

Novel NMR-Technology to Assess Food Quality and Safety

Markus Link, Manfred Spraul, Hartmut Schaefer, Fang Fang, Birk Schuetz

Abstract—High Resolution NMR Spectroscopy offers unique screening capabilities for food quality and safety by combining non-targeted and targeted screening in one analysis.

The objective is to demonstrate, that due to its extreme reproducibility NMR can detect smallest changes in concentrations of many components in a mixture, which is best monitored by statistical evaluation however also delivers reliable quantification results.

The methodology typically uses a 400 MHz high resolution instrument under full automation after minimized sample preparation.

For example one fruit juice analysis in a push button operation takes at maximum 15 minutes and delivers a multitude of results, which are automatically summarized in a PDF report.

The method has been proven on fruit juices, where so far unknown frauds could be detected. In addition conventional targeted parameters are obtained in the same analysis. This technology has the advantage that NMR is completely quantitative and concentration calibration only has to be done once for all compounds. Since NMR is so reproducible, it is also transferable between different instruments (with same field strength) and laboratories. Based on strict SOP's, statistical models developed once can be used on multiple instruments and strategies for compound identification and quantification are applicable as well across labs.

Keywords—Automated solution, NMR, non-targeted screening, targeted screening.

I. INTRODUCTION

High Resolution NMR Spectroscopy offers unique screening capabilities for food quality and safety by combining non-targeted and targeted screening in one analysis [1]. The objective is to demonstrate, that due to its extreme reproducibility and transferability NMR can detect smallest changes in concentrations of many components in a mixture, which is best monitored by statistical evaluation however also delivers reliable quantification results.

II. MATERIALS AND METHODS

The SGF-Profiling is providing a full ^1H -NMR-spectrum for each sample. In principle, all NMR spectrometers are suitable for this analysis. A 400 MHz spectrometer with

Markus Link is Business Development Manager at Bruker BioSpin GmbH, Rheinstetten, 76287 Germany (phone: 00491726396940; fax: 0049721516191094; e-mail: markus.link@bruker-biospin.de).

Manfred Spraul, Director Business Development & NMR Application at Bruker BioSpin GmbH, Rheinstetten, 76287 Germany (e-mail: Manfred.spraul@bruker-biospin.de).

Hartmut Schaefer, Fang Fang and Birk Schuetz are within the Applications Team, at Bruker BioSpin GmbH, Rheinstetten, 76287 Germany.

proton optimized detection and automatic sample changing was defined as the basis of the procedure. Strict SOP's have been established for minimized sample preparation, storage, measurement and processing.

For sample preparation only a predefined buffer has to be added. For cloudy sample a centrifugation has to be done prior to buffer addition. Concentrates are diluted to reach a predefined Brix value. 540 μl of sample material is mixed with 60 μl buffer (containing phosphate buffer in D_2O) to obtain a final sample pH of 3 (e.g. for fruit juice and wine).

The ^1H -NMR spectral acquisitions are carried out by using a modified version of the NOESYGPPRID pulse sequence, with standardized continuous wave pre-saturation of the water resonance (25 Hz field), during the relaxation delay.

For fruit juice time domain data were collected into 64 K data points. A recycle time of ~ 8 sec was covering a sweep width of $\text{SW}=20\text{ppm}$. An exponential window function, with $\text{LB}=0.3$ Hz, was used prior to Fourier transformation of the data with zero filling into 64 K frequency domain points.

Data processing with standard spectrometer software were performed.

Baseline and phase correction were carried out using an automated processing program. Measurement procedure runs under complete control of a laboratory management system using barcodes for every sample.

For wine, acquisition conditions only vary in the solvent suppression mode, using a shaped pulse with 8 suppression frequencies on each individual solvent line with a pre-saturation field of 3 Hz per frequency.

Per registered spectrum, different interpretation routines are performed. Each interpretation of data is carried out by uni- and multivariate statistical analysis to reference models based on authentic samples (non-targeted mode). The system is calibrated with a reference database containing spectra of representative samples covering the natural variability of the examined sample type. The quality of interpretation depends on representativeness and the number of reference spectra. The databases for the food analysis are built up using certified authentic samples of juices from the industrial process. All spectra have to be acquired under identical conditions to ensure comparability. For each sample a fully signed documentation including origin and condition of processed fruits, as well as the applied technology is available to guarantee traceability [2].

From the large range of available multivariate statistical methods applied in context of metabonomics data analysis [3], [4], different possibilities of comparison can be used. A

multitude of NMR spectral parameters can be entered into mathematical models. The combination of methods can improve sample characterization and in some cases final judgment. Individual components are identified and quantified using information from a reference compound NMR spectral base (targeted mode).

Besides in-house developed scripts for MatLab, data analysis was performed mainly with the software package AMIX. For chemo metrical data analysis, spectra are segmented into equidistant chemical shift regions, the so-called buckets. Respective segment integrals are calculated in order to set up so called bucket tables for input into Principal Component Analysis, combined with discriminant analysis. Bucketing parameters were adjusted to the specific aspects investigated.

III. RESULTS AND DISCUSSION

The SGF Profiling for fruit juice represents a heterogeneous collection of cascading statistical models which can be applied consecutively to one single spectrum, such as specific models for multi fruit type separation, fruit type differentiation between citrus varieties (e.g. *Citrus sinensis* and *Citrus reticulata*), differentiation of product categories (e.g. orange juice and orange juice made from concentrate), or characterization of compositional differences for two groups of similar products (e.g. apple juice concentrates from Poland and China). Different answers presented as graphical or numerical results can be compiled in an analytical report by suitable software [5]. Only an exemplary extract of the possibilities can be presented here. In a cloud representation of PCA output as well as in classification, the position of the examined sample spectrum is indicated by a star.

An orange juice concentrate is from a Brazilian producer has been allocated to the common group, Orange/Mandarin and has been separated from other fruit types.

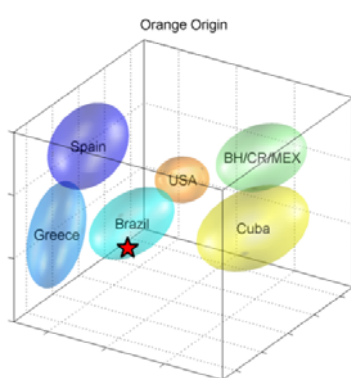


Fig. 1 Orange Juice Origin as determined by rapid NMR screening

The same sample is projected to differentiation of geographic origin, which is a relevant commercial aspect with regard to price and quality. The control of product type is important in some cases, to detect economic frauds, e.g. if a label says direct juice, however in reality re-diluted

concentrate was used. Therefore a special model was developed to solve such question.

All models comparing different groups apply similarity calculations in order to classify any sample. Such models are mainly used to check declared specificities of any product in comparison with defined alternative possibilities. The Soft Independent Modelling of Class Analogy is comparing a spectrum only with one specific reference spectra set, in order to determine if spectral sequences with low variability in the reference group. This can indicate a deviation from the target population, without comparing to any defined alternative. The same spectrum, as per previous figures, is calculated here.

An addition of *Citrus reticulata* (mandarin, clementine) in orange juice is not allowed in Europe. Such an addition is considered as an adulteration. A Ridge Regression curve is calculated based on pure orange and mandarin juice samples, for which spectra have been combined mathematically with different proportions. Value 0 corresponds to 100% pure *Citrus sinensis*, value 1% to 100% pure *Citrus reticulata*. In the laboratory, different mixtures of juices used for modelling, were prepared and measured with the calibration system. The share of *reticulata* is given on the x-coordinate. The results close to the linearity curve indicate good prediction and are in line with verification by DNA-testing and conventional analysis.

Similar models, as shown for orange juice products are developed for other juice products as well as for wine, honey and edible oil, too.

Furthermore, using the same spectra again, quantification can be carried out. Considering that analytical errors for both methods could be added when comparing NMR results and conventional analysis by enzyme test kit and/or HPLC, the NMR precision is more than sufficient for a pre-screening prior to conventional analysis.

Quantification of multiple compounds can be performed with one calibration and reference database to finished goods (bottled consumer goods). Furthermore, other models are transferable like the control of fruit content in products, with less than 100% fruit content such as drinks and nectars.

For better performance of compound identification, in case of signal overlap, a rapid 2D J-Resolved spectrum [6] is recorded as part of the standard routine.

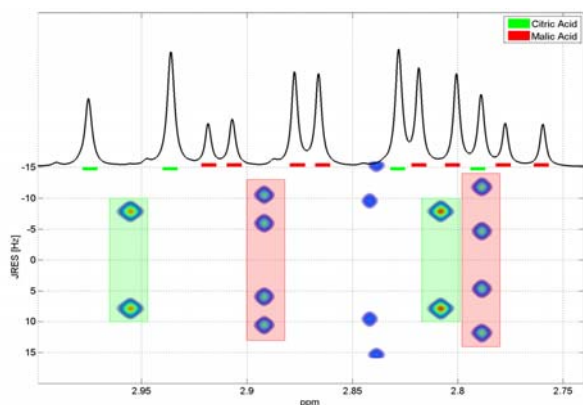


Fig. 2 Rapid 2D J-Resolved spectrum recorded during the automatic measurement procedure

Fig. 2 shows a region of the 1D (NOESY) and 2D (JRES) spectra of a pear juice. Signals of Citric Acid (columns 1 and 3) and Malic Acid (columns 2 and 4) are separated in the 2D-spectrum.

Having established the statistical and quantification methodology, it is possible to expose the technology described for fruit juices to other food material by exchanging the underlying spectral database from juice for example to **wine**. The knowledge base for quantification in this case has to be modified as well to represent chemical shift variation of the compounds to be quantified, as they are observed in a different matrix, which can also produce different signal overlay problems. Applying the statistical technology to wines allows e.g. to differentiate variety, geographical origin and vintage.

The second release of the WineScreener™, offering a major increase in result parameters in quantification and in statistical analysis. For example for a German Riesling 116 parameters are automatically generated from one wine sample measurement. Results are generated; those either are not available with conventional analysis or need multiple conventional methods to run. Multiple methods also mean different sample preparation procedures are needed, such increasing the cost of the analysis substantially. Therefore the WineScreener™ produces results at lowest cost per sample and per parameter. Since quality and authenticity are ever increasing in importance for the consumer, the NMR method comes in time.

For this 400 MHz push-button system, which can also perform juice screening, standard operation procedures for wine analysis have been defined and optimized, that also include sample preparation. Due to the highest reproducibility of NMR, statistical analysis is extremely powerful, allowing detecting smallest changes or many parameters at a time. This enables for example the determination of geographical origin of wines first of all on a country wide basis, but also for sub-areas within a country. For German Riesling a differentiation from Austrian Riesling is given, in addition the German origin can be further subdivided into the major Riesling growing areas like Rheinhessen, Baden and Württemberg.

Besides the geographical origin, also the grape variety is an important parameter, defining also the price of the wine. Worldwide variety models have been developed for Riesling, Sauvignon Blanc and Chardonnay in white wine and Merlot, Shiraz, Cabernet Sauvignon and Tempranillo in red wine (Fig. 3). For wines from Germany and Austria further varieties are parts of the analysis. In the same way vintage can be differentiated for German and Austrian wines. The verification part of the WineScreener™ result contains the function of being able to detect all types of NMR visible deviations, be they known or unknown and therefore contributes not only to product quality, but also to product safety.

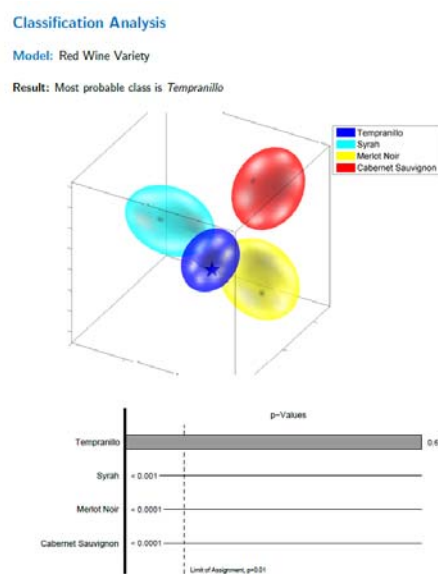


Fig. 3 International red wine variety model

Quantification of compounds in the wine delivers 56 values in the report, which is generated automatically. For each value the distribution of concentrations in the corresponding authentic model is given. This allows drawing conclusions also from quantitative parameters not defined for example in the OIV (Organisation International Du Vin) and such produces additional criteria for top quality of the wine.

The two examples given indicate the potential of NMR in food quality and safety, being transferable from one material to another applying the corresponding normal sets. Investigations have also been made on olive oil and other edible oils as well as honey [7].

IV. CONCLUSIONS

SGF-Profiling is a NMR-based high throughput screening for fruit juices and wines. The combination of chemo metric modelling with molecule specific signal treatment makes it a powerful tool for food quality assessment. Specific quality aspects and adulterations can be identified by applying a number of statistical tests on the same spectrum. Fruit and

product type differentiation, adulteration by sugar or acid addition, geographical origin and fruit mixtures are predictable as well as grape variety, vintage and geographical origin. Moreover, a list of substances being present in fruit juice and wines is quantifiable. The application of SGF-Profiling in routine controls is enhancing the control density and the amount of detected quality deviations. The pre-selection carried out allows choosing well targeted conventional analysis for a sample. In many cases, it can save higher analytical costs. Therefore the SGF-Profiling is useful and extremely economic in cases where samples need a very fast analytical answer and where a large number of samples should be tested and can be transferred to other food materials as shown.

ACKNOWLEDGMENT

The Riesling Sample Set was obtained from Prof. Dr. Fischer from DLR Neustadt Germany

REFERENCES

- [1] H.-J. Hofsommer, "Fruit Processing. New Analytical Techniques for Judging the Authenticity of Fruit Juices – an overview", in *Fruit Processing*, 1999, pp. 471-479
- [2] S. Koswig, "Fruit Processing, determination of foreign fruit types and fruit varieties - Analysis. Evaluation and Practical Problems", in *Fruit* November/December 2006. pp. 401-407
- [3] Vandeginste B.G.M. et al., "Handbook of Chemometrics and Qualimetrics", Elsevier, 1998, Vol. 2.
- [4] T. Hastie et al. "The Elements of Statistical Learning – Data Mining, Inference and Prediction", Springer, 2001
- [5] Rinke P. et al., "Fruit Processing – An ¹H-NMR-technique for high throughput screening in quality and authenticity control of fruit juice and fruit juice raw material- SGF-Profiling", 2007, pp. 10-18
- [6] S. Braun et al., "150 and More Basic NMR Experiments", Wiley-VCH, 1998
- [7] Lachenmeier et al. *Journal of Agriculture and food chem.* 57, 2009, pp. 7194-7199W.