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DIVISION OF CHEMISTRY

The Relation of the Spectro Vitamin A and Carotene Content of Butter to its Vitamin A Potency Measured by Biological Methods

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS
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The estimation of the vitamin A potency of a feed by the usual method of feeding it to rats is a long and expensive process. For this reason, chemical methods are also used. It is highly desirable to know the relation between the results obtained by the chemical methods and by the rat methods, so that the results secured by the much shorter and less expensive chemical methods can be interpreted in terms of biological units. From the determination of the biological potency, of carotene, and of spectro-vitamin A in 32 samples of butter, it was found that the number of Sherman-Munsell units calculated from the chemical analyses, by use of one equation, differed in 21 samples by 4 units or less from the number of Sherman-Munsell units actually found by feeding the samples to rats. In 11 additional samples of butter fat the differences were greater than 4 units but most of them within reasonable agreement with what could be expected. There were three samples for which the vitamin A potency calculated from the analyses was much higher than the potency found by biological methods. Equally good agreement was found with another equation. Both the equations closely express the relation between the carotene and spectro-vitamin A as determined by chemical analyses and its vitamin A potency as measured by the Sherman-Munsell method, but there are some exceptions, and this must be remembered when making an interpretation of such analyses. Moreover, in making the analyses of the butter, contact with rubber or cork may give too high results. Equations to show the relation between the carotene and spectro-vitamin A and International units of vitamin A potency are also given. A number of analyses of butter given by other workers are calculated to Sherman-Munsell units of vitamin A and also to International units.

A unit of vitamin A in butter, determined chemically, is apparently more efficient biologically than a unit of vitamin A in cod liver oil determined in the same way.

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BULLETIN NO. 560 FEBRUARY, 1938

THE RELATION OF THE SPECTRO-VITAMIN A AND CAROTENE CONTENT OF BUTTER TO ITS VITAMIN A POTENCY MEASURED BY BIOLOGICAL METHODS

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Butter is a valuable food and contains vitamin A. The vitamin A potency of butter has been found to depend upon the quantity of vitamin A stored by the cow, the quantity of vitamin A in the daily ration of the cow and, when the vitamin A in the daily ration is insufficient, the time elapsed since this ration was first fed. The vitamin A potency of butter has been chiefly determined by biological methods, in which rats were used. Such methods require a depletion period of several weeks, followed by a period of 5 to 8 weeks in which the butter to be tested is fed. Biological methods for vitamin A therefore require considerable time and equipment, and are expensive. The results are usually expressed in terms of Sherman-Munsell units per gram or International units per gram.

The vitamin A potency of butter is due partly to vitamin A itself and partly to carotene (2, 16). Carotene can be used by the animal for the same purpose as vitamin A or converted into vitamin A in the animal body. Carotene can be determined by chemical methods (15, 16). Vitamin A may be determined by measuring the intensity of the blue color when solutions of antimony trichloride are brought in contact with vitamin A, or by measuring the quantity of light absorbed at 328 millimicrons when the light is passed through a solution containing the vitamin A (2, 10). Unfortunately, other substances besides vitamin A produce the blue color with antimony in chloride or absorb light at 328 $m\mu$. In spite of this disadvantage both methods have been extensively use. This bulletin deals only with the method which uses the absorption of light.

The chemical determinations of carotene and vitamin A are much more rapid and less expensive than the determination of vitamin A potency by biological methods. For this reason, and because these methods are being used, it is desirable to know the relation between the results of the chemical and the biological methods of analysis. We need to know by what procedure and with what degree of accuracy we can calculate International units or Sherman-Munsell units of vitamin A potency from the results of the estimation of vitamin A and carotene by chemical methods. It is the object of this publication to discuss these relations.

The absorption of light by vitamin A may be measured by means of a spectrograph equipped with a photometer, or in case of cod liver oil by means of a special instrument called a vitameter. The estimation of carotene and vitamin A in butter by the spectrographic method has been made by Gillam et al. (10, 11, 17) in England, Baumann et al. (2, 3) in Wisconsin, and by workers in this laboratory (19). Baumann and Steenbock (2) com-

pared their results by the spectrographic method with results by the biological method on butters believed to be similar, and estimated that 20 micrograms of vitamin A are equivalent to 66 biological units.

The spectrographic method or the vitameter is extensively used for the estimation of vitamin A in cod liver oils, other fish liver oils, and their concentrates. Altho the method is rapid and convenient, the relation between the vitamin A so determined and the International units of vitamin A have not yet been agreed upon. Biological methods are still the official methods of the U. S. Pharmaceutical Association. According to Barthen and Leonard (1), sufficient data are presented by them to warrant the adoption (by the U. S. P.) of a spectrophotometric method for the vitamin A content of cod and other fish liver oils as an alternate for the biological assay. According to these workers, and also according to Holmes et al. (12) and Wilkins (18), each worker should standardize his vitameter by means of U. S. P. reference cod-liver oil.

Samples and Methods Used

The samples of butter used had been collected by O. C. Copeland of the Division of Dairy Husbandry for work published in Texas Bulletin 536 (9), and the vitamin A potency was estimated by means of rats, by Mr. Ray Treichler, using the method previously described (4, 6, 8, 9). Fourteen samples of the butter had been secured at various intervals from two cows which had first been depleted in vitamin A and then placed upon good grass pasture. Eighteen samples were composite samples of butter from 3 groups of 3 cows, each of which had been fed on a good dairy feed and received its vitamin A from yellow corn or yellow corn and alfalfa leaf meal. The vitamin A potency fed to the cows averaged 7,000, 170,000 and 340,000 Sherman-Munsell units per day respectively. The vitamin A potency of the butters from these cows ranged from 4 to 62 Sherman-Munsell units per gram. Carotene and vitamin A were estimated as described below. Eleven other butters were used later.

The carotene in the butter fat was estimated with the aid of a Keuffel and Esser spectrophotometer. The melted fat was placed in a 1 cm. tube and the density at 470 and 480 millimicrons read against an empty tube. The readings were made by Miss Mary Anna Grimes of the Division of Rural Home Research. The amount of carotene was calculated by multiplying the density by appropriate factors, which had previously been worked out for pure carotene dissolved in butter fat. This method has already been described in full (16).

The Spectrographic Method for Vitamin A in Butter

For convenience, the name "spectro-vitamin" is given to vitamin A estimated by means of the spectrograph. The method used was based upon the procedure reported by Gillam (10, 11) and also used by Baumann and Steenbock (2), which requires the determination of the amount of light absorbed at 328 millimicrons by the unsaponifiable residue dissolved in methanol. The apparatus used was provided with interchangeable ground

glass joints, and all necessary seals were made with gypsum. Five grams of butter, in a 300 cc. flask attached to a condenser by a No. 20 interchangeable glass joint, were refluxed in a stream of nitrogen for 30 minutes with 50 cc. of aldehyde-free 12% alcoholic potash. Fifty cc. of water were then added and the mixture cooled to 4° C. and transferred to a 1 liter pear-shaped separatory funnel. Then 50 cc. of ether, and next 150 cc. of cold water were added. The ether layer was then drawn off and the aqueous alcoholic fraction extracted 3 more times with 15 cc. portions of ether. The combined ether solutions were washed repeatedly with cold water until free from alkali, dried over anhydrous sodium sulphate, and placed immediately in a 300 cc. flask attached to a Claissen distillation tube by means of a No. 20 interchangeable joint. The side tube of this Claissen tube was connected to an ordinary condenser to which was attached a side-neck flask, connected to a vacuum pump. The ether was distilled off in nitrogen with reduced pressure. The residue was taken up in as small amount as possible of absolute methanol, and the impurities crystallized out by cooling for 2 hours at —8° C. with an ice-salt mixture. The cold solution was filtered, the residue washed with cold methanol, the filtrate made up to 10 cc. and preliminary photographs made at several settings of the photometer. If necessary, the solution was diluted, and photographs made at several more photometer settings. The photographs were made with a density between 0.6 and 1.1 at 328 millimicrons with a depth of 1 or 2 cm. If the density was too low, 10 gm. of butter were used in a second determination, with the use of corresponding larger quantities of the reagents.

The absorption spectra were photographed through a Bausch and Lomb medium quartz spectrograph equipped with a photometer reading in density, and a silver electrode. A solution of the same reagents made in a manner similar to that of the vitamin A solution was used in the comparison tube.

The methanol solution of the unsaponifiable matter contained some carotene. Although its maximum absorption is at about 450 millimicrons, carotene absorbs light to some extent at 328 millimicrons. Since no data to correct for this absorption were available to us, Table 1 was prepared. Solutions of purified carotene in methanol were assayed for carotene by comparing them colorimetrically against 0.1 per cent potassium bichromate, and the absorption at 328 millimicrons was determined in the same solution with the spectrograph. The amount of carotene in the solution of unsaponifiable material from the butter being tested was estimated by colorimetric comparison against the bichromate. The correction in density for carotene was read from Table 1 and subtracted from the density obtained on the butter solution.

Table 1. Density (D) of carotene in methanol at 328 mu. with 2 cm. depth of absorbing liquid

Carotene, parts per million	Correction of D	Carotene, parts per million	Correction of D
1	0.03	11	0.41
2	0.06	12	0.46
3	0.10	13	0.50
4	0.13	14	0.55
5	0.16	15	0.60
6	0.20	16	0.65
7	0.24	17	0.70
8	0.28	18	0.75
9	0.32	19	0.80
10	0.36		

The spectro-vitamin A content in parts per million of the butters was calculated from the density corrected for carotene by the method used by Baumann and Steenbock (2). The Beer Lambert equation $E = 1/cd\ D$ was used, in which E is the extinction coefficient, d the depth of the absorption cell, c the concentration of vitamin A, and D the density of absorption. The value of 1600 of Carr and Jewell, was used for E, since this value was also used by Baumann and Steenbock and by Gillam. Since 1600 is the density of a 1% solution of the pure vitamin A in a 1 cm. absorption cell, a solution containing 1 part per million would have a density of $1600 \times .0001$ or 0.16. Thus, a solution in a 1 cm. cell having a density of 1.0 would contain 6.25 parts per million of vitamin A. This gives a very simple method of calculating the results.

Holmes and Corbet (12A) have recently prepared a crystalline preparation of vitamin A much stronger than the above, with an extinction value of 2100 instead of 1600.

The method we used for preparing the unsaponifiable residue is similar to those used by most of the previous workers. However, some workers used chloroform instead of methanol to dissolve the unsaponifiable residue for the final analysis. Our work shows that vitamin A is very stable in the methanol and quite unstable in chloroform. Table 2 shows that at room

Table 2. Stability of vitamin A in chloroform and in methanol at room temperature

Mg. vitamin A concentrate in 100 cc.	Density at 328 at start	Density at 328 after standing 7 days	Loss of vitamin A per cent
In chloroform 0.80	1.1	.7	36
0.64	0.9	.4	55
0.48	0.7	.3	55
0.32	0.6	.2	67
0.16	0.3	.1	70
In methanol 0.80	1.1	1.1	0
0.64	.9	.9	0
0.48	.7	.7	0
0.32	.4	.4	0
0.16	.3	.3	0

temperature a chloroform solution containing 0.8 milligrams of a vitamin A concentrate per 100 cc., lost 36 per cent of the vitamin in a week, and even

greater amounts in more dilute solutions, while a methanol solution lost none in 7 days. Cooling the methanol solution to —8° C. with an ice-salt mixture, is necessary in order to remove some materials other than vitamin A that absorb light at 328 mμ. The unsaponifiable residue from 10 gram samples of butter was dissolved in 10 cc. methanol and cooled to —8° C. The precipitates were dissolved in 10 cc. of methanol and had densities varying from 0.2 to 0.6 for a 2 cm. depth. In a number of tests, no additional material which absorbed at 328 mμ could be removed by a subsequent cooling to a temperature as low as —72°. The butter may still contain material not vitamin A which absorbs light at 328 millimicrons. Baumann and Steenbock (2) discarded all analyses of butter in which the absorption at 280 exceeded that at 328 millimicrons.

Very large quantities of material absorbing at 328 mμ could be dissolved by alcohol or ether from either cork stoppers or rubber stoppers. The density obtained in some experiments with cork and rubber was as much as 1.2.

Some samples of methyl alcohol, ethyl alcohol, and ethyl ether contained practically no material absorbing at 328 millimicrons but other samples contained appreciable amounts. The reagents should be tested and purified by distillation if necessary. Solvents that have come in contact with cork or rubber require purification. The ether and alcohols should also be free from aldehydes or peroxides, as has been pointed out by Baumann and Steenbock (2).

Values for the density ranging from 0.6 to 1.1 were found to be the most desirable to use when photographing the absorption line. Values above 1.1 required such a long exposure that they were impracticable. When the values were below 0.6 the percentage of error of reading increased.

Little or no destruction of spectro-vitamin A was found to occur. Several tests with known quantities of vitamin A gave recoveries of 91% to 100%. Baumann and Steenbock (2) likewise found no destruction of vitamin A to occur.

The Relation of Units of Vitamin A Potency to the Parts Per Million Of Vitamin A and Carotene

As a basis for equations which show the relation of spectro-vitamin A and carotene to vitamin A potency, we have the estimations of vitamin A potency with rats by the Sherman-Munsell method, and the estimations of the carotene, and the spectro-vitamin A, in 32 samples of butter, as given in Tables 3 and 4. According to our previous results (8), 1 microgram of carotene dissolved in Wesson oil is equal to 1.4 Sherman-Munsell rat units. In the calculations presented here, it is assumed that carotene in butter has this value, though there is some evidence (10) that a small part (6%) of the supposed carotene is xanthophyll, and also that carotene in butter may have a higher vitamin A potency than carotene in oil (15). If one part per million of carotene is equal to 1.4 Sherman-Munsell units of vitamin A potency per gram, in order to find the number of Sherman-Munsell rat units due to vitamin A, the parts per million of carotene should be multi-

Table 3. Vitamin A potency in Sherman-Munsell units, spectro-vitamin A and carotene in butter fat from depleted cows placed on pasture

Days on pasture	Carotene. Parts per million	Vit. A potency by biological tests Sherman-Munsell units per gram	Vit. A potency as calculated due to carotene Sherman-Munsell units per gram	Vit. A potency as calculated due to vit. A Sherman-Munsell units per gram	Spectro-vit. A by analysis. Parts per million	Units vit. A potency equal to one microgram of spectro-vitamin A
Cow 301						
0	0.53	12	.7	11.3	2.1	5.38
1	0.78	20	1.1	18.9	3.7	5.11
2	3.03	30	4.2	25.8	6.5	3.97
3	3.73	50	5.2	44.8	5.5	8.15
4	4.79	40	6.7	33.3	5.8	5.74
7	6.13	33	8.6	24.4	3.3	7.39
14	8.03	40	11.2	28.8	4.6	6.26
Cow 311						
0	0.44	12	.6	11.4	2.7	4.22
1	0.84	18	1.2	16.8	3.6	4.67
2	2.43	40	3.4	36.6	5.1	7.17
3	4.43	40	6.2	33.8	5.5	6.15
4	6.09	40	8.5	31.5	6.0	5.25
7	7.45	25	10.4	14.6	3.3	4.42
14	9.94	40	13.9	26.1	5.3	4.92
Average of 5 underscored						7.02

Table 4. Vitamin A potency in Sherman-Munsell units, spectro-vitamin A and carotene in butter from cows on yellow corn and alfalfa leaf meal

Weeks on experiment	Carotene. Parts per million	Vit. A potency by biological test Sherman-Munsell units per gram	Vit. A potency as calculated due to carotene Sherman-Munsell units per gram	Vit. A potency as calculated due to vit. A Sherman-Munsell units per gram	Spectro-vit. A by analysis. Parts per million	Units vit. A potency equal to one microgram of spectro-vitamin A
Fed yellow corn						
0	16.69	43	23.4	19.6	9.1	2.15
1	7.87	33	11.0	22.0	3.5	6.29
5	2.04	14	2.9	11.1	2.6	4.27
9	1.13	10	1.6	8.4	2.5	3.36
13	0.76	7	1.1	5.9	1.7	3.47
17	0.36	4	.5	3.5	1.5	2.33
Fed yellow corn and alfalfa leaf meal						
0	9.71	50	13.6	36.4	7.1	5.13
1	10.46	33	14.6	18.4	3.8	4.84
5	5.55	20	7.8	12.2	3.1	3.94
9	4.06	25	5.7	19.3	3.2	6.03
13	3.46	14	4.8	9.2	2.6	3.54
17	3.92	12	5.5	6.5	2.7	2.41
Fed yellow corn and alfalfa leaf meal						
0	9.82	62	13.7	48.3	7.0	6.90
1	9.50	43	13.3	29.7	4.2	7.07
5	5.07	28	7.1	20.9	3.1	6.74
9	4.53	23	6.3	16.7	3.3	5.06
13	4.94	20	6.9	13.1	2.9	4.52
17	3.58	10	5.0	5.0	3.1	1.61
Average of the 5 underscored						6.61

plied by 1.4 and the product subtracted from the total Sherman-Munsell units. The difference is the potency due to vitamin A.

If we assume that all the spectro-vitamin A is actually vitamin A and divide the number of Sherman-Munsell units due to vitamin A by the parts per million of spectro-vitamin A, the values given in the last columns of Tables 3 and 4 are secured. One unit of spectro-vitamin A is equal to from 3.97 to 8.15 Sherman-Munsell units for the butters in Table 3 and from 1.61 to 7.07 units for those listed in Table 4. This is a wide variation.

The samples were next arranged in 3 groups according to their spectro-vitamin A content. In 16 samples containing from 1.5 to 3.3 parts per million, one part per million of spectro-vitamin A equals on the average 4.3 Sherman-Munsell units per gram. In 6 samples containing from 3.5 to 4.6 parts per million, one part per million spectro-vitamin A averages 5.7 Sherman-Munsell units per gram. In the remaining 10 samples containing from 5.1 to 9.1 parts per million of spectro-vitamin A, one part per million spectro-vitamin A averages 5.5 Sherman-Munsell units per gram. The average value of the spectro-vitamin A in terms of Sherman-Munsell units is lower with the group containing less than 3.3 parts per million than with the other two groups. The variations of the factors between the samples within each group is less than when all the samples are considered in one group.

The relation discussed above may be expressed by the following equation:

$$\text{Equation A: } U = D\,S + 1.4\,C.$$

Here U is the Sherman-Munsell units per gram, S the spectro-vitamin A in parts per million, C the carotene in parts per million, and D is 4.3 when the spectro-vitamin A is 3.4 parts per million or less, or 5.6 when it is more than 3.4 parts per million. This method of calculating Sherman-Munsell units of vitamin A from the chemical value will be compared with a method described below.

Another method of expressing the relation between the spectro-vitamin A and the biological vitamin A potency is based on the following considerations. If vitamin A is considered to be a single chemical compound, the highest values in terms of Sherman-Munsell units should represent the pure compound, while the lower values given in Tables 4 and 5 may be due to the presence of substances which absorb light at the same wave length but have no vitamin A potency. It has already been shown that such substances may be present. These highest values are underscored. When the five highest values in Table 4 are averaged, one part per million of spectro-vitamin A equals 6.6 Sherman-Munsell units of vitamin A potency per gram. When the 5 highest in Table 3 are averaged, one unit of spectro-vitamin A potency is equal to 7.0 Sherman-Munsell units. These two averages are remarkably close considering the different sources of the butter from which they are derived.

From the above considerations we assume that one unit of pure vitamin A equals 6.8 Sherman-Munsell units, and that lower values are due to the presence of pseudo-vitamin A. The vitamin A was calculated for all the samples from the vitamin A potency in Sherman-Munsell units after deduc-

tion of that part assumed to be due to carotene. This was done by dividing the number of Sherman-Munsell units by 6.8. The pure vitamin A so calculated was subtracted from the amount found by actual analysis and the difference is assumed to be due to substances that absorb light at 328 millimicrons other than vitamin A, which for the sake of brevity and also because it is calculated by the same factors used for vitamin A, we will call pseudo-vitamin A. The pseudo-vitamin A so calculated for the various samples of butter is given in Table 5.

The pseudo-vitamin A given in Table 5 ranges from 6.2 to —1.1 parts per million in the 32 samples, the minus value being due to errors in the analytical or biological analysis. Excluding the unusually high value of 6.2, the range is from 2.7 to —1.1, while the average variation for the 31 samples (from the algebraic mean) is 0.84. The average of the 5 groups taken separately, range from 0.58 to 1.23. There is more regularity in the values within group 2 and group 5 than in the other groups. Since there are only 5 minus values in the 32 samples, it is evident that subtraction of the average value of the pseudo-vitamin A from the total spectro-vitamin A will increase the accuracy of the calculation for 27 of the butters. The Sherman-Munsell units may be calculated by the equation:

Equation B: $U=(S-0.8)\ 6.8+1.4C$.

Here U is the Sherman-Munsell units per gram, S the spectro-vitamin A in parts per million, and C the carotene in parts per million.

Relation of Vitamin A Potency Calculated From the Chemical Analyses to the Potency Found by the Biological Tests

Two equations A and B express the relation of the chemically estimated carotene and spectro-vitamin A to the biological vitamin A potency. Equation A uses two factors; one derived from the average relation between the spectro-vitamin A when it is 3.4 parts per million or lower, and the biological vitamin A potency, and other factor from the relation of the spectro-vitamin A when it is higher than 3.4 parts per million. The other, Equation B, is based on the assumption that one unit of pure vitamin A is equal to 6.8 Sherman-Munsell units. Correction is made for impurities (pseudo-vitamin A) by subtraction of the average value 0.8 from the spectro-vitamin A. The spectro-vitamin A and carotene in samples of butter have been calculated to Sherman-Munsell units of vitamin A potency by these two equations, and the results are compared with the Sherman-Munsell units obtained by the biological tests with rats in Tables 6 and 7.

When comparing the calculated results with those found biologically, it is well to remember that the biological methods for estimating vitamin A are not highly accurate. An error of 10 to 20 per cent may be expected and even greater deviations may occur. The calculations were carried to two decimal places, but the calculated potency is put down in whole numbers, since the biological estimation is not accurate to even this extent.

Table 5. Calculated pseudo-vitamin A in butter (parts per million)

Laboratory Number	Spectro-vit. A calculated from Sherman-Munsell units of vitamin A	Spectro-vit. A by analysis	Pseudo-vit. A (by difference)
Group 1			
40930	2.9	9.1	6.2
40961	3.2	3.5	.3
41131	1.6	2.6	1.0
41256	1.2	2.5	1.3
41299	.9	1.7	.8
41384	.5	1.5	1.0
Average			1.77
Average, first sample excluded			0.88
Group 2			
40921	5.4	7.1	1.7
40951	2.7	3.8	1.1
41124	1.8	3.1	1.3
41231	2.8	3.2	.4
41265	1.4	2.6	1.2
41362	1.0	2.7	1.7
Average			1.23
Group 3			
40952	7.1	7.0	—.01
40955	4.4	4.2	—.2
41132	3.1	3.1	0
41218	2.5	3.3	.8
41282	1.9	2.9	1.0
41385	.7	3.1	2.4
Average			.65
Group 4			
39124	1.7	2.1	.4
39127	2.8	3.7	.9
39128	3.8	6.5	2.7
39131	6.6	5.5	—1.1
39132	4.9	5.8	.9
39134	3.6	3.3	— .3
39138	4.2	4.6	.4
Average			.56
Group 5			
39125	1.7	2.7	1.0
39126	2.5	3.6	1.1
39129	5.4	5.1	— .3
39130	5.0	5.5	.5
39133	4.6	6.0	1.4
39135	2.1	3.3	1.2
39139	3.8	5.3	1.5
Average for group			.91
Average of all			1.01
Average of all, first sample excluded			0.84

Table 6. Vitamin A in Sherman-Munsell units per gram as found by biological tests of butter and as calculated from the analyses by Equation A and Equation B

Laboratory number	Date samples collected for analyses	Calculated from spectro A		Calculated from carotene units	Total calc. vit. A potency		Vit. A potency found units	Difference between calc. and found	
		Equation B	Equation A		Equation B	Equation A		Equation B	Equation A
Group 4	Cow 301, depleted and placed on grass pasture Nov. 20.								
39124	Nov. 20, 1933	8.8	9.0	.7	10	10	12	2	2
39127	Nov. 22, 1933	19.7	20.7	1.1	21	22	20	—1	—2
39128	Nov. 28, 1933	38.8	36.4	4.2	43	41	30	—13	—11
39131	Nov. 24, 1933	32.0	30.8	5.2	37	36	50	13	14
39132	Nov. 25, 1933	34.0	32.5	6.7	41	39	40	—1	1
39134	Nov. 28, 1933	17.0	14.2	8.6	26	23	33	7	10
39138	Dec. 5, 1933	25.8	25.8	11.2	37	37	40	3	3
Group 5	Cow 311, depleted and placed on grass pasture Nov. 20.								
39125	Nov. 20, 1933	12.9	11.6	.6	14	12	12	—2	0
39126	Nov. 22, 1933	19.0	20.2	1.2	20	21	18	—2	—3
39129	Nov. 23, 1933	29.2	28.6	3.4	33	32	40	7	8
39130	Nov. 24, 1933	32.0	30.8	6.2	38	37	40	2	3
39133	Nov. 25, 1933	35.4	33.6	8.5	44	42	40	—4	—2
39135	Nov. 28, 1933	17.0	14.2	10.4	27	25	25	—2	0
39139	Dec. 5, 1933	30.6	29.7	13.9	45	44	40	—5	—4

Table 7. Vitamin A in Sherman-Munsell units per gram as found by biological tests of butter and as calculated from the analyses by Equation A and by Equation B

Lab. No.	Feed	Weeks on Exp.	Calculated from spectro-vit. A		Calculated from carotene	Total calculated vit. A potency		Vit. A potency found	Difference between calc. and found	
			Equation B	Equation A		Equation B	Equation A		Equation B	Equation A
Group 1										
40930	Yellow corn............	0	56.4	51.0	23.4	80	74	43	—37	—31
40961	Yellow corn............	1	18.4	19.6	11.0	29	31	33	4	2
41131	Yellow corn............	5	12.2	11.2	2.9	15	14	14	— 1	0
41256	Yellow corn............	9	11.6	10.8	1.6	13	12	10	— 3	— 2
41299	Yellow corn............	13	6.1	7.3	1.1	7	8	7	0	— 1
41384	Yellow corn............	17	4.8	6.5	.5	5	7	4	— 1	— 3
Group 2										
40921	Yellow corn and 3 pounds alfalfa meal...........	0	42.8	39.8	13.6	56	53	50	— 6	— 3
40951	Yellow corn and 3 pounds alfalfa meal...........	1	20.4	21.3	14.6	35	36	33	— 2	— 3
41124	Yellow corn and 3 pounds alfalfa meal...........	5	15.6	13.3	7.8	23	21	20	— 3	— 1
41231	Yellow corn and 3 pounds alfalfa meal...........	9	16.3	13.8	5.7	22	20	25	3	5
41265	Yellow corn and 3 pounds alfalfa meal...........	13	12.2	11.2	4.8	17	16	14	— 3	— 2
41362	Yellow corn and 3 pounds alfalfa meal...........	17	12.9	11.6	5.5	18	17	12	— 6	— 5
Group 3										
40952	Yellow corn and 6 pounds alfalfa leaf meal.......	0	42.2	39.2	13.7	56	53	62	6	9
40955	Yellow corn and 6 pounds alfalfa leaf meal.......	1	23.1	23.5	13.3	36	37	43	7	6
41132	Yellow corn and 6 pounds alfalfa leaf meal.......	5	15.6	13.3	7.1	23	20	28	5	8
41218	Yellow corn and 6 pounds alfalfa leaf meal.......	9	17.0	14.2	6.3	23	21	23	0	2
41282	Yellow corn and 6 pounds alfalfa leaf meal.......	13	14.3	12.5	6.9	21	19	20	— 1	1
41385	Yellow corn and 6 pounds alfalfa leaf meal.......	17	15.6	13.3	5.0	21	18	10	—11	— 8

By Equation A, 21 of the 32 calculated values differ 4 or less units from the units found, 6 differ 5 to 8 units, 4 differ 9 to 14 units, and 1 differs 31 units. By Equation B, 20 differ 4 units or less from the units found, 8 differ 5 to 8 units, 3 differ 10 to 14 units, and 1 differs 37 units. In a large percentage of the samples, the agreement is as close as could be expected. With about 12 per cent of the samples, the agreement is poor. The two methods of calculation give practically the same results.

Carotene, spectro-vitamin A, and Sherman-Munsell units were determined on 11 additional samples of butter not included in the 32 previously discussed. The data regarding these butters, and the relation between the Sherman-Munsell units calculated and those found, are given in Table 8. In this work, 5 of the 11 calculated values differ 4 or less units from the units found, 2 differ 6 to 7 units, 2 differ 10 to 14 units, and 2 differ 20 to 37 units by both methods of calculation. Some wide differences occur.

Table 8. Additional samples of butter vitamin A in Sherman-Munsell units per gram as found by biological tests of butter and as calculated by Equation A and Equation B

Lab. No.	Carotene parts per million	Spectro-vit. A parts per million	Sherman-Munsell units found per gram	Total calc. vit. A potency Sherman-Munsell units		Difference between calculated and found	
				Equation B	Equation A	Equation B	Equation A
42315	7.0	9.3	66	68	62	2	— 4
42316	2.2	5.7	33	36	35	3	2
42573	3.6	4.6	30	31	31	1	1
42766	19.1	11.8	67	101	93	34	26
42767	18.1	9.0	75	81	76	6	1
42768	15.2	7.9	56	70	66	14	10
42771	14.0	6.2	60	56	54	— 4	— 6
45261	6.4	3.9	18	30	31	12	13
45263	2.7	6.4	43	42	40	— 1	— 3
45270	4.3	8.7	30	67	55	37	25
45438	1.9	4.8	23	30	30	7	7

Equation A is slightly better than Equation B. Either of the equations may be used with a fair degree of accuracy to calculate the determinations of carotene and spectro-vitamin A to Sherman-Munsell units. While most of the units calculated from the chemical analyses can be expected to agree closely with the biological potency, a small proportion of the chemical analyses may give much too high values for the vitamin A potency. The occasional high values may be due to the presence of unusually high quantities of substances which absorb light at 328 millimicrons but are not vitamin A (pseudo-vitamin A), or due to the inaccuracy of the biological analysis. The quantity of these substances may depend upon the quantity in the feed of the cow, as well as upon other factors. Feeds contain substances of this kind. When the method previously described for vitamin A is applied to feeds containing no vitamin A, appreciable quantities of substances which absorb light at 328 millimicrons are secured. Some analyses of this kind (calculated to pseudo-vitamin A) are given in Table 9. Besides occurring in rubber and cork, pseudo-vitamin A occurs in corn, dried yeast, and other materials.

Table 9. Pseudo-vitamin A in some feeds as determined by method for vitamin A

Kind of feed	Pseudo-vitamin A parts per million
Corn bran	5.2
Corn, yellow	4.8
Cottonseed meal (43% protein)	3.1
Milo head chop	4.3
Oats	7.3
Rice bran	26.3
Rice polish	16.9
Wheat bran	3.8
Wheat gray shorts	4.4
Yeast	21.2

In our rat colony, one Sherman-Munsell unit of vitamin A has been found equal to 1.2 International units (8). In order to convert the Sherman-Munsell units into International units, it is, therefore, only necessary to multiply them by 1.2.

Equation A becomes

(C) $I\ U = 1.2\ (DS + 1.4C)$.

Equation B becomes

(D) $I\ U = (S - .8)\ 8.16 + 1.68C$.

Units of Vitamin A Potency of Butter Calculated From Analyses by Other Workers

The vitamin A potency in Sherman-Munsell units and International units of some analyses of butter reported by Baumann and Steenbock (3) and by Gillam et al. (10, 17) have been calculated by means of Equation B and the results are given in Tables 10 and 11. The calculated units seem rea-

Table 10. Vitamin A potency of butter fat, calculated from work of Baumann et al.

Breed and season	Carotene average parts per million	Spectro-vitamin A Average parts per million	Sherman-Munsell units per gram (calc.)	International units per gram (calc.)
Ayrshire, March samples, winter ration	4.8	8.4	58	70
Guernsey	10.3	6.8	55	66
Holstein	5.2	10.2	71	85
Jersey	7.1	7.1	53	64
Brown Swiss	6.0	7.8	56	67
Ayrshire, June samples, winter ration	4.9	6.9	48	58
Guernsey	7.8	5.1	40	48
Holstein	4.3	10.1	69	83
Jersey	5.5	5.3	38	46
Brown Swiss	5.6	6.8	49	59
Ayrshire, July samples, green ration	5.5	12.2	85	102
Guernsey	17.0	8.5	76	91
Holstein	6.6	15.1	106	127
Jersey	10.7	11.5	88	106
Brown Swiss	9.8	13.8	102	122

Table 11. Vitamin A potency calculated from analyses of Gillam et al.

	Carotene, parts per million	Spectro-vit. A parts per million	Sherman-Munsell units per gram (calc.)	International units per gram (calc.)
Winter average, stall fed				
Shorthorn	2.5	5.8	38	46
Ayrshire	2.7	6.6	43	52
Friesian	3.5	6.1	41	49
Guernsey	7.3	5.4	42	50
Summer average, grazing				
Shorthorn	2.9	8.4	56	67
Ayrshire	4.1	11.8	81	97
Friesian	4.7	12.1	83	100
Guernsey	11.4	9.5	75	90
Devon cows				
Control	.9	2.9	15	18
40 lbs. A. I. V. grass silage	3.0	5.5	36	43
70 lbs. A. I. V. grass silage	3.7	4.8	32	38
70 lbs. A. I. V. grass silage and 4 lbs. dried grass	4.3	5.5	38	46

sonable when compared with the units found in other samples by biological methods, and when the vitamin A potency of the feed eaten in each case is considered.

The calculated vitamin A potency of the July samples of butter fat of Baumann et al. (3) are quite high. The results may appear too high, but do not look unreasonable when it is considered that the cows received about 1,000 milligrams of carotene a day, equal to about 1,400,000 Sherman-Munsell units or 1,680,000 International units of vitamin A. The June samples were also from cows receiving high quantities of vitamin A (212 to 274 mg. carotene per day) and resemble those from Texas cows on pasture. The calculated vitamin A potencies of the butter fats reported by Gillam et al. are much lower than for the Wisconsin samples. The quantity of carotene fed is not known, but the results are in accordance with the known history of the samples.

Biological Value of Vitamin A of Butter Compared With That of Cod Liver Oil

It is interesting to compare the apparent biological value of spectro-vitamin A in cod liver oil with that in butter. According to Holmes et al. (12) the average "E value" of U. S. P. standard cod liver oil is 1.61. This oil thus contains about 1,006 parts per million of spectro-vitamin A (on the assumption of 1,600 as the E value of pure vitamin A) and as one gram contains 3,000 U. S. P. units, one microgram of spectro-vitamin A equals nearly 3 U. S. P. units of vitamin A potency. A similar value of 3 units per microgram is given for pure crystalline vitamin A (12A), although the biological value is less if the E value of 2,100 also given is taken into consideration. For butter, however, one unit of spectro-vitamin A may be equal to as much as 8 (Equation D) U. S. P. units of vitamin A potency. The significance of these differences remains to be ascertained. Perhaps butter contains a different vitamin A from that in cod liver oil.

SUMMARY

1. An improved method for the spectrographic estimation of vitamin A in butter fat is described.
2. Analyses of 32 samples of butter fat were used to develop two equations that show a definite relationship between the amount of vitamin A and carotene found by the spectrographic method, and the number of Sherman-Munsell rat units. These equations are:

   Equation A: $U=DS+1.4C$
   Equation B: $U=6.8\,(S-0.8)+1.4C$

   U is the number of Sherman-Munsell units per gram, S the spectro-vitamin A in parts per million, and C the carotene in parts per million. In Equation A, D is 4.3 when the spectro-vitamin A is less than 3.4 parts per million and 5.6 when it is 3.4 or more parts per million.

   Sherman-Munsell units can be converted into International units by multiplying by 1.2. Equation A then becomes

   (C): $U=1.2\,(DS+1.4C)$, and Equation B becomes
   (D): $IU=8.16\,(S-0.8)+1.68C$. IU is the number of International units.
3. In testing Equation B, it was found that with 21 out of the 32 samples of butter fat analyzed the calculated Sherman-Munsell units differed from the number of Sherman-Munsell units actually found by less than 4 units. Larger differences were found in the other 11 samples, but the agreement was as good as could be expected in all but 3 or 4 samples. In an additional 11 samples the agreement in most cases was also good. Similar results were found with Equation A.
4. Spectographic estimations of vitamin A in samples of butter fat analyzed by other laboratories in this country and by laboratories in England were calculated to International units and the results obtained are in reasonable agreement with what could be expected when the carotene in the feeds received by the cows is considered.
5. Vitamin A in butter may have a higher biological value than that in cod liver oil.

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