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Non-conventional parameters for quality evaluation of refined oils with special reference to commercial class olive oil

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Abstract

Forty olive oil samples (refined olive oil plus virgin olive oil) were tested to check the possibility of using recent non-conventional analytical parameters to assess quality. The routine analyses prescribed by the EEC Reg. No 2568/91 and subsequent extensions were carried out on all samples. The analytical techniques used included silica gel column chromatography for the separation of polar compounds from oil, and their subsequent high performance size-exclusion chromatography (HPSEC) analysis to quantify the oxidation, polymerisation and hydrolysis product classes. Based on the results obtained, it seems logical to use the percent oligopolymer content to measure the level of oxidation in commercial class olive oil. The diglyceride level can provide information about the extent of hydrolytic degradation.

Keywords: Refined oils; Olive oil; Quality evaluation; Non-conventional parameters; HPSEC analysis

1. Introduction

The indices commonly used to assess the degree of oxidation of edible oils and fats are the peroxide value (PV) that measures primary oxidation, the specific absorption at 232 nm (K_{232}), related to the content of conjugate dienes, which are formed as a result of oxidation and during the bleaching phase in oil refinement, and the *p*-anisidine value (*p*-AV) that is used to assess secondary oxidative degradation. However, these analyses only partial quantify the phenomenon and do not seem to satisfy the major need to know the actual state of oil oxidation, based on different phases of initiation, propagation and termination of the radical mechanisms. The level of hydrolysis of a fat is generally ascertained through the determination of percent of free fatty acids (FFA).

Over the past few years, the use of silica gel column chromatography has allowed separation and determination of polar compounds (PC) from oils, according to the IUPAC methodology (1987a). PC consist of substances of higher polarity than unaltered triglycerides. By means of the high-performance size-exclusion chromatography (HPSEC) analysis of PC it has been possible to quantify the percentages of oxidation, polymerisation and hydrolysis product classes that help to characterise the edible oils and fats under study. Moreover, triglyceride oligopolymers (PTG) are considered by different authors to be reliable indicators of oxidative deterioration due to their high stability and low volatility (Paradais & Navar, 1981; Stevenson, Waisey-Genser, & Eskin, 1984; White & Wang, 1986).

The purpose of the present work has been to consider both the traditional analytical parameters of official methods (Official Journal of the European Communities, 1991) and other non conventional ones, introduced more recently, for testing their use in the quality evaluation of refined vegetable oils, with special reference to commercial class olive oil.

2. Materials and methods

Forty samples of 1 l glass bottles of commercial class olive oil, purchased in different supermarkets, were tested. Following the EEC regulation No 356/92 (Official Journal of the European Communities, 1992), the commercial

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class olive oil is obtained by blending refined olive oil and virgin olive oils other than "*lampante*".

The EEC regulation does not, however, specify the amount of virgin olive oil to add to refined oil except as ascertained in a previous paper (Gomes, Caponio, Baiano, De Pilli, Bilancia, & Delcuratolo, 2002), that the mean amount of virgin olive oil added is less than 20%. For the sake of clarity, the reader is reminded that "virgin" olive oil is oil obtained from olives (*Oleaceae*, *Olea europaea* L) by mechanical or other physical means without any further refinement excect filtration. *Lampante* olive oil is a virgin olive oil with more than 3.3% of FFA and/or unpleasant flavour and/or an evident oxidation state. It is only sold after refining. "Refined" olive oil is oil obtained by refining *lampante* olive oil.

Upon arrival at the laboratory, the samples were stored in a freezer at −20 °C until they were analysed. Each sample was thawed at room temperature and then submitted to routine analyses for a first characterisation, which consisted in the determination FFA, PV, UV spectrophotometry (Official Journal of the European Communities, 1991) and measurement of the *p*-AV (IUPAC, 1987b). PC were determined in each sample, by means of silica gel column chromatography, as described by the IUPAC method (1987a). PC were submitted to HPSEC to determine PTG, oxidised triglycerides (ox-TG) and diglycerides (DG). The chromatographic system consisted of a Perkin-Elmer pump series 10, a 7125 S sample injector (Rheodyne), a 50 μl injector loop and a series of three PL-gel columns (Perkin-Elmer, Beaconsfield, Great Bretain) 7.5 mm i.d.×30 cm length. A PL-gel guard column (Perkin-Elmer, Beaconsfield, Great Bretain) of 7.5 mm i.d.×5 cm length was used. The detector was a differential refractometer connected to an integrator. CH_2Cl_2 for HPLC was the elution solvent at a flow rate of 1.0 ml/min.

Peak identification in each chromatogram and quantitative determinations of the classes of compounds under investigation were performed as described elsewhere (Gomes, 1992; Gomes & Caponio, 1999). Linear regression analysis was carried out and the Pearson's product–moment correlation coefficients, among traditional and more recent quality indices, were evaluated.

3. Results and discussion

Table 1 shows synthetically, in terms of mean, standard deviation (S.D.) and minimum and maximum values, the determinations made on the tested samples, by the routine analyses of the Official Methods as well as more recent types of PC determinations and their subsequent analysis by HPSEC.

As shown in Table 1, all determinations of routine analyses fall within the prescribed limits (Official Journal of the European Communities, 1991). However, the PV, as an analytical parameter to define primary oxidation, is of great interest for virgin oils but is negligible if applied to olive oil. Actually, this oil is mostly refined and it is well known that, in recently refined oils, hydroperoxides are nearly absent, as they are degraded and/or transformed during processing. This is also the case for the measure of FFA. This determination is used to test the hydrolytic degradation but it is known that, in oil refinement, FFA are removed during neutralisation so that the percentage FFA loses its relevance and at best can be used at most to assess the effectiveness of the neutralisation phase.

Table 1
Analytical characteristics of commercial olive oils

Determinations	Values			
	Min.	Max.	Mean	S.D.
FFA (%)	0.05	1.01	0.40	0.24
PV (meq/kg)	3.0	13.6	7.4	2.6
K_{232}	1.58	2.98	2.28	0.34
p-AV	3.62	11.55	6.41	2.07
PC (%)	3.00	6.86	5.27	0.78
ox-TG (%)	0.56	1.37	0.95	0.19
PTG (%)	0.20	1.18	0.61	0.24
PTG (%)+ox-TG (%)	0.96	2.34	1.55	0.34
DG (%)	1.66	4.77	3.20	0.53

FFA, free fatty acids; PV, peroxide value; K_{232}, specific absorption at 232 nm; *p*-AV, *p*-anisidine value; PC, polar compounds; ox-TG, oxidized triglycerides; PTG, triglyceride oligopolymers; DG, diglycerides.

Table 1 shows that PC amount, to 5.27% (S.D.=0.78) on average. The PC provide an overall indication of the oxidative and hydrolytic degradation, as they consist of FFA, partial glycerides and products deriving from the oxidation and polymerisation of triglycerides (Gomes & Caponio, 1998). PC also include polar components from virgin olive oil.

The mean value of PTG amounts to 0.61% (S.D.=0.24) and is lower than the quantities found in refined seed oils prior to their use for frying (Cuesta, Sanchez-Montero, & Sanchez-Muniz, 1995; Dobarganes, Perez-Camino, & Marquez-Ruiz, 1988; Rojo & Perkin, 1987; Sanchez-Muniz, Cuesta, & Garrido-Polonio, 1993). It is already proven that PTG are mostly formed during the refining process of vegetable oils, in quantities which depend—the processing conditions being equal—on the state of oxidation of crude oil (Gomes, 1989; Gomes & Caponio, 1998).

The mean value of ox-TG was 0.95% (S.D.=0.19); ox-TG are another major class of degradation substances, as they include all forms of oxidation of triglycerides. The levels observed for this class of compounds are usually lower than those found in refined seed oils (Cuesta et al., 1995; Dobarganes et al., 1988; Sanchez-Muniz et al., 1993).

Lastly, the mean value of PTG+ox-TG, which represents the degree of overall oxidation of oil, is

1.55% (S.D. = 0.34). It has been proved that PTG and ox-TG act in oils as pro-oxidants and as precursors of volatile oxidation products (Frankel, Neff, Selke, & Brooks, 1988; Yoon, Jung, & Min, 1988); this is why the simultaneous measure of these two classes of compounds is useful to predict the shelf life of oil.

To assess the applicability of the non-conventional parameters above cited, we have checked whether assuming the amount of PTG to be new analytical index of the oxidative deterioration implies a correlation between the latter and traditional parameters. For this purpose, Figs. 1 and 2 show the values of K_{232} and *p*-AV respectively, of each sample, as a function of the relative percentage content of PTG. Clearly, there is a weak correlation at $P < 0.05$ in the case of K_{232} and at $P < 0.001$ in the case of *p*-AV. Hence it may be stated, in relation to olive oil, that PTG are significantly correlated with the two traditional analytical parameters commonly used to measure the level of oxidation. Fig. 3 shows that there is a positive weak correlation at $p < 0.05$ also between K_{232} and *p*-AV. No correlation was found, however, between K_{232} and the percentage content of ox-TG or between the latter and *p*-AV (Figs. 4 and 5). This could be explained by the fact—experimentally observable—that, during refinement, a variable portion of ox-TG is transformed into PTG

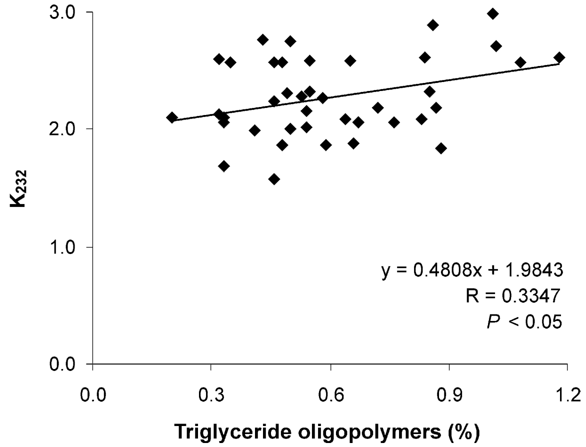

Fig. 1. Values of K_{232} plotted against the percentage contents of triglyceride oligopolymers.

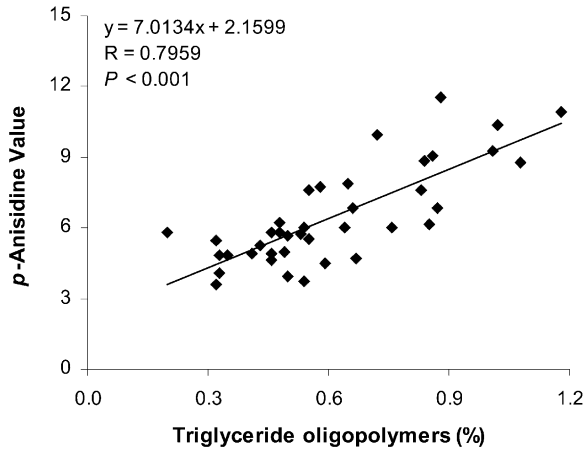

Fig. 2. *p*-Anisidine values plotted against the percentage contents of triglyceride oligopolymers.

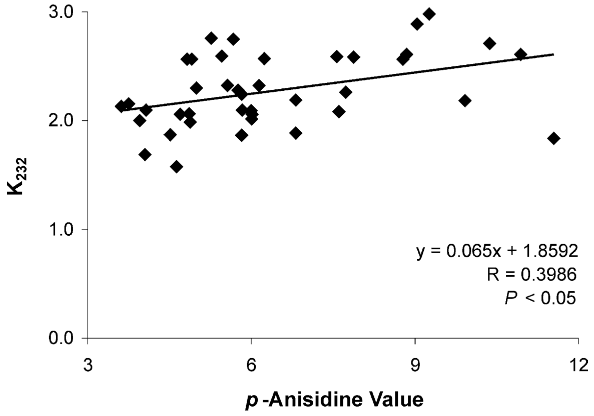

Fig. 3. Values of K_{232} plotted against the *p*-anisidine values.

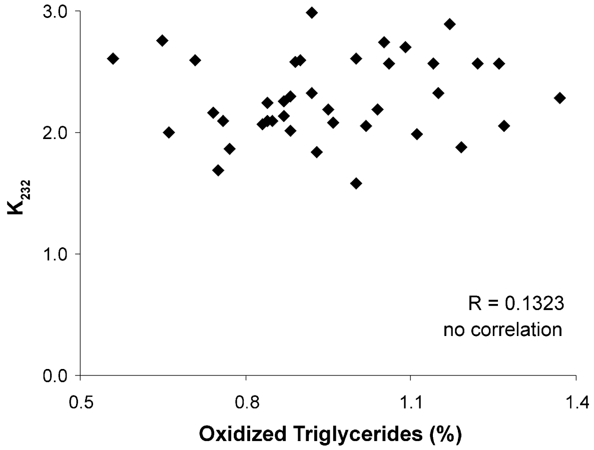

Fig. 4. Values of K_{232} plotted against the percent contents of oxidised triglycerides.

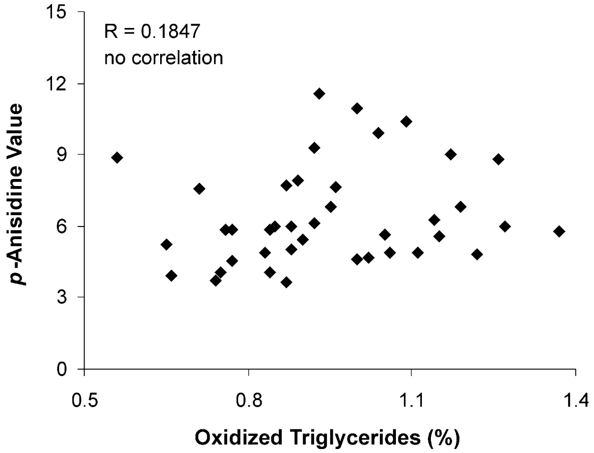

Fig. 5. *p*-Anisidine values plotted against the percent contents of oxidised triglycerides.

(Gomes & Caponio, 1996; Gomes and Caponio, 1998). The drop in ox-TG during refinement leads to the conclusion that they are not final products of oxidation and cannot be correlated with the existing level of oxidation in oil. As a consequence there is also a lack of correlation between ox-TG and PTG.

Another index that might be envisaged for quality evaluation is the overall level of oil oxidation, which is given by the sum of percentages of ox-TG and PTG. As shown in Figs. 6 and 7, there is a positive correlation between this index and *p*-AV at $P<0.001$ and with K_{232} at $P<0.05$. The argument that there is a correlation between total oxidation and the two traditional parameters under consideration, which has not been found between the ox-TG and the latter, as previously stated, does confirm the assumption that ox-TG are partially involved in polymerisation reactions, so that the sum PTG + ox-TG rather than only ox-TG can effectively reflect the existing degree of oxidation.

In the case of the olive oil samples considered, it seems that the traditional analyses of *p*-AV and K_{232} are effective enough to measure the degree of existing degradation; however, it should be noted that these indices are largely affected by the refining procedures (Amati, Mincuzzi, & Losi, 1969; Hastert, 1992; Tiscornia, Forina, & Evangelisti, 1982), the technological

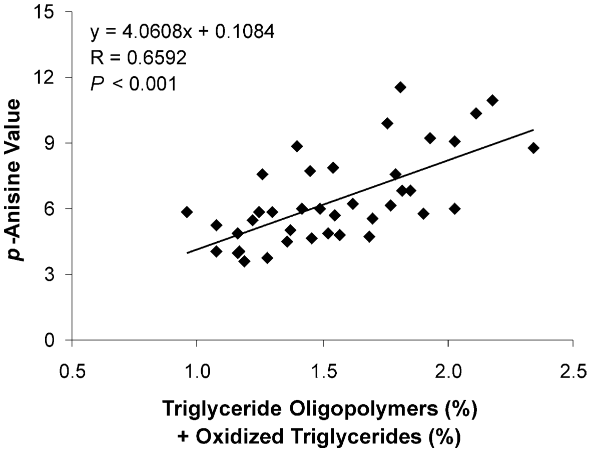

Fig. 6. *p*-Anisidine values plotted against the percent of triglyceride oligopolymers plus percent of oxidised triglycerides.

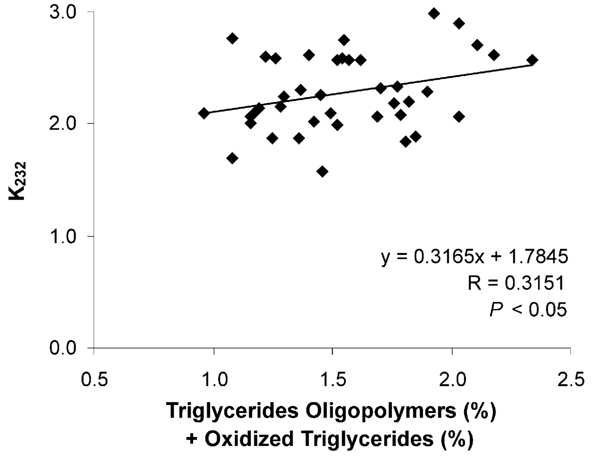

Fig. 7. Values of K_{232} plotted against the percent of triglyceride oligopolymers plus percent of oxidised triglycerides.

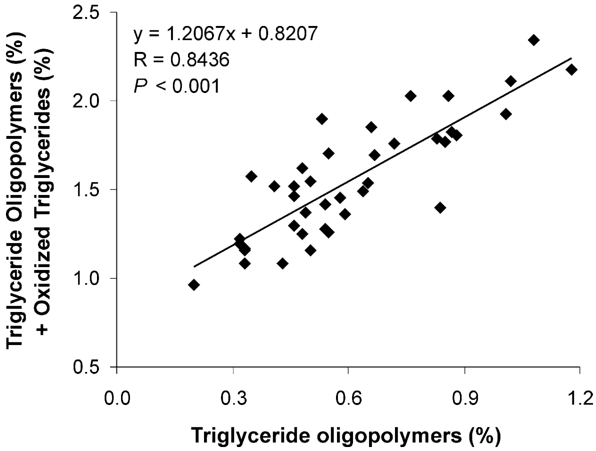

Fig. 8. Percent of triglyceride oligopolymers plus percent of oxidised triglycerides plotted against the percent of triglyceride oligopolymers.

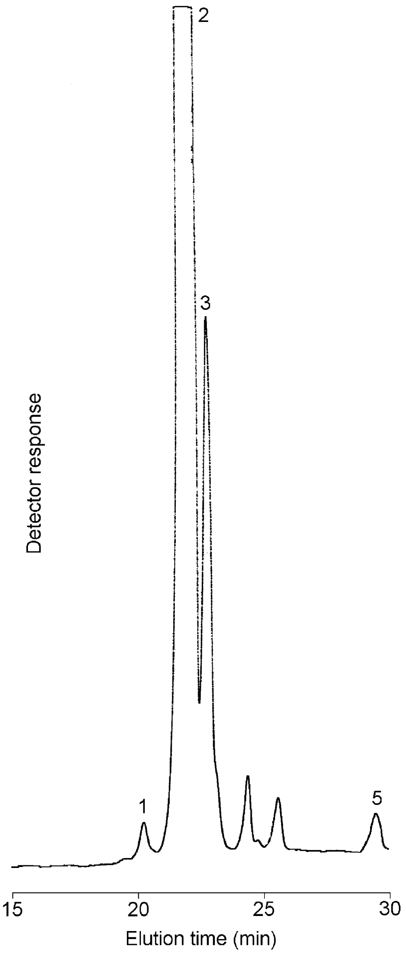

Fig. 9. Direct HPSEC of an olive oil. (1) Triglyceride oligopolymers; (2) triglycerides; (3) diglycerides; (5) free fatty acids.

innovations (Morchio, Di Bello, Mariani, & Fedeli, 1988) and, at least for the spectrophotometric analysis, by the utilisation, not allowed by law, of substances able to reduce the specific absorptions (Amelotti, Catelani, & Conti, 1981; Benedetti, Buoncristiani, & Salvadarini, 1966). This means that there can be samples for which the above traditional analyses lose their analytical value. On the other hand, as shown in Fig. 8, there is an excellent correlation ($P<0.001$) between the PTG percentage and the overall level of oxidation (PTG% + oxTG%); so it seems that oxidative deterioration can be indicated by the PTG percentage only. This would imply a large simplification of the analytical procedure, if the only point of interested were the assessment of the oxidative degradation, because it is possible to quantify PTG by submitting oil directly to the HPSEC analysis, thus preventing preliminary separation of PC from oil and their subsequent HPSEC analysis. Fig. 9 shows a chromatogram obtained by submitting a commercial olive oil sample to the HPSEC analysis directly. Moreover, based on the literature, the PTG, consisting of stable final products of oxidation, are increased, not only by the level of oxidation of crude oil subject to refinement, as already indicated, but also by the "forcing" effected during the bleaching and/or by higher temperatures used in the subsequent deodorisation (Eder, 1982; Gomes, 1988; Hoffman, 1989), thus underlining the goodness of the choice made.

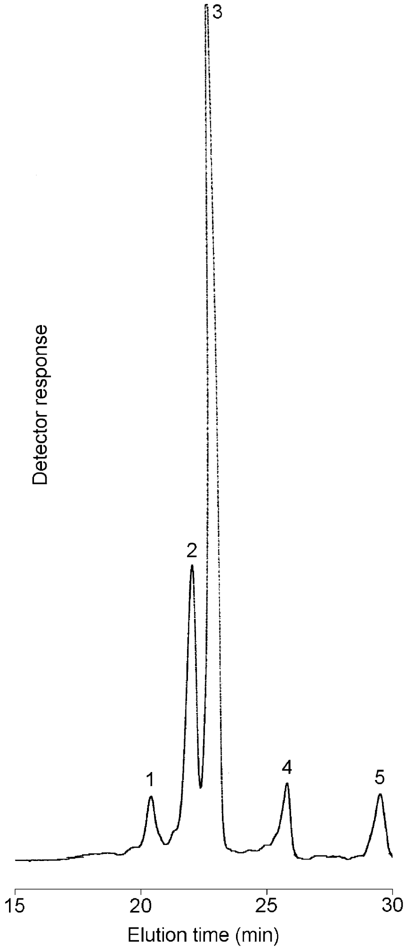

Fig. 10. HPSEC of polar compounds in an olive oil. (1) Triglyceride oligopolymers; (2) oxidised triglycerides; (3) diglycerides; (4) free sterols and triterpene diols; (5) free fatty acids.

Another point concerning quality is the assessment of hydrolytic degradation. It has already been stated that the FFA cannot be used for this purpose. The EEC Regulation No. 2568/91 (Official Journal of the European Communities, 1991) establishes a maximum value of 0.5% for refined olive oil. On the other hand, through the HPSEC analysis of PC it is possible to resolve and quantify the DG, as shown in Fig. 10, thus obtaining another quality indicator able to measure the existing level of hydrolysis more reliably in olive oil samples.

4. Conclusions

From the results obtained and based on the literature, it seems appropriate, in formulating the quality evaluation of refined vegetable oils, notably the commercial class olive oil, to overlook the routine analyses used so far and use the PTG percentage to measure the level of oxidation. The diglyceride level can provide information on the extent of hydrolytic degradation.

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