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PECTASE IN DOYENNÉ BOUSSOCH PEARS AND CHANGES IN THE QUANTITY OF THE ENZYME DURING DEVELOPMENT

BY

C. WEURMAN

Central Institute for Nutrition Research T.N.O., Utrecht.
Laboratorium voor Algemene Plantkunde, Plantenphysiologie en Pharmacognosie, Univ. Amsterdam

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INTRODUCTION

The evidence for the occurrence of pectase in apples and pears is somewhat confusing. According to SMOCK and NEUBERG (1950) the presence of the enzyme in apples has never been proved satisfactorily. KERTESZ (1951) states that he and his collaborators could not prove its occurrence in the same fruit. Neither apples nor pears are mentioned by MATUS (1948) in a detailed survey of the literature on the occurrence of pectase in plants. EGGENBERGER (1949), on the other hand, is of the opinion that proof of the occurrence of the enzyme in these fruits is available in the older work of MEHLITZ with SCHEUER (1934) and MAASS (1935). In the experiments of these investigators strong gels were formed when sap from cherries and some berry-fruits was added to 2 % apple-pectin solutions. For apples and pears, however, only small gel fragments and precipitates were found in prolonged experiments where other factors could have influenced the results. Yet in the still earlier work of SLOEP (1928) and of BERTRAND and MALLÈVRE (1894, 1895) considerable gel formation with apple extracts was obtained and later the same experimental results were published by TSEREVITINOV and ROZANOVA (1934). An explanation for this diversity of opinion as to the occurrence of pectase in fruit is given elsewhere (WEURMAN, 1952). Recently POLLARD and KIESER (1951) measured the activity of pectase in apples by determining the methanol liberated by the enzyme but apart from the unconvincing experiments of MEHLITZ and co-workers mentioned above, no other information on the occurrence of pectase in pears has appeared.

In the course of experiments to investigate the changes in pectin in pears during development and ripening it became of interest to consider the possible interference of pectase with these changes.

METHODS

The presence of pectase in Doyenné Boussoch pears was demonstrated by the formation of gels when extracts of the fruit were added

to pectin solutions. The enzyme could be precipitated by addition of $(NH_4)_2SO_4$ to these extracts and the activity of the dried preparation measured titrimetrically.

Changes in the quantity of the enzyme in the fruit during the development were estimated by recording the increase in the viscosity of extract-pectin mixtures. The following procedure was followed.

Samples of at least 15 pears were picked at various dates during the development. The fruit was washed, peeled, cored, grated and ground in a mortar with quartz. To 15.00 g of the pulp 52.5 ml of 0.1 mol Na_2HPO_4 was added so as to raise the pH of the mixture to 8 and thus loosen the adsorption of the enzyme on the cell structure (cf. a.o. MACDONNELL JANSEN and LINEWEAVER (1945). The suspension was left for $^1/_2$hr at 30° C, centrifuged and the supernatant filtered. 20.0 ml of the filtrate (extract 'E') was well mixed with 35.0 ml of 0.25 % B.P. pectin * solution in

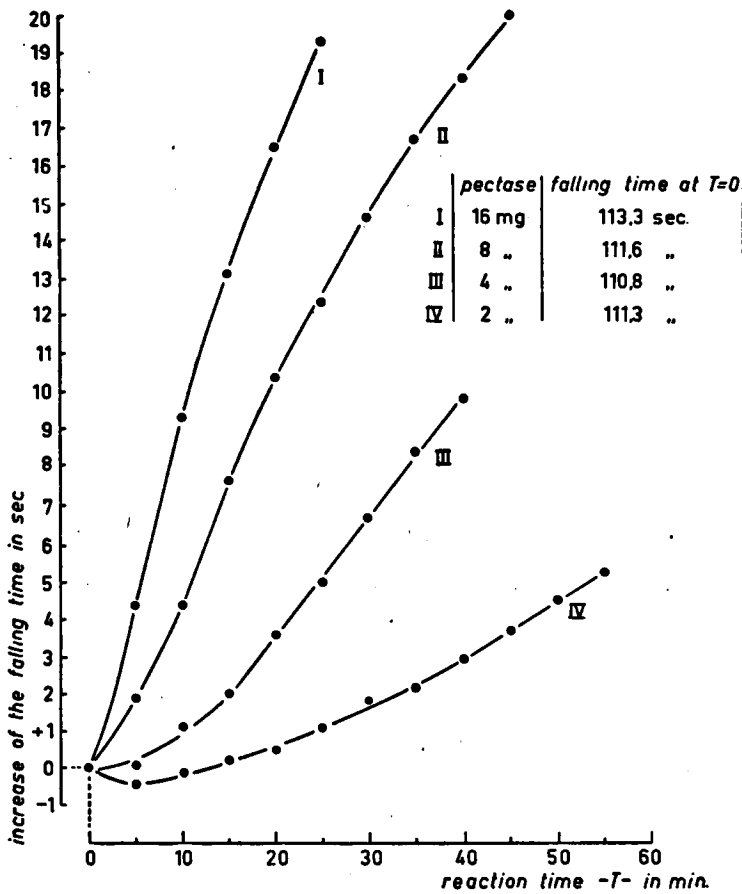

Fig. 1. The increase in viscosity of a pectin solution caused by pectase 50 ml 0,20% B.P.-pectin in 0,067 mol. phosphate buffer pH 6,0; 5 ml distilled water containing different amounts of pectase. Temp. 25° C

* Properties of the pectin, indicated by 'B.P.' are given elsewhere (WEURMAN, 1954).

0.067 mol phosphate buffer pH = 6.0. The pH of the reaction mixture was 6.55 ± 0.05; no change in the pH took place during the experiments. The increase in viscosity was measured using a Hoeppler falling ball viscosimeter at 25.00 ± 0.02° C. The results of the experiments are presented in the form of graphs in which the increase in the falling time of the ball indicating the increase in viscosity, is plotted against time. Control experiments were carried out with boiled extracts.

It is known that the increase in viscosity caused by the action of pectase on pectin is influenced by cations of higher valency, especially Ca- ions. The enzyme itself is activated by these ions in a complicated way (cf. a.o. LINEWEAVER and BALLOU (1945), MACDONNELL a. cow. (l.c.), PITHAWALA, SAVUR and SREENIVASAN (1948). Quite apart from their action on the enzyme, they play a role in gel formation, as was

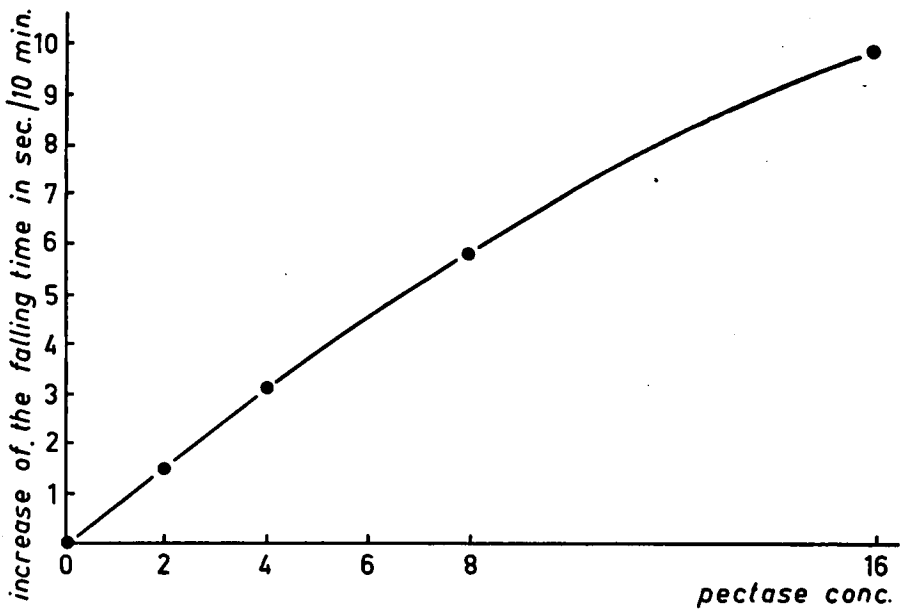

Fig. 2. The relation between the maximum increase in viscosity of a pectin solution per unit of time and the amount of pectase added to the medium. Data from graphs of fig. 1; explanation in text

pointed out clearly by KERTESZ (1951, p. 361). Because of these complications, objections can be made against the viscosity method when it is used to study the kinetics of the enzyme reaction. When used however only with the object of assessing the total activity of the pectase present in extracts, the method, besides being sensitive and consuming little time, is extremely useful as is shown in the following experiments.

Different quantities of pectase, prepared from orange flavedo according to MACDONNELL a. cow. (l.c.) are dissolved in 5 ml distilled water and added to 50 ml samples of an 0.20 % B.P.-pectin solution in 0.067 mol phosphate buffer pH 6.0. The increase in viscosity is measured following the method described above; the results are presented in Fig. 1. Some of the complications mentioned find ex-

pression in the form of the curves for which it will be diffiuclt to gvie a satisfactory kinetic explanation. When however, as is done in Fig. 2, the tangents of the straight part of the curves are plotted against the concentration of pectase in the mixture, it is evident from the resulting graph that the enzyme can be estimated quantitatively when the concentration is not too high.

Experimental results

a. The presence of pectase in pears and the isolation of the enzyme

The presence of pectase in extracts of pears picked in the early stages of development can easily be shown by the formation of gels. When 25 ml. extract "E" from pears picked in the middle of June was added to 100 ml of a 1 % B.P.-pectin solution, a strong, firm gel set after 3 hrs. With extracts of pears of later stages of development the setting of the gels was delayed while at the same time the firmness of the gels decreased. No gel formation was found any more with extracts of pears picked after the middle of August.

Although only a very weak pectase activity can be expected to exist in these fruits, it was found still possible to isolate the enzyme from pears picked in the beginning of September and kept in cold storage (2° C) for some time. Using the method of MacDonnell a. cow. (l.c.) 500 mg air-dry pectase was obtained from 400 g. tissue residue after centrifuging the pulp of these pears. The activity, measured by titration of the acid liberated from pectin by the enzyme following the method of Kertesz (1951; p. 362; 20° C i.s.o. 30° C) was found to be 12.0 P.M.U. (pectin methyl esterase units; Kertesz, 1951 p. 364 and Kertesz, 1937).

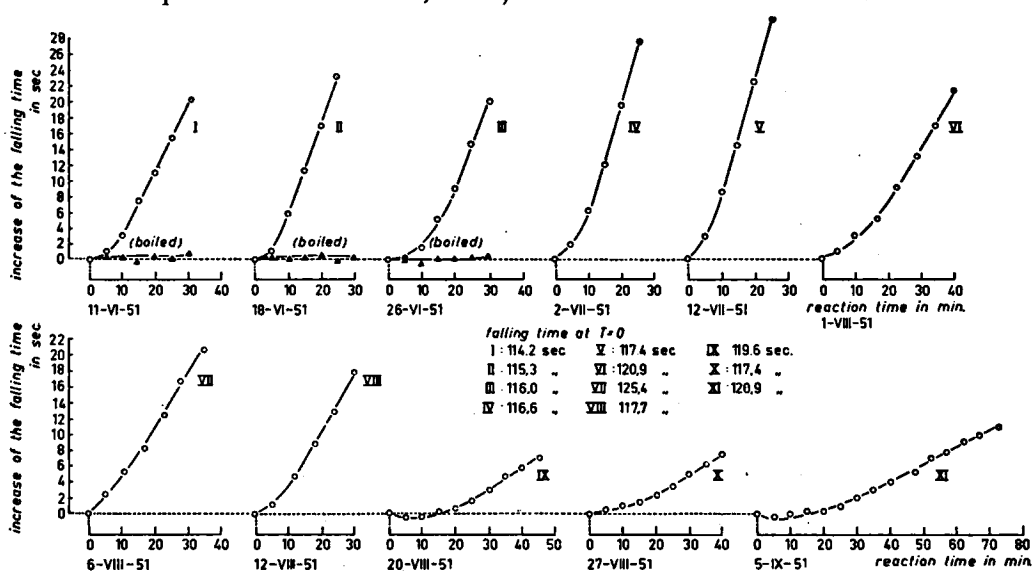

Fig. 3. The increase in viscosity of pectin solutions after addition of pectase containing extracts from pears. 35 ml 0,25% B.P.-pectin in 0,067 mol. phosphate buffer pH 6,0; 20 ml extract. Temp. 25° C

b. Changes in the activity of pectase during the development

By measuring the rate of increase in viscosity of pectin-extract "E" mixtures according to the method described above, the changes in the activity of the pectase in the fruit during its development were investigated. The results of the experiments are given in fig. 3. It is seen that the activity of the pectase in young fruits is much greater than in older ones since the curves are much steeper in the case of the former.

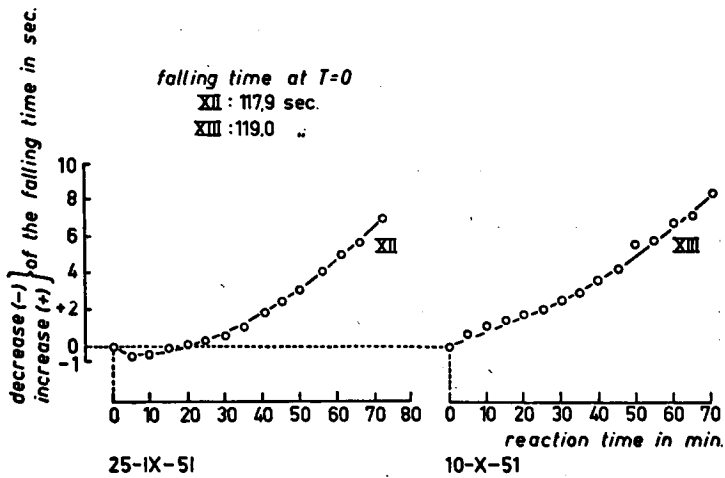

Fig. 3a. Explanation as fig. 3

In fig. 4 this is shown more clearly by plotting the gradient of the straight part of the curves against the date of picking; the activity is high in the early stages of development, starts to fall off rapidly in the second half of July and straightens out to a very low level at about two weeks before the commercial picking time (early September).

In this respect it might be of interest to mention the results of some

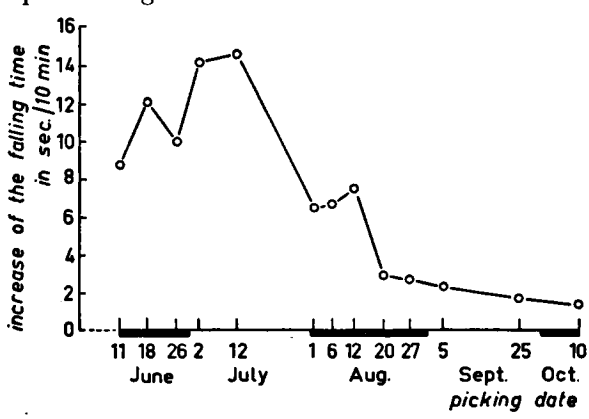

Fig. 4. Pectase activity in Doyenné Boussoch pears during development

experiments in which the behaviour of the enzyme was investigated in fruit kept in storage at low temperature (2° C) and in fruit ripened directly after picking at a date where the pectase activity was still at an intermediate level.

It was found that the activity of the enzyme did not change appreciably after picking either on ripening or storage; the activity level of the enzyme in the fruit at the moment of picking thus seems to be stabilized by the separation from the tree. In fig. 5 experimental data

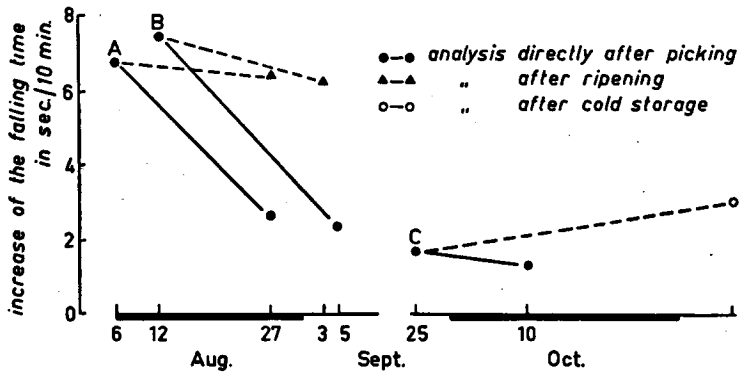

Fig. 5. Changes in the activity of pectase in pears during their development and after storage (dotted lines)

are presented. For comparison the decrease of the activity in fruits left on the tree is given in the same graph.

It is suggested that a possible explanation may be found here for the difficulties encountered with the ripening after cold storage of pears picked in too early stages of development. Confirmation of the experimental results however is considered desirable.

Discussion

In the preceding paragraphs it was shown that the rate of increase in viscosity of pectin-extract mixtures changed during the development of pears and it was concluded that the activity of the pectase present in the fruit changed accordingly. As mentioned above, the character of the reactions however on which the method of determination is based is rather complex. Therefore it should be considered whether changes in the amount of Ca in solution in the fruit might not have produced the results of our experiments.

In fig. 6 the influence of $CaCl_2$ added in different amounts to a pectin-pectase solution on the increase in viscosity is shown. The quantity of pectase used in these experiments is chosen such that the rate of increase in viscosity when no Ca is added is comparable with the rate found in fruit picked at the end of September. It is seen that the addition of Ca to the reaction mixture produces an increase in the rate. In fig. 7 this increase is plotted against the $CaCl_2$ added.

We may assume that the total amount of Ca per unit weight of fruit does not change considerably during the development. If our

results were caused by changes in the concentration of the Ca-ions in the extracts a low amount of soluble Ca should be present in the fruit in which a low "pectase activity" was found and vice versa.

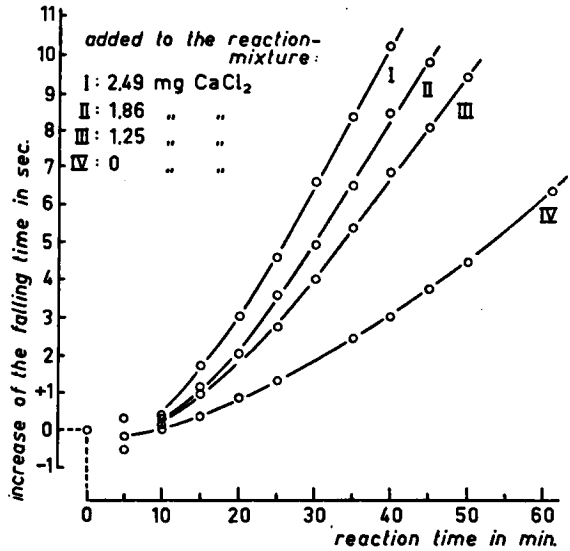

Fig. 6. The effect of Ca on the increase in viscosity of a pectin solution caused by pectase
50 ml 0,20% B.P.-pectin in 0,067 mol. phosphate buffer pH 6,0; 5 ml distilled water containing 3 mg pectase; 5 ml $CaCl_2$ solution containing different amounts of the salt. Temp. 25° C

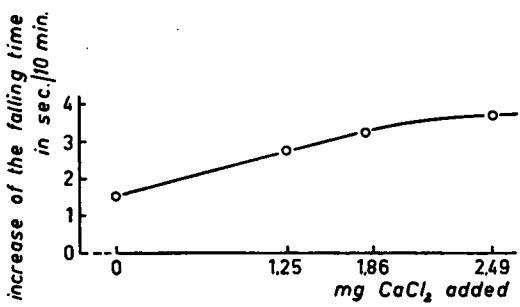

Fig. 7. The relation between the maximum increase in viscosity of a pectin solution per unit of time caused by pectase and the amount of Ca added to the medium. Data from graphs of fig. 6

In pears picked at 5/IX and kept in cold storage for about four months (presumably showing a low pectase activity) soluble, insoluble and total Ca was determined as oxalate in the ash by titration with $KMnO_4$. In the extract "E" from 100 g pulp 4.4 mg Ca was found (soluble Ca); in the residue 2.6 mg (insoluble Ca) and in 100 g pulp 7.2 mg (total Ca). Thus, even in old fruit with a low pectase activity most of the Ca is present in solution.

In the experiments to determine the activity of the pectase during the development the amount of extract "E" taken was derived from approximately 5 g of pulp and therefore contained only 1/20 of the amounts of Ca found above. From these considerations and the results presented in fig. 7 and fig. 4 it is clear that the changes in the rate of increase in viscosity during the development cannot be attributed to changes in the Ca-content of the extracts used in the experiments.

Summary

The presence of pectase in Doyenné Boussoch pears was demonstrated by gel tests and the activity of the isolated enzyme was estimated titrimetrically.

By measuring the rate of increase in viscosity of mixtures of pectin solutions and pear extracts, changes in the pectase content of the fruit during the development were investigated.

The activity of the enzyme was found to be high in the young fruit, then to start falling off rapidly at about the middle of July and to straighten out to a low level some two weeks before the commercial picking time.

Possible changes in the Ca content of the extracts used in the experiments were shown not to be responsible for the results obtained.

The fact that the pectase content of the fruit does not decrease after picking as it does when left on the tree suggests an explanation for some of the difficulties encountered with the ripening of pears after cold storage.

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