THE JOURNAL OF THE American Oil Chemists' Society

Volume 34 MAY, 1957 No. 5

Provitamin A Activity and Stability of β-Carotene in Margarine

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AS A COLORING AGENT in margarine and other fats, β-carotene is widely used throughout the world. In many countries it also serves as a source of provitamin A activity to supplement the vitamin A fortification. Table I presents a survey of current fortification practices in some countries where carotene is used. The vitamin A levels listed are those prescribed by law or applied voluntarily in the industry. In Denmark, England, and Australia vitamin A fortification is mandatory at the levels indicated. In the United States and Switzerland vitamin A fortification is optional; but levels are prescribed by law to justify a label declaration of a fortified product. Where it is known that carotene can be considered a source of provitamin A activity, the level or the basis of calculation of potency is indicated in the table.

In the United States margarine is colored at present by one of three materials: carotene, annatto extract, or FD&C Yellows 3 and 4. Harris (1), recently reviewing pigments for food fats, concludes that, since carotene is biologically active and can contribute significantly toward the vitamin A activity, it is the yellow color of choice. Twelve United States margarine manufacturers and five shortening manufacturers were using carotene in 1957 as compared to one margarine manufacturer in 1953.

The chemical synthesis of β-carotene (2) on a production scale from citral of lemon grass oil follows large-scale production of vitamin A (3), likewise synthesized from lemon grass oil. Both compounds have a role in the edible fat and oil industry. Synthetic vitamin A has been shown to be admirably suited for fortification of fatty-type foods (4). Animal assay data confirm full biological activity for vitamin A of synthetic origin (4, 5).

The commercial development of pure, synthetic, all-*trans* β-carotene as a coloring agent and source of vitamin A for margarine, shortening, and other foods prompted the investigation of its provitamin A activity and stability. The U.S.P. rat curative growth assay was chosen for determination of provitamin A activity since this type of test provided the basis for the original establishment of the relative potencies of β-carotene and vitamin A.

The International Unit (or U.S.P. unit) is equivalent to 0.6 microgram of all-*trans* β-carotene and 0.344 microgram of all-*trans* vitamin A acetate (0.3 microgram of vitamin A alcohol). These values were established by the Expert Committee on Biological Standardization of the World Health Organization (6) in 1949 after careful statistical evaluation of collaborative rat bioassays of β-carotene and vitamin A. The first study, conducted largely in Great Britain (7), yielded a potency of 3,200,000 I.U. per gram for vitamin A alcohol as against 1,667,000 I.U. per gram for all-*trans* β-carotene. The second collaborative assay was sponsored by the United States Pharmacopoeia (8) in 1946–7 with 12 laboratories participating. Based on the above potency for β-carotene, the potency of vitamin A alcohol was found to be 3,333,000 I.U. per gram. Thus by present standards one microgram of vitamin A alcohol is recognized as equivalent in biological activity to 2 micrograms of all-*trans* β-carotene.

There are numerous reports in the literature indicating that the provitamin A activity of β-carotene is dependent on the specific conditions of the rat assay. For example, Koehn (9) and Burns *et al.* (10, 11) found β-carotene as active as vitamin A on a weight basis when the rats were given 1 mg. of α-tocopherol per day. Hove (12) however demon-

TABLE I
Vitamin A Fortification of Margarine

Country	Vitamin A potency	Comment on carotene
United States	15,000 I.U./lb.	β-carotene a permissible coloring; also used for vitamin A activity (0.6 mcg. = 1 U.S.P.U.)
Austria	20,000 I.U./kg.	β-carotene a permissible coloring.
Denmark	16,000 I.U./kg.	Carotene sources used exclusively for color; supplies vitamin A activity at 4,000 I.U./kg.[a]
England	760–940 I.U./oz. (minimum & maximum)	β-carotene a permissible coloring; (0.75 mcg. = 1 I.U.).
Finland	20,000 I.U./kg.	β-carotene a permissible coloring.
Germany	20,000 I.U./kg.	β-carotene a permissible coloring.
Sweden	25,000 I.U./kg.	β-carotene a permissible coloring. When carotene sources are used, supplies 5,000 I.U./kg.[a]
Netherlands	20,000 I.U./kg.	β-carotene a permissible coloring.
Norway	20,000 I.U./kg.	β-carotene a permissible coloring.
Switzerland	20,000–60,000 I.U./kg.[b]	β-carotene a permissible coloring; also used for victim A activity (0.6 mcg. = 1 I.U.).
Australia	30,000–40,000 I.U./kg.	β-carotene a permissible coloring.

[a] Plus the vitamin A value. [b] The two levels are prescribed for margarine labeled "contains vitamin A" and "rich in vitamin A," respectively.

strated that this increased activity was caused primarily by the omission of yeast from the diet. Using 8% dried yeast, Hove (12) found β-carotene to have only 40 to 50% of the activity of vitamin A on a weight basis, but in the absence of yeast 70 to 90%. Deuel *et al.* (13) reported that feeding β-carotene in margarine enhanced its activity for rat growth by as much as 30% relative to vitamin A, which was unaffected by the carrier. It was demonstrated by High and Wilson (14) that B_{12}-deficient rats do not store as much vitamin A in the liver when fed high levels of carotene as rats receiving adequate vitamin B_{12} (55 mcg./kg. of diet). Mayfield and Roehm (15) found increased rat liver storage of vitamin A due to B_{12} added to diets with certain yeasts but not with others. These authors (16) also reported the effects of ascorbic acid on liver stores of vitamin A in rats fed β-carotene; the extent of storage was dependent on the sex of the rats and on the relative and absolute quantities of ascorbic acid and carotene fed. Hebert and Morgan (17) and Swick and Baumann (18) found that the addition of tocopherol increased tissue stores of vitamin A in rats fed β-carotene but had no effect when vitamin A was fed.

It is evident from the reports cited that the utilization of β-carotene by the rat is strongly dependent on the experimental conditions. For the purpose of evaluating synthetic β-carotene it appears preferable therefore to use a standard assay procedure, such as the U.S.P. method (19), insure the adequacy of the diet in α-tocopherol and vitamin B_{12}, compare with natural β-carotene, and use U.S.P. vitamin A acetate as the standard.

Experimental

Biological Assays. Crystalline, synthetic, all-*trans* β-carotene, micropulverized crystalline synthetic all-*trans* β-carotene (24%) vegetable oil-suspension, a crystalline natural carotene product, and various brands of margarine fortified in the manufacturer's plant with vitamin A and β-carotene were tested against the U.S.P. vitamin A standard in a series of three rat-curative, growth assays.

Procedure. Sprague-Dawley male rats were used with 10 to 12 to a group for each of 2 levels of sample. The standard growth response curve was based on 2 or 3 levels of the U.S.P. vitamin A acetate standard fed every 4 days in 0.2 milliliter of diluent; the middle level in assay 1 and both levels of standard in assay 2 were run in duplicate. Carotene was fed in a similar manner; the levels were determined on the basis of spectrophotometric and/or colorimetric assays of the products tested. The assays followed the U.S.P. method (19) except for the inclusion of tocopherol and vitamin B_{12} in the diet and the use of margarine for the dosing solutions. These changes, which are detailed below, were designed to favor the utilization of carotene (9, 10, 11, 14, 17, 18).

Since the margarine samples had to be fed with little dilution, all samples and standard were diluted in vitamin A-free margarine to keep the effect, if

TABLE II

U.S.P. Rat-Curative, Growth Assays of β-Carotene

Material fed	No. of rats	Dosing oil [a]	Dosage per rat/4 days	Gain in weight after 28 days (grams)	Vitamin A activity/g. of carotene product fed		Percentage of International Standard [b]
					I.U.	±*S.E.*(%)	
Assay 1			*I.U.*				
U.S.P. Reference Standard Vitamin A	12	M	3.19	37.6			
	11	M	4.69	59.7			
	10	M	4.69	57.0			
	13	M	6.91	83.2			
Crystalline Carotene			*Mcg.*				
Natural [c]	10	M	2.66	31.7	1,200,000	8.2	
	10	M	3.90	51.1			
Synthetic,[d] Lot 4458	12	M	1.91	37.5	1,790,000	5.1	107
	11	M	2.81	65.7			
Synthetic, Lot 4457	10	M	1.91	37.7	1,540,000	7.5	92
	11	M	2.81	51.2			
Synthetic, Lot 4458	9	C	1.91	35.4	1,520,000	6.7	91
	7	C	2.81	51.4			
Assay 2			*I.U.*				
U.S.P. Reference Standard Vitamin A	8	M	3.00	33.9			
	9	M	3.00	39.8			
	8	M	4.41	61.6			
	10	M	4.41	59.4			
			Mcg.				
Synthetic carotene, 24% micropulverized suspension in oil, Lot 4677 (400,000 I.U./g.)	9	M	7.98	47.7	460,000	6.7	115
	9	M	11.73	74.9	(1,920,000)[e]		
Assay 3			*I.U.*				
U.S.P. Reference Standard Vitamin A	9	C	3.00	35.4			
	10	C	4.41	61.7			
			Mcg.				
Synthetic carotene, 24% micropulverized suspension in oil, Lot 4677 (400,000 I.U./g.)	8	C	7.98	42.5	408,000	6.6	102
	9	C	11.73	66.2	(1,700,000)[e]		

[a] M = margarine, C = cottonseed oil.
[b] 0.6 microgram of β-carotene = 1 I.U. or 1 g. = 1,667,000 I.U.
[c] Natural crystalline carotene product with colorimetric assay equivalent to 1,200,000 I.U./g.
[d] Synthetic all-*trans* carotene from Hoffmann-La Roche Inc., meeting the following specifications for the U.V. spectrum in cyclohexane:

Wavelength	$E^{1\%}_{1cm.}$
340 mμ	140
Max. 454–455 mμ	2,450
Min. 469–471 mμ	1,970
Max. 482–484 mμ	2,150

[e] Corrected potency per gram of all-*trans* β-carotene.

TABLE III

U.S.P. Rat-Curative, Growth Assays of Commercial Margarines with Added Vitamin A and β-Carotene

Material fed [a]		No. of rats	Dosage per rat/4 days	Gain in weight after 28 days (grams)	Vitamin A activity per pound of margarine					Ratio: $\frac{\text{Biological}}{\text{Colorimetric}} \times 100$
					Biological total		Colorimetric			
							Vitamin A	Carotene [b]	Total	
Assay 1			*I.U.*		*I.U.*	±*S.E.*(%)	*I.U.*	*I.U.*	*I.U.*	(%)
U.S.P. Reference Standard Vitamin A		12	3.19	37.6						
		11	4.69	59.7						
		10	4.69	57.0						
		13	6.91	83.2						
Brand of margarine	Type of carotene		Margarine lb. x 10^{-4}							
A	Natural	12	2.76	61.5	18,400	5.8	11,000	5,700	16,700	110
		12	4.06	91.3						
A	Synthetic	12	2.76	55.2	16,600	5.1	10,300	5,600	15,900	104
		11	4.06	84.7						
B	Synthetic	12	2.11	61.2	26,000	5.8	18,600	7,800	26,400	99
		12	3.12	96.0						
Assay 2			*I.U.*							
U.S.P. Reference Standard Vitamin A		8	3.00	33.9						
		9	3.00	39.8						
		8	4.41	61.6						
		10	4.41	59.4						
Brand of margarine (all contain synthetic β-carotene)			Margarine lb. x 10^{-4}							
C		8	1.79	36.1	17,100	4.9	11,900	5,800	17,700	97
		10	2.64	64.0						
D		8	1.79	32.4	15,000	5.7	10,700	5,800	16,500	91
		9	2.64	51.7						
E		10	1.79	45.7	18,500	6.0	12,900	6,200	19,100	97
		9	2.64	63.4						
F		10	1.79	39.7	18,200	5.2	12,900	6,500	19,400	94
		10	2.64	68.7						
G		9	1.79	36.7	17,100	5.5	12,300	5,500	17,800	96
		9	2.64	63.1						
H		8	1.79	37.6	17,800	5.8	11,200	5,000	16,200	110
		10	2.64	64.4						
I		9	1.79	39.1	18,100	5.0	12,400	5,400	17,800	102
		10	2.64	67.3						Average 100

[a] Each letter refers to a different manufacturer. [b] 0.6 microgram of β-carotene = 1 I.U. or 1 gram = 1,667,000 I.U.

any, of the diluent constant. Cottonseed oil (Wesson Oil) was compared to margarine as the diluent for the vitamin A standard and a sample of synthetic carotene in one assay to test for possible augmentation of the provitamin A potency of carotene by margarine reported by Deuel *et al.* (13). In addition to the U.S.P basal diet, vitamin B_{12} (55 mcg./kg. diet) and tocopherols (0.15 mg./rat/day of mixed tocopherols in assay 1 and 0.75 mg. of dl-α-tocopherol per rat per day in assays 2 and 3) were fed to all groups.

Crystalline carotene products under test were dissolved and diluted in vitamin A-free margarine or cottonseed oil in the laboratory. The carotene oil suspension was diluted similarly. At the time of dosing the margarine dilutions were placed in an oven at 40°C. just long enough to liquefy the carrier. Cottonseed oil dilutions were treated in the same manner. Fresh dilutions were made weekly, gassed with CO_2 after use, and stored in the refrigerator. Commercially fortified margarine samples likewise were stored under refrigeration.

RESULTS

1. *Assays of Carotene Only.* The results of three separate assays of carotene only are summarized in Table II. The potencies were calculated per gram of carotene product. Samples of all-*trans*, synthetic β-carotene were compared directly to the International Standard value for β-carotene. All of the experimental values are close to the accepted value (1 gram = 1,667,000 I.U.), and the standard errors are relatively small. The average potency of the three synthetic carotene samples dosed in margarine (1,750,000 I.U. per gram) against the vitamin A standard in the same carrier is almost identical with the value of 1,700,000 I.U. per gram in assay 3, where both carotene and vitamin A were dosed in cottonseed oil. Hence these data provide no evidence for enhancement of the provitamin A activity of carotene fed in margarine, such as reported by Deuel *et al.* (13). These differences in findings may be due to variations between laboratories in basal diets, dosing schedules, volumes of margarine fed, etc.

2. *Assays of Commercial Margarines with Added Vitamin A and β-Carotene.* The results of two bioassays are summarized in Table III, which includes a comparison with potencies calculated from colorimetric assays for vitamin A and carotene. Vitamin A in margarine was determined by Carr-Price assay, using a modification of the U.S.P. method (20), which includes saponification and triple extraction with ether followed by petroleum ether. The water-washed extract is dried with sodium sulfate and evaporated, and the residue is diluted in chloroform for colorimetric reading with an internal standard in an Evelyn Colorimeter with a 620 filter.

Carotene was measured colorimetrically in a solution of margarine in Skellysolve B, clarified, if necessary, by shaking with Johns-Manville Hyflo

and centrifugation. A 440 filter was used in the Evelyn Colorimeter.

Luckmann *et al.* (21) have reported in great detail on comparative spectrophotometric, colorimetric, and biological assays for vitamin A and carotene in margarine.

In calculating activity of carotene from colorimetric assays, micrograms of β-carotene are converted into International Units, using the factor 0.6 mcg. = 1 I.U. For each margarine sample the bioassay is in close agreement with the sum of the colorimetric values for vitamin A and carotene. The standard errors are small in all cases.

3. *Statistical Treatment.* Calculations of potency and standard error for each individual sample were made by the method of Bliss (22), modified to take into account the differences in the number of animals in the various groups. To ascertain the over-all average potency of synthetic, all-*trans* β-carotene relative to vitamin A based on the three assays shown in Table II, the data were tested and found to be homogeneous by the chi-square test (22). Hence they were combined to yield an average potency for β-carotene of 1,730,000 I.U. per gram with a standard error of 3.5%.

The data presented in Table III were also subjected to the chi-square test and proved to be homogeneous. The weighted, mean biological potency is 18,400 I.U. per pound with a standard error of 630 units (3.4%). This average of the bioassays is in close agreement with the average colorimetric value of 18,350 I.U. per pound for vitamin A and carotene. By subtracting the average colorimetric vitamin A assay (12,420 I.U. per pound) from the average biological potency of vitamin A and β-carotene, a value of 5,980 I.U. per pound is obtained for the biological potency of the carotene (3,560 micrograms or 5,930 I.U. as determined colorimetrically). This corresponds to 1,680,000 I.U. per gram of β-carotene. This value, although only an approximation because of the lack of comparative colorimetric and bioassay values for the vitamin A present, nevertheless is practically the same as the average value found in assays of carotene only.

In a recent report to the Institute of Food Technologists, Espoy (23) stated that bioassays of β-carotene yielded higher activity than the International Standard value, namely, that only 0.40 to 0.45 microgram rather than 0.60 microgram is required to supply 1 I.U. of vitamin A activity. As detailed previously, the conditions of the biological test have a determining influence on the potency of carotene relative to vitamin A. There is, as yet, no general agreement on this subject. Hence at present there would appear to be no compelling reason for changing the present relation as defined by the International Standards.

STABILITY OF SYNTHETIC β-CAROTENE

1. *In Vegetable Oil Suspensions.* Crystalline β-carotene, while insoluble in water, is not soluble to a great extent in oil. Solubility of β-carotene in vegetable oils approximates 0.08%. However, to obtain a proper color in margarine, a solubility of only 0.0008% (5,000 to 6,000 I.U. of vitamin A activity per pound) is required. Hence oil solubility is ample for practical usage.

In the shipment and storage of the β-carotene vitamin A vegetable-oil concentrates in sealed cans as used in the United States for coloring and fortifying a batch of margarine, it is desirable that the concentrates be held to a small volume. Hence the carotene concentration greatly exceeds the solubility of carotene in the oil of the concentrate, and the concentrates consist of suspensions of fine carotene crystals in a solution of vitamin A in vegetable oil. The stability of β-carotene in these β-carotene, vitamin A, vegetable-oil concentrates in hermetically sealed, tin-plated, sanitary cans is of interest.

An experimental batch of 75-g. cans was set up, one half of which contained synthetic β-carotene and vitamin A acetate in cottonseed oil without antioxidant addition (regular); the other half was prepared with added α-tocopherol (experimental). Samples were submitted for carotene assay after canning and after storage. The results of these tests are summarized in Table IV. The data illustrate the excellent stability of carotene in the β-carotene, vitamin A, vegetable-oil concentrates used commercially for the simultaneous coloring and fortification of margarine.

TABLE IV

Stability of β-Carotene in Carotene, Vitamin A, and Vegetable-Oil Concentrates in Hermetically Sealed Cans

	Regular (no antioxidant)		Experimental (tocopherol added)	
	(mg. carotene/g.)	*(I.U./g.)*	*(mg. carotene/g.)*	*(I.U./g.)*
After canning	14.2	23,700	14.2	23,700
42 days, 113°F.	14.2	23,700	14.2	23,700
3 months, 75°F.	14.2	23,700	14.3	23,800
3 months, 86°F.	14.3	23,800	14.1	23,500
3 months, 98°F.	14.2	23,700	14.2	23,700

2. *In Margarine.* Margarines fortified with vitamin A and β-carotene by the manufacturer were stored for 2 and 6 months at 40°F. and 75°F., respectively. Samples were also stored for similar periods but alternately at 40°F. and 75°F. every 24 hrs. Vitamin A and carotene assays were carried out by colorimetric methods as described previously. The stability data for both substances are summarized in Table V. In all cases the stability of both vitamin A and β-carotene is excellent. Average losses for the β-carotene are only slightly greater than for vitamin A. Melnick *et al.* (24) also found by physico-chemical assays slightly greater losses for β-carotene than for vitamin A in margarine and excellent stability of both compounds in margarine. Deuel and Greenberg (25) confirmed the excellent stability of vitamin A in margarine by means of rat bioassays. The present results confirm the findings of these authors that both vitamin A and β-carotene are almost completely stable in margarine for the normal shelf-life of the product.

Summary

Samples of synthetic β-carotene have been assayed for vitamin A activity by the rat-curative, growth method against vitamin A acetate and compared with natural carotene. The U.S.P. XIV diet was modified by the addition of vitamin B_{12} and α-tocopherol, which have been reported to enhance carotene utilization. Doses of vitamin A and carotene were given in cottonseed oil and in margarine; but, contrary to the report of Deuel *et al.* (13), no significant increase was observed in the utilization of carotene fed in margarine. The samples tested include crystalline

TABLE V

Retention of Vitamin A and β-Carotene in Commercial Margarine

Manufacturer	Form of vitamin A	Initial assay	After 2 Months' Storage			After 6 Months' Storage		
			40°F.	40°F. & 75°F.	75°F	40°F.	40°F. & 75°F.	75°F
			(U.S.P. Units of Vitamin A Activity per Gram)					
J	Vitamin A	13,900	12,000 (86)	12,000 (86)	11,300 (81)	14,700 (106)	13,500 (97)	13,600 (98)
	Carotene	6,400	6,700 (105)	6,300 (98)	6,000 (94)	6,100 (95)	5,900 (92)	5,500 (86)
	Vitamin A	13,300	13,100 (99)	13,300 (100)	13,100 (99)	.....	.....	.....
	Carotene	6,100	5,900 (97)	5,900 (97)	5,900 (97)	.....	.....	.....
K	Vitamin A	13,800	14,400 (104)	13,500 (98)	13,800 (100)	.....	.....	.....
	Carotene	5,300	5,200 (98)	5,100 (96)	4,900 (93)	.....	.....	.....
	Vitamin A	12,300	13,100 (107)	12,700 (103)	12,300 (100)	.....	.....	.....
	Carotene	5,300	5,100 (96)	5,100 (96)	4,900 (93)	.....	.....	.....
L	Carotene	6,100	.....	.....	.....	6,100 (100)	5,700 (93)	5,200 (85)
M	Vitamin A	11,000	.....	.....	.....	10,600 (96)	.....	10,100 (92)
	Carotene	5,200	.....	.....	.....	4,800 (92)	.....	4,800 (92)
	Vitamin A	10,300	.....	.....	.....	10,600 (103)	.....	10,400 (101)
	Carotene	5,200	.....	.....	.....	5,000 (96)	.....	5,300 (102)
	Vitamin A	13,400	13,400 (100)	13,400 (100)	13,800 (103)	13,100 (98)	12,800 (94)	.....
	Carotene	5,200	5,050 (96)	5,200 (100)	4,850 (92)	5,300 (102)	4,700 (91)	.....
N	Vitamin A	13,350	.....	.....	.....	11,900 (89)	12,000 (90)	11,100 (83)
	Carotene	5,950	.....	.....	.....	5,400 (91)	5,400 (91)	5,300 (89)
	Vitamin A	12,700	13,000 (102)	12,500 (98)	11,800 (93)	13,600 (107)	12,800 (101)	11,800 (93)
	Carotene	6,400	6,300 (98)	5,800 (91)	5,800 (91)	6,700 (105)	6,300 (98)	5,300 (83)
O	Vitamin A	14,200	13,900 (98)	12,800 (90)	13,200 (93)	13,400 (94)	13,700 (97)	12,700 (89)
	Carotene	5,850	5,900 (101)	6,100 (104)	5,800 (99)	5,900 (101)	5,800 (99)	5,500 (94)
	Vitamin A	13,500	13,100 (97)	12,300 (91)	12,600 (93)	14,200 (103)	13,500 (100)	12,800 (95)
	Carotene	6,050	6,200 (103)	6,200 (103)	5,900 (98)	5,700 (94)	5,100 (84)	5,200 (86)
	Vitamin A	11,800	11,600 (98)	11,000 (93)	10,500 (89)	12,000 (102)	12,800 (108)	10,900 (92)
P	Carotene	5,750	5,700 (99)	5,600 (97)	5,400 (94)	5,600 (97)	5,500 (96)	4,900 (85)
	Vitamin A	13,500	13,000 (96)	13,800 (102)	13,800 (102)	12,400 (92)	12,800 (95)	11,500 (85)
	Carotene	6,000	5,700 (95)	5,700 (95)	5,400 (90)	6,100 (102)	5,900 (98)	.....
	Vitamin A	11,800	11,400 (97)	12,000 (102)	11,600 (98)	12,000 (102)	11,800 (100)	.....
	Carotene	5,550	5,300 (96)	5,200 (94)	5,100 (92)	5,700 (103)	5,500 (99)	5,200 (94)
	Vitamin A	12,300	12,000 (98)	13,100 (107)	12,300 (100)	12,100 (98)	12,100 (98)	12,300 (100)
	Carotene	5,050	4,900 (97)	4,900 (97)	4,800 (95)	5,400 (107)	5,050 (100)	4,500 (89)
Q	Vitamin A	12,400	12,500 (101)	12,600 (102)	13,100 (106)	12,100 (98)	11,700 (95)	10,900 (88)
	Carotene	5,500	5,600 (102)	5,600 (102)	5,400 (98)	5,300 (96)	5,200 (95)	4,700 (86)
	Vitamin A	13,000	12,900 (99)	14,200 (109)	11,400 (87)	11,500 (88)	12,600 (97)	11,300 (87)
	Carotene	6,500	6,400 (99)	6,300 (97)	5,900 (91)	6,300 (97)	6,200 (95)	5,400 (83)
	Average							
	Vitamin A		(99)	(99)	(96)	(98)	(98)	(92)
	Carotene		(99)	(98)	(94)	(99)	(95)	(89)

NOTE: Figures in parentheses are % retention based on initial assay value.

all-*trans* β-carotene, micropulverized all-*trans* β-carotene in an oil suspension, and a series of 10 commercial margarines fortified with vitamin A. and carotene in a ratio of about 2 I.U. of vitamin A to 1 I.U. of carotene.

In terms of vitamin A activity in the rat bioassay, the average potency of β-carotene in three separate bioassays of crystalline carotene was found to be 1,730,000 I.U. per gram with a standard error of ±3.5%. Thus in these assays 1 I.U. of vitamin A activity was found to be equivalent to 0.58 mcg. of all-*trans* β-carotene, a value in essential agreement with 0.6 mcg., the presently accepted International Standard. For margarine samples containing vitamin A and β-carotene, the average vitamin A activity in 2 bioassays was found to be very close to that calculated from the colorimetric assays, using the factor for β-carotene, 0.6 mcg. = 1 I.U. The fact that other workers have reported higher provitamin A activity for β-carotene in the rat bioassay indicates the dependence of the results on the particular conditions of the bioassay.

The stability of vitamin A and β-carotene in commercially prepared margarines stored at 40°F. and 75°F. was studied by accepted colorimetric procedures. Average retention values of 94% or better were obtained in margarines stored two months when the vitamin activity was supplied either from β-carotene or from vitamin A.

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[Received October 12, 1956]