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Research Article

The Influence of the Purification Method on the Molecular Mass and Hydrodynamic Behaviors of Apple Pectin

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Abstract: This study investigates the influence of purification methods on the molecular weight and hydrodynamic properties of pectin polysaccharides (PPs) obtained from apple pomace using the flash hydrolysis method. PPs were purified from the hydrolysate solution through alcohol precipitation (AP) and dia-ultrafiltration (DUF). The molecular weight and molecular weight distribution (MWD) were analyzed using high-performance size exclusion liquid chromatography (HPLC) equipped with an online differential refractometer and viscometer. Hydrodynamic parameters, including intrinsic viscosity ([η]) and hydrodynamic radius (R_h (w)), were determined using the ASTRA 5.3.4.20 software. The application of the DUF method during hydrolysate purification yielded superior molecular parameters, with the polydispersity coefficient (M_w/M_n) decreasing by nearly half. Additionally, increased DUF cycles significantly improved [η] and R_h (w) values from 92.7 to 107.7 g/mL and from 9.7 to 11.0 nm, respectively. The M_w-R_h (w) plot suggests that pectin polysaccharide (PP) macromolecules predominantly exhibit shapes resembling segmented rods or swollen coils, with slopes of 0.65 and 0.64 for both purified samples. The combined double-cycle ultrafiltration and ethanol precipitation process, using an optimal pectin solution-to-ethanol (EtOH) ratio of 1:2, emerged as a more effective purification strategy. This approach enhances the molecular weight and hydrodynamic properties of pectin, which are essential for various applications. Furthermore, the DUF method reduces the reliance on large quantities of expensive, flammable alcohol and eliminates the need for vacuum evaporation, resulting in a cost-effective, sustainable, and environmentally friendly pectin production process.

***Keywords*:** pectin polysaccharides, purification, ethanol precipitation, dia-ultrafiltration, hydrodynamic radius, intrinsic viscosity, molecular weight distribution

1. Introduction

Food waste and by-products are now recognized as a major global problem that threatens the long-term sustainability of the food supply chain. The Food and Agriculture Organization of the United Nations estimates that about one-third of all food produced globally is wasted each year. Of the 1.5 billion tons of fruits and vegetables

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produced annually, about 0.5 billion tons are either wasted or converted into by-products, such as peels, seeds, shells, pods, pulp, etc., during processing.[1]

These wastes or by-products can be converted into valuable raw materials such as polysaccharides, polyphenols, essential oils, dietary fiber, resins, flavor compounds and pigments, instead of being incinerated or dumped in landfills.[2]

Pectin polysaccharides (PPs) are plant cell wall polymers that are widely used in the food and pharmaceutical industries as a stabilizer and emulsifier to improve product stability, as a composite material - a carrier of drugs and food nutrients due to their biosafety, gelling, and emulsifying abilities.[2-10] The demand for obtaining pectin from new sources is constantly growing. PPs are commonly used in the food industry as an additive to products such as jams, jellies, low-calorie products, stabilizing acidified dairy products, thickeners and emulsifiers. In the pharmaceutical industry, PPs are used to prepare drugs that lower cholesterol and blood sugar levels and to treat gastrointestinal diseases. PPs have shown high efficiency in wound healing and have a synergistic effect on drugs in the treatment of cancer.[3-11] Pectin-type polysaccharides as natural and non-toxic substances are used in the food and medical industries due to their biological, analgesic, anti-hepatitis B virus (HBV) and immunological activities.[6-10]

Until now, polysaccharide extraction in industry and research has been mainly carried out in acidic conditions at high temperatures and long hydrolysis times, while some biologically active substances are always present in the extracts (hydrolysates). Previous studies have shown that the parameters of the external solution during hydrolysis-extraction of PPs significantly affect their structure and biological activity.[12,13]

The use of PPs is expanding in many other sectors of the food and pharmaceutical industries, such as the creation of drug carriers, coatings, food ingredients and films, paper substitutes, foams, etc.[4-12] Due to such a diverse use of pectin, there is an urgent need to study other unconventional sources or modifications of existing sources to obtain pectin with the desired quality by rationally modifying pectin using chemical and enzymatic treatment.

The versatility of PPs is related to the structural diversity of these cell wall polymers. Structurally, pectin polymers are heteropolysaccharides mainly composed of three distinct subdomains: homogalacturonan (HG), rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II). The linear subdomain of HG consists of α-1,4-linked galacturonic acid (GalA) residues and carboxyl groups that can be methyl esterified at C6 of the pyranose ring. RG I consists of repeating [→α-D-GalpA-1,2-α-L-Rhap-1,4→] disaccharide units substituted at the rhamnose residues by heterogeneous side chains consisting of galactose, arabinose, and xylose residues. RG II has more complex branches, consisting of 12 different types of glycosyl residues linked on a backbone of seven to nine GalA residues.[4,14,15]

The main sources of commercial pectin are citrus fruits, apples and by-products formed after their processing, including citrus peel and apple pomace. Alternative sources of pectin include cocoa husks, sunflower heads, sugar beet, pumpkin, watermelon, pear and potato pulp.[3,16] Apple pomace contains about 10-15%, and citrus peel contains about 20-30% pectin on a dry matter basis, while sunflower head residues and sugar beet contain about 10-20% pectin on a dry weight basis.[2,3,15-17]

Commercial pectin production traditionally relies on vacuum evaporation and alcohol precipitation (AP), which requires large quantities of expensive and flammable alcohol, resulting in high production costs and limiting the widespread commercial use of refined pectin. Our recent study introduces an innovative dia-ultrafiltration (DUF) process implemented in a pilot plant as a cost-effective, green, and environmentally friendly alternative for pectin production.[18] Comparative analysis of the two methods indicates that DUF significantly enhances flux ($p < 0.05$), achieves higher pectin purity, and effectively separates the main pectin backbones. Moreover, DUF yields pectin with a higher molar mass (M_w) and reduced polydispersity (M_w/M_n) compared to the conventional AP method.[18,19]

The functionality of polysaccharides largely depends on their molecular weight and their hydrodynamic behavior in solution. The characteristics of PPs in this regard are important since they can potentially be used in the production of industrial products. The purpose of the work is to study the effect of the method of purifying the pectin hydrolysate solution extracted from apple pomace on the molecular weight and hydrodynamic properties of PPs in solution.

2. Material and methods

2.1 *Raw materials and reagents*

Apple pomace was obtained from red apples of Faizobod district, Republic of Tajikistan, by squeezing fruits and

dried at 60 °C for 6 hours. All used reagents were purchased from Sigma-Aldrich (USA). All solutions were prepared using double distilled water.

2.2 *Methods*

2.2.1 *Hydrolysis and extraction*

PP was extracted by flash hydrolysis-extraction method in an autoclave under pressure (Figure 1) for a short period.[19] Dried apple pomace is swollen in water, washed with water and hydrolyzed at 130 ℃ for 5 min with hydrochloric acid in a ratio of raw material: acid 1:20, at pH 2.0. The pressure in the autoclave is automatically controlled by a steam generator (steam generator MBA-20D, USA) under 1.5 atm (22.0 psi).

Figure 1. Flash hydrolysis system

2.2.2 *Purification and analysis*

Further isolation of PP fractions was carried out in two ways: AP and dia-ultrafiltration (DUF) purification and ultrafiltration concentration,[19] followed by isolation of PP from the solution with EtOH. The precipitation was performed in the ratio of PPs solution and EtOH 1:1, 1:2, and 1:3. The DUF purification was carried out in the cross-flow ultrafiltration system (*Krossflo*, Spectrum Labs.com, USA) in two cycles. The PPs were isolated from the concentrated solution by Acl in the same manner as before (Figure 2).

The content of galacturonic acid (GA) and the degree of its methyl esterification (DM) were analyzed according to the analytical methods.[17]

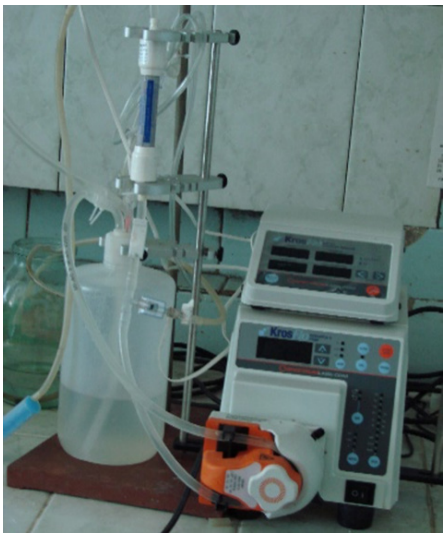

Figure 2. Krossflo DUF system

2.2.3 *Determination of molar mass and molar mass distribution*

The molecular weight (M_w) and molecular weight distribution of the pectin samples were analyzed by high-performance size-exclusion liquid chromatography (HPLC) using a high-pressure delivery system (Waters, USA), a built-in 2-channel vacuum degasser connected in series with a differential viscometer model ViscoStar (Wyatt Technology, USA), a differential refractometer (RI 2410, Waters, USA), two size-exclusion columns PL-Aquagel OH-60 and OH-40, an automatic sample injection system (717 Plus Auto Injector, Waters), see Figure 3.

Figure 3. High-performance size-exclusion liquid chromatography system

Dry pectin samples (~2 mg/ml) were dissolved in the mobile phase (0.05 M $NaNO_3$), centrifuged at 20,000 g for 30 min and filtered through a membrane filter (Millex HV 0.22 μm, Millipore Corp., USA). The flow rate and injection volume were 0.8 ml/min and 100 μl, respectively. Samples were analyzed on the same day and in triplicate.

The PPs fraction output from the column was detected sequentially using ViscoStar and RI 2410 detectors. The electronic outputs of both detectors were connected to separate serial ports of the same personal computer so that data could be simultaneously acquired and processed by ASTRA 5.3.4.20 (Wyatt Technology) and Breez (Waters) software.

The columns were calibrated using a series of Pullulan standards (Showa Denko K.K., Japan) with M_w values of 788 kDa; 667 kDa; 404 kDa; 112 kDa; 47.3 kDa and 22.8 kDa, respectively. M_w, M_n and M_z values for pectins were obtained using a universal calibration. The average value of the refractive index increment with concentration (dn/dc) was sourced from Muhidinov et al.[17] and was equal to 0.134 ml/g.

3. Results and discussion

3.1 *Influence of purification method on the value of molar mass and molecular weight distribution*

Aside from the previous study,[19] where apple pectin was obtained from apple sources in the Mumiobod region of Tajikistan using the flash method at temperatures ranging from 100 °C to 130 °C for 5-7 minutes, with purification conducted under pilot plant conditions, this study aims to investigate the detailed effects of two purification methods (alcohol precipitation and DUF methods) on the molecular weight and hydrodynamic properties of pectin obtained under distinct hydrolysis conditions (130 °C for 5 minutes) from the apple pectin of Faizobod region (APF).

Figure 1 shows the size-exclusion chromatograms (SEC) and molecular weight distribution (MWD) curves of the APF sample obtained by flash hydrolysis-extraction at 130 °C for 5 min and purified by EtOH precipitation at different ratios of PP hydrolysate solution and EtOH. The entire sample was eluted within the free/total volume of the chromatographic column. For all samples, one broad peak is visible at a volume of 12.5-18 ml. It is obvious from the MWD curves that the difference in molecular weight and their distribution for each pectin sample is very insignificant (Figure 4).

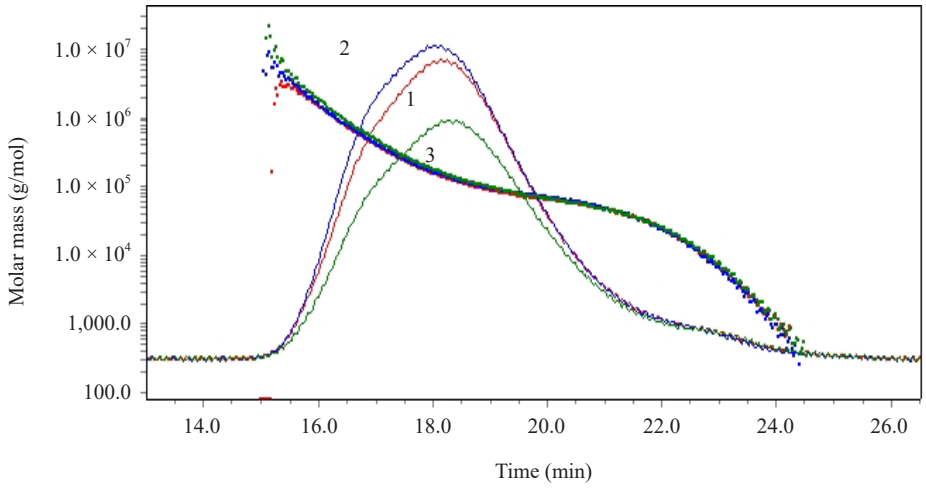

Figure 4. HPLC chromatograms and MWD curves of apple PP obtained by the flash method at 130 °C for 5 min, purified by the AP method: 1: APF-130-5 (1:1); 2: APF-130-5 (1:2) and 3: APF-130-5 (1:3)

A similar picture is observed from the chromatogram of PP samples obtained by the flash method at 130 °C for five minutes with one, two and three cycles of DUV purification (Figure 5).

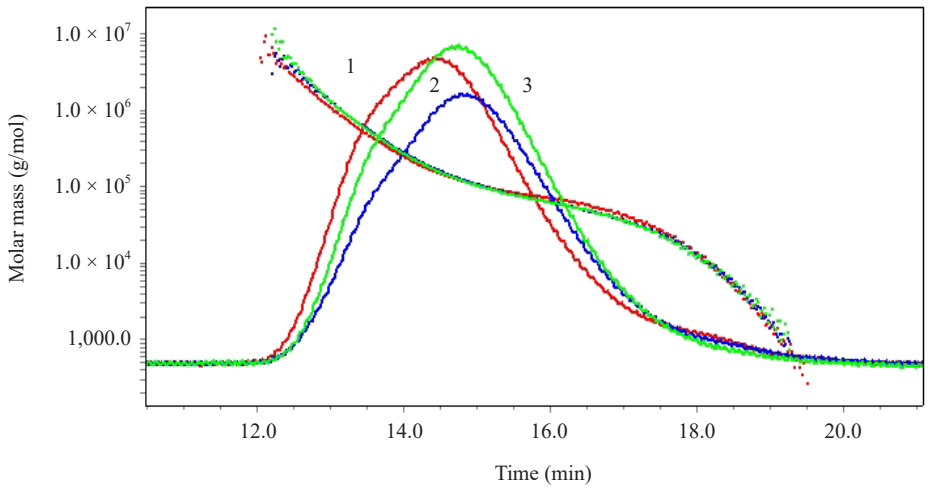

Figure 5. HPLC chromatograms and MWD curves of apple PP obtained by the flash method at 130 °C for 5 min, purified by the DUF method: 1: APF-130-5 AP 1:2; 2: APF-130-5 DUF1 and 3: APF-130-5 DUF2

The SEC profile of the PP samples chromatograms obtained by both purification methods presents a monomodal and relatively broad (10-1,000 kDa) molecular weight distribution.

Figures 6 and 7 show differential MWD curves, from which the molecular weight maxima of the PP samples purified by the AP and DUV methods are clearly visible.

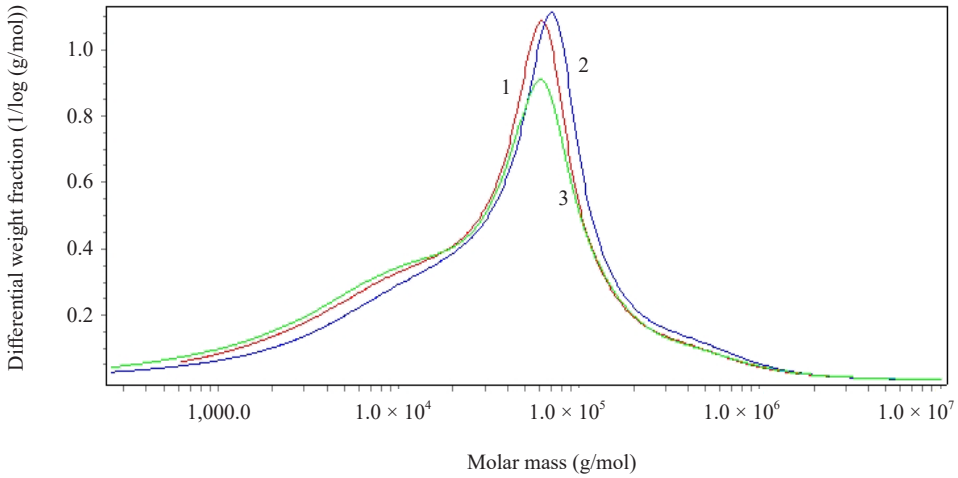

Figure 6. Differential curves of MWD of apple PPs obtained by the flash method at 130 °C for 5 min, purified by the AP method: 1: APF-130-5 (1:1); 2: APF-130-5 (1:2) and 3: APF-130-5 (1:3)

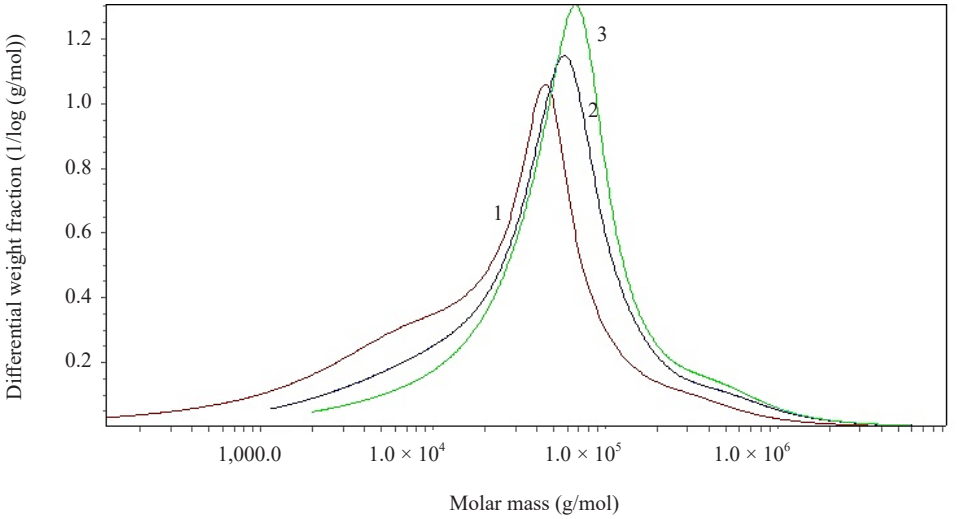

Figure 7. Comparative differential curves of MWD of apple PPs obtained by the flash method at 130 °C for 5 min and purified by the AP and DUF methods: 1: APF-130-5 AP 1:2; 2: APF-130-5 DUF1 and 3: APF-130-5 DUF2

If, in the first method, the maximum value of the molecular weight of PP occurs at a hydrolysate solution and EtOH ratio of 1:2, while in the case of DUF purification, a double cycle is considered optimal for this pectin. Moreover, DUF purification enhances the fractions with higher molecular weight (Figure 7, lines 2 and 3), thereby improving the overall quality of pectin (Table 1). The weight-average molecular weight (M_w) and the polydispersity index (M_w/M_n) of the pectin samples, determined using the ASTRA 5.3.4.20 program based on the chromatogram profiles (Figures 4 and 5), are presented in Table 1. Additionally, Table 1 includes data on the galacturonic acid (GA) content and the degree of methylation (DM) of pectin polysaccharides (PP).

From the data in Table 1, it is evident that the yield of pectin increases proportionally with the increase in the ratio of the hydrolysate solution to the EtOH; probably, self-precipitation of low-molecular fractions in the pectin gel occurs. This leads to a decrease in the content of the main component of PP, the HG fraction in pectin macromolecules. Meanwhile, with an increase in the multiplicity of the DUF process, the value of the PPs yield decreases due to the splitting off and filtration of low-molecular components (sugars, oligosaccharides, proteins, polyphenolic substances and other mineral compounds), which leads to a noticeable increase in the HG subdomains, which affect the functional

characteristics and quality of pectin as the gelling agent.

Table 1. PP yield (%), GA content (%), DM (%), microgel content (MG), molecular weight (M_w), polydispersity index (M_w/M_n) of pectin samples and their hydrodynamic properties

	PP Yield, %	GA, %	DM, %	MG, %	M_w, kDa	M_w/M_n	M_z e^{-3} Da
APF-130-5 EtOH (1:1)	13.5	69.2	80.4	15.4	97.4	10.12	757.3
APF-130-5 EtOH (1:2)	**15.7**	**67.5**	**81.3**	**17.4**	**108.6**	**15.7**	**784.9**
APF-130-5 EtOH (1:3)	20.4	63.8	80.8	22.1	99.7	16.3	1,038.0
APF-130-5 DUF1 (1:1)	12.0	67.2	82.4	12.6	119.3	7.9	1,126.0
APF-130-5 DUF1 (1:2)	**13.6**	**70.2**	**81.3**	**17.6**	**99.5**	**9.05**	**737.0**
APF-130-5 DUF1 (1:3)	17.4	65.8	80.4	34.5	67.4	6.6	433.2
APF-130-5 DUF2 (1:1)	10.3	68.6	81.4	22.1	128.1	4.6	800.8
APF-130-5 DUF2 (1:2)	**12.8**	**71.5**	**82.3**	**19.0**	**119.4**	**7.3**	**955.9**
APF-130-5 DUF2 (1:3)	15.5	70.2	81.0	21.4	76.5	6.4	855.4

Analysis of the M_w and polydispersity index (M_w/M_n) values, with the AP method, shows that with an increase in the concentration of the precipitant, i.e. the ratio of pectin hydrolysate solution and precipitant from 1:1 to 1:2 the M_w value increases from 97.4 to 108.6 kDa, and at a ratio of 1:3 it decreases to a value of 99.7 kDa. In this case, a hydrolysate solution: EtOH ratio of 1:3, the M_w/M_n index of PPs macromolecules is high (16.3), which indicates the coprecipitation of low-molecular products of hydrolysis. The amount of aggregated particles-microgels (MG) in these pectin samples is more than 15.4%, and with an increase in the hydrolysate solution, the EtOH ratio increases to 22.1%. This indicates the ability of PP chains under these conditions to aggregate through hydrogen bonds. The z value of the average molecular weight (M_z) also increases in parallel with the increase in the amount of MG, which confirms the hypothesis put forward early[20] about the relationship between the M_z value and the degree of aggregation of PPs macromolecules.

Using the DUF purification method of the hydrolysate solution, the data on the molecular parameters of PP have somewhat superior results: M_w of the PP samples from the first stage of the dialysis and ultrafiltration process (DUF1) to the second stage (DUF2) increases: 99.5 kDa and 119.4, respectively, for the PPs isolated from the solution with EtOH at the optimal solution and precipitant ratio of 1:2. And in this case, a further increase in the concentration of the precipitant during the isolation of PP from the concentrate solution (1:3) leads to a decrease in the M_w value. If the M_w value of PP samples obtained by the primary DUF process with single and triple EtOH precipitation decreases from 119.3 kDa to 67.4 kDa, at the same time, at the secondary DUF process, M_w accordingly drops from 128.1 kDa to 76.5 kDa. These observations indicate that PP macromolecules are released from low-molecular fractions of poly- and oligosaccharides by the DUF process. Moreover, the polydispersity index decreases almost 2 times, which confirms the above hypothesis. Moreover, the reliability of this statement was confirmed by the data of 1D and 2D nuclear magnetic resonance (NMR) spectroscopy obtained for apple pectin from another variety of apples on a pilot plant.[19] It is also evident from the data in Table 1 that the M_z value during the second DUF process decreases from 1126 kDa to 800 kDa, which indicates the advantage of this purification method compared to the EtOH precipitation method.[19] Consequently, the use of a high ratio of PP hydrolysate and EtOH solutions (1:3) is undesirable since it leads to a loss in pectin quality.

As was stated earlier,[13] based on the complexity of the structural organization of PP in the cell wall of plants, the process of hydrolysis-extraction of these polysaccharides is a heterogeneous reaction of parallel processes. Taking into account the heterogeneity of pectin macromolecules (linear chains HG and branched RG I),[19] it should be stated that the hydrolysis reaction occurs more intensively in branched sections of pectin chains rich in neutral sugars, and the regions

of PPs with a high content of GA (HG-region). Moreover, the PP fraction is stabilized by intermolecular bonds, either through hydrogen bonding or calcium ions, extracted in its native state, and remains practically unchanged until the end of the hydrolysis process. This makes it possible to regulate not only the hydrolysis process,[17,18] but also the purification method (extraction), which allows obtaining PPs with specified physicochemical characteristics. Thus, from the analysis of molar mass and their distribution in the process of purification of pectin hydrolysates, it was possible to find optimal conditions for obtaining PP with the desired properties.

3.2 *Influence of purification method on the value of hydrodynamic parameter of PP in solution*

Hydrodynamic properties are other important technological parameters of pectin that determine the shape and size of polymer chains in solution, affecting the characteristics of PPs in addition to M_w and MWD. These applicable parameters evaluate the quality of the final product when using pectin as a thickener and gelling agent in the food and pharmaceutical industries. The most common and simple method for determining the hydrodynamic properties of polymers is the intrinsic viscosity [η] and the diffusion coefficient in a dilute solution. [η] of a solution of a high-molecular substance has the dimension of a specific volume and serves as a measure of additional energy losses associated with the rotation of macromolecules in a flow. Owing to the SEC method in combination with a differential viscometric detector and the ASTRA software (Wyatt Technology), it is possible to determine not only the intrinsic viscosity online but also to determine the viscometric radius (R_η close to hydrodynamic radius-R_h), which provides information on the shape and size of a macromolecule in solution (Table 2).

Table 2. Values of hydrodynamic parameters of PP ([η], ml/g and R_η (w), nm)

	M_w, kDa	[η], ml/g	R_η (w), nm
APF-130-5 AP (1:1)	97.4	87.8	8.9
APF-130-5 AP (1:2)	**108.6**	**91.6**	**9.4**
APF-130-5 AP (1:3)	99.7	72.9	8.2
APF-130-5 DUF1 (1:1)	119.3	92.4	9.7
APF-130-5 DUF1 (1:2)	**99.5**	**85.3**	**9.0**
APF-130-5 DUF1 (1:3)	67.4	63.5	7.3
APF-130-5 DUF2 (1:1)	128.1	107.7	11.0
APF-130-5 DUF2 (1:2)	**119.4**	**99.6**	**10.1**
APF-130-5 DUF2 (1:3)	76.5	66.6	7.7

As can be seen from the data in Table 2, as the concentration of the precipitant increases, the value of [η] passes through a maximum and is within the range of 87.8, 91.6, 72.9 ml/g. It is followed by a change in the values of M_w (108.6; 99.5; 119.4) and the size of the molecule (R_η (w)) 8.9; 9.4 and 8.2 nm, respectively. After applying the DUF purification method, the following changes in the values of M_w, [η], and (R_η (w)) are observed: during the first DUF cycle, the value of these parameters increases, then monotonically decreases with further precipitation of PPs with an increase in the ratio of the hydrolysate to EtOH solutions; further with an increase in the DUF multiplicity, the values of [η] and R_η (w) increase significantly from 92.7 to 107.7 g/ml and 9.7 to 11.0 nm, respectively. Applying the DUF removes low-molecular substances entangled in PP chains, which positively affects their properties. Tables 1 and 2 show significantly different values of pectin quality parameters (GA content (%), DM (%), microgel content (MG), molecular weight (M_w), polydispersity index (M_w/M_n), and their hydrodynamic properties) in range of pectin solution to EtOH ratio values. From the analysis of molar weight and hydrodynamic properties, it follows that the optimal ratio of hydrolysate: EtOH

solutions is equal to 1:2, and the multiplicity of DUF in two cycles is sufficient for the effective isolation of PP from purities in obtaining pectin sample with the desired properties.[18]

The overall analysis of molar mass and hydrodynamic parameter values resulting from the AP and DUF purification methods indicates that the latter method is preferable. The optimal pectin quality parameters can be achieved through a combination of two DUF cycles and a reduced quantity of alcohol. It should be noted that isolating PP through alcohol precipitation alone requires significantly more precipitant compared to the amounts needed after DUF purification. Following DUF purification, the hydrolysate solution undergoes ultrafiltration, reducing its volume by two to three times and consequently requiring less precipitant. Therefore, the DUF method eliminates the need for large quantities of expensive and flammable alcohol and vacuum evaporation, resulting in a low-cost, green, and environmentally friendly pectin production process.

The hydrodynamic behavior results of the studied PP obtained by the ASTRA software are in good agreement with the hydrodynamic parameters of PP found by other methods.[19-22] The average size of branched pectin calculated using size separation technologies in combination with number, mass, and molecular weight sensitive detectors[22] was 7.2 nm, and most pectin samples showed a major peak around this region. Pumpkin pectin was reported to be distributed in two distinct size fractions with a peak at R_η ~ 10 nm and M_w around 70 kDa and another fraction with higher molecular weight and sizes.[20] The difference may be due to the processes of formation of these polysaccharides in the cell wall by any or all of the different species, the degree of maturity, and the method of PPs extraction.[14,23]

3.3 *Influence of purification method on shape and size of PP macromolecules*

One of the most important properties of polymer molecules is their ability to fold and change conformation from a rod-like shape to a spherical shape as the polymer chain length increases. To determine the conformational characteristics of polymer chains, various physical methods have been developed that measure the translational and rotational friction of macromolecules. These methods include viscometry, sedimentation, isothermal translational diffusion, dynamic light scattering, electrical and dynamic birefringence, among others.

The M_w, [η], and (R_η (w)) data obtained using the ASTRA software allow us to estimate the shape of the molecule based on their measured values - dependence of the hydrodynamic radius versus the molecular weight of PPs (conformational plot). The slope of this graph (Figures 8 and 9) allows us to estimate the shape of a homogeneous polymer[24] and is a measure of its compactness.

Furthermore, the slope values in this graph represent averages of all the shapes present and do not necessarily measure the molecular conformations of the individual molecules present. In the case of a heterogeneous polymer such as pectin, the relationship between slope and molecular shape is somewhat more complex, as shown in Figures 8 and 9.

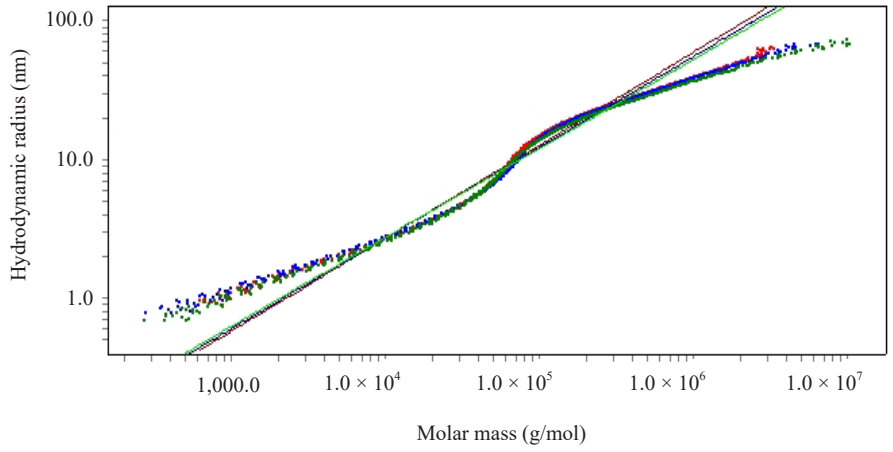

Figure 8. Dependence of the hydrodynamic radius on the molecular weight of APF-130-5 AP: APF-130-5 EtOH 1:1 (red line, b = 0.67), APF-130-5 EtOH 1:2 (blue line, b = 0.65) and APF-130-5 EtOH 1:3 (green line, b = 0.64)

Figure 8 shows a dependence of the hydrodynamic radius versus the molecular weight of PPs obtained by the flash method and purified with EtOH (APF-130-5 EtOH). The graph clearly shows that isolated PP macromolecules, depending on their hydrodynamic size, are distributed over three areas in the form of low-, medium- and high-molecular fractions. The estimated average slopes with the coefficient "*b*" are very close and equal to 0.67, 0.65, and 0.64 for PP samples extracted by EtOH precipitation at a hydrolysate solution: EtOH ratio of 1:1, 1:2, and 1:3, respectively. This indicates that in solution, PP macromolecules are predominantly in the form of segmented rods or swollen coils.

Figure 9 shows a conformational graph for PP samples obtained by the DUF purification method. The curve character is almost the same; the values of the coefficient "*b*" are equal to 0.64 for PPs purified by one cycle of DUF and 0.63 for PPs isolated by an additional DUF method. As follows from the numerical values of the coefficient "*b*" from the conformational graph, the sizes of the apple PPs macromolecule do not differ from each other.

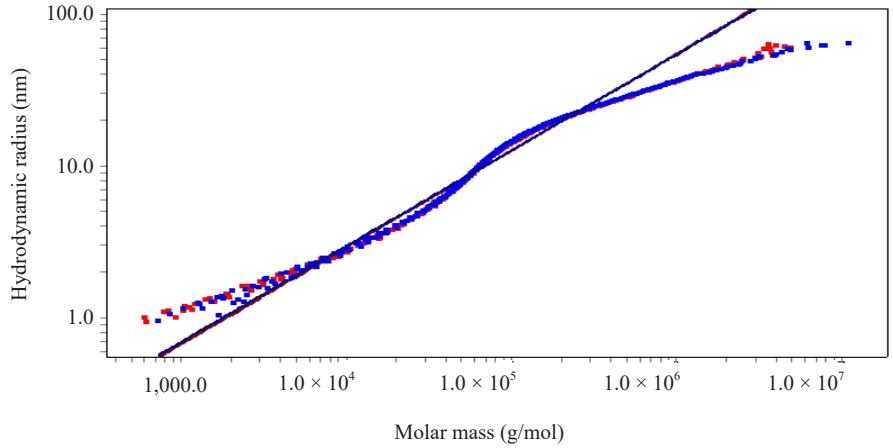

Figure 9. Dependence of the hydrodynamic radius (R_η) on the molecular weight of APF-130-5 DUF: APF-130-5 DUF 1 (b = 0.64), the second, APF -130-5 DUF 2 (b = 0.63)

3.4 *Impact of hydrodynamic parameters in PP structure-function relationship*

It is known that the molecular structure of pectin polyelectrolytes is much more complex, which can be influenced not only by the extraction mode, methods of its isolation and purification but also by the DM of carboxyl groups, the ability of macromolecules to interchain aggregation, which is important to control during production processes.[4,6,13-17,19-21,25,26]

Fishman et al.[25] for the first time, using atomic force microscopy, showed that orange PP is a mixture of spherical and linear molecules in the form of rods, curved, segmented and branched rods.[13] In addition, these linear molecules can assemble into a network, a form that is close to a compact sphere.

Recently, Alba et al.[26] have studied the influence of DM, pH, temperature and concentration on the macromolecular behavior of pectin in solution. Small-angle X-ray scattering supplemented with atomic force microscopy and molecular dynamics were used to investigate the structure, shapes and sizes of PPs in solution. Two structural forms were observed, characterized by a series-connected cluster (aggregates) of 100 to 200 nm in size. The second level of structure arises from single biopolymer chains with a radius of gyration (R_g) from ~6 to 42 nm.

The radius of gyration (R_g) and the hydrodynamic radius (R_h) are both measurements of the size of a molecule, but they differ in how they are calculated. The value of R_g and R_h for the semi rods and coiled molecules is almost the same. The value of R_g is higher for the linear polymers. The ratio R_g/R_h is inversely related to the Flory-Fox parameter and can be thought of as a measure of how well the molecule is drained by the solvent. Molecules that are easily drained have small viscosimetric radii and Flory-Fox parameters.

The development of a series of in vitro and in silico macromolecular spatial models for PP shows that the chain flexibility increases with increasing DM and at acidic pH, while hydrogen bonding is the responsible thermodynamic

driving force for cluster formation. High DM type of PPs produce structures of lower fractal dimension with less efficient packing. The numerical values of the conformational parameter "*b*" obtained in this work are in good agreement with this hypothesis for high DM apple PPs.

Neckebroeck et al.[27] also studied the emulsifying and stabilizing mechanisms of the subdomain properties of the structure of a number of PP extracts (apple, carrot, tomato and onion) depending on their structure and molecular weight. The evaluation of the emulsifying and stabilizing potential of PP samples in oil-in-water emulsion included the study of their ability to reduce interfacial tension along with the study of the storage stability of pectin-stabilized emulsions. This work showed that pectin samples extracted from plants of different origins exhibit different structural properties, which leads to different emulsification and emulsion stabilization potential. They showed that the molar mass of the polymer potentially plays an important role in its structure-function relationship. The main structural differences between these pectin samples were the DM of the pectin fractions, the hydrodynamic radius (R_h) and the M_w value. In addition, the R_h value at pH 6.0 decreased significantly (twofold) for isolated pectin fractions.

4. Conclusion

The extraction of PPs is a physicochemical process involving hydrolysis, dissolution of pectin polymers, and the diffusion of macromolecules from plant tissues under the influence of various processing parameters. Additionally, the isolation and purification stages of PPs, aimed at removing low-molecular-weight hydrolysis products from the solution, play a crucial role in ensuring the quality of the extracted compound. Understanding the physical properties of PPs, including their molar mass and hydrodynamic behavior in solution, is essential for evaluating pectin quality and optimizing technological processes. The findings indicate that the intrinsic viscosity, molecular mass, and hydrodynamic size of PP macromolecules are highly sensitive to the conditions applied during extraction and isolation.

The analysis of the influence of these parameters under various extraction and purification conditions can lead to the production of pectin with tailored properties for specific applications. The flash hydrolysis method and the efficient extraction method - DUF prevent prolonged contact of raw materials with the hydrolyzing agent and high temperatures, thereby minimizing the potential degradation of pectin molecules in the solution.

The overall analysis of molar mass and hydrodynamic parameter values resulting from AP and DUF purification methods indicates that the latter is preferable. The DUF method achieves optimal pectin quality parameters through a combination of two DUF cycles and reduced alcohol usage. Notably, isolating PP via AP alone requires significantly more precipitant compared to the DUF method. Following DUF purification, the hydrolysate solution undergoes ultrafiltration concentration, reducing its volume by two to three times and thereby requiring less precipitant. Furthermore, the DUF method avoids the need for large quantities of expensive and flammable alcohol and vacuum evaporation, resulting in the production of a high-quality product while making pectin production a cost-effective, green, and environmentally friendly process.

Despite these advancements, the functional properties of PPs remain unclear, necessitating further research to elucidate the relationship between pectin structure and function, particularly its role in plants. The quality characteristics of many plant-based food products, especially their textural and rheological properties, are heavily influenced by the pectin content and composition, as well as the type of raw material processing employed. This study has demonstrated the conformational behavior of apple pectin during its isolation from hydrolysate solution using two practical methods, addressing its industrially and biologically significant applications. These findings pave the way for the rational development of advanced biomaterials based on this valuable biopolymer.

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Author contributions

Zumratov A. Kh. conducted the experiments and processed the data. Nasriddinov A. S. provided scientific leadership and contributed to data acquisition and processing. Ismoilov I. B. developed the methodology, processed the data, and performed the technical revision of the text. Ashurov A. I. contributed to the methodology, data processing, and reviewed and corrected the manuscript. Kholov Sh. Yo. worked on the methodology and data processing. Mukhidinov Z. K. provided scientific leadership and was responsible for writing, reviewing, editing, and revisions. Khalikov D. Kh. supervised the research and contributed to conceptual design.

Conflict interest

The authors declare no conflict of interest regarding the publication of this article.

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