NMR METABOLOMICS IN FOOD AUTHENTICATION

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Abstract- NMR (Nuclear Magnetic Resonance) represents the elective technique for structure elucidation of molecules and is particularly suited to analyze complex mixtures like foods. NMR provides the identification of a wide range of chemical compounds simultaneously, revealing potential markers or disclosing sophisticated frauds. The great economic values of high quality or guaranteed foods demands highly refined characterization tools. In this context metabolomics represents a privileged approach for modern analytical studies in the next decades.

Keywords**:** *NMR, Metabolomics, Food, Authentication*

1. INTRODUCTION

Food authentication process is generally considered a great concern, involving several aspect like geographical or genetic origin, fraud detection, compositional content etc, but essentially the compliance with the label declaration. In addition, the production methods and the processing technologies employed should not affect the quality attributes of high value foods, like PDO (Protected Designation of Origin), PGI (Protected geographical indication), and TSG (Traditional specialities guaranteed) products. Consumers, producers and regulators are involved at different levels with different efforts, providing standards and analytical procedures aimed to verify the final quality and safety of the foods. European Union legislation reserved specific names for foods and beverages of a particular quality, introducing regulatory framework to protect authentic products and trying to minimize unfair and misleading competitions from non-genuine or counterfeit products. During these last decades NMR demonstrated its capability in providing comprehensive metabolic profiles that would be useful in authenticity determination [1-3]. In the present study, some products like Traditional Balsamic Vinegar of Modena (TBVM), coffee, tomato paste, and saffron have been investigated with this aim.

2. EXPERIMENTAL

NMR spectra were recorded on Bruker AV600 and Bruker DMX500 spectrometers (Bruker Biospin GmbH, Rheinstetten, Karlsruhe, Germany) operating at 14.09 T and 11.7 T respectively and equipped with a 5mm reverse probe with z-gradient. Spectra were processed with TOPSPIN software v3.5 (Bruker Biospin GmbH, Rheinstetten, Karlsruhe, Germany) and multivariate statistical analysis was performed with SIMCA P v13 (Umetrics AB, Umea Sweeden). More details are available in the relative references.

3. RESULTS AND DISCUSSION

3.1. Traditional Balsamic Vinegar of Modena

TBVM is a PDO product (Reg. CE n° 812/2000) made only by cooked must, aged in wooden barrels for at least 12 years before sale; it could also reach more than 25 years of ageing, giving rise to the so called “extra old” product. During the ageing process, cooked must experience several chemical modifications, like sugar degradation, acetylation process, migration of aroma molecules from barrels etc. Official analytical controls are very easy to be overcome by clever fraudulent actors of these very valuated product. NMR allowed to evaluate and to quantify the different ratio of isoforms of the two main saccharides present in the cooked must, glucose (G) and fructose (F), providing the correct evaluation of the authenticity of TBVM. Investigations of “authentic” and suspicious TBVM highlighted a possible NMR protocol to detect the non-observation of the disciplinary rules that must be fulfilled for PDO products. Natural occurring of fructose isoforms are in both pyranosidic (P) and furanosidic (F) mojeties, with 4.8%, 23%, 2.4% and 69.8% for αFF, βFF, αFP and βFP respectively while glucose is present in only pyranosidic mojety with

37% and 63% for αGP and βGP respectively. During the cooking process, the most abundant fructose isoform, βFP, experience a dramatic reduction and the almost total absence of water does not allow the tautomeric re-equilibration process, thus constituting a label for the traditional process used for PDO production of TBVM Fig. 1. Because of this, αFP isoform remain observable in authentic TBVM, in particular C2αFP. This evaluation and the deviation for chemical shift experienced by C2αFF and C3βFF larger than 1 and 0.5 ppm respectively, indicated fraudulent product shows the expansion of the ^{13}C NMR anomeric region for authentic and fraudulent TBVM [4,5].

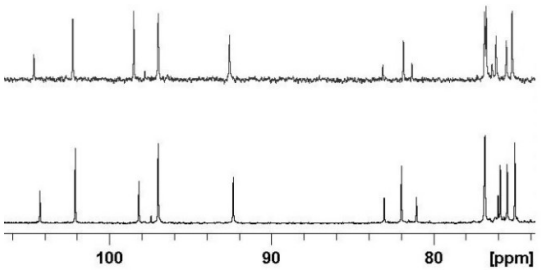

Fig. 1 Expansion of the ^{13}C spectrum of a TBVM (bottom) and a fraudulent TBVM (top)

3.2. Roasted coffee

Coffee represents the second commodity in the world from the economical point of view, involving Africa, Asia and Latin America as the main exporting partners. Among more than 100 different species recognized so far**,** two are the most relevant and widely cultivated**:** *Coffea arabica*and *Coffea canephora*var. robusta (commonly known as arabica and robusta), accounting for about 65% and 35% of the total world production respectively. Differences in environmental, growing conditions, methods of processing and drying between arabica and robusta, led to a sweet and floral/fruity pronounced flavour profile in arabica and a strong and cocoa flavour in robusta. The price gap between the two species has significantly widened in the last years leading to an increase of unlawfully replace of high quality arabica coffee with the cheaper robusta. The differentiation between the two species is possible considering green or roasted coffee beans on the basis of their size, shape and, for the green ones, also by the colour; conversely the identification and the quantification of arabica and robusta in roasted and ground coffee blends results very arduous. To achieve this purpose, certified arabica and robusta roasted coffee beans of different geographical origin, ground following the commercial requirements and processed under different roasting conditions were used to accurately prepared blendsof ground coffee with arabica composition ranging between 0 and 100% in weight. The ^{1}H NMR data of the water extracts were used to create an OPLS(Orthogonal Projection to Latent Structures) model that showed samples well-arranged according to their compositional properties, complying the percentage of arabica, along the first parallel component (Fig. 2).

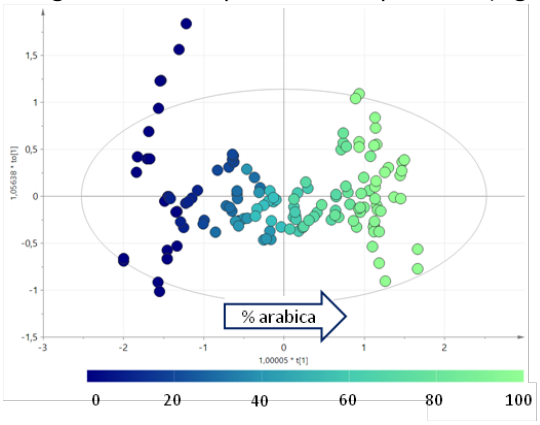

Fig. 2 OPLS score plot of water extracts of *C. arabica*and*C. canephora*var. robusta.

On the basis of this model, test set samples constituted by arabica and robusta blends of known composition were investigated to check the predictive performance of the OPLS model obtaining a very good agreement between predicted and real percentage composition on the basis of the multiple chemical components. The presented patented approach, by considering several chemical compounds simultaneously, prevents possible misled declaration or labelling about the composition that could occur performing methods based on single marker detection more prone to frauds and to erroneous evaluations [6,7].

3.3. Tomato paste

Italy is considered a worldwide leader in processed tomato quality, nevertheless several tons of triple-concentrated tomato paste were imported from developing countries and mainly from the biggest tomato producer, China. Many potential frauds regarding the real origin of tomato products could be made, and for this reason a growing interest from both consumers and producers about food

geographical characterization and authenticity is increasing presently. Following these requests more than 100 samples of double (DC) and triple (TC) concentrated tomato paste from Italy and China produced in 2007 and 2008 were analyzed by ^{1}H NMR. Initially a PCA (Principal Component Analysis) analysis was performed only on NMR data of TC samples of 2007 showing a clear separation of samples according to their geographical origin. The S-plot of the corresponding OPLS-DA (Orthogonal Projection to Latent Structures - Discriminant Analysis) highlighted citrate as characteristic for Chinese samples. Considering that it is well known that citrate can be added for pH correction and to avoid bacteria growth also if not allowed in these products, a new PCA model was performed excluding this variable. Again a clear discrimination of samples was achieved showing aspartate and glutammine as characteristic for Chinese samples while Italian one were characterized by larger amounts of glucose and fructose content. Successively DC and TC samples produced in 2007 and 2008 were analyzed together to evaluate the influence of the production year on the geographical discrimination of samples. PCA led to a clear sample differentiation according to the origin irrespective of the concentration rate of samples (double- and triple-concentrated tomato paste) and the tomato production year, at least for 2007 and 2008 by plotting the first two PCs. Only the third latent component routed a possible sample discrimination according to the production year for each country considered separately indicating a higher influence by the production year in sample discrimination. To maximize the differences between Italian and Chinese samples and to highlight the metabolites characteristic for each class of samples, an OPLS-DA model was performed on all samples (Fig. 3) [8,9]. Interestingly the corresponding S-plot highlighted the samediscriminant metabolites showed by considering only samples produced in 2007.

The OPLS-DA model performed by considering only samples produced in 2007 was therefore used to predict the origin of a test set samples produced in both 2007 and 2008 obtaining a correct classification for more than 95% of samples.

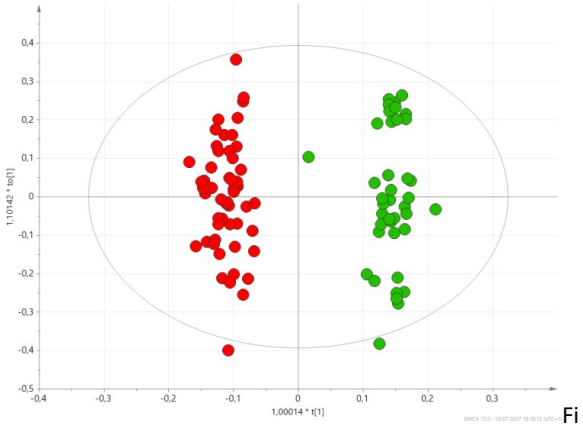

Fig. 3 OPLS-DA score plot performed considering DC and TC concentrated tomato paste from Italy (green) and China (red) produced in 2007 and 2008.

3.4. *Saffron*

The high market value of saffron has made this product very attractive for adulteration practices. Saffron is the dried stigma of *Crocus sativus* L. flowers and can be sold in powder or in stigma form. Powder form represent the more distributed form of saffron on the international market and it is the most prone form to be frauded. In this sense, synthetic colorants, ground other vegetal tissues of saffron or of plant species close to saffron, salts or other increasing weight material could be added for this practice. Saffron sold as stigma (filaments) is slightly more difficult to be altered, but still addition of other saffron tissues or vegetal parts to filaments could be possible. ISO 3632-2 trade standard [10] is the official test for authenticity of saffron, even though it shows several limitations [11,12]. The NMR based metabolomic approach allowed the investigation of the secondary metabolites of saffron extracts, showing powerful characteristics in detecting frauds. In recent works, the potentiality of NMR was already demonstrated in quantifying Sudan dye I-IV intentionally added to authentic saffron samples [13], as well as adulteration of saffron with plants material [14]. In the following example, a commercial saffron sample, sold in powder, has been investigated by a multistep workflow exploiting HPLC, UV-Vis, FT-IR and ^{1}H NMR to uncovered a challenging case of saffron adulteration [15]. In particular, the sophisticated practice adopted, achieved the total substitution of saffron with tartrazine and sunset yellow, along with propane-1,2-diol, propan-2-ol and acylglycerols (Fig 4).

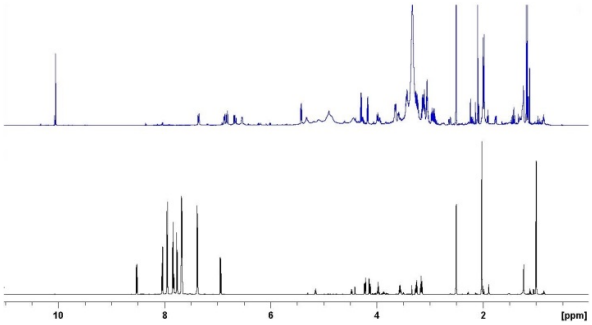

Fig. 4 ^{1}H NMR spectra of authentic saffron (top trace) and adulterated samples (bottom trace) in DMSO.

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REFERENCES

[1] R. Consonni, L.R. Cagliani, Nuclear Magnetic Resonance and Chemometrics to Assess Geographical Origin and Quality of Traditional Food Products.Adv. Food Nutr.Res. 59 (2010) pp. 87-165.

[2] M. Spraul, M. Link, H. Schaefer, F. Fang, B. Schuetz, "Wine analysis to check quality and authenticity by fully-automated 1H NMR", Proc. 38th World Congress of Vine and Wine (Part 1), July 5-10, 2015, Mainz, Germany, vol. 5, pp. 02022 1-5.

[3] M. Spraul, B. Schuetz, P. Rinke, S. Koswig, E. Humpfer, H. Schafer et al., NMR-Based Multi Parametric Quality Control of Fruit Juices: SGF Profiling. Nutrients.1 (2009) pp. 148-155.

[4] R. Consonni, L.R. Cagliani, S. Rinaldini, A. Incerti, Analytical method for authentication of traditional balsamic vinegar of Modena. Talanta.75 (2008) pp. 765-769.

[5] R. Consonni, Patent MI2007A001489 (2007).

[6] L.R. Cagliani, G. Pellegrino, G. Giugno, R. Consonni, Quantification of Coffea arabica and Coffea canephora var. robusta in roasted and ground coffee blends, Talanta. 106 (2013) pp. 169-173.

[7] R. Consonni, L.R. Cagliani, Patent n. MI2012A001281 (2012).

[8] R. Consonni, L.R. Cagliani, M. Stocchero, S. Porretta, Triple concentrated tomato paste: discrimination between Italian and Chinese products, J. Agric. Food Chem. 57 (2009) pp. 4506-4513.

[9] R. Consonni, L.R. Cagliani, M. Stocchero, S. Porretta, Evaluation of the production year in Italian and Chinese tomato paste for geographical determination using O2PLS models, J. Agric. Food Chem. 58 (2010) pp. 7520-7525.

[10] ISO (2010). International Standard ISO 3632–2: Saffron (Crocus sativus L.) Test Methods. Geneva: The International Organization for Standardization.

[11] R. Consonni, S.A. Ordoudi, L.R. Cagliani, M. Tsiangali, M.Z. Tsimidou, On the traceability of commercial saffron samples using 1H NMR and FT-IR metabolomics, Molecules special issue: Saffron (Crocus sativus L): omics and other techniques in authenticity, quality and bioactivities studies, Molecules. 21 (2016) pp. 286-299.

[12] S. Ordoudi, M. de los Mozos Pascual, M.Z. Tsimidou, On the quality control of traded saffron by means of transmission Fourier-transform mid-infrared (FT-MIR) spectroscopy and chemometrics, Food Chem. 150 (2014) pp. 414-421.

[13] E.A. Petrakis, L.R. Cagliani, P.A. Tarantilis, M.G. Polissiou, R. Consonni. Sudan dyes in adulterated saffron (Crocus sativus L.) identification and quantification by 1H NMR, Food Chem. 217 (2017) pp. 418-424.

[14] E.A. Petrakis, L.R. Cagliani, M.G. Polissiou, R. Consonni. Evaluation of saffron (Crocus sativus L.) adulteration with plant adulterants by 1H NMR metabolite fingerprinting, Food Chem. 173 (2015) pp. 890-896.

[15] S.A. Ordoudi, L.R. Cagliani, D. Melidou, M.Z. Tsimidou, R. Consonni. Uncovering a challenging case of adulterated commercial saffron, Food Control.81 (2017) pp. 147-155.