Influence of the parameters of hydrolysis-extraction high-temperature process on epyyield, physico-chemical and molecular mass characteristics of pectin substances

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Abstract. The effect of the duration of high-temperature hydrolysis-extraction on the yield and parameters of pectin from various sources has been studied. It is shown that the variation in the duration of the process leads to a phased conversion of high-molecular aggregated pectin macromolecules into samples enriched with galacturonic acid units with a narrow molecular weight distribution. The results give an additional opportunity to directly control the hydrolysis-extraction process in order to obtain the desired product in high yield and specified parameters.

1. Formulation of the problem
Pectin substances are contained in the cell wall of all higher flowering plants and are one of the most complex and dynamically structured class of biopolymers. Their structure and properties depend on a number of both internal (type, preliminary preparation, term and conditions of storage of plant raw materials, etc.) and external factors (duration of the hydrolysis-extraction process, temperature, pH of the hydrolysing agent, etc.) [1]. Unique gel-forming and sorption properties of pectin substances cause their wide application in various areas of food industry and medicine [2, 3]. This trend is increasing every year, in turn, increasing the need for the production of pectin. Currently, in the market of pectins and pectin-containing products, the needs of consumers significantly exceed the offers of producers. The reason for this is the fact that most of the existing technologies for the production of pectin substances are based on the use of strong acids (HCl, HNO3, H3PO4, H2SO4), aluminum chloride, ammonium hydroxide, a mixture of alcohol with acid and alcohols of various concentrations. The production process takes place in aggressive media with variations in extraction and hydrolysis time from 3 to 6 hours and a general process cycle of up to 12 hours or more, which causes significant energy costs. Isolation of water-soluble pectins is carried out with the help of huge amounts of ethyl alcohol [4]. These technological factors significantly increase the cost of the targeted products, making them virtually inaccessible to a wide range of consumers.

Also it should be noted that the available experimental, computational, model methods for research of multicomponent physicochemical systems [5-10] don’t cover the whole variety of the proceeding processes. In this connection, the task arises of developing modern energy and resource-saving technologies that allow regulate the process of obtaining pectin substances with the specified structure and properties.
2. Methodology
Pomace, sunflower heads, pumpkin fruits, stems and leaves of rhubarb were used as a raw material for pectin substances. Hydrolysis-extraction was carried out by the method developed by us [11-17]. The process proceeded at 120 °C, a pressure of 1.5 atm., the hydraulic module 1:20. As a hydrolyzing agent, a solution of HCl with pH = 2.0 was experimentally determined. The duration of the hydrolysis-extraction process varied from 3 to 10 minutes. Water-soluble pectin substances were isolated according to the method described in [18, 19]. For the samples obtained, physicochemical characteristics were determined: the content of galacturonic acid, the degree of esterification by the methods described in the works, respectively [20, 21]. The mass-average (Mw), number-average (Mn) molecular weights (MW), z-average MW (Mz) and polydispersity index (Mw / Mn) were determined by high-performance liquid chromatography (HPLC) [22, 23].

3. Results and Discussion
Earlier, we showed that the hydrolysis-extraction method under the influence of high temperature and pressure leads to a significant increase in the yield of pectin substances, positively affecting their quality [11, 14]. But, the parameters of the process for obtaining pectin, in particular the duration of extraction, are selected depending on the characteristics of the processed raw materials. In this connection, the effect of the duration of the hydrolysis-extraction process under the influence of high temperature and pressure on the yield and parameters of pectin substances was studied.

Table 1. The yield of pectin substances of different raw materials (% with respect to the weight of the feedstock), the content of galacturonic acid and the degree of esterification of the samples (% relative to the mass of the PV)

| Time, min | Apple | Sunflower | Rhubarb | Pumpkin |
|----------|-------|-----------|---------|---------|
|          | Yeld  | GA        | DE      | Yeld    | GA     | DE | Yeld    | GA     | DE | Yeld     | GA     | DE |
| 3        | 21,50 | 68,40     | 54,35   | 19,00   | 70,00  | 38,88 | 15,00   | 78,00  | 49,44 | 2,24     | 72,00  | 84,51 |
| 5        | 23,60 | 70,20     | 52,17   | 24,68   | 74,00  | 26,37 | 17,50   | 81,60  | 50,00 | 1,77     | 75,00  | 81,69 |
| 7        | 26,30 | 72,00     | 50,55   | 29,71   | 76,00  | 23,91 | 18,00   | 84,00  | 51,14 | 3,77     | 70,80  | 82,35 |
| 10       | 26,80 | 74,40     | 50,55   | 32,00   | 76,20  | 21,98 | 18,98   | 85,20  | 52,27 | 3,92     | 61,20  | 84,29 |

The content of galacturonic acid in pectin substances varies according to a similar pattern, with the exception of pumpkin pectin. With the increase in the duration of the hydrolysis-extraction process, the change in the content of GA units in pumpkin pectin is extreme. In this case, the maximum is for a 5-minute extraction. Further, the content of HA decreases, reaching 61.20% at the end of the process, which gives grounds to suggest that the process of decomposition of the pectin macromolecule of this type of raw material is uneven. For other sources of raw materials, as the duration of the process grows, the pectin substances are continuously enriched with GA units. It should be noted that the PS rhubarb is characterized by a high content of galacturonic acid units, which indicates the predominance of linear homogalacturonan chains in pectin macromolecules of this type of raw material.

The value of the degree of esterification remains practically unchanged. Exceptions are the pectin substances of the sunflower heads, the value of which with the increase in the duration of the process is somewhat reduced. The reason for this can be the de-esterification of the methyl groups of the galacturonic acid units and the breakdown of the macromolecule of the polysaccharide matrix, especially the esterified GA residues (Table 1).

Calculation of molecular and hydrodynamic parameters of pectin substances was carried out using the software ASTRA 5.3.4.13 and Breez (Waters). The change in the molecular mass parameters of
various pectins is of a dissimilar character and indicates the influence of the raw material source on the properties of the target products.

In particular, for pectin substances of apples and sunflower, the molecular weight and the polydispersity index decrease with increasing duration of the hydrolysis-extraction process under the influence of high temperature and pressure. This fact, as well as low values of yield of PS in three-minute hydrolysis, indicate extraction at the beginning of the process of native, yet not degraded pectin macromolecules. Further increase in the duration of the process leads to the destruction of aggregated pectin molecules with a simultaneous decrease in the polydispersity of the samples, which indicates the enrichment of the pectin substances in the hydrolysis-extraction process by linear homogalacturonan chains.

Table 2. Molecular-mass parameters of pectin substances of apples, sunflower heads and rhubarb.

| Time, min | Apple | Sunflower | Rhubarb |
|----------|-------|-----------|---------|
|          | Mw e | Mw/Mn | Mz e | AG* | Mw e | Mw/Mn | Mz e | AG* | Mw e | Mw/Mn | AG* |
| 3        | 560  | 11,5  | 7736 | 31,5 | 5195 | 70    | 86660| 36,5 | 98   | 2,4   | 9,9  |
| 5        | 370  | 8     | 7310 | 7,5  | 175  | 5     | 1120 | 33,5 | 114  | 2,3   | 9,8  |
| 10       | 190  | 6,7   | 3806 | 15   | 98   | 1,5   | 351  | 19   | 127  | 7,5   | 6,34 |
| 60**     | 134  | 3,8   | 1133 | 23,15| 142  | 3,22  | 1692 | 40   | 268  | 4,8   | 5,3  |

**aggregated fraction;  
** samples were obtained at atmospheric pressure, temperature 85 °C, hydromodule 1:20, duration 60 minutes, continuous stirring, pH = 1.2

From the differential molecular weight distribution (MWD) curves shown in Figures 1 and 2, it can be seen that at the beginning of the process the pectin substances of apples and sunflower have a broad MMP. But, with increasing duration of hydrolysis, high-molecular aggregates gradually turn into samples with a narrow molecular-mass distribution. Further increase in the duration of the process to 10 min leads to the formation of molecules with a bimodal distribution. If the low temperature and long duration of the process lead to the production of low molecular weight pectin substances with a narrow molecular weight distribution, the effect of high temperature and pressure even in a short period of time exerts a stronger effect on the degradation of the polysaccharide matrix of these raw materials.

The rhubarb pectin substances obtained at atmospheric pressure, on the other hand, have a higher molecular mass and are more polydisperse than those obtained under the influence of high temperature and pressure. With increasing duration of the MMP process, the rhubarb pectin becomes broad, and the molecular weight and polydispersity values increase. Taking into account the high value of Mw of pectin obtained at 60 min. and low temperature, it can be concluded that the high molecular weight fractions of pectins in the rhubarb are localized in the secondary cell wall. In addition, PS of rhubarb are different from samples obtained from other sources of raw materials in that they are more thermodabile and are degraded at 120 °C, even in a short period of time (Table 2, Figure 3).

For pectin substances of pumpkin, regardless of the method of production and the duration of the process, a bimodal molecular weight distribution is characteristic (Table 3, Figure 4). The appearance of a second peak in the differential MWD curves indicates the presence of a second low-molecular fraction, which indicates the heterogeneity of the structure of the PV of the given feedstock.
Figure 1. Differential curves of the molecular weight distribution of the pectin substances of apples obtained at $T = 85 \, ^\circ C$ (1) and under the influence of high temperature and pressure ($2 - t = 3 \, \text{min}, \, 3 - t = 5 \, \text{min}, \, 4 - t = 10 \, \text{min}$)

Figure 2. Differential curves of the molecular weight distribution of pectin substances of sunflower obtained at $T = 85 \, ^\circ C$ (1) and under the influence of high temperature and pressure ($2 - t = 3 \, \text{min}, \, 3 - t = 5 \, \text{min}, \, 4 - t = 10 \, \text{min}$)

Figure 3. The curves of the molecular weight distribution of rhubarb pectin substances obtained at $T = 85 \, ^\circ C$ (1) and under the influence of high temperature and pressure ($2 - t = 3 \, \text{min}, \, 3 - t = 5 \, \text{min}, \, 4 - t = 10 \, \text{min}$)

Figure 4. Differential curves of the molecular weight distribution of pectin substances of pumpkin, obtained under the influence of high temperature and pressure ($1 - t = 3 \, \text{min}, \, 2 - t = 5 \, \text{min}, \, 3 - t = 7 \, \text{min}, \, 4 - t = 10 \, \text{min}$)

Table 3. Molecular-mass and hydrodynamic parameters of pectin substances of pumpkin

| Time, min. | \(\#\) | Mw \(c^3\), kDa | Mw/Mn | Mw \(c^3\), kDa | [\(\eta\)]*, ml/g | \(R_h\)*, nm | b*** | \(AG_c\), % |
|-----------|--------|-----------------|-------|-----------------|------------------|----------|------|---------|
| 60        | 1      | 673,90          | 1,93  | 2069,00         | 747,0            | 39,7     | 0,57 | 17,70   |
|           | 2      | 72,02           | 1,20  | 82,99           | 251,0            | 13,2     | -    | -       |
| 3         | 1      | 661,00          | 1,80  | 1267,00         | 689,4            | 39,0     | 0,61 | 37,50   |
|           | 2      | 65,71           | 1,15  | 74,29           | 162,4            | 11,2     | -    | -       |
| 5         | 1      | 769,50          | 3,40  | 3969,00         | 375,1            | 33,1     | 0,47 | 40,00   |
|           | 2      | 58,58           | 1,20  | 69,01           | 125,8            | 10,0     | -    | -       |
| 7         | 1      | 622,10          | 1,86  | 1317,00         | 471,8            | 33,4     | 0,56 | 21,60   |
|           | 2      | 62,61           | 1,28  | 77,84           | 153,3            | 10,9     | -    | -       |
| 10        | 1      | 403,50          | 1,56  | 619,40          | 492,4            | 33,1     | 0,50 | 34,95   |
|           | 2      | 41,72           | 1,31  | 48,64           | 180,0            | 10,2     | -    | -       |

*viscosity
** hydrodynamic radius;
*** coefficient, found from the curve of the dependence of the hydrodynamic radius and the molecular mass, which determines the conformation of the macromolecule in the solution.
The main fraction of pumpkin pectin substances obtained in the low temperature process has a high molecular weight of 673900 kD and Mz (2069000 kD), a low polydispersity index (Mw / Mn = 1.93), and a low aggregation capacity. At the beginning of the hydrolysis-extraction process under the influence of high temperature and pressure, the pumpkin pectin substances have molecular weight and polydispersity values close to those of samples obtained at low temperature. But, at the same time, there is an almost twofold decrease in the Mz index and an increase in the aggregation capacity of the main fraction of pectin substances. With an increase in the duration of the hydrolysis-extraction process, the change in the molecular weight of both Pumpkin PT fractions is extreme. The maximum is 5 minutes. Similarly, the Mz index and the polydispersity of the samples change.

The value of the coefficient b also has a high value, regardless of the method for producing the pumpkin pectin, with the exception of samples obtained by 5-minute extraction under the influence of high temperature and pressure. The average values of the coefficient b for the mixture of extracted pectins are found to indicate the conformation of random coils with an elongated shape. The PS macromolecules obtained with a 5-minute extraction have a compact conformation, because of their high aggregation, as indicated by the high values of AG and Mz.

An analysis of the data obtained shows that the appearance of maxima in the change in the content of the elements of galacturonic acid and the molecular mass parameters in the region of 5 minutes indicates that up to this time value the cell wall decays and the extraction of native pectin macromolecules. With the further increase in the duration of the process, along with the continuing disintegration of elements of the cell wall, the decomposition of already extracted high molecular molecules begins.

4. Conclusion
The studied effect of the duration of the hydrolysis-extraction process under the influence of high temperature and pressure gives additional information on the structure of the pectin macromolecule of various plant materials. The revealed regularities can serve as a basis for development of technology for obtaining target products with specified properties.

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