Effect of an Antisense Pectin Methylesterase Gene on the Chemistry of Pectin in Tomato (*Lycopersicon esculentum*) Juice†

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Pectins in tomato (*Lycopersicon esculentum*) juice processed from transgenic fruits with reduced levels of pectin methylesterase activity and from control fruits were characterized for total uronic acid, degree of methoxylation (DOM), and molecular mass. The molecular weights of pectins isolated from transgenic fruit juice are much higher than the molecular weights of pectins isolated from control fruit juice under all processing conditions. Depending on processing conditions, juice from transgenic fruits contained 30–50% higher amounts of total uronic acids and had 25–250% higher DOM compared to juice from control fruits.

Keywords: *Lycopersicon esculentum; tomato juice; pectin methylesterase; transgenic*

INTRODUCTION

Pectins are primarily polymers of D-galacturonic acid with a linear chain of (1→4)-D-α-galacturopyranosyl-uronic acid containing partially methylesterified carboxyl groups. Pectins significantly contribute to the consistency of tomato products (Becker et al., 1972; Bhasin and Bains, 1987). Pectin methylesterase (PME), an enzyme that accumulates during fruit development and ripening, demethoxylates fruit pectin (Roberts, 1990). Demethoxylated pectins, being a better substrate for polygalacturonase (PG), are more susceptible to degradation during processing of fruit juice and adversely affect the quality of tomato products. In tomato processing, pectolytic enzymes are inactivated by heat to protect the pectic substances against degradation. Pectolytic enzymes liberated during crushing, however, act very quickly; and 100% retention of pectic substances is not possible even under the best practical commercial conditions of rapid heating of the crushed tomatoes (Wagner and Miers, 1967; Miers et al., 1967). Further, degradation of pectins by pectolytic enzymes during ripening and harvest of fruit cannot be controlled by any of these methods. With advancements in biotechnology, however, it has become possible to modify the plants at the genetic level to control the activity of pectolytic enzymes in vivo (Schuch et al., 1991; Tieman et al., 1992).

We have introduced an antisense PME gene under the control of cauliflower mosaic virus 35S promoter into tomato plant (Tieman et al., 1992). Fruits from these transgenic tomato plants exhibit greatly reduced levels of PME (<10% of wild-type cultivar Rutgers at the same activity) but ripened normally (Tieman et al., 1992). This paper reports the quality of pectin present in the juice processed from these transgenic fruits along with a segregating line with 0 copy of the introduced gene (azygous) and wild-type Rutgers. Effects of different processing conditions have also been reported.

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MATERIALS AND METHODS

Red ripe fruits from wild-type Rutgers, transgenic 3781^, and azygous 3781^ (a segregating line of 3781^ with 0 copy of the introduced gene) were processed into juice by cold break, hot break, and microwave heating methods. In hot break methods, as the product was crushed, it was continuously heated to 88 °C in an open steam-jacketed kettle. The process took about 3 min. The crushed fruits were passed through a small laboratory finisher (Langsenkamp Co., Model 185S) fitted with a 0.56 mm screen to remove skin and seeds. In microwave processing, the tomato fruits were quartered and heated in a microwave oven (General Electric Co. Model 4JE 1465 H001, 1.4 kW) at high setting for 4 min, rotated and heated for another 4 min, and then processed into juice. The juice was heated for 2 min in the microwave before canning. In the cold break method, the processed juice was allowed to stand for 1 h at 22 °C for extended action of pectolytic enzymes before heating and canning. Pectin from processed juice was extracted as ethanol-insoluble solids (Tieman et al., 1992) and used for determination of total uronic acids (Ahmed and Labavitch, 1977), degree of methoxylation (DOM) (Maness et al., 1990), and molecular mass (Tieman et al., 1992). EDTA-soluble pectins were extracted from ethanol-insoluble solids using 50 mM sodium acetate buffer (pH 4.5) containing 40 mM EDTA at 20 °C for 4 h and used for gel filtration column chromatography using a Sepharose CL4B column as described by Tieman et al. (1992). Fractions (0.4 mL) were analyzed for uronic acid content according to the method of Filisetti-Cozzi and Carpita (1991). The experiment was carried out in triplicate with independent extracted samples with reproducible results. Estimation of the molecular mass of column fractions was made with blue dextran (2000 kDa), branched dextrans with molecular mass ranging between 17.7 and 500 kDa, and bromophenol blue (670 Da) (Sigma Chemical Co., St. Louis, MO). It may, however, be noted that dextrans may not have conformations similar to those of pectin polymers; therefore, the values shown in Figure 1 are merely an estimation of molecular mass.

RESULTS AND DISCUSSION

Total uronic acid present in tomato juice processed from transgenic, azygous, and wild-type Rutgers fruits using cold break, hot break, and microwave heating methods are given in Table 1. Juice from transgenic fruits contains significantly ($p < 0.05$) higher amounts of total uronic acids compared with juice from azygous and wild-type Rutgers; the percentage increase ranges from 35 to 50%. Pectins from transgenic fruits also have

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Table 1. Effect of Reduced PME Activity on Total Uronic Acid and Degree of Methylesterification of Pectin in Processed Tomato Juice[a]

	uronic acid, μmol/mg of cell wall			degree of methylesterification, %		
processing method	Rutgers	azygous	transgenic	Rutgers	azygous	transgenic
cold break	0.57 ± 0.01^{Bc}	0.56 ± 0.01^{Bb}	0.76 ± 0.03^{Ac}	14.1 ± 1.0^{Cc}	18.6 ± 1.5^{Bc}	51.4 ± 0.8^{Ac}
hot break	0.64 ± 0.01^{Ca}	0.69 ± 0.00^{Ba}	0.96 ± 0.02^{Aa}	50.1 ± 1.1^{Ca}	51.8 ± 1.2^{Ba}	63.4 ± 0.9^{Aa}
MW heating	0.59 ± 0.01^{Bb}	0.57 ± 0.02^{Bb}	0.83 ± 0.02^{Ab}	39.1 ± 2.9^{Cb}	43.4 ± 0.4^{Bb}	55.9 ± 2.4^{Ab}

[a] Rutgers is the parental cultivar of tomato used for creating transgenic plant. Azygous and transgenic represent segregated progenies of transformant 3781 (Tieman et al., 1992) containing 0 and 2 copies of the introduced PME antisense RNA gene, respectively. Cold, hot, and MW represent cold break, hot break, and break after microwave heating of tomatoes, respectively. Upper case letters represent difference between samples from different genotypes processed under the same conditions; lower case letters represent difference between samples from the same genotype processed under different conditions. Figures with same letters are not significantly different. Figures with different letters are significantly different at 95% confidence.

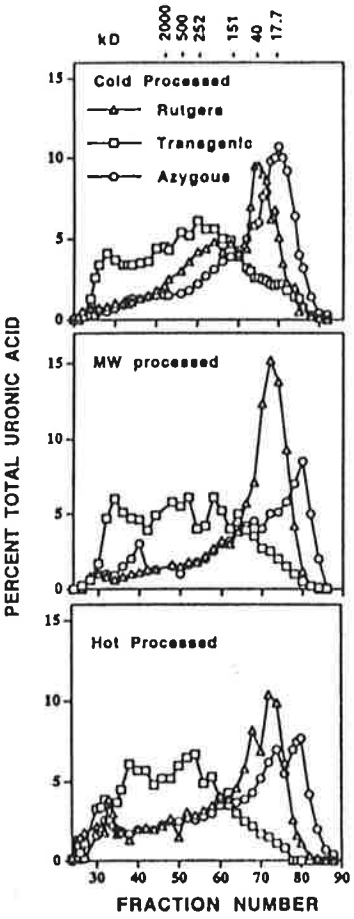

Figure 1. Gel filtration chromatographic analysis of EDTA-soluble polyuronides from Rutgers, azygous, and 3781 homozygous fruit juice processed by cold break, microwave (MW), and hot break methods.

significantly higher ($p < 0.05$) DOM, especially the cold break processed tomatoes. Pectins in cold break processed juice showed over 250% increase in DOM, while pectins in hot break and microwave heating processed juice exhibited about 25% increase in DOM as a result of lower PME activity in transgenic fruits. A marked increase in the molecular size of pectin in juice from transgenic tomatoes was obtained under all processing conditions. The majority of pectins present in transgenic fruit juice are of much higher molecular mass than those present in juice from wild-type or azygous fruits. This was true for even cold break processed juice (Figure 1). The observed increase in DOM of pectin from transgenic fruit juice is the result of its reduced PME activity. By demethoxylating pectins, PME facilitates their depolymerization by PG by forming better substrates. Higher DOM of pectin reduces the depolymerization of pectin by PG, resulting in a higher amount of pectin with higher molecular mass in transgenic fruit juice. Increased uronic acid is likely due to reduced binding of methoxylated pectins to cell walls.

Method of processing influences the amount and DOM of pectin present in the juice from wild-type fruit. Hot processed juice from all three genotypes contains higher amounts of pectin with higher DOM and higher molecular mass compared to the cold processed juice (Table 1 and Figure 1). This is expected as pectin-degrading enzymes are heat inactivated in hot processed juice. In cold processed juice, the enzyme activity is not inhibited, leading to degradation of pectins. Total pectin content, DOM, and molecular mass of pectins even in cold processed transgenic fruit juice are higher compared to those of hot processed juice from wild-type Rutgers, indicating the effectiveness of the introduced gene in protecting the quality of pectin in transgenic tomato fruits.

Since the nature of pectin influences the quality of processed tomato products, the increased molecular mass and DOM of pectin in transgenic tomato fruit juice is highly desirable and of economic significance to the tomato processing industry.

LITERATURE CITED

Ahmed, A. E.; Labavitch, J. M. A simplified method for accurate determination of cell wall uronide content. *J. Food Biochem.* **1977**, *1*, 361–365.

Becker, R.; Miers, J. C.; Nutting, M. D.; Dietrich, W. C.; Wagner, J. R. Consistency of tomato products. 1. Effects of acidification on cell walls and cell breakage. *J. Food Sci.* **1972**, *37*, 118–125.

Bhasin, U. R.; Bains, G. S. Study of pectolytic factors and processing in relation to rheological characteristics of tomato juice. *J. Food Sci. Technol.* **1987**, *24*, 247–253.

Filisetti-Cozzi, T. M. C. C.; Carpita, N. C. Measurement of uronic acids without interference from neutral sugars. *Anal. Biochem.* **1991**, *197*, 157–162.

Maness, N. O.; Ryan, J. D.; Mort, A. J. Determination of degree of methylesterification of pectins in small samples by selective reduction of esterified galacturonic acid to galactose. *Anal. Biochem.* **1990**, *185*, 346–352.

Miers, J. C.; Wagner, J. R.; Sanshuck, D. W. Consistency of tomato products. II. Effect of pH during extraction on tomato juice consistency. *Food Technol.* **1967**, *21*, 923–926.

Roberts, K. Structure at the plant cell surface. *Curr. Opin. Cell Biol.* **1990**, *2*, 920–928.

Schuch, W. J.; Kanczler, J.; Robertson, H. G.; Tucker, G. D.; Bright, S.; Bird, C. Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. *HortScience* **1991**, *26*, 1517–1520.

Tieman, D. M.; Harriman, R. W.; Ramamohan, G.; Handa, A. K. An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. *Plant Cell* **1992**, *4*, 667–679.

Wagner, J. R.; Miers, J. C. Consistency of tomato products. I. The effect of tomato enzyme inhibition by additives. *Food Technol.* **1967**, *21*, 920–923.

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