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PECTINASE IN PEARS

BY

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INTRODUCTION

During the ripening of many fruits the amount of insoluble pectin in the tissue decreases while the quantity of the soluble form increases. In the last stages of the ripening process the soluble pectin itself is broken down to galacturonic acid.

However, with the exception of the tomato (McCOLLOCH and KERTESZ, 1948; idem 1949) hardly any experimental evidence has been brought forward to explain the nature of these transformations. JOSLYN, MIST and LAMBERT (1952) presented some evidence for the occurrence of pectinase (P.G.) in benzoated apple juice stored at 0° C for one month. JOSLYN and SEDLEY (1940) found a marked decrease in the estimated amount of pectin present in the pulp of apples and citrus fruit when comparing the amount directly after preparation and after leaving the pulp for definite periods of time. They attributed this decrease to pectinase, present in the pulp. Objections were made against the method used since micro-organisms (KERTESZ, 1951, p. 342) or, possibly, pectase (McCOLLOCH and KERTESZ, 1949) could have produced the same experimental results. In fact, since many attempts to prove the presence of pectinase in apples by reductometric methods have been unsuccessful and it was found that pectin can be broken down *in vitro* to its basic units by ascorbic acid with or without the addition of peroxides to the reaction mixture, KERTESZ (1943) tentatively proposed a non-enzymic mechanism to explain these transformations. Elsewhere WEURMAN (1953) has shown the presence of a thermolabile inhibitor for pectinase in extracts from pears. The inhibitor could be isolated and some of its properties were studied. It is considered that any pectinase, if present in the fruit, would be indetectable in the presence of the inhibitor. At the same time it was found that at low pH values pectinase was more strongly adsorbed on solid cell material than the inhibitor (unpublished data).

It was decided to use this difference to try and prepare extracts and pulp residues in which the ratio of enzyme and inhibitor was shifted in favour of the former and thus to prove the presence of pectinase in the fruit.

THE PRESENCE OF PECTINASE DEMONSTRATED VISCOSIMETRICALLY

To prove the presence of pectinase in pears the property of the enzyme to lower the viscosity of pectic acid solutions was used (RAHMAN and JOSLYN, 1953; MOTTERN and HILLS, 1946; DEUEL and WEBER, 1945; WEITNAUER, 1946; JERMYN and TOMKINS, 1950).

The pulp of Doyenné Boussoch pears of different stages of ripeness, prepared by grinding the grated tissue down in a mortar with quartz, was brought to pH 7.5 by the addition of 0.5 N NaOH. The adsorption of the enzyme on cell walls is thus weakened. The pulp was filtered through silk cloth and the filtrate left overnight after the addition of toluol (HILLS, OGG and SPEISER, 1945). It is essential to leave the crude filtrate for some time. The pectase present in the filtrate (WEURMAN, 1954) was found to lower the pH by almost two units, usually causing gel formation and thus indicating that a marked demethoxylation of the pectin had taken place. If the methoxyl content of the pectin is not reduced to as small a value as possible the pectase would interfere in the following experiments by raising the viscosity of the mixture and might thus mask the decrease in viscosity caused by any pectinase present. The filtrate was again adjusted to pH 7.5 and was filtered through paper under weak suction. Samples of the filtrate were then added to pectic acid solutions pH 4.0 and the changes in viscosity of the reaction mixture taking place during the next 30 min at 25.00 $\pm$ 0.02° C were recorded with the use of a Hoeppler viscosimeter.

In a number of experiments a marked decrease in viscosity was found indicating that pectinase was present in the filtrate. A representative example of such an experiment is given in Fig. 1. The change in the viscosity is expressed as the change in the falling time of the ball

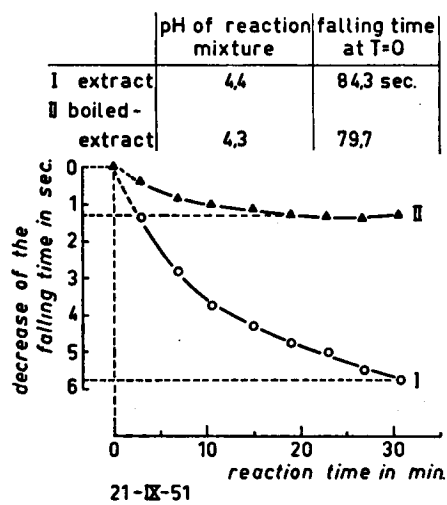

Fig. 1. The effect of an extract from pears on the viscosity of a pectic acid solution. 30 ml 2% M.P.Z.-pectic acid solution in water, pH 4.0; 20 ml pear extract; temp. 25° C.

in the viscosimeter tube and is plotted against the reaction time. The decrease still found with the boiled extract can be attributed to salt effects.

In other experiments with fruit of a different degree of ripeness no decrease in the viscosity of the reaction mixture took place (Fig. 2) and it was considered of interest to investigate the relation between the occurrence of a measurable pectinase activity and the degree of ripeness of the fruit.

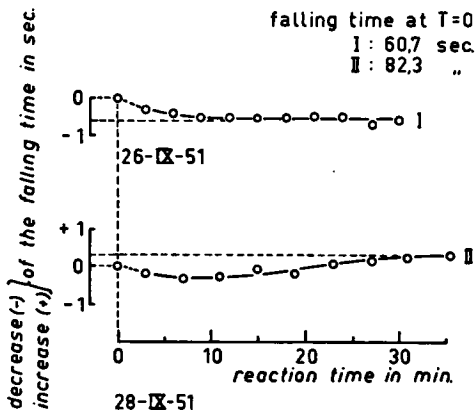

Fig. 2. The effect of an extract from pears on the viscosity of a pectic acid solution. Experimental conditions as Fig. 1.

Extracts were prepared from fruit of which the degree of ripeness was assessed accurately in advance and experiments were carried out in the way described above. The results are presented in Table I. It is seen that the presence of pectinase in the fruit could only be demon-

TABLE I

Relation between the occurrence of detectable pectinase activity and the degree of ripeness in pears

Decrease in viscosity (+) no decrease (—)	Degree of ripeness		Colour of the fruit
—	1	unripe	green
—	2	„	light-green
—	3	almost ripe	greenish light-yellow
—	3	„ „	„ „ „
+	4	ideal ripe	light-yellow
+	4	„ „	„ „
+	4	„ „	„ „
+	4	„ „	„ „
+	4	„ „	„ „
+	4	„ „	„ „
+	4-5	„ „	light-yellow:ellow
—	5	just over ripe	yellow
—	6	over ripe	orange-yellow

strated in a very short period of the ripening process when the fruit was considered "ideal ripe." This suggests a connection between the pectinase activity and the well known fact of the very short period of optimum consumption quality in pears.

We are of the opinion that a greater amount of pectinase is present in the extracts than is suggested by the rate of the decrease in viscosity found in the experiments, for it should be remembered that in these extracts pectinase inhibitor is present as well. It has been shown previously (WEURMAN, 1953) that addition of increasing quantities of the isolated inhibitor to mixtures of pectic acid and commercial pectinase caused an increasing inhibition of the enzyme up to a certain maximum. Beyond this the residual breakdown of the pectic acid was not influenced by further additions of inhibitor. It may well be that only such residual breakdown was found to take place in the experiments presented. An experiment, shown in Fig. 3 favours this

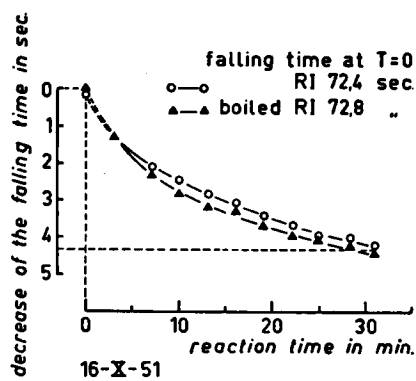

Fig. 3. The effect of an addition of pectinase inhibitor, RI, on the decrease in viscosity of a pectic acid solution caused by pear extract.
30 ml 2% M.P.Z.-pectic acid solution in water, pH 4.5; 20 ml pear extract; 5 ml solution containing 50 mg RI; temp. 25° C.

suggestion. Active and heat-deactivated pectinase inhibitor was added to a mixture of pectic acid and pear extract. The extract was found to contain pectinase as shown by the decrease in viscosity, but no difference in the rate of the decrease was found whether active or deactivated inhibitor was added indicating that a maximum inhibition had already taken place in the mixture. However, another explanation is possible for the fact that the pectinase activity in the extracts is not affected by the addition of inhibitor. As was shown previously (WEURMAN, 1953) the activity of the "depolymerase" prepared from tomatoes according to MCCOLLOCH and KERTESZ (loc. cit.) was not influenced by the pectinase inhibitor isolated from pears and it thus might well be that the pectinase from pears is identical with the tomato-depolymerase.

THE PRESENCE OF PECTINASE DEMONSTRATED REDUCTOMETRICALLY

The presence of pectinase in pears was also demonstrated by measuring the increase in reducing power of pectin solutions left in contact with the pulp. It was considered advisable in these experiments to first remove as much of the enzyme inhibitor as possible.

Finely minced pulp of healthy, over-ripe Doyenné Boussoch pears was centrifuged, the residue stirred in 5 times its volume of distilled water and the pH brought to 3.8 by adding small amounts of 0.5 NHCl. The suspension was again centrifuged and two samples (P and W) of the residue, weighing 80.0 g were taken. Sample P was mixed with 130 ml of a solution of purified pectin (2,25 g Z.M.P.-pectin in 250 ml distilled water; for properties of the pectin see WEURMAN, 1953), 1.00 g sodium benzoate, 2.60 ml 0.5 NHCl to bring the pH to 4.0 and 3.20 ml distilled water (final volume 80.0 + 135.80 ml). Sample W, the blank of the experiment, was mixed with 130 ml distilled water, 1.00 g sodium benzoate and 5.80 ml 0.5 NHCl, by which again the pH was brought to 4.0 (final volume 80.0 + 135.80 ml).

Both samples were divided in two equal parts (a and b). P.a. and W.a. were centrifuged directly after preparation and the reducing power of 5 ml samples of the filtered supernatant was measured according to WILLSTÄTTER and SCHUDEL (1918). P.b. and W.b. were left at 30° C for 3 days; the pH was kept constant by the addition of 0.1 N NaOH while distilled water was added to keep the volumes equal. After 3 days the reducing power was measured as in the other samples. The results of the determinations (average of 4 closely agreeing determinations) are given in Table II.

TABLE II
Explanation in text

Sample	Time (days)	Substrate	0.1 N $Na_2S_2O_3$/5ml filtrate
P.a.	0	pectin	3.73 ml
P.b.	3	„	3.35 „
W.a.	0	water	3.77 „
W.b.	3	„	3.76 „

It is seen that an increase in reducing power was only found when purified pectin was added to the reaction mixture (P.a., P.b.). Since 1 mg galacturonic acid is equal to 0.097 ml 0.1 N thiosulphate in reducing power, 3.8 mg galacturonic acid was formed in 5 ml filtrate of the reaction mixture originating from approximately 35 mg pectin (water content of the original centrifuged residue taken into account). So 10 % of the pectin added to the tissue residue was broken down to galacturonic acid by the pectinase present in the fruit tissue.

It should be mentioned that the precipitates formed on addition of acetone to the filtrates of P.a. and P.b. were repeatedly found to differ markedly from each other. The breakdown of pectin was thus easily perceptible in P.b. These observations are very convincing for

the experimentor working in this field as an indication for the presence of an active pectinase in the medium (KERTESZ, 1951, p. 343).

SUMMARY

Finely minced tissue of Doyenné Boussoch pears was either extracted with dilute acid or treated with alkali prior to extraction to obtain pulp residues or extracts in which the ratio of pectinase inhibitor to pectinase has been changed in favour of the enzyme. The presence of pectinase in either the pulp residue or the extract was then demonstrated by reductometric and viscosimetric methods.

REFERENCES

DEUEL, H. and F. WEBER, Helv. Chim. Acta, *28*, 1089–1110, (1945).
HILLS, C. H., C. L. OGG, and R. SPEISER, Ind. Engng. Chem., Anal. Ed., *17*, 507–510, (1945).
JERMYN, M. A. and R. G. TOMKINS, Biochem. Journ., *47*, 437–442, (1950).
JOSLYN, M. A., S. MIST and E. LAMBERT, Food Technol., *6*, 133–139, (1952).
JOSLYN, M. A. and A. SEDKY, Plant Physiol. *15*, 675–687, (1940).
KERTESZ, Z. I., Pectic Substances, New York–London, 1951.
KERTESZ, Z. I., Plant Physiol., *18*, 308–309, (1943).
MCCOLLOCH, R. J. and Z. I. KERTESZ, Arch. Biochem., *17*, 197–199, (1948).
MCCOLLOCH, R. J. and Z. I. KERTESZ, Food Technol., *3*, 94–96, (1949).
MOTTERN, H. H. and C. H. HILLS, Ind. Engng. Chem., *38*, 1153–1156, (1946).
RAHMAN, M. B. and M. A. JOSLYN, Food Res., *18*, 308–318, (1953).
WEITNAUER, G., Helv. Chim. Acta, *29*, 1382–1399, (1946).
WEURMAN, C., Acta Bot. Neerland., *2*, 107–121, (1953).
WEURMAN, C., Acta Bot. Neerland., *3*, 100–107 (1954).
WILLSTÄTTER, R. and G. SCHUDEL, Ber., Deut. Chem. Ges., *51*, 781, (1918).