 1975). Therefore, these oils have to be refined. Refining eliminates certain factors, such as an excess of acidity or the presence of altered compounds responsible for undesirable organoleptic attributes. However, other compounds with higher molecular weight than fatty acids, such as triglyceride polymers, oxidized triglyceride monomers, or diglycerides, cannot be readily eliminated and remain in the oil as indicators of the original degradation (Dobarganes et al., 1988a, 1989).

Classical indices such as peroxide value and free fatty acids give a partial indication of the oil quality. In contrast, the concentration of minor glyceride compounds in an oil is an objective guide to its quality. If oil extraction has been perfectly controlled and continuous immediately before analysis, the amount of polar compounds indicates the extent of alteration of the oil in the fruit before extraction. In the present work we study the influence of different conditions of storage of olive fruits cv. Picual on the composition of the virgin oil extracted from them.

MATERIALS AND METHODS

Biological Material. Olive (*Olea europaea* cv. Picual) fruits grown in Seville, Spain, were used. Fruits (126 kg) from 20-year-old trees were picked in the first days of December at the ripening stage. Harvesting was done manually. Before storage, fruits were mixed to ensure a homogeneous sampling.

Fruit Storage. Five different storage conditions were assayed: three in controlled atmosphere (CA) at 5 °C and 90–96% relative humidity (RH) (air + 3% CO_2; 5% O_2 + 3% CO_2; 5% O_2 + <1% CO_2) and two in air [one in the environment (temperatures and RH of 6–17 °C and 60–70%, respectively) and the other in cold room at 5 °C and 90–96% RH. Relative humidity at 5 °C (air and CA) was maintained using humidifiers.

The three CA storage conditions were maintained in plastic containers of 60 × 40 × 40 cm, impermeable to the gases, and closed with methacrylate plates. Gastightness was achieved in each case by sealing the top with a pure rubber seal and woodworker's clamps. The two storage conditions with air atmosphere were also maintained in the same kind of containers, but they were kept open in a cold room (5 °C) or outside the laboratory (environment).

Four trays, each containing 6 kg of fruit, were put in each container. Sampling was carried out after 15, 30, 45, and 60 days of storage. Each tray represented a sampling date.

An additional sample of 6 kg was taken for the initial assay (time zero).

In the CA containers, the concentration of respiratory gases was monitored and controlled manually each day. An infrared gas analyzer (Servomex 1400, range 0–10%) for carbon dioxide and a paramagnetic gas analyzer (Servomex 1400) for oxygen were used. The corrections of atmospheric composition were made by injection of pressurized N_2 and CO_2 and/or air. Excess of CO_2 was corrected by bubbling the gas through an aqueous solution of 2 N KOH.

Oil Extraction. Oil was extracted using an Abencor analyzer. This consisted of three basic elements: mill, thermobeater, and pulp centrifuge (Martínez Suárez et al., 1975). The mill was fitted with changeable sieves to give different grades of milling. After milling, the paste was beaten for 20 min and 200 mL of water was added. The beating was maintained for a further 10 min. The centrifuge was basket-type, revolving at 3500 rpm, having a central orifice at the bottom as liquid outlet. The operation time of this machine was 1 min. The oil–wastewater mixture was divided by decantation.

Determination of Total Polar Compounds. The samples of virgin olive oil, obtained according to the procedure described above, were evaluated in nonpolar and polar compounds in triplicate. The method was that proposed by IUPAC (Waltking and Wessels, 1981) for the determination of total alteration in fats used for frying, with the following modifications: 1.0 g (±1 mg) of oil was dissolved in about 5 mL of a mixture of hexane/diethyl ether (90/10 v/v) and transferred to a chromatography column (30 × 1.8 cm i.d.) containing 20 g of silica (silica gel 60, Merck, of 0.063–0.200-mm particle size and 70–230 mesh) 5% deactivated with distilled water and resuspended in the hexane/diethyl ether mixture. The first fraction (nonpolar compounds) comprised unaltered triglycerides and the majority of the unsaponifiable matter (hydrocarbons). It was eluted from the rest of the polar compounds using 150 mL of the same solvent mixture at a flow rate of 2 mL/min. Next, the polar compounds were collected with 150 mL of diethyl ether at the same flow rate. This second fraction contained compounds with polarity higher than that of triglycerides: triglyceride polymers, oxidized triglyceride monomers, diglycerides, monoglycerides, and free fatty acids. Both fractions were determined gravimetrically once the solvent had been evaporated.

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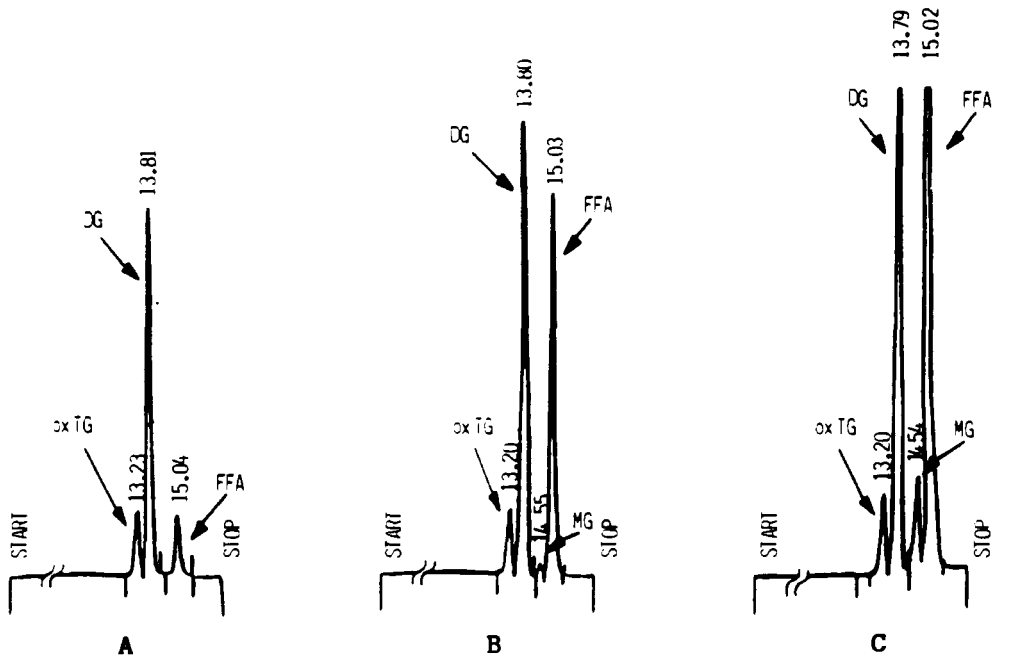

Figure 1. Distribution of polar compounds in oils from (A) initial olives, (B) olives stored for 60 days at 5 °C, and (C) olives stored for 60 days between 6 and 17 °C. Abbreviations: oxTG, oxidized triglyceride monomers; DG, diglycerides; MG, monoglycerides; FFA, free fatty acids.

Table I. Evolution of Total Polar Compound Concentrations[a] in Oils Extracted from Stored Olive Fruits

time, days	storage conditions (°C/% CO_2/% O_2)				
	6–17/0/21	5/0/21	5/3/20.6	5/3/5	5/<1/5
0	16.0 ± 4.2	16.0 ± 4.2	16.0 ± 4.2	16.0 ± 4.2	16.0 ± 4.2
15	29.0 ± 4.2	22.9 ± 4.2	23.0 ± 8.5	17.0 ± 2.8	21.1 ± 1.4
30	377.5 ± 4.9	25.4 ± 1.4	26.0 ± 0.0	22.0 ± 0.0	26.1 ± 4.2
45	425.8 ± 18.4	32.4 ± 0.4	40.9 ± 5.7	34.9 ± 5.6	33.0 ± 8.5
60	439.7 ± 17.0	41.1 ± 2.8	78.6 ± 8.5	58.2 ± 2.8	55.9 ± 7.1

[a] Milligrams per gram of oil. Means ± SD of three determinations.

Table II. Evolution of Diglyceride Concentrations[a] in Oils Extracted from Stored Olive Fruits

time, days	storage conditions (°C/% CO_2/% O_2)				
	6–17/0/21	5/0/21	5/3/20.6	5/3/5	5/<1/5
0	10.8 ± 2.9	10.8 ± 2.9	10.8 ± 2.9	10.8 ± 2.9	10.8 ± 2.9
15	21.2 ± 3.0	17.2 ± 0.0	17.1 ± 6.4	12.4 ± 2.1	15.6 ± 1.1
30	135.0 ± 1.8	17.0 ± 0.9	18.5 ± 0.0	15.9 ± 0.0	19.4 ± 3.2
45	102.4 ± 5.2	23.3 ± 3.1	25.8 ± 3.6	24.3 ± 4.0	24.0 ± 6.2
60	83.5 ± 3.2	26.0 ± 1.8	36.8 ± 3.7	31.6 ± 1.5	33.0 ± 4.2

[a] Milligrams per gram of oil. Means ± SD of three determinations.

Separation and Quantification of Individual Polar Compounds by High-Performance Size Exclusion Chromatography (HPSEC). The individual polar compounds were determined quantitatively using HPSEC (Dobarganes et al., 1988b; Pérez-Camino et al., 1991). The procedure is summarized as follows: chromatograph, Konik Model 500 A (Konik S. A., Barcelona, Spain); detector, refractive index Hewlett-Packard Model 1037 A (Hewlett-Packard, Pittsburgh, PA); integrator, Hewlett-Packard Model 3390; columns, two of PL-gel (5 μm), Hewlett-Packard 100 and 500 Å connected in series; flow, 1 mL/min tetrahydrofuran; sample, dissolved in tetrahydrofuran at a concentration of 15 mg/mL; injection, loop of 10 μL. Three chromatograms showing the efficiency of the separation are shown in Figure 1.

Responses of each compound measured by HPSEC with a refractive index detector differed little from each other. The quantity of each compound in the sample of oil is obtained taking into account the weight of the total polar fraction and the percentage of the peak corresponding to the compound.

RESULTS AND DISCUSSION

Table I shows the total polar compound content of the oils extracted from olives stored under the storage conditions tested. The increase taking place between days 15 and 30 in olives stored in air at ambient temperature was the most remarkable result found. The olives stored at 5 °C underwent a gradual increase in concentration of total polar compounds, with hardly any difference up to day 30. At day 45, a distinct change was seen in the atmospheres tested. At 5 °C the mean value in polar compounds of the olives stored in 3% CO_2 was greater than that of those stored in air at the same temperature.

Analysis and Evolution of Each Component of the Polar Fraction. As high temperature was not used either in storing the fruit or in obtaining the oils, polymerization compounds were not formed (peaks with Rt < 13 min; Figure 1). The amounts of oxidized triglyceride monomers ranged between 2.8 and 3.8 mg/g of oil, and there were no general differences either among the types of storage or between beginning and end of the process. A slight increase in the presence of oxidized triglyceride monomers was seen only in the final fortnight in the oil from fruits stored at environmental temperature.

As Table II shows, by 30 days there was a remarkable increase in diglyceride content in the oil extracted from olives stored at ambient temperature. This shows that lipase activity, from either the fruits or the parasitic microorganisms, increased drastically during this period. After 30 days, there was a continuous decrease in the concentration of diglycerides, probably because the rate of their decomposition after 30 days was greater than their formation.

At 5 °C, diglyceride accumulation slowly increased linearly during the whole storage period tested. The oils from fruit stored in 3% CO_2 and air showed the highest mean concentration of these molecules. In the cold, lipase activity may be more inhibited, so that the triglyceride concentration in the medium always remains saturant and diglycerides do not reach a competitive level.

Table III shows the change in concentration of monoglycerides. These molecules, the same as diglycerides, are intermediate metabolites of triglyceride hydrolysis. There-

Table III. Evolution of Monoglyceride Concentrations[a] in Oils Extracted from Stored Olive Fruits

time, days	storage conditions (°C/% CO_2/% O_2)				
	6–17/0/21	5/0/21	5/3/20.6	5/3/5	5/<1/5
0	nd	nd	nd	nd	nd
15	nd	nd	nd	nd	nd
30	11.7 ± 0.1	0.4 ± 0.0	nd	nd	nd
45	7.2 ± 0.4	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
60	4.7 ± 0.1	0.5 ± 0.0	1.8 ± 0.2	1.4 ± 0.1	1.0 ± 0.1

[a] Milligrams per gram of oil. Means ± SD of three determinations. nd, not determined.

Table IV. Evolution of Free Fatty Acid Concentrations[a] in Oils Extracted from Stored Olive Fruits

time, days	storage conditions (°C/% CO_2/% O_2)				
	6–17/0/21	5/0/21	5/3/20.6	5/3/5	5/<1/5
0	2.4 ± 0.6	2.4 ± 0.6	2.4 ± 0.6	2.4 ± 0.6	2.4 ± 0.6
15	5.2 ± 0.8	3.6 ± 0.4	3.5 ± 1.2	2.8 ± 0.4	3.2 ± 0.2
30	227.6 ± 3.0	6.0 ± 0.4	5.0 ± 0.0	3.4 ± 0.0	4.2 ± 0.6
45	312.4 ± 15.6	5.8 ± 0.8	11.8 ± 1.6	7.6 ± 1.2	6.4 ± 1.6
60	347.7 ± 13.4	12.2 ± 0.8	36.2 ± 3.7	22.2 ± 1.0	19.3 ± 2.4

[a] Milligrams per gram of oil. Means ± SD of three determinations.

fore, it is not surprising that the changes in both are similar. However, their concentration is very low in absolute terms. It has been demonstrated that, during plant development, there are cellular mechanisms which prevent the accumulation of monoglycerides (Flack, 1976; Krog and Lauridsen, 1976). Lenzen et al. (1976) demonstrated the toxic effect of lysophospholipids on biological membranes. Monoglycerides, with similar molecular structure, could have the same properties. In fact, the existence of an enzyme specifically responsible for their hydrolysis in castor-oil plant endosperm has been demonstrated (Muto and Beevers, 1974).

The fact that the free fatty acids are end products of the acylglycerol hydrolysis steps explains their increase in concentration throughout storage (Table IV). Coinciding with the spectacular increase in di- and monoglycerides, an even greater increase was seen in free fatty acids between days 15 and 30 in the oil from olives stored at ambient temperature.

As the molecular weight of a monoglyceride is only a little greater than that of a fatty acid, and a diglyceride is approximately double, the mass of fatty acids that would have previously accompanied the di- and monoglycerides formed can be estimated. In virgin olive oils we would expect that each 2 mg of diglyceride corresponds to 1 mg of fatty acids (Dobarganes et al., 1988a). If hydrolysis continues, the determination of a certain weight of monoglyceride would imply that double that weight of free fatty acids has been liberated. In the oil from olives stored at ambient temperature, the concentration of fatty acids after 30 days is appreciably higher than that expected theoretically. In contrast, both after 15 days of this treatment and during the whole development of the others, the mass of free fatty acids found is approximately that estimated from the amount of mono- and diglycerides present. This means that, in the former case, most of the fatty acids come from complete degradation of the triglycerides, the product of lipases with nonspecific action, such as those produced by the fungus *Aspergillus niger* (Fukumoto et al., 1963). This supports the concept that the generalized increase in total polar compounds from hydrolysis and the proliferation of fungal infection are interdependent. In the second case, the lipolytic action was probably carried out by the lipases with specific action on esterifications 1 and 3 of the glycerin skeleton (Brockerhoff and Jensen, 1974). The increase, either of the total polar compounds in this type of storage compared with the rest or in the proportion of fatty acids found, compared with that expected from the mass of mono- and diglycerides found experimentally. This may be a consequence of low-temperature inhibition of lipase activity.

The results show the advantages of storing olive fruit at 5 °C between harvest and processing, especially if this period is prolonged for more than 2 weeks. On the other hand, variations in storage atmosphere do not afford additional advantages.

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