Structuring foods to improve the delivery of bioactives and nutrients

Dr Didier DUPONT

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
- Second largest French public research organisation with a staff of nearly 11000 and a budget of 680 millions euros
- Largest European organisation for agricultural research
Multidisciplinary and multi-scale approaches, reinforced by two high-caliber facilities

Dairy Platform (Iso 9001)  Biological Resource Center (Iso 9001)

145 staff  25 PhD students

- Structuration / disintegration mechanisms of food matrix: from structural characterization to digestion
- Dairy processing and cheese making: toward sustainable dairy systems
- Microbial interaction: food matrix and host cell

Please visit http://www6.rennes.inra.fr/stlo_eng
By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health.
Our goals

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

Bioactivities
- Bioactive peptides
- Amino acids
- Fatty acids
- Minerals…

Gut Immune System
The digestive process

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**The Gastric Phase**

- **α-amylase**: Chewing and deglution
- **esophagus**: Storage, grinding and mixing in the stomach
- **stomach**: Pepsin, Gastric lipase, HCl, Fasted pH 1.3-2.5
- **duodenum**: pH 6.5-6.8
- **jejenum**, **ileum**: Small intestine
- **large intestine**: Nutrient absorption, Intestinal transit
- **blood**: Nutrient absorption

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**Gastric Phase**

Gastric phase = a very complex but crucial step for the whole digestion process

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*From Roger Lentle, Massey Univ. NZ*
The structure of dairy products modulate the bioavailability of dietary amino acids

Barbé F.¹, Ménard O.¹, Le Gouar Y.¹, Buffière C.², Famelart M.-H.¹, Laroche B.³, Le Feunteun S.⁴, Rémond D.² and Dupont D.¹

¹ INRA STLO Rennes, France
² INRA UNH Clermont-Ferrand, France
³ INRA MIA Jouy-en-Josas, France
⁴ INRA GMPA Grignon, France
Objective: to compare kinetics of digestion of dairy products of identical composition but different structure

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) → Mean Retention Time in the stomach
Time of residence in the stomach

Ultra Low Heat powder

unheated milk ("raw" milk)

96 min

heated milk

96 min

148 min

124 min

352 min

? min

rennet gel

pH 6.6

pH 6.6

pH 4

pH 4

stirred acid gel
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

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

β-lg
Bioactive peptides released during digestion differ from one matrix to another

More than 4000 peptides were identified in the gut lumen!!

<table>
<thead>
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<th>Protein</th>
<th>Sequence</th>
<th>Activity</th>
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<td>1-23</td>
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<td>Mullally et al. (1997)</td>
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</tbody>
</table>

- 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
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**

gghrelin (gastrointestinal hormone → appetite stimulation)

- milk gelation:
  - postprandial ghrelin concentration = satiety?

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Barbé et al. Food Chem 2013
Highly cited paper
In silico model of transit and absorption

Better understanding of the food behaviour in the stomach

Predictive model??

Le Feunteun et al. Food Bioprocess Tech 2014
Differential behaviour of acid/rennet gels in gastric conditions

- Acid/Rennet gel: identical composition, similar rheological properties and pore size
- Time of residence in the stomach (Acid 148 min /Rennet 352 min)
- How can we explain this difference? Dynamic in vitro digestion of the 2 gels

Ménard et al. Food Chem 2014

DIDGI® StoRM® software

- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl

Emptying : Elashoff’s model

Stomach

- Pancreatin
- Bile
- Simulated intestinal fluid
- NaHCO₃

Small intestine

Emptying : Elashoff’s model
Behaviour of acid and rennet gels in the stomach during *in vitro* dynamic digestion

Formation of a strong coagulum with rennet gel $\rightarrow$ slow down the gastric emptying of caseins

The structure that a food adopts in the stomach is essential to understand its digestion
Understanding the mechanisms of dairy gel particles degradation in the stomach

Floury J.¹, Cardoso Bianchi T.L.¹, Thévenot J.¹, Dupont D.¹, Jamme F.², Lutton E.³, Panouillé M.³, Boué F.³, Le Feunteun S.⁴

¹ INRA STLO Rennes, France
² SOLEIL Synchrotron Gif-sur-Yvette, France
³ INRA GMPA Grignon, France
Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter.

DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light.

✿ Allow the imaging of protein intrinsic fluorescence with a UV microscope.
Kinetics of gel particles disintegration: comparison of rennet/acid gel
Can the food structure be used to control the delivery of bioactives?

Pineda-Vadillo C., Le Gouar Y., Menard O., Guerin-Dubiard C., Nau F., Dupont D.

INRA STLO Rennes, France
Evaluate the effect of the food matrix structure on the release and bioavailability of DHA during *in vivo* digestion.

<table>
<thead>
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<th>SANG</th>
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<tr>
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<td>+24 h</td>
<td>+48 h</td>
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**YUGULAR CATHETER**

**DUODENAL CANULE**

DHA bioavailability

De visu characterization
Proteolysis
DHA quantification

pH
Food matrices of identical composition but different structure

1. OMELETTE (Egg yolk and egg white MIXED and COOKED)

2. HARD BOILED- EGGS (Egg yolk and egg white NOT MIXED and COOKED)

3. MOUSSE (FOAMED RAW Egg white mixed with RAW egg yolk)

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

DHA recovery after cooking: Omelette (99.4%) Boiled-eggs (104%) Mousse (91.7%)
Quantification of DHA in plasma

Delivering DHA through omelette increases DHA bioavailability
Infant formulas
Can we create lipid structures biomimetic of the native fat globule?

Le Huërou-Luron I.\textsuperscript{1}, Bouzerzour K.\textsuperscript{2}, Ferret-Bernard S.\textsuperscript{1}, Ménard O.\textsuperscript{2}, Le Normand L.\textsuperscript{1}, Perrier C.\textsuperscript{1}, Le Bourgot C.\textsuperscript{1}, Jardin J.\textsuperscript{2}, Bourlieu C.\textsuperscript{2}, Carton T.\textsuperscript{3}, Le Ruyet P.\textsuperscript{4}, Cuinet I.\textsuperscript{4}, Bonhomme C.\textsuperscript{4}, Dupont D.\textsuperscript{2}

\textsuperscript{1} INRA ADNC Rennes, France
\textsuperscript{2} INRA STLO Rennes, France
\textsuperscript{3} BIOFORTIS, Saint-Herblain, France
\textsuperscript{4} LACTALIS, Retiers, France
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

**Formula T1**
- Interface 100 % Proteins
- 100% vegetable oil

**Formula T2**
- Interface 100 % phospholipids
- 100% vegetable oil

**Formula T3**
- Interface 100 % phospholipides
- 40% vegetable oil + 60% milk fat

*Natural milk fat globules* (0.2 – 10 μm, mean diameter ~ 4 μm)

**Milk fat globule membrane (MFGM)**
- Xanthine oxidase
- Phospholipids
- Glycosylated polypeptide
- Butyrophilin

**Milk fat globule membranes (MFGM)**
- Electrostatic potential: -11 to -13 mV
Can the composition of Infant Formula modulate the physiological response of the neonate?

**Automatic meal delivery** (10 meals/day)

- **Veg**
- **Veg + PL**
- **Dairy Fat + PL**

**Rehydration at 20%**

**Mesenteric Lymph Nodes (MLN)**

**Collect of effluents and tissues**

**Proximal Jejunum**

**Median Jejunum**

**Ileum**

**Effluents:**
- SDS-PAGE
- Elisa

**Tissues:**
- Morphometry
- Enzyme Activities
- Intestinal Permeability
- Local immune response
- Microbiota

**Slaughtering after**
- 7 days
- 28 days
  (90 min postprandial)

**+ Mother-fed piglets**
**MF = + control**
Protein Digestion

Milk Proteins better resist to intestinal digestion in the presence of dairy fat

⇒ Modification of the interface

(Granger et al 2005; Davies et al, 2001)
Secretory activity of MLN

Interferon-γ (Th1 pro-inflammatory)

Interleukine-10 (Th2 anti-inflammatory)

Milk lipids ➔ maturation of the piglet’s immune system more similar than with sow’s milk

The composition/structure of the infant formula «orientates» the microbiota

More Proteobacteria with milk fat /
More Firmicutes with plant oil

Conclusion

Food structure regulates the kinetics of digestion and the release of nutrients and bioactives in the bloodstream.

Gastric emptying rate will highly depend on the structure that the product will adopt in the stomach cavity.

Understanding the mechanisms of food particle breakdown in the stomach is critical to control the structure a food will adopt in gastric conditions.

Being able to design food structures for controlling the kinetics of hydrolysis of macronutrients will allow to obtain food particularly adapted to specific population.

- Overweight/diabetic
- Elderly/Athletes

Release Rate
The Bioactivity & Nutrition team

Head
Didier DUPONT - Senior Scientist

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

Technicians
Gwenaëlle HENRY
Yann LE GOUAR

Engineers
Julien JARDIN
Olivia MENARD
Jordane OSSEMOND

PhD students
Samira de OLIVEIRA (2013-2016)
Lucie LORIEAU (2016-2019)
Linda LEROUX (2016-2019)
Manon HIOLLE (2016-2019)
Yohan REYNAUD (2016-2019)

Masters students


Improving health properties of food by sharing our knowledge on the digestive process

COST Action FA1005

Dr. Didier DUPONT, Senior Scientist, INRA, France

INFOGEST

June 2011 – May 2015
340 scientists - 130 institutes – 37 countries
We are pleased to announce the next

5th International Conference on Food Digestion

in Rennes, France, 4-6 April 2017