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Softening of Commercial Cucumber Salt-Stock in Relation to Polygalacturonase Activity[a]

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A sensitive method is described for detecting a pectin-splitting enzyme in commercial cucumber brines. The enzyme compares similarly in chemical behavior to polygalacturonase and has been found present in cucumber brines from various brining areas and correlated with softening of the salt-stock.

During the brine fermentation of cucumbers for manufacture of pickles, the industry is often faced with an uncontrollable problem of spoilage known as "softening." At such times the cucumbers, referred to as salt-stock, soften to a varying extent. They may either lose their firmness completely, becoming mushy; or they may develop softening to such a minor degree that it is hardly noticeable even to an experienced plant operator. In some years, the spoilage is far more serious and widespread than in others. The economic loss to the industry is considerable when soft salt-stock has to be discarded or, at best, used in low-quality products.

The commercial brining operation has been described *(5)* as follows: "The cucumbers are brined in wooden vats ranging in capacity from 100 to 1,200 bushels. The vats, after being filled with cucumbers, are fitted with false heads made of wooden boards, and salt brine of a given concentration is added to a level a few inches above the head. Next, dry salt is added on the false head of the vat to maintain the initial concentration, which otherwise would become diluted by the water from the cucumbers. The initial brine strength used ranges from about 8 to 10%, depending upon the individual plant concerned. In most instances the brine concentration is gradually raised so that a holding strength of about 16 to 18% is reached after about six weeks. Under these conditions an active acid fermentation resulting from the growth of salt-tolerant, acid forming bacteria begins within a day or so after the cucumbers are brined and may continue for about six weeks. The preserving effect of the brine is due chiefly to the combined action of salt and the developed acidity." Under most commercial conditions, the acidity developed falls in the range of 0.4 to 0.9%, calculated as lactic acid; the pH is generally depressed below 3.8 *(5, 11)*.

It is generally accepted that softening is seldom detected until after the active fermentation period. The softening action has been considered to be the result of action by pectin-splitting enzymes on the pectin composing the middle lamella of cucumber tissue. The chemistry of pectin and its association in fruits and vegetables has recently been the subject of three review articles *(2, 13, 19)*. Phaff and Joslyn *(21)*, in their review on pectic enzymes, concluded that there were two principal types; pectinesterase (*syn.* pectase) which catalyzes the de-esterification of pectin by removal of the methoxyl groups, and polygalacturonase (*syn.* pectinase) which catalyzes the glycosidic hydrolysis of pectin or pectic acid.

The present study considers principally the detection of pectin-destroying enzymes in commercial cucumber fermentations. There was no information found in the literature dealing with softening of cucumbers brined under commercial conditions and the enzymes involved. A method which would permit the early detection of such enzyme systems would prove valuable for the pickle industry for it would permit the early forecasting of the probable final quality of salt-stock. Likewise such a method would benefit the bacteriologist conducting research in brine fermentations for it would serve as a measure of the softening activity of organisms encountered. For the chemist in fermentation research, the proposed method would aid in developing softening control procedures. The enzyme responsible for the destruction of pectin is polygalacturonase; hence, at the start of these experiments polygalacturonase was considered to be associated with cucumber softening. The results to follow substantiate this assumption.

Selection of Method

Phaff and Joslyn *(21)* list five general methods for measuring activity of polygalacturonase on pectin solutions: Increase in reducing substances measured as aldoses; decrease in calcium pectate precipitate; decrease in alcohol precipitate; marked drop in viscosity of pectin solution; and decrease in optical rotation.

Some of these methods have definite limitations for application to cucumber brines. The methods *(9, 16, 17, 23)* with the exception of the drop in viscosity of pectin solution that have been reported depend on a strong glycosidic hydrolysis within a range of time extending from a few minutes to several hours. Hydrolysis at this rate would require relatively high concentrations of the enzyme in order to be effective. The softening of cucumbers is extremely slow compared to such a reaction and therefore one would expect relatively small amounts of enzyme in the brine. There is further reason to believe that softening is not a complete splitting of the pectic substances in the cucumber to galacturonic acid, but rather only a depolymerization of the protopectin molecule. This is indicated by the work of Fabian and Johnson *(6)* who found that the pectin content of

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mushy, as well as firm salt-stock cucumbers, was the same when measured as calcium pectate. A similar relationship has been shown in connection with the ripening and softening of peaches *(1)*.

Jansen and MacDonnell *(9)*, studying the change in viscosity of pectin by polygalacturonase, reported that a 50% change in viscosity of a 1.0% pectin solution hydrolyzed by the enzyme, or by heat, occurs with only a 2% breakage of the glycosidic bonds. Kertesz *(14)* observed that a 1% hydrolysis by the enzyme on a pectin solution would cause about 35% reduction in the viscosity. He pointed out that the decrease in viscosity was more rapid than the increase of aldehydic reducing groups. Viscosity changes of a 1% pectin solution was used by Mottern and Hills *(17)* as a measurement of the enzyme activity in tomato extract.

At the start of the present work several workers *(3, 12, 22)* in the field of pectin and pectic enzymes suggested that the viscosity method might prove the most satisfactory for indicating low concentrations of the enzymes. Principal emphasis has been placed on developing such a method for brines, although the method described by Jansen and MacDonnell *(9)* for measuring liberated aldehydic groups as aldoses was also investigated. This method was more accurate than the viscosity method and more specific for high concentrations of the enzyme, but it was found that there was not sufficient enzyme in a polygalacturonase-active brine to give a measurable reduction of a standard iodine solution using either pectin or pectic acid. It was evident from these observations that this method was not suitable for the present work. Details of the work with this method are therefore not included in this report.

Viscosity Method of Measurement

Materials. The pectin[d] used in the experiments described was a commercial citrus product of highest quality, recommended by the research staff *(12)* of the California Fruit Growers Exchange as the most desirable material for the work. The polygalacturonase enzyme system used was the product Pectinol M[e]. As this product contains 90% glucose as diluent the quantities of the enzyme in the standards were one-tenth the quantity of the product used. Studies of the Pectinol enzyme by Fish and Dustman *(7)* and Jansen and MacDonnell *(9)* have shown the principal enzyme to be polygalacturonase with small amount of pectinesterase being also present. More recently Lineweaver, Jang, and Jansen *(15)* have reported several more enzymes including cellulase and amylase to be present in the Pectinols.

Procedure. Pectin solutions of low concentrations exhibit viscosities of relative large value. The pectin molecule being a high polymer of galacturonic acid may serve as an indicator for measuring extremely small quantities of polygalacturonase. The method used in this study was essentially that of measuring the decrease in the size of the pectin molecule as the result of enzyme activity. The change in viscosity offers a means of measuring the extent of depolymerization.

Studies of the effect of the concentration, temperature, pH, and presence of salts on the viscosity of pectin solutions have demonstrated *(4, 18)* that the viscosity of dilute solutions increases with concentration to 0.5%, and also increases with decreasing temperatures. Further, the viscosity was found to be at the minimum at pH 6. On the basis of previous work, it was recognized that it would be necessary to buffer the pectin solution at the desired pH, and to control temperature and the concentration of pectin to insure accurate viscosity measurements.

Pectin solution. A 3% pectin solution yielding a relative viscosity about 40 was used as a standard. It was prepared in 0.5N sodium citrate—citric acid buffer solution at pH 4.0. The buffer solution was heated to 50° C.; the pectin was added gradually during mixing with an electric stirrer; and the suspension was then treated 1 minute in a Waring Blendor. To insure uniformity the solution was passed through several thicknesses of cheesecloth. Microbial growth was inhibited by the addition of 0.1 milliliter of toluene to each 100 milliliters of solution which was stored in a tightly stoppered flask in the refrigerator at 5° C.

Polygalacturonase standard solutions. These were prepared from accurately weighed quantities of commercial Pectinol. A 10,000 microgram per milliliter (1.0%) solution in distilled water was prepared freshly each time and proper dilution standards were made for each desired concentration.

Cucumber brines. Samples were taken from vats of fermenting cucumbers at commercial salting stations with the aid of a stainless steel tube reaching to the approximate center of the vat. They were stored in bottles of approximately 250-milliliter capacity, preserved with 0.5 milliliter toluene, and held at 5° C. The polygalacturonase-active brine used in most of the experiments is designated as H-10.

Viscosity measurements. Twenty-five milliliters of 3% pectin solution were placed in a 50-milliliter Erlenmeyer flask. To this was added either 5 milliliters of the standard polygalacturonase solution or the cucumber brine with suspected enzyme activity. The two liquids were thoroughly mixed and this flask was designated as *A*. A second flask *B*, to be used as a control, contained the above two solutions except that the enzyme solution (or brine) was heated in an 80° C. water bath for 10 minutes and cooled before it was placed in the pectin solution. The heat inactivates the enzyme system but permits the salt and other constituents to be present as in flask A. To inhibit microbial growth, two drops of toluene were put in each flask and stoppered tightly. The flasks were placed in a constant tmeperature incubator and held for viscosity readings.

A 20-milliliter volumetric pipette was used to measure the dropping-time of the pectin solutions. The stem

[d] Obtained from California Fruit Growers Exchange, Ontario, California, under the name "Pectinum NF VIII." Analysis: Free of sugars; hydrolyses to galacturonic acid, 88-90%; methoxyl 9.7%, and pH 3.7 to 4.0. A pectin suitable for enzyme test in pickle brines should be specified.

[e] Pectinol M is a commercial enzyme manufactured and supplied by the Röhm and Haas Company, Philadelphia, Pa.

Mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.

had been cut about 10 centimeters from the bulb and fire-polished. The inside diameter of the opening was 2.11 millimeters and the dropping time, measured with an electric precision stop-clock, was 0.05 minutes for water as compared to an average 2.00 minutes for 3% pectin solution. The pectin solution was drawn into the pipette with a rubber suction bulb attached to the top. The flow time was measured from the graduation mark on the upper stem to an etched mark 5 millimeters from the bulb on the lower stem. This pipette was easily cleaned and dried between each viscosity reading with hot water, ethyl alcohol, and acetone. (See Figure 1).

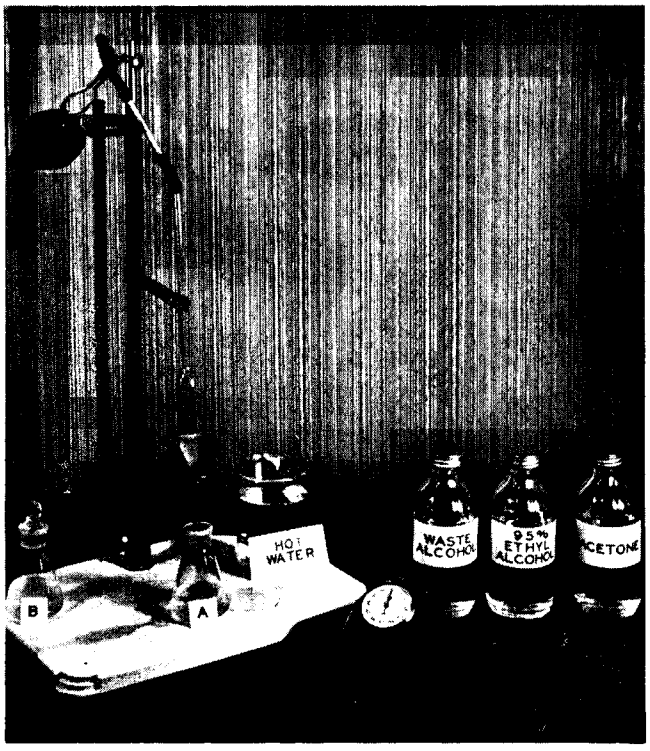

FIG. 1. Apparatus for determining viscosity of pectin solution showing pipette with suction bulb attached. Flask A contains pectin solution plus enzyme solution and flask B pectin solution plus heated enzyme solution. The hot water, waste alcohol, and acetone are used in order listed to clean and dry the pipette between each viscosity measurement. The stop-watch (or an electric precision stop-clock) is used to measure the flow time of pectin solution from the pipette.

The pectin solution — enzyme solution mixtures (Flasks A and B) were brought to a temperature of 26° C. in a constant temperature bath and viscosities were read at 26° C. ± 1° C. The equipment permitted making measurements with such speed and accuracy that reading time variation was within ± 0.01 of a minute.

The activity of the polygalacturonase is expressed as a loss in viscosity of the pectin and active enzyme solution mixture, A, over the pectin and inactive enzyme solution mixture, B, calculated as follows:

$$\frac{(B-A)}{B} \times 100 = \text{Percent loss in viscosity}$$

The results of using different concentrations of polygalacturonase ranging from 0 to 4.00 micrograms per milliliter are shown in Table 1. The reactions were at

TABLE 1

Effect of Polygalacturonase on the Viscosity of Pectin Solution Buffered at pH 4.0 and Incubated at 30° C.

Polygalacturonase*	Loss in Viscosity	
	3-day Incubation	6-day Incubation
μ per ml.	*percent*	*percent*
0.00	0	0
0.04	2	7
0.08	9	14
0.12	15	19
0.16	17	25
0.20	19	29
0.40	23	35
0.60	28	38
0.80	32	43
1.20	39	47
1.60	41	51
2.00	46	55
2.50	46	56
3.00	48	59
4.00	52	62

* Reaction mixture; 5 ml. enzyme solution and 25 ml. of 3% pectin solution, average relative viscosity of heated control, 30.3.

30° C. temperature and buffered at pH 4.0, and the viscosities were determined after 3- and 6-day incubation periods. A definite rate of change in the viscosity was observed with these levels of enzyme solution but the relation was not linear. For example, a 6-day incubation using 0.12 microgram of polygalacturonase

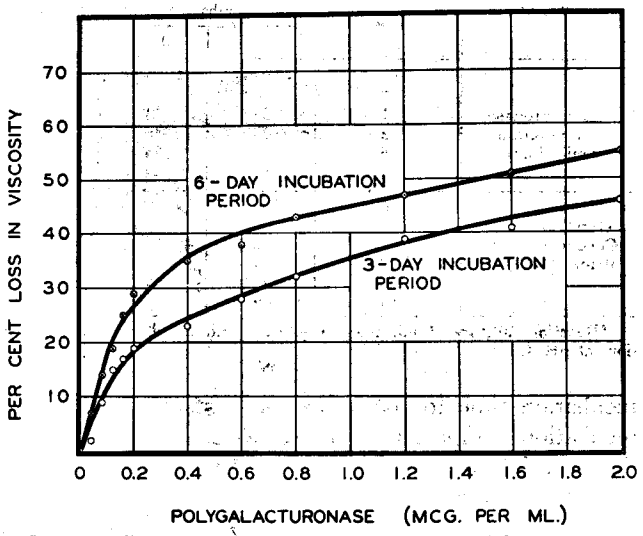

FIG. 2. Effect of polygalacturonase (Pectinol M) on the viscosity of pectin solution.

standard gave a change in viscosity of 19% whereas 5 times that amount, 0.60 microgram, gave 38%, only twice that change in viscosity. Figure 2 is a graphic presentation of the data in Table 1. A sharp break in the viscosity curve was noted between 0.20 and 0.40 microgram per milliliter enzyme solution. The most accurate part of the curve was that between 0 and 0.20 microgram enzyme solution using the 6-day incubation period.

Four concentrations of the "active" cucumber brine (H-10), shown in Table 2, gave results similar to those for standard polygalacturonase. The percent change in viscosity was 20% for 1 milliliter of brine diluted to 5 with water and was 38% for an undiluted 5 milliliter sample. The reaction mixture, as to pH, pectin concentration, incubation temperature and time, was the same as for the standard polygalacturonase.

TABLE 2

Effect of Various Concentrations of Cucumber Brine, H-10 on the Viscosity of Pectin Solution, Buffered at pH 4.0 and Incubated at 30° C.

Cucumber Brine (H-10)*	Water Added	Loss in Viscosity
ml.	*ml.*	*percent*
1	4	20
2	3	28
4	1	32
5	0	38

*Reaction mixture contained 5 ml. brine-water solution and 25 ml. of 3% buffered pectin solution.

Factors Influencing Enzyme Activity and Relative Viscosity

Influence of temperature and period of incubation. The effect of 20°, 30°, and 40° C., incubation temperatures and length of time ranging from 1 to 10 days was observed on both the commercial polygalacturonase and the "active" cucumber brine (H-10). As shown in Table 3 the loss in viscosity increased with the length of

TABLE 3

Influence of Temperature, and Incubation Period on Enzyme Activity of Polygalacturonase and "Active" Cucumber Brine in Pectin Solution

Enzyme	Incubation Time	Loss in Viscosity* Incubation Temperature		
		20° C.	30° C.	40° C.
	days	*percent*	*percent*	*percent*
Polygalacturonase (2.00 microgram per milliliter)	0	0	0	0
	1	23	23	24
	3	32	46	39
	6	44	56	53
	10	50	62	56
"Active" Cucumber Brine (H-10)	0	0	0	0
	1	5	12	17
	3	14	29	28
	6	27	39	39
	10	30	57	53

*Reaction mixture, 5 ml. enzyme solution and 25 ml. 3% pectin solution, at pH 4.

incubation time for both enzyme systems. The 30° C. incubation temperature favored greater viscosity loss than the 20° or 40° C.; yet there was not too great a difference between the 30° and 40° C. temperatures.

It was observed that depolymerization may occur due to the influence of temperature in the absence of enzymic activity. This degradation would not introduce a large error as the results are based on a ratio of two pectin solutions held at the same temperature. It was found that in a 10-day incubation period, a pectin solution with relative viscosity of 43 lost 1% at 20° C., 3% at 30° C., and 18% at 40° C. Even though this loss in viscosity was compensated, it was not considered desirable to use the 40° C. incubation temperature. Because of these observations, a 6-day incubation period at 30° C. was adopted for measuring the enzymic activity.

Adjustment of pH. Five different pH levels were tested with the buffered citrate-citric acid-pectin solution. The results are given in Table 4 for polygalacturonase and "active" brine (H-10). The relative viscosity decreased as the pH of the solutions was increased from 2.5 to 6.3. Similar results are reported by other investigators *(4, 18)*. The maximum change in viscosity caused by polygalacturonase and brine enzyme occurred at pH 4.0, which was approximately the same as the optimum pH given by others *(16, 20, 21, 22)*. From the results it was evident that the enzyme in the brine and the commercial polygalacturonase responded in the same manner to pH adjustment.

TABLE 4

Effect of pH on Loss in Viscosity of Pectin Solution by Polygalacturonase and Active Cucumber Brine (Vat H-10)

pH	Relative Viscosity†	Loss in Viscosity* Polygalacturonase (μ per ml.) 0.04	0.20	2.00	pH	Relative Viscosity†	Loss in Viscosity* Active Brine Vat H-10
		percent	*percent*	*percent*			*percent*
2.5	48	6	18	47	2.7	60	22
4.0	30	8	28	54	3.7	39	38
5.0	29	4	16	44	4.5	35	39
5.8	25	3	7	37	5.3	34	37
6.3	18	2	6	25	5.5	29	28

* Incubation time 6 days at 30° C.; reaction mixture contained 5 ml. enzyme solution and 25 ml. 3% pectin solution.
† Viscosity of flask B.

Influence of Sodium Chloride. Pallman et al. *(20)* observed that a number of chloride salts in low concentrations activated polygalacturonase. They further found that the maximum activity was obtained at 3.0 milliequivalents (0.017% NaCl) regardless of whether sodium or potassium chloride was used. In cucumber fermentations, the salt concentration may range from 5 to 18% by weight.

Tests were made to study the effect of 0, 5, 10, 15 and 20% sodium chloride solutions on the viscosity of pectin, and on the activity of 0.20 and 2.00 micrograms per milliliter of polygalacturonase upon pectin. The data given in Table 5 confirm other reports *(4, 18)* that increasing salt concentration raises the relative viscosity of the pectin solution. The loss in viscosity is markedly reduced at the 0.20 microgram per milliliter level of the enzyme in salt solutions greater than 10%.

In a second experiment (reported also in Table 5), a cucumber brine was inactivated by heat and polygalacturonase at two levels was added; 0.20 and 2.00 micrograms per milliliter. The cucumber brine con-

TABLE 5

*Influence of Salt on the Viscosity of 3 Percent Pectin Solution With and Without Polygalacturonase, and Upon Heated Cucumber Brine With the Enzyme**

Sodium chloride concentration†	Relative viscosity of Pectin Solution	Loss in Viscosity Polygalacturonase μ/ml. 0.20	2.00
percent by wt.		*percent*	*percent*
0	26	29	54
5 (20°)**	27	28	55
10 (40°)	33	16	52
15 (58°)	37	10	46
20 (75°)	40	8	39
F-271 Cucumber Brine			
16 (60°)	42	12	38

* Incubation time 6 days at 30° C. reaction mixture contained 5 ml. enzyme solution and 25 ml. 3% pectin solution.
† Standard polygalacturonase solution prepared with salt solutions or brine to desired concentrations.
** Salometer equivalents as percent saturation with sodium chloride.

tained 16% salt and the results are very similar to the salt-enzyme solution test.

In a third experiment an "active" cucumber brine (H-10) was increased in salt content from 13.3% to 21.3% (50° to 80° sal.). The loss in viscosity at 13.3% salt was 40% as compared to 42% with 21.3% salt.

These observations show that high concentrations of salt will not inactivate or precipitate polygalacturonase. This is in disagreement with the report by Fabian and Johnson *(6)* who concluded that the enzymes [protopectinase which is closely related to polygalacturonase *(21)*, and pectase] produced by *B. mesentericus fuscus* became inactive in salt concentrations above 2 or 3%. It is doubtful whether they were working with the polygalacturonase enzyme that is found in commercial cucumber brines.

Pectinesterase in cucumber fermentations. During the testing of brines for polygalacturonase activity it was observed that in a number of cases a gel formed in the flask containing pectin solution plus unheated brine (Flask A) but that no gel was ever found in the pectin solution mixtures plus heated brine (Flask B). The gel formation was attributed to the action of pectinesterase which de-esterifies the pectin and to the presence of polyvalent cations.

A further test for the esterase was made by the examination of several brines using the method of Hills and Mottern *(8)*. Brine F-100, with negative polygalacturonase activity, contained 16 times the amount of the esterase as the active polygalacturonase brine (H-10). The following values expressed as milligrams methoxyl per 30 minutes per milliliter were obtained: Cucumber brine F-100, 1.0; cucumber brine H-10, 0.06; fresh tomato extract (high in pectinesterase) 14.6. It has been reported *(9)* that partial de-esterification must take place before glycosidic hydrolysis, and the simultaneous action of polygalacturonase and pectinesterase will speed the glycosidic hydrolysis of the pectin. Since no attempt has been made to separate the two enzymes in this study, the speed of the reactions cannot be taken as a measurement of polygalacturonase but a measurement of pectin-hydrolysis. The objective of the study was to correlate pectic enzymes in the fermentations to softening of cucumber salt-stock. Further, it has been shown *(10)* that high concentrations of crude pectinesterase caused inhibition of the polygalacturonase. However, this inhibitory effect was not noticed with the purified esterase. The "gel" brines reported in the present study indicate that when calcium or polyvalent cations are present the naturally occurring esterase may exhibit an inhibitory effect on polygalacturonase action (see addendum).

An interesting observation was made with several vats of cucumbers that were extremely moldy when brined. Mold growth occurred during transportation of fresh cucumbers that were improperly refrigerated in

TABLE 6

Correlation of Polygalacturonase Activity in Commercial Cucumber Fermentations With Salt-Stock Firmness

Plant and Vat No.	Fermentation Age	Polygalacturonase Activity Measured by Loss in Viscosity of Pectin Solution	Firmness of Cucumber Salt-Stock*	Chemical Properties of the Brines			Chemical Properties of the Brines		
				Salt Concentration	pH	Total Acidity as Lactic	Vat Capacity	Variety	Size ††
Plant F.†	*days*	*percent*	*pounds*	*percent*		*percent*	*bushels*		
139 1	27	54	7	15	3.30	0.80		Producer	1's and 2's
2	46	43		17	3.40	0.78			
271 1	41	33	8	16	3.50	0.42		Producer	1's and 2's
2	60	19		18	3.45	0.35			
262 1	43	0	16	16	3.85	0.28	(600-800)	Producer	1's and 2's
2	62	0		18	3.95	0.28			
100 1	43	0	16	17	4.00	0.27		Producer	1's and 2's
2	75	0		18	4.10	0.26			
Plant C.									
H2	26	33	Mushy	13	3.50	0.72	700	Producer	1's
H10	30	39	Mushy	13	3.45	0.80	700	Producer	1's
G42	32	38	Mushy	14	3.40	0.65	700	Producer	1's
G58	30	21	Mushy	14	3.40	0.73	700	Producer	1's
Plant O.									
20-18	69	0	20	19	3.90	0.42	1000	Producer and Model	Mixed
21-3	80	0	18	19	4.00	0.28	400	Earliest of All	Mixed
21-14	80	0	16	19	4.20	0.31	800	Producer and Model	Mixed
Plant T.									
10**	132	8	8	18	3.25	0.51	500	National and Model	Mixed
115**	60	7	13	14	3.20	0.82	500	National	2's
77	96	0	17	16	3.30	0.81	500	National	1's and 2's
Plant W.									
252**	122	9	Mushy	20	3.12	0.44	700	National	1's and 2's
126	135	4	11	21	2.85	0.98	700	Model	1's and 2's
124	134	1	13	19	3.15	0.62	700	Model	1's and 2's
Plant M.									
401	111	13	12	17	3.30	0.74	600	.....	1's and 2's
504	87	5	11	17	3.20	0.59	600	Producer	Mixed
210	117	2	13	18	3.30	0.48	210	Model	2's and 3's

* Firmness indicated in pounds resistance using USDA fruit tester with 5/16 inch tip. Values represent average of 10 cucumbers No. 2 size (1⅛ to 1½ inches diameter) tested at stem and blossom ends for each lot. Values 16 to 20 pounds are considered firm and values of 13 and below inferior. All lots pressure tested after a 2 to 4 months' curing period in brine.

† Two brine samples representing two different ages of fermentation were taken from the vats at Plant F.

** Brine from vats drained off and new brine added prior to test. Undoubtedly part of enzyme was lost.

†† No. 1, up to 1⅛ inches in dia.; No. 2 up to 1½ inches; No. 3 above 1½ inches. Mixed refers to all three sizes.

transit. A polygalacturonase viscosity test made 1 day after cucumber salting indicated a very high enzyme activity. The enzyme activity dropped to a very weak test on a 2-day sample and a 3-day sample gave a firm gel test. After a 3-month curing period, the cucumber salt-stock from these vats was very firm in texture thereby substantiating the opinion that polygalacturonase activity was not pronounced.

Addition of Polygalacturonase to Cured, Commercial Salt-Stock

Polygalacturonase from Pectinol (200 μ/ml. brine) was added to cured, cucumber salt-stock which was approximately 1 year old. This material was considered very firm and had an average pressure test, as indicated by the USDA fruit tester, of 18. The salt-stock brine was at pH 4.0 and 18% salt. The enzyme reduced the firmness of the stock 50% in 42 days and 66% in 62 days as compared to the control. This was evidence that polygalacturonase will soften commercially cured cucumber salt-stock.

As the Pectinols contain other enzymes *(15)*, a purified polygalacturonase, supplied by Mr. E. F. Jansen of the Western Regional Research Laboratory, Albany, California, was also added to salt-stock (4 μ/ml.) and produced softening.

Polygalacturonase in Commercial Cucumber Brines

Twenty fermentations, representing six commercial pickling plants in five southern states, were tested for polygalacturonase activity using the viscosity method. The results given in Table 6 were typical of the range in activity of 239 commercial vat fermentations studied. They represented considerable variation in cucumber sizes, cucumber varieties, vat capacities, salt treatments, ages of fermentations, and acid production. Fourteen of the 20 brines gave positive loss in viscosity of the pectin solution. The firmness of cucumber salt-stock from the 14 vats ranged from soft and mushy to only a slight loss in firmness as indicated by the USDA fruit pressure tester. The salt-stock from the six polygalacturonase-negative vats was of very good quality with respect to firmness. Summary of enzyme activity of commercial brines is given in Table 7.

TABLE 7

Summary of Enzyme Activity Studies of Commercial Brines

Item	Polygalacturonase Activity	
	Positive	Negative
239 Brines Examined	74 (31%)	165 (69%)
9 States Sampled	6	8
19 Salting Sta. Sampled	6	18
% Loss in Viscosity	5-54	0
Firmness of Cured Stock	Mushy—13 lbs.	16-22 lbs.
Brine Strength	10-21%	12-19%
Total Acidity as Lactic	0.2-1.2%	0.2-0.9%
Brine pH	3.2-3.9	3.4-4.0

Conclusions

Two methods for measuring the polygalacturonase activity on pectin solutions were tested: (1) Loss in viscosity of a standard pectin solution, and (2) liberation of aldehydic groups by hydrolysis. The viscosity method was capable of detecting enzyme activity in extremely low concentrations. The enzyme concentration of the cucumber fermentations was too low to liberate aldehydic groups at sufficient rate to be measurable with iodine solution.

A polygalacturonase-like enzyme was found in commercial cucumber fermentations and was considered responsible for softening of salt-stock under commercial conditions. The enzyme found was active in brines from numerous commercial brining stations and was commonly found under conditions of salt concentration, acidity, pH, and temperature which prevailed at plants operating in various parts of the United States.

The polygalacturonase-like enzyme from cucumber fermentations and the commercial polygalacturonase (Pectinol) reacted alike to adjustments of pH, temperature, salt, and time of incubation. The maximum activity of both enzyme systems was at pH 4.0 and 30° C. Increasing the salt concentration up to 21% by weight (80° salometer) did not inactivate polygalacturonase in either the commercial preparation or the active brine.

Firm, cured, cucumber salt-stock from commercial source was softened by the addition of polygalacturonase to the brine.

The relative viscosity of the standard pectin solution was influenced by pectin concentration, pH, temperature and addition of sodium chloride. All of these factors must be controlled to insure accurate measurements, and for making quantitative comparison.

The enzyme pectinesterase was found in certain cucumber fermentations and in such cases produced a gel with pectin in the presence of polyvalent ions.

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Addendum

After this article was written, it was observed that with certain brines, the pectinesterase produced a gel in the presence of polygalacturonase. This reaction obviously blocked the determination for the polygalacturonase by the viscosity test. To eliminate the gelling action of pectinesterase, the brines, containing a few drops of toluene as a preservative, should first be incubated at 40° C. for 24 to 48 hours. The test is then carried out as described in the article.

LITERATURE CITED

1. APPLEMAN, C. O., AND CONRAD, C. M. Pectic constituents of peaches and their relation to softening of the fruit. Maryland Agr. Expt. Sta. *Bull.* **283** (1926).

2. BAKER, G. L. High-polymer pectins and their de-esterification. *Advances in Food Research* **1**, 395, Academic Press Inc., New York, N. Y. (1948).

3. BAKER, G. L. *Personal Communication* (1948).

4. BAKER, G. L., AND GOODWIN, M. W. Fruit jellies—X. The role of pectin-6. Viscosity of dilute pectin solutions as affected by metallic salts and pH. Del. Agr. Expt. Sta. *Bull.* **216**, *Tech.* **23** (1939).

5. ETCHELLS, J. L., AND JONES, I. D. Characteristics of lactic acid bacteria from commercial cucumber fermentations. *J. Bact.* **52**, 593 (1946).

6. FABIAN, F. W., AND JOHNSON, E. A. Experimental work on cucumber fermentation. Mich. Agr. Expt. Sta. *Tech. Bull.* **157** (1938).

7. FISH, V. B., AND DUSTMAN, R. B. The enzyme activities of Pectitnol A on pectin and other substances. *J. Am. Chem. Soc.* **67**, 1155 (1945).

8. HILLS, C. H., AND MOTTERN, H. H. Properties of tomato pectase. *J. Biol. Chem.*, **168**, 651 (1947).

9. JANSEN, E. F., AND MACDONNELL, L. R. Influence of methoxyl content of pectic substances on the action of polygalacturonase. *Arch. Biochem.* **8**, No. 1, 97 (1945).

10. JANSEN, E. F., MACDONNELL, L. R., AND JANG, R. Simultaneous actions of polygalacturonase and pectinesterase on pectin. *Arch. Biochem.* **8**, No. 1, 113 (1945).

11. JONES, I. D., AND ETCHELLS, J. L. Physical and chemical changes in cucumber fermentation. *Food Industries* **15**, Jan. 62 (1943).

12. JOSEPH, G. H. *Personal communication* (1947).

13. JOSLYN, M. W., AND PHAFF, H. J. Recent advances in the chemistry of pectic substances. *Wallerstein Lab. Comm.* **10**, No. 29, 39 (1947).

14. KERTESZ, Z. I. Pectic Enzymes. IV. Structural considerations in connection with the enzymic hydrolysis of pectins. *J. Am. Chem. Soc.* **61**, 2544 (1939).

15. LINEWEAVER, H., JANG, R., AND JANSEN, E. F. Specificity and purification of polygalacturonase. *Arch. Biochem.* **20**, No. 1, 137 (1949).

16. MATUS, J. Untersuchungen über die aktivität der Pektinase. *Ber. Schweiz. Bot. Ges.* **58**, 319 (1948).

17. MOTTERN, H. H., AND HILLS, C. H. Low ester pectin from apple pomace. *Ind. Eng. Chem.* **38**, 1153 (1946).

18. OWENS, H. S., LOTZKAR, H., MERRILL, R. C., AND PETERSON, M. Viscosities of pectin solutions. *J. Am. Chem. Soc.* **66**, 1178 (1944).

19. PALLMAN, H., AND DEUEL, H. Übersicht über die chemie und physik der pektinstoffe und besprechung der neueren literatur. 1937-1946. *Chimia* **1**, 27 (1947).

20. PALLMAN, H., MATUS, J., DEUEL, H., AND WEBER, F. Über die aktivität der pektinase. *Rec. Trav. Chim. Pays-Bas.* **65**, 633 (1946).

21. PHAFF, H. J., AND JOSLYN, M. A. The newer knowledge of pectic enzymes. *Wallerstein Lab. Comm.* **10**, No. 30, 133 (1947).

22. REED, G. Personal Communication. (1948).

23. WEBER, F., AND DEUEL, H. Über die bewertung von filtrationsenzymen. Mitt. Lebensm. Hyg. **36**, 368 (1945).