Antioxidant Capacity of Oat (*Avena sativa* L.) Extracts. 2. In Vitro Antioxidant Activity and Contents of Phenolic and Tocol Antioxidants

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Oat milling fractions were examined for concentrations of total phenolics, tocols, and phenolic acids and in vitro antioxidant activity to determine their potential as dietary antioxidants. Methanolic extracts of pearling fractions, flour and aspirations from flaking, and trichomes had high, intermediate, and low antioxidant activities, respectively, evaluated by the β-carotene bleaching method. Pearling fractions were also highest in total phenolics and tocols. *p*-Hydroxybenzoic acid, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, and ferulic acid were identified and quantified by HPLC. Three avenanthramides and an unidentified ferulate derivative were also detected. Total phenolic content was significantly correlated with antioxidant activity, and regression equations that predicted antioxidant activity from phenolic and tocol concentrations were calculated. Antioxidant activity, evaluated by β-carotene bleaching, was correlated with measures of oxygen radical absorbance capacity and low-density lipoprotein oxidation. These data indicate a potential for oat products, especially those enriched in outer layers of the groat, to contribute to dietary intakes of antioxidant phytonutrients.

Keywords: *Avena sativa; oat; antioxidant activity; phenolic acids; tocols*

INTRODUCTION

Antioxidants are important in the prevention of human disease. Compounds with antioxidant activity may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents, and quenchers of singlet-oxygen formation (Andlauer and Fürst, 1998), thereby protecting the body from degenerative diseases such as cancer (Bailey and Williams, 1993). Hydroxycinnamates such as ferulic acid, caffeic acid, and *p*-coumaric acid inhibit low-density lipoprotein (LDL) oxidation (Castelluccio et al., 1996), potentially protecting the body from atherosclerosis (Wayner et al., 1985).

Phenolic compounds with antioxidant activity, such as flavonoids and derivatives of cinnamic and benzoic acids, have been identified in oats, but few quantitative data are available (Durkee and Thivierge, 1977; Collins, 1986, 1989; Duve and White, 1991; Dimberg et al., 1993; Xing and White, 1997). The effects of processing on the content and activity of potential antioxidative compounds in oats are less well characterized (Dimberg et al., 1996).

Methods for extraction (methanol, ethanol, and ethyl acetate), identification of compounds (TLC, GC/MS, and HPLC), and evaluations of antioxidant activity [oxygen radical absorbance capacity (ORAC), Rancimat, DNA oxidation, LDL oxidation] vary considerably, making comparisons between studies difficult. Further considerations for measuring antioxidant activity include time and expense and relating in vitro results with potential in vivo activity.

The objectives of this study were to (1) measure the antioxidant activities of methanol extracts of oat fractions by using a simple, inexpensive in vitro test to determine which fractions had the greatest activities, (2) compare those activities with other measures of antioxidant activity, ORAC and LDL oxidation, (3) separate phenolic compounds and tocols in the extracts by HPLC and determine the identities of the major components and their concentrations, and (4) use multiple regression analysis to predict antioxidant activities from component concentrations.

MATERIALS AND METHODS

Sample Preparation. Oat fractions were prepared by The Quaker Oats Co. Samples were collected from steamed and kiln-dried groat flour, green (undried) groat flour, trichomes (groat hairs), green groat pearlings (5.00% of groat weight), steamed and kiln-dried groat pearlings (1.66, 2.97, and 7.14% of groat weight), and aspirations from the flaking process. All samples (~75−215 g) were extracted with methanol (500 mL) for 2 h with stirring. The samples were filtered (Whatman No. 1 paper), and the residue was washed with methanol (200 mL). Combined extracts were concentrated under vacuum and adjusted to a standard final volume (80 mL). Extracts were analyzed for inhibition of LDL oxidation and ORAC by a research team at The Jean Mayer USDA Human Nutrition Research Center on Aging in Boston, MA (Handelman et al., 1999). Subsamples were subsequently tested by us for antioxidant activity in the form of inhibition of coupled autoxidation of linoleic acid and β-carotene, total phenolic contents, individual phenolics content, and tocol content.

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Antioxidant Activity Assay. Antioxidant activity of all extracts was determined by measuring the coupled autoxidation of β-carotene and linoleic acid (Marco, 1968; Miller, 1971; Lee et al., 1995). Samples were diluted with methanol to the equivalent of 10 mg of starting material per 40 μL. β-Carotene (2 mg) was dissolved in 20 mL of chloroformm, and 3 mL was added to 40 mg of linoleic acid and 400 mg of Tween 40. Chloroform was removed under a stream of nitrogen gas. Oxygenated deionized water (100 mL) was added and mixed well. Aliquots (3 mL) of the β-carotene/linoleic acid emulsion were mixed with 40 μL of sample and incubated in a water bath at 50 °C. Oxidation of the emulsion was monitored spectrophotometrically (Shimadzu UV160U recording spectrophotometer) by measuring absorbance at 470 nm over a 60-min period. Control samples contained 40 μL of methanol in place of the extract. Degradation over time is nonlinear; therefore, antioxidant activity is expressed as percent inhibition relative to the control after a 60-min incubation using the equation

$$\mathrm{AOA} = 100(\mathrm{DR_C} - \mathrm{DR_S})/\mathrm{DR_C}$$

where AOA is the antioxidant activity, DR_C is the degradation rate of the control $= \ln(a/b)/60$, DR_S is the degradation rate of the sample $= \ln(a/b)/60$, a is the initial absorbance at time 0, and b is the absorbance at 60 min.

Total Phenolic Content. A determination of total phenolic content, measured as gallic acid (GA) equivalents (mg kg^{-1}), was made with the Folin−Ciocalteu phenol reagent (Ragazzi and Veronese, 1973). One-milliliter samples were diluted to 25% of the original concentration with methanol, and 0.5 mL of Folin−Ciocalteu phenol reagent (2.0 N) and 3.0 mL of Na_2CO_3 (200 g L^{-1}) were added in the given order. The mixtures were vortexed and the reactions allowed to proceed for 15 min at room temperature. The mixtures were then diluted with 10 mL of deionized water and centrifuged for 5 min at 1250*g*, and the absorbance was measured at 725 nm. Methanol was used as a control in the place of the sample. Gallic acid equivalents were determined from a standard concentration curve.

Identification and Quantitation of Phenolic Compounds. Phenolic components of extracts were separated using HPLC (Gilson). Samples (20 μL) were eluted from a C_{18} column (Alltech Econosphere, 5 μm pore size, 150 × 4.6 mm) using a 1−40% linear gradient of acetonitrile (pH 2.8 with acetic acid) over a 75-min period at a flow rate of 1 mL min^{-1} and detected at 290 nm. Compounds were identified by comparing retention times and absorption spectra (200−360 nm) with known samples using a Shimadzu Model SD-M10VP diode array detector. Quantities of identified compounds were determined using external standards and expressed as milligrams per kilogram of extracted material.

Identification and Quantitation of Tocols. An aliquot of each sample extract was evaporated under vacuum at 40 °C. The tocols in the residues were redissolved in 2 mL of hexane and filtered, and aliquots were analyzed by HPLC as previously described (Peterson and Qureshi, 1993). Briefly, a Waters μPorosil 10-μm particle size, 3.9 × 300 mm, column was used with an isocratic Gilson HPLC, using 0.5% 2-propanol in hexane as the mobile phase. Flow rate was 1.3 mL min^{-1}, and peaks were detected with a Shimadzu RF-535 fluorescence monitor using an excitation wavelength of 295 nm and emission wavelength of 330 nm. Peaks were identified by retention time compared to a tocotrienol-rich fraction of palm oil (PORIM, Malaysia) that contains α-tocopherol and α-, γ-, and δ-tocotrienols. They were quantitated by integration of peak areas with Gilson's 712 software and compared against α- and γ-tocopherol standards. Tocotrienols were quantitated against the corresponding tocopherol standard; β-tocopherol and β-tocotrienol were quantitated against γ-tocopherol. Total tocols are reported as the sum of tocopherols and tocotrienols.

Statistical Analyses. Correlation and regression analyses were performed using the CORR and REG procedures of SAS, respectively (SAS Institute, 1990). Multiple-component models for maximizing R^2 were developed with the RSQUARE and MAXR methods of REG.

Table 1. Antioxidant Activity and Total Phenolic and Tocol Concentrations of Oat Fractions

		mg kg^{-1}	
fraction	AOA[a]	TPC[b]	tocols[c]
trichomes	47.4 ± 4.3[d]	139 ± 13	14.6 ± 1.1
dried groat flour	52.6 ± 5.0	89 ± 7	11.7 ± 0.5
green groat flour	60.8 ± 5.5	156 ± 14	21.3 ± 1.9
pearlings, 1.66%	73.4 ± 3.1	342 ± 37	52.0 ± 1.6
pearlings, 2.97%	77.6 ± 3.1	337 ± 43	67.7 ± 5.4
pearlings, 7.14%	74.6 ± 2.7	274 ± 22	54.3 ± 2.7
green pearlings, 5%	75.3 ± 2.9	324 ± 31	69.5 ± 3.5
aspirations	56.5 ± 6.1	107 ± 2	11.1 ± 2.4

[a] Antioxidant activity, percent inhibition of autoxidation of linoleic acid/β-carotene emulsion. [b] Total phenolic concentration. [c] Sum of tocopherols and tocotrienols. [d] Standard error of the mean.

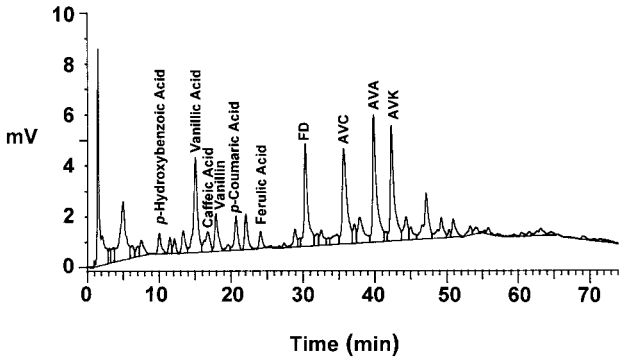

Figure 1. Separation of phenolic compounds from pearling sample by HPLC on C_{18} column using a 1−40% acetonitrile gradient, pH 2.8, 1 mL min^{-1} flow rate, detected at 290 nm.

RESULTS

Antioxidant Activity. Pearling samples had the highest antioxidant activity (73.4−77.6%), followed by flour (52.6−60.8%), aspirations (56.5%), and trichomes (47.4%) (Table 1). Antioxidant activity of green pearling samples did not differ from that of dried pearlings, whereas green groat flour (60.8%) had greater activity than dried groat flour (52.6%). The in vitro antioxidant activity test values were significantly positively correlated ($P \leq 0.05$) with three other measures of antioxidant activity, LDL-AAPH ($r = 0.71$), LDL-Cu^{2+} ($r = 0.88$), and ORAC-AAPH ($r = 0.72$), conducted on the same extracts and reported by Handelman et al. (1999). The correlation with ORAC-Cu^{2+}/H_2O_2 ($r = 0.46$) was also positive, but not significant.

Total Phenolic Content. Total phenolic content was highest in pearlings (274−342 mg kg^{-1}), followed by green groat flour (156 mg kg^{-1}), trichomes (139 mg kg^{-1}), aspirations (107 mg kg^{-1}), and dried groat flour (89 mg kg^{-1}) (Table 1). Total phenolic content was significantly correlated ($P \leq 0.05$) with antioxidant activity ($r = 0.93$), LDL-AAPH ($r = 0.82$), LDL-Cu^{2+} ($r = 0.98$), and ORAC-AAPH ($r = 0.79$). Trichomes were lower in antioxidant activity relative to their phenolic content as compared to the other samples.

Phenolic Compounds. All samples except the trichomes had qualitatively similar HPLC chromatograms (Figure 1). *p*-Hydroxybenzoic acid, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, and ferulic acid were identified by comparing retention times and spectra from the diode array detector with authentic standards.

Table 2. Soluble Phenolic Components of Oat Fractions

fraction	HBA[a]	VA	CA	VAN	PCA	FA	AVA	AVK	AVC	FD[b]
trichomes	39 ± 0.6[c]	0 ± 0.0	2 ± 0.3	7 ± 0.0	2 ± 0.0	3 ± 0.1	12 ± 0.2	9 ± 0.0	5 ± 0.1	1 ± 0.0
dried groat flour	7 ± 1.8	5 ± 0.0	1 ± 0.0	1 ± 0.0	1 ± 0.0	1 ± 0.0	14 ± 0.1	12 ± 0.0	13 ± 0.0	1 ± 0.0
green groat flour	2 ± 0.7	11 ± 0.2	3 ± 0.6	1 ± 0.0	1 ± 0.0	1 ± 0.0	25 ± 0.4	27 ± 1.4	22 ± 0.6	1 ± 0.2
pearlings, 1.66%	29 ± 0.1	15 ± 0.5	5 ± 0.1	4 ± 0.1	1 ± 0.0	1 ± 0.0	55 ± 7.3	47 ± 8.1	48 ± 6.7	7 ± 0.8
pearlings, 2.97%	30 ± 1.1	20 ± 1.0	4 ± 0.9	4 ± 0.7	1 ± 0.0	2 ± 0.1	68 ± 6.8	61 ± 4.1	56 ± 6.6	7 ± 0.6
pearlings, 7.14%	20 ± 2.8	13 ± 0.6	3 ± 0.4	3 ± 0.2	1 ± 0.0	1 ± 0.0	50 ± 0.4	43 ± 1.0	44 ± 1.7	5 ± 1.0
green pearlings, 5%	10 ± 0.8	16 ± 1.2	5 ± 0.0	2 ± 0.2	1 ± 0.1	1 ± 0.0	50 ± 0.4	51 ± 2.2	40 ± 0.2	4 ± 0.3
aspirations	16 ± 1.9	11 ± 1.0	2 ± 0.1	3 ± 0.5	1 ± 0.2	1 ± 0.2	5 ± 1.3	4 ± 0.8	3 ± 0.1	4 ± 0.9

[a] HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CA, caffeic acid; VAN, vanillin; PCA, *p*-coumaric acid; FA, ferulic acid; AVA, avenanthramide A; AVK, avenanthramide K; AVC, avenanthramide C; FD, ferulate derivative. [b] FD is reported as ferulic acid equivalents. [c] Measures are mg kg^{-1} ± standard error of the mean.

One peak with a spectrum matching (+)-catechin and (−)-epicatechin was detected, but its retention time did not match that of either flavan-3-ol. The proton NMR spectrum indicated that this compound contained a ferulate ring, and thus it is being referred to as FD (ferulate derivative) until identification is complete. Three peaks were detected with retention characteristics, UV spectral properties, and NMR spectral properties of avenanthramides (Collins and Mullin, 1988; Collins, 1989; Dimberg et al., 1996). NMR analysis identified two of the peaks as *N*-(4′-hydroxycinnamoyl)-5-hydroxyanthranilic acid [AVA of Collins and Mullin (1988); AV4 of Dimberg et al. (1996)] and *N*-(3′4′-dihydroxycinnamoyl)-4-hydroxyanthranilic acid [AVK of Collins and Mullin (1988)]. Identification of the third peak is tentative pending verification by mass spectroscopy and/or NMR, but it is probably AVC (Collins and Mullin, 1988). In a previous paper (Emmons and Peterson, 1999), AVA, AVC, and AVK were designated AV4, AV3, and AV1, respectively, using the terminology of Dimberg et al. (1996). Compounds that were identified and had standards available were quantified as milligrams per kilogram of starting material (Table 2). FD was analyzed and presented as ferulic acid equivalents (mg kg^{-1}).

The avenanthramides, caffeic acid, FD, and vanillic acid were the phenolics that were significantly correlated ($P \leq 0.05$) with antioxidant activity ($0.74 \leq r \leq 0.95$). Caffeic acid, FD, and the avenanthramides were significantly correlated with LDL-Cu^{2+} ($0.83 \leq r \leq 0.92$), caffeic acid, AVA, and AVK were significantly correlated with LDL-AAPH ($0.72 \leq r \leq 0.86$), and caffeic acid and AVK were significantly correlated with ORAC-AAPH ($r = 0.92$ and 0.74, respectively). No individual phenolic concentrations were correlated with ORAC-Cu^{2+}/H_2O_2.

Tocols. Pearling fractions (dried or green) were higher in total tocols than all other fractions (Table 1). Dried groat flour was lower in tocols than the green groat flour. Trichomes were lower in tocols than green groat flour but higher than dried groat flour and aspirations. Aspirations and dried groat flour had the lowest total tocols. Total tocols were significantly correlated ($P \leq 0.05$) with antioxidant activity, ORAC-AAPH, LDL-AAPH, and LDL-Cu^{2+} ($0.77 \leq r \leq 0.95$).

Regression Analysis. Regression analysis was performed to determine individual and combinations of phenolic compounds and total tocols that would explain the greatest amount of variation in the antioxidant activity measures (Table 3). Other combinations of one to four variables predicted the activities determined by the several methods almost as well as those shown. The best single-phenolic component model (AVK) accounted for 90% of the variation in AOA. None of the other methods were predicted as well by a single component.

Table 3. Regression Analysis of Phenolic and Tocol[a] Models for Antioxidant Methods

method	model[b]	R^2
AOA	0.5 AVK + 48.4	0.90
	0.5 AVK − 2.9 FA + 52.5	0.93
	0.4 AVK + 1.8 FD − 0.2 HBA + 49.7	0.96
	0.4 AVC + 2.6 FD + 6.2 VAN − 1.2 HBA + 45.9	0.98
	0.4 TT + 48.2	0.90
ORAC-AAPH	1.4 CA − 0.1	0.84
	1.9 CA − 0.3 FD − 0.1	0.93
	1.3 CA − 0.2 VA + 0.05 AVC + 0.6	0.97
	1.2 CA − 0.6 FA − 0.2 VA + 0.04 AVA + 1.3	1.00
	0.06 TT + 1.9	0.59
ORAC-Cu^{2+}/H_2O_2	2.8 CA − 4.4	0.53
	5.0 CA − 1.8 FD − 4.6	0.92
	4.9 CA − 1.6 FD − 0.5 VAN − 3.2	0.94
	2.4 CA − 0.3 VA + 0.8 AVK − 0.7 AVA − 1.7	0.98
LDL-AAPH	1.2 CA − 1.3	0.75
	0.3 AVK − 0.2 AVC − 0.1	0.86
	0.6 CA + 0.2 AVK − 0.2 AVC − 0.8	0.92
	1.7 VAN − 3.1 FA + 0.7 AVK − 0.6 AVA − 0.3	0.97
	0.06 TT + 0.08	0.71
LDL-Cu^{2+}	0.06 AVA + 0.3	0.84
	0.4 CA + 0.04 AVA − 0.4	0.91
	0.6 FD − 0.2 VA + 0.05 AVA + 0.1	0.99
	0.8 FD − 0.3 CA − 0.2 VA + 0.06 AVK + 0.4	0.99
	0.06 TT + 0.3	0.89

[a] Sum of tocopherols and tocotrienols = TT. [b] The best significant ($P \leq 0.05$) phenolic model for each method is given for one, two, three, and four variables.

The best two-component phenolic models contained caffeic acid or AVK as positive variables and ferulic acid, FD, or AVC as negative variables. These models improved R^2 for all methods as compared to one-component models. Caffeic acid and FD were the most prominent variables in the three-component models, followed by AVK, AVC, and vanillic acid. Caffeic acid and AVK were always positive variables. Caffeic acid, vanillic acid, AVK, and AVA were most prominent in the four-component models; AVK was always a positive variable, and vanillic acid always negative. The methods were predicted better by three- and four-component phenolic models than by the one- and two-component models. However, AOA, LDL-Cu^{2+}, and ORAC-AAPH were predicted well by the single phenolics AVK, caffeic acid, and AVA, respectively.

AOA and LDL-Cu^{2+} were predicted well by total tocols (Table 3). Two-component models containing a phenolic compound and total tocols (mixed model) predicted antioxidant activity better than the best two-component

Table 4. Regression Analysis of Phenolic and Tocol[a] Mixed Models for Antioxidant Methods

method	two-component model[b]	R^2	three-component model[c]	R^2
AOA	0.4 TT − 3.5 FA + 53.0	0.95	0.3 TT + 1.8 FD − 0.2 HBA + 49.9	0.98
	0.4 TT − 5.6 PCA + 54.3	0.94	0.3 TT + 1.4 FD − 1.4 VAN + 50.9	0.97
	0.4 TT + 0.4 VA + 46.9	0.94	0.4 TT + 0.3 VA − 3.0 FA + 51.2	0.97
	0.4 TT − 1.1 VAN + 51.4	0.93	0.4 TT + 0.5 VA − 0.2 HBA + 49.3	0.97
ORAC-AAPH	0.01 TT + 1.3 CA − 0.01	0.85	0.03 TT + 1.6 CA − 0.4 FD + 0.1	0.96
	0.01 TT − 0.2 VA + 2.4	0.72	0.03 TT + 1.3 CA − 0.1 VA + 0.5	0.95
			0.01 TT + 1.3 CA − 0.04 HBA + 0.6	0.89
			0.01 TT + 1.3 CA − 0.2 VAN + 0.5	0.87
ORAC-Cu^{2+}/H_2O_2			0.1 TT − 1.9 FD + 4.4 CA − 4.1	0.94
			0.1 TT + 3.0 CA − 0.6 VA − 2.6	0.86
LDL-AAPH	0.03 TT + 0.8 CA − 1.0	0.81	0.1 TT + 0.7 CA − 0.1 VA − 0.6	0.88
	0.08 TT − 0.1 VA + 0.4	0.80	0.1 TT + 0.9 CA − 0.3 FD − 0.9	0.88
	0.1 TT − 0.1 AVC + 0.1	0.79	0.1 TT + 0.2 AVK − 0.2 AVC	0.88
	0.1 TT − 0.1 AVA + 0.2	0.77	0.1 TT + 0.7 CA − 0.1 AVC − 0.9	0.87
LDL-Cu^{2+}	0.1 TT + 0.03 HBA − 0.2	0.95	0.04 TT + 0.5 FD − 0.1 VA + 0.1	0.99
	0.1 TT + 0.2 VAN − 0.3	0.94	0.04 TT + 0.3 CA + 0.03 HBA − 0.7	0.98
	0.04 TT + 0.2 FD − 0.1	0.94	0.1 TT + 0.5 VAN − 0.9 FA + 0.01	0.98
	0. 4 TT + 0.3 CA − 0.2	0.92	0.04 TT + 0.3 CA + 0.2 VAN − 0.7	0.97

[a] Sum of tocopherols and tocotrienols = TT. [b] Models given are significant ($P \leq 0.05$) and have improved R^2 from the best single-phenolic or tocol model. [c] Models given are significant ($P \leq 0.05$) and have improved R^2 from the best two-component mixed model.

phenolic model for AOA and LDL-Cu^{2+} (Table 4). The best three-component mixed model was an improvement over the best three-component phenolic model only for AOA.

DISCUSSION

The total phenolic contents of the four pearling fractions were more than double those of the other fractions, indicating a seed coat/aleurone/subaleurone location for many of the phenolic compounds. The slight decrease in total phenolic content of the 7.14% pearlings, relative to the 2.97 and 1.66% pearling samples, indicates their declining concentration toward the interior (starchy endosperm) of the kernel. Fulcher (1986), using histochemical techniques, noted a high concentration of phenolic compounds in the bran layers, especially the aleurone cell walls. Phenolic concentration was considerably reduced in the dried groat flour sample relative to the green groat flour. This indicates that the drying process may either degrade some of the phenolic compounds or render them less soluble. A comparison of the total phenolic concentration of the green pearlings (5%) with the mean of the 2.97 and 7.14% dried pearlings indicates a similar, though less pronounced, trend.

The very high correlation between total phenolic content and AOA indicates that the phenolic compounds may be responsible for a large proportion of the antioxidant activity. Only the trichome sample had an AOA lower than expected on the basis of its total phenolic content. This may indicate that some of the phenolics of the trichomes were inactive as antioxidants, or there may be counteracting prooxidants in trichomes.

The predominant phenolic compounds were the avenanthramides, *p*-hydroxybenzoic acid, and vanillic acid. The sums of the components that were identified and quantified (Table 2) accounted for 45−75% of the total phenolic content measured for each fraction. Testing of individual components with the Folin−Ciocalteu phenol reagent revealed that the components, at equal concentrations, do not contribute equally to the total phenolic content measure. For example, caffeic acid at 5 mg kg^{-1} is equivalent to 7 mg kg^{-1} GA equivalents, whereas vanillic acid at 10 mg kg^{-1} or catechin at 100 mg kg^{-1} is equivalent to 7 or 68 mg kg^{-1} GA equivalents, respectively. The concentrations we report compare favorably with the results of Dimberg et al. (1986), who found similar levels of avenanthramides (21−62 mg kg^{-1}) and some of the phenolic acids in three oat cultivars. Dimberg et al. (1986) reported a prominent unidentified peak that eluted in a similar position to our FD.

Pearlings produced by removing 2.97% of the total groat weight had higher levels of avenanthramides than pearlings composed of fewer outer layers (1.66% groat weight) or pearlings that may include a portion of the endosperm (7.14% groat weight). Aspirations, groat flour, and trichomes removed from groats contained much lower levels of avenanthramides than pearlings, indicating that these components are localized mainly in the aleurone layer of the groat. Processing of groats to concentrate this layer should increase the antioxidant content of the product.

Three other methods of measuring antioxidant activity, the inhibition of Cu^{2+}- or AAPH-induced LDL oxidation and ORAC with AAPH as a peroxyl radical generator, were significantly correlated with total phenolic content. Mechanisms by which oat phenolics can provide protection in these assays are discussed by Handelman et al. (1999). Individual phenolic acids such as caffeic acid and vanillic acid are positively associated with increased antioxidant activity and have antioxidant activity in in vitro linoleic acid oxidation systems (Dimberg et al., 1993). Each of the identified components, with the exception of *p*-coumaric acid, showed antioxidant activity at concentrations within the range found in the oat extracts when tested individually with the in vitro antioxidant activity measure used for the oat extracts (data not shown). Avenanthramides, substituted *N*-cinnamoylanthranilate alkaloids isolated only from oats (Collins, 1989), have also shown antioxidant activity in an in vitro linoleic acid oxidation system (Dimberg et al., 1993). Three avenanthramides, present in high concentrations, were correlated (r = 0.94) with increased antioxidant activity in this study.

Antioxidant activity of oat fractions, as measured by the coupled autoxidation of linoleic acid and β-carotene (AOA), was significantly correlated to ORAC-AAPH and inhibition of LDL oxidation by Cu^{2+} or AAPH, as

measured at the USDA Jean Mayer Human Nutrition Research Center on Aging (Handelman et al., 1999). Therefore, we propose that this method could be used as a quick and inexpensive screen for antioxidant potential of samples before they are tested by more extensive methods.

From the regression analysis it can be concluded that the total antioxidant activity of a sample is due to a complex mixture of several antioxidant and pro-oxidant compounds. Each antioxidant method measures inhibition of oxidation due to different chemical reactions and so is affected by different combinations of tocols and phenolic compounds. The models presented here optimized R^2 for each method, and many models could explain >80% of the variance in antioxidant activity, especially those containing three or four components. Caffeic acid, AVC, AVK, FD, vanillic acid, and vanillin, alone and in combinations with other phenolics, appear to be the most important phenolics in determining antioxidant activity in the oat fractions we studied. Caffeic acid was associated with the two ORAC methods and inhibition of LDL oxidation induced by AAPH, whereas AVA was associated with inhibition of LDL oxidation induced by Cu^{2+} and the coupled autoxidation of linoleic acid and β-carotene (AOA). Due to high correlations between many phenolic compounds, many combinations are possible that would give good predictions of antioxidant activity. Total tocols, alone and in combinations with phenolics, were significantly associated with AOA, ORAC-AAPH, and LDL oxidation induced by AAPH and Cu^{2+}.

ABBREVIATIONS USED

Antioxidant Methods: AOA, antioxidant activity measured as percent inhibition of coupled autoxidation of linoleic acid and β-carotene; ORAC-AAPH, oxygen radical absorbance capacity assay using 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator; ORAC-Cu^{2+}/H_2O_2, oxygen radical absorbance capacity assay using Cu^{2+}/H_2O_2 as a hydroxyl radical generator; LDL-AAPH, inhibition of cholesterol ester hydroperoxide formation from AAPH-induced LDL oxidation; LDL-Cu^{2+}, inhibition of cholesterol ester hydroperoxide formation from Cu^{2+}-induced LDL oxidation. **Phenolic Components**: TPC, total phenolic content; AVA, AVC, and AVK, avenanthramides A, C, and K, respectively; CA, caffeic acid; FA, ferulic acid; FD, unidentified peak with ferulate NMR proton spectrum; HBA, *p*-hydroxybenzoic acid; PCA, *p*-coumaric acid; VA, vanillic acid; VAN, vanillin.

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