

Oat Tocols: Saponification vs. Direct Extraction and Analysis in High-Oil Genotypes¹

David M. Peterson,^{2,3} Camille M. Jensen,² David L. Hoffman,⁴ and Birgitta Mannerstedt-Fogelfors⁵

ABSTRACT

Cereal Chem. 84(1):56–60

Tocols are natural antioxidants that occur in grains that may benefit human and animal health. Therefore, it is important to accurately measure their concentrations in foods and feeds and to determine how genetics and growing environment can influence their levels. The first objective was to evaluate saponification versus direct extraction for the analysis of tocals in oat (*Avena sativa* L.). The second was to determine the effects of growing environment, hulled versus hulless phenotype, and genetic background on tocol concentration, and to see whether tocol and lipid concentrations were associated. For the first objective, oat grain samples from two locations were either extracted by saponification or directly with methanol, and extracts were analyzed by HPLC. The saponification

method increased yield by $\approx 25\%$ and was less time-consuming, so it was adopted for the second objective. For the second objective, oat genotypes were developed by crossing high-oil parents from Iowa State University with hulled and hulless cultivars adapted to arid Western environments. These were grown at Aberdeen and Tetonia, ID, and the tocals and lipid concentrations were analyzed at Madison, WI. There were significant effects of growing environment, genotype, and the presence or absence of hulls on tocol concentrations. Tocol and lipid concentrations were not correlated. Progeny of crosses involving the genotype IA91098-2 had tocol concentrations that exceeded both parents.

Tocals (tocopherols and tocotrienols) exist as lipid-soluble compounds in cereal grains and other sources and are known generically as vitamin E. There are four homologues of tocopherol, α , β , γ , and δ , which have different methyl substitutions on the chroman ring. A similar series exists for the tocotrienols. The tocotrienols have three double bonds in the phytyl side chain, whereas the tocopherol side chain is fully saturated (Kamal-Eldin and Appelqvist 1996). Tocals inhibit lipid oxidation in cells and thus may serve to stabilize foods, in addition to their role as vitamins in humans and livestock. Their biological activity results from their ability to donate phenolic hydrogen atoms to free radicals, thus breaking destructive chain reactions. The activities of the various homologues have varied substantially in both in vitro and in vivo systems (Packer 1995; Kamal-Eldin and Appelqvist 1996), so it is important in evaluating various plant sources to measure the concentration of each homologue.

There are a few reports on tocol concentrations in oat (Slover et al 1969; Lasztity et al 1980; Barnes 1983; Piironen et al 1986; Peterson and Qureshi 1993; Bryngelsson et al 2002). All reports agree that α -tocotrienol is the major homologue, followed by α -tocopherol. Various other homologues are found in minor or trace amounts. A survey of 12 genotypes grown in three locations in the United States found a range of 9.1–18.5 mg kg⁻¹ for the concentration of α -tocotrienol, and 7.2–9.6 mg kg⁻¹ for α -tocopherol (Peterson and Qureshi 1993). A strong correlation ($r^2 = 0.83$) between tocotrienol concentration and oil concentration was found in a group of oat genotypes with an unusually high oil content range of 69–181 g kg⁻¹ (Peterson and Wood 1997). A similar result was found in a study of seven Swedish cultivars with a range of 62–135 g kg⁻¹ of oil (Bryngelsson et al 2002). Recently,

oil bodies were isolated from oat aleurone and germ, and their intrinsic association with tocals was confirmed by analysis (White et al 2006).

Typically, tocals have been extracted from oat grains with methanol (Peterson and Qureshi 1993) and separated by high-performance chromatography on normal-phase silica columns (Kamal-Eldin et al 2000). Recently, Panfili et al (2003) demonstrated a higher yield of tocals from barley by using a hot saponification procedure as compared with direct methanol extraction. A single oat sample in this study yielded 72.1 mg kg⁻¹ of total tocals using the hot saponification procedure, which is higher than previous reports for oat.

The first objective of our study was to compare the saponification procedure with the direct methanol extraction for oat to determine whether a higher yield was consistently obtained, as had been shown for barley (Panfili et al 2003). Then, using the best procedure, we examined a group of oat genotypes that were developed as high-oil lines, but whose oil contents were closer to normal than the lines previously examined by Peterson and Wood (1997). We wanted to determine whether the correlations between tocals and oil content that were found in the extremely high-oil oat genotypes were also present in oat with oil contents closer to the normal range. Also, we wanted to compare levels of lipid and tocals in hulled and hulless genotypes and to determine whether the levels were influenced by the growing location.

MATERIALS AND METHODS

Plant Material

The oat grain used for the methods comparison was harvested from plots grown at Aberdeen, ID, and Madison, WI, in 2003 as part of a larger experiment to determine genotype and environment effects on several constituents. Samples were selected to include a wide range of tocol concentrations. Plants were grown using normal cultural conditions for the area. Duplicate extractions of each sample were analyzed.

The grain used for the analysis of tocals and lipid was from hulled and hulless high-oil (HO) lines that were developed by David Hoffman (Table I). The lines were developed by crossing locally adapted cultivars with high-oil genotypes derived from a recurrent selection program at Iowa State University (Frey and Holland 1999). The HO lines were picked for preliminary yield trials as F₄ plants and the 02HO lines as F₅ plants. Lines were selected for replicated yield trials on the basis of yield, shatter,

¹ Cooperative investigation of the U.S. Department of Agriculture, Agricultural Research Service, and the University of Wisconsin Agricultural Experiment Station. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

² USDA, ARS, Cereal Crops Research Unit, 502 Walnut St., Madison WI 53726.

³ Corresponding author. E-mail: dmpeter4@wisc.edu

⁴ USDA, ARS, Small Grains and Potato Germplasm Research Unit, 1691 S 2700 W, Aberdeen ID 83210, deceased.

⁵ Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, S-75007, Uppsala, Sweden.

lodging, protein, and oil data from the preliminary trial. The hulled and hullless lines were grown together in completely randomized blocks in 2004 at two locations in Idaho: Aberdeen (four replicates) and Tetonia (three replicates). Aberdeen is a mid-elevation (1,350 m) arid site averaging 230 mm of precipitation, mostly coming during the winter months. During the growing season, plants experience moderately hot days and cool nights. The plots were irrigated as needed. Tetonia is a high-elevation (1,885 m) site with warm days and cold nights. The average annual precipitation is 508 mm, coming mostly during the winter. The plots were not irrigated. Single analysis was performed for each extract.

Laboratory Methods

The oat grain from hulled genotypes was dehulled with an impact dehuller; groats and hulls were separated by air aspiration; and groats were sorted by hand to remove broken kernels. The groats and hullless kernels were ground in an ultracentrifugal mill (Retsch ZM-1, Brinkman Instruments Co.) to pass a 0.5-mm screen.

For direct extraction, freshly ground 0.5-g samples were extracted twice with 7 mL of methanol at room temperature for 20 min in screw-capped test tubes on an oscillating shaker. The tubes were centrifuged at 1,000 × g for 6–7 min and the supernatants decanted into conical test tubes. The combined solvents were evaporated in a SpeedVac (ThermoSavant) at 45°C. The residues were dissolved in 2 mL of hexane and stored at –20°C in capped microfuge tubes. For extraction with saponification, the procedure of Panfili et al (2003) was followed, except that it was scaled down to 0.5-g samples. Freshly ground 0.5-g samples were combined with 0.5 mL of KOH (600 g L⁻¹), 0.5 mL of 95% ethanol, 0.5 mL of NaCl (10 g L⁻¹) and 1.25 mL of pyrogallol (60 g L⁻¹ ethanol) in screw-capped test tubes and incubated at 70°C for 25 min. After cooling in an ice bath, 3.75 mL of NaCl (10 g L⁻¹) was added. The suspensions were extracted twice with 3.75 mL of hexane/ethyl acetate (9:1, v/v), the organic phases were combined and evaporated to dryness in the SpeedVac at 45°C. The residues were dissolved in 1% 2-propanol in hexane before analysis.

The tocots were separated by HPLC using an isocratic system with a 5 µm 4.6 × 250 mm silica column (Alltech) and a mobile phase of 5 mL L⁻¹ of 2-propanol in hexane. The flow rate was 1 mL min⁻¹, and 50-µL aliquots of the samples were injected. Peaks were detected by fluorescence with an excitation at 295 nm and emission at 330 nm. The α-tocopherol and α-tocotrienol peaks

were identified by retention time, and peak areas were integrated and compared with an α-tocopherol standard (Matrea) because pure α-tocotrienol is not commercially available. α-Tocotrienol exhibits the same quantitative fluorescence response as does α-tocopherol (Thompson and Hatina 1979). Minor tocot components were not integrated, as they account for a very small percentage of total tocots in oat (Peterson and Qureshi 1993). Peak areas of the standard and of a check sample of barley did not show evidence of a loss of column efficiency during the course of the experiment. Lipids were extracted twice with petroleum ether (10 mL/1.0 g) for 1 hr at room temperature. Supernatants obtained by centrifugation at 2,000 × g were decanted into tared flasks, and the solvent evaporated in a fume hood overnight. The flasks were oven dried (30 min at 40°C), cooled, and weighed.

RESULTS AND DISCUSSION

The total tocot yield from 19 samples had a range of 13–32 mg kg⁻¹ with direct extraction (Fig. 1). This was a greater range than was previously reported for a group of 12 oat genotypes (19–30 mg kg⁻¹) (Peterson and Qureshi 1993). The saponification method out-yielded direct extraction for total tocots in 18 of the 19 samples analyzed (range = 16–50 mg kg⁻¹). The saponification method yielded mean values of 118% of α-tocopherol, 129% of α-tocotrienol, and 126% of total tocots as compared with the direct extraction method, all of which were significant differences (Table II). The methods were highly correlated (*r* = 0.82 for total tocots).

The Swedish cultivar, Freja, had the highest yield at 50 mg kg⁻¹ with the saponification method. This concentration was much lower than the value of 72 mg kg⁻¹ that Panfili et al (2003) reported for one unspecified oat sample. By contrast, our previous results with 30 genotypes of barley with a range of 42–80 mg kg⁻¹ tocots by direct extraction (Peterson and Qureshi 1993) were similar to results of Panfili et al (2003) with barley (75.7 ± 13.2 mg kg⁻¹). This indicates that their result with one oat sample was unusually high. Earlier, Piironen et al (1986) used a room temperature saponification method to extract tocots from three commercial rolled oat samples, and they reported a mean of 32 mg kg⁻¹ of total tocots. This value was nearly identical to the mean value we obtained by saponification, and lends support to our suggestion that the high tocot concentration of the sample analyzed by Panfili et al (2003) was unusual.

TABLE I
Genotypes and Pedigrees for Aberdeen and Tetonia Trials

Hulled		Hullless	
Genotype	Pedigree	Genotype	Pedigree
HO-55	IA91001-2/Powell	HO-144	IA91001-2/92Ab1
HO-59	IA91001-2/Powell	HO-161	IA91001-2/92Ab1
HO-66	IA91098-2/Monida	HO-243	IA91098-2/Lamont
HO-284	IA91324-2/Powell	HO-261	IA91098-2/Lamont
HO-286	IA91324-2/Powell	HO-265	IA91098-2/Lamont
HO-293	IA91324-2/Powell	HO-384	IA91324-2/Lamont
HO-305	IA91098-2/Powell	HO-435	IA91324-2/Lamont
02HO-139	Maverick/IA91324-2	02HO-33	Lamont/IA91001-2
02HO-142	Maverick/IA91324-2	02HO-38	Lamont/IA91001-2
02HO-146	Maverick/IA91324-2	02HO-83	Lamont//IA91098-2/Lamont
02HO-168	Monida/IA91098-2	02HO-116	Provena//IA91001-2/Provena
02HO-209	IA91001-2/Powell//Powell	02HO-223	92Ab1/IA91098-2
IA91001-2	Parent	02HO-230	92Ab1/IA91098-2
IA91098-2	Parent	02HO-247	IA91001-2/92Ab1//92Ab1
IA91324-2	Parent	92Ab1	Parent
Maverick	Parent	Lamont	Parent
Monida	Parent	Provena	Parent
Powell	Parent		
Ajay	Check		
Dal	Check		
OT-50	Check		

Saponification hydrolyzes triacylglycerides, phospholipids, and other lipid esters and converts the fatty acids into hydrophilic soaps. These remain in the aqueous phase during the subsequent extraction of the tocols into the organic phase. It has been suggested that the decreased load of material extracted into the organic phase and subsequently injected into the analytical column may improve the selectivity of detection (Lang et al 1992). However, fluorescence detection is more sensitive and selective than absorbance, and it is not clear that lipid materials would interfere materially with tocol detection. Conversely, the harsh alkaline conditions during saponification may partially degrade the unsaturated tocotrienols (Chow et al 1969). It has also been suggested that the soaps produced by saponification may help solubilize the tocols (Ueda and Igarashi 1987). Panfili et al (2003) suggested that the increased yield of barley tocols by saponification might be due to the hydrolysis of tocol esters. However, Chow et al (1969) found no evidence for tocol esters in barley or oat. Another possibility is that the alkaline conditions of the saponification would open up the cell wall matrix and release tocols that might be encumbered within the cellulose fibrils. Further work is needed to determine the reasons for the increased yield by saponification.

Based on these empirical results, we decided to use the saponification method for our second experiment. Although saponification was an extra step in the procedure, the overall time required was actually lowered because evaporation of the hexane-ethyl acetate solvent was considerably faster than evaporation of methanol.

The analysis of variance for the genotypes grown in trials at Aberdeen and Tetonia, ID, showed significant differences between genotypes and trials for each parameter in both hulled and hulless populations (Table III). There were significant trial × genotype interactions for α-tocotrienol and total tocols in the hulless population, but there were no significant interactions for the hulled population.

The lipid concentrations of groats from the hulled genotypes at Aberdeen ranged from 114 g kg⁻¹ for IA91001-2 to 69 g kg⁻¹ for Ajay, with a mean of 92 g kg (Fig. 2). IA91001-2 was a high-oil parent, and Ajay a normal-oil check cultivar. The corresponding range for hulled genotypes at Tetonia was 107 g kg⁻¹ for IA91001-2 to 68 g kg⁻¹ for Ajay, with a mean of 85 g kg⁻¹. The lipid concen-

tration range for hullless genotypes at Aberdeen was 112 g kg⁻¹ for 02HO-223 to 74 g kg⁻¹ for Provena, with a mean of 95 g kg⁻¹, and at Tetonia, 97 g kg⁻¹ for HO-243 to 66 g kg⁻¹ for Provena, with a mean of 81 g kg⁻¹. The trials at Aberdeen produced higher lipid concentrations than those at Tetonia for both hulled and hullless genotypes. At Aberdeen, the hullless population had a slightly higher average lipid concentration than the hulled population, whereas at Tetonia, the hulled population averaged higher than the hullless population.

The total tocol concentrations of groats from the hulled genotypes at Aberdeen ranged from 47.0 mg kg⁻¹ (HO-66) to 34.4 mg kg⁻¹ (HO-305), with an average of 40.7 mg kg⁻¹. At Tetonia, tocol concentrations of the hulled genotypes ranged from 38.3 mg kg⁻¹ (HO-293) to 25.9 mg kg⁻¹ (Powell), with an average of 31.1 mg kg⁻¹. For the hullless genotypes, the ranges were 46.7 mg kg⁻¹ (HO-265) to 34.1 mg kg⁻¹ (Lamont) at Aberdeen with an average of 39.2 mg kg⁻¹ and 36.0 mg kg⁻¹ (HO-265) to 18.5 mg kg⁻¹ (02HO-223) at Tetonia, with an average of 24.1 mg kg⁻¹. The tocol concentrations were much higher at Aberdeen than at Tetonia for both hulled and hullless populations. At Aberdeen, both hulled and hullless populations had nearly identical means, but at Tetonia, the hulled population had a much higher level of tocols than the hullless population. α-Tocotrienol comprised, on average, 75–77% of the total tocols. One might have expected that in the absence of a protective hull, the hullless genotypes would have higher levels of antioxidant tocols but that was not the case in this study.

In a recent study, the total tocol concentrations of groats from 33 hulled oat genotypes grown at Aberdeen, ID, averaged 24.4, 33.5, and 27.9 mg kg⁻¹ in three different growing seasons (Peterson et al 2005). The same genotypes grown at Tetonia, ID, contained 26.2, 26.7, and 19.9 mg kg⁻¹ in the same years. It is apparent from the current and previous experiment that plants grown at Tetonia had lower tocols than those grown at Aberdeen in three of the four growing seasons that were compared.

There were no significant correlations between the tocol concentrations and lipid concentration in any of the trials with the exception of weak correlations between lipids and α-tocotrienol and total tocols in the Aberdeen hullless trial ($R^2 = 0.15$ and 0.13 , respectively, $P < 0.01$). This is in contrast to the very high correlations observed by Peterson and Wood (1997) in Iowa-grown oat

TABLE II
Comparison of Saponification vs. Direct Extraction on Yield (mg kg⁻¹) of Tocols from Oat^{a,b}

Tocol	Saponification	Direct Extraction	<i>t</i>	<i>r</i>
α-Tocopherol	8.0 ± 3.5	6.8 ± 2.3	2.68*	0.88
α-Tocotrienol	20.3 ± 5.2	15.7 ± 4.1	7.18**	0.84
Total tocol	28.3 ± 7.4	22.5 ± 5.4	5.88**	0.82

^a Nineteen oat samples analyzed in duplicate by two methods. Mean values were compared using a paired *t*-test.

^b Mean ± standard deviation. *,** indicate significance at $P < 0.05$ and 0.01 , respectively.

TABLE III
Analysis of Variance for Parameters of Hulled and Hulless Oat Genotypes (G) from Trials (T) at Aberdeen and Tetonia, ID

Source	df	Mean Squares			
		α-Tocopherol	α-Tocotrienol	Total Tocol	Lipid
Hulled					
T	1	52.1**	2,466**	3,234**	21.4**
G	20	12.6**	48.3**	75.8**	7.43**
T × G	20	1.01	19.3	24.5	0.91
Hulless					
T	1	209**	3,990**	6,030**	45.3**
G	16	4.7**	65.0**	95.8**	4.81**
T × G	16	1.51	27.9**	40.3**	0.51

^a **, indicates significance at $P < 0.01$.

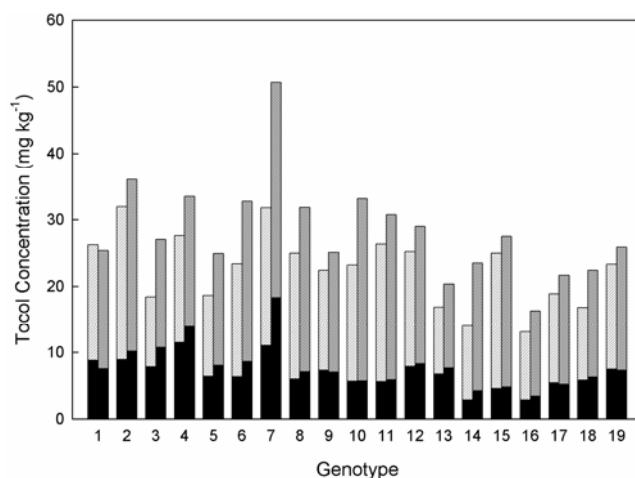


Fig. 1. Tocol yields of 19 oat samples by direct methanol extraction and saponification. In each pair of bars, the first represents direct extraction, the second represents saponification. The lower portion of each bar indicates α-tocopherol yield and the upper portion indicates α-tocotrienol. Data are means of duplicate analyses. Genotypes grown at Madison WI were 1, OT368; 2, Cayuse; 3, Whitestone; 4, Celsia; 5, LAO597-066; 6, Vista; 7, Freja; 8, Belle; 9, Kaufmann. Genotypes grown at Aberdeen ID were 10, Stormogul; 11, Cayuse; 12, Betania; 13, Whitestone; 14, Belle; 15, Matilda; 16, LAO597-066; 17, SA98606; 18, Betania; 19, Freja.

genotypes with lipid concentration range of 69–181 g kg⁻¹. These very high-oil genotypes were produced by nine cycles of recurrent selection from a base population that was developed using high-oil *A. sterilis* and *A. sativa* genotypes crossed with locally adapted (Iowa) cultivars (Branson and Frey 1989; Frey and Holland 1999). The different results between the two experiments could have resulted either from the different growing environments (Idaho vs. Iowa) or from the different lipid concentration ranges in the populations.

Three of these same very high-oil genotypes from the Iowa State recurrent selection program were crossed with Idaho-adapted cultivars to create the high-oil lines in the present study. However, the three Iowa genotypes used as parental checks (IA91001-2, IA91098-2, and IA91324-2) produced much lower oil concentrations when grown in Idaho than had been reported from Iowa (Frey and Holland 1999). Tocol concentrations in these genotypes were also much higher in groats produced in Iowa (Peterson and Wood 1997) than in those from Idaho. Therefore, it appears that the environment has a strong influence on both oil and tocol levels of these unusual genotypes.

The association between oil and tocol concentrations that was reported by Peterson and Wood (1997) could be a result of either gene linkage or epigenetic effects. If the genes affecting oil and tocol concentrations were linked, one would expect to see a correlation in the current experiment, even though the expression of these genes was attenuated as compared with the Iowa-grown

material reported earlier by Frey and Holland (1999). Alternatively, if high oil levels induce the expression of higher tocols, perhaps the oil levels in the current experiment were not above a threshold level needed to cause tocol concentrations to increase.

The high-oil parents from the Iowa program that were used in the present study were also among the highest in tocol concentrations, with a few exceptions as noted below. The tocol concentrations of the progeny genotypes were typically equal to one of the parents or intermediate between them. However, three hullless genotypes (HO-243, HO-265, and O2HO-223) from the Aberdeen trial had tocol concentrations higher than the high-tocol parent, which in each case was IA91098-2. This increased level of tocols was not observed in the Tetonia trial for these or any other hullless genotypes. Among the hulled genotypes, HO-66 and O2HO-168 had higher levels of tocols than either parent, the former in both trials, the latter only at Aberdeen. The high-tocol parent for these genotypes was also IA91098-2. Therefore, it appears that IA91098-2 has potential as a useful parent in a breeding program to develop high-tocol cultivars or lines. However, the variable expression of the high-tocol trait between environments must be addressed before a practical breeding program is undertaken. Also, it should be noted that the genotypes in this study were selected for high oil. If selections had been made for high tocol concentration, it is possible that other transgressive segregates might have been identified from progeny of IA91098-2 or of the other two high-oil parents.

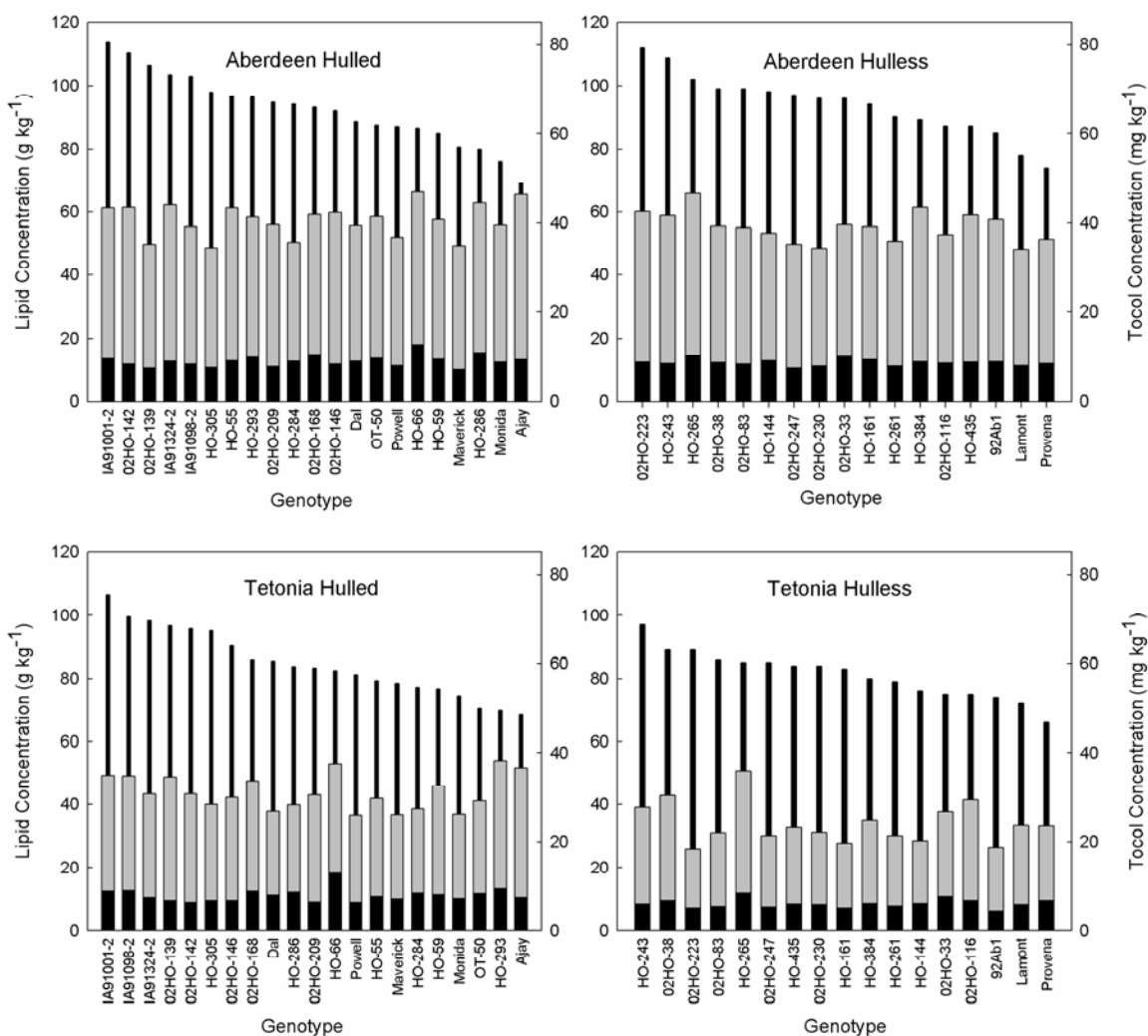


Fig. 2. Lipid and tocol concentrations of oat genotypes from four trials. Data are averages of four replicates at Aberdeen and three replicates at Tetonia, ID. Narrow black bars represent lipid concentrations. Wide black bars are α -tocopherol concentrations. Wide gray bars are α -tocotrienol concentrations. Genotypes are arranged in descending order for lipid concentration in each trial.

CONCLUSIONS

The saponification method of extracting tocopherols from ground oat has advantages over the previously used direct methanol extraction method. The yield of tocopherols was improved by $\approx 25\%$, and the time involved was reduced significantly. The growing environment had significant effects on concentrations of lipids and tocopherols in some hulled and hullless oat genotypes. Concentrations of both were markedly higher in the irrigated, higher-yielding Aberdeen environment than in the cooler rain-fed plots at Tetonia. Tocopherol concentrations of hulled and hullless genotypes averaged the same at Aberdeen, but at Tetonia, hulled genotypes had higher tocopherol concentrations. Lipid and tocopherol concentrations were not significantly correlated, unlike a previous report with extremely high-oil oat genotypes. Most of the genotypes had tocopherol concentrations that were equal to one of the parents or intermediate between them, but several whose high-oil parent was IA91098-2 had higher tocopherol concentrations than either parent. IA91098-2 might be a useful parent in an experiment to boost tocopherol levels by hybridization.

ACKNOWLEDGMENTS

The skilled technical assistance of Lauri Herrin is gratefully acknowledged.

LITERATURE CITED

- Barnes, P. J. 1983. Cereal tocopherols. Pages 1095-1100 in: Progress in Cereal Chemistry and Technology, Proc. 7th World Cereal and Bread Congress. J. Holas and J. Kratochvil, eds. Elsevier: Amsterdam.
- Branson, C. V., and Frey, K. J. 1989. Recurrent selection for groat oil content in oat. *Crop Sci.* 29:1382-1387.
- Bryngelsson, S., Mannerstedt-Fogelfors, B., Kamal-Eldin, A., Andersson, R., and Dimberg, L. H. 2002. Lipids and antioxidants in groats and hulls of Swedish oats (*Avena sativa* L.). *J. Sci. Food. Agric.* 82:606-614.
- Chow, C. K., Draper, H. H., and Csallany, S. 1969. Method for the assay of free and esterified tocopherols. *Anal. Biochem.* 32:81-90.
- Frey, K. J., and Holland, J. B. 1999. Nine cycles of recurrent selection for increased groat-oil content in oat. *Crop Sci.* 39:1636-1641.
- Kamal-Eldin, A., and Appelqvist, L.-Å. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* 31:671-701.
- Kamal-Eldin, A., Gørgen, S., Pettersson, J., and Lampi, A.-M. 2000. Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. *J. Chromatog. A* 881:217-227.
- Lang, J. K., Schillaci, M., and Irvin, B. 1992. Vitamin E. Pages 153-195 in: Modern Chromatographic Analysis of Vitamins, 2nd Ed. Chromatographic Science Series Vol. 60. A. P. DeLeenheer, W. E. Lambert, and H. J. Neils, eds. Marcel Dekker: New York.
- Lásztity, R., Berndorfer-Kraszner, E., and Huszár, M. 1980. On the presence and distribution of some bioactive agents in oat varieties. Pages 429-445 in: Cereals for Food and Beverages. G. E. Inglett and L. Munck, eds. Academic Press: New York.
- Packer, L. 1995. Nutrition and biochemistry of the lipophilic antioxidants: Vitamin E and carotenoids. Pages 8-35 in: Nutrition, Lipids, Health, and Disease. A. S. H. Ong, E. Niki, and L. Packer, eds. AOCS: Champaign, IL.
- Panfilii, G., Fratianni, A., and Irano, M. 2003. Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *J. Agric. Food Chem.* 51:3940-3944.
- Peterson, D. M., and Qureshi, A. 1993. Genotype and environmental effects on tocopherols of barley and oats. *Cereal Chem.* 70:157-162.
- Peterson, D. M., and Wood, D. F. 1997. Composition and structure of high-oil oat. *J. Cereal Sci.* 26:121-128.
- Peterson, D. M., Wesenberg, D. M., Burrup, D. E., and Erickson, C. A. 2005. Relationships among agronomic traits and grain composition on oat genotypes grown in different environments. *Crop Sci.* 45:1249-1255.
- Piironen, V., Syväoja, E.-L., Varo, P., Salminen, K., and Koivistoinen, P. 1986. Tocopherols and tocotrienols in cereal products from Finland. *Cereal Chem.* 63:78-81.
- Slover, H. T., Lehmann, J., and Valis, R. J. 1969. Vitamin E in foods. Determination of tocopherols and tocotrienols. *J. Am. Oil Chem. Soc.* 48:417-420.
- Thompson, J. N., and Hatina, G. 1979. Determination of tocopherols and tocotrienols in foods and tissues by high performance liquid chromatography. *J. Liq. Chromatogr.* 2:327-344.
- Ueda, T., and Igarashi, O. 1987. Effect of coexisting fat on the extraction of tocopherols from tissues after saponification as a pretreatment for HPLC determination. *J. Micronutr. Anal.* 3:15-25.
- White, D. A., Fisk, I. D., and Gray, D. A. 2006. Characterisation of oat (*Avena sativa* L.) oil bodies and intrinsically associated E-vitamins. *J. Cereal Sci.* 43:244-249.

[Received April 13, 2006. Accepted October 12, 2006.]