

C-hordein as reference material in gluten detection

Xin Huang

Grain Technology Group,
Department of Food and Nutrition,
University of Helsinki, Finland

xin.huang@helsinki.fi

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Procedure for gluten detection in gluten-free products

Raw products
(intact proteins)



Food Matrix



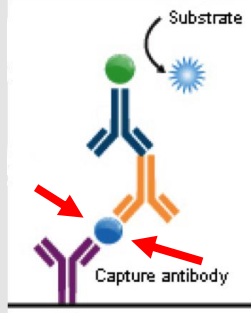
Hydrolysed or fermented food
(small peptides)



Aqueous alcohol
with reducing
agents

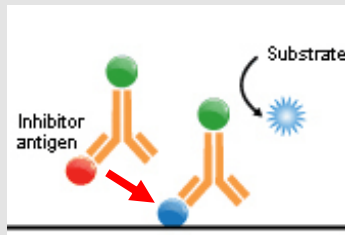
Extraction

Aqueous
alcohol



Sandwich ELISA

Antibody
recognition



Competitive ELISA

Gliadin
standard

Reference
material

Hydrolysates
mixture
(wheat, barley
and rye)

Conversion factor
 $\times 2$

gliadin:glutenin
(1:1)

Gluten
concentration

Conversion
factor $\times 2$

Gluten-free oats contamination

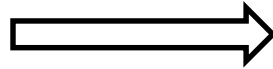
Barley grown in same area



Oat



Gluten-free?

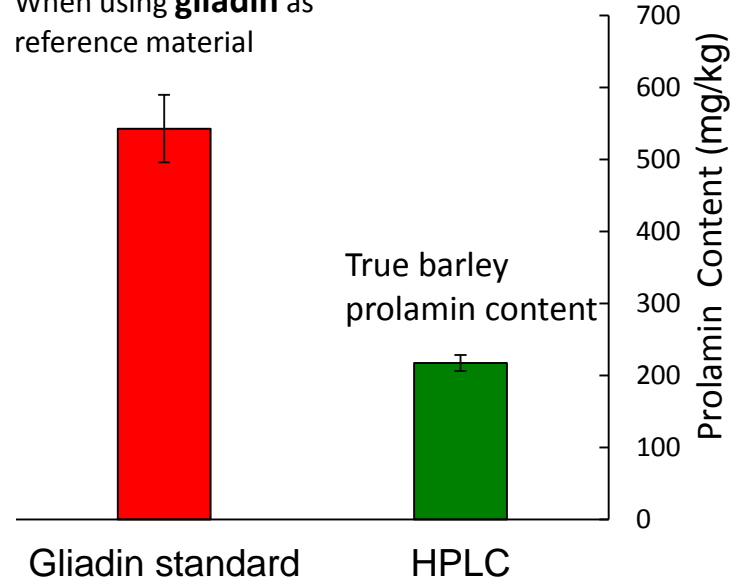


R5 antibody

- ☐ R5 raise against rye secalin
- ☐ Mainly recognise QQPF
- ☐ Codex 118-1979

Overestimation $\times 2.5 (\times 2) = 5$

When using **gliadin** as reference material



- ☐ Barley contamination in oats 7-30 times overestimation (Kanerva 2006)
- ☐ Barley contamination in maize 1.8 times overestimation (Mujico 2008)
- ☐ Total hordein cv.Sloop 15 times over gliadin (Tanner 2013)

Why?

- ❑ Barley and wheat prolamins compositions are different.
- ❑ **A separate barley hordein is needed for calibration**

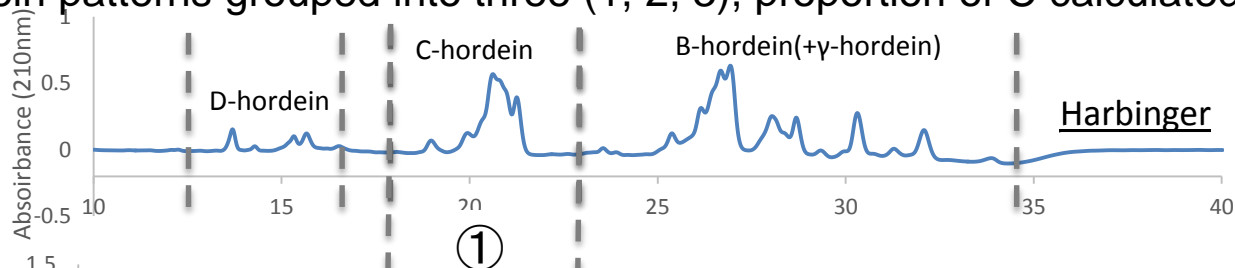
| | Wheat | Barley | Prolamin Working Group (PWG) gliadin (86%/protein) |
|-----------|---|----------------------------------|--|
| Gliadins | α/β -gliadin ω -gliadins γ -gliadins | C-hordeins γ -hordeins | α/β -gliadins (42%) ω -gliadins (11%) γ -gliadins (47%) |
| Glutenins | LMW-glutenins HMW-glutenins | B-hordeins D-hordeins | LMW-glutenins ? HMW-glutenins ? |

Hordein composition and profile in RP-HPLC

- ❑ 28 barley cultivars collected from Nordic countries, and their prolamins separated on C8 column
- ❑ Based on C-hordein patterns grouped into three (1, 2, 3), proportion of C calculated

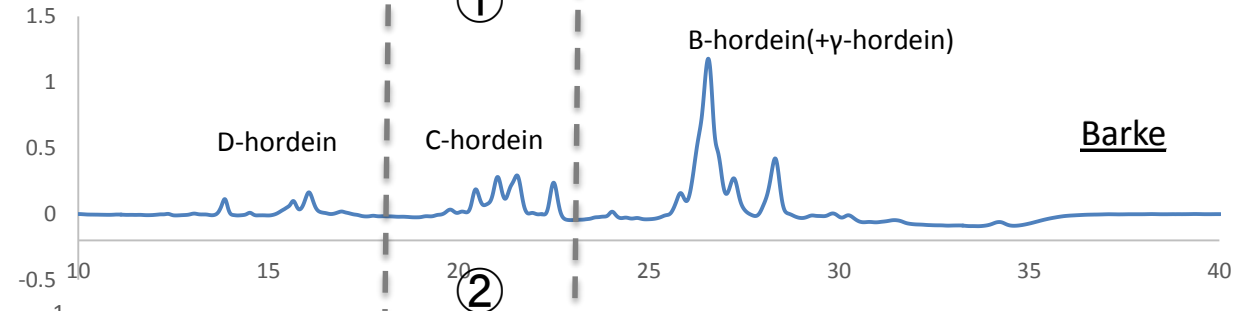
①

Harbinger, Streif
KWS Asta, Overture
Jorma



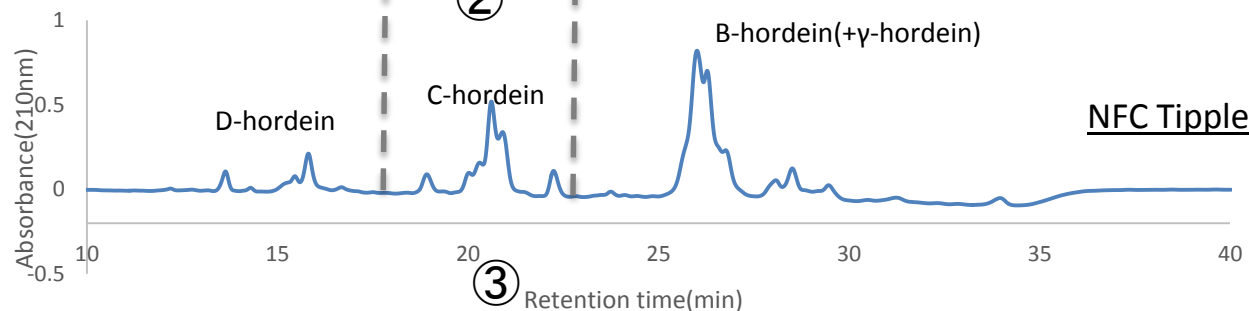
②

Barke, Brage, Vilde,
Polartop, Fairytale,
Wolmari, Voitto, Elmeri,
Marthe, Einar, Edvin,
Aukusti, Xanadu, Scarlett,
Toria, Annabel, Minttu,
SW Mitja, Lacey



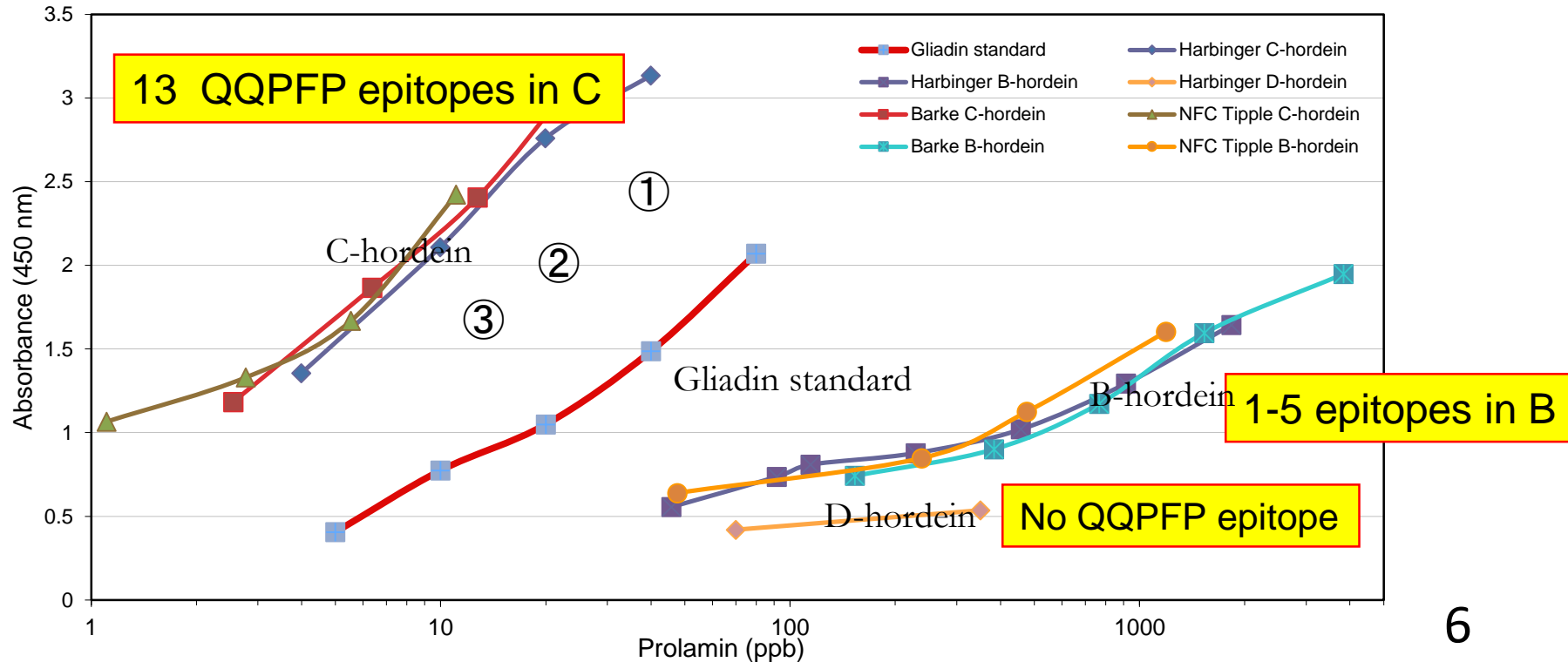
③

NFC Tipple, Saana,
Tocada, Jyvå, Propino



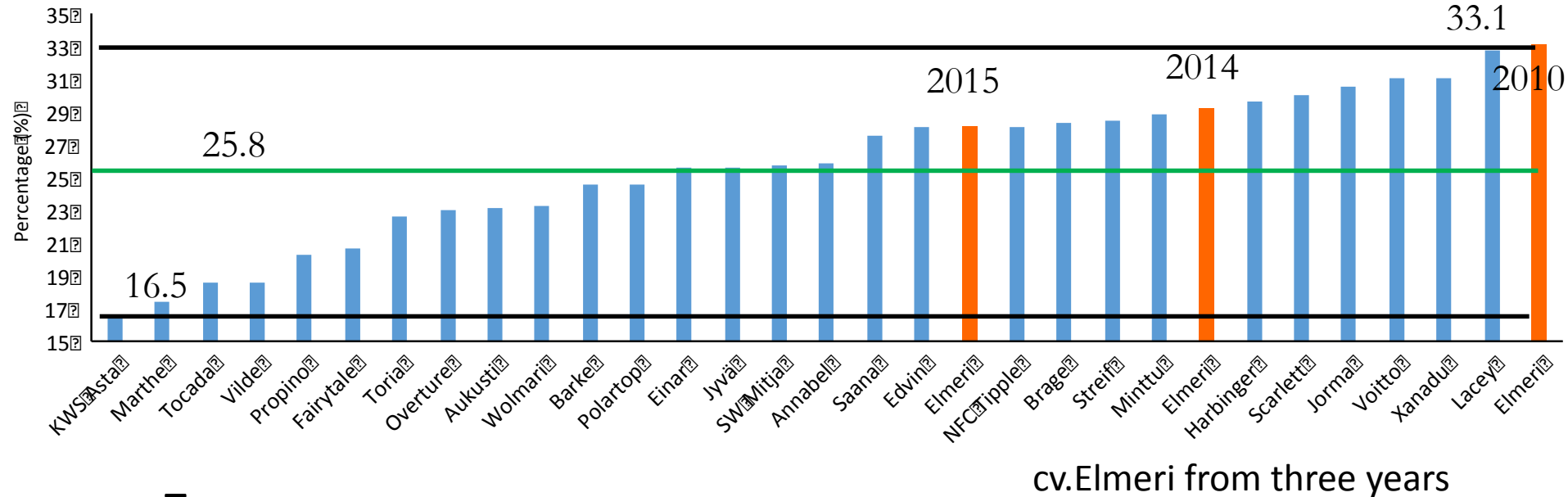
Hordein subunits against R5

- ❑ C-hordein is more reactive than B- and D-hordeins against R5
- ❑ The three HPLC patterns of C-hordeins do not differ against R5



C-hordein proportion of total hordein in the 28 cultivars

- ❑ The proportion ranges from 16.5% to 33.1%
- ❑ Cultivar and environment affect the proportion
- ❑ The average proportion is around 26%

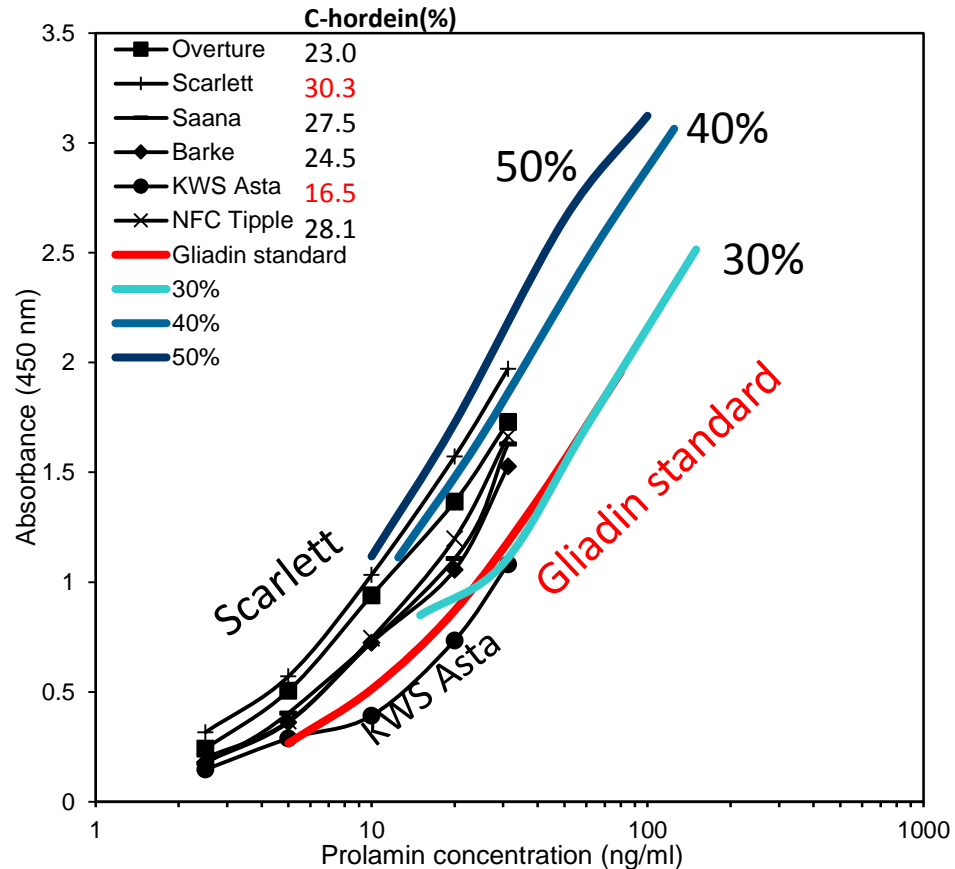


A hordein standard strategy

- ❑ A total hordein as reference material (cultivar mixtures? one cultivar? solubility? conversion?)
- ❑ Main recognition comes from C-hordein, why not just C-hordein to present the whole hordein?
- ❑ A preparative cation exchange chromatography to isolate C-hordein

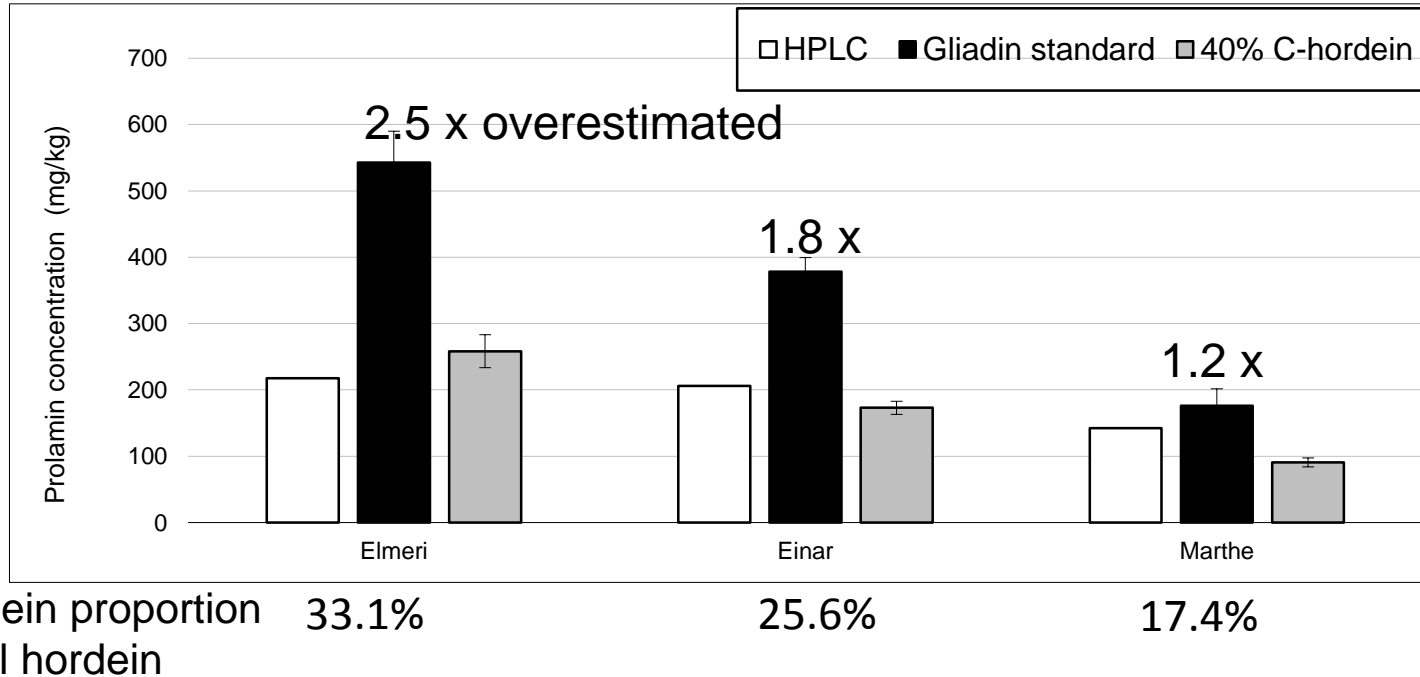
How much C-hordein?

- ❑ Purified total hordeins from cultivars plot against C-hordein standard
- ❑ **40% C-hordein** as reference material



GF oat spiked with **three** barley cultivars

- ❑ The higher C-hordein proportion, the more overestimation
- ❑ 40% C-hordein calibrated prolamin content, no conversion factor



How about wheat?

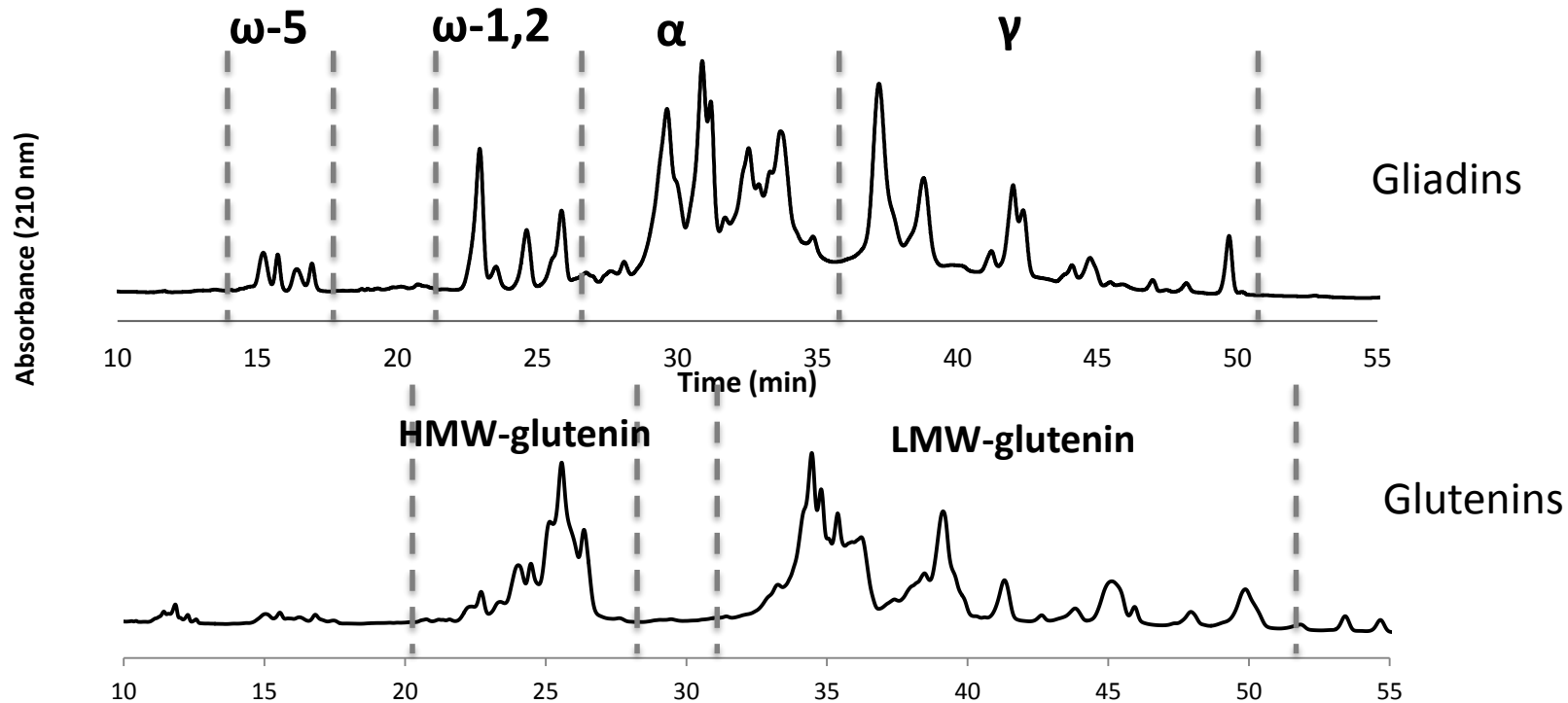
- ☐ PWG **gliadin standard**, 28 wheat cultivars from UK, France and Germany in 1999, **not updated**
- ☐ not pure enough as gliadin, **not proved by IRMM**
- ☐ Another cultivar mixture every some years?
- ☐ MoniQA association --> one wheat cultivar

- ☐ Wheat gliadin standard not good for barley, but one type of barley prolamin for wheat calibration?

27 Collected wheat cultivars popular in 2016 based on yield

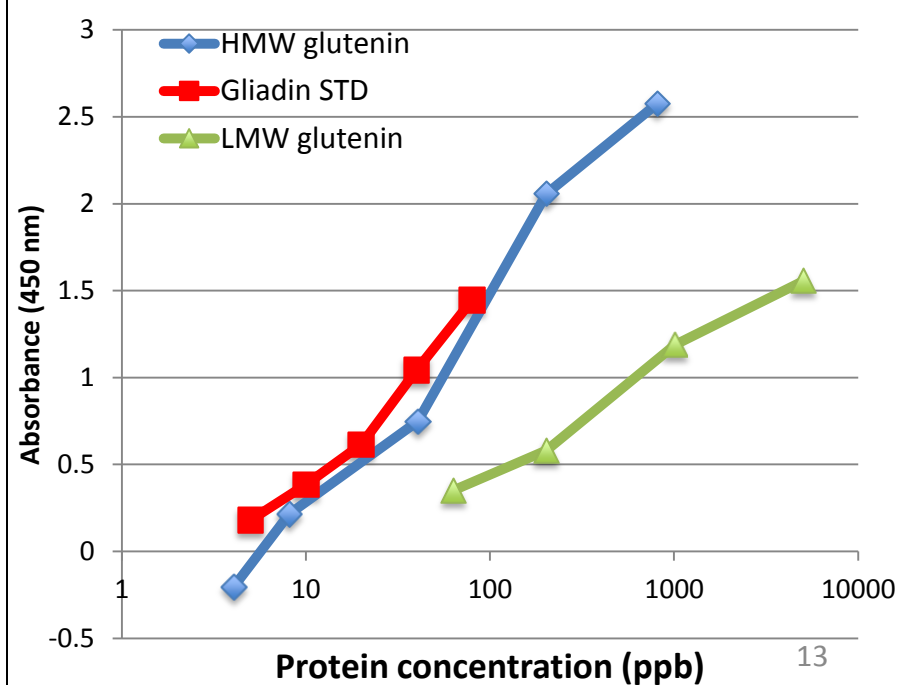
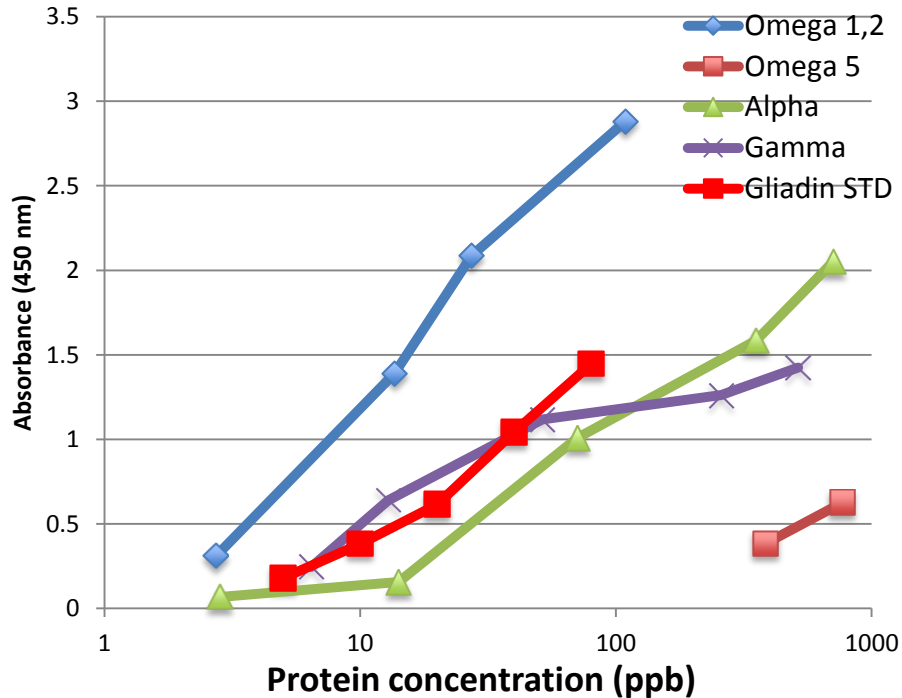
- UK (Siskin, Lili, Crusoe, Zulu, Claire, Revelation, Britannia)
- France (Cellule, Apache)
- Germany (Julius, Kerubino)
- Finland (Anniina, Amaretto, Quarna)
- Sweden (Julius, Brons, Hereford)
- Canada (Brandon, Stettler, Foremost, Penhold)
- Australia (Gregory, Lancer, Spitfire, Suntop, Mace)

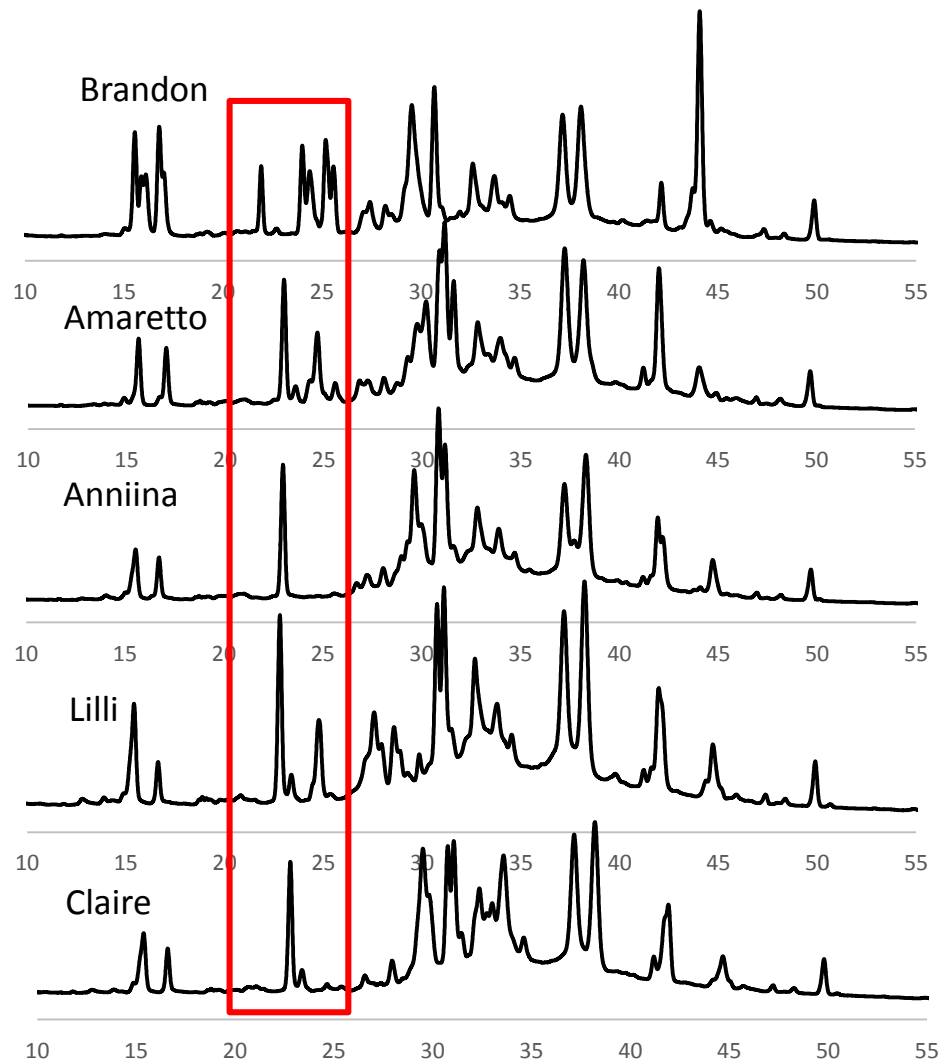
RP-HPLC separation of gluten proteins from cv.Crusoe



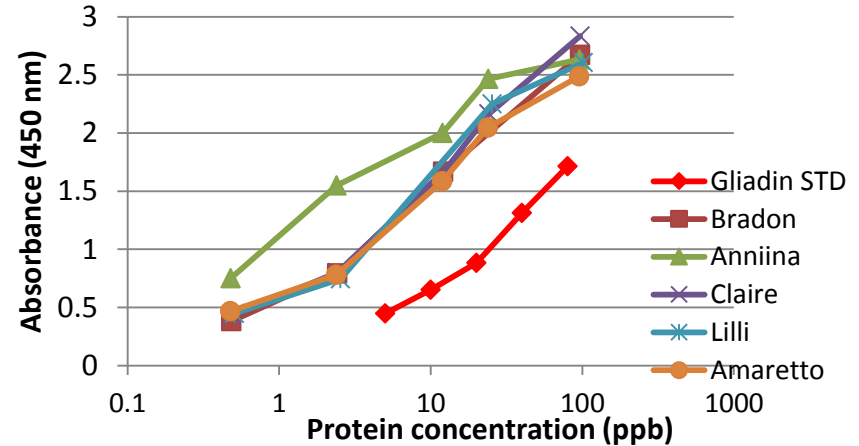
Gluten proteins against R5 antibody

- ❑ ω -1,2 gliadins had the most recognition against R5 antibody
- ω -1,2 gliadins = C-hordeins (PQQPFPQQ), ω -5 gliadins (FPQQQ)
- ❑ Basically, all gluten proteins have responses against R5 antibody





Types of omega-1,2 gliadins from five cultivars and their R5 responses



- ☐ ω -1,2 gliadins from four cv react similarly, expect one cultivar with one HPLC peak.
- ☐ ω -1,2 gliadins R5 response less stable than C-hordeins among cultivars.

| | Omega 1,2 (%) | Omega 5 (%) | Alpha (%) | Gamma (%) |
|------------|--------------------------|------------------------|----------------------|----------------------|
| Lili | 14.5 | 4.2 | 65.2 | 14.8 |
| Bradon | 14.0 | 10.8 | 52.3 | 19.7 |
| Cellule | 13.2 | 9.9 | 66.4 | 7.6 |
| Stettler | 12.6 | 7.3 | 56.5 | 22.3 |
| Crusoe | 12.5 | 2.8 | 68.6 | 15.0 |
| Patras | 12.0 | 5.1 | 64.4 | 17.1 |
| Amaretto | 11.1 | 3.7 | 68.7 | 15.3 |
| Kerubino | 11.1 | 3.3 | 65.7 | 18.7 |
| Quarna | 10.6 | 4.7 | 69.6 | 15.1 |
| Britannia | 10.0 | 4.6 | 70.0 | 15.5 |
| Suntop | 9.8 | 3.1 | 71.5 | 15.6 |
| Apache | 9.5 | 4.0 | 75.1 | 8.8 |
| Lancer | 9.3 | 4.4 | 67.5 | 17.9 |
| Julius SE | 9.2 | 2.8 | 71.8 | 14.3 |
| Julius GER | 8.9 | 3.9 | 70.4 | 15.5 |
| Mace | 8.8 | 8.1 | 63.5 | 18.4 |
| Penhold | 8.6 | 4.9 | 69.2 | 17.2 |
| Revelation | 8.0 | 4.1 | 71.3 | 15.5 |
| Claire | 7.8 | 3.6 | 73.8 | 13.7 |
| Siskin | 7.8 | 4.3 | 69.3 | 16.9 |
| Foremost | 7.4 | 2.6 | 69.1 | 19.3 |
| Gregory | 7.4 | 4.5 | 69.7 | 17.7 |
| Brons | 7.1 | 2.9 | 71.8 | 16.1 |
| Zulu | 6.6 | 3.8 | 74.7 | 13.8 |
| Anniina | 6.3 | 3.5 | 74.4 | 14.8 |
| Spitfire | 6.1 | 5.1 | 71.6 | 16.3 |
| Hereford | 5.7 | 4.0 | 75.1 | 15.2 |

Gliadin composition

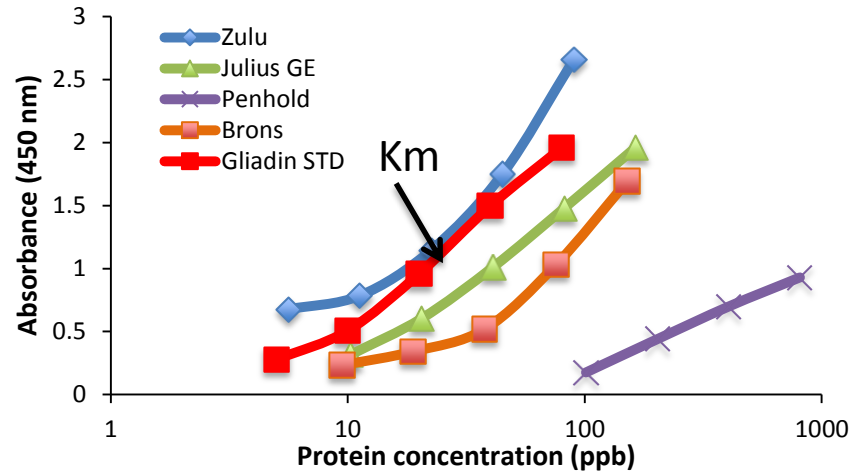
- ❑ ω -1,2 gliadins of total gliadin in wheat cultivars ranges from 5.7% to 14.5%, average 9.1%.
- ❑ C-hordein of total hordein in barley cultivars ranges from 16.5% to 33.1%.

Cultivar Km

| | |
|------------|-------|
| Zulu | 22.5 |
| Lancer | 51.4 |
| Crusoe | 51.5 |
| Cellule | 59.2 |
| Lili | 64.9 |
| Hereford | 66.0 |
| Quarna | 66.8 |
| Revelation | 73.7 |
| GER-Julius | 77.9 |
| Siskin | 78.1 |
| Hereford | 79.4 |
| Anniina | 82.0 |
| Claire | 83.7 |
| Britannia | 93.5 |
| Spitfire | 100.1 |
| Kerubino | 102.7 |
| Apache | 103.1 |
| Amaretto | 110.3 |
| SWE-Julius | 118.0 |
| Gregory | 142.9 |
| Bradon | 158.0 |
| Brons | 274.8 |
| Penhold | 314.0 |

| | |
|---------------|-------|
| Cultivar | |
| Average | 103.2 |
| Gliadin STD | 63.7 |
| 10% C-hordein | 100.5 |
| 20% C-hordein | 27.9 |
| 30% C-hordein | 15.2 |

Isolated gluten and their R5 responses

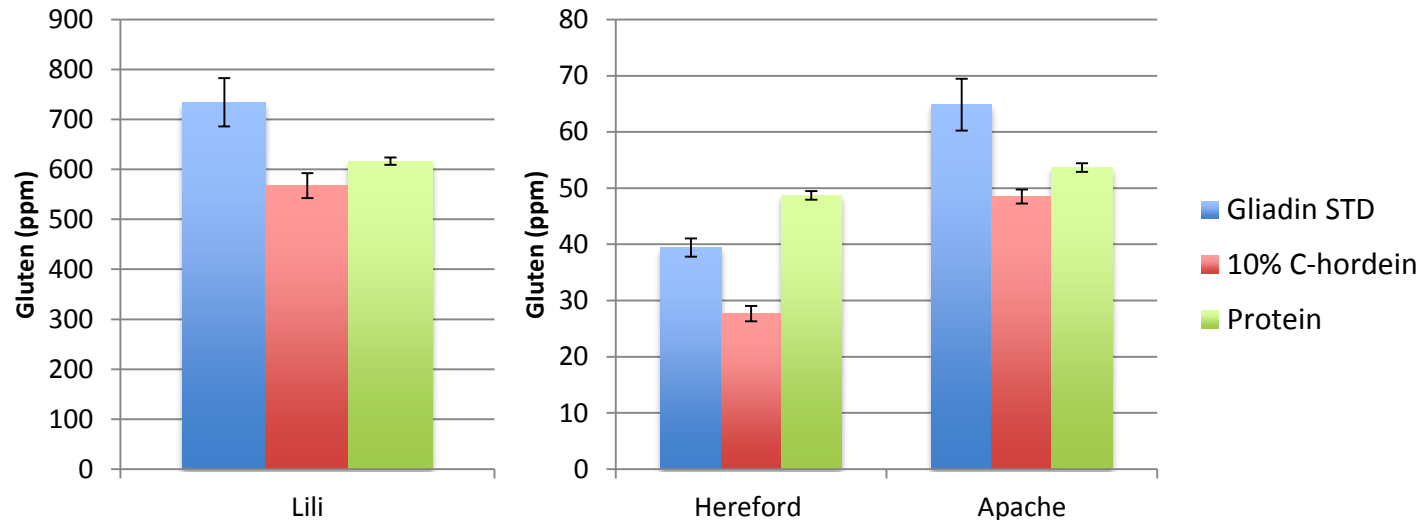


- ❑ The response expressed as Dissociation constant (Km): the substrate concentration that yield half of enzyme velocity maximum (Vmax)
- ❑ Isolated gluten R5 responses vary a lot among cultivars
- ❑ The total gluten react with R5 does not entirely correlate with omega-1,2 gliadin proportion, for the complexity of gluten composition.

❑ Use 10% C-hordein as reference to represent the whole gluten

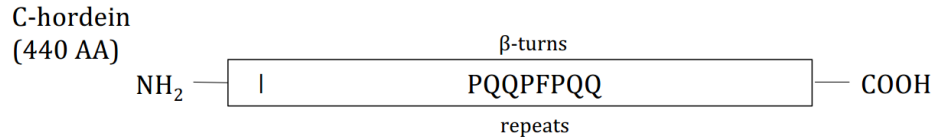
Spiking wheat in oats

- ❑ Spiking wheat flour in gluten-free oat flour.
- ❑ Calibrated with 10% C-hordein, no conversion factor
- ❑ Gliadin standard and conversion factor 2
- ❑ Wheat flour-nitrogen content*5.7=protein content → * 80% gluten



Conclusion-Benefits of C-hordein

- ❑ Gliadin standard is not good for barley gluten calibration
- ❑ Wheat standard itself requires update



- ❑ Good substrate for R5 antibody, repeats of epitopes
- ❑ Constant reactivity
- ❑ Good solubility, no cysteine, no aggregation problem
- ❑ Possibility to adjust, for barley and wheat

Acknowledgements

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- ☐ Ass.Prof. Tuula Sontag-Strohm
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