A STUDY OF THE CAROTENOID PIGMENTS OF WHEAT AND FIVE TYPES OF RUST SPORES

A Thesis submitted
to the Committee on
Post-Graduate Studies of
the University of Manitoba

By

Harold Johannson

In partial fulfilment of the requirements for
the Degree of Master of Science

May, 1935.

ACKNOWLEDGEMENTS

The author expresses his gratitude to Dr. W. F. Geddes, Chemist in Charge, Dominion Grain Research Laboratory, Winnipeg, for the suggestion of the problem and for his continued interest and help in its solution.

He wishes to express his sincerest thanks and appreciation to Mr. D. S. Binnington, National Research Council, for his invaluable assistance in all phases of the study.

Thanks are also due to Mr. L. D. Sibbitt for his help in preparing the photographs of the spectral distribution curves.

TABLE OF CONTENTS

A STUDY OF THE CAROTENOID PIGMENTS OF WHEAT AND FIVE TYPES OF RUST SPORES

INTRODUCTION

The visual colour of flour or its products can be attributed to some five factors, namely: (1) granulation, (2) moisture, (3) bran particles, (4) foreign matter, and (5) carotenoid pigments.

Granulation exerts a considerable influence on the colour of flour. When the particles are large and coarse the flour looks dull and grey, but as they become finer the flour appears whiter. This effect of granulation is due to the difference in the refraction of light by the dense particles and the air surrounding them and also to the large particles casting shadows.

The effect of moisture is pronounced and can be shown by the familiar Pekar test. Here, with a decrease of grade, the flour becomes darker. According to Bertrand and Mutermilch (1907), the joint action of a glutenase and tyrosinase results in the formation of a brown pigment.

The third factor is bran particles, and is apparent in the case of the milling of "red" wheats.

Foreign matter such as dirt, soil and smut spores necessarily affect the colour, depending of course on the amount present.

The last factor contributing to flour colour is carotenoid pigmentation. The content of these pigments will determine

the yellowness of a flour. Monier-Williams (1912) concluded that carotene was the major pigment of flour. The quantity present was found by Winton (1911) to vary considerably with the type of wheat.

It should be noted the first four factors mentioned may be eliminated or controlled during the process of milling, but the pigments present in the endosperm will appear in the flour. These again have to be reduced by bleaching, due to the public demand for white bread. In contrast to this, semolina for macaroni manufacture should be highly pigmented. Thus great importance is attached to this property of wheat, and a measure of this yellow colour becomes necessary, but before a method can be truly satisfactory the actual pigments present need to be determined.

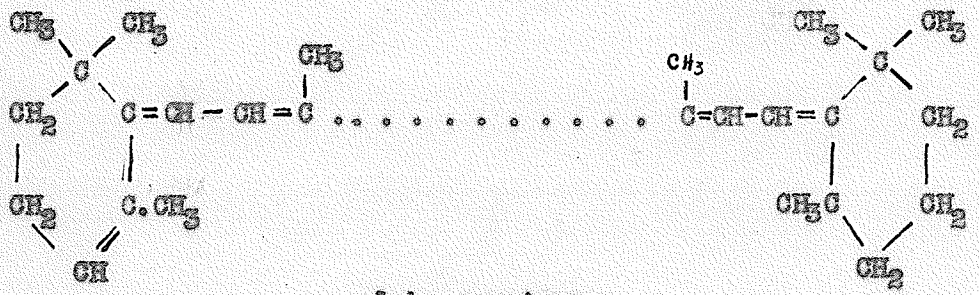
A review of the literature on wheat and flour pigments is rather confusing, since it shows pronounced differences of opinion. However, before proceeding further it might be as well to review briefly the properties of Carotene and Xanthophyll and then to consider the work done on wheat pigments with these in mind.

SUMMARY OF THE PROPERTIES OF CAROTENE AND XANTHOPHYLL

(1) Carotene

Willstalter and Mieg (1907) first settled the problem of its formula and showed it to be a hydrocarbon $C_{40}H_{56}$. Quite recently it has been found that this pigment is composed of two isomers, alpha, an optically active form, and beta, optically inactive. The question of the structure is not definitely settled but the following formulae are generally accepted:

beta carotene



alpha carotene

Table I shows some of the properties of the two isomers.

Table I

Property	α form	β form	Workers
Melting point	174-5°(corr.)	182° (uncorr.)	Kuhn & Lederer
Optical rotation	$[\alpha]^{18}_{645} = 328$	optically inactive	Kuhn & Lederer
in benzene	$[\alpha]^{20}_{cd} = 365$		Karrer & Morf.
Solubility in hexane at 0°.	2.94 mgm/cc.	1.09 mgm/cc	Kuhn & Lederer
Points of maximum intensity of absorption bands in CS_2	511 mμ. 478 mμ	521 mμ 485.5 mμ	Kuhn & Lederer

Carotene forms highly coloured solutions in ether, chloroform, petroleum ether, benzene carbon tetrachloride and carbon disulphide as well as in ethereal and fatty oils and oleic acid. The colour will vary according to the concentration, from yellow to golden yellow, except in the case of carbon disulphide where it is orange red to blood red.

Carotene is characterized by the ease with which it takes up oxygen. On oxidation the melting point falls and the pigment changes markedly in properties. Schertz (1925) investigated the stability in various solvents and found that absolute ethyl alcohol or petroleum ether solutions are extremely stable if kept in an ice box.

A most important property is that of adsorption. According to Tswett, carotene is not adsorbed by calcium carbonate, whereas xanthophyll is. This fact forms the basis of his so-called chromatographic analyses. Schertz has found some discrepancies in the literature and suggests more work be done before it be accepted.

Solutions of carotene exhibit characteristic absorption spectra, and it is this property which is usually used in its identification. The concentration of carotene in natural products is extremely low and, therefore, isolation as a crystalline product is difficult.

McNicholas (1931) published the spectral distribution curves for carotene and xanthophyll, and the changes in these curves with oxidation. At that time the existence of the isomers of carotene was not known, but recent workers have suggested that his prepartion was composed of approximately 30% of the alpha form. Throughout this study, McNicholas' pigment curves have been used as a basis of comparison,

because only in the last few weeks have the absorption spectra of the isomers become available.

(2) Xanthophyll

Xanthophyll $C_{40}H_{56}O_2$ is a pigment usually associated with carotene and very similar to it in its behaviour and properties.

There are some solubility differences of the pure pigments. Schertz's figures are given in Table II.

Table II

Solubility expressed in mgms. per litre.

Solvent	Xanthophyll	Carotene
Petroleum ether	9.5	626.0
Absolute ethyl alcohol	201.5	15.5
Absolute methyl alcohol	134.9	-
Pure anhydrous ether	952.0	1005.0

The figures for carotene are determined on the pigment extracted from carrot roots and this is composed of approximately 30 per cent of the alpha form.

These figures do not hold in the presence of fats, which are generally present in extracts of plant material. Hence the difficulty in separating the two on the basis of solubility differences if lipoid matter is present.

The absorption spectra is very similar to carotene, so much so, that early workers have mistaken one for the other. The bands are shifted slightly toward the blue end of the spectrum with respect to carotene.

Karrer and his co-workers (1930) have shown that xanthophyll is very commonly found as esters of the fatty acids. These have been

named helenien and physalien. They are widely found in plants and have many of the properties of true fats. The esters are said to be more soluble in hydrocarbon solvents than in alcohols.

REVIEW OF WORK ON WHEAT PIGMENTS

Monier-Williams (1912) compared the absorption spectra of a petroleum ether extract of flour with that of carrot carotene and assumed them to be identical.

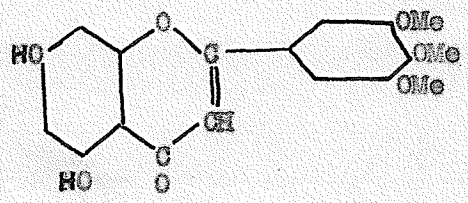
Palmer (1922) identified xanthophyll in wheat bran and stated that in this bran, xanthophyll constituted the major and carotene the minor fraction of the yellow pigments.

Coward (1924) stated that no carotene nor xanthophyll could be found in the grain of a variety of English wheat known as Carter's "Red Stand-up". The method of extraction consisted of saponification with 20% KOH on a steam bath for several hours, under a reflux condenser, then extracted with alcohol and the pigments transferred to ether and then petroleum ether. The xanthophyll was separated by adsorption in a Tswett calcium carbonate tower. This hot saponification may have been too drastic a treatment, and led to oxidation and consequent destruction of the carotenoids.

Kent-Jones and Herd (1927) used alkaline methanol for extraction and concluded that the major pigment was xanthophyll. Their solutions were brownish-yellow in alkaline solution and nearly colourless in acid solution. This identification is questionable, since xanthophyll is greenish-yellow in that solvent and not brownish. Similarly, this pigment is not known to exhibit a colour change except in strong acid solution.

Ferrari (1929) compared the absorption spectra of gasoline extracts of flour with that of carrot carotene and concluded that they were the same, the position of the bands agreeing closely with the results of Willstätter and Stoll's work (1928) on that pigment. The bands of the flour extracts were, however, slightly shifted toward the violet end of the spectrum. Now, it should be noted that bands of xanthophyll are displaced slightly more toward the violet end of the spectrum than carotene, but Ferrari concluded that it was not the presence of this pigment which caused the shift in the absorption bands of his flour extracts. He thought it to be due to a density difference between petroleum ether solution of the pure pigment and the flour extract, and also that material other than pigments were dissolved.

Anderson and Perkin (1931) isolated an ether soluble flavone pigment from wheat; this pigment they called "Tricin". The subject of phenolic pigments in wheat have been little studied, since this subject is very new. Anderson (1932) presented the following formula for tricin.



Markley (1933), in a private communication, presented a method for a separation of the pigments of wheat, which he claimed to be reasonably quantitative. His conclusions were that xanthophyll was the major pigment present and carotene the minor. The method will be described in detail later but can be explained briefly here. The extraction is accomplished by shaking with naphtha for 18 hours, saponifying with alkaline methyl alcohol and centrifuging to break the

emulsion. The carotene is contained in the naphtha while the flavone pigments and xanthophyll are in the methanol layer. After dilution with water the alcoholic layer is extracted three times with ether. Markley asserts that the first extraction contains xanthophyll, and the other two, mixtures of xanthophyll and flavone. It would hardly seem probable that the first treatment should extract only the carotenoid. Further separation of the original alcoholic extracts yielded two more flavone fractions.

Markley is not definite as to the identity of the flavone fractions except one, which he calls "Tricin". He considers, however, that carotene and xanthophyll constitute 90 to 95 per cent of the absorption of light at 435.8 mu.

(Note After completion of this work the following references to wheat pigments were found:-

Bowden and Moore (1933) isolated xanthophyll from the oil of the embryo of wheat. They studied the spectral absorption and other properties of this oil and found it to be similar to xanthophyll from other sources.

Schulerud (1933), in determining the absorption spectra of alcoholic extracts of bran, found the bran pigment to be very similar to, if not identical with, Anderson's "Tricin".)

PROBLEM

It is apparent, from the review of the literature, that it is not settled whether carotene or xanthophyll constitute the major pigment of wheat. Also, there was found in this laboratory some evidence of the presence of one or more other pigments. This study was undertaken with the object of investigatin these points and of studying the effect

of different solvents on ground wheat, with a view to determining a method of separation of the pigments on this basis. Also, a small quantity of some four types of rust spores, high in carotenoid pigments, became available, which had been grown under two sets of conditions, presence and absence of light, and differed considerably in appearance. Thus a preliminary investigation of these differences was planned.

Later, the details of Markley's method became available and it was thought advisable to study it further.

APPLICATION OF THE SPECTROPHOTOMETER TO THE PROBLEM

By means of this instrument, it is possible to measure the relative amount of light transmitted in any wavelength by a cell containing a pigmented solution. Solutions of carotenoids have the greatest absorption of light in the blue to violet end of the spectrum, and therefore measurements are made in this range, since small differences will be apparent in the relative light absorption.

Transmission is defined as the ratio of the light passing through the last surface of the cell to the light incident on the first surface of the cell.

Transmittancy is then given by the relationship

$$\text{Transmittancy} = \frac{\text{Transmission of Solution}}{\text{Transmission of Solvent}}$$

Transmissivity is the "b" root of the transmittance where "b" is the length of the layer of liquid in centimetres

$$t = \sqrt[b]{T}$$

Specific Transmissivity is the "bc" root of the transmittance, i.e. $= \sqrt[bc]{T}$

where c = concentration in cgms per litre.

$$\text{Specific Transmissive Index } (K) = -\frac{\log T}{bc}$$

The equation $bcK = -\log T$ is known as Beer's Law and which Schertz (1925) found to hold true for dilute solutions of carotene. Thus knowing the value of K and the cell length, one can calculate the concentration of pigment present.

A further application of the instrument to this work is the identification of pigments by means of their spectral distribution curves. If their transmittancies are determined throughout a portion of the spectrum, and these values plotted against wavelength, a curve characteristic of the pigment is obtained.

DESCRIPTION OF THE SPECTROPHOTOMETER

Due to the great importance of the instrument in this study, a detailed description would not be amiss. It will be considered under two headings, namely: the spectrometer and the photometer.

(a) Spectrometer

This is of the constant deviation type where the collimator and telescope are permanently fixed at right angles to each other and the prism rotated, (Fig.1). The prism is quadrangular and is equivalent to two 30° prisms and a totally reflecting prism. The degree of rotation is measured on an drum, on which the wavelength scale is engraved. The illumination for the drum and the photometer scale is provided by a small lamp attached directly to the instrument. The collimator has an adjustable entrance slit permitting variation in the length of the field and intensity of illumination. A shutter eye-piece allows a rectangular section of any desired width to be viewed, and serves to limit the portion of the spectrum to be observed.

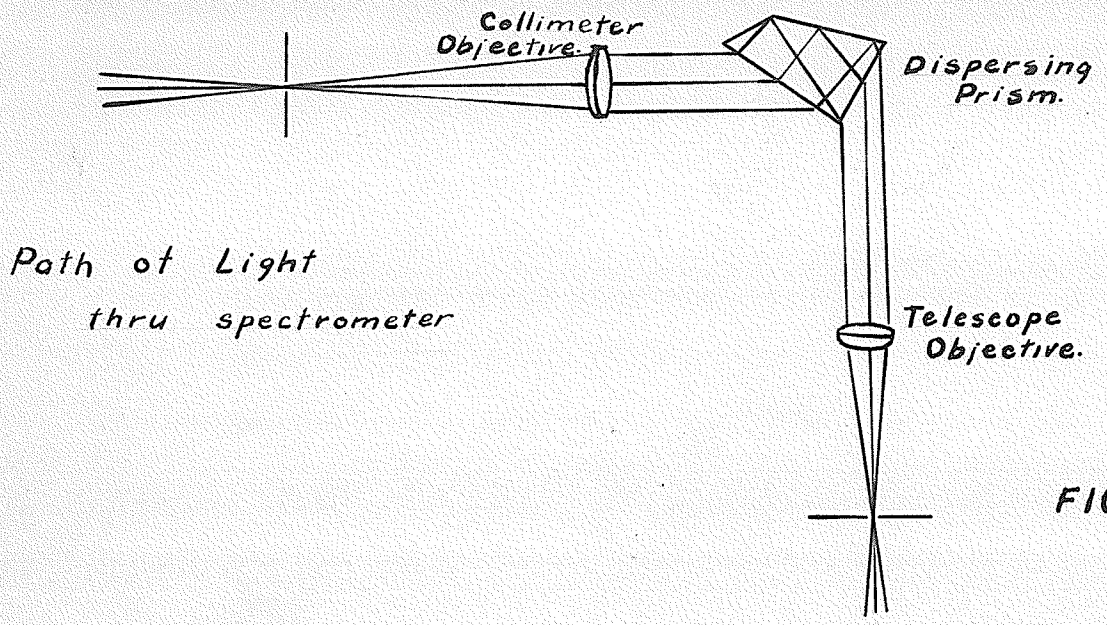


FIG. 1.

(b) Photometer

This part is of the Martens polarization type where the two beams of light are polarized at right angles to each other. The intensity of the two beams are adjusted by means of the rotation of a nicol prism, the movement of which is read on a divided circle. One quadrant of tnis circle is graduated in densities, a second in transmission percentage, and the third and fourth in angular degrees.

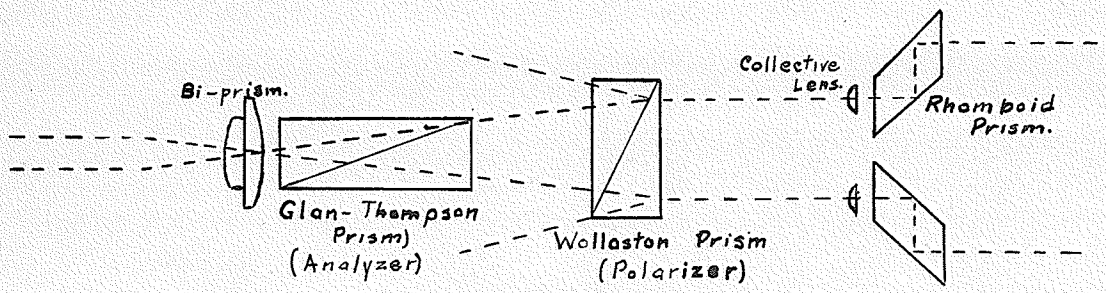


FIG. 2.

The beams of light which have passed through the cells are brought close together by two rhomboid prisms, (Fig. 2). The correct optical imagery is obtained by the two collective lenses. By means of the Wollaston prism, each beam is split into two parts, vibrating at right angles to each other. One part of each beam is lost within the instrument, the remaining parts, polarized mutually perpendicular, are brought by means of the biprism

and telescope lens to form the two halves of the photometric field which is viewed by the eye.

Since the two beams of light reaching the eye are polarized at right angles to each other, a rotation of the nicol will increase the brightness of one-half of the field while decreasing the other half, one becoming a maximum when the other becomes extinct and vice versa. It can be seen from this arrangement that a single rotation of the analyzer will result in the complete extinction of each field, or in four extinction or zero points, i.e., where the two halves of the field will be matched. The field that is seen is rectangular in shape and is of the type known as the juxtaposed spectra, the width of which is controlled by the shutter eye-piece.

The light source for concentration determinations is a mercury arc housed in a large sphere coated on the inside with magnesium oxide. This gives a very white even reflecting surface. The light passes out through a small ground-glass window placed slightly off centre, so as to prevent any direct illumination. For measurements of light absorptions at different wavelengths a Mazda photoflood lamp is substituted.

The speciment tubes are held in saddles which are adjustable vertically to bring them into correct alignment in the light paths. Two lenses then serve to focus these two parallel beams on the rhomboid prisms of the photometer.

OPERATION OF THE SPECTROPHOTOMETER

The first step in determining the transmittancy of solutions is to turn on the mercury arc lamp. This light source is used since it gives a light of very high intensity with a band which can be isolated at wavelength 435.8 mu. It is found necessary to allow the lamp to become hot before

readings are taken. If this is not done, droplets of mercury obscure the field and the intensity is lower. Five minutes is usually sufficient time for warming. The instrument is balanced without tubes by adjusting the lamp so that the photometric field is equally balanced at 4358 Å, reading 100% transmittancy. The balance is then checked by inserting first empty cells, then filling with solvent.

One cell is filled with the solvent and the other cell with the solution and placed in the saddles of the instrument. The photometer is adjusted so that the two halves of the field are of equal intensity. The percentage transmittancy is then read directly from the scale. The operation is repeated five times and then the tubes are interchanged in position. Five more readings are taken, the final value being the average of the ten determinations. If there is any inequality of illumination, this will be eliminated by interchanging the tubes, and similarly will avoid error in readings, due to polarization in the sample.

In obtaining data for a spectral distribution curve, the arc lamp is replaced by a Mazda "Photoflood" lamp which serves as a continuous source. The wavelength drum is set to a definite wavelength and 10 readings taken, 5 in each position, as previously mentioned. The drum is set again to another wavelength and 10 readings taken, and so on, until the entire visible spectrum is covered. Whether the intervals of wavelength are large or small will depend on whether the transmittancies are changingrapidly or not. For example, in the case of carotene solutions or wheat extracts, readings are taken at intervals of 100 A from 5400 A down to 5000 A because the change is quite gradual, but from 5000 to 4200 A at intervals of 50 A, since the change is rapid. The process of obtaining a spectral distribution curve is rather lengthy and great care should,

therefore, be exercised that evaporation of the solution does not occur.

Certain precautions must be noted in making these measurements. Some solvents such as petroleum ether, ethyl ether, which are very volatile, have a tendency to seep around the edges of the cover-glasses at the ends of the cell. This results in smearing of the outside of the end plates and prevents accurate work. It is, therefore, absolutely essential to have perfectly clean polished surfaces before making measurements.

Another factor which contributes to inaccuracy is the presence of any turbidity in the extract. The light is scattered by the suspended particles and results in transmittancy values which are too low.

MATERIALS AND METHODS

The material used throughout this study was Garnet wheat. This particular variety was selected since it has a moderately high pigment content.

All the grinding was performed in a Wiley mill, the product being reduced until it would entirely pass a sieve with circular holes measuring 1/2 mm. in diameter.

Method of Preparing Extracts

The method used was that outlined by Ferrari (1933) with slight modifications, and is as follows:

> "Twenty grams of the ground sample are weighed into a 250 cc. glass stoppered bottle and 100 cc. of solvent added from a pipette. The bottle is shaken frequently for one hour and allowed to stand in the dark overnight. In the morning it is re-shaken, allowed to settle, and the supernatant liquid decanted into 100 cc. lipless

centrifuge tubes, closed with rubber caps, and centrifuged at approximately 2200 r.p.m. for thirty minutes. The clear extract is siphoned off with a capillary bore siphon, having the shorter end bent upwards.

Solvents

Naphtha - This is a petroleum distillate known commercially as varnish makers' naphtha. The distillation range is given in Table III.

Table III.

Initial Boiling Point 316°F.

Temperature Range °F.	Per cent distilled over %
316 - 324	10
324 - 326	10
326 - 328	10
328 - 330	10
330 - 333	10
333 - 336	10
336 - 340	10
340 - 346	10
346 - 355	10
355 - 398 (end point)	9
Residue	0.5
Loss	0.5

93:7 Solvent refers to 93 parts by volume of the naphtha described above and 7% absolute ethyl alcohol.

90:10 Solvent refers to 90 parts by volume of naphtha and 10 parts of absolute ethyl alcohol.

Petroleum Ether - This solvent is a fraction, having a distillation range of 30 to 55°C. However, the petroleum ether used in the recrystallization of carotene had a range of 30 to 40°C.

Ethyl Ether - was anhydrous, being dried over sodium and distilled.

The other solvents, such as chloroform, carbon disulphide, pyridine, etc. were Merck's "reagent" chemicals.

ISOLATION OF CAROTENE FROM CARROTS

A common method for the identification of pigments in natural products is a comparison of the absorption spectra of the extract with that of a pure pigment or mixtures of pigments. For this reason it was decided to isolate pure carotene and compare its spectra with that of ground wheat extracts. Further, a determination of the specific transmissive index (K) for the pure pigment might serve as a check on the spectrophotometer

The method used was essentially that developed by Schertz, with some minor modifications.

Thirty pounds of carrots were carefully scrubbed and cleaned with a brush, and cut with a sharp knife into slices approximately 4 mm. thick. These were laid out on wire trays and given a preliminary drying in an air oven for about 48 hours at 45°C. A blast of air was introduced into the oven to increase the speed of drying. The carrots were given a final drying in a vacuum oven at 50°C. for 4 hours, and reduced to a fine powder in a Wiley mill, with a $\frac{1}{8}$ mm. sieve. The weight of the dry, ground powder was 1217 grams. It should be noted that thorough drying is necessary before grinding in a mill of this type, otherwise clogging of the machine will result.

The powder was poured into a wide glass tube, 5 feet long, the stem of which was attached to a large suction flask. A plug of cotton was placed in the stem to prevent the powder from sifting through during the extraction. Low boiling petroleum ether (33 - 55°C.) was added and allowed to stand in contact with the powder overnight. In the morning slight suction was applied, more solvent added and this repeated until the filtrate was practically colourless. This solution was protected at all times from direct sunlight and was covered as much as possible. In all, there were some seven litres of dark red petroleum ether solutions. The bulk

of this solvent, however, was recovered by distillation under reduced pressure, using a block tin condenser. During the distillation, dry carbon-dioxide was bubbled through the solution by means of a fine capillary. This prevented bumping and oxidation of the pigment, and helped in evaporation of the solvent.

The percolate was reduced to a volume of about 250 cc. and transferred to a beaker, the final concentration being made in a vacuum desiccator as shown below:

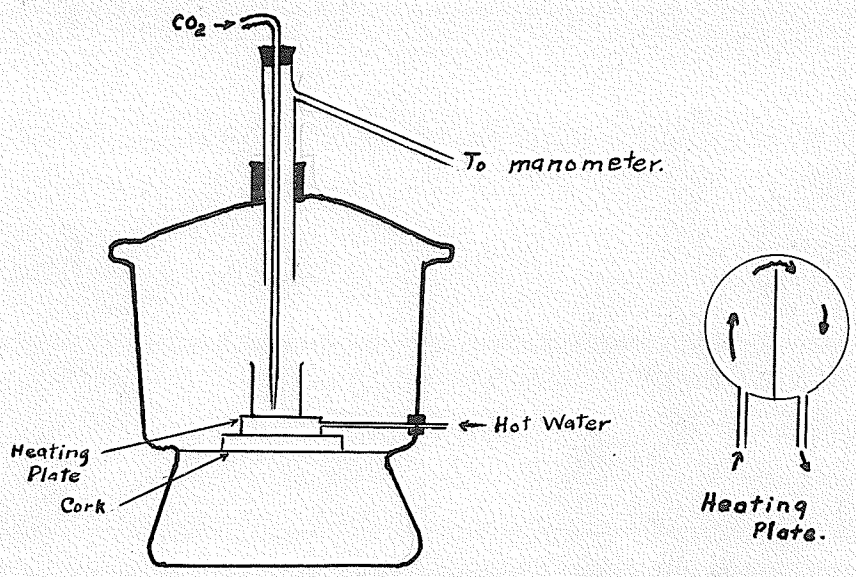


Fig. 3.

The atmosphere with this arrangement was always one of carbon-dioxide and petroleum ether vapor, thus reducing the chances of oxidation. Warming was continued by passing water at 50°c. through the heating plate. Then, in the final stages, when the volume was very small, the warm water supply was replaced by ice water. This cooling effect, combined with the evaporation of the solvent, proved sufficient to initiate crystallization. The solution

was filtered on hardened filter paper under gentle suction and the mother liquor used to rinse the beaker in which the precipitation took place, in order to remove the crystals of carotene that adhered to the sides. The crystals were dissolved in the least possible amount of carbon disulphide on the same filter and allowed to drain into a small distilling flask, and successive small quantities of absolute alcohol, up to approximately 75 cc., were added with shaking. Reduced pressure was applied and shaking continued by hand until the volume was reduced to approximately one-third. This process took about 2½ hours.

The suspension was again filtered and the flask in which the precipitation took place was rinsed with the filtrate and a little petroleum ether. Suction was applied to free the crystals from solvent, and small quantities of petroleum ether used to wash them. This solvent helped to free the carotene crystals from fats and waxes.

The crystals were redissolved in carbon disulphide, but the precipitation this time was accomplished by the addition of 60-75 cc. petroleum ether. This last precipitation was employed to remove any alcohol of crystallization. The concentration of the solution was made under reduced pressure in the vacuum desiccator as before. The crystals were collected on hardened filter paper and washed several times with small portions of petroleum ether and dried in an ordinary vacuum desiccator for one hour over "Hyvac" oil.

The crystals were in the form of thin plates, some as long as 4 mm., reddish-violet in colour and possessing a very high metallic lustre. The melting point was 173-174°C.

DETERMINATION OF THE SPECIFIC TRANSMISSIVE INDEX

Schertz (1925) stated that the specific transmissive index (K) is the most reliable criterion of purity, more so than the melting point. The K value changes on oxidation of the pigment and hence is a sensitive test of the purity of the material.

The crystals were dissolved in a mixture of 93% of varnish-makers' naphtha and 7% absolute alcohol. This solvent was chosen, since it is used in making carotene determinations on flour, and the value for K is necessary for these calculations.

A weighed quantity was dissolved and various dilutions of the stock solution were made. The transmittancies were determined at 435.8 mu. using a 10-cm. cell and the calculations from the equation $bcK = -\log T$ or $K = -\frac{\log T}{bc}$.

Table IV.

Concentration cgms. per litre	% Transmittancy	$-\log_{10} T$	K.
.0180	44.50	.35164	1.9535
.0560	20.32	.69208	1.9224
.0510	9.20	1.03621	1.9189
.0720	4.27	1.36957	1.9021

If the mean is taken of the last three values, K = 1.9145. This is in excellent agreement with previously determined figures -- Schertz, 1.9148 and Ferrari, 1.9165.

It should be noted that the accuracy of the instrument is greatest in the region 4 to 30% transmittancy and for that reason the high K value for .0180 cgms. per litre was discarded.

SPECTRAL DISTRIBUTION CURVES OF THE EXTRACTED PIGMENT

A further comparison was made, namely, that of the spectral distribution curve of the pigment extracted from carrots, with that published by McNicholas (9) for carotene. The first curve was made with a petroleum ether solution. It can be seen from the accompanying graph (Fig.3) that it is shifted somewhat toward the end of the spectrum, although the shape is practically identical. However, McNicholas' work was done on an ethyl ether solution, so it was concluded that the shift might be due to a solvent effect. To verify this, data for curves (Tables V, VI and VII) were obtained for the extracted carotene in ethyl ether and naphtha alcohol (95:7) as well. (Fig.4).

Explanatory Note

The data for the following curves have all been recalculated to the same basis as McNicholas' carotene curve (3.03% at 4358 Å), so as to make them comparable, since they would not be of the same concentration. In the tables, the columns are headed T%, which are the actual transmittancy values obtained, and T'% the recalculated figure.

In some of this work both 5 and 10 cm. cells have been used to help increase the accuracy. When the readings become too low, using the larger cell, the solution is transferred to the 5 cm. tube which will result in less absorption of light and higher transmittancies In such cases, the figures are calculated to the corresponding 10 cm. cell-readings with a special slide rule and finally to 3.03 basis.

Table V

Data for Spectral Distribution Curves of Extracted Carotene and Values Recalculated to 3.03% Transmittancy at 4358 Å.

Petroleum Ether Solution.

Wavelength in millimicrons	T%	T'%
510	86.2	85.3
505	77.5	75.95
500	57.7	55.2
495	36.5	33.65
490	17.49	15.25
485	7.56	6.15
480	4.41	2.72
475	3.41	2.32
470	3.10	2.72
465	4.40	2.98
460	4.34	3.38
455	3.28	2.61
450	2.18	1.61
445	1.97	1.47
440	2.62	1.96
435	4.08	3.15
430	5.22	4.08
425	5.81	4.70
420	7.60	6.16
415	9.20	7.60
410	10.95	8.75

Table VI.

Data for Spectral Distribution Curve of Extracted Carotene and Values Recalculated to 3.03%, Transmittancy at 4558 Å.

Ethyl Ether Solution.

Wavelength in millimicrons	T%	T'%
505	77.00	73.0
500	58.20	52.0
495	33.15	28.40
490	19.40	14.80
485	9.26	5.70
482	6.69	3.85
475	5.59	3.10
470	4.64	2.48
468	4.72	2.51
465	4.77	2.58
460	4.36	2.30
455	3.90	2.00
454	3.28	1.62
452	2.74	1.30
450	2.60	1.26
448	2.91	1.40
446	3.22	1.58
445	3.41	1.70
440	4.67	2.50
435	6.90	4.00
430	9.90	6.18
425	13.13	8.65
420	18.16	12.70

Table VII.

Data for Spectral Distribution Curve of Extracted Carotene and Values Recalculated to 3.03% Transmittancy at 4558 Å.

Naphtha-Alcohol Solution.

Wavelength in millimicrons	T %	T'%
515	79.0	74.75
510	67.5	60.20
505	47.7	38.40
500	30.2	21.35
495	16.3	9.63
493	13.1	7.25
490	8.2	3.95
485	5.7	2.50
482	4.86	-
478	4.86	2.03
476	4.94	2.08
474	5.37	2.20
472	5.66	2.51
470	5.67	2.51
468	5.58	2.45
466	5.37	2.23
464	4.85	2.05
462	4.38	1.77
460	4.15	1.70
455	2.97	1.07
453	2.86	1.02
450	3.04	1.09
447	3.44	1.32
445	3.82	1.52
442	4.85	2.02
440	5.22	2.21
435	6.82	3.14
430	7.40	3.47
425	8.80	4.36
420	11.02	5.80
415	15.42	8.95
410	20.20	12.65

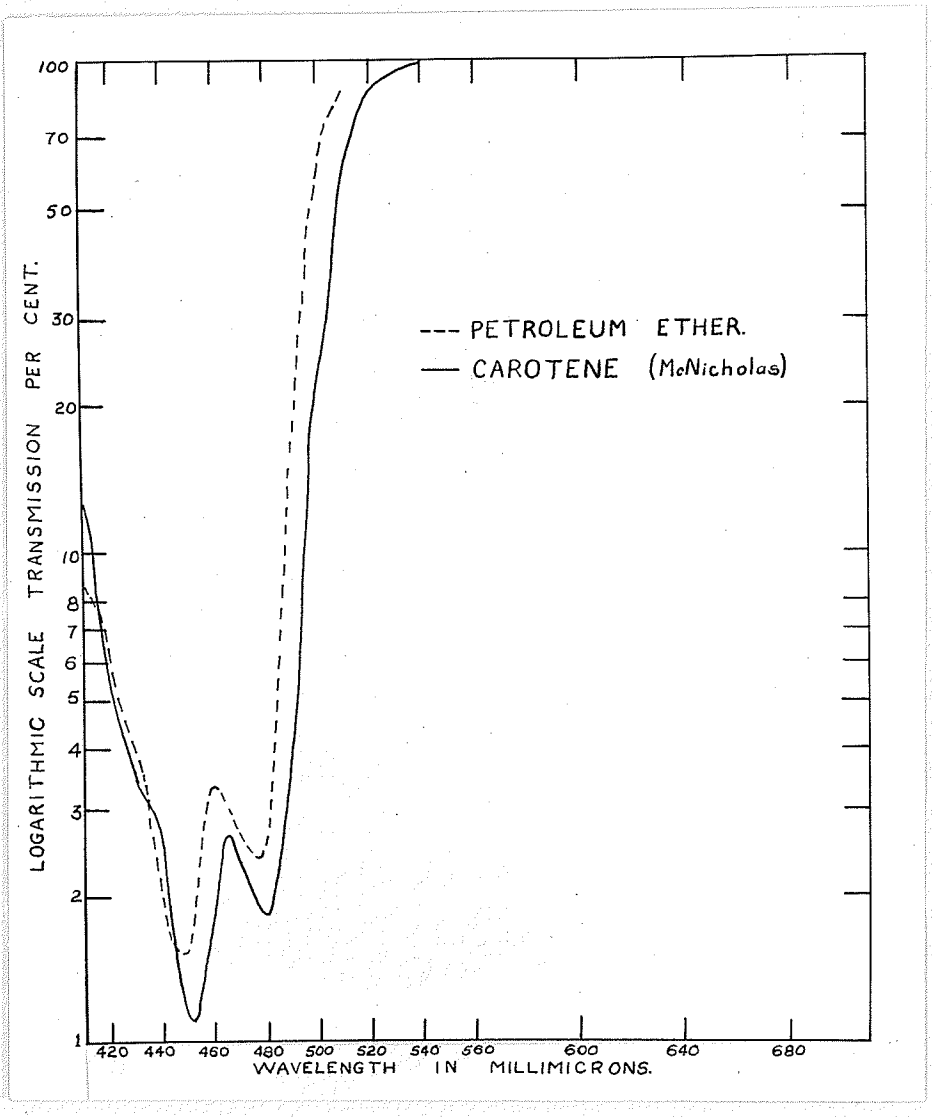

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
--- PETROLEUM ETHER.
— CAROTENE (McNicholas)
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.

FIG. 4.

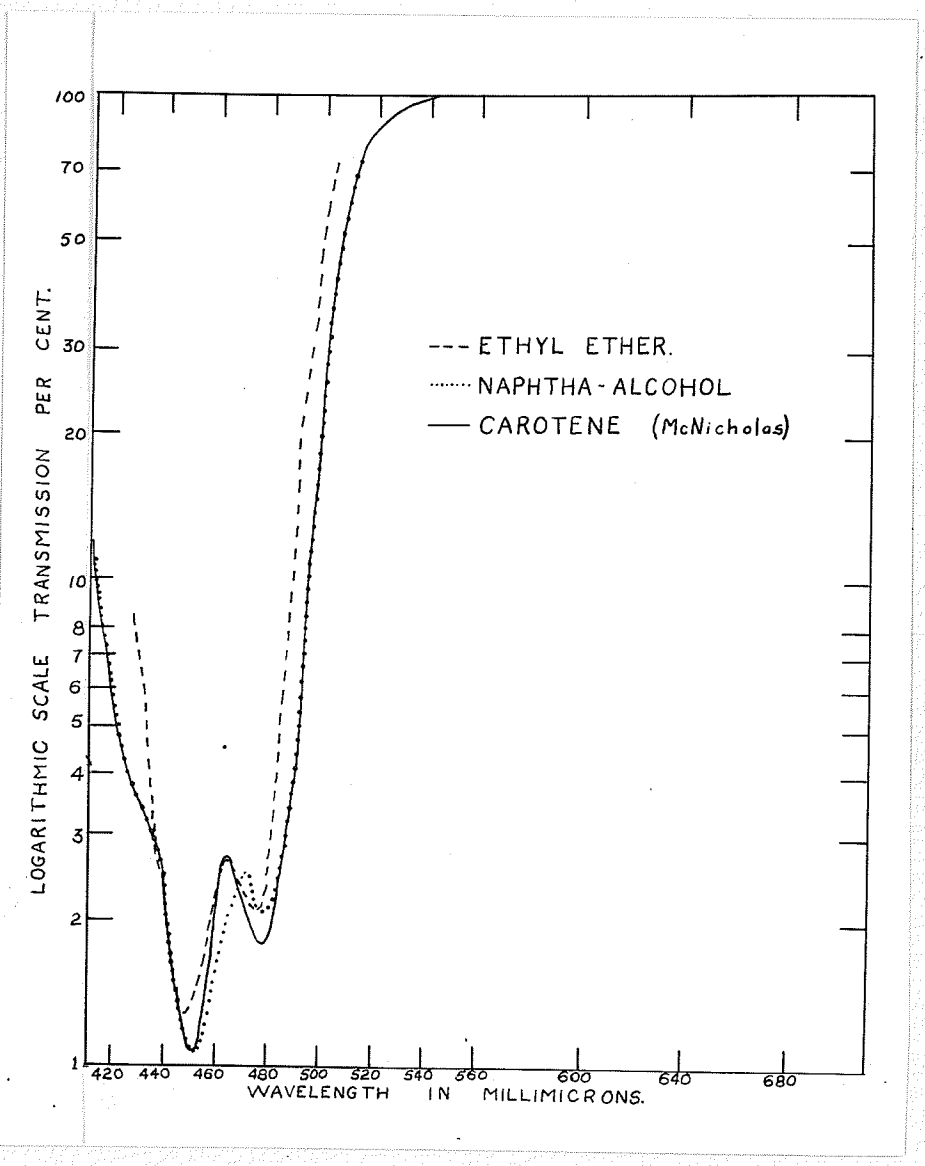

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
--- ETHYL ETHER.
..... NAPHTHA - ALCOHOL
—— CAROTENE (McNicholas)

FIG. 5

Discussion of the Curves

Petroleum Ether Solution

The shape of the curve for carotene in this solvent is practically identical with McNicholas' carotene curve, but it is shifted approximately 50 A towards the blue end of the spectrum. The depth of the troughs are not as deep as the comparison curve, but this is probably due to a difference in concentration and not to oxidation since the K value agrees so well.

Naphtha-Alcohol Solution

This curve follows the comparison curve exactly at the extremities of the bands but the maxima and minima at 4510 and 4650 Å are shifted toward the red.

Ethyl Ether Solution

The curve for this solution seems more compressed than the others but the maxima and minima are identical with McNicholas' carotene solution.

PREPARATION OF WHEAT EXTRACTS WITH A VARIETY OF ORGANIC SOLVENTS

It was thought that the prepartion of extracts of wheat, using a series of organic solvents and their spectral study, might yield some useful information, to determine whether any one solvent would extract only one pigment and hence serve in making a separation. The following solvents were chosen and extract prepared according to Ferrari's method, in each case using a 20-gram sample and 100 cc. of solvent, (except pyridene - 10 gr. and 50 cc.), absolute ethyl and methyl alcohols, chloroform, ethyl ether, petroleum ether, acetone, glacial acetic acid, pyridene, carbon-tetrachloride, carbon disulphide, ethyl acetate, benzine, naphtha, and naphtha-alcohol (90:10).

The concentrations of these solutions as measured by their

transmittancies at 4358 Å, are given in Table IV. The determinations with each solvent were made in duplicate, and figures given are the means of the two.

The extract prepared with glacial acetic acid had to be discarded since it was impossible to clarify, even after repeated centrifuging.

The colour of all these solutions was a greenish yellow, with the exception of the carbon disulphide extract, which was decidedly reddish, and the pyridene extract a dark yellow. This colour difference of the carbon disulphide solution can be explained by the fact that the absorption bands of carotene in this solvent are shifted considerably toward the red end of the spectrum, and hence appears reddish. The colour of the pyridene extract, however, would suggest that pigments other than carotene and xanthophyll were being removed. The spectral distribution curve should yield some information on this point.

Table VIII.

Solvent	Transmittancies
Absolute ethyl alcohol	3.30
Absolute methyl alcohol	2.72
Chloroform	5.66
Ethyl ether	4.30
Petroleum ether	18.50
Acetone	5.10
Pyridene	.15
Carbon tetrachloride	22.40
Carbon disulphide	8.04
Ethyl acetate	7.80
Benzine	9.30
Varnish makers' naphtha	11.29
Naphtha-alcohol (90:10	

These figures would tend to show that pyridene extracts the most pigment, and then ethyl and methyl alcohol, using this method as an index of concentration.

Spectral Distribution Curves

The data for the curves of these extracts are given in Tables IX to XV. As was previously mentioned, there was found in this laboratory some evidence to show the presence of a pigment or pigments other than carotene or xanthophyll. This was indicated by an absorption band near the red end of the spectrum. For this reason, readings were taken from 7000 to 6400 A at intervals of 100 Angstroms in each case. The transmittancy values from 6400 down to 5400 changed so slightly that it was almost impossible to distinguish any difference with the usual intervals of wavelength. Therefore, it was assumed that the values between these points might be joined by a straight line. Readings were taken at intervals of 100 A down to 5000 A and from that wavelength down to 4200 at intervals of 50 Å.

The data for each curve are given under two headings, T and T'%, the former referring to the actual transmittancy percentages obtained and the latter to the recalculated values.

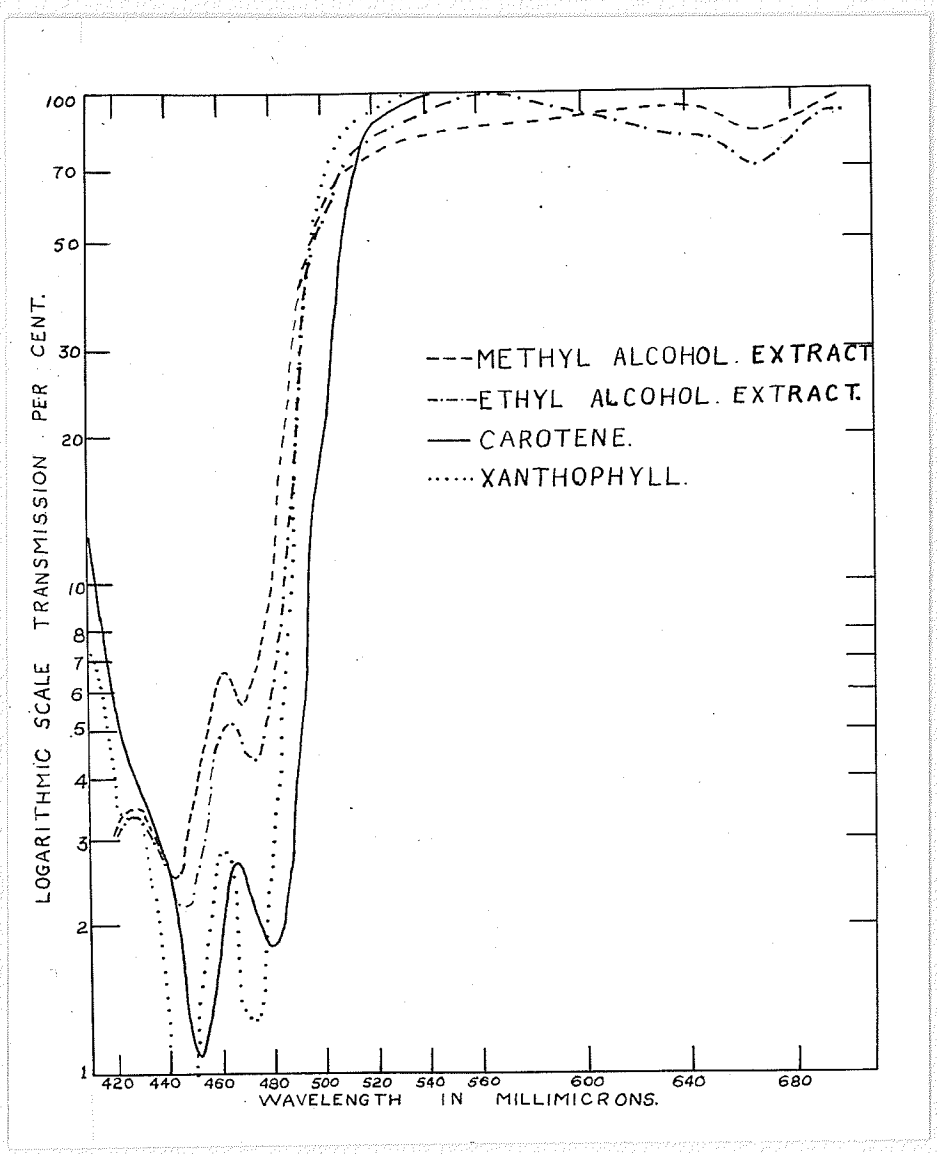

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
---METHYL ALCOHOL. EXTRACT
-·-·-ETHYL ALCOHOL. EXTRACT.
—— CAROTENE.
····· XANTHOPHYLL.
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.

FIG. 6.

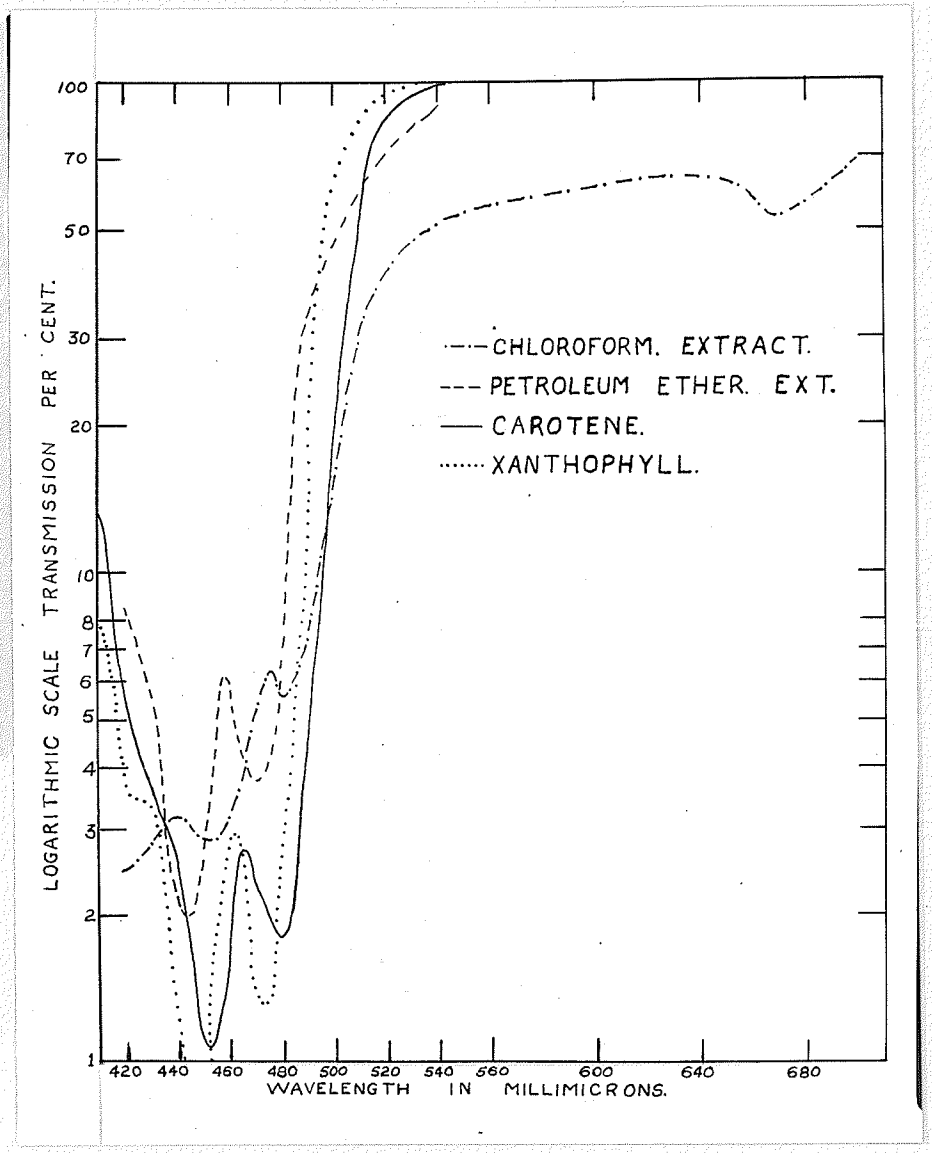

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
CHLOROFORM. EXTRACT.
PETROLEUM ETHER. EXT.
CAROTENE.
XANTHOPHYLL.

FIG. 7.

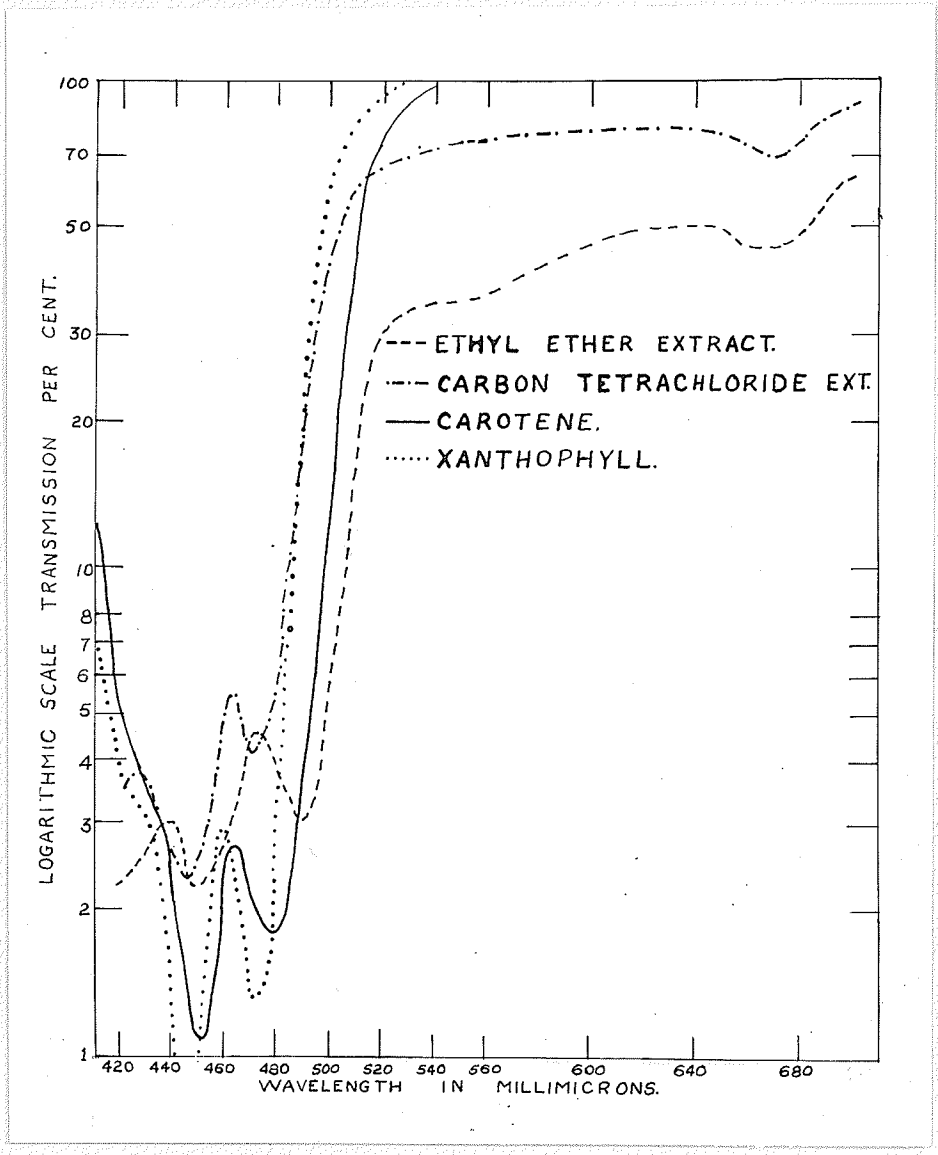

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
ETHYL ETHER EXTRACT.
CARBON TETRACHLORIDE EXT.
CAROTENE.
XANTHOPHYLL.

FIG. 8.

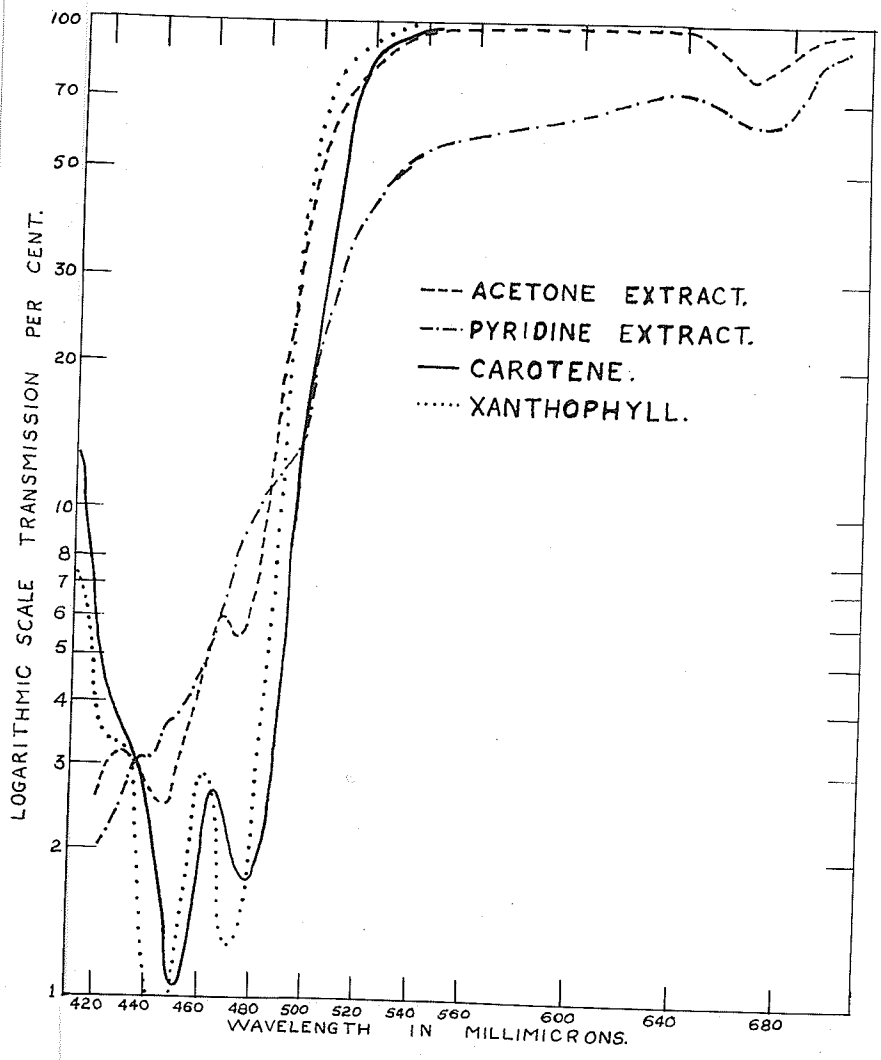

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
--- ACETONE EXTRACT.
-·- PYRIDINE EXTRACT.
— CAROTENE.
····· XANTHOPHYLL.

FIG. 9.

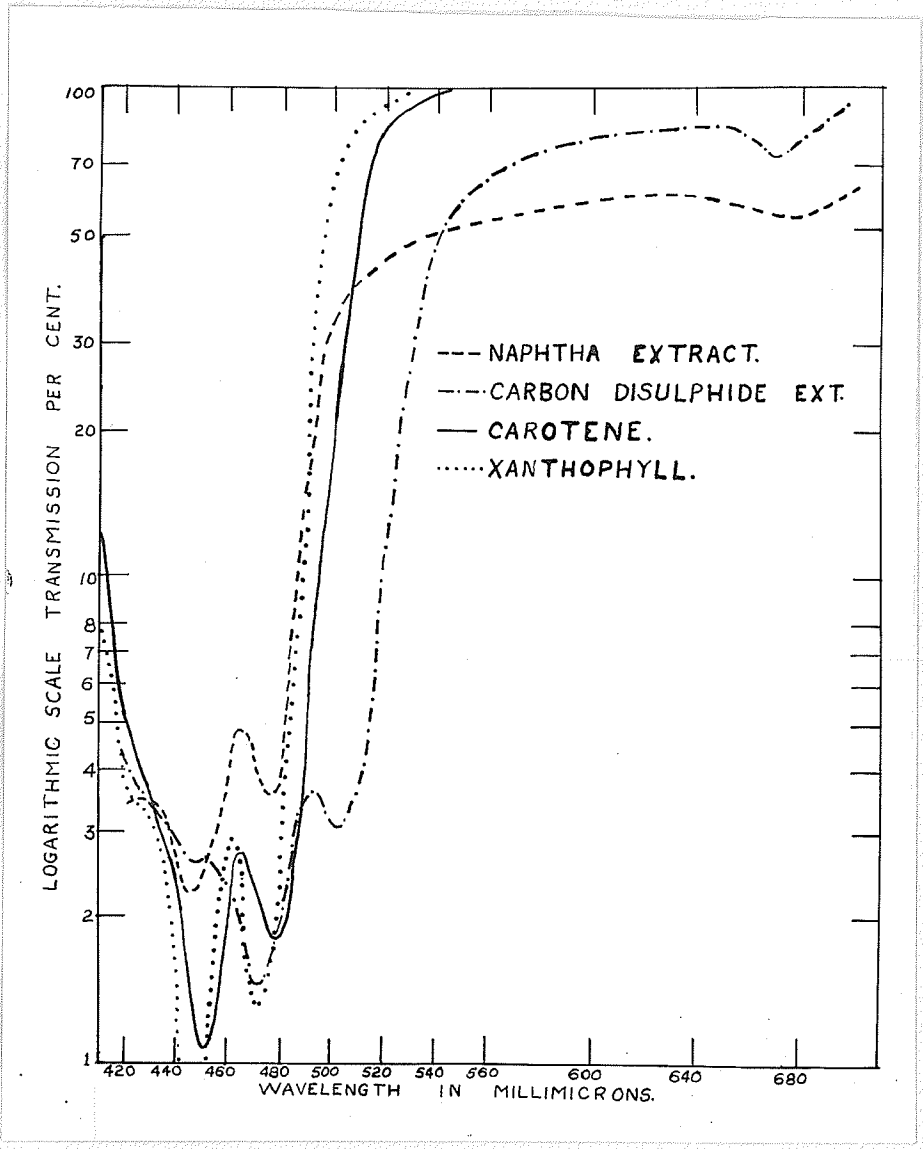

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
--- NAPHTHA EXTRACT.
-·-·- CARBON DISULPHIDE EXT.
— CAROTENE.
····· XANTHOPHYLL.

FIG. 10.

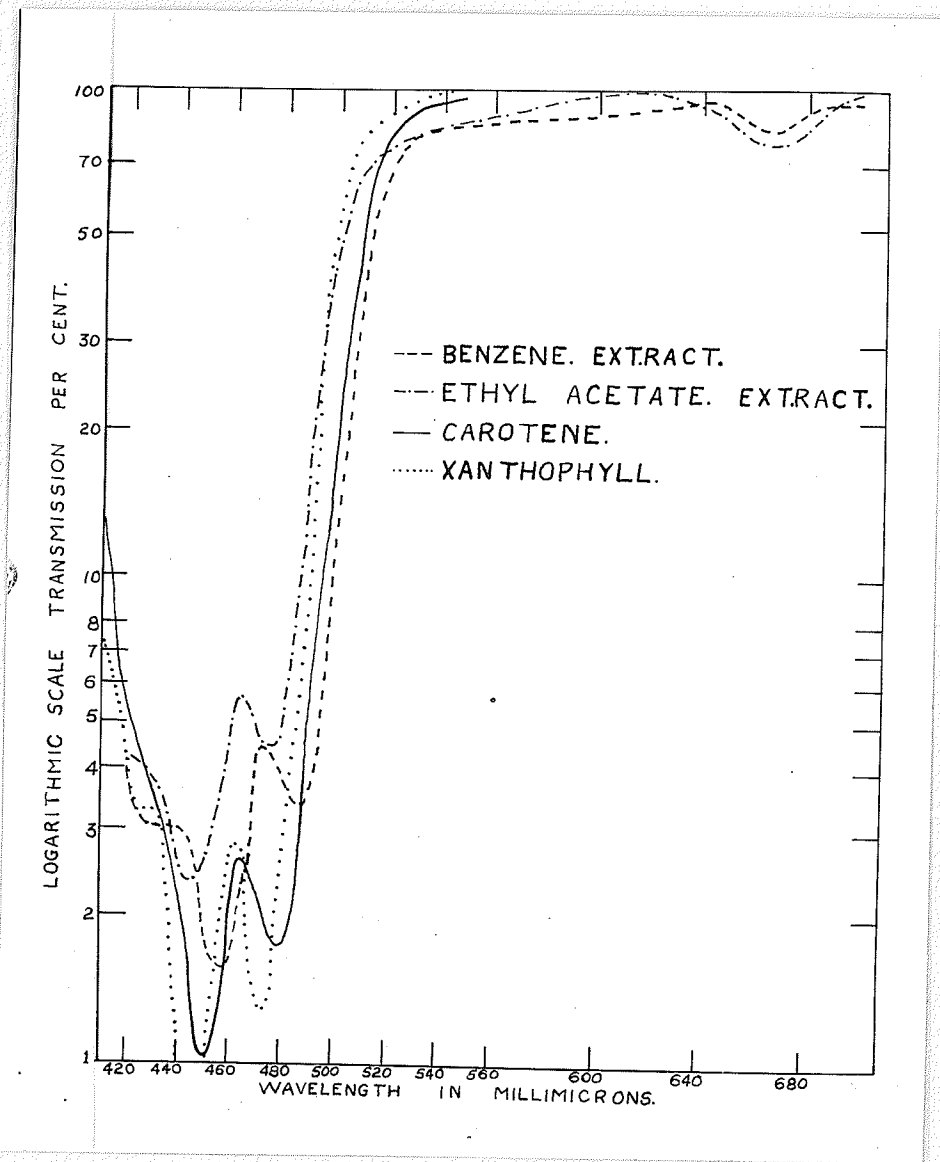

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
--- BENZENE. EXTRACT.
-·- ETHYL ACETATE. EXTRACT.
— CAROTENE.
····· XANTHOPHYLL.

FIG. 11.

Table IX

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents.

Wavelength in millimicrons	Absolute Ethyl Alcohol extract		Absolute Methyl Alcohol extract	
	T%	T'%	T%	T'%
700	91.2	90.8	94.8	94.2
690	90.4	89.9	91.4	90.6
680	79.0	78.1	85.7	84.5
670	72.7	71.5	81.3	79.8
660	74.4	73.3	83.4	82.0
650	80.9	80.0	89.1	88.2
640	83.4	82.6	92.2	91.5
540	93.8	93.5	80.6	81.0
530	89.5	89.0	79.6	80.0
520	83.3	82.5	74.4	74.9
510	75.7	74.6	69.7	70.3
505	67.4	66.0	64.9	65.5
500	56.8	55.0	57.7	58.4
495	43.6	41.8	46.8	47.6
490	25.2	24.5	34.3	33.2
485	14.10	12.80	21.1	21.80
480	7.57	6.62	12.05	12.65
475	5.26	4.55	7.27	7.75
470	5.05	4.35	5.52	5.85
465	5.73	4.95	5.68	6.08
460	5.62	4.87	5.94	6.35
455	4.36	3.72	4.97	5.35
450	3.16	2.66	3.67	3.96
445	2.58	2.15	2.59	2.82
440	2.94	2.47	2.24	2.43
435.8	3.59	3.05	2.80	3.05
430	4.09	3.46	3.05	3.30
420	3.74	3.17	2.76	3.00

Table X

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents.

Wavelength in millimicrons	Chloroform Extract T%	Chloroform Extract T'%	Ethyl Ether Extract T%	Ethyl Ether Extract T'%
700	76.1	69.4	88.2	87.5
690	77.1	62.2	86.4	85.6
680	66.4	57.9	77.8	76.6
670	60.9	51.5	70.7	69.2
660	64.5	55.6	74.0	72.6
650	68.8	60.7	78.6	77.4
640	70.4	62.6	81.4	80.3
540	62.8	54.4	75.4	74.1
530	57.5	48.5	73.3	71.9
520	52.2	42.7	72.8	71.4
510	42.3	32.4	64.2	62.4
505	35.6	24.0	58.0	56.0
500	25.7	16.85	49.5	47.3
495	18.3	10.80	38.6	36.4
490	13.23	7.05	24.2	22.1
485	11.36	5.80	15.50	11.90
480	10.84	5.43	7.99	6.80
475	12.00	6.22	5.50	4.58
470	10.94	5.50	5.06	4.19
465	8.97	4.25	6.48	5.47
460	7.21	3.19	6.04	5.04
455	6.24	2.65	4.46	3.67
450	6.54	2.81	3.06	2.48
445	6.89	3.00	2.86	2.28
440	7.19	3.19	3.32	2.68
435.8	6.90	3.03	3.74	3.03
430	6.51	2.68	4.52	3.72
420	5.92	2.47	4.26	3.49

Table XI

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents

Wavelength in millimicrons	Petroleum Ether Extract		Acetone Extract	
	T%	T'%	T%	T'%
700			97.8	97.4
690			97.9	95.6
680	Readings remained		90.1	88.6
670	constant from 700 -		81.6	79.1
660	540 mm.		82.8	80.4
650			91.7	91.5
640			98.2	97.9
540	94.3	86.4	95.8	95.2
530	93.5	84.5	91.3	90.7
520	87.1	70.8	84.2	82.0
510			74.6	71.3
505	84.3	62.2	68.3	64.4
500	79.8	56.9	56.9	52.2
495	71.8	43.7	41.2	35.9
490	85.0	44.0	26.6	20.7
485	74.0	22.0	16.11	12.10
480	60.0	7.80	10.04	7.03
475	52.4	3.95	8.02	5.42
470	51.6	3.68	8.28	5.62
465	52.9	4.15	8.67	5.95
460	57.4	6.22	7.05	4.65
455	54.0	4.58	5.68	3.65
450	48.7	2.74	4.87	2.62
445	45.5	1.95	3.92	2.37
440	46.7	2.18	4.32	2.65
435.8	49.7	3.03	4.93	3.03
430	55.5	5.28	5.01	3.15
420	60.9	8.35	4.05	2.44

Table XII

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents

Wavelength in millimicrons	Pyridene Extract		Carbon Tetrachloride Extract	
	T%	T'%	T%	T'%
700	89.6	87.8	86.2	59.0
690	85.1	80.9	85.9	57.0
680	68.9	65.4	82.3	48.2
670	65.7	61.9	81.2	46.0
660	68.4	64.8	81.5	46.8
650	72.5	69.3	82.1	48.0
640	75.6	72.6		
540	58.4	54.1	75.0	34.4
530	55.3	50.8	74.6	33.8
520	46.6	41.8	72.8	30.8
510	39.2	34.3	65.9	21.3
505	29.4	24.8	57.8	13.0
500	21.7	17.4	51.0	8.23
495	17.13	13.32	31.0	4.20
490	15.27	11.70	28.0	3.05
485	14.51	11.00	28.2	3.25
480	10.53	9.42	31.8	4.34
475	9.33	8.75	32.3	4.51
470	7.56	6.92	32.3	4.53
465	6.04	5.58	28.8	3.29
460	5.27	4.85	25.8	2.42
455	4.56	4.10	24.3	2.07
450	4.03	3.63	24.8	2.21
445	3.81	3.50	27.2	2.82
440	3.24	2.98	27.8	2.97
435.8	3.35	3.03	28.0	3.03
430	2.68	2.47	26.4	2.59
420	2.15	1.94	25.2	2.28

Table XIII.

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents.

Wavelength in millimicrons	Carbon Disulphide Extract		Ethyl Acetate Extract	
	T%	T'%	T%	T'%
700	95.2	93.0	98.2	97.3
690	88.7	83.8	94.8	92.2
680	82.9	75.8	87.9	82.2
670	78.4	69.9	84.2	77.1
660	85.0	78.7	86.1	79.7
650	88.3	83.2	90.4	86.0
640	89.1	84.4	93.6	90.5
540	62.8	50.4	86.2	80.3
530	43.2	29.0	84.3	77.8
520	25.0	11.50	82.8	75.8
510	10.97	3.84	76.5	67.5
505	9.45	3.10		
500	9.70	3.20	57.5	44.3
495	10.46	3.60	43.9	29.9
490	10.22	3.47	29.5	16.6
485	9.60	3.16	18.64	8.50
480	6.85	1.92	12.67	4.83
475	5.74	1.48	11.84	4.33
470	5.73	1.47	12.95	4.97
465	6.69	1.86	14.31	5.75
460	7.80	2.32	12.16	4.53
455	8.27	2.55	9.72	3.26
450	8.39	2.59	8.02	2.45
445	8.38	2.59	7.50	2.23
440	9.01	2.88	8.99	2.93
435.8	9.36	3.03	10.40	3.03
430	9.53	3.12	11.06	3.95
420	11.78	4.29	11.58	4.22

Table XIV

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents.

Wavelength in millimicrons	Benzene Extract		Naphtha Extract	
	T%	T'%	T%	T'%
700	96.4	94.5	70.6	61.9
690	96.3	94.4	67.0	57.6
680	92.6	89.0	64.5	54.7
670	88.5	83.2	65.8	55.8
660	88.6	83.5	66.6	57.2
650	94.4	91.6	67.0	58.8
640	96.8	95.2		
540	88.8	83.5	59.8	49.3
530	86.6	80.4	59.8	49.3
520	78.4	69.2	56.5	45.6
510	54.3	39.6	52.0	40.6
505	40.4	25.3		
500	25.0	12.25	55.5	38.0
495	16.04	6.23	46.0	27.9
490	11.17	3.61	30.8	14.45
485	10.80	3.42	20.4	7.35
480	11.74	3.98	14.15	4.02
475	12.99	4.53	13.13	3.52
470	11.68	3.88	15.03	4.45
465	8.63	2.45	15.60	4.72
460	6.56	1.61	13.90	3.91
455	6.56	1.61	11.48	2.87
450	7.59	2.02	10.30	2.39
445	9.57	2.87	9.80	2.20
440	9.95	3.03	10.90	2.62
435.8	9.97	3.04	12.50	3.28
430	9.93	3.01	12.90	3.46
420	11.78	3.90	12.60	3.32

Table XV

Data for Spectral Distribution Curves of Extracts of Ground Garnet Wheat prepared with Different Solvents.

Wavelength in millimicrons	Naphtha-Alcohol extract (90-10)		Petroleum-Ether-Alcohol extract (90:10)	
	T%	T'%	T%	T'%
700	69.2	69.7	87.0	88.5
690	64.5	65.2	83.5	84.3
680	60.0	60.8	76.2	78.2
670	54.7	55.4	70.0	73.2
660	56.5	57.2	72.5	75.5
650	59.3	60.0	78.5	81.7
640	62.5	63.0	85.5	87.2
640	51.8	52.6	72.5	75.5
630	51.5	52.3	72.5	75.5
620	48.8	48.9	72.5	75.5
610	45.5	46.4	67.8	71.2
600	33.7	34.5	57.6	61.9
595	24.8	25.6	44.8	49.5
590	15.4	16.1	32.0	36.9
585	8.55	9.10	16.0	20.1
580	5.03	5.40	7.50	10.55
575	4.45	4.80	3.85	5.75
570	4.83	5.10	3.10	4.78
565	5.18	5.50	3.72	5.60
560	4.86	5.00	4.26	6.39
555	3.28	3.52	3.51	5.33
550	2.30	2.51	2.31	3.68
545	2.06	2.25	1.68	2.77
540	2.63	2.87	1.55	2.61
535.8	2.90	3.15	1.92	3.08
530	3.02	3.29	2.28	3.52
525	3.81	3.84	2.70	4.10
520	3.60	3.83	2.53	3.88

Discussion of Curves

Ethyl Alcohol Extract

This shows a very definite band at the red end, with a maxima at approximately 6700 A. This band belongs neither to carotene nor xanthophyll. The maxima and minima of the bands at the blue end correspond exactly to xanthophyll (McNicholas), but the transmittancies from 4300 A on decrease in value instead of increasing as they do for carotene or xanthophyll. This band at the blue end would also indicate the presence of non-carotenoid pigment. The general outline of the curve would seem to show that the pigment extracted by this solvent is essentially xanthophyll. The question arises whether the bands at 6700 and 4300 are due to one or to two pigments.

Methyl Alcohol Extract

This shows a band at 6700 A as did the previous extract. The bands in the blue region are very close to those of xanthophyll, though they are shifted very slightly towards the lower wavelengths. It is questionable whether the difference is significant. A band with a maxima at 450 is also evident. The general slope of the curve is that of xanthophyll. This conclusion is to be expected, considering the solubility relationship. Pure carotene is almost insoluble in methyl alcohol.

Chloroform Extract

It shows the previously mentioned bands at 6700 A, at the red end. The general form is that of carotene. The depths of the troughs are much less than that of the pure pigment but this is probably due to the difference in pigment concentration. This extract, as the two previous ones, therefore shows a mixture of carotenoid and non-carotenoid pigments.

Ethyl Ether Extract

This extract exhibits a curve showing the band at the red end

with a maxima at approximately 6700 A, a band at the blue end of the spectrum as well as general carotenoid characteristics. The position of the maxima and minima agreeing very well with that of xanthophyll. Thus, from this evidence ethyl ether extracts a carotenoid predominantly xanthophyll, and pigments which show bands at the blue and red ends of the visible spectrum.

Petroleum Ether Extract

The curve for this extract shows it to be entirely carotenoid in character, the bands in the blue and red ends being entirely absent. The work of Schertz on the pure pigments shows that carotene to be very soluble in this solvent but xanthophyll only slightly. The maxima of the bands of this extract, however, coincide exactly with those of xanthophyll. The shape of the curve is like carotene, since there is no flattening of the curve between 450 - 420 millimicrons. Therefore, it is likely that the pigment extracted is essentially carotene and the shift in the curve is due to a solvent effect, since the comparison curve is in ethyl ether. Material other than pigment extracted by the petroleum ether might also contribute to the shift.

Acetone Extract

The curve for this extract shows a definite band, both at the blue and red ends. The maxima and minima of the main portion coincide with those of xanthophyll. Therefore, this solvent extracts carotenoid and non-carotenoid material.

Pyridene Extract

The general shape of the curve for this extract is very different from the rest. At the red end it shows a band having a maxima at 670 mu. The transmittancies gradually fall from this point, similar to a carotenoid curve, down to about 480 millimicrons. Then, instead of the usual troughs

and crests, the curve merely drops off more gradually. Thus it would seem that the shape is due predominantly to non-carotenoid material, but that small amounts of carotenoid pigments were present to shift the shape of the curve where their absorption bands belonged.

Carbon Tetrachloride Extract

The shape of the curve for this extract is predominantly carotenoid, though bands are also present at 4700 Å and 4300 Å. The position of the central bands would suggest carotene since the band at 4520 Å agrees closely, but the absorption band at 4900 Å is shifted approximately 100 Å toward the higher wavelengths. No explanation can be offered for this peculiarity.

Carbon Disulphide Extract

The curve shows a band at the red end but not at the blue end. From this fact one may conclude that the band at 670 is separate from the band at 430 and due to two different substances. The general form is carotenoid but shifted approximately 30 mμ. toward the red end of the spectrum. The curve flattens from 455 to 445 and then rises to 440 where there seems to be almost another flat portion before the final rise. Perhaps the flattening from 455 to 445 might be due to xanthophyll.

Ethyl Acetate Extract

Here again there is a band at 670 but none at 430. The general form of the curve follows exactly that of xanthophyll, having a flattening from 430 to 420. Thus, this solvent would seem to extract essentially xanthophyll and a pigment having a band at 670.

Benzene Extract

The curve for this extract has a band at 670 and a general form similar to carotene. However, from 440 to 430 there is a flattening and then an abrupt rise in transmittancy to 420.

Naphtha Extract

This extract shows a band at 670 and also at 430. The general shape is that of xanthophyll. This solvent, therefore, extracts predominantly xanthophyll and non-carotenoid material with bands at the red and blue ends.

As can be seen from the discussion of these curves, it is very difficult to say definitely what pigments are present. The presence of a second pigment in solution will undoubtedly modify the shape of the first curve and the extent of this change will depend on the concentration of the pigment added. Thus, with a complex system as is present in wheat, with at least two carotenoids and most likely two or more non-carotenoid pigments present, it is hard to judge. Also, oxidation must complicate the position and shape of the curves, since very probably this process or change has occurred.

From the data presented, the following solvents extract predominatly carotene: carbon tetrachloride, petroleum ether, chloroform and benzene, and xanthophyll removed by ether absolute ethyl and methyl alcohols, acetone, ethyl acetate and naphtha. Pyridene, on the other hand, shows a non-carotenoid spectral distribution curve.

A PRELIMINARY STUDY OF THE CAROTENOID PIGMENTS OF FIVE TYPES OF RUST SPORES.

Five different types of rust spores, grown by the Dominion Grain Research Laboratory were available for this study. Each type of spore was produced under two sets of conditions, namely, the presence and absence of light.

The object of the present investigation is to determine the content of carotenoid pigments, together with some information regarding the nature of the various pigments present. The amount of material available was very limited, and it was therefore found necessary to limit the latter part of the investigation to a determination of the spectral distribution curves, supplemented by a few special tests. Isolation of the pigments is not feasible with such small amounts of material.

Methods Employed

Rupture of the spores is necessary before the pigments can be extracted with the usual solvents. In the preliminary experiments this was accomplished by grinding with sand in an agate mortar. This method was later modified by placing a weighed amount of the spores in a well stoppered bottle, adding 50 ccs. of solvent and a quantity of solid glass beads. The bottle and contents were then shaken mechanically for 24 hours. At the expiration of this time, the extract was made up to definite volume to replace solvent lost by evaporation, centrifuged, and the clear extract siphoned off into small stoppered containers.

The transmittancy of this extract was first determined at 4358 A.U. by the method of Schertz for carotene. This was later followed by a determination of the spectral distribution curve, employing a "Photoflood Lamp" bulb as a source of white light.

The study was made originally with petroleum ether as a solvent but was later repeated using varnish makers' naphtha. This was done partly on account of some discrepancies in the data, and partly because petroleum ether is very volatile and evaporates from the cells during the time readings are being taken.

[Concentration of the pigments was calculated from the following formula:

$$bck = - \log T$$

Where b = length of cell in centimetres

c = concentration

k = specific transmissive index of carotene at 4358 A.U.

T = transmittancy

k for carotene is 1.9148 (Schertz)]

Table XVI

Type of Spore	Pigment expressed as carotene p.p.m. of sample (Solvent - Naphtha)	Pigment expressed as carotene p.p.m. of sample (Solvent - Petroleum ether)
Grey Uncovered	23.4	13.0
" Covered	16.8	26.8
White Uncovered	214.7	127.6
" Covered	56.0	60.8
Yellow Uncovered	993.1	1253.8
" Covered	713.2	807.5
Antique Brown Uncovered	518.8	217.0
" " Covered	505.2	692.3
Normal Uncovered	944.9	-
" Covered	565.9	-

All readings were taken using a 10-cm. cell.

It will be noted that the "uncovered" spores exhibit a uniformly higher pigment content the the "covered" samples. In the prior invesitgation this condition did not hold. As the total pigment content is of the same general order, it is therefore unlikely that this difference between the two sets of results can be attributed to the change in the solvent used, and it is felt that the samples in the previous study must have been either labelled incorrectly or become "mixed" in the course of the laboratory work.

An examination of the ratios $\frac{\text{uncovered}}{\text{covered}}$ (Table XVII), indicate that no definite relationship exists between the pigment content of different types of spores grown under the two sets of conditions.

TABLE XVII.

Type of Spore	Ratio $\frac{\text{uncovered}}{\text{covered}}$
Grey	1: .72
White	1: .26
Yellow	1: .72
Antique Brown	1: .97
Normal	1: .59

Spectral Distribution Curves

The data from which these curves were plotted were obtained by making a series of transmittancy measurements at appropriate wavelengths throughout the region of the visible spectrum where appreciable absorption takes place. All the readings were made using a cell thickness of 10 cm. Data are given in Tables XVIII to XXII and curves in Figures 12 to 16.

As a basis for comparison, the spectral distribution curve for pure carotene has been plotted on each graph. This curve has been calculated from data by McNichols (Bureau of Standards Journal of Research, Vol.7, p.171, 1931). In order that the curves for the rust spores may be directly comparable,

they have been recalculated to the same transmittancy at 4358 Å. as the McNicholas curve (3.03%). This method of handling the results is only valid if the pigment present is essentially carotene, but, as pointed out in connection with the determination of concentration, it provides some basis of comparison, and is the best available at the present time.

A major difficulty in the interpretation of these curves resides in the fact that at the time McNicholas determined his values, the existence of two isomeric carotenes was not known, and his material undoubtedly consisted of a mixture of alpha and beta carotenes. A search of the more recent literature yields very little information regarding the spectral properties of the two isomers, other than the fact that the edge of the principal absorption band for beta carotene is shifted approximately 100 Å, towards the red end, as compared with that of alpha carotene. Unfortunately, this type of data is purely qualitiative, and it would appear highly desirable that accurate spectral distribution curves for the two isomers be determined. Another difficulty resides in the fact that practically no information is available regarding the effect of solvent on the nature of these curves. It is, of course, known that carotene in carbon bisulphide, for example, shows a different set of bands from those exhibited in, say, petroleum ether, but practically nothing is known regarding the shifting effect of various petroleum fractions, or for instance the addition of varying amounts of ethyl alcohol. In spite of these difficulties, however, certain comparison can be made and inferences drawn accordingly.

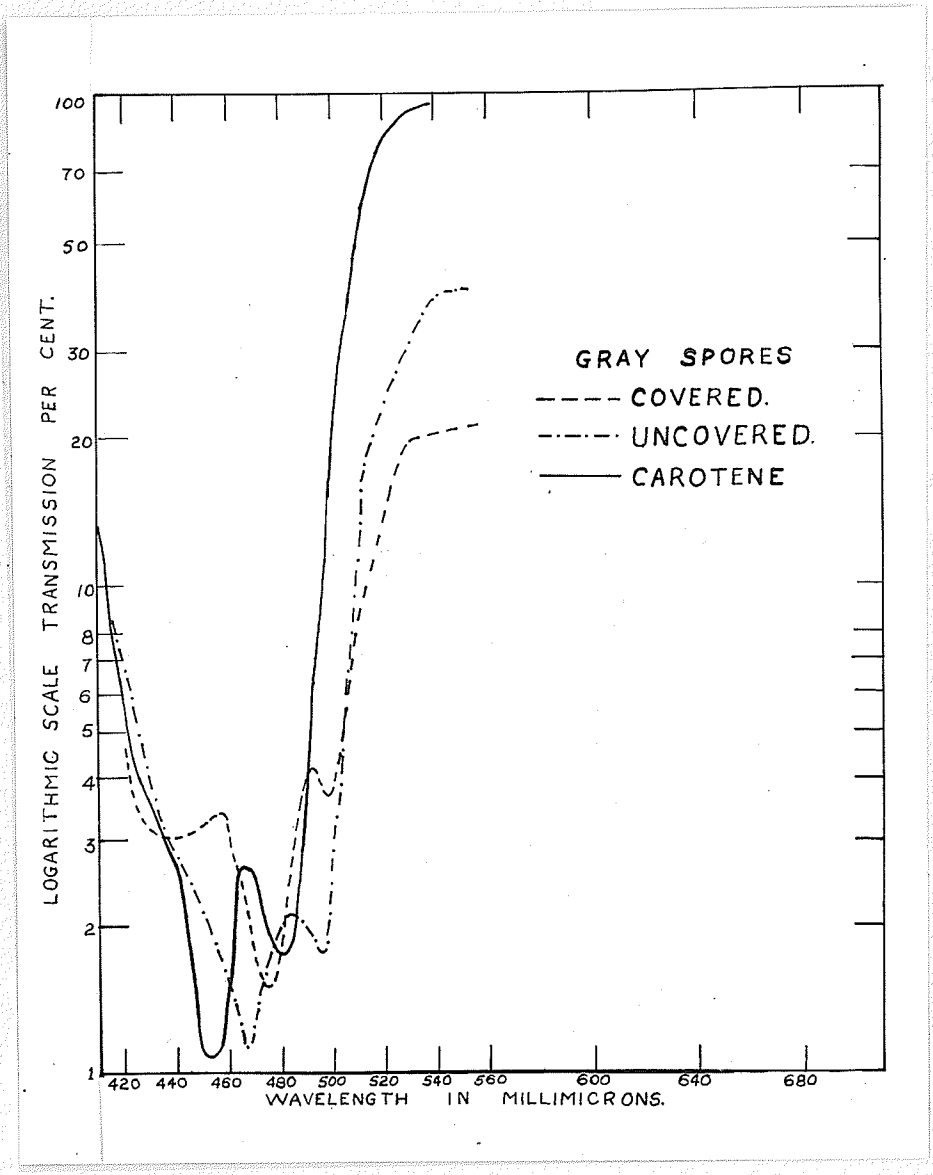

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
GRAY SPORES
COVERED.
UNCOVERED.
CAROTENE

FIG. 12.

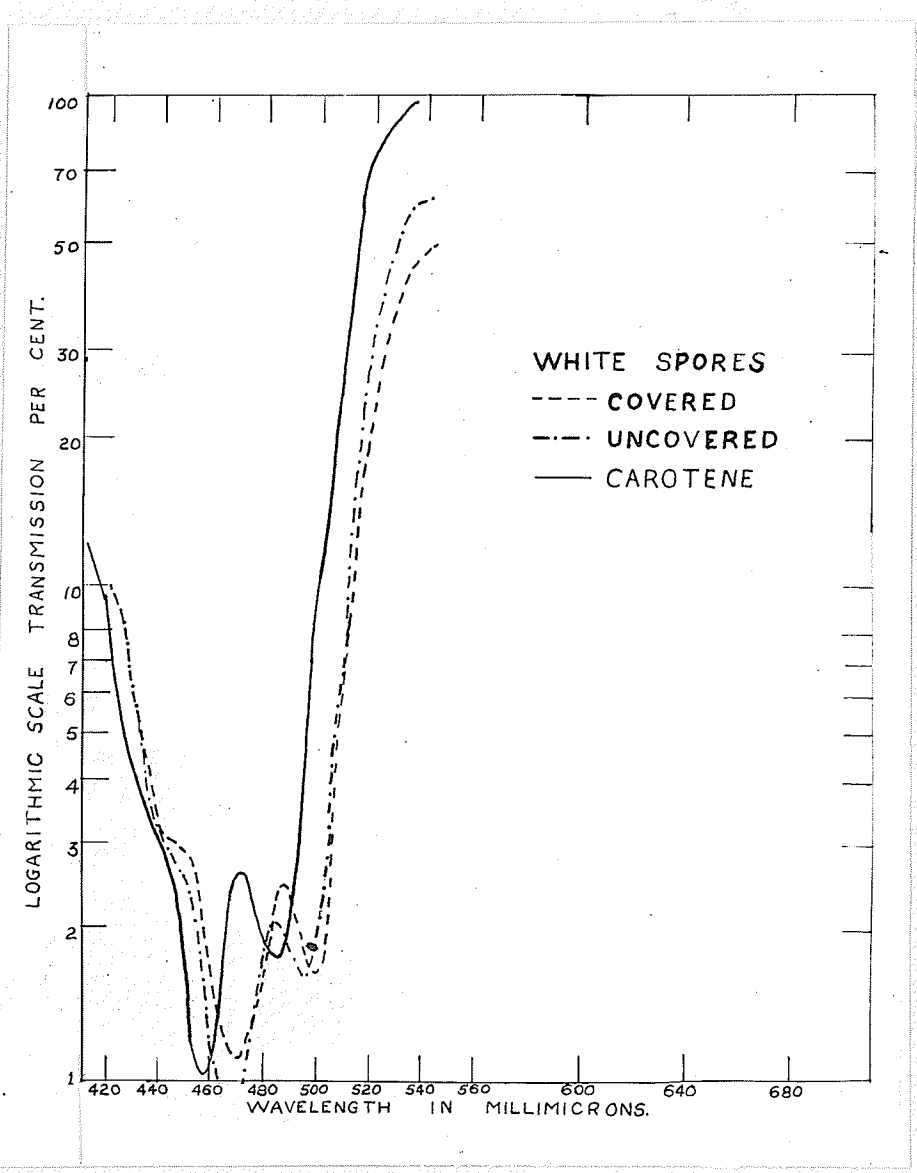

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
WHITE SPORES
COVERED
UNCOVERED
CAROTENE

FIG. 13.

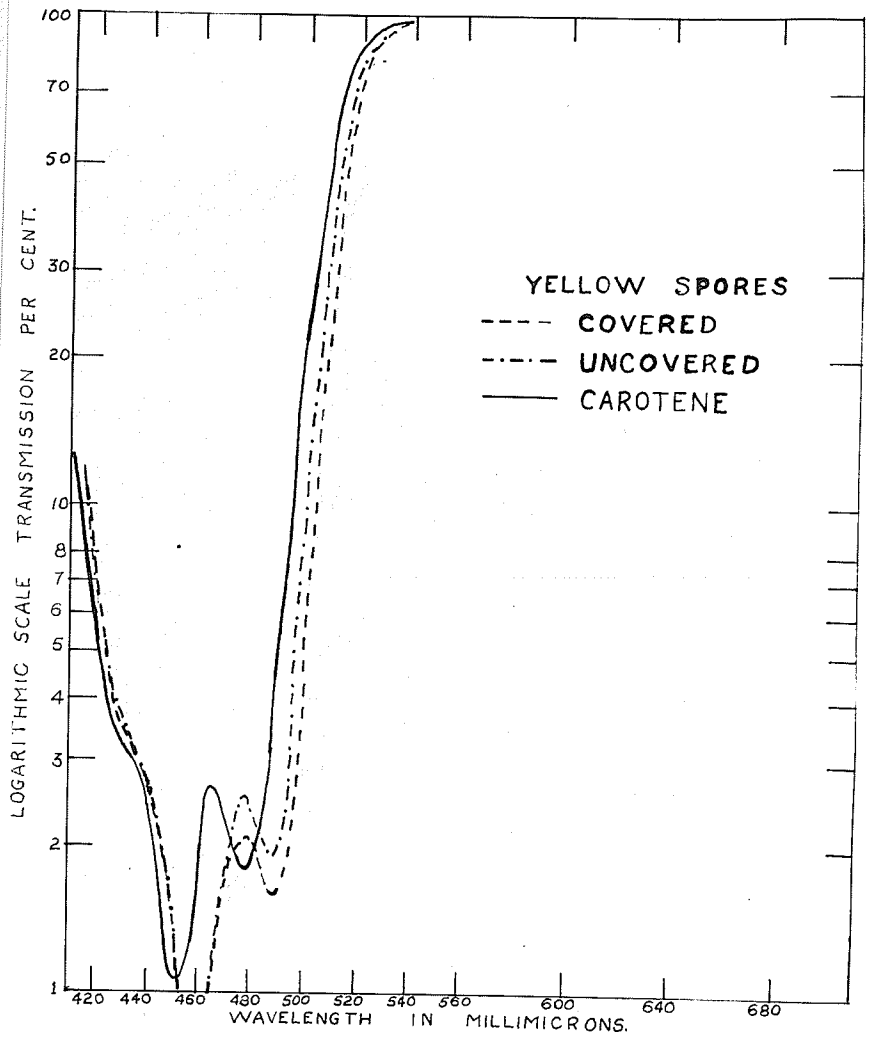

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
YELLOW SPORES
COVERED
UNCOVERED
CAROTENE

FIG. 14.

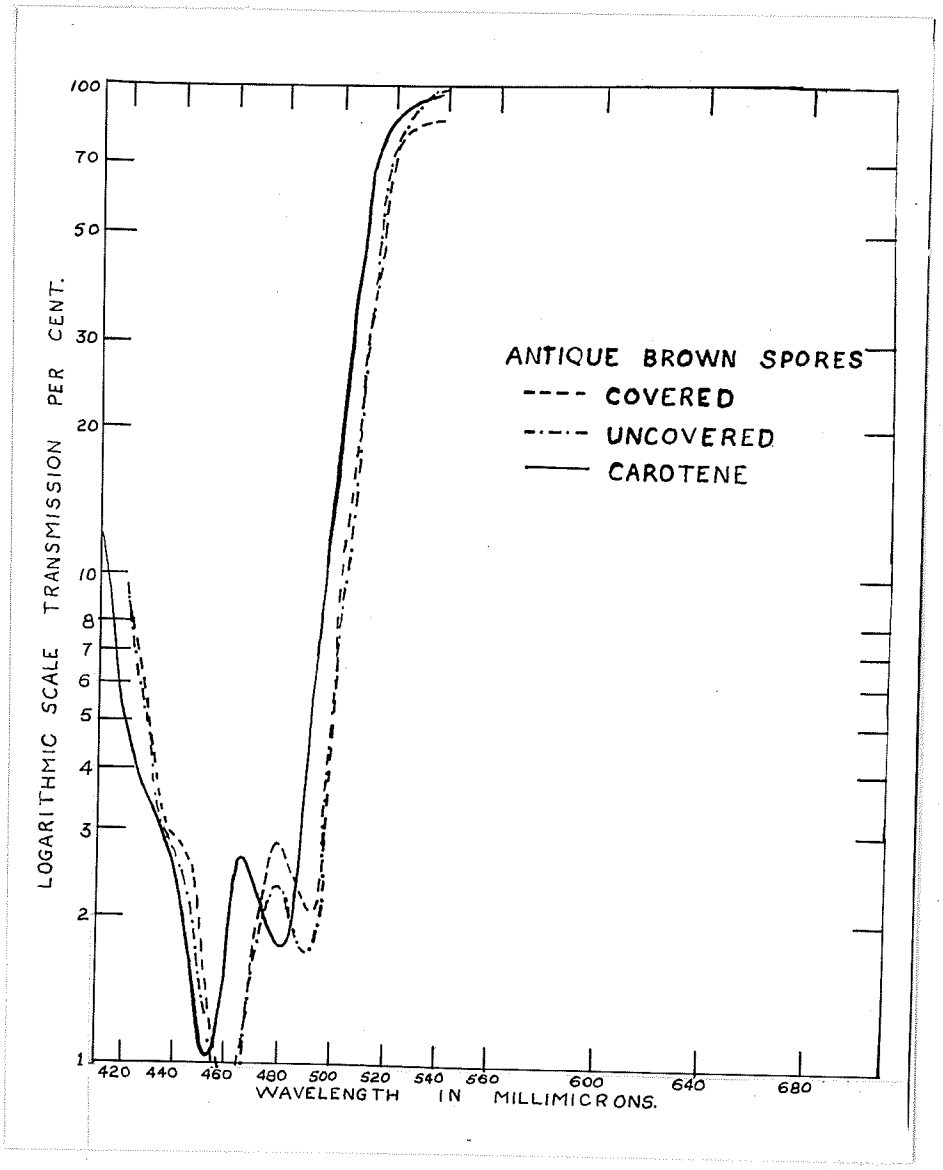

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
ANTIQUE BROWN SPORES
---- COVERED
-·-·- UNCOVERED
—— CAROTENE

FIG. 15.

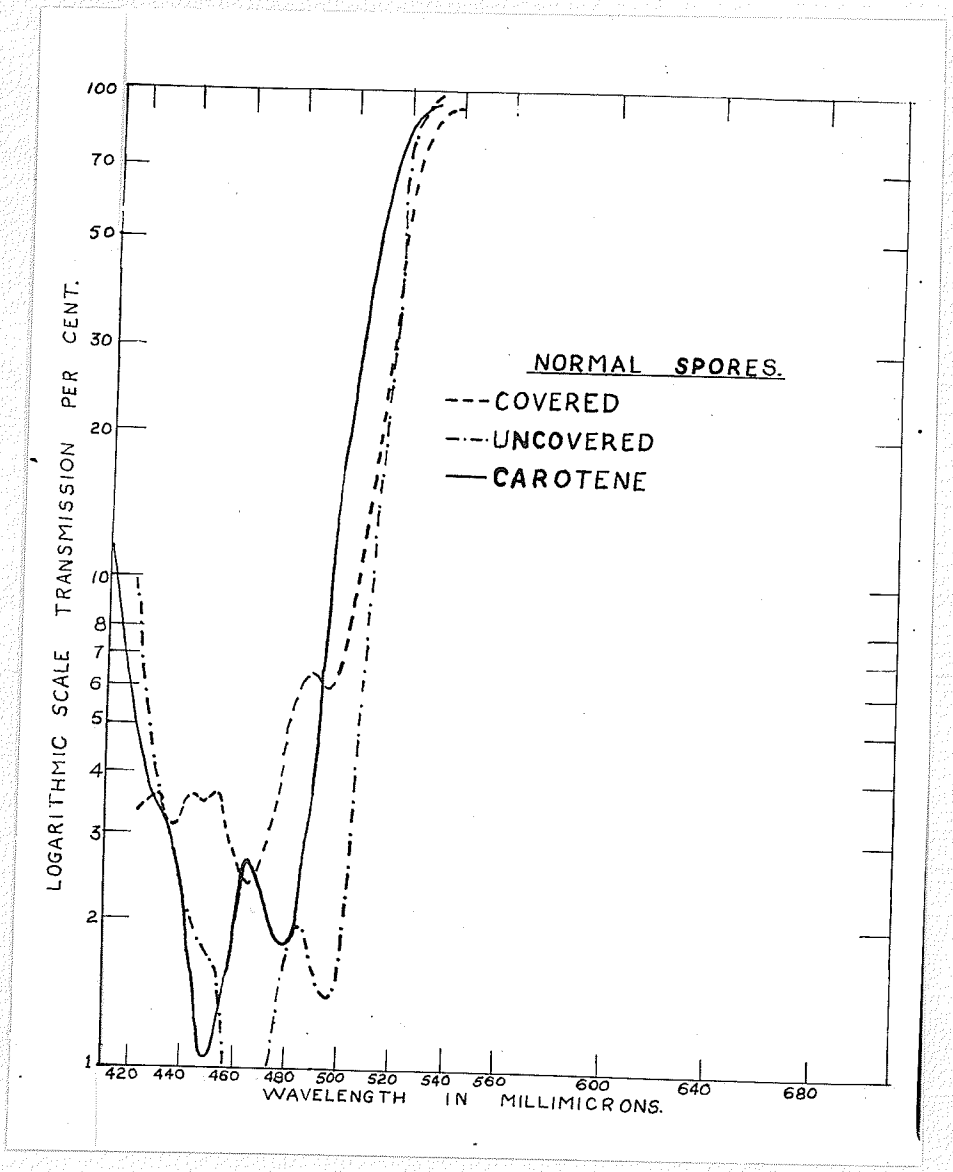

NORMAL SPORES.
---COVERED
-·-·-UNCOVERED
——CAROTENE
LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.

FIG. 16.

Data for Curves

Table XVIII

Grey Spores

"Uncovered"			"Covered"		
Wavelength in millimicrons	T%	T'%	Wavelength in millimicrons	T%	T'%
560	80.3	38.4	560	80.8	21.2
550	80.5	38.8	540	80.5	20.6
540	80.1	38.0	530	80.3	20.2
530	75.9	30.0	520	76.5	14.2
525	74.1	27.0	515	75.9	11.03
520	71.4	23.0	510	71.5	8.65
515	65.9	16.2	505	67.1	5.45
510	59.4	14.65	500	63.5	3.65
507.5	53.4	6.45	495	64.0	3.88
505	49.9	4.82	490	64.2	3.95
502.5	46.7	3.60	485	61.0	2.73
500	44.6	2.95	480	58.9	2.11
497.5	41.0	2.04	475	56.4	1.53
495	39.3	1.71	470	57.1	1.69
490	40.7	1.98	460	62.5	3.24
485	41.12	2.08	450	62.1	3.10
480	41.22	2.09	440	61.8	3.00
475	38.74	1.69	430	62.0	3.08
470	37.72	1.42	420	65.7	4.69
465	35.76	1.12			
460	38.45	1.54			
450	41.68	2.20			
445	43.06	2.53			
440	44.20	2.83			
435	45.00	3.08			
430	47.00	3.71			
425	50.36	5.13			
420	53.90	6.75			
415	56.64	8.38			

Table XIX

White Spores

"Uncovered"			"Covered"		
Wavelength in millimicrons	T%	T'%	Wavelength in millimicrons	T%	T'%
580	78.1	63.75	540	79.0	49.5
570	79.1	65.2	530	76.5	45.0
560	74.8	58.9	520	67.6	31.1
550	74.6	58.6	515	62.6	24.75
540	76.2	60.9	510	51.9	14.17
530	74.9	59.1	508	48.4	11.5
525	66.3	47.3	506	44.3	8.83
520	60.2	39.6	504	41.9	7.46
515	52.6	31.0	500	34.0	4.02
510	40.7	19.43	497.5	28.4	2.36
505	25.8	8.45	495	25.5	1.70
500	17.16	4.02	490	25.4	1.68
497.5	14.29	2.89	485	27.6	2.17
495	12.19	2.17	480	28.87	2.41
490	10.44	1.62	475	26.3	1.88
485	10.44	1.62	470	23.54	1.31
480	11.84	2.07	465	22.10	.11
475	11.64	1.99	460	22.16	1.12
470	9.76	1.44	455	24.4	1.50
465	7.45	.88	450	27.7	2.18
460	6.75	.74	445	30.34	2.88
455	8.44	1.11	440	29.72	2.68
450	10.62	1.69	435	31.22	3.10
445	13.26	2.55	430	34.32	4.10
440	14.11	2.81	425	38.76	5.48
435	14.81	3.08	420	42.74	7.98
430	16.17	3.62			
425	19.85	5.23			
420	25.52	8.50			
415	28.83	10.37			

Table XX

Yellow Spores

"Uncovered"			"Covered"		
Wavelength in millimicrons	T%	T'%	Wavelength in millimicrons	T%	T'%
540	96.4	94.2	540	98.5	96.6
530	95.3	92.4	530	94.0	87.0
520	85.8	77.9	520	89.7	78.2
515	69.9	55.8	510	62.3	34.1
510	52.3	34.8	500	27.87	5.50
505	34.3	17.5	495	18.45	2.17
500	19.91	7.22	490	15.88	1.53
495	11.56	2.98	485	16.93	1.77
490	8.58	1.85	480	18.15	2.08
485	9.39	2.13	475	16.98	1.78
480	10.40	2.51	470	15.55	1.46
475	9.23	2.07	465	12.98	.96
470	7.68	1.53	460	11.13	.68
465	5.92	1.00	455	12.66	.92
460	5.10	.79	450	15.48	1.45
455	5.57	.90	445	18.58	2.18
450	7.64	1.52	440	20.46	2.73
440	10.56	2.58	435.8	21.51	3.03
430	12.84	3.53	430	22.80	3.48
425	16.78	5.47	425	27.30	5.23
420	22.20	7.40	420	34.16	8.73
415	26.90	11.80			

Table XXI

Antique Brown Spores

"Uncovered"			"Covered"		
Wavelength in millimicrons	T%	T'%	Wavelength in millimicrons	T%	T'%
540	98.5	96.6	540	94.5	85.3
530	96.3	91.7	530	94.0	84.5
520	87.3	73.3	520	84.2	62.5
510	58.5	29.4	510	61.3	26.23
500	26.8	4.95	500	36.0	6.17
495	18.80	2.20	495	26.2	2.58
490	16.58	1.68	490	24.0	2.02
485	17.25	1.80	485	25.4	2.38
480	19.33	2.32	480	27.13	2.83
475	18.70	2.17	475	25.62	2.41
470	15.52	1.40	470	22.43	1.69
465	12.85	.92	465	18.45	.99
460	11.66	.75	460	17.60	.87
455	13.48	1.02	455	19.16	1.09
450	16.36	1.39	450	25.35	1.88
445	19.00	2.25	445	26.56	2.68
440	20.60	2.70	440	27.15	2.88
435.8	21.68	3.03	435.8	27.83	3.03
430	24.13	3.87	430	31.50	4.27
425	28.98	5.90	425	36.75	6.45
420	36.41	9.90	420	42.66	9.77

Table XXII

Normal Spores.

"U n c o v e r e d"			"C o v e r e d"		
Wavelength in millimicrons	T%	T'%	Wavelength in millimicrons	T%	T'%
540	99.0	98.2	540	93.5	90.7
530	97.3	95.1	530	82.8	76.0
520	66.8	42.0	520	56.6	43.8
510	25.75	8.48	510	36.06	14.18
500	10.56	1.62	500	14.90	6.33
495	9.30	1.53	495	13.92	5.73
490	10.43	1.64	490	14.85	6.30
485	11.50	1.97	485	14.63	6.15
480	10.30	1.61	480	12.70	5.00
475	8.05	1.02	475	9.85	3.46
470	6.05	.61	470	8.00	2.57
465	5.46	.51	465	7.53	2.32
460	6.15	.65	460	8.08	2.60
455	8.30	1.09	455	9.60	3.32
450	10.53	1.67	450	10.03	3.53
445	10.98	1.80	445	9.92	3.48
440	12.26	2.21	440	9.90	3.47
435.8	14.85	3.03	435.8	9.00	3.03
430	18.22	4.52	430	10.07	3.58
425	22.83	6.82	425	9.66	3.37
420	29.42	10.90	420	9.45	3.25

Discussion of Curves

Grey Spores

The curves for "covered" and "uncovered" differ considerably as was the case in the earlier study. The curve for the covered spores shows a decided third band in the blue violet that is not characteristic of either carotene or xanthophyll. No indication of this band exists in the "uncovered" extract. No definite conclusions can be drawn from this data regarding the nature of the pigments present.

White Spores

The curves for both "covered" and "uncovered" spores are essentially identical, and entirely at variance with those obtained in the prior study. They are, however, in excellent agreement with the curves obtained in an earlier and preliminary study. The characteristics are definitely those of carotene with a strong shift to the red end, indicating a considerable percentage of the beta isomer. A slight suggestion of xanthophyll is apparent in the covered spores.

Yellow Spores (Previously named Orange).

The curves for both "covered" and "uncovered" are essentially identical and very similar to those obtained previously; the shift towards the red end is more strongly marked, however, indicating a larger percentage of beta carotene. No definite indication of xanthophyll exists.

Antique Brown Spores

The curves for both "covered" and"uncovered" spores are essentially identical, and very similar to those obtained previously. The shift towards the red end is quite marked, suggesting a preponderance of beta carotene. The high intensity of the second band suggests some xanthophyll. Further evidence of this will be noted in the appearance of a third band

(flattening) in the case of the covered spores.

Normal Spores

These were not included in the prior investigation and, therefore, no comparison can be made. The curves for "covered" and "uncovered" differ markedly, the latter exhibiting definite beta carotene characteristics. The curve for the "covered" spores suggests both carotene and xanthophyll but also shows the presence of a third pigment possessing a band in the blue violet very similar to that of the grey spores.

EXPERIMENTS ON THE PERCOLATION OF A COLUMN OF GROUND WHEAT WITH A SERIES OF SOLVENTS.

From the information gathered in this study of the extracts prepared with different solvents, it was thought that a separation might be effected by percolating a column of wheat with a series of solvents. For instance, petroleum ether presumably takes out only carotene, then that pigment would be removed and xanthophyll would be left, along with the pigments that give rise to the red and blue bands.

The first series tried was petroleum ether, followed by ethyl acetate and then acetone, (Fig.17 and Table XXIII). The second was petroleum ether, benzene, ethyl acetate and acetone, (Figs. 18 and 19, and Tables XXIV and XXV).

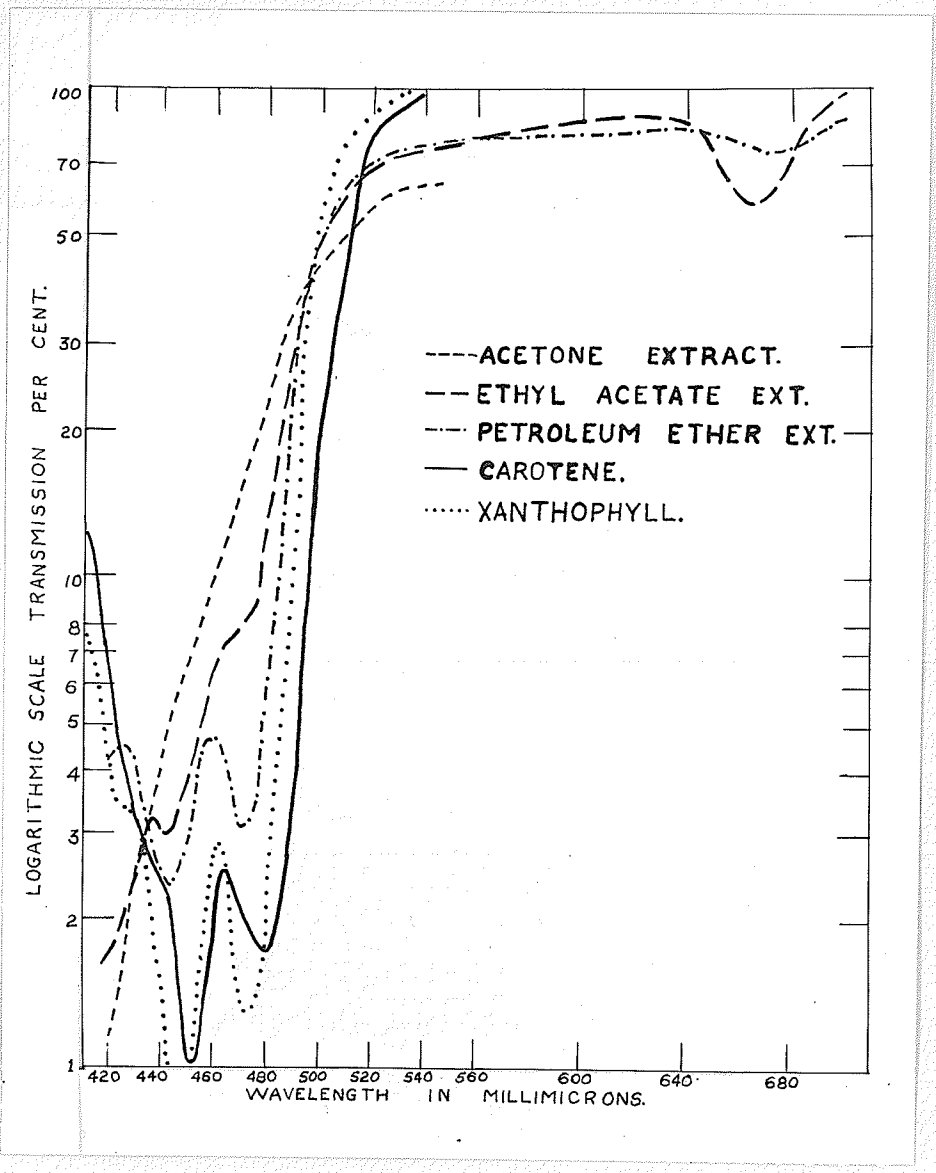

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
----ACETONE EXTRACT.
— —ETHYL ACETATE EXT.
-·-·- PETROLEUM ETHER EXT.
—— CAROTENE.
····· XANTHOPHYLL.

FIG. 17.

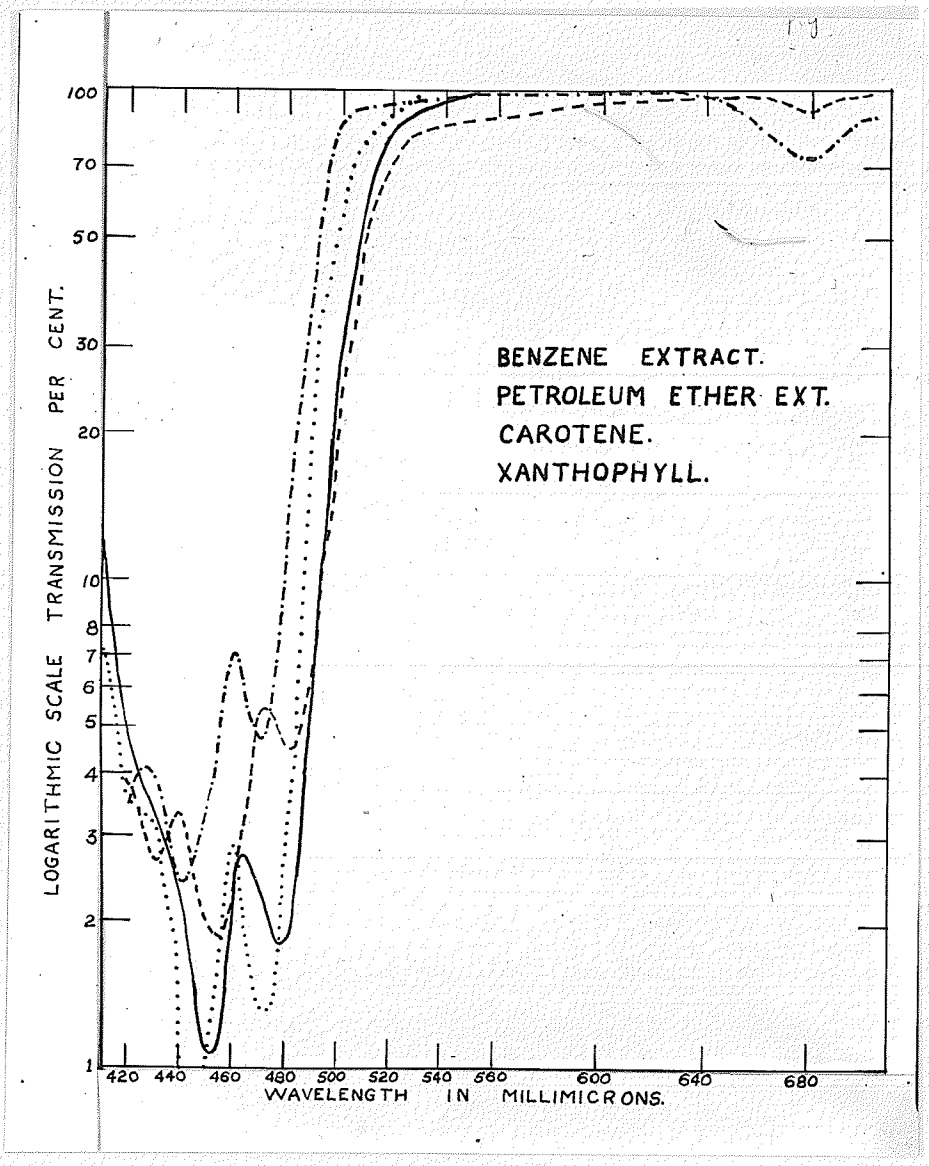

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
BENZENE EXTRACT.
PETROLEUM ETHER EXT.
CAROTENE.
XANTHOPHYLL.

FIG. 18.

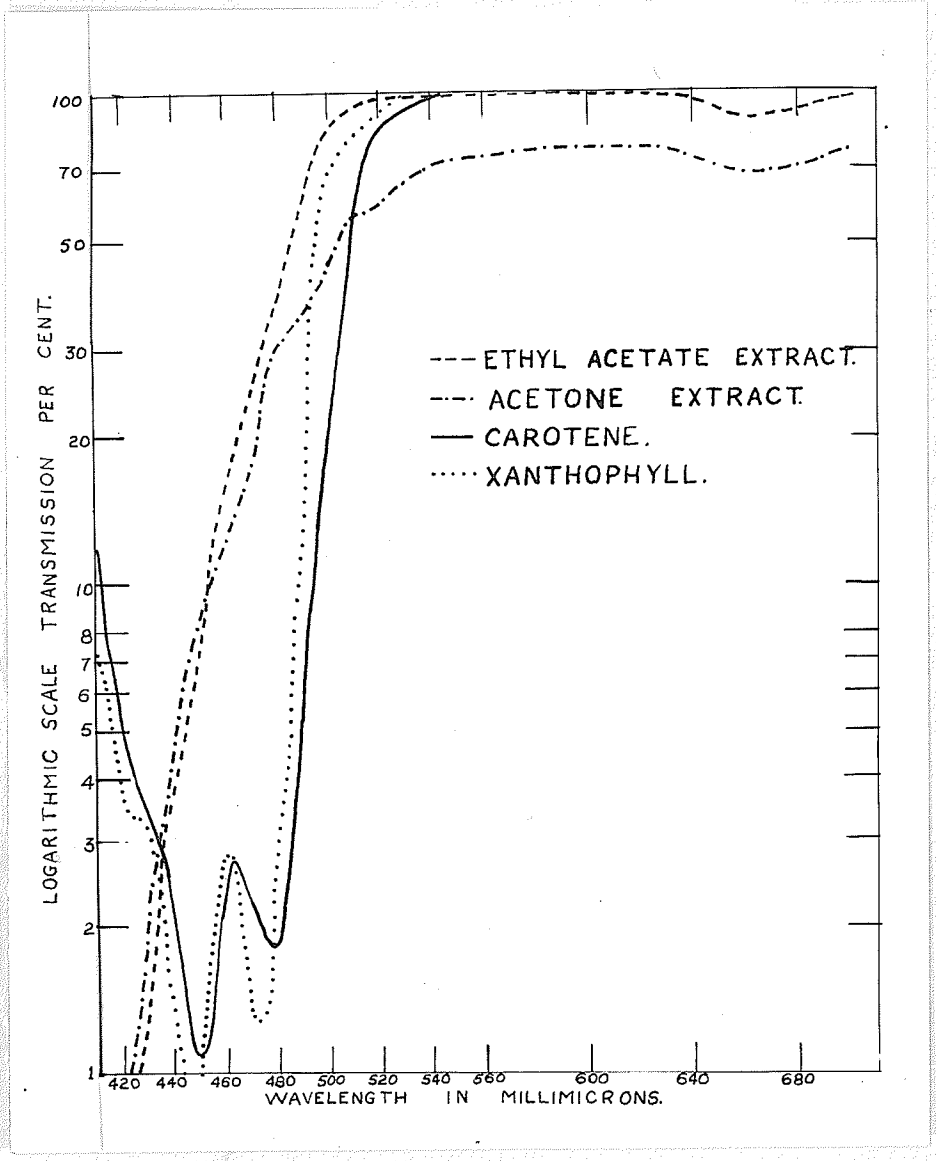

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
---ETHYL ACETATE EXTRACT.
-·-· ACETONE EXTRACT.
— CAROTENE.
····· XANTHOPHYLL.

FIG. 19.

Table XXIII

Wavelength in millimicrons	Petroleum Ether extract		Ethyl Acetate extract		Acetone extract	
	T%	T'%	T%	T'%	T%	T'%
700	87.5	85.8	98.5	98.2	-	-
690	84.0	82.2	93.5	92.2	-	-
680	77.0	74.7	78.7	74.8	-	-
670	76.0	74.1	66.0	60.2	-	-
660	80.5	78.5	65.7	60.0	-	-
650	83.5	82.1	77.5	73.2	-	-
540	79.0	77.0	78.8	74.7	75.0	64.2
530	74.3	72.2	76.0	75.8	75.0	64.2
520	72.7	70.2	75.0	70.4	71.5	59.6
510	66.5	63.4	67.7	62.2	65.5	52.2
505	62.5	59.2	63.2	57.1	62.0	47.9
500	55.0	51.5	57.3	50.7	59.0	44.4
495	42.0	38.0	49.5	42.4	55.5	40.4
490	29.75	25.9	36.7	29.4	51.0	35.5
485	16.80	13.75	27.3	20.5	43.7	28.1
480	8.85	6.70	18.1	12.4	37.2	21.8
475	5.00	3.58	13.6	8.80	33.0	18.20
470	4.50	3.16	12.54	7.90	29.0	14.50
465	6.10	4.48	11.95	7.50	25.4	12.20
460	7.00	4.74	10.82	6.62	23.0	10.45
455	6.20	4.52	8.60	5.00	19.9	8.40
450	4.20	2.93	6.75	3.73	17.6	6.92
445	3.45	2.37	5.45	2.87	15.0	5.40
440	3.70	2.57	5.80	3.10	12.8	4.42
435.8	4.30	3.03	5.70	3.03	11.32	3.03
430	6.00	4.36	4.60	2.32	8.90	2.42
425	6.20	4.51	3.65	1.77	6.75	1.58
420	5.85	4.25	3.40	1.61	5.42	1.13

Table XXIV.

Wavelength in millimicrons	Petroleum Ether extract		Benzene extract	
	T%	T'%	T%	T'%
700	97.7	89.2	99.5	99.2
690	96.2	83.0	98.0	97.6
680	93.5	73.0	92.7	91.4
670	94.0	74.6	96.2	95.6
660	96.3	83.5	99.0	98.8
650			100.0	99.9
540	Transmittancy		88.0	86.0
530			85.0	82.5
520	approximately 100%.		80.5	77.4
510			60.0	54.6
505	98.2	91.5	46.2	40.1
500	97.5	88.5	38.5	32.3
495	93.0	70.4	15.30	12.30
490	83.5	41.8	8.90	6.75
485	73.6	22.7	6.25	4.55
480	63.4	11.05	6.32	4.60
475	56.4	6.27	7.20	5.55
470	52.9	4.60	7.05	5.20
465	55.2	5.65	4.85	3.43
460	57.4	6.83	3.40	2.31
455	54.9	5.52	2.70	1.79
450	50.5	3.68	3.17	2.13
445	47.2	2.66	4.07	2.82
440	46.2	2.40	4.75	3.36
435.8	48.5	3.03	4.35	3.03
430	51.5	4.03	3.82	2.62
425	51.7	4.12	4.75	3.35
420	50.0	3.50	5.45	3.90

Table XXV

Wavelength in millimicrons	Ethyl Acetate extract		Acetone extract	
	I%	T%	I%	T%
700	97.0	77.0		
690	96.3	75.3		
680	94.1	70.7		
670	93.5	68.5		
660	93.7	69.3		
650	94.0	70.3		
540	94.5	72.5	100.0	97.5
530	93.5	68.5	100.0	97.5
520	91.0	59.0	100.0	97.5
510	90.5	57.0	99.0	94.5
505	90.0	55.4	99.0	94.5
500	86.7	44.7	98.0	89.0
495	84.5	38.8	96.0	79.0
490	83.0	35.1	92.0	68.5
485	83.0	35.1	89.0	51.6
480	81.5	31.7	85.0	39.8
475	78.0	24.8	82.0	33.4
470	75.0	19.85	78.0	24.4
465	70.5	14.0	76.0	21.05
460	68.0	11.45	72.0	15.50
455	66.7	10.30	69.0	12.20
450	63.0	7.43	66.0	9.43
445	62.0	6.80	59.5	5.23
440	59.0	5.18	57.0	4.12
435.8	55.7	3.03	54.0	3.03
430	50.0	2.04	46.0	1.22
425	44.0	1.00	43.0	.84
420	40.0	.58	40.0	.55

First Series

One hundred grams of ground wheat were placed in a tube, the lower end of which was drawn out and attached to a suction flask. A wad of cotton was placed in the bottom of the tube to prevent sifting. The petroleum ether was added from the top and slight suction applied to hasten the percolation. It was necessary to add approximately 500 ccs. before the yellow colour was removed. Before the ethyl acetate was added, air was drawn through the column to remove the previous solvent. 200 ccs. of ethyl acetate were added, and a highly coloured extract was obtained, with a slightly greener tinge than that of the petroleum ether. Most of the pigment, however, was removed in the first 75 - 100 ccs. Finally, 100 ccs. of acetone were added but this was so faintly coloured that it was passed through the column twice. This last extract was concentrated before reading in the spectrophotometer.

The data for the curves in this series are given in Table XXIII and the curves in Fig. 17.

Petroleum Ether Extract

Unlike the previous curve for the petroleum ether extract, this one shows the bands at the red and blue ends of the spectrum. The only explanation that can be offered for this difference is the fact that 20 grams were used in one case and 100 grams in the other, and hence more pigment was extracted.

Ethyl Acetate Extract

This curve shows a very pronounced band at the red end, in fact than any of the previous extracts. Evidently the pigment responsible for this band is present in greater concentration. From 5400 A the transmittancies fall off as in a typical carotenoid curve, but from

5750 to 5650 A there is a shift toward the red end instead of a definite absorption band. There is also indication of a band at approximately 5450 - 5420, which corresponds to the position of one maxima of the xanthophyll curve. Finally, the transmittancy falls from 4370 down to 4200 A.

Obviously a carotenoid is present, although to some extent its presence is masked by some other pigment.

Acetone Extract

The curve is definitely non-carotenoid. The transmittancies fall off steadily from 5400 to 4200. There is no evidence of any band at the red end, since the transmittancy of the solution remains practically constant from 7000 to 5400.

Second Series

Petroleum Ether Extract

This solvent again shows bands at the red and blue ends, but the position of the maxima and minima are most peculiar, since they correspond exactly with those of xanthophyll. Considering the solubility of this pigment in petroleum ether, this relationship is not to be expected and is very probably due to the fact that the curve for carotene and xanthophyll in petroleum ether is not in the same position as in ethyl ether.

Benzene Extract

This shows the band at the red end as well as a typical carotene curve. At the blue end there is a most peculiar condition which has not been encountered previously, i.e., the transmittancy falls from 4400 to 4300 A and then rises again to 4200. Thus, this solvent seems to extract essentially carotene and some other pigments which give rise to other bands.

Ethyl Acetate and Acetone Extracts.

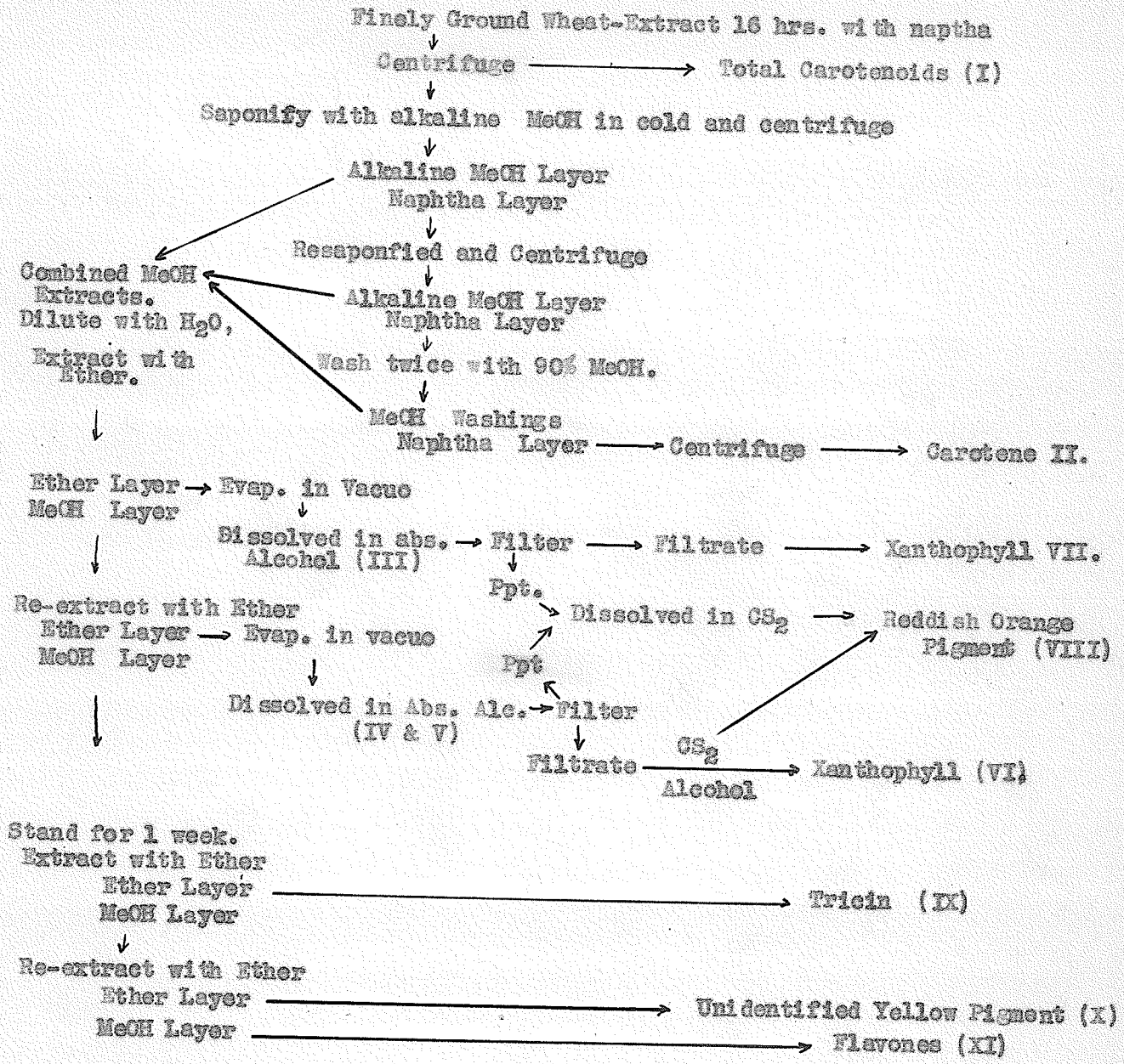
These are considered together, since they seem to be essentially the same. Both show indications of the band at the red end of the spectrum and then, from 5400 A on, the transmittancies fall gradually. It is doubtful whether these shifts in the acetate curve are significant. Now, it is possible that this shape of curve might be due to the pigment which is represented by the band with a maxima at 4700 A.

From these curves it can be seen that a series of solvents employed for a separation of the pigments in so complex a system is of no value. No one solvent will extract only one pigment. As already mentioned, the presence of fats seriously upsets the ordinary solubility relationships and probably account for the variable results obtained.

STUDIES ON MARKLEY'S METHOD FOR THE SEPARATION OF WHEAT PIGMENTS.

In the review of the literature it was mentioned that Markley (10) had devised a method for the separation of the pigments of wheat, based on Willstatter and Stolls' method. Since a separation of this kind was the ultimate aim of this investigation, it was decided to repeat and study this work.

Method.


Finely Ground Wheat-Extract 16 hrs. with naptha
Centrifuge
Total Carotenoids (I)
Saponify with alkaline MeOH in cold and centrifuge
Alkaline MeOH Layer
Naphtha Layer
Resaponfied and Centrifuge
Combined MeOH Extracts.
Dilute with H_2O,
Extract with Ether.
Alkaline MeOH Layer
Naphtha Layer
Wash twice with 90% MeOH.
MeOH Washings
Naphtha Layer
Centrifuge
Carotene II.
Ether Layer
MeOH Layer
Evap. in Vacuo
Dissolved in abs. Alcohol (III)
Filter
Filtrate
Xanthophyll VII.
Ppt.
Dissolved in CS_2
Reddish Orange Pigment (VIII)
Re-extract with Ether
Ether Layer
MeOH Layer
Evap. in vacuo
Ppt
Dissolved in Abs. Alc. (IV & V)
Filter
Filtrate
CS_2
Alcohol
Xanthophyll (VI)
Stand for 1 week.
Extract with Ether
Ether Layer
MeOH Layer
Tricin (IX)
Re-extract with Ether
Ether Layer
MeOH Layer
Unidentified Yellow Pigment (X)
Flavones (XI)

The method in detail is as follows:

" 100 ccs. of the naptha extract (I) of Seek mill ground wheat is shaken with 50 cc. 11% KOH in 85% methanol for 40 minutes in a shaking machine. The emulsion is poured into centrifuge tubes and centrifuged at moderate speed until a clean separation is formed. The naphtha layer is then siphoned back and shaken again for 20 min. with 50 ccs. of 11% KOH in methanol. It is separated again as above, and twice extracted with 90% methanol. The naphtha extracts (II) are recentrifuged at very high speed to clarify them.

The various methanol washings are combined, re-extracted with naphtha and diluted with water. They are then extracted with 50 ccs. ether. This extract is a very intense bright yellow (III). The alcoholic solution is again extracted with ether, this time using 100 ccs. This extract is a decided orange red colour (IV). The extraction is repeated a third time, using 100 ccs. ether; this extract is pale orange-red (V). Extracts III, IV and V, are evaporated overnight at room temperature in vacuo, and the next morning slowly raised to 60°C. until all the ether is driven off. 10 ccs. of absolute alcohol are added to each. Extracts IV and V give large quantities of a chocolate-brown precipitate, which is filtered off. The filtrates are combined, since they appear identical in coloration (VI). The chocolate-coloured precipitate is apparently a sterol with pigment adsorbed upon it. This pigment could only be dissolved in CS_2(VIII), the solution being orange-red in colour. Allowing for a shifting of the bands in this solvent, this pigment possesses very similar absorption to Schulerud's flavone extracts.

The alcoholic solution of extract (III) is made up to 100 ccs. with absolute alcohol (VII). This gives a bright lemon-yellow extract with no

brownish tints.

The original alcoholic extracts of the naphtha extract upon standing for a week were noticed to be nearly as intensely coloured as before they were extracted with ether to practically complete removal of colour. A further ether extract yielded a brownish extract. A second re-extraction with ether yielded a small quantity of lemon-yellow pigment, which reacted negatively to the phenol reagent, while the first did."

This procedure was followed exactly and all the extracts were examined spectrophotometrically, using a 10-cm. cell. The data for the curves are given in Tables XXVI to XXIX and Figures 20 to 23. All the data have been calculated to the basis of McNicholas' carotene curve (Transmittancy 3.03% at 4358 Å), except the two xanthophyll fractions which have been recalculated to the same basis as the xanthophyll curve of McNicholas (Transmittancy 2.10% at 4358 Å).

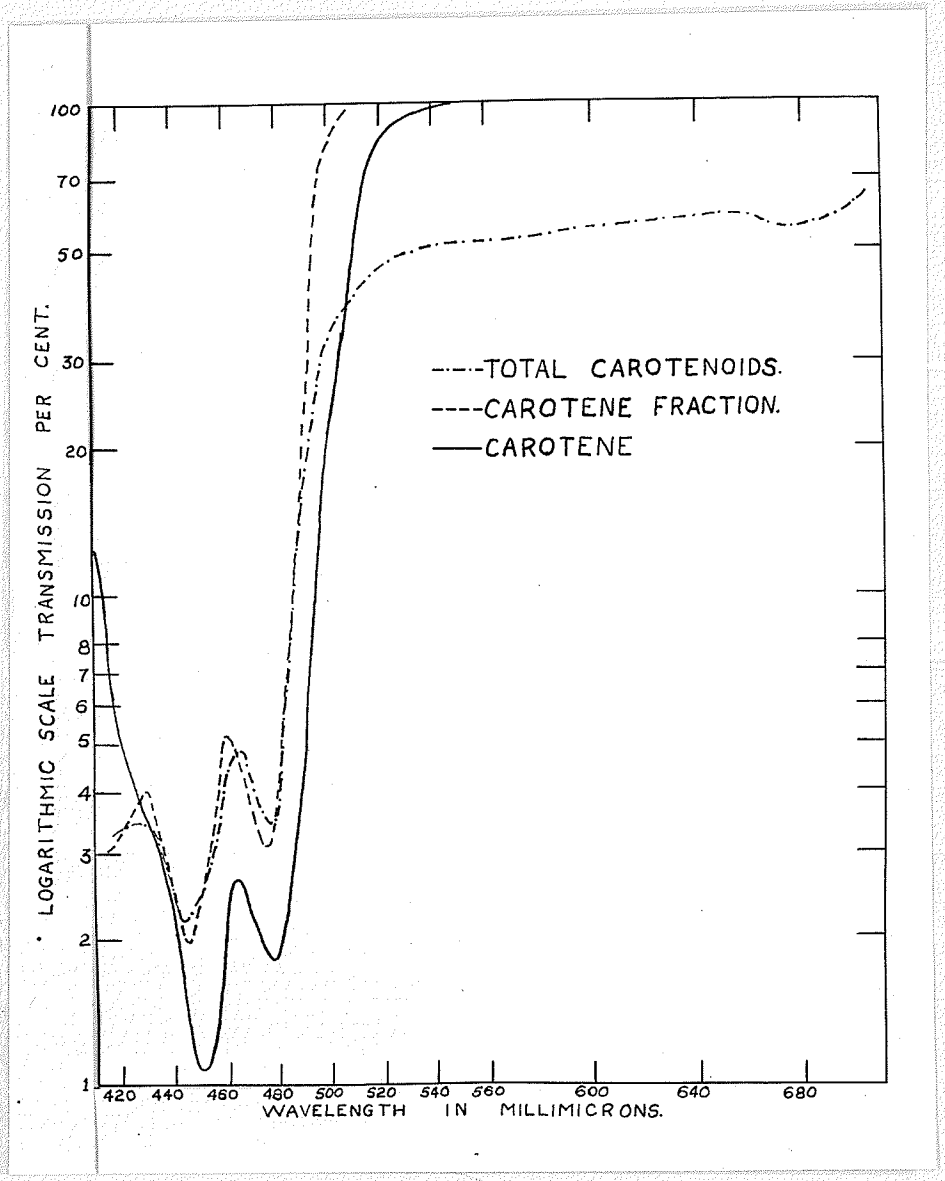

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
-·-·-TOTAL CAROTENOIDS.
----CAROTENE FRACTION.
——CAROTENE
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.

FIG. 20

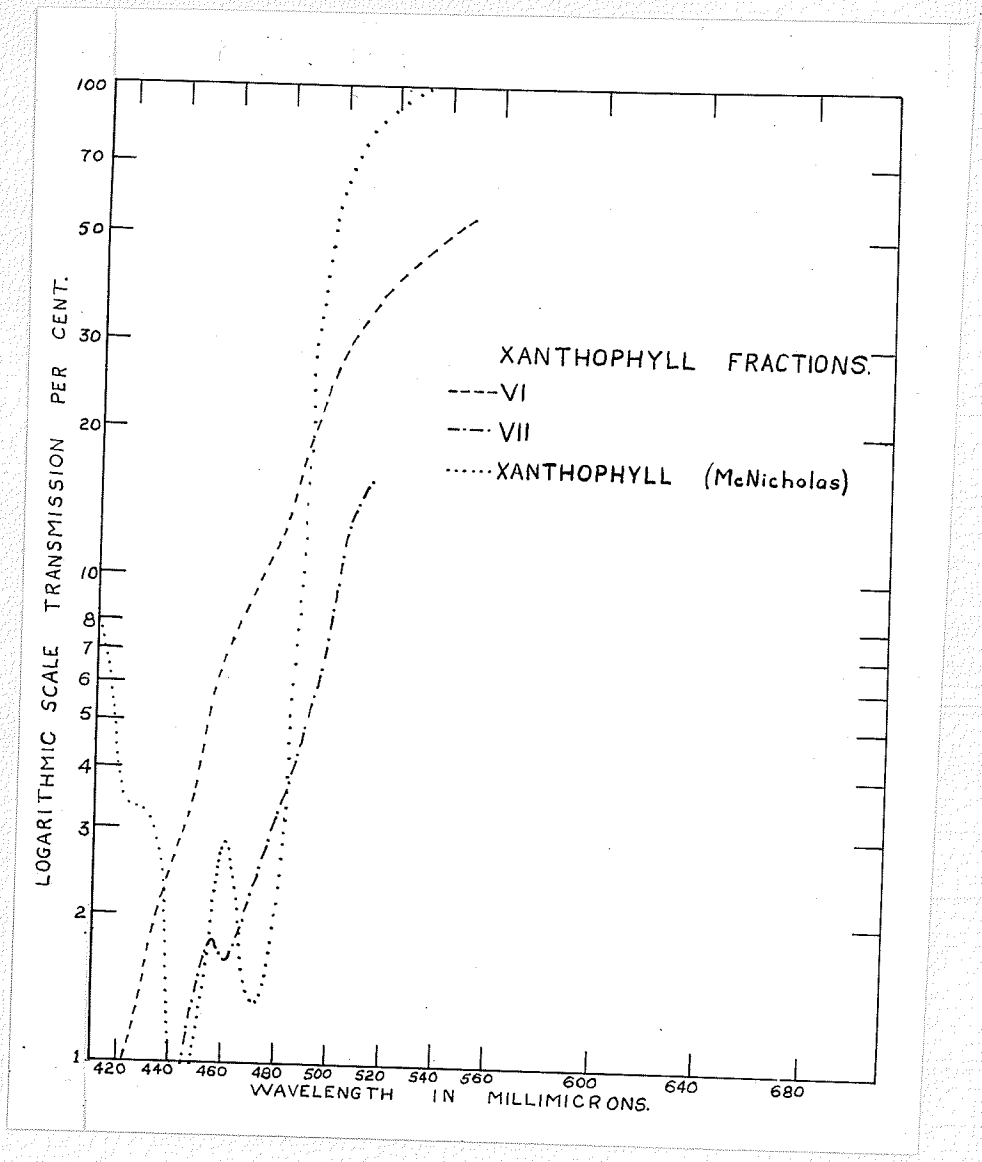

LOGARITHMIC SCALE TRANSMISSION PER CENT.
100
70
50
30
20
10
8
7
6
5
4
3
2
1
420
440
460
480
500
520
540
560
600
640
680
WAVELENGTH IN MILLIMICRONS.
XANTHOPHYLL FRACTIONS.
VI
VII
XANTHOPHYLL (McNicholas)

FIG. 21.

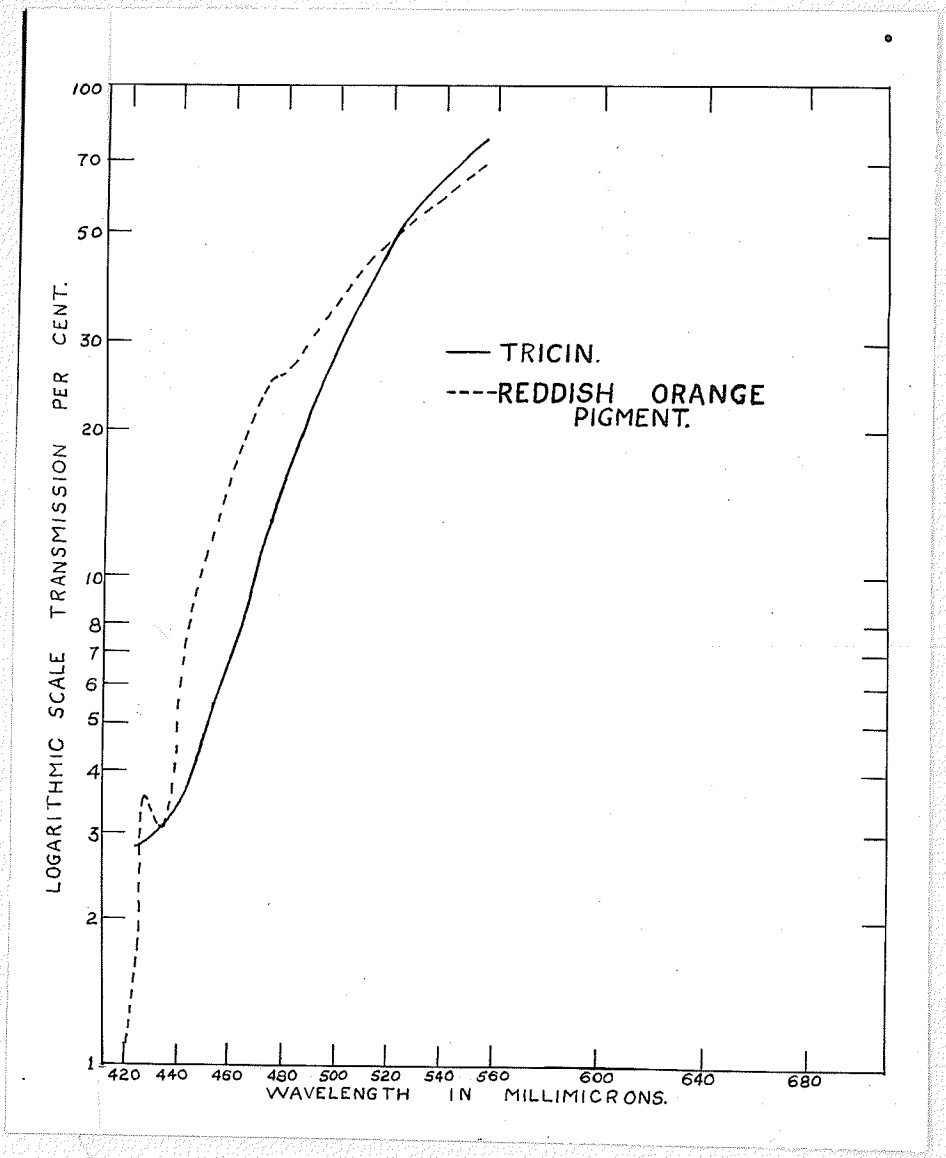

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.
TRICIN.
REDDISH ORANGE PIGMENT.

FIG. 22.

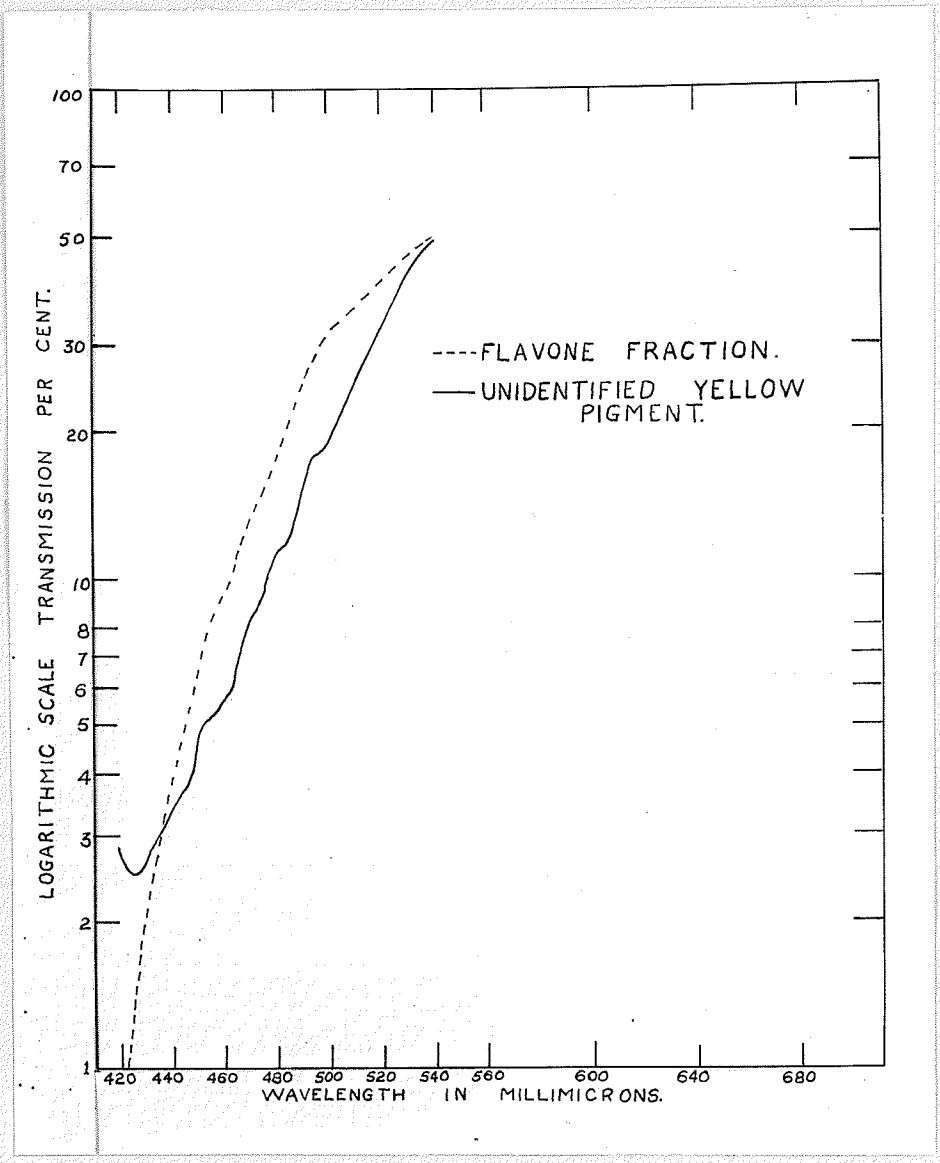

100
70
50
30
20
10
8
7
6
5
4
3
2
1
LOGARITHMIC SCALE TRANSMISSION PER CENT.
----FLAVONE FRACTION.
——UNIDENTIFIED YELLOW PIGMENT.
420 440 460 480 500 520 540 560 600 640 680
WAVELENGTH IN MILLIMICRONS.

FIG. 2.3

Data for Curves of Markley's Method.

Table XXVI

Wavelength in millimicrons	Total Carotenoids (I)		Carotene Fr. (II)	
	T%	T'%	T%	T'%
700	70.7	61.9		
690	67.0	57.6		
680	64.5	54.7		
670	65.8	55.8		
660	66.0	57.2		
650	67.0	58.8		
540	59.8	49.3	100	100
530	59.8	49.3	100	100
520	56.5	45.6	100	100
510	52.0	40.6	99	99
500	55.5	38.0	97.5	80.0
495	46.0	27.9	91.8	48.0
490	30.8	14.45	82.8	21.6
485	20.4	7.35	73.5	8.2
480	14.15	4.02	67.3	4.05
475	13.13	3.52	65.0	3.05
470	15.05	4.45	67.0	3.90
465	15.60	4.72	68.5	4.65
460	15.90	5.91	69.5	5.20
455	11.48	2.87	65.6	3.28
450	10.30	2.39	63.5	2.51
445	9.80	2.20	61.6	1.98
440	10.90	2.62	63.8	2.62
435.8	11.9	3.03	64.7	3.03
430	12.9	3.46	67.3	4.03
425	12.9	3.46	66.3	3.58
420	12.6	3.32	65.2	3.13

Table XXVII

Wavelength in millimicrons	Xanthophyll Fr. (VII) T%	Xanthophyll Fr. (VII) T'%	Xanthophyll Fr. (VI) T%	Xanthophyll Fr. (VI) T'%
700				
690				
680				
670				
660				
650				
540	82.5	14.9	67.2	47.8
530	82.5	14.9	64.2	45.9
520	82.3	14.9	60.8	39.7
510	82.5	14.9	55.5	33.5
500	78.7	9.33	50.5	28.1
495	75.5	6.20	46.2	23.8
490	74.2	5.18	40.8	18.9
485	72.2	3.95	37.1	15.85
480	71.0	3.38	32.3	12.25
475	68.8	2.49	29.3	10.22
470	68.2	2.25	27.5	9.08
465	66.0	1.64	26.6	8.57
460	66.5	1.78	24.0	7.05
455	64.3	1.27	22.2	6.08
450	61.5	.82	19.36	4.89
445	60.5	.69	15.2	3.01
440	59.0	.55	14.16	2.65
435.8	60.5	.69	12.50	2.10
430	60.5	.69	10.80	1.60
425	60.5	.69	8.83	1.12
420	60.5	.69	7.83	.89

Table XXVIII

Wavelength in millimicrons	Reddish-Orange Pigment (VIII)		Tricin (IX)	
	T%	T'%	T%	T'%
700				
690				
680				
670				
660				
650				
540	58.5	59.4	73.3	63.9
530	54.0	55.0	67.5	56.7
520	48.0	49.0	62.2	49.2
510	41.1	42.1	51.5	38.4
500	36.6	37.6	44.5	31.1
495	34.2	35.2	39.7	26.4
490	30.4	31.4	35.2	22.2
485	27.0	28.0	31.8	19.22
480	24.6	25.5	27.4	15.49
475	24.3	25.2	24.4	13.10
470	22.1	23.0	21.1	10.62
465	18.50	19.40	17.75	8.27
460	15.06	15.91	16.05	7.18
455	12.50	13.23	14.15	5.97
450	9.50	10.15	11.90	4.63
445	7.46	8.03	10.82	4.05
440	5.00	5.42	9.50	3.37
435.8	2.75	3.03	8.86	3.03
430	3.20	3.51	8.65	2.92
425	1.46	1.62	8.45	2.82
420	.71	.81	8.38	2.79

Table XXIX

Wavelength in millimicrons	Unknown Yellow Pigment (X)		Flavones (XI)	
	T%	T'%	T%	T'%
700				
690				
680				
670				
660				
650				
540	77.4	48.5	48.2	49.2
530	75.8	42.4	44.2	45.2
520	66.8	32.0	39.8	40.8
510	61.8	25.6	34.8	35.8
500	55.0	18.5	30.6	31.6
495	54.8	18.3	26.6	27.6
490	49.3	13.57	23.8	24.8
485	47.0	11.82	18.50	19.40
480	46.3	11.38	15.60	16.42
475	42.5	8.93	14.48	15.28
470	41.3	8.22	12.10	12.82
465	37.5	6.28	10.25	10.95
460	35.8	5.52	8.68	9.31
455	35.5	5.30	7.33	8.83
450	33.2	4.42	5.75	6.21
445	31.2	3.71	4.65	5.05
440	30.2	3.40	3.78	4.12
435.8	29.0	3.03	2.75	3.03
430	27.7	2.67	1.88	2.10
425	27.0	2.49	1.16	1.30
420	26.5	2.83	.61	.70

Discussion of the Curves

Carotene Fraction

The position of the maxima and minima agree very well with the carotene curve of McNicholas, but at the blue end there is an absorption band which does not belong to carotene. Therefore, the fraction cannot be wholly carotene, although it must be composed almost entirely of that pigment, since the position of the other bands agree so well.

Xanthophyll Fraction

Extract (VII), according to Markley, is composed of a pigment which is xanthophyll or a mixture of xanthophylls, while extract (VI) is a mixture of flavone type pigment and xanthophyll. However, from the data obtained here, this is not the case. The curve for extract (VI) shows the transmittancies dropping steadily from 5400 A to 4200 A and with slight shifts, perhaps due to some xanthophyll being present. Extract (VII) has a curve approximately the same shape, except that it shows a small band with a maxima at 4620 Å. This, however, does not correspond to a band of xanthophyll according to the xanthophyll curve (McNicholas). From these data, one can see that extract (VI) is not composed of xanthophyll. It is more probable that both extracts contain, essentially, other pigments, presumably flavones and xanthophyll only in a minor part.

Little can be said about the curves of the other extracts, since curves for flavones and triein are not available for comparison. They all show a similarity in shape. The extract labelled "Triein" has a very smooth curve, the transmittancies falling gradually, and finally the curve flattens from 4300 to 4200 Å. The others, the yellow pigment, reddish orange pigment, and the fraction called "flavones" all have some irregularities.

Whether these irregularities or shifts in some cases are significant, or whether they are due to errors in reading, is uncertain.

Discussion of the Method

From the data and curves obtained it can be seen that the method gives a comparatively pure carotene fraction after the saponification, but it does not yield a pure xanthophyll fraction. There is not a good enough separation of the carotenoid from the flavones, the difficulty probably being due to the high solubility of both the pigments in ether. Considering the last part of the method, the separation of the flavone, it does not seem logical that continued ether extraction of the methyl alcohol layer should yield different entities. The spectral distribution curves of these fractions would seem to substantiate this, since they are all so similar in shape.

COMPARISON OF THE CAROTENE FRACTION OF EXTRACTS PREPARED WITH NAPHTHA AND NAPHTHA-ALCOHOL.

In routine determinations of carotene on ground wheat, the 90:10 solvent has been used. It has been known that naphtha extracts less pigment, but it was uncertain whether the addition of the alcohol increased the amount of carotene extracted. Markley's method gives a reasonably pure carotene; therefore, it was decided to use it to investigate this point.

The separation was carried through with both solvents, in duplicate, down to the carotene fraction. In each case 100 ccs. of extract was used for saponification. The data, as determined by transmittancy measurements at 4358 Å, are as follows, expressed as parts per million:-

	A	B
Naphtha-Alcohol	1.62	1.74
Naphtha	1.00	1.00

This shows definitely that the 90:10 solvent extracts more carotene than the naphtha. The methyl alcohol layer, using 90:10, was a dark brownish yellow, while the MeOH layer with naphtha was a pale lemon-yellow colour. Thus, the mixed solvent also extracts more pigment other than carotene, probably flavones.

SUMMARY AND CONCLUSIONS

Carotene was extracted from carrot roots by the method developed by Schertz with some minor modifications. This product was a mixture of the two isomeric forms, alpha and beta, having a melting point of 174°C. The specific transmissive index of the pigment in a mixture of 93% varnish makers' naphtha and 7% absolute ethyl alcohol was found to be 1.9145, which is in excellent agreement with previously determined figures of Schertz (1.9148), and Ferrari (1.9165). The spectral distribution curves were made on three solvents, ethyl ether, petroleum ether and naphtha-alcohol, and found to differ, due to a solvent effect on the position of the bands. Petroleum ether solution, when compared with the curve for carotene of McNicholas determined in ethyl ether, is shifted towards the blue end, while in the other two solvents, towards the red end of the spectrum.

A series of extracts of ground Garnet wheat was prepared with different solvents, and their spectral distribution curves studied.

These showed that carbon tetrachloride, chloroform, petroleum ether and benzene removed essentially carotene; and ethyl ether, absolute methyl and alcohols, acetone, ethyl acetate and naphtha removed essentially xanthophyll. However, in most cases, non-carotenoid pigments were extracted as well, which showed bands at approximately 6700 A and 4300 A. Pyridene differed from the rest in exhibiting a totally non-carotenoid spectral distribution curve.

Different types of rust spores grown under conditions of both presence and absence of light have been investigated for their content of carotenoid pigments. In all cases, the spores produced under normal light conditions, show a higher pigment content. Some differences also occur between the spectral distribution curves of the spores grown under contrasting conditions. In general, however, the curves are decidedly similar to those obtained in the earlier study and indicate similarity in the nature of the pigments present, the principal exception to this being the so-called "white" spores, the pigment content of which is quite definitely carotene.

The only spores which exhibit bands that are not characteristic of carotene or xanthophyll are the "normal covered" and "grey covered", both of which show a very similar band in the blue-violet. Further work is necessary before any suggestion can be offered as to the nature of the third pigment.

A column of ground wheat was percolated with a series of solvents, one after the other, in the hope of separating the pigments. This proved unsuccessful, since it appears that no one solvent will extract a single pigment and leave the rest. Lipoids are probably responsible for this difficulty. Hence separation by solvents cannot be effected without previous saponification of the material.

It should be noted that there is a pronounced solvent effect on the position of the absorption bands of a pigment, as shown by the spectral distribution curves of the extracted carotene in ethyl ether, petroleum ether and naphtha alcohol. Therefore, the method of comparison of the curves of extracts with solutions of pure pigments, as employed in this study, is only correct if the solvents are the same in both cases. Unfortunately, the data for spectral distribution curves of the pure pigments in different solvents are not available, but this work should be done before studies of this type are continued.

A study of a method of pigment separation devised by Markley, was made. This showed that a relatively pure carotene fraction could be obtained. The spectral distribution curve of the fraction was very similar to the McNicholas' carotene curve, although it exhibited a non-carotenoid band at 4300 Å. The xanthophyll fraction, however, is not satisfactory since it also contains flavone pigments. The various flavone pigment fractions yield spectral distribution curves of an essentially similar nature. Then, considering that these fractions are all ether extracts of a methyl-alcohol solution, it is unlikely that they represent separate and distinct pigments. However, the knowledge of these pigments is so scant that no definite conclusions can be drawn in this respect.

Using Markley's method to obtain a carotene fraction, the 90:10 solvent was compared with naphtha. The former was found to extract considerably more carotene, although it also removed more of the other pigments.

About the time that the work described in this thesis was completed, a series of papers was published by Miller (11,12,13 and 20), and associates, outlining a precise photo-electric method of spectrophotometry in the visible, together with improved methods of preparation and spectral

distribution curves for the carotene isomers, xanthophyll and lycopene.

Unfortunately, the only data available at the time this study was made were that of McNicholas (9). However, comparisons have been made between the data presented by Miller (13) and that of McNicholas (9) and it was found that the curve for beta carotene is practically identical with that of McNicholas' carotene. This proves that his preparation, and also the one made in this study are almost wholly composed of the beta isomer. Also, Miller's work shows the position of the maxima and minima of the alpha carotene curve to be almost identical with that of leaf xanthophyll.

From this information it seems probable that a spectral distribution curve of a crude extract of flour or wheat is not sufficient to determine the carotenoids present, and that a separation must first be made, since differentiation of the curves of the pigments is so critical.

BIBLIOGRAPHY

1. Andersen, J.A.
1932 The yellow coloring matter of Khapli wheat. II. The constitution of tricetin. Can. Jour. Res. 7: 285-292.

2. Bailey, C.H.
1925 Chemistry of wheat flour. Chem. Cat. Co., New York.

3. Ferrari, C.G., and Bailey, C.H.
1929 The carotenoid pigments of flour. Cereal Chem. 6: 218-240.

4. _____ and Bailey, C.H.
1929 The determination of carotin in flour. Cereal Chem. 6:347-71.

5. _____
1933 Spectrophotometric determination of the carotenoid pigment content of wheat flour. Cereal Chem. 10: 277-287

6. Geddes, W.F., Binnington, D.S., and Whiteside, A.G.O.
1934 A simplified method for the determination of carotene in flour extracts. Cereal Chem. 11:1-24

7. Hanna, W.F.
1931 Studies on the nature of rust resistance in wheat. Can. Jour. Res. 4: 134-147.

8. Kent-Jones,
1924 Modern cereal chemistry. North. Pub. Co., Liverpool, England.

9. McNicholas, H.J.
1931 The visible and ultra-violet absorption spectra of carotin and xanthophyll and the changes accompanying oxidation. Bur. Stand. Jour. Res. 7: 171-193.

10. Markley, M.C. and Bailey, C.H.
1935 Nature of the pigments of the gasoline extract of wheat. Cereal Chem. 12:33-39.

11. Miller, E.S.
1934 The application of quantitative spectral analyses to binary mixtures of the common carotenoids. Plant Phys. 9:681-684.

12. _____
1935 A rapid and accurate quantitative method for the determination of the common carotenoids: Analyses of alpha and beta carotene and leaf xanthophyll in thirteen plant tissues. Jour. Amer. Chem. Soc. 57:347-349.

13. _____
1935 Improved methods for the purification of the common carotenoids, and the quantitative measurement of their absorption spectra. Bot. Gaz. 96: 447-467.

14. Optical Society of America
1922 Report of the committee on colorimetry for 1920-21.

15. Palmer, L.S.
1922 Carotenoids and related pigments. Chem. Cat. Co.

16. Routh, I.B.
1933 Recent advances in the chemistry of vitamins. Jour. Chem. Ed. 10.

17. Schertz, F. M.
1923 The quantitative determination of carotene by means of the spectrophotometer and the colorimeter. Jour. Agr.Res. 26:383-400.

18. _____
1925 Some physical and chemical properties of carotin and the preparation of the pure pigment. Jour.Agr.Res. 30:469-474.

19. _____
1925 Some physical and chemical properties of xanthophyll and the preparation of the pure pigment. Jour.Agr.Res. 30: 575-585.

20. Zscheile, F.P., Hogness, T.R., and Young, R.F.
1934 The precision and accuracy of a photoelectric method for comparison of the low light intensities involved in measurement of absorption and fluorescence spectra. Jour. Phys. Chem. 38: 1-11.

21. Annual Review of Biochemistry,
1932 Vol. 1.

22. Annual Review of Biochemistry,
1933 Vol. 2.