Determination of the Diglyceride Content in Greek Virgin Olive Oils and Some Commercial Olive Oils by Employing $^{31}$P NMR Spectroscopy

Penelope Fronimaki, Apostolos Spyros, Stella Christophoridou, and Photis Dais*

NMR Laboratory, Department of Chemistry, University of Crete, 714 09 Iraklion, Crete, Greece

In this study, the diglyceride contents of 96 samples of virgin olive oils from the regions of Crete, Lesvos, Messinia, Pilion, Zakynthos, Halkidiki, and Iria, 15 samples of commercial extra virgin and pure olive oils, and 3 samples each of refined olive oils and pomace oils were determined by a facile method introduced in a previous publication. This method is based on the phosphorylation of the free hydroxyls of the diglycerides with 2-chloro-4,4,5,5-tetramethyldioxaphospholane and the integration of the appropriate peaks in the $^{31}$P NMR spectra. This preliminary study showed interesting trends in the diglyceride content of the virgin olive oils from the various regions of Greece that can be used as simple criteria to assess the olive oil characteristics. Analysis of variance has been carried out for the diglyceride content of each region in an attempt to detect possible differences in the diglyceride levels among the various regions. Finally, the relationship between the ratio of 1,2-diglycerides to the total amount of diglycerides and the total amount of diglycerides has been used to monitor the quality of virgin olive oils, commercial olive oils, refined olive oils, and pomace oils.

KEYWORDS: Diglycerides; virgin olive oils; phosphitylation; $^{31}$P NMR

INTRODUCTION

Diacylglycerols, usually termed diglycerides (DGs), are minor constituents of virgin olive oils accompanying the major triacylglycerol or triglyceride (TG) components. They are found as 1,2-diglycerides (1,2-DGs) and 1,3-diglycerides (1,3-DGs). Originally, 1,2-DGs arise from the incomplete biosynthesis of triacylglycerols (T), whereas a second source of 1,2-DG formation is the limited enzymatic hydrolysis (lipolysis) of TGs (2). On the other hand, 1,3-DGs are considered to be secondary products resulting from the isomerization of 1,2-DGs during the extraction process and continued during the storage of the olive oil (3–6). Therefore, freshly made virgin olive oil from healthy olive fruits are expected to contain almost solely 1,2-DGs, the concentration of which decreases during storage while the 1,3-DG content and the total DG concentration increase. In this respect, the concentration levels of both 1,2-DGs and 1,3-DGs may be indicative of the olive oil freshness. From these facts, it has been suggested (3, 4, 7) that the ratio of 1,3-DGs to 1,2-DGs and the ratio of 1,2-DGs to the total amount of diglycerides [$D = 1,2$-DGs/(1,2-DGs + 1,3-DGs)] are useful indices to assess the age and quality of olive oils. Although no official regulations have been established regarding the DG content of the various olive oil grades, fresh extra virgin olive oils of the same olive variety are expected to have the lowest ratio 1,3-DGs/1,2-DGs and the highest value for the parameter $D$.

The natural content of DGs in fresh virgin olive oils does not exceed 1–3% depending on the olive fruit ripeness and olive fruit variety (3, 4, 7, 8). In refined olive oils, the level of diglycerides (mainly 1,3-DGs) is higher (4–5%), and it goes up to 15–20% in pomace oils (3, 4, 6, 9). Also, larger amounts of DGs are obtained in neutralized oils produced from starting materials with high levels of free fatty acids or when they are extracted with solvents from olive husks and then refined through industrial processes. In this respect, the content of 1,2-DGs and 1,3-DGs can provide a good discrimination between virgin olive oils and low-quality olive oils.

The quantitative determination of diglycerides in olive oils has been carried out by using several chromatographic techniques, such as gas chromatography (10, 11), high-performance size exclusion chromatography (12, 13), and high-performance liquid chromatography (14). In recent years, high-resolution $^1$H and $^{13}$C NMR spectroscopy have been applied effectively for the characterization of mono- and diglycerides in olive oil samples (4, 7, 8, 15, 16).

In a previous publication (17) we have introduced a facile NMR method to determine the mono- and diglyceride composition in olive oils. This method is based on the derivatization of the labile hydrogens of the hydroxyl groups of the diglycerides and/or monoglycerides with 2-chloro-4,4,5,5-tetramethyldioxaphospholane (I) according to the reaction shown in Scheme 1 and the use of the $^{31}$P chemical shifts to identify the labile centers. Compound I reacts rapidly and quantitatively under mild conditions with the hydroxyl groups.

This analytical approach will be used in the present study to detect and quantify the diglyceride content of a large number...
of virgin olive oil samples from different regions of Greece and additional samples of commercial olive oils, refined olive oils, and pomace oils. The diglyceride content determined in these classes of edible oils proves to be a useful index to detect virgin olive oil quality.

MATERIALS AND METHODS

All solvents were of analytical grade and were purchased from Aldrich. The derivatizing reagent I was synthesized from pinacol and phosphorus trichloride in the presence of triethylamine following the method described in the literature (18).

Olive Oil Samples. The virgin olive oil (VOO) samples were obtained from four regions of Crete (Sitia, Kolimbari, Iraklion, and Peza) and the regions of Lesvos, Zakynthos, Messinia, Ilia, Halkidiki, and Pilion. The VOO samples were provided by the local cooperatives and produced according to the same method of extraction (centrifugation). Virgin olive oils were extracted within 48 h after harvesting and stored immediately at ~20 °C. The majority of the VOO samples considered in this study came from the same olive variety (Koroneiki). A few samples were derived from different varieties of olive fruits (Kolovi and Adramitiani from Lesvos, green olives from Halkidiki, and a local variety from Pilion). The oil samples were collected during the periods 2000—2001. Additional olive oil samples were collected from the four regions of Crete during the period of 1999—2000. The date of extraction of the olive oils was different in the various locations (November to February). All of the Greek olive oils were virgin according to the official analytical methods and limits (19, 20). Commercial olive oils were purchased from supermarkets. The commercial olive oils are classified according to their labels as extra virgin olive oils (EVOO) and pure olive oils (POO). Refined olive oils and pomace oils were obtained from a small local company.

Sample Preparation. A stock solution (10 mL) composed of pyridine and CDCl₃ in a 1.6:1.0 volume ratio containing 0.6 mg of pomace oils were obtained from a small local company. Olive oils (EVOO) and pure olive oils (POO). Refined olive oils and Commercial olive oils were purchased from supermarkets. The commercial olive oil samples was placed in a 5 mm NMR tube. The required volume of the stock solution (0.4 mL) and the reagent (15 μL) were added. The reaction mixture was left to react for ~0.5 h at room temperature. Upon completion of the reaction, the solution was used to obtain the 31P NMR spectra.

NMR Experiments. 31P NMR spectra were obtained on a Bruker AMX500 spectrometer operating at 202.6 MHz for the phosphorus-31 nucleus at room temperature. To neglect NOE effects, the inverse gated decoupling technique was used. Typical spectral parameters for quantitative studies were as follows: 90° pulse width, 12.5 μs; sweep width, 10 kHz; relaxation delay, 30 s; memory size, 16K (zero-filled to 32K). Line broadening of 1 Hz was applied, and drift correction of transients, 64.

RESULTS AND DISCUSSION

NMR Method. 1H NMR spectroscopy and 13C NMR spectroscopy have been used previously to detect DGs in olive oils (4, 7, 8, 15, 16). Although these magnetic resonance methods are quantitative and do not require any pretreatment of the samples, they are not so effective as 31P NMR spectroscopy under certain circumstances. The unambiguous detection and quantification of the DG resonances using 1H NMR require spectrometers operating at very high magnetic field strengths (≥14.1 T or 600 MHz in terms of the proton Larmor frequency). At lower magnetic field strengths, the DG resonances are overlapped by the strong resonances of the triglycerides (15, 16), thus calling into question the ability of this magnetic resonance method to provide a reliable quantitative determination of these minor components at lower magnetic field strengths. On the other hand, the large range of chemical shifts (~800 ppm) reported for the 31P nucleus ensures a good separation of the diglyceride signals. This is shown in Figure 1, which depicts the 1H and 31P NMR spectra of an olive oil sample at two magnetic field strengths. The excellent resolution between the 31P chemical shifts permits a reliable detection of the phosphitylated mono- and diglycerides, even at a magnetic field strength of 9.4 T or 162.1 MHz (Figure 1b). No monoglyceride resonances appear in the proton spectrum, and an extensive overlap of the diglyceride resonances with those of the strong α,α′ proton resonances of the triglycerides and their 13C satellites is observed even at 11.7 T or 500 MHz (Figure 1c). The situation becomes worse at even lower magnetic field strengths (compare panels c and d of Figure 1).

Apart from the wide range of the 31P chemical shifts, the 100% natural abundance of the 31P nucleus and its high sensitivity, which is only ~15 times less than that of the proton nucleus, make the 31P NMR experiments a reliable analytical tool to determine very low amounts of the order of micromoles. These properties of the 31P nucleus should be contrasted with the low natural abundance and sensitivity of the 13C nucleus, which in addition is characterized by long relaxation times. Thus, quantitative 13C NMR experiments require lengthy accumulations and long relaxation delays to achieve a satisfactory signal-to-noise ratio. It is worth mentioning that an overnight 13C NMR experiment performed for an olive oil sample gave a value of 0.77 for the ratio D, which is in excellent agreement with the value of 0.78 obtained from the 31P NMR experiment in 20 min. This result, also, shows that no decomposition or isomerization of the diglycerides occurs during the NMR experiment.

Another advantage of the 31P NMR method of analysis relative to the proton and carbon NMR experiments is the introduction of an internal standard, usually cyclohexanol, in the reaction mixture. The presence of the standard of known amount allows the determination of the absolute concentration of the phosphitylated product II (Scheme 1), avoiding thus normalization conditions.

Apart from the diglyceride signals, the 31P NMR spectrum in Figure 1 shows additional peaks owing to 1-monoglyceride and total sterols (STE). The peak denoted CH belongs to the internal standard cyclohexanol.
Diglycerides in Virgin Olive Oils

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Figure 1. Expansions of the diglyceride region of (a) 202.6 MHz and (b) 162.1 MHz 31P NMR spectra and (c) 500 MHz and (d) 400 MHz 1H NMR spectra of a virgin olive oil sample. The 31P NMR spectra show peaks that belong to 1-monoglyceride and total sterols (STE). The peak denoted CH is due to the internal standard cyclohexanol. In the proton spectrum the peaks are assigned as follows: 1, CH; 2, α-CH₂; 5, α′-CH₂ of 1,2-diglyceride; 3, CH; 4, CH₂ of 1,3-diglyceride; *, 13C satellites of triglyceride signals.

Analysis of the Olive Oil Samples. The integrals of the appropriate resonances in the 31P NMR spectra of the olive oil samples at 202.6 MHz (Figure 1) were used to determine quantitatively the amounts of 1,2-DGs, 1,3-DGs, and total DGs and the ratio D. The results of such an analysis for the virgin olive oils from the various regions of Greece are summarized in Table 1. The same parameters for the commercial olive oils are depicted in Table 2. Also, Table 2 contains the diglyceride content and D values of three samples of refined olive oil and three samples of pomace oils. Moreover, Tables 1 and 2 contain the acidity values of the olive oils determined from the peak of the phosphitylated free fatty acids, which appears at δ 134.8.

Tables 3–5 compare the mean values and other basic statistical parameters of the total DG, 1,2-DG, and 1,3-DG concentrations, respectively, for the virgin olive oils from the various regions of Greece, excluding Messinia, Zakynthos, and Pilion because fewer samples were obtained from those locations. Careful inspection of Tables 1 and 3–5 reveals some interesting trends in the diglyceride content of the Greek olive oils for the period 2000–2001.

1. The total amount of DGs in all VOO samples ranges from 1 to 3% (Table 1). On the basis of the means of the total amount of DGs (Table 3), the seven regions can be grouped into three classes. The first class contains the regions of Sitia and Peza with an average mean value of total DGs of 1.66%; the second class includes the regions of Ili, Lesvos, and Halkidiki with an average mean value of 2.01%; and the third class contains the regions of Kolymbari and Iraklion with an average mean value of 2.21%. The regions of Messinia, Zakynthos, and Pilion with two to three samples each show average values of 1.57, 2.30, and 2.48, respectively.

2. The samples from the regions of Kolymbari, Iraklion, Lesvos, and Halkidiki show a rather large dispersion of the total DGs, whereas the virgin olive oils from Sitia, Ili, and Peza are characterized by a rather uniform concentration of total DGs (Table 3).

3. The majority of the Greek VOO samples show a higher content of 1,2-DGs than 1,3-DGs (Table 1). Only two samples (12 and 19) from Kolymbari and one sample (1) from Zakynthos (period 2000–2001) show a lower amount of 1,2-DGs. Inspection of Table 4 shows that only one group of regions contains about the same amount of 1,2-DGs (Sitia, Kolymbari, Peza, and Halkidiki with an average mean value of 1.46%). The oils of the remaining regions show higher amounts of 1,2-DG, with the highest value (2.93%) observed in one sample (1) from Pilion (Table 1). There is no uniformity in the 1,2-DG content of the various regions, except perhaps for the olive oils from Sitia (period 2000–2001).

4. The 1,3-DG content is quite small for most virgin olive oils, and it ranges from ~0.1 to ~1.0% (Table 1), except for the three samples from Kolymbari (12 and 19) and Zakynthos (1). The lowest levels of 1,3-DGs are observed in the olive oils from Sitia, Peza, Iraklion, Ili, and Messinia and the highest in the olive oils from Zakynthos and Kolymbari (Tables 1 and 5).

5. The ratios of 1,2-DG to the total amount of diglycerides (D) are high, ranging on the average from 0.92 (samples from Sitia and Ili) to 0.62 (samples from Kolymbari). Only three samples (period 2000–2001), two from Kolymbari (12 and 19) and one from Zakynthos (1), show a lower ratio D, which is about half of the ratios observed in the rest of the olive oil samples (Table 1).

6. The olive oils from Sitia, Peza, Iraklion, Messinia, and Ili show a uniformity of the ratios D, whereas variations in the ratios are observed in the samples from Kolymbari (0.47–0.73), Lesvos (0.63–0.94), Pilion (0.61–0.92), Halkidiki (0.65–0.95), and Zakynthos (0.44–0.71).

Comparing the olive oils from the four regions of Crete collected in two consecutive time periods, 1999–2000 and 2000–2001 (Tables 1 and 3–5), we observe that the olive oils from Kolymbari do not show any difference in the diglyceride content (and the ratio D) with year of harvesting. However, the
samples from the other three regions are characterized by distinct differences in the 1,2-DGs, 1,3-DGs, total DGs, and the ratios D. For instance, the olive oils from Iraklion of the period 2000–
2001 show a lower mean content of 1,3-DGs, a higher mean content of 1,2-DGs and total DGs, and a higher mean value of the ratio D than the corresponding compositional parameters of the samples extracted in the period 1999–2000. This observation indicates that the composition of fresh virgin olive oils in diglycerides from the same region may differ from one year to another. However, further analyses with samples of olive oils derived from several crop seasons in each region are necessary to establish the levels of the diglyceride content.

The commercial olive oil samples that are labeled as virgin show lower amounts of 1,2-DGs than 1,3-DGs (Table 2).

Moreover, the ratios D are lower than those of the virgin olive oils from the various regions of Greece, ranging from 0.32 to 0.48. Nevertheless, one sample (EVOO2) is clearly distinguished from the others as shown by a higher value of the ratio D (0.64). On the other hand, the commercial pure olive oils show the highest amounts of 1,3-DGs and total DGs, the lowest amount of 1,2-DGs, and the lowest value for the ratio D among all of the olive oil samples studied (compare Tables 1 and 2). The total amount of DGs exceeds the value of 3%, ranging between 3.40 and 6.15%. The same trends are observed in the DG content and the ratio D for the refined olive oils and pomace oils (Table 2).

For virgin olive oils, the amount of DGs and the ratio D depend on several factors, such as the olive variety, the ripeness
of the olive fruit, the environment, and the storage life of the product. The effect of the olive variety on the amount of diglycerides and the ratio \( D \) cannot be distinguished easily from the effect of the environment. However, the present data do not show any significant changes due to the olive variety. For instance, the ratios \( D \) for the varieties Adramitiani (0.84) and Kolovi (0.81) from Lesvos are not different, whereas differences are observed in the values of the ratio \( D \) of virgin olive oils extracted from the same variety (Koroneiki) in the same or different regions (Table 1).

The effect of the degree of olive ripeness is reflected on the diminution of the amount of 1,2-DGs and the ratio \( D \) as the olive fruit becomes ripe. Recent studies (3, 7) have shown that unripe olives are characterized by larger amounts of 1,2-DGs than overripe olive fruits, whereas normal ripeness results in an intermediate amount of 1,2-DGs. This factor is not known for the present olive oil samples except for one sample (1) from Pilion, which was produced from unripe olive fruits and therefore is characterized by the highest amount of 1,2-DGs. The variation of the amount of 1,2-DGs and the ratios \( D \) observed for the samples originated from the region of Sitia as compared to those from the other three regions of Crete may show different degrees of ripening.

The final factor of storage life is not effective for the present virgin olive oils, because the samples were collected shortly after extraction. However, storage seems to have an effect on commercial olive oils, which in addition are of unknown origin. At any rate, the lower amounts of 1,2-DGs than 1,3-DGs and the low ratios \( D \) of the commercial virgin olive oils indicate that their quality is in general inferior to that of the virgin olive oils obtained from the various regions of Greece. These conclusions are illustrated graphically in Figure 2, where the ratios \( D \) are plotted against the total amount of DGs for all of the olive oils summarized in Tables 1 and 2.

The majority of the olive oils from the various regions of Greece are clearly gathered at the upper left corner of the plot (high \( D \) values and low total DGs), indicating that they are fresh virgin olive oils. The commercial virgin olive oils are characterized by low \( D \) values, so their position in the graph depends on the total DGs. Olive oils with low total DGs (<3%) tend to fall in the lower left corner, whereas olive oils with higher total DGs (>3%) tend to cluster in the lower right corner of the plot (Figure 2). At any rate, the present commercial virgin olive oils do not fall at the upper left corner of the plot, a fact that can be explained by their low quality and/or their long shelf life. The tendency of the commercial pure olive oils to gather at the lower right corner of the plot is pronounced (Figure 2). These samples are clearly not virgin. The three samples from Kolymi (12 and 19) and Zakynthos (1) can be classified as virgin olive oils of inferior quality as they fall in the same region of the plot with the commercial virgin olive oils. The commercial virgin olive oil (EVOO2) with a higher ratio \( D \) (0.64)

### Table 2. Commercial Olive Oils, Refined Olive Oils, and Pomace Oils: 1,2-DGs, 1,3-DGs, Total DG Content, Ratio \( D \) (1,2-Diglyceride Content to the Total Amount of Diglycerides) and Acidity Values

<table>
<thead>
<tr>
<th>sample</th>
<th>1,2-DG (%)</th>
<th>1,3-DG (%)</th>
<th>total DGs (%)</th>
<th>( D )</th>
<th>acidity</th>
</tr>
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<tbody>
<tr>
<td>EVOO1</td>
<td>0.70</td>
<td>1.43</td>
<td>2.13</td>
<td>0.33</td>
<td>0.39</td>
</tr>
<tr>
<td>EVOO2</td>
<td>1.58</td>
<td>0.87</td>
<td>2.45</td>
<td>0.64</td>
<td>0.42</td>
</tr>
<tr>
<td>EVOO3</td>
<td>1.72</td>
<td>2.03</td>
<td>3.11</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>EVOO4</td>
<td>0.34</td>
<td>0.08</td>
<td>0.43</td>
<td>0.11</td>
<td>0.115</td>
</tr>
</tbody>
</table>

### Table 3. Statistical Parameters for the Total DGs of Virgin Olive Oils of Various Regions of Greece

<table>
<thead>
<tr>
<th>region</th>
<th>mean</th>
<th>variance</th>
<th>min</th>
<th>max</th>
<th>range</th>
<th>median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitia</td>
<td>2.28</td>
<td>0.085</td>
<td>1.91</td>
<td>2.59</td>
<td>0.68</td>
<td>2.22</td>
</tr>
<tr>
<td>Sitia</td>
<td>2.14</td>
<td>0.63</td>
<td>1.50</td>
<td>1.94</td>
<td>0.44</td>
<td>1.675</td>
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</table>

### Table 4. Statistical Parameters for the 1,2-DG Concentration for Virgin Olive Oils of Various Regions of Greece

<table>
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<th>variance</th>
<th>min</th>
<th>max</th>
<th>range</th>
<th>median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitia</td>
<td>1.62</td>
<td>0.004</td>
<td>1.53</td>
<td>1.71</td>
<td>0.18</td>
<td>1.62</td>
</tr>
</tbody>
</table>

### Table 5. Statistical Parameters for the 1,3-DG Concentration for Virgin Olive Oils of Various Regions of Greece

<table>
<thead>
<tr>
<th>region</th>
<th>mean</th>
<th>variance</th>
<th>min</th>
<th>max</th>
<th>range</th>
<th>median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitia</td>
<td>0.66</td>
<td>0.08</td>
<td>0.39</td>
<td>0.97</td>
<td>0.58</td>
<td>0.51</td>
</tr>
</tbody>
</table>

appears at the same region with the virgin olive oils. Finally, the refined and the pomace oils tend to the lower right corner of the graph as expected.

Analysis of Variance (ANOVA). The previous qualitative observations lead to useful conclusions about the levels of DGs of virgin olive oils from various regions of Greece and classify the quality of olive oils on the basis of their ratio $D$. However, to make this study more efficient, it is necessary to carry out an advanced statistical test to examine the possibility that the DG content in the present virgin olive oils reflects the effect of geographical origin. Therefore, the amount of the total DGs and the ratio $D$ were analyzed with one-way analysis of variance.

Eighty-nine VOO samples were selected from the regions of Sitia, Kolymbari, Peza, Iraklion, Ilia, Lesvos, and Halkidiki, excluding Messinia, Zakynthos, and Pilion because fewer than five samples were obtained from those locations. Table 6 summarizes the results when the variability between the oils of the various regions was compared. In all cases, the selected variables were significantly different for each group (regions), indicating that the null hypothesis should be rejected, and the olive oil sample means within each group do differ significantly.

Next, a post-hoc comparison of the means will be used to determine whether the differences were significant. We have avoided the least significant difference (LSD) test because its efficiency in multiple comparisons has been questioned in the past (22). Moreover, this test applies for groups having equal numbers of measurements within each group. Therefore, we decided to apply a more conservative test for unequal numbers of measurements, the Tukey HSD test for unequal samples sizes (Spjotroll/Stoline test) (23). The test for the total DGs variable for the oils of the period 1999−2000 showed no significant differences ($P > 0.05$) among the means of all olive oil samples of the period 2000−2001 for the various regions, except perhaps between the means of Sitia and those of Kolymbari and Iraklion. Significant differences were observed between the means of the ratios $D$ of the oils (collected during the period 2000−2001) from Kolymbari and those from the other regions, which, however, do not differ significantly from each other. Also, significant differences are observed in the means of the ratios $D$ with the year of harvest. Oils from the same region of Crete at two consecutive years of production were differentiated at the 0.05 significance level. On the basis of this analysis, we conclude that at this level of significance ($P < 0.05$), and using the Tukey HSD test, only samples with differences in the mean values of the ratio $D > 0.15$ unit can be discriminated.

Nevertheless, the present results demonstrate that the DG content cannot be used alone to classify virgin olive oils according to their geographical origin. In addition to the DG

![Figure 2](image-url)
content, several other parameters (e.g., fatty acid composition and sterols) should be considered to reach this goal.

**Acidity Values.** Assuming that the acidity value is due to only the free fatty acids liberated from the hydrolysis of TGs to DGs, a linear relationship between the total DGs and the acidity value would be expected. However, a plot of the acidity value against the total DGs of the virgin olive oils in Table 1 gave a poor correlation ($r = 0.68$). A much better correlation ($r = 0.89$) is observed between the acidity value and the amount of 1,3-DG (Figure 3). In general, the concentration of the free fatty acids is expected to increase with increasing concentration of 1,3-DGs. The consumption (isomerization) of 1,2-DGs produced during the hydrolysis of TGs to 1,3-DGs shifts the equilibrium of the hydrolysis toward the products and hence to an increase in the concentration of the acids.

**Conclusions.** This study demonstrates the efficiency of the $^{31}$P NMR technique to detect and quantify the diglyceride content in olive oils. $^{31}$P NMR spectra with a significant signal-to-noise ratio are obtained in 20 min, allowing, in addition to diglycerides, the detection of the minor monoglyceride constituents of olive oil. Moreover, no decomposition of the phosphitylated diglycerides is observed for several hours at room temperature after derivatization.

Also, this study has led to some useful conclusions about the diglyceride levels of virgin olive oils from various regions of Greece. Although the sampling made in the present work cannot be considered complete, and it should be extended in future studies, the present results support earlier conclusions that diglycerides can be a useful index to classify the quality and freshness of olive oils.

**LITERATURE CITED**