COMMUNICATIONS

Pectinesterase Activity as a Function of pH, Enzyme, and Cation Concentrations

Equations are presented that predict the activity of pectinesterases as a function of cation, and enzyme concentrations and pH. Although the equations are empirical, they are statistically significant and quantitatively represent kinetic differences between plant and fungi pectinesterases.

Pectinesterase is an enzyme that catalyzes the hydrolysis of the methylester groups found in the pectin chain. It is commonly found in fruits and vegetables and it is also synthesized by various microorganisms. Both pectinesterases are activated by the presence of cations, specifically Na^+ and Ca^{2+}. However, for a constant cation concentration, their optimum pH values differ markedly, being in the acidic range for microorganisms and neutral or slightly alkaline range for higher plants. Numerous results have been published that qualitatively show this behavior, and the reader is referred to various summaries that cover the existing literature (Davies, 1963b; Hofstee, 1960; Kertesz, 1951; Reed, 1966; Reid, 1961). The present work was motivated by the desire to obtain quantitative results that could be used to predict the influence of pH and cation concentration on the activities of the various plant and fungal pectinesterases.

EXPERIMENTAL

Materials. The two pectinesterases studied were commercial preparations: a freeze-dried tomato concentrate from Worthington Biochemical Corp. and a Rohm and Haas fungi concentrate, Pectinol 42-E. As substrate an unstandardized rapid set pectin (A/S Kovenhavns Pektinfabrik, Denmark) was employed, with a percent esterification of 13.02, determined by standard techniques (Owens *et al.*, 1952).

Procedure. The enzyme activity was tested by the potentiometric method of Hills and Mottern (1947), employing a Beckman Model K automatic titrator, modified as to have a constant temperature bath at 30° C. All measurements were made at this temperature. The 0.02 *N* NaOH consumed by the enzymatic reaction was followed, employing a microburette and an electric laboratory clock. The initial pectin concentration was 0.5% by weight, unless otherwise indicated. A 1 *M* NaCl solution was used as the cation source. Data were taken every 0.5 to 1 min up to 5 to 30 min, depending on the reaction rate. To obtain the enzyme activity only the linear part of the relation between volume of alkali consumed *vs.* time was considered. The experimental data were fitted by a linear least squares digital program, from which the slope was obtained. Activity is expressed as units of pectinesterase activity, a unit being equal to the removal of one methoxy group per minute. A blank (without enzyme) run was done every time pH and cation concentration were changed, and the result was used to correct the normal (with enzyme) run. More details on the equipment and procedures can be found elsewhere (Mayorga, 1969).

RESULTS AND DISCUSSION

In order to have a clear understanding of the effect of each variable upon the activity and also their possible interrelations, it was decided to employ a 3^3 factorial experimental design (Davies, 1963a). The levels for each variable were chosen in such a way as to be within the range of values already reported in the literature. These values are summarized in Table I for the two enzymes. The results of this design are tabulated in Table II for the tomato pectinesterase and in Table III for the fungi pectinesterase. In order to select the statistically significant first and second order effects among the variables, a variance analysis was done for the two sets of data, according to standard techniques (Davies, 1963a). The activities were then correlated as a function of these significant parameters, and a model was developed employing a multiple regression digital program (Dykstra, 1963), from which the value of the constants was obtained.

The regression model for the tomato pectinesterase was:

$$\begin{aligned}ACT = {} & -1572.33 - 8.93\,(CEN) + 8.25\,(CEN)^2 - \\ & 587.44\,(SAL) + 439.37\,(PH) - 29.93\,(PH)^2 + \\ & 76.97\,(PH)\,(SAL) \quad (1)\end{aligned}$$

For the fungi pectinesterase:

$$\begin{aligned}ACT = {} & -61.93 - 1.52\,(CEN) + 50.69\,(SAL) + \\ & 29.12\,(PH) - 3.49\,(PH)^2 + 0.61\,(CEN)\,(PH) - \\ & 126.79\,(PH)\,(SAL)^2 + 17.16\,(SAL)^2\,(PH)^2 \quad (2)\end{aligned}$$

Where:

ACT = activity, units $\times 10^3$
CEN = enzyme concentration, mg of concentrate
SAL = cation concentration, molarity
PH = Solution pH.

The activity values predicted by these two models are also shown in Tables II and III for the two enzymes. The standard error of estimate was equal to 1.404 for the tomato enzyme and 0.362 for the fungi pectinesterase.

In Equation 1, the linear factor for enzyme concentration, the quadratic for pH, and the second order pH and salt effect are significant to a 1% level according to an F test (Davies,

Table I. Values of the Variables

Variable	Levels 1	2	3
Tomato Pectinesterase			
CEN, mg	1.5	2.0	2.5
SAL	0.10*M*	0.15*M*	0.20*M*
PH	7.0	7.5	8.0
Fungi Pectinesterase			
CEN, mg	1.0	6.0	11.0
SAL	0.05*M*	0.10*M*	0.15*M*
PH	3.5	4.0	4.5

Table II. Tomato Pectinesterase Activity Units × 10^3

	pH								
	7.0			7.5			8.0		
	SAL			SAL			SAL		
CEN mg	0.10*M*	0.15*M*	0.20*M*	0.10*M*	0.15*M*	0.20*M*	0.10*M*	0.15*M*	0.20*M*
1.5	37.32	35.76	32.81	40.63	40.97	42.19	37.60	36.24	39.27
	37.04	34.60	32.18	43.58	43.07	42.56	35.16	36.58	37.99
2.0	47.96	45.36	40.61	54.36	52.43	55.32	44.74	44.12	47.71
	47.02	44.59	42.16	53.56	53.06	52.55	45.14	46.56	47.98
2.5	60.80	57.12	56.26	67.47	70.39	65.53	60.54	58.92	62.59
	61.13	58.70	56.27	67.67	67.17	66.66	59.25	60.67	62.09

Note: For every set of conditions, the first reported value corresponds to the experimental result, the other one has been calculated by Equation 1.

Table III. Fungi Pectinesterase Activity Units × 10^3

	pH								
	3.5			4.0			4.5		
	SAL			SAL			SAL		
CEN mg	0.05*M*	0.10*M*	0.15*M*	0.05*M*	0.10*M*	0.15*M*	0.05*M*	0.10*M*	0.15*M*
1.0	0.54	0.50	0.67	1.26	1.15	1.35	1.97	2.79	2.15
	0.00	0.59	0.21	1.59	2.39	2.01	1.66	2.52	2.27
6.0	2.92	3.16	3.38	6.76	6.71	7.00	7.10	10.09	8.48
	2.86	3.64	3.26	6.16	6.95	6.58	7.74	8.60	8.35
11.0	5.91	6.06	6.16	11.16	11.82	11.89	12.69	15.34	13.51
	5.91	6.69	6.30	10.72	11.51	11.14	13.82	14.68	14.43

See Note in Table II.

1963a). The rest are significant to a 5% level. In Equation 2, all the factors are significant to a 1% level, except the linear for enzyme concentration and the second order salt square and pH square, which are significant at a 5% level.

Equations 1 and 2 show that the activity of both enzymes is affected quite differently by the concentration and pH variables. For a constant enzyme and cation concentrations, the optimum pH values can be calculated from Equations 1 and 2 by standard differential calculus techniques. For the tomato pectinesterase the result is:

$$PH = \frac{439.37 + 76.97\,(SAL)}{59.86} \qquad (3)$$

For the fungi pectinesterase:

$$PH = \frac{29.12 + 0.61\,(CEN) - 126.79\,(SAL)^2}{6.98 - 34.32\,(SAL)^2} \qquad (4)$$

Substituting into these formulas the corresponding values from Table I, the results shown in Table IV are obtained. Two major differences can be observed. First the optimum pH for the fungi pectinesterase is a function of the enzyme concentration as well as of the cation concentration. Second, for the tomato pectinesterase, an increase of twice the cation concentration corresponds to an increase of about 0.1 pH unit. The same tendency is observed for the fungi pectinesterase, but the increase is less as the enzyme concentration diminishes.

These results apply to the range of conditions explored in this work and only for the two enzymes tested. A generalization of these models would be made if similar results are obtained with enzymatic preparations from different plants and vegetables and various microorganisms (bacteria and fungi). However, this study would demand a great deal of effort, not only due to the large number of possible sources of pectinesterases, but because of the enzyme concentration steps needed so as to carry out the tests with as pure as possible enzyme extracts, as recommended by Dixon and Webb (1964). This scheme would take all appropriate parameters into account, such as those reported by Hultin *et al.* (1966) on the banana fruit, where they found the possible existence of three different pectinesterases in the fruit.

In conclusion, this work is limited in scope and application but points out, quantitatively, the different behavior of two pectinesterases with respect to enzyme and cation concentration and pH.

Table IV. Optimum pH Values

Tomato Pectinesterase

SAL	PH
0.10	7.47
0.15	7.53
0.20	7.59

Fungi Pectinesterase

	CEN		
SAL	1.0	6.0	11.0
0.05	4.27	4.71	5.15
0.10	4.29	4.75	5.20
0.15	4.33	4.82	5.31

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LITERATURE CITED

Davies, O. L., "The Design and Analysis of Industrial Experiments," pp. 247–250, 332–6, Hafner Publishing Co., New York, 1963a.
Davies, R., Microbial Extracellular Enzymes, their uses and some factor affecting their formation, in C. Rainbow, Rose A. H., "Biochemistry of Industrial Microorganisms," pp. 118, 133, Academic Press, New York, 1963b.
Dixon, M., Webb, E. C., "Enzymes," pp. 10–25, Academic Press, New York, 1964.
Dykstra O., Multiple Regression Package, Program 1620/6.0.043, IBM Library, 1963.
Hills, C. H., Mottern, H. H., *J. Biol. Chem.* **168,** 651 (1947).
Hofstee, B. H. J., Fatty acid esterases of low eserine sensitivity and related enzymes, in: P. D. Boyer, Lardy, H., Myrback, K., "The Enzymes," Vol. 4, pp. 499, Academic Press, New York, 1960.
Hultin, H. O., Sun, B., Bulfer, J., *J. Food Sci.* **31,** 320 (1966).
Kertesz, Z. I., "The Pectic Substances," Interscience Publishers Ltd., pp. 360–374, London, 1951.
Mayorga, H., "Pectinesterasas de Hongos y Plantas; influencia de ciertas variables en su actividad," Tesis, Universidad de San Carlos, Guatemala, Septiembre, 1969.
Owens, H. S., McCready, R. M., Shepherd, A. D., Schultz, T. H., Pippen, E. L., Swenson, H. A., Miers, J. C., Erlandsen, R. F., Maclay, W. D., "Methods used at WRRL for extraction and analysis of pectic material," United States Department of Agriculture, Document AIC-340, June, 1952.
Reed, G., "Enzymes in Food Processing," pp. 83–87, Academic Press, New York, 1966.
Reid, W. W., Preparation, properties, and assay of pectin-degrading enzymes, in: "Production and application of enzyme preparations in food manufacture," pp. 35–42, *Soc. Chem. Ind.*, (London), Monograph No. 11, 1961.

Héctor Mayorga
Carlos Rolz

Central American Research Institute (ICAITI)
Guatemala, Central America

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