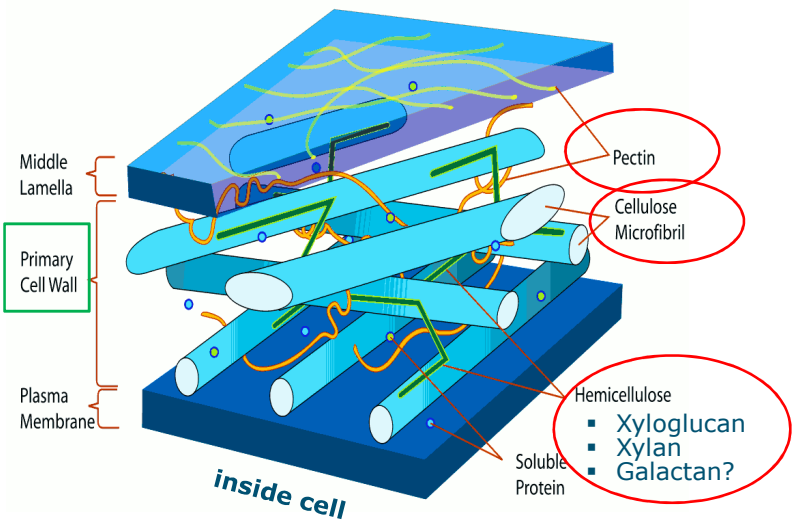
Physiciochemical characteristics of pectin as dietary fiber and its role in colonic health

Henk A. Schols
Laboratory of Food Chemistry

WAGENINGEN UNIVERSITY & RESEARCH

CARBOHYDRATE COMPETENCE CENTER

Primary cell wall - architecture



WAGENINGEN UNIVERSITY & RESEARCH

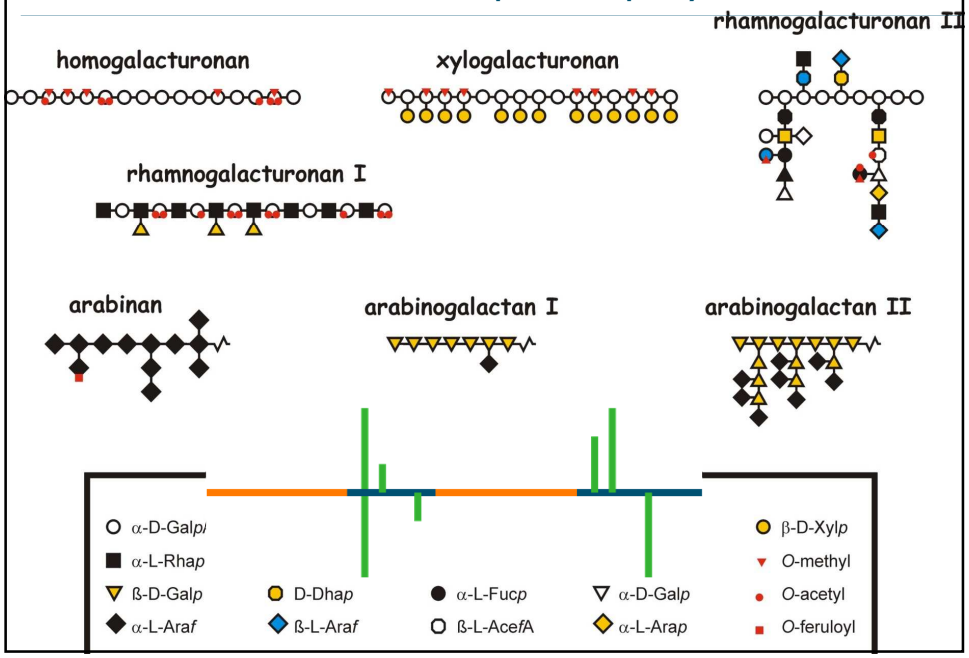
McCann et al. 1992 J. microsc., 166, 123-136

Pectin plays an important role as:

- Cell Wall component
  - Determine (partly) texture of tissues
  - Change during ripening of e.g. fruits (endogenous enzymes)
  - Change during processing due to depolymerization, de-esterification, solubilization, etc.
  - Present in by-products from agro industry
- Ingredient for food industry
  - Modification during extraction and down-stream processing
  - Thickener and gelling agent
  - Stabilizer in fruit and milk beverages
- Health promoting component
  - Lowering cholesterol levels
  - Dietary Fiber & fermentation
  - Immuno modulating

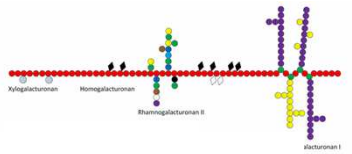
WAGENINGEN UNIVERSITY & RESEARCH

Schematic structure of pectic polysaccharides



Occurrence and proportion of the various structural elements of native pectin in apple, sugar beet and soybean

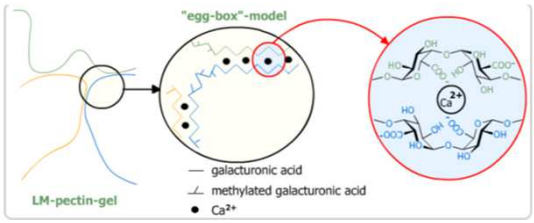
	soybean meal	sugar beet pulp	apple
total polysaccharide (w/w % dm)	16	67	20
pectic substances (% of total PS)	59	40	42
structural element (% of pectic substances)			
homogalacturonan	0	29	36
xylogalacturonan	21	<1	4
rhamnogalacturonan II	4	4	10
rhamnogalacturonan backbone	15	8	4
arabinan	60	46	27
arabinogalactan I		12	20
arabinogalactan II	0	0	+



Same building blocks!!
However, 'always' different fine-structure and different relative amounts!

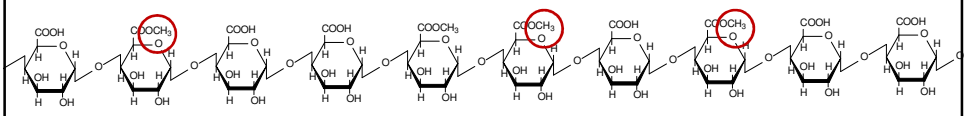
Pectin as food ingredient

- HM Pectin: sugar-acid gel
  - Sugar lowers water activity and increase chain-chain interaction (= gel)
  - Low pH reduces acidity – less interaction – more chain-chain interaction
- LM Pectin
  - Calcium-pectate gel
  - Low sugar jams



WAGENINGEN
UNIVERSITY & RESEARCH

Parameters influencing food applications of pectins

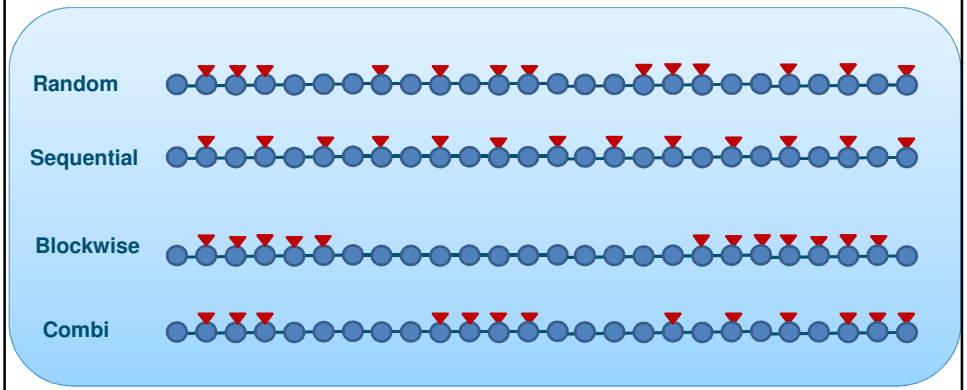


- Raw material used
- Extraction conditions used
  - Down stream modification (HM vs LM pectin, amidation)

- Molecular weight
- Degree of methyl esterification
  (and acetyl groups if present)
- Distribution of methyl esters
  (and acetyl groups if present)

- Calcium-sensitivity
- Solubility

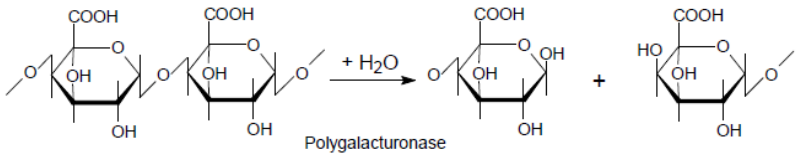
Homogalacturonan key parameters: Level and distribution of methyl esters



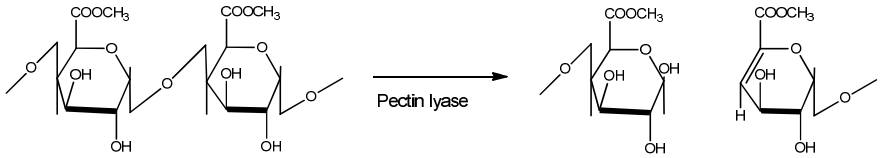
Pectin functionalities are strongly determined by level and distribution of the methyl esters

Characterisation of pectins using enzymatic fingerprinting

Use of polygalacturonase (PG) and pectine lyase (PL) to degrade pectins into diagnostic oligomers



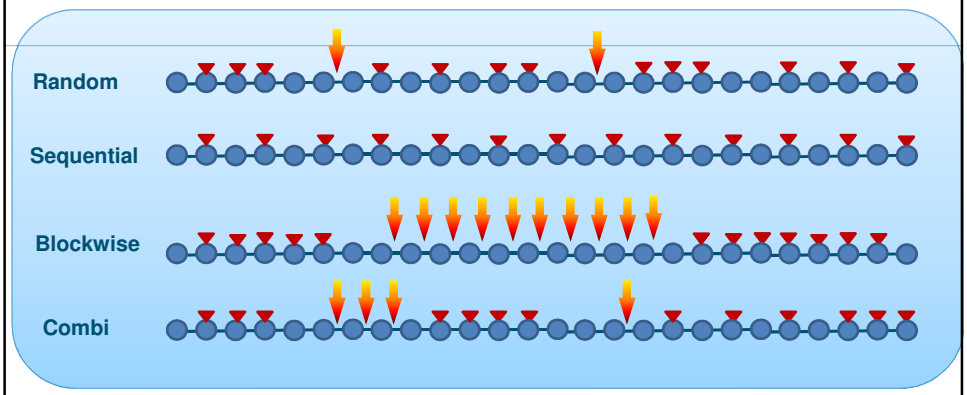
PG: Release of saturated galacturonic acid oligomers



PL: Release of unsaturated galacturonic acid oligomers

WAGENINGEN UNIVERSITY & RESEARCH

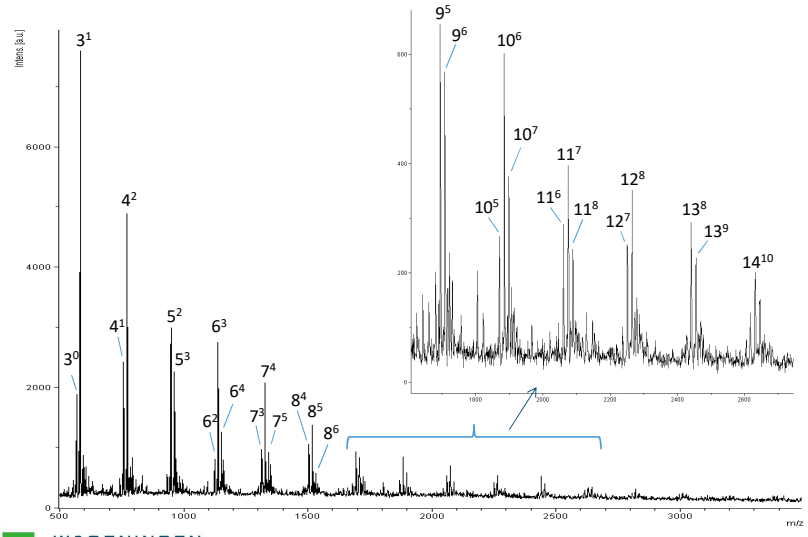
Homogalacturonan: Finger printing of methyl ester groups using PG



= Galacturonic acid (GalA) = methyl-GalA = site of attack endo-PG

WAGENINGEN UNIVERSITY & RESEARCH

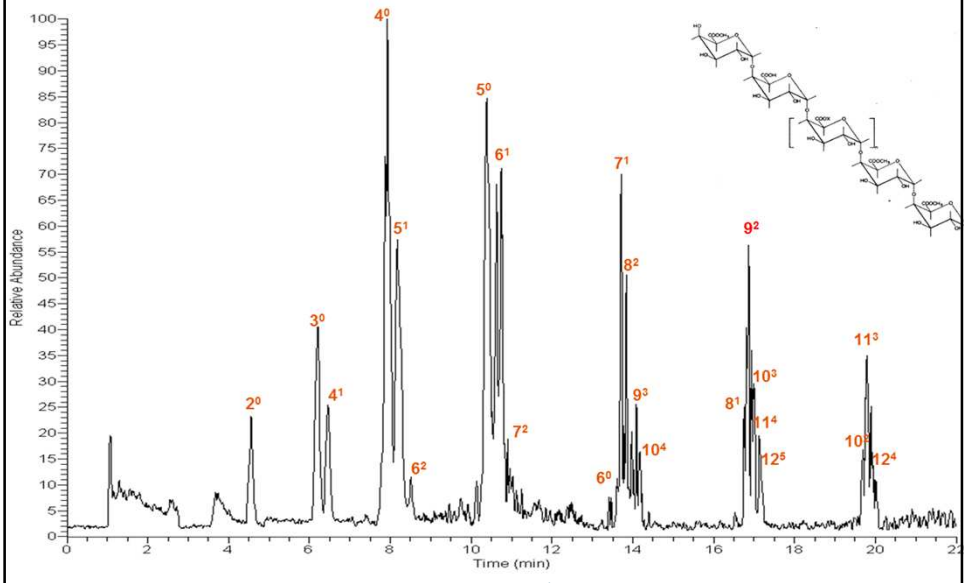
MALDI TOF MS of random esterified DM70 pectin digested by endo-PG



WAGENINGEN UNIVERSITY & RESEARCH

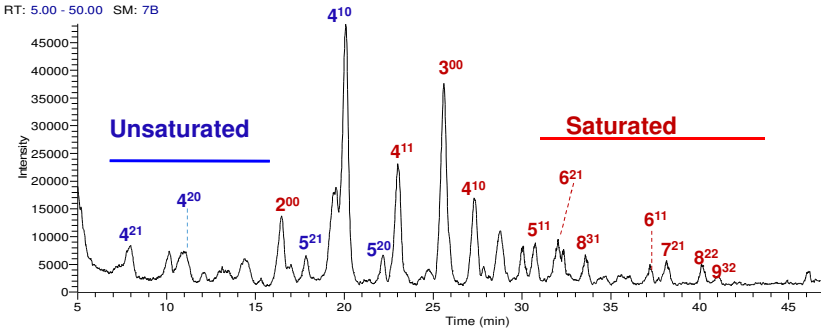
4^2= $GalA_4$ 2 methyl

HILIC-Iontrap-MS^n method: methyl-esterified pectin oligomers



5^1= $GalA_5$ 1 methyl

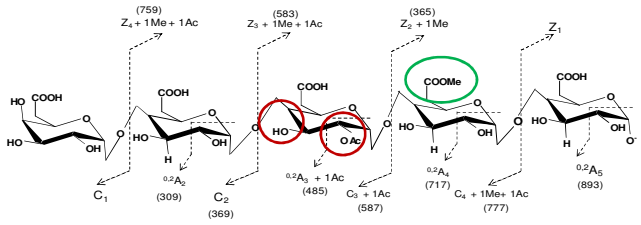
HILIC-Iontrap-MSn method is also capable to separate and identify complex mixtures of oligomers after PG and PL digestion of Sugar Beet Pectin



Annotation: 4^{10}= 4 galacturonic acid unit; 1 methyl group. 0 acetyl groups

WAGENINGEN UNIVERSITY & RESEARCH

HILIC-MS/MS fragmentation enables the structure elucidation of SBP oligomers

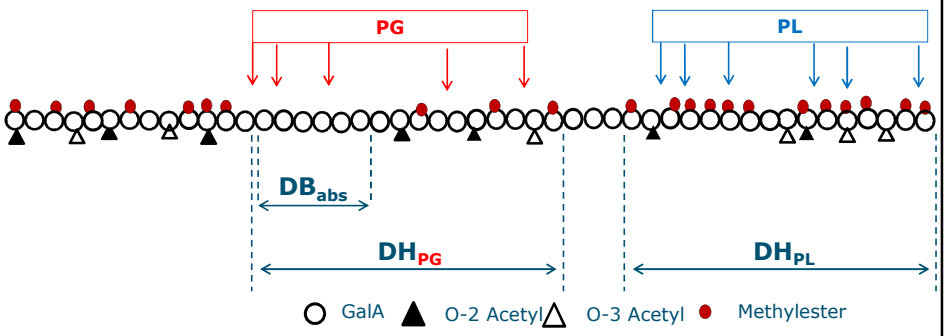


5^{11} (*m/z* 953)

Precise annotation of methyl ester and O-2 and O-3 acetyl group position by ring cleavage MS/MS fragmentation

Introduction of descriptive parameters

Degree of Blockiness and Degree of Hydrolysis



DB does quantify unsubstituted mono-, di- and tri GalA oligomers as released by PG

DH_{PG} does also include PG released methyl esterified segments of the pectin

DH_{PL} does quantify PL released highly methyl esterified / acetylated oligomers from the pectin

Pectin is a dietary fibre

Official AACC definition of dietary fibre

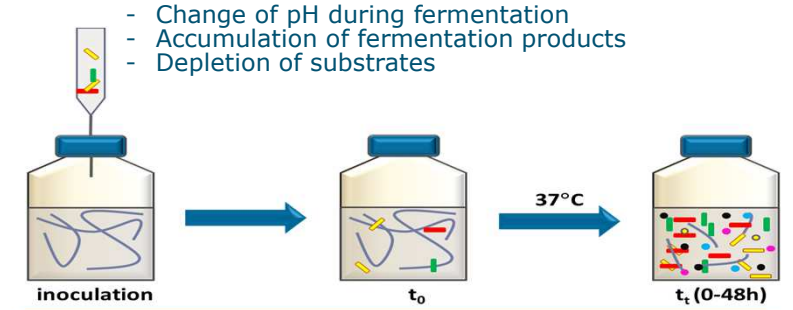
- Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and adsorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.

WAGENINGEN UNIVERSITY
WAGENINGEN UR

Batch fermentation of DF

+ Simple, easy to prepare, 'one bottle' fermentation.
+ Buffer-regulated pH
+ Possibility to do fermentation with small amount of substrate.
+ Fast, a lot of substrates can be tested simultaneously.

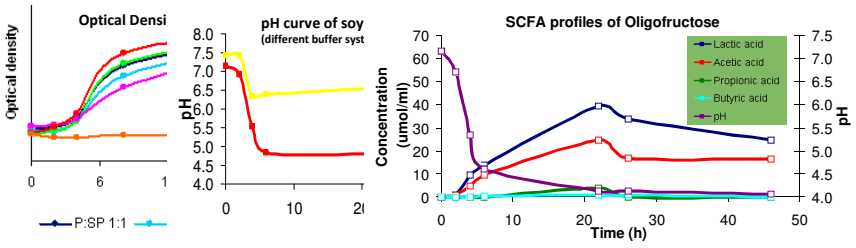
- Change of pH during fermentation
- Accumulation of fermentation products
- Depletion of substrates



WAGENINGEN UNIVERSITY & RESEARCH

Monitoring *in vitro* fermentation (human fecal inoculum)

- Optical Density
- pH curve
- Short Chain Fatty Acids
- Remaining dietary fibers (after hydrolysis)
- NEED TO ANALYSE INDIVIDUAL MOLECULES



WAGENINGEN UNIVERSITY & RESEARCH

In vivo piglet trial

Soluble pectin supplementation to diet

citrus pectin

LMP/HMP
3%

+

wheat based diet +
Soy bean meal

+

wheat AX

+

TiO_2
0.25%

autoclaved
soybean meal

Soy pectin 3%

+

wheat AX

+

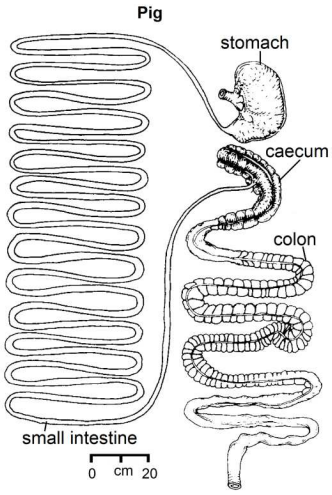
TiO_2
0.25%

+

wheat based diet

WAGENINGEN
UNIVERSITY & RESEARCH

Sample and data collection



Gastrointestinal tract of pig (Stevens et al., 1998).

Digesta present in:

- Ileum
- Proximal colon (**pCol**)
- Mid colon (**mCol**)
- Distal colon (**dCol**)

Analysis of :

- Dietary Fibre + fragments
- Short Chain Fatty Acids
- Digestibility marker
- Microbiota composition

WAGENINGEN
UNIVERSITY & RESEARCH

Constituent monosaccharide composition of total NSP in the digesta

	CONT				LMP			
	Ileum	pCol	mCol	dCol	Ileum	pCol	mCol	dCol
DM	16.2	16.2	23.9	29.1	14.4	15.7	19.1	22.2
Protein	23.8	28.8	36.4	34.2	22.2	21.9	24.7	26.1
Starch	7.4	6.1	1.1	0.7	11.9	7.3	6.2	5.1
LMW-sugars	0.5	t	t	t	0.9	t	t	t
NSP	38.9	32.7	29.8	29.6	33.3	32.3	31.4	31.3
Molar proportion (mol%)								
Glc	*38*	*38*	*37*	*37*	*44*	*38*	*39*	*39*
Xyl	*25*	*30*	*35*	*35*	*18*	*29*	*31*	*31*
Ara	*15*	*15*	*15*	*16*	*11*	*15*	*14*	*15*
UA	*6*	*8*	*6*	*5*	*15*	*10*	*7*	*6*
Gal	*9*	*5*	*4*	*4*	*8*	*5*	*5*	*5*
Man	*4*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
Rha	*1*	*2*	*1*	*1*	*1*	*2*	*2*	*2*
A/X	0.62	0.51	0.45	0.46	0.62	0.51	0.47	0.47

WAGENINGEN UNIVERSITY & RESEARCH

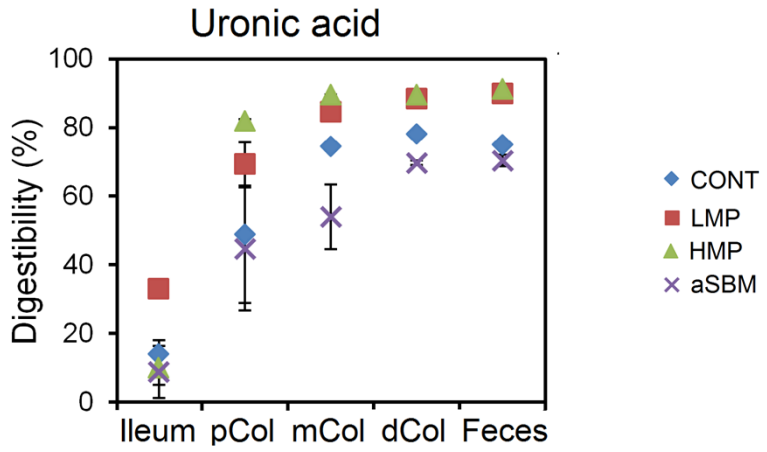
Constituent monosaccharide composition of total NSP in the digesta

	CONT				LMP				HMP				aSBM			
	Ileum	pCol	mCol	dCol	Ileum	pCol	mCol	dCol	Ileum	pCol	mCol	dCol	Ileum	pCol	mCol	dCol
DM	16.2	16.2	23.9	29.1	14.4	15.7	19.1	22.2	13.6	18.6	20.0	23.9	14.0	13.6	16.6	20.7
Protein	23.8	28.8	36.4	34.2	22.2	21.9	24.7	26.1	23.1	22.8	26.3	31.3	18.8	23.2	26.3	30.3
Starch	7.4	6.1	1.1	0.7	11.9	7.3	6.2	5.1	7.3	5.7	4.8	2.1	8.4	5.8	3.4	2.1
LMW-sugars	0.5	t	t	t	0.9	t	t	t	0.9	t	t	t	1.0	t	t	t
NSP	38.9	32.7	29.8	29.6	33.3	32.3	31.4	31.3	34.2	35.0	31.8	32.2	39.7	35.4	33.6	31.2
Molar proportion (mol%)																
Glc	*38*	*38*	*37*	*37*	*44*	*38*	*39*	*39*	*33*	*40*	*39*	*37*	*38*	*38*	*37*	*38*
Xyl	*25*	*30*	*35*	*35*	*18*	*29*	*31*	*31*	*17*	*30*	*33*	*34*	*24*	*29*	*31*	*33*
Ara	*15*	*15*	*15*	*16*	*11*	*15*	*14*	*15*	*12*	*15*	*15*	*15*	*16*	*16*	*15*	*15*
UA	*6*	*8*	*6*	*5*	*15*	*10*	*7*	*6*	*24*	*7*	*5*	*6*	*7*	*7*	*7*	*6*
Gal	*9*	*5*	*4*	*4*	*8*	*5*	*5*	*5*	*9*	*4*	*4*	*4*	*9*	*6*	*5*	*5*
Man	*4*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*3*	*2*	*1*	*2*	*4*	*3*	*2*	*2*
Rha	*1*	*2*	*1*	*1*	*1*	*2*	*2*	*2*	*1*	*1*	*2*	*1*	*1*	*1*	*1*	*1*
A/X	0.62	0.51	0.45	0.46	0.62	0.51	0.47	0.47	0.72	0.50	0.46	0.45	0.71	0.54	0.48	0.45

- Starch remains more dominantly present in pectin diets
  - Digestibility depends on pectin methyl esterification and complexity
- Different dietary fibers are fermented differently
- For quantitative data → digestibility marker was added

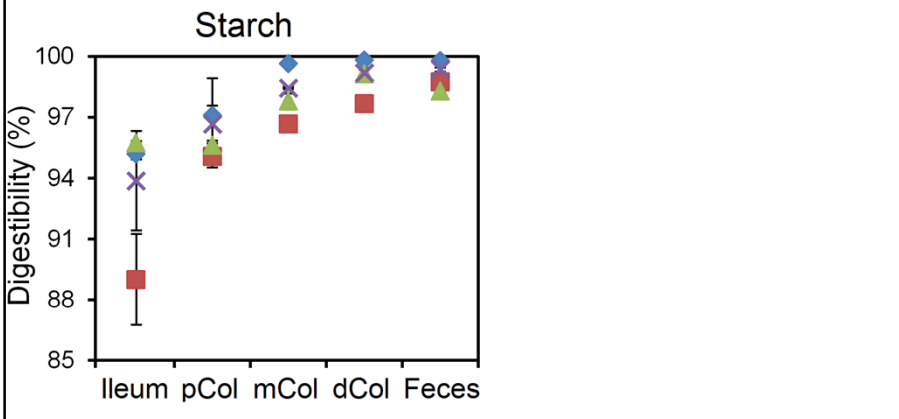
WAGENINGEN UNIVERSITY & RESEARCH

Apparent digestibility of pectin



- Pectin is highly fermentable.
  - Fermentation profile is different for LM and HM pectin
- Although same soy pectin structures are present in CONT and aSBM, pectins present are differently fermented → soluble vs insoluble soy pectin

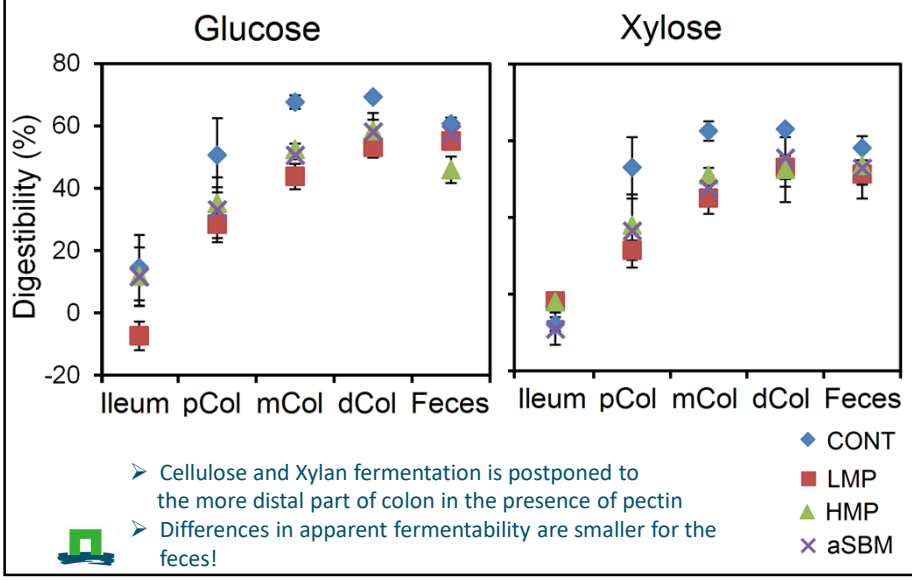
Apparent digestibility of starch and Dietary Fibre (NSP)



- Starch digestion is slightly lower in presence of pectin
  - Digestibility depends on pectin methyl esterification
- Also Dietary Fibre (NSP) fermentation is postponed to distal part of colon in the presence of pectin

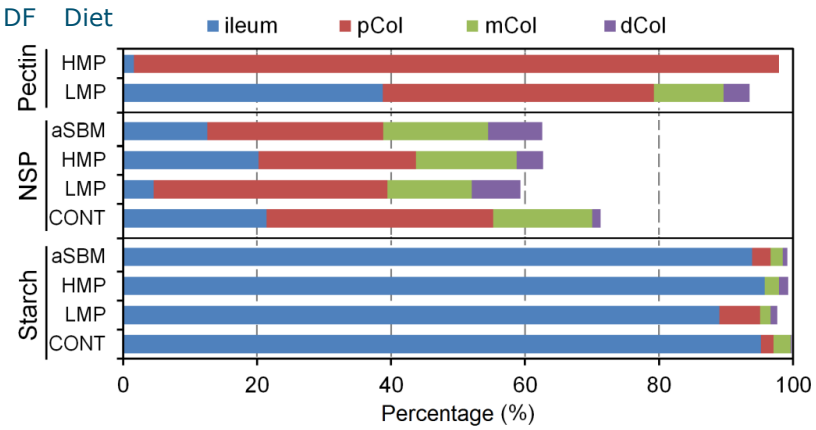
Apparent digestibility of glucose and xylose

(for cellulose and cereal xylan respectively)



- Cellulose and Xylan fermentation is postponed to the more distal part of colon in the presence of pectin
- Differences in apparent fermentability are smaller for the feces!

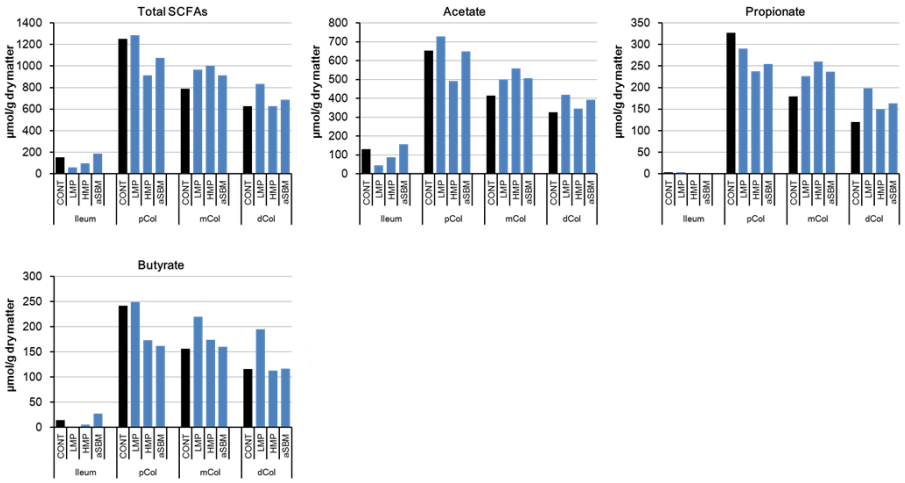
Summary: w/w percentage of digested starch, pectin and NSP in different sections



CONT: control diet;
LMP: low methyl-esterified pectin enriched diet;
HMP: high methyl-esterified pectin enriched diet;
aSBM: autoclaved soybean meal enriched diet.

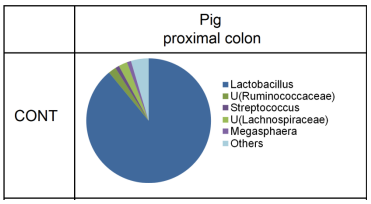
WAGENINGEN UNIVERSITY & RESEARCH

Concentration of SCFAs in digesta of piglets fed with different diets



WAGENINGEN
UNIVERSITY & RESEARCH

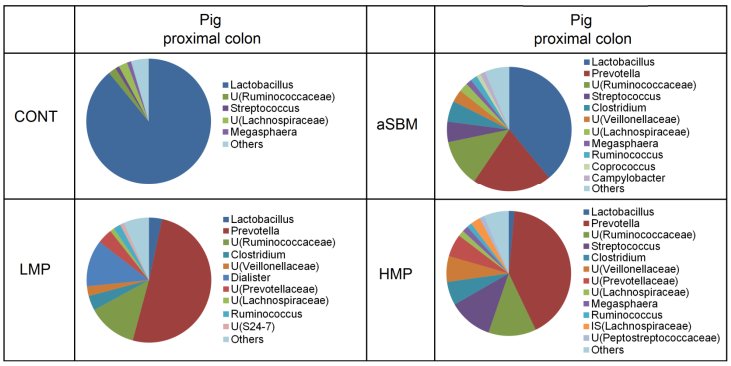
Relative abundance of microbiota at the genus level



Lactobacillus
Prevotella
U(Ruminococcaceae)
Megasphaera

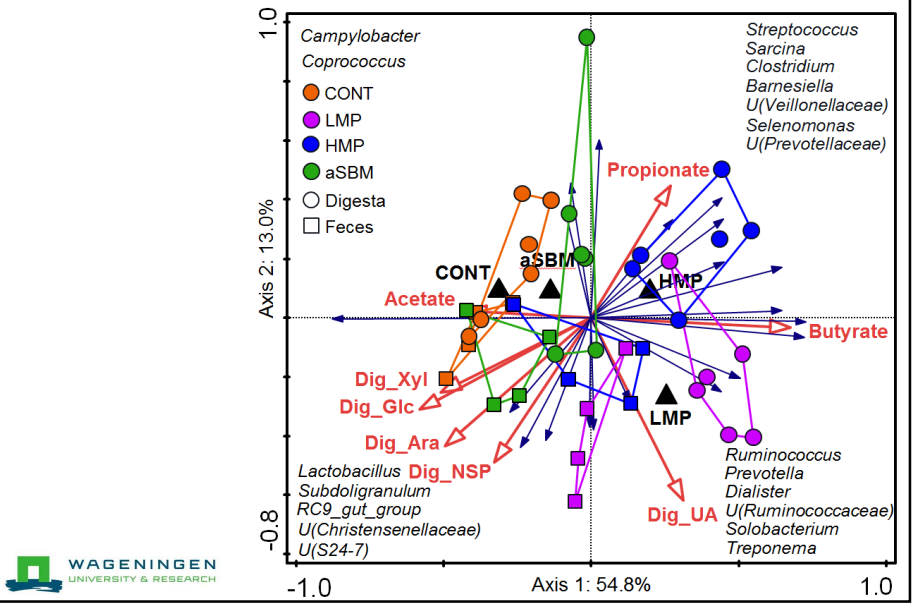
Microbiota pattern, typical for plant eaters

Relative abundance of microbiota at the genus level



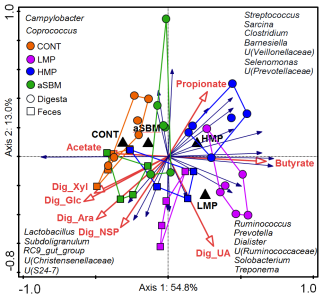
Lactobacillus ↓
Prevotella ↑
U(Ruminococcaceae) ↑
Megasphaera ↑

Link between microbiota, SCFAs and fiber digestibility



WAGENINGEN
UNIVERSITY & RESEARCH

Link between microbiota, SCFAs and fiber digestibility



- Colonic digesta from LMP- and HMP- and aSBM-fed pigs clustered separately from the samples obtained from CONT-fed pigs.
- *Prevotella*, *Dialister* and unclassified microbes in the family Ruminococcaceae correlated with the LMP diet.

- *Prevotella* in the colonic digesta of LMP- and HMP-fed pigs correlated with the production of butyrate and propionate.
- The digestibility of uronic acid correlated with the production of butyrate and genera *Prevotella, Dialister* and unclassified microbes in the family Ruminococcaceae.

WAGENINGEN
UNIVERSITY & RESEARCH

Conclusions

- Pectins were rapidly fermented in intestinal tract of piglets
- Different fermentation characteristics for pectins, depending on methyl esterification
- Solubilized soy pectin was differently fermented than untreated/insoluble soy pectin
- Supplementation of pectins shifted the fermentation site of other DFs to more distal parts
- Starch mainly utilized in small intestine! However, fermentation speed may depend on other fibres present
- Pectin supplementation could be a strategy to control fibre fermentability over the entire colon
- Pectin also have shown to influence the immune system !

Thank you for your attention !

Acknowledgements

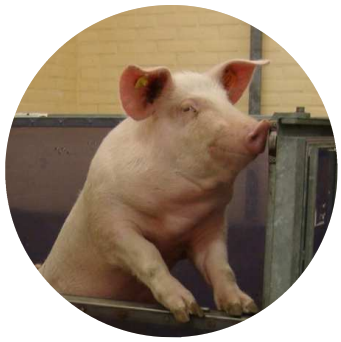
Animal Nutrition WU
Carol Souza da Silva
Guido Bosch

Food Chemistry WU
Melliana Jonathan Christiane Rosch
Lingmin Tian Harry Gruppen

Host Microbe Interactomics WU
Marjolein Meijerink Nico Taverne
Jerry Wells

Microbiology WU
Klaudyna Borewicz Hauke Smidt

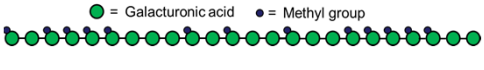
University Medical Center Groningen
Neha Sahasrabudhe Leonie Vogt
Paul de Vos



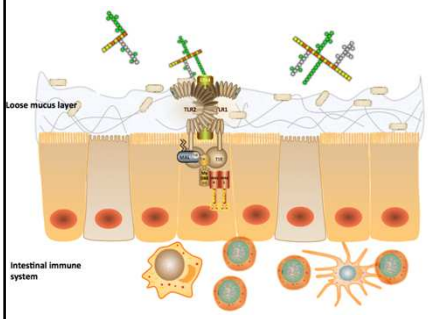
WAGENINGEN UNIVERSITY & RESEARCH

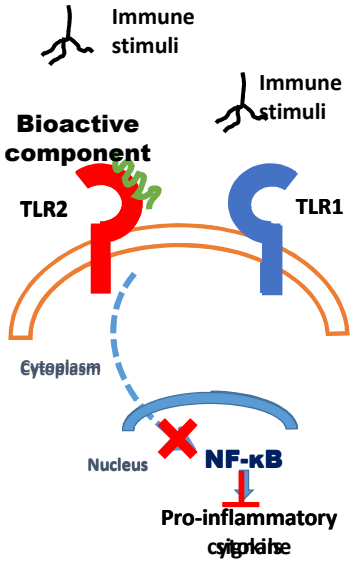
UMCG

Pectin immune modulating properties



Different for different pectins

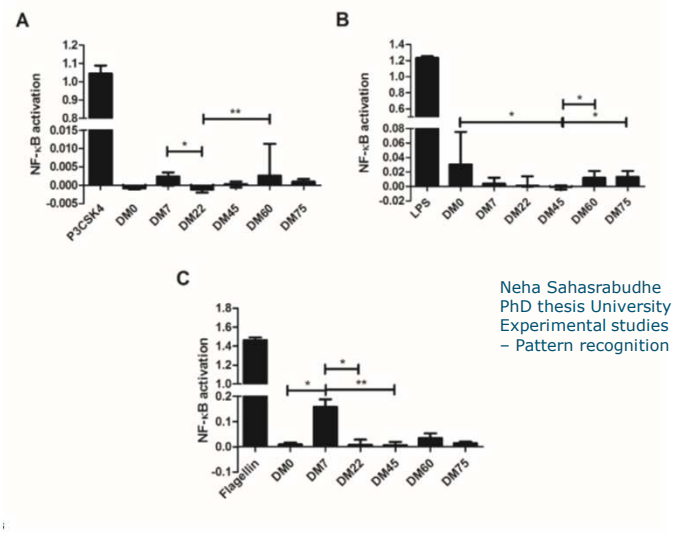




WAGENINGEN UNIVERSITY & RESEARCH

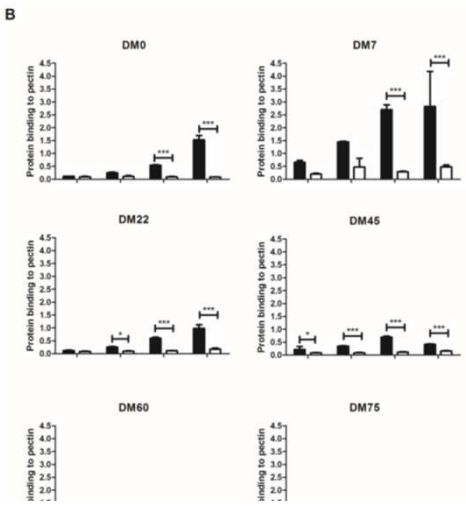
Pectin does not activate TLR2, TLR4 and TLR5.

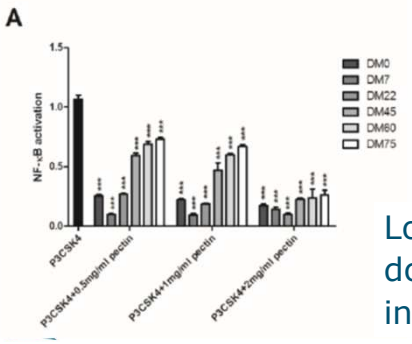
TLR activation represented as NF-κB activation of HEK-BlueTM TLR



Neha Sahasrabudhe
PhD thesis University Medical Center Groningen
Experimental studies on dietary fibers
– Pattern recognition receptor interactions

Low DM pectin inhibits TLR2-TLR1 by directly binding to the TLR2 ectodomain

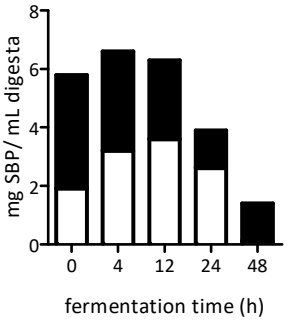




Low DM pectin reduces the doxorubicin induced inflammation in mice

Increase of immune activity during the in vitro batch fermentation of sugar beet pectin

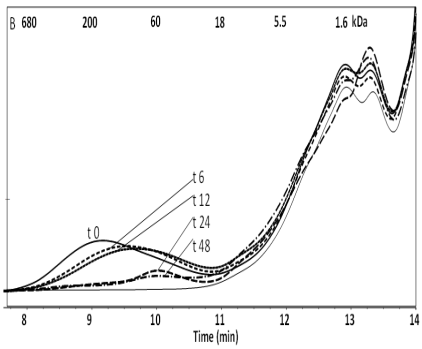
Amounts of soluble (black) and insoluble (white) sugar beet pectin present in batch fermentation digesta



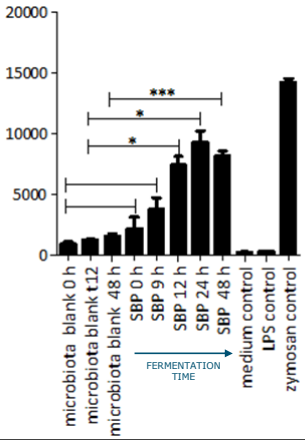
WAGENINGEN UNIVERSITY & RESEARCH

Increase of immune activity during the in vitro batch fermentation of sugar beet pectin

HPSEC polysaccharide profile of SBP (0, 6, 12, 24, 48 h) fermentation digesta



TNF-acytokine production by Bone Marrow Dendritic Cells of TLR2/4 knockout mice



WAGENINGEN UNIVERSITY & RESEARCH