Structuring Food to Improve the Delivery of Bioactives

Didier DUPONT, French National Institute for Agricultural Research (INRA), Rennes, FRANCE
The National Institute for Agronomic Research

- Set up in 1946
- A public, scientific and technological establishment
- Under the joint authority of the Ministries of Agriculture and Research
- Staff of nearly 11000 and a budget of 700 M€
- Largest European organisation for agricultural research, 2nd largest in the world
- Working on Food, Nutrition, Agriculture and the Environment
Milk & Egg Science & Technology

Our disciplinary skills
- Biochemistry
- Microbiology/ Mol Biology
- Process & technology

Our facilities
- Mass spectrometry
- Confocal microscopy, AFM
- Quantitative PCR
- ITC, Biacore 3000
- Technology platform, Spray-drying tower
- Biological Resource Centre

Model systems

In situ systems

140 people
25 PhD students
7 private companies
Increasing our knowledge on food digestion to increase our knowledge on the effect of food on human health
Our goals

To model these phenomena in order to develop a reverse engineering approach

To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion

To determine the impact of the structure of food matrices on these mechanisms

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Models available at INRA for simulating digestion

In vitro static models (infant, adult)
- Dupont et al. 2010ab, Mol Nutr Food Res
- Minekus et al. 2014 Food Funct

In vitro dynamic models (infant, adult, elderly)
- Menard et al. 2014, Food Chem
- Sanchez et al. 2015 Food Res Int

In silico models
- Le Feunteun et al. 2014 Food Bioprocess Tech

Human models
- De Oliveira et al. 2016 Am J Clin Nutr
- De Oliveira et al. 2017 Clin Nutr

Animal models
- Barbé et al. 2013, 2014 Food Chem
- Le Huerou-Luron et al. 2016 Eur J Nutr

\[ \Phi_{12} = k_{12\text{whey}} \times (V_1 - m_{\text{whey}} \times \alpha) + k_{12\text{aggr}} \times m_{\text{aggr}} \times \alpha \]
Example 1
Food structure and delivery of bioactive peptides
The food matrices

Fat-free matrices: 40 g/L caseins, 10 g/L whey proteins, 95 g/L lactose and minerals + marker of the meal transit (Cr\(^{2+}\)-EDTA) → Gastric emptying half-time
The multi-canulated mini-pigs

6 minipigs (20 ± 1kg)

1 catheter: abdominal aorta

6 minipigs × 6 matrices × 8 sampling times after ingestion = 288 plasma samples collected

2 cannulas: end of stomach and mid-jejunum

6 minipigs × 6 matrices × 8 sampling times after ingestion × 2 sampling sites = 576 effluent samples collected
Impact of food structure on gastric emptying half-time

- Ultra Low Heat powder
- Unheated milk ("raw" milk) → 96 min → Rennet gel, pH 6.6
- Heated milk → 352 min → Rennet gel, pH 6.6
- 96 min → 148 min → Acid gel, pH 4
- 124 min → Stirred acid gel, pH 4

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**Milk proteins in the duodenum (ELISA)**

**Casein**
- Intense and early peak with milk/ lower and delayed with gels
- Intermediate behaviour with stirred gel
- Low concentrations with rennet gel but casein release tends to increase over time

**β-Lg**
- Only traces of milk proteins found in the jejunum
- Dairy products remain highly digestible

Barbé et al. 2013, 2014 Food Chem
The liquid-gel transition

2) effect on absorption

- milk gelation: → delayed proteins transit → delayed AA absorption
- maximal AA concentration in the plasma

3) potential effect on satiety

ghrelin (gastrointestinal hormone → appetite stimulation)

Kinetics of proteolysis and amino acid bioavailability are driven by gastric emptying
Bioactive peptides released during digestion differ from one matrix to another.

More than 16,000 milk peptides identified in the gastrointestinal tract of pigs.

<table>
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- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
  - Kinetics of release are different

But identifying a bioactive peptide in the lumen does not prove that it will exert a biological action!

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**Protein Sequence Activity Reference**

4 20 50 105 165 225 315

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**Acid Gel**

**Rennet Gel**

- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
  - Kinetics of release are different

**Barbé et al. 2014**

**Food Res Int**
Example 2
Food structure and delivery of phytochemicals
Development of bioactive-enriched food to fight the risk of developing a metabolic syndrome

6 M€ European project  
2015-2019  
26 partners

Development of BEF

3 egg products  
3 dairy products  
Synergism

+ AC  + BG  + DHA  
+ DHA + AC  + DHA + BG

(40 mg AC) (Yubero et al, 2012)  
(250 mg DHA) (EFSA, 2010)  
(3 g BG) (EFSA)

Sensory analysis

2 egg products  
2 dairy products

Selection of BEF

1 egg product  
1 dairy product

Characterization of the BEF

Stability of bioactives  
Microbiological safety  
Nutritional profile

Large multicentric 3-month intervention study (250 volunteers)
**In vitro** digestion to determine the effect of the food matrix on the delivery of anthocyanins

**Oral phase**
- Mix 1:1 with Simulated Salivary Fluid (SSF) and saliva amylase (75 U/mL)
- 2 min, pH 7

**Gastric Phase**
- Mix 1:1 with Simulated Gastric Fluid (SGF) and pepsin (2000 U/mL)
- 2h, pH 3

**Intestinal Phase**
- Mix 1:1 with Simulated Intestinal Fluid (SIF) and enzymes
  - Pancreatin (based on trypsin 100 U/mL) or pure enzymes
  - Bile (10mM)
- 2h, pH 7

**Infogest consensus in vitro digestion model**

**PATHWAY-27**

+ AC Dairy and egg matrices

Control solution
Soluble = Bioaccessible

Almost no AC were degraded

Stable but insoluble

Pineda-Vadillo et al. 2015 *Food Res Int*

Food matrix affects AC bioaccessibility

Protective effect of the food matrix towards intestinal digestion of AC
Example 3
Food structure and delivery of DHA
FOOD MATRICES
Same composition but different structure

OMELETTE: Egg yolk and egg white MIXED and WELL COOKED

HARD-BOILED EGGS: Egg yolk and egg white NOT MIXED and WELL COOKED

MOUSSE: FOAMED RAW Egg white + RAW egg yolk. MIXED

Egg yolk: egg white proportion as in real eggs
500g /intake 1.74 g of DHA

Recovery DHA after cooking
Omelet (99.4%) Egg (104%) Mousse (91.7%)
DHA bioaccessibility and bioavailability

**Duodenum**

- **OMELET**
- **MOUSSE**
- **EGG**

**Plasma**

- **OMELET**
- **MOUSSE**
- **EGG**

DHA bioavailability (Area Under the Curve) : Mousse = Hard-boiled Eggs < Omelette (p<0.005)

The food matrix regulates DHA bioavailability
Improving health properties of food by sharing our knowledge on the digestive process

International Network

Dr. Didier DUPONT, Senior Scientist, INRA, France

June 2011 – May 2021
350 scientists - 130 institutes – 38 countries
Chair
Didier Dupont - France

Vice-chair
Alan Mackie - UK

**In vitro/in vivo correlations**
WG1

**In vitro semi-dynamic model of digestion**
WG2

**Models for specific populations**
WG3

**Digestive lipases and lipid digestion**
WG4

**Digestive amylases and starch digestion**
WG5

**In silico models of digestion**
WG6

**The “Mind-the-Gap” group**

Didier Dupont
Alan Mackie
Uri Lesmes
Myriam Grundy
Nadja Siegert
Choi-Hong Lai
Guy Vergeres

www.cost-infogest.eu
We are pleased to announce the next

6th International Conference on Food Digestion

in Granada, Spain, April 2019
Key Takeaways

► Understanding the mechanisms of food digestion is a necessary step to strengthen our knowledge on the relationships between food and human health.

► The effect of food structure on bioactive and nutrient bioavailability can only be demonstrated by comparing foods with identical composition (but different structure).

► Food structure drives the kinetics of nutrient/bioactive release in the GI tract and the bioavailability. Gastric emptying can be a key parameter.

► An international effort is needed to harmonize the digestion models used by the scientific community.
The Bioactivity & Nutrition team

**Head**
Didier DUPONT - Senior Scientist

**Scientists**
- Rachel BOUTROU – Junior Scientist
- Amélie DEGLAIRE – Lecturer
- Juliane FLOURY – Lecturer
- Catherine GUERIN - Lecturer
- Joëlle LEONIL -- Senior Scientist
- Françoise NAU – Professor
- Frédérique PEDRONO – Lecturer
- Jonathan THEVENOT – Post-doc

**PhD students**
- Lucie LORIEAU (2016-2019)
- Linda LEROUX (2016-2019)
- Manon HIOLLE (2016-2019)
- Yohan REYNAUD (2016-2019)

**Technicians**
- Gwenaëlle HENRY
- Yann LE GOUAR
- Nathalie MONTHEAN

**Engineers**
- Julien JARDIN
- Olivia MENARD
- Jordane OSSEMOND

**Masters students**
- Lucie LORIEAU
- Linda LEROUX
- Manon HIOLLE
- Yohan REYNAUD
Thank you for your kind attention!