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Pectin methylesterase activity and other factors affecting pH and titratable acidity in processing tomatoes

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ABSTRACT

Pectin methylesterase (PME) rapidly hydrolyzes pectin methylesters in cold break tomato juice to form methanol and to increase titratable acidity (TA). Within 30 min, the juice methanol concentration increased from 35 to over 400 μg g^{-1} while the pH dropped from 4.45 to 4.20. On average, cold break juice pH values were 0.21 U lower and TAs 12.4 μeq g^{-1} higher than those for hot break juices prepared from the same tomatoes. The greater TA in the cold break juices equalled the amount of methanol formed, consistent with both resulting from PME activity in the cold break juice. For 16 cultivars evaluated, there was a correlation between the methanol content of the cold break juices and the consistency of hot break juices, which could be the result of different pectin contents in the different cultivars. Differences in the citric, glutamic, and malic acid contents between these cultivars were small.

1. Introduction

In California, approximately 10–12 million metric tons of processing tomatoes are grown annually and processed to make a number of different products, such as juice, soup, ketchup and sauces. The largest portion is thermally processed and concentrated into tomato paste, which is typically stored for up to 1 year, then diluted for formulation of ketchup, sauces, and other value-added products. Many variations in the quality of the paste can occur, depending on factors such as the cultivar of tomatoes used, the finisher screen size and, most importantly, the break temperature (the temperature to which the tomatoes are initially heated). An additional portion of the harvested fruit (approx. 25%) is peeled and processed into products such as whole peeled and diced tomatoes. The majority of the diced tomato is bulk-packaged for use as ingredients in the manufacture of other products, such as salsa, spaghetti and pizza sauce.

Two important quality attributes of processing tomatoes are pH and titratable acidity (TA). Tomatoes typically have sufficient acidity to maintain a pH below 4.6 and, accordingly, are not classified as a low acid food. Because of this tomatoes do not require the more drastic thermal treatments required of foods classified as low acid for the destruction of spoilage microorganisms, to ensure food safety. Industrial processors of tomatoes in California typically specify a pH of 4.2 or 4.3 in their processed products, to ensure a margin of safety. The acidity of the fruit is also important as a contributor to the flavour of the tomato products.

Citric acid is the most abundant acid in tomatoes and the largest contributor to the total TA (Paulson & Stevens, 1974; Stevens, 1972). The decrease in TA and rise in pH that occurs with maturity and over-maturity are due to a loss of citric acid (Anthon, LeStrange, & Barrett, 2011). Two other acids that contribute to the TA are glutamic and malic acids. Glutamic acid is present in tomatoes at levels comparable to that of citric acid and may be an important contributor to tomato flavour (Fuke & Shimizu, 1993). Malic acid is usually present at much lower levels than is citric acid but the ratio of malic to citric has been reported to vary between different tomato cultivars (Stevens, 1972; Suarez, Rodriguez, & Romero, 2008). The levels of these acids in modern processing tomato cultivars, and the extent of variation between cultivars have not been reported.

Tomato processing conditions can also affect tomato juice pH. It is well known that, once a tomato is homogenised, the enzymes pectin methylesterase (PME) and polygalacturonase (PG) become extremely active, causing rapid pectin breakdown. To avoid this enzymatic loss of pectin, the majority of tomatoes undergo the hot-break process, where the tomatoes are rapidly heated to >90 °C immediately after fruit disintegration, to thermally

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inactivate PME and PG and prevent the enzymatic breakdown of pectins (Barrett, Garcia, & Wayne, 1998; Luh & Daoud, 1971; McColloch, Nielsen, & Beavens, 1950). The hydrolysis of pectin by PME and PG is an important issue for tomato processors because the loss of pectin reduces juice viscosity. Less widely recognised is the fact that the hydrolysis of pectin methylesters by PME will also alter the pH and TA of the tomato juice because of the additional galacturonic acid residues produced by methylester hydrolysis. Tomato juice prepared without heating, commonly referred to as a cold break juice, has been shown to have greater acidity and a lower pH than a hot break juice produced from the same tomatoes (Stadtman, Buhlert, & Marsh, 1977). While it is generally accepted that pectin hydrolysis occurs in a cold break juice, there are few published reports that clearly illustrate just how quickly these enzymatic reactions will occur after fruit homogenisation. In particular, the speed and extent to which PME de-esterifies pectin in a tomato homogenate have not been previously shown. With enzymatically active cell wall material, pectin de-esterification by PME was reported to occur within 10 min (Koch & Nevins, 1989). The rate of de-esterification in tomato juice produced from whole tomatoes has not been reported.

Here we have examined, in greater detail, the changes in pH and TA caused by PME activity occurring after tomato homogenisation. We have also measured the difference in pH and TA between hot and cold break juices with a much larger sample size than those analysed previously, to obtain a more accurate estimate of the magnitude of the changes in pH and TA due to PME activity. In addition, we have measured the citric, glutamic and malic acid contents in juice from a number of processing tomato cultivars, to determine whether there is significant variation in acid composition between cultivars, and to estimate the relative contributions of different acids to the measured total TA.

2. Materials and methods

2.1. Materials

All chemical reagents were obtained from Sigma (St. Louis, MO).

2.2. Tomatoes

In 2009, 16 processing tomato cultivars were grown in five counties in California. For each cultivar, two replicate plantings were made in each county, resulting in a total of 10 replicate plantings for each cultivar and an overall total of 160 samples. In 2010, 16 cultivars were again grown but only four counties were used, resulting in a total of eight replicate plantings for each cultivar and an overall total of 128 samples. Of the 16 cultivars grown in 2010, seven were the same as in 2009 and nine were different. Ripe fruit was harvested as described previously (Garcia & Barrett, 2006).

2.3. Preparation and evaluation of juices

Preparation of hot and cold break tomato juices were as described previously (Barrett, Weakley, Diaz, & Watnik, 2007). Individual tomatoes were cut in half, lengthwise, and the halves separated to form two samples of approximately 1300 g each. Cold break juice was prepared from one of the samples by homogenising the tomato halves in a blender. A hot break juice was prepared from the other 1300 g sample by heating in a 1400 W commercial microwave oven for 6 min at 100% power, followed by 6 min at 50% power. Both juices were analysed for titratable acidity, pH and soluble solids; Bostwick juice consistency was determined on the hot break juice only. Assays were performed as described (Barrett et al., 2007). Cold break juices were allowed to stand at room temperature for at least 1 h between blending and analysis.

2.4. Methanol assay

Methanol concentrations in the cold break juices were determined using alcohol oxidase and Purpald, as described previously (Anthon & Barrett, 2004). To determine the rate of methanol formation in a tomato homogenate, a single roma type tomato (cultivar not known) was homogenised in a blender; then, at various times after the start of homogenisation, 0.1 ml aliquots of juice were removed and mixed with an equal volume of 0.1 M phosphate buffer (pH 2.0) containing 1 g l^{-1} of sodium lauryl sulphate. We have previously shown that this buffered detergent solution inactivates PME, preventing any further methanol formation (Anthon & Barrett, 2010). To estimate the methanol concentration in the fruit prior to homogenisation additional tomatoes were homogenised directly in an equal volume of the buffered detergent solution, to prevent any PME activity from occurring. These juice samples were analysed for methanol, using alcohol oxidase and Purpald, as described previously (Anthon & Barrett, 2004). Control experiments showed that sodium lauryl sulphate, at the concentrations used here, did not inhibit alcohol oxidase or interfere with the determination of methanol.

2.5. Organic acids

Supernatants were prepared from these juices by centrifuging at 15,000*g* for 5 min. Suitable dilutions of these supernatants were analysed for citric, malic and glutamic acids using enzyme kits (R-Biopharm, Marshall, MI) in a 96-well plate format, as described (Vermeir, Nicolai, Jans, Maes, & Lammertyn, 2007). It has been shown that acid levels, determined with these kits on crude tomato juice supernatants, show excellent agreement with HPLC analysis (Vermeir et al., 2007). Phosphate concentrations were determined by the method of Ames (1966) with the reagent volumes modified to a final volume of 0.2 ml for use in a 96-well plate format.

2.6. Statistical methods

Means, standard deviations, and correlations were calculated using statistical functions in Microsoft Excel.

3. Results and discussion

3.1. Methanol and acid formation by PME

It is well known that, when a tomato is disintegrated without heating, PME is released into the juice and rapidly hydrolyzes the methylesters in the pectin, producing methanol and polygalacturonic acid. This polygalacturonic acid can then be further hydrolysed into oligomers and monomers of galacturonic acid by PG. The speed with which PME de-esterifies pectins in a cold break juice was determined by homogenising a tomato in a blender and allowing the juice to stand at room temperature. A typical time course shows that methanol was rapidly produced in the tomato homogenate (Fig. 1). The initial level of methanol in the fruit, prior to homogenisation, was determined by homogenising additional tomatoes under conditions that rapidly inactivate PME, preventing any pectin ester hydrolysis. A methanol content of 35 ± 11 μg g^{-1} (n = 4) was determined, which is similar to what we have found previously (Anthon & Barrett, 2010). From this initial level, the methanol concentration in the homogenate increased to 198 μg g^{-1} by 2 min and to more than 400 μg g^{-1} by 30 min. In parallel with this increase in methanol concentration, was a

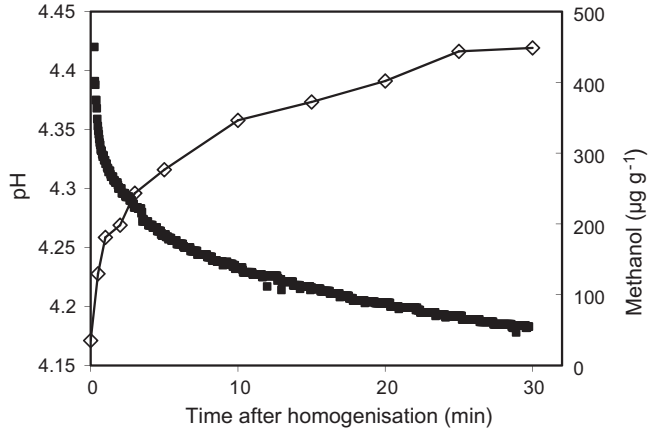

Fig. 1. Decrease in pH (■) and increase in methanol content (◇) in a cold break tomato juice.

decrease in the pH. From an initial pH of about 4.45 the pH dropped to less than 4.20 after 30 min. The pH change after homogenisation cannot be determined exactly because, by the time the first pH measurement could be made (15 s after the start of homogenisation), the pH was already rapidly dropping. In addition to producing methanol, the hydrolysis of methylesters by PME results in the formation of acidity (Hagerman & Austin, 1986; Kertesz, 1937). The drop in pH, in parallel with the increase in methanol concentration, is consistent with a de-esterification of pectin by PME in the tomato juice.

Unlike in a cold break tomato juice, in a hot break tomato juice, the tomatoes are heated to inactivate PME and other enzymes prior to homogenisation, preventing any breakdown of the pectins in the juice. Microwave heating of whole fruit prior to homogenisation is a commonly used method for preparing hot break juices. We have previously shown that such microwave hot break juice contains no greater amount of methanol than that found in the intact fruit, indicating a complete inactivation of PME (Anthon & Barrett, 2010). The magnitude of the pH change resulting from PME activity in cold-broken tomatoes was determined by comparing the pH values of hot and cold break tomato juices prepared from the same tomatoes. Tomatoes were cut in half lengthwise, and a microwave hot break juice was prepared from one set of halves and a cold break juice from the other set. A plot of pH values of the hot break juices versus those of the cold break juices showed that the two were correlated ($r^2 = 0.77$) but, as expected, because PME was inactivated in the hot break juice, the pH values of the hot break juices were always higher (Fig. 2A). The pH of the cold break juices ranged from 4.04 to 4.65 while the hot break juices ranged from 4.31 to 4.86. The average differences in pH between the hot and cold break juices were 0.23 ± 0.06 for the juices prepared in 2009 and 0.19 ± 0.03 for the juices prepared in 2010 (Table 1).

Cold break juices had higher TA than had hot break juices. The TA values in the cold break juices ranged from 35 to 68 μeq g^{-1} versus 25 to 56 μeq g^{-1} in the hot break juices (Fig. 2B). The average differences in TA between the hot and cold break juices were 12.4 ± 2.24 μeq g^{-1} in 2009 and 12.4 ± 1.33 μeq g^{-1} in 2010 (Table 1). These values are equal to about one third of the total TA measured for the hot break juices. Put another way, about one quarter of the total measured TA in the cold broken tomato juices was from acids formed enzymatically after the tomatoes were homogenised. The average methanol concentration in the cold break juices prepared in 2010 was 11.5 ± 1.12 μmol g^{-1} (Table 1). This amount of methanol is essentially equal to, on a molar basis, the amount of in TA in the cold break juices formed after homogenisation (12.4 ± 1.33 μeq g^{-1}), as would be expected if both are the result

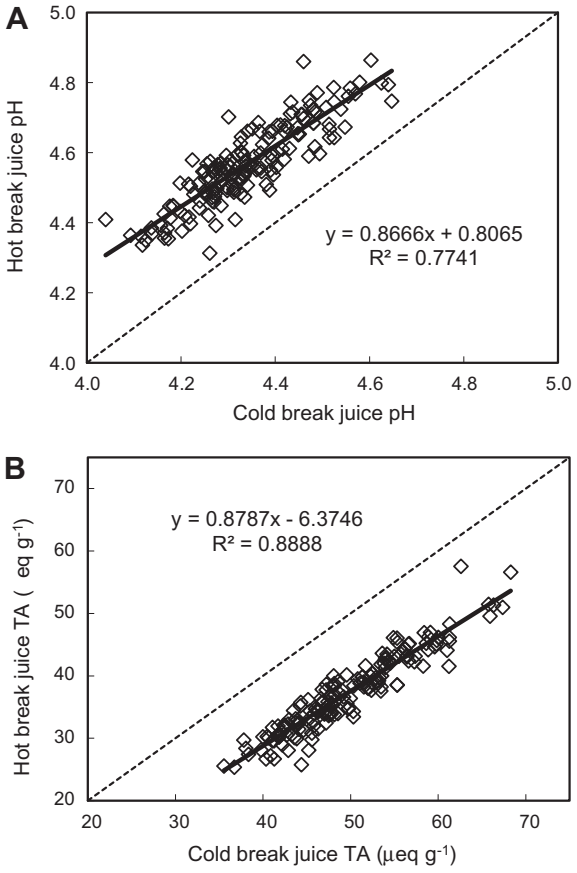

Fig. 2. pH and TA for hot and cold break juices prepared in 2009. (A) pH (B) TA.

Table 1
Differences in pH and TA between hot and cold break juices. Methanol was measured on the 2010 cold break samples only.

	Year	Cold break	Hot break	Difference
pH	2009	4.34 ± 0.12	4.57 ± 0.12	−0.23 ± 0.06
	2010	4.31 ± 0.08	4.51 ± 0.09	−0.19 ± 0.03
TA (μeq g^{-1})	2009	49.8 ± 6.7	37.3 ± 6.3	12.4 ± 2.3
	2010	51.4 ± 6.2	38.9 ± 5.7	12.4 ± 1.3
Methanol (μmole g^{-1})	2010	11.5 ± 1.1		
Brix	2009	5.15 ± 0.54	5.13 ± 0.45	0.02 ± 0.32
	2010	5.38 ± 0.53	5.37 ± 0.48	0.02 ± 0.16

of PME activity. Total soluble solids contents were not different between the hot and cold break juices (Table 1).

Stadtman et al. (1977) were the first to report that hot break tomato juices have higher pHs and lower TAs than have cold break juices, and that these differences were the result of PME activity occurring after fruit homogenisation. However, they found that the average difference in pH between hot and cold break juices was about 0.10 pH units and the average difference in TA was 5.0 μeq g^{-1}. These differences are only half as large as those found here. In a more recent study, hot and cold break juices were prepared from processing tomato cultivars similar to those used here. Surprisingly, no difference in pH or TA between the hot and cold break juices were noted (Akbudak, 2010). In both of these previous instances the tomatoes were disintegrated then heated by conventional thermal processing. It is possible that these hot break methods were less effective than the microwave procedure that we used

here for rapid enzyme inactivation. This could have allowed for more PME activity to occur in their hot break juices prior to complete enzyme inactivation, increasing the TAs of these juices and resulting in less apparent difference between their hot and cold break juices. Consistent with this, in both of these previous studies, the TA values of their hot break juices were mostly greater than 50 μeq g^{-1}, which is higher than the range of 30–50 μeq g^{-1} found here (Fig. 2).

3.2. Cultivar differences

The average amounts of methanol in the cold break juices were different for different tomato cultivars. Cultivars with the greatest amount of methanol also showed the largest difference in TA between hot and cold break juices, consistent with both resulting from PME activity in the cold break juice (Table 2). Processing tomato cultivars are known to vary in pectin content, which could account for the variable amounts of methanol formed in the cold break juices from different cultivars. Pectin content is also an important determinant of juice consistency. Cultivars with high pectin content generally produce hot-break juices with high gross viscosities. Gross viscosities of tomato juices are typically measured with a Bostwick consistometer, in which the distance a defined volume of juice flows in 30 s is determined. A low Bostwick value indicates a high gross viscosity. Thus cultivars with high pectin content would be expected to produce hot break juices with low Bostwick values and cold break juices with high methanol content. Consistent with this, for the 16 cultivars of tomatoes analysed, there was a significant correlation ($p < 0.001$) between the Bostwick values of hot break juices and the methanol contents of cold break juice prepared from the same tomatoes (Fig. 3). Cultivars with low Bostwick values contained more methanol in their cold break juices and had larger differences in TA between hot and cold break juices than had cultivars with high Bostwick values (Table 2). The extent to which the pectin galacturonic acid residues are methylesterified can vary for pectins from different sources. Thus, an assumption (in using the methanol concentration of the cold break juice as a measure of the pectin content in the fruit) is that this degree of pectin methylesterification does not vary appreciably between different tomato cultivars. The good correlation between methanol content and juice consistency suggests that such is the case. A possible practical application for this correlation would be as a screening tool in selecting for consistency in processing tomatoes cultivars.

Table 2
Cultivar differences for methanol content of cold break juices, TA differences between hot and cold break juices, and Bostwick values of hot break juices for juices prepared in 2010. Cultivars are ranked from highest to lowest methanol content.

Cultivar	Methanol (μmol g^{-1})	TA diff. (μeq g^{-1})	Bostwick (cm)
H 5608	13.5	14.7	12.9
H 8504	12.8	14.0	13.0
UG 19406	12.8	13.9	14.0
H 5508	12.3	12.8	13.5
CXD 282	12.2	12.8	13.5
H 9780	12.0	13.0	13.5
Nun 6385	11.8	12.9	13.9
HMX 7885	11.7	12.8	15.2
CXD 255	11.5	12.9	14.8
H 4007	11.2	12.5	16.4
AB 3	10.7	11.6	16.9
BQ 163	10.6	11.9	17.3
Nun 6394	10.4	10.6	16.6
AB 2	10.3	11.0	18.1
BQ 205	10.2	10.5	17.9
SUN 6366	9.5	10.1	18.4

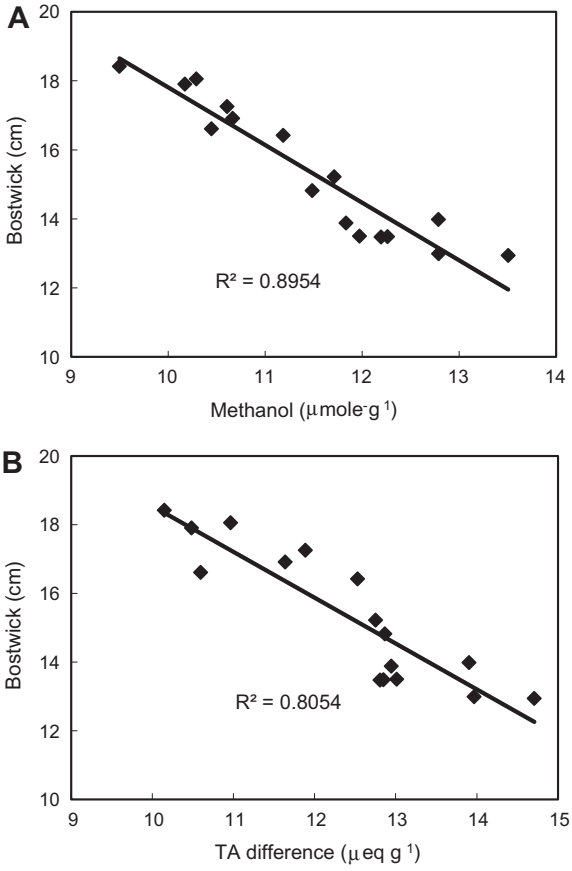

Fig. 3. (A) Correlation between average Bostwick values of hot break juices and the average methanol contents of cold break juices for 16 cultivars of tomatoes. (B) Correlation between average Bostwick values of hot break juices and the average difference in TA between hot and cold break juices prepared from 16 cultivars of tomatoes.

The pH of the tomato is an important fruit quality measure that is often reported in cultivar evaluations or in studies on growing conditions (Gautier et al., 2008; Loiudice et al., 1995; Oke, Ahn, Schofield, & Paliyath, 2005; Suarez et al., 2008). In nearly all cases, the measurements are made on cold-break juices with no attempt to inactivate PME prior to homogenisation. Authors have not routinely reported how quickly pH was measured after homogenisation nor whether this time interval was consistent for different samples. In tomatoes, due to the high activity of PME, the length of this time interval will affect the measured pH (Fig. 1). The pH values reported thus do not represent the pH of the intact tomato, nor will they accurately reflect the pH to be expected if the tomato is to be processed by hot-break methods. Since the amount of acidity formed in cold break juices were different for different cultivars (Table 2), meaningful comparisons of TA and pH between cultivars are best obtained from hot break juices.

3.3. Organic acids

To assess the influence of individual organic acids on the pH and TA of hot break tomato juice, the concentration of citric, malic, and glutamic acids were measured for the 128 juice samples analysed in 2010. In agreement with others (Loiudice et al., 1995; Paulson & Stevens, 1974; Stevens, 1972; Suarez et al., 2008), citric acid was the most abundant acid with an average of concentration of 2.54 mg g^{-1} (range 1.29–3.76 mg g^{-1}). Glutamic acid was present at a concentration similar to that of citric acid, with an average concentration of 2.11 mg g^{-1} (range of 1.00–3.21 mg g^{-1}). The

Table 3
Average pH and acid contents of hot break juices from 16 processing tomato cultivars grown in 2010. Values are mean ± SD (n = 8).

Cultivar	pH	TA (μeq g^{-1})	Citric acid (mg g^{-1})	Malic acid (mg g^{-1})	Glutamic acid (mg g^{-1})	Phosphate (μmol g^{-1})
UG 19406	4.37 ± 0.05	47.5 ± 5.60	3.12 ± 0.45	0.36 ± 0.08	2.24 ± 0.38	2.73 ± 0.39
H 8504	4.39 ± 0.06	46.3 ± 4.16	2.92 ± 0.51	0.30 ± 0.07	2.43 ± .050	2.46 ± 0.43
H 5508	4.45 ± 0.05	44.3 ± 3.06	3.09 ± 0.50	0.37 ± 0.04	1.94 ± 0.19	2.29 ± 0.65
AB 2	4.43 ± 0.09	43.8 ± 3.85	2.81 ± 0.53	0.36 ± 0.04	1.80 ± 0.45	2.39 ± 0.45
BQ 205	4.46 ± 0.06	42.6 ± 4.44	2.89 ± 0.57	0.32 ± 0.06	2.16 ± 0.41	2.62 ± 0.60
H 9780	4.46 ± 0.04	42.4 ± 4.45	2.84 ± 0.39	0.31 ± 0.06	2.18 ± 0.49	2.78 ± 0.38
BQ 163	4.51 ± 0.06	42.3 ± 4.36	2.74 ± 0.57	0.30 ± 0.06	2.21 ± 0.29	2.90 ± 0.67
AB 3	4.49 ± 0.06	41.5 ± 3.89	2.98 ± 0.50	0.33 ± 0.04	1.80 ± 0.38	2.67 ± 0.68
H 5608	4.51 ± 0.06	37.8 ± 4.35	2.93 ± 0.42	0.31 ± 0.06	1.71 ± 0.34	2.16 ± 0.99
H 4007	4.58 ± 0.07	37.0 ± 4.91	2.83 ± 0.53	0.37 ± 0.05	1.90 ± 0.34	2.48 ± 0.26
SUN 6366	4.54 ± 0.06	35.6 ± 3.51	2.41 ± 0.48	0.29 ± 0.06	2.16 ± 0.39	2.26 ± 0.45
CXD 255	4.51 ± 0.06	35.1 ± 3.48	2.09 ± 0.50	0.28 ± 0.04	2.55 ± 0.45	2.81 ± 0.39
Nun 6385	4.54 ± 0.06	33.7 ± 2.79	2.46 ± 0.48	0.27 ± 0.05	2.02 ± 0.45	2.56 ± 0.41
Nun 6394	4.58 ± 0.07	32.6 ± 2.97	2.34 ± 0.40	0.25 ± 0.04	1.99 ± 0.38	2.70 ± 0.30
CXD 282	4.57 ± 0.08	32.0 ± 2.78	2.11 ± 0.38	0.32 ± 0.03	2.13 ± 0.17	2.20 ± 0.51
HMX 7885	4.72 ± 0.07	28.2 ± 2.58	2.11 ± 0.37	0.31 ± 0.03	1.89 ± 0.33	2.83 ± 0.35

average malic acid concentration was much lower at 0.31 mg g^{-1} (range 0.187–0.508 mg g^{-1}). Inorganic phosphate ions will also influence the TA and pH. Average phosphate concentration was 2.41 μmol g^{-1} (range of 1.31–3.64 μmol g^{-1}) in these juices.

From the measured concentration of each individual acid, such as citric acid, the known pKa's of this acid, and the measured pH of the juice, a value can be calculated for the amount of TA in the juice attributable to this acid (Paulson & Stevens, 1974). This calculated TA can then be used to determine the portion of the total TA, measured titrimetrically on the same juice, attributable to this acid. From such calculations, it was found that citric acid was the greatest contributor to the total TA. For the 128 juices examined in 2010, the portion of the total measured TA attributable to citric acid ranged from 33% to 90%, with an average of 57%. Glutamic acid, although present at nearly the same concentration as citric acid on a weight basis, accounted for a much lower percentage of the measured TA, between 6% and 26% with an average of 15%. The smaller contribution to total TA from glutamic versus citric acid simply reflects the presence of the three carboxylic acid groups in citric acid. Similar calculations for malic acid and phosphate ions showed that their average contributions to the total TA were 5% and 6%, respectively. The sum of these calculated TAs was, on average, equal to 83% of the measured TA. The remaining 17% of the measured TA must be accounted for by acids not measured.

Differences were apparent in pH, TA, and organic acid composition between tomato cultivars. The average hot break juice TA ranged from a high of 47.5 to a low of 28.2 μeq g^{-1} for the 16 cultivars examined. Average juice pH ranged from 4.37 to 4.72 (Table 3). As would be expected, cultivars with the highest TA also had the lowest pH. A correlation analysis showed that the pH and TA were highly correlated with each other (r^2 = 0.85). Correlation analysis also showed that both pH and TA were significantly correlated with the citric acid content ($p < 0.0001$) but not the malic acid, glutamic acid or phosphate content of the hot break juices. These results are in agreement with our earlier finding that the increase in pH and decline in TA with fruit maturity paralleled a decline in citric acid content but not changes in either the malic or glutamic acid content or the concentration of phosphate ions (Anthon et al., 2011).

Concern over a recent trend towards rising pH values in tomatoes arriving at processing plants in California suggests that selecting for cultivars with higher acidity and lower pH values may be desirable. The correlation between citric acid content and both pH and TA indicates that screening for increased citric acid content may be a useful way to select for higher TA and lower pH. An advantage of screening for citric acid is that by using the enzymatic assays in a 96-well microplate format, many samples can be rapidly screened. Unlike measurements of pH and TA, which are confounded by enzymatic activity occurring after the fruit is homogenised and thus require making a hot break juice, citric acid could potentially be measured in a cold break juice.

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References

Akbudak, B. (2010). Effects of harvest time on the quality attributes of processed and non-processed tomato varieties. *International Journal of Food Science and Technology, 45*, 334–343.

Ames, B. N. (1966). Assay of inorganic phosphate, total phosphate and phosphatases. *Methods in Enzymology, 8*, 115–118.

Anthon, G. E., & Barrett, D. M. (2004). Comparison of three colorimetric reagents in the determination of methanol with alcohol oxidase. Application to the assay of pectin methylesterase. *Journal of Agricultural and Food Chemistry, 52*, 3749–3753.

Anthon, G. E., & Barrett, D. M. (2010). Changes in pectin methylesterification and accumulation of methanol during production of diced tomatoes. *Journal of Food Engineering, 97*, 367–372.

Anthon, G. E., LeStrange, M., & Barrett, D. M. (2011). Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes. *Journal of the Science of Food and Agriculture, 91*, 1175–1181.

Barrett, D. M., Garcia, E., & Wayne, J. E. (1998). Textural modification of processing tomatoes. *CRC Review Food Science and Nutrition, 38*, 173–258.

Barrett, D. M., Weakley, C., Diaz, J. V., & Watnik, M. (2007). Qualitative and nutritional differences in processing tomatoes grown under commercial organic and conventional production systems. *Journal of Food Science, 72*, pp. C441–C451.

Fuke, S., & Shimizu, T. (1993). Sensory and preference aspects of umami. *Trends in Food Science and Technology, 4*, 246–261.

Garcia, E., & Barrett, D. M. (2006). Assessing lycopene content in California processing tomatoes. *Journal of Food Processing and Preservation, 30*, 56–70.

Gautier, H., Diakou-Verdin, V., Benard, C., Reich, M., Buret, M., Bourgaud, F., et al. (2008). How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *Journal of Agricultural and Food Chemistry, 56*, 1241–1250.

Hagerman, A. E., & Austin, P. J. (1986). Continuous spectrophotometric assay for plant pectin methylesterse. *Journal of Agricultural and Food Chemistry, 34*, 440–444.

Kertesz, Z. I. (1937). Pectic enzymes. I. The determination of pectinmethoxylase activity. *Journal of Biological Chemistry, 121*, 589–598.

Koch, J. L., & Nevins, D. J. (1989). Tomato fruit cell wall: use of purified tomato polygalacturonase and pectinmethylesterase to identify developmental changes in pectins. *Plant Physiology, 91*, 816–822.

Loiudice, R., Impembo, M., Laratta, B., Villari, G., Lo Voi, A., Siviero, P., et al. (1995). Composition of San Marzano tomato varieties. *Food Chemistry, 53*, 81–89.

Luh, B. S., & Daoud, H. N. (1971). Effect of break temperature and holding time on pectin and pectic enzymes in tomato pulp. *Journal of Food Science, 36*, 1030–1043.

McColloch, R. J., Nielsen, B. W., & Beavens, E. A. (1950). Factors influencing the quality of tomato paste II. Pectic changes during processing. *Food Technology, 4*, 339–342.
Oke, M., Ahn, T., Schofield, A., & Paliyath, G. (2005). Effects of phosphorus fertilizer supplementation of processing quality and functional food ingredients in tomato. *Journal of Agricultural and Food Chemistry, 53*, 1531–1538.
Paulson, K. N., & Stevens, M. A. (1974). Relationships among titratable acidity, pH and buffer composition of tomato fruit. *Journal of Food Science, 39*, 354–357.
Stadtman, F. H., Buhlert, J. E., & Marsh, G. L. (1977). Titratable acidity of tomato juice as affected by break procedure. *Journal of Food Science, 42*, 379–382.
Stevens, M. A. (1972). Citrate and malate concentrations in tomato fruits: Genetic control and maturational effects. *Journal of the American Society for Horticultural Science, 97*, 655–658.
Suarez, M. H., Rodriguez, E. R., & Romero, C. D. (2008). Analysis of organic acid content in cultivars of tomatoes harvested in Tenerife. *European Food Research and Technology, 226*, 423–435.
Vermeir, S., Nicolai, B. M., Jans, K., Maes, G., & Lammertyn, J. (2007). High-throughput microplate enzymatic assays for fast sugar and acid quantification in apple and tomato. *Journal of Agricultural and Food Chemistry, 55*, 3240–3248.