JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

NMR-Based Metabolomics for Organic Farming Traceability of Early Potatoes

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Supporting Information

ABSTRACT: ^{1}H HRMAS-NMR spectroscopy was successfully used to determine the metabolic profiles of 78 tubers obtained from three early genotypes grown under organic and conventional management. The variation in total hydrogen, carbon, and nitrogen contents was also assessed. A PLS-DA multivariate statistical analysis provided good discrimination among the varieties and cropping systems (100% unknown samples placed in a cross-validation blind test), suggesting that this method is a powerful and rapid tool for tracing organic potatoes. As a result of the farming system, the nitrogen content decreased by 11−14% in organic tubers, whereas GABA and lysine accumulated in the organic tubers of all clones. Clear variations in primary metabolites are discussed to provide a better understanding of the metabolic pathway modifications resulting from agronomical practices.

KEYWORDS: *Solanum tuberosum L., HRMAS-NMR, PLS-DA, CHN*

INTRODUCTION

Metabolomics has been extensively applied to investigate how "genotype x environment" interactions influence the overall physiological and biochemical status of plants.[1,2] At present, no single technique can identify all metabolites within an organism (100 000−200 000[3]). ^{1}H NMR spectroscopy is one of the most promising nontargeted methods for analyzing a wide range of metabolites in biological systems because any chemical compound that contains protons produces signals. Some successful applications of this technique in food science include fingerprinting analysis (e.g., coffee[4] and wine[5]), biomarker identification,[6] and stress-related metabolic shifts.[7] Further improvement has been provided using high resolution magic angle spinning-NMR (HRMAS-NMR) spectroscopy, which does not require sample extraction and purification and is thus less invasive.[8] The potential influence of sample preparation on peak variation, often reported as a serious limitation in these types of studies, has consequently been removed.[9] It is also possible to achieve high-resolution spectra by removing the band extension due to dipolar interactions and magnetic susceptibility within the sample. However, the complex and large data sets obtained from "-omics" approaches greatly increase the requirements for data mining resources, that is, mathematical systems suitable for properly handling high-content information. This difficulty can be overcome by employing exploratory statistical tools, such as PCA (principal component analysis) and PLS-DA (partial least-squares discriminating analysis).

Many foods have been characterized by HRMAS-NMR coupled with a multivariate analysis strategy.[10] A similar metabolomics approach has also been widely used in potato (*Solanum tuberosum* L.): several glycoalkaloids,[11] solanidine, acetylleptidine,[12] diterpenoids,[13] and starch[14] were isolated, and the NMR signals were assigned. Studies of metabolic pathway modifications in genetically modified tubers have proposed adopting an integrated strategy.[15,16]

Accounting for a total production of more than 300 million metric tons worldwide, potatoes is the third most important crop after rice and wheat, as reported by the International Potato Center (CIP; Lima, Peru). The edible tuber contains mainly water (∼79%), carbohydrates (∼18%, predominantly starch), proteins (∼2%), lipids (0.1%), and organic acids (0.4−1%).[17] Health-related compounds, such as glycoalkaloids, which are known as antinutrients, carotenoids,[18] and many different polyphenols, such as anthocyanins,[19] are also present. Similar to many other foods, an increasing acreage of worldwide potato production is shifting toward organic agriculture because of its assumed relevant economic impact with social and political implications as well as because of the detailed European agricultural policies (2009/128/CE Directive). Although public opinion agrees that organic foods are healthier than conventional ones, little scientific evidence is currently available.[20] Environmental factors and genotype may greatly influence the yield and chemical composition of tubers,[21] but how agricultural techniques affect chemical composition, especially from a nutritional and toxicological perspective, is not fully understood.

Received: July 8, 2013
Revised: October 24, 2013
Accepted: October 25, 2013
Published: October 25, 2013

dx.doi.org/10.1021/jf402961m | J. Agric. Food Chem. 2013, 61, 11201−11211

In addition, although many potato-breeding companies are funding programs aimed at obtaining cultivars specifically suitable for organic agriculture, the varieties most widely cultivated under the current organic regime in Europe have not been specifically selected for this purpose.

One example is "Ditta", which is used in this study and is characterized by a good resistance to *Globodera rostochiensis* and late blight. Market demand prompted the commercialization of early and late-maturing varieties to make foods available almost year round, and the consumption of organic potatoes has been specifically extended to traditional early cycles, typically requiring high chemical inputs during farming. The present study analyzed three early genotypes using the strategy recently described by Ritota et al.[1,22] and Valentini et al.[10] Targeted approaches for assessing organic and conventional farming have previously been reported, using vitamin C and total protein or nitrate content as potential markers for assessing the cultivation system,[20,23,24] but the results remain controversial. Data about the impact of agricultural practices on tuber yield; quality; and nitrate, carbohydrate; and protein contents are also available.[25,26] However, a reliable method for evaluating the cultivation system under which tubers are produced has not yet been documented, and a metabolomics-based approach has not been presented.

This work aimed to elucidate some features of organic early potato tubers and to lay the groundwork for a model to clearly distinguish the farming system. This was performed by determining metabolic fingerprints using ^{1}H HRMAS-NMR spectroscopy coupled with multivariate analysis, as a method of testing the stability of the tuber composition for a given clone under different crop management systems.

MATERIALS AND METHODS

The experimental design and descriptions of the climatic data, soil, crop protection, fertilization treatments, diseases, and yield measurements have been previously described in Lombardo et al.[25] Disease-free "seed" tubers (not presprouted) of three tetraploid *S. tuberosum* L. clones were planted in January 2007: a firm-texture Austrian variety, Ditta (Agrico, NV, Netherlands), and two experimental CRA breeding clones, MN 1404 O5 (Pioneer x AC Belmont) and ISCI 4F88 (Spunta x MN 1326G2). They were grown at a density of 5.33 plants m^{-2} in a farm near Siracusa, Southern Italy (37°01′N, 15°12′38.9E, 30 m above sea level). Both organic (Regulation EEC No 2092/91 and 834/2007) and conventional management systems were applied, and factors affecting the comparison between the two farming systems were controlled, as suggested in Kumpulainen et al.[27] The field trial was planned as randomized block designs. Each plot (42×45 m^2) held 101 plants, and a replicated experimental design of three blocks for each variety and treatment was used. The same nitrogen regime (50 kg ha $^{-1}$ N in presowing and 83 kg ha $^{-1}$ N at the tuber induction) was applied, with synthetic fertilizer in the conventional system and with torrefied bones, meat meals, and feathers in the organic system. The farm had been managed under an organic system for 6 years before the trial was started, after a crop rotation with potato, zucchini, and wheat. At harvesting, 78 tubers, corresponding to 13 biological replicates of all groups (3 varieties × 2 cultivation systems), were randomly picked, washed, peeled, freeze-dried in liquid nitrogen, ground with a homogenizer (Stomacher 400 Pbi, Waring Blender) to a uniform consistency and stored at −80 °C until analysis. Three samples out of 13 for each group were analyzed separately for a blind cross-validation test.

Determination of Total Carbon, Hydrogen and Nitrogen (CHN). Sixty ground samples were loaded (1.5 g) into aluminum foil cups and analyzed using a LECO Truspec CHN analyzer. All setting parameters followed the "Organic Application Note" (LECO). Data analysis was performed using the NetOp software, and the results were expressed as the percentage of dry weight. A Student's *t* test for independent samples (Statistica 9 StatSoft, Inc.) was performed.

^{1}H HRMAS-NMR Analysis. NMR characterization was performed using the HRMAS-NMR approach with a Bruker Avance 400 spectrometer (BioSpin, Germany), operating at a proton frequency of 400.13 MHz and equipped with a HRMAS 4 mm dual channel probe head; the samples were spun at 7 kHz. The ^{1}H NMR spectra were referenced to the methyl group signal at δ 0.00 ppm of TSP, that is, 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt, and the ^{13}C NMR spectra were referenced to the TSP δ 0.00 ppm. The samples were prepared by inserting 5 mg of freeze-dried material into a 4-mm HRMAS rotor with a 50-μL spherical insert. Approximately 40 μL of 0.01 M D_2O phosphate buffer, pH 7.2, with 0.5% TSP was then added.

The ^{1}H HRMAS-NMR spectra were acquired using a water suppression pulse sequence, noesypr1D (Bruker library), 32 K data points over a 4807 Hz spectral width and adding 256 transients. A recycle delay of 2 s was used, the 90° pulse length was 5.0 μs, and the saturation of water residual signal was achieved by irradiating during the recycle delay at 4.70 ppm. Each FID (free induction decay) was Fourier transformed with 64 K data points and manually phased and base-lined. A line-broadening factor equal to 0.3 Hz was applied to the FID prior to FT. The ^{13}C-HRMAS-NMR spectra were acquired with the power-gated decoupling sequence, zgpg30 (Bruker library), using a 30° flip angle pulse of 5.0 μs. The experiments were performed using 64 K data points over a 22 123 Hz (∼220 ppm) spectral width by adding 64 K transients with a recycle delay of 3 s. Each spectrum was FT transformed with 128 K data points and manually phased and base-lined, and a line-broadening factor of 0.5 Hz was applied to the FID prior to FT. The ^{1}H−^{1}H TOCSY (total correlation spectroscopy) experiment was performed in the TPPI phase-sensitive mode, with a 4807-Hz spectral width in both dimensions, 100 ms of spin−lock time of 4500 Hz, 2 K data points in f_2 and 1 K increments in f_1, each with 32 scans. The ^{1}H−^{13}C HMQC spectra were acquired in the TPPI phase-sensitive mode, with a 4807-Hz spectral width in the f_2 dimension and a 15083-Hz spectral width in f_1. Two K data points in f_2 and 256 increments in f_1, each with 64 scans, were used.

Data Analysis and Statistics. All spectra were manually phased, baseline-corrected, and aligned using the ACD lab 8.0 software (Advanced Chemistry Development, Inc., Canada). Alignment of spectra was performed by referring to the TSP signal. The water residue signal and random noise regions were removed from the ^{1}H spectra by applying the dark region method available in the ACD lab 8.0 software. The NMR spectra were divided into spectral bins with widths of 0.04 ppm using the ACD bucketing method within the ACD lab 8.0 software, The area under each bin was integrated and normalized with respect to the sum of all integrals, which was set equal to 100. The resulting data matrices, consisting of rows that reflect observations/samples and columns that represent variables, were used as input variables for the statistical analysis.

An exploratory multivariate analysis using principal component analysis (PCA) was performed on the entire data set to reveal any inherent data clustering and to identify any possible outliers. A Student's *t* test was performed on the score matrix to determine which PC contributed significantly to the discrimination. In addition, the reduced and normalized NMR data set was imported into MATLAB (version 7.1, The Mathworks, Natick, MA). The data were mean-centered for each variable. Unit variance was scaled and analyzed using in-house routines and PLS_Toolbox (version 4.2, eigenvector Research, 2007).

Following PCA, a multivariate and supervised statistical analysis, PLS-DA, was performed. This technique visualizes the data structures by reducing the data set complexity to find predictive and latent variables through the application of a priori knowledge. NMR data were analyzed using the partial least-squares projections to latent structures−discriminant analysis.[28] The determination of the optimal number of latent variables, that is, LVs, was conducted by evaluating the variance captured and statistics, in particular, by considering parameters such as the REsidual Sum of Squares ($RESS^2$), the PREdicted Sum of Squares (PRESS), Q^2, and the cumulative Sum of Squares X and Y (SSQX and SSQY, respectively).[29] PRESS is a measurement of the predictive ability of the constructed model, whereas SSQ is related to the model's goodness of fit. Q^2 represents the default parameter used in the PLS-DA discriminations and focuses on how well the class label can be predicted

Table 1. Percentage of Total Nitrogen, Carbon, and Hydrogen of cv. Ditta, MN 1404 05, and ISCI F488[a]

	Ditta			MN 1404 05			ISCI F488		
%	conventional	organic		conventional	organic		conventional	organic	
carbon	41.21 ± 0.25	41.09 ± 0.23	*	41.07 ± 0.15	40.90 ± 0.29	*	40.94 ± 0.23	41.18 ± 0.15	*
hydrogen	5.95 ± 0.01	5.94 ± 0.03	*	6.05 ± 0.02	5.90 ± 0.04	*	5.94 ± 0.01	5.97 ± 0.01	*
nitrogen	1.73 ± 0.01	1.49 ± 0.01	**	1.84 ± 0.01	1.64 ± 0.01	**	2.03 ± 0.01	1.76 ± 0.01	**

[a]Data reported are the average of 10 replicates, and their variability was measured as standard deviation. Note: *$p > 0.01$; ** $p < 0.01$.

from new data.[30] By using the SSQ and PRESS parameters and the Q^2 criterion as a guideline ($Q^2 > 0.05$),[30] it is possible to evaluate the optimal number of components for a model with a good fitting, a high predictive ability, and reduced overfitting. The obtained model was validated using the Venetian blinds cross-validation method. An analysis of variance (ANOVA) was performed as a control test on the entire data set (MATLAB version 7.1).

RESULTS

Carbon, Hydrogen, and Nitrogen. Table 1 reports the means and standard deviations of the clones and farming systems and shows that the CHN trend of the three genotypes was very similar. The mean values of total carbon or hydrogen in the tubers did not reveal any significant differences between the different types of farming, and the coefficient of variation (CV) was <0.8% for all values. The mean values of total nitrogen content measured in the organic tubers of the three genotypes were significantly lower in comparison with tubers from conventional farming ($p < 0.01$).

Assignment of the ^{1}H HRMAS-NMR Spectrum. The assignment of important metabolites in the 1H-HRMAS-NMR spectrum is reported in Figure 1. Intense peaks are visible in the middle field region, within the range 3.5−4.5 ppm, typically attributed to carbohydrates, which usually account for more than 80% of the potato tuber dry weight. The assignments of ^{1}H and ^{13}C chemical shifts based on one-dimensional and two-dimensional TOCSY experiments, supported by chemical shifts reported in the literature,[16] allowed the unequivocal identification of several metabolites (Table 2). Only very small differences in chemical shifts from those reported in literature were found, and they were most likely due to different experimental conditions, such as the medium viscosity, magnetic susceptibility, and pH. Signals belonging to D-glucose were clearly distinguished in the spectra on the basis of the chemical shifts of the anomeric CH (Table 2) and most likely belong to amylose and amylopectin because in potato, most of the D-glucose units form linear and branched polysaccharides, amylose and amylopectin, respectively. Signals from organic acids, especially malate, citrate and fumarate, are visible, in agreement with their high levels in potato tubers, and range from 0.4% to 1% of fresh weight.[17] Valine, leucine, isoleucine, threonine, alanine, asparagine, glutamate, glutamine, cysteine, and tryptophan were identified. Aliphatic amino acids were found in the high field region from 0.5 to 3.5 ppm, and ^{1}H chemical shifts of aromatics, which could be assigned to tyrosine and tryptophan, were detected. In addition, peaks between 7.44 and 7.48 ppm with low intensities were assigned to high-molecular-weight aromatics, possibly polyphenols.

Classification of the Cultivars According to Agronomic Farming. PLS-DA models for the organic and conventional samples were constructed. Table 3 reports the values for the number of LVs, PRESS, Q^2, and SSQX and SSQY. Both obtained models are based on 2 LVs: LV1 explains 24% and 20% for conventional and organic samples, respectively, whereas LV2 accounts for 22% and 16% of the captured variance. According to Table 3, sample classification between cultivars is very good, especially considering the conventional cropping system. The mean centers of distribution are quite far apart, with LV2 capable of distinguishing MN 1404 05 from the other genotypes. The score plots, which graphically represent the sample groupings, clearly show that the discrimination between cultivars became less defined when they were grown under organic management (Figure 2).

Classification of the Cultivation Systems According to Variety. According to the CV, PCA applied to the collected ^{1}H HRMAS-NMR spectra was not significant in sample discrimination. The **T**-score matrix and the *t* values measured for the first 10 PCs indicate that none of the PCs were useful in sample classification (Supporting Information Table S1).

A PLS-DA classification model was successfully obtained to discriminate between the agronomical practices for each investigated genotype. A model with a good fitting, a high predictive ability, and reduced overfitting was obtained (Table 4). A very good classification was also gained, as shown in Figure 3 (A, B, and C), where the data set is projected on the space spanned by only the first latent variable. The organic samples were consistently associated with positive values of LV1, whereas the conventional ones lie along the negative part of the LV1 axis. The reliability of the constructed models was also evaluated: six samples for which only the cultivar was known were run as a blind test. They were all placed in the correct sets, supporting the robustness of the PLS-DA models.

The variable importance in projection (VIP) scores and the regression coefficients of the PLS-DA model were inspected to identify the most relevant metabolites contributing to the observed discrimination. The VIP score of a predictor expresses the share of the individual variable to the definition of the F-latent vector model. Because of the normalization used in the definition of the VIP, variables presenting values less than 1.5 were not considered to contribute significantly to the model. Table 5 lists the 15 most important buckets contributing to the classification and the relative *W* values, weight factors for the VIP, useful for indicating the plot containing higher quantities of the corresponding metabolite. When *W* is positive, the compound is more abundant in organic potatoes, whereas negative *W* values indicate that it is more abundant in conventional samples. For example, conventionally grown tubers contained larger amounts of simple and complex carbohydrates (β- and α-glucose, amylose). The *W* loading plots are shown in Figure 3 (D−F). In Ditta, proline and carbohydrates had the largest VIPs; their negative *W* value shows their abundance in conventional tubers. In MN 1404 O5, the largest VIP was observed for alanine, isoleucine, and lysine (their positive *W* indicates their presence in larger amounts in organic samples) as well as for valine, which is the most relevant metabolite in ISCI F488 sample classification. The high VIPs for lysine and y-aminobutyric (GABA) are common to all the genotypes, indicating their large incidence in discriminating the cultivation systems. All organically grown

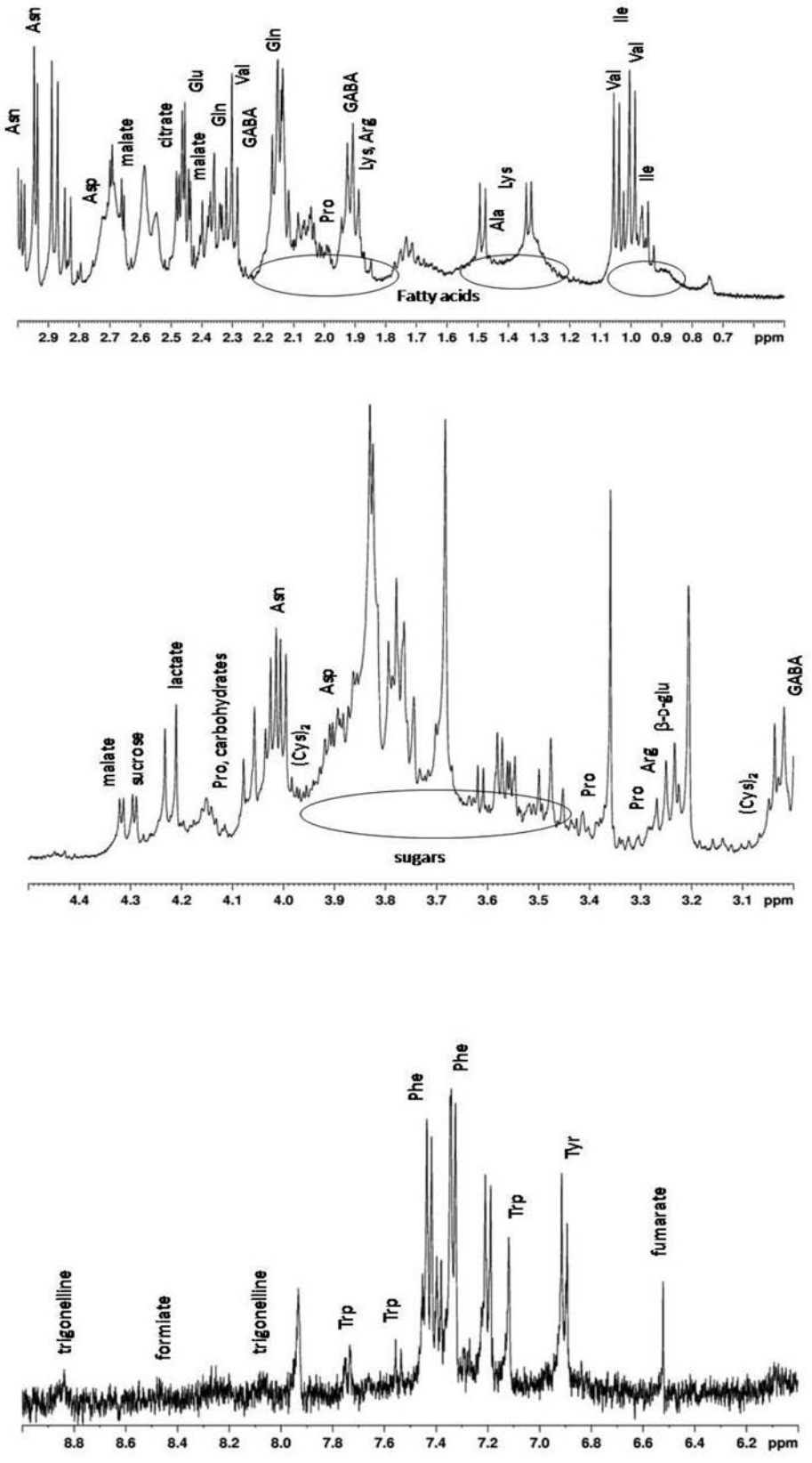

Figure 1. [1]H HRMAS-NMR spectrum of potato tubers' (cv. Ditta) reporting assignment of important metabolites and representative expansions of the most relevant regions: high, middle, and low field regions, from top to bottom.

clones also had a decreased concentration of citric acid and fumaric acid, as shown in Table 5, with a trend similar to that of hexoses. A variety-specific behavior of malic acid revealed a divergence between Ditta and ISCI F488.

Table 2. ^{1}H and ^{13}C Chemical Shifts of Assigned Metabolites Obtained from TOCSY Experiment[a]

compd	assignment	^{1}H (ppm)	multiplicity [J (Hz)]	^{13}C (ppm)
		Carbohydrates		
β-glucose (β-Glc)	CH-1	4.65	d [7.92]	
	CH-2	3.25	dd	
	CH-3	3.52		
	CH-4	3.39		
	CH-5	3.46		
	CH_2-6.6′	3.87; 3.72		
α-glucose (α-Glc)	CH-1	5.22	d [3.82]	
	CH-2	3.55		
	CH-3	3.71		
	CH-4	3.40		
	CH-5	3.84		
	CH_2-6.6′	3.83; 3.84		
		Organic Acids		
citric acid	α,γ-CH	2.57	dd [HMQC]	45.64
	α′,γ′-CH	2.68	dd [HMQC]	45.64
malic acid (Mal)	α-CH	4.31	dd [10.40; 3.02]	
	β-CH	2.68	dd [15.41; 3.00]	43.21
	β′-CH	2.39	dd [15.38 10.41]	43.21
formic acid	HCOOH	8.46	s	
fumaric acid	α,β-CH═CH	6.52	s	
lactic acid (Lac)	α-CH	4.12		69.01
	β-CH_3	1.33		20.75
piruvic acid	β-CH_3	2.36		
		Amino Acids		
alanine (Ala)	α-CH	3.77		51.39
	β-CH_3	1.48	d [7.24]	
arginine (Arg)	α-CH	3.77		
	β-CH_2	1.92		
	γ-CH_2	1.69		
	δ-CH_2	3.25	t [7.04]	
asparagine (Asn)	α-CH	4.01	dd	50.70
	β-CH	2.80	dd [14.10; 8.0]	34.3
	β′-CH	2.98	dd [14.08; 4.4]	34.3
aspartate (Asp)	α-CH	3.87		
	β,β′-CH_2	2.66		
γ-aminobutyrate acid (GABA)	α-CH_2	2.29	t [7.30]	34.00
	β-CH_2	1.91	m	
	γ-CH_2	3.02		
glutamate (Glu)	α-CH	3.77		54.10
	β-CH	2.05	m	27.01
	β′-CH	2.10		27.01
	γ-CH_2	2.36	m	33.65
glutamine (Gln)	α-CH	3.77		54.48
	β,β′-CH_2	2.15	m	26.13
	γ-CH	2.47	m	
isoleucine (Ile)	α-CH	3.63		60.08
	β-CH	1.98		
	γ-CH	1.26		
	γ′-CH	1.48		
	γ-CH_3	1.02	d [7.00]	
	δ-CH_3	0.95		
leucine (Leu)	α-CH	3.74		
	β-CH_2	1.75		
	γ-CH	1.69		
	δ-CH_3	0.96	d [6.25]	
	δ′-CH_3	0.94	d [6.25]	
lysine (Lys)	α-CH	3.78		
	β-CH_2	1.89		
	γ-CH_2	1.48		
	δ-CH_2	1.70		

dx.doi.org/10.1021/jf402961m | J. Agric. Food Chem. 2013, 61, 11201−11211

Table 2. continued

compd	assignment	^{1}H (ppm)	multiplicity [J (Hz)]	^{13}C (ppm)
		Amino Acids		
	ε-CH_2	3.01		
methionine (Met)	α-CH	3.81		
	β-CH_2	2.12		
phenylalanine (Phe)	*o*-CH	7.34		
	m-CH	7.41		
	p-CH	7.37		
proline	α-CH	4.14		
	β-CH	2.36		
	β'-CH	2.08		
	γ-CH_2	2.00		
	δ–δ'-CH	3.38		
serine (Ser)	α-CH	3.84		
	β,β'-CH_2	3.96		
threonine (Thr)	α-CH	3.61		
	β-CH	4.25		
	γ-CH_3	1.34		
tryptophan (Trp)	CH-4, ring	7.73	d [8.04]	
	CH-5, ring	7.20		
	CH-6, ring	7.28		
	CH-7, ring	7.56		
tyrosine (Tyr)	CH-2.6, ring	7.20	d [8.50]	
	CH-3.5, ring	6.91	d [8.50]	
valine (Val)	α-CH	3.62		
	β-CH	2.27	m	
	γ-CH_3	1.00	d [7.09]	
	γ'-CH_3	1.05	d [7.09]	
		Fatty Acids		
unsaturated fatty acids	CH_3	0.97		
	CH_2	2.03		
	CH═CH	5.11		
		Other Metabolites		
choline	α-CH_2	3.93		
	β-CH_3	3.43		
	N–CH_3	3.11	s	
solanine	CH_3	0.96		
	CH_2	1.03		
	CH_2	1.24		
	CH_2	1.29		
	CH_2	1.31		
	CH_2	1.43		
	CH_2	1.49		
hydroxycinnamic		7.80		
		7.45		
		7.10		
		6.90		
		6.05		
cytidine	N–CH	7.73	d [7.36]	
	NH_2–C–CH	6.12		
	O–CH–N	5.88		
betaine	N–$(CH_3)_3$	3.89		
	N–CH_2	3.26		
ethanol	CH_3	1.18	t [7.00]	16.90
	CH_2	3.65	q	57.88
methanol	CH_3	3.36	s	49.29
N,*N*-dimethylformamide	CO–H	7.91	s	
sterols		0.73	b	10.43

[a] ^{1}H and ^{13}C chemical shifts refer to TSP signal; in both cases, δ = 0.00 ppm.

We also observed many different amino acids: arginine, glutamine, alanine, isoleucine, valine, tryptophan, leucine, glutamate, tyrosine, aspartate, and cysteine. Their relative composition and accumulation largely changed in response to

Table 3. Variance-Captured and Statistics-Obtained Values (SSQ, PRESS, Q^2) of PLS-DA Model for the First Five Latent Variables (LVs) for Samples Cropped in Conventional and Organic Regime

	conventional				organic			
LV	SSQX	SSQY	PRESS^2	Q^2	SSQX	SSQY	PRESS^2	Q^2
1	**0.2323**	**0.3957**	**14.4972**	**0.5168**	**0.1963**	**0.3732**	**16.2181**	**0.4594**
2	**0.3304**	**0.7996**	**7.2927**	**0.3966**	**0.3423**	**0.7470**	**9.0175**	**0.2807**
3	0.417	0.8631	8.8538	−1.2089	0.3910	0.8697	9.8925	−0.9553
4	0.5011	0.9012	9.1277	−2.3342	0.4438	0.9433	7.9279	−2.0417
5	0.5367	0.9414	8.6513	−3.3795	0.5053	0.9671	7.1026	−5.2582

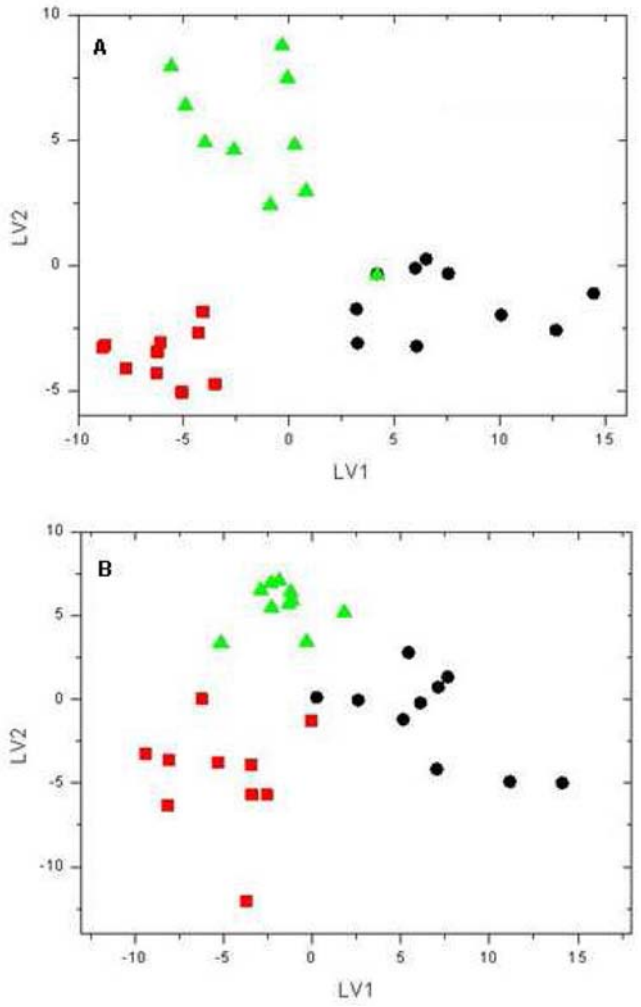

Figure 2. PLS-DA score plots of potato tubers cv. Ditta (circles), MN 1404 05 (triangles), and ISCI F488 (squares) grown under conventional (A) and organic (B) systems.

the crop management system and cultivar, with the exception of lysine, which was always highly concentrated in organic tubers. It was also possible to assign peaks relative to fatty acids accumulating differentially in all the clones. No clear indication for the differential accumulation of interesting health-valuable compounds, such as glycoalkaloids or ascorbic acid, was obtained. Traces of hydroxycinnamic acid and intermediates of the shikimic acid biosynthetic pathway and phenylpropanoid metabolism were identified, but their content was lower in organic potatoes (data not shown).

Table 4. Variance-Captured and Statistics-Obtained Values (SSQ, PRESS, Q^2) of PLS-DA Model for the First Five Latent Variables (LVs) Used to Classify the Cropping Regime

LV	SSQX	SSQY	PRESS^2	Q^2
		Ditta		
1	**0.1174**	**0.7179**	**10.661**	**0.4669**
2	0.2376	0.8888	9.5179	−2.3743
3	0.3282	0.9647	8.1561	−6.3362
4	0.4129	0.9877	7.7076	−20.8643
5	0.5303	0.9945	7.306	−58.3539
		MN 1404 05		
1	**0.1625**	**0.7539**	**6.9537**	**0.6523**
2	0.2444	0.8944	7.6897	−2.1244
3	0.3169	0.950	9.5528	−8.0503
4	0.4008	0.9809	10.9137	−20.8215
5	0.5526	0.9905	12.0483	−62.2298
		ISCI F488		
1	**0.1957**	**0.8187**	**5.4202**	**0.729**
2	0.3336	0.9314	3.7643	−1.0764
3	0.3922	0.9773	2.9124	−3.2446
4	0.4419	0.9911	3.1642	−12.9428
5	0.5325	0.9952	3.2031	−34.8823

DISCUSSION

^{1}H HRMAS-NMR combined with PLS-DA analysis was successfully applied for classifying cultivars according to the cultivation system based on the tubers' chemical composition. The Q^2 values, reported in Table 3, revealed that the tubers' metabolomes were dependent on the crop management strategies and on genetic determinants. The resulting score plots show that the investigated genotypes under study, not selected for organic cultivation, separate better under traditional than under organic management (Figure 2); this result suggests a stronger adaptation to the former system, under which the tubers fully express their varietal qualitative distinctness. The majority of clones currently cultivated under the organic regime were not specifically selected for this purpose. This preliminary study shows the suitability of the NMR-based metabolomics approach for differentiating tuber varieties cultivated under the same environmental conditions and as a possible tool for discriminating between different potato clones. In addition, these results highlighted the importance of breeding programs dedicated to the development of cultivars specifically suitable for organic agriculture.

Intriguingly, a validated statistical model attributing an unknown sample to the correct farming type irrespective of the variety was built in the present study. The predictive ability was good for all varieties, confirming the ability of this method to discriminate between different crop managements systems (Table 4). This provides the basis for further analysis utilizing

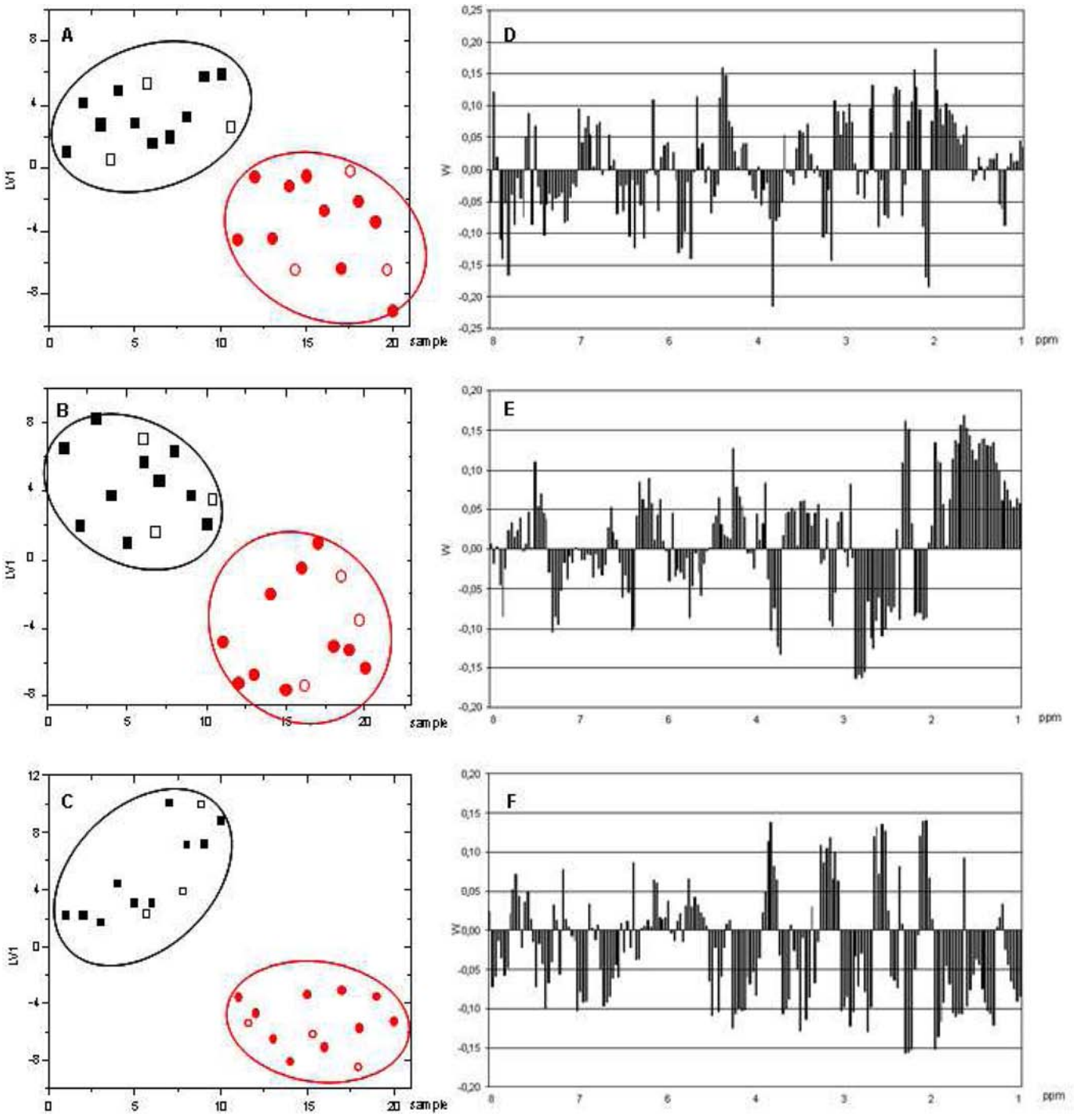

Figure 3. PLSDA and W score plots (left and right columns, respectively) for classification according to the farming system for Ditta, MN (top), 1404 05 (middle), and ISCI F488 (bottom). Circles identify samples grown under conventional and squares under organic system. Empty symbols represent samples used for blind validation test.

a higher number of samples to obtain a robust model capable of identifying the farming system with high confidence. This study confirms that a whole-metabolome analysis should be preferred[23,25] to a single biochemical marker, which cannot be fully exhaustive and replicable because of the strong "genotype x environment" interaction.

This study also highlights the potential of ^{1}H HRMAS-NMR for characterizing the metabolite profiles of mature organically and conventionally produced potato tubers and for determining the impact of the farming method. Fertilizers, pesticides, location, soil type, and year can all affect the chemical composition of the tuber; moreover, cultivation system differences can affect plant metabolism and mineral absorption. As expected, our study showed that the metabolome of the tuber is strongly influenced, and primary metabolites were the most modulated (Table 5). The tubers analyzed were clearly characterized by the observed shifts in their metabolic profiles, and they exhibited similar trends in metabolite variation and a strong influence of variety. Our findings show that GABA and lysine, which are commonly present at high levels in potato tubers, were the metabolites most sensitive to crop management among the investigated genotypes, whereas the other metabolites

Table 5. Metabolites Relevant for Discrimination between Samples Cropped with Organic and Conventional Systems[a]

Ditta				MN 1404 05				ISCI F488			
ppm	VIP	metabolite	*W*	ppm	VIP	metabolite	*W*	ppm	VIP	metabolite	*W*
4.12	8.4295	Pro, carbohydrates	−0.2152	1.48	5.1890	Ala, Ile, Lys	0.1689	2.28	3.6521	Val	0.1562
1.92	6.4876	Arg, Lys, GABA	0.1888	2.96	4.8888	Asn	−0.1639	2.24	3.6244	unknown	0.1556
2.00	6.1499	Pro	−0.1838	2.88	4.8004	Asn	−0.1624	2.20	3.4325	Gln	0.1514
2.04	5.1829	fatty acids	−0.1688	2.28	4.7554	Val, GABA	0.1616	1.88	3.4261	Lys GABA	0.1513
3.32	3.7051	Pro	−0.1427	2.92	4.6388	Asn	−0.1596	2.00	2.9122	Ile	−0.1395
2.16	3.0725	Gln	0.1299	2.84	4.3479	Asp, Asn	−0.1546	2.04	2.8630	Ile, Glu, Fatty acid	−0.1383
2.44	3.0558	Gln, malic acid	0.1296	1.44	4.2391	Ile	0.1526	4.12	2.8154	β-FF	−0.1371
1.88	2.8268	Arg, Lys, GABA	0.1246	1.24	3.4792	unknown	0.1383	1.84	2.7647	unknown	0.1359
6.54	2.8198	fumaric acid	−0.1245	1.88	3.2823	Lys, GABA	0.1343	2.60	2.7424	citric acid	−0.1353
2.40	2.7830	Gln, malic acid	0.1237	4.00	3.2413	$(CyS)_2$, Asn	−0.1335	2.68	2.5945	citric, malic acids	−0.1316
8.46	2.6649	formic acid	0.1210	1.28	3.2298	fatty acids	0.1332	2.80	2.5281	unknown	0.1299
2.48	2.5441	Gln	0.1182	2.72	2.8746	Asp	−0.1257	3.72	2.4845	Lys, Leu	0.1288
3.28	2.1372	Arg	0.1084	4.04	2.7653	carbohydrates	−0.1233	2.56	2.4008	citric acid	−0.1266
3.44	2.0534	β-Glc	−0.1062	1.32	2.3082	fatty acids, Thr	0.1126	4.64	2.3225	β-Glc	−0.1246
3.08	1.9271	$(Cys)_2$	0.1029	2.76	2.3024	Asp	−0.1125	3.04	2.2603	GABA, Lys	0.1229

[a]For simplicity we report the first 15 signals. Note: VIP (Variable Importance in Projection) represents the contribution of the individual variable to the definition of the F-latent vector model. VIP < 1.5 has not been considered to contribute significantly to the model. W is the weight factor of VIP.

were genotype-dependent. Proline accumulated only in organic tubers of cv. Ditta, most likely because of lower potassium availability in organic soil.[31] On the other hand, both GABA and lysine accumulated significantly more in the organic tubers of all three clones, with high VIP values.

GABA is most likely the most interesting because it plays a role in complex interconnections of several pathways linking amino acid metabolism to TCA (GABA shunt). Fait et al.[32] proposed considering the GABA shunt as an integral part of TCA and the conversion of glutamate into GABA as a primary response to changing environmental conditions, as well as a possible index of a decreased respiratory rate. However, a possible key role of GABA in the observed carbon and nitrogen partitioning is hypothetical because all conventional tubers also showed a low level of lysine and a high level of sugars. Although a direct role of lysine as a regulatory signal has never been demonstrated in plants, Stepansky et al.[33] suggested that lysine catabolism is induced when sugar levels are too high to inhibit the conversion of amino acids into sugars.

Table 5 also shows a notable variation in organic acid concentrations. We can rule out the idea that time, storage conditions, and herbicides, which were not used for weed control in our study, could have influenced organic acid concentrations.[34] The increase in fumaric acid in conventional tubers is consistent with previous studies reporting an accumulation of fumaric acid under higher nitrate availability.[35] Organically grown Ditta tubers also showed an increase in malic acid accumulation, a reduction in starch, and a decrease in dry matter and total yield, all of which are parameters commercially important for tubers.[25] Studies on transgenic tomato fruit have shown that a higher malate content is associated with lower levels of starch and carbohydrates,[36] providing a possible explanation for our results. The final outcome could be that, depending on the variety considered, changes in malic acid levels could influence starch accumulation and yield in a similar way.

The consumer perception is that organic foods are healthier and safer than conventional foods.[37] However, the evidence supporting their effective higher value is currently inconclusive and needs to be corroborated.[38] Previous studies have reported an increase in vitamin C levels in response to organic farming, but this evidence was not always replicable in different growing seasons.[23,25,37] Our results did not show any significant difference in vitamin C content between the two cultivation systems, although a recent analysis with ^{1}H HRMAS-NMR found that vitamin C could discriminate between the geographic origins of sweet pepper[1]. An explanation could be that its content was lower than the detection limit of this technique, usually between 1 and 0.01 mM.

According to our results, the impact of harmful or nutritional compounds in organic tubers was negligible; thus, there is no strong evidence for the health value of products obtained by organic managements. Further research on this topic is required. However, our results suggest some interesting considerations. The presence of acrylamide in potato-based products is frequently debated and is strictly correlated with the presence of asparagine and reducing sugars due to the Maillard reaction occurring during heat processing. A strong decrease in the levels of asparagine and carbohydrates was observed only in organic tubers of MN 1404 05, and the former was one of the most discriminating metabolites for the cultivation system for this clone. The obtained results confirm the importance of potato variety selection for reducing the formation of acrylamide[39] and putatively identify MN 1404 05 as the genotype for further investigation. Surprisingly, our analysis also detected formic acid in organic tubers of Ditta (Table 5). This acid is usually employed in hive defense from bee varroa.[40] We therefore believe that it was present in bee products used in organic farming (bee glue), as previously reported, although no analytical evidence supports this hypothesis.

CHN analysis revealed a lower N content in organic tubers, most likely related to the use of feathers, torrefied bones and meat meals rather than synthetic fertilizers, and a similar total carbon content, in agreement with Maggio et al.[21] and Hoefkens et al. [24] (Table 1). According to the theory of carbon/nitrogen balance, we would expect carbohydrate accumulation under organic farming due to a compensation for lower N absorption. However, our experimental results did not correspond to this expectation. A possible explanation is that all organically grown potato plants exhibited to some extent the symptoms of *Phytophtora infestans* infection, with a high susceptibility at the 15th week after planting (1, in the Malcomson 1−9 scale; data not shown). Plants might actually suffer from reduced photo-

synthetic CO_2 assimilation under abiotic or biotic stress, with decreased translocation of sucrose in tubers. In addition, the carbon compounds might be preferentially partitioned in differentiation-related processes, according to the growth-differentiation balance hypothesis. Irrespective of the reasons behind the decrease in carbohydrates in organic potatoes, their content is regarded as a commercially important quality trait of tuber, although relatively little is known regarding the physiological importance of carbohydrates for tuber metabolism.

In conclusion, this study represents, to our knowledge, the first investigation involving a wide-range metabolic profiling in potatoes conducted using ^{1}H HRMAS-NMR. We provide, for the first time, the basis for constructing a validated and robust experimental model that can efficiently distinguish potato tubers according to the cultivation system. ^{1}H HRMAS-NMR can be used as a powerful, reliable and rapid method for discriminating between both varieties and cultivation systems, bypassing time-consuming and expensive extraction and purification steps and resulting in a more comprehensive and replicable strategy in comparison with the use of biochemical markers.

■ ASSOCIATED CONTENT

Supporting Information

T matrix obtained by PCA model for the first 10 PCs built for Ditta, MN 1404 05, and ISCI F488, from top to bottom is listed in Table S1 (confidence range of 0.05); *t* values, cumulative variance, and total variance explained are also reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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