Oxidation, Flavor, and Texture of Walnuts Reduced in Fat Content by Supercritical Carbon Dioxide

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ABSTRACT: English walnuts are popular because of their good taste, high n-3 FA content, and reported hypocholesterolemic effects. However, walnuts have a high fat content (~70% w/w) that is highly polyunsaturated, which contributes to oxidative instability. The objectives of this study were: (i) to use supercritical carbon dioxide (SC-CO_2) extraction to decrease the total fat content of walnuts, and (ii) to determine the effects of SC-CO_2 lipid extraction on the oxidative stability, flavor, and textural characteristics of the reduced-fat walnuts. The fat content of English walnut pieces was reduced by 25 and 40% with a pilot-scale SC-CO_2 extraction system. Full-fat, 25-, and 40%-reduced-fat walnuts were stored at 25 and 40°C for 8 wk. FA profiles were similar for residual oil in all treatments, and the profiles did not change with storage. PV and volatile compounds were significantly greater ($P < 0.05$) in full-fat walnuts than in reduced-fat walnuts at both storage temperatures. A trained sensory panel judged the reduced-fat walnuts to be less astringent and to have less walnut and rancid flavors. Full-fat walnuts had greater hardness than reduced-fat walnuts by both sensory and instrumental texture profile analyses. In general, reducing the relative fat contents of walnuts by 25% improved oxidative stability and maintained a high level of consumer acceptance.

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KEY WORDS: Flavor, oxidation, oxidative stability, supercritical carbon dioxide extraction, texture, walnuts.

During the past several years, walnuts have gained popularity because of their good taste, high n-3 FA content (linolenic acid), and reported hypocholesterolemic (1,2) and antihypertensive effects (1). One negative aspect of walnut consumption is the relatively high total fat content of this commodity (~70% w/w). In addition, the highly polyunsaturated nature of walnut lipids makes them prone to oxidative instability. Despite the oxidative problem, oil extracted from walnuts is sold and enjoyed as a high-quality specialty oil in many parts of the world (3). Even though there is interest in walnut oil as a commodity, the remaining walnut meat generally is not considered as a potential food, likely because of the residues left from the extraction solvent. Recent studies in supercritical carbon dioxide (SC-CO_2) extraction of many seeds, cereals, and walnuts (4) may pave the way for using the walnut pieces as a food. Indeed, SC-CO_2 extraction has been used as a tool to reduce fat in other foods (5,6), but little attention has been paid to the FA profile, oxidative stability, and flavor attributes of the reduced-fat products. The objectives of this study were to use SC-CO_2 to decrease the total lipid content of walnuts and to determine the effects of SC-CO_2 lipid extraction on the oxidative stability, flavor, and textural characteristics of the reduced-fat walnuts.

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MATERIALS AND METHODS

Premium walnut pieces were provided by Diamond Walnut Growers, Inc. (Stockton, CA). The walnuts were randomly divided into three treatment groups: 25% reduced-fat (fat extracted by SC-CO_2), 40% reduced-fat (fat extracted by SC-CO_2), and full-fat (no fat extraction). The 25 and 40% reduced-fat walnuts represent relative reductions of the total fat content (i.e., 25% less total fat than the full-fat walnut pieces). Preliminary data from our laboratory suggested that oil reductions greater than 40% resulted in high levels of walnut fracture and product loss.

SC-CO_2 extraction. The pilot-scale semicontinuous SC-CO_2 extraction system located at the National Center for Agricultural Utilization Research (Peoria, IL) and described by Friedrich *et al.* (7) was used. Extractions for each reduction level (25 and 40%) were performed in duplicate at 10,000 psi (68.9 MPa) and 80°C with a CO_2 flow rate of 0.15 kg/min. The walnuts were flushed with nitrogen and stored at –20°C prior to analysis. Preliminary experiments to determine feasibility were performed using a custom-assembled supercritical fluid extraction system (8) at operating pressures up to 10,000 psi and temperatures ranging from 45 to 80°C.

Total fat content. The Bligh and Dyer method (9) with modifications was employed for determining total fat content. Walnuts (25 g) were homogenized using a Polytron homogenizer (Kinematica GmbH, Luzern, Switzerland; distributed by Brinkmann Instruments, Westbury, NY) with 90 mL of water, 100 mL of methanol, and 50 mL of chloroform for 2 min. An additional 50 mL of chloroform was added to the mixture and blended for two more minutes. The homogenate was centrifuged at 500 × *g* for 10 min, the water (upper) layer removed by aspiration, and the remaining homogenate vacuum-filtered through Whatman No. 1 filter paper (Maidstone, England), re-extracted with 10 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 50 mL of chloroform. Solvent was removed by rotary evaporation under vacuum at 30°C.

Analytical procedures. Tocopherol content was determined by HPLC according to the AOCS Official Method Ce 8-89 (10). FAME were prepared and analyzed as described by Hammond (11). Moisture content was determined according to the 2-h oven-drying method described in AOCS Official Method Ba-38 (10). Crude protein was determined by using a PerkinElmer Series II Nitrogen Analyzer 2410 (PerkinElmer Corp., Norwalk, CT). Nitrogen content was multiplied by a factor of 6.25 to estimate crude protein content.

Color measurements. Color was measured using a Color Difference Meter (Model D25A-2; Hunter Laboratories, Inc., Reston, VA). Walnuts with the pellicles removed were placed in a petri dish, and the readings were recorded by rotating the sample dish in 90° increments. A total of six samples were evaluated in triplicate for each experimental replication.

Hardness measurement. The hardness of fresh walnut meats was evaluated using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY). A 1.2-cm square was cut from walnut pieces, and the thickness was measured using calipers. Cut pieces were grouped according to thickness with 8 and 9 mm used for analysis. A 1.5" (3.81 cm) anvil was used to determine 50% compression at a compression rate of 0.5 mm/s. A total of 15 samples were evaluated and the results averaged for each extraction replicate to account for surface differences and to reduce SE among experimental replications.

Scanning electron microscopy (SEM). Internal walnut structures were prepared for SEM by physically snapping the edges of freshly broken walnut pieces. Samples were mounted with aluminum stubs using carbon adhesive tape. Silver paint was applied around sample edges to promote conductivity and aid in attachment. The samples were sputter-coated using a Denton Desk II Cold Sputter/etch coating unit (Denton Vacuum, Inc., Morestown, NJ). Walnuts were viewed using a gold/palladium (60:40) alloy target, and images were collected with a JEOL 5800LV scanning electron microscope.

Storage studies. SC-CO_2-extracted (25 and 40% reduced-fat) and full-fat walnuts were randomly divided for storage under two different conditions, 25 and 40°C, both in the dark for 8 wk. Walnuts were placed in aluminum pans and loosely covered with plastic wrap during storage.

Oxidation measurements. PV were measured weekly using the modification of AOCS method Cd 8-53 described by Crowe *et al*. (12). For PV analysis, oil was extracted by small-scale SC-CO_2 extraction [FastFat9 (HT), ISCO Inc., Lincoln, NE], in which ground walnut (2.5 g) was placed in the extraction vessel and extracted at 10,000 psi (68.9 MPa) for 35 min at a flow rate of 5.0 mL/min with chamber and restrictor temperatures of 85 and 80°C, respectively. All PV analyses were performed in duplicate and the results averaged.

Volatile oxidation compounds were measured at 0, 4, and 8 wk of storage by solid phase microextraction (SPME), a technique previously applied to measuring lipid degradation volatiles in vegetable oils (13). Walnuts (5 g) were ground in a Waring blender at high speed for 10 s and placed in a two-necked round-bottomed flask. One neck of the flask contained a small external fan designed to circulate the headspace above the sample. The SPME device was inserted through the other neck. The flask was placed in a 50°C water bath, where the sample was equilibrated for 5 min prior to exposure of the SPME fiber (100 µm polydimethyl siloxane) for 30 min. The SPME fibers were thermally desorbed for 1 min in the inlet of a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) at 230°C and were 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; Bellefonte, PA). The gas chromatograph oven was held for 3 min at an initial temperature of 30°C, and 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. 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.

Sensory analysis. Walnuts from each storage condition were evaluated by a trained 10-person panel comprising Iowa State University faculty and students with previous experience in evaluating stored oils and/or high-fat foods. Sensory panelists were familiarized with evaluation methods during three 1-h training sessions using walnut samples oxidized at different levels, AOCS Recommended Practice Cg 2-83 reference samples for rancid and painty flavor (10-8), texture references for hardness, and examples of astringent products. At weeks 0, 4, and 8, samples were presented in duplicate at two different sittings and evaluated using a 15-cm line scale, with the low end of the scale indicating none and the high end indicating extreme for each attribute (rancidity, paintiness, astringency, and hardness). Sample evaluation was performed by panelists in individual sensory booths under red light. Additionally, consumer acceptance testing was conducted for the 25% reduced-fat and full-fat walnuts by 33 untrained panelists using a 9-point balanced hedonic scale.

Statistical analysis. Statistical analysis were performed by ANOVA with repeated measures and Pearson's correlation using the General Linear Model procedures of SAS 6.06 (Cary, NC) to determine significant differences among treatments at $P < 0.05$.

RESULTS AND DISCUSSION

Walnut extraction. Preliminary experiments using a custom-assembled supercritical fluid extraction system (8) demonstrated the feasibility of SC-CO_2 lipid extraction from walnut pieces, with lipid reduction levels between 25 and 40% (w/w). Lipid extraction beyond 40% of total fat resulted in product fracture and powdering of the walnut pieces. Initial studies also demonstrated that gradual pressurization and depressurization were necessary to minimize damage to the walnut pieces.

Fat and protein percentages of full-fat, and 25 and 40% reduced-fat walnuts were significantly ($P < 0.05$) different

among treatments (data not shown). Total fat (w/w) contents decreased from 69% for the full-fat walnuts to 51 and 41%, respectively, for the approximately 25 and 40% reduced-fat walnuts, with expected commensurate increases in the relative protein percentages of 14% in the full-fat walnuts to 21 and 27% in the 25 and 40% reduced-fat walnuts, respectively. Moisture contents (3.0%) were similar among all three walnut fat levels. FA compositions of the residual walnut oil were not significantly different among the three treatments. The approximate FA compositions were: palmitic acid, 6.9%; stearic acid, 2.0%; oleic acid, 16.5%; linoleic acid, 60.9%; and linolenic acid, 13.7%. Alexander *et al.* (5) reported significant effects of SC-CO_2 extraction conditions on the FA composition of TAG from pecans. The solubilities of individual FA differ in CO_2 (8); however, the relatively low ratio of saturated/unsaturated FA within the TAG structure in walnuts likely made fractionation difficult, particularly under the extraction conditions used in this study, which were designed to optimize extraction time.

Tocopherol content. Both total tocopherol and individual tocopherol isomer contents were significantly lower in both the reduced-fat walnut treatments than in the full-fat walnuts, except for α-tocopherol, which was similar (Table 1). Previous studies, using SC-CO_2 extraction in soybeans, reported only limited extraction of tocopherols and suggested that lower tocopherol levels may be partly responsible for the decreased oxidative stability of SC-CO_2-extracted oils (14). The SC-CO_2 procedure extracted lipids from the outer portion of the walnut first. The outer area of the walnut may have a relatively greater concentration of tocopherols that naturally attenuates surface oxidation, thus explaining why greater relative reductions (based on the amount of oil removed) in tocopherol occurred in the 25% reduced-fat product. To our knowledge, however, distributions of tocopherols within walnuts have not been measured.

PV. PV for the full-fat walnuts increased faster than the PV for both of the reduced-fat walnut treatments when stored at both 25 and 40°C (Figs. 1A and 1B). The 25% reduced-fat walnuts had a significantly greater PV after 5 wk at 40°C than did the 40% reduced-fat walnuts. The differences between the two reduced-fat treatments were not significant throughout the 25°C storage period. Despite the highly unsaturated nature of walnut oil, the PV were relatively low at both storage temperatures. After reaching a PV of ~15 meq/kg, a decline in PV was noted in both the full-fat and 25% reduced-fat walnuts at 40°C. Similar trends in oxidizing walnuts have been reported by other investigators (15,16). At this point during oxidation, the rate of peroxide degradation to secondary oxidation compounds evidently was greater than the rate of peroxide formation, a fact supported by the sharp increase in hexanal contents (see next paragraph) between 4 and 8 wk for both the full-fat and 25% reduced-fat treatments (Figs. 2A and 2B).

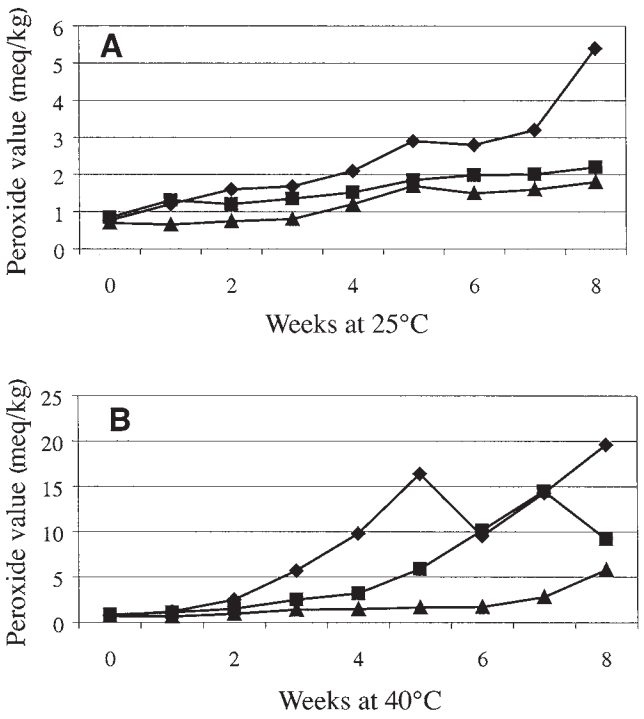

FIG. 1. PV of walnuts stored at (A) 25°C and (B) 40°C for 8 wk. (◆) Full-fat, (■) 25% reduced-fat, and (▲) 40% reduced-fat English walnuts.

Volatile oxidation compounds. Volatile oxidation compounds were present initially at similar levels among all walnut treatment groups (data not shown). Most compounds increased significantly with time at both storage temperatures for each of the three walnut fat levels and were generally significantly greater in the full-fat walnuts. Hexanal was the primary volatile oxidation product measured in all three walnut treatments. Linoleic acid is the predominant FA in walnuts; therefore, the relatively high levels of hexanal are expected because this compound is formed primarily from the breakdown of linoleic acid hydroperoxides (17). The amount of hexanal formed by 8 wk at 25°C and generally after 4 wk at 40°C was significantly greater in the full-fat walnuts than in both reduced-fat treatments (Figs. 2A and 2B). After 4 wk at both temperatures, the 40% reduced-fat walnuts were significantly lower in hexanal content than the other two treatments.

Sensory evaluation. Human sensory scores for walnuts stored at 25 and 40°C for both rancidity (r = 0.85 at 25°C; r = 0.87 at 40°C) and paintiness (r = 0.95 at 25°C; r = 0.89 at 40°C) correlated well with hexanal formation at both 25 and

TABLE 1
Total and Individual Tocopherol Content of Walnuts

Walnut treatment (% fat reduction of walnuts)	Tocopherol content (ppm)[a]			
	Total	Alpha	Gamma	Delta
Full-fat	202[a]	8.4[a]	139[a]	12.8[a]
25%	108[b]	5.6[a]	93[b]	7.5[b]
40%	108[b]	4.4[a]	96[b]	7.9[b]

[a]Values within a column with the same superscript letter are not significantly different, $P > 0.05$.

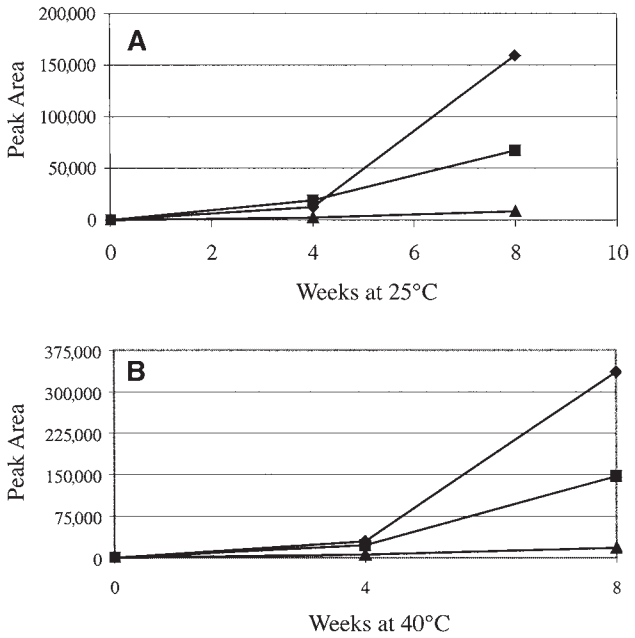

FIG. 2. Hexanal content of walnuts stored at (A) 25°C and (B) 40°C for 8 wk, expressed as relative peak areas. ◆ Full-fat, ■ 25% reduced-fat, ▲ 40% reduced-fat.

40°C, with both oxidized flavor and hexanal content increasing with increased walnut fat content (Table 2). No significant differences in rancidity or paintiness were found between the 25 and 40% reduced-fat walnuts stored at 40°C. Astringency scores were significantly lower in both the 25 and 40% reduced-fat walnuts than in the full-fat walnuts at all sampling times. The lower astringency scores were likely the result of $SC\text{-}CO_2$ extraction of ellagic acid from the walnut pellicle (18). Consumer acceptance hedonic scores of 25% reduced-fat walnuts were not significantly different ($P > 0.05$) from full-fat walnut scores (6.5 and 7.0, respectively). Consumer acceptance testing was not performed on the 40% reduced-fat walnuts because descriptive panel sessions indicated obvious differences between these walnuts and the full-fat walnuts.

Partial removal of fat from the walnuts by using $SC\text{-}CO_2$ extended walnut shelf life based on PV, hexanal, and human sensory data. Partial fat removal also has been reported to increase the shelf life of pecans (19) and peanuts (20). The authors suggested that the decrease in the available lipid substrate was responsible for the increased stability of these commodities. Because exposure of lipids to oxygen is an important oxidation mechanism, it is also feasible that the removal of lipids from the external surface of the walnut pieces, where they are most accessible to oxygen and hence oxidation, resulted in increased oxidative and flavor stability of the partially defatted walnuts.

Texture—sensory and instrumental. Texture of the fresh walnut meats, as determined by both human sensory and instrumental analyses, significantly decreased in hardness with decreasing walnut fat content (Figs. 3A and 3B). Instrumental and sensory evaluations of hardness were strongly correlated among all three walnut treatment groups ($r = 0.95$).

TABLE 2
Sensory Attributes of Walnuts Stored at 25 and 40°C

		Walnut treatment (% fat reduction of walnuts)		
Sensory attribute	Time (wk)	Full-fat	25%	40%
Stored at 25°C				
Rancidity[a]	0	0.0[a]	0.0[a]	0.0[a]
	4	2.5[a]	0.3[b]	0.4[b]
	8	4.3[a]	1.8[b]	1.1[b]
Paintiness[a]	0	0.0[a]	0.0[a]	0.0[a]
	4	2.5[a]	1.1[b]	0.4[b]
	8	4.3[a]	2.3[b]	1.9[b]
Astringency[a]	0	8.9[a]	3.9[b]	3.3[b]
	4	8.4[a]	3.5[b]	3.7[b]
	8	8.9[a]	3.8[b]	3.2[b]
Stored at 40°C				
Rancidity[a]	0	0.0[a]	0.0[a]	0.0[a]
	4	3.7[a]	1.6[b]	0.9[b]
	8	8.2[a]	2.0[b]	2.7[b]
Paintiness[a]	0	0.0[a]	0.0[a]	0.0[a]
	4	3.8[a]	1.8[b]	1.1[b]
	8	7.7[a]	3.5[b]	2.8[b]
Astringency[a]	0	8.9[a]	3.9[b]	3.3[b]
	4	8.5[a]	4.1[b]	3.0[b]
	8	8.7[a]	3.4[b]	3.5[b]

[a]Values within a row with the same superscript letter are not significantly different, $P > 0.05$. Scoring: 0 = none, 15 = extreme; $n = 10$ panelists.

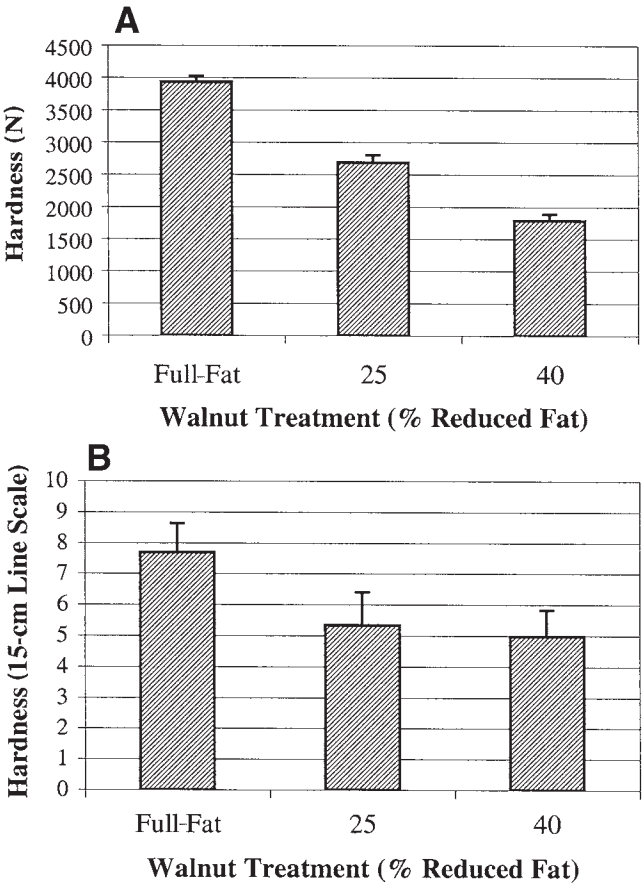

FIG. 3. Hardness of walnuts as measured (A) with a Texture Analyzer and (B) by sensory evaluation (0 = not hard, 15 = extremely hard). Error bars represent SD.

Significant decreases in breaking intensity and hardness measured by rheometry for partially defatted peanuts (6), and decreased hardness and increased fracturability of supercritically extracted pecans (21) were reported previously. These differences in hardness may be understood by viewing scanning electron micrographs, which show clear differences in cell wall structure among each of the walnut treatments (Figs. 4A–C). Cell walls of the full-fat walnuts were intact (Fig. 4A), cell walls of the 25% reduced-fat walnuts (Fig. 4B) were distorted but remained intact, whereas the 40% reduced-fat walnuts (Fig. 4C) displayed evidence of cell wall collapse. Preliminary studies in our laboratory indicated unacceptably high levels of product loss with greater than 40% fat reduction in walnuts, indicating probable complete cell wall collapse at these fat reduction levels.

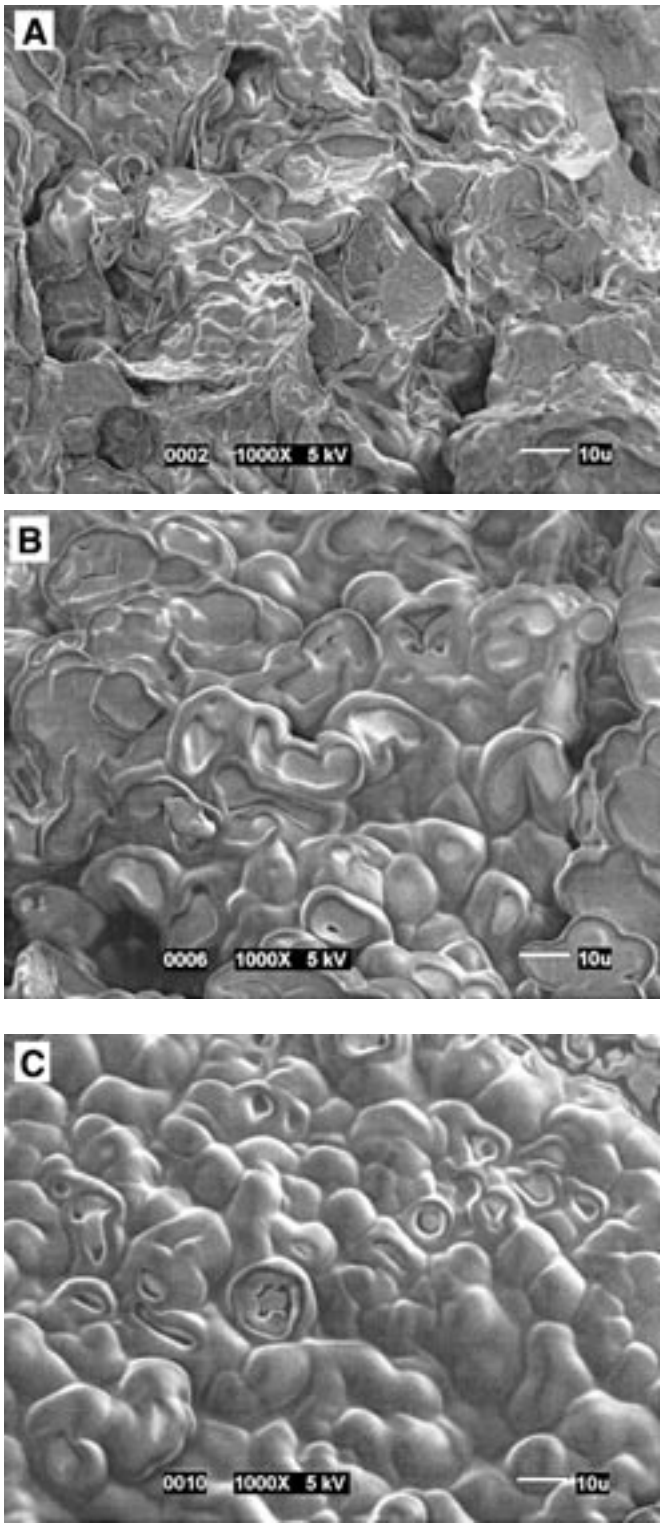

FIG. 4. Scanning electron micrographs of walnuts: (A) full-fat, (B) 25% reduced-fat, and (C) 40% reduced-fat.

TABLE 3
Hunter Color Values of Walnuts

Walnut treatment (% fat reduction of walnuts)	Hunter color values[a]		
	L	a	b
Full-fat	40.9[b]	1.3[a]	11.7[a]
25%	60.0[a]	1.2[a]	12.3[a]
40%	64.9[a]	1.2[a]	13.6[a]

[a]Values within a column with the same superscript letter are not significantly different, $P > 0.05$.

Color analysis. Color of walnuts was affected ($P < 0.05$) by SC-CO_2 extraction (Table 3). The L-values, indicating lightness/darkness of a surface, were significantly lower in the full-fat walnuts than in the reduced-fat treatments, meaning that the reduced-fat walnuts had a whiter appearance. Hunter a- and b-values were not significantly different among all walnut treatments. Similar color changes with SC-CO_2 extraction of pecans and peanuts have been reported by other investigators (22,23). The extracted walnut oil had an amber color, which may have contributed to the darker color of the walnuts prior to SC-CO_2 extraction of the oil and accompanying lipid-soluble pigments.

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