

Evaluation of the State of Oxidation of Olive–Pomace Oils. Influence of the Refining Process

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The aim of this investigation was to trace the fate of oxidation levels in crude olive–pomace oil during refining. Seven series of samples were examined. Each series comprised samples of the unprocessed crude oil and of the same oil collected after each step of the refining process. The analyses were performed by means of silica gel column chromatography and high-performance size-exclusion chromatography. The data obtained showed that triglyceride oligopolymers increased during refining, especially during bleaching and deodorization, and that the levels of these compounds were on average ~4-fold greater in the refined oils than in the crude oils. Conversely, oxidized triglycerides decreased by an average of 49% as the oil was processed into a refined oil. When the percent values of oxidized triglycerides were plotted against the percent values of oligopolymers, a linear trend of the experimental data was depicted for all the series examined. The regression lines computed revealed correlation between the two classes. This indicated that one of the reasons oxidized triglycerides decreased during olive–pomace oil refining was related to the occurrence of polymerization reactions. It was finally suggested that the content of oligopolymers triglycerides may be usefully employed as a reliable analytical index for estimating the level of oxidation of a freshly refined oil.

Keywords: *HPSEC; triglyceride oligopolymers; oxidized triglycerides; olive–pomace oils; refining process*

INTRODUCTION

Crude olive–pomace oils are extracted from olive pomaces by means of a solvent. Olive pomace is a byproduct of virgin olive oil processing and is of varying importance to all of the countries of the Mediterranean basin where olives are grown (Oleaceae, *Olea europea*).

Crude olive–pomace oil is often very acid, colored, and oxidized. Intensive refining is thus required to make it suitable for human consumption.

Industrial refining eliminates a number of undesirable substances (free fatty acids, malodorous substances, and excess amounts of coloring substances) and brings about the degradation and/or transformation of hydroperoxides, while some signs of the original oxidative and hydrolytic degradation remain. Refining leads to the presence of and/or changes in classes of compounds which seem to be related to how processing is effected (Eder, 1982) and to the state of oxidation and hydrolysis of the crude oil used (Gomes, 1989). These classes of compounds include triglyceride oligopolymers and oxidized triglycerides which are currently the subject of a considerable amount of research as they are believed to be reliable indices of oxidative degradation (Pérez-Camino et al., 1990; Hopia, 1993a).

There are very few studies in the literature concerning the evolution of the oxidation and polymerization products during the refining of vegetable oils. In particular, little or no information exists on olive–

pomace oil, in spite of the fact that the high level of oxidation present in the crude oil and the intense refining to which it is subject may produce greater amounts of triglyceride oligopolymers that may be potentially dangerous to human health.

The influence of olive–pomace drying and oil extraction with solvent on the state of oxidation of crude olive–pomace oils was explored in a previous paper (Gomes and Caponio, 1997). This paper will focus on the effect of the single steps of the refining process on the state of oxidation and hydrolysis of olive–pomace oil. The aim of this investigation was to improve our understanding of these phenomena and to better define the nutritional quality of pomace oil.

MATERIALS AND METHODS

A total of seven series of samples were examined. Each series comprised samples of the unprocessed crude olive–pomace oil and samples of the same oil after the steps of conventional refining (neutralization, bleaching, and deodorization). Dewaxing is also part of the refining process of pomace oils, but since it does not entail heating, it has no impact on the formation of triglyceride oxidation and polymerization products and has thus been disregarded in our investigation.

The processing conditions were the ones generally used for these oils (Bernardini, 1982a,b). Large amounts of bleaching earth, up to 5%, were required to remove the intense green color.

The samples were of ~250 mL each and were collected in 1995 and 1996 from several processing plants located in the south of Italy. The samples were representative of large stocks of pomace oil undergoing refining. After some time, samples were also collected from other stocks of oil being processed at the same plant to study the influence the oxidation of the crude

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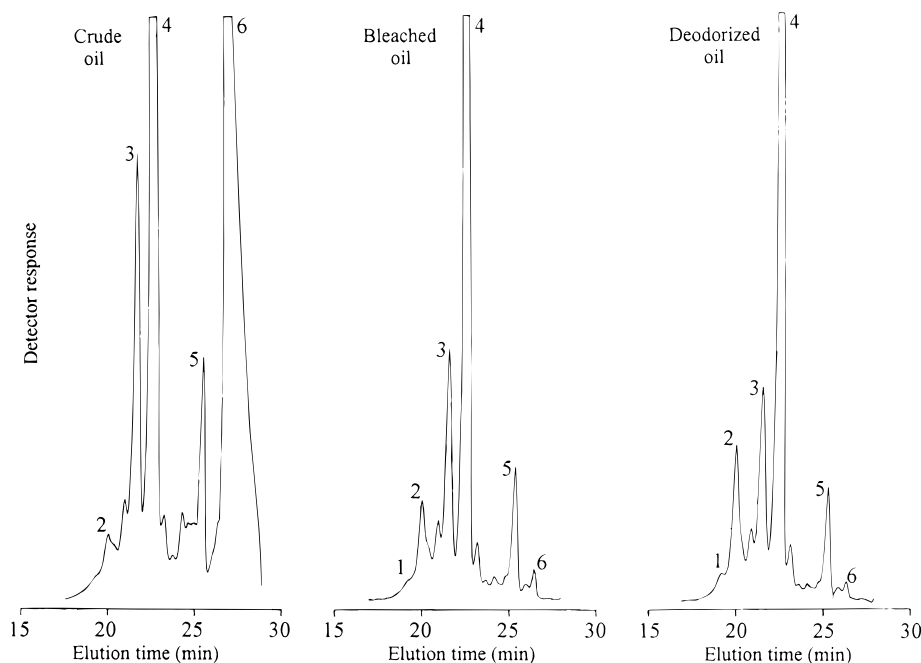


Figure 1. HPSEC analysis of polar compounds derived from an olive-pomace oil during refining: (1) triglyceride trimers; (2) triglyceride dimers; (3) ox-TG; (4) diglycerides; (5) free sterols and triterpene diols; (6) FFA.

oil had on the chemical characteristics of the refined oil when the same processing technology was used.

The samples were kept in a freezer at -20°C from the moment they reached the laboratory until they were analyzed. Each sample was left to thaw at room temperature and then underwent routine analyses for a first characterization, namely determination of percent free fatty acids (FFA), peroxide value (PV), UV spectrophotometry (*Off. J. Eur. Communities*, 1991), and measurement of the *p*-anisidine value (*p*-AV) (IUPAC, 1987a).

Polar compounds (PC) were determined in each sample by means of silica gel column chromatography as described by the IUPAC method (IUPAC, 1987b). PC were submitted to high-performance size exclusion chromatography (HPSEC) to determine oxidized triglycerides (ox-TG), oligopolymers, and partial glycerides.

The chromatographic system consisted of a Perkin-Elmer pump, series 10, a 7125 S sample injector (Rheodyne), a $50\ \mu\text{L}$ injector loop, and a series of three PL-gel columns (Perkin-Elmer Ltd., Beaconsfield, Great Britain) of $0.75\ \text{cm i.d.} \times 30\ \text{cm}$ length. The columns were packed with highly cross-linked styrene divinylbenzene copolymers with a particle diameter of $5\ \mu\text{m}$ and pore diameters of 500, 500, and $100\ \text{\AA}$, respectively. A PL-gel guard column (Perkin-Elmer Ltd.) of $7.5\ \text{mm i.d.} \times 5\ \text{cm}$ length was used. The detector was a differential refractometer (Shimadzu RID 6A, Shimadzu Corp., Japan) connected to an integrator. The elution solvent used was $\text{CH}_2\text{-Cl}_2$ for HPLC at a flow rate of $1.0\ \text{mL/min}$.

The procedures for identifying the peaks on each chromatogram and for the quantitative assessment of the classes of compounds under investigation were carried out as described elsewhere (Gomes, 1992).

RESULTS AND DISCUSSION

Figure 1 shows the HPSEC chromatograms of polar compounds derived from an olive-pomace oil during the refining process. The HPSEC method we used allowed us to measure the oligopolymers, oxidized triglycerides, and diglycerides as well as to separate free sterols and triterpene diols (peak 5) from the FFA (peak 6). This enabled us to obtain a better chromatographic separation and to acquire more data for sample characterization (Gomes and Caponio, 1996). As can be seen in

Table 1. Repeatability of the Determination of Polar Compounds, Oxidized Triglycerides, Diglycerides, and Oligopolymers in Crude Olive-Pomace Oil and in the Same Oil after Each Step of the Refining Process

	crude	neutralized	bleached	deodorized
polar compounds				
mean	17.68	8.79	7.55	7.37
SD	0.613	0.326	0.318	0.352
CV%	3.47	3.71	4.21	4.78
oxidized triglycerides				
mean	2.53	2.25	1.45	1.20
SD	0.060	0.082	0.023	0.025
CV%	3.36	3.64	1.59	2.08
diglycerides				
mean	5.29	5.13	4.83	4.69
SD	0.178	0.143	0.125	0.101
CV%	3.36	2.79	2.59	2.15
oligopolymers				
mean	0.30	0.48	0.85	1.09
SD	0.004	0.013	0.015	0.019
CV%	1.33	2.71	1.76	1.74

Figure 1, peak 5 gradually decreases during refining as was to be expected since part of the free sterols are lost in the process (Hoffman, 1989). According to its elution time, the peak between peaks 2 and 3 presented a molecular weight of ~ 1200 , which some authors assume to be ascribable to diglyceride dimers (Perrin, 1989).

The measurements of the triglyceride oligopolymers, oxidized triglycerides, and diglycerides were shown to be reproducible by analyzing a series of samples during refining seven times. The results are reported in Table 1. As may be noted, the coefficient of percent variation (CV%) of PC is $<5\%$, while the CV% values of ox-TG, diglycerides, and oligopolymers range from 3.64% to 1.33%, thus showing that the method used has a good degree of precision.

Table 2 shows the results of the determinations made on all of the samples examined. As may be noted, the chemical characteristics of the crude olive-pomace oils are similar to the ones reported in a previous paper for these oils (Gomes and Caponio, 1997).

Table 2. Characteristics of Olive-Pomace Oils during Refining

sample	FFA (%) of oleic acid)	diglycerides (%)	PV (mequiv/kg)	<i>p</i> -AV	<i>K</i> ₂₃₂	<i>K</i> ₂₇₀	ΔK	PC (%)	ox-TG (%)	total triglyceride oligopolymers (%)
processing plant 1, sample series A										
crude	10.80	5.08	11.0	17.39	4.575			18.27	2.03	0.26
neutralized	0.42	4.58	13.4	14.54	3.893			8.11	2.00	0.31
bleached	0.48	4.56	2.1	9.06	4.122			7.20	1.39	0.72
deodorized	0.29	3.88	0.2	8.13	4.358	1.737	0.133	6.36	1.11	1.03
processing plant 1, sample series B										
crude	8.06	7.49	15.0	17.44	4.476			18.18	2.06	0.45
neutralized	0.20	5.45	14.8	19.70	3.924			8.71	2.10	0.54
bleached	0.35	5.44	4.4	11.02	4.352			8.07	1.54	0.87
deodorized	0.15	4.88	1.2	11.22	4.618	1.803	0.141	7.73	1.01	1.33
processing plant 1, sample series C										
crude	7.35	6.29	12.8	15.16	4.367			16.28	2.22	0.20
neutralized	0.22	4.61	12.5	14.41	3.628			7.71	2.21	0.37
bleached	0.42	4.51	1.5	8.01	3.845			7.38	1.53	0.81
deodorized	0.25	4.35	0.4	8.06	3.574	1.766	0.137	7.18	1.49	1.08
processing plant 2										
crude	8.16	5.56	10.6	17.00	4.292			16.55	2.46	0.29
neutralized	0.22	5.09	10.8	14.79	3.791			8.16	2.42	0.35
bleached	0.23	4.91	3.7	10.32	3.639			7.66	1.60	0.72
deodorized	0.29	4.12	0.5	7.92	4.005	1.760	0.144	7.10	1.36	1.16
processing plant 3										
crude	9.98	7.58	15.2	14.70	4.545			20.40	2.22	0.44
neutralized	0.16	6.02	14.1	14.03	3.681			8.38	2.19	0.60
bleached	0.25	5.78	2.9	10.58	3.566			8.30	1.56	0.86
deodorized	0.11	5.37	0.7	5.63	3.594	1.354	0.151	7.76	1.10	1.36
processing plant 4										
crude	9.17	5.29	12.7	19.69	4.551			17.68	2.53	0.30
neutralized	0.16	5.13	13.0	17.76	3.434			8.79	2.25	0.48
bleached	0.34	4.83	2.5	10.52	3.777			7.55	1.45	0.85
deodorized	0.16	4.69	0.6	5.48	3.729	1.395	0.132	7.37	1.20	1.09
processing plant 5										
crude	14.05	10.65	14.9	16.36	4.900			28.45	3.10	0.43
neutralized	0.54	7.34	12.6	17.13	3.789			12.44	3.18	0.52
bleached	0.89	6.25	1.9	10.03	5.638			10.04	1.35	1.10
deodorized	0.21	5.84	0.3	5.65	4.244	1.666	0.157	8.74	1.00	1.30

PC decreased by an average of 54% (range = 50.3–58.9) in the neutralized oil due to removal of the FFA. A further, slight, decrease was observed during the subsequent phases of bleaching and deodorization. PC are made up of substances with a higher polarity than unaltered triglycerides and mainly comprise FFA, partial glycerides, and triglyceride oxidation and polymerization products. Hence, the PC levels found in refined olive-pomace oils provided an overall indication of oxidative and hydrolytic degradation, with values ranging from 6.36% to 8.74% and a mean value of 7.46%.

Table 2 shows that the ox-TG remained practically unchanged during neutralization, although they decreased considerably during bleaching and deodorization. The levels of ox-TG in the refined olive-pomace oils ranged from 1.00% to 1.49% with a mean value of 1.18%. This is an interesting group of compounds as it includes all forms of triglyceride oxidation. There are very few reports in the literature on the fate of ox-TG during the refining of edible vegetable oils. Some authors recently considered the issue mainly by resorting to pilot plants; for some of them the data obtained showed no clear trend in the levels of ox-TG during processing (Hopia, 1993b), while others concluded that the ox-TG remain virtually unchanged during processing due to their low volatility (Pérez-Camino et al., 1993). In this paper, focused on the refining of olive-pomace oils, the samples were collected after each step of the refining process from different pomace processing plants. The results obtained showed that, irrespective

of the specific processing techniques used at each single plant, ox-TG decreased by 49% on average (range = 33–68%) as the crude oil was processed into a refined oil. This confirmed the findings we reported in a previous paper on the refining of olive oil (Gomes and Caponio, 1996).

Oligopolymers were present in very small amounts in the crude oils and increased slightly during neutralization due to removal of the FFA, which causes them to concentrate. They increased more substantially during bleaching due to the catalytic effect of the earths and during deodorization, which is the most important thermal step of the refining process. On the whole, oligopolymers increased by an average of 3.7 times as the crude oil was processed into refined oil (range = 3.0–5.4). The amounts of oligopolymers were no doubt greater than the amounts present in refined olive oils (Gomes, 1994) and were similar to the oligopolymer contents of some refined seed oils (Gomes and Catalano, 1988; Dobarganes et al., 1988; Sanchez-Muniz et al., 1993).

Figure 1 and, better still, the data presented in Table 2 show that the decrease in ox-TG was concomitant to an increase in polymerization products. When the percent values of ox-TG were plotted against the percent values of oligopolymers, a linear trend of the experimental data was depicted for all series of samples examined. The regression lines computed yielded coefficients of correlation with $p < 0.01$ for the series from plant 5 and for series A and B from plant 1 and with p

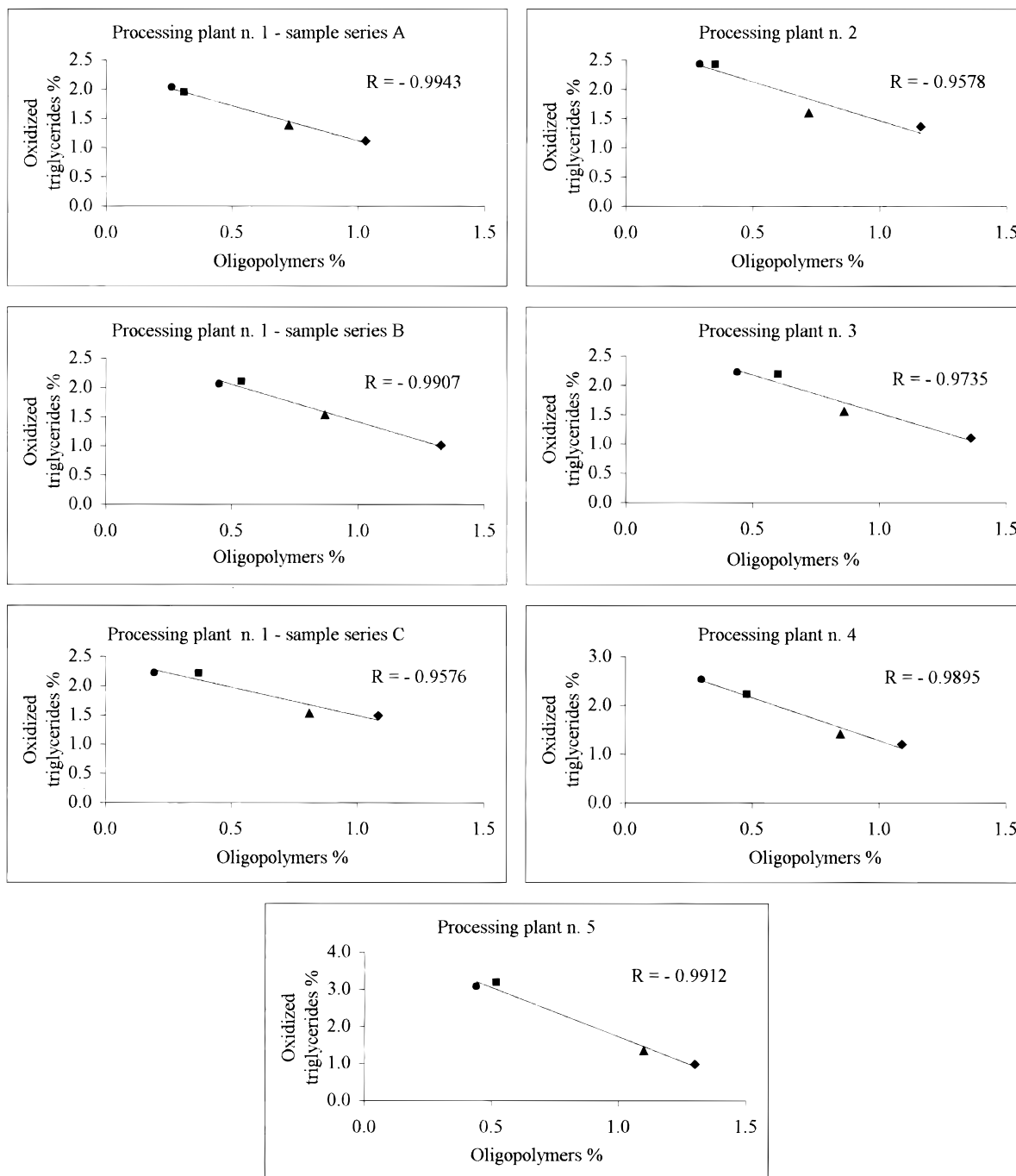


Figure 2. Percent values of ox-TG plotted against percent values of oligopolymers during refining: ●, crude oil; ■, neutralized oil; ▲, bleached oil; ◆, deodorized oil.

< 0.05 for all other series (Figure 2). The fact that the correlation existed supported the hypothesis that one reason ox-TG declined during vegetable oil refining was the occurrence of polymerization reactions.

Careful consideration of the refined olive-pomace oils reported in Table 2 indicates that the determinations provided for by EC regulation 2568/91 (1991) and subsequent extensions yielded values which fall within the ranges established for this commercial class of oil, thus illustrating how these analyses are of little value in assessing the degree of oxidation and hydrolysis of freshly refined oils. FFA are removed during neutralization, and hydroperoxides are degraded and/or transformed during refining, while the spectrophotometric indices partly depend on how the refining process is

carried out (Tiscornia et al., 1982). Table 2 also shows that the *p*-AV value decreases during processing and does not appear to be suitable for measuring the level of oxidation of a freshly refined product. Oligopolymers are the only class of compounds that increases. It therefore seems that the level of oligopolymers may be used as an index of the oxidative degradation of refined oil. In any case, an even more accurate evaluation of the degree of oxidation can be obtained by measuring oligopolymers and ox-TG at the same time because part of the latter are involved in polymerization reactions and part of them remain in the oil. It has also been demonstrated that ox-TG and oligopolymers act as pro-oxidants in oils (Yoon et al., 1988) and are precursors of volatile oxidation products (Frankel et al., 1988).

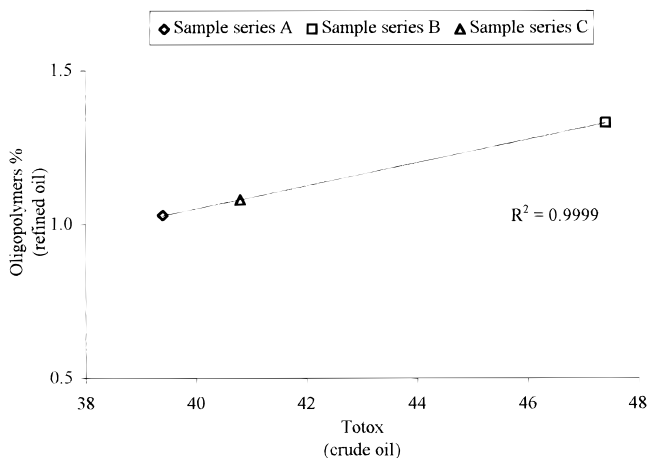


Figure 3. Contents of oligopolymers in refined olive-pomace oils plotted against TOTOX (total oxidation = 2PV + *p*-AV) of the corresponding crude oils.

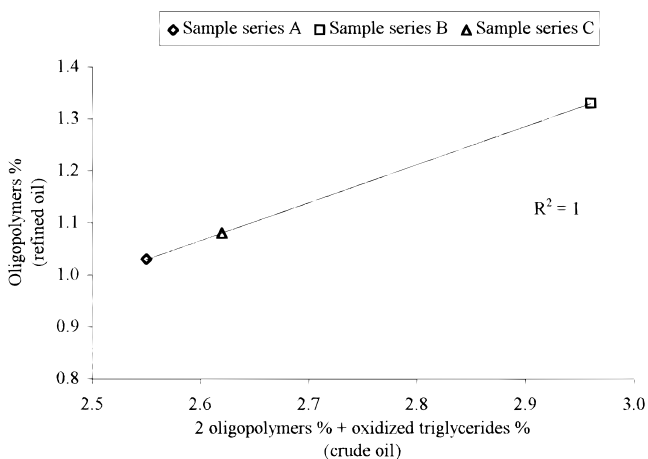


Figure 4. Oligopolymeric contents of refined olive-pomace oils plotted against twice the percent of oligopolymers plus percent of ox-TG of the corresponding crude oils.

Hence, the concomitant measurement of the two classes of compound concurs to estimating the shelf life of an oil.

The influence the state of oxidative degradation of the crude oil had on the chemical characteristics of oils that had been refined using the same technology was studied by analyzing sample series A–C from processing plant 1 over time. Once the data in Table 2 were processed, significant linear correlations were shown to exist between the total oxidation index of the crude oils, TOTOX (2PV + *p*-AV), and the oligopolymer content of the refined oils ($R^2 = 0.9999$; Figure 3) as well as between twice the percent content of oligopolymers plus the percent value of ox-TG of the crude oils and the content of oligopolymeric substances of the refined oils ($R^2 = 1$; Figure 4). It was interesting to note how the same result was obtained both when degradation of the crude oil was evaluated by means of conventional determinations and when more complex analyses, which measured specific classes of oxidative degradation products, were performed. The fact that we examined only three series of samples may not allow us to draw final conclusions, but one may infer that the high values of R^2 are indicative of a general trend, namely, that the formation of oligopolymeric substances in oils that have been refined using the same technological procedures depends on the overall level of oxidative degradation of the crude oil. Hence, we may reasonably state that the

percent value of oligopolymers can be used as a reliable index of oxidative degradation of freshly refined oils.

Finally, as may be seen from Table 2, diglycerides decreased in all of the series due to intense refining. Determination of these compounds in the refined oils was instrumental in determining hydrolytic degradation.

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