A CHROMATOGRAPHIC STUDY OF THE CAROTENOIDS OF CORN

by

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INTRODUCTION

With the single exception of yellow corn, the cereal grains are all practically devoid of vitamin A value. Although its chief coloring matter has no vitamin A potency, yellow corn is an important source of vitamin A because of its content of carotene and cryptoxanthin. Analysis for vitamin A potency would, therefore, be a measure for comparing various feed corns.

At present, the most reliable assay for vitamin A is a biological one which depends upon the increased growth effected by the feeding of the substance under investigation to a group of test animals previously brought to a condition of avitaminosis A. This method, being biological, is quite long and tedious and is subject to all the errors and inconsistencies of biological responses.

The need, therefore, of a rapid, accurate chemical assay is manifest. Attempts have been made to estimate vitamin A content based upon a color reaction (Carr-Price reaction) with a dry chloroform solution of antimony trichloride. However, this reaction is curtailed in its applicability in that all carotenoid pigments

including those not vitamin A-active give the test although in different degrees.

It is known that certain of the carotenoid pigments are progenitors of vitamin A. Euler, Demole, Karrer, and Walker (4) have pointed out that vitamin A activity of a feed parallels carotene content. If it were possible to determine qualitatively and quantitatively the various carotenoid pigments of a feed, then, by assigning the proper vitamin A value to each of the constituents present, one should arrive at an accurate vitamin A measure of the feed itself. It was with this objective that the present investigation was undertaken. Obviously, before final credence can be given to this chemical assay it must be verified by standard biological methods.

THE CAROTENOID PIGMENTS OF CORN

Bogert (1) has defined a carotenoid as a nitrogen-free polyene pigment, consisting wholly or chiefly of a long acyclic chain of carbon atoms united in an uninterrupted sequence of conjugated double bonds, which system of conjugations function as the chromophore.

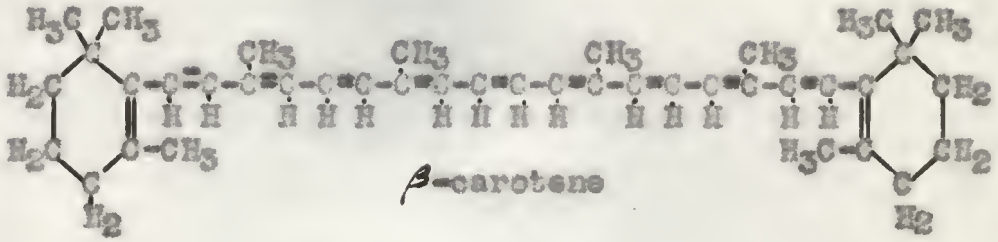
Some sixty such compounds have been identified and characterized.

The carotenoids are commonly classified according to their composition: the hydrocarbons (carotene, lycopene, etc., $C_{40}H_{56}$) which are readily soluble in ether and petroleum ether and quite insoluble in aqueous alcohol, and the oxygen-containing carotenols (xanthophylls) which usually contain hydroxyl groups.

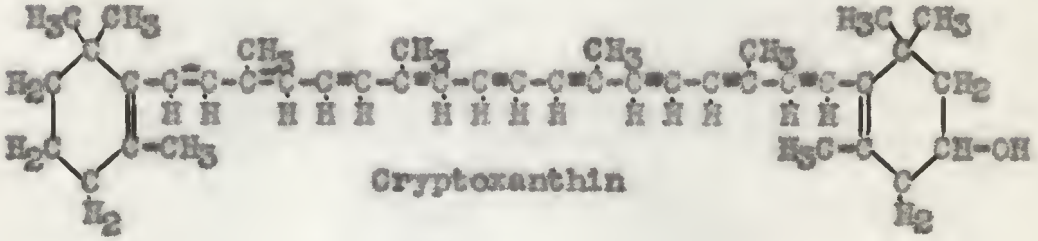
Though as many as sixty carotenoids have been reported to date, only four of the pigments of plant origin, α-, β-, and γ-carotene (isomeric hydrocarbons), and cryptoxanthin (a xanthophyll) together with a single zoocarotenoid of unknown structure (echinenone from sea urchin (21)) have been found to possess vitamin A activity. β-carotene has twice the vitamin A potency of each of the others.

From the standpoint of this investigation, an artificial classification of the pigments of corn based on vitamin A-activity is, in many respects, a more convenient one. The formulae of β-carotene (14) and cryptoxanthin (19), the pigments responsible for the vitamin A potency of yellow corn are given for comparison with the formula of vitamin A itself as established by Heilbron, Heslop, Morton, and Webster (8), Karrer, Morf,

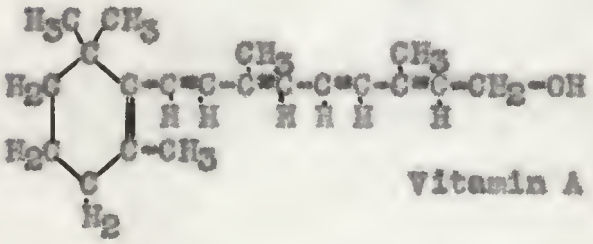
and Schopp (11, 12):



β-carotene



Cryptoxanthin

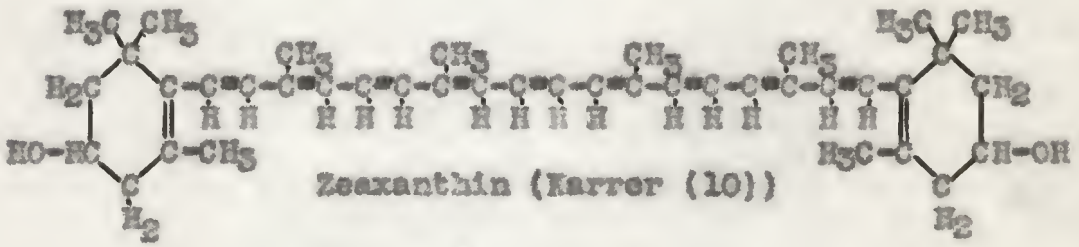


Vitamin A

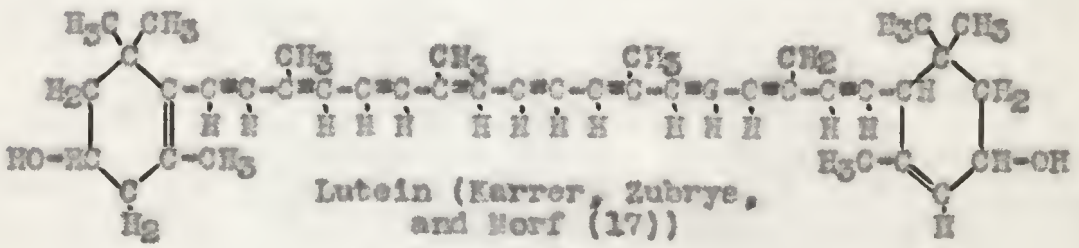
Until recently corn was known to have at least three carotenoid pigments, β-carotene, cryptoxanthin, and zeaxanthin. Prior to 1934, only one pigment, zeaxanthin, had been isolated from corn (Karrer, Salomon, and Wehrli, (13); Karrer, Wehrli, and Helfenstein, (16)). However, on the basis of the vitamin A activity of yellow corn, the presence of carotenes had been predicted (Euler, Demole, Karrer, and Walker, (4)).

Kuhn and Grundmann (20) had shown that fresh corn does not contain more than traces of carotene but does contain considerable amounts of cryptoxanthin. Buxton (2) reported that cryptoxanthin constitutes approximately ninety per cent of the vitamin A-active components of corn.

Lutein (leaf xanthophyll) is the most common xanthophyll and is always found associated with chlorophyll *a* and *b* in every green plant. Zeaxanthin, its isomer, is the pigment present in largest amounts in corn. Although lutein had never been associated with corn, the results of this investigation warrant its consideration. Both zeaxanthin and lutein are inactive as provitamin A. Their formulae are given:



Zeaxanthin (Karrer (10))



Lutein (Karrer, Zubrys, and Morf (17))

It is interesting to note the structural relationship of the corn pigments. Cryptoxanthin is monohydroxy-β-carotene, zeaxanthin is the dihydroxy- derivative, while lutein in the dihydroxy-α-carotene.

SEPARATION AND DETERMINATION OF CAROTENOIDS

All methods for the determination of carotenoids are based on the differential distribution of the pigments in a petroleum-alcohol system. In such a partition, carotenes, lycopene, cryptoxanthin, and xanthophyll esters are found to be epiphasic, being forced into the petroleum phase by dilution of the alcohol with water. Saponification and washing with ninety per cent methanol removes all of the xanthophylls except cryptoxanthin.

Typical of such methods is the Peterson, Hughes, and Freeman modification of the Guilbert method (24) for the determination of carotene. It is described briefly somewhat as follows:

The finely ground material is saponified with alcoholic potash, filtered, and the residue washed with alcohol until the filtrate comes through colorless. The filtrate is extracted repeatedly with

Skellysolve B (b.p. 40-60°) until a test portion of the extract becomes colorless when washed completely with 90 per cent methanol. The petroleum fractions are combined and washed with water until free of alkali. The xanthophylls are removed by extracting with 90 per cent methanol until the wash alcohol comes off colorless. The skellysolve solution containing the carotene is washed with water to remove the alcohol and dried by filtering through anhydrous sodium sulfate into a volumetric flask. The solution is then brought to volume and the concentration determined spectrophotometrically (Peterson, Hughes, and Freeman (24)) or colorimetrically (Fraps (6)).

This method and other similar methods are applicable when either carotene or cryptoxanthin is the sole petroleum-soluble pigment present, but is unsuitable for provitamin A assay when both are present, since no provision is made for the separation of the two from mixtures.

Kuhn and Grundmann (19) have pointed out that cryptoxanthin can be removed from a mixture of cryptoxanthin and β-carotene in petroleum ether solution by washing with ninety-five per cent methanol.

CHROMATOGRAPHY

Adsorbents

Workers in the field of carotenoid chemistry are indebted to Tswett (29) for the discovery that if a carotenoid mixture in carbon disulfide, benzene, or petroleum ether is poured through an evenly packed column of various powdered materials, a chromatographic distribution of the pigments into definite zones is accomplished by a preferential adsorption of the pigments by the material. This finding made possible rapid progress in a field handicapped for lack of methods for separating such closely related pigments.

In the choice of an adsorbent one proceeds somewhat empirically. Theoretically, any pulverized material may be used as an adsorbent providing it is insoluble in the solvent to be used and providing it exercises no destructive effect upon the solvent or on the adsorbed materials. In practice, one selects not the most "active" adsorbent but rather one of medium adsorbability. This gives good retention of the components which, by washing with pure solvent, are carried through

the column with moderate speed and thus permits the mechanical separation of the layers and facilitates the elution of the single components from the adsorbent.

Tswett tested over one hundred adsorbents. Several authors champion a particular adsorbent, the most commonly used materials being (32):

Aluminum Oxide (also hydrated). Alumina is perhaps the most widely used adsorbent because of its usefulness in aqueous as well as anhydrous media. The preparations of different workers in the field as well as the commercial grades vary widely in their adsorptive power, so that the quality of the material should always be exactly defined.

Aluminum oxide of the firm Merck "standardized according to Brockmann" proves to be an adsorbent of remarkably constant properties, since it is brought to a constant water-content by heating (allegedly in a stream of CO_2) and is brought to a desirable activity by a study of its behavior toward a suitable, pure pigment. This product is, however, not cheap and is often used in combination with ordinary commercial varieties, particularly since it is too active for certain purposes.

It may be observed that the standardized Al_2O_3 of Cahn and Phipers (3) reacts more strongly alkaline

than ordinary varieties, and precautions must be observed if an alkaline-sensitive substance is to be studied.

A very important development in the technique of activation was developed by Ruggli and Jensen (26) who washed the oxide with tap water and then strongly heated the material. For most purposes a single treatment will suffice but may be repeated.

Very frequently it is found that the surface of an Al_2O_3 is too active and must be de-activated. This is attained, according to Heilbron and Phipers (9), by washing Brockmann standardized Al_2O_3 with methanol and drying in air. Another common method for decreasing the activity of an adsorbent is to adulterate the adsorbent with some inactive material such as silicious earth.

Magnesium Oxide. This has been recommended by Euler and Gard (5) and especially by Strain (27). It has different activities depending on the mode of preparation. If the metal is simply burned, the oxide possesses only slight activity. This is also true of the product obtained from the carbonate by heating at a higher temperature. Best results have been obtained by the removal of water from magnesium hydroxide at a moderate temperature. An American product (Micron

brand magnesium oxide, No. 2641, California Chemical Corporation, Newark) is recommended. Ordinary market material is suitable for most purposes.

Calcium Hydroxide. According to Karrer and Walker (15), ordinary commercial slaked lime is a fine adsorbent and possesses, over the more frequently used oxide, the advantage of cheapness. For the study of water-containing liquids it can, of course, not be used.

Calcium Carbonate. Calcium carbonate, used often by Tswett, is a mildly active adsorbent. Moist preparations work poorly and must be heated a few hours at 150°.

For some purposes, it may be advisable to use more than one adsorbent in the same column, one above the other.

Chromatographic Techniques

Tswett columns are prepared by inserting a wad of cotton into the constricted stem of a pyrex glass tube (15 cm. x 2 cm.), then adding small portions of the dry adsorbent and mechanically pressing it tightly and uniformly with the aid of suction from below. Care must be exercised to insure that the edges are packed

as firmly as the center of the column otherwise the pigments will adsorb in inverted cone-shaped zones which will hinder the mechanical separation of the individual fractions. Frequently the joints between successive portions of adsorbent become very pronounced as soon as the medium is added and detract from the appearance of the column. These packing lines may be minimized by loosening the packed material to a depth of about 3 mm. before each successive addition of adsorbent.

In preparing a chromatogram, the solution is concentrated to a few milliliters by reduced pressure distillation free from air, and is then poured directly on to the column. The flask is rinsed thoroughly with several small portions of the solvent and these added to the column. The column is developed by drawing pure solvent through it under suction. It is recommended by Miller (23) that the entire process be carried out in an inert atmosphere to guard against oxidation of the pigments in air.

After the pigments have separated sufficiently, the column is divided into the individual zones mechanically. Pigments may be removed from the separated fractions by elution of the adsorbent with alcohol. After filtering, the concentration may be determined spectrophotometrically

in a suitable solvent.

The position which a pigment takes in a column is always the same for a given mixture. The higher oxygenated pigments are the more strongly adsorbed (occupy the uppermost portion of the column) and arrange themselves in order of decreasing oxygen content. In another series--hydrocarbons, for example--the least saturated pigment adsorbs first.

Winterstein and Stein (31) have made a study of the relationship of chemical constitution, structure, and behavior on a column. The following partial adsorption series of the carotenoids from a solution of petroleum ether is taken from the work of Winterstein (30):

Most strongly adsorbed	Fucoxanthin	$C_{40}H_{56}O_6$	Alcohols on $CaCO_3$
	Violaxanthin	$C_{40}H_{56}O_4$	
	Flavoxanthin	$C_{40}H_{56}O_3$	
	Zeaxanthin	$C_{40}H_{56}O_2$	
	Lutein	$C_{40}H_{56}O_2$	
	Cryptoxanthin	$C_{40}H_{56}O$	
	Lycopene	$C_{40}H_{56}$	Hydrocarbons on Al_2O_3
	-carotene	$C_{40}H_{56}$	
	-carotene	$C_{40}H_{56}$	
Most weakly adsorbed	-carotene	$C_{40}H_{56}$	

By using columns of very small diameter (about 1 mm.), Strain claims to have been able to separate as little as 0.0015 mg. of carotene from carrots into the alpha and beta isomers.

Gillam and El Ridi (7) caution that in chromatographic methods care should be taken to differentiate between genuine separations and transformations in the pigment caused by the adsorption process.

ABSORPTION SPECTRA

Like other compounds containing conjugated systems of double bonds, the carotenoids absorb light of definite wave lengths. These absorption properties of the carotenoids are typical and lend themselves ideally to the identification and quantitative determination of the carotenoid pigments. Spectrophotometric methods are particularly applicable to the pure pigments as separated by fractional chromatographic adsorption.

Recent literature treats absorption spectra of the various pigments by reporting the points of maximum absorption (extinction or absorption maxima) which are independent of concentration or stratum thickness. The absorption maxima of the pigments of corn are given in

Table 1.

The absorption of light is usually represented by a curve obtained when the logarithm of the specific absorption is plotted against the wave length. However, the preferred method is the one usually used by Miller (22) in which the specific absorption coefficient (α), rather than its logarithm, is plotted as the ordinate. Thus from Beer's law:

$$I_x = I_o\, 10^{-\alpha cx}$$

$$\alpha = \frac{\log_{10} I_o/I_x}{cx}$$

$$\alpha = D/cx \quad \text{where D (optical density)} = \log_{10} I_o/I_x$$

I_o = intensity of light transmitted by solvent cell
I_x = intensity of light transmitted by solution cell
x = thickness of solution layer in centimeters
c = concentration of carotenoids in grams per liter

Kuhn and Grundmann (19) stated "cryptoxanthin may be easily mistaken for β-carotene because its absorption spectrum is practically identical with it and zeaxanthin" (See also Table 1). Absorption spectra, therefore, are not applicable to the positive identification of the corn carotenoids; however, other properties namely, position of adsorption on columns, and adsorption

behavior when mixed with known pigments, prove the identity of the pigment. In this study, therefore, the spectrophotometer was used primarily to determine the optical density of the solutions at maximum absorption. From this the concentration of the solution could be calculated using the specific absorption coefficients (α's) of the literature. The specific absorption coefficients for the different pigments as given in Table 2 are, for the most part, those obtained by interpolation from the absorption curves of various authors.

Table 1. Absorption maxima of the pigments of corn

Solvent	β-carotene			Cryptoxanthin			Zeaxanthin		
CS_2	521	485.5		519	483	452	519	483	450
$CHCl_3$	497	466		497	463	433	494	462	429
C_5H_5OH (abs.)				486	452	424	483	451	423
Petroleum ether	483.5	452	426	485.5	452	424	483	451	
Hexane	482	451		484	451	423			

Table 2. Specific absorption coefficients of the carotenoids (25). (Wave length in Angstroms).

Pigment	Solvent	1st Maximum	Minimum	2nd Maximum
Flavoxanthin	Ethanol	4370	4540	4670
	α	(229)	(116)	(234)
Zeaxanthin	Ethanol	4530	4690	4810
	α	(244)	(200)	(218)
Lutein	Ethanol	4465	4625	4750
	α	(257)	(182)	(234)
	Ether	4450	4600	4740
	α	(260)	(182)	(234)
Cryptoxanthin	Ethanol	4520	4700	4820
	α	(254)	(209)	(226)
	Heptane (2)	4555	4735	4845
	α	(252)	(207)	(221)
β-carotene	Heptane (2)	4515	4720	4805
	α	(240)	(196)	(213)
	Skelly-solve (24)	4500	4700	4800
	α	(238)	(200)	(212)

EXPERIMENTAL PROCEDURE

The corn used in this research was an ordinary yellow corn (referred to as Stock sample) such as might have been purchased at any feed store. After considerable preliminary experimentation, this method for determining pigment distribution which is an extension of the Peterson, Hughes, and Freeman modification of the Guilbert method was devised:

One hundred grams of the finely ground sample was saponified by refluxing with 400 cc. of saturated alcoholic potash (10% KOH) for thirty minutes. The saponified mixture was cooled and filtered on a sintered-glass funnel. The residue on the funnel was washed alternately with 50 cc. portions of skellysolve and alcohol until the filtrate came through colorless. All filtrates were combined in a separatory funnel and the skellysolve was induced to separate by dilution with 100 cc. of water. The hypophase was drawn off and extracted repeatedly with 50 cc. portions of skellysolve until a single extraction contained no pigment which was not removable with ninety per cent methanol.

The petroleum fractions were combined and washed

with 90% methanol until the methanol wash came off colorless. The methanolic wash and the alcoholic residue were retained to be examined for xanthophylls. Final traces of alcohol were washed from the epiphase with water and the skellysolve solution was dried by filtering through anhydrous sodium sulfate.

The petroleum solution was concentrated to 5-10 cc. by reduced pressure distillation and then chromatographed under nitrogen. A mixture of magnesium oxide (Micron Brand No. 2641) and silicious earth (1:1) was used for the adsorbent. The distilling flask was rinsed with a few milliliters of skellysolve and this added to the column. Pure solvent was then drawn through the column under suction until the two zones which developed (see Plate I) had drawn apart sufficiently so that they could be separated mechanically. Separation was accomplished by simply loosening the adsorbent through a given zone with a square-ended spatula and transferring the material to a 150 ml. beaker. The pigments, thus differentiated, were freed from the adsorbent by elution with ethanol, filtered, and the solid material washed thoroughly with alcohol. The pigments were re-extracted with skellysolve, washed with water, and finally dried by filtering through anhydrous sodium sulfate into a

volumetric flask. The solutions were made to volume and their concentrations calculated from the optical densities at 4500 Angstroms as determined on a Bausch and Lomb visual spectrophotometer. At a wave length of 4500 Å. the extinction coefficients of β-carotene and cryptoxanthin are identical and have a value of 238.

For the determination of the xanthophylls, the methanolic washes and the alkaline residue were extracted completely with ether. (Before the ether would form a layer it was necessary to dilute greatly with water.) The ether was completely removed by reduced pressure distillation. The same techniques as described above were employed in the chromatographic study of the xanthophylls except that dichloroethane was substituted for skellysolve as the solvent.

Again the column zones were separated, eluted with alcohol, filtered, and re-extracted back into ether, in which medium the concentration was determined again spectrophotometrically using the extinction coefficients for the respective pigments in ethanolic solution as reported in the literature (See Table 2).

Finally, this method was applied to the determination of the pigment distribution of seven select samples of seed corn from different parts of the United States.

The results are tabulated in Table 3.

DISCUSSION AND CONCLUSIONS

This was intended as an investigation of the vitamin A-potentialities of a corn. Consequently it concerned itself primarily with the petroleum phasic pigments, β-carotene and cryptoxanthin. As a result, the various experimental techniques were tried on the petroleum fractions of corn. Certain technical conclusions can be drawn from this preliminary experimentation:

(1) Seven days' saponification at ice box temperatures gave the same β-carotene-cryptoxanthin distribution as did one-half hour's refluxing. (2) Ordinary distillation and reduced pressure distillation under nitrogen as compared with reduced pressure concentration had little effect.

(3) Different adsorbents coupled with different methods of activation gave the same results except under extreme conditions. Calcium carbonate (Buxton, 2) and aluminum oxide (Kuhn and Brockmann, 18) were ineffective in retaining cryptoxanthin and β-carotene unless they had been previously activated at 250° C. for a period of ten hours. Both of these materials possessed

the additional disadvantage of extreme fluffiness and presented a mechanical difficulty in packing the adsorption column firmly. Strain's magnesium oxide activated at temperatures from -27° C. to 300° C. had the same adsorptive effects on the corn pigments; however, on a magnesium oxide column which had been activated at 530° C., there was a visible bleaching of the carotenoids as they entered the column, β-carotene being affected most.

Only two zones were obtained on chromatographing the petroleum extract of corn (Plate I, Fig. 1). This had been anticipated since it was known that cryptoxanthin and β-carotene are present in corn and that both are petroleum phasic. Conclusive identification of the pigments was effected however, not by their absorption spectra, but by their adsorption properties. Column-pure β-carotene from alfalfa when added to a petroleum extract of corn adsorbed with the least strongly adsorbed corn pigment as is readily discernible in Plate II, Fig. 4. There was frequently encountered in this study, a faint zone which preceded β-carotene in the column and was inseparable from it; this may have been the pseudo-carotene of Gillam and El Ridi (7).

EXPLANATION OF PLATE I

Fig. 1. A chromatogram of the petroleum-soluble pigments of corn.

Fig. 2. A chromatogram of the corn xanthophylls.

PLATE I

Fig. 1

Fig. 2

EXPLANATION OF PLATE II

Fig. 3. A chromatogram of the petroleum-soluble pigments of corn.

Fig. 4. A chromatogram of the petroleum-soluble pigments of corn to which had been added some column-pure β-carotene from alfalfa.

PLATE II

Fig. 3

Fig. 4

The absorption spectra of cryptoxanthin and β-carotene of corn vary from those of the same pigments in heptane as given by Buxton (2). These curves vary most in the region of the second maximum and it has been assumed in calculating concentrations of solutions that the curves are real at the first maximum. Zechmeister and Tuzson (33) have shown that solvent effects alone may distort the absorption spectrum of a pigment and that this distortion is greater in the longer wave length regions. It might therefore be assumed that these different curves are inherent properties of the corn extracts and have not been induced during columnation.

Contrary to anticipations based on statements in the literature to the effect that zeaxanthin is the xanthophyll of corn, a chromatographic study of an ether extract of the alcoholic pigments of corn revealed the presence of not one but four xanthophyllic carotenoids (Plate I, Fig. 2). Taken in the order of their adsorption on a column, they are:

A strongly-adsorbed pigment whose absorption spectrum shows maxima at 4550 and 4475 Å. and falls off rapidly in the longer wave lengths. This pigment constitutes from 4-14 per cent of the total pigments of

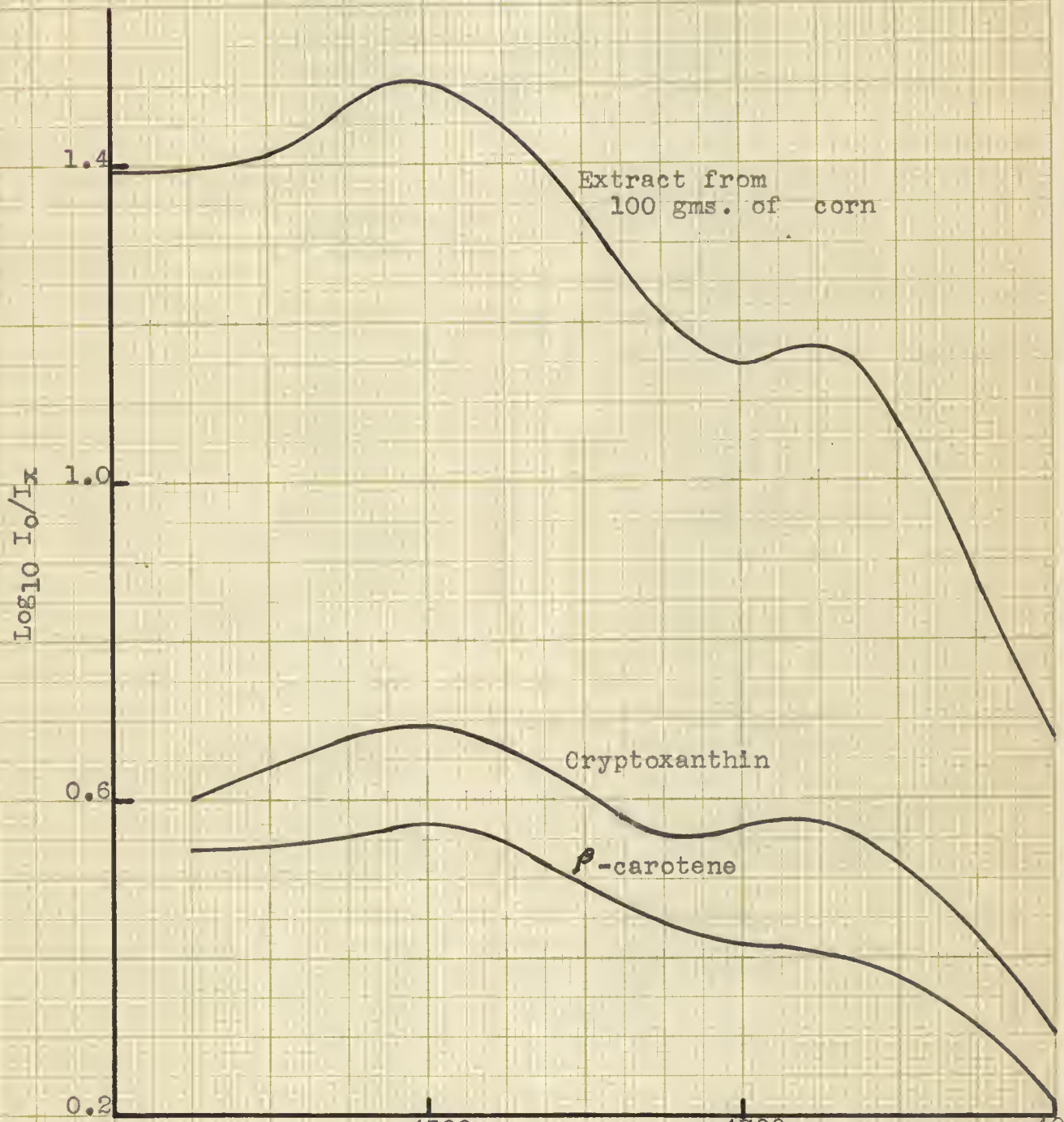
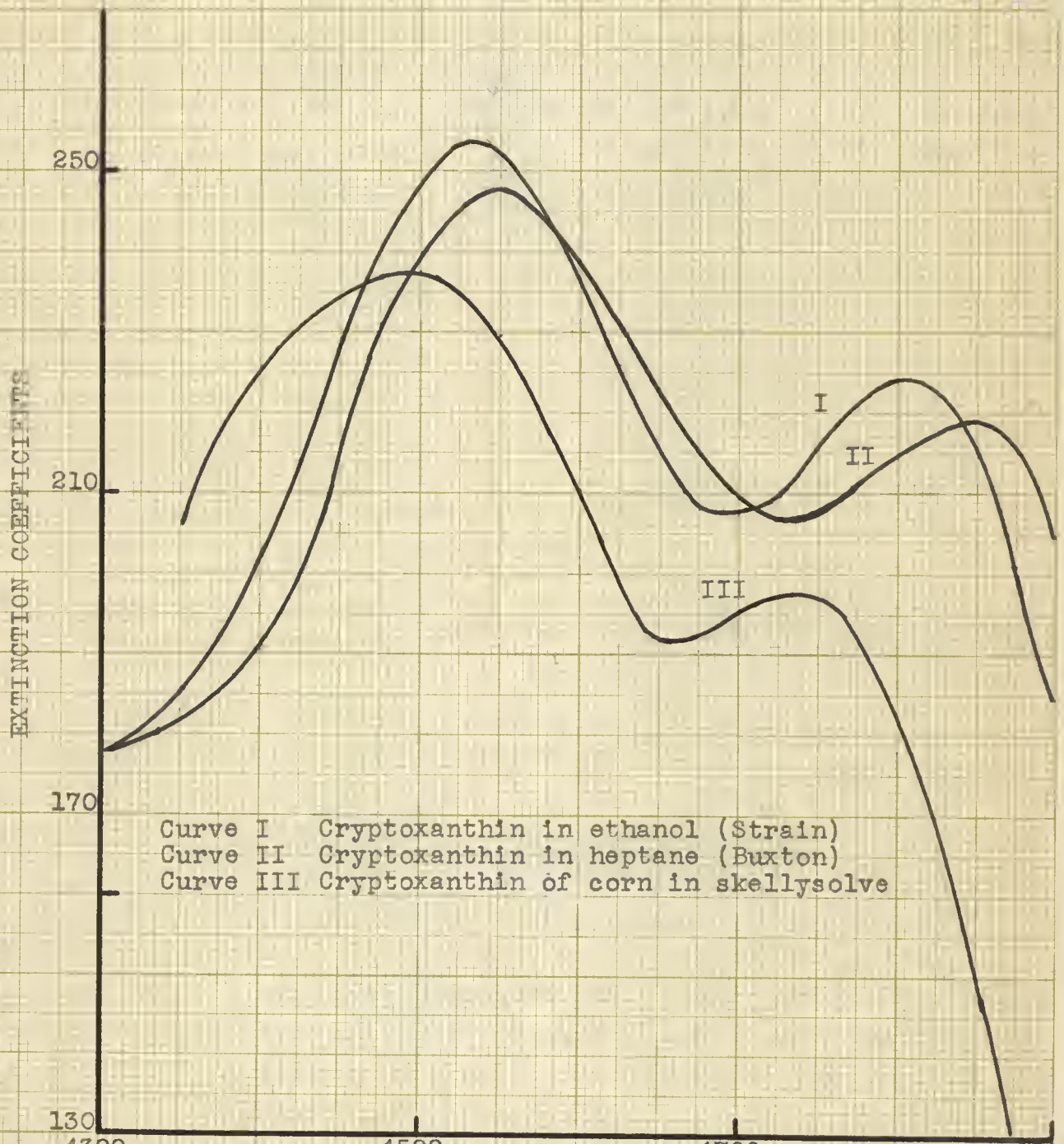


Fig. 5. Curves of the petroleum-soluble pigments of corn.



Curve I Cryptoxanthin in ethanol (Strain)
Curve II Cryptoxanthin in heptane (Buxton)
Curve III Cryptoxanthin of corn in skellysolve

Fig. 6. Absorption spectra of cryptoxanthin.

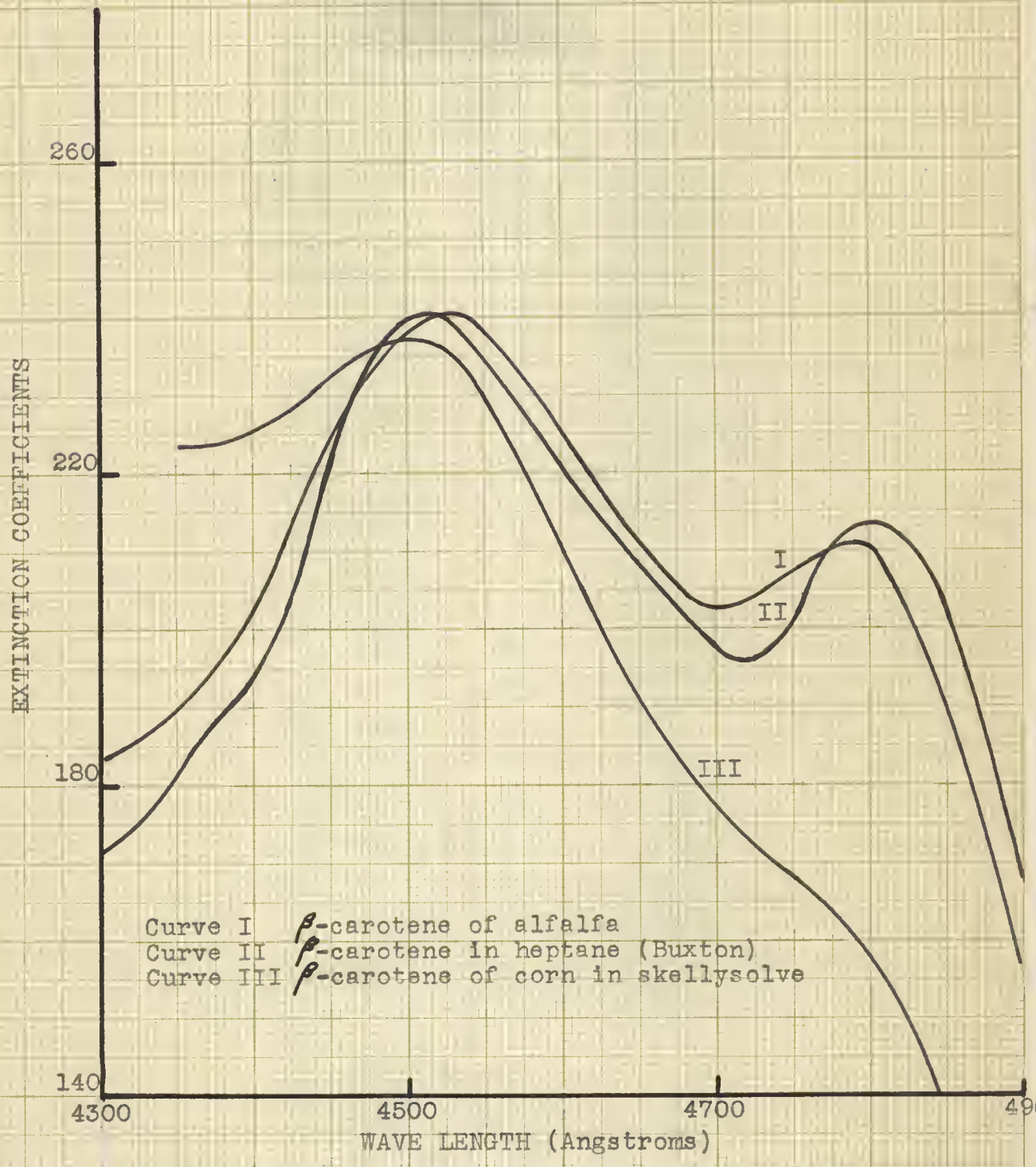


Fig. 7. Absorption spectra of β-carotene.

FURTHER EXPLANATION OF FIG. 8

——— Xanthophylls of corn in ether.

– – – – – Xanthophylls in ethanol by Strain, except for lutein whose absorption spectrum is for ether solution.

The extinction coefficients of the corn pigments are based on the assumption that the specific absorption coefficients of the pigments in ether and in ethanol are identical at 4500 Å.

The left ordinates give the values of the extinction coefficients for zeaxanthin and cryptoxanthin, while the extinction coefficients for the upper fraction and for lutein are to the right of the graph.

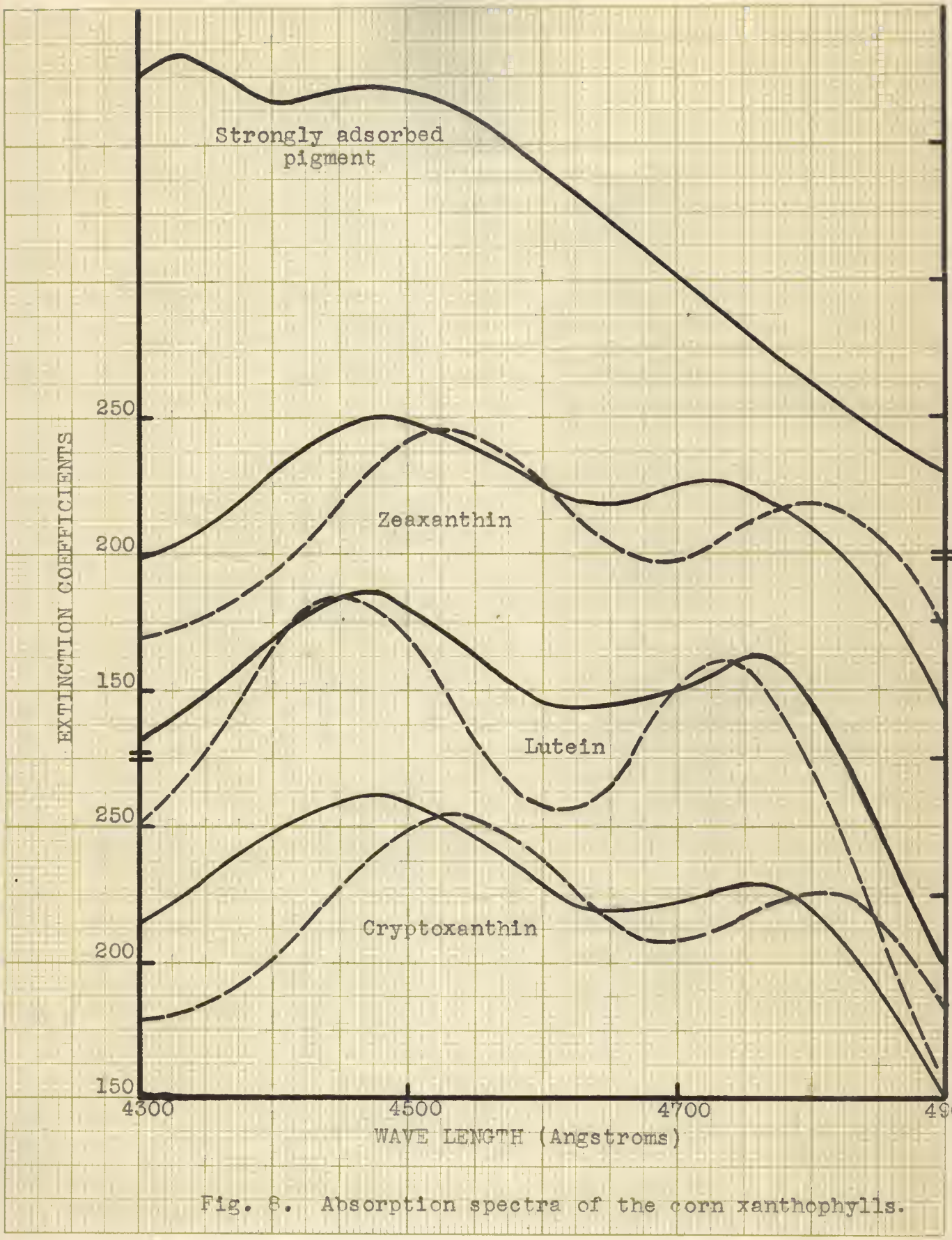


Fig. 8. Absorption spectra of the corn xanthophylls.

of corn based on the assumption that the sum of the various column fractions equals the total extractable pigment of corn.

The position of adsorption, color, and absorption spectrum of the second zone indicated that it was zeaxanthin. In addition, a color reaction of a solution of this pigment in dichloroethane with sulfuric acid was the same as that of zeaxanthin. However, instead of constituting by far the bulk of the corn carotenoids as reported in the literature, these results showed it to be present in corn only to the extent of about thirty-one per cent.

Lutein adsorbs below zeaxanthin on columns. The third column fraction of corn xanthophylls gave an absorption spectrum which resembled that given by Strain for lutein in ether solution. Furthermore, a qualitative test for lutein (Strain, 28) based on a color reaction of a dichloroethane solution with sulfuric acid was also given by this corn fraction, which constituted about 34% of the total corn carotenoids.

A solution of the least strongly adsorbed xanthophyll of corn when added to the column-separated petroleum-soluble cryptoxanthin and re-adsorbed on a column, gave a single line which showed them to be identical.

Table 3. Distribution of the carotenoids of corn.
The percentage distribution of the pigments is based on the assumption that the sum of the individual constituents accounts for all of the extractable pigments of corn.

Sample	Petroleum-Soluble		Alcohol-Soluble Pigments			
	Cryptoxanthin (mg/100g)	β-carotene (mg/100g)	Strongly adsorbed pigment	Zeaxanthin (mg/100g)	Lutein (mg/100g)	Cryptoxanthin (mg/100g)
Stock	0.291	0.239	0.175	0.486	0.657	0.054
%	15.46	12.79	9.30	25.82	33.82	2.87
CE 2865	0.179	0.167	0.250	0.520	0.569	0.131
No. 8 %	9.85	9.18	13.77	28.63	31.36	7.21
CE 2870 Midland (Anderson Co.)	0.148	0.146	0.093	0.523	0.572	0.030
%	9.77	9.64	6.15	33.87	37.80	1.98
CE 2873 Reid Yellow	0.060	0.185	0.100	0.510	0.830	0.232
Dent %	3.13	9.65	5.21	26.60	43.28	12.10
CE 2875 Maya	0.389	0.202	0.083	0.531	0.475	0.045
Golden %	22.62	11.70	4.81	30.79	27.51	2.61
CE 2876	0.118	0.136	0.168	0.598	0.472	0.089
US 44 %	7.49	8.46	10.47	37.75	29.95	5.68
CE 2885	0.158	0.093	0.097	0.566	0.406	0.085
US 13 %	11.25	6.61	6.90	40.60	28.90	6.05
CE 2887 Iowa 939	0.172	0.077	0.137	0.381	0.516	0.119
%	12.27	5.49	9.78	27.19	36.80	8.50

In fact, from 10-60 per cent of the cryptoxanthin was shown to have been retained in the alcoholic fraction. The presence of cryptoxanthin among the xanthophylls immediately disqualifies any chemical vitamin A-assay which concerns itself only with the petroleum-soluble pigments. Table 4 gives the total and relative amounts of cryptoxanthin and β-carotene in these corn samples and includes the calculated vitamin A potencies of the corn. This chemical assay lacks biological verification.

Table 4. Relative distribution of vitamin A-potent carotenoids of corn.

Sample	Cryptoxanthin		Carotene	Vitamin A
	Absolute (mg. per 100 gms.)	Percentage	Absolute (mg. per 100 g.)	(Calc. in Internat'l units/100 g.)
Stock	0.345	59.1	0.239	685
CE 2865	0.310	65.0	0.167	536
CE 2870	0.178	54.9	0.146	391
CE 2873	0.292	61.2	0.185	551
CE 2875	0.434	68.2	0.202	698
CE 2876	0.207	60.4	0.136	398
CE 2885	0.243	72.3	0.093	358
CE 2887	0.291	79.0	0.077	371

SUMMARY

1. Some cryptoxanthin is found in the alcoholic fraction of corn extract; therefore, any provitamin A-assay which concerns itself exclusively with the petroleum fraction is inadequate.

2. The relative amount of β-carotene in corn is greater than has been previously reported. The β-carotene:cryptoxanthin distribution is about 1:2.

3. Lutein, previously unreported in corn, is shown to be one of the predominant corn carotenoids.

4. The presence of an unidentified, strongly-adsorbed xanthophyll of corn is reported for the first time.

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