An exploratory NMR nutri-metabonomic investigation reveals dimethyl sulfone as a dietary biomarker for onion intake

Hanne Winning,*[a] Eduvigis Roldán-Marín,[bc] Lars O. Dragsted,[b] Nanna Viereck,[a] Morten Poulsen,[d] Concepción Sánchez-Moreno,[c] M. Pilar Cano[c] and Søren B. Engelsen[a]

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The metabolome following intake of onion by-products is evaluated. Thirty-two rats were fed a diet containing an onion by-product or one of the two derived onion by-product fractions: an ethanol extract and the residue. A 24 hour urine sample was analyzed using ^{1}H NMR spectroscopy in order to investigate the effects of onion intake on the rat metabolism. Application of interval extended canonical variates analysis (ECVA) proved to be able to distinguish between the metabolomic profiles from rats consuming normal feed and rats fed with an onion diet. Two dietary biomarkers for onion intake were identified as dimethyl sulfone and 3-hydroxyphenylacetic acid. The same two dietary biomarkers were subsequently revealed by interval partial least squares regression (PLS) to be perfect quantitative markers for onion intake. The best PLS calibration model yielded a root mean square error of cross-validation (RMSECV) of 0.97% (w/w) with only 1 latent variable and a squared correlation coefficient of 0.94. This indicates that urine from rats on the by-product diet, the extract diet, and the residue diet all contain the same dietary biomarkers and it is concluded that dimethyl sulfone and 3-hydroxyphenylacetic acid are dietary biomarkers for onion intake. Being able to detect specific dietary biomarkers is highly beneficial in the control of nutritionally enhanced functional foods.

Introduction

Onions (*Allium cepa*) constitute a part of the daily diet for most of the world's population. Nutritionally, onion properties have been widely reported, indicating beneficial health effects. Most of the beneficial health effects have been related to the onion anti-oxidantic, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory, antimicrobial, prebiotic and cardiovascular protective properties.[1–3] The main bioactive compounds in onion related to the onion beneficial health effects include flavonols, particularly quercetin and quercetin glucosides,[4] soluble fibers, fructooligosaccharides and organosulfur compounds.[5–9] Organosulfur compounds have become subject of many investigations due to their potential chemopreventive and antioxidant effects.[9–11] For example, the *S*-methyl sulfoxide isolated from *Allium cepa* has been shown to have a lipid-lowering effect in cholesterol-fed rats.[12] The metabolism of onion is not yet fully understood, but cycloalliin, an organosulfur compound found in garlic and onion, initiates several biological activities and its metabolite, (3*R*,5*S*)-5-methyl-1,4-thiazane-3-carboxylic acid, has been found in urine after intravenous or oral administration to rats.[13] Boyle and co-workers found a significant decrease in the level of human urinary 8-hydroxy-2′-deoxyguanosine after ingestion of an onion meal.[6]

Worldwide, large amounts of onion disposal are produced from the production of onions. Because onion is toxic for many animals, this waste product cannot be utilized in the general feeding industry. Onion waste can be stabilized as a useful onion by-product which can act as an antioxidant or antibrowning agent. With respect to the health beneficial effect of onion, developing dietary supplements or nutritionally enhanced functional foods including onion could be highly beneficial. When developing nutritionally enhanced functional foods, it requires understanding of the mechanisms of prevention and of protection in order to utilize and document the potential nutritional effects of an onion supplement. The identification of biologically active molecules as potential dietary biomarkers leads to a greater understanding of biochemical pathways and potentially allows objective quantification of onion intake in mammalian metabolomic studies[14] and eventually in human metabolomic studies.

In contrast to **metabolomics**, which focuses on high-throughput characterization of low molecular weight metabolites in order to obtain a complete molecular profile of the measured biological sample, **metabonomics** focuses at measuring how the molecular profile (metabolome) responds to external factors. High-resolution nuclear magnetic resonance (NMR) (most often proton, ^{1}H, NMR) has emerged as a powerful non-invasive technique for metabonomic studies due to its ability to simultaneously detect a large number of compounds in a rapid high-throughput manner that requires little sample manipulation.[15] ^{1}H NMR spectroscopy is widely used to study the metabolic

[a]*University of Copenhagen, Faculty of Life Sciences, Dept. of Food Science, Quality & Technology, Rolighedsvej 30, 1958 Frederiksberg C, Denmark*
[b]*University of Copenhagen, Faculty of Life Sciences, Dept. of Human Nutrition, Centre for Advanced Food Studies, Rolighedsvej 30, 1958 Frederiksberg C, Denmark*
[c]*Department of Plant Foods Science and Technology, Instituto del Frío, Consejo Superior de Investigaciones Científicas (CSIC), Ciudad Universitaria, José Antonio Novais 10, E-28040 Madrid, Spain*
[d]*National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark*

variation in biofluids and its capabilities for metabonomics is well established.[16] It has become more and more common to combine investigations of complex NMR spectra with advanced multivariate data analysis such as chemometrics in order to extract systematic latent information from the complex biological NMR spectra. Such analysis requires a minimum of assumptions and the relationships may be visualized intuitively. Already in the early nineties, principal component analysis (PCA)[17] was introduced to classify ^{1}H NMR spectra of urine by the Nicholson group at Imperial College (UK).[18] This group also introduced the definition of metabonomics as: 'understanding the metabolic responses of living systems to pathophysiological stimuli *via* multivariate statistical analysis of biological ^{1}H NMR spectroscopic data'.[19] Subsequently, the term metabonomics has been broadened to 'metabolic processes studied by ^{1}H NMR spectroscopy of biofluids' and thus also includes nutrition studies.[20]

Urine is often used as a biological fluid for metabonomic investigations due to the easy and non-invasive possibilities of collecting repeated samples, the variable metabolite composition, and the often higher metabolite concentrations achieved relative to blood plasma.[21] The fact that the urine profiles are generated and analyzed without *a priori* assumptions about the metabolic and physiological processes involved allows several hypotheses to be tested simultaneously, as well as new hypotheses to be generated from unexpected associations. In nutritional metabonomics, one often has to deal with large variability in the samples compared to the changes of interest induced by the nutritional intervention. This often large variation is caused by biological variation, experimental inhomogeneity or inadequate sample procedure (sample preparation, time of sampling and storage). Therefore, the inter- and intra-individual metabolite variance within a normal population has to be evaluated qualitatively and quantitatively before conclusions can be made. In rodents, it has been determined that species, strain, genetics, sex, age, hormone concentrations, diurnal cycles, diet, temperature, stress and gut microflora all contribute to the metabolic composition of the urine of the animals.[22] However, it is known that human volunteers in dietary metabonomic studies frequently do not report all their medication or food supplements[23] and it is therefore highly desirable to gain objective knowledge about the true diet of a test person. Investigations using animals as a model-system make it possible to investigate biomarkers under controlled conditions.

A rodent study was recently conducted to evaluate possible health effects after feeding with an onion by-product and two derived onion fractions.[24] In this study, the effect of onion intake on antioxidant enzymes, DNA damage, and gut environment in healthy rats was investigated and it was found that the onion by-product and the onion sub-fractions have no genotoxicity, may support antioxidative defense and alter the functionality of the rat gut microbiota. The purpose of the work presented here is to investigate the effect of onion intake on urine composition of the same rats with explorative metabonomic analysis using ^{1}H NMR spectroscopy and chemometrics. Onion contains both soluble and insoluble compounds. Therefore, the onion product was fractionated into two fractions: an ethanol extract, rich in fructooligosaccharides; and the residue, the insoluble matrix. In this way, potential onion dietary biomarkers in the onion by-product investigated can be either located in the extract or left in the residue.

Experimental

Onion and rat study

The onion product used to feed the studied animals is a freeze-dried onion by-product produced from a pasteurized onion paste (*Allium cepa* L. *cepa*, 'Recas') produced at Instituto del Frío (CSIC, Madrid, Spain). The onion by-product was fractionated into an ethanol/water soluble extract which is 70% (w/w) of the by-product and the rest, the dry residue, which is 30% (w/w) of the by-product. The extraction and the rat study were carried out at the National Food Institute, Technical University of Denmark (Søborg, Denmark). The onion extraction procedure and the animal study are detailed as described elsewhere.[24] Briefly, 32 male rats of the inbred strain Fisher 344 were divided into four groups of eight rats and fed four weeks either a control feed, a control feed supplemented with 10% of onion by-product, a control feed supplemented with 7% of onion extract or a control feed supplemented with 3% onion residue (Table 1). The 10% dose was sufficient to elicit physiological effects but not high enough to cause any adverse effects in the rats. The amounts of onion fractions to be added to the feed were chosen taking into account the content of the dietary fiber fructans in each fraction obtained in the extraction of the onion by-product.[24] Consequently, the two onion fractions are supplemented to the feed in concentrations which match the concentration of the by-product. Due to the experimental design, the extract and the residue added should be similar to the by-product, provided that the extraction is complete. The animals were housed 2 × 2 in Macrolon cages, in the same room under the same experimental conditions. The control group was fed with an isocaloric diet, substituting onion sugars with sucrose and onion fiber with starch.

Table 1 Composition of rat feed. For detailed composition of diet see Roldán-Marín *et al.*[24]

	Composition/g per kg feed			
	Control group	Onion by-product group	Onion extract group	Onion residual group
Onion by-product	0	100	0	0
Onion extract	0	0	70	0
Onion residue	0	0	0	30
Control feed	1000	900	930	970
Total feed	1000	1000	1000	1000

Urine samples

Urine samples were collected for a period of 24 h. Two milliliters of 1 mM NaN_3 were added to the urine sample test tubes before the urine was collected and the tubes were kept at 0 °C. The urine volume was recorded and samples were frozen in portions at −80 °C for further analysis. Prior the ^{1}H NMR analysis, the thawed urine samples were prepared by centrifugation at 1200 *g* for 10 min, 340 μl of the supernatant were transferred to NMR tubes followed by addition of 170 μl of 100 mM phosphate buffer solution (H_2O, D_2O, TSP-d4 (per-deuterated 3-trimethylsilyl propionate sodium salt), NaN_3, pH 7.4) to reduce the pH range of the samples. TSP-d4 was added to act as an internal chemical shift reference (δ^1H 0.0), D_2O was added to provide a lock signal for the NMR spectrometer and NaN_3 was added as a preservative. The urine samples were prepared to run in a random order.

^{1}H NMR measurements

^{1}H NMR spectra were recorded for the 32 urine samples. The spectra were acquired on a Bruker Avance Ultra Shield 400 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at 400.13 MHz using a broad band inverse probe head. Data were accumulated at 300 K employing a pulse sequence using pre-saturation of the water resonance during the recycle period followed by a composite 90° pulse[25] with an acquisition time of 2.04 s, a recycle delay of 5 s, 128 scans and a sweep width of 8012.82 Hz, resulting in 32 000 complex data points. All samples were automatically tuned, matched and shimmed. Prior to Fourier transformation, each FID was apodized by Lorentzian line broadening of 1.0 Hz and zerofilled once and the corresponding spectra were manually phased and automatically baseline corrected. Receiver gain was automatically set. Prior to the chemometric analysis the raw proton NMR spectra data matrix to be investigated had the dimensions (32 × 65 536) but was reduced to 32 202 data points (10–0.2 ppm) excluding spectral areas with no signals. All spectra were aligned (rigid movement) in proportion to the TSP signal at 0.0 ppm. Furthermore, due to insufficient (unequal) depression of the water signal, the area from 5.00 to 4.50 ppm was removed. It also proved necessary to normalize spectra in proportion to the total sum of the spectra in order to remove the large concentration differences of the urine samples. Normalization of urinary metabolic data is best considered as a data transformation which minimizes inter-sample variation due to differences in gross urinary concentration between samples caused by volume and dry matter differences. Furthermore, two 2D NMR experiments – total correlation spectroscopy (TOCSY) and heteronuclear single quantum coherence (HSQC) spectra – were acquired on urine from a rat fed the onion by-product supplement. These experiments were used for assignment of selected signals. The 2D NMR spectra were recorded using the Bruker pulse sequences mlewphpr and hsqcgpph (mixing time of 60 ms).[26,27] The 2D NMR spectra were referenced to TSP-d4 at 0.0 ppm before data analysis. Besides the spectra of the rat urine, ^{1}H NMR spectra of the three different onion fractions were obtained. One milligram of each of the onion fractions was suspended in 1 ml D_2O solution with added 10% TSP-d4. Acquisition parameters were similar to the one used for the urine NMR spectra. The ^{1}H NMR spectrum of dimethyl sulfone was measured with the pure chemical compound dissolved in the 100 mM phosphate buffer solution (pH 7.4) and with the same acquisition parameters as the urine ^{1}H NMR spectra.

Chemometric analysis and software

Multivariate data analysis in the form of PCA and partial least squares regression (PLS)[28] was applied to obtain optimal quantitative and qualitative information from the measured spectra. PCA is the primary tool for investigation of large bilinear data structures for the study of trends, groupings and outliers. By means of PCA it is possible to find the main variation in a multidimensional data set by creating new linear combinations, principal components (PCs), from the underlying latent structures in the raw data. PLS is a multivariate calibration method by which two sets of data, X and y, are related by means of regression. The purpose of PLS is to establish a linear model of latent variables (LVs), which enables the prediction of a reference value y (slow measurement) from the measured spectrum X (fast measurement). Furthermore, extended canonical variates analysis (ECVA)[29] models were applied for classification of feed groups. Canonical variates analysis (CVA)[30,31] is a method for estimation of directions in space that maximizes the differences between groups of samples. However, CVA cannot deal with highly collinear data such as spectroscopic data, where the number of variables is much larger than the number of samples. The ECVA method solves this problem by the use of PLS in the inner part of CVA and thereby allowing for the analysis of highly collinear data.[29] In order to improve the calibration models and to investigate the influential areas of the spectra, interval PLS (iPLS) and interval ECVA (iECVA) were employed.[32] iPLS is an extension of PLS which develops local PLS models on a number of sub-intervals of the full-spectrum region. The main advantage of iPLS is that it provides an overall picture of the relevant information in different spectral sub-divisions, thereby facilitating interpretations and removing interferences from other regions. iECVA works similarly to the iPLS model.

Scaling or other pre-transformations of NMR data are often necessary before the data analysis in order to assure that all signals are influencing the model. In this study, pareto-scaling was used as scaling method applied to the NMR data before the further data analysis. Pareto-scaling reduces the relative importance of large values, but keeps the data structure partially intact. Each variable is divided by the square root of the standard deviation of the column values.[33] Due to the low number of samples, all of the calibration models were validated using cross-validation (CV) with five segments, leaving out one segment at a time from which the root mean square error of cross-validation (RMSECV) was calculated as a measure of the prediction error. This validation method without using an independent test set is known to be slightly optimistic.[34] However, great consistency with permutation tests was obtained for all models, and besides, it was not the scope of the current study to optimize PLS correlations.

The spectra were analyzed using the chemometric software LatentiX 2.0 (www.latentix.com, Latent5, Copenhagen, Denmark), PLS Toolbox 4.11 (Eigenvector Research, Manson, Washington, USA), and MATLAB 7.6 2008a (The MathWorks,

Inc., Natrick, Massachusetts, USA). Regression (iPLS) and the iECVA model were performed in MATLAB using iToolbox and the ECVA Toolbox version 2.02, respectively (all available at www.models.life.ku.dk).

Results and discussion

The ^{1}H NMR spectra of the three onion products fed to the rats are shown in Fig. 1. There are both similarities and differences in the ^{1}H NMR spectra of the three onion products, illustrating both the complexity and the difference between the three onion fractions before they are metabolized by the rats. The spectra reveal diets high in fructans (3.5–6 ppm) with significant amount of aromatic (6–9 ppm) compounds. The ^{1}H NMR spectrum of the extract differs from the spectrum of the residue by more intense signals in the aromatic region. On the other hand, the residue spectrum has slightly more intense signals in the high-field region of the spectrum compared to the ^{1}H NMR spectra of the two other fractions. The spectrum of the (ethanol) extract differs from the spectra of the by-product and the residue by a triplet at δ 1.18 ppm which is assigned to the CH_3 signal from residual ethanol. From the spectra, it is difficult to assure that the by-product spectrum equals the residue plus the extract spectrum.

Fig. 2 shows the average of ^{1}H NMR rat urine spectra of each of the feed groups. The spectra appear very similar despite the different feeding schemes. The ^{1}H NMR spectra of urine typically contain thousands of sharp lines from predominantly low molecular weight metabolites except for one broad band at δ 5.8 ppm from urea.[35–44] The spectra display a wide range of metabolites such as aromatics, aliphatic compounds, sugars, amino acids and other metabolites. However, from this global investigation of the raw data, no obvious difference in the urine profile of the three onion diets can be detected.

In order to investigate possible metabolic differences between the different feeding schemes, a PCA model was established on the full ^{1}H NMR spectra of the 32 urine samples. However, the PCA model was not able to distinguish between the four different feeding groups or to group the samples in an onion and a control group (Fig. 3). No separate clustering was observed for any of the four classes, indicating that the variance between and within classes is similar at least for the two most important principal components.

In order to scrutinize the spectra for signals able to distinguish between the control feed and the different onion-fed groups (by-product, extract and residue), iECVA was carried out on the urine spectra (Fig. 4) using 20 equally sized sub-intervals. Indeed, two interesting intervals were found by iECVA which were able to improve the misclassifications rate significantly. The best interval, 6.50–6.95 ppm, was able to reduce the number of misclassifications from 11 to 2 (Fig. 4). The interval includes signals from some of the aromatic compounds in the urine. The second best interval from 2.98–3.42 ppm was able to decrease the number of misclassifications to 3. The signals in two intervals selected by iECVA are shown in Fig. 5, colored according to the feed group.

The signal at δ 3.15 ppm in one of the selected regions in Fig. 5 shows that the urine spectra with the highest signal intensity at 3.15 ppm is the urine from rats fed with a diet which contained a 10% supplement of onion by-product. In contrast, the urine from rats fed the control diet (without onion) shows no signal in this area. This indicates that the signal also contains quantitative information on onion *dose*. The signal with a chemical shift of δ 6.8 ppm shows (to a lesser extent) the same pattern. This signal matches the spectral profile of 3-hydroxyphenylacetic acid, when matched in an NMR spectral base (BBIOREFCODE) containing 535 compounds found in urine. The correlation between the signal at δ 3.15 ppm and one of the signals from the aromatic protons in 3-hydroxyphenylacetic acid at 6.8 ppm is 0.94, which indicates a connection between the signals. They could be from protons within the same metabolite; however, the signal from the CH_2 group in 3-hydroxyphenylacetic acid is expected to be located at approximately 3.75 ppm. Instead, this could indicate that the 3-hydroxyphenylacetic acid and the compound which has signal at δ 3.15 ppm are both involved in the metabolism of onion. The correlation coefficient between the signal at 6.80 ppm

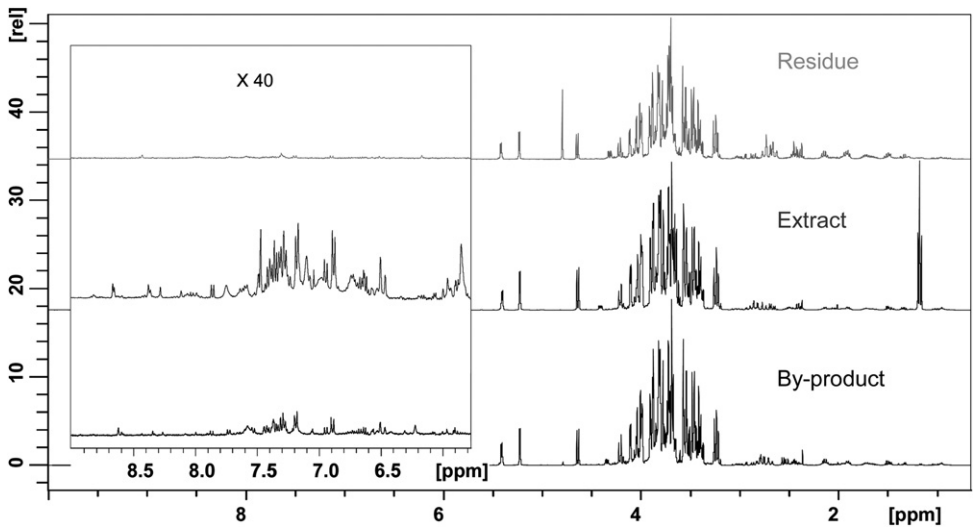

Fig. 1 ^{1}H NMR spectra of onion by-product, onion extract and onion residue dissolved in D_2O included 1 mg/ml TSP-d4.

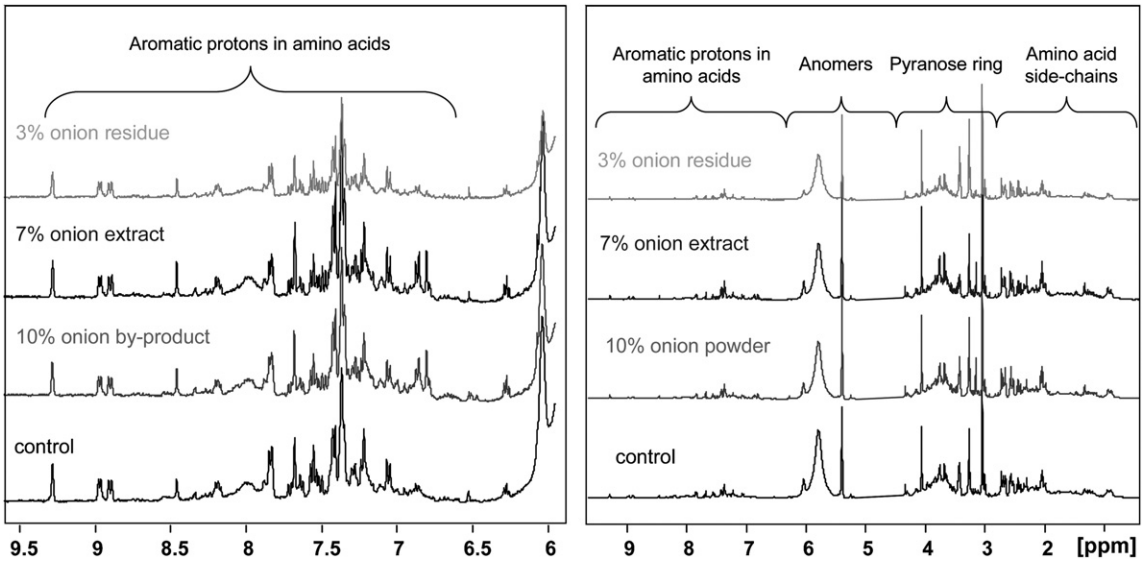

Fig. 2 ^{1}H NMR spectra of averaged rat urine from each of the four dietary groups. The water signal is removed and the aromatic region magnified by a factor of 100.

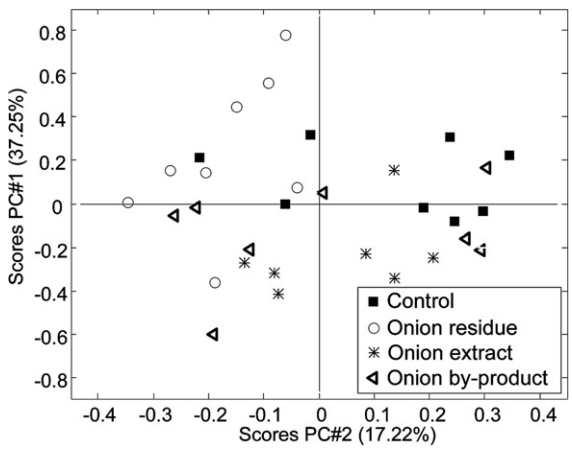

Fig. 3 PCA model (PC1 *versus* PC2) of pareto-scaled ^{1}H NMR spectra of 32 urine samples of rats from the four different feeding schemes.

and the doublet at 6.86 ppm is slightly lower: 0.89, which might indicate that other compounds also have a signal at this chemical shift.

In order to investigate quantitative information regarding onion intake (*onion dose*) the ^{1}H NMR spectra were analyzed using PLS relating to the ^{1}H NMR spectra and the onion dose (0, 3, 7 and 10%). Variable selection using iPLS was applied to find which regions of the ^{1}H NMR spectra of urine that include quantitative information about the onion dietary biomarkers. The prediction error of the full-spectrum model was 1.56% (w/w), as illustrated by the dashed line in Fig. 6A. Two intervals were found which were able to improve the prediction error significantly: 6.50–6.95 ppm and 2.98–3.42 ppm (marked in Fig. 6A). The optimal interval is 2.98–3.42 ppm which results in a prediction error of 1.12% using only 3 LVs. Adding the interval around 6.8 ppm results in a further reduction of the error to 0.97% (w/w) using only one LV. The actual *versus* predicted plot in Fig. 6B shows a simple one-component PLS model obtained on the two selected intervals, revealing that the NMR urine spectra contain univocal quantitative information about onion dose.

The two optimal spectral regions found by iPLS are exactly the same intervals as found by the iECVA. This indicates that it is the same quantitative information which is extracted by the iECVA and iPLS. Unfortunately, the experimental design used has dose and fraction confounded which makes it impossible to decide which effect is modeled, even if the ^{1}H NMR spectra should hold information about both features. In theory, it should be possible to mathematically remove the information about dose and retain the information about fraction. This can be done by orthogonalization where the vector describing the dose response is withdrawn from the data.[45] Indeed, it was tested in this study, but the orthogonalization approach led to a rather overfitted classification (results not shown).

In the interval of 2.98–3.42 ppm, one signal seems particularly important. The signal has chemical shift δ of 3.15 ppm. Based on 2D experiments (TOCSY and HSQC) of the urine sample and NMR measurements of the pure compound measured under exactly the same conditions as the urine, this signal was identified as the methyl protons (CH_3) in dimethyl sulfone (Fig. 7). Indeed, this symmetric compound has only one signal in the ^{1}H NMR spectrum, and no cross-peak in the TOCSY spectrum. Therefore the assignment of the signal is difficult and should be further verified. From the HSQC experiment, the chemical shift of the corresponding ^{13}C was found to be at 44 ppm, which also indicates assignment to dimethyl sulfone.

Dimethyl sulfone is an oxidation product of dimethyl sulfoxide (DMSO) and it is highly possible to find DMSO in urine as a result of an onion diet because onions contains many sulfoxides.[12] It has previously been shown that DMSO is metabolized to dimethyl sulfone in humans and rats.[46] DMSO is a universal solvent and has the characteristic property that it is able to penetrate the skin. DMSO is an industrial solvent

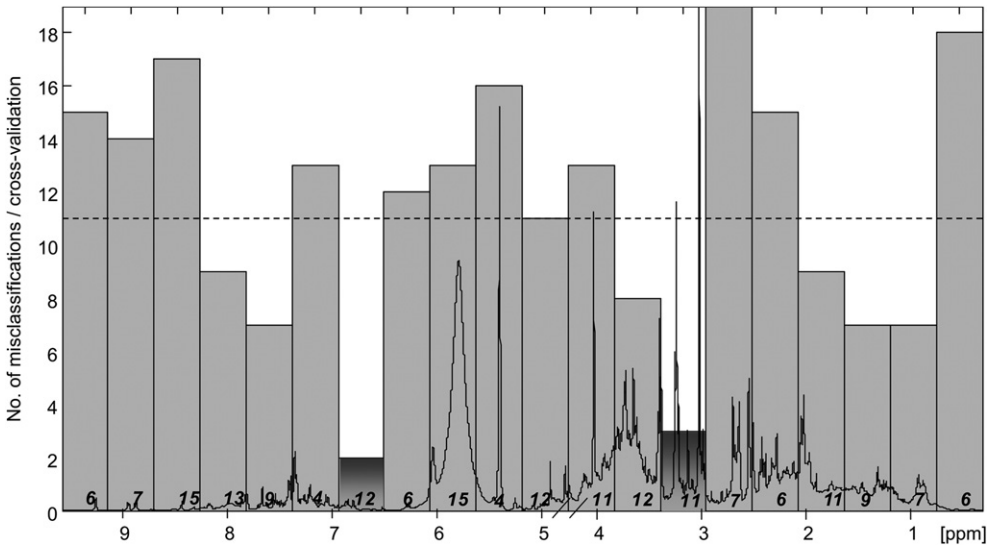

Fig. 4 iECVA plot ^{1}H NMR spectra of urine from onion-fed rats indicating the two best intervals for lowest number of misclassifications. The dotted line is number of misclassifications (11 for 11 LVs) for the global model and the italic numbers are optimal LVs in the interval model.

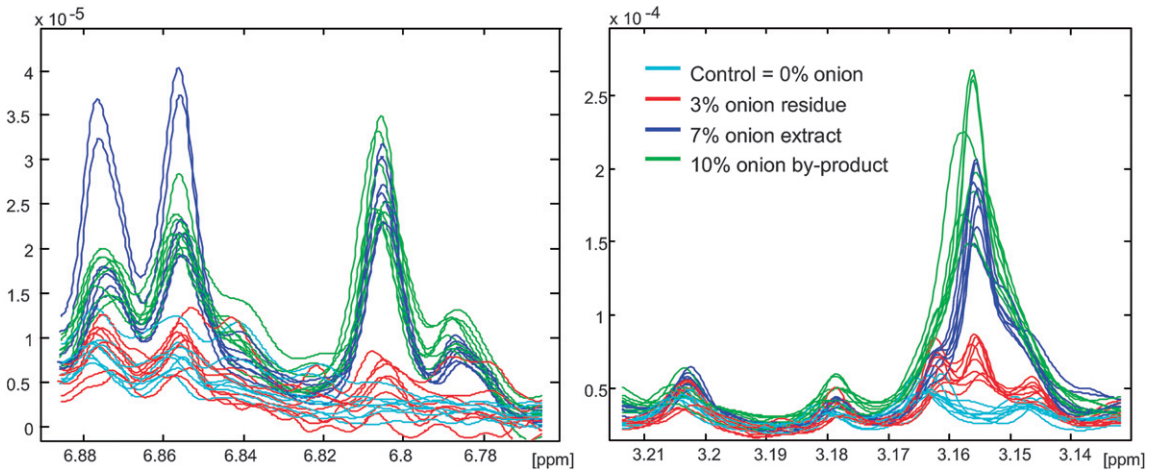

Fig. 5 Selected spectral regions from the iECVA model of the ^{1}H NMR spectra of urine from onion-fed rats, revealing difference in signal intensity for each onion fraction.

approved by the FDA for treatment of interstitial cystitis (bladder inflammation) and side effects include reports of onion odor breath. Several reports have suggested that DMSO may also be effective in inhibiting cholesterol-induced atherosclerosis in experimental animals.[47–49] Dimethyl sulfone has been reported in human sweat,[50] in urine following asparagus consumption[51,52] and in cow's milk from pasture-fed cows.[53] The compound therefore seems to originate from sulfur-rich herbs and plant foods but there may be a genetic element involved in its formation in humans. Dimethyl sulfone has recently been linked with the occurrence of skin cancer.[54,55] Gallagher and co-workers[55] found that skin cancer patients showed significantly higher levels of dimethyl sulfone in the skin measured by gas chromatography/mass spectrometry (GC-MS). An NMR study has also found detectable levels of dimethyl sulfone normally present in the blood and cerebrospinal fluid, suggesting that it derives from dietary sources, intestinal bacterial metabolism, and the body's endogenous methanethiol metabolism.[56]

The good correlation between onion dose and the NMR spectra of urine shows that the onion dietary biomarker is present in all fractions and is equally distributed in the fractions of the by-product. Apparently, the concentration of the dietary biomarker is proportional to the onion dose intake independent of the fed onion fraction. That dimethyl sulfone is present in all urine fractions may be due to the extraction procedure which was not able to eliminate the dietary biomarker from the by-product to the extract. The by-product consisted of intact cell walls which may be the reason why all dimethyl sulfone was not removed from the residue. Another explanation could be that the compound is only partly soluble in ethanol. A third explanation may be that dimethyl sulfone is a degradation product of more lipid-soluble organosulfur compounds from onion, and that these compounds were only weakly soluble in 60% ethanol.

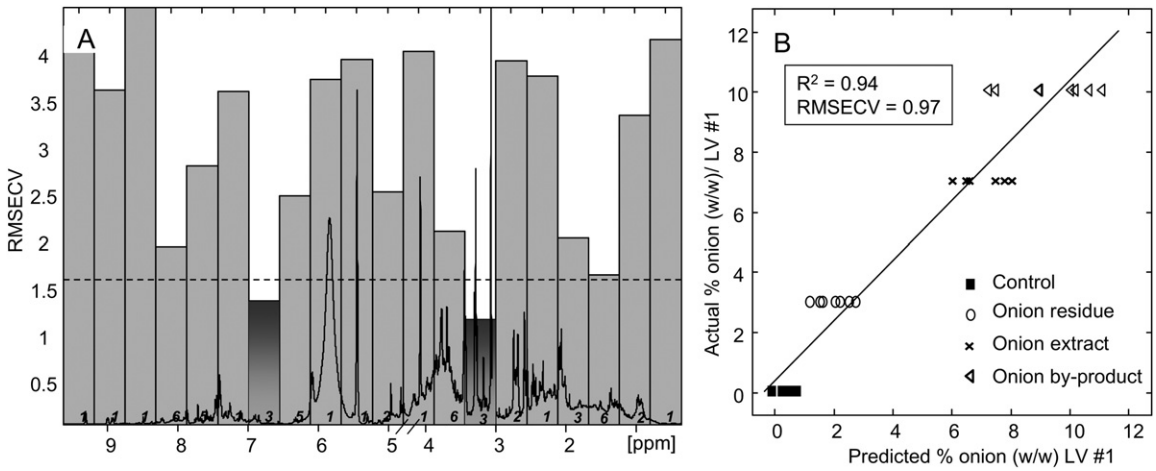

Fig. 6 (A) iPLS plot of the prediction of % (w/w) onion in feed obtained on ^{1}H NMR spectra of urine from onion-fed rats. Dashed line is RMSECV (7 LVs) for the global model. Italic numbers are optimal LVs in the interval model. The two best intervals are highlighted. (B) Actual *versus* predicted plot of PLS model of onion dose and urine from onion-fed rats, performed on the highlighted intervals (A).

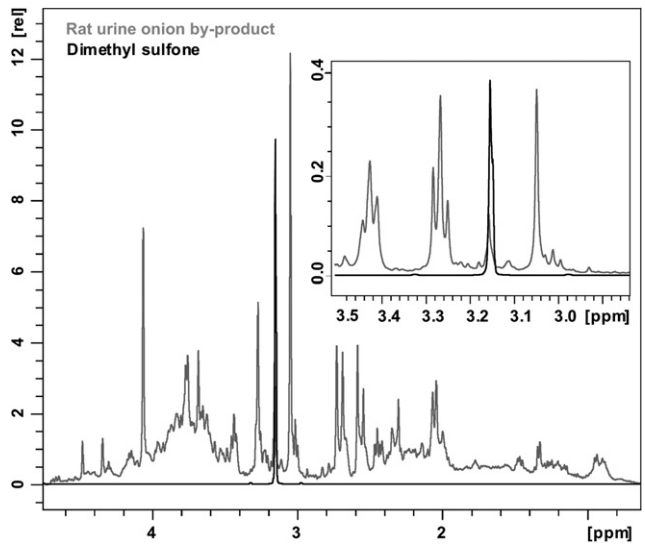

Fig. 7 ^{1}H NMR spectrum of selected urine from rat fed an onion by-product diet overlapped with a spectrum of pure dimethyl sulfone.

Conclusions

Two onion dietary biomarkers were identified as being dimethyl sulfone and 3-hydroxyphenylacetic acid. Quantitative PLS models showed that the onion dose responded the quantitative information in the urine ^{1}H NMR spectra primarily due to the dietary biomarker dimethyl sulfone. This indicated that urine from rats fed with the two fractions (extract and residue) of the onion by-product and rats fed with the onion by-product all contain the dietary biomarker and that the dietary biomarker is present in all fractions and in the same concentrations as the doses. Therefore, it was possible to identify an objective dietary biomarker for onion intake but not for the different onion products. Clearly, the dimethyl sulfone ends up in all fractions and is therefore a dietary biomarker for onion intake. Being able to quantify the dietary intake can be very beneficial as a control in diet intervention studies. The self-reported dietary intake in forms of food frequency questionnaires has been the dietary assessment method used most frequently in large-scale studies. This is primarily because it is easy to administer, it is less expensive than other dietary assessment methods, and it provides a rapid estimate of usual intake.[57] However, there exists a great problem in using food frequency questionnaires because self-reports of food intake are not accurate and sometimes misleading. McKeown *et al.* showed that correlations between 24 h urinary nitrogen excretion and dietary intake from the food frequency questionnaire were as low as 0.25.[58] Clearly, a potential exists in using the onion dietary biomarker in various nutrition studies. We are now in the process of carrying out a human study with an onion product in order to verify the usefulness of this marker and to determine whether genetic variability or variations in gut flora might affect its usefulness in humans.

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