Contribution of Chemical Components of Cornicabra Virgin Olive Oils to Oxidative Stability. A Study of Three Successive Crop Seasons

M.D. Salvador*, F. Aranda, and G. Fregapane

Universidad de Castilla - La Mancha, Facultad de Ciencias Químicas,
Departamento de Química Analítica y Tecnología de Alimentos, 13071 Ciudad Real, Spain

ABSTRACT: Oxidation is the primary cause of virgin olive oil quality deterioration. This paper presents a correlation between oxidative stability, as determined by the Rancimat method, and some chemical components involved in the oxidation process of a set of 74 Cornicabra virgin olive oils obtained from three successive crop seasons (94/95 to 96/97). Results showed a clear influence of total polyphenols on virgin olive oil stability, with linear regression coefficients which were similar for the three seasons studied, and a much lower contribution of α-tocopherol and unsaturated fatty acids, mainly linoleic acid. A significant effect dependent on the crop season was also observed.

Paper no. J8920 in *JAOCS 76,* 427–432 (April 1999).

KEY WORDS: Cornicabra variety, oxidative stability, virgin olive oil.

Virgin olive oil's characteristic aroma, taste, and color distinguish it from other edible vegetable oils. Its excellent organoleptic and nutritive properties, together with the current tendency of consumers to select the least-processed foods, have prompted a reassessment of its consumption in the regular diet. It is therefore a matter of great concern for the olive oil industry to preserve its product without loss of these positive attributes. Furthermore, it would be very useful to be able to predict olive oil stability and shelf life from a single, or a few, analytical or quality parameters.

Deterioration of oil quality is mainly due to oxidation caused by the high reactivity of free radicals to fatty acids (1). Although rapid methods of assessing the oxidative stability of oils, like the Rancimat apparatus (2), which correlates well with the active oxygen method (3), have been introduced, none of the established quality criteria has been reported to correlate well with the shelf life of olive oil.

Oxidative stability must be related to the concentration of some olive oil chemical components, in particular unsaturated fatty acids and natural antioxidants; however, the exact role of individual compounds has not been yet firmly established.

*To whom correspondence should be addressed at Universidad de Castilla - La Mancha, Facultad de Ciencias Químicas, Departamento de Química Analítica y Tecnología de Alimentos, Campus Universitario s/n, 13071 Ciudad Real, Spain.
E-mail: asalvador@qata-cr.uclm.es

The relative rate of oxidation of oleate, linoleate, and linolenate was reported to be 1, 40–50, and 100 times, respectively (1), and it was suggested that oil stability be evaluated by means of a formula that includes monounsaturated, linoleic, and linolenic acid content, denominated Oxidative Susceptibility (4).

It has been reported by many authors that virgin olive oils with high polyphenol content are more resistant to autoxidation (5–12). Oxidative stability correlated mainly with the concentration of hydrophilic phenols, which were evaluated colorimetrically with the Folin Ciocalteau reagent; however, tyrosol, the main phenol present in olive oil, showed practically no antioxidant effect (13). Furthermore, three new natural secoiridoid compounds, derivatives of oleuropeine and ligstroside that possess antioxidant activity, have been isolated from virgin olive oil (14–16). As a result, although the level of phenolic compounds has been related to oil stability, it is not yet sufficiently clear what compounds are responsible for the antioxidant activity.

Tocopherols in virgin olive oil are important for their antioxidant nutritional quality (17,18). The existence of a correlation between tocopherols and oil stability has not been well established (19). Although the synergistic effect of these compounds on polyphenol antioxidant activity has been demonstrated (20), no good correlation between oxidative stability, determined by Rancimat method, and total tocopherols has been found in virgin olive oils (4,12).

It has been known since the 1960s that 4-methyl-sterols isolated from the unsaponifiable fraction of wheat germ and corn oils inhibit oxidation of linoleic acid during frying (21). Though the main olive oil sterol is β-sitosterol, oils that contain up to 9% of Δ5-avenasterol have been found (22), and the antioxidant activity of this compound has been studied by Williamson (23).

Chlorophyll and carotenoid compounds also play an important role in the oxidative stability of processed foodstuffs due to their antioxidant nature in the dark and prooxidant activity in the light (24). The photooxidative activity of chlorophylls and their derivatives *via* singlet oxygen formation has been demonstrated in experiments on decolored olive oils (25).

This paper presents a correlation between oxidative stability, determined by the Rancimat method, and some chemical

variables involved in the oxidation process of a set of 74 Cornicabra virgin olive oils obtained from three successive crop seasons (1994/95 to 1996/97). It is a preliminary study prior to deeper analyses of the relationship between stability (and/or antioxidant activity) and individual olive oil chemical components, in an attempt to establish a correlation between olive oil stability and shelf life, and a single, or a few, analytical or quality parameters.

The Cornicabra variety used in this study covers an area of 300,000 Ha, mainly located in the center of Spain, and accounts for 14% of the total production; the fat yield of the fruit is 23% of fresh weight and its oil is valued for its high stability and good sensory characteristics (26).

MATERIALS AND METHODS

Oil samples. Virgin olive oil samples ($n = 74$) of Cornicabra variety were collected from industrial oil mills located in the production areas of Toledo and Ciudad Real (Castilla-La Mancha) during the crop seasons 1994/95, 1995/96, and 1996/97. Samples were filtered with anhydrous Na_2SO_4 and stored at 4°C in darkness using amber glass bottles without head space until analysis.

Analytical methods. Determination of free acidity, peroxide index, ultraviolet light (UV) absorption characteristics, and fatty acid composition were carried out following the analytical methods described in Regulations EEC/2568/91 and EEC/1429/92 of the Commission of the European Union (27).

Free acidity, given as percentage of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) with ethanolic potash.

Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq/kg), was determined as follows: a mixture of oil and chloroform/acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution.

K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, with an UV spectrophotometer (Hewlett-Packard, HP 8452A), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Oxidative stability was evaluated by the Rancimat method since it is a fast and reliable analytical procedure (3). Stability was expressed as the oxidation induction time (hours) measured with the Rancimat 679 apparatus (Metrohm, Herisau, Switzerland), using an oil sample of 3.5 g, warmed to 98°C, and an air flow of 10 L/h.

Phenolic compounds were isolated by three successive extractions of a solution of oil in hexane with a water/methanol mixture (60:40). To a suitable aliquot of the combined extracts, the Folin-Ciocalteau reagent (Merck, Darmstadt, Germany) was added and the absorption of the solution at 725 nm was measured. Values are given as milligrams of gallic acid per kilogram of oil (28).

Tocopherols were evaluated following the AOCS Method Ce 8-89 (29). A solution of oil in hexane was analyzed by high-performance liquid chromatography (Waters 2690) on a silica gel Lichrosorb Si-60 column (particle size 5 µm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain), that was eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 mL/min. A fluorescence detector (Waters 470) with excitation and emission wavelengths set at 290 and 330 nm, respectively, was used.

Chlorophyll and carotenoid compounds (mg/kg) were determined at 472 and 670 nm in cyclohexane using the specific extinction values, by the method of Minguez-Mosquera *et al.* (30).

For the determination of fatty acid composition, the methyl esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potash, and analyzed by gas chromatography (GC) with a Hewlett-Packard 5890 chromatograph equipped with a flame-ionization detector. A fused-silica column (50 m length × 0.25 mm i.d.) coated with SGL-1000 phase (0.25 µm thickness; Sugerlabor) was used. Helium was used as carrier gas with a flow through the column of 1 mL/min. The temperature of the injector and detector was set at 250°C, whereas the oven temperature was 210°C. An injection volume of 1 µL was used.

Sterols (%) were determined by GC (Hewlett-Packard 5890) with a capillary column (25 m length × 0.25 mm i.d.) coated with SGL-5 (0.25 µm thickness; Sugerlabor). Working conditions were as follows: carrier gas, helium; flow through the column, 1.2 mL/min; injector temperature, 280°C; detector temperature, 290°C; oven temperature, 260°C; injection volume 1 µL.

Analytical determinations were carried out at least in duplicate.

Statistical analysis. Statistical analysis was performed using the NCSS 6.0 statistical software (31). Differences were considered statistically significant when probability was greater than 95% ($P < 0.05$).

RESULTS AND DISCUSSION

The large majority of the values obtained for free acidity, peroxide value, and UV absorption characteristics, with the exception of acidity in twelve 1994/95 samples, complied with the regulations of the European Union Commission (EEC/656/95 and posterior modification EEC/656/95) for the highest category "extra" virgin olive oils.

Pearson's correlation coefficients between oxidative stability (dependent variable) and some analytical determinations studied (independent variables) are summarized in Table 1. Olive oil samples from the crop seasons were treated as three separate data sets taking into account that, as previously observed for the same olive variety (32), the harvest year may affect oil classification. A high correlation, consistent through the three seasons, was observed for total polyphenols content (from 0.873 to 0.927). The correlation obtained with each of the three years studied was higher than that with the total number of olive oil samples. A good direct correlation between oxidative stability and total polyphenols content determined by a colorimetric method has been reported by many authors (6–8,10–12).

TABLE 1
Pearson's Correlation Coefficients Between Oxidative Stability (dependent variable) and Some Analytical Determinations (independent variables) for Cornicabra Virgin Olive Oil Samples Obtained from Three Crop Seasons (n = 74)

Variable	1994/95	1995/96	1996/97	Total
Free acidity	0.1700	–0.6701	–0.5960	–0.5323
Peroxide value	–0.4284	–0.3341	0.5729	–0.2025
K_{232}	–0.2498	–0.0759	0.6081	–0.2371
K_{270}	–0.2730	–0.0163	0.4147	–0.0508
Oxidative stability	1.0000	1.0000	1.0000	1.0000
Chlorophylls	0.1081	0.5376	0.5763	0.4360
Carotenoids	0.1237	0.5524	0.6405	0.4718
Polyphenols	0.9190	0.9269	0.8728	0.8374
α-Tocopherol	0.6284	0.8708	0.5097	0.6048
C18:1	–0.0208	–0.2486	0.7718	0.0042
C18:2	0.0004	–0.3111	–0.8741	–0.3036
C18:3	0.2497	0.6511	0.2450	0.2975
β-Sitosterol	0.0128	0.6667	0.2519	0.3079
Δ5-Avenasterol	–0.1040	–0.5790	–0.1332	–0.1956

α-Tocopherol concentration showed a much lower correlation with oxidative stability (0.510–0.870). Although this component apparently has only a low effect on oxidative stability, the synergistic effect of α-tocopherol on polyphenol antioxidant activity has been demonstrated (20).

The high correlation observed for α-tocopherol in the year 1995/96 (0.870) could be explained by the similar correlation obtained that particular year between α-tocopherol and polyphenols: 0.837 (Table 2). In a similar way, the correlation with polyphenols could explain the high correlation observed between oxidative stability and oleic and linoleic acid in the season 1996/97, as well as with other oil compounds. Pearson's correlation coefficients between polyphenols and some chemical components are shown in Table 2.

Linear regression obtained for oxidative stability vs. total polyphenols content in Cornicabra virgin olive oils is shown in Figure 1 and Table 3. The three resulting lines, one for each crop season, are almost parallel, with a slope ranging from 0.288 to 0.355, whereas the intercept increased from the season 1994/95 to 1996/97 (from –20.4 up to 18.9). This means that with similar polyphenol content, the oxidative stability of the oils increased from season 1994/95 to 1996/97. This effect may be explained if we consider that a statistically significant difference was observed in many analytical and quality parameters with respect to the crop season for this variety (32). By way of example, Figure 2 shows distribution plots for free acidity and peroxide value for the three years studied. It should also be noted that the season 1994/95 was characterized by several preceding years of drought.

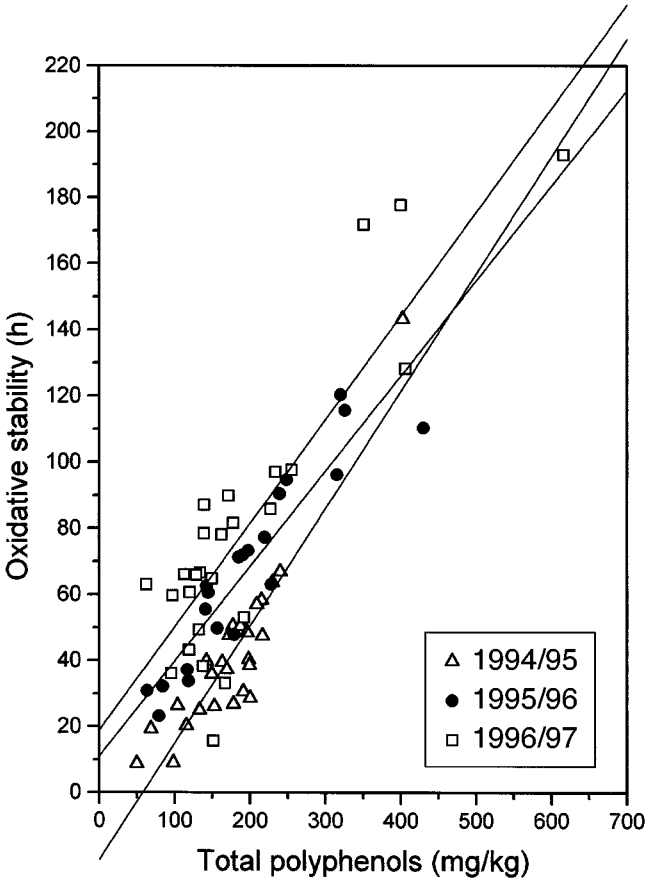

FIG. 1. Linear regression of oxidative stability vs. total polyphenol content for Cornicabra virgin olive oil samples obtained from three crop seasons (n = 74).

TABLE 2
Pearson's Correlation Coefficients Between Polyphenols and Some Other Analytical Determinations for Cornicabra Virgin Olive Oil Samples Obtained from Three Crop Seasons (n = 74)

	1994/95	1995/96	1996/97	Total
Free acidity	0.1169	–0.6181	–0.5108	–0.3584
Peroxide value	–0.4531	–0.3022	0.4161	–0.0760
K_{232}	–0.0720	0.1376	0.7195	0.1067
K_{270}	–0.0412	0.0161	0.3872	0.1310
Oxidative stability	0.9190	0.9269	0.8728	0.8374
Chlorophylls	0.0573	0.4063	0.4628	0.3893
Carotenoids	0.1519	0.4195	0.5189	0.4261
Polyphenols	1.0000	1.0000	1.0000	1.0000
α-Tocopherol	0.6054	0.8373	0.4315	0.5297
C18:1	–0.0352	–0.5046	0.5091	–0.0421
C18:2	0.0742	–0.0588	–0.7065	–0.2342
C18:3	0.3526	0.7225	0.2856	0.3511
β-Sitosterol	0.1071	0.6885	0.0723	0.3232
Δ5-Avenasterol	–0.1091	–0.6275	0.0423	–0.2304

A new Pearson's correlation analysis was carried out specifying polyphenol content as a partial variable (NCSS 6.0). The linear influence of this variable is removed by sweeping it out of the matrix. This provides a statistical adjustment to

TABLE 3
Linear Regression of Oxidative Stability vs. Total Polyphenol Content for Cornicabra Virgin Olive Oil Samples Obtained from Three Crop Seasons (n = 74)

Crop Season	Slope	Intercept	r^2
1994/95	0.355 ± 0.031	–20.4 ± 5.7	0.845
1995/96	0.288 ± 0.027	11.1 ± 5.8	0.859
1996/97	0.314 ± 0.036	18.9 ± 8.2	0.762
Total	0.327 ± 0.025	1.1 ± 5.3	0.701

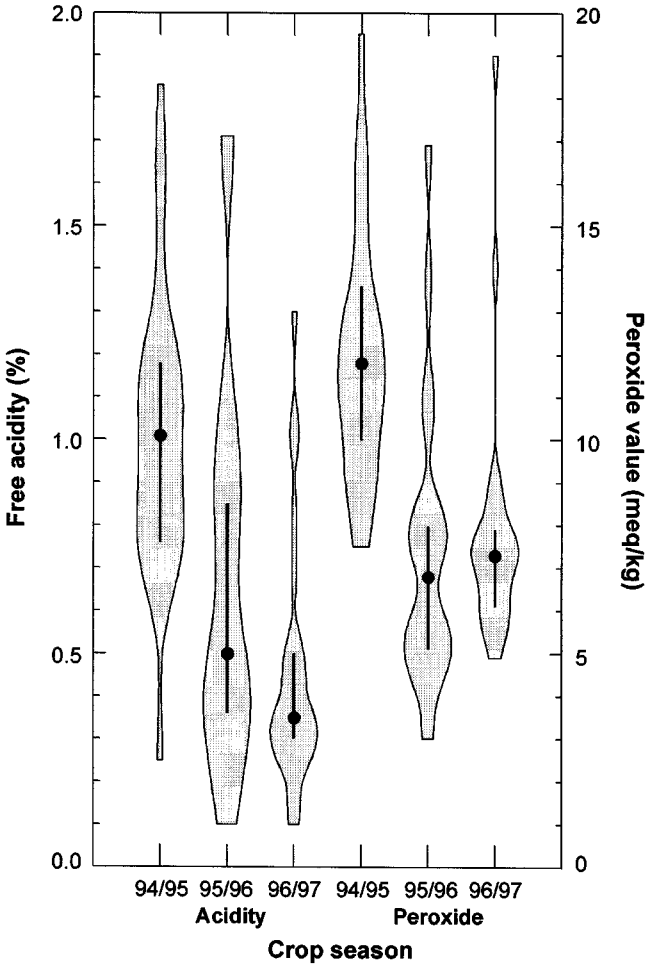

FIG. 2. Distribution plots for free acidity and peroxide value for Cornicabra virgin olive oil samples obtained from three crop seasons (n = 74).

the remaining variables using multiple regression and allows the analysis of the relationship between oxidative stability and other components like α-tocopherol and unsaturated fatty acids (Table 4). By this means it was observed that a good relationship with oleic and linoleic acids was maintained in the season 1996/97 (0.780 and –0.745, respectively); a similar correlation showed up for the season 1995/96.

TABLE 4
Pearson's Correlation Coefficients Between Oxidative Stability and Unsaturated Fatty Acids or α-Tocopherol for Cornicabra Virgin Olive Oil Samples Obtained from Three Crop Seasons (n = 74)

	1994/95	1995/96	1996/97	Total
Partial variable: polyphenols				
Polyphenols	0.0000	0.0000	0.0000	0.0000
α-Tocopherol	0.2298	0.4618	0.3023	0.3477
C18:1	0.0294	0.6763	0.7796	0.0721
C18:2	–0.1722	–0.6848	–0.7452	–0.2024
C18:3	–0.2014	–0.0715	–0.0092	0.0069
OS[a]	–0.1907	–0.6692	–0.7453	–0.1811
R18[b]	–0.1777	–0.6894	–0.7584	–0.1753
Partial variables: polyphenols and α-tocopherol				
Polyphenols	0.0000	0.0000	0.0000	0.0000
α-Tocopherol	0.0000	0.0000	0.0000	0.0000
C18:1	0.1266	0.7774	0.7761	0.3581
C18:2	–0.3078	–0.7659	–0.7147	–0.4172
C18:3	–0.3441	–0.3583	–0.2416	–0.3444
OS[a]	–0.3429	–0.7923	–0.7178	–0.4484
R18[b]	–0.3287	–0.8068	–0.7319	–0.4450

[a]Oxidative susceptibility (4).
[b]Polyunsaturated/monounsaturated ratio of C_{18} fatty acids. See text.

With respect to unsaturated fatty acid content of olive oil, some authors have suggested a relationship between oxidative stability and the oxidative susceptibility (OS) of the oil, where OS is equal to monounsaturated fatty acids + 45 × linoleic acid +100 × linolenic acid (4). In a similar way, we wanted to verify the validity of a relationship between stability and the polyunsaturated/monounsaturated ratio of the C18 fatty acids series (R18), where R18 is equal to (C18:2 + n C18:3)/C18:1 (since oleic acid showed a positive correlation with oxidative stability). The factor n indicates the difference in the velocity of the autoxidation reaction of the two polyunsaturated fatty acids. Some authors have indicated that this factor is close to 2 (1). Nonlinear regressions were performed; the best n value obtained for the olive oil samples analyzed was between 1.5 and 5 and was dependent on the crop season. However, no important variations in the resulting r^2 value were observed when the value of 2 was used. In all cases, similar correlation has been obtained for linoleic acid (C18:2), OS, or R18 (Table 4).

TABLE 5
r^2 Values Obtained in the Linear Regression Between Oxidative Stability and Polyphenols, α-Tocopherol and/or Unsaturated Fatty Acids for Cornicabra Virgin Olive Oil Samples Obtained from Three Crop Seasons (n = 74)[a]

Variable	1994/95	1995/96	1996/97	Total
Polyphenols	0.844	0.859	0.762	0.701
Polyphenols and α-tocopherol	0.853	0.889	0.783	0.737
Polyphenols and C18:2	0.849	0.925	0.894	0.713
Polyphenols and OS	0.850	0.922	0.894	0.711
Polyphenols and R18	0.849	0.926	0.899	0.710
Polyphenols, α-tocopherol and C18:2	0.867	0.954	0.894	0.783
Polyphenols, α-tocopherol and OS	0.870	0.959	0.895	0.790
Polyphenols, α-tocopherol and R18	0.869	0.961	0.8995	0.789

[a]For abbreviations see Table 4.

TABLE 6
Multiple Linear Regression Between Oxidative Stability and Polyphenols, R18, and α-Tocopherol for Cornicabra Virgin Olive Oil Samples Obtained from Three Crop Seasons (n = 74)[a]

	Polyphenols	R18	α-Tocopherol	Intercept	r^2
		Polyphenols and R18			
1994/95	0.358 ± 0.031	−2.24 ± 2.53		−5.9 ± 17.4	0.849
1995/96	0.301 ± 0.02	−8.01 ± 1.98		74.0 ± 16.2	0.926
1996/97	0.191 ± 0.032	−29.39 ± 5.27		244.3 ± 40.8	0.899
Total	0.322 ± 0.025	−3.51 ± 2.34		27.1 ± 18.1	0.710
		Polyphenols, α-tocopherol and R18			
1994/95	0.315 ± 0.038	−4.54 ± 2.72	0.128 ± 0.070	−0.4 ± 16.6	0.869
1995/96	0.212 ± 0.027	−8.33 ± 1.48	0.174 ± 0.044	59.1 ± 12.6	0.961
1996/97	0.190 ± 0.033	−28.65 ± 5.69	0.060 ± 0.152	229.5 ± 56.0	0.900
Total	0.234 ± 0.028	−9.78 ± 2.35	0.306 ± 0.060	37.9 ± 15.7	0.789

[a]For abbreviations see Table 4.

When the variable α-tocopherol content was included in the multivariate regression on top of that of total polyphenols, the Pearson's correlation coefficient between oxidative stability and unsaturated fatty acids (oleic acid, linoleic acid, OS, or R18) increased in seasons 1995/96 and 1994/95 and was maintained in 1996/97 (Table 4). This effect could indicate that α-tocopherol is a variable, like polyphenols and unsaturated fatty acids, to take into account in the study of the relationship between oxidative stability and chemical composition of olive oil.

The increase in the linear regression correlation parameter r^2 considering one variable (polyphenols), two variables (polyphenols and α-tocopherol or unsaturated fatty acids), and three variables is shown in Table 5. A small increase in the correlation was obtained in 1994/95 with the introduction of a second variable in the regression equation; also, with all three variables the maximum observed increase was only 0.026, which gave a final r^2-value of 0.870. Higher increases were observed in the seasons 1995/96 and 1996/97. In the former, the r^2 improved by 0.067 and 0.102 with the addition of R18 and both R18 and α-tocopherol, respectively, with a final value of 0.926 and 0.961 in 1995/96. In the latter season (1996/97), on the other hand, the introduction of R18 raised the correlation by 0.137 and practically no additional benefits were obtained by addition of the variable α-tocopherol. Small differences only were observed using linoleic acid, OS, or R18 as unsaturated fatty acid variable in the regression equation.

Regression equations for two (polyphenols and R18) and three (polyphenols, α-tocopherol, and R18) variables are reported in Table 6. The effect due to the crop year mentioned above is visible here as well, in particular in the different values obtained for the intercept. This effect needs to be studied and defined because it may be possible to obtain a single equation, valid for any crop season, with a fixed relationship between the two or three major variable coefficients (polyphenols, α-tocopherols, and/or unsaturated fatty acids) to predict oxidative stability.

Results obtained in this work show that multivariate statistical evaluation of some olive oil chemical characteristics and quality criteria may be important in predicting the stability of this valuable natural product. However, additional research on a larger number of samples from other crop seasons and varieties is required to determine the effect of olive oil chemical components on oxidative stability, to develop a predictive equation for oxidative stability based on a few chemical compounds, and to study the relationship between oxidative stability and olive oil shelf life.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Spanish Comision Interministerial de Ciencia y Tecnología (CICYT) for supporting this research project ALI95-0197-CO2-02. Thanks are due to F. Alba, F. Gutierrez, and A. Cert (Instituto de la Grasa, Sevilla) for useful suggestions.

REFERENCES

1. Frankel, E.N., Chemistry to Autoxidation: Mechanism, Products and Flavor Significance, in *Flavor Chemistry of Fats and Oils,* edited by D.B. Min. and T.H. Smouse, American Oil Chemists' Society, Champaign, 1985, pp 1–37.
2. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* AOCS Press, Champaign, 1991, Method Cd 12-53.
3. Gutiérrez, F., Determinación de la Estabilidad Oxidativa de Aceites de Oliva Vírgenes: Comparación Entre el Método A.O.M. y el Método Rancimat, *Grasas Aceites 40:*1–5 (1989).
4. Cert, A., J. Alba, M. León-Camacho, W. Moreda, and M.C. Pérez-Camino, Effects of Talc Addition and Operating Mode on the Quality and Oxidative Stability of Virgin Oils Obtained by Centrifugation, *J. Agric. Food Chem. 44:*3930–3934 (1996).
5. Vazquez, A., C. Janer, and M.L. Janer, Polifenoles Naturales y Estabilidad del Aceite de Oliva, *Grasas Aceites 26:*14–18 (1975).
6. Gutierrez, R., C. Janer, M.L Janer, F. Gutierrez, and A. Vazquez, Relación Entre los Polifenoles y la Calidad y Estabilidad del Aceite de Oliva Virgen, *Ibid. 28:*101–106 (1977).
7. Gutfinger, T., Polyphenols in Olive Virgin Oils, *J. Am. Oil Chem. Soc. 58:*966–968 (1981).
8. Tsimidou, M., G. Papadopoulus, and D. Boskow, Phenolic Compounds and Stability of Virgin Olive Oil. Part I, *Food Chem. 45:*141–144 (1992).
9. Di Giovacchino, L., M. Solinas, and M. Miccoli, Aspetti Qualitative e Quantitative Delle Produzione Olearie Ottenute Dalla

Lavorazione Delle Olive con Differenti Sistemi di Estrazione, *Riv. Ital. Delle Sost. Grasse Vol. LXXI:*587–594 (1994).
10. Di Giovacchino, L., M. Solinas, and M. Miccoli, Effect of Extraction Systems on the Quality of Virgin Olive Oil, *J. Am. Oil Chem. Soc. 71:*1189–1194 (1994).
11. Angerosa, F., and L. Di Giovacchino, Natural Antioxidants of Virgin Oil Obtained by Two and Tri-phases Centrifugal Decanters, *Grasas Aceites 47:*247–254 (1996).
12. Baldioli, M., M. Servili, G. Perretti, and G.F. Montedoro, Antioxidant Activity of Tocopherols and Phenolic Compounds of Virgin Olive Oils, *J. Am. Oil Chem. Soc. 73:*1589–1593 (1996).
13. Papadopoulus, G., and D. Boskow, Antioxidant Effect of Natural Phenols on Olive Oil, *Ibid. 68:*669–671 (1991).
14. Montedoro, G.F., M. Servili, M. Baldioli, and E. Miniati, Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 1. Their Extraction, Separation and Quantitative and Semiquantitative Evaluation by HPLC, *J. Agric. Food Chem. 40:*1571–1576 (1992).
15. Montedoro, G.F., M. Servili, M. Baldioli, and E. Miniati, Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 2. Initial Characterization of the Hydrolyzable Fraction, *Ibid. 40:*1577–1580 (1992).
16. Montedoro, G.F., M. Servili, M. Baldioli, R. Selvaggi, E. Miniati, and A. Macchioni, Simple and Hydrolizable Compounds in Virgin Olive Oil. 3. Spectroscopic Characterization of the Secoiridoid Derivatives, *Ibid. 41:*2228–2234 (1993).
17. Gracian, J., and G. Arevalo, Adición de Tocoferoles a Aceites de Oliva Refinados con Vistas al Mejoramiento de su Estabilidad Oxidativa y de sus Cualidades Dietéticas, *Grasas Aceites 22:*177–184 (1971).
18. Karmal-Eldin, A., and L.A. Appelqvist, The Chemistry and Antioxidant Properties of the Tocopherols and Tocotrienols, *Lipids 31:*671–701 (1996).
19. Shmoulvich, V.G., Interrelation of Contents of Unsaturated Fatty Acid and Vitamin E in Food Product Lipids, *Appl. Biochem. Microbiol. 30:*547–551 (1994).
20. Papadopoulus, G., M. Tsimidou, and D. Boskow, Stability of Virgin Olive Oil: Assessment of Natural Antioxidants and Other Related Factors, in *Food Flavors, Ingredients and Composition,* edited by G. Charalambous, Elsevier Publishers, B.V., Amsterdam, 1993, pp. 321–326.
21. Maestro Duran, R., and R. Borja Padilla, Actividad Antioxidante de Esteroles y Ácidos Organicos Naturales, *Grasas Aceites 44:*208–212 (1993).
22. Boskow, D., and D. Morton, Changes in the Sterol Composition of Olive Oil on Heating, *J. Sci. Food Agric. 26:*1149–1153 (1975).
23. Williamson, E., The Antioxidant Activity of Δ5-Avenasterol, Ph.D. Thesis, University of Reading, United Kingdom, 1988.
24. Gutierrez, F., J. Garrido, L. Gallardo, B. Gandul, and M.I. Minguez, Action of Chlorophylls on the Stability of Virgin Olive Oil, *J. Am. Oil Chem. Soc. 69:*866–871 (1992).
25. Mínguez-Mosquera, M.I., B. Gandul, and J. Garrido, Pigments Present in Virgin Olive Oil, *Ibid. 67:*192–196 (1990).
26. Barranco, D., L. Rallo, M. Uceda, and M. Hermoso, Fichas Varietales del Olivo, Cornicabra. Unaproliva 1, nº3 (1994).
27. European Union Commission. Regulation EEC/2568/91 on the Characteristics of Olive and Olive Pomance Oils and Their Analytical Methods. Annex II: Determination of Acidity Value. Annex III: Determination of Peroxide Index. Annnex IX: Spectrophotometric Test of UV Absorption, *Off. J. Eur. Commun. L248:*6–36 (1991) Regulation EEC/1429/92 on the Modifications to the Regulation EEC/2568/91. *Ibid.* (1992).
28. Vázquez, A., C. Janer, and M.L. Janer, Determinación de los Polifenoles del Aceite de Oliva, *Grasas Aceites 24:*350–357 (1973).
29. Determination of Tocopherols and Tocotrienols in Vegetable Oils and Fats by HPLC, *Official Methods and Recommended Practices of the American Oil Chemists' Society,* American Oil Chemists' Society, Champaign, 1989, Official Method Ce 8-89.
30. Mínguez-Mosquera, M.I., L. Rejano, B. Gandul, A.H. Sanchez, and J. Garrido, Color-Pigment Correlation in Virgin Olive Oil, *J. Am. Oil Chem. Soc. 68:*322–337 (1991).
31. *Statistical Solutions,* Number Cruncher Statistical System (NCSS) 6.0, Cork, Ireland, 1997.
32. Salvador, M.D., F. Aranda, and G. Fregapane, Chemical Composition of Spanish "Cornicabra" Virgin Olive Oil from 1995/96 and 1996/97 Crops Extracted with Three Different Systems, *J. Am. Oil Chem. Soc. 75:*1305–1311 (1998).

[Received June 25, 1998; accepted November 29, 1998]