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Influence of Salt (NaCl) on Pectinolytic Softening of Cucumbers [a]

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SUMMARY

Experimental packs of pasteurized cucumbers were treated with pectinase from three sources under controlled conditions with respect to temperature, pH, acidity, salt concentration, and absence of microbial development. The enzyme-treated lots revealed that as the salt content of the cucumbers increased, their firmness likewise increased according to a first-order reaction. Based on cucumber softening data obtained by use of the three pectinases, tabulated information is presented which permits an estimate of the relative degree of softening that may be expected in curing brines at different salt concentrations.

In the commercial brining of cucumbers for salt-stock pickles, salt (NaCl) concentration is most important for determining the nature of the fermentation and for preserving the stock for long periods of time. The initial salt concentrations may range from 6.5 to 10% depending on the individual pickle plant, but the final concentration is about the same for all plants: 16% salt at the end of the fermentation period (11). The influence of the initial salt concentration on the amount of total acid formed in the fermenting brine, along with the corresponding pH values, has been established (15). Etchells and Jones (12) demonstrated that the use of a low salt brine (about 5% NaCl) gave rapid formation of a high amount of acid and a low pH, whereas, the use of increasingly higher brine strengths (10% NaCl and above) gave slower rates of acid formation, together with increasing rates of gas evolution and resultant bloater spoilage (hollow cucumbers).

Acid-forming bacteria and yeasts are the principal groups of organisms usually found during the active fermentation of cucumbers. Their population changes and identification in commercial brines have been studied extensively by Etchells and associates (4, 8–10, 12, 13). Brining procedures at commercial plants which use high initial salt concentrations were reported (4) to favor large populations of fermentative yeast species and to be chiefly responsible for the bloater formation.

In 1958, Etchells *et al* (7) reviewed the earlier investigations as well as the more recent studies dealing with the nature of softening-type spoilage of cucumber salt-stock. It has been established (1, 2, 5–7) that softening is caused by the pectin-splitting fungal enzyme pectinase (complex enzyme system of which one component of the pectic enzyme mixture is polygalacturonase); cellulase is also associated with softening in the commercial brines. These studies definitely implicated filamentous fungi as the actual causative agent responsible for softening of cucumbers under commercial conditions. Further, the hydrolytic enzymes pectinase and cellulase were reported to be introduced into the brines chiefly by way of fungus-laden flowers that remain attached to the cucumbers and to a lesser extent by the cucumber fruit. The enzymes are produced in the flowers prior to entering the brine and not by fungal growth during the fermentation.

Mechanical removal of flowers from cu-

[a] This study was carried out under a cooperative project with the Departments of Animal Industry and Horticulture of the North Carolina Agricultural Experiment Station.

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cumbers or draining off the brine after 36 hours are procedures recommended (6) and used by the pickle industry to reduce the softening enzymes in the brines.

In 1950, Bell *et al* (1) reported 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 μg/ml of polygalacturonase upon pectin. In those studies the method of assay caused a fivefold dilution of the salt-enzyme solutions; so the final salt concentrations above were lower, and salt influence on enzyme action reversible. They reported that increasing the salt concentration levels raised the relative viscosity of the pectin solution and reduced the enzyme activity as measured by percent loss in viscosity. They concluded that high concentrations of salt did not completely denature polygalacturonase.

It has been generally believed by experienced commercial operators in the pickle industry that the use of low-salt-brining procedures for cucumbers will result in more softening losses than the use of higher brine strengths (11, 12). Scientific reports are not available to substantiate these beliefs; therefore, the present investigation was undertaken to study the influence of salt concentration on the pectinolytic softening of cucumbers. The tests were conducted in the laboratory where controlled conditions could be maintained.

MATERIALS AND METHODS

Experimental Cucumbers

Fresh-pack (pasteurized) cucumbers were made during the 1957 and 1958 growing season at a commercial plant located in Ayden, North Carolina. These experimental packs consisted of 12 jars (1 quart capacity) each of the individual salt treatments calculated to equalize at 0, 4, 8, 12, 16 and 20% NaCl for the 1957 pack; and, at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 20% NaCl for the 1958 pack. The experimental packs were prepared by first weighing the salt and placing it into each jar, then packing the jars with 14 to 16 washed cucumbers, Model variety, 1 to 1⅛ inches in diameter. For the 1957 pack, a cover liquor containing 19 ml of 85% lactic acid per gallon of water was poured onto the cucumbers to approximately one-half inch from the top of the jar; for the 1958 pack, the acid was increased to 23 ml per gallon. These jars were then sealed, coded, and pasteurized in a continuous commercial unit using steam which gave an internal product temperature of 165° F; the jars were promptly cooled with water to less than 100° F and cased according to code numbers. The final equalization of the lactic acid was in a range of 0.20 to 0.24% and the brine pH was 3.8 to 4.1; the 1958 pack was slightly lower in pH and higher in acidity than the 1957 pack, but it was in the given range. A few jars spoiled in the 0 to 4% salt treatments at pH 4.0 but a sufficient number of jars in these particular lots were free of spoilage to conduct the enzyme tests.

Pectinase Enzyme Sources

A commercial concentrated pectinase enzyme 46 AP (Lot No. 32) of fungal origin, was used.[c] One percent aqueous solution of this enzyme was sterilized by Seitz filtration and decimal dilutions prepared so that one ml would give the desired concentration per treatment (Table 1).

Purified polygalacturonase (PG), prepared from fungal pectinase at the Western Utilization Research and Development Division, USDA, Albany, California (16), was kindly supplied by E. F. Jansen of that laboratory. The purified enzyme was used in the 1958 and 1959 experiments at 1.0 ppm and 0.1 ppm respectively.

Four fungi, previously isolated and studied (7), were also used as pectinase sources. The fungi were grown on the surface of White's mineral broth plus pectin (7) and after two weeks incubation at 28° C, the clear filtrate was separated from the cells, diluted with 9 parts of water, and 1 ml of the 1:10 dilution added per jar of cucumbers (Table 2). Ten drops of toluene were added to each jar as a preservative.

Measurement of Cucumber Firmness

The USDA Fruit Pressure Tester (17) was used to measure cucumber firmness (2). For this study, each pressure test value represents the average of 10 cucumbers, each with a single center punch and recorded to the nearest pound resistance to the 5/16-inch tip of the instrument. Firmness values of less than 3 lb are not measurable with this tester. Adjective "Firmness Ratings" for salt-stock cucumbers of 1 to 1⅛ inches diameter assigned to pressure test values have been reported (2) as follows: *Very firm,* 18 lb and above; *Firm,* 14 through 17 lb; *Inferior,* 11 through 13 lb; *Soft,* 5 through 10 lb; *Mushy,* 4 lb and below. This information is given to assist the reader in interpreting the data in Tables 1 and 2.

[c] Supplied by Rohm and Haas Co., Philadelphia, Penna.

TABLE 1

INFLUENCE OF PECTINASE ENZYME AND SODIUM CHLORIDE CONCENTRATIONS ON CUCUMBER FIRMNESS

Enzyme	Enzyme concentration	Cucumber firmness [1] Sodium chloride concentration in % 0	1	2	3	4	5	6	8	10	12	16	20
	ppm	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*
(1958 Experiment)													
Control	0	14.4	.....	.....	.....	14.4	.....	.....	14.7	.....	14.6	14.3	14.6
46AP	0.01	<3	.....	.....	.....	9.5	.....	.....	10.5	.....	12.5	12.7	13.8
46AP	0.10	<3	.....	.....	.....	9.9	.....	.....	12.4	.....	13.1	13.3	13.4
46AP	1.00	<3	.....	.....	.....	4.2	.....	.....	8.2	.....	8.9	10.6	11.7
46AP	10.00	<3	.....	.....	.....	<3	.....	.....	5.0	.....	7.0	9.5	10.5
46AP	10.00 [2]	<3	.....	.....	.....	6.9	.....	.....	8.6	.....	11.2	11.7	12.7
PG	1.00	<3	.....	.....	.....	<3	.....	.....	5.6	.....	8.1	10.5	9.6
PG	1.00 [2]	<3	.....	.....	.....	7.3	.....	.....	10.1	.....	11.6	13.2	13.0
(1959 Experiment)													
Control	0	17.0	15.9	16.9	16.4	16.0	16.1	16.6	16.6	17.1	16.8	17.0	16.5
46AP	0.01	7.9	.....	13.7	14.1	12.9	14.7	.....	14.3	15.2	15.2	14.4	15.5
46AP	0.10	3.8	5.8	7.1	9.2	11.9	.....	.....	13.6	14.0	14.7	12.0	14.1
46AP	1.00	<3	<3	3.3	3.9	6.8	12.4	9.3	10.9	13.2	12.7	13.0	13.8
46AP	10.00	<3	<3	<3	<3	<3	3.3	3.7	5.6	8.4	10.1	11.5	12.2
46AP	10.00 [2]	<3	<3	4.8	6.8	10.8	10.2	11.8	11.8	14.5	14.5	14.8	15.7
PG	0.10	<3	<3	4.2	4.2	9.7	9.9	11.5	12.2	13.8	15.6	15.1	15.9

[1] Pressure test readings were taken after 1-month incubation at 30° C except where 7 days is indicated. Value of <3 = too soft to pressure test and the cucumbers ranged from very soft to mushy. Firmness values for controls averaged 14.5 lb in 1958 and 16.6 lb in 1959.

[2] Incubated 7 days.

RESULTS AND DISCUSSION

Softening Activity of Pectinase 46 AP and PG

Experimental cucumber packs (one-quart jars) with six levels of salt (0, 4, 8, 12, 16 and 20%) were treated in 1958 with increasing concentrations of 46 AP pectinase calculated to equalize at 0.01, 0.1, 1.0 and 10.0 ppm. An additional salt series was treated with purified polygalacturonase calculated to equalize at 1 ppm. The experimental lots of cucumbers together with a matched control salt series (without added enzyme) were incubated at 30° C. Cucumber firmness in pounds was recorded for the 10 ppm level of 46 AP enzyme and 1 ppm level of the PG enzyme after 1-week incubation and for all treatments after one-month incubation (Table 1, top-half). The firmness values for the control cucumber samples ranged from 14.3 to 14.7 (av 14.5); no change in firmness was demonstrated by increasing the salt concentration alone. For the added enzyme treatments, the cucumbers from the higher salt levels gave higher firmness readings. Cucumber firmness for all enzyme treatments without salt was less than 3 lb. Increasing levels of 46 AP pectinase decreased the firmness of the cucumbers within each salt concentration. An increase of incubation time for the two enzyme treatments (10 ppm of 46 AP and 1.0 ppm of PG) gave decreasing firmness of the cucumbers within each salt treatment. In comparing the action of pectinases from 2 sources, 1 ppm PG reduced cucumber firmness in the same order of magnitude with the different salt levels as did 10 ppm 46 AP and at about the same rate.

A second experiment was conducted in 1959 with double the number of salt concentration levels used in the experiment just described. The 46 AP enzyme was used at the same concentrations but the PG enzyme level was reduced to 0.1 ppm. Cucumber firmness values as shown in the lower part of Table 1 are the results of these tests.

In general, the results obtained for the second experiment were in agreement with those from the first. It was again demonstrated that the use of increasingly higher salt concentrations gave correspondingly higher values for cucumber firmness. Also, an increase in enzyme concentration resulted in a decrease in cucumber firmness at all levels of salt concentration.

The action of 1 ppm PG enzyme on the firmness of cucumbers at different salt strengths calculated from the data in Table 1, is presented in Figure 1. The rate of

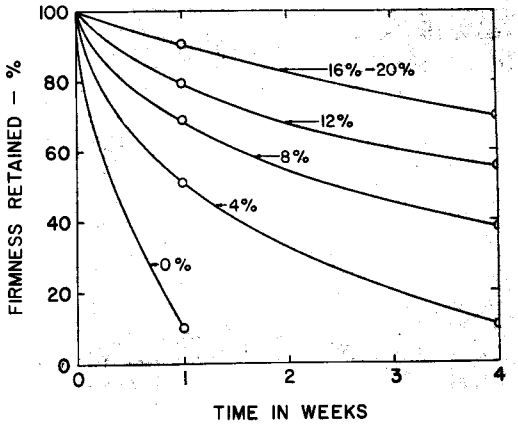

Fig. 1. Softening action of polygalacturonase enzyme (1 ppm) on cucumbers at different salt concentrations (in %).

enzyme action was very rapid at the lower salt levels and a higher degree of cucumber firmness was retained at the higher salt concentrations after 4 weeks' storage. However, it is emphasized that even with 16 to 20% salt the cucumbers lost 30% of their initial firmness in 4 weeks.

Softening Activity of Pectinase from Cell-Free Fungal Filtrates

Four fungi—*Fusarium roseum, F. oxysporum, F. solani* and *Ascochyta cucumis*—were among the 10 most frequently isolated species from the cucumber plant (*Cucumis sativus* L.) and represented 27% of 1032 fungus isolations obtained by Etchells *et al* (7). The 10 most frequently isolated species were shown (7) to produce pectinase and to soften cucumber tissue in a 2.5% salt brine acidified to pH 3.7. In the current study, 1-ml amounts of a 1:10 dilution of the cell-free filtrate from 14-day growth in broth from each of the above-named species were

added to a series of jars of cucumbers containing 0, 4, 8, 12, 16 and 20% salt. The final concentration of fungal filtrate per quart jar of cucumbers and brine was approximately 100 ppm.

The firmness values for the fungal filtrate experiment after 1-month incubation at 30° C are presented in Table 2. The filtrates of the 4 species reduced cucumber firmness to a greater extent in the lower salt levels than in the higher. These results were essentially the same as those from the pectinase enzyme experiments. If the data for the action of the fungi filtrates on cucumber firmness (Table 2) are plotted together with those for 10 ppm of pectinase 46 AP and 1 ppm PG, it is apparent that there is little difference in the softening behavior of the 3 enzyme sources toward cucumber tissue (Fig. 2). Further, the cucumbers retained more firmness as the salt strength was increased. If the log of the salt concentration is plotted against percent cucumber firmness retained, a straight line is obtained. This suggests that pectinolytic softening is a first-order reaction. Such data for pectinase 46 AP and the fungal filtrates are given in Figure 3.

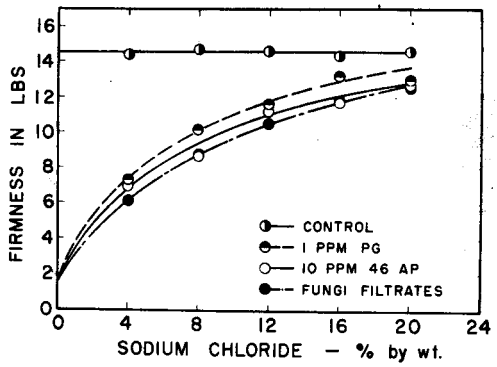

FIG. 2. Softening action of pectinase enzymes on cucumbers at different salt concentrations (in %).

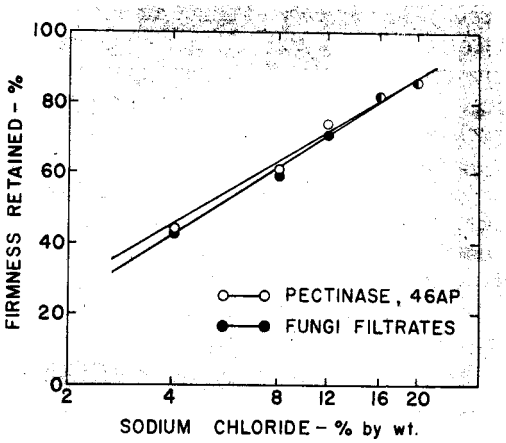

FIG. 3. Relationship of salt to pectinase activity on cucumber firmness.

SALTING-OUT ENZYMES

Enzymes have the chemical structure and general characteristics assigned to proteins. In the present study different sources of the pectinase enzymes were exposed to increasing concentrations of sodium chloride. Thus, the mention of a few general characteristics of enzymes, particularly their relation to several salts, is in order to form a basis of

TABLE 2

INFLUENCE OF CELL-FREE FUNGAL FILTRATES AND SODIUM CHLORIDE CONCENTRATIONS ON CUCUMBER FIRMNESS

Fungal filtrate added [1]		Cucumber firmness [2] Sodium chloride concentration in %					
		0	4	8	12	16	20
species	*no.*	*lb*	*lb*	*lb*	*lb*	*lb*	*lb*
Fusarium							
F. roseum	C-653	.....	9.0	9.8	11.3	12.3	12.9
F. oxysporum	C-214	<3	4.1	8.5	11.4	12.7	12.2
F. solani	C-775	.....	4.4	7.7	9.6	11.3	12.8
Ascochyta cucumis	C-1048	.....	6.7	8.9	9.2	10.7	12.2
Average		<3	6.1	8.7	10.4	11.8	12.5
Control		14.4	14.4	14.7	14.6	14.3	14.6

[1] Cell-free filtrate from 14-day-old growth in White's mineral broth plus pectin. One ml of a 1:10 dilution of the filtrate added to each quart jar of cucumbers.

[2] Pressure test readings were taken after 1-month incubation at 30° C.

explanation as to the influence of sodium chloride on enzymatic softening of cucumber tissue presented in this paper.

Pallmann *et al* (19) observed that a number of chloride solutions activated pectinase; maximum activity was obtained at 3.0 milliequivalents (0.017% NaCl) regardless of whether sodium or potassium chloride was used. The presence of small amounts of salt is necessary for the solution of proteins or enzymes in water. However, when increasingly larger quantities of very soluble salts, such as $(NH_4)_2SO_4$ and Na_2SO_4, and to a lesser extent NaCl, $MgSO_4$ and K_3PO_4, are added to enzyme solutions, the solubility of the enzyme decreases (3, 18, 20). At some definite salt concentration, the enzyme is almost completely precipitated from solution, and this technique is used for enzyme and protein isolation studies.

In general, precipitation of an enzyme by salt is most complete at or near the isoelectric pH of the specific enzyme. This would be at a pH value where the positive charges would equal the negative with a net charge of zero. Under such conditions, the protein or enzyme would not migrate in an electric field. Dixon and Webb (3) give the following general equation for enzyme solubility in concentrated salt solutions: $\log S = B\text{-}KI$, where S is solubility, I is the ionic strength, B and K constants. B depends greatly on temperature and pH, but K is independent of these factors. This equation tends to explain the log relationship of salt concentration to per cent firmness of cucumbers as reported in Figure 3 herein.

TABLE 3

RELATIVE PECTINOLYTIC SOFTENING OF CUCUMBERS AS INFLUENCED BY INCREASING SALT (NaCl) CONCENTRATIONS

Salt concentration		Relative [1] softening activity
salometer	by wt	
degrees	%	%
<5	<1.3	>90
6	1.6	82
8	2.1	76
10	2.6	70
12	3.2	65
14	3.7	60
16	4.2	56
18	4.8	53
20	5.3	50
22	5.8	47
24	6.4	44
26	6.9	42
28	7.4	40
30	8.0	38
32	8.5	36
34	9.0	34
36	9.5	32
38	10.1	30
40	10.6	29
42	11.1	28
44	11.7	26
46	12.2	25
48	12.7	24
50	13.2	23
52	13.8	22
54	14.3	21
56	14.8	20
58	15.4	19
60	15.9	18
65	17.2	15
70	18.6	12
>75	>19.8	<10

[1] Expressed as relative % loss of cucumber firmness to be expected at different salt strengths by the action of a constant pectinase level when compared to non-enzyme-treated controls.

RELATIVE PROTECTION OF CUCUMBER FIRMNESS AGAINST SOFTENING ACTIVITY OF PECTINASE

In order to develop a relative rate of pectinolytic softening of cucumbers, an overall cucumber firmness average at each salt treatment was calculated; this required consideration of the data in Tables 1 and 2. The averages were made in terms of percent retained firmness of the cucumbers and plotted against the log of salt concentration expressed as percent saturation of NaCl (degrees salometer). Table 3 was prepared from this graph and is proposed to be used as a guide to estimate the protective action of various percentages of salt against softening enzyme activity. For example, if the pectinase level was sufficiently high (= to 1 ppm 46 AP) and remained constant in 38° and 20° salometer fermentations, then the relative degree of pectinolytic softening expected would be 30 and 50%, respectively, of original cucumber firmness, indicating that cucumbers brined at the lower brine strength would be about 20% softer.

A commercial cucumber fermentation usually does not have a fixed concentration of salt other than at the time the vats are filled, headed and covered with brine. Brining procedures, as a rule, provide for a gradual increase in brine strength during the first 4 to 6 weeks after filling the vats. It would be difficult indeed to predict the softening rate under such conditions other than to point out that the results of these experiments indicate a decrease of pectinolytic softening would be expected where higher initial brine strengths were employed. However, if brining procedures were modified to substantially increase the initial cover brine, such practice would, as mentioned in the introduction, be at the risk of increasing bloater formation (hollow cucumbers) resulting from a more gaseous fermentation.

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LITERATURE CITED

1. Bell, T. A., J. L. Etchells, and I. D. Jones. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. *Food Technol.*, **4**, 157.
2. Bell, T. A., J. L. Etchells, and I. D. Jones. 1955. A method for testing cucumber salt-stock brine for softening activity. U. S. Dept. Agr., *ARS-72-5,* 15 pp.
3. Dixon, M., and E. C. Webb. 1958. *Enzymes.* Academic Press, Inc., New York, N. Y.
4. Etchells, J. L., and T. A. Bell. 1950. Classification of yeasts from the fermentation of commercially brined cucumbers. *Farlowia,* **4**, 87.
5. Etchells, J. L., T. A. Bell, and I. D. Jones. 1955. Studies on the origin of pectinolytic and cellulolytic enzymes in commercial cucumber fermentations. *Food Technol.,* **9** (3), 14.
6. Etchells, J. L., T. A. Bell, and I. D. Jones. 1955. Cucumber blossoms in salt-stock mean soft pickles. *Research and Farming,* (N. C. Agr. Expt. Sta.), **13** (1–4), 14.
7. Etchells, J. L., T. A. Bell, R. J. Monroe, P. M. Masley, and A. L. Demain. 1958. Populations and softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. *Appl. Microbiol.,* **6**, 427.
8. Etchells, J. L., F. W. Fabian, and I. D. Jones. 1945. The *Aerobacter* fermentation of cucumbers during salting. Mich. Agr. Expt. Sta. *Tech Bull. 200.*
9. Etchells, J. L., and I. D. Jones. 1943. Bacteriological changes in cucumber fermentation. *Food Inds.,* **15** (2), 54.
10. Etchells, J. L., I. D. Jones, and W. M. Lewis. 1947. Bacteriological changes during the fermentation of certain brined and salted vegetables. U. S. Dept. Agr. *Tech Bull. 947.*
11. Etchells, J. L., I. D. Jones, and T. A. Bell. 1950-51. Advances in cucumber pickling. *Yearbook of Agr.* (U. S. Dept. Agr.) 229. U. S. Government Printing Office, Washington, D. C.
12. Etchells, J. L., and I. D. Jones. 1951. Progress in pickle research. *Glass Packer,* **30** (4), 264; (5) 358.
13. Fabian, F. W., C. S. Bryan, and J. L. Etchells. 1932. Experimental work on cucumber fermentation. Mich. Agr. Expt. Sta. *Tech. Bull. 126.*
14. Fabian, F. W., and L. J. Wickerham. 1935. Experimental work on cucumber fermentation. VIII. Genuine dill pickles—A biochemical and bacteriological study of the curing process. Mich. Agr. Expt. Sta. *Tech. Bull. 146.*
15. Jones, I. D. 1940. Salting of cucumbers: influence of brine salinity on acid formation. *Ind. Eng. Chem.,* **32**, 858.
16. Lineweaver, H., R. Jang, and E. F. Jansen. 1949. Specificity and purification of polygalacturonase. *Arch. Biochem.,* **20**, 137.
17. Magness, J. R., and G. F. Taylor. 1925. An improved type of pressure tester for the determination of fruit maturity. U. S. Dept. Agr. Dept. Circ. 350.
18. Neilands, J. B., and P. K. Stumpf. 1955. *Outlines of Enzyme Chemistry.* John Wiley and Sons, Inc., New York, N. Y.
19. Pallmann, H., J. Matus, H. Deuel, and F. Weber. 1946. Über die aktivität der pektinase. *Rec. trav. chim. Pays-Bas,* **65**, 633.
20. West, E. S., and W. R. Todd. 1955. *Textbook of Biochemistry.* 2nd ed. The Macmillan Co., New York, N.Y.