more dilute solution. However, the solubility of all the curds definitely decreases with time, being on an average 0.0020, 0.00145 and 0.0012 $N$ for one day, one week and one and one-half months, respectively, all four curds being then 0.0012 $N$ with respect to total palmitate in solution.

The solubilities recorded by Bennett[4] were the mean of total palmitate and total sodium, but it is now clear that these two quantities should have been kept separate since alkalinity and solubility change independently, and are very different for different curds.

The alkalinity is most striking. Since 1910 we have had occasional indications that the formation of curd is accompanied by the development of alkali. The mother liquors of the stronger curds are now found to be more alkaline than any actual solution of soap is ever found to be, and the alkalinity is maintained. The alkalinity of a 1 $N$ curd is ten times and that of a $N/4$ curd twice that of a dilute curd. Furthermore, the alkalinity increases with time for all the stronger curds. The sodium palmitate from which the curds were derived was exactly neutral in composition. Hence curding is accompanied by definite and appreciable hydrolysis, possibly an example of membrane equilibrium or ion exchange on the exposed surface of the fibers. The $p$H of a fifteen-year old specimen of $N/20$ sodium palmitate was 9.2.

## Summary

Curd fibers of sodium palmitate sorb methylene blue to about the same extent when dye is added to fibers already formed and to those which form in presence of the dye. Since in both cases time is required to complete the sorption, it is evident that sorption is largely external upon the surface of the excessively fine primary ultramicroscopic crystalline fibers. After a week the weight and number of dye molecules sorbed exceeds the weight and number of sodium palmitate molecules. Thereafter the sorption distinctly diminishes and after a year may be only one-third, without any indication that a final value is yet attained, although the fibers must still be less than 100 Å. in diameter.

The solubility of curd fibers formed from concentrated solution is at first only slightly greater than those from dilute solutions, but all diminish to about the same extent with time.

The alkalinity of the stronger curds is far greater than that of any soap solution and it is ten times larger for a 1 $N$ curd than for a 0.05 $N$ curd, although the latter falls in the course of years to $p$H 9.2.

STANFORD UNIV., CALIF. RECEIVED JUNE 26, 1939

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[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NEW YORK STATE AGRICULTURAL EXPERIMENT STATION]

# Pectic Enzymes. IV. Structural Considerations in Connection with the Enzymic Hydrolysis of Pectins[1]

BY Z. I. KERTESZ

The high viscosity of aqueous solutions of pectins attracted attention over a hundred years ago but no satisfactory theory has been proposed as yet to explain the physical properties of pectins in the light of our present knowledge of their chemical composition. The final disproval of Ehrlich's tetragalacturonic acid theory[2] paved the way for renewed activity in this field. By the use of various concentrations of ethanol,[3] arabans and galactans[4] may be removed from the crude pectin preparations. The resulting compound (a true pectin composed almost exclusively of galacturonic acid anhydride residues with $CH_3O$ present as ester in most carboxyl groups) lends itself well for investigations of the relation between physical and chemical properties.

Without having conclusive evidence on hand and reasoning mostly by analogy, the chains of galacturonic acids have been regarded by most investigators as being responsible for the viscosity of pectins. Accordingly the loss of viscosity under certain conditions has been attributed to the decomposition of the polygalacturonic acid chains.

(1) Approved by the Director of the New York State Agricultural Experiment Station as Journal Paper No. 327, April 1, 1939. Presented at the Baltimore meeting of the American Chemical Society, April, 1939.

(2) Ehrlich, *et al.*, *Biochem. Z.*, **168**, 263 (1926); **169**, 13 (1926); **212**, 162 (1929); etc.

(3) Henglein and Schneider, *Ber.*, **69**, 309 (1936); Schneider, *et al.*, *ibid.*, **69**, 2530 (1936); **69**, 2537 (1936); **70**, 1611 (1937); **70**, 1617 (1937); **71**, 1553 (1938).

(4) Hirst and Jones, *J. Chem. Soc.*, 454-460 (1939).

Based upon such reasoning Mehlitz and Scheuer[5] proposed a method for the determination of the enzymic hydrolysis of pectins by the changes which occur in the viscosity.

A study of the enzymic decomposition of pectins has been in progress at this Laboratory for several years.[6] The results obtained here never indicated a close relationship between changes in the viscosity and the decomposition as measured chemically. A systematic study of this problem was therefore undertaken to explain this discrepancy. The results obtained are presented herewith.

## Experimental Methods

The viscosity determinations were performed at 30.0°, using Ostwald viscosity pipets. In the experiments yielding the data presented in Fig. 1 and Fig. 3 the viscosity determinations were made as follows. When the

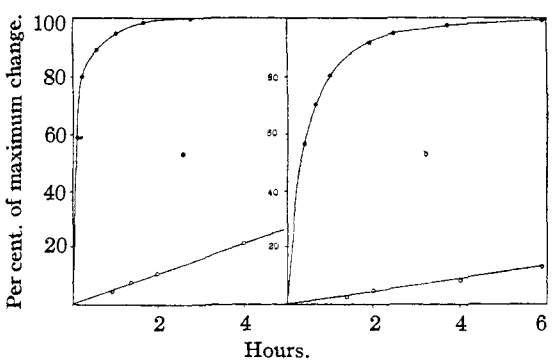


Fig. 1.—Changes in the viscosity (●), and reducing power (○), of pectin solutions during hydrolysis by 0.10% enzyme (a) and 0.05% enzyme (b).

reaction mixtures were made up from the prewarmed components and mixed, 3 cc. was pipetted immediately into the Ostwald pipet, which had been placed in the bath more than half an hour earlier. The determinations were started about three minutes later. The values obtained for the relative viscosity represent the average value for the period required for the outflow. In constructing the viscosity curves in Fig. 1 and Fig. 3 the viscosity values were taken as representing this average viscosity at the time of starting of the viscosity determination plus half of the time needed for the outflow.

In the case of the experiments demonstrated in Figs. 2 and 3 the pectin solution was heated in a bath of boiling water. To obtain the viscosity values for Fig. 2 the volumetric flasks holding the pectin solution were cooled at the end of the heating period and the contents made up to the original volume.

The reaction mixtures were composed of 100 cc. of pectin solution of *p*H 3.2 plus 10 cc. of 1% (filtered) commercial pectinase.[7] Only 10 cc. of 0.5% enzyme was used in obtaining Fig. 1b. Experiments reported in this paper have been repeated with different pectins giving essentially the same results. The data presented herein have been obtained on purified citrus pectin. The concentrations of the solutions used in Figs. 1, 2, and 3 were 0.854, 0.686, and 0.849%, respectively.

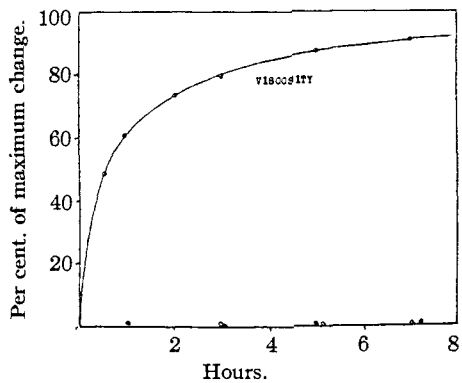


Fig. 2.—Changes in the viscosity (◑), reducing power (○), and methoxyl content (●), of a pectin solution upon heating.

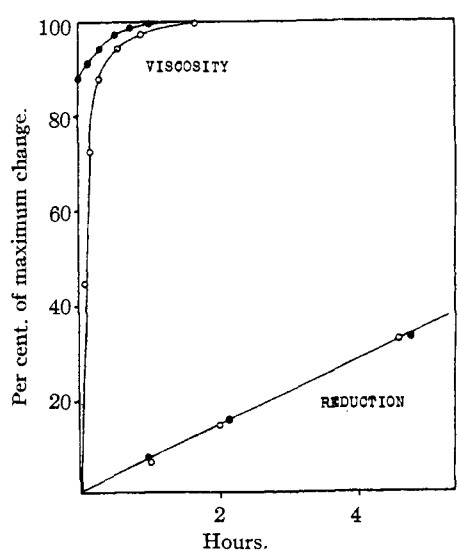


Fig. 3.—Changes in the viscosity and reducing power of unheated (○), and heated (●), pectin solutions upon enzymic hydrolysis.

The Willstätter–Schudel hypoiodide method[8] was used for the determination of the increase in the reducing power resulting from the activity of the pectin-polygalacturonase. It is essential to keep the limitations of this method in mind[9] in order to obtain dependable results. The enzymic demethoxylation was followed by titrating 10-cc. samples

(5) Mehlitz and Scheuer, *Biochem. Z.*, **268**, 355 (1934).
(6) Kertesz, *Ergebnisse Enzymforschung*, **5**, 233 (1936).
(7) For the commercial pectinase used in these experiments the author is indebted to the Röhm and Haas Company of Bristol, Penna. It was produced by precipitation with ethanol from a water extract from *Aspergillus sp.* grown on special media.
(8) Willstätter and Schudel, *Ber.*, **51**, 780 (1918).
(9) Myrbäck and Örtenblad, *Svensk Kem. Tidskrift*, **50**, 72 (1938).

of the reaction mixture with 0.1 *N* sodium hydroxide, using phenolphthalein indicator and adding 5 cc. more alkali. The excess of the alkali was titrated after one hour's standing at room temperature. One cc. of alkali consumed for the saponification equals 3.1 mg. of $CH_3O$ split off.

The pectic enzymes available at this time are mostly mixtures of several enzymes acting upon pectin. In spite of the attempts made in this Laboratory and elsewhere,[10] no method is available as yet for the quantitative separation of the enzymes present in commercial pectinases. As a result, the action of several enzymes upon pectins is proceeding simultaneously in the reaction mixtures. The most important known chemical change occurring during the enzymic decomposition of pectins is the hydrolysis of the polygalacturonic acid chains by the pectin-polygalacturonase. Another enzyme which is present and may influence the viscosity as well as the velocity of the hydrolysis is pectin-methoxylase,[11] an esterase responsible for the splitting off of the $CH_3O$ groups attached to most COOH groupings of pectins. It is not held likely that at the *p*H of the reaction mixtures the demethoxylation would have had a significant effect on the changes in viscosity. Furthermore, the changes which may have been caused by this enzyme would be negligible as compared with those caused by the pectin-polygalacturonase. As will be discussed later in this paper, a 1% hydrolysis of the polygalacturonic acid chains would cause approximately 33% reduction in the viscosity. A 1% demethoxylation on the other hand will cause only about 1% loss of the viscosity caused by the presence of the methyl esters.

## Results and Discussion

To study the relation between the changes which occur in the viscosity and reducing power during enzymic hydrolysis, pectin solutions were exposed to the action of a commercial pectinase in two different concentrations. The results presented in Fig. 1 are expressed as percentages of the maximum possible changes. Complete hydrolysis of the structure into galacturonic acid would cause a reduction of 100, while the low initial reduction was taken as 0. Similarly the difference between the time of outflow of the original reaction mixtures and those completely decomposed was taken as 100 and the decrease observed as a fraction thereof. There is an abrupt change in the viscosity of the mixtures in the first few minutes of the reaction. The reducing power is increasing slowly and (up to the extent of decomposition shown in the figure) at a constant rate.

It is necessary at this time to consider briefly the interrelation of the changes followed by the determination of the viscosity on the one hand and by the increase in the reducing power on the other. In pectin as well as in the case of other polysaccharides some heterogeneity must be assumed with regard to the size of the molecules present (in whatever sense the word molecule may be understood). This heterogeneity is even more increased upon a limited degree of cleavage of the chains and the "average" values calculated will depend on the method of measurement as well as on the method of calculation. Determinations of the increase in the reduction (as well as all other end-group methods and also freezing point and osmotic pressure determinations) give averages defined by the expression[12]

$$M_n = \frac{1}{\Sigma f_i/M_i}$$

where $f_i$ is the fractional weight of the constituent of molecular weight $M_i$ and the summation is to be applied to all constituents present. This is the "number average." On the other hand, viscosity measurements give an average value for the size of the molecule according to the expression

$$M_w = \Sigma f_i M_i$$

This is a "weight average." Calculations with the possible degradation products of homogeneous polymers (as pectin is thought of, for instance) indicate that upon hydrolysis the "weight average" molecular weight or chain length as measured by the viscosity will decrease slower than the "number average" measured by reduction. A mathematical consideration of this problem will be presented elsewhere, but it may be cited here that a 1% increase in the reduction in the case of pectin should result in a halving of the "number average" molecular size while the "weight average" (and thus according to Staudinger the viscosity) should decrease only about 33%. As stated before, the experimentally observed decrease in the viscosity is more rapid than the corresponding change in the reducing power would allow according to the calculations.

If the observed changes in the viscosity are actually caused by the changes in the size of the polygalacturonic acid chains, then any decrease in the viscosity should be accompanied by a corresponding increase in the reducing power. It is known that the viscosity of pectin solutions decreases upon heating.[13] To obtain information on this matter pectin solutions were heated (at *p*H 3.2) to 100° and the changes in the viscos-

(10) Rothschild, *Enzymologia*, **5**, 359 (1938).
(11) Kertesz, *J. Biol. Chem.*, **121**, 589 (1937).
(12) Kraemer and Lansing, *J. Phys. Chem.*, **39**, 153 (1935).
(13) Myers and Baker, Univ. of Delaware Expt. Sta. Bull. No. 149 (1927).

ity (as determined at 30.0° ) and reducing power and methoxyl content followed. The results, shown in Fig. 2, prove conclusively that there is no correlation between the changes in the viscosity and reducing power. There is no indication that polygalacturonic acid chains were split during the heating of the pectin solution.

There also appeared some possibility that the decrease of the viscosity may be caused by progressive demethoxylation of the pectin. It has been shown by von Fellenberg,[14] and more recently by the author,[15] that the viscosity of pectin solutions will decrease upon extensive demethoxylation. Tests conducted during the heating and shown in Fig. 2 proved that there was no demethoxylation whatsoever during the period of heating used in this experiment.

If the viscosity is not a property of the chemical polygalacturonic acid chains, the velocity of the enzymic hydrolysis of a pectin solution in which the viscosity has been lowered by heat should be identical with the velocity of hydrolysis of the original unheated pectin solution. That this indeed is the case is shown in Fig. 3 where "reduction" represents the hydrolysis of both the original pectin solution and that heated for five hours to 100° before the enzyme was added. The viscosity changes are also indicated in the figure, showing that there was some additional decrease in the viscosity of the heated solution "b" during the hydrolysis. Determinations of the progress of changes in the reducing power of the heated and unheated solutions showed that the changes were identical in both mixtures. The progress of demethoxylation, not shown in the figure, was also identical. These observations make it even more probable that no chemical changes are induced by the heating of the pectin solution. In other words, the viscosity is not directly associated with the chemical structure of pectin.

It is obvious, therefore, that changes in the viscosity may not be used as a measure of enzymic decomposition.[16] The changes occur only in the early part of the hydrolysis. In the case of many pectins of "low quality" or of those which are degenerated by heat treatment, there would be but little change at all in the viscosity upon enzymic decomposition.

(14) Von Fellenberg, *Biochem. Z.*, **85**, 118 (1918).

(15) Kertesz, *Food Research*, **3**, 481 (1938).

(16) The measurement of the viscosity of a pectin solution may nevertheless give valuable information concerning its usefulness for various practical purposes where its colloidal properties are important [Baker, *Food Ind.*, **6**, 305 (1934)].

Assuming for the present that all aldehyde groups which are set free during the enzymic hydrolysis show the reduction characteristic of galacturonic acid, the conclusion must be drawn that the viscosity is not directly caused by the polygalacturonic acid molecules. The formula $[(G)_m]_n$ presents a possible explanation of this observation. The polygalacturonic acids $(G)_m$ containing $m$ galacturonic acid anhydride residues form a secondary aggregate or polymer by the association of $n$ polygalacturonic acid chains. The viscosity of pectins is mostly caused by the aggregate $[(G)_m]_n$ which is destroyed by heating. The units of $(G)_m$ show no additional reducing power when $n$ is lowered to approach 1. On the other hand, if the galacturonic acid chains $(G)_m$ are attacked by the enzyme, the secondary structure falls apart. It is reasonable to assume that the formation of aggregates from the $(G)_m$ units is a property of polygalacturonic acid chains of certain minimum length. With the disturbance of this primary structure by reducing the size of the chemical unit, the secondary polymer is automatically destroyed.

This hypothesis explains why the viscosity in enzyme hydrolyzed pectin solutions decreases more rapidly than one would expect from the molecular size and the viscosity calculated therefrom by Staudinger's method. It also accounts for the observed decrease in the viscosity of heated pectin solutions without an increase in the reducing power, *i. e.*, without splitting of the polygalacturonic acid chains $(G)_m$.

From the trend of the viscosity curves obtained during this work one may draw the conclusion that the polygalacturonic acid structure $(G)_m$ also possesses some viscosity higher than that corresponding to a solution of galacturonic acid of equal concentration (see Fig. 2). This is indicated also when pectin is hydrolyzed by low concentrations of pectin-polygalacturonase, but its viscosity is relatively low when compared to that of the complete structure $[(G)_m]_n$.

It is of interest to note that a similar double structure has been found to exist in the case of starches. Baird, Haworth and Hirst[17] reported the disaggregation of starch without decomposing the chemical unit. Later Hassid and Dore[18] isolated a natural starch which showed low viscosity and appeared to be composed of unaggre-

(17) Baird, Haworth and Hirst, *J. Chem. Soc.*, 1201 (1935).

(18) Hassid and Dore, THIS JOURNAL, **59**, 1503 (1937).

gated starch molecules. From the work reported in this paper it seems likely that the structure of the large pectin molecule is similar to that of certain starches. On the other hand, Schneider and Fritschi[19] claim that pectin has a "macro molecule" bound with main valences only and is not an aggregate of the chemical units. The observations made at this Laboratory are not in favor of their contention.

There is no information available on the relative reducing power of polygalacturonic acids containing different numbers of galacturonic acid anhydride residues. The formula proposed for polygalacturonic acid by Morell, Baur and Link[20] is generally accepted and if true would indicate a direct proportionality between the number of molecules in the polymer and its reducing power. In case of starch the presence of non-reducing aldehyde groups has been assumed[21] but without conclusive evidence to that effect. The low reducing power observed in purified pectins is believed by the author to be a characteristic property of the pectin molecule. In spite of many attempts made at this Laboratory, no pectin preparation entirely free of reducing power has been obtained as yet. The significance of the natural reducing power of pectins will be the subject of a later contribution.

Assuming that the aldehydic reducing groups are tied up or masked in some manner in higher polymers of galacturonic acid, this would only modify and not eliminate the postulated secondary structure. Until evidence of this fact is presented, it seems more likely that the aggregates of polygalacturonic acid molecules are held together by means of secondary valence attractions not affecting the reducing power of the terminal aldehydic groups of the chemical polygalacturonic acid units.

(19) Schneider and Fritschi, *Ber.*, **69**, 2540 (1936).
(20) Morell, Baur and Link, *J. Biol. Chem.*, **105**, 1 (1934).
(21) See a discussion of this matter in Hanes, *The New Phytologist*, **36**, 101 (1937).

## Summary

1. When pectin solutions are enzymatically decomposed the viscosity decreases more rapidly than corresponds to the increase in reducing power.

2. The viscosity of pectin solutions can be reduced by heat without any increase in the reducing power. This heat-degenerated pectin is decomposed by enzymes at the same velocity as the original unheated pectin solution.

3. These results are explained by postulating a structure $[(G)_m]_n$ for pectin in which $(G)_m$ is a polygalacturonic acid. The $n$ units of polygalacturonic acids form a secondary aggregate which is mostly responsible for the viscosity of pectin.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

# The Action of Chlorine on Thiocyanates

BY TREAT B. JOHNSON AND IRWIN B. DOUGLASS[1]

This paper is contributed as a report of an investigation carried out recently in this Laboratory on the development of new methods for synthesis of sulfonyl chlorides. The recent interest in the chemotherapy of sulfanilamide and related compounds has stimulated a demand for new and improved methods of synthesizing sulfonyl chlorides. We have reported previously on a method of formation of chlorides of this type by interaction of chlorine with isothioureas,[2] and also the action of chlorine on other types of sulfur combinations.[3]

Observations on the chemical action of anhydrous chlorine on the lower alkyl thiocyanates have been made by Cahours,[4] Riche,[5] and James,[6] but none of the workers identified a sulfonyl chloride among the various reaction products described. Cahours mentioned obtaining by the chlorination of methyl thiocyanate a heavy yellow oil which solidified in contact with ammonia. If the experimental conditions employed by him

(1) Sterling Professorship of Chemistry Research Assistant, 1937–1938.
(2) Johnson and Sprague, THIS JOURNAL, **58**, 1348 (1936); *Science*, **83**, 528 (1936); Sprague and Johnson, THIS JOURNAL, **59**, 1837 (1937); **59**, 2439 (1937).
(3) Johnson and Douglass, THIS JOURNAL, **60**, 1486 (1938).
(4) Cahours, *Ann.*, **61**, 96 (1847).
(5) Riche, *ibid.*, **92**, 357 (1854).
(6) James, *J. Chem. Soc.*, **51**, 268 (1887).