PAPER

METABOLOMICS STUDY OF CEREAL GRAINS REVEALS THE DISCRIMINATIVE METABOLIC MARKERS ASSOCIATED WITH ANATOMICAL COMPARTMENTS

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ABSTRACT

This study used NMR-based metabolomics to compare the metabolic profile of different anatomical compartments of cereal grains i.e. bran and endosperm in order to gain further insights into their possible role in the beneficial health effects of whole grain products (WG). Polar water-soluble metabolites in 64 bran and endosperm, samples from rye and wheat were observed using 600 MHz NMR. Bran samples had higher contents of 12 metabolites than endosperm samples. A comparative approach revealed higher contents of azelaic acid and sebacic acid in bran than in endosperm. In a pilot study, the consumption of WG rye bread (485 g) caused NMR signals in 24h urine corresponding to azelaic acid. The relatively high abundance, anatomical specificity, pattern of metabolism, urinary excretion in human, antibacterial, and anticancer activities suggest further studying of azelaic acid when exposure to WG or beneficial effects of WG are investigated.

- Keywords: Metabolomics, NMR, wholegrain, rye, wheat, metabolites, biomarkers -

INTRODUCTION

Epidemiological studies have consistently shown that intake of whole grain (WG) can protect against the development of chronic diseases (SLAVIN *et al.* 2001), e.g. type 2 diabetes (T2D) (DE MUNTER *et al.* 2007, MURTAUGH *et al.* 2003), cardiovascular disease (CVD) (FLINT *et al.* 2009; JACOBS *et al.* 2007; MELLEN *et al.* 2008), and certain cancers (CHAN *et al.* 2007, HAAS *et al.* 2009; LARSSON *et al.* 2005; SCHATZKIN *et al.* 2008). The American Association of Cereal Chemists provided the following scientific and botanical definition of WG in 1999: "whole grain shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical component-the starchy endosperm, germ and bran-are present in the same relative proportion as they exist in the intact caryopsis" (International 1999). Whole grains are a rich source of fiber and bioactive compounds, including tocopherols, B vitamins, minerals, phenolic acids, and phytoestrogens (FARDET, 2010). It is generally recognized that the synergistic action of compounds mainly present in the bran and germ fractions of cereals accounts for the protective effects of WG products (FARDET, 2010; LIU, 2007). Recently, the composition and the diversity of bioactive compounds in different anatomical components of cereal grains have been systematically investigated in a large number of different species and varieties within the HEALTHGRAIN project (NYSTROM *et al.* 2008; SHEWRY *et al.* 2010; WARD *et al.* 2008). However, that project screened the cereal samples for bioactive compounds already documented in cereals using a targeted approach, and made no comparison of the untagged profile of the metabolites in different compartments of cereal grains.

Metabolomics is an untargeted approach in which the profile of metabolites in a biospecimen is measured using high-throughput analytical methods, e.g. NMR and mass spectrometry (LENZ and WILSON, 2007; NICHOLSON and WILSON 2003). We have used this approach previously to examine the complex physiological/biochemical effects of WG rye products in humans (MOAZZAMI *et al.* 2012; MOAZZAMI *et al.* 2014; MOAZZAMI *et al.* 2011). The aim of the present study was to search for the discriminative metabolites in the two major anatomical compartments in cereal grain, endosperm and bran, using an untargeted NMR-based metabolomics approach and with the emphasis on wheat and rye to gain further insights into their possible role in the beneficial health effects of whole grain products. NMR analysis can potentially provide characteristic structural data, which can be used for elucidation and eventual identification of unknown compounds found to discriminate between the metabolic profiles of bran and endosperm in cereals.

MATERIALS AND METHODS

Serial sample collection and extraction

A total of 64 cereal samples, comprising 18 wheat endosperm, 24 wheat bran, 8 rye endosperm, and 14 rye bran were obtained from the HEALTHGRAIN (WARD *et al.* 2008) project or from a local market. The endosperm and bran samples originated from HEALTHGRAIN projects were from the same grain sample material and therefore were matched (Wheat samples n = 18; and rye samples n = 8). The HEALTHGRAIN project rye varieties (and populations) included potugaise-3, potugaise-6, Haute Loire, Grandrieu, Nikita, Rekrut, Dankowskie-Zlote, and Lovaszpatonai-1. The details about rye varieties are given in NYSTROM *et al.* (2008). The HEALTHGRAIN project wheat varieties included Disponent, Herzog, Tommi, Campari, Tremie, San Pastore, Gloria, Spartanka, Avalon, Claire, Malacca, Maris Huntsman, Rialto, Riband, Obriy, CF99105, Chinese-Spring, and Cadenza. The details about wheat varieties are given by SHEWRY *et al.* (2010). All rye and wheat varieties were grown in the field at Martonvasar, Hungary, in 2005. Full details of the site including soil type, mineral composition, and weather condition has been given by SHEWRY *et al.* (2010).

All samples were milled, and 0.5 g milled material was extracted in 5 mL Milli-Q water for 18 h. The samples were then centrifuged (5 min-1500 g), and 2 mL supernatant was extracted, mixed with 8 mL ethanol and centrifuged (15 min-1,500 g) in order to precipitate the soluble viscose polymers. A 5 mL portion of the ethanol supernatant was dried using an evacuated centrifuge (Savant, SVC 100H, Savant Instrument INC, NJ) and dissolved in phosphate buffer (280 μL, 0.25 mol/L, pH 7.0), D2O (40 μL), and sodium-3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionate solution (TSP, 30 μL, 23.2 mmol/L) (Cambridge Isotope Laboratories, Andover, MA). The mixture was then used for 1H NMR analysis. An internal standard was added to the mixture in order to ensure semi-quantitative measurements of metabolites captured by 1H NMR. For 2D NMR analysis the mixture was freeze-dried and dissolved in D2O before analysis.

Human experiment and the preparation of urine sample for NMR analysis

In a pilot study, a male subject (age 35; BMI = 23.4) consumed refined wheat bread 485 g for 6 days (breakfast 2 portions, lunch 1 portion, dinner 1 portion). On day six, 24-hour urine was collected. On day seven, he substituted the 485 g refined wheat bread with 485 g of whole grain rye bread and the urine was collected for 24 hours. During the seven days of experiment, any other cereal products were avoided. The choice of consuming refined wheat bread *vs* whole grain rye bread was made to

replicate the condition of previous human interventions in which refined wheat bread was used as the control diet (MOAZZAMI *et al.* 2011; MOAZZAMI *et al.* 2012; BONDIA-PONS *et al.* 2013). The refined wheat bread was prepared from commercial refined wheat flour and whole grain rye bread was prepared from commercial whole grain rye flour. The whole trial was repeated twice, in two different times. This study complied with the Helsinki Declaration, as revised in 1983. The urine samples were kept in -80°C freezers before analysis. The urine samples (500 μL) were mixed with phosphate buffer (250 μL, 0.25 M, pH 7.0) containing 5 mmol/L sodium-3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionate (TSP) (Cambridge Isotope Laboratories, Andover, MA) as an internal standard. Resulting solutions were centrifuged to remove particulate matter. The supernatant was then transferred into 5-mm NMR tubes for 1H NMR analysis. For 2D-NMR analysis, 600 μL of the supernatant was freeze-dried and dissolved in 600 μL D_2O before 2D-NMR analysis.

NMR measurements and the identification of signals

The 1H NMR analyses (cereal extracts and human urine) were performed on a Bruker spectrometer operating at 600 MHz (Karlsruhe, Germany). 1H NMR spectra were obtained using zgesgp pulse sequence (Bruker Spectrospin Ltd.) at 25°C with 128 scans and 65,536 data points over a spectral width of 17942.58 Hz. Acquisition time was 1.82 s and relaxation delay was 4.0 s. The NMR signals which were found discriminating between different anatomical compartments were identified primarily using the NMR Suite 7.1 library (ChenomX Inc, Edmonton, Canada), Human Metabolome Data Base and Biological Magnetic Resonance Data Bank. In the event of multiplicity, the identity was confirmed with 2D NMR. In human experiment, the identity of phytochemical in the urine originating from the cereals in the diet was also confirmed using 2D-NMR. Phase-sensitive TOCSY and COSY with presaturation (2k × 512 experiments) were performed with 32 scans and a spectral width of 7195 Hz for both F1 and F2. The mixing time for TOCSY was 80 ms. HSQC was performed using 32 scans and a spectral width of 7211 Hz and 250002 Hz for proton and carbon, respectively. All cereal extracts and urine samples were reconstituted in D2O before 2D NMR analysis.

The 1H NMR spectra data (cereal extracts) were processed using Bruker Topspin 1.3 software and were Fourier-transformed after multiplication by a line broadening of 0.3 Hz and referenced to TSP at 0.0 ppm. Spectral phase and baseline were corrected manually. Each spectrum was integrated using Amix 3.7.3 (Bruker BioSpin GmbH, Rheinstetten) into 0.01 ppm integral regions (buckets) between 0.5-10 ppm, in which area between 4.60-5.18 ppm containing residual water was removed. Each spectral region was then normalized to the intensity of internal standard (TSP).

Statistical analysis

Principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were performed using SIMCA-P+ 12.0.1 software (UMETRICS, Umeå, Sweden) after centering and pareto-scaling of the data as previously described (MOAZZAMI *et al.* 2011). The presence of outliers was investigated using PCA-Hotelling T2 Ellipse (95% CI) and the normality of multivariate data was investigated using the normal probability plot of the PCA model. Variable influences on projection (VIP) values of the OPLS-DA model were used to determine the most important discriminative NMR bucket (signals). NMR buckets (signals) with VIP > 1 for which the corresponding jack-knife-based confidence intervals were not close to or including zero were considered discriminative. The significance of OPLS-DA model was tested using cross-validated ANOVA (CV-ANOVA), which assesses the reliability of OPLS models (CV-ANOVA $p<0.05$ means the OPLS-DA model is reliable) (ERIKSSON *et al.* 2008).

The absolute concentrations of metabolites with corresponding NMR signals that were found to be discriminative in OPLS-DA were calculated from the NMR spectra using the NMR Suite 7.1 profiler (ChenomX Inc, Edmonton, Canada) and internal standard after correction for overlapping signals. The absolute concentrations of the discriminative metabolites were further investigated using ANOVA in the case of normal distribution, and the Mann-Whitney test when the distribution was skewed (Anderson-Darling test, $p<0.05$).

RESULTS AND DISCUSSION

PCA model was fitted using NMR spectral data (buckets) obtained for the bran and endosperm extracts. Three outliers were identified and excluded from the data set based on PCA-Hotelling T2 Ellipse (95% CI). The first and the second component explained 67.4% and 18.1% of spectral variation (R2X) respectively (figure not shown). An OPLS-DA model was fitted including three predictive and six orthogonal components. The first, second, and third predictive components explained 61%, 15.2%, and 1.0% of spectral variation respectively (model parameter: R2Y=0.937; Q2Y=0.876; Cross-validated ANOVA p-value = 2.98 × 10-38) (Fig. 1).

The first component in each model basically separated the bran samples obtained from rye

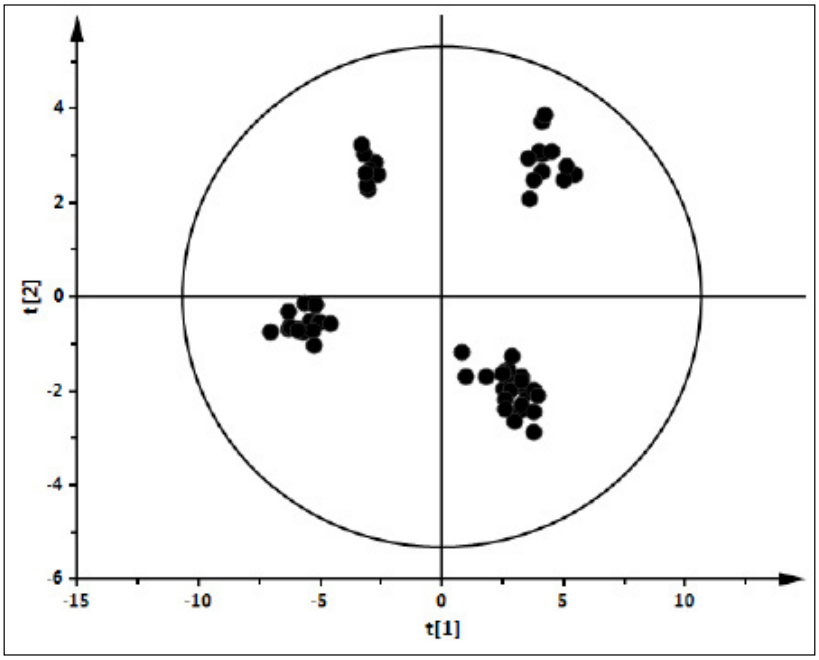

Fig. 1 - OPLS-DA separated different anatomical compartments of cereal grains based on their profile of metabolites measured using NMR. The model parameters were as follow: R2Y=0.937; Q2Y=0.876. Cross-validated ANOVA p-value = 2.98 × 10-38. Score t[1] (component 1) and score t[2] (component 2) are new variables summarizing the X-variables (the intensity of NMR signals corresponding to metabolites).

and wheat from the endosperm samples (Fig. 1, Table 1). The second component separated rye samples (both endosperm and bran samples) from wheat samples (Fig. 1; Table 2). Bran samples contained a higher content of 12 metabolites and four unknown signals than endosperm samples (Table 1), and their contents contributed to composing the first predictive component of the OPLS-DA model. The content of eight metabolites and five unknown signals changed along the second predictive component of the OPLS-DA model (Table 2) separating wheat samples i.e. both anatomical compartments from rye samples. The concentrations of all eight metabolites were found higher in wheat compared with rye. The metabolic signature of wheat and rye samples acquired from the local market did not deviate from those acquired from HEALTHGRAIN project as all sample tightly accumulated in their corresponding species-compartment cluster (Fig. 1). The absolute concentrations of metabolites that were found to differ between different anatomical compartments and species were calculated from NMR spectra and further investigated using ANOVA or the Mann-Whitney test. A total of 12 metabolites were found to differ between different species and different anatomical compartments in the same species, e.g. bran compared *vs* endosperm (Table 3; Fig. 2).

Table 1 - Discriminative metabolites along the first predictive component of the OPLS-DA model (n = 64)[1,2].

Metabolite	NMR signal (ppm)[3]	VIP (Confidence interval)[4]
Azelaic acid & sebacic acid[5]	1.304 ; 1.530 ; 2.179	2.6 (0.67) ; 1.6 (0.41) ; 1.6 (0.40)
Acetate	1.928	1.3 (0.26)
Alanine	1.494	1.1 (0.08)
Betaine	3.269	6.9 (0.59)
Choline	3.206; 4.085	4.4 (0.46); 2.3 (0.24)
Citrate	2.545	1.4 (0.13)
Isoleucine	0.942	1.0 (0.18)
Leucine	0.964	1.4 (0.24)
Malate	2.663	1.1 (0.31)
Maltose	3.280; 3.727; 3.997	1.4 (0.24); 4.4 (0.49)
Succinate	2.412	1.3 (0.16)
Unknown signals[6]	3.778; 4.054; 4.155; 4.020	2.3 (1.07); 2.3 (0.18); 2.2 (0.23); 4.6 (0.32)

[1]OPLS-DA Score scatter plot: the first component separated the bran samples (right) from the endosperm samples (left). All metabolites present in higher concentrations in bran. The model parameters for three predictive component fitted were as follow: R2Y=0.937; Q2Y=0.876. Cross-validated ANOVA p-value = 2.98 × 10-38; [2]Wheat endosperm (n = 18), wheat bran (n = 24), rye endosperm (n = 8), and rye bran (n = 14); [3]One NMR signal from the corresponding spectral bucket with the highest VIP values was reported when several buckets covered a distinct NMR signal; [4]NMR signals with VIP > 1 for which the corresponding jack-knife-based confidence intervals were not close to or including zero were considered discriminative; [5]Concentration equivalent of azelaic acid; [6]Unknow signals are located in sugar region.

Table 2 - Discriminative metabolites along the second predictive component of the OPLS-DA model (n = 64)[1,2].

Metabolite	Loading[3]	NMR signal (ppm)[4]	VIP (Confidence interval)[5]
Azelaic acid & sebacate[6]	-	1.304; 1.545; 2.179	3.3 (0.34); 2.1 (0.25); 1.9 (0.20)
Betaine	-	3.269; 3.904	5.2 (0.84); 3.1 (0.39)
Choline	-	3.206; 4.085	3.5 (0.42); 1.9 (0.15)
Citrate	-	2.545	1.0 (0.18)
Leucine	-	0.964	1.1 (0.16)
Maltose	-	3.278; 3.727; 5.257	6.1 (0.92); 3.2 (0.49); 1.0 (0.78)
Succinate	-	2.414	1.1 (0.23)
Unknown signals[7]	+	3.778; 4.054; 4.155; 3.915; 4.043	3.7 (0.75); 1.7 (0.25); 1.6 (0.21); 2.4 (0.46); 2.7 (0.32)

[1]OPLS-DA Score scatter plot: the second component separated the wheat samples (below) from the rye samples (above). The model parameters for three predictive component fitted were as follow: R2Y=0.937; Q2Y=0.876. Cross-validated ANOVA p-value = 2.98 × 10-38; [2]Wheat endosperm (n = 18), wheat bran (n = 24), rye endosperm (n = 8), and rye bran (n = 14); [3]Loadings: (+): higher concentration in rye samples. (-): higher concentration in wheat samples; [4]One NMR signal from the corresponding spectral bucket with the highest VIP values was reported when several buckets covered a distinct NMR signal; [5]NMR signals with VIP > 1 for which the corresponding jack-knife-based confidence intervals were not close to or including zero were considered discriminative; [6]Concentration equivalent of azelaic acid; [7]Unknow signals are located in sugar region.

Table 3 - Absolute concentrations of metabolites (μmol/g) found to be discriminative along the first and second predictive components[1].

	Concentration μmol/g (mean ± SD)			
Metabolite	1 : Rye endosperm	2 : Rye bran	3 : Wheat endosperm	4 : Wheat bran
Azelaic acid & sebacic acid	0.68 ± 0.21[a]	1.70 ± 0.27[a]	0.70 ± 0.16[a]	4.32 ± 1.25[b]
Acetate	1.19 ± 0.26[a]	3.98 ± 4.08[b]	0.73 ± 0.32[c]	2.70 ± 0.91[d]
Alanine	0.40 ± 0.07[a]	1.81 ± 0.62[b]	0.38 ± 0.13[a]	1.25 ± 0.47
Betaine	10.52 ± 2.80[a]	28.23 ± 6.77[b]	3.70 ± 2.13[c]	34.53 ± 9.79[d]
Choline	0.78 ± 0.13[a]	6.70 ± 1.09[b]	1.12 ± 0.25[c]	6.91 ± 1.18[d]
Citrate	0.55 ± 0.05[a]	5.25 ± 1.55[b]	0.62 ± 0.29[c]	4.93 ± 1.72[b]
Isoleucine	0.15 ± 0.03[a]	0.58 ± 0.21[b]	0.16 ± 0.03[a]	0.48 ± 0.13[b]
Leucine	0.37 ± 0.10[a]	1.52 ± 0.48[b]	0.35 ± 0.08[a]	1.40 ± 0.35[b]
Malate	6.04 ± 0.85[a]	6.22 ± 3.21[b]	7.43 ± 2.73[a]	10.24 ± 5.47[a]
Maltose	17.22 ± 0.182[a]	24.35 ± 8.46[b]	0.86 ± 1.52[c]	21.14 ± 7.27[b]
Succinate	0.54 ± 0.08[a]	1.34 ± 0.70[b]	0.43 ± 0.15[a]	1.43 ± 0.47[a]

[1]ANOVA was performed for betaine, succinate, citrate, alanine, leucine, isoleucine, and maltose. Mann-Whitney test was performed for malate, acetate, and choline. Metabolite means followed by different letters are significantly different (p<0.05). (Mean ± SD).

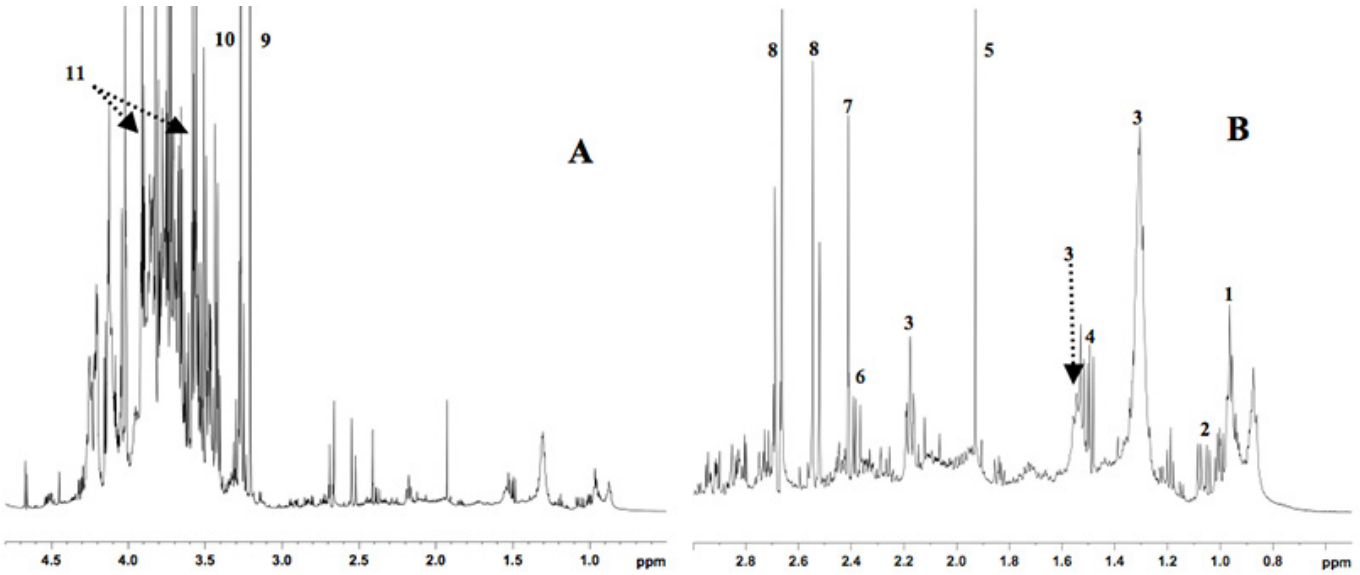

Fig. 2 - (A) A typical 1H NMR spectrum from rye bran polar extract and (B) magnified region 0.5 - 3.0 ppm. Annotated metabolites: Leucine (1), isoleucine (2), Azelaic acid and sebacic acid (3), alanine (4), acetate (5), malate (6), succinate (7), citrate (8), choline (9), betaine (10) and sugar region (11).

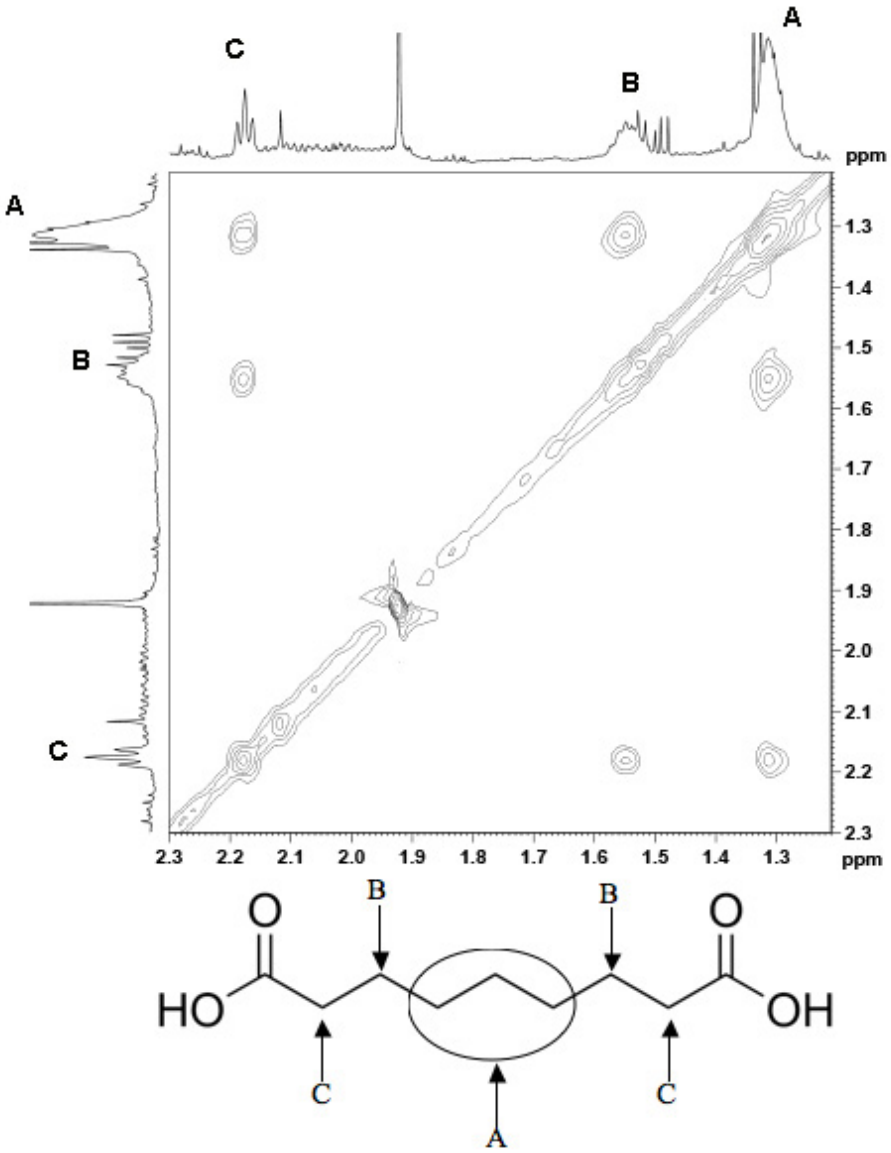

Fig. 3 - TOCSY NMR spectrum of typical rye bran polar extract presenting coupling between a multiple at 1.304 ppm (A), a multiple at 1.545 ppm (B), and a triplet at 2.179 ppm (C), which belong to azelaic acid and sebacic acid, and the assignment of the corresponding -CH2- groups on Azelaic acid molecule.

Multivariate statistical analysis (OPLS-DA model) also included signals discriminating between bran and endosperm, which appeared as a multiplet at 1.304 ppm, a multiplet at 1.545 ppm, and a triplet at 2.179 ppm (Fig. 2; Table 1). Using 2D NMR and spiking with authentic standard, these signals were assigned to two saturated, straight-chain dicarboxylic acids, namely azelaic acid (C9H16O4) and sebacic acid (C10H18O4). TOCSY NMR indicated that these signals were in the same spin system (Fig. 3). COSY NMR also confirmed coupling between (-CH2-) signals at 1.304 ppm and 1.545 ppm, and between (-CH2-) signals at 1.545 ppm and 2.179 ppm. No coupling to a CH3 group was observed on the TOCSY and COSY spectra, confirming dicarboxylic structure. The carbon chemical shifts were assigned from coupling to the corresponding hydrogen in HSQC NMR. There was a cross-peak between protons at 1.304 ppm and carbon at 31.484 ppm, between protons at 1.545 ppm and carbon at 28.926 ppm, and between protons at 2.179 ppm and carbon at 40.735 ppm. After applying new processing consisting of lining broadening (-1) and Gaussian broadening (0.6), the triplet at 2.179 appeared to be two overlapping triplets from azelaic acid and sebacic acid, the chemical shifts of which deviated from each other by 1.54 Hz. The identity of azelaic acid was further confirmed using authentic standard. The molar concentration of total dicarboxylic acids (azelaic + sebacic acid) was then calculated and expressed as equivalent to azelaic acid (Table 3).

No signal corresponding to azelaic acid was detectable in the 24h urine of a male subject af-

ter the consumption of refined wheat bread (485 g). However, after the consumption of whole grain rye bread (485 g), NMR signals corresponding to azelaic acid with similar coupling pattern as azelaic acid in the bran extract were detected in 24h urine (Fig. 4).

The concentrations of azelaic acid and sebacic acid were found to be higher in wheat and rye bran compared with the corresponding endosperm. In addition, wheat bran had higher dicarboxylic acid contents than rye bran (Table 3). To our knowledge this is the first study to report comparative differences in these dicarboxylic acids in different anatomical compartments of wheat and rye. In humans, 60 and 17 % of the administered dosage of azelaic acid and sebacic acid, respectively, are excreted in the urine within the first 12 hours (PASSI *et al.* 1983). It has been suggested that the dicarboxylic acids are to some extent subjected to β-oxidation, since dicarboxylic acids found in serum and urine possess 2, 4, or 6 carbon atoms shorter than the corresponding administered dicarboxylic acids (PASSI, NAZZARO-PORRO, PICARDO, MINGRONE and FASELLA 1983). Recently BONDIA-PONS *et al.* using metabolomics approach have shown that azelaic acid beside alkylresorcinols metabolites and enterolactone are the most discriminate metabolites, and are found in higher concentration urine after the intervention with whole grain rye bread compared with refined wheat bread (BONDIA-PONS *et al.* 2013). The present study showed that the main source of azelaic acid detected in the urine is bran, and that azelaic acid is found in both wheat and rye.

The relatively high abundance, anatomical specificity and localization in bran, pattern of metabolism, and previous findings regarding the identification of azelaic acids as discriminative metabolite in the urine after whole grain *vs* refined grain consumption (BONDIA-PONS *et al.* 2013) suggest that these dicarboxylic acids can be further investigated as biomarkers of exposure to WG products. Further studies are needed to investigate the correlation between azelaic acid concentration and alkylresorcinols concentration, which are validated biomarkers of WG intake (Ross 2012) in different biofluids after the intake of whole grain products. Little is known about the possible metabolic effects of the dicarboxylic acids in mammals. However, azelaic acid is known for its antibacterial (YU and VAN

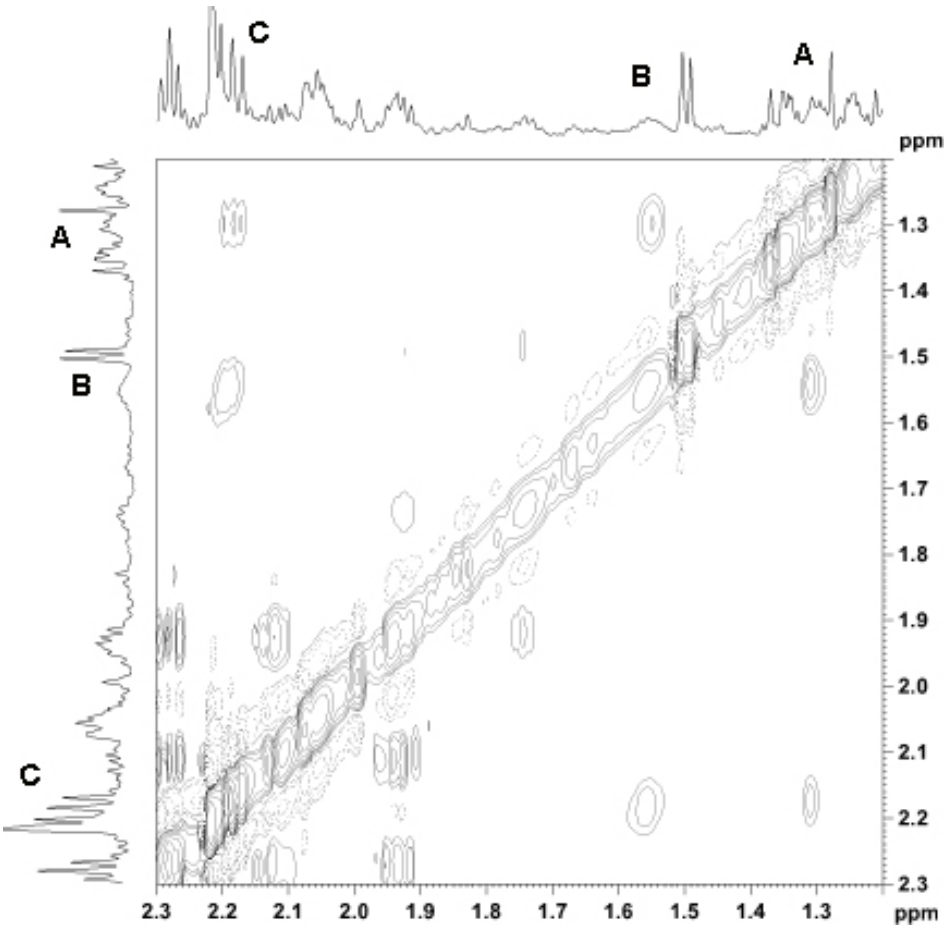

Fig. 4 - TOCSY NMR spectrum of 24 hour urine of a 35 year old man after the consumption of 485 g whole grain rye bread. The pattern of coupling between signals at 1.304 ppm (A), at 1.545 ppm (B), and at 2.179 ppm (C) was similar to that observed in rye bran polar extract.

SCOTT 2004) and anticancer activities (MANOSROI *et al.* 2007), which might contribute to the benefits attributed to WG intake.

Three amino acids i.e. alanine, isoleucine, and leucine, were also present in higher concentrations in bran than in endosperm. These amino acids gave rise to sharp and distinct NMR signals, distinguishing them from the amino acids in proteins, which possess broad NMR signals. In addition, higher contents of succinate, citrate, and malate were observed in bran than in endosperm. These metabolites are associated with the citric acid cycle and central carbon metabolism. The higher levels of amino acids and citric acid may indicate higher metabolic activities in bran compared with endosperm.

Consistent with previous studies, we observed higher levels of betaine and choline in bran than in endosperm (BRUCE *et al.* 2010). In addition, wheat bran had higher levels of betaine than rye bran. Circulating betaine is reported to be increased postprandially in animal models (BERTRAM *et al.* 2009; YDE *et al.* 2012) and in fasting plasma of humans after a 6-8 week intervention with WG rye products (MOAZZAMI *et al.* 2011; MOAZZAMI *et al.* 2012). Betaine acts as a methyl donor in the betaine-homocysteine methyl transferase reaction (BHMT-R), which converts homocysteine and betaine to methionine and N,N-dimethylglycine (DELGADO-REYES and GARROW 2005). Recently, in two separate human studies, we observed an increase in BHMT-R, as indicated by higher N,N-dimethylglycine levels (MOAZZAMI *et al.* 2011; MOAZZAMI *et al.* 2012) and lower homocysteine levels (MOAZZAMI *et al.* 2011), after a 6-8 week intervention with WG rye products compared with refined wheat products, which highlights the metabolic effects of betaine located in the bran of cereals.

In the present study, we used NMR-based metabolomics as an untargeted approach to gain further insights into the metabolic profile of different anatomical compartments of cereal grains. NMR profiling covers metabolites with μmol/g concentration. NMR also proved useful for identification and structural determination of unknown metabolites associated with different anatomical compartments.

ABBREVIATIONS

BHMT, betaine homocysteine methyl transferase; BHMT-R, betaine-homocysteine methyl transferase reaction; CVD, cardiovascular disease; OPLS-DA, orthogonal partial least squares-discriminant analysis; PCA, principal component analysis; T2D, type 2 diabetes; WG, whole grains.

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M.C. and A.A.M. wrote the manuscript. M.C. performed the NMR analysis and metabolomics data analysis. A.G. performed the optimization of extraction method. A.A.M. had primary responsibility for the final content. All authors read and approved the final manuscript and there is no conflict of interest.

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