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

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Tomato Product Quality from Transgenic Fruits with Reduced Pectin Methylesterase

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ABSTRACT

Juice from transgenic tomato (*Lycopersicon esculentum* cv Rutgers) fruits with reduced levels of pectin methylesterase (PME) activity due to the expression of a PME antisense gene exhibited improvement in quality. The percentage increase in juice from transgenic fruits over that of juice from wild type Rutgers ranged between 5.1–5.3 for total solids, 3.8–6.1 for soluble solids, 70-80 for efflux viscosity, 180–220 for serum viscosity and about 50 for precipitate weight ratio. Time of harvest had no effect on quality of juice. Ketchup prepared from transgenic fruit juice had a lower Bostwick value, reduced serum separation and high serum viscosity compared to ketchup from parental Rutgers.

Key Words: tomato juice, ketchup, transgenic fruits, pectin methylesterase, consistency

INTRODUCTION

TOMATO is the second largest fruit crop in dollar value in the USA and other parts of the world. The annual worldwide production of processing tomato fruits is more than 20 million tons with >50% in the US. More than 80% of tomatoes are consumed in the form of processed products such as juice, paste, puree, ketchup, sauce, etc. (Gould, 1992). Sensory qualities and hence marketability of tomato products depend upon their consistency. Products with low consistency may be sold at lower prices or graded unacceptable (Kertesz and Loconti, 1944; McColloch and Kertesz, 1949; Crandall and Nelson, 1975). Consistency of tomato products depends upon the amount and the quality of pectic substances (Luh et al., 1954; McColloch, 1952; McColloch et al., 1952). Inability to inactivate pectin methylesterase (PME) and other pectolytic enzymes can result in altered consistency of tomato products (Miers et al., 1967; Twigg, 1959). Inactivation of these enzymes during tomato processing is generally achieved by high temperature although pH and additives have also been used (McColloch, 1952; Wagner and Miers, 1966). Pectolytic enzymes liberated during crushing, act very quickly and total retention of pectic substances is never obtained even under the best practical commercial conditions of rapidly heating crushed tomatoes (McColloch and Kertesz, 1949; Twigg, 1959). Further, degradation of pectins by pectolytic enzymes during fruit ripening and between harvest and processing cannot be controlled by any of these methods. However, modification of plant gene expression by antisense or co-suppression has made it possible to control the activity of pectolytic enzymes *in vivo* (Dellapenna et al., 1990; Schuch et al., 1991; Tucker et al., 1992).

We introduced an antisense PME gene under the control of cauliflower mosaic virus 35S promoter into tomato (Tieman et al., 1992). The fruits from transgenic plants had greatly reduced levels of PME activity (<10% of normal tomato cultivar Rutgers at the enzyme activity level), ripened normally, showed higher degree of pectin methylesterification, increased pectin size, decreased EDTA soluble pectins, and an increase in soluble solids in raw juice (Tieman et al., 1992). Our objective was to determine quality characteristics of the processed juice and ketchup prepared from these genetically modified fruits by different processing methods and compare such characteristics with juice and ketchup from non-transgenic wild type Rutgers and azygous fruits.

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MATERIALS & METHODS

Plant material

Tomato plants of wild type 'Rutgers', tarnsgenic 3781^ and azygous 3781^ genotypes were grown in the field at O'Neall Memorial Research farm (Tippecanoe County, Indiana) in a randomized complete block design with four replications for each cultivar. Each row contained 10 plants spaced 46 cm apart, and rows were spaced 91 cm apart. The plot consisted of an experimental and two guard rows for each block with the same line grown in the experimental and guard row of each block. All fruits at the red ripe stage were harvested weekly. Uniformly ripened, fresh and healthy tomatoes from fields were brought to the pilot plant, sorted and washed under tap water. The tomatoes were processed into juice by cold break, hot break and microwave heating methods.

Fruit processing

For cold break processing of tomato fruits, a small commercial extractor (Langsenkemp Co. Model 185S) was used to crush and express tomatoes through a 9.5 mm screen. The crushed fruits were passed through a small laboratory finisher fitted with a 0.56 mm screen to remove seeds and skin. The juice was canned in 303 × 406 enameled cans. The sealed cans were heated in boiling water bath for 20 min, cooled rapidly under running tap water and stored at 4°C for further analysis. For hot break, the crushed tomato fruits were continuously heated to 88°C in an open steam jacketed kettle. The hot juice was then passed through a finisher fitted with 0.56 mm screen to remove seeds and skin. The rest of the procedure was the same as in the cold break method. For microwave (MW) processing, the tomatoes were quartered and heated in a microwave oven (General Electric Co. Model 4JE 1465 H001, 1.4 KW) at high heat setting for 4 min, rotated and heated for another 4 min, and then processed into juice as described earlier in the cold break method. The juice was heated for 2 min in the microwave oven before canning. The other steps were the same as described for the cold break method.

Preparation of tomato ketchup

Tomato ketchup was prepared from hot break processed tomato juice using the procedure and formulation described by Gould (1992). Briefly, the juice from respective genotypes was concentrated to 33° Brix by evaporating water in an open steam jacketed kettle. Sugar (4.5%) and salt (1.6%) were added at the beginning while vinegar (5% acidity) was added at the end of the processing. Spices, Clove (0.045%), All spice (0.60%), Cyanne (0.05%), and Garlic powder (0.025%), were packed together in a cheese cloth pouch while onions were chopped in a blender and packed in a separate pouch. The pouches were put into the kettle containing juice. At the end, pouches were squeezed and removed. Ketchup was filled hot into 1000 mL clean glass bottles with screw caps.

Analytical methods

To determine the pH of the tomato juice and ketchup, a Corning glass electrode pH meter (Model 125) was used. For titratable acidity, 10g of product was mixed with 50 mL of boiled and cooled distilled water in a 100 mL glass beaker, and titrated with 0.1N NaOH solution to pH 8.1 ± 0.05 using pH meter. Results were reported as percent citric acid. Total solids were determined following the NCA vacuum oven drying procedure (Lamb, 1977). About 15 mg of diatomaceous earth was dried in an aluminum dish at 110°C for 30 min, cooled in a desiccator and weighed. Nearly 10-g sample was weighed accurately into a dish and placed on a boiling water bath until samples appeared dry. It was then dried in a vacuum oven to constant weight at 70°C under a vacuum of 76 cm Hg. The dish was cooled in a desiccator, weighed, and the percent total solids calculated. For soluble solids, aliquots of serum were obtained by centrifugation and filtration of the juice and ketchup (Lamb, 1977). These were dried using the same technique employed for total solids determination. The serum viscosity was determined using a size 100-Canon Fenske viscometer at 25°C in a constant temperature water bath. Four independent readings were taken using 7 mL of serum for each sample. The standard flow time for 7 mL of distilled water at 25°C was 64.6 sec. Efflux viscosity of tomato juice was measured with a standard Libby pipette for tomato juice at 25 ± 0.5°C. Bostwick consistency was determined with Bostwick consistometer by measuring the distance (cm) product flowed in 30 sec when both product and consistometer had equilibrated to 25°C. Values were reported in cm. Serum separation in ketchup was determined using the method of Cardec et al. (1985). Ketchup (15 mL) was placed with a syringe in a stainless steel cone (42 mesh, 60°) and serum collected in a graduated cylinder for 5 min. The volume collected was recorded ± 0.1 mL. Precipitate weight ratio was determined following the method of Takada and Nelson (1983).

RESULTS

Effect of reduced PME on quality attributes of processed juice

Fruits from transgenic 3781^ (homozygous for a PME antisense gene), azygous 3781^ (a segregating line of 3781^ with 0 copies of introduced gene), and wild type Rutgers were processed by cold break, hot break and MW methods and quality attributes of the processed products were determined. Juice from transgenic fruits contained higher levels of both total and soluble solids compared to that from azygous and wild type Rutger fruits (Table 1). Although the method of processing influenced levels of both total and soluble solids in the processed tomato juice from all cultivars, the juice from transgenic fruits always contained higher total and soluble solids compared to that from azygous and wild type fruits. Depending on processing conditions, overall increase in total solids in juice from transgenic fruits compared to that from azygous and wild type Rutger fruits ranged from 5.1–5.3% and soluble solids increased by 3.8–6.1%. Similar results have been reported in transgenic tomato fruits with reduced PME (Tieman et al., 1995).

Reduced PME activity in transgenic fruits resulted in increase in efflux and serum viscosities of the processed juice from transgenic tomato fruits. Increase in efflux viscosity ranged between 70–80%, while in serum viscosity it ranged between 180–220% in the processed juice from transgenic fruits over that of juice from azygous and wild type Rutger fruits (Table 1). Hot processed juice from all genotypes had the highest efflux and serum viscosity, followed by MW and cold break juice. For transgenic fruit juice, both efflux and serum viscosities were about 60% and 200% higher even after cold break compared to hot break juice from azygous and wild type Rutger fruits (Table 1). Precipitate weight ratio (PPT), an indicator of better processing attributes of tomatoes (Takada and Nelson, 1983), was ~50% higher for juice from transgenic fruits compared to that from non-transgenic fruits (Table 1).

Low levels of PME activity increase the degree of methoxylation of fruit pectins (Tieman et al., 1992). We determined changes in titratable acidity and pH of the processed juice from all 3 genotypes. Although pH of the processed juice from transgenic fruits was slightly higher than that of wild type Rutgers and azygous fruits, the increase did not affect processing of the juice (Table 1). Methods of processing had no effect on the pH of the processed juice. A slight increase in pH of transgenic tomato fruit with low PME has been reported by Tieman et al. (1995). Although, titratable acidity of the processed juice was similar for the three genotypes for a particular processing method, a slight reduction in titratable acidity was observed in cold processed compared to hot and MW processed juices.

Table 1—Quality characteristics of tomato juice from wild type and transgenic Rutgers fruits using various processing conditions

Variable	Processing condition[b]	Genotype[a] Rutgers	3781^ Azygous	3781^ Homozygous
Total Solids (% Fr. wt)	Cold	6.83 ± 0.09	6.84 ± 0.03	7.18 ± 0.03[d]
	Hot	7.36 ± 0.13	7.07 ± 0.08	7.74 ± 0.05[c]
	MW	6.91 ± 0.03	6.60 ± 0.14[c]	7.28 ± 0.04[d]
Soluble Solids (% Fr. wt)	Cold	6.18 ± 0.10	6.09 ± 0.08	6.56 ± 0.04[d]
	Hot	6.63 ± 0.15	6.60 ± 0.05	6.96 ± 0.06[c]
	MW	6.33 ± 0.04	6.15 ± 0.06[c]	6.57 ± 0.04[e]
Precipitate Weight ratio	Cold	9.25 ± 0.13	9.51 ± 0.33	13.56 ± 0.18[e]
	Hot	10.67 ± 0.13	11.04 ± 0.21	15.51 ± 0.13[e]
	MW	11.22 ± 0.22	9.73 ± 0.07[e]	16.66 ± 0.26[e]
Serum Viscosity (Sec)	Cold	73.33 ± 0.23	73.75 ± 0.53	219.66 ± 10.21[e]
	Hot	93.33 ± 2.42	103.91 ± 2.07[c]	262.91 ± 10.75[e]
	MW	77.33 ± 0.59	74.75 ± 0.16[c]	258.58 ± 14.90[e]
Efflux Viscosity (Sec)	Cold	28.00 ± 1.04	28.11 ± 1.12	43.17 ± 4.50[e]
	Hot	30.67 ± 1.04	31.04 ± 1.21	58.16 ± 2.30[e]
	MW	29.11 ± 2.11	29.16 ± 1.07	50.28 ± 1.72[e]
pH	Cold	4.25 ± 0.02	4.28 ± 0.01	4.35 ± 0.02[e]
	Hot	4.28 ± 0.01	4.27 ± 0.01	4.33 ± 0.01[d]
	MW	4.27 ± 0.02	4.21 ± 0.01[c]	4.35 ± 0.02[d]
Acidity (% citric acid)	Cold	0.49 ± 0.01	0.53 ± 0.03[e]	0.50 ± 0.01
	Hot	0.53 ± 0.01	0.55 ± 0.01[c]	0.53 ± 0.01
	MW	0.51 ± 0.01	0.57 ± 0.01[e]	0.50 ± 0.01

[a] 3781^ Azogous and 3781^ Homozygous represent the segregated progenies of transformant 3781^ containing 0 and 2 copies of the introduced PME antisense RNA gene, respectively.

[b] Cold, Hot and MW represent cold-break, hot-break, and break-after microwaving tomatoes, respectively.

[c] Significantly different from Rutgers ($P<0.05$).

[d] Significantly different from Rutgers ($P<0.01$).

[e] Significantly different from Rutgers ($P<0.001$).

Effect of growing conditions on quality of processed juice

Fruits from all three genotypes were harvested throughout the growing season and processed to determine the effects of different climatic conditions on the quality of juice from transgenic fruits. As shown (Fig. 1), efflux and serum viscosity of the processed juice from a particular genotype remained similar for the fruits harvested throughout the growing season, with much higher values for the juice obtained from transgenic fruits. Levels of total and soluble solids, and PPT value remained higher in juice from transgenic fruits compared to wild type Rutgers and azygous fruits (data not shown). Collectively, results indicated that the effects of introduced PME antisense gene were maintained throughout the growing season.

Characteristics of ketchup from transgenic tomatoes with low PME

Hot break juice from fruits of three genotypes was concentrated and processed into ketchup. To compare the quality of ketchup, levels of soluble solids were kept constant. About 10-fold increase in serum viscosity was obtained in ketchup made from transgenic fruits compared to that made from wild type Rutger and azygous fruits (Table 2). Bostwick value of ketchup made from transgenic fruits was much lower than that from azygous and wild type fruits (Table 2). Serum separation decreased over twofold in ketchup from transgenic fruits compared to that from azygous and wild type fruits (Table 2). A large increase in brookfield consistency index was found in ketchup made from transgenic fruits (Table 2). Detrimental changes in pH or titratable acidity were not observed in ketchup made from transgenic fruits.

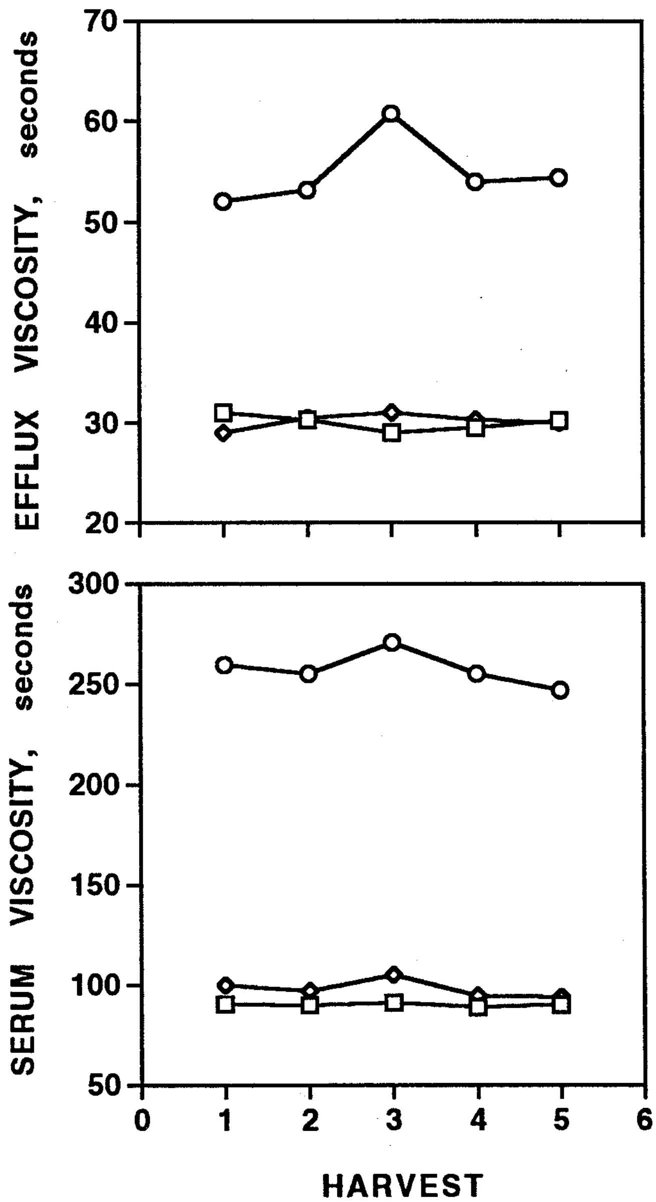

Fig. 1—Effect of harvesting time on efflux and serum viscosities of processed juice from wild type Rutgers (◇), azygous 3781 (□) and transgenic 3781 (○). Ripe tomato fruits from successive harvest during 1992 were processed by hot break and efflux and serum viscosities determined as described under experimental section. Harvesting dates were August 27 (Harvest 1), September 3 (harvest 2), September 10 (harvest 3), September 17 (harvest 4), and September 26 (harvest 5), respectively.

Table 2—Quality characteristics of tomato ketchup from wild type and transgenic tomatoes

	Genotype[a]		
	Rutgers	3781^ Azygous	3781^ Homozygous
Total solids	35.57 ± 0.27	34.50 ± 0.21	34.00 ± 0.10
Soluble solids	33.00 ± 0.10	33.00 ± 0.00	33.00 ± 0.00
pH	4.22 ± 0.01	4.23 ± 0.00	4.32 ± 0.02
Acidity (% citric acid)	1.85 ± 0.03	1.92 ± 0.02	1.70 ± 0.03
Viscosity			
Serum (sec.)	548.7 ± 27	514.9 ± 21	5400 ± 102
Bostwick (Distance in cm in 30 sec.)	4.62 ± 0.17	4.07 ± 0.06	3.12 ± 0.13
Serum separation	2.75 ± 0.13	2.35 ± 0.10	0.92 ± 0.05
Brookfield consistency index at			
33 Brix°	1.93	2.25	3.47
25 Brix°	0.96	1.00	1.56

[a] Fruits from each genotype were processed by hot-break and processed juice was used to make ketchup. Genotype details are as in Table 1.

DISCUSSION

THE EXACT MECHANISM of solids level increase in transgenic fruit juice is not clear. Some of the increase may be due to highly esterified pectins of transgenic fruits which did not bind to the cell wall. The observed increase in serum and efflux viscosity of juice from transgenic tomato fruits was most likely due to pectin changes as a result of reduced levels of PME activity (Tieman et al., 1992). Also, increase in degree of methyl esterification would inhibit polygalacturonase (PG) activity, resulting in increased product quality (McColloch et al., 1950; Luh et al., 1954). Transgenic fruits have pectin with 20–40% higher degree of methoxylation and contain pectins of higher molecular weight compared with wild type Rutgers (Tieman et al., 1992). Increase in the consistency of tomato products due to higher degrees of methoxylation and higher molecular weights of pectins has been reported (McColloch and Kertesz, 1949; Bhasin and Bains, 1987; Schuch et al., 1991; Kramer et al., 1992). McColloch and Kertesz (1949) observed that tomato juice with high consistency had pectin with higher degrees of methoxylation while that with lower consistency contained pectin with lower degrees of methoxylation. Increase in juice consistency and serum viscosity from PG antisense tomato fruits has been reported by Schuch et al. (1991) and Kramer et al. (1992). Small increases in the pH of juice from transgenic fruits may partly be due to increased degree of methoxylation of pectin. This would reduce the number of -COOH groups resulting in an increase in pH of the product. However, other environmental factors influence pH and acidity of tomato fruits as well (Davies and Hobson, 1981).

Higher viscosity of heat processed compared to cold processed juice was expected as heat processing inactivates pectolytic enzymes leading to higher retention of pectic substance. Bhasin and Bains (1987) reported that hot break juice had higher consistency due to high molecular weight pectin with high degree of methoxylation. Kertesz and Loconti (1944) observed that heating raw tomato fruits prior to, or immediately after crushing, inactivated pectic enzymes which could adversely affect consistency of the product. Our data demonstrated that in-vivo reduction of PME activity can provide very effective means to improve viscosity of juice without heat inactivation of pectolytic enzymes.

Takada and Nelson (1983) reported a correlation between PPT value and Bostwick and efflux viscosity of tomato products. Our study also showed a correlation between Bostwick and efflux viscosity and PPT value. We also found a linear relationship between PPT value and serum viscosity ($r^2=0.979$), described by the equation, $Y = 33 X\text{-}255$ (X, PPT value; Y, serum viscosity). Low Bostwick values and higher serum viscosity of ketchup prepared from transgenic fruit juice could be due to the nature and amount of pectin present. Lower serum separation may also be due to higher pectin content which would bind more water in the ketchup reducing serum separation (Thakur et al., 1995).

It is evident from the data presented that by inhibiting PME activity we could improve many desirable characteristics of products from tomato fruits. Soluble solids and consistency are among the most important quality characteristics of tomato products. A large, highly significant increase in solids and consistency would be of great interest and economic significance to the tomato processing industry.

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