# Diacylglycerols in the evaluation of virgin olive oil quality*

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**RESUMEN**

**Diacilgliceroles en la evaluación de la calidad de aceite de oliva virgen.**

Se han analizado muestras de aceite de oliva virgen dentro del primer mes después de la extracción y después del primer año; también se han analizado muestras de aceite de oliva comestible y lampante antes y después de la neutralización de los ácidos libres.

Además de los parámetros usuales de calidad se han evaluado igualmente los contenidos e isómeros de posición de los diglicéridos.

Se encontró que los aceites de oliva extra vírgenes, incluso los analizados después del primer año, aún satisfacían al menos dos de los siguientes cinco requisitos: a) diglicéridos totales ≤ 2.0%; b) 1,2–diglicéridos≤ 1.5%; c) 1,3–diglicéridos ≤ 0.4%; d) diglicéridos totales x 1,3–diglicéridos ≤ 1.0; e) diglicéridos totales/1,3–diglicéridos > 5.0.

Se sugiere por tanto que para ser definidos como aceites vírgenes extra, deben cumplir con al menos dos de los parámetros anteriores, además de con las características establecidas por las Regulaciones Números 2568/91 y 183/93 de la CE.

*PALABRAS-CLAVE: Aceite de oliva virgen — Calidad — Diacilglicerol.*

**SUMMARY**

**Diacylglycerols in the evaluation of virgin olive oil quality.**

Virgin olive oil samples were analyzed within one month after extraction and after one year; edible and lampante olive oil samples were analyzed before and after neutralization of free acids.

In addition to the usual quality parameters, the amount and position isomers of diglycerides were equally assessed.

It was found that extra virgin olive oils, even through analyzed after one year, still meet at least two of the following five requirements: a) total diglycerides ≤ 2.0%; b) 1,2–diglycerides ≤ 1.5%; c) 1,3–diglycerides ≤ 0,4%; d) total diglycerides x 1,3–diglycerides ≤1.0; e) total diglycerides/1,3–diglycerides > 5.0.

It is therefore suggested that for extra virgin oils to be defined, they should comply with at least two of the above parameters, in addition to the characters prescribed for by EC Regulations Nos. 2568/91 and 183/93.

*KEY-WORDS: Diacylglycerol — Quality — Virgin olive oil.*

## 1. INTRODUCTION

Following the enactment of E. C. regulation No. 2568/91, as partially amended by No. 183/93, differentiation of the three categories of *virgin* olive oil by chemical analysis is based mostly on the free acidity percentage in oils.

Given the present state of knowledge, this parameter for evaluation does not appear to characterize oil quality to a sufficient degree since it is a known fact that it is technically possible to reduce the acidity level by allowing poor quality oils to be classified as "*extra*" olive oil categories.

Having compared the findings of several teams of tasters, we would say that even the panel-test does not seem to be sufficiently reliable to differentiate among the three categories of *virgin* oils. In any case, an evaluation based solely on a panel test represents a subjective expression which can only be verified by means of a further objective expression.

Therefore it appears necessary to identify more analytical elements capable to yield precise information in addition to that provided for by the aforementioned E. C. Regulation and, at any rate, irrespective of any deacidification treatment.

With this aim in mind, it was considered appropriate to investigate partial glycerides.

Work on these compounds of the saponifiable fraction of olive oil started in 1965 (Catalano M., 1965; Vázquez Roncero A. et al., 1965) and is still under way. Increasingly refined analytical techniques have been used and many fundamental studies have been contributed (Catalano M., 1972; D'Alonzo R. P. et al., 1982; Mariani G. et al., 1985; Leone et al., 1988; Amelotti et al., 1989; Catalano M. et al., 1991; Frega et al., 1993). However, the quality of virgin oils could not be defined by these studies, centered upon parameters relative to partial glycerides; nor was it possible to differentiate the three olive oil commercial categories from one another.

That was precisely the goal of the work reported here.

## 2. EXPERIMENTAL

Samples of virgin olive oil with different percent acidity, obtained from selected highly specialized Italian olive growing areas, were submitted to chemical analysis.

All samples were taken directly during processing at oil industries equipped with olive oil mechanical extration systems.

In addition to the usual quality parameters (acidity, peroxide number, U. V. absorption), (NDG 1976), mono- and diglycerides were also determined and were differentiated with respect to both molecular weight and isomeric positions.

HRGC analysis was performed under the following conditions: fused silica capillary column SE 52,25 m. x 0.32 mm (l x i.d.); film 0.15 μm; programmed temperature from 180°C to 360°C, 6°C/min, isotherm at 360°C for 10

min; injector on column with secondary cooling; FID at 400°C; carrier hydrogen; dehydrated oil (on anhydrous $Na_2SO_4$) treated with silylating reagents (HMDS + TMCS + Pyridine, 3:1:9 v.v.: 100 µl/ mg oil, SUPELCO Chromat. Products, 1994, Supelchem S.r.l.- Milano), at room temperature for 15 min; detector response factors calculated by means of standard calibration mixture (monoolein 33%, diolein 33%, triolein 33%; diolein consist of 85% 1,3–isomer and 15% 1,2–isomer – Sigma, St. Luis 1994).

The research was conducted on a large number of virgin oil samples, all analysed within one month from extration. Some of them were again analysed one year later, after storage either at the oil processing industry or in the laboratory; where the oil was kept in the dark and at different temperatures: room temperature, 4°C and –6°C.

Lastly, a considerable number of samples of oils classified as *edible* and *lampante* were analysed before and after neutralizing in the laboratory. Neutralization was performed with a 10% acqueous solution of NaOH, then washed with water containing 2% NaCl until pH 7.00; phases separation was obtained by centrifugation.

Data for total diglycerides and for the two isomeric forms 1,2– and 1,3–diglycerides were processed statistically to give an immediately appreciable evaluation.

## 3. RESULTS AND DISCUSSION

The results obtained by analysing 227 samples of freshly produced virgin olive oil are shown synthetically in tables I and II where only the diglyceride contents of the following oil categories are given: *extra virgin*, *virgin*, *edible* or *lampante* oil. Table I shows the most significant statistical parameters, table II also shows percent frequencies.

Results obtained for monoglycerides and the composition both of monoglycerides and diglycerides were omitted; also omitted were common parameters of quality, due to their limited interest in connection with the above described aims.

Overall, the data show the remarkable significance of total diglycerides (TD) and of 1,2– (1,2d) and 1,3– (1,3d) diglycerides as characteristic parameters of virgin oil quality. The amounts observed in *extra virgin* oils were ≤2.0% for total diglycerides in 99.4%≤1.5% for 1,2–diglycerides in 97.7%, and ≤0.4% for 1,3–diglycerides in 100% of the samples. Also the product TD x 1,3d appears to be important as 100% of the samples show values ≤1.0. Lastly, values >5.0 for TD/1,3d were recorded in 86.3% of the *extra virgin* samples.

A clearcut difference is also observed between *extra*

*Table I.* ***Virgin*** **olive oil. Parameters describing diglycerides percent**

| Acidity% | Nº. of samples | Mean | S.D. | Min. | Max. | Range | V.C.% |
|---|---|---|---|---|---|---|---|
| | | | Total Diglycerides (TD) | | | | |
| ≤1.0 | 175 | 1,1 | 0,4 | 0,2 | 2,2 | 2,0 | 33,7 |
| 1.1-2.0 | 20 | 2,0 | 0,4 | 1,1 | 2,8 | 1,7 | 22,0 |
| >2.0 | 32 | 3,5 | 1,2 | 2,2 | 6,5 | 4,3 | 35,1 |
| all | 227 | 1,5 | 1,0 | 0,2 | 6,5 | 6,3 | 67,3 |
| | | | 1,2-Diglycerides (1,2 d) | | | | |
| ≤1.0 | 175 | 1,0 | 0,3 | 0,2 | 1,8 | 1,6 | 30,6 |
| 1.1-2.0 | 20 | 1,4 | 0,4 | 0,6 | 2,3 | 1,7 | 32,4 |
| >2.0 | 32 | 1,7 | 0,5 | 0,9 | 3,3 | 2,4 | 29,6 |
| all | 227 | 1,1 | 0,4 | 0,2 | 3,3 | 3,1 | 38,3 |
| | | | 1,3-Diglycerides (1,3 d) | | | | |
| ≤1.0 | 175 | 0,1 | 0,1 | 0,0 | 0,4 | 0,4 | 113,3 |
| 1.1-2.0 | 20 | 0,6 | 0,4 | 0,3 | 1,8 | 1,5 | 67,1 |
| >2.0 | 32 | 1,8 | 1,1 | 0,5 | 5,0 | 4,5 | 60,7 |
| all | 227 | 0,4 | 0,7 | 0,0 | 5,0 | 5,0 | 172,1 |
| | | | TDx 1,3d | | | | |
| ≤1.0 | 175 | 0,1 | 0,2 | 0,0 | 0,9 | 0,9 | 138,5 |
| 1.1-2.0 | 20 | 1,2 | 1,0 | 0,3 | 4,3 | 4,0 | 81,1 |
| >2.0 | 32 | 7,6 | 7,5 | 1,2 | 32,5 | 31,3 | 98,8 |
| all | 227 | 1,3 | 3,8 | 0,0 | 32,5 | 32,5 | 292,4 |

*Table II.* **Diglycerides of *virgin* olive oils. % Frequences**

| % diglycerides | extra virgin | virgin | edible or lampante | all |
|---|---|---|---|---|
| | | Total Diglycerides (TD) | | |
| <1,0 | 41,7 | 0,0 | 0,0 | 32,1 |
| 1,0-1,5 | 42,8 | 15,0 | 0,0 | 34,5 |
| 1,5-2,0 | 14,9 | 35,0 | 0,0 | 14,5 |
| 2,0-2,5 | 0,6 | 40,0 | 25,0 | 7,5 |
| 2,5-3,0 | 0,0 | 5,0 | 21,8 | 3,5 |
| >3,0 | 0,0 | 5,0 | 53,2 | 7,9 |
| | | 1,2-Diglycerides (1,2 d) | | |
| <1,0 | 56,0 | 20,0 | 6,3 | 45,8 |
| 1,0-1,5 | 41,7 | 45,0 | 34,3 | 41,0 |
| 1,5-2,0 | 2,3 | 25,0 | 34,3 | 8,8 |
| >2,0 | 0,0 | 10,0 | 25,1 | 4,4 |
| | | 1,3-Diglycerides (1,3 d) | | |
| <0,2 | 85,7 | 0,0 | 0,0 | 66,0 |
| 0,2-0,4 | 14,3 | 45,0 | 0,0 | 15,0 |
| 0,4-1,0 | 0,0 | 45,0 | 25,0 | 7,6 |
| 1,0-2,0 | 0,0 | 5,0 | 40,6 | 6,2 |
| 2,0-3,0 | 0,0 | 0,0 | 18,8 | 2,6 |
| >3,0 | 0,0 | 5,0 | 15,6 | 2,6 |
| | | TDx 1,3 d | | |
| <1,0 | 100,0 | 75,0 | 0,0 | 83,7 |
| 1,0-5,0 | 0,0 | 20,0 | 43,8 | 7,9 |
| 5,0-15,0 | 0,0 | 5,0 | 34,4 | 5,3 |
| >15,0 | 0,0 | 0,0 | 21,9 | 3,1 |
| | | TD/ 1,3 d | | |
| <5,0 | 13,7 | 70,0 | 100,0 | 30,8 |
| 5,0-10 | 29,1 | 30,0 | 0,0 | 25,1 |
| 10-20 | 44,0 | 0,0 | 0,0 | 33,9 |
| >20,0 | 13,1 | 0,0 | 0,0 | 10,1 |
| Nº. analyzed samples | 175 | 20 | 32 | 227 |

*virgin* oils on the one hand, and *edible* and *lampante* oils with respect to the abovementioned parameters; instead, samples *class*ifiable as *virgin* oils reveal values ranging with the limits given for *extra virgin* oils, with significant percent frequencies. Therefore, such parameters provide an important tool for differentiating virgin oils in *class* I from those of the last two *class*es, though not for identifying differences with respect to *class* II, particularly if obtained by accurate blending.

Some *extra virgin* and *virgin* oil samples were analysed in the fresh state, then stored in the dark for one year in full and accurately stopped glass bottles, at room temperature (18–25 °C), and at 4 °C and –6 °C. Percent frequencies within the limits mentioned above with reference to diglycerides are given table III.

It was decided that the study should not be protracted for more than one year because of the known tendency of olive oil to autoxidize in time, up to an extent that can be evaluated by means of specific parameters.

Many more observations could be made. Let us just point out that both the *extra virgin* and the *virgin* oil show a remarkable decrease in the frequency of samples with amounts of 1,3–diglycerides ≤0.4% and of samples presenting a ratio TD / 1,3d > 5.0 due to considerable amounts of 1,2–diglycerides changing over to 1,3–diglycerides. This happens mostly with oils stored at

*Table III.* **Glyceridic composition of *extra virgin* olive oils analyzed fresh and after 1 year storage at different temperature in the dark. % Frequences**

| Investigated parameters | limit value | fresh oils | after 1 years at room temperature | at +4ºC | at -6ºC |
|---|---|---|---|---|---|
| total diglycerides | ≤2.0% | 99.4 | 96.7 | 77.3 | 87.5 |
| 1,2-diglycerides | ≤1.5% | 97.7 | 100.0 | 100.0 | 100.0 |
| 1,3-diglycerides | ≤0.4% | 100.0 | 10.0 | 45.4 | 62.5 |
| tot. diglyc. x 1,3-diglycerides | ≤1.0 | 100.0 | 26.7 | 77.3 | 87.5 |
| tot. diglyc. / 1,3-diglycerides | >5.0 | 86.3 | 0.0 | 22.7 | 25.0 |

*Table IV.* **Glyceridic composition of *extra virgin* olive oils stored for 1 year at to room temperature in the dark vs. *edible* or *lampante* oils considered as such or after neutralization. % Frequences**

| Investigated parameters | limit value | *Extra virgin* oils after 1 yr. at room temp. | *edible* or *lampante* oils as such | neutralized |
|---|---|---|---|---|
| total diglycerides | ≤2.0% | 96.7 | 2.8 | 8.2 |
| 1,2-diglycerides | ≤1.5% | 100.0 | 36.1 | 97.2 |
| 1,3-diglycerides | ≤0.4% | 10.0 | 2.8 | 0.0 |
| tot. diglyc. x 1,3-diglycerides | ≤1.0 | 26.7 | 2.8 | 0.0 |
| tot. diglyc. / 1,3-diglycerides | >5.0 | 0.0 | 5.6 | 0.0 |
| Nº. analyzed samples | | 30 | 36 | 36 |

*Table V.* **Olive oil samples with the parameters: TD≤2.0%; 1,2-d≤1.5%; 1,3-d≤0.4%; TDx 1,3-d≤1.0%; TD/ 1,3-d>5.0**

| Characteristic of oils | Parameter Number 0 | | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nº. of samples | % | Nº. of samples | % | Nº. of samples | % | Nº. of samples | % | Nº. of samples | % | Nº. of samples | % |
| *extra virgin* | | | | | | | | | | | | |
| fresh | - | - | - | - | - | - | - | - | 26 | 15 | 149 | 85 |
| after 1 yr. at room temp. | - | - | - | - | 21 | 70 | 6 | 20 | 4 | 10 | - | - |
| *virgin* | | | | | | | | | | | | |
| fresh | 6 | 30 | 1 | 5 | 5 | 25 | 7 | 35 | 1 | 5 | - | - |
| after 1 yr. at room temp. | - | - | 10 | 91 | 1 | 9 | - | - | - | - | - | - |
| *edible or lampante* | | | | | | | | | | | | |
| as such | 26 | 72 | 10 | 28 | - | - | - | - | - | - | - | - |
| *edible or lampante* | | | | | | | | | | | | |
| neutralized | 1 | 3 | 35 | 97 | - | - | - | - | - | - | - | - |

room temperature. Instead, in the oils kept at low temperature, the changes, though still noticeable, decrease as storage temperature decreases. In any case, the limits given above in respect of total diglycerides and 1,2–diglycerides for *extra virgin* oils stored at room temperature for one year are still applicable for their characterization.

Lastly, 36 samples of *edible* and *lampante* olive oils were analysed before and after neutralizing in the laboratory with NaOH at 60 °C by simulating the condition normally observed by the oil processing industry in the deacidification process. The percent frequencies of the samples falling within the limits already reported for diglycerides are given in table IV, along with the data concerning *extra virgin* oils stored for one year at room temperature.

There are clearcut differences between the latter and *edible* or *lampante* oils, particularly in total diglycerides and TD x 1,3d. Our investigation also indicates that neutralized oil does not show any noteworthy change in total diglycerides, 1,3–diglycerides, TD x 1,3d and TD / 1,3d. This finding suggests that oil with >2% acidity, even if treated only by neutralization, still retain their diglyceride content practically unmodified. Therefore, also under these conditions, diglycerides may represent a useful element for olive oil quality judgement, especially if all the parameters describing them are taken into account, namely TD; 1,2d; 1,3d; TD x 1,3d; TD / 1,3 as shown in table V in a summary form.

## 4. CONCLUSIONS

The results of the analyses concerning total diglycerides and their isomeric forms obtained on a large number of olive oil samples *(extra virgin, virgin, edible* and *lampante)* contribute new knowledge in connection with a more effective evaluation of the objective chemical qualities of a virgin olive oil.

*Extra virgin* oils differ but slightly from *virgin* oils, particularly if their quality is only slightly lower than in *class* I; conversely, there is an obvious difference vis-à-vis the *edible* and the *lampante* oils, even if previously subjected to neutralizing treatment.

Consequently, on the basis of our findings, it appears that *extra virgin* olive oils that may have been analysed after storing at room temperature for up to one year, can only be ascribed to such category if they meet at least two of the five following characteristics in their composition, all of them concerning diglyceride percentages: a) total diglycerides ≤ 2.0%; b) 1,2–diglycerides ≤ 1.5%; c) 1,3–diglycerides ≤ 0.4%; d) total diglycerides x 1,3–diglycerides ≤ 1.0; e) total diglycerides/1,3-diglycerides > 5.0.

In conclusion, it appears correct to believe that virgin olive oil, even if stored for one year, cannot be rated as *extra virgin* unless it meets at least two of the five above mentioned parameters. Conversely, when more than two such parameters are complied with then the investigated *extra virgin* oil may be regarded as meeting the best requirements of high chemical quality.

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