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SOME CHARACTERISTICS OF SUNFLOWER PROTOPECTIN DECOMPOSITION PRODUCTS

D. Kh. Khalikov, Z. K. Mukhiddinov, Kh. Kh. Avloev, and F. T. Abdusamiev

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The decomposition kinetics of protopectin from sunflower heads are studied using yields, monosaccharide composition, and viscosity of the acid hydrolysis products. The process involves the successive reactions protopectin → microgel → pectin → oligosaccharide.

The production of pectins (PS) from biomass has been discussed in numerous studies including monographs and reviews [1, 2]. However, the sequence of chemical processes in protopectin (PP) decomposition has been insufficiently studied owing to the difficulty of identifying the actual chemical reactions during hydrolysis of plant cells. Nevertheless, the mechanism of PP degradation by acid catalysts must be known in order to optimize the parameters for producing PS with a high content of galacturonic acid (GA) units in the polysaccharide macromolecule.

Our goal is to study the kinetics of sunflower (*Helianthus annuus* L.) PP hydrolysis in order to develop the optimal method for producing high-quality pectin. It is currently thought that PS are grafted polymers, the main chain of which includes rhamnose units in small quantities in addition to GA units. Other neutral sugars (NS) may be found in the side branches [3]. In particular, the primary structure of macromolecular purified apple pectin isolated by us from industrial samples consists of rhamnogalacturonane [4].

The hydrolysis of plant PP under ordinary conditions produces in solution mixtures of acidic and neutral polysaccharides that differ in both primary structure and molecular mass. The ratio between reaction components, in turn, is a function of the process parameters and the characteristics of the starting material. The principal problem in controlling the hydrolysis is directing the reaction toward enrichment of the solution in the acidic components, PS. This problem could be solved mainly by obtaining quantitative data for the rate constants of the decomposition of PP monosaccharide units.

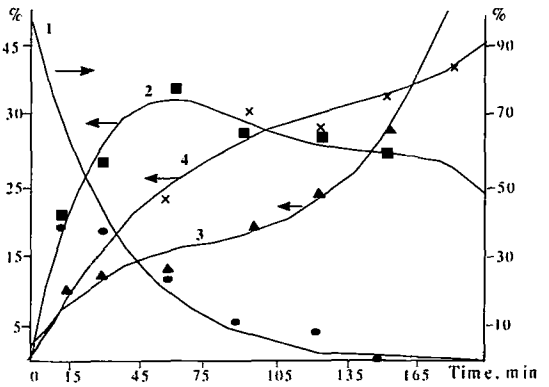

Fig. 1. Decomposition kinetics of sunflower heads PP (1) and yield of acid-hydrolysis products: MG (2), PS (3), and OS (4). Initial pH 1.2, temperature 85°C, hydromodulus 20.

V. I. Nikitin Institute of Chemistry, Academy of Sciences of the Republic of Tadzhikistan, Dushanbe, fax (3772) 21 49 11. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 581-585, September-October, 1999. Original article submitted October 16, 1998.

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Figure 1 shows the PP decomposition kinetics and yields of microgel (MG), PS, and oligosaccharides (OS) as a function of hydrolysis time. The points on the curves indicate the fraction of PP components distributed in the given fraction at the given hydrolysis time. The process is distinguished by a peak in the MG content in the hydrolysate solution. The PS and OS contents gradually increase whereas that of cellulose PP decreases to zero as the hydrolysis time increases.

The results can be explained by assuming that PP hydrolysis and the subsequent decomposition of the products follow the scheme:

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

Following this scheme, we calculated the contants K_1 and K_2 for the first two reaction steps using the following equations [5]:

$$\omega_{max\,(MG)} = \alpha[(e^{-r\ln r/(r-1)} - e^{-\ln r/(r-1)})]/(r-1),$$
$$K_1 = \ln r/[t_{max}(r-1)],$$
$$K_2 = r K_1,$$

where $\omega_{max\,(MG)}$ is the maximum MG content (%), t_{max} is the time to reach the maximum MG content (min), and α is the starting PP content (taken as 100%).

Using the experimental values $\omega_{max\,(MG)} = 37.5\%$ and $t_{max} = 60$ min (see Fig. 1) and Eqs. (2-4), we set $r = 0.962$, which made it possible to calculate K_1 and K_2 as $2.83 \cdot 10^{-4}$ and $2.72 \cdot 10^{-4}$ sec^{-1}, respectively. The similarity in the values of K_1 and K_2 is interesting and apparently is explained by the similarity in the PP and MG hydrolysis mechanisms and an averaging of the decomposition constants of bonds formed by the GA and NS units. The corresponding constants K_1 and K_2 were also calculated for the chemical bond decomposition of GA, total NS, and their individual components in order to find the reactivity of the acidic and neutral PP components.

The experimental results necessary for this are given in Figs. 2 and 3. The contents of these components during the hydrolysis, like for MG, also attain peak values. The constants K_1 and K_2 were calculated from Eqs. (2-4) using the approach described above. The results are listed in Table 1. The quantities α were calculated for the plateau using yield of the corresponding component as a function of hydrolysis time. As an example, Fig. 4 shows the yield as a function of hydrolysis time for GA and total NS.

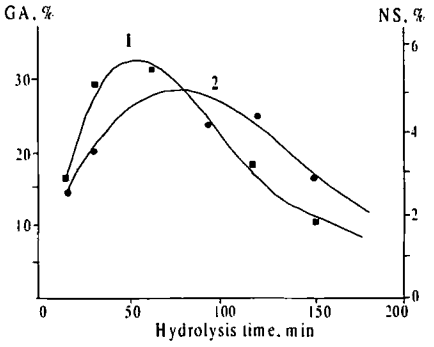

Fig. 2

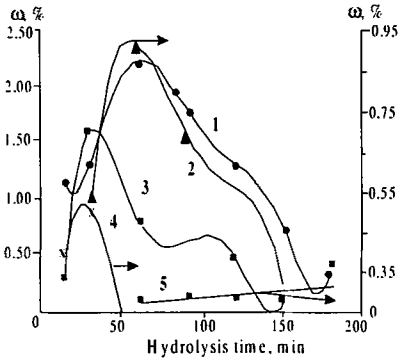

Fig. 3

Fig. 2. GA concentration (1) and total NS (2) in MG as a function of sunflower heads PP hydrolysis time.

Fig. 3. Change of neutral monosaccharide content (ω) in MG as a function of hydrolysis time: Rha (1), Gal (2), Man (3), Xyl (4), Ara (5).

Table 1 shows that r is less than unity for GA, Rha, Xyl, and Man whereas it is greater than unity for NS and Gal. This indicates that the corresponding bonds involving the first group of the monosaccharide units are decomposed more easily in PP than in MG; in the second instance, less easily. Quantitative hydrolysis rate constants for Glc could not be estimated owing to its high content in OS. Apparently this is due to the formation of Glc not only from PP but also from other cellulose components. The diagram of yield as a function of hydrolysis time for Man has two maxima (Fig. 3) with r less and greater than unity, respectively. This means that the Man units in both PP and MG occupy at least two energy states and are not hydrolyzed simultaneously. Figure 3 also shows that the Ara units in MG are practically not hydrolyzed. The increase in its

TABLE 1. Parameters of Eqs. (2-4) for MG and its Monosaccharide Components·

Name	W_{max}, %	t_{max}, min	α, %	r	10^4K_1, sec	10^4K_2, sec
MG	37.500	60.0	100.00	0.9620	2.83	2.72
GA	26.940	65.8	41.80	0.2302	4.83	1.11
NS	4.900	75.0	32.54	4.2560	0.99	4.21
Rha	2.230	60.0	4.73	0.5795	3.60	2.09
Xyl	0.510	26.5	1.17	0.6970	7.49	5.22
Gal	0.922	55.0	3.00	1.4055	2.54	3.58
Man	1.620	36.5	2.14	0.1134	11.21	1.27
Man	0.680	102.5	2.14	1.3230	1.41	1.86
Glc	1.075	61.2	-	-	-	-

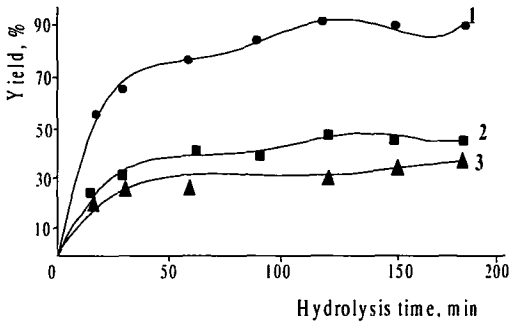

Fig. 4. Decomposition of PP (1) and its acidic (2) and neutral (3) components as a function of hydrolysis time.

fraction during the reaction, like for GA, is due to the decrease in the content of the other neutral monosaccharides. These processes suggest that the GA content in MG is stabilized and reaches more than 80% after about 1 h. The degree of esterification of GA units remains constant (52-57%).

The relative constancy of the MG content and the stability of the GA units to the hydrolyzing agents may be due to the presence of Ca ions in MG. In fact, all MG samples contain Ca ions, the content of which is practically independent of the hydrolysis time and constant (4.04 ± 10%). The ratio of the equivalent mass of GA [ω(GA)] and Ca ions [ω(Ca)], which was about unity, was calculated using the data for GA and Ca-ion content in MG. This most likely indicates that MG chains are bonded to each other through carboxyl groups and Ca ions to form secondary structures similar to the "egg-carton" model [6]. The structures are apparently a part of the PP core unit that is released in native form into the hydrolysate solution. This model for sunflower PP, in contrast with apple PP [7], is more clearly applicable. The intermolecular bonds in PP and MG are destroyed by acid. This releases Ca ions that accumulate in the OS fraction,

In this instance HCl not only acts as a catalyst but also is directly involved in the chemical reaction. The gradual increase in the pH of the hydrolysate solution from 1.2 to 1.55 at the end of the reaction is consistent with this. The experimental data were treated assuming a second-order irreversible reaction, the equations for which gave the following rate constants for extraction of Ca ions for PP, MG, and PS: $K(PP) = 1.20\times10^{-5}$, $K(MG) = 0.35\times10^{-5}$, $K(PS) = 0.86\times10^{-3}$ l/(mol·sec), respectively.

The relatively low value of K for MG compared with the analogous value for PP and PS undoubtedly indicates that its structure is stable. This is apparently due to the high uniformity with which the intermolecular bonds are formed. Mainly the side chains composed of NS units and portions of the chain with irregular Ca bridges are decomposed. The MG structure is homogeneous not only with respect to monosaccharide content but also molecular mass. This is confirmed by the lack of variation in the characteristic viscosity ($[\eta] = 2.10$ dl/g) upon PP hydrolysis that was measured for MG samples freed beforehand of Ca ions by binding them to EDTA with subsequent membrane purification.

The PS composition is enriched during the whole hydrolysis in GA units, which reach their maximum content at the end of the process but do not reach the value found for MG. The PS composition undergoes complicated changes as the

hydrolysis time increases. Arabinose and xylose accumulate in addition to the GA. This suggests that arabinoxylogalacturonane forms, with the xylose units most probably in the side chains of macromolecule. For PS, [η] decreases during the reaction, in contrast with that for MG. It reaches values from 4.20 to 1.50 dl/g and is stable at this level for 1.5 h.

The OS fraction contains mainly low-molecular-weight fragments of neutral polysaccharides. A natural consequence of the total hydrolysis is the enrichment of the OS fraction in low-molecular-weight fragments mainly of NS and Ca ions.

Thus, the experimental data obtained by us on the qualitative and quantitative contens of the decomposition products of sunflower PP make it possible to control the hydrolysis for preparing PS with specific physicochemical parameters.

EXPERIMENTAL

We used ground sunflower heads that were treated with hot water (85°C) in a 1:20 ratio for 30 min with decantation three times. This isolates 42% of the water-soluble substances that contain 4% PS and have low chemical indicators: GA content, 44.4%; degree of esterification, 74.5%; characteristic viscosity [η] = 0.14 dl/g. The dried sunflower heads samples were extracted with isopropyl alcohol, providing another 5.6% of alcohol-soluble substances.

The cellulose purified this way was hydrolyzed using HCl at a starting pH 1.2 with a source—hydrolysate volume ratio 1:20 and various hydrolysis times. The hydrolysate solutions were separated from the cellulose and separated into three fractions by successive centrifugation at 7200 g, precipitation with a three-fold excess of isopropanol, and drying of the remaining mother liquor. These fractions were called the MG, PS, and OS, respectively. The contents of GA, NS, and Ca ions in each fraction were determined.

The monosaccharide composition of a fraction was determined by GLC using an internal standard [8]. The characteristic viscosity of soluble fractions was measured in 1% NaCl in an Ubellohde viscosimeter. The GA content in the reaction products was determined by the carbazole method [9]; the moisture content, by derivatography; the methoxy group content, by titration. The content of Ca ions in the fractions was found by titration with 0.05 N EDTA using erichrome black T in ammonium buffer as indicator [10].

REFERENCES

1. N. P. Shelukhina, R. Sh. Abaeva, and G. B. Aimukhamedova, *Pectin and its Preparation Parameters* [in Russian], Ilim, Frunze (1997).
2. L. N. Gentschev, *Ind. Obst. Gemusever.*, **73**, 17 (1998).
3. K. Keegrstra, W. Kenneth, W. Talmoedge, W. D. Bauer, and P. Alberseim, *Plant Physiol.*, **51**, 188 (1973).
4. Z. K. Mukhiddinov, D. Kh. Khalikov, E. E. Grigor'eva, and V. P. Panov, *Khim. Prir. Soedin.*, 91 (1993).
5. Ya. I. Gerasimov et al., *Course of Physical Chemistry* [in Russian], 2nd Ed., Khimiya, Moscow (1973), Vol. 2, p. 32.
6. M. L. Jarvis, *Plant Cell. Environ.*, **7**, 153 (1984).
7. D. Kh. Khalikov, Z. K. Mukhiddinov, M. G. Asoev, and V. A. Degtyarev, *Khim. Prir. Soedin.*, 787 (1994).
8. Z. K. Mukhitdinov, D. Kh. Khalikov, and V. A. Degtyarev, *Khim. Prir. Soedin.*, 455 (1990).
9. E. A. McComb and R. M. McCready, *Anal. Chem.*, **24**, 1630 (1952).
10. A. G. Loginov, A. G. Voskresenskii, and I. S. Solodnym, *Analytical Chemistry* [in Russian], Prosceshchenie, Moscow (1975).