An Integrative Approach to Understanding the Ripening of Mould-Ripened Cheeses

Steve Labrie
Institut sur la nutrition et les aliments fonctionnels (INAF)
Centre de recherche en sciences et technologie du lait (STELA)
Université Laval
Québec city, Canada
I. The case of mould-ripened cheeses
   1. The role of the eukaryotic organisms.
   2. The Non- Starter Yeast and Moulds (NSYM).

II. A polyphasic and integrative approach to understanding the ripening
   1. Molecular quantification.
   2. The metatranscriptome.
   3. The volatilome.
   4. The contribution of the milk’s native microflora.
I. The case of mould-ripened cheeses
The major fungal players

- *Penicillium camemberti*
  
  - Filamentous mould conferring the white velvet characteristic to the cheese.
  
  - Considered a domesticated form of *P. commune*
  
  - Produce NH$_3$ causing alkaninization at the surface.
  
  - Participate in flavor formation through the production of methyl cetone, esters, aldehydes, lactones and volatile sulfur compounds
The major fungal players

• *Geotrichum candidum*

  • Formally a yeast

  • Could show a dimorphic phenotype depending on the strain and the cheese conditions

  • Consume lactate and produce ammoniac, resulting in cheese alkalinization

  • Aids *P. camemberti* colonization

  • Flavor formation
Also inoculated

• **Debaryomyces hansenii**
  • Lactose, lactate, citrate assimilation
  • Easily colonizes the cheese and competes with other fungi

• **Kluyveromyces lactis / K. marxianus**
  • Lactose assimilation
  • Aroma production

• **Yarrowia lipolitica**
  • Often found as secondary microflora
  • Tributyrin hydrolysis
  • Lactose, galactose, lactate assimilation
  • Aroma production
Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec

Karine Lavoie · Marilyne Touchette · Daniel St-Gelais · Steve Labrie
Some milk contains interesting yeasts

### Producer A

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>June Isolate</th>
<th>June CFU/ml</th>
<th>July Isolate</th>
<th>July CFU/ml</th>
<th>August Isolate</th>
<th>August CFU/ml</th>
<th>September Isolate</th>
<th>September CFU/ml</th>
<th>October Isolate</th>
<th>October CFU/ml</th>
<th>Average CFU/ml</th>
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<tbody>
<tr>
<td><em>Candida glaeosa</em></td>
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<td>9.1E+02</td>
<td>20</td>
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<td>13</td>
<td>1.3E+03</td>
<td>10</td>
<td>5.0E+02</td>
<td>18</td>
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<td>4.1E+03</td>
<td>23</td>
<td>5.8E+02</td>
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<table>
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<tr>
<th>Molds</th>
<th>June Isolate</th>
<th>June CFU/ml</th>
<th>July Isolate</th>
<th>July CFU/ml</th>
<th>August Isolate</th>
<th>August CFU/ml</th>
<th>September Isolate</th>
<th>September CFU/ml</th>
<th>October Isolate</th>
<th>October CFU/ml</th>
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<td>2</td>
<td>5.0E+01</td>
<td>7.5E+01</td>
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Total yeasts and molds        16 1.6E+03 12 6.0E+02 18 1.8E+03 9 4.1E+03 25 6.3E+02 1.8E+03
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<thead>
<tr>
<th>Isolates</th>
<th>Source</th>
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<td>LMA-519</td>
<td>Cheese 1* (Milk a) Apr 3</td>
<td>67</td>
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<td>1</td>
<td>12</td>
<td>13</td>
<td>3</td>
<td>10</td>
</tr>
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</table>
II. A polyphasic and integrative approach to understanding the ripening
Ripening culture quantification → Activity of the fungal microflora

Effect on the cheese aroma

Identification of the Y&M secondary microflora
Molecular quantification vs. traditional microbiology

FIG 2 Microbial count on acidified PDA medium (pH 3.5) of the *K. lactis* LMA-437 (●), *G. candidum* LMA-436 (□), and *P. camemberti* (▲) ripening culture.

FIG 3 Comparison of the evolution of the mycelium biomass (×) and the surface microbiota quantification of a soft-cheese model curd using a TaqMan real-time qPCR method. The ripening culture contained *K. lactis* LMA-437 (●), *G. candidum* LMA-436 (□), and *P. camemberti* (▲).
Changing the ecosystem

FIG 3 Comparison of the evolution of the mycelium biomass (×) and the surface microbiota quantification of a soft-cheese model curd using a TaqMan real-time qPCR method. The ripening culture contained *K. lactis* LMA-437 (●), *G. candidum* LMA-436 (■), and *P. camemberti* (▲).

FIG 5 Comparison of the evolution of the mycelium biomass (×) and the surface microbiota quantification of a soft-cheese model curd using a TaqMan real-time qPCR method. The ripening culture contained *K. lactis* LMA-437 (●), *D. hansenii* LMA-1019 (○), *G. candidum* LMA-436 (□), and *P. camemberti* (▲).
Studying the metatranscriptome of a simple industrial mould-ripened cheese

• Industrial cheese inoculated using only *Penicillium camemberti* and *Geotrichum candidum*

• Identification of the genes expressed using a metatranscriptomic approach

• Gene expression monitored at seven sampling points during the ripening
Proteolysis

Caseins

Proteases

Peptides and Amino acids

Cell wall

Elimination

Amino acid

Lyases

Thiols

Transamination

Decarboxylation

Reduction

Aromatic compounds

Figure adapted from Mariley, 2004
Study of the organic volatile compounds produced by the native yeast of Quebec province terroir milk

• Selection of 5 Non-Starter Yeast species from raw milk and cheese:
  • *Saccharomyces servazii*
  • *Pichia fermentans*
  • *Pichia jadinii*
  • *Issatchenkia orientalis*
  • *Rhodotorula mucilaginosa*

• Ripening cultures:
  • *Geotrichum candidum*
  • *Penicillium camemberti*
Sample preparation

• Camembert-type slurries

• Inoculation of slurries using yeast alone or in combination with standard ripening culture.

• Ripening conditions:
  • 10°C and 90% humidity, 10 days.
  • 4°C and 90% humidity, up to day 35.

\[ R. \text{mucilaginosa} + G. \text{candidum} + P. \text{camemberti} = \text{Sample} \]
Volatile compound extraction using SPME-GC-MS

- **SPME-GC-MS**: *Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry*

- Day 14
- Day 21
- Day 28
Comparison of COV products of two yeasts

- *I. orientalis* (day 35) : ___________
- *S. servazzii* (day 35) : ___________

Heptanal:
- Fatty, oily
- Powerful rancid

1-hexanol:
- Green herb

1-heptanol:
- Planty
- Oily

2-phenylethyl acetate:
- Raisin-pineapple
- Honey

3-methyl butanoic acid
- Rotten fruit
- Sweat

Isoamyl alcohol:
- Bitter, burnt, harsh

Phenylethyl alcohol:
- Floral
- Rose
Conclusions

• **Non-Starter Yeast and Molds (NSYM)**
  • Potentially a source of new aroma and flavors

• **What to expect?**
  • New genetic methods unravel the complexity of the mould-ripened cheese ecosystem
  • A more precise picture of the ripening activities will become available
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