Improvement and validation of milk fatty acid predictions using mid-infrared spectrometry

H. Soyeurt[1,2,*], S. McParland[3], D.P. Berry[3], E. Wall[4], N. Gengler[1,2], F. Dehareng[5], and P. Dardenne[5]

[1] University of Liège, Gembloux Agro-Bio Tech - GxABT, Gembloux, Belgium
[2] National Fund for Scientific Research, Brussels, Belgium
[3] Teagasc Moorepark Dairy Production Rsearch Centre, Cork, Ireland
[4] Scottish Agricultural College, Penicuik, UK
[5] Walloon Agricultural Research Center – CRA-W, Gembloux, Belgium

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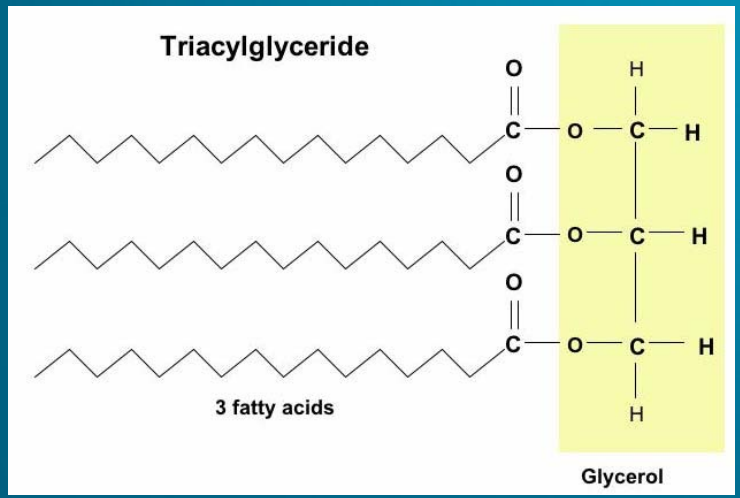
Fatty Acids

- Generally, 2.5 to 7.0% of fat in bovine milk
- 96% of fat is composed by triglycerides
  - Groups of fatty acids (FA):
    - **Saturated** (SAT): 70%
    - Unsaturated (UNSAT): 30%
      - **Monounsaturated** (MONO): 25%
      - **Polyunsaturated** (POLY): 5%



Langara, 2008

Measurement

- Gas chromatography:
  - Major advantage: accuracy
  - Major disadvantages:
    - Expensive reagents
    - Time consuming
    - Skilled staff

Measurement

- Gas chromatography:
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➔ Find an alternative method

Measurement

- Gas chromatography:
  - Major advantage: reliability
  - Major disadvantages:
    - Expensive reagents
    - Time consuming
    - Skilled staff

- Mid-InfraRed (MIR) spectrometry:
  - Fast analysis (up to 500 samples/hour)
  - Cheap analysis
  - Used in routine milk recording

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Collection of samples in Belgium

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Collection of samples in Belgium

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<u>High variability</u>:

→ *For milk recording scheme*:

- March 2005 to December 2007
- 475 cows in 8 herds
- 6 dairy breeds

→ *From milk payment scheme*

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Collection of samples in Belgium

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

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Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=239)

Collection of samples in Belgium

Analysed by Mid-Infrared (MilkoScan FT6000)

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Chromatographic analysis

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PLS approach was used to estimate the calibration equations

Methodology

- 4 methods were tested:
  - (1) Partial Least Squares regressions (PLS)

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  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file:
    - Spectra provided by different spectrometers for the same milk samples

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Methodology

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- 4 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file (REP)
  - (3) PLS + first derivative applied to the spectra:
    - Correction of baseline drift

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Methodology

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- 4 methods were tested:
  - (1) Partial Least Squares regressions (PLS)
  - (2) PLS + repeatability file (REP)
  - (3) PLS + first derivative (DER)
  - (4) PLS + DER + REP

Collection of samples in Belgium

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=239)

PLS approach was used to estimate the calibration equations

Internal validation by cross-validation

External validation by adding new samples

Collection of samples in Belgium

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=239)

PLS approach was used to estimate the calibration equations

Internal validation by cross-validation

Cross-validation :

- 20 groups

Collection of samples in Belgium

Analysed by Mid-Infrared (MilkoScan FT6000)

Spectra were exported

Selection of interesting samples by Principal Component Approach

Chromatographic analysis

Mid-Infrared spectrum

CALIBRATION SET (N=239)

PLS approach was used to estimate the calibration equations

362 new samples :

- Collected in Belgium, Ireland and Scotland
- Between April 2008 and August 2009
- from several breeds and cows

External validation on independent new samples

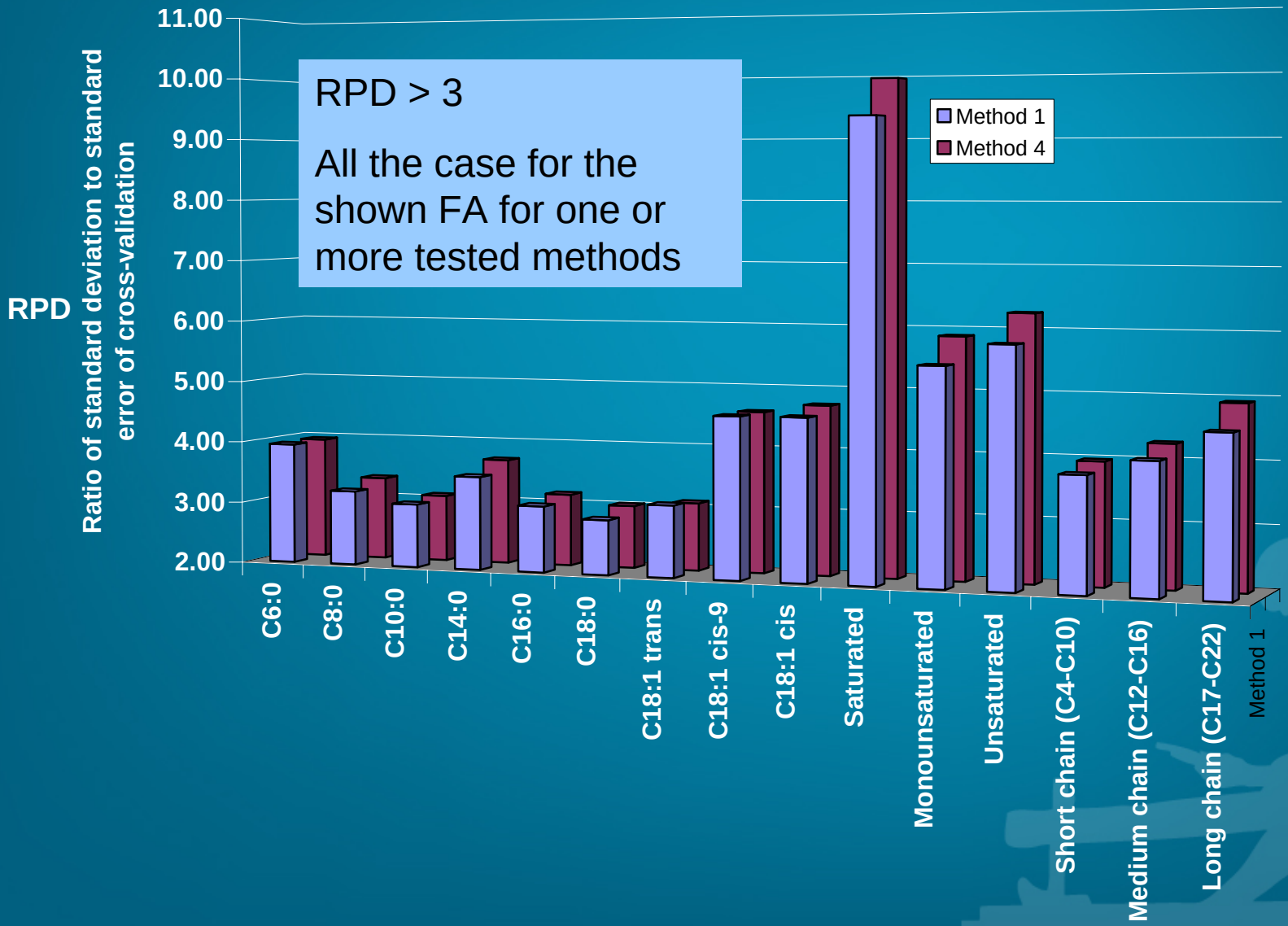
Most Interesting Results

Constituent	N=239 Mean	SD
C6:0	0.08	0.02
C8:0	0.05	0.02
C10:0	0.12	0.04
C14:0	0.48	0.14
C16:0	1.29	0.42
C18:0	0.49	0.23
C18:1 trans	0.15	0.09
C18:1 cis-9	0.89	0.36
C18:1 cis	0.96	0.37
Saturated	2.98	0.85
Monounsaturated	1.26	0.43
Unsaturated	1.46	0.48
Short chain (C4-C10)	0.39	0.11
Medium chain (C12-C16)	2.19	0.64
Long chain (C17-C22)	1.86	0.69

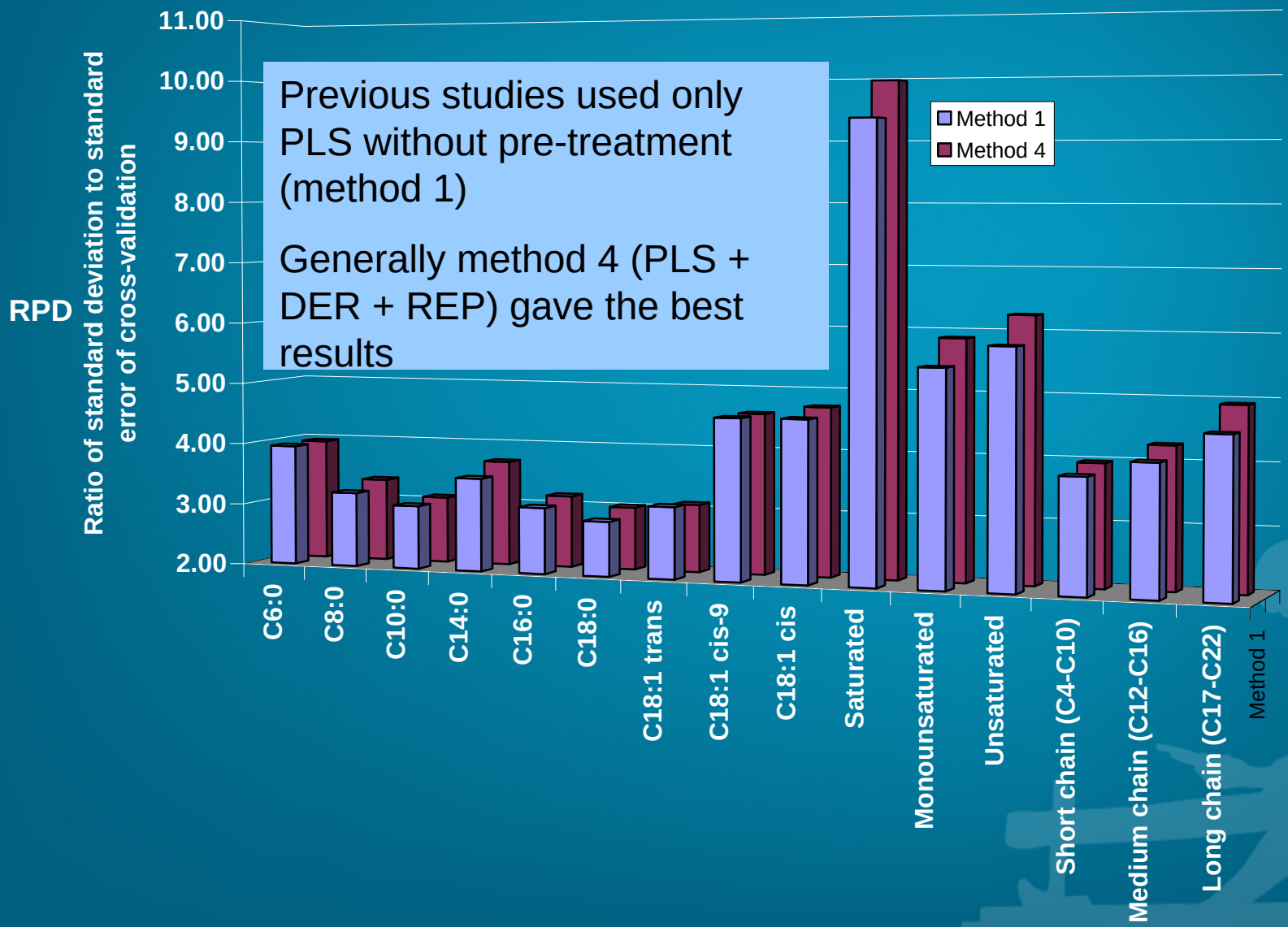
High variability of FA :

Coefficient of variation (100/mean * SD) ranged from 25% to 60%.

Most Interesting Results



Most Interesting Results



Most Interesting Results

	Method 4	
	R^2cv (N=239)	R^2v (N=362)
C6:0	0.94	0.90
C8:0	0.91	0.81
C10:0	0.89	0.73
C14:0	0.93	0.90
C16:0	0.90	0.90
C18:0	0.89	0.72
C18:1 trans	0.90	0.49
C18:1 cis-9	0.95	0.91
C18:1 cis	0.96	0.93
Saturated	0.99	0.98
Monounsaturated	0.97	0.95
Unsaturated	0.97	0.96
Short chain	0.94	0.93
Medium chain	0.95	0.94
Long chain	0.96	0.95

R^2cv and R^2v confirms the ability of MIR to predict some FA directly in milk

Most Interesting Results

g/dl of milk	RPD (N=239) 1 (*)	2 (*)	3 (*)	4 (*)
C6:0	3.95	**4.02**	3.89	3.95
Total C18:1 trans	**3.16**	3.09	3.05	3.09
C18:1 cis-9	4.61	**4.68**	4.35	4.6
Saturated	9.34	**10.01**	9.55	9.95

RPD = ratio of SD to the standard error of cross-validation; 1 = PLS; 2 = PLS+REP; 3 = PLS+DER; 4 = PLS+DER+REP

Some FA could be better predicted using another method

Conclusion

- MIR can be used to quantify some FA directly on milk
- Prediction of fat and prediction of FA are decorrelated → interest to use specific equations to quantify FA (data not shown)
- Improvement of accuracy by:
  - Using a first derivative and a repeatability file to develop calibration equations
  - OR chosing the most appropriated method based on the studied FA

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