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Pectinesterase in the Cucumber[1]

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INTRODUCTION

There are two principal enzymes responsible for pectin degradation of fruits and vegetables. They are pectinesterase (*syn.*: pectase, pectinmethylesterase, pectinmethoxylase), which catalyzes the deësterification of pectin; and, polygalacturonase (*syn.*: pectinase), which catalyzes the glycosidic hydrolysis of pectin and pectic acid. This study deals with the enzyme pectinesterase (PE) and the possible part it plays in the type of spoilage known as softening which occurs during the manufacture of cucumber salt-stock for pickles. The details concerning salt-stock manufacture have been given by others (1,2,3) and will not be repeated here.

In a recent report Bell, Etchells, and Jones (4) observed a correlation between the polygalacturonase activity of commercial cucumber brines and the softening of salt-stock removed from these brines. They further reported pectinesterase to be present in the brines from both firm and soft lots of cucumbers. Jansen and MacDonnell (5) and Matus (6) have observed that the glycosidic hydrolysis of pectin must first be preceded by deësterification of the pectin to pectinic acids. This puts considerable importance on the part pectinesterase may play in the destruction of the pectin of cucumbers in relation to salt-stock soften-

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ing. The presence of the esterase alone in the brine would not necessarily be a causative agent, but together with the glycosidase enzyme system, softening of salt-stock could readily take place.

Many fruits and vegetables are known to contain pectinesterase, but the cucumber (*Cucumis sativus*) apparently has not been studied in this respect. An investigation of various tissues of this plant is therefore in order to determine if they are contributing sources of the enzyme in the commercial fermentations. The pectinesterase of the fruit of the tomato plant (*Lycopersicon esculentum*) was also studied for comparison. Following the first report on the esterase enzyme by Fremy (7) in 1840, there have been numerous publications (8–18) dealing both with its occurrence in plant tissues, and with its mode of action. Some of the good sources of pectinesterase are tomatoes, pea vines, alfalfa, eggplant, orange flavedo, and tobacco.

Methods of pectinesterase analysis are based on measurement of the rate of liberation of carboxyl groups as determined with a standard alkali. In 1936, Kertesz (9) offered the first quantitative method of measurement, replacing the qualitative test based on the rate of a pectate-gel formation. His method has been modified and improved by Lineweaver and Ballou (10), MacDonnell *et al.* (11), and Hills and Mottern (13).

Procedure

Outline of Work

The investigation of pectinesterase was undertaken for the following reasons: (*a*) It was desired to establish whether the cucumber is a source of the enzyme and, if so, to determine the characteristics of the enzyme under varying conditions of pH, temperature, and sodium chloride concentration; (*b*) to trace the enzyme quantitatively through the developmental stages of the cucumber plant and fruit and of the tomato fruit; (*c*) to measure the rate of diffusion of the enzyme from cucumbers of different size and variety into brine under laboratory conditions which did not permit fermentation; and (*d*) to measure the enzyme activity in brines and cucumbers during fermentation under conditions typical of the commercial pickling industry.

Method of Pectinesterase Assay

The technique as described by Hills and Mottern (13) was followed very closely and it is summarized below: Into a 600-ml. beaker were added 200 ml. of a 1% pectin[2] solution, 5 ml. of 0.2 *M* sodium oxalate solution, the enzyme extract, sufficient

[2] Pectin NF was used and was obtained from the California Fruit Growers Exchange, Ontario, California. Mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.

2 *M* sodium chloride solution to make the substrate 0.15 *M* with respect to this salt, and distilled water to make a total volume of 500 ml. The amount of sodium chloride solution added to the reaction mixture varied due to the amounts of enzyme extract added. The latter ranged from 10 to 50 ml. The reaction mixture for enzyme measurements was carried out in a water bath at 30°C., electrically stirred and connected with a Beckman pH meter. Standard 0.1 *N* or 0.5 *N* sodium hydroxide solutions were used to hold the pH constant, and for measurements of deësterification.

Pectinesterase Unit

The number of milliequivalents of ester bonds hydrolyzed per minute per unit volume or weight of enzyme material at pH 7.5, 30°C., and with 0.15 *M* sodium chloride when acting on a 0.4% pectin solution is designated as a unit of pectinesterase. This unit is very similar to that described by Lineweaver and Ballou (10) with symbol PE μ, and Hills and Mottern (13) with symbol *K*. Unless otherwise specified the conditions set forth above were followed in this study.

Extract

The freshly picked cucumbers, tomatoes, and other plant tissues tested were washed with cool tap water, graded for well-developed, healthy fruits and tissues, assorted to respective sizes, and frozen in airtight cans at −10°C. For analysis the plant material was partially thawed and ground in a Waring Blendor with a small amount of distilled water to which sufficient sodium chloride was added to make the tissue–water mixture 2% with respect to this salt. The pH of the blended material was determined, and those below pH 6.0 were adjusted to this value with 1 *N* sodium hydroxide. The juices were extracted through heavy, fruit-press cloths, made to known volume, and stored under toluene at 5°C. The ash content of the green cucumber is very low, so there was no attempt made to purify the PE enzyme extract or free it from naturally occurring minerals. The salt-stock cucumbers and dry seeds were blended directly without freezing. Sodium chloride was not added in the enzyme extraction from salt-stock. This extraction method is very similar to that recommended by Hills and Mottern (13) for pectinesterase from tomatoes.

Discussion of Results

The Presence and Properties of Cucumber Pectinesterase

Samples of small green cucumbers gave measurements of pectinesterase activity in sufficient amounts (approximately 0.300×10^{-2} units/ml.) to accurately measure and study. Since the properties of this enzyme in plant materials other than that of the cucumber have been so completely studied by other investigators (10,11,13,14,18), their techniques were easy to apply to this study. The properties of cucumber pectinesterase were found to be very similar to those reported for orange flavedo (11) and tomato (13), although slight differences were observed for optimum pH and for electrolyte stimulation. The opti-

TABLE I

Effect of Pectinesterase Activity on Pectin at Various pH Levels

pH of reaction mixture[a]	Relative activity %	
7.5	100	
7.0	97	
6.0	64	
5.0	20	
4.0	1	(37)[b]
3.5	0	(6)[b]

[a] Reaction mixture was at 30°C., 0.15 *M* NaCl (includes NaCl of enzyme extract) and 0.4% pectin.

[b] NaCl in substrate doubled to 0.30 *M* concentration.

mum pH for cucumber esterase activity was 7.5 and maximum electrolyte concentration was between 0.15 and 0.20 *M* sodium chloride in pectin substrate (see Tables I and II). The salt concentration of the substrate was shown to be very important, and at lower pH levels increasing amounts produced higher activities. For example, at pH 4.0 and 0.15 *M* sodium chloride concentration, the activity could hardly be detected, but by doubling the salt content of the substrate the esterase activity was approximately one-third that observed at the optimum pH of 7.5. Salt activation at low pH values has been demonstrated with orange flavedo (11) and tomato (13) esterases.

The increase in the rate of enzyme reaction, with increasing temperatures at intervals of 10 degrees from 20° to 60°C. (Q_{10}), is slightly less than that observed with tomato pectinesterase (13). The Q_{10} values for cucumber pectinesterase are 1.38 for 20–30°C.; 1.39 for 30–40°C.; 1.29

TABLE II

Effect of Sodium Chloride on Pectinesterase Activity at pH 7.5

Sodium chloride[a] concentration *M*	Relative activity %
0.05	80
0.10	89
0.15	98
0.20	100
0.30	85
0.60	51
1.00	14

[a] NaCl of enzyme extract included.

for 40–50°C.; and 1.24 for 50–60°C. Denaturation of cucumber esterase becomes apparent when temperatures rise above 60°C., and complete inactivation takes place at 70°C. for 10 min.

The enzyme extract was very stable at pH 6.0 when stored at 5°C. This is similar to the behavior described for tomato pectinesterase (13). When the extract was at room temperature, and at pH levels of 2.0, 3.0, 4.0, 5.0, and 6.0, the per cent retention was greatest in those which approached pH 6.0 (Table III). At room temperature, complete inactivation was observed at pH 2.0 in 1 week, and at pH 3.0 in 1 month.

Comparison of Cucumber and Tomato Pectinesterase with Respect to Concentration During Development of Plant Tissue

Fresh leaves, petioles, stems, flowers, fruits, and dry, mature seeds of the cucumber variety Producer were tested for pectinesterase activity (Table IV). The seeds, petioles, stems, and large fruits were considerably lower in activity than the leaves, flowers, and small fruits.

TABLE III

Per Cent Retention of Pectinesterase Activity at Five Different pH Levels

Storage time	pH level of storage					
	6.0*	6.0	5.0	4.0	3.0	2.0
Retention after 1 week, %	100	98	94	48	1.4	0
Retention after 1 month, %	100	77	67	18	0	0

* Stored in refrigerator at 4°C.; all others stored at 25°C. Pectinesterase activity determined at optimum conditions.

The enzyme activity on a weight basis (PE units/g.) of the fruit decreased very rapidly with increase in size and with fruit development. The activity on an individual whole-fruit basis remained approximately the same, with no steady increase or decrease in amount.

The pectinesterase content of the tomato covering the complete period of fruit development is reported and illustrated in Fig. 1. On a weight basis (PE units/g.), the green tomatoes of sizes less than 10 g. increased more than four times in activity during development to the 100–150 g. weight range, and ten times in activity to complete ripeness. On an individual fruit basis, the enzyme content of the tomato increased

over 300 times during the period it developed from a very small green fruit to a fully ripe tomato. This substantiates earlier reports (14,16) that pectinesterase was present in higher concentrations in ripe tomato fruits than in the green ones. However, the PE content of the fruit throughout the developmental stages, which includes the green to ripe, has not previously been clearly indicated.

From these data with the cucumber and tomato, it is evident that the pectinesterase activity of the two fruits is decidedly different in the rate of enzyme production during fruit development. The enzyme content in the cucumber remains more or less constant throughout the

TABLE IV

Pectinesterase Activity of Extracted Cucumber Tissue

Cucumber tissue tested (Producer variety)	Pectinesterase units × 10^3		
	Extracted juice	Cucumber[a]	Whole cucumber[a]
	per ml.	*per g.*	*each*
Seeds	0.009	0.090	—
Leaves	0.237	0.395	—
Petioles	0.079	0.117	—
Stems	0.109	0.109	—
Flowers			
staminate	0.025	2.155	0.10
pistillate and embryo unpollinated	0.074	0.870	0.34
pollinated	0.153	1.092	0.55
Green whole fruit, range in sizes, *g.*			
5–10	0.342	0.339	3.05
10–25	0.327	0.302	5.45
25–50	0.183	0.160	5.34
50–75	0.136	0.126	8.16
75–100	0.059	0.054	4.92
100–150	0.029	0.026	3.13
150–200	0.027	0.025	4.05
200–300	0.028	0.024	5.60
Ripe yellow fruit, *g.*			
300–500	0.008	0.008	4.00

[a] Data converted from analysis of extracted juice.

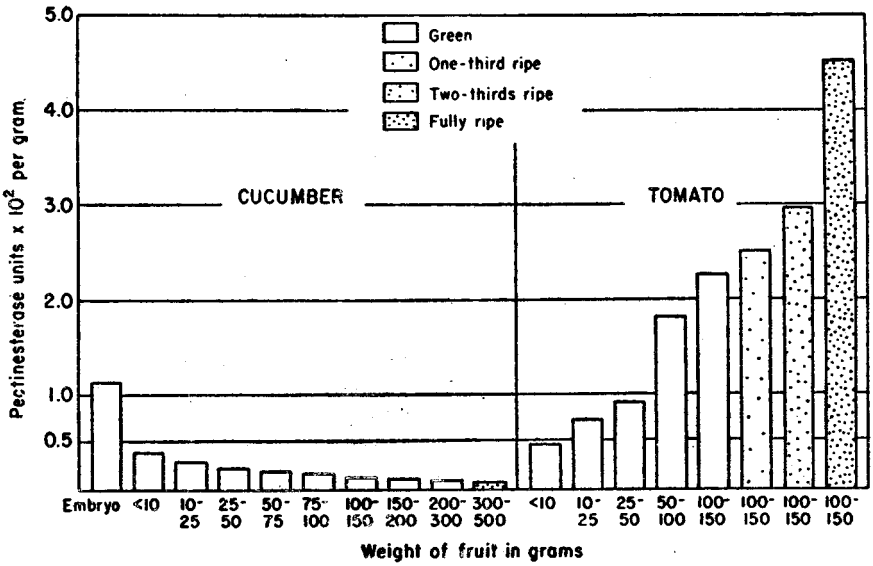

FIG. 1. The pectinesterase content of the cucumber and tomato according to fruit size and ripeness.

growth period; whereas, in the tomato, the enzyme content increases very rapidly. This is illustrated in Fig. 1.

Cucumber Pectinesterase Diffusion into Brine

The rate of pectinesterase diffusion into the brine was determined on four sizes each of four pickling varieties of cucumbers, namely, Producer, Model, National, and Earliest of All. The green cucumbers were brined according to size and variety in No. 10 tin cans; toluene was added to prevent fermentation. The brining treatment for all lots was identical. The ratio of cucumber to brine was of the order of 90 ml. of 13.2% sodium chloride solution to each 100 g. of cucumber. After 1 week the brine concentration had dropped to 6.6 ± 0.2% salt and was considered equalized. At this time the brine and the extracted pressed juice from the cucumbers were adjusted to pH 6.0 and stored at 5°C. with toluene as a preservative. No additional salt was added in the enzyme extraction of the cucumbers.

The brines and extracts of the fruit of different size and variety were

examined for esterase activity; the data are presented in Table V. The diffusion of pectinesterase from the cucumbers into the brine was related to cucumber size; the smaller the cucumber, the more complete was equalization. In all four varieties, the fruit of the smallest size-range showed the highest enzyme activity/g. tissue. This is the same behavior as shown in Table IV. There was no significant difference in the rate of diffusion or amount of activity between varieties. It is of interest to point out that the activity was higher in the extracts ob-

TABLE V

The Pectinesterase Activity of Four Sizes of Four Cucumber Varieties as Measured on the Brines and Pressed Juices after One-Week Equalization Period Without Fermentation[a]

Variety of cucumber	Extract tested	Pectinesterase units × 10^3/ml.			
		Cucumber sizes (dia. in inches)			
		0.75–1 in.	1–1.5 in.	1.5–2 in.	2–2.5 in.
Producer	Brine	0.124	0.016	0.009	0.004
	Juice	0.428	0.204	0.104	0.059
Model	Brine	0.066	0.041	0.011	0.005
	Juice	0.418	0.223	0.093	0.059
National	Brine	0.116	0.034	0.008	0.004
	Juice	0.515	0.268	0.109	0.062
Earliest of All	Brine	0.055	0.017	0.005	0.001
	Juice	0.485	0.286	0.129	0.075

[a] Stored at room temperature, approx. 25°C.

tained by the diffusion experiment (Table V) than in those from the straight extraction experiment (Table IV). The difference is attributed to the use of a greater amount of sodium chloride in the diffusion experiment. It appears that increasing the salt content gave a more complete elution of the enzyme from the cucumber pulp.

Commercial Cucumber Fermentations

Samples of brine and cucumbers from vats of approximately 700-bushel capacity undergoing fermentation were selected. They were

taken from two vats each of three commercial sizes of pickling cucumbers, namely, size No. 1 (small), size No. 2 (medium), and size No. 3 (large). Further division was subsequently made between the sizes on a weight basis. The pectinesterase activity was determined on the brines and extracts of the cucumbers (Table VI). The enzyme activity of these samples was measurable, but much lower than that found in the diffusion experiment described earlier, or in the extract of the fresh fruit of the Producer variety. There was no difference in activity between extracts or brines of cucumbers of the different sizes obtained from the commercial operation at the period of fermentation when sampling took place.

TABLE VI

Pectinesterase Activity of Commercially Brined Cucumbers After 43–47 Days of Fermentation

Cucumber				Pectinesterase units × 10^3	
Size No.[a]	Diameter	Range	Average weight	Cucumber	Brine
	in.		*g.*	*per g.*	*per ml.*
1	< 1	Small	6	0.016	0.021
		Medium	16	0.016	
		Large	30	0.017	
2	1–1.5	—	48	0.011	0.007
3	1.5–2	Small	75	0.012	0.005
		Large	107	0.016	0.007

[a] Represents samples from two vats each of size Nos. 1, 2, and 3.

The low pectinesterase activity was considered to indicate an evidence of enzyme inactivation during the elapsed fermentation period. Inactivation was attributed to the instability of the enzyme under the conditions which prevailed with respect to the acidity and temperature of the brine. The brines in the vats sampled may be characterized with respect to pH, acidity, salt content, and temperature as follows: pH, 3.5–3.7; acidity as lactic, 0.28–0.34%; sodium chloride, 15.8%; temperature, 25–30°C. It was shown (Table III) that the stability of cucumber pectinesterase stored under similar conditions in the laboratory was very poor. For example, after 1 month storage at pH 4.0 and 25°C., retention in activity was only 18%. It is evident that pectines-

terase activity is greatly reduced under commercial conditions. To determine the amount of cucumber pectin converted to pectinic or pectic acids within the fruit in commercial fermentations would be of further interest in studies on this problem.

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Summary and Conclusions

The seeds, leaves, petioles, stems, flowers, and fruit of the pickling cucumber (*Cucumis sativus*) were found to contain the deësterifying pectic enzyme, pectinesterase. The optimum for activity of this enzyme (pH, 7.5, and NaCl, 0.15–0.20 *M*) was very similar to that reported for orange flavedo and tomato esterase.

The rate of development of the enzyme in the cucumber and tomato (*Lycopersicon esculentum*) was found to be different. In the cucumber, the esterase content remains fairly constant throughout fruit development, but in the tomato, the enzyme content increases very rapidly, to over 300 times its initial amount.

The diffusion of the pectinesterase from four varieties of pickling cucumbers into a 13.2% sodium chloride brine, without fermentation, was more rapid from the small-sized cucumbers than from the large. There were no real differences in esterase content among the four varieties studied.

Under commercial conditions, six vats of cucumbers undergoing fermentation were observed for the presence of pectinesterase. The activity in both the extracted cucumber juice and in the brine was low as compared to that observed in the nonbrined, green cucumbers. The low activity was attributed chiefly to enzyme inactivation caused by the acid resulting from the lactic fermentation.

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