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Sugar and polyol compositions of some European olive fruit varieties (*Olea europaea* L.) suitable for table olive purposes

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Abstract

An experimental investigation was carried out on olive fruits of *Douro*, *Hojiblanca*, *Cassanese*, *Taggiasca* and *Thasos* cultivars, to assess free sugar and polyol compositions and their changes during ripening and processing. TMS ethers of sugars from olive pulp were analysed by GC and GC–MS and identification of each sugar component was obtained by comparison of retention times and mass spectra with those of authentic compounds. Quantitation of sugars was performed on mixture whose tautomeric equilibria were stable. Glucose, fructose and galactose were the main sugars found in olive pulp. Appreciable quantities of mannitol were also present. Sucrose and inositol were present in very low concentrations. A significant correlation ($r = 0.941$) between mannitol and oil contents was found. Sugar content decreased from green to cherry and black colour, according to the stage of ripeness of fruits. In processed olive-fruits, sugar contents varied among olive cultivars according to processing conditions.

Keywords: Sugars; Sugar alcohols; *Olea europea* L.; Table olive; Oil content; Processing

1. Introduction

Olea europaea L. is one of the most important and widespread crop of the Mediterranean basin. Most of the olive production is destined for olive oil; however, a considerable part of it is processed to different types of olives for direct human consumption. According to statistical data (IOOC, 1998), worldwide production of table olives is about 1,000,000 tons. The European Union countries contribute about 50% of this amount.

A series of transformations occurs in olive fruit during both ripening and processing. Sugars are the main soluble components in olive tissues and play an important role, providing energy for metabolic changes. They are important components of the cell-wall, related to the textural properties (Jiménez, Guillén, Sánchez, Fernández-Bolaños & Heredia, 1995) of the fruit and act as precursors for olive oil biosynthesis (Donaire, Sánchez-Raya, López-Gorge & Recalde, 1977). In table olive processing sugars act as carbon source for microorganisms (Tseng & Montville, 1990; 1992) for producing secondary metabolites responsible for good characteristics and the distinctive flavour of the commodities.

Most of the determinations of sugars from a variety of plant material have been performed by the GC technique and detected as methyl, acetyl, TMS ethers, and oxime-TMS ethers (Churms, 1990; Mason & Slower, 1971; Robards & Whitelaw, 1986; Sweeley, Bentley, Makita, & Wells, 1963; Van Den, Biermann & Marlett, 1986) or by the HPLC technique (Falqué-López & Fernández-Gómez, 1996). GC is suitable for such studies since it combines great sensitivity with high resolution (Laker, 1980). As with carbohydrate analyses, GC has been used to determine polyol concentrations (Makinen & Soderling, 1980).

Although sugar composition in olive tissues has been studied (Donaire, Sánchez-Raya, López-Gorge & Recalde, 1975; Fernández-Bolaños, Fernández Díez, Rivas Moreno, Gil Serrano & Pérez Romero, 1982; 1983), little information exists on the effects of variety, origin and maturity and the changes related to processing, so a better knowledge of these factors may contribute to significant developments in technology, safety and quality of table olives.

The aim of this work was to focus on the changes of sugars occurring during ripening and processing of some European olive varieties, in order to provide a more solid basis for selecting processing techniques, considering their nutritional value and their role in fermentation processes.

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2. Materials and methods

2.1. Samples

In Table 1 the sample code, the basic characteristics and the geographical origin of olive samples are shown. Olive-fruits (*Olea europaea* L.) of *Douro*, *Hojiblanca*, *Cassanese*, *Taggiasca* and *Thasos* cultivars, were used. The fruits of *Hojiblanca* cultivar were supplied at green, cherry and black stages of ripeness by Agro Sevilla Aceitunas SCA (Spain). *Douro*, *Thasos*, *Taggiasca* and *Cassanese* olives were harvested when the fruits reached a black surface colour and were supplied by Macarico Lda (Portugal), Institute of Technology of Agricultural Products (Greece), Fratelli Carli (Italy) and Oleificio Gabro SNC di Brogna (Italy), respectively.

2.2. Chemicals

Sugar standards (arabinose, fructose, glucose, galactose, mannitol, inositol and sucrose) were purchased from Sigma (St. Louis, MO). All the reagents were obtained from Carlo Erba (Milan, Italy) and were of analytical grade.

2.3. Determination of oil content

Oil content was determined by extracting dry material with 40–60°C petroleum ether using a Soxhlet apparatus. The extract was dried at 70°C and weighed.

2.4. Extraction and determination of free sugars and polyols

Free sugars and polyols of the olive fruits were extracted and purified according to the method described by Bianchi and Pozzi (1994), using D-arabinose solution (0.03 mg/ml) as an internal standard. Extracts were dried and converted into trimethylsilyl ethers with a silylation mixture made up of pyridine, hexamethyldisilazane and trimethylchlorosilane (2:1:1) at 60°C for 1 h under stirring. The solvent was then removed under a stream of nitrogen and TMS derivatives were kept in isoctane for GC and GC–MS analyses

2.5. GC analysis

The samples were analysed in a Carlo Erba gas-chromatograph equipped with a flame ionisation detector and a HP 1 capillary column (Hewlett-Packard, Palo Alto, CA) of 30 m×0.32 mm (i.d.), 0.10 μm film thickness. The oven temperature was programmed from 70 to 90°C at 20°C/min and up to 300°C at 4°C/min. Hydrogen was used as carrier gas at a column pressure of 35 kPa. The samples were injected by the ''on column'' mode.

2.6. GC–MS analysis

A Hewlett-Packard (HP) GC-5890 interfaced to a MSD-5970 was used for analysis. A DB5 capillary column (J&W Scientific, Folsom, CA) of 30 m×0.32 mm i.d.×0.25 μm film thickness, was directly introduced into the ion source, which was operating in the electron impact mode (EI). Other conditions and parameters were as follows: interface 320°C, and the chromatographic conditions were the same as that for GC analysis; helium was the carrier gas with a head pressure of 35 kPa; ion source temperature 200°C and ionization energy 70 eV; electron current 0.3 mA.

2.7. Identification and calculations

Individual sugars and polyols were identified by comparison of spectra and retention times of peaks with those of TMS derivatives of authentic compounds. Preliminary analyses of control olive samples indicated no arabinose peak in the free sugar profile, so this compound was used for quantitative determinations of sugars. TMS

Table 1
Basic characteristics of table olive samples

Sample code	Variety	Origin	Stage of ripening	Processing
D1	Douro	Portugal	Black fruits	Fresh olives
D2	Douro	Portugal	Black fruits	Darkened olives by lye, air oxidation and ferrous lactate treatments, followed by sterilization
H1	Hojiblanca	Spain	Green fruits	Fresh olives
H2	Hojiblanca	Spain	Cherry fruits	Fresh olives
H3	Hojiblanca	Spain	Black fruits	Fresh olives
H4	Hojiblanca	Spain	Black fruits	Darkened olives by lye, air oxidation and ferrous gluconate treatments, followed by sterilization
Ca1	Cassanese	Italy	Black fruits	Fresh olives
Ca2	Cassanese	Italy	Black fruits	Oven-dried olives
T1	Taggiasca	Italy	Black fruits	Fresh olives
T2	Taggiasca	Italy	Black fruits	Naturally Greek style olives in brine
Th1	Thasos	Greece	Black fruits	Fresh olives
Th2	Thasos	Greece	Black fruits	Dry salted olives

ethers of reducing sugars gave multiple peaks due to the presence of tautomeric and isomeric forms (Nikolov & Reilly, 1983); therefore mutarotation equilibrium data were used for quantitation. Each sugar standard and the olive extracts were equilibrated in aqueous solution for 24 h to achieve mutarotational equilibrium (Saura-Calixto, Canellas & Garcia-Raso, 1984). Equilibrated sugars were evaporated to dryness, then derivatized and analysed. The amount of each sugar present in the olive extract was calculated as follows:

$$\text{sugar (mg/100 g)} = \frac{C_s \cdot W_a \cdot 100}{k_s \cdot C_a \cdot W_s}$$

where k_s, correction factor; C_s, integrator counts for the sugar peak; C_a, integrator counts for the internal standard, D-arabinose; W_a, weight (mg) of D-arabinose added to the sample and W_s, weight (g) of sample based on dry matter.

3. Results and discussion

The physical and chemical characteristics of fresh olive varieties are shown in Table 2. All the cultivars had a good pulp/stone ratio, except for *Taggiasca* cultivar (T1) that showed the lowest pulp percentage. In *Hojiblanca* cultivar (H1, H2 and H3), oil content increased during the ripening process. *Douro* (D1) and *Thasos* (Th1) had the lowest and the highest oil amounts, respectively.

Table 2
Physico-chemical characteristics of fresh olive fruits

Sample	Fruit weight (g)	Stone weight (g)	Pulp/stone ratio	Oil content dry wt.(%)
D1	4.1	0.60	5.83	52.0
H1	3.2	0.64	4.02	55.8
H2	3.6	0.64	4.60	56.0
H3	4.8	0.68	6.00	59.2
Ca1	3.7	0.64	4.78	60.1
T1	2.4	0.70	2.42	60.9
Th1	3.6	0.78	4.90	64.8

Because of the wide variation in the amounts of individual sugars in olive-fruits, preliminary analyses were performed to determine the optimum sample size and the attenuation required for each sugar peak. As a measure of linearity, mixtures containing varying amounts of each sugar and polyol, plus a fixed amount of internal standard, derivatized and chromatographed under identical conditions, were analysed. Plots of the peak area ratios (sugar/arabinose) versus the weight ratios (sugar/arabinose) gave essentially straight lines. The slopes (k), as calculated by the method of least squares, were used to correct for variations in response. The values of k for each sugar were: fructose 0.85; galactose 0.98; glucose 0.59; mannitol 1.56; inositol 1.40 and sucrose 0.83. Correlation coefficients, found for all standards, ranged between 0.997 and 0.999, except for mannitol and inositol whose coefficients were 0.991 and 0.992, respectively.

Results of the GC determinations are given in Table 3. The major free sugars in fresh olive-pulp were glucose, fructose and galactose, while sucrose was detected at low concentrations. Results expressed in mg/100 g on dry weight basis for glucose, fructose and galactose ranged from 2052 to 5420, from 134 to 1196 and from 85 to 1360, respectively. In addition, olive samples contained two polyols, mannitol and inositol; the latter was present at small levels, except for fresh olives from *Thasos* cultivar (Th1) that showed the highest values of mannitol (1801 mg/100 g dry wt.) and inositol (256 mg/100 g dry wt.). Such significant differences in concentrations probably reflect the metabolic behaviour of each cultivar in relation to the genotype and to different climatic and environmental conditions.

A linear trend between mannitol and oil content was observed (Fig. 1). Regression analysis showed a good correlation coefficient ($r = 0.941$; $P < 0.05$), which supports the closeness of the linear model. The relative

Table 3
Sugars and polyols in unprocessed and processed olive fruits[a]

Sample	Fructose	Galactose	Glucose	Sucrose	Mannitol	Inositol
D1	1196	1360	5420	24	384	67
D2	52	28	190	4	59	8
H1	134	319	5414	440	544	96
H2	502	93	4276	73	541	46
H3	852	85	3551	59	798	11
H4	20	5	60	2	95	3
Ca1	741	1287	3859	13	1122	85
Ca2	357	147	1646	9	969	70
T1	259	681	2815	130	981	18
T2	20	112	228	22	377	16
Th1	838	817	2052	4	1801	256
Th2	116	156	902	2	930	113

[a] Each value is the mean of duplicate analyses expressed in mg/100 g dry wt. pulp. Coefficient of variation was normally under 10% for all sugars.

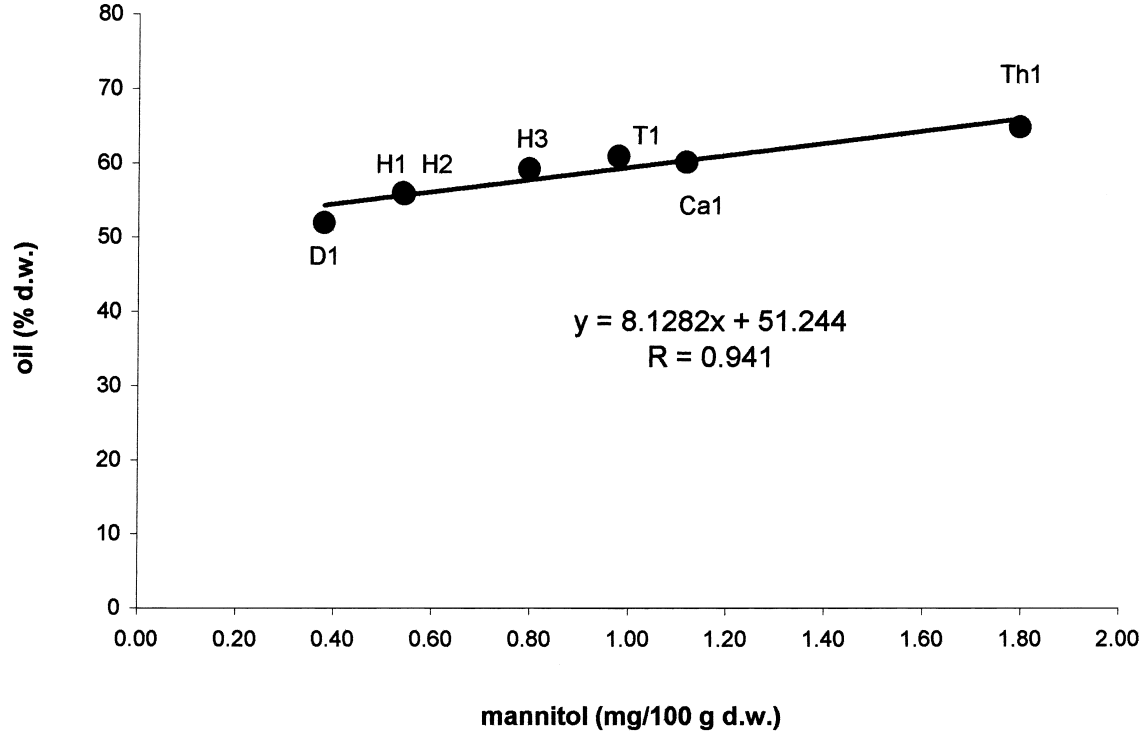

Fig. 1. Relationship of mannitol values and oil content of olive varieties.

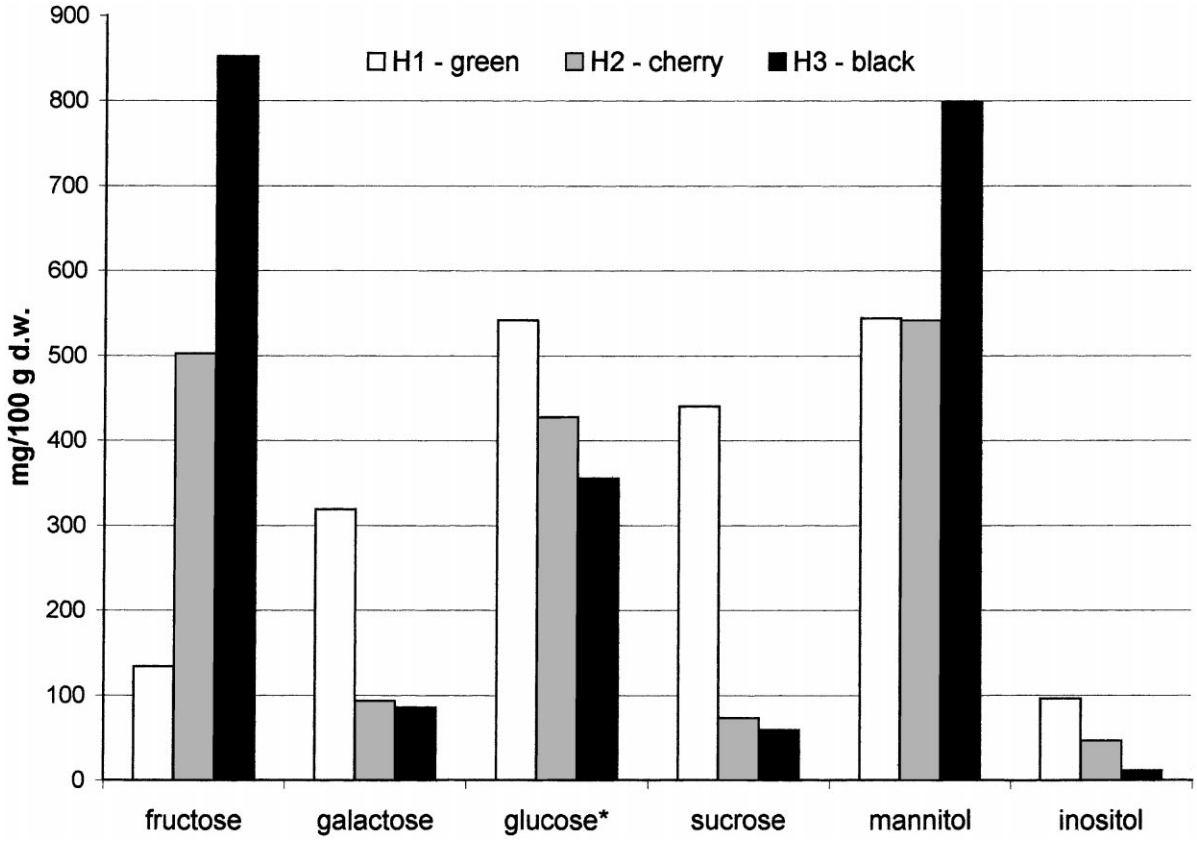

Fig. 2. Sugar and polyol compositions of Hojiblanca cultivar during ripening. Each value is the mean of duplicate analyses and the coefficient of variation was normally under 10% for all sugars. * = the value must be multiplied by 10.

amount of mannitol in the fruit might indicate the potential of a cultivar for oil biosynthesis. Thus, the mannitol in the olive, as well as other polyols in many other higher plants, might be of specific importance in the metabolic transformation and synthesis of the fruit storage material (Fernández Díez, 1975; Lewis & Smith, 1967; Trip, Krotkov & Nelson, 1964). *Thasos* cultivar (Th1) seems to have these characteristics.

In *Hojiblanca* cultivar (Table 3; H1, H2 and H3 samples), glucose, the principal free sugar detected, decreased gradually during maturation, probably because it was metabolised via acetyl CoA to oil (Stumpf, 1983); galactose and sucrose diminished markedly when the fruit colour surface changed from green to cherry, while fructose increased considerably during the ripening process. The highest amount of mannitol detected in black fruits of *Hojiblanca* cultivar (H3) confirms the linear correlation between oil and polyols contents (Fig. 2).

During processing and product storage, sugar composition showed great qualitative and quantitative variations, depending on the variety and according to the industrial process (Table 3).

Olive fruits of *Douro* (D2) and *Hojiblanca* (H4) cultivars, processed by the Californian system, which consists of a progressive darkening by air oxidation of the lye-treated olives, followed by iron salts (ferrous gluconate or lactate) treatment and by a final step of autoclaving, showed a marked decrease of sugar content, by 25 and 29 times from the initial values, respectively, suggesting that processing operations, such as lye, washing and sterilisation treatments, caused high sugar loss in olive-fruit. Glucose was the major sugar quantified, followed by galactose and fructose. Mannitol and inositol contents decreased during processing, but showed minor changes with respect to the initial contents.

Naturally Greek style olives of *Taggiasca* cultivar (T2) after 6 months of storage in brine, also showed a diminution in sugar content, because of bacterial metabolism, with mannitol being the major compound detected, followed by glucose and galactose.

Cassanese (Ca2) olive fruits processed by the "Ferrandina method" (Marsilio, Lanza, Campestre & De Angelis, 2000) were also analysed. Oven-dried treatments diminished the total amount of sugars and polyols, but this was minor with respect to the initial value, if compared with the other processing technologies. The same behaviour was also observed in dry-salted olives of *Thasos* cultivar (Th2). This latter sample showed a minor decrease of total sugar content, due to the loss of water and, probably, due to a consequent slowdown of metabolic pathways.

4. Conclusion

Data resulting from this work improve the fundamental knowledge about the composition and evolution of free sugars and polyols in olive fruits, whose determination is very important to explore varietal and growth differences and to optimise the production process of table olives in relation to different available processing technologies.

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