Evaluation of Vitamin E by HPLC in a Variety of Olive-Based Foodstuffs

Antonio López, Alfredo Montaño*, and Antonio Garrido

Food Biotechnology Department, Instituto de la Grasa (Consejo Superior de Investigaciónes Científicas), Seville, Spain

ABSTRACT: A survey of vitamin E levels in a wide variety of olive-based foodstuffs was conducted. Vitamin E was determined by normal-phase HPLC. The only form of vitamin E found in all commercial presentations of table olives was α-tocopherol, with an average content of 3.1 mg/100 g edible portion. A very low content (<0.4 mg/100 g edible portion) of γ-tocopherol was found in most of the samples analyzed. The main sources of variation of vitamin E were olive cultivars and commercial presentations. Processing type (Spanish style green olives, directly brined olives, ripe olives) had a limited influence. Irrespective of the elaboration style, the Gordal cultivar was the poorest with respect to the vitamin E content. On the other hand, all commercial presentations based on the Hojiblanca cultivar had high contents of vitamin E. The results of this study may be used by the industry for requirements of nutritional labeling or by nutritionists to estimate vitamin E intakes in diets that include table olives.

Paper no. J10987 in *JAOCS 82*, 129–133 (February 2005).

KEY WORDS: Directly brined olives, HPLC, olive cultivars, ripe olives, Spanish-style green olives, table olives, α-tocopherol, γ-tocopherol, vegetable products, vitamin E.

Vitamin E is thought to function primarily as a chain-breaking antioxidant that prevents the propagation of lipid peroxidation. Current dietary patterns appear to provide sufficient vitamin E to prevent deficiency symptoms such as peripheral neuropathy. Estimates of vitamin E intake are underreported, owing in part to underreporting of the amounts of dietary fat consumed and the lack of specificity of sources in the diet (1). Currently, the Recommended Dietary Allowance (RDA) is based only on the α-tocopherol form of vitamin E, which represents a change from previous recommendations. Other naturally occurring forms of vitamin E (β-, γ-, and δ-tocopherols and the tocotrienols) do not contribute toward meeting the vitamin E requirement because (although absorbed) they are not converted to α-tocopherol by humans. The RDA for both men and women is 15 mg (35 μmol)/d of α-tocopherol (1).

Table olives are the main fermented vegetable product in Western countries. They constitute an important part of the Mediterranean diet. The world production of table olives reached 1,450,000 tonnes in the 2000/2001 season (2). Of the vitamins found in table olives, tocopherols are present in relatively high amounts. Assuming the α-tocopherol content as that reported by the USDA (3), one serving of 100 g of the edible portion of green table olives can provide about 25% of the RDA of vitamin E.

Industrial elaboration of table olives is restricted to only a few types or styles, each of which can be found in various commercial presentations. The most common are: green olives, Spanish or Sevillian style; directly brined olives, turning color or naturally black olives; and ripe olives, California style. In brief, the procedure for preparing green, Spanish-style olives consists of treating the fruits with a dilute NaOH solution, followed by water washes, and brining. In brine, olives undergo a lactic acid fermentation. The commercial presentations of green olives are numerous and include the use of many stuffing materials (4). Untreated olives (green, turning color, or naturally black) are directly brined after picking, where they undergo a limited fermentation and lose some of their natural bitterness. As the market demands, olives are sorted, graded, and packed. In some commercial presentations, they can be broken or cut along their higher longitudinal diameter and/or seasoned with natural products or their flavors (4). Olives for producing ripe olives (by alkaline oxidation) are first preserved in an aqueous solution (brine, acidic water, etc.) and darkened throughout the year. Darkening consists of several treatments with dilute NaOH solutions and water washes, with aeration between them. Darkened olives are immersed in a lactate or gluconate iron solution and packed in light brine. Their commercial presentations are limited to plain (whole), pitted, sliced, and, sometimes, olive paste (4).

The number of cultivars devoted to table olives around the world is limited. In Spain, Gordal, Manzanilla, Hojiblanca, Cacereña, Carrasqueña, Verdial, Aloreña, and Arbequina are the most popular. Their compositions differ from one to another (5). Furthermore, during conditioning for the different commercial presentations, the product suffers new modifications due to pitting, slicing, and stuffing. The diverse stuffing materials may produce variations in the composition of their respective final products.

The aim of this work was a systematic investigation of the content of vitamin E in table olives, taking into account cultivars, processing methods, and commercial presentations.

EXPERIMENTAL PROCEDURES

Samples. The study was carried out in 67 composite samples from different commercial presentations. Each composite

*To whom correspondence should be addressed at Food Biotechnology Department, Instituto de la Grasa (CSIC), Avda. Padre García Tejero, 4, Seville, Spain. E-mail: amontano@cica.es

sample was made up of three to eight units (cans, jars, or plastic pouches), depending on their sizes, from one to five elaboration companies, and different packing dates, according to their availability on market shelves. The average time from packing was about 3 mon. Producers kindly supplied those not available in the local markets. Samples of Spanish-style green olives were of the following cultivars: Gordal (plain, pitted, seasoned, and stuffed with formulated red pepper strips, natural red pepper, almond, cucumber, onions, garlic, and jalapeño, as well as a mixture of olives and formulated red pepper strips called salads), Manzanilla (plain, pitted, sliced, sliced "salads," anchovy-flavored, plain seasoned, "gazpachas," pitted "salads," "alcaparrado," and stuffed with formulated red pepper strips, formulated anchovy strips, formulated and marinated anchovy strips, natural red pepper, almond, almond and red pepper, formulated salmon strips, formulated tuna strips, onions, capers, garlic, hazelnut, hot pepper, formulated hot pepper strips, "piquillo" pepper, lemon, ham, anchovy, formulated orange strips, cheese, formulated "jalapeño" strips, and formulated garlic strips), Carrasqueña (pitted, pitted salads, and "alcaparrado"), and Hojiblanca (plain, pitted, sliced, and stuffed with formulated pimento strips). Directly brined olives included Gordal (broken seasoned turning color), Manzanilla (turning color just in brine, seasoned turning color, and biologic), Hojiblanca (seasoned), Arbequina (seasoned), Aloreña (seasoned prepared from fresh and stored olives), and Verdial (seasoned broken). Samples of directly brined olives were intended only for local markets. Finally, ripe olives were of the Gordal (plain), Manzanilla (pitted), Carrasqueña (plain and pitted), Hojiblanca (plain, pitted, and sliced), and Cacereña (plain, pitted, and sliced).

Chemicals. Acetone, *n*-hexane, and propan-2-ol (Romil, Cambridge, United Kingdom) were of HPLC grade. Diethyl ether (Carlo Erba, Milano, Italy), ethanol (Merck, Darmstadt, Germany), and BHT (Sigma, Diesenhofen, Germany) were of analytical reagent grade. A tocopherol kit consisting of α,β-,γ-, and δ-tocopherol was obtained from Merck. Stock standard solutions (100 μg/mL) were prepared in *n*-hexane. A mixed standards working solution (5 μg/mL of each tocopherol) was prepared from the stock solutions by dilution with *n*-hexane. Control of stock standard solutions was carried out spectrophotometrically as suggested by IUPAC (6).

Analysis of tocopherols. Freeze-dried samples (8 g) were homogenized, in an UltraTurrax homogenizer for 1 min, with 25 mL water plus 50 mL of acetone (containing 0.1% BHT as an antioxidant). The resulting homogenate was vacuum-filtered, and the residue was again extracted three to four times to remove all color. Diethyl ether (150 mL) containing 0.1% BHT was added to the pooled extracts in a separatory funnel of 1 L. The solvents were mixed, water (100 mL) was added, and the phases were allowed to separate. The aqueous hypophase was extracted twice with portions (50 mL) of diethyl ether. The pooled ether extracts were washed three or four times with water (100 mL) and transferred to a volumetric flask (250 mL), which was then filled to the mark with diethyl ether. An aliquot (2 mL) was evaporated under nitrogen, ethanol (2 mL) was added, and evaporation was continued to remove traces of water. The residue was dissolved in 2 mL *n*-hexane and then filtered through a 0.45-μm filter prior to injection into the HPLC system for analysis of tocopherols. All manipulations were carried out under gold fluorescent lighting. Tocopherols were determined in a Waters 2690 Separations Module (Waters Assoc., Milford, MA), a JASCO FP-920 fluorescence detector (JASCO Corp., Tokyo, Japan) (excitation wavelength, 290 nm; emission wavelength, 330 nm), and a computer with Waters Millenium 32 Chromatography Manager, version 3.00. Filtered extract (10 μL) was injected onto a Spherisorb W silica column (250 × 4.6 mm i.d., 5 μm; Teknokroma, Barcelona, Spain) using propan-2-ol in *n*-hexane (0.5:99.5, vol/vol) as the mobile phase at a flow rate of 1 mL/min. All analyses were performed at least in duplicate.

Determination of fat. Fat content was determined using the Soxhlet technique as described previously (5).

Statistical data analysis. Statistica software version 6.0 (StatSoft Inc., Tulsa, OK) was used for data processing. The General Linear Model (GLM) was used to test the effects of cultivars, elaboration styles (green, ripe, or directly brined), and commercial presentations. Simultaneous confidence intervals were established according to the Scheffé test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Chromatographic conditions used for analysis of vitamin E in the present work were the same as those described for vegetable oils and fats (6), which permit a good separation of the different naturally occurring forms of this vitamin α-, β-, γ-, and δ-tocopherols and the tocotrienols). The only tocopherol found in all commercial presentations of table olives was α-tocopherol. γ-Tocopherol was found in most of the samples analyzed, but its concentration was always significantly lower than that of α-tocopherol. β-Tocopherol and δ-tocopherol were not detected in any sample. This is in agreement with Hassapidou *et al.* (7), who analyzed the tocopherol composition in Greek table olives. Although the presence of tocotrienols was not investigated in detail in the present work, in general no chromatographic peaks were observed at the retention times where tocotrienols should elute according to the bibliography in Reference 8 for equivalent chromatographic conditions. α-Tocotrienol (0.5–1.0 mg/100 g pericarp) has been reported to be present in raw black olives, but only in trace amounts after processing (7).

The frequency distributions (histograms) of contents of α- and γ-tocopherol in mg/100 g edible portion (e.p) in all samples of table olives are shown in Figure 1. The highest proportion of the table olive samples (23.3%) had between 3.5 and 4.0 mg α-tocopherol/100 g e.p. In the case of γ-tocopherol, the highest percentage (41.8%) of samples was in the range 0.1–0.2 mg/100 g e.p. As pointed out earlier, the RDA is currently based only on the α-tocopherol form of vitamin E. Therefore, all results mentioned from now on correspond to the α-tocopherol content only.

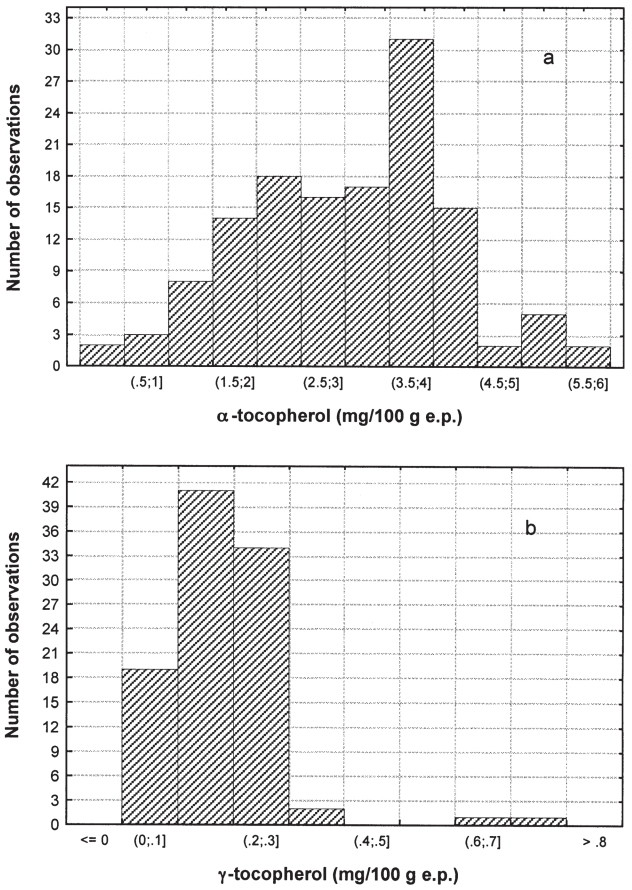

FIG. 1. Histograms for the distribution of α-tocopherol (a) and γ-tocopherol (b) in commercial table olives. e.p., edible portion.

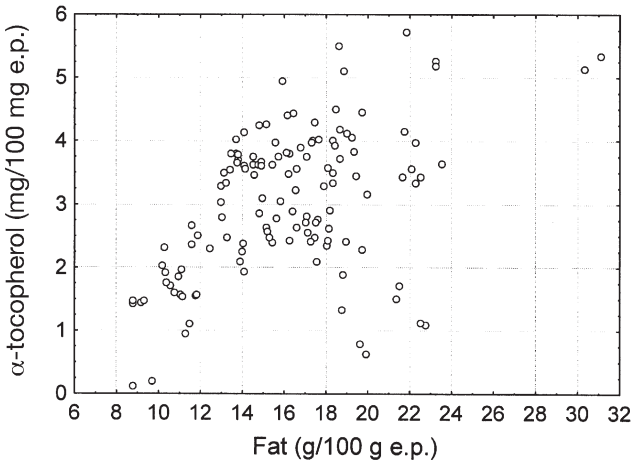

FIG. 2. Relationship between α-tocopherol content and fat content for all commercial presentations of table olives (n = 131). e.p., edible portion.

An overall comparison between elaboration styles by GLM demonstrated (data not shown) that there was no statistical difference ($P < 0.05$) in their α-tocopherol contents. The average content was 3.1 mg α-tocopherol/100 g e.p. This value is quite close to the α-tocopherol content reported in some fresh vegetables such as broccoli (9) and higher than values (<1.0 mg/100 g e.p.) reported for other pickled vegetables such as pickled garlic, cucumbers, capers, sauerkraut, and jalapeño peppers (3,10). There was a positive and highly significant ($P < 0.001$) correlation between α-tocopherol content and fat content (Fig. 2) when all samples were included in the statistical analysis, but fat content accounted for less than 25% of the variance in α-tocopherol content ($R^2 = 0.225$).

Vitamin E content in green, Spanish-style olives. Table olives of the Gordal cultivar showed the lowest α-tocopherol content (mean of about 2.0 mg/100 g e.p.). The highest average content (3.7 mg/100 g e.p.) was found for the Hojiblanca cultivar, followed by Manzanilla (3.3 mg/100 g e.p.). Results for commercial presentations are presented in decreasing order within each cultivar (Table 1). Five groups were established within both the Gordal and Manzanilla cultivars. The highest concentration of α-tocopherol was found in Gordal olives stuffed with almond or Manzanilla olives stuffed with hazelnut. It appears that the presence of almond or hazelnut as an ingredient results in increasing contents of vitamin E. This is consistent with the relatively high levels of α-tocopherol in nuts (11). On the other hand, commercial presentations with ingredients such as vegetables (e.g., capers, hot peppers) or animal products (e.g., tuna, salmon), which present low to moderate levels of α-tocopherol, had lower contents of vitamin E. The last group within the Manzanilla cultivar was made of one commercial presentation, namely, that based on sliced olives. The slicing operation, carried out after the pitting operation, appears to contribute to oxidative losses of vitamin E. The commercial presentations from the Carrasqueña and Hojiblanca cultivars were gathered in two groups each (Table 1).

When a simple commercial presentation such as pitted olives, which is found in all four cultivars, was used for comparison between cultivars (data not shown), one can see that the presentation made from the Hojiblanca cultivar had the highest levels of vitamin E. Comparison is affected by the form of expression of α-tocopherol content (mg/100 g e.p. or mg/100 g fat). Thus, presentation from the Gordal cultivar showed practically the same α-tocopherol content, expressed as mg/100 g fat, as the presentation from the Manzanilla cultivar (13.32 vs. 13.28), but the difference was significant when the α-tocopherol content was expressed as mg/100 g e.p. (1.57 vs. 2.35). This is due to the lower fat content of the Gordal cultivar (11.79 vs. 17.69%). A statistically significant relationship ($P < 0.001$) was observed between the α-tocopherol content and fat content of Spanish-style products (data not shown), but fat content accounted for a low proportion of the variance in α-tocopherol content ($R^2 = 0.396$; $n = 93$).

Vitamin E content in directly brined olives. Compared with the Spanish style, the number of commercial presentations in this case was rather limited, although a greater diversity of olive cultivars in this style was found. The highest contents of vitamin E were found in presentations made from the Hojiblanca and Arbequina cultivars, whereas the Gordal cultivar had the lowest level. The high fat content of the Arbequina cultivar (30.7%)

TABLE 1
α-Tocopherol Content in Spanish Style Green Olives According to Cultivars and Commercial Presentations

Cultivar	mg α-tocopherol/100 g e.p.			
Commercial presentation[a]	Mean[b]	SE	CL_{low}[c]	CL_{high}[c]
Gordal				
Stuffed with almond	3.82	0.13	3.56	4.10
Seasoned	2.59	0.13	2.32	2.86
"Salads"	2.07	0.09	1.88	2.27
Pitted, stuffed red pepper strips, stuffed natural peppers, stuffed "jalapeño" peppers	1.67	0.06	1.54	1.81
Stuffed cucumber, stuffed onions, or garlic	1.32	0.07	1.16	1.47
Manzanilla				
Stuffed with hazelnut	5.23	0.24	4.74	5.72
Plain, anchovy-flavored, stuffed natural red pepper, stuffed almond	4.46	0.12	4.22	4.71
Seasoned, "salads," stuffed pepper strips, anchovy strips, "alcaparrado," onions, "piquillo" pepper, hot peppers, hot pepper strips, fresh anchovy, lemon	3.58	0.07	3.43	3.73
Pitted, "gazpachas," slices "salads," stuffed cheese, garlic strips, "jalapeño" strips, orange strips, ham, garlic, capers, pepper + almond, tuna, salmon strips	2.63	0.07	2.50	2.78
Slices	1.61	0.24	1.12	2.10
Carrasqueña				
Pitted	3.65	0.18	3.17	4.14
"Salads" and "alcaparrado"	2.12	0.12	1.78	2.46
Hojiblanca				
Stuffed with pimento strips	3.67	0.34	2.84	4.51
Plain, pitted and "salads"	3.64	0.19	3.15	4.12

[a]"Strips" are made of a paste of alginate or carragenate and the stuffing material.
[b]Mean value(s) of the commercial presentation(s).
[c]Confidence limits of means for $P \leq 0.05$. e.p., edible portion.

contributing to the vitamin E content, expressed as mg α-tocopherol/100 g e.p., is noteworthy. The relationship between α-tocopherol content and fat content for the samples ($n = 18$) in this style was not significant.

Vitamin E content in ripe olives (darkened by oxidation). These olives also had relatively high contents of vitamin E (Table 3). These results must be highlighted since this style of olives requires several lye treatments and water washes, with aeration, between them. Furthermore, with the exception of the Gordal cultivar, concentrations of α-tocopherol were similar to those found in the same cultivar/commercial presentation prepared as Spanish-style olives. The regression of fat content on

TABLE 2
α-Tocopherol Content in Directly Brined Table Olives According to Cultivars and Commercial Presentations

Cultivar	mg α-tocopherol/100 g e.p.			
Commercial presentation	Mean[a]	SE	CL_{low}[b]	CL_{high}[b]
Gordal				
Broken seasoned turning color	0.72	0.10	0.50	0.94
Manzanilla				
Turning color, seasoned turning color	3.49	0.07	3.34	3.65
Biologic	3.88	0.10	3.66	4.10
Hojiblanca				
Seasoned turning color	5.31	0.10	5.09	5.53
Arbequina				
Seasoned	5.25	0.10	5.03	5.47
Aloreña				
Seasoned fresh	1.61	0.10	1.39	1.83
Seasoned after storage	1.11	0.10	0.89	1.33
Verdial				
Broken seasoned	3.47	0.10	3.26	3.70

[a]Mean value(s) of the commercial presentation(s).
[b]Confidence limits of means for $P \leq 0.05$. e.p., edible portion.

TABLE 3
α-Tocopherol Content in Ripe (darkened by oxidation) Olives According to Cultivars and Commercial Presentations

Cultivar	mg α-tocopherol/100 g e.p.			
Commercial presentation	Mean[a]	SE	CL_{low}[b]	CL_{high}[b]
Gordal				
Plain	0.17	0.32	0.00	0.86
Manzanilla				
Pitted	2.26	0.32	1.57	2.95
Carrasqueña				
Plain and pitted	4.08	0.23	3.59	4.57
Hojiblanca				
Plain, pitted, and slices	3.96	0.19	3.56	4.36
Cacereña				
Plain, pitted, and slices	3.42	0.19	3.02	3.82

[a]Mean value(s) of the commercial presentation(s).
[b]Confidence limits of means for $P \leq 0.05$. e.p., edible portion.

α-tocopherol content (data not shown) was significant as well ($P < 0.01$; $R^2 = 0.359$; $n = 20$). The lowest content of α-tocopherol in the ripe olive commercial presentations was found in the Gordal cultivar, as was the case for green or directly brined olives, whereas the highest contents were found in the Hojiblanca and Carrasqueña cultivars. The content of vitamin E reported by the USDA (3) for ripe olives (1.65 mg α-tocopherol/100 g e.p.) is within the range found in the present survey.

ACKNOWLEDGMENTS

The Spanish Government (AGL-2003-00779) and Junta de Andalucía supported this work (throughout financial support to group AGR-125). We also thank Amparo Cortés for technical assistance.

REFERENCES

1. Food and Nutrition Board, Institute of Medicine, the National Academies, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*, National Academies Press, Washington, DC, 2000.
2. International Olive Oil Council (IOOC), Draft Final Balance, Season 2001/2002, 88th IOOC Meeting, Madrid, 2003.
3. USDA, Agricultural Research Service, *USDA Nutrient Database for Standard Reference, Release 17* (2004) Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp/Data (accessed September 2004).
4. Garrido, A., M.J. Fernández-Díez, and R.M. Adams, *Table Olives. Production and Processing*, Chapman & Hall, London, 1997.
5. Fernández-Díez, M.J., R. Castro, A.G. Fernández, F.G. Cancho, F.G. Pellissó, M.N. Vega, A.H. Moreno, I.M. Mosquera, L. Rejano, M.C.D. Quintana, *et al.,* Biotecnología de la Aceituna de Mesa, CSIC, Madrid, 1985.
6. IUPAC, Determination of Tocopherols and Tocotrienols in Vegetable Oils and Fats by High Performance Liquid Chromatography, in *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn., Blackwell Scientific, Oxford, 1992.
7. Hassapidou, M.N., G.D. Balatsouras, and A.G. Manoukas, Effect of Processing upon the Tocopherol and Tocotrienol Composition of Table Olives, *Food Chem. 50*:112–114 (1994).
8. Eitenmiller, R.R., and W.O. Landen, *Vitamin Analysis for the Health and Food Sciences*, CRC Press, Boca Raton, FL, 1999, pp 109–148.
9. Ching, L.S., and S. Mohamed, α-Tocopherol Content in 62 Edible Tropical Plants, *J. Agric. Food Chem. 49*:3101–3105 (2001).
10. Casado, F.J., A. López, L. Rejano, A.H. Sánchez, and A. Montaño, Nutritional Composition of Commercial Pickled Garlic, *Eur. Food Res. Technol. 219:*355–359 (2004).
11. Maguire, L.S., S.M. O'Sullivan, K. Galvin, T.P. O'Connor, and N.M. O'Brien, Fatty Acid Profile, Tocopherol, Squalene, and Phytosterol Content of Walnuts, Almonds, Peanuts, Hazelnuts and the Macadamia Nut, *Int. J. Food Sci. Nutr. 55*:171—178 (2004).

[Received November 9, 2004; accepted February 1, 2005]