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FEATURED ARTICLE

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Eugenio Aprea, Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), S. Michele a/A, Italy.

In this article, the author compares two mass spectrometric techniques: gas chromatography-mass spectrometry (GC-MS) and Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) for monitoring the evolution of volatile organic compounds in dehydrated porcini mushrooms during their shelf life.

NMR SOLUTIONS

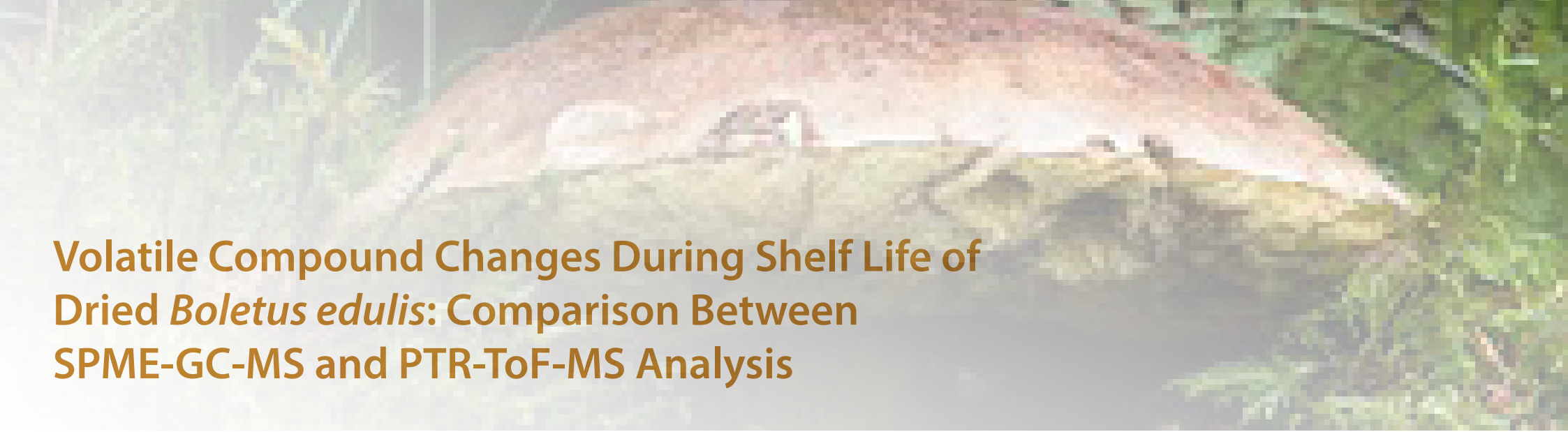
Protein-Detected NMR-Based Methods

Jesús Jiménez Barbero, Scientific Director, CIC bioGUNE, Bizkaia, Spain.

RESEARCH ROUND-UP

Raman Spectroscopy as a Tool for the *in situ* Study of Three Lichens Species from Antarctica and Brazil

Luiz Fernando Cappa de Oliveira, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil.



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Introduction

The purpose of our study was the investigation of the evolution of volatile compounds in dehydrated porcini mushroom during the shelf life. Although considered a stable product dried porcini flavour changes during storage. The study was developed in collaboration with a local distributor of dried mushrooms, interested to know more about its product during the commercial shelf

life (up to 12 months) and evaluate new methods to assist quality control and product development.

Results from Research

We monitored for the first time the evolution of volatile compounds emitted by dried porcini during commercial shelf life (12 months at 20 °C) and accelerated shelf life (12 months at 37 °C) by two mass spectrometric-based techniques: gas

chromatography-mass spectrometry (GC-MS) and Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS). We obtained new and interesting results both from a scientific and technological point of view. By using GC-MS, we identified 66 volatile compounds, 36 of which were reported for the first time in dried porcini. In contrast, PTR-ToF-MS allowed hundreds of MS peaks to

be monitored adding information to unknown compounds or to compounds that are not easily measurable by SPME-GC-MS such as ammonia and small volatile amines. Results of our work show that short periods at temperatures higher than 20 °C induce drastic changes in the volatile profiling of dried porcini during shelf life.

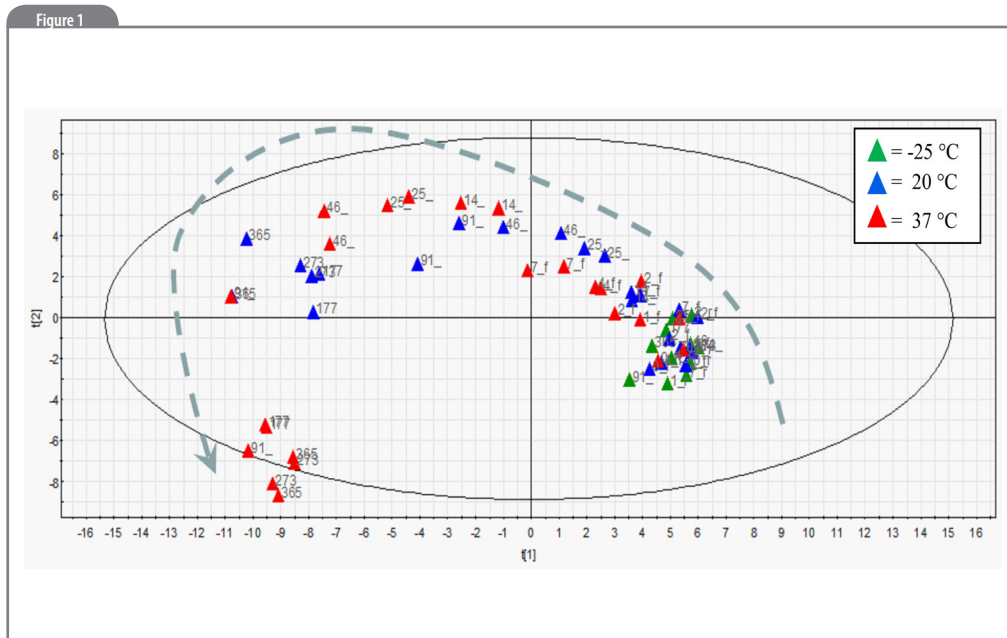


Figure 1: PCA of the headspace VOCs of dried porcini stored at different temperatures. Numbers indicate days of storage. The grey arrow indicates the direction of the observed time evolution.

Conclusion

Altogether we have a comprehensive view of the volatile compounds released by porcini mushrooms and of the effects of storage conditions. Being volatile compounds responsible for flavour perception, we now have a better insight into the key characteristics of this valuable and high-priced product.

We can now also rapidly evaluate

raw material and storage conditions. Our data indicate, for instance, that even short periods at temperatures higher than 20 °C should be avoided in order to preserve the initial volatile profile as much as possible (and thus the flavour) of dried porcini during shelf life. This is not trivial because storage temperatures often exceed this value.

This work also demonstrates the advantages of using both GC–MS and PTR–ToF–MS in order to increase analytical capabilities in terms of productivity and analytes covered.

Reference

1. Eugenio Aprea, Andrea Romano, Emanuela Betta, Franco Biasioli, Luca Cappellin, Marco Fanti and Flavia Gasperi, *Journal of Mass Spectrometry*, 50 (1), 56–64, (2015).



Eugenio Aprea completed a degree in Food Science and Technology at the University of Naples “Federico II”, Italy and received his PhD in Natural Science at the University of Innsbruck, Austria, specializing in trace gas analysis focused on food-related and environmental topics.

Since 2001 he has worked at the Istituto Agrario di S. Michele all’Adige (now Fondazione Edmund Mach).

In 2004 he was visiting researcher (Marie Curie) at the Nutrition and Food Biotechnology Centre at the University College of Cork, Ireland. From 2004 to 2005, he was research assistant at the University of Innsbruck working on PTR-MS. Currently, he is a staff member of the Sensory Quality research group at Fondazione Edmund Mach working on the characterization of food product properties related to sensory perceptions.

He has co-authored 85 papers (65 with IF; September 2015), 12 book chapters and over 160 contributions at conferences. He is Editorial Board Member of “The Open Agriculture Journal” (since 2007), “Recent Patents on Food, Nutrition & Agriculture” (since 2008), and “Current Nutrition & Food Science” (since 2013).

NMR Solutions: Protein-Detected NMR-Based Methods

Jesús Jiménez Barbero, Scientific Director, CIC bioGUNE, Bizkaia, Spain.

NMR-based screening is a powerful experimental technique that can be exploited by employing either ligand- or receptor-based methods [V. Roldós, F.J. Cañada and J. Jiménez-Barbero, *Chembiochem.*, 12, 990-1005 (2011)]. We have already described several protocols operating from the ligand's perspective (see previous *NMR Solutions*). Now, we will illustrate the typical scheme employed when analysing binding from the protein's point-of-view.

Receptor-based NMR methods detect and measure some specific NMR parameters of the protein signal resonances in the presence and lack of putative ligands [M. Pellecchia, D. S. Sem and K. Wuthrich, *Nat. Rev. Drug Discovery*, 1, 211–219 (2002)]. NMR parameters are very sensitive to changes in the chemical environment of the nuclei under analysis, from chemical shifts to relaxation times. Provided that the assignment of the resonances of the target receptor is known, ligand-induced changes in chemical shifts (δ) of different protein cross-peak signals resonances can be directly visualized on the primary sequence, or even in the secondary or tertiary structure of the macromolecular receptor. Indeed, the most commonly used parameter

is the chemical shift (δ), usually monitored through heteronuclear 2D-methods. In fact, the protocol consists of following the chemical shift perturbations of key protein NMR signals in the presence of the added ligand. In principle, for protein-detected NMR studies of small proteins, chemical shift variations of specific proton resonance(s) of the receptor, as induced by the presence of the ligand, might suffice for proper monitoring of the binding event. However, signal overlapping in homonuclear 1H-1H 2D NMR spectra (TOCSY, NOESY) becomes a major problem for polypeptides above 50–60 residues. Therefore, heteronuclear experiments (HSQC or its variants) are the method of choice. In principle, either 1H-13C or 1H-15N correlation

methods could be employed. In these cases, stable-isotope labelling (^{13}C , ^{15}N) is imperative. Isotope labelling enables monitoring of ligand binding by observation of the chemical shift perturbations on the detected 1H/ ^{13}C / ^{15}N signals, being the observed perturbations associated to the ligand-binding event.

Different aspects should be taken into consideration when performing these experiments. The application of NMR methods to proteins demands specific physicochemical properties of the protein target that might pose difficult challenges. First, milligram quantities of soluble, non-aggregated protein must be over-expressed in the proper culture medium (bacteria, yeast, cells, etc) and purified.

Therefore, suitable expression

hosts must be found that permit the required isotope enrichment (e.g. ^{13}C , ^{15}N , ^2H), which is critical for the resonance assignment process of large (>30000 Da) protein targets. Therefore, the experimental access to the required data might be rather expensive. After sufficient quantities of labelled receptor are available, it must be ensured that the sample is stable in solution for the time required for the sequential resonance signal assignment process. Typically, at least one week of measurement time might be necessary. Once the data are collected, the key cross peaks that will be monitored should be assigned. Although new data acquisition approaches promise to accelerate resonance assignment, it can still be a lengthy process (at least

weeks) for relatively large proteins (>30000 Da) routinely encountered in research. Signal assignment is crucial since then, by identifying chemical shift perturbations in the assigned protein resonances, not only are ligands identified, but also the binding sites at the protein structure are localized in a straightforward manner. Moreover, localization of binding sites may also make it possible to distinguish immediately between specific and nonspecific binding. Unlike ligand-based methods, receptor-based NMR methods do not rely on fast exchange to retrieve bound state information. In this case, and contrary to ligand-based NMR techniques, observation of the interaction process may be possible for a larger range of affinities, from millimolar to nanomolar [T. Peters, B. Meyer, *Angew. Chem., Int. Ed.*, 42, 890–918 (2003)]. Monitoring the chemical shift perturbations of the protein signals in the absence and in the presence of different ligand concentrations permits the characterization of both higher and lower affinity hits, including the estimation of the binding affinity, provided that a carefully designed titration experiment is carried out. Preferably, HSQC experiments should be performed using a variety of ligand/protein ratios. Therefore, even having access to a good concentration (ca. 0.1 mM) of the receptor, at least one day

of measurement is required to obtain good data. As for any 2D NMR experiment, for every ligand/protein ratio, a given number of fids (at least 128-256) have to be recorded to achieve the required digital resolution in the evolution dimension (f1), which can be further expanded using linear prediction algorithms. The number of scans is dictated by the amount of material available and by the sensitivity of the NMR instrument. A minimum magnet corresponding to a 600 MHz 1H NMR Larmor frequency is required (for proteins up to 20 kDa). For larger systems, bigger magnets are required. The NMR instrument to be employed depends on a variety of factors: first, and more importantly, on the accessibility to a high-field spectrometer, depending on the complexity and the degree of overlapping of the heteronuclear NMR spectrum of the protein. Therefore, the availability of very high-field magnets and especially the possible access to instruments equipped with cryo-probes allows NMR experiments to be performed with minute amounts of sample. For instance, nowadays, the use of cryo-probes allows protein amounts to be used in the micromolar range. In any case, there is a limit in the size of the protein, which can be easily analysed by heteronuclear NMR, of approximately 40 kDa. In this case, additional labelling of the backbone and side chain



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C-H with deuterium is advisable. Nevertheless for protein oligomers, due to symmetry reasons, receptor molecules displaying larger molecular sizes can also be monitored. For large molecules with this or beyond this size, relaxation is very fast. Thus, line broadening becomes important, and most of the NMR signals show very little intensity and may even disappear below the noise level. There are some heteronuclear experiments that have been devised to minimize the intensity losses due to relaxation. The most popular ones are TROSY (transverse relaxation-optimized spectroscopy, K. Pervushin, R. Riek, G. Wider, K. Wüthrich, *Proc Natl Acad Sci USA*, 94, 12366-12371 (1997) and CRINEPT-like techniques (cross-correlated relaxation enhanced polarization transfer, R. Riek, G. Wider, K. Pervushin and K. Wüthrich, *Proc Natl Acad Sci USA*, 96, 4918-4923 (1999)).

As standard, 0.5 mL of 0.05–0.2 mM of the receptor (protein) is used in this experiment. The ligand is usually present in 1–20 fold molar over the concentration of protein binding sites. The selected ligand/protein ratio depends on the binding affinity and on the kinetics of the exchange reaction. If possible, three or four molar ratios should be tested. First, specific heteronuclear NMR spectra of the free protein is separately recorded and analysed. Secondly, the protein sample is titrated using a concentrated stock solution of the

ligand in the corresponding buffer. The possible existence of chemical shift perturbations are carefully monitored to assess binding for the different ligand/protein molar ratios. As usual, the binding ability or the activity of the protein should be monitored before and after performing the NMR experiments. For extracting sound conclusions, the data collected after a significant loss of binding or specificity should be used with extreme caution, since the architecture of the binding could have changed. This fact is relatively easy to detect by the heteronuclear experiments.

Jesús Jiménez Barbero is currently Scientific Director of CIC bioGUNE, Bizkaia, Spain. Previously he was a Full professor, and Head of the Department of Chemical and Physical Biology, at the University Autónoma in Madrid. Professor Jiménez Barbero has been the recipient of the Spanish Royal Society of Chemistry Janssen-Cilag Award in Organic Chemistry (2003), the Bruker NMR Prize of the Spanish Royal Society of Chemistry (2008), and the International Whistler Award in Carbohydrate Chemistry (2010). He has over 400 papers in refereed journals, five patents, and supervised 16 PhD theses, given more than 100 Invited or Plenary talks at international scientific conferences and institutions and been a member or chair of the Scientific and/or Organizing Committees of more than 30 Symposia. Professor Jiménez Barbero is currently President of the Royal Society of Chemistry of Spain and is or has been a member of the Editorial and/or Advisory Boards of numerous scientific journals.



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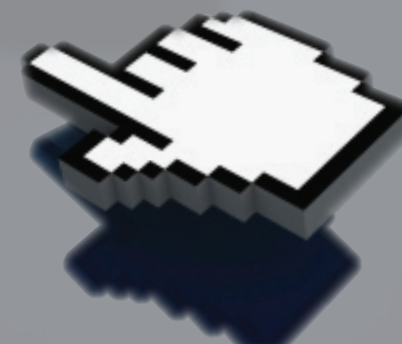
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Research Round-Up

Raman Spectroscopy as a Tool for the *in situ* Study of Three Lichens Species from Antarctica and Brazil

Luiz Fernando Cappa de Oliveira,
Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil.



Luiz Fernando Cappa de Oliveira from the Spectroscopy and Molecular Structure Group, Department of Chemistry, Universidade Federal de Juiz de Fora, Brazil discusses his research into the use of Raman spectroscopy as a tool for the *in situ* study of three lichens species from Antarctica and Brazil.

What was the purpose of your research?

My research deals with the use of Raman spectroscopy to analyse a varied type of chemical samples ranging from standard chemicals to the most complex systems such as lichens, marine corals and sponges, food, pigments, rocks and meteorites, natural products (leaves, barks, seeds and flowers), astrobiology and so on. Sometimes we are able to analyse them using information based only on the spectroscopic data and sometimes we are able to make use of other auxiliary methods

such as chemometrics or analytical techniques, such as chromatography or nuclear magnetic resonance, for instance.

This kind of use is based on the unique principle that Raman spectroscopy can enable us to assign the vibrational modes of the molecules that are part of the system that we are investigating, and are related to the molecular geometry of each one of the compounds. It is important to notice that Raman spectroscopy is a non-destructive analytical technique that can be used with different laser lines to

avoid fluorescence or thermal decomposition, and water is not a problem as a component of the system. The technique can be used for solids, liquids, gases, surfaces, for bulk analysis, and for macro or micro samples. By using a microscope, it is useful for a very small quantity of sample.

What were the key results from your research?

We were able to analyse the most different chemicals that are part of a versatile living system, such as carotenes or other conjugated

polyenes in natural products, lichens, marine corals or sponges, and to identify what kind of conjugated polyenes they are composed of: if they are carotenes, carotenoids or polyenals, which are different chemical structures with different metabolic routes promoted by the living systems. Data acquired about such chemical structures led to gaining knowledge about different strategies of life for each one of the living species. In the case of the recent paper published in the *Journal of Raman Spectroscopy* [1], for example, we were able to identify not

Figure 1



Figure 1: Main research from the group. For a larger version, click [here](#).

only conjugated polyenes but also other polyphenol structures. This is very unusual as the lichen in question makes use of all these chemicals as antioxidant agents in its metabolism. In general, we were able to demonstrate that Raman spectroscopy is one of the most powerful techniques to analyse chemicals in samples without destroying them. Furthermore, we

were able to give them back to the owner (a museum, for example). In the case of living systems, we were able to provide a very powerful technique to analyse specific metabolites, such as conjugated polyenes or polyphenols to understand what their role in the metabolism of such species is. In the end, we were able to analyse any chemicals, in any condition,

with varying temperature, pressure or other parameters that could be important, in any kind of physical state: liquid, gas or solid.

What does this actually mean?

It means that Raman spectroscopy is the solution for the world's problems!

Reference

1. Rafaella F. Fernandes, Adriano A. Spielmann and Luiz Fernando C. de Oliveira, *J. Raman Spec.*, 46(1), 70-75 (2015).



Luiz Fernando Cappa de Oliveira was born in Santos, São Paulo state, Brazil in 1959. He graduated in Chemistry in 1983 at the Chemistry Institute of Universidade Estadual Julio de Mesquita Filho – UNESP, where he also obtained his Masters in Inorganic Chemistry in 1986. He obtained his PhD at the Chemistry Institute from Universidade de São Paulo - USP in 1991, in the area of Physical Chemistry. Since 1995 he has been a teacher and researcher at the Chemistry Department at Universidade Federal de Juiz de Fora, UFJF, specializing in Physical Chemistry and Molecular Spectroscopy disciplines. Nowadays he is Professor of Molecular Spectroscopy at UFJF.

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Contacts

Dean Graimes
Publishing Director
dean.graimes@sepscience.com
tel. +44 203 490 6949

David Hills
Scientific Director
david.hills@sepscience.com
tel. +44 1270 628551

Janet Kelsey
Editor
janet.kelsey@sepscience.com
tel. +44 1270 619936

Jeroen Reiniers
Commercial Director
jeroen.reiniers@sepscience.com
tel. +65 6408 9751

Jackie Tan
Conference Manager
jackie.tan@sepscience.com
tel. +44 203 490 6949

Jason Maddocks
Online Content Manager
jason.maddocks@sepscience.com
tel. +44 1270 629496

Etsuko Shearer
Japan Representative
Etsuko Ikeda
etsuko.ikeda@sepscience.com
tel. + 81 (0)90 4195 5509