The Pectic Substances of Mature John Baer Tomatoes

By Z. I. Kertesz and R. J. McColloch

NEW YORK STATE AGRICULTURAL
EXPERIMENT STATION
GENEVA, N. Y.

BULLETIN NO. 745 AUGUST, 1950

ABSTRACT

THE various pectic substances present in tomatoes are known to play an important part in the characteristics of the fresh fruit and in the quality of tomato products.

Although the total amount of pectic substances in mature tomato fruit is now fairly well established, little seems to be known of the various types of pectic materials, especially pectinic acids, which make up the bulk of the total pectic constituents. In addition to obtaining information on this point, an attempt was made in the present study to establish whether tomatoes which are just slightly underripe or overripe (as judged mostly from color) will show any significant differences in pectic constituents.

Within the narrow maturity range studied, the John Baer tomatoes used in this study do not show definite trends of practical importance in their pectic constituents. This further strengthens the assumption that color is not a reliable indicator of the true maturity of the tomato fruit.

The pectinic acids isolated from the tomatoes were characterized by their solubility behavior, uronide and methoxyl content, and solution viscosity.

The proteins of tomatoes become easily incorporated into pectic preparations. About one-fourth of the pectinic acids, obtained by extraction with ammonium oxalate, showed average methyl ester contents which would put this fraction into the class of low-ester (low-methoxyl) pectinic acids.

This finding is of significance in explaining the reactivity of the pectic constituents with polyvalent ions as, for instance, with calcium used in firming canned whole tomatoes.

A publication of the
New York State Agricultural Experiment Station
Geneva, N. Y.,
New York State College of Agriculture
A unit of the State University of New York
At Cornell University

BULLETIN No. 745

THE PECTIC SUBSTANCES OF MATURE JOHN BAER TOMATOES[1]

Z. I. KERTESZ AND R. J. McCOLLOCH[2]

INTRODUCTION

THE large increase in the production of tomatoes and the manufacture of tomato products during the past 25 years has brought about increased interest in the chemical composition and biochemical behavior of the tomato fruit. The majority of the investigations noted below dealt with components which affect the commercial value of tomatoes by influencing color, texture, and nutritional properties of the fresh fruit and of various tomato products.

For some time this Station has been concerned with the pectic constituents of tomatoes. While present in comparatively small proportions, these compounds seem to play an important role in the texture and firmness of fresh fruit as well as of canned tomatoes, and also influence the consistency of tomato products such as juice, puree, and paste (10, 22).[3] During these investigations, the lack of detailed information on the occurrence of the various types of pectic materials in tomatoes became apparent, together with the need for a more exact characterization of pectic components[4] by their chemical composition and solubility behavior.

The reports of Conrad (3), and Appleman and Conrad (1) called attention for the first time to the important role of pectic constituents in fresh and canned tomatoes. Appleman and Conrad made a study of both the transformations which occur in the pectic constituents during maturation and changes which are caused by canning operations. They found that the pectinic acid content, designated by them as pectin and extracted by cold ammonium citrate solution and water, increased while the content of protopectin, extracted by boiling 0.5 per cent ammonium citrate solution and boiling N/30 hydrochloric acid, decreased as the fruit passed through the green-mature, pink,

[1]These investigations were in part supported by a grant from the American Can Company.

[2]Now located at the Laboratory of Fruit and Vegetable Chemistry, United States Department of Agriculture, Pasadena, California.

[3]Reference made by number to Literature Cited, page 14.

[4]The nomenclature adopted by the American Chemical Society in 1944 (9) is used throughout this paper.

and red ripe stages of development. Some transformation of protopectin into more easily soluble pectinic acids was observed during the canning process and a further slight change in the same direction was noted when the cans were allowed to stay warm after canning.

Subsequent work by Kassab (7), LeCrone and Haber (12), Bohart (2), and Loconti and Kertesz (13) indicated that there is a great variation in the proportion of total pectic substances in tomatoes.

Paech (20) investigated the relation between softening, color formation, and changes in the pectic constituents in ripening tomatoes and found that the proportion of soluble pectin (pectinic acids) changed little after the fruit turned light green, while the proportion of acid-soluble pectic substances (protopectin) decreased throughout the season and reached a level equal to that of the pectinic acids as the fruit turned red.

None of these investigations dealt with the methyl ester content of the pectinic acids obtained from tomatoes. The recent emphasis on the practical application of low-ester pectins (5) suggested that the behavior of the pectic constituents of tomatoes might at times be governed by their low ester content. This assumption was made more probable by the presence in tomatoes (8) of a very active pectin-methylesterase (pectase).

The present paper deals with the results of investigations of the pectic constituents of unripe, ripe, and overipe John Baer tomatoes.

EXPERIMENTAL METHODS

The fresh John Baer tomatoes used in these experiments were grown on the Station farm and were gathered in October, 1944. Three classes of tomatoes with respect to the degree of ripeness were selected, according to the following arbitrary criterion: Unripe tomatoes were taken as those which, though partially red, still contained some small patches of green coloration of the skin. Ripe tomatoes were taken as those fully red ripe and still firm. Overripe tomatoes were taken to be those which were dark red and had definitely begun to soften to such an extent as to make damage in transit under commercial conditions probable. Cracked and blemished tomatoes were carefully excluded.

The tomatoes selected for these three groups on any given day were weighed and the weights recorded. The fruit in each sample was then cut separately with a stainless steel paring knife into a

sufficient volume of boiling 95 per cent alcohol to give a final concentration of 70 per cent alcohol in the mixture. The cut thin slices were allowed to drop directly into the boiling alcohol to minimize the possibility of enzyme action which might alter the original character of the tomato pectins. This operation was performed in batches of tomatoes weighing less than 1 kg per batch.

After completion of the cutting, the mixture was boiled for a few seconds and was then allowed to cool and to stand overnight. Next day the solids were separated by squeezing the mixture through a rayon cloth. The alcohol-insoluble solids were dehydrated by letting them stand submerged in 95 per cent alcohol. They were then drained, rinsed with some more 95 per cent alcohol, pressed out, and rubbed in a large porcelain mortar until dry. The dry samples were extracted with anhydrous ether. Considerable color was removed by these latter extractions, but the losses in the weights of the samples were insignificant. The samples were again dried in air, stored in brown bottles, and designated as alcohol-insoluble solids (AIS). They were given the code letters A, B, and C, indicating that they were obtained from unripe, ripe, and overripe tomatoes, respectively.

For the preparations of the different pectic fractions, exactly 25-gram quantities of each unripe (A), ripe (B), and overripe (C) AIS were weighed out and extracted twice for 2 hours with 800 ml of water at 30°C. The extracts were combined and made up to 2 liters. The residues were then each extracted twice with similar volumes of 0.5 per cent ammonium oxalate at 30°C. Finally, the residues from the oxalate treatments were extracted twice with 0.05 N HCl at 85°C.

These extracts, containing the water, oxalate-, and acid-soluble fractions from the AIS representing underripe, ripe, and overripe tomatoes were then each separately precipitated by the addition of 2 volumes of 95 per cent alcohol (final concentration about 67 per cent). Enough hydrochloric acid was added to the mixtures to give a final normality of 0.05 N.

The alcohol precipitates were filtered on sharkskin filter paper and washed with acidified alcohol followed by pure 95 per cent alcohol. The precipitates were scraped off the paper and dried in air by grinding in a mortar. The last traces of alcohol were removed by letting the dried precipitates equilibrate over water in a desiccating jar at reduced pressure for several days (6). They were then re-dried over phosphorus pentoxide under high vacuum at 85°C for 4 hours. The dry samples were weighed and stored in air-tight bottles for

further analysis. The nine fractions obtained were identified as the "prepared alcohol precipitates" of the water (A-1, B-1, C-1), oxalate (A-2, B-2, C-2), or acid (A-3, B-3, C-3) fractions of the AIS samples A, B, and C.

The methoxyl contents of the "prepared alcohol precipitates" were determined by the modified volumetric Zeisel method (18). The determinations were made on 10-mg samples and the titrations made with 0.020 N sodium thiosulfate from a microburette.

The uronic acid contents of the "prepared alcohol precipitates" were determined by the method of Whistler, Martin, and Harris (23). Exactly 0.2000-gram samples were used and the carbon dioxide evolution was measured every hour for 8 hours. The average carbon dioxide evolved per hour after the first 5 hours was taken to represent the hourly formation of carbon dioxide by nonuronide materials. The corresponding correction was therefore subtracted from the total carbon dioxide liberated in 5 hours to obtain that liberated from the uronic acids. The pectic acid contents of the samples were calculated by multiplying the (corrected) mg of carbon dioxide liberated by 4.0. The pectinic acids were found by adding 17/31 of the weights of methoxyl in the samples of prepared alcohol precipitates to the weights of pectic acids. The factor 17/31 was used to correct for the 1 hydroxyl group lost when the methyl ester is metathetically formed from a carboxyl group and a methoxyl radical.

For the determination of relative viscosity, 0.1-gram samples of the "prepared alcohol precipitates" were weighed out and then dissolved as completely as possible by adding 75 ml of hot distilled water with rapid stirring. The dissolved samples were cooled, filtered, and made up to 100 ml in a manner to contain 0.9 per cent sodium chloride and to be at pH 2.0. The viscosities were determined on small portions of these solutions at 30°C in a Cannon-Fenske-Ostwald viscosimeter. Duplicate aliquots of the remaining portions of these solutions were submitted to calcium pectate determinations (21). The relative viscosities of the solutions found were plotted against the concentration of calcium pectate and the relative viscosities of 0.1 per cent solutions obtained by extrapolation. The tests showed that under these conditions, the relative viscosity is nearly a straight line function of concentration in the range encountered. It is assumed that the viscosities of solutions as measured under such conditions are indicative of the comparative average molecular weights of the pectinic acids.

Nitrogen was determined by the Nessler method and expressed as protein nitrogen (N × 6.25) on the assumption that protein would be the primary nitrogenous compound present in these alcohol-extracted samples. The crude fiber content of the AIS samples was also determined by employing the accepted official method (19) and definition.

RESULTS

The results obtained are given in Tables 1, 2, and 3. The results in Table 3 are those obtained by direct determinations of the materials specified. The values given in Table 2 (exclusive of crude fiber) were derived from results in Table 3 on the basis of the percentages of "prepared alcohol precipitates" extracted from the alcohol-insoluble solids. The results in Table 1 are expressed on the basis of the fresh tomatoes and were derived from Table 2 on the basis of the percentages of the alcohol-insoluble solids (AIS) in the fresh tomatoes.

It should be pointed out again that the term "pectinic acids" refers to the sum of the polyuronides obtained by carbon dioxide determinations on the tomato constituents precipitable by 67 per cent alcohol, plus 17/31 of the respective Zeisel methoxyl content. The pectinic acids present in these samples were also determined by the calcium pectate method. The results by this latter method paralleled the results obtained by the carbon dioxide determination but ran 10 to 40 per cent higher and qualitative tests showed that in many cases the calcium pectates contained some substances in addition to polyuronides. Such observations were also reported by Appleman and Conrad (1). Rather than using various methods for correcting the calcium pectate values (1, 4), we preferred to determine the pectic constituents by the carbon dioxide method on the alcohol-precipitated material.

DISCUSSION

Table 1 shows the chemical composition of the three samples of tomatoes of slightly different maturities. The proportion of alcohol-insoluble constituents was very similar in the three samples. As shown in Table 2, about 10 per cent of the AIS consisted of pectic substances. The crude fiber contents of the three samples were similar and represented 27.5 to 30.4 per cent of the AIS. The protein fraction (derived by multiplying the nitrogen content by 6.25) was also uniform and represented 25.8 to 27.8 per cent of the AIS.

Turning now to the pectic constituents, it is apparent from Table 3 that the oxalate-soluble fraction was practically constant while the

TABLE 1.—INFORMATION ON THE JOHN BAER TOMATOES USED FOR THE PREPARATION OF PECTINIC ACIDS.

SAMPLE	NO. OF SAMPLES TAKEN	TOTAL NO. OF FRUITS USED	FRESH WEIGHT OF FRUIT, GRAMS	WEIGHT OF ALCOHOL-INSOLUBLE PORTION, GRAMS	ALCOHOL-INSOLUBLE FRACTION, PER CENT	PERCENTAGE PECTINIC ACIDS* SOLUBLE IN			PROTEIN (ALCOHOL-INSOLUBLE N × 6.25), PER CENT	CRUDE FIBER, PER CENT
						WATER	OXALATE	ACID		
	1	2	3	4	5	6	7	8	9	10	11
Unripe	3	22	3,466	52.0	1.50	0.080	0.044	0.048	0.172	0.38	0.43
Ripe	7	56	8,112	130.0	1.60	0.062	0.040	0.032	0.134	0.44	0.44
Overripe	5	28	4,297	64.5	1.50	0.061	0.041	0.060	0.162	0.42	0.46

*See Tables 2 and 3 for details.

TABLE 2.—COMPOSITION OF THE HOT 70 PER CENT ALCOHOL-INSOLUBLE FRACTION OF TOMATOES.

CODE, SAMPLE	PERCENTAGE ALCOHOL PRECIPITABLE FRACTION EXTRACTED BY SUCCESSIVE EXTRACTIONS WITH				TOTAL POLYURONIDE (BY CO_2) PER CENT*	TOTAL PECTINIC ACIDS, PER CENT*	AV. METHOXYL CONTENT OF 6, PER CENT	AV. DEGREE OF ESTERIFICATION OF 6, PER CENT	PROTEIN (ALCOHOL-INSOLUBLE, N × 6.25), PER CENT	ASH, PER CENT	CRUDE FIBER, PER CENT	SUM OF 6, 9, 10, AND 11, PER CENT
	Water 1	Oxalate 2	Acid 3	Total 4	5	6	7	8	9	10	11	12
A Unripe	7.50	3.56	7.45	18.50	10.84	11.44	9.66	59	25.8	5.9	28.9	71.9
B Ripe	5.73	3.13	5.87	14.73	7.95	8.40	10.15	62	27.2	6.2	27.5	69.3
C Overripe	6.39	3.34	8.70	14.43	10.27	10.82	9.43	58	27.8	6.5	30.4	75.5

*See Table 3 for details.

TABLE 3.—COMPOSITION OF ALCOHOL-PRECIPITATED PREPARATIONS FROM WATER, OXALATE, AND ACID EXTRACTS FROM THE ALCOHOL-INSOLUBLE PORTIONS OF UNRIPE (A), RIPE (B), AND OVERRIPE (C) TOMATOES.

CODE	EXTRACTANT	POLYURONIDE (BY CO_2), PER CENT	Methoxyl, PER CENT	PECTINIC ACID (FROM 1 AND 2), PER CENT	METHOXYL ON BASIS OF 3, PER CENT	DEGREE OF ESTERIFICATION OF 3, PER CENT	RELATIVE VISCOSITY, (0.1 PER CENT SOLUTION)	PROTEIN (ALCOHOL-INSOLUBLE N × 6.25), PER CENT	ASH, PER CENT	SUM OF 3, 7, AND 8, PER CENT
		1	2	3	4	5	6	7	8	9
A-1	Water	66.0	8.9	70.0	12.5	77	1.57	3.4	6.11	79.5
A-2	Oxalate	79.8	4.6	81.9	5.6	34	1.67	3.3	2.69	87.9
A-3	Acid	41.0	3.6	42.6	8.5	52	1.39	29.5	5.86	78.0
B-1	Water	63.4	8.2	67.1	12.2	75	1.98	3.3	4.56	75.0
B-2	Oxalate	78.2	5.0	80.5	6.2	38	1.53	3.1	4.28	87.9
B-3	Acid	31.8	3.9	33.6	11.6	71	1.73	31.6	6.45	71.7
C-1	Water	59.8	7.5	63.2	11.9	73	1.93	3.4	10.24	76.8
C-2	Oxalate	79.4	4.9	81.6	6.0	37	1.26	3.5	7.38	92.5
C-3	Acid	43.6	4.3	45.5	9.5	58	1.80	32.3	6.16	84.0

water- and acid-soluble fractions were irregular. The results are in better agreement with those of Paech (20) than with the findings of Appleman and Conrad (1). True enough, the latter authors used the calcium pectate method (21) which they, as well as we, have found unreliable. Yet, these differences cannot be accounted for on the basis of the methods used in determining the pectic substances, i.e., carbon dioxide evolution vs. calcium pectate, since the calcium pectate determination made along with these investigations showed the same general trends as were found by the more accurate and less ambiguous uronide-carbon dioxide method.

It is more likely that the differences are due to either one or a combination of two other possible factors. The first one might be the procedure used for preparing the alcohol-insoluble solids from the fresh tomatoes. The previous authors macerated the tomatoes in a meat grinder and allowed the resulting pulp to drop into hot alcohol. This procedure might allow more time for any pectolytic factors in the tomato tissue to act before inactivation occurred than our method of slicing the tomatoes directly into boiling alcohol. Since the activity of the pectolytic factors present increases markedly with the ripening of the tomato fruit, their results may reflect in part this increase in activity.

The second, and perhaps more significant, factor affecting these results may be found in the latitude possible in defining ripe, unripe, and overripe tomatoes. The previous authors extended their definition from wholly green tomatoes as unripe to disintegrating tomatoes as overripe. There is serious doubt whether the extent of red coloration is a dependable index of progressive internal chemical changes typical of the maturing tomato fruit. The definitions for the three sets of samples given above are such that they include a much narrower range of maturity than has been covered by previous students of pectic changes in tomatoes. However, our main purpose was to establish the variations in the pectic fractions of tomatoes of the type now delivered to processing plants.

Table 2 shows the composition of the AIS obtained from the three sets of samples. Here the differences between the amount of material extracted by the various solvents and precipitated by alcohol and the polyuronide contents calculated from the carbon dioxide obtained from these same samples are noteworthy (columns 4 and 5). The average methoxyl contents of the pectinic acids were not greatly different. It is of importance to note that protein made up one-fourth

of these samples. This clearly indicates the fallacy of using alcohol precipitation in the determination of the pectic constituents of plant materials. The ash content of these preparations was fairly low considering that a direct alcohol precipitation without the use of added acid was applied in their isolation. The constituents listed in Table 2 (pectinic acids, protein, ash, and cellulose) accounted for 69.3 to 75.6 per cent of these samples.

Table 3 shows the results of the actual polyuronide determinations on the individual fractions extracted by water, oxalic acid, and hydrochloric acid and precipitated by alcohol ("prepared alcohol precipitates"). The low uronide contents of all samples, but especially of those obtained by acid extraction, is quite surprising. In these latter samples the protein content was high. Column 9, in which the percentages of these various components were combined, indicates that the low polyuronide content of the acid-extracted samples was mainly due to their high protein content. The variations of the totals may be due mainly to two factors. First of all, in such calculations, there is a distinct possibility of enlarging by multiplications the various experimental errors. Secondly, the use of the factor 6.25 for tomato protein is arbitrary. While it is fairly certain that in this type of material, which has been extracted with alcohol, the bulk of the nitrogen found is derived from proteins, there is no assurance that the factor 6.25 holds for these compounds. Unfortunately there is no specific information available on this point at the present time.

Table 3 shows that there appear to be three characteristic pectinic acid fractions present in the tomato fruit. The water-soluble fractions are characterized by having high methoxyl contents and comparatively high molecular weights, as judged by the relative viscosities of the solutions. The acid-soluble fractions are characterized by lower methoxyl content and the oxalate-soluble fractions by yet lower methoxyl contents. If relative viscosity can be accepted as a criterion of the comparative molecular weights, it appears from column 5 of Table 3 that the molecular weights of the water-soluble pectinic acids reach a maximum at the ripe stage and that the molecular weight of the acid-soluble fraction increases while that of the oxalate fraction appears to decrease steadily. Columns 6, 7, and 8 of Table 1 seem to indicate that the acid-soluble pectinic substances are increasing at the expense of the water- and oxalate-soluble fractions.

The results indicate a comparatively high average degree of esterification in the pectinic acids isolated from these three sets of tomatoes

(Table 2). Pectinic acids of an average degree of esterification of about 60 per cent could not be expected to play an important part in the calcium firming of tomatoes (11), a reaction which involves the formation of water-insoluble acid calcium pectinates (13). Taking the average degrees of esterification at face value, such a reaction with calcium could only be expected to occur after some additional de-esterification by the naturally present enzyme, pectin-methyl-esterase (8). On the other hand, a perusal of the data in Table 3 indicates that in all three samples of tomatoes the oxalate-soluble fraction of the pectinic acids showed a degree of esterification of 38 per cent and below. Such pectinic acids are known to form insoluble complexes with calcium and, therefore, some degree of firming might occur due to the reaction between this fraction and the calcium even without any additional de-esterification. In fact, the solubility in ammonium oxalate indicates that these fractions might have been made insoluble in water by the presence of polyvalent ions in the tomatoes.

The present study was made to serve as the starting point of more extensive investigations into the fate of the pectic constituents of tomatoes during the manufacturing of tomato products. This latter work soon led to some observations which cast considerable doubt on the usefulness and, indeed, validity of the present methods of pectin chemistry. For instance, the alcohol-insoluble solids found in two corresponding samples of tomato juice prepared by the "hot break" and the "cold break" methods (10) contained 11.3 per cent and 6.6 per cent polyuronides, respectively, by the carbon dioxide method (23), while the corresponding determinations on the same materials by the calcium pectate method (15) gave 8.3 per cent and 0.8 per cent, respectively. This apparent inconsistency led to the discovery of other pectolytic factors (14, 15, 16, 17) which will be dealt with elsewhere. It also emphasized the difficulties in our definition of pectic substances and led to the omission of the use of the calcium pectate method in the present study.

SUMMARY

The alcohol-insoluble fractions were prepared from three large lots of tomatoes representing slightly unripe, ripe, and slightly over-ripe fruit as judged by the extent of coloration and softening. These fractions were extracted by water, oxalic acid, and hot dilute hydrochloric acid and the extracted materials precipitated by acidulated

alcohol. The latter samples were analyzed for polyuronides by the carbon dioxide evolution method and for methoxyl groups by the Zeisel method. The nitrogen and ash contents and the viscosity in a 0.1 per cent solution of all samples were also determined.

These studies indicate that the pectic constituents do not show definite trends in either quantity or composition during the period from just before until just after the peak of optimum ripeness for processing. Three characteristic types of pectinic acids were found to be present which were distinguished by their solubilities in the three solvents used. These fractions showed characteristic differences in their methoxyl contents and relative average molecular weights as indicated by viscosity measurements.

The extracted pectinic acids when precipitated by acidulated 67 per cent alcohol gave precipitates which contained up to two-thirds of nonuronide impurities, mostly proteins.

LITERATURE CITED

1 Appleman, C. O., and Conrad, C. M. The pectic constituents of tomatoes and their relation to the canned products. *Maryland Agr. Exp. Sta. Bul. 291. 1927.*

2 Bohart, G. S. Chemical studies of raw products used for canning. *The Canner*, **82,** *113. 1936.*

3 Conrad, Carl M. A biochemical study of the insoluble pectic substances in vegetables. *Amer. Jour. Bot.*, **13,** *531–547. 1926.*

4 Heinze, Peter H., and Appleman, C. O. A biochemical study of curing process in sweet potatoes. *Plant Physiol.*, **18,** *548–555. 1943.*

5 Hills, Claude H., White, Jonathan W. Jr., and Baker, George L. Low-sugar jellying pectinates. *Proc. Inst. Food Tech.*, **1942,** *47–58. 1942.*

6 Jansen, E. F., Waisbrot, S. W., and Rietz, E. Errors in Zeisel methoxyl values for pectin due to retained alcohol. *Ind. and Eng. Chem., Anal. Ed.*, **16,** *523–524. 1944.*

7 Kassab, M. A. Observations on the pectin content of California tomatoes. *Fruit Prod. Jour.*, **11,** *69–70. 1931.*

8 Kertesz, Z. I. Pectic enzymes, II: Pectic enzymes of tomatoes. *Food Res.*, **3,** *481–487. 1938.*

9 ————, Baker, George L., Joseph, Glenn H., Mottern, H. H., and Olsen, Aksel G. Report of the committee for the revision of the nomenclature of pectic substances. *Chem. Eng. News*, **22,** *105. 1944.*

10 ————, and Loconti, J. D. Factors determining the consistency of commercial canned tomato juice. *New York State Agr. Exp. Sta. Tech. Bul. No. 272. 1944.*

11 ————, Tolman, T. G., Loconti, J. D., and Ruyle, E. H. The use of calcium in the commercial canning of whole tomatoes. *New York State Agr. Exp. Sta. Tech. Bul. No. 252. 1940.*

12 Le Crone, F., and Haber, E. S. Changes in the pectic constituents of tomatoes in storage. *Iowa State Col. Jour. Sci.*, **1,** *467–476. 1933.*

13 Loconti, J. D., and Kertesz, Z. I. Identification of calcium pectate as the tissue-firming compound formed by treatment of tomatoes with calcium chloride. *Food Res.*, **6,** *499–508. 1941.*

14 MacDonnell, L. R., Jansen, Eugene F., and Lineweaver, Hans. The properties of orange pectinesterase. *Arch. Biochem.*, **6,** *389–401. 1945.*

15 McColloch, R. J., and Kertesz, Z. I. An unusually heat-resistant pectolytic factor from tomatoes. *Arch. Biochem.*, **17,** *197–199. 1948.*

16 ————, ————. Recent developments of practical significance in the field of pectic enzymes. *Food Tech.*, **3,** *84–86. 1949.*

17 Mottern, H. H., and Hills, Claude H. Low ester pectin from apple pomace. *Ind. and Eng. Chem.*, **38,** *1153–1156. 1946.*

18. Niederl, Joseph B., and Niederl, Victor. Micromethods of quantitative analysis. *New York: John Wiley and Sons, Inc. 1938.*

19 Official and Tentative Methods of Analysis. *Washington: Assoc. Off. Agr. Chem.* Ed. 6. *1945.*

20. Paech, K. Pflanzenphysiologische Grundlagenforschung. *Landw. Jahrb.*, **85,** *653–660. 1938.*

21 Poore, H. D. Recovery of naringin and pectin from grapefruit residue. *Ind. and Eng. Chem.*, **26,** *637–639. 1934.*

22 Smith, H. R. Consistency of tomato catsup. *The Canning Trade*, **54,** *14. 1931.*

23 Whistler, Roy L., Martin, Albert R., and Harris, Milton. Determination of uronic acids in cellulosic materials. *Jour. Res. Nat. Bur. Standards*, **24,** *13–23. 1940.*