Innovating special nutrition

Dr Schär

Suitablility and Safety Aspects of Cereals and Pseudocereals for Gluten-Free Foods

Prof. Dr. Peter Koehler
German Research Centre for Food Chemistry, Freising, Germany

Different Meanings of Gluten

Gluten (Latin = glue)

1. Starch industry
   - Gluten: water-insoluble by-product of starch preparation
   - From wheat: vital gluten → food, non-food, feed applications
   - From maize: corn gluten → feed
2. Cereal chemistry
   - Gluten: proteins (techno-functional) from wheat gluten
3. Coeliac disease
   - Gluten: Coeliac-active proteins/peptides from wheat, rye, barley and their crossbreeds and possibly oats
   - Defined in the Codex Alimentarius-Standard for gluten-free foods

Prolamins: alcohol-soluble part of gluten

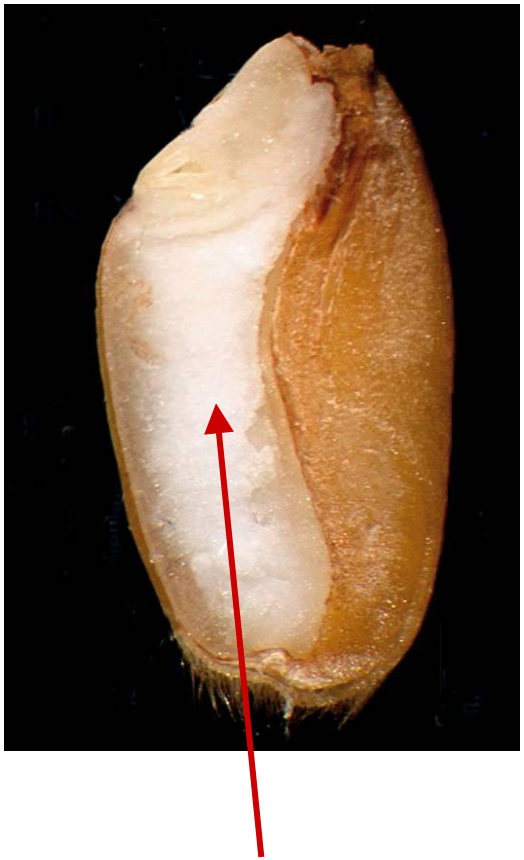
Glutelins: alcohol-insoluble part of gluten

Cereals and Pseudocereals

- Cereals: Plants belonging to the familiy of the *Poaceaea* (grasses) that are used for human nutrition
  - Wheat, rye, barley
  - Oats
  - Maize, rice sorghum
- Pseudocereals: Plants not belonging to the *Poaceae* but are used and processed like cereals:
  - Buckwheat, amaranth, quinoa
- Coeliac-safe raw materials
  - Are **gluten-free 'by nature'** (maize, rice sorghum, buckwheat, amaranth, quinoa)
  - Derive from gluten-containing cereals and have been been **rendered gluten-free** (e.g. wheat starch)
  - Must not be **contaminated** with gluten by any of the gluten-containing grains

Gluten Proteins

Source: Kampffmeyer Food Innovation



Endosperm

- Are **storage proteins**, which are only present in the starchy endosperm of the kernel
- Several hundred components
- 5 - 10 % of kernel dry mass
- Approximately 80 % of total kernel protein
- Only function: supplying the germ with nitrogen and amino acids during germination
- Poor nutritional value (low content of essential amino acids)
- Gluten proteins of wheat are responsible for the unique baking performance of wheat flour

Classification of Gluten Proteins

- Recently, amino acid sequences of all storage protein types of wheat, rye and barley have been determined

Protein type	Wheat	Rye	Barley
Prolamins	ω-gliadins	ω-secalins	C-hordeins
(monomeric)	α-gliadins	-	-
	γ-gliadins	γ-40k-secalins	γ-hordeins
Glutelins	HMW-glutenins	HMW-secalins	D-hordeins
(polymeric)	LMW-glutenins	γ-75k-secalins	B-hordeins

- Oats: Up to date no commonly accepted classification!

Safety Aspects

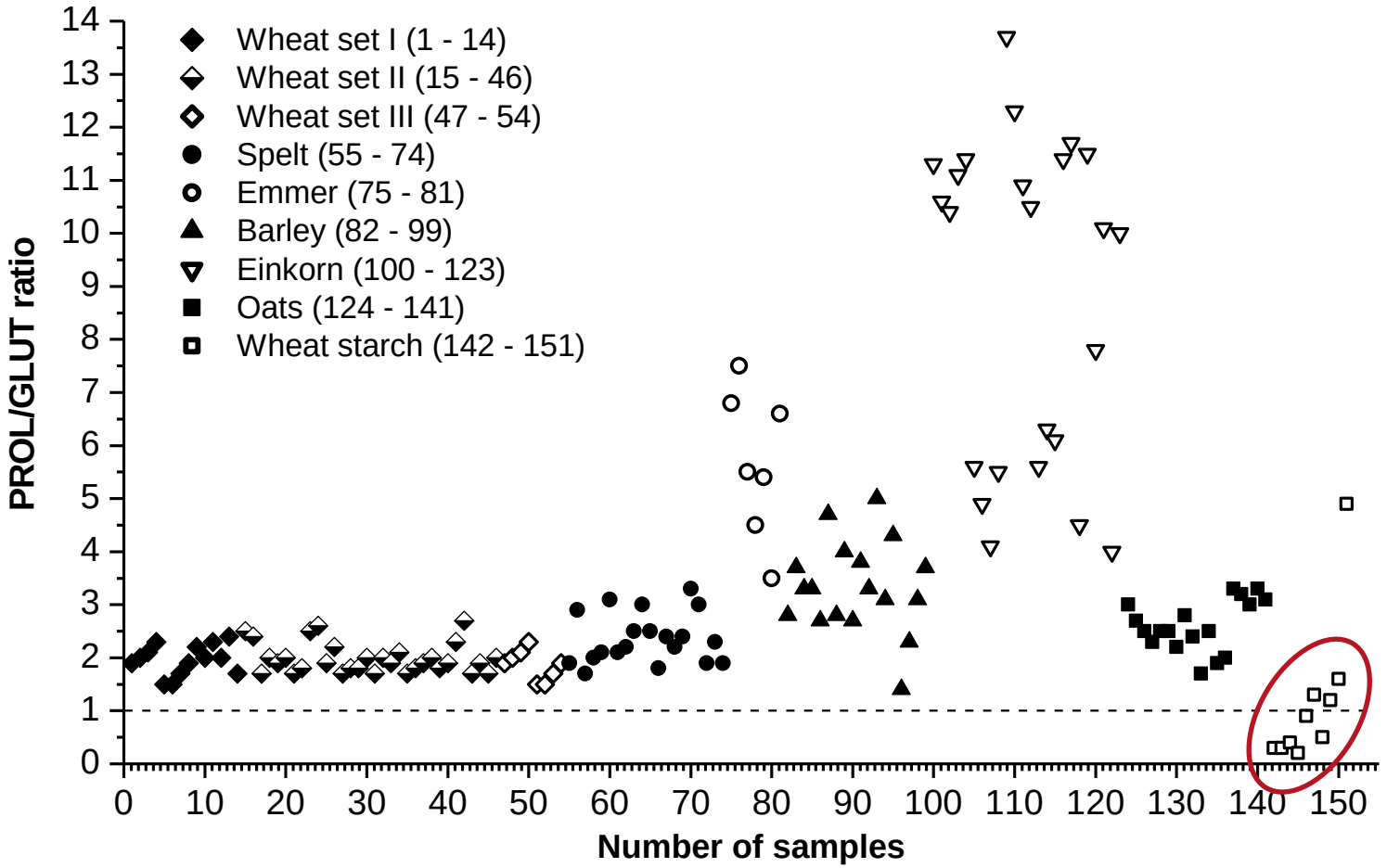
- Codex Alimentarius and Regulation (EG) No. 41/2009
  - Thresholds: 20 mg gluten/kg and 100 mg gluten/kg
- Suitable methods are required to quantify the gluten content of (gluten-free) foods
- Requirements for the analysis of the gluten content of foods
  - **Methods** that are sensitive enough to quantify gluten concentrations well below 20 mg/kg and suitable for routine analysis
  - An **independent reference method** to verify the routine method
  - A **reference material** for method calibration with distinct protein(type) or peptide content to convert the measured signal into prolamin/gluten concentration

label for gluten-free foods

State-of-the-Art of Gluten Analysis

- A **reference material** for method calibration is available, but is not certified
- **Methods** with sufficient sensitivity are available (ELISA)
- **Reference methods** are available, but they are not (yet) suitable for food analysis
- Currently no accepted method to determine the gluten content directly by measuring **all** responsible proteins
- Gluten is currently determined by quantifying prolamins and multiplying the prolamin content by **factor 2** to obtain the gluten content

Prolamin/Glutelin Ratios



- PROL/GLUT ratios < 1 only occur in starch samples

Wieser and Koehler, 2009

Gluten Analysis – Methods

- Real-time PCR
  (Sandberg et al., 2003; Zeltner et al., 2009; Mujico et al., 2011)
  - ☺ Specific detection of wheat, rye, barley and oats
  - ☺ Q-PCR of wheat with a limit of detection (LOD) around 1.5 mg gliadin/kg
  - ☺ Screening method for the presence of gluten-containing cereals
  - ☹ Not suitable for heated or partially hydrolyzed samples
  - ☹ Detects DNA and not protein (→ no gluten quantitation possible!)

Gluten Analysis – Methods

- MALDI-TOF mass spectrometry of intact proteins
  (Camafeita et al., 1998; Hernando et al., 2003)
  - ☺ Detection of the characteristic mass profiles of prolamins
  - ☹ Not sensitive enough with a LOD around 100 mg gliadin/kg

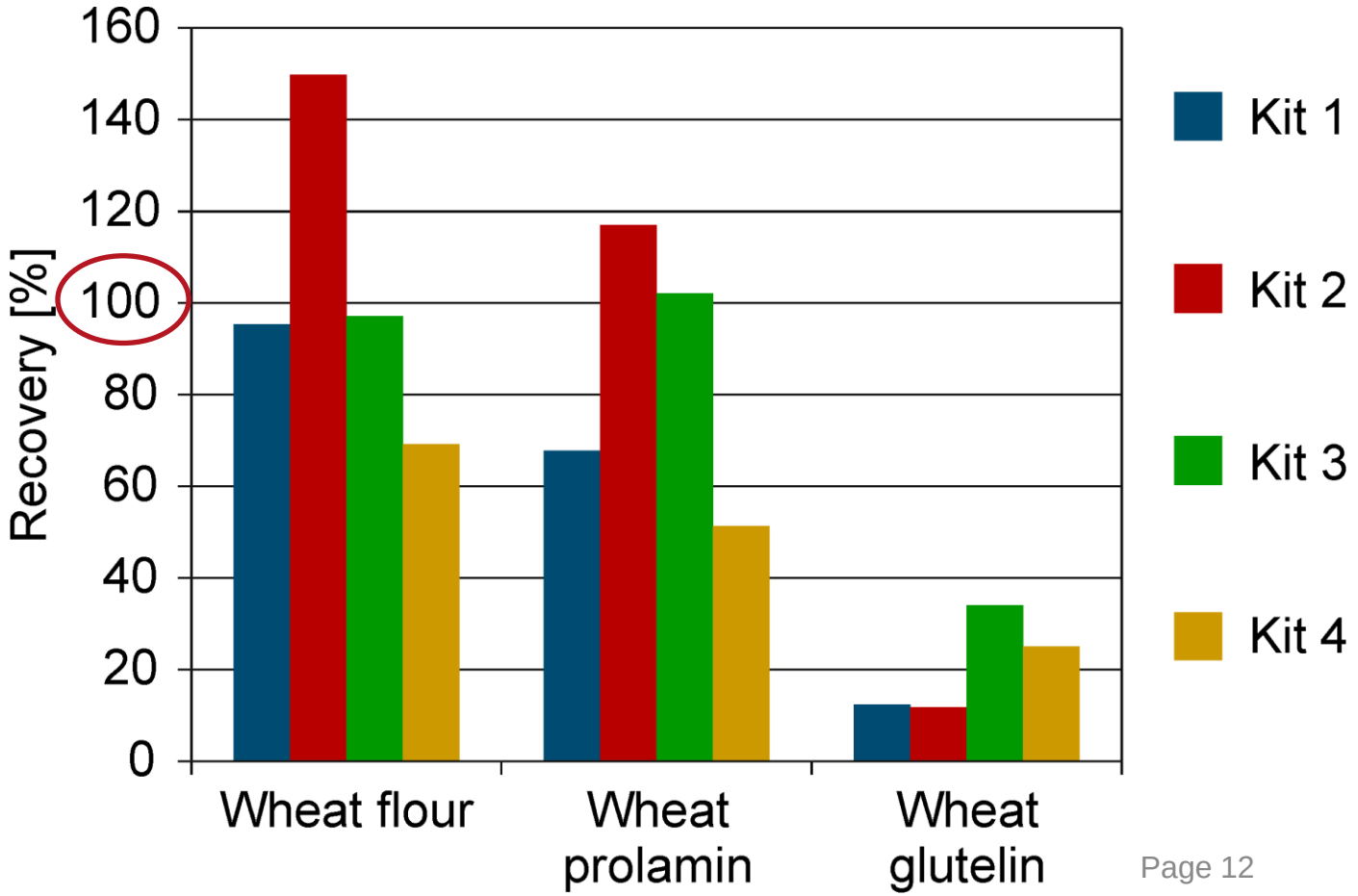
- LC-MS/MS of enzymatic digests of gluten proteins
  (Weber et al., 2009; Sealey-Voyksner et al., 2010)
  - ☺ Highly sensitive detection of peptides with LODs below 0.03 mg/kg
  - ☺ Potential for being a references method for gluten quantiation
  - ☹ Difficult calculation of the gluten content from the amount of peptide
  - ☹ Currently no comprehensive method for wheat, rye, barley, and oats
  - ☹ Expensive and sophisticated equipment necessary
  - ☹ Stable isotope labeled internal standards required

Gluten Analysis – Methods

- Immunochemical Methods (enzyme-linked immunosorbent assays, ELISA) (Valdes et al., 2003; Morón et al., 2008; Mujico et al, 2012)
  - ☺ Sufficient sensitivity with LODs of 1.5 mg gliadin/kg
  - ☺ Fast and suitable for routine analysis
  - ☺ No special equipment needed
  - ☺ Ridascreen® Gliadin ELISA based on the R5-antibody has been approved as “Official First Action” method by AOAC International and is currently endorsed as a ‘Type 1 Method’ by Codex Alimentarius
  - ☹ Strongly dependent on the reference protein for calibration
  - ☹ Only determination of specific prolamin types
  - ☹ Calculation of the gluten content from the prolamin content
  - ☹ Different sensitivity of kits for different cereal species
  - ☹ Different sensitivity depending on the antibody used

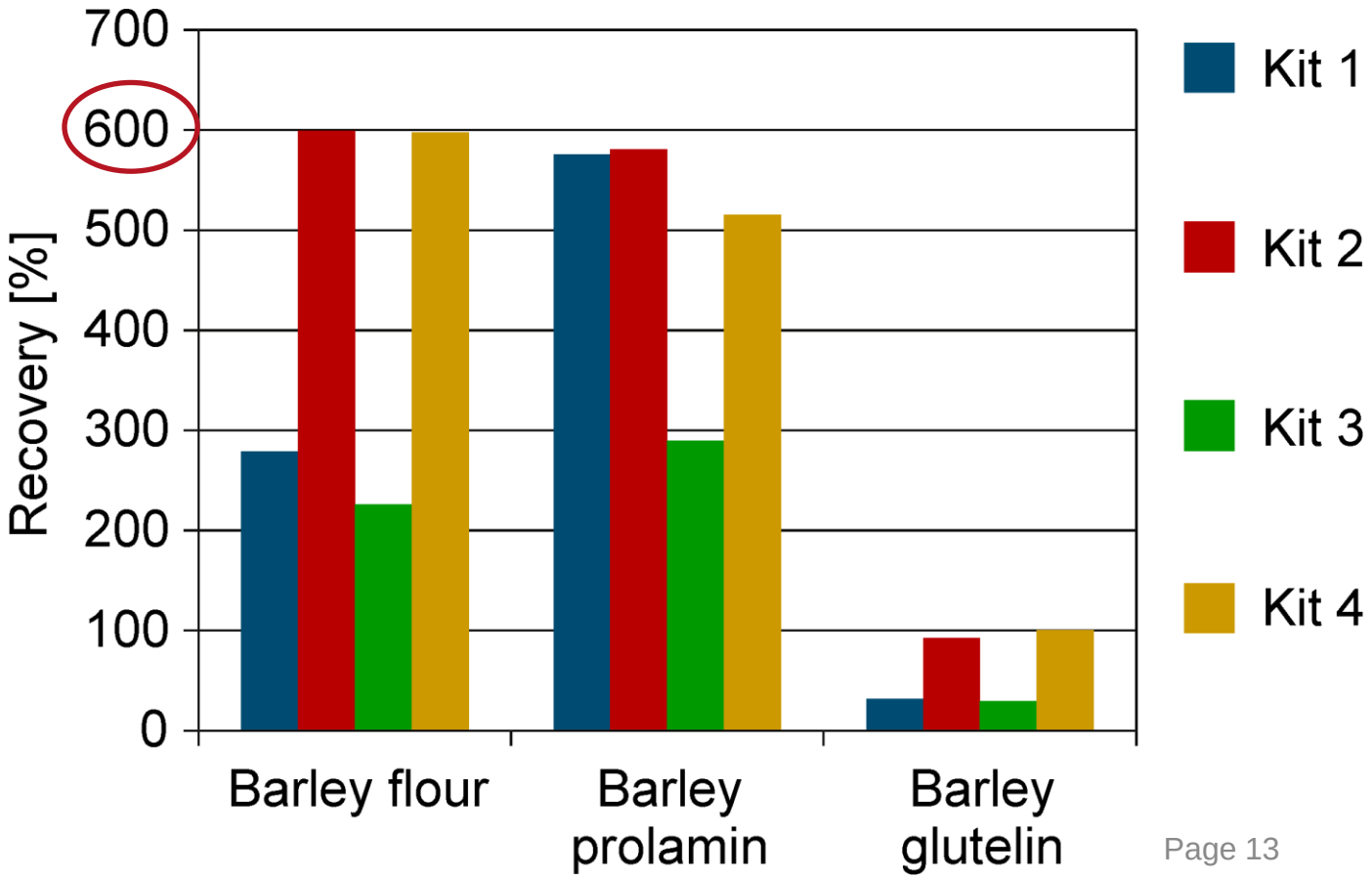
Wheat: Recovery With Different ELISA Kits

- Prolamin target content: 30 mg/kg (= 100 %)



Barley: Recovery With Different ELISA Kits

- Prolamin target content: 30 mg/kg (= 100 %)



Gluten Analysis – State-of-the-Art

- RP-HPLC-UV (Wieser et al., 1998; Wieser and Koehler, 2009)
  - ☺ Real gluten (prolamin + glutelin) content can be determined
  - ☺ Absolute quantitation with any protein reference possible
  - ☺ Routine application possible
  - ☺ Provides basic data on gluten composition
  - ☹ Not sensitive enough with a limit of quantitation around 250 mg/kg
  - ☹ Possible interferences of other proteins present in food samples
    → limited to raw materials due to low selectivity

Perspectives – Future Developments

- New protein reference(s) suitable for **each** method needed
- ELISA
  - New antibodies
  - Detection of **all** protein types ($\Sigma_{(types)}$ = gluten)
  - Specificity for different cereal species?
  - Improvement of gluten quantitation in fermented foods
- Non-immunochemical methods
  - LC-MS analysis of storage proteins (Σ = gluten) or peptides?
  - How to report peptide concentrations?
  - Selection of suitable sequences (toxic/non-toxic but conserved epitopes)
  - Absolute quantitation without stable isotope labeling?
  - Gel permeation chromatography with fluorescence detection (**GP-HPLC-FLD**) of gluten proteins (autofluorescence or labeling)

Wheat Starch: Comparison GP-HPLC-FLD and ELISA

- Quantitative data [mg/kg]; $Gluten_{ELISA} = 2 \times Gliadin_{ELISA}$

Sample	Gluten (GP-HPLC-FLD)	Gluten (ELISA competitive)	Gluten (ELISA Sandwich)	Gliadin/ Glutenin
Gf $W1_{(f)}$	7	16	8	n.c.*
Gf $W3_{(f)}$	43	32	14	0.48
Gf $W5_{(f)}$	26	10	6	n.c.*
$W1_{(t)}$	26	46	16	1.02
$W3_{(f)}$	52	22	20	0.30
$W4_{(f)}$	250	104	46	0.38
$W5_{(f)}$	31	20	16	0.91
$W7_{(f)}$	43	170	66	2.38
$W8_{(f)}$	10189	11590	11904	3.19
$W9_{(t)}$	< 5	n.c.*	n.c.*	n.c.*
$W11_{(t)}$	800	298	414	1.08

* not calculable; (f) = food grade; (t) = technical; Gf W = wheat starch labelled as gluten-free; W = wheat starch with no specification of gluten content

Conclusions

- Gluten analysis is an analytical challenge because
  - gluten has an extremely complex composition and
  - gluten from different cereals species shows homologies but also distinct differences
- ELISA methods are state-of-the art in gluten analysis
- Unprecise gluten quantitation due to calculation on the basis of a fixed prolamin/glutelin ratio of 1
- New antibodies for both prolamins and glutelins would enable analytical determination of the gluten content instead of calculation
- Need for independent analytical methods to confirm ELISA results: LC-MS, HPLC-Fluorescence Detection
- The determination of the "true" gluten content of many (gluten-free) foods appears to be not possible to date and remains a challenge!

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You for your attention!