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SOME KINETIC FEATURES OF THE HYDROLYSIS OF PROTOPECTIN

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Some features of the kinetics of the hydrolysis of apple protopectin have been studied on the basis of an analysis of monosaccharide residues of the pectin substances and determinations of characteristic viscosity. It has been shown that the fine regulation of the physicochemical parameters of the hydrolysis of protopectin leads to the formation of pectin substances with different properties.

The development of conditions for performing the hydrolysis of the protopectin of plant raw material is a fairly complex problem since the production of pectin substances of given structural parameters and defined physicochemical properties requires the very accurate and well-coordinated regulation of numerous parameters of the process. Many investigations and also monographs and reviews [1-3] having mainly a technological direction have been devoted to this question. The complexity of the problem is increased even further by the circumstance that the optimization of the hydrolysis of protopectin depends substantially on the type of plant raw material and its variety and ripeness [4].

In view of this, it appeared of interest to consider the hydrolysis of protopectin as a combination of parallel and successive chemical reactions taking place in a heterogeneous system. The number of publications in this directions is limited. In particular, in [5] the hydrolysis of protopectin is considered as a successive two-stage reaction.

On the basis of the analysis of the amount of uronide component in the initial, intermediate, and final hydrolysis products, it has been shown that the kinetics of the process follow a successive first-order reaction and the change in the amount of intermediate product has an extremal nature.

It must also be mentioned that, in the decomposition of protopectins, the individual components of the cell wall, especially hemicelluloses, also undergo hydrolysis to different degrees according to the conditions of the process. As a result, the first stage of the process gives rise to a complex mixture consisting of acidic and neutral polysaccharides which, in their turn, may undergo further decomposition.

The aim of the present work was a qualitative description of the hydrolysis of apple protopectin with respect to the changes in the amounts of all the monosaccharide residues of the reaction products.

The hydrolysis of protopectins has been analyzed on the basis of the basis of the kinetics of two successive monomeric reactions: Protopectin $\xrightarrow{K_1}$alcohol-precipitated pectin $\xrightarrow{K_2}$alcohol-soluble substances.

For annual combination of the hydrolysis parameters (time, temperature, and pH), we determined the amount of remaining protopectins z(t), alcohol precipitated pectins y(t), and alcohol-soluble substances q(t). The results obtained are shown in Table 1. Thus, change in the protopectin contents from the starting material results from an exponential rule. As seen in Fig. 2, the dependence of the data on polylogarithmic mesure rectifies, while nondependence on the pH value extrapolates to a single point. For this data we can determine the contents of protopectin in the starting material (A_0), determined as 27.4%.

Using the first-order equation

$$y(t) = A_0 e^{-K_1 t},$$

we determined K_1 values, which were $0.27 \cdot 10^{-4}$ s^{-1}, $0.96 \cdot 10^{-4}$ s^{-1}, and $1.63 \cdot 10^{-4}$ s^{-1} at pH 2.4, 1.8, and 1.32, respectively. These results show that under otherwise identical conditions with a fall in the pH the rate of hydrolysis of protopectin rises considerably. In particular, with a fall in the pH of approximately unity, the value of K_1 increases by almost an order of magnitude. However, it must be mentioned that the values of K_1 and A_0 obtained are gross and consist of averaged values of all the bonds undergoing hydrolysis under the given experimental conditions.

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TABLE 1. Monosaccharide Compositions of the Alcohol-Precipitated Pectins as Functions of the pH of the Solution and the Time of Hydrolysis

pH	t, min	Monosaccharide composition of the pectin substances, wt.%							
		Rha	Ara	Xyl	Man	Gal	Glc	$\Sigma_{n.c}$	Gal A
2.45	20	1.5	25.6	–	–	4.6	1.2	33.0	65.0
	40	1.6	19.0	–	–	3.7	1.0	25.3	74.0
	60	1.7	15.0	–	–	2.1	0.5	19.3	80.0
	90	1.8	6.4	–	–	1.0	0.1	9.3	89.0
	150	2.0	0.1	–	–	0.5	0.3	2.9	95.0
1.80	20	1.9	18.5	0.5	–	4.3	3.8	29.0	68.4
	40	4.31	6.71	0.70	–	1.31	1.87	14.9	81.4
	65	1.07	5.86	0.25	–	2.10	2.98	12.3	85.4
	90	1.79	5.34	0.45	–	2.38	1.94	11.9	86.5
	120	0.98	3.60	0.41	–	1.67	1.42	8.08	90.2
	180	0.78	2.24	0.20	–	1.48	–	4.7	93.6
		3.8	41.0	0.82	1.13	4.7	46.0		
1.32	20	1.23	2.53	1.20	–	5.14	11.19	21.3	76.2
	40	1.02	..47	0.61	0.29	5.29	8.3	17.5	80.3
	60	1.37	1.92	1.45	0.30	5.12	8.18	16.2	81.0
	90	1.18	1.345	1.17	0.247	4.03	5.73	14.45	85:0
	120	1.05	0.53	1.04	0.237	2.90	4.17	10.7	89.6
	180	1.25	1.88	1.31	0.15	4.2	3.27	12.9	85.6

TABLE 2. Apparent Diffusion Coefficient (*D*) of Alcohol Precipitated Pectin as a Function of the pH of the Solution and the Time of Hydrolysis of Apple Pomace

Time of hydrolysis of the protopectin, min	D, cm^2·sec^{-1}		
	pH 2.45 $D \cdot 10^8$	pH 1.80 $D \cdot 10^8$	pH 1.32 $D \cdot 10^8$
20	2.4542	10.023	34.133
40	7.6892	18.340	48.066
60	–	24.928	–
65	–	–	62.182
70	8.220	–	–
90	–	27.875	73.600
100	12.043	–	–
120	–	–	64.400
150	–	–	–
180	–	–	–

Radius of a swollen particle of pomace $r_0 = 0.136$ mm.

We determined the quantitative levels of all the monosaccharides in the alcohol-precipitated pectin (Table 1). Within the framework of the present paper, the use of the results given in Table 1 to calculate the value of K_2 does not appear possible, since the value of K_1 for the cleavage of a concrete bond is lacking. We have limited ourselves to a qualitative consideration of the hydrolysis of protopectin. As we have shown [6], the alcohol-precipitated components of a hydrolysate of the plant cells

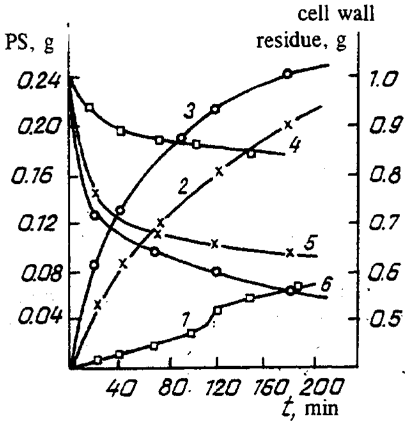

Fig. 1. Yields of pectin (*1*, *2*, *3*) and of the residual mass of the cell wall of apples (*4*, *5*, *6*) as a function of the time of hydrolysis at various pH values of the solution: pH = 2.45 (*1*, *4*); 1.80 (*2*, *5*); 1.32 (*3*, *6*); initial weight of dry pomace — 1 g.

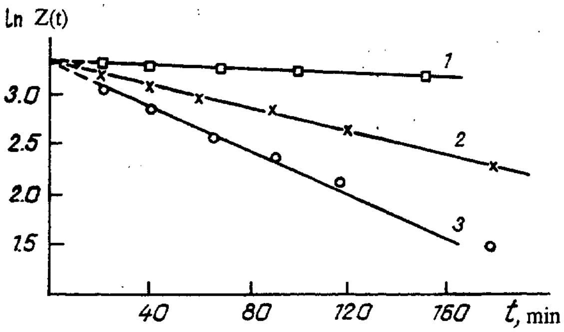

Fig. 2. The dependence of ln Z(t) on the time of hydrolysis of apple pomace at various values of the solution: pH 2.45 *1*); 1.80 (*2*); 1.32 (*3*); *T* = 85°C.

consist of a mixture of acidic and neutral polysaccharides, and the macromolecules of the former contain monosaccharide residues in addition to *D*-galacturonic acid units [7]. The results given in Table 1 showed that at a constant temperature and pH the amount of *D*-galacturonic acid in the pectin increased and the amount of neutral sugars decreased. Consequently, the hydrolysis process, having begun in the protopectin, also continued in solution, and the bonds formed by the neutral monosaccharide residues underwent the greatest breakdown. This led to an enrichment of the pectin with *D*-galacturonic acid units (Fig. 3).

Also important are the dissimilar rates of cleavage of the bonds formed by different monosaccharides. As can be seen from Fig. 4, with an increase in the time of hydrolysis there was a change in the composition of the neutral polysaccharides as a result of a fall in the number of residues of the neutral monosaccharides arabinose and glucose, because of which the composition was enriched with rhamnose and galactose. At the same time, the amount of xylose remained practically unchanged. This means that the most labile bonds in this case are those formed by arabinose and glucose residues which, as has been shown [8] form the bulk of the neutral polysaccharides and polysaccharide components of the polygalacturonan molecule in the form of side-chains. On the other hand, the same results give grounds for considering that rhamnose, galactose, and xylose are components of the macromolecules of acidic polysaccharides and therefore possess considerable resistance to the action of hydrolyzing agents.

On the basis of what is shown in Table 1 and Fig. 4, the impression might be created that the greater the time of hydrolysis the better is the quality of the pectins obtained, since the amount of *D*-galacturonic acid then rises. However, the properties of the pectin substances are determined to a considerable degree not only by their chemical structure but also by their molecular mass.

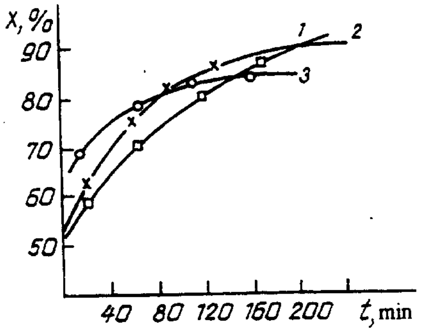

Fig. 3

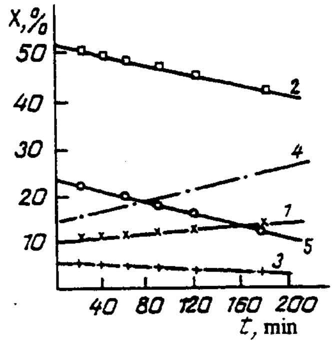

Fig. 4

Fig. 3. Dependence in the change in the amount of galacturonic acid in the alcohol-precipitated pectin on the time of hydrolysis at pH 2.45 (*1*); 1.80 (*2*); and 1.32 (*3*); $T = 85°C$.

Fig. 4. Change in the composition of the neutral sugars of the PS as a function of the time of hydrolysis of apple pomace: *1*) Rha; *2*) Ara *3*) Xyl; *4*) Gal; *5*) Glc; $T = 85°C$

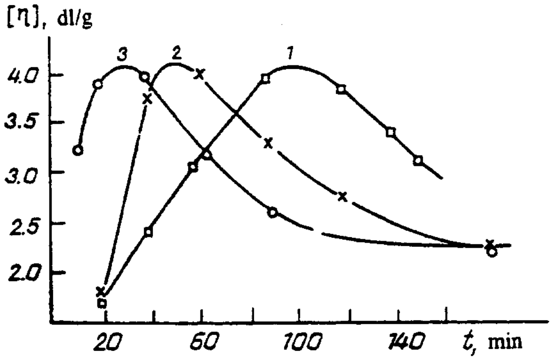

Fig. 5. Dependence of the characteristic viscosity of the PSs on the time of hydrolysis. Hydrolysis conditions: pH = 2.45 (*1*), 1.8 (*2*); 1.32 (*3*); $T = 85°C$.

Figure 5 shows the dependence of the characteristic viscosity of the pectin on the time of hydrolysis. All the curves, which were obtained at different pH values of the solution, are of extremal nature, and with a rise in the pH the value of the maximum shift in the direction of an increase in the time of hydrolysis.

It is important that the values of the characteristic viscosity in the region of the maximum are practically independent of the pH, which shows an identity of the chemical mechanism of hydrolysis, an increase in the concentration of the acid only accelerating the process taking place.

It must also be added that the hydrolysis of the plant cell is the third stage of the hydrolysis of the polysaccharides of the matrix. An argument in favor of this hypothesis, in addition to the constancy of the characteristic viscosities in the region of the maximum, is provided by the results of a study of the diffusion of pectins from the plant cell phase into the solution (Table 2). The coefficients of diffusion increased both with a fall in the pH and with an increase in the time of hydrolysis. Such a nature of the change in *D* is due to a decrease in the dimensions of the macromolecule under the action of the above-mentioned factors.

Thus, a qualitative consideration of the extraction of pectin substances from the plant cell permits the conclusion that the establishment of physicochemical laws and the derivation from them of an equation for the processes involved in the hydrolysis of protopectin leads to the production of pectin substances with given properties.

EXPERIMENTAL

The hydrolysis of plant raw material was studied mainly for the case of apple pomace of the autumn variety, 1989 harvest. Before hydrolysis, the apple pomace was repeatedly washed with cold and hot water until water-soluble substances and neutral mono- and polysaccharides had been eliminated. The apple pomace prepared in this way was dried and comminuted to a particle size of 1-1.6 mm. The hydrolysis and extraction of the pectins was conducted under the action of hydrochloric acid at 85°C, at pH values of solutions of 2.45, 1.80, and 1.32, and a liquor ratio of 1:25. The pectin was isolated from the solution by precipitation with ethanol and was dried in vacuum.

The complete acid hydrolysis of the pectins, and analysis for the amounts of galacturonic acids and neutral sugars were carried out by the procedure described in [6].

The coefficient of diffusion *D* was calculated in accordance with the procedure described in [9].

REFERENCES

1. N. P. Shelukhina, R. Sh. Abaeva, and G. B. Aimukhamedova, Pectin and the Parameters of its Production [in Russian], Frunze (1987).
2. N. S. Karpovich, L. V. Donchencho, V. V. Nelina, et al., Pectin: Production and Use [in Russian], Urozhai, Kiev (1989), p. 86.
3. L. N. Gentschev, Industriale Obst- und Gemuseververtung, **73**, Nos. 1/2, 17 (1988).
4. D. Seipp, Gartenbauwissenschaft, **43**, No. 5, 222 (1978).
5. I. N. Panchev, N. A. Kritchev, and C. Kratchanov, Carbohydr. Polym., **11**, 193 (1989).
6. Z. K. Mukhiddinov, D. Kh. Khalinkov, and V. A. Degtyarev, Khim. Prir. Soedin., 455 (1990).
7. Z. K. Mukhiddinov, D. Kh. Khalikov, É. K. Mukhiddinov, D. Kh. Khalikov, E. V. Grigor'eva and V. P. Panov, Khim. Prir. Soedin., 91 (1993).
8. J. A. De Vries, A. G. J. Voragen, F. M. Rombouts, and W. Pilnik, in: Chemistry and Function of Pectins, M. L. Fishman and J. J. Jen (eds.), ACS Symposium Series 310, p. 38.
9. L. K. Shataev, N. N. Kuznetsova, and G. É. Elkin, Carboxylic Cations in Biology [in Russian], Nauka, Leningrad (1979).