PECTOLYTIC ENZYME ACTIVITY IN VARIOUS PARTS OF THE CUCUMBER PLANT AND FRUIT

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PECTOLYTIC ENZYME ACTIVITY IN VARIOUS PARTS OF THE CUCUMBER PLANT AND FRUIT[1]

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Introduction

Each year the pickle industry suffers considerable economic loss caused by an uncontrollable type of spoilage known as softening of cucumber salt-stock. Recently, BELL *et al.* (1) identified a causative agent in commercial pickle brines to be a pectin-splitting enzyme, related to polygalacturonase, and gave a method for measuring its activity in the brines. Before the pickle industry can expect to have reliable methods for controlling or preventing spoilage caused by enzymatic softening, the source of the pectin-splitting enzyme must be investigated.

The present study deals principally with the extent of pectolytic enzyme activity in the seeds, flowers, petioles, stems, and fruit of several varieties and sizes of pickling cucumbers (*Cucumis sativus* L.). No attempt was made fully to characterize the pectolytic enzyme. The work indicated that certain parts of the plant and the mature whole fruit may be the source of the softening enzyme rather than microbial activity as heretofore supposed.

As ripe tomatoes (*Lycopersicon escu-*

[1] This study was carried out under a co-operative project with the Department of Horticulture of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina.

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lentum Mill.) are known to contain a pectolytic enzyme, the study included measurements on Marglobe variety as a check on the viscosity method and also to determine whether any differences occurred during development of the enzyme system in fruits of the two plants.

General methods of commercial salting of cucumbers for pickles have been described by ETCHELLS and JONES (2) and by others (3, 19) and will not be given in this report.

Nomenclature

The nomenclature of the pectolytic enzymes which catalyze the hydrolysis of the glycosidic bonds of pectin and also cause a reduction in viscosity of pectin or pectic acid has been reviewed recently (12, 15, 18). These enzymes are referred to as pectinase and polygalacturonase. More recently (13), an enzyme slightly different from polygalacturonase has been reported in ripe tomatoes and named pectic acid-depolymerase.

In the present study the general term "pectolytic enzyme" is used. Although the enzyme is similar to polygalacturonase, its characteristics are not yet fully known. When cucumber salt-stock becomes soft, the glycosidic hydrolysis of pectin or pectic acid to galacturonic acid would not necessarily have to be complete. Only a slow and partial depolymerization of the pectin molecule would be required. The work of FABIAN and JOHNSON (4) indicates that this is true because they found the total pectin content of both mushy and firm salt-stock pickles to be the same when measured as calcium pectate. The glycosidic enzyme system found in the brines from soft salt-stock, or in the extracts of the cucumber and tomato tissues reported here, may be only a depolymerase and, if so, would not hydrolyze pectic acid to galacturonic acid.

Review of literature

There are numerous sources of the pectolytic enzymes. A recent article by MATUS (12) lists sixty-six specific sources for pectinase, including bacteria, actinomycetes, molds, fungi, higher plants, and animals. It is doubtful if all sources above are correct, since methods for testing the enzyme vary with different workers, and much of the work was done without the newer knowledge of pectin and pectic enzymes. As an example, the pectic enzyme from *B. mesentericus fuscus*[3] claimed by FABIAN and JOHNSON (4) to be responsible for softening of cucumber salt-stock could not be the polygalacturonase-like enzyme system found in soft-cucumber brines or in the commercial pectinols (1), because of the outstanding differences in chemical properties (e.g., pH, acidity, and salt tolerance of the enzyme systems).

The extensive studies (5, 7, 8, 10, 16) with the polygalacturonase enzyme system have been based on molds as the source. Because the present study deals with a pectolytic enzyme active in varieties of pickling cucumbers and in tomatoes, sources from higher plants reported in the literature are of greater interest.

In 1921 PATON (17) demonstrated that Easter lily pollen contained pectinase. In summarizing, he pointed out the following: "Histological examination shows that in most instances pollen tubes make their way between the walls of adjacent cells rather than penetrating them. We should expect therefore to find most frequently not a cytase- or cellulose-digesting enzyme, but rather a pectinase ca-

[3] BERGEY'S *Manual of Determinative Bacteriology* (6th ed., 1948) lists *B. mesentericus fuscus* as a synonym for *B. subtilis*.

pable of digesting the pectin of the inner lamella."

In 1938 KERTESZ (9) found polygalacturonase activity in ripe tomatoes but stated that it apparently was absent in the green tomato. The method he used to indicate glycosidic hydrolysis was based on a decrease in alcohol precipitate of a pectin solution when acted upon by the tomato extract. He was not satisfied with the change in viscosity of a pectin solution for measuring polygalacturonase activity. MACDONNELL *et al.* (11) found polygalacturonase activity in ripe tomatoes and observed an increase in reducing groups liberated from pectic acid as determined by the WILLSTÄTTER-SCHUDEL hypoiodite method. They observed that tomato extract was twice as active at pH 4.0 as compared with its activity at pH 5.6. MOTTERN and HILLS (14) also observed polygalacturonase activity in tomato extract and found a decrease in viscosity of 0.9–1.2% per hour when 1.0 ml. was added to 200 ml. of a 1% pectin solution at pH 4.0 and at 30° C. Recently, MCCOLLOCH and KERTESZ (13) reported a polygalacturonase-like enzyme in ripe tomatoes and named it pectic acid-depolymerase. They considered this enzyme to be different from polygalacturonase of mold origin because depolymerization of pectic acid was not complete to galacturonic acid and the tomato enzyme was more heat-resistant.

Material and methods

EXTRACTION.—Freshly picked cucumber and tomato plant tissues were washed with cool tap water, graded for well-developed healthy fruits, and assorted to respective sizes. Four varieties of cucumber—Producer, Model, National, and Packer—and the Marglobe variety of tomato were represented.

The cucumber and tomato fruits were then frozen in sealed cans and held at −10° C. Later, they were partially thawed and were ground with small amounts of distilled water in a Waring Blendor with 2% sodium chloride. The pH values of the blended materials were then 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 leaves, flowers, petioles, stems, and dry seeds[4] of the cucumbers and the embryo and flower of the tomato were blended directly without freezing. The extraction procedure was similar to that used by HILLS and MOTTERN (6) for pectinesterase of tomatoes.

PECTOLYTIC ENZYME MEASUREMENTS.—The method described by BELL *et al.* (1) was used, which, briefly, is as follows: Two 25-ml. amounts of approximately 3% pectin[5] solution, having a relative viscosity of about 30 and buffered at pH 4.0 with sodium citrate–citric acid, were placed in 50-ml. Erlenmeyer flasks. Five milliliters of the extracted juice were placed in one flask, and 5 ml. of the same extract which had been heated to 80° C. in a water bath for 10 minutes to inactivate the enzyme were placed in the other. After an incubation period of 6 days at 30° C., the dropping time from the pipette and apparatus previously described (1) was determined. The measurement of activity was expressed as percentage loss in viscosity of the unheated extract over that of the control. Values so obtained are not a linear quan-

[4] Kindly supplied by Mr. FRANK B. FAUST, Manager, Associated Seed Growers, Atlanta, Georgia.

[5] Pectin NF was used and was obtained from California Fruit Growers Exchange, Ontario, California. The mention of trade-name products does not imply that they are indorsed or recommended by the Department of Agriculture over other similar products not mentioned.

titative measurement of enzyme activity, as demonstrated previously (1).

Where gel formation interfered with the viscosity test, the extracted juices were treated to destroy the pectinesterase prior to the enzyme determinations. This was done by incubating the extract at pH 3.0 for 24 hours at 40° C. These conditions used for destroying pectinesterase also cause some reduction in activity of the enzyme responsible for loss in viscosity of pectin. This was demonstrated by lower values for controls which were obtained upon treating non-gel samples.

Results and Discussion

CUCUMBERS.—From the results of measurements of pectolytic enzyme activity in the cucumber materials (table 1), it appears that the cucumber itself may be an important contributing factor to softening.

Glycosidic hydrolysis as indicated by percentage loss in viscosity of a pectin solution was demonstrated in many parts of the cucumber plant and fruit (table 1). Extracts from dry cucumber seeds were strongly positive; losses in viscosity of the pectin solution ranged from 44 to 72%. Extracts of leaves, petioles, and stems for the variety examined were all negative for enzyme activity. Staminate flowers and the pollinated pistillate flowers of two varieties, Producer and National, were strongly positive; but the unpollinated were negative. This would indicate that the pectolytic enzyme is produced by the staminate flowers and transmitted in the pollen grains by insects to the pistillate flowers.

Green, whole fruits were weak to negative in enzyme activity, whereas the ripe, whole fruits of the Producer, Model, and National varieties were strongly positive. Seeds in the yellow to orange-colored ripe fruits were more mature, and the enzyme activity most likely ties in with the seed development. This may offer an explanation for the soft-center type of spoilage found in large-sized salt-stock made from cucumbers approaching maturity. It is of added interest that the

TABLE 1

PECTOLYTIC ENZYME ACTIVITY AND PH OF EXTRACTED CUCUMBER TISSUE

Cucumber tissue tested	pH	% pectolytic enzyme activity as loss in viscosity*
Seeds (10% extract):		
Producer var.	6.5	44
Model var.	6.2	68
National var.	6.4	50
Packer var.	6.4	72
Leaves, Producer var.†	7.0	0
Petioles, Producer var.	6.4	0
Stems, Producer var.	6.0	0
Flowers (approx. 2% extract):		
Staminate		
Producer var.	6.3	91
National var.	.....	88
Pistillate and embryo		
Unpollinated		
Producer var.	7.40	0
National var.	7.40	0
Pollinated		
Producer var.	7.20	89
National var.	7.00	84
Green whole fruit:†		
Producer var.		
Range in sizes (gm.)		
5–10	6.1	12
10–25	5.9	9
25–50	5.8	9
50–75	5.7	7
75–100	5.8	0
100–150	5.7	0
150–200	5.8	1
200–300	5.2	22
Ripe whole fruit:		
Producer var.	4.4	56
Model var.	4.5	43
National var.	4.4	53

* Degree of pectolytic activity: 0–3%, negative; 4–10%, weak; 11–30%, moderate; above 30%, strong. The percentage loss in viscosity values is not a linear quantitative measurement.

† The extracted juice with pectin solution gave a gel caused by the enzyme pectinesterase. To avoid this, extracted juice was adjusted to pH 3.0, incubated at 40° C. for 24 hours, and then tested on pectin solution for enzyme activity.

pH value of the extracted fruit decreased as the fruit developed—the value for the small (green) fruit was 6.1, whereas for the ripe it was 4.4. Pectolytic activity was found in the extracts of mature fruits where the pH of 4.4 is near the optimum for polygalacturonase activity (12, 18) and also for the softening enzyme (1) of commercial brines.

These findings put an altogether new light on the problem of softening of commercial cucumber brine stock. Heretofore, microbial activity in the brine has been assumed to be the sole enzyme source.

TOMATOES.—Pectolytic enzyme activity was absent in the embryos with flowers, and in six stages of development of the green fruit of the Marglobe tomato starting with fruit of less than 10 gm. in weight to those of 150 gm. Fruits in the three stages of ripeness, as indicated by red skin color of one-third, two-thirds, and all red, were moderately to strongly positive.

This work confirms other reports (9, 11, 13, 14) that ripe tomatoes contain a polygalacturonase-like enzyme. However, the absence of this enzyme in the early developmental stages and in the green fruit has not previously been clearly demonstrated. Sizable quantities of green tomatoes are brined yearly by the pickle industry for use as dills, relishes, cut pickle, and pasteurized products. The absence of the softening enzyme in the green fruit may, in part, account for the lack of difficulty experienced by industry in brining this commodity.

Summary

1. The cucumber plant and fruit (*Cucumis sativus*) have been found to be a source of a pectolytic enzyme as measured by a loss in viscosity of a pectin solution.

2. The enzyme of the cucumber was strongly active in the seeds, staminate and pollinated pistillate flowers, and ripe fruit but was not found in the unpollinated flowers, leaves, petioles, and stems.

3. Enzyme activity was weak to negative in the eight stages of cucumber development of immature fruit.

4. Pectolytic enzyme activity was absent in the green tomato (*Lycopersicon esculentum*) including the embryo with flowers and six stages of green-fruit development.

5. High activity of the enzyme was found in the red ripe tomato fruit.

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