



Sustainable sheep milk: exploring udder health traits and antioxidant activity

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The complex world of antioxidants....

- Nature of the antioxidant
- Variety of assays
- Interpretation of the results

Which are our objectives?

- To characterize sheep milk for the antioxidant activity
- To investigate the ***factors*** affecting the antioxidant activity in sheep milk

Breed, parity, stage of lactation

Milk composition

Udder health proxies

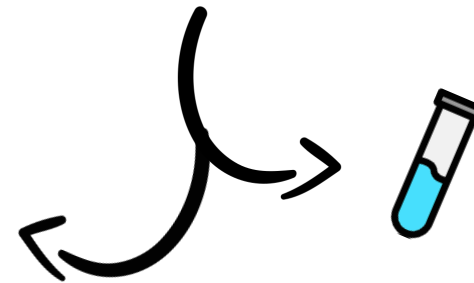
Materials & Methods

Sampling

- Project: SHEEP4CHEESE
- Area: Asciano (Italy)
- Ewes: 792
- Breeds: Massese, Comisana
- Sampling: November 2021 - March 2023



Lab analyses



Non-enzymatic antioxidant assays:
Ferric reducing antioxidant power (**FRAP**)
2-diphenyl-1-picryl-hydrazyl-hydrate (**DPPH**)

Composition:
fat, protein, casein,
lactose, SCS, minerals

Materials & Methods

Milk composition

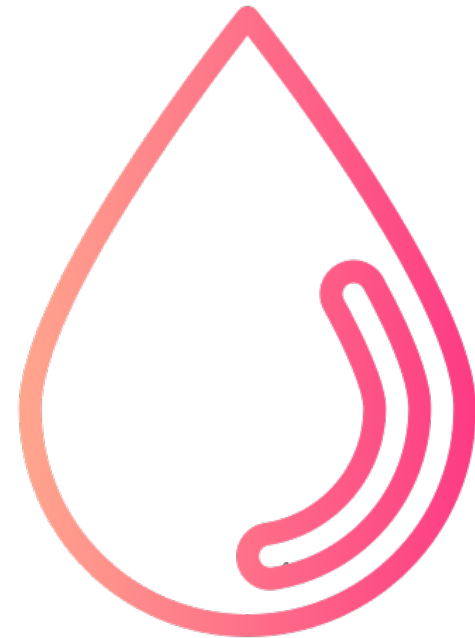
- fat, protein, casein (%) (MIR spectroscopy)
- somatic cell count (SCC) (Fluorocitometry) → $SCS = \log_2(SCC/100) + 3$

Minerals (X-ray fluorescence)

- Ca, Mg, Na, Cl (mg/kg)

Antioxidant assays (Vis-NIR spectroscopy)

- FRAP: μM eq. ascorbic acid/mL of milk; *action*: transfer of e^- ; *abs*: 593 nm
- DPPH: % of inhibition; *action*: transfer of e^- / H^+ ; *abs*: 515 nm

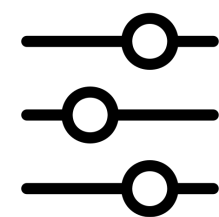


Materials & Methods

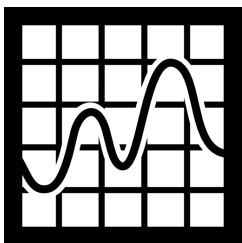
Statistical analysis

Generalized Additive Mixed Model (GAMM)

FRAP & DPPH =



Breed (2 cl.)
Parity (2 cl.)



Days in milk
Milk Yield
Protein × fat
Casein × SCS
Ca × Mg
Na × Cl
Sampling × breed

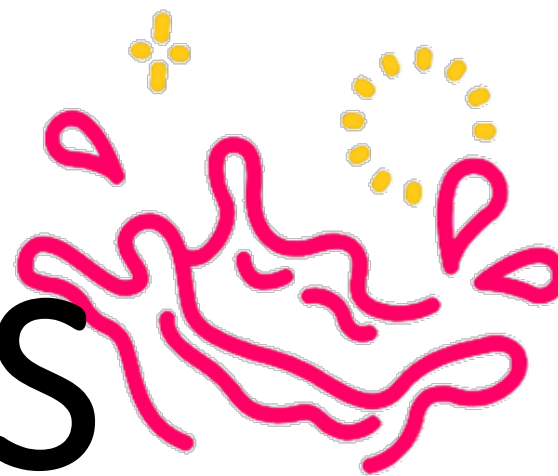


Parametric terms



Smooth functions

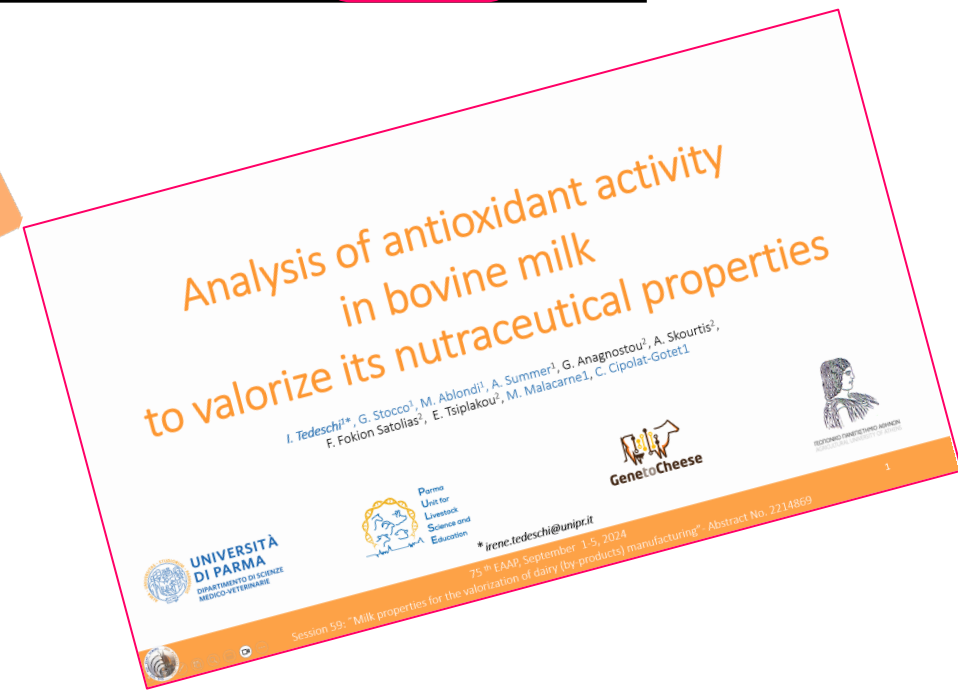
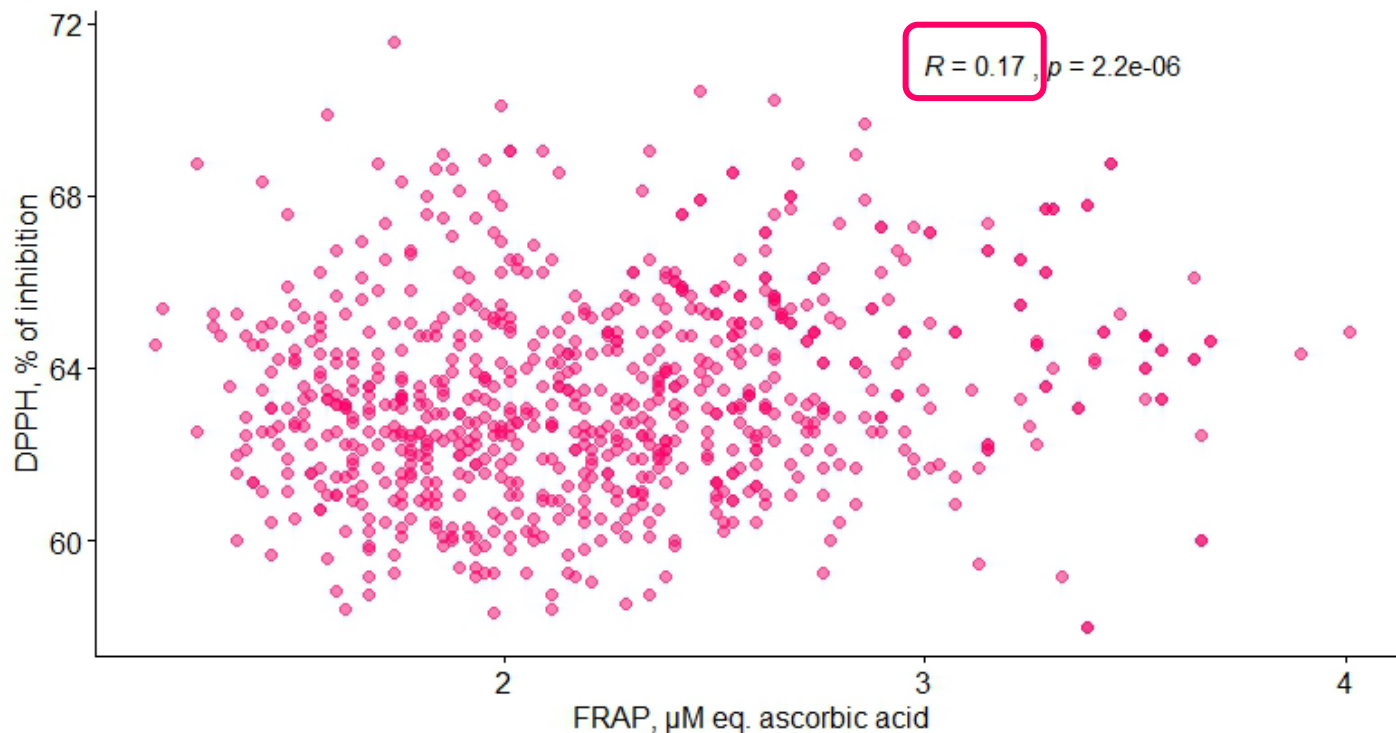
RESULTS



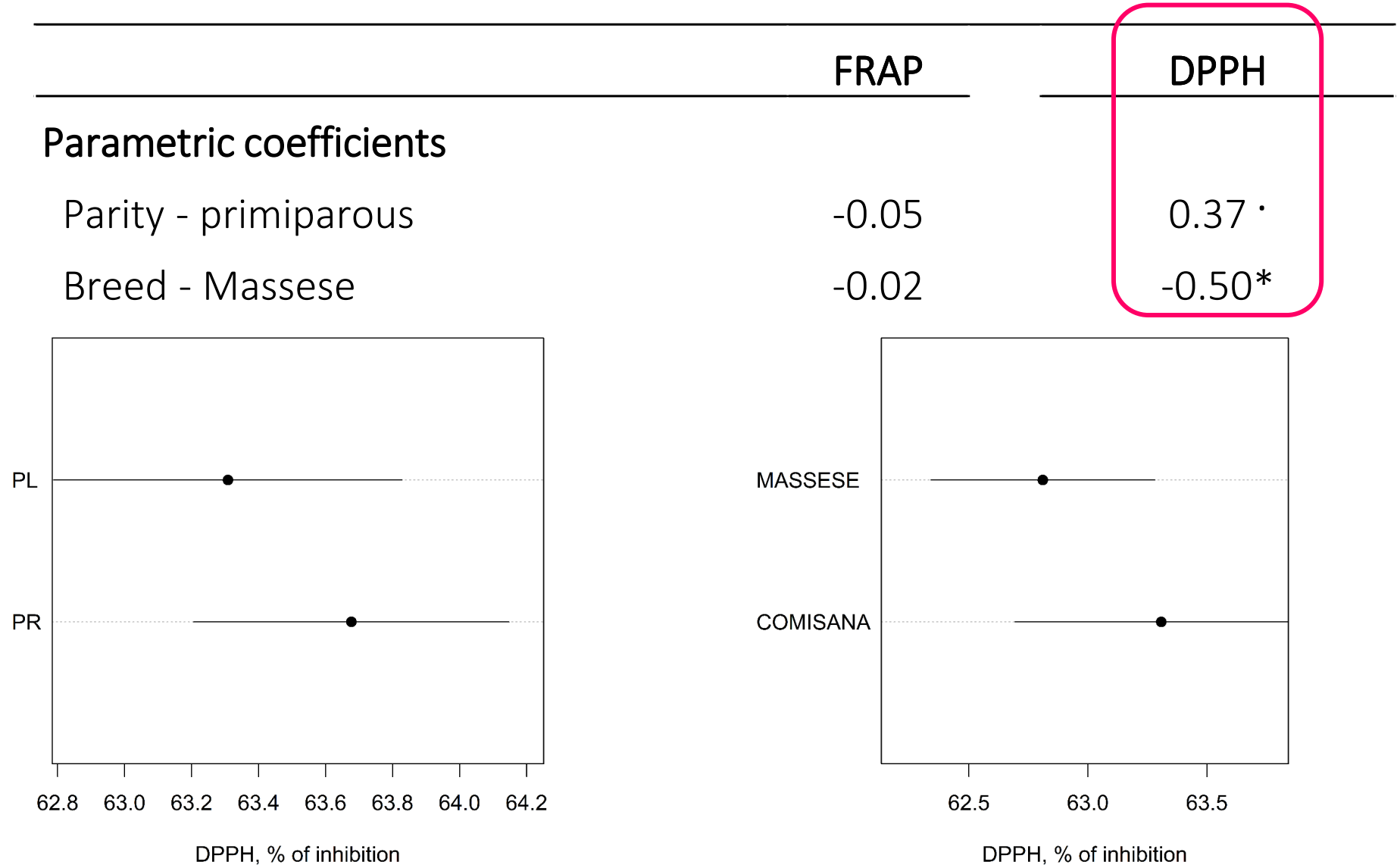
Descriptive statistics...

Trait	Mean	SD	CV, %
FRAP, μM eq. ascorbic acid/mL of milk	2.24	0.54	24
DPPH, % of inhibition	63.4	2.29	4

... & Pearson's correlation



Summary of GAMM



*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; · = $P < 0.1$

Summary of GAMM

	FRAP	DPPH
Parametric coefficients		
Parity - primiparous	-0.05	0.37 ·
Breed - Massese	-0.02	-0.50*
Smooth terms, edf		
DIM	2.05*	2.63
MY	2.37	1.00
Protein × fat	4.72***	2.12**
Casein × SCS ³	2.00*	2.46
Ca × Mg	2.00*	9.36 ·
Na × Cl	11.32 ·	6.66
Adjusted R²	0.50	0.43

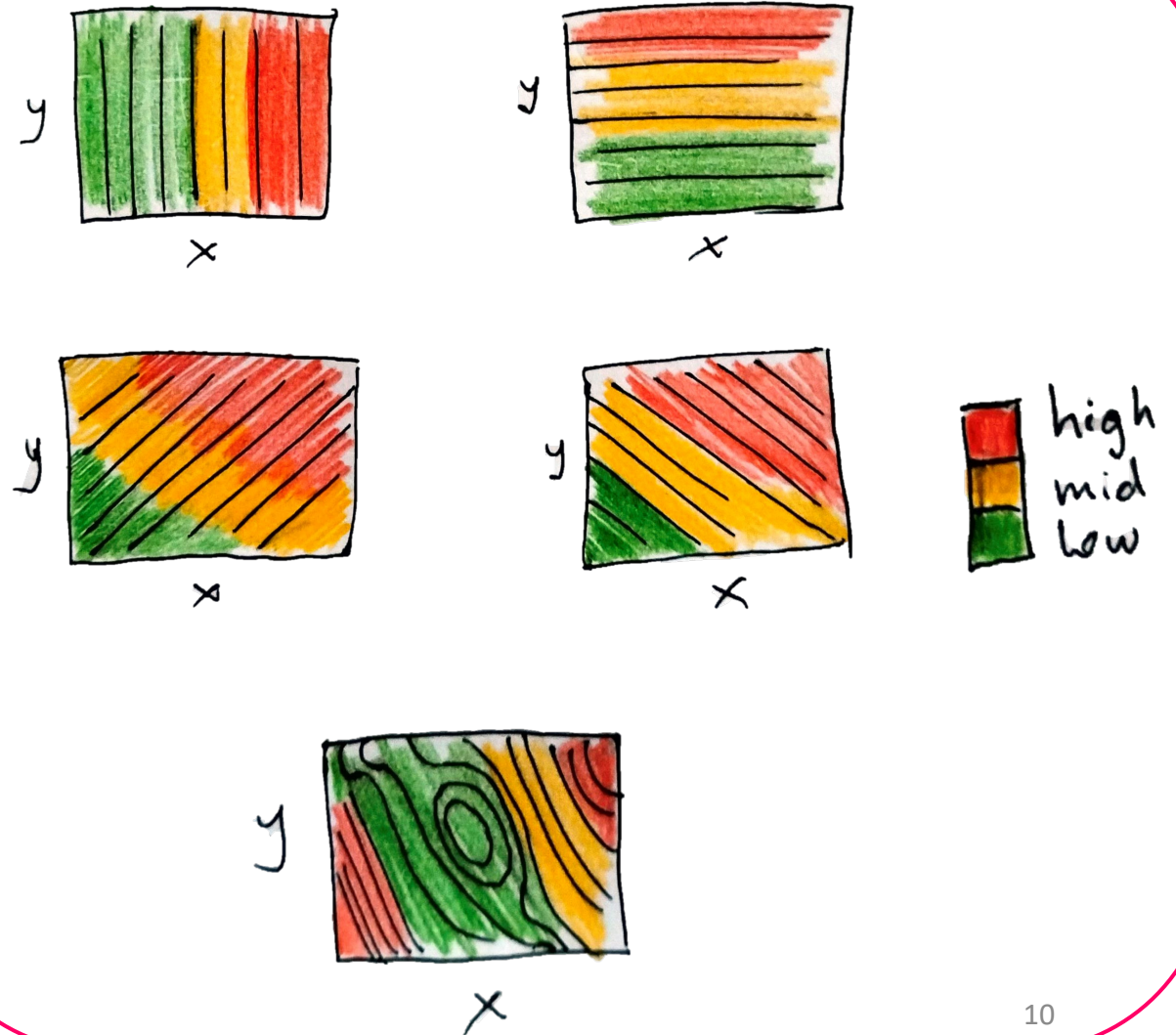
Type of relationship
 edf = 1 *linear*
 1 > edf ≤ 2 *weakly non-linear*
 edf > 2 *highly non-linear*

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; · = $P < 0.1$

How are smooth terms visualized ?

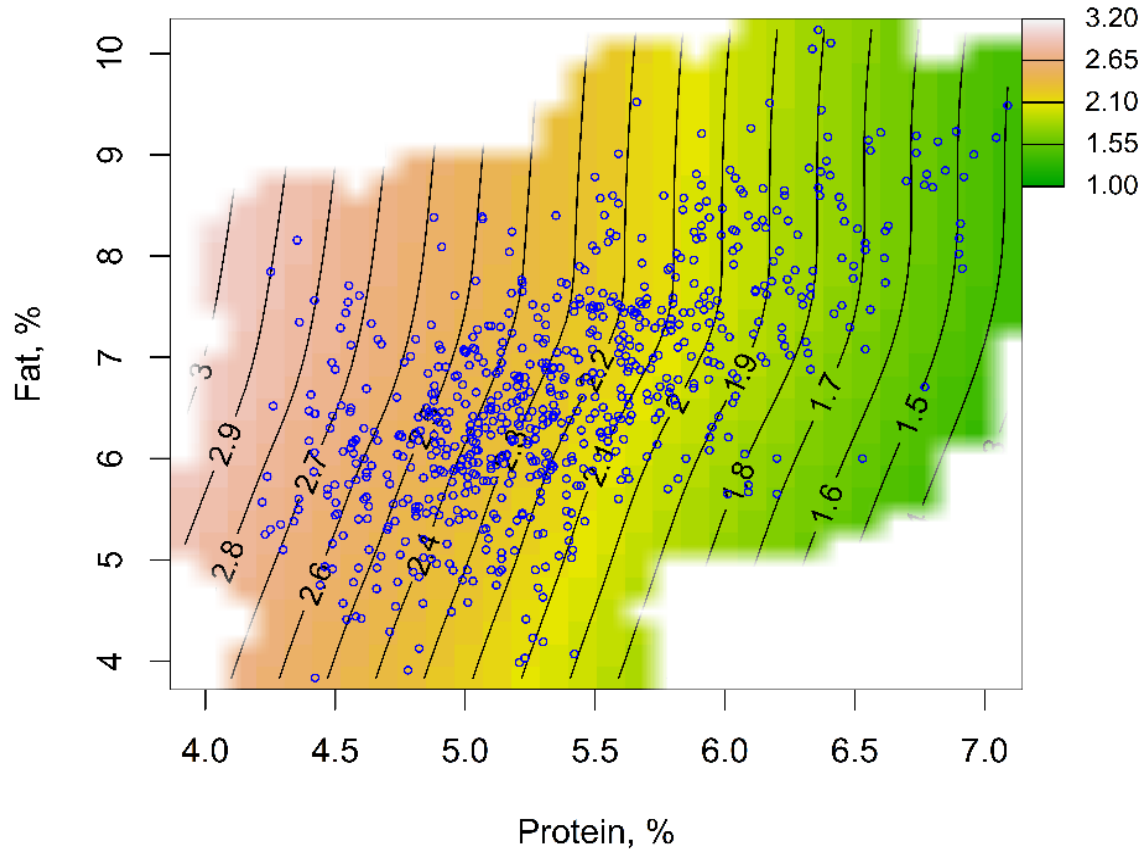
The contour plots

- To display the relationship between 3 variables in 2D: 2 independent (X and Y) and 1 dependent (Z)
- To show values of the Z variable (contour lines) for combinations of the X and Y variables
- Colored bands represent ranges of the Z variable

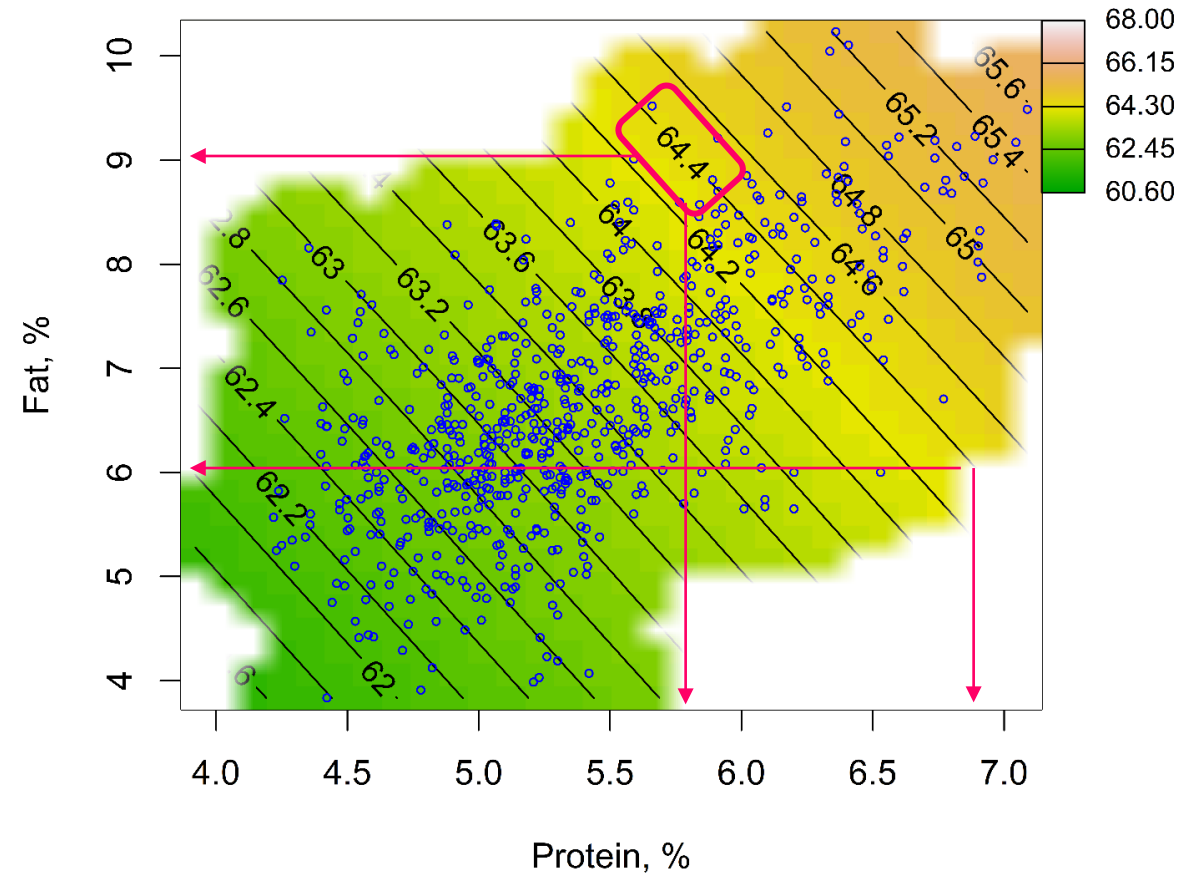


Effect of protein × fat

FRAP, μM eq. ascorbic acid/mL of milk

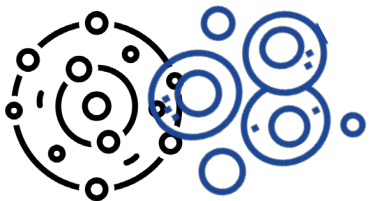
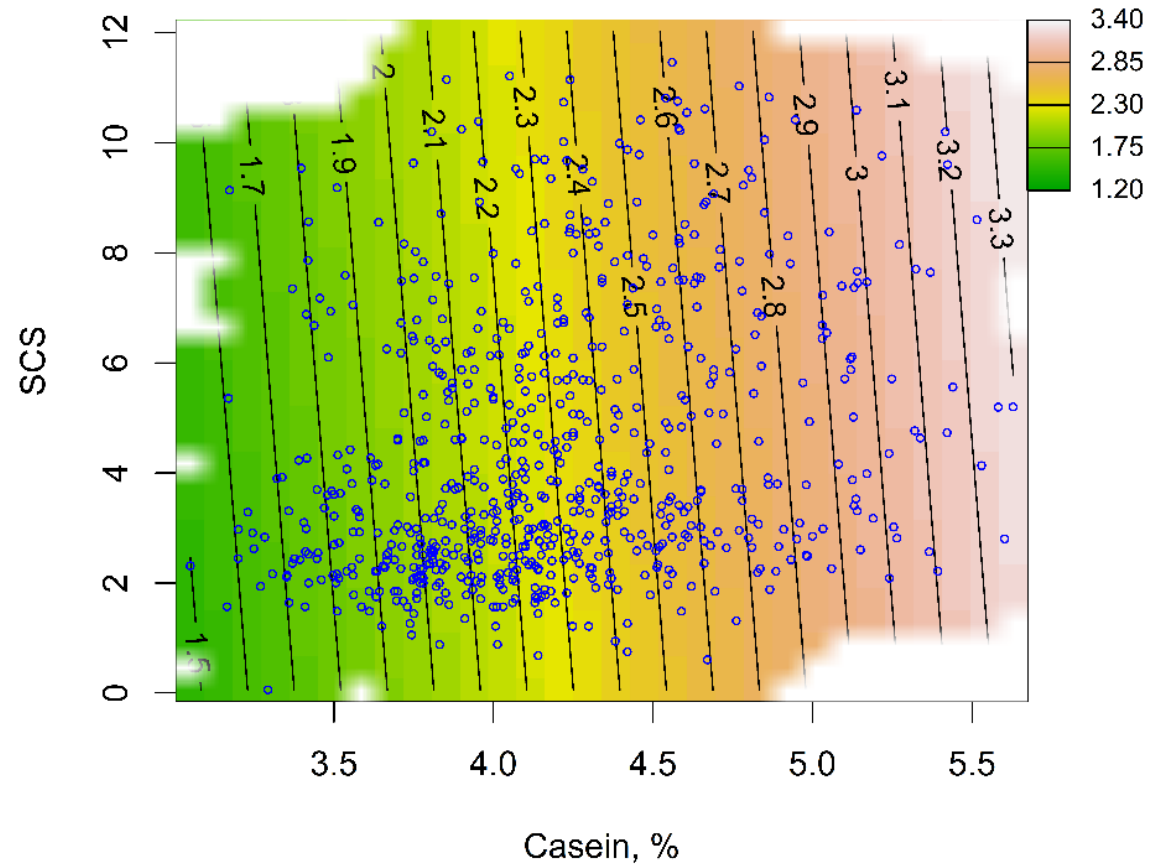


DPPH, % of inhibition



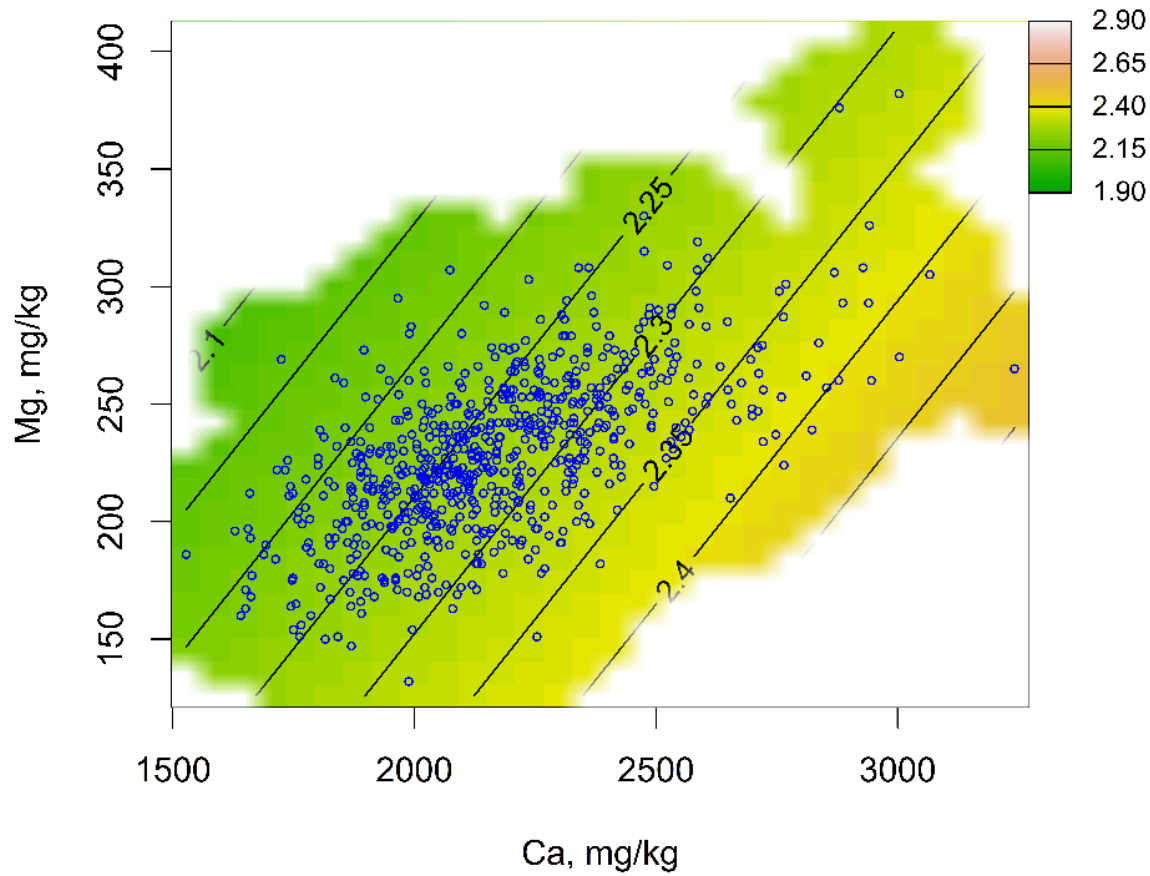
Effect of casein × SCS

FRAP, μM eq. ascorbic acid/mL of milk

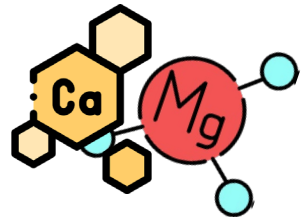
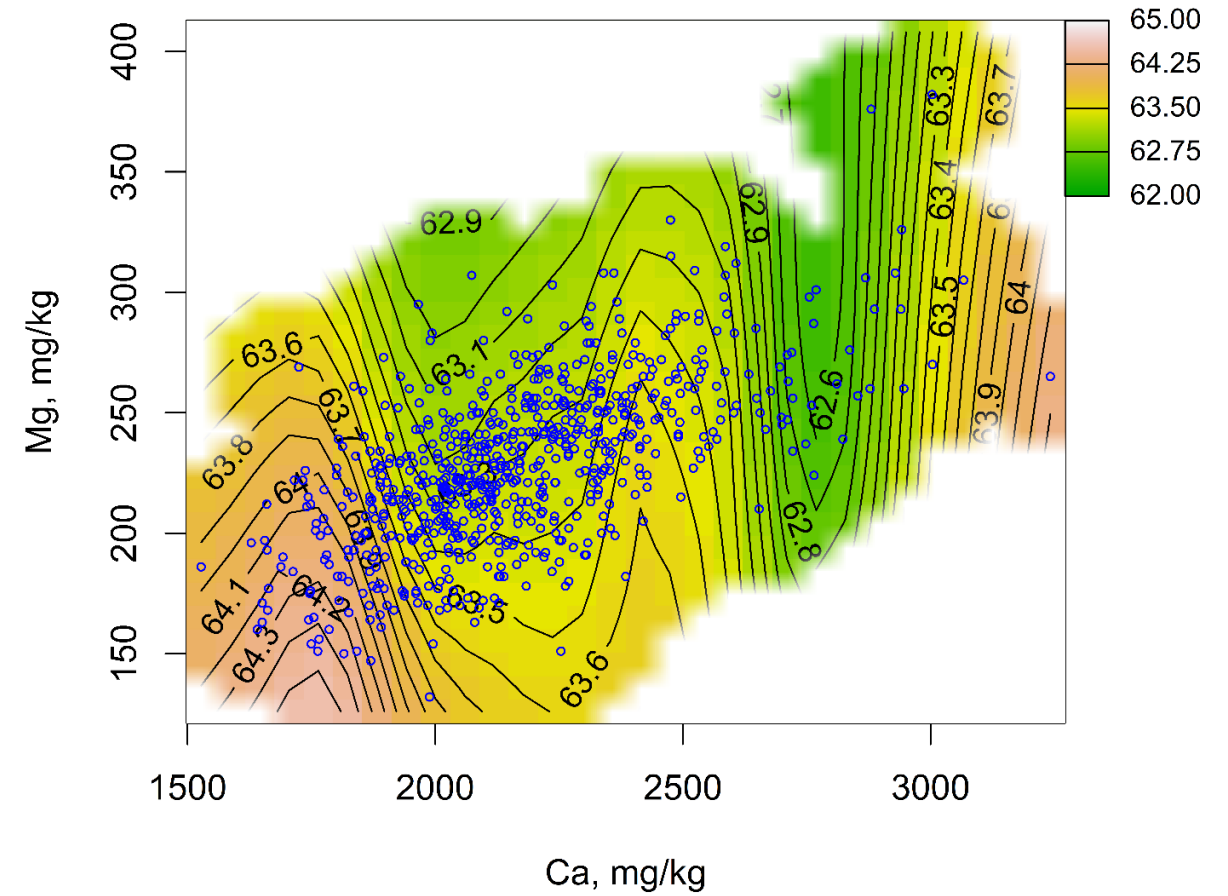


Effect of Ca × Mg

FRAP, μM eq. ascorbic acid/mL of milk

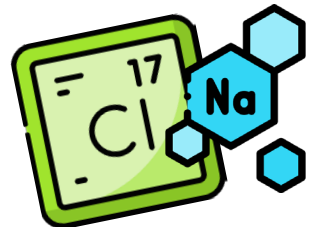
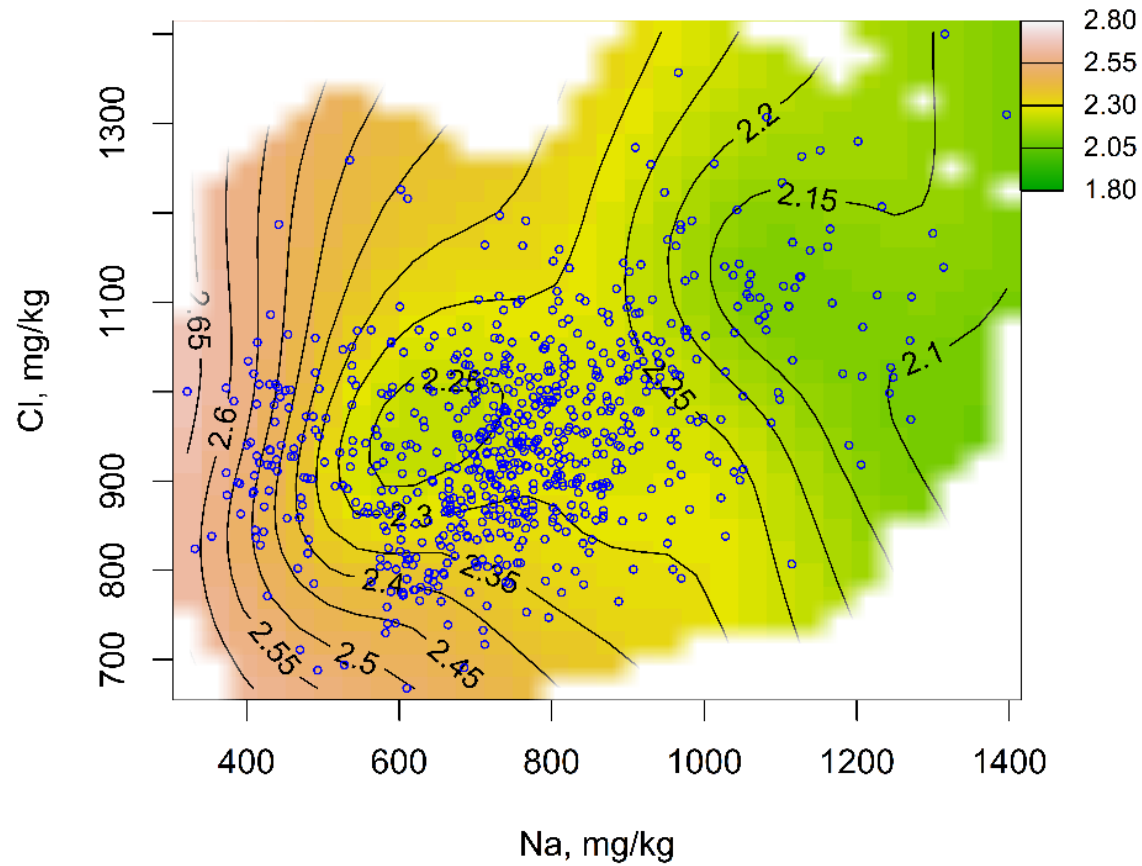


DPPH, % of inhibition



Effect of Cl × Na

FRAP, μM eq. ascorbic acid/mL of milk

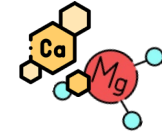


Conclusions

Nature of the antioxidants and udder health



The antioxidant activity is described by the interactions among milk components, and by the udder health condition



Complementarity of assays

The use of different assays is useful to understand the antioxidant potential of milk

Thank you for your attention!

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