Cellulase in High Pigment and Crimson Tomato Fruit[1]

Francis E. Sobotka and Alley E. Watada[2]
West Virginia University, Morgantown

Abstract. Cellulase activity increased with softening of ripening fruit and differed among the tomato lines. Fruit containing genes for high pigment had lower cellulase activity and did not soften as rapidly as the commercial lines.

The phenomenon of fruit softening has been related to hydrolysis of pectic substances (4) and to a lesser extent to a reduction in the number of cellulose microfibrils (10). The extent of cellulose degradation in ripening tomato fruit has not been fully investigated; however, a cellulase enzyme has been isolated from tomato which is capable of hydrolyzing carboxymethylcellulose to glucose (5). Hall (5, 6) observed that cellulase activity increased with ripening of tomato fruit and the activity of the insoluble fraction was higher than that of the soluble fraction. Dickenson and McCollum (2) postulated that the cellulase(s) could act concomitantly with pectic enzymes in fruit softening. Thus, activation and intensity of cellulase activity could have an effect on the rate and degree of fruit softening. In contrast, Hobson (8) concluded that cellulase plays a minor role, if any, in fruit softening. The findings presented here do not agree with those of Hobson and indicate that cellulase is involved in fruit softening.

Materials and Methods

Firmness and cellulase activity of fruit of several tomato lines were analyzed at different stages of maturity. The tomato lines used were the commercial cvs. Manhattan and West Virginia 63, and the breeding lines designated as the 'T-lines'. that contain genes for high pigment (hp), crimson characteristic (cc), or both high pigment and crimson characteristic (hp cc). Analyses were made on fruit harvested at the mature green and breaker stages and on fruit held for various periods at 22°C after harvest at the breaker stage. Stages of maturity and ripeness were based on criteria described by Lampe[3] and Workman et al (13). Cellulase activity of the pericarp and placental tissues was measured separately in 'Manhattan' and 'T-3640' (hp cc) fruit.

Fruit firmness was measured with the Pressure Load Firmness Meter as described by Sobotka[4].

Cellulase was extracted from macerate of 10 fruit as described by Hobson (8). The enzyme was precipitated by adding enough crystalline ammonium sulfate while stirring to make a 0.8 saturated solution. After 30 minutes at 2°C the solution was centrifuged at 10,000g for 20 minutes at 0°C. The supernatant was decanted and the precipitated enzyme redissolved in 50 ml 0.02 M acetate buffer, pH 5.0. The resulting enzyme solution was centrifuged at 0°C and the supernatant was retained for enzyme determination.

Cellulase activity was determined by the viscometric method of Bell et al. (1) with modification. Merthiolate at a concn of 0.01% was used instead of toluene as a substrate preservative. Merthiolate did not act as an enzyme inhibitor.

Cellulase activity is expressed in units/mg protein: 100 units of cellulase activity equaled a 50% loss in viscosity after 20 hr of incubation with 1% carboxymethoxycellulose 7M, (Hercules Powder Company, Wilmington, Del.) as a substrate (pH 5.0) at 30°C. Total protein was determined by the method of Itzhaki (9). Absorbance was converted to mg protein by using bovine serum albumin as the standard. Cellulase activity was calculated also on a fresh wt basis and the result was similar to that using protein as the base.

Results

Rate of fruit softening differed among the tomato lines (Table 1). Softening occurred most rapidly with 'West Virginia

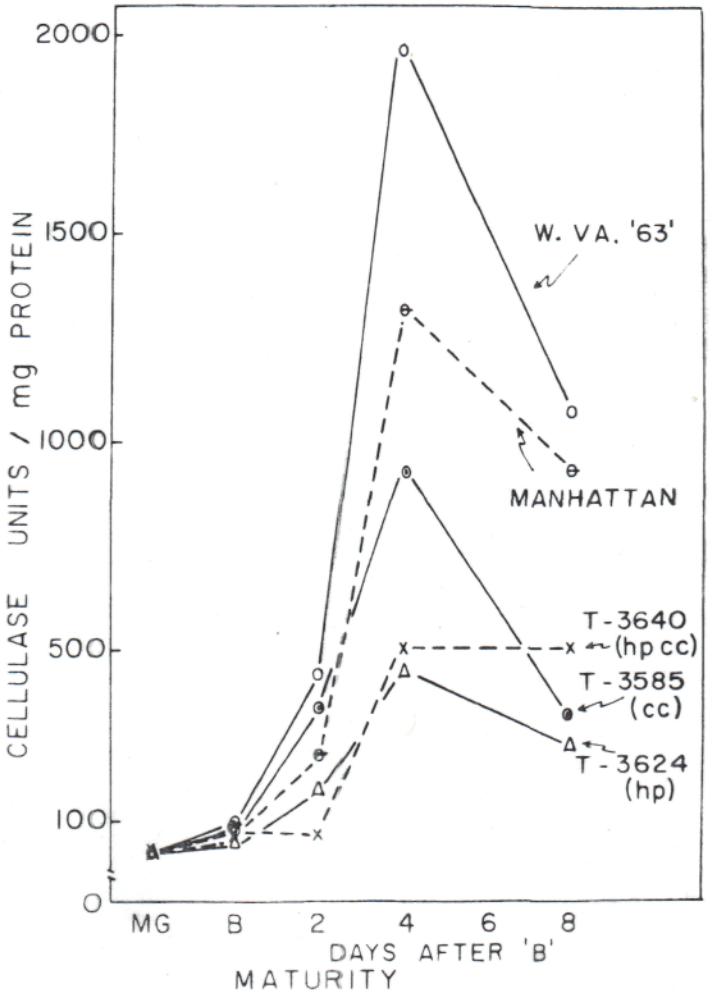

Fig. 1. Cellulase activity of ripening fruit of several tomato lines.

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[2]Present address: Hort. Crops Res. Br., MQD, ARS, USDA, Beltsville, Maryland.

[3]Lampe, Craig. 1968. Studies of fruit quality of several cultivars of *Lycopersicon esculentum* L. Master's Thesis, West Virginia University, Morgantown. 51 p.

[4]Sobotka, Francis E. 1970. Physical and biochemical studies of the softening process of several cultivars of *Lycopersicon esculentum* L. Master's Thesis, West Virginia University. 92 p.

Table 1. Compression reading (g/cm) of ripening fruit of several tomato lines as measured with the Pressure Load Firmness Meter.

	Maturity[1]					
	M. G.	B	2	4	6	8
Manhattan	4888 a[2]	4550 b	2751 c	1461 d	1386 d	1033 e
T-3640 (hp cc)	6063 a	5909 a	4181 b	2693 c	2543 c	2210 d
W. Va. '63	7026 a	4664 b	2503 c	1785 d	1162 e	894 f
T-3624 (hp)	7229 a	4489 b	2828 c	2596 c	1995 d	1507 e
T-3585 (cc)	6198 a	4694 b	2828 c	2241 d	1735 e	1286 f

[1]M. G., mature green; B, breaker; and number refers to days after "B".

[2]Means within each row followed by the same letter are not significantly different at the 5% level (Duncan's New Multiple Range Test).

63' fruit and least rapidly with 'T-3640' (hp cc) fruit. The compression values of these 2 lines dropped 87 and 64% respectively by the eighth day following breaker stage. The other lines lost about 79% of the compression during the same period. On the eighth day following breaker stage the compression values of 'T-3640' (hp cc) and 'T-3624' (hp) were significantly greater than those of other lines.

Cellulase activity increased with fruit ripening and differed among the lines (Fig. 1). Maximum activity in all lines was exhibited by the fourth day at 22°C following breaker stage. The enzyme activity of the commercial line was 2 to 3 fold greater than that of the T-lines containing genes for high pigment. Cellulase activity of 'T-3585' (cc) was intermediate and significantly different from that of other lines.

The cellulase activity differed greatly within the fruit (Table 2). The activity increased with ripening in both tissues and in 'Manhattan' the sharp increase was noted 2 days earlier in the placental tissue than in pericarp. In both lines, the maximum activity was 4- to 5-fold greater in the placental tissue than in pericarp. The maximum activity noted in the placental tissue of 'T-3640' (hp cc) was significantly lower than that of 'Manhattan' placental tissue but significantly higher than that of 'Manhattan' pericarp tissue.

Table 2. Cellulase activity of the placental and pericarp tissues of 'Manhattan' and 'T-3640' (hp cc) fruit during ripening.

		Maturity[1]				
		M. G.	B	2	4	8
Placental	Manhattan	27.41 a[2]	28.25 a	386.5 a	2133.10 a	1950.75 a
	T-3640 (hp cc)	7.20 a	26.30 a	76.97 a	775.55 a	855.20 b
Pericarp	Manhattan	17.16 a	59.29 a	95.53 b	484.75 c	310.10 c
	T-3640 (hp cc)	19.48 a	13.11 a	40.56 a	206.47 d	175.35 c

[1]M. G., mature green; B, breaker; and number refers to days after "B".

[2]Means within a column followed by the same letter are not significantly different at the 5% level and Duncan's New Multiple Range Test.

Discussion

Tomato lines with firmer fruit exhibited lower cellulase activity than those with softer fruit. The commercial lines had softer fruit and a higher max cellulase activity than the T-lines. Highest firmness readings were obtained with 'T-3640' (hp cc) fruit which had the lowest cellulase activity. Hobson (8) found that in the 'Potentate' and 'Harbinger' cultivars cellulase activity was not correlated with fruit firmness. His data showed that the cultivar with firmer fruit had higher cellulase activity than that with the softer fruit which is in contrast to our findings.

The rapid rate of softening that occurred within the first 6 days was closely associated with the change in cellulase activity. The coefficients of correlation between softening and cellulolytic activity of up to the fourth day ranged from 0.69 to 0.79 among the tomato lines studied. These significant coefficient values suggest that cellulase plays a role in fruit softening at a specific stage of ripening. In contrast, Hobson (8) concludes that cellulase plays an insignificant role in fruit softening. His conclusion, based on findings that cellulase activity was not associated with firmness of the cultivars or significantly related to fruit softening, was not in agreement with our findings.

The reason for the discrepancy between Hobson's (8) and our study is not known. The enzyme was extracted by his procedure, but the activity was assayed viscometrically rather than analysis of reducing groups. The former method was considered to be more accurate (3) (Errikson, 1969). On the other hand, as noted by Hobson, studies have shown that viscometric and reducing group assay give similar results of cellulase activity (7, 11) (Hash, Levison, 1950). Perhaps differences in tomato cultivars used for study may account for the discrepancy.

Cellulase is not the only enzyme responsible for fruit softening. This is indicated by the sharp decrease in firmness and insignificant increase in cellulase activity that occur in the very early stage of ripening. Other enzymes, such as the pectic hydrolyzing enzymes, are also responsible for softening of the fruit. As with cellulase activity, the polygalacturonase activity of 'Manhattan' and 'West Virginia 63' was shown to be significantly greater than that of 'T-3640' (hp cc) or 'T-3624' (hp) (12). This would suggest that firmness and long shelf life[3] of 'T-3640' is partly due to low activity of these hydrolyzing enzymes.

Literature Cited

1. Bell, T. A., J. L. Etchells, and Ivan D. Jones. 1955. A method for testing cucumber salt stock brine for softening activity. *Publ. U.S. Dept. of Agr.* ARS-72-5.
2. Dickenson, D. B., and J. P. McCollum. 1964. Cellulase in tomato fruits. *Nature* 203:525-526.
3. Errikson, Karl-Erik. 1969. New methods for the investigation of cellulase. Cellulase and their application. Robert A. Gould, Editor. *Amer. Chem. Soc.* Washington, D. C. 470 p.
4. Foda, Y. H. 1957. Pectin changes during ripening as related to flesh firmness in the tomato. Ph.D. Dissertation, University of Illinois. 68 p.
5. Hall, Chesley B. 1963. Cellulase in tomato fruits. *Nature*

200:1010-1011.
6. _____. 1964. Cellulase activity in tomato fruits according to portion and maturity. *Bot. Gaz.* 125:156-157.
7. Hash, John H., and Kendall W. King. 1958. On the nature of the β-glucosidases of *Myrothecium verrucaria*. *J. Biol. Chem.* 232:381-393.
8. Hobson, G. E. 1968. Cellulase activity during the maturation and ripening of tomato fruit. *J. Food Sci.* 33:588-592.
9. Itzhaki, Ruth F., and D. M. Gill. 1964. A microbiuret method for estimating proteins. *Anal. Biochem.* 9:401-410.
10. Kertesz, A. I., M. Eucore, and G. Fox. 1959. A study of apple cellulose. *Food Res.* 24:14-19.
11. Levinson, Hillel S., and Elwyn T. Reese. 1950. Enzymatic hydrolysis of soluble cellulose derivatives as measured by changes in viscosity. *J. Gen. Physio.* 33:601-628.
12. Sobotka, Francis E., and Alley E. Watada. 1970. Changes in polygalacturonase activity in fruits of different tomato cultivars. *Proc. W. Va. Acad. Sci.* In Press.
13. Workman, M., H. K. Pratt, and L. L. Morris. 1957. Studies on the physiology of tomato fruit. I. Respiration and ripening behavior at 20 degrees C as related to date of harvest. *Proc. Amer. Soc. Hort. Sci.* 69:352-365.