

Oxidative Stability of Walnut Oils Extracted with Supercritical Carbon Dioxide

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ABSTRACT: Supercritical carbon dioxide (SC-CO₂) was used to partially defat walnuts, and the oxidative stability of the extracted walnut oils was assessed. The SC-CO₂-extracted oils were less stable during accelerated storage in the dark than was pressed walnut oil, as determined by PV, headspace analysis by solid-phase microextraction, and sensory methods. The SC-CO₂-extracted oils, however, exhibited greater photo-oxidative stability than did pressed walnut oil by all of these methods, possibly because of the presence of chlorophyll in the pressed oil. Oxidative stability indices and tocopherol contents were significantly lower in the SC-CO₂-extracted oils than in pressed oil.

Paper no. J10100 in *JAOCs* 80, 575–578 (June 2003).

KEY WORDS: Oil oxidation, oxidative stability, supercritical carbon dioxide extraction, walnut oil.

In other work conducted in our laboratory, supercritical carbon dioxide (SC-CO₂) was used to decrease the total fat content of walnuts by 25 and 40% (1). Walnut oil is prized as a specialty oil because of its characteristic flavor and aroma and its potential health benefits (2,3). It is therefore a valuable co-product from this SC-CO₂ process. Several studies have reported decreased thermal oxidative stability, at 60°C, of SC-CO₂-extracted soybean, sunflower, corn, and cottonseed oils (4–6); however, SC-CO₂-extracted perilla oil was found to be more stable during storage at 50°C than either hexane-extracted or mechanically pressed oil (7). Savage *et al.* (8) measured the oxidative stability of various walnut oils based on Rancimat data (evaluated at 110°C), and reported significant differences among oils from different walnut cultivars. To our knowledge, the photo-oxidative stability (photosensitivity) of SC-CO₂-extracted oils has not been evaluated. This information may be particularly important in the case of walnut oils, which are often stored in clear glass bottles.

The objective of this study was to compare the stabilities of SC-CO₂-extracted walnut oil with commercially pressed oil under photo-oxidative and accelerated dark conditions.

MATERIALS AND METHODS

Premium walnut pieces and commercially pressed oil from premium walnuts, all from the same cultivars, were obtained

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from Diamond Walnut Growers Inc. (Stockton, CA). Walnut pieces were subjected to oil extraction by using the pilot-scale semicontinuous SC-CO₂ extraction system located at the National Center for Agricultural Utilization Research (Peoria, IL) (9). The work was done in tandem with a study evaluating the impact of fat reduction on walnut quality (1); thus, the treatments were designed to create walnuts with two levels of oil reduction: 25 and 40% reduction of total fat from the walnuts. The oil treatments used in the current study, then, were those extracted from the premium walnut pieces to create the walnuts with 25 and 40% relative fat reduction, plus the commercially pressed oil. Oil extractions for the SC-CO₂ treatments (25% SC-CO₂ and 40% SC-CO₂) were performed in duplicate at 10,000 psi and 80°C, using analytical grade CO₂ with a CO₂ flow rate of 0.33 lb/min. The walnut oils were flushed with nitrogen and stored at –20°C prior to analysis. The commercially pressed oil treatment was divided into two parts so that the storage could be replicated.

Storage conditions. Walnut oils from each extraction were divided to provide replicate samples and were stored at 60°C in the dark and 35°C in the light (540 lx). For each storage condition, 100-mL beakers containing 50 mL of oil (surface area to volume ratio 0.03 mm²/mL) were loosely covered with plastic wrap and stored in a constant temperature oven for 15 d. Oxidative stability indices (OSI) were determined by the AOCS standard method Cd 12b-92 (10) with an Oxidative Stability Instrument (Omnion, Rockland, MA) operating at 100°C.

Analytical procedures. PV were conducted on the oils stored under each condition every 3 d using the modification of the AOCS method Cd 8-53 described by Crowe and White (11). FAME were prepared and analyzed as outlined by Hammond (12). Tocopherols were measured by HPLC according to AOCS method Ce 8-89 (10). Chlorophyll was determined according to AOCS method Cc 13i-96 (10). All analyses were performed in duplicate at time zero and the data averaged for each experimental replication.

Volatile analysis. Two milliliters of oil was placed in a 20-mL glass vial sealed with a Teflon/silicone septum. After equilibration of the oil sample for 5 min at 50°C, the 100 µm poly-(dimethylsiloxane) solid-phase microextraction fiber (SPME) (Supelco Inc., Bellefonte, PA) was exposed to the vial headspace for 30 min at 50°C. The volatile compounds collected on the poly(dimethylsiloxane) fiber were thermally desorbed for 1 min in the inlet of the gas chromatograph (Hewlett-Packard 5890 Series II GC; Palo Alto, CA) with an inlet temperature of 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 column temperature of the gas chromatograph was held for 3 min at an initial temperature of 30°C and then was increased to a final temperature of 210°C at 6°C/min with a final hold time of 5 min. Peaks were detected by an FID held at 220°C. 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 from 10^0 to 10^{-4} $\mu\text{L/mL}$. Standards of hexanal (98% pure) and nonanal (96% pure) were obtained from AccuStandard, Inc. (New Haven, CT); standards of *t*-2-pentenal (95% pure), *t*-2-heptenal (90% pure), *t,t*-2,4-heptadienal (90% pure), 1-octen-3-ol (98% pure), and *t,t*-2,4-decadienal (85% pure) were purchased from Aldrich Chemical Company (Milwaukee, WI). The compound *t,c*-2,4-heptadienal was identified by MS, using a Hewlett-Packard 5970 mass-selective detector in place of the FID, and the response of *t,t*-2,4-heptadienal was used for its quantitation. All peaks were identified by comparing their retention times and mass spectra with those of known compounds. Volatile compounds in each replicate oil treatment were analyzed in duplicate at time zero and 15 d, and the data from each day averaged.

Sensory evaluation. Five panelists with previous walnut and oil evaluation experience were trained in two sessions for familiarity in identifying off-flavors in oxidized oils. During training, panelists were presented with walnut oils autoxidized to various degrees and standard references for "painty" and "rancid" flavors in oils according to AOCS method Cg-2-83 (10). The term "off-flavor" was agreed upon by the panelists to be the most appropriate descriptor for the overall oxidized flavor changes in walnut oil during oxidation. Prior to sensory evaluation, samples were placed in screw-capped glass vials and equilibrated to 25°C in the dark. Samples were evaluated using a 15-cm line scale with "no off-flavor" and "extreme off-flavor" as anchors. Samples from each experimental replication were evaluated in duplicate on day 0 and day 15, and the data from each day averaged.

Statistical analysis. Data were analyzed by ANOVA with repeated measures using the General Linear Model procedure of SAS 6.06 (Cary, NC). Significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Analyses of the three walnut oil samples, pressed and SC-CO₂-extracted from 25 and 40% walnut-lipid reductions, showed no significant differences in FA composition among the oils. The approximate percentages (by GC percentage area) of each FA were: palmitic, 8.1%; stearic, 1.5%; oleic, 16.7%; linoleic, 61.2%; and linolenic, 12.5%. Previous studies in our laboratory indicated no differences in the FA composition of pressed- and SC-CO₂-extracted walnut oils (13). The presence of a high level of PUFA in walnut oil is a significant contributor to its oxidative instability as also noted in soybean oil (14).

Two oil storage conditions were used in this study: 60°C storage in the dark to measure autoxidation, and 35°C storage under fluorescent light to measure photo-oxidation. Oil oxida-

tion was monitored by several methods, each selected to measure different types of products. The PV was used to measure hydroperoxides as initial oxidation products. Volatile analysis was indicative of breakdown products of hydroperoxides (secondary oxidation products), which are important in rancid or off-flavor development. Finally, the OSI was used to provide a rapid, convenient measure to predict overall stability.

The PV of the SC-CO₂-extracted oils were significantly greater than that of the pressed oil at all sampling times except time zero during storage at 60°C for 14 d (Fig. 1; exact ANOVA data not shown). As expected, given the relatively high levels of PUFA, the PV of all oils increased rapidly under accelerated storage conditions. During storage at 35°C in the light, however, the SC-CO₂ oils exhibited significantly greater stability at all sampling times based on PV than did the pressed oil (Fig. 2; exact ANOVA data not shown). No significant differences in PV were measured between the SC-CO₂-extracted oils within each storage condition. The greater rate of oxidation in the SC-CO₂-extracted oils stored at 60°C may have been a result of the greatly reduced levels of tocopherols in these oils (Table 1). Alternatively, a previous study showed a reduction in phospholipid content of oil from SC-CO₂-extracted soybean flakes, which was thought to decrease positive synergistic effects of tocopherols with phospholipids (4). In that study, SC-CO₂-extracted oils were much less stable than hexane-extracted oils, despite high levels of tocopherols in the SC-CO₂-extracted oils. The level of chlorophyll present in the pressed sample, although relatively low (4 ppm; chlorophyll was not detectable in the SC-CO₂-extracted oils), probably decreased the photo-oxidative stability of that oil. Hall and Cuppett (15) found that low levels of chlorophyll (4.21 ppm) were sufficient to cause a slight increase in the rate of photo-oxidation in soybean oil. The increased stability of SC-CO₂-extracted oils to light-induced oxidation is important because specialty oils, such as walnut, are generally stored in clear glass bottles on store shelves, making them susceptible to photo-oxidation. Other factors (in this study) that can influ-

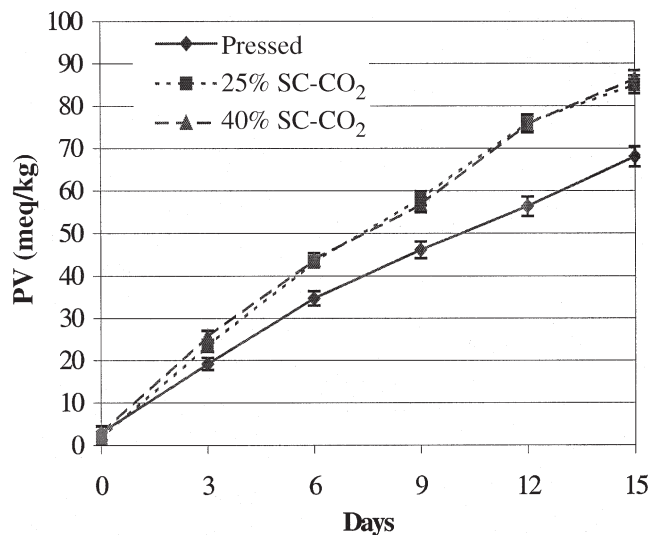


FIG. 1. PV of walnut oil stored at 60°C in the dark. SC-CO₂, supercritical carbon dioxide.

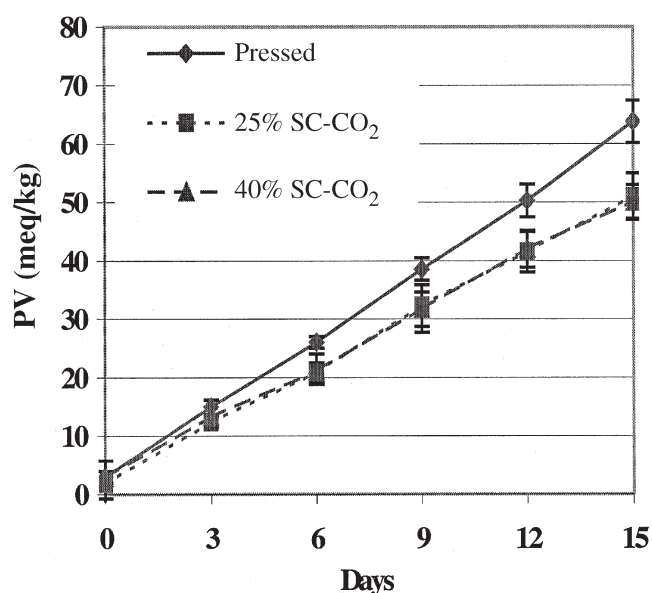


FIG. 2. PV of walnut oil stored at 35°C in the light. For abbreviation see Figure 1.

ence oil quality include trace metals, FFA, water content, and presence of nonlipid components in the oils.

Total tocopherol levels and levels of individual tocopherol homologs were significantly greater in the pressed than in either of the SC-CO₂-extracted oils (Table 1). Total and γ -tocopherol levels of the 40% SC-CO₂ walnut oils were significantly greater than levels in the 25% SC-CO₂-extracted walnut oil, but the practical differences were probably not important. The longer extraction time for the 40% SC-CO₂-extracted oil than for the 25% SC-CO₂-extracted oil probably facilitated the slightly greater levels of tocopherol. The γ -homolog was the predominant tocopherol in all three oils. The levels of total and individual tocopherol homologs measured in the pressed oil were similar to those reported in the literature (8). But β -tocopherol, which has been reported at low levels (<10 ppm) in several walnut cultivars (8), was not detected. Tocopherols are important antioxidants, which act by donating a hydrogen to peroxy radicals in the propagation step leading to the formation of hydroperoxides and by reacting with alkoxy radicals, thereby inhibiting further decomposition of hydroperoxides to volatile secondary oxidation compounds (16). Pro-oxidant effects of high tocopherol levels have been demonstrated in soybean oil; however, this effect has not been examined in walnut

TABLE 1
Content of Total and Individual Isomers of Tocopherol in Fresh (day 0) Walnut Oils

Oil type	Tocopherols ^a (ppm)			
	Total	Alpha	Gamma	Delta
Pressed	373.0 ^a	28.3 ^a	306.0 ^a	38.4 ^a
25% SC-CO ₂ ^b	32.0 ^b	1.7 ^b	27.9 ^b	2.4 ^b
40% SC-CO ₂ ^b	38.5 ^c	2.0 ^b	34.1 ^c	2.4 ^b

^aValues in the same column with different superscript letters are significantly different ($P < 0.05$).

^bOils obtained from walnuts where the oil was reduced by 25 or 40% with supercritical carbon dioxide (SC-CO₂) extraction.

oils. Savage *et al.* (8) reported a significant relationship between total tocopherol contents combined with the level of oil unsaturation and oxidative stability, as determined by a single-point PV of walnut oils from various cultivars.

Because of the relatively high levels of PUFA present in the oils, an OSI temperature of 100°C rather than 110°C was used to slow oxidation slightly and enhance differences between the oils. Both of the SC-CO₂-extracted oils had significantly lower OSI (2.5 and 1.2 h for the 25 and 40% SC-CO₂-extracted oils, respectively) than did the pressed oil (12.3 h). Given the ~10 times greater level of tocopherols present in the pressed oil, it is not surprising that the OSI was considerably greater in this oil. The mechanism for oxidation of oils at 100°C differs from the mechanism at 60°C (16); however, the OSI findings are consistent with the results of 60°C storage in the dark, in which the pressed oil was more stable than the SC-CO₂-extracted oils. The walnut oil from the 40% SC-CO₂-extraction had a significantly lower OSI than did the 25% SC-CO₂-extracted oil, despite greater levels of tocopherols in the 40% SC-CO₂ oil, again suggesting that factors other than tocopherol content influence stability. Both SC-CO₂-extracted walnut oils, however, had extremely low OSI values, despite the utilization of lower testing temperatures than typically used for OSI.

The primary volatile compounds identified from SPME oil headspace analysis are shown in Table 2. Hexanal, a product of linoleic acid degradation, was the primary volatile compound in all oils after storage under both conditions. Relatively large amounts of *trans,trans*- and *trans,cis*-2,4-heptadienal were measured in all three oils stored at 60°C in the dark. This volatile compound is a decomposition product of linolenic acid, which makes up about 12.5% of the FA in walnut oil. Nonanal, which is formed primarily from oleic acid, was greater after light than dark storage. The rate of oleic acid autoxidation, which ultimately leads to the formation of volatile compounds, is much lower than that of PUFA, such as linoleic or linolenic acid, ~1:10.3:21.6 for oleic/linoleic/linolenic acids, respectively (17). Photo-oxidation of oleic acid, however, occurs at a much higher rate than does autoxidation of oleic acid because of singlet oxygen addition directly to the double bond during photo-oxidation (16).

Initial sensory evaluation of the walnut oils at time zero indicated that the SC-CO₂-extracted oils had a significantly lower off-flavor intensity than did the pressed walnut oil (Table 3). After the completion of storage at 60°C in the dark, both SC-CO₂-extracted walnut oils had significantly greater off-flavor intensity scores than did the pressed oil. In contrast, after completion of storage at 35°C in the light, the pressed walnut oil had a significantly greater off-flavor intensity score than did the SC-CO₂-extracted oils. The flavor intensity scores correspond with the levels of hexanal measured in each of the oils following completion of storage under both the light and dark conditions (Table 2). Total volatile and hexanal contents have been correlated with sensory evaluation of other oils (18). Recently, a significant correlation was found between undesirable sensory scores and the total amount of volatile compounds isolated by SPME (19).

TABLE 2
Volatile Compound Contents (ppm) of Walnut Oils Stored at 60°C in the Dark and 35°C in the Light

Volatile compound	60°C in the dark						35°C in the light					
	Pressed		25% SC-CO ₂ ^a		40% SC-CO ₂ ^a		Pressed		25% SC-CO ₂ ^a		40% SC-CO ₂ ^a	
	0 d ^b	15 d	0 d	15 d	0 d	15 d	0 d	15 d	0 d	15 d	0 d	15 d
<i>t</i> -2-Pentenal	0.2	2.9	ND ^c	4.5	ND	5.1	0.2	6.3	ND	5.5	ND	6.1
Hexanal	0.4	19.5	ND	40.2	ND	45.6	0.4	30.6	ND	21.2	ND	25.3
<i>t</i> -2-Heptenal	0.2	4.6	ND	14.3	ND	11.2	0.2	5.1	ND	4.6	ND	5.2
<i>t,t</i> -2,4-Heptadienal	1.4	7.4	ND	17.5	ND	14.2	1.4	2.6	ND	1.5	ND	1.3
<i>t,c</i> -2,4-Heptadienal	1.7	11.1	ND	22.4	ND	30.1	1.7	3.0	ND	2.3	ND	3.1
1-Octen-3-ol	0.3	3.3	ND	5.1	ND	5.6	0.3	7.1	ND	4.2	ND	3.4
Nonanal	1.0	1.5	ND	1.8	ND	2.1	1.0	12.6	ND	8.1	ND	7.9
<i>t,t</i> -2,4-Decadienal	0.5	9.1	ND	17.6	ND	19.5	0.5	4.4	ND	3.3	ND	2.7

^aSee Table 1 for description of oil treatments.

^bAll oils tested after 0 and 15 d of storage.

^cND, not detectable.

TABLE 3
Sensory Evaluation Off-Flavor Scores^{a,b} of Walnut Oils

Day	Pressed ^c	25% SC-CO ₂ ^c	40% SC-CO ₂ ^c
0	3.0 ^a	1.0 ^b	1.1 ^b
15 (60°C dark storage)	10.1 ^b	14.6 ^a	13.2 ^a
15 (35°C light storage)	14.2 ^a	11.6 ^b	12.2 ^b

^aA score of 0 = no off-flavor and 15 = extreme off-flavor.

^bValues in the same row with different superscript letters are significantly different ($P < 0.05$).

^cSee Table 1 for description of oil treatments.

Oil extraction by SC-CO₂ has been associated with greater thermal oxidative instability of the oils than by solvent or extruder extraction methods (4–6). Compared with pressed walnut oil, the thermal oxidative stability of both of the SC-CO₂-extracted walnut oils examined in this study was less as determined by multiple types of oxidation measurements, including PV, OSI, and volatile-compound and sensory analyses. The SC-CO₂-extracted oils showed increased stability to photo-oxidation as determined by PV, volatile, and sensory analyses.

ACKNOWLEDGMENTS

The authors thank Gary List and Scott Taylor (USDA/ARS/NCAUR) for assistance in extracting the walnuts with the SC-CO₂ system. Journal Paper No. J-19550 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3768, and supported by the Hatch Act and State of Iowa funds.

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[Received March 5, 2002; accepted May 12, 2003]