Improving taste and flavor in dairy product through milk analysis of free fatty acid by Mid-infrared (MIR) spectrometry

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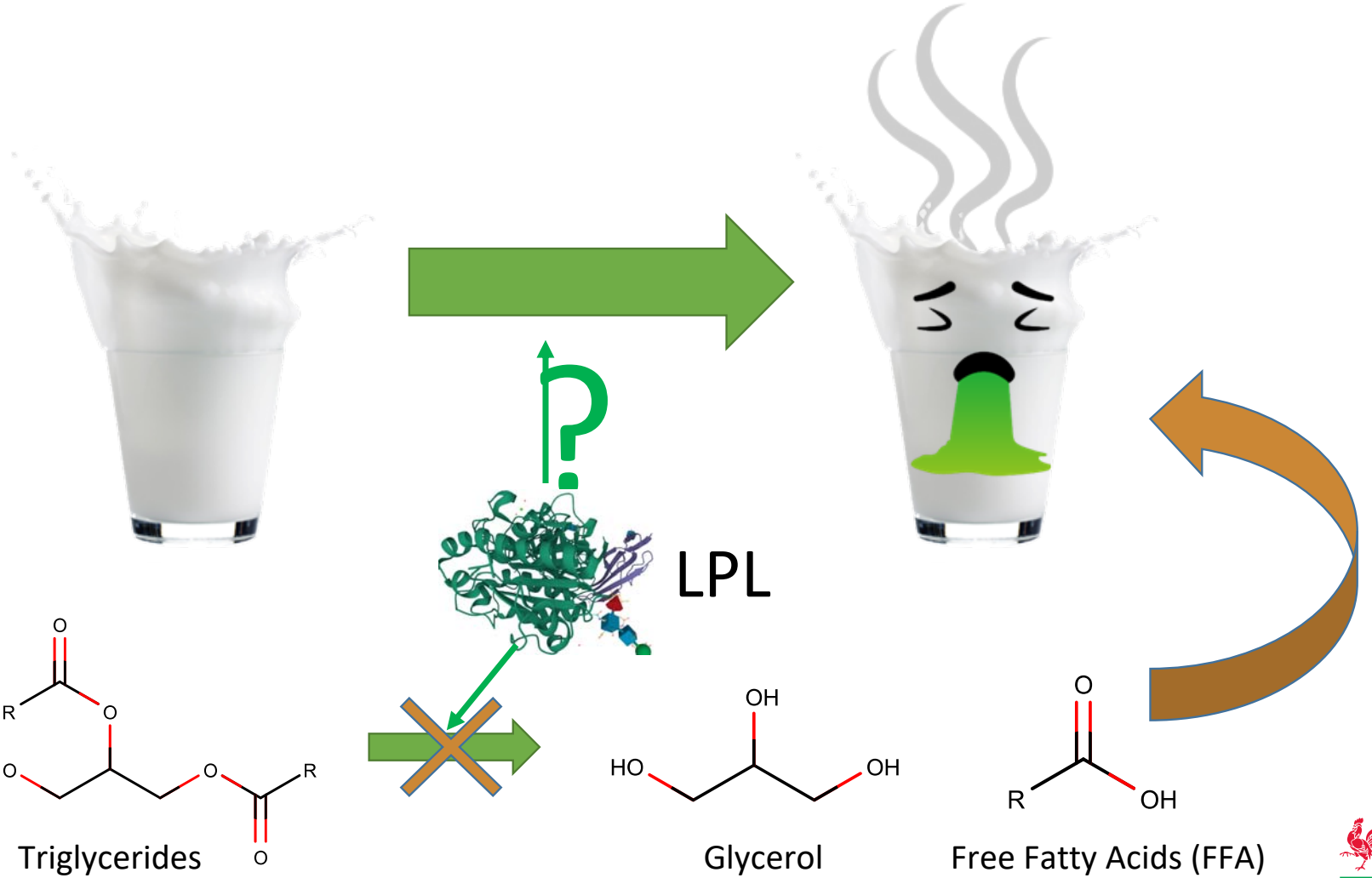
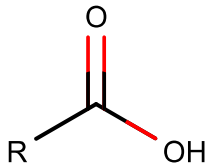
Introduction: how it's happen ?



Problematic and objectives

- Problem → the appearance of taste defects **after milking during cooling in the milk tank**

- This problem is economically **costly**
- Not all free fatty acids have the same impact, mainly the **short-chain free fatty acids (SFFA)**



R= 4 Carbon → C4
R= 16 Carbon + 1 unsaturation → C16:1

- Currently methods can only estimate lipolysis through an overview of the acidity of the milk fat → Not representative of the taste

- New method to determine FFA → GC-MS/MS
- mEq/100g of Fat → 0.28g of C18:0 → Our work mg/L
- ***The objectives of the project***

1) Development of free fatty acid prediction equations

2) Advice activities in the field of milk taste and aroma

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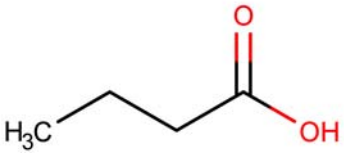
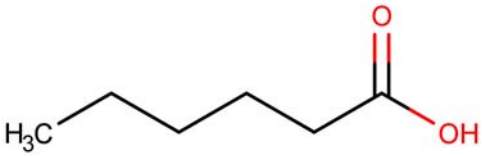
Effect on the taste quality of dairy products

Butyric acid (C4:0)



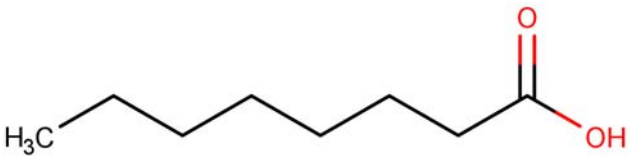
Rancid taste

Caproic acid (C6:0)

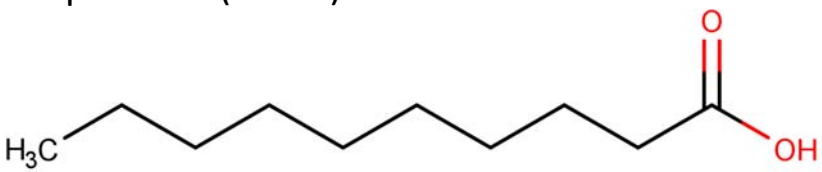


Rancid taste

Caprylic acid (C8:0)



Capric acid (C10:0)



Rancid taste

Other FFA → effect on the milk transformation
Inhibition of bacteria C12:0 → C18:3

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Lipolysis treshold

Lipolyse milk from 1.2 mEq/100g of fat

Rancid butter from 1.5 mEq/100g of fat
Other problem Fatty acid oxidation
35 mg/l of C4 and 26 mg/l of C6

Soap and rance taste
No threshold define

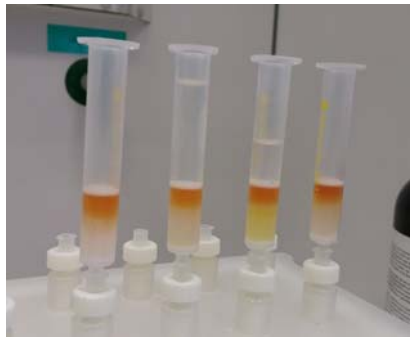
Could have a positive or a negative effect
FFA precursor of cheese aroma
Cheese with moderate lipolyse: <2%
Soft cheese: 5 to 10%
Blue cheese: >20%

Gas Chromatography (GC) Analysis

Sampling

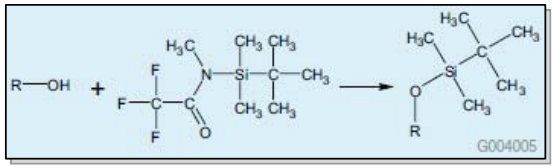
Lab

Extraction



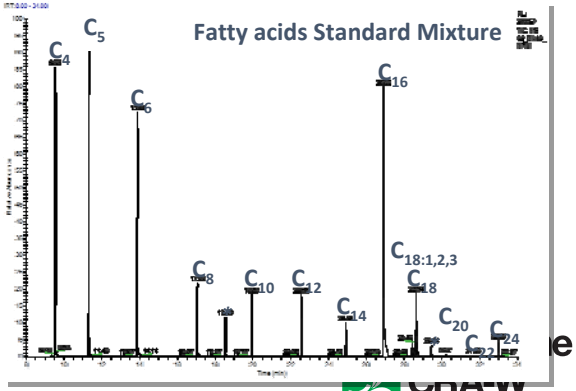
2 extraction with 50/50
Diethylether/n-hexane

Derivatization



GC MS/MS Injection

GC integration



Free fatty acids
References

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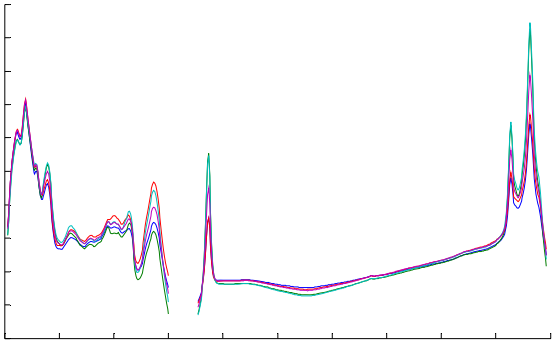
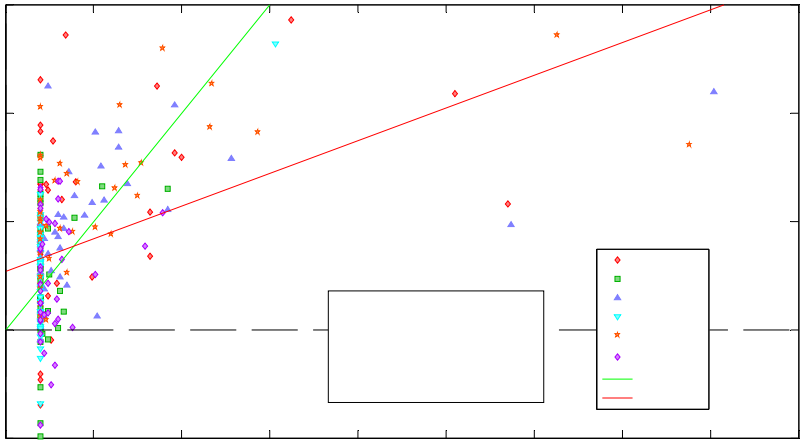
Spontaneous lipolysis

- Different breed were collected: Montbéliarde, Holstein, Brown Swiss, DP Belgian Blue, Simmental
- Few bulk milk per country were collected

- Spectra were standardized through EMR method (Grelet et al 2015)



- First, 250 samples were collected
- Model built were too bad for short chain FFA +/- R^2=0.20 → lack of available data
- Short chain free fatty acid are under LOQ

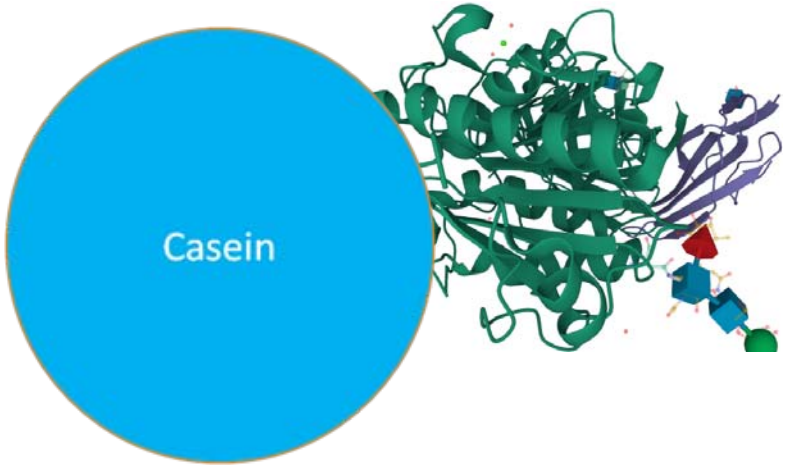


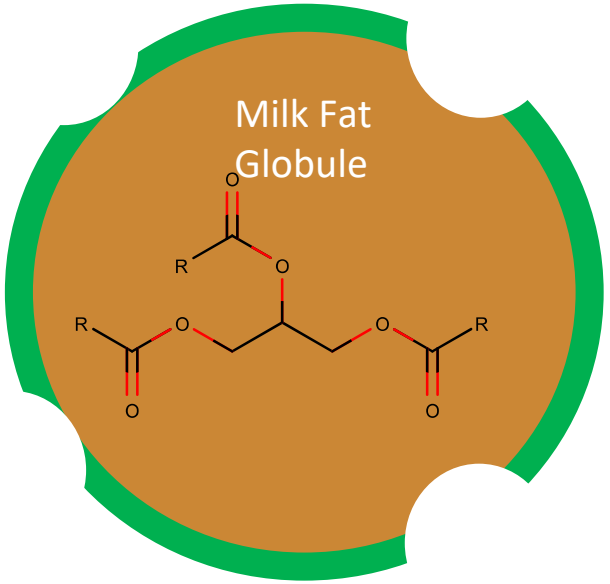
Find a new way to induce lipolysis ???

Grelet, C., Pierna, J. F., Dardenne, P., Baeten, V., & Dehareng, F. (2015). Standardization of milk mid-infrared spectra from a European dairy network. *Journal of dairy science*, *98*(4), 2150-2160.

B: Induced lipolysis

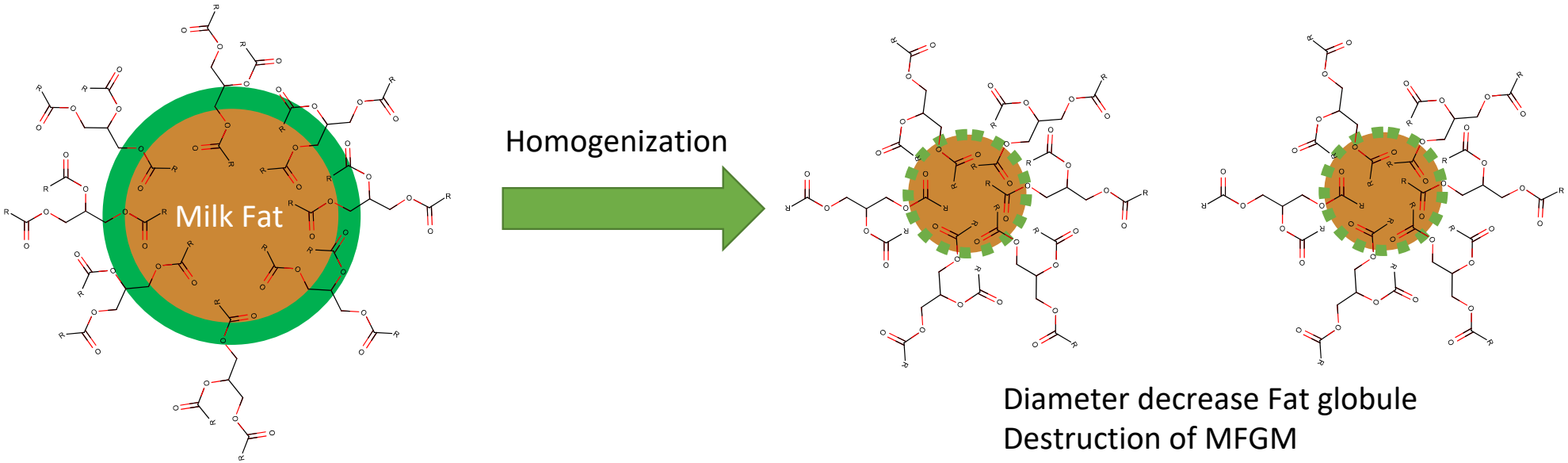
- Induced lipolysis is related to ***Mechanical stress*** of the milk
- The LPL is clearly link to the caseine of the milk
- Shaking lead to a link break of the caseine and the LPL





B: Induced lipolysis: Homogenization

- For the induced lipolysis, the size distribution of the fat is important
- Homogenization is achieved by pumping milk through small openings under very high pressure



Diameter decrease Fat globule
Destruction of MFGM
→ Triglycerides more available

B: Induced lipolysis: Homogenization

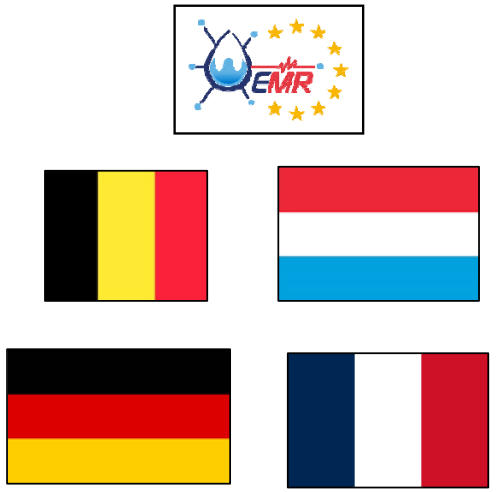
- Test made with 5 different milk
- Milk are store at 4°C
- Different time after homogenization + 2h, 4h, 12h and 48h of storage

(mg/L)	Milk 1			Milk 5		
	C4	C6	C8	C4	C6	C8
Homogenized	35.6	12.2	13.2	24.3	11.7	14.7
Storage 2H	62.6	28.9	15.1	102.3	41.6	17.3
Storage 4H	75.0	26.0	14.9	97.6	35.2	15.1
Storage 12H	69.5	24.5	18.1	88.7	32.6	9.8
Storage 48H	217.2	76.8	25.6	325.6	114.5	38.0

Best way to increase the concentration of the SCFFA

Sampling

Type of sample	Number
Spontanneous lipolysis	330
Bubbling sample	25
Storage 0H	15
Storage 1/2H	261
Storage 1H	66
Storage 2H	50
Storage 4H	25
Storage 12H	14
Storage 24H	10

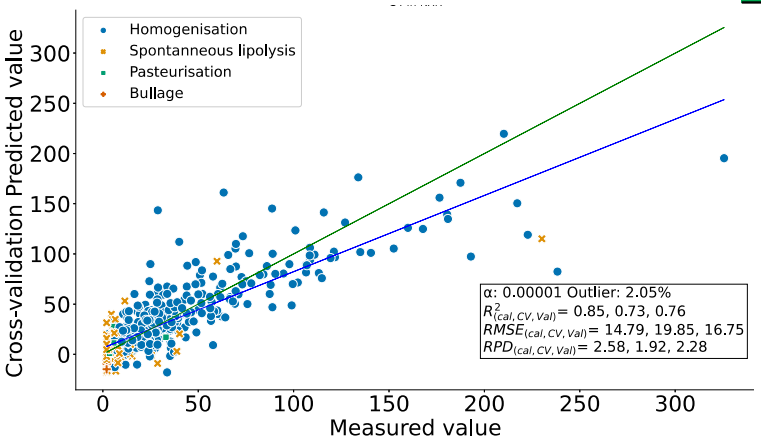


A total of 796 samples

Final models

A) **Models were built** for free fatty acid using several algorithms: Principal Component Regression (PCR), Partial Least Square regression (PLSr), Elasticnet regression (ENR), Kernel Ridge regression (KRR) and Support Vector Machine-Regression (SVM-R)

B) **A cross validation 10 groups** subset and random external validation with **20%** of total sample → evaluate models



C4 model including spontaneous lipolysis and homogenized sample with kernel Ridge Regression

Modelling performances of each Free Fatty acid with the best algorithm based on lowest RMSE from the external validation

FFA	Algo	R^2_{cal}	R^2_{cv}	R^2_{val}	$RMSE_{cal}$	$RMSE_{cv}$	$RMSE_{val}$	RPD_{cal}	RPD_{cv}	RPD_{val}
C4	KRR	0.85	0.73	0.76	14.79	19.85	16.75	2.58	1.92	2.28
C6	ENR	0.77	0.68	0.57	9.74	11.58	12.70	2.10	1.77	1.61
C8	SVM-R	0.85	0.54	0.40	4.95	8.71	9.72	2.61	1.48	1.33
C10	KRR	0.59	0.52	0.38	8.88	9.62	9.15	1.56	1.44	1.51
C12	PCR	0.35	0.31	0.08	6.45	6.65	6.34	1.24	1.21	1.26
C14	KRR	0.52	0.40	0.02	9.91	11.06	11.04	1.44	1.29	1.29
C16	KRR	0.50	0.36	0.20	22.40	25.25	21.82	1.41	1.25	1.45
C18	PLS	0.70	0.59	0.49	11.18	12.99	12.48	1.81	1.56	1.62
SCFFA	ENR	0.83	0.72	0.77	29.74	38.27	36.22	2.42	1.88	2.08
MCFFA	ENR	0.67	0.45	0.40	23.63	30.40	31.04	1.73	1.34	1.29
LCFFA	KRR	0.49	0.34	0.27	37.56	42.92	51.11	1.40	1.17	1.17

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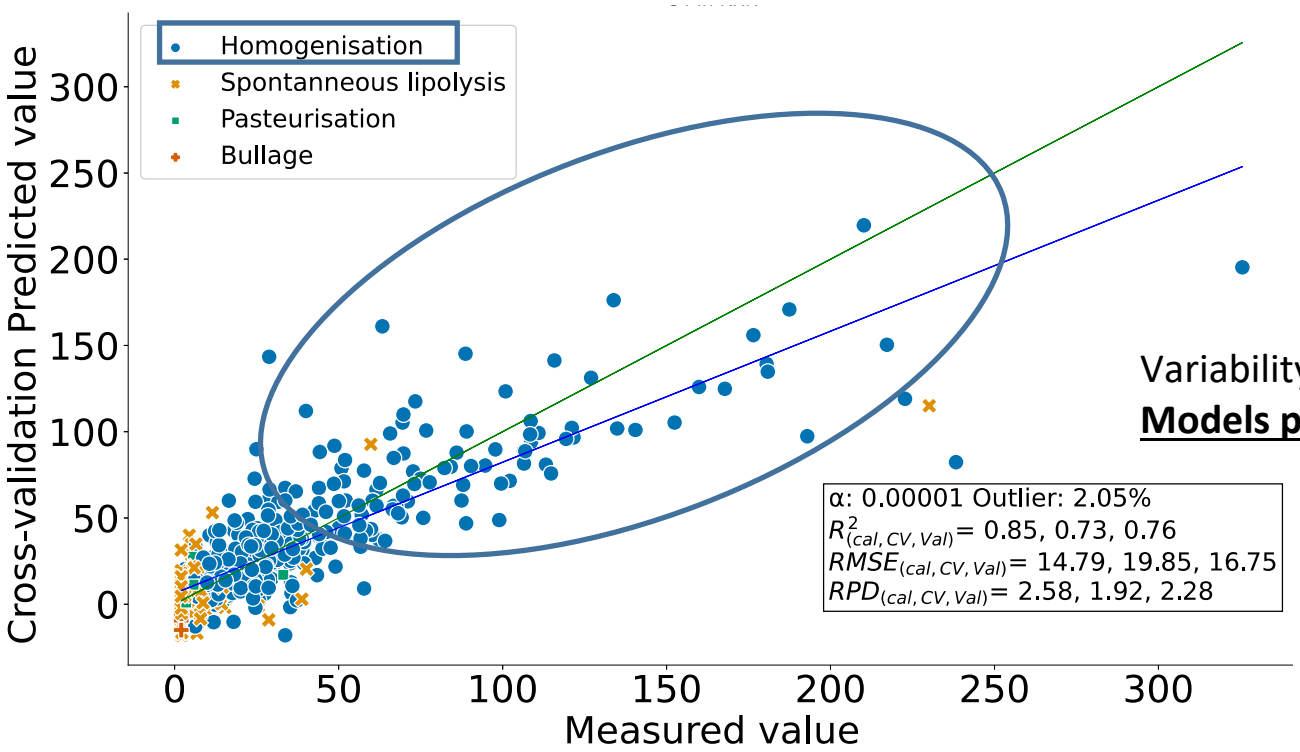
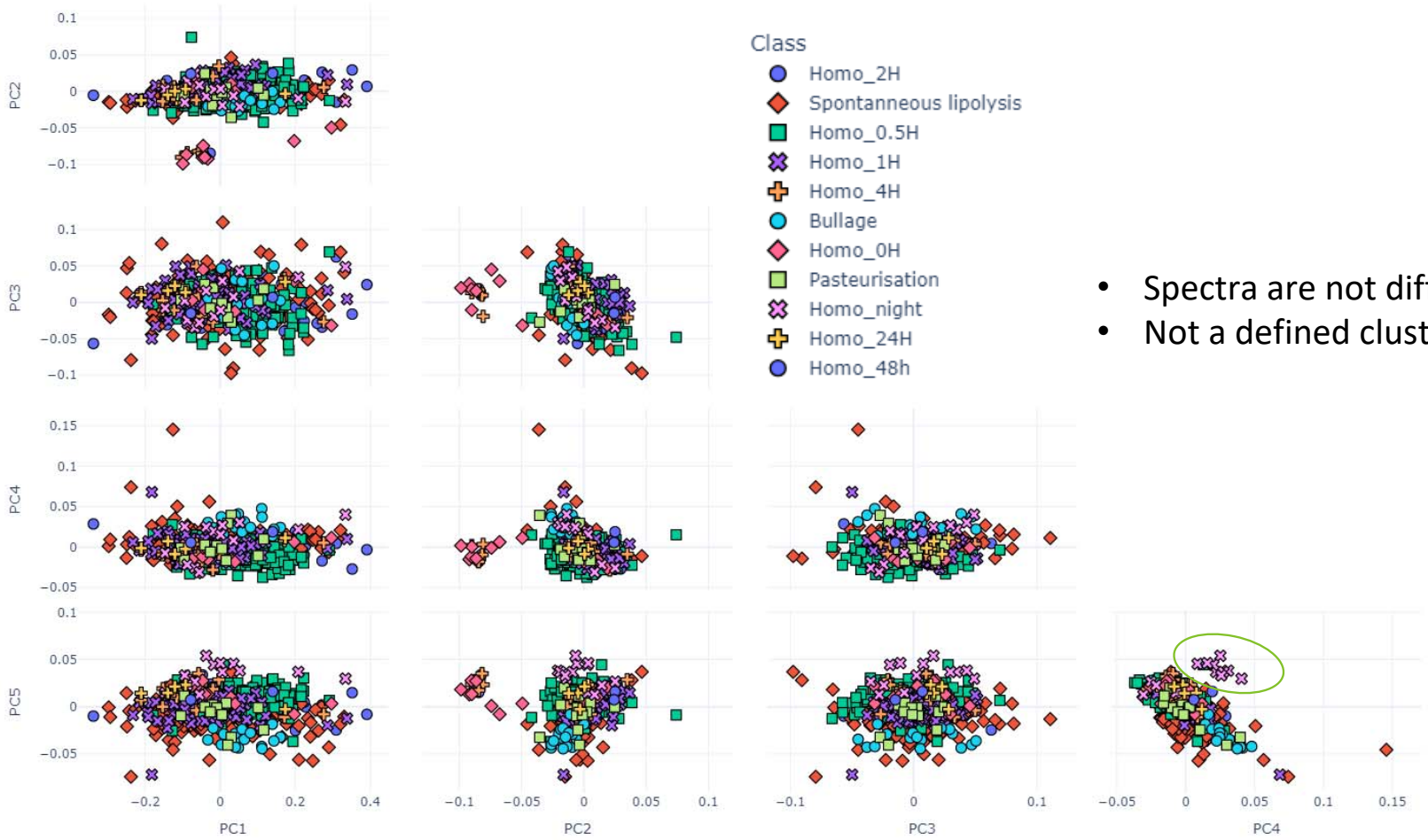
Final models



Variability mainly come form storage after homogenisation

Models predict storage after homogenization or FFA ?

PCA Analysis



- Spectra are not different
- Not a defined cluster with PCA

10 milk

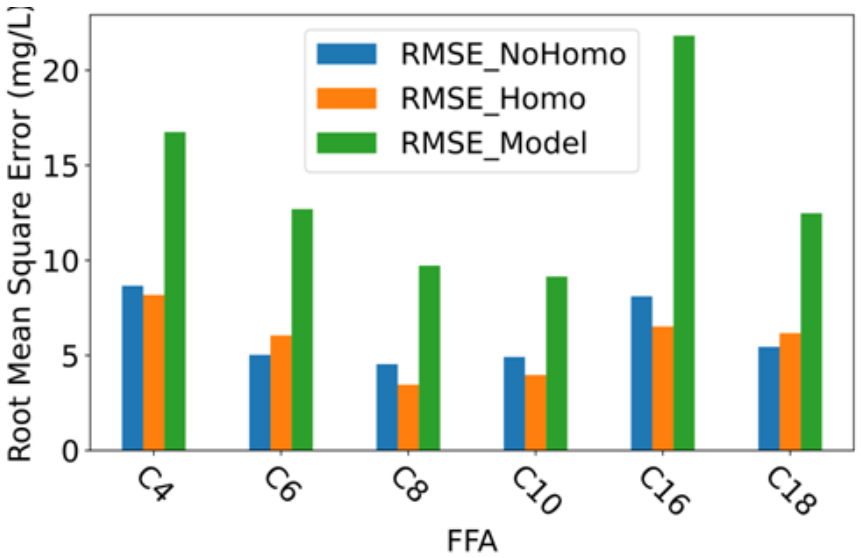
Pasteurisation 72°C
+ samples divide in 2

2x10 Pasteurized milk

Storage 4h after Homogenization

Mid infrared → FFA Prediction
GC-MS/MS → FFA Reference

Mid infrared → FFA Prediction
GC-MS/MS → FFA Reference



- As the **RMSE** in homogenized pasteurized milk is **small** → time after homogenization is **not** predicted

Discriminant models

FFA	Algo	Threshold (Q3)	Accuracy	Specificity	Sensitivity
C4	PLS-DA	31.31	0.83	0.87	0.71
C6	PLS-DA	24.60	0.84	0.88	0.74
C8	PLS-DA	16.46	0.83	0.87	0.74
C10	SVM	17.20	0.87	0.92	0.71
C12	PLS-DA	11.08	0.77	0.78	0.74
C14	PLS-DA	15.72	0.77	0.76	0.78
C16	PLS-DA	45.60	0.76	0.75	0.81
C18	SVM	38.50	0.83	0.84	0.81
C18:1	PLS-DA	16.19	0.64	0.65	0.62
C18:2	SVM	3.30	0.80	0.87	0.52
SCFFA	PLS-DA	75.14	0.84	0.88	0.73
MCFFA	PLS-DA	44.90	0.81	0.83	0.75
LCFFA	PLS-DA	103.23	0.72	0.70	0.76

3 algorithm were utilised in order to make discriminant analysis: **PLS-DA**, Random Forest **(RF)** and Support Vector Machine **(SVM)**
Threshold were arbitraly choosen of **75%** of the percentile
Performances are calculated

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Discriminant models

FFA	Algo	Threshold (Q3)	Accuracy	Specificity	**Sensitivity**
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C6	PLS-DA	24.60	0.84	0.88	**0.74**
C8	PLS-DA	16.46	0.83	0.87	**0.74**
C10	SVM	17.20	0.87	0.92	**0.71**
C12	PLS-DA	11.08	0.77	0.78	**0.74**
C14	PLS-DA	15.72	0.77	0.76	**0.78**
C16	PLS-DA	45.60	0.76	0.75	**0.81**
C18	SVM	38.50	0.83	0.84	**0.81**
C18:1	PLS-DA	16.19	0.64	0.65	**0.62**
C18:2	SVM	3.30	0.80	0.87	**0.52**
SCFFA	PLS-DA	75.14	0.84	0.88	**0.73**
MCFFA	PLS-DA	44.90	0.81	0.83	**0.75**
LCFFA	PLS-DA	103.23	0.72	0.70	**0.76**

3 algorithm were utilised in order to make discriminant analysis: **PLS-DA**, Random Forest **(RF)** and Support Vector Machine **(SVM)**
Threshold were arbitraly choosen of **75%** of the percentile
Performances are calculated

Accuracy and Specificity is better in SVM than PLS-DA
→ Main Goal of the algorithm is to correctly classified high value and avoid false positif

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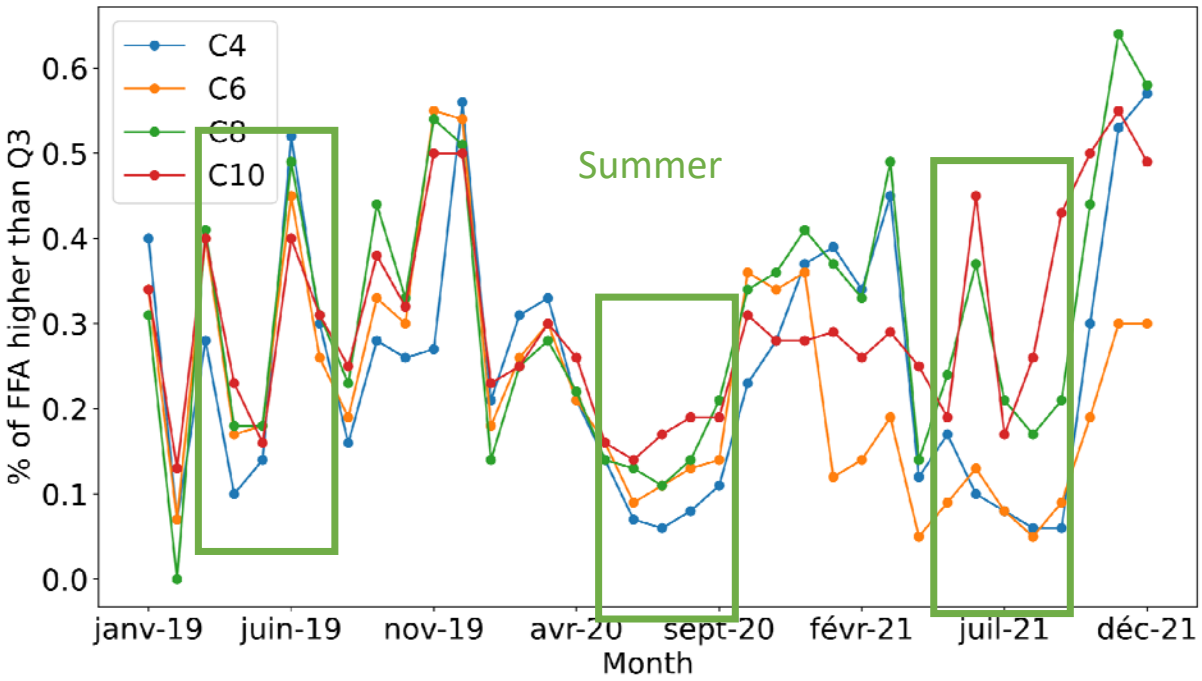
Large scale prediction

Applied on the Luxembourg DB 2 Year 2019-2021 on **569197** spectra
Month representation of barn with at least 1 cows higher than the threshold (Q3)



The 4 free fatty acids follow more or less the same trend

Minimum in 2020 Warmer spring and summer

Hypothesis: grazing lead to a better taste of the milk

Conclusion & outlook

- Conclusion

- Models better with storage time after Homogenization
- Storage time after Homogenization really increased the lipolysis

- The model predict FFA and not time after Homogenization through PCA, large scale prediction and Pasteurization test
- Good model for SCFFA
- Discriminant model works

- Outlook

- **Sensory Threshold** for milk unfit for consumption
- Fine tuned threshold Q3 is not perfect
- New Validation with real sample

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Thank you for you attention

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partners and sub - partners of the project

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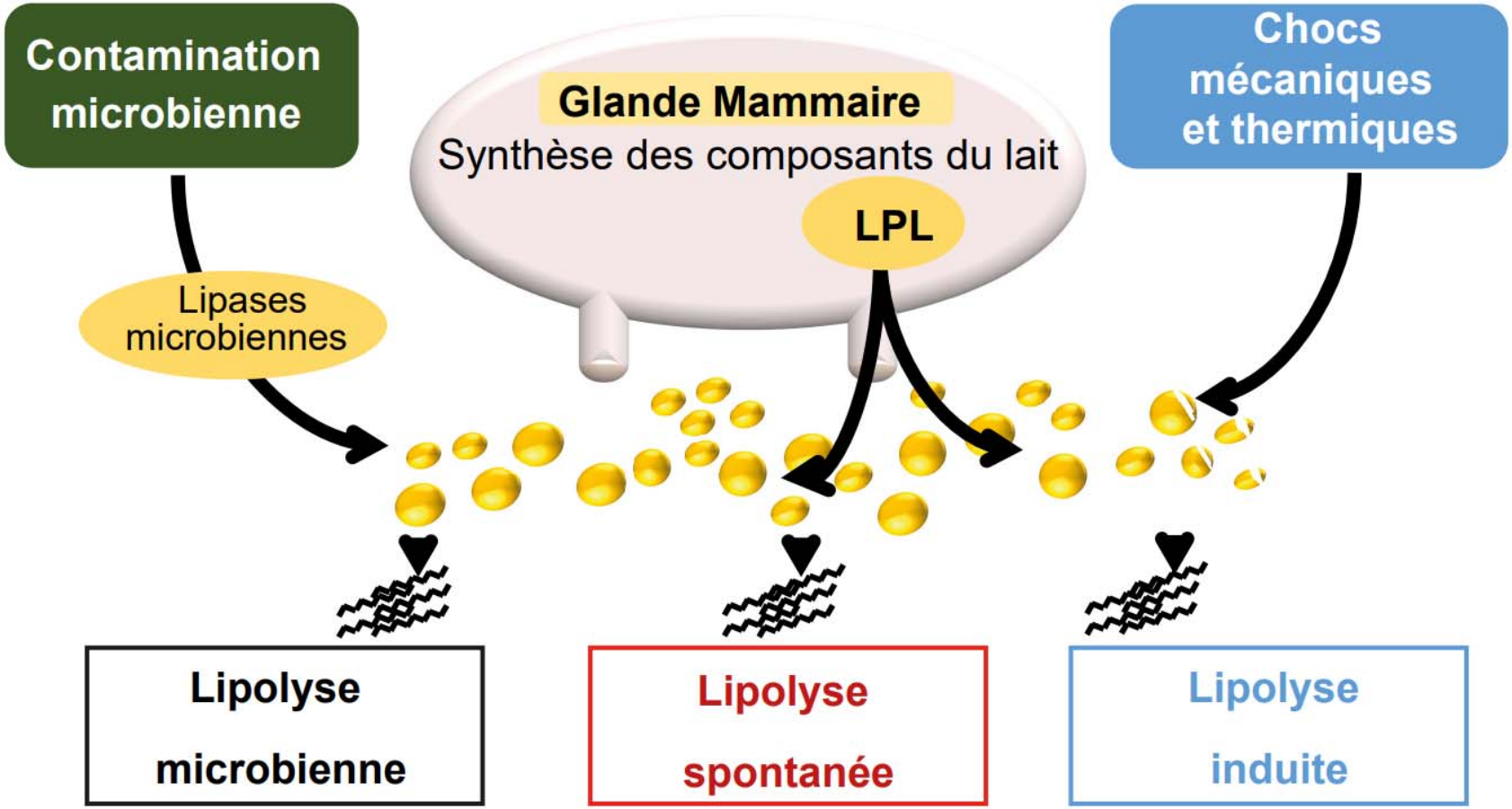
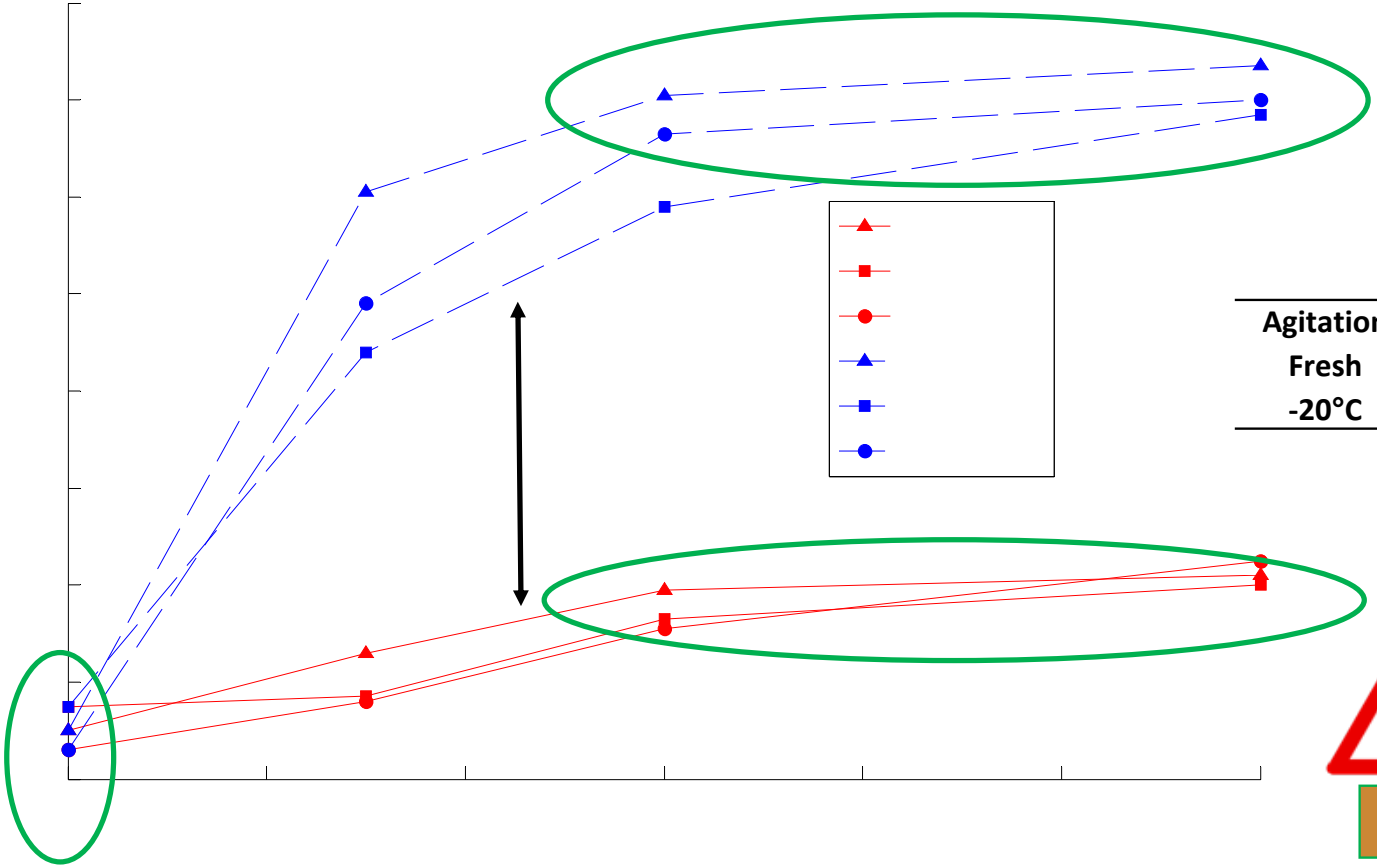
Introduction: kind of lipolysis



VANBERGUE, Elise, POULET, Jean-Louis, PEYRAUD, Jean-Louis, *et al.* Le point sur la lipolyse du lait de vache: facteurs de variations et mécanismes biochimiques. *INRAE Productions Animales*, 2020, vol. 33, no 1, p. 41-52.

B: Induced lipolysis: Vortex and freezing



Agitation	C4 (mg/l)	C6 (mg/l)	C8 (mg/l)
Fresh	106%	71%	150%
-20°C	456%	348%	515%

Mir and Frozen milk are not compatible

Freezing unusable

B: Induced lipolysis: Bubbling

- Bubbling during 2 min at 0.4 bar
- With Nitrogen to avoid any oxidation

	C4	C6	C8	C10	C12	C14	C16	C16:1	C18	C18:1	C18:2
Milk 16	<2	<3.5	<2.5	3.75	<5.5	4.52	13.63	<3	12.66	<4.5	<3
Milk 17	<2	<3.5	<2.5	<3.5	<5.5	<3.5	12.8	<3	15.77	<4.5	<3
Milk 18	<2	<3.5	<2.5	<3.5	<5.5	<3.5	10.76	<3	13.2	<4.5	<3
Milk 19	<2	<3.5	<2.5	<3.5	<5.5	4.93	13.31	<3	12.72	4.55	<3
Milk 20	<2	<3.5	<2.5	<3.5	<5.5	<3.5	10.69	<3	13.6	<4.5	<3
Availability	35%	15%	35%	55%	10%	65%	100%	0%	100%	45%	0%

- Bubbling not induced a lot of lipolysis
- Bad news → easy to perform and compatible with Mid-IR

Discriminant models

Applied on the Luxembourg DB 2 Year 2019-2021 on 569197 spectra
Pourcentage higher than the threshold (Q3)

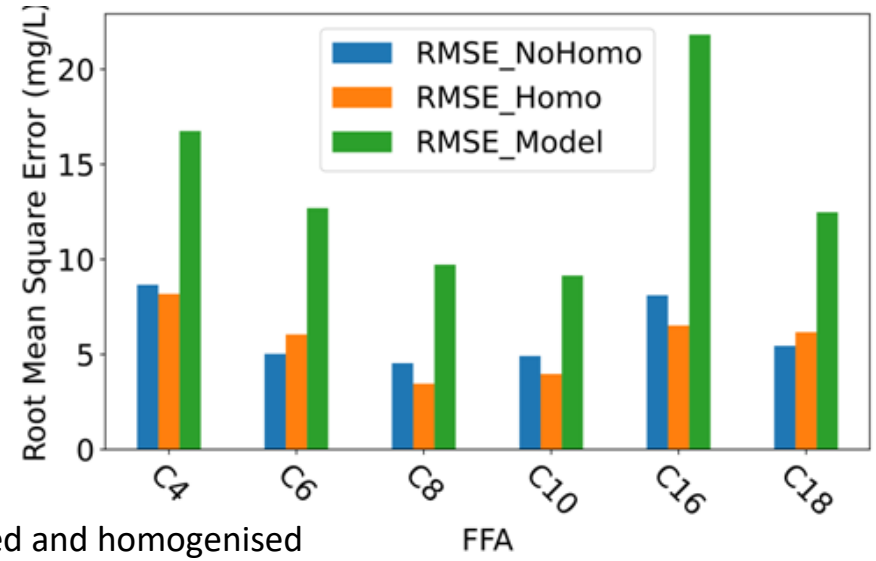
	PLS-DA	RF	SVM	SVM-PLS
C4	30.8	2.8	2.2	15.0
C6	16.5	3.4	3.8	10.5
C8	16.2	1.7	7.9	13.8
C10	19.7	2.9	11.3	21.0
C12	34.8	5.4	10.1	12.7
C16	24.9	6.6	19.3	12.1
C18	8.3	8.3	24.6	23.0
SCFFA	19.7	2.6	4.3	14.7
MDFFA	26.1	4.6	8.6	30.5
LCFFA	24.1	5.3	6.1	4.1

PLS-DA: 1271
RF: 1412
SVM: 596
SVM-PLS: 11
→ samples higher in each FFA

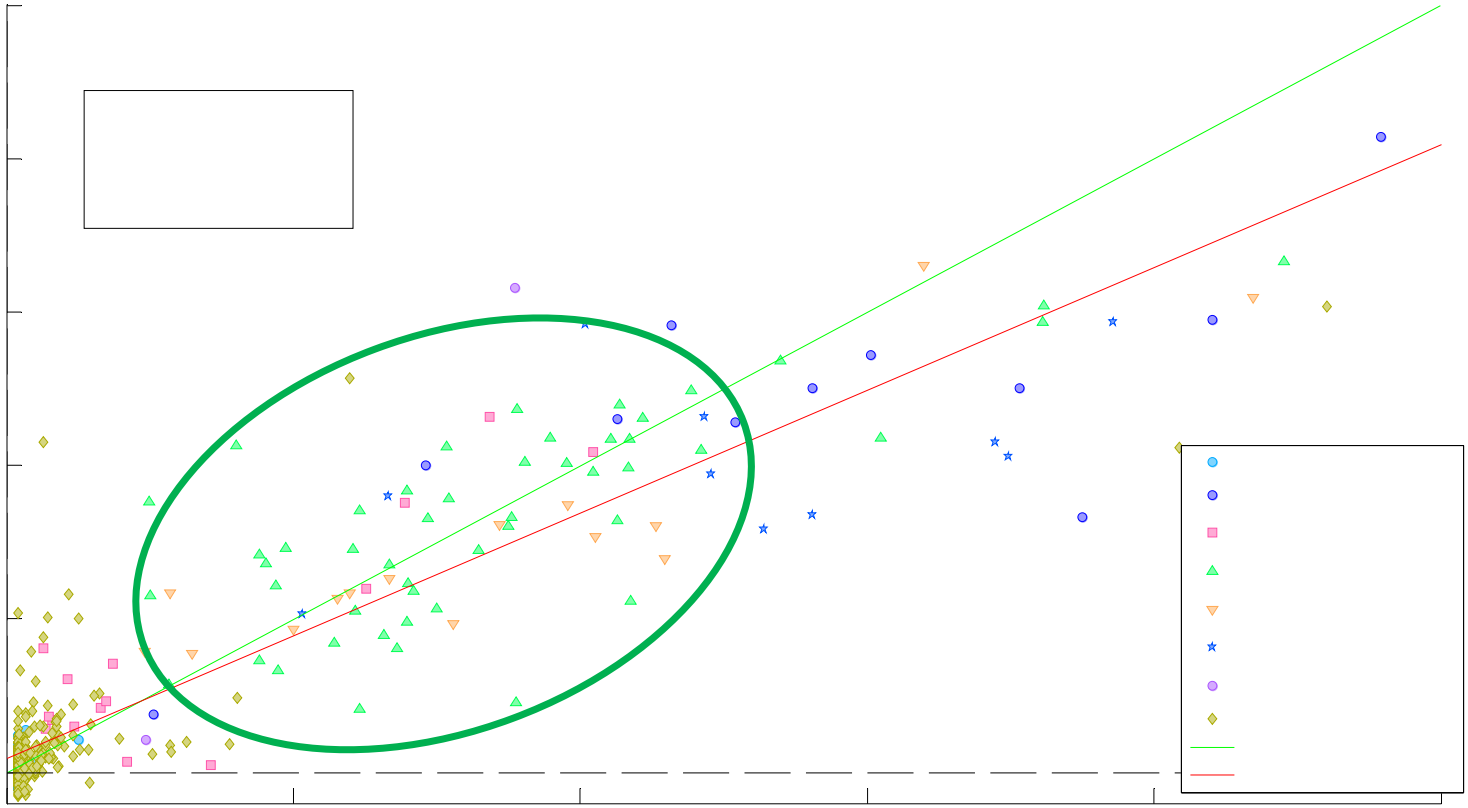
Pasteurisation milk

Problem: Is it the time after homogenisation predict?
Check: 10 milk samples are pasteurised then extracted directly and spectrally, the other sample is homogenised for 4 hours then spectrally extracted. → graph
Reference value (GC-MS/MS): Around 20% → Pasteurisation work, lipolysis is inhibited after pasteurisation!
What about predictions? After 4h after homogenization, if the model predict time, the errors value between reference data and predicted data should be very strong (more than 100%), However this is not the case RMSE is under the errors of the model → Model Predict FFA

	RMSE_NoHomo	RMSE_Homo	RMSE_Model
C4	8.66	8.18	16.75
C6	5.03	6.05	12.70
C8	4.54	3.46	9.72
C10	4.92	3.97	9.15
C16	8.11	6.52	21.82
C18	5.45	6.17	12.48

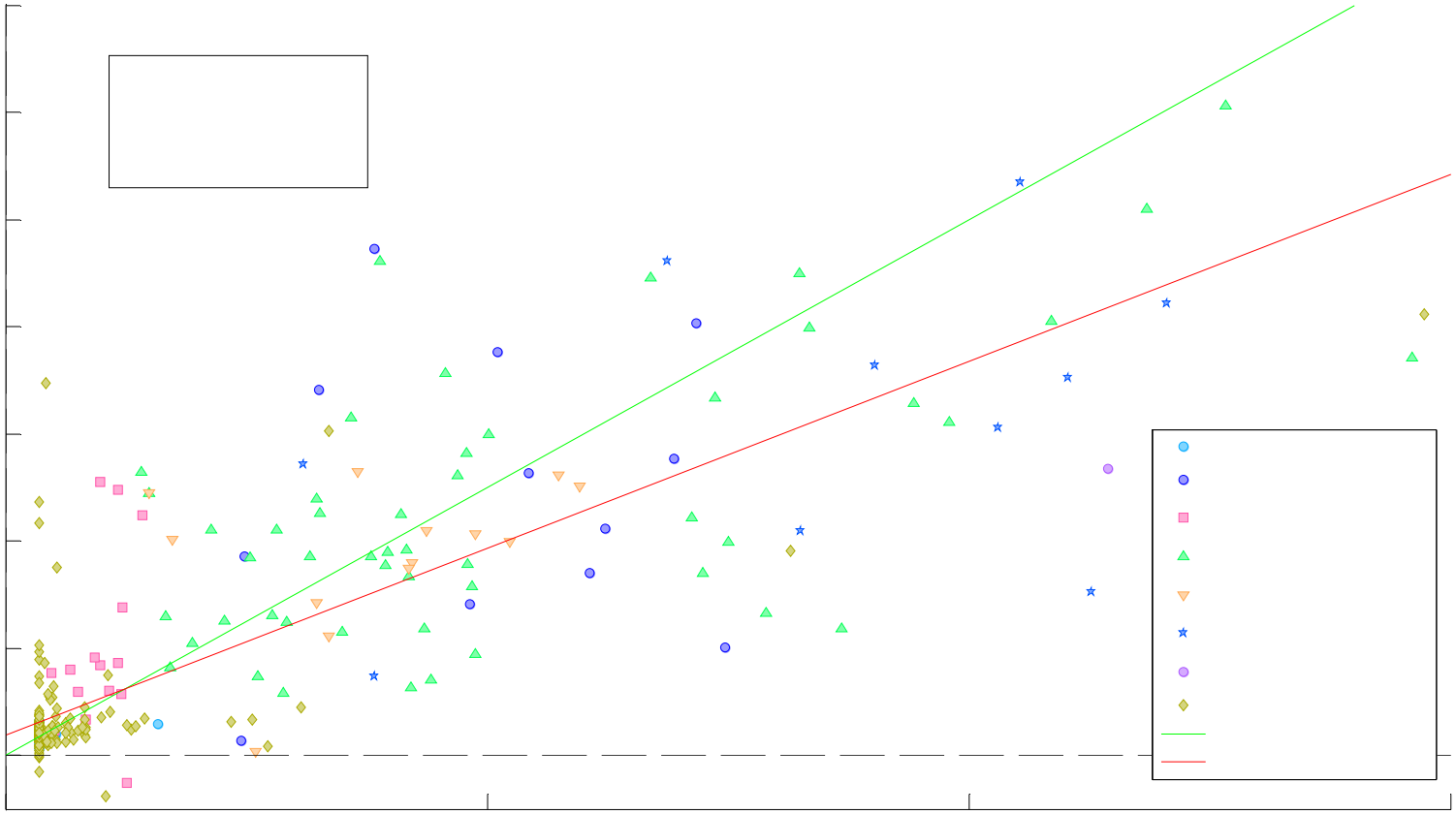


1. Error seems to be equal between pasteurised milk: non-homogenised and homogenised
2. AS the Error in homogenised pasteurised milk is small → homogenisation time is not predicted



SVM Parameter
E-SVM algoritm
X-block conversion: pls 13
N-fold CV split: 6
10 outlier
SD 46.4 → RPD 2.43

Classic PLS 13 → R^2 0.733 RMSE 24.00
With N-fold 10 → R^2 0.830 RMSE 19.09
With Nu-SVR algo → R^2 0.809 RMSE 20.24
PCA 13 → R^2 0.66 RMSE 27.29



SVM Parameter
E-SVM algoritm
X-block conversion: pls 9
N-fold CV split: 6
8 outlier
SD 25.5 → RPD 1.75

PLS: $R^2$0.583 RMSE 16.56

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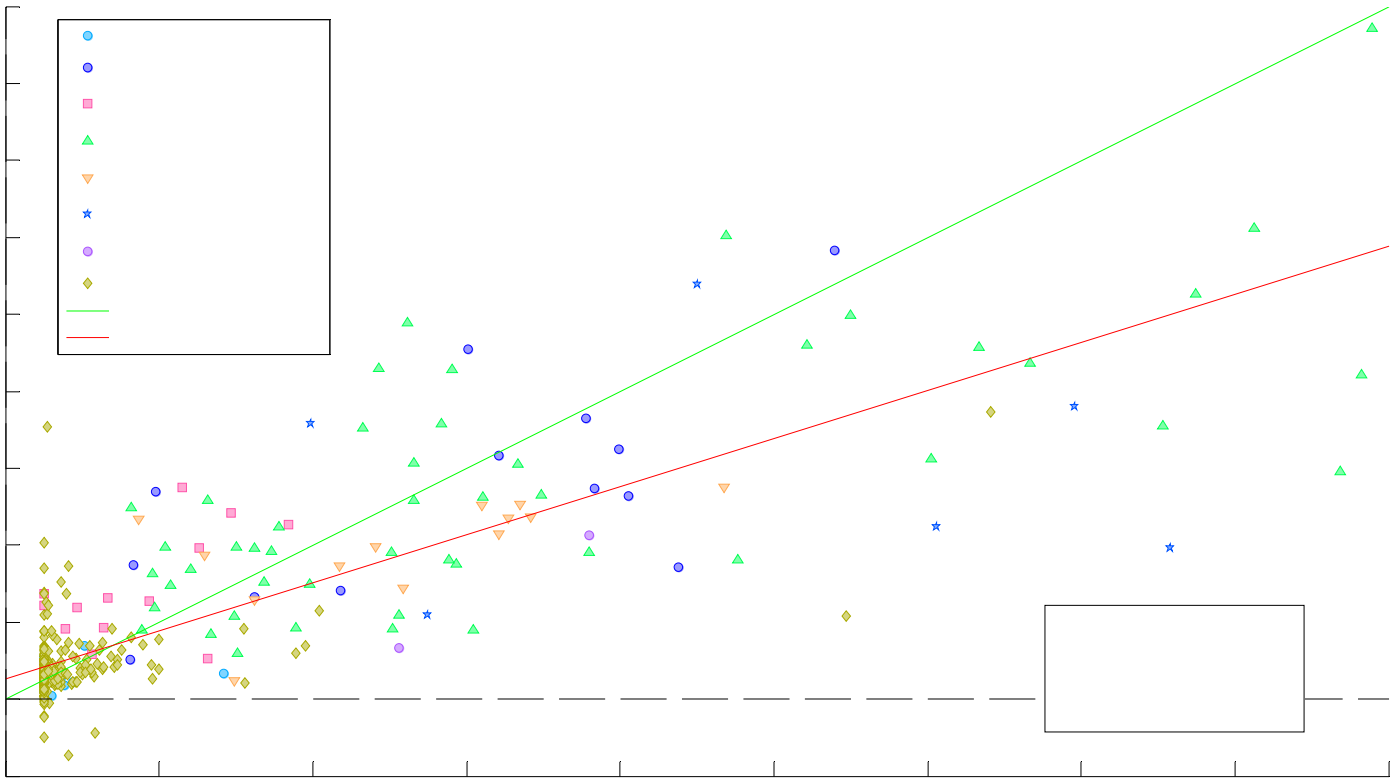
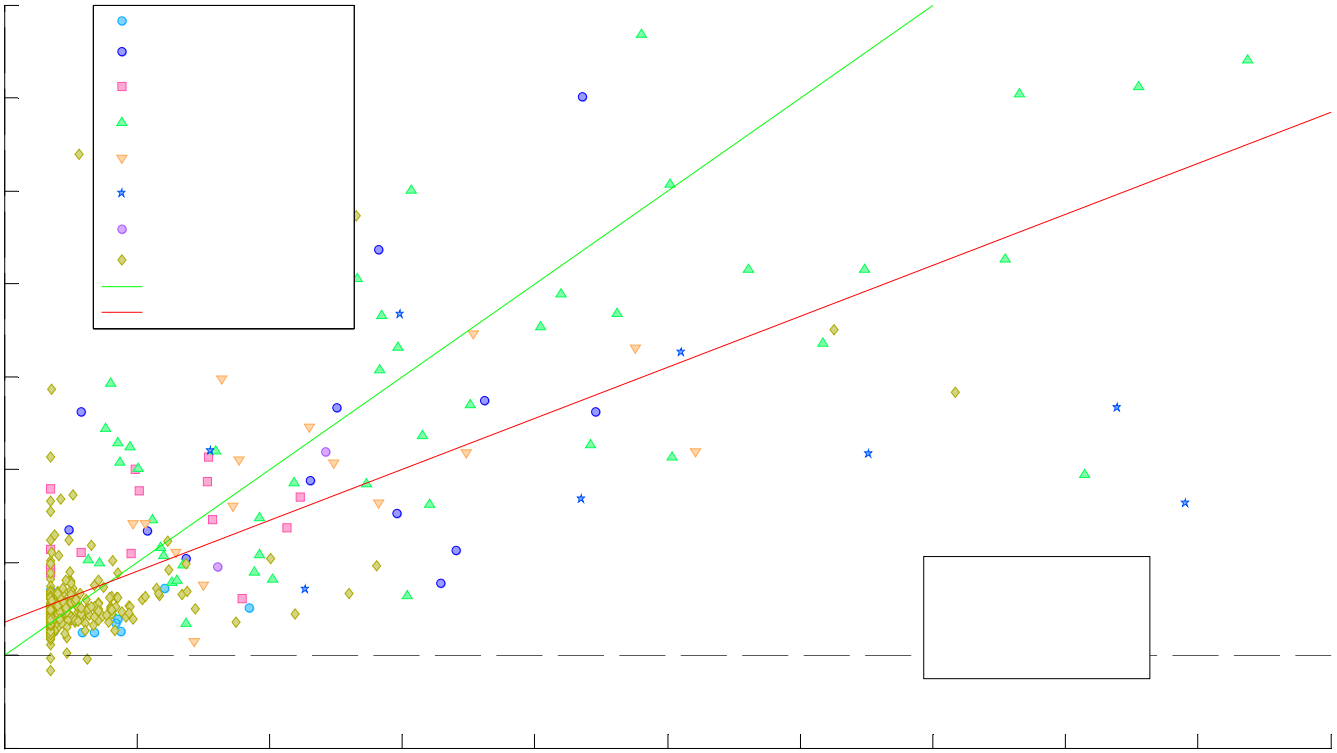


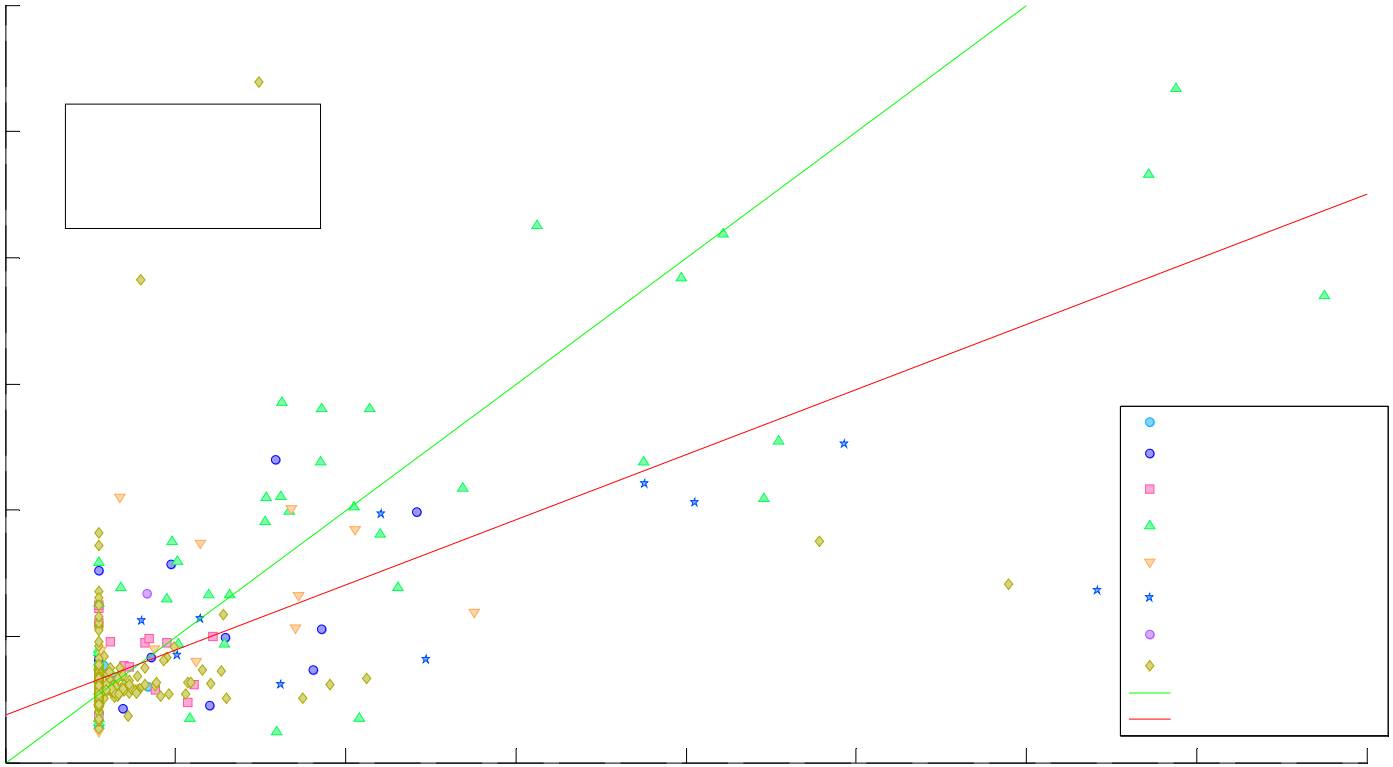
SVM Parameter
Nu-SVM algoritm
X-block conversion: pls 10
N-fold CV split: 6
13 outlier
SD 15.5 → RPD 1.74

PLS: R^2 0.557 RMSE 10.42



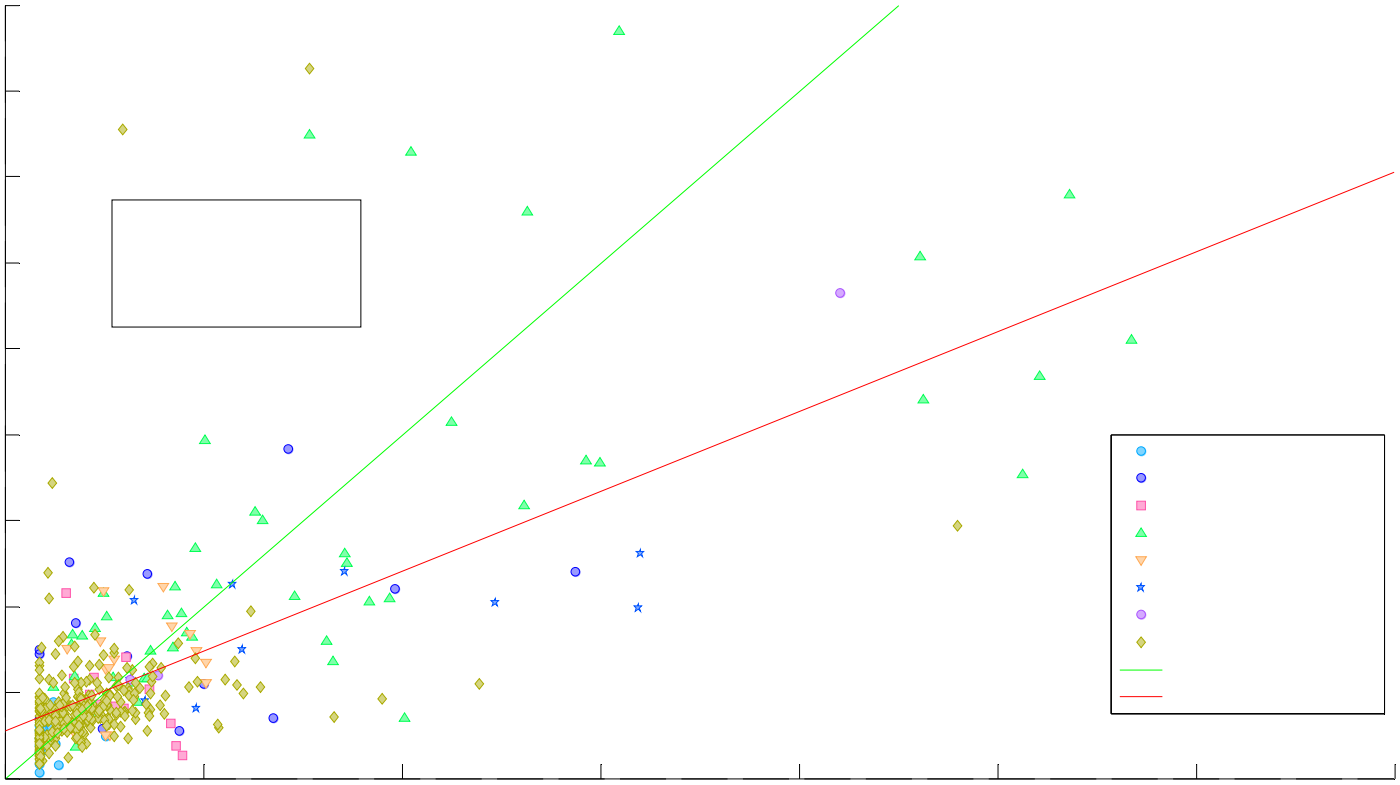
SVM Parameter
E-SVM algoritm
X-block conversion: pls 10
N-fold CV split: 6
8 outlier
SD 15.0 → RPD 1.50

PLS: R^2 0.475 RMSE 11.06



SVM Parameter
E-SVM algoritm
X-block conversion: pls 13
N-fold CV split: 6
7 outlier
SD 9.25 → RPD 1.43

PLS: R^2 0.438 RMSE 6.94



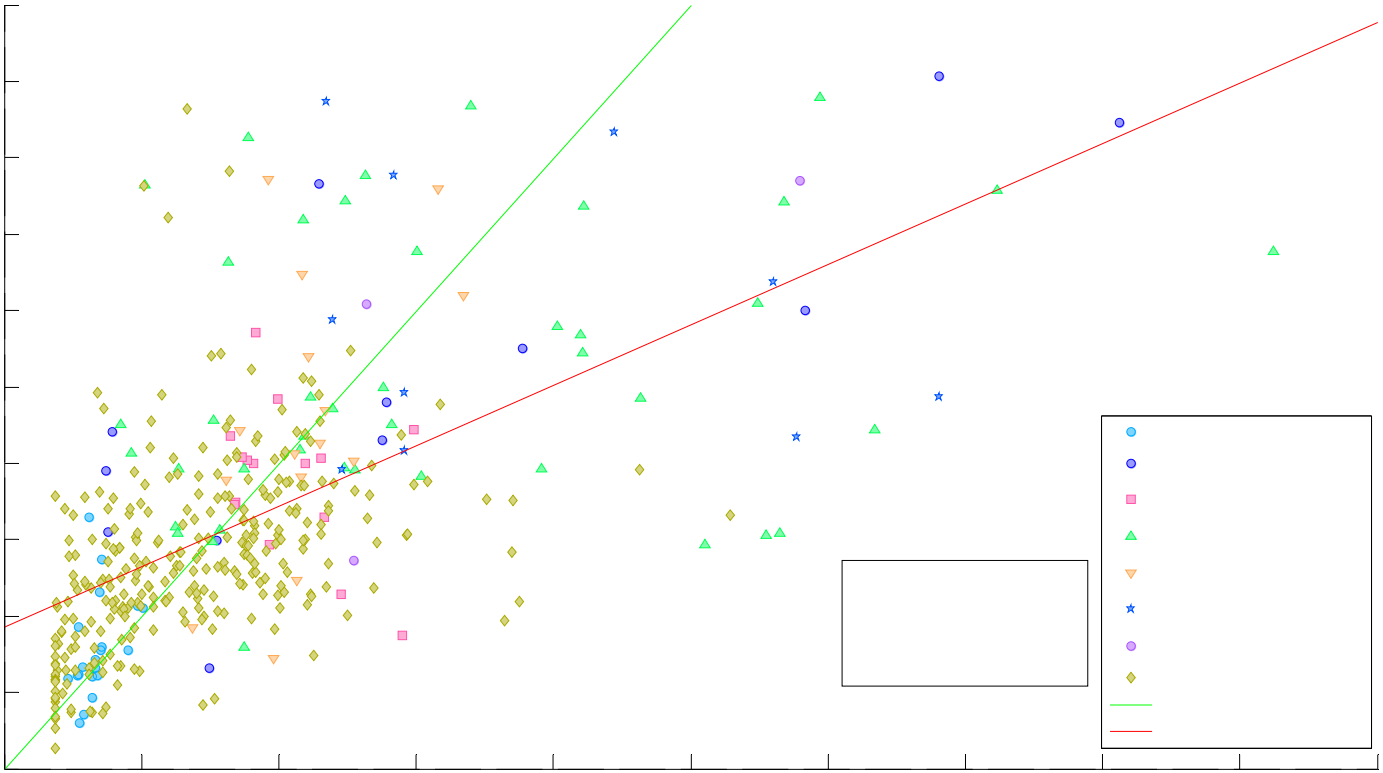
SVM Parameter
E-SVM algoritm
X-block conversion: pls 13
N-fold CV split: 6
1 outlier
SD 13→ RPD 1.06

PLS: R^2 0.41 RMSE 12.93

- Homogenization of milk
- Milk storage at 4°C during 12h
- Vortex 30 sec of milk 6-10

Difference (%) of FFA between sample not homogenized and homogenized

	C4	C6	C8	C10	C12	C14	C16	C16:1	C18	C18:1
Milk 1	2611%	1950%	1411%	745%	77%	284%	174%	0%	19%	23%
Milk 9	4509%	1883%	1520%	1174%	339%	1541%	1616%	113%	520%	2234%
Milk 10	511%	530%	592%	433%	128%	293%	292%	0%	63%	110%



SVM Parameter
E-SVM algoritm
X-block conversion: pls 10
N-fold CV split: 6
15 outlier
SD 26 → RPD 1.26

PLS: R^2 0.277 RMSE 22.68

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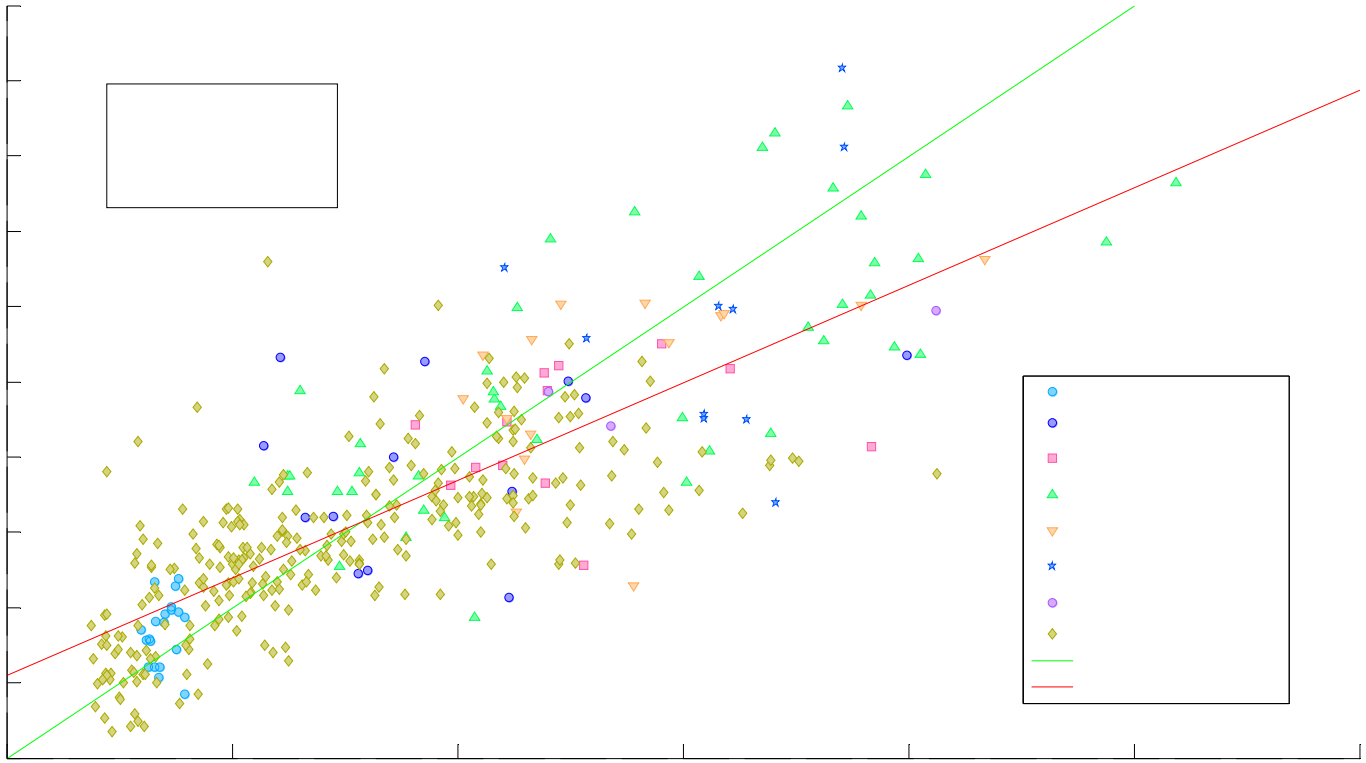
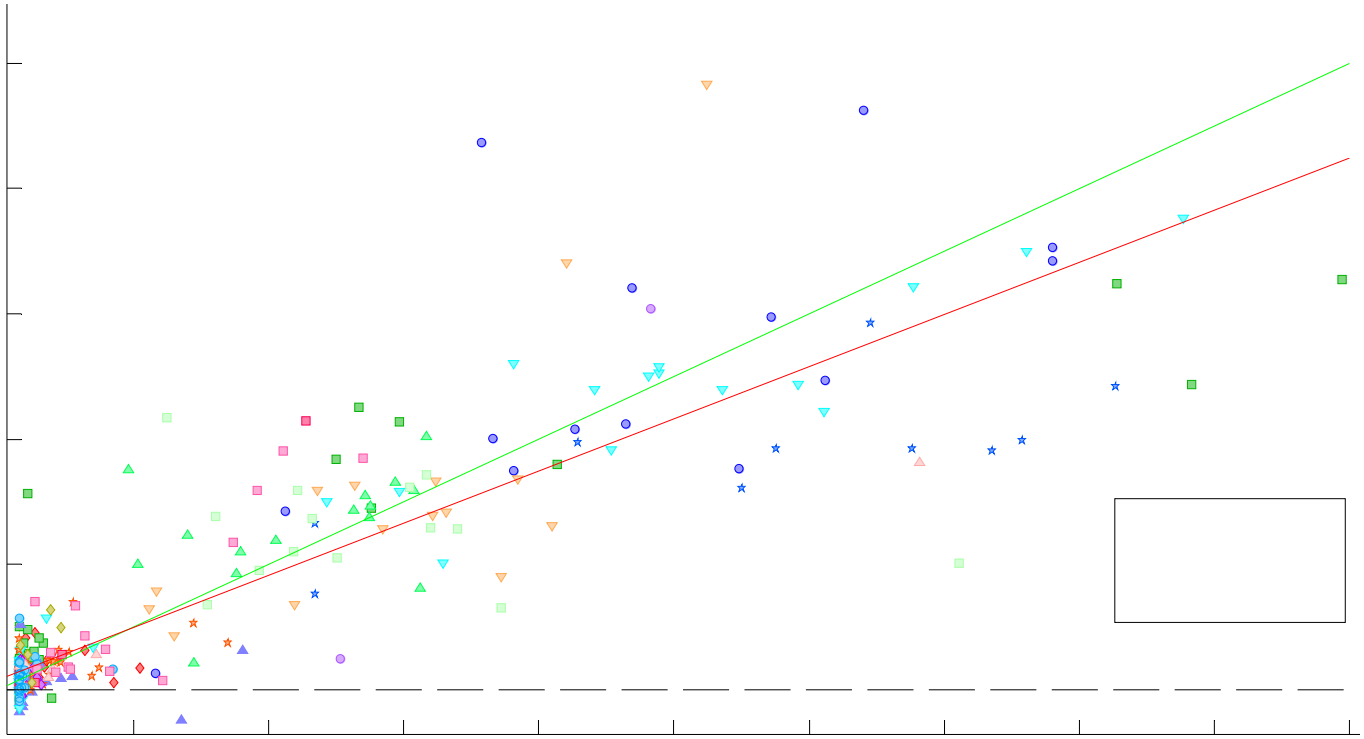


SVM Parameter
E-SVM algoritm
X-block conversion: pls 13
N-fold CV split: 6
19 outlier
SD 19 → RPD 1.66

PLS: R^2 0.59 RMSE 12.23

Volatile Free Fatty acid (VFFA)



SVM Parameter
E-SVM algoritm
X-block conversion: pls 13
N-fold CV split: 6
19 outlier
SD 93.25 → RPD 2.18
Cost 10000

PLS 0.789 RMSE 42.93

Data of spontaneous lipolysis

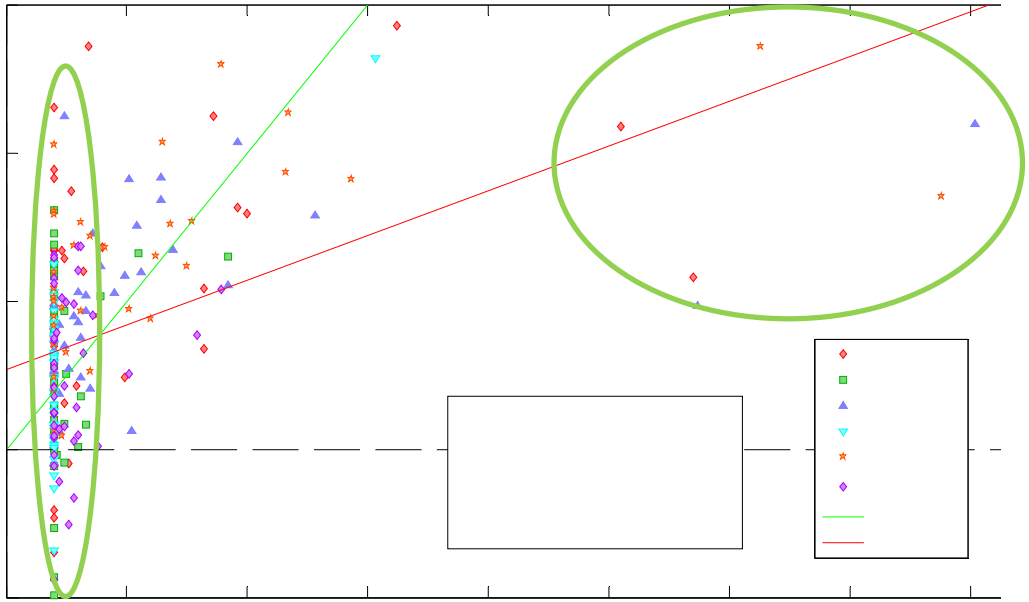
Percentage of usable data → Higher than the Limit of the quantification (LOQ)

	C4	C6	C8	C10	C12	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20
Belgium	43%	21%	43%	31%	0%	52%	98%	0%	100%	50%	0%	0%	0%
Germany 1	26%	2%	7%	43%	21%	100%	100%	21%	100%	90%	31%	2%	45%
Germany 2	74%	36%	50%	74%	40%	100%	100%	38%	100%	95%	93%	5%	93%
Luxemburg	3%	3%	28%	58%	33%	85%	100%	5%	100%	73%	18%	3%	13%
France 1	55%	48%	64%	90%	38%	100%	100%	29%	100%	100%	57%	10%	40%
France 2	52%	7%	10%	2%	2%	29%	52%	7%	98%	40%	2%	0%	0%
Total	42%	20%	34%	50%	22%	78%	92%	17%	100%	75%	34%	3%	32%

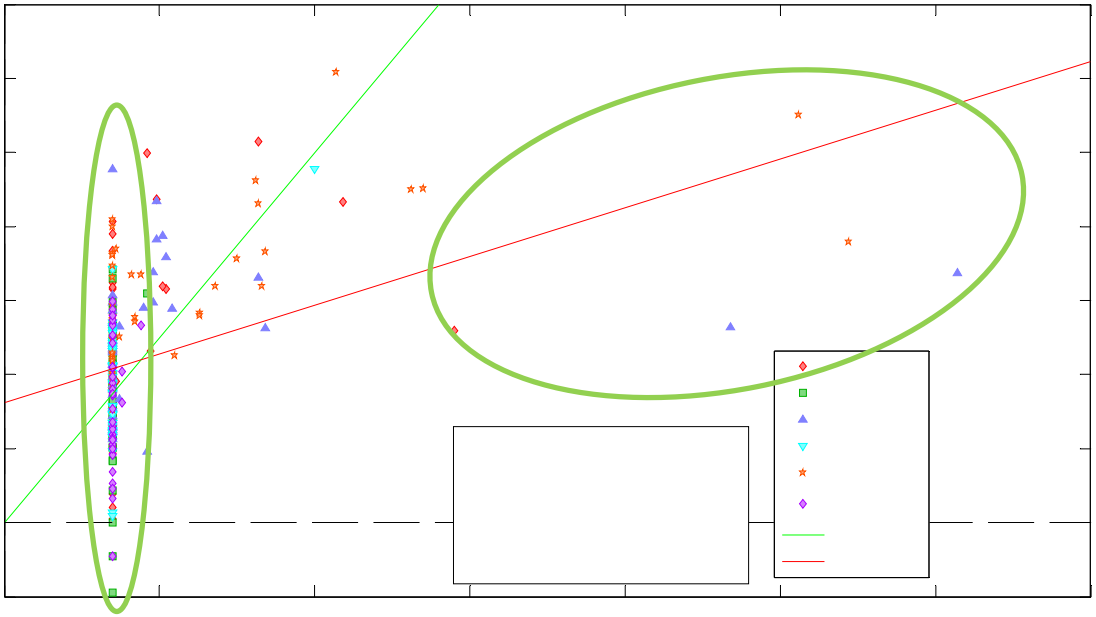
→ Lack of available data

Short free fatty acid models

C4



C6



- The data under the Limit of Quantification (LOQ) is not usable
- Not enough high data, predictions are underestimated

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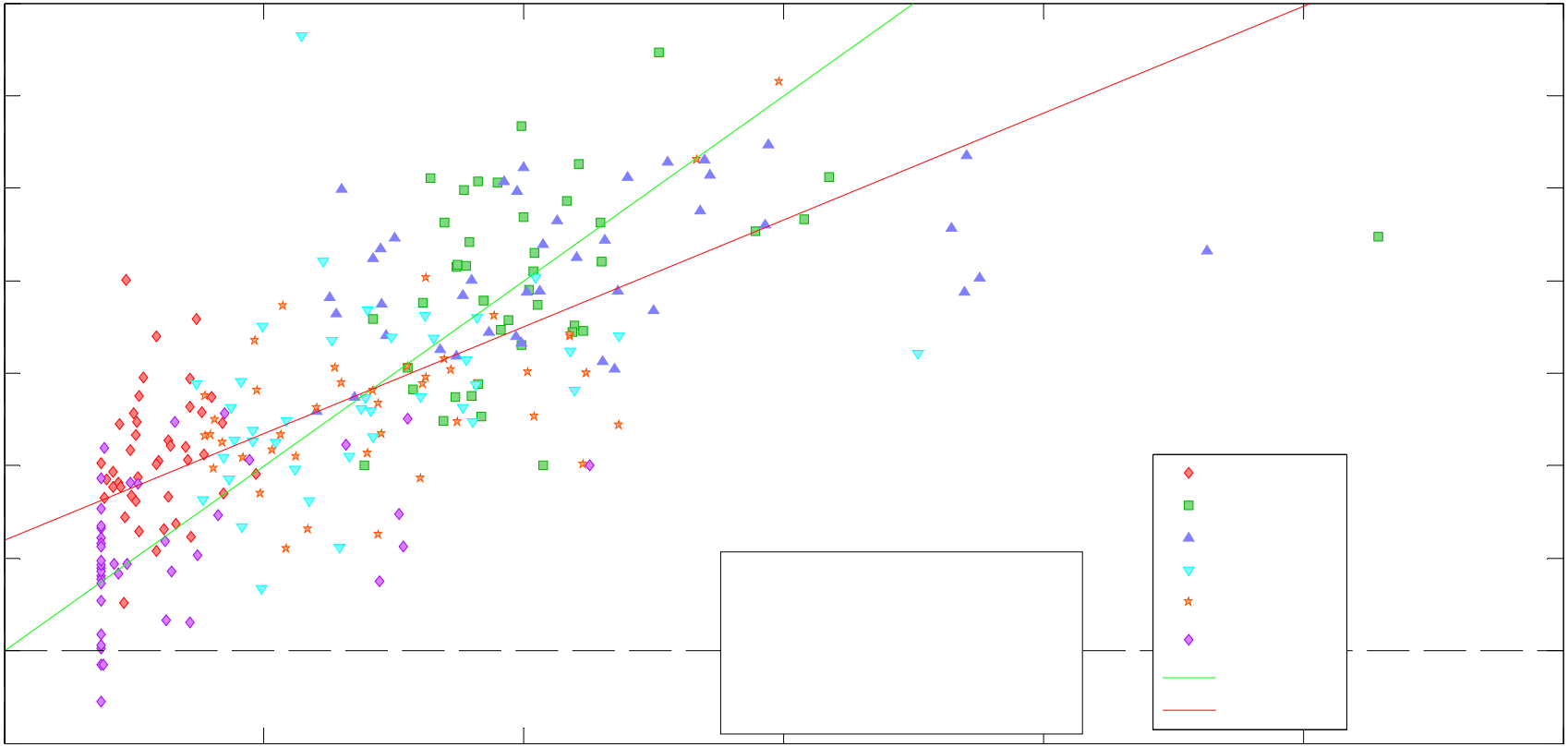
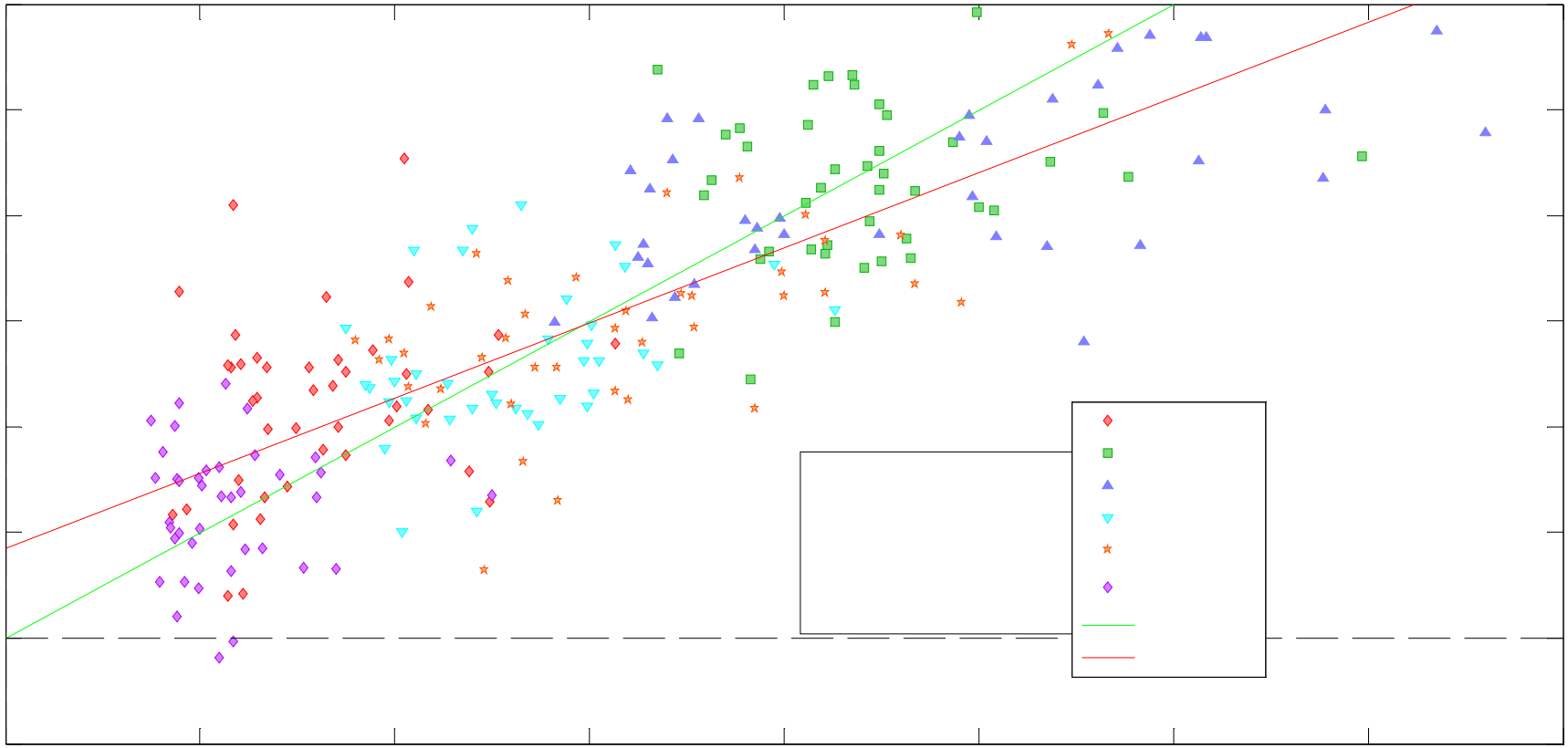
C16



C18



Performance of free fatty acid models

	Mean (mg/L)	SD (mg/L)	R^2	RMSECV	RPD
C4	3.83	5.13	0.2	4.71	1.09
C6	4.38	3.29	0.16	3.14	1.05
C8	3.32	2.28	0.23	2.04	1.12
C10	5.12	3.35	0.21	3.04	1.10
C12	6.14	1.97	0.1	1.96	1.01
C14	8.27	5.75	0.32	4.86	1.18
C16	27.95	16.72	0.503	11.9	1.41
C16:1	3.31	1.23	0.12	1.19	1.03
C18	30.21	16.74	0.66	9.02	1.86
C18:1	13.75	19.85	0.26	17.21	1.15

- Bad R^2
- The performance of the FFA prediction is dependent from the concentration of the corresponding fatty acid

Big problem of availability of the data, need variability

B: Induced lipolysis: Homogenization

- Test made with 5 different milk
- Samples directly after homogenization
- Samples after homogenization + 0.5h 2h, 4h, 12h and 48h of storage

(mg/L)	Milk 1			Milk 5		
	C4	C6	C8	C4	C6	C8
Homogenized	35.6	12.2	13.2	24.3	11.7	14.7
Storage 2H	62.6	28.9	15.1	102.3	41.6	17.3
Storage 4H	75.0	26.0	14.9	97.6	35.2	15.1
Storage 12H	69.5	24.5	18.1	88.7	32.6	9.8
Storage 48H	217.2	76.8	25.6	325.6	114.5	38.0

- 552 samples:
  - 15 Homo 0H
  - 123 Homo 0.5H
  - 50 Homo 2H
  - 15 Homo 4H
  - 14 Homo night
  - 10 Homo 24H
  - 300 spontaneous lipolysis
  - 25 bubling sample

Homogenization samples coming from 4 country: Luxemburg, Belgium, France and Germany

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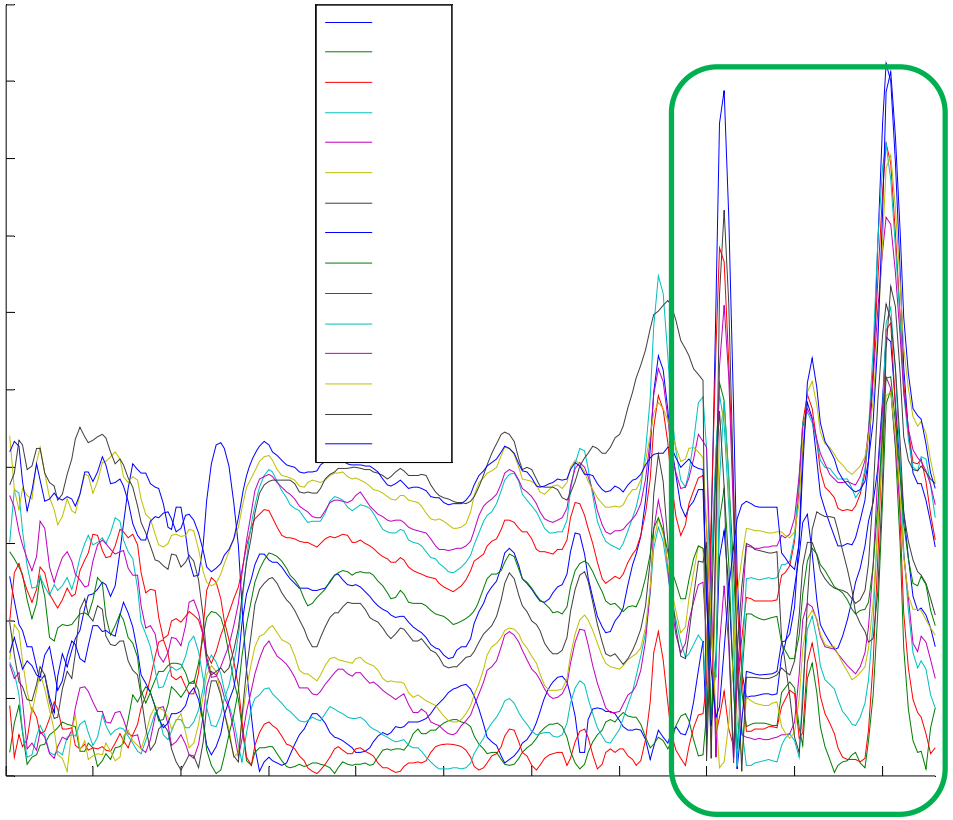
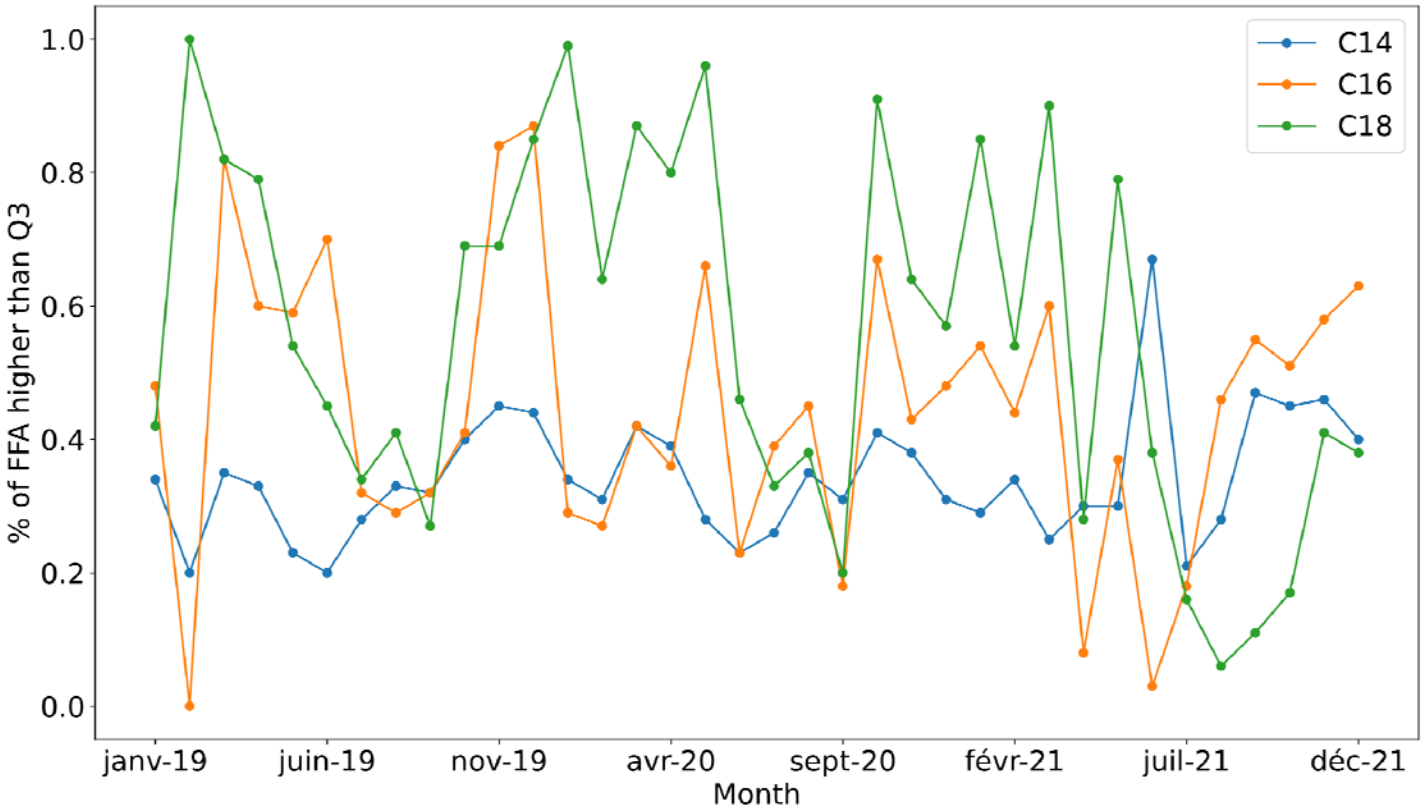
Spectra differences between non homogenized milk and homogenized milk



Sample	RMSE_Mean
Lux-1	0.0035
Lux-2	0.0015
Lux-3	0.0017
Lux-4	0.0020
Lux-5	0.0027
Lux-6	0.0029
Lux-7	0.0039
Lux-8	0.0049
Lux-9	0.0045
Lux-10	0.0060
Lux-11	0.0066
Lux-12	0.0066
Lux-13	0.0075
Lux-14	0.0073
Lux-15	0.0077

Test sur 15 lait après 2h homogenization + pasteurization: spectral differences ?



Classification is much higher for C16 and C18 → more related to spontanneous lipolysis