Changes in the Concentration of Carotenes of Ripening Homestead Tomatoes[1]

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Abstract. The concentration of 7 carotenoids were determined at 6 maturity stages in Homestead tomatoes as related to the U.S.D.A. Classification Standards of Tomato Maturity. The concentration of 5 of the pigments increased through all the stages of maturity. Two of the pigment concentrations increased through several stages of maturity and then decreased.

INTRODUCTION

In studies involving the color characteristics of tomatoes, it is desirable to know the course of development of the various carotenoids. Considerable work has been reported concerning the development of carotenes in tomatoes, but no work has been reported correlating analysis of the various carotenes with U.S.D.A. Classification Standards of Tomatoes (9). Porter and Lincoln (6) reported the carotenes and the genetic control of carotene synthesis of several tomato varieties. Rabourn and Quackenbush (8) studied the carotenes of immature and mature tomatoes and found that phytoene, phytofluene and lycopene are not present in green tomatoes. Zscheile (10) has determined the absorption spectra of tomato puree and has made spectroscopic studies of carotene extracts. The concentration of the various pigments was not related to the outside color. Goodwin and Jamikorn (2) followed in tomatoes the concentration of phytofluene, alpha-carotene, beta-carotene, zeta-carotene, neurosporene and lycopene as a function of time and temperature. The rate of ripening and color formation is influenced by so many internal and external factors that time and temperature alone cannot be used to predict the pigment composition of tomatoes.

Both in the processing industry and in research with tomatoes it is desirable to estimate the internal pigment composition on the basis of external factors. An attempt has been made to follow the development of the various pigments as a function of the U.S.D.A. Color Classification Standards of Tomatoes (9). A study was therefore initiated using the Homestead variety. Analyses were made for phytoene, phytofluene, alpha-carotene, beta-carotene, zeta-carotene, gamma-carotene, and lycopene at 6 stages of maturity in the ripening tomatoes.

MATERIALS AND METHODS

Three 80-lb lots of green Homestead tomatoes were purchased from a local produce house, with 2 lots being of the spring crop

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and 1 lot of the fall crop. The 3 lots were ripened at 22°C under a cover of Kraft paper which allowed ripening under subdued light. According to McCollum (4) tomatoes ripened at a consistent temperature under bright light have a higher carotene content than when ripened in the dark at the same temperature. The tomatoes were allowed to ripen until the desired maturity stage was obtained and then were analyzed. The 6 maturity stages used were green, breakers, turning pink, light red and red (9).

In the green stage, the surface of the tomato is light to dark green. The breakers stage consists of a definite break from green to tannish yellow, with not more than 10% pink or red on the surface. The turning stage is when 10 to 30% of the surface is tannish yellow, pink or red. At the pink stage from 30 to 60% of the surface is pink or red. Light red maturity stage is when over 60% of the surface is pinkish-red or red, but not over 50% is red. In the red stage of maturity, over 50% of the surface is red.

When the desired maturity was reached, a 1 to 2 kg sample was removed from the lot and blended in a Waring Blendor[4] with 1000 ml of water. Four g of $CaCO_3$/1000 g of tomatoes were added to the blended slurry to precipitate the pectins. Methyl alcohol 2:1 (V/W) and filter aid (10% Hyflo Super-Cel by weight of tomatoes) were added to the blended tomatoes. From this step on the procedure for extraction and saponification was similar to Purcell's (7). The slurry was filtered through paper coated with filter aid on a Buchner funnel. The methanol-water filtrate was discarded and the mat was scraped into a large container and extracted with acetone-hexane (1:1) repeatedly until no further color could be obtained from the mat. The acetone-hexane extract was washed with water to remove the acetone.

The hexane extract was saponified by shaking for 5 minutes with 1/8 volume methanol saturated with KOH. After 1 hour, the hypophase which had formed was discarded. The hexane epiphase was washed twice with 100 ml of 90% methanol to remove the oxygenated carotenoids (1, 5). The hexane extract was washed with water to remove the alcohol and alkali. An aliquot was taken of the hexane extract and the optical density was determined at 450 and at 502 mμ (3) and used to obtain the concentration of the lycopene present before chromatography.

A rotary evaporator was used to concentrate the hexane extract to a convenient volume to allow crystalization of the lycopene. After filtering and washing, the crystalized lycopene was redissolved in hexane. This procedure greatly simplified chromatography of the remaining pigments.

The hexane extract was column chromatographed on MgO (Fisher Sea Sorb 43) and Hyflo Super-Cel (1:1 W/W) (7). The column (40×2.5 cm) was developed with increasing amounts of acetone in hexane up to 10%. This allowed the elution of phyto-

[4]Mention of trade products and brands does not imply that they are endorsed or recommended by the United States Department of Agriculture over similar products and brands not mentioned.

fluene, alpha-carotene and beta-carotene as separate fractions. The column was extruded and the remaining carotene pigments were cut from the column. The pigment bands were washed from the column material using acetone-hexane (1:1). The separated pigments in the acetone-hexane extract were washed 3 times with water to remove the acetone. Where necessary, the hexane extract was reduced to a small volume on a rotary evaporator and re-chromatographed. This provided for the complete separation of the pigments.

The lycopene obtained from the chromatographic column was added to the solution of crystalized lycopene to obtain the total concentration of lycopene present.

The phytoene was evaporated to a small volume and was chromatographed on an alumina column (27×1 cm). The phytoene was eluted from the column with 5% ether-hexane and collected in 5 ml samples on a fraction collector.

Spectrometric curves and amounts of the separated pigments were obtained by using a Cary Model 11 spectrophotometer. The relative amounts of the separated pigments were determined by using the known specific absorption coefficients given in Table 1.

Results and Discussion

The results of the analyses of the carotenoid pigments from the 3 lots of fruits at the 6 maturity stages were averaged and reported in Table 2. These values were all determined after chromatography except for lycopene, which was determined before and after chromatography. The values for lycopene obtained after chromatography were 12 to 25% lower than the values before chromatography. Lime, *et al.* (3) also reported a loss of 10.3% lycopene using very rapid column chromatography. This loss is attributed to incomplete elution of the lycopene from the column material and loss through manipulation of the lycopene crystals separated before chromatography. Therefore, the values for the chromatographed lycopene were not reported and the values reported in Table 2 were determind before chromatography.

Alpha-carotene and beta-carotene were the only carotenes that were found at the mature green stage of maturity. None of the non-cyclic carotenes were found at this stage. Phytofluene, zeta-carotene, gamma-carotene and lycopene were found at the breakers stage. Phytoene was not present until the turning stage. Lycopene,

Table 1.—Wave length (mμ) absorbency index.

Pigments	Wave length (mμ)	Absorbency[a]	Reference
Phytoene	286	85	(8)
Phytofluene	348	150	(6)
Alpha-carotene	446	275	(6)
Beta-carotene	451	250	(3)
Zeta-carotene	400	220	(6)
Gamma-carotene	460	270	(6)
Lycopene	502	320	(6)

[a]Absorbency index at specified wave length, 1 cm cell thickness and 1 mg/ml concentration.

Table 2.—Mean carotenoid pigments of Homestead tomatoes at 6 stages of maturity.

Stage	Micrograms per gram fresh weight						
	Phytoene	Phytofluene	Alpha-Carotene	Beta-Carotene	Zeta-Carotene	Gamma-Carotene	Lycopene
Mature green	—	—	0.014 ± 0.004[a]	1.17 ± 0.09	—	—	—
Breakers	—	0.036 ± 0.01[a]	0.036 ± 0.002	1.81 ± 0.07	0.015 ± 0.001	0.16 ± 0.02	0.41 ± 0.04
Turning	0.77 ± 0.18[a]	0.14 ± 0.02	0.021 ± 0.006	2.15 ± 0.06	0.12 ± 0.05	0.40 ± 0.06	0.44 ± 0.05
Pink	1.51 ± 0.35	0.33 ± 0.07	0.019 ± 0.009	2.35 ± 0.19	0.19 ± 0.01	0.58 ± .05	9.01 ± 0.71
Light red	3.31 ± 1.2	0.81 ± 0.06	0.017 ± 0.001	2.98 ± 0.46	0.50 ± 0.08	0.65 ± 0.08	22.3 ± 2.6
Red	8.77 ± 1.5	2.25 ± 0.07	0.014 ± 0.002	2.50 ± 0.39	0.58 ± 0.09	0.70 ± 0.09	82.8 ± 4.5

[a]Standard error of the mean.

phytoene, phytofluene, zeta-carotene and gamma-carotene increased in concentration through all the stages of maturity. Alpha-carotene increased in concentration until the breakers stage, then decreased as the fruit became red. Beta-carotene increased in concentration through all the stages of maturity until the light red stage, then decreased.

The data in Table 2 suggest that each carotene may follow a course of development and accumulation not dependent upon other carotenes. Precursor-product relationships expected from a scheme of sequential dehydrogenation and ring formation to form alpha and beta carotenes are not obvious. The changes in the concentration of alpha and beta carotenes may be explained by assuming that the rate of synthesis is constant and independent of the precursor concentration while the rate of destruction increases with maturity. Conversely, the rate of ring closure may decrease in the late stages of maturity while the rate of destruction is constant. Thus the data do not conclusively support any concept of the interrelationship between carotenes.

Data obtained from the 3 ripening series on pigment concentration demonstrate reproducibility of selection when tomatoes are selected according to maturity standards. The data obtained should give the processor and researcher greater confidence in the use of classification standards as a means of determining corresponding maturity.

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