XXVI ENCONTRO NACIONAL
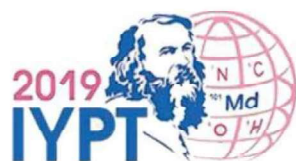Sociedade Portuguesa de Química

150 ANOS PARA 118 ELEMENTOS
A TABELA PERIÓDICA

XXVI ENCONTRO NACIONAL

DA SOCIEDADE PORTUGUESA DE QUÍMICA

24, 25 E 26 DE JULHO DE 2019
FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO

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UNIVERSIDADE DO PORTO

AMINO ACIDS PROFILE FOR ASSESSING SERRA DA ESTRELA CHEESE PRODUCERS

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OBJECTIVES

Serra da Estrela cheeses collected from 6 producers have typical chemical characteristics that allows its discrimination?

↓

Samples VARIABILITY:
produced during different time periods; the raw milk from different animals; cheese processing may slightly differ between cheesemaking producers.

↓

Cheeses' amino acids profile

ANALYSIS

Characterization of individual amino acids constituents by UHPLC-MS/MS
(Dionex Ultimate 3000 UPLC instrument from Thermo Scientific, USA)

↓

diode-array detector and mass detector Linear Ion Trap LTQ XL mass spectrometer
(ThermoFinnigan, San Jose, CA, USA) with an ESI source

chromatographic separation with a U-VDSpher PUR C18-E 100mm×2.0 mm id, 1.8 µm column (VDS optilab, Germany) at 40 °C

mobile phase: (A) 0.1% (v/v) formic acid in water and (B) 0.1% (v/v) formic acid in acetonitrile/water (50:50, v/v)

multistep gradient program with flow rate of 0.40 mL/min and an injection volume of 5 µL

data acquisition with Xcalibur®data system
(ThermoFinnigan, San Jose, CA, USA).

Correctly classify cheese samples according to their producer (dependent variable)

↓

Amino acids quatification → 10 nmol/µL standard solutions
Calibration of each amino acid within the range of 0–7 nmol/µL → $R^2 > 0.98$

↓

Independent variables → amino acids matrix data (centered and scaled)

Linear discriminant analysis → supervised pattern recognition method

↓

Internal evaluation was considered using k-folds (10 folds)
(minimize model over-fitting reducing the risk of over-optimistic results)

↓

Meta-heuristic simulated annealing variable selection algorithm
(selection of amino acids with the optimal discrimination performance)

11 amino acids selected

asparagine, cysteine, glutamic acid,
histidine, leucine+isoleucine, lysine,
phenylalanine, proline, threonine,
tryptophan, tyrosine

CONCLUSION

The obtained models in the cross-validation allowed an average classification accuracy of 90%, while the best model allowed correct classifications with a predictive sensitivity (proportion of positive correct classifications) and specificity (proportion of negatives correct classifications) greater than 95%.

SAMPLING

6 cheese certified producers located in different municipalities of the region of Serra da Estrela

↓

24 cheeses were collected and samples stored -20°C until analysis

↓

Cheeses with **45 maturation days**

↓

5.0 g of cheese + 10 mL of water:acetonitrile (50:50) (v/v) with 3.0 mM N-Acetyl-L-Tyrosine (internal standard) solution
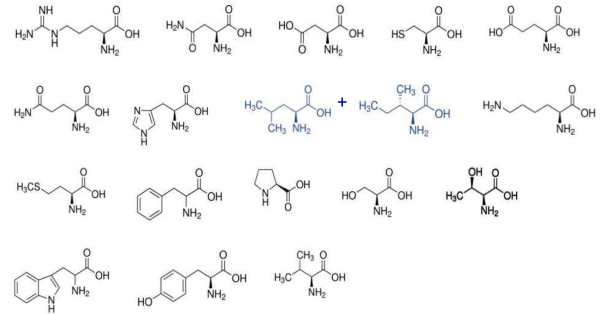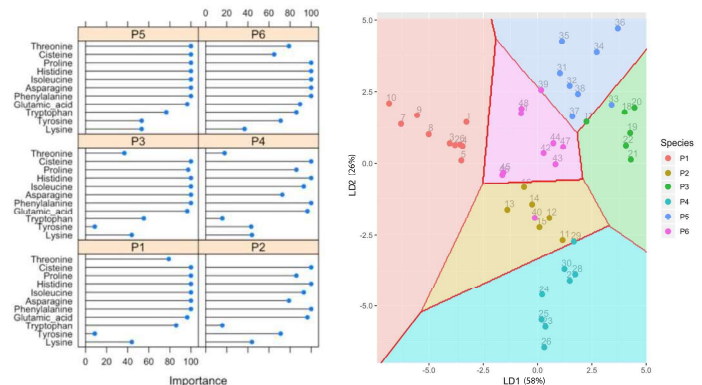
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Sonicated for 10 min at room temperature (20 °C)

↓

Centrifuged at 4°C at 10.000 rpm for 10 min
(Heraeus Multifuge X1R – Thermo Fisher Scientific)

↓

Filtered through 0.2 µm nylon membrane filter
(Whatman PURADISC 25 NYL) and stored at -4 °C.

RESULTS

Quantitative amino acid profile in cheese → 17 amino acids

arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, leucine+isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine