

Looking for a peptide signature to distinguish high and low-digestible genotypes

Mélanie Lavoignat, Angéla Juhász, Utpal Bose, Thierry Sayd, Christophe Chambon, Miguel Ribeiro, Gilberto Igrejas, Sébastien Déjean, Catherine Ravel, Emmanuelle Bancel



INRAE

17^e ICC International Cereal and Bread Congress
E. Bancel

UNIVERSITÉ,
Clermont
Auvergne



17th ICC International Cereal and Bread Congress

"Healthy Cereal diets from sustainable food systems"



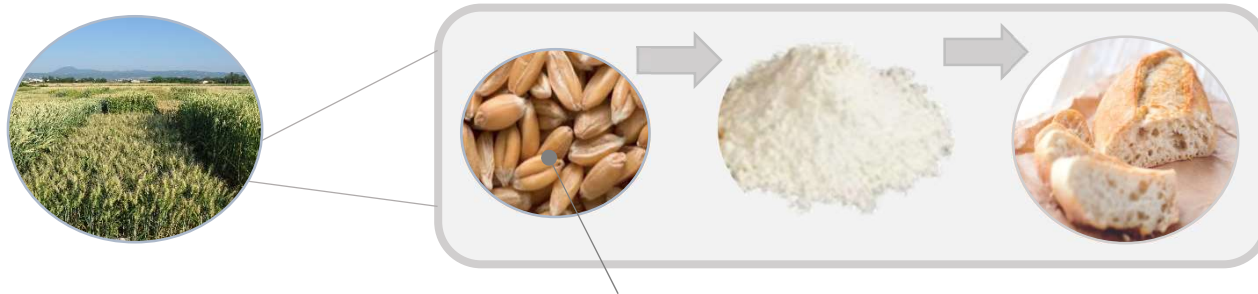
Diapositive 1

UB0

If Emmanuelle will present, then it will be good to keep only Emmanuelle's name on the title slide and mention our name in the acknowledgement.

Bose, Utpal (A&F, St. Lucia); 2024-04-19T00:43:38.604

🌿 Dual role of gluten proteins – bread quality and health



8-15 % Proteins

Bread wheat

Almost 20 % of nitrogen intakes for adults



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Johansson et al., 2013, Scherf et al. 2016, Shiferaw et al., 2013

☘ Dual role of gluten proteins – bread quality and health



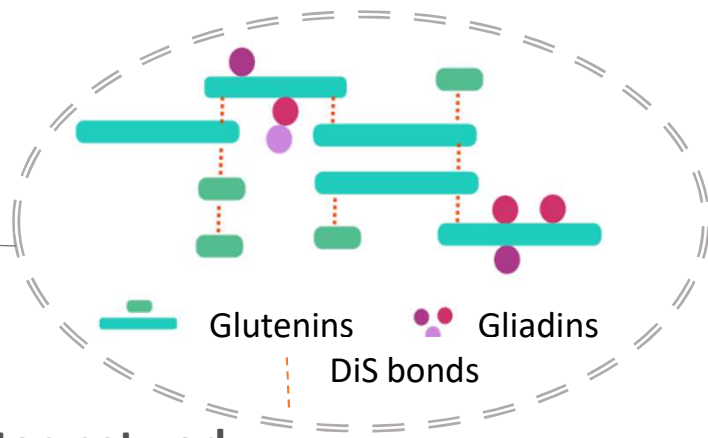
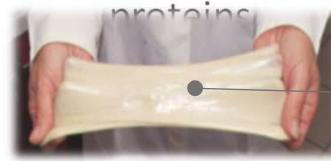
Bread wheat

Almost 20 % of nitrogen intakes for adults

8-15 % Proteins

80 % Gluten proteins

20% Non-gluten proteins



Polymeric gluten network



**Essential for processing...
but**



...linked to health issues



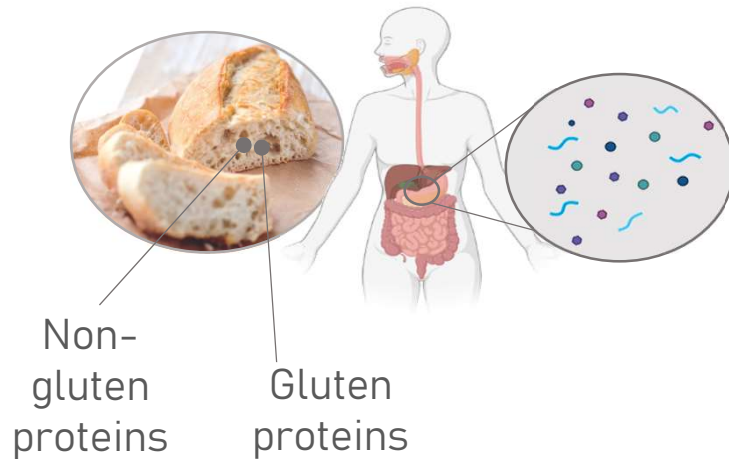
INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Johansson et al., 2013, Scherf et al. 2016, Shiferaw et al., 2013

Partially digested gluten epitopes can trigger immune reactions



- Wheat proteins are partially resistant to gastrointestinal enzyme hydrolysis
- Intact peptides reach the intestinal epithelium
- Lead to reactions involving immune mechanisms



INRAE

17^e ICC Internaional Cereal and Bread Congres. Nantes, France.2024

E. Bancel

Lamacchia et al. 2014, Scherf et al. 2016

p. 4



Genetic variability for bread protein digestibility



Field trials

17 *T. aestivum* cultivars were grown at Clermont-Ferrand (CF) and Estrées-Mons (EM) in France. Grain and flour were phenotyped.



Bread-making
Yeast fermentation



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024
E. Bancel

Lavoignat et al, 2023

p. 5



Genetic variability for bread protein digestibility

Lavoignat et al, 2023



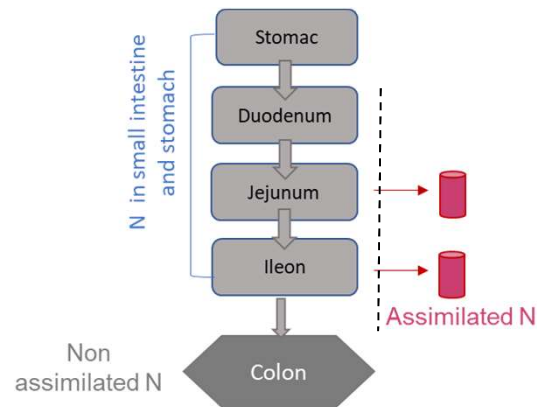
Field trials

17 *T. aestivum* cultivars were grown at Clermont-Ferrand (CF) and Estrées-Mons (EM) in France. Grain and flour were phenotyped.

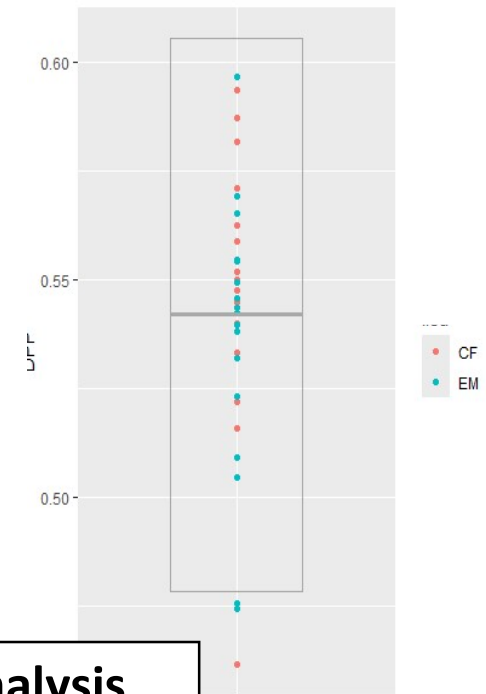
In-vitro digestion (2h)



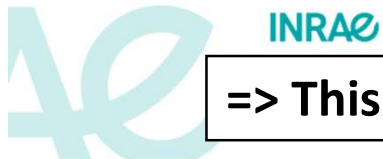
Partial Protein Digestibility of bread proteins



$$PPD = N_{\text{assimilated}} / N_{\text{total}}$$



=> This work provided the samples for a peptidomic analysis



Two key questions



- (1) What is the peptidomic landscape from bread digestion?
- (2) Is there a peptide signature related to the protein digestibility?

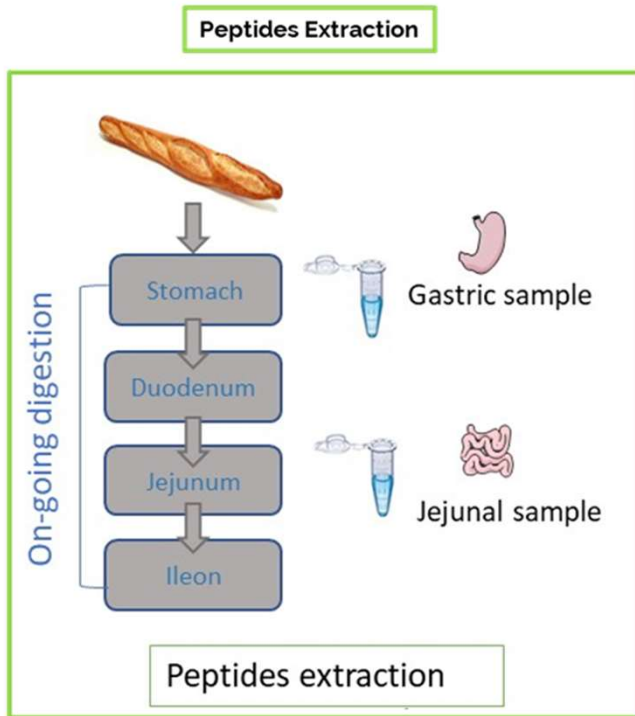


INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Our approach - The peptidomics workflow

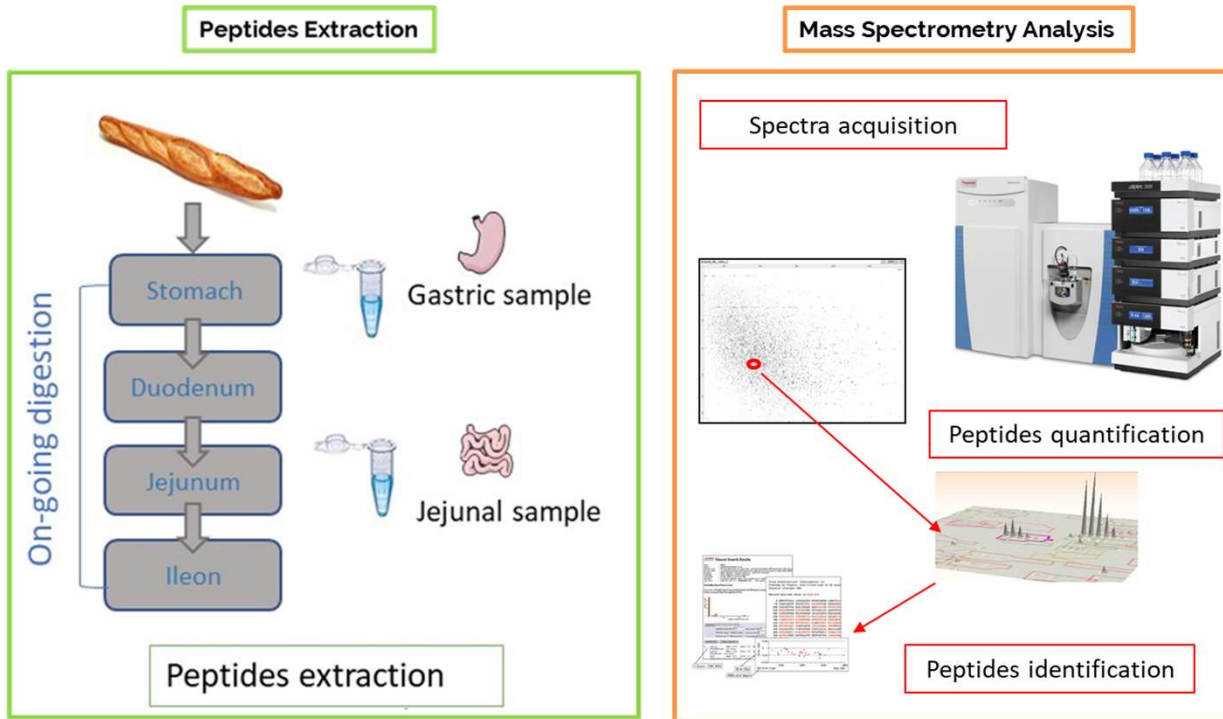


INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Our approach - The peptidomics workflow

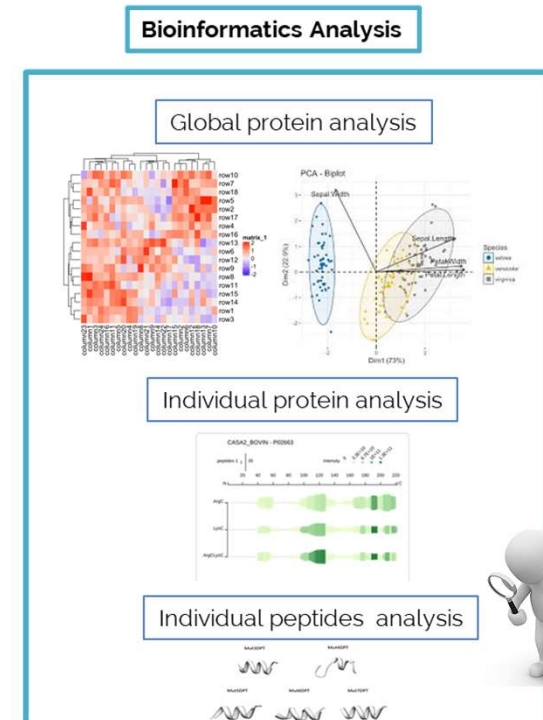
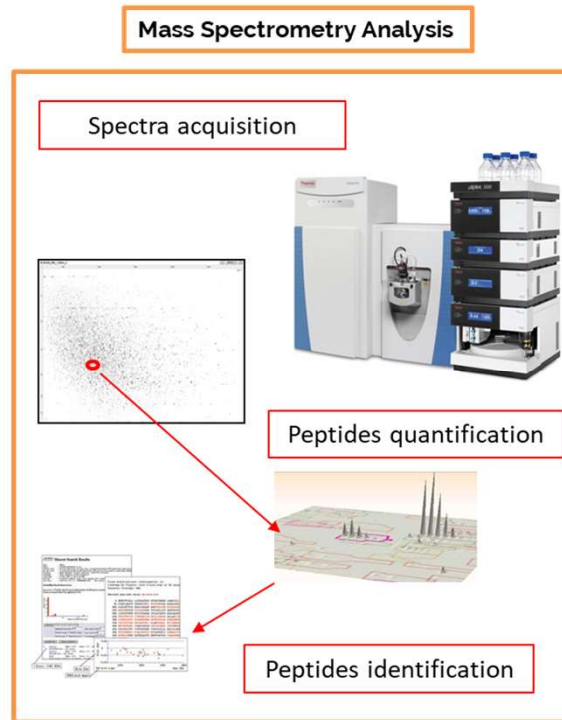
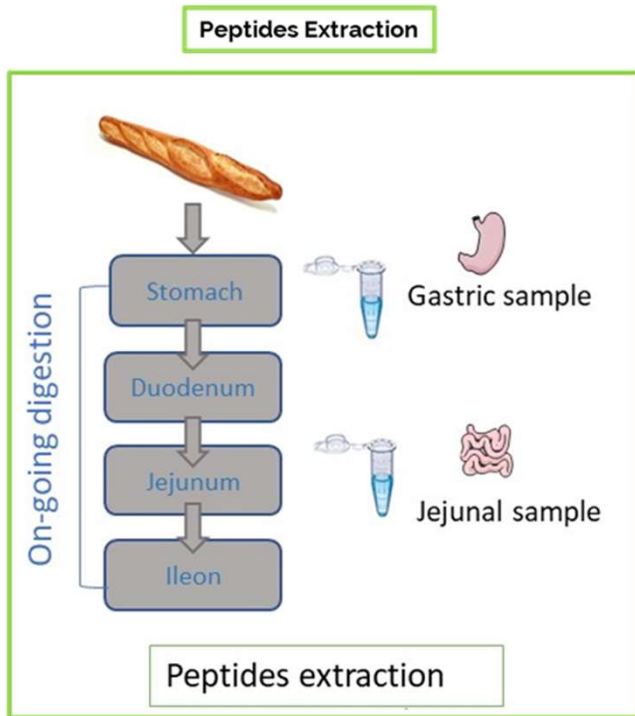


INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Our approach - The peptidomics workflow



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

The first question



(1) What is the peptidomic landscape from bread digestion?

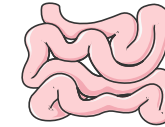
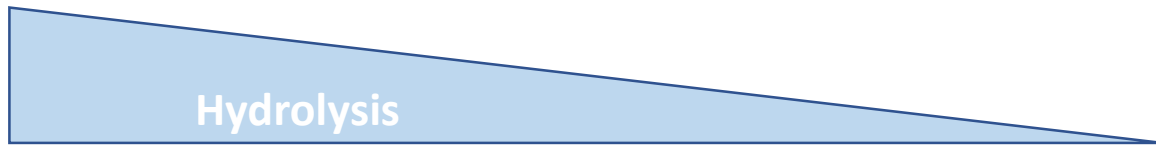


INRAE

17° ICC Internaional Cereal and Bread Congres. Nantes, France.2024

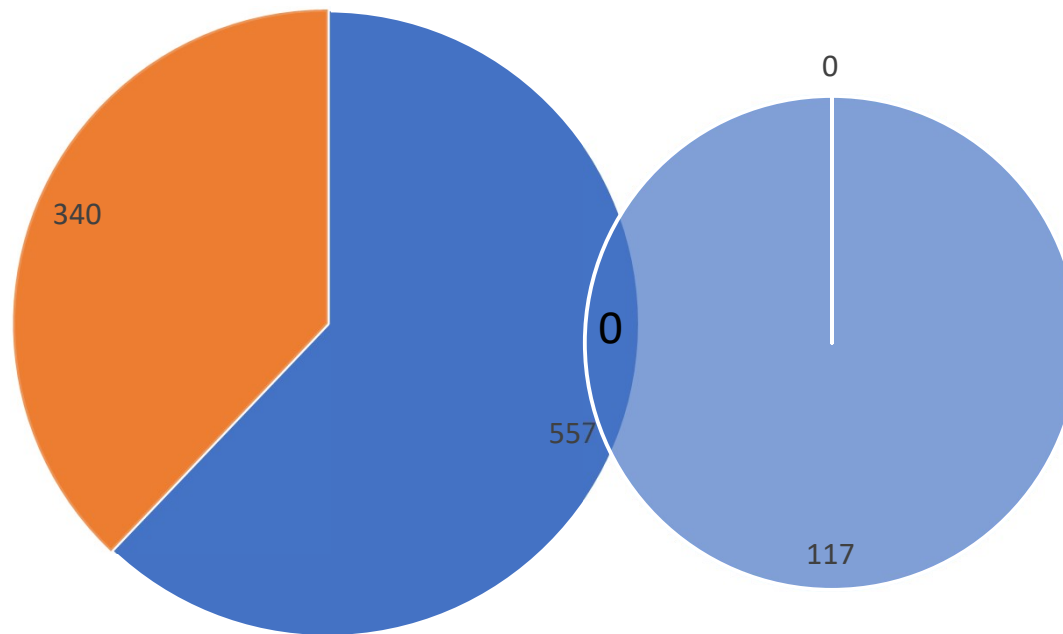
E. Bancel

(1) What is the peptidomic landscape from bread digestion ?



987 gastric peptides

117 jejunal peptides



■ assigned ■ not assigned



INRAE

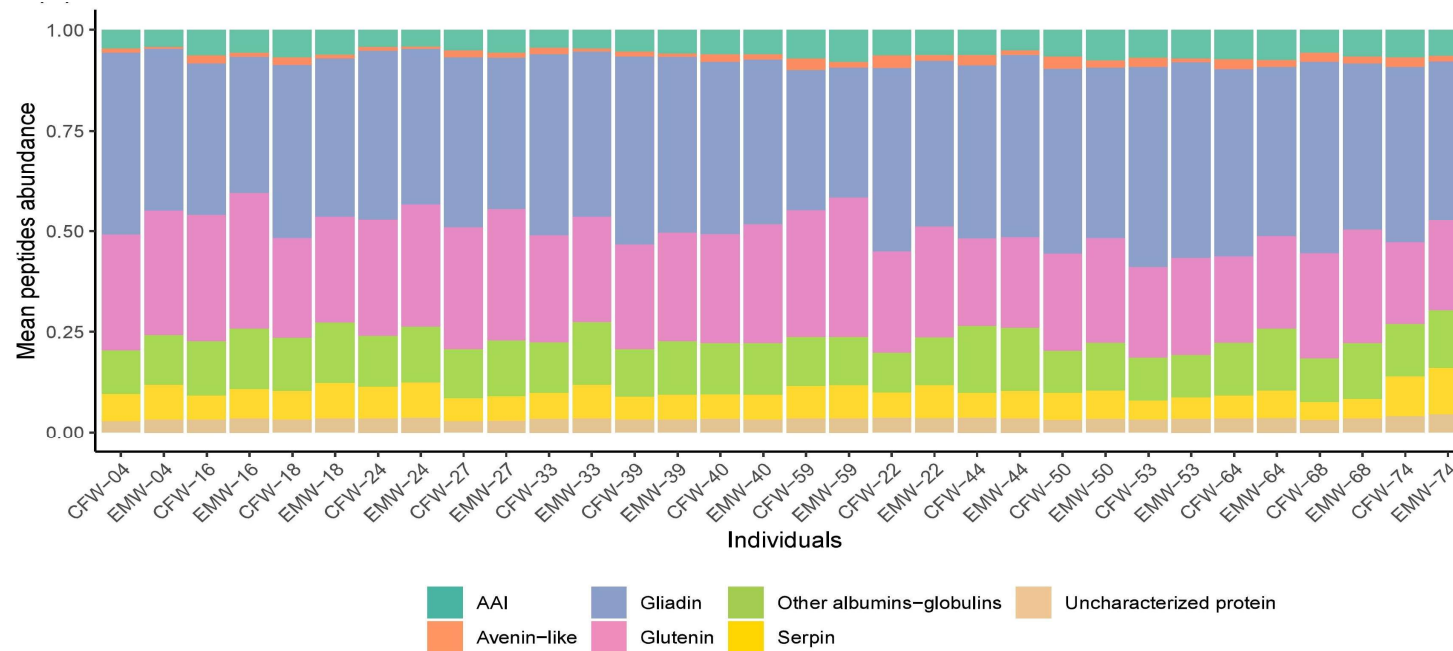
17^e ICC Internaional Cereal and Bread Congres. Nantes, France.2024

E. Bancel

(1) What is the peptidomic landscape from bread digestion?



Gastric peptides (987)



➤ The protein family origin of the gastric peptides is diverse



INRAE

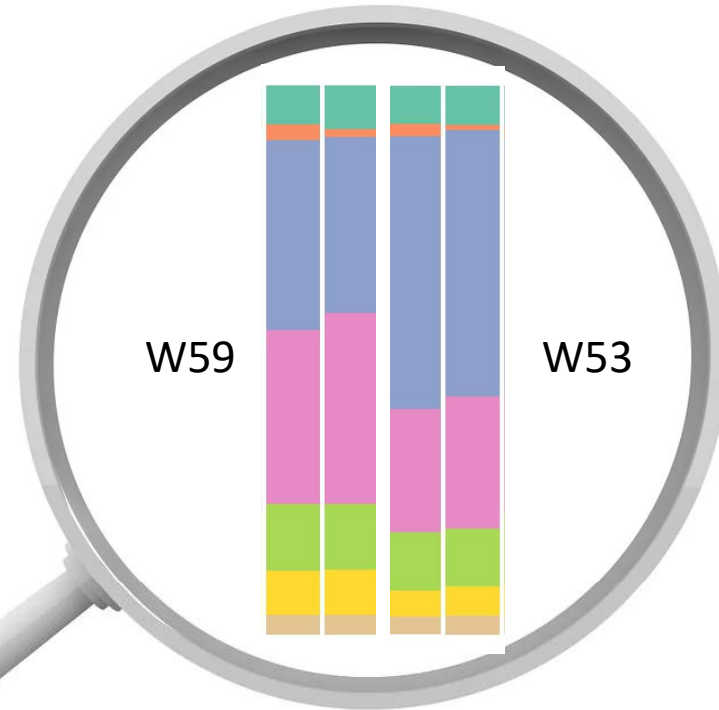
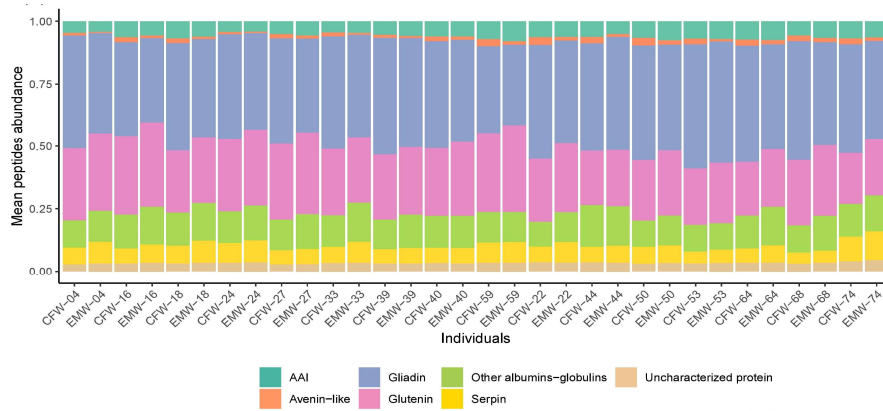
17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(1) What is the peptidomic landscape from bread digestion?



Gastric peptides (987)



- The **protein family origin** of the gastric peptides is diverse
- Location- and genotype-dependent variabilities are identified



INRAE

17° ICC Internaional Ce

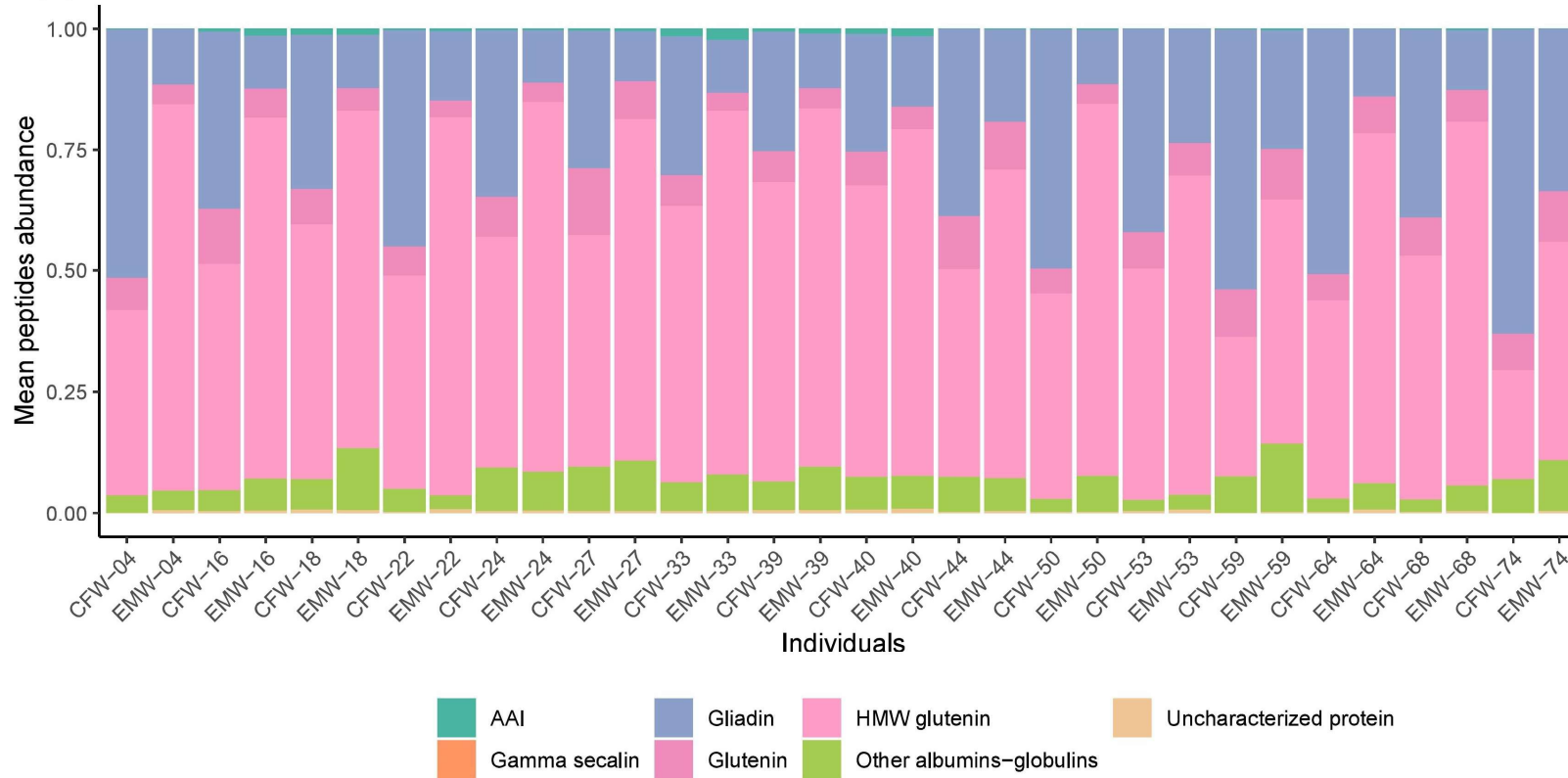
E. Bancel



(1) What is the peptidomic landscape from bread digestion ?



Jejunal peptides (117)



➤ The jejunal peptides were almost exclusively derived from **storage proteins**



INRAE

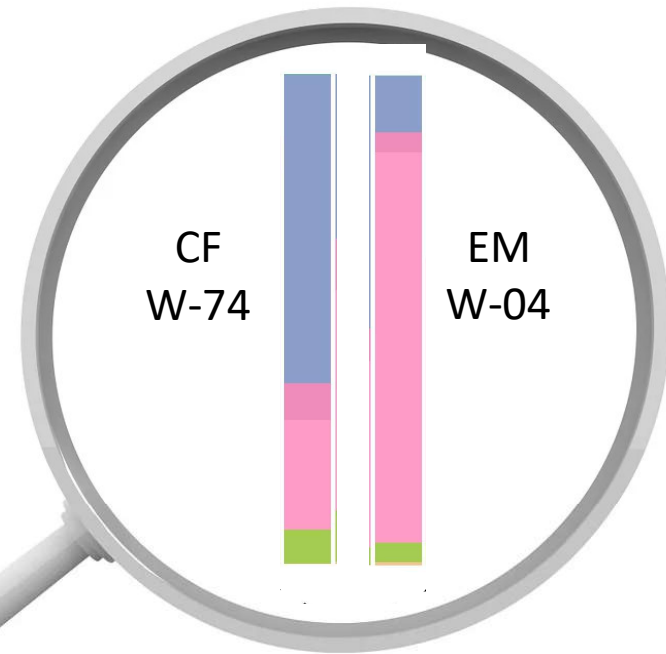
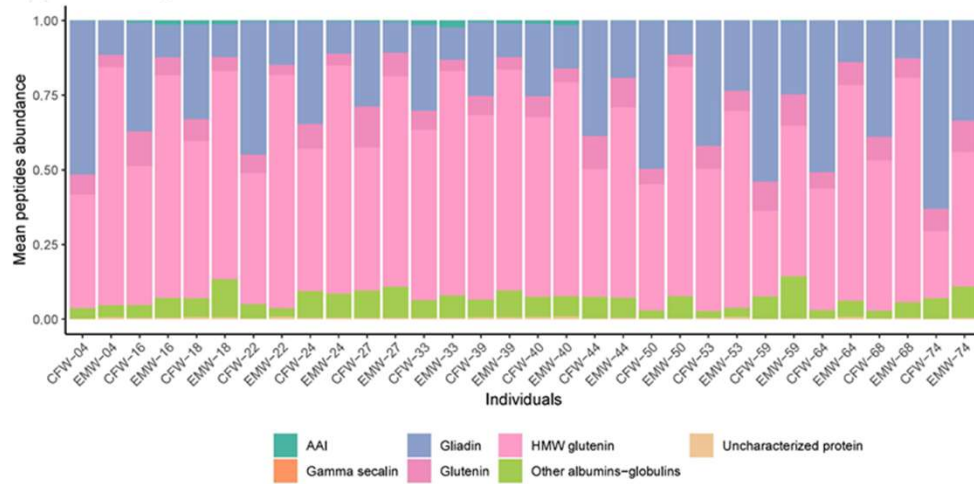
17° ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(1) What is the peptidomic landscape from bread digestion ?



Jejunal peptides (117)



➤ The jejunal peptides were almost exclusively derived from **storage protein**

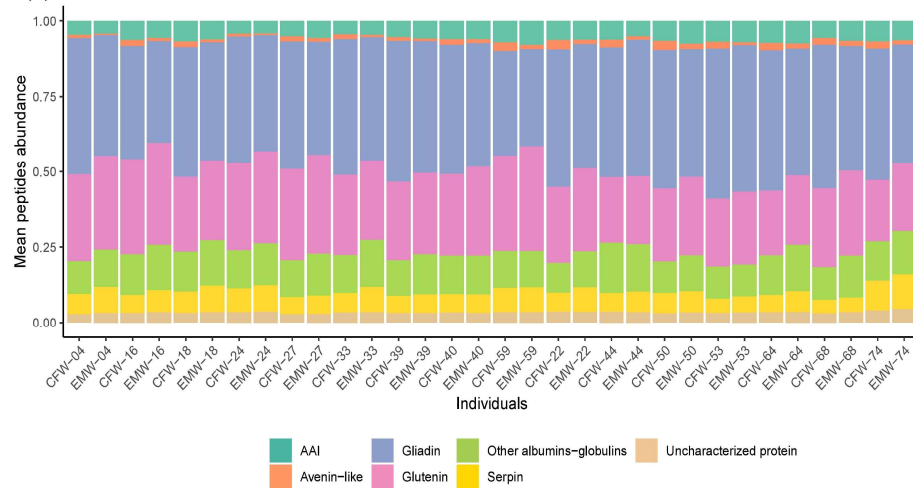
➤ Location- and genotype-dependent variabilities are identified



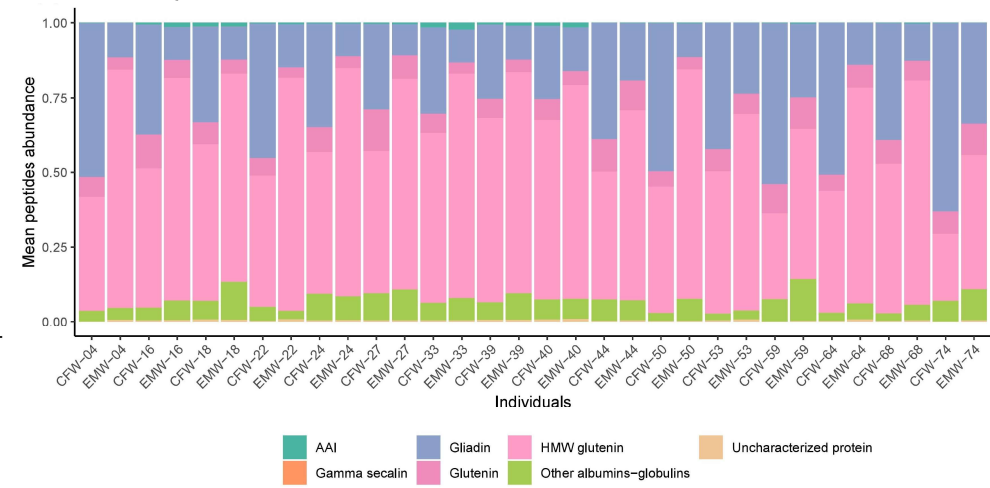
(1) What is the peptidomic landscape from bread digestion ?



Gastric peptides (987)



Jejunal peptides (117)



- The gastric and jejunal peptides containing proteins detected during digestion depends on the genotype and may be sensitive to environmental changes as is the storage protein composition of the flour



INRAE

17° ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(1) What is the peptidomic landscape from bread digestion ?



Epitope mapping analysis of the detected gastric and jejunal peptides



	Total	Celiac T-cell				Food allergen			
		Intact		Partial		Intact		Partial	
Gastric+jejunal	1104	33	3%	35	3%	168	15%	250	23%
Gastric	957	27	3%	11	1%	80	8%	174	18%
Jejunal	117	6	5%	24	21%	88	75%	76	65%

UB0

Number and percentage of intact and partially digested gastric and jejunal peptide mapped to

- **Celiac epitopes (blue)**
- **Food allergen epitopes (orange)**



INRAE

17^e ICC Internaional Cereal and Bread Congres. Nantes, France.2024

E. Bancel

Diapositive 18

UB0

Please check this word? Is it "jejunal" or "Jejujal"

Bose, Utpal (A&F, St. Lucia); 2024-04-19T01:12:57.108

(1) What is the peptidomic landscape from bread digestion ?



Epitope mapping analysis of the detected gastric and jejunal peptides



	Total	Celiac T-cell				Food allergen			
		Intact	3%	Partial		Intact		Partial	
Gastric+jejunal	1104	33	3%	35	3%	168	15%	250	23%
Gastric	957	27	3%	11	1%	80	8%	174	18%
Jejunal	117	6	5%	24	21%	88	75%	76	65%

UBO

Number and percentage of intact and partially digested gastric and jejunal peptide mapped to

- **Celiac epitopes (blue)**
- **Food allergen epitopes (orange)**

➤ More peptides are related to food allergen

➤ In %, more intact peptides for celiac epitopes in the gastric phase than in the jejunal suggesting partial hydrolysis

➤ In %, more partial peptides for food allergen epitopes in the gastric phase

➤ 5% still intact in the jejunum

➤ but 75% still intact in the jejunum



Diapositive 19

UB0

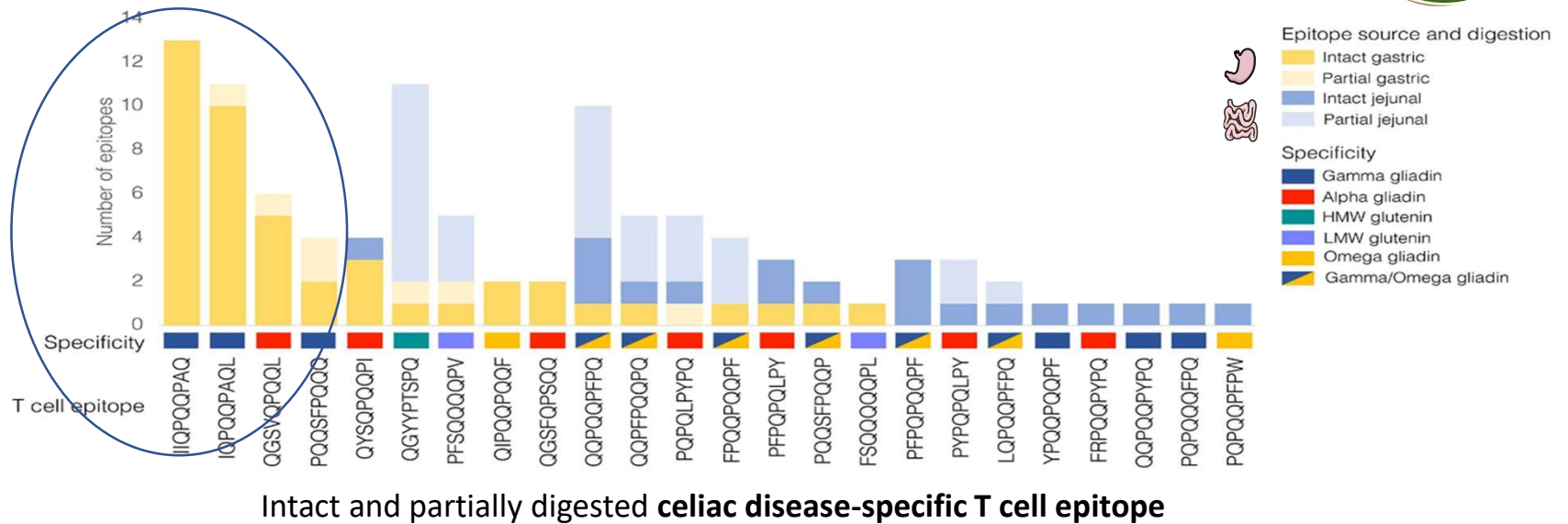
Please check this word? Is it "jejunal" or "Jejujal"

Bose, Utpal (A&F, St. Lucia); 2024-04-19T01:12:57.108

(1) What is the peptidomic landscape from bread digestion?



Epitope mapping analysis of the detected gastric and jejunal peptides



- The gastric peptides were enriched in intact, γ - and α -gliadin-specific T cell core epitope, which were digested in the intestinal phase



INRAE

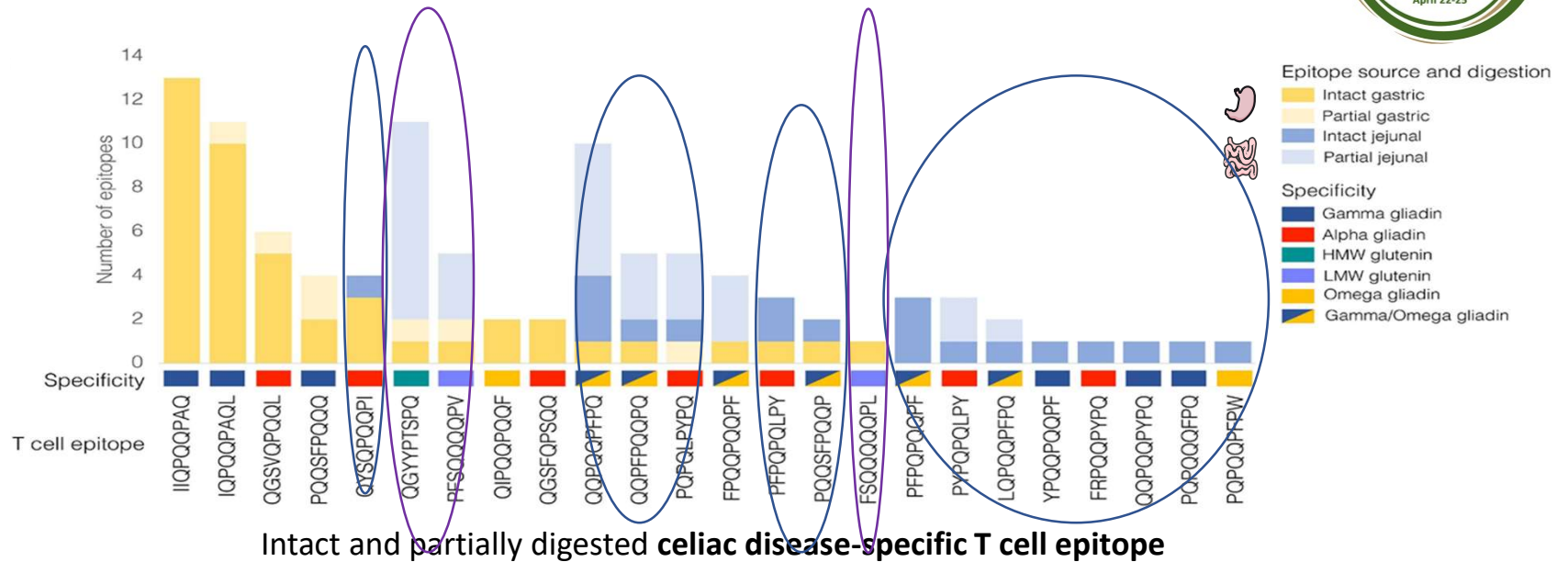
17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(1) What is the peptidomic landscape from bread digestion?



Epitope mapping analysis of the detected gastric and jejunal peptides



- The gastric peptides were enriched in intact, γ - and α -gliadin-specific T cell core epitope, which were digested in the intestinal phase
- All intact epitopes in the jejunum came from gliadins
- Epitope from glutenins did not reach intact the jejunum



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(1) What is the peptidomic landscape from bread digestion?

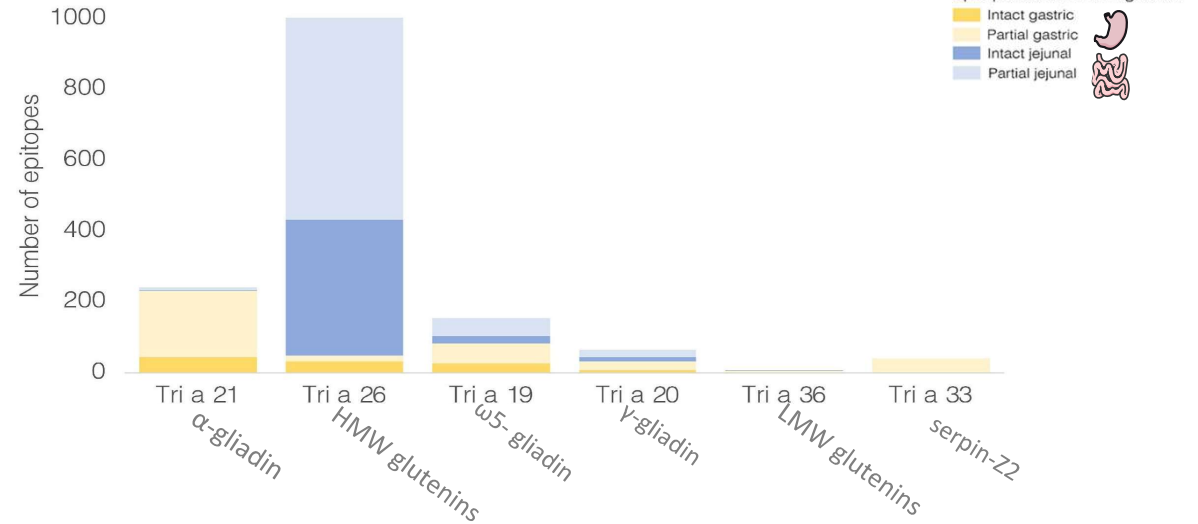


Epitope mapping analysis of the detected gastric and jejunal peptides



Compartment	Food allergen			
	Intact		Partial	
Gastric + Jejunal (n=1104)	168	15%	250	23%
Gastric (n=987)	80	8%	174	18%
Jejunal (n=117)	88	75%	76	65%

Number and percentage of gastric and jejunal peptides mapped to **food allergens epitopes**



- A large proportion of the HMW glutenins (Tri a 26) and a fraction of ω5- gliadin (Tri a 19) and γ-gliadin (Tri a 20) allergen epitopes are **intact in gastric and jejunal** fractions



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

The second question



(2) Is there a peptide signature to distinguish high- and low-digestible genotypes?



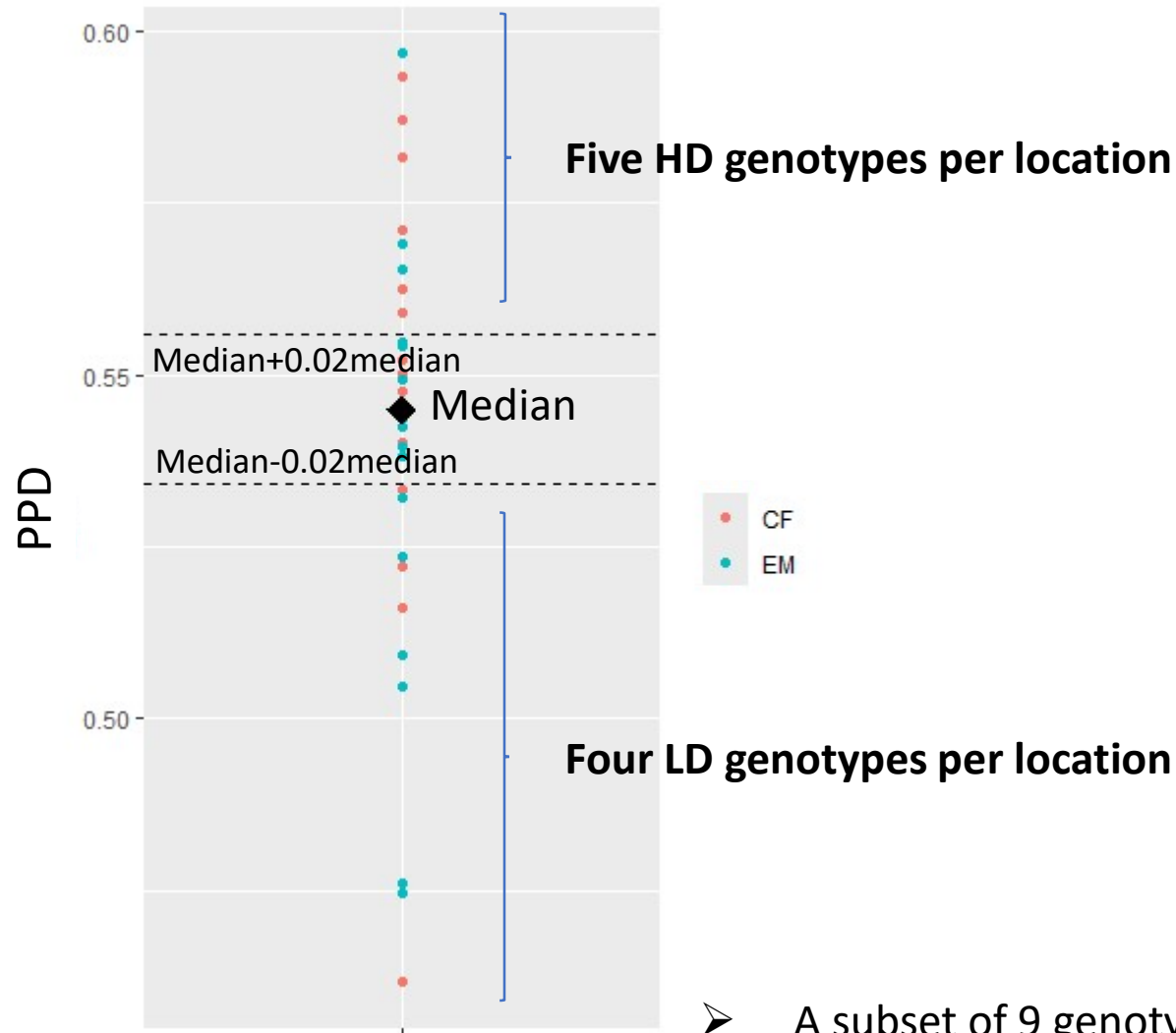
INRAE

17° ICC Internaional Cereal and Bread Congres. Nantes, France.2024

E. Bancel



Selected wheat cultivars



➤ A subset of 9 genotypes from the set used to describe the peptide landscape from the digestion

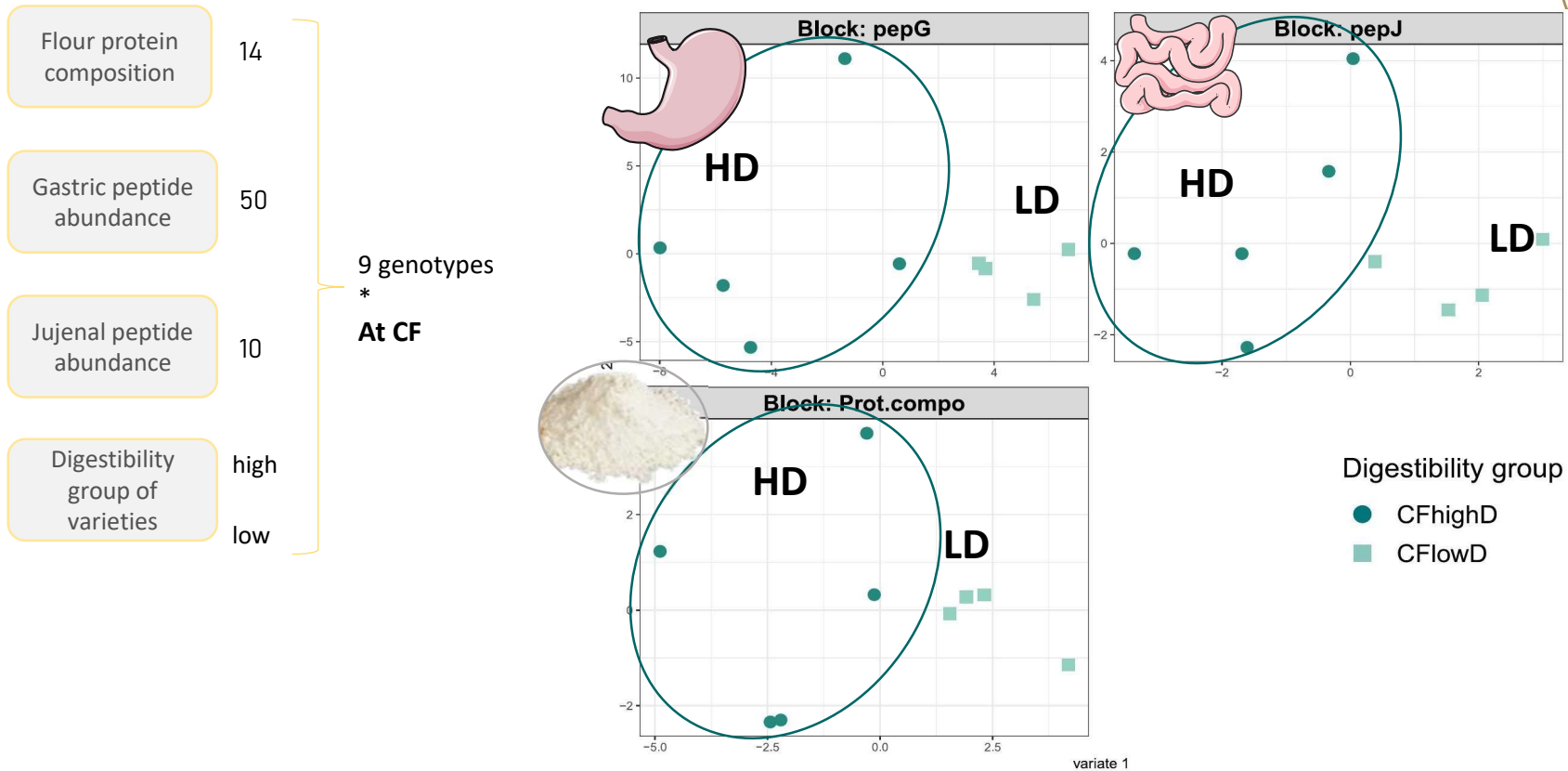


INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(2) Is there a peptide signature to distinguish high- and low-digestible genotypes?



- Gastric and jejunal peptides discriminated HD and LD genotypes
- Flour protein composition discriminated HD and LD genotypes

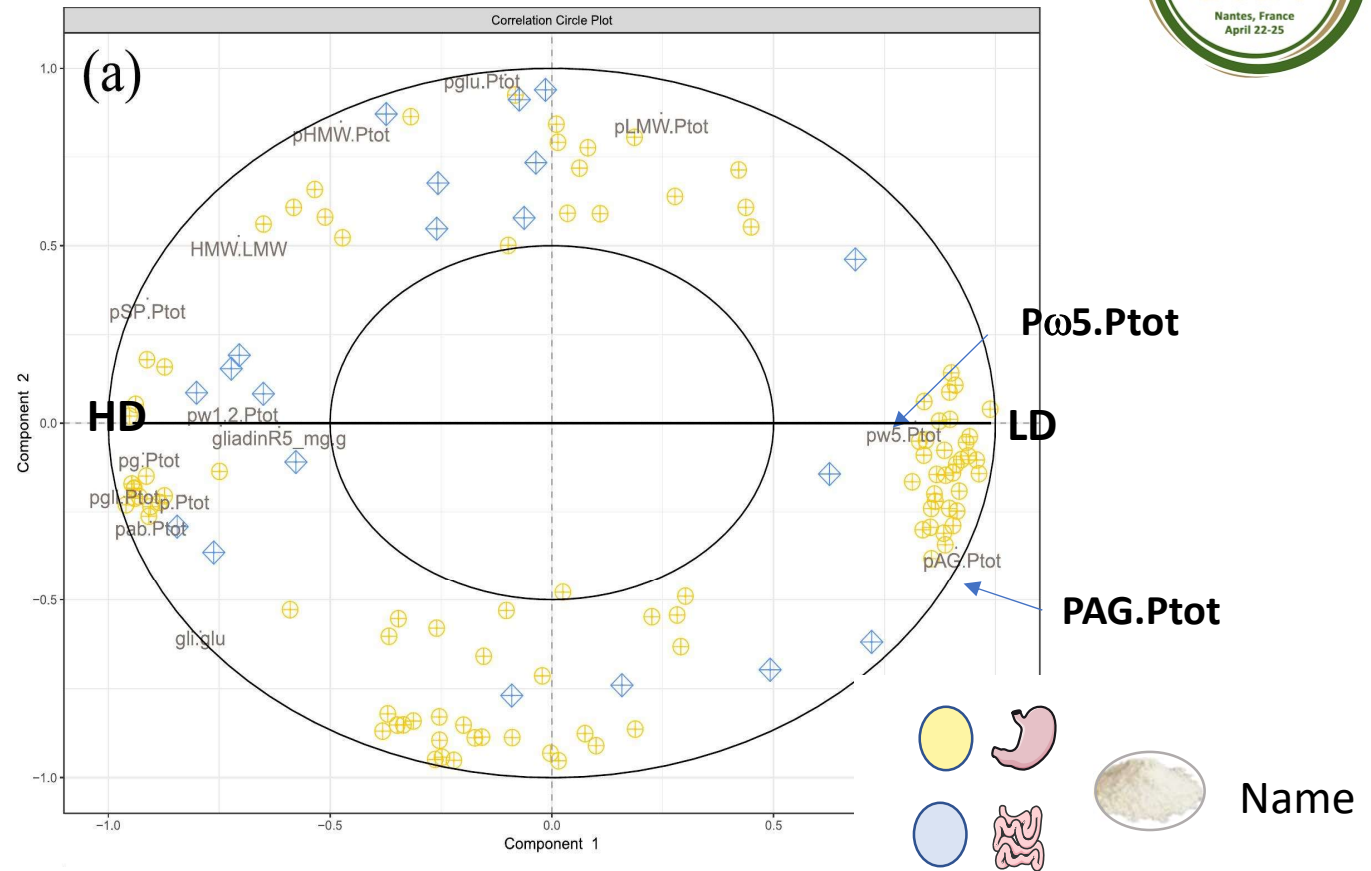


(2) Is there a peptide signature to distinguish high- and low-digestible genotypes?



- Flour protein composition 14
- Gastric peptide abundance 50
- Jujunal peptide abundance 10
- Digestibility group of varieties high low

9 genotypes *
At CF



➤ A high proportion of **albumins-globulins** and **ω 5-gliadins** in the flour were associated with **LD genotypes**

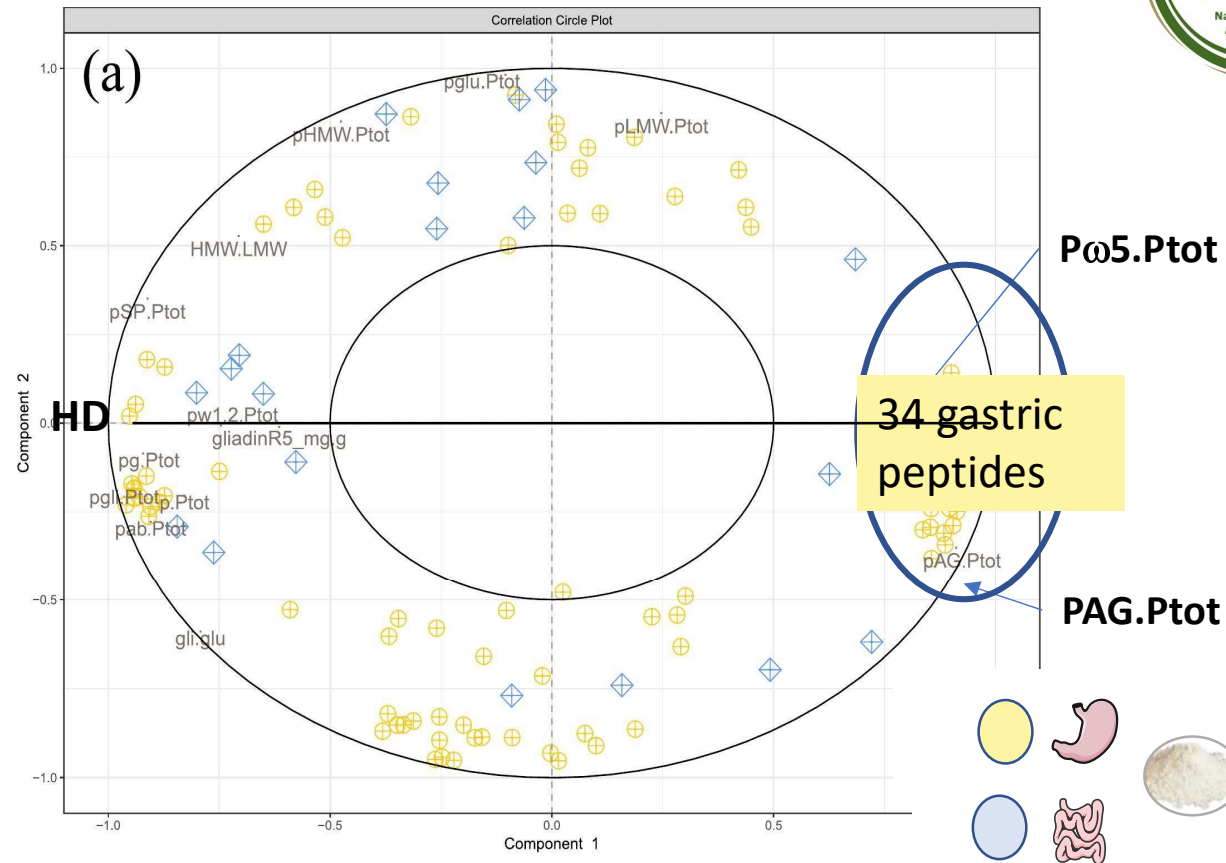


(2) Is there a peptide signature to distinguish high- and low-digestible genotypes?



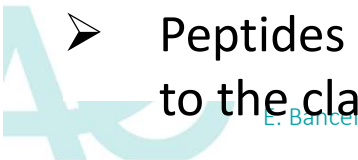
- Flour protein composition 14
- Gastric peptide abundance 50
- Jujenal peptide abundance 10
- Digestibility group of varieties high
- low

9 genotypes *
At CF



➤ A high proportion of **albumins-globulins** and **ω 5-gliadins** in the flour were associated with **LD genotypes**

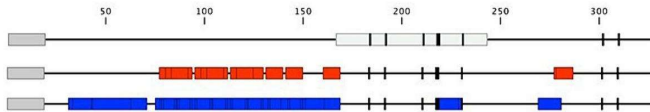
➤ Peptides projected with **one class of flour protein** did not necessarily map to the class of protein it was projected with



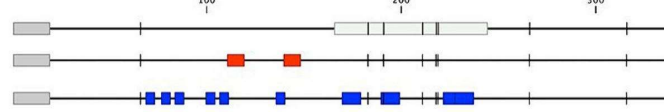
(2) Which peptides explain a low or a high digestibility cultivar?



Gamma gliadin (A0A4D6Q653)



LMW glutenin (Q6QGV8)



Pfam domains

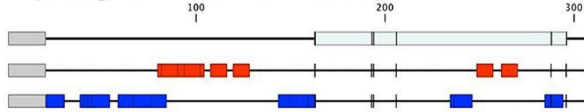
- Gliadin
- Tryp_alpha_amyl
- Glutenin_HMW

| Cysteine residue

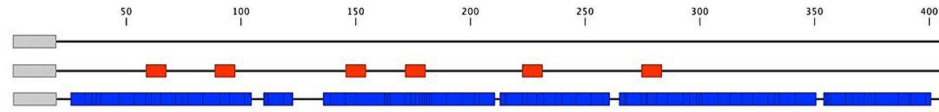
Epitopes

- CD T cell core
- Food allergy

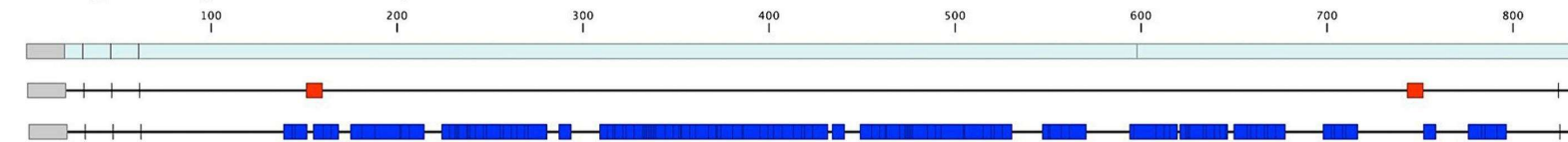
Alpha gliadin (A0A0K2QJJ9)



Omega gliadin (A0A2U8JD20)



HMW glutenin (A0A0H4PMT1)



→ Information relevant from the database with allergic and celiac epitopes
Immune Epitope Database and Analysis Resource (IEDB, <https://www.iedb.org/>).



INRAE

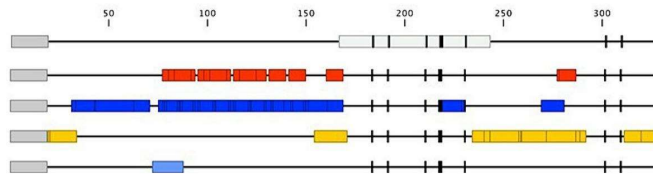
17° ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

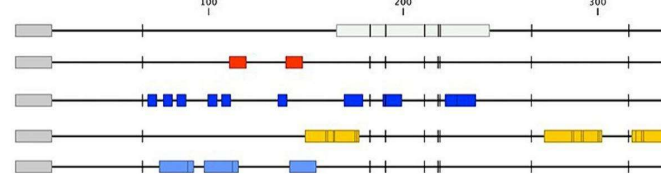
(2) Which peptides explain a low or a high digestibility cultivar?



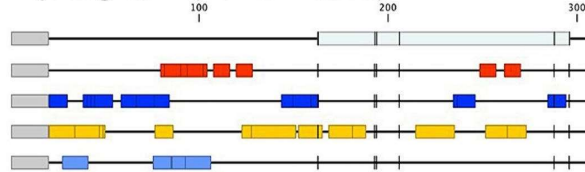
Gamma gliadin (A0A4D6Q653)



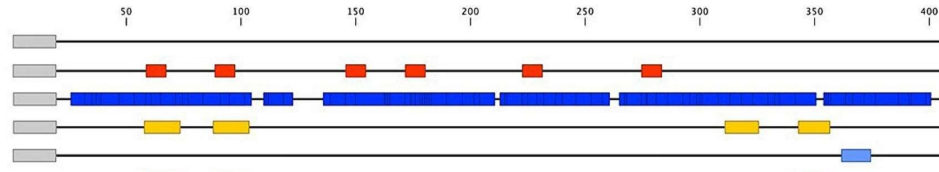
LMW glutenin (Q6QGV8)



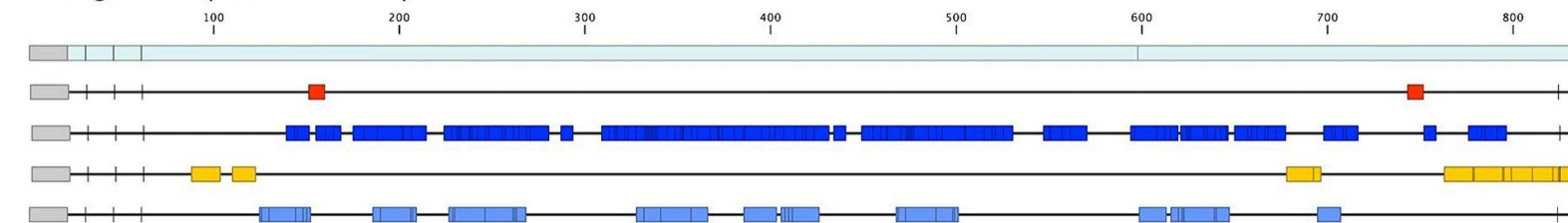
Alpha gliadin (A0A0K2QJJ9)



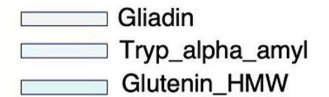
Omega gliadin (A0A2U8JD20)



HMW glutenin (A0A0H4PMT1)

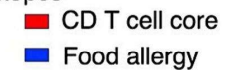


Pfam domains



| Cysteine residue

Epitopes

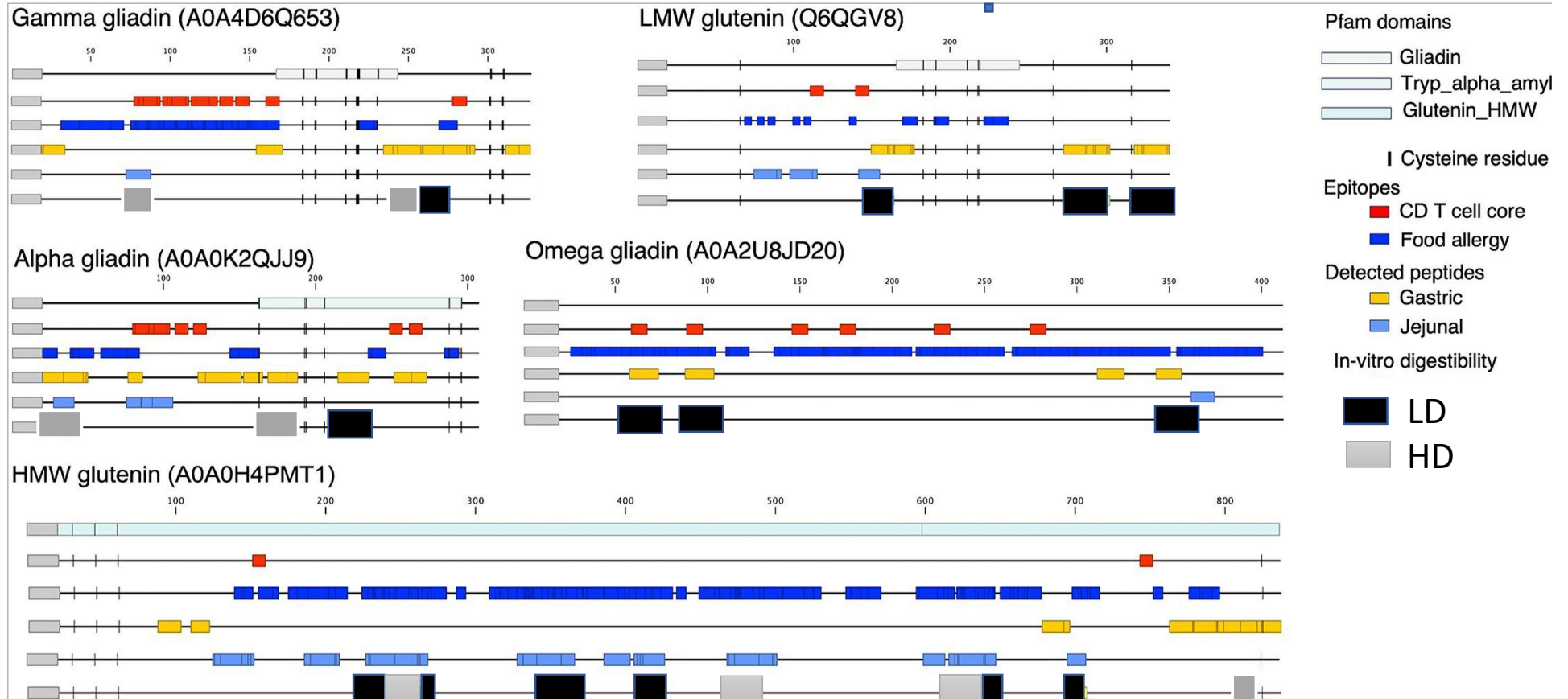


Detected peptides



- Gastric and jejunal peptides generally mapped either at the N- and C-termini or close to the conserved Pfam domains (not for jejunal peptides from HMW glutenin)
- Jejunal peptides more frequently mapped to the epitope-rich repetitive regions of storage proteins

(2) Which peptides explain a low or a high digestibility cultivar?



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

(2) Which peptides explain a low or a high digestibility cultivar?



- Peptides associated with high or low digestibility genotypes differed in abundance.
- They did not differ in mass, sequence length, amino acid composition or epitope diversity. The celiac or allergen epitopes were noted not to be higher in low-digestible genotypes.





More info:



Food Chemistry
Volume 448, 1 August 2024, 139148



Peptidomics analysis of in vitro digested wheat breads: Effect of genotype and environment on protein digestibility and release of celiac disease and wheat allergy related epitopes

Mélanie Lavoignat^a, Angéla Juhász^b, Utpal Bose^{b c}, Thierry Sayd^d, Christophe Chambon^d,
Miguel Ribeiro^{e f}, Gilberto Igrejas^{f g h}, Sébastien Déjeanⁱ, Catherine Ravel^a  ,
Emmanuelle Bancel^a

DOI: [10.1016/j.foodchem.2024.139148](https://doi.org/10.1016/j.foodchem.2024.139148)



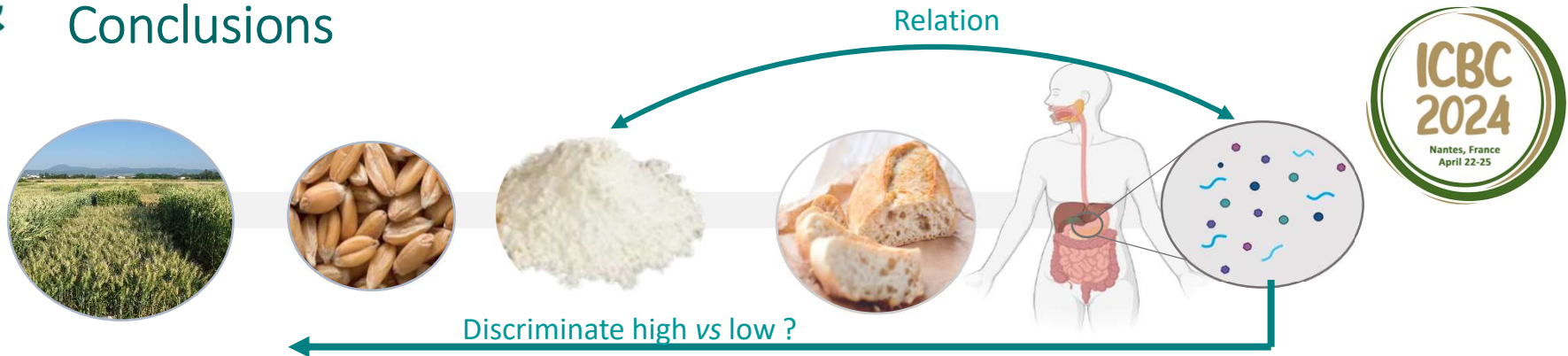
INRAE


17^e ICC International Cereal and Bread Congress. Nantes, France. 2024

E. Bancel



Conclusions



 This study established a **peptidomic and epitope diversity map** of digested bread and provided new insights and correlations between genotypes, digestibility and wheat sensitivities such as celiac disease and wheat allergy.

 No signature was found to characterize peptides from LD or HD genotypes.

However, we have to keep in mind that the digestibility of bread proteins is not only explained by the cultivar. The process induces protein modifications from flour to bread. Food processing can then determine their accessibility for enzymatic hydrolysis.

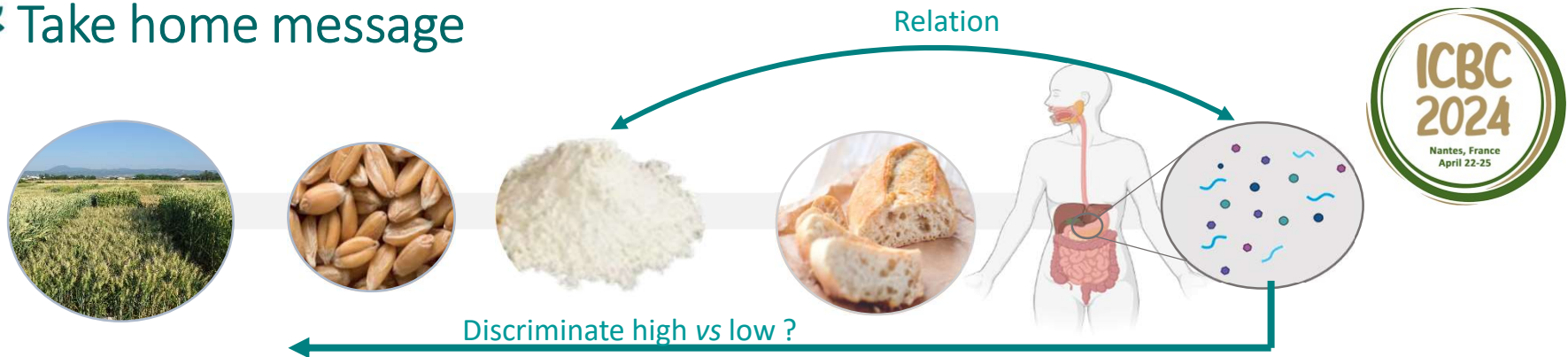


INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Take home message



- Peptidomic composition in the stomach is related to flour protein content and composition
- Digested peptide abundances vary between HD vs LD genotypes
- Fingerprint of celiac epitopes indicates they were intact in the gastric phase
- Peptides associated with LD genotypes were not enriched in epitopes



INRAE

17^e ICC International Cereal and Bread Congress. Nantes, France.2024

E. Bancel

Acknowledgments



**Mélanie Lavoignat, Thierry Sayd,
Christophe Chambon & Catherine Ravel**



Institut de mathématiques de Toulouse : **Sébastien Déjean**



University of Trás-os-Montes and Alto Douro : **Miguel Ribeiro & Gilberto Igrejas**



Edith Cowan University : **Angéla Juhász**



CSIRO : **Utpal Bose**

Funding

Research National Agency & French funds
to support Plant Breeding (FSOV)
GlutNsafe (2016Y)
ANR17-CE21-0009 GlutN

Australian Research Council
Centre of Excellence for
Innovations in Peptide and
Protein Science (CE200100012)
Coeliac Australia (G1005443)



17^e ICC International Cereal and Bread Congress. Nantes, France.2024
E. Bancel

Thank you for your attention



INRAE

17^e ICC International Cereal and Bread Congress
E. Bancel

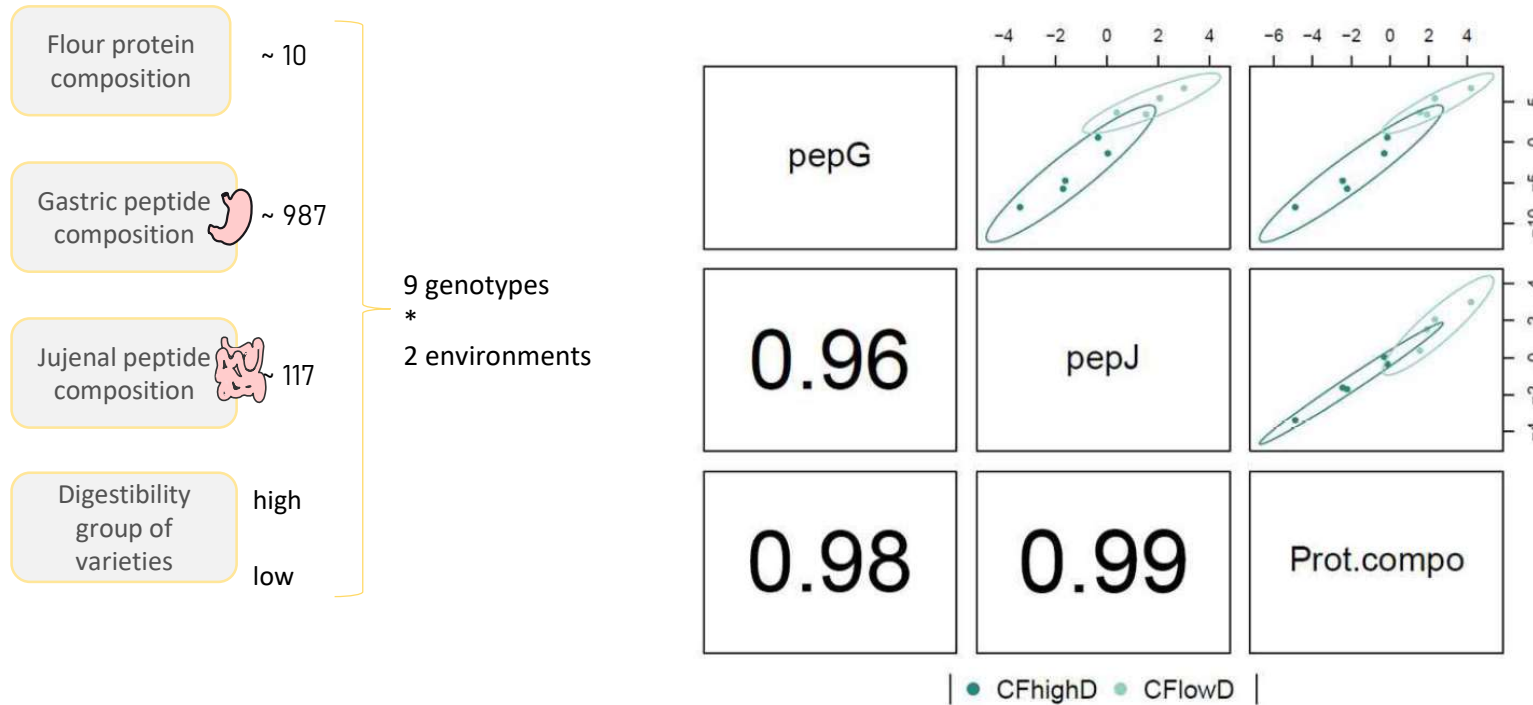
UNIVERSITÉ, France.2
Clermont
Auvergne



17th ICC International Cereal and Bread Congress
"Healthy Cereal diets from sustainable food systems"



(1) What is the peptidomic landscape from bread digestion ?

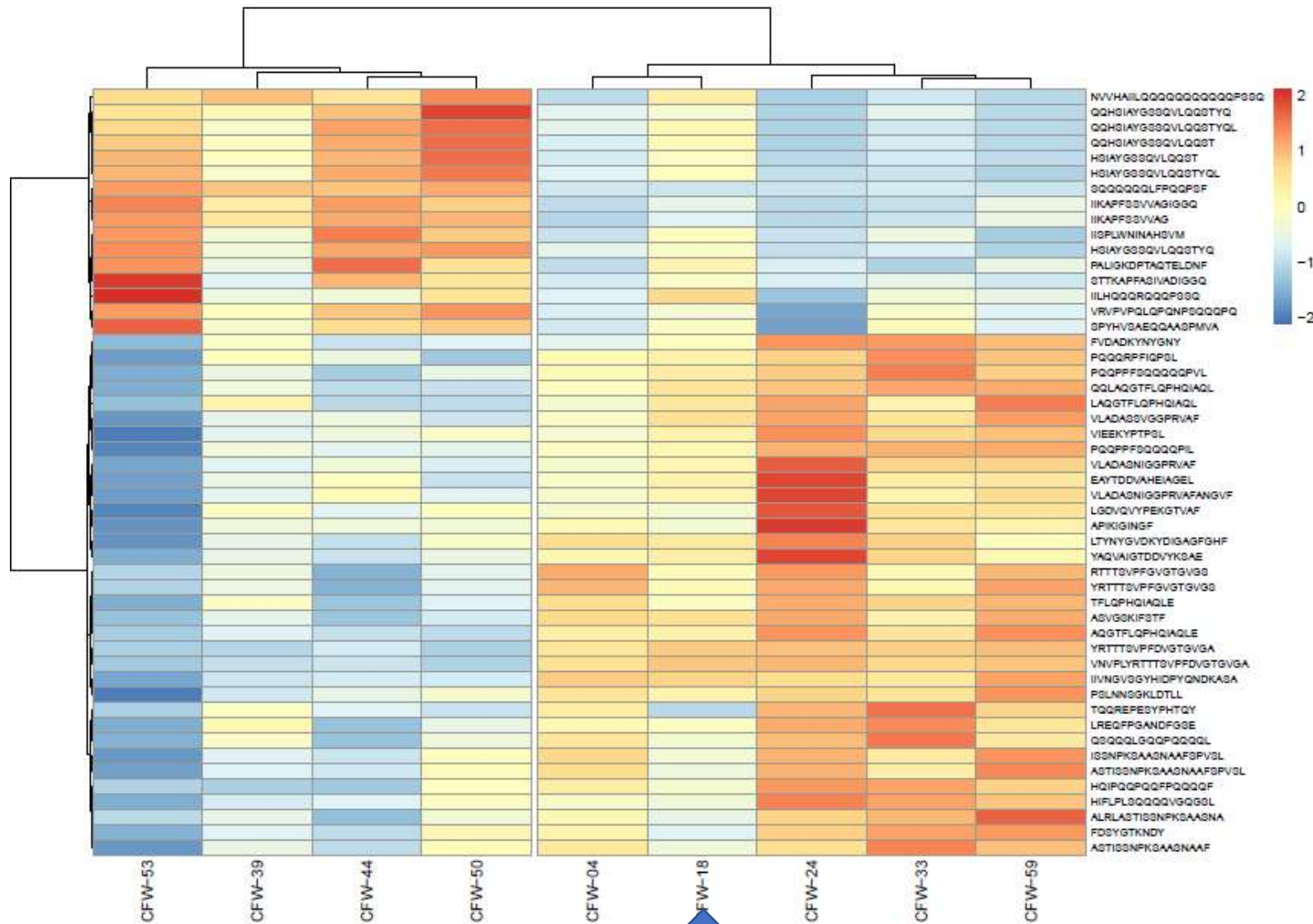


- The gastric and jejunal peptide abundances were strongly linked to flour protein content and composition



INRAE

(2) Is there a peptide signature to distinguish high- and low-digestible genotypes?



INRAE High Digestibility

Low Digestibility

17^e ICC International Cereal and Bread Congress, Nantes, France 2024

E. Bancel

