# Creation of universal MIR calibrations by standardization of milk spectra: example of fatty acids

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## Context and objectives

- Fatty acid (**FA**) content of cow milk can be predicted by Mid-Infrared (**MIR**), however to build **robust** MIR equations, **large spectral datasets** with samples covering the maximum chemical variability is needed
- The sharing of data is a potential solution, but due to the **existing differences between each MIR instrument**, a step of **spectral standardisation** is necessary
- The goal of this research is to correct the differences in instrumental responses from different types and brands of MIR instruments, by using a standardisation method, in order to build and use common MIR calibrations predicting fatty acids content of milk

## Fatty acids analysis and standardisation of MIR instruments

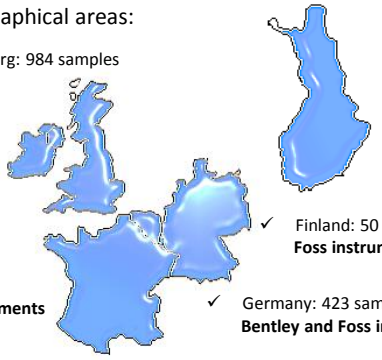
- **1827** milk samples selected, based on spectral variability, and analyzed in MIR with Foss (FT+, FT6000) and Bentley (FTS) instruments and in GC to obtain fatty acid profile (since 2006).
- Dataset covering large variability of breeds (17), seasons, feeding systems and geographical areas:

✓ Belgium and Luxembourg: 984 samples
**Foss instruments**

✓ UK: 106 samples
**Foss instruments**

✓ Ireland: 144 samples
**Foss instruments**

✓ France : 150 samples
**Bentley and Foss instruments**

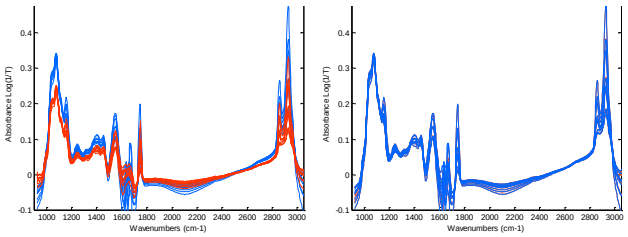


✓ Finland: 50 samples
**Foss instruments**

✓ Germany: 423 samples
**Bentley and Foss instruments**

- Method used: Piecewise Direct Standardisation
- Milk MIR spectra from all instruments are mathematically corrected to match the instrumental response of a reference instrument, via analysis of common milk samples

*Fig 1. Graphical view of spectra from a Bentley instrument (red) and spectra from the reference instrument (blue), before (left) and after standardisation (right)*




- Partial Least Square method (PLS) used to build calibrations
- **1371** samples used as calibration dataset (including 66 Bentley)
- **456** samples used as validation dataset (including 22 Bentley)

## Results and discussion

- Standardisation allows to bring all the spectra from different instruments into the reference instrument spectral format, and to group them into a common database
- Common FA equations where created for all instruments, including Foss and Bentley
- Validation results (with Foss and Bentley), show the good ability of the equations to be used on all instruments (even on Bentley standardized spectra)

*Tabl 1. Statistical parameters of FA equations, calibration and validation*

| | Calibration using 1371 samples | | | | | Validation (456 samples) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Constituents (g/100 ml milk) | # terms | Mean | SD | SEC | $R^2c$ | SEP | $R^2p$ | RPDp | Use |
| Fat | 7 | 3.906 | 0.981 | 0.007 | 1.00 | 0.007 | 1.00 | 140.11 | +++ |
| Total of Saturated FA | 8 | 2.694 | 0.757 | 0.072 | 0.99 | 0.082 | 0.99 | 9.23 | +++ |
| Total of Mono-unsaturated FA | 10 | 1.073 | 0.344 | 0.059 | 0.97 | 0.066 | 0.97 | 5.20 | ++ |
| Total of Poly-unsaturated FA | 11 | 0.159 | 0.045 | 0.021 | 0.79 | 0.023 | 0.75 | 1.97 | - |
| Total of Unsaturated FA | 10 | 1.233 | 0.379 | 0.064 | 0.97 | 0.076 | 0.96 | 4.99 | + |
| Total of Short chain FA | 9 | 0.348 | 0.097 | 0.025 | 0.93 | 0.027 | 0.92 | 3.59 | + |
| Total of Mid chain FA | 11 | 1.988 | 0.606 | 0.104 | 0.97 | 0.123 | 0.95 | 4.93 | + |
| Total of Long chain FA | 10 | 1.579 | 0.498 | 0.110 | 0.95 | 0.126 | 0.95 | 3.95 | + |
| Total of branched FA | 9 | 0.091 | 0.026 | 0.013 | 0.77 | 0.015 | 0.64 | 1.74 | - |
| Total of odd FA | 10 | 0.156 | 0.041 | 0.016 | 0.84 | 0.019 | 0.76 | 2.14 | 0 |
| Total of trans FA | 13 | 0.159 | 0.068 | 0.029 | 0.82 | 0.036 | 0.76 | 1.90 | - |
| C4:0 | 11 | 0.104 | 0.029 | 0.008 | 0.93 | 0.009 | 0.90 | 3.21 | + |
| C6:0 | 8 | 0.072 | 0.021 | 0.006 | 0.91 | 0.007 | 0.89 | 2.97 | 0 |
| C8:0 | 9 | 0.047 | 0.014 | 0.004 | 0.91 | 0.005 | 0.88 | 2.78 | 0 |
| C10:0 | 12 | 0.111 | 0.035 | 0.010 | 0.92 | 0.011 | 0.89 | 3.17 | + |
| C12:0 | 12 | 0.134 | 0.043 | 0.011 | 0.93 | 0.013 | 0.90 | 3.29 | + |
| C14:0 | 9 | 0.447 | 0.123 | 0.030 | 0.94 | 0.036 | 0.91 | 3.42 | + |
| C14:1 cis | 10 | 0.038 | 0.014 | 0.008 | 0.71 | 0.009 | 0.63 | 1.59 | - |
| C16:0 | 10 | 1.192 | 0.400 | 0.091 | 0.95 | 0.106 | 0.92 | 3.77 | + |
| C16:1 cis | 10 | 0.065 | 0.024 | 0.013 | 0.73 | 0.016 | 0.63 | 1.51 | - |
| C17:0 | 8 | 0.027 | 0.008 | 0.003 | 0.81 | 0.005 | 0.67 | 1.60 | - |
| C18:0 | 12 | 0.394 | 0.137 | 0.056 | 0.84 | 0.064 | 0.84 | 2.14 | 0 |
| C18:1 cis9 | 9 | 0.744 | 0.261 | 0.061 | 0.95 | 0.068 | 0.95 | 3.84 | + |
| Total C18:1 trans | 13 | 0.125 | 0.056 | 0.025 | 0.80 | 0.032 | 0.73 | 1.75 | - |
| Total C18:1 cis | 9 | 0.801 | 0.286 | 0.063 | 0.95 | 0.070 | 0.95 | 4.09 | + |
| Total C18:1 | 10 | 0.931 | 0.314 | 0.060 | 0.96 | 0.071 | 0.96 | 4.42 | + |
| C18:2 cis9, cis12 | 10 | 0.061 | 0.022 | 0.011 | 0.75 | 0.014 | 0.65 | 1.56 | - |
| C18:2 cis 9, Trans 11 | 10 | 0.028 | 0.020 | 0.010 | 0.74 | 0.013 | 0.69 | 1.54 | - |
| Total C18:2 | 9 | 0.096 | 0.026 | 0.014 | 0.71 | 0.017 | 0.63 | 1.55 | - |
| C18:3 cis9, cis12, cis 15 | 11 | 0.020 | 0.008 | 0.004 | 0.69 | 0.005 | 0.60 | 1.56 | - |
| Total of omega 3 | 11 | 0.026 | 0.010 | 0.006 | 0.68 | 0.006 | 0.64 | 1.68 | - |
| Total of omega 6 | 11 | 0.103 | 0.028 | 0.014 | 0.74 | 0.018 | 0.66 | 1.57 | - |

*Legend*
# terms = number of terms (latent variable)
SD = standard deviation of reference values
SEC = standard error of calibration
$R^2c$ = $R^2$ of calibration
SEP = standard error of prediction
$R^2p$ = $R^2$ of prediction
RPDp = ratio of SD/SEP. See RPD class

| RPD | | Class | Application | Symbol |
|---|---|---|---|---|
| 0 | 2 | Very poor | Allows to compare groups of cows, distinguish high or low values | - |
| 2 | 3 | Poor | Screening | 0 |
| 3 | 5 | Fair | Qualitative screening | + |
| 5 | 6.5 | Good | Quality control | ++ |
| 6.5 | + | Excellent | As precise as reference value | +++ |

## Conclusion

- Standardisation allows to merge MIR milk spectra from different instruments together
- **Common equations were built and can be used in routine by all MIR instruments participating to the standardisation**

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