

# DISCRIMINATION BETWEEN SLOVENIAN COW, GOAT, AND SHEEP MILK AND CHEESE BASED ON ELEMENTAL AND STABLE ISOTOPE COMPOSITION

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Due to increased consumer requirements and quality expectations, there exist an increasing interest in demand for high quality dairy products with clear geographical origin, such as product with a Protected Denomination of Origin (PDO) or protect geographical indication (PGI). The consumption of dairy product is steadily increasing in recent years, rendering the proof of provenance a vital issue in food and consumer protection. The Protected Designation of Origin (PDO) trademark has been assigned to numerous local products based strictly on their area of origin, while PGI covering dairy products has at least one said stage that takes place in a certain area.

**Our study includes a combination of elemental and stable isotope analysis of milk and cheese from cow, goat and sheep provided from several farms from different regions of Slovenia to identify the patterns that allow us to distinguish among cow, goat and sheep milk and cheese and to discriminate milk and cheese according to their region of production.**

## SAMPLING

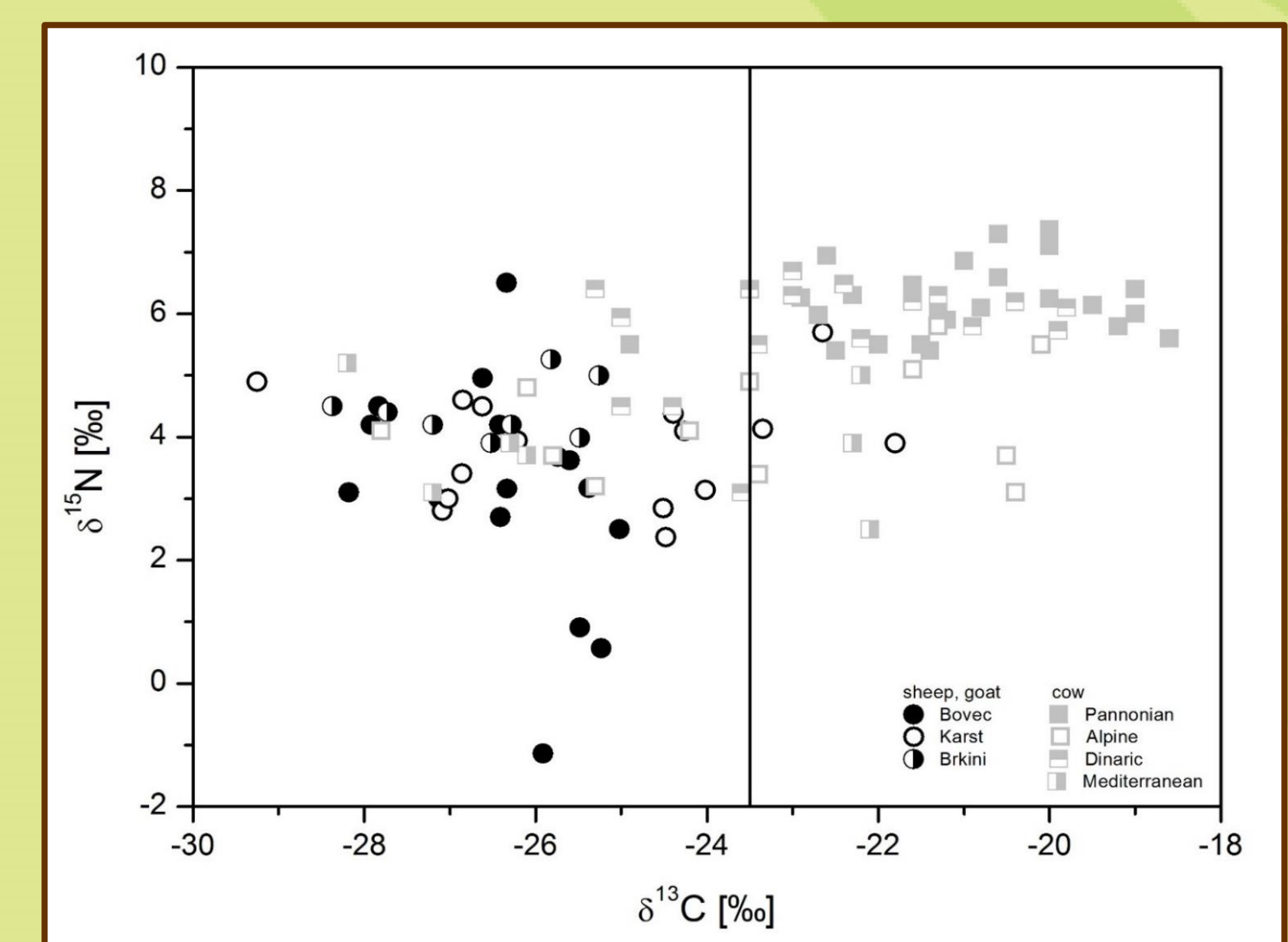
### Milk samples:

- 76 samples of cow milk from Alpine, Dinaric, Pannonian and Mediterranean
- 11 samples of goat milk and 35 samples of sheep milk from Bovec (Alpine), Karst, Vipava, Brkini (Mediterranean), central Slovenian region (Dinaric)
- 7 samples with mixed origin; sheep-goat-cow (1 sample), sheep-goat (2 samples), sheep-cow (2 samples) and goat-cow (2 samples)

### Cheese samples:

- 15 samples of sheep cheese
- 6 samples of goat cheese
- 9 samples of cow cheese

Two types of cheese have EU PDO status: Bovški sheep cheese (Bovški ovčji sir) and cow cheese (Tolminc) and one local PDO status Kraški sheep cheese (Kraški ovčji sir).



**Figure 1:** Stable isotope composition of carbon and nitrogen in casein for milk samples from different regions in Slovenia.  $\delta^{13}\text{C}$  values greater than  $-23.5\text{‰}$  indicating the presence of maize in the diet. Most of these samples are from Pannonian region. The lowest  $\delta^{13}\text{C}$  values are observed in the Alpine and Mediterranean region where animal feed is based on grass and hay.

## ANALYTICAL METHOD

### Stable isotope analysis

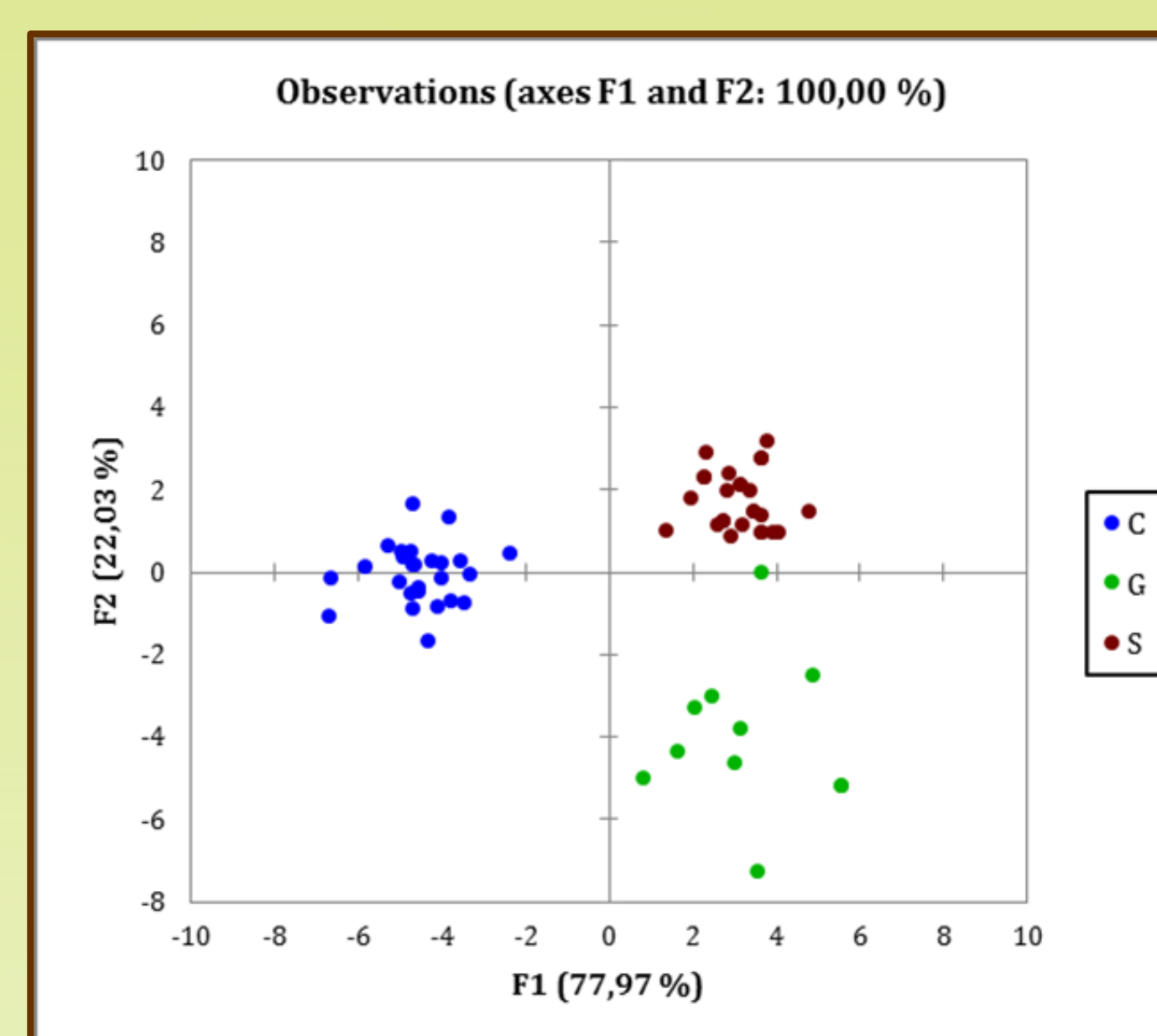
- determination of  $\delta^{18}\text{O}$  in milk with IsoPrime MultiFlow Bio equilibration unit (GV Instruments)
- determination of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  in casein with IsoPrime 100 –Vario PYRO Cube (OH/CNS)

### Elemental analysis

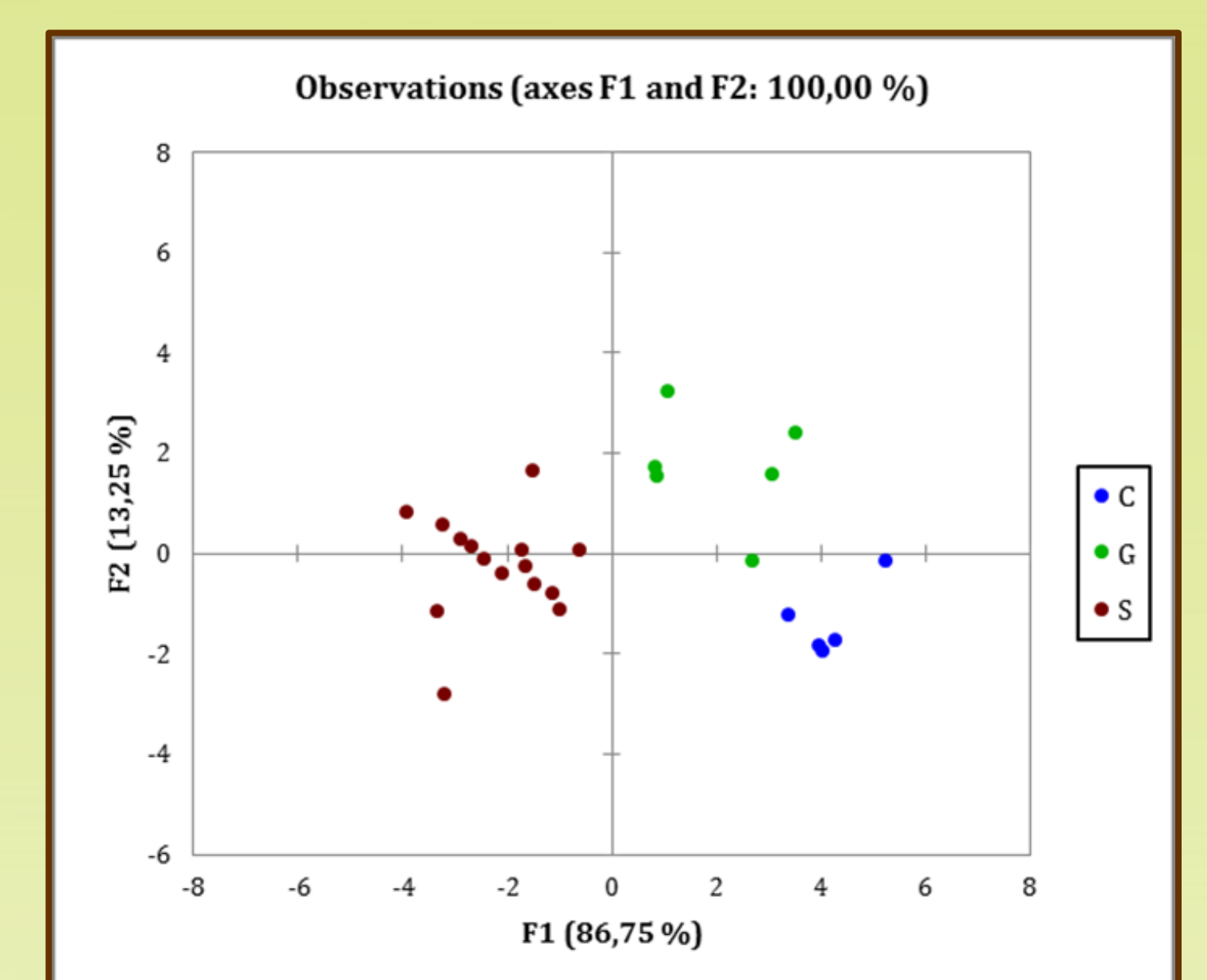
- determination of macro- and micro-elements (P, S, Cl, K, Ca, Zn, Br, Rb, Sr) on a freeze-dried samples by X-ray fluorescence spectrometry (XRF)

### Statistical analysis

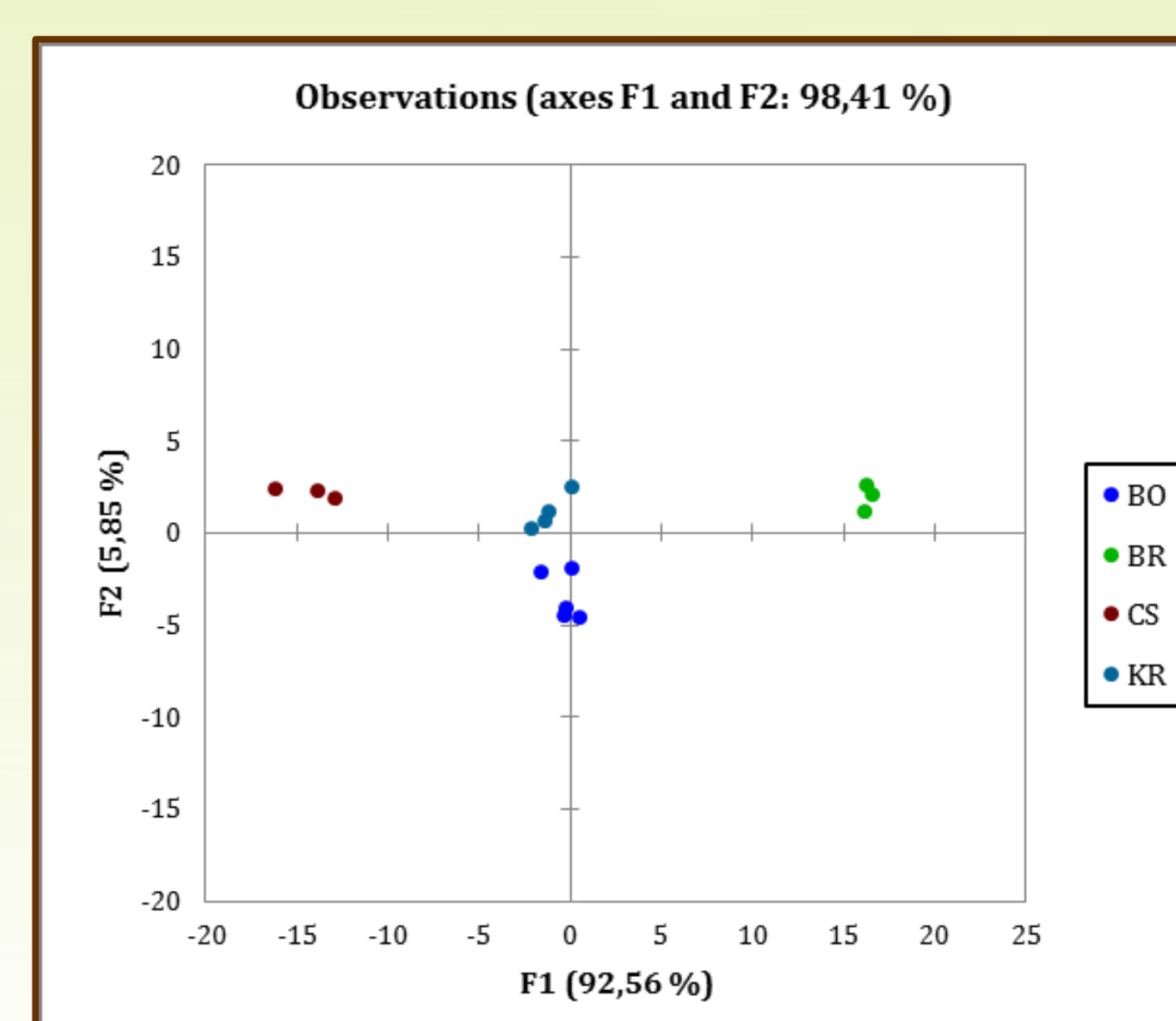
- calculations and multivariate analysis were carried out using the XLSTAT software package (Addinsoft, New York, USA).



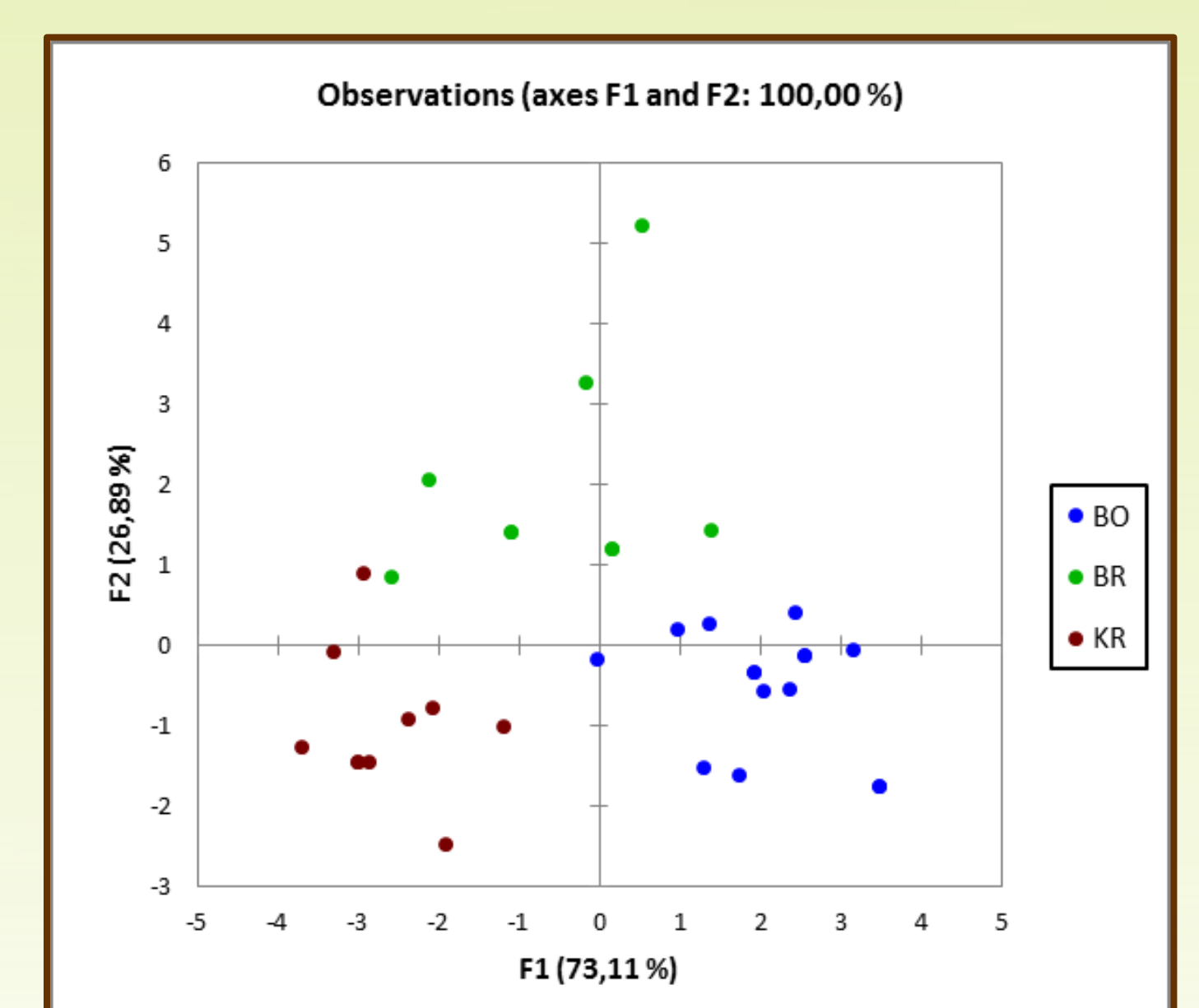
**Figure 2:** Classification of the milk samples according to the different species origin (cow (C), goat (G), and sheep (S)). Stable isotopes in a combination with elemental composition is the most appropriate method for differentiate between cow, sheep and goat milk. The most significant variables were K, Cl, P and  $\delta^{18}\text{O}$  in function 1 and Cl, S, P, and Zn in function 2.



**Figure 3:** Discrimination of three type specie origin of cheese (cow (C), goat (G), and sheep (S)). The major parameters that discriminate the three cheese types were in the first function: Ca, Zn, S and Cl and Zn, Ca, P and  $\delta^{15}\text{N}$  in the second one.



**Figure 5:** Discrimination of sheep cheese according to production location (BO-Bovec, BR-Brkini, CS-Central Slovenia, KR-Karst). The classification functions reveal that all Bovec, Karst, Central Slovenia and Brkini samples were correctly classified (100%) into their original group.



**Figure 4:** Discrimination of sheep milk according to production origin (BO-Bovec, BR-Brkini, KR-Karst). The classification functions reveal that all Bovec and Karst samples were correctly classified (100%) into their original group, while for samples of Brkini origin 90% correct classification was achieved.

## CONCLUSION

The first systematic characterization of authentic Slovenian cow, sheep and goat milk and cheese were defined using a combination of different isotopic ratios of major bioelements ( $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{34}\text{S}/^{32}\text{S}$ ) and elemental composition. This preliminary study highlights the most important parameters, which in DA classification differentiate milk and cheese according to animal species and geographical origin and can contribute towards supporting the existence of a Protected Designation of Origin (PDO). PDO products are important for the promotion of the local products and in addition to the contribution to local economy, they contribute to the promotion of the culture of the given community as well.

## Acknowledgements

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