ISO Fd Food ERA Chair

Safety
Quality
Traceability

2nd ISO-FOOD

Symposium FROM

FOOD SOURCE TO HEALTH

BOOK of ABSTRACTS

Portorož, Slovenia **April 24 – 26, 2023**

Institut
"Jožef Stefan"
Ljubljana, Slovenija

MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA LJUBLJANA SLOVENIJA 2004

Authentication and Geographical Characterisation of Italian Grape Musts through Glucose and Fructose Carbon Isotopic Ratios Determined by LC-IRMS

M. Perini[1],*, S. Pianezze[1], R. Larcher[1]

(1) Fondazione Edmund Mach (FEM), Via E. Mach 1, 38098 San Michele all'Adige, Italy
*matteo.perini@fmach.it

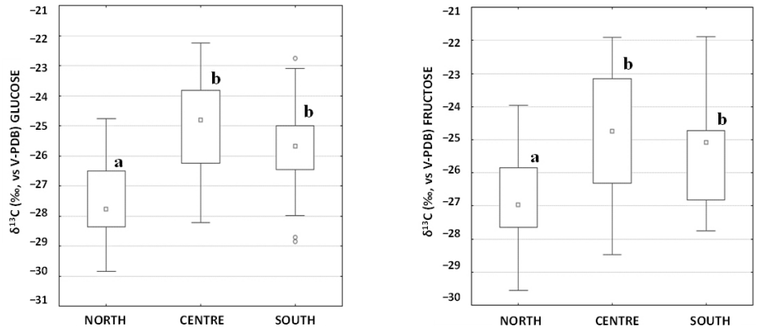


Figure 1. Box-Whisker plot of the glucose and fructose LC-IRMS analysis divided by the origin of the sample (north, centre or south Italy). Different letters identify statistically different groups according to the ANOVA ($p < 0.01$).

The authenticity of grape musts is normally checked through the stable isotopic analysis of carbon ($\delta^{13}C$) after fermentation and distillation by following the official OIV MA AS-312-06 method. Unfortunately, it presents some issues that are difficult to overcome. Grape must samples can only be analysed after they have been fermented to obtain ethanol. The process must be carried out under careful control of the fermentation to avoid the presence of unwanted by-products arising from a premature fermentation interruption. Moreover, if the musts have been preserved by the addition of sulphur dioxide (SO_2), they must undergo an additional step to eliminate the SO_2, which would affect the fermentation. Once the product has been fermented, the ethanol must be separated using specific distillation columns (such as the Cadiot ones) making it possible to obtain ethanol free of isotopic fractionation with a minimum alcohol degree of 95% vol.

In this study, the alternative use of a technique based on $\delta^{13}C$ isotopic analysis of the major sugars of the grape must by liquid chromatography coupled with isotope ratio mass spectrometry (LC-IRMS) is provided. In LC–IRMS, analytes are separated on an LC system and consecutively oxidized in an online reactor to CO_2, which is required for the determination of compound-specific carbon isotopic ratios. This technique has been already used in the study of matrices such as wine [1], ethanol [1,2], glycerol [2], and honey [3] to detect fraudulent alterations of their natural composition such as the addition of exogenous sugars to the products. The LC-IRMS allows a single separation of the individual components of a sample and makes it possible to determine their $\delta^{13}C$ values online, avoiding both the disadvantages of off-line methods and the disadvantages of methods requiring a derivatization step (such as GC-C-IRMS), causing the addition of extra carbons.

In order to discriminate between musts from different areas of Italy, a preliminary dataset was considered; the $\delta^{13}C$ isotopic ratios of glucose and fructose of around 100 authentic Italian must samples from 16 different sampling regions were analysed. In addition, the $\delta^{13}C$ variability in authentic and fake must (added with increasing percentages of exogenous sugars) has been explored and tested to verify their validity as fraud detectors. The two analysed parameters, ranging from –29.8‰ to –21.9‰, are well correlated ($R^2 = 0.7802$) and the northern Italian regions showed significantly more negative $\delta^{13}C$ values for both sugars than the rest of the dataset (Figure 1). By using the LC-IRMS technique, the addition of exogenous sugars, such as fructose and glucose from C_4 photosynthetic cycle plants, is easily detectable as it modifies the $\delta^{13}C$ of the individual sugars.

References

[1] F. Guyon, L. Gaillard, M.-H. Salagoïty, B. Médina (2011) *Analytical and Bioanalytical Chemistry*, 401 (5), 1551-1558.

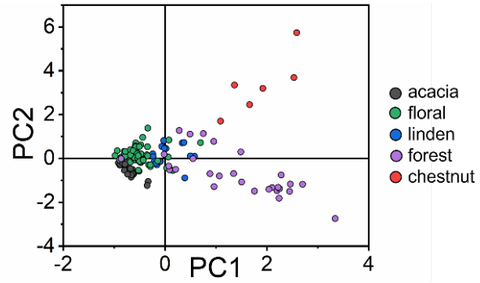
[2] A. I. Cabañero, J. L. Recio, M. Rupérez (2008) *Rapid Communications in Mass Spectrometry*, 22 (20), 3111-3118.

[3] L. Elflein, K.-P. Raezke (2008) *Apidologie*, 39 (5), 574-587.

Differentiation of the Mineral Content of Slovenian Honeys by Botanical Origin Using Principal Component Analysis

M. Kolar[1],*, J. Imperl[1], A. Pavlin[1], D. Kočar[1]

(1) Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia
*mitja.kolar@fkkt.uni-lj.si



Honey is a substance derived from nectar and secretions from different plant species and is produced by honeybees. It is mixture of various types of sugars and water, which combined represent up to 95 % of the total weight. The remainder is made up from proteins, amino acids, vitamins, and minerals [1].

The composition of honey, presence and ratios of its components, is indicative of the botanical (plant species) and geographical origin (geology, climate) [2,3].

Mineral content in honey is very low (below 0.2 %) and is somewhat linked to its colour – lighter honeys have lower metal content, while darker honeys contain higher levels of minerals. Despite this it has been shown that the mineral content is dependent on the nectar or secretion source of the honey and thus it is possible to differentiate honeys by their botanical origin [2,3].

To achieve this an elemental analysis of many honey samples is done and the results are statistically evaluated (e.g., Principal Component Analysis) [1-4].

The present study focused on Slovenian honeys of many types and the possibility to differentiate them by their botanical origin based on the mineral content. For this purpose, 142 honey samples of five varieties (floral, forest, acacia, linden, and chestnut) were collected and digested in a microwave digestion system using a mixture of concentrated nitric(V) acid and hydrogen peroxide. Concentrations of elements in the sample solutions were measured by ICP-MS.

The mass percentages of 24 elements were determined, some were found in expectedly significant quantities (Na, Mg, K, Ca, and Fe), while most in low levels or below the detection limit.

A selection of 12 elemental profiles (B, Mg, Al, K, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Ba) were used for PCA analysis to distinguish between the honey varieties. The first three principal components were calculated, covering 42.6 %, 18.3 %, and 10.2 % of the variance, respectively. A 2D score and loading plot and a 3D score plot were graphed.

Successful clustering of honey types was accomplished. Floral, acacia and linden honeys are close to the origin and are discernible from each other. Floral and acacia honeys are in compact clusters, while linden honeys are spread out. Forest and chestnut honeys are grouped in a wider, oval shape and reach away from the origin at different angles.

Acknowledgements

The work was supported by Slovenian Research Agency within the program P1-0143 and project J4-454.

References

[1] L. Strojnik, J. Hladnik, N. C. Weber, D. Koron, M. Stopar, E. Zlatić, D. Kokalj, M. Strojnik, N. Ogrinc (2021) *Foods*, 10 (7), 1550.

[2] P. J. H. Dunn, D. Malinovsky, G. Holcombe, S. Cowen, H. Goenaga-Infante (2021) *Rapid Communications in Mass Spectrometry*, 35 (20), e9177.

[3] R. A. Werner, W. A. Brand (2021) *Rapid Communications in Mass Spectrometry,* 15 (7), 501-519.