EFFECT OF ENVIRONMENTAL FACTORS UPON THE COLOR OF THE TOMATO AND THE WATERMELON[1]

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(WITH SEVENTEEN FIGURES)

Introduction

A contribution to the knowledge of the physiological mechanism involved in the production of color in fruits of commercial value is of considerable advantage and usefulness at the present time. A premium is always paid for desirably colored products, both in the canning and in the fruit and market gardening industries. The natural color of ripe fruits may be due to pigments of very diverse nature, produced by the protoplast either in the living plastid, in the cell sap, or deposited in the cell wall. The plant physiologist is particularly interested in the biochemical changes which are responsible for the development of these varied pigments, the conditions under which they are produced, and their ultimate fate in the animal body. At the present time the layman is fast becoming familiar with the fact that certain of these yellow plant pigments are precursors of vitamin A and play an important rôle in human nutrition.

The chlorophyll of higher plants is produced in special organs of the living cell called chloroplasts. According to ZIRKLE (**63**) first mention of these "green granules" was made by COMPARETTI (**13**) in 1791. The living nature of these bodies, however, was not established until almost a century later when SCHIMPER (**51**) proved that they arise by division of preexisting structures and gave them the name "plastids." The pigments of the green leaf were first extracted and given the name "chlorophyll" by PELLETIER and CAVENTOU (**50**) in 1818, but it was not until 1864 that the first separation of the chlorophyll complex into green and yellow components was made by STOKES (**55**). In a paper before the Royal Society he stated: "I find the chlorophyll of land plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties. The green substances yield solutions exhibiting a strong red fluorescence; the yellow substances do not." It is to this man that science owes the discovery of the still widely used method of partition between two immiscible solvents. He stated definitely, "For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation." Unfortunately, this discovery was overlooked by

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plant physiologists and did not come into general use until after BORODIN (**11**) isolated two different types of crystals from the yellow constituent. In 1906 TSWETT (**57**) introduced the chromatographic method for the separation of the component pigments, which also is widely used today. Furthermore, it was TSWETT who introduced the term "carotenoid" and proposed grouping the several yellow pigments found in leaves under this name. ARNAUD (**9**) some years previously had investigated the yellow pigment accompanying chlorophyll and discovered that it was identical with the carotene isolated from the carrot (*Daucus carota* Linn.) by WACKENRODER (**58**) in 1831. ARNAUD first established the hydrocarbon nature of the pigment and proposed a relationship between the yellow pigments and chlorophyll, suggesting a rôle for them similar to that of hemoglobin. It is interesting to note that recently JAVELLIER, ROUSSEAU, and EMERIQUE (**28**) examined one of ARNAUD'S preparations sealed in a tube of hydrogen and found it to possess biological activity after a period of forty years.

In 1913 MONTEVERDE and LUBIMENKO (**44**) presented a spectrocolorimetric method for the estimation of plastid pigments and studied their transformation in living plant tissue. In the same year WILLSTÄTTER and STOLL (**61**) applied the method of splitting a complex molecule into cleavage products. By purification and further study of these products they were able to reconstruct the chlorophyll molecule and by this means obtained proof of its constitution. During the course of these investigations WILLSTÄTTER and MIEG (**60**) obtained the two yellow pigments as secondary products in extracting chlorophyll from nettle leaves and established their empirical formulae. The observation that carotenoids may act as oxygen carriers or absorbers led WILLSTÄTTER to introduce this concept into the photosynthesis mechanism, as a means of reducing chlorophyll b and keeping the a and b components in equilibrium. In 1927 ZECHMEISTER (**8**) showed by catalytic hydrogenation that carotene contained eleven ethylenic linkages and was a bicyclic compound. Lycopene was found by the same investigator to contain thirteen double bonds and to be an unsaturated aliphatic molecule. Lycopene was first discovered in the fruit of the tomato (*Lycopersicum esculentum* Mill.) by MILLARDET (**40**) in 1876, although it did not receive the name lycopene until 1903 when SCHUNCK (**52**) presented a paper before the Royal Society of London. WILLSTÄTTER and ESCHER (**59**) isolated this red pigment from Italian tomatoes in 1910 and suggested its hydrocarbon nature. Today both carotene and lycopene are recognized as polyene hydrocarbons and PALMER (**49**) suggests a new system of nomenclature for the entire group. Furthermore, KUHN (**32**) has shown that the color of certain carotenoid compounds depends upon a chain of more than four conjugated double bonds and presented a method of separation of carotenoid pigments which has been developed further by MILLER (**41**). The fact that the smell

of violets was apparent on the oxidation of carotene led KARRER (**3**) to believe the odor due to the presence of β-ionone rings. Subsequent investigation proved the symmetrical structure of carotene and that both ends of the hydrocarbon chain were cyclic and identical. Lycopene was found to add thirteen molecules of hydrogen, forming perhydrolycopene and by a series of reactions lycopene was shown to be a symmetrical acyclic hydrocarbon carrying thirteen double bonds, eleven of which are conjugated. The deep color of lycopene adds weight to KUHN'S (**33**) observation on crocetin and bixin that deep color requires more than four conjugated double bonds. Furthermore, KARRER accomplished the difficult synthesis of perhydrovitamin A and then was able to present the structural formula of vitamin A. Previous to this time OSBORNE and MENDEL (**48**) and MCCOLLUM and DAVIS (**38**) had found the A factor to be present in cod liver oil and in butter. STEENBOCK (**54**) brought out the fact that only products containing certain yellow pigments had vitamin A potency. DRUMMOND (**17**) repeated the work using pure carotene and obtained negative results due to the solvent used. It remained for EULER (**2**) and KARRER (**3**) independently of each other, to harmonize these apparently discordant results and to prove β-carotene to be the parent substance which is converted to vitamin A by the animal body. Numerous contributions by these and other investigators have shown the existence in plants of a series of polyene hydrocarbons, a series of polyene alcohols, and several polyene carboxylic acids. The carotenoid pigments are of wide distribution in nature and are responsible for a great number of colors in all plant organs. They are known to exist together and in combination with other organic pigments, so that fruit or flower color is likely to consist of a pigment complex, variation being due to the balance between the various pigments involved.

The marked effect of temperature upon the production of lycopene was first discovered by DUGGAR (**18**) in 1913. This remarkable contribution showed conclusively that a temperature of 30°–37° C. clearly inhibited the development of lycopene both in detached fruit and in fruit growing on the vine. The factors for reddening were not destroyed by the high temperature, for upon return to a temperature of 20° C. lycopene formation proceeded rapidly. Lycopene formation follows the destruction of the chlorophyll but certain other changes which take place within the cells remain unknown. Lycopene formation, however, does not necessarily follow decomposition of chlorophyll as HARVEY (**27**) has recently shown. The chemical reactions and the relationship between the pigments, the source from which the pigments are derived, the physiological importance of each component, are questions which remain little understood and for which quest is being carried on throughout the world. Lycopene formation occurs only at temperatures above 10° C. and below 37° C. Chlorophyll decomposition

requires a temperature above 15° C. and is removed less rapidly at 40° C. than at 24° C. In addition, HARVEY (**26**) has shown that chlorophyll decomposition by ethylene can occur only when the cells are in an active metabolic state. The presence of fungi, slight mechanical injuries, or certain other conditions prevent the balancing action of ethylene. Therefore, it is likely that the decomposition of chlorophyll does not take place by a single enzyme mechanism, but that the whole actively metabolic system of the cell is required. The same is indicated for lycopene formation.

Extensive reviews of the literature are found in monographs by KARRER (**3**), ZECHMEISTER (**8**), LEDERER (**4**), MAYER (**5**), BERGMANN (**1**), and PALMER (**6**).

Determination of color

The subject of color is a most fascinating one for scientist and layman alike. Man has been color-conscious for a long time, the word itself being very old. Like many old words, the word "color" has been used to convey different meanings. As a household word, its meaning is vastly different from its use by the physicist.

Color depends on the constituents of white light which are not absorbed but which are reflected. Color sensations in the normal individual depend on the responses of the retina of the eye to different wave lengths of light. If the light has one definite wave length, the color produced will have a pronounced hue. When Sir ISAAC NEWTON passed white light through a glass prism, he obtained a continuous spectrum containing all the spectral hues, which may be described as red, orange, yellow, green, blue, and violet. However, NEWTON recognized their objective physical meaning, saying plainly, "The rays, to speak properly, are not coloured. In them is nothing else than a certain power or disposition to stir up a sensation of this or that colour. . . . So colours in the object are nothing but a disposition to reflect this or that sort of rays more copiously than the rest." NEWTON also knew that any color could be matched by mixing the component blue, green, and red portions in proper proportions. If the primary spectral colors, *i.e.*, light of wave lengths of 460, 530, and 650 mμ be mixed in varying proportions, any hue whatsoever can be produced. The visible spectrum is arbitrarily divided into six broad regions (fig. 1), but the color of the spectrum varies continuously throughout its length. The physical difference between red, green, or blue is one of wave length. The unit commonly used for specifying this difference is the millimicron. Table I shows the approximate wave lengths of these six broad regions.

In the analysis of color the eye is not a good analytical instrument. It gives a confused sensation owing to the inability clearly to distinguish between the three components of color, particularly brightness and purity. When a color is measured spectroscopically, such confusion is eliminated.

TABLE I

SPECTRAL REGIONS IN TERMS OF WAVE LENGTH

	$m\mu$
Violet	400–450
Blue	450–500
Green	500–570
Yellow	570–590
Orange	590–610
Red	610–700

If the light from a reflected surface is measured by a colorimetric method, the eye perceives only those wave lengths to which it is sensitive. Different eyes are sensitive in different degrees to different wave lengths. In such a procedure different observers may obtain different results. Also, when a color is observed under varying quality of illumination, the sensation produced under each light will be different. Colorimetric measurements, therefore, are not dependent on the light source alone, but also on the idiosyncrasies of human vision. Spectrophotometry, on the other hand, provides a method for completely specifying the stimulus of color independent of material color standards, or of abnormalities of the observer's vision and other personal eccentricities. The color of a reflecting surface can be determined with sufficient precision, and the analysis of any color can be plotted in the form of a spectrophotometric curve, as in figure 1. Thus, two or more colors may be compared by comparing their curves, each of which constitutes a permanent record of the color and does not require the maintenance of a sample of the color.

The color of green tomato fruits is due to the complex chlorophyll mixture within the chloroplast. The fact that yellow pigments accompany chlorophyll has been known since the fundamental work of STOKES in 1864. Upon the decomposition of chlorophyll at temperatures from 30°–37° C., carotenes, xanthophylls, and certain yellow pigments give the tomato its characteristic color. If the temperature during the ripening period is held below 30° C., unknown changes take place within the actively metabolic cells, resulting in the formation and accumulation of lycopene, the carmine-red isomer of carotene. The color changes due to the physical mixture of the various pigments can be satisfactorily determined by spectrophotometric reflection measurements. Thus, optics furnishes the plant physiologist with an accurate method of color measurement, and enables him to interpret color changes owing to the fact that there are two or more pigments present in physical mixture rather than in chemical combination.

In this work reflection measurements from the surface of the fruit were taken with a Keuffel and Esser direct-reading spectrophotometer. The re-

flection measurements thus obtained were plotted in the form of a spectrophotometric curve and also converted into terms of the red, green, and violet excitation values. From these values the dominant wave length and percentage of purity were read from a copy of the chart as published in the report of the committee of the Optical Society of America (**56**) on colorimetry for 1920–21. The percentage of relative brightness was also computed so that the colors of fruits are given in numerical expression of the three attributes of color, namely, hue, saturation, and brilliance.

In describing the color stimulus in terms of the psychological sensation which it produces, all of these attributes of color must be considered. The Committee of the Optical Society of America (**56**) suggests the following definitions: "Brilliance is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white." Brilliance is expressed as percentage of relative brightness. The percentage of brightness of a color defines that proportion of the total amount of white light falling upon it that the color is capable of reflecting or transmitting. All colors except black exhibit brilliance. The brightness of absolute black is zero, that of pure white 100 per cent.

"Hue is that attribute of certain colors in respect of which they differ characteristically from the gray of the same brilliance and which permits them to be classed as reddish, yellowish, greenish, or bluish." Hue is expressed as dominant wave length.

"Saturation is that attribute of all colors possessing a hue, which determines their degree of difference from a gray of the same brilliance." Saturation is expressed as percentage of purity. All colors which exhibit a hue must also exhibit a saturation. Grays have zero percentage purity. Saturation determines the degree in which a color possesses hue; thus, the percentage purity defines how red or how green, or how yellow a color is. Purity and chroma are synonymous terms.

For example, spectrophotometric reflection measurements show the color of a ripe tomato as having a brightness of 11.8 per cent. a dominant wave length of 609 mμ, and a purity of 24.5 per cent. The spectrophotometric curve is presented in figure 1; the dominant wave length shown by the vertical line falls in the orange-red region of the spectrum, and the observer pictures an attractive red fruit. A red and a pink may have the same dominant wave length but will differ in percentage of purity; pink, being an unsaturated red, will have a lower percentage of purtiy. A pure spectral red has a purity of 100 per cent. On the other hand, the red will have a lower percentage of brightness owing to the lesser proportion of white in it. Where the curve is an approximate straight line parallel to the abscissa, the color is said to be gray. The horizontal line of zero relative energy will represent black, whereas the line of 100 relative energy will represent white.

Curve 2 is a spectral reflection curve of a Marglobe tomato ripened at 32° C. and which appears to the eye as a brilliant yellow. In numerical terms the color of this fruit would be described as possessing a brightness of 33.5 per cent., a dominant wave length of 581.5 mμ, and a purity of 59 per cent. Curve 3 is plotted from reflection measurements taken from a typical Marglobe tomato picked in the so-called "mature green" stage. The color of this fruit is described as having a brightness of 32.9 per cent., a dominant wave length of 570 mμ, and a purity of 53 per cent. Such a color indicates a yellowish green in which the green components of chlorophyll to a large extent mask the yellow ones.

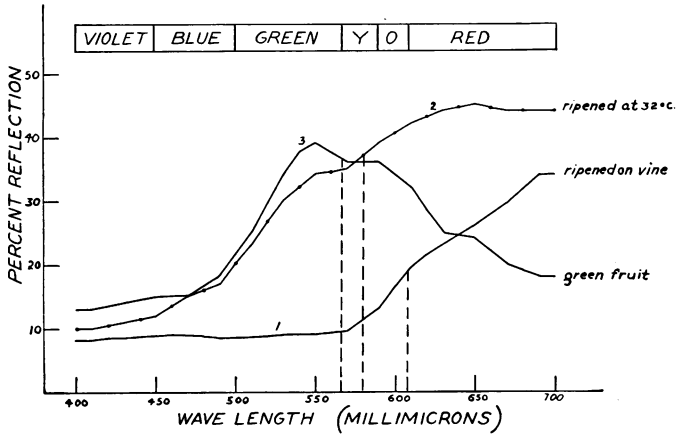

FIG. 1. Color analysis of Marglobe tomatoes.

This method of color analysis was chosen because the spectrophotometer is an instrument of precision by means of which the attributes of color can be measured accurately. As previously pointed out, the eye is not an analytical instrument, and, with advance in color measurement, spectrophotometers will undoubtedly become more readily available in the future.

Effect of temperature

EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN TOMATO

In an attempt to ripen quickly some tomato fruits which were gathered green to avoid losing the fruits from frost DUGGAR (**18**) made the discovery that those fruits ripened at a temperature of 20° C. in the dark for seven days had a much redder color than those stored in an incubator at a temperature of 35° C. for an equal length of time. This observation led to a series of carefully planned and executed experiments on greenhouse-grown Earliana tomatoes. Later similar experiments were conducted on species of *Capsicum* and *Momordica*. In all cases fruits stored at temperatures above 30° C. and

below 37° C. showed very little production of lycopene and were distinctly yellow in color. The same results were obtained when only half-grown fruits were tried and also when the experiments were extended to cover fruits developing on the vine. These facts led DUGGAR to conclude that the optimum temperature range for lycopene formation is narrow and that its optimum coincided with the optimum for growth or perhaps was a few degrees lower. Furthermore, the factors for reddening were not destroyed by a temperature of 37° C., for when such fruits were returned to favorable temperature of 20° C., lycopene development proceeded and in four or five days the yellow fruits became red. EULER, KARRER, KRAUS, and WALKER (**20**) confirmed the observation that temperatures above 30° C. produce tomato fruits which are yellow in color. They suggest that the yellow pigment so induced is not

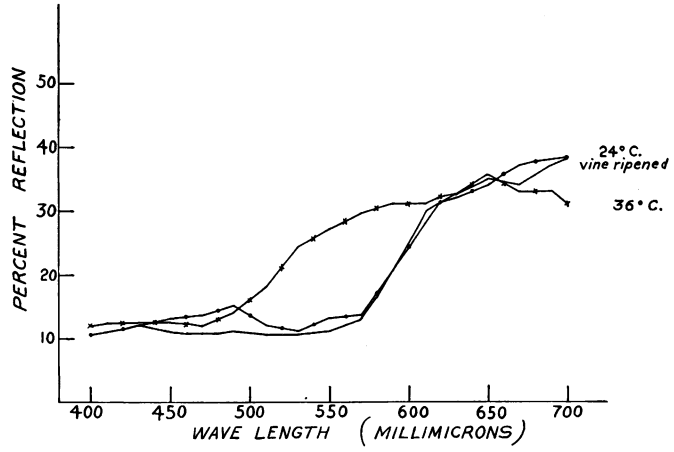

FIG. 2. Effect of temperature (24°, 36° C.).

of a carotenoid nature but is possibly a flavone or flavinol. However, the last named authors make the clear assumption that lycopene formation is an enzymatic process and that lycopene synthesis does not occur at temperatures above 30° C. because of the destruction of the unknown enzyme.

In the experimental work herein reported the author used Bonny Best and Marglobe varieties of tomatoes. The fruits were picked green and selected for uniformity in size and in approximate age from blossoming time. The fruits were then stored for twelve to twenty-four hours at 20° C. and again finally selected for experimental work. The fruits were placed in darkness in constant temperature cases of two and one-quarter cubic feet capacity and the temperatures were held at 24°, 28°, 32°, 36°, and 40° C. (± 1° C.). Reflection measurements were taken with a Keuffel and Esser spectrophotometer at the close of a seven-day period in the case of series 1 and 4, and at the end of a nine-day period in series 2 and 3. Series 2, 3, and 4 served also

as checks in the work on the effect of ethylene, of varying concentration of oxygen, and of light respectively. Moisture was supplied by evaporation from open dishes in each constant temperature case. Reflection measurements were taken from three representative tomatoes in each case. To obtain a flat surface for reflection a section of the pericarp wall 22 mm. in diameter was removed with a sharp cork borer and this section mounted in the 25-mm. cell which accompanies the instrument. Such a section when flattened filled the cell and gave a uniform reflection surface. Measurements were recorded at 20-mμ intervals from 450 to 690 mμ respectively. Since the analytical work requires a reading at 10 mμ intervals from 400 to 700 mμ respectively, assumed readings were interpolated or extrapolated as necessary. However, the sensitivity of the eye below 450 and above 690 mμ is so

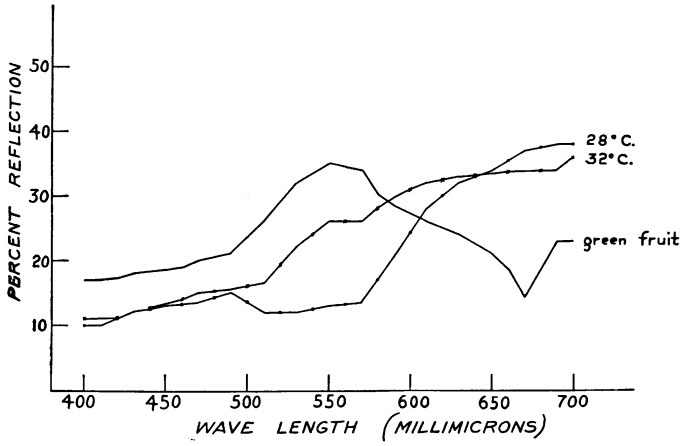

FIG. 3. Effect of temperature (28°, 32° C.).

low that an assumed value is sufficiently accurate. The percentage of reflection was recorded, and the red, green, and violet sensations and the relative luminosity as compared to the luminosity of noon-day sun were calculated with the aid of a special Keuffel and Esser slide rule. From these data the brightness, dominant wave length, and purity of each sample were calculated and are presented in table II.

The most remarkable observation in respect to the effect of temperature (fig. 4) is the great difference in percentage of brightness and percentage of purity of samples ripened at 24° and 28° C. as compared with those ripened at 32° and 36° C. This difference is even more pronounced when dominant wave length is considered. Fruits ripened at 32° and 36° C. show a dominant wave length of from 500 to 588 mμ, appearing bright yellow, while those ripened at 24° and 28° C. show a dominant wave length of from 595.5 to 609.5 mμ and appear a deep orange-red to the eye. Spectral reflection curves are presented in figures 2 and 3.

When the bright yellow fruits ripened at 32° and 36° C. are returned to a temperature 20° C., they rapidly turn orange-red and show a dominant wave length of 592.5 to 597.0 mμ. After four days at 20° C., such fruits are as red as normal fruits ripened on the vine and show a dominant wave length of from 600 to 610 mμ.

A second remarkable effect of temperature of great importance is the fact that both Bonny Best and Marglobe varieties of tomatoes when ripened at 40° C. do not turn yellow but remain green, *i.e.*, chlorophyll decomposition is prevented. Frequently brown spots of various size and shape develop after four or five days and such fruits in which normal metabolic activity is at least seriously checked are quickly attacked by bacteria and other fungi. Fruits ripened at 40° C. for seven days show a dominant wave length of

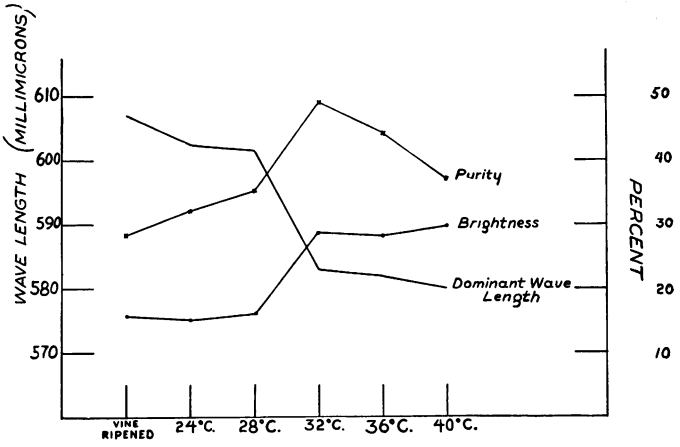

FIG. 4. Effect of temperature on purity, brightness, and dominant wave length.

578.5 mμ, a brightness of only 22.96 per cent., and a purity of 22.5 per cent. and appear a dull green to the eye. This low purity as compared with normal green fruit indicates unsaturation, *i.e.* a gray-green of low brilliance, having a larger proportion of white admixed. When these fruits ripened at 40° C. are transferred to a temperature of 20° C., they do not produce lycopene as do the fruits ripened at 32° or 36° C. but very quickly undergo internal breakdown. Such fruit are particularly subject to attack by *Bacillus carotovorus, Phoma* sp., *Rhizopus nigricans,* and other saprophytic organisms. The normal metabolic activity of such fruits is seriously interfered with, resulting in the more or less complete disorganization of the plasma structure. With this disorganization the complete destruction of the whole cellular mechanism for lycopene formation takes place. This great difference which is produced when the temperature is changed from 36° to 40° C. is remarkable and with other previously mentioned reasons indicates that

TABLE II

EFFECT OF TEMPERATURE UPON PRODUCTION OF COLOR IN TOMATO

SERIES 1 + 4–9 DA., 2 + 3–7 DA.	BRIGHTNESS				DOMINANT WAVE LENGTH				PURITY			
	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 1	SERIES 2	SERIES 3	SERIES 4
Vine ripened fruit	16.33	17.68	16.93	11.80	610.0	605.0	605.0	609.0	25.0	32.0	31.5	24.5
Green fruit	29.91	30.45	26.26	31.21	565.0	570.0	577.5	562.0	35.0	40.0	37.0	40.0
24° C.	16.93	16.74	15.73	11.38	608.0	600.0	600.0	602.5	23.0	30.0	32.5	33.0
28° C.	15.89	17.91	16.06	14.17	609.5	600.0	595.5	604.0	22.0	35.0	46.0	38.5
32° C.	25.36	22.32	30.81	36.83	582.5	588.0	582.0	580.0	45.5	42.5	57.0	51.0
36° C.	25.69	26.02	33.39	28.31	582.0	580.0	583.5	582.5	50.0	32.0	51.2	44.0
40° C.	22.96	33.65	32.74	30.21	578.5	580.0	581.0	579.0	22.5	39.0	42.0	45.0
32° C. + 2 da. 20° C.	19.02	.....	.....	.....	597.0	.....	.....	.....	29.00	.....	.....	.....
32° C. + 4 da. 20° C.	16.63	.....	.....	.....	605.0	.....	.....	.....	30.00	.....	.....	.....
36° C. + 2 da. 20° C.	18.51	.....	.....	.....	592.5	.....	.....	.....	42.00	.....	.....	.....
36° C. + 4 da. 20° C.	17.35	.....	.....	.....	600.0	.....	.....	.....	32.00	.....	.....	.....

lycopene formation is not a simple enzymatic change but, like the decomposition of chlorophyll, is an internal change which can take place only in actively metabolic cells, and emphasizes the physiological inequalities of different temperature ranges.

Effect of temperature upon development of color in watermelon

Both Karrer (3) and Zechmeister (8) state that lycopene is the red pigment in the watermelon (*Citrullus vulgaris* Schrad.). A series of experiments was planned to determine the effect of temperature upon lycopene production in this fruit. It was found to be impossible to hold green watermelon fruits at temperatures of 24° to 40° C. for more than a few days. Fruits which were removed from the vine were quickly attacked by saprophytic fungi and were reduced to a decayed mass in a relatively short time. One lot of melons, variety Winter Queen, were successfully held at 24°, 28° and 32° C. for fifteen days.

The spectrophotometric data for these fruits, as seen in the accompanying table, show that in fifteen days the fruits have undergone very little change regardless of the temperature at which they were held. This difference in behavior between the watermelon and the tomato led to further attempts to ripen watermelon fruits under temperature control. All other attempts to artificially ripen watermelons off the vine gave negative results because of fungus attack. Removal of fruits with ten to fifteen feet of vine, trial feeding of immature melons with glucose, and several other attempts proved futile. However, it was deemed practical to move temperature equipment to the field. Three fruits of variety Arikara were held at controlled temperature for nineteen days. One fruit was kept in the dark at 18° to 22° C., another was kept at 33° to 37° C. in the light, and a third melon was held in the same higher temperature case but in the dark. The color analyses of such fruits show close agreement, brightness varying from 14.62 to 16.43 per cent., dominant wave length from 592 to 595 mμ, and purity from 44 to 50 per cent. (table III).

These data indicate that perhaps the same mechanism for lycopene formation does not exist in the watermelon as in the tomato. A shift in temperature from 20° to 37° C. did not result in the prevention of lycopene formation as in the case of the tomato,the red pigment production continuing without apparent interruption regardless of the temperature. Either lycopene formation proceeds in a different fashion in the watermelon than in the tomato or else we are dealing with a red pigment other than lycopene. Bilger (10) has recently shown that the red pigment from the Japan red pepper differs in chemical composition from lycopene or from the Zechmeister-Cholnoky red pigment from paprika, and that this new pigment is not an isomer of carotene. However, Brown (12), working with the red

TABLE III

EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN WATERMELON

	BRIGHTNESS	DOMINANT WAVE LENGTH	PURITY
	Variety Winter Queen		
		mμ	
Green fruit	31.45	585.0	16.0
24° C. 15 days	28.34	589.0	16.0
28° C. 15 "	21.06	588.0	23.0
32° C. 15 "	31.27	584.5	17.0
36° C. 15 "	data not available due to decay		
40° C. 15 "	data not available due to decay		
	Variety Arikara (vine ripened)		
18°–22° C. 19 days, dark	16.43	592.0	50.0
33°–37° C. 19 days, light	14.62	595.0	44.0
33°–37° C. 19 days, dark	15.84	595.0	44.0

pigment of the Perfection pimento (*Capsicum annuum*), finds it to be identical with the capsanthin isolated by ZECHMEISTER and CHOLNOKY (**62**) from the Hungarian paprika. Quantitative data in regard to the identification of the red pigment of the watermelon is needed. Data derived from breeding experiments with both the watermelon and tomato indicate that red is invariably dominant to yellow in hybrids. The genetic ratios obtained suggest that a complex group of factors are present and that they must be in subtle balance. Furthermore, certain unknown metabolic factors are at play which are capable of producing changes within the organism. MUNSELL (**45**) has shown that the red fleshy portion of fresh watermelon tissue when fed to rats as a sole source of vitamin A produces a satisfactory unit gain over an eight-week period. This would lead to the belief that either certain yellow carotenoids must be present in the tissue of the watermelon and that the red pigment would seem to be superimposed on the yellow, as is the case in the tomato, or that certain other antiophthalmic-anti-infective substances are present. Both VON EULER and KARRER state that of all the naturally occurring carotenoids only the carotenes have a provitamin A effect. It seems likely, therefore, that such changes in color in the tomato due to temperature, or lack of such changes in the watermelon, as herein reported, are not the result of a single enzymatic factor but that they are the result of a subtle balance of conditions occurring in the actively metabolic, living cells.

Effect of ethylene upon the production of color in the tomato

Observations in regard to the physiological activity of ethylene go back to the early work on the effect of illuminating gas on plants. GIRARDIN (**23**) was the first worker to describe injury to street trees due to illuminating gas

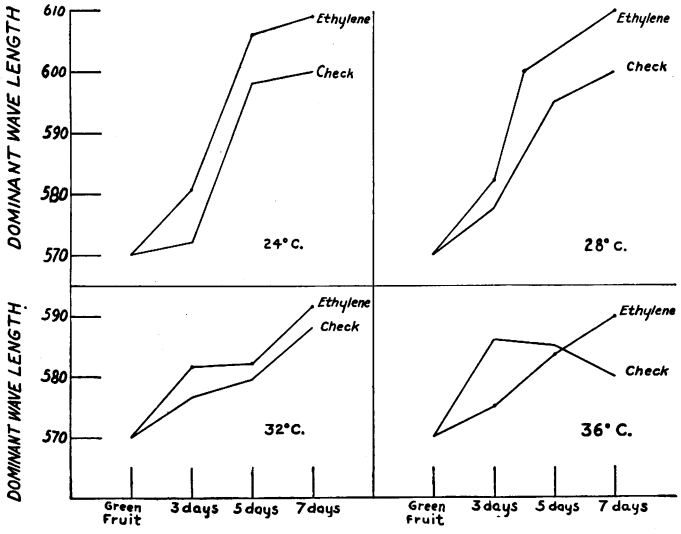

FIG. 5. Effect of ethylene at different temperature.

and KNY (**31**) was the first worker to conduct experimental work on the injurious nature of illuminating gas to maple and linden trees. As early as 1884 MOLISCH (**42**) observed that the linear growth of corn roots was retarded by low concentration of illuminating gas. In 1901 NELJUBOW (**46**) obtained a horizontal curvature of pea and bean seedlings with a concentration of but one part ethylene to one million parts of air. CROCKER and KNIGHT (**15**) determined that the injurious effect of illuminating gas on the flowering of carnations was due to the amount of ethylene which it contained. Later, MOLISCH (**43**), and CROCKER and KNIGHT (**30**) demonstrated that the

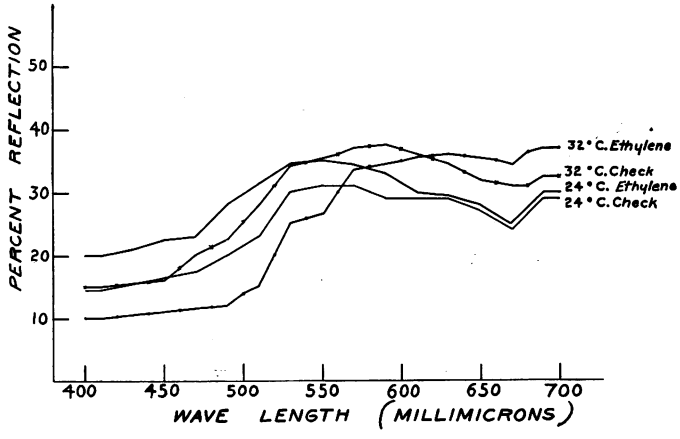

FIG. 6. Effect of ethylene (3 days, 24°, 32° C.).

physiological activity of tobacco smoke was due to the small amount of ethylene present.

SIEVERS and TRUE (**53**) observed the effects of the products of incomplete combustion of kerosene and other substances on the rate of coloring of citrus fruits. DENNY (**16**), trying empirically the substances in pure form which might cause this acceleration of color, was the first investigator to use ethylene for coloration of citrus fruits. By the use of ethylene, self-blanching varieties of celery were blanched by HARVEY (**24**) in six days with a concentration of ethylene of one part to one thousand at a temperature of 18° C. For the dark green varieties a longer time (ten to twelve days) was required. Positive results with ethylene on the ripening of tomatoes and bananas were also obtained by HARVEY (**26**) and successful commercial methods were worked out and established.

Recently ELMER (**19**) discovered that certain volatile substances from several varieties of apples caused an inhibition of growth of potato sprouts. GANE (**22**) was able to identify analytically the active volatile substance as ethylene, the amount produced being very small. NELSON and HARVEY (**47**), using the leaf epinasty method, were also able to show that Golden self-blanching celery produces in normal metabolism some gaseous or volatile compound and that the decomposition of chlorophyll is accomplished by some process similar to that by which celery is commonly blanched artificially by ethylene.

TABLE IV

EFFECT OF ETHYLENE ON PRODUCTION OF COLOR IN TOMATO

	BRIGHTNESS		DOMINANT WAVE LENGTH		PURITY	
	ETHYLENE	CHECK	ETHYLENE	CHECK	ETHYLENE	CHECK
Vine ripened	.....	17.68	.....	605.0	.....	32.0
Green fruit	.....	30.45	.....	570.0	.....	40.0
24° C. 3 days	29.07	32.61	580.5	572.0	39.5	28.0
28° C. 3 "	26.00	32.27	582.0	577.5	43.0	28.5
32° C. 3 "	28.11	33.94	581.5	576.5	58.0	47.0
36° C. 3 "	38.37	21.60	575.0	586.0	40.5	23.5
24° C. 5 days	17.26	16.66	606.0	598.5	29.0	36.5
28° C. 5 "	14.57	16.72	600.0	595.0	36.0	30.5
32° C. 5 "	30.50	34.03	582.0	579.5	47.5	39.0
36° C. 5 "	34.78	28.45	583.5	585.0	45.0	44.0
24° C. 7 days	15.26	16.74	609.0	600.0	31.5	30.0
28° C. 7 "	14.71	17.91	610.0	600.0	32.0	35.0
32° C. 7 "	23.01	22.32	591.5	588.0	44.0	42.5
36° C. 7 "	20.43	26.02	590.0	580.0	40.5	32.0

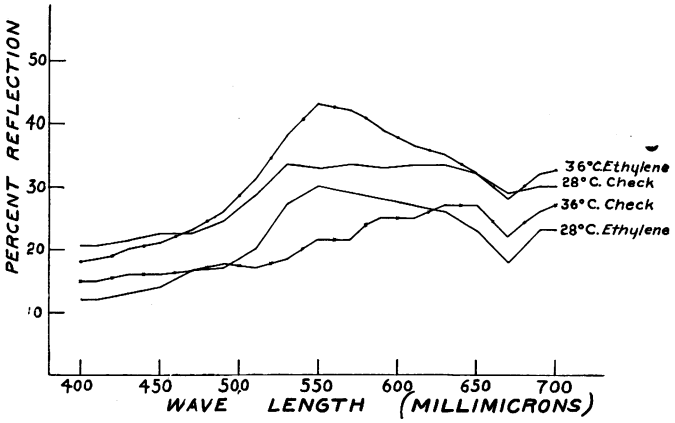

FIG. 7. Effect of ethylene (3 days, 28°, 36° C.).

The production of the red color of the tomato is due to the formation of lycopene within the actively metabolizing cells of the fruit. For this red color to be manifest the chlorophyll must first be decomposed. The data presented in table IV show that, under the influence of ethylene, the decomposition of chlorophyll is hastened and that the production of lycopene quickly follows, providing the temperature is suitable.

Bonny Best tomatoes were used in this work and received ethylene gas at the rate of one part per thousand on three successive days. The fact that ethylene-ripened fruits are redder than untreated ones in the same length of time is clearly demonstrated when dominant wave length is plotted against time, as shown in figure 5. However, it should be clearly stated that lycopene formation does not take place at temperatures of 32° or 36° C. whether

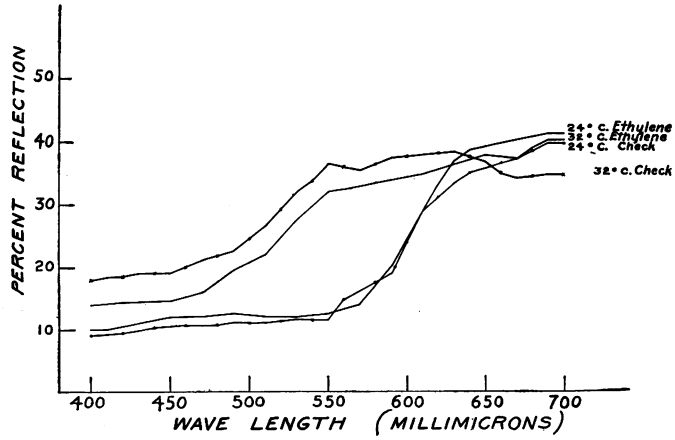

FIG. 8. Effect of ethylene (5 days, 24°, 32° C.).

treated with ethylene or not. Undoubtedly, some of the commercial failures to hasten the reddening of tomatoes by the use of ethylene are due to the fact that lycopene formation has such a narrow temperature range. Fruits ripened at 32° C. or above possess a dominant wave length of 575 to 591.5 mμ and a relatively high purity. Such fruits appear a bright yellow to the eye. When these yellow fruits are returned to a temperature of 20° C. to 28° C., lycopene formation occurs rapidly. However, such yellow fruits can be maintained in the yellow condition for from two to three weeks by storage at a temperature of 2° C. Evidently lycopene formation does not necessarily follow chlorophyll decomposition, and if lycopene is formed from colorless chlorophyll decomposition products, its synthesis involves a mechan-

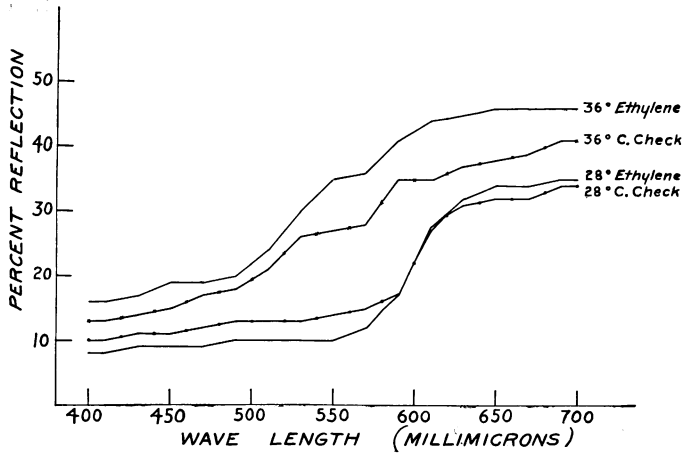

FIG. 9. Effect of ethylene (5 days, 28°, 36° C.).

ism different from the mechanism responsible for chlorophyll decomposition. The data in table IV (plotted in fig. 5) also show that ethylene hastens lycopene formation as well as chlorophyll decomposition. The ethylene-treated fruits possess a dominant wave length of 600 to 606 mμ at the end of a five-day-ripening period whereas the dominant wave length of those fruits ripened without ethylene varies from 595 to 598.5 mμ and appears more orange-red to the eye than those of the ethylene group (figs. 8, 9). Likewise, at the end of a seven-day period the ethylene-ripened fruits show a higher dominant wave length and thus would appear redder to the eye (figs. 10, 11).

Little is actually known in regard to the mode of action of ethylene in the living cell. A review of the literature shows that ethylene is exceedingly active and effects a great diversity of reactions. It is capable of markedly modifying the growth rate of meristematic tissue; of producing intumescences in many different plant tissues; of bringing about premature abscission of leaves; of producing specific epinastic response of petioles at a

minimum concentration of but one part in ten millions; of greatly increasing the oxidation rate in tissues exposed to it even in very low concentrations; of giving increase in soluble sugars and amino acids, at the expense of the insoluble carbohydrates, proteins, and fats; and of hastening the process of chlorophyll decomposition. Data presented above adds weight to the latter statement and, in addition, show that lycopene formation is materially hastened by ethylene, provided a suitable temperature is maintained.

Effect of varying concentrations of oxygen upon production of color in tomato

In agreement with EULER (**2**) and KARRER (**20**), LUBIMENKO (**36, 37**) holds the view that the process of ripening in the tomato is an enzymatic

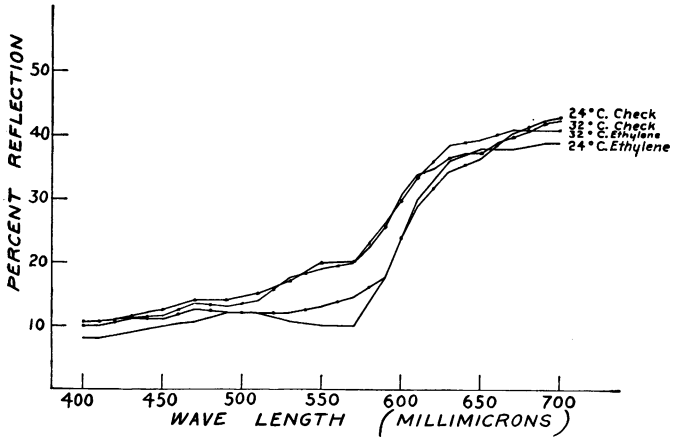

FIG. 10. Effect of ethylene (7 days, 24°, 32° C.).

one. However, he maintains that three separate processes take place in ripening. In the first period when the synthetic reaction is not crowded by oxidation, the accumulation of chlorophyll takes place. Later, oxidation processes predominate, whereby the chlorophyll together with the yellow components are destroyed and transformed into colorless unknown materials. His third postulate has to do with the color-producing activity of the plastid and the accumulation of a red carotenoid pigment within the cells. Whether or not these oxidation processes represent a part of what actually takes place in the cell remains to be seen. Preliminary work on ripening tomatoes in oxygen and other gases indicated that lycopene formation occurs only under conditions favorable for normal metabolism. Green tomatoes placed under pure carbon dioxide and nitrogen gases undergo no development of lycopene regardless of the temperature at which they are held. After five to seven days, such fruits undergo very serious internal breakdown, followed by rapid putrefaction. When the oxygen supply is

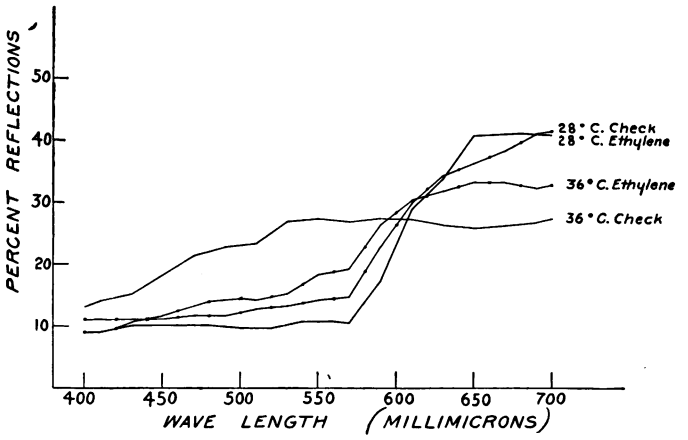

FIG. 11. Effect of ethylene (7 days, 28°, 36° C.).

inadequate, the formation of lycopene does not take place even under favorable temperature conditions. However, chlorophyll decomposition goes on uninterruptedly at low concentrations of oxygen, and at temperatures of 24° and 28° C., as well as at 32° and 36° C., yellow tomatoes are produced (figs. 12–16). Table V shows the dominant wave length of such yellow fruits to be 581.5, 578, 580.5, and 585 mμ at 24°, 28°, 32°, and 36° C. respectively.

Fruits ripened at low and high oxygen concentrations respectively were placed in the constant-temperature cases previously mentioned but within desiccators of approximately three and one-half liters capacity, four fruits being placed in each. Low oxygen concentrations were maintained by keeping the fruits in closed desiccators, which were ventilated with a stream of air for five minutes each twenty-four hours. High oxygen concentration was

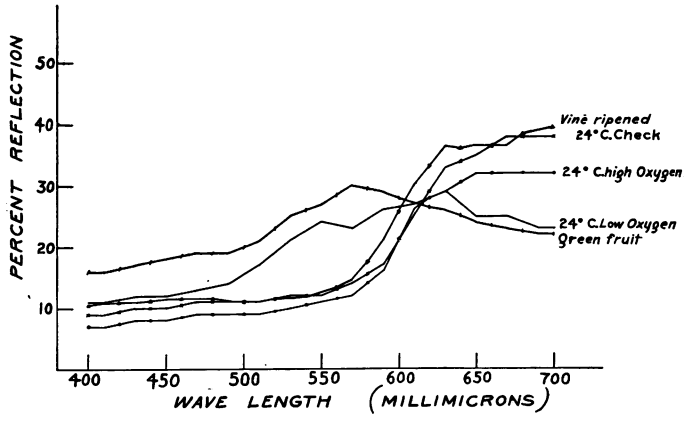

FIG. 12. Effect of oxygen concentration, 24° C.

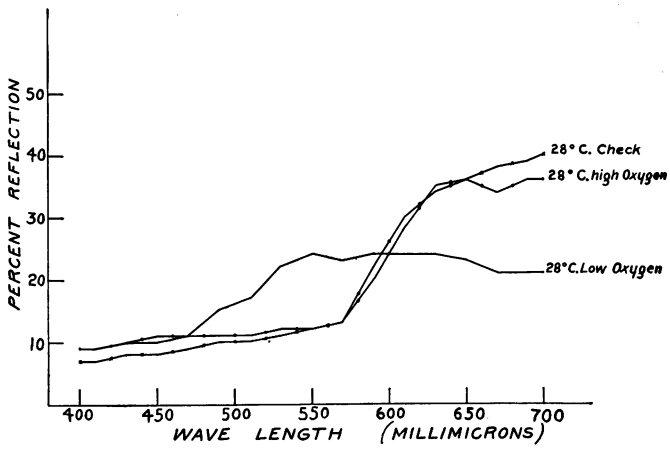

FIG. 13. Effect of oxygen concentration, 28° C.

maintained by slow, constant bubbling of a mixture of 50 per cent. air and 50 per cent. oxygen through the desiccators, the mixture being prepared in and liberated to the desiccators from individual gasometers.

Yellow fruits which were produced under low concentrations of oxygen reddened when returned to the air at 20° C. for four or five days, as also did the yellow fruits which were ripened at 32° and 36° C. in the high oxygen chambers. The close agreement of the dominant wave lengths of fruits ripened at 32° C. is remarkable, the fruits ripened in a low concentration of oxygen having a dominate wave length of 580.5 mμ, the fruits ripened at a high concentration of oxygen having a dominant wave length of 579.5 mμ, and the fruits ripened in air, not being confined to desiccators, having a dominant wave length of 582.0 mμ (fig. 14). The same is true of

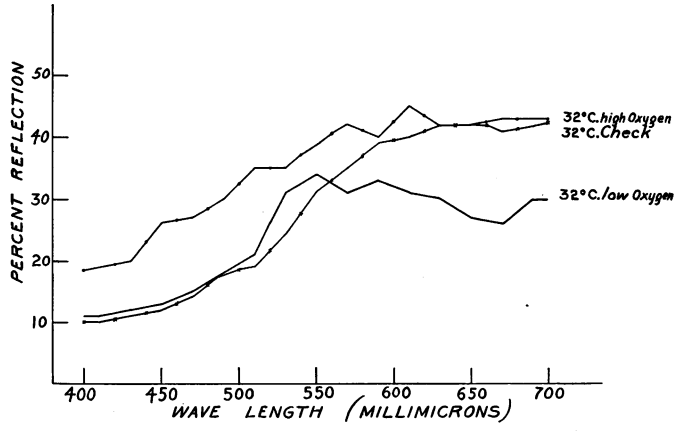

FIG. 14. Effect of oxygen concentration, 32° C.

TABLE V

EFFECT OF VARYING CONCENTRATION OF OXYGEN ON PRODUCTION OF COLOR IN TOMATO

	DOMINANT			BRIGHTNESS WAVE LENGTH			PURITY		
	LOW 0_2	HIGH 0_2	CHECK	LOW 0_2	HIGH 0_2	CHECK	LOW 0_2	HIGH 0_2	CHECK
Vine ripened	.....	.....	16.93	.....	.....	605.0	.....	.....	31.5
Green fruit	.....	.....	26.26	.....	.....	577.5	.....	.....	37.0
24° C. 7 da.	22.84	14.18	15.73	581.5	598.5	600.0	44.0	40.0	32.5
28° C. 7 da.	22.06	16.76	16.06	578.0	601.0	595.5	49.0	35.0	46.0
32° C. 7 da.	27.79	38.68	30.81	580.5	579.5	582.0	52.0	35.0	57.0
36° C. 7 da.	23.55	21.10	33.39	585.0	583.5	583.5	54.0	43.0	51.5

those fruits held at 36° C. having a dominant wave length of 585.0, 583.5, and 583.5 in low oxygen, high oxygen, and air respectively (fig. 15). This indicates that chlorophyll decomposition goes on at both low and high concentrations of oxygen but that the formation of lycopene is prevented when the oxygen supply is decreased, as in the case of fruit ripened under the partial anaerobic conditions of the experiment. That temperatures above 30° C. inhibit the production of lycopene is again apparent, which together with the before mentioned observation that suitable quantities of oxygen must be present for lycopene formation to take place indicates that beyond all reasonable doubt, the production of lycopene is a process which can take place only when the cells are actively metabolizing and that it seems to be more than a simple enzymatic change.

Effect of light upon the production of color in the tomato

The discovery by HARVEY (27) of the importance of light as a factor in the decomposition of the carotenoids present in butter and in the peel of

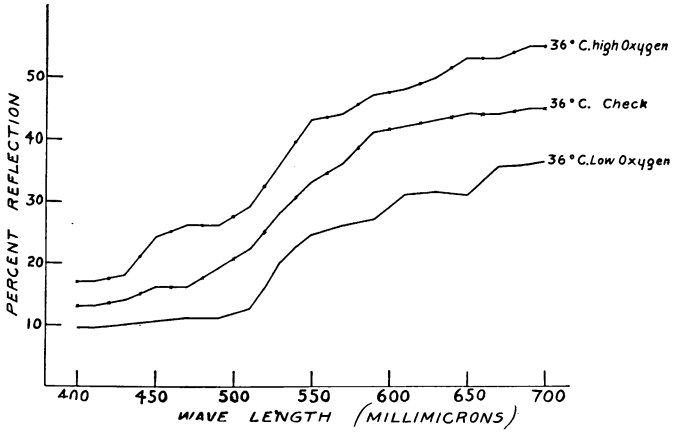

FIG. 15. Effect of oxygen concentration, 36° C.

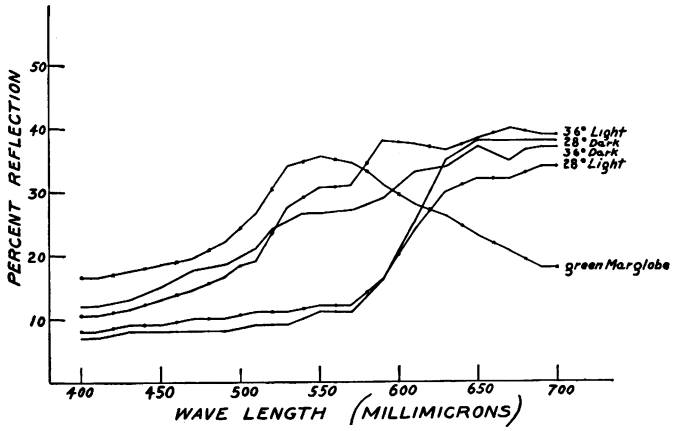

FIG. 16. Effect of light, 28°, 36° C.

orange fruits in the presence of benzaldehyde led to a series of experiments in which Marglobe tomatoes picked in the full-grown, green stage were ripened at temperatures of 24°, 28°, 32°, and 36° C. in total darkness and illuminated for twenty-four hours daily. The light source was a 1000-watt Mazda bulb giving an illumination of 1540 to 1620 foot-candles as measured by the Weston photronic cell, model 603. That chlorophyll decomposition is hastened by light is not denied but from the data presented in table VI lycopene formation is shown to proceed equally well in either light or darkness, provided the fruits are maintained in the presence of air and at a suitable temperature, namely, 20° to 28° C. (figs. 16, 17). Also, light may affect the yellow carotenoid constituents of the tomato, but the data indicate that temperature is the limiting factor in lycopene production. This also seems to hold true for the decomposition of other carotenoids. It was considered that light absorbed by chlorophyll and by carotenoid pigments might activate the decomposition of these pigments owing to the energy absorption,

TABLE VI

EFFECT OF LIGHT ON PRODUCTION OF COLOR IN TOMATO

	BRIGHTNESS		DOMINANT WAVE LENGTH		PURITY	
	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK
Vine ripened fruit	11.80	.....	609.0	.....	24.5	.....
Green fruit	31.21	.....	562.0	.....	40.0	.....
24° C. 9 da.	11.68	11.38	604.0	602.5	30.0	33.0
28° C. 9 da.	14.70	14.17	599.0	604.0	34.0	38.5
32° C. 9 da.	33.30	36.83	581.0	580.0	48.0	51.0
36° C. 9 da.	30.27	28.31	581.5	582.5	54.0	44.0

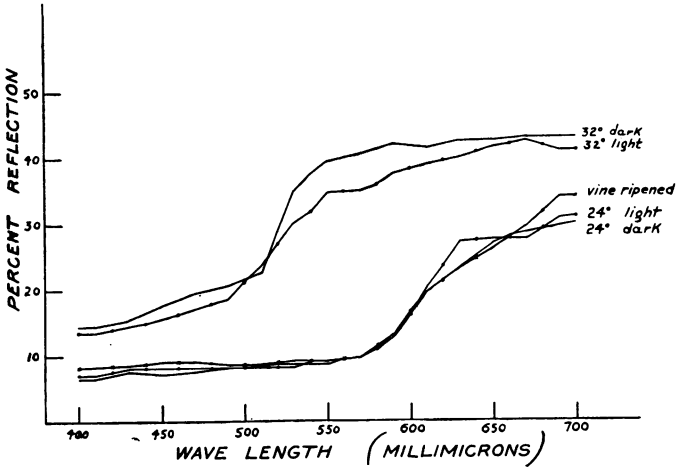

FIG. 17. Effect of light, 24°, 32° C.

but the data here presented indicate that this physical factor does not accelerate chlorophyll decomposition or the removal of carotenoids from the fruit in an appreciable amount. The data of HARVEY were obtained in the presence of benzaldehyde which may form active peroxides on light exposure of wave lengths which are absorbed by the carotenoids, and thus lead to their rapid decomposition. The tomato evidently does not produce such aldehydes as appear in the seeds of certain rosaceous plants. However, further explanation must await further progress in the explanation of certain internal changes which take place within the plasma complex of the living plastid.

Summary

1. The optimum temperature for lycopene formation in the tomato was found to be 24° C. Lycopene is not formed at temperatures above 30° C. When tomatoes are ripened at 32° to 38° C., bright yellow fruits are produced. When such yellow fruits are returned to 20° to 24° C., lycopene is developed normally.

2. Chlorophyll decomposition in tomato fruits is prevented by a temperature of 40° C. or higher. Such fruits remain green and neither produce lycopene when returned to a temperature of 20° to 24° C. nor the yellow pigments when returned to 32° C.

3. In the case of the watermelon, a shift in temperature from 20° to 37° C. did not check the production of the red pigment, indicating that the same mechanism for lycopene formation does not exist in the watermelon as in the tomato. It seems likely that color changes in the tomato due to temperature, or lack of such changes in the watermelon, are not the result

of a single enzymatic factor but are the result of a subtle balance of conditions occurring in the actively metabolic cells.

4. Ethylene hastens lycopene formation as well as chlorophyll decomposition in the tomato, provided a suitable temperature is maintained.

5. Lycopene formation is prevented when the oxygen supply is decreased, chlorophyll decomposition going on uninterruptedly within the 24° to 36° C. temperature range.

6. Chlorophyll decomposition is hastened by light, but lycopene formation proceeds equally well in either light or darkness provided the fruits are maintained in the presence of air and at a suitable temperature.

The work here reported was performed in the Laboratory of Plant Physiology, University of Minnesota, and the writer wishes to acknowledge the assistance and encouragement of Professor R. B. HARVEY. Thanks are also due Professor R. A. GORTNER, University of Minnesota, for loan of the spectrophotometer, and to the Keuffel and Esser Co., Hoboken, New Jersey, for their helpful suggestions.

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LITERATURE CITED

1. BERGMANN, E. Die chemische Erforschung der Naturfarbstoffe. Ergebn. Physiol. **35**: 158–300. 1933.
2. EULER, H. VON. Die biochemischen und physiologischen Wirkungen von Carotin und Vitamin A. Ergebn. Physiol. **34**: 360–405. 1932.
3. KARRER, P. Carotinoide. Ergebn. Physiol. **34**: 812–847. 1932.
4. LEDERER, E. Les Carotenoides des Plantes. Paris. 1934.
5. MAYER, F. Chemie der organischen Farbstoffe. Zweiter Band. Natürliche organische Farbstoffe. Julius Springer. Berlin. 1935.
6. PALMER, L. S. Carotenoids and related pigments. Amer. Chem. Soc. Monographs. New York. 1922.
7. TSWETT, N. Chlorophylls in the plant and animal world (Russian). Warsaw. 1910.
8. ZECHMEISTER, L. Carotinoide: ein biochemischer Bericht über pflanzenliche und tierische Polyenfarbstoffe. Julius Springer. Berlin. 1934.
9. ARNAUD, A. Recherches sur la composition chimiques de la carotine, sa fonction chimique, et sa formule. Compt. Rend. Acad. Sci. (Paris) **102**: 1119–1122. 1886.
10. BILGER, L. N. The composition and properties of certain red and yellow plant pigments. Bull. Basic Sci. Res. **3**: 37–46. 1931.

11. BORODIN, J. Ueber krystallinische Nebenpigmente des Chlorophylls. Bull. Acad. Imp. Sci. St. Petersburg **28**: 328–350. 1883.
12. BROWN, W. L. A contribution to the chemistry of pepper pigments. Jour. Biol. Chem. **110**: 91–94. 1935.
13. COMPARETTI, A. Prodromo de fisica vegetabile. Padova, 1791. (Citation by ZIRKLE, **63**.)
14. CROCKER, W., HITCHCOCK, A. E., and ZIMMERMAN, P. W. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. **7**: 231–248. 1935.
15. ————, and KNIGHT, L. I. Effect of illuminating gas and ethylene upon flowering carnations. Bot. Gaz. **46**: 259–276. 1908.
16. DENNY, F. E. Effect of ethylene upon respiration of lemons. Bot. Gaz. **77**: 322–329. 1924.
17. DRUMMOND, J. C. Researches on the fat-soluble accessory substance. I. Observations upon its nature and properties. Biochem. Jour. **13**: 81–102. 1919.
18. DUGGAR, B. M. Lycopersicin, the red pigment of the tomato, and the effect of conditions upon the development. Washington Univ. Studies **1**: 22–45. 1913.
19. ELMER, O. H. Growth inhibition of potato sprouts by the volatile products of apples. Science n.s. **75**: 193. 1932.
20. EULER, H., KARRER, P., *et al.* Zur Biochemie der Tomatenfarbstoffe. Helv. chem. Acta. **14**: 154–167. 1931.
21. FERRARI, C. G. Spectro-photometric determination of the carotinoid content of wheat flour. Cereal Chem. **10**: 277–287. 1933.
22. GANE, R. Production of ethylene by some ripening fruits. Nature **134**: 1008. 1934.
23. GIRARDIN, W. Einfluss des Leuchtgazes auf die Promenaden und Strassenbäume. Jahresb. Agr. **7**: 199–200. 1864.
24. HARVEY, R. B. A new method for blanching celery. Minnesota Hort. **53**: 41–43. 1925.
25. ————. Blanching celery. Minnesota Agr. Exp. Sta. Bull. 222. 1925.
26. ————. Artificial ripening of fruits and vegetables. Minnesota Agr. Exp. Sta. Bull. 247. 1928.
27. ————. Unpublished data.
28. JAVELLIER, M., ROUSSEAU, S. and EMERIQUE L. La composition chimique des tissus dans l'avitaminose A: phosphose, extrait lipoidique, cholesterol. Compt. Rend. Acad. Sci. (Paris) **188**: 580–582. 1929.
29. KARRER, PAUL. The chemistry of vitamins A and C. Chem. Rev. **14**: 17–30. 1934.
30. KNIGHT, L. I., and CROCKER, WM. Toxicity of smoke. Bot. Gaz. **55**: 337–371. 1913.

31. KNY, L. Um den Einfluss des Leuchtgazes auf die Baumvegetation zu prüfen. Bot. Zeit. **29**: 852–854, 867–869. 1871.
32. KUHN, R., and BROCKMANN, H. Bestimmung von Carotinoiden. Zeitschr. physiol. Chem. **206**: 41–64. 1932.
33. ————, and WINTERSTEIN, A. Über die Konstitution des Pikrocrocins und seine Beziehung zu den Carotin-Farbstoffen des Safrans. Ber. d. chem. Ges. **67**: 344–357. 1934.
34. KUHN, R., and WINTERSTEIN, A. Thermischer Abbau der Carotin-Farbstoffe. Ber. d. chem. Ges. **65**: 1873–1880. 1932.
35. LAAN, P. A. VAN DER Der Einfluss von Aethylen auf die Wuchsstoffbildung bei *Avena* und *Vicia*. Rec. Trans. Bot. Néerland **31**: 691–742. 1934.
36. LUBIMENKO, V. N. Transformation of plastid pigments in living plant tissue (Russian). Mem. Acad. Sci. Petrograd **33**: 275–91. 1916.
37. ————. Les pigments des plastes et leur transformation dans les tissus vivants de la plante. Rev. Gén. Bot. **40**: 23–29, 88–94, 146–155, 226–243, 303–320, 372–384. 1928.
38. MCCOLLUM, E. V., and DAVIS, MARGUERITE The necessity of certain lipins in the diet during growth. Jour. Biol. Chem. **15**: 167–175. 1913.
39. MACGILLIVRAY, J. H. Tomato color as related to quality in the tomato canning industry. Indiana Agr. Exp. Sta. Bull. 350. 1931.
40. MILLARDET, A. Note sur une substance colorante nouvelle découverte dans la tomate. Nancy, 1876. (Abstract in Bot. Jahresb. **4**: 783. 1876.)
41. MILLER, E. S. Improved methods for the purification of the common carotenoids and quantitative measurements of their absorption spectra. Bot. Gaz. **96**: 447–467. 1935.
42. MOLISCH, H. Über die Ablenkung der Wurzeln von iher normalen Wachstumsrichtung durch Gaze (Äerotropismus). Sitzungsber. kais. Akad. Wiss. Wien **90**: 111–196. 1884.
43. ————. Über den Einfluss des Tabakrauches auf die Pflanze. Teil I. Sitzungsber. kais. Akad. Wiss. Wien **120**: 3–30. 1911.
44. MONTEVERDE, N. A., and LUBIMENKO, V. N. Récherches sur la formation de la chlorophyll chez les plantes. Bull. Acad. Sci. Petrograd Ser. 6, **7**: 1007–1028. 1913.
45. MUNSELL, H. E. The vitamin A, B, C, and G content of watermelon. Jour. Home Econ. **22**: 680–85. 1930.
46. NELJUBOW, D. Über die horizontale Nutation der Stengel von *Pisum sativum* und einiger anderen Pflanzen. Bot. Centr. **10**: 128–139. 1901.

47. NELSON, R. C., and HARVEY, R. B. The presence in self-blanching celery of unsaturated compounds with physiological action similar to ethylene. Science n.s. **82**: 133–134. 1935.
48. OSBORNE, T. B., and MENDEL, L. B. The influence of butter-fat on growth. Jour. Biol. Chem. **16**: 423–437. 1913.
49. PALMER, L. S. The biological and chemical nomenclature for the carotenoides. Science n.s. **79**: 488–490. 1934.
50. PELLETIER, J., and CAVENTOU, J. B. Sur la matière verte des feuilles. Ann. Chim. Phys. Ser. 2, **9**: 194–196. 1818.
51. SCHIMPER, A. F. W. Ueber die Gestalten der Stärkebildner und Farbkörper. Bot. Centralbl. **11**: 175–178. 1882.
52. SCHUNCK, C. A. The xanthophyll group of yellow coloring matters. Proc. Roy. Soc. London **72**: 165–176. 1903.
53. SIEVERS, A. F., and TRUE, R. H. A preliminary study of the forced curing of lemons as practiced in California. Bur. Plant Industry Bull. 232. 1912.
54. STEENBOCK, H. White corn *vs.* yellow corn and a probable relation between the fat soluble vitamin and yellow plant pigments. Science n.s. **50**: 352–353. 1919.
55. STOKES, G. G. On the supposed identity of biliverdin with chlorophyll, with remarks on the constitution of chlorophyll. Proc. Roy. Soc. London **13**: 144–145. 1864.
56. TROLAND, L. E., *et al.* Report of the Colorimetry Committee of the Optical Society of America. 1920–21. Jour. Opt. Soc. Amer. **6**: 527–596. 1922.
57. TSWETT, M. Adsorptionsanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls. Ber. d. bot. Ges. **24**: 384–393. 1906.
58. WACKENRODER, H. Über das Oleum radicis. Dauci Aetherum, das Carotin, den Carotenzucher und den officinellen suceus Dauci, usw. Diss. de Anthelminticis Göttigen (1826): Geiger's Mag. Pharm. **33**: 144–172. 1831.
59. WILLSTÄTTER, R., and ESCHER, H. H. Über den Farbstoff der Tomate. Zeit. physiol. Chem. **64**: 47–61. 1910.
60. ————, and MIEG, W. Über die gelben Begleiter des Chlorophylls. Ann. Chem. **355**: 1–28. 1907.
61. ————, and STOLL, A. Untersuchungen über Chlorophyll. Julius Springer. Berlin. 1913.
62. ZECHMEISTER, L., and CHOLNOKY, L. VON. Untersuchungen über den Paprika-Farbstoff. I. Ann. **454**: 54–71. 1927; II. Ann. **455**: 70–81. 1927; III. Ann. **465**: 288–299. 1928; IV. Ann. **478**: 95–111. 1930.
63. ZIRKLE, C. Structure of the chloroplast in certain higher plants. Amer. Jour. Bot. **13**: 301–320, 321–341. 1926.