MOLAR MASS CHARACTERISTICS OF CHERRY TREE EXUDATE GUMS OF DIFFERENT SEASONS

GINA AMARIOAREI, MARIA LUNGU,* SORIN CIOVICĂ

"Gheorghe Asachi" Technical University, Faculty of Chemical Engineering and Environmental Protection, Department of Natural and Synthetic Polymers,
71 A, Mangeron Bvd., 700050, Iasi, Romania
**Apollonia University of Iasi, 11, Pacurari Str., 700511, Iasi, Romania*

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The polysaccharide components of cherry tree exudate gum (*Prunus cerasus*, *Prunus avium*) belong to the arabinogalactan group. There are structural variations in the proportion of monosaccharides, molar ratio and glycosidic linkages, which determine the properties and use of exudate gums. In this paper, Size Exclusion Chromatography (SEC/GPC) is applied for determining polydispersity and molecular mass (Mw) of the cherry tree exudate gum of *P. avium*, *P. cerasus* and egg plum (*P. domestica*).
The intrinsic viscosity values of cherry tree EAL gum solutions were also obtained, confirming the existence of a compact internally crosslinked structure of the exudate gum polysaccharide.
The results of Brookfield viscosity characterization outline the essential characteristics of the cherry gum exudates.

Keywords: exudate *Prunus* gums, SEC/GPC chromatography, molecular weight, polydispersity

INTRODUCTION

Natural gums have been used as thickening and stabilizing agents from the oldest times and are known to possess a unique range of functionalities.[1] They have been studied extensively using different techniques: hydrophobic affinity chromatography,[2-5] anion-exchange chromatography,[3] gel-permeation chromatography,[6] high-performance size-exclusion chromatography[5] and flow-field flow fractionation.

The arabic gum is the most studied representative of the arabinogalactan class, due to its practical and industrial importance. The arabic gum was separated into three fractions by applying the hydrophobic affinity chromatography method.[4,5] Usually, the arabic exudate gum has a very low protein content (0.35%), and is referred to as arabinogalactan (AG), representing 88.4% of the total gum and having a molecular weight of 3.8×10^5 Da. The second fraction representing 10.4% of the total gum is AG–protein complex (AGP) with a molecular mass of 1.45×10^6 Da and a protein content of 11.8%. The smallest fraction (1.2% of total gum) is represented by a low molecular weight glycoprotein (GP), with a molecular mass of 2.5×10^5 Da and a protein content of 47.3%.[7]

The *Prunus* genus belongs to the *Rosaceae* family and includes several fruit-bearing trees e.g. the peach (*P. persica*), damson (*P. insitia*), egg plum (*P. domestica*), cherry (*P. avium*, *P. cerasus*, *P. virginiana*), and almond (*P. amygdalus*). These species produce exudate gum as a result of a disease (gummosis) on their fruit and trunk, especially after mechanical injury followed by microbial attack of *Polyporus* sp.[8] Gums are produced by pathogenic degradation of certain cells or whole plant tissues. Gum production starts in certain cell groups derived from cambium, which are not differentiated into xylem cells and remain in the parenchymatous state, and then spreads to the adjacent differentiated xylem tissues, where the walls are autolyzed, so that the gum is formed inside the cambium. The gum swelling may break the cortex of the cherry tree and the gum exudes through this split.[9]

The polysaccharide components of cherry tree exudate gum (*P. cerasus*, *P. avium*) belong to the arabinogalactan group[10] and include arabinose (Ara), xylose (Xyl), galactose (Gal), glucopyranosyluronic acid (GlcpA), 4-Me-GlcpA, with lower amounts of rhamnose (Rha) and mannose (Man). There are structural variations based on the proportion of monosaccharides and glycosidic linkages. The molar ratio of monosaccharides D-glucuronic A, D-Gal, D-Man, L-Ara, D-Xyl, L-Rha in egg plum tree exudate gum is 1:2:1:3:0.023:0 and in cherry tree exudate gum 1:2:1:6:3:0.[11] The peach tree exudate gum polysaccharide was found to be composed of Ara, Xyl, Man, Gal and glucuronic acid (and its 4-O-methyl derivative) in 36:7:2:42:13 molar ratio.[12] Advanced hydrolysis of plum gum and cherry gum (which can be achieved by heating their aqueous solutions in acidic or alkaline reaction medium) leads to D-galactose and other aldobionic acids, consisting of a residue of D-glucuronic acid and lower amounts of D-mannose, linked together through C_1–C_2 β-glycosidic linkages.[11]

The chemical composition and molecular weight (Mw) of the gums of *P. avium* and *P. cerasus* aqueous extract (EA) and lyophilized aqueous extract (EAL) have not been thoroughly investigated.

This paper analyses the characteristics of cherry tree exudate gum of *P. avium* and *P. cerasus* by using Size Exclusion Chromatography and viscosity measurement to make a comparison with the results obtained for other group members (arabinogalactan group) and with two other important exudate gums (karaya and tragacanth gum).

EXPERIMENTAL

Materials and methods

Fresh cherry tree exudate gums of *P. cerasus* and *P. avium* were collected in spring, summer and winte, in a Romanian fruit-growing area, from the tree trunk as partially dried transparent tears. The gum free of biological impurities was dried and kept in contact with air. After that it was ground to a fine powder, allowing faster hydration and homogeneous dispersion.

A gum solution of 2% (w/v) in water was prepared by stirring for 16 h at room temperature. The EA sample was obtained by removing insoluble fragments by filtration, and then the solution was lyophilized (sample EAL).

Determination of molecular weight (Mw) and polydispersity

The GPS/SEC technique is frequently used for the analysis of polymers and biological materials, and is based upon the molecular size of the solute.

EA and EAL were first dissolved in water and then diluted with 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 solution (pH 7) as eluent to a final concentration of 0.10 mg/ml. Both solutions were filtered through a 0.22 μm filter (Millipore) and GPC/SEC analysed by using a Varian gel permeation chromatography PL 120 system, with three columns (3×PL aquagel-OH 300×7.5 mm^2) and with a differential refractive index detector (DRI).

The analysis parameters were the following: flow rate = 1 ml/min, t = 25 °C, sample injection volume = 100 μl and run time analysis = 40 min. For calibration, polyethylene glycol standards with MW between 895500 and 106 were used. The molecular mass distribution was calculated with the Cirrus GPC computing device.

Viscosity measurement

Viscosity measurement of spring EAL *P. cerasus* cherry gum solution with a 5% concentration (w/v) was carried out by using a Brookfield DV-I+4 spindle 3 equipment at 50 and 100 rpm at 23 °C, the EAL solution native pH being ≈ 6.

The measurement was repeated after the solution was heated to 45 °C and then cooled to 23 °C.

In another series of measurements, a 2% solution was used to investigate the influence of pH on the viscosity. The applied pH values were the following: 4.00, 7.18, 11.47; the values were chosen randomly.

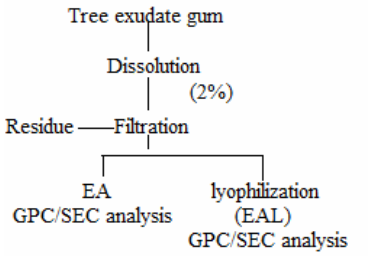

Figure 1: Extraction scheme of cherry tree exudate gum (*P. cerasus*, *P. avium*)

Viscosity measurement: intrinsic viscosity

Summer EAL exudate cherry gum of *P. cerasus* and *P. avium* were dissolved in water and measurements were done after 24 h. Viscometric measurements were carried out using an Ubbelohde viscometer V_2, with K = 0.003 at 25 ± 0.01 °C. Several dilutions were made *in situ*. The intristic viscosity [η] is determined by using a Huggins plot of η_{sp}/c versus c, where c is concentration and η_{sp} is specific viscosity.

RESULTS AND DISCUSSION

The molecular weight distribution profile and the average molecular weight of EA gums depend on the tree species and on the season when they were collected (Figure 3, Table 1).

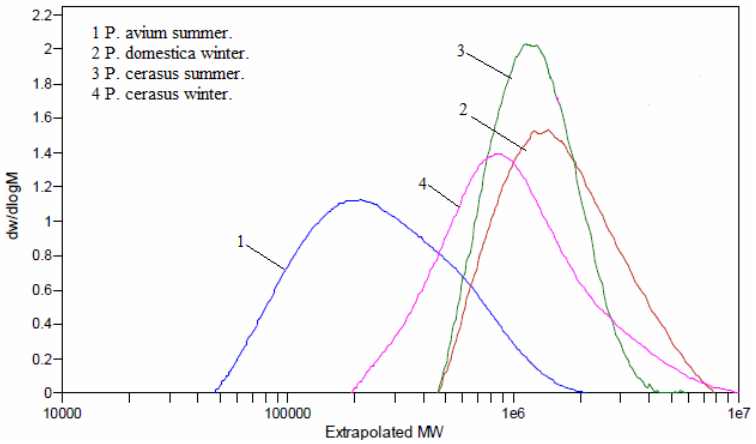

Figure 2: GPC/SEC molecular mass distribution profile of EA exudate gum

Table 1
Molecular weight parameters of *P. cerasus*, *P. avium*, *P. domestica* gum of different seasons, determined by GPC/SEC chromatography

Nr	Exudate gum	M_w/M_n	$M_p \times 10^5$ g/mol	$M_n \times 10^5$ g/mol	$M_v \times 10^5$ g/mol	$M_z \times 10^5$ g/mol	$M_w \times 10^5$ g/mol	Mz+1 g/mol
1	*P. avium* summer (EA)	1.57	2.47	1.90	2.78	4.75	2.99	6.71
2	*P. domestica* winter (EA)	1.36	14.37	13.09	16.96	24.89	17.8	33.18
3	*P. cerasus* summer (EA)	1.18	12.89	11.00	12.73	15.67	13.0	18.67
4	*P. cerasus* winter (EA)	1.61	8.86	7.39	10.97	20.89	11.9	34.16
5	*P. avium* summer EAL	1.4	0.47	0.38	0.53	0.94	0.56	16.13
6	*P. cerasus* summer EAL	2.5	3.88	2.01	4.26	16.39	5.08	32.71
7	*P. cerasus* winter EAL	1.8	0.39	0.29	0.48	1.25	0.54	2.34

The process of molecule solvation or aggregate formation because of the H-bonds, observed for karaya gum, probably determined by the presence of acetyl groups in the structure,[13] cannot be excluded.

The polydispersity (M_w/M_n) of *P. cerasus* winter EA gum is larger in comparison with the other EA gums analysed. It can be observed that the characteristics decrease significantly for *P. cerasus* summer gum – from 1.61 to 1.18.

The average molecular weight decreases in the following order: *P. domestica* winter, *P. cerasus* summer, *P. avium* summer and from summer to winter in the case of *P. cerasus*.

P. cerasus cherry tree EAL spring exudate has the highest polydispersity index value in comparison

with the other lyophilized exudate gums. The lyophilisation process diminishes the (M_w/M_n) value of *P. avium* summer gum, but increases significantly that of *P. cerasus* gum. The average molecular weight also decreases, being correlated with polydispersity modification and depending on tree species and season.

A comparison of the molecular mass of *P. cerasus* cherry tree gum with those of tragacanth gum (8.4×10^5 Da), karaya gum (16×10^6 Da),[13] arabic gum ($5\text{-}6.5\times10^5$ Da) and *P. cerasus* peach tree exudate gum (5.61×10^6 Da)[12] could indicate *a priori* the area of application, if this property is connected with rheological properties.[17]

The results of the GPC/SEC analysis of EAL samples are presented in Figure 3.

The profile of molecular weight distribution curves (Figures 2 and 3) demonstrates the evolution of the analysed molecular parameters of the EA and EAL gum solutions. The molecular mass of the EA was influenced by the associations of molecular chains existing in the sample, after lyophilization these associations were destroyed – the effective diameter of the macromolecule being smaller it diffused more easily into the column, so the molecular mass of the gums had different values.

Table 2
Molecular weight and the corresponding intrinsic viscosity of *P. cerasus* and *P. avium* cherry tree exudate gum EAL

Cherry tree exudate gum EAL	$M_n\times10^5$ (g/mol)	$M_w\times10^5$ (g/mol)	M_w/M_n	k	[η] (dl/g)
P. avium	0.38	0.56	1.47	0.30	3.84
P. cerasus	2.01	5.08	2.52	0.95	7.08

The real molecular mass can be considered closer to the values of EAL.

The interaction between the chains in solution may affect the elution profile and the molar masses can be overestimated[16] (e.g. the most evident is the case of *P. cerasus* winter with great differences between EA and EAL). Differences can be also observed in the other gums analyzed.

In Figures 2 and 3, depending on the type of gum, the width of the molecular mass distribution curve of EA and EAL gums highlights the existence of a large population of macromolecule chains – a phenomenon that is more pronounced as a result of lyophilization, as shown in Figure 3.

Intrinsic viscosity was determined using the Huggins plot and a second method of extrapolation, the Kraemer plot of $(\ln \eta_{rel})/c$. Summarized data are presented in Table 2.

The extraction process (solubilization and filtration) of cherry tree exudate gum being done in water, it interfered with the high native proportion of neutral sugar side-chains, and therefore resulted in a highly branched structure and formation of aggregates (similar to arabic gum and related to other exudate gums),[14,15] which led to the distortion of molecular mass characteristics and to the dependence of viscosity on concentration.

The intrinsic viscosity values of cherry tree EAL gum solutions confirm the existence of a compact internally crosslinked structure of the exudate gum polysaccharide.

Table 3
The influence of pH on Brookfield viscosity characteristics of 2% EAL (mPa.s)

Speed (rpm)	pH 4.00	pH 7.18	pH 11.47
50	56.6	75.00	54.6
100	65.8	79.00	61.1

Table 4
The dynamic viscosity characteristics of 5% EAL (mPa.s)

Speed (rpm)	Before heating	After heating
50	290	270
100	260	240

The volume of the aggregates was smaller than the total volume that could be occupied by the cherry tree exudate gum polisaccharide chains as individual coils.[15]
The pH of a 2% EAL cherry tree exudate gum solution varies between 5 and 6, and the viscosity remains quite stable over a broad range of values (pH 4-11) (Table 3), this behavior being similar with that of tragacanth gum.[18] The heating of the dispersion at 45 °C followed by cooling at 25 °C do not affect significantly the viscosity of *P. cerasus* cherry tree gum EAL (Table 4).

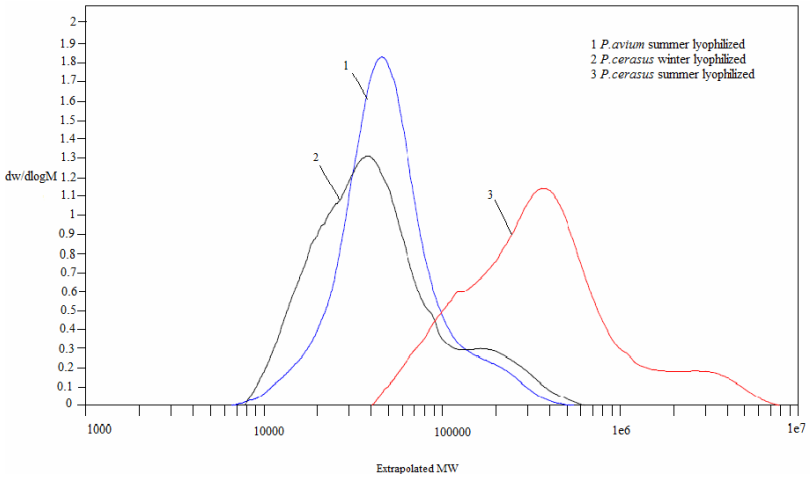

Figure 3: GPC/SEC molar mass distribution of EAL cherry tree gums of different seasons: 1 – *P. avium* summer lyophilized, 2 – *P. cerasus* winter lyophilized, 3 – *P. cerasus* summer lyophilized

CONCLUSION

The molar mass distribution and polydispersity of cherry tree exudate gums vary from one season to another, and also from a species to another. Generally, lyophilization of gum solutions decreases the average molecular weight in correlation with polydispersity modification.

The average molecular weight of these gums is of the same order of magnitude with other extensively used exudate gums, such as arabic, karaya and tragacanth gum.

The correlation of molecular mass of *P. cerasus* cherry gum with that of the other exudate gums may give an indication as to future industrial use and its performance in such applications.

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