



**Bruker BioSpin**



## SGF Profiling™ – NMR Based Fruit Juice Quality Control

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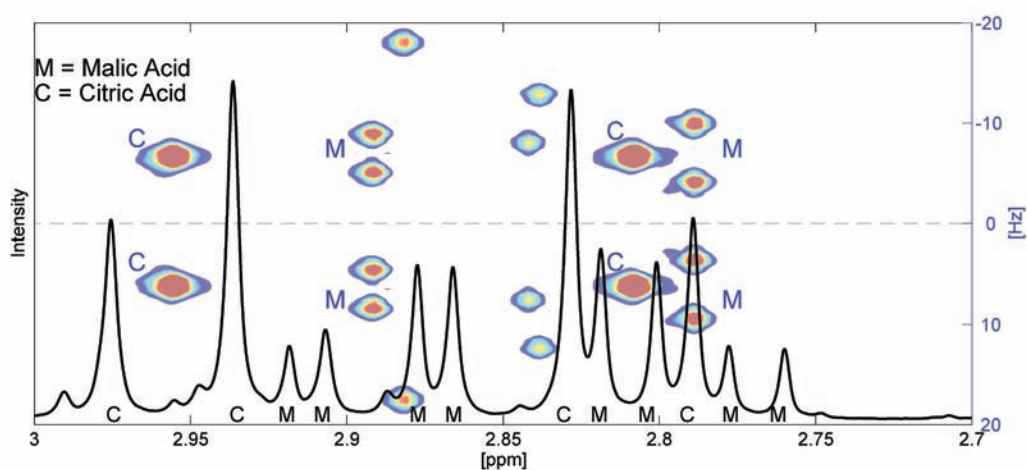
Traditionally, NMR has been perceived as a tool for structure verification, elucidation and purity analysis. However, driven by the needs of the emerging field of Metabonomics/Metabolomics, NMR has rapidly expanded in recent years into the areas of mixture analysis and screening applications. These developments were facilitated by high-throughput sample changing technology (either sample tube or flow-injection methods), integrated sample preparation, and the improved quality of digital spectrometers in general.

Today, NMR is an established tool in a wide range of metabonomics-related applications, including drug toxicity and efficacy screening with animal models, clinical diagnosis of inborn errors of metabolism, and general health status screening in the context of epidemiological research, to name but a few examples. In such studies, hundreds of samples may have to be screened per day with regard to the identity and concentration of selected metabolites as well as for between-sample comparison of spectral patterns using multivariate statistics to obtain classification and discrimination information. NMR is a particularly well-suited detector for the analysis of biological fluids, deriving truly quantitative and structural information while featuring high throughput (a 1D spectrum measured in a few minutes), excellent reproducibility, and minimal sample preparation (typically, only addition of buffer).

In this report we demonstrate that the principles established for NMR applications in metabonomics have been successfully transferred to yet another but closely related field of mixture analysis, i.e. quality control of beverages. A corresponding method for fruit juice analysis, developed in a joint effort by Bruker BioSpin GmbH and SGF International e.V. has been introduced recently under the name SGF Profiling. The system is fully automated with respect to sample transfer, measurement, data analysis and reporting and is set up on an AVANCE™ III 400 flow-injection NMR spectrometer. For each juice a multitude of parameters related to quality and authenticity are evaluated simultaneously from a single data set acquired within a few minutes. This multi-marker/multi-aspect NMR screening approach features low cost-per-sample and is highly competitive with conventional and targeted juice quality control.

Initially, the new NMR-based method was developed as a cost- and time-efficient prescreening tool to identify suspicious samples which may have a quality or authenticity issue and hence require more detailed conventional analysis. However, after having established a spectral database, which currently contains spectra from more than 3000 reference juices, including ca. 800 fully authentic samples taken by SGF inspectors on site, one can clearly see that the potential of NMR analysis goes far beyond our original intentions.

## NMR Spectroscopy



Peach puree screening by NMR at 400 MHz. The expanded region shows a 1D NOESY presat (water-suppressed) spectrum overlaid on a fast J-resolved 2D spectrum. The J-resolved experiment provides easy identification of multiplets for citric acid (C) and malic acid (M), and the 1D spectrum provides quantitation.

## Analytical Instrumentation

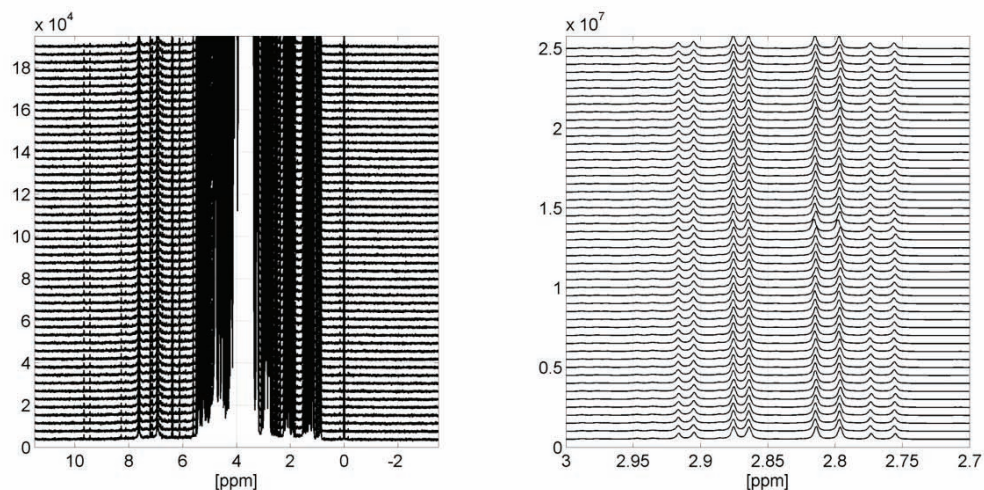
SGF Profiling™ is based on a flow-injection AVANCE™ III 400 NMR spectrometer which offers low-cost-per-sample capability. Each sample needs minimal preparation effort: for clear juices only the buffer addition (90 % juice / 10 % buffer) is needed, other juices like orange juice need to be centrifuged before. The buffer also contains D2O for locking and sodium azide to suppress microorganism activity.

The fully automated acquisition procedure includes the adjustment of the temperature, tuning and matching, locking, shimming and the optimization of the pulses and presaturation power for each individual sample. Two NMR experiments are executed: a modified 1D-version of the 2D-NOESY sequence

with well-defined water presaturation to allow quantitative evaluation even close to water signal and a fast 2D J-Resolved which is used for safe identification of the NMR signal. With modern NMR instruments the baseline correction is obsolete and other processing calculations such as phase corrections and referencing are done in full automation.

This setup yields in excellent spectra quality and reproducibility. The figure below shows 50 spectra obtained under the conditions described including sample preparation. It can be seen that phase and baseline are perfectly set in the left part and the signal positions are absolutely stable as seen in the expansion on the right side (signals of malic acid in apple juice).

## Reproducibility



Reproducibility test on one apple juice 50 times injected with automatic preparation, measurement and processing. Left: whole spectrum, right: expansion of malic acid signals.

## Data Analysis and Reporting

Currently the typical batch size used by SGF is between 50 and 120 samples in one measurement session. Therefore, automatic data analysis and report generation is a mandatory feature of SGF Profiling. In general, several key compounds must be identified and quantified, and a cascade of statistical tests and classification and discrimination steps must be applied to scan for multiple aspects of sample quality.

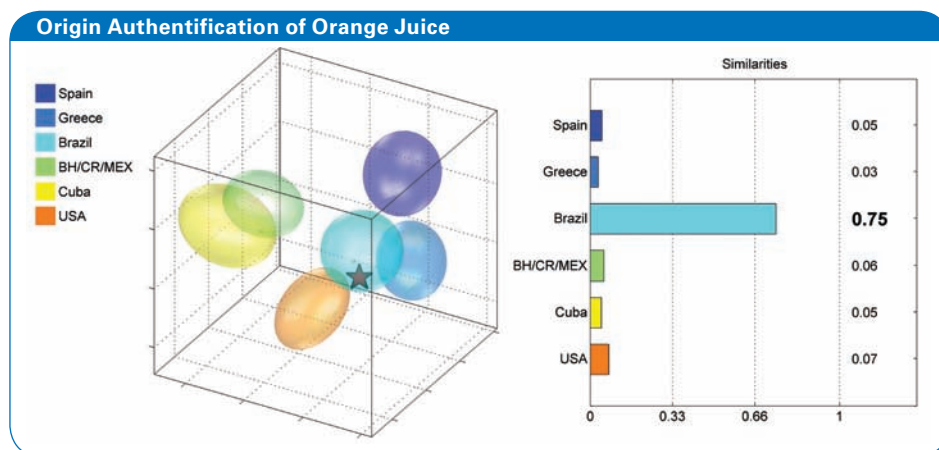
In comparison with reference standards, specific deviations in the concentration of a particular compound or in the profile of a specific combination of compounds may indicate characteristic quality and authenticity problems, such as the addition of sugar. Therefore, concentrations of compounds are of primary interest for the food chemist in the classical juice assessment procedure. Thus, NMR spectroscopy provides a clear advantage in methodology since it is possible to quantify many compounds from just one measurement with high correlations to the reference methods, in addition to providing data for the statistical evaluations explained below. At the moment, SGF Profiling provides absolute concentrations for more than 20 different compounds (depending on the type of juice), i.e. sucrose, glucose, fructose, proline, alanine, 5-hydroxymethyl-furfural (HMF), ethanol, methanol, acetone, phlorin and the acids malic, citric, lactic, fumaric, quinic, succinic, citramalic, formic, benzoic, acetic, and galacturonic. Furthermore, various useful relationships between compounds concentrations are calculated, e.g. the ratio glucose/fructose or the ratio of sucrose to total sugars.

In addition to the quantification, an exhaustive statistical analysis is applied to the data. In metabonomics the classification and/or verification of samples are major objectives. Our approach is, first, to exactly identify the sample by cascading classification models and, second, to validate the sample with respect to its most qualificatory group (e.g., apple juice concentrate from Poland). This reduces the variance of the validation models and, therefore,

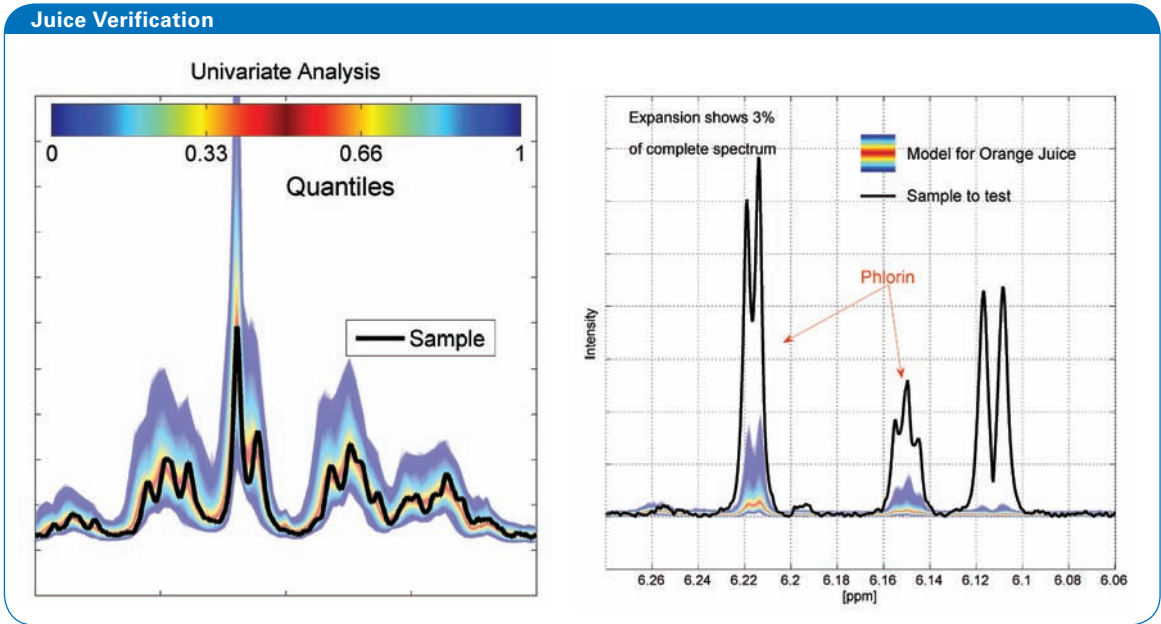
increases their discriminatory power. The foundation of the statistical analyses is our large reference database of more than 3000 samples of more than 30 different types of fruit juices from more than 50 countries.

As a first step, it is reasonable to estimate the type of fruit. The global model can differentiate between apple, orange/mandarin, sour cherry, pineapple, black currant, passion fruit, lemon, grapefruit, banana and grape. Of course, this information is usually provided with the sample's metadata and is rarely a reason for reclamation, except when orange juice (*Citrus sinensis*) is mixed with mandarin juice (*Citrus reticulata*). The latter is often cheaper so that some companies add it to orange juice without declaration (not allowed in Europe). Our NMR method can detect the addition of mandarin juice at a level of 10% or more and therefore allows a screening prior to DNA- or other conventional analyses.

With regard to orange juice, more specialized models can distinguish between direct juice and juice from concentrates and can detect the origin of the fruit. The figure below shows the results for the estimation of origin for a particular orange juice sample. The possible sources or groups included in the model are Spain, Greece, Brazil, Belize/Mexico/Costa Rica, Cuba and USA. Other important origins like Argentina or South Africa will be added when more reference samples are available. A 3D projection of the discrimination model space shows the ellipsoids of probability for each source, and the sample of interest represented by a red star. Similarity factors are calculated in the complete discrimination space and provide the probabilities for the estimation of source (the juice is most likely from Brazil). Up to now, we have developed detailed classification models for orange juice, as shown, apple juice (origin; concentrate vs. direct juice), sour cherry and pineapple. The underlying statistical method is a combination of PCA (principal components analysis) and discrimination analysis. The accuracy is checked via cross-validation and Monte Carlo analyses.



Estimation of the origin of an orange juice. Left: 3D projection of the model space with the ellipsoids of 95% probability for the various origin groups; the star represents the actual sample analyzed. Right: Calculated probabilities that the sample originates from one of the sources included in the model.



Verification of fruit juice samples. Left: apple juice, the 400 MHz <sup>1</sup>H spectrum in the region near 2 ppm (black trace) is plotted over a quantiles plot (color) of the model spectra set (univariate analysis of apple juice at 2 ppm). Right: orange juice, unusual high amount of phlorin indicates the usage of orange peel.

After the determination of the most likely group assignment, the sample is verified in two steps. First, a univariate analysis compares each spectral region of interest with the reference data set and detects deviations in compound concentrations. The second approach is a multivariate analysis for detecting deviations which are not apparent in a univariate analysis. If both methods give the same positive result, the sample is considered "representative" and has successfully passed the prescreening trial. In this case there is no need to examine the sample further.

Another important method for verification, in particular for market samples, is the estimation of the fruit content of the juice. Conventionally, this is done by quantifying selected compounds and minerals and comparing these amounts with reference distributions. In NMR screening, hundreds of variables can be measured on the basis of just one spectrum; hence, we can use regression analysis to estimate the fruit content. Our results have shown that such an analysis yields results with a relative accuracy of about 10% for more than 95% of the samples.

### Reporting

**NMR Sample Profile**  
B-KS-05-038035-2007-12-02-2008/A

Sample provided by: Customer opt  
Sample: Orange juice  
Inspection Lot Number: 218  
Inspection Lot Date: 11.13.2007  
Start Date: 11.13.2007  
Sample Reception Date: 11.13.2007  
Sample Reception Qty: 7821  
Packaging: bottle  
Measuring Date: 14. Jan. 2008 10:58:58

**Results Summary**

Type of Analysis	Reference ID	Result	Value	Unit
Classification Model	10001	OK	05.43	781
Isotopic Analysis	10001	OK	0.00	0.00
Glucose Model	10001	OK	0.00	0.00
Orange - Minerals	10001	OK	0.00	0.00
Orange Concentrate Effect	10001	OK	0.00	0.00
Orange Stage	10001	OK	0.00	0.00
Validation Model	10001	OK	0.00	0.00
Mineral Analysis (05.4)	10001	OK	0.00	0.00
Fruit Content (05)	10001	OK	84%	0.00
Quantification	10001	OK	0.00	0.00

**Quantification Results**

Compound	Concentration	Unit	Flag
ERYTIC	10		
ethanol	10		
methanol	10		
glycerol	10		
ascorbic acid	10		
malic acid	10		
citric acid	10		
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Bruker JuiceScreener™ delivering push-button NMR based quality control of fruit juices.

## Conclusion and Outlook

In this report we have introduced the SGF Profiling™ method for the authentication, verification and quality control of fruit juices. In addition to the quantification of a large array of characteristic compounds, this fully automated NMR screening technique uses statistical models for the estimation of fruit content or the origin of the juice. This analysis tool can show known and unknown deviations from normality. Currently, routines are under development to identify unknown deviations by constructing spectral patterns which can be compared to Bruker's reference compound database for biofluids and food materials (BBIOREFCODE).

The reference juice database is updated regularly, so that additional or improved statistical models will be available in the near future for fruit type, fruit origin or other discriminatory factors. Thus, the predictive power of the SGF Profiling method will be refined and increased over time.

The examples presented here for the screening of fruit juices can also be seen as proof-of-principle for other upcoming applications. The same workflow (preparation, measurement, processing, reporting) and underlying mathematical methods can be easily transferred to other quality control applications, such as the screening of milk, wine or beer. Furthermore, all future metabonomics screening techniques, including clinical diagnosis using biofluids (e.g. urine, plasma, CSF, bile) will benefit from the experience obtained with these fruit juice applications.



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Bruker BioSpin is continuing to revolutionize the design, manufacture and distribution of life science, process control and analytical research tools based on magnetic resonance core technology. Bruker BioSpin is the worldwide technology and market leader in NMR, EPR and preclinical MRI.

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