Effects of Herbs on Hydrolytic and Oxidative Degradation of Olive Oil in Canned Tomatoes

Antonietta Baiano[a,*], Tommaso Gomes[b], and Carla Severini[a]

[a]Department of Food Science, University of Foggia, 71100 Foggia, Italy, and [b]Dipartimento di Progettazione e gestione dei sistemi agro-zootecnici e forestali, section of Industrie Agro-Alimentari, University of Bari, 70126 Bari, Italy

ABSTRACT: The effects of adding mixtures of herbs such as garlic, laurel, and marjoram on selected chemical indices of olive oil from canned dried tomatoes were studied for various storage periods of up to 1 yr. Conventional analytical indices measured included acidity, PV, and *p*-anisidine value. Flavored samples showed kinetic constant values that were significantly ($P = 0.001$) higher than unflavored ones, whereas oligopolymer and oxidized TG and DG contents were similar to or slightly higher than in the unflavored samples compared with those with herbs. The addition of the mixture of herbs slowed polymerization reactions but did not inhibit TG oxidation. Discrepancies between the results obtained by conventional analyses and high-performance size-exclusion chromatography indicated that the former approaches were insufficient to determine oxidative degradation of oil as a result of interferences from compounds in the food matrix.

Paper no. J10782 in *JAOCS 82*, 759–765 (October 2005).

KEY WORDS: Canned vegetables, HPSEC, hydrolytic degradation, olive oil, oxidation, preserved foods.

Studies concerning the chemical quality of pure olive oil (1–11) are more prevalent than those about oxidative and hydrolytic degradation of oils added to preserved foods (12–18). Some of these studies have identified and characterized added oil in canned foods. Specific identification of the oils is difficult since there are interactions between the food matrix and the oil. In the case of canned tuna, the identification is made easier by the low linoleic acid content of the tuna oil (<2%) compared with the components of added olive oil (5–13%) (12). For this reason, the addition of other vegetable or animal oils with olive oil in foods would produce an increase in the linoleic acid content so that it would be greater than 13.5%, which is the limit fixed by the "Fat and Derivative Rules" for olive oil (19). According to Bizzozero and Carnelli (15), since the influence of fish fat is negligible, the evaluation of both the oleic acid *trans*-isomers and the sum of linoleic and linolenic *trans*-isomers could be used to ascertain the authenticity of oil added to canned tuna and mackerel. Gomes *et al*. (18) investigated the chemical indices of oils from 30 packaged vegetables and fish. In canned fish, the transfer of fat from the food matrix to the oil is significant, since it reduces the percent oleic acid value and increases FA having a high M.W. and a high degree of unsaturation. With vegetables preserved in oil, the lipid transfer from the food matrix to the oil is negligible.

Studies have been carried out on the influence of processing and storage on chemical parameters and minor compounds of extra virgin olive that is used as a packing oil in preserved foods. Paganuzzi *et al.* (13,14) reported the results of analyses of packing oil of foods in glass containers. In different samples, acidity increased and exceeded 1% after 9 mon of storage, whereas the PV exceeded 20 mequiv O_2/kg after 1 mon. The K_{232} value exceeded 2.4 after 3–6 mon, whereas K_{270} and ΔK were higher than 0.20 and 0.01, respectively, after 1 mon.

Numerous studies have considered the antioxidant effects of spices and herbs added to pure oil. According to Farag *et al*. (20), the antioxidant activity of essential clove oil is greater than that of thyme. Sage, cumin, rosemary, thyme, and cloves show a decreasing order of ability to reduce antioxidant activity of linoleic acid (21). Different terpenic compounds isolated from oregano show varying antioxidant activities (22). According to Protogeras *et al.* (23), some terpenic compounds of sage (especially carnosol) reduce antioxidant activity of olive and corn oils. Both thyme and laurel oils (whose main compounds are terpenes) have a strong antioxidant activity for protecting soy oil (24). Marjoram is effective against peanut oil oxidation (25). The antioxidant activity of some spices and herbs was also studied with sunflower oil (6). The most effective spices, in decreasing order, were basil, parsley, mint, sage, rosemary, garlic, laurel, onion, marjoram, and hot pepper.

No references have been found concerning the antioxidant effect of spices and herbs on oils added to preserved foods. The main purpose of adding spices and herbs to foods canned in oil is to give special flavors to these products. However, spices and herbs also slow down the oxidative degradation of oils.

The objective of this work was to evaluate the effects of herbs on the oxidative and hydrolytic degradation properties of olive oil in canned tomatoes.

MATERIALS AND METHODS

Preparation of tomatoes canned in oil. Preparation of the samples was carried out according to traditional recipes. In brief, dried salted tomatoes were blanched in boiling white vinegar for 30 s, then drained and dried. Samples were then divided into two aliquots, and 2 g of marjoram (moisture 7.9%), 0.5 g of laurel (moisture 4.5%), and 2 g of garlic (moisture 70.0%) were added to half of them. Samples were placed in 280-g capacity

*To whom correspondence should be addressed at Department of Food Science, University of Foggia, Via Napoli 25, 71100 Foggia, Italy.
E-mail: a.baiano@unifg.it or antonellabaiano@tiscali.it

glass containers filled with a commercial olive oil, hermetically sealed with metal caps, and submitted to heat pasteurization (74°C, 15 min), followed by rapid cooling to room temperature. This heat treatment is recommended by the U.S. Food and Drug Administration (26) for canned foods having a pH lower than 4.6. To simulate retail conditions, storage was carried out for 12 mon at room temperature.

Analyses. The oils were separated from the vegetable matrix immediately following pasteurization and at 0.5, 1, 3, 6, and 12 mon of storage. Separated oils were filtered over anhydrous sodium sulfate and stored in glass tubes with screw caps at −20°C until analyzed. The original commercial olive oil was also analyzed as a control.

(i) FA composition, and $C_{18:1}$ and $C_{18:2} + C_{18:3}$ *trans-isomer contents.* These were determined as follows. FAME were prepared according to the 2568/91 EEC Regulation (27) and submitted to GC analysis. The GC system consisted of a GC 8560 Mega 2 (Fisons Instruments, Milan, Italy) equipped with a WCOT (wall-coated open tubular) fused-silica capillary column (Chrompack, Middelburg, The Netherlands), 25 m, 0.32 mm i.d., 0.5 μm film thickness. The oven temperature was held at 200°C. An FID, connected to an integrator, was used (T = 300°C). The carrier gas was helium, which was applied at a flow rate of 2 mL/min. The peaks on the chromatograms were identified by comparison with methyl ester standards (Sigma Chemical, St. Louis, MO). The *trans*-isomer analyses were carried out using a Silar 10 capillary column (Chrompack), 50 m, 0.32 mm i.d., 0.20 μm film thickness, using the GC conditions reported in the 1429/92 EEC Regulation (28).

(ii) Acidity, PV, spectrophotometric indices, p-anisidine values (p-AV), and polar compounds. Acidity was expressed as g of oleic acid per 100 g of oil, according to the AOCS Official Method Cd 3d-63 (29); PV were expressed as mequiv active oxygen per kg of oil, according to AOCS Official Method (29); spectrophotometric indices (K_{232}, K_{270}, and ΔK) were determined according to AOCS Official Method Ch 5-91 (29). *p*-AV were determined according to IUPAC method 2504 (30); polar compounds (PC) were determined according to IUPAC method 2507 (30).

PC were analyzed by high-performance size-exclusion chromatography (HPSEC) to determine oligopolymers, oxidized TG, and DG. The chromatographic system consisted of a PerkinElmer pump, series 10; a 7125 S sample injector (Rheodyne); a 50 μL injector loop, and three PL-gel columns of 0.75 mm i.d. × 30 cm length (PerkinElmer Ltd., Beaconsfield, United Kingdom) in series. The columns were packed with highly cross-linked styrene divinylbenzene copolymers with a particle diameter of 5 μm and pore diameters of 500, 500, and 100 Å. A PL-gel guard column (PerkinElmer Ltd.) of 7.5 mm i.d. × 5 cm length was used. The detector was a differential refractometer (refractive index detector, RID-6A; Shimadzu Corp., Osaka, Japan) connected to an integrator. The elution solvent was HPLC-grade CH_2Cl_2 (purity = 99.9%; Baker, Deventer, The Netherlands) applied at a flow rate of 1.0 mL/min.

Peaks on the chromatograms were identified by using polystyrene standards (Supelco, Milan, Italy) of known molecular masses (M.W. = 4000 and 2000 g/mol), and with tristearin, distearin, and monostearin standards (Sigma-Aldrich, Milan, Italy). For each standard, the elution volume was measured under the same conditions as used in our analyses. The logarithm of MW as a function of elution volume was plotted, and the line of best fit was drawn by using the least-squares method. From the elution volume of each separated peak in a chromatogram, the corresponding MW was obtained (2). Known amounts of oligopolymers, oxidized TG, and DG were obtained by preparative gel permeation chromatography of polar compounds derived from a refined peanut oil and then used as standards according to the HPSEC method. The amount collected for each standard, corresponding to a given class of compounds, was used to prepare a stock solution in CH_2Cl_2 and also containing different concentrations after successive dilutions. These solutions were analyzed by HPSEC following the analytical method developed by Gomes (2). The calibration curves were obtained by plotting the amounts of standards (μg) that had been injected into the HPSEC system loop against the areas of the corresponding chromatogram peaks (31).

Analyses were carried out in duplicate; the difference between the samples did not exceed 6%. Kinetic constants (*K*) and significance (*P*) were also calculated from the linear regression analyses of the curves representing acidity, PV, *p*-AV, spectrophotometric indices, total PC, and the different classes of PC.

RESULTS AND DISCUSSION

The FA composition of the commercial olive oil used in filling the canned product was determined to confirm that it was olive oil (Table 1). The FA composition was in accordance with the "Fat and Derivative Rules" for olive oil (19); i.e., the following percent FA composition was determined: $C_{14:0}$, 0.05; $C_{16:0}$, 12.79; $C_{16:1}$, 1.22; $C_{18:0}$, 2.25; $C_{18:1}$, 73.96; $C_{18:2}$, 8.47; $C_{18:3}$, 0.66; $C_{20:0}$, 0.26; $C_{20:1}$, 0.12; $C_{22:0}$, 0.07; $C_{24:0}$, 0.20. Furthermore, myristic, linolenic, arachic, eicosenoic, behenic, and lignoceric acid contents were below the European legal upper limits (27). Table 1 also the contents of $C_{18:1}$ and $C_{18:2} + C_{18:3}$ *trans*-isomers of both the original oil and that which was separated from the vegetable matrix after 12 mon of storage. The percentages of these *trans*-isomers were within the limits fixed for the pure oil (27), and neither the pasteurization nor the storage induced noticeable changes.

TABLE 1
$C_{18:1}$ and $C_{18:2} + C_{18:3}$ *trans* Isomer Contents of the Original Olive Oil and the Oils Separated from the Canned Vegetable Matrix After 12 mon of Storage (*n* = 2)

Oils	$C_{18:1}$ *trans* (%)[a]	$C_{18:2} + C_{18:3}$ (%)[a]
Original olive oil	0.05	< 0.05
Olive oil from unflavored, canned tomatoes after 12 mon of storage	0.05	< 0.05
Olive oil from flavored, canned tomatoes after 12 mon of storage	0.05	< 0.05

[a]Percentage of the total FA composition.

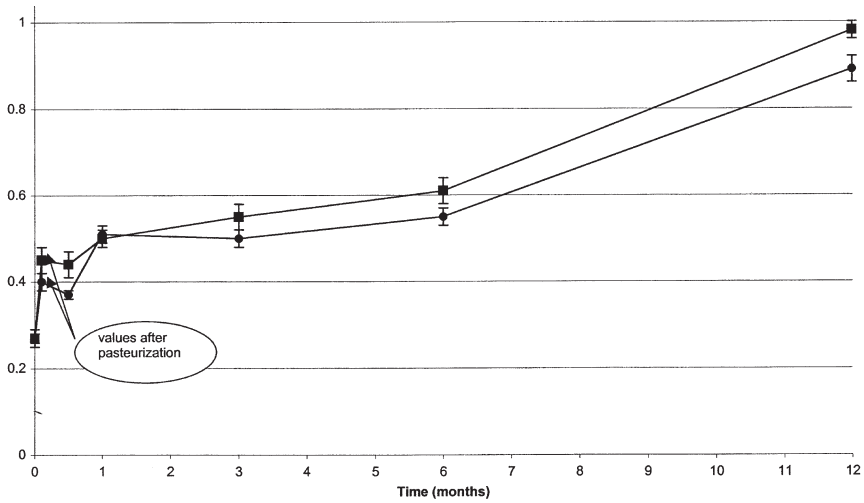

FIG. 1. Acidity percentages, expressed as g of oleic acid/100 g of fat, of oils added to canned tomatoes. (●) Olive oil from pasteurized, unflavored tomatoes; (■) olive oil from pasteurized, flavored tomatoes.

Acidity (%), which is a measure of the FFA and therefore is traditionally used to evaluate hydrolytic reactions, increased from 0.27 to 0.89% for unflavored samples and to 0.98% for the flavored ones after 12 mon of storage (Fig. 1). Kinetic constants were 0.0422 and 0.0475, respectively, at a P level of 0.001. With all conditions, which were the same for both types of samples, these differences could be explained by the presence of herbs (garlic, in particular) that affected moisture content. Despite the contact with a moist vegetable matrix (tomatoes), acidity values can be considered satisfactory. Also, after 12 mon of storage, changes in acidity values were less than 1%. The upper European legal limit for pure olive oil is 1.5%.

Figure 2 shows the trends in PV of both series of samples. For all samples, PV reached a maximum at 3 mon of storage and then decreased. This behavior can be explained by the initial increase in hydroperoxides (odorless, flavorless compounds, produced during the primary step of oxidation) and their successive breakdown to give aldehydes and ketones responsible for the off-flavors (secondary oxidation) and nonvolatile compounds such as oligopolymers. The PV of all samples were always lower than the European legal limits fixed for the pure olive oil (15 mequiv O_2/kg). The kinetic constants (calculated on the rising part of the curves) of flavored samples (1.803) were higher than K of the unflavored tomatoes (1.497). Since there was a faster increase in hydroperoxide compounds detected in the flavored oils, the addition of herbs would seem to have a significant pro-oxidant effect ($P = 0.05$).

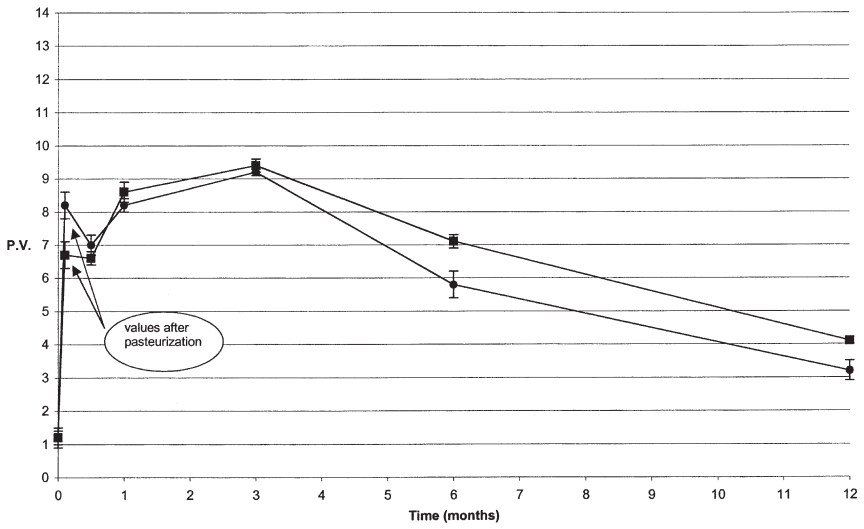

FIG. 2. Peroxide values, expressed as mequiv O_2/kg of fat, of oils added to canned tomatoes. (●) Olive oil from pasteurized, unflavored tomatoes; (■) olive oil from pasteurized, flavored tomatoes.

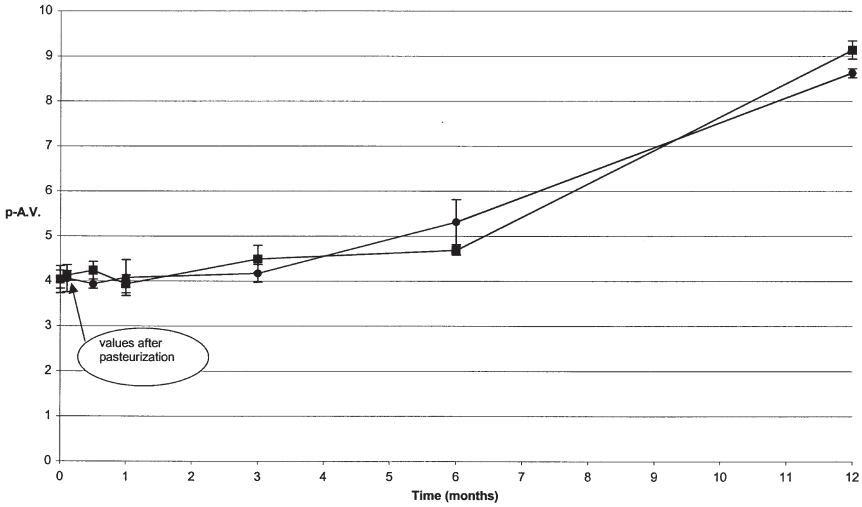

FIG. 3. *p*-Anisidine values (p-AV) of oils added to canned tomatoes. (●) Olive oil from pasteurized, unflavored tomatoes; (■) olive oil from pasteurized, flavored tomatoes.

Secondary oxidation was measured as the *p*-AV. An increase in *p*-AV usually corresponds to a decrease in PV. From a graphical point of view, the addition of herbs would seem not to affect this parameter (Fig. 3) whereas the kinetic constants were significantly ($P = 0.001$) different (0.3914 for flavored samples vs. 0.3750 for unflavored). Data concerning secondary oxidation confirm those related to primary oxidation and also the prooxidant effect of the mixture of marjoram, laurel, and garlic.

Spectrophotometric constants are another measure of oil oxidation. Doubly conjugated bonds, which absorb at 232 nm, are indices of primary oxidation whereas triply conjugated bonds, absorbing at 270 nm, are indices of secondary oxidation. In Figure 4 the trends of spectrophotometric indices as a function of the storage time are shown. All the spectrophotometric indices were below the European legal limits set for pure olive oil (3.40, 1.00, and 0.13, for K_{232}, K_{270}, and ΔK, respectively). Furthermore, instead of a constant increase, these indices showed the lowest values after a month of storage. Kinetic constants found for the unflavored samples (0.0222, 0.0151, and 0.0006 for K_{232}, K_{270}, and ΔK, respectively) were higher than those of the flavored ones (0.0207, 0.0073, and 0.0004) and seem to indicate an antioxidant effect of the herb mixture used.

Concerning the PC, pasteurization and storage caused a

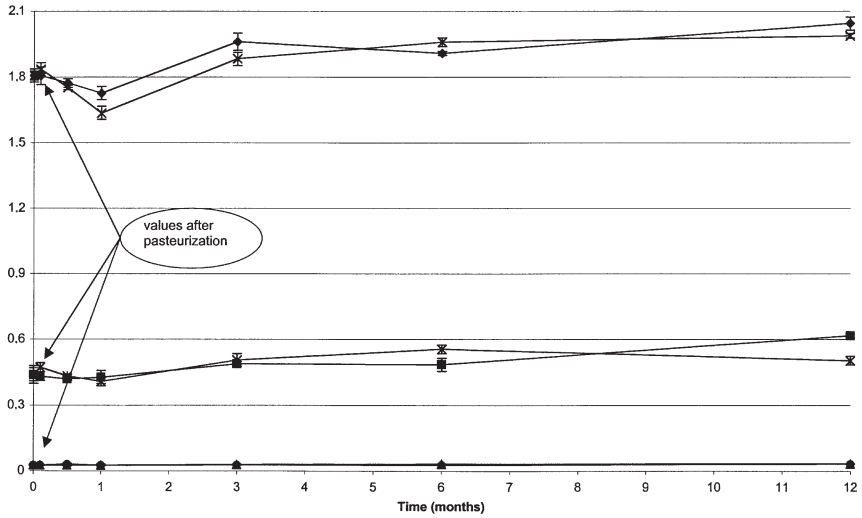

FIG. 4. Spectrophotometric indices of olive oils added to canned tomatoes. (◆) K_{232} olive oil from unflavored, canned tomatoes; (×) K_{232} olive oil from flavored canned tomatoes; (■) K_{270} olive oil from unflavored, canned tomatoes; (✻) K_{270} olive oil from flavored, canned tomatoes; (▲) ΔK_{270} olive oil from unflavored canned tomatoes; (●) ΔK_{270} olive oil from flavored, canned tomatoes.

TABLE 2
Percentages of Total Polar Compounds and the Different Classes of Polar Compounds (n = 2)

Total polar compounds	Oligopolymers	Oxidized TG	DG	FFA	Others	Time pre-, post-heat pasteurized	Total polar compounds	Oligopolymers	Oxidized TG	DG	FFA	Others
Olive oils from heat-pasteurized, unflavored canned tomatoes							Olive oils from heat-pasteurized, flavored canned tomatoes					
4.26	0.19	0.49	3.04	0.27	0.27	**Prepasteurized**	4.26	0.19	0.49	3.04	0.27	0.27
4.71	0.19	0.62	3.19	0.40	0.31	**Postpasteurized, 1 d**	4.74	0.19	0.61	3.19	0.45	0.30
4.81	0.19	0.66	3.30	0.37	0.29	**2 wk**	4.65	0.19	0.62	3.10	0.44	0.30
5.03	0.19	0.68	3.35	0.51	0.30	**1 mon**	4.82	0.19	0.65	3.19	0.50	0.29
4.93	0.20	0.73	3.28	0.50	0.22	**3 mon**	4.76	0.20	0.72	3.07	0.55	0.22
5.01	0.20	0.78	3.23	0.55	0.25	**6 mon**	5.06	0.22	0.76	3.23	0.61	0.24
5.80	0.28	0.81	3.56	0.89	0.26	**12 mon**	5.69	0.24	0.79	3.44	0.98	0.24

small increase in oligopolymers, oxidized TG, DG, and FFA. PC are mainly made up of products of TG oxidation, polymerization, and hydrolysis. HPSEC analyses measure TG oligopolymers, oxidized TG, and DG. Oligopolymers are mainly found in refined oils (32) although small amounts may also form in virgin olive oils through oxidation (3). The amount of TG oligopolymers present in an oil is considered a reliable index of oxidative degradation (3,33,34). Oxidized TG comprise all of the products of TG oxidation and are thus another important class of substances to determine whether there has been oxidation of an oil. The determination of DG enables a more complete measurement of hydrolytic degradation than does free FFA alone. The sum of percent TG oligopolymers and oxidized TG represents the degree of total oxidation of an oil since it is made up of the substances from oxidation and polymerization of TG.

The percentages of total PC and their different classes for all the oils studied are reported in Table 2. Total PC and their different classes (especially oligopolymers) showed only small increases during storage, as demonstrated by their low K values. Hydrolytic and oxidative degradation did not seem to affect these samples. The analysis of PC gave different results when compared with those obtained by the above mentioned conventional methods. In fact, unflavored samples showed DG, oligopolymer, and oxidized TG kinetic constants higher than those observed for those flavored samples (Table 3). In particular, the addition of herbs seemed to inhibit strongly the formation of oligopolymers, with only a minor influence on the formation of oxidized TG. This could mean that the mixture of garlic, laurel, and marjoram, in the quantity used, did not inhibit oxidation but lowered polymerization reactions.

Figure 5 shows the trends of total oxidation (sum of oligopolymers and oxidized TG). One can see that most of the parameters studied increased during the first months of storage. This was followed by a decreased rate of increase for hydrolytic degradation and oxidation.

The results obtained from this study demonstrate that, to evaluate the quality of added olive oil during the shelf life of canned vegetables, the use of the conventional chemical analyses is not sufficient and can even give inaccurate results because of interferences and interactions with the other ingredients and compounds from the food matrix. HPSEC is a better technique for investigating the hydrolytic and oxidative state of oils. FFA analysis, PV, spectrophotometric indices, and p-AV indicate the pro-oxidant activity of the added herbs. HPSEC analysis demonstrates that the addition of herbs does

TABLE 3
Kinetic Constants and Related Significance (P) (n = 2)

Mean K values	Unflavored	P	Flavored	P
DG	0.027	0.05	0.0261	0.01
Oligopolymers	0.0069	0.001	0.0044	0.001
Oxidized TG	0.0193	0.05	0.0191	0.05
Oligopolymers + oxidized TG	0.0262	0.01	0.0235	0.01
Total polar compounds	0.0922	0.01	0.0924	0.001

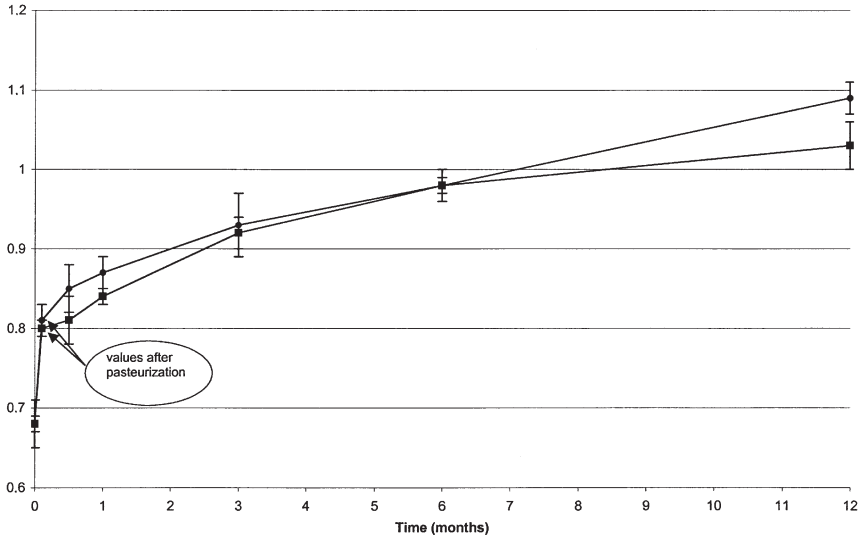

FIG. 5. Percentages of oligopolymers + oxidized TG in oils added to canned tomatoes. (●) Olive oils from pasteurized, unflavored, canned tomatoes; (■) olive oils from pasteurized, flavored canned tomatoes.

not significantly affect the oil properties, apart from the inhibition of polymer formation. The choice of a good quality oil, the use of small containers (to avoid a prolonged heat treatment), and the application of a properly controlled heat pasteurization process result in a canned product with improved shelf life.

REFERENCES

1. Morchio, G., A. Di Bello, C. Mariano, C., and E. Fedeli, Individuazione di particolari oli rettificati in oli vergini di oliva [Revelation of Particular Refined Oil in Virgin Olive Oil], *Riv. Ital. Sostanze Grasse 66*:251–257 (1989).
2. Gomes, T., Oligopolymer, Diglyceride and Oxidized Triglyceride Contents as Measures of Olive Oil Quality, *J. Am. Oil Chem. Soc. 69*:1219–1223 (1992).
3. Gomes, T., A Survey of the Amounts of Oxidized Triglycerides and Triglyceride Dimers in Virgin and "Lampante" Olive Oils, *Fat Sci. Technol. 97*:368–372 (1995).
4. Gomes, T., I contenuti di oligopolimeri di trigliceridi e trigliceridi ossidati dell'olio di oliva. Possibilità di una migliore caratterizzazione delle diverse classi merceologiche [Triglyceride Oligopolymer and Oxidized Triglyceride Contents of Olive Oil. How Can the Different Commerical Grades Characterization Be Improved], *Riv. Ital. Sostanze Grasse 72*:345–349 (1995).
5. Proto, M., L'importanza della determinazione degli acidi grassi *trans*-isomeri negli oli di oliva e di semi di girasole, *Ind. Aliment. 31*:769–770 (1992).
6. De Felice, M., T. De Leonardis, and S. Comes, L'aromatizzazione degli oli alimentari. Effetti sull'autossidazione [The Aromatization of Edible Oils. Effects on Auto-oxidation], *Ibid. 32*:249–253 (1993).
7. Di Cesare, L.F., G. Sansovini, M. Riva, and A. Schiraldi, Produzione di oli di oliva aromatizzati all'aglio—Influenza dei metodi di produzione convenzionali e dei trattamenti con microonde sui componenti solforati dell'aglio degli oli e sulla loro stabilità all'ossidazione [The Influence of Traditional and Microwave Technology on Garlic Sulfur Compounds in Garlic Aromatized Oils and on the Stability of Olive Oil to Oxidation], *Ibid. 32*:825–835 (1993).
8. Alloggio, V., F. Caponio, and T. De Leonardis, Influenza delle tecniche di preparazione della pasta di olive sulla qualità dell'olio. Nota I. Profilo quali-quantitativo delle sostanze fenoliche, mediante HPLC, in olio d'oliva vergine della cv. Ogliarola Salentina, *Riv. Ital. Sostanze Grasse 73*:355–360 (1996).
9. Alloggio, V., and F. Caponio, Influenza delle tecniche di preparazione della pasta di olive sulla qualità dell'olio. Nota II. Evoluzione delle sostanze fenoliche e di alcuni parametri di qualità in funzione della maturazione delle drupe in olio d'oliva vergine della cv. Coratina [The Influence of Olive Paste Preparation Techniques on the Quality of Olive Oil. Note II. Evolution of Phenolic Substances and of Some Quality Parameters Referred to the Ripening of Drupes in Virgin Olive Oil from the Coratina cv.], *Ibid. 74*:443–447 (1997).
10. Paganuzzi, V., F. De Iorgi, F., and A. Malerba, Influenza dell'invecchiamento e della temperatura su alcuni parametri previsti dal Reg. CEE n. 2569/91 sull'olio di oliva [Influence of Aging and Temperature on Some Parameters Provided by EEC Regulation N. 2568/91 on Olive Oil], *Riv. Ital. Sostanze Grasse 74*, 231–239 (1997).
11. Fogliano, V., A. Ritieni, S.M. Monti, G. Randazzo, C. Maresca, D. Della Medaglia, and R. Sacchi, Attività antiossidante dei composti fenolici dell'olio extra-vergine di oliva, in *Ricerche e Innovazioni nell'Industria Alimentare* (Chiriotti Editori)*, Vol. III*, Pinerolo, Italia, 1998, pp. 512–515.
12. Cucurachi, A., L'olio d'oliva di copertura del tonno [Olive Oil for Tunafish Canning], *Riv. Ital. Sostanze Grasse 43*:335–342 (1966).
13. Paganuzzi, V., F. De Iorgi, and A. Malerba, Sull'olio di oliva vergine extra impiegato in conserve alimentari confezionati in contenitori di vetro Nota 1—Variazione di alcuni parametri chimico-fisici al mutare delle condizioni di produzione e nel corso dell'invecchiamento, *Ibid. 72*:529–537 (1995).
14. Paganuzzi, V., F. De Iorgi, and A. Malerba Sull'olio di oliva vergine extra impiegato in conserve alimentari confezionati in contenitori di vetro Nota 2—Variazione del contenuto di alcuni componenti minori al mutare delle condizioni di produzione e nel corso dell'invecchiamento, *Ibid. 73*:409–415 (1995).
15. Bizzozero, N., and L. Carnelli, Composizione acidica e trans in saturazione dell'olio di copertura di sgombri e tonni conservati in scatola [Fatty Acid Composition and *trans* Unsaturation of

the Covering Oil of Canned Mackerels and Tunas], *Ind. Aliment. 35*:680–683 (1996).
16. Cavallaro A., N. Bizzozero, L. Carnelli, and P. Renon, Composizione acidica e *trans* insaturazione dell'olio di copertura di sardine conservate in scatola [Fatty Acid Composition and trans Unsaturation of the Covering Oil of Canned Sardines], *Ind. Aliment. 35*:801–805, 812 (1996).
17. Mucciarella, M.R., and V. Marsilio, Influenza del processo tecnologico di preparazione e della conservazione sulle caratteristiche chimico-fisiche dell'olio di oliva vergine extra usato come copertura di conserve vegetali in vasetti di vetro [Fatty Acid Composition and *trans* Unsaturation of the Covering Oil of Canned Sardines], *Riv. Ital. Sostanze Grasse 74*:105—112 (1997).
18. Gomes, T., F. Caponio, A. Baiano, and T. De Pilli, Misura della degradazione ossidativa ed idrolitica di olio di oliva utilizzato come copertura in conserve alimentari, *Ibid. 75*:77–82 (1998).
19. Fat and Derivative Rules, Caratteristiche degli Oli Vergini di Oliva, dell'Olio di Oliva e dell'Olio di Sansa di Oliva. Stazione Sperimentale per le Industrie degli Oli e dei Grassi [Characteristics of Virgin olive Oil, Olive Oil and Olive Husk Oil], Milan, Italy, 1994.
20. Farag, R.S., A.Z.M.A. Badei, and G.S.A. El Baroty, Influence of Thyme and Clove Essential Oils on Cottonseed Oil Oxidation, *J. Am. Oil Chem. Soc. 66*:800–804 (1989).
21. Farag, R.S., A.Z.M.A. Badei, F.M. Hewedi, and G.S.A. El Baroty, Antioxidant Activity of Some Spice Essential Oils on Linoleic Acid Oxidation in Aqueous Media, *Ibid. 66*:792–799 (1989).
22. Vekiari, S.A., C. Tzia, V. Oreopolou, and C.D. Thomopoulos, Isolation of Natural Antioxidants from Oregano, *Riv. Ital. Sostanze Grasse 70*:25–28 (1993).
23. Protogeras, J., V. Oreopolou, and C. Tzia, Natural Antioxidants from *Salvia triloba* for Vegetable Oils, *Ibid. 75*:507–509 (1998).
24. Zygadlo, J.A., A.L. Lamarque, D.M. Maestri, and N.R. Grosso, Empleo de aceites esenciales como antioxidantes naturales [Use of Essential Oils as Natural Antioxidants], *Grasas Aceites 46*:285–288 (1995).
25. Maestri, D.M., J.A. Zygadlo, A.L. Lamarque, D.O. Labuckas, and C.A. Guzmán, Effect of Some Essential Oils on Oxidative Stability of Peanut Oil, *Grasas Aceites 47*:397–400 (1996).
26. U.S. FDA, Food and Drug Administration: Acidified Foods, *Code of Federal Regulation*, title 21, volume 2. Cite: 21*CFR*114 (2002).
27. *Official Journal of European Community L 248* (5 September): 2568/91 EEC Regulation (1991).
28. *Official Journal of European Community L 150* (26th May): 1429/92 EEC Regulation (1992).
29. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1993.
30. IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn., Blackwell Scientific Publications, Oxford, United Kingdom, 1987.
31. Gomes, T., and F. Caponio, Effort to Improve Quantitative Determination of Oxidation and Hydrolysis Compound Classes in Edible Vegetable Oils, *J. Chromatogr. 844*:77–86 (1999).
32. Eder, S.R., The Formation of Artifacts During Deodorization of Fats and Oils, *Fette Seifen Anstrichm. 84*:136 (1982).
33. Paradise, A.J., and W.W. Navar, Evaluation of New Methods for the Assessment of Used Frying Oils, *J. Food Sci. 46:*449–451 (1981).
34. White, P.J., and Y. Wang, A High Performance Size-Exclusion Chromatographic Method for Evaluating Heated Oils, *J. Am. Oil Chem. Soc. 63*:914–920 (1986).

[Received January 12, 2004; accepted August 10, 2005]