Nestlé Good food, Good life

ENFiber
European Network for Valorising Food Processing Waste into Sustainable Fibers

Analytics for characterization of food carbohydrates & dietary fibers

Dr. Carolina O. Pandeirada (COP)

(carolina.oliveirapandeirada@rd.nestle.com)

Specialist in Carbohydrates

Department of Proteins & Carbohydrates Analytics

Nestlé Research (Switzerland)

Tartu (Estonia), 05.05.2026


Nestlé Research in Lausanne, Switzerland. Pic: Nestlé

Dietary Fiber (DF) definitions

❖ Dietary fiber, as defined by the American Association of Cereal Chemists International (AACCI) (now Cereals & Grains Association):

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

❖ **Several other definitions of DF have been subsequently published, e.g.:**

'Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Added fiber consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Total fiber is the sum of dietary fiber and added fiber.' (Food Nutrition Board (FNB) of the Institute of Medicine of the National Academies (USA) (2001))

'Dietary fiber consists of carbohydrate polymers with **ten or more** monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, and; synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.' (2009 by the Codex Alimentarius Commission (CAC))

AOAC Methods

Table 1. AOAC (OMA), AACCI, ICC, and Codex official methods for the analysis of dietary fiber and what they claim to measure

AOAC OMA	AACC	CODEX Type	ICC	What OMA claims to measure
985.29	32-05.01	I	-	Total dietary fibre in foods
991.42	32-20.01	I	-	Insoluble dietary fibre in foods and food products
991.43	32-07.01	I	-	Total, soluble and insoluble dietary fibre in foods
993.19	-	I	-	Soluble dietary fibre in foods and food products
994.13	32-25.01	I	-	Total Dietary Fibre (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin)
2001.03	32-41.01	I	-	Dietary fibre containing supplemented resistant maltodextrin (RMO)
993.21	-	I	-	Total dietary fibre in food and food products with ≤ 2% starch
2009.01	32-45.01	I	-	Total dietary fiber in foods (INTDF)
2011.25[a]	32-50.01	I	-	Insoluble, soluble and total dietary fibre in foods (INTDF)
2017.16[a]	32-60.01	I	185	Total dietary fibre in foods and food ingredients (RINTDF)
2022.01[a]	-	-	191	Insoluble, soluble and total dietary fibre in foods (RINTDF)
995.16	32-23.01	II	166	Beta-glucan in oats
997.08	32-31.01	II		Fructans in food products
999.03	32-32.01	III		Fructans in foods
2000.11	32-28.01	II		Polydextrose in foods
2001.02	32-33.01	II		*trans*-Galactooligosaccharides (TGOS) in selected food products
2002.02	32-40.01	II		Resistant starch in starch and plant materials

[a] Not included in the original Codex list of accepted DF methods.

Journal of AOAC INTERNATIONAL, 2023, Vol.106, No.4
OMA : Official methods of analysis

AOAC Method: OMA 991.43 – Total, soluble and insoluble DF in Foods – enzymatic/gravimetric method

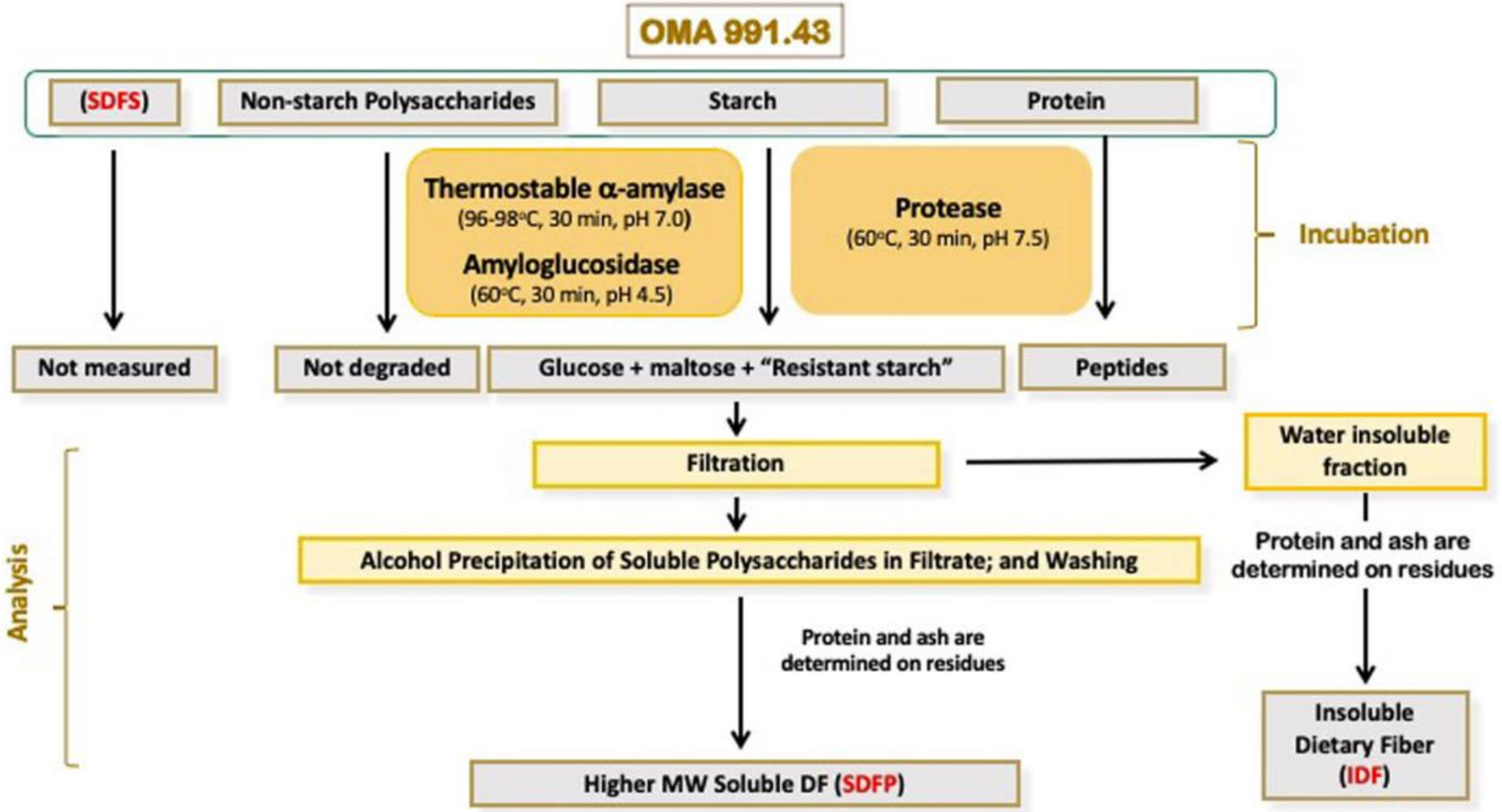


Figure 2. Schematic representation of the steps involved in OMA 991.43.

Journal of AOAC INTERNATIONAL, 2023, Vol.106, No.4
SDFS : **S**oluble **D**ietary **F**iber that remains **S**oluble in 78% EtOH.
SDFP : **S**oluble **D**ietary **F**iber that remains **P**recipitates in 78% EtOH.
IDF : **I**nsoluble **D**ietary **F**iber

Research and Development

AOAC Method: OMA 2001.03 – TDF & High Mw & Low Mw – enzymatic/gravimetric method & liquid chromatographic determination

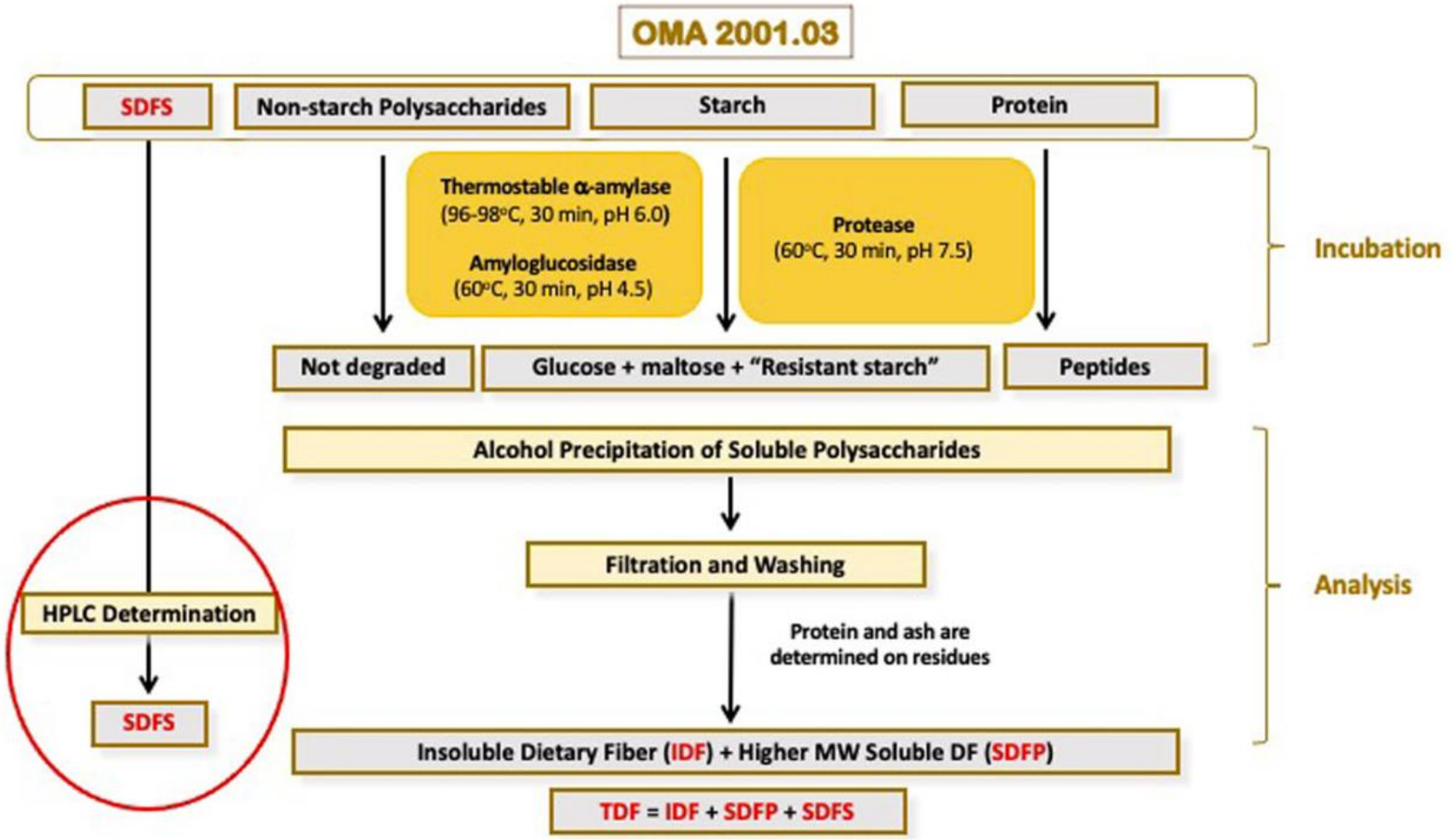


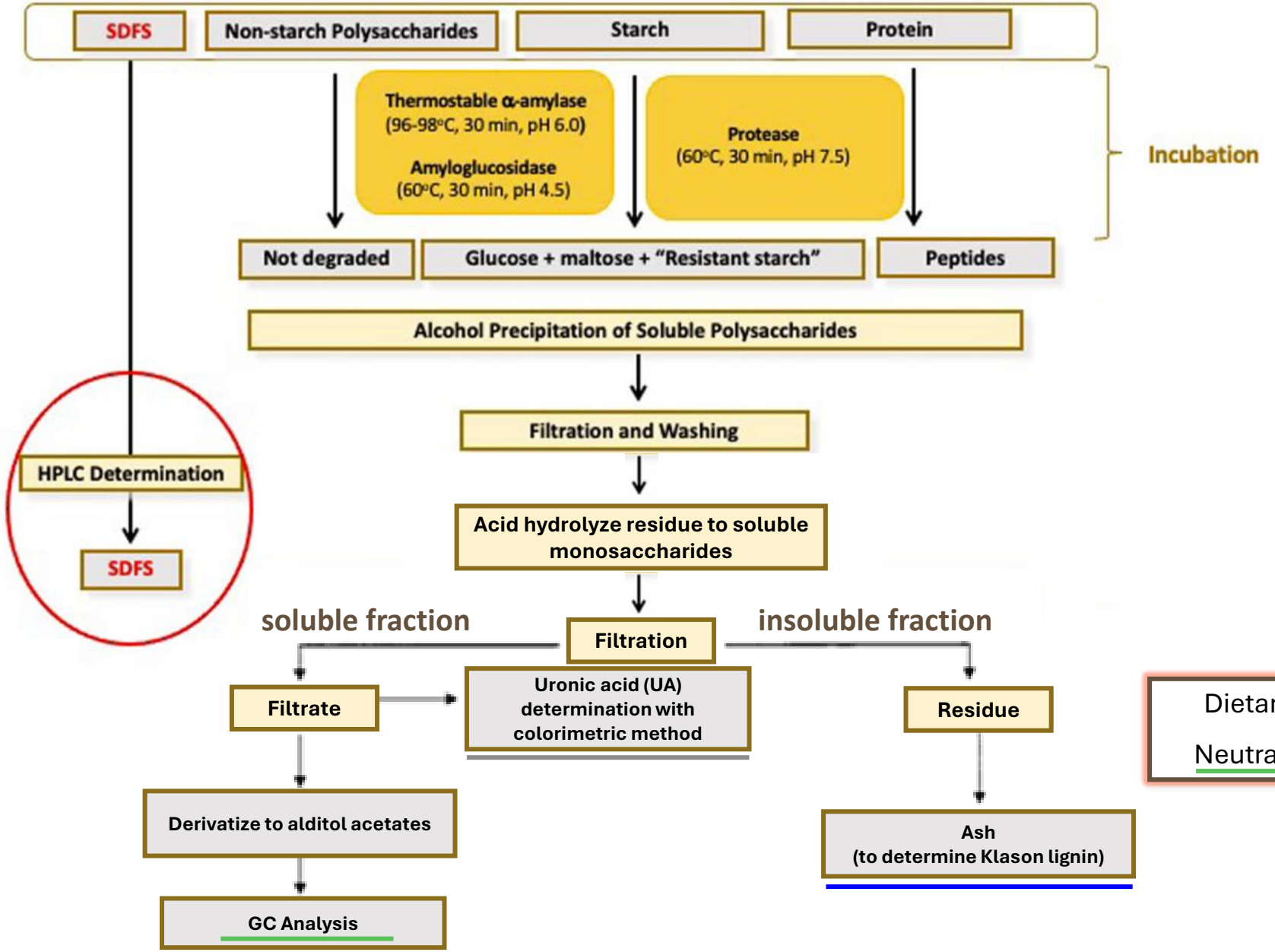
Figure 3. Schematic representation of the steps involved in OMA 2001.03.

Journal of AOAC INTERNATIONAL, 2023, Vol.106, No.4

SDFS : **S**oluble **D**ietary **F**iber that remains **S**oluble in 78% EtOH.
SDFP : **S**oluble **D**ietary **F**iber that remains **P**recipitates in 78% EtOH.
IDF : **I**nsoluble **D**ietary **F**iber
TDF : **T**otal **D**ietary **F**iber
Mw : **M**olecular **w**eight

Research and Development

AOAC Method: OMA 994.13 – TDF: Neutral & Acidic sugars + Klason Lignin



Dietary fiber (IDF+SDFP) =

Neutral Sugars + UA - Lignin

Research and Development

Analytical characterization of carbohydrate/DF features

Extractions...

Purified Carbohydrate
(mono- to poly-saccharide)

Structural Analysis

(mono- to poly-)

(mono-)
Strong acid hydrolysis
or
Derivatization

(mono-)
Methylation with CH_3I
1. Hydrolysis
2. Derivatization

(mono- to poly-)

(non-sugar)
Alkaline hydrolysis

Sample pre-treatment

	HPSEC-RI/MALLS/Visc	HPAEC-PAD	GC(-FID)	GC(-MS)	UPLC(-MS)	NMR	(HS-)GC HPLC NMR
Analytical technique							
Structural feature	**Mw**	**Sugar composition**		**Type of glycosidic linkages**		**Sequence of sugars, position** and **configuration; Non-sugar substituents**	**Non-sugar substituents** (e.g. Methyl-esters and acetyl groups)

Couture et al 2024, *Nature Protocols,* 19, 3321–3359.

Wang et al 2021, *Journal of Food Composition and Analysis,* 96, 103715.

Research and Development

Structural reconstruction of polysaccharides using oligosaccharides

Purified Polysaccharide (PS)

Highly PS specific & pure enzymes

Partial depolymerization

Oligosaccharides

OS retain the PS structural information

Sample pre-treatment

Monitor depolymerization
HPSEC(-RI/MALLS/Visc)

Flourescent labeling

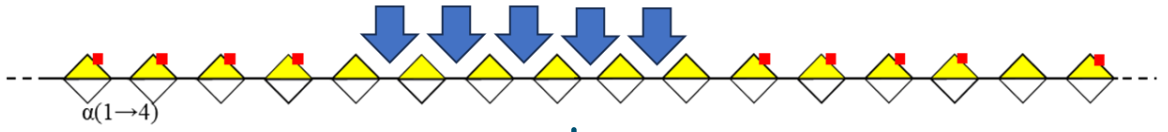
Labeled Oligosaccharide

Sample pre-treatment

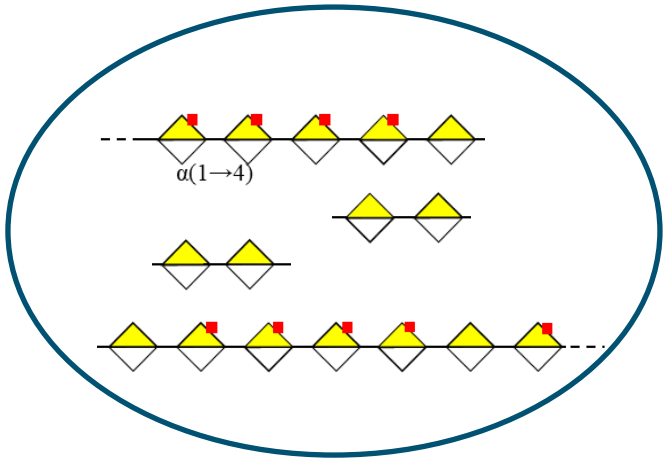
LC(detectors): HPAEC(-PAD), HILIC(-ELSD/UV/MS), PGC(-ELSD/UV/MS) if UV is used
CE-LIF
MS: MALDI-TOF MS and ESI-MS
LC-MS: HILIC- or PGC-ESI-MS
NMR

Analytical technique

Homogalacturonnan



Enzymatic digestion (endo-polygalacturonase; endo-PG)



GalA-oligomers

Puzzling back

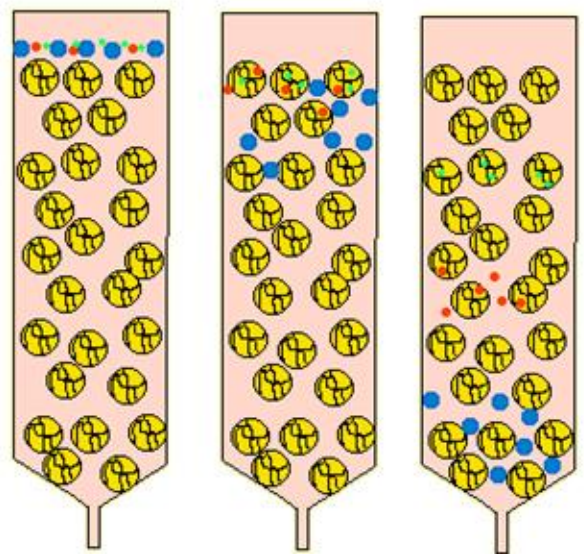
Site of action of endo-PG

GalA ■ *O*-Methyl

Research and Development

Analytics to characterize oligosaccharides

- **Method to <u>monitor polysaccharide depolymerization:</u> HPSEC-RI/MALLS/Visc**



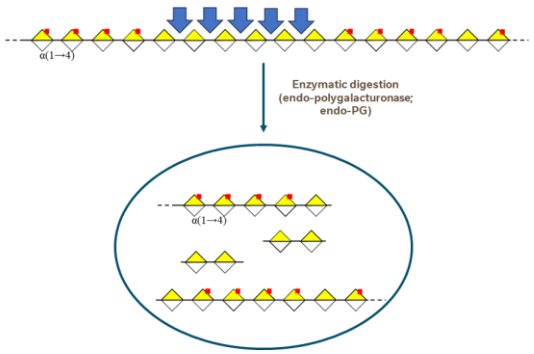
Elution in HPSEC

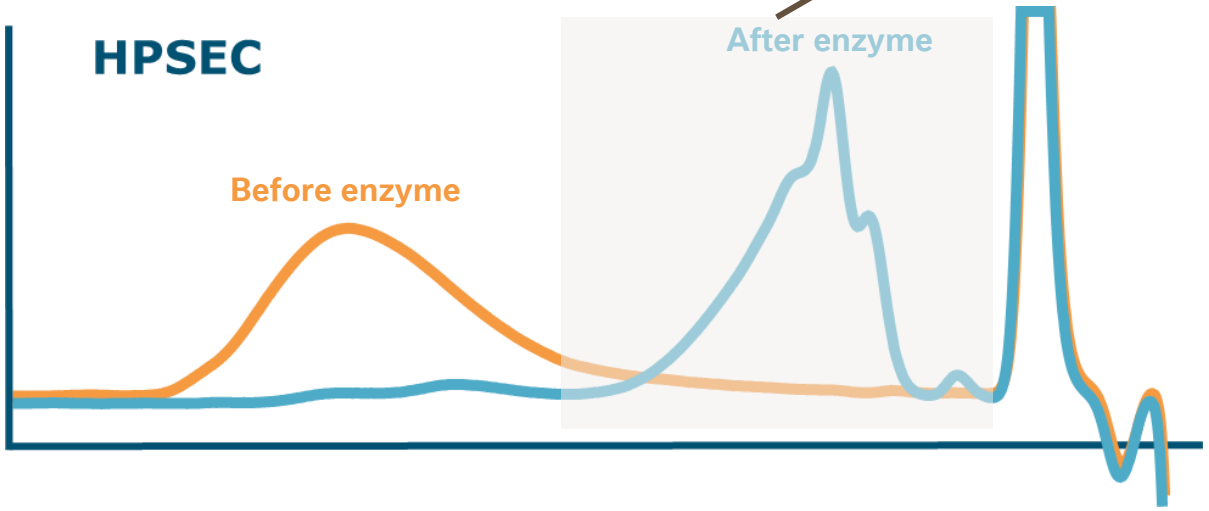
Too large to enter pores:
- ➢ Elutes first

Can enter some pores:
- ➢ Elutes second

Enters many pores:
- ➢ Elutes last

Isocratic elution using a buffer

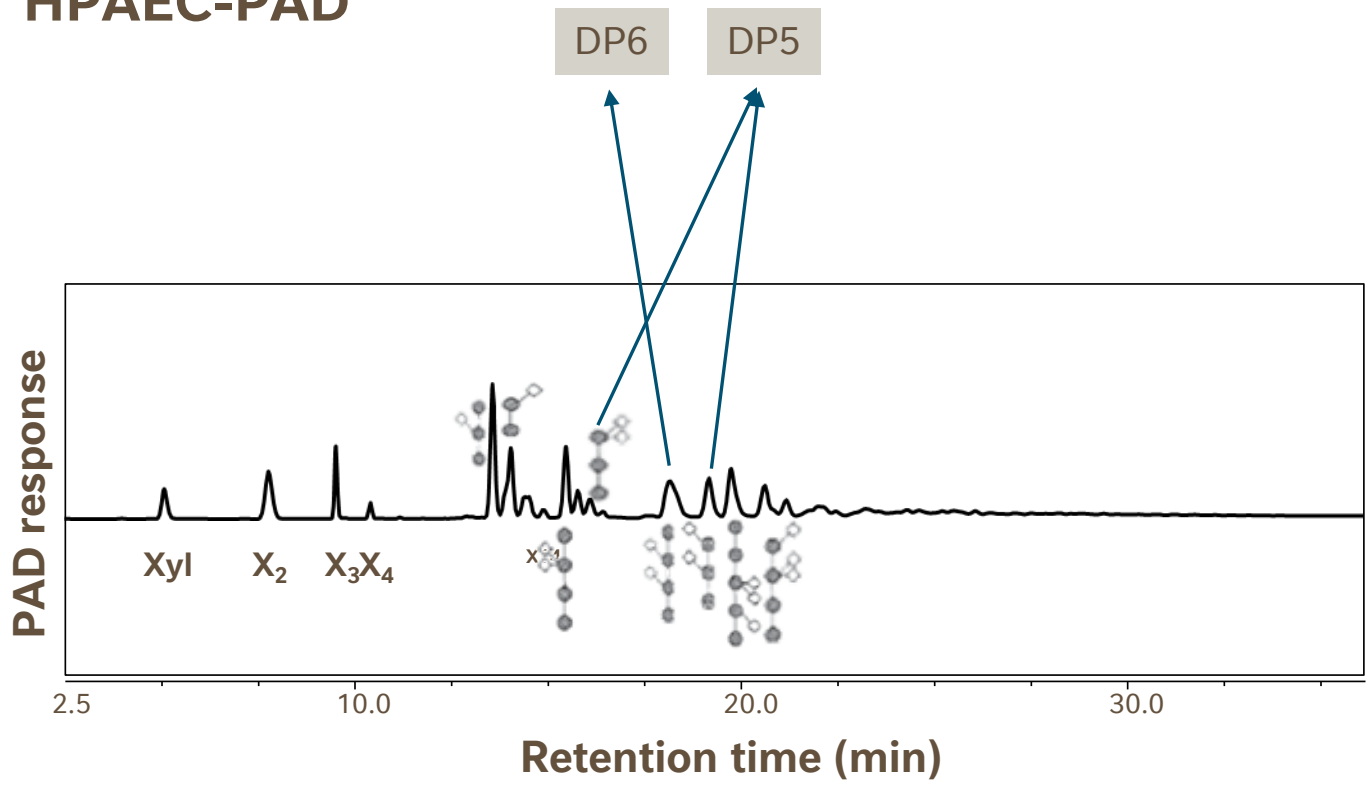




Adapted slide kindly provided by Prof Emeritus Henk A. Schols (FCH-WUR)

Analytics to characterize oligosaccharides

- **Method to identify & quantify oligosaccharides (OS):**
  - **HPAEC-PAD**



Endo-xylanase digestion of wheat **arabinoxylan**

Arabino-xylo-oligosaccharides (A)XOS

HPAEC-PAD profile

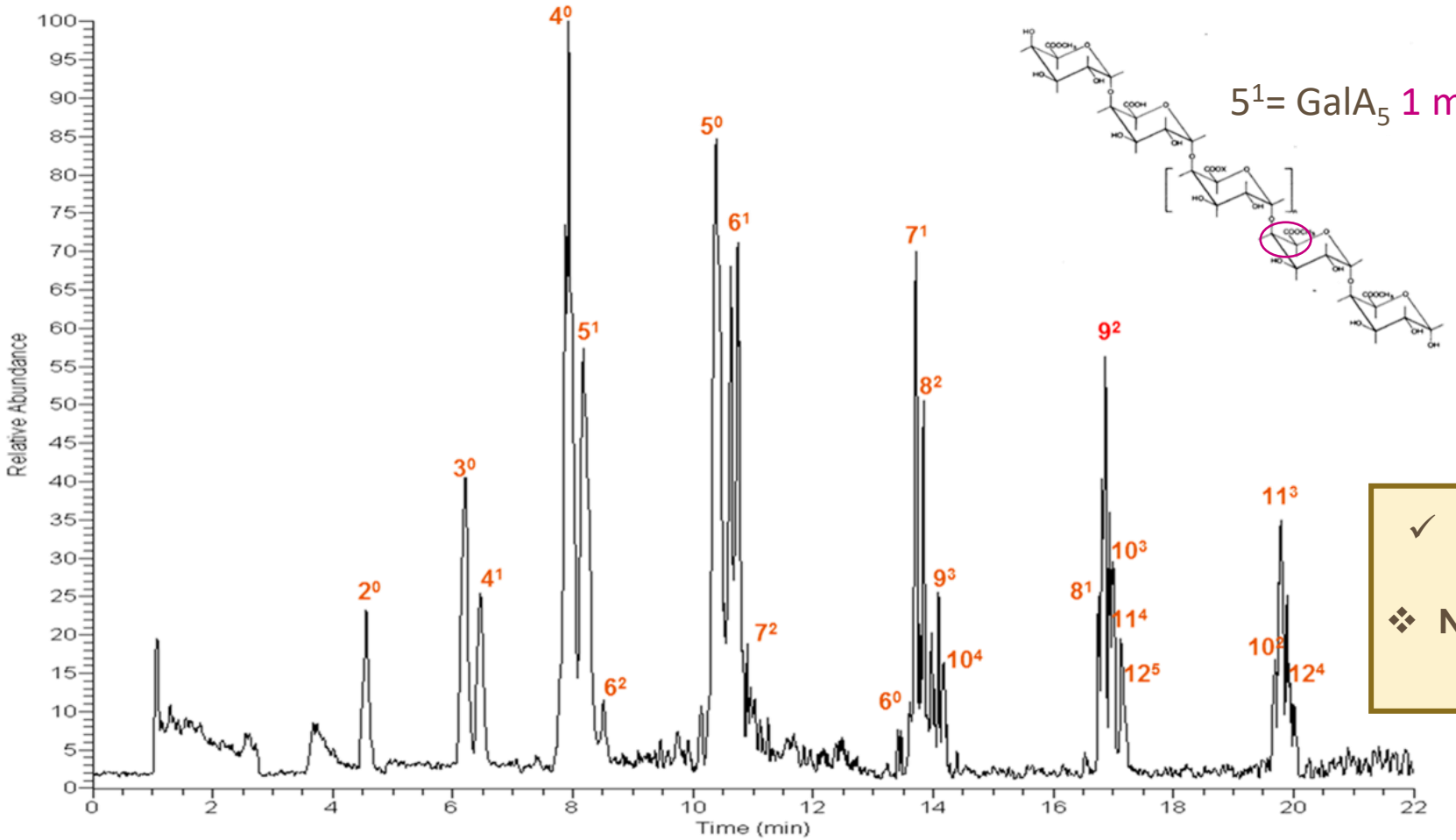
❖ **Identification of OS requires the use of look-alike oligosaccharide standards, i.e. (A)XOS.**
✓ **Separation of isomers is possible.**

Unpublished data within the scope of the work published by Pandeirada et al., 2022, *Carbohydrate Polymers,* 276, 118795.
HPAEC-PAD : **H**igh **P**erformance **A**nion-**E**xchange **C**hromatography coupled to **P**ulse **A**mperometric **D**etection.
DP : **D**egree of **P**olymerization

Research and Development

Analytics to characterize oligosaccharides

- **Method to <u>identify oligosaccharides:</u>**
  - **HILIC-LC-ESI-Iontrap-MS of methyl-esterified pectin oligomers**



5^1= $GalA_5$ 1 methyl

✓ **Isomers could have been separated and distinguished in LC-ESI-MS.**

❖ **No quantification can be attained with MS.**

Jermendi et a., 2022, *Carbohydrate Polymers,* 277, 118813.

HILIC : **H**ydrophilic **L**iquid **I**nteraction **C**hromatography

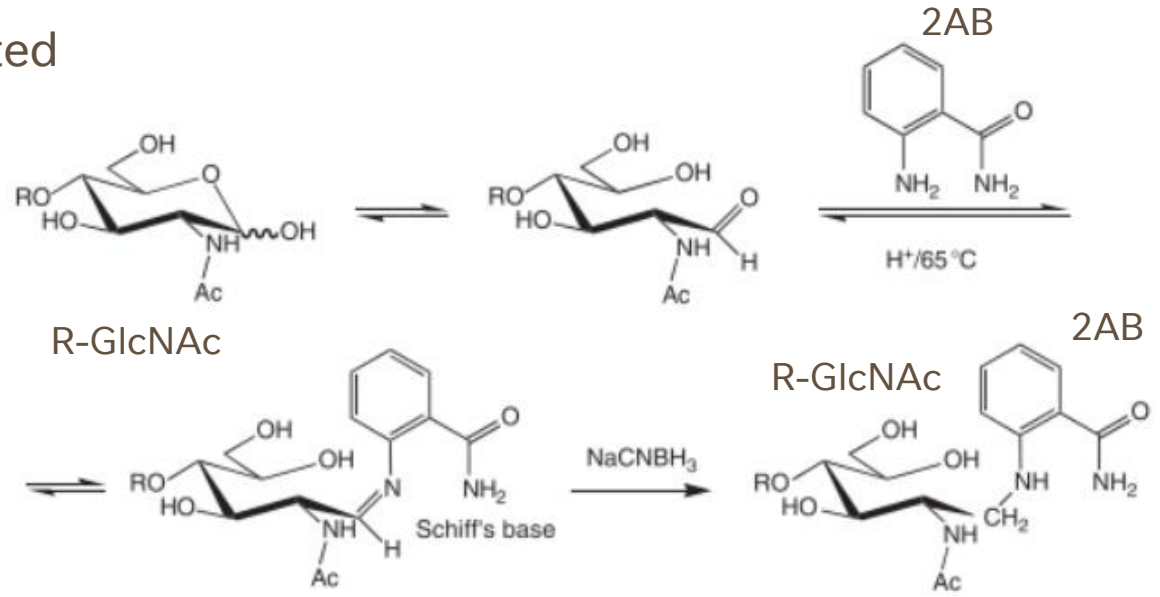
GalA : **G**alacturonic **A**cid

Research and Development

Analytics to characterize oligosaccharides

- **Method to <u>identify & quantify oligosaccharides:</u>**
  - **HILIC-LC-ESI-Iontrap-MS/UV**

- Carbohydrates lack the presence of a conjugated system to absorb light.
- Reductive amination reaction with 2AB:



- ➢ Presence of a reducing end sugar;
- ➢ Yield of derivatization;
- ✓ Fluorescent label allows quantification (mol/mol basis);

Excitation 330 (373)nm
Emission 420 (448)nm

Determination of β-Galactooligosaccharides (GOS) in Infant Formula and Adult Nutritionals: Single Laboratory Validation, First Action 2021.01
D. Cuany, F. Andestion, X. Fontannaz, T. Benet, V. Spichtig and S. Austin
J. AOAC Int. 2022 Vol. 105 Issue 1 Pages 142-158

Quantitative determination of human milk oligosaccharides in faecal matter
T. Benet, A. Dardinier, H. L. P. Tytgat and S. Austin
Anal Biochem 2025 Pages 115845

Determination of Seven Human Milk Oligosaccharides (HMO) in Infant Formula and Adult Nutritionals: First Action 2022.07
T. Bénet, N. Frei, V. Spichtig, D. Cuany and S. Austin
Journal of AOAC INTERNATIONAL 2024 Vol. 107 Issue 2 Pages 286-302

Research and Development

Overview of methods

Analytical technique	Mono-saccharides	Oligo-saccharides	Poly-saccharides	α/ß configuration	Mw/DP	Detection	Derivatization/ Labelling	Quantification
HPSEC	√	√	√	X	√/x	RI/MALLS/Visc	X	+/-
GC	√	X	X	X	X	FID MS	√	+ (internal standard)
HPAEC	√	√	X	X	x/√	PAD	X	+ (standards)
NMR	√	√	√	√	X	-	X	+
HILIC	√	√	X	+/-	x/√	ELSD UV MS	x √ x	- + (label) -
PGC	√	√	X	+/-	x/√			
MALDI-TOF	X	√	X	X	x/√	MS	X	-
ESI	+/-	√	X	X	x/√	MS	X	-

Nestlé Good food, Good life

Acknowledgements

- Prof Emeritus Henk A. Schols (Wageningen University & Research (WUR), The Netherlands)
- Dr E. Jermendi (WUR)
- ETH Zürich – Food Biochemistry
- Nestlé Institute of Food Safety & Analytical Sciences
- Dr Sean Austin (Nestlé R&D)
- Proteins & Carbohydrates Analytics Department (Nestlé R&D)

Nestlé Good food, Good life

ENFiber
European Network for Valorising Food Processing Waste into Sustainable Fibers

Analytics for characterization of food carbohydrates & dietary fibers

Dr. Carolina O. Pandeirada (COP)

(carolina.oliveirapandeirada@rd.nestle.com)

Specialist in Carbohydrates

Department of Protein & Carbohydrate Analytics

Nestlé Research (Switzerland)

Tartu (Estonia), 05.05.2026

Thank you for your attention!!!

Nestlé Research in Lausanne, Switzerland. Pic: Nestlé