Studies on the Alcohol-Insoluble Solids of Chico III and Homestead-24 Tomatoes

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Alcohol-insoluble solids (AIS) tended to be associated with the shape of tomato. The levels of AIS were highest in the pear-shaped tomatoes and lowest in the round tomatoes. Protein levels were generally lower in the AIS from cultivars with high AIS levels. Total pectins of the pectic fractions and composition of the various pectic fractions in AIS did not differ between Chico III and Homestead-24 tomatoes except the water-soluble pectin was higher in the Chico III AIS. Galacturonic acid was the principal component in the pectin of Chico III and Homestead-24 tomatoes. Other sugars identified as components of the pectins were galactose, xylose, ribose, arabinose, and rhamnose. The viscosity of 1% suspensions of AIS of the two was 88 cP for Chico III and 55 cP for Homestead-24. Enzymatic digestion of 1% suspensions of AIS resulted in changes in the viscosity. Pronase caused an increase in viscosity while cellulase and pectinase caused a decrease in viscosity. The changes in viscosity of suspensions for Chico III AIS were greater than for Homestead-24. These results indicate a possible relationship between the proteins, pectins, and cellulose in the overall viscosity of tomato products.

Alcohol-insoluble solids (AIS) affect the consistency of pureed tomato products (Kertesz and McColloch, 1950; Janoria et al., 1975), and the effect has been attributed to various constituents of those solids. Pectic substances are involved in the consistency of tomato juice (Kertesz and Loconti, 1944). McColloch et al. (1950) suggested that the protein fraction of the AIS could be important to the consistency of tomato products. The high molecular weight substances associated with the alcohol-insoluble solids contribute to the viscosity (Foda and McCollum, 1970). Whittenberger and Nutting (1957) have reported that cellulose was the single substance most closely related to viscosity. Whittenberger and Nutting (1958) indicated that the cellulose material responsible for the viscosity was located in the cell walls and concluded that consistency was maximum when both the cell walls and pectin were present in quantity. The consistency depended largely on the quantity, shape, and degree of subdivision of the cell walls, and the character of this material was determined by the presence of pectins. Foda and McCollum (1970) reported that proteins and cellulose contribute to the consistency of tomato products and attributed the viscosity of juice primarily to its insoluble fraction. The AIS are affected by tomato phenotype (Stein and Brown, 1975; McColloch et al., 1950). Those authors have reported that "pear"-shaped tomatoes generally yielded more viscous products and had the highest AIS. Chico cultivar, a "pear"-shaped cultivar and forerunner of the Chico III, has been shown to possess superior processing characteristics to Homestead-24 which is a round-shaped tomato (Stephens et al., 1970). Stein and Brown (1975) observed that the total extractable pectins in the AIS were highest in the round tomatoes. They indicated that the type, not the total quantity, of pectins might determine the overall consistency of the products.

Possibly a complex interaction among the various constituents of the AIS of tomatoes is responsible for the consistency. The purpose of our investigation was to ascertain whether compositional differences in the AIS between the "pear" and "round" types of tomatoes could be responsible for the attributed differences in their processing characteristics.

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MATERIALS AND METHODS

Preparation of Tomato Sample. Chico III and Homestead-24 cultivars were selected for use in this study due to their differences in shape and processing characteristics. The Chico III and Homestead-24 tomato cultivars were grown by the Texas Agricultural Experiment Station, Weslaco, Tex. Five other cultivars were obtained from Texas Agricultural Experiment Station plots or from local producers and all were harvested at the same stage of ripeness. Fully red ripe samples were handpicked to avoid damaged and nonuniform fruit and were carefully washed. Whole tomato samples (about 600 g) were selected, frozen solid with liquid nitrogen, finely crushed in a heavy cloth bag, sealed in cans, and stored at −20 °C until AIS were prepared. Quick freezing of the tomato tissue prior to crushing aided in preventing the degradation of pectic substances during the crushing process.

The crushed, frozen samples were weighed and slowly added to boiling 95% ethanol (2 parts ethanol to 1 part tomatoes) in a blender equipped with a stainless steel blender vessel and heavy duty heating straps to keep the alcohol near boiling during constant blending. The macerate was blended for 2 min, placed in a 2-L beaker, heated to boiling, and allowed to stand overnight. The weight was adjusted to the original sample weight by carefully removing the excess supernatant. Macerated samples were sealed in quart jars and stored at −20 °C for subsequent analysis.

Preparation of Alcohol-Insoluble Solids (AIS). We determined AIS according to the methods of McColloch et al. (1950) but used a Soxhlet extractor. About 50 mL of tomato macerate was carefully weighed into an extraction thimble, extracted six times with 95% ethanol, and then extracted with diethyl ether until the extractant was colorless. The tomato AIS was dried in air then dried in a vacuum oven at 70 °C, cooled, and weighed. AIS from large batches (about 300 g) of tomato macerate were prepared in a similar manner using a large Soxhlet extractor: the large batches of AIS were used for extraction of the pectic substance.

Protein levels in the AIS were determined by the methods of the AOAC (1970) using a conversion factor %N × 6.25.

Extraction of Pectic Substances. Pectic substances were fractionated from a weighed sample (about 2 g) of AIS according to their solubility in water, 0.2% ammonium

oxalate, 0.05 N hydrochloric acid, and 0.05 N sodium hydroxide by exhaustive extraction at 70 °C. Four extractions for 1 h of 200 mL each were sufficient to extract the pectic substances. Extracts were combined, concentrated to 300 mL with a rotary evaporator, and adjusted to a pH of 5.5 by dropwise addition of concentrated hydrochloric acid or sodium hydroxide as needed. Pectic substances in the concentrated extract were determined by the carbazole–hexuronic acid–sulfuric acid method of Dische (1946).

Precipitation of Pectic Substances. Pectic substances were precipitated from combined extracts of each fraction by adding 2 volumes of 95% ethanol containing 0.05 N hydrochloric acid. Final concentration of ethanol in the precipitating mixture was about 60%. The gelatinous precipitate which formed was filtered through a nylon cloth and washed three times with 70% ethanol and pressed dry after each washing to remove residual 70% ethanol entrapped in the gelatinous mass. The precipitated pectin was dried in a vacuum oven at 70 °C.

Enzymatic Hydrolysis of Pectic Substance. Each fraction of the purified pectin was enzymatically hydrolyzed essentially according to the method of Wiley and Tavakoli (1969). Each pectin precipitate was suspended in 300 mL of the extracting medium for that fraction, and the pH of the medium with pectin was adjusted to 5.5 by dropwise addition of 6 N hydrochloric acid or 6 N sodium hydroxide as needed. About one-half the weight of a 1% solution of Pectinol-100D was added to each fraction, layered with toluene, and allowed to hydrolyze for 96 h at room temperature. Then the hydrolysate was filtered through shark skin filter paper and concentrated to about 2.0 mL under vacuum. A twofold volume of 95% ethanol was added to precipitate the enzyme protein. The precipitate was collected by centrifugation at 12 000*g* for 30 min in a Sorvall RC2B refrigerated centrifuge and the supernate was freeze-dried.

Silylation and Gas–Liquid Chromatography. The products of enzymatic hydrolysis of the pectic fractions were silylated by adding 0.25 mL of Tri-Sil-Z reagent (mixture of trimethylsilylimidazole in pyridine) to a known weight, usually 10–30 mg, of the product. The mixture was shaken and allowed to stand at room temperature for 17 h for complete derivitization. For gas–liquid chromatography of the trimethylsilyl derivatives we used a Perkin-Elmer Model 900 gas chromatograph equipped with dual flame ionization detectors and a Leeds and Northrup Speedomax G recorder. The derivitized mixture (1 mL) was injected into a 6 ft × $^1/_8$ in. coiled, stainless steel column packed with Chromosorb W, 60-80 mesh, coated with 3% SE-52. The temperature was programmed at 2 °C/min from 140–200 °C with an injection port and detector temperatures of 260–250 °C, respectively. Helium was used as the carrier gas at a rate of 40 mL/min.

Standards and Identification. The monosaccharides and uronides occurring in each pectic fraction were identified according to their relative positions on the gas chromatogram and by the use of paper chromatography (Hamerman et al., 1955) with authentic standard compounds. The standards used in gas–liquid chromatography were prepared like unknown samples and allowed to muturotate for 24 h prior to freeze-drying. Relative concentrations were calculated to the area under each peak on the gas–liquid chromatogram; areas were determined by the use of a planimeter.

Viscosity of AIS Suspensions. For measurement of viscosity suspensions of Homestead-24 and Chico III AIS suspensions, 3 g of AIS was suspended in 300 mL of water in a 600-mL beaker, maintained at 70 °C, and allowed to extract for 2 h. The suspension was cooled to room temperature. Sufficient samples were prepared to allow for duplicates of each treatment and control. Initial viscosity was measured at zero time. Duplicates of each suspension were digested with 300 mg of cellulase, 75 mg of Pronase (a nonspecific proteolytic enzyme), and 3.0 g of pectinase at room temperature. The enzymes, from commercial sources, were of the highest purity available. At 2 and 4 h after treatment and twice daily until 70 h had elapsed, viscosity was measured in centipoise (cP) with a Brookfield Model LVT viscometer equipped with a No. 2 spindle operated at 60 rpm and 25 °C.

The data was statistically evaluated, where applicable, according to the procedures of Steel and Torrie (1960).

Table I. Alcohol-Insoluble Solids (AIS) and AIS Protein Levels in Tomato Cultivars with Different Shaped Fruit

Cultivar	Shape	% AIS[a]	% protein in AIS
Chico	Pear	1.71[a]	23.47[a]
Chico III	Pear	1.66[a]	22.62[a]
Chico Grande	Large Pear	1.45[b]	24.59[b]
La Bonita	Plum	1.75[a]	26.36[bc]
Saladette	Plum	1.51[b]	26.78[bc]
Homestead-24	Round	1.40[b]	28.96[c]
Monte Grande	Round	1.22[c]	27.46[c]

[a] Values in the table represent means of data collected over a 3-year period except for La Bonita and Saladette which represent 2 years' data. AIS values are expressed as percent of fresh weight. Means in same column with the same superscript are not different.

RESULTS AND DISCUSSION

AIS and protein levels in the AIS for seven cultivars of tomatoes with different shapes appear in Table I. Our results confirmed those of McColloch et al. (1950), who reported AIS values of 1.9% for "pear"-shaped and 1.3% for "round" tomatoes compared with 1.69 and 1.31%, respectively, for those types in our study. AIS in La Bonita, a plum-shaped tomato, was comparable with that of pear-shaped tomatoes and higher than the other plum cultivar tested. AIS in Chico Grande, a large pear-shaped cultivar, was much lower than the pear and only slightly higher than the round types. All data support observations by McColloch et al. (1950) that the shape of tomatoes influences the AIS levels. The size of the tomato might also have influenced the AIS levels, because the round cultivars and the Chico Grande (a large pear) are much larger than the pear- and plum-shaped tomatoes, which are of similar size. Janoria et al. (1975) showed that AIS had a strong correlation with viscosity of the juice and tomato phenotypes.

Because the protein content of AIS is high, McColloch et al. (1950) suggested that proteins could be involved in the consistency of tomato products. Williams and Bevenue (1954) reported the protein content of AIS as 17%, but in our tests, the protein content of AIS ranged from 22.6% for the Chico III to 29.0% for the Homestead-24. The AIS levels were generally inversely related to percent protein in the AIS. La Bonita, however, had a high AIS value (1.75%) and intermediate level (26.8%) of protein in the AIS. Protein in the AIS was highest in the round tomatoes, but on a fresh weight basis did not differ among the various cultivars.

The total extractable pectic substances in the AIS of Homestead-24 were slightly higher, but not significantly, in Homestead-24 than that in the Chico III cultivar (Table II). On a fresh weight basis, however, total pectic substances were 0.38% for Chico III and 0.36% for Home-

Table II. Levels of Alcohol-Insoluble Solids (AIS) and Pectic Substances in Chico III and Homestead-24 Tomatoes[a]

	Chico III, %	Homestead-24, %
Alcohol insoluble solids[b]	1.66*	1.40*
Total pectic substances[c]	22.9	26.2
H_2O soluble	10.6*	12.3*
Oxalate soluble	4.4	5.0
HCl soluble	2.8	3.4
NaOH soluble	5.1	5.5

[a] Asterisk indicates means significantly different at the 5% level of probability. [b] Alcohol-insoluble solids percent of fresh weight, based on duplicate determinations on 60 samples of Chico III and 40 samples of Homestead-24 tomatoes over a 3-year period. [c] Percent of alcohol-insoluble solids.

stead-24 considering that Homestead-24 AIS represents 1.31% of the fresh tomato weight compared with 1.69% AIS in Chico III. The water-soluble fraction was higher in the Homestead-24 AIS than in the Chico III tomatoes. Sodium hydroxide, hydrochloric acid, and ammonium oxalate soluble fractions were not different between cultivars.

Galacturonic acid, galactose, xylose, ribose, arabinose, and rhamnose were, in order with decreasing concentration, the major components of the pectic substances of both tomato cultivars (Table III). Glucose was identified, in traces, in some of the fractions, but was not confirmed as a component or a possible contaminant. Tavakoli and Wiley (1965) found D-glucuronic acid and D-glucose to be components of apple cell wall polysaccharides, but we did not detect them in tomato AIS pectins. We found rhamnose and ribose to be constituents of tomato pectic substances but were not reported as components of apple AIS (Tavakoli and Wiley, 1965). Stevens and Paulson (1976) have recently reported ribose and glucose to be components of tomato AIS. These authors also found mannose to be a component of tomato pectins and did not report the presence of rhamnose. Galacturonic acid, the major component and only uronide present in the pectic substances from each pectic fraction, ranged from 86.06 to 93.74% of the total components identified and quantified. Galacturonic acid was slightly lower in the water soluble than in other fractions. Galactose was the monosaccharide in highest concentration in all pectic fractions and ranged from 3.19 to 7.81% of the total components identified. The levels of each of the components found in pectic substances of each solubility fraction differed slightly within each cultivar; however, there were very few differences in the composition of each fraction between cultivars. The galactose levels in the sodium hydroxide soluble fraction were higher for Chico III (7.02%) than for the Homestead-24 (3.41%). Tavakoli and Wiley (1965) detected no differences in the saccharide and uronide content in apple AIS except for glucuronic acid and glucose, neither found in tomato pectic substances, even though the apples varied in firmness between samples.

Janoria et al. (1975) indicated that AIS was highly correlated with the viscosity of tomato juice. Our data show that AIS values were higher for Chico III than for Homestead-24 tomatoes; however, the pectin levels in the AIS are slightly higher for Homestead-24. Thus, the total pectin in the tomatoes apparently did not differ between the two cultivars assuming that all of the pectin was precipitated by the 80% ethanol in sample preparation. Our observations agree with those of Stephens et al. (1970) for fresh Homestead-24 and Chico (a cultivar closely related to Chico III) tomatoes.

In the 1% suspension of ether-extracted AIS the initial viscosity was 84–88 centipoise (cP) for Chico III and only 50–54 cP for Homestead-24. These data indicated that the levels of AIS alone were not responsible for the reported (Stephens et al., 1970) differences in processing characteristics of the fruit of the two cultivars.

Enzyme treatment caused changes in the viscosity of 1% suspensions of AIS (Table IV). Pronase decreased viscosity during the first 4 h of enzyme action and then increased viscosity. Viscosity of control samples decreased slightly and then increased slightly; however, the change was much greater in the enzyme-treated samples. Foda and McCollum (1970) noted a similar pattern when whole

Table III. Monosaccharides and Uronides in Solubility Fractions of Pectic Substances from Alcohol-Insoluble Solids of Chico III and Homestead-24 Tomatoes[a]

	Weight %							
	Chico III pectic fractions				Homestead-24 pectic fractions			
Component	H_2O	Oxal.	HCl	NaOH	H_2O	Oxal.	HCl	NaOH
Galacturonic acid	86.29	91.70	90.70	90.47	86.06	93.74	90.79	93.52
Galactose	7.73	4.21	6.73	7.02	7.81	3.19	5.97	3.41
Xylose	2.27	1.13	1.03	1.28	2.08	0.78	0.87	1.82
Ribose	1.58	1.44	0.69	0.49	1.76	1.07	0.85	0.53
Arabinose	1.58	1.26	0.57	0.57	1.63	1.08	0.87	0.62
Rhamnose	0.63	0.33	0.29	0.28	0.66	0.24	0.65	0.11

[a] Data are means of duplicate determinations on extractions with each solvent from eight AIS samples of each cultivar.

Table IV. Effect of Cellulase, Pronase, and Pectinase on the Viscosity of 1% Suspensions of Alcohol-Insoluble Solids from Chico III and Homestead-24 Tomatoes

	Viscosity, cP							
	Control		Cellulase		Pronase		Pectinase	
Time, h	C-111	HS-24	C-111	HS-24	C-111	HS-24	C-111	HS-24
0	88	52	88	54	85	49	84	50
2	82	50	67	40	80	44	50	36
4	79	48	67	40	84	46	49	35
20	92	51	61	39	120	55	43	32
28	95	54	62	38	143	67	40	33
45	104	56	61	37	134	80	36	30
52	104	56	59	35	142	85	35	28
70	108	59	59	36	133	88	31	25

tomato juice was treated with Pronase. The increase in viscosity after Pronase digestion was greater in Chico III AIS than in Homestead-24 suspensions.

Enzymatic digestion with cellulase and pectinase caused a decrease in the viscosity of a 1% suspension of AIS from both cultivars. Pectinase decreased the viscosity to a greater extent than did cellulase. This may be attributed to the cellulase contamination inherent in commercial pectinase preparations. As was the case with Pronase, the change in viscosity was greater in the suspensions of Chico III AIS.

The AIS levels tended to be indicative of the shape of tomato. The levels of protein and pectin in Chico III and Homestead-24 AIS were not sufficiently different to account for the previously noted differences in characteristics (Stephens et al., 1970); however, protein levels in the AIS were generally lower in cultivars with high AIS. The compositions of the various pectic fractions of Chico III and Homestead-24 AIS were not different. Viscosity of 1% suspensions was considerably higher for Chico III than Homestead-24. This shows that AIS content alone does not explain the attributed differences. Enzymatic digestion of AIS suspensions of the Chico III and Homestead-24 tomatoes indicated a combination of factors may affect the character of processed tomato products.

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Lipids of *Penicillium roqueforti*. Influence of Culture Temperature and Age on Unsaturated Fatty Acids

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The lipids of *Pencillium roqueforti* were influenced by culture age and temperature. Maximum lipid accumulation in the mycelium occurred at a culture age of 120 h at 25 °C. During the log growth phase the relative proportions of both protein and polar lipid decreased, while carbohydrate and triglycerides increased. Palmitic, stearic, and oleic acids increased to a peak level at 120 h of incubation and then declined, whereas linoleic acid increased throughout the incubation period. A decrease in the growth temperature resulted in a decrease in the amount of all components in the mycelium. The relative proportions of protein, polar lipid, and free fatty acid increased while triglycerides decreased as growth temperatures were lowered. As temperature was decreased from 25 to 11.5 °C, linoleic acid content increased from 20.6 to 26.0%, and linolenic acid from 0 to 9.1% of total fatty acids. These increases occurred principally in the polar lipids. The increase in linoleic acid during the log growth phase occurred almost entirely within the polar lipids, whereas during the stationery phase, the increase occurred mostly within the triglycerides.

Penicillium roqueforti is the principal microorganism involved in the biochemistry of blue-type cheeses where lipid catabolism and oxidation are essential events in the ripening process (Kinsella and Hwang, 1976, 1977). However, there is little information available regarding the composition and metabolism of the lipids of mycelium of *P. roqueforti* and the factors which affect these.

There are data available on the lipids of other fungi (Weete, 1974), though most analyses are not comprehensive. The effects of changes in growth temperature on fungal lipid content and fatty acid composition have been investigated (Kates and Baxter, 1962; Meyer and Bloch, 1963; Brown and Rose, 1969; Kates and Paradis, 1973). The effects of culture aging on composition of fungi have been reported in relation to protein and carbohydrate (Gottlieb and VanEtten, 1964), lipid content (Weete et al.,

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