Physico-chemical and molecular-mass parameters of pectin polysaccharides obtained under high temperatures and pressures

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Abstract. Two different methods are used to obtain pectic polysaccharides from food industry waste: traditional and hydrolysis-extraction method under the influence of high temperature and pressure in a short period of time. It is shown that the application of the new method allows to increase the yield of the target products, to optimize the physico-chemical characteristics and the molecular-mass parameters of pectin.

1. Formulation of the problem
Various fruit and vegetable extracts remaining in bulk after production of juices, as well as sunflower heads can become a promising source for the production of pectin polysaccharides, the trend of which in various areas of industry and medicine is increasingly increasing. The preparation of a pectin is a multi-step process involving steps with different functions. The main stages are:
- preparation of raw materials,
- hydrolysis-extraction,
- separation of hydrolyzate solution from cell wall residues,
- purification of the hydrolyzate,
- isolation of water-soluble fraction of pectic polysaccharides,
- drying to the powder state of the finished product,
- in some cases - modification of pectic polysaccharides.

According to most researchers, the main role is played by the hydrolysis-extraction stage. The quality and properties of the target products depend on the way it is carried out, on the choice of parameters at this stage [1-3]. As a consequence, the problem arises of developing modern technologies for the production of pectic polysaccharides with a regulated structure and properties. The available experimental and calculation-theoretical studies about influence of technological process conditions on the physico-chemical and other properties of products [4-9] don’t allow to solve this problem, unfortunately. The main reason of it is a complexity of raw materials composition, obtained products, physico-chemical processes taking place during of pectin production, etc.

In connection with this, the purpose of this study was a comparative study of the physico-chemical and molecular-mass parameters of pectins of various origins obtained by the traditional method [10,
11] and the hydrolysis-extraction method under the influence of high temperature and pressure [12-18].

2. Methodology

Most of the existing methods for the preparation of pectic polysaccharides are based on the principle of hydrolysis-extraction of plant material in the static regime. The process thus proceeds in closed systems, at a temperature of 90-100 °C, a duration of 1-4 hours and continuous mixing. These static conditions provide for the extraction of a significant amount of pectic polysaccharides, but pectin macromolecules diffusing from the plant cell to the solution are exposed to excessive hydrolyzing agent and temperature, which leads to their degradation, deterioration of the physico-chemical parameters, and loss of basic properties. In this paper, the traditional method of hydrolysis-extraction in static mode (Method 1) [10, 11] was used as a reference standard to evaluate the effectiveness of the new developed hydrolysis-extraction method under the influence of high temperature and pressure (Method 2). The raw materials used pomace, pumpkins, stems and leaves of rhubarb, peach, beet pulp, mandarin crusts and sunflower heads.

The traditional method (Method 1): Hydrolysis-extraction of plant raw materials is carried out in closed systems, at a process temperature of 85 °C for 1-4 hours and continuous stirring. The obtained extract is separated from the fiber residues, neutralized and a water-swelling fraction, conventionally called microgel (MG), is isolated by centrifugation at a speed of 4000-5000 rpm. Subsequent precipitation from the remaining solution with a three-fold volume of ethanol, water-soluble components, conditionally named pectin substances (PS) are isolated and evaporation of the mother liquor yields low molecular weight oligosaccharide (OS) residues [10, 11].

Hydrolysis-extraction method under the influence of high temperature and pressure (Method 2): The plant for the production of pectic polysaccharides is presented in Figure 1. The essence of the method is as follows: the equilibrium swollen plant material, placed in the unit, a hydrolyzing agent with the necessary pH value is added, the lid is sealed and the steam is started. The lid is closed tightly and steam is started. The pressure in the installation is automatically controlled by the steam injector. The time of hydrolysis is read by the stopwatch from the moment the temperature is reached. Hydrolysis in this case can be carried out by varying the temperature from 100 to 140°C, pressure from 1 to 3 atm., Duration from 3 to 10 minutes. In this case, the effect of temperature and pressure ensures a high yield of the desired product without the use of strong acids. It has been experimentally established that the optimal pH of the hydrolysing agent is 2.0 at the same time. Also, the duration of the hydrolysis-extraction process is significantly shortened from 60 to 3-10 minutes [12-18].

The hydrolyzate solution is further divided into MH, MF and OS by the above-described procedure. The content of the elements of galacturonic acid (GA) in pectic substances and the degree of esterification of carboxyl groups of GA (DE) were determined by the methods described in [19, 20], respectively.

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) [21, 22]. Depending on the viscosity of the PV solution, the dried sample was dissolved in 0.05M NaNO3 to a final concentration of 1 or 2 mg/ml, centrifuged at 20,000 g for 20 min, separating the aggregated fraction and filtered through a membrane filter (0.45 μm, Millipore Millex-HN). A 0.1-0.2 ml solution was automatically introduced into the chromatograph. The flow rate of the eluent was 0.8 ml/min. The solvent delivery system consisted of a two-channel vacuum degasser, a high-pressure pump (Waters 1515 Isocratic Pump) and an auto injector (717 Plus Auto Injector, Waters). Two columns containing PL-Aquagel OH40 and PL-Aquagel OH60 were used to separate the PV. MM pectin substances were determined simultaneously using the software ASTRA 5.3.4.13 (Wyatt Technology) and Breez (Waters). Values for Mw were obtained by HPLC using a universal calibration using the molecular standards of Pullulan (Showa Denko K.K., Japan).
3. Results and Discussion

The yield of pectic polysaccharides depends not only on the source of raw materials, but also on the method of their preparation (Table 1). When using the hydrolysis-extraction method under the influence of high temperature and pressure, the yield of pectin substances and oligosaccharides increases significantly for all the types of raw materials studied.

The main physico-chemical parameters determining the properties of pectic polysaccharides are the content of the elements of galacturonic acid, the degree of esterification and the molecular weight. According to the literature, the effect of high temperature and pressure often leads to a decrease in the content of GA [23]. But the use of the new hydrolysis-extraction method, due to the shortening of the extraction process, does not adversely affect this parameter, on the contrary, the maximum content of galacturonic acid is observed precisely under the influence of high temperature and pressure (Table 1). The degree of esterification, which largely determines the scope of pectic polysaccharides, does not undergo significant changes depending on the hydrolysis-extraction method.

![Figure 1. Installation for hydrolysis-extraction under the influence of high temperature and pressure](image)

1 - autoclave; 2 - steam generator; 3 - the hermetic cover; 4 - pressure and temperature sensor; 5 - swollen raw material in hydrolyzate solution.
Table 1. Comparative values of the yield of MG, MF, OS*, degree of esterification and the content of galacturonic acid in pectin substances

| The raw material | MG   | PS   | OS   | GA  | DE   |
|------------------|------|------|------|-----|------|
|                  | M1   | M2   | M1   | M2  | M1   | M2  |
| Apple            | 3.60 | 2.60 | 23.60| 24.65| 28.00| 70.00|
| Quince           | 1.40 | 4.50 | 12.40| 25.90| 56.50| 66.00|
| Apricot          | 1.22 | 3.76 | 7.05 | 37.92| 69.19| 66.00|
| Lemon            | 2.00 | 2.06 | 20.06| 23.80| 45.84| 72.24|
| Orangegrits      | 0.80 | 1.00 | 24.00| 27.10| 32.30| 77.76|
| Rhubarb          | 1.11 | 3.18 | 17.50| 35.03| 29.32| 81.60|
| Peach            | 0.86 | 4.28 | 7.20 | 37.85| 43.36| 65.28|
| Pumpkin          | 1.19 | 8.68 | 3.77 | 73.71| 76.92| 64.80|
| Orange peel      | 0.48 | 1.00 | 16.25| 18.75| 62.40| 64.00|
| Citrus           | 0.68 | 1.57 | 18.30| 24.69| 29.97| 64.80|
| Sunflower        | 1.20 | 8.20 | 24.68| 12.80| 7.50 | 56.40|
| Beet             | 1.00 | 1.60 | 10.44| 48.14| 48.96| 75.60|

*M1 (Traditional method) - T = 85 °C, hydromodule 1:20, duration 60 minutes, continuous stirring, pH = 1.2; M2 (Method of hydrolysis-extraction under the influence of high temperature and pressure) - T = 120 °C, P = 1.5 atm., hydromodule 1:20, duration 5 min, pH = 2.0.

There is no clear regularity in the change in the molecular mass parameters of pectin polysaccharides, which requires further study in this field (Table 2, Figure 2). In particular, pectin samples obtained from apples, apricots and pumpkins under the influence of high temperature and pressure are resistant to elevated temperature, have a higher molecular weight compared to similar samples obtained by the traditional method. In these pectins, anomalies associated with the aggregation of macromolecules are observed. As can be seen, the more Mz of pectin, the more its molecules are subject to aggregation, with the exception of lemon pectin. Most likely, this fact is explained by the peculiarities of the structure of the pectin macromolecule of this type of raw material.

Table 2. Molecular Weight Characteristics of PS*

| The raw material | Mw·e\(^{-3}\), kD | Mz·e\(^{-3}\), kD | Mw/Mn | AG, % |
|------------------|-------------------|-------------------|--------|-------|
|                  | M1    | M2    | M1    | M2    | M1    | M2    | M1    | M2    |
| Apple            | 134.0 | 309.4 | 1133  | 4473  | 3.80  | 9.50  | 23.5  | 20.0  |
| Apricot          | 114.5 | 718.0 | 755   | 12500 | 4.70  | 10.90 | 18.5  | 8.5   |
| Quince           | 176.0 | 112.4 | 7309  | 613   | 4.35  | 2.80  | 25.0  | 8.5   |
| Peach            | 243.8 | 65.5  | 2103  | 145   | 6.30  | 3.07  | 14.5  | 23.0  |
| Lemon            | 107.8 | 82.3  | 267   | 387   | 2.89  | 9.31  | 18.5  | 16.0  |
| Pumpkin          | 673.9 | 999.7 | 2069  | 4112  | 1.93  | 2.74  | 17.7  | 40.0  |
| Rhubarb          | 268.0 | 114.0 | 1479  | 338   | 4.80  | 2.30  | 26.5  | 49.0  |
| Sunflower        | 142.4 | 181.0 | 1793  | 1374  | 3.22  | 5.00  | 40.0  | 33.5  |

*Mw is the weight average of MW; Mn - number average MW; Mz is the z-average MW; Mw / Mn is the polydispersity index; AG - aggregated fraction
4. Conclusion
Thus, the new developed method of hydrolysis-extraction under the influence of high temperature and pressure allows to increase the yield of pectic polysaccharides, to improve their quality, and to significantly shorten the duration of the production process, to soften the pH of the hydrolyzing agent, which will lead to a reduction in the costs of pectin production and environmental improvement.

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Figure 2. The curves of the molecular weight distribution of pectins on HPLC using a combination of viscosimetric and refractometric detectors
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