Developing Flavours in Soft Cheeses

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Technological diagram
e.g. Camembert

Milk pH=6.6-6.8
Proteins >31.5g/L Fat

Ca Cl₂ addition (1ml/ml of a 10% CaCl₂ solution),
Starters, addition of rennet when pH=6.3
(20ml/100 liters)

CaCl₂ addition

Starters,
addition of rennet when pH=6.3
(20ml/100 liters)

curd

Drained curd
Salted curd
Dry salting (PDO) or Brine
Dried curd
Packaging
Ripening
Ready to eat

Moulding

Return after 30min, 2h,
3h after end of moulding
pH at moulding ??

T° < 37° C in tank,
30° C in air
T° =34° C
18h for PDO

T° =14° C, 85% RH

T° =12-14° C, 13 days, 98% RH
T° =8-10° C
2 weeks, 95 HR
## Summary of the differences between lactic curd and rennet curd

<table>
<thead>
<tr>
<th>Curd mainly lactic</th>
<th>Curd mainly rennet</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Acidification and only then draining</td>
<td>• Draining and then acidification</td>
</tr>
<tr>
<td>• pH 4.5</td>
<td>• pH 5.2</td>
</tr>
<tr>
<td>• Residual Lactose</td>
<td>• No residual lactose</td>
</tr>
<tr>
<td>• Deminéralisation</td>
<td>• High minéral concentration</td>
</tr>
<tr>
<td>• Low buffer capacity</td>
<td>• Strong buffer capacity</td>
</tr>
<tr>
<td>• Weak Interactions between proteins</td>
<td>• Strong interactions between caseins</td>
</tr>
<tr>
<td>• High moisture level</td>
<td>• Low moisture level</td>
</tr>
<tr>
<td>• Fragile</td>
<td>• Ferm and elastic</td>
</tr>
<tr>
<td>• Small cheeses</td>
<td>• Big cheeses</td>
</tr>
</tbody>
</table>
PLAN

Introduction

1. Texture

2. Malt and Fruity flavours

3. Cabbage and Garlic flavours

4. Goaty, Blue cheese to Soapy flavours

5. Mushroom flavours

6. Plastic

Conclusions
Mass transfer in soft cheese ripening

- Context: Important changes in curd, composition, structure, aspect, texture, colour and taste due to biological activity and transport phenomena.

Leclercq Perlat et al, 2004
Lactose and lactate changes during ripening

Leclercq-Perlat et al, 2012
**Microbial growth during ripening**

**DESACIDIFICATION**

**SURFACE**

Fungi:
- Yeasts
- Geotrichum
- Penicillium

**INSIDE**

Fungi + ripening bacteria:
- Micrococcinae: Micrococcus, Arthrobacter, Brevibacterium
- Corynebacterinae: Corynebacterium
- Staphylococci

**MATURATION**

PH = 5.8

Time
pH Change at the surface and in the core of a Camembert cheese
Buffer capacity

- Buffer capacity can be defined as the resistance to pH change, low pH decrease the buffer capacity with strong consequences on texture.

- Low pH at moulding (pH= 6.1 (traditional camembert) to 5.2 (lactic washed rind))
  - Makes easier the pH rise after the *Penicillium* growth, gives creamy underind after a while
  - Makes the cheese center tough and chalky
Evaluation of the ripening efficiency on the base of texture change

RH : 88%
Triangles : 16° C
Circles : 12° C
Squares : 8° C

RH : 92%

Leclercq-Perlat et al, 2012
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Formation of fruity notes directly from fat

Coconut, apricot, peach

Proposed Mechanism for δ-lactones production from triglycerides containing 5-hydroxy acids. R1=(CH2)n-CH3; R2 and R3=CO-(CH2)n-CH3 (Alewijn et al. 2007)
Malt and Fruity flavours

They are mainly coming from amino acid breakdown

These flavour compounds are produced early in the ripening process

Yeast are mainly responsible of these flavours

At the end of ripening, some bacteria may reinforce these flavour notes especially in washed rind cheeses
PROTEINS

<table>
<thead>
<tr>
<th>PEPTIDES</th>
<th>Proteases,</th>
<th>aminopeptidases, carboxypeptidases</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>AMINO ACIDS</th>
<th>transaminases</th>
<th>decarboxylase</th>
<th>lyases</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-ceto acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehydes, alcools, acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole, phenol, styrene sulfur compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
OXIDATIVE DEGRADATION OF AMINO ACIDS

Amino Acid

TRANSAMINASE

NH₂

DECARBOXYLASE

CO₂

α-ceto acids

Amines

Fruity notes

NADH

NAD⁺

Aldehydes

NH₂

1/2O₂

Branched chain alcohols

Branched chain acids

ESTERS
OXIDATIVE DEGRADATION OF AMINO ACIDS

**TRANSAMINASE**

\[ \text{Amino Acid} \rightarrow \alpha\text{-ceto acids} \]

**DECARBOXYLASE**

\[ \text{Amino Acid} \rightarrow \text{Amines} \]

\[ \text{Amino Acid} \rightarrow \text{Branched chain alcohols} \]

\[ \text{Amino Acid} \rightarrow \text{Branched chain acids} \]

\[ \text{Amino Acid} \rightarrow \text{Aldehydes} \]

\[ \text{Aldehydes} \rightarrow \text{CO}_2 \]

\[ \text{CO}_2 \rightarrow \text{NADH} \]

\[ \text{NADH} \rightarrow \text{NAD}^+ \]

\[ \text{NAD}^+ \rightarrow \text{NH}_2 \]

\[ \frac{1}{2}\text{O}_2 \rightarrow \text{NH}_2 \]

\[ \text{NH}_2 \rightarrow \text{ESTERS} \]

**Malt Flavours**
The amino acids breakdown is the main source of malt and fruity notes:

- aldehydes (malt and chocolate notes),

- alcools, esters, thioesters (fruity notes)
Starters sources of malt and fruity notes

- aldehydes (malt and chocolate notes) :
  
  *Debaryomyces hansenii*,
  *Staphylococcus xylosus*

- alcools, esters, thioesters (fruity notes) :
  
  *Kluyveromyces lactis*,
  *Geotrichum candidum*
12°C; rh=95%

4°C; rh=?; packaged

METHYL 3 BUTANAL

Ripening time (days)
Olfactive thresholds of esters

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Aromatic Notes</th>
<th>perception threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>Solvent, fruity</td>
<td>5 ppm&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 ppm&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl Propionate</td>
<td>Pineapple</td>
<td>9,9 ppba</td>
</tr>
<tr>
<td>Ethyl Butyrate</td>
<td>Pineapple</td>
<td>0,13 à 45.10&lt;sup&gt;4&lt;/sup&gt; ppba</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,6 ppm&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl Hexanoate</td>
<td>pineapple, banana</td>
<td>1 ppba</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0,85 ppm&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoamyle acetate</td>
<td>pear, banana</td>
<td>2 ppba</td>
</tr>
<tr>
<td>2-phenylethyl acetate</td>
<td>floral, rose</td>
<td>18,5 ppm&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-phenylethyl propionate</td>
<td>floral, fruity</td>
<td>16,8 ppm&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: in water; <sup>b</sup>: in oil or butter; <sup>c</sup>: in a cheese base.
Thioester formation

- AA → CH₃SH
- Sugars, FFA, AA → Acyl CoA
- Enzyme?
- Acyl S Me + CoASH
**Geotrichum candidum** is able to produce thioesters (Berger et al, 1999)
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Conclusions
Degradation of amino acids also leads to:

- phenols (animal notes, role in ewes milk cheeses)

- sulfur compounds (garlic and cabbage notes—important especially in smear ripened cheeses)
Relations between sulfur amino acid metabolism and flavour production.
Relations between sulfur amino acid metabolism and flavour production

Cystéine \xrightarrow{\text{Transamnisation}} \text{Mercaptolactate} \xleftrightarrow{\text{}} \text{Mercaptopyruvate} \xrightarrow{\text{Transamnisation}} \text{Methionine} \xrightarrow{\text{Transamnisation}} \text{KMBA} \xrightarrow{\text{}} \text{HMBA}

\text{H}_2\text{S} + \text{Pyruvate} \xrightarrow{?} \text{Mercapto-ethanal} \xrightarrow{?} \text{Mercapto-ethanol} \xrightarrow{\text{Transamnisation}} \text{Methional} \xrightarrow{\text{NADH}^+} \text{Méthionol} \xrightarrow{\text{NAD}^+} \text{Polysulfides}

\text{Garlic and cabbage like}
Olfactive thresholds of sulfur compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>In water</th>
<th>In oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS</td>
<td>0.3*</td>
<td>1.2*</td>
</tr>
<tr>
<td>DMDS</td>
<td>18.69+</td>
<td>2.5*</td>
</tr>
<tr>
<td>DMTS</td>
<td>0.23+</td>
<td>2.5*</td>
</tr>
<tr>
<td>DMQS</td>
<td>0.06+</td>
<td>ND*</td>
</tr>
</tbody>
</table>

*Kubickova & Grosch, 1997

+ Our results
Micro-organisms able to produce sulfur compounds

- **Geotrichum candidum**

- **Hafnia alvei**

- **Coryneform bacteria:**
  - *Brevibacterium linens, B. aurantiacum*
  - *Micrococcus sp.*

- **Staphylococcus equorum and S. lentus**

- **Lactobacilli**
Propriétés aromatiques de Camembert fabriqués avec *P. camemberti* avec et sans *G. candidum*
(Molimard et al, 1996)

![PCA plot](chart.png)

- Intensity, Blue Cheese
- Cardboard, plastic
- Cabbage, cow shed
- Butter, Milk, Cream, mushroom
- Propriétés aromatiques de Camembert fabriqués avec *P. camemberti* avec et sans *G. candidum* (Molimard et al, 1996)
12°C; rh=95%  

4°C; rh=?; packaged

Ripening time (days)

DMDS

Cheese rind

Inner mass

AgroParisTech
PLAN

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TRIGLYCERIDES

SATURATED FATTY ACIDS

UNSATURATED FATTY ACIDS

HYDROXY ACIDS, OXO-ACIDS, HYDROPEROXY-ACIDS

Lipases
Effect of fat on the sensory properties

- Texture factor (thickener, lubricant, emulsifier, …)
- Flavour support
- Flavour precursor
Lipid degradation

Step 1 lipolysis: It can be a limiting step (Goat cheese, Blue cheese)

Step 2: fatty acid oxidation (usually not the limiting step)
### Level of lipolysis in different cheeses

<table>
<thead>
<tr>
<th>Fat Acidity (mg of FFA/100 g MG)</th>
<th>Cheddar - Emmental</th>
<th>Gouda</th>
<th>Parmesan, Camembert</th>
<th>Roquefort</th>
<th>Bleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.25%</td>
<td>~0.2-1%</td>
<td>~1.5%</td>
<td>~4-5%</td>
<td>8-10%</td>
<td>18-25%</td>
</tr>
<tr>
<td>Fat Acidity (G)</td>
<td>1.5-2.0</td>
<td>0.8-16*</td>
<td>6-8</td>
<td>20</td>
<td>30-40</td>
</tr>
<tr>
<td>mg of FFA/cheese g</td>
<td>0.4-5</td>
<td>~5</td>
<td>12-15</td>
<td>25-30</td>
<td>50</td>
</tr>
</tbody>
</table>
## B- Lipolysis in blue Cheeses compared to other types of cheeses

### Table 3  Typical concentration of free fatty acids (FFA) in different cheese varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>FFA (mg kg(^{-1}))</th>
<th>Variety</th>
<th>FFA (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabrales</td>
<td>33200</td>
<td>Gruyere</td>
<td>1500</td>
</tr>
<tr>
<td>Danablu</td>
<td>32600</td>
<td>Brie</td>
<td>1300</td>
</tr>
<tr>
<td>Roquefort</td>
<td>32400</td>
<td>Cheddar</td>
<td>1000</td>
</tr>
<tr>
<td>Parmesan</td>
<td>5000</td>
<td>Camembert</td>
<td>700</td>
</tr>
<tr>
<td>Provolone</td>
<td>2100</td>
<td>Mozzarella</td>
<td>360</td>
</tr>
</tbody>
</table>
Specific fatty acids are goaty

- 4-ethyl-2octenoic acid
- 4-Ethyl octanoic acid (threshold 6 ppb against 0.9 ppm for decanoic acid)
- 4-methyl octanoic acid (threshold 20 ppb against 3.4 ppm for the decanoic acid)
Free fatty acid changes during camembert cheese ripening

In soft cheeses volatil FFA increase global flavour intensity

BUT too many fatty acids gives soapy flavours
Blue Cheese Flavours – in mould ripened cheese it is giving flavour intensity

Beta-oxidation

Methyl ketones

2n-1 C

Secondary alcool

Fruity

Blue Cheese Flavours – in mould ripened cheese it is giving flavour intensity


Blue Cheese Flavours – in mould ripened cheese it is giving flavour intensity

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Conclusions
Triglycerides
Phospholipids...

Lipases

Saturated FFA

Desaturases

Unsaturated fatty acids

Lipoxigenases

Hydroperoxides (R-O-O-H)

Hydroperoxide lyases

Alcohols, aldehydes, ou acids, unsaturated

Oxidation COOH Terminale (β-oxidation)

Méthyl ketones 2n-1 carbons, Acids with 2n-2C

Secondary alcohols
Oxydation of a pentadiene motive through a Lipoxigenase

Linoleic acid

\[ \text{Lipoxigenase} \quad O_2 \]

Hydroperoxides of linoleic acid (HPODEs)

- (8-HPODE)
- (9-HPODE)
- (10-HPODE)
- (13-HPODE)
This pathway gives mushroom, metallic, geranium like, vegetal like flavours

Spontaneous oxydation (rare in cheese) but mainly activated by moulds (Penicillium camemberti mainly)
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Conclusions
Effect of ripening temperature on the styrene production by *P. camemberti* on a model curd

After 1 week at the culture temperature

And 3 weeks at the storage temperature

![Graph showing the effect of ripening temperature on styrene production](image)
Context: Important changes in curd, composition, structure, aspect, texture, colour and taste due to biological activity and transport phenomena.
Plastic flavour tested with a panel of 20 trained judges (camemberts made with 4 strains of *Penicillium*, with and without strains of *G. candidum*)
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SURFACE

DESACIDIFICATION

Uptake:
- residual sugars, citrate and lactate
- proteolysis, lipolysis
- flavours (alcohols, aldehydes, esters)

pH=5.8

MATURATION

Proteins ➔ amino-acids

Flavours (sulfurs, thioesters, phénols)

Triglycerides ➔ FFA

Flavours (methyl ketones, alcohols, sulfur compounds, unsaturated carbonyles)

INSIDE

www.agroparistech.fr
CONCLUSIONS

- Fungi and Bacteria are able to produce a large diversity of flavour compounds which will determine the cheese flavour notes perceived.

- Understanding the origin of the flavour notes
  - Understanding may help to manage flavor defects
  - May help to choose the starters

- The parameters used in the technology (management of the relative humidity, temperature, ventilation) will not only change the growth of the different species of the microbial ecosystem but also the physiology of some of the micro-organisms.