# What Are We Eating?

## Food quality analysis using NMR

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**What ends up on our plates? We used to think we knew – until we were disabused of this notion in early 2013. Instead of beef, there had been large-scale use of processed horsemeat, especially in frozen products and mincemeat. Although this posed no hazard to health, the damage was enormous, since many products had to be taken off supermarket shelves. As customers, we are prepared to pay more for higher quality goods than for substandard products. Yet we also count on suppliers not just to promise us a high standard of quality but also to deliver on that promise. With the techniques and methods offered by modern food analysis, food quality can be analysed to a high degree of precision. Previously, such types of quality analysis were possible only by investing a great deal of time and money, however – nor could they be conducted on an industrial scale. Today, NMR spectroscopy offers us a method for conducting low-cost, high-throughput quality analysis work, thereby guaranteeing the availability of high-quality, affordable food on our plates.**

On the subject of food, the term "quality" is of particular importance – for both customers and manufacturers. At the same time, "quality" does not always mean the same thing to the consumers and producers of food. While manufacturers necessarily view the host of legal requirements governing ingredients and maximum concentrations of harmful substances as quality parameters, modern consumers are more interested in factors such as the authenticity of the food they purchase – whether this authenticity refers to variety, biological processing, or geographical origin. Customers are certainly happy to pay a higher price for food that is organically produced and sourced from a precisely defined region. While the analytical verification of food varieties is possible using genetic analysis (for example), geographical origin can be determined by looking at isotopes. Yet these methods are highly complex and therefore permit analysis only by random sampling. As this year's horsemeat scandal [1] has already demonstrated, customer opinion is highly sensitive to the counterfeiting of foodstuffs – even in cases where it presents absolutely no danger to their health. While consumers and retail suffer, such issues also have a major impact on the producers who unwittingly processed counterfeit raw materials.

## The power of NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy can offer a helping hand here: this is a technique that, in a similar form – magnetic resonance imaging (MRI) – is also used for applications in medicine. With NMR spectroscopy, the response of a magnetically active atomic species – e.g. $^{1}H$ or $^{13}C$ – to a radio frequency pulse is measured in a strong external magnetic field. The signals produced allow to draw conclusions about the chemical environment of these atoms and hence the identity of the substance under analysis. Since NMR spectroscopy is the only method that permits the characterisation of both the structure and the dynamic state of molecules, one of the uses of the technique is to investigate issues of bio-medical relevance. These include studying the molecular basis for food allergies, the principles of bacterial transcription – for the development of new antibiotics – or the origins of prion diseases [2–5]. Last but not least, NMR spectroscopy is a quantitative method: thanks to the modern technology now available, NMR is capable of identifying and quantifying docens of molecule species in parallel of molecule species in one parallel operation. Furthermore, the technical advances made in recent years have ensured that the analyses obtained from NMR spectroscopy are extremely reproducible: even deviations in concentrations in the region of 1:1,000,000 can be reproduced with unmatched reliability.

NMR spectroscopy therefore is ideally suited for the rapid and comprehensive testing of food quality [6]. In a single measurement taking just a few minutes, several dozen ingredients can be identified and quantified, without previous chromatographic separation of the foodstuff under investigation. With NMR spectroscopy, more samples can be investigated and more critical parameters can be measured than was possible using earlier methods in the field of food analysis. Those involved in manufacturing and processing food thereby receive a rapid, cost-effective ingredient profile – of their raw materials, for example – which contributes to a decisive improvement in food safety and thus to increased consumer confidence.

One unique feature of NMR spectroscopy is the reproducibility of the spectra in terms of the position and intensity of the signals. To date, NMR spectroscopy has also developed into one of the most important methods for the comprehensive study of metabolites – in the field of metabonomics – and degradation products in food. To this end, groups of samples are analysed using statistical methods – such as principal component analysis (PCA),

for example – to detect differences between them. Statistical models can be constructed on the basis of the differences thereby discovered (i.e. the principal components, PC): by making comparisons with these models, new, unknown samples can be matched with a variety, an origin, or a year of production, for example – as is already possible for wines [7]. Even the manufacturing process used – such as for fruit juices – can still be detected in the end product [8]. While the majority of analytical methods only detect parameters that have been specifically targeted, the use of reference spectra comparisons means NMR spectroscopy also permits the detection of unknown counterfeiting incidents or deviations in quality.

Recently, we successfully applied NMR spectroscopic analysis to differentiate boar and sow meat in the final product, alongside the exact grilling method used.

## Boar or sow?

To create the statistical models, a database was set up containing NMR spectra obtained from authentic samples of boar and sow meat, as well as from pork chops that had been cooked under carefully controlled conditions either on a wood-fired grill, a gas-powered grill or an electric grill. All of the pieces of meat had been frozen following slaughter or preparation and stored at -20 °C. For the NMR analysis, fat-free samples weighing about 1 g were removed from the pieces of meat while still frozen, and ground up in liquid nitrogen using a MM400 mixer mill (Retsch GmbH). Perchloric acid (70 %, C. Roth) was added to these samples to prevent their degradation by enzymes. Insoluble components of the meat extract were removed using centrifugation. Sodium hydroxide was used to set the sample's pH value to 7.0. As internal standards, 10 % $D_2O$ (99.8 %, Sigma-Aldrich) and traces of trimethylsilyl propionate (d6-TSP, Eurisotop) were added. These samples were then sequentially measured using standardised pulse programs (noesygppr1d; Bruker Biospin GmbH) at 298 K and a measuring frequency of 600 MHz using a Bruker Avance II+ spectrometer (Bruker BioSpin GmbH). The spectra were processed automatically using TopSpin 3.2 before then receiving a statistical analysis with AMIX 3.9.14 (both Bruker BioSpin GmbH). Overall, the spectrum was subdivided into 294 buckets, which were then input as principal component analysis variables. Substances were identified by consulting the literature [9], the HMDB database [10] and the BBioRef-Code database (pH 7.0, Bruker BioSpin GmbH). Since the statistical analysis process interprets the most minimal differences, a precise and reproducible procedure is essential when preparing samples.

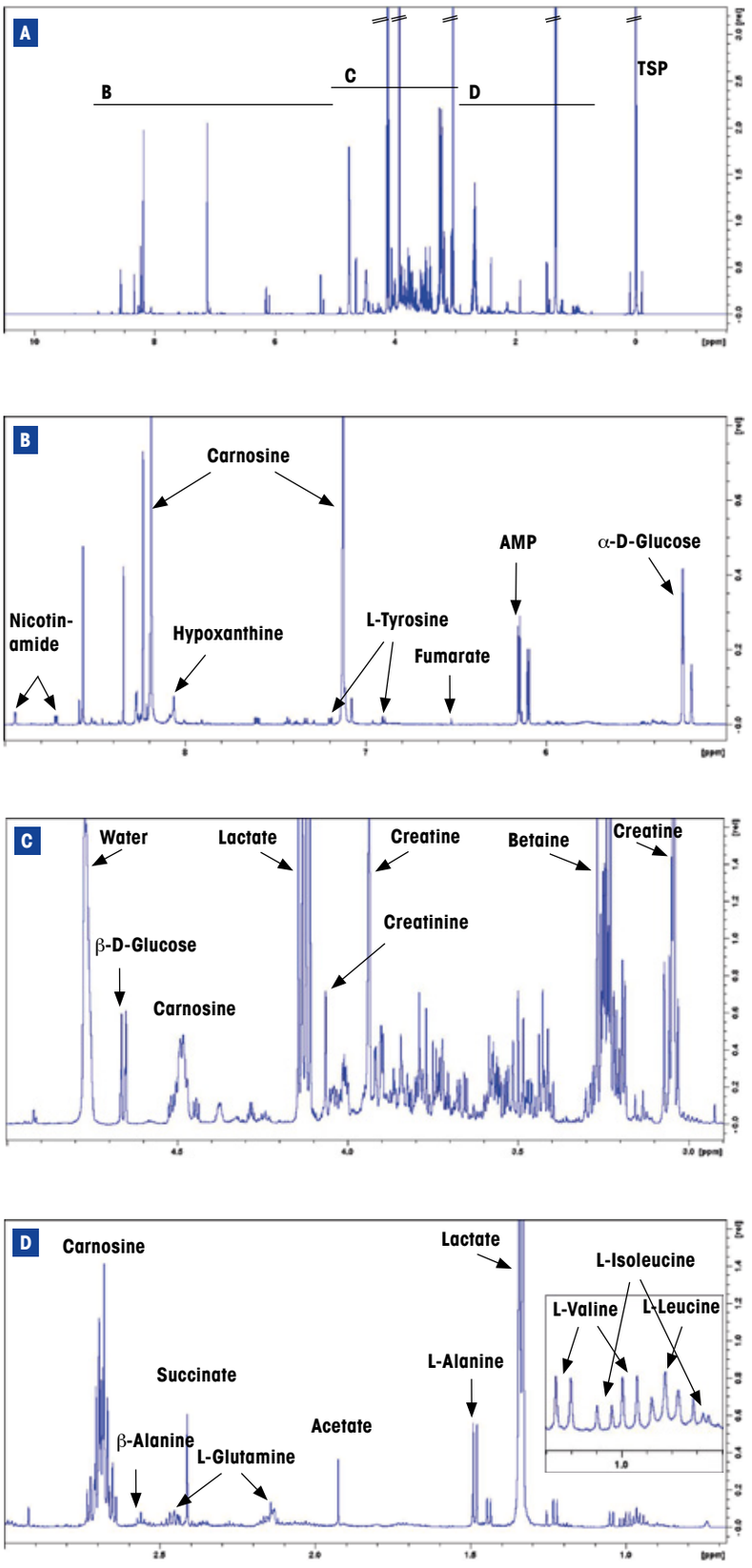


**Fig. 1 (A)** 600 MHz $^1$H-NMR spectrum of a representative pork meat sample (perchloric acid extract, sow); the x-axis shows the NMR chemical shift in ppm, while the y-axis shows the respective signal intensity in relative units. The signal at 0 ppm corresponds to the internal standard (TSP), acting as a reference for the chemical shift and the signal intensity. For better presentation of the matched signals, three enlargements are shown: **(B)** 4x enlargement of intensity, section 9–5 ppm; **(C)** and **(D)** 2x enlargement in each case, sections from 5–2.9 ppm and 2.9–0.7 ppm.

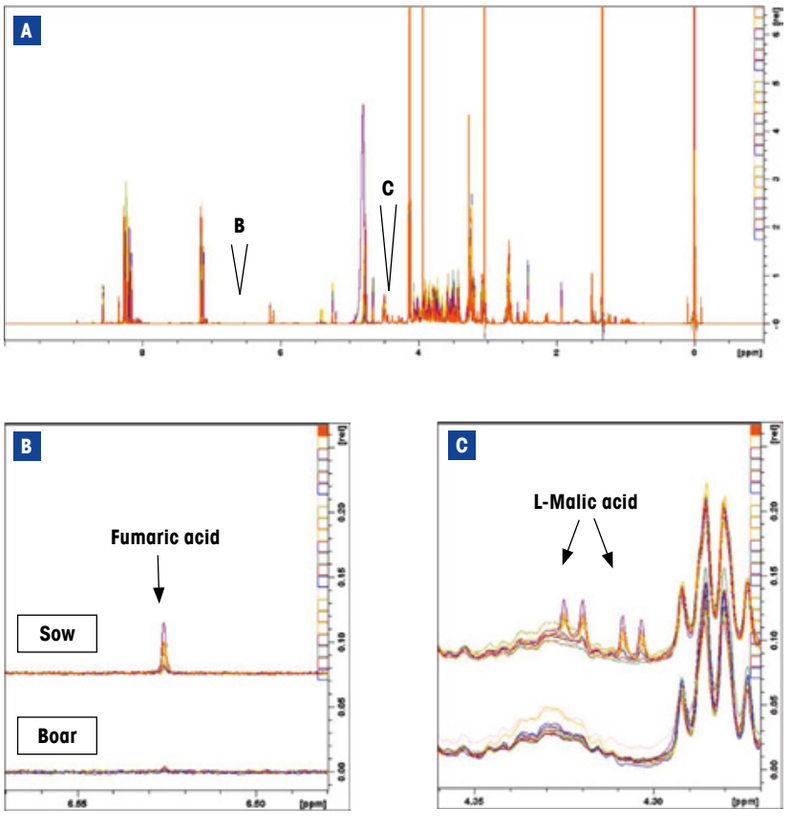


**Fig. 2 (A)** Superimposition of 22 600-MHz NMR pork meat spectra, 11 samples each from boar and sow. The differences between the sow and boar samples are visible only at high magnification. Figures B and C show that the spectra from sow meat exhibit additional signals, such as the fumaric acid singlet at 6.526 ppm **(B)** and the double doublet for malic acid at 4.314 ppm **(C)**.

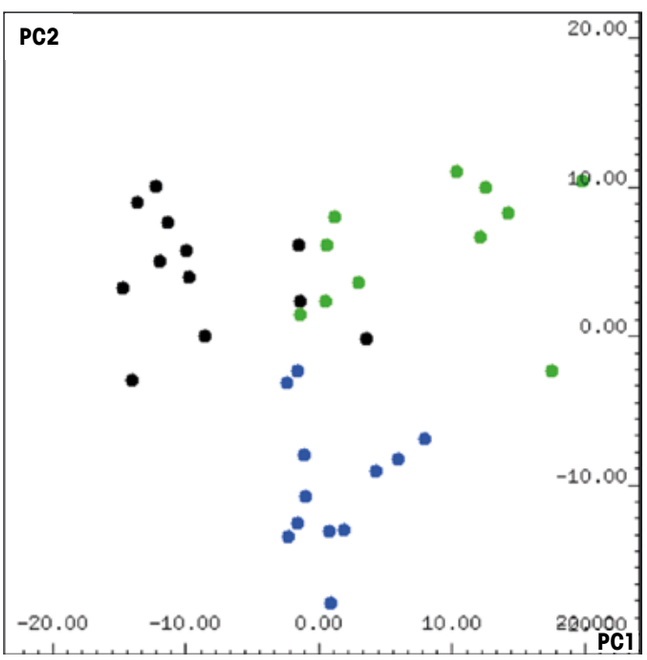


**Fig. 3** Depiction of principal components (principal components PC 1 vs. PC 2) for the $^{1}$H-NMR spectra of perchloric acid spectra from 32 grilled meat samples. Blue: wood-fired grill; black: electric grill; green: gas-powered grill. Together, the principal components PC1 and PC2 describe 51.05 % of total variance. To create the bucket table, a spectral window of 10 to 0 ppm was utilised. Following the exclusion of highly-variable regions, the selected bucket size of 0.03 ppm yielded a total of 294 statistical variables.

## The magic of metabonomics

The differentiation of boar and sow was conducted in the course of the development of a rapid test to identify the presence of skatole in boar meat – a compound that is responsible for the familiar "boar taint" in pork. The first step in this process requires the automated differentiation of sow meat and boar meat. The $^{1}$H-NMR spectrum of a representative pork meat sample can be recorded in a single measurement taking just 2 m (fig. 1). Numerous substances of relevance for quality, present at hugely different concentrations (from % to ppm), can be identified immediately. Although NMR spectra from sow and boar show no immediate differences (fig. 2A), their enlargements (2B, 2C) do reveal discrepancies in the spectra from sow meat and boar meat – such as in the concentration of the metabolites fumaric acid and L-malic acid. These and other differences can be used to match up unknown samples. In principle, the substances used to distinguish between samples do not need to be known a priori. In analysing grilled meat prepared using different grilling techniques (wood stove, gas, or electric grill), the distinction can be made even without knowing the chemical nature of the distinguishing characteristics used. The NMR spectra of the grilled meat samples were investigated using principal component analysis. The corresponding scores plot of the principal components PC1 and PC2, which alone constitute 51 % of the variance in the spectra, shows that the three different grilling methods are clearly presented in the metabolites spectrum and – even by using the comparatively simple method of principal component analysis – can already be easily distinguished (fig. 3). Accordingly, the cooking method method used can also be proven for an unknown sample – reliably, quickly, and cost-effectively. Although the identity of the substances enabling the statistical differentiation of the cooking methods is initially unknown, it can be revealed by further analyses – including the combination of mass spectrometry with advanced NMR methods (iMetabonomics, i.e. integrated metabonomics) – if it is of interest to do so.

We have shown that NMR spectroscopy is not only capable of determining a wealth of quality-relevant substances from a meat sample, but can also identify the slaughtered animal's sex and the method used for cooking. Work continues apace on the creation of models supporting the advanced analysis of meat samples.

NMR spectroscopy permits the analysis of foodstuffs to determine their authenticity, their purity, and many other quality characteristics. With our modern methodological

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studied technical chemistry in combination with business administration at the University of Linz, completing his doctorate there in 1999. This was followed by a period of post-doc work at The Scripps Research Institute, La Jolla, CA, before joining the Department of Biopoylmers at the University of Bayreuth in 2000, where he completed his habilitation in 2006 in biophysical chemistry. In 2008 he served as interim head of the Department of Biochemistry at the University of Bayreuth. A member of the Research Centre for Bio-Macromolecules (RC BIOmac) at the University of Bayreuth since 2010, he has also been adjunct professor there since 2013. He is also CEO of ALNuMed GmbH. His research interests include NMR methods for the characterisation of flexible proteins, NMR-based food analytics, particularly of beverages, meat and plant-based foodstuffs, as well as the application of combined analysis methods.

## Paul Rösch

studied physics at the universities of Karlsruhe and Heidelberg before receiving his doctorate at the Max Planck Institute for Medical Research in Heidelberg. This was followed by a post-doc at the University of Pennsylvania Medical School in the USA, and a post as research assistant at the Max Planck Institute for Medical Research in Heidelberg. In 1989, he completed his habilitation in biophysics at the University of Heidelberg. He has been head of the Department of Biopolymers since 1990 and Executive Director of Research Centre for Bio-Macromolecules (RC BIOmac) at the University of Bayreuth. His key areas of research are NMR-based biomedical structural research, focusing in particular on the molecular basis of food allergies and research into bacterial transcription as a target for new antibiotics.

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completed undergraduate studies in biochemistry at the University of Bayreuth, completing his bachelor's dissertation on the NMR-based metabonomics of pork and grilled meat in 2013 at the RC BIOmac at the University of Bayreuth.

## Felix Brauer

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expertise and the more advanced analysis techniques currently being researched, many food scandals – from milk adulterated with melamine to the horsemeat furore – could have been prevented in an efficient, cost-effective way. NMR spectroscopy has developed into an essential method for increasing consumer safety – ensuring consumers can be certain that the product described on the packaging is in fact the meal that ends up on their plates.

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Literature
[1] 2013 horsemeat scandal in Europe: http://en.wikipedia.org/wiki/Horsemeat_scandal
[2] Burmann, B.M et al. (2010) Science 328, 501-504
[3] Burmann, B.M et al. (2012) Cell 150, 291-303
[4] Berkner, H. & Rösch, P. (2009) Biospektrum 06.09, 618–620
[5] Camilloni ,C. et al. (2012) Biophys J 102, 158–167
[6] Schwarzinger, S. & Rösch, P (2012) GIT Labor-Fachzeitschrift 4/2012, 214–215
[7] Godelmann, R. et al. (2013) J Agric Food Chem 61, 5610–5619
[8] Spraul, M. et al. (2009) Nutrients 1(2), 148–155
[9] Jung, Y. et al. (2010) J. Agric. Food Chem. 58 (19), 10458–10466
[10] Wishart, D.S. et al. (2013) Nucleic Acids Res. 41(D1), D801–807

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