Comparison of Oil and Phytosterol Levels in Germplasm Accessions of Corn, Teosinte, and Job's Tears

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Seeds of 49 accessions of corn (*Zea mays* ssp. *mays*), 9 accessions of teosinte (*Zea* species that are thought to be ancestors and probable progenitors to corn), and 3 accessions of Job's tears (*Coix lacryma*), obtained from a germplasm repository, were ground and extracted with hexane. Whole kernel oil yields and levels of four phytonutrients (free phytosterols, fatty acyl phytosterol esters, ferulate phytosterol esters, and γ-tocopherol) in the oils were measured. Among the seeds tested, oil yields ranged from 2.19 to 4.83 wt %, the levels of ferulate phytosterol esters in the oil ranged from 0.047 to 0.839 wt %, the levels of free phytosterols in the oil ranged from 0.54 to 1.28 wt %, the levels of phytosterol fatty acyl esters in the oil ranged from 0.76 to 3.09 wt %, the levels of total phytosterols in the oil ranged from 1.40 to 4.38 wt %, and the levels of γ-tocopherol in the oil ranged from 0.023 to 0.127 wt %. In general, higher levels of all three phytosterol classes were observed in seed oils from accessions of *Zea mays* ssp. *mays* than in seed oils from accessions of the other taxonomic groups. The highest levels of γ-tocopherol were observed in teosinte accessions.

Keywords: *Phytosterols; lipids; tocopherols; sterols*

INTRODUCTION

We have previously reported that corn fiber oil (oil extracted from the fiber fraction from corn wet milling) contains high levels of three phytosterol classes: free phytosterols (1−2 wt %), fatty acyl phytosterol esters (4−9 wt %), and ferulate phytosterol esters (4−6 wt %) (*1*). More recently we have reported the variation in the levels of these phytosterol classes in modern corn hybrids (*2*). Others (*3*, *4*) have reported that the most abundant molecular species of ferulate phytosterol ester (FPE) in corn was sitostanol-ferulate ester, which comprised ∼70% of the total FPE. Kondo et al. (*5*, *6*) reported high levels of ferulate phytosterol ester (also mostly in the form of sitostanol-ferulate ester) in Job's tears (*Coix lacryma*). Teosinte is a taxonomic grouping including several *Zea* species and subspecies that are thought to be ancestors and probable progenitors of corn (*7*). Because of the growing importance and value of phytosterols as natural cholesterol-lowering nutraceuticals (*8*), the current study was undertaken to survey, for the first time, the levels of FPE and other phytosterol classes in seed oils from accessions of corn (*Zea mays* ssp. *mays*), teosinte, and Job's tears.

MATERIALS AND METHODS

Seeds of *Zea mays* ssp. *mays*, teosinte (including seven annuals, *Z. mays* var. *parviglumis*, *Z. mays* var. *huehuetenangensis*, and *Zea diploperennis* and four accessions of *Z. mays* ssp. *mexicana*; and two perennial teosintes, *Zea perennis* and *Zea diploperennis*), and Job's tears (*Coix lacryma* ssp. *jobi*) were obtained from the North Central Regional Plant Introduction Station, U.S. National Plant Germplasm System, USDA, ARS, Ames, IA, in 1998. Accessions were selected by Mark Millard, Maize Curator, Iowa State University, to meet the requirements of the study. Air-dried seeds were ground to 20 mesh with a Wiley mill (Thomas Scientific, Philadelphia, PA), extracted with hexane (duplicate samples, each consisted of 4 g of ground seeds/40 mL of hexane), and quantitatively analyzed via normal phase HPLC with evaporative light-scattering detection, as previously described (*1*). The yield of oil was measured gravimetrically. The HPLC retention times of phytosterol fatty acyl esters (St:E), triacylglycerols (TAG), γ-tococopherol (γ-toc), free phytosterols (St), and ferulate phytosterol esters (FPE) were 1.8, 4.0, 20.1, 21.2, and 26.5 min, respectively. Each sample was analyzed by duplicate injections, and the mean and standard deviation were reported.

RESULTS AND DISCUSSION

Comparing first the extractable oil values for seeds of the 61 accessions, the levels ranged from 2.19 to 4.83 wt % (Table 1). The levels of oil in two of the three Job's tears (*C. lacryma*) accessions were the highest of all samples, but oil levels among the third Job's tears accession, the 9 teosinte accessions, and the 49 *Z. mays* ssp. *mays* accessions were all in the 2.1−3.6% range.

All accessions in Table 1 are listed in order of increasing levels of total phytosterols in the oils, within each of the three groups. In oils from the *Z. mays* ssp. *mays* accessions, the range of total phytosterols was from about 1.8 to 4.4 wt %, with a mean value of 2.77 wt %. The values of total phytosterols in the oils from teosinte and *Coix* accessions showed little variation and averaged ∼1.6% for both groups.

When the levels of FPE (a relatively rare phytosterol class, thought to be unique to grains) (*3*, *4*) in seed oils of the 61 accessions were compared, large variations were observed. One goal of the current study was to determine whether Job's tears (*5*, *6*) is a better source of FPE than corn. The results clearly indicate that both contain significant levels of FPE, but almost all of the

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10.1021/jf010280h CCC: $20.00
Published on Web 07/13/2001

Table 1. Yield and Composition of Oil from Corn and Other Species

genus species subspecies	accession	source, country	plant name	% oil (in seeds)	FPE (wt % of oil)	St:E (wt % of oil)	St (wt % of oil)	total sterols (wt % of oil)	γ-toc (wt % of oil)
Zea mays ssp. *mays*	PI 484595	Mexico	Puebla 32	4.52 ± 0.06	0.147 ± 0.003	0.96 ± 0.10	0.70 ± 0.09	1.81	0.035 ± 0.006
	Ames 19467	Mexico	Guerrero 3	4.12 ± 0.08	0.270 ± 0.004	0.76 ± 0.02	0.82 ± 0.01	1.85	0.039 ± 0.003
	Ames 19558	Mexico	Mexico 37	4.10 ± 0.43	0.185 ± 0.001	1.05 ± 0.10	0.66 ± 0.06	1.90	0.024 ± 0.002
	Ames 19625	Mexico	Oaxaca 28	3.77 ± 0.17	0.211 ± 0.007	0.95 ± 0.05	0.75 ± 0.03	1.91	0.023 ± 0.002
	PI 484704	Mexico	Jalisco 44	4.14 ± 0.05	0.197 ± 0.002	1.11 ± 0.02	0.75 ± 0.01	2.06	0.045 ± 0.000
	PI 484401	Mexico	Aquascaleinte 8	4.07 ± 0.70	0.182 ± 0.024	1.11 ± 0.01	0.78 ± 0.01	2.07	0.029 ± 0.002
	PI 484718	Mexico	Jalisco 103	4.09 ± 0.01	0.161 ± 0.001	1.21 ± 0.01	0.75 ± 0.00	2.12	0.034 ± 0.002
	PI 217404	Argentina	Argentine Pop	3.97 ± 0.03	0.243 ± 0.002	1.24 ± 0.02	0.72 ± 0.00	2.20	0.035 ± 0.000
	PI 571675	Peru	Madre Dios 46	4.07 ± 0.04	0.127 ± 0.004	1.32 ± 0.01	0.81 ± 0.01	2.26	0.047 ± 0.002
	Ames 19481	Mexico	Guanajuato 36	3.64 ± 0.10	0.228 ± 0.006	1.28 ± 0.03	0.76 ± 0.02	2.27	0.032 ± 0.000
	PI 587132	Indiana	Sg 1533	2.24 ± 0.04	0.502 ± 0.000	0.88 ± 0.00	0.91 ± 0.00	2.29	0.083 ± 0.000
	PI 587137	Michigan	Ms71	3.46 ± 0.01	0.153 ± 0.001	1.22 ± 0.01	0.94 ± 0.00	2.31	0.083 ± 0.000
	PI 443972	Columbia	Antioquioa 556	3.42 ± 0.09	0.273 ± 0.045	1.15 ± 0.05	0.90 ± 0.02	2.32	0.065 ± 0.000
	PI 213697	Pennsylvania	Lanc. Sure C	3.59 ± 0.03	0.219 ± 0.005	1.29 ± 0.03	0.88 ± 0.02	2.39	0.038 ± 0.002
	PI 445252	Columbia	Narino 625	3.36 ± 0.01	0.192 ± 0.018	1.30 ± 0.00	0.91 ± 0.01	2.40	0.052 ± 0.002
	PI 390840	Peru	Chunco	3.00 ± 0.04	0.166 ± 0.001	1.18 ± 0.02	1.11 ± 0.01	2.46	0.065 ± 0.002
	PI 444741	Columbia	Choco 356	3.13 ± 0.20	0.234 ± 0.015	1.16 ± 0.07	1.09 ± 0.04	2.48	0.058 ± 0.002
	PI 587140	Minnesota	A632	2.63 ± 0.05	0.142 ± 0.004	1.33 ± 0.01	1.02 ± 0.01	2.49	0.069 ± 0.000
	PI 255978	Rhode Island	Rhode Island White	3.37 ± 0.01	0.303 ± 0.011	1.41 ± 0.01	0.82 ± 0.02	2.53	0.035 ± 0.000
	PI 587138	Minnesota	A554	3.54 ± 0.06	0.172 ± 0.002	1.43 ± 0.02	0.95 ± 0.00	2.55	0.090 ± 0.004
	PI 311237	Virginia	Hickory King	2.96 ± 0.09	0.361 ± 0.008	1.23 ± 0.04	0.97 ± 0.03	2.56	0.040 ± 0.000
	Ames 19097	Iowa	B73 X Mol7	2.73 ± 0.16	0.258 ± 0.011	1.41 ± 0.07	0.94 ± 0.05	2.61	0.042 ± 0.000
	PI 488974	Mexico	Puebla 42	3.08 ± 0.06	0.315 ± 0.004	1.45 ± 0.01	0.89 ± 0.00	2.66	0.036 ± 0.000
	PI 503732	Peru	Lambayeque 46	3.56 ± 0.01	0.195 ± 0.003	1.54 ± 0.15	1.00 ± 0.01	2.74	0.035 ± 0.002
	PI 503844	Peru	Piura 196	3.37 ± 0.07	0.201 ± 0.006	1.51 ± 0.06	1.05 ± 0.03	2.76	0.040 ± 0.000
	PI 485347	Peru	Lima 13	3.82 ± 0.01	0.190 ± 0.005	1.80 ± 0.01	0.84 ± 0.00	2.83	0.027 ± 0.000
	PI 503717	Peru	Lambayeque 25	3.57 ± 0.00	0.234 ± 0.000	1.49 ± 0.00	1.15 ± 0.00	2.87	0.048 ± 0.002
	PI 390833	Peru	W-C 1082	3.18 ± 0.04	0.218 ± 0.011	1.57 ± 0.04	1.09 ± 0.00	2.88	0.054 ± 0.000
	PI 587127	Indiana	H105W	2.43 ± 0.02	0.174 ± 0.003	1.61 ± 0.00	1.15 ± 0.00	2.93	0.081 ± 0.000
	PI 550473	Iowa	B73	2.63 ± 0.01	0.330 ± 0.003	1.51 ± 0.01	1.13 ± 0.00	2.97	0.111 ± 0.003
	PI 280853	Wisconsin	Silver King	3.07 ± 0.01	0.341 ± 0.001	1.58 ± 0.07	1.07 ± 0.02	2.99	0.054 ± 0.000
	PI 214198	Manitoba	Northwest. den	2.74 ± 0.09	0.404 ± 0.001	1.53 ± 0.05	1.08 ± 0.03	3.01	0.056 ± 0.002
	PI 445504	Columbia	Tolima 378	2.57 ± 0.06	0.384 ± 0.003	1.50 ± 0.00	1.14 ± 0.00	3.02	0.077 ± 0.003
	PI 444125	Columbia	Boyaca 406	3.48 ± 0.11	0.227 ± 0.008	1.98 ± 0.01	0.81 ± 0.00	3.02	0.050 ± 0.007
	PI 213730	Arizona	Sel. From A	2.87 ± 0.02	0.352 ± 0.001	1.48 ± 0.07	1.21 ± 0.02	3.04	0.046 ± 0.002
	PI 558532	Missouri	Mol7	2.40 ± 0.02	0.434 ± 0.002	1.61 ± 0.01	1.08 ± 0.01	3.12	0.078 ± 0.003
	PI 587135	Iowa	IA5125	3.34 ± 0.03	0.383 ± 0.021	1.77 ± 0.02	0.97 ± 0.00	3.12	0.127 ± 0.001
	PI 452058	Illinois	Reids yellow den	2.54 ± 0.01	0.244 ± 0.007	1.79 ± 0.01	1.14 ± 0.01	3.17	0.081 ± 0.003
	PI 550522	Tennessee	T232	3.12 ± 0.04	0.241 ± 0.003	2.11 ± 0.03	0.91 ± 0.00	3.26	0.072 ±0.004
	PI 550467	Iowa	B37	2.75 ± 0.01	0.247 ± 0.003	1.87 ± 0.00	1.15 ± 0.00	3.27	0.040 ± 0.000
	PI 494085	Peru		2.91 ± 0.01	0.186 ± 0.002	1.91 ± 0.01	1.19 ± 0.02	3.29	0.052 ± 0.002
	PI 558518	Missouri	Mo15W	3.44 ± 0.14	0.087 ± 0.001	2.39 ± 0.11	0.82 ± 0.03	3.30	0.088 ± 0.002
	PI 445082	Columbia	Narino 369	2.77 ± 0.01	0.334 ± 0.006	2.07 ± 0.02	1.02 ± 0.01	3.42	0.068 ± 0.002
	PI 390842	Peru	Pardo	2.65 ± 0.03	0.357 ± 0.008	1.68 ± 0.02	1.39 ± 0.01	3.43	0.052 ± 0.002
	Ames 22639	Wisconsin	Country Gent.	4.45 ± 0.06	0.198 ± 0.001	2.46 ± 0.01	0.80 ± 0.01	3.45	0.027 ± 0.003
	PI 445414	Columbia	Santander S-371	2.51 ± 0.00	0.329 ± 0.006	1.91 ± 0.01	1.24 ± 0.01	3.48	0.059 ± 0.003
	Ames 1785	Texas	Shoepeg	2.62 ± 0.02	0.839 ± 0.015	1.56 ± 0.01	1.28 ± 0.02	3.68	0.056 ± 0.003
	PI 444731	Columbia	Choco 339	2.67 ± 0.04	0.375 ± 0.002	2.42 ± 0.03	1.17 ± 0.01	3.97	0.070 ± 0.000
	PI 550490	North Dakota	ND246	3.01 ± 0.03	0.305 ± 0.005	3.09 ± 0.06	0.98 ± 0.02	4.38	0.093 ± 0.000
means of 49 accessions of corn (*Z. mays* ssp. *mays*)				**3.25** ± **0.59**	**0.266** ± **0.122**	**1.53** ± **0.45**	**0.97** ± **0.17**	**2.77** ± **0.57**	**0.055** ± **0.023**
Zea mays var. *parviglumis*	PI 384061	Mexico	El Salado	3.47 ± 0.06	0.049 ± 0.001	0.88 ± 0.01	0.47 ± 0.01	1.40	0.082 ± 0.001
Zea mays ssp. *mexicana*	Ames 8083	Mexico	Ames 8083	2.79 ± 0.15	0.083 ± 0.012	0.80 ± 0.02	0.56 ± 0.00	1.44	0.083 ± 0.004
	PI 566687	Mexico	Maiz de Huiscato	3.54 ± 0.04	0.047 ± 0.000	0.84 ± 0.00	0.55 ± 0.01	1.44	0.076 ± 0.001
	PI 566684	Mexico	Acece	2.63 ± 0.10	0.062 ± 0.004	0.86 ± 0.03	0.58 ± 0.00	1.50	0.081 ± 0.001
	PI 566674	Mexico	Maicillo	2.34 ± 0.66	0.083 ± 0.011	0.89 ± 0.03	0.61 ± 0.05	1.58	0.093 ± 0.006
Zea diploperennis[a]	PI 441931	Mexico	1375	2.19 ± 0.17	0.094 ± 0.007	0.83 ± 0.00	0.70 ± 0.01	1.62	0.074 ± 0.006
Zea luxurians	PI 441933	Guatemala	G-5	2.78 ± 0.26	0.112 ± 0.010	0.94 ± 0.02	0.59 ± 0.01	1.64	0.047 ± 0.003
Zea mays var. *huehuetenangensis*	Ames 21880	Guatemala	G-120	2.76 ± 0.08	0.051 ± 0.001	1.17 ± 0.01	0.63 ± 0.01	1.85	0.084 ± 0.002
Zea perennis[a]	Ames 21875	Mexico	VIII.B. 11	2.32 ± 0.02	0.081 ± 0.001	1.29 ± 0.01	0.70 ± 0.00	2.07	0.106 ± 0.002
means of 9 accessions of teosintes (*Z. mays* and other *Zea* species)				**2.76** ± **0.48**	**0.073** ± **0.023**	**0.94** ± **0.17**	**0.60** ± **0.07**	**1.62** ± **0.22**	**0.081** ± **0.016**
Coix lacryma ssp. *jobi*	Ames 14529	Japan	UI 7528	3.03 ± 0.27	0.101 ± 0.006	0.79 ± 0.02	0.54 ± 0.01	1.43	0.045 ± 0.000
	PI 324509	Brazil		4.78 ± 0.25	0.107 ± 0.005	0.098 ± 0.00	0.58 ± 0.02	1.67	0.030 ± 0.003
	PI 320865	Brazil		4.83 ± 0.17	0.119 ± 0.002	0.96 ± 0.03	0.61 ± 0.01	1.69	0.030 ± 0.001
means of 3 accessions of Job's tears (*Coix lacryma* ssp. *jobi*)				**4.21** ± **1.03**	**0.109** ± **0.009**	**0.91** ± **0.10**	**0.58** ± **0.04**	**1.60** ± **0.14**	**0.035** ± **0.009**

[a] Perennial teosintes.

corn accessions contain much higher levels of FPE than Job's tears (or teosinte). The current results are consistent with our previous report that the total kernel extract of three modern corn hybrids exhibited a range of FPE concentration of 0.351−0.411 wt %, using identical extraction and analysis methods (*9*). The 49 *Z. mays* ssp. *mays* accessions included in this study (Table 1) contained a wide range of FPE values, ranging from a low of 0.087 wt % to a high of 0.839 wt %, with a mean value of 0.27 wt %. Teosinte and *Coix* accessions also showed variability in FPE levels, but on average contained much lower levels of FPE (0.07 and 0.11 wt %, respectively).

The levels of St:E in the kernel oils from accessions of *Z. mays* ssp. *mays* ranged from 0.76 to 3.09 wt %, with an average value of ∼1.5 wt %. Again, these values were somewhat higher than those found in oils from the teosinte and *Coix* accessions (average values of ∼0.9 wt % for both groups, with low levels of variability).

The levels of free phytosterols (St) in the oils from all three groups of accessions were lower than those of the other two classes of sterol conjugates and were more consistent (less variability) among accessions. The levels of St in the oils of *Z. mays* ssp. *mays* accessions averaged ∼1%, whereas the levels in the oils of teosinte and *Coix* accessions each averaged only ∼0.6%.

Because the HPLC-ELSD method that was employed for phytosterol analyses also separated and quantified γ-tocopherol, and because this is the only vitamin E derivative that occurs in significant levels in corn (*10*), its levels were also reported in Table 1. The levels of γ-tocopherol ranged from 0.023 to 0.127 wt %, and the only apparent trends were that the lowest levels occurred in the *Coix* accessions and that teosinte samples had the highest average levels (0.08%). We recently reported that heat pretreatment of corn fiber caused a 10-fold increase in the levels of γ-tocopherol, and we suggested some possible explanations for this phenomenon (*10*). Although in the current study γ-tocopherol was measured in extracts of whole ground kernels instead of ground corn fiber, we anticipate that heat pretreatment of ground corn would probably also increase the levels of extractable γ-tocopherol.

The primary purpose of this study was to examine the natural variation in the phytosterol and γ-tocopherol contents of kernel oil prepared from various accessions of corn, teosinte, and Job's tears. The results will help to assess the potential of these accessions for use in future breeding studies with selected germplasm lines in an effort to produce new corn hybrids with acceptable agronomic traits *and* high levels of phytosterols. If these hybrids were then commercialized, grown, and wet milled, it is anticipated that the resulting fiber fractions could potentially yield a corn fiber oil that contains higher phytosterol levels than can be obtained with current corn hybrids.

The relatively high levels of total phytosterols in the kernel oils from some accessions studied here would suggest a potential for a commercial "whole kernel oil" that could contain physiologically significant levels of phytosterols in a practical daily serving of oil (1−3 g of phytosterols per day is currently considered to be the amount needed to significantly lower the level of serum cholesterol) (*11*, *12*). Such an oil, which could potentially be produced at a modified dry grind corn-to-ethanol plant, would have a significantly higher commercial value than a commodity corn oil.

ACKNOWLEDGMENT

We thank Michael Powell for expert technical assistance. We also thank Mark Millard, Department of Agronomy, Iowa State University, for assistance in selecting and obtaining germplasm samples.

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Received for review March 2, 2001. Revised manuscript received May 16, 2001. Accepted May 16, 2001. Mention of brand or trade name does not constitute an endorsement by the U.S. Department of Agriculture or the University of Illinois above others of a similar nature not mentioned.

JF010280H