

## Cell walls polysaccharides of rose hips

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**Abstract:** *The content of alcohol insoluble solids (AIS), polysaccharide composition of cell walls and uronic acids content calculated as galacturonic acid in rose hips (Rosa villosa (Rosa pomifera Herrm) 'Karpattia', Rosa canina L., Rosa rugosa Thunb.). Rose hips were extracted by means of pectin sequential extraction. Rose achenes contained more alcohol insoluble solids than flesh. The main saccharide building cell walls of rose fruit was glucose from cellulose. The achenes were rich in xylose and the flesh was rich in arabinose. The content of galacturonic acid in flesh was 2 to 3 times higher than in the case of achenes. The largest polysaccharide fraction both in whole fruit and achenes was Concentrated Alkali-Soluble Polysaccharides fraction, while in flesh it was Water Soluble Pectins fraction. Chelating Agent Soluble Pectins fraction was the smallest in the flesh, achenes and the whole hypanthium. Differences in the polysaccharide composition, the content of AIS and galacturonic acid were found, depending on the species of rose hips. In addition, differences in composition were found in the case of wild growing and controlled cultivation hips. Rose hips achenes contain more total dietary fiber than the flesh, but the flesh contains more pectin fraction (soluble dietary fiber) while the achenes contain more cellulose (insoluble dietary fiber).*

**Keywords:** *rose hips; AIS; polysaccharides composition; galacturonic acid; sequential pectins extraction; plant cell wall; gas chromatography.*

### Introduction

Rose (*Rosa* L.) is a genus of shrubs belonging to the Rosaceae family, which includes numerous species of fruit trees and shrubs, wild growing, and cultivated for medicinal and decorative purposes. The genus *Rosa* includes about 120 plant species, which can be found almost everywhere in Europe, Asia Minor, and North Africa, mainly in the zone of warm and cool temperate climate in the northern hemisphere [1-3]. Among the most common species occurring in Poland

practically all over the country, there are *Rosa villosa* (*Rosa pomifera* Herrm), *Rosa canina* L., *Rosa rugosa* Thunb. [1, 2]. The dog rose (*R. canina*) is a particularly common and best-known species of rose [3, 5].

Commonly called the fruit, is, in fact, a pseudofruit (hypanthium (*p.* hypanthia). It is only underneath the colorful, fleshy pile that hard achenes, which are the correct fruit, are found. Flesh composes usually about 71%, and achenes about 19% of the total weight of the fruit [3].

Rose pseudofruit is a valuable source of vitamin C and ranges from 130 to 6700 mg/100 g [6]. The amount of vitamin C can be up to 40 times higher than in citrus fruits [7]. Three dog rose fruits are able to cover the daily requirement for vitamin C of the human body. In addition to L-ascorbic acid (KA), the fruit also contains dehydro-L-ascorbic acid (DHA), which is a product of L-ascorbic acid oxidation [3, 6]. The dog rose hips contain over 130 compounds, including tocopherols, amino acids, pectin, waxes, tannins, flavonoids, anthocyanins, phytosterols, carotenoids, organic acids, minerals, essential oils and oils with a high content of unsaturated fatty acids. Rose fruits, especially their achenes, contain large amounts of dietary fiber, including pectin [3, 4, 6-10].

Polysaccharides from the walls of plant cells play an important role in the food we consume as they compose dietary fibre [11]. The main components of plant cell walls are pectins, cellulose, and hemicelluloses [11, 12]. Pectins are a component of water-soluble dietary fiber. Their main properties include slowing down the process of sugar absorption from the gastrointestinal tract, as a result of coating the mucosa, which in turn, it slows the stomach emptying process and prevents the intestine from supplying too much sugar. This mechanism is important for people with diabetes, as the slower absorption of sugar reduces the need for insulin [13]. Homogalacturonan (HG) is the dominant type of polysaccharide that forms the structure of the pectin molecule. This compound is a linear polymer that is built from  $\alpha$ -D-galacturonic acid residues connected by  $\alpha$ -1  $\rightarrow$  4 glycosidic bonds [14-16]. Ramnogalacturonan I (RG I) accounts for about 20-35% of the structure of pectic substances found in the plant cell wall. RG I is made up of at least 100 D-galacturonic acid and L-rhamnose residues staggered, which form  $\alpha$ -D-galacturonopyranosyl- (1,2) - $\alpha$ -L-rhamnopyranose disaccharide, whose subsequent units connect with each other using 1,4-O-glycosidic bonds. The most common side chains are monomers or oligomers of  $\alpha$ -L-arabinofuranose and  $\beta$ -D-galactopyranose [14-16]. Ramnogalacturonan II (RG II) is a polysaccharide with a branched structure. Xylogalacturonan (XG) is a branched polymer whose main chain is composed of D-galacturonic acid residues connected with each other by  $\alpha$ -1  $\rightarrow$  4 glycosidic bonds [14-16]. Hemicellulose and cellulose are composing insoluble dietary fiber and are responsible for increasing peristalsis [17]. In terms of chemical structure, hemicellulose can be divided into two groups. The first consists of hexose saccharides (D-mannose, D-glucose, D-galactose) with a simplified formula ( $C_6H_{10}O_5$ )<sub>n</sub>, and the second includes pentose saccharides (D-xylan, D-arabinose)

with the general formula  $(C_5H_8O_4)_n$ . Hemicelluloses are characterized by solubility in dilute alkaline solutions, but they do not dissolve in water. The building blocks of hemicelluloses are xylans, mannans, galactans, arabinogalactans and galactomannans [18, 19]. Both fractions of dietary fibre are a substrate for bacterial fermentation in the colon [17].

Apple pomace, citrus albedo (the white part of the peel), beet pulp and sunflower shells are by-products of the agri-food industry and are a source of pectic substances (over 15% in dry matter). Apple pomace obtained in the juice industry contains 15% to 20% pectin, citrus – 30% to 35% and are the main source of pectin extraction. Among other sources of pectin, the following may be important: tomato skins 32%, watermelon seeds 20% [20], chicory root 13.1%, onion skins 13%, leek leaves 15%, lupine 1.5-7% [21]. Rose fruits can be considered as a valuable source of dietary fiber and may be an interesting alternative for obtaining pectins. On the other hand, dietary fiber present in rose may be responsible for beneficial activity of rose fruits on human health.

The aim of the study was to enrich the knowledge about the polysaccharide composition of dietary fiber which can be obtained from rose fruits.

## Experimental

### Materials

Rose hips from species: (*Rosa villosa* (*Rosa pomifera* Herrm) 'Karpattia', *Rosa canina* L., *Rosa rugosa* Thunb.) were obtained from the Research Institute of Horticulture at fruits maturity (September 2018). Standard fertilization was used during plants growth. Additionally, *R. canina* fruits growing wildly in Pabianice were collected. A part of the fresh fruits (every cultivar 3 kg) was divided into achenes and flesh, immediately. Whole rose fruits, achenes and flesh were subjected to a freeze-dried process (Christ Alpha 1-2LDplus, Martin Christ, Germany, 48 h, 0.20 mbar). The dried material was crushed with a mill using liquid nitrogen. Dry matter determination was performed at 105 °C for 3 hours, the dry matter of the samples was 97-99%.

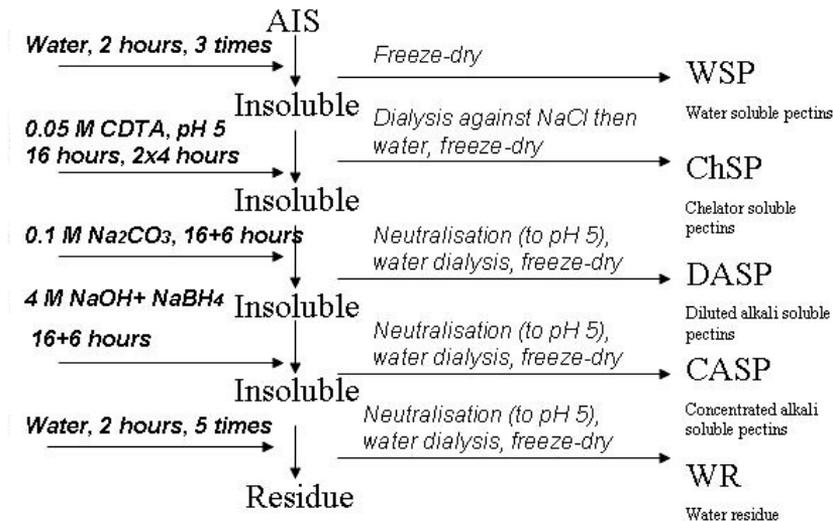
### Methods

#### *Alcohol Insoluble Solids (AIS)*

Alcohol insoluble solids were prepared according to Renard [22]. Approximately 5 g of powdered samples (3 rose cultivars; whole dried pseudofruits, separated achenes and separated flesh) were mixed with 50 ml of 70% ethanol. Incubation was carried out for 1 hour at room temperature. After incubation, the samples were filtered using a vacuum pump, washed several times with 70% ethanol. Then, the samples were washed twice with 25 ml of acetone/water/acetic acid mixture at 60:39:1 ratio, acetone/water mixture at 80:20 ratio and 100% acetone. The resulting solids were dried at 40°C and weighed. All in duplicate.

### Sequential polysaccharide extraction

Extraction was performed according to Kosmala et al. [23] (Figure 1). AIS samples (1.5 g) were extracted three times with 45 ml of water (2 h, 25°C) and the solids were separated by centrifugation from the supernatant. The supernatants from each extraction were combined and freeze-dried (WSP, Water Soluble Pectins). The residue was extracted with 45 ml of 0.05 mol/l CDTA at pH 5 (16 h + 2 × 4 h, 25°C), centrifuged, supernatants were combined and dialyzed in dialysis tubing MWCO 12400 (Sigma-Aldrich, Poland) against 0.1 mol/l NaCl solution and then against water (ChSP, Chelating Agent Soluble Pectins). The residue was extracted with 0.1 mol/l Na<sub>2</sub>CO<sub>3</sub> (16 h + 6 h, 20°C), the supernatants were combined and neutralized with acetic acid to pH 4-5 and then dialyzed against water and freeze-dried (DASP, Diluted Alkali-Soluble Pectins). The residue was extracted with 4 mol/l NaOH + 1 g/l NaBH<sub>4</sub> (16 h + 8 h, 20°C) supernatants were combined and neutralized with acetic acid to pH ~ 4, then dialyzed against water and freeze-dried (CASP, Concentrated Alkali-Soluble Polysaccharides). The residues were extracted with 45 ml portions of water until the alkaline reaction was absent. The supernatants were combined, neutralized to pH 4-5, dialyzed against water and freeze-dried (Water residue). The solid residue after extraction was also freeze-dried (Residue).



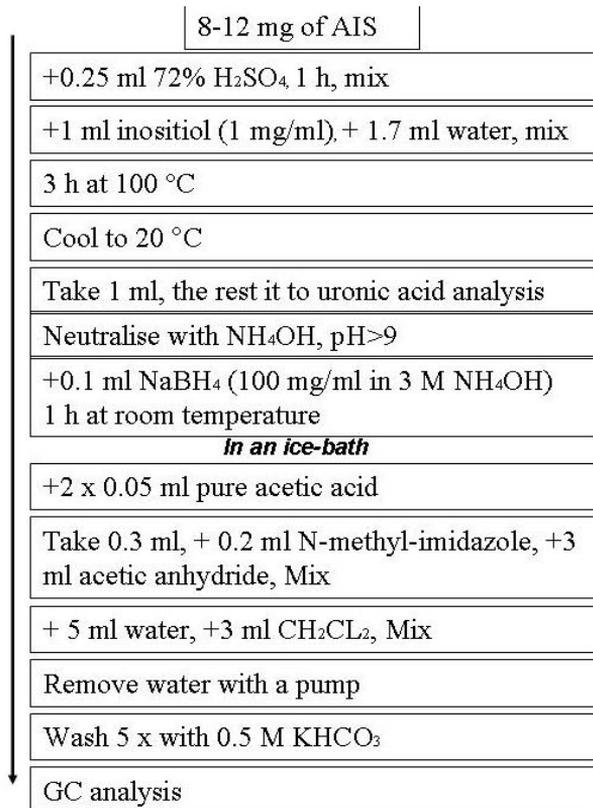
**Figure 1.** Sequential pectin extraction

### Determination of polysaccharides content in the fruit

#### a) Cell walls hydrolysis

Hydrolysis was carried out according to Kosmala et al. [24] (Figure 2). Approximately 8-12 mg AIS were weighed into test tubes, 250 µl of 72% H<sub>2</sub>SO<sub>4</sub> solution was added and mixed. The samples were incubated for 1 hour at room

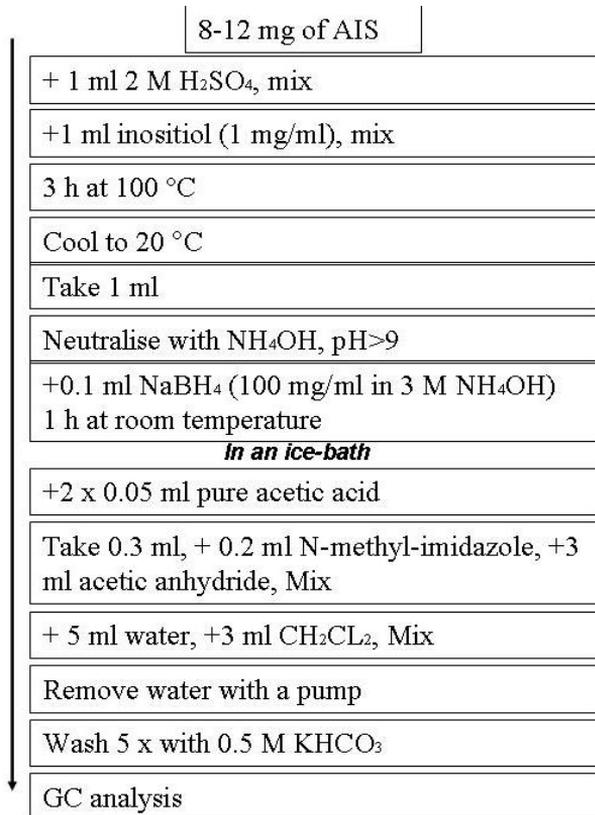
temperature, then 1 ml of inositol (1 mg/ml) solution and 1,7 ml of distilled water were added. The samples were heated at 100°C for 3 hours and then cooled to room temperature. The tubes were transferred into 1 ml of each sample and neutralized with 0.3 ml of 25% NH<sub>4</sub>OH to make the solution pH greater than 9. Then, 0.1 ml of NaBH<sub>4</sub> solution (100 mg/ml in 3 mol/l NH<sub>4</sub>OH) was added and allowed to stand for one hour at room temperature. After incubation, 0.05 ml of pure acetic acid was added twice to the samples. 1 ml of sample was taken and transferred to a glass test tube. Then 0.2 ml of N-methyl-imidazole and 3 ml of acetic anhydride were added. Then 5 ml of cold distilled water and 3 ml of CH<sub>2</sub>Cl<sub>2</sub> were added to the samples and mixed. The resulting aqueous phase was removed and the organic phase was washed four times with 5 ml of 0.5 mol/l KHCO<sub>3</sub> solution. The solutions remaining after washing were dissolved in 0.5 ml of CH<sub>2</sub>Cl<sub>2</sub> and analyzed by GC. The procedure allowed total of cellulose hydrolysis into glucose. CGlc – cellulosic glucose was calculated as total glucose minus non-cellulosic glucose (NGlc). All in triplicate.



**Figure 2.** Cell wall hydrolysis with pre-hydrolysis in 72% sulphuric acid, 1 hour to hydrolyze cellulose

### b) Pectins hydrolysis

Approximately 8-12 mg AIS were weighed into test tubes, 1 ml of inositol solution at a concentration of 1 mg/ml was added and mixed (Figure 3). Then 1 ml of 2 mol/l H<sub>2</sub>SO<sub>4</sub> solution was added and the contents were mixed. The samples were heated at 100°C for 3 hours. The subsequent hydrolysis procedure was the same as the procedure described in (a). The procedure did not allow total cellulose hydrolysis into glucose but only hydrolyzed pectins and hemicelluloses giving NGlc – non-cellulosic glucose. All in triplicate.



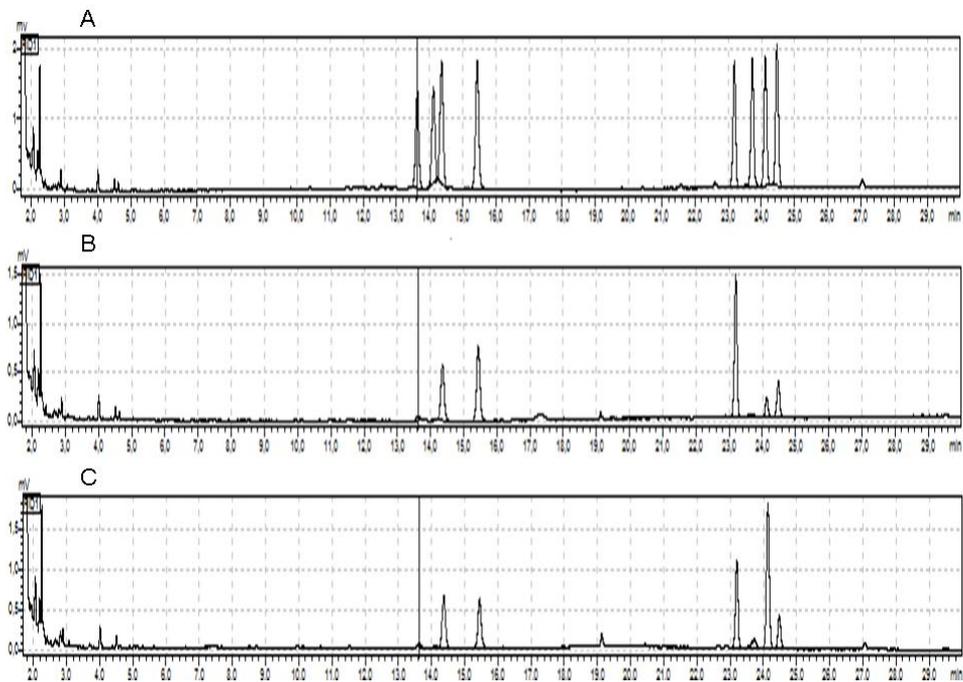
**Figure 3.** Pectins hydrolysis (Saeman procedure)

### c) Gas chromatography

The separation was performed using the Shimadzu GC – 2010 Plus chromatograph (Tokyo, Japan) (Figure 4). The chromatograph was equipped with the AOC – 20i autosampler and Hydrogen Peak Scientific hydrogen generator (Inchinnan, Scotland, UK). The gases used for the separation were hydrogen, helium, and air. The chromatographic separation was performed on a Zebtron ZB-5 column (Phenomenex, USA) with dimensions of 30 m × 0.25 mm × 0.25 μm.

Detection was carried out using a flame ionization detector (FID). The gas flow rate for the FID detector was respectively: 30 ml/min for helium, 40 ml/min for hydrogen and 400 ml/min for air, respectively. The analysis was performed in the temperature gradient: 15 minutes to 170°C, then 200°C at 6°C/min, then 10 minutes at 200°C and cooling to the initial temperature for 10 minutes. The temperature of the injection was 250°C, and the injection volume was 1 µl in Split mode (ratio 1:25). The sample flow rate through the column was 1.53 ml/min (total flow of 42.8 ml/min, purge flow 3.0 ml/min). Identification of the sugar alditols contained in the samples was made on the basis of the peak retention times on the chromatograms of the samples with the chromatograms of the standard solutions (rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, all Sigma-Aldrich, Poland). The sugars concentrations were calculated as follows: standard sugar concentration divided by (standard sugar area divided by inositol area of the same run) multiplied by (analyte area divided by inositol area of the same run);

$C_x = [C_w / (A_w / A_{\text{inositol1}})] \times (A_x / A_{\text{inositol2}})$ . Inositol was used as internal standard (Sigma-Aldrich, Poland).



**Figure 4.** GC-FID chromatographs. At 13.6 min rhamnitol peracetyl; 14.1 min fucitol peracetyl; 14.4 min arabinitol peracetyl; 15.4 min xylitol peracetyl; 23.2 min inositol peracetyl; 23.7 min mannitol peracetyl; 24.1 min glucitol peracetyl; 24.5 min galacitol peracetyl. A – standard mixture, B – *Rosa canina* Saeman procedure, C – *Rosa canina* with pre-hydrolysis

### **Galacturonic acid**

The analysis was carried out according to Blumenkrantz and Asboe-Hansen [25]. In tubes, 0.5 ml of the sample (obtained by procedure a) was mixed with 3 ml of 0.0125 mol/l sodium tetraborate reagent in concentrated sulphuric acid (96%) and carefully mixed. The tubes were placed in a thermostatic water bath at 80°C for 20 minutes. After this time, the reaction was stopped by placing the tubes in a water bath until the room temperature was reached. Then 50 µl of MHDP solution was added, the contents of tubes were mixed and absorbance was measured at a wavelength  $\lambda = 520$  nm exactly after 10 minutes (at spectrum maximum). Galacturonic acid was used as a standard (Sigma-Aldrich, Poland). All in triplicate. Calibration curve:  $ABS = 0.01x + 0.0852$ ,  $R^2 = 0.9933$ .

### **Statistical analysis**

One way anova with Duncan test (Statistica 12, Statsoft) were used.

## **Results and Discussion**

### **Alcohol insoluble solids (AIS)**

In all analyzed rose species, the amount of AIS was the highest for achenes, and the lowest for flesh (Table 1). The highest amount of AIS was found in achenes of wild *R. canina* which amounted to 842 mg/g, and the smallest in the flesh of *R. villosa* 'Karpattia', for which the AIS content was 268 mg/g. The flesh of the *R. canina* hip from the cultivation contained much more AIS than the fruit pulp growing wild. In contrast, the achenes of cultivated *R. canina* contained a smaller amount of AIS than the fruits of this rose growing wild. The content of alcohol insoluble solids for the whole fruit of these rose was comparable. For the pulp and whole fruit of *R. villosa* 'Karpattia', the smallest amount of AIS was demonstrated compared to other analyzed species, meanwhile, the achenes of this species contained more AIS than others and their content was comparable to the AIS content of wild *R. canina* achenes. Comparing the obtained results with the results of studies carried out on other fruits, it can be seen that rose hips have a much higher share of AIS – from 480 ( 'Karpattia') to 597 mg/g (*R. canina* wild), compared to plum fruit, in which the AIS content is 17.7-24.9 mg/g fresh weight, which corresponds to AIS content from 122-154 mg/g dry matter [24], or cherry fruit, for which it is in the range of 78-97 mg/g dry matter [23]. In the studies of Milala et al. [4] on the *R. villosa* 'Karpattia' hips, it was shown that the dried pulp contained from 27.5 to 29.7 g/100g of dietary fiber, and achenes from 70.5 to 72.1 g/100 dry matter. This confirms the results presented in the article and it can be concluded that the fruits of rose are an exceptionally rich source of dietary fiber. In addition, the achenes contain much more dietary fiber than the flesh.

### **Composition of alcohol insoluble solids**

The main saccharide in AIS was glucose derived from cellulose hydrolysis (Table 1) (Figure 4). Its highest content was 199 mg/g for the *R. rugosa* achenes, and the lowest for the flesh of the wild *R. canina*, for which it amounted to 79 mg/g. The content of non-cellulosic glucose was much lower than that

cellulosic glucose and most of it was detected in the flesh, where the largest amount was 30 mg/g for wild *R. canina*, and the lowest in achenes where it was 5 mg/g for both *R. canina* wild growing and from controlled cultivation.

In the achenes samples of all the analyzed roses, xylose was the second most abundant sugar after cellulosic glucose. The highest content of xylose was 168 mg/g for *R. canina* achenes from controlled cultivation and 141 mg/g for wild *R. canina* achenes. In *R. rugosa* achenes its content was 131 mg/g and in achenes of *R. villosa* 'Karpattia' 130 mg/g. The xylose content was the highest for wild *R. canina* and the lowest for wild species and was 88 and 59 mg/g respectively.

In flesh for all studied rose species, the second most dominant saccharide after cellulosic-glucose was arabinose. The content of this saccharide was the highest for *R. canina* from cultivation and the lowest for *R. rugosa*. For all analyzed samples the mannose content was at a similar level both for the flesh, achenes and the whole fruit. In the case of samples of *Rosa canina* from controlled cultivation, the most mannose was found in the achenes, for samples of *R. rugosa* and *R. villosa* 'Karpattia' in the flesh and for samples of wild *R. canina* both in the flesh and in the whole fruit.

The flesh of the analyzed rose species contained about twice as much galactose as the whole fruit and much more than the achenes. The highest amount of galactose was found in the flesh of *R. villosa* 'Karpattia' where its content was 66 mg/g, and the lowest content was found in the achenes of wild growing *R. canina* – 5 mg/g. Fucose content in all analyzed samples did not exceed 2 mg/g, and in achenes of *R. villosa*, 'Karpattia' and wild *R. canina* wild was not detected.

**Table 1.** AIS content [mg/g], polysaccharide composition and galacturonic acid content [mg/g AIS] in the rose hips

	AIS yield n=2	Rha n=3	Fuc n=3	Ara n=3	Xyl n=3	Man n=3	Gal n=3	NGlc n=3	CGlc n=3	GalA n=3
CW	563 ±4de	4 ±1bcd	2 ±1ab	51 ±19bcd	59 ±29cd	13 ±5	27 ±8bcdef	14 ±1cd	117 ±47cd	92 ±16bcd
CF	453 ±1e	7 ±0ab	2 ±1a	92 ±20a	38 ±10de	15 ±1	47 ±10ab	19 ±4c	137 ±28abc	111 ±27b
CA	773 ±25bc	4 ±1cd	1 ±1abc	19 ±2de	168 ±9a	16 ±3	12 ±2def	5 ±0d	188 ±13ab	30 ±9g
RW	550 ±14e	2 ±1d	1 ±1abc	18 ±0de	70 ±14c	10 ±2	12 ±1def	18 ±3c	111 ±10cd	71 ±13def
RF	403 ±8g	6 ±3abc	2 ±1ab	61 ±17abc	32 ±8de	21 ±8	31 ±9bcde	25 ±5b	151 ±51abc	141 ±9a
RA	749 ±18c	3 ±1cd	1 ±0abc	22 ±6de	131 ±5b	16 ±1	14 ±4cdef	6 ±1d	199 ±18a	50 ±1fg
VW	480	5	1	40	71	7	32	15	128	78

	±11f	±1abc	±0abc	±10cde	±1c	±2	±8bcd	±1c	±27abc	±15cde
<b>VR</b>	269	9	2	83	33	15	66	15	140	116
	±5i	±3a	±1a	±33ab	±14de	±7	±26a	±1c	±64abc	±6b
<b>VA</b>	808	3	0	17	130	13	11	6	155	56
	±44b	±0cd	±0bc	±3de	±13b	±4	±2ef	±1d	±20abc	±3efg
<b>WW</b>	597	3	1	40	88	11	20	8	133	97
	±31d	±0cd	±0abc	±0cde	±0c	±0	±0cdef	±2d	±2abc	±19bc
<b>WF</b>	341	4	1	67	21	11	38	30	79	108
	±1h	±1bcd	±0abc	±26abc	±6e	±5	±14bc	±5a	±21c	±24b
<b>WA</b>	842	2	0	9	141	7	5	5	150	33
	±36a	±0d	±0c	±1e	±14ab	±0	±0f	±1d	±19abc	±7g
<b>P</b>	0.00	0.002	0.029	0.000	0.000	0.104	0.000	0.000	0.011	0.000

CW – *Rosa canina* whole, CF- *R.canina* flesh, CA – *R. canina* achenes, RW – *Rosa rugosa* whole, RF – *R.rugosa* flesh, RA – *R. rugosa* achenes, VW – *Rosa villosa* whole, VF – *R.villosa* flesh, VA – *R. villosa* achenes, WW – wild *Rosa canina* whole, WF – wild *R.canina* flesh, WA – wild *R. canina* achenes. Mean values ± standard deviation, AIS – alcohol insoluble solids, Rha – rhamnose, Fuc – fucose, Ara – arabinose, Xyl – xylose, Man – mannose, Gal – galactose, NGlc – non-cellulosic glucose, CGlc – cellulosic glucose, GalA – galacturonic acid, values in columns marked with different letters are different at  $P > = 0.05$ .

The highest amount of monosaccharides was found in the achenes from the controlled cultivation of *R. canina* – 413 mg/g AIS, while the lowest amount for the flesh of this species wild growing. For *R. rugosa* and *R. canina*, both wild and cultivated, the highest amount of monosaccharides was found in the achenes, while in the case of *R. villosa* ‘Karpattia’, the highest number of monosaccharides was found in the flesh. From all analyzed samples, wild *R. canina* flesh had a significantly lower content of monosaccharides than the pulp of other studied species, however, in the case of the whole fruit, the highest content was found. Rose fruits may be considered a high source of dietary fiber, that is also confirmed by other research [4].

The polysaccharide composition of rose hips, in comparison to other fruits, differs in the number of individual saccharides. For example, rose hips contain less galactose – from 12 (*R. rugosa*) to 32 mg/g (*R. ‘Karpattia’*) in comparison to plum fruits, in which its amount is 84-164 mg/g, depending on the variety [24]. Rose fruits are characterized by a higher presence of xylose in their composition – from 59 mg/g in the case of wild *R. canina* to 88 mg/g in the case of *R. canina* from cultivation, and in plum fruits its amount is in the range of 11-18 mg/g [24]. The content of other monosaccharides and their ratio in both fruits is similar. Compared to cherry fruit, in which the xylose content is from 9 to 13 mg/g [23], rose hips have a much higher xylose content, up to 88 mg/g in the case of wild *R. canina*. The content of arabinose in rose hips is lower – from 18 mg/g for *R. rugosa* to 51 mg/g for *R. canina* from cultivation, and in cherry fruits is in the range of 66-72 mg/g. The ratio and content of other saccharides in rose and cherry fruits are very similar.

The flesh samples were characterized by the highest content of uronic acids, calculated as galacturonic acid, in all the analyzed samples. In the samples of the whole fruit their content was slightly lower, and the lowest for the samples of achenes. The highest content of galacturonic acid was detected in *R. rugosa* flesh and was 141 mg/g AIS, and the lowest in *R. canina* achenes from cultivation for which it was 30 mg/g AIS (Table 1).

Analyzing the obtained results of uronic acid content, calculated as galacturonic acid, it can be concluded that the flesh of rose hips contains about 3 times more uronic acids than the achenes. Higher content of galacturonic acid indicate high content of pectins, as galacturonic acid is the main component [14-16]. Only for *R. villosa* 'Karpattia' samples, the flesh was found to contain twice as much galacturonic acid as the achenes.

The content of galacturonic acid in rose hips is lower than in other fruit species. Rose hips contain about 2.5 to 4 times less galacturonic acid than plum fruits, for which its content is 222-387 mg/g [24] but plums are known for a very high content of dietary fiber. For cultivated *R. canina*, *R. rugosa* and *R. villosa* 'Karpattia' most of the polysaccharide components in AIS are contained in the fruit flesh. However, the percentage differences between the flesh and the achenes are very small.

Only in the case of *R. canina* 'Karpattia' the flesh contains much more polysaccharide components – 48%, and achenes – 39%. The flesh of this species was characterized by the highest amount of polysaccharide components, while the lowest amount was determined for the whole *R. rugosa* fruit. For the flesh and achenes of *R. canina*, *R. rugosa* and *R. villosa* 'Karpattia' the percentage of polysaccharide components were higher than for the whole fruit. However, for the wild *R. canina* sample, the most polysaccharide components were found in the whole fruit.

### ***Sequential polysaccharide extraction***

The main pectin fraction in *R. canina* hips was alkaline soluble polysaccharides (CASP), whose content was 230 mg/g (Table 2), which a fraction of hemicelluloses. The second fraction was water soluble pectin (WSP), which amounted to 191 mg/g AIS, which is a fraction of pectin that is the easiest to extract. The content of the fraction of weak alkaline soluble pectins (DASP) was 67 mg/g, and the residue from the extraction of residual 'hairy' regions of pectin (Water residue) was 93 mg/g. The lowest content was detected in the case of fractions of pectins soluble in the chelating agent (ChSP) and amounted to 46 mg/g. The residue after sequential pectin extraction was 450 mg/g, the residue composes mostly of cellulose, an insoluble part of dietary fiber.

**Table 2.** The content of pectin fractions in rose flesh, achenes and whole fruit

	<i>Rosa canina</i> flesh	<i>Rosa canina</i> achenes	<i>Rosa canina</i> whole fruit
Fraction	Yield [mg/g]		
WSP	294	51	191
ChSP	62	27	46
DASP	128	42	67
CASP	268	196	230
Water residue	88	55	93
Residue	241	666	450

WSP – Water soluble pectins, ChSP – Chelating agent soluble pectins, DASP – Diluted alkali soluble pectins, CASP – Concentrated alkali soluble polysaccharides.

In the rose flesh fraction, the pectin fraction of WSP was the largest part of pectin and amounted to 294 mg/g, while the ChSP fraction was the lowest – 62 mg/g. For the seed sample, the CASP fraction was the main fraction, its content was 196 mg/g, and the lowest amount, similarly to the flesh, was the ChSP fraction – 27 mg/g. The amount of CASP fraction in the flesh, whose content was 268 mg/g, was slightly lower than the content of WSP fraction, which was the main pectin fraction. The content of Water residue fraction was higher in the flesh, where it amounted to 88 mg/g, and for achenes, its content was equal to 55 mg/g.

In the flesh of the *R. canina* hip, there is about 6 times more WSP fraction than in the achenes of this plant. There is more than twice as much ChSP fraction in the flesh as in the achenes and 3 times as much DASP fraction. Achenes contain most of the Residue fraction, which consists mostly of cellulose. Its amount was 666 mg/g for achenes, 450 mg/g for whole fruit and 241 mg/g for flesh. The extraction residue (Residue) is the largest part of the composition of achenes and the whole *R. canina* fruit, it amounts to 64.2% and 41.8%, respectively (Figure 3). In flesh fraction, the highest percentage share of the total pectin fraction is the WSP fraction – 27.2%. The lowest content for flesh, achenes and the whole fruit is the ChSP fraction, which is respectively 5.7%, 2.6%, and 4.3%.

Both in the whole fruit, achenes, and flesh, the CASP fraction constitute about 20% of all polysaccharide fractions. The content of ChSP fraction is less than 5.7% and the content of Water residue fraction is less than 8.6%. The biggest differences in terms of fraction content are noticeable in the case of WSP and DASP fractions for flesh and achenes. Flesh contains 27.2% of WSP fraction and 11.8% of DASP fraction, while the achenes contain only 4.9% of WSP fraction and 4.1% of DASP fraction.

The separation of rose hips into flesh and achenes fraction allowed on obtaining dietary fiber preparations with different composition. Seed fraction was mostly composed of cellulose, the main component of insoluble dietary fiber, and

the content of pectic galacturonic acid was significantly lower than in flesh fraction. Also in the seed fraction hemicelluloses built from xylans were in higher quantity [18, 19]. In the flesh fractions there was more soluble dietary fiber represented by higher content of galacturonic acid and also arabinose, rhamnose, galactose, which are a building blocks of homogalacturonan and rhamnogalacturonan I [14-16].

*R. canina* fruits differ significantly in the content of individual pectin fractions in comparison to other fruits. In the whole rose fruit, there is much more WSP fraction than in cherry fruit (68 mg/g) [23] or plum (86-117 mg/g depending on the variety) [24]. In the case of ChSP fraction in the rose hips its content is lower than in case of cherry (222 mg/g) and plum (63-154 mg/g). The content of DASP fraction in plum and cherry fruits is much higher than in *R. canina* hips, respectively 204-308 mg/g and 158 mg/g. In the composition of pectin fractions in rose hips, the content of CASP fractions is comparable to the content of this fraction in plums (138-223 mg/g) and cherries (181 mg/g). The *R. canina* hips are characterized by a slightly higher content of Water residue fraction in comparison to cherries (58 mg/g) and a much lower content in comparison to plums (123-136 mg/g). In fruit, the residue from sequential extraction of pectins is about 3 times higher than in the cherry (161 mg/g) or plum (143-171 mg/g). The results suggest that by simple separation of rose hips into flesh and seed fraction we can obtain dietary fiber preparation with different composition of soluble and insoluble fraction, with flesh containing more the first fraction (more pectins) and achenes containing more of the second fraction (more cellulose and hemicelluloses). As rose fruits are a rich source of dietary fiber it may be worth considering as an alternative source of pectins. Especially, as flesh fraction was proven to be a high source of pectin fractions (WSP, ChSP, DASP).

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