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Varietal Differences in the Vitamin E Content of Corn

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The relationship of maturation time of corn grain to its α-tocopherol and γ-tocopherol contents was examined. Whether expressed as α-tocopherol/g of fat or α-tocopherol/g of dry weight of corn, there was no correlation between α-tocopherol content and the time required to reach maturation. γ-Tocopherol, however, declined, as time to maturity increased. The amount of γ-tocopherol was 1–4 times that of α-tocopherol. However, because of the reportedly low biological activity of γ-tocopherol, the calculated vitamin E activity in corn was independent of the time required to reach maturity.

Corn breeding has produced many changes in corn grain which have been beneficial to both the corn grower and livestock producer, such as pest resistance, hardiness for particular climatic conditions, increased protein quality, and shorter maturation times. Introduced with some of these improvements may be some other changes which affect the nutrient value of the corn in positive or negative ways. Since corn is a major constituent of many diets for livestock and is a primary provider of vitamin E in those diets before supplementation, we are concerned with the vitamin E content of corn and the potential effects of genetic alteration of the plant on the vitamin E content of corn grain.

Within a variety of corn, the vitamin E content increases as the corn matures (Contreras-Guzman et al., 1982). This raises the question of whether the vitamin E content of varieties requiring less time to reach maturity is less than that of those requiring longer times. Since the faster maturing varieties are popular in some regions, such an effect could be expected to result in reduced vitamin E levels in formulated feeds. To test this hypothesis, we analyzed the α-tocopherol and γ-tocopherol levels of different varieties of corn grain with maturation times ranging from 97 to 138 days.

MATERIALS AND METHODS

Corn. Triplicate samples of 42 varieties of corn were obtained from a major corn breeding company. All of the corn was grown in 1983 in Iowa on the company's test plots. The harvested corn was dried at 35 °C for 5 days, whereupon it was shelled. The dried grain was packaged and mailed to our laboratory where it was stored at −4 °C until it was analyzed. Thus, the observed differences in the vitamin E contents of the varieties should not be attributable to differences in conditions of growing, handling, or storage. Immediately before analysis, samples of corn grain were finely ground in a laboratory sample mill.

Determination of α- and γ-Tocopherols. Samples of ground corn were saponified by addition of equal volumes of 50% KOH and ethanol containing 10% pyrogallol (antioxidant) and heating to 70 °C for 15 min. Tocopherols were extracted from the saponified samples with hexane containing 0.2% BHT. The hexane extracts were evaporated to dryness under nitrogen and reconstituted in ethanol.

Tocopherols were separated by HPLC with a C-18 column with methanol–water (96:4, v:v) as the mobile phase. α- and γ-tocopherols were detected by fluorescence (excitation 291 nm, emission 330 nm) with a flow cell attachment and were quantitated by comparison to commercially available standards.

Fat. Fat was extracted from ground corn by ether reflux in a Goldfisch apparatus and was quantitated gravimetrically.

RESULTS AND DISCUSSION

The relationships of the α- and γ-tocopherol contents of the grain to maturation time are shown in Figure 1. Whether the results are expressed per unit dry matter or per unit fat, the α-tocopherol level did not vary significantly ($P > 0.05$) as a function of maturation time. γ-Tocopherol, on the other hand, decreased significantly ($P < 0.01$) as maturation time increased. Although the level of γ-tocopherol in most samples was much higher than that of α-tocopherol, the total vitamin E activity was largely attributable to the α-tocopherol content, as the γ form is much less biologically active (Bieri and Evarts, 1974). In order to consider the biological relevance of these physical measurements of tocopherol contents, they were converted to USP units of vitamin E activity (1 USP unit being equal to the formerly used International Unit). This was done by multiplying the γ-tocopherol values by 0.1 to convert them to equivalents of α-tocopherol and multiplying the

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Table I. Fat, Tocopherol, and Vitamin E Content of Corn

variety code	days to maturity	fat, % dry matter	α-tocopherol, mg/kg[a]	γ-tocopherol, mg/kg[a]	vitamin E activity	
					USP units[b]/kg	USP units[b]/lb
1	97	4.74	19.2	65.7	38.4	17.5
2	99	4.12	5.5	69.2	18.5	8.4
3	100	4.25	22.4	61.1	42.5	19.3
4	105	3.72	10.0	45.6	21.7	9.9
5	105	3.88	6.2	59.7	18.1	8.2
6	105	4.14	10.3	57.7	23.9	10.9
7	106	3.60	16.7	61.0	34.0	15.5
8	107	4.10	5.9	49.5	16.2	7.4
9	107	3.85	11.3	49.3	24.2	11.0
10	107	3.62	17.9	62.7	36.0	16.4
11	109	3.76	14.4	53.2	29.4	13.4
12	109	4.40	20.0	45.9	36.6	16.6
13	110	4.52	19.0	40.5	34.3	15.6
14	110	4.11	10.5	50.0	23.1	9.7
15	110	3.56	13.4	59.5	28.8	13.1
16	112	3.44	12.3	42.6	24.7	11.2
17	114	3.42	11.1	32.2	21.3	9.7
18	114	3.88	12.9	51.0	26.8	12.2
19	114	3.99	13.1	65.1	29.2	13.2
20	114	3.87	13.2	43.6	26.2	11.9
21	115	4.06	16.8	83.2	37.4	17.0
22	115	4.42	13.5	69.9	30.5	13.9
23	118	3.69	12.5	52.8	26.5	12.0
24	118	4.07	15.6	40.9	29.3	13.3
25	121	3.84	15.6	32.7	28.1	12.8
26	121	3.96	11.0	40.2	22.4	10.2
27	122	4.22	5.9	45.3	15.5	7.0
28	122	4.17	6.6	45.5	16.6	7.5
29	123	4.19	18.6	38.1	33.4	15.2
30	124	4.06	16.1	33.6	29.0	13.2
31	124	4.70	18.2	46.4	34.0	15.5
32	126	3.64	10.6	29.8	20.2	9.2
33	127	3.94	9.4	34.9	19.2	8.7
34	129	4.20	16.3	27.1	28.3	12.9
35	131	4.39	19.3	31.5	33.5	15.2
36	131	4.22	9.4	36.2	19.4	8.8
37	131	4.08	10.7	33.7	21.0	9.5
38	132	3.55	15.8	25.6	27.4	12.5
39	132	5.06	16.5	36.0	29.9	13.6
40	136	3.90	16.4	22.9	27.8	12.6
41	136	4.80	22.0	23.4	36.3	16.5
42	138	4.17	12.7	40.1	24.9	11.3
mean		4.05	13.7	46.1	27.3	12.4
std dev		0.37	2.9	14.1	6.7	3.1

[a] Corn dried to 89% dry matter. [b] Calculated from 1.49 × (α-tocopherol/kg + 0.1 × γ-tocopherol/kg).

sum of the α-tocopherol value and equivalents for each sample by 1.49 (United States Pharmacopoeial Convention, 1980). (Bieri and Evarts (1974) reported a range of biopotency of *d*-γ-tocopherol of 6–16% of that of α-tocopherol, depending on the particular vitamin E deficiency syndrome being treated. Ten percent is recommended by the Committee on Dietary Allowances (National Research Council, 1980) for calculation of α-tocopherol equivalents from γ-tocopherol.) The contribution of the tocotrienols, which were also detected in the corn samples, was ignored, as we had no standards for their quantitation. D-α-Tocotrienol has a reported biopotency of about 20% of that of α-tocopherol (Scott, 1978), and consequently, the vitamin values reported here may be slightly low. The tocopherol measurements, expressed as mg of tocopherol per kg of air-dried corn (i.e., 89% dry matter), and the calculated vitamin E activities are shown in Table I.

Vitamn E activity was not significantly ($P > 0.05$) correlated with maturation time or with fat content. However, vitamin E values differed significantly ($P < 0.001$) among varieties. The calculated vitamin E activity varied from 15.5 to 42.5 USP units per kg of air-dried corn, with the mean being 27.3 ± 6.7 (standard deviation) USP units per kg.

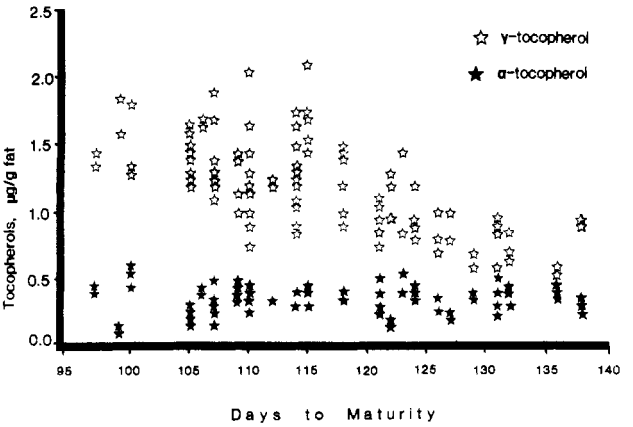

Figure 1. Relationship of α- and γ-tocopherol contents of corn grain and the time required for corn to reach maturity, based on 42 different varieties. Each point represents the mean of duplicate determinations.

In studies of the vitamin E content of corn grain, others have reported great variations among varieties (Weber, 1984; Gavrilovic, 1982), variation within the same varieties between different growing seasons (Gavrilovic, 1982), and

Table II. Vitamin E Analyses of Corn

samples	range, USP units/kg	mean, USP units/kg	ref
15 inbreds	9.0–37.7[a]	21.4[a]	Weber, 1984
11 samples from 5 geographical regions	3.2–12.0[a]	8.9[a]	Coret et al., 1983
4 inbreds	31.7–35.5[a]	34.0[a]	Contreras-Guzman et al., 1982
4 inbreds	1.9–12.2[c] 12.2–16.8[d]	6.2 15.0	Gavrilovic, 1982
17 samples from 7 states	17.0–52.2[b]	29.7[b]	Bunnell et al., 1968
10 samples from 7 states	16.1–31.4[b]	22.8[b]	Herting and Drury, 1969

[a] Calculated from data of cited paper by using the formula 1.49 × mg of (α-tocopherol/kg + 0.1 × mg of γ-tocopherol/kg). [b] Calculated by using 1.49 × mg of α-tocopherol/kg. [c] 1980 crop year. [d] 1981 crop year.

variations among samples from different geographical regions (see Table II, Cort et al., 1983; Bunnell et al., 1968; Herting and Drury, 1969).

Most feed tables for use in the formulation of livestock feeds list the vitamin E content of corn from 20–26 USP units per kg (National Research Council, 1980, 1979, 1978, 1977, 1975). The average from the present study falls slightly above this range. However, as can be seen from Table II, many other reported averages are lower. Furthermore, an average value does not indicate the very great variation which may occur among different samples of corn. Hence, the use of the average vitamin E values listed in current feed tables may at times significantly overestimate the vitamin E contribution of corn to a diet.

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Isolation of N^2-[γ-L-(+)-Glutamyl]-4-carboxyphenylhydrazine in the Cultivated Mushroom *Agaricus bisporus*

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N^2-[γ-L-(+)-Glutamyl]-4-carboxyphenylhydrazine (GCPH) has been isolated from *Agaricus bisporus*, the cultivated mushroom of commerce of the Western hemisphere. The compound was purified by using a combination of cation-exchange and reverse-phase high performance liquid chromatography and the structure confirmed by using fast-atom bombardment (FAB) mass spectrometry and FAB/tandem mass spectrometry. The average level of GCPH in the four samples analyzed was 42 ± 3 μg/g of wet mushroom.

INTRODUCTION

The mushroom of commerce in the Western hemisphere, *Agaricus bisporus*, has been shown to contain up to 400 ppm of agaritine "N^2-[γ-L-(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine" (GHPH), (Levenberg, 1961; Kelly et al., 1962). We subsequently found agaritine in locally purchased mushrooms in levels up too 700 ppm (Ross et al., 1982).

Although GHPH does not induce tumors in mice (Toth et al., 1981; Toth and Sornson, 1984) "over 50 hydrazine derivatives have induced tumors in laboratory animals" (Toth, 1984). The aim of our continuing studies is to identify biosynthetic hydrazine precursors or products of agaritine that are present in this commercial mushroom.

Shütte et al. (1972) have examined the incorporation of isotopically labeled compounds into agaritine by *Agaricus bisporus*. They found incorporation of radioactivity into agaritine from shikimic acid, glutamic acid, and *p*-aminobenzoic acid at levels of 0.14%, 0.68%, and 4.1%, respectively. LaRue (1977) subsequently postulated a biosynthetic scheme leading to GHPH invoking *p*-hydrazinobenzoic acid (HB) and N^2-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine (GCPH) as intermediates.

We recently reported that HB is present in *Agaricus bisporus* at an average level of 10.7 ± 2.0 μg/g of mushroom (wet weight) (Chauhan et al., 1984). We now report the successful isolation, identification, and quantitation

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