Impact of Extraction Method on Yield of Lipid Oxidation Products from Oxidized and Unoxidized Walnuts

Tammy D. Crowe[1], **Troy W. Crowe**[1], **Lawrence A. Johnson, and Pamela J. White***

Food Science and Human Nutrition Department and Center for Crops
Utilization Research, Iowa State University, Ames, Iowa 50011

ABSTRACT: The objective of this study was to measure and compare differences in oxidized products of oil extracted from unoxidized and oxidized walnuts using five different extraction methods: (i) mechanical pressing, or solvent extraction with (ii) hexane, (iii) methylene chloride, (iv) chloroform/methanol, or (v) supercritical carbon dioxide (SC-CO_2). Of the extraction methods evaluated, only chloroform/methanol and methylene chloride provided reasonable results for all parameters measured (total lipid yield, FA profile, PV, conjugated dienes, FFA content, and volatile content); however, chloroform/methanol extracted significantly greater levels of volatile compounds. The SC-CO_2 extraction with purified gas was simple and accurate for all data except collection of volatile compounds, as these materials are lost during the lipid extraction. Pressing was neither quantitative nor qualitative, and hexane extraction retrieved significantly lower levels of volatiles than the other methods, except for SC-CO_2.

Paper no. J9996 in *JAOCS 79*, 453–456 (May 2002).

KEY WORDS: Extraction method, extraction of oxidized products, lipid oxidation, oxidized products, solvent extraction of walnuts, supercritical fluid extraction of walnuts, volatile compounds in walnuts, walnut oxidation.

The qualitative and quantitative extraction of lipids is imperative to the success of the analytical procedure in examining the shelf life of high-fat foods, such as walnuts. The quality of fat-containing foods may be assessed by measuring indices of lipid oxidation, such as PV, conjugated dienes, FFA, and volatile compounds. The methods employed to extract lipids from oxidized foods may influence the values obtained for these oxidative products. Extraction conditions, e.g., temperature, time, and type of solvent, have been shown to affect sensory quality (1), volatile content (2), and oxidative stability of the extracted oil (3, 4).

For laboratory-scale extraction, using chloroform/methanol as the extraction solvent (5,6) is generally considered optimal for removal of all lipid classes (7). But direct comparisons of this and other methods, including solvent-free extraction techniques such as pressing and supercritical CO_2 (SC-CO_2), with regard to complete removal of oxidation products and potential artifact production during extraction, have not been published. The purpose of this study was to compare the oxidation products present in the oil extracted from both unoxidized and oxidized walnuts by several different methods.

MATERIALS AND METHODS

Food grade premium walnut pieces were obtained from Diamond Walnut Growers, Inc. (Stockton, CA) and stored at –20°C prior to use. For oxidized samples, walnuts were stored on loosely covered aluminum trays at 60°C in the dark for 8 d. Following extractions, oil samples were stored at –20°C under nitrogen. All extractions were performed in triplicate.

Extraction procedures. Published methods were used for extraction of lipids after grinding or homogenizing by the method suggested for that extraction process.

(i) Methylene chloride extraction. Lipids were extracted according to the method described by O'Keefe *et al.* (8) with modifications. For each extraction, 100 g of walnuts and 300 mL of methylene chloride were blended for 1 min at high speed in a Waring blender. The slurry was vacuum-filtered through Whatman No. 1 filter paper (Maidstone, England), and re-extracted with 200 mL methylene chloride. Solvent was removed by rotary evaporation under vacuum at 40°C.

(ii) Chloroform/methanol extraction. The Bligh and Dyer method (6) with modifications was employed. Walnuts (50 g) were homogenized using a PowerGen Model 125 Homogenizer with a 10 × 195 mm sawtooth generator (Fisher Scientific, Pittsburgh, PA) with 180 mL of water, 200 mL of methanol, and 100 mL of chloroform for 2 min. An additional 100 mL of chloroform was added to the mixture and blended for 2 min more. The homogenate was centrifuged at 500 × *g* for 10 min, the upper layer was removed by aspiration, and the bottom layer was vacuum-filtered through Whatman No. 1 filter paper, re-extracted with 200 mL of chloroform, and refiltered. The chloroform/lipid extract was passed through anhydrous sodium sulfate (Na_2SO_4), and the Na_2SO_4 was rinsed with 100 mL of chloroform. Solvent was removed by rotary evaporation under vacuum at 30°C.

(iii) Hexane extraction. A pilot-scale hexane extraction method described by Shen *et al.* (9) was used. For each

---

[1] Present address: Kraft Foods, Battle Creek, MI 49016.

*To whom correspondence should be addressed at Food Science and Human Nutrition Dept., Iowa State University, 2312 Food Sciences Bldg., Ames, IA 50011-1060. E-mail: pjwhite@iastate.edu extraction, 700 g of walnuts previously ground for 1 min at high speed in a Waring blender was placed in a glass vessel and extracted at 60°C at a ratio of 2:1 (hexane/walnuts). Walnuts from each replication were extracted six consecutive times with fresh hexane for 6 min of recirculation, plus 3 min of drainage, and the collected solvent was pooled. The total extraction time was approximately 45 min. Walnut oil was desolventized in a rotary evaporator under vacuum at 60°C.

(iv) SC-CO_2 extraction. Walnut pieces were ground at high speed in a Waring blender to pass through a 10-mesh screen, and a weighed amount (2.5 g) was placed inside an extraction vessel. Diatomaceous earth (0.5 g) was placed above and below the sample. The extraction vessels were placed in a FastFat9(HT) (ISCO Inc., Lincoln, NE) supercritical fluid extractor, where the contents were extracted with either welding grade (Praxair Inc., Danbury, CT) or Coleman grade CO_2 (Air Products Inc., Allentown, PA) at 10,000 psi for 35 min, a CO_2 flow rate of 5.0 mL/min, and chamber and restrictor temperatures of 85 and 80°C, respectively. The extracted oil was collected into an evaporator flask fitted with a glass wool plug and cooled in an ice bath. A total of 20 runs were performed, with the oil pooled for each of the triplicate extractions.

(v) Pressing. Walnuts (20 g) were placed between two 25 × 25 cm aluminum plates and mechanically pressed at ambient temperature (Black Brothers Laminating and Gluing Equipment, Mendota, IL) at 1,000 psi for 2 to 3 min. Extracted oil was collected using a rubber spatula and filtered through cheesecloth prior to storage. A total of 10 presses were performed, with the oil pooled for each of the triplicate extractions.

Analytical methods. PV and conjugated dienes were determined by the American Oil Chemists' Society (AOCS) official methods Cd 8-53 and Ti 1a-64, respectively (10). FFA were measured according to a modification of method Ca 5a-40 (10) proposed by Rukunudin *et al.* (11) using a 0.5-mL oil sample. Total oil content was determined gravimetrically after the extract was dried to a constant weight as outlined in method Ca 2c-25 (10). FA were prepared for analysis as FAME as previously described (12). All analyses were performed in duplicate and the results averaged.

Volatile analyses were performed using solid-phase microextraction (SPME) as described by Snyder *et al.* (13) with modifications. Briefly, a 2-mL sample of oil with 1 ppm dodecane added as an internal standard was placed in a 20-mL clear glass vial from Supelco Inc., Bellefonte, PA with a Teflon-lined silicone septum. The vial was placed in a 50°C water bath and allowed to equilibrate for 5 min. The SPME fiber (polydimethylsiloxane; Supelco Inc.) was exposed to the headspace for 30 min, thermally desorbed in the inlet of a Hewlett-Packard 5890 Series II GC (Palo Alto, CA) at 230°C, and transferred in helium at 1.7 mL/min onto a Supelco SPB-1 fused-silica capillary column (30 m, 0.25-mm i.d., 0.25 µm film thickness). The gas chromatograph was held for 3 min at an initial temperature of 30°C, increased to a final temperature of 210°C at 6°C/min with a final hold time of 5 min. Volatile compounds were detected by an FID held at 220°C. For MS, a Hewlett-Packard 5970 mass-selective detector was used in place of the FID. Peaks were identified by comparing their retention times and mass spectra with those of known compounds. Standard response curves were prepared for the major volatile compounds in oxidized oils using authentic standards added to stripped soybean oil (sparged at 50°C with helium for 16 h) in concentrations ranging from 10^{-3} to 10^{-7}.

Statistical analysis. Statistical analyses were performed using the General Linear Model Procedures of SAS 6.06 (1991) (SAS, Cary, NC). Tukey's (HSD) Studentized *t*-test was used to test for significant differences between mean values of analytical procedures among treatments, with significance established at a level of $P < 0.05$.

RESULTS AND DISCUSSION

The oil yields by each of the extraction methods are presented in Table 1. As expected, oil extraction by pressing did not result in a quantitative yield. Oil recoveries from walnuts by using organic solvents and SC-CO_2 were not significantly different from each other. Previously, similar results were reported for oil yields from SC-CO_2 extraction when compared with ether/hexane extraction (14), and acid hydrolysis/solvent extraction (15).

FA composition was not influenced by extraction method, with similar relative percentages of the predominant FA in walnut oil achieved by all methods (palmitic 6.5%, stearic 2.6%, oleic 15.4%, linoleic 60.2%, linolenic acids 14.5%, and other 0.8%; data not shown). Also, there were no significant differences in FA composition of the oxidized vs. unoxidized walnuts among extraction methods.

Values for primary oxidation products (PV and conjugated dienes) were not significantly different among the solvent extraction methods used in this study, except for welding grade SC-CO_2. Calvo *et al.* (4) reported that oxidative stability of supercritically extracted sunflower oil could be improved by

TABLE 1
Quantitative Yields (w/w) of Oil Extraction of Unoxidized Walnuts by Various Methods

Extraction method	Total lipids[a] (%)	Extraction method	Total lipids[a] (%)
Hexane	67.2[a]	Chloroform/methanol	69.9[a]
Methylene chloride	69.8[a]	SC-CO_2 (Coleman[b])	68.2[a]
Pressing	32.2[b]	SC-CO_2 (welding[b])	68.2[a]

[a] Values within the two columns marked "total lipids" with the same superscript letter are not significantly different, $P < 0.05$.
[b] Coleman (high-purity) and welding grade CO_2.

TABLE 2
PV, Conjugated Dienes, FFA, Hexanal Content, and Total Volatiles (integrator counts) of Oil Extracted by Various Methods from Unoxidized and Oxidized Walnuts

	PV (meq/kg)	Conjugated dienes (%)	FFA (%)	Hexanal (ppm)	Total volatiles (count × 10^{-5})
Unoxidized walnuts[a]					
Hexane	0.91^{b}	0.13^{a}	0.20^{a}	0	2.8^{a}
Methylene chloride	1.01^{b}	0.12^{a}	0.26^{a}	0	7.1^{b}
Pressing	0.06^{a}	0.11^{a}	0.24^{a}	0	10.3^{b}
Chloroform/methanol	1.02^{b}	0.12^{a}	0.27^{a}	0	14.5^{c}
SC-CO_2 (Coleman)	0.99^{b}	0.13^{a}	0.23^{a}	ND[b]	ND
SC-CO_2 (welding)	1.58^{c}	0.19^{b}	0.22^{a}	ND	ND
Oxidized walnuts[a]					
Hexane	9.41^{b}	0.50^{a}	0.40^{b}	90.9 $(19.1)^{a}$	5.3^{a}
Methylene chloride	8.61^{b}	0.53^{a}	0.23^{a}	252.7 $(5.8)^{b}$	25.4^{b}
Pressing	5.02^{a}	0.46^{a}	0.27^{a}	554.0 $(10.3)^{c}$	31.5^{b}
Chloroform/methanol	10.43^{b}	0.50^{a}	0.29^{a}	627.2 $(2.5)^{d}$	160.0^{c}
SC-CO_2 (Coleman)	8.73^{b}	0.51^{a}	0.28^{a}	ND	ND
SC-CO_2 (welding)	12.37^{c}	0.82^{b}	0.59^{c}	ND	ND

[a] Values within a column for each category (unoxidized or oxidized) with the same letter are not significantly different, $P < 0.05$.
[b] ND, not detectable. Values in parentheses are percentages relative to total peak area.

using ultra-pure CO_2. The increase in primary oxidation products would be expected because of oxidation caused by CO_2 impurities. The PV from oxidized walnut oil extracted with chloroform/methanol tended to be greater than the PV obtained by using the other solvent extraction methods and SC-CO_2 (Coleman), possibly because it is more efficient than the other solvents in extracting polar oxidation products. PV obtained from pressed walnuts for both unoxidized and oxidized walnuts were significantly lower than PV obtained for the other extraction methods (Table 2). Oil extraction by pressing was not quantitative and therefore may not have resulted in a representative sample, particularly in the presence of polar compounds such as hydroperoxides.

Among the extraction methods evaluated, conjugated diene values were significantly greater in both oxidized and unoxidized walnuts oils extracted with welding grade CO_2 (Table 2). No significant differences in conjugated diene values of oil from the unoxidized or oxidized walnuts were measured among the other five extraction methods (Table 2).

Unlike the results from the other analytical tests, the level of FFA in the lipids from unoxidized walnuts extracted with SC-CO_2 (welding) was not different from the FFA levels obtained by the other extraction methods (Table 2). In general, oxidation of the walnut oil would not result in hydrolytic cleavage of the FA from the glycerol backbone of the TAG, so the FFA level would not be expected to change very much with walnut oxidation. Some enzymes present in the walnuts, however, might catalyze the FA cleavage (16), as would water. FFA values were not different among extraction methods for the unoxidized walnuts; however, for oxidized walnuts, oil extracted with SC-CO_2 (welding) had significantly greater FFA than did hexane-extracted oils, which had significantly greater FFA than did oils from all other extraction methods (Table 2). The relatively high extraction temperature and length of solvent exposure to the walnuts during the hexane extraction method may have either solubilized more of the FFA that were present or actually accelerated hydrolytic rancidity. The latter seems likely given that levels of FFA in oil from the oxidized walnuts were increased only after extraction with either hexane or welding grade CO_2, and because impurities in the welding grade CO_2, particularly water, might increase hydrolytic rancidity. As expected, FFA were greatest in the lipids from oxidized walnuts extracted using the lower-quality, welding grade CO_2.

Chloroform/methanol was the most effective among all methods for extraction of total volatile compounds, particularly from the oxidized walnuts (Table 3). Similar increases in hexanal, an important marker of oxidative flavor deterioration, with walnut oxidation was observed for both methylene chloride and pressing (Table 3). The total volatile content of the hexane-extracted walnut oil was lower by approximately an order of magnitude in both the unoxidized and oxidized samples, most likely indicating volatile loss during the extraction or desolventizing step. No volatiles were detected in oils extracted using SC-CO_2 from either the unoxidized or oxidized walnuts. King *et al.* (2) also reported relatively few volatile compounds in the lipid fraction from SC-CO_2-extracted raw beef samples using milder SC-CO_2 extraction conditions than those presented here. These extraction conditions strip the sample of volatiles as a result of the procedure. If desired, the volatile compounds could be captured for further analysis. Several investigators have reported various methods for the collection of volatile compounds from lipids extracted using SC-CO_2, including Tenax traps (2), direct extraction to a gas chromatograph (17), and extraction into ethanol (18).

Of the extraction methods evaluated, only chloroform/methanol and methylene chloride provided reasonable results for all parameters (total lipid yield, FA profile, PV, conjugated dienes, FFA, and volatile content). The Bligh and Dyer chloroform/methanol extraction and the pilot-scale hexane-extraction methods were the most time consuming, in terms of both time (~2 h) performing the extractions and cleanup of equipment and glassware. SC-CO_2 extraction using high-purity (Coleman grade) CO_2 was the most rapid and efficient method of oil extraction, requiring only grinding of the sample and relatively little cleanup. SC-CO_2 extraction offers the additional advantage of being a solvent-free method. But special procedures would be required for the collection of volatile compounds when using this method, and quantitative extraction of lipids may require sample-specific extraction conditions for optimization, such as changes in extraction temperatures, pressures, and time (14). Several researchers have found SC-CO_2 to be a suitable replacement for traditional organic solvent extractions for determination of total lipids and analysis of specific lipid components (14,19–21), particularly in comparison with results obtained with less environmentally harmful nonpolar solvents.

ACKNOWLEDGMENTS

Journal paper No. J-19370 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3768, and supported by Hatch Act and State of Iowa funds.

REFERENCES

1. Morales, M.T., and R. Aparicio, Effect of Extraction Conditions on Sensory Quality of Virgin Olive Oil, *J. Am. Oil Chem. Soc. 76*:295–300 (1999).
2. King, M.F., B.L. Hamilton, M.A. Matthews, D.C. Rule, and R.A. Field, Isolation and Identification of Volatiles and Condensible Material in Raw Beef with Supercritical Carbon Dioxide Extraction, *J. Agric. Food Chem. 41*:1974–1981 (1993).
3. Kamal-Eldin, A., and L.A. Appelqvist, The Effects of Extraction Methods on Sesame Oil Stability, *J. Am. Oil Chem. Soc. 72*:967–969 (1995).
4. Calvo, L., M.J. Cocero, and J.M. Diez, Oxidative Stability of Sunflower Oil Extracted with Supercritical Carbon Dioxide, *Ibid. 71*:1251–1254 (1994).
5. Folch, J., M. Lees, and G.H. Sloane-Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).
6. Bligh, E.G., and W.J. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*:911–917 (1959).
7. Carpenter, D.E., J. Ngeh-Ngwainbi, and S. Lee, Lipid Analysis, in *Methods of Analysis for Nutrition Labeling*, edited by D.M. Sullivan and D.E. Carpenter, AOAC International, Arlington, VA, 1993, pp. 85–104.
8. O'Keefe, S.F., V.A. Wiley, and D.A. Knauft, Comparison of Oxidative Stability of High- and Normal-Oleic Peanut Oils, *J. Am. Oil Chem. Soc. 70*:489–492 (1993).
9. Shen, N., W. Fehr, L. Johnson, and P. White, Oxidative Stabilities of Soybean Oils with Elevated Palmitate and Reduced Linolenate Contents, *Ibid. 74*:299–302 (1997).
10. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D. Firestone, 4th edn., American Oil Chemists' Society, Champaign, 1989.
11. Rukunudin, I.H., P.J. White, C.J. Bern, and T.B. Bailey, A Modified Method for Determining Free Fatty Acids from Small Soybean Oil Sample Sizes, *J. Am. Oil Chem. Soc. 75*:563–568 (1998).
12. Hammond, E.G., Organization of Rapid Analysis of Lipids in Many Individual Plants, in *Modern Methods of Plant Analysis*, New Series, Vol. 12, Essential Oils and Waxes, edited by H.F. Linskens and J.F. Jackson, Springer-Verlag, Berlin Heidelbert, New York, 1991, pp. 321–330.
13. Snyder, J.M., J.W. King, and Z. Zhang, Comparison of Volatile Analysis of Lipid-Containing and Meat Matrices by Solid-Phase Micro- and Supercritical Fluid-Extraction, in *Flavor Analysis: Developments in Isolation and Characterization*, edited by C.J. Mussinan and M.J. Morello, ACS Symposium Series 705, American Chemical Society, Washington DC, 1998, pp. 107–115.
14. King, J.W., F.J. Eller, J.M. Snyder, J.H. Johnson, F.K. McKeith, and C.R. Stites, Extraction of Fat from Ground Beef for Nutrient Analysis Using Analytical Supercritical Fluid Extraction, *J. Agric. Food Chem. 44*:2700–2704 (1996).
15. Eller, F.J., and J.W. King, Supercritical CO_2 Extraction of Fat: Comparison of Gravimetric and GC-FAME Methods, *Ibid. 46*:3657–3661 (1998).
16. Mate, J.I., M.E. Saltveit, and J.M. Krochta, Peanut and Walnut Rancidity: Effects of Oxygen Concentration and Relative Humidity, *J. Food Sci. 61*:465–472 (1996).
17. Snyder, J.M., J.W. King, and K.S. Nam, Determination of Volatile and Semivolatile Contaminants in Meat by Supercritical Fluid Extraction/Gas Chromatograph/Mass Spectrometry, *J. Sci. Food Agric. 72*:25–30 (1996).
18. Fadel, H., F. Marx, A. El-Sawy, and A. El-Ghorab, Effect of Extraction Techniques on the Chemical Composition and Antioxidant Activity of *Eucalyptus camaldulensis* var. *brevirostris* Leaf Oils, *Z. Lebensm. Unters. Forsch. A 208*:212–216 (1999).
19. Taylor, S.L., J.W. King, and G.R. List, Determination of Oil Content in Oilseeds by Analytical Supercritical Fluid Extraction, *J. Am. Oil Chem. Soc. 70*:437–439 (1993).
20. Lembke, P., and H. Englehart, Development of a New SFE Method for Rapid Determination of Total Fat Content in Food, *Chromatographia 35*:509–516 (1993).
21. Hopper, M.L., J.W. King, J.H. Johnson, and A.A. Serino, Multivessel Supercritical Fluid Extraction of Food Items in the Total Diet Study, *J. Assoc. Off. Anal. Chem. 78*:1072–1079 (1995).

[Received July 6, 2001; accepted February 5, 2002]