INHIBITION OF PECTINASE AND CELLULASE BY CERTAIN PLANTS[1]

THOMAS A. BELL,[2] JOHN L. ETCHELLS,[2] CARLOS F. WILLIAMS,[3] AND WILLIAM L. PORTER[4]

Introduction

Pectic and cellulosic substances are the most abundant organic materials in fruits and vegetables and their chemical changes have been related to texture and quality in food processing (**11**). The softening of brined cucumbers under commercial conditions was demonstrated (**6**) to be caused by pectinolytic and cellulolytic enzymes which were introduced into the fermentation by mold-laden flowers attached to the green cucumbers. The need for adequate control of enzymatic softening of cucumbers has resulted in several reports by the authors (**3, 7, 8**).

BELL and associates (**2, 4, 8**) have demonstrated the presence of naturally occurring pectinase and cellulase inhibitor(s) in the leaves of grape (*Vitis*). The inhibitor content of leaves of the muscadine group (*V. rotundifolia* Michx.) was much higher than for those of the Concord variety (*V. labrusca* L.). The grape-leaf inhibitor(s) (GLI) for pectinase and cellulase was characterized as a water-soluble, heat-stable, high-molecular weight organic substance, which was not dialyzable through a cellophane membrane against water. Increasing concentrations of GLI caused a reduction of pectinase enzyme activity approaching 100% inhibition; reduction of cellulase activity was also obtained, but the percentage of inhibition was dependent on the enzyme source used. ETCHELLS *et al.* (**8**) reported the inhibition of the two softening enzymes in small-scale cucumber fermentations by the use of grape leaves. Furthermore, increasing levels of grape-leaf extract gave increasing firmness to the cucumbers that had been treated with cucumber flowers as a source of both enzymes. The use of grape leaves was not considered practical for commercial-scale fermentations, but the results provided a most promising lead for the chemical control of the softening enzymes—pectinase and cellulase.

Studies have continued on the chemical nature of the water-soluble enzyme inhibitor in grape leaves. Recent findings by PORTER, SCHWARTZ, BELL, and ETCHELLS (unpublished data) have revealed that the pectinase inhibitor in the leaves is a tannin-like substance which is precipitated by caffeine, nicotine sulfate, and gelatin. Inhibition of pectic enzymes by tannins and other phenolic compounds has been reported by others (**5, 9, 10**). Some tannins, such as gambir tannin, do not inhibit pectinase (**9**), however. In 1958 (**4**) and 1960 (**2**) the authors reviewed the pertinent literature on pectinase and cellulase inhibitors as well as that for certain other hydrolytic enzyme systems such as amylase; therefore, these areas will not be covered herein.

The present study was initiated to screen the water-soluble extracts of leaves of a wide variety of plants for their ability to inhibit pectinase and cellulase.

Material and methods

LEAF EXTRACTIONS.—Mature leaf samples, about 200 gm. each, from the different plants were collected, with two exceptions, from the greenhouses and the Method Horticultural Station, North Carolina State College and vicinity. The papaya and pomegranate leaves were supplied by Dr. F. P. GRIFFITHS, U.S. Fruit and Vegetable Products Laboratory, Weslaco, Texas. There were 71 samples representing 61 species in 32 different families. Classification followed BAILEY (**1**). Because the muscadine grape group was known to contain the pectinase and cellulase inhibitor, ten varieties and two breeding lines of this plant were included in the tests. The fresh leaves from all plants were washed in tap water, air dried to original weight, then stored in polyethylene freezer bags at $-10°$ C. About ten leaf samples at a time were removed from the freezer for extraction and enzyme inhibitor tests. This procedure was repeated until all plants were screened against four enzyme preparations. Aqueous extracts from each frozen leaf sample were obtained as follows: 20 gm. of shredded leaves were blended in 400 ml. of distilled water for 3 minutes; the slurry was pressed through several thicknesses of cheesecloth and the extract clarified by centrifugation for 15 minutes at 3000 r.p.m. The supernatant extract was either tested immediately or preserved with ten drops of toluene

[1] This study was carried out under a co-operative project between the North Carolina Agricultural Experiment Station and the U.S. Food Fermentation Laboratory of the Southern Utilization Research and Development Division, ARS, USDA. Published with the approval of the Director of Research, North Carolina Agricultural Experiment Station, as Paper no. 1312 in the Journal Series.

[2] U.S. Food Fermentation Laboratory, ARS, USDA, Raleigh, North Carolina.

[3] Department of Horticulture, North Carolina Agricultural Experiment Station, Raleigh, North Carolina.

[4] Plant Products Laboratory, Eastern Utilization Research and Development Division, ARS, USDA, Philadelphia, Pennsylvania.

and stored overnight at 4° C. When leaf extracts interfered with the enzyme tests, such as causing gel formation with the polypectate solution, the extracts were dialyzed in a cellophane membrane for 4 hours against water. The grape-leaf inhibitor had been found to be non-dialyzable under such conditions.

PECTINASE AND CELLULASE SOURCES.—Crude enzyme solutions were prepared from partially dried cucumber flowers (CF) as previously described (**2, 4**). The flowers from Model variety cucumbers (1–1⅛ inches in diameter) were collected at a pickle manufacturing plant in North Carolina and frozen in polyethylene freezer bags for later enzyme extraction. Two commercial enzymes were also used, pectinase 46AP and cellulase 19AP, supplied by Rohm and Haas Company, Philadelphia, Pennsylvania.

MEASURING ENZYME ACTIVITY AND INHIBITION.—The enzyme systems measured were pectinase, which hydrolyzes the glycosidic bonds of pectic acid, and cellulase, which hydrolyzes the glucosidic bonds of a soluble cellulose derivative (sodium carboxymethylcellulose). The viscosity methods as reported in earlier papers by BELL and co-workers (**2–4**) were used for measuring activity for both enzyme systems. Standard enzyme reaction curves were used to convert percentage loss in viscosity to units of activity. A value of 100 units was established to equal 50% loss in viscosity in 20 hours of an enzyme–substrate mixture at 30° C. and buffered at pH 5.0. Plant-leaf extracts were tested for inhibition by mixing two parts of enzyme solution with one part of leaf extract; water controls were used in place of extract and/or enzyme solutions. Enzyme activity was usually in the range of 350-400 units. The reduction in pectinase and cellulase activity was expressed in percentage loss in activity of the enzyme control as caused by the plant-leaf extract (fig. 1).

Results and discussion

Pectinase and cellulase activities from cucumber flowers were shown in previous reports (**2, 4**) to be reduced by increasing levels of GLI in a first-order type reaction. As shown in figure 1, a plot of the logarithm of grape-leaf concentration (muscadine grape) against percentage reduction of enzyme activity (either pectinase or cellulase) gives a straight line from about 10% to 95%. For comparative purposes in measuring the degree of pectinase and cellulase inhibition by the different plant extracts, a linear scale of enzyme inhibition is indicated on figure 1. Thus, for each plant a 5% (fresh-weight) extract is compared to the GLI. Reduction of enzyme activity from 0% to 25% is considered doubtful to negative; 25% to 60% equals 1+ (weak); 60% to 80%, 2+ (moderate); 80% to 90%, 3+ (strong); and greater than 90% equals 4+ (very strong inhibition).

Table 1 gives the results of testing the different leaves for their ability to inhibit two sources of pectinase and two of cellulase. About one-half the plant-leaf extracts were negative to doubtful for all four enzyme preparations. The twenty-nine plant species which were positive for inhibition of one or more enzymes were scattered among the different families, although the number of positives in the Rosaceae may be of significance; for example, blackberry, raspberry, and rose contained moderate to strong enzyme inhibition except for cellulase 19AP, which was not inhibited.

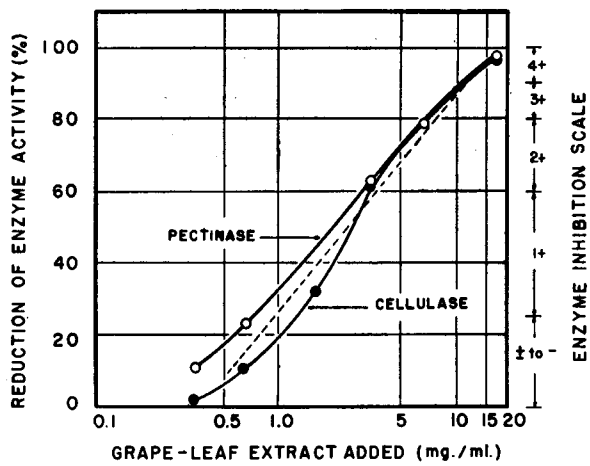

FIG. 1.—Increasing concentrations (mg/ml) of grape-leaf extract as related to reduction of activity of cucumber-flower (CF) pectinase and cellulase. For comparing enzyme inhibition by other plant leaf samples, a scale from − to 4+ is shown on right for converting per cent enzyme reduction to a linear scale unit.

PECTINASE INHIBITION.—Of the twenty-nine species of plants which demonstrated pectinase inhibition, nineteen inhibited the enzyme from both sources. The following eight species gave strong to very strong inhibition and would be considered as good sources of the pectinase inhibitor: muscadine grape, persimmon, dogwood, blackberry, blueberry, raspberry, rose, and sericea.

CELLULASE INHIBITION.—Fewer plant species demonstrated cellulase inhibition than had demonstrated pectinase inhibition. With cellulase (CF), although fourteen species were positive, nine of these were only weakly inhibitory. Further, none of the species equaled the inhibitory action of the muscadine grape. Five species of plants (muscadine, persimmon, dogwood, blueberry, and sericea) were considered good sources of the inhibitor; these species also gave strong pectinase inhibition. Only two species were positive against cellulase 19AP: muscadine

TABLE 1

PECTINASE AND CELLULASE INHIBITION BY PLANT-LEAF EXTRACTS[a]

SCIENTIFIC NAME	COMMON NAME	DEGREE OF ENZYME INACTIVATION[b] Pectinase 46AP	Pectinase CF	Cellulase 19AP	Cellulase CF
Acer rubrum L.	Red maple	2+	1+	–	1+
Antirrhinum majus L.	Snapdragon	–	1+	–	–
Capsicum frutescens L.	Bell pepper	2+	–	–	–
Carya illinoensis Koch.	Stuart pecan	2+	1+	–	–
Chaenomeles japonica Lindl.	Flowering quince	–	1+	–	–
Cornus florida L.	Flowering dogwood	4+	3+	–	3+
Dianthus caryophyllus L.	Carnation	1+	–	–	–
Diospyros virginiana L.	Persimmon	4+	4+	1+	3+
Euphorbia pulcherrima Willd.	Poinsettia	–	1+	–	–
Fragaria chiloensis Duchesne	Albritton strawberry	2+	2+	–	1+
Ipomoea batatas Lam.	Sweet potato	1+	–	–	–
Iris hybrid	German iris	2+	–	–	–
Lespedeza cuneata Don.	Sericea	3+	3+	–	3+
Ligustrum lucidum Ait.	Privet	2+	1+	–	–
Parthenocissus quinquefolia Planch.	Virginia creeper	2+	2+	–	–
Pelargonium hortorum	Geranium	2+	1+	–	1+
Persea americana Mill.	Avocado	2+	1+	–	–
Phaseolus vulgaris L.	Snap bean	–	1+	–	–
Prunus domestica L.	Plum	2+	1+	–	–
Prunus persica Batsch.	Hale Harrison peach	2+	–	–	–
Punica granatum L.	Pomegranate	1+	1+	–	1+
Pyrus communis L.	Pear	2+	–	–	–
Rosa odorata Sweet.	Tea rose	4+	2+	–	1+
Rubus hybrid	Carolina blackberry	3+	2+	–	1+
Rubus strigosus Michx.	Latham raspberry	3+	2+	–	1+
Thea sinensis L.	Tea	1+	1+	–	1+
Vaccinium ashei	Rabbiteye blueberry	2+	4+	–	3+
Vitis labrusca L.	Concord grape	1+	2+	–	1+
Vitis rotundifolia Michx.[c]	Muscadine grape	4+	4+	2+	4+

[a] In addition to species listed in table which gave positive results with one or more enzyme preparations, the following species were tested and gave negative to doubtful results for all four enzyme preparations: *Althea rosea* Cav., *Begonia semperflorens* Link and Otto, *B. carpreolata* L., *Brassica oleracea* L., *Carica papaya* L., *Chrysanthemum hortorum*, *Citrus limon* Burm. f., *Coffea arabica* L., *Coleus blumei* Benth., *Curcurbita maxima* Duchesne, *Elaeagnus pungens* Thunb., *Ficus carica* L. (Celeste), *Gardenia jasminoides* Ellis, *Hedera helix* L., *Hibiscus syriacus* L., *Ilex cornuta* Lindl. (Burfordii), *Ipomoea purpurea* Lam., *Lonicera japonica* Thunb., *L. tartarica* L., *Lycopersicon esculentum* Mill., *Magnolia grandiflora* L., *Matthiola incana* R. Br., *Morus alba* L., *Nandina domestica* Thunb., *Nicotiana tabacum* L., *Petunia hybrida* Vilm., *Prunus cerasus* L. (Montmorency), *Pueraria thunbergiana* Benth., *Pyrus malus* L. (Lodi), *Sainpaulia ionantha* Wendl., *Trifolium repens* L., *Tagetes patula* L.

[b] See text or figure 1 for meaning of symbols.

[c] Ten varieties and two breeding lines of muscadine grape were tested; inhibitory action was same for all. Varieties were Burgaw, Creek, Creswell, Hunt, wild sp., Scuppernong, Thomas, Topsail, Yuga, and Willard. Tetraploid forms of Yuga and Thomas were equal in enzyme inhibition to diploid forms.

grape and persimmon. It is not readily understood why extracts failed to actively inhibit cellulase 19AP, but this enzyme system has been shown (2) to be rather heat stable and composed of several enzyme components which are not clearly defined.

INHIBITOR CONCENTRATIONS IN SELECTED PLANT SPECIES.—To demonstrate the first-order type reaction as previously shown for the muscadine grape inhibitor (fig. 1), four plant-leaf samples were tested further. Persimmon, dogwood, raspberry, and sericea, together with muscadine grape, were assayed in decreasing concentration (mg/ml) of their plant-leaf extracts against pectinase (CF) and cellulase (CF). The results shown in table 2 for the different plant-leaf extracts, when compared to the percentage of enzyme inactivation, will support a first-order reaction as shown for grape leaves in figure 1.

The sericea leaves were air-dried at 55° C. for 24 hours and there was only slight loss in the inhibitor as compared to the fresh-leaf samples (table 2). The inhibitor substance in grape leaves has been shown (4) to be reduced more than one-half when dried under such conditions.

TABLE 2

PECTINASE AND CELLULASE (CF) INHIBITION BY DECREASING CONCENTRATIONS OF PLANT-LEAF EXTRACTS

PLANT	CONCENTRATION OF PLANT EXTRACT ADDED (MG/ML)	ENZYME INACTIVATION Pectinase (%)	Cellulase (%)
Vitis rotundifolia (muscadine grape)	16.7	95	99
	3.3	62	66
	0.7	28	35
Diospyros virginiana (persimmon)	16.7	86	99
	3.3	69	92
	0.7	18	50
Cornus florida (dogwood)	16.7	87	84
	3.3	55	50
	0.7	21	.....
Rubus strigosus (raspberry)	16.7	63	69
	3.3	37	38
	0.7	10	16
Lespedeza cuneata (sericea)	16.7	88 (87)[a]	83 (73)
	3.3	64 (53)	36 (27)
	1.6	46 (31)	21 (19)
	0.3	14 (12)	6 (12)

[a] Data in parentheses are per cent enzyme inactivation by air-dried samples calculated on fresh-leaf sample basis for comparative purposes.

Summary

1. The water-soluble leaf extracts from 61 plant species in 32 families were screened for their ability to inhibit two hydrolytic fungal enzymes, cellulase and pectinase.

2. Leaf extracts from 29 species inhibited pectinase and extracts from 14 inhibited cellulase.

3. The leaves from muscadine grape, persimmon, dogwood, blueberry, sericea, blackberry, raspberry, and rose were considered good sources for the pectinase inhibitor as measured against cucumber-flower pectinase and a commercial pectinase.

4. In general, cellulase inhibition by the different plant species was less pronounced than that observed for pectinase. The first five species listed in the previous paragraph gave strong inhibition of cucumber-flower cellulase. Muscadine grape and persimmon were the only two species which inhibited the commercial cellulase enzyme 19AP, and then only moderate to weak inhibition was obtained.

The authors gratefully acknowledge the assistance of Dr. W. W. G. SMART, JR., Department of Animal Industry and Dr. W. A. COPE, Department of Field Crops, North Carolina Agricultural Experiment Station, for supplying the sericea samples and for suggesting the use of this plant as an inhibitor source.

LITERATURE CITED

1. BAILEY, L. H. Manual of Cultivated Plants. Macmillan Co., New York, 1949.
2. BELL, T. A.; AURAND, L. W.; and ETCHELLS, J. L. Cellulase inhibitor in grape leaves. BOT. GAZ. 122:143–148. 1960.
3. BELL, T. A.; ETCHELLS, J. L.; and JONES, I. D. A method for testing cucumber salt-stock brine for softening activity. Publ. U.S. Dept. Agr. ARS-72-5. 1955.
4. BELL, T. A., and ETCHELLS, J. L. Pectinase inhibitor in grape leaves. BOT. GAZ. 119:192–196. 1958.
5. COLE, M. Oxidation products of leuco-anthocyanins as inhibitors of fungal polygalacturonase in rotting apple fruit. Nature 181:1596–1597. 1958.
6. ETCHELLS, J. L.; BELL, T. A.; and JONES, I. D. Studies on the origin of pectinolytic and cellulolytic enzymes in commercial cucumber fermentations. Food Technol. 9(3): 14, 16. 1955.
7. ETCHELLS, J. L.; BELL, T. A.; MONROE, R. J.; MASLEY, P. M.; and DEMAIN, A. L. Populations and softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. Appl. Microbiol. 6:427–440. 1958.
8. ETCHELLS, J. L.; BELL, T. A.; and WILLIAMS, C. F. Inhibition of pectinolytic and cellulolytic enzymes in cucumber fermentations by Scuppernong grape leaves. Food Technol. 12:204–208. 1958.
9. HATHWAY, D. E., and SEAKINS, J. W. T. The influence of tannins on the degradation of pectin by pectinase enzyme. Biochem. Jour. 70:158–163. 1958.
10. POLLARD, A.; KIESER, M. E.; and SISSONS, D. J. Inactivation of pectic enzymes by fruit phenolics. Chem. and Ind. (London) 1958:952. 1958.
11. WEIER, T. E., and STOCKING, C. R. Histological changes induced in fruits and vegetables by processing. Adv. in Food Res. 2:297–342. 1949.