ACTIVITY REPORT 2020

For the implementation of the Postdoctoral Project **PD 90/2020**

Advanced chemometric methods applied for authentication and traceability of Transylvanian agriproducts - *AGRICHEM*

Stage 1. Assessing the elemental and isotopic profile of mushrooms, using ICP-MS and IRMS, and experimental data processing using classical and advanced chemometric techniques

(September - December 2020)

Summary of the stage

In this first stage of the project, 57 samples of mushrooms were analyzed from isotopic and multielemental point of view (29 samples of gălbiori and 28 samples of hribe). The obtained results were processed using classical chemometric methods (ANOVA - analysis of variance and linear analysis discriminant - LDA) and advanced chemometric methods (artificial neural networks - ANN). The ANOVA analysis showed the elements that can differentiate the two species of mushrooms studied. Moreover, the LDA led to a model, validated, based on three predictors. ANN highlighted the predictors with the highest contribution to the model, which offer a prediction rate of 94.4% ($\delta^{13}C$, Ag and Ni).

Content of the scientific and technical report (RST)

1. Wild mushrooms samples collection, samples preparation for IRMS and ICP-MS analysis.
2. Fusion and pretreatment of experimental data.
3. Classical and advanced chemometric data processing.
4. Results dissemination.

In this stage of the project a total of 57 mushrooms samples were collected belonging to two different species. Mushroom samples were collected in individual bags, labeled with the appropriate species, area and date of sampling and transported to the laboratory as soon as possible.

The preparation of the samples for multielemental analysis using ICP-MS technique was performed as follows: the mushroom samples were washed with distilled water and then dried in an oven at 105 °C for 24 hours. The obtained samples were ground in a very fine powder and a quantity of 0.2 g of this powder was used in the digestion step. This stage was performed according to the method presented in the literature by Dospatliev L. (Dospatliev & Ivanova, 2017). Samples were digested using a mixture of HNO_3 (65%) and H_2O_2 (30%), 6: 1, (v / v).

The samples preparation for isotopic content analysis using IRMS technique was carried out as follows: the water from the fresh mushrooms was extracted using cryogenic distillation,

without isotopic fractionation. The values of $\delta^{18}O$ and $\delta^{2}H$ in the water extracted from the mushrooms were determined using an elemental analyzer DLT-100 Los Gatos Research. To determine $\delta^{13}C$, the samples were dried at 55 °C for 24 hours and analyzed using a dual-operated Delta V Advantage mass spectrometer (Thermo Scientific).

Values are expressed as δ (‰), for $\delta^{13}C$ versus Vienna Pee Dee Belemite, for $\delta^{18}O$ versus Vienna Standard Mean Ocean Water, and for $\delta^{2}H$, using the expression:

$$\delta X = \frac{R_{sample} - R_{standard}}{R_{standard}}$$

where R_{sample} is the isotopic ratio of the sample, and $R_{standard}$ is the isotopic ratio of the standard.

The mean values of the macroelements P and Na showed higher values in the hribe samples, in contrast to K and Mg which showed higher mean values in the galbiori. Regarding the toxic elements, As, Hg, Cd and Pb had higher average values in hribe compared to those of galbiori. The other elements had different variations in the two species of analyzed mushrooms, for example the average value of Cu and Mn were higher in the galbiori samples, compared to Zn and Mn, which had higher average values in the hribe.

The isotopic composition generally provides information about the climatic and geographical conditions (temperature, humidity, precipitation, altitude) of the analyzed sample. Deuterium values varied between -84.20 ‰ and -1.20 ‰, for $\delta^{13}C$ the range was -27.30 ‰ and -22.30 ‰, and for $\delta^{18}O$ the range of variation was between -12.10 ‰ and 3.00 ‰.

The experimental results obtained from the IRMS and ICP-MS analysis were transferred to a single data file, which constituted the working matrix for further chemometric processing. The initial processing consisted in the elimination of some elements that were below the detection limit for more than half of the analyzed samples and which otherwise could have led to erroneous statistical results.

After applying ANOVA, the following markers were highlighted: $\delta^{13}C$ (p=0.001), P (p=0.016), Zn (p=0.010), As (p=0.001), Ag (p=0.001), Cd (p=0.001), Hg (p=0.001). The linear discriminant analysis (LDA) provided a model which led to an initial classification as well as cross-validation of 94.7%. Difference from an ideal classification is due to the wrong attributes samples (one sample of galbiori and two of hribe). The distribution of the samples can be observed in Figure 1.

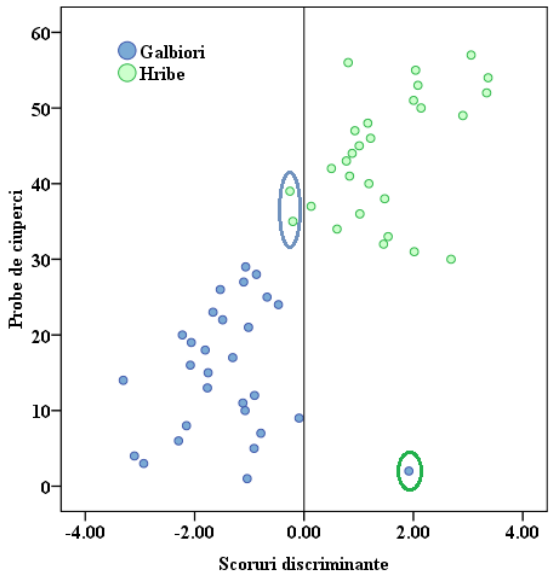

Figure 1 Mushrooms distribution obtained after LDA, based on $\delta^{13}C$, P and K

The predictors on the basis of which this distribution was obtained are: $\delta^{13}C$, P and K. It can be observed that the average values for hribe ($\delta^{13}C$=- 24.4821 ‰, P=12297.39 µg/g, K=45665.71 µg/g) are higher for all three predictors, compared to the average values for galbiori ($\delta^{13}C$=-26.0448 ‰, P=7233.01 µg/g and K=65047.81 µg/g).

Results dissemination for this stage of the project consisted of:

✓ The initiation of ***project web page*** https://www.itim-cj.ro/PNCDI/agrichem/

✓ ***Experimental data set***, with isotopic and multielemental data, for authentic mushrooms samples

✓ ***Chemometric model*** developed based on specific markers for mushrooms traceability

✓ Scientific ***ISI article***: **Ioana Feher**, Dana Alina Magdas, Cezara Voica, Gabriela Cristea, Costel Sarbu, Fuzzy Divisive Hierarchical Associative-Clustering Applied to Different Varieties of White Wines According to Their Multi-Elemental Profiles, Molecules, *2020*, *25*(21), 4955-4965 (**ISI = 3.267**)

✓ ***Activity stage report***

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03.12.2020 ***Dr. Ioana Feher***