New Parameters and Analytical Challenges for Milk Recording by Fourier-Transform Mid-Infrared Spectrometry (FTMIR)

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Why mid-infrared?

• Advantages of **InfraRed Spectrometry**:
  – Fast
  – No destructive method
  – Environmentally friendly

• Near infrared vs. mid-infrared (MIR):
  – **MIR**: high sensitivity to the chemical environment due to the fundamental absorptions of molecular vibrations (Belton, 1997)
  – **NIR**: much more complex structural information related to the vibration behavior of combination bonds (Cen and He, 2006)
• **MIR spectrum**: absorptions of IR at frequencies correlated to the vibrations of specific chemical bonds within a molecule (Coates, 2000)

• Typical chemical composition (Smith, 1996)

Figure 1: MIR spectrum of milk (Sivakesava and Irudayaraj, 2002)

<table>
<thead>
<tr>
<th>Frequency Range (cm(^{-1}))</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1700 – 1500 cm(^{-1})</td>
<td>N-H</td>
</tr>
<tr>
<td>1200 – 900 cm(^{-1})</td>
<td>C-O</td>
</tr>
<tr>
<td>3000-2800 cm(^{-1})</td>
<td>C-H</td>
</tr>
<tr>
<td>1450-1200 cm(^{-1})</td>
<td>COOH</td>
</tr>
</tbody>
</table>
Why mid-infrared?

• Other advantages of MIR spectrometry:
  – Largely used by milk labs to quantify the major components of milk
  – Milk samples collected for the milk payment or for the routine milk recording are analyzed by MIR
General aim

• **Aim**: Development of management and selection tools useful for the dairy sector including dairy industry and dairy farmers in the current economic context

• **How?**
  – Direct use of the results obtained from calibration equations which predict the contents of specific milk components
Interest

Animal Health
- Fatty acids, minerals, lactoferrin, lactose, β-hydroxybutyrate, acetone...

Herd Management
- Urea, fat, protein, lactose...

Nutritional Quality of Milk
- Fatty acids, lactoferrin, minerals...

Technological Quality of Milk
- Traits related to cheese making (casein, dornic acidity, coagulation time...)

Environment
- Fatty acids, methane emission through fatty acid contents, urea

Biodiversity
- Changes in milk composition

Hygienic Quality of Milk
- Antibiotics, somatic cells... (less important for milk recording)
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MIR spectrum
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- Traits related to cheese making (casein, dornic acidity, coagulation time...).
Common traits

- Fat content
- Protein content
- Urea
- Lactose
- Casein
- Free fatty acids

Recent studies showed that the MIR spectrometry is currently **under-used**
**Collection of milk samples**

**Prediction:**
- Fat
- Protein
- Lactose
- …

**Calibration equations**

**MIR spectrometer**

**Raw data = Spectra**
Principle

Collection of milk samples

Development of new equations

Prediction:
- Fat
- Protein
- Lactose
- ...

Calibration equations

Raw data = Spectra

(Foss, 2008)
Few examples...

Fatty acids, minerals, lactoferrin, ketone bodies, cheese-making properties...
Fatty Acids (FA)

• Recent studies confirmed the ability of MIR to predict FA in milk (g/dl of milk):
• Lower ability to predict FA content in fat (g/100g of fat)
• New results obtained in the RobustMilk project (www.robustmilk.eu)
  – Multi-breeds, multiple countries and multiple production systems
• All studied FA have a RPD (SD/SECV) greater than 2
Fatty Acids

<table>
<thead>
<tr>
<th>Constituent (g/dl of milk)</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>RPD</th>
<th>SECV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated FA</td>
<td>496</td>
<td>2.40</td>
<td>0.80</td>
<td>15.7</td>
<td>0.0513</td>
</tr>
<tr>
<td>Monounsaturated FA</td>
<td>491</td>
<td>1.06</td>
<td>0.37</td>
<td>8.9</td>
<td>0.0411</td>
</tr>
<tr>
<td>Polyunsaturated FA</td>
<td>499</td>
<td>0.16</td>
<td>0.05</td>
<td>2.6</td>
<td>0.0204</td>
</tr>
<tr>
<td>Unsaturated FA</td>
<td>492</td>
<td>1.22</td>
<td>0.41</td>
<td>9.6</td>
<td>0.0428</td>
</tr>
<tr>
<td>Short chain FA</td>
<td>486</td>
<td>0.31</td>
<td>0.11</td>
<td>6.7</td>
<td>0.0165</td>
</tr>
<tr>
<td>Medium chain FA</td>
<td>496</td>
<td>1.78</td>
<td>0.60</td>
<td>6.5</td>
<td>0.0928</td>
</tr>
<tr>
<td>Long chain FA</td>
<td>495</td>
<td>1.52</td>
<td>0.57</td>
<td>6.5</td>
<td>0.0875</td>
</tr>
</tbody>
</table>


This study will be presented in details at ADSA conference in July at Denver (USA)
Minerals

- First results were published by Soyeurt et al., 2009

<table>
<thead>
<tr>
<th>mg/l de lait</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SECV</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>87</td>
<td>1,333</td>
<td>260</td>
<td>95</td>
<td>2.74</td>
</tr>
<tr>
<td>K</td>
<td>61</td>
<td>1,336</td>
<td>168</td>
<td>136</td>
<td>1.24</td>
</tr>
<tr>
<td>Mg</td>
<td>61</td>
<td>110</td>
<td>18</td>
<td>11</td>
<td>1.68</td>
</tr>
<tr>
<td>Na</td>
<td>87</td>
<td>403</td>
<td>107</td>
<td>64</td>
<td>1.68</td>
</tr>
<tr>
<td>P</td>
<td>87</td>
<td>1,093</td>
<td>127</td>
<td>50</td>
<td>2.54</td>
</tr>
</tbody>
</table>

- Current study confirmed these results with a larger database (more than 100 samples)
Lactoferrin

<table>
<thead>
<tr>
<th>mg/l de lait</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SECV</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin</td>
<td>57</td>
<td>253</td>
<td>206</td>
<td>86</td>
<td>2.39</td>
</tr>
</tbody>
</table>

- Milk glycoprotein involved in the immune system defenses
- Preliminary results published in 2007
- Validation in the RobustMilk project ([www.robustmilk.eu](http://www.robustmilk.eu)) on more than 3,000 data
Ketone Bodies

- **Acetone**: Hansen (1999) and Heuer et al. (2001)
- De Roos et al. (2007) studied also 2 other ketone bodies (N spectral data > N sample)

<table>
<thead>
<tr>
<th>mMol</th>
<th>N</th>
<th>Mean</th>
<th>SECV</th>
<th>R²c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>1,063</td>
<td>0.146</td>
<td>0.184</td>
<td>0.72</td>
</tr>
<tr>
<td>β-hydroxybutyrate</td>
<td>1,069</td>
<td>0.078</td>
<td>0.065</td>
<td>0.62</td>
</tr>
</tbody>
</table>

De Roos et al., 2007
# Cheese-Making

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>R²cv</th>
<th>SECV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Titrable acidity (SH°/50ml)</strong></td>
<td>De Marchi et al., 2009</td>
<td>1,063</td>
<td>3.26</td>
<td>0.43</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Rennet coagulation time (min)</strong></td>
<td>De Marchi et al., 2009</td>
<td>1,049</td>
<td>14.96</td>
<td>3.84</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Dal Zotto et al., 2008</td>
<td>74</td>
<td>15.05</td>
<td>3.78</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>De Marchi et al., 2009</td>
<td>1,064</td>
<td>6.69</td>
<td>0.12</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Titrable acidity (D°)</strong></td>
<td>Colinet et al., 2010(*)</td>
<td>203</td>
<td>16.22</td>
<td>2.01</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Curd firmness (mm)</strong></td>
<td>Dal Zotto et al., 2008</td>
<td>74</td>
<td>32.43</td>
<td>7.95</td>
<td>0.45</td>
</tr>
</tbody>
</table>

(*) These results will be presented by Colinet at «New Technologies » session on Friday at 10:50 am
General aim

• **Aim**: Development of management and selection tools useful for the dairy sector including dairy industry and dairy farmers in the current economic context

• **How?**
  – Direct use of the results obtained from calibration equations which predict the contents of specific milk components
  – Integration of these infrared predictions in specific models taken into account the variability of these values in order to extend the number of possible valorizations
Few examples...
Urea

Month of test

HTD MU level (mg/L)

Expected HTD MU level
Observed HTD MU level

Bastin et al., 2009
Fatty Acids

• Potential used of FA predictions (*milk labs*):
  – FA predicted from *bulk tank milk*:
    • Separate scheme of milk collecting
    • Subsidy given in Belgium by a dairy company for milk with higher unsaturated FA
  – FA predicted from *individual cows* (*Milk recording*)
    • To discard cows
    • Animal selection programs
      – Most interesting bulls and dams
      – Possible internationalization based on relationships among animals
Fatty Acids

• Animal selection program for FA
  – RobustMilk project (www.robustmilk.eu)
  – Heritable trait with sufficient genetic variability
    • Saturated FA: +/- 44% (more than fat content)
    • Monounsaturated FA: +/- 22%
  – Feasability of genetic selection
    • Genetic evaluation for cows in first lactation
    • Results will be presented at INTERBULL session (Nicolas Gengler on Wednesday at 8:00 am) and at ADSA conference in Denver
Results of the genetic evaluation for 1,993 bulls with a sufficient number of Walloon daughters with known FA information.
Fatty Acids

The most interesting bulls for the considered fat content

\[ y = 0.1966x + 0.2596 \]
\[ R^2 = 0.8528 \]
Conclusions

• MIR is currently under used in practice
• New parameters predictable by MIR exist with potential interests for milk recording:
  – Directly MIR predictions
  – Models to offer specific valorizations for dairy industry (farmers, dairy companies, breeding associations...)

But ... (analytical challenges)

- The **MIR equation used should be validated** on the considered cow population:
  - Breed differences can appear
  - Milk from bulk tank is less variable than milk samples collected from individual cows
- Currently, it is possible to **implement externally** the new equations thanks to the recording of spectra
  - Make sure that the variability of the spectral data used for the prediction was taken into account in the calibration set used to build the calibration equation
- The **accuracy of the MIR prediction should be tested** regularly by the use of reference samples
  - Since January 2008, MIR FA predictions is implemented in the Walloon milk lab ➔ a maintenance is realized using milk samples with known contents of FA
But ... (computational challenges)

• The number of studied traits will increase
  – Some traits are correlated → for the development of specific valorizations for breeders, it will be important to know the relationships among studied traits
    • e.g., fatty acids vs. protein, ...
  – The optimum of content for the studied trait can be different following the considered aim
    • e.g., high lactoferrin in milk interesting for human health vs. Milk sample with high content of lactoferrin can be produced by a sick cow → take into account the natural variation of each studied trait

→ multiple traits models → high computational cost
A lot of work to do ...
Collaborators for our researches

- **GxABT**: Nicolas Gengler - Valérie Arnoald - Catherine Bastin - Alain Gillon - Sylvie Vanderick
- **CRA-W**: Frédéric Dehareng - Pierre Dardenne
- **Comité du Lait**: Didier Veselko - Emile Piraux
- **AWE**: Carlo Bertozzi - Laurent Laloux - Xavier Massart
Thank you for your attention

MIR spectrometer

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