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### The state of the art about the development of midinfrared based fatty acids predictions and their applications along the dairy food chain

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### Abstract

Milk mid-Infrared (MIR) spectrometry has been used since the 1980's to analyse milk components. Today advanced analytical techniques and powerful data processing tools allow additional value to be derived from this spectral information. For instance, milk MIR spectra can be used to quantify the fatty acid (FA) composition in dairy milk. Research into milk FA prediction has the potential to significantly impact the dairy chain, from analysis of the nutritional value of milk and also in management benefits to the farmer. However, the application of this technology at the farm level is currently limited. The ExtraMIR project aims to reduce that gap and support the dairy chain in future market needs. This research investigates the variability and reliability of reference data sets for statistical FA modelling to predict the milk FA composition using MIR spectra. Various published FA models from 8 different countries (Belgium, USA, Netherlands, Italy, Australia, Canada, France and China) are analysed using the R square mean-centred cross validation ranking method (Grelet et al., 2021), also taking into account the reference sample size. The variations in accuracy of the Fatty Acid prediction models, specifically for the individual FA C12:0, ranged from 0.92 in the best examples to 0.71 in the poorest examples. This suggests that models analysed are highly imprecise and only for use in detecting extreme values. This knowledge will be combined with the various practical applications of ExtraMIR analysis in the field, where FA data is fed back into the agricultural industry, to aid and benefit farmers in on-farm management and future proofing milk production. Typically, the main constraint between application of FA models across different countries arises from the variability within farming systems. This is due to differences in climate, nutrition and breed. With research visits to Belgium, New Zealand and Canada not only were these constraints clear to witness but also the demands within the agricultural markets were different. These differences in demand generated research focuses specific to the needs of the national agricultural industries which further deviated from the aspirations of the international agricultural focus areas. This differing of objectives can also give rise to innovation within research areas and dairy consultancy organisations. Given that the benefits of milk FA's can be used as an indicator of ration quality of cattle feed, animal health and welfare concerns, environmental footprint of milk production and the technological properties of milk. This will contribute to closing the gap between



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the existing extensive research and the application in the field from advisory service providers.

### Introduction

Milk MIR spectra can be used to quantify the fatty acid (FA) composition in dairy milk. The applications in the agricultural Research into milk FA prediction offers potential benefits to the dairy industry, including at the farm level, through analysis of the nutritional value of milk. However, the application of this technology at the farm level is currently limited.

The purpose of this study is to investigate the variability and reliability of reference data sets for statistical FA modelling to predict the milk FA composition from MIR spectra. The application potential is analysed using the R square mean-centred cross validation ranking method published by Grelet *et al.* (2021).

## Material and methods

The reliability of data sets used in 6 different published research papers, in 6 different countries, focused on statistical FA modelling predictions, was analysed using a classification table developed for mean-centred cross-validation of RPD, relative RMSE and R<sup>2</sup>. For the purpose of analysis the data sets were converted to R<sup>2</sup>, allowing for comparison between the studies. The ranking method of Grelet *et al.* (2021) was used to analyse the models. Phenotypes including fine milk components, blood components, status of dairy cows and technological properties of milk were used in order to perform a non-supervised K-means Near Neighbour (KNN) clustering of models, with seven clusters, following 3 parameters: their mean-centred cross-validation RPD, relative RMSE and R<sup>2</sup>.

### Results and discussion

The seven groups of models are recorded below in Table 1, with the range of their performance indicators and their interpretation for potential applications.

### Model Analysis 1

The interpretation and cluster groupings from Table 1 have been applied to a study conducted by Grelet at al (2021) in Belgium which looked into large scale phenotyping in the dairy sector using milk MIR spectra. Table 2 contains the fatty acid and the subsequent interpretation of the reference material.

When applying the interpretations from the classification groupings to the R<sup>2</sup> values given in Table 2, the quality of the reference data suggests that the grouped fatty acids have a strong correlation, falling within the any allocation and quality control classification and that the applications in the field would be well received and factually accurate. However, C18:2c9t11 has ranked poorly and should not be used as part of any research other than to detect extreme values.

Cluster	RPDcv	Relative RMSEcv	R²cv	Interpretation for application
1	> 6	<5%	> 0.97	Any application
2	4.2 - 6	<10%	0.94 - 0.97	Quality control Quantitative
3	3 - 4.2	<10%	0.89 - 0.94	screening
4	2 - 3	<25%	0.74 - 0.89	Rough screening Allows to compare groups,
5	1.5 - 2	<25%	0.55 - 0.74	discriminate high or low values Highly imprecise, can be used to detect extreme
6	1.5 -2	>25%	0.55 - 0.74	values Not
7	< 1.5		< 0.55	recommended

Table 1. Characteristics of the 7 K-mean clusters resulting from the classification of 57 milk MIR models following their mean-centred cross-validation RPD, relative RMSE and R2 (adapted from Grelet et al., 2021).

## The interpretation and cluster groupings have Table 1 have also been applied to a study conducted by Rutten *et al.* (2009) in the Netherlands, which looked into prediction bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. Table 3 contains the summer and winter milk analysis correlations alongside the cluster group ranking.

Table 3 shows that the reference data used in this study ranked very low again the cluster group ranking scores and averages around group 4 which would suggest that the data should only be used as a method of rough screening. There are some higher scores in the groupings of 2 and 3 which would then be useable as quality control and quantitative screenings.

The interpretation and cluster groupings have Table 1 have also been applied to a study conducted by Ferrand-Calmels *et al.* (2014) in France which investigated the prediction of fatty acid profiles in cow milk by mid-infrared spectrometry. Table 4 contains the comparison of methods used to develop calibration equations on the MilkoScan FT6000 analyser data for FA cow milk (g/100 mL of milk) on the validation set.

#### Model Analysis 2

Model Analysis 3

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		Cluster Group	-
Phenotype (Fatty Acid)	R²cv	Ranking	Interpretation
SAT FA (g/dL)	0.99	1	Any application
C18:1cis9 (g/dL)	0.95	2	Quality control
LCFA (g/dL)	0.95	2	Quality control
MCFA (g/dL)	0.97	2	Quality control
MONO FA (g/dL)	0.97	2	Quality control
Tot18:1cis (g/dL)	0.95	2	Quality control
Total _C18:1 (g/dL)	0.96	2	Quality control
UNSAT (g/dL)	0.97	2	Quality control
C10 (g/dL)	0.91	3	Quantitative screening
C12 (g/dL)	0.92	3	Quantitative screening
C14 (g/dL)	0.93	3	Quantitative screening
C16 (g/dL)	0.94	3	Quantitative screening
C4 (g/dL)	0.93	3	Quantitative screening
C6 (g/dL)	0.91	3	Quantitative screening
C8 (g/dL)	0.91	3	Quantitative screening
SCFA (g/dL)	0.93	3	Quantitative screening
C17 (g/dL)	0.80	4	Rough screening
C18 (g/dL)	0.84	4	Rough screening
Odd Fatty Acids (g/dL)	0.83	4	Rough screening
PUFA (g/dL)	0.77	4	Rough screening
Total Trans (g/dL)	0.80	4	Rough screening
18:1 trans (g/dL)	0.79	4	Rough screening
			Allows to compare groups,
C14:1 (g/dL)	0.68	5	discriminate high or low
			values
			Allows to compare groups,
C16:1c (g/dL)	0.73	5	discriminate high or low
			values
			Allows to compare groups,
C18:2c9c12 (g/dL)	0.72	5	discriminate high or low
			values
			Allows to compare groups,
C18:3c9c12c15 (g/dL)	0.68	5	discriminate high or low
			values
			Allows to compare groups,
FA isoanteiso (g/dL)	0.75	5	discriminate high or low
			values
			Allows to compare groups,
Omega3 (g/dL)	0.66	5	discriminate high or low
			values
			Allows to compare groups,
Omega6 (g/dL)	0.72	5	discriminate high or low
			values
			Allows to compare groups,
Tot18:2 (g/dL)	0.69	5	discriminate high or low
			values
			Highly imprecise, can be
C18:2c9t11 (g/dL)	0.74	6	used to detect extreme
			values

Table 2. Details of the 7 K-mean clusters resulting from the classification of 57 milk MIR models following their normalised cross-validation RPD, relative RMSE and R2 (Grelet et al., 2021).



				Fatty	y Acids -	Milk (g/dL)				
Trait	AA	AA Cluster Group Ranking	WM	WW Cluster Group Ranking	WS	WS Cluster Group Ranking	SS	SS Cluster Group Ranking	SW	SW Cluster Group Ranking
C4:0	0.91	3	0.83	4	0.78	4	0.77	4	0.82	4
C6:0	96.0	2	0.89	3	0.89	4	0.89	3	0.90	3
C8:0	0.94	2	0.85	4	0.84	4	0.84	4	0.85	4
C10:0	0.92	3	0.75	4	0.81	4	0.81	4	0.76	4
C12:0	0.85	4	0.61	5	0.71	5	0.73	5	0.62	5
C14:0	0.94	3	0.81	4	0.84	4	0.85	4	0.82	4
C16:0	0.94	2	0.85	4	0.75	4	0.79	4	0.83	4
C18:0	0.82	4	0.58	5	0.64	5	0.70	5	0.59	9
C18:1 cis-9	0.92	3	0.69	5	0.79	4	0.81	4	0.68	9
C18:1 cis-11	0.27	7	0.18	7	0.15	7	0.23	7	0.12	7
C18:1 trans-4-8	0.48	7	0.23	7	0.13	7	0.20	7	0.15	7
C18:1 trans-9	0.53	7	0.32	7	0.27	7	0.29	7	0.27	7
C18:1 trans-11	0.63	9	0.26	7	0.09	7	0.17	7	0.18	7
C18.2 cis-9,12	0.36	7	0.19	7	0.14	7	0.17	7	0.15	7
C18.2 cis-9, trans-11	0.58	3	0.27	7	0.14	7	0.20	7	0.13	7
C18:3 cis-9,12,15	0.45	7	0.19	7	0.22	7	0.23	7	0.14	7
C6-C12	0.95	2	0.81	4	0.87	4	0.87	4	0.82	4
C14-C16	0.97	2	0.90	3	0.81	4	0.85	4	0.89	3
C18u	0.94	2	0.69	5	0.77	4	0.80	4	0.68	5
Ratio SFA:UFA	0.91	3	0.43	7	0.65	5	0.59	5	0.38	7

- AA Calibration in half of all data and validation in the other half of all data.
- WW Calibration in half on the winter data and calidation in the other half of the winter data.
- WS Validation of the midel from scenario WW in all summer data.
- SS Calibration in half of the summer data and validation in the other half of the summer data.
- SW Validation of the model scenario SS in all winter data.

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Table 4. Comparison of the methods used to develop calibration equations on the MilkoScan FT6000 analyser data for FA in cow milk (g/100mL) on the validation set (Ferrand-Calmels et al., 2014).

Comparison of the methods used to develop call	bration equations (	on the MilkoScna.	FT6000 Analyzer	·(Food Electric A/	S Hillerod, Denmarl	k) data for FA inv	20WS milk									
Fatty Acid	PLS R <sup>2</sup> Clust	er Group Ranking	AGIPLS R <sup>2</sup> Ch	ster Group Ranki	ng AG2PLS R <sup>2</sup> Chu	ister Group Rank	ing Elastic net R <sup>2</sup> Clust	er Group Ranking.	LASSO R <sup>2</sup> Clusté	er Group Ranking	Ridge Regression R2 Cl	uster Group Ranking	First Derivative + PLS R <sup>2</sup> C	Cluster Group Ranking	Wavelet + PLS R <sup>2</sup> C	uster Group Ranking
C40	0.93	2	093	2	0.93	3	0.87	4	0.00	3	50	4	0.92	3	0.94	2
C60	96.0	2	960	2	0.97	_	0.93	3	0.93	3	75:0	4	0.96	2	0.96	2
C80	16.0	1	860	1	0.97	_	0.89	3	0.00	3	0.75	4	0.97	-	0.97	
C100	0.95	2	0.95	2	96.0	2	0.84	4	0.85	4	0.62	5	0.96	2	0.96	2
C12/0	96.0	2	960	2	960	2	0.82	4	0.84	4	0.48	1	0.97	1	0.96	2
C14.0	0.95	2	0.95	2	0.95	2	0.84	4	0.86	4	0.74	5	0.96	2	0.95	2
C160	0.94	2	0.92	3	0.93	3	0.86	4	0.87	4	0.71	5	0.93	3	0.92	3
C18.0	0.85	4	0.89	3	0.85	4	0.76	4	0.77	4	97:0	1	0.85	4	0.87	4
Totaltrans 18:1	0.85	4	0.83	4	0.83	4	0.71	5	0.71	5	06.0	1	0.88	4	0.87	4
cis-9 C18:1	0.97	1	60	1	0.97	-	0.85	4	0.00	3	0.38	2	0.98	1	0.96	2
Totalcis C18:1	0.97	-	60	-	96:0	2	0.84	4	0.90	3	0.37	٢	0.98	1	0.95	2
TotalC18:1	0.97	-	60	-	96:0	2	0.84	4	0.88	4	0.38	٢	0.98	1	0.96	2
cis-9, cis-12 C182 (linoleic acid)	0.78	4	9/20	4	0.75	4	0.58	5	0.61	5	0.45	٢	0.80	5	0.80	4
cis-9, trans-11 C18:2 (conjugated linoleic acid)	0.83	4	0.83	4	0.83	4	0.58	9	0.71	5	0.21	٢	0.87	4	0.78	4
C183n-3 (inolenic acid)	0.86	4	69'0	5	0.25	٢	0.54	٢	0.58	9	06:0	٢	0.82	5	0.85	4
SFA	1.00	_	1.00	_	0:99	_	0.96	2	0.96	2	16:0	3	1.00	_	0.99	_
MUFA	0.98	_	860	_	0.97		0.84	4	0.89	3	0.41	٢	0.99	_	0.97	
PUFA	0.78	4	0.81	4	0.81	4	0.71	5	0.73	5	0.53	٢	0.87	4	0.82	4
trans FA	0.86	4	0.86	4	0.86	4	0.74	5	0.74	4	0.26	٢	0.90	3	0.88	4
n-3	0.81	4	0.79	4	0.81	4	1910	5	0.65	5	0.26	2	0.86	4	150	4

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acids groupings in the any application interpretation but also some scores are within group 5 which are less reliable and suitable for comparing groups and discriminating

	-		-
		Cluster	
	<b>D</b> 2	Group	
Fatty Acid	R <sup>2</sup>	Ranking	Interpretation
04.0	0.00	-	Allows to compare groups, discriminate high or
C4:0	0.66	5	low values
C6:0	0.38	$\frac{1}{7}$	Not recommended
C8:0	0.37	(	Not recommended
040.0	0.00	~	Allows to compare groups, discriminate high or
C10:0	0.00	3 7	Iow values
011.0	0.21	1	Allowe to compare groupe, discriminate high or
C12.0	0.71	5	Allows to compare groups, discriminate high of
C12.0	0.71	7	Not recommended
C14:0	0.19	1	Pough scrooning
014.0	0.00	4	Allows to compare groups, discriminate high or
C14·1	0.61	5	low values
014.1	0.01	Ũ	Allows to compare groups, discriminate high or
C15 <sup>.</sup> 0	0.61	5	low values
C16:0	0.86	4	Rough screening
01010	0.00		Highly imprecise, can be used to detect extreme
C16:1	0.62	6	values
C17:0	0.53	7	Not recommended
C17:1	0.31	7	Not recommended
			Highly imprecise, can be used to detect extreme
C18:0	0.73	6	values
			Allows to compare groups, discriminate high or
C18:1 in-9 trans	0.60	5	low values
C18:1 in-9 cis	0.79	4	Rough screening
C18:2n-6 trans	0.17	7	Not recommended
			Allows to compare groups, discriminate high or
C18:2n-6 cis	0.62	5	low values
			Highly imprecise, can be used to detect extreme
C18:3n-3	0.58	6	values
			Allows to compare groups, discriminate high or
C18:2 cis-9,cis-12	0.65	5	low values
C22:6n-3	0.22	7	Not recommended
SFA	0.94	2	Quality control
MUFA	0.84	3	Quantitative screening
	0.00	_	Allows to compare groups, discriminate high or
PUFA	0.66	5	low values
UFA	0.84	4	Rough screening
Chart Chain	0.70	5	Allows to compare groups, discriminate high or
Short-Chain	0.72	5	
Long Chain	0.90	3	Quantitative screening
Long-Chain	0.83	4	Rough screening

Table 5. Fitting statistics of each prediction equation estimating fatty acid concentrations using the model development data sets expressed as g/100g of milk (Fleming et al., 2017)

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high or low values. The most successful method of regression in this study was AG1 PLS R<sup>2</sup>, scoring very well throughout on most of the individual fatty acids.

# **Model Analysis 4** The interpretation and cluster groupings have Table 1 have also been applied to a study conducted by Fleming *et al.* (2017) in Canada, which investigated predicting milk fatty acid content with mid-infrared spectroscopy in Canadian dairy cattle, using differently distributed model development sets. Table 5 contains the statistics of each prediction equation estimating fatty acid concentrations using the model development data sets expressed as g/100g of milk.

The data in Table 5 shows high variability in the classification grouping, with no fatty acids falling into the number 1 grouping. There are also several fatty acids that fall into the category of not recommended, which would suggest that the reference data set used in this study does not have a good fit in to the regression model used.

### Model Analysis 5

The interpretation and cluster groupings in Table 1 have also been applied to a study conducted by Wang *et al.* (2017) in Australia, which investigated the use of mid-infrared spectrometry to predict milk fatty acid, energy balance and methane emissions. Table 6 contains the Pearson correlations between milk fatty acids and energy balance derived using individual cow data and the prediction accuracy using MIR data on the fatty acids compared with the cluster group rankings.

Table 6 contains no classification groups 1, 2 or 3 which means that the categories of any application for quality control and quantitative screening have been removed from the analysis. The fatty acids groupings are largely focused around 4 and 5 which would fall into the rough screening and group comparison and discrimination high or low value categories. This study therefore would not be accurate enough to use in the field but would be of use as a general screening method based on the reference data set that was used.

### Model Analysis 6

The interpretation and cluster groupings in Table 1 have also been applied to a study conducted by Zhao *et al.* (2022) in China based on the prediction of milk fatty acid content by mid-infrared spectroscopy in Chinese Holstein cows. Table 7 contains the best prediction accuracy of prediction models for each fatty acid expressed as g/100g of milk.

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Individual		Cluster Group	
Fatty Acid	R <sup>2</sup>	Ranking	Interpretation
Un-identified	0.54	7	Not recommended
			Allows to compare groups.
C4:0	0.73	5	discriminate high or low values
C6 <sup>.0</sup>	0.78	4	Rough screening
C8:0	0.76	4	Rough screening
00.0	0.10		Allows to compare groups
C10·0	0.72	5	discriminate high or low values
010.0	0.72	U U	Allows to compare groups
C10·1	0.61	5	discriminate high or low values
010.1	0.01	0	Allowe to compare groups
C12·0	0.72	5	discriminate high or low values
012.0	0.72	0	Highly impression can be used to
014 inc	0.69	C	Highly imprecise, can be used to
C 14 ISO	0.68	Ö	
014.0	0.70	r	Allows to compare groups,
C14:0	0.73	5	discriminate nign or low values
0111	0.50	0	Highly imprecise, can be used to
C14:1	0.56	6	detect extreme values
		_	Allows to compare groups,
C15 iso	0.68	5	discriminate high or low values
			Highly imprecise, can be used to
C15 anteiso	0.55	6	detect extreme values
			Allows to compare groups,
C15:0	0.72	5	discriminate high or low values
			Allows to compare groups,
C16 iso	0.69	5	discriminate high or low values
C16:0	0.74	4	Rough screening
			Allows to compare groups,
C16:1	0.62	5	discriminate high or low values
C17 iso	0.53	7	Not recommended
C17 anteiso	0.49	7	Not recommended
			Highly imprecise, can be used to
C17:0	0.61	6	detect extreme values
C17:1	0.52	7	Not recommended
C18:0	0.80	4	Rough screening
			Allows to compare groups,
C18:1 t9	0.65	5	discriminate high or low values
			Highly imprecise, can be used to
C18:1 t10	0.59	6	detect extreme values
			Highly imprecise, can be used to
C18:1 t11	0.58	6	detect extreme values
			Allows to compare groups.
C18:1 cis	0.63	5	discriminate high or low values
C18:1 c9	0.51	7	Not recommended
			Allows to compare groups.
C18:1 c11	0.65	5	discriminate high or low values
	0.00	Ŭ	Highly imprecise can be used to
C18·2 n6	0.56	6	detect extreme values
	0.00		Highly imprecise can be used to
C18·3 n3	0.57	6	detect extreme values
C20.0	0.37	4	Rough screening
020.0	0.15		Allows to compare groups
C20.1 c11	0.68	5	discriminate high or low values
020.1011	0.00	5	Highly imprecise can be used to
	0.65	6	detect extreme values
	0.00		

Table 6. Pearson correlations between milk fatty acids and energy balance derived using individual cow data and the prediction accuracy using MIR data on the fatty acids.

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Table 7. Best prediction accuracy of different prediction models for each fatty acid expressed as g/100g of milk (Zhao et al., 2022).

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This study has highlighted that there is in some cases a lack in application value of the reference material. This has long been an issue in the industry where there is a disconnect between the amount of research that is being done and the actual application in the dairy industry. The general low scores using the cluster grouping method would suggest that there are some strengths in the research and that some studies are very positive for individual fatty acids or grouped fatty acids although few are good for both.

The variability between research studies in each country also reinforces the industry opinion that it is difficult to replicate the work of others between countries. Reference data sets are often unique to each country with nutrition, climate and milk system all leading to discrepancies between the milk quality observed in each country.

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Conclusions

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