Effects of Free Fatty Acids on Oxidative Stability of Vegetable Oil

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ABSTRACT: The effect of free fatty acid (FFA) content on the susceptibility to thermooxidative degeneration of vegetable oils was determined by Rancimat analysis. A prooxidant effect of FFA was observed in all filtered oils, independently of lipidic substrate and of its state of hydrolytic and oxidative alteration. The intensity of this effect was related to FFA concentration, but regression analysis of the experimental data did not show a general correlation law between FFA concentration and induction time (I_t). Different results were obtained for freshly processed virgin olive oils, characterized by postpressing natural suspension–dispersion: opposite behavior was observed of FFA content as regards oxidative stability, depending on the presence of suspended-dispersed material. This fact is of interest because the dispersed particles play a double stabilizing effect on both oxidative and hydrolytic degradation. These results showed that avoidance of oil filtration is highly desirable to extend olive oil's shelf life.

Paper no. J8835 in *JAOCS 76,* 325–329 (March 1999).

KEY WORDS: Oil oxidation, Rancimat test, vegetable oil acidity, virgin olive oil stability.

Fats, oils, and lipid-containing foods are oxidized by air contact at different rates, and this results in sensory and nutritional degradations. One of the most important parameters that influences lipid oxidation is the degree of unsaturation of its fatty acids. The presence of natural compounds having different chemical structures that exhibit antioxidant activity may also affect the oxidative rate (1–7). Another lipid alteration is lipid hydrolysis, with consequent free fatty acid (FFA) generation, by chemical or enzymic action. This phenomenon is of particular interest in water-containing lipidic matrices, such as butter and virgin olive oils during olive processing.

Although the original causes and the consequences of oxidative and hydrolytic degradation processes are quite different, they seem to interact with each other and contribute to the reduction of the oil shelf life. In fact, several papers (8–10) have been published on the prooxidant action of FFA. It seems to be exerted by the carboxylic molecular group, which accelerates the rate of decomposition of hydroperoxides (9).

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This study reports detailed data on the effect of FFA content on the susceptibility to thermooxidative degeneration of several vegetable oils. Oxidative stability was evaluated by accelerated tests performed with a Rancimat apparatus (11–22). Although the Rancimat induction time (I_t) values cannot be directly converted into real shelf life terms, rapid methods are needed to compare oxidative stability of fats and oils since I_t at ambient storage conditions is too long for practical determination. Accelerated methods employ one or more of the various prorancidity factors (temperature, air flow, ultraviolet light, catalysts) to speed up the deterioration and shorten the stability test period. In order to find a possible correlation between Rancimat test data and shelf life, two approaches were tried. The first was based on the Arrhenius-type correlation between I_t and temperature (13,16). However, mechanistic changes may occur at lower temperatures, resulting in deviation from a linear relationship between log I_t and temperature with consequent misleading predictions of the shelf life of the oils. The second one was based on the effective correlation between I_t and real storage conditions (19,22). In this case results are not generally applicable: the regression coefficient depends on the oil type and experimental conditions (oil surface area exposed to atmosphere, depth of the oil). Moreover, lack of predictive ability may be due to the presence of phospholipids and volatile antioxidants, such as butylated hydroxytoluene (BHT).

Even though accelerated methods are affected by this limitation, they are a useful tool for comparison of lipid oxidative rates when only one parameter is changed, such as FFA concentration, temperature, or addition of specific components or organic extracts.

EXPERIMENTAL PROCEDURES

Analytical grade reagents and solvents were supplied by Carlo Erba (Milano, Italy).

The following vegetable oils were analyzed: cold-pressed high oleic sunflower oil (sample 1), supplied by AL.FA. Olii Crudi S.r.l. (Fabro, Italy); refined grapeseed oil (sample 2), purchased in a market; refined olive oil (sample 3), supplied by Carapelli S.p.A. (Firenze, Italy); six virgin olive oils—and three filtered ones (samples 4, 5, and 6), purchased in a market, and three freshly produced artisanal oils (samples 7, 8, and 9), characterized by postpressing suspended-dispersed materials.

Modified oil matrices were prepared by bleaching or filtration and/or by adding FFA or methyl oleate to the original oils as follows: (i) Between 0 and 3% of FFA was added to the sunflower, grapeseed, and refined olive oils. FFA was obtained by saponification (NGD method C39-1976) (23) of a portion of the corresponding oils. For sunflower oil, FFA was also purified by thin-layer chromatography (TLC) on 200 × 200 × 0.5 mm silica gel G (Stratochrom SI; Carlo Erba) and eluted with *n*-hexane/diethyl ether (60:40, vol/vol); the corresponding TLC band was scraped off and extracted with chloroform. (ii) Between 0 and 3% of pure oleic acid (Sigma Chimica) was added to the sunflower and virgin olive oils. (iii) Between 0 and 3% of methyl oleate (Sigma Chimica, Milano, Italy) was added to one of the virgin olive oils (sample 6). (iv) Cloudy virgin olive oil (sample 7) was filtered with a Sartorius pressure filter SM 16249 (Firenze, Italy), using two different types of filters (paper filter and 0.45 µm Teflon membrane). Afterward, between 0 and 3% of pure oleic acid was added to the filtered oils. (v) Cloudy virgin olive oil (sample 8) was filtered with a 0.45 µm Teflon membrane, and known amounts of pure oleic acid (between 0 and 3%) were added. (vi) Cloudy virgin olive oil (sample 9) was bleached with clay and treated with increasing amounts of pure oleic acid. Bleaching was performed by adding 5% (w/w) of bleaching clay (Prolit PN, Caffaro S.p.A., Milano, Italy) to an oil/*n*-hexane solution (1:1, vol/vol), which was stirred for 1 h at room temperature. The bleached oil was recovered by centrifugation, and solvent was removed at 40°C by using a vacuum rotary evaporator. (vii) Membrane-filtered sample 5 was treated with the same number of moles of tridecanoic, palmitic, and oleic acid (Sigma Chimica).

The acid value (AV) (NGD method C10-1976) (23) and the peroxide value (PV) (NGD method C35-1976) (23) of the original oils were measured. Two replicates of each determination were performed. Increasing AV required for the tests were obtained by adding stoichiometric amounts of FFA to 10 g of oil; in order to verify the actual values reached, the initial and final acidities were measured on 3 g of oil.

The accelerated oxidative test was performed, in three replicates, on all original and modified oil samples by using a Metrohm Rancimat model 679 (Herisau, Switzerland). Five grams of each sample was employed; the heater temperature was set at 110°C; the rate of air flow through the samples was adjusted to 20 L/h; and the volatile reaction products were caught into a trap containing 60 mL of distilled water. Glassware was scrupulously cleaned according to the following procedure. Reaction vessels and antifoam rings were heated for 2 h with a 10% solution of Extran MA 01 (Merck, Darmstadt, Germany), followed by cooling and rinsing with tap water, acetone, and distilled water. Conductivity cells and electrodes were soaked overnight with detergent solution and then rinsed with tap water, acetone, and distilled water. All glassware was oven-dried at 80°C before use.

Regression analysis of the experimental data was performed by the Prism program (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSION

Figures 1, 2, and 3 show the results of FFA addition to oils on the Rancimat induction time. FFA addition shortened the I_t of all vegetable oils, as previously reported (8–10). TLC-purified FFA, obtained from high-oleic sunflower oil (Fig. 2), allowed exclusion of any anti- or prooxidant activity which might be due to the presence of trace amounts of unsaponifiable constituents that could have been extracted with the FFA during saponification. On the other hand, no significant effect of the TLC silica powder on the oil stability was detected. This was verified by adding to sunflower oil the dry extract obtained by washing TLC adsorbent with chloroform.

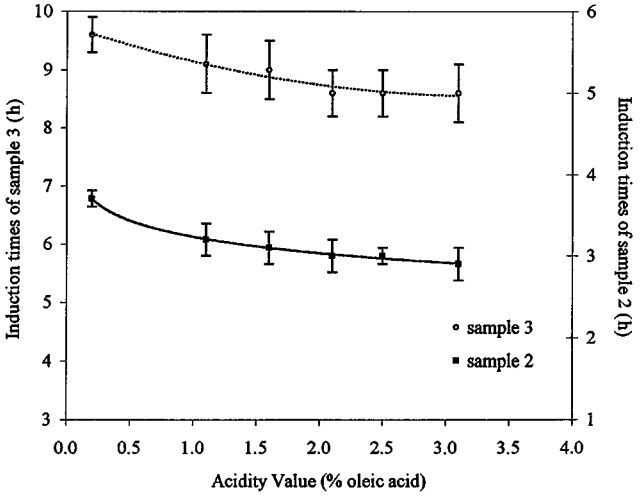

FIG. 1. Thermooxidative behavior (Rancimat Test) of refined grapeseed oil (sample 2) and refined olive oil (sample 3) with increasing amounts of free fatty acid (FFA) obtained by saponification of a portion of the corresponding oils added. Error bar amplitude matches mean ± SD of three replicates.

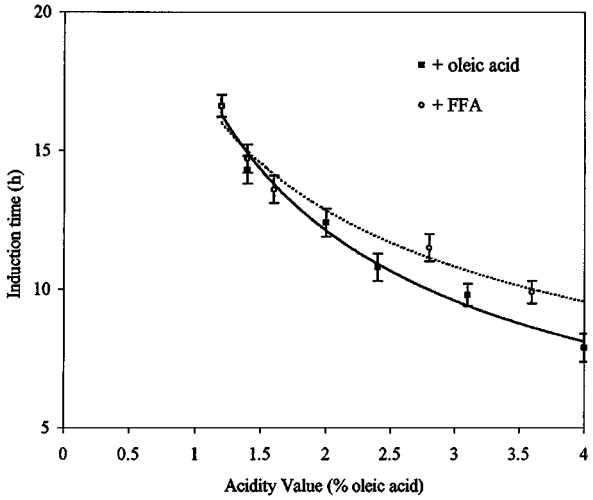

FIG. 2. Thermooxidative behavior (Rancimat Test) of sunflower oil (sample 1) with increasing amounts of oleic acid or FFA obtained by saponification added. Error bar amplitude matches mean ± SD of three replicates. For abbreviation see Figure 1.

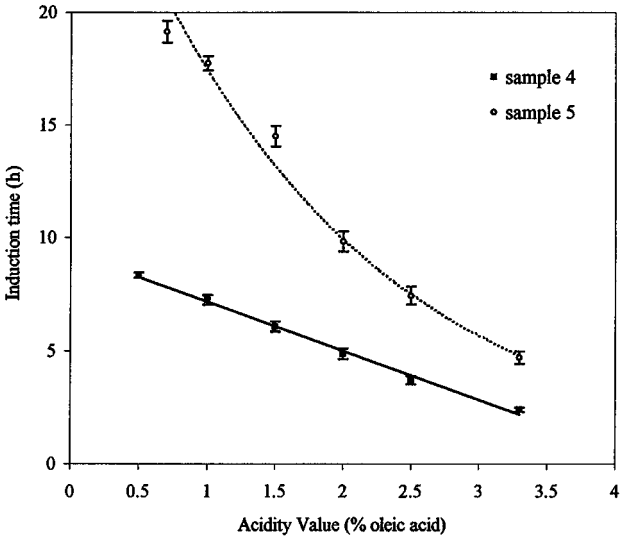

FIG. 3. Thermooxidative behavior (Rancimat Test) of filtered virgin olive oils (samples 4 and 5) with increasing amounts of oleic acid added. Error bar amplitude matches mean ± SD of three replicates.

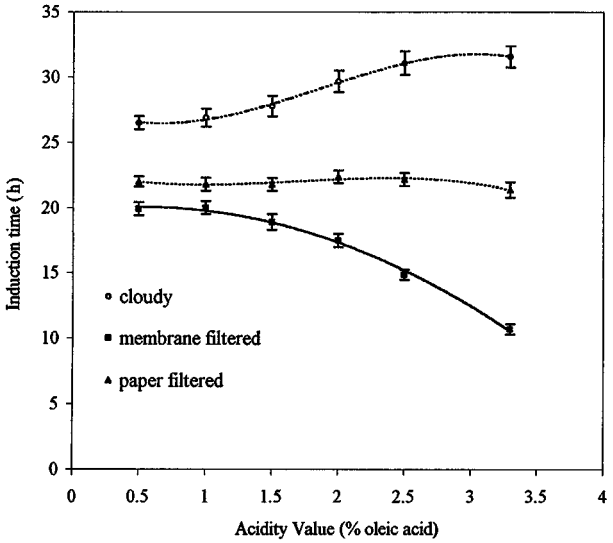

FIG. 5. Thermooxidative behavior (Rancimat Test) of untreated, paper-filtered, and Teflon-filtered (0.45 µm) cloudy virgin olive oil (sample 7) with increasing amounts of oleic acid added. Error bar amplitude matches mean ± SD of three replicates.

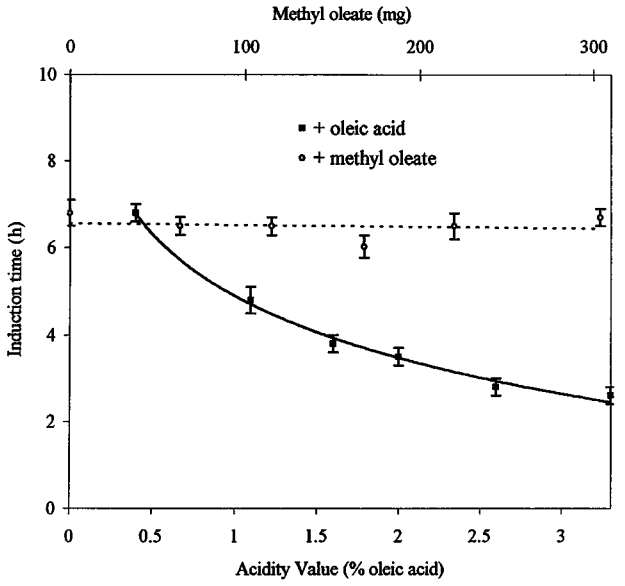

FIG. 4. Thermooxidative behavior (Rancimat Test) of a virgin olive oil (sample 6) with increasing amounts of oleic acid and methyl oleate added. Error bar amplitude matches mean ± SD of three replicates.

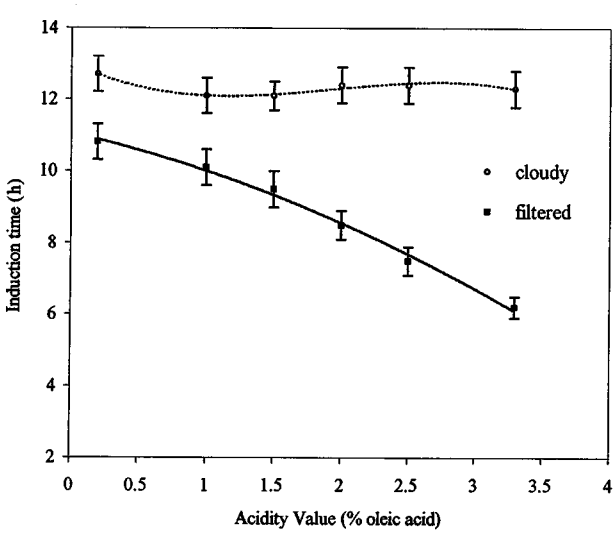

FIG. 6. Thermooxidative behavior (Rancimat Test) of untreated and Teflon-filtered (0.45 µm) cloudy virgin olive oil (sample 8) with increasing amounts of oleic acid added. Error bar amplitude matches mean ± SD of three replicates.

The prooxidant effect of the free carboxylic group was confirmed when a fatty acid methyl ester was added to a filtered virgin olive oil (Fig. 4). Indeed, the conversion of the carboxylic group into a methyl ester nullified the prooxidant activity.

Different results were obtained for freshly processed virgin olive oils (Figs. 5–7), characterized by postpressing natural suspension–dispersion: in two samples opposite behavior was observed to those observed in the other vegetable oils, while sample 8 showed a Rancimat I_t unrelated to FFA content. These trends, however, became similar to those of the other oils when the suspended-dispersed material was totally eliminated by filtration (Figs. 5 and 6) or treatment with bleaching clays (Fig. 7). For all clear vegetable oils studied, a prooxidant effect of FFA was observed independently of lipidic substrate and of its state of hydrolytic and oxidative alteration (Table 1). The intensity of this effect was related to FFA concentration, but regression analysis of the experimental data was not able to find a general correlation law between FFA concentration and I_t (Table 2).

The suspended-dispersed material of cloudy virgin oil exerted a positive stabilizing effect; in fact, its elimination produced shorter I_t. For the cloudy oils, an opposite behavior was observed of FFA content on susceptibility to thermooxidative

TABLE 1
Quality Parameters of Original Oil Samples

#	Oil	AV[a]	PV[b]	I_t[c]
1	Cold-pressed sunflower oil	1.21 ± 0.02	5.4 ± 0.2	16.6 ± 0.4
2	Refined grapeseed oil	0.20 ± 0.01	2.3 ± 0.2	3.7 ± 0.1
3	Refined olive oil	0.21 ± 0.02	1.9 ± 0.1	9.6 ± 0.3
4	Extra virgin olive oil (filtered)	0.49 ± 0.02	23.1 ± 0.9	8.3 ± 0.1
5	Extra virgin olive oil (filtered)	0.71 ± 0.02	4.4 ± 0.2	19.1 ± 0.5
6	Extra virgin olive oil (filtered)	0.41 ± 0.01	31.2 ± 0.7	6.8 ± 0.2
7	Extra virgin olive oil (cloudy)	0.51 ± 0.02	5.9 ± 0.3	26.5 ± 0.5
8	Extra virgin olive oil (cloudy)	0.19 ± 0.01	19.3 ± 0.5	12.5 ± 0.7
9	Extra virgin olive oil (cloudy)	0.42 ± 0.01	5.3 ± 0.3	26.7 ± 0.8

[a]AV, acid value (% oleic acid); mean ± SD of two replicates.
[b]PV, peroxide value (meq O/kg); mean ± SD of two replicates.
[c]I_t, induction time (h); mean ± SD of three replicates.

degeneration, depending on the presence of suspended-dispersed material. These effects balanced out for sample 8 and the oil filtered by paper (Fig. 6). On the other hand, the antioxidative effect of the suspended particles was lower than that of the dispersed particles, which may be due to the higher surface/dimension ratio of the dispersed particles, since they had a smaller diameter than the suspended ones. The amino or imino groups of lignin derived from olive nut are primarily accountable for cloudy material. It could be hypothesized that, after reacting with FFA, these amino or imino groups liberated the phenolic groups that were previously bonded to hydrogen, a state in which they were not able to display any antioxidant activity. In fact, in a previous paper (24) it was demonstrated that FFA bonded to dispersed particles and precipitated with them as a brown-colored residue. Therefore, suspended and dispersed particles that constitute the veiling of a virgin olive oil may play a double stabilizing role with regard to both oxidative and hydrolytic degenerations. These effects are so positive that avoidance of oil filtration is highly desirable in order to extend olive oil's shelf life. Also, it is important to minimize hydrolytic degradation of the oil by enzymic catalysis, which is related to several parameters such as quality of olives, storage conditions of olives before processing, and time required for the separation of oil during olive processing.

Table 3 displays the effect of the same number of moles of different FFA on the oxidative stability of the same substrate (sample 5). Chainlength had no significant effects on I_t, whereas chain unsaturations made the oil more susceptible to thermooxidative degeneration.

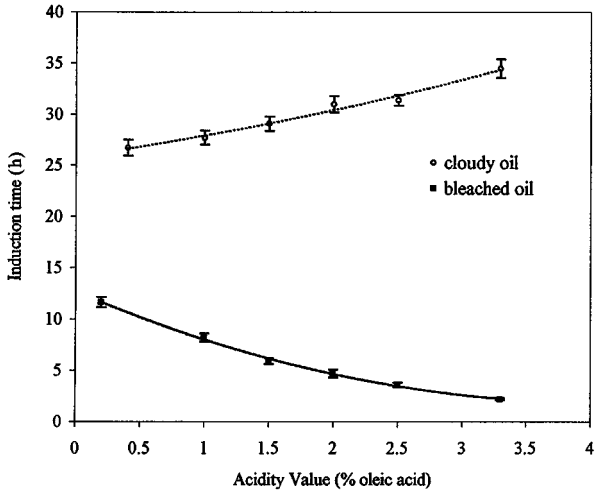

FIG. 7. Thermooxidative behavior (Rancimat Test) of untreated and bleached cloudy virgin olive oil (sample 9) with increasing amounts of oleic acid added. Error bar amplitude matches mean ± SD of three replicates.

ACKNOWLEDGMENTS

This work was supported by funds of the Ministry of University and of Scientific and Technological Research of Italy (MURST, 40%).

TABLE 2
Regression Analysis of the Experimental Data

Sample #[a]	Added acid	Regression type	R^2
1	FFA from saponification	Power	0.97
1	Oleic acid	Power	0.98
2	FFA from saponification	Logarithmic	>0.99
3	FFA from saponification	Binomial	0.96
4	Oleic acid	Exponential	0.99
5	Oleic acid	Linear	>0.99
6	Oleic acid	Logarithmic	>0.99
7	Oleic acid	Binomial	>0.99
8	Oleic acid	Binomial	>0.99
9	Oleic acid	Binomial	>0.99

[a]All oil samples were perfectly clear. FFA, free fatty acids.

TABLE 3
Effect of Different FFA on the Oxidative Stability of a Virgin Olive Oil (sample 5)

	Quantities		
Acid	mg	mmol	I_t (h)[a]
Tridecanoic	113.8	0.531	15.0 ± 0.1
Palmitic	136.1	0.531	14.3 ± 0.3
Oleic	150.0	0.531	10.1 ± 0.2

[a]Mean ± SD of triplicate analyses. For abbreviation see Table 2.

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[Received March 31, 1998; accepted December 10, 1998]