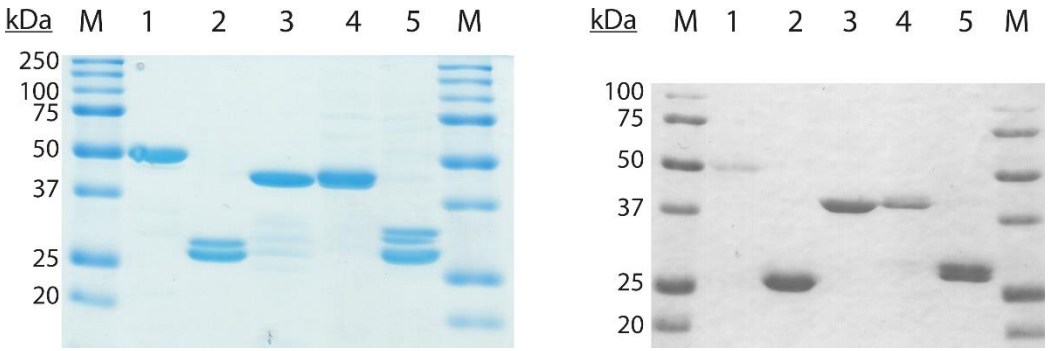
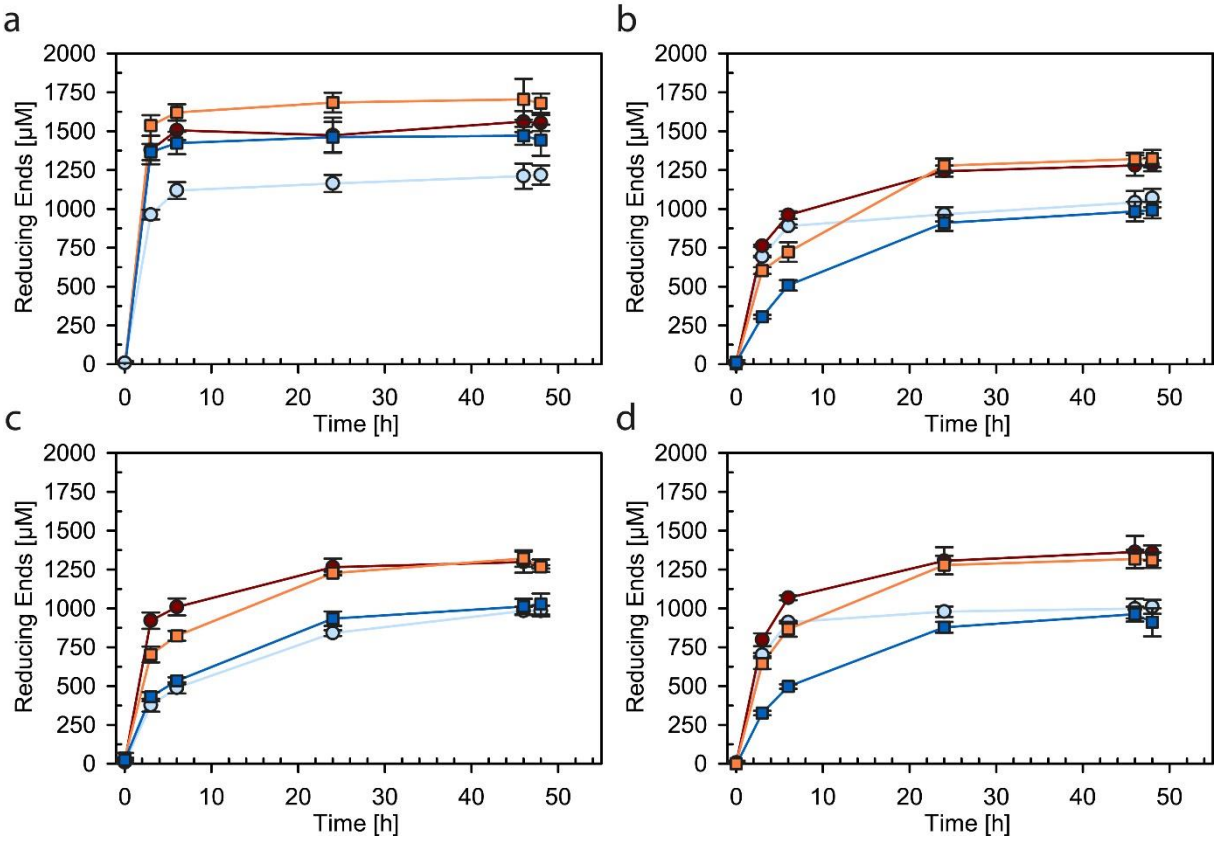
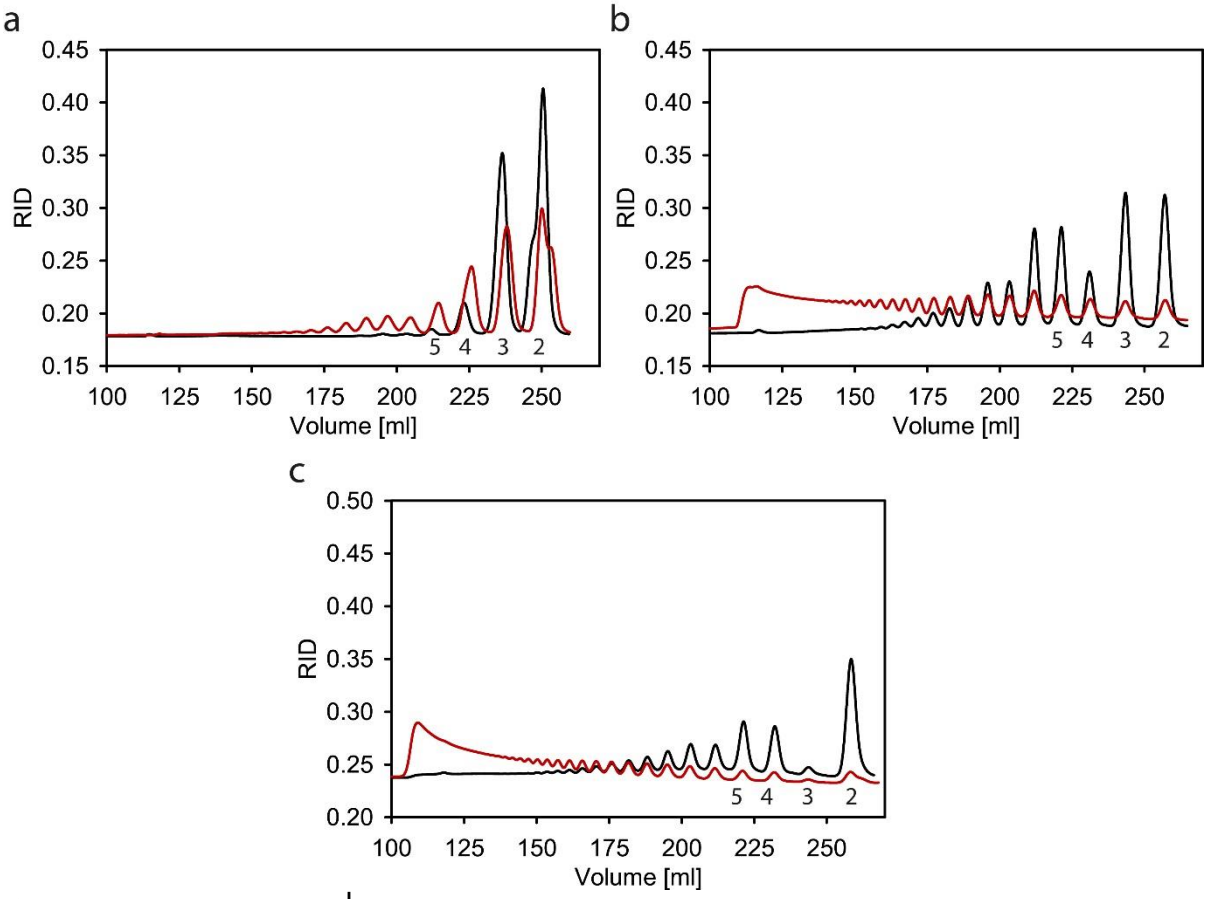


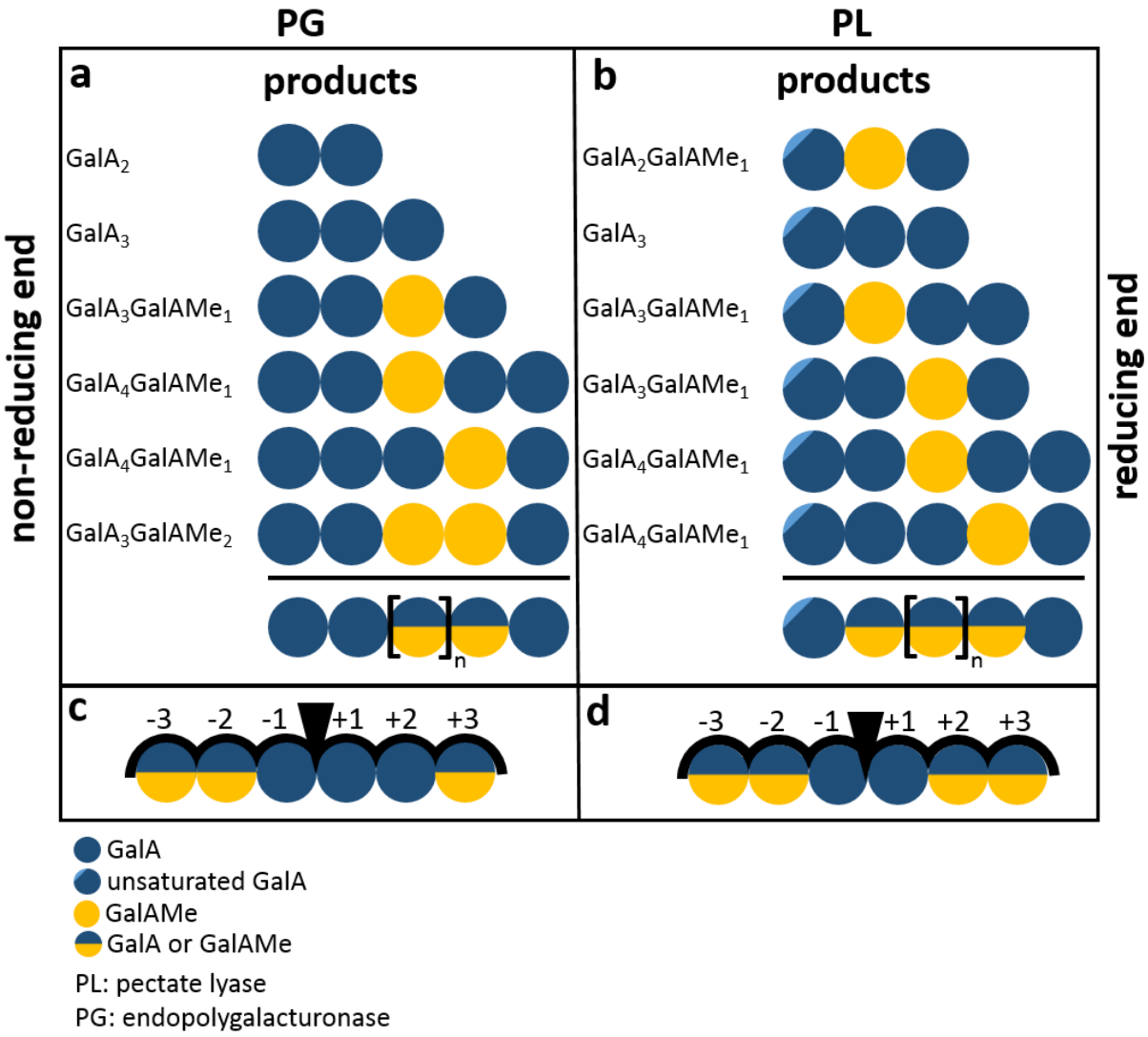
Supplementary Figure 1 | SDS-PAGE (left) and corresponding Western-Blot (right) of ChiB (1), CSN-174 (2), CSN-7M (3), CSN-MN (4), and CSN-MHKI (5). The enzymes were synthesized using *E. coli* Rosetta2 (DE3) [pLysSRARE2] production strains carrying the corresponding pET-22b(+)::StrepII constructs. Six µg of purified protein was separated on the gel and stained with Serva Blue G250 (SERVA Electrophoresis GmbH, Germany) or via chemiluminescence with a *Strep*-Tactin® horseradish peroxidase conjugate (IBA, Germany) after blotting. Precision Plus Protein™ All Blue (Bio-Rad Laboratories GmbH, Germany) was used as protein standard (M).



Supplementary Figure 2 | Hydrolysis of a series of chitosans (DA 11% (light blue), DA 19% (red), DA 35% (orange), and DA 50% (dark blue)) with (a) CSN-174, (b) CSN-7M, (c) CSN-MN and (d) CSN-MHKI. Samples were incubated for 48 h at 37°C and mild shaking. To check for end-point cleavage, fresh enzyme was added after 46 h. At each time point, reducing end concentrations were determined.



Supplementary Figure 3 | Distribution of the degree of polymerization (DP) of oligomers produced with (a) CSN-174, (b) CSN-MN, and (c) CSN-MHKI. The size distributions of hydrolysates of chitosans with F_A 0.19 (black) and 0.50 (red) were analysed by Size Exclusion Chromatography (SEC) utilizing Refractive Index Detection (RID). For this purpose, the chitosans were hydrolysed with chitosanases until maximal cleavage was reached. Numbers below the peaks indicate the corresponding DPs as verified using UHPLC-ESI-MS. CSN-7M was not analysed, as it shows the same specificity like CSN-MN (compare Figures 3 and 4).



Supplementary Figure 4 | Products (up to DP 5) and specificities of an endo polygalacturonidase (PG) from *Aspergillus* sp. and a pectate lyase (PL) from *Aspergillus niger* on partially methyl esterified pectins used as substrates. The products of (a) PG and (b) PL were used to identify the subsite specificities (c and d) of the two enzymes for methyl esterified and non-methyl esterified GalA units in the homogalacturonan chain.

Supplementary Table 1 | CSN-174 products after the hydrolysis of chitosan with F_A 0.11-0.50. Shown are the molar fractions of each oligomer with an abundance of ≥ 1% and their possible pattern of acetylation. When two or more patterns are given, sequencing of the oligomer was not complete. Data was gained by quantitative sequencing.

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.11	67.2%	DD	65.1%	DD	56.4%	DD	66.8%	DD
	16.0%	DA	14.2%	DA	19.1%	DA	14.7%	DA
	7.0%	DDA	10.7%	DDA	13.3%	DDA	7.3%	DDA
	3.4%	ADD	3.5%	ADD	3.0%	ADD	2.4%	ADD
	1.2%	AD	1.2%	ADDA	1.5%	AD	2.4%	DDD
	1.1%	ADDA	1.1%	ADA	1.4%	ADA	1.1%	ADDA
	1.1%	ADA			1.2%	ADDA	1.1%	AD
0.19	48.4%	DD	47.3%	DD	41.1%	DD	53.1%	DD
	21.8%	DA	20.5%	DA	24.1%	DA	22.9%	DA
	10.0%	DDA	13.5%	DDA	15.0%	DDA	9.3%	DDA
	5.9%	ADD	5.5%	ADD	4.4%	ADD	3.3%	ADD
	2.9%	ADDA DADA	2.8%	ADA	3.3%	ADA	2.6%	ADAD ADDA
	2.8%	ADA	2.3%	ADDA. DADA	2.8%	ADDA	2.5%	ADDA DADA
	2.6%	ADAD ADDA	2.2%	ADAD ADDA	1.8%	AD	2.0%	ADA
	1.7%	AADD	1.5%	AADD	1.6%	AADA	1.4%	AADD
	1.4%	AADA	1.3%	AADA	1.4%	AADD	1.4%	DAD
	1.2%	AD	1.0%	AD	1.0%	DAD	1.4%	AADA
							1.3%	AD
0.35	29.8%	DD	31.3%	DD	27.9%	DA	30.3%	DD
	27.3%	DA	27.2%	DA	22.5%	DD	28.3%	DA
	10.1%	DDA	12.3%	DDA	16.7%	DDA	10.7%	DDA
	7.2%	ADD	6.3%	ADD	6.2%	ADA	5.1%	ADD
	5.6%	ADA	4.6%	ADA	6.0%	ADD	4.7%	ADA
	4.2%	ADDA	4.4%	ADDA DADA	5.1%	ADDA	4.6%	ADDA
	3.8%	AADD	4.1%	ADAD ADDA	3.2%	AADA	3.2%	AADA
	3.1%	AADA	2.7%	AADD	2.8%	AADD	2.6%	AADD
	1.4%	AD	2.6%	AADA	1.9%	AD	1.9%	DAD
	1.4%	AADAD AADDA	1.5%	DAD	1.8%	AADDA	1.9%	AADDA
	1.3%	DADA	1.3%	AD			1.5%	AD
	1.3%	AADDA DAADA	1.3%	AADDA			1.2%	AAADD
	1.2%	DAD	1.2%	DAAD DADA			1.1%	AAADA
0.50	25.5%	DA	24.6%	DA	26.3%	DA	32.9%	DA
	14.5%	DD	13.4%	DD	12.0%	ADA	17.8%	DD
	12.2%	ADA	12.0%	ADA	11.6%	DDA	10.1%	ADA
	8.3%	AADA	11.2%	DDA	11.4%	DD	8.7%	AADA
	6.9%	ADD	7.2%	AADA	8.0%	AADA	7.7%	DDA
	6.8%	DDA	6.4%	ADD	7.4%	ADAD ADDA	7.2%	ADAD ADDA
	6.7%	ADDA DADA	6.3%	ADDA DADA	7.2%	ADDA DADA	7.0%	ADDA DADA
	6.6%	ADAD ADDA	6.2%	ADAD ADDA	5.3%	ADD	4.7%	ADD
	4.2%	AADD	3.3%	AADD	3.0%	AADD	4.0%	AADD
	4.0%	AAADD AADAD AADDA	2.7%	AAADA	2.9%	AAADA	2.3%	DAD
	3.2%	AAADA	2.4%	AADDA DAADA	2.6%	AADDA	1.5%	ADAD DAAD
	2.6%	AADDA DAADA DADAA	2.0%	AADAD AADDA	1.4%	AAADD	1.5%	AD
	2.0%	AAADD AADAD DAAAD	1.9%	DAD	1.4%	AD	1.3%	DAAD DADA
	1.3%	AD	1.5%	AAADD	1.2%	AAAADA		

D: GlcN; A: GlcNAc.

Supplementary Table 1 | Continued

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.50	1.0%	AAAADA	1.3%	DAAD DADA				
			1.2%	ADAD. DAAD				
			1.2%	AD				

D: GlcN; A: GlcNAc.

Supplementary Table 2 | CSN-7M products after the hydrolysis of chitosan with F_A 0.11-0.50. Shown are the molar fractions of each oligomer with an abundance of ≥ 1% and their possible pattern of acetylation. When two or more patterns are given, sequencing of the oligomer was not complete. Data was gained by quantitative sequencing.

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.11	53.8%	DD	56.1%	DD	57.2%	DD		
	16.1%	DDD	14.5%	DDD	14.5%	DDD		
	6.6%	ADDD	6.1%	ADDD	6.2%	ADDD		
	6.5%	DADDD	5.3%	DADDD	5.6%	DADDD		
	2.8%	ADD	3.3%	DADD	3.0%	ADD		
	2.5%	DADD	3.0%	ADD	3.0%	DADD		
	2.1%	ADADD DAADD	1.9%	DDADD	2.2%	ADADD DAADD		
	1.7%	DDADD	1.9%	ADADD ADDAD	1.6%	DDADD		
	1.5%	ADADD ADDAD	1.0%	ADADDD ADDADD	1.4%	AADDDD ADADDD ADDADD DAADDD DADADD DDAADD		
	1.4%	DDAD			1.4%	DDAD		
	1.0%	ADADDD ADDADD ADDDAD			1.4%	DAADDD DADADD DADDAD DADDDA DDAADD DDADAD DDADDA DDDAAD DDDADA		
					1.3%	ADADD ADDAD		
					1.2%	DAADD DADAD		
0.19	43.6%	DD	44.0%	DD	44.8%	DD		
	11.1%	DDD	12.1%	DDD	11.3%	DDD		
	8.7%	ADDD	9.1%	ADDD	8.3%	ADDD		
	7.2%	DADDD	7.7%	DADDD	7.1%	DADDD		
	4.2%	DADD	4.5%	ADADD DAADD	4.8%	DADD		
	3.9%	ADD	3.8%	ADD	4.2%	ADADD DAADD		
	3.9%	DDADD	3.3%	DADD	3.8%	ADADD ADDAD		
	3.5%	ADADD ADDAD	3.2%	DDADD	3.5%	ADD		
	2.6%	ADADDD DAADDD	3.1%	ADADD ADDAD	3.4%	DDADD		
	2.2%	DAADD DADAD	2.5%	DAADD DADAD	2.2%	ADADDD DAADDD		
	1.9%	ADADDD ADDDAD	1.8%	ADADDD DAADDD	2.1%	ADADDD ADDADD ADDDAD ADDDDA		
	1.6%	DAADDD DADDAD	1.7%	DAADDD DADADD DADDAD	1.7%	DAADD DADAD		
	1.3%	DDAD	1.6%	DDAD	1.4%	DAADDD DADADD DADDAD DADDDA		
	1.1%	AADDD	1.2%	ADADDD ADDADD ADDDAD	1.4%	ADDAD DADAD		
			1.1%	ADDADD ADDDAD DADADD DADDAD	1.3%	ADDADD ADDDAD ADDDDA DADADD DADDAD DADDDA		
			1.1%	ADDAD DADAD	1.3%	ADDADD ADDDAD DADADD DADDAD DDAADD DDADAD		
					1.0%	DDAD		

D: GlcN; A: GlcNAc.

Supplementary Table 2 | Continued

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.35	20.9%	DADDD	20.5%	DADDD	19.2%	DADDD		
	18.6%	DD	18.7%	DD	17.8%	DD		
	12.2%	ADDD	12.1%	ADDD	17.8%	DDD		
	10.5%	DDD	10.2%	DDD	9.7%	ADDD		
	7.0%	DDADD	6.8%	DDADD	5.7%	DDADD		
	4.9%	DADD	5.5%	ADADD DAADD	4.5%	ADADDD DAADDD		
	3.9%	DAADDD DADADD	4.1%	DADD	4.4%	DDADDD		
	3.8%	ADADDD DAADDD	4.1%	ADADDD DAADDD	4.0%	AADDD ADADD DAADD		
	3.3%	DDADDD	3.5%	DAADD DADDA	3.3%	DAADDD DADADD		
	3.2%	ADADD	3.1%	DDADDD	3.1%	ADDDD		
	3.0%	DAADD	3.0%	AADDD	3.0%	DAADD DADAD DADDA DDAAD DDADA		
	2.2%	AADDD	2.6%	ADADD ADDDA	2.5%	DDDD		
	1.6%	ADD	2.6%	DAADDD DADADD DADDAD	2.4%	DADD		
	1.5%	DDDDD	2.1%	ADADDD ADDADD ADDDAD	2.1%	AADDD ADADD ADDAD ADDDA		
	1.1%	ADDADD DADADD	1.6%	ADD	1.8%	ADADDD ADDADD		
	1.1%	ADADDD ADDADD	1.5%	DDDDD	1.4%	ADD		
					1.0%	DDDDD		
0.50	17.0%	DADDD	20.7%	DADDD	17.1%	DADDD		
	13.6%	ADDD	14.2%	DD	15.4%	DD		
	13.4%	DD	12.0%	ADDD	12.1%	ADDD		
	7.9%	DAADD	10.3%	DDD	8.9%	DDD		
	5.8%	DAADDD DADADD DADDAD DADDDA	8.9%	ADADDD DAADDD	6.7%	ADADDD DAADDD		
	5.4%	ADADDD DAADDD	7.0%	DAADDD DADADD	5.3%	DADD		
	5.4%	DADD	6.9%	AADDD ADADD ADDAD ADDDA	4.7%	DAADD		
	5.0%	DDD	6.6%	AADDD ADADD DAADD	4.1%	DAADDD DADADD		
	4.7%	DDADD	4.5%	DDADD	3.9%	ADADDD ADDADD		
	4.4%	ADADD	3.9%	DAADD DADAD DADDA DDAAD DDADA	3.7%	AADDD		
	3.9%	AADDD	3.5%	DADD	3.7%	DDADD		
	2.6%	ADD	3.3%	ADDAD DADAD DDAAD	3.4%	ADADD		
	2.4%	DDADDD	2.6%	DDADDD	3.4%	ADD		
	2.0%	ADDADD ADDDAD DADADD DADDAD DDAADD DDADAD	2.6%	ADADDD ADDADD	2.1%	DDADDD		
	2.0%	ADDADD ADDDAD ADDDDA DADADD DADDAD DADDDA	2.6%	ADD	1.3%	ADDADD DADADD		
	1.7%	AAADDD	1.1%	DDDDD	1.1%	ADDDD		
	1.6%	ADADDD ADDADD ADDDAD ADDDDA	1.0%	AAADDD AADADD ADAADD	1.1%	DDDDD		

D: GlcN; A: GlcNAc.

Supplementary Table 2 | Continued

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.50	1.5%	DDDDD	1.0%	DDDD	1.0%	ADAADD ADADAD ADADDA DAAADD DAADAD DAADDA		
	1.1%	AADD						
	1.0%	DDDAD						
	1.0%	AAADD						

D: GlcN; A: GlcNAc.

Supplementary Table 3 | CSN-MN products after the hydrolysis of chitosan with F_A 0.11-0.50. Shown are the molar fractions of each oligomer with an abundance of ≥ 1% and their possible pattern of acetylation. When two or more patterns are given, sequencing of the oligomer was not complete. Data was gained by quantitative sequencing.

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.11	37.8%	DD	35.2%	DDD	39.5%	DD	49.3%	DD
	28.1%	DDD	33.2%	DD	25.0%	DDD	30.8%	DDD
	9.2%	ADDD	9.8%	DADDD	8.5%	ADDD	7.2%	ADDD
	8.7%	DADDD	5.3%	ADDD	6.6%	DADDD	3.8%	DADD
	2.6%	DDADD	4.0%	DDADDD	4.0%	DADD	3.3%	DDA
	2.3%	DADD	2.1%	DADD	2.6%	DDADD	1.3%	ADD
	1.9%	DDADDD	2.0%	DDADD	2.6%	ADD		
	1.6%	ADADDD DAADDD	1.2%	ADADDD DAADDD	1.7%	ADADDD DAADDD		
	1.4%	ADD	1.1%	DDA	1.6%	DAADDD DADADD		
	1.3%	DAADDD DADDAD	1.0%	DAADDD DADDAD DADDDA	1.1%	DDADDD		
	1.0%	ADADD ADDAD			1.1%	ADADD ADDAD ADDDA		
					1.0%	ADADD DAADD		
0.19	29.4%	DD	27.5%	DDD	32.9%	DD	32.7%	DD
	21.4%	DDD	27.4%	DD	18.5%	DDD	21.2%	DDD
	11.5%	ADDD	13.4%	DADDD	13.7%	ADDD	14.7%	DADDD
	11.3%	DADDD	9.0%	ADDD	10.4%	DADDD	10.5%	ADDD
	3.9%	DADD	4.7%	DDADDD	4.8%	DADD	3.7%	DDADD
	2.5%	ADADDD DAADDD	3.0%	DDADD	4.5%	ADADD DAADD	3.1%	DADD
	2.2%	ADD	2.5%	DADD	3.7%	ADD	1.8%	DDA
	2.1%	DDADDD	2.5%	ADADDD DAADDD	2.6%	ADADD ADDDA	1.7%	ADDAD DADAD
	2.1%	ADADDD ADDADD ADDDDA	1.9%	DAADDD DADADD	2.5%	DDADD	1.6%	DAADD DADAD DADDA
	2.0%	DDADD	1.5%	ADADD DAADD	2.3%	DAADD DADDA	1.5%	AADDD
	2.0%	DAADDD DADADD DADDDA	1.1%	ADD	1.4%	DDAD	1.4%	ADADD ADDAD ADDDA
	1.8%	ADADD DAADD	1.0%	AADDD	1.0%	DAD	1.3%	ADD
	1.7%	DAADD DADAD DADDA	1.0%	DDDDA			1.0%	DDDDD
	1.4%	ADADD ADDAD ADDDA	1.0%	ADADD ADDDA				
	1.3%	DDAD	1.0%	ADADDD ADDADD				
	1.1%	ADDADD DADADD						
	1.0%	AADDD						
	1.0%	DDDAD						
	1.0%	ADDAD DADAD						
0.35	20.4%	DD	20.3%	DD	20.8%	DD	23.5%	DD
	16.4%	ADDD	17.0%	DDD	14.3%	ADDD	16.2%	DADDD
	11.3%	DADDD	15.7%	DADDD	11.4%	DADDD	14.5%	ADDD
	11.2%	DDD	13.9%	ADDD	8.9%	DDD	13.0%	DDD
	5.8%	ADADDD DAADDD	5.8%	ADADDD DAADDD	6.3%	DADD	5.6%	ADADD DAADD
	4.2%	DADD	4.3%	DAADDD DADADD	6.1%	ADADD DAADD	5.0%	DAADD DADDA
	3.9%	ADADDD ADDADD ADDDDA	3.9%	DADD	5.7%	ADADDD DAADDD	4.7%	DDADD
	3.6%	DAADDD DADADD DADDDA	3.8%	DDADDD	4.7%	ADADD ADDAD ADDDA	4.2%	DADD
	3.6%	ADD	3.5%	DDADD	4.6%	ADD	3.0%	AADDD
	3.5%	DDADD	3.5%	AADDD	4.2%	DAADDD DADADD DADDAD	2.1%	ADD

D: GlcN; A: GlcNAc.

Supplementary Table 3 | Continued

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.35	3.2%	ADADD DAADD	2.6%	ADADDD ADDADD	3.3%	DAADD DADAD DADDA	2.1%	ADADD ADDDA
	3.2%	DAADD DADAD	2.4%	ADADD DAADD	3.0%	ADADDD ADDADD ADDDAD	1.8%	DDA
	3.1%	ADADD ADDAD	2.3%	DAADD DADDA	2.3%	DDADD	1.6%	DDAD
	3.1%	ADDAD DADAD	1.8%	ADD	2.2%	AADDD	1.5%	ADDDA DADDA
	2.9%	AADDD	1.2%	ADDADD DADADD	2.1%	ADDAD DADAD DDAAD	1.4%	DDDDD
	1.8%	ADDADD ADDDDA DADADD DADDDA			1.9%	ADDAD ADDDA DADAD DADDA	1.0%	ADAD ADDA
	1.7%	DDADDD			1.9%	DDAD		
	1.5%	DDAD			1.5%	ADDADD ADDDAD DADADD DADDAD		
	1.5%	ADDADD DADADD DDAADD			1.2%	AADADD AADDAD AADDDA ADAADD ADADAD ADADDA		
	1.4%	ADDDDA DADDDA DDADDA			1.2%	ADAD DAAD		
	1.1%	ADAD ADDA			1.2%	ADDDAD DADDAD DDADAD		
	1.0%	ADAD DAAD			1.0%	ADDADD DADADD DDAADD		
0.50	15.7%	DD	15.2%	ADDD	15.7%	DD	17.4%	ADDD
	15.2%	ADDD	14.1%	DD	15.1%	ADDD	17.4%	DD
	8.9%	ADADD DAADD	13.6%	DADDD	8.4%	DADDD	13.3%	DADDD
	7.7%	ADADD ADDAD	10.7%	DDD	8.0%	ADADDD DAADDD	10.0%	DDD
	7.3%	DADDD	9.2%	ADADDD DAADDD	7.6%	ADADD DAADD	7.6%	DAADD DADAD DADDA
	5.9%	DADD	6.9%	AADDD	6.9%	ADADDD ADDADD	7.0%	AADDD
	5.9%	ADD	5.8%	DAADDD DADADD	6.5%	DADD	6.6%	ADADD DAADD
	5.1%	AADDDD ADADDD ADDADD ADDDDA	4.5%	ADADDD ADDADD	6.0%	DAADD DADAD	4.3%	DADD
	4.8%	DAADDD DADADD DADDDA DDAADD DDADDA	4.2%	DADD	5.8%	ADD	3.1%	ADD
	4.8%	AADDDD ADADDD DAADDD	3.4%	DAADD DADAD DADDA	5.7%	ADADD ADDAD	3.1%	ADADD ADDAD ADDDA
	4.7%	DAADD DADAD	3.0%	DDADDD	4.4%	DDD	2.6%	ADDDA DADDA
	4.7%	DDD	2.8%	ADD	4.3%	AADDD	2.2%	DDA
	4.4%	ADDADD DADADD DDAADD	2.7%	ADADD DAADD	4.1%	ADDAD DADAD	2.0%	DDADD
	4.2%	AADDD	2.0%	ADADD ADDAD ADDDA	3.0%	ADAADD	1.8%	ADDA DADA
	3.5%	ADDAD DADAD	1.9%	DDADD	2.7%	DAADDD DADADD	1.6%	ADAD ADDA
	2.7%	ADAD DAAD	1.7%	ADDAD DADAD	1.9%	AADD	1.6%	AADD
	2.6%	ADAD ADDA	1.5%	AADADD AADDAD ADAADD ADADAD ADDAAD DAAADD DAADAD DADAAD	1.8%	ADAD DAAD	1.5%	ADDAD DADAD
	1.8%	DDAD	1.0%	DDA	1.6%	ADAD ADDA	1.3%	AAADD
	1.7%	ADAADD ADADAD ADADDA	1.0%	ADDDA DADDA	1.6%	ADDADD DADADD	1.1%	DA

D: GlcN; A: GlcNAc.

Supplementary Table 3 | Continued

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.50	1.6%	AADADD ADAADD DAAADD	1.0%	AAADDD	1.5%	AAADDD	1.1%	DDAD
	1.5%	DDADD	1.0%	AADADD AADDAD AADDDA	1.4%	DDADD	1.0%	DAAD DADA
	1.3%	AAADDD			1.2%	DDADDD		
	1.1%	AADD			1.1%	DDAADD		
	1.1%	DAAADD DAADAD DAADDA			1.0%	AAADD		
	1.0%	AADDAD ADADAD DAADAD						

D: GlcN; A: GlcNAc.

Supplementary Table 4 | CSN-MHKI products after the hydrolysis of chitosan with F_A 0.11-0.50. Shown are the molar fractions of each oligomer with an abundance of ≥ 1% and their possible pattern of acetylation. When two or more patterns are given, sequencing of the oligomer was not complete. Data was gained by quantitative sequencing.

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.11	61.6%	DD	62.7%	DD	60.1%	DD	65.2%	DD
	13.1%	DADD	12.9%	DADD	11.5%	DADD	9.0%	DADDD
	11.4%	DADDD	11.5%	DADDD	10.9%	DADDD	8.1%	DADD
	3.7%	ADDD	3.3%	ADDD	3.0%	ADDD	2.8%	DA
	2.0%	DAADDD DADADD	2.0%	DAADDD DADADD	2.4%	DAADDD DADADD	2.7%	ADDD
	1.5%	ADD	1.3%	ADD	2.2%	DAADD DADAD DADDA	1.8%	DDD
	1.5%	ADADDD DAADDD	1.3%	ADDADD DADADD	1.5%	ADADDD DAADDD	1.7%	DADDA
	1.3%	ADDADD DADADD	1.1%	ADADDD DAADDD	1.3%	ADDADD DADADD	1.0%	DAADD DADAD
	1.0%	AADDD	1.0%	ADADD DAADD	1.2%	DDADD		
					1.2%	DA		
					1.1%	DAD		
					1.1%	ADD		
0.19	48.2%	DD	51.9%	DD	48.7%	DD	53.6%	DD
	18.6%	DADD	17.9%	DADD	12.9%	DADD	13.4%	DADDD
	11.0%	DADDD	12.8%	DADDD	11.2%	DADDD	12.1%	DADD
	5.0%	ADDD	4.2%	ADDD	5.5%	ADDD	4.4%	ADDD
	3.9%	DAADDD DADADD	2.5%	DAADD	3.9%	DAADDD DADADD	3.6%	DAADD DADDA
	2.6%	ADD	2.3%	ADD	3.1%	ADDADD DADADD	2.5%	ADDDA DADDA
	2.3%	ADDADD DADADD	2.1%	AADDD	3.0%	ADD	2.1%	ADADD DAADD
	2.2%	ADADDD DAADDD	1.1%	DDADD	2.5%	ADADD ADDAD DAADD DADAD	1.7%	DA
	2.1%	AADDD			2.2%	DAADD DADAD DADDA	1.7%	AADDD
	1.7%	DAADD DADAD			1.7%	DAD	1.3%	ADD
					1.6%	ADADDD DAADDD	1.0%	DDADD
					1.5%	AADDD	1.0%	ADADD ADDDA
					1.2%	DDADD		
0.35	28,5%	DD	33,2%	DD	32,0%	DD	37,2%	DD
	26,7%	DADD	23,0%	DADD	20,4%	DADD	18,7%	DADD
	9,9%	DADDD	11,8%	DADDD	13,5%	DADDD	14,5%	DADDD
	5,5%	DAADDD DADADD DADDDA	5,3%	DAADDD DADADD	6,7%	DAADDD DADADD DADDAD	7,2%	DAADD DADDA
	5,3%	ADDD	5,1%	DAADD DADAD	4,3%	DAADD	5,8%	ADADD DAADD
	4,9%	DAADD	5,0%	ADDD	3,8%	AADDD	4,2%	ADDD
	4,1%	ADD	4,0%	AADDD	3,6%	ADDADD DADADD	3,6%	AADDD
	4,1%	ADADDD DAADDD	3,2%	ADADDD DAADDD	3,4%	ADDD	3,1%	ADDDA DADDA
	3,8%	AADDD	3,2%	ADD	2,9%	ADADDD DAADDD	2,1%	ADD
	2,4%	ADDADD DADADD	2,8%	ADDADD DADADD	2,0%	ADD	1,6%	ADADD ADDDA
	1,8%	ADADDD ADDADD ADDDDA	2,1%	DDADD	1,5%	DADDA	1,4%	DA
	1,5%	AADD	1,2%	AADD	1,4%	DAD	1,3%	AADD
	1,3%	ADADD			1,3%	ADDDAD DADDAD	1,1%	DADA
	1,2%	AADADD ADAADD DAAADD			1,2%	ADADDD ADDADD ADDDAD	1,0%	DDADD
					1,1%	AADD	1,0%	DDDDD
					1,1%	AADADD DAAADD		

D: GlcN; A: GlcNAc.

Supplementary Table 4 | Continued

F_A	oligomer composition							
	replicate 1		replicate 2		replicate 3		replicate 4	
0.50	29.5%	DADD	23.1%	DADD	22.6%	DADD	49.5%	DD
	22.2%	DD	19.4%	DD	19.4%	DD	25.7%	DADD
	9.0%	DAADD	7.9%	DAADD	9.8%	DAADD DADAD	5.4%	ADDD
	8.1%	DADDD	7.7%	DADDD	6.8%	DADDD	4.3%	ADD
	7.0%	AADDD	7.4%	DAADDD DADADD	6.7%	ADDD	3.4%	ADDA DADA
	6.3%	ADD	6.6%	ADDADD DADADD	5.3%	DAADDD DADADD DADDAD	3.0%	DA
	5.9%	ADDD	6.2%	ADDD	4.4%	ADD	2.9%	AADD
	4.2%	AADD	6.0%	ADD	3.8%	AADDD	2.2%	ADAD ADDA
	2.6%	ADADD	5.9%	AADDD	3.7%	ADADDD DAADDD	1.9%	DDAD
			3.8%	AADD	2.7%	ADAADD ADADAD DAAADD DAADAD	1.8%	DAAD DADA
			2.3%	ADADD	2.7%	AADADD ADAADD DAAADD	1.6%	DDDD
			1.6%	AADADD	2.5%	ADADD ADDAD		
			1.6%	ADADDD DAADDD	2.4%	ADDADD DADADD		
			1.6%	DAAADD	2.4%	ADADDD ADDADD ADDDAD		
			1.0%	DDAD	2.1%	AADD		
					1.8%	DAD		
					1.7%	DAAD DADA		
					1.7%	ADAD DAAD		
					1.5%	ADDDAD DADDAD		
					1.5%	ADAADD ADADAD ADDAAD		
					1.5%	DAAADD DAADAD DADAAD		
					1.1%	ADDDD		

D: GlcN; A: GlcNAc.

Supplementary Table 5 | Relative abundancies (molar fraction) of patterns at the reducing and non-reducing ends of oligomers in the early phase of hydrolysis (15 min). Oligomers were produced from chitosans with F_A 0.11-0.50 using CSN174, CSN-7M, CSN-MN, and CSN-MHKI. Shown are the mean values with standard deviations of three independent measurements of three independent enzyme batches.

	reducing ends unknown pattern	reducing end dyad frequencies			
		AA	AD	DA	DD
CSN-174 F_A 0.11	0.0 ± 0.0%	0.1 ± 0.1%	1.1 ± 0.1%	9.8 ± 6.2%	89.0 ± 6.3%
CSN-174 F_A 0.50	0.0 ± 0.0%	1.1 ± 1.3%	4.4 ± 1.1%	37.4 ± 6.4%	57.1 ± 6.4%
CSN-7M F_A 0.11	0.5 ± 0.5%	0.5 ± 0.6%	2.1 ± 0.4%	1.8 ± 0.6%	95.5 ± 0.9%
CSN-7M F_A 0.50	0.7 ± 0.9%	0.4 ± 0.2%	4.7 ± 1.6%	1.7 ± 0.5%	93.2 ± 1.7%
CSN-MN F_A 0.11	0.0 ± 0.0%	0.7 ± 1.0%	1.4 ± 1.5%	0.9 ± 0.9%	97.0 ± 2.3%
CSN-MN F_A 0.50	0.7 ± 1.0%	0.6 ± 0.9%	3.8 ± 5.3%	2.3 ± 3.2%	93.3 ± 9.4%
CSN-MHKI F_A 0.11	0.0 ± 0.0%	0.1 ± 0.1%	0.7 ± 0.4%	9.0 ± 12.0%	90.2 ± 12.4%
CSN-MHKI F_A 0.50	8.5 ± 11.2%	4.1 ± 4.2%	5.9 ± 5.5%	6.0 ± 4.3%	83.9 ± 13.8%
	non-reducing ends unknown pattern	**non-reducing end dyad frequencies**			
		AA	AD	DA	DD
CSN-174 F_A 0.11	0.0 ± 0.0%	0.0 ± 0.1%	1.4 ± 0.9%	6.0 ± 1.8%	92.6 ± 2.4%
CSN-174 F_A 0.50	0.0 ± 0.0%	1.5 ± 1.1%	4.1 ± 1.2%	12.7 ± 4.9%	81.7 ± 4.5%
CSN-7M F_A 0.11	0.4 ± 0.5%	0.3 ± 0.3%	1.4 ± 0.1%	6.9 ± 1.1%	91.4 ± 1.2%
CSN-7M F_A 0.50	0.0 ± 0.0%	0.6 ± 0.3%	3.0 ± 1.0%	21.5 ± 2.9%	74.9 ± 3.8%
CSN-MN F_A 0.11	0.2 ± 0.2%	0.7 ± 1.0%	0.4 ± 0.3%	2.7 ± 3.6%	96.2 ± 3.5%
CSN-MN F_A 0.50	0.0 ± 0.0%	0.9 ± 1.3%	2.6 ± 3.7%	9.6 ± 13.6%	86.6 ± 18.6%
CSN-MHKI F_A 0.11	0.0 ± 0.0%	0.0 ± 0.1%	1.4 ± 0.5%	6.8 ± 1.1%	91.9 ± 1.6%
CSN-MHKI F_A 0.50	8.1 ± 11.5%	4.1 ± 4.2%	5.3 ± 3.0%	14.4 ± 9.3%	76.3 ± 15.5%

D: GlcN; A: GlcNAc.

Supplementary Table 6 | Pattern distribution of mono-acetylated oligomers with DP 3-6 produced with CSN-7M. Given are the molar fractions of each oligomer at the end point of hydrolysis. Shown are the mean values with standard deviations of three independent measurements of three independent enzyme batches.

oligomer	pattern of acetylation	mole fraction			
		F_A 0.11	F_A 0.19	F_A 0.35	F_A 0.50
D_4	DDDD	0.4 ± 0.0%	0.5 ± 0.0%	1.2 ± 0.8%	0.8 ± 0.2%
D_2A_1	DDA	0.4 ± 0.0%	0.4 ± 0.0%	0.3 ± 0.0%	0.4 ± 0.2%
	DAD	0.4 ± 0.0%	0.4 ± 0.0%	0.2 ± 0.0%	0.6 ± 0.5%
	ADD	3.0 ± 0.1%	3.7 ± 0.2%	1.5 ± 0.1%	4.5 ± 2.7%
D_3A_1	DDDA	0.3 ± 0.0%	0.3 ± 0.0%	0.4 ± 0.0%	0.3 ± 0.2%
	DDAD	1.2 ± 0.2%	1.3 ± 0.3%	0.3 ± 0.2%	0.4 ± 0.0%
	DADD	2.9 ± 0.3%	4.1 ± 0.6%	3.8 ± 1.1%	5.2 ± 1.3%
	ADDD	6.3 ± 0.2%	8.7 ± 0.3%	11.3 ± 1.1%	11.2 ± 2.4%
D_4A_1	DDDDA	0.0 ± 0.0%	0.1 ± 0.1%	0.6 ± 0.1%	0.0 ± 0.1%
	DDDAD	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.3 ± 0.5%
	DDADD	1.8 ± 0.1%	3.5 ± 0.3%	6.5 ± 0.6%	3.4 ± 1.8%
	DADDD	5.8 ± 0.5%	7.4 ± 0.3%	20.2 ± 0.7%	13.7 ± 7.5%
	ADDDD	0.1 ± 0.1%	0.2 ± 0.2%	1.3 ± 1.3%	0.2 ± 0.1%
D_5A_1	DDDDDA	0.0 ± 0.0%	0.0 ± 0.0%	0.1 ± 0.1%	0.0 ± 0.0%
	DDDDAD	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.1%	0.0 ± 0.0%
	DDDADD	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.2 ± 0.3%
	DDADDD	0.0 ± 0.0%	0.1 ± 0.2%	3.6 ± 0.6%	1.7 ± 1.2%
	DADDDD	0.0 ± 0.0%	0.0 ± 0.0%	0.1 ± 0.1%	0.1 ± 0.1%
	ADDDDD	0.0 ± 0.0%	0.0 ± 0.1%	0.4 ± 0.4%	0.1 ± 0.1%

D: GlcN; A: GlcNAc.

Supplementary Table 7 | MS parameters for tandem MS of re-*N*-acetylated ^{18}O-labelled chitosan oligomers. Shown is an exemplary setup, as the elution time was adjusted for each set of measurement.

Elution time [min]	Target mass [m/z]	Mass [m/z]	Sequence	Width	Cut-Off	Amplitude [%]
0-4	200	-	-	-	-	-
4-7.5	420	430.2	A1D1	2	116	85
7.5-11.5	630	633.2	A2D1	2	171	90
7.5-11.5	630	636.2	A1D2	2	172	90
11.5-15.5	840	836.3	A3D1	1.5	226	100
11.5-15.5	840	839.3	A2D2	1.5	227	100
11.5-15.5	840	842.3	A1D3	1.5	227	100
15.5-19	1040	1039.4	A4D1	1.5	281	100
15.5-19	1040	1042.4	A3D2	1.5	281	100
15.5-19	1040	1045.4	A2D3	1.5	282	100
15.5-19	1040	1048.4	A1D4	1.5	283	100
19-23.5	1240	1242.5	A5D1	1.5	335	110
19-23.5	1240	1245.5	A4D2	1.5	336	110
19-23.5	1240	1248.5	A3D3	1.5	337	110
19-23.5	1240	1251.5	A2D4	1.5	338	110
19-23.5	1240	1254.5	A1D5	1.5	339	110

D: GlcN; A: GlcNAc.

Supplementary Table 8 | List of pectin samples used.

Pectin substrate	Abbreviation	Supplier	DM
polymeric pectin C	PC	Carl Roth GmbH (Karlsruhe, Germany)	≈ 70%
polymeric pectin from apple	PAP	Carl Roth GmbH (Karlsruhe, Germany)	≈ 38%
polymeric pectin from citrus peel	PCP	Fluka (Buchs, Switzerland)	63-66%

DM: degree of methyl esterification.

Supplementary Table 9 | List of pectin degrading enzymes used.

Enzyme	Abbreviation	Supplier
endo-Polygalacturonanase from *Aspergillus niger*	PG	Megazyme (Bray, Ireland) Lot No. MPG00901
Pectate lyase from *Aspergillus sp*.	PL	Megazyme (Bray, Ireland) Lot No 10801

Supplementary Table 10 | HILIC parameters for LC- MS measurements of pectin oligomers.

time [min]	eluent
0-2.5	100% A
2.5-15	linear gradient to 100% B
15-17	100% B
17-17.5	linear gradient to 100% A
17.5-20	100% A