Lipid and protein release during in vitro digestion of different cheese types

Sylvie Turgeon
STELA Dairy Research Centre
Institute of nutrition and functional foods
Science enhancing nutrition!

INAF
Institute on nutrition and functional foods

STELA Dairy Research Centre
The expertise

Biomolecules

- Cytotoxicity
- Embryo culture
- Antioxydant capacity, antimutagenic activity
- Stimulation/immune system, inflammatory response
- Antimicrobial and antiviral action
- CACO cells
- Animal studies (diabetes, obesity, cardiovascular disease, neurology…)

-omic technologies

Technologies and processes

- TNO dynamic gastrointestinal model

Clinical trials in nutrition
Menu

• Food structure and nutritional properties
• Role of digestion process
• Examples:
  • Milk proteins
  • Yoghurt
  • Cheeses...

• Conclusion:
  • Consider food in addition to nutrient content...
Food structure and nutritional properties: Almonds...

Physical form influences nutrients digestion and absorption

Role of mastication:
availability of nut lipids is largely dependent on the mechanical fracture of their cell walls
Fecal fat excretion was significantly higher after 10 chews than after 25 and 40 chews (both $P < 0.05$)

Roasting of almonds leads to differences in the distribution of protein in the stomach and to the gastric emptying of protein

Cassidy et al 2009 Am J Clin Nutr 89: 794-800
Food Matrix impact on macronutrients nutritional properties:

study for delivery system development but also understanding traditional food nutrients release

Turgeon and Rioux, 2011 Food Hydrocolloids 25: 1915-1924
Digestion process vs Metabolic Responses

Bioaccessibility

Glycemia/insulinemia

Protein use for muscle

Effect on satiety
Rate of food digestion - examples

- Influenced by the protein type

Rate of appearance of total leucine in plasma

△ Whey protein

- Casein

Fast vs. Slow protein

Boirie et al, 1997
Rate of food digestion- examples

• Influenced by the protein type
• Food matrix impact

Food matrix: interactions between components
  - as found naturally;
  - influenced by processing steps.
Rate of food digestion - examples

- Influenced by protein type
- Food matrix impact

Rate of appearance of leucine in plasma

Barbé et al. Food Chem. 136: 1203
**Satiating properties of yogurt formulation: Yogurts composition (in vitro / in vivo)**

<table>
<thead>
<tr>
<th>%</th>
<th>CTRL 2.8:1</th>
<th>C+WP 1.5:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solid</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Fat</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proteins</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Casein</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>WP</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Fibre</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Criteria:**

- Close to commercial products
- Sensory properties (taste, appearance, viscosity)
- Products stability (syneresis, gel formation)
Satiating properties of yogurt formulation

- 20 healthy men
- Age: 32.4 ± 9.1 years
- BMI: 20-30 kg/m²
- Stable weight (6 months preceding the study)
- No metabolic disease and use of medication susceptible to interfere with the outcome of the study.
Subsequent food intake after ad-libitum buffet when yogurts were consumed as a snack

- Increased level of WP-induced satiety
- The total caloric intake (meal+snack) was negative

Is it related to a different digestion pattern?  Or ≠ AA content
**In vitro digestion model**

**Digestion steps (37 °C)**

- 9g of dairy products
  - Oral (2 min)
    - 12mL saliva
  - Gastric (60 min)
    - 24mL gastric solns
  - Duodenal (60 min)
    - 24mL duodenal solns
    - 12mL bile
    - 4mL NaHCO$_3$

**Sample analysis**

- Matrix degradation
  - After centrifugation
    - Pellet
    - Supernatant
  - Protein solubilization
Composition of the pellet at the end of the gastric and duodenal digestion steps (in vitro)

<table>
<thead>
<tr>
<th>Protein and dry matter weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastric</strong></td>
</tr>
<tr>
<td>C+WP</td>
</tr>
<tr>
<td>CTRL</td>
</tr>
<tr>
<td><strong>Duodenal</strong></td>
</tr>
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<td>C+WP</td>
</tr>
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www.ulaval.ca
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<th>Duodenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C+WP</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>CTRL</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>C+WP</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>CTRL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What about Cheese?
Reduced 5% increase in Energy

**Source of SFA**

- Mixed: -17%
- Plant: -38%
- Butter: -17%
- Meat: +48%
- Dairy: -38%
- Total: -29%

**Hazard Ratio for CVD**

Reduced ↔ Increased

*De Oliveira Otto et al Am J Clin Nutr 2012;96:397–404*

*Follow-up 10 yrs*
Cheese vs. butter lowers LDL-C

13% of energy from cheese or butter

# Cheese vs. butter lowers LDL-C

**TABLE 1**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Cheese</th>
<th>Butter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum 4:0–12:0</td>
<td>14.7</td>
<td>14.3</td>
</tr>
<tr>
<td>14:0</td>
<td>10.5</td>
<td>10.7</td>
</tr>
<tr>
<td>16:0</td>
<td>27.1</td>
<td>29.2</td>
</tr>
<tr>
<td>18:0</td>
<td>11.6</td>
<td>10.7</td>
</tr>
<tr>
<td>18:1n–9²</td>
<td>20.9</td>
<td>20.3</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>18:3n–3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Others</td>
<td>10.9</td>
<td>10.5</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td>834</td>
<td>19</td>
</tr>
</tbody>
</table>

1. Analyzed by Qlip, Leusden, Netherlands.
2. Including 18:1trans12.
Whole foods vs. nutrients

- Fat
- Minerals
- Protein
- Vitamins
- Carbs
- Bacteria
- Sterols
- Moisture

Food structure
Goal of our work

Understand the role of dairy products composition and microstructure on food matrix disintegration and the kinetics of protein/lipid digestion using an *in vitro* digestion model

1- comparison of commercial cheeses digestion
2- impact of Ca and lipid contents
Cheese digestion experiments

Commercial cheeses:

- Cheddar (C)
- Light Cheddar (LC)
- Mozzarella (M)
- Light Mozzarella (LM)

Cheese particle size
2.4 ± 0.5 mm before digestion
# Composition and Texture of cheeses

<table>
<thead>
<tr>
<th>Cheeses</th>
<th>C</th>
<th>LC</th>
<th>M</th>
<th>LM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>33.5  \textsuperscript{a}</td>
<td>21.7  \textsuperscript{b}</td>
<td>20.0  \textsuperscript{c}</td>
<td>14.4  \textsuperscript{d}</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>23.4  \textsuperscript{b}</td>
<td>29.1  \textsuperscript{a}</td>
<td>28.4  \textsuperscript{a}</td>
<td>30.3  \textsuperscript{a}</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>7.9   \textsuperscript{c}</td>
<td>18.7  \textsuperscript{a}</td>
<td>13.3  \textsuperscript{b}</td>
<td>18.6  \textsuperscript{a}</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.5   \textsuperscript{b}</td>
<td>0.7   \textsuperscript{a}</td>
<td>0.7   \textsuperscript{a}</td>
<td>0.7   \textsuperscript{a}</td>
</tr>
</tbody>
</table>

Letters: significantly different $p<0.05$
**In vitro digestion model**

**Digestion steps (37 °C)**
- 18g of dairy products
  - Oral (2 min)
    - 12mL saliva
  - Gastric (120 min)
    - 24mL gastric solns
  - Duodenal (60 min)
    - 24mL duodenal solns
    - 12mL bile
    - 4mL NaHCO₃

**Sample analysis**
- After centrifugation
  - Pellet
  - Supernatant
- Matrix degradation
- Protein solubilization

Shear rate at 30 s⁻¹
Disintegration of cheese at end of each digestion steps

- Cheese disintegration was slower with lower fat content in cheese
- Cheddar presented the fastest cheese disintegration
Nutrients release from cheese at the end of each digestion steps

Cheddar presented the fastest protein and lipid release
Preliminary disintegration model for cheeses (Fang et Turgeon, 2014)

- Cheese texture:
  - Cheddar: Decrease in Hardness, Cohesiveness, Disintegration
  - Mozzarella: Decrease in Hardness, Cohesiveness

- Cheese composition:
  - Increase in Fat content
Impact of calcium on lipid release

• Interactions Calcium – Lipids
  - Lipolysis accelerated
  - Reduced Absorption

Insolubles Ca salts

Ca$^{2+}$

Lumière intestinale

Gouttelette de TAG

Lipase

Colipase

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Ayala Bribiesca, 2014
Cheddar cheeses with fat fractions and Ca

- Experimental cheeses
  - Three fat fractions
  - Two salting conditions
    - Addition of CaCl₂

- *in vivo study*
  - Rats: Wistar
  - Training
    - To eat cheese
    - Timing
  - Postprandial lipemia
    - Triglycerides
    - Cholesterol
    - Free Fatty acids

<table>
<thead>
<tr>
<th>Fraction HB</th>
<th>Salting</th>
<th>Ca (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olein ↓SF</td>
<td>NaCl</td>
<td>7136</td>
</tr>
<tr>
<td>Control</td>
<td>or</td>
<td></td>
</tr>
<tr>
<td>Stearine ↑SF</td>
<td>NaCl + CaCl₂</td>
<td>9466</td>
</tr>
</tbody>
</table>

Ayala-Briebiesca, Britten, Turgeon, 2014, subm.
Results: *in vivo study*

- Cheese ingested in 15 minutes
- Lipemia
  - Interactions calcium-lipids
    - Effect on lipolysis
      - Lipemia ↑ with Ca
      - Fast Absorption of olein
    - Effect on precipitation
      - Lipemia ↓ with Ca + FA-SLC
      - Slower Absorption of stearine

Ayala-Briebiesca, Britten, Turgeon, 2014, subm.
Importance to consider **food matrix** and not only components

- Components and structure in interaction
- Need to understand the mechanism

**To develop delivery systems**
**but more importantly**
**to better know traditional foods as cheese**
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