Characteristics and rheological properties of ammonium oxalate extracted rosehip pectin

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Abstract. Rosehip fruits are an abundant source of bioactive compounds with enormous application in food technology, because of its nutritional and healthy properties. However, isolation and characterization of rosehip pectin remained challenges, because its properties depend on extraction procedure. Therefore, this study aims to evaluate the characteristics of ammonium oxalate-extracted rosehip pectin and its rheological behavior. Dried rosehip fruits (cultivar Plovdiv 1) were used for alcohol-insoluble solids (AIS) preparation. Rosehip pectin was extracted from AIS using 0.5% aqueous ammonium oxalate. The obtained polysaccharide was characterized with a degree of esterification 68%, degree of acetylation 1.0%, anhydrouronic acid content 87% and yield 13%. FTIR spectrum confirmed the pectin structure with typical bands, especially in fingerprint region showing the presence of α-(1→4)-bond between the galacturonic acid units. To the best of our knowledge, some physicochemical properties of rosehip pectin were evaluated for the first time. The rheological studies showed the non-Newtonian, shear-thinning and weak thixotropic behavior. The rosehip pectin demonstrated good swelling properties (22 ml/g), higher water holding capacity (6.04 g water/g sample), than oil-holding capacity (2.44 g oil/g sample). The obtained results highlighted that rosehips are rich in pectin with valuable rheological properties, revealing their potential use in food technology. Key words: Rosehip fruits, pectin extraction, rheological properties.

1. Introduction

The genus Rosa (family Rosaceae) comprises about 200 species, as among them Rosa canina L., is well-known and mostly utilized one, because of its phytochemical profile and biological activity [1,2]. Rosehip fruits are a rich source of vitamins (especially vitamin C), organic acids, polyphenols (phenolic acids, flavonoids, proanthocyanidins, catechin, etc.), tocopherols, carotenoids, sugars, polysaccharides (mainly pectins), lipids (fatty acids and galactolipids), macro- and microelements [2-5]. Due to the rich phytochemical profile of its fruits, R. canina demonstrates many health promoting properties. Its fruits and extracts from them are used as antioxidant [6,7], inhibition of cancer cell proliferation, the prevention of ageing and cardiovascular, against some diseases as influenza, infections, inflammatory diseases, chronic pain, and ulcer [8].

In food technologies rosehip fruits traditionally used for infusions, beverages, jams, jellies, and marmalades production, as well as additives in yoghurt and breads [1,9,10].
Its fruits are a rich source of pectin, that improve the technological and functional properties of the final product. Therefore, the investigation about yield, extraction process and characteristics of rosehip pectin remain challenges. Ognyanov et al. [11] isolated pectin with galacturonic acid content (45.5%) and degree of methylesterification (62%) from rosehip by 1% aqueous citric acid extraction. In their study homogalacturonan is the main building block of the extracted pectin and consists of long methylesterified/acyetylated GalA sequences interspersed with small blocks of non-methyl-esterified GalA units [11]. Taneva et al. [12] also reported for highly esterified pectin obtained by oxalate extraction from wild growing and cultivated rosehip fruits (peel and seeds) [12]. However, the information about some physicochemical and rheological properties of rosehip pectin is still not full, missing or unavailable. Therefore, the aim of the current study is to evaluate the characteristics of ammonium oxalate-extracted rosehip pectin and its rheological behavior.

2. Material and Methods

2.1. Plant material
Fresh rosehip fruits cultivar Plovdiv 1 were collected from Pomorie (Bulgaria) during the autumn of 2016. The plant material was dried at 40°C and used in a dry state for pectin isolation. The moisture content of rosehip fruits was determined by drying at 105°C for four hours. The moisture content of initial rosehip fruits was 42.25%, while in dry fruits moisture content was 8.90%. All other reagents and solvents were of the analytical grade and they used without further purification.

2.2. Isolation of pectin from rosehip
The dried rosehip pulp and cover were separated from seeds and they were finely ground in laboratory homogenizer in particle size between 0.2 to 0.5 mm. The ration between pulp and seeds was 58%:42% dw. The ground material was successively extracted with petroleum ether, n-hexane and finally with 95% ethanol in a Soxhlet extractor to remove pigments and waxes. The residue was dried and then it was used for preliminary washed with 70% ethanol acidified with 2% hydrochloric acid to obtain alcohol-insoluble solids (AIS). The extraction of pectin from AIS was performed as previously described procedure using 0.5% ammonium oxalate extraction [12].

2.3. Characterization of rosehip pectin
Degree of esterification and anhydrouronic acid content were determined by titration methods [13]. Degree of acetylation was determined by the hydroxamic acid reaction using β-D-glucose pentaacetate as a standard [14].

2.3.1. Homogeneity and molecular weight of rosehip pectin
The analysis was performed on an HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) with a column Shodex OH pack 806 M (ID 8×300 mm), (Shodex Co., Tokyo, Japan) operating at 30°C and an RI detector (VWR Hitachi Chromaster, 5450, Japan). The elution was performed with the mobile phase 0.1 M NaNO₃ with a flow rate of 0.8 ml/min [15]. Rosehip pectin (5 mg/ml) was filtered through 0.45 μm filter PTFE45/25 mm (Isolab, Germany), and it was injected. The standard curve was built with the different pullulan standards with known molecular weight (P-5, P-10, P-20, P-50, P-100, P-200, P-400, and P-800, Showa DENKO, Japan) and it was used for calculation.

2.3.2. Fourier transformed-infrared (FTIR) spectroscopy
The FT-IR spectrum of rosehip pectin was recorded on a Nicolet FT-IR Avatar Nicolet (Thermo Science, USA) in KBr pellet in the range of 4000–400 cm⁻¹ with a resolution of 4 cm⁻¹ after 132 scans. The absorption was reported in wavenumbers (cm⁻¹).
2.4. Functional properties

2.4.1. Swelling properties
Swelling properties of rosehip pectin were analyzed as follows: 100 mg pectin sample was hydrated with 10 ml distilled water in a calibrated cylinder (1.5 cm in diameter) at 25°C. The bed volume was recorded after 18 h and the results were expressed as volume per g pectin dry weight [16].

2.4.2. Water-holding capacity (WHC) and oil-holding capacities (OHC)
Rosehip pectin (100 mg) was weighted into a tared 50 ml polypropylene centrifuge tube, then 10 ml deionized water or sunflower oil was added, respectively. The test tubes were closed tightly and then they were vigorously mixed. The samples were stored for 24 h at 20°C before centrifuging at 3500 rpm for 15 min. The excess of water or oil was decanted and the tubes inverted for 1 h at 20°C. The tubes were weighed and dried at 105°C to the constant weight. The WHC was expressed as the grams water held by 1 g of the sample. For OHC the results were expressed as the grams of oil held by 1 g of the sample [17].

2.5. Rheological analyses

2.5.1. Flow curves
Flow curves of rosehip pectin solution were carried out according to the procedure reported by Krystyjan et al. [18]. The water solutions of pectin int the concentrations of 0.5%; 1.0%; 2.5%, and 5% (w/w) was mixed at 25°C for 24 h. The RS6000 (Gebrueder Haake GmbH, Karlsruhe, Germany) rheometer in CR mode with a CC26 Ti measuring system and 1.9 gap, at 25°C was used. The rate of shear was raised from 0 to 100 s⁻¹, over a 5-min period and a subsequent decrease in the shear rate from 100 to 0 s⁻¹, also over 5 min. The flow curves obtained were described by Ostwald de-Waele rheological models:

\[ \tau = K \cdot (\dot{\gamma})^n \]

Where: \( \tau \) is shear stress (Pa), K is a consistency coefficient (Pa·sⁿ), \( \dot{\gamma} \) is shear rate (s⁻¹), and \( n \) is flow behavior index (-). The areas of hysteresis loops were calculated using RheoWin software.

2.5.2. Time sweep test
A time sweep test was carried out by the used parallel plates PP60 Ti, in 2-mm gap as a geometry of the system. All measurements were performed in the range of the linear viscoelastic region and at a frequency of 1 Hz. The temperature of the experiment was 25°C and time 10 min.

2.6. Statistics
The experimental data were subjected to an analysis of variance, at the confidence level of \( p=0.05 \), using Statistica v. 8.0 software (Statsoft, Inc., Tulsa, OK, USA). Fisher test was used for the determination of statistically significant differences.

3. Results and discussion

3.1. Characterisation of rosehip pectin
Physicochemical characteristics of pectin extracted from the rosehip fruits of cultivar Plovdiv 1 by ammonium-oxalate extraction were summarized in table 1. Rosehip pectin was obtained in a yield 13% with a high degree of esterification (68.1%) and low degree of acetylation (1.0%). The Anhydrouronic content was also high (87.6%). Similar to our reports for high degree of esterification were reported [11,12], however, in ammonium-oxalate extraction the degree of acetylation of rosehip pectin was ten times lower than 1.0% citic acid extracted one [11]. The resulting rosehip pectin characterized as highly methyl esterified as apple, orange and lemon pectin with a close degree of esterification (63-69%) and
low degree of acetylation below 2% [19]. These characteristics should influence the functional properties of rosehip pectin as potential good gelling agent and weak emulsifier.

**Table 1.** Characterization of rosehip pectin.

| Characteristics                          | Rosehip pectin |
|------------------------------------------|----------------|
| Yield, %                                 | 13.01±0.60     |
| Degree of esterification, %              | 68.12±0.50     |
| Anhydrouonic content, %                  | 87.67±1.21     |
| Degree of acetylation, %                 | 1.00±0.20      |
| Weight molecular weight (M_w), kDa       | 218.74         |
| Number molecular weight (M_n), kDa       | 189.16         |
| Polydispersity index (M_w/M_n)           | 1.16           |
| Swelling properties, ml/g                | 22.00±0.10     |
| Water-holding capacity, g water/g sample | 6.04±0.11      |
| Oil-holding capacity, g oil/g sample     | 2.44 ±0.05     |

Moreover, some functional properties of rosehip pectin were evaluated (table 1). The swelling properties of rosehip pectin were 22 ml water/g sample and these values showed its ability to absorb water. The obtained results were comparable with the reported data for citrus pectin (19 to 21 ml/g sample) [20]. The water holding capacity of rosehip pectin was better than its oil-holding capacity. The similar trend was reported previously in leek and fumaria pectin [21,22]. Oil holding capacity of rosehip pectin (2.44±0.25 g oil/g) was in a good agreement with reported values for water-soluble polysaccharides from *Rosa roxburghii* Tratt fruits (3.29±0.38 g oil/g) [20] and pectin from fumaria [21].

3.2. Homogeneity and molecular weight of rosehip pectin

The molecular size of the isolated rosehip pectin was evaluated by high-performance liquid chromatography-size-exclusion chromatogram (HPLCSEC), as the weight average (M_w) molecular weights, and polydispersity index (M_w/M_n) were determined. The elution profiles of the rosehip pectin revealed a rather broad peak beginning at 8.3 min (figure 1). M_w distributions representing populations of polymers of high molecular weights. The polydispersity index (M_w/M_n) was calculated as well (1.16) (table 1). HPSEC elution pattern also suggested the appearance of additional small peaks, which eluted after the main peak at retention time between 13.0 min and 16.5 min. These peaks could be ascribed to small nondialyzable HG sequences (large oligomers) or neutral oligomers. Our results were close to previous report for ammonium oxalate extracted pectin from fumaria [21].

![Figure 1](image_url)

**Figure 1.** High-performance liquid chromatography-size-exclusion chromatogram (HPLCSEC) of molecular weight distribution of rosehip pectin.

In comparison with previous reports for different molecular weight populations in the range of 10–100 kDa for rosehip pectin isolated by 1% aqueous citric acid extraction [11], our study demonstrated...
higher values for molecular weight after ammonium oxalate extraction (table 1). Therefore, the extraction under acidic condition could lower the molecular weight of the polymer.

3.3. FTIR spectroscopy
In the FTIR spectrum of rosehip pectin the typical bands for pectin were observed (figure 2). A broad band at 3400 cm\(^{-1}\) was assigned to stretching of hydroxyl groups, an absorption at 1743 cm\(^{-1}\) was due to C=O stretching vibration of methyl-esterified carboxyl groups, the absorption at 1637 cm\(^{-1}\) was caused by C=O stretching vibrations of ionic carboxyl groups. In addition the bands at 1236 cm\(^{-1}\) that correspond to the acetyl (–COCH\(_3\)) groups. The bands at 1145, 1105 and 1016 cm\(^{-1}\) indicated the pyranose structure. The band at 920 cm\(^{-1}\) was assigned to \(\rho(CH_3)\) methyl group in complex ester, the bands 888 cm\(^{-1}\) and 760 cm\(^{-1}\) were due to \(\alpha\)-D-galactopuranosyl units in \(\delta_1\) conformation, while 833 cm\(^{-1}\) was typical for ring vibration. Similar bands were reported early for 0.25% oxalate extracted rosehip, celery and fumaria pectin [11, 23, 24]. Other bands at 667, 619, 535, 514, 502 cm\(^{-1}\) were in accordance with the vibrations of pyranose rings [25]. Moreover, some typical bands were reported in the FTIR spectrum of Romanian rosehip fruits [26].

![Figure 2. FTIR spectrum of ammonium oxalate extracted rosehip pectin.](image)

3.4. Rheological studies
The flow curves of rosehip pectin solutions were shown (figure 3), while table 2 presented the parameters of the rheological model, that was fitted to the flow curves. The area of the hysteresis loop was also calculated. The flow curves of pectin solutions were typical for non-Newtonian fluids, shear thinning, the viscosity of which decreased with increasing shear rate [18, 22, 27, 28]. In the case of the rosehip pectin solutions, an increase in the value of the shear stress was observed with the increase in the pectin concentration in the mixture. In the range of pectin concentration from 0.5 to 2.5%, the increase of shear stress was small, only at 5% concentration there was a sharp increase of this parameter. In order to precisely characterize the properties of the tested solutions, the Ostwald de-Waele rheological model was adapted to the obtained flow curves. The flow index \(n\) and the consistency coefficient \(K\) were determined (Table 2). The values of the determination coefficient \(R^2\) were high, close to unity, which proves a good fit of the rheological model to the obtained flow curves (table 2). In turn, the flow index (\(n\)) describes the deviation from the Newtonian fluid, for which it takes the value equal 1. If the \(n>1\) fluids are shear-thickening, while \(n<1\) fluids are pseudoplastic (shear-thinning) [28]. The values of the flow index of 0.5 and 1.0% solutions of rosehip pectin were at the same, close to unity level (0.96), proving a slight deviation from the Newtonian flow. At higher pectin concentrations, the value of flow index decreased and reached the value of 0.56 at the highest pectin concentration. It confirmed their shear-thinning nature. Such behavior has also been noticed for pectin isolated from tamarillo fruit [29] and leek pectin [22].
The K parameter is a measure of the viscosity of the fluids, the higher is the value, the greater the viscosity of the tested substances. The consistency coefficient of rosehip pectin solutions increased with increasing polysaccharide concentration in the mixture (table 2). At a low concentration of pectin in the solutions (0.5-2.5%), the differences were small, hence their viscosity was low. There was a clear increase in the viscosity of the mixture above the concentration of 5%. According to Guimarães et al. [30] and Ognyanov et al. [22] at low concentration of pectin, homogeneously dispersed particles do not interact with each other because the distance between them is too great. As the pectin concentration increases, the viscosity increases due to the increase in hydrogen bonding with the hydroxyl groups, as well as the distortion in the velocity pattern of the liquid of hydrated molecules [30].

Figure 3. Flow curves of rosehip pectin in different concentrations.

Table 2. Rheological parameters of rosehip pectin solutions at 0.5-5.0% concentrations.

| Concentration of pectin (%) | K (Pa·s^n) | Ostwald de-Waule model | R^2       | Area of hysteresis loop (Pa/s) |
|-----------------------------|------------|------------------------|-----------|-------------------------------|
| 0.5                         | 0.01 ± 0.00^b | 0.96 ± 0.00^a         | 0.9998 ± 0.0000 | 0.25 ± 0.01^b |
| 1.0                         | 0.16 ± 0.05^b | 0.96 ± 0.00^a         | 1.0000 ± 0.0000 | 0.82 ± 0.00^b |
| 2.5                         | 0.24 ± 0.05^b | 0.84 ± 0.00^b         | 1.0000 ± 0.0000 | 0.77 ± 0.21^b |
| 5.0                         | 7.71 ± 0.00^a | 0.55 ± 0.00^c         | 0.9999 ± 0.0001 | 291.00 ± 2.83^a |

Parameters in columns denoted with the same letters (a, b, etc.) do not differ statistically at the level of confidence p=0.05.

The results in the area of the hysteresis loops of the rosehip pectin solutions were presented in table 2. It was observed that samples with low concentration of pectin showed weak thixotropic properties, that increased with the solid concentration increases (table 2). Due to shearing, weak physical bonds break, which affects the breakdown of the internal structure. When the shear forces are released, the damaged structure is rebuilt. The rate of structure recovery is higher when the amount of forming bonds is significant [31]. From a technological point of view, thixotropy is of great importance in the food industry, as it is related to the formation and destruction of the internal structure of medium, and therefore indicates the level of mixture stability.
Figure 4 shows the modulus of elasticity $G'$ and viscosity $G''$ of 5% rosehip pectin solution. The mechanical modules remained constant, furthermore loss modulus ($G''$) was larger than the modulus of elasticity ($G'$) over the entire time range. According to Steffe [32] such tendency point to dilute solution behavior, as well as the stability of the tested mixture over time. Moreover, research proves that the system is not a gel. As claimed Oakenfull and Scott [33] the main interactions that keep the gel structure of pectin is hydrogen bonding, however, it is insufficient to overcome the entropic barrier to gelation. The pectin gelation process depends on many factors. In case of highly methyl-esterified pectin gelling structure is forming at acidic medium (pH<3.5) and at a significant amount of sugar by hydrophobic interactions and hydrogen bonding. In the case of low-esterified pectin gelation occurs primarily in the presence of Ca$^{2+}$ ions through the formation of "egg-box" junction zones with the free carboxylic groups from the independent pectin chains [22].

4. Conclusion
In the current study the physicochemical properties and rheological behavior of rosehip pectin obtained by ammonium oxalate extraction were evaluated. Isolated rosehip pectin characterized as high molecular with a high degree of esterification and low degree of acetylation. To the best of our knowledge this is the first report about swelling properties, water holding capacity, oil-holding capacity and rheological behavior of its aqueous solutions. The obtained results characterized rosehip pectin with good swelling properties, better oil holding capacity, and its behavior as non-Newtonian and weak thixotropic effect. All these results demonstrated the future potential of the application of rosehip pectin in food technology and pharmacy for development of functional foods and neutraceuticals with improved rheological properties.

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