

EFFECTS OF SUB-OPTIMAL RIPENING TEMPERATURES

ON TOMATO FRUIT QUALITY AS DETERMINED

BY OBJECTIVE MEASUREMENT

BY

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## ABSTRACT

Controlled environment experiments were conducted to study the influence of four night/day temperature regimes; 17.8/25.6, 7.2/18.3, 4.4/15.6 and 2.8/13.9°C on the quality of tomato fruits, Lycopersicon esculentum Mill. c.v. Early Red Chief, measured objectively at three harvest dates. Temperature effects on vegetative and reproductive growth and fruit cracking were also examined. In addition, the stability of carotenoid pigments of macerated and cubed fruit stored at -20° for 0, 10, 20 and 40 days was studied.

Low air temperatures decreased plant growth, caused chlorosis of vegetative growth, and reduced the frequency of fruit cracking but had little effect on fruit weight. Flower formation continued at all temperatures with the exception of the 2.8/13.9 environment while fruit set occurred only at the two highest thermal regimes.

Fruits harvested at 17.8/25.6 were considerably lower in total solids, reducing sugars and titratable acidities and had substantially higher pH values than fruit exposed to 7.2/18.3, 4.4/15.6 and 2.8/13.9. Temperature had little or no effect on fruit refractive indices and total pectic substances. The failure of total pectic substances to reflect the apparent firmness differences between treatments indicates that total pectic substances are not a satisfactory index of this quality parameter.

Surface and internal lightness and yellowness declined with

increasing temperatures and later harvests, while redness values increased. Fruit harvested at 17.8/25.6 attained a full red coloration in 7 days, while those exposed to 7.2/18.3 required about 14 days to reach a comparable level of colour development. Fruit exposed to 2.8/13.9 were of inferior colour as evidenced by high  $L$  and  $b_L$  values and low  $a_L$  values.

The high degree of association between lightness and yellowness values under all treatment conditions suggests that surface colour and, to a lesser extent, internal colour can be adequately specified in terms of a constant and two, rather than three, variables.

The high overall correlation coefficient obtained between surface and internal  $Lb/a$  ratios immediately indicated the possibility of utilizing surface  $Lb/a$  ratios to predict internal colour. Temperature and harvest dates influenced the relationship between internal and surface colour ratios as evidenced by the decrease in correlation coefficients with higher temperatures and later harvests.

The effect of decreasing temperatures on tomato colour was found to be largely a function of temperature effects on lycopene synthesis. Colour values showed marked changes as total carotene concentrations increased up to about 55  $\mu\text{g/g}$  fresh weight. Continued increases above this level were not accompanied by parallel changes in surface or internal colour.

Temperatures and harvest dates affected all pigment concentrations with the exception of  $\gamma$ -carotene and, for the most part,  $\beta$ -carotene.

The temperature regimes ranked in order of decreasing fruit quality were as follows: 7.2/18.3; 17.8/25.6; 4.4/15.6; 2.8/13.9.

Although of satisfactory coloration, fruits harvested at 17.8/25.6 were rated below the 7.2/18.3 fruit for reasons of lower dry matter, sugar and acid contents.

Storage duration had little effect on carotenoid concentrations of cubed samples. In macerated samples, phytoene, phytofluene and  $\beta$ -carotene concentrations decreased with storage time. When fresh samples were analysed, all pigment concentrations with the exception of lycopene were found to be much lower in macerated than in cubed samples.

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## INTRODUCTION

The tomato (Lycopersicon esculentum Mill.) is a warm climate species of sub-tropical origin, grown extensively in the United States (California, Florida, Texas, Indiana, Maryland, New Jersey), Mexico, Spain, Netherlands and Australia. Cool temperatures represent one of the dominant factors limiting the distribution of the crop in Canada, and consequently production is largely confined to areas in southern Ontario, notably the counties of Essex and Kent.

Tomatoes destined for fresh market consumption are harvested at stages of maturity ranging from mature green to firm red, while those destined for processing are usually harvested at a full red condition. Low temperatures during the harvest period not only influence the yield, but also the quality of the fruit. Prolonged exposure of vine-ripening fruit to temperatures below about 10°C results in chilling injury and hence fruit of inferior or unacceptable quality.

Many studies have been carried out to assess the effects of ripening temperatures on fruit quality constituents; however, a wide number have not considered the diurnal character of the temperature factor. In most instances, detached fruit have been used to study the influence of ripening temperatures on physical and chemical components. The use of the latter experiments as a basis of prediction of fruit quality under glasshouse or field conditions may be invalidated to some degree by virtue of the fact that continuing translocation between the vine and fruit during the ripening process has been eliminated as an

intrinsic factor.

Colour is one of the most important and complex attributes of fruit quality and hence considerable attention has been directed towards its characterization and measurement. Although colour grading of tomatoes is based, almost without exception, on visual appraisal, emphasis in recent years has been placed on objective measurement by photoelectric tristimulus colorimetry. The inter-relationships of the tri-coordinate colour values during the ripening of tomato fruits has received only limited investigation. A knowledge of the relationship between these values under a wide range of environmental conditions is imperative in the derivation of meaningful colour indices.

The chemical analysis of fruit tissues during maturation affords the opportunity of studying changes in chemical constituents and their possible relationships; in many cases, analytical data can be utilized as indices of quality. For example, carotenoid analysis of tomatoes not only permits identification and quantification of pigments, but also indicates the particular pigments relevant to colour and permits an approximate evaluation of colour quality.

Quality improvement through selective breeding and improved cultural practices requires a comprehension of the physical and chemical potentialities of the plant material against a background of specific environments.

The primary objectives of the present study were to determine the effects of sub-optimal air temperatures at three specific harvest dates on tomato fruit quality as indicated by colour, pigments, pH, titratable acidity, sugars, refractive index, total pectic substances and dry matter.



## LITERATURE REVIEW

### Tomato Quality

Food quality has been defined as "the composite of those characteristics that differentiate individual units of a product and have significance in determining the degree of acceptability of that unit to the user" (Kramer, 1965).

Attributes of quality have been measured by sensory, chemical and instrumental means. For purposes of standardization and uniformity in quality evaluation, emphasis has been placed on the development and utilization of objective methods for the measurement of various quality attributes (Kramer, 1951, 1966; Nauman, 1965; Mackinney et al., 1966).

Of the characteristics contributing to the quality of tomatoes and tomato products, colour, texture, and flavour have been considered as major criteria of product acceptance. Objective measurements to determine the quality of tomatoes have included: colour, pigments, firmness, pectic substances, pH, total acidity, sugars, soluble solids, total solids and ascorbic acid. (Underwood, 1950; Forshey and Alban, 1954; Kattan et al., 1957; Yamaguchi et al., 1960).

### Tomato Colour

Colour has not only been found to constitute an important component and measure of fruit quality, but also is recognized as contributing significantly to the grade of raw and processed tomato products.

Colour has been found to serve as the most reliable index of tomato maturity. McCollum (1956) pointed out that age, size and appearance

before incipient coloration, were not accurate measures of maturity. He concluded that although carotenoid production varied in relation to other ripening changes, colour served as the best indicator of ripeness. He therefore suggested the breaker stage as a good base line in sampling for ripeness. The breaker stage may be defined as that point at which the green fruit first shows visual evidence of yellow to orange pigmentation. Coloration usually initiates at the styler region.

### Tomato Colour Measurement

Colour is regarded as one of the most difficult quality factors to evaluate, particularly in view of the fact that the development of measuring methods has presented individual problems with each food product (Robinson et al., 1952). Desrosier (1954) concluded that tomato colour quality was most accurately estimated by evaluation of the extracted juice rather than by surface or cross-sectional examination. Yeatman and Sidwell (1958) found that the resultant colour of a blend of several tomatoes was biased in the direction of the redder fruit and hence concluded that individual fruits were best for colour determination.

### Subjective Methods

Tomato colour has been subjectively assessed by direct visual inspection, or more commonly, with the assistance of reference guides including standard colour plates, three-dimensional models, colour handbooks and colour dictionaries. For example, the "practically uniform good red colour" of Canada Fancy grade canned tomatoes is defined as "a colour typical of red or reddish varieties in which not less than 95 per cent of the surface areas of the tomatoes are as red as "Tomato Red" (Plate 3, I-12) and not more than 5 per cent of the surface areas may be as yellow as or possess less red

than Plate 4, F-12 as illustrated in Maerz and Paul's Dictionary of Colour" (Canada Department of Agriculture, 1966).

Spinning disc colorimeters employing Munsell notations have been used extensively for tomato colour measurement (MacGillivray, 1928, 1931a, 1931b, 1937, 1948; Mitchell, 1935; Nickerson, 1946; Kramer and Smith, 1946; Robinson et al., 1952; Gould, 1953). The "good colour" and "reasonably good colour" of Canada Fancy and Canada Choice tomato juice and concentrated tomato juice are specified in terms of Munsell colour discs (Canada Department of Agriculture 1966). Although the use of spinning disc colorimeters constitutes an improvement over the aforementioned procedures, the final judgement remains subjective and hence, for example, Kramer et al. (1959) reported that evaluations of the same samples by different laboratories resulted in a variation of as much as two colour grade points. Carasco (1937) indicated preference for the Lovibond Tintometer in the colour grading of canned and dried tomatoes but this instrument does not appear to be used extensively for tomato colour measurements.

The accuracy of subjective evaluations may be regarded as being dependent upon such factors as normality of observer vision, observer fatigue, colour uniformity of the sample, surface gloss, size and shape of the fruit, product wetness, internal cell structure, particle distribution and sample environment including quality and direction of illumination. (MacGillivray, 1931a; Hunter, 1942; Smith and Huggins, 1952; Mavis and Gould, 1954; Wegener et al., 1957; Mackinney and Little, 1962).

#### Objective Methods

Objective techniques for tomato colour determination have included chemical analysis, reflectance spectrophotometry, and photoelectric

tristimulus colorimetry. The latter represents a most rapid and convenient means of measurement.

The Hunter Color and Color Difference Meter (C.D.M.) (Hunter, 1948, 1958) has perhaps been the most widely applied instrument in tomato colour measurement. The Hunter C.D.M. was designed as a photoelectric tristimulus colorimeter that incorporated source filter photo-cell combinations approximating the Commission Internationale de l'Eclairage (C.I.E.)  $\bar{x}$ ,  $\bar{y}$ ,  $\bar{z}$ , standard observer functions and computer circuitry, thereby permitting psychologically significant colour estimation without elaborate calculation (Hunter, 1942, 1958; Hunter Assoc. Lab. Inc., 1966). In other words, the Hunter instrument measures colour physically and directly translates the stimulus in terms of three visually meaningful scales, data from which can be used for calculating values which correlate well with the hue, saturation and lightness of visual perception. The Hunter L scale measures lightness, the  $a_L$  scale redness when positive, gray when zero and greenness when negative, and the  $b_L$  scale yellowness when positive, gray when zero and blueness when negative. Hunter (1958) presented the following equations for the conversion of Hunter C.D.M. values into C.I.E. values:

$$\begin{aligned} L &= 100Y^{\frac{1}{2}} \\ a_L &= 175Y^{-\frac{1}{2}} (1.02X - Y) \\ b_L &= 70Y^{-\frac{1}{2}} (Y - 0.847Z) \end{aligned}$$

The C.I.E. system was designed to provide for the mathematical expression of colour data in terms of the absolute (X,Y,Z) or fractional (x,y,z) amounts of the red, green and blue primaries necessary for the imaginary standard observer to effect a match with a given sample (Davis and Gould, 1955; Clydesdale, 1969).

Although Hunter values do not directly constitute measures of the physiological colour attributes, Younkin (1950a, 1950b) and Davis and Gould (1955) developed methods for converting Hunter measurements of tomato purees into Munsell renotation terminology.

The Munsell system was produced for the specification of colour in terms of three visually uniform scales namely, hue, value and chroma. However, an examination of the Munsell colours confirmed irregularities in spacing and hence revised notations referred to as Munsell renotations were derived (Newhall, 1940; Newhall et al., 1943; Nimeroff, 1968).

#### Assessment of Measuring Methods and Data Reduction

Evaluations of methods of colour measurement have been based on the degree of association with complementary colour scoring by experienced observers. In discussing the relationship of visual estimates and measured values, Hunter (1942) summarized factors which could interfere with the correlation. Kramer (1952) stated that a correlation coefficient of .90 between a measurement method and observer specification constituted excellent agreement, while a correlation coefficient of  $<.80$  was indicative of an unsatisfactory method.

In terms of tomato colour measurement, the performance of the Hunter C.D.M. was rated superior to subjective procedures, chemical methods, reflectance spectrophotometry and the use of the Photovolt Reflection Meter, the Agtron and the Purdue Colour-Ratio Meter, for reasons of speed, accuracy and precision (Kramer, 1950; Robinson et al., 1952; Desrosier, 1954).

Younkin (1950) found that the C.D.M. could detect differences in puree colours that were not visually apparent.

Data reduction has taken the form of utilization of the one or more measured variables for the computation of single value colour indices through the use of multiple regression equations, charts or nomographs (Kramer, 1951).

Numerous colour indices have been proposed for the specification of raw or processed tomato colour. MacGillivray (1931a) formulated a colour index based on the Munsell System. His colour rating formula was:  $(13.00 - \text{Chroma Number}) + \text{Hue}$ . Kramer (1950) derived a regression equation where colour score =  $12.6 + .553a - 1.478b$ . Robinson et al. (1952) found that Hunter  $a_L/b_L$  ratios were "a convenient and accurate method of expressing the colour of tomato juice within the brightness and chromaticity ranges normally encountered". Halsey and Jamison (1958) observed a high correlation between visual scoring of tomato colour and  $a/b$  ratios. In contrast, Mavis and Gould (1954) reported that the  $Lb/a$  expression gave superior correlations with subjective (U.S.D.A.) colour evaluations of tomato pulp. Yeatman and Sidwell (1958) confirmed Younkin's (1954) opinion that  $a/b$  values did not fully characterize tomato juice colour. Younkin (1954) pointed out that since the concentrations and types of pigments varied with variety, environment and stage of maturity, each colour attribute could affect the appearance thus necessitating incorporation of all three dimensions of colour in the calculation of indices.

In concurrence with Younkin's views, Yeatman et al. (1960) derived a formula for computing raw tomato juice colour which was capable of distinguishing differences between the samples obtained from fruit ripened under a wide range of conditions. The formula

$$\text{Tomato Colour} = \frac{2000 \cos \theta}{L} \quad \text{where } \cos \theta = \frac{a_L}{\sqrt{a_L^2 + b_L^2}} \quad (\text{Hunter notations}),$$

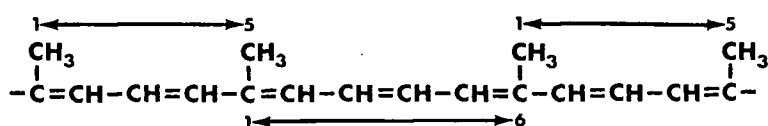
was found to provide the best relationship ( $r = .95$ ) between visual colour scores and instrumental evaluations. Based on this function, Hunter and Yeatman (1961) developed the Direct-Reading Tomato Colorimeter.

In the measurement of processed tomato juice colour, it was found that the  $Lb/a$  ratio was substantially superior to the  $\frac{2000 \cos \theta}{L}$  formula (Yeatman, 1969).

### Carotenoids

The colour of ripening tomatoes has been found to be due largely to the presence of a complex carotenoid pigment system; the colour appearance are conditioned by the particular pigment types and concentrations; these in turn are determined by genetic constitution and environment.

Carotenoids have been described as yellow to red fat-soluble polyenes (usually  $C_{40}$  compounds) composed of isoprene units arranged such that central methyl groups occupy 1:6 positions relative to each other while all other methyl side chains occupy 1:5 positions viz:



The series of conjugated C=C double bonds constitutes the chromophoric system of the carotenoids (Karrer, 1948; Goodwin, 1952, 1955; Commission of the

Nomenclature of Biological Chemistry, 1960).

### Spectral Properties

The wavelength and intensities of the carotenoid absorption maxima (generally three) have been found to be a function of the number of conjugated double bonds. The colour properties of carotenoids are dependent upon a chromophoric system of greater than four conjugated double bonds. Thus, for example, the 'colourless carotenoid' phytoene with three conjugated double bonds, shows no absorption maxima in the visible region of the spectrum, whereas lycopene (in hexane) with eleven conjugated bonds absorbs predominantly at 443, 472, and 504 m  $\mu$  (Weedon, 1965). In addition, solvent effects and structural variations in carotenoid molecules have been shown to profoundly influence the spectral properties. In this connection, Karrer and Jucker (1950) have outlined a series of empirical relationships existing between carotenoid structure and spectra.

Zechmeister (1962) has reviewed the effect of trans-cis isomerization on the absorption spectra of carotenoids. Zechmeister and Polgar (1943) observed that in the conversion of the all-trans into a mixture of cis-trans isomers, colour intensity decreased. The spectral curves in the visible region were altered as follows:

- 1) the extinction values and the degree of fine structure decreased;
- 2) the absorption maxima shifted towards shorter wavelengths.

### Carotenoids of Tomato Fruits

Jensen (1967) has stated that approximately 180 carotenoids are known to occur naturally. Curl (1961) reported isolating 22 xanthophyll components from tomato fruits of which the major proportion consisted of lutein, violaxanthin and neoxanthin. The carotenoids of tomatoes have been found



to consist predominantly of carotenes (Kuhn and Grundmann, 1932). Trombly and Porter (1953) listed 19 carotenes obtained from tomato extracts. The two polyenes (colourless carotenoids) phytoene and phytofluene are common to tomatoes (Porter and Zscheile, 1946a; Rabourn and Quackenbush, 1953; Tomes, 1963).

McCollum (1955) showed that the colour of red tomatoes was dependent upon the total carotenoids as well as the ratio of the dominant pigments, lycopene and  $\beta$ -carotene. McCollum noted that although  $\beta$ -carotene represented only two to ten percent of the total carotenoids, it exerted a pronounced effect on colour.

Pigment distribution studies indicated that the concentration of total carotenoids was highest in the outer pericarp while  $\beta$ -carotene quantities were greatest in the locular region (McCollum 1955).

Polar differences have been studied by Ellis and Hamner (1943) and McCollum (1955) who found that although coloration was usually initiated in the apical end, carotene content was greatest in the proximal region of the ripe fruit. To compensate for polar and morphological differences McCollum (1955, 1956) recommended that in sampling for colour and/or pigment studies equal and opposite fruit sectors should be used when whole fruits were not available.

#### Specification of Tomato Colour Based on Pigment Studies

A number of investigators have attempted to characterize tomato colour on the basis of carotenoid content. Hunter and Yeatman (1961) indicated that because several different pigments combined to produce the colour of tomatoes, the colour potential could not be satisfactorily measured by a simple procedure such as the absorption of a single pigment at a single wavelength. Davis (1949) used the light absorption data from acetone

extracts as an index of colour. Kramer and Smith (1946) observed a good correlation between transmittance readings at 485 millimicrons of tomato benzene extracts and organoleptic colour ratings. Younkin (1954) pointed out that the methods of Kramer and Smith (1946) and Davis (1949) assumed that the relative amounts of the principal pigments were constant when in fact Went et al., (1942) and Porter and Zscheile (1946b) had established that such was not the case. McCollum (1953) described a method in which the total carotenoid to  $\beta$ -carotene ratio was calculated as a chemical index of colour; however, he had noted earlier (McCollum, 1944) that several factors (unspecified) other than total pigments influenced the colour values of tomato juice to an unknown extent.

MacGillivray (1948) and Robinson et al. (1952) stated that since tomato colour was also dependent upon factors other than carotenoid quantity, specification of colour based on pigment concentrations was not recommended. Further, MacGillivray (1948) concluded that colour data could be more accurately interpreted if expressed in terms of colour attributes.

### Firmness

Kattan (1957) pointed out that firmness ranked second only to colour as a quality parameter in fresh market and processing tomatoes. Firmness has been regarded as an important factor in the mechanical harvesting, handling, shipping and marketing of tomatoes (Sayed et al., 1966). Firmness of the fresh fruit determines to a large extent the textural properties of processed tomatoes. For example, Luh et al. (1960) showed that juices made from soft-ripe tomatoes were thinner in consistency than those made from firm-ripe fruit. Moghrabi (1958) has stated that catsup yields may vary by as much as 25 percent depending on firmness of fruit.

### Firmness Measurements

Several instruments have been devised for firmness measurements of tomato fruits. Fischer and Sengbush (1935) measured firmness by placing the fruit under a cork attached to a fulcrum. A weight was shifted across the beam until the fruit was seen to split. The readings were found to be influenced by fruit diameter and furthermore, the exact time of splitting was difficult to establish. West and Snyder (1938) measured firmness of tomato fruits with a Chatillon penetrometer. The method used by Paech (1938) in which a plunger was forced against the fruit wall until the fruit was punctured, required a gradual increase in pressure until penetration occurred.

Hamson (1952a) criticized earlier methods as being too slow and inaccurate. The Cornell pressure tester developed by Hamson (1952a,b) was designed to measure firmness in a manner similar to hand compression. Since firmness was determined by compression at a single point on the fruit, the measurement position presented a problem particularly in large loculed fruit. Kattan (1957a,b) overcame this difficulty by devising an instrument (Firm-o-meter) based on a multi-point compression principle. A force was applied to a chain encircling the fruit and the resultant strain was measured on a scale graduated from zero to ten. The scale readings were inversely related to firmness. The scale values were not influenced by differences in fruit diameter as also found by Hamson (1952a) relative to the Cornell pressure tester. Further, their data indicated significant agreement with subjective firmness evaluations.

Garrett et al. (1960) evaluated a number of instruments and found that the Asco Firmness Meter showed exceptionally good agreement with panel

measurements.

Ang et al. (1963) compared panel firmness ratings of canned whole tomatoes with Kramer Shear press measurements. The correlation coefficients between the shear press with the Universal cell attachment and subjective evaluations were .88 and .92 for the two experiments carried out.

### Pectic Substances

The literature on pectic substances is characterized by conflicting terminology, broad definitions, and in many cases, questionable methods. Kertesz (1951) and Joslyn (1962) have therefore emphasized the difficulties in the interpretation and evaluation of many of the earlier research reports.

Pectic substances may be described as colloidal polyuronide macromolecular complexes consisting mainly of D-galacturonic acid residues linked together by  $\alpha$ -1,4 glycosidic bonds to form a linear polymer. The carboxyl groups of the chain are O-methylated to varying degrees. (Bonner, 1950; Kertesz, 1951, 1963).

It has been suggested that certain sugars and their derivatives are covalently attached to the polygalacturonide backbone and could well form an intimate part of the main chain (Albersheim, 1965). Evidence quoted in support of this proposal includes studies by Aspinall and Canas-Rodriguez (1958) who confirmed the presence of L-rhamnose, L-arabinose and D-galactose in pectic acid isolates of the sisal plant (Agave sisalana). Further, Aspinall and Fanshawe (1961) detected L-rhamnose, L-arabinose, D-galactose and traces of L-fucose, 2-O-methyl-L-fucose, 2-O-methyl-D-xylose and a number of oligosaccharides on partial hydrolysis of pectic acid

extracted from alfalfa (Medicago sativa).

Pectic substances of higher plants have been found to occur in the intercommunicating cellulose interstices of primary cell walls and have been noted as constituting the major component of the middle lamella (Bailey, 1938). Pectic constituents of the middle lamella are believed to consist primarily of water-insoluble calcium and magnesium pectates which act to cement adjacent cells together thus imparting firmness to tissues (Bonner, 1950).

Pectic substances are generally classed as protopectins, pectinic acids and pectic acids. Pectic substances are also frequently designated in terms of the solvent systems used for their extractions.

#### Relationship of Pectic Substances to Kinesthetic Properties of Plant Materials

Although present in limited amounts, the quantities and nature of pectic constituents have been found to contribute considerably to the firmness of fresh tomatoes, the structural integrity or wholeness of the canned product and the consistency of tomato purees, pastes, catsups, sauces and juices (Kertesz and McColloch, 1950; Kertesz, 1951; Luh et al., 1954).

Hamson (1952a) correlated instrumental firmness measurements with the various pectic fractions of firm and soft tomato lines. He noted that water-soluble, ammonium oxalate-soluble, and acid-soluble pectins were significantly correlated with firmness at the five percent level, while total pectins were significant at the one percent level. In addition, it was found that firm varieties were consistently higher in all pectic fractions and it was therefore concluded that marked quantitative differences in the uronide

constituents were associated with differences in firmness. Haber and LeCrone (1933) observed that the softening of tissues was accompanied by a conversion of insoluble into soluble forms of pectin. Moghrabi (1958) found that the pectic change most closely associated with softening of fresh tomato fruits was the decrease in protopectin. Foda (1957) and Sayed et al. (1966) also concluded that the protopectin constituents appeared to be the fraction contributing most to the firmness of fresh tomatoes.

In reference to processed tomatoes, Appleman and Conrad (1927) observed a relationship between the pectin to protopectin ratio of fresh tomatoes and the amount of disintegration of the whole canned product. Deshpande et al., (1965) found that firmness of canned tomatoes was significantly correlated with total pectic constituents, with the ratio of carbonyl to pectic content, and with mineral content. The results indicated that a high content of pectic substances, large molecular size, and low methoxyl content of pectic constituents would result in improved firmness. Kertesz (1940) observed that the firmness of canned whole tomatoes could be improved by the addition of calcium salts, presumably because the calcium reacted with the pectic acid present in the fruit to form a gel which supported the cell structure.

Rooker (1930) was one of the first workers to emphasize the significance of pectic substances in tomato products other than the whole canned fruit. McColloch et al. (1950) indicated that lower pectin contents due to enzymatic activity during processing generally resulted in tomato pastes of lower consistency.

Tomato juice has been described as a diphasic system composed of serum and suspended particles. Pectic substances in the fluid phase were

found to play an important role in determining the serum viscosity and hence, consistency. Pectic compounds in the suspended particles were also regarded as contributing to juice consistency, but to a lesser extent. (Kertesz and Loconti, 1944; Kertesz, 1951).

#### Changes in Pectic Substances with Ripening

Appleman and Conrad (1927) reported changes in pectic substances with maturation and noted that protopectin predominated in green fruit but as ripening progressed, partial hydrolysis resulted in the decrease of protopectin and the corresponding increase in soluble pectin. The loss of pectate from the middle lamella is considered to cause softening of the fruit as maturation progresses. Kertesz and McColloch (1950) studied pectic transformations during ripening but found no indication of any definite quantitative or compositional trend. Moghrabi (1958) pointed out that these negative findings could possibly be explained on the basis that the fruit used in the study had advanced beyond the maturity stage where significant changes in pectic constituents could be expected and further, that low field temperatures may have complicated the results. Dalal et al. (1965, 1966) found that total pectin concentrations were highest in green fruit between one and three inches in diameter and gradually decreased with increasing coloration. Soluble pectin increased throughout all maturity classes studied. Although Woodmansee et al. (1959) found no consistent changes in the soluble pectin fraction of unripe, ripe and over-ripe fruits, a number of researchers (Haber and LeCrone 1933; Luh et al. 1960) have demonstrated the gradual increase in this fraction, and its subsequent slight decline with fruit deterioration.

McClendon et al. (1959) found that free galacturonic acid increased by a factor of 10 during ripening but the amount present in ripe tomatoes was small relative to the total uronide fraction. Spenser (1965) indicated that the small amount present could not account for the decrease in soluble pectic substances during the ripening period. However, she reasoned that once free galacturonic acid was formed, it was further metabolised and hence, did not accumulate.

The exact manner in which pectic substances are degraded is incompletely understood. Spenser (1965) pointed out that changes in pectic substances during ripening involve depolymerization, demethylation and deacetylation. Stier et al. (1956) stated that some evidence suggested protopectin depolymerization into soluble pectin followed by de-esterification and hydrolysis into smaller units.

The degradation of pectic substances during ripening has been attributed to two classes of pectyolytic enzymes, namely, polygalacturonases (PGs) and Pectinesterases (PEs). Polygalacturonase enzymes act to cleave  $\alpha$ -1, 4'-glycosidic linkages and can be subdivided into three types. Type I PGs are believed to split glycosidic bonds at random and to preferentially attack pectic substances of high molecular weight and low methoxy content. Type II PGs are considered to show preference for highly esterified polygalacturonides. Type III PGs are believed to hydrolyse pectic substances systematically beginning at one end of the molecule. Pectinesterases catalyse the removal of methyl ester groups from pectic substances. (Deuel and Stutz, 1958).

Kertesz (1938) emphasised the importance of pectic enzymes in affecting the quality of tomato products. He noted the rapid increase in



PE with ripening, and attributed the decrease in viscosity of cold pressed tomato juice to demethoxylation caused by extensive PE activity.

#### Other Constituents of Tomato Quality

Quality is dependent upon physical and chemical components and a wide number of compositional studies have been carried out on tomato fruits (Bohart, 1940; Winsor and Massey, 1958; Winsor et al., 1962a,b; Hanna, 1961; Woodmansee et al., 1959; Simandle et al., 1966; Yamaguchi et al., 1960; Lee and Sayre, 1946; Thompson, 1965; Thompson et al., 1962; Dalal et al., 1965, 1966).

McCollum (1956) pointed out that the composition of tomato fruits varies with such factors as maturity, light exposure, temperature, morphological structure and position on the plant. He further stated that "Where the constituents of quality are dependent upon so many factors, differences between progenies or treatments are difficult to measure without the utmost care in sampling".

The majority of studies appear to indicate that pH, sugars, total solids and soluble solids tend to increase while total acidity decreases with ripening. The literature on the pattern of changes during ripening presents a somewhat variable picture. Janes (1941) reported that titratable acidity reached a maximum at the orange stage of maturity and subsequently decreased. Rosa (1925, 1926), Ulrich and Renac (1950) and Winsor et al. (1962a,b) found that titratable acidity was highest at the mature green or breaker stages. Janes (1941) reported that sugar contents were maximal at the orange stage of ripening and thereafter decreased slightly. Winsor et al. (1962a,b) and Rosa (1925, 1926) concluded that sugars generally increased from

the mature green to fully red ripe stages. It is probably that the variability in findings can be partially explained in terms of the factors cited by McCollum (1956).

#### Flavour:

Flavour is an agglomeration of sensations, the perception of which may involve the sense of taste and smell and the chemical sense (Crocker, 1945; Gorman, 1964).

The physiological complexity of flavour has precluded the development of adequate objective procedures for the thorough characterization of the taste and odour of food materials. Panels provide the most satisfactory method of flavour assessment. However, Kramer (1966) pointed out that certain flavour dimensions could be evaluated, at least in part, by chemical and instrumental means. For example, sourness can be indicated by pH or total acid values, while sweetness can be approximated by sugar content or refractive indices (Kramer, 1951, 1965).

Sugars and organic acids (predominantly glucose, fructose, citric and malic acid) are regarded as the constituents primarily responsible for the taste of tomatoes, while largely unknown volatile compounds account for tomato odour (Simandle et al., 1966; Dalal et al., 1966; Lower and Thompson, 1966, 1967; Winsor, 1966).

Hamdy and Gould (1962) suggested the possibility of using  $\alpha$ -keto glutaric to citric acid and  $\alpha$ -amino-nitrogen to citric acid ratios for the objective determination of processed tomato flavour.

Scott and Walls (1947) found that the sugar/total acidity ratios of ripe fruit and processed juice were in close agreement with

organoleptic flavour ratings. Juices with high sugar/acid ratios were bland, lacked sharpness in taste, and tended to be "flat". Juices with low ratios were sharp and acid.

Forshey and Alban (1954) considered that tomato fruits with pH ranges of 4.05 to 4.15, titratable acidities of .45 to .55 per cent, and reducing sugars of 3.20 to 3.50 per cent were of high quality. Fruits with higher pH's or lower titratable acidities, and lower reducing sugars were rather "flat" and tasteless. Similarly, Winsor (1966) found that fruits low in titratable acidity and/or low in sugar content were of inferior taste. It was stated that the best quality fruits were those high in both sugars and organic acids. Winsor noted that the taste of fruit at the orange-red stage of maturity was superior to other maturity classes because of the relatively high sugar content and adequate titratable acidity level.

Dalal et al. (1966) concluded that the inferior flavour of greenhouse-grown tomatoes as compared to field-ripened fruit could be attributed to the lower concentrations of organic acids, sugars and volatile reducing substances found in the former.

### Temperature

#### Effect of Temperature on Colour and Pigment Concentrations during Ripening

Duggar (1913) discovered the effect of temperature on lycopene synthesis when he observed that constant temperatures of 30 to 37°C resulted in yellow- to orange-coloured fruit. In addition, Duggar indicated that temperatures below 10°C sharply inhibited lycopene development. These findings were substantiated by Rosa (1926) who studied the relationship

of temperature to the ripening of green tomatoes as indicated by pigment development. Rosa found that at temperatures of 4 and 8°C, no red, and little yellow coloration occurred, thereby establishing a lower limit of 8°C for lycopene production. However, it was noted that fruit at the turning stage of maturity did become red when stored for 18 days at 8°C. Pigment development was slow and incomplete at 11°C, most rapid at 25°C and inhibited at temperatures above 30°C. Hence, it was evident that ripening temperature not only affected the final colouring, but also the rate of ripening. Wright et al. (1931) found that at 15.6°C mature green tomatoes required from 19 to 30 days to ripen. An abrupt fall-off in ripening rate was noted below 15.6°C in all varieties studied. At 10°C mature green fruit required about 40 days to ripen and were pale in colour. Fruit held at 4.4°C failed to colour and showed considerable low temperature injury after 10 days. The lowest ripening temperature providing good colour and flavour development was 12.8°C. In comparable studies Ayres and Peirce (1960) found that the rate of ripening proceeded in the order: ambient room temperature, 25, 20, 15, 10°C.

Vogele (1937) noted large differences in per cent brightness, per cent purity and dominant wavelength as calculated from spectral reflectance curves of fruit ripened at 32 and 36°C. He found that the optimum temperature for lycopene synthesis in tomatoes was 24°C, and that fruit exposed to 40°C remained green in spite of a subsequent return to lower temperatures. Vogele further noted that lycopene formation did not necessarily follow chlorophyll decomposition as previously supposed.

MacGillivray (1934) studied coloration of field tomatoes as affected by diurnal temperature fluctuations and concluded that although

day temperatures considerably exceeded  $32^{\circ}\text{C}$ , lycopene formation was permitted because of lower night minimal temperatures. Yamaguchi et al. (1960) and Sayre et al. (1953) observed a similar result when tomatoes were exposed to variable daily temperatures.

Sayre et al. (1953) compared tomatoes ripened under various diurnal temperature regimes and found essentially no difference in Hunter a/b external colour ratios or pigment concentrations between vine- and self-ripened fruit. However, Hood (1959) found that in the variety Tiny Tim, lycopene development occurred up to  $32^{\circ}\text{C}$  in fruit on the vine whereas the red pigmentation was retarded in detached fruit at temperatures above  $21^{\circ}\text{C}$ .

Hanson (1921) noted that ripening fruit progressed through a series of changes from green to whitish green (indicating chlorophyll destruction); to light yellow as carotene synthesis was initiated; and then through orange to red as the lycopene concentration increased.

Although earlier researchers had observed colour changes in tomatoes and attempted to relate these to carotenoid synthesis (Duggar, 1913; Rosa, 1926; MacGillivray, 1934), little attention could be given to comprehensive investigations on quantitative and qualitative polyene changes as influenced by maturity, temperature and other factors until the development of reliable carotenoid extraction and chromatographic resolution techniques (Strain, 1934; Zscheile et al., 1942; Zscheile and Porter 1947).

Went et al. (1942) studied the carotenoid contents of individual tomatoes ripened on plant under different temperatures and lighting conditions and found it impossible to compare individual values for the differently treated fruits due to high fruit to fruit variability within

treatments as indicated by a concomitant  $26.5^{\circ}\text{C}$  day -  $20^{\circ}\text{C}$  night investigation. They concluded that the physiological condition of the plants rather than the various  $20^{\circ}\text{C}$  -  $26.5^{\circ}\text{C}$  temperature combinations controlled carotenogenesis in attached fruit. It was further noted that a concentration change in one carotenoid was generally paralleled by similar behaviour in the other fractions. In detached fruit, Went and his co-workers not only confirmed the temperature effects of lycopene synthesis, but also carried out a quantitative study of 10 other carotenoid fractions as affected by ripening temperatures. The majority of the polyene fractions were substantially lower in quantity when fruits were stored at  $33^{\circ}\text{C}$  as compared to  $26.5^{\circ}\text{C}$ .

Meredith and Purcell (1966) determined quantitative changes in carotenoids in ripening Homestead tomatoes at six different maturity stages. The fruit were permitted to ripen at  $22^{\circ}\text{C}$  under reduced light until the required maturity stage (green, breakers, turning, pink, light red and red) was attained whereupon one-to two-kilogram samples were analyzed. Mature green tomatoes were found to contain only  $\alpha$  and  $\beta$ -carotene whereas fruit at the breaker stage also contained phytofluene,  $\zeta$ -carotene,  $\gamma$ -carotene and lycopene. Phytoene appeared at the turning stage of maturity. All pigments progressively increased in concentration with the exception of  $\alpha$ -carotene which gradually decreased with ripening beyond the breaker point and  $\beta$ -carotene which increased up to the light red stage and thereafter decreased somewhat.

Goodwin and Jamikorn (1952) studied the effect of  $0^{\circ}$ ,  $15^{\circ}$ ,  $30^{\circ}$  and  $37^{\circ}\text{C}$  temperatures on carotenoid biosynthesis in excised green tomatoes

showing evidence of colour change, at ripening intervals of three, six, nine, and twelve days. Temperatures inhibiting lycopene synthesis ( $0^{\circ}\text{C}$ , and  $37^{\circ}\text{C}$ ) were also found to cause a corresponding marked suppression in phytofluene,  $\zeta$ -carotene and neurosporene production. Lycopene, phytofluene,  $\zeta$ -carotene, and neurosporene tended to appear simultaneously during ripening; however, subsequent accumulation rates differed substantially.  $\alpha$ - and  $\beta$ -carotene syntheses were reduced at  $0^{\circ}\text{C}$  but only slightly inhibited above  $30^{\circ}\text{C}$ . It was concluded that  $\alpha$ - and  $\beta$ -carotene syntheses in tomato fruits were largely independent of the Porter-Lincoln pathway (Porter and Lincoln, 1950) and possibly coincided with a mechanism found in leaves.

Porter and Lincoln (1950) postulated that the biosynthesis of carotenoids proceeds through successive dehydrogenation reactions and ring closures, thus:

tetrahydrophytoene  $\rightarrow$  phytoene  $\rightarrow$  phytofluene  $\rightarrow$   $\zeta$ -carotene  $\rightarrow$  neurosporene  
 lycopene  $\rightarrow$   $\gamma$ -carotene  $\rightarrow$   $\beta$ -carotene  $\rightarrow$   $\alpha$ -carotene.

Interpretations of temperature effects on the pattern of carotenoid development have been complicated by genetic, physiological and additional aspects. Thompson et al. (1962) pointed out the difficulty in the distinction between environmental and heritable effects unless adequate sampling and testing techniques are applied.

Genetic involvement in temperature effects on carotenoids was emphasized by Tomes et al. (1956, 1958). In temperature inhibition studies with normal red strains, Tomes (1963) reaffirmed the finding that lycopene reduction was not accompanied by a significant decrease in  $\beta$ -carotene.

This suggested that the former pigment did not represent a precursor of  $\beta$ -carotene. However, in the strains High Beta and Intermediate Beta, all carotenoid fractions examined including  $\beta$ -carotene were highly inhibited at temperatures exceeding  $30^{\circ}\text{C}$ .

#### Effect of Temperature on Firmness and Pectic Constituents

The rate of decomposition of pectic substances and fruit softening have been shown to be directly related to temperature. Carre (1922), in one of the first extensive works on pectic changes during storage, found that low temperatures decreased the rate of pectic breakdown in apples.

Haber and LeCrone (1933) measured the changes in pectic substances of mature green (at point of incipient coloration) and fully ripe tomatoes stored at  $2.2$ ,  $10$ , and  $21.1^{\circ}\text{C}$  for extended periods. He found that the rate and extent of change in pectic constituents was proportional to temperature. At  $21.1^{\circ}\text{C}$  there was an increase in soluble pectin at the expense of protopectin up to three weeks storage but thereafter protopectin, soluble, and total pectins decreased due to deterioration of the fruit. At lower temperatures, the rate and extent of changes were considerably reduced. At  $2.2^{\circ}\text{C}$  protopectin contents tended to remain constant while the soluble pectin fraction showed a slight increase.

Moghrabi (1958) studied rates of change in pectic constituents and firmness of tomatoes as affected by temperature and maturity. Samples were harvested at the time of incipient colouring and placed under constant temperatures of  $15$ ,  $20$ ,  $30$ , and  $35^{\circ}\text{C}$  for periods of three, six, nine and twelve days. The most effective temperature to retard softening, particularly in the earlier phases of ripening, was  $15^{\circ}\text{C}$ . The softness of fruit stored for nine days at  $15^{\circ}\text{C}$  was found to be equivalent to that of fruit stored



for three days at 20°C. Loss of firmness proceeded in the following order: 30, 35, 20, 15°C. Protopectin contents were found to drop sharply at all temperatures during the first three days with the highest rate of loss occurring at 15 and 20°C and the lowest at 35°C. The rate of decrease of the oxalate-extracted fraction at different temperatures varied during ripening. At 12 days, these losses varied directly with increasing temperature. Total pectins, as obtained by summation, decreased during ripening. The effect of temperature on total pectic contents was not significant for the variety W.R-3 but fruits of the Stokesdale variety exposed to 35°C were consistently higher in total pectin than those exposed at 15, 20 and 30°C.

Foda (1957) found that tomatoes ripened at 15°C softened more readily than at 30°C. Protopectin also decreased more rapidly and water soluble pectin accumulated. The ammonium oxalate fraction (low methoxyl pectin) was higher at 30°C due to a more favourable temperature for pectin-estrerase activity. Partial inactivation of protopectinase at 30°C was indicated.

#### Temperature Effects on Other Components of Quality

Rosa (1926) determined compositional changes at four-day intervals in green tomatoes stored under various constant temperatures. He noted that total solids and total acids decreased rapidly in fruit held at 25°C, while sugars increased slightly during the first four days and then gradually decreased. At 19°C, compositional changes were less rapid and less extensive. At 4 and 12°C, total solids and total acids showed little change with time, but sugar concentrations slowly increased throughout the treatment period.

Craft and Heinze (1954) concluded that temperature and duration of storage had little or no effect on total acidity, soluble solids, and pH.

Lingle et al. (1965) placed tomato plants in growth chambers maintained at day-night temperatures of 30-30, 30-25, 30-20, 30-15, 30-10, 30-5, and 23-17°C. Fruits at the canning-ripe stage of maturity were analyzed for various factors. Results indicated that pH and titratable acidity were not influenced by night temperature. Total and soluble solids were lowest in fruit held at 5°C and highest at 15 and 20°C night temperatures.

Wedding and Vines (1959) studied the effects of temperature on the development of hormone set fruit and found that soluble solids and titratable acidity were significantly lower at 30-15°C day/night temperatures compared to 25-15 or 20-10°C.

Haber (1931) carried out a detailed study that included the influence of temperature on tomato acid contents. He found that low temperatures (2.4, 4.4 and 10°C) greatly retarded the rate of acid changes in stored fruit. The total acidities of green mature tomatoes following four to five weeks storage were essentially the same for the three low storage temperatures. The total acidities of green mature fruit stored at 21.1°C decreased rapidly and fell to much lower levels than those of corresponding fruit held at lower temperatures. The rate and extent of pH change varied directly with temperature and duration of storage. The pH increase was most marked and rapid at 21.1°C.

Hall, (1968) exposed tomato fruits at the incipient colour stage to temperatures of 3.3, 7.2 or 10°C for zero, four or eight days with or without a subsequent six day storage period at 21.1°C. The titratable acidity of pericarp portions of fruit held at 3.3°C was significantly higher than that of fruits stored at 7.2 or 10°C. The highest pericarp acidities occurred after four days at low temperatures. Acidities of

pericarp and locular portions of fruit chilled at four or eight days followed by six days at  $21.1^{\circ}\text{C}$  were higher than those of control fruits ripened without prior chilling. It was considered that low temperatures delayed ripening and so delayed acid decomposition.

#### Low Temperature Injury

Chilling injury has been described as a physiological disorder common to many fruits and vegetables when exposed to non-freezing temperatures below about  $10^{\circ}\text{C}$  for an adequate period either before or after harvest. A loss or reduction in ripening capacity and other characteristics has been found to be symptomatic of low temperature injury (Lewis 1961).

Wright et al. (1931) carried out experiments to study chilling effects on tomatoes at various temperatures. Previously there was indication that when tomatoes were exposed to temperatures of  $4.4^{\circ}\text{C}$  or less for a very short time, the fruit was rendered incapable of ripening when reinstated to a more suitable environment. Diehl (1924) showed that tomatoes could tolerate exposure to near freezing temperatures for as long as five days without injury, and furthermore, that less damage resulted when chilling occurred at the turning point as opposed to the mature green stage of maturity. Wright et al. (1931) found that exposure of fruit to  $0^{\circ}\text{C}$  for one to four days did not prevent subsequent ripening at  $21.1^{\circ}\text{C}$ , but the rate of ripening was retarded to a considerable extent. Fruit stored for eight days at  $0^{\circ}\text{C}$  and then replaced into a  $21.1^{\circ}\text{C}$  environment for a period of 19 days showed very incomplete coloration and extensive decay.

Lewis (1961) indicated that the extent of chilling injury was proportional to the length of exposure to low temperatures and increased with decreasing chilling temperatures.

## MATERIALS AND METHODS

### Plant Growth

Tomato plants (cultivar Early Red Chief) grown in a greenhouse, were transplanted from flats into 9.5 litre plastic pails four to five weeks after seeding. The plants were staked and then trimmed periodically to remove excess lateral shoots and terminal growth.

Between October and March supplemental lighting was provided (cool white fluorescent; intensity approximately 2.7 klux [250 ft. c.] at plant level; 14 hr. photoperiod). The additional lighting was found to be necessary in preliminary growth trials to prevent flower abortion.

The plants were watered daily with Hoagland's solution (macro-nutrients only) (Hewitt, 1966), from age six weeks to the termination of each experiment. This was found to circumvent blossom end rot problems which occurred when plants received only water each day, with or without nutrient solution once weekly.

Approximately two weeks before the fruit were expected to begin ripening (evaluated in preliminary trials), 16 plants selected on the basis of overall condition, adequate numbers of fruit, and adequate size of fruit, were divided into four uniform groups (Fig. 1). Each group was then transferred from the greenhouse to a Percival Model PGC-78 growth chamber in which plant allocation was random.

Growth chamber photoperiods of 15 hours per 24 hour cycle were maintained. Light from a combination of 16 cool white fluorescent lamps and 10, 40 watt tungsten filament lamps provided an intensity of about 12.9 klux at a distance of 20 cm below the light barrier.



Fig. 1. Greenhouse Grown Plant Immediately Prior to Placement in a Growth Cabinet

The growth cabinets were operated with a daily 17.8-25.6°C minimum-maximum temperature programme until a number of fruit attained the breaker point. At this time, individual fruit were tagged and temperature treatments initiated.

#### Establishment of Temperature Programmes

Thermographic charts covering the period September 8 through October 5 for 1964, 1965 and 1966 from Summerland, B.C. were evaluated in order to establish an average daily temperature curve (Figure 2) on which the temperature regimes for the study were based. Only those days for which the temperatures approximated a typical diurnal fluctuation (85 per cent of all days) were considered in the determination of this average daily temperature curve.

The initiation and termination of the maximum temperature period was obtained by derivation of the mean times at which a temperature 2°F (1.1°C) below the daily maximum occurred (12.33 p.m. - 4.47 p.m.). Similarly, the minimum period was located by calculating the mean times for which the temperature was 1.1°C above the daily minimum (2.12 a.m. - 6.27 a.m.). The average daily maximum and minimum temperatures were obtained for the specific period.

Four temperature programmes (Figure 3) with selected minimum-maximum temperatures of:

1. 17.8 - 25.6°C
2. 7.2 - 18.3
3. 4.4 - 15.6
4. 2.8 - 13.9

were patterned after the calculated curve. Growth chambers equipped with

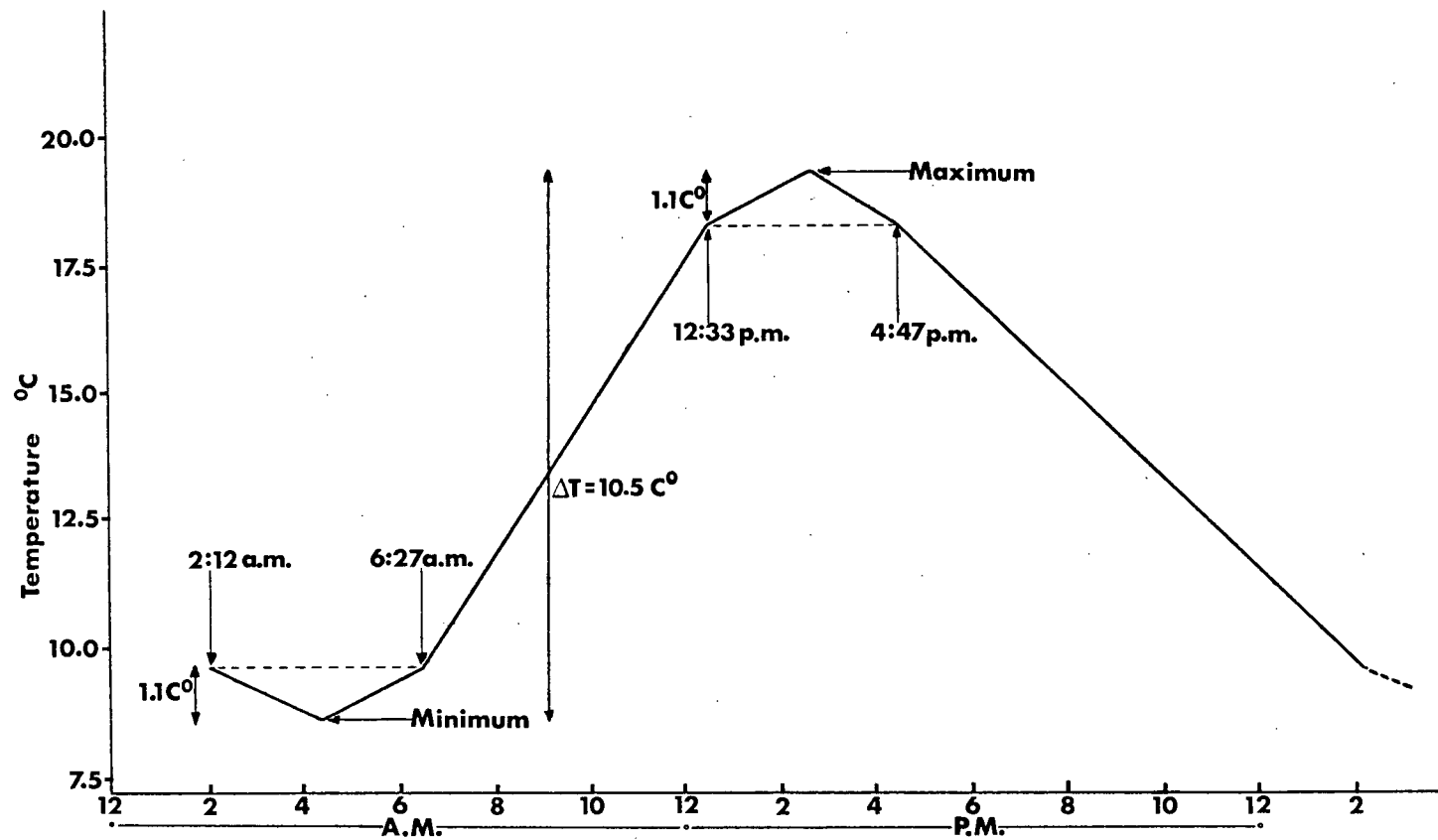


Fig. 2. Average Daily Temperature Curve

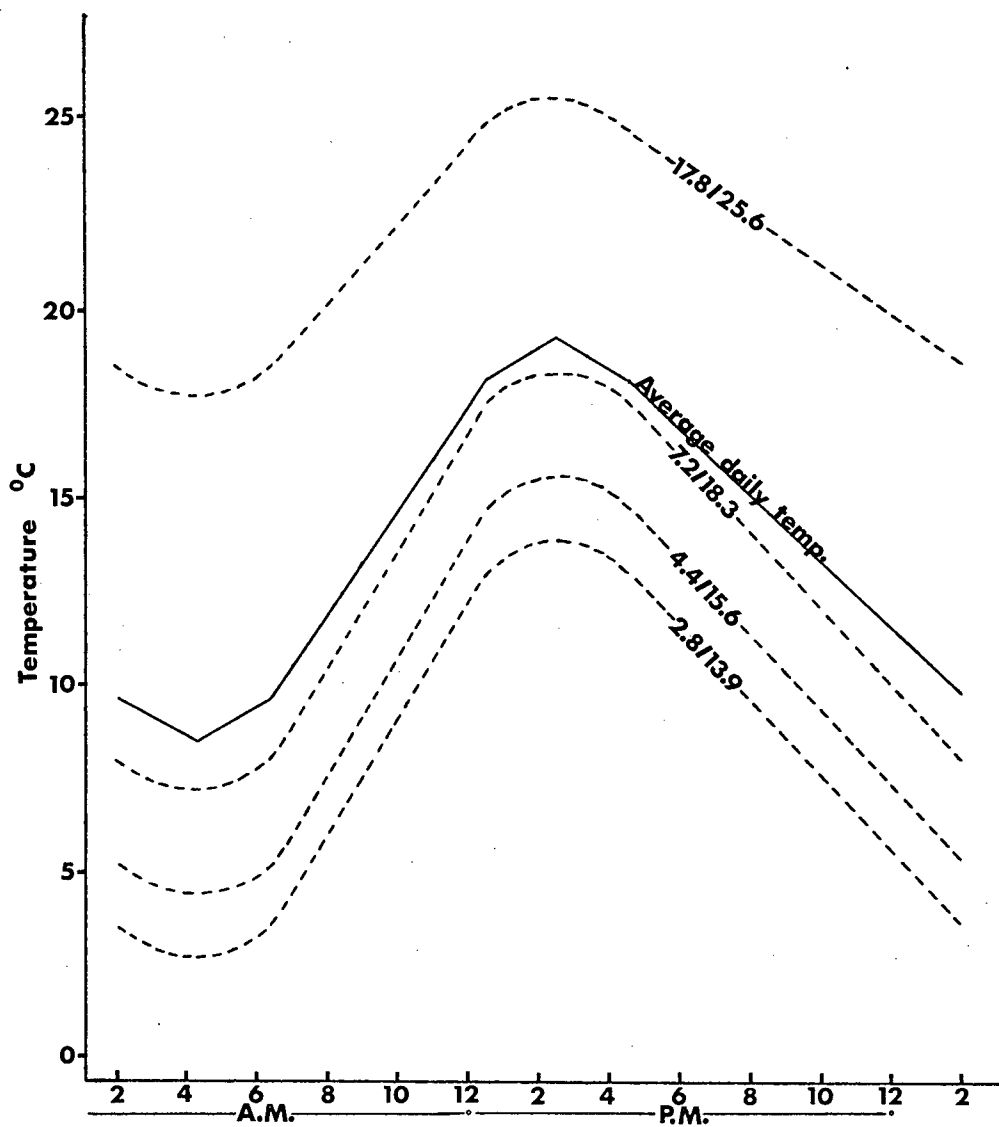


Fig. 3. Temperature Programmes Used in Growth Chamber Experiments



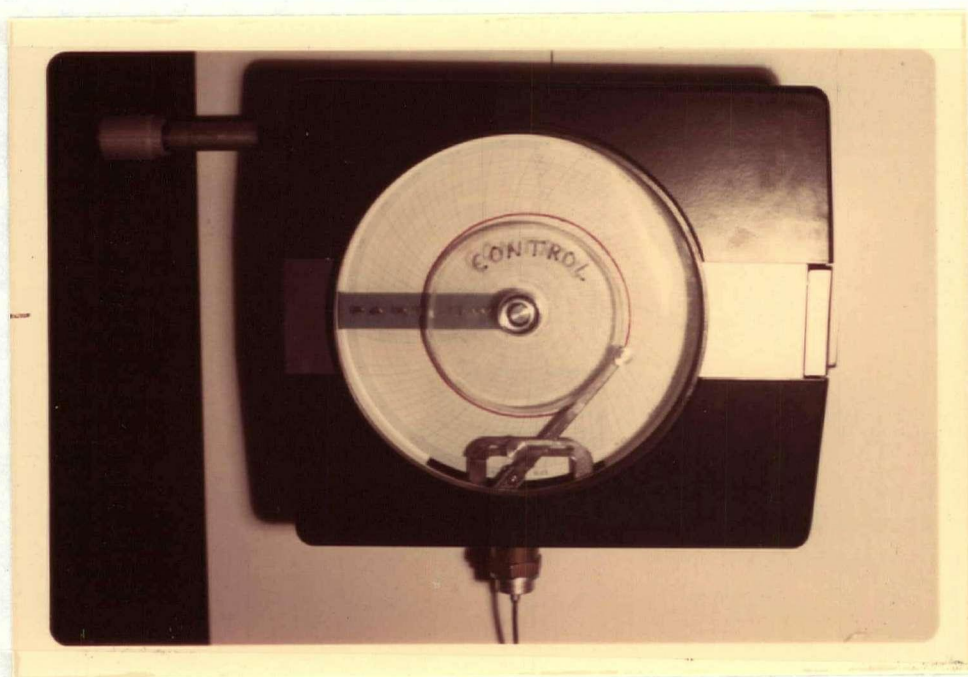


Fig. 4. Growth Chamber Temperature Programmer in Operation  
for the 17.8/25.6 Regime

temperature programmers were then fitted with plexiglass cams (Figure 4) describing the derived daily temperature curves.

### Experimental Design

Growth chamber experiments were arranged as split-plot designs with a minimum of three replications. Four chambers representing the four different temperature regimes constituted a run or replicate. Each chamber contained four plants from which two fruits per plant were harvested at seven, fourteen, and twenty-one days following the onset of incipient coloration (breaker point).

### Analytical Sequence

Individual fruits were weighed and analyzed as shown in Figure 5, except that pigment studies were based on pooled samples. Fruits that weighed less than 90 grams were not analyzed and another fruit was selected to ensure that all plants were represented by two fruits per harvest date.

#### 1. External Colour:

The Hunterlab Colour and Colour Difference meter was standardized using a black enamel-coated plexiglass plate containing a central 1 1/16" bevelled aperture above which was placed a tomato red reference tile # D33C-221. The instrument was calibrated to read:  $L = 10.0$ ,  $a_L = 7.7$  and  $b_L = 2.8$ . The red tile was then replaced with a fruit and three readings taken at about 120° spacings on the lower half of the tomato ensuring that the blossom end scar was not included. Average  $L$ ,  $a_L$  and  $b_L$  values were reported.

Following the removal of two cubes for subsequent pectin and pigment studies, the remaining portion of the fruit was blended for one minute. A sufficient amount of the macerated material was placed in a

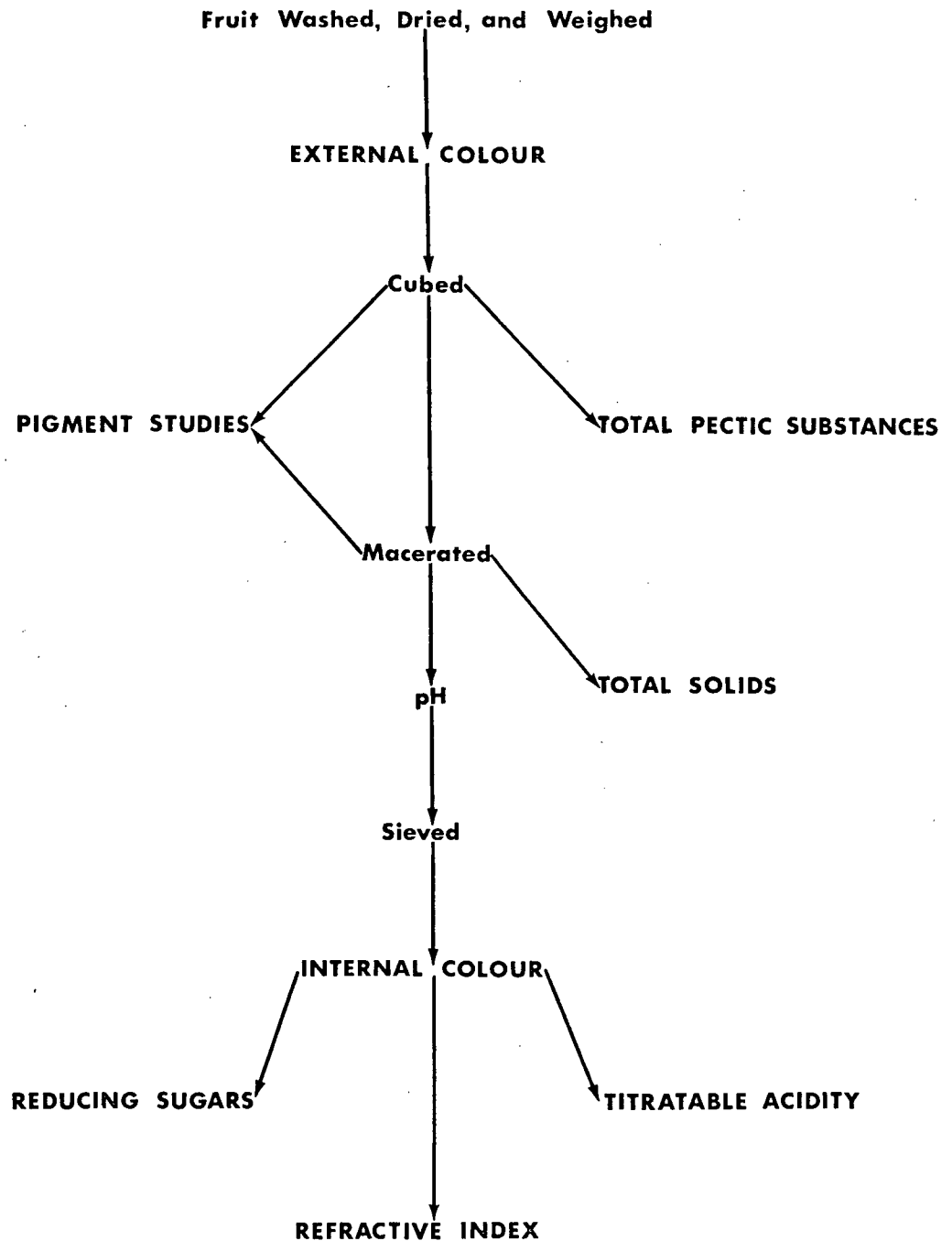


Fig. 5. Sequence of Analysis

sample jar and stored at  $-20^{\circ}\text{C}$  for dry matter determination.

## 2. pH:

pH was determined with a Radiometer PMH 26 pH meter previously standardized with a pH 4.0 buffer. The macerate was strained through a 1 mm. mesh sieve to remove skin and seed fragments.

## 3. Internal Colour:

Fifty grams of sample was then immediately placed into a plastic sample tray and colour readings taken on the Hunterlab Colour and Colour Difference meter adjusted to  $L = 25.1$ ,  $a_L = 25.5$  and  $b_L = 11.9$  with reference standard # D33C-221.

## 4. Total (Titratable) Acidity:

Ten grams of freshly blended material was placed in a 250 ml beaker with 150 ml of distilled water and titrated with 0.1N NaOH to pH 8.1.

## 5. Refractive Index:

A small amount of material was filtered under pressure through Whatman No. 2<sup>V</sup> filter paper and the refractive index of the filtrate determined at  $20^{\circ}\text{C}$  on a Bausch and Lomb refractometer.

## 6. Reducing Sugars:

The Lane and Eynon copper reduction method as described by Ruck (1963) was applied to extracts prepared according to the National Cannery Association (N.C.A.) Laboratory manual (1956).

## 7. Total Solids:

A modification of the N.C.A. (1956) method for total solids determination was used. Approximately 10 grams of thawed, blended sample was placed in pre-desiccated 9 x 2 cm aluminum moisture dishes, covered, and quickly weighed on an analytical balance. Excess moisture was driven off

over a water bath and the samples were then placed in a vacuum oven operated at 70°C and 25 to 27 inches (125 to 75 mm) mercury. Air was permitted to enter the oven at the rate of four to six bubbles per second, after having been double dried through sulfuric acid. At the end of three hours, the air flow rate was increased to about 8-10 bubbles per second and the samples removed from the oven one hour later. The moisture dishes were covered, cooled overnight in a desiccator, and final weights obtained.

Results, as indicated by the initial 50 samples, were sufficiently reproducible so as to obviate the necessity of further duplicate analyses.

#### 8. Total Pectic Substances:

Twenty to thirty gram tomato cubes were weighed, placed in 125 ml 95% ethyl alcohol, and stored at 0°C prior to analysis. The material was blended for five minutes and filtered on a Buchner funnel using Whatman No. 1 filter paper. The residue was washed with additional 95% ethanol. The residue plus filter paper was then macerated with 200 ml 0.5% Versene and the pH adjusted to 11.5. The remainder of the preparation procedure and the spectrophotometric determination were carried out as described by McComb and McCready (1952a, b).

#### 9. Pigment Analyses:

The eight samples representing the same maturity from a given growth cabinet were thawed after short term -20°C storage and equal weights combined. Thirty grams of pooled material were blended for two to three minutes with 100 ml 75 - 60 v/v acetone-hexane. The mixture was filtered through a Buchner funnel and the residue washed with 50 ml acetone-

hexane solution. The residue plus filter paper was then macerated with a small amount of the solvent system and re-filtered thereby providing a very rapid and efficient means of pigment extraction. The crude extract was purified as described by Tomes (1963).

An absorption spectrum was obtained for the purified carotenoid solution using a Unicam SP 800 recording spectrophotometer (Fig. 6). The concentration of the extract (expressed as lycopene) was determined in order to monitor column recoveries. Column recoveries averaged 87%.

Immediately prior to chromatography the sample was concentrated to about 10 ml in a flash evaporator at 40°C. An aliquot of the concentrated extract was then spotted on a 10x 1.5 cm magnesium oxide - Hyflo SuperCel 1:1 w/w column (Sephadex K 15/30 brand). Duplicate columns were run by stepwise elution beginning with 0% acetone in hexane through to 18% acetone-hexane. Elution of the final pigments bands was accelerated by the addition of small amounts of 95% ethanol in acetone-hexane.

The fractions collected were washed four times with large volumes of distilled water and their spectra obtained.

Pigment concentrations were calculated according to the following formula:

$$C = \frac{TVE \times 10^4}{E_{1\text{cm}}^{1\%} \times Sv \times W} ((OD_1 \times EV_1) + \dots + (OD_k \times EV_k))$$

where C = concentration in  $\mu\text{g/gm}$  fresh weight

OD = optical density at the specified wavelength (refer to Table 1)

$E_{1\text{cm}}^{1\%}$  = extinction coefficient

EV = eluent volume

SV = volume of extract spotted on column

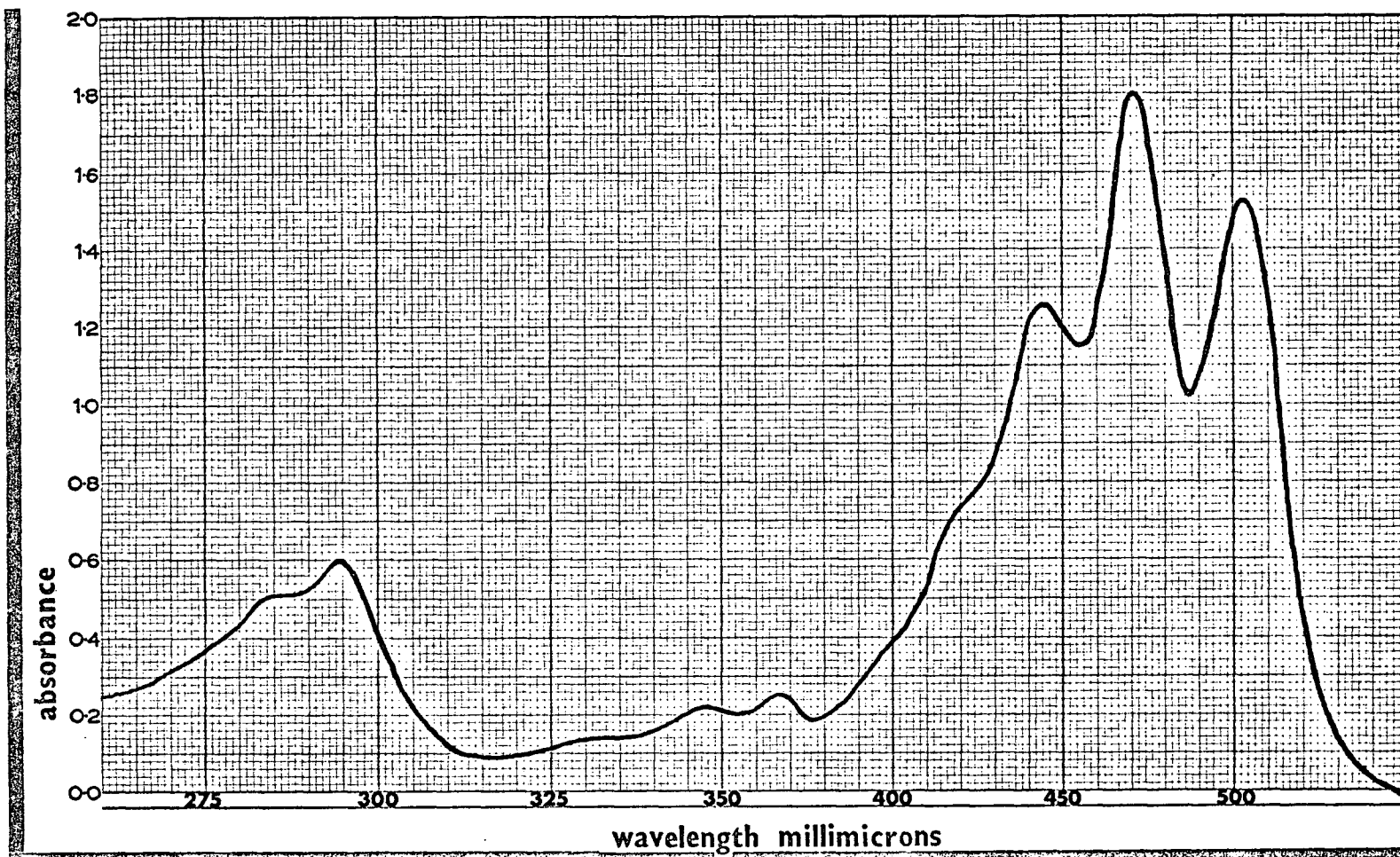


Fig. 6. Typical Absorption Spectrum of a Carotenoid Extract Prior to Chromatography

TABLE 1

Data Used in Quantitative Determination of Pigments

<u>Pigments</u>	$E_{1\text{ cm}}^{1\%}$	<u>Wavelength (m<math>\mu</math>)</u>	<u>Reference</u>
Phytoene	850	286	Rabourn and Quackenbush 1953
Phytofluene	1500	348	Porter and Lincoln 1950
$\alpha$ -Carotene	2750	446	Porter and Lincoln 1950
$\beta$ -Carotene	2500	451	Lime <u>et al.</u> 1952
$\xi$ -Carotene	2200	400	Porter and Lincoln 1950
$\delta$ -Carotene	3210	456	Tomes 1963
$\gamma$ -Carotene	2700	460	Porter and Lincoln 1950
Neurosporene	2990	438	Tomes 1963
Lycopene	3200	502	Porter and Lincoln 1950



W = weight of tomato sample

TVE = total volume of extract after concentration

Pigment identities were established by

1. the position of absorption bands on the column
2. comparison of absorption spectra in hexane and chloroform with known samples or reported spectra
3. thin layer co-chromatography where authentic samples were available

#### Carotenoid Stability Study:

Pigment decomposition during sample storage was suspected because of inconsistencies in the data. An experiment was therefore designed in order to assess the effects of storage form and duration on carotenoid concentrations.

Fruit from tomato plants grown under greenhouse conditions were harvested 14 days following breaker point. Longitudinal sections of 20 to 30 grams were cut from each of three to five fruits, sealed in containers and placed in  $-20^{\circ}\text{C}$  storage. The remaining portions of the fruits were combined, macerated for  $2\frac{1}{2}$  - 3 minutes, 30 gram samples weighed out (required 2 - 3 minutes) and stored as above. Pigment analyses were conducted on fresh samples, and on pooled material stored for 10, 20 and 40 days.

## RESULTS

### Effect of Controlled Environment on Vegetative Growth

Effects of temperature on vegetative growth and development were readily observed (Fig. 7a,7b). Plants exposed to the 17.8/25.6°C night/day diurnal temperature system exhibited rapid and prolific shoot growth. Shoots initiated before and particularly during the controlled environment period were very thin and showed extreme elongation under growth chamber conditions. Emergent leaves were somewhat reduced in size. The upper vegetative canopy remained green, while extensive chlorosis and abscission of lower leaves was noted. In contrast, plants grown at 7.2/18.3 showed a growth pattern more characteristic of plants held under greenhouse conditions. Elongation of both existing and newly developed shoots did not appear to be abnormal. The apparent quantity of vegetative material produced at 7.2/18.3 was less than that produced under the 17.8/25.6 temperature regime. In addition, the death of lower leaves was reduced considerably. At 4.4/15.6, shoot formation was negligible, while growth of older shoots was sharply inhibited. The die-back of lower leaves was very limited. Shoot initiation and growth did not occur at 2.8/13.9. The 2.8/13.9 environment was obviously below that required for plant survival over an extended period.

Low temperatures increased the degree of chlorosis (Fig. 7a, 7b). Little or no discoloration was attributable to temperature within the 17.8/25.6 treatment. At 7.2/18.3 a limited amount of discoloration was evident. At 4.4/15.6 considerable chlorosis, largely restricted to the



Fig. 7a. Plant Responses to Controlled Environment Treatments of 40 Days Duration.  
 Temperatures: 17.8/25.6 left, and 7.2/18.3 right.  
 Insert: Flowering and Fruit Set at 7.2/18.3.

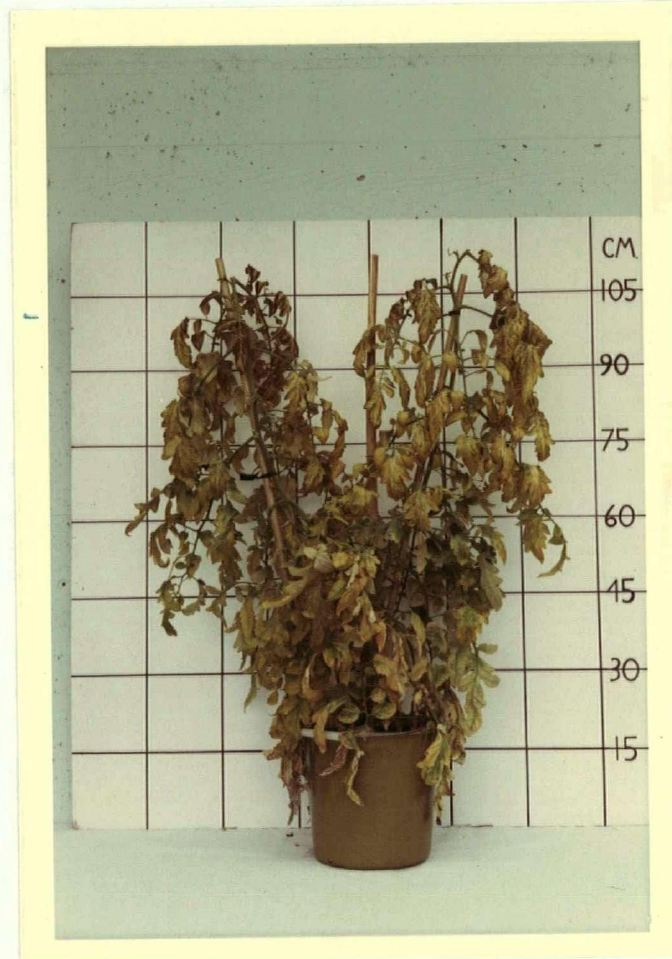


Fig. 7b. Plant Responses to Controlled Environment Treatments of 40 Days Duration.  
Temperatures: 4.4/15.6 left, and 2.8/13.9 right.



upper portion of the plants was readily noted. At 2.8/13.9 chlorophyll destruction was general and severe.

#### Effect of Controlled Environment on Reproductive Growth

Flowering and fruit set continued at the two highest temperatures (Fig. 7b). Some flowering was evident at 4.4/15.6 but no new fruit were produced. No flowering occurred at 2.8/13.9.

The tomato fruits were found to be very prone to cracking under growth chamber conditions (Table 2). A very high incidence of radial and concentric cracking was observed at 17.8/25.6. The frequency of cracking decreased with lower temperatures. Cracking was most prevalent at the 21 day harvest.

Fruit tended to be smallest at 17.8/25.6 and weights were somewhat higher at the two intermediate temperatures but these differences were not significant (Table 3).

#### Effects of Ripening Temperatures on some Chemical Characteristics of Tomato Fruits

Initially, experiments were designed with a minimum of three replications. However, growth chamber malfunctions resulted in unequal replication between the different temperature regimes (Table 4) with respect to dry matter, refractive index, reducing sugar, pH, and titratable acidity data analyses. In these instances, statistical analyses were carried out on the data from the two complete replicates  $R_3$  and  $R_5$  (Tables 5 and 6).

The raw data provided by replicate  $R_5$  were not considered to be truly indicative of the influence of temperature during ripening

Table 2. Effect of Temperature on the Frequency of Fruit Cracking at Three Harvest Dates

Temperature	Harvests (days from breaker point)		
	7	14	21
17.8/25.6	35.7*	75.6	95.7
7.2/18.3	23.4	41.7	66.0
4.4/15.6	14.7	12.5	48.5
2.8/13.9	15.2	17.6	40.6

\*Each figure represents the percent of fruit having one or more cracks and is derived from a minimum sample size of 32 fruit.

Table 3. Effect of Temperature on Fruit Weight at Three Harvest Dates

Temperature	Harvests (days from breaker point)		
	7	14	21
17.8/25.6	165.6*	160.6	162.4
7.2/18.3	174.6	183.9	172.5
4.4/15.6	177.8	186.5	175.3
2.8/13.9	164.9	169.3	170.8

\*Each value is the mean of three replicates. The means were not significantly different according to Duncan's multiple range test ( $P = .05$ ).

Table 4. Specification of Controlled  
Environment Temperature  
Replicates

Temperature	Replicate				
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
17.8/25.6	+	+	+	+	+
7.2/18.3	+	+	+	+	+
4.4/15/6	-	+	+	-	+
2.8/13.9	-	-	+	+	+

+ experiment completed

- experiment not completed because of  
equipment malfunction



Table 5. F Values and the Significances of Main Effects and Interactions on Chemical Characteristics of Tomato Fruits

Treatment Effect	Variable					
	Dry Matter	Refractive Index	Reducing Sugars	pH	Titratable Acidity	Pectins
Temperature	1.82	.54	3.01	10.17*	5.80	2.03
Harvest	.42	.09	1.41	66.13*	5.92	1.19
T x H	1.76	1.10	1.30	8.16*	.47	.64

+ The analyses of variance were based on replicates  $R_3$  and  $R_5$ , except in the case of pectins where three complete replicates were used.

\* Only the F values for pH were significantly different ( $P = .05$ )

Table 6. Effect of Temperature on Chemical Characteristics of Tomato Fruits at 3 Harvest Dates

Variable	Temperature	Harvests (days from breaker stage)		
		7	14	21
Dry Matter (per cent)	17.8/25.6	6.08* a	6.07 a	6.13 a
	7.2/18.3	6.69 a	6.50 a	6.43 a
	4.4/15.6	6.69 a	6.62 a	6.44 a
	2.8/13.9	6.75 a	6.53 a	6.19 a
Refractive Index	17.8/25.6	1.3406 a	1.3407 a	1.3409 a
	7.2/18.3	1.3411 a	1.3413 a	1.3412 a
	4.4/15.6	1.3410 a	1.3413 a	1.3410 a
	2.8/13.9	1.3414 a	1.3412 a	1.3406 a
Reducing Sugars (per cent)	17.8/25.6	2.99 a	2.81 a	2.69 a
	7.2/18.3	3.11 a	3.19 a	3.09 a
	4.4/15.6	3.27 a	3.08 a	3.06 a
	2.8/13.9	3.38 a	3.26 a	2.88 a
pH	17.8/25.6	4.37 a	4.48 a	4.66 a
	7.2/18.3	4.26 b	4.38 ab	4.45 b
	4.4/15.6	4.24 b	4.31 b	4.41 b
	2.8/13.9	4.22 b	4.30 b	4.40 b
Titratable Acidity (ml 0.1 N NaOH)	17.8/25.6	6.91 a	5.73 b	4.96 b
	7.2/18.3	8.42 a	7.09 ab	6.13 ab
	4.4/15.6	8.31 a	7.32 a	6.47 a
	2.8/13.9	8.68 a	7.65 a	6.75 a
Pectins (per cent A.U.A.)	17.8/25.6	.276 a	.260 a	.272 a
	7.2/18.3	.304 a	.277 a	.276 a
	4.4/15.6	.296 a	.288 a	.289 a
	2.8/13.9	.314 a	.306 a	.296 a

\* Each value is the mean of 2 replicates except for total pectins which are means of 3 replicates. Figures followed by the same letter within a particular measurement and harvest are not significantly different at  $P = .05$ , according to Duncan's multiple range test.

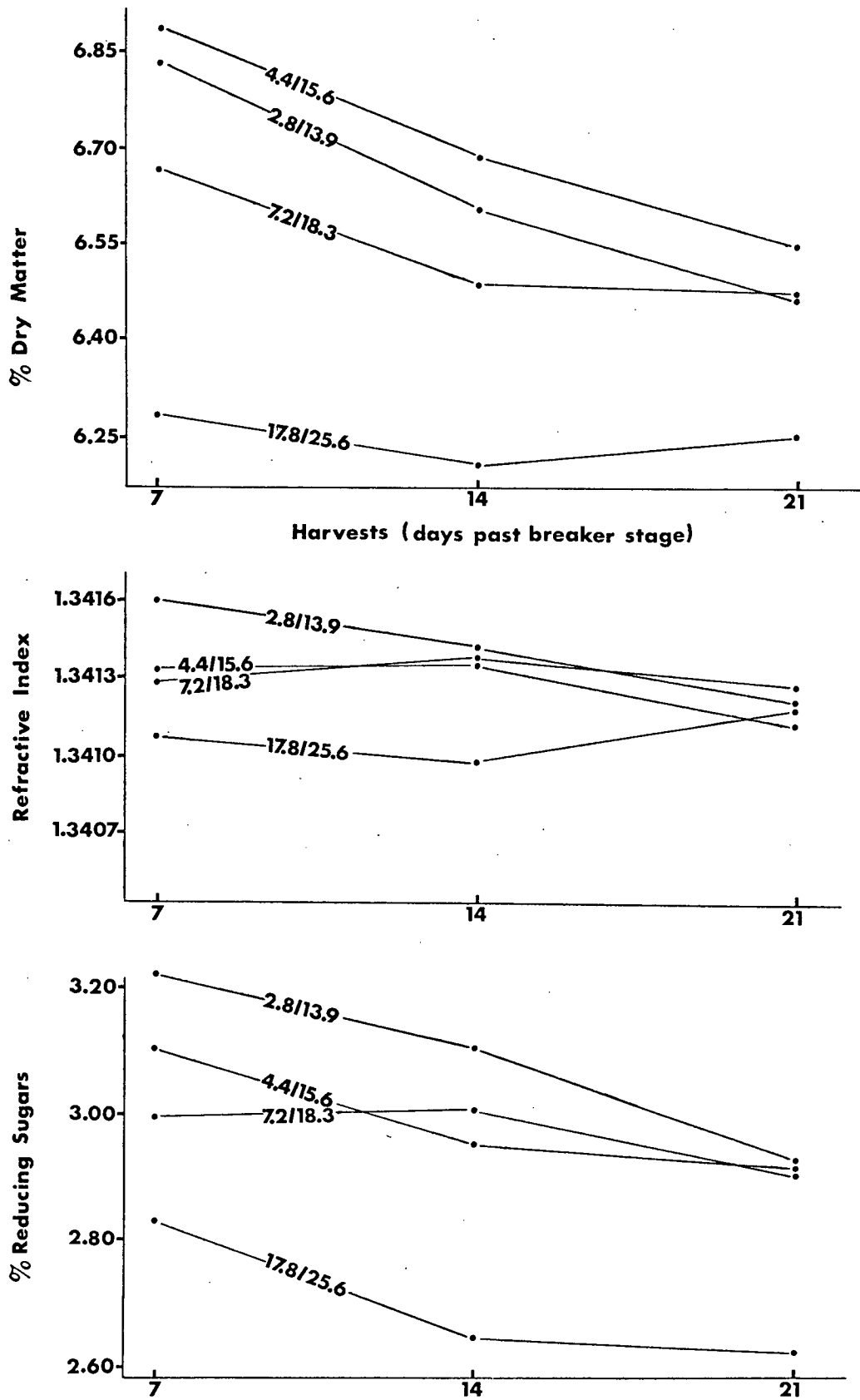


Fig. 8a. Influence of Temperature and Harvest Dates on Dry Matter, Refractive Index, and Reducing Sugars of Tomato Fruits

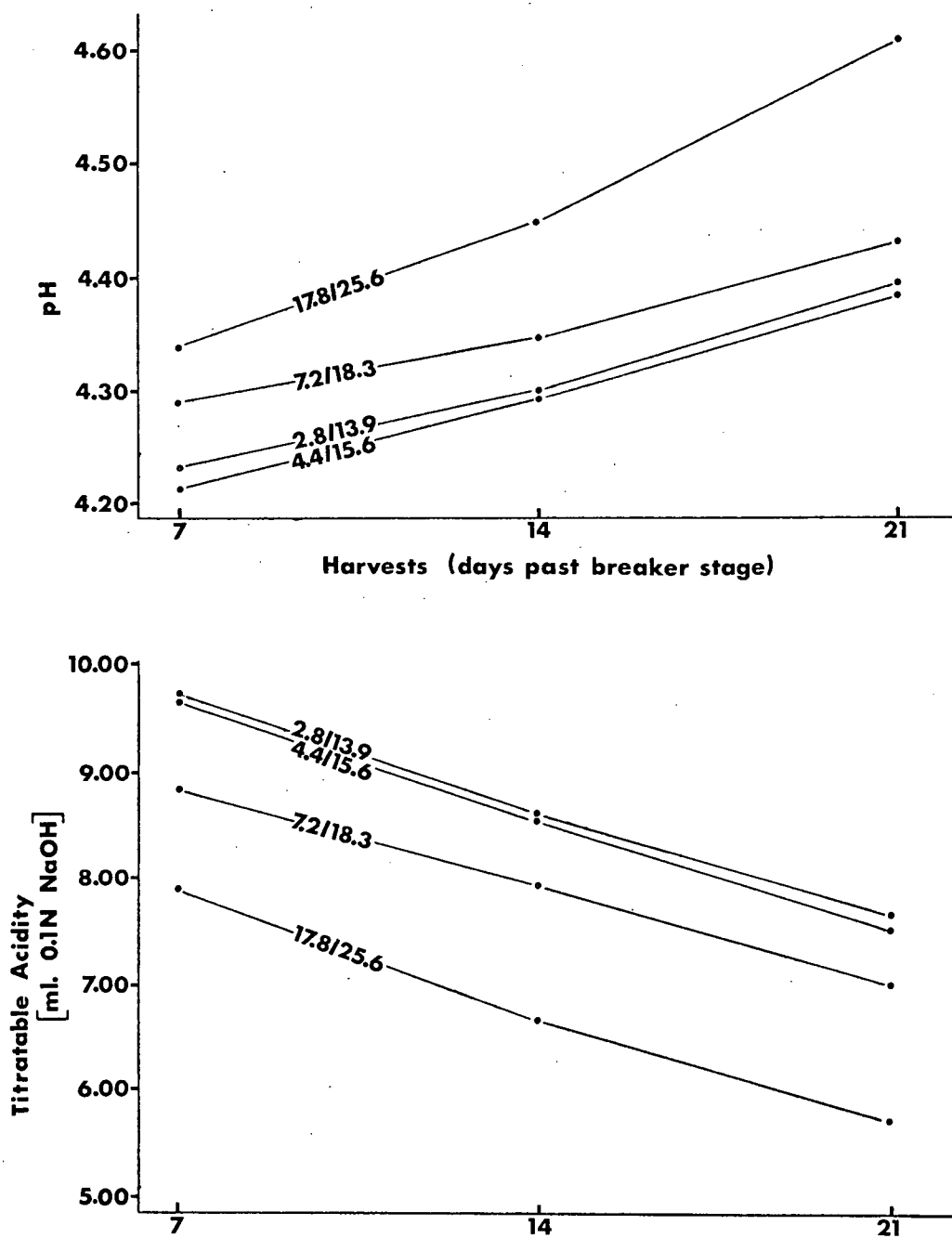


Fig. 8b. Influence of Temperature and Harvest Dates on pH and Titratable Acidity of Tomato Fruits

on several factors, particularly titratable acidity, hence supplementary graphs were prepared. The use of means derived from all data exclusive of missing values was not satisfactory as experimental runs (reps.) were significantly different in almost every case regardless of the manner in which valid analyses of variance could be done. Table 4 indicates the possible combinations of replications and temperatures which can be used in the analyses of variance. Thus, a general linear hypothesis computer programme (Sampson, 1968) was utilized to provide prediction values for the missing data. The temperature x harvest date means which included predicted data were then computed and graphically presented (Fig. 8a and 8b).

#### Dry Matter

The dry matter contents of tomato fruits were lowest at 17.8/25.6 for each of the three harvest dates (Fig. 8a). In addition, the per cent dry matter tended to decrease with later harvests at all temperatures except 17.8/25.6. The per cent dry matter showed no evidence of change with harvest dates at 17.8/25.6. The analysis of variance using only replicates  $R_3$  and  $R_5$  did not confirm the apparent temperature and harvest date effects on the per cent dry matter.

#### Refractive Index

Temperature and harvest dates appeared to have little or no effect on fruit refractive indices (Fig. 8a). The statistical analysis (Table 5 and 6) also showed that the refractive index measurements did not change significantly with different temperatures or harvest dates.

### Sugars

Preliminary analyses demonstrated that sucrose, when present, occurred only in trace quantities. Hence, subsequent analyses were done only for reducing sugars. The per cent reducing sugars were lowest at 17.8/25.6 and tended to increase with lower temperatures (Fig. 8a). The reducing sugar contents of fruit showed a tendency to decrease with later harvests. Again the statistical analysis with replicates  $R_3$  and  $R_5$  indicated that temperature and harvest effects on sugar contents were non-significant (Table 5 and 6).

### pH

The pH of tomato fruits increased as the temperature and the number of days to harvest increased (Fig. 8b). The pH of fruit held at 17.8/25.6 was significantly higher than that of fruit at 2.8/13.9, 4.4/15.6 and 7.2/18.3 harvested at a comparable 7 day interval (Table 6). At 14 days, the pH's of 17.8/25.6 and 7.2/18.3 fruit did not differ significantly but pH's at 17.8/25.6 were significantly higher than those at the two lowest temperatures. At 21 days, the pH's of fruit maintained at the three lowest temperatures were considerably below those of fruit subjected to the 17.8/25.6 temperature.

### Titratable Acidity

Titratable acidity decreased with higher temperatures and later harvests (Table 8b). The titratable acidity values of fruit held at 2.8/13.9 and 4.4/15.6 were almost identical and decreased at the same rate with increasing harvest times. According to the analysis of

variance of replicates  $R_3$  and  $R_5$ , titratable acidity was not significantly influenced by temperature or harvest date (Table 5). However, when 17.8/25.6, 7.2/18.3, and 4.4/15.6 data were analyzed using  $R_2$ ,  $R_3$  and  $R_5$ , temperature and harvest main effects were highly significant. These data also indicated that titratable acidity was significantly lower at 17.8/25.6 irrespective of the harvest level. Further, differences between the 7- and 21-day harvests were demonstrated for the three higher temperature regimes.

#### Pectic Substances

Total pectic substances (per cent anhydrouronic acids) were highest at 2.8/13.9 and tended to decrease with higher temperatures. The pectic substances showed a slight decrease with later harvest dates. Statistical analysis of the data showed that neither temperature nor harvest dates significantly influenced the total pectin contents of fruits (Table 5 and 6).

#### Effect of Ripening Temperatures on Fruit Colour

##### External Colour

Both the temperature and the number of days past the breaker point strongly influenced the surface colour of fruit (Fig. 9). At 17.8/25.6 a minimum period of about 7 days beyond the breaker stage was required to obtain full red fruit. At 7.2/18.3, fruit required approximately 14 days following incipient coloration to attain a full red condition. At 4.4/15.6, a near full red was reached only after 21 or more days. At 2.8/13.9, fruit did not surpass a bright orange stage

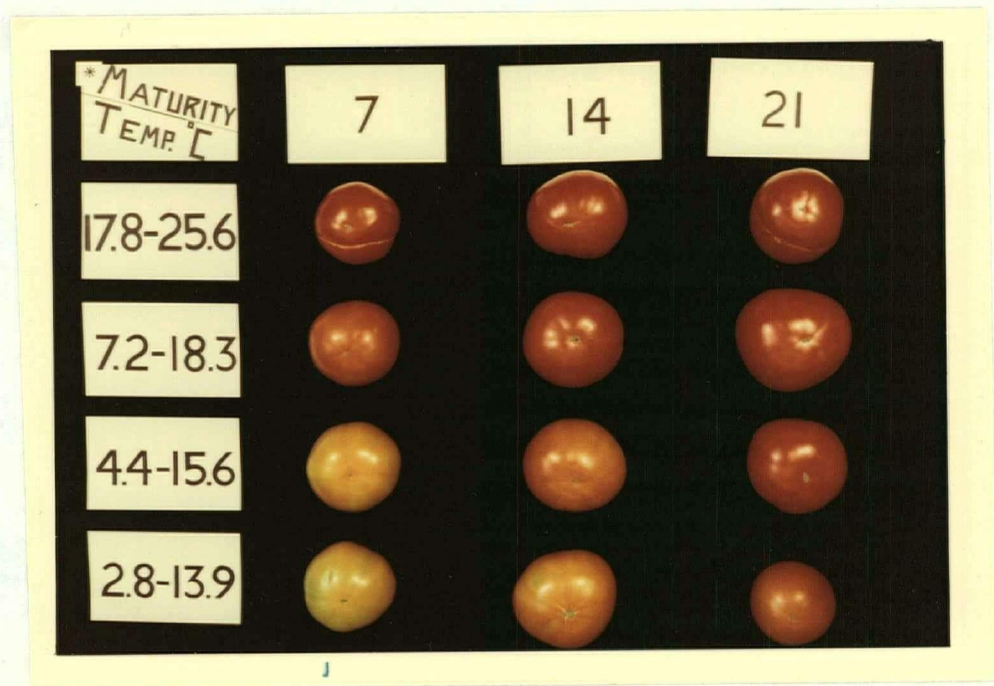
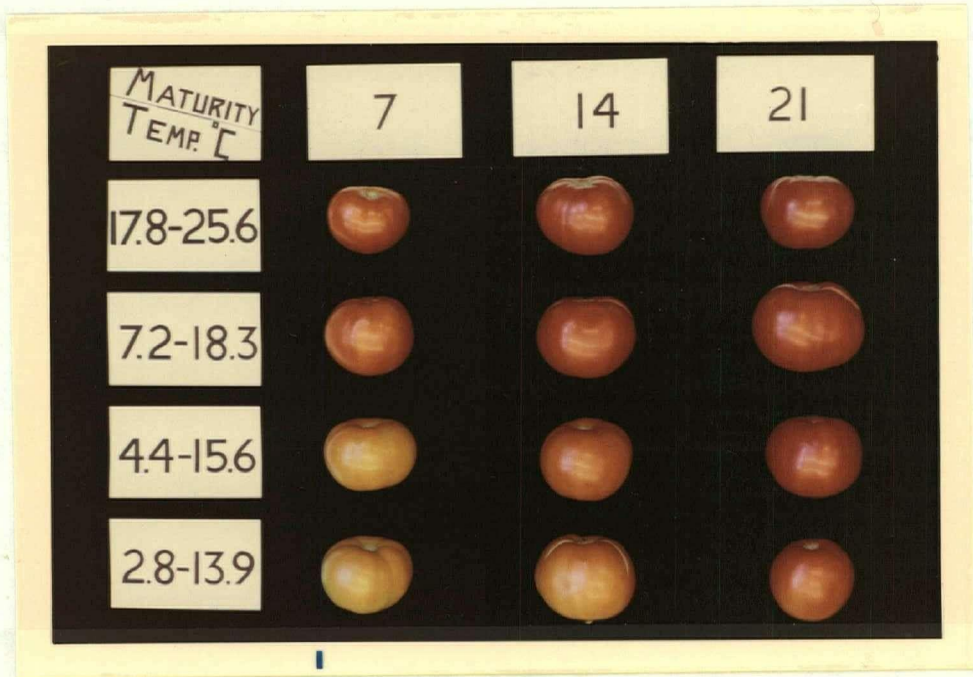


Fig. 9. Temperature-Maturity Tomato Colour Gradients.  
Above: Side View. Below: Blossom End View.



of coloration.

Coloration was less uniform at lower ripening temperatures. At the 7-day harvest at 2.8/13.9 and to a lesser extent at 4.4/15.6, fruit frequently possessed large green areas.

Hunter L,  $a_L$  and  $b_L$  external colour values were highly significantly affected by temperature and harvest dates (Table 7 and 8). The temperature x harvest interactions were significant in all cases. Duncan's multiple range tests were done in such a manner that the effects of each temperature at each harvest level could be assessed (Table 9).

Surface lightness, as measured by the Hunter L scale, increased with decreasing temperatures at all three harvest times. At 7.2/18.3, 4.4/15.6 and 2.8/13.9, lightness decreased with increasing harvest times. However, at 17.8/25.6, harvest dates had no effect on surface lightness. Hunter  $a_L$  values, a measure of sample greenness to redness, showed a considerable change with temperature. At 7 days, fruit redness increased progressively with increasing temperatures. At 14 days, surface redness intensified from 2.8/13.9 to 4.4/15.6 and 7.2/18.3. Hunter  $a_L$  values of fruit from 17.8/25.6 were significantly lower than those of corresponding 14 day fruit from 7.2/18.3. At 21 days,  $a_L$  values were significantly greater at the two intermediate temperatures.

When examined as a function of the harvest time, sample redness was noted to increase at 2.8/13.9 and 4.4/15.6. At 7.2/18.3, surface redness increased up to 14 days and thereafter decreased slightly. At 17.8/25.6,  $a_L$  values decreased somewhat with each successive harvest date.

Hunter  $b_L$  values were inversely related to both temperature and harvest time. The data show that the yellow component of the tomato

Table 7. Analysis of Variance for L<sub>Ext</sub> Colour Values to Indicate the Complete Partition of Variation in the Split Plot Analysis

Source	D.F.	Sum Sq	Mean Sq.	Error	F
Run	2	12.78	6.39	Pot/RT	8.29**
Temp	3	340.79	113.60	R x T	104.52**
R x T	6	6.52	1.08	Pot/RT	1.41 n.s.
Pot/RT	36	27.77	.77		1.98*
Age	2	141.50	70.74	A x R	84.67**
A x R	4	3.34	.83	A x P/RT	1.72 n.s.
A x T	6	27.19	4.53	A x R x T	5.37**
A x R x T	12	10.12	.84	A x P/RT	1.73 n.s.
A x P/RT	72	35.03	.48		1.25 n.s.
Error	144	56.21	.39		
Total	287	661.27			

n.s. Not Significant  
 \* Significant at P = .05  
 \*\* Significant at P = .01

Table 8. F values and the significances of Main Effects and Interactions on Fruit Colour

Treatment Effect	External Colour			Internal Colour		
	L	a	b	L	a	b
Temperature	104.52**	11.51**	117.97**	116.04**	24.96**	112.01**
Harvest	84.67**	28.89**	87.39**	15.06*	39.80**	8.81*
T x H	5.37**	24.95**	4.06*	4.98**	6.90*	7.40**

\* Significant at P = .05

\*\* Significant at P = .01

Table 9. Effect of Temperature on Surface Colour

Colour Scale	Temperature	Harvests (days from breaker stage)		
		7	14	21
L	17.8/25.6	12.86*c	12.42d	12.30c
	7.2/18.3	14.80b	13.38c	12.91c
	4.4/15.6	16.30a	14.34b	13.81b
	2.8/13.9	16.26a	15.25a	14.56a
a	17.8/25.6	10.47a	10.15b	9.45b
	7.2/18.3	9.69a	11.33a	10.84a
	4.4/15.6	7.77b	10.74ab	11.29a
	2.8/13.9	6.25c	8.68c	10.09b
b	17.8/25.6	4.40c	3.97d	3.88d
	7.2/18.3	5.84b	4.86c	4.45c
	4.4/15.6	6.84a	5.70b	5.29b
	2.8/13.9	6.72a	6.25a	5.83a

\* Each value is the mean of 3 replicates. Means followed by the same letter within a particular measurement and harvest did not differ significantly according to Duncan's multiple range test at the 5% level.

colour was greatest at the 7-day harvest and was maximal at lower temperatures.

### Internal Colour

Temperature and harvest main effects and the T x H interactions on internal colour measurements were significant in all cases (Table 8). Since the temperature x harvest interactions were significant, the data were re-analyzed for simple effects. The similarities in the Duncan's test results for the L, and  $a_L$  and  $b_L$  mean values for pureed and whole fruit samples (Table 9 and 10) suggested that internal colour changes tended to closely parallel those of fruit surfaces.

The lightness of 7 and 14 day samples was highest at 2.8/13.9 and 4.4/15.6, lower at 7.2/18.3 and lowest at 17.8/25.6. At 21 days, internal L values decreased with successively higher temperature treatments.

Hunter  $a_L$  values showed a marked dependence on temperature, particularly at the 7-day harvest. Sample redness attained a maximal level in 7 days at 17.8/25.6. At 14 and 21 days, fruit macerates from the 17.8/25.6 and 7.2/18.3 treatments were substantially redder than corresponding samples derived from fruits held at lower temperatures.

Sample yellowness increased with lower temperatures at all three harvests. The highest  $b_L$  values were found at 2.8/13.9 at 7 days, while the lowest values were at 17.8/25.6 at 7 and 14 days.

The internal L,  $a_L$  and  $b_L$  values were not influenced by the selected harvest times at 17.8/25.6 in contrast to the effect at the other temperature regimes.

When fruit colour was expressed in terms of Lb/a ratios (Fig.10),

Table 10. Effect of Temperature on Internal Colour

Colour Scale	Temperature	Harvest Interval (days from breaker stage)		
		7	14	21
L	17.8/25.6	32.83*c	30.75c	31.90d
	7.2/18.3	44.03b	36.11b	34.55c
	4.4/15.6	48.84a	42.06a	41.68b
	2.8/13.9	49.54a	46.20a	45.04a
a	17.8/25.6	28.21a	28.34a	27.78a
	7.2/18.3	20.72b	26.67a	26.68a
	4.4/15.6	13.23c	21.98b	23.76b
	2.8/13.9	9.67d	16.85c	18.94c
b	17.8/25.6	11.26c	11.40d	12.74c
	7.2/18.3	16.34b	13.16c	12.73c
	4.4/15.6	19.09a	15.62b	15.79b
	2.8/13.9	21.05a	18.45a	18.10a

\* see footnotes Table 9

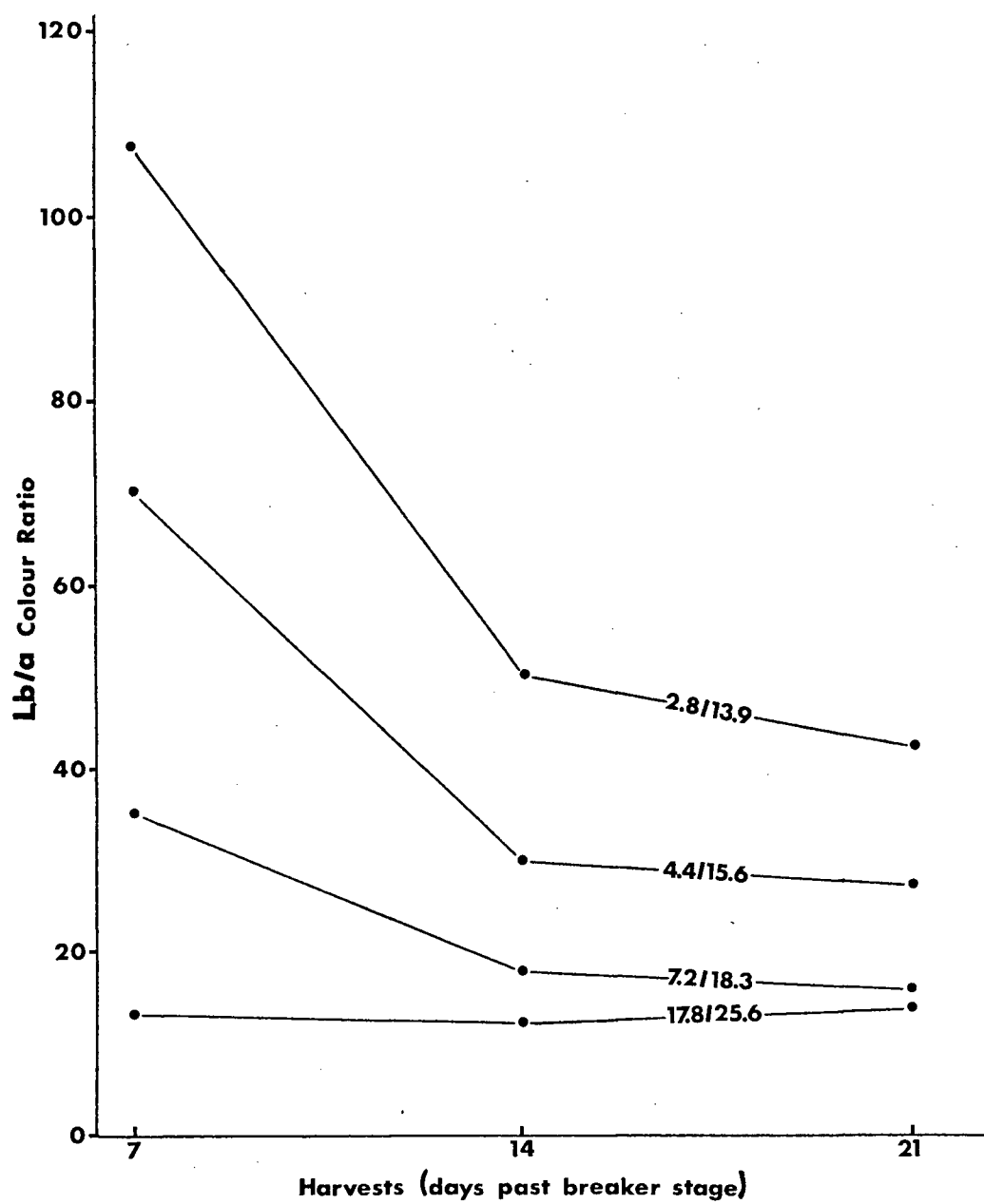


Fig. 10. Influence of Temperature on Internal Colour Ratios

it was readily apparent that temperature effects were most pronounced at the 7-day harvest. From 7 to 14 days, Lb/a ratios showed a very marked decline at 2.8/13.9 and 4.4/15.6, a lesser rate of decrease at 7.2/18.3 and little or no change at 17.8/25.6. From 14 to 21 days, Lb/a ratios dropped appreciably at 2.8/13.9, declined only slightly at 4.4/15.6 and 7.2/18.3, and showed a small rise at 17.8/25.6. The 14-day 17.8/25.6 treatment had the lowest ratio.

#### Relationships between Colour Variables

When the Hunter values for surface colour were graphically compared, a number of inter-relationships between the  $L$ ,  $a_L$  and  $b_L$  readings during the ripening process became immediately apparent. The  $L_{ext}$   $b_{ext}$  graph (Fig. 11a) demonstrated that the relationship between surface lightness and yellowness was essentially linear and that decreases in surface lightness were consistently associated with losses in sample yellowness. An overall correlation coefficient of .97 was obtained. Further, a high degree of association between  $L_{ext}$  and  $b_{ext}$  values persisted over all temperature and maturity ranges (Table 11).

When  $L_{ext}$  and  $a_{ext}$  values were graphed, a parabolic curve resulted (Fig. 11b). The decrease in surface lightness from a maximum magnitude of about 16 to about 14 was accompanied by a substantial rise in external redness. Surface redness was maximal when a lightness value of near 13.5 was attained. A further decrease in lightness was then accompanied by a drop in sample redness. An overall correlation of .76 was obtained. The correlation coefficient decreased noticeably



TABLE 11  
Correlation Coefficients for External and Internal  
Colour Variables

Temperature °C	Harvests (days from breaker stage x	External			Internal		
		y	L	L	a	L	L
		b	a	b	b	a	b
17.8/25.6	7	.94	.30	.37	.67	.24	.55
	14	.82	.67	.85	.84	.16	.15
	21	.82	.65	.64	.84	.10	.28
7.2/18.3	7	.90	.35	.29	.66	.45	.56
	14	.88	.31	.32	.79	.28	.10
	21	.91	.83	.77	.81	.63	.44
4.4/16.5	7	.94	.26	.33	.71	.30	.37
	14	.91	.21	.33	.85	.48	.34
	21	.95	.75	.62	.81	.62	.70
2.8/13.9	7	.93	.46	.32	.34	.23	.47
	14	.97	.63	.70	.64	.66	.47
	21	.94	.56	.39	.85	.79	.74

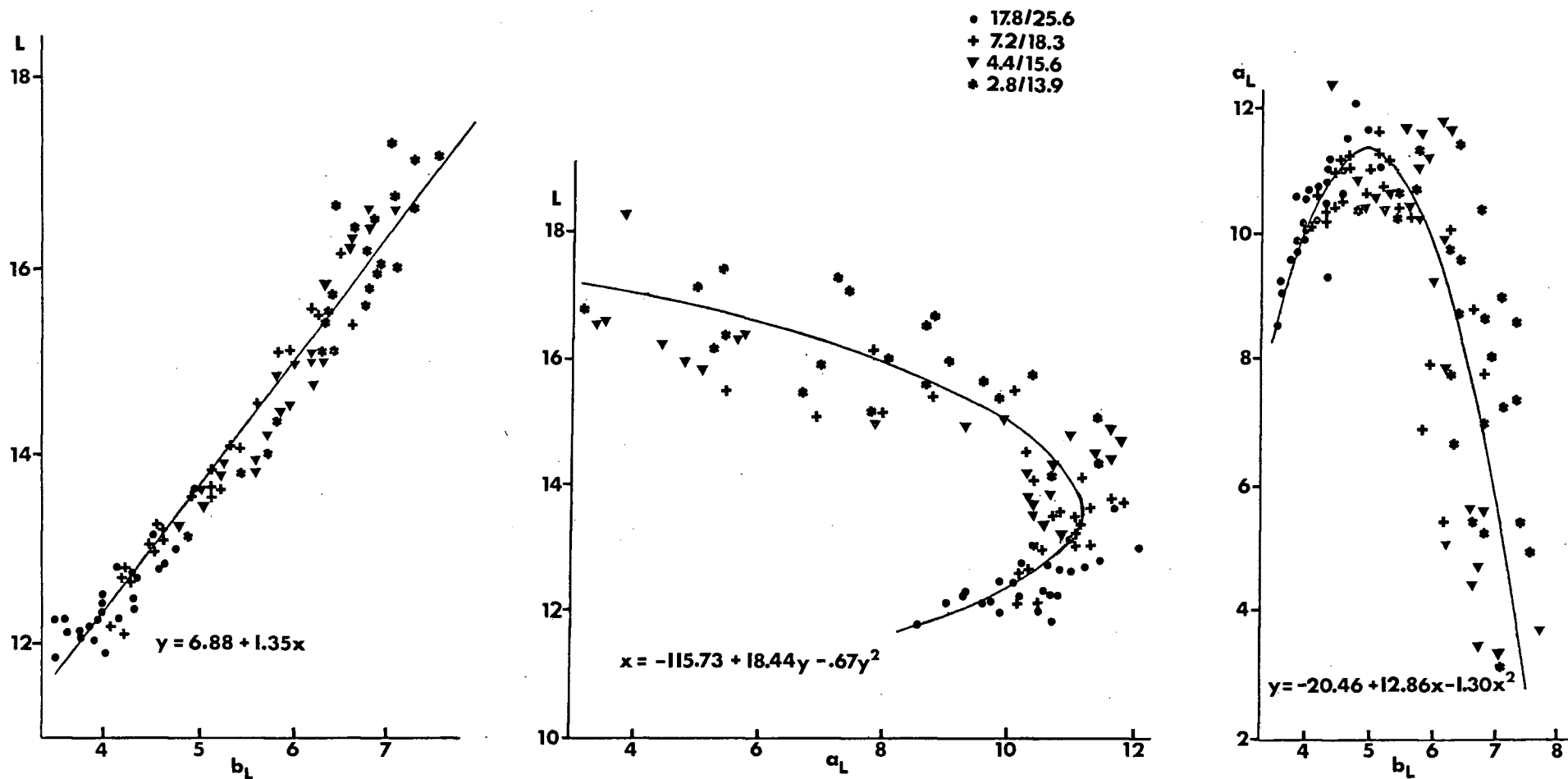


Fig. 11a-c. Relationships Between Surface  $L$ ,  $a_L$ , and  $b_L$  Values

\* Points on the graphs constitute data from one replicate.

\*\* Equations based on three replicates.

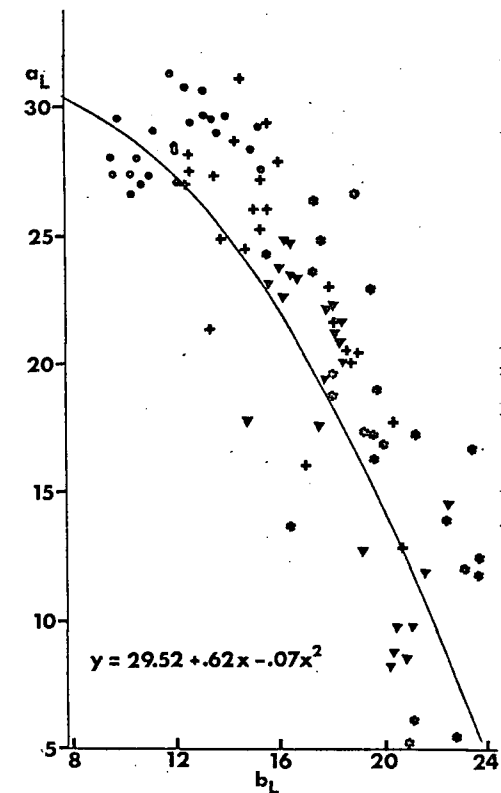
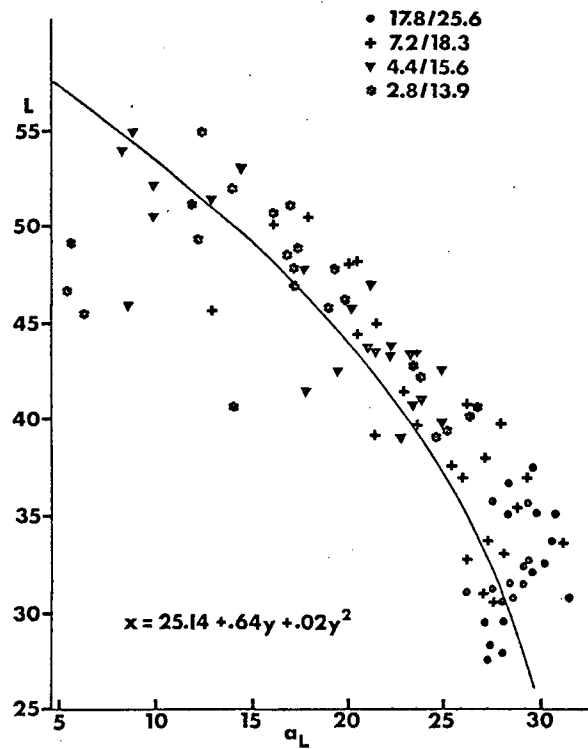
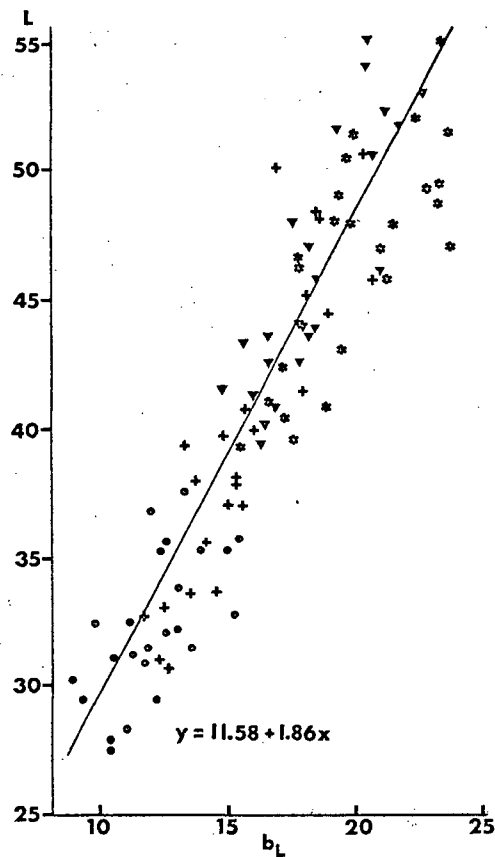


Fig. 12a-c. Relationships Between Internal L,  $a_L$ , and  $b_L$  Values

\* Points on the graphs constitute data from one replicate.

\*\* Equations based on three replicates.

Table 12. Correlation Coefficients for Surface and Internal Colour Measurements at Four Temperatures

x	$L_{ext}$		$a_{ext}$		$b_{ext}$		$Lb/a_{ext}$
y	$L_{int}$	$Lb/a_{int}$	$a_{int}$	$Lb/a_{int}$	$b_{int}$	$Lb/a_{int}$	$Lb/a_{int}$
17.8/25.6	.27**	.14 n.s.	.08 n.s	.05 n.s.	.12 n.s.	.27*	.40**
7.2/18.3	.69**	.67**	.63**	-.68**	.63**	.67**	.76**
4.4/15.6	.76**	.69**	.85**	-.88**	.68**	.63**	.85**
2.8/13.9	.72**	.60**	.80**	-.65**	.80**	.61**	.82**

n.s. Not Significant  
 \* Significant at P = .05  
 \*\* Significant at P = .01

Table 13. Correlation Coefficients for Surface  
and Internal Lb/a Indices

Temperature	Harvests (days past breaker stage)		
	7	14	21
17.8/25.6	.59**	.31*	.49**
7.2/18.3	.68**	.68**	.58**
4.4/15.6	.75**	.68**	.54**
2.8/13.9	.70**	.67**	.65**

\* Significant at  $P = .05$

\*\* Significant at  $P = .01$

with higher temperatures and later harvests (Table 11).

The relationship between surface  $a_L$  and  $b_L$  measurements was also curvilinear (Fig. 11c). As fruit ripened, the rapid increase in surface redness was accompanied by a moderate fall in yellowness.

The  $b_{ext}$  values declined most rapidly when surface redness was maximal. An overall correlation coefficient of .54 was obtained.

When internal  $L$ ,  $a_L$ ,  $b_L$  graphs and regression results (Fig. 12a, b, c and Table 11) were compared to the foregoing several differences were noted. The  $L_{int}$  to  $b_{int}$  plots were linear as anticipated; however, lower correlation coefficients were obtained. Although satisfactory parabolic functions could be fitted to the internal  $L$  to  $a_L$  and  $a_L$  to  $b_L$  relationships, definite tendencies towards linearity were observed. The equations  $x = 25.14 + .64y + .02y^2$  and  $y = 29.52 + .62x - .07x^2$  provided the best-fits ( $r = .77$  and  $.82$  to the  $L$  to  $a_L$  and  $a_L$  to  $b_L$  data respectively).

Although the  $L_{ext}$ ,  $a_{ext}$ ,  $b_{ext}$  variables correlated poorly with the corresponding internal colour measurements at 17.8/25.6, the correlations improved substantially with lower temperatures (Table 12). When the internal and external  $Lb/a$  colour ratios were related by regression analysis a correlation coefficient of .88 was obtained for all data. While the correlation between surface and internal colour increased with decreasing temperatures it tended to decrease with time following incipient coloration (Table 13). External and internal colour appeared to be best related at 4.4/15.6 at 7 days ( $r = .75$ ).

### Effect of Temperature on Carotenoid Development During Ripening

Pigment analyses of Early Red Chief tomato fruits established the presence of phytoene, phytofluene,  $\alpha$ -carotene,  $\beta$ -carotene,  $\xi$ -carotene,  $\gamma$ -carotene, neurosporene, and lycopene. A compound with spectral and adsorptive characteristics resembling those of  $\delta$ -carotene was found in small quantities in a number of samples, however the presence of  $\delta$ -carotene could not be confirmed.

The analyses of variance of data for all variables, as exemplified by Table 14, indicated that in most instances polyene production was sensitive to both temperature and ripening period (Table 15).

In general, pigment concentrations were maximal under the 17.8/25.6 diurnal temperature regime (Table 16, Part II). Phytoene, phytofluene and  $\xi$ -carotene quantities decreased progressively with lower temperatures. Temperature main effect means showed that neurosporene and lycopene concentrations were lowest at 2.8/13.9 and 4.4/15.6, and highest at 17.8/25.6.  $\alpha$ -Carotene concentration was inversely related to temperature.  $\gamma$ -Carotene synthesis was not affected by temperature.  $\beta$ -Carotene production was largely independent of temperature.

In the majority of cases, carotenoid concentrations increased with ripening time (Table 16, Part II). However,  $\alpha$ -carotene amounts were maximal at 7 days past incipient coloration and minimal at the 21 day harvest. Neither  $\beta$ -carotene nor  $\gamma$ -carotene development was significantly influenced by fruit maturity.

From the more detailed information provided in Table 16, Part I; and Fig. 13a, 13b and 13c, a number of interesting situations were evident.

Table 14. Analysis of Variance for Carotenoid  
Temperature - Harvest Date Study

Source	D.F.	Sum Sq.	Mean Sq.	Error	F
Rep	2	.60	.30		.02 n.s.
Temp	3	7376.80	2458.93	T x R	38.75 **
T x R	6	380.78	63.46		4.90 **
Harv	2	2479.30	1239.65		95.74 **
T x H	6	150.10	25.02		1.93 n.s.
Error	16	207.18	12.95		
Total	35	10594.76			

Variable: Lycopene

n.s. Not Significant  
 \* Significant at P = .05  
 \*\* Significant at P = .01



Table 15. F values and the significances of Main Effects on Pigment Concentrations

Treatment Effect	Pigment							
	Phytoene	Phytofluene	$\alpha$ -Carotene	$\beta$ -Carotene	$\xi$ -Carotene	$\gamma$ -Carotene	Neurosporene	Lycopene
Temperature	58.36**	174.01**	55.47**	4.11 n.s.	207.18**	.90 n.s.	6.86*	38.75**
Harvest	82.28**	263.55**	7.88*	.85 n.s.	54.66**	.12 n.s.	10.14**	95.74**
T x H	7.16**	2.40 n.s.	1.27 n.s.	1.48 n.s.	.48 n.s.	.59 n.s.	.81 n.s.	1.93 n.s.

n.s. Not Significant

\* Significant at P = .05

\*\* Significant at P = .01

Table 16. Temperature and Harvest Effects on Pigment Concentrations

Temperature	Harvest	Pigment Concentration ( $\mu\text{g/g f. wt.}$ )							
		Phyto- toene	Phyto- fluene	$\alpha$ -Caro- tene	$\beta$ -Caro- tene	$\xi$ -Caro tene	$\gamma$ -Caro tene	Neuro- porene	Lyc- pene
17.8/25.6	7	18.14*b	5.92 b	0.02 a	2.97 a	1.30 b	0.64 a	0.31 a	43.50 c
	14	23.05 a	7.87 a	0.02 a	2.18 b	1.70 a	0.51 a	0.32 a	57.68 b
	21	19.19 b	8.23 a	0.02 a	2.19 b	1.94 a	0.51 a	0.37 a	64.77 a
7.2/18.3	7	11.82 b	3.99 c	0.06 a	3.81 a	0.67 b	0.65 a	0.23 a	24.33 c
	14	18.31 a	6.44 b	0.05 a	3.57 a	1.12 a	0.73 a	0.24 a	43.27 b
	21	19.07 a	7.23 a	0.04 a	3.45 a	1.21 a	0.69 a	0.26 a	51.52 a
4.4/15.6	7	6.51 c	2.26 c	0.08 a	3.22 a	0.37 c	0.56 a	0.12 b	16.10 b
	14	12.30 b	4.82 b	0.06 ab	3.24 a	0.67 b	0.62 a	0.16 ab	27.60 a
	21	15.97 a	6.03 a	0.04 b	3.61 a	0.91 a	0.63 a	0.21 a	31.87 a
2.8/13.9	7	3.91 c	1.26 c	0.07 a	3.56 a	0.25 b	0.54 a	0.08 b	9.30 b
	14	9.93-b	3.67 b	0.07 a	3.73 a	0.53 a	0.61 a	0.14 ab	20.49 a
	21	13.19 a	4.61 a	0.06 a	3.67 a	0.73 a	0.56 a	0.17 a	24.19 a
Main Effects	Level								
Temperature	17.8/25.6	20.13 a	7.34 a	0.02 c	2.45 b	1.65 a	0.55 a	0.33 a	55.32 a
	7.2/18.3	16.40 b	5.89 b	0.05 b	3.61 a	1.00 b	0.69 a	0.24 ab	39.71 b
	4.4/15.6	11.59 c	4.37 c	0.06 ab	3.36 a	0.65 c	0.60 a	0.16 b	25.19 c
	2.8/13.9	9.01 d	3.18 d	0.07 a	3.65 a	0.50 d	0.57 a	0.13 b	21.49 c
Harvests	7	10.10 b	3.36 c	0.06 a	3.39 a	0.65 c	0.60 a	0.19 c	23.31 c
	14	15.90 a	5.70 b	0.05 ab	3.18 a	1.01 b	0.62 a	0.22 b	37.26 b
	21	16.85 a	6.53 a	0.04 b	3.23 a	1.20 a	0.60 a	0.25 a	43.09 a

\* Each value is the mean of three replicates, except for  $\alpha$ -carotene, where means of two replicates are reported. Means in the same column within a particular temperature or main effect element, sharing the same letter did not differ significantly according to Duncan's multiple range test ( $P=.05$ ).

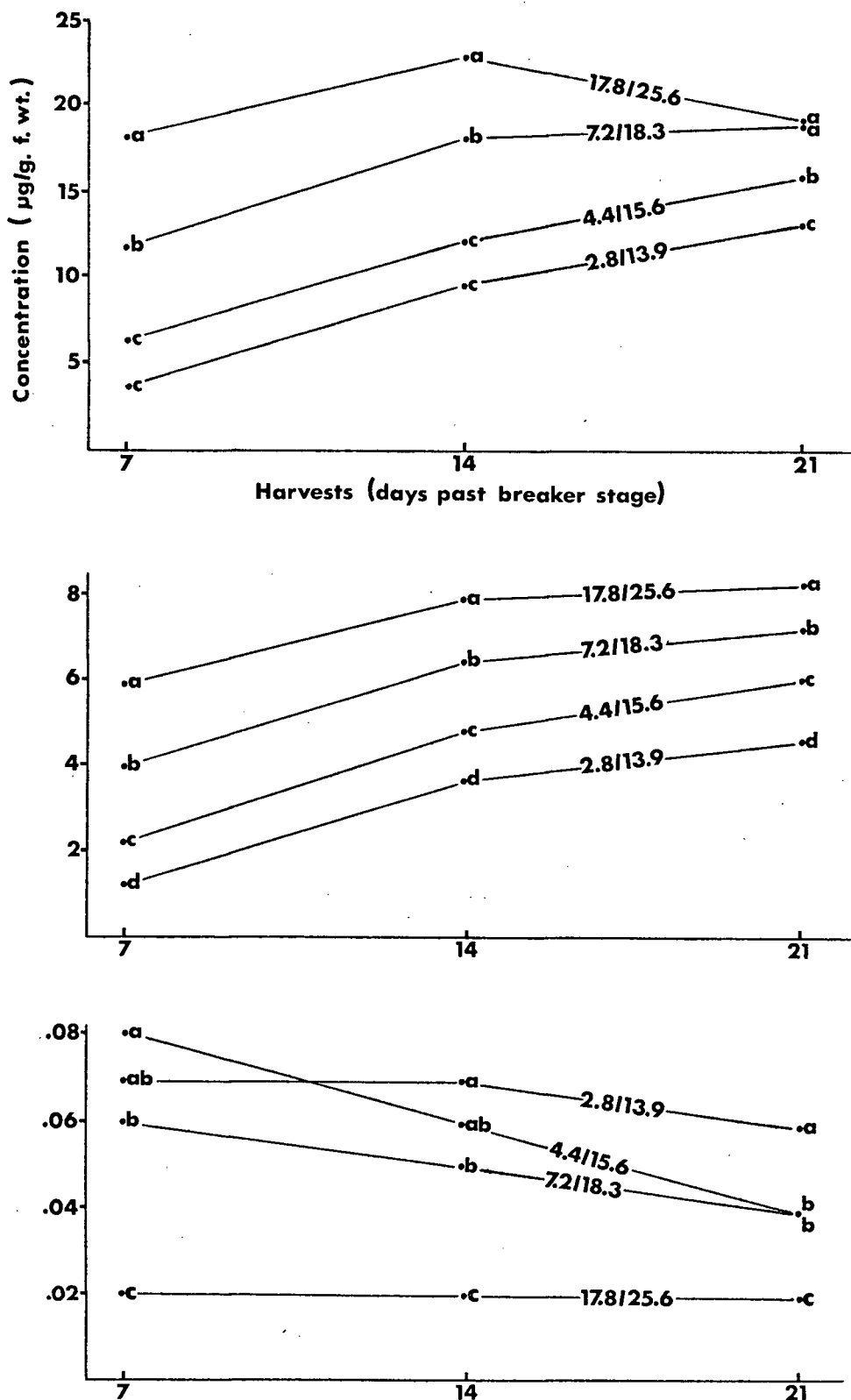


Fig. 13a. Influence of Temperature at Three Harvest Dates on Carotenoid Pigments. Top: Phytoene. Centre: Phytofluene. Bottom:  $\alpha$ -carotene.

\* Points on the same graph within a particular harvest, sharing the same letter did not differ significantly according to Duncan's multiple range test ( $P=0.05$ ).

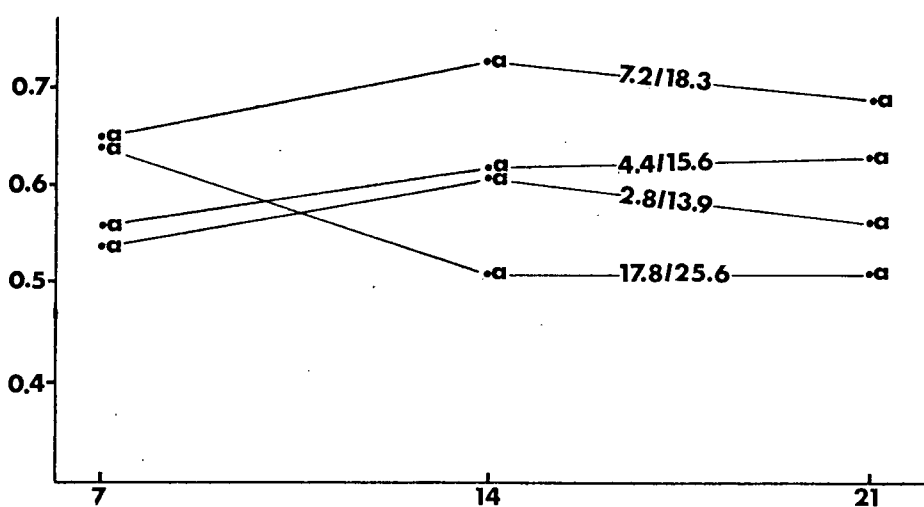
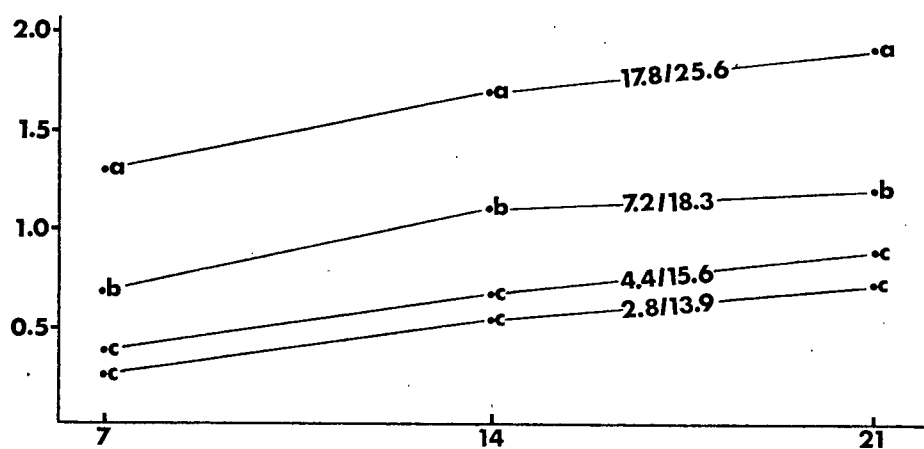
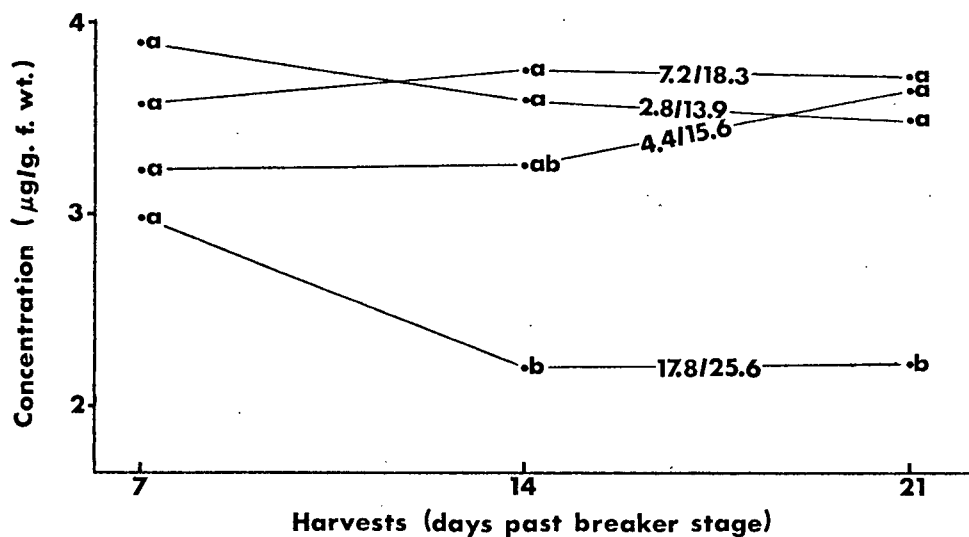


Fig. 13b. Influence of Temperature at Three Harvest Dates on Carotenoid Pigments. Top:  $\beta$ -carotene. Centre:  $\zeta$ -carotene. Bottom:  $\gamma$ -carotene.

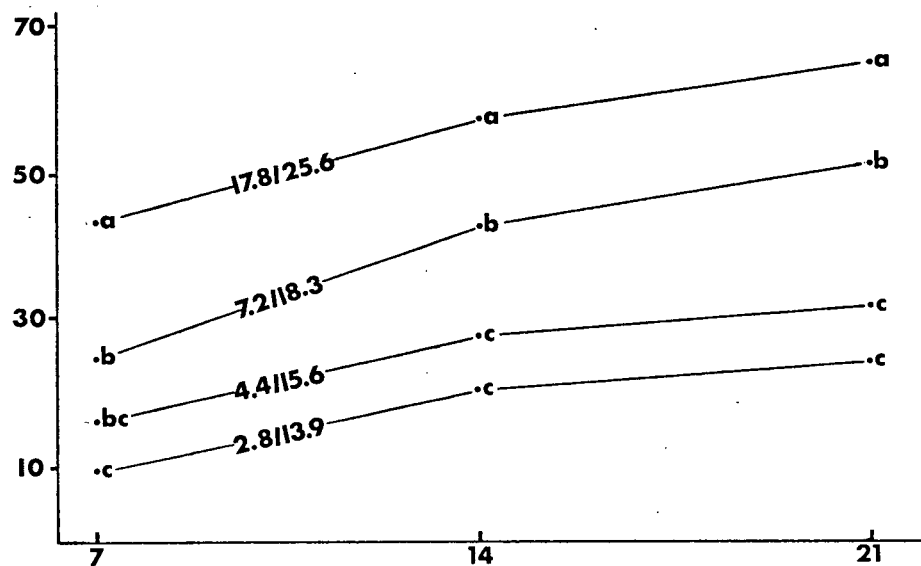
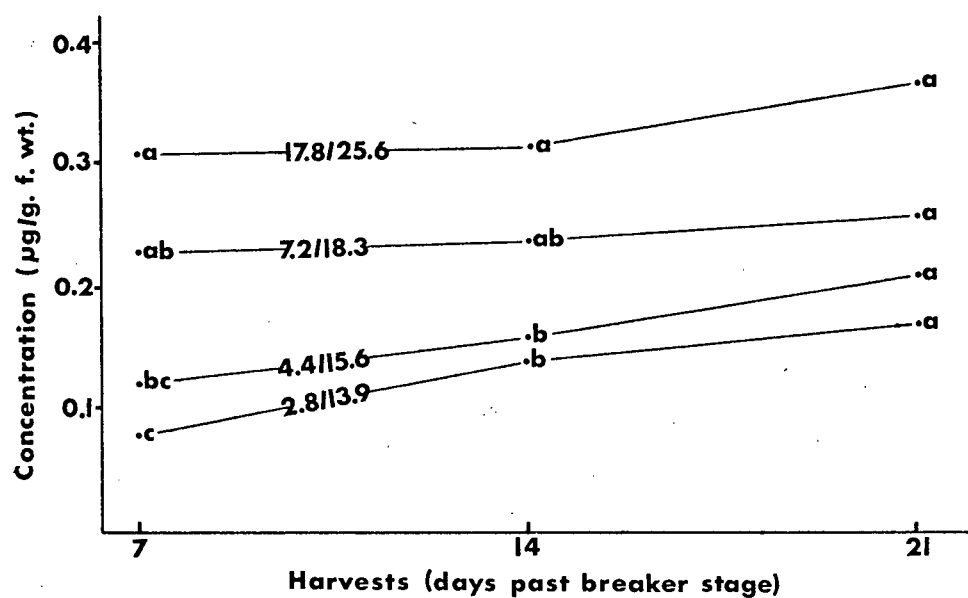


Fig. 13c. Influence of Temperature at Three Harvest Dates on Carotenoid Pigments. Top: Neurosporene. Bottom: Lycopene.

In most instances, both the temperature and harvest factors behaved in an approximately similar manner at all levels considered. Phytoene, phytofluene,  $\xi$ -carotene, neurosporene and lycopene quantities tended to be lowest at the 7-day, 2.8/13.9 treatment and highest at the 21-day, 17.8/25.6 treatment. However, a highly significant T x H phytoene interaction was demonstrated. Data for phytoene indicated a marked increase in the compound with maturity within the 2.8/13.9 and 4.4/15.6 temperature systems. At 7.2/18.3, the phytoene concentration increased significantly between 7 and 14 days but thereafter did not change appreciably. At 17.8/25.6, the concentration of this polyene was maximal at 14 days and showed a significant decrease between 14 and 21 days.

The pattern of change in  $\alpha$ -carotene quantity was not uniform at all temperatures.  $\alpha$ -Carotene concentrations were low and constant at 17.8/25.6, were significantly greater and relatively consistent at 7.2/18.3 and 2.8/13.9, but decreased rapidly with time at 4.4/15.6.

No other anomalous situations were evident with respect to pigment changes with time and temperature. In reference to phytoene, phytofluene,  $\xi$ -carotene, neurosporene and lycopene, it was noted that the lower the temperature the greater the difference in pigment concentrations between the 7-, 14-, and 21-day harvests.

#### Carotenoid Stability Study

From the first growth chamber temperature experiments on tomato pigments, it was concluded that certain irregularities in the carotenoid results, for example, the extremely erratic fluctuations in phytoene and

phytofluene concentrations, could only be attributed to some variation in the sampling and/or analytical methods. A critical review of these procedures showed that both cubed and macerated samples had been used for the analyses and further that the  $-20^{\circ}$  pre-analytical storage periods had ranged from 10 to 66 days. Thus, a study on the influence of these two variables on tomato carotenoids was initiated.

In the initial experiments, fruits of comparable maturity were prepared and analysed as previously described (see Methods and Materials), except that the fresh samples consisted of macerated material only. When the fresh, and the 10-day macerated, and the 10-day cubed fractions were compared, it became obvious that the fresh sample was only equivalent to a "0-day fresh macerated sample". In succeeding experiments, the 0-day fresh cubed sample was derived by placing a quantity of freshly cut sectors directly into the extraction solvent prior to blending.

The analysis of variance for main effects demonstrated that storage form was a major factor affecting pigment concentrations, while storage duration and the main effect interaction appeared to be of minor consequence (Table 17).

In comparing the pigment contents of cubed and macerated samples at the same storage times, it was evident that cubed samples retained much higher levels of pigments in the majority of instances. Differences in the lycopene quantities of cubed and macerated samples at the 0-, 10-, 20-, and 40-day storage periods were not statistically significant, as was the case with  $\gamma$ -carotene at the 10-day level and  $\beta$ -carotene at 0 days.

When storage duration was evaluated independently for each of

Table 17. F values and the significances of Main Effects and Interactions  
for Pigment Losses During Storage at -20°C

Treatment Effect	Variable					
	Phytoene	Phytofluene	$\beta$ -Carotene	$\xi$ -Carotene	$\gamma$ -Carotene	Lycopene
Storage Duration	3.27 n.s.	.16 n.s.	1.77 n.s.	2.54 n.s.	.46 n.s.	1.95 n.s.
Storage Form	100.67 **	151.28 **	122.24 **	269.92 **	90.11 **	.53 n.s.
D x F	.80 n.s.	.01 n.s.	2.69 n.s.	.07 n.s.	.05 n.s.	.20 n.s.

n.s. Not Significant

\*\* Significant at P = .01



Table 18. Effect of Sample Condition and Storage Duration on Carotenoid Concentrations

Sample Condition	Storage Period (days at -20°C)	Pigments (µg/g f.wt.)					
		Phyto- toene	Phyto- fluene	β-Caro- tene	ζ-Caro- tene	γ-Caro- tene	Lyco- pene
Macerated	0	16.00a *	2.22a	2.86a	0.39a	0.59a	67.05a
	10	12.49b	1.91b	2.72a	0.30ab	0.55a	68.53a
	20	9.50bc	1.78bc	2.66a	0.20b	0.55a	66.30a
	40	7.59c	1.59c	2.45a	0.21b	0.56a	63.92a
Cubed	0	26.44a	9.37a	3.38a	1.43a	0.88a	69.33a
	10	25.71a	9.28a	3.30a	1.34a	0.86a	68.03a
	20	24.76a	9.26a	3.30a	1.19a	0.82a	67.12a
	40	23.44a	8.86a	3.42a	1.25a	0.85a	65.04a

\* Each value is the mean of 3 replicates. Means within the same column and sample type sharing the same letter did not differ significantly according to Duncan's multiple range test (P = .05)

the two sample forms, no effects on phytoene, phytofluene,  $\beta$ -carotene,  $\zeta$ -carotene,  $\gamma$ -carotene or lycopene in cubed samples were apparent. For macerated samples,  $\beta$ -carotene,  $\gamma$ -carotene and lycopene contents were not significantly reduced by the length of the storage period; however, there were appreciable decreases in the levels of phytoene, phytofluene and  $\zeta$ -carotene (Table 18).

Pigment concentrations of cubed samples decreased only slightly with storage time, while those of macerated samples generally showed a greater rate of loss. With the exception of phytoene, pigment losses tended to be linear with time. Phytoene degradation in macerated samples was most rapid during the first 20 days of storage.

## DISCUSSION

### Temperature Effects on Vegetative Growth

Light and temperature conditions exerted a pronounced influence on vegetative growth and development. Verkerk (1964) noted that stem and side-shoot growth in greenhouse-grown tomato plants was more rapid and resulted in longer internodes at 23/18 d/n as compared to lower temperatures. However, this could not account for the extreme elongation of shoots observed in the present experiments in the 17.8/25.6°C growth chamber treatment. Since the 17.8/25.6 temperature regime was considered to be near optimal for vegetative growth, the thinness and extreme elongation of shoots was deemed to be largely due to the light factor. The dense upper vegetative canopy effectively reduced light penetration resulting in extensive chlorosis and abscission of lower leaves. Light meter readings of less than 0.5 to 0.7 klux were not uncommon at soil surface levels.

The fact that elongation of both existing and newly developed shoots appeared to be normal at 7.2/18.3 can be explained on the basis that the effect of vegetative growth on the light factor was eliminated by lower growing temperatures.

The 4.4/15.6 and 2.8/13.9 temperatures were sub-optimal for vegetative growth and hence the quantity of vegetative material produced under these regimes was far less than that at the two highest temperatures.

In the present study, extensive chlorosis of tomato leaves was observed at 4.4/15.6 and particularly 2.8/13.9. One of the earliest

studies of temperature effects on chlorophyll content was carried out by Nightingale (1933) who noted that the quantity of chlorophyll in tomato leaves decreased following a 10-day exposure to 13°. More recently, McWilliam and Naylor (1967) and Alberda (1969) found that chlorophyll accumulation in Zea mays L. was inhibited at low temperatures. McWilliam and Naylor (1967) pointed out that chlorosis at low temperatures may be due to an inhibition of synthesis of chlorophyll precursors. They concluded that at 16° the final stage of chlorophyll synthesis in corn was prolonged and that this coupled with exposure to high light intensities caused photodestruction of the pigment at a faster rate than its synthesis.

#### Temperature Effects on Fruiting

Porte (1952) pointed out that tomato fruits continue to increase in size during ripening and that during the four day period prior to the turning point fruits increase in size by approximately 12 per cent. The onset and rate of ripening at 17.8/25.6 in the present studies was very rapid and hence lower fruit weights were recorded. In contrast, fruit held at 7.2/18.3 and 4.4/15.6 remained in a green condition for longer periods of time and continued to increase in size. Fruits harvested at 7.2/18.3 and 4.4/15.6 were about 10 per cent larger than those at 17.8/25.6 however the differences were not significant.

The fact that differences between experimental runs were highly significant indicated that there was a strong pre-treatment

effect. The mean weights for the three replicates (harvested fruit only) were 140.4, 177.9 and 197.7 grams. The 140.4 g. mean weight was derived from plants which had been grown in the greenhouse during the winter season. At this time, greenhouse conditions were somewhat less than optimal for plant growth and fruit development. It was also found that during the November to March period, fruit sets averaged only 12 per plant as opposed to 22 per plant during the summer months.

Tomato varieties vary greatly in their ability to set fruit under low temperatures (Curme, 1962). The cultivar Early Red Chief was noted in the present experiments to set fruit at 17.8/25.6 and 7.2/18.3. Although flowering did occur at 4.4/15.6 no subsequent fruit development was observed. The 2.8/13.9 temperature was sub-minimal for flowering and fruit setting.

#### Fruit Cracking

A very high incidence of fruit cracking was noted under growth chamber growing conditions with the frequency of cracking being greatest at higher temperatures and later harvests. The higher occurrence of cracking at the 21 day harvest is consistent with the comments of Voisey and Lyall (1965) who stated that red-ripe fruits were more susceptible to cracking than immature fruit.

The differences in the frequencies of cracking between the four treatments could not be associated with differences in relative humidities between the growth chambers. At 2.8/13.9, fruit surfaces

were frequently moist with condensation unlike fruit grown under higher temperatures and yet the frequency of cracking was lowest under the former regime.

The higher occurrence of cracking at the 17.8/25.6 and 7.2/18.3 temperatures may have been due to the daily fluctuations in soil moisture levels although the plants did not wilt. Voisey and Lyall (1966) stated that a sudden uptake of water after a dry period resulted in the expansion of fruit contents which in turn caused the skin to stretch and fracture. At lower temperatures, soil moisture contents remained more uniform and a lower incidence of cracking was recorded.

#### Effect of Ripening Temperatures on Chemical Characteristics of Tomato Fruits

The supplementary graphs were considered to be somewhat more representative of the effects of temperature and harvests on the per cent dry matter, refractive index, per cent reducing sugars, pH and total acidity of tomato fruits than the statistical analyses for the reason that the graphs utilized a substantially greater amount of data. Furthermore, in a number of instances, the statistical analyses using replicates  $R_3$  and  $R_5$  failed to show significant differences where obvious and consistent differences existed when all data were examined. This was primarily due to the fact that replicate  $R_5$  contained a large proportion of extreme values. For example, titratable acidity values for  $R_5$  were considerably lower than those for any other replicate. In addition, the total acidity values decreased only slightly with harvest dates in  $R_5$  whereas other replicates indicated a sharp decline with

later harvests.

Differences between experimental runs were significant for all compositional factors, again suggesting a pre-treatment effect. This was not entirely unexpected since plants used in the growth chamber experiments were selected from material grown in the greenhouse throughout the year. Forshey and Alban (1954) have shown that the pH, titratable acidity and sugar contents of greenhouse ripened fruits vary markedly with the growing season.

The per cent dry matter tended to decrease with later harvests except at 17.8/25.6, where the solids content remained unchanged. Previously, Thompson et al. (1962) noted that the total solids content of fruit stored at 18.3° decreased from 7 to 14 days past the turning stage. Hall (1966) found that the total solids content showed little change from 6 to 12 days past the mature green stage, but subsequently declined. In contrast, Yamaguchi et al. (1960) reported increases in total solids with ripening under field conditions, however, their results may have been complicated by a pre-harvest precipitation factor.

In the present study, the per cent dry matter generally increased with lower temperatures. The per cent dry matter of 17.8/25.6 treated fruit was considerably lower than that of fruit exposed to the 7.2/18.3, 4.4/15.6 and 2.8/13.9 temperatures. Similar findings were reported by Sayre et al. (1953).

Temperatures and harvest dates had little effect on fruit refractive indices. The data on harvest effects is consistent with the findings of Hanna (1961) and Hall (1966) who reported that soluble

solids of fruits did not change significantly during ripening. In the majority of studies, refractometer measurements are expressed as per cent sucrose, and termed "soluble solids". Thompson et al. (1962) noted that soluble solids showed a significant decrease with ripening. Moghrabi (1958) observed marked increases in soluble solids with ripening but attributed the increases mainly to losses in fruit fresh weight. Wedding and Vines (1959) noted that soluble solids increased with lower ripening temperatures whereas Craft and Heinze (1954) showed that storage temperatures had no effect on soluble solids.

The soluble and total solids contents of tomato fruits are of importance in determining the yield of concentrated tomato products, (MacGillivray and Clemente, 1956; Yamaguchi et al., 1960; and Lower and Thompson, 1966). Lower and Thompson (1966), have stated that an increase of 0.2 per cent in soluble solids of raw fruit is regarded to be of economic significance in tomato processing. Underwood (1950) has stressed the importance of solids contents in influencing the consistency of tomato juice. From these comments, it is therefore, evident that fruits ripened at 17.8/25.6 were the least suitable for the manufacture of tomato products.

The per cent reducing sugars were highest at the three lower temperatures. Learner and Wittwer (1952) pointed out that lower night temperatures reduce respiration rates and therefore favour carbohydrate accumulation.

Sugar contents showed only a slight tendency to decrease with later harvests. Earlier Craft and Heinze (1954) reported that, in most



instances, sugars tended to increase from 4 to 9 days past the mature green stage and then decreased from 9 to 14 days. Other studies (Yamaguchi et al., 1960; Winsor et al., 1962) have shown a continued increase in sugar contents with maturation.

Since higher sugar contents are associated with good flavour quality, fruit harvested at the three lowest temperatures would be considered somewhat superior to 17.8/25.6 treated fruit.

The pH of tomato fruits was found to increase with later harvests as demonstrated earlier by Yamaguchi et al. (1960) and Hanna (1961). The pH of fruits was also observed to be temperature dependent with the highest values occurring at 17.8/25.6 and the lowest at 4.4/15.6 and 2.8/13.9. No pH differences existed between fruit exposed to 4.4/15.6 and 2.8/13.9. The pH of fruit held at 17.8/25.6 tended to increase more rapidly with later harvests than those subjected to lower temperatures.

These findings are contrary to those reported by Craft and Heinze (1954). They exposed mature green tomatoes to temperatures of 0, 4.4, 8.9, 18.3, and 23.9 for 4, 9, and 14 days and concluded that temperature and storage duration had no effect on fruit pH. Similarly, Lingle et al. (1965) noted that night temperatures ranging from 5 to 30° had little or no effect on the pH of tomato fruits. The titratable acidity was influenced by both temperature and harvest dates. The total acidity declined with harvests in the study carried out by Hall (1966). Winsor et al. (1962b) was unable to establish a consistent trend in acidity changes of fruit walls following the stage of initial

coloration, however, Winsor et al. (1962a) did find that the total acidity of juices expressed from whole fruits declined with ripening.

The highest titratable acidity values were found at 2.8/13.9 and 4.4/15.6. At higher temperatures, lower total acidity values were evident. Haber (1931), Wedding and Vines (1959) and Hall (1968) also noted that lower temperatures resulted in higher total acidities. The fact that total acidities were highest and almost identical at 2.8/13.9 and 4.4/15.6 suggests a close approach to the minimum temperature required for the metabolism of organic acids.

From the data it was readily apparent that pH increased uniformly as titratable acidity decreased.

Much attention has been devoted to the study of pH and total acidity in tomatoes because of the importance of acidity in determining the quality of raw tomatoes and processed products. Lambeth et al. (1966), Gould (1957), and Leonard et al. (1959) have pointed out the requirement of high acidity in facilitating a reduction of processing times and temperatures thereby permitting improved colour, flavour, texture, and ascorbic acid retentions. Lower and Thompson (1967) indicated that pH's above 4.5 and total acidities below .35 per cent were not conducive to the maintenance of high processing quality.

In the present study, pH values exceeding 4.5 and titratable acidity values of less than .35 were encountered only at 17.8/25.6 at 21 days and occasionally at 14 days. This indicated that fruits harvested at 17.8/25.6 at 7 days and at lower temperatures irrespective of harvests could be processed with a minimal loss in quality.

Total pectic substances showed only a slight tendency to decrease with higher temperatures and later harvests. Previously, Moghrabi (1958) found that there was very little difference between the total pectic contents of fruit held at 15 and 20°. Kertesz and McColloch (1950) and Woodmansee et al. (1959) were unable to demonstrate any consistent quantitative changes in pectic constituents with ripening while Dalal et al. (1965, 1966) observed a minor decrease in total pectins between the large green and red ripe stages of maturity. Foda (1957) and Moghrabi (1958) noted that total pectic substances decreased considerably during ripening, but this resulted primarily from changes occurring during the first 3 to 6 days following the turning stage. In the present study, fruits were harvested at 7, 14, and 21 days past the turning point and thus, with the possible exception of the 2.8/13.9 treatments, a major decrease in total pectic substances with harvests was not anticipated.

The important role of pectic substances in influencing the textural properties of tomato fruits is well recognized. Forshey and Alban (1954) made use of total pectic content as a chemical index of fruit textural quality. Foda (1957) noted that protopectin contents and instrumental measurements of firmness of tomato inner pericarp tissues were correlated to the extent of .93 to .99, however, this was not necessarily indicative of the firmness of whole fruits. Deshpande et al. (1965) and Sayed et al. (1966) observed highly significant correlations between firmness measurements of canned and whole tomatoes respectively, and total pectic substances, but in no case did the

correlation coefficients exceed .71. In the present study, treatment effects on total pectic substances were non-significant, and yet, based on subjective assessments, differences in fruit firmness were considered to exist. Since a chemical analysis does not account for such factors as pericarp thickness, carpel size, number and arrangements, it is doubtful that measurements based on an analysis of fruit constituents alone would serve as an adequate objective measure of tomato firmness.

#### Effect of Temperature on Surface Colour

The Hunterlab Color and Color Difference Meter equipped with a D25L circumferential lighting unit but used for small area evaluation provided a satisfactory means for the assessment of the surface colour of tomato fruits. In spite of a reduction in instrument sensitivity due to the decrease in the viewing area, differences in surface redness, yellowness and lightness were readily detected. Although the CDM-D25L is designed for the measurement of flat surfaces and utilizes a four inch diameter specimen area (Hunter Associates Laboratory, 1966) the fact that 1 1/16" curved surface readings of a series of yellow to orange to red specimens were highly correlated with four inch diameter flat surface colour readings indicate that the modified instrument can be used to measure adequately the surface colour of tomato fruits.

External redness was clearly dependent on the ripening temperature and the number of days from the turning point to harvest. The highest  $a_L$  values were attained at the 17.8/25.6 temperature regime in the 7 day harvest, at 7.2/18.3 in the 14 day harvest, at 4.4/15.6 and 2.8/13.9 in the 21 day harvest indicating that the rate of red color-

ation of fruit surface accelerates with increasing temperatures. These findings are consistent with those of Ayres and Peirce (1960) who observed that fruits ripened at  $10^{\circ}$  required greater than 30 days to attain a uniform red state while fruits ripened at  $20^{\circ}$  coloured in 7 to 10 days.

Hall (1968) noted that coloration at  $20^{\circ}$  assumed a curvilinear relationship with respect to time, being most rapid between 3 and 5 days following the turning stage. With the apparent exception of the 17.8/25.6 regime, a similar relationship was observed when  $a_L$  values were related to the 7, 14, and 21 day harvest. Fruit held at 17.8/25.6 for 7 days after the turning point had already advanced through the main phase (breaker stage to uniform red) in the coloration sequence and hence showed little subsequent change in  $a_L$  readings, whereas fruit stored at 2.8/13.9, 4.4/15.6 and to a lesser degree, 7.2/18.3 showed a continued increase in redness from 7 to 21 days. The effect of the lower temperatures was to extend the period of maximum colour change beyond the 7 day harvest.

Temperatures during ripening not only influenced the rate but also the extent to which  $L$ ,  $a_L$ , and  $b_L$  changes took place. The decreases in sample redness with lower thermal regimes were directly attributable to the inhibitory effect of temperatures on carotenoid synthesis. The highest  $a_L/b_L$  values, indicative of good colour development, occurred at 17.8/25.6 and 7.2/18.3. The slightly less intense coloration of 21 day - 4.4/15.6 fruit was reflected by lower  $a_L/b_L$  readings. The maximum value of the hue component of 2.8/13.9 treated fruit was considerably lower than those of 21 day fruit at higher temperatures. The best colour development at 2.8/13.9 was a bright orange. These findings are generally in agreement with those of Sayre et al. (1953) who noted that the best

coloration occurred at 23.9/15.6 d/n, while fruits ripened at 15.6/7.2 d/n were fairly red but not of sufficient intensity to rate U.S. No. 1 in colour. Similarly Ayres and Peirce (1960) noted that the fruits stored at 10° tended to be more pink than red in hue. The results of the present experiments are not in full agreement with the constant temperature study carried out by Wright et al. (1931). They reported that the lowest temperature to facilitate good colour development was 12.8°.

The present study made use of diurnal temperature systems and hence both the minimum and maximum temperatures as well as their duration became factors of importance. The results suggest that MacGillivray's (1934) conclusion (confirmed by Sayre et al. 1953, and Yamaguchi et al. 1960) that lower night temperatures permitted satisfactory coloration when day temperatures exceeded the maximum for lycopene development can be expanded to include the situation where night temperatures fall below that necessary for lycopene synthesis while day temperatures remain within the range required for adequate pigmentation.

Temperature exerted a pronounced effect on surface yellowness values at all harvest dates. Hunter  $b_L$  readings were greatest under low temperatures and showed a decline with ripening time. The increase in  $b_L$  values with lower temperatures was indicative of the progressive delay in the ripening process.

The differences in  $b_L$  values for the various treatments were attributable to changes in carotenoid constituents. The distinctly yellow appearance of fruit at low temperatures and earlier harvest dates was due to a preponderance of  $\beta$ -carotene. McCollum (1955) noted that a  $\beta$ -carotene content of 2 to 10 per cent of the total carotenoids was more than suffi-

cient to alter the chromaticity of tomato fruits. At 2.8/13.9 - 7 days,  $\beta$ -carotene constituted approximately 25 per cent of the total carotenoids.

The decrease in surface yellowness with ripening might be expected to be paralleled by substantial losses in  $\beta$ -carotene, however, when carotenoid analyses were carried out this did not prove to be the case.  $\beta$ -carotene concentrations at 7.2/18.3, 4.4/15.6 and 2.8/13.9 were not only comparable but showed little consistent evidence of decreasing with later harvests. The reports of Goodwin and Jamikorn (1952), Thompson et al. (1965), Dalal et al. (1966), and Meridith and Purcell (1966) demonstrate that  $\beta$ -carotene quantities usually increase from the mature green or breaker stages up to the pink or light red stages of maturity.

It is thus obvious, that as chlorophyll decomposition in mature green fruits occurs, the colouring effect of  $\beta$ -carotene becomes important.  $\beta$ -carotene continues to represent the main pigment contributing to the yellowness of the fruit, but with further ripening, lycopene concentrations become a critical factor. As lycopene quantities increase expression of  $\beta$ -carotene decreases. Hence the redness rather than yellowness component becomes the dominant factor governing the appearance of fruits.

Surface lightness changes closely paralleled those of  $b_{ext}$  under all treatment conditions. The highest lightness values were found at the lowest temperatures and the earliest harvests. The visually apparent darkening of fruits with ripening was confirmed by lower  $L_{ext}$  readings.

The advantages in using a tristimulus colorimeter for the determination of pigment concentrations are numerous. Such instruments have previously been reported for the specification of carotenoid concentrations in sweet potatoes and squash and also the pigment (predominantly anthocyanins)

contents in cranberry products (Francis, 1969).

Surface redness might be expected to constitute a measure of the concentration of red pigments in tomato fruits, however, the analytical data indicated that this was not entirely the case. At low temperatures, increases in surface  $a_L$  values coincided with increases in lycopene concentrations, but at the two highest temperatures increases in lycopene quantities were not associated with a consistent change in  $a_L$  values. Since the  $a_{ext}$  readings did not reflect the lycopene contents of red-ripe fruits, measurement of surface redness as a basis of prediction of carotenoid concentrations would be of questionable value. Thompson et al. (1965) noted that Hunter values of raw pericarp, locule and whole fruit juices confirmed their carotenoid data in most respects, but failed to distinguish quantitative carotenoid differences in 14 day - 18.3° ripened crimson from either high pigment or standard red tomatoes.

#### Internal Colour and the Relationship to Surface Colour

The effects of temperature and harvest dates on internal colour were similar to those on surface colour values. In both instances,  $L$  and  $b_L$  values increased with lower temperatures and decreased with later harvests. Slight differences between surface and internal  $L$ ,  $a_L$  and  $b_L$  measurements were evident where conditions permitted over-ripening. Thus at 17.8/25.6 internal  $a_L$  values remained at a maximal level for all harvest dates while external  $a_L$  values declined somewhat from 7 to 21 days. Similarly,  $a_L$  internal values remained constant and maximal at 7.2/18.3 from 14 to 21 days while surface redness declined.



Although internal and surface colour could not be compared in terms of absolute values because of the differences in measurement techniques, it was noted that fruit stored at 2.8/13.9 for 7 days had an internal redness of only 33.7 per cent of that of 17.8/25.6, 7-day fruit while surface redness was 59.7 per cent of that of 17.8/25.6, 7-day fruit. At 2.8/13.9, 14-days, internal and surface  $a_L$  readings were equivalent to 59.5 and 85.5 per cent of the corresponding 17.8/25.6, 14-day fruit. Similarly, internal and surface yellowness values of fruits at 2.8/13.9, 7 days were 1.9 and 1.5 times greater than  $b_L$  values at 17.8/25.6, 7 days. This indicated that at lower temperatures internal colour development lagged behind that of fruit surfaces. Ayres and Peirce (1960) also noted that colour within the fruit did not keep pace with surface colour under low temperature conditions.

Correlation studies showed that surface redness, yellowness and lightness were best associated with the internal variables at lower temperatures. The lower correlations at 7.2/18.3 and the non-significant relationships at 17.8/25.6 indicate that independent  $L$ ,  $a_L$  and  $b_L$  surface values are of negligible value in estimating the internal colour at higher temperatures. When internal and external  $Lb/a$  ratios were compared, a very high overall correlation coefficient was obtained suggesting that surface colour expressed as the  $Lb/a$  ratio could be used to accurately specify internal colour. The highest  $Lb/a$  correlation coefficients obtained for the four temperature treatments were found at 2.8/13.9 and 4.4/15.6. Although the  $Lb/a$  correlation coefficient at 17.8/25.6 was highly significant it was evident that surface colour could not be used

to predict accurately the internal colour at this temperature. Desrosier (1954) has stated that surface colour is as good a measure of internal colour as cross-sectional colour but can occasionally be misleading.

When  $Lb/a$  ratios were correlated for each of the temperature and harvest treatments, the coefficients were found to be highly significant with one exception. The highest correlation coefficients occurred at lower temperatures and earlier harvests demonstrating that both the ripening temperature and harvest date influenced the relationship between surface and internal colour of tomatoes. In no case did the correlation coefficients exceed .80.

Part of the difficulty in correlating surface and internal colour may have been due to the fact that raw juice samples were not deaerated prior to colour evaluation. Previously, Yeatman and Sidwell (1958) reported that although deaeration improved the colour of tomato juices "the improvement was so constant and predictable that it was found logical to discontinue deaeration". When aerated and deaerated samples were tested this was found not to be entirely the case. The effect of aeration was greatest but least predictable on sample L values ( $r = .51$ ). In contrast, the correlation coefficients for the  $a_L$  and  $b_L$  values were .96 and .77.

#### Association between Tristimulus Colour Coordinates of Tomato Fruits.

Comparatively few studies have been done on possible relationships between the colour coordinates of tomato fruits. Asselbergs et al. (1961) determined the colour range of a series of raw juices derived from

tomato fruits of various ripeness levels using a spectrophotometer equipped with a digital tristimulus integrator. They observed a very high linear correlation ( $r = .90$ ) between luminous reflectance  $Y$  and  $x$ -chromaticity measurements. They considered the high correlation between "lightness" and "redness" to be an important characteristic of the colour range of raw tomato juice. In the present study, internal lightness was also noted to decrease with increasing redness, but a correlation coefficient of only .77 was observed between the Hunter  $L$  and  $a_L$  values. Sample aeration may have represented a factor contributing to this lower degree of association. The difference in the correlation coefficients may also have been due to the fact that the range of juice colours used by Asselbergs et al. included only light, medium and dark red samples, whereas a substantially wider range of colours was encountered in the present growth chamber experiments.

When surface  $L$  and  $a_L$  values were examined, the relationship was found to be non-linear, particularly at higher temperatures and later harvests.

Yeatman et al. (1960) and Yeatman (1969) studied the distribution of raw tomato juice colours in terms of Hunter  $L$ ,  $a_L$  and  $b_L$  dimensions. The data presented by Yeatman et al. (1960) indicate the existence of a relationship between sample lightness and yellowness values. In the present study, a good linear relationship between juice lightness and yellowness was demonstrated, except where conditions resulted in poor colour development. Surface  $L$  and  $b_L$  values correlated to the extent of .97. Further, a high degree of association between surface lightness and yellowness persisted over the extreme range of treatments.

These findings indicated that the surface colour, and to a lesser extent, the internal colour of tomato fruits could be very adequately specified through the use of two ( $a_L$ , and  $b_L$  or  $L$ ), rather than three colour scales. The high degree of association between  $L$  and  $b_L$  values would also permit the simplification of three variable colour indices.

The  $a_L$ ,  $b_L$  chromaticity diagram given by Ayres and Peirce (1960) for a range of immature to over-ripe tomatoes illustrates that, with ripening, the decrease in  $b_L$  values for outer wall and skin portions, and for section walls was accompanied by a marked increase in sample redness values. The present studies showed similar relationships between redness and yellowness values up to about the red stage of ripeness. The results of Ayres and Peirce demonstrated that, with over-ripening, redness values continued to increase with no change or an increase in  $b_L$  values. In the present growth chamber experiments, over-ripening was found to be accompanied by a continued decrease in internal  $b_L$  and an increase in internal  $a_L$  values. Further, surface  $b_L$  and  $a_L$  values declined with ripening beyond the red stage, giving rise to a parabolic relationship between surface redness and yellowness.

#### Effect of Temperatures and Harvests on Fruit Carotenoids

Lycopene and  $\beta$ -carotene represented the dominant carotene pigments in the tomato fruits at all temperatures and harvests. Lycopene generally accounted for greater than 80 per cent of the total carotene content. The increase in the proportion of lycopene to total carotenes with increasing temperatures and later harvests was largely a reflection

of treatment effects on lycopene concentrations in the absence of corresponding changes in  $\beta$ -carotene contents. Thus, at 17.8/25.6 at 21 days, lycopene constituted 92.8 per cent of the total carotene fraction, as opposed to 67.4 per cent at 2.8/13.9. Data presented by Tomes (1963) and Meredith and Purcell (1966) show that lycopene constitutes in excess of 90 per cent of the total carotenes of ripe red varieties.

$\alpha$ -Carotene,  $\gamma$ -carotene, neurosporene and  $\zeta$ -carotene were present only in small amounts. Previously, Porter and Lincoln (1950) reported that  $\alpha$ -carotene is present in commercial varieties usually in trace quantity only, while  $\gamma$ -carotene is found in red tomato varieties in concentrations rarely exceeding 5  $\mu\text{g/g}$ .

Substantial quantities of phytoene and phytofluene were encountered in the majority of treatments, particularly where conditions were conducive to adequate ripening. Comparable concentrations for ripe fruits of a number of other red varieties have been reported by Tomes (1963) and Thompson et al. (1965).

Temperatures and/or harvest dates influenced the concentrations of all pigments isolated with the exception of  $\gamma$ -carotene. Phytofluene,  $\zeta$ -carotene, neurosporene and lycopene increased while  $\alpha$ -carotene concentrations declined with harvests. Phytoene concentrations increased with later harvests except at 17.8/25.6 from 14 to 21 days, where a significant decrease was observed. The findings concur with those of Meredith and Purcell (1966) except that they observed continued increases in  $\gamma$ -carotene and phytoene during ripening. Since many of the fruits harvested at 17.8/25.6 at 21 days showed evidence of

deterioration, it seems probable that the lower phytoene concentrations resulted from oxidative decomposition.

The  $\beta$ -carotene contents of fruit at 17.8/25.6 and 7.2/18.3 tended to decrease with harvests, but a similar trend was not evident at lower temperatures. Dalal et al. (1965) and Meredith and Purcell (1966) found that  $\beta$ -carotene increased up to the light red or pink stage of maturity and thereafter decreased.

Phytoene, phytofluene,  $\xi$ -carotene, neurosporene and lycopene concentrations were highest at 17.8/25.6 and decreased progressively with lower temperatures. The low concentrations of carotenoid pigments at 2.8/13.9 indicated a nearness to the minimal temperatures for carotenoid synthesis.

Earlier workers (Rosa, 1926, Wright et al., 1931), using constant temperature systems established lower limits of between 4.4 and 8° for lycopene development. Went et al. (1942) found that fruit stored at 2° showed no evidence of lycopene synthesis. From the present study, it was readily apparent that, in spite of very low night temperatures, the higher day temperatures did permit carotenoid synthesis to a limited extent.

$\alpha$ -Carotene contents were inversely related to temperature in contrast to the results presented by Goodwin and Jamikorn (1952). They found the  $\alpha$ -carotene concentrations, not only increased with ripening, but were somewhat higher at 15° as compared to 0°. From their temperature maturity study, Goodwin and Jamikorn (1952) concluded that the Porter-Lincoln pathway was only of minor importance in the synthesis of  $\alpha$ -carotene

and  $\beta$ -carotene. In the present study, it was found that  $\alpha$ -carotene,  $\beta$ -carotene, and  $\gamma$ -carotene did not respond to temperatures and harvests in accordance to the treatment effects on the other carotenoids. Thus, the results not only seem to support the contention of a different pathway for  $\alpha$ -carotene and  $\beta$ -carotene, but also tend to suggest the possibility that  $\gamma$ -carotene production may be independent of the Porter-Lincoln pathway.

#### Relationship of Carotenoids to Tomato Colour

Since lycopene and  $\beta$ -carotene generally accounted for 95 per cent or more of the total carotene fraction, the other carotenoid pigments were of little consequence in influencing fruit colour.

At 2.8/13.9 and 4.4/15.6, particularly at the 7-day harvests,  $\beta$ -carotene represented a substantial portion of the total carotenes. For this reason, and owing to its high extinction value,  $\beta$ -carotene would therefore have contributed greatly to the fruit colour. Hence, the high proportion of  $\beta$ -carotene to lycopene was associated with fruit of a yellow to orange hue.

At higher temperatures,  $\beta$ -carotene concentrations tended to decline with delayed harvest, while lycopene quantity increased, and thus the significance of  $\beta$ -carotene in influencing fruit colour decreased. There was a marked increase in colour intensity and a great change in hue as lycopene concentrations increased up to about 32  $\mu\text{g/g}$  fresh weight. Between 32 and 43  $\mu\text{g/g}$  colour intensity increased to a lesser extent and fruit changed from an orange to red hue. When lycopene concentrations exceeded about 40  $\mu\text{g/g}$ , Hunter internal and external values and ratios showed little appreciable change.

Fruit harvested at 7.2/18.3 - 14 days and 17.8/25.6 - 7 days were judged to have a satisfactory full red coloration. At these treatments the mean total carotene contents were 49  $\mu\text{g/g}$ . Compensating for column losses, then actual concentrations of about 56  $\mu\text{g/g}$  would have existed. It is thus apparent, that satisfactory red coloration requires a total carotene concentration in excess of 55  $\mu\text{g/g}$  of which lycopene accounts for approximately 90 per cent of the carotenes.

#### Effects of Sample Condition and Storage Duration on Carotenoid Pigments

Little research has been directed towards the study of the carotenoid pigments of tomato tissues as affected by aeration, and/or low temperature factors. Yeatman(1959) reported that the colour of whole red tomato fruits remained stable at  $-10^{\circ}$  for  $1\frac{1}{2}$  years; however, colour is not a good criterion of the carotenoid status of fruits, especially where carotene contents exceed 55  $\mu\text{g/g}$ .

In the present study, storage form was found to be the main factor affecting carotenoid concentrations. Cubed samples retained much higher levels of phytoene, phytofluene,  $\beta$ -carotene,  $\xi$ -carotene and  $\gamma$ -carotene than macerated tissues. Lycopene content was not affected by sample maceration. Chichester and Nakayama (1965) commented that lycopene in tomato fruits is relatively stable even under the most adverse conditions.

The greatest loss of carotenoids in macerated tissues occurred before storage at  $-20^{\circ}$ . The high loss of carotenoids excepting lycopene at 0 days storage was largely attributable to the inclusion of oxygen during the  $2\frac{1}{2}$  - 3 minute blending phase prior to solvent extraction.



The pigments in order of sensitivity to decomposition were phytofluene,  $\zeta$ -carotene, phytoene,  $\gamma$ -carotene,  $\beta$ -carotene and lycopene. Thus, the relatively common procedure of blending tomato fruits for pigment analysis prior to extraction with solvents cannot be recommended.

The carotenoid concentrations of cubed samples showed only a slight tendency to decrease with storage at  $-20^{\circ}$  demonstrating that pigment contents of intact tomato tissues were reasonably stable. Phytoene, phytofluene and  $\zeta$ -carotene losses were considerable in stored macerated tissues but this was most likely due to oxidative losses before the temperatures of the tissues reached  $-20^{\circ}$ . It is therefore evident that carotenoid analysis should be preferably carried out on fresh material or samples stored for a minimal time period. Where samples cannot be analysed immediately, whole fruit or fruit sections rather than blended samples should be stored.

## SUMMARY AND CONCLUSIONS

Controlled environment experiments were conducted to study the effects of air temperatures at harvest dates of 7, 14, and 21 days past the breaker stage on tomato fruit quality. Emphasis was placed on the assessment of temperature effects, notably low temperatures, on fruit colour, the pigments responsible for the colour characteristics, and the relationships of colour parameters to each other during the ripening process. In addition, controlled temperature effects on vegetative and reproductive growth and fruit characteristics were examined. An investigation was also undertaken to determine the effects of sample maceration and storage duration at  $-20^{\circ}$  on carotenoid concentrations of tomato tissues. From the results of these experiments the following conclusions can be drawn:

1. Decreasing temperatures suppressed vegetative growth. The  $4.4/15.6^{\circ}\text{C}$  night/day thermal regime was definitely sub-optimal for vegetative growth while the  $2.8/13.9$  temperature system was sub-minimal for vegetative growth and development. Prolonged exposure to low temperatures caused extensive and severe chlorosis of leaves.
2. The minimal temperature for flowering is lower than that required for fruit set. Flowering continued at  $4.4/15.6$  but was prevented at  $2.8/13.9$ . The critical minimum temperatures for fruit set in the cultivar Early Red Chief lies between  $7.2/18.3$  and  $4.4/15.6$ . The substantially higher frequency of fruit cracking at  $17.8/25.6$  was probably due to an indirect effect of temperature on soil moisture levels.
3. The total solids contents of fruits were lowest at  $17.8/25.6$  and showed

only a slight tendency to decrease with delayed harvest. Treatment effects on fruit refractive indices were largely negative demonstrating that ripening temperatures had little effect on a net change in total soluble constituents.

4. The per cent reducing sugars generally showed a slight decline with delayed harvest. Sugar contents were lowest at 17.8/25.6 presumably because of respiratory losses.
5. The acidity of fruits was dependent on both temperature and harvest dates. Titratable acidity declined while pH values increased with later harvests and higher temperatures. The higher titratable acidity and lower pH values with decreasing temperatures indicated that organic acid losses through respiration were progressively retarded by lower temperatures.
6. There were only very minor differences in the total pectic substances of fruits between the various treatment levels. Since appreciable firmness differences existed, it may be concluded that total pectic substances do not serve as a suitable index of the textural properties of whole fruit.
7. Temperatures and times of harvest had a pronounced influence on surface and internal fruit colour. Surface and internal lightness and yellowness decreased while redness values increased with higher temperatures and later harvests. Temperatures influenced the rate and extent to which colour changes took place. Fruit showed a full red colour development at 17.8/25.6 in 7 days, and at 7.2/18.3 in 14 days or less. The near full red coloration of fruit harvested at 4.4/15.6

at 21 days was reflected in somewhat higher Lb/a ratios than those obtained at 17.8/25.6 and 7.2/18.3 at 21 days. Fruit exposed to 2.8/13.9 ranged from yellow to bright orange depending on the harvest date.

8. Changes in fruit colour were due almost exclusively to temperature and harvest effects on lycopene synthesis. At low temperatures and particularly at the 7-day harvests,  $\beta$ -carotene constituted a substantial portion of the total carotenes and exerted a pronounced influence on the colour of fruits. The importance of  $\beta$ -carotene decreased progressively with increasing temperatures and later harvests. A total carotene content of approximately 55  $\mu\text{g/g}$  fresh weight was required for satisfactory red coloration. Total carotene contents in excess of 55  $\mu\text{g/g}$  had little effect on colour.
9. Temperatures and harvests influenced the relationships between surface and internal colour measurements. Surface L,  $a_L$  and  $b_L$  readings and Lb/a ratios were best related at low temperatures and earlier harvests. In spite of the very high overall correlation between surface and internal Lb/a ratios, the fact that the correlation coefficients were considerably lower where temperatures facilitated satisfactory colour development indicates that surface colour cannot be conclusively relied upon in the colour grading of tomatoes at the processing level. Surface colour cannot be used to specify accurately the internal colour of fully ripened tomatoes.
10. The consistently high correlation coefficients between surface lightness and yellowness values demonstrated that one measurement could be used to

determine both lightness and yellowness. Thus, three parameter surface colour indices may be specified by the use of two measurements and a constant, the constant accounting for the relationship between  $L$  and  $b_L$  values.

11. Temperatures exerted a marked influence on phytoene, phytofluene,  $\zeta$ -carotene, neurosporene and lycopene contents but had little or no effect on  $\beta$ -carotene and  $\gamma$ -carotene concentrations.  $\alpha$ -Carotene concentrations were highest at the earliest harvest at the lowest temperature. The decrease in phytoene with over-ripening at 17.8/25.6 between 14 and 21 days may have been due to oxidative decomposition.  
The fact that  $\alpha$ -carotene,  $\beta$ -carotene and  $\gamma$ -carotene concentrations did not respond to temperature and harvest effects in the same manner as did the other pigments seems to support the contention that more than one pathway is involved in the biosynthesis of carotenoids.
12. In spite of higher dry matter, sugar contents, total acidity; lower pH values; and slightly higher pectic contents and refractive indices, fruit ripened at 4.4/15.6 and 2.8/13.9 were of inferior quality because of reduced colour development. Fruits ripened at 7.2/18.3 and 17.8/25.6 were of high colour quality. However, fruits ripened at 7.2/18.3 were of superior total quality for reasons of higher acid, sugar and dry matter contents. The treatment providing the best quality as indicated by objective measurements was 7.2/18.3 at 14 days.
13. Carotenoids of intact tissues were relatively stable when stored from 10 to 40 days at  $-20^{\circ}\text{C}$ . Macerated tissues stored at  $-20$  contained substantially lower concentrations of all carotenoids except for lycopene.

The major portion of the losses found in macerated tissue occurred during the blending procedure rather than during low temperature storage periods. For this reason, maceration of tomato fruits at room temperature prior to extraction cannot be recommended when analysing tissues for carotenoid constituents.

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