The recoveries obtained were entirely satisfactory (Table III) and the values determined chemically on 10 premixes and 18 broiler mashes agreed very well with those found by microbiological assay (*2*). Correlation coefficients of 0.904 and 0.971 were obtained between the microbiological and the chemical method for the feed samples and premixes respectively. Average values of 28.4 mg. per pound (range 24 to 34.3 mg. of niacin per pound) in the mashes and 2061.0 mg. per pound (range 1613 to 2322 mg. of niacin per pound) in the premixes were observed.

The method described employs a smaller sample than that suggested in the AOAC procedure (1 ounce). The potassium permanganate decolorization procedure gives extracts of lighter color than those of untreated samples, even after precipitation of protein. While the superiority of the sample blank employed in this procedure over that employed in the AOAC procedure may be questionable, it is the authors' experience that cyanogen bromide does contribute toward the blank. They have consequently preferred to use all reagents rather than to omit the cyanogen bromide reagent for the sample blank measurement.

The smaller quantities of sample employed, the smaller dilutions, and the greater stability of reagents, make this method adaptable to routine use.

Table III. Recovery of Niacin Added to Representative Feed and Premix Samples

Sample	Niacin in 2-G. Sample, γ	Niacin Added to 2-G. Sample, γ	Niacin Determined, γ	Recovery, %
Mixed feed	126	50	180.0	108.0
		100	230.0	94.0
		150	275.0	99.3
	112	50	160.0	96.0
		100	210.0	98.0
		150	264.0	101.3
Premix	7061	1500	8500.0	95.9
		3000	10050.0	99.5
	8700	1500	10160.0	97.3
		3000	11600.0	96.6
			Av.	98.59

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SWEET POTATO PIGMENTS

Relationship of Tristimulus Colorimeter Readings to Carotenoid Pigments in Sweet Potatoes

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Changes in the carotenoid content have been found to be a sensitive index of the response of sweet potatoes to various growing and storage conditions. If this index is to be widely used, a rapid method of measuring these changes is needed. Because carotenoids are the pigments responsible for the color of the flesh it seemed likely that the Hunter color and color-difference meter might be used to measure these changes and thereby provide the fast method needed. Coefficients of correlation between total carotenoids of sweet potatoes, as determined chemically, and the values obtained with the Hunter meter are statistically highly significant, but are not sufficiently close to give assurance that even relatively large differences will be detected.

THE FLESH OF SWEET POTATOES may range in color from creamy white through yellow and orange to salmon-pink, depending on the concentration of carotenoid pigments. β-Carotene is the principal carotenoid in varieties having deeply colored flesh, and as the precursor of vitamin A it is the most important pigment in all varieties from the nutritional standpoint. Genetic factors are largely responsible for differences in carotenoid concentration between varieties, but other factors may readily and markedly affect the concentration of the pigments within a variety. Preharvest conditions may result in a lower or a higher concentration at harvest (*3*) or may influence the rate of accumulation after harvest (*6*). Environmental conditions after harvest may result in an increase, a decrease, or the maintenance of a rather uniform amount during storage (*4*).

Changes in the carotenoid concentration thus appear to be a sensitive indicator of the response of sweet potatoes to various environmental conditions, perhaps more sensitive than the subjective indicators commonly used. A major deterrent to its use for this purpose is the long, laborious, chemical procedure now used to measure these pigments and the changes they undergo. Edmond, Garrison, Wright, Woodward, Steinbauer, and Deonier (*2*) reported visual changes in the flesh color, but evalutions of such changes are difficult and only major changes or differences are likely to be detected. If a rapid, objective method

that would accurately detect small differences in carotenoid concentration could be found, these changes could be used in routine studies. A short method would also expedite other work involving measurements of carotenoid pigments.

A short method should permit the pigments to be measured *in situ*, since extraction in itself is a rather lengthy procedure. The Hunter color and color-difference meter was designed for the rapid and precise measurement of color and small color differences and its sensitivity to small differences in color is widely recognized and accepted. It has been used with some success with several food products (*1*, *7*, *8*, *11*, *12*, *14*, *15*). More recently, since collection of data for this paper was completed, other workers (*9*, *10*, *13*) have reported favorably on the use of this instrument in the rapid estimation of the carotenoid contents of varieties and breeding lines of sweet potatoes. The work reported here was undertaken to determine the accuracy and consistency with which this instrument indicates small differences in carotenoid concentration and the feasibility of using it to measure the response of sweet potatoes to environmental conditions as shown by changes in carotenoid contents.

Materials and Methods

Selection of a surface, or surfaces, to be tested with the Hunter meter for comparison with the chemical values is complicated by the wide variation in the distribution of carotenoids within sweet potatoes. The carotenoids may be fairly uniformly distributed throughout the root. They may be heavily deposited near the stem end with a definite decrease toward the root end or they may be deposited so as to form bands of darker colored tissue interspersed with lighter areas, or lighter areas within a darker field. The average carotenoid concentration in one root may be two or three times that in another root of the same variety and treatment (*5*). Variations within a root may exceed this amount within a distance of a few millimeters (*4*). Homogenizing for greater uniformity does not appear feasible, because of the major effects that environmental conditions have on tissue oxidation and discoloration rate.

A cross section about midway between the two ends, and near the maximum diameter of the root would seem the logical place to expect a concentration of carotenoids near the average for the whole root. Additional surfaces, such as at quarter-lengths, might be expected to reflect the higher and lower concentrations in those areas with a mean equal to that at the center. Any additional readings would correspondingly increase the time required by the method with little assurance of a corresponding increase in accuracy. Multiple readings per root consequently were ruled out as being of less value than single readings on more roots to compensate for differences between roots.

In order to use all sized roots in the U. S. No. 1 grade, the smaller opening of the specimen support furnished with the instrument was selected for use when illuminating the exposed area. Approximately half of the roots in the U. S. No. 1 grade of these varieties could not have been used with the larger opening. Limited tests with the larger opening did not result in a higher correlation coefficient between the Hunter meter values and the carotenoid contents than did the smaller opening. Color values were read directly from the three potentiometer rheostat scales, *Rd*, *a*, and *b*, previously balanced with the appropriate color standard as noted below.

Sweet potatoes of two varieties, Yellow Jersey with creamy white or light-yellow flesh and Orange Little Stem with deep-orange or salmon-pink flesh, were used in these studies. Individual roots were cut crosswise through their greatest diameter, placed immediately upon the specimen support and the color readings made on the freshly cut surface. Total carotenoids of these same roots were then determined chemically (*4*). The results were compared with the Hunter meter values. Samples consisted of individual roots and groups of five roots; the latter were compared with the average of the five sets of meter readings on the individual roots making up the sample. Samples composed of 10 replicates of five roots each were analyzed at harvest and after storage by the two methods to determine whether changes shown by chemical analysis would also be shown in the Hunter meter values. The carotenoid content is reported on the fresh-weight basis at the time of sampling.

The Yellow Jersey variety was tested with both a white (*Rd* = 81.6, *a* = plus 6.0, *b* = plus 4.0) and a yellow (*Rd* = 27.9, *a* = minus 3.1, *b* = plus 35.0) standard and the Orange Little Stem with a red (*Rd* = 32.0, *a* = plus 32.7, *b* = plus 11.1) and the yellow standard. After several tests it appeared that the readings taken with the white standard were more closely correlated with carotenoid contents in the Yellow Jersey variety, and the red standard more closely in the Orange Little Stem variety. Only these two standards were used in the later tests. Because of the similarity of results with the two varieties and because the Hunter meter values were in general correlated slightly better with the carotenoid contents in Orange Little Stem only that variety is included in this report.

Results

The accuracy with which the Hunter meter indicates the total carotenoid contents may be judged by comparing the Hunter meter values directly with the chemical values. The meter values may be converted to other values representing dominant wave length, purity, or some other quality, and the converted values can then be compared with the chemical values. Coefficients of correlation *r*, between total carotenoids, as determined chemically, and several of the Hunter meter values were calculated (Table I). Most of these are statistically highly significant but not very close. Much of the variation in the meter values is not directly related to the variation in the carotenoid contents as found by chemical analysis. Coefficients of determination (Table I) indicate that at best only 61% (r^2 times 100) is so related in the individual root samples, and only 17% in the multiple root samples.

The meter values showing the highest correlation both for individual root samples and for multiple root samples were the multiple correlation *Rd*, *a*, *b* and *a*/*b*. *Rd*, *a*, *b* was slightly higher for individual root samples, and of the same value as *a*/*b* for multiple root samples. Because of the variation in carotenoid contents between roots, multiple roots must be taken to ensure a representative sample, and the simpler *a*/*b* correlation coefficient should serve about as well as the more complicated *Rd*, *a*, *b* one.

Roots with the same or nearly the same carotenoid contents varied widely in meter values, and conversely roots with the same or nearly the same meter values varied widely in carotenoid contents.

Table I. Coefficients of Correlation, *r*, and Coefficients of Determination, $r^2 \times 100$, between Total Carotenoids and Hunter Meter Values of Orange Little Stem Sweet Potatoes

	r		$r^2 \times 100$	
	60 individual root samples	*90 multiple root samples*	*60 individual root samples*	*90 multiple root samples*
Rd	−0.74[a]	−0.40[a]	55	16
a	+0.75[a]	+0.26[b]	56	7
b	+0.09	+0.009	1	..
Rd, *a*, *b*	+0.78[a]	+0.41[a]	61	17
a/*b*	+0.74[a]	+0.41[a]	55	17
ΔE	−0.60[a]	−0.02	36	..
Dominant wave length	+0.72[a]	+0.33[a]	52	11
Purity	+0.42[a]	+0.33[a]	18	11

[a] Coefficient of correlation significant at 1% level.
[b] Coefficient of correlation significant at 5% level.

This is shown in Figure 1, where the Hunter *a/b* values are plotted against total carotenoids. The *a/b* values gave one of the highest coefficients of correlation, yet the scatter diagram shows wide disagreement between the meter values and the carotenoid values. This is also shown in Figure 2, where average values from three tests, using 10 replicates of five roots each per sample in each test, are plotted against time in storage. Total carotenoids showed a highly significant increase during storage in each test, but the meter values gave little or no indication of the increase.

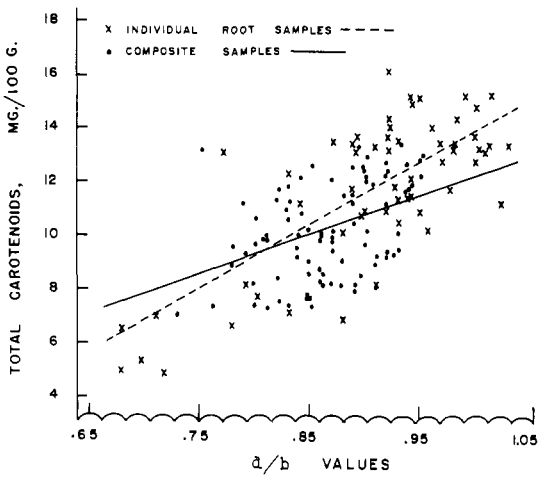

Figure 1. Regression lines and scatter diagrams of total carotenoids

Hunter a/b values for individual and for composite samples of Orange Little Stem sweet potatoes

Discussion

The results reported show that there are highly significant correlations between the total carotenoid contents of sweet potatoes and the values obtained with the Hunter meter. However, the correlations are far from perfect, and may be of limited value where other than major differences are concerned. Composite samples of sweet potatoes analyzed at harvest and after storage showed an increase in carotenoid content from 8.0 to 11.6 mg. per 100 grams (a difference of 1.36 mg. was significant at the 1% level); the Hunter meter values showed little change during this period. This left the problem of a quick method for determining small differences still unsolved.

Why the Hunter meter failed to indicate the carotenoid contents more closely is somewhat conjectural. It was noted that roots varying rather widely in carotenoid contents showed a better correlation between carotenoids and the Hunter meter values than roots varying less. Of the 60 individual roots plotted in Figure 1, 15 of those most closely approaching the *Rd* value of the reference standard varied relatively little in carotenoid contents and showed no significant correlation between carotenoid contents and either of the meter values. The 15 departing most widely from the reference standard varied much more in carotenoid contents and showed a highly significant correlation with the *Rd* and *a* values of the Hunter meter. In the storage studies the 10 replicates within a composite sample usually showed a significant correlation between the meter values and carotenoid contents. Only when the variation in carotenoid was less than normal did they fail to do so.

Because roots varying relatively little in carotenoid contents failed to show significant correlations, perhaps differences in other characteristics may influence absorbency more than slight differences in carotenoid contents. Moisture, texture, composition, or other characteristics may be involved. These may have changed appreciably during storage. Some indications of internal breakdown, sometimes called pithy breakdown, were observed in February, and were readily evident in April. However, the effect of the breakdown was not great enough to cause consistent differences in the correlation coefficients within replicates of composite samples taken at harvest and after storage.

Even though other characteristics may have influenced absorbency to some degree, it seems extremely unlikely that the failure in this case is primarily due to inability of the instrument to detect differences of the magnitude dealt with in this work. A far more likely explanation is that the Hunter meter indicated differences in carotenoid concentration at the exposed surfaces, but that these surfaces often did not closely approximate the average concentration in the whole root. In general a root rich in carotenoids would have more of the pigments at the exposed surface than one poor in carotenoids, and the meter values would show a correlation even though the concentration at the exposed surfaces might vary appreciably from the average in both roots. But where the differences are relatively small, the concentration at the exposed surface would need be fairly close to the average for the instrument to differentiate correctly between roots of different concentrations. Otherwise, errors would inevitably result. The magnitude of the undetected increases in carotenoids in the stored sweet potatoes leads one to question whether the exposed surfaces, regardless of how or where taken, can safely be relied upon for a reasonably accurate indication of the average content of the whole root.

The carotenoids appear to be more uniformly distributed in some varieties than in others. Also, they may be more uniformly distributed at harvest than after storage, and the difference may be particularly noticeable in a variety with light-colored flesh. In Yellow Jersey a deep yellow circular band often occurred just beneath the skin surface of the stored roots, and the bands were usually wholly or partially outside the area exposed to the photocell. With more nearly uniform distribution within the root one would expect the meter values to agree more closely with the chemical values than in roots where the distribution was more uneven. This, together with the wider differences between varieties and selections, might explain the more favorable results reported by other workers. Of the two varieties used in this study the Orange Little Stem is much richer in carotenoids. Visually the pigments appear to be more uniformly distributed in the Orange Little Stem after storage than in the Yellow Jersey. However, the accuracy with which the Hunter values reflected the chemical values in the two varieties was about the same, and the differences in distribution between the two varieties may have been more apparent than real.

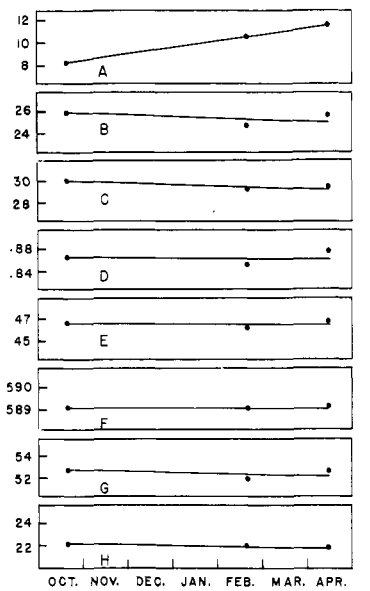

Figure 2. Total carotenoids and Hunter meter values of Orange Little Stem sweet potatoes before and after storage

A. Total carotenoids, mg./100 grams
B. Hunter *a* scale values
C. Hunter b scale values
D. a/b values
E. Hunter *Rd* scale values
F. Dominant wave length, mμ
G. Purity *H*Δ*E*

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EGG PROTEIN MIGRATION

Protein Distribution in Fresh and Stored Shell Eggs from Hens Fed Crude Cottonseed Oil

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Hens fed rations containing 2.5% crude cottonseed oil produce eggs that develop pink whites and large salmon-colored yolks after 6 months or more of cold storage. Protein migrates from the white to the yolk. The composition of the migrating protein was calculated from changes in protein distribution in whites and yolks of fresh and stored shell eggs as determined by paper electrophoresis. The migrating protein contained ovalbumin, conalbumin, and lysozyme, but no ovomucoid or ovoglobulin. Livetin migrated from yolk to white. The lipovitellenin band from 6-month-old eggs moved almost three times as far as that from fresh eggs during electrophoresis. Part of the lipovitellin was converted to a protein which behaved similarly to lipovitellenin under the conditions used for electrophoresis.

HENS FED RATIONS containing crude cottonseed oil produce eggs that develop viscous pink whites and large salmon-colored yolks after 6 months or more of cold storage (*11*). Migration of protein and water from the white to the yolk also occurs and is probably caused by a weakened vitellin membrane (*1*). Conalbumin migrates from white to yolk and iron from yolk to white (*10*). Evans, Bandemer, Davidson, and Schaible (*7*) reviewed the literature on pink white and salmon yolk discoloration of eggs.

Evans *et al.* (*6*) calculated the composition of the protein that migrated from the white to the yolk during storage of shell eggs from hens fed crude cottonseed oil, on the basis of the weight of protein that migrated, the methionine, cystine, and serine contents of the white and yolk proteins of fresh and 6-month-old eggs, and literature values (*9*) for the contents of these amino acids in the individual egg white proteins.

A more direct determination was desired of the amounts of different egg white proteins that migrate from the white to the yolk during storage of eggs produced by hens fed crude cottonseed oil. Evans and Bandemer developed procedures for the quantitative determination of the different proteins in egg white (*4*) and egg yolk (*5*). These methods have been used to determine the protein composition of whites and yolks in fresh and stored eggs; and these data were then used in calculations.

Experimental

Twelve pullets were housed in laying cages and fed a ration containing 2.5% of crude cottonseed oil. The basal ration to which the oil was added consisted of ground corn 34.5%, ground oats 20.0%, wheat bran 15.0%, flour middlings 10.0%, dehydrated alfalfa 3.0%, meat scraps 3.0%, dried milk 2.0%, fish meal 2.5%, soybean oil meal 2.5%, ground oyster shell flour 5.0%, steamed bone meal 1.5%, salt 0.6%, and fish oil (2000 units of vitamin A and 400 units vitamin D) 0.4%. Chicks hatched in April 1956 were placed in the laying cages as pullets at about the time they started to lay in September. Eggs were gathered twice daily, marked with pullet number and date, and placed in cold storage at 0° C. or used within three days as fresh eggs for protein separations.

Eggs from each of 11 pullets were studied fresh and after 6 months of cold storage. A sample consisted of two consecutive eggs from a pullet, and three samples (or six eggs) were used from each pullet for each period. Whites of two eggs carefully separated from the yolks were combined, weighed, mixed briefly in the Waring Blendor, and stored in the refrigerator until used. The two yolks were combined, weighed, mixed with an equal weight of 10% sodium chloride solution, and stored in the refrigerator until used. Eggs were saved for the fresh egg samples on Friday, Saturday, and Sunday of each week. Two eggs from each pullet were then used on Monday morning.

Protein distributions in the whites and yolks were determined by the procedures of Evans and Bandemer (*4*, *5*). Duplicate samples of egg whites from four pullets were used for electrophoresis on Monday afternoon and from another four on Tuesday afternoon. Yolk sam-