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A METHOD FOR TESTING CUCUMBER SALT-STOCK BRINE FOR SOFTENING ACTIVITY

This publication gives step-by-step directions for testing commercial cucumber brines for the softening type spoilage caused by a pectinolytic and cellulolytic (C_x) agents (enzymes). These instructions are directed to workers in quality control laboratories of food plants and the results of the tests should give an early forecast as to possible salt-stock spoilage caused by softening. The tests may also be applied to genuine dills and overnight dills.

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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A METHOD FOR TESTING CUCUMBER SALT-STOCK BRINE FOR SOFTENING ACTIVITY

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INTRODUCTION

Spoilage known as "softening" of commercial cucumber salt-stock is a serious problem in the pickle industry. In some years, softening is far more severe and widespread than in others. The economic loss to the industry is considerable when soft salt-stock must be discarded or, at best, used in low-priced products.

The nature of the softening of commercial salt-stock has been studied by the authors (1, 2, 3).[2] The principal softening agent found in salt-stock brine was similar in chemical behavior to a pectinolytic enzyme called polygalacturonase (pectinase). The presence of this enzyme in cucumber brines was correlated with the softness of the salt-stock and the latter condition was attributed to the breakdown of the pectic substances of the cucumber. These substances act as cementing material between the cells of fruits and vegetables and play an important role in firmness or texture quality.

Only recently, a second softening agent in the form of a cellulolytic (C_x) enzyme (7) has been found by the authors (3) in cucumber brines. Jansen and Seegmiller (5) have informed us that they, too, have found this cellulolytic (C_x) enzyme in a cucumber brine in California. However, the exact role of this enzyme in the softening mechanism has not been clearly indicated at this time. Thus, two softening agents (pectinolytic and cellulolytic enzymes) may be determined, using much of the same procedure but with different substrates; sodium pectate for the pectin-splitting enzyme and sodium carboxymethylcellulose for the cellulose splitting enzyme (C_x).

[1] One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture.

[2] Numbers in parenthesis refer to Literature Cited, page 8.

It is the purpose here to provide specific and detailed directions for making the tests for pectinolytic and cellulolytic enzymes, including a list of the materials needed and a description of the apparatus used. Notable improvements over previous reports (1, 2) have been made as to shortening the time required to complete the tests and increase accuracy in measuring softening activity. The use of these tests should prove to be of value to the pickle industry in supplying information on possible softening of cucumbers in individual vats. An early forecast of the final firmness should aid plant operators in processing salt-stock before softening deterioration becomes a serious economic loss.

MATERIALS

Apparatus. In Appendix I a list of "Special Equipment" and "Regular Equipment" is tabulated for the convenience of the laboratory worker. These items of laboratory equipment may be purchased from scientific supply companies. Some of this equipment is pictured in Figure 1.

Reagents. The reagents used for making the tests, together with methods of preparation, are listed in Appendix I.

SAMPLING OF BRINE FROM COMMERCIAL VATS

It is best to obtain a brine sample from the approximate center of the container (vat, tank, barrel or keg). This may be accomplished (4) by using a 4 to 6' length of 3/16" stainless steel tubing (sealed at one end with lead or solder and perforated with several 1/16" holes for a distance of 6 to 8" from the sealed end) which is inserted through an opening in the false head, down into the brine toward the center of the vat. The brine sample is withdrawn through a previously attached piece of rubber tubing into a No. 1, two-hole rubber stopper and two short lengths of glass tubing--one for the rubber tubing leading from the stainless steel sampling tube, and the other for a suction bulb to start the siphoning action. To avoid contamination, the 12 oz. bottle is filled with the brine sample, emptied, filled a second time and emptied before taking the final sample in an 8 to 12 oz. jar or bottle. One-half ml. of toluene (as a preservative) is added to the brine sample and the bottle tightly capped. If samples are to be stored for several days, they should be put in a refrigerator. Samples sent through the mail should be preserved with toluene.

PROCEDURE FOR PECTINOLYTIC ENZYME TEST

Follow these three steps in making the test:

1. Dialysis of the brine samples.
2. Preparation and measuring flow time of Ostwald-Fenske viscosity pipettes with pectate solution (PS) and dialyzed sample.
3. Calculation of softening activity units.

Figure 1. Constant temperature bath containing Ostwald-Fenske pipettes for making pectinolytic and cellulolytic enzyme tests. (See Appendix I for list of equipment shown in this figure.)

Dialysis of the Brine Samples. To prevent a gel reaction with the pectate solution, the salt from the cucumber brine must be removed. This is accomplished by the use of cellophane tubing as a dialyzing membrane, and the softening enzymes remain in the tubing for the viscosity tests. Dialysis has been proven to be a better procedure to prevent gel formation than the previously recommended method (2) of incubation of the brine.

About 25-ml. amounts of the brines to be tested are placed in cellophane tubing as follows: Cut the cellophane with scissors into 8-inch lengths; wet the strips in water and open by rubbing the tubing between thumb and index finger. To form bags for the brine, seal one end by twisting several times then making a tight knot. The brine samples are poured into the cellophane bags and the tops closed without trapping air by twisting and winding a small rubber band about the cellophane. The bags containing the brine samples should be checked for possible leaks before proceeding further. Each sample is suspended by a clamp and string with its identification number from a large iron ring into a 3 to 5 gallon pail, wide-mouth glass jar, or similar container. The iron ring is clamped to a ring stand or suitable support. Cool tap water is then run into the container from the bottom by use of rubber tubing connected to water outlet, permitting the water to completely cover the cellophane bag-samples and overflow the container. The rate of flow for the water should be about one gallon per 5 minutes, for a period of 3 hours. The tap water is then poured out of the container and replaced with distilled water. The samples are submerged for 1 hour in distilled water; this completes the dialysis. The samples are then removed from the ring, the cellophane tubes opened by removing the rubber bands, and the dialyzed brine samples transferred to 30-ml. test tubes, containing 5 drops of toluene.

Preparation and Measuring Flow Time of Ostwald-Fenske Viscosity Pipettes with Pectate Solution and Dialyzed Samples. For each brine sample to be tested, suspend a viscosity pipette in the 30°C water bath and measure 5 ml. of pectate solution into each pipette. After allowing approximately 10 minutes for the pectate solution to reach water bath temperature, start with the first dialyzed sample and measure accurately 1 ml. into the first viscosity pipette. Mix the sample with the pectate solution in the pipette by placing the suction air-flow tube from the vacuum pump in contact with the large opening of the pipette. By suction, move the pectate-sample mixture to the upper bulb and with a stopwatch or electric stopclock measure the flow time from the top to the bottom etched marks. The filling, mixing, and flow time reading should be made within two minutes. This flow time observation is called the "Initial" reading. Repeat this operation for each dialyzed sample. Place one drop of toluene into each viscosity pipette and stopper.

The flow time for each viscosity pipette sample should be made at Initial, 20-hour and 44-hour periods. If the sample is extremely active (loss of 50% viscosity--see calculation of softening activity) at the 20-hour period, then it is not necessary to make further readings.

The Ostwald-Fenske pipettes must be thoroughly cleaned after each use and this is best accomplished by forcing hot tap water through them by use of rubber tubing. The pipettes are then rinsed with ethyl alcohol, next with acetone, and drying is completed by drawing air through them by use of the vacuum pump.

Calculations of Softening Activity Units (Pectinolytic). The flow time in seconds for each pectate-sample is recorded at the initial (A), 20-hour period (B_{20}) and 44-hour period (B_{44}), and the softening activity first expressed as loss in viscosity of the pectate-sample (or pectinol solution). The average flow time of water (W) in the viscosity pipettes is 4.4 seconds, so this is applied in the equation and calculated as follows:

$$\frac{A - B_{20}}{A - W} \times 100 = \text{Percent loss in viscosity at 20-hr. period}$$

For 44-hr. period, use B_{44} to replace B_{20} in equation.

Example: If a dialyzed brine sample gives an initial flow time with pectate solution of 35.2 seconds and a flow time at the 20-hour period of 31.4 seconds, and at the 44-hour period of 27.7 seconds, then:

$$\text{Loss in viscosity at 20-hr. period} = \frac{35.2-31.4}{35.2-4.4} \times 100 = \frac{3.8}{30.8} \times 100 = 12.3\%$$

$$\text{Loss in viscosity at 44-hr. period} = \frac{35.2-27.7}{35.2-4.4} \times 100 = \frac{7.5}{30.8} \times 100 = 24.3\%$$

If various concentrations of pectinol solutions are allowed to react with the pectate solution, and their percent loss in viscosity for a given length of time is plotted against the concentration, a curve is produced. Also, a similar curve is produced when a given concentration of the pectinol solution or an active cucumber brine is plotted against reaction time periods, starting the flow time readings at the initial period and making them frequently until approximately 75% loss in viscosity is reached. When the loss in viscosity values are plotted against the log of the concentration or of the time, these data are nearly linear except below the 10% value.

Softening activity units are proposed which are linear to enzyme concentrations. Here, 100 units of activity equals 50% loss in viscosity of 1.0% pectate-pectinolytic enzyme solution at 30°C, pH 5.0, for 20-hour period. For the convenience of the laboratory worker a Conversion Table and Figure are given in Appendix II to make conversions from loss in viscosity values to pectinolytic activity units. The table gives the units for both 20- and 44-hour reaction periods, whereas the figure refers only to the 20-hour reaction period.

To complete the above example, using the Conversion Table where loss in viscosity for 20 hours was 12.3%, and for 44 hours was 24.3%, the pectinolytic activity for this brine sample using the nearest whole numbers is 15 units for the 20- or 44-hour period. If the units are not the same for the two reaction periods, then an average should be recorded as the final value for softening activity.

PROCEDURE FOR CELLULOLYTIC ENZYME TEST

The same three steps as given for the Pectinolytic Test are followed, except in Step 2. Here a 1.2% solution of sodium carboxymethylcellulose (CMC) is substituted for the pectate solution. The procedure for the cellulolytic test becomes quite simple once the brine sample has been examined for the pectinolytic agent. For example, if the sample has been dialyzed, then this test starts with Step 2 using the dialyzed sample.

In Step 3 (calculation of softening activity units), the percent loss in viscosity is calculated for 20- and 44-hour readings as illustrated by the previous example for the pectinolytic test. In our experience the 20-hour reaction period has been sufficient for most samples.

Using similar experimental technique as for pectinolytic activity units, a Conversion Table for cellulolytic activity has been developed (Appendix II). This table may be used to convert percent loss in viscosity for 20-hour reaction times directly to cellulolytic activity units which are linear in relationship.

DISCUSSION

Correlation of Pectinolytic and Cellulolytic Enzyme Activity Units with Firmness of Cucumber Salt-Stock. The authors have tested several hundred commercial and experimental brine samples obtained from the major cucumber brining areas of the country. In general, there has been a very good correlation between pectinolytic activity of the brines and the soft texture of the salt-stock cucumbers from such brines. In our experience (6), the USDA Fruit Pressure Tester has proven to be a very reliable instrument for measuring salt-stock firmness. It is much more sensitive than the hand for detecting losses in firmness, particularly in the range of 20 to 30%. Salt-stock may have lost as much as 50% of its firmness before it is readily detected by hand. For further information on the use of the pressure tester, salt-stock and firmness ratings corresponding with the pressure data, see Appendix III.

The softening units obtained for a given period during the fermentation must be taken into account before correlation of salt-stock firmness with pectinolytic activity can be made. There will be some samples where enzyme activity will not correlate with the firmness ratings. One of the reasons for this might be a loss in enzyme concentration caused by leaky vats. Also, there is some indication of enzyme inactivation before or after sampling. In cases where there is no correlation between enzyme activity and firmness, repeated tests should be made.

The authors (3) have shown that where salt-stock softening was induced by adding cucumber flowers to vats at the start of the fermentation, the pectinolytic and the cellulolytic activities were highest from 1 to 4 days and dropped rather sharply after 10 to 14 days. Generally, the enzyme activity after this period leveled off and then dropped very gradually until the salt-stock was used.

Frequency of Testing Brines. At least 3 tests are desirable and these should be made on each vat at the following periods:

Test Number	*Age of Fermentation in Days*
1	1 to 4
2	10 to 14
3	more than 30
4	Additional test if there is no correlation between firmness and enzyme activity.

The periods for testing the brines will vary to some extent for different brining areas of the country as well as for different plant procedures for brining. Both the pectinolytic and the cellulolytic tests are helpful and in time, one might use such data in explaining the sources (fungi, yeasts, bacteria, cucumber fruit) of the softening agents. If time does not permit making both tests, then the pectinolytic enzyme test should be the one most frequently followed. The first test (1-4 days) is very important because it gives an early forecast of the pectinolytic and cellulolytic activities brought into the vat on the cucumbers and other materials. The results of early enzyme activities may be 4 or 5 times greater than those of later tests.

The second (10-14 days), third (more than 30 days) and fourth tests are important because the softening agents are more constant during this period and this gives the activity levels during fermentation and storage.

For enzyme tests made after the 10th day (age of fermentation) an "Activity Rating" for the units are as follows:

Pectinolytic or Cellulolytic Units	*Activity Ratings*
0-10	negative to weak
11-25	moderately active
26-40	strong
41-60	very strong
more than 60	extremely strong

Suggested Use of the Test for Other Brined Foods. Genuine dill pickles and partly fermented, refrigerated dills undergoing softening have been tested and their lack of firmness correlated with positive enzyme tests. A similar softening type of spoilage occurs in other brined foods such as kraut, olives, tomatoes, onions, peppers and cauliflower. Workers responsible for the firmness quality of these commodities may find the enzyme test useful.

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APPENDIX I

SPECIAL EQUIPMENT

Item		Quantity Needed
1.	Constant Temperature Bath composed of 1 each of the following items: *Jar*, glass cylinder type, 12" diameter and 12" high. *Base*, with 3 rods for 12" diameter jar. *Electronic control unit* with thermo-regulator and 1 heating unit suitable for 12" diameter jar which will maintain temperature at 30° ± .1°C. *Stirrer*, electric for 12" jar. *Holder* for 12 Ostwald-Fenske viscosity pipettes, consists of 14" diameter circular press board properly cut to hold the pipettes approximately 1" from large opening supported by No. 8 rubber stopper. (see Figure I.)	1
2.	*Viscosity Pipettes*, Ostwald-Fenske, uncalibrated size No. 300.	12
3.	*Brine Sampling Tube* (as described under "Sampling Brine from Commercial Vats").	1
4.	*Vacuum Pump*, electric equipped with two traps in the suction line to prevent toluene or acetone from reaching the pump. The traps may be made with a heavy glass bottle (approximately 1 liter) containing 10 or 20 SAE grade motor oil. The suction lines should enter the bottle through a two-hole rubber stopper and with the use of glass tubing; the suction line going from the bottle should extend below the surface of the oil and the line to the pump extend only through the stopper. (In place of the electric vacuum pump, an *Aspirator type filter pump* operated by entrainment of air by a water or steam jet may be used. The two traps in the suction line would not be necessary in using an aspirator type vacuum pump.)	1
5.	*Pressure Tester*, USDA Fruit -- from D. Ballauf Manufacturing Company[3], 619 H Street, N. W., Washington 1, D. C.	1

[3]Mention of trade products or companies does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over similar products or companies not mentioned.

REGULAR EQUIPMENT

Item		Quantity Needed
1.	*Balance* with weights (accuracy ± .05 gms).	1
2.	*Bottles*, sampling, 8-12 oz. capacity with screw caps, tin or rubberized liner. (Paraffined liners not satisfactory for samples stored with toluene.)	50
3.	*Burner, Bunsen*, with iron support, ring and wire gauze (or electric hot plate).	1
4.	*Cheese cloth*, 10 yards.	1
5.	*Clamps*, pinch cock, flat jaws, nickel-plated brass.	12
6.	*Container*, 3-5 gallon, stainless steel, enamel pail, or glass jar, 12" diameter.	1
7.	*Cylinder*, graduated 500 ml. capacity.	1
8.	*Dialyzer Tubing*, seamless cellulose, inflated diameter 3/4", 100 ft. rolls.	2
9.	*Distilling Apparatus*, water.	1
10.	*Flasks*, Erlenmeyer, 2000 ml. capacity.	2
11.	*Pipettes*, volumetric or serological, 5-ml. capacity.	5
12.	*Pipettes*, volumetric or serological, 1-ml. capacity.	25
13.	*Rack* or support, metal, for test tubes.	1
14.	*Ring*, iron with clamp.	1
15.	*Rods*, glass stirring, 10 to 12" in length.	5
16.	*Stop Watch* or electric precision stop clock, graduated in 1/10 sec.	1
17.	*Stoppers*, rubber, No. 2 size.	100
18.	*Stoppers*, rubber, No. 8 size for viscosity pipettes.	50
19.	*Stoppers*, cork, size for test tubes.	100
20.	*Stoppers*, cork, size for viscosity pipettes.	50
21.	*Test tubes*, 20-30 ml. capacity.	100
22.	*Thermometers*, general laboratory (range -10° to +110°C).	2
23.	*Tubing*, rubber, 1/4-inch bore, 1/8-inch wall.	25 ft.
24.	*Waring Blendor*[3].	1

[3]Mention of trade products or companies does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over similar products or companies not mentioned.

CHEMICALS

Item

1. Sodium Polypectate Product No. 24[3], (SP) from Sunkist Growers, Ontario, California.
2. Pectinol M[3] (polygalacturonase). From Rohm-Haas Company, Philadelphia, Pennsylvania.
3. Cellulose Gum (Sodium Carboxymethylcellulose CMC-70-M type)[3]. From Hercules Powder Company, Cellulose Products Department, Wilmington, Delaware.
4. Ethyl Alcohol 95%.
5. Acetone CP.
6. Toluene CP.
7. Sodium Hydroxide CP.
8. Citric Acid CP.

Preparation of Sodium Polypectate (SP) Solution. A 1.2% sodium polypectate (referred to herein as SP or pectate solution) in a sodium hydroxide-citric acid buffer solution at pH 5.0 is used. Weigh out 2.00 grams of sodium hydroxide and 5.3 grams of citric acid and dissolve in 1500 ml. of distilled water. Heat the solution over a stove or Bunsen burner until the temperature reaches 50° to 60°C. Pour 500 ml. of this warm solution into a Waring Blendor and add 6 grams of SP gradually, in a sprinkling manner, and at the same time stirring the liquid surface with a glass rod. Blend the suspension of sodium polypectate and buffer solution for approximately 1 minute, or until it is free from lumps. To insure uniformity of the solution, pour it through two thicknesses of cheese cloth. The remaining 1000 ml. of warm buffer solution is blended in 500 ml. amounts with 6.00 grams of SP in the same manner as described above for the first 500 ml. of blending. After the combined pectate solutions are allowed to cool, add 5 ml. of toluene and store in a tightly stoppered flask or bottle in the refrigerator. In our experience, the refrigerated pectate solution has kept in good condition for at least 3 months.

Preparation of Sodium Carboxymethylcellulose Solution. A 1.2% CMC-70M solution in sodium hydroxide-citric acid buffer solution at pH 5.0 is used. This solution is prepared in the same manner as the SP solution, except 6.0 grams of CMC are used in place of the 6.0 grams of SP.

Preparation of Pectinol (Polygalacturonase) Standard Solution. Weigh out 1 gram of commercial Pectinol and dissolve in 100 ml. of distilled water. Decimal dilutions (e.g., 10 ml. of 1% solution in 90 ml. of water, etc.) may be made and used for trial experiments to give experience in measuring softening activity. (See Procedure for Pectinolytic Test.)

[3]Mention of trade products or companies does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over similar products or companies not mentioned.

APPENDIX II

CONVERSION TABLE I

LOSS IN VISCOSITY (%) TO PECTINOLYTIC ACTIVITY UNITS

LOSS IN VISCOSITY	PECTINOLYTIC ACTIVITY		LOSS IN VISCOSITY	PECTINOLYTIC ACTIVITY	
	20 HRS.	44 HRS.		20 HRS.	44 HRS.
Percent	*Units*	*Units*	*Percent*	*Units*	*Units*
0	0	0	28	40	19
1	1	0	29	42	20
2	2	1	30	44	21
3	3	1	31	46	22
4	4	2	32	48	23
5	6	3	33	51	24
6	7	3	34	52	25
7	8	4	35	55	26
8	9	4	36	57	27
9	10	5	37	60	28
10	12	6	38	63	30
11	13	6	39	65	31
12	15	7	40	68	32
13	16	8	41	71	34
14	17	8	42	74	35
15	18	9	43	77	37
16	20	10	44	80	38
17	22	10	45	82	39
18	23	11	46	86	41
19	25	12	47	90	43
20	26	12	48	93	44
21	28	13	49	96	46
22	29	14	50	100	48
23	31	15	55	122	58
24	32	15	60	146	69
25	34	16	65	182	87
26	36	17	70	225	107
27	38	18	75	280	133

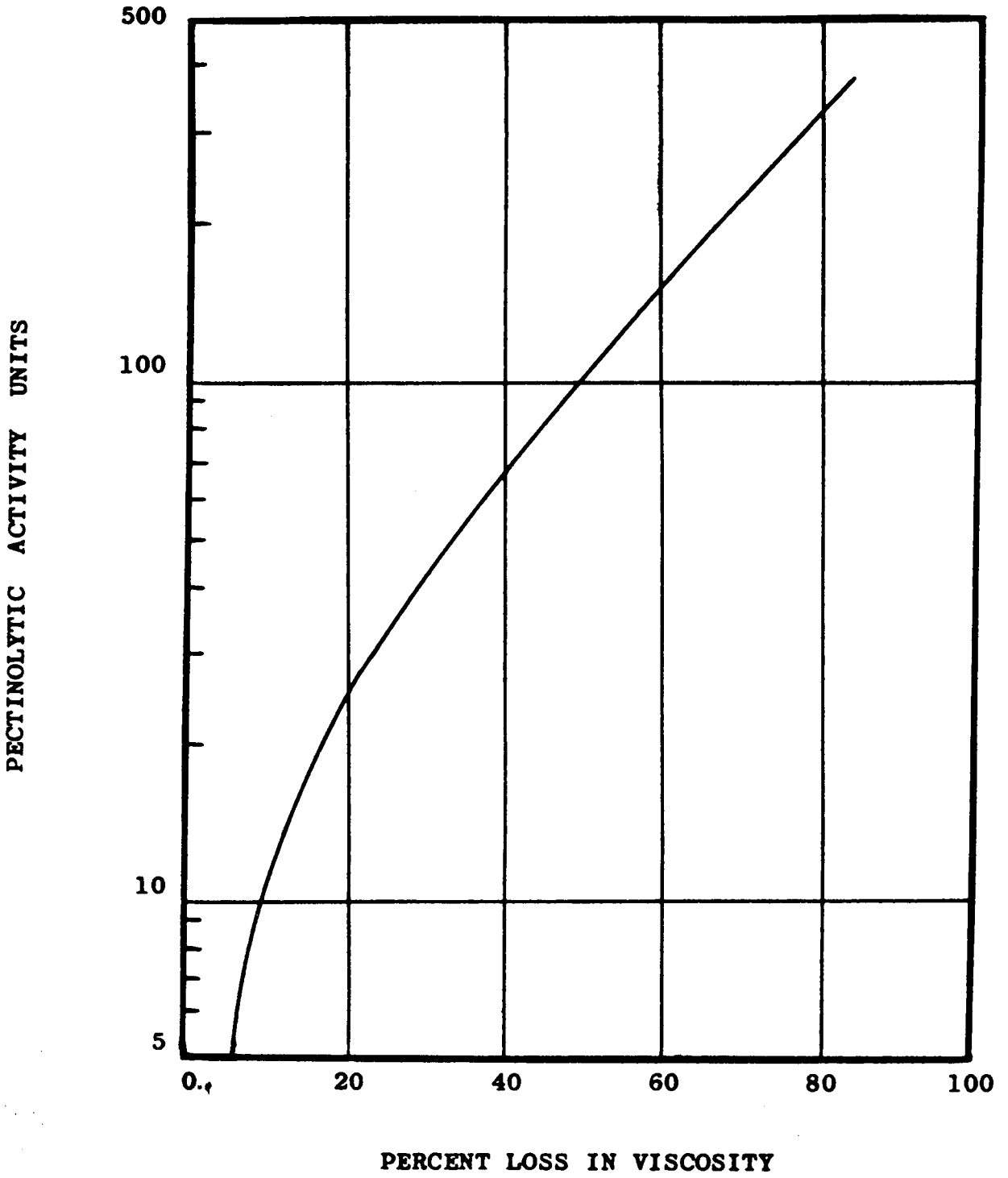

Figure 2. The log relationship of the pectinolytic activity units as plotted against percent loss in viscosity.

CONVERSION TABLE 2

LOSS IN VISCOSITY (%) TO CELLULOLYTIC ACTIVITY UNITS

LOSS IN VISCOSITY	CELLULOLYTIC ACTIVITY AT 20-HR. PERIOD	LOSS IN VISCOSITY	CELLULOLYTIC ACTIVITY AT 20-HR. PERIOD
Percent	*Units*	*Percent*	*Units*
0	0	28	33
1	1	29	35
2	2	30	37
3	3	31	39
4	4	32	41
5	4	33	43
6	5	34	45
7	6	35	48
8	7	36	50
9	8	37	52
10	9	38	55
11	10	39	58
12	11	40	60
13	12	41	64
14	13	42	66
15	14	43	70
16	15	44	74
17	17	45	78
18	18	46	82
19	19	47	86
20	20	48	90
21	22	49	95
22	23	50	100
23	25	55	124
24	27	60	160
25	28	65	230
26	29	70	330
27	31	75	470

APPENDIX III

The USDA Fruit Pressure Tester and Tentative "Firmness Ratings" for Cured Cucumber Salt-Stock

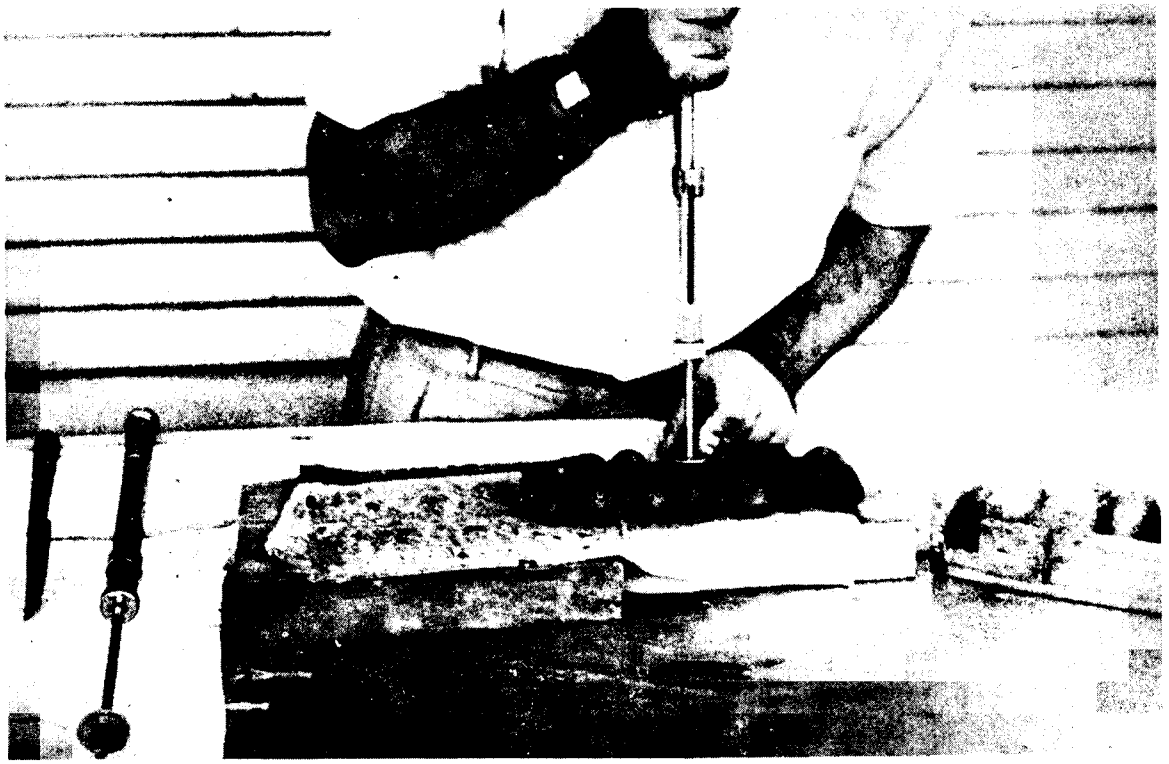

Figure 3. Measuring firmness of cured salt-stock with the USDA Fruit Pressure Tester.

Pressure Test Values (in pounds) Converted to Firmness Ratings for No. 1 size (1 - 1 1/8" diameter) Cured Salt-Stock

Pressure Test[1]	*Firmness Rating*
18 lbs. and above	Very firm
14 through 17 lbs.	Firm
11 through 13 lbs.	Inferior
5 through 10 lbs.	Soft
4 lbs. and below	Mushy

[1]Pressure test value is the firmness to the nearest pound resistance to 5/16" tip of the USDA Fruit Pressure Tester. This value should be the average for 20 cucumbers, each with a single center punch. The cured cucumber salt-stock used for testing should be selected for uniform size (1 - 1 1/8"); 3 carpel development; fairly straight; and free from bloaters, crooks, and nubs.