Distribution of Tocopherols Within the Corn Kernel

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Abstract

The concentration of tocols and tocotrienols in the germ, endosperm and pericarp of the corn kernel was determined after hand dissection of four dent corn hybrids—normal, early maturing, high oil and high lysine (opaque-2). Among these hybrids the germ fraction contained 70–86% and the endosperm fraction contained 27–11% of the tocopherols extracted from the whole grain. The endosperm fractions contain all of the measurable tocotrienols occurring in whole corn. In the four hybrids analyzed, the germ fraction contained 94–96% of the α-tocopherol extracted from the whole grain. Concentration of α-tocopherol was the highest in the germ fraction of the opaque-2 hybrid.

Introduction

The mechanical separation of cereal grain leads to products with varying tocopherol content. Herting and Drury (1) state that the observed decrease in α-tocopherol content of processed cereals may be explained by both oxidation during processing and variation in tocopherol content among the cereal fractions. The distribution of tocopherols within the corn kernel has not been reported previously. It has been generally accepted that since the germ fraction is high in oil, this fraction contains the most significant portion of tocopherols.

Hall and Laidman (2) determined the distribution of tocopherols in hand-dissected wheat fractions. Their study showed that the endosperm fraction of the wheat grain had more than 90% of the tocotrienols and more than 50% of the tocopherols isolated from the whole grain.

We investigated the concentration of tocols and tocotrienols in hand-dissected corn fractions and the contribution each fraction made to the tocopherol content of the whole grain.

Materials

Normal dent: Agricultural Engineering Farm, University of Illinois, Urbana, Ill., 1968 crop, commercial single cross (B37TMS × H84) (Oh43RF × A619).

Early maturing dent (C-14): Bear Hybrid Corn Co., Inc., Decatur, Ill., 1965 crop, variety designation: C-14.

High-oil dent (Alexho): Illinois Agricultural Experimental Station, Urbana, Ill., 1968 crop, single cross Alexho 144(55) × R802A.

High-lysine dent (opaque-2): Alumni Seed Foundation, Purdue University, Lafayette, Ind., 1967 crop, single cross $W64AO_2 \times B37O_2$.

Experimental Procedures

Each hybrid sample was steeped in distilled water for 20–30 min before hand dissection into germ, endosperm and pericarp. The pericarp fraction also contained the tip cap. After each sample was ground to 40 mesh in a Wiley mill, intermediate model, the corn lipids were extracted immediately with ethanol according to the procedures outlined by Quaife and Harris (3). The corn lipids were saponified and those nonsaponifiable were isolated by the procedure described by the Analytical Methods Committee, Society for Analytical Chemistry, London (4). The thin layer chromatographic (TLC) method of Whittle and Pennock (5) for the isolation and separation of tocols and tocotrienols was used with some modifications. TLC plates were impregnated with fluorescein indicator by the addition of 0.5 ml of 0.05% aqueous disodium fluorescein (Distillation Products Industries) for each plate to the aqueous slurry of Silica Gel G according to Stahl (E. Merck AG, Darmstadt, Germany). The plates (20 cm × 20 cm × 375 mμ) were then spread, air dried and held at 110 C for 1 hr.

The nonsaponifiable corn lipids from each sample were dissolved in benzene (200 μl) and the solution was applied as a 16 cm line centered 2 cm from the bottom of a TLC plate. One centimeter from each side of the plate was spotted a mixture of 5 μg each of α- and γ-tocopherol. The plate was developed in chloroform in an equilibrated stainless-steel tank covered with a green-tinted glass plate. The developed

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TABLE I

Concentration of Tocopherols in Hand-Dissected Corn Fractions[a]

Dent hybrid		Proportion of kernel, %	α-T[b]	α-T-3[b]	γ-T	γ-T-3	δ-T	Total
Normal								
	Endosperm	80.8	0.7	8.7	1.9	18.9	....[c]	30.2
	Germ	11.4	162	....	378	.....	9.6	550
	Pericarp[d]	7.8	7.5	Trace	20.4	Trace	....	27.9
C-14								
	Endosperm	80.7	0.7	6.0	1.9	8.7	....	17.3
	Germ	11.2	129	....	332	.....	8.9	470
	Pericarp[d]	8.1	5.1	Trace	17.2	Trace	....	32.3
Alexho								
	Endosperm	76.7	0.7	4.5	1.0	7.2	....	13.4
	Germ	16.6	140	....	339	.....	10.4	489
	Pericarp[d]	6.7	6.8	Trace	18.7	Trace	....	25.5
Opaque-2								
	Endosperm	79.8	0.5	5.6	1.3	3.8	....	11.2
	Germ	11.6	194	....	388	.....	7.3	589
	Pericarp[d]	8.6	6.8	Trace	19.3	Trace	....	26.1

[a] All data are expressed in micrograms of tocopherol per gram of dry matter.
[b] T, tocopherol; T-3, tocotrienol.
[c] An ellipsis in each case signifies that this tocopherol was not observed to be present.
[d] These fractions contain both the tip cap and the pericarp.

TABLE II

Tocopherol Contribution of Corn Fractions to the Whole Kernel as Calculated[a]

	Dent hybrid	α-T	α-T-3	γ-T	γ-T-3	δ-T	Total
Normal							
	Endosperm	0.6	7.0	1.5	15.3	....[b]	24.4
	Germ	18.5	....	43.2	.....	1.1	62.8
	Pericarp	0.6	....	1.6	.....	....	2.2
	Whole calc'd.[c]	19.7(22%)[d]	7.0(8%)	46.3(52%)	15.3(17%)	1.1(1%)	89.4
	Whole found	19.0(20%)	8.4(9%)	50.8(53%)	17.0(18%)	Trace	95.2
C-14							
	Endosperm	0.6	4.8	1.5	7.0	....	13.9
	Germ	14.5	....	37.4	.....	1.0	52.9
	Pericarp	0.4	....	1.4	.....	....	1.8
	Whole calc'd.	15.5(23%)	4.8(7%)	40.3(59%)	7.0(10%)	1.0(1%)	68.6
	Whole found	14.7(22%)	6.2(9%)	38.6(56%)	8.8(13%)	Trace	68.3
Alexho							
	Endosperm	0.7	4.5	1.0	7.2	....	13.4
	Germ	24.6	....	56.3	.....	1.8	82.7
	Pericarp	0.5	....	1.3	.....	....	1.8
	Whole calc'd.	25.8(26%)	4.5(5%)	58.6(60%)	7.2(7%)	1.8(2%)	97.9
	Whole found	29.4(26%)	5.3(5%)	70.0(61%)	9.3(8%)	Trace	114.0
Opaque-2							
	Endosperm	0.4	4.5	1.0	3.0	....	8.9
	Germ	22.5	....	45.0	.....	0.8	68.3
	Pericarp	0.6	....	1.7	.....	....	2.3
	Whole calc'd.	23.5(30%)	4.5(6%)	47.7(60%)	3.0(4%)	0.8(1%)	79.5
	Whole found	25.1(26%)	5.6(6%)	64.4(65%)	4.3(4%)	Trace	99.4

[a] All data are expressed in micrograms of tocopherol per gram of whole grain.
[b] An ellipsis signifies negligible contribution.
[c] By addition of the grain fraction contributions.
[d] The figures in parentheses are percentages of the total tocopherol content of the whole grain.

chromatogram was removed and allowed to dry in dim light. The standards along each side of the TLC plate aided in locating the tocopherol zones under a shortwave UV lamp. Each tocopherol zone was collected with a Desaga micro zone collector fitted with an extraction thimble. The tocopherol was eluted from the adsorbent with portions of ethanol (10 × 1 ml) and the eluate transferred to a 10 ml volumetric flask. A portion was removed to determine tocopherol content by the Emmerie-Engel method (4). Determinations were made on duplicate corn samples. To separate the tocols from the tocotrienols, the remaining portion of the eluate was concentrated under reduced pressure below 45 C. The residue was dissolved in benzene (200 μl) and this solution was applied to a TLC plate exactly as described above. This chromatogram was developed in 20% diisopropyl ether-80% petroleum ether in an unequilibrated stainless-steel tank covered with a green-tinted glass plate. After the chromatogram was removed from the tank and dried in dim light, the tocol and tocotrienol zones were located under a shortwave UV lamp. Each tocopherol zone was collected and the tocopherol eluted from the adsorbent as previously described. The ratio of tocol to tocotrienol was determined by the Emmerie-Engel method (4) and the concentration of each tocopherol in the corn samples calculated. Recovery of the tocols, α- and γ-tocopherol, by this procedure was 83 ± 1% and was not so complete as reported by Whittle and Pennock (5).

The tocotrienols were characterized by hydrogenation of the pure tocotrienols or mixtures of tocotrienols with tocols according to Quaife and Harris (6). In both cases the hydrogenation led to only one product corresponding to the tocol. The identity of each tocol was confirmed by dianisidine, phosphomolybdic acid, and Emmerie-Engel sprays.

Results and Discussion

Hand dissection of the four hybrids into three fractions (germ, endosperm and pericarp) and subsequent analysis of each fraction for tocopherol concentration led to the results compiled in Table I. All calculations are made on a dry weight basis. Although concentration of tocopherol was high in the germ fractions from all four hybrids, the germ fraction of the opaque-2 hybrid had the highest amount. The endosperm fraction from the normal dent showed the highest tocopherol concentration of the hybrids. No marked difference was observed in tocopherol distribution or concentration among the four pericarp fractions.

The contribution each grain fraction made to the tocopherol content of the whole grain was calculated from the proportion of the kernel that each fraction contributed to the dry weight of the whole grain (Table II). By summation of the tocopherol contributions made by each grain fraction the concentration of tocopherols in the whole grain has been calculated. The difference between tocopherol concentration in the whole grain and that calculated may be explained by the following factors: (a) Mechanical loss of dry matter during hand dissection, (b) oxidative degradation of tocopherols during hand dissection, and (c) natural variation of tocopherol concentration within the corn samples.

In Table III is compared the percentage of tocopherols found in each fraction of these hybrids. The endosperm and germ fractions contain 97–98% of the tocopherol present in the corn kernel. The endosperm's

TABLE III

Percentage of Tocopherols Found in Each Fraction of the Corn Kernel

Dent hybrid	Endosperm, %	Germ, %	Pericarp, %
Normal	27	70	3
C-14	20	77	3
Alexho	14	84	2
Opaque-2	11	86	3

TABLE IV

Distribution of α- and γ-Tocopherol Within the Corn Kernel

Fraction	Normal, %		C-14, %		Alexho, %		Opaque-2, %	
	α-T	γ-T	α-T	γ-T	α-T	γ-T	α-T	γ-T
Endosperm	3	3	4	4	3	2	2	2
Germ	94	93	94	93	95	96	96	94
Pericarp	3	3	3	3	2	2	3	4

tocopherol content, which varies from 27% in normal dent to 11% in the opaque-2 hybrid, represents all of the measurable tocotrienols found in the corn kernel.

The germ fraction contributes from 70% in normal dent to 86% in the opaque-2 hybrid to the tocopherol content of the whole grain. This fraction of all four hybrids contains 94–96% of the α-tocopherol, as well as 93–96% of the γ-tocopherol present in the corn kernel (Table IV). Only the germ fraction was observed to contain δ-tocopherol.

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