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Vitamin E Content of Feedstuffs Determined by High-Performance Liquid Chromatographic Fluorescence

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A method is described for the extraction and high-performance liquid chromatographic separation and quantitation of naturally occurring tocopherols and tocotrienols in feedstuffs. Validation of the method in feedstuffs is also reported including reproducibility, linearity, recovery, and precision. A survey of U.S. feedstuffs was performed by using this developed method, and the results are presented showing the α-, β-, γ-, and δ-tocopherols and the α- and γ-tocotrienols of animal feedstuffs collected in five major areas of the United States. Assay results from 77 samples are included.

There are eight naturally occurring forms of vitamin E: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Various analytical techniques have been used to detect, separate, and quantitate these compounds. Bunnell et al. (1968) reported a comprehensive survey of the α-tocopherol content of feedstuffs in which gas–liquid chromatography was used for the determination of α-tocopherol in alfalfa and secondary magnesium phosphate chromatography followed by two-dimensional thin-layer chromatography used for the separation and determination of α-tocopherol and α-tocotrienol. Bieri et al. (1970) and Ames (1971) reported the use of colorimetric methods to determine α-tocopherol and total tocopherols. Other significant contributors to vitamin E analytical methodology include Lovelady (1973), Tangney et al. (1978), and Slover (1971). Bunnell (1971) provides a thorough review of the development of analytical procedures for vitamin E.

The advent of high-performance liquid chromatography (HPLC) as an accepted technique for vitamin analysis has provided the analytical chemist with methods that offer advantages in speed, accuracy, and specificity over those available in the past. Abe et al. (1975), Vatassery et al. (1978), and Carpenter (1979) have reported the HPLC separation of α-, β-, γ-, and δ-tocopherol. Thompson and Hatina (1979) used HPLC to separate and determine the four tocopherols and α-, β-, and γ-tocotrienol in a variety of tissues and foods. Manz and Philipp (1981) reported a method for the determination of α-tocopherol and α-tocotrienol in animal feeds and human foodstuffs. Other applications of HPLC in vitamin E analysis include the work of Eriksen (1980), Cohen and Lapointe (1980), and Widicus and Kirk (1979).

The purpose of this investigation was to determine the tocopherol and tocotrienol contents of a wide variety of feedstuffs by using HPLC. The separation and identification of all eight vitamin E isomers were achieved. However, the quantitation of β- and δ-tocotrienol was not reported due to the instability and insufficient purity of available standards. The procedure has proved itself to be reliable and suitable for routine laboratory use including the determination of the α-tocopheryl acetate content of mixed feeds.

MATERIALS AND METHODS

High-Performance Liquid Chromatography. The HPLC system used consisted of a Model 950 HPLC pump (Tracor Inc.), a Model 650-10 LC fluorescence detector (Perkin Elmer Corp.) with excitation at 294 ± 2 nm and emission at 325 ± 2 nm, a Model 7120 injector (Rheodyne) equipped with a 20-μL loop, and a normal-phase Chromegasphere SI 60, 5 μm, column, 15 cm × 4.6 mm i.d. (E. S. Industries). Chromatograms were recorded and peak areas determined by using a Model 3390 integrator (Hewlett-Packard). The mobile phase was 2.5% (v/v) tetrahydrofuran in isooctane with a flow rate of 1.5 mL/min. The injection volume was 20 μL. All solvents used

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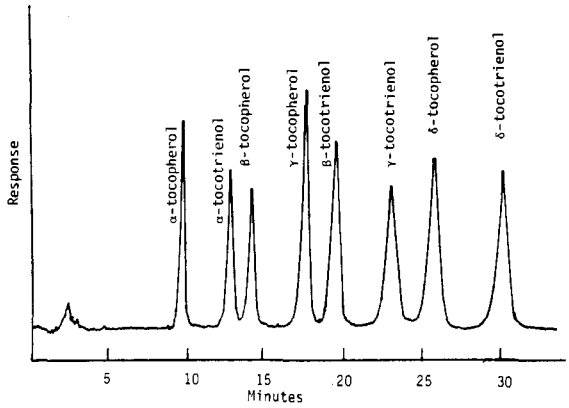

Figure 1. Chromatogram of standard solutions: α-tocopherol, 0.60 μg/mL; α-tocotrienol, 0.68 μg/mL; β-tocopherol, 0.76 μg/mL; β-tocotrienol, 1.31 μg/mL; γ-tocopherol, 1.70 μg/mL; γ-tocotrienol, 1.20 μg/mL; δ-tocopherol, 1.69 μg/mL; δ-tocotrienol, 1.48 μg/mL.

were chromatographic grade (Burdick and Jackson). The ascorbic acid used was USP-FCC. All other reagents used were reagent grade.

Standards. The following standards were used: α-tocopherol in sealed ampules (Eastman Organic Chemicals); β-tocopherol, isolated and purified from wheat germ oil (Sigma Chemical Co.); γ- and δ-tocopherol, and α-, β-, γ-, δ-tocotrienol in sealed ampules were obtained from F. Hoffmann-La Roche & Co., Basel, Switzerland.

Procedure. *Sample Preparation.* Samples of 250 g (mash, corn, pellets, grain, etc.) were ground to pass through a No. 30 sieve and were mixed thoroughly. Five-gram portions of the ground samples were accurately weighed, transferred into cellulose thimbles, placed on a Soxhlet containing 100 mL of alcohol, and extracted for 90 min. Samples of fat were saponified directly without preextraction. The sample extracts were refluxed with 2 g of ascorbic acid for 2–3 min. Ten grams of potassium hydroxide pellets was added and the samples were saponified for an additional 30 min.

The saponified extracts and two 40-mL water rinses were transferred into a 500-mL separatory funnel and extracted 2 times with 100 mL of petroleum ether (bp 36–58 °C). The extracts were combined and washed with several 100-mL portions of water until free of alkali. The petroleum ether extracts were filtered through 20 g of sodium sulfate and the separatory funnel was rinsed with two 20-mL portions of petroleum ether. The extracts were evaporated almost to dryness in a water bath at 45 °C under a stream of nitrogen and the residues dissolved in 10 mL of isooctane.

If necessary, the samples were diluted with isooctane to obtain a concentration of about 1 μg/mL. This solution was used for HPLC analysis.

Standard Preparation. Individual standards were accurately weighed, dissolved, and diluted with isooctane to obtain a concentration of about 1 μg/mL.

Calculations. Aspl × 454 × C × f/(Astd × 1000) = concentration of tocopherols (mg/lb) where C = standard concentration (μg/mL), f = dilution factor, Aspl = area of sample peak, Astd = area of standard peak, 454 = grams per pound, and 1000 = conversion from micrograms to milligrams.

DISCUSSION

The chromatographic separation of tocopherol and tocotrienol standards is presented in Figure 1. Typical chromatograms of tocopherols and tocotrienols extracted from feed ingredients are shown in Figure 2. The reproducibility of the HPLC system was determined by injecting one sample of α-tocopherol (1.42 μg/mL) 6 times with a relative standard deviation of 0.9% obtained. Response was linear for α-tocopherol within the range of 0.2–2.0 μg/mL with a correlation coefficient of 0.9996 obtained from regression analysis. The average recovery of α-tocopherol added to soybean meal, corn, alfalfa, and cottonseed meal was 99.4%. Individual recovery data and base-line values for the feeds used are shown in Tables I and II. The precision of the method was determined by analyzing one sample 6 times with a relative standard deviation of 2.7% obtained.

The tocopherol and tocotrienol values determined for 77 samples of 12 important feedstuffs are presented in Table III. The separation of the tocopherols is essential since each isomer has a specific vitamin E potency equivalent. α-Tocopherol, which has the greatest vitamin E activity, is the dominant isomer in feed grains. There-

Table I. Recovery of α-Tocopherol[a] Added to Feed[b]

level, mg/lb	amount added, mg/5 g of feed	amount recovered, mg/5 g of feed	recovery, %
2	0.0296	0.0290	98.0
	0.0240	0.0240	100.0
	0.0224	0.0226	100.9
	0.0274	0.0269	98.2
	0.0220	0.0227	103.2
			av: 100.1
5	0.0547	0.0546	99.8
	0.0542	0.0567	104.6
	0.0480	0.0474	98.8
	0.0449	0.0437	97.3
	0.0490	0.0520	106.1
	0.0480	0.0465	96.9
			av: 100.6
10	0.0720	0.0715	99.3
	0.0960	0.0941	98.0
	0.1090	0.1100	100.9
	0.1080	0.1080	100.0
			av: 99.6
20	0.224	0.208	92.9
	0.240	0.237	98.8
	0.218	0.217	99.5
			av: 97.1
			total av: 99.4

[a] Solutions of α-tocopherol in alcohol added to feed at the indicated levels. [b] Soybean meal containing 0.002 mg of α-tocopherol/g.

Table II. Recovery of α-Tocopherol Spray Dried[a] from Feed[b]

sample, mg/lb, added		mg/lb recovered	% recovery
corn	11.3	11.5	101.8
corn	12.5	12.7	101.6
corn	15.2	14.9	98.0
alfalfa	11.4	11.1	97.4
alfalfa	9.9	9.5	96.0
alfalfa	11.3	11.1	98.2
cottonseed meal	9.8	9.9	101.0
			av: 99.1
corn	21.1	20.2	95.7
corn	22.5	22.8	101.3
corn	22.4	22.8	101.8
alfalfa	18.4	18.7	101.6
alfalfa	18.6	18.6	100.0
alfalfa	21.3	21.4	100.5
cottonseed meal	20.8	20.1	96.6
			av: 99.6

[a] Accurately weighed amounts of α-tocopherol spray dried added to feed samples. [b] Base-line values: corn, 6.2 mg of α-tocopherol/lb; alfalfa, 8.8 mg of α-tocopherol/lb; cottonseed meal, 4.3 mg of α-tocopherol/lb.

Table III. Vitamin E Survey (Values in mg/lb)

	territory[a]	α-tocopherol	α-tocotrienol	β-tocopherol	γ-tocopherol	γ-tocotrienol	δ-tocopherol
corn	10	3.5	3.6		19.1	11.0	
corn	10	4.6	3.1		18.0	6.2	
corn	13	5.5	3.6		17.4	6.4	
corn	46	6.7	3.8		13.4	6.8	
corn	10	5.3	4.5		25.0	6.9	
corn	10	3.0	2.4		19.0	8.2	
grower corn	10	4.2	3.1		15.6	4.5	
whole corn	18	6.8		0.27	9.1	2.7	
yellow corn	49	3.5	1.8		10.6	4.8	
whole corn	49	0.90	2.2	0.24	12.7	3.6	
whole corn	49	4.3	2.3	0.16	13.6		
soybean meal	10	1.3			15.2	trace	2.0
soybean meal	10	1.1			13.4	trace	2.7
soybean meal	46	0.57			6.1	trace	0.9
soybean meal	13	0.69			7.7	trace	1.3
soybean meal	10	0.85			10.5	trace	1.7
soybean meal	10	1.0			8.0	trace	2.4
soybean meal	10	0.84			5.8	trace	1.5
soybean meal	10	0.75			6.3	trace	1.8
soybean meal	13	0.61			3.6	trace	0.9
soybean meal	18	0.59			2.4		1.9
soybean meal	49	0.79			5.8	trace	1.9
soybean meal	49	0.49			5.0	trace	1.7
soybean meal	49	0.45			2.7		1.1
soybean meal	49	0.38			2.6		1.5
soybean meal	49	0.40			1.4		
fish meal	13	1.6					
fish meal	46	3.6					
fish meal	46	0.18					
fish meal	49	3.5					
fish meal	49	1.3					
fish meal (60%)	49	3.7					
meal and bone meal	10	NMA[b]					
meal and bone meal	46	NMA					
cottonseed meal	46	8.3			3.1	0.35	
cottonseed meal	10	5.5			8.0	1.1	
cottonseed meal	10	5.3			7.7	1.0	
cottonseed meal	10	1.9			5.2	0.63	
cottonseed meal	49	0.51			2.2	1.0	
cottonseed meal	49	1.0			2.4		
cottonseed meal	49	2.0			2.4		
corn gluten meal	10	2.7	20.9		10.0		
corn gluten meal	10	6.6	22.4		16.7		
corn gluten meal	13	6.0	25.5		17.8		
corn gluten meal	18	2.3	5.3		6.2	10.7	trace
corn gluten meal	49	2.3	6.9		5.4		0.43
oats	10	2.0	10.0				
oats	10	2.1	9.7				
oats	49	3.4	4.8	0.35			
rolled oats	46	3.6	9.0	0.48			
whole oats	49	3.2	3.6		trace		
whole oats	49	2.6	3.6	0.26			
whole oats	18	3.1	2.8	0.27			
alfalfa dehydrated	46	29.0			2.4	2.8	trace
alfalfa dehydrated	10	16.4			1.7	1.5	trace
alfalfa dehydrated	10	35.0			15.3	4.2	6.0
alfalfa dehydrated	13	21.0			3.0	trace	trace
alfalfa sun-cured	10	24.0					
alfalfa meal	49	12.5			1.6		
alfalfa meal	18	14.5					
alfalfa meal	49	38.0		0.41	3.3		
alfalfa pelleted	49	13.7			6.4		
milo	10	3.0	trace		6.2		
milo	49	1.9	0.57		7.5		
barley	10	4.4	12.9	0.68	1.7		
barley	10	3.6	10.5	0.30	1.4	1.5	
barley crimped	10	3.2	7.9	0.33	1.5		trace
crimped barley	18	2.2	3.9		0.79		
wheat	46	3.7	1.2	2.2			
wheat	10	5.5	1.3	2.4			
wheat	10	5.3	1.4	2.6			
whole wheat	49	2.3	0.32	1.1			
animal fat	13	5.1	trace				
animal fat (poultry)	46	9.3	trace	0.11			
animal fat	49	1.5		0.12	3.0		0.73
animal fat	49	2.5			8.3		
animal fat	49	1.2			3.5		trace

[a] Territories: 10 = California, Nevada, and Utah; 13 = Iowa; 46 = Alabama and Tennessee; 18 = Pennsylvania, New York, and New England; 49 = Ohio, Indiana, Kentucky, and Michigan. [b] NMA = no measurable amount.

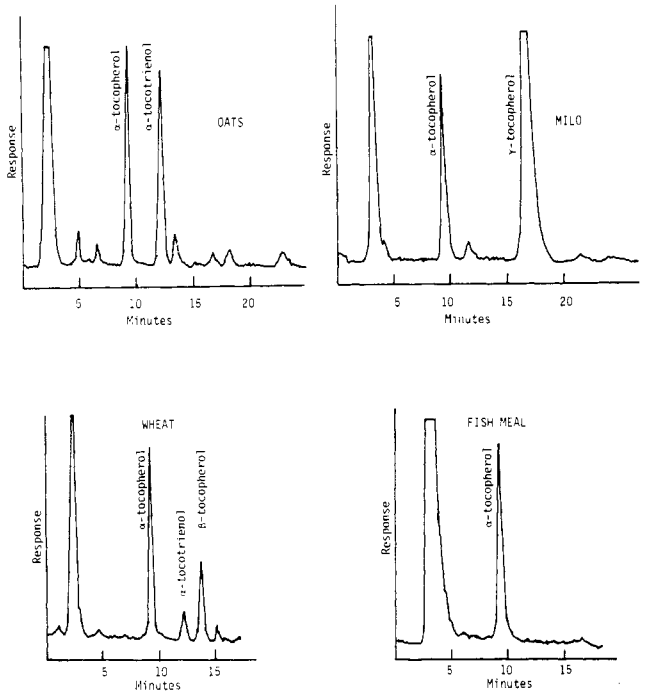

Figure 2. Chromatograms of extracts from typical feedstuffs.

fore, it is important to know the natural α-tocopherol content present in feedstuffs when recommending levels of vitamin E supplementation in animal rations.

The α-tocopherol values obtained by HPLC were lower in most cases than values reported by Bunnell et al. (1968) for corn, corn gluten meal, oats, barley, and wheat. These grains contain significant amounts of α-tocotrienol. The determination of α-tocopherol using secondary magnesium phosphate chromatography reported by Bunnell et al. (1968) does not separate α-tocopherol from α-tocotrienol, thus accounting for the high values obtained. On the other hand, for feedstuffs containing no α-tocotrienol such as soybean meal, fish meal, cottonseed meal, and animal fat, values obtained by HPLC are comparable to those of Bunnell et al. (1968). α-Tocopherol and α-tocotrienol values obtained by Slover (1971) on corn, barley, and oats are lower than those reported here. These low values are most likely due to GC methods that require extensive sample cleanup and derivatization with the possibility of metal-catalyzed oxidation of the tocopherols. The HPLC method reported requires a short extraction with no significant loss of tocopherol as proven by recovery data. Separation of each tocopherol and tocotrienol isomer was also obtained.

The importance of vitamin E in animal nutrition is well established. A recent study published by Adams (1982) using the method described herein showed that many cattle feedlot rations have low α-tocopherol content. These low levels of α-tocopherol intake were reflected in low plasma tocopherol levels found in cattle, indicating a need for vitamin E supplementation.

The data presented in Table III provide a comprehensive update of the tocopherol contents of major feedstuffs in the United States.

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Registry No. α-Tocopherol, 59-02-9; β-tocopherol, 148-03-8; γ-tocopherol, 7616-22-0; δ-tocopherol, 119-13-1; α-tocotrienol, 1721-51-3; γ-tocotrienol, 14101-61-2.

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