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Isolation and characterization of plant cell wall material from rose hip fruits

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In the present study, plant cell wall material from rose hip fruits was isolated as alcohol-insoluble solids. Chemical composition of the cell wall material and initial fruits (edible part) was investigated and compared. The amount of polyphenols (flavonoids), pigments, lipids, vitamin C was removed in a different extent during hot alcohol/acetone treatment. In contrary, proteins, polysaccharides and some polyphenols (condensed tannins) were co-precipitated due to the dehydration effect of alcohol, which was the main factor that restricted the extractability of 'contaminants'. In addition, carbohydrates (mainly pectins and cellulose) were found out to be the main constituents of the 'purified' rose hip cell wall preparation.

Keywords: Rose hip, Plant cell walls, Alcohol-insoluble solids, Characterization, Polysaccharides.

INTRODUCTION

Rose hip (RH) is widely distributed in particular regions of Europe (Balkan Peninsula), Asia (the Middle East), South and North America [1]. Fruits are mainly used for commercial production of jams, jellies, marmalades, tea, soups, food additives and functional ingredients. In traditional folk medicine RH is applied for curing infections, osteoarthritis and for the treatment of the common cold [2].

The RH fruits also attract attention as rich sources of low-molecular weight nutraceutical components, such as vitamins (C, B, E), carotenoids, phenols, essential oils, fatty acids and minerals, which have already been recognized as bioactive [3-8]. Although the impacts of low molecular weight biologically active substances are extensively studied, it seems that they are not responsible for all health beneficial effects.

Some of the studies dealing with RH fruits refer to the occurrence of polysaccharides [3, 5, 6]. However, the obtained polysaccharides were not fully characterized and therefore further investigation of the polymer composition and structure of the RH fruit cell walls is required. In addition, thorough knowledge of the composition of RH cell walls is crucial and essential for better understanding and assessing the quality of the fruits and their suitability for technological processing.

An extensive study of the RH polysaccharide fractions is related to the preparation of plant cell walls 'relatively' pure from intracellular components. It should be noted that the methods of cell wall preparation depend on the amounts of intracellular compounds in the initial plant material (starch, protein, polyphenols, etc.), the stages of ripeness, and used anatomical parts of the plant. Alcohol-insoluble solids (AIS) have usually been used as a source of cell wall material (CWM) by various researchers [9-12]. Generally, AIS are prepared by immersing the tissue into a hot aqueous ethanol solution, followed by blending the mixture to disrupt the material and further solubilisation of low-molecular weight compounds. After filtration the residue is washed with absolute ethanol and acetone or petroleum ether. The advantage of this method for cell wall preparation is the fast inactivation of the most endogenous enzymes, which could alter polymers structure. However, dehydration with organic solvents is known to influence the solubility of the polysaccharides and additionally facilitate the formation of co-precipitate (artifacts) between cell wall components [9, 12].

In the current study a method for isolation of plant cell walls as AIS from RH fruits is described and the results of chemical composition analysis of the cell wall material and initial fruits are presented and compared.

EXPERIMENTAL

Plant material

The RH fruit material (with orange-red colour of the fruits) was obtained from a local producer (Smolyan, the Rhodope Mountains, Bulgaria). The material was enclosed in polyethylene bags and stored frozen (−18 ºC) before further treatment.

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Preparation of cell wall material

Cell wall material was obtained using the hot alcohol insoluble solids method. Extractions were carried out in a glass flask (4 L) placed in an incubator as follows: 200.0 g of chopped and freeze-dried rose hip fruits stripped of seeds were transferred into 2.0 L of ethanol/water 80:20 (v/v) solution (solid:liquid ratio = 1:10 w/v) pre-heated at 70 ºC. The obtained mixture was kept at 70ºC under vigorous shaking (every 10 min) for 1 h and then heating was discontinued. The resulting material was allowed to cool down and it was left overnight at room temperature. Then the mixture was filtered through a nylon cloth to remove the solid particles. The same procedure was repeated as incubation was carried out for 1 h. After filtration, the insoluble residue was washed with ethanol/water 80:20 (v/v) (70ºC) solution (solid:liquid ratio = 1:5 w/v) for 1 h. Finally, the solid was washed with acetone (solid:liquid ratio = 1:4, w/v) at 30ºC for 1 h. The solid material was vacuum-filtrated and additionally squeezed from excess of solvent through a cloth.

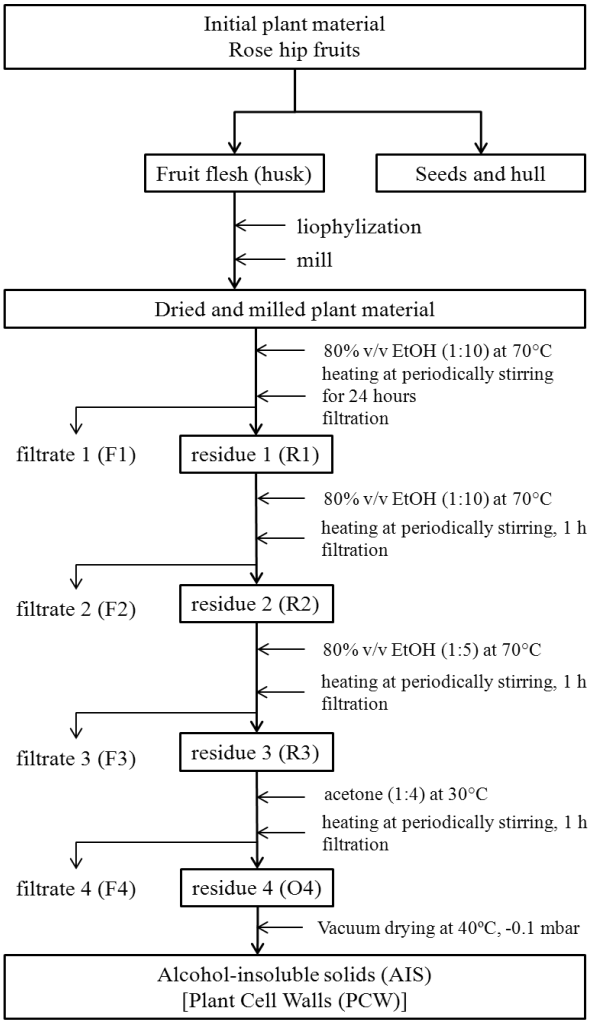


Fig. 1. Scheme of cell wall material preparation from rose hip fruits.

The obtained alcohol-insoluble residue was further vacuum-dried (40ºC, -0.1 mbar) to a constant weight. The general scheme of the cell wall material preparation from RH fruits followed in this investigation is presented in Fig. 1.

Moisture and ash content

For the moisture content, ground samples (~1.5 g) were dried in an automated moisture analyzer (KERN®DLB, Germany) at 105ºC until a constant weight. Results were used for dry solid calculation. Ash content was determined as the pulverized samples (0.5-2 g) were placed in a crucible, ignited in a muffle furnace at 550ºC to a constant weight.

Total carotenoids content

The total carotenoids were determined using acetone as a solvent according to Lichtenthaler and Wellburn [13]. For extraction of lycopene and *β*-carotene the method with acetone/petroleum ether solvents was used as previously described by Georgé *et al*. [14]. The concentrations of lycopene and *β*-carotene were calculated using the equations of Lime *et al*. [15].

Vitamin C content

The amount of ascorbic acid in the RH fruits was determined according to the 2,6-dichloroindophenol titrimetric method of AOAC [16].

Total carbohydrate content and monosaccharide composition analysis

Before analysis the samples were submitted to a prehydrolysis treatment with 72% (w/w) H_2SO_4 at 30 °C for 1 h, followed by a hydrolysis step with 1 M H_2SO_4 at 100 °C for 3 h. The total carbohydrates content in the hydrolyzates was assayed by the phenol-sulfuric acid method according to DuBois *et al*. [17] using glucose (10-80 μg/ml) as a reference. Absorbance was measured at 490 nm. The standard curve was constructed with glucose and galacturonic acid mixture (1.5:1.0) and was used for result calculation (AIS material). Individual neutral sugar composition of the obtained plant cell walls was determined [18] by gas chromatography using inositol as an internal standard. The constituent sugars released were analysed as their volatile alditol acetates [19]. The uronic acid content was determined by an automated colorimetric *m*-hydroxyl-diphenyl assay [20] using an auto-analyser Skalar San^{++} system (Skalar Analytical BV, Breda, The Netherlands). Galacturonic acid (12.5–100.0 μg/ml) was used for the calibration curve. The methylpentoses content was evaluated by the thioglycolic acid-sulphuric

acid method with L-Rha and L-Fuc (1:1) as standards [21].

Cellulose content

The cellulose determination was performed according to the Kürschner-Hoffer gravimetric method with a modification [22]. Briefly, a sample (0.5 g) was boiled (30 min) with 25 ml of acetic-nitric reagent (acetic acid:H_2O:HNO_3 = 8:2:1 v/v) in a round-bottom flask. After cooling the insoluble residue was filtrated through a sintered glass filter (G3) under vacuum, washed successively with hot acetic-nitric reagent, then with deionized water to neutral pH, ethanol (96% v/v) and finally with an excess of petroleum ether. The obtained residue was dried in a laboratory ventilated oven at 50ºC to a constant weight.

Protein content

The crude protein content of the samples was estimated by the micro-Kjeldahl method [23]. The nitrogen expressed as ammonia content in the digested samples was determined by the acetylacetone-formaldehyde colorimetric method using ammonium sulphate as a standard [24]. For calculation of crude protein, a value of the nitrogen-to-protein conversion factor of 6.25 was used.

Total lipids content

The powdered dried RH fruits (without seeds) (14.0-15.0 g) were packed in a cellulose thimble. An exhaustive extraction with petroleum ether (with boiling point: 40–60°C) (500 ml) was carried out for 8 h in a Soxhlet extractor. The obtained crude extract was dried under vacuum and its weight was used for calculation of the lipids content on the basis of dry weight of the samples.

Lignin determination

The lignin content of the prepared AIS was evaluated by two different analytical methods: the Klason lignin gravimetric method (KL) [25] and the spectroscopic acetyl bromide lignin (ABL) method [26].

Total phenols content

Freeze-dried fruits (edible part) or AIS (0.5 g) were mixed with 40 ml of 80% acetone in 0.2% formic acid and extracted at room temperature on a magnetic stirrer for 1 h. Then the samples were centrifuged (6000 g, 20 min) and supernatants were collected. Total phenolics were determined according to the method of Singleton and Rossi with Folin–Ciocalteu's reagent [27]. Gallic acid (10-200 μg/mL) was employed as a calibration standard and the results were expressed as mg gallic acid equivalents (GAE) per g dry weight.

Flavonoids and condensed tannins content

Freeze-dried fruits (edible part) or AIS (1.0 g powder) were mixed with 40 ml of 80% ethanol in 0.5% formic acid. Extraction was conducted on a magnetic stirrer at room temperature for 1 h. The samples were centrifuged (6000 g) for 20 min and clear supernatants were used for total flavonoids and tannins content analysis. The total flavonoids content was determined according to the method of Chang *et al*. [28] with $AlCl_3$ reagent. The results were presented as μg equivalents quercetin (QE) per g dry weight according to the calibration curve constructed with quercetin dihydrate (10-200 mg/L).

The methylcellulose precipitation assay was performed according to Sarneckis *et al*. [29] for determination of condensed tannin concentration. (-) Epicatechin standard (≥ 90% HPLC, Fluka) aqueous solutions (10-200 mg/L) were used for calibration curve construction.

Antioxidant activity assays

Oxygen Radical Absorbance Capacity (ORAC) assay. ORAC was measured according to the method of Ou *et al*. [30] with some modifications described by Denev *et al*. [31]. ORAC analyses were carried out on a FLUOstar OPTIMA plate reader (BMG Labtech, Germany). A fluorescence filter with excitation wavelength of 485 nm and emission wavelength of 520 nm was used.

DPPH radical-scavenging ability. The extraction process was carried out with 100 % acetone in an ultrasonic bath [32]. DPPH assay was performed as described by Ivanov *et al*. [33]. The results were expressed as micromoles of Trolox equivalents (TE) per gram (μmol of TE/g) dry weight.

RESULTS AND DISCUSSION

Characterization of initial RH fruits

The current study started with removing of the edible part (fruit flesh) from seeds and hull. Mean fruit weight was 1.93 g that was based on randomly weighed 1 kg of fruits. The fruit flesh was the main usable part, as its proportion was 61.5%. The seeds and hull represented 38.5% of the fresh fruits weight. Average number of seeds in one fruit was 17 (from 100 fruits). Further, these parts were not subjects of interest and analysis. The results for yields and chemical composition of initial RH fruits are summarized in Table 1. The dry matter content of the investigated fruit flesh was 47% which was

comparable with the previous study by Bonev *et al*. [34] who reported 43.4%. Rosu *et al*. [35] found that the dry matter varied among rose species and they reported that *Rosa canina* L. Str. species had a slightly higher value (49.9%) than in our study.

Table 1. Yield, chemical composition and antioxidant capacities of initial RH fruits and isolated plant cell wall material (AIS)[a].

	Initial RH fruits	Plant cell wall material (AIS)
Yield (% w/w)	-	57
Dry matter (% w/w)	47	97
Ash (% w/w)	5.0	2.0
Crude protein (% w/w)	3.5	4.6
Total lipids (% w/w)	0.77	0.37
Total carbohydrates (% w/w)	34	52
Uronic acids (% w/w)	9.6	16.2
Cellulose (% w/w)	7.0	11.0
Methylpentoses (% w/w)	1.8	2.3
Lignin content (% w/w)		
Klason	-	8.0
ABSL	-	5.1
Vitamin C content (mg/100 g)	610	35
Total phenolics (mg GAE/g)	91	54
Total flavonoids (μg QE/g)	262	52
Total tannins (μg CE/g)	178	136
Total carotenoids (μg/g)	119.0	3.0
Lycopene	63.6	1.3
β-Carotene	54.0	1.5
ORAC (μmol TE/g)	2094.3	1372.9
DPPH (μmol TE/g)	2493.6	1870.8

[a] Values are the average of two replicates.

Initial RH fruit flesh was characterized with 5.0% ash content that was higher than the value previously found by Bonev *et al*. [34] (2.22%), but lower compared with the investigation of Demir and Özcan [5] for two RH samples from Turkey (7.35% and 6.48%).

The crude protein content of fruit flesh was evaluated to be 3.5% and it was in close agreement with the data of Michev *et al*. [36], but lower compared to these of Demir and Özcan [5] – 6.7 and 8.4%. According to the literature, the protein content of RH ranged between 2.3 and 4.58% dry matter [37] depending on maturity stage, agro-climate condition, and even correlated with altitude [35].

Our result for the lipid content (0.77%) was slightly lower than those reported by other authors who have shown for *R. canina* – 1.78% total fat content and 1.6 and 1.2% crude oil of two RH species [5]. In comparison with data in the literature, our result was in very good agreement with reports for fully ripened *R. canina* fruits (0.70-0.80%) [36].

It was shown that carbohydrates were the main constituents of the RH fruits based on dry matter (34%). Uronic acids and cellulose were accounted for nearly 10% and 7.0% of the dry matter, respectively. In addition, methylpentoses (Rha and Fuc) content was 1.8% and combined with those of uronic acid and cellulose occupied nearly 18.4%. Therefore, the soluble sugars should be accounted for the rest. The other authors who studied different RH species showed that cellulose content varied between 2.1% and 9.7% [34].

Vitamin C was recognized as one of the most important components of RH fruits based on our results. Its concentration was estimated to be 610 mg/100 g dry weight. This value was comparable with those found by Ercisli [6] who reported 727-943 mg/100 g ascorbic acid. Interestingly, higher levels of vitamin C content (1358 mg/100 g) were determined by Dimitrov and Bonev [4] for different RH species (31 sp.). In another study, Demir and Özcan [5] found considerably higher levels of vitamin C (2365 and 2712 mg/100 g).

It is well known that RH has been proposed as a source of health-promoting natural pigments, such as carotenoids [2, 3, 38]. In our study we found 119 μg/g total carotenoids content which value was comparable with the lower amounts of carotenoids (101.24 and 190.29 μg/g) detected in an earlier harvesting period [38]. Further, the concentrations of *β*-carotene (54.0 μg/g) and lycopene (63.6 μg/g) were found to constitute nearly 98% of the carotenoid content, which was similar to previous investigations [38]. With regard to previous studies [3], the total carotenoids content determined in *Rosa* species showed a wide range from 78 to 568 μg/g, which is in line with our results.

Initial RH had a high proportion of total phenolic content. The investigated species contained 91 mg GAE/g which was in close agreement with the reports for the same species [6] (96 mg GAE/g), but higher than reported by Denev

et al. [39] (56 mg/g). Our result was also higher than those determined by Demir *et al*. [40] who found variation in different *Rosa* species – between 31.08-52.94 mg/g. Among widely distributed polyphenols are the flavonoids, which consist of flavones, flavanols, anthocyanins and proanthocyanidins. Because of this it was of interest to evaluate the total flavonoids and condensed tannins content. The investigated species contained 262 μg QE/g total flavonoids. This amount was lower than that obtained in recent studies [41], but it was of the same order (0.63 mg QE/g). The authors have also demonstrated a quantitative variation of phenolics between two RH species due to the different extraction solvent used. According to them, it seems that flavonoids of RH fruits can be totally extracted using water as an extraction solvent rather than a highly concentrated organic solvent (methanol, ethanol, acetone, etc.) as used in the traditional extraction methods. Further, the measured concentration of total tannins in our case was 178 μg CE/g. Not enough studies discussed the quantitative amount of phenols and most of them have been focused on proanthocyanidin aglycones and glycosides characterization [42]. The tannins content was comparable to that in the report of Cunja *et al*. [43] who found values for proanthocyanidin trimer 1-3 ranging between 119.6-181.4 μg/g. In contrary, Taneva *et al*. [8] measured higher levels (3.86%, 3.76%, and 1.46%) for total tannin content in water, 50% (v/v) and 70% (v/v) ethanol extracts, respectively, demonstrating that water was a better solvent for higher recovery of tannins. In addition, tannin content may vary widely during the growing season in particular anatomical plant parts, as Hashidoko [44] has noted that condensed tannins were more abundant in the underground parts of the plant and the polar phenol fraction contained higher amounts of catechin oligomers and polymers.

The antioxidant activity of *R. canina* hips was evaluated by ORAC and DPPH methods (Table 1). Initial RH sample showed high antioxidant activity (ORAC – 2094.3 μmol TE/g; DPPH – 2493.6 μmol TE/g). ORAC value was higher than earlier determined [7] – 1873.5 μmol TE/g.

Characterization of isolated plant cell wall material

The second main objective of our research was the isolation of plant CWM as AIS and its chemical characterization. We were particularly interested in how the chemical composition changed after plant material treatment with organic solvents. For easier comparison the results are presented in Table 1. During extraction of initial RH material with hot aqueous ethanol (70ºC) and acetone, 43% of the dry matter was solubilized. The AIS fraction represented 57% (w/w) of the lyophilized plant material. Although there were earlier studies concerning the carbohydrate constituents of the cell walls of suspension cultures from hip fruits [45, 46], the yields of the AIS have not been reported before, and cannot be therefore compared. The result of the AIS content in the present study was lower comparing with data from our earlier experiments with RH fruits (76.1%) [47]. It should be pointed out that we followed a different procedure for AIS preparation and the starting RH material was differently prepared.

Ash content (2 %) was reduced to 60% due to aqueous organic solvent treatment.

Interestingly, the AIS had a high content of crude protein (4.6%) by reason of the co-precipitation of soluble protein with polysaccharides during alcohol treatment. In addition to the dehydration effects of aqueous alcohol and acetone, AIS was isolated from freeze-dried hips fruits material. Nevertheless, it is well-known, and also expected in our case, that AIS methods have advantage of cell wall enzymes inactivation. Also, initial RH fruit material seems not to be a rich source of protein such as in a case of protein-rich soybean. Because of this we did not consider the necessity of additional extraction steps for removing co-precipitated proteins with chemical (detergents, PAW), enzyme (or both) treatment. This is consistent with the study of Renard [12] who found that the nitrogen content was significantly higher in the AIS procedures than in other methods and among these it was significantly higher in AIS prepared from freeze-dried material.

Total lipids content was reduced by nearly 52% compared to the initial content, logically due to organic solvent treatment.

Independently of the fact that lignin is typical for the second cell walls, rose hip fruits are composed of lignified elements. It was important to adapt an analytical method for lignin quantification in isolated plant cell walls. Reviews critically emphasized the relative advantages of the different methods published [25, 48, 49]. The most frequently used methods for lignin estimation are the classical gravimetric Klason and the spectroscopic ABL one. Klason lignin levels are determined as the amount of acid-insoluble material remaining after sulphuric acid (Seaman) hydrolysis. Despite the method simplicity, it should be noted that there are some weak points related to overestimation of lignin content due to protein and polysaccharide components, Maillard products, humin precipitates and lignin-like phenolic matter

formed during prolonged treatment with acids. Also, condensed tannins and tannin-protein complexes could affect the results [48].

ABL procedure requires isolation of plant cell wall with a hot solvent, then its lignin constituent is solubilized into acetyl bromide/acetic acid solution followed by absorbance reading at 280 nm. This method seems to be a good alternative to the traditional gravimetric one, since it has been found that high protein content and carbohydrates did not interfere with lignin quantification [49]. However, higher amounts of non-lignin substances (precipitated and/or wall-bound) like phenolic compounds (tannins, acids, etc.) could have partial contribution to the total lignin content, since they have coincidental absorption spectra [48]. In this work, the lignin value using Klason method was higher (8.0%) than the ABL value (5.1%). The higher value could be attributed to the above mentioned plant 'contaminants'. In the literature, various data on the content of lignin in AIS of different fruits and vegetables can be found. Voragen *et al*. [50] reported that raspberry, pineapple, pear, carrot, cucumber, and especially cherry were rich in crude lignin (49-169 g/kg). They have also stated that the lignin content could be overrated by the method used. Another future research will be focused on the isolation of lignin with acidic 1,4-dioxane and determination of some basic chemical characteristics.

Vitamin C content was reduced by more than 94% in the AIS material and a value of 35 mg/100 g was measured. The result could find its explanation taking into account that various factors, including light, oxidation, metal ions, alkaline pH and high temperature, affected the stability and content of vitamin C [51]. During plant cell wall preparation, vitamin C was simultaneously extracted and degraded due to temperature, light and oxygen exposure. The similar tendency was observed with the total carotenoid pigments that seemed to be totally extracted. Their amount decreased by more than 97% compared to the initial plant material, probably due to better solubility in the organic solvent (acetone) used during cell wall isolation and instability in front of light, thermal treatment and oxygen. Lycopene and β-carotene contents were minimized (1.3 μg/g and 1.5 μg/g).

There was a tendency for reduction in the levels of polyphenol components in AIS, as well. The total phenolic content was reduced by 41% or nearly 60% was recovered (54 mg GAE/g). Higher recovery could be due to the highly concentrated organic solvent (hot aqueous ethanol (70ºC, 80%) and acetone) used in plant cell wall isolation. Pure solvent led to co-precipitation of complex carbohydrate polymers in the cell walls that limited the polyphenol accessibility and the efficiency of its extraction. Therefore, optimization of the extraction solvent is needed through reduction of ethanol concentrations (< 80%). This could alter the plant structure by swelling the matrix, enabling the solvent to more completely penetrate the plant material and solubilize extractable (non-wall-bound) phenolic compounds. On the other hand, the aqueous solvent would remove some additional water-soluble wall constituents (pectin). The possibility for interactions between intracellular polyphenols and plant cell walls components (protein, polysaccharides) should not be forgotten which could also affect extractability regardless of the extractants.

It was demonstrated that flavonoids were more fully removed from the initial RH material. Sequential treatment with different solvents was a good approach for removing the low-molecular weight flavonoids from the wall, as the total content was reduced by 80% and the measured value was 52 μg QE/g. Also, there was a slight decrease (24%) in the level of condensed tannins in AIS (136 μg CE/g) suggesting that only a fraction of the tannins was extracted during AIS preparation. Probably, some of the non-extracted tannins were highly polymerized, wall-bound or co-precipitated in the highly concentrated organic solvents used. These findings were consistent with those of previous studies. For instance, Renard [12] noted that there was a clear trend for higher procyanidin content in the cell walls, when it was isolated as AIS. Earlier work [52] showed that 50% aqueous methanol is a poor solvent for proanthocyanidin polymers, whereas highly polymerized proanthocyanidin forms (DP > 10) dominated in the extracts of RH fruits. According to these authors unextractable proanthocyanidins were with an average DP higher than 14 [53]. The degree of polymerization may have a great influence on the extractability of condensed tannins. Regarding the antioxidant activity, there was a slight decrease in ORAC (34%) and DPPH (25%) activity, probably as a result of low-molecular weight components removal. Higher activities might be correlated with residual unextractable and co-precipitated phenolic compounds.

It was found that carbohydrates were the main constituents of the RH AIS (Table 1). The total carbohydrates represented nearly 52% of the AIS dry material. Further indication for cell wall polysaccharide types present in AIS could be obtained after neutral sugar composition analysis (Table 2). The major sugar moiety in RH AIS was glucose (19.1%) followed by galacturonic acid –

16.2% (Table 1). Cellulose was accounted for 11% of the dry matter (Table 1), suggesting that residual glucose (8.1%) constitutes the hemicellulose fraction (xyloglucan, etc.).

Table 2. Neutral sugar composition of RH cell wall material (% w/w)*.

Monosaccharides	Plant cell wall material (AIS)
Rha	0.4
Ara	4.1
Gal	4.0
Glc	19.1
Xyl	2.7
Fuc	0.8
Man	2.0
Total	33.1

*Values are the average of two replicates.

In addition, AIS was composed of generally recognized pectin monosaccharides. Amongst them, lower amounts of arabinose (4.1%), galactose (4.0%), mannose (2.0%) and xylose (2.7%) were found. Rhamnose content represented only 1.2% of the total neutral sugars, but it was indicative of pectin ramified region presence. In conclusion, the sugar composition of the AIS revealed the presence of different types of polysaccharides, such as pectin, cellulose and hemicelluloses. Due to the higher purity of the AIS used, the result of the neutral sugar composition content in the present study was higher compared with data from our earlier experiments with RH fruits [47]. It must be noted that we followed a different procedure for AIS preparation and the starting RH material was differently prepared. Additional comparison of our results with data in the literature cannot be done since the sugar composition of the RH AIS has not been previously reported. Such preparation 'virtually' free of contaminants could be effectively used to study major cell wall carbohydrate polymers in a further investigation.

CONCLUSIONS

Several conclusions may be drawn from the application of AIS as a method for plant cell wall preparation. The choice of the initial plant material treatment is strictly individual and it should depend on the major chemical constituents. Also, amounts of different chemical constituents are removed in different extents. There is no perfect method for the simultaneous and complete removal of all interfering substances without affecting the interaction between cell wall components. Therefore, we consider that the preparation of CWM is a matter of compromise with the aims of investigation. To the best of our knowledge, there are no published reports on the method for isolation of CWM from rose hip as AIS. Moreover, the current study is among the few ones presenting more detailed information about the chemical composition of fruit AIS.

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ИЗОЛИРАНЕ И ХАРАКТЕРИСТИКА НА РАСТИТЕЛНИ КЛЕТЪЧНИ СТЕНИ ОТ ШИПКОВИ ПЛОДОВЕ

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(Резюме)

В настоящото изследване бяха изолирани растителни клетъчни стени от шипкови плодове под формата на алкохолно-неразтворима част. Химичният състав на клетъчно-стенния материал и на изходните плодове (ядлива част) беше изследван и сравнен. Количеството на полифенолни вещества (флавоноиди), пигменти, липиди и витамин С беше отстранено в различна степен по време на обработката с горещ алкохол/ацетон. Противно на това, протеините, полизахаридите и някои полифеноли (кондензирани танини) бяха утаени поради дехидратиращия ефект на алкохола, който е и основният фактор, ограничаващ екстрахирането на „контаминантите“. В допълнение, въглехидратите (главно пектин и целулоза) бяха основните компоненти на „пречистения“ клетъчно-стенен материал от шипкови плодове.