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The effect of neutral sugar distribution on the dilute solution conformation of sugar beet pectin

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Abstract

A partially degraded sugar beet pectin (C) was found to be heterogeneous in composition with neutral sugar-rich fractions of both high and low hydrodynamic radii. A neutral sugar-poor fraction was found at intermediate hydrodynamic radii. In this paper we demonstrate using both conformation zoning and the global analysis method that fractions of sugar beet pectin rich in neutral sugar side chains (RG-I regions) are less flexible than those rich in galacturonic acid (HG regions).

Key words: Sugar beet *(Beta vulgaris)* pectin; neutral sugar distribution; conformation; persistence length (L_p); mass-per-unit length (M_L)

1. Introduction

Dilute solution properties of pectins such as viscosity also depend on chemical composition, degree of esterification, solvent environment (*i.e.* salt concentration, sugar concentration and pH) together with temperature (Oakenfull, 1991). Hydrodynamic studies based on intrinsic viscosity ($[\eta]$), sedimentation coefficient ($s^0_{20,w}$), radius of gyration (r_g) and weight average molecular weight (M_w) have focussed on qualitative/ semi-quantitative methods of estimating the overall conformation based around "power law" Mark-Houwink-Kuhn-Sakurada relations which link intrinsic viscosity, sedimentation coefficient and radius of gyration with molar mass $[\eta] \propto M^a$, $s^0_{20,w} \propto M^b$ and $r_g \propto M^c$, where a, b and c have defined values for specific conformation types. The translational frictional ratio, f/f_o, conformation zoning (Pavlov, Rowe, & Harding, 1997; Pavlov, Harding, & Rowe, 1999) and the persistence length, L_p (Kratky and Porod, 1949) have also been used to estimate dilute solution conformation. A picture of a semi-flexible conformation for citrus pectins irrespective of degree of esterification (and charge) has emerged from these studies (Anger and Berth, 1985; Axelos, Lefebvre, & Thibault; Axelos, & Thibault, 1991, Berth, Anger, & Linow, 1977; Harding, Berth, Ball, Mitchell, & Garcìa de la Torre., 1991; Garnier, Axelos & Thibault, 1993; Malovikova, Rinaudo, & Milas, 1993; Cros, Garnier, Axelos, Imbery, & Perez, 1996; Braccini, Grasso, & Perez, 1999; Morris, Foster, & Harding, 2000, 2002; Morris, García de la Torre, Ortega, Castille, Smith, & Harding, 2008; Fishman, Chau, Kolpak, & Brady, 2001; Fishman, Chau, Hoagland, & Hotchkiss, 2006; Noto, Martorana, Bulone, & San Biagio, 2005). Sugar beet, apple and flax pectins have also been demonstrated to have semi-flexible structures (Ralet, Crépeau, Lefebvre, Mouille, Höfte, & Thibault, 2008; Axelos, & Thibault, 1991; Cros, *et al.*, 1996; Morris, Ralet, Bonnin, Thibault, & Harding, 2010), however a purified RG-I fraction has a random coil conformation (Morris, *et al.*, 2010). Pectin molecular weight and chain flexibility are also important in mucoadhesive interactions and pharmaceutical applications (Nafee, Ismail, Boraie, & Mortada, 2007; Morris, Kök, Harding, & Adams, 2010).

In an earlier paper (Morris & Ralet, 2012) we have demonstrated the use of size exclusion chromatography coupled to refractive index (RI) and UV detectors (SEC-RI-UV) to visualise heterogeneity in the neutral sugar content of pectin fractions. This suggests that the overall conformation approach may not be applicable to heterogeneous pectins *e.g.* sugar beet; apple and flax and in this second paper in the series we will estimate the dilute solution conformation of fractions of different chemical composition.

2. Experimental

2.1. Materials and Methods

2.1.1. Acid extraction of sugar beet pulp (Pectin C)

The alcohol insoluble residue (AIR) (5 g) from sugar beet pulp was obtained as described in Levigne, Ralet, & Thibault (2002) and the resultant pectin (yield 350 mg/g) will be referred to as pectin C.

2.1.2. SEC-MALLS-RI-UV-DPV

High performance size exclusion chromatography (HPSEC) was performed at room temperature on a system consisting of a Shodex OH SB-G guard column (Showa Denko, Tokyo, Japan) followed by in series (Shodex OH-Pak SB-805 HQ and Shodex OH-Pak SB-804 HQ) eluted with 50 mM sodium nitrate buffer containing 0.02 % sodium azide as an antibacterial agent at a flow rate of 42 ml/h. The eluent was detected on-line by:

1. SpectroMonitor 3000 variable wavelength UV detector at 325 nm (LDC/Milton Roy, Paris, France)
2. MiniDawn light scattering (LS) detector (Wyatt, Santa Barbara, U.S.A.)
3. T-50A differential pressure viscometer (DPV) (Viscotek, Huston, U.S.A.)
4. ERC 7515A differential refractometer (RI) (Sopares, Gentilly, France)

The refractive index increment, *dn/dc* was taken to be 0.146 ml/g (Chapman, Morris, Selvendran, & O'Neill, 1987).

3. Results and Discussion

3.1. Physical characterisation of pectin C

Acid extracted pectin is heterogeneous with respect to molar mass, intrinsic viscosity and composition (Oosterveld, Beldman, Schols & Voragen, 1996; Morris, *et. al.*, 2010; Morris & Ralet, 2012) (see **Figure 2** Morris & Ralet, 2012). The use of UV absorbance (325 nm) allows the visualisation of those populations of pectic molecules, which are substituted with ferulic acid *i.e.* the arabinan, galactan or arabinogalactan side chains present on the RG-I region of sugar beet pectin as has been shown previously (Oosterveld, *et al.*, 1996; Morris, *et. al.*, 2010; Morris & Ralet, 2012). In the previous paper in this series (Morris & Ralet, 2012) we demonstrated using on-line RI and UV detection how the neutral sugar composition of a pectin molecule varies during elution. We will now demonstrate how this change in chemical composition affects the physical properties (*e.g.* molar mass and intrinsic viscosity) of the pectin and how this impacts on conformation.

3.1.1. Molar mass and intrinsic viscosity

A molar mass and intrinsic viscosity of 270000 g/mol and 375 ml/g, respectively (**Table 1**) are in general agreement with previous studies on sugar beet pectins (Levigne, *et. al.*, 2002; Morris, *et. al.*, 2010). Furthermore, pectin C is polydisperse ($M_w/M_n = 2.15$, where M_w and M_n are the weight and number average molar masses, respectively) and there are wide range of different conformations (**Figures 1** and **2**). In this case the slope of this Mark-Houwink-Kuhn-Sakurada (MHKS) plot has no sensible meaning as this approach is only valid for a series of homologous (or pseudo-homologous) polymers. However what we can do is estimate the conformation at randomly selected points (indicated in red in **Figure 2**) and correlate this information with the chemical composition at each point.

3.1.2. Global analysis method (HYDFIT)

The linear flexibility of polymer chains can also be represented quantitatively in terms of the persistence length, L_p of equivalent *worm-like chains* (Kratky, & Porod, 1949) where the persistence length is defined as the average projection length along the initial direction of the polymer chain. In the case of a theoretical perfect random coil $L_p = 0$ and for the equivalent extra-rigid rod $L_p = \infty$, although in practice limits of ~ 1 nm for random coils (*e.g.* pullulan) and 200 nm for a extra-rigid rod (*e.g.* xanthan) are more appropriate (Harding, 1997).

The persistence length, L_p and mass per unit length, M_L can be estimated using Multi-HYDFIT program (Ortega, & García de la Torre, 2007) which considers data sets of intrinsic viscosity and molar mass. It then performs a minimisation procedure (see Ortega, & García de la Torre, 2007) finding the best values of M_L and L_p satisfying the Bushin-Bohdanecky (Bushin, Tsvetkov, Lysenko, & Emel'yanov, 1981; Bohdanecky, 1983) equation (equation 1).

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2} \tag{1}$$

where Φ is the Flory-Fox constant (2.86 x 10^{23} mol^{-1}) and A_0 and B_0 are tabulated coefficients (Bohdanecky, 1983)

$$M_L = \frac{m}{l} \tag{2}$$

m and l are the average molar mass and length of the average monomeric unit.

Flexibility can be estimated from the ratio of L_p/M_L (nm^2mol/g) which decreases with increasing flexibility (Patel, Morris, Ebringerová, Vodenicarová, Velebny, Ortega, García de la Torre, & Harding, 2008; Morris, *et. al.*, 2010). The "average" conformation for pectin C is shown in **Table 1.** These results are consistent with previous findings which suggest that sugar beet; apple and flax pectins have flexible structures (Axelos, & Thibault, 1991; Cros, *et. al.*, 1996; Ralet, *et. al.*, 2008; Morris, *et. al.*, 2010). In order to further demonstrate the differences in conformation across the elution profile we have superimposed the Mark-Houwink-Kuhn-Sakurada plot for this pectin if we were to assume the entire population had the same "average" conformation (**Figure 2**). The slope of the plot is 0.61 indicating a semi-flexible conformation.

When we go on to look at the pectin conformation at each of the 20 selected slices we can see that fractions rich in neutral sugars are more flexible than those richer in galacturonic acid (**Table 2**). This is consistent with the findings that HG–rich regions are considerably stiffer (Cros, *et al.*, 1996; Braccini, *et. al.*, 1999; Noto, *et. al.*, 2005; Morris, *et al.*, 2010).

3.1.3. Conformation zoning (Normalised scaling relations)

Pavlov, Harding & Rowe (1999) described a procedure to represent the conformation of polymers in solution based on the relationship between their molar mass, intrinsic viscosity and mass per unit length, M_L. A plot of lof $[\eta]M_L$ versus $10^8 M_w/M_L$ enables an estimate of the overall solution conformation of a macromolecule in solution: where Zone A represents an extra rigid rod, Zone B a rigid rod, Zone C a semi-flexible coil, Zone D a random coil and Zone E a globular or branched structure. In this case we have taken the mass per unit length calculated previously using the HYDFIT algorithm (Ortega, & García de la Torre, 2007). As we can see from **Figure 3** the pectins with higher neutral sugars are generally more flexible and those which are richer in HG fractions are less flexible this is in agreement with findings on sugar beet pectin sub-fractions (Morris, *et. al.*, 2010), although all fractions are less flexible than we expected. This is most likely due the estimation of flexibility relying solely on the variation of intrinsic viscosity with molar mass rather than using a suite of hydrodynamic techniques (Morris**,** Patel, Picout, Ross-Murphy, Ortega, Garcia de la Torre, & Harding, 2008; Kök, Abdelhameed, Ang, Morris, & Harding, 2009). There also seems to be a semi-quantitative relationship between L_p/M_L (nm^2mol/g) and the conformation as estimated by conformation zoning in that the transition from rigid rod to semi-flexible coil seems to occur at ~ 0.01 nm^2mol/g (**Figure 4** and **Table 2**). The only exception to this is shown in italics in **Table 2**.

4. Conclusions

In this paper we demonstrate using both conformation zoning (Pavlov, *et. al.*, 1999) and the global analysis method (Ortega, & García de la Torre, 2007) that fractions of sugar beet pectin rich in neutral sugar side chains (RG-I regions) are more compact than those rich in galacturonic acid (HG regions). Therefore as a consequence we should be aware that any measurements on a non-fractionated material will only give the “weight-average” molar mass, intrinsic viscosity or conformation and may not necessarily be representative of the distribution of pectin molecules. This has repercussions for their functional properties.

5. Acknowledgements

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Legends to Figures

Figure 1 The change in molar mass (▬) and intrinsic viscosity (▬) with elution volume for sugar beet pectin C.

Figure 2 Mark-Houwink-Kuhn-Sakurada plot (▲) for sugar beet pectin C. Twenty points on this plot were chosen at random for further conformational analysis (■). We have superimposed the corresponding plot for this pectin (■) if we were to assume the entire population had the same "average" conformation. The slope of the plot is 0.61 consistent with a semi-flexible conformation.

Figure 3 Normalised scaling plot of $[\eta]M_L$ versus M_w/M_L (adapted from Pavlov, *et. al.,* 1999) where the neutral sugar content for each pectin C fraction is indicated (in red). Zone A: extra rigid rod; Zone B: rigid rod; Zone C: semi-flexible; Zone D: random coil and Zone E: globular or branched.

Figure 4 A plot of L_p/M_L versus estimated neutral sugar content. On this plot a larger value of L_p/M_L is consistent with a less flexible conformation. Based on the conformation as estimated by conformation zoning there seems to be a semi-quantitative relationship and the transition from rigid rod to semi-flexible coil seems to occur at ~ 0.01 nm^2mol/g.

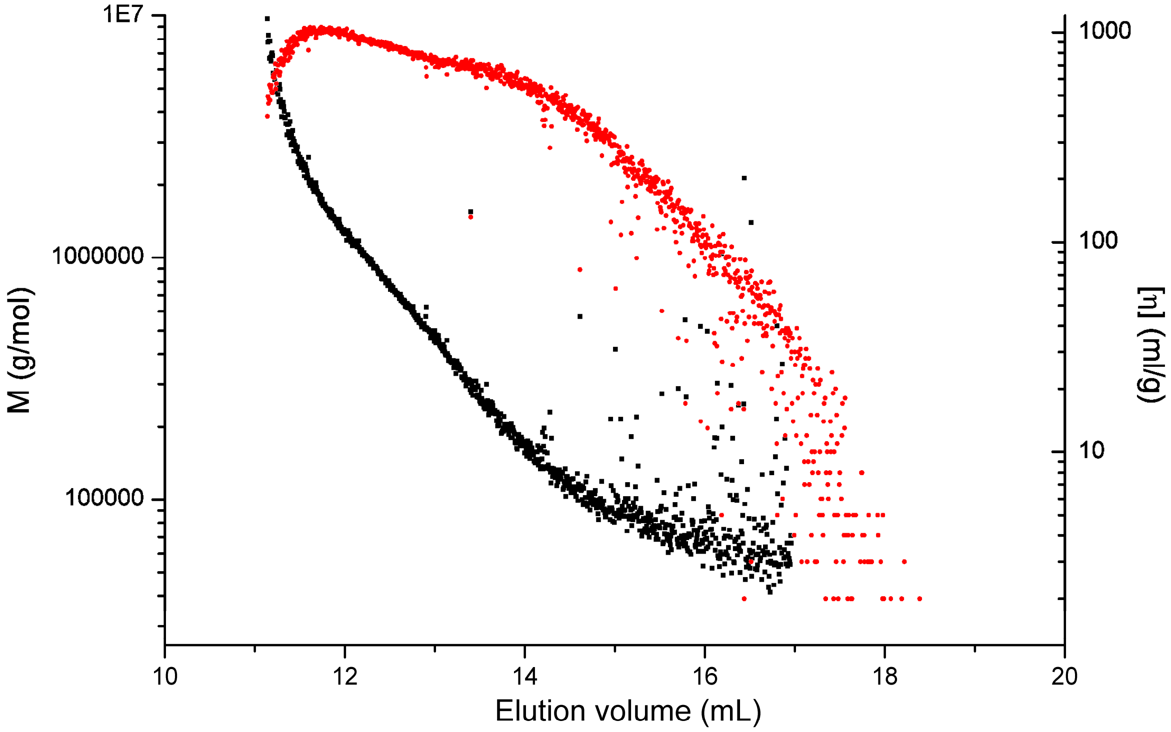
Table 1 Physical and conformational properties of sugar beet pectin C

Pectin	**M_w, g/ mol (MALLS)**	**$[\eta]_w$, ml/ g (DPV)**	**M_L, g/ mol nm**	**L_p, nm**	**L_p/M_L, nm^2mol/g**	**Zone (A-E)**
C	(2.7 ± 0.2) x 10^5	375 ± 10	330 ± 30	3.9 ± 0.4	0.012 ± 0.002	C

Table 2 Chemical, physical and conformational properties for randomly selected pectin fractions

Elution Volume, ml	**M, g/mol**	**[η], ml/g**	**L_p, nm**	**M_L, g/ mol nm**	**Neutral sugars, mol %**	**L_p/M_L, nm^2mol/g**	**Zone (A-E)**
11.19	9.68 x 10^6	398	2.0	630	59.2	0.0032	C/D
11.22	7.26 x 10^6	506	3.4	810	60.1	0.0042	C
11.26	5.81 x 10^6	605	3.4	670	64.9	0.0051	C
11.27	4.85 x 10^6	707	1.5	260	67.6	0.0058	D
11.28	6.30 x 10^6	542	3.4	740	66.8	0.0046	C
11.39	3.88 x 10^6	757	5.4	750	66.7	0.0072	C
11.50	2.92 x 10^6	883	5.4	610	68.9	0.0088	C
11.59	2.43 x 10^6	935	8.3	800	69.4	0.0104	B/C
11.81	1.64 x 10^6	1042	9.8	720	66.5	0.0135	B
12.31	9.83 x 10^5	901	13	800	59.9	0.0163	B
12.51	7.83 x 10^5	873	16	820	56.8	0.0195	B
13.00	5.00 x 10^5	716	16	760	47.4	0.0209	B
13.04	4.52 x 10^5	751	22	840	46.1	0.0263	B
13.16	4.04 x 10^5	713	26	880	43.3	0.0294	B
13.46	*3.08 x 10^5*	*636*	*4.4*	*280*	*37.8*	*0.0157*	*C*
13.55	2.60 x 10^5	671	18	610	36.8	0.0294	B
14.35	1.30 x 10^5	467	11	430	31.9	0.0256	B
14.93	9.23 x 10^4	307	24	600	28.4	0.0397	B
16.09	7.17 x 10^4	86	4.9	520	33.6	0.0094	C
16.71	5.50 x 10^4	50	5.4	650	53.3	0.0083	C

*errors in all estimates are appoximately 10%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20
Elution volume (mL)

Figure 1

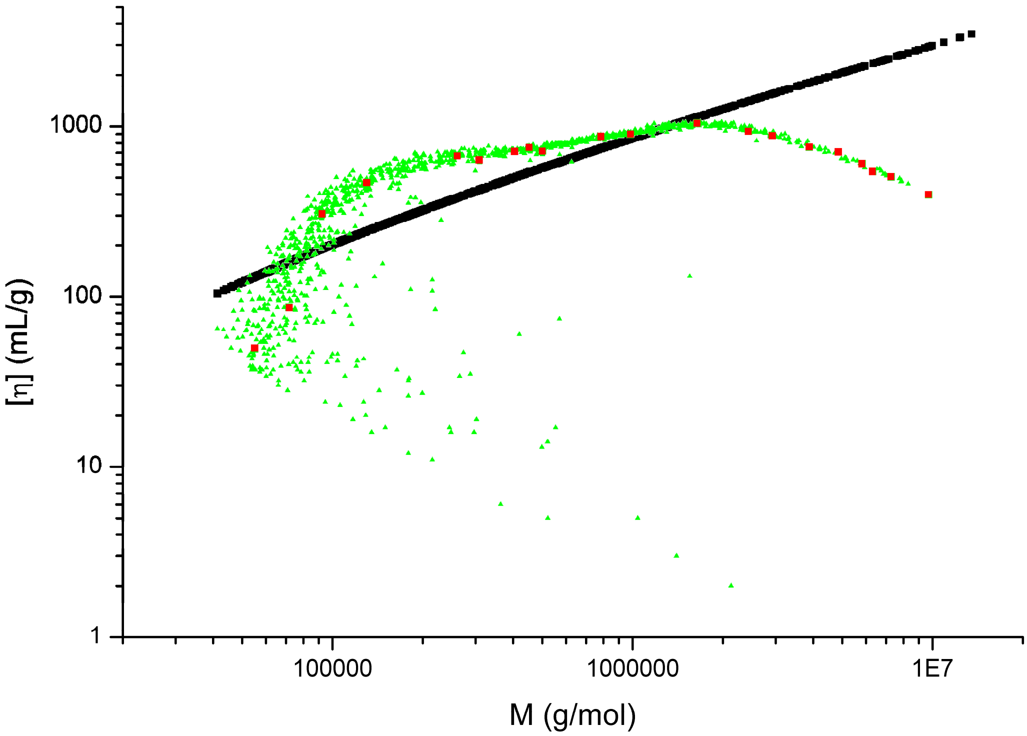
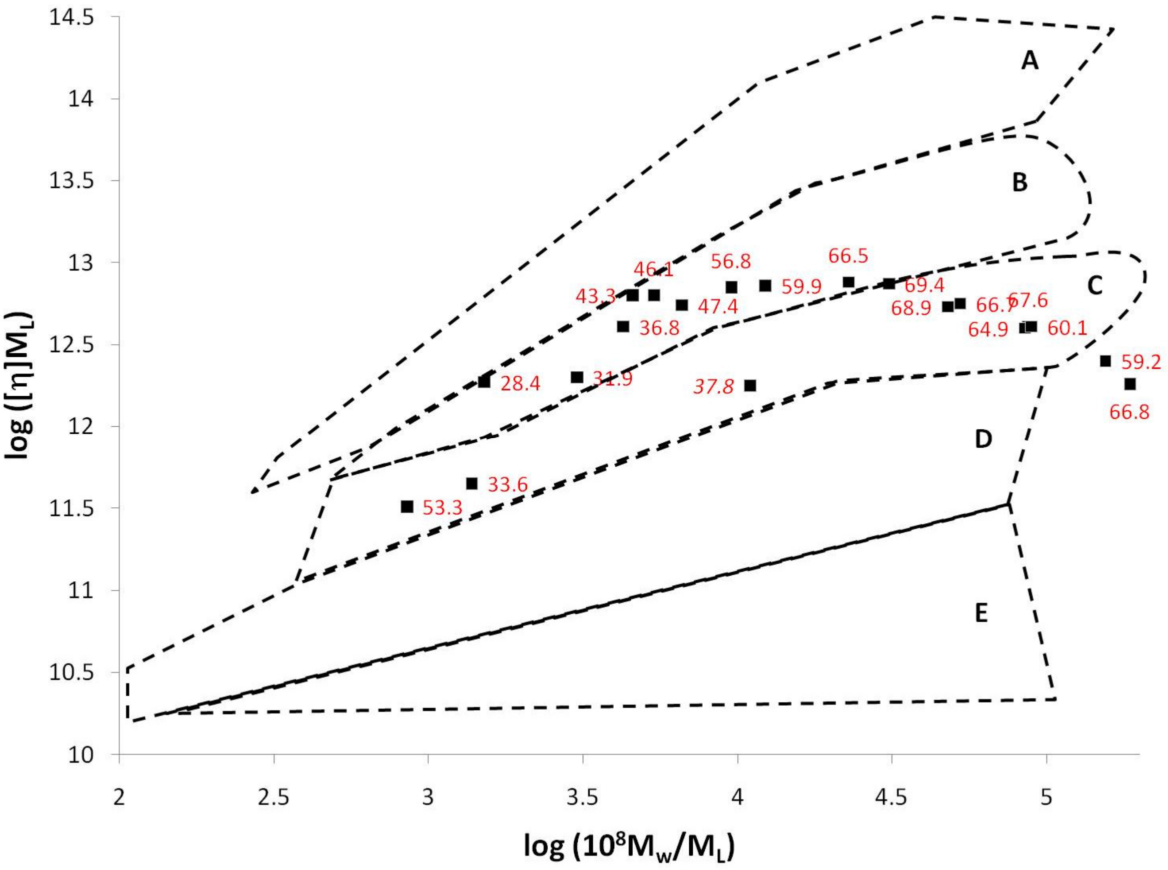


Figure 2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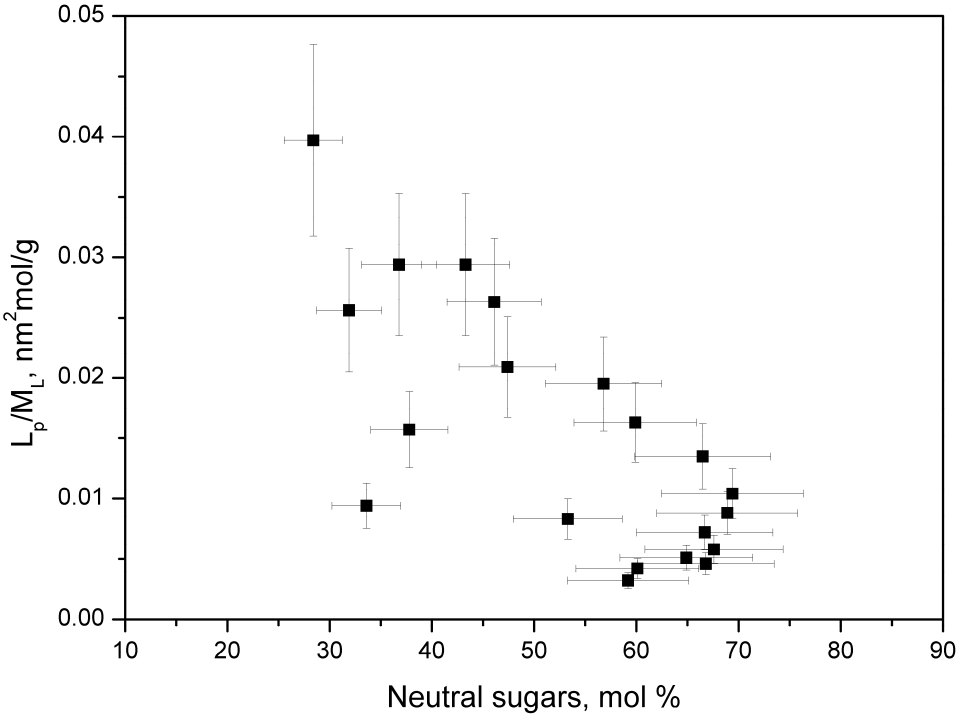
log ([η]M$_L$)
log (10^{8}M$_w$/M$_L$)

Figure 3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80
90
Neutral sugars, mol %

Figure 4