QUANTIFICATION OF WHEY IN FLUID MILK USING CONFOCAL RAMAN MICROSCOPY AND ARTIFICIAL NEURAL NETWORK

Roney Alves da Rocha¹, Igor Moura Paiva², Virgílio de Carvalho dos Anjos¹, Marco Antônio Moreira Furtado², Maria José Valenzuela Bell¹

¹ Physics Department, Federal University of Juiz de Fora, Minas Gerais, Brazil

² Pharmacy Department, Federal University of Juiz de Fora, Minas Gerais, Brazil
OUTLINE

- Motivation
- Materials and methods
- Raman results
- PLS and ANN results
- Conclusions
Brazil and Minas Gerais: 2014

Brazil: 37 billion liters of milk
Minas Gerais: 10 billion liters

Usual frauds:
- Water
- Whey
- Caustic soda
- Formaldehyde
- Urea

... Minas Gerais
RECOMMENDED TECHNIQUES:

- HPLC to evaluate the GMP (Glycomacropeptides):
  - it is time consuming (it takes 1 or 2 weeks to have the results), needs sample preparation, mail, etc.
  - So, in most of cases milk has already been commercialized and consumed when the fraud is evidenced.

- We are proposing an on-line procedure, with Raman equipment.
MATERIALS AND METHODS

- Fluid milk from a local farm (Marvin), Protein (3.5%, fat 3.0% and carbohydrate (5.0%).
- Sweet whey was prepared by enzimatic coagulation using chymosin
- Adulterated samples were prepared with different levels of whey addition (in %): 0; 0.25; 0.50; 0.75; 1; 1.5; 2; 2.5; 3;4;5;10;15;...90;95;100% (30 levels of adulteration)
- Samples for Raman measurements were drops of 1 μl of milk+whey, pipetted on a microscope slide in circles with diameter of 2 cm.
Confocal Raman Microscopy

\[ \lambda = 514.5 \text{ nm} \]

Ar+ laser

Ti:Sa laser

He-Ne laser

Horiba Jobin Yvon T64000

Diameter of the drops: 2 cm (1\(\mu\)l)
Diameter of the laser spot: 1\(\mu\)m

Measurements were performed at room temperature
- No baseline correction
- No normalization
- No smoothing
- No filters

Each spectrum is composed by the average of 3 spectra obtained at different points of the sample
Partial Least Squares (PLS)

Percentage of actual fraud

$R^2 = 0.9923$

Fraction of whey added (actual) (%) vs. Fraud actual, %

Fraction of whey added (predicted) (%) vs. Fraud predicted, %

Partial Least Squares (PLS)
ARTIFICIAL NEURAL NETWORK

\[
\begin{align*}
1 & \rightarrow 0 \text{ Bias} \\
X_1, X_2, \ldots, X_{715} & \rightarrow 1, 2, \ldots, n \\
W_{01}, W_{11}, W_{12}, W_{1n}, \ldots, W_{n1}, W_{nn} & \\
V_1, V_2, \ldots, V_n & \\
\% \text{ Fraud} & \\
\end{align*}
\]

\( n=19 \)

SAS JMP software, version 11
ARTIFICIAL NEURAL NETWORK

ANN, training data

R²=0.9999

Percentage of actual fraud

ANN, validation data

R²=0.9999

Percentage of actual fraud
**Conclusions**

- Raman is a very sensitive technique that has potential applications for on-line studies in dairy products.
- No baseline correction or smoothing of the spectra were required.
- Milk can be analyzed without pretreatments.
- ANN is a very robust statistical tool and has good perspectives to be used for whey detection and other adulterants. It does not require a high computational time.
- Response time was about 10 minutes, but can be reduced to few seconds according to the Raman equipment.
ACKNOWLEDGEMENTS

UFJF, and the Brazilian agencies CAPES, FAPEMIG, CNPQ for financial support
CONTACT

- Maria Jose Valenzuela Bell
- Physics Department, UFJF, Minas Gerais, Brazil

E-mail: mjbell@fisica.ufjf.br

Thank you for your attention