

# PROTEIN FINGERPRINTING BY CAPILLARY ELECTROPHORESIS WITH ULTRAVIOLET ABSORPTION DIODE ARRAY DETECTION FOR DIFFERENTIATION OF QUINOA VARIETIES



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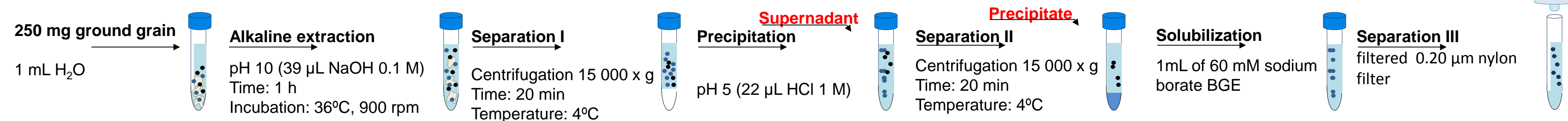
## Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an andean grain with more than 3,000 ecotypes recognized for its exceptional nutritional properties. In western countries, where it is sold as a gluten-free protein-rich super food with a broad amino acid spectrum, quinoa trade and consumption is rapidly expanding. Quinoa is consumed as a whole grain or after different processing methods (e.g. extrusion), but it is also milled to produce high-value flour, which is susceptible to adulteration. In consequence, there is a growing interest in developing novel analytical methods to expand the knowledge regarding quinoa composition. In this study, we developed a rapid and simple capillary electrophoresis-ultraviolet adsorption diode array detection (CE-UV-DAD) method to obtain characteristic multiwavelength electrophoretic profiles of protein extracts from different quinoa grain varieties (black, red, white from Peru and royal white from Bolivia). Then, advanced chemometric methods (i.e. multivariate curve resolution alternating least squares, MCR-ALS, followed by principal component analysis, PCA, and partial least squares discriminant analysis, PLS-DA) were applied to deconvolute the components present in the CE-UV-DAD electropherograms and classify the different quinoa varieties according to their differential protein composition.

## Experimental

### A) Protein Extraction

Legend: Bran (blue), Water (light blue), Protein (black), Soluble bran components (grey)



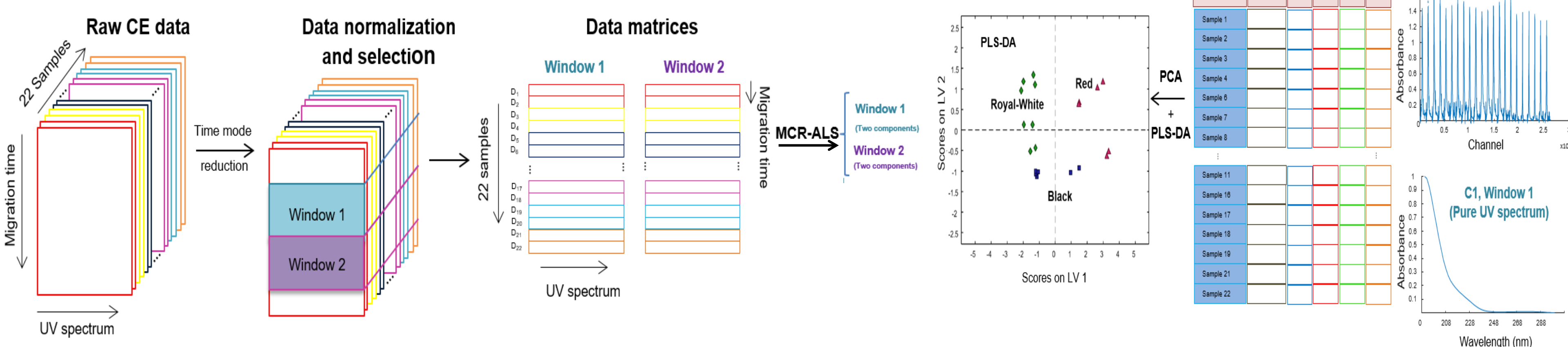
### B) CE-DAD

- ✓ Protein extracts were dissolved in BGE
- ✓ Sample injection: 50 mbar, 10 s
- ✓ Separation voltage: +25 kV
- ✓ Wavelength range: 190-400 nm



- ✓ Postconditioning (930 mbar): water (3 min)
- ✓ Between injections (930 mbar): 0.5% SDS (2 min), water (3 min), 1 M NaOH (3 min), water (3 min) and BGE (3 min).
- ✓ Activation (930mbar): 1 M HCl (20 min), water (20 min), 1 M NaOH (20 min), water (20 min) and BGE (20 min).

### C) Data Analysis



## Results

### RAW CE-DAD ELECTROPHEROGRAMS

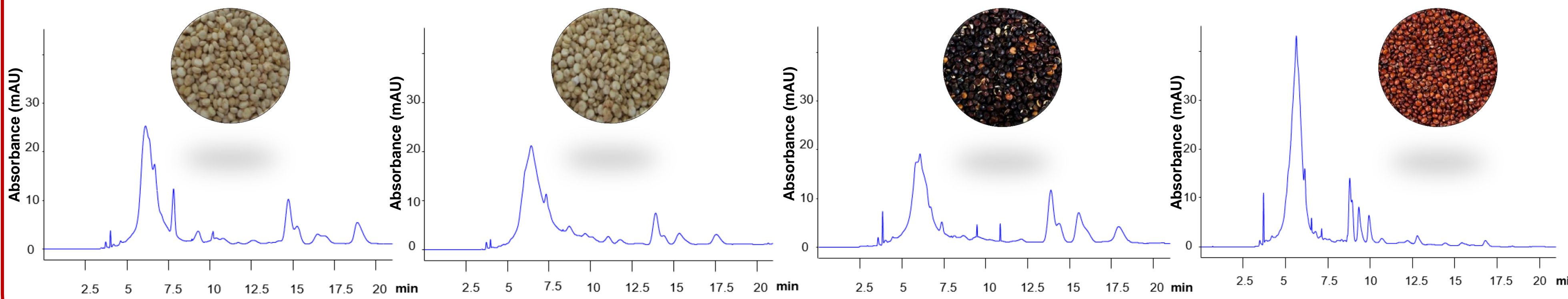
$\lambda = 214 \text{ nm}$

Royal Quinoa (Ro)

White Quinoa (W)

Black Quinoa (B)

Red Quinoa (R)



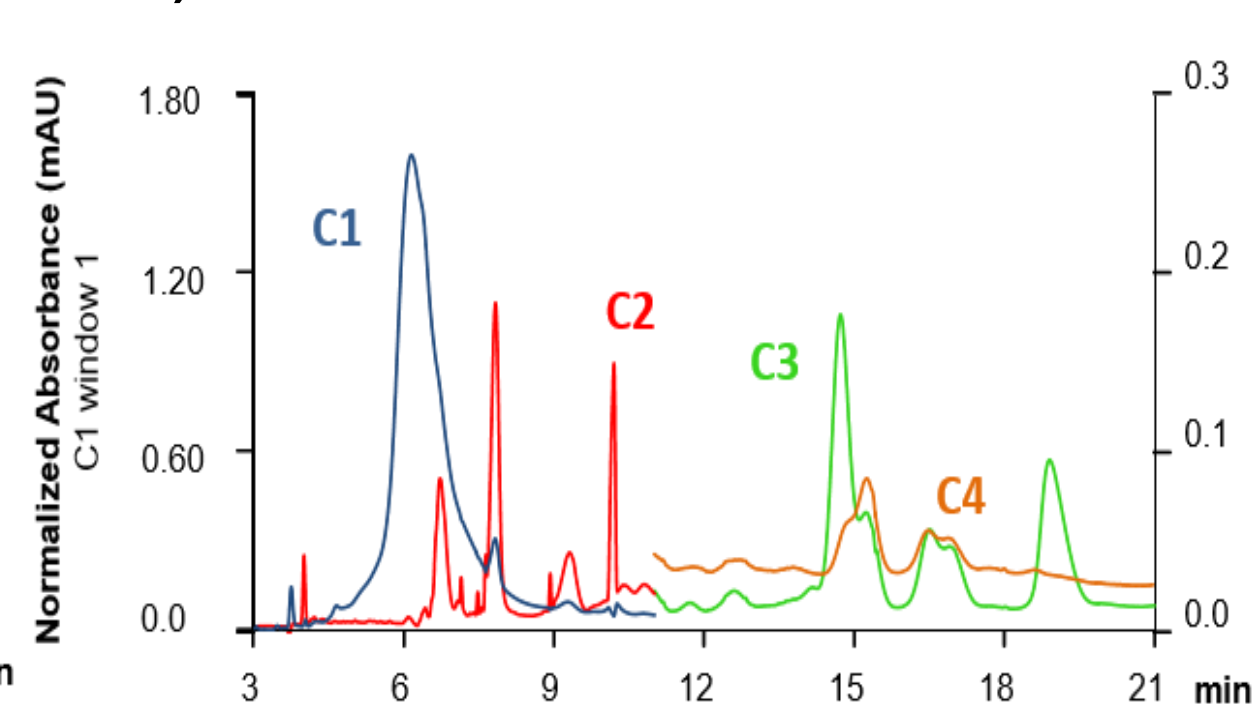
Complex CE-DAD electrophoretic profiles

### MCR-ALS DECONVOLUTION

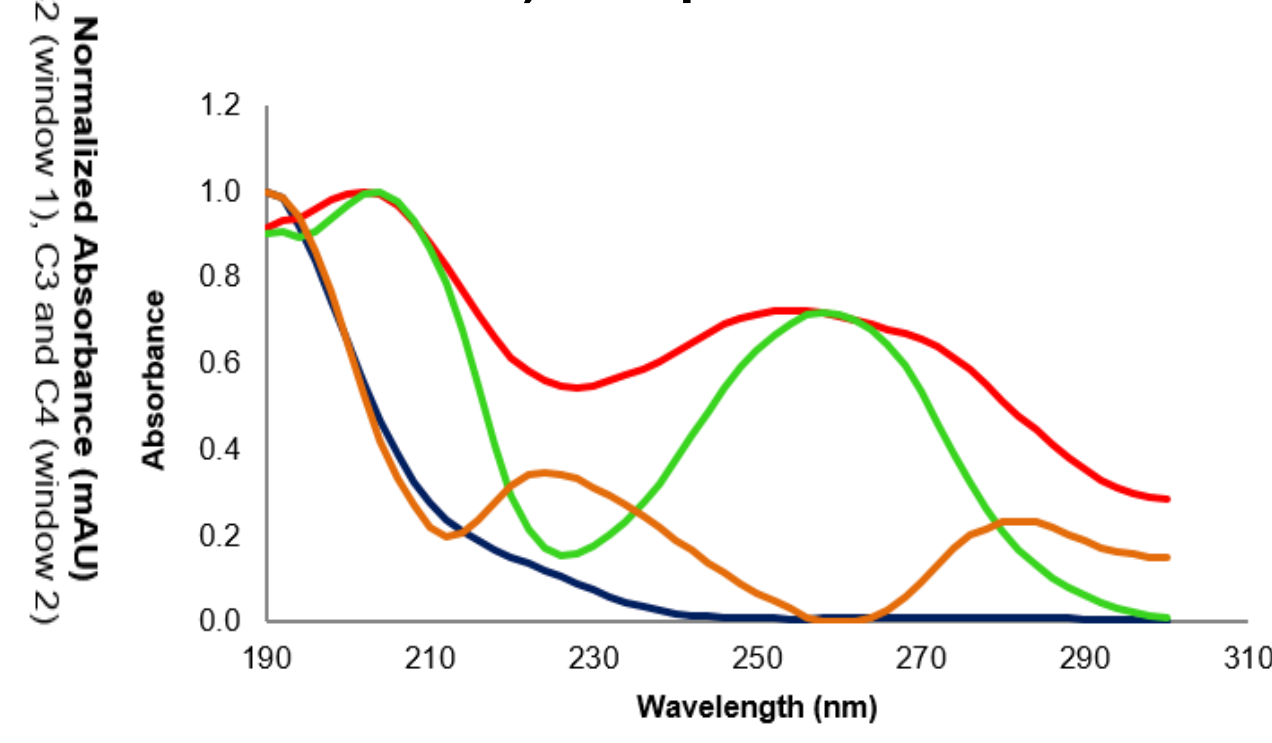
A) Royal Quinoa Raw Electropherogram

B) MCR-ALS (deconvoluted components)

i) Concentration Profiles



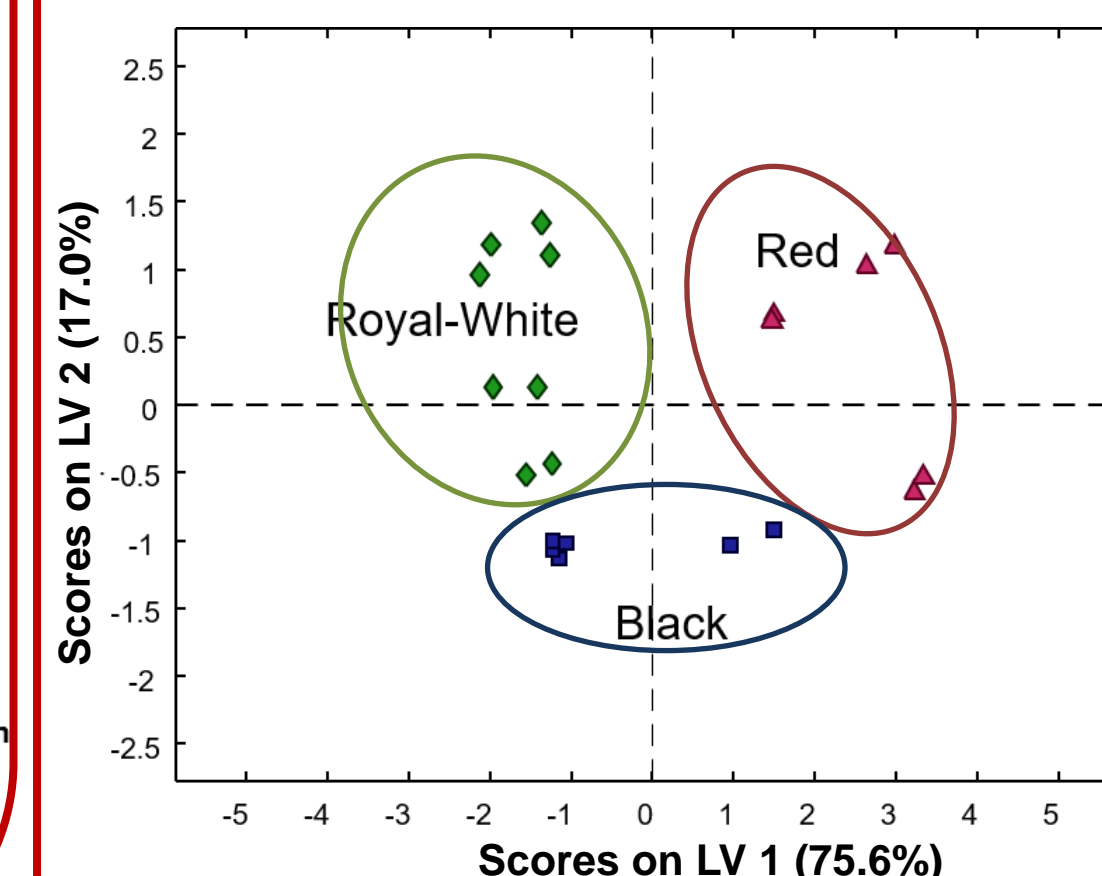
ii) UV Spectra



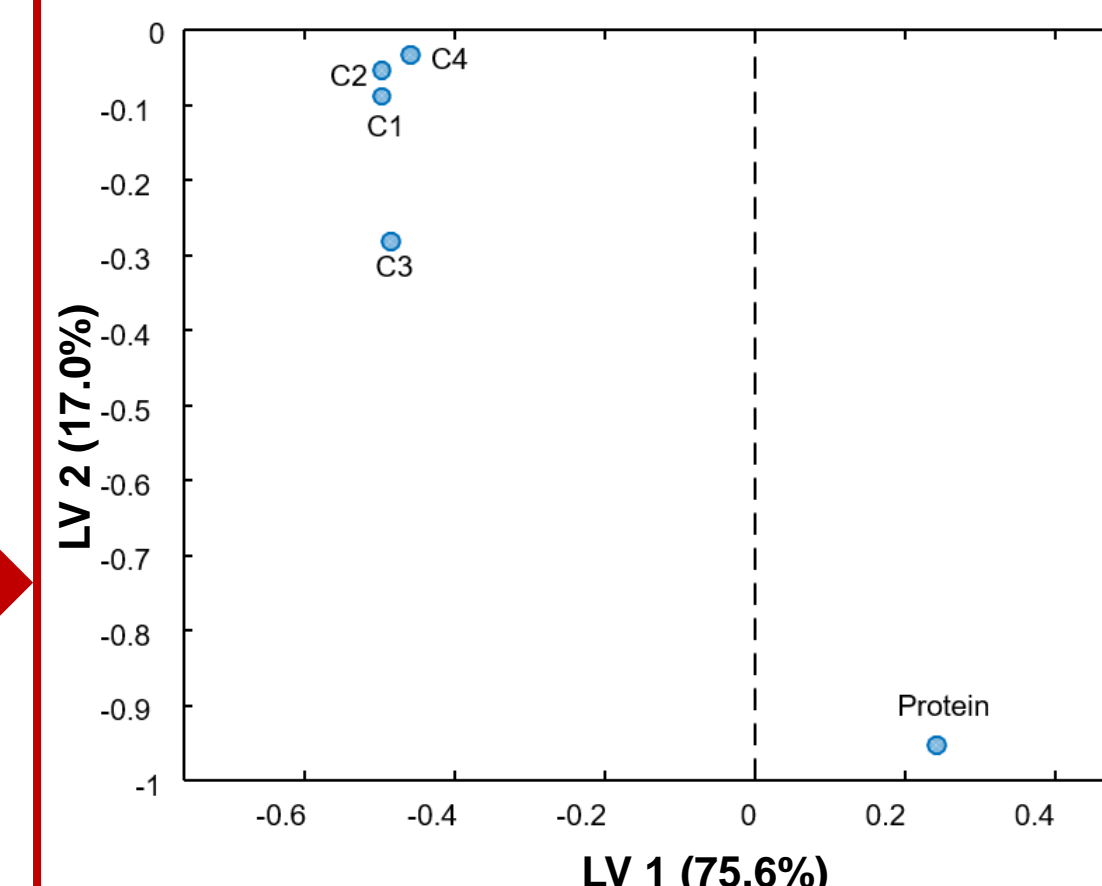
C2, C3 and C4 do not show the typical UV spectra in proteins

### PLS-DA ANALYSIS

A) PLS-DA. Scores Plot

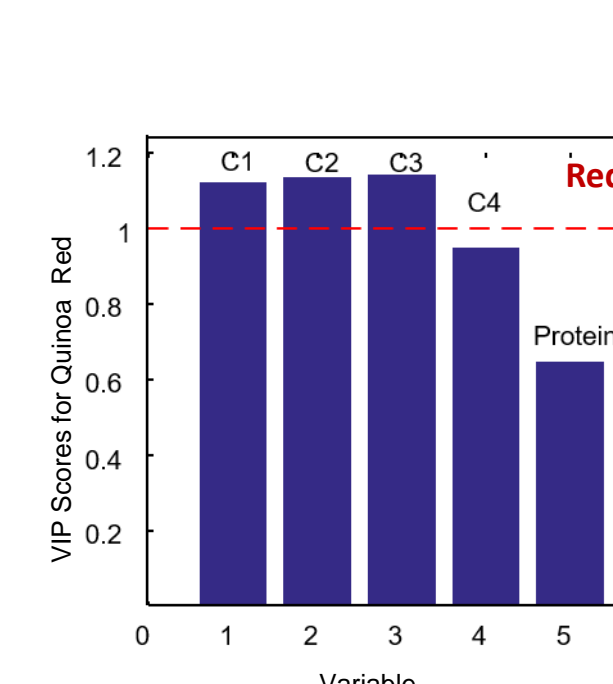
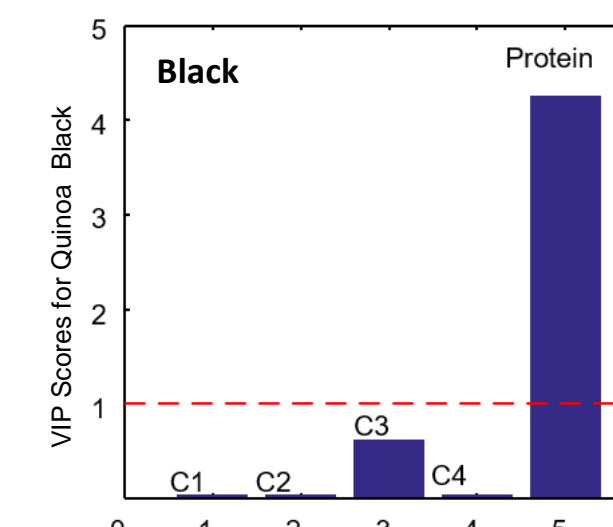
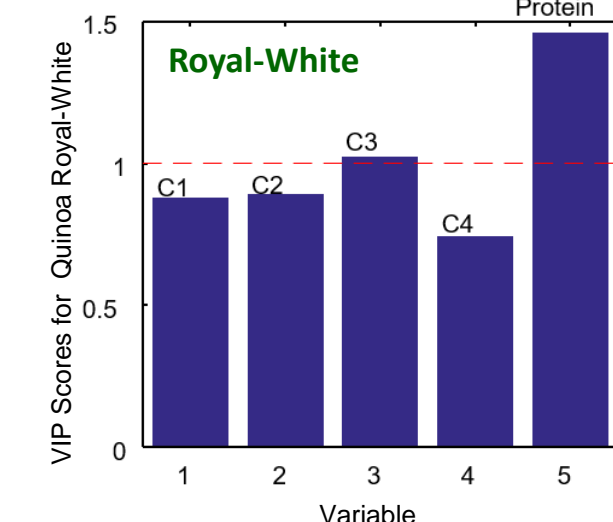


B) PLS-DA. Loadings Plot



Two latent variables allow classifying the different quinoa varieties

C) PLS-DA. VIP Plots



C4 is the only variable non critical for differentiation (VIP<1)

## References

- [1] R. Galindo-Luján, L. Pont, V. Sanz-Nebot, F. Benavente. *Food Chemistry* 341 (2021) 128207.
- [2] L. Pont, I. Compte, V. Sanz-Nebot, J. Barbosa, F. Benavente. *Food Anal. Methods* 13 (2020) 325-336.
- [3] L. Pont, F. Benavente, J. Jaumot, R. Tauler, J. Alberch, S. Ginés, J. Barbosa, V. Sanz-Nebot. *Electrophoresis* 37 (2016) 795-808.
- [4] A. Barroso, E. Giménez, F. Benavente, J. Barbosa, V. Sanz-Nebot. *Talanta* 160 (2016) 614-623.

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## Conclusions

- ✓ A CE-DAD method to obtain multiwavelength electrophoretic fingerprints was applied to protein extracts from B, R, and Ro quinoa samples.
- ✓ Deconvolution with MCR-ALS allowed the resolution of the most relevant components in the electrophoretic profiles, which showed characteristic UV-spectra.
- ✓ The areas of the 4 resolved components and the total protein content determined by the Kjeldahl method were considered for multivariate data analysis PCA and partial least squares discriminant analysis (PLS-DA).
- ✓ PCA allowed detecting two white quinoa outlier samples and defining three sample classes (i.e. B, R and W-RO quinoa).
- ✓ PLS-DA allowed classifying of quinoa varieties based on protein fingerprinting and could be used for simple and enhanced quality control of quinoa-containing foodstuff.