

Session 33.

Mediterranean buffalo farming for sustainable milk and meat production

Room: Reggiana – Palazzo Affari 2nd Floor
Chair: Neglia / Niero



Assessment of infrared spectroscopy for the quantification of protein fractions in buffalo milk

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The 75th EAAP Annual Meeting

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Outline

- Introduction
- Aim
- Materials & Methods
- Results & Discussion
- Conclusions



In Italy

- ✓ **Campania and Lazio regions**

90% of Italian buffalos



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In Italy

- ✓ **Campania and Lazio regions**

90% of Italian buffalos



- ✓ Buffalo milk is mainly processed into **Mozzarella di Bufala Campana PDO**



Very soft and tasty
Rich in milk and flavours
Exported all over the world

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Important factors affecting buffalo milk processability for cheesemaking process

✓ **Milk acidity**

→ pH levels and titratable acidity

✓ **High calcium content**

→ improve curd firmness and texture of final product

✓ **Somatic cell**

✓ **High fat and protein content**

→ contribute to cheese yield, structure and flavor

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Buffalo milk protein fractions

REFERENCE METHOD

PREDICTIVE METHOD

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Buffalo milk protein fractions

REFERENCE METHOD



Reverse-phase HPLC (**RPHPLC**)



- ✓ High precision and sensitivity
 - ✓ Expensive equipment
 - ✓ Specialized personnel
 - ✓ Time-consuming

PREDICTIVE METHOD

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Buffalo milk protein fractions

REFERENCE METHOD



Reverse-phase HPLC (**RPHPLC**)



- ✓ High precision and sensitivity
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PREDICTIVE METHOD



Mid-infrared spectroscopy (**MIRS**)



- ✓ Non-destructive technique
- ✓ Non-specialized personnel
 - ✓ Cost-effective
 - ✓ Rapid analysis

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Few data available for **buffalo milk protein composition**.



Interest to have a deeper and quiker analytical methods for:

- Investigating **variation** of buffalo milk protein fractions
- **Modifying milk protein** composition
- Altering **frequency** of specific protein **genetic variants**



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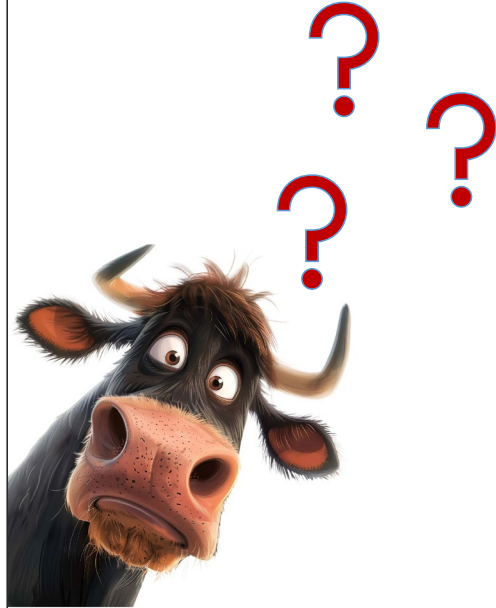
→ Results & Discussion

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With the present study we would like to:

1. Determine protein fractions in buffalo milk



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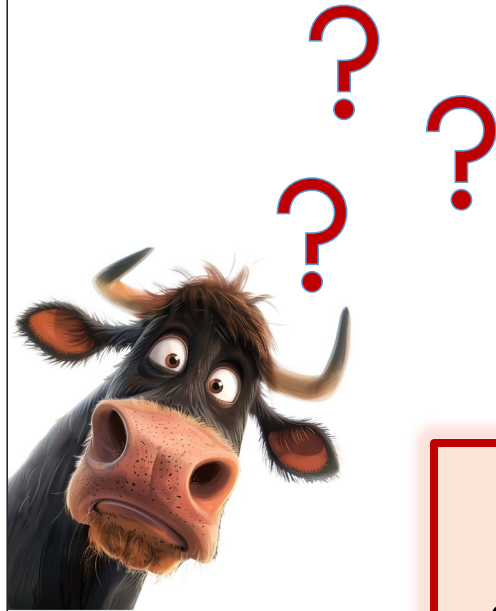
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With the present study we would like to:

1. Determine protein fractions in buffalo milk



2. Assess the effectiveness of mid-infrared spectroscopy to predict milk protein composition

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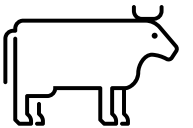


Area of Mozzarella di Bufala Campana PDO cheese



Sample collection

401 Italian Mediterranean buffaloes



7 commercial herds



Each buffalo was sampled once



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$n = 401$



MilkoScan™ 7 RM (FOSS)



MILK COMPOSITION:

- ✓ Fat, protein, casein and lactose (%)
- ✓ Urea (mg/dL)
- ✓ SCC (cells/mL)



SPECTRA COLLECTION

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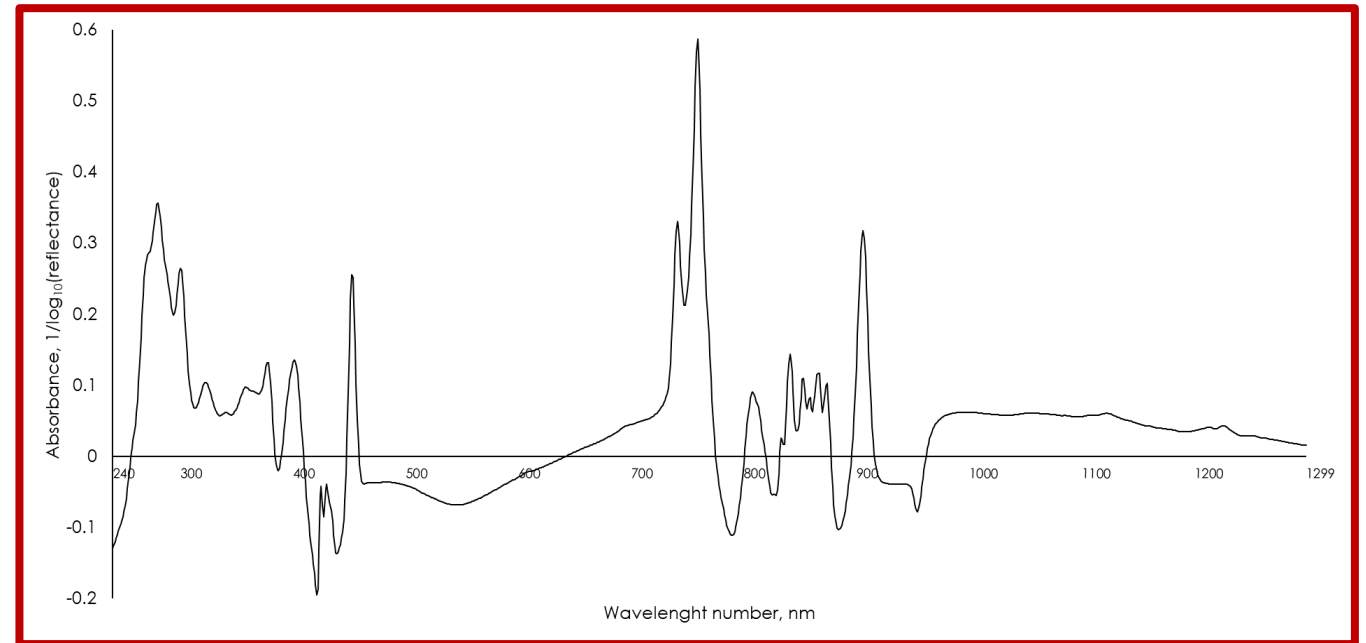
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SPECTRA COLLECTION

Mid-infrared average raw spectra of buffalo milk samples



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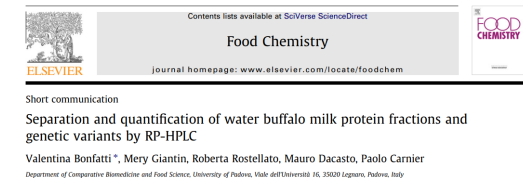


Reversed-phase HPLC
(Agilent Technologies)

MILK PROTEIN COMPOSITION:

- ✓ κ -CN
 - ✓ α_{s2} -CN, α_{s1} -CN
 - ✓ β -CN
 - ✓ α -LA
 - ✓ β -LG
- mg/mL

Bonfatti et al., 2013

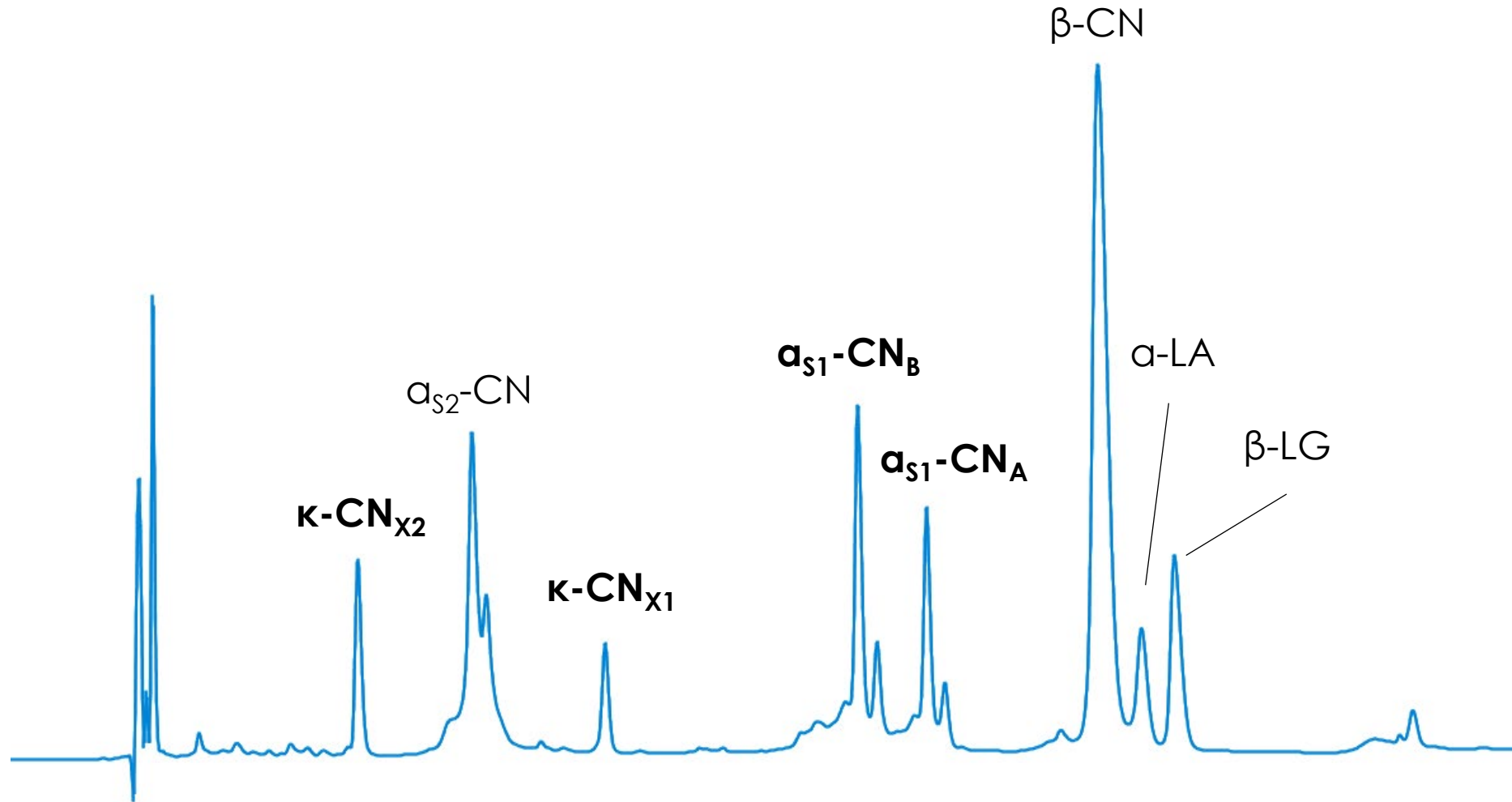


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Chromatograms of individual sample of buffalo milk with different κ - and α_{s1} -CN genetic variants



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Chemometric analysis

→ Prediction models were built through **PLS regression analysis**.

WinISI 4 software 

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Chemometric analysis

- Prediction models were built through **PLS regression analysis**.
- The prediction equations obtained were validated using a **5-fold cross-validation** using 3 rounds of outliers elimination.

WinISI 4 software **ISI**

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Chemometric analysis

- Prediction models were built through **PLS regression analysis**.
- The prediction equations obtained were validated using a **5-fold cross-validation** using 3 rounds of outliers elimination.
- The **best model** of each trait was chosen based on:
 - ✓ **LF** selected to minimize the RMSE of cross validation;
 - ✓ **R²** in cross validation;
 - ✓ **RPD** in cross validation.

WinISI 4 software **ISI**

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Descriptive statistics¹ of milk quality traits

Milk quality traits ²	N	Mean	SD	CV, %	Minimum	Maximum
Fat, %	380	7.26	1.79	24.63	2.10	13.70
→ Protein, %	383	<u>4.56</u>	0.40	8.69	3.24	5.84
Casein, %	383	3.71	0.38	10.36	2.57	4.91
Lactose, %	377	4.66	0.25	5.34	3.75	5.29
Urea, mg/dL	381	47.40	16.12	34.01	3.87	99.22
SCS, units	382	3.28	1.68	51.41	-0.64	8.26

¹SD: standard deviation; CV: coefficient of variation.
²SCS: somatic cell score, calculated as $SCS = 3 + \log_2(SCC/100,000)$, where SCC is somatic cell count.

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Descriptive statistics¹ of milk protein fractions

Milk protein fractions ² , mg/mL	N	Mean	SD	CV, %	Minimum	Maximum
Total protein	387	55.54	11.36	20.46	31.86	89.15
Total CN	388	47.29	10.20	21.57	27.23	83.63
κ-CN	387	7.56	2.28	30.19	2.40	15.64
α _{s2} -CN	388	7.41	1.58	21.33	3.01	13.68
→ α _{s1} -CN	388	<u>18.78</u>	4.38	23.34	8.96	35.35
→ β-CN	388	<u>13.50</u>	3.12	23.15	6.83	24.73
Total WP	388	8.35	1.91	22.90	3.49	14.79
α-LA	386	2.17	0.50	23.20	0.87	3.61
β-LG	387	6.15	1.50	24.36	2.35	10.79

¹SD: standard deviation; CV: coefficient of variation.

²CN: casein; WP: whey protein.

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Fitting statistics¹ of prediction model

Milk protein fractions ² , mg/mL	N	% outliers	SE _{cv}	R ² _{cv}	LF
→ Total protein	351	8.83	7.98	<u>0.51</u>	13
→ Total CN	351	8.83	6.99	<u>0.53</u>	12
κ-CN	361	6.23	1.65	0.44	11
α _{s2} -CN	358	7.01	2.77	0.45	12
α _{s1} -CN	365	5.20	2.07	0.56	11
β-CN	352	8.57	1.81	0.60	12
Total WP	350	9.10	1.37	0.38	14
α-LA	354	8.05	0.37	0.44	13
β-LG	354	8.05	1.16	0.34	14

¹N: number of samples; SE_{cv}: standard error of prediction of cross-validation; R²_{cv}: coefficient of determination of cross-validation; LF: latent factors.

²CN: casein; WP: whey protein.

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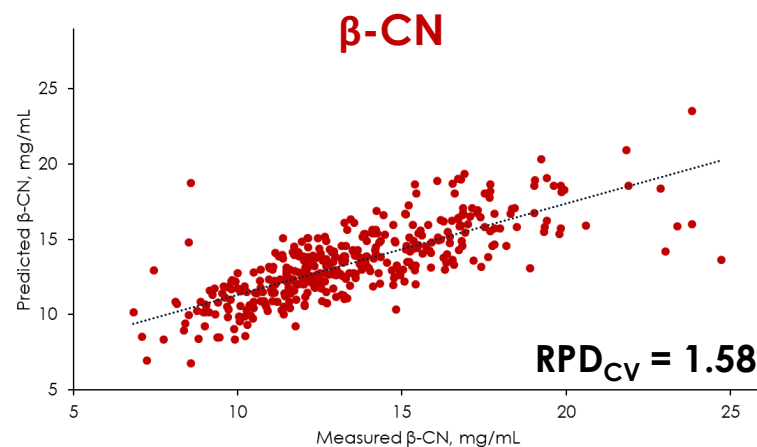
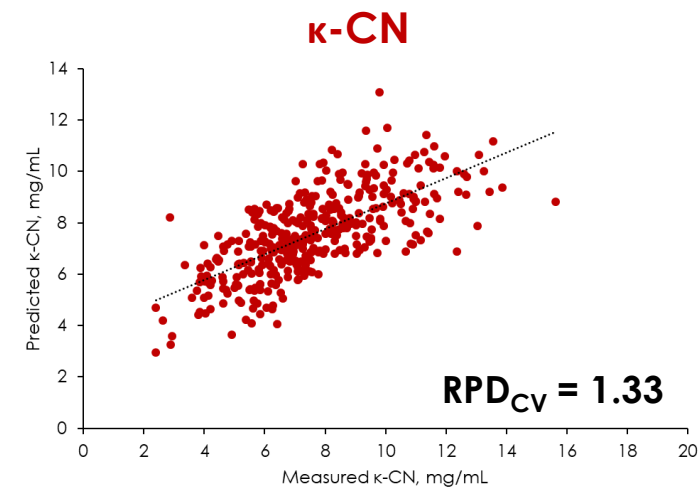
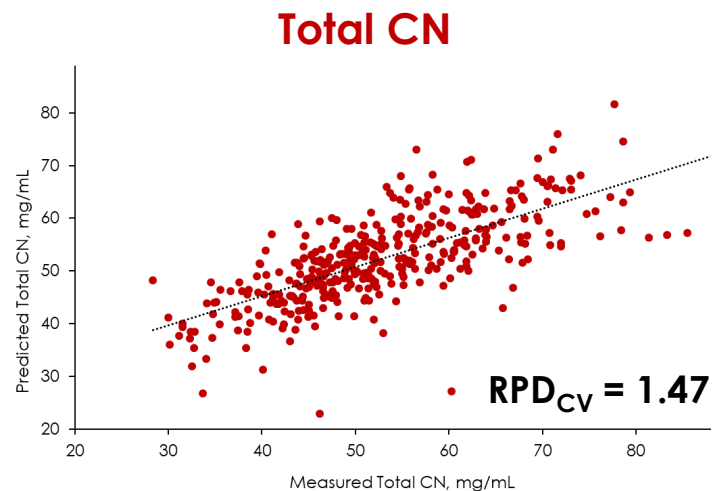
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Measured through RP-HPLC vs predicted through MIRS

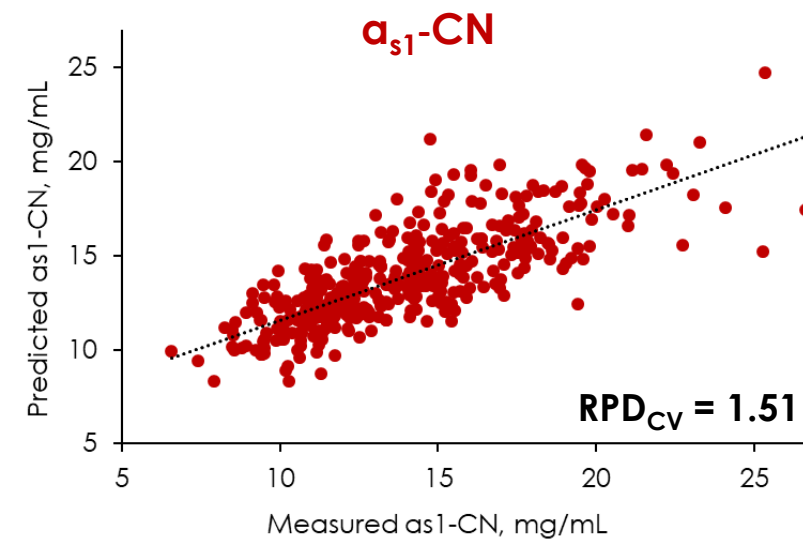
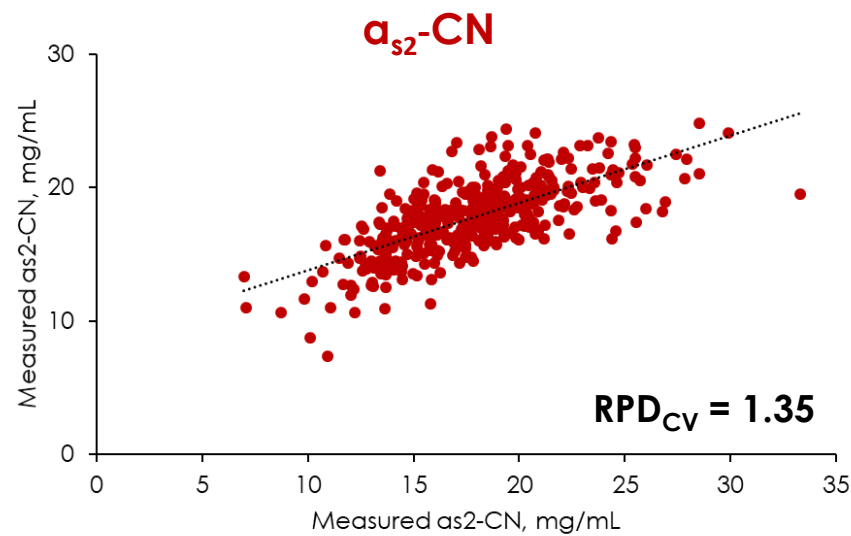


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Measured through RP-HPLC vs predicted through MIRS



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- ✓ Better results were obtained for **total protein, total CN, α_{s2} -CN and β -CN.**
- ✓ Further research should be addressed to improve the accuracy of mid-infrared spectroscopy models by **increasing the number of samples in calibration** and **considering alternative chemometric approaches.**
- ✓ These results can be used to **genetically enhance milk technological traits in Mozzarella di Bufala Campana PDO area.**



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Thank you for your attention!

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