Chemical characteristics of pectin; how pectin affects our immunity

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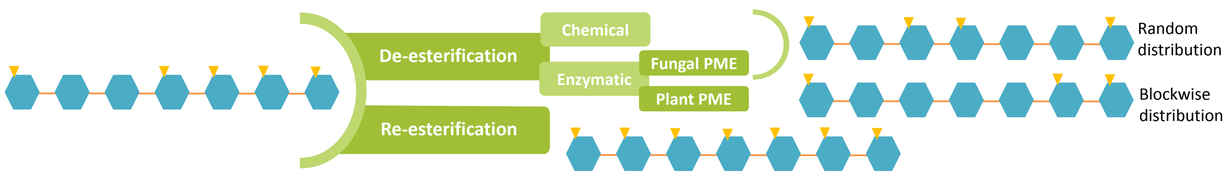
Background

Pectin is a dietary fibre that might be essential for the prevention of Western diseases. Recently, evidence has been found that health beneficial effects of pectin are highly dependent on its chemical structure. The effects go beyond prebiotic effects of pectin as dietary fibre and might influence the immune system. Typical characteristics of pectins include level and distribution of methyl esters and molecular weight. Next to the methylesterification (DM) other structural properties may influence the effects of pectins. In this study **we aim to fully characterize and tailor pectins in order to enhance the immune barrier function.**

Tailoring pectins

Pectins are modified in multiple ways. They can be re-esterified, de-esterified chemically or enzymatically using Pectin Methylesterase (PME), and their molecular weight can be lowered without changing other chemical properties.



Characteristics of modified pectins

Ball milling successfully lowers molecular weight, but does not change the chemical properties of orange pectin. After re-esterification and consequent de-esterification with alkali the neutral sugar levels have slightly altered and the molecular weight is lowered.

Table 1. Sugar composition and molecular weight of a parental pectin and modified pectins.
O = Orange, c = commercial, bm = ball milled, re/de-a = re-esterified and consequently de-esterified using alkali. Numbers e.g. 36 represent the DM values (moles of methanol/100 moles of galacturonic acid). O64c = commercial Orange pectin DM 64

Parental sample	WUR code	mol%						w/w%	Mw
	DM	Rha	Ara	Xyl	Gal	Glc	Uronic acid	Total	kDa
O64c	**O64c**	0.4	7.0	0.4	7.2	1.3	84	86	92
	O60bm	0.7	6.4	0.3	7.4	1.5	84	83	11
	O63re/de-a	0.4	0.1	0.3	6.1	1.5	92	80	22

Fingerprinting of modified pectins

Pectins are digested into diagnostic oligomers by Pectin Lyase (PL) and Polygalacturonase (PG).

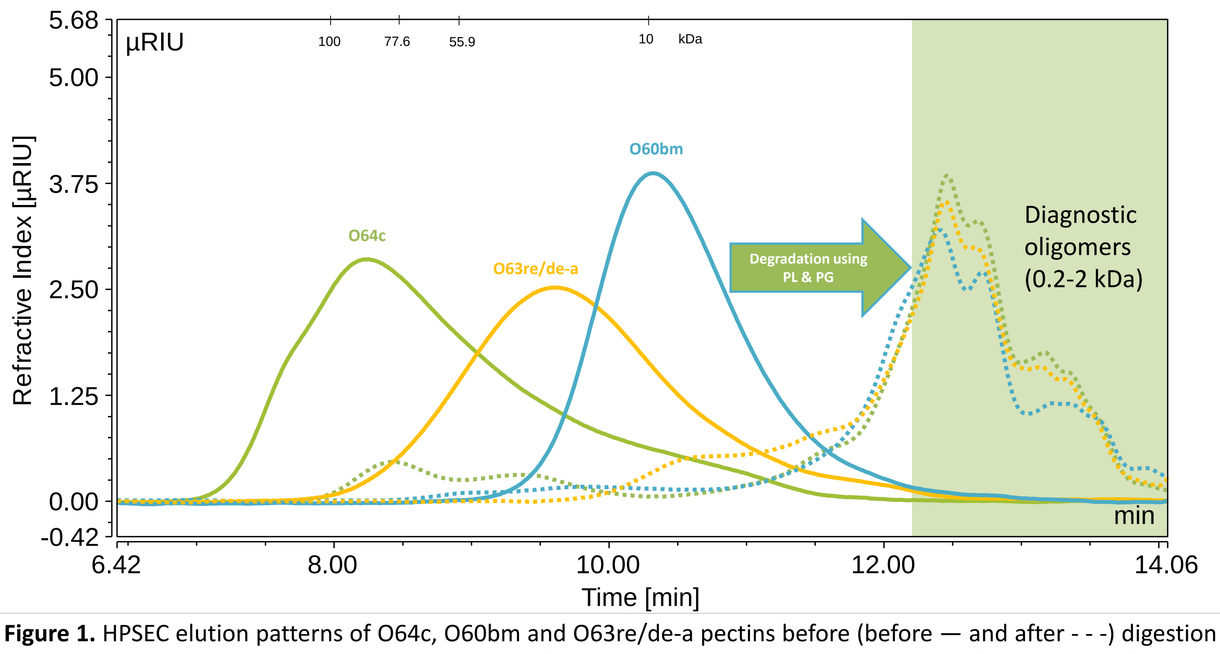


Figure 1. HPSEC elution patterns of O64c, O60bm and O63re/de-a pectins before (before — and after - - -) digestion by homogalacturonan degrading enzymes: PL and PG. Molecular masses of pectin standards (in kDa) are indicated.

Diagnostic oligomers after digestion

The HILIC-LC-MS elution patterns of three pectins illustrate that the main degradation products are present in the digests in different ratios, demonstrating different methylester distribution in the same DM pectins.

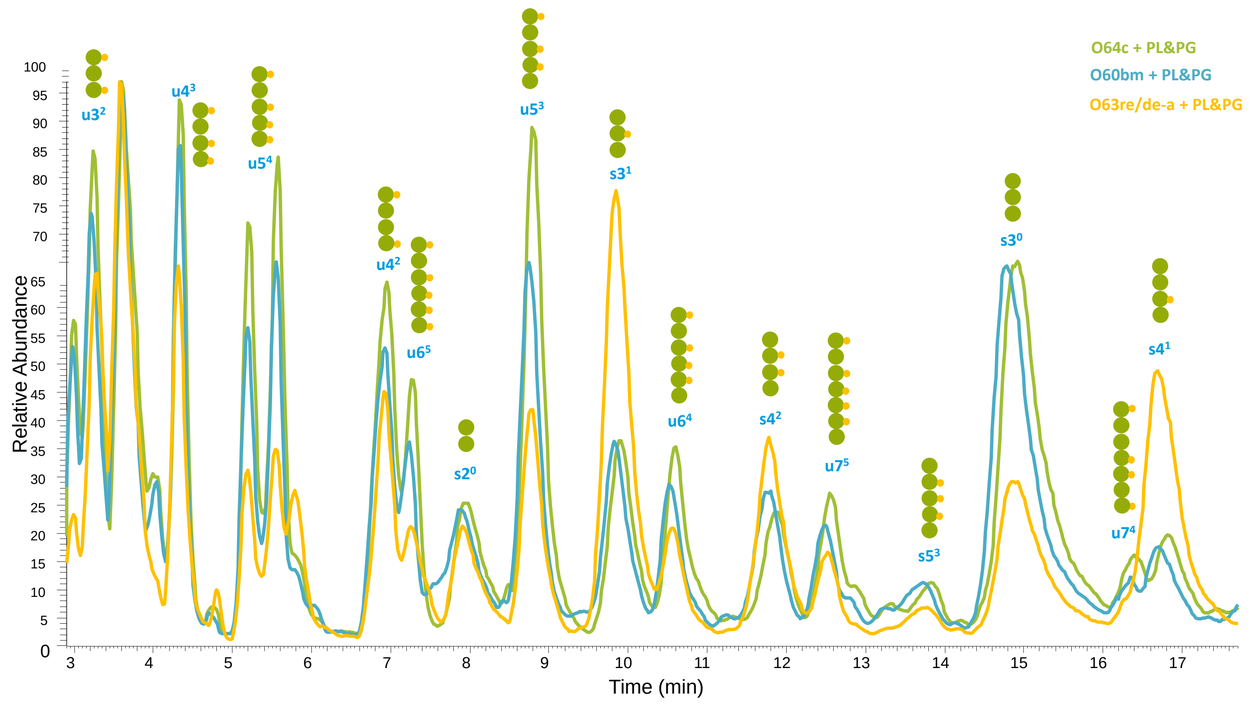


Figure 2. HILIC elution pattern of O64c, O60bm and O63re/de-a pectins digested by PL and PG, HG degrading enzymes. Peak annotation: 4^1: DP 4 with 1 methylester. u: unsaturated GalA; s: saturated GalA. ● = GalA, ●• = methylesterified GalA

Degree of blockiness and immune activity

By calculating the DB the methylester distribution over the HG backbone can be revealed. The higher the DB the more blockwise the methylester distribution is. It can be seen that by chemical modification of the pectin the DB becomes more randomly distributed and the TLR modulatory effects become lower. By ball milling the DB and the modulatory effects are slightly decreasing.

Table 2. Degree of Blockiness and absolute Degree of Blockiness related to TLR2 activation and TLR2/1 inhibition. DB is calculated as non-methylesterified GalA monomer, dimer, trimer released by the enyzmes expressed as percentage of the total amount of nonmethylesterified GalA residues present. The absolute Degree of B lockiness (DB_{abs}) is related to the total GalA residues present in the pectin.

Parental sample	WUR code	DB	DB_{abs}	TLR2 activation	TLR2/1 inhibition
O64c	**O64c**	37	13	29	45
	O60bm	34	13	23	34
	O63re/de-a	20	7	9	9

Conclusion

- Pectin structure can be tailored in a defined way.
- Molecular weight plays a minor role in TLR2 activation and TLR2/1 inhibition.
- Re-esterification and consequent de-esterification can result in a highly randomly distributed pectin which has a major effect on TLR2 activation and TLR2/1 inhibition.

References

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