

Influence of the Type of Hydrolysis on the Intrinsic Viscosity of *Lithraea Molleoides* Fruit Gum

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Abstract

Lithraea Molleoides fruit Gum (LMFG) is obtained from the total hydrolysis of the fruit. The hydrolysis used are three, thermal (LMFG-T), basic (LMFG-B) and acid (LMFG-A). The aim of hydrolysis is bond breaking and de-esterification of polysaccharide, with the consequent increase in solubility and decrease in molecular weight. Intrinsic viscosity measurement is standard for polymers and biopolymers, as well as being inexpensive. Through intrinsic viscosity, in addition, the hydrodynamic radius, molecular weight, shape factor, etc. can be determined. The objective of this work is to be able to evaluate the different hydrolysis through intrinsic viscosity measurements and, in turn, to study the performance of each one of them.

Keywords: *Lithraea Molleoides* fruit Gum, intrinsic viscosity, molecular weight, hydration value.

Introduction

Lithraea molleoides (Vell.) Engl. (Anacardiaceae), popularly called “molle”, “drinking molle”, “white molle”, “sweet molle”, and “chichita”. This plant is traditionally used in infusions as digestive and diuretic. This species develops in the phytogeographic region of the southern Chaco and specifically our study focuses on the province of San Luis, Argentina. In preliminary studies we have observed that the *Lithraea molleoides* fruit shows diuretic and gastro protective activity and is used as a natural sweetener in “mate” intake (Garro et al., 2015).

The intrinsic viscosity is determined by the Solomons-Ciuta

equation through measurements of flow times in capillary viscometers and the density of biopolymeric solutions in the Newtonian zone (Masuelli, 2018a). The intrinsic viscosity measurement used is single-point method by Solomons-Ciuta equation; from this is calculated the molecular weight, hydrodynamic radius, form factor, etc. (Masuelli & Illanes, 2014a).

The following equation corresponds to the Solomon-Ciuta 1962 simple point method,

$$[\eta] = \frac{1}{c} \sqrt{2\eta_{sp} - 2 \ln \eta_r} \quad (1)$$

where c is polymer concentration (g/cm³), η_r is relative viscosity,

η_{sp} is specific viscosity, and $[\eta]$ is intrinsic viscosity (cm^3/g). The $\eta_{sp} = \eta_r - 1$, and $\eta_r = \eta_r \rho_0 t_0$, where ρ t are density (g/cm^3) and draining time (s) of solution, and ρ_0 t_0 corresponding at pure solvent (Masuelli, 2013a).

Mark 1938 and Houwink 1940, M-H, links the intrinsic viscosity with molecular weight, and this equation is applicable to many biopolymers and synthetic polymers, is specialty used for molecular weight determination. The “ k ” and “ a ” parameters both vary with the nature of the biopolymer solution, temperature, pH, ions and dissolvent (Masuelli, et al. 2013b). The calculation of M-H parameters (Masuelli et al., 2013b, Masuelli, 2014b) is carried out by the plot representation of the following equation:

$$\ln[\eta] = \ln k + a \ln MW_v \quad (2)$$

The exponent “ a ” is a function of biopolymer shape, and varies from 0.5 to 2.0. These constants can be determined experimentally by measuring the intrinsic viscosity of several biopolymer samples for which the molecular weight (Masuelli, 2011).

The M-H exponent bears the signature of a biopolymer chain’s three-dimensional conformation in the solvent environment: a values from 0 reflect a rigid sphere in an ideal solvent; those from 0.5–0.8 a random coil in a good solvent; and from 0.8–2.0 a rod like conformation (Masuelli, 2011 and Masuelli & Sansone, 2012).

The hydrodynamic radius (R_H) is given by the Einstein relation (Masuelli, 2013c),

$$M[\eta] = v_{a/b} N_A \frac{3}{4} \pi (R_H)^3 \quad (3)$$

and

$$v_{a/b} = \frac{[\eta]}{V_s} \quad (4)$$

$v_{(a/b)}$ is called the Einstein viscosity increment, and V_s is specific volume (cm^3/g) (Harding, 1997).

The corresponding value of “hydration” of the molecule, δ (g/g), is defined by,

Regarding the Mark-Houwink parameters, the value of “ k ” shows a good affinity of the solvent with LMFG, being the highest for acid hydrolysis. Regarding the shape parameter “ a ” and the shape factor ($v_{a/b}$), this is very similar to each other, highlighting the quasi-sphericity of the biopolymer in solution.

Gums	$[\eta]$ (cm^3/g)	k (cm^3/g)	a	M (g/mol)	R_H (nm)	$v_{a/b}$	δ (g/g)
LMFG-T	22.83	0.0435	0.5141	195000	14.23	2.57	8.20
LMFG-B	12.43	0.0325	0.5240	85000	9.00	2.62	4.05
LMFG-A	62.40	0.0623	0.5200	589000	28.64	2.60	23.33

Table 1: Hydrodynamic values of gums.

As previously described, the objective of hydrolysis is to generate polysaccharides of lower molecular weight and soluble in water or in another solvent at constant temperature. Similar case is observed in alcayota gum (Zanon & Masuelli 2018) and chañar brea gum (Masuelli et al 2018b).

The hydrodynamic radius (R_H) and the hydration value (δ) decrease for the same reason in addition to the fact that the molecular weight also decreases. Another aspect not less is that for the same hydrolysis, but different reagent, be it hydrochloric acid or

$$\delta = \frac{V_s - \bar{v}}{\rho_0} \quad (5)$$

and

$$\bar{v} = \left(1 - \frac{\partial \rho}{\partial c}\right) / \rho_0 \quad (6)$$

where \bar{v} is the partial specific volume (cm^3/g) (Curvale et al. 2008).

Experimental

The *Lithraea molleoides* fruit was collected in San Francisco del Monte de Oro ($32^\circ 36' 00''$ S, $66^\circ 07' 30''$ W) in the province of San Luis, Argentina, with voucher number: UNSL # 533. The fruit was washed and selected, then dried for 24 hours in an oven at 60°C . All fruit (husk and seed) was ground and separate to 50 mesh. All hydrolysis was performed at a concentration of NaOH 0.001M (Anhedra, Argentina) or HCl (Ciccarelli, Argentina) at 80°C for 6h. The soluble fraction was precipitated with etanol/water 70/30 and separated by filtration, the filtrate was concentrated at 60°C for 8h, and dried at 60°C for 24h.

Solutions were prepared at 0.25% wt. in distilled water. Viscosity was measured with an Ubbelohde 1C viscometer (IVA, Argentina) and density with an Anton Paar DMA35N densimeter (Austria).

Results and Discussion

According to the intrinsic viscosity and molecular weight data calculated (see Table 1), it can be deduced that the most energetic hydrolysis is the basic one, since these parameters decrease with respect to thermal and acid hydrolysis. This phenomenon can be explained due to the nature of the biopolymer, a polysaccharide rich in hydroxyl and carboxyl groups, where the carboxyls group incorporate Na^+ , increasing solubility and also favoring greater bond breaking. In the case of acid hydrolysis, this causes a greater unwinding of the polysaccharide, which favors a greater availability of groups that are solubilized with the consequent increase in these properties as well as in the expressed values of R_H and hydration value.

sodium hydroxide, the salt formed in the latter is more soluble in water. A consistent phenomenon is the increase of the partial specific volume (\bar{v}) and is reasonable due to the increase of the hydration value. Finally, it can be said that K_H values show that water is a good solvent for example in pectins (Ruano et al. 2020).

Conclusions

In what is observable from the intrinsic viscosity data on the different hydrolysis of the fruit of *Lithraea molleoides*, it can be ensured that the basic hydrolysis affects the polysaccharide in a way, obtaining a low molecular weight (85,000 g/mol). Regarding acid hydrolysis, it seems to be the most efficient and gives a molecular weight of 589,000 g/mol, and thermal hydrolysis of 189,000 g/mol. The shape factor is between $2.57 \geq v_{a/b} \leq 2.62$, which indicates that the LMFG in aqueous solution has a spheroidal shape, as confirmed by its value of “a” close to 0.5. The hydration value is low compared to other gums, and its highest value is for LMGF-A, mainly based on its large molecular weight and the great ability to associate with hydroxyl groups.

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