QUALITATIVE AND QUANTITATIVE DETERMINATION OF SWEET POTATO PIGMENTS

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INTRODUCTION

It is an established fact that the principal pigment of the sweet potato is Beta-carotene. Since Beta-carotene is a vitamin A precursor the amount of Beta-carotene in sweet potatoes has undergone extensive investigation. Few attempts have been made to identify the pigments of the sweet potato other than Beta-carotene, but there have been some investigations of their carotene/total pigment ratio. There are some variations in reported values of the carotene/total pigment ratio.

Some varieties of sweet potatoes, after baking, have an undesirable brown color. It is believed that one or more of the non-carotene pigments of the sweet potato might cause this undesirable color.

With these considerations in mind an investigation was undertaken to determine the identity of the non-carotene pigments of the sweet potato, and some possible causes for the variations in reported values of the carotene/total pigment ratio.

REVIEW OF LITERATURE

Carotenoids are the yellow to deep red or purple, nitrogen-free, polyene pigments containing a chromophoric system of conjugated double bonds. Carotenoid hydrocarbons are called carotenes. Xanthophylls are the oxygen derivatives of carotenes; they may be alcohols, ketones, hydroxy-ketones, ethers, aldehydes, esters of carotenoid alcohols, acids, or esters of carotenoid acids.

Botanists and chemists have exibited an interest in the carotenoid pigments since the days of Berzelius, but they were originally hampered by the lack of good methods of separation. Fundamental advances in carotenoid chemistry became possible in 1906 when Tswett, a Polish botanist, put forth his original proposals for adsorption analysis. This technique is more generally termed chromatography. Tswett extracted green leaves with a light petroleum fraction and passed the extract through a column of finely powdered calcium carbonate contained in a vertical glass tube. From his observations of the various green and yellow zones Tswett declared, against all opposition, that chlorophyll was a mixture of two components and that leaf carotene was not a chemical entity, but a mixture of two or more homologues.

Tswett's work with carotenoids was generally disbelieved and it was not until 1931 that Kuhn, Winterstein and Lederer successfully introduced chromatography into the preparative

field of the polyene pigments. Until that time practically nothing was known of the structure of the carotenoids. Today the structure and physical properties of a considerable number of the carotenoids have been definitely determined, owing primarily to the work of Karrer, Kuhn, Zechmeister, Strain and many other workers. Extensive use and improvements in Tswett's chromatographic technique have made this rapid advance in carotenoid chemistry possible. The chromatographic technique for the isolation of carotenoid pigments is described in Strain's (21) Leaf Xanthophylls, Strain's (20) Chromatographic Adsorption Analysis, and Zechmeister and Cholnoky's (25) Principles and Practice of Chromatography.

Carotenoids are widely distributed in the plant kingdom. They are found in the leaves of all green plants and in many primitive plant forms such as bacteria, yeasts, and algae. Usually more than one carotenoid occurs in a given plant and usually both carotenes and xanthophylls are present. Certain plants may contain a particular carotenoid in relative abundance, and thus may serve as a good source for its preparation. Strain (19) states that a number of carotenoid pigments have been found in most or all higher plants thus far examined. They are: cryptoxanthin, lutein, zeaxanthin, violaxanthin, flavoxanthin, neoxanthin, and Beta-carotene. Strain further states that of the chloroplast pigments, the xanthophylls are subject to the greatest variation. Different xanthophylls predominate in plants belonging to different classes and many plants do not contain a single xanthophyll in common.

Carotenoids are relatively insensitive to alkali. Thus carotenoid esters are often hydrolyzed in alkali to free the carotenoid alcohols or acids. They are sensitive, however, to acids, heat, light, and air oxidation. It has been found that, in general, the xanthophylls are more unstable than carotenes. They also show with relative ease the phenomena of isomerization. Zechmeister (24), (26) has studied extensively cis-trans isomerization of Beta-carotene. He has prepared some ten isomers, determined their absorption spectra, and given them names that are prefixed by neo (e.g. Neo-Beta-carotene B and Neo-Beta-carotene U). Pauling (18) concluded that only certain of the double bonds are available for cis-trains isomerization, Beta-carotene thus having only 20 possible cis-trans isomers instead of a possible 1,056.

In 1936 Matlack (12) reported the presence of Beta-carotene in sweet potatoes which fact has been confirmed by many workers. He also reported the presence of another pigment which he believed to be violaxanthin. In 1945 Kemmerer, Fraps, and Meinke (11) reporting on the constituents of crude carotene of some human foods indluded sweet potatoes in their research. Using three varieties they found an average of 86 per cent Beta-carotene, 4 per cent neo-Beta-carotene B, and 10 per cent impurity A. Impurity A is the term that they used for unidentified pigments. They reported the absence of neo-Beta-carotene U and Alpha-carotene in sweet potatoes. They used a column of calcium hydroxide for the adsorbent. The pigments were eluted and

the amounts were read on a KWSZ colorimeter. They used the total amount of pigment recoverable from the column as 100 per cent rather than the amount present before chromatographing. They stated that the recovery in all but six samples was over 90 per cent. This seemed to stimulate interest in the pigments of the sweet potato other than Beta-carotene. There have been no other reports in the literature on the identity of any of the non-carotene pigments, but there are several published articles on the carotene/total pigment ratio of sweet potatoes.

In 1946 and again in 1948 Ezell and Wilcox (1), (2) reported that the carotene/total pigment ratio varies among and within different varieties of sweet potatoes, and that this ratio increases with greater concentration of total pigments. Their values ranged from a ratio of about 30 per cent for sweet potatoes having less than one mg. total pigment per hundred gms. of potatoes to 88 per cent for varieties having six mgs. per hundred gms. They used the Wall and Kelley (23) method of extracting and determining the carotene. The total pigments were determined before chromatographing, and the carotene after chromatographing. Both were read in a colorimeter using a 440 millimicron filter. In 1949 Miller, Melamoy, Mikell, and Hernandez (15) reported on the carotene/total pigment ratio of fourteen varieties of sweet potatoes. The lowest value obtained was 89 per cent and four varieties had 101, 102, 103, and 106 per cent. In no case was total pigment less than 5 mgs. per hundred gms. The method of carotene analysis used was that of O'Connor, Heinzelman, and Jefferson (17) in which cold ethyl alcohol is

used for extraction, dicalcium phosphate in the adsorption column for the purification, and 2,2,4-trimethylpentane as the solvent in the spectrophotometric measurements.

PART I

QUALITATIVE DETERMINATION OF SWEET POTATO PIGMENTS

Equipment. The chromatographic adsorption tubes were obtained from the Scientific Glass Apparatus Company of Bloomfield, New Jersey. They were fitted with ground glass joints for ease of adsorbent removal, with the male connection sealed with a perforated glass plate. The large column, 43 X 270 mm., was used principally for the concentration of pigments. The small columns, 19 X 200 mm., were used for separation of the pigments.

The absorption spectra were obtained by the use of a Beckman model DU photoelectric quartz spectrophotometer.

Adsorbents. Numerous adsorbents were tried. Calcium carbonate was discarded because of the difficulty of preparing it in an active form. Calcium hydroxide was found to adsorb the non-carotene pigments at the top of the column. It adsorbed too strongly to allow an adequate separation, but was useful for concentrating the pigments. Magnesium oxide, activated dicalcium phosphate, and zinc carbonate were found to be the best adsorbents for this type of experiment. Zinc carbonate was used in most of the experiments because of its ready availability and the fact that it needed no activation.

Experimental Methods. The pigments from a number of varieties of sweet potatoes were extracted with a mixture of two volumes of ethanol and one volume of petroleum ether (Skellysolve

B. B. P. 60°-70° C.) in Waring blendors. Water was added to the extract until two phases separated. The alcohol-water layer was then washed free of pigments with small quantities of petroleum ether. Alcohol was removed from the combined petroleum ether extracts by washing with water. The extract was dried over anhydrous sodium sulfate. This extract was chromatographed on a column, (38 X 270 mm.) composed of a one to one mixture of zinc carbonate and diatomaceous earth (Johns-Manville Hyflo Super-Cel). Carotene was washed into the eluate with petroleum ether. The pigments remaining on the column were eluted with a 2 per cent by volume solution of alcohol in petroleum ether. Two green bands, probably chlorophylls, were not eluted by the alcohol-petroleum ether solution. The petroleum ether (total volume 200 mls.) was washed free of alcohol with water and dried over anhydrous sodium sulfate.

Four columns (19 X 200 mm.) of the zinc carbonate-diatomaceous earth mixture were prepared by applying suction, and successively tamping small quantities into place. Ten mls. of petroleum ether were added to each of the columns followed by 25 mls. of the extract. The columns were developed with petroleum ether for a period of eleven hours. A bright yellow band followed by an orange band slowly moved down the columns, and passed into the eluate in about two hours time. The pigments were collected separately for determination of their absorption spectra. After developing the columns for eleven hours the pigments had separated into two distinct bands, a lower yellow band and an orange band just above it. Some brownish-orange pigment

remained adsorbed near the top of the columns. The columns were extruded and the parts containing the pigments were divided into six fractions. The pigments in each fraction were eluted with a two per cent solution of alcohol in petroleum ether.

A number of earlier experiments were run, but this was the most successful experiment. Pertinent data from these earlier experiments will be brought out in the section on results and discussion.

Pigments of sweet potato varieties 10x3-128 and 30x43-10 were chromatographed separately and the absorption curves for the non-carotene pigments were determined to see if there was any appreciable difference in the two varieties, and to get a comparison with Beta-carotene and violaxanthin.

Results and Discussion. As already noted, the presence of Beta-carotene in sweet potatoes reported by Matlack (12) has been confirmed by many other workers. Matlack also reported the presence of another pigment which he believed to be violaxanthin. The absorption curves for Beta-carotene, violaxanthin, and the non-carotene pigments of the sweet potato are shown in Figure 1. This indicates that the unknown pigment or mixture of pigments is not violaxanthin, although it does not preclude the possibility of the non-carotene pigments being a mixture of pigments of which violaxanthin is one.

The absorption curve for the bright yellow pigment that was washed through the column after developing the column for two hours with petroleum ether is given in Figure 2. Upon rechromatographing it formed only one zone indicating that it was a chromatographically homogenous compound. This pigment was separated

and the results reproduced in three different experiments. Maxima were at 455 and 434 millimicrons.

Figure 4 shows the absorption curve for the yellow pigment that was fixed on the column after developing the chromatogram for 11 hours. Of the six fractions into which the column was divided, the lower two were found to be identical. Maxima were at 427 and 402 millimicrons. The absorption curve for the orange pigment just above this yellow pigment is given in Figure 5. Maxima were at 452, 427, and 402 millimicrons. This pigment may have been contaminated with some of the yellow pigment that was adsorbed just below it since the maxima of 427 and 402 millimicrons were repeated.

Table 1 lists the absorption maxima of the carotenoid pigments that might be expected in sweet potatoes. The absorption maxima of the pigments found in this investigation are also listed for purposes of comparison. The absorption maxima of the unknown pigments isolated from sweet potatoes do not check with that of any of the listed carotenoid pigments or with that of any of the other carotenoid pigments found in the literature.

Two pigments were obtained in a state of chromatographic homogeneity as shown by failure of repeated absorption and elution to modify the spectral properties. The non-carotene pigments of the sweet potato would logically be expected to be carotenoids because of their chromatographic behavior and the nature of the other pigments present. The absorption maxima of the pigments that were isolated did not correspond with those of the described carotenoid pigments. The pigments isolated could possibly have been isomerization products of carotenoids. Isomerization of

the samples was avoided as much as possible by refrigerating, avoiding heating methods for concentration, using brown flasks when available, and keeping in tightly stopered flasks when not being studied.

Table 1

Comparison of Absorption Spectra of Common Carotenoid Pigments and the Pigments Isolated from Sweet Potatoes in This Work

Pigment	Absorption maxima in millimicrons		
Beta-carotene[a]	483.5	452	424
Violaxanthin	472	442.5	417.5
Lutein	476	446.5	420
Zeaxanthin	482.5	451.5	423
Crytoxanthin	484	451	423
Flavoxanthin	450	422	
Neoxanthin	466	436	
Pigment Mixture[b]	452	427	
Unknown Pigment	452	427	
Unknown Pigment	455	434	
Unknown Pigment	427	402	
Unknown Pigment	452	427	402

[a](13), (21) Solvent petroleum ether B.P. 70°-80° C.

[b]Experimentally determined. Solvent-petroleum ether B.P. 60°-70° C.

FIGURE I
A COMPARISON OF THE ABSORPTION SPECTRA OF BETA-CAROTENE, VIOLAXANTHIN AND THE NON-CAROTENE PIGMENTS OF THE SWEET POTATO.

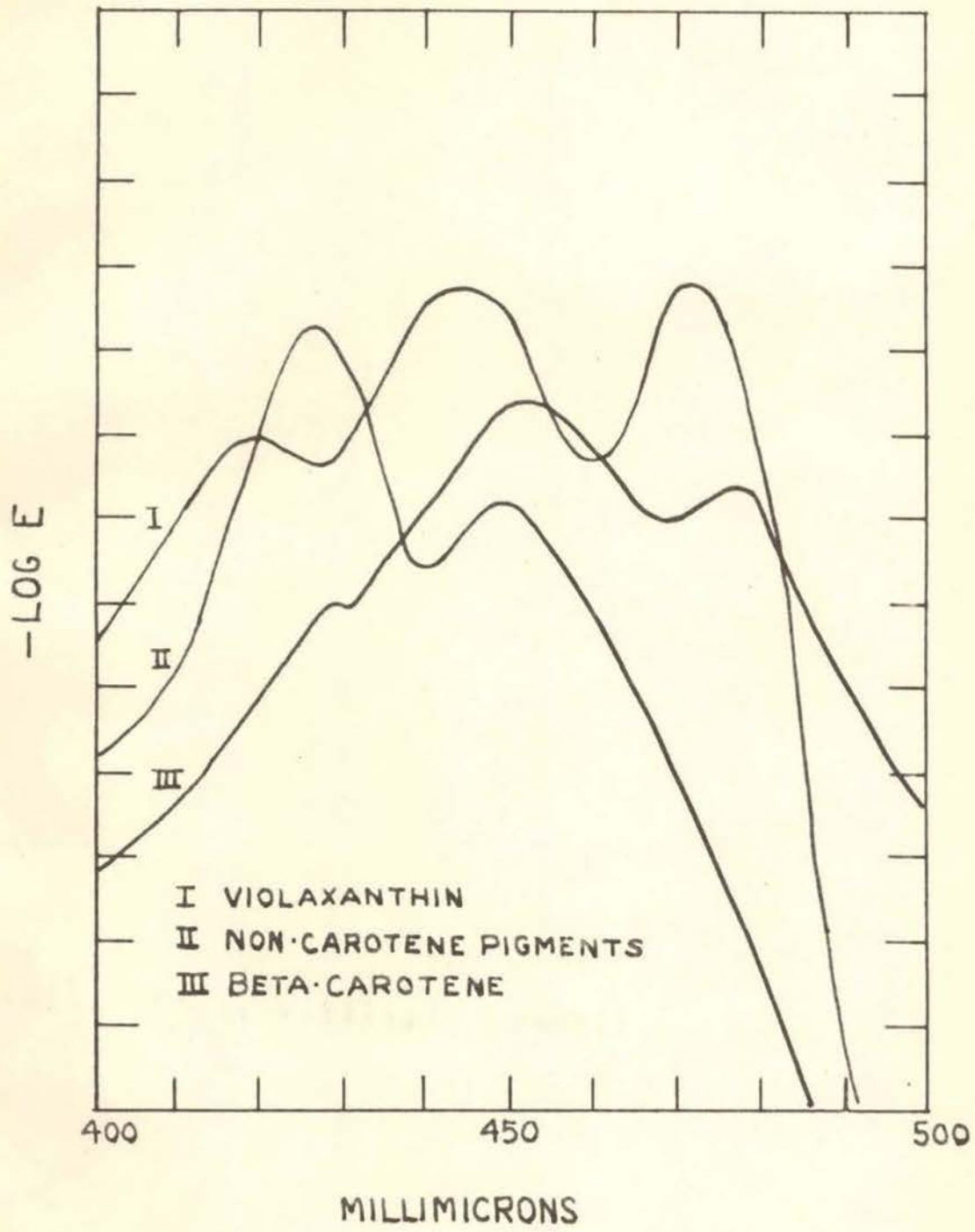


FIGURE II

ABSORPTION SPECTRA OF THE YELLOW PIGMENT OBTAINED AFTER DEVELOPING THE CHROMATOGRAM FOR TWO HOURS WITH PETROLEUM ETHER

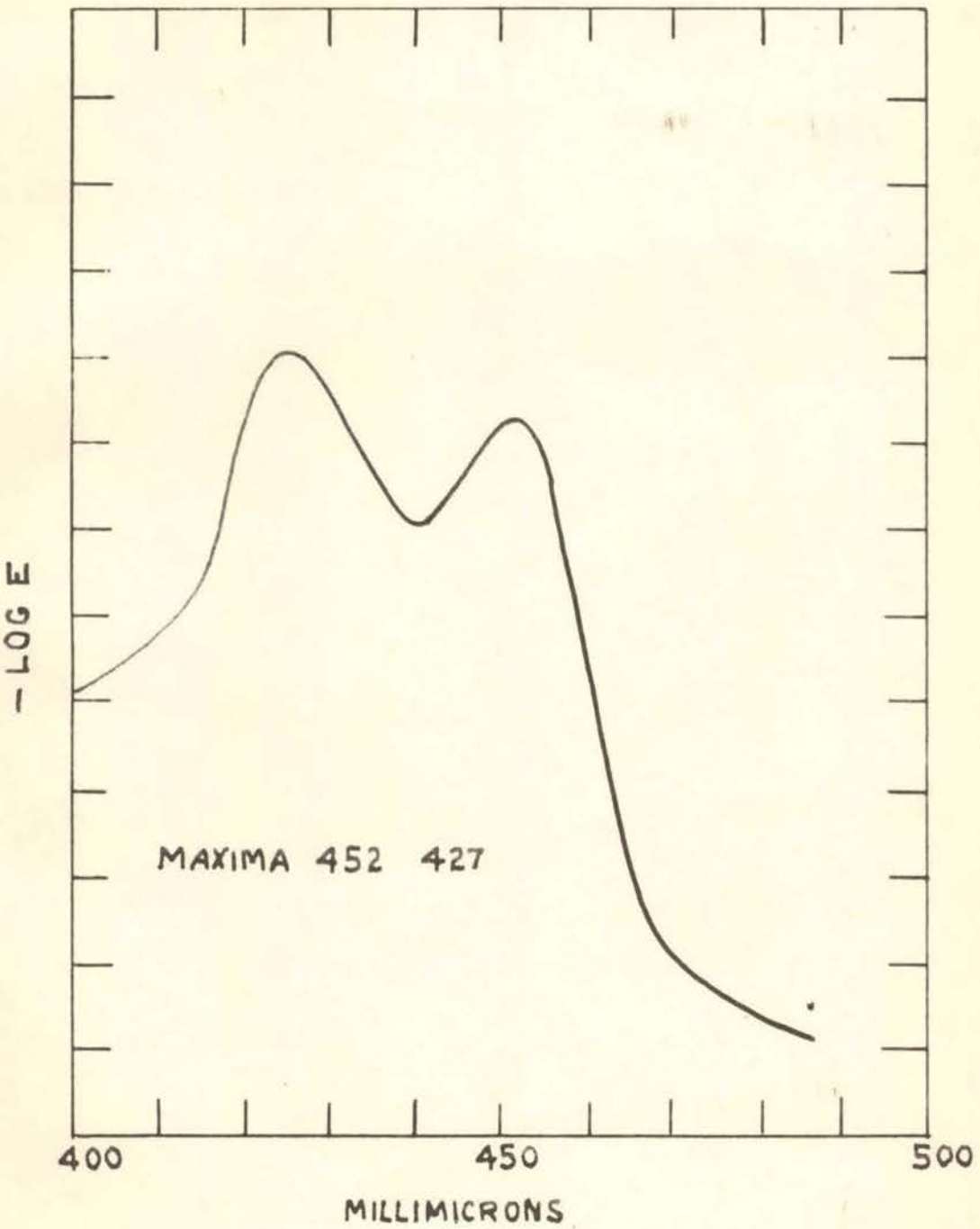


FIGURE III

ABSORPTION SPECTRA OF THE ORANGE PIGMENT OBTAINED AFTER DEVELOPING THE CHROMATOGRAM FOR TWO HOURS WITH PETROLEUM ETHER

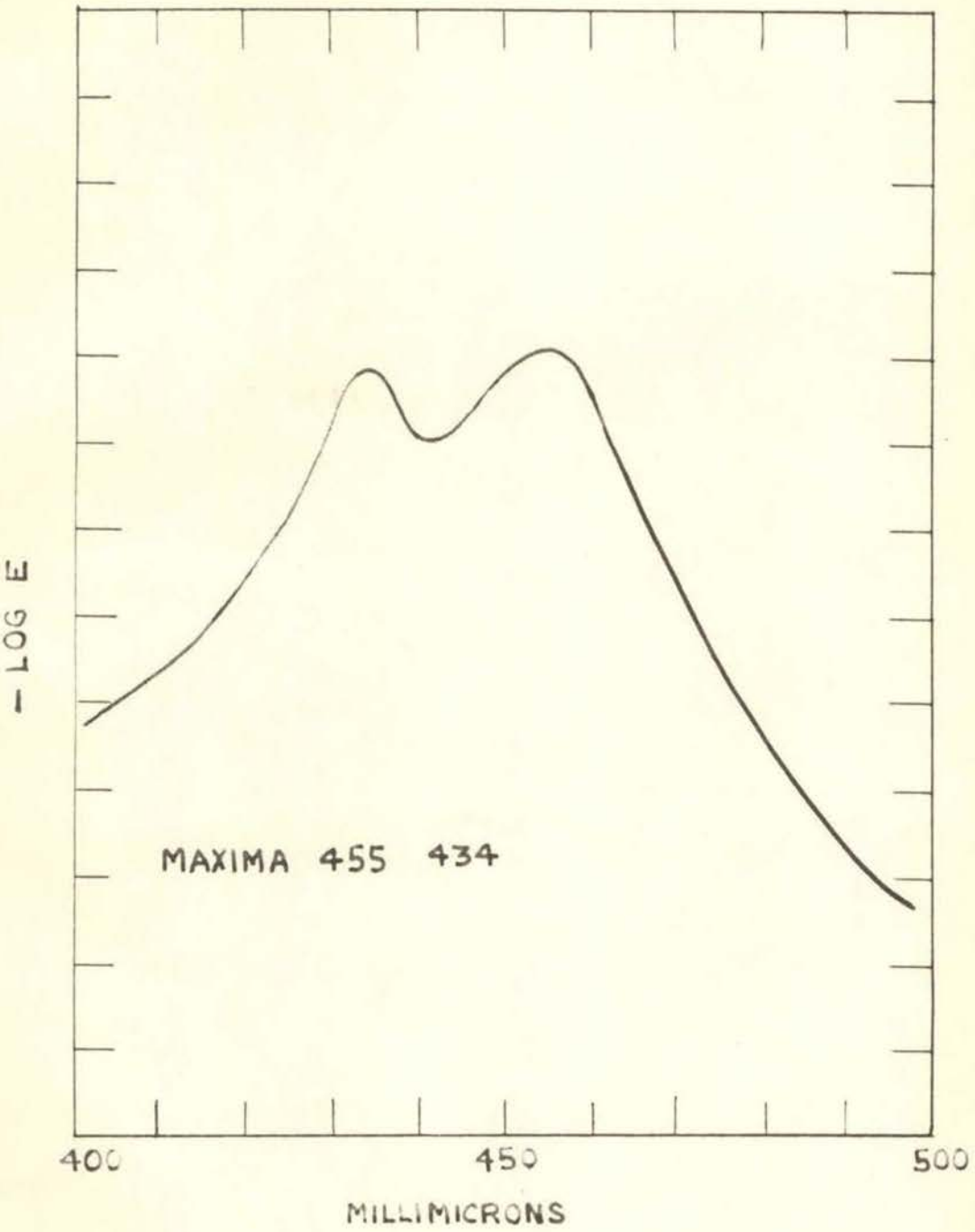


FIGURE IV

ABSORPTION SPECTRA OF THE YELLOW PIGMENT OBTAINED AFTER DEVELOPING THE CHROMATOGRAM FOR ELEVEN HOURS WITH PETROLEUM ETHER

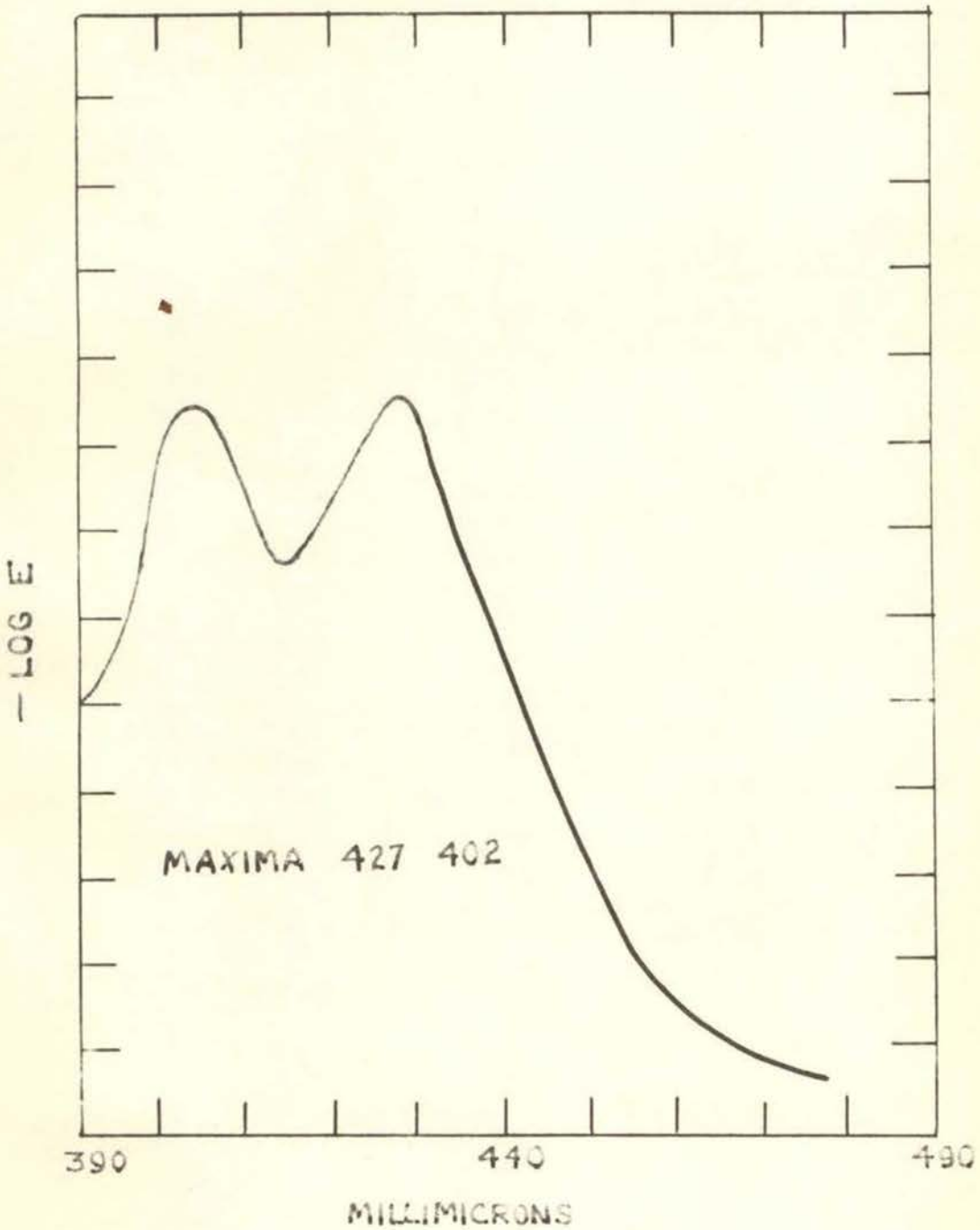
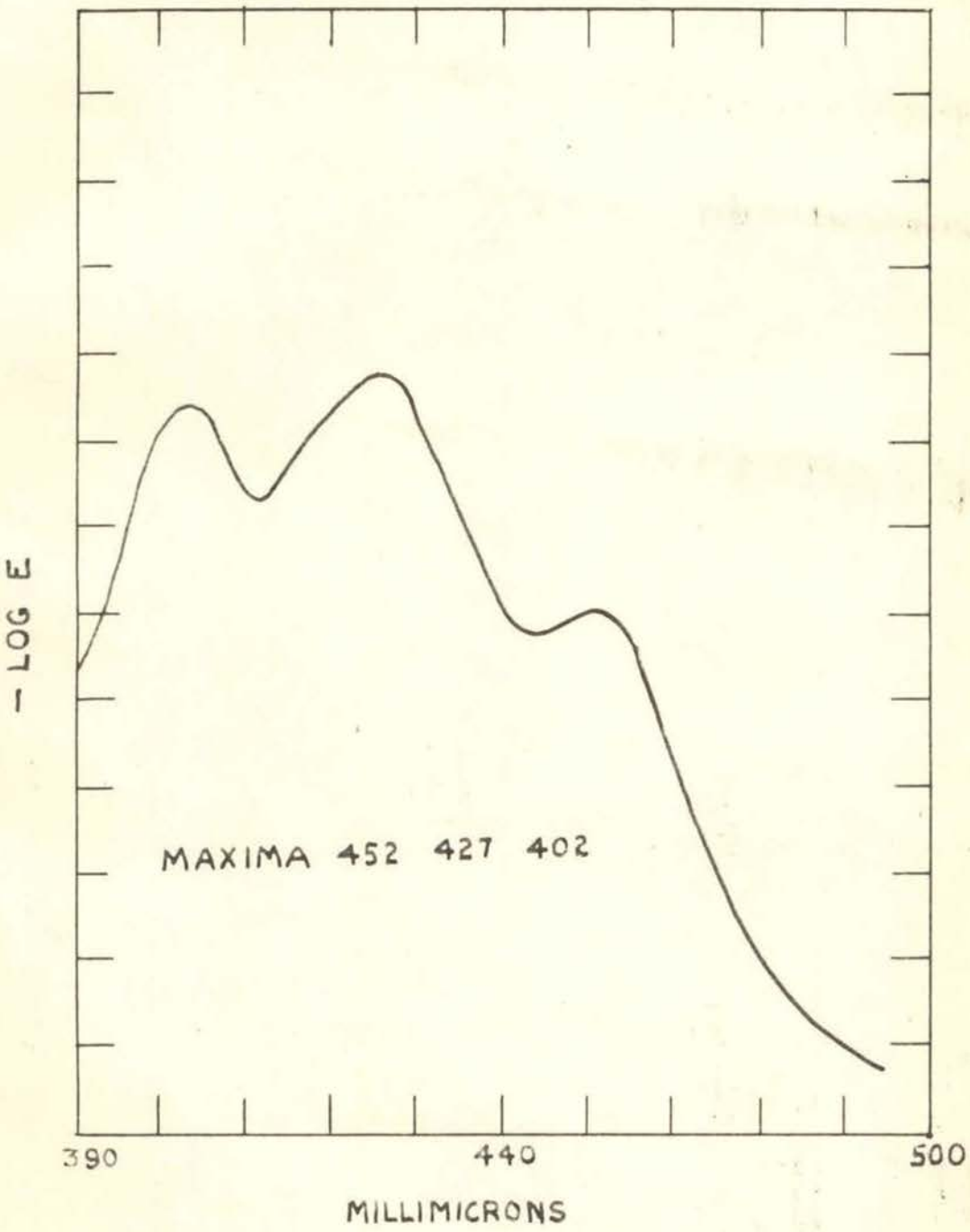


FIGURE V.

ABSORPTION SPECTRA OF THE ORANGE PIGMENT OBTAINED AFTER DEVELOPING THE CHROMATOGRAM FOR ELEVEN HOURS WITH PETROLEUM ETHER



PART II

QUANTITATIVE DETERMINATION OF SWEET POTATO PIGMENTS

The objects of these experiments were to determine in sweet potatoes: (1), the amount of carotene, total petroleum ether soluble pigments, carotene/total pigment ratio; and (2), the effect of reading total pigments before and after chromatographing.

For this experiment 20 varieties of sweet potatoes were furnished by Dr. H. B. Cordner of the Oklahoma Agricultural Experiment Station. They consisted of standard and new varieties developed by Dr. Cordner and associates. They were grown at Perkins, Oklahoma in a light sandy soil and were harvested about November 1, 1949. They were stored until June 1, 1950 before being analyzed.

Methods. The Moore and Ely (16) method of analysis for carotene was used in this work. Each of ten roots was halved longitudinally, and one of the halves from each root was ground and thoroughly mixed together. Two ten-gram samples of this mixture were quickly weighed on a torsion balance and placed in Waring blendor cues. From this point the samples were treated as separate entities. Another ten-gram sample of this mixture was weighed into a platinum crucible for moisture determination. This sample was dried overnight at 105° C.

One hundred mls. of 95 per cent ethanol and 60 mls. of petroleum ether (Skellysolve B. B.P. 60°-70° C.) were quickly

added to the sample in the blendor. The blendor was started, and 95 per cent alcohol was added until the mixture foamed. The blendor was then allowed to run for five minutes. The sample was quantitatively transferred to a flask, and if necessary, was stored overnight in a refrigerator. The liquid from the extraction was poured off into a 500 ml. separatory funnel. Enough water was added to cause a separation between the alcohol and ether layers, and the alcohol phase were washed successively with three to five 30 to 35 ml. portions of petroleum ether, each of which was added to the original ether extract. The ether phase was washed eight times with water to remove all traces of alcohol. Anhydrous sodium sulfate was added to this extract containing the pigments to remove the water. This extract was stored overnight under refrigeration if necessary.

The petroleum ether extract was made up to a known volume in a graduated cylinder. A ten-ml. aliquot was made up to 50 mls. for a direct reading of total pigments. A twenty-ml. aliquot was chromatographed using an equal parts by volume mixture of activated dicalcium phosphate and glucose (Dyno, Corn Products Refining Co.) as the adsorbent. Upon washing with petroleum ether the carotene passed through the column into the eluate which was made up to a final volume of 100 mls. The pigments remaining on the column were eluted with a 2 per cent by volume solution of alcohol in petroleum ether, and made up to a volume of 100 mls. with petroleum ether.

These solutions were read in an Evelyn photoelectric colorimeter using a filter having an absorption maximum of 440 milli-

microns. The amounts of carotene, total pigments, and non-carotene pigments were determined from a standard curve prepared from a solution of 90 per cent Beta and 10 per cent Alpha-carotene.

Results and Discussion. As indicated by Table 1 there is considerable error in determining the carotene/total pigment ratio that probably does not fall strictly into the category of mechanical errors. Duplicate samples of variety 10x3-128 showed a carotene/total pigment ratio of 91 per cent when calculated by dividing the carotene value by that of the carotene plus non-carotene pigments, whereas the carotene/total pigment ratio was 101 per cent when calculated by dividing the carotene value by that of total pigments before chromatographing. This same phenomenon was encountered in variety 1x9-20m where the ratios were 88 and 99 per cent respectively for the two methods. Table 2 shows that for variety 10x3-128 the actual amount of non-carotene pigments present was as high as in any other variety. The opposite effect is observed in variety Okla. 35. For this variety the carotene/total pigment (carotene plus non-carotene pigments) was 89 per cent, while the carotene/total pigments before chromatographing ratio was 81 per cent. (Table 2). The latter effect could be caused by a loss of pigments on the chromatographic column, such as the presence of appreciable quantities of chlorophylls which are not eluted with the other non-carotene pigments.

Out of fourteen varieties analyzed by Miller et al. (15) four varieties were found to have a carotene/total pigment ratio of over 100 per cent on freshly harvested samples. Their method of determination of this ratio was to divide the amount of carotene by the amount of total pigments before chromatographing.

Ezell and Wilcox (2) reported a carotene/total pigment ratio for the Porto Rico variety of 81 per cent, while Miller et al. (15) reported a ratio of 97 per cent for this variety. Miller suggested that the difference might be due to the actual difference in the chemical composition of the roots grown in different regions. The value of 84 per cent found in this experiment agrees well with that of Ezell and Wilcox. In some more recent work that is to be published in the Southern Cooperative Bulletin series, Fieger (3) found a value of 85 per cent for the Porto Rico variety while Peterson and Sherwood found a value of 81 per cent for this variety. The carotene/total pigment ratio of Porto Rico variety as reported by four different groups of workers from different regions is in fair agreement. The methods of measurement were with filter type instruments using standard curves prepared from a solution of 90 per cent Beta and 10 per cent Alpha-carotene. The exact explanation for the high results for the Porto Rico variety and Miller's generally high results obtained for all varieties is not known. The most likely explanation is a difference in methods of determining the amount of total pigment. Miller et al. stated that they used the method of O'Connor, Heinzelman, and Jefferson (17) whereby a spectrophotometric measurement is made at 450 and 476 millimicrons. An average of these two values are taken, and should agree within 0.2 to 0.3 per cent. The absorption curve of the non-carotene pigments of the sweet potato (Figure 1) reveals a considerable drop in absorption from 450 to 476 millimicrons which would make one wonder if it was possible to achieve such accuracy using this method.

It would be expected that the amount of total pigments calculated in Beta-carotene units would be greater at 450 millimicrons than the amount would be at 476 millimicrons. Different results might also be expected from standard curves prepared using different filters. (Carotene is usually determined from a standard curve using a filter that absorbs maximally at 440 millimicrons, however 460 millimicrons is sometimes used.)

Ezell and Wilcox (1), (2) reported that as the amount of total pigments increased the amount of carotene increased much more rapidly than the other pigments. In the five varieties which they tested, total pigments ranged from 0.4 mg. per 100 gms. to 5.5 mg. per 100 gms. No direct comparison was possible with their date due to the fact that the sweet potatoes tested in this experiment have a generally greater concentration of total pigments. An examination of Table 3 indicates that in the varieties selected for this experiment the amount of carotene does not necessarily increase more rapidly than the amount of non-carotene pigments.

Table II

Amounts of Pigments in Sweet Potato Varieties and Their Carotene/Total Pigment Ratio.[a] (Duplicate Analysis)

Variety	Total Pigment[b]	Carotene	Non-Carotene Pigment	Carotene Total Pigment[c]	Carotene Total Pigment[b]
Nancy Gold	7.3	6.4	0.7	90	88
	7.5	6.4	0.9	88	85
15 Self 6	11.8	10.9	1.3	89	92
	13.3	11.7	1.4	89	88
Okla 13	10.2	8.6	1.0	90	84
	10.2	8.7	1.2	88	85
10x3-126	11.0	10.1	1.7	86	92
	12.2	10.2	1.6	86	84
10x3-128	16.6	17.0	1.7	91	102
	15.5	15.5	1.6	91	100
1x9-20m	7.3	7.2	1.0	88	99
	6.6	6.6	0.8	89	100
Okla 24	13.9	12.7	1.0	93	91
	13.9	12.5	1.3	91	90
18x36-6	9.2	8.3	1.1	88	90
	9.5	8.3	0.8	91	87
47x46-10	6.2	5.4	0.5	92	87
	14.9	13.3	1.4	91	89
3x10-310	13.3	11.9	1.1	92	90
	13.3	12.4	1.5	89	93
Okla 25	12.4	10.4	1.5	87	84
	12.2	10.0	1.5	87	82
30x43-10	18.8	17.0	1.3	93	90
	19.9	18.0	1.3	93	90
6x23-24	7.3	6.2	0.8	89	85
	7.1	6.3	0.7	90	89
10x6-4	9.3	7.9	1.0	89	85
	9.9	8.7	0.9	91	88
38x31-8	12.0	10.7	1.3	95	89
	10.2	9.0	1.0	90	88

[a] Amounts are expressed as mg./100 grams, fresh weight. Ratios are given as percentages.

[b] Before chromatographing.

[c] Total pigment calculated by adding carotene and non-carotene pigment values.

Table II--Continued

Variety	Total Pigment	Carotene	Non-Carotene Pigment	Carotene Total Pigment	Carotene Total Pigment
18x31-8	12.4	10.9	1.2	90	88
	12.8	11.3	1.2	90	88
36x24-7	9.3	8.0	0.8	91	86
	8.3	7.2	0.8	90	87
B2934	2.0	1.2	0.5	71	60
	1.4	1.0	0.5	75	71
Porto Rico	7.5	6.6	0.9	88	88
	8.8	7.1	0.9	89	81
Okla 35	9.0	7.3	1.0	88	81
	10.8	8.8	0.9	91	81

Table III

Amount of Carotene and Carotene/total Pigment Ratios[a]

Variety	Carotene	Carotene Total Pigment[b]	Carotene Total Pigment[c]
Nancy Gold	27.6	89	87
15 Self 6	55.2	89	90
Okla 13	31.5	89	85
10x3-126	38.7	86	88
10x3-128	57.1	91	101
1x9-20m	26.4	89	99
Okla 24	52.0	92	91
18x36-6	33.5	90	89
47x46-10	43.5	91	89
3x10-310	49.5	90	91
Okla 25	36.2	87	83
30x43-10	71.7	93	90
6x23-24	26.0	89	87
10x6-4	31.9	90	87
38x31-8	33.9	93	89
18x31-8	43.4	90	88
36x24-7	33.5	91	86
B293	3.7	69	65
Porto Rico	23.0	88	84
Okla 35	32.0	89	81

[a]Amounts are expressed as mg./100 grams, dry weight. Ratios are given as percentages.

[b]Total pigment calculated by adding carotene and non-carotene pigment.

[c]Before chromatographing.

SUMMARY

Experiments were conducted seeking to identify the non-carotene pigments of the sweet potato. The chromatographic behavior of the unknown pigments and the nature of the other pigments known to be present would lead one to believe that the non-carotene pigments are carotenoids. Although two pigments were obtained in a state of chromatographic homogeneity their absorption spectra did not correspond with that of any of the carotenoid pigments found in the literature.

The carotene/total pigment ratio of 20 varieties of sweet potatoes were determined and results indicate that there may be considerable error in present methods for determining this ratio. Carotene/total pigment ratios were determined by dividing the amounts of carotene by the amounts of total pigment before chromatographing and by dividing the amounts of carotene by the amounts of total pigment secured by the addition of carotene and non-carotene values. In two varieties the amounts of total pigment determined by the addition of carotene and non-carotene values were greater than the amounts of total pigment determined before chromatographing. The data makes it difficult to believe that this phenomenon could be caused by experimental errors.

An accurate method of determining carotene/total pigment ratios and the identity of the non-carotene pigments of the sweet potato would seem to warrant further investigation.

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