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PHYSICOCHEMICAL PRINCIPLES OF PLANT-CELL PROTOPECTIN DECOMPOSITION BY ACID CATALYSTS

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Research results published in 1990-2002 on acid hydrolysis of apple protopectin and sunflower calathides were reviewed. Decomposition products and the biological activity of the resulting pectinic substances were identified.

Key words: protopectin, pectinic substances, microgel, monosaccharides.

Hydrolysis conditions of plant protopectin (PP) are difficult to develop because numerous process parameters must be finely and mutually regulated in order to produce pectinic substances (PS) with given structural parameters and well defined physicochemical properties. Several monographs and reviews [1-3] on this problem are mainly technological in nature. The complexity of the problem is increased because the type of actual chemical reactions during PP hydrolysis depends substantially on the type of plant material, its quality, ripeness, storage conditions [4], and process parameters. Also, knowledge of the PP decomposition mechanism by acid catalysts is useful for optimizing the reaction parameters for producing pectin and developing the technology leading to the production of the final product with a high content of galacturonic acid (GA) units. This is important for forminhg a polymer network structure or imparting gel-like properties, owing to which PS are widely used in the food and pharmaceutical industries [3].

Several new biologically active properties of PS have been discovered, in particular, the manifestation by plant food fiber of various capabilities related to lipid metabolism [5], which is due to a certain extent to the swelling of one of its components, PS. Our research results showed that PS isolated from peach, apricot, and quince intensify bile production by the liver [6]. They actively participate in bile catabolism, increase the synthesis of total bile acids (TBA) and phospholipids, and decrease formation of cholesterol and bilirubin. Pectin can be used in therapy of gallstones (GS), especially early in their formation [7]. When used to protect the mucous lining of the intestine from the harmful effect of toxic metabolites and antibacterial preparations, PS in combination with antibiotics significantly accelerate treatment of patients with acute intestinal infection [8].

Considering these properties of PS, special investigations are needed on the formation of their basic structural component, copolymers of polygalacturonic acid, during hydrolysis.

We reviewed results of research on the hydrolysis of PP from apple and sunflower calathide that was performed on the assumption that its decomposition is a combination of parallel and successive chemical reactions occurring in a heterogeneous system.

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I. Features of Apple PP Decomposition by HCl

A mathematical model describing pectin yield (Y_1) and relative viscosity (Y_2) of the solution on experimental conditions was developed at an early stage in order to prepare pectin of a given molecular weight [9]. In particular, this model for orange pressings obeys the following equations:

$$Y_1 = 9.37 + 0.63x_2;\ Y_2 = 0.75 - 0.53x_1 + 0.34x_2 - 0.26x_3, \quad (1)$$

where x_1 is the acid concentration; x_2, temperature; and x_3, reaction time.

During an investigation of the yield and quality of pectin extracted by 0.5% HNO_3 from apple pressings as functions of temperature and processing time, a kinetic model was proposed [10] for the sequential conversion of PP Z(t) at rate constant k_1 into soluble pectin Y(t) and decomposition of the latter to low-molecular-weight oligosaccharides (OS). This process for a first-order unimolecular reaction is described by the differential equation:

$$dY(t)/dt = k_1Z(t) - k_2Y(t), \quad (2)$$

where k_1 is the rate constant for conversion of PP into soluble pectin and k_2 is the rate constant for pectin decomposition.

Without pectin decomposition ($k_2 = 0$), the yield of pectin with time is:

$$Y(t) = A_0(1-e^{-kt}), \quad (3)$$

where A_0 is the initial uronide content of the pectin component in the starting material.

Hence, we find the condition for maximal yield of pectin:

$$Y_{max} = A_0k_2k_1^{-1}\exp\{(k_1/k_2)/(1-k_2/k_1)\}. \quad (4)$$

It was found [10] that the rate constant for apple-pectin extraction varies from $2.57\cdot10^{-4}$ sec^{-1} at 60°C to $5.63\cdot10^{-4}$ sec^{-1} at 80°C. The yield of pectin is greatest (9.31%) at 80°C with a 68-minute extraction.

It should be noted that the relatively high yield at high temperature and the long processing time do not mean that the resulting pectins are of high quality because the molecular weight, degree of esterification, and other properties can decrease under these conditions. However, reducing the temperature increases significantly the processing time. Therefore, temperature regulation and hydrolysis time are not sufficient to produce high-quality pectin in high yield. The starting material must also be additionally treated, in particular, with an enzyme or ultrasound for preliminary softening of the plant cells. Plant material containing pectin, in particular, apple and citrus pressings and pomegranate, was used to show [11] that hydroacoustic treatment with a cavitation intensity of 0.4-2.1 at a 1:5 ratio can not only increase significantly the yield of PS but also increase their molecular weight. The kinetics of PP hydrolysis are described by the first-order equation:

$$\lg (C/C_0) = -kt, \quad (5)$$

where C_0 is the total amount of pectin in the starting material and C is the pectin content in the extract.

The quantity k (0.031) and activation energy (~20 kJ/mol) indicate that acid hydrolysis occurs according to the equations:

$$PP + H_3O^+ \rightarrow PP\text{-}H^+ + H_2O$$

$$PP\text{-}H^+ + H_2O \rightarrow \text{pectin} + \text{cellulose} + \text{monosaccharide} + H_2O$$

Since the pH of the reaction mixture is less than 4.0, it is obvious that acid hydrolysis occurs via cavitation activation of water to form active H_3O^+ cations. This is consistent with the dependence of the hydrolysis rate constant on the cavitation intensity (Qk): k decreases from $5.17\cdot10^{-4}$ sec^{-1} to $3.67\cdot10^{-4}$ sec^{-1} as Qk changes from 1.1 to 0.4 [11].

Thus, the description of the hydrolysis of plant material based on the kinetics of an irreversible first-order reaction, although not expressing completely the actual processes occuring during PP decomposition, enables the experimental results to be generalized.

In general, pectin extraction can be viewed as a successive heterogeneous chemical reaction occurring in two phases, the swelled pressings and the external solution, that includes diffusion of the hydrolyzing reagent into the cell wall, its adsorption, PP hydrolysis, diffusion of pectin macromolecules into the hydrolysate solution, and decomposition of the polymer chain.

Sorption of Hydrolyzing Reagent by Plant Pressings. The sorptive properties of PP located in the cell wall for the hydrolyzing reagent are due to interaction forces that depend on the degree of polarization of various functional groups. In particular, the degree of ionization of carboxylic acids affects both the diffusion and chemical processes. Certain characteristics of the functional groups can be found by studying the sorption of acid in the cell wall [12].

The kinetics and isotherms for HCl absorption by apple pressings (Fig. 1) indicate that equilibrium is established quickly. The pH was measured 24-30 h after contact of the pressings with the solution for greater accuracy.

The equilibrium adsorption constants (k_θ) were calculated from the sorption isotherms by using the Langmuir equation. The thermodynamic parameters of HCl sorption by apple pressings were estimated using the equation

$$k_\theta = \theta/C_e(1-\theta), \tag{6}$$

where $\theta = (a/a_m)$ is the degree of filling, a is HCl adsorption, a_m is the limiting adsorption, and C_e is the equilibrium concentration of HCl.

The free-energy change of the sorption process was calculated using differential values for k_θ and their subsequent integration over the whole range of θ:

$$\Delta G^0 = -RT \ln k_\theta. \tag{7}$$

Figure 2 shows the differential values of $\ln k_\theta$ as a function of the degree of filling (θ). It can be seen that $\ln k_\theta$ increases with increasing θ. However, as a rule, the content and activity of water in the plant cell wall changes during sorption under actual conditions. The process itself may occur cooperatively. Therefore, we investigated the swelling of apple pressings over a broad pH range. The degree of swelling of apple pressings remains constant as the pH changes from 1.3 to 2.5 at 3.4 g/g. This indicates that these parameters are not coupled.

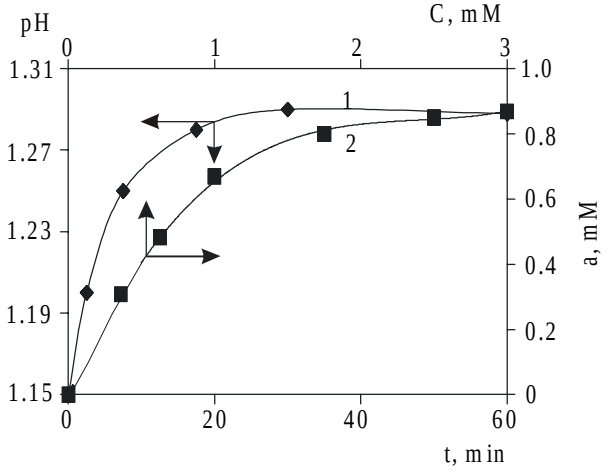

Fig. 1.

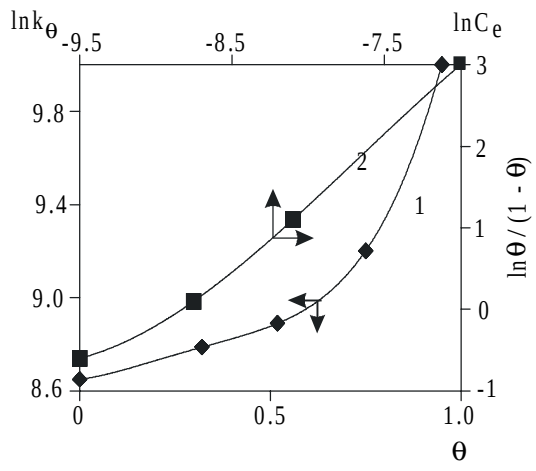

Fig. 2.

Fig. 1. Kinetics (1) and isotherm (2) of HCl sorption by apple pressings.

Fig. 2. Apparent HCl adsorption constant ($\ln k_\theta$) as a function of degree of filling (θ) by pressings of acid (1) and $\ln\theta(1-\theta)$ as a function of the logarithm of the equilibrium concentration (C_e) (2) of HCl in solution with apple pressings at 20°C.

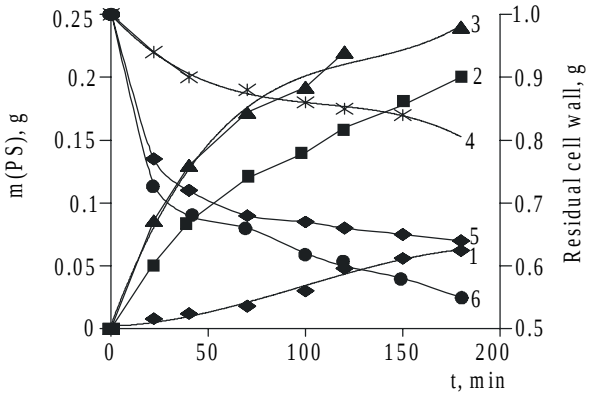

Fig. 3.

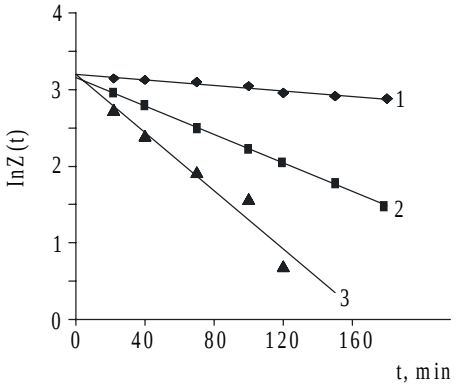

Fig. 4.

Fig. 3. Yield of pectin (1-3) and residual mass of apple cell wall (4-6) as functions of hydrolysis time at various solution pH values and 1 g starting mass of dry pressing. pH: 2.45 (1, 4); 1.80 (2, 5), 1.32 (3, 6).
Fig. 4. The value lnZ(t) as a function of hydrolysis time of apple pressings at 85°C and various pH values: 2.45 (1), 1.80 (2), 1.32 (3).

We write Eq. (6) in the following form in the presence of a cooperative effect [13]:

$$k_\theta = \theta/[C_e^n(1-\theta)], \tag{8}$$

where n is the Hill parameter.

If n > 1, the process has a cooperative nature; for n = 1, this effect is missing. If a cooperative effect is present, its contribution to the free-energy change of the system can be estimated from the Wyman formula [14]:

$$G_n^0 = -RT \int_{\theta=0}^{\theta=1} (1-1/n)\{d\theta/[\theta(1-\theta)]\}. \tag{9}$$

The Hill parameter (n) was calculated over the whole range of filling of the plant cell by acid based on a plot of $\ln\theta(1-\theta)$ as a function of $\ln C_e$ according to the Hill equation. Then the value was used to estimate the free energy component due to cooperativity [Eq. (8)]. The quantities $\Delta G°$ and $\Delta G_n°$, equal to -21.8 and -6.2 kJ/mole, were calculated from the integral values of $\ln k_\theta$ and Eq. (8).

Thus, sorption of HCl by apple pressings is due to the negative free energy change of the process, which has a cooperative nature.

Hydrolysis of PP by apple cell can be represented as successive decomposition of PP and intermediate pectin to relatively low-molecular-weight OS. Based on this assumption, the intermediate and final products were separated [15] as alcohol-precipitated and alcohol-soluble fractions. Analytical data for the content of uronide and neutral sugar (NS) units in the starting, intermediate, and final products from PP hydrolysis showed that the kinetics of the process are consistent with a successive first-order reaction whereas the change in the amount of intermediate (pectin) lies at an extreme.

PP hydrolysis was analyzed using the kinetics of the two sequential unimolecular reactions: PP $\xrightarrow{k_1}$ alcohol-precipitated pectin $\xrightarrow{k_2}$ alcohol-soluble compounds.

For each combination of hydrolysis parameters (time, temperature, and pH), the amounts of remaining PP z(t), alcohol-precipitated pectin y(t), and alcohol-soluble compounds q(t) were determined (Fig. 3). This gives a straight line in semilogarithmic coordinates and extrapolates to a single point regardless of pH (Fig. 4). The PP content in the starting material (A_0), 27.4%, was determined using these data.

The quantity A_0 was used in the first-order kinetic equation

$$Z(t) = A_0\exp\{-k_1 t\},$$

to calculate k_1 values of $0.27\cdot10^{-4}$ sec^{-1}, $0.96\cdot10^{-4}$ sec^{-1}, and $1.63\cdot10^{-4}$ sec^{-1} at pH values of 2.45, 1.8, and 1.32, respectively.

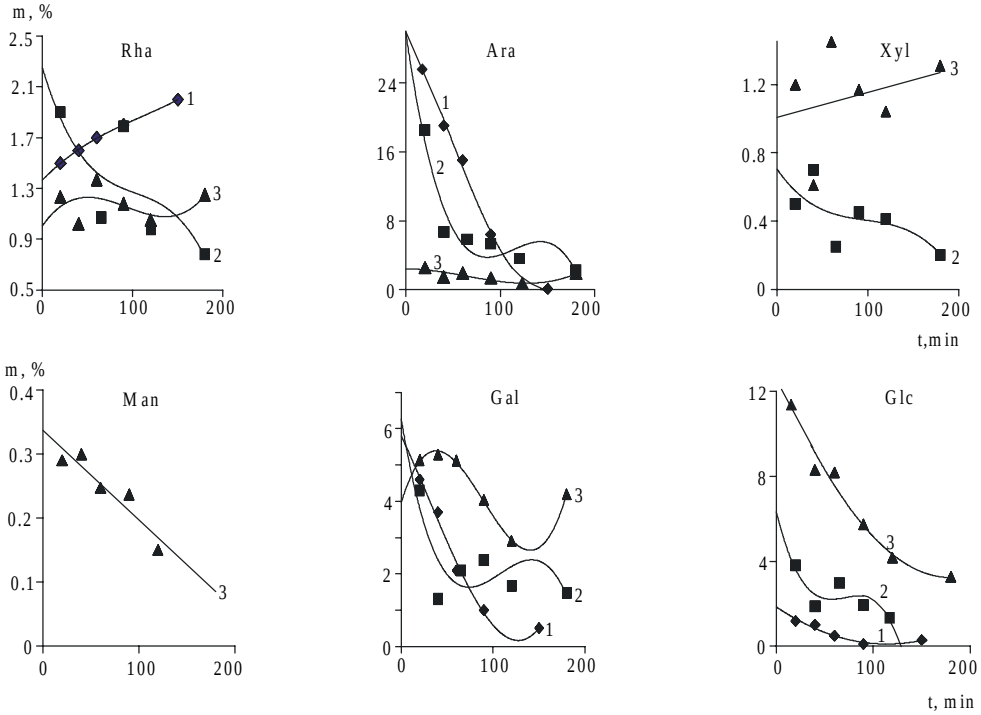

Fig. 5. Monosaccharide composition of apple pectins precipitated by alcohol as a function of initial solution pH and hydrolysis time. Hydrolysis temperature 85°C. pH: 2.45 (1), 1.8 (2), 1.32 (3).

Therefore, the rate of PP hydrolysis increases significantly as the pH decreases under otherwise equal conditions. In particular, k_1 increases by almost an order of magnitude as the pH decreases by about one unit. However, it must be noted that k_1 and A_0 are empirical and averaged over all bonds that are hydrolyzed under these experimental conditions.

The quantitative composition of all monosaccharides (Fig. 5) and the content of galacturonic acid (GA) units (Fig. 6) were determined in pectin precipitated by alcohol. We will restrict ourselves to a qualitative examination of PP hydrolysis. We previously demonstrated [16] that the alcohol-precipitated components of plant-cell hydrolysate consist of a mixture of acidic and neutral polysaccharides. Macromolecules of the former, in addition to D-GA units, contain monosaccharide units [17]. The content of D-GA in the pectin increases at constant temperature and pH whereas the content of NS decreases (Fig. 5).

Therefore, the hydrolysis that started in PP continues in solution. Bonds formed by neutral groups of monosaccharides are decomposed most. This enriches the pectin in D-GA units (Fig. 6).

The different decomposition rates of bonds formed by different monosaccharides are also important. As the hydrolysis time increases, the composition of the neutral polysaccharides changes as a result of a decrease in the number of neutral monosaccharide groups: arabinose, mannose, galactose, and glucose. As a result, the composition is enriched in rhamnose and xylose. Bonds formed by arabinose, galactose, and glucose are the most labile in this instance. As has been demonstrated [16], these make up the bulk of the neutral polysaccharides and polysaccharides in the polygalacturonan macromolecules as side branches. On the other hand, these results suggest that the acidic polysaccharide macromolecules contain rhamnose and xylose. Therfore, they are very stable to the action of hydrolyzing reagents.

The research results (Figs. 5 and 6) give the impression that the longer the hydrolysis time, the better the quality of the resulting pectins since the content of D-GA increases. However, the properties of the PS are determined to a large extent by the molecular weight in addition to the chemical structure. Figure 7 shows the characteristic viscosity of pectin as a function of hydrolysis time. The curves give maxima at the various pH values. The maximum shifts toward increasing hydrolysis time as the pH increases. It is important that the value of the characteristic viscosity is practically independent of pH. This indicates that the hydrolysis mechanism is identical and increasing the acid concentration only accelerates the process.

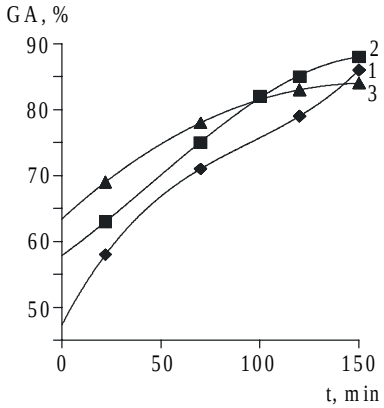

Fig. 6.

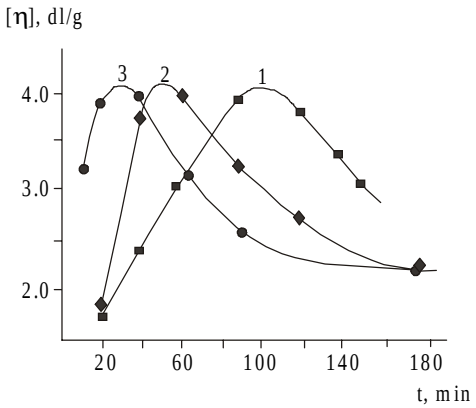

Fig. 7.

Fig. 6. Content of GA in pectin precipitated by alcohol as a function of hydrolysis time at 85°C and pH: 2.45 (1), 1.80 (2), 1.32 (3).

Fig. 7. Characteristic viscosity of PS as a function of hydrolysis time at 85°C and pH: 2.45 (1), 1.8 (2), 1.32 (3).

Thus, the features of plant-cell PP decomposition using apple pressings as an example lead to the conclusion that PP decomposition can be controlled and the method of producing pectin with given properties can be optimized by simple regulation of the physicochemical parameters of the hydrolysis.

II. Features of Sunflower Calathide PP Decomposition by Acid Hydrolysis

Purified and ground sunflower calathides were hydrolyzed using HCl at a starting pH of 1.2 at a raw material:hydrolysate ratio of 1:20 for various reaction times. The hydrolysates were separated from the cell matter and divided into three fractions by successive centrifugation at 7200 rpm, precipitation by a three-fold amount of isopropanol, and drying of the remaining mother liquor. After dividing, they were arbitrarily called microgel (MG), PS, and OS. The contents of GA, NS, and Ca ions were determined for each fraction. The monosaccharide composition of the fraction was determined by GC using an internal standard [18].

Table 1 lists the kinetics of sunflower PP decomposition and yields of MG, PS, and OS [19]. A distinguishing feature of this process is the peak in the change of MG content in the hydrolysate. It can also be seen that the contents of PS and OS gradually increase with increasing hydrolysis time whereas the cellular PP decreases to zero.

The results can be explained by assuming that the monosaccharide units from chains of PP and its decomposition products redistribute after hydrolysis into the observed fractions according to the reaction sequence [20]:

$$PP \rightarrow MG \rightarrow PS \rightarrow OS$$

Considering this sequence, the data were processed using equations derived for describing the kinetics of a sequential first-order reaction [21]:

$$\omega_{max(mg)} = [a/(1-r)](\exp\{-r\ln r/(r-1)\}-\exp\{\ln r/(r-1)\}, \quad (10)$$

$$k_1 = t_{max}\ln[r/(r-1)], \quad (11)$$

$$k_2 = rk_1, \quad (12)$$

where ω_{max} (mg) is the maximal content of monosaccharide in MG (%), t_{max} is the reaction time at which the monosaccharide in MG reaches a maximum (min), and *a* is the initial monosaccharide content in the PP.

TABLE 1. Distribution of Monosaccharide Units in Pectin Fractions After Various Hydrolysis Times

Hydrolysis time, min	Fraction	Component content, %											Yield
		Rha	Ara	Xyl	Man	Gal	Glc	GalA	ΣHC	ΣCax	Ca	Moisture	
15	MG	1.130	-	0.390	0.290	0.58	0.34	14.81	2.73	17.54	0.88	1.46	22.44
	PS	0.420	-	0.440	-	0.77	1.98	5.11	3.61	8.72	0.42	1.04	10.44
	OS	-	-	-	0.610	1.22	14.04	5.58	15.87	21.45	1.79	1.88	25.11
	Total	1.550	-	0.830	0.900	2.57	16.36	25.50	22.21	47.71	3.09		57.77
30	MG	1.330	-	0.500	1.610	0.53	1.04	19.96	5.01	24.97	1.13	1.93	28.00
	PS	0.520	-	0.620	-	1.85	0.82	6.44	3.81	10.25	0.42	1.30	12.00
	OS	-	-	-	0.700	1.83	15.09	3.60	17.62	21.22	1.29	1.91	24.44
	Total	1.850	-	1.120	2.310	4.21	16.95	30.00	26.44	56.44	2.84		64.44
60	MG	2.230	0.290	0.100	0.830	0.91	1.11	30.95	5.47	36.42	1.57	2.27	39.74
	PS	0.310	0.082	0.260	-	2.68	0.38	7.63	3.71	11.34	0.45	1.45	13.25
	OS	1.570	4.150	1.300	1.990	2.03	5.60	3.65	16.64	20.29	1.42	1.61	23.50
	Total	4.110	4.522	1.660	2.820	5.62	7.09	42.23	25.82	68.05	3.44		76.49
90	MG	1.780	0.290	0.030	0.620	0.69	0.72	23.90	4.13	28.03	1.32	2.57	32.89
	PS	0.230	0.054	0.290	-	4.14	0.32	10.84	5.03	15.87	0.62	2.15	18.67
	OS	6.450	4.000	1.860	2.690	2.59	10.47	3.67	28.06	31.73	1.87	2.24	35.53
	Total	8.460	4.344	2.180	3.310	7.42	11.51	38.41	37.22	75.63	3.81		87.09
120	MG	1.300	0.290	0.020	0.500	0.56	0.63	26.22	3.30	29.52	1.33	1.70	31.55
	PS	0.220	0.049	0.110	-	2.94	0.35	16.43	3.67	20.10	0.83	2.50	23.55
	OS	6.450	2.060	1.160	2.010	2.23	9.87	4.64	23.78	28.42	1.72	2.34	32.53
	Total	7.970	2.399	1.290	2.510	5.73	10.85	47.29	30.75	78.04	3.88		87.63
150	MG	0.740	0.220	-	0.100	0.31	0.39	16.92	1.76	18.68	0.85	1.22	20.69
	PS	0.170	0.067	0.300	-	4.14	0.20	23.30	4.88	28.18	0.89	3.19	32.00
	OS	7.320	1.550	0.670	1.540	2.28	12.56	6.05	25.92	31.97	2.53	2.58	37.82
	Total	8.230	1.837	0.970	1.640	6.73	13.15	46.27	32.56	78.83	4.27		90.51
180	MG	0.360	0.380	-	-	0.52	0.18	22.85	1.44	24.29	1.11	1.65	27.50
	PS	0.230	0.123	0.450	-	2.32	0.17	15.52	3.29	18.81	0.88	2.27	21.60
	OS	8.290	1.080	0.310	1.620	1.41	18.91	7.19	31.62	38.81	1.97	2.21	42.74
	Total	8.880	1.583	0.760	1.620	4.25	19.26	45.56	36.35	81.91	2.85		91.84

A value of r that satisfies Eq. (10) was selected using experimental values of ω_{max} (mg), t_{max}, and *a* calculated by summing the component contents in all fractions in the area of the plateau (Table 1) and using Eqs. (10)-(12) and computer analysis [22]. This made it possible to calculate numerical values of k_1 and k_2. The corresponding k_1 and k_2 were calculated for decomposition of the GA chemical bond, the total NS, and their separate components (Figs. 8 and 9) in order to calculate the reactivity of the acidic and neutral PP components toward acid hydrolysis. Plots of the change of content of these components during the hydrolysis give maxima. Using the method described above, k_1 and k_2 were calculated using Eqs. (10)-(12) (Table 2).

The quantities *a* were calculated from the area of the plateau in the yield of the corresponding components as functions of the hydrolysis time.

The quantity r is less than unity for GA, Rha, Xyl, and Man; greater, for total NS and Gal. Therefore, bonds involving the first group of the monosaccharides are easier to decompose in PP than in MG. In the second instance, the opposite is true. The quantitative rate constants for Glc hydrolysis could not be estimated because of its high content of OS versus that expected. Apparently this was due to their formation from not only PP but also starch of other cellular components. For Rha, Gal, and Man, the plot of yield as a function of hydrolysis time has two and three maxima with the corresponding ω_{max} and t_{max} values (Table 2 and Fig. 9). Whereas the r values for Rha and Gal are greater than zero and similar to each other, the values for Man are greater and less than zero. This means that Man in both PP and MG is found in at least two energy states that are hydrolyzed at different rates. It was also shown that the Ara content in MG is very small and that its increase during the reaction, like for GA, is due to a reduction in the content of other neutral monosaccharides. These processes cause the GA content in the MG to be stabilized and reach >80% if the process is carried out for about one hour. The degree of GA esterification also remains unchanged, up to 52-57%.

TABLE 2. Parameters of Equations (1.7) for MG and its Monosaccharide Components

Component	ω_{max}, %	t_{max}, %	a, %	r	$10^4 k_1$, sec^{-1}	$10^4 k_2$, sec^{-1}
MG	37.500	60.00	100.00	0.9620	2.83	2.72
NS	4.900	75.00	32.54	4.2560	0.99	4.21
GA	26.940	65.81	41.80	0.2302	4.83	1.11
Rha	2.057	67.30	4.73	0.7007	2.94	2.06
Rha	2.230	60.00	4.73	0.5795	3.60	2.09
Xyl	0.500	26.52	1.17	0.7290	7.33	5.34
Gal	1.113	50.00	3.00	0.9830	3.36	3.30
Gal	0.922	55.00	3.00	1.4055	2.54	3.58
Glc	1.075	61.20				
Xyl	0.510	26.52	1.17	0.6970	7.49	5.22
Man	1.620	36.50	2.14	0.1134	11.21	1.27
Man	0.830	61.20	2.14	0.8960	2.88	2.58
Man	0.680	102.50	2.14	1.3230	1.41	1.86

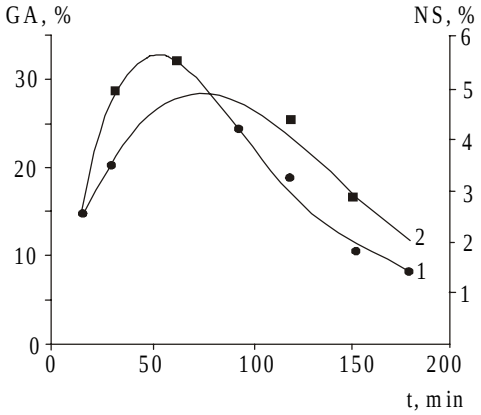

Fig. 8.

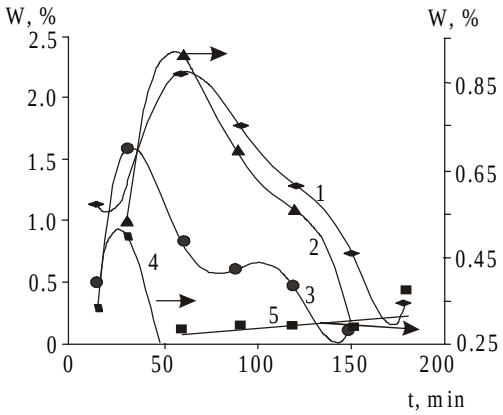

Fig. 9.

Fig. 8. Content of GA (1) and NS (2) in MG as functions of hydrolysis time of sunflower-calathide PP.
Fig. 9. Change of neutral monosaccharide units (W) in MG as a function of hydrolysis time. Rha (1), Gal (2), Man (3), Xyl (4), Ara (5).

The PS composition is enriched in GA units during the whole hydrolysis and reaches a maximum at the end of the process but is less than MG. As the hydrolysis time increases, the PS composition undergoes complicated changes during which they and GA units are enriched in Ara and Xyl units. This leads to the conclusion that arabinoxylogalacturonan is extracted whereas Ara units are most likely found on the sidechains of the macromolecule.

The OS fractions occur mainly in the low-molecular-weight fractions of neutral polysaccharides. A natural consequence of the overall hydrolysis is the enrichment of the OS fraction in low-molecular-weight units, mainly NS.

Thus, experimental data for the qualitative and quantitative compositions of sunflower PP decomposition products lead to the conclusion that the features of the acid hydrolysis are analogous to apple PP decomposition by acid catalysts. However, the decomposition products of apple and sunflower PP are different. This is due mainly to the GA and NS ratio and the degree of esterification of the carboxlates, which is significantly less in sunflower PS. The high content of free carboxylates in sunflower pectin leads to the formation of chains crosslinked by polyvalent metal ions.

III. Effect of Hydrolysis Parameters on Microelement Composition and Hydrodynamic Properties of Sunflower Pectins

The molecular weight (MW) or characteristic viscosity [η] is one of the principal parameters of pectin polysaccharides, owing to which they acquire gel-like properties. Many equations relating [η] and the MW have been described. However, literature data for pectins do not always correspond with each other. It can be assumed that the main reason for this behavior of PS is the presence in them of various types of microelements that form intermolecular bonds to the PS carboxylates and lead to the formation of various three-dimensional structures. Among the elements involved in this process, calcium, magnesium, and aluminum are especially important. The composition and content of these elements depend on the source from which the PS were prepared. Sunflower and Swiss-chard PP are traditionally considered sources that are rich in heavy and toxic elements. Development of a method to remove them is a separate industrial issue.

Specimens were ashed to analyze the microelement composition of pectins obtained from sunflower calathides (SFC). Pectin obtained under analogous conditions from apple pressings (AP) was used for comparison. The composition and structure of AP were found by GC and ^{13}C NMR spectroscopy.

According to spectral analysis [23], the content of all elements in both types of pectin comply with medical-biological requirements and health standards. The calcium contents of SFC and AP were compared. In SFC, the Ca^{2+} content reaches 4% whereas it is <0.1% in AP. SFC contains about an order of magnitude less Mg^{2+} ions than Ca^{2+} whereas these elements in AP are present in identical amounts. The contents of heavy elements such as Pb ($1\cdot10^{-4}$% standard), Cd ($1\cdot10^{-5}$%), Cu ($1\cdot10^{-3}$%), and Zn ($3\cdot10^{-3}$% standard) are also within the standard rangese for both types of pectins whereas the Hg standard ($1\cdot10^{-5}$%) is lower than the detection limit of the spectral instrument. Arsenic ($5\cdot10^{-5}$%) was not detected in AP. Its content in SFC was above the allowed dose. Therefore, additional processing is necessary to lower its level. This problem was successfully solved by us by hydrolyzing sunflower calathide PP in an acid—salt system with subsequent purification of the hydrolysate by ultrafiltration.

Considering the high content of Ca^{2+} in SFC, we bound the Ca^{2+} with Trilon B (EDTA) from MG and PS and then removed the complex from solution by membrane filtration in order to obtain reproducible results for the [η] measurements. The viscosity as a function of concentration gave a straight line, in agreement with the high correlation coefficients (R^2 = 0.96-0.99). These data and the results shown in Fig. 10 indicate that the characteristic viscosity [η] of MG pectin components is practically constant during hydrolysis at 2.126 ± 0.067 (**1**).

It should be considered that Ca^{2+} in plant PP cell walls forms intermolecular bonds to carboxylates of polygalacturonic acid with a three-dimensional structure that is modeled as an "egg carton" [24]. Keeping in mind the nonuniformity of the monosaccharide composition of the pectin macromolecules, it should be confirmed that the hydrolysis is more extensive in the parts of the polymeric chains that are rich in NS whereas the PP fragments with a high content of GA that are stabilized by intermacromolecular bonds through Ca^{2+} are solubilized in the native state and remain practically unchanged until the hydrolysis is complete. This is confirmed by the monosaccharide composition of MG, in which the content of GalA > 80% [15], the constant [η] during the reaction [23] (Table 1, Fig. 10, 1), and the sharp drop of the Huggins constant for MG in the initial stages of hydrolysis and its constant value during subsequent reaction stages (Fig. 10, 5). It is important that $K\eta'$ decreases from a value greater than unity to less than 0.5. This is indicative of a change in the solvent quality from poor to good. Since the solvent in both instances is the same, the changes occurring in the behavior of the polymer confirm the assumption about the change of MG mononsaccharide composition. The degree of MG swelling increases owing to the change of MG structure during the hydrolysis and the improvement in solvent quality (Fig. 10, 3). However, its ability to swell decreases owing to a reduction in the fraction of acidic monosaccharide units in the starting material (**4**).

The content of Ca^{2+} in addition to the monosaccharide composition has a significant effect on the ability of MG to swell, as noted above. The degree of swelling of the specimens increases as the content of Ca^{2+} in the MG decreases (if they are bound by Trilon B). This is due to a decrease in the number of intermolecular bonds formed by Ca^{2+}.

PS are more susceptible to hydrolyzing reagents than MG because of the nonuniformity of the monosaccharide composition and the high content of NS (GalA content = 50-60%). As a result, the fraction of NS decreases or the GalA content increases by 10-15%, the amount of Ca^{2+} decreases by ~25% (Table 1), and [η] decreases (Fig. 10, 2). For the studied processing times, the last parameter is described by the logarithmic equation $[\eta] = -1.2022 \ln(t) + 7.2903$ with a correlation coefficient R^2 = 0.9685. This indicates that [η] in the initial hydrolysis stages is 7.290 and decreases after 180 min of reaction to 1.047 dl/g. This corresponds to a decrease of [η] by seven times. The results indicate that Ca^{2+} stabilizes and preserves the native structure of PS and that high-quality pectin can be produced by subsequent removal of these ions.

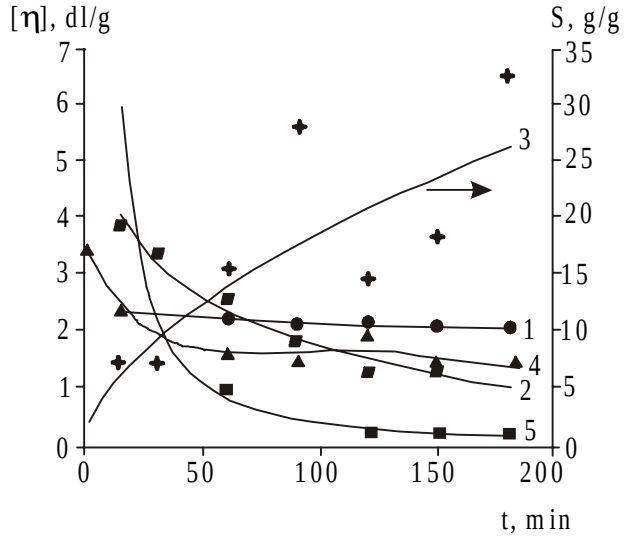

Fig. 10. Change of characteristic viscosity ([η]), MG (1), PS (2), degree of swelling (S) of MG (3), cell residuals (4), and Huggins coefficient (K′) (5) as functions of hydrolysis time of sunflower-calathide PP at pH 1.2 and 85°C.

Thus, the principal parameters of PS are the monosaccharide composition, the content of Ca^{2+}, and the molecular weight of the linear chains. These parameters are closely interrelated and each combination of them corresponds to a certain structure for the final product. This makes it possible to produce PS with given physicochemical characteristics by fine regulation of the hydrolysis.

IV. Effect of Supporting Electrolyte on Hydrolysis of Sunflower PP

Acid—base catalysis is known to be accelerated not only by acid and base but also by anions and undissociated acids and bases, including water. Therefore, the reaction rate at a given concentration of reagents is equal to the sum of the rates due to the activity of all catalytic species. According to this assumption, the overall rate of a catalytic reaction is equal to the sum of the rates due to each of the components [25]:

$$\vartheta = k_{H^+}[H_3O^+] + k_{A^-}[A^-] + k_M[HA] + k_{OH^-}[OH]^- + k_W[H_2O]. \quad (13)$$

We introduce the designation below to account for the effect of water

$$\vartheta_0 = k_{H^+}[H_3O^+] + k_{OH^-}[OH^-] + k_W[H_2O]. \quad (14)$$

Then

$$\vartheta = \vartheta_0 + k_{A^-}[A^-] + k_M[HA] \quad (15)$$

or, multiplying and dividing the last term by $[A^-]$, we obtain:

$$\vartheta = \vartheta_0 + \{k_{A^-} + k_M[HA]/[A^-]\}[A^-]]. \quad (16)$$

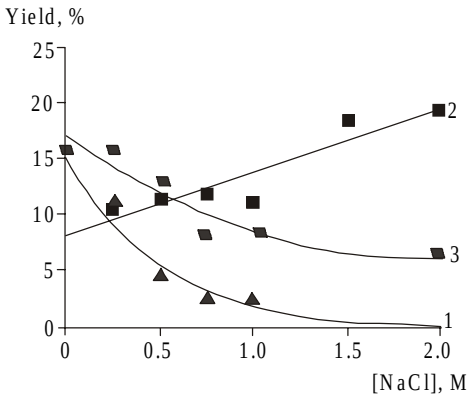

Fig. 11.

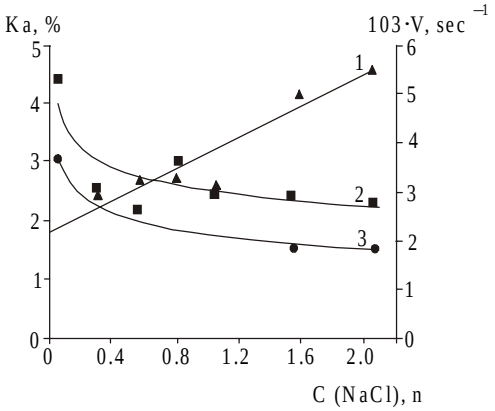

Fig. 12.

Fig. 11. Yield of MG (1), PS (2), and OS (3) as functions of NaCl concentration in hydrolysis solution at pH 1.2, 85°C, and hydrolysis time 60 min.

Fig. 12. Effect of NaCl on rate of formation of PS (1) and Ka for PS (2) and MG (3).

Equation (16) shows that the hydrolysis rate changes linearly with increasing concentration of the salt that gives the anion A^-. The effect of NaCl on hydrolysis of sunflower calathide PP was studied at pH 1.2 and 85°C by varying the NaCl concentration from 0 to 2 g-eq/L for a reaction time of 60 min (specimens numbered KP-60). The last parameter was selected considering that, as we showed previously [19], the yield of practically all MG components is maximal in the absence of a low-molecular-weight salt.

Figure 11 shows the yields of MG, PS, and OS at various NaCl concentrations. It is noteworthy that the PS yield increases smoothly with increasing NaCl concentration whereas the yields of MG and OS decrease.

One of the principal reasons for the effect of a low-molecular-weight salt on the MG yield is apparently the destruction of intermolecular bridges form by Ca^{2+} and the conversion of MG into PS. The reduction of MG and OS yields with increasing NaCl concentration leads to a significant increase in the PS yield. Figure 12 plots the rate of PS formation as a function of NaCl concentration. According to Eq. (16), this function gives a straight line and the rate of PP hydrolysis $[k_{A^-} + k_M([HA]/[A^-])]$ in the studied NaCl concentration range increases by ~2.5 times. The quantity ν_0 and the expression in parentheses in Eq. (4) were calculated from the slope and the intercept of the straight line. These were 2.1750 and 1.5817, respectively, with a correlation coefficient of 0.8581. Considering that we used HCl as the catalyst, the second term in the parentheses can be neglected because the concentration of undissociated HCl is insignificant in solution owing to its high degree of dissociation. Therefore, the slope of the straight line for the rate as a function of NaCl concentration gives to a first approximation the rate constants of hydrolysis (k_A = 1.5817) in the presence of chloride.

Thus, anions of the low-molecular-weight salt in addition to the acid can definitely contribute to the acceleration of PP hydrolysis.

The catalytic activity of the counterion creates an additional possibility for regulating the hydrolysis and adjusting the structural parameters of the PS components.

The effect of the low-molecular-salt on the hydrolysis of sunflower PP is evident not only in the change of yield and increased overall rate but also in the structural features of the monosaccharide products of the decomposition.

The change of acidic (K_a) and esterified (K_e) products in MG and PS hydrolysis indicates that K_a in MG decreases with increased process time and, consequently, K_e increases (Figs. 12 and 13). Apparently the main reason for this phenomenon is connected with the fact that the carboxylates are liberated from the intermolecular bonds formed by Ca^{2+} during PP hydrolysis under the influence of the low-molecular-weight salt. This makes the uronide components of the polysaccharide available to the hydrolyzing reagents and apparently also to the anions. This is the reason for the decomposition of the macromolecules at these sites of the chain. In PS, K_A also decreases. However, in contrast with MG, K_e decreases rather sharply (Fig. 13). This can be explained by the catalytic action of the carboxylates of PS chains during de-esterification. The lack of this effect in MG is explained most likely by the binding of the carboxylates to Ca^{2+}.

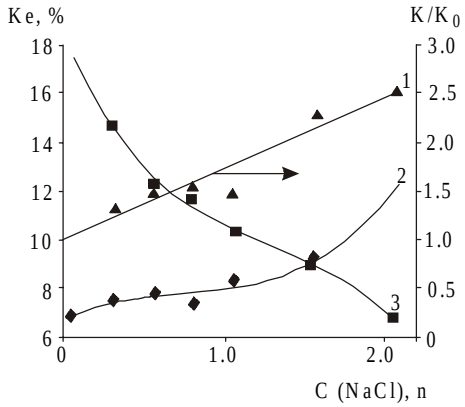

Fig. 13.

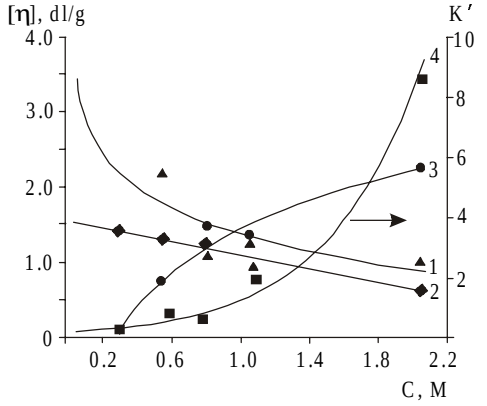

Fig. 14.

Fig. 13. Effect of NaCl on relative increase of the rate of formation of PS (1), K_e for MG (2), and PS (3).

Fig. 14. Effect of NaCl on characteristic viscosity (1, 2) and Huggins constant (3, 4). MG, 1.3; PS, 2.4.

As the ionic strength of the hydrolysate solution increases, the characteristic viscosity of both MG and PS decreases (Fig. 14). For MG and PS without NaCl under otherwise equal conditions, [η] is 2.2 and 2.5 dl/g, respectively [20], whereas with NaCl these values decrease almost linearly to 1.1 and 0.7 dl/g (Fig. 14). At first glance, this is due to a decrease of the MW of the pectin. It should be noted that it is also possible that this effect is due to a change in the chemical parameters of the pectin macromolecules that cause a change in the conformation of the polymeric chain. This is indicated, in particular, the increase of the Huggins parameter for MG and PS with increasing solution ionic strength. As a result of an increase of K′ or a solvent of lower quality (Fig. 14), the PS chain becomes compact. This decreases [η]. Considering that we used HCl as the catalyst, the second term in the expression (in parentheses) can be neglected since the concentration of undissociated HCl is insignificant in solution owing to the high degree of HCl dissociation.

Thus, addition of a low-molecular-weight salt to the solution during acid hydrolysis of sunflower PP can significantly increase the rate and change the molecular and structural parameters of the corresponding decomposition products. The experimental results with sunflower PP in the presence of NaCl are useful for producing high-quality pectin.

V. Effect of Solution Acidity on Hydrolysis of Sunflower PP

Several investigations have addressed the development of conditions for hydrolyzing sunflower PP using acid catalysts [26, 27]. This includes the effect of an acid—salt system that leads to the production of several cross-linked and linear polysaccharides that differ in the molecular structure of the polymeric chains and the composition of the repeating unit [28]. One of the principal parameters responsible for producing PS with a regular molecular structure is the solution pH. The role of the acid is considered to be its effect as a catalyst [29]. However, the change of solution pH during hydrolysis of sunflower PP has been studied [22]. It was concluded that the acid is involved as a catalyst and a reagent. Therefore, the role of the acid during decomposition of sunflower PP must be studied in more detail.

The hydrolysis of sunflower calathide PP was investigated at initial pH_0 values of 1.05, 1.2, and 1.4 at 85°C and various processing times.

It was demonstrated that the dynamics of the MG, PS, and OS yields at all pH values are analogous to those described above (section II). For PS and OS, the yield increases smoothly with increasing acid concentration. For MG at all initial acidities (pH_0), the yield passes through a maximum with increasing hydrolysis time.

Table 3 shows experimental results for MG yield (ω_{max}, %) relative to PP content, hydrolysis time near the maximum (t_{max}, min), and the calculated $r = k_2/k_1$, k_1, and k_2. The change of these parameters with increasing initial pH_0 follows no particular trend. This is not due to the selection of r [19] because the difference between the maximum MG yields that are found experimentally and by calculation differ insignificantly (10^{-5}-10^{-6} units, Table 3). Another reason may be the fact that r and the calculated k_1 and k_2 do not characterize actual reactions but reflect a certain averaged value of several reaction parameters. Apparently the principal reason is the pH change during the reaction.

TABLE 3. Effect of Initial Solution Acidity on Numerical Values of Parameters in Eqs. 10-12

pH_o	t_{max}, min	ω_{max}, %	$\omega_{max(exp)}$-$\omega_{max(calc)}$	r	k_1, min^{-1}	k_2, min^{-1}
1.05	129.3	29.36	-0.000035	1.5246	0.0062	0.0095
1.2	64.65	39.01	0.000065	0.8873	0.0164	0.0146
1.4	83.67	18.18	0.0000023	3.2614	0.0062	0.0203

The solution pH may rise during the hydrolysis because the acid is consumed in extracting Ca^{2+} from PP [30, 31]. This is illustrated by Eq. (17):

$$–COO–Ca–OOC– + 2H_3O^+ \rightarrow 2 –COOH + Ca^{2+} + 2H_2O. \quad (17)$$

This assumption was validated by analyzing experimental results using the equation for a second-order irreversible reaction. The constants for all components of the hydrolysate decreased sharply at the start of the process and then stabilized at constant values (e.g., for MG). The change of the constants was unexpected and could be explained by structural changes of the corresponding specimens that were caused by other catalytic reactions occurring simultaneously with the extraction of Ca^{2+}.

The ratio of the constants indicates that Ca^{2+} ions are liberated slowly from MG. This causes significant accumulations of MG during the reaction.

Another function of the acid is its catalytic action. The first step in the catalytic decomposition of PP consists of acid adsorption by plant cells [32].

The catalytic action of the acid on PP decomposition can be described by the following equations [21]:

$$PP + K \xleftarrow{k_1, k_2} PPH^+*, \quad (18)$$

$$PPK* \xrightarrow{k_3} MG + PS + K, \quad (19)$$

$$-d[MG]/dt = k_3k_1[PP][H^+]/(k_2[MG] + k_1[PP]), \quad (20)$$

$$k_1[PP] << k_2[MG], \quad (21)$$

$$-d[MG]/dt = k_3k_1[PP][H^+]/k_2[MG]. \quad (22)$$

Equations (18)-(22) show that an active protonated complex of PP forms first. Then this complex decomposes into MG and PS or reverts back to starting material. According to this scheme, there should be a direct dependence of the PP decomposition rate on the catalyst concentration.

In fact, the PP decomposition rate was observed to be directly proportional to the ratio $[PP][H^+]/[MG]$ at all starting pH_0 values.

Thus, the effect of the solution acidity on PP hydrolysis is a combination of processes occurring in parallel. These are catalytic reactions and extraction of metal ions from the polymer framework to form polysaccharides with linear and branched structures.

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