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Oil Stability Prediction by High-Resolution ^{13}C Nuclear Magnetic Resonance Spectroscopy

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^{13}C NMR spectra of oil fractions obtained chromatographically from 66 vegetable oils were obtained and analyzed to evaluate the potential use of those fractions in predicting oil stabilities and to compare those results with oil stability prediction by using chemical determinations. The oils included the following: virgin olive oils from different cultivars and regions of Europe and north Africa; "lampante" olive, refined olive, refined olive pomace, low-erucic rapeseed, high-oleic sunflower, corn, grapeseed, soybean, and sunflower oils. Oils were analyzed for fatty acid and triacylglycerol composition, as well as for phenol and tocopherol contents. By using stepwise linear regression analysis (SLRA), the chemical determinations and the ^{13}C NMR data that better explained the oil stability determined by the Rancimat were selected. These selected variables were related to both the susceptibility of the oil to be oxidized and the content of minor components that most contributed to oil stability. Because ^{13}C NMR considered many more variables than those determined by chemical analysis, the predicted stabilities calculated by using NMR data were always better than those obtained by using chemical determinations. All these results suggest that ^{13}C NMR may be a powerful tool to predict oil stabilities when applied to chromatographically enriched oil fractions.

KEYWORDS: Antioxidant activity; high-resolution ^{13}C NMR; oil analysis; oil characterization; oil stability; phenols; tocopherols; vegetable oils; virgin olive oil

INTRODUCTION

Oxidative rancidity is by far the most important complex of chemical reactions that limits the shelf life of oils (*1*, *2*). It is a great economic concern to the food industry because it leads to the development of various off-flavors and off-odors which render these foods unacceptable or reduce their shelf life. Therefore, for many decades these reactions have been extensively studied, and oxidative stability remains as an important parameter in evaluating the quality of fats and oils (*3*−*5*).

To estimate the susceptibility of an oil to oxidation, it is usually subjected to an accelerated oxidation test under standardized conditions so that the signs of deterioration are revealed within several hours or days. Examples of such tests are the Schaal test (oil maintained at 60 °C) and the Swift stability test (oil kept at 97.8 °C and aerated continuously) (*1*, *3*). The extent of oxidation is then measured by sensory and chemical tests such as peroxide value, ultraviolet absorption, or oxygen uptake, among others. More recently, special instruments have been developed for the automation of the Swift test, namely the Rancimat apparatus and the oil stability index (OSI) apparatus. These apparatuses have gained acceptance because they produce results in the form of induction periods much faster than in the case of the Schaal test. However, these methods have also drawbacks (*6*, *7*), and there is not a universally accepted methodology that produces reliable results in a short time period.

Because oil stability varies from one oil to another and depends on its triacylglycerol composition as well as on the presence of different minor components in the oils, different attempts have been carried out to quantify these components in the oils and to correlate these results with the stability of the corresponding oils. Thus, the presence of phenols (*8*−*12*), tocopherols (*13*, *14*), and other compounds (*15*−*17*) in the oils have been profusely studied. On the basis of these data, the prediction of oil stabilities should be suitable.

The major problem with these attempts is that oil stability does not depend exclusively on the concentration of one component. Thus, different components should be determined, some of them by means of procedures that are laborious and time-consuming. Therefore, there is a need to develop new analytical techniques that can afford acceptable results in a short time. One spectroscopic technique with a high potential in this field is high-resolution nuclear magnetic resonance (NMR) spectroscopy. Both ^{1}H and ^{13}C NMR allow the simultaneous determination of different compounds present in the oils (*18*−*20*). In addition, recent studies from this laboratory have allowed us to increase the potentiality of this technique by prior concentration of the minor components of the oils (*21*, *22*). This process is carried out by the chromatographic elimination of a significant proportion of the unmodified triacylglycerols. The

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Table 1. Fatty Acid Composition of Analyzed Oils[a]

	FA (%)						
oil group	16:0	16:1	18:0	18:1	18:2	18:3	others
virgin olive (36)	11.62 ± 2.16^{b}	0.73 ± 0.32^{b}	2.87 ± 0.51^{b}	76.10 ± 5.64^{b}	7.26 ± 3.93^{b}	0.64 ± 0.07^{b}	0.80 ± 0.17^{b}
olive (4)	10.78 ± 0.72^{b}	$0.69 \pm 0.13^{b,c}$	2.81 ± 0.22^{b}	76.36 ± 2.89^{b}	8.01 ± 2.32^{b}	0.60 ± 0.02^{b}	0.76 ± 0.18^{b}
olive pomace (6)	10.52 ± 1.91^{b}	$0.67 \pm 0.34^{b,c}$	2.83 ± 0.37^{b}	74.85 ± 1.31^{b}	$9.52 \pm 1.46^{b,d}$	0.52 ± 0.21^{b}	$1.09 \pm 0.14^{b,e}$
LE rapeseed (2)	4.95 ± 0.02^{c}	0.12 ± 0.12^{c}	1.82 ± 0.26^{c}	58.48 ± 2.50^{c}	23.55 ± 0.55^{c}	7.04 ± 2.27^{c}	4.05 ± 1.18^{c}
HO sunflower (3)	4.16 ± 0.14^{c}	0.09 ± 0.01^{c}	3.97 ± 0.13^{d}	75.71 ± 2.30^{b}	14.20 ± 2.05^{d}	0.06 ± 0.01^{b}	1.81 ± 0.09^{d}
corn (3)	10.62 ± 0.09^{b}	0.11 ± 0.01^{c}	2.02 ± 0.09^{c}	28.92 ± 0.50^{d}	56.55 ± 0.59^{e}	0.73 ± 0.05^{b}	$1.06 \pm 0.07^{b,e}$
grapeseed (3)	6.60 ± 0.20^{c}	0.05 ± 0.04^{c}	4.17 ± 0.27^{d}	18.77 ± 2.66^{e}	69.40 ± 3.06^{f}	0.23 ± 0.05^{b}	0.77 ± 0.42^{b}
soybean (4)	11.43 ± 0.23^{b}	0.07 ± 0.04^{c}	3.99 ± 0.27^{d}	$22.44 \pm 1.46^{d,e}$	55.14 ± 1.17^{e}	5.56 ± 0.90^{d}	$1.37 \pm 0.15^{d,e}$
sunflower (5)	6.66 ± 0.35^{c}	0.12 ± 0.02^{c}	4.32 ± 0.45^{d}	28.11 ± 2.45^{d}	59.09 ± 2.75^{e}	0.07 ± 0.01^{b}	1.62 ± 0.24^{d}

[a] Data are mean values ± SD. The number of oils in each group is indicated in parentheses after group name. Abbreviations: FA, fatty acid; HO, high-oleic; LE, low-erucic. [b−f]Means in the same column with different superscripts are significantly different ($p < 0.05$).

chromatographic fraction obtained in this way contains many polar components that contribute to oil stability. As a continuation of those studies, the present investigation was undertaken to study the application of ^{13}C NMR spectroscopy to the prediction of oil stabilities.

MATERIALS AND METHODS

Materials. Sixty-six samples were analyzed in this study. These included 36 virgin olive oils from different different cultivars and regions of Europe and north Africa (specifically Spain, Italy, Greece, and Tunisia), 1 "lampante" olive oil, 3 refined olive oils, 6 refined olive pomace oils, 2 low-erucic rapeseed oils, 3 high-oleic sunflower oils, 3 corn oils, 3 grapeseed oils, 4 soybean oils, and 5 sunflower oils. Most of the samples were obtained from our Institute's experimental oil mill (Instituto de la Grasa, Seville, Spain), the Institute's Department of Analysis, the Institute's Pilot Plant, or Koipe S. A. (Andujar, Jaén, Spain). In addition, some of the refined oils were prepared and refined in our laboratory using a laboratory-scale apparatus described previously by Dobarganes et al. (*23*). This procedure included degumming with phosphoric acid, neutralization with sodium hydroxide, bleaching with bleaching earth (Trisyl) for 10 min at 90 °C, and deodorization under vacuum (1 mm) at 250 °C for 3 h.

Oil Fractionation. Triplicate samples of the oils were fractionated by column chromatography using 19 g of silica gel 60 (particle size 0.063−0.200 mm) as absorbent, which was obtained from Merck (Darmstadt, Germany) and used without any previous treatment. The column was prepared in the elution solvent (hexane/diethyl ether, 87:13). The oil (6 g) was dissolved in 10 mL of the same solvent and introduced into the column. Most nonpolar compounds, including a significant portion of the triacylglycerols, were eluted with 100 mL of the elution solvent and were discarded. The oil fraction, containing polar compounds as well as a small part of triacylglycerols and other nonpolar compounds, was then eluted with 100 mL of acetone.

Oil Analysis. Oils were analyzed to determine their stabilities and for fatty acid and triacylglycerol composition, as well as for the presence of some components with recognized antioxidant activity (i.e., phenols and tocopherols). Oil stability was determined by the Rancimat method (Metrohm Co., Basel, Switzerland) at 110 °C. A continuous air stream (15 L/h) was passed through the heated sample and the volatiles were absorbed in a conductivity cell. Conductivities were continuously monitored until a sudden rise signified the end of the induction period (IP). IPs were determined (in hours) by the method of tangents to the two parts of the kinetic curve (*15*). Fatty acid composition was determined by capillary gas chromatography using the fatty acid methyl esters obtained by oil saponification and esterification (*24*). Triacylglycerol composition was determined by gas chromatography (*25*). Phenols were extracted by solid-phase extraction chromatography (*26*) and determined with the Folin−Ciocalteau reagent (*27*). Results are expressed as tyrosol equivalents. Tocopherols were determined by high-performance liquid chromatography with fluorimetric detection (*28*).

NMR Spectroscopy. ^{13}C NMR spectroscopy was performed on a Bruker AC 300P (Bruker Instruments, Inc., Karlsruhe, Germany) operating at 75.4 MHz. The oil fractions obtained by column chromatography as described above were evaporated, dissolved in 700 μL of $CDCl_3$ (containing 0.03% vol/vol tetramethylsilane), introduced into a 5-mm NMR tube, and acquired as described previously (*22*). The concentration of the solutions acquired was 0.17−2.47 mg/μL (*21*). Free induction decays were transformed by using absolute intensity, and chemical shifts were related to the signal for tetramethylsilane (δ 0 ppm). The solvent $CDCl_3$ was used as the internal standard for height intensity and to correct for small changes in field homogeneity. A total of 135 peaks at the same chemical shifts/positions were selected, and peak heights were recorded for use in the data analysis of intensity patterns. The recorded intensities for each oil were collected in a matrix, with each row containing all 135 peaks of one spectrum. This matrix also included oil stability, triacylglycerol composition, fatty acid composition, phenol content, and tocopherol content, determined as described above. The values used in the data analysis were the average of the three replicates obtained for each oil. No further preprocessing of the data was performed.

Data Analysis. Statistical data analysis was performed with the SPSS for Windows (version 11.0.1) statistical package (SPSS Inc., Chicago, IL). Analytical results are expressed as mean values ± SD. Statistical comparisons between two groups were made using Student's t test. With several groups, ANOVA was used. When significant F values were obtained, group differences were evaluated by the Student−Newman−Keuls test (*29*). Significance level was $p < 0.05$ unless otherwise indicated. Stepwise linear regression analysis (SLRA) was applied to the data matrix, prepared as described above, to select the variables (chemical determinations or NMR signals) that better explained the oil stability determined by the Rancimat.

RESULTS

Oil Analysis. Oils were analyzed to determine their stabilities and for fatty acid and triacylglycerol composition, as well as for the presence of some components with recognized antioxidant activity (i.e., phenols and tocopherols). Fatty acid compositions of the oil groups analyzed are reported collectively in **Table 1**. As expected, virgin olive, olive, olive pomace, low-erucic rapeseed, and high-oleic sunflower oils had oleic acid as the major fatty acid. On the contrary, the major fatty acid in corn, grapeseed, soybean, and sunflower oils was linoleic acid. The relative amounts of other fatty acids depended on the oil analyzed. Thus, olive oils had important amounts of palmitic and linoleic acids, high-oleic sunflower oil was rich in linoleic acid, and low-erucic rapeseed was rich in linoleic and linolenic acids. Soybean oils were also rich in linolenic acid, and had also important amounts of oleic and palmitic acids. Corn, grapeseed, and sunflower oils were also rich in oleic and palmitic acids.

These results were confirmed when triacylglycerol composition was determined (**Table 2**). Thus, virgin olive, olive, olive pomace, and high-oleic sunflower oils had OOO as the main

Table 2. Triacylglycerol Composition of Analyzed Oils[a]

	TAG (%)								
oil group	POO	POL	PLL	SOO	OOO	OOL	OLL	LLL	others
virgin olive	28.07 ± 1.86^{b}	$5.66 \pm 2.91^{b,c}$	1.53 ± 0.77^{b}	5.24 ± 1.14^{b}	41.80 ± 6.69^{b}	5.78 ± 2.42^{b}	2.67 ± 0.70^{b}	1.81 ± 0.40^{b}	7.44 ± 2.13^{b}
olive	27.49 ± 0.48^{b}	$5.33 \pm 1.43^{b,c}$	1.00 ± 0.13^{b}	5.05 ± 0.53^{b}	42.23 ± 3.80^{b}	6.64 ± 1.88^{b}	3.31 ± 0.42^{b}	2.24 ± 0.09^{b}	6.73 ± 0.87^{b}
olive pomace	27.29 ± 3.17^{b}	$6.14 \pm 0.79^{b,c}$	2.00 ± 0.61^{b}	5.07 ± 0.54^{b}	40.41 ± 4.16^{b}	7.52 ± 1.68^{b}	2.64 ± 0.79^{b}	1.90 ± 0.19^{b}	7.02 ± 1.73^{b}
LE rapeseed	$9.13 \pm 0.01^{c,d}$	$6.68 \pm 0.42^{b,c,d}$	3.81 ± 0.57^{c}	2.56 ± 0.30^{c}	34.07 ± 1.96^{b}	21.41 ± 0.47^{c}	14.01 ± 0.61^{c}	6.95 ± 0.44^{c}	1.40 ± 0.26^{c}
HO sunflower	11.83 ± 0.28^{c}	2.07 ± 0.22^{b}	1.31 ± 0.33^{b}	8.91 ± 0.18^{d}	60.28 ± 0.75^{c}	5.56 ± 0.72^{b}	5.08 ± 0.70^{d}	3.26 ± 1.06^{b}	1.71 ± 0.34^{c}
corn	$7.14 \pm 0.47^{d,f}$	14.90 ± 0.58^{e}	15.69 ± 0.33^{d}	1.18 ± 0.11^{c}	5.94 ± 0.18^{d}	15.62 ± 0.07^{d}	19.91 ± 0.87^{e}	13.87 ± 1.22^{d}	5.77 ± 0.54^{b}
grapeseed	2.49 ± 0.28^{e}	$9.37 \pm 0.59^{c,d}$	16.79 ± 2.05^{d}	1.70 ± 0.54^{c}	3.69 ± 1.64^{d}	7.93 ± 1.61^{b}	22.29 ± 1.75^{f}	26.04 ± 3.62^{e}	$9.68 \pm 0.24^{b,d}$
soybean	$5.06 \pm 0.27^{e,f}$	14.37 ± 0.63^{e}	18.42 ± 0.84^{e}	1.88 ± 0.26^{c}	4.26 ± 0.60^{d}	9.19 ± 0.62^{b}	17.32 ± 0.45^{g}	17.64 ± 0.64^{f}	11.87 ± 0.56^{d}
sunflower	3.94 ± 0.49^{e}	10.78 ± 0.94^{d}	11.66 ± 1.26^{f}	2.02 ± 0.26^{c}	6.85 ± 1.24^{d}	14.41 ± 1.24^{d}	25.09 ± 1.34^{h}	16.34 ± 1.60^{g}	8.92 ± 1.12^{b}

[a] Data are mean values ± SD. The number of oils in each group is indicated in **Table 1**. Abbreviations: HO, high-oleic; LE, low-erucic; TAG, triacylglycerol. Fatty acid residues in TAG: L, linoleic; O, oleic; P, palmitic; S, stearic. [b−h]Means in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3. Stability and Phenol and Tocopherol Contents of Analyzed Oils[a]

oil group	stability (h)	phenol (ppm)	α-tocopherol (ppm)	β-tocopherol (ppm)	γ-tocopherol (ppm)	δ-tocopherol (ppm)
virgin olive	23.4	156.0	196.8	3.3	16.0	0.0
	(43.0–1.6)	(409.4–29.0)	(371.3–1.0)	(46.3–0.0)	(33.6–0.0)	(0.2–0.0)
olive	9.7	45.2	114.4	1.2	13.3	0.0
	(13.0–6.3)	(70.4–33.0)	(222.1–6.7)	(2.4–0.0)	(14.6–12.0)	(0.0–0.0)
olive pomace	13.5	41.0	219.6	15.0	10.0	0.0
	(23.5–6.0)	(56.9–29.1)	(519.1–1.9)	(35.0–0.0)	(24.5–0.0)	(0.0–0.0)
LE rapeseed	4.7	40.6	212.2	0.0	375.4	18.0
	(8.0–1.3)	(44.5–36.6)	(387.4–36.9)	(0.0–0.0)	(468.7–282.0)	(26.3–9.8)
HO sunflower	14.7	22.9	926.5	37.5	0.0	0.0
	(16.6–13.0)	(24.4–20.0)	(974.9–878.1)	(40.6–34.4)	(0.0–0.0)	(0.0–0.0)
corn	9.2	42.2	322.1	12.8	1042.8	49.9
	(9.5–9.0)	(55.0–30.1)	(369.4–252.0)	(14.0–10.8)	(1154.8–830.5)	(64.9–33.9)
grapeseed	4.4	26.5	439.2	35.6	23.2	0.0
	(5.3–3.3)	(31.6–23.4)	(713.0–299.5)	(54.6–20.2)	(29.5–18.3)	(0.0–0.0)
soybean	5.2	24.6	124.9	21.9	833.4	392.1
	(6.0–4.6)	(28.3–23.0)	(188.3–79.0)	(28.2–16.1)	(1010.7–671.0)	(437.0–307.7)
sunflower	3.0	23.2	845.7	40.2	6.4	0.1
	(5.3–1.2)	(27.0–19.5)	(977.6–743.5)	(51.2–34.2)	(11.8–4.5)	(0.4–0.0)

[a] Data are mean values and the range is indicated in parentheses. The number of oils in each group is shown in **Table 1**. Abbreviations: HO, high-oleic; LE, low-erucic.

triacylglycerol, followed by POO. Low-erucic rapeseed oil also had OOO as the main triacylglycerol, but as a consequence of their relatively high content of linoleic acid (**Table 1**), OOL and OLL were the following two major triacylglycerols. High linoleic oils showed a different triacylglycerol pattern, and LLL, OLL, OOL, POL, and PLL were major triacylglycerols in these oils.

Differently from fatty acid and triacylglycerol compositions, which were relatively homogeneous for the studied oil groups, stability and phenol and tocopherol contents depended much more on the estate of the individual oils. For this reason the obtained results have been summarized indicating means and ranges in **Table 3**. As expected, the highest stabilities usually corresponded to virgin olive oils, which have a low content of polyunsaturated fatty acids and significant amounts of the natural antioxidants analyzed. Other oils had different stabilities that depended on fatty acid composition and on the amount of natural antioxidants.

Virgin olive oils had the highest phenol contents because they were the only crude oils studied. It is well-known that phenol losses occur during the refining process (*27*), and, therefore, refined vegetable oils usually had less than 50 ppm phenols (expressed as tyrosol equivalents), according to the previously described data for vegetable oils (*30*). Although all refined vegetable oils had low phenol values, the highest values were obtained for low-erucic rapeseed and corn oils, according to the data found by HPLC (*31*).

Tocopherol values also depended on the estate of single samples, although some tendencies were also observed among the different oil groups assayed. Thus, sunflower oils were very rich in α-tocopherol, whereas γ-tocopherol was the major tocopherol in low-erucic rapeseed, corn, and soybean oils. δ-Tocopherol was not very common, although it was high in soybean oils. All these data are also in agreement with previously reported composition data of edible oils (*32*).

^{13}C NMR Spectra of Oil Fractions. ^{13}C NMR spectra of oil fractions were much more complex than those obtained for complete oils. They exhibited carbonyl carbons between 177.8 and 172.8 ppm (signals P1−P13), olefinic carbons between 141.0 and 121.0 ppm (signals P14−P29), glycerol carbons between 72.1 and 61.0 ppm (signals P33−P44), and aliphatic carbons between 58.0 and 11.8 ppm (signals P45−P135). These signals corresponded to the carbon atoms of the different components present in the isolated fractions: triacylglycerols, polymeric triacylglycerols, oxidized triacylglycerols, diacylglycerols, monoacylglycerols, and free fatty acids, as well as the various minor polar components of the oils (sterols, fatty alcohols, phenols, etc.). A detailed analysis of obtained spectra and the assignments of the corresponding signals was described previously (*22*). A selected list of the signals of interest in the present study, along with their corresponding assignments, is presented in **Table 4**.

Prediction of Oil Stabilities by Using Their Fatty Acid and Triacylglycerol Composition Data and Their Phenol and Tocopherol Contents. By using SLRA it was possible to select the chemical determinations that better explained the oil stability determined by the Rancimat. The summary of the model is presented in **Table 5**. When applied to the 66 oils analyzed,

Table 4. Chemical Shifts and Assignments of ^{13}C NMR Signals Selected by SLRA

signal	δ (ppm)	assignment[a]	signal	δ (ppm)	assignment[a]	signal	δ (ppm)	assignment[a]
P1	177.75	CA (FFA1)	P46	57.30	UK	P85	31.48	UK
P5	173.80	CA (1,2DAG1α)	P52	52.25	UK	P102	27.79	UK
P16	132.17	OL	P56	48.03	UK	P103	27.33	UK
P18	129.97	OL (O10; L9)	P58	45.76	ST	P117	22.69	Oω2; Sω2
P19	129.81	OL	P65	39.32	UK	P122	21.06	ST
P34	72.04	GL (1,2DAG2)	P72	36.13	STchain	P128	18.99	ST
P35	71.69	ST3, HD	P74	35.66	UK	P130	18.20	UK
P37	69.96	UK	P78	34.06	S2α	P131	14.26	UK
P38	68.85	GL (TAG2)	P79	34.00	O2α; L2α; ST	P134	11.94	ST
P40	64.99	GL (1,3DAG1/3; 1MAG3)	P80	31.93	Sω3			
P42	62.17	GL (1,2DAG3; 2MAG1/3)	P82	31.86	ST			

[a] Assignments are abbreviated with carbon type followed by compound and carbon number, if known. Abbreviations: CA, carbonyl; DAG, diacylglycerol; FFA, free fatty acid; GL, glycerol; HD, hydroxyderivative; L, linoleic; MAG, monoacylglycerol; O, oleic; OL, olefinic; S, saturated; ST, sterol; TAG, triacylglycerol; UK, unknown. Greek letter ω is used when carbons are numbered beginning by the methyl end. Greek letter α is used for positions 1 and 3 in triacylglycerols.

Table 5. Summary of SLRA Applied to the Chemical Determinations[a]

all analyzed oils				only virgin olive oils			
model	selected variables	correlation	typical error	model	selected variables	correlation	typical error
1	Ph	0.685	9.38626	1	OOL	0.621	10.12684
2	Ph, POL	0.769	8.31267	2	OOL, αT	0.816	7.58486
3	Ph, POL, OOL	0.787	8.09086	3	OOL, αT, PLL	0.863	6.72616
4	Ph, POL, OOL, γT	0.805	7.84367	4	OOL, αT, PLL, Ph	0.883	6.34834
5	Ph, POL, OOL, γT, αT	0.828	7.48522				
6	Ph, POL, OOL, γT, αT, P	0.851	7.07847				
7	Ph, POL, γT, αT, P	0.843	7.17867				
8	Ph, POL, γT, αT, P, PLL	0.857	6.94202				

[a] Abbreviations: Ph, phenols; αT, α-tocopherol; γT, γ-tocopherol. Fatty acids and fatty acid residues in TAG: L, linoleic; O, oleic; P, palmitic; S, stearic.

stability could be predicted ($r = 0.857$, $p < 0.0001$) by using phenol (Ph), α-, and γ-tocopherol (αT and γT, respectively), palmitic (P), and POL and PLL contents. As expected, the predictive variables were related both to the fatty acid composition of the oils and the content of natural antioxidants. The regression equation was

$$\text{predicted stability} = -0.496 + 6.02\cdot10^{-2}\cdot\text{Ph} - 3.707\cdot\text{POL} + 1.48\cdot10^{-2}\cdot\gamma\text{T} + 1.97\cdot10^{-2}\cdot\alpha\text{T} + 2.549\cdot\text{P} + 0.782\cdot\text{PLL}$$

Figure 1A shows the plot of the stabilities calculated for the 66 analyzed oil samples against the stabilities determined by the Rancimat.

When SLRA was applied only to the virgin olive oil group, the correlation was better ($r = 0.883$, $p < 0.0001$) and less variables were selected. In fact, the predictive variables were phenol, α-tocopherol, OOL, and PLL contents. The regression equation was

$$\text{predicted stability} = 20.466 - 2.118\cdot\text{OOL} + 8.58\cdot10^{-2}\cdot\alpha\text{T} - 4.253\cdot\text{PLL} + 2.97\cdot10^{-2}\cdot\text{Ph}$$

Figure 1B shows the plot of the stabilities calculated for the 36 analyzed virgin olive oil samples against the stabilities determined by the Rancimat.

Prediction of Oil Stabilities by Using ^{13}C NMR Data of the Chromatographically Obtained Oil Fractions. Analogously to chemical determinations, by using SLRA it was possible to select the ^{13}C NMR signals that better explained the oil stability determined by the Rancimat. The model is summarized in **Table 6**. When applied to the 66 oils analyzed, stability could be predicted ($r = 0.914$, $p < 0.0001$) by using intensities of P1, P18, P52, P56, P78, P79, P82, and P103 signals. The regression equation was

$$\text{predicted stability} = 32.782 - 7.293\cdot\text{P79} + 94.805\cdot\text{P52} - 5.925\cdot\text{P1} + 2.837\cdot\text{P18} - 4.898\cdot\text{P78} - 54.839\cdot\text{P56} - 4.522\cdot\text{P82} + 5.382\cdot\text{P103}$$

Figure 2A shows the plot of the stabilities calculated for the 66 analyzed oil samples against the stabilities determined by the Rancimat.

When SLRA was applied only to the virgin olive oil group, the correlation was better ($r = 0.99991$, $p < 0.0001$), and oil stability could be predicted by using intensities of P5, P16, P19, P34, P35, P37, P42, P46, P56, P58, P65, P72, P74, P79, P80, P85, P102, P117, P122, P128, P130, P131, and P134 signals (model no. 29). The regression equation was

$$\text{predicted stability} = 61.169 - 11.729\cdot\text{P79} - 6.191\cdot\text{P80} - 57.091\cdot\text{P122} + 18.290\cdot\text{P128} - 57.804\cdot\text{P134} + 52.949\cdot\text{P35} - 26.623\cdot\text{P102} - 12.841\cdot\text{P130} - 36.454\cdot\text{P37} - 44.716\cdot\text{P46} + 0.611\cdot\text{P117} + 31.360\cdot\text{P58} - 26.846\cdot\text{P65} + 30.970\cdot\text{P19} - 30.902\cdot\text{P56} + 30.639\cdot\text{P131} - 29.788\cdot\text{P16} + 19.962\cdot\text{P72} - 3.636\cdot\text{P5} + 3.232\cdot\text{P34} - 3.386\cdot\text{P42} - 1.870\cdot\text{P85} + 5.081\cdot\text{P74}$$

Nevertheless, very good correlations were also attained with fewer signals (**Table 6**). **Figure 2B** shows the plot of the stabilities calculated using the model no. 29 (**Table 6**) for the 36 analyzed virgin olive oil samples against the stabilities determined by the Rancimat.

DISCUSSION

High-resolution NMR is considered among the most powerful techniques yet described for the analysis of vegetable oils (*18*–

Table 6. Summary of SLRA Applied to the NMR Data[a]

all analyzed oils				only virgin olive oils			
model	selected variables	correlation	typical error	model	selected variables	correlation	typical error
1	P79	0.715	8.78899	1	P79	0.781	7.98543
2	P79, P40	0.816	7.32278	2	P79, P80	0.852	6.80835
3	P79, P40, P52	0.840	6.93453	3	P79, P80, P38	0.895	5.87169
4	P79, P40, P52, P1	0.858	6.60384	4	P79, P80, P38, P122	0.929	4.96248
5	P79, P40, P52, P1, P18	0.877	6.22603	5	P79, P80, P38, P122, P128	0.941	4.62455
6	P79, P40, P52, P1, P18, P78	0.892	5.90633	6	P79, P80, P38, P122, P128, P134	0.951	4.26491
7	P79, P52, P1, P18, P78	0.891	5.89795	7	P79, P80, P38, P122, P128, P134, P35	0.960	3.92739
8	P79, P52, P1, P18, P78, P56	0.899	5.72045	8	P79, P80, P38, P122, P128, P134, P35, P102	0.968	3.60287
9	P79, P52, P1, P18, P78, P56, P82	0.907	5.55670	9	P79, P80, P38, P122, P128, P134, P35, P102, P130	0.973	3.37186
10	P79, P52, P1, P18, P78, P56, P82, P103	0.914	5.40419	10	P79, P80, P38, P122, P128, P134, P35, P102, P130, P37	0.980	2.98043
				11	P79, P80, P38, P122, P128, P134, P35, P102, P130, P37, P46	0.985	2.65521
				12	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46	0.984	2.66223
				13	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117	0.988	2.37926
				14	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58	0.990	2.15759
				15	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65	0.993	1.90889
				16	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19	0.995	1.68766
				17	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P78	0.996	1.45583
				18	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P78, P56	0.997	1.22331
				19	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P78, P56, P131	0.998	1.04240
				20	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P78, P56, P131, P16	0.999	0.8993
				21	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16	0.999	0.91372
				22	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124	0.999	0.69863
				23	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124, P72	0.999	0.60821
				24	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124, P72, P5	1.000	0.52853
				25	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124, P72, P5, P34	1.000	0.43666
				26	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124, P72, P5, P34, P42	1.000	0.38532
				27	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124, P72, P5, P34, P42, P85	1.000	0.33665
				28	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P124, P72, P5, P34, P42, P85, P74	1.000	0.28360
				29	P79, P80, P122, P128, P134, P35, P102, P130, P37, P46, P117, P58, P65, P19, P56, P131, P16, P72, P5, P34, P42, P85, P74	1.000	0.29576

[a] Chemical shifts and assignments of variables are given in **Table 4**.

22, *33*). Thus, it has been used for determination of oil genuineness, quality, and geographical and varietal origin. Nevertheless, it had not been yet employed for oil stability prediction, more likely as a consequence of the difficulty of extracting information of the minor components present in the oils which are playing a major role in oil stability. The above results show, however, that, if minor components in the oils are concentrated, ^{13}C NMR is a powerful tool to determine oil stability.

Predicting oil stabilities with a good accuracy always needs the determination of fatty acid composition as well as the content of the various oil components which contribute to oil stability. SLRA allowed selection of those components that better explained the observed stability. Thus, when SLRA was applied to the 66 analyzed oils, the chemical determinations selected were phenol, α- and γ-tocopherol, palmitic acid, and POL and PLL contents. These variables defined the susceptibility of the oil to be oxidized and the minor components that most contributed to oil stability. Surprisingly, linolenic acid content, which is considered the most easily oxidizable fatty acid, was not selected. This is a consequence of the many factors that contribute to oil stability, and, thus, the oils with the highest linolenic acid contents (low-erucic rapeseed and soybean oils) did not exhibit the lowest observed stabilities.

Oil stabilities in virgin olive oils were also well predicted and, analogously to the broad sample size described above, the susceptibilities of triacylglycerols to be oxidized (OOL and PLL contents) and the presence of natural antioxidants in virgin olive oils (phenol and α-tocopherol contents) explained the observed stabilities.

Analogous conclusions were also obtained when SLRA was applied to ^{13}C NMR data. Thus, the variables selected were related to fatty acid composition (for example, P18, which is related to the C-10 in oleic acid and C-9 in linoleic acid; P78, which is related to C-2 of saturated fatty acids in the position α of triacylglycerols; or P79, which is related to C-2 of oleic

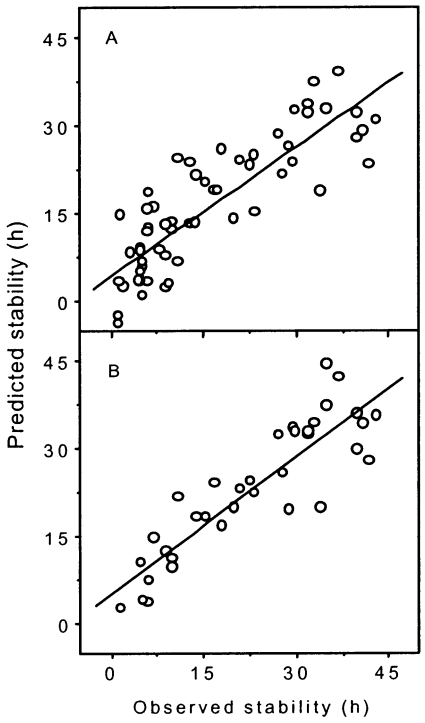

Figure 1. Plot of observed Rancimat stabilities against stabilities predicted by SLRA using the chemical determinations obtained from (A) the 66 oils analyzed in this study and (B) the 36 virgin olive oils analyzed.

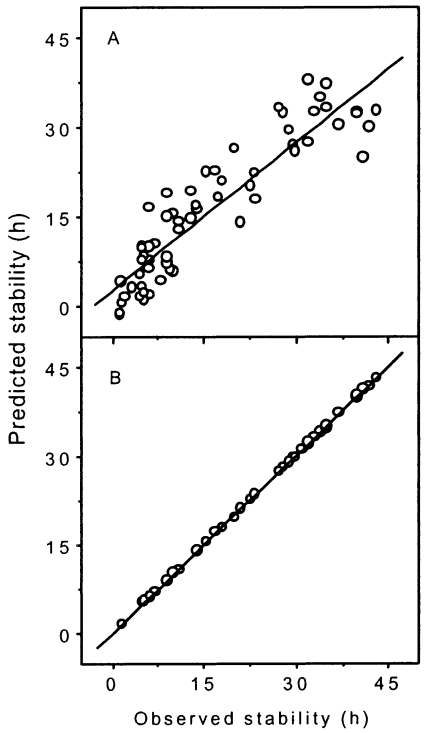

Figure 2. Plot of observed Rancimat stabilities against stabilities predicted by SLRA using the ^{13}C NMR data obtained from (A) the 66 oils analyzed in this study and (B) the 36 virgin olive oils analyzed.

and linoleic acids in the position α of triacylglycerols) (**Table 4**), as well as to the presence of minor components in the oils with antioxidant characteristics (for example, P79 and P82 are related to the sterol content in the oils). In addition, SLRA also selected other variables that should also play a role in oil stability, some of which have been assigned (P1, for example, which is related to free fatty acid content) and others, for which assignments are unknown at present (P52, P56, and P103). Because ^{13}C NMR considers many more variables than those determined by chemical analysis, the predicted stabilities calculated by using NMR data were always better than those obtained by using chemical determinations.

Analogous results were obtained when oil stabilities of the virgin olive oil group were predicted by using ^{13}C NMR data. In this case, the predicted oil stabilities did not differ from those determined experimentally, and these results were much better than any other attempt previously carried out in the literature to predict oil stabilities on the basis of chemical analyses and with independence of the number of variables considered (*34*, *35*). All these results suggest that ^{13}C NMR may be a powerful tool to predict oil stabilities when applied to chromatographically enriched oil fractions.

LITERATURE CITED

(1) Kristott, J. Fats and oils. In *The Stability and Shelf life of Food*; Kilcast, D., Subramaniam, P., Eds.; CRC Press: Boca Raton, FL, 2000; pp 279−309.

(2) Nawar, W. W. Lipids. In *Food Chemistry*, 3rd edition; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 225−319.

(3) Warner, K., Eskin, N. A. M., Eds. *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*. AOCS Press: Champaign, IL, 1995.

(4) Frankel, E. N. *Lipid Oxidation*. The Oily Press: Dundee, UK, 1998.

(5) Min, D. B.; Lee, H. O. Chemistry of lipid oxidation. 30 years of progress. In *Flavor Chemistry*; Teranishi, R., Wick, E. L., Hornstein, I., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 175−187.

(6) Frankel, E. N. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci. Technol.* **1993**, *4*, 220−225.

(7) Frankel, E. N.; Meyer, A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925−1941.

(8) Tovar, M. J.; Motilva, M. J.; Romero, M. P. Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *J. Agric. Food Chem.* **2001**, *49*, 5502−5508.

(9) García, J. M.; Yousfi, K.; Mateos, R.; Olmo, M.; Cert, A. Reduction of oil bitterness by heating of olive (*Olea europaea*) fruits. *J. Agric. Food Chem.* **2001**, *49*, 4231−4235.

(10) Silva, F. A. M.; Borges, F.; Ferreira, M. A. Effects of phenolic propyl esters on the oxidative stability of refined sunflower oil. *J. Agric. Food Chem.* **2001**, *49*, 3936−3941.

(11) Briante, R.; Cara, F. I.; Tonziello, M. P.; Febbraio, F.; Nucci, R. Antioxidant activity of the main bioactive derivatives from oleuropein hydrolysis by hyperthermophilic β-glycosidase. *J. Agric. Food Chem.* **2001**, *49*, 3198−3203.

(12) Brenes, M.; Hidalgo, F. J.; García, A.; Ríos, J. J.; García, P.; Zamora, R.; Garrido, A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oils. *J. Am. Oil Chem. Soc.* **2000**, *77*, 715−720.

(13) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532−2538.

(14) Kulas, E.; Ackman, R. G. Different tocopherols and the relationship between two methods for determination of primary oxidation products in fish oil. *J. Agric. Food Chem.* **2001**, *49*, 1724−1729.

(15) Gutiérrez, F.; Fernández, J. L. Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of "extra" quality. *J. Agric. Food Chem.* **2002**, *50*, 571−577.

(16) Zamora, R.; Alaiz, M.; Hidalgo, F. J. Influence of cultivar and fruit ripening on olive (*Olea europaea*) fruit protein content, composition, and antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4267−4270.
(17) Psomiadou, E.; Tsimidou, M. On the role of squalene in olive oil stability. *J. Agric. Food Chem.* **1999**, *47*, 4025−4032.
(18) Sacchi, R. High-resolution NMR of virgin olive oil. In *Magnetic Resonance in Food Science. A View to the Future*; Webb, G. A., Belton, P. S., Gil, A. M., Delgadillo, I., Eds.; Royal Society of Chemistry: Cambridge, UK, 2001; pp 213−226.
(19) Mannina, L.; Patumi, M.; Proietti, N.; Bassi, D.; Segre, A. L. Geographical characterization of Italian extra virgin olive oils using high-field ^{1}H NMR spectroscopy. *J. Agric. Food Chem.* **2001**, *49*, 2687−2696.
(20) Spyros, A.; Dais, P. Application of ^{31}P NMR spectroscopy in food analysis. I. Quantitative determination of the mono- and diglyceride composition of olive oils. *J. Agric. Food Chem.* **2000**, *48*, 802−805.
(21) Zamora, R.; Gómez, G.; Dobarganes, M. C.; Hidalgo, F. J. Oil fractionation as a preliminary step in the characterization of vegetable oils by high-resolution ^{13}C NMR spectroscopy. *J. Am. Oil Chem. Soc.* **2002**, *79*, 261−266.
(22) Zamora, R.; Gómez, G.; Hidalgo, F. J. Classification of vegetable oils by high-resolution ^{13}C NMR spectroscopy using chromatographically obtained oil fractions. *J. Am. Oil Chem. Soc.* **2002**, *79*, 267−272.
(23) Dobarganes, M. C.; Pérez-Camino, M. C.; Márquez-Ruiz, G.; Ruiz-Méndez, M. V. New analytical possibilities in quality evaluation of refined oils. In *Edible Fats and Oils Processing: Basic Principles and Modern Practices*; Eriksson, D. R., Ed.; American Oil Chemists' Society: Champaign, IL, 1990; pp 427−429.
(24) American Oil Chemists' Society. *Official Methods and Recommended Practices*, 5th ed.; AOCS: Champaign, IL, 1999; method Ce 1e-91.
(25) Carelli, A. A.; Cert, A. Comparative study of the determination of triacylglycerol in vegetable oils using chromatographic techniques. *J. Chromatogr.* **1993**, *630*, 213−222.
(26) Pirisi, F. M.; Cabras, P.; Cao, C. F.; Migliorini, M.; Muggelli, M. Phenolic compounds in virgin olive oil. 2. Reappraisal of the extraction, HPLC separation and quantification procedures. *J. Agric. Food Chem.* **2000**, *48*, 1191−1196.
(27) Satue, M. T.; Huang, S.-W.; Frankel, E. N. Effect of natural antioxidants in virgin olive oil on oxidative stability of refined, bleached, and deodorized olive oil. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1131−1137.
(28) American Oil Chemists' Society. *Official Methods and Recommended Practices*, 5th ed.; AOCS: Champaign, IL, 1999; method Ce 8-89.
(29) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 7th ed.; Iowa State University Press: Ames, IA, 1980.
(30) Zambiazi, R. C.; Przybylski, R. Effect of endogenous minor components on the oxidative stability of vegetable oils. *Lipid Technol.* **1998**, *10*, 58−62.
(31) Bernwieser, I.; Sontag, G. Determination of phenolic acids in vegetable oils using HPLC coupled with multielectrode detection. *Z. Lebensm.-Unters. Forsch.* **1992**, *195*, 559−561.
(32) Bockisch, M. *Fats and Oils Handbook*; AOCS Press: Champaign, IL, 1998; p 112.
(33) Vlahov, G. Application of NMR to the study of olive oils. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *35*, 341−357.
(34) Aparicio, R.; Roda, L.; Albi, M. A.; Gutiérrez, F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.
(35) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. Antioxidant activity of tocopherols and phenolic compounds in virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

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