DSC Determination of Thermally Oxidized Olive Oil

E. Vittadini[a], J.H. Lee[a], N.G. Frega[b], D.B. Min[a], and Y. Vodovotz[a,*]

[a]Food Science & Technology Department, The Ohio State University, Columbus, Ohio 43210, and [b]Dipartimento di Scienze Alimentari e della Nutrizione, Facoltà di Agraria, Università degli Studi di Ancona, 60131 Ancona, Italy

ABSTRACT: The feasibility of using DSC as an analytical method to evaluate the autoxidation of olive oil at 50°C and thermal oxidation at 93 and 180°C in 10-mL airtight vials was studied. DSC peak enthalpy and peak crystallization temperatures were compared with headspace oxygen depletion and headspace volatiles in oxidized oil samples. A single crystallization peak was found in olive oil. The crystallization peak shifted to lower temperatures, and the enthalpy associated with this phase transition decreased as the exposure time increased at 93 and 180°C. DSC peak enthalpy in olive oil at 50, 93, and 180°C showed correlations of 0.84, 0.91, and 0.95, respectively, with headspace oxygen depletion in sample bottles. Correlation of DSC initial peak temperature with headspace oxygen depletion was 0.53, 0.87, and 0.95 at 50, 93, and 180°C, respectively. Correlations of DSC peak enthalpy and initial peak temperature with headspace volatiles at 180°C were 0.95 and 0.97, respectively. These results indicate that DSC is a good analytical method to determine the oxidative stability of olive oil at frying temperature.

Paper no. J10508 in *JAOCS 80*, 533–537 (June 2003).

KEY WORDS: DSC, headspace oxygen content, headspace volatile compounds, olive oil oxidation.

DSC monitors the changes of physical or chemical properties of a material as a function of temperature by detecting the heat changes associated with phase transitions such as crystallization and glass transition (1,2). DSC compares the rate of heat flow of a sample to that of an inert reference material as both are heated or cooled. DSC thermograms are characterized by endothermic and/or exothermic peaks whose area is proportional to the enthalpy gained or lost by the material undergoing phase transition. The phase transitions take place over a specific temperature range depending on the sample compositions and physical properties (1).

DSC has been used in lipid chemistry in the characterization of melting and crystallization of pure edible oils (3), heat of fusion and crystallization (4), the fat liquid/solid ratio (5), polymorphic forms (6,7), and the quantification of oil in fried potatoes (8) and olives (9). Pioneering works in the use of thermal analysis (10,11) indicated the feasibility of using thermogravimetry, pressure differential calorimetry, and differential thermal analysis to determine the oxidative stability of oil. Tan and Che Man and their coworkers reported the systematic use of DSC to study the quality of vegetable oils. These researchers studied the correlation of DSC analysis with standard chemical methods for fat oxidation such as total polar compounds (12,13), iodine value (13,14), percentage of FFA, anisidine value, and PV (13). In all cases, DSC parameters were found to correlate well with the standard chemical methods. Gloria and Aguilera (8) also reported a good correlation of DSC results with both the amounts of total polar compounds and the viscosity and color of heated oils. Isothermal DSC was used to predict the oxidative stability of vegetable oil (16), suggesting that this technique could be a valuable tool for routine analysis in the oil industry. DSC also has been proposed to be a valuable tool for characterization and identification of vegetable oils.

DSC has significant advantages compared to the classical chemical methods, including small sample size (<20 mg), minimal sample preparation, no use of chemical agents or solvents, short experimental times, and simplicity of operation.

Olive oil has been widely consumed in Europe, and the demand for olive oil products has increased among ethnic groups, gourmets, and consumers of health and diet foods due to its possible health benefits and disease prevention (17). A rapid, reproducible, and sensitive analytical technique is needed to monitor the thermal stability of olive oil during processing. DSC studies on the autoxidation and thermally induced oxidation of olive oil at different temperatures have not been reported in the literature.

The objective of this work was to evaluate DSC as an analytical method to evaluate the autoxidation at 50°C and thermal oxidation of olive oil model systems at 93 and 180°C.

EXPERIMENTAL PROCEDURES

Materials. Fresh extra virgin olive oil from Tuscany was donated by the University of Ancona, Italy, and kept refrigerated in the dark until samples were prepared. A manual solid-phase microextraction (SPME) fiber holder unit, 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), serum bottles, the fiber assembly holder, silicone-coated rubber septa, and aluminum caps were purchased from Supelco (Bellefonte, PA).

Heat treatment. Olive oil (2 g) was placed in a 10-mL vial (25 × 40 mm, 20 mm diameter from Supelco, Inc.) and sealed

*To whom correspondence should be addressed at Food Science & Technology Department, The Ohio State University, Parker Food Science and Technology Bldg., 2015 Fyffe Ct., Columbus, OH 43210.

E-mail: vodovotz.1@osu.edu

airtight with a silicone-coated rubber septum and an aluminum cap. The oxidative stability of olive oil by autoxidation was studied at 50°C for 1, 3, 6, 9, 14, and 28 d. The accelerated oxidation stability of olive oil was studied at 93°C for 2, 4, 8, 12, 24, 72, 144, and 216 h. The oxidative stability of olive oil at frying conditions was studied at 180°C for 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 72, 144, and 216 h. Samples were prepared in duplicate.

All samples were allowed to cool to room temperature for 30 min after thermal treatment and then either analyzed immediately or placed in a –20°C freezer until used.

DSC analysis. The 8–15 mg of oil were placed into an open aluminum pan and analyzed with a DSC 2920 (TA Instruments, New Castle, DE). Samples were equilibrated at 20°C for 1 min and then scanned at a 2°C/min rate to –60 or –70°C. At least duplicate analyses were carried out for each sample. A single and slow scanning rate (2°C/min) was selected to ensure thermal equilibrium of the sample and to allow for comparison among samples (18).

Headspace oxygen analysis. The headspace oxygen in sample bottles of olive oil was determined by injecting 100 µL headspace gas of samples into a GC equipped with a thermal conductivity detector. A column (1.8 m × 0.32 cm) packed with 60/80 Molecular Sieve 13 X (Alltech Associates, Inc., Deerfield, IL) was used. The flow rate of hydrogen was 20 mL/min. Temperatures of the oven, injector, and thermal conductivity detector were 40, 120, and 150°C, respectively.

Headspace volatile compound analysis by SPME. The 65 µm PDMS/DVB trapped the headspace volatile compounds in the sample bottles for 30 min at 30°C in a water bath. The isolated volatile compounds by PDMS/DVB were desorbed for 2 min at the injector port of a gas chromatograph with an FID.

GC conditions. The Hewlett-Packard 5890 gas chromatograph was equipped with a 0.75 mm i.d. glass injection liner, an FID, and a 30 m × 0.25 mm i.d., 1.0 µm film, DB-5, from J&W Scientific (Folsom, CA). The oven temperature was held at 40°C for 2 min and increased from 40 to 160°C at the rate of 6°C/min and from 160 to 210°C at 8°C/min. The injector and detector temperatures were 250 and 300°C, respectively. The flow rate of nitrogen carrier gas was 1.0 mL/min.

RESULTS AND DISCUSSION

DSC analysis. A typical DSC thermogram of fresh olive oil during cooling from room temperature to –60°C at a scanning rate of 2°C/min is shown in Figure 1. DSC analysis of olive oil samples was carried out upon cooling from a melted state to achieve high analytical reproducibility (18). Samples undergoing cooling showed an exothermic phase transition due to crystallization of the oil such as that shown in Figure 1 for fresh olive oil. The crystallization peak in the DSC thermogram showed an initial temperature of crystallization (T_0), the peak maximum temperature of crystallization (T_p), the end peak temperature of crystallization (T_f), and the peak area as enthalpy of crystallization (Fig. 1). The peak line shape of the fresh sample was Gaussian-like. The CV of the T_0, T_p, T_f, and area in the DSC thermogram of fresh olive oil were 0.5, 0.7, 0.6, and 0.8%, respectively, which shows good reproducibility of the DSC analysis.

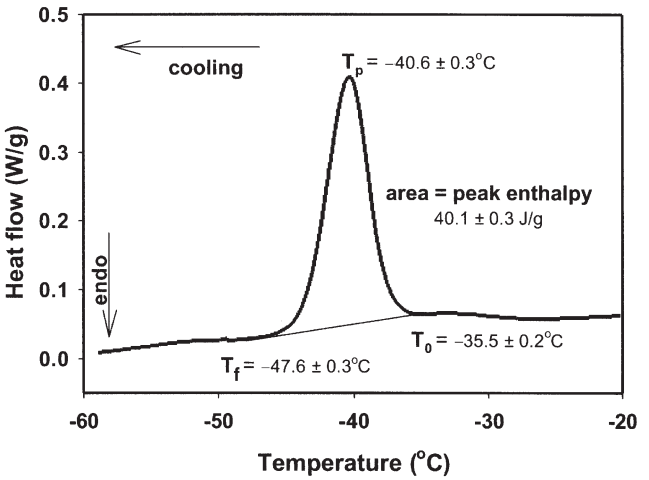

FIG. 1. Typical DSC thermogram of fresh olive oil during cooling from room temperature to –60°C at a scanning rate of 2°C/min. The parameters used to characterize the peak are shown. T_p, peak maximum temperature of crystallization; T_f, end peak temperature of crystallization; T_0, initial temperature of crystallization.

DSC thermograms of olive oil at 50°C representing autoxidation and at 93 and 180°C for thermal oxidation are shown in Figures 2A, 2B, and 2C, respectively. A shift of the crystallization peak toward lower temperatures was observed in all samples as a consequence of oxidation, which agrees with previous reports on seed oils (8,13). The shift in T_0 of the crystallization peak toward lower temperatures was found to be a function of the oxidation conditions. Samples held at 93 and 180°C showed a more evident shift of the crystallization peak at shorter times of exposure. A substantial shift of the crystallization peak was observed in the sample held at 180°C after only 1 h of thermal treatment (Fig. 2C), compared to 144 h in the sample held at 93°C (Fig. 2B). A very slight shift was observed in the sample held at 50°C for 28 d (672 h).

The crystallization peak of oil held at 93 and 180°C not only shifted to lower temperatures but also was subject to a line shape change. The peak spanned a greater temperature range and decreased in heat flow (height) with increasing length of the high-temperature treatment. A similar phenomenon was also reported for seed oils (8,13).

The effects of storage time at 50, 93, and 180°C on the peak enthalpy and the initial temperature of crystallization of the olive oil in the DSC thermogram are shown in Figures 3 and 4, respectively. The enthalpy of the crystallization of olive oil decreased with increasing length of high-temperature treatment (Fig. 3), indicating that a smaller amount of sample was crystallized under the experimental conditions (assuming no change in the latent heat of crystallization of the sample upon oxidation). Samples held at 180°C showed a marked decrease in peak enthalpy immediately after exposure to oxidative conditions. On the other hand, samples held at 50 and 93°C

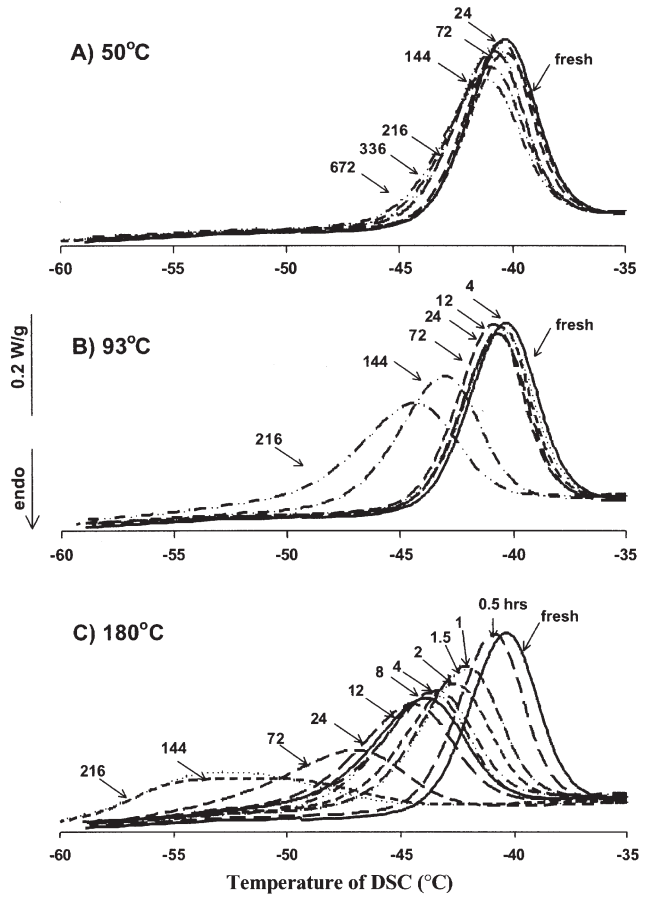

FIG. 2. DSC thermograms of olive oil during storage at 50 (A), 93 (B), and 180°C (C) in the dark. The number of hours of storage at 50, 93, and 180°C is shown on each thermogram.

showed an initial plateau with small changes in DSC crystallization enthalpy followed by a decrease in enthalpy that indicates little (or no) degradation of the oil at these lower oxidative temperatures (Fig. 3). A similar trend was also found for T_0 of the olive oil. T_0 decreased as the olive oil was oxidized, more perceptibly and quickly in the samples held at 180°C and more gradually, after an initial constant plateau, for the samples held at 93 and 50°C (Fig. 4).

The shift of the crystallization peak to lower temperatures (Fig. 4) and the decrease in crystallization enthalpy (Fig. 3) could be due to the depletion of TG, the increase of the FFA content, and/or the increase of viscosity during oxidation (12,15). Moreover, the ability of the crystallizing molecules to come in contact and align to form a crystal detectable under the DSC experimental conditions may have been hindered by the presence of charged and more disordered molecules (increase of polar compounds and low M.W. compounds) and by the increased viscosity of the oxidized olive oil (8).

To assess the feasibility of using DSC to quantify the effect of autoxidation of oils at various temperatures, thermally treated olive oil was also subjected to traditional chemical methods of analysis.

Headspace oxygen and headspace volatile compound analyses. The depletion of headspace oxygen content commonly has been used to determine the degree of oxidation of oils (19). The effects of storage time at 50, 93, and 180°C on the headspace oxygen content of olive oil in the dark are shown in Figure 5. As expected, the headspace oxygen content in airtight sample bottles decreased as storage time increased (Fig. 5). Fast oxygen depletion was observed in the thermally oxidized olive oil (from 21 to 7% within 12 h at 180°C and from 21 to 8.5% in 144 h at 93°C), whereas headspace oxygen depletion occurred very slowly in the 50°C sample (1.5% decrease over 28 d). The depletion of headspace

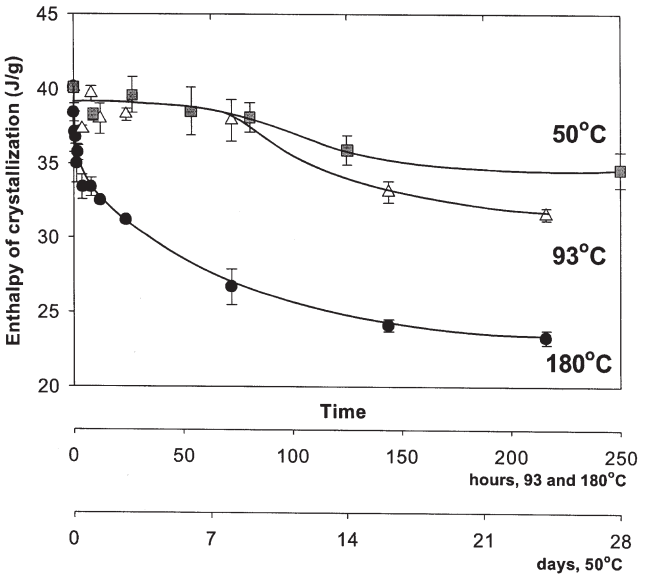

FIG. 3. Effects of storage days at 50°C, and hours at 93 and 180°C, on the peak enthalpy of olive oil (DSC analysis).

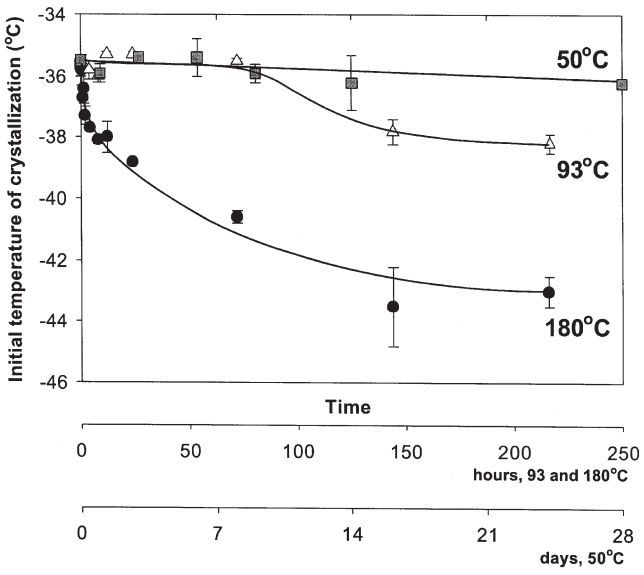

FIG. 4. Effects of storage days at 50°C, and hours at 93 and 180°C, on the initial temperature of crystallization (T_0) of olive oil (DSC analysis).

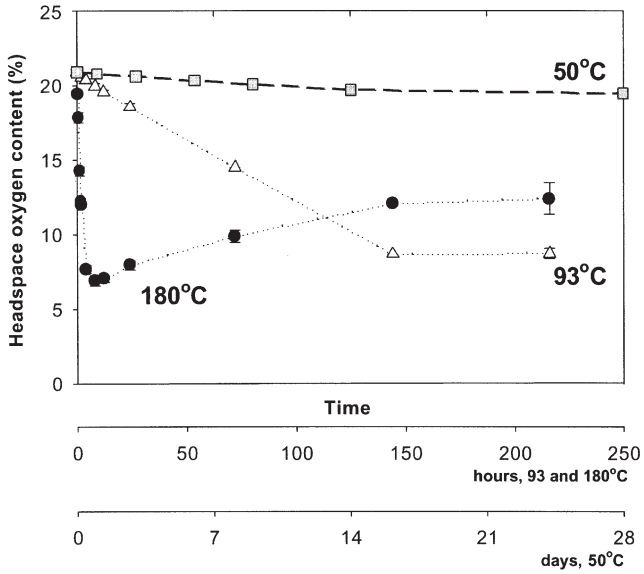

FIG. 5. Effects of storage days at 50°C, and hours at 93 and 180°C, on the headspace oxygen content of olive oil in the dark.

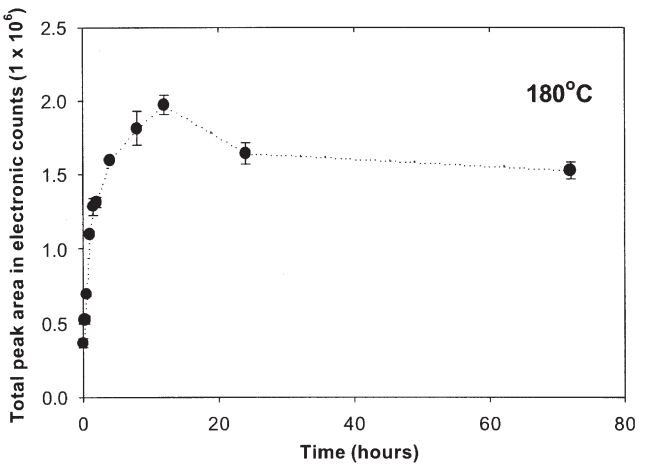

FIG. 6. Effects of storage hours at 180°C on the headspace volatile compounds of olive oil in the dark.

oxygen in airtight olive oil sample bottles is due to the reaction between unsaturated FA and oxygen.

An interesting observation was the increase of headspace oxygen in oil samples held at 180°C as storage times increased from 12 to 216 h (Fig. 5). This was attributed to the oxygen generated by chemical degradation of oil components containing the peroxyl moiety as shown in the following reactions:

$$2ROOH \rightarrow H_2O_2 \rightarrow H_2O + 1/2\ O_2 \quad [1]$$

$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2 \quad [2]$$

The radical elimination of a peroxy radical resulted in the release of headspace oxygen as a leaving group (20). The rate of oxygen formation became higher than the oxygen depletion rate only after 12 h at 180°C, and the overall headspace oxygen content increased.

Analysis of headspace volatile compounds has been used to determine the degree of oxidation in oil (21), and it has been applied in this study to follow the oxidation of olive oil at 180°C (Fig. 6). The headspace volatile compounds increased as the storage time increased up to 12 h and then decreased (Fig. 6) as expected, and followed the trend observed in the headspace oxygen content results at 180°C (Fig. 5). The decrease of headspace volatile compounds at 180°C from 12 to 72 h may be due to the higher formation rate of nonvolatile compounds from volatile compounds through polymerization, compared to the formation rate of volatile compounds in closed-bottle model systems (22).

Correlation of DSC analysis and headspace oxygen or volatile compounds analysis. Correlations (R^2) of DSC peak enthalpy and initial temperature of crystallization with headspace volatile compounds and headspace oxygen content in oxidizing olive oil samples are shown in Table 1.

Linear R^2 of DSC peak enthalpy and DSC initial temperature of crystallization with headspace volatile compounds were 0.95 and 0.97, respectively, in airtight vials at 180°C up to 12 h (Table 1). DSC peak parameters (enthalpy and T_0) have a very good correlation with the depletion of headspace oxygen content. Bimodal linear correlation ($R^2 \geq 0.95$) was found in the samples stored at 180°C in the 0 to 12 and 12 to 216 h ranges (Table 1), reflecting the decrease and subsequent increase of headspace oxygen in this sample. Linear correlations were found for the olive oil samples held at 50 and 93°C over the range of time considered in this study (Table 1). DSC enthalpy showed a better correlation to the headspace oxygen depletion than DSC peak temperature. A possible explanation

TABLE 1
Correlation of Headspace Oxygen Content and Volatile Compounds with DSC Peak Enthalpy (enthalpy) and DSC Initial Temperature of Crystallization (T_0) of Olive Oil Bottles During Storage[a]

		Enthalpy	R^2	Time	T_0	R^2	Time
Headspace oxygen (%)	180°C	$y = -61.2 + 2.1x$	0.95	0–12 h	$y = 204.3 + 5.21x$	0.95	0–12 h
	180°C	$y = 25.6 - 0.6x$	0.99	12–216 h	$y = -29.6 - 1.0x$	0.98	12–216 h
	93°C	$y = -34.6 + 1.4x$	0.91	0–216 h	$y = 151.2 + 3.7x$	0.87	0–216 h
	50°C	$y = 10.6 + 0.3x$	0.84	0–672 h	$y = 61.4 + 1.2x$	0.53	0–672 h
Volatile compounds (1×10^6)	180°C	$y = 8.99 - 0.21x$	0.95	0–12 h	$y = 17.50 - 0.44x$	0.97	0–12 h

[a]y: headspace oxygen (%) for the headspace oxygen analysis; electronic counts (1×10^6) for the volatile compound analysis. x: storage time (days for 50°C and hours for 93 and 180°C).

is that peak enthalpy is a parameter representing the phase transition over the entire temperature range, and it reflects the properties and the composition of the whole sample. Initial peak temperature of crystallization, on the other hand, describes the crystallization temperature of specific components in the sample, but it does not reflect the actual composition of the sample. The change in molecular conformation of oxidizing lipids does not affect the entire sample simultaneously; it is a more gradual process, and some nonoxidized molecules will crystallize at the same temperature as the fresh sample. More extended chemical degradation of the sample such as polymerization may be needed to induce a shift in the initial peak temperature.

DSC can be used as an analytical tool in determining the oxidative stability of olive oil, and it is expected to show similar behavior in other vegetable oils undergoing thermal oxidation. The DSC method has many advantages compared to standard chemical methods for determining the oxidative stability of oil including: short experimental times, limited sample preparation, small sample size, no use of solvents, simplicity to operate, and good reproducibility. This study shows that DSC is a reproducible analytical method and can be used to evaluate the oxidative stability of the thermally oxidized oils such as the deep-fat frying process at 180°C.

REFERENCES

1. Wonderlich, B., *Thermal Analysis*, Academic Press, New York, 1990.
2. Ma, C., V.R. Harwalkar, and T.J. Maurice, Instrumentation and Techniques of Thermal Analysis in Food Research, in *Thermal Analysis of Foods*, edited by V. Harwalkar and C.Y. Ma, Elsevier Science, New York, 1990, pp. 1–16.
3. Kaiserberger, E., DSC Investigation of the Thermal Characterization of Edible Fats and Oils, *Thermochim. Acta 151*:83–90 (1989).
4. Sessa, D., Derivatization of Cocoa Butter Equivalent from Jojoba Transesterified Ester *via* a Differential Scanning Calorimetry Index, *J. Sci. Food Agric. 72*:295–298 (1996).
5. Toro-Vazquez, J., E. Dibildox-Alvarado, V. Herrera-Coronado, and M. Charo-Alonso, Tryglyceride Crystallization in Vegetable Oils, in *Engineering and Food for the 21st Century*, edited by J. Welti-Chanes, G.V. Barbosa-Canovas, and J.M. Aguilera, CRC Press, Boca Raton, 2002, pp. 105–123.
6. Chapman, G.M., E.E. Akehurst, and W.B. Wright, Cocoa Butter and Confectionery Fats. Studies Using Programmed Temperature X-Ray Diffraction and Differential Scanning Calorimetry, *J. Am. Oil Chem. Soc. 48*:824–830 (1971).
7. Lawler, P., and P. Dimick, Crystallization and Polymorphism of Fats, in *Food Lipids: Chemistry, Nutrition, and Biotechnology*, edited by C. Akoh and D.B. Min, Marcel Dekker, New York, 1998, pp. 229–250.
8. Aguilera, J., and H. Gloria, Determination of Oil in Fried Potato Products by Differential Scanning Calorimetry, *J. Agric. Food Chem. 45*:781–785 (1997).
9. Iannotta, N., C. Oliviero, G.A. Ranieri, and N. Uccella, Determination of the Oil Content in Olives by the DSC Technique, *Eur. Food Res. Technol. 212*:240–243 (2001).
10. Hassel, R., Thermal Analysis: An Alternative Method of Measuring Oil Stability, *J. Am. Oil Chem. Soc. 53*:179–181 (1976).
11. Buzas, I., E. Kurucz, and J. Hollo, Study of the Thermoxidative Behavior of Edible Oils by Thermal Analysis, *Ibid. 56*:685–688 (1979).
12. Tan, C., and Y.B. Man, Quantitative Differential Scanning Calorimetric Analysis for Determining Total Polar Compounds in Heated Oils, *Ibid. 76*:1047–1057 (1999).
13. Tan, C., and Y.B. Man, Differential Scanning Calorimetric Analysis for Monitoring the Oxidation of Heated Oils, *Food Chem. 67*:177–184 (1999).
14. Haryati, T., Y.B. Che Man, A. Asbi, H.M. Ghazali, and L. Buana, Determination of Iodine Value of Palm Oil by Differential Scanning Calorimetry, *J. Am. Oil Chem. Soc. 74*:939–942 (1997).
15. Gloria, H., and J.M. Aguilera, Assessment of the Quality of Heated Oils by Differential Scanning Calorimetry, *J. Agric. Food Chem. 46*:1363–1368 (1998).
16. Tan, C.P., Y.B. Che Man, J. Selamat, and M.S.A. Yusoff, Comparative Studies of Oxidative Stability of Edible Oils by Differential Scanning Calorimetry and Oxidative Stability Index Methods, *Food Chem. 76*:385–389 (2002).
17. Harwood, J.L., and P. Yaqoob, Nutritional and Health Aspects of Olive Oil, *Eur. J. Lipid Sci. Technol. 104*:685–697 (2002).
18. Tan, C., and Y.B. Che Man, Differential Scanning Calorimetric Analysis of Palm Oil, Palm Oil-Based Products, and Coconut Oil: Effect of Scanning Rate Variation, *Ibid. 76*:89–102 (2002).
19. Min, D.B., T.L. Li, and H.O. Lee, Effects of Processing Steps on the Contents of Minor Compounds and Oxidation of Soybean Oil, *Adv. Exper. Med. Biol. 434*:161–180 (1998).
20. Gardner, H.W., Reactions of Hydroperoxides—Products of High Molecular Weight, in *Autoxidation of Unsaturated Lipids*, edited by H.W. Chan, Academic Press, London, 1987, pp. 51–93.
21. Doleschall, F., Z. Kemény, K. Recseg, and K. Kodblacvári, A New Analytical Method to Monitor Lipid Peroxidation During Bleaching, *Eur. J. Lipid Sci. Technol. 104*:14–18 (2002).
22. Warner, K., Chemistry of Frying Fats, in *Food Lipids*, edited by C.C. Akoh, and D.B. Min, Marcel Dekker, New York, 1998, pp. 167–180.

[Received December 2, 2002; accepted February 27, 2003]