Cortisol determination in dairy cows hairs by Near-infrared (NIR), Mid-infrared (MIR) and Raman spectroscopy.

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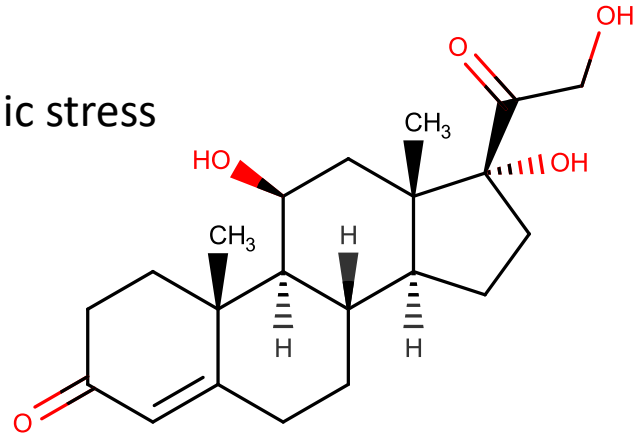
Introduction

Biomarkers of chronic stress ?

Grelet et al 2022 has determine two biomarkers of chronic stress

- hair cortisol
- glycated protein (fructosamine)



- 1047 cows hair samples were collected and analysed by ELISA and spectroscopy technique → Cortisol determination
- 134 coming from a stress experimentation → 3 spectroscopy techniques
- 913 without experimentation and coming from “Supposed stress farm” → best spectroscopy technique

The objective of this work is to evaluate the possibility of hair cortisol determination through vibrational spectroscopy
And maybe helping to directly classify if a cow is stressed or not

Grelet, C., Dries, V. V., Leblois, J., Wavreille, J., Mirabito, L., Soyeurt, H., ... & HappyMoo Consortium. (2022). Identification of chronic stress biomarkers in dairy cows. *Animal*, *16*(5), 100502.

Experiment

Stress group (severe overstocking for 4 weeks) + punctual unusual events

Control group normal situation for 4 weeks

	Number of cows	Place	Feed bunk
Stressed	15	<5 m²	7 places
Unstressed	15	>10 m²	>15 places

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Experiment: Hair preparation

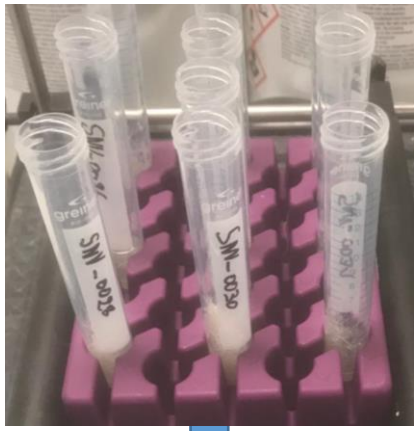
Sampling

Shaving cow's tail

Sieving

Porosity 400 → 250 → 200µm

Cleaning



3.5ml isopropanol
Vortex: 2min
5d drying

Grinding

Ball: 20mm
Time: 5min
Frequency: 22Hz

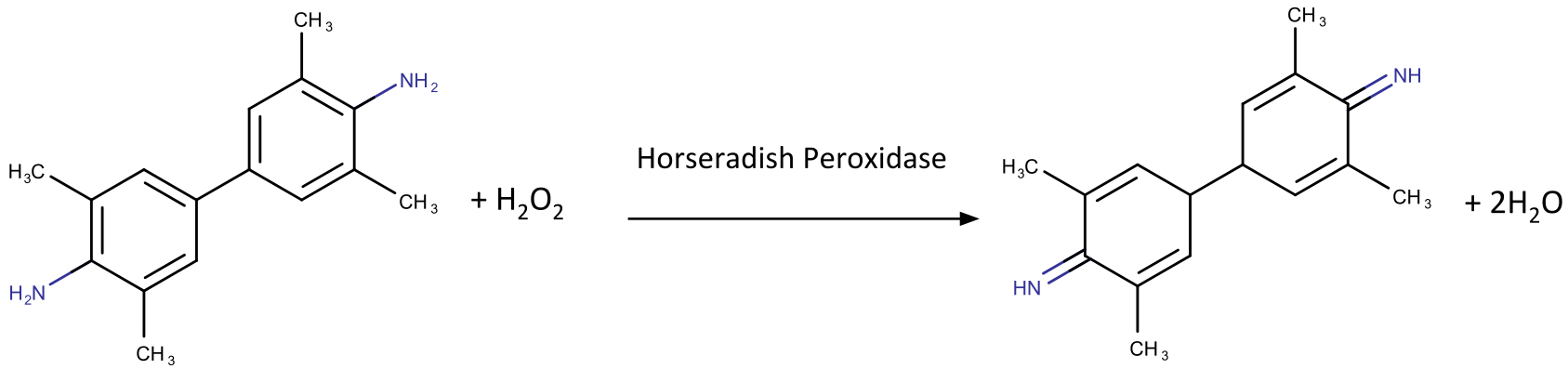
Cortisol extraction

1) 50 mg of Hair+1.5ml methanol
2) Extraction 18h 30°C
3) Centrifuge 7000 RPM 2min
4) 0.75ml of supernatant
5) Dry under vacuum
6) Complete with 0.25mL of Elisa Buffer

Spectroscopic analysis

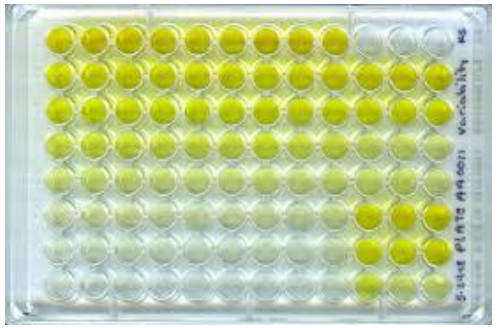
ELISA: Cortisol determination

Enzyme-Linked Immuno Sorbent Assay



Colourless

Blue compound



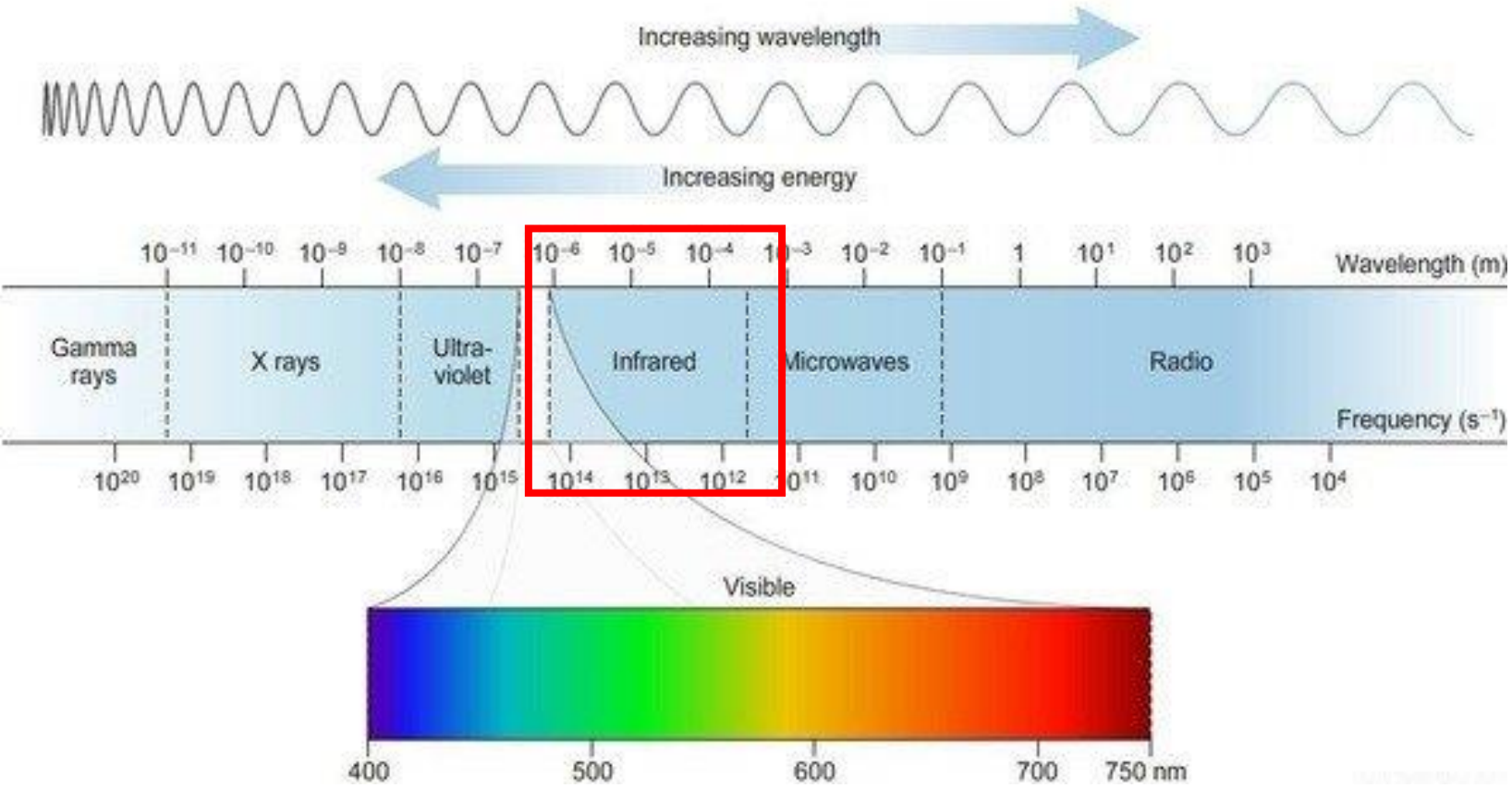
After acidic condition: =NH → $=NH_2^+$ → Yellow colour

Optical reading at 450nm

Cortisol inhibit Horseradish Peroxidase → [Cortisol] is inversely proportional to the appearance of the color

Spectroscopy: analysis

Vibrationnel spectroscopy



Focus on infrared wavelength

Near infrared (NIR): 12500 < f < 4000 cm^{-1}

Mid infrared (MIR): 4000 < f < 200 cm^{-1}

Raman: 4000 < f < 50 cm^{-1}

NIR is higher in energy so it will be more focus groupment, skeleton vibration

MIR & Raman less energy focus on the bonding vibration

Spectroscopy: Apparatus

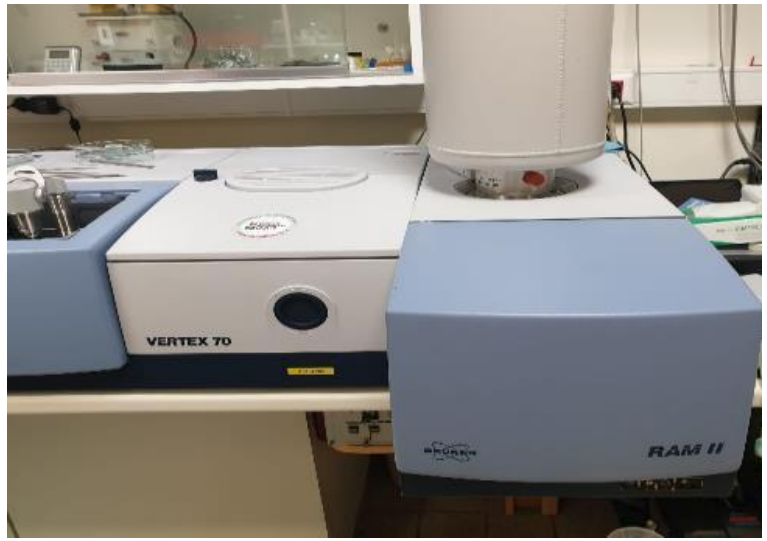
NIR

Bruker MPA spectrometer

MIR

→ ATR Platinium

Raman



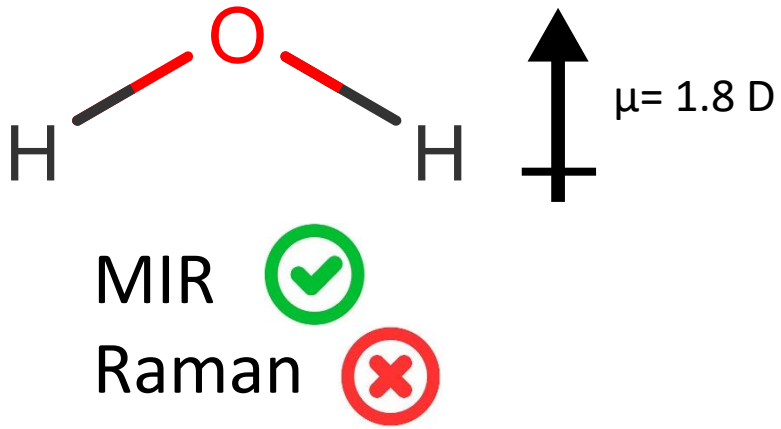
Bruker VERTEX 70

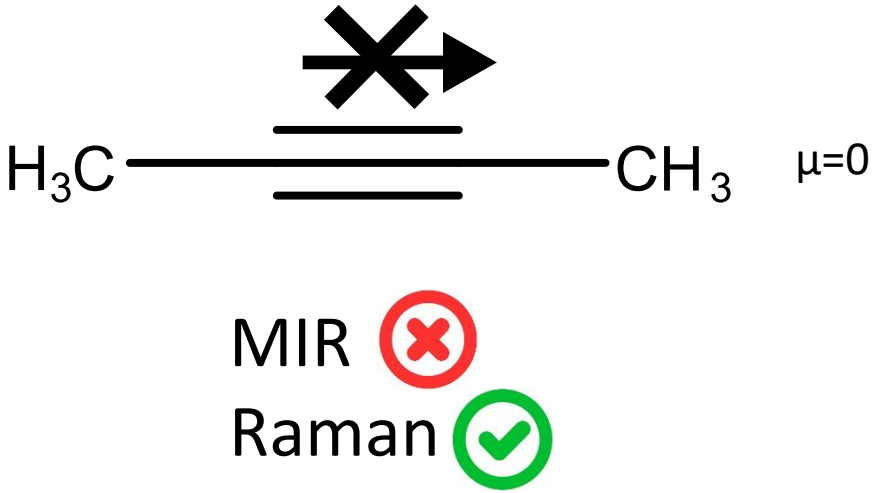
→ RAM II

Vibrationnel Spectroscopy: complementary of Mir and Raman

MIR and Raman has approximately the same excitation energy → focus on molecular structure
Both techniques will accept different vibration modes

- MIR will be related to the dipolar moment of the molecule
- Raman will be related on the polarizability of a molecule



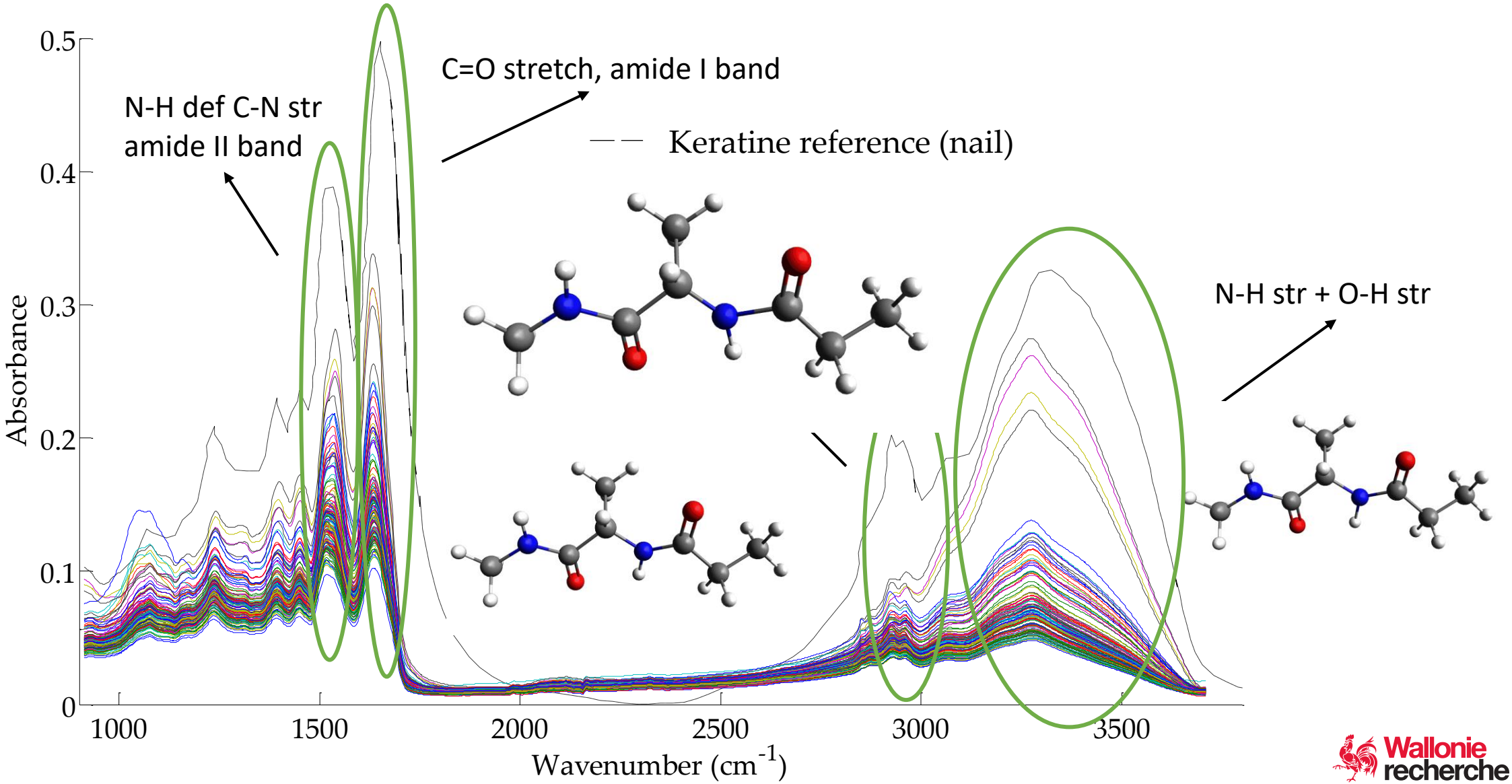


MIR → Heteronuclear bond: C=0, C-N, C-Cl

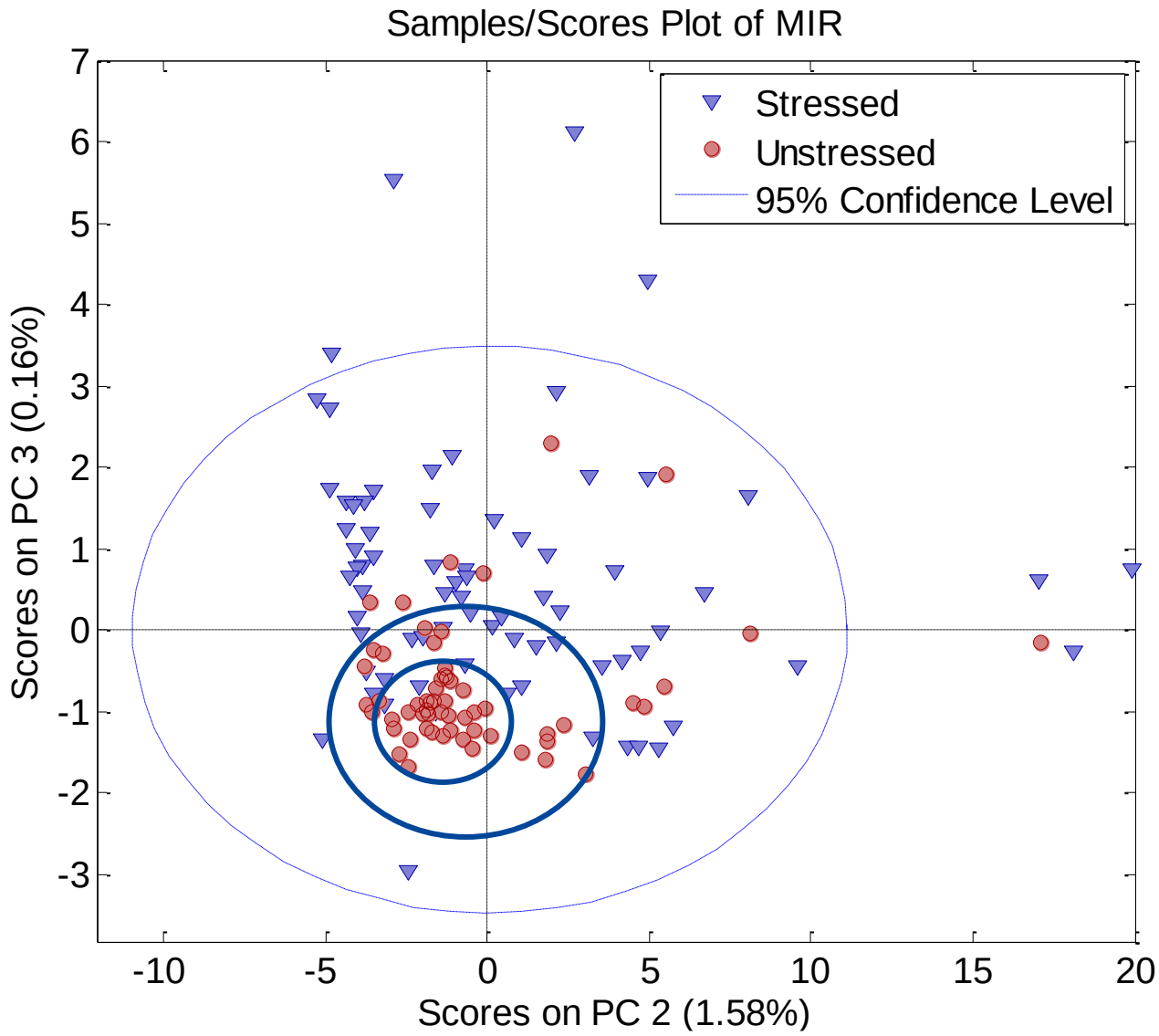
Raman → Homonuclear bond: C-C, C=C, C-S

Complementary information of MIR and Raman on molecular levels

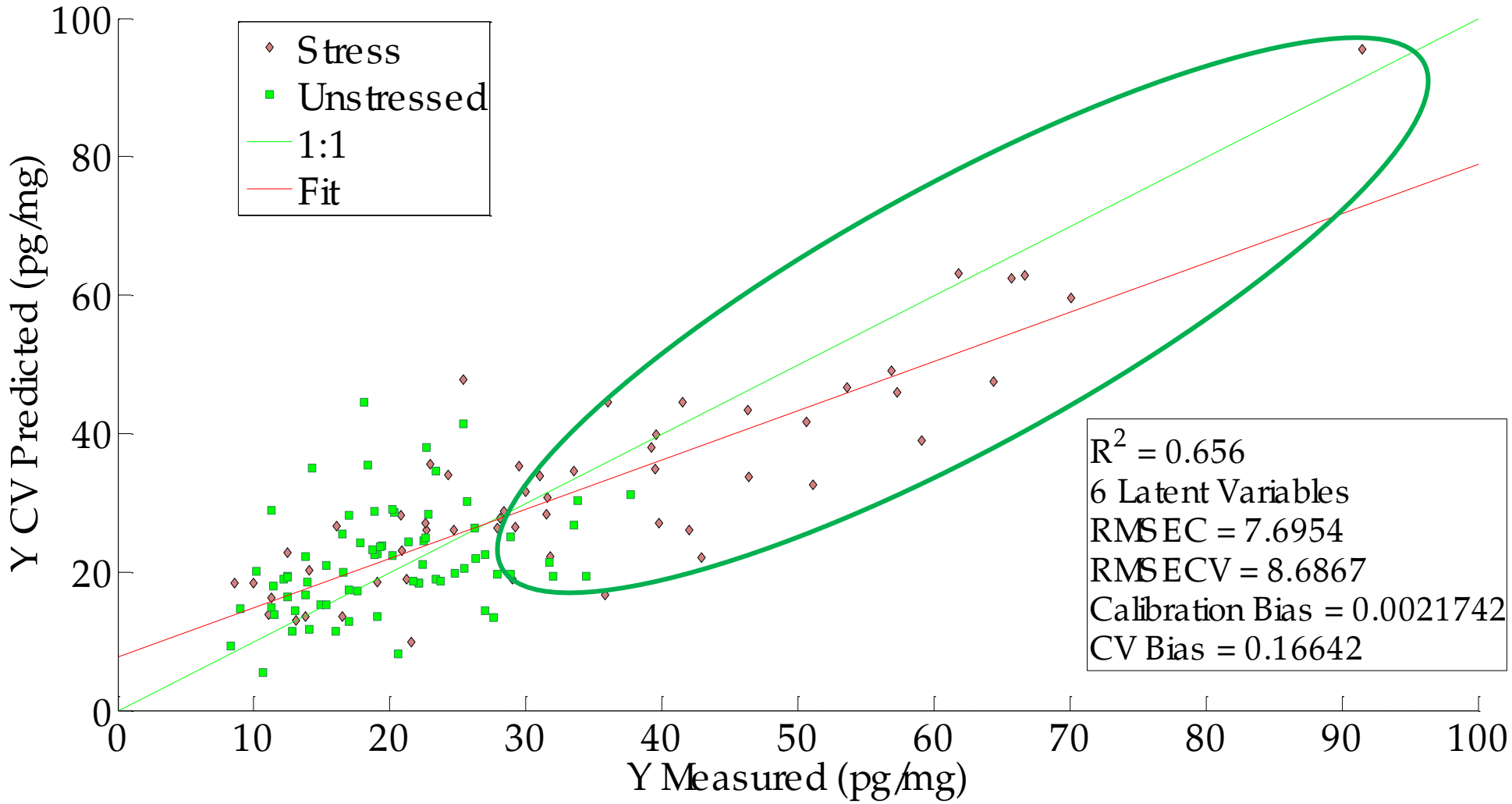
MIR: Spectra


N-H def C-N str
amide II band
C=O stretch, amide I band
Keratine reference (nail)
N-H str + O-H str
Absorbance
0.5
0.4
0.3
0.2
0.1
0
1000
1500
2000
2500
3000
3500
Wavenumber (cm-1)

MIR: PCA

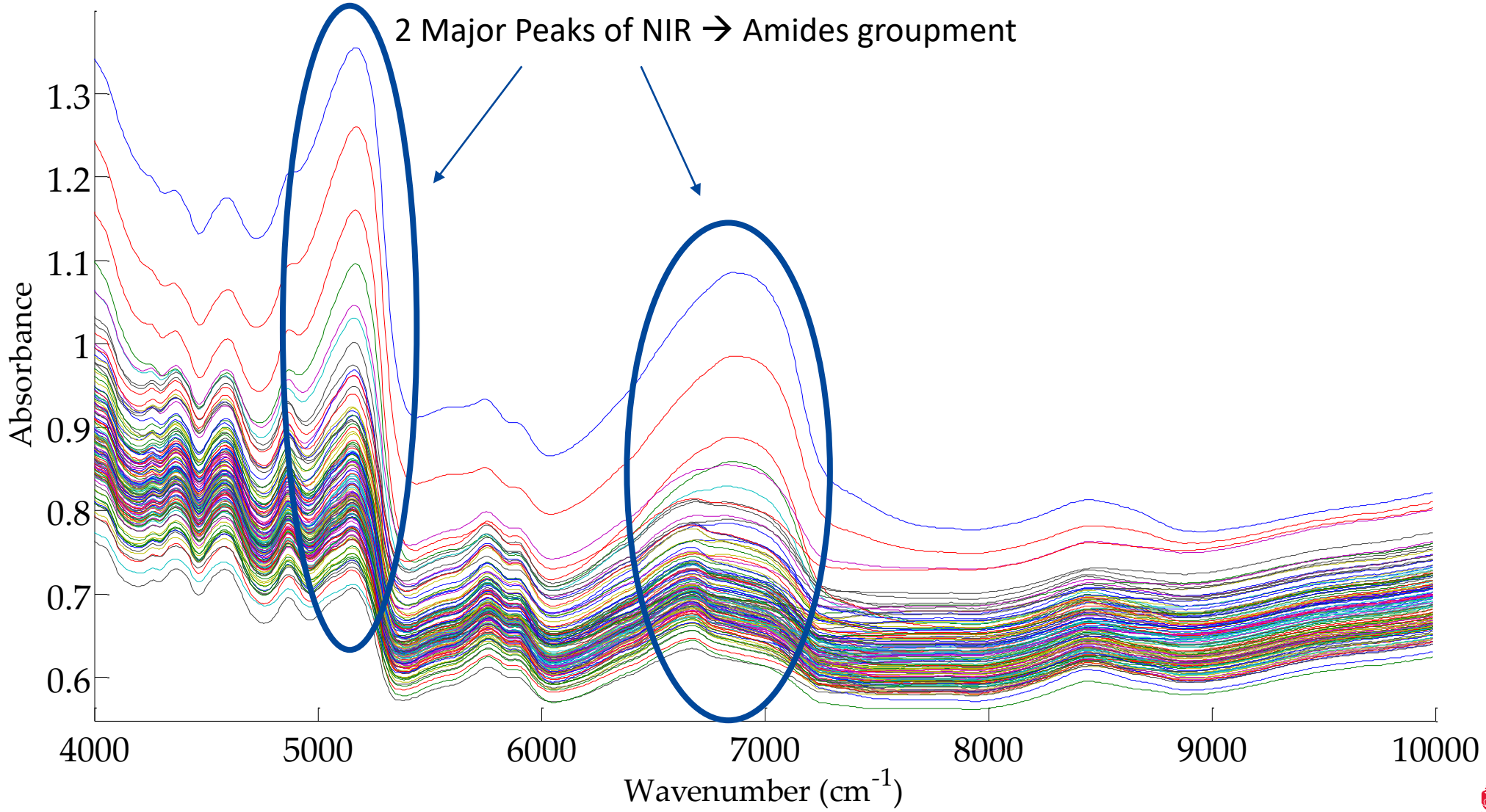

Samples/Scores Plot of MIR
Stressed
Unstressed
95% Confidence Level
Scores on PC 3 (0.16%)
Scores on PC 2 (1.58%)
7
6
5
4
3
2
1
0
-1
-2
-3
-10
-5
0
5
10
15
20

MIR: PLS

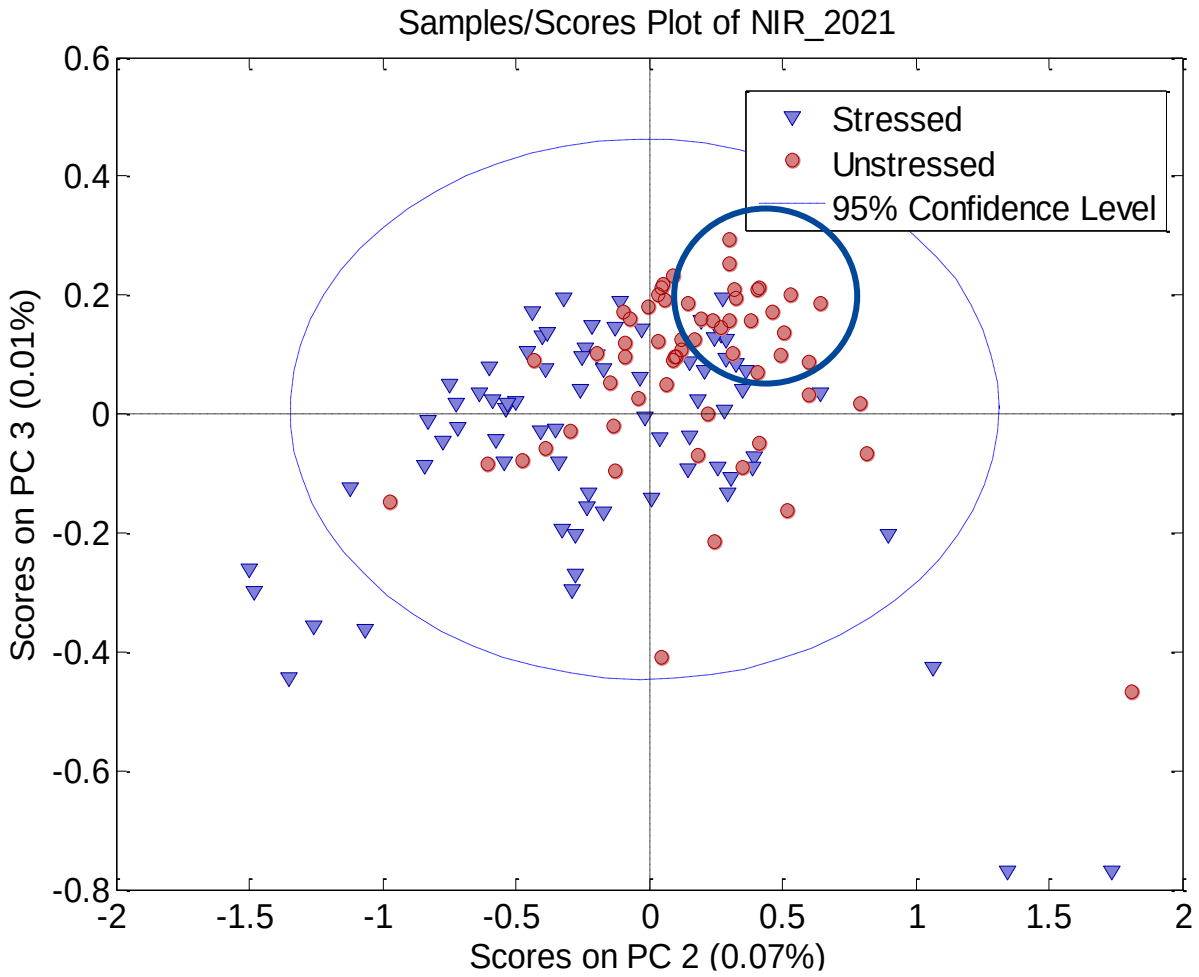


SNV + first derivative 5% of outliers

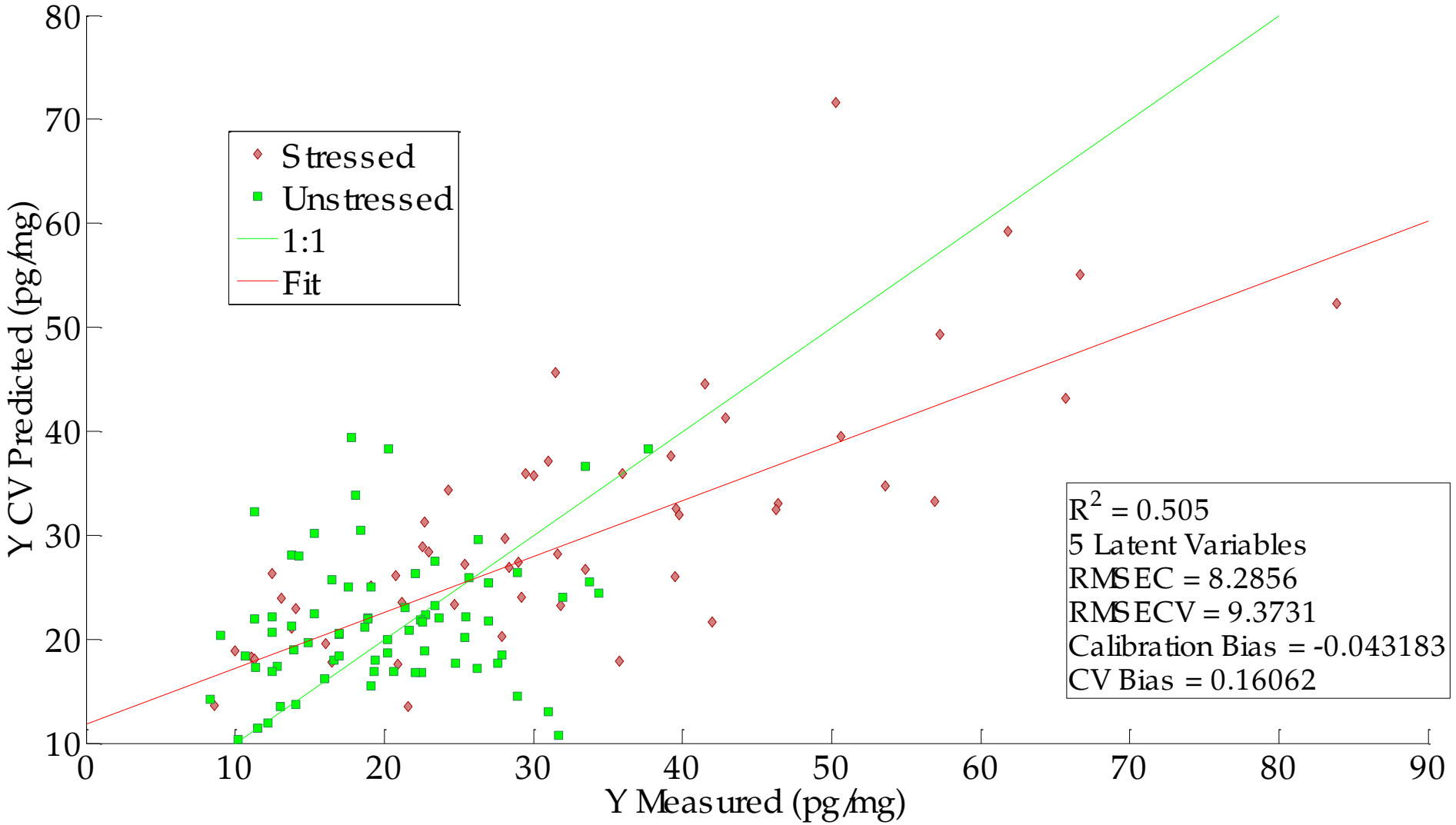
NIR: Spectra


2 Major Peaks of NIR → Amides groupment
Absorbance
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
4000
5000
6000
7000
8000
9000
10000
Wavenumber (cm-1)

NIR: PCA

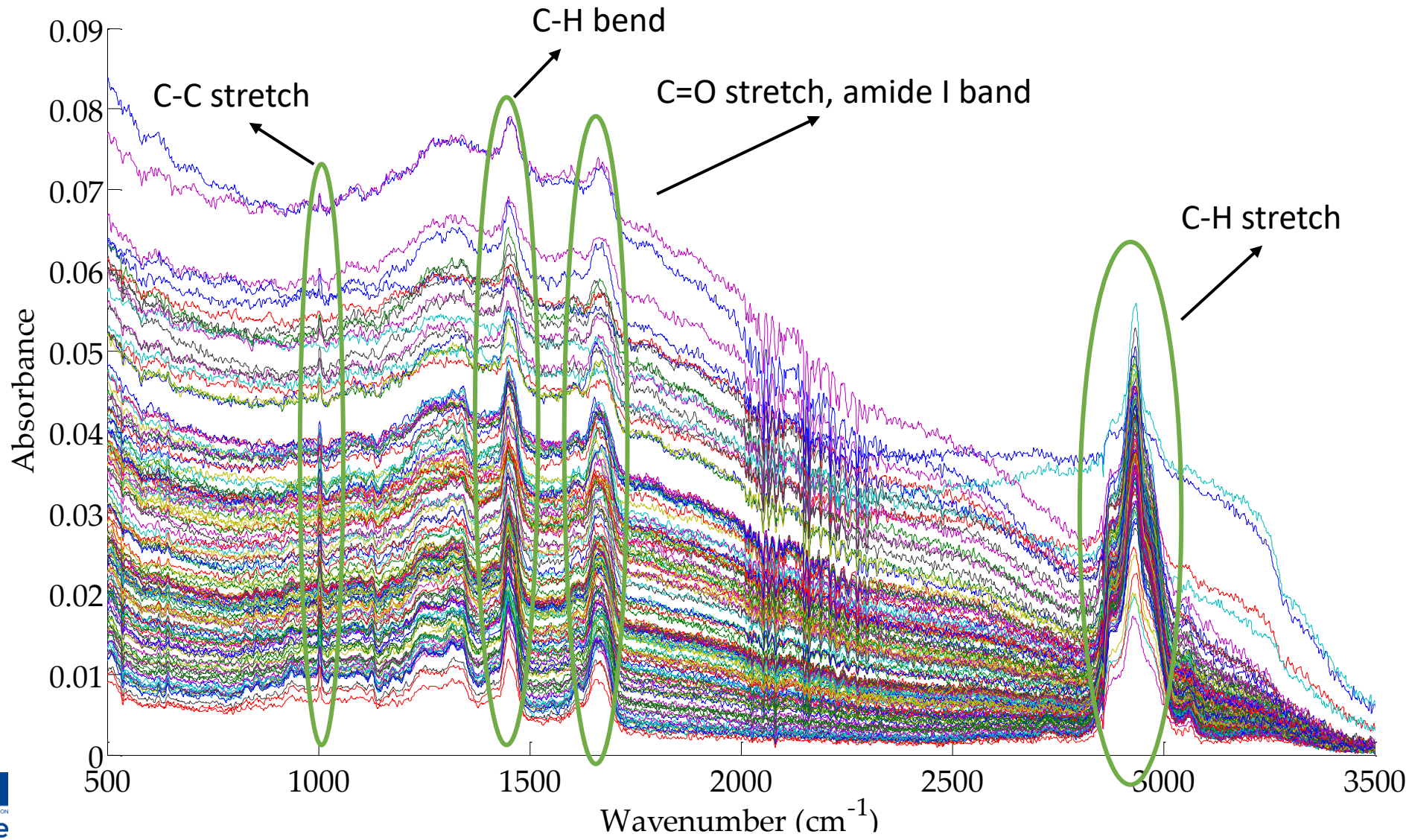

Samples/Scores Plot of NIR_2021
Stressed
Unstressed
95% Confidence Level
Scores on PC 3 (0.01%)
Scores on PC 2 (0.07%)
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2

NIR: PLS

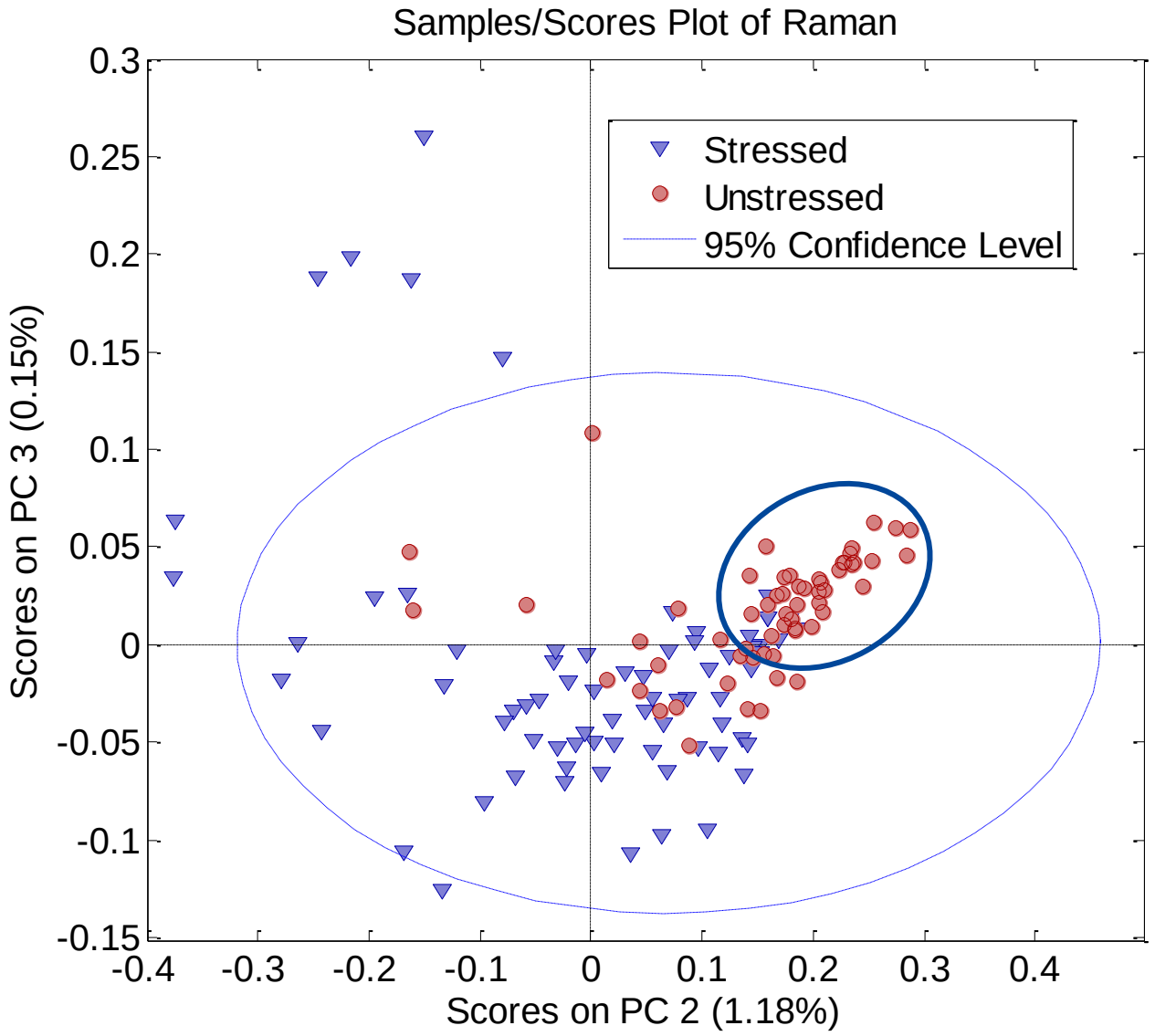


SNV + first derivatif of spectra and delete 5% of outliers

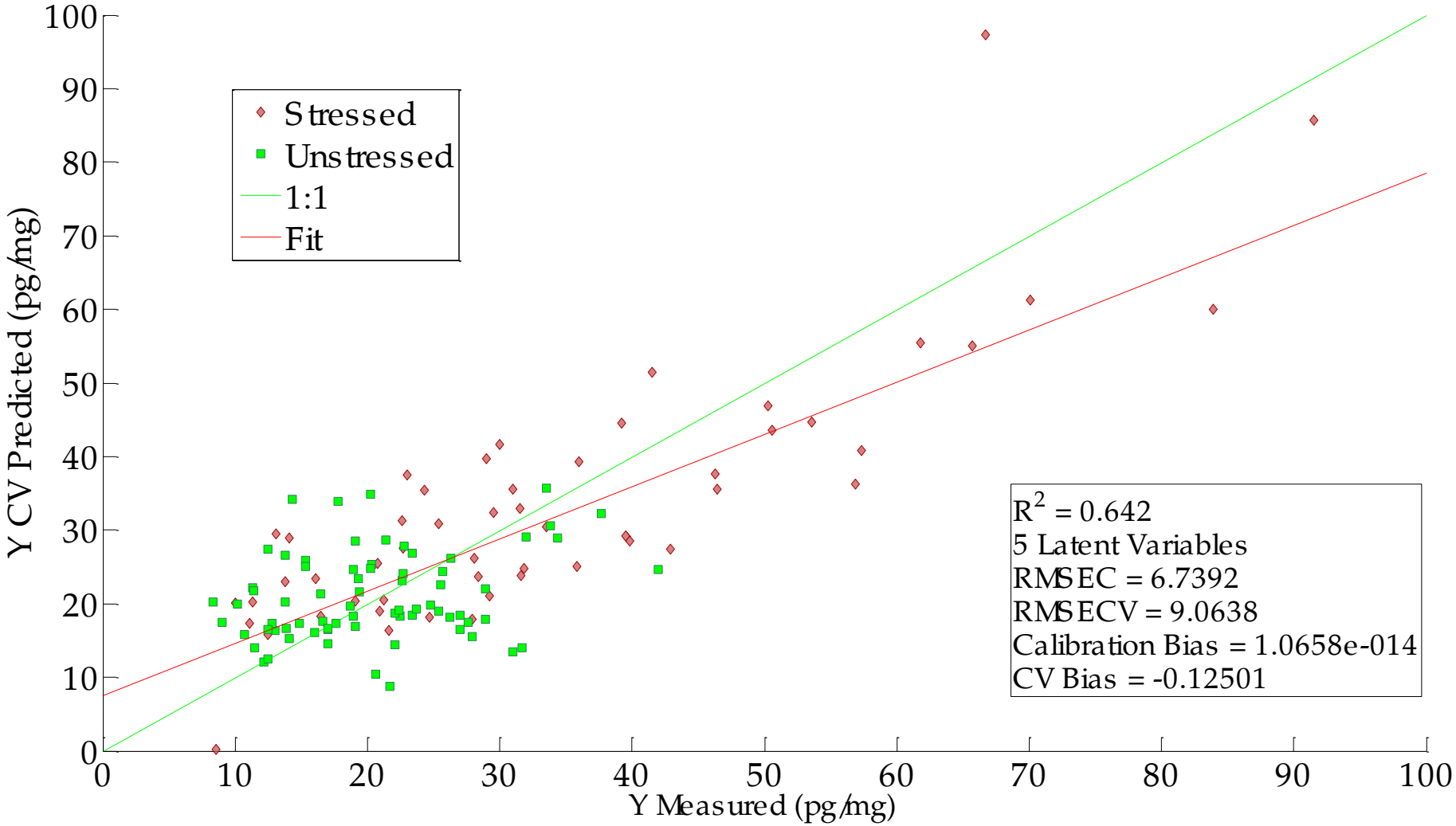
Raman: Spectra



Raman: PCA


Samples/Scores Plot of Raman
Stressed
Unstressed
95% Confidence Level
Scores on PC 3 (0.15%)
Scores on PC 2 (1.18%)
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4

Raman: PLS



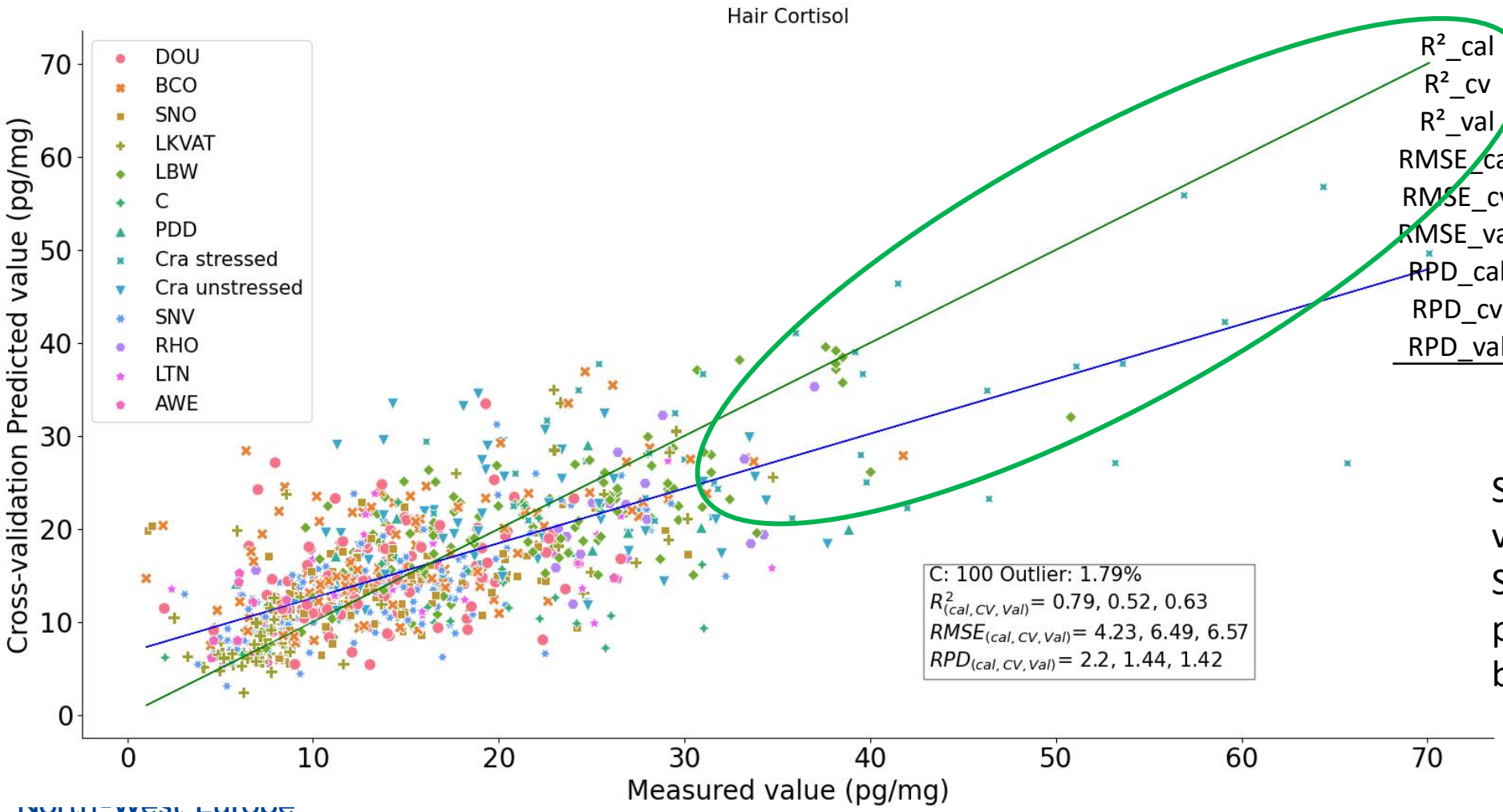
Autoscale + delete 6% of outliers

Large-scale sampling descriptive statistics

- 1047 Hair samples were collected in 12 DHI
- Analyzed in MIR analysis and ELISA
- Different farm were selected where different stress factor were supposed
- Any experimentation were done expect for CRA-stressed

Hair Cortisol(pg/mg)	Count	Mean	Std	Min	25%	50%	75%	Max
Global	1047	17.34	10.32	1.02	10.04	14.95	22.54	91.50
AWE	18	10.36	8.04	3.70	6.02	7.16	8.60	28.91
BCO	143	15.11	7.65	1.02	10.13	13.29	19.24	49.70
Convis	37	19.29	6.92	2.04	14.84	20.18	24.01	33.23
Cra stressed	58	36.22	19.01	8.60	22.63	31.55	49.32	91.50
Cra unstressed	75	20.09	6.74	8.30	14.60	19.10	24.25	37.70
DOU	120	13.69	4.63	1.99	10.38	13.17	16.60	26.57
LBW	165	22.16	8.95	7.23	15.23	21.53	27.70	52.08
LKVAT	91	11.24	7.70	2.50	6.79	7.94	11.75	34.75
LTN	34	15.87	8.31	2.39	8.05	15.52	23.95	34.71
PDD	13	20.69	10.06	5.86	11.23	24.80	25.20	38.85
RHO	27	24.88	7.11	6.16	23.43	25.55	27.93	37.00
SNO	98	16.20	8.61	1.09	11.61	14.81	19.19	68.41
SNV	168	12.47	5.82	2.70	8.17	11.79	16.11	32.23

Large-scale prediction: modelling



	PCR	PLS	ElasticNet	KRR	SVM-R
R^2_cal	0.48	0.46	0.61	0.53	0.79
R^2_cv	0.44	0.41	0.47	0.47	0.52
R^2_val	0.56	0.44	0.58	0.57	0.63
RMSE_cal	6.43	6.25	5.51	6.10	4.23
RMSE_cv	6.71	6.58	6.47	6.49	6.49
RMSE_val	7.18	7.02	7.02	7.06	6.57
RPD_cal	1.39	1.37	1.60	1.46	2.20
RPD_cv	1.33	1.30	1.37	1.38	1.44
RPD_val	1.24	1.22	1.26	1.26	1.42

SVM-R is better with an external validation

Similar tendency and performances are found than before

North-West Europe
HappyMoo
European Regional Development Fund

Wallonie recherche CRA-W

- Conclusion

- This preliminary work has demonstrated that the hair cortisol could be determine by the spectroscopy technique
- MIR spectrum seems to be the most accurate method to determine cortisol
- Enlarge the dataset keep the same performance modelling
- The chronic stress could have an effect to the structure of the hair

- Outlook

- Enlarge the dataset: Stressed cows
- Applied on meat cows
- Classification with different algorithms
- Do the analysis without any treatment of the Hair
- With portable spectrometer

Thank you for your attention

Any questions ?

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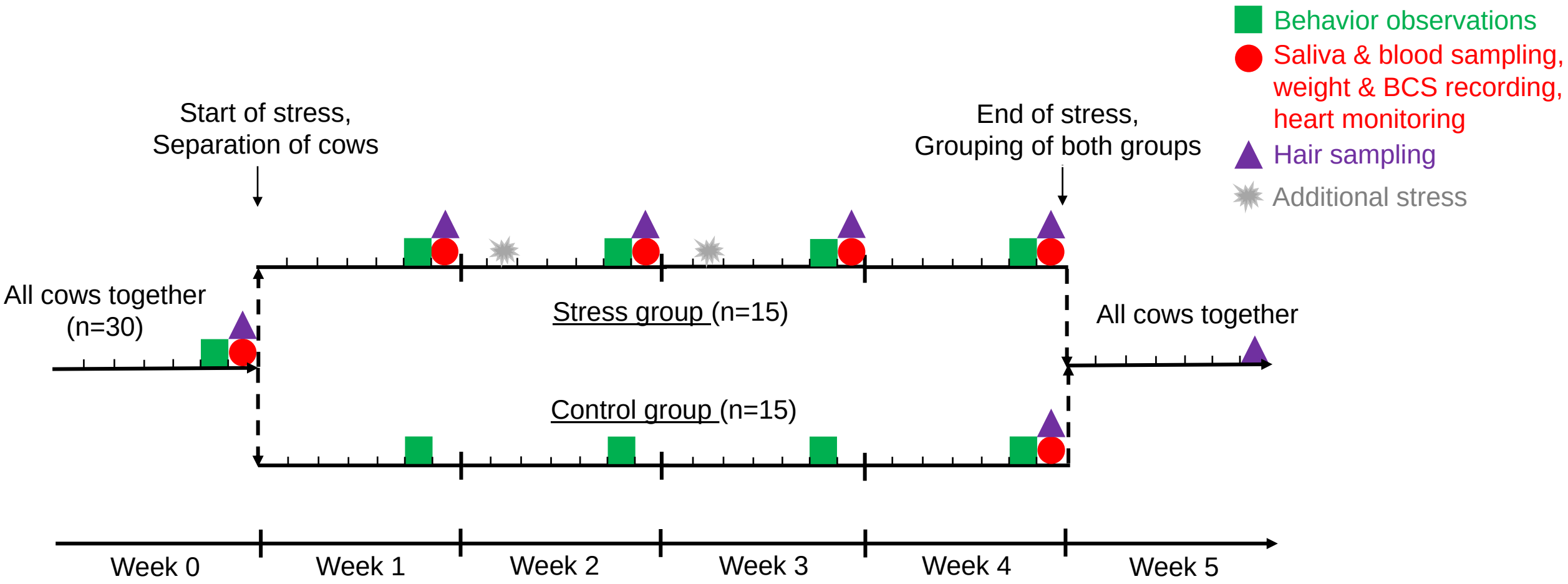
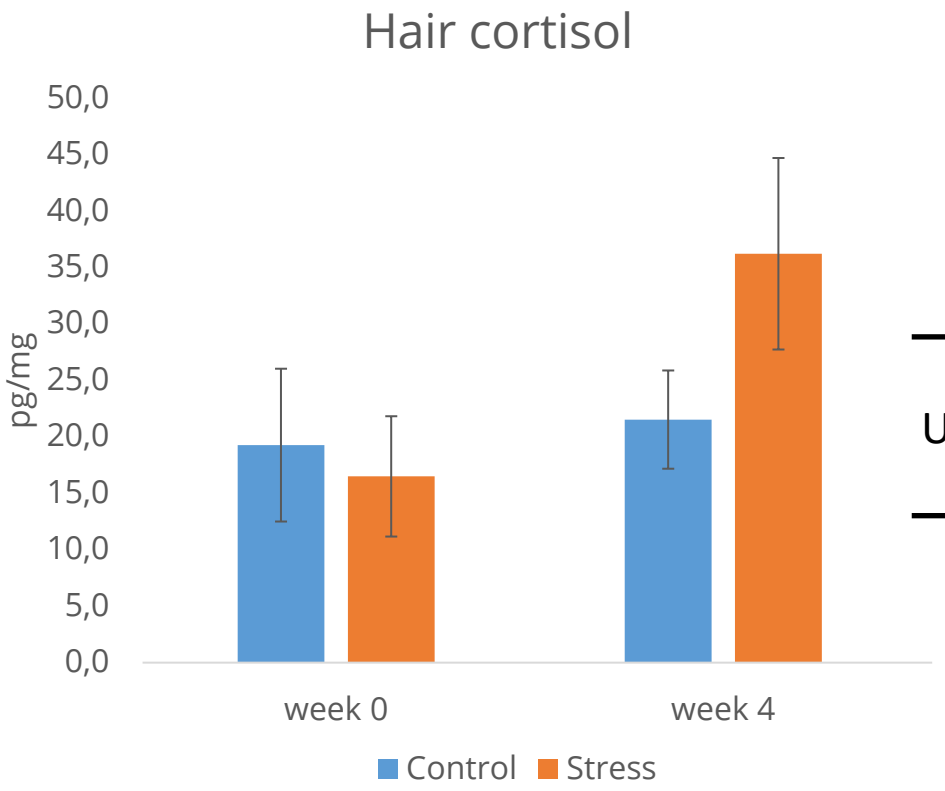
Experiment



Grelet, C., Dries, V. V., Leblois, J., Wavreille, J., Mirabito, L., Soyeurt, H., ... & HappyMoo Consortium. (2022). Identification of chronic stress biomarkers in dairy cows. *Animal*, *16*(5), 100502.

Results: ELISA



(pg/mg)	Week 0	Week 1	Week 2	Week 3	Week 4
Unstressed	19.3				21.5
Stressed	16.5	24.8	43.3	23.1	52.0

Grelet, C., Dries, V. V., Leblois, J., Wavreille, J., Mirabito, L., Soyeurt, H., ... & HappyMoo Consortium. (2022). Identification of chronic stress biomarkers in dairy cows. *Animal*, *16*(5), 100502.

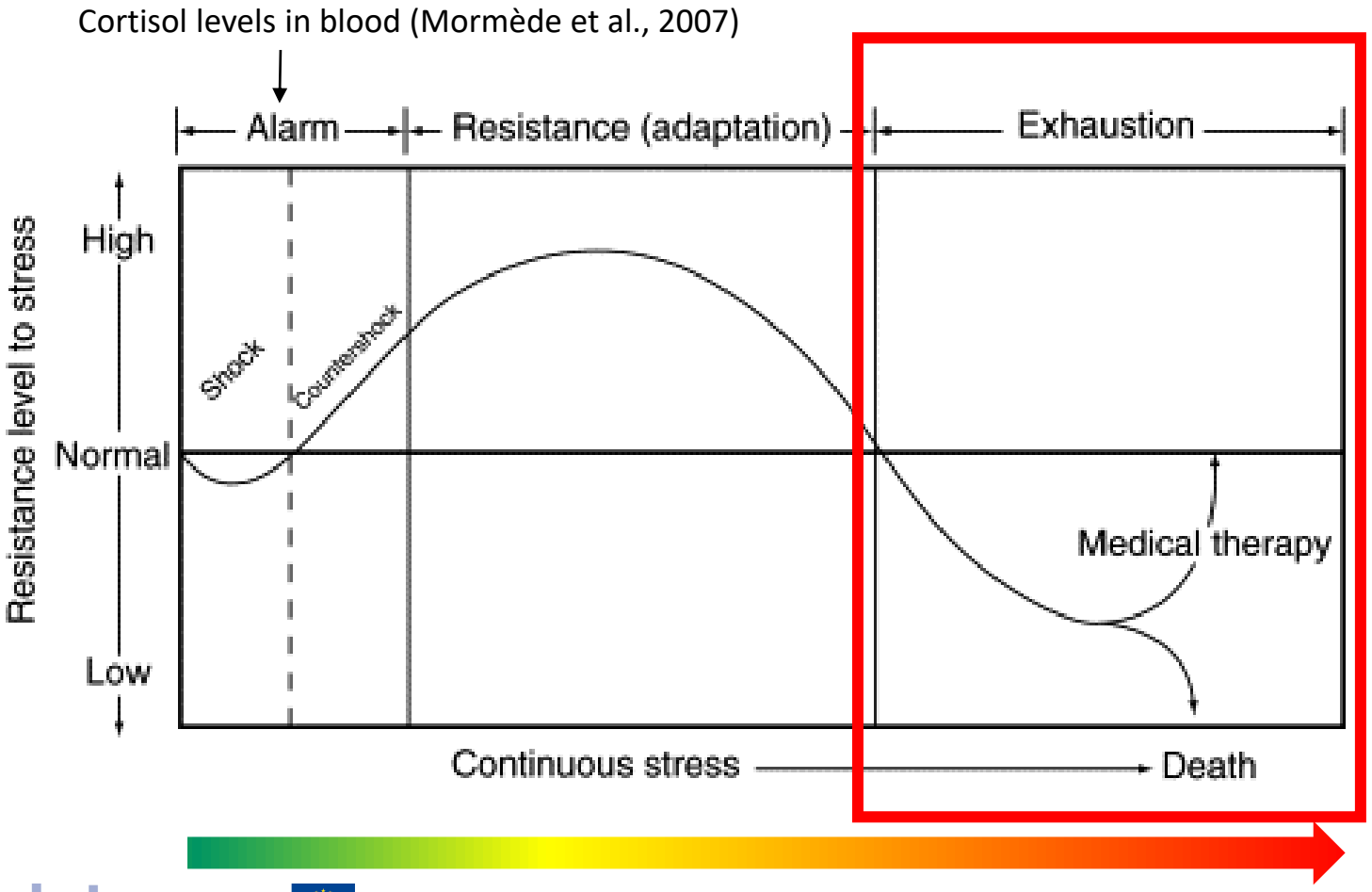
Cortisol: mecanism

- Intro: Différence stress aigue et chronique
- Intro: Explication Elisa NIR MIR Raman
- M&M Préparation des poils et schema experimental du stress
- Resultats: PCA: NIR, MIR Raman
- Outlook validation externe

- https://www.researchgate.net/figure/The-different-Amide-contributions-to-the-IR-bending-region-are-reported-with-different_fig3_268748998

Introduction: chronic stress

"stress is the non-specific response of the body to any demand made upon it" (Selye, 1976)



↗ susceptibility to metabolic, inflammatory and infectious diseases (Moberg et al., 1980; Romero, 2004).

↗ fertility troubles (Dobson and Smith, 2000; Walker et al., 2008)

↘ growth disturbances (Elsasser et al., 1995)

↘ weight (Mormède et al., 2007)

↘ milk production (Tallo-Parra et al., 2018)

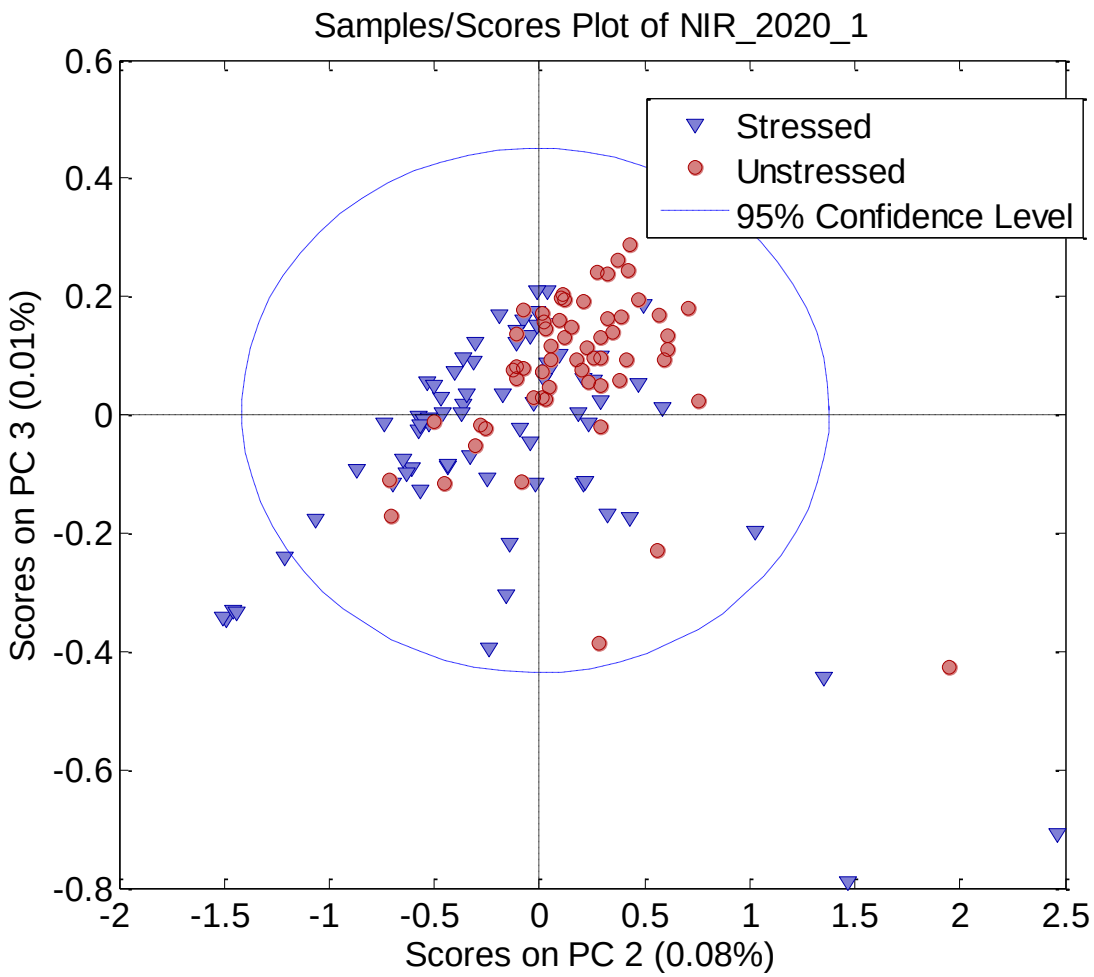
↘ production and economics of farms,

↘ welfare of cows

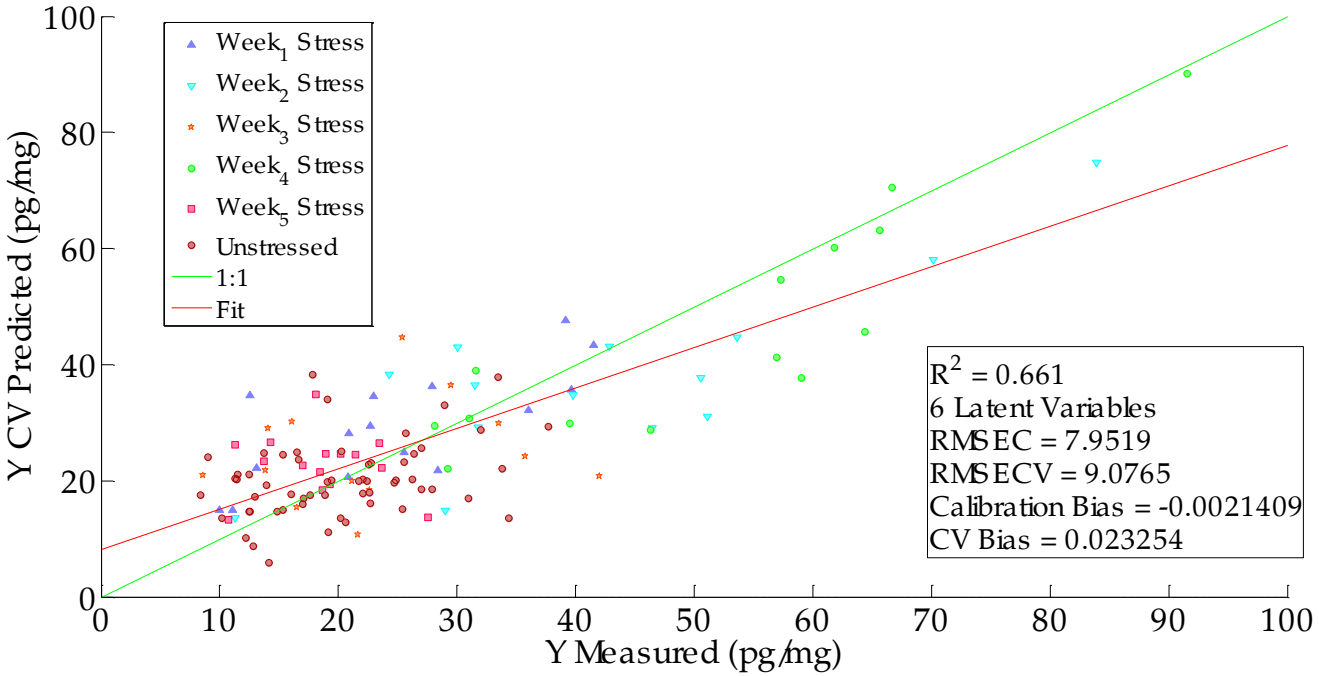
↘ societal perception of dairy production

Figure of General adaption syndrome (from A.C. Brown, C.I. Waslien, in Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003)

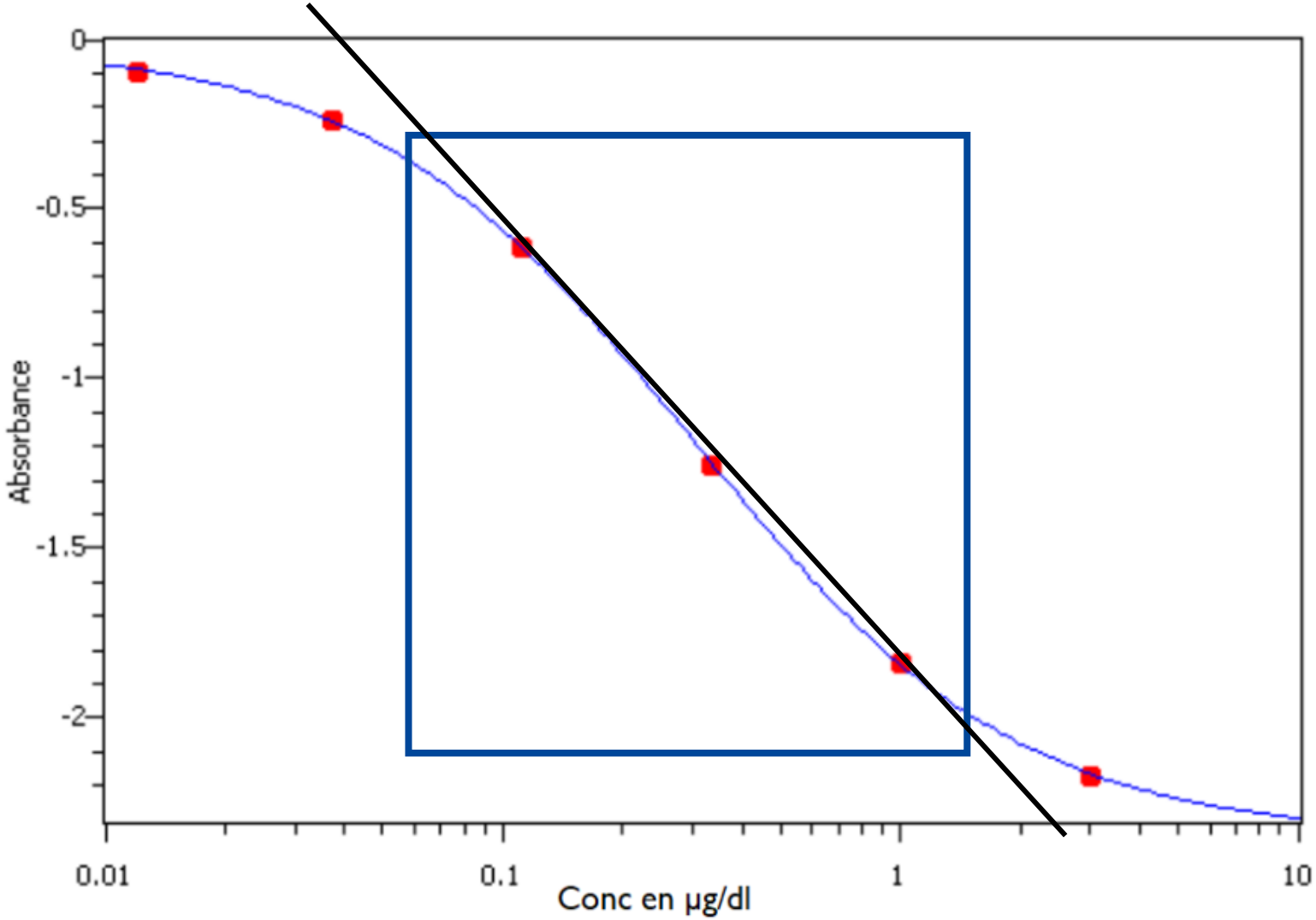
NIR 2020

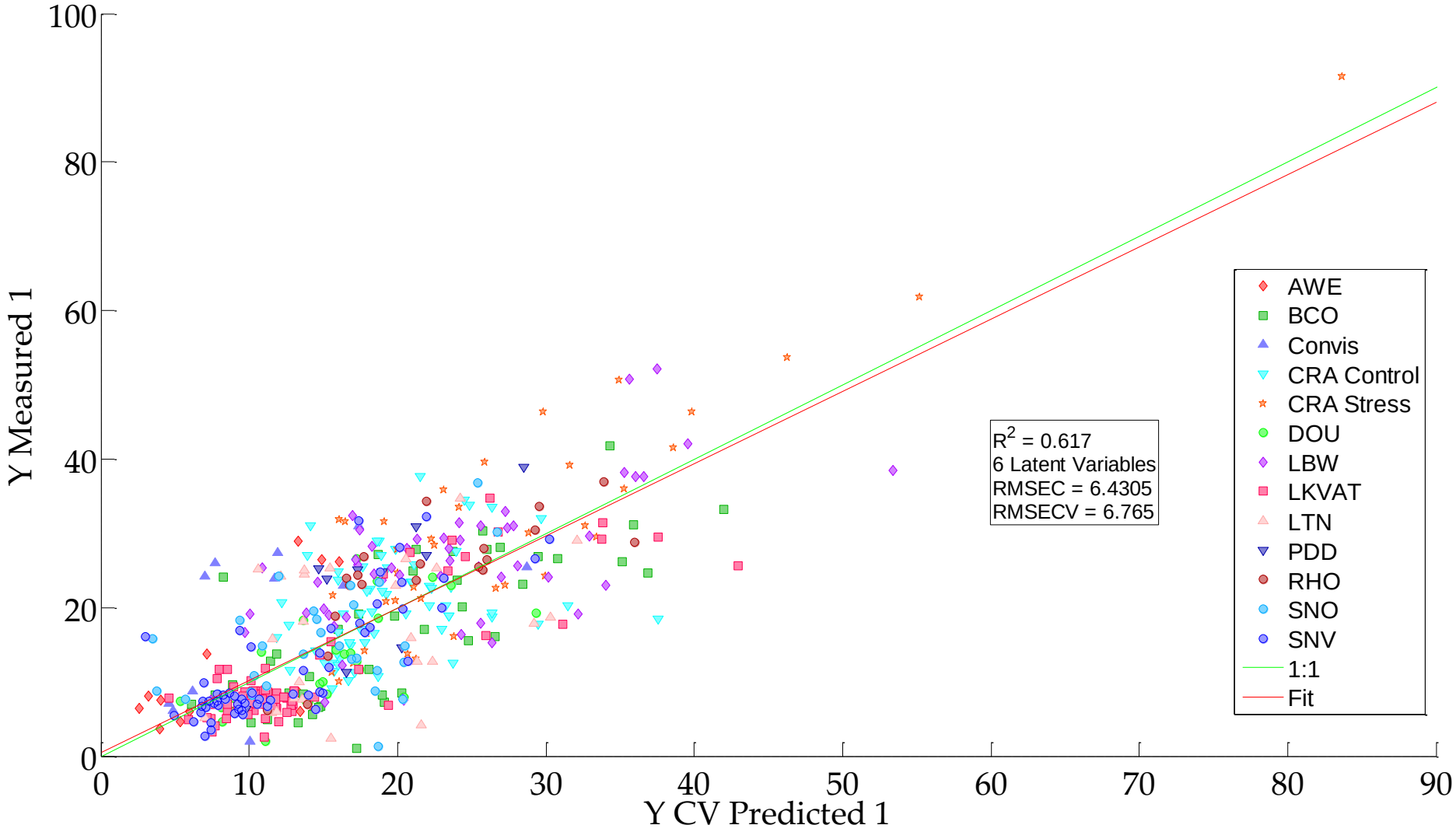


NIR 2020



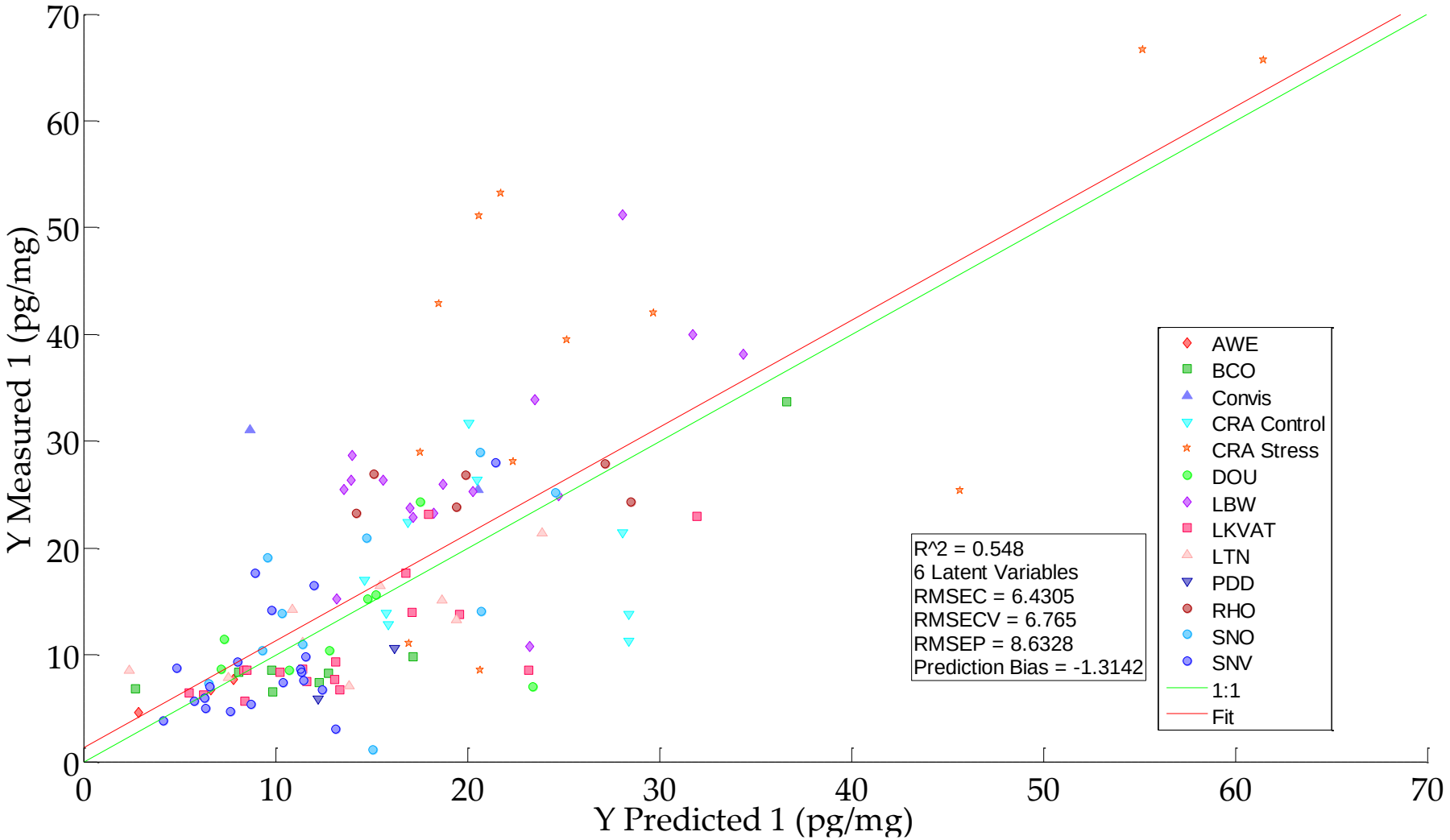
ELISA: Cortisol determination





Pretreatment
SNV+Savgol
derivative

Outlier: 21
SD: 10.9
RPD: 1.61


Y Measured 1 (pg/mg)
Y Predicted 1 (pg/mg)
0
10
20
30
40
50
60
70
R^2 = 0.548
6 Latent Variables
RMSEC = 6.4305
RMSECV = 6.765
RMSEP = 8.6328
Prediction Bias = -1.3142
AWE
BCO
Convis
CRA Control
CRA Stress
DOU
LBW
LKVAT
LTN
PDD
RHO
SNO
SNV
1:1
Fit