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Quantitation of the Main Constituents of Some Authentic Grape-Seed Oils of Different Origin

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This paper describes the composition of 30 grape-seed oils obtained from France, Italy, and Spain during 2002−2003. Oils were extracted from the seeds using small-scale industrial solvent extraction equipment and analyzed in their unrefined state using standard methods for fatty acids, fatty acids in the triacylglycerol 2-position, tocopherols and tocotrienols, triglycerides, sterols, steradienes, and iodine value. Values for the composition of the sterols, triglycerides, fatty acids, iodine value, and tocopherol composition were generally in good agreement with the results of previous similar surveys. Steradienes (stigmastadiene, campestadiene, stigmastatriene, and campestatriene) were detected in the oil and were probably formed from sterols during the extraction process.

KEYWORDS: Grape-seed oil; *Vitis vinifera*; fatty acids; triacylglycerol 2-position; tocopherols; tocotrienols; triacylglycerols; sterols; steradienes; iodine value

INTRODUCTION

Grape-seed oil is obtained from the seeds left following pressing of the juice from grapes for wine making. These contain only 6−20 wt % of oil, which is usually extracted with solvent and refined before use. World production of grapes totaled just over 60 000 000 metric tons in 2003, with France, Italy, and Spain being the major world grape producers each providing just over 10% of the total (*1*), but it is not clear what proportion of the seeds from these crops was used in oil manufacture. Oil extraction is becoming increasingly popular as a means of using this waste material from the wine industry, and the oil is gaining in popularity for culinary and other purposes.

We have recently reported the results of surveys of authentic hazelnut and walnut oils obtained from nuts and seeds collected in the major-producing countries (*2*, *3*). This paper describes the results for a study of grape-seed oils obtained and analyzed under similar conditions. The survey reported covers a wide range of analytes in samples collected from France, Spain, and Italy, countries which supply the bulk of the grape seeds for oil production in Europe. The samples were collected as seeds at the source, and the oil was extracted in the U.K. A full audit trail was established to ensure the authenticity of the samples. Oil was extracted under conditions very similar to those used in industry and analyzed using official methods with full quality assurance procedures.

This report updates data reported in a previous comprehensive survey (*4*) and is based on a larger number of samples from each country with country of origin authenticity assured by collection by the authors. The information supports and updates the data available in the Codex standard (*5*).

Most earlier surveys of grape-seed oil composition are limited and based on laboratory extraction of oil and chromatography on packed columns. Most have concentrated on the fatty acid (FA) composition (*6*−*14*), and many describe only the principal FA (*6*, *7*, *9*, *12*), analyzed without official or validated methods (*7*−*9*, *11*−*13*). The data show that the content of linoleic acid is high (usually 70−75%) and that of saturated acids low.

Earlier reports of the sterol composition (*15*, *16*) are considerably more limited in terms of sample numbers, range of sterols, and quality assurance. Very little more information is available regarding the content of tocopherols and tocotrienols (*4*, *17*−*20*). The major tocol is γ-tocotrienol (*4*, *19*, *20*), often present at over 500 mg/kg although the levels are extremely variable. The single paper dedicated to tocopherols in grape-seed oil (*18*) provides data for only a single sample. One publication (*19*) shows α-tocopherol levels to more than double on refining while those of the coeluted γ-tocopherol and β-tocotrienol decrease by the same factor.

Data regarding other components such as 2-position FA and triacylglycerols are much more limited (*4*).

This work presents the first collection of comprehensive data derived from statistically significant numbers of samples, extracted using procedures used in the oil industry, and analyzed using modern official methods with comprehensive quality assurance procedures. This publication provides a reliable database for use in dietary studies and authenticity testing.

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MATERIALS AND METHODS

Grape Seeds. Grape seeds were collected by the authors directly from wine producers in France, Italy, and Spain. Two of the samples from France and one from Italy were collected from the year 2000 harvest and the remainder from the year 2001 harvest. Samples were obtained from the French regions Gironde (5 samples), Vallon Pont d'Arc (2), and Macon, Aude, and Loire Atlantique (1 each); from the Italian regions Faenza (8 samples) and Petralia (Sicily, 2); and from the Spanish regions Catalunya (3 samples), Castilla-La Mancha (2), Navarra (2), and Zaragoza, Aragon, and Comunidad Valenciana (1 each).

As grape seeds were not available at the vineyards where the grapes were grown, they were collected from wine-making sites. It could not be assured that each batch of seeds was of the same variety or from a single vineyard, and in some cases it was not possible to confirm the grape color. Some samples were mixtures from both red and white grapes. All seeds were in good condition on delivery although some contained residues assumed to be dried pulp or skin.

The seeds were packed on collection in secure heavy-duty polyethylene containers and sealed with numbered tamper-proof plastic tags with a unique number that was used at all stages from collection through analysis, allowing a detailed audit trail to be established. They were stored at 4 °C prior to transport to the extraction plant. The oil was extracted from the grape seeds by solvent extraction with hexane and stored in polypropylene bottles under nitrogen at 4 °C prior to analysis. The oil was not bleached, deodorized, or otherwise refined. Normal cleaning procedures were carried out between samples to ensure that there was no adventitious mixing of different samples.

Standards. Standards of the sterols stigmasterol (95%) and sitosterol (containing campesterol), and of fatty acid methyl esters, were obtained from Sigma, Poole, U.K. The steradienes stigmasta-3,5-diene (69%) with campesta-3,5-diene (13%) and stigmasta-3,5,22-triene (97%) were obtained from Chiron AS, Trondheim, Norway. The internal standard cholesta-3,5-diene (95%) was obtained from Sigma. Tocopherols and tocotrienols (>95%) were obtained from Merck Biosciences, Schwalbach, Germany. The CBR reference sample RM162 (soya and maize oil) was purchased from the Community Bureau of Reference, Brussels, Belgium.

Determination of Composition. Analytical methods used for the determination of sterols (demethylsterols), steradienes, FA, iodine value, the composition of FA in the triacylglycerol (TAG) 2-position, triacylglycerol carbon number (TCN), and tocopherols and tocotrienols were as described previously (*2*, *3*).

RESULTS AND DISCUSSION

Oil Extraction. The industry solvent extraction process was not necessarily exhaustive, and therefore it was concluded that the amount of oil in the grape seeds could not be quantified. Solvent extraction left about 1% of residual hexane in the oil, but this level would have negligible effect on the determination of the composition of the oil.

Quality Assurance. For the data reported all results for the replicate injection and analysis samples were within the warning limits of Shewart control charts and all results for the reference materials were within specified limits.

Sterol Composition. Limited tables for the fatty acid, demethylsterol, and tocopherol content of grape-seed oils are provided in the current Codex standard (*5*).

The sterol composition of grape-seed oils expressed in milligrams per 100 g of oil is given in **Table 1**. The total levels range from 258 to 1125 mg/100 g with an average value of 571 mg/100 g. These values are in reasonable agreement with those published elsewhere (*4*, *15*). In agreement with recent (*4*) workers we measured a wide range of sitostanol in all samples, reports of the presence of this sterol being absent from most earlier surveys, possibly through coelution on less efficient chromatographic systems.

Table 1. Sterol Composition of Grape-Seed Oils (mg/100 g)

	10 samples each from		
	France	Italy	Spain
cholesterol	0.9–5.1	0.8–4.8	1.2–4.7
cholestanol	nd[a]	nd	nd
brassicasterol	nd	nd	nd
24-methylene-cholesterol	0.5–4.2	0.2–4.2	0.4–2.2
campesterol	27.0–94.4	26.3–84.1	34.6–67.0
campestanol	nd–3.5	nd–3.3	1.1–2.8
stigmasterol	34.5–96.1	28.5–92.0	43.6–75.9
Δ7-campesterol	0.7–3.4	nd–3.7	0.5–3.8
Δ5,23-stigmasta-dienol	nd–4.2	0.9–5.6	1.2–3.8
clerosterol	1.9–8.5	1.7–6.1	2.4–5.6
β-sitosterol	208.7–823.5	172.3–682.6	316.3–632.2
sitostanol	8.6–40.4	9.3–35.3	15.0–39.2
Δ5-avenasterol	1.4–19.3	4.6–21.4	2.8–4.9
Δ5,24-stigmasta-dienol	nd–5.3	nd–3.3	1.1–4.7
Δ7-stigmastenol	1.7–19.3	2.9–37.1	5.1–10.0
Δ7-avenasterol	nd–6.9	nd–8.1	nd–2.3
total	323.3–1125.1	257.6–961.2	444.9–849.2

[a] nd = less than 0.1.

Table 2. Sterol Composition of Grape-Seed Oils (%)

	sample		
	France	Italy	Spain
cholesterol	0.2–0.8	0.1–1.0	0.3–0.7
cholestanol	nd[a]	nd	nd
brassicasterol	nd	nd	nd
24-methylenecholesterol	0.1–0.5	0.1–0.9	0.1–0.4
campesterol	8.2–10.1	6.6–10.2	7.6–9.0
campestanol	nd–0.5	nd–0.4	0.2–0.4
stigmasterol	7.9–11.3	5.4–11.0	7.0–12.3
Δ7-campesterol	0.1–0.4	nd–1.0	0.1–0.4
Δ5,23-stigmastadienol	nd–0.8	0.3–0.8	0.2–0.5
clerosterol	0.5–1.0	0.4–0.6	0.5–0.8
β-sitosterol	68.9–76.0	66.9–74.7	69.7–77.4
sitostanol	2.4–4.7	3.1–4.4	3.5–4.8
Δ5-avenasterol	0.3–2.1	1.0–4.5	0.4–1.0
Δ5,24-stigmastadienol	nd–1.2	nd–0.8	0.1–1.0
Δ7-stigmastenol	0.4–3.2	0.7–4.3	0.8–2.1
Δ7-avenasterol	nd–0.6	0.2–1.6	nd–0.3

[a] nd = less than 0.1.

Table 3. Steradiene Composition of Grape-Seed Oils (mg/kg)

	sample		
	France	Italy	Spain
campestadiene	nd[a]–0.35	nd–0.44	0.03–4.48
stigmastadiene	0.75–1.85	nd–3.9	0.14–6.69

[a] nd = less than 0.05.

The sterol composition of grape-seed oils expressed as percentage of total sterols is given in **Table 2**.

Values for the sterols' percent composition were generally in good agreement with the Codex standard (*5*) and results published elsewhere (*4*, *15*, *16*). In contradiction to one earlier survey (*4*) we found no brassicasterol. Slightly higher proportions of campesterol (10−13%) have been reported in five Italian oils (*16*).

Steradiene Composition. The steradiene composition of the grape-seed oils is given in **Table 3**.

Stigmastadiene and campestadiene were detected in almost all of the grape-seed oils, at levels up to 6.69 mg/kg. The 3,5-

Table 4. Fatty Acid Composition of Grape-Seed Oils (%)

	France	Italy	Spain
myristic acid C14:0	nd[a]–0.1	0.1	nd–0.1
pentadecanoic acid C15:0	nd	nd	nd
palmitic acid C16:0	6.6–8.4	7.9–9.6	7.1–11.6
palmitoleic acid C16:1	0.1–0.2	0.1–0.2	0.1
margaric acid C17:0	nd–0.1	0.1	0.1
heptadecenoic acid C17:1	nd	nd	nd
stearic acid C18:0	3.5–4.5	3.9–4.6	3.5–5.4
oleic acid C18:1	14.0–17.6	17.2–20.9	16.2–20.0
linoleic acid C18:2	69.3–74.6	63.1–69.0	61.3–70.2
linolenic acid C18:3	0.4–1.8	0.4–0.8	0.3–0.6
arachidic acid C20:0	0.1–0.9	0.2–1.7	0.2–0.4
eicosenoic acid C20:1	nd–0.4	nd–0.4	0. 2
behenic acid C22:0	0.1–0.5	nd–0.4	nd–0.5
docosenoic acid C22:1	nd–0.1	nd	nd
lignoceric acid C24:0	nd–0.1	nd–0.3	nd–0.3

[a] nd = less than 0.1.

stigmastadiene levels were frequently above the limit of 0.15 mg/kg acceptable for extra virgin olive oil according to EC regulations (*21*). There have been no previous reports of the analysis of grape-seed oils for sterol degradation products. Although steradiene formation is usually ascribed to acid-earth bleaching or deodorization, it has been established that such compounds can be formed simply on heating olive oil (*22*) or on heat drying the solid residues used to prepare olive pomace oils (*23*). The steradienes found in the grape-seed oil might have been formed either during heating of the seeds to dry them, during the solvent extraction, or during a filtration stage. The levels are unlikely to be related to the variety, type, or origin of the grape. It is of interest that the proportion of 3,5-campestadiene to 3,5-stigmastadiene is somewhat higher than the proportions of the parent sterols (campesterol and sitosterol). This might indicate that the relative rates of formation or loss differ from those encountered during bleaching.

Fatty Acid Composition. The fatty acid composition of the grape-seed oils is given in **Table 4**.

The results fell within similar ranges to the literature reports (*6*, *9*−*13*); however, others have reported values both higher and lower than these ranges. Thus five samples of unspecified origin had higher 16:1 (0.4−0.5%) and lower C18:3 (0.2−0.3%) and C20:1 (0.1%) (*16*). Three varieties of Egyptian oils had higher values for C16:0 (9.6−11.9%) and C18:1 (19.4−24.6%) with low C18:2 (61.3−61.6%) and with C18:3 not detected (*9*). Ten varieties from Sicily had considerably higher proportions of C14:0 (1.6−8.0%) and C16:0 above 10% in seven samples (average 11.2%) (*8*). Fourteen varieties from Romania had generally lower levels of C16 (5.9−8.3%,average 6.8%), C18:0 (2.7−4.1%, average 3.4%), 18:1 (10.7−16.0%, average 13.6%), and C18:3 (not detected) but higher levels of C18:2 (71.1−76.1%, average 74.5%) (*7*).

The ranges of C18:3 and C20:0 were widened by one sample of oil from French seeds that had values considerably higher than the remaining samples (C18:3 at 1.8% and C20:0 at 0.9% compared with C18:3 at 0.4−1.0% and C20:0 at 0.12−0.3%), these results being confirmed by reanalysis.

Iodine Value. The mean calculated iodine values of grape-seed oils ranged from 124 to 143. For France the range was 136−143, for Italy 129−136, and for Spain 124−138. The results were comparable to those reported elsewhere (*4*, *9*, *10*) with a slightly narrower range.

Fatty Acid in the Triacylglycerol 2-Position Composition. The triacylglycerol 2-position fatty acid composition of grape-seed oils is given in **Table 5**.

Table 5. Triacylglycerol 2-Position Fatty Acid Composition of Grape-Seed Oils (%)

	France	Italy	Spain
myristic acid C14:0	nd[a]	nd–0.1	nd–0.1
palmitic acid C16:0	0.5–2.4	0.6–2.2	1.2–3.1
palmitoleic acid C16:1	0.1–0.2	0.1–0.2	0.1–0.2
margaric acid C17:0	nd–1.0	nd–0.1	nd–0.7
stearic acid C18:0	0.2–1.2	0.3–1.0	0.5–1.6
oleic acid C18:1	20.4–32.1	24.9–30.9	21.0–31.3
linoleic acid C18:2	62.9–75.7	64.6–72.8	63.4–76.0
linolenic acid C18:3	0.3–3.2	0.3–2.8	0.2–1.4
arachidic acid C20:0	nd	nd–0.1	nd–0.1
eicosenoic acid C20:1	nd	nd–0.1	nd
erucic acid	nd	nd–0.3	nd
nervonic acid	nd	nd–0.1	nd
others	nd–1.6	nd–2.0	nd–0.7

[a] nd = less than 0.1.

Table 6. Triacylglycerol Carbon Number (TCN) Composition of Grape-Seed Oils (%)

	France	Italy	Spain
TCN 48	nd[a]–0.5	nd–0.6	nd–0.3
TCN 50	0.7–2.6	1.3–3.7	1.1–1.9
TCN 52	19.3–23.9	20.7–27.1	21.0–23.7
TCN 54	73.9–78.9	68.1–77.0	73.9–77.1
TCN 56	nd–1.7	nd–1.5	0.7–1.5
TCN 58	nd	nd	nd
TCN 60	nd	nd	nd

[a] nd = less than 0.1.

The values are in good agreement with those reported for a range of grape-seed oils (*15*), but three oils from three varieties of Egyptian grape seeds contained considerably higher levels of C16:0 (9.6−12.8%) and C18:0 (4.5−6.2%) and lower levels of C18:2 (61.2−61.6%) (*24*). Combined levels of C16:0 and C18:0 in most of the samples exceeded the national limit of 1.5% established in Italy for grape-seed oil (*25*). However, this limit was equally exceeded by 10 of 15 grape-seed oils studied previously (*4*), indicating the need for comprehensive surveys to provide accurate databases for the setting of meaningful limits.

Triacylglycerol Carbon Number Composition. The triacylglycerol carbon number composition (TCN) of the grape-seed oils is given in **Table 6**.

The results for most triglycerides match the profiles reported elsewhere (*4*, *26*, *27*). The proportion of TCN48 is theoretically too high for the proportion of C16 fatty acids determined, and some of the response may be due to coeluted steryl ester. Very similar ranges of both C16 fatty acids and TCN48 have been reported elsewhere (*4*).

Tocopherol Composition. The tocopherol composition of grape-seed oils is given in **Table 7**.

The total tocopherol content ranged from 63 to 1208 mg/kg, considerably wider than the Codex range (240−410 mg/kg) and similar to some literature values (*4*) but considerably lower than four samples from Germany that contained over 2500 mg/kg (*17*, *18*). Levels of the individual tocopherols were extraordinarily wide ranging, typically from undetectable to over 100 mg/kg regardless of country. Thus in 12 of the 30 samples the major tocopherol was the α-isomer, in 10 samples it was the *β*-isomer, in another 6 it was *γ*-tocopherol, and in 2 it was the Δ-isomer. This variation was observed in the previous major survey (*4*) but otherwise made the comparison of these data with the published literature (*17*, *19*, *20*) of limited value, particularly as the literature reports describe very few samples.

Table 7. Tocopherols and Tocotrienol Composition of Grape-Seed Oils (mg/kg)

	France	Italy	Spain
α-tocopherol	18–229	14–160	nd[a]–75
β-tocopherol	nd–109	nd–133	nd–127
γ-tocopherol	nd–61	nd–119	nd–168
Δ-tocopherol	nd–47	nd	nd–69
α-tocotrienol	nd–163	nd–352	nd–60
β-tocotrienol	nd–67	nd–22	nd–125
γ-tocotrienol	nd–500	nd–785	nd–399
Δ-tocotrienol	nd	nd	nd–82
total	127–706	63–1208	240–542

[a] nd = less than 10 mg/kg.

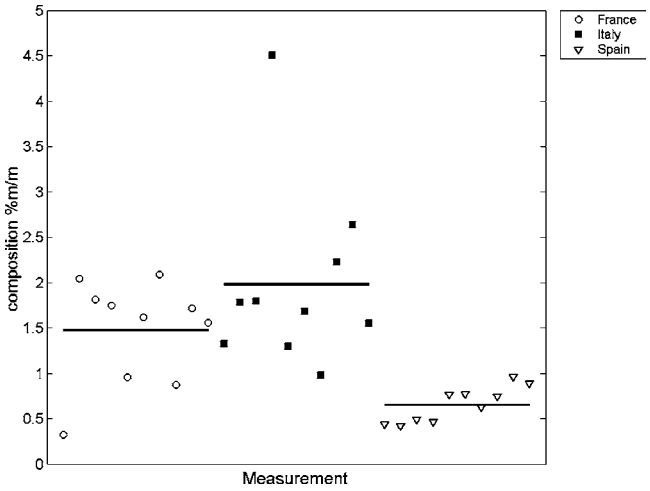

Figure 1. ANOVA of values for Δ5-avenasterol data.

Of the tocotrienols the γ-isomer predominated, often at levels in excess of the tocopherols. However, there was again a substantial variation, and in fact single samples from France and Italy and three samples from Spain did not contain any detectable γ-tocotrienol, the Spanish samples having relatively high levels (92−125 mg/kg) of β-tocotrienol.

Country of Origin. The data were examined using the CSL-Metabolab visualization tool as described previously (*2*) to identify differences between samples from the three countries and also between oils from the seeds of red and white grapes. However, as the samples were mixtures from different vineyards and the varieties not known, the significance of these analyses is reduced.

ANOVA was able to distinguish between oils from Spanish and Italian grape seeds based on data for Δ5-avenasterol (**Figure 1**) and for French and Italian oils based on the C18:2 fatty acid data (**Figure 2**).

Univariate statistics indicated Δ5-avenasterol, Δ7-avenasterol, fatty acids C16:0 and C16:1, 2-position fatty acids C18:1 and C18:2, and triacylglycerol CN52 as having greater than 99% significance for indicating grape-seed country of origin.

Analysis was made using an ANOVA on all data, looking for cases where at least one country had a significant difference, and using pairwise *t*-tests for all pairs of countries and all data, looking for differences between individual pairs of countries. The most significant result was obtained for Δ7-avenasterol that separated most Italian samples from French and Spanish ones (**Figure 3**).

The same approach was unable to identify factors with greater than 99% significance for grape-seed color, but cholesterol, Δ7-campesterol, and γ-tocotrienol had greater than 95% significance. ANOVA applied to γ-tocotrienol gave almost complete separation of red and white grape-seed oils **(Figure 4**), but this information would probably be lost in refined oils.

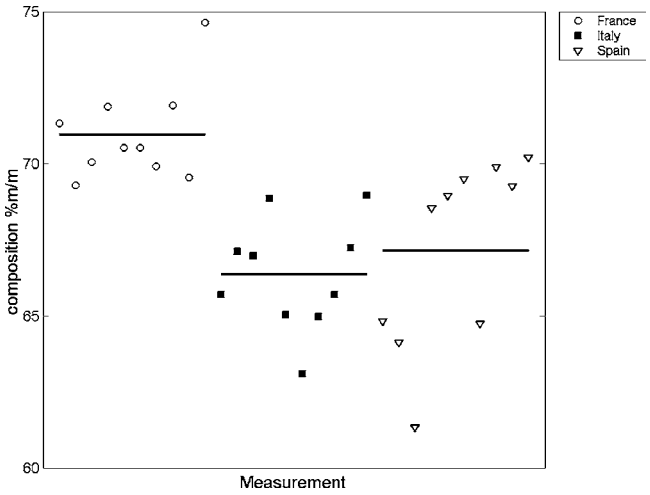

Figure 2. ANOVA of values for C18:2 fatty acid data.

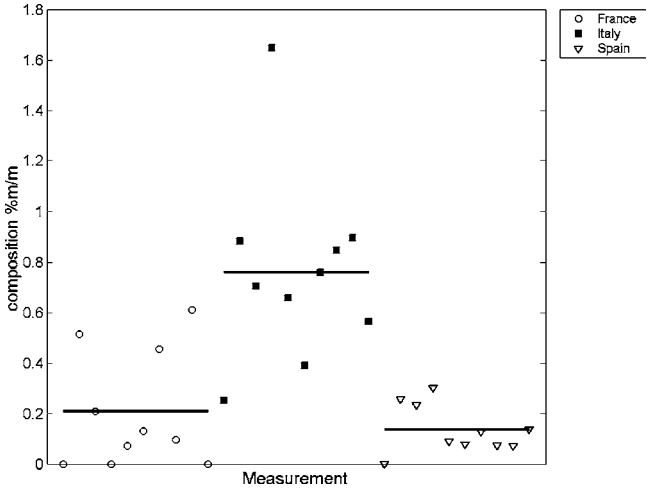

Figure 3. ANOVA of values for Δ7-avenasterol data.

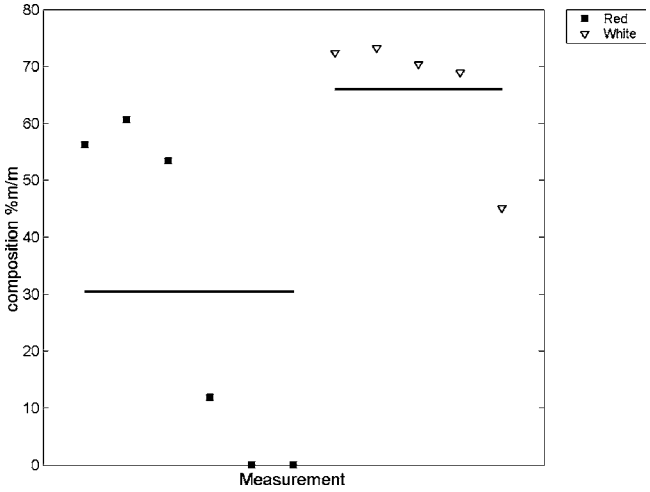

Figure 4. ANOVA of values for γ-tocotrienol data.

Figure 4 ANOVA of Values for γ-Tocotrienol Data. A multivariate principal components analysis was performed using all variables. The data were both mean centered and scaled to unit variance, and 10 PC scores were calculated, but the analysis was unable to distinguish oils effectively between the countries of origin or the seed colors.

Factors Affecting the Composition of Grape-Seed Oil. Several factors contribute to measured changes in composition of vegetable oils. The oil composition is likely to have been affected by variety, growing location, maturity, and the composition of the soil. Grape-seed oil composition can be changed by, for example, drying and tannin-removal operations (*20*). It was not possible to identify the grape varieties or precise areas of origin because the seeds were by necessity collected from winemakers and not the vineyards where they were grown. It is likely that several of the samples analyzed comprised mixtures of seeds from the region described, as was evidenced by the mixture of grape colors in some samples.

Handling of the grape seeds prior to collection could have affected their composition. The times between harvest of the seeds and collection prior to dispatch to the laboratory were not available. Although delays between collection and transfer to the laboratory were minimized, some delays were encountered during the oil extraction operations.

The oil was analyzed as it was obtained from the extraction plant, although it would normally be refined before sale for food use. The poor yield from the seeds did not provide sufficient oil for the industrial refining procedure, and it was felt that this processing could not be reproduced in the laboratory. The results have not been adjusted to account for any residual solvent in the grape-seed oil as the "dilution" of the oil by solvent residues would not have any significant effect on any of the results as reported.

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