

Alpha-Tocopherol Content of Cereal Grains and Processed Cereals

David C. Herting and Emma-Jane E. Drury

Although natural grains often contain appreciable quantities of vitamin E (α -tocopherol), little information is available concerning the content in cereals as consumed in the human diet. The α -tocopherol content of cereal grains and cereals derived therefrom was determined by using thin-layer chromatography on alumina. Average contents in whole corn, wheat, oats, and rice were 1.53, 0.87, 1.54, and 0.35 mg. per 100 grams, respectively. Up to threefold variation among samples of the same natural grain was undoubtedly influenced by where the plant was grown, the time of harvest, and the stability after harvest. The processing of grain

by flaking, shredding, puffing, and other procedures to produce cereals usually resulted in extensive loss of vitamin E, sometimes as much as 90%. Analyses of samples of the same type of cereal from different processors showed up to fivefold variation, which reflects not only the variation in the original grain but probably also differences in processing technique. The lipid content of the grain was usually reduced by processing, but the distribution of fatty acids was essentially unchanged. The extensive loss of vitamin E during processing of grains to cereals suggests the advisability of restoring nutritive value by fortification of the finished cereal.

Most cereal grains contain appreciable quantities of vitamin E activity (principally α -tocopherol) and have often been assumed to be important contributors of α -tocopherol to the human diet. Perhaps overlooked with the passage of time has been the increasing degree of processing of these grains prior to consumption, particularly by western civilizations.

Vitamin E content before and after processing of grains has not been directly evaluated in the United States. Comparison of α -tocopherol levels in whole grain (Bunnell *et al.*, 1968; Harris *et al.*, 1950; Herting and Drury, 1963, 1967) with scattered and limited values for α -tocopherol content of grain products (Bunnell *et al.*, 1965; Dicks-Bushnell and Davis, 1967; Harris *et al.*, 1950) strongly suggests not only loss during processing but also increased loss with increased processing (Ames, 1969).

Conclusions based on such data are clouded by the appreciable variation which occurs naturally among samples of the same type of grain. Described here are studies designed to provide data showing average values and variability for α -tocopherol in the four major cereal grains, to obtain representative values for the α -tocopherol content of various types of processed cereals, to obtain direct data on the α -tocopherol content of input grain and output processed cereals, and to determine the effect of processing on fatty acid distribution.

METHODS AND MATERIALS

Samples. Samples of grain were obtained from grain suppliers in various parts of the United States. Corn came from at least seven states, wheat from at least six states, oats from at least three states, and rice from at least three states. Processed cereals as well as brown and white rice were purchased in local grocery stores. Attempts were made to obtain samples containing only one grain and representative of as many different types of processing as possible but not confounded by the addition of extra fat or antioxidant. The latter two conditions were not always met. In addition, excellent cooperation by five grain processors provided input grains and finished products representing a variety of processing conditions.

Preparation of Samples. All samples were ground either in

a hammer mill or with a mortar and pestle and were extracted according to Quaife and Harris (1948), either in equipment as described by them or in a Soxhlet apparatus. An aliquot of the petroleum ether (b.p. 60° to 71° C.) extract was evaporated under nitrogen in a tared beaker to measure total lipid. The remaining petroleum ether extract was concentrated and saponified, and the nonsaponifiable fraction recovered, as described by Herting and Drury (1963).

Chromatography of Tocopherols. The tocopherols were separated in dim light by two-dimensional thin-layer chromatography on unactivated alumina Eastman Chromagram sheets (No. 6062) (Herting and Drury, 1967). Solvent systems of benzene-diethyl ether (90 to 10) for the first dimension and light petroleum ether (b.p. 35° to 60° C.)-diisopropyl ether (80 to 20) (Whittle and Pennock, 1967) for the second dimension were generally used, although minor adjustments in solvent polarity were necessary for several samples during the study. Recoveries of the two major tocopherols, α - and γ -, from the two-dimensional system were consistent [$81.2 \pm 0.9\%$ (S.E.) and $80.8 \pm 1.0\%$ (S.E.), respectively], although not as complete as those observed by Whittle and Pennock (1967). The only difficulty with this method was experienced when the presence of butylated hydroxyanisole interfered with the measurement of γ - or β -tocopherol.

Colorimetric Measurement. The method of Tsen (1961) was adapted for measurement of small amounts of tocopherol. The tocopherol spot was cut out of the sheet, placed in a flat-bottomed 8-dram vial, and covered by 1.4 ml. of an ethanolic solution of bathophenanthroline ($3.0 \times 10^{-3}M$) (G. Frederick Smith Chemical Co.). The vial was capped and shaken for 15 seconds by hand or with a vibratory mixer. After standing for 15 minutes, a 1.0-ml. aliquot from this solution was transferred to a 15-ml. centrifuge tube, and 0.3 ml. of an ethanolic solution of ferric chloride ($2 \times 10^{-3}M$) (J. T. Baker Chemical Co.) was added with mixing. Exactly 15 seconds after the last drop of the ferric chloride was added, 0.3 ml. of an ethanolic solution of H_3PO_4 (0.172M) (86%, J. T. Baker Chemical Co.) was added with mixing to complex residual ferric ion. The salmon-pink color was stable in a normally lighted room for at least 90 minutes. A reagent blank was prepared for each set of samples, using an uncontaminated section of the alumina sheet.

A portion of the solution was transferred to a microcell (1-cm. light path, capacity 1.3 ml.), and the absorbance was measured in a Beckman DU spectrophotometer at 534 $m\mu$.

Biochemical Research Laboratories, Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N. Y. 14603

Table I. Tocopherol Content of Corn and Processed Corn

Form	Samples	Lipid, %	Tocopherols, Mg./G. Lipid		α-Tocopherol	
			α	γ	Mg./100 g.	% loss
Whole grain (yellow)	10 ^a	4.08 (3.61-4.50)	0.38 (0.27-0.55)	1.24 ^b (0.97-1.95)	1.53 (1.08-2.11)	—
Whole grain (white)	1	4.04	0.24	0.76	0.98	—
Grits (yellow)	2	0.89 (0.42-1.36)	0.34 (0.23-0.44)	1.06 (0.61-1.51)	0.35 (0.10-0.60)	77
Flakes	A	0.38 ^c	0.20	0.41	0.08	95
	B	0.38	0.40	1.56	0.15	90
	C	0.25	0.11	0.50	0.03	98
Puffed	D	0.65 ^d	0.14	0.52	0.09	94
Shredded Meal	E	0.28	0.31	0.92	0.08	95
Yellow	F	0.70	0.59	1.29	0.42	73
White	G	1.38	0.47	1.27	0.64	35

^a Includes 7 samples reported previously (Herting and Drury, 1963).

^b One sample also contained 0.12 mg. α-tocotrienol per gram lipid.

^c Includes unknown amount of unknown vegetable oil.

^d Includes unknown amount of unknown hydrogenated vegetable oil.

Table II. Tocopherol Content of Wheat and Processed Wheat

Form	Samples	Lipid, %	Tocopherols, Mg./G. Lipid			α-Tocopherol	
			α	β	α T-3 ^a	Mg./100 g.	% loss
Whole grain	9 ^b	2.08 (1.10-2.66)	0.45 (0.21-0.80)	1.06 (0.82-1.68)	0.06 (0.0-0.20)	0.87 (0.50-1.18)	—
Flakes	H	1.30	0.19	0.48	0 ^c	0.25	71
	I	1.62	0.33	1.05	0.09	0.54	38
Puffed	J	2.03	0.34	0.91	0	0.68	22
	K	1.47	0.32	1.16	0	0.47	46
Shredded	L	1.08	0.15	0.89	0	0.17	80
	M	1.13	0.27	0.43	0.18	0.31	64
	N	0.96 ^d	0.09	^e	0	0.09	90
Meal	O	1.04	0.10	1.27	0	0.11	87
Flour ^f	P	1.26	0.06	0.62	0	0.07	92

^a α-Tocotrienol.

^b Includes one sample reported previously (Herting and Drury, 1967).

^c Also contained 0.04 mg. δ-tocopherol per gram lipid.

^d Includes trace amount of corn oil added as vehicle for BHA.

^e Measurement precluded by interference from BHA.

^f 74% extraction.

Table III. Tocopherol Content of Oats and Processed Oats

Form	Samples	Lipid, %	Tocopherols, Mg./G. Lipid			α-Tocopherol	
			α	γ	α T-3 ^a	Mg./100 g.	% loss
Whole grain	4	5.12 (4.28-5.93)	0.30 (0.23-0.41)	0.01 (0.0-0.03)	0.06 (0.0-0.16)	1.54 (1.30-1.76)	—
Whole grain (stored 6 years)	1	4.22	0.10	0	0.04	0.42	73
Granular	Q	3.64	0.02	0.07	0.06	0.08	95
	R	2.02 ^b	0.04	0.10	0	0.09	94
Shredded Meal	S	3.02	0.02	0.02	0	0.08	95
Rolled	T	6.49	0.20	0.03	0.08	1.33	14
	U	6.20	0.15	0.02	0.04	0.94	39
	V	6.43	0.28	0.01	0.05	1.81	(18) ^c
1-Minute Instant	W	6.94	0.29	0.01	0.10	2.04	(32) ^c
	X	6.99	0.27	^d	0.07	1.86	(21) ^c

^a α-Tocotrienol.

^b Includes unknown amount of unknown hydrogenated vegetable oil.

^c Apparent % gain.

^d Not measured because of interference from BHA.

Chromatography of Fatty Acids. The lipid from the total lipid determination was converted to methyl esters by treatment either with BF₃ in methanol (Metcalf and Schmitz, 1961) or with CHCl₃-methanol-H₂SO₄ (100:100:1) at 170° C. (Peisker, 1964). The methyl esters were analyzed with a Perkin-Elmer 900 instrument equipped with dual 6-foot × 0.250-inch glass columns packed with either Gas-Chrom P (60- to 80-mesh) coated with 7.5% diethylene glycol succinate (w./w.) and 7.5% butanediol succinate (w./w.) or acid-washed, silanized Chromosorb W (80- to 100-mesh) coated with 15%

diethylene glycol succinate (w./w.). The columns were programmed at 10° per minute from 100° to 200° C. with temperatures of 220° for both the flash heater and the flame ionization detector. A positive helium pressure on the column of 50 p.s.i. resulted in a flow rate of 60 ml. per minute. Peak areas were measured by Disc integration.

RESULTS AND DISCUSSION

Contents of lipid and tocopherols in all samples of grain and processed grain are reported for corn, wheat, oats, and rice in

Table IV. Tocopherol Content of Rice and Processed Rice

Form	Samples	Lipid, %	Tocopherols, Mg./G. Lipid		α -Tocopherol	
			α	γ	Mg./100g.	% loss
Whole (rough) grain	1	2.26	0.16	0.17	0.35	—
Whole grain, parboiled	1	2.00	0.07	0.20	0.15	57
Dehulled (brown)	3	2.47	0.10	0.16	0.26	26
		(2.16–2.82)	(0.05–0.15)	(0.03–0.27)	(0.13–0.42)	
Dehulled, parboiled	1	2.37	0.06	0.14	0.15	57
Milled (white)	4	0.65	0.16	0.42	0.10	71
		(0.55–0.82)	(0.09–0.20)	(0.32–0.56)	(0.05–0.12)	
Milled, parboiled	3	0.60	0.14	0.13	0.06	83
		(0.22–0.89)	(0.05–0.27)	(0.04–0.25)	(0.04–0.08)	
Grits	1	0.32	0.11	0.30	0.04	89
Expanded	Y	0.55	0.03	0.04	0.01	97
Puffed	Z	0.23	0.31	0.44	0.07	80
	AA	0.28	0.26	0.49	0.07	80
	BB	0.82	0.06	0.10	0.05	86
	CC ^a	2.19	0.05	0.65	0.12	66
Shredded	DD	0.11	0.16	0.16 ^b	0.02	94
Meal	EE	0.63	0.16	0.43	0.10	71

^a Contains coating of unknown composition.

^b Sample also contained 0.06 mg. δ -tocopherol per gram lipid.

Tables I to IV. Although values for α -, β -, and γ -tocopherols and α -tocotrienol (α -T-3) are included, those for α -tocopherol are of primary interest. Average contents for α -tocopherol of 1.53, 0.87, and 1.54 mg. per 100 grams of whole corn, wheat, and oats, respectively, are similar to those reported by Bunnell *et al.* (1968), but our value of 0.26 mg. per 100 grams of dehulled rice is appreciably lower than their value of 1.35 mg. per 100 grams of brown rice. Up to threefold variation in α -tocopherol content was observed among samples of the same grain. This is undoubtedly influenced by many factors such as the strain of plant, where the grain was grown, the time of harvest, and the stability after harvest. Although most

of our samples were obtained within one year after harvest, one sample of oats (Table III) had been stored for 6 years and showed a low level of α -tocopherol.

Grain products were selected to represent various types of processing. On the basis of the average α -tocopherol contents of whole grains, virtually any type of processing resulted in less vitamin E in the product at the retail level. For corn, losses ranged from 35% for white meal to 98% for brand C flakes (Table I). Wheat products showed losses from 22% for puffed brand J to 92% for flour (Table II). Manufacture of oatmeals resulted in relatively little or no decrease of vitamin E (Table III), but more extensive processing increased losses to

Table V. Tocopherol Content of Grains and Derived Products

Grain	Form	Sample	Lipid, %	α -Tocopherol		
				Mg./G. lipid	Mg./100 g.	% loss
Corn	Yellow grain	—	3.61	0.39	1.42	
	Yellow meal	F	0.70	0.59	0.42	70
	White grain	—	4.04	0.24	0.98	
	White meal	G	1.38	0.47	0.64	35
	Yellow grits	—	1.36	0.44	0.60	
	Flakes cereal	B	0.38	0.40	0.15	75
	Yellow grits	—	0.42	0.23	0.10	
	Shredded cereal	E	0.28	0.31	0.08	20
Wheat	Whole grain	—	2.27	0.37	0.83	
	Flour ^a	P	1.26	0.06	0.07	92
	Whole grain	—	2.33	0.21	0.50	
	Puffed cereal	K	1.47	0.32	0.47	6
	Whole grain	—	2.66	0.24	0.63	
	Puffed cereal	J	2.03	0.34	0.68	(8)
	Whole grain	—	1.79	0.48	0.86	
	Shredded cereal	N	0.96 ^b	0.09	0.09	90
Oats	Whole grain	—	5.43	0.31	1.70	
	Meal	V	6.43	0.28	1.81	(6)
Rice	Milled grain	—	0.55	0.19	0.11	
	Puffed cereal	Z	0.23	0.31	0.07	36
	Milled grain (parboiled)	—	0.89	0.09	0.08	
	Puffed cereal	BB	0.82	0.06	0.05	38
	Milled grain	—	0.63	0.20	0.12	
	Puffed cereal	AA	0.28	0.26	0.07	42
	Milled grain (parboiled)	—	0.70	0.05	0.04	
	Expanded cereal	Y	0.55	0.03	0.01	75
	Milled grits	—	0.32	0.11	0.04	
Shredded cereal	DD	0.11	0.16	0.02	50	

^a 74% extraction.

^b Includes trace amount of corn oil added as vehicle for BHA.

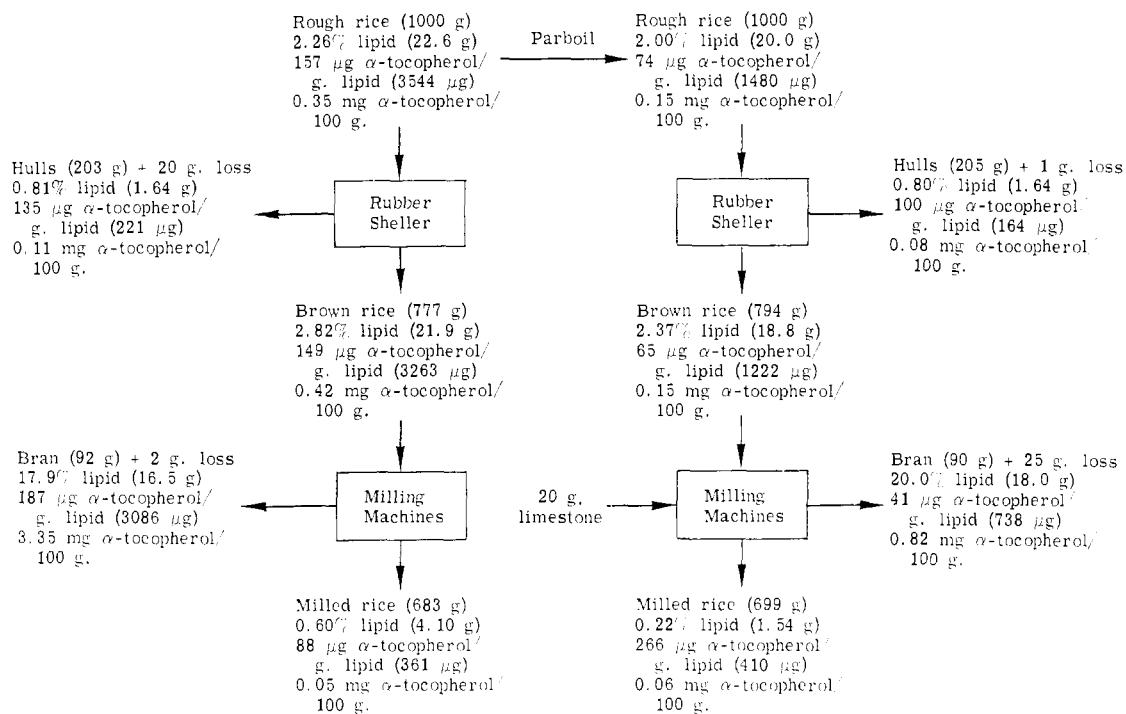


Figure 1. Lipid and α -tocopherol in rice fractions

about 95%. Rice cereal products consistently showed more than 70% loss of α -tocopherol during production (Table IV).

For each grain, several brands of one type of cereal were analyzed to ascertain the variation that might occur among products from different processors. Thus, three brands of corn flakes (Table I) showed from 0.03 to 0.15 mg. of α -tocopherol per 100 grams, three brands of shredded wheat (Table II) showed from 0.09 to 0.31 mg., three brands of rolled oats (Table III, T,U,V) showed from 0.94 to 1.81 mg., and four brands of puffed rice (Table IV) showed from 0.05 to 0.12 mg. This variation among different brands of the same type of cereal probably reflects some variation in processing techniques in addition to the variation in the original grain.

To determine more directly the losses of tocopherol during processing, the cooperation of five processors was obtained in providing input grain and output grain products taken on the same day. α -Tocopherol contents for such paired samples (Table V) showed some loss in most cases, varying from 20% for a shredded corn cereal to 92% for the wheat flour. Oatmeal showed no loss of α -tocopherol, probably because only the hull is removed and the processing is mild. Corn meal, in comparison, is usually made following removal of both hull and most of the germ, resulting in less α -tocopherol in the product as consumed. Among the more highly processed cereals, removal of some of the fractions of the grain is followed by flaking, shredding, puffing, or expansion. In general, these processes resulted in relatively greater loss of tocopherol, although the puffed products were inconsistent. The puffing of wheat caused no loss, whereas the puffing of rice reduced α -tocopherol content by approximately 40%.

Losses of tocopherol during the production of flour have been studied in England for some years (Moran, 1959). The value for 74% extraction flour (Table V) suggests an appreciably greater loss than that observed by Moran (1959) for flour of a similar extraction but cannot be compared directly because Moran's values were based on total reducing materials. Even this low level of α -tocopherol may be further reduced when the flour is baked into bread (Moore *et al.*, 1957).

The most comprehensive study involved the production of milled rice from rough rice both before and after parboiling (Figure 1). The flow diagram shows that the bulk of the α -tocopherol originally present in the rough rice was lost in the bran fraction during the milling of brown rice, leaving very little α -tocopherol in the white rice. Parboiling introduced another variable and resulted in a significant loss of α -tocopherol even before any additional processing. Of the α -tocopherol remaining in the parboiled brown rice, more than half was lost during the milling process. Sechi and Rossi-Manaresi (1958) have also shown that "reasonably well-milled rice" (essentially the endosperm) retained only a "trace" of the total tocopherol originally in their sample of brown rice (1.31 mg. per 100 grams).

Evaluation of data up to this point has relied on content of α -tocopherol per 100 grams of product as consumed. Examination of changes in tocopherol content *per gram of lipid* (Tables I through V) shows that the concentration was often higher in the product than in the original grain and that the changes in the various tocopherols were not always the same. This is not necessarily surprising, because net changes in tocopherol are influenced by many factors. Different degrees of fractionation of the grain precede different types of processing. Furthermore, there are different levels of tocopherol in the various fractions of the kernel, and the proportion of α -tocopherol to total tocopherol also varies from fraction to fraction (Hall and Laidman, 1968; Moran, 1959). Comparison of data on rice with those of Moran (1959) on wheat also suggests that both the qualitative and quantitative distribution of tocopherols among the various fractions differ among grains, and it seems reasonable to assume that distribution among the fractions may well vary even within samples of the same grain. Adding to these factors the possible direct destruction of α -tocopherol by various cooking and drying procedures makes it difficult to distinguish clearly between loss of tocopherol by removal and destruction of tocopherol by processing.

Compounding these uncertainties is the lack of data for

Table VI. Distribution of Fatty Acids in Lipids from Grains and Processed Grains

Grain	Form	Samples	Fatty Acids, % ^a										
			<14	14:0 ^b	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20 (unsat)	>20
Corn	Whole	4			15.1	0.1	1.8	27.7	53.3	0.7	0.8	0.2	
	Grits	2			19.8	0.2	1.5	16.9	58.6		2.7	Tr ^c	
	Meal	2			20.9	0.2	1.5	20.7	54.4	0.5	1.7	Tr	
	Cereal	3			15.9	0.3	2.4	23.6	52.3	0.7	3.8	1.2	
Wheat	Whole	7			18.2	0.2	0.7	16.2	59.8	2.8	1.4	0.2	0.2
	Meal	1			21.9	Tr	0.8	10.0	64.4		2.9		
	Cereal	5			18.9	0.3	0.9	17.8	57.2	0.8	3.7	0.2	
	Flour	1			19.1	0.5	0.6	11.8	64.6		3.2	0.3	
Oats	Whole	4	Tr	0.1	18.7	0.1	1.6	38.4	38.2	1.3	0.9	0.6	
	Meal	5	Tr	Tr	17.1	0.4	1.3	38.2	40.6	1.3	0.5	0.3	
	Cereal	1	0.6	0.1	16.8	0.4	1.5	38.4	41.3		0.9	0.2	
Rice	Whole	1			28.4	Tr	2.7	37.6	31.2	Tr			
	Brown	3		1.0	27.5	Tr	2.0	43.0	25.1	1.0	0.1	0.2	
	Milled	4	0.1	0.9	24.0	0.1	2.5	29.6	41.2	1.1	0.4		
	Grits	1		0.7	24.1	0.3	1.8	30.6	40.8		1.3	0.3	
	Meal	1	1.1	0.9	21.7	Tr	1.3	29.4	42.2	0.2	2.0	1.3	Tr
	Cereal	5	Tr	0.7	21.6	0.2	1.4	34.4	39.1	1.0	1.1	0.4	0.1

^a % by weight from peak areas.

^b First number shows carbon atoms; second number shows double bonds.

^c Tr = trace.

Table VII. Relationship between α -Tocopherol and Polyunsaturated Fatty Acids in Wheat and Processed Wheat

Form	Sample	Lipid, %	PUFA, ^a G./G. Lipid	α -Tocopherol, Mg./G. Lipid	α -Tocopherol-PUFA, ^a Mg./G.	
Whole grain	1	2.66	0.54	0.24	0.44	
	2	1.79	0.62	0.48	0.77	
	3	2.56	0.59	0.31	0.53	
	4	2.13	0.64	0.47	0.73	
	5	2.27	0.61	0.37	0.61	
	6	2.00	0.59	0.59	1.00	
	7	2.33	0.57	0.21	0.37	
Meal	O	1.04	0.61	0.10	0.16	
	Cereal	I	1.62	0.56	0.33	0.59
		J	2.03	0.54	0.34	0.63
		K	1.47	0.51	0.32	0.63
		L	1.08	0.57	0.15	0.26
M		1.13	0.58	0.27	0.47	
Flour	P	1.26	0.61	0.06	0.10	

^a Polyunsaturated fatty acids.

stability of tocopherol in processed cereals between the time of manufacture and the time of consumption. Data from these laboratories (Herting and Drury, 1963) showed 35% loss of α -tocopherol during storage of ground corn at room temperature for 6 months, confirming at least qualitatively the earlier report by Kodicek *et al.* (1959) of approximately 36% loss of α -tocopherol during storage of maize for approximately 12 weeks. Rothe *et al.* (1958) observed approximately 50% reduction in tocopherol of white and whole grain flours and about 25% loss in wheat germ during storage for 80 days at 37° C. Correspondingly, prolonged shelf life of processed cereals may be accompanied by additional losses of α -tocopherol, particularly if no antioxidant is added either to the cereal or to the packaging material.

An important factor in vitamin E nutrition is the influence of polyunsaturated fatty acids (PUFA), and the ratio between α -tocopherol and PUFA has been proposed as a criterion of nutritional status (Harris and Embree, 1963). Our values for fatty acids in grains and grain products (Table VI) showed that the distribution of PUFA after processing usually resembled that in the whole grain. Since the processing of grains some-

times increased and sometimes decreased the content of α -tocopherol per gram of lipid, the α -tocopherol-PUFA ratio was correspondingly sometimes increased and sometimes decreased.

To illustrate the variation which may occur in this ratio for one type of cereal grain, the specific contents of PUFA and of α -tocopherol in samples of wheat and wheat products are given in Table VII. The α -tocopherol-PUFA ratio varied 10-fold, ranging from 0.10 in a sample of flour to 1.00 in a sample of whole wheat.

It seems clear that the processing of grains to grain products as consumed by the American people is accompanied in most cases by appreciable losses of vitamin E, and that these data suggest more strenuous processing in recent years has increased the loss. Rubin (1966) and Harris (1968) considered both the possible need for supplementary vitamin E and its suitability for inclusion in the enrichment formula of white flour. This concept could well be extended to include processed cereals, as proposed by Ames (1969). Farinaceous foods would seem to be an appropriate choice for restoration, because they are available at relatively low cost, are amenable

to supplementation without undue changes in color and flavor, and are widely accepted and consumed by people of relatively low socio-economic status.

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