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at the Technical University of Munich

Tracking the fate of gluten peptides during food processing

Dr. Katharina Scherf

IV SIAN – Symposium Food & Nutrition
Rio de Janeiro, Brazil, June 17, 2019

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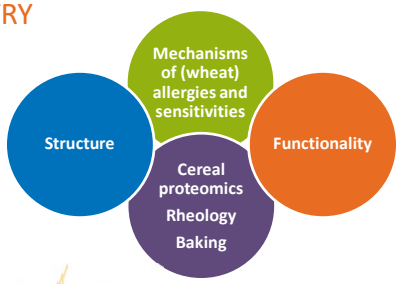
FUNCTIONAL BIOPOLYMER CHEMISTRY

Key research questions:

- How do different factors influence **structure**-**functionality**-**bioactivity** relationships between and within biopolymers as well as between **biopolymers** and the **human gastrointestinal** and **immune systems**?
- Why does the **prevalence** of celiac disease, non-celiac gluten sensitivity (NCGS) and allergies **increase** within the population?



CD-damaged small intestinal mucosa | healthy

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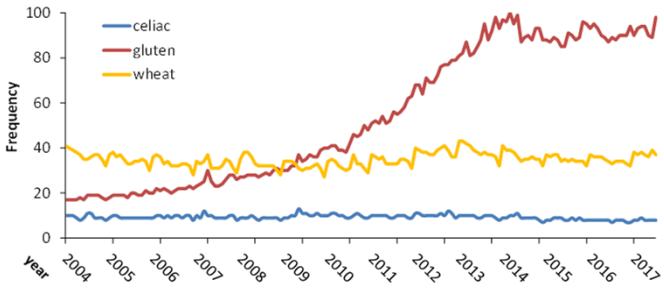
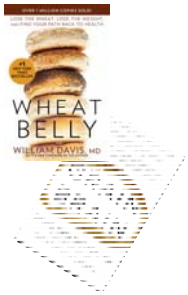
DOES WHEAT MAKE US… SICK, FAT, STUPID and ADDICTED?





- Wheat consumption is associated with a variety of health risks
- Consumers are unsure what to believe

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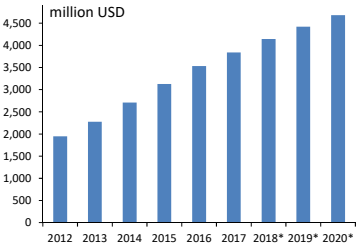
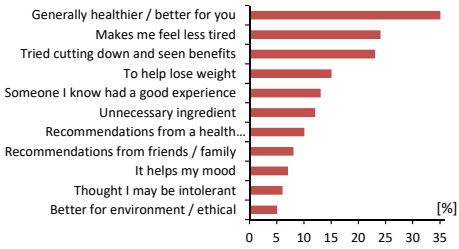
GLUTEN-FREE MARKET

Gluten-free product retail sales worldwide



Reasons why consumers reduce gluten intake (UK, 2015)



- 11% of consumers look to reducing gluten intake (UK, 2015)
- Among consumers reducing gluten intake, 66% have no sensitivity to gluten whereas 30% (think they) are (UK, 2015)

Statista.com, Jan. 22, 2019

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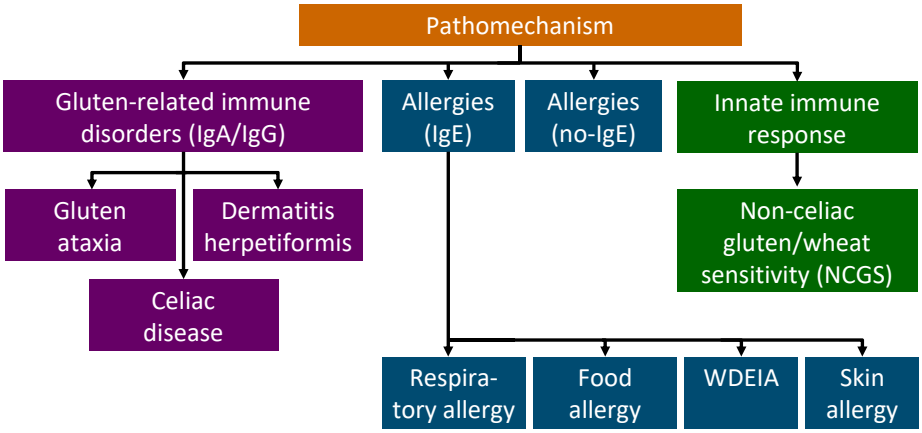
WHEAT/GLUTEN SENSITIVITIES



Modified from Sapone et al., 2012; EG: eosinophilic gastroenteritis, EoE: eosinophilic esophagitis, WDEIA: Wheat-dependent exercise-induced anaphylaxis

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CELIAC DISEASE

Chronic inflammation of the small intestine caused by lifelong intolerance to dietary gluten from wheat, rye and barley

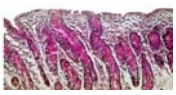
Prevalence: ≈ 1 % of the population

Causes:

- Genetics: HLA-DQ2 or HLA-DQ8 positive
- **Gluten** from wheat, rye and barley, maybe oats
- Further factors:
  - Infections (rotavirus, adenovirus 12)
  - Changes of gut microbiota
  - Hygiene hypothesis

Therapy: lifelong, strict gluten-free diet

healthy



CD-damaged small intestinal mucosa

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CAUSES: GLUTEN

- Storage proteins of wheat, rye and barley
- Content: 5 - 10 g/100 g flour
- Wheat gluten: responsible for good baking quality
- Two components:

  Prolamins (gliadins): monomeric proteins

  Glutelins (glutenins): polymeric proteins
- **Cause for CD**
- Rich in proline and glutamine
  → resistant to human gastrointestinal enzymes
- Long peptides reach the small intestinal mucosa
  → activation of the human immune system

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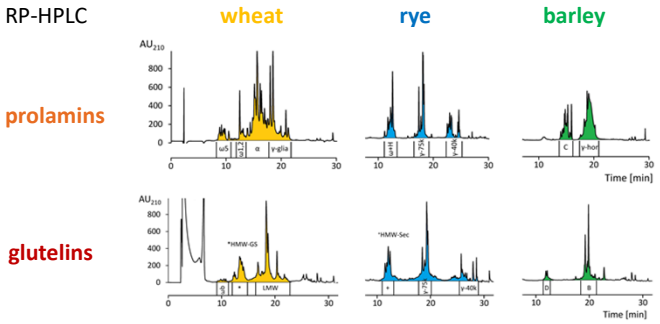
CAUSES: GLUTEN

Albumins	Globulins	Prolamins	Glutelins
water-soluble	salt-soluble	alcohol-soluble	alcohol-insoluble
metabolic proteins		storage proteins (gluten)	

RP-HPLC



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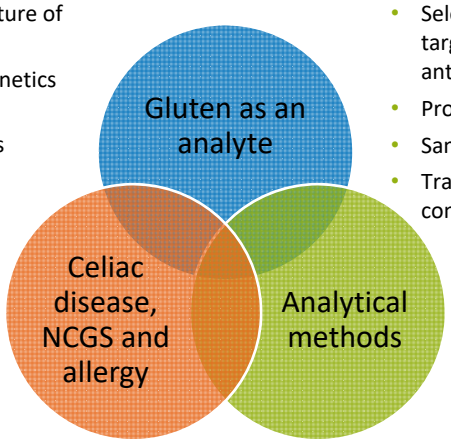
CHALLENGES OF GLUTEN ANALYSIS

- Gluten: complex mixture of 100+ proteins
- Variability (due to genetics and environment)
- Protein modifications during processing



- Selection of relevant target epitopes and antibodies
- Protein polymorphism
- Sample preparation
- Trace analysis in complex food matrices

Patients react individually

- to different proteins and epitopes
- with variable sensitivities

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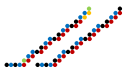
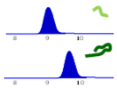
FUNCTIONAL BIOPOLYMER CHEMISTRY

Recent research

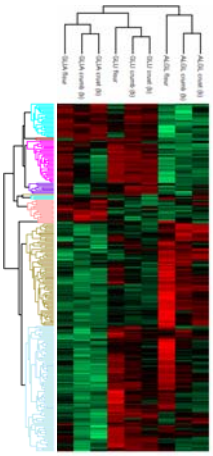
Targeted LC-MS/MS methods to quantitate



- the 33-mer peptide



- gluten using isolated gluten protein types as reference materials



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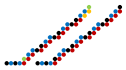
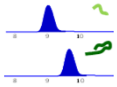
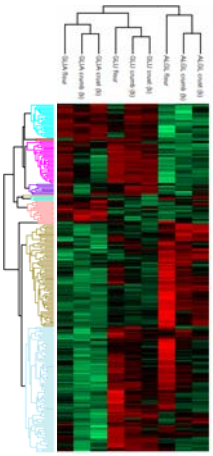




Targeted LC-MS/MS methods to quantitate

- the 33-mer peptide
- gluten using isolated gluten protein types as reference materials



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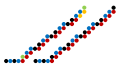
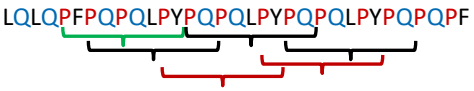


TARGETED LC-MS/MS METHOD TO QUANTITATE THE 33-MER



T-cell epitopes:
DQ2.5-glia-α1a
DQ2.5-glia-α1b
DQ2.5-glia-α2 [1,2]

- 3 T-cell epitopes, 6 overlapping copies in total
- Initiation of a strong immune response ("immunodominant" CD-active peptide)[3]
- 768 results for a literature search (Science Direct, "33 mer", June 05, 2019)
  - 26 results for the year 2019 so far

→ Frequently used in clinical and analytical model studies

[1]Arentz-Hansen, Körner et al., 2000 [2]Sollid et al., 2012 [3]Shan et al., 2002 [4]Morón et al., 2008

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THE 33-MER AS A MODEL SYSTEM

A	Pepsin [1,2]	L↓QLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	✗
B	Trypsin [1]	LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	✗
C	Chymotrypsin (h) [1]	LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	✗
D	Chymotrypsin (l) [1]	L↓QL↓QPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	✗
E	Barley malt extract [3]	LQ↓LQP↓FPQPQLP↓YPQPQLP↓YPQPQLP↓YPQPQP↓F	✓
F	EP-B2 [4]	LQ↓LQPFPQPQ↓LPYPQPQ↓LPYPQPQ↓LPYPQPQPF	✓
G	Triticain-α [5]	LQL↓Q↓PFPQPQ↓LP↓YPQPQ↓LP↓YPQPQ↓LP↓YPQPQ↓PF	✓
H	Dipeptidyl peptidase IV [6]	LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	✗
I	Aspergillopepsin [6]ª	LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	✗
J	AN-PEP [7]	LQLQP↓FP↓QPQLP↓YP↓QPQLP↓YP↓QPQLP↓YP↓QPQP↓F	✓
K	SC-PEP [8]	LQLQPFPQPQLP↓YP↓QPQLP↓YP↓QPQLP↓YP↓QPQPF	✓
L	MX-PEP [8]	LQLQP↓FPQP↓QLP↓YPQPQLP↓YPQPQLP↓YPQPQPF	✓
M	FM-PEP [7,8]	LQLQP↓FPQPQLP↓YP↓QPQLP↓YP↓QPQLP↓YP↓QPQP↓F	✓
N	*Rothia mucilaginosa* [9]	LQLQPF↓P↓QPQ↓LPY↓PQPQ↓LPY↓PQPQ↓LPY↓PQ↓PQ↓PF	✓
O	Pseudolysin [10]	LQ↓LQPFPQPQ↓LPYPQPQ↓LPYPQPQ↓LPYPQPQPF	✓
P	LAB PepN + PepX + PepO [11]	LQ↓L↓QP↓FP↓QPQ↓LP↓YP↓QPQ↓LP↓YP↓QPQ↓LP↓YP↓QP↓QPF	✓
Q	*Rhizopertha dominica* [12]	LQLQPFP↓QPQLP↓YP↓QPQLP↓YP↓QPQLP↓YPQP↓QPF	✓
R	Kuma030 [13]	LQLQPFPQPQ↓LPYPQPQ↓LPYPQPQ↓LPYPQPQPF	✓

Scherf et al., 2018

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SEQUENCE ALIGNMENT OF α-GLIADINS

```
          1         11        21        31        41        51
α1   Q9M4M3 VRVPVPQLQP QNPSQQQPQE QVPLMQQQQQ FPGQQEQFPP QQPYPHQQPF PSQQPYPQPQ
α2   Q9M4L6 VRVPVPQLQP QNPSQQQPQE QVPLVQQ-QQ FPGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α3   Q9M4M0 VRVPVPQLQP QNPSQQQPQE QVPLVQQ-QQ FLGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α4   Q9M4M2 VRVPVPQLQP QNPSQQQPQE QVPLVQQ-QQ FLGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α5   Q9M4M1 VRVPVPQLQP QNPSQQQPQE QVPLVQQ-QQ FLGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α6   Q9M4M6 VRVPVPQLQP QNPSQQQPQE QVPLMQQQQQ FPGQQERFPP QQPYPHQQPF PSQQPYPQPQ
α7   Q9M4M4 VRVPVPQLQL QNPSQQQPQE QVPLVQE-QQ FPGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α8   Q9M4L9 VRVPMPQLQP QDPSQQQPQE QVPLVQQ-QQ FLGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α9   Q9M4M5 VRVTVPQLQP QNPSQQQPQE QVPLVQQ-QQ FLGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
α10  Q9M4L8 VRVPVPQLQP QNPSQQHPQE QVPLVQQ-QQ FLGQQQSFPP QQPYPQPQPF PSQQPYLQLQ
α11  Q9M4L7 VRVPVPQLQP QNPSQQQPQE QVPLVQQ-QQ FLGQQQPFPP QQPYPQPQPF PSQLPYLQLQ

          61        71        81        91        101       111         ...265-290
          PFP-PQ---- ---------- LPYPQTQPFP PQQPYPQPQP QYPQPQQPIS QQQAQQ----
          PFPQPQLPYP QPQLPYPQPQ LPYPQPQPFR PQQPYPQSQP QYSQPQQPIS QQQQQQQQQQ
          PFPQPQ---- ---------- LSYSQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQQQ
          PFPQPQ---- ---------- LSYSQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQQQ
          PFPQPQ---- ---------- LSYSQPQPFR PQQLYPQPQP QYSQPQQPIS QQQQQQQQQQ
          PFP-PQ---- ---------- LPYPQTQPFP PQQPYPQPQP QYPQPQQPIS QQQAQQ----
          PFPRPQ---- ---------- LPYPQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQ--
          PFPQPQ---- ---------- LPYSQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQQQ
          PFPQPQ---- ---------- LPYSQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQQQ
          PFPQPQ---- ---------- LPYLQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQQQ
          PFPQPQ---- ---------- LPYSQPQPFR PQQPYPQPQP QYSQPQQPIS QQQQQQQQQ-
```

Scherf et al., 2018

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RATIONALE & AIMS

BLAST search in UniProtKB database

- 17 protein sequences from *Triticum aestivum* and 3 from *T. spelta* contain the 33-mer peptide (out of 587 α-gliadin entries for *Triticum* sp.)
- Only 1 protein sequence with evidence at protein level (P18573) from the Norwegian wheat cultivar Mjølner

Quantitative data on contents of 33-mer peptide in different wheat cultivars is unavailable

→ Development of a stable isotope dilution assay (SIDA) combined with LC-MS/MS to quantitate the 33-mer peptide in wheat flours

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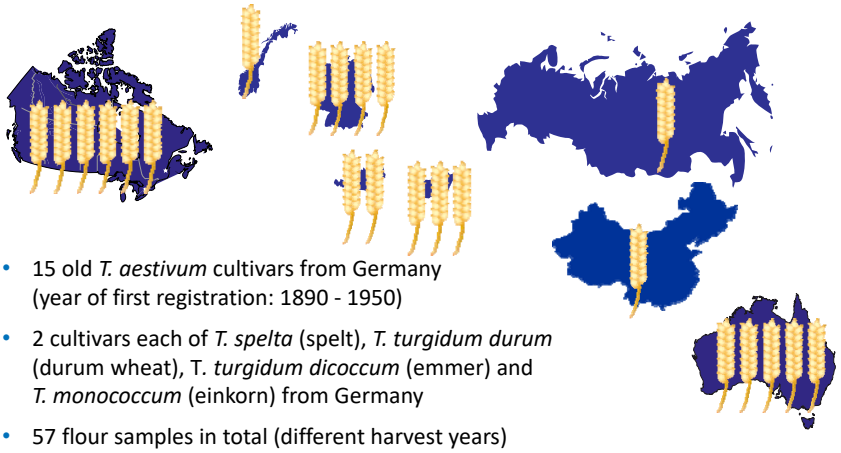
COLLECTION OF WHEAT FLOURS

- 23 modern *Triticum aestivum* (common wheat) cultivars from around the world



- 15 old *T. aestivum* cultivars from Germany (year of first registration: 1890 - 1950)
- 2 cultivars each of *T. spelta* (spelt), *T. turgidum durum* (durum wheat), T. *turgidum dicoccum* (emmer) and *T. monococcum* (einkorn) from Germany
- 57 flour samples in total (different harvest years)

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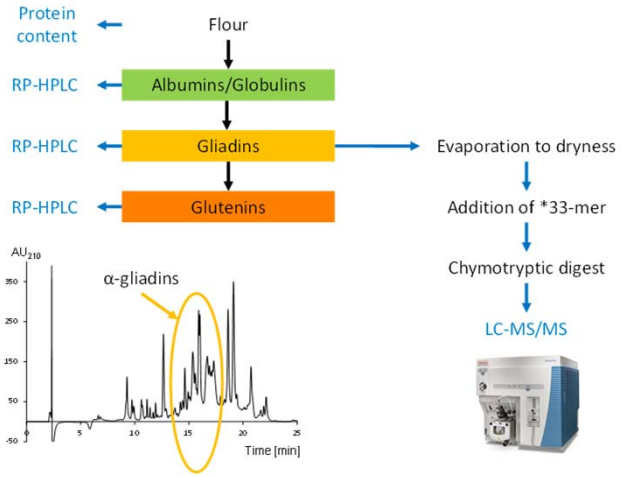
FLOUR CHARACTERIZATION & SAMPLE PREPARATION



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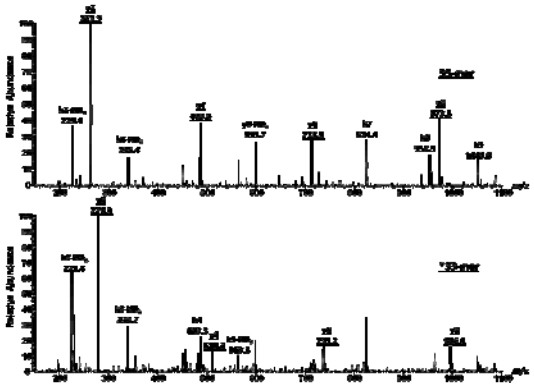
LC-MS/MS METHOD

LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF (33-mer, Analyt)

LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF (*33-mer, Standard)

F: $^{13}C_9{}^{15}N$-phenylalanine,
P: $^{13}C_5{}^{15}N$-proline



Peptide	Precursor ion *m/z* (charge)	Product ion *m/z* (1+)
33-mer	**979.0 (4+)**	**263.3 (y2)**
	1305.2 (3+)	488.9 (y4)
		713.5 (y6)
		973.5 (y8)
*33-mer	**987.0 (4+)**	**279.0 (y2)**
	1316.0 (3+)	510.3 (y4)
		735.2 (y6)
		996.0 (y8)

Response factor: 0.999

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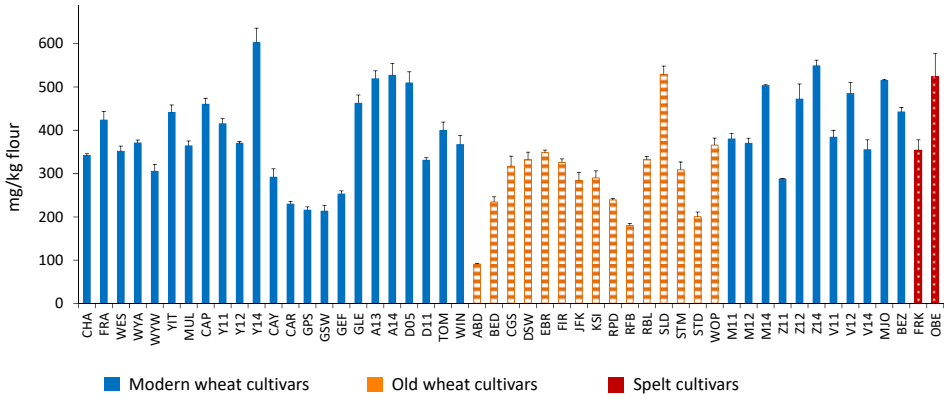
33-MER CONTENTS IN FLOURS



Schalk et al., 2017b

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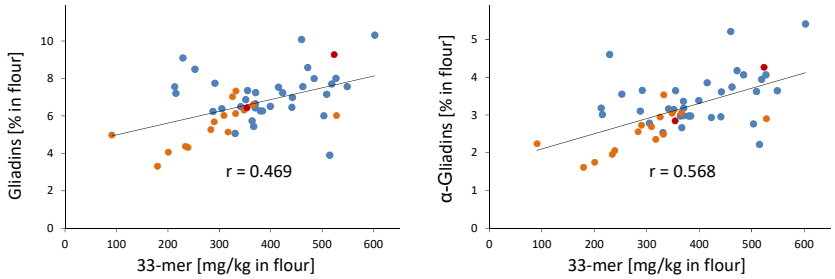
33-MER CONTENTS IN FLOURS

Average content of 33-mer (n = 51):

- 368 ± 109 mg/kg flour range: 91 - 603 mg/kg flour
- 11.7 ± 3.1 mg/g α-Gliadin range: 4.1 – 23.2 mg/g α-gliadin

Harvest year has a greater influence on 33-mer contents than cultivar

Correlations:



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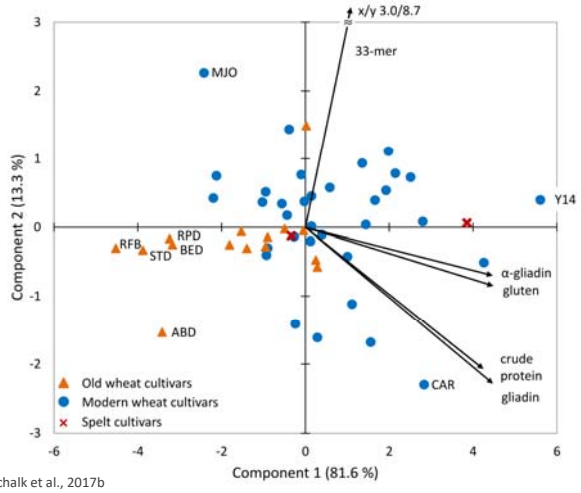
PRINCIPAL COMPONENT ANALYSIS



Schalk et al., 2017b

→ **No obvious cluster formation** between modern and old wheat cultivars

→ **No correlations** between contents of 33-mer and those of α-gliadins, gliadins, gluten or crude protein

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CONCLUSIONS – 33-MER PEPTIDE

- All 23 modern and 15 old wheat and 2 spelt cultivars contained the 33-mer
- Contents of 33-mer (n = 51) ranged from
  - 91 - 603 µg/g flour and
  - 4.1 - 23.2 mg/g α-gliadin
- No or weak correlation between contents of 33-mer and gliadins or α-gliadins
- Harvest year had a greater influence on 33-mer contents than cultivar
- Contents of 33-mer were not suitable to differentiate old from modern wheat cultivars
- Durum wheat, emmer and einkorn did not contain the 33-mer (< LOD)

→ **First study to report accurate quantitative values for 33-mer contents in a set of 57 wheat flours**

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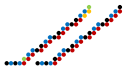
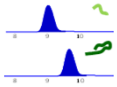


- the 33-mer peptide



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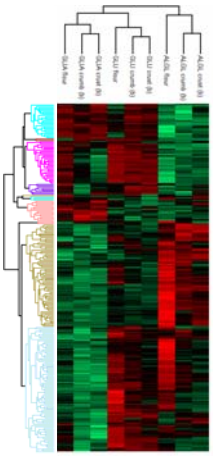

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GLUTENFREE PRODUCTS: LEGISLATION

International: Codex Alimentarius

Codex Standard 118-1979 (2015)

Threshold for gluten in gluten-free products: **20 mg/kg** of the product

- Gluten analysis: immunologic or other method
- Antibody should react with protein fractions that are toxic to persons intolerant to gluten
- Validated methods calibrated against a **certified reference material**
- Limit of detection: ≤ 10 mg gluten/kg
- Method for gluten determination: ELISA R5 Mendez Method

Codex Standard 1-1985 (2010)

Mandatory declaration of gluten-containing cereals on product labels (allergen labeling)

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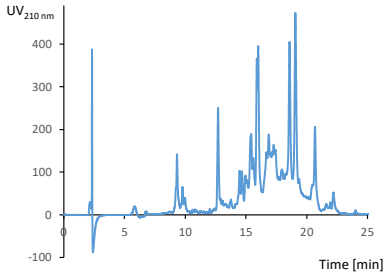
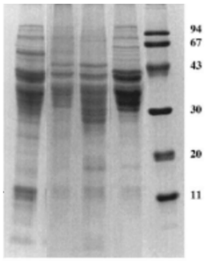
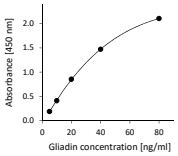
REFERENCE MATERIAL FOR GLUTEN

- No certified reference material available
- No reference material for glutelins, gluten or flour
- **PWG-gliadin** (isolated from a mixture of 28 European wheat cultivars)







Van Eckert et al., 2006; Scherf & Poms, 2016

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PWG-GLIADIN

- ☺ Purified, homogeneous and completely soluble (60% ethanol)
- ☺ Extensively characterized
- ☺ Stability is monitored regularly
- ☺ Representative for European wheat cultivars
- ☺ Used to calibrate ELISA test kits and other analytical methods

- ☹ Contains only gliadins
- ☹ Gluten content is calculated from the gliadin content
- ☹ Limited supply
- ☹ Reproducible production of a new batch is problematic

→ Initiative to prepare new reference materials for gluten

Van Eckert et al., 2006; Scherf & Poms, 2016

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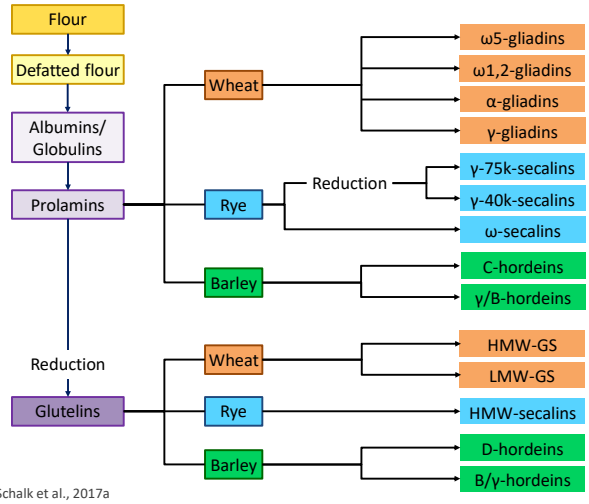
REFERENCE MATERIALS FOR GLUTEN ANALYSIS



Schalk et al., 2017a

Uses:

- Calibration of gluten analytical methods, e.g., LC-MS/MS
- Identification of novel target sequences for gluten detection by ELISA
- Characterization of ELISA antibody reactivities
- Studies on pathogenic mechanisms in CD, NCGS and WDEIA

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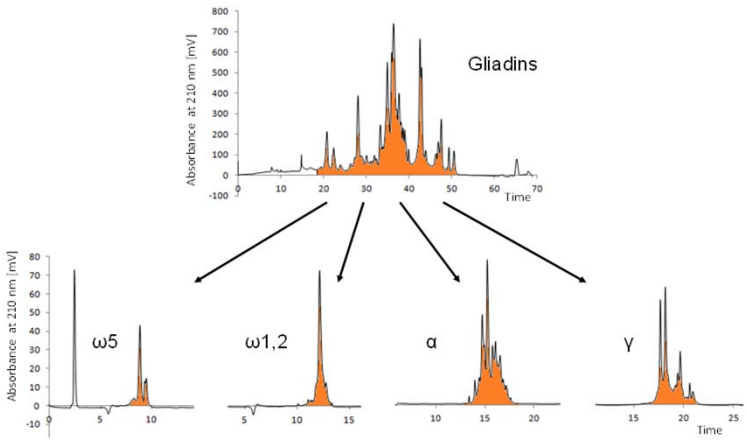
REFERENCE MATERIALS FOR GLUTEN ANALYSIS



Schalk et al., 2017a

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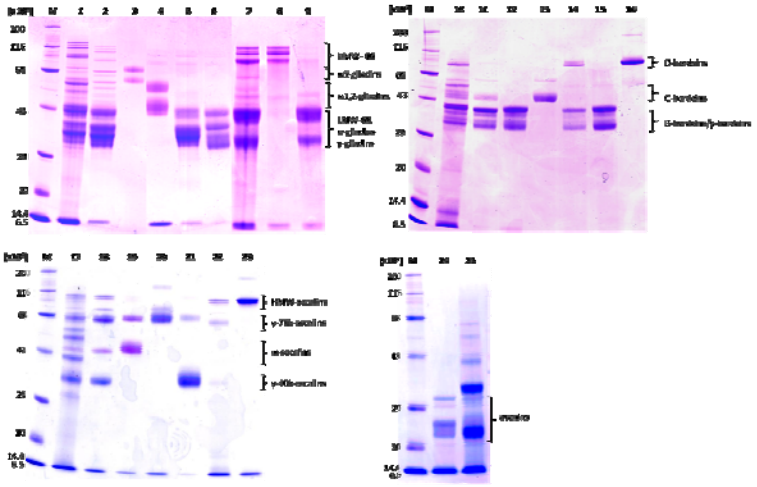
REFERENCE MATERIALS FOR GLUTEN ANALYSIS



Schalk et al., 2017a

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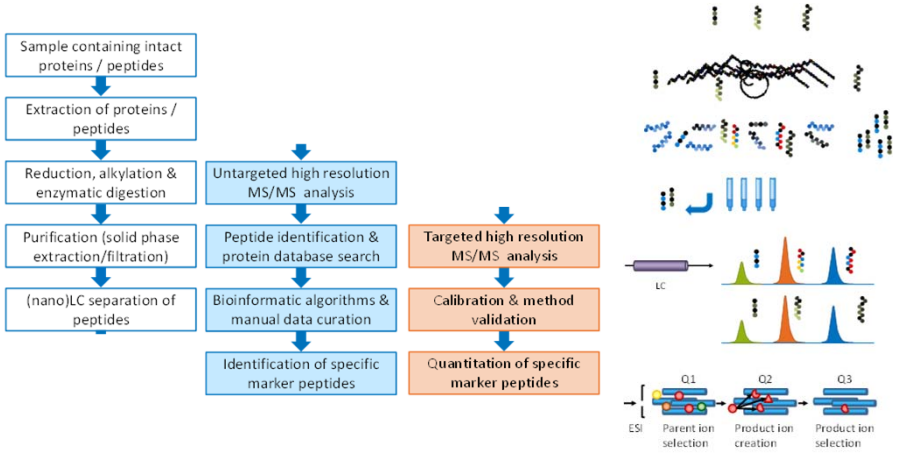
PROTEOMICS WORKFLOW



Schalk et al., 2017a

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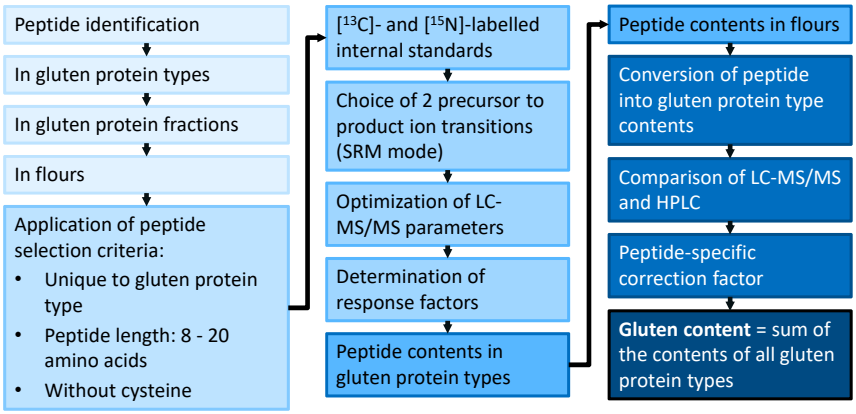
STRATEGY FOR TARGETED LC-MS/MS



Schalk et al., 2018a,b

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SELECTED GLUTEN MARKER PEPTIDES

	Amino acid sequence	Protein type	Peptide concentration [µg/mg]*
P1	QQQPLPPQQTFPQQPL	LMW-GS	10.8 ± 0.2
P2	GQQPQQQQL	LMW-GS	11.9 ± 0.3
P3	VQQQIPVVQPSIL	LMW-GS	4.9 ± 0.0
P4	SIILQEQQQGF	LMW-GS	8.9 ± 0.4
P5	LQPGQGQQGY	HMW-GS	5.3 ± 0.4
P6	TASLQQPGQGQQGHYPASL	HMW-GS	3.3 ± 0.1
P7	HVSVEHQAASL	HMW-GS	7.5 ± 0.3
P8	ASIVAGIGGQ	γ-Gliadins	18.7 ± 2.0
P9	NIQVDPSGQVQW	γ-Gliadins	16.8 ± 2.2
P10	LQPQQPQQSFPQQQQPL	γ-Gliadins	2.0 ± 0.2
P11	**LQLQPFPQPQLPYPQPQPF**	α-Gliadins	5.9 ± 0.0
P12	FQPSQQNPQAQGF	α-Gliadins	3.9 ± 0.1
P13	RPQQPYPQPQPQY	α-Gliadins	9.5 ± 0.2
P14	QQYPQQQPSGSDVISISGL	ω5-Gliadins	11.3 ± 0.1
P15	GSSLTSIGGQ	ω1,2-Gliadins	5.4 ± 0.5
P16	FPHQSQQPF	ω1,2-Gliadins	0.8 ± 0.0

	Amino acid sequence	Protein type	Peptide concentration [µg/mg]*
P17	AIDTRVGV	γ/B-Hordeins	39.0 ± 2.5
P18	QQPQPQQGQQQQVPQSVF	γ/B-Hordeins	35.2 ± 0.3
P19	**AQQQPSIEEQHQL**	γ/B-Hordeins	3.2 ± 0.1
P20	GGGLTTEQPQGGKQPF	D-Hordeins	5.9 ± 0.5
P21	TQQKPGQGYNPGGTSPL	D-Hordeins	48.8 ± 1.7
P22	IIPQQPQQPFPLQPHQPY	C-Hordeins	32.8 ± 0.0
P23	RQLNPSSQEL	C-Hordeins	26.3 ± 0.3
P24	**VQQQPPFVQQEQPF**	Avenins	13.2 ± 0.8
P25	DPSEQYQPYPEQQEPF	Avenins	6.4 ± 0.4
P26	LQPQLQQQL	Avenins	13.4 ± 1.4
P27	**ASIETGIVGH**	γ-75k-Secalins	3.4 ± 0.1
P28	SQLEVVRSL	γ-75k-Secalins	1.0 ± 0.0
P29	QQFPQQPQQPFPQQPL	γ-75k-Secalins	0.9 ± 0.0
P30	RQLNPSEQEL	ω-Secalins	0.5 ± 0.0
P31	AQQPEQLISQQPFPL	ω-Secalins	2.1 ± 0.0
P32	LTSPQQPGQGQQGY	HMW-Secalins	0.6 ± 0.1
P33	STSPRQPGQGQQEY	HMW-Secalins	9.3 ± 2.0

Schalk et al., 2018a,b

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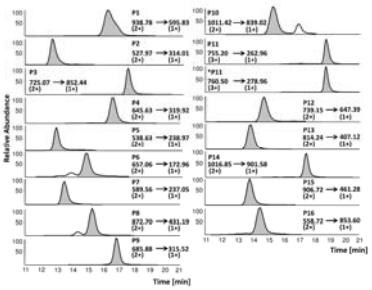
TARGETED LC-MS/MS METHOD TO QUANTITATE GLUTEN



33 marker peptides

of which

16 for **wheat**

7 for **rye**

7 for **barley**

3 for **oats**

of which

4 CD-active peptides

Peptide	[^{13}C]- and [^{15}N]-labeled peptides	Grain type
33-mer	LQLQPFPQPQLPYPQPQLPYPQPQLPYPQ**PQPF**	Wheat
ISTD 11	LQLQPFPQPQLPYPQPQ**PF**	Wheat
ISTD 19	AQQQPSIEEQHQ**L**	Barley
ISTD 24	VQQQPPFVQQEQP**F**	Oats
ISTD 27	ASIETGI**VG**H	Rye

Schalk et al., 2018a,b

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TARGETED LC-MS/MS METHOD TO QUANTITATE GLUTEN

	Protein type	Peptide / gluten protein type [µg/mg] (LC-MS)	Peptide / wheat flour [µg/g] (LC-MS)	Gluten protein type / wheat flour [%] (LC-MS)	Gluten protein type / wheat flour [%] (RP-HPLC)	Recovery of LC-MS/MS compared to RP-HPLC [%]
P1	LMW-GS	10.8 ± 0.2	29.4 ± 0.2	0.24 ± 0.03	1.99 ± 0.02	12.0
P2	LMW-GS	11.9 ± 0.3	24.1 ± 0.4	0.19 ± 0.01		9.6
P3	LMW-GS	4.9 ± 0.0	21.3 ± 0.7	0.41 ± 0.02		20.5
P4	LMW-GS	8.9 ± 0.4	224.6 ± 16.7	2.37 ± 0.18		119.2
P5	HMW-GS	5.3 ± 0.4	90.6 ± 1.2	1.62 ± 0.08	0.83 ± 0.02	195.2
P6	HMW-GS	3.3 ± 0.1	< LOD	< LOD		-
P7	HMW-GS	7.5 ± 0.3	86.3 ± 7.9	1.08 ± 0.04		129.5
P8	γ-Gliadins	18.7 ± 2.0	639.4 ± 26.11	3.19 ± 0.09	1.85 ± 0.15	172.3
P9	γ-Gliadins	16.8 ± 2.2	477.3 ± 33.6	2.66 ± 0.39		143.9
P10	γ-Gliadins	2.0 ± 0.2	16.1 ± 1.7	0.76 ± 0.08		41.1
P11	α-Gliadins	5.9 ± 0.0	137.2 ± 13.7	2.19 ± 0.22	2.91 ± 0.30	75.3
P12	α-Gliadins	3.9 ± 0.1	18.5 ± 0.7	0.45 ± 0.03		15.3
P13	α-Gliadins	9.5 ± 0.2	8.7 ± 0.2	0.09 ± 0.01		3.0
P14	ω5-Gliadins	11.3 ± 0.1	25.6 ± 2.4	0.20 ± 0.02	0.51 ± 0.02	39.9
P15	ω1,2-Gliadins	5.4 ± 0.5	86.2 ± 2.9	1.50 ± 0.12	0.67 ± 0.09	224.1
P16	ω1,2-Gliadins	0.8 ± 0.0	< LOD	< LOD		-

n = 3, mean ± standard deviation

Schalk et al., 2018a,b

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TARGETED LC-MS/MS METHOD TO QUANTITATE GLUTEN

Protein type		Peptide / wheat starch [µg/g]	Gluten protein type / wheat starch [µg/g]	Gluten content in wheat starch [µg/g]		
		LC-MS	LC-MS	LC-MS	GP-HPLC-FLD	R5 ELISA
Wheat starch 6				**117.5 A**	**103.6 B**	**82.5 C**
P4	LMW-GS	0.9 ± 0.1	92.2 ± 20.1			
P8	γ-gliadins	0.9 ± 0.1	25.3 ± 9.7			
Wheat starch 15				**2665.7 A**	**6543.3 B**	**7022.0 B**
P4	LMW-GS	8.5 ± 0.6	755.7 ± 56.6			
P7	HMW-GS	7.7 ± 1.1	743.7 ± 107.8			
P8	γ-gliadins	19.0 ± 2.5	554.2 ± 71.8			
P11	α-gliadins	2.3 ± 0.2	479.4 ± 40.2			
P15	ω1,2-gliadins	0.7 ± 0.1	132.7 ± 10.8			

Different superscript letters denote significant differences (ANOVA, Tukey's test, $p < 0.05$) between results of different methods within one sample, n = 3, mean ± standard deviation, GP-HPLC-FLD, gel permeation high performance liquid chromatography with fluorescence detection, Scherf et al., 2016, R5 ELISA, Ridascreen Gliadin, R-Biopharm, Darmstadt, Germany

Schalk et al., 2018a,b

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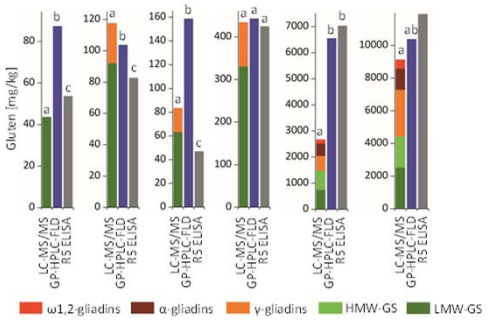
TARGETED LC-MS/MS METHOD TO QUANTITATE GLUTEN

Wheat starches

W1 W2 W3 W4 W5 W6



GP-HPLC-FLD, gel permeation high-performance liquid chromatography, different letters designate significant differences between the methods (ANOVA, $p < 0.05$)

Gluten

LC-MS/MS: sum of all gluten protein types

GP-HPLC-FLD: sum of prolamins and glutelins

R5 ELISA: prolamin content × 2

Calculations from peptide to protein contents based on defined amounts of gluten protein types as reference materials

Schalk et al., 2018a,b

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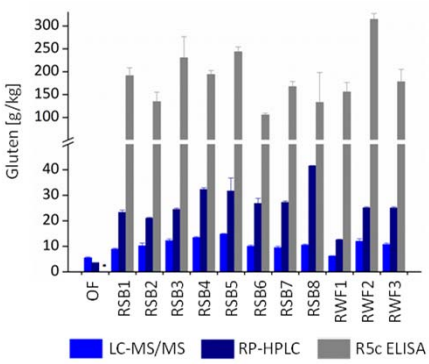
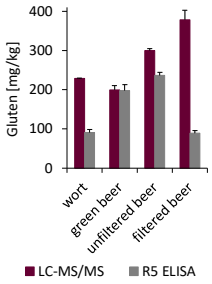
TARGETED LC-MS/MS METHOD TO QUANTITATE GLUTEN

Rye samples



Beer samples



RP-HPLC, reversed-phase high-performance liquid chromatography, different letters designate significant differences between the methods (ANOVA, $p < 0.05$), OF, oat flour, RSB, rye semolina bran, rye wholemeal flour

Calculations from peptide to protein contents based on defined amounts of gluten protein types as reference materials

Schalk et al., 2018a,b

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LC-MS/MS TO QUANTITATE GLUTEN – CON'S

- Choice of enzyme for gluten digestion influences the results
- Potential loss of gluten peptides during clean-up
- Choice of LC-MS setup influences the results
  - Acquisition mode, untargeted vs. targeted analysis
  - Data evaluation (bioinformatics, lack of curated plant protein databases)
- Targeted LC-MS analysis only looks at pre-selected peptides
  - Only CD-immunogenic peptides?
  - No detection in case of amino acid substitution/deletion/insertion
- Conversion of peptide contents back to **gluten** content
- Expensive and specialized instrumentation
- High-level of expertise required

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LC-MS/MS TO QUANTITATE GLUTEN – PRO'S

- Differentiation between wheat, rye, barley and oats
- Identification of peptide (protein) sequences in the sample
- Applicable to samples from which gluten was partially removed by processing, e.g. beer or sourdough samples
- Highly sensitive detection of gluten peptides with limits of quantitation down to 0.03 mg peptide/kg
- Accurate targeted quantitation of specific peptides using stable isotope labeled peptide standards
- Versatility: possibility of using different LC-MS setups
- Untargeted analyses generate a huge amount of data in a comparatively short time that can be evaluated in different ways
- Possibility of detecting gluten and allergens in one run (multi-methods)

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CONCLUSIONS – LC-MS/MS FOR GLUTEN ANALYSIS

- First LC-MS method to use well-defined reference proteins to detect all gluten protein types
- Targeted LC-MS:
  - Every peptide needs its own labeled standard for accurate results
  - So far, no comprehensive method for all known CD epitopes
- Further advances in LC-MS instrumentation: high throughput analyses generating a huge amount of data
- Better curation of plant protein databases is needed
- Conversion of peptide contents to **gluten** remains challenging

→ **LC-MS/MS: complementary to ELISA, especially for samples where ELISA is known to experience difficulties**

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THANK YOU … TO YOU FOR YOUR ATTENTION!

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