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Research Article

Isolation and Characterization of Pectic Polysaccharide Fraction from *In Vitro* Suspension Culture of *Fumaria officinalis* L.

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In the current study, an acidic polysaccharide from the *in vitro* suspension culture of *Fumaria officinalis* L. was obtained by extraction with 0.8% (w/v) aqueous ammonium oxalate. The polysaccharide fraction mainly consisted of galacturonic acid (41.0%), followed by galactose (7.3%) and arabinose (5.6%). This suggests the presence of arabinogalactan side chains in the rhamnogalacturonan-I segment of the studied pectin, which was mainly built up by homogalacturonan segments. The pectin was evaluated as low-methyl-esterified (45.0%) with degree of acetylation 3.4%. The polymer fraction was consisted of different molecular weight populations in the range of 6–600 kDa. The high amount of 4-L-hydroxyproline (11.7% of total protein) and the specific positive reaction to Yariv's phenylglycoside reagent indicated the presence of an arabinogalactan protein in the cell walls. The functional properties of the polysaccharide fraction were evaluated, as it possessed better water-holding capacity than oil-holding capacity. The studied pectin demonstrated significant foaming ability and promising emulsifying properties in a concentration 1%. Therefore, the isolated polysaccharide fraction could be successfully used as emulsifier and foaming agent in food products and pharmaceutical supplements.

1. Introduction

Fumaria officinalis L., known as common fumitory, drug fumitory, or earth smoke, is a plant of *Fumaria* genus that belongs to the Fumariaceae family. It is widely distributed in particular regions of Europe (e.g., the Balkan Peninsula, Mediterranean region). In Bulgarian flora, the genus is represented by 10 species [1]. In traditional folk medicine, common fumitory is mostly used as antihypertensive, diuretic, hepatoprotectant, and for treatment of skin rashes [2].

The presence of isoquinoline alkaloids, the most important of which is protopine, is mainly associated with the biological activity of *Fumaria* spp. In a previous study, the protocol for *Fumaria* spp. callus culture preparation was developed, and the possibility for protopine production was evaluated [1, 3]. Additionally, some of the factors affecting callus induction were also studied [3]. The biological activity is associated not only with alkaloids, but polyphenol components are also found to affect the activity. In a previous study, the total polyphenol content and antioxidant activity of five Bulgarian *Fumaria* spp., including *F. officinalis* L., were evaluated [4]. Moreover, detailed descriptions of pharmacological activities of polysaccharides isolated from herbs were subject of many reports [5, 6]. Most of these activities have been observed in pectic polysaccharides and hemicelluloses. Therefore, the polysaccharides are very important active ingredients in the cell walls, which are responsible for the biological activity. However, information about the presence

and chemical composition of the polysaccharides constituting the primary cell wall are missing. In our view, special attention must be paid to the pectic polysaccharides.

Generally, pectic substances are physiologically active structural polysaccharides of the plant cell wall, which play an important role in the middle lamella. To date, it is widely accepted that the pectic polysaccharides are composed of homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) regions and highly complex rhamnogalacturonan-II (RG-II) fragment. HG consists of an unbranched molecule composed of α-(1 → 4)-linked D-galacturonic acid (D-Gal*p*A) [7]. The Gal*p*A residues can be methyl-esterified at *C*-6 and carry acetyl groups at *O*-2 and/or *O*-3. RG-I is a side-chain-containing polysaccharide, whose backbone consists of [→4-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1→] disaccharide repeating units [7]. The side chains in the RG-I region are mainly composed of arabinans, galactans, and/or arabinogalactans of varying length and composition [8]. RG-II has a HG backbone substituted by four side chains (defined as A–D), consisting of different sugars, including uronic acids, rhamnose, and rare monosaccharides such as apiose, 2-*O*-methyl-fucose, 2-*O*-methyl-xylose, aceric acid, 3-deoxy-D-*manno*-2-octulosonic acid (Kdo), and 3-deoxy-D-*lyxo*-heptulosaric acid (Dha) [9]. The structure of pectic substances is extremely complex and depends on factors such as raw material origin and extraction conditions used [10]. The best-known uses of pectin are mainly in the food industry as gelling, thickening, and stabilizing ingredients [11]. Because of this, the study of some physicochemical characteristics and rheological properties are of great importance and are required for a further successful application.

Therefore, the current study describes the isolation of a pectic polysaccharide fraction from the walls of suspension-cultured cells of *Fumaria officinalis* L., its primarily chemical characterization, and some physicochemical properties as well.

2. Materials and Methods

2.1. Plant Material. F. officinalis L. plants were collected in May 2013 from their natural habitats near Sozopol, the Black sea region, Bulgaria. The plants were identified by the references of the Herbarium of the Institute of Biodiversity and Ecosystem Research in Sofia (SOM) and the Herbarium of the University of Sofia "St. Kliment Ohridski." A voucher specimen of *F. officinalis* L. (SOM 1030) was deposited in SOM [12].

2.2. Callus Induction. F. officinalis L. callus induction was initiated on a Murashige and Skoog medium and supplemented with sucrose and plant growth regulators 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine, as previously described [1]. The obtained calli separated from explants were cultivated in petri dishes with a subculturing period of 21 days.

2.3. Suspension Cultures. Suspension cultures were obtained from selected callus lines of *Fumaria officinalis* L., as previously described [1]. The suspension liquid was filtered from the suspension culture and then the culture was freeze-dried.

2.4. Alcohol-Insoluble Solid Preparation. Before isolation of the polysaccharide, the alkaloids (mainly protopine) were removed from the lyophilized biomass by 95% (v/v) ethanol extraction, as previously described by Georgieva et al. [1]. The residual biomass was lyophilized and then used for the further isolation of the alcohol insoluble solids (AIS). AIS were obtained as follows: 250 g of lyophilized residual biomass was stirred overnight with 5 L 95% (v/v) ethanol, acidified with 10 mL conc. HCl, then vacuum filtration was performed and the residue was washed with 95% (v/v) ethanol to neutral pH value. The obtained residue was dried and used for further polysaccharide isolation.

2.5. Polysaccharide Isolation. AIS from the dried suspension culture *F. officinalis* (35 g) were extracted with 1 L 0.8% (w/v) aqueous ammonium oxalate by a continuous stirring for 1 h at 75°C on a magnetic stirrer. The obtained extract was filtered through a nylon cloth, and the residue was extracted again under the abovementioned conditions with 600 mL 0.8% (w/v) aqueous ammonium oxalate. Both filtrates after chelating extraction were combined, cooled down, and then precipitated by the addition of 1.2 L cold 95% (v/v) ethanol. The resulting precipitate was collected by filtration. Further purification of the fraction was done by redissolution of the precipitate in distilled water and centrifugation. The clear supernatant was dialyzed at 4°C (72 h, MWCO 3500 Da) against distilled water and then it was filtrated and freeze-dried to yield the purified fraction.

2.6. Monosaccharide Composition. The pectic polysaccharide was analyzed for its neutral sugar composition by gas chromatography [13], using inositol as an internal standard. The samples were treated with 72% (w/w) H_2SO_4 for 1 h at 30°C, followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100°C. The released neutral sugars were converted to volatile alditol acetates prior to be analyzed by gas chromatography [14].

2.7. Uronic Acid Content. The total uronic acid content of pectin fraction was determined by an automated *m*-hydroxydiphenyl assay using an autoanalyser Skalar San^{++} system (Skalar Analytical BV, Breda, Netherlands) controlled by FlowAccessV3 software (version 3.1.18), and galacturonic acid (12.5–100.0 μg/mL) was used for calibration curve construction [15]. The uronic acid content of AIS material was estimated as described by Ahmed and Labavitch [16]. In brief, the AIS sample was solubilized by dispersing in 72% (w/w) H_2SO_4 for 1 h at 30°C, followed by a hydrolysis step with 1 M H_2SO_4 for 3 h at 100°C. An aliquot of hydrolyzate was taken for the analysis. Differential determination of GalA and GlcA was carried out by the 3,5-dimethylphenol colorimetric method according to Scott [17], as GalA and D-glucurono-6,3-lactone were used as standards.

2.8. Protein and L-Hydroxyproline Content. Protein content was analyzed by Bradford's assay using bovine serum

albumin as a standard [18]. Hydroxyproline was assayed following the method of Kivirikko and Liesmaa [19] after hydrolysis of the sample (5 mg) at 110°C with 6 M HCI in sealed tubes for 16 h. *cis*-4-L-Hydroxyproline was used as a standard. The reagent preparation and methodology were carried out as described by York et al. [20].

2.9. Total Phenol Content. Total phenols were determined with the Folin-Ciocalteu reagent using ferulic acid as a standard [21].

2.10. Degree of Methyl-Esterification (DM) and Degree of Acetylation (DAc). Pectin sample (1 mg/mL) was saponified with 0.5 M NaOH, and after neutralization, the released methanol was quantified using combined enzymatic/colorimetric method as described elsewhere [22]. DAc was determined by the hydroxamic acid reaction method, using β-D-glucose pentaacetate as a standard [23].

2.11. High-Performance Size-Exclusion Chromatography (HPSEC) Analysis of Molecular Weight Distribution. The separation was performed on a HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) system equipped with a column Shodex OH-pack 806 M (i.d. 8 mm × 300 mm), (Shodex Co., Tokyo, Japan) and a refractive index detector (VWR Hitachi Chromaster, 5450, Japan). The sample (3 mg/mL dissolved in 0.1 M $NaNO_3$) was injected (20 μL) and eluted with aqueous 0.1 M sodium nitrate solution at a flow rate of 0.8 mL/min. The column was maintained at 30.0 ± 0.1°C [24]. The samples were passed through a 0.45 μm syringe filter, PTFE 45/25 mm (Isolab, Germany) before injection. The standard curve built with 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) was used for calculation.

2.12. Yariv Test. The presence of arabinogalactan proteins (AGPs) was detected by the single radial gel diffusion test as described by Van Holst and Clarke [25].

2.13. Qualitative Test for 3-Deoxy-D-Manno-2-Octulosonic Acid (Kdo). The qualitative estimation of polysaccharide-linked Kdo was performed by the periodate-thiobarbituric acid colorimetric assay described by Karkhanis et al. [26]. A modified method of York et al. was used [27]. In brief, the sample (2 mg) was firstly hydrolyzed for 30 min at 95°C in 1 mL of 0.1 M H_2SO_4. The hydrolysate was cooled into an ice water bath and then it was centrifuged for 10 min at 4°C (14,000 rpm). Further, sodium metaperiodate solution (40 mM in 62.5 mM H_2SO_4, 0.25 mL) was added to the supernatant (0.5 mL), and the mixture was allowed to stand for 20 min at room temperature. The oxidation was stopped by adding sodium sulfite solution (2.0% in 0.5 M HCl, 0.3 mL). The sample was vortex-mixed till the disappearance of brown color and after that freshly prepared aqueous 2-thiobarbituric acid solution (0.6%, 0.5 mL) was added. The mixture was incubated in a boiling water bath for 15 min and immediately after that DMSO (1 mL) was added to each test tube. After cooling to room temperature, the absorbance of the pink color was measured at 548 nm against a reagent blank prepared with distilled water instead of sample hydrolysate. Lavender polysaccharide PSC-2 fraction and chPS-L2 subfraction were used as positive control [28].

2.14. Fourier Transform Infrared (FTIR) Spectroscopy. The sample (4 mg) was mixed with spectroscopic grade KBr and then was pressed into a pellet. FT-IR spectrum was collected on a Nicolet Avatar 330 (Thermo Electron Corp., USA) spectrometer. The spectrum was recorded over a wavenumber range of 4000–400 cm^{-1} at 132 scans with a spectral resolution of 4 cm^{-1}.

2.15. Physicochemical Properties

2.15.1. Viscosity Determination. Kinematic viscosity (υ, $mm^2 \cdot s^{-1} = cSt$) was measured with a Ubbelohde capillary viscometer (Ref. no. 513 10), Capillary I with constant K printed on the viscometer ($K = 0.01607$) and it was calculated by the following equation:

$$\upsilon = K(t - \vartheta), \tag{1}$$

where K is constant; t is the average flow time, s, and ϑ is the Hagenbach correction for t, s.

2.15.2. Swelling Properties. The swelling properties of the pectin were evaluated as previously described by Robertson et al. [29]. In brief, the dried pectin (100 mg dry weight) was hydrated in 10 mL distilled water in a calibrated cylinder (1.5 cm diameter) at room temperature. After equilibration (18 h), the bed volume was recorded and expressed as volume·g^{-1} original substrate dry weight.

2.15.3. Water-Holding and Oil-Holding Capacity. The water-holding and oil-holding capacities of the pectin were determined in duplicate as described [30]. The samples (100 mg) were placed into preweighed 50 mL polypropylene centrifuge tubes and 10 mL deionized water or sunflower oil was then added. The tube was tightly closed, and the contents were vigorously mixed. They were held for 24 h at 20°C before centrifugation at 3500 rpm for 15 min; the excess water or oil was decanted, and the tubes were inverted for 1 h at 20°C. The tubes were then weighed and dried at 105°C to constant weight.

2.15.4. Foam Ability and Foam Stability. The foaming properties of the isolated pectin were studied by a stirring/shaking method [31]. The series of different concentrations of pectin solutions were prepared (0.5; 1.0, 1.5, and 2.0% w/w). All foam tests were performed in duplicate. Reproducibility of the results was typically expressed as mean ± 10%. The foam ability (FA, %) was determined as an aliquot of 15 mL pectin solution whipped in a graduated 50 mL cylinder by hand for 60 s. The foam ability was determined by volume increase (%) immediately after shaking and was calculated by

$$\text{FA, \%} = \frac{(V_1 - V_2)}{V_1} \times 100, \tag{2}$$

where V_2 is the volume of pectin solution immediately after whipping, and V_1 is the volume of solution before whipping.

The foam stability (FS, %) is characterized by the volume of entrapped air, still remaining in the foam after a certain period of time, $t > 0$. The foam stability was defined as the volume of the foam that remained after 60 min at room temperature (20°C) and was expressed as a percentage of the initial foam volume. The test was performed as described by Ivanov et al. [32]. The foam stability was given by the parameter percentage volumetric foam stability

$$\text{FS, \%} = (V_{1\text{foam}} - V_{0\text{foam}}), \quad (3)$$

where "$V_{0\,\text{foam}}$" is the volume of the formed foam; "$V_{1\,\text{foam}}$" is the volume of the foam change with the time (t). Foam stability over time was assessed by measuring the foam volume from 1 to 60 min.

2.15.5. Emulsion Properties. The emulsion capacity and emulsion stability of the 50/50 water in oil model emulsion systems, containing the studied pectin, were evaluated [31]. The concentration of studied pectin was 1.0% w/w. It was dissolved with 0.15 M NaCl by stirring on a magnetic stirrer at 45°C to 50°C for 2 hours. The concentration of the oil phase was 50% w/w. Sunflower oil was purchased from the local market. Twenty milliliters of pectin solution (1.0%, w/w) was homogenized with 20 mL sunflower oil for 5 min at 10000 rpm by using a homogenizer (Ultra Turrax IKA T18 Basic, Germany).

Centrifugal Test. The emulsion stability was evaluated by centrifugation at 3000 × g (Hettich EBA 20, Germany) for 20 min. The height of the emulsified layer was measured. The emulsifying ability (EA) was calculated as a ratio of the height of the emulsified layer and the height of the total content of the tube and presented in percentages (%):

$$\text{EA}(\%) = \frac{\text{EPV}_1}{\text{WV}_1} \times 100, \quad (4)$$

where EPV_1 is the volume of the emulsion phase, and WV_1 is the total volume of the system.

Turbidimetric Method. The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined as modified by Diniz et al. [33]. The emulsions (1 g) were transferred in volumetric flasks at 0 and 5 min after homogenization and diluted 100-fold. The absorbance of the diluted solutions was measured at wavelength 540 nm using Camspec-M, 107, the UK spectrophotometer. For calculation of the emulsifying activity index (EAI) and emulsion stability (ESI), the absorbance values measured immediately after agitation (A_0) and 5 min (A_5) after emulsion formation were used. The emulsifying activity index ($m^2 \cdot g^{-1}$) and emulsion stability index (min) were calculated with

$$\text{EAI} = \frac{2 \times 2.303 \times A_0 \times D}{c \times l \times (1 - \varphi) \times 1000}, \quad (5)$$

Table 1: Yield and chemical characterization of ammonium oxalate-extracted polysaccharide fraction from suspension culture of *Fumaria officinalis* L. (g/100 g dw)[a].

Yield (g/100 g dw)	7.0
Protein	4.1
Hyp	0.48 (11.7)[d]
Total phenols	0.3
Total sugars	71.0
Uronic acids	45.6
GalA	41.0 (61.0)[b]
GlcA	—
Neutral sugars	25.4
Rhamnose	2.0 (3.1)[b]
Galactose	7.3 (10.5)
Arabinose	5.6 (9.5)
Glucose	7.6 (11.0)
Mannose	0.3 (0.4)
Fucose	0.3 (0.5)
Xylose	2.3 (3.9)
Degree of methylation	45.0[c]
Degree of acetylation	3.4[c]

[a]Values are the average of two replicates. [b]Values in brackets represent the sugar composition in mol%. [c]Moles methanol or acetyl per 100 moles of galacturonic acid. [d]Hyp content as part of total protein content.

$$\text{ESI} = \frac{A_0}{A_0 - A_5} \times t, \quad (6)$$

where c is the initial concentration; φ is the volume fraction of oil used in the emulsion (0.50); D is the dilution factor used (100); t is 5 min, and A_0 and A_5 are the diluted emulsion absorbance at times (0 and 5) in min.

Microscopic Test. For microstructure determination of the prepared emulsion, microscope system (microscope A. KRÜSS OPTRONIC, Germany, equipped with a USB-camera connected to a personal computer) was used. For this purpose, the emulsion was placed on a glass slide and its structure was observed at two magnifications: 400x and 1000x.

3. Results and Discussion

3.1. Isolation and Characterization of Pectin. An extensive study of polysaccharides is related to the preparation of "relatively" pure from intracellular component cell walls. As a first step, we prepared AIS as a source of cell wall material from dry cell suspension culture of *Fumaria officinalis* L. Further, the composition analysis showed that the AIS consisted of uronic acids (10.0% w/w), suggesting the presence of acidic-type polysaccharides. The yield and chemical characteristics of the obtained ammonium oxalate fraction are shown in Table 1. The ammonium-oxalate extraction solubilized 32% of the total uronic acids, present in the AIS, and the obtained fraction was characterized as a pectic-type polysaccharide preparation having 45.6% uronic acids (Table 1). It

Table 2: Sugar molar ratios.

Fraction	GalA/total neutral sugars	Rha/GalA	Ara + Gal/Rha	Ara/Gal	HG	RG-I	HG/RG-I
Ammonium oxalate	2.2	0.05	6.4	0.9	57.9	26.3	2.2

could be observed that a relatively small part of the total cell walls were recovered (7.0%). Therefore, most of the polyuronides (68%), probably ester cross-linked within the cell wall matrix, were still contained in the residue from the polysaccharide extraction. Our data for yield, uronic acid content, and recovery of the fraction could not be compared with literature data because we report information about them for the first time. However, Chambat et al. [34] have isolated ammonium oxalate-soluble galacturonic acid-containing polymers from *Rosa glauca* cell walls cultured *in vitro*. Similarly, the polymer fraction has been mainly composed of uronic acids (50%), and 21% of the total polyuronides have been extracted. It should be noted that the different extraction conditions such as temperature, pH, extraction time, and solid/liquid ratio affected the polysaccharide yield. The lower yield of polysaccharide isolated from suspension-cultured *Fumaria* cell walls reported here could be explained with nonconventional ammonium oxalate extractant, its concentration 0.8% (w/v), extraction time, temperature, and maturation of the cell wall material.

3.2. Monosaccharide Composition. The extracted pectin was assessed using sugar composition analysis. The neutral sugar composition of *Fumaria* pectin was summarized in Table 1. The overall analytical data obtained on the polysaccharide composition reveal that it was made up of 45.6% uronic acids and 25.4% neutral sugars. The investigated fraction was consisted mostly of ordinary pectic monosaccharides. Amongst them, galacturonic acid (41.0%) and galactose (7.3%) were the main components followed by glucose (7.6%) and arabinose (5.6%). Galactose and glucose were present in nearly equal molar amounts. In addition, we performed analysis for separately determination of D-glucuronic acid (Glc*p*A) and galacturonic acid because RG-I backbone substitution with Glc*p*A has been reported [35]. Glucuronic acid was not detected, probably of its very low levels (<3%) and the sensitivity of the method. Therefore, Gal*p*A rather than Glc*p*A was assumed to be the predominant hexuronic acid. Interestingly, the high level of glucose (30.0% of total neutral sugars) could be attributed to the residual starch or much less coextracted hemicellulose. Moreover, in this case, ammonium oxalate treatment could solubilize part of the cellulose polymers closely connected to the pectin. The L-rhamnose content was 2.0%, which represented 7.8% of the total neutral sugars. The presence of rhamnose combined with galactose and arabinose is typical for pectins with a RG-I backbone branched with arabinan, galactan, and/or arabinogalactan side chains [5]. Minor amounts of mannose (0.3%), fucose (0.3%), and xylose (2.3%) were also detected, suggesting the presence of small proportion of xylogalacturonan regions and/or RG-II. In order to investigate the presence of Kdo, an important ketosidic component in lipopolysaccharides and RG-II, in the obtained fraction, the thiobarbituric acid assay was performed. As a result, it could be noticed that the fraction contained significantly low amount of thiobarbiturate-positive substances (+0.060 absorbance unit/2 mg) compared to the lavender PSC-2-positive control (+0.342 unit/2 mg). In our previous study, it was shown that RG-II fragments were present in lavender PSC-2 and chPS-L2 fractions [28]. This indicated that Kdo was present in minor amounts in polysaccharide-bound form, and only a very low amount of RG-II segments constituted the acidic fraction. The total sugar content of this fraction was 71.0% (w/w), and some noncarbohydrate cell wall components were also found. The presence of oxalic-acid salts as a result of difficult removal of chelating agents from extraction mixture could not be excluded since it was previously reported [36, 37]. It appeared that the obtained acidic fraction contained a minor complex of polyphenolic components (0.3%) and some proteins (4.1%). The presence of protein component suggested that galactose and arabinose could also originate from highly branched arabinogalactan type II-forming AGP complexes which were confirmed by the strongly positive reaction with Yariv's reagent. Interestingly, the high amount of 4-L-hydroxyproline (11.7% of total protein) measured was an indication for the presence of some structural Hyp-rich glycoprotein (extensin) or AGPs. Such components could be incorporated in the networks of other cell wall constituents (pectin, cellulose, etc.) or covalently linked to them. Interestingly, AGP and extensin are broadly distributed in tissue and cell suspension cultures, and it is thought to function in various aspects of plant growth and development [38]. In another study, it was found that continuous ammonium oxalate treatment and chlorite oxidation of tomato suspension-culture walls solubilized a high proportion of the extensin protein along with a considerable amount of pectin [39].

Summarizing the sugar composition data as sugar molar ratios, a useful bit of information, could be observed (Table 2). Despite giving an "average" value, these values provide us with a view of the complexity and structure of the extracted polysaccharide. The values also allow us to compare our sample with literature data, where very often the same approach is done [40]. Keeping in mind Gal*p*A content (61.0 mol%), higher calculated value (2.22) of the ratio of Gal*p*A to neutral sugars showed that pectin contained longer and more linear HG fragments with minor neutral sugar residues attached. The molar ratio rhamnose: Gal*p*A is indicative for both the presence and contribution of RG-I blocks within the pectin backbone. RG-I consists of alternating rhamnose and Gal*p*A unit, and the ratio between them within RG-I backbone is 1 : 1. The considerably lower (0.05) ratio found for the fraction showed that the pectic population contained only low amounts of RG-I segments. However, the RG-I segments present had a relatively high ratio

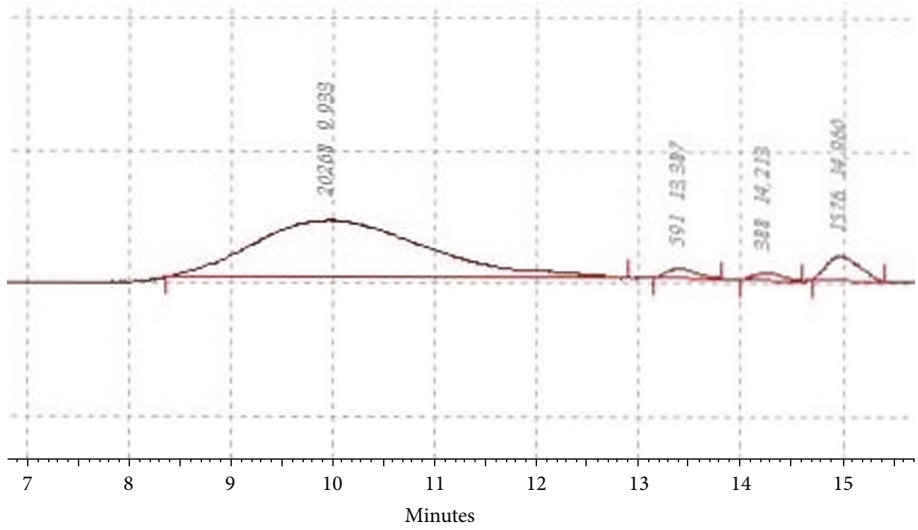


Figure 1: HPSEC elution pattern of ammonium oxalate-extracted common fumitory pectin. Pullulan standards (6200–805,000 Mw range) were used to estimate the molecular weights.

(Ara + Gal)/Rha (6.4). This ratio approximately indicates the average side-chain length (branching), pointing that the ammonium oxalate-extracted pectin contained a low level of long-side chains. In the neutral sugar side-chain segments, relative amounts of Ara and Gal were nearly equal (Ara/Gal = 0.9) showing that the extracted pectin was constructed of arabinogalactan as side chains of RG-I fragments. When using the equations suggested by M'sakni et al. [41], the calculated RG-I (mol%) and HG (mol%) contents were 26.3% and 57.9%, respectively. The amount of HG fragments was 2.2 times larger than that of RG-I. This could indicate that the pectin fraction is composed of high-proportion HG fragment, as a main building block, in contrast to the RG-I blocks. Incidentally, this was confirmed in the primary walls of suspension-cultured cells of *R. glauca* by Chambat and Joseleau [42].

Our results were in good agreement with previous reports on neutral monosaccharide composition typical for pectins. According to them, rose hip petal pectin, extracted with mixture of 0.25% solutions of ammonium oxalate and oxalic acid, was consisted mainly of rhamnose, arabinose, galactose, next to minor amounts of xylose, mannose, and glucose [43]. Fucose was not detected as a component of pectin. The variation in sugar composition could be explained with different plant material and extraction condition used.

3.3. Degree of Methyl Esterification and Degree of Acetylation. The polymer fraction was characterized as low-methyl-ester pectin with degree of esterification 45%. Interestingly, the reported methyl-ester content was in agreement with this observed for oxalate-soluble rose hip pectin previously obtained by Khodzhaeva et al. [44]. The pectin was also characterized with low degree of esterification (31.9%). The reason for this could be the use of ammonium oxalate, which is a weak organic acid and calcium-chelating agent. Therefore, low-methyl-esterified and noncovalently linked HG fragments held in the cell walls by calcium ions could be solubilized. Moreover, the study by Pinheiro et al. [45] revealed that the concentration of chelating agent, such as citric acid, was a very significant factor influencing the degree of methyl-esterification of highly esterified pectin from passion fruit peels.

The pectin sample contains also some other substituents such as acetyl groups. The acetyl content was found to be only 0.4% w/w, and the calculated DAc value (3.4 mol%) was relatively low, probably due to the used extractant. It should be indicated that DAc was expressed as acetyl content per D-Gal*p*A unit assuming that only D-Gal*p*A residues were acetylated. However, some previous studies reported that not only D-Gal*p*A residues in HG region but also L-Rha*p* and D-Gal*p*A units in the RG-I fragment could be partially *O*-acetyl-substituted [46, 47]. Additionally, it is well known that the high acetyl content is responsible for the poor gelling and rheological properties of pectic polysaccharides. Typically, apple and citrus pectins are characterized as low-acetylated. In contrast, sugar beet, leek, potato, pear, and plum exhibited higher acetyl-ester content [10, 48].

3.4. Molecular Weight Distributions. The molecular weight distribution pattern of the isolated acidic fraction is shown in Figure 1. The pectin was eluted early as a rather broad peak beginning at 8.5 min. Generally, Figure 1 shows that this fraction consisted of different populations. The molecular weight distribution of the main peak ranged between high (>600 kDa) and relatively low molecular weight compounds (>6 kDa). The polydispersity index was calculated as well (1.19). 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 15.5 min. These peaks could be ascribed to small nondialyzable HG sequences (large oligomers) or neutral oligomers. Probably, these oligomers may come from the partial hydrolysis of other carbohydrate polymers constituting the cell walls or separated from the pectin macromolecules as a result of "trimming" effect of ammonium oxalate during the extraction. To our

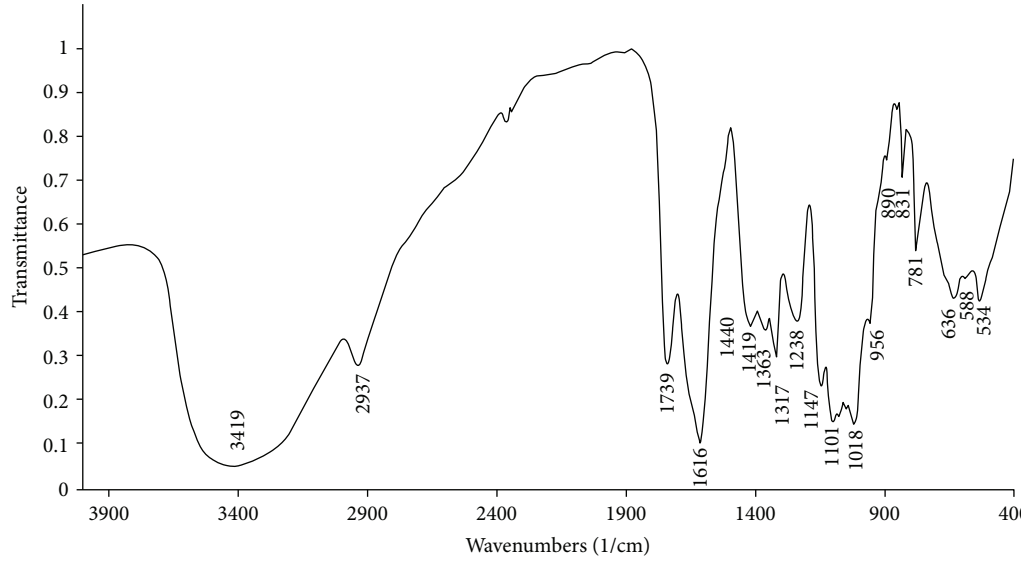


Figure 2: FT-IR spectrum of polysaccharide isolated from *in vitro* culture of common fumitory.

Table 3: Characteristic infrared bands shown by various functional groups.

Characteristic IR frequencies, cm^{-1}	Experimental IR frequency, cm^{-1}	Assignments
3200–3490	3419	ν(OH); intramolecular H-bonds
2933–2981	2937	ν_{as}(CH)(CH_2)
1750–1745	1740	ν(C = O) stretching vibration of alkyl ester
1640–1600	1616	COO^- antisymmetric stretching vibration, polygalacturonic acid; $\delta(H_2O)$
1400–1410	1419	COO^- symmetric stretching vibration
1330–1320	1317	Ring vibration
1243	1244	ν(C-O)
1125–1162	1147	ν_{as}(C-O-C), glycoside
1100–1093	1101	ν(C-O) ring
1019–1014	1018	ν(C-O), ν(C-C) (C2-C3, C2-O2, C1-O1 pectin)
950–960	954	C-O bending vibration
975	975	γ(OH)
888	889	α-D-Gal*p* C1
833–832	832	Ring vibration
760	765	α-D-Glc*p* C1
540	534	Polygalacturonic acid
441	441	C-O-C torsion deformation in methyl polygalacturonate

knowledge, there are no published data for molecular weight distribution of pectin extracted with ammonium oxalate from *in vitro* cultured *Fumaria* sp. However, in comparison with a previous study, the value of 27.3 kDa for oxalate soluble rose hip petal pectin was calculated [43].

3.5. Fourier Transform Infrared (FTIR) Spectroscopy. The FT-IR spectrum of the studied polysaccharide is presented on Figure 2. The typical bands for pectic polysaccharides (Table 3) were found, and most of the bands coincided with some previous reports for celery, tomato, and citrus pectins [24, 49, 50].

The strong band at 3400 cm^{-1} was assigned to the ν (O–H) stretching vibrations of hydrogen-bonded and free hydroxyl groups. The band at 2937 cm^{-1} was attributed to ν (C–H) stretching of CH_2 groups [24]. The region from 1800 to 1500 cm^{-1} was considered as important and particularly useful for define esterification degree. The broad bands at 1760–1730 cm^{-1} originated from vibration of esterified groups [51]. The signal at 1749 cm^{-1} was typical for C = O stretching vibration of methyl-esterified carbonyl groups in pectin: ν $(C=O)_{COOH}$ ($COOCH_3$) of Gal*p*A. The bands at 1631 cm^{-1} and 1550 cm^{-1} corresponded to the absorption of the carboxylate anions ν_{as} (O=C–O) of nonesterified

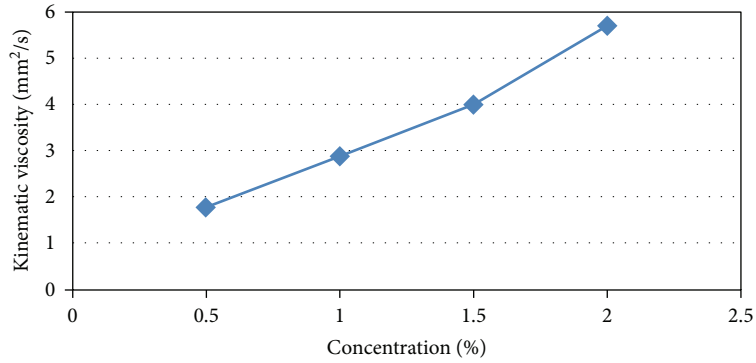


Figure 3: Kinematic viscosity of different pectin concentration from *in vitro* cultures of *Fumaria officinalis*.

Table 4: Functional properties of pectin isolated from *in vitro* cultures of *Fumaria officinalis*.

Fraction	Swelling properties, mL water/g sample	Water-holding capacity (WHC), 1 g water/g sample	Oil-holding capacity (OHC), g oil/g sample
Ammonium oxalate	14.0	10.8	2.4

groups in pectins. They were partly overlapped with a very intense and sharp band at 1616 cm^{-1}. The latter was assigned to asymmetric stretching vibration of carboxylate anion ν_{as} (COO^{-}) distinguished from C=O stretching band of esters. Similarly, the band at 1440 cm^{-1} associated with ν_s (O=C–O) vibration of nonesterified groups in pectin was also overlapped with more intensive peak at 1419 cm^{-1}, which is typical for ν_s (COO^{-}) of salts of pectic acid. Moreover, bands typical for deformation vibrations of absorbed water δ (H_2O) (the signal around 1650 cm^{-1}) could also influence on the bands in the region from 1600 cm^{-1} to 1700 cm^{-1}, similar to the report of Chylinska et al. [50]. The bands observed in the region at 1319–1380 cm^{-1} were due to ν (C–C) and scissors δ (CH_2) vibration. The strong band at 1238 cm^{-1} associated with the vibrations δ (C–H) of pectic acid salt was also observed. Further analysis of the spectrum in the 1200–1000 cm^{-1} region showed specific bands typical for a pyranose ring vibration. Bands at 1147 cm^{-1}, 1101 cm^{-1}, and 1018 cm^{-1} were associated with the vibrations involving ν (C–O–C) glycosidic bond, ν (C–C), ν(C–O), and δ (OCH) bending in the ring, respectively. This indicated the predominance of the HG segments in the fraction. Additionally, bands that appeared at 1077 cm^{-1}, 1047 cm^{-1}, and 890 cm^{-1} might be attributed to β-arabinogalactan and β-glycosidic linkage presence [52]. A weak band at 918 cm^{-1} which was obscured by 956 cm^{-1} could be attributed to the deformation scissor vibrations of δ (CHH) and δ (COH) bonds. The γ (C–OH) stretching vibrations of side groups overlapping with γ (OH) of the ring gave a strong band at 831 cm^{-1}. This band together with 956 cm^{-1} was considered for characteristic of α-glycosidic linkage and a presence of RG-I molecule [50]. In addition, similar to celery pectin spectrum [24] and in the FT-IR spectrum of pectin fraction from *F. officinalis* suspension culture, the bands at 889 cm^{-1} and 765 cm^{-1} typical for α-D-Glc*p* or α-D-Gal*p* in C1 conformation were found.

3.6. Functional Properties

3.6.1. Viscosity Determination. The kinematic viscosity increased with the increase of pectin concentration (Figure 3). The obtained values of kinematic viscosity of *Fumaria* pectin ranged from 1.8 to 5.8 mm^2/s (cSt). The resulting values even at the highest concentration were lower than orange pectin obtained after different extractant [53]. The highly concentrated pectin solutions (2%) could not be applied to high-pressure homogenizer; therefore, the concentration of pectin used to prepare emulsions in this study was limited to be less than 1.5% (w/w).

3.6.2. Water-Holding Capacity and Oil-Holding Capacity. The water-holding capacity of the isolated pectin was 10.8 g water/g (Table 4). The values were significantly higher in comparison to water-soluble polysaccharides from *Rosa roxburghii* Tratt fruits (0.25 g water/g) [53], polysaccharides from carob flour (1.4 g water/g) [54], and insoluble locust bean fraction (5.65 g water/g) [55] but close to WHC of citrus pectin (8–10 g water/g) and apple fibers (9.36 g water/g) [56, 57]. This could be explained by the high uronic acid content or HG presence in the sample. However, our values were lower than some other pectins (37.84 g water/g) [55] and sunflower pectin (57 g water/g organic material) [58]. The oil-holding capacity of pectin isolated from *in vitro* cultures of *F. officinalis* was calculated to be 2.4 g oil/g (Table 4). Our results for OHC was near to this shown by water-soluble polysaccharides from *Rosa roxburghii* Tratt fruits (3.19 oil/g sample) [53] and higher than OHC for oat starches (0.29–0.34 g oil/g sample) [59]. This polysaccharide fraction possessed better WHC than OHC.

The swelling properties are shown in Table 4. It was found that this pectin sample was capable of absorbing quantity of water (14 mL water/g sample). It was lower than a

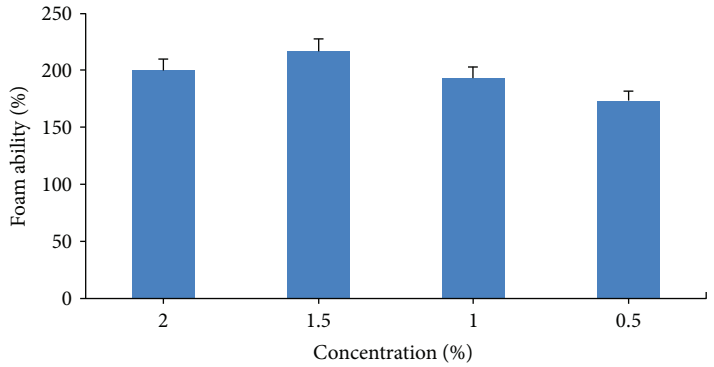


Figure 4: Foaming ability of different pectin concentration isolated from *in vitro* cultures of *Fumaria officinalis*.

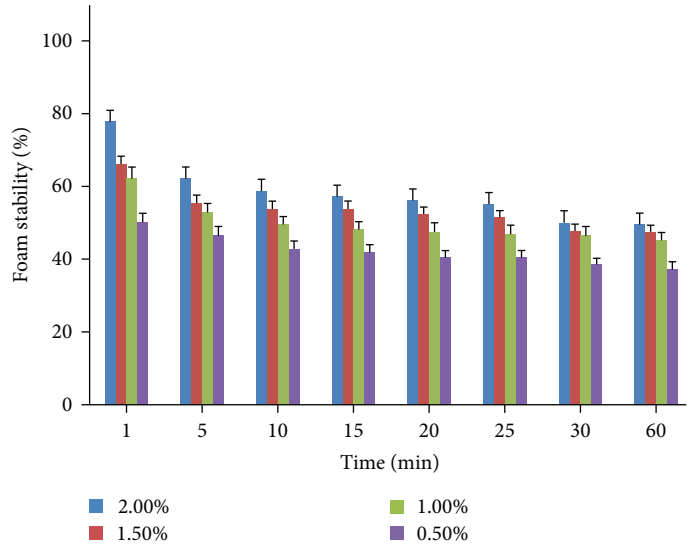


Figure 5: Foaming stability of different pectin concentration isolated from *in vitro* cultures of *Fumaria officinalis*.

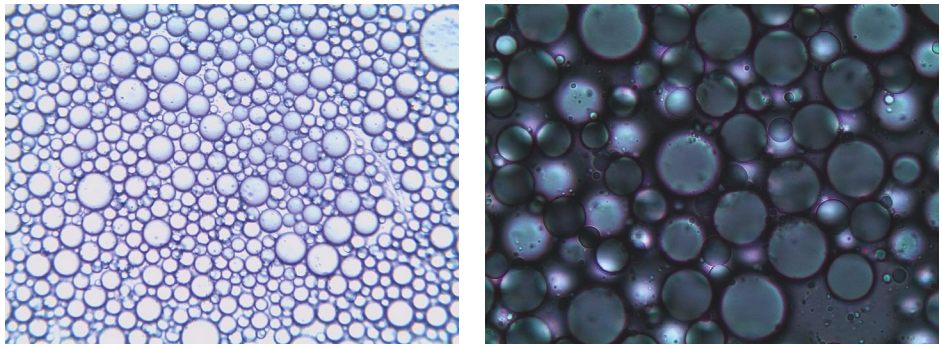
previous report for citrus pectin [56] that was in the range from 19 to 21 mL/g and higher than swelling properties of carob flour polysaccharide 11.4 mL/g [54].

3.6.3. Foaming Properties. The foaming ability and foaming stability of foams prepared with different concentration of pectin fraction are presented in Figures 4 and 5, respectively. It was found that this pectin demonstrated high foaming ability above 150% (Figure 4) and formed stable foams even in low concentration of 0.5%. Pectin isolated from *in vitro* culture of *F. officinalis* with concentrations 0.5 to 2.0% showed high FS, which after 30 min decreased with 50%. The foams did not disappear even after 60 min as the foaming stability of pectin solutions remained in the range from 40 to 50% of the original volume (Figure 5). The high foaming ability could be explained with the presence of protein bound to carbohydrate chains. Generally, FC and FS increased with increasing the concentration of pectin in solutions. Similar trends were reported in FS of foams prepared with different polysaccharides from *Rosa roxburghii* Tratt fruits and *Trigonella foenum-graecum* [53].

Table 5: Stability of emulsions of pectin isolated from *in vitro* cultures of *Fumaria officinalis*.

Type	Centrifugal test Separated phase, % ±0.1			Turbidimetric method	
	Oil	Water	Emulsion	EAI, $m^2\ g^{-1}$	ESI, min
Pectin	0.0	63.2 ± 0.1	36.8 ± 0.2	2763.6 ± 0.1	10.0 ± 0.1

3.6.4. Emulsifying Properties. The EAI and ESI are considered as indexes for evaluation of emulsifying properties of investigated samples. For the first time together with foaming properties, model 50/50 water in oil emulations prepared with *Fumaria* pectin was studied (Table 5). The results demonstrated promising emulsifying properties of isolated pectin. After centrifugation none oil phase separation was observed, only water and emulsion layers were presented. The EAI of pectin (36.8%) was lower than the reported values for potato pectins (44.97%–47.71%), sugar beet pectin extracted by acids and the commercial citrus pectin (44.87%), and apple pectin (45.34%) [60, 61]. Our data for EA were similar to



(a) (b)

Figure 6: Microscopy images of the structure of model oil/water emulsions prepared with pectin. (a) 400x and (b) 1000x magnifications.

emulsions prepared with grapefruit pectin [62]. In comparison with 50% emulsion prepared with 0.4% amidated pectin that showed emulsion stability (94%), our emulsions possessed lower stability [63].

Additional characteristic of the obtained 50/50 emulsions was done by a microscopic analysis. The microscopy images of 50/50 emulsions prepared with pectin from common fumitory *in vitro* culture are shown in Figure 6. The stability of prepared emulsions was observed for a period of 1 day at room temperature. The emulsion stability was evaluated by droplet size determination and their distribution in the continuous phase [64]. The average diameter of emulsion droplets was calculated. The image of emulsion with two magnifications (400x and 1000x) showed a homogeneous structure with approximately equal droplet size ($d = 12.5\ \mu$m) (Figure 6). The particle sizes were homogeneously distributed in the whole emulsion volume. It can be considered that the small particles sizes were associated with better dispersion system stability. The separation of oil phase was not observed, and the coalescence process was not found at all (Figure 6). It was demonstrated that the type of pectin strongly affected the emulsion droplet size. Highly methylated pectin was considered to be the best emulsifier for nanosized emulsions due to its high hydrophobic portion in the molecules. Using high concentration of pectin could reduce the emulsion droplet size [65]. In our case, this low methyl-esterified pectin from *Fumaria* (45% DM) showed good emulsifying properties. Some reports demonstrated a strong correlation between rheological properties (emulsion stability) and DM of the pectin [66]. Additionally, the protein moiety, feruloyl, and acetyl groups play a major role in pectin emulsifying activities, while the emulsion-stabilizing properties of the polymer are controlled by the HG fragments and the neutral sugar side chains of the RG-I [67]. In our case, the promising emulsifying properties of the polysaccharide could be explained with the presence of protein and some acetyl esters, respectively. Additionally, EC and ES of polysaccharides also depended on sugar composition, the residual aqueous components of the hydrophobic protein, or rheological characteristics [68]. The protein and hydrophobic acetyl groups of pectin can act as anchors on the oil particle surface, thus decreasing the surface tension [69].

4. Conclusion

For the first time, we showed the presence of acidic fraction in the cell walls of suspension culture of *Fumaria officinalis* L. The fraction predominantly consisted of linear HG segments and characterized as typical low-esterified pectic polysaccharide. Moreover, AGPs as side-chains of RG-I backbone were determined. In addition, the isolated pectin showed significant foaming ability as it formed stable foams. Its water-holding capacity dominated over oil-holding capacity. Pectin in concentration 1% could be successfully used as stabilizer of 50/50 emulsions. All these findings for common fumitory pectin could be useful for further investigation regarding biological activity, food application, and medicine.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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