Identification and Function of Antioxidants from Oat Groats and Hulls

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ABSTRACT: Antioxidant components of methanolic extracts of groats and hulls from Ogle oats were identified and quantified by using gas chromatography–mass spectrometry (GC–MS) and gas chromatography after *N,O*-bis(trimethylsilyl)acetamide derivatization. Ferulic, *p*-coumaric, vanillic, *p*-hydroxybenzoic, 4-hydroxyphenylacetic acids and vanillin and catechol were quantified in groat and hull extracts. Additionally, caffeic acid in groat extracts, and *o*-coumaric, sinapic and salicylic acids in hull extracts were quantified. Extracts from groats and hulls at levels of 0.05, 0.1, 0.2, and 0.3% w/w, based on total phenolic content, were added to soybean oil, and their antioxidant effectiveness was compared with that of 0.02% w/w tertiary butylhydroquinone (TBHQ) and a control (no additives) at 60°C in the dark by measuring peroxide values. Antioxidant activities of both extract sources increased with increased concentration. During 20 d of storage, the groat extract (0.3%) was not significantly different from TBHQ after day 16, and hull extracts (0.2 and 0.3%) were not significantly different from TBHQ on day 20. Oils containing pure phenolics at the same concentrations measured in the groat and hull extracts oxidized more quickly than did oils containing the extracts.
JAOCS 74, 303–307 (1997).

KEY WORDS: Antioxidants, GC–MS identification, oats, peroxide values, phenolics.

Lipid oxidation is a great concern of the food industry. Adding antioxidants to oils and fats is one of the principal ways to retard oxidation, and synthetic antioxidants are widely used in the food industry. Because of the possible toxic effects of synthetic antioxidants, many natural antioxidants have been employed to prevent oxidation. Oats have been used as a source of natural antioxidants for many years since Peters and Musher (1) used oat flour to prevent rancidity of lard and oils. More recently, solvent extraction has been the major method used to isolate oat antioxidants. Daniels and Martin (2), Daniels *et al.* (3), and Daniels and Martin (4,5) used light petroleum ether to defat the whole oat kernel and diethyl ether to extract the phenolic compounds. Durkee and Thivierge (6) used aqueous ethanol to extract the phenolic acids from oat seed meal. Sosulski and other researchers (7,8) extracted free phenolic acids and soluble phenolic acid esters from oats and other cereals by using methanol–acetone–water as the solvent. Collins and coworkers (9,10) used aqueous methanol to extract phenolics from oat groats and hulls. Duve and White (11) compared eight separate solvent systems for extraction of antioxidants from oat groats and hulls. They found that the greatest antioxidant activities were obtained with methanolic extracts. Tian and White (12) purified the methanolic extracts by using a silica gel column.

Some phenolics were identified in oat flour by using paper chromatography and thin-layer chromatography (TLC) (3,6). Sosulski *et al.* (8) reported the occurrence of some phenolic acids from debranned oat flour extracts. Tian and White (12) used *N,O*-bis(trimethylsilyl)acetamide (TMSA) derivatization (13) followed by gas chromatography–mass spectrometry (GC–MS) to identify ferulic and caffeic acids from oat groats.

Comparably less research has been done on phenolics in oat hulls. By using TLC and paper chromatography, Taketa (14) and Vogel (15) found some phenolics in oat hulls. However, little has been done on identifying and quantifying phenolics from oat hulls by using GC and GC–MS. The objectives of this study were to improve the extraction of antioxidants from oat groats and hulls [previously described (12)] from our laboratory, to identify and quantify phenolic antioxidants in the extracts by TLC, GC, and GC–MS, and to test the antioxidant activity in soybean oil (SBO).

EXPERIMENTAL PROCEDURES

Extraction of oat groat and hull antioxidants. "Ogle" oats (*Avena sativa* L.) were 1994 certified stock grown in Story County, IA. A Wintersteiger dehuller, type LD 180 (Gesellschaft m.b.H. & Co., Ried, Austria) was used to dehull the oats. Broken groats were removed by hand before milling. A grist mill (Magic Mill III Plus, Salt Lake City, UT) was used to grind both oat groats and hulls at a medium-size setting.

Antioxidants from oats were extracted by the methods of Duve and White (11) and Tian and White (12); however, more solvent was used in the current procedure. Oat groats or hulls (200 g) were extracted with methanol at room temperature

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(25°C) with constant stirring for 24 h. The solvent was changed every day for 7 d. Solvent amounts used were as follows: day 1, 1.4 L; days 2 and 3, 1.2 L; and days 4–7, 800 mL.

Determination of total phenolic contents. Total phenolic content of the extracts was tested by using methods (9.110 through 9.112) of the Association of Official Analytical Chemists (AOAC) (16).

Fractionation of oat extracts by column chromatography before TLC. The crude extracts were fractionated through a column (25 × 250 mm) of Silica Gel 60 (70–230 Mesh ASTM; EM Science, Gibbstown, NJ) to produce a purified extract. The silica gel was activated overnight at 120°C. Five milliliters of methanolic crude extract were applied to the column, which was then eluted with 500 mL chloroform/ethanol/acetic acid (49:1:1, vol/vol/vol). The eluent was collected in ten 50-mL fractions which were evaporated to 1 mL each.

*TLC to identify the chemical composition of the oat extracts***.** The chemical composition of the oat extracts was tentatively determined by following a modified procedure of Pratt and Miller (17). Nine sprays were used to help identify the composition of the oat extracts (Table 1).

Fractionation of oat extracts by column chromatography before identification and quantification of phenolics by GC and GC–MS. The crude extracts were fractionated through a column (35 × 500 mm) of Silica Gel 60 (70–230 Mesh ASTM; EM Science, Gibbstown, NJ) previously activated at 120°C. Fifteen milliliters of extract were applied to the column, which was then eluted with 1 L of ethyl acetate: hexane (3:1, vol/vol). The eluent was collected in twenty 50-mL fractions, and similar fractions (tested by TLC) were combined to make 10 fractions, each of which was evaporated to 1 mL.

GC and GC–MS to identify and quantify the chemical composition of the oat extracts. The crude extracts and each fraction from column chromatography were derivatized with TMSA and analyzed by GC and GC–MS by using the method of Pometto and Crawford (13). Briefly, TMSA derivatization was accomplished by adding 100 µL of dioxane, 10 µL of pyridine, and 50 µL of TMSA to the vial, which contained 1 to 3 g oat extract. The compound 3,4-dimethoxybenzaldehyde (0.1% wt/vol) in 1,4-dioxane was used as an internal standard. The vials were incubated in a Reacti-Therm III heating module (Pierce Chemical Company, Rockford, IL) at 35°C for exactly 2 h from the time of TMSA addition. Twenty-three standards (Sigma Chemical Company, St. Louis, MO) also were converted to TMSA derivatives and used as known compounds on GC–MS. The standards used were: ferulic, caffeic, vanillic, syringic, gentisic, *trans*-cinnamic, protocatechuic, *o*-, *m*-, and *p*-coumaric, *p*-hydroxybenzoic, salicylic, 4-hydroxyphenylacetic, homovanillic, homogentisic, sinapinic, gallic and β-resorcylic acids; and vanillin, protocatechualdehyde, catechol, acetosyringone, and sinapinaldehyde.

After derivatization, the extracts were injected into the GC and GC–MS (GC, Hewlett-Packard 5890 Series II, equipped with a flame-ionization detector and split/splitless injector, MS, Hewlett-Packard 5970 Series Mass Selective Detector; Kennett Square, PA). A SPB-1 fused-silica capillary column of 0.25 mm × 30 m × 0.25 µm film thickness (Supelco Inc., Bellefonte, PA) was used, and program parameters were as follows: injector temperature, 240°C; detector temperature, 280°C; initial oven temperature, 120°C; initial time, 2 min; rate, 20°C/min; final temperature, 260°C; and final time at 260°C, 10 min. For the flame-ionization detection, the carrier gas was helium, and the flow rate was 20 mL/min with 1 to 2 mL/min flowing through the column. The excess gas was split off before reaching the column.

Oil storage for testing the antioxidative effect of oat extracts. The extracts were evaluated for antioxidant activity in refined, bleached, and deodorized SBO obtained from Archer Daniels Midland Co. (Des Moines, IA). The oil contained no additives except for citric acid. All tests were run in duplicate oil samples. SBO (10 g) containing purified extracts of oat groats and hulls at concentrations of 0.05, 0.1, 0.2 and 0.3%, based on total phenolic content of the extracts, were stored in 50-mL open beakers at 60°C in the dark for 20 d. Additional treatments included tertiary butylhydroquinone (TBHQ) (0.02%) and a control containing no additives. The oils were sampled every two days and peroxide values (PV, method described later) were measured. SBO was chosen for testing the antioxidative activity of the extracts because SBO is easily oxidized. Storage tests were conducted at 60°C in the dark instead of 30°C in the light because the antioxidative effects of previously tested groat extracts were more pronounced under the former conditions (12). The percentage inhibition of the treatment (Table 2) was calculated on day 20 by this formula: [(PV of control – PV of treatment)/(PV of control)] × 100.

Oil storage for testing the antioxidative effect of pure phenolics. The amounts and proportions of phenolics identified in the original extracts of the oat groats and hulls were tested in SBO. For example, SBO (10 g) containing the same, or half the amounts and proportions of phenolics as the most effec-

TABLE 1
Sprays Used and Results in Antioxidant Identification by Thin-Layer Chromatography

Sprays	Color	Identified components
$FeCl_3$–$K_3Fe(CN)_6$	Blue	Phenolics
$FeCl_3$	Brown	Phenolics without di-, tri-hydroxy groups
NH_4OH–$AgNO_3$	Brown	Reducing compounds
DPNA[a]	Brown	Phenolics with free *o*-, *p*-OH group
Na_2CO_3	Fluorescent color change	Phenolics with free OH group
I_2 vapor	Brown	Sugar mercaptals, alcohols, hexanoic acids, glycerides, *N*-acylamino sugars, neutral and acid polysaccharrides
Aniline oxalate	Yellow	Uronic acid
p-Anisidine	Light brown	Deoxy sugars and aldohexoses
p-Anisidine HCl	Brown	Aldopentoses

[a]Diazotized *p*-nitroaniline.

TABLE 2
Peroxide Values (PV) and Percentage Inhibition of Oat Extracts Toward Soybean Oil Oxidation on Day 20

		Groat extract at					Hull extract at			
	Control[a]	0.05%	0.1%	0.2%	0.3%	Control[a]	0.05%	0.1%	0.2%	0.3%
PV (meq/kg)	151	21.5[b]	17.8[b]	16.7[b]	2.4[c]	91.6	12.0[b]	6.8[c]	4.2[d]	3.3[d]
Inhibition (%)	—	85.8[c]	88.2[c]	89.0[c]	98.4[b]	—	87.0[d]	92.6[c]	95.5[b]	96.4[b]

[a]Control contains only soybean oil with no added extract. Means with the same letter (b–d) within the same category of a row are not significantly different.

TABLE 3
Peroxide Values (PV) and Percentage Inhibition of Oat Extracts and Pure Phenolics Toward Soybean Oil Oxidation on Day 20

			Pure phenolics[b] in groat extract at			Pure phenolics[b] in hull extract at	
	Control[a]	Groat extract 0.3%	0.3%	0.15%	Hull extract 0.3%	0.3%	0.15%
PV (meq/kg)	211	4.5[d]	38.7[b]	26.6[b,c]	13.2[c,d]	45.6[b]	37.9[b]
Inhibition (%)	—	97.9[b]	81.7[e]	87.4[d]	93.8[c]	78.4[f]	81.9[e]

[a]Control contains only soybean oil with no added extract. Means with the same letter (b–f) within the same category of a row are not significantly different.
[b]The amount and proportion of the phenolics identified in 0.3 or 0.15% of the original extracts.

tive amount of the extract were stored in 50-mL open beakers at 60°C in the dark for 20 d. For comparison, the optimal level of the extracts and a control containing no additives were included. Tests were run in duplicate oil samples. The percentage inhibition of the treatments (Table 3) on day 20 was calculated by the formula just shown.

PV. The PV of the oils were analyzed at the time of sampling by using the Stamm test as modified by Hamm *et al.* (18). The PV of the oils were run in duplicate and the results were averaged.

Data and statistical analysis. All data are the average of two replicate experiments. The least square means of the PV were calculated by using the Statistical Analysis System (19). The significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Determination of total phenolic content (TPC). The TPC of crude and purified groat and hull extracts were determined. The results are the average of several determinations. The hull had about 560 mg/kg TPC, which was higher than that of the groat (400 mg/kg). Purified extracts had TPC values similar to those of the crude extracts, indicating that few, if any, phenolics were removed during purification of the extracts through silicic acid.

Tian and White (12) obtained TPC values of only 30 mg/kg from oat groat. The current results gave much greater TPC values, likely because greater solvent-to-groat ratios of 6:1 vol/wt to 7:1 vol/wt were used during the first 3 d, compared with the 4:1 vol/wt used previously.

TLC to identify the chemical composition of the oat extracts. The TLC and sprays described in Table 1 were used to tentatively identify in the groat and hull extracts the types of antioxidants and the nature of compounds bound to them. Results showed the presence of phenolics and other components, such as sugars, alcohols, acids and glycerides, in both groat and hull extracts. The results were similar to those reported in previous studies (11,12).

GC and GC–MS to identify the chemical composition of the oat extracts. By using GC–MS and GC, ferulic, *p*-coumaric, caffeic, vanillic, *p*-hydroxybenzoic, and 4-hydroxyphenylacetic acids, and vanillin and catechol were identified and quantified in the groat extracts (Table 4). Sosulski *et al.* (8) found ferulic, *p*-coumaric, caffeic, vanillic, *p*-hydroxybenzoic, (*p*-hydroxyphenyl)acetic, syringic, sinapic, and protocatechuic acids in debranned oat flour extracts, with ferulic acid being present in the greatest amount. They found total phenolic acids in oat flour of 87 mg/kg, which was less

TABLE 4
Concentrations of Monophenolics Found in the Ground Groats and Hulls as Determined by GC and GC–MS[a]

Phenolic	Ground groats (mg/kg)	Ground hulls (mg/kg)
Ferulic acid	147.2	142.3
p-Coumaric acid	44.9	59.7
Caffeic acid	16.8	—
Vanillic acid	16.1	24.3
p-Hydroxybenzoic acid	3.5	50.0
Vanillin	3.4	54.2
4-Hydroxyphenylacetic acid	0.6	4.6
Catechol	trace	0.1
o-Coumaric acid	—	6.9
Sinapic acid	—	5.6
Salicylic acid	—	3.1
Total	232.5	350.8

[a]Calculation based on 6% of moisture in both ground groats and hulls. GC–MS, gas chromatography–mass spectrometry.

than that found in the current study (233 mg/kg). Durkee and Thivierge (6) also found ferulic, *p*-coumaric, vanillic, *p*-hydroxybenzoic, and sinapic acids in oat seed meal, also with ferulic acid having the greatest concentration. They identified phenolic acids by using specific color reactions, R_f values on TLC, and comparison with authentic compounds using paper chromatography. The present study agrees with both of these papers in that ferulic acid occurred in the greatest amount. In addition to phenolic acids, vanillin, and catechol were found in oat groats in the present study.

Ferulic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, *o*-coumaric, sinapic, 4-hydroxyphenylacetic and salicylic acids, and vanillin and catechol were identified and quantified in the hull extracts (Table 4). Among those, *o*-coumaric, 4-hydroxyphenylacetic and salicylic acids, and vanillin and catechol were reported for the first time. Fatty acids, such as hexadecanoic and tetradecanoic acids, also were found in the oat extracts in this study. Taketa (14) and Vogel (15) found ferulic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, sinapic, and syringic acids in oats by using TLC and paper chromatography; however, the amounts were not quantified. Some of the monophenolics may come from the fragments of glycosides after derivatization under these conditions. The current study, however, did not evaluate the native form of the phenolics in oat extracts.

Oil storage for testing the antioxidative effect of oat extracts. Preliminary studies of the extracts in SBO showed that oat extracts had little antioxidative effect on SBO at concentrations less than 0.05% w/w (based on TPC). Oat extracts at 0.05, 0.1, 0.2, and 0.3% w/w in oil were used in further studies.

Figure 1 shows the PV development during storage at 60°C in the dark of a control SBO with no additives, oat groat extracts (Fig. 1A), oat hull extracts (Fig. 1B), or with TBHQ. After day 8, differences among treatments began to occur, with the control oxidizing very quickly. During the study, the oil with 0.3% groat extract was not significantly different from the oil with TBHQ. Oils containing 0.2 and 0.3% hull extract were not significantly different in PV from the oil with TBHQ. Table 2 shows the percentage of inhibition of oat extracts toward SBO oxidation based on the PV difference from the control on day 20. Percentage of inhibition increased as the concentration of groat and hull extracts increased. Groat extract at 0.3% and hull extract at 0.2 and 0.3% had significantly more inhibition than did the other treatments. These results were different from those of Duve and White (11), in which no significant differences in PV occurred among the control and the SBO containing groat and hull extracts during storage at the same conditions. These differences may be because lesser concentrations (<0.1% extracts) were used in the previous study. Tian and White (12) reported that SBO samples containing groat extracts (0.01, 0.02, and 0.03%) were not significantly different from SBO containing TBHQ. The concentrations of extracts in the latter study were effective at seemingly lower concentrations than in the present study; however, concentrations were based on TPC, and the TPC in the current study were 13 times greater than in the study by Tian and White (12). Therefore, the actual amounts of extract that were effective were about the same.

Oil storage for testing the antioxidative effect of pure phenolics. Figure 2 shows the PV development during storage at 60°C in the dark of (i) a control SBO (no additive), and of SBO containing (ii) oat hull extract (0.3%), (iii) oat groat ex-

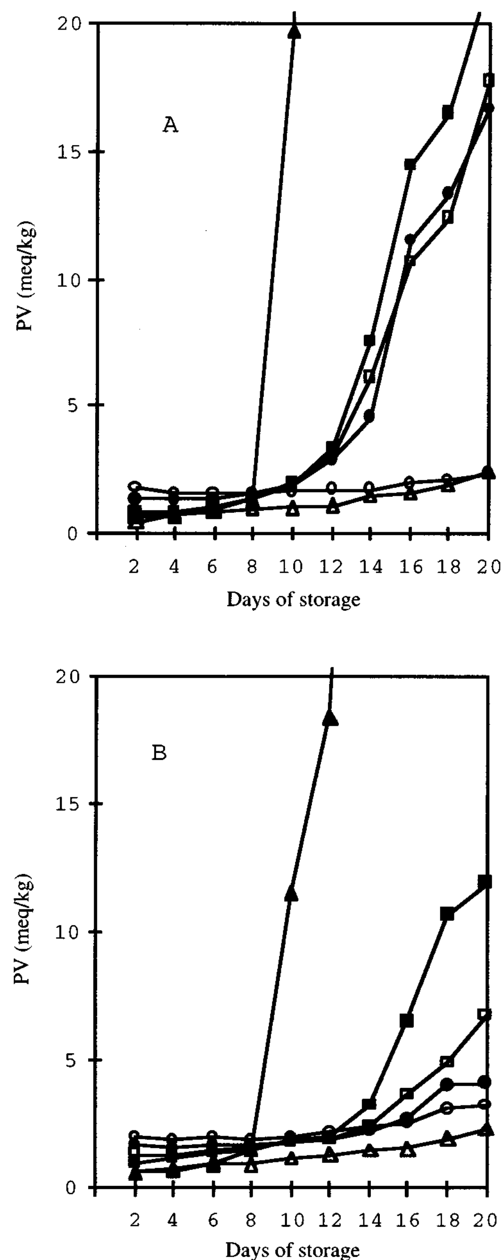

FIG. 1. Peroxide values (PV) for soybean oil (SBO) stored at 60°C. (A) ▲, SBO (no additives); △, SBO with 0.02% TBHQ; ■, SBO with 0.05% oat groat extract; □, SBO with 0.1% groat extract; ●, SBO with 0.2% groat extract; ○, SBO with 0.3% groat extract. (B) Peroxide values for SBO stored at 60°C. ▲, SBO; △, SBO with 0.02% TBHQ; ■, SBO with 0.05% oat hull extract; □, SBO with 0.1% hull extract; ●, SBO with 0.2% hull extract; ○, SBO with 0.3% hull extract; TBHQ, tertiary butylhydroquinone.

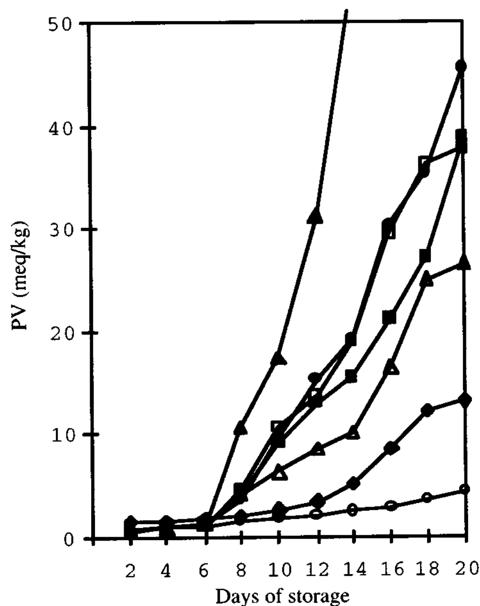

FIG. 2. Peroxide values (PV) for SBO at 60°C. ▲, SBO; ○, SBO with 0.3% oat groat extract; ◆, SBO with 0.3% oat hull extract; SBO with the amount and proportion of the phenolics identified in 0.3% groat extracts (■); 0.15% groat extracts (△); 0.3% hull extracts (●); 0.15% hull extracts (□). For abbreviations, see Figure 1.

tract (0.3%), (iv) the amounts and proportions of phenolics identified in 0.3% groat extracts, (v) the amounts and proportions of phenolics identified in 0.15% groat extracts, (vi) the amounts and proportions of phenolics identified in 0.3% hull extracts, and (vii) the amounts and proportions of phenolics identified in 0.15% hull extracts. The PV of the control was significantly greater than that of all other treatments. There were no significant differences between treatments containing different amounts of pure phenolics for both hulls and groats. The PV of treatments containing the groat and hull extracts were significantly less than all other treatments and the control. The percentage inhibition of the pure phenolics and oat extracts toward SBO oxidation on day 20 are shown in Table 3. Groat and hull extracts both had significantly more inhibition of oxidation than the pure phenolics at the same amounts and proportions. These results indicate the possible presence of additional, unmeasured antioxidants in the extracts, such as flavonoids and other phenolics. The groat extract had a greater antioxidative effect than did the hull extract at the same concentration. This may be because of the presence of caffeic acid, which was only found in the groat extract, but not the hull extracts. In previous tests of methyl linoleate in the lipophilic solvent, dodecane, and under strong oxidizing conditions, caffeic acid had a much greater antioxidative efficiency than did *p*-hydroxybenzoic, sinapic, and salicylic acids (20). The latter three compounds occurred in much greater concentration or only occurred in oat hulls. Both extracts, however, dramatically reduced the formation of peroxides during storage of the soybean oil for 20 d.

ACKNOWLEDGMENTS

We thank Dr. Kenneth Frey, professor, Department of Agronomy, Iowa State University, for providing the oats for this research project. This is Journal Paper J-16843 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 3128.

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[Received April 30, 1996; accepted October 17, 1996]