

Food Analysis to Check Quality, Safety and Authenticity by Full-Automated ¹H-NMR

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Abstract. Full-automated high resolution ¹H-NMR spectroscopy offers unique screening capabilities for food quality and safety by combining non-targeted and targeted screening in one analysis (15 - 20 minutes from acquisition to report). Full-automated high resolution ¹H-NMR (400 MHz) has found its way into the quality control of food and beverages over the last years. NMR reproducibility allows statistical investigations e.g. for detection of variety, mixing of varieties, geographical origin and adulterations, where smallest changes of many ingredients at the same time must be recorded. The non-targeted approach to the data allows detecting even unknown deviations, if they are visible in the ¹H-NMR spectra of e.g. fruit juice, wine, edible oils or honey. The same data acquired in high throughput mode are also subjected to quantification of multiple compounds. Reproducibility and transferability of the solutions shown are user-, instrument- and laboratory-independent. The method has been proven on fruit juices and wine, where so far unknown frauds could be detected. In addition conventional targeted parameters are obtained in the same analysis. This technology has additionally the advantage that NMR is completely quantitative and concentration calibration only has to be done once for all compounds.

Keywords: Non-targeted screening; targeted screening; detection of frauds; user-, instrument- and lab-independent method; detection of e.g. geographical origin, varieties

1. Introduction

High Resolution NMR Spectroscopy offers unique screening capabilities for food quality and safety by combining non-targeted and targeted screening in one analysis to supplement conventional analysis [1], [2]. 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.

2. Materials and Methods

The SGF-Profiling is providing a full ¹H-NMR-spectrum for each sample. A 400 MHz spectrometer with 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.

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.

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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 characterisation and in some cases final judgement. Individual components are identified and quantified using information from a reference compound NMR spectral base (targeted mode).

3. 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, see Figure 1, such as specific models for multi fruit type separation [5], 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 characterisation 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. 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.

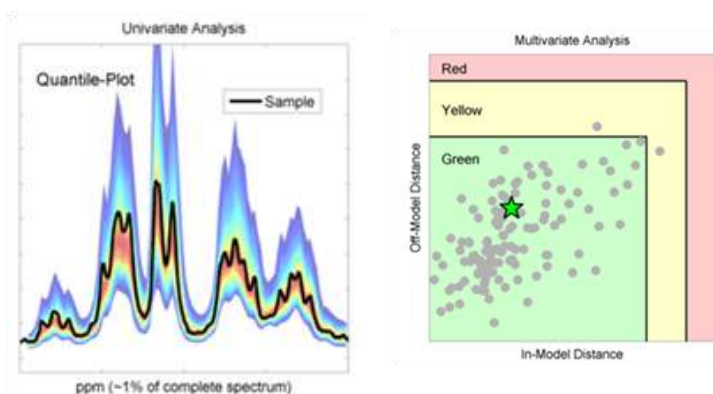


Fig. 1: Left: Univariate Analysis vs. Multivariate Analysis in NMR based Food Screening; Right: Multivariate Analysis in NMR-based food screening

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.

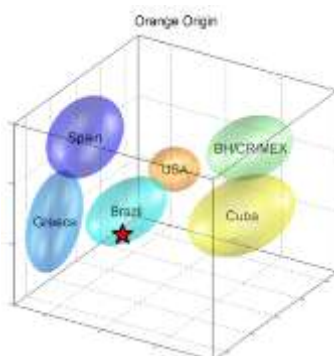


Fig. 2: Orange Juice Origin as determined by rapid NMR screening

The same sample is projected to differentiation of geographic origin, which is a relevant commercial aspect with regard to price and quality. The orange juice in Figure 2 has been determined as Brazilian orange juice. 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.

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.

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.

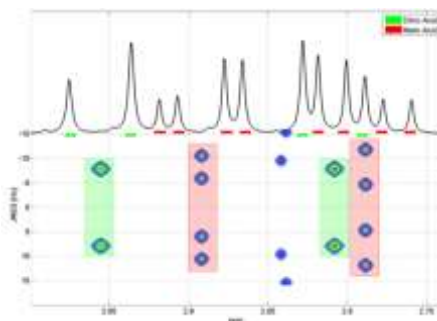


Fig. 3: Rapid 2D J-Resolved spectrum recorded during the automatic measurement procedure

Figure 3 shows a region of the ID (NOESY) and 2D (JRES) spectra of a pear juice. Signals of Citric Acid (green) and Malic Acid (red) are separated in the 2D-spectrum.

3.1. Extension to Food and Drink Materials Other Than Fruit Juice

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 instance for a German Riesling 116 parameters are automatically generated from one wine sample measurement. Several of those parameters are either 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. The full potential is shown in Figure 4 for a German Riesling.

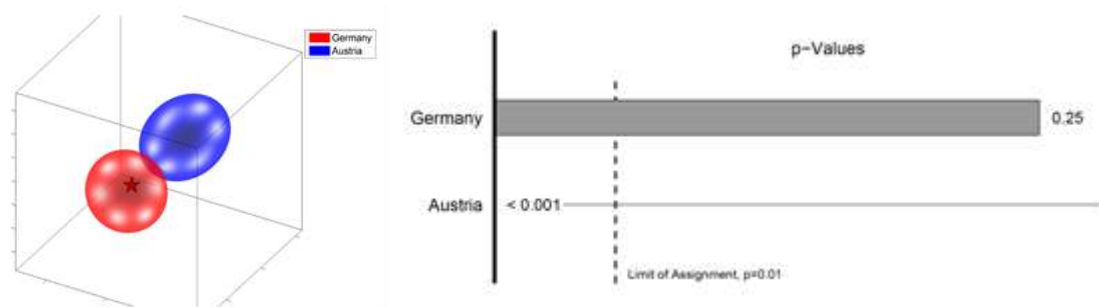


Fig. 4: Result of a German Riesling presented in graphical form with probability values

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 (Figure 5). 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 contribute not only to product quality, but also to product safety.

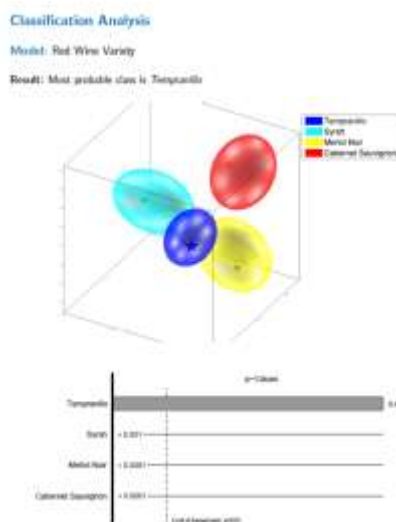


Fig. 5: International red wine variety model

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. Figure 6 shows an extract from the quantification part of the wine report that is generated automatically after every measurement. LOQ`s are also given besides the absolute concentration values for each compound. In the distribution curves on the right side, the black bar indicates the actual sample on top of the NMR derived concentration profile.

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 and other edible oils as well as honey.

Targeted Analysis

In the following tables the results of the quantitative analysis are given. Parameters labelled with * are calculated parameters.

Standard Parameters:		Amino Acids:							
Compound	Designation Pa	Compound	Value	Unit	LOQ	Official Reference	Wine-Profiling™		
	Compound					Flag	min	max	NMR Reference Database
total alcohols	acetic acid	4-aminobutanoic acid	<130	mg/L	120	○	-	-	not available
total alcohols	acetic acid	alanine	<36	mg/L	36	○	-	-	not available
ethanol	ethanoate	arginine	<150	mg/L	150	○	-	-	not available
ethanol-v*	formic acid	proline	222	mg/L	150	○	-	-	not available
glycerol	formic acid	(Poly)phenols:							
glucose	gluconic acid	Compound	Value	Unit	LOQ	Official Reference	Wine-Profiling™		
fructose	glutamic acid					Flag	min	max	NMR Reference Database
glucose/fru	malic acid	caffeic acid	20	mg/L	15	○	-	-	not available
sucrose	malic acid	epicatechin	<30	mg/L	30	○	-	-	not available
arabinose	HMF	gallic acid	<25	mg/L	25	○	-	-	not available
total sugar	isoflural	shikimic acid	22	mg/L	20	○	-	-	not available
total ferment		trimesic acid	13	mg/L	10	○	-	-	not available
tartaric acid	Compound	Stabilising Agents:							
malic acid	methanol	Compound	Value	Unit	LOQ	Official Reference	Wine-Profiling™		
lactic acid	1,3-propanediol					Flag	min	max	NMR Reference Database
citric acid	2,3-butanediol	benzoic acid	<10	mg/L	10	●	-	0 **	not available
energy value	2-methyl-propanol	acetic acid	<10	mg/L	10	●	-	200 **	not available
bead units	2-phenylethanol	sulphuric acid	<30	mg/L	30	○	-	-	not available
carbohydrat	3-amethoxy-benzal	Sources for Reference Values							
	acetaldehyde	a) EU-Verordnung							
	glycerol/ethanol	b) Hersteller: OENO 10/2004							
		c) Weinverordnung (Rahmang. 21. April 2006)							

Fig. 6: Excerpt of an identification/quantification table of a WineScreener report

4. 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.

5. Acknowledgements

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