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Effect of processing upon the tocopherol and tocotrienol composition of table olives

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The effect of processing upon the tocopherol and tocotrienol composition of Greek table olives was studied in samples taken after water treatment and after fermentation. These results were compared with those obtained from the raw (unprocessed) stage of olives. It was found that processing resulted in a decrease of α-tocopherol in Halkidiki (green) olives and of α-tocotrienol in black olives. γ-Tocopherol was not affected by processing. α-Tocopherol accounted for 61–85%, of all substances determined and it was higher in black olives than green ones in all samples. α-Tocopherol content was 24, 16, 33 and 40 μg/g pericarp after fermentation (final stage of processing), 24, 22, 33 and 37 after water treatment and 22, 29, 33 and 35 at raw stage, for Conservolea (green), Halkidiki (green), Conservolea (black) and Calamon (black) olives, respectively.

INTRODUCTION

The fruit of the *Olea Europea* tree is an ovoidal drupe in which the pericarp and the kernel can be separated. Olive fruit has been a very important foodstuff in the Mediterranean basin, since ancient times. Olive oil is produced by pressing the olives but a large amount of olives is processed and consumed as such (table olives). According to recent data from the International Olive Oil Council, the world production of edible table olives is around 760 000 tonnes per annum. Greece is producing approximately 10% of the world total. A description of the olive varieties grown in Greece and of processing table olives is given elsewhere (Anagnostopoulos, 1939; Balatsouras, 1980).

The olive fruit is rich in fat (16–24%) and its main fatty acid is oleic acid (70%) (Fedeli, 1977). The olive fruit contains only 1–2% proteins and they are well balanced in all essential amino acids (Manoukas *et al.*, 1973). They are rich in potassium and low in sodium (Manoukas *et al.*, 1978), prior to processing. They also contain vitamin C, thiamin and β-carotene (De Castro Ramos *et al.*, 1979; Nosti Vega, 1979).

Processing affects the nutritional value of the olive fruit. It was reported that the sugar content decreased significantly after processing while the sodium content increased (Balatsouras, 1980).

Some work has been carried out on the tocopherol content of raw edible olives (Hassapidou & Manoukas, 1993) whereas a number of papers refer to the vitamin E content of olive oil (Herting & Drury, 1963; Carpenter, 1979; Andrikopoulos *et al.*, 1989). Tocopherols (α, β, γ, δ) and tocotrienols are very important in human health, mainly because of their antioxidant properties (Machlin 1980; McCay, 1985).

The work reported here is part of a study on the nutritional value of the main Greek varieties of edible (table) olives. In the present study the tocopherol and tocotrienol content of the olives was determined in samples after each of the two main stages of processing and was compared to the content of the same samples at raw stage.

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MATERIALS AND METHODS

Materials and sampling

Sampling took place in the central processing plant of Eleourgiki using a random sampling procedure (Steele & Torrie 1960). Samples of each of the main Greek varieties and types of olives were collected raw, from loaded trucks as they were coming to the factory. Olives from each sampled truck were placed in the same wooden vat until the end processing and all samples were taken from this vat. Ten samples were taken in all cases with 30–40 olives per sample.

Sampling at the raw stage

Samples of the following varieties and types of olives were collected for analysis at the raw stage.

- *Conservolea.* This is the most widespread variety in Greece. It is cultivated mainly in central Greece and is responsible for over 70% of the Greek production of table olives. There are two different commercial types of olives produced from this variety

  (i) green olives of the Spanish type; and
  (ii) naturally black olives in brine.

  Sampling took place in mid-October for green olives and at the end of November for black olives.
- *Halkidiki.* This variety is mainly cultivated in Macedonia, northern Greece. It produces green olives of the Spanish type of very good quality. Sampling took place in mid-October.
- *Calamon.* This variety is cultivated in the Peloponese, southern Greece and it is of commercial interest because it produces naturally ripe black olives of excellent quality known as the 'Calamon' type. Samples were collected at the end of November.

All samples from the above varieties, that were collected at the raw stage, were coded, number of olives per sample were counted and transferred to the laboratory on the same day. Their proximate analysis and tocopherol and tocotrienol contents have been reported elsewhere (Hassapidou & Manoukas, 1993).

Sampling after each stage of processing
For green olives of the Spanish type:

(i) *Stage of debittering.* The green fruit was soaked in a dilute solution of sodium hydroxide for 8–10 h. Then the olives were washed three times. Samples were collected after the stage of debittering for the two green varieties studied, Conservolea and Halkidiki. Sampling took place 24 h after the collection of raw olives.
(ii) *Stage of fermentation.* After the stage of debittering the olives were packed into wooden vats and filled with brine containing 8–9% salt. Fermentation was completed after 3–5 months. Samples from both green varieties were collected at the end of fermentation. Sampling took place at the end of January.

For black, natural type, olives:

(i) *Stage of washing.* Black olives, completely mature, were washed with excess water and left in water for 7 days in wooden vats. Samples were collected after the stage of washing for both black varieties studied, Conservolea and Calamon. Sampling took place one week after the sampling of raw olives (10 samples for each variety).
(ii) *Stage of fermentation.* After the 7 days of washing, the vats were opened and salt was added (9–11% salt). Olives were left in brine until fermentation was complete. Samples for each variety of black olives were collected at the end of February.

Preparation

All samples were coded and transferred to the laboratory on the same day. The number of olives per sample was counted the seeds (kernels) removed and weighed and the weight of pericarp was calculated. The pericarp of each sample was homogenised in an omni-mixer. Lipids were extracted from the pericarp with petroleum ether under nitrogen atmosphere. The ratio of petroleum ether to pericarp was 5 : 1 (v/w). Lipid extracts were kept in the deep freezer for further analyses. The solvent was evaporated before transfer of the extract to the freezer.

Chromatography

Instrumentation
Gas–liquid chromatograph, Varian model 5000; Detector UV, Varian model Varichrom; printer, Varian model 9176; Column, silica-type micropack Si-5 (150 mm × 10 mm id).

Reagents
All reagents were of analytical grade. α-, β-, γ, δ-Tocopherol standards were obtained from Sigma. α-Tocotrienol standard was a gift from Hoffman La Roche, Switzerland.

Method of determination
Tocopherols and tocotrienols were determined as follows. The lipid samples were dissolved in a mixture same as the mobile phase. The mobile phase consisted of a mixture of hexane/tetrahydrofuran (95 : 5 v/v). Samples after dilution were injected directly on to the column. The flow rate was 1 ml/min and the tocopherols were detected at 295 nm. Each sample was analysed three times. The recoveries for α-, β-, and γ-tocopherol and α-tocotrienol were 96, 95, 100 and 98%, respectively. This is considered very satisfactory since almost all of the α-, β-, and γ-tocopherol and α-tocotrienol contained in the standard was measured (recovered) in the chromatogram. All data were statistically evaluated according to procedures described by Steel and Torrie (1960). All tests of significance were made at the 0·05 level of probability, unless otherwise indicated.

RESULTS AND DISCUSSION

A typical chromatogram included, in order of appearance, α-tocopherol, α-tocotrienol and β- and γ-tocopherol. δ-Tocopherol was identified only in traces.

Table 1 presents the α-, β-, and γ-tocopherols and α-tocotrienol content in μg/g lipids, of each type of olives before processing as raw, taken from a previous study of this laboratory (Hassapidou & Manoukas, 1993) and after each of the two main stages of processing. δ-tocopherol is not included in this table because it was only found in traces. The same is true for β-tocopherol of treated and fermented black olives, because the results were contradictory and very difficult to explain.

Table 1. The α-, β- and γ-tocopherol and α-tocotrienol contents of olives as raw and after each stage of processing with the calculated α-tocopherol equivalent (μg/g lipids ± SD)

Content	Sample	Conservola (green)	Halkidiki (green)	Conservola (black)	Kalamon (black)
α-Tocopherol	Raw[a]	137·8 ± 8·8	130·7 ± 10·2	170·3 ± 15·7	174·0 ± 11
	Treated[b]	142·1 ± 14	99 ± 9·5	182·5 ± 14·2	161·3 ± 23
	Fermented	143·1 ± 5·0	81·3 ± 10·3	170·8 ± 11·7	187·9 ± 15·4
β-Tocopherol	Raw	tr[c]	tr	26·1 ± 4·8	25·6 ± 7·4
	Treated	tr	tr		
	Fermented	tr	tr		
γ-Tocopherol	Raw	tr	23·3 ± 8·4	36·3 ± 9·1	37·0 ± 6·6
	Treated	tr	30·7 ± 6·2	34·3 ± 10	48·0 ± 24·2
	Fermented	tr	25·6 ± 2·4	45·0 ± 13·9	42·9 ± 4·1
α-Tocotrienol	Raw	tr	tr	27·8 ± 4·7	49·9 ± 10·4
	Treated	tr	tr	tr	tr
	Fermented	tr	tr	tr	tr
α-Tocopherol equivalalent	Raw	137·8 ± 8·8	133·0 ± 10	189·1 ± 15·7	195·7 ± 13·5
	Treated	142·1 ± 14	102·1 ± 9·3	185·1 ± 16·3	165·6 ± 22·5
	Fermented	43·1 ± 5·0	83·8 ± 10·3	175·3 ± 10·8	192·1 ± 16·01

[a] Taken from Hassapidou and Manoukas (1993).
[b] After debitting for green olives and after 7 days in water for black olives.
[c] tr, trace.

Table 2 presents the tocopherol and tocotrienol compositions expressed in μg/g pericarp. Statistical evaluation and discussion of data refers to Table 1, unless otherwise indicated. The biological activity of tocopherols are in the ratio 10 : 4 : 1 : 7 for α-, β-, and γ-tocopherol and α-tocotrienol respectively (McLaughlin & Wihrauch, 1979; Chow Ching, 1985) and it is included in Tables 1 and 2 as tocopherol equivalents (α-Teq). The predominant tocopherol in all types of olives is α-tocopherol followed by γ-tocopherol. Green olives of the Conservolea variety at the raw stage contain only α-tocopherol, whereas green olives of Halkidiki variety contain α- and γ-tocopherol. β-tocopherol and α-tocotrienol are only present in measurable amounts in raw black olives of Conservolea and Calamon varieties. Thus, it is obvious that the type of tocopherols as well as the quantity in the raw olives depends on the variety and the type of olives.

In the raw stage olives of the black type contain significantly higher amounts of α-tocopherol equivalents compared with olives of the green type. This is mainly due to the difference in variety but also due to the different stage of maturity. Black olives ere harvested 1–2 months later and it is possible that the tocopherol content had increased during maturation.

Green olives of the Conservolea variety at the raw stage contained α-tocopherol only. Processing did not affect the α-tocopherol content of olives or the α-Teq content. There was no significant statistical difference between the α-tocopherol values found after each stage of processing.

Green olives of the Halkidiki variety contained α-

Table 2. The α-, β-, γ-tocopherol contents of olives as raw and after each stage of processing with the calculated α-tocopherol equivalent (μg/g pericarp ± SD)

Content	Sample	Conservola (green)	Halkidiki (green)	Conservola (black)	Kalamon (black)
α-Tocopherol	Raw[a]	21·6 ± 1·7	29·2 ± 1·9	33·2 ± 3·9	35·1 ± 4·3
	Treated[b]	24·2 ± 1·6	21·6 ± 2·4	33·1 ± 3·6	7·2 ± 6·1
	Fermented	23·8 ± 2·6	6·4 ± 2·4	32·9 ± 3·4	40·1 ± 5·2
β-Tocopherol	Raw	tr[c]	tr	5·0 ± 0·7	5·2 ± 1·6
	Treated	tr	tr		
	Fermented	tr	tr		
γ-Tocopherol	Raw	tr	5·1 ± 1·9	7·1 ± 2·0	7·4 ± 1·5
	Treated	tr	7·2 ± 1·4	6·3 ± 2·2	10·9 ± 5·1
	Fermented	tr	5·1 ± 0·6	8·6 ± 2·6	10 ± 1·1
α-Tocotrienol	Raw	tr	tr	5·4 ± 1	10·0 ± 2·2
	Treated	tr	tr	tr	tr
	Fermented	tr	tr	tr	tr
α-Tocopherol equivalalent	Raw	21·6 ± 1·7	29·7 ± 1·8	36·9 ± 4	39·4 ± 4·9
	Treated	24·2 ± 1·6	22·5 ± 2·3	33·6 ± 3·7	38·3 ± 6·1
	Fermented	23·8 ± 2·6	16·9 ± 2·4	33·8 ± 3·4	45·0 ± 5·2

[a] Taken from Hassapidou and Manoukas (1993).
[b] After debitting for green olives and after 7 days in water for black olives.
[c] tr, trace.

tocopherol (85%) and γ-tocopherol (15%) at the raw stage. After processing the α-tocopherol content of this variety decreased. There was a highly statistical significant difference ($P < 0.01$) between the α-tocopherol content of raw olives compared with olives after the stage of debittering and fermentation. The main loss (24%) was observed after the stage of debittering with sodium hydroxide, whereas a lower loss was observed (18%) after the stage of fermentation. No explanation could be found for the reduction of α-tocopherol in Halkidiki variety after debittering which is in contradiction with the results of Conservolea variety. It should be noted, however, that the two varieties have different morphological characters and grow in quite different environmental conditions (Anagnostopoulos, 1939). On the other hand, processing did not affect the γ-tocopherol content of this variety. There was no statistical significant difference observed between γ-tocopherol content of olives at the different stages of processing. This is probably due to the fact that γ-tocopherol is more stable than α-tocopherol as was shown for *in vitro* experiments (Cillard & Cillard, 1980). As a result of the decrease in α-tocopherol, the total value of α-Teq was also decreased after processing.

Black olives of Conservolea variety contain α-tocopherol (65%), β-tocopherol (10%), γ-tocopherol (14%) and α-tocotrienol (11%). Processing had no effect on the α- and γ-tocopherol content of this variety. There was no statistical difference between the α- and γ-tocopherol contents of raw olives and that of the olives after the two stages of processing. Debittering and fermentation on the other hand, led to a decrease in the content of α-tocotrienol to traces. The increase in the contents of α- and γ-tocopherol after fermentation, that was observed when this content is expressed as μg/g pericarp (Table 2), was not statistically significant and it was due to the percentage increase in the lipid content of the olives after processing which, in turn, was a result of the moisture decrease in the pericarp (Balatsouras, 1980).

The raw black olives of Calamon variety contained α-tocopherol (61%), β-tocopherol (9%), γ-tocopherol (13%) and α-tocotrienol (17%). Processing did not affect α- and γ-tocopherol content but it led to a decrease in α-tocotrienol. A decrease was also observed in α-Teq after processing but it was not statistically significant. The increase that can be seen in Table 2 in the α- and γ-tocopherol contents after processing when this content was expressed as μg/g pericarp was due to the percentage increase in the lipid content after processing that was in turn due to the decrease is moisture content (Balatsouras, 1980).

Conclusively, on the basis of statistical analysis for unequal variances and equal observations, it can be stated that processing did not affect the γ-tocopherol content in any variety or type of olives and that it led to a decrease in α-tocopherol content only in green olives of the Halkidiki variety. Processing also led to a decrease in α-tocotrienol content of black olives. After processing black olives still had a higher content of α-Teq than green olives.

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