JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Metabolic Discrimination of Mango Juice from Various Cultivars by Band-Selective NMR Spectroscopy

Masanori Koda, Kazuo Furihata, Feifei Wei, Takuya Miyakawa, and Masaru Tanokura*

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

ABSTRACT: NMR-based metabolic analysis of foods has been widely applied in food science. In this study, we performed discrimination of five different mango cultivars, Awin, Carabao, Keitt, Kent, and Nam Dok Mai, using metabolic analysis with band-selective excitation NMR spectra. A combination of unsupervised principal component analysis (PCA) with low-field region ^{1}H NMR spectra obtained by band-selective excitation provided a good discriminant model of the five mango cultivars. Using F_2-selective 2D NMR spectra, we also identified various minor components in the mango juice. Signal assignment of the minor components facilitated the interpretation of the loading plot, and it was found that arginine, histidine, phenylalanine, glutamine, shikimic acid, and trigonelline were important for classification of the five mango cultivars.

KEYWORDS: *NMR, selective excitation, metabolic profiling, nondestructive analysis, mango*

INTRODUCTION

Verification of food authenticity (e.g., verification of the country of origin, botanical origin, and adulteration of foods) is important for food safety and has become more important due to the globalization of markets. Therefore, numerous analytical methods have been proposed for and applied to the determination of food authenticity. The most widely used methods for food authenticity are DNA analysis,[1] trace element analysis,[2] stable isotope analysis,[3] and metabolic analysis.[4−6] Metabolic analysis can be applied to various foodstuffs, including processed foods, and has also been applied to the study of food processing or food microbiology, as well as to food science in general.[7] For metabolic analysis, various methods of component analysis are employed, including gas chromatography (GC), liquid chromatography−mass spectrometry (LC-MS), capillary electrophoresis−time-of-flight mass spectrometry (CE-TOF-MS), and nuclear magnetic resonance (NMR) spectroscopy. Among them, NMR has two primary advantages: (i) the sample preparation is simple and easy, which minimizes the change in the chemical composition and the loss of minor components during sample preparation; and (ii) various organic chemical species (e.g., sugars, lipids, amino acids, and organic acids) are detected simultaneously. Therefore, many NMR-based metabolic analyses have been performed.[8−10] In addition, metabolic profiling with ^{1}H NMR spectra has potential for efficient screening in high-throughput.

Minor components in food frequently play an important role in the food characterization. For example, minor components in olive oil are used for identifying geographical origin;[11] minor components in honey are used for identifying botanical origin;[12,13] and minor sugars are used for detecting adulteration of syrup.[14] However, many foodstuffs contain dominant major components (e.g., sugars, lipids, ethanol, and acetic acid). The signals of these major components frequently obscure those of the minor components, or make it difficult to detect the minor components due to the problem of dynamic range. Therefore, solvent extraction or separation is frequently performed to remove major components from the sample preparation. On the other hand, band-selective excitation is one of the most efficient methods to detect minor components sensitively without separation. Band-selective excitation for the analysis of minor components in foods has been performed using selective 1D NMR spectroscopy, and the effectiveness of this technique has been demonstrated.[15,16] However, conventional F_1-selective 2D NMR spectroscopy[17] frequently generates undesired t_1 noises from major components, which obscure the signals in the 2D NMR spectra and limit the receiver gain, making the analysis of minor components difficult. Therefore, we recently developed F_2-selective two-dimensional (2D) NMR spectroscopy to obtain high-quality 2D spectra of minor components in foods without separation for food analysis.[18] F_2-selective 2D NMR spectroscopy, which excites along the F_2-axis, can suppress undesired t_1 noises and provide high-quality 2D NMR spectra.

In this study, we measured band-selective NMR spectra of minor components in the mango juices from five cultivars (Awin, Carabao, Keitte, Kent, and Nam Dok Mai) to discriminate them. Among the many mango fruits and juices that are imported to Japan, these five mango cultivars are accessible, and cultivar Carabao is less expensive than the other cultivars. When analyzing a whole fruit, it is relatively easy to discriminate what the cultivar is. In juice, on the other hand, it can be difficult to discriminate what cultivar was used; furthermore, juice has the possibility of adulteration. Therefore, a discrimination method of mango cultivars is important for assessing food authenticity. Since all mango juices contain mainly water and sugars, it seems that the minor components are most important for the discrimination of the cultivars. Low-field region (6−10 ppm) ^{1}H NMR spectra have not been used

Received: October 12, 2011
Revised: January 14, 2012
Accepted: January 15, 2012
Published: January 16, 2012

dx.doi.org/10.1021/jf2041438 | J. Agric.Food Chem. 2012, 60, 1158−1166

ordinarily very much due to the weakness of the signal intensity, but those obtained by the band-selective excitation are expected to provide useful information about minor components in the mango juice rapidly. As a result, the band-selective excitation provided high-quality ^{1}H NMR spectra of minor components in the low-field region, and the combination of the low-field region NMR spectra and an unsupervised principal component analysis (PCA) provided a good classification model for the five mango cultivars. F_2-selective 2D NMR spectra provided high-quality signals of minor components containing exchangeable amine protons, and more than 30 different metabolites were identified, facilitating interpretation of the PCA.

MATERIALS AND METHODS

Materials and Sample Preparation. Five cultivars of 11 mango fruits, for a total of 55 fruits, were used in this study. Fruits of the mango cultivars Awin (origin Taiwan), Carabao (origin Philippines), Keitt (origin USA, California), Kent (origin Mexico), and Nam Dok Mai (origin Thailand) were obtained from a local market. Because all the fruits did not ripen at the time of purchase, the fruits were left to ripen sufficiently at room temperature. After ripening (based on smell and appearance), each mango was ground into a pulp and then centrifuged at 26 000 × *g* for 10 min at 4 °C. The supernatant was used as mango juice. The mango juices were stored at −20 °C until NMR analysis.

For the signal assignment, D_2O (99.9%, 70 μL; Shoko Co., Ltd., Tokyo, Japan) was added to the mango juice (630 μL), and then the sample (700 μL) was mixed and transferred to a 5 mm NMR tube. A very small amount of 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was added to the sample as a chemical shift reference.

For the multivariate analysis, the pH value was adjusted using oxalate buffer to minimize chemical shift changes. The sample preparation was as follows: the mango juice (5 mL) was transferred into a 15 mL centrifuge tube, and the pH value was adjusted to ∼4.2 using 6 M HCl or 4 M NaOH. Oxalate buffer (300 mM, pH 4.20, 210 μL) and D_2O (containing 0.25 mM DSS and 0.25 mM histidine as chemical shift references, 70 μL) were then added to the mango juice (420 μL). The sample (700 μL) was mixed and transferred to a 5 mm NMR tube.

In determining a rapid and accurate pH adjustment method for practical use, we found that pH adjustment with buffer only was favorable for dealing with many samples, but a 7-fold greater volume of buffer than sample was needed to make an accurate pH adjustment; this volume made it difficult to detect minor components. On the other hand, accurate pH adjustment using HCl or NaOH was time-consuming. Consequently, the most efficient pH adjustment method was a combination of rough pH adjustment with HCl or NaOH and subsequent addition of the oxalate buffer.

NMR Spectroscopy. All NMR spectra were obtained at 20 °C on a Varian Unity INOVA-500 spectrometer. The water signal was suppressed by a presaturation method.

The selective excitation was achieved using a double pulse field gradient spin echo (DPFGSE)[19] approach and a refocusing band-selective 180° pulse with uniform response and phase (Re-Burp).[20] The pulse lengths of Re-Burp covering 6.0−10.5 ppm, 6.3−10.5 ppm, and −0.2 to 3.1 ppm were 2.0 ($\gamma H_1/2\pi$ = 3.2 kHz), 2.1 ($\gamma H_1/2\pi$ = 3.1 kHz), and 2.9 ($\gamma H_1/2\pi$ = 2.4 kHz) ms, respectively. The F_2-selective 2D NMR spectra were observed according to the previously described method,[17] with the excitation along the F_2-axis. The DPFGSE was incorporated just before free induction decay (FID).

The acquisition parameters of the ^{1}H NMR spectra were as follows: spectral width, 8 000 Hz; number of data points, 32 k; acquisition time, 2.048 s; and delay time, 2.0 s. For multivariate analysis, the receiver gain was set at 16 dB (nonselective excitation) or 52 dB (selective excitation), and the number of scans was 128 (nonselective excitation) or 256 (selective excitation), respectively. The acquisition parameters of total correlation spectroscopy (TOCSY) were as follows: spectral width, 6 000 Hz; number of t_2 data points, 2 048; number of t_1 data points, 512; number of scans, 16; acquisition time, 0.341 s; delay time, 2.0 s; field strength of MLEV-17 spin-lock pulse, 7.1 kHz; length of trim pulse, 2 ms; and mixing time, 80 ms. The acquisition parameters of double-quantum filtered correlation spectroscopy (DQF-COSY) were as follows: spectral width, 6 000 Hz; number of t_2 data points, 2 048; number of t_1 data points, 512; number of scans, 16; acquisition time, 0.341 s; and delay time, 2.0 s. The acquisition parameters of nuclear Overhauser effect spectroscopy (NOESY) were as follows: spectral width, 6 000 Hz; number of t_2 data points, 2 048; number of t_1 data points, 512; number of scans, 16; acquisition time, 0.341 s; delay time, 2.0 s; and mixing time, 800 ms. The acquisition parameters of ^{1}H−^{13}C heteronuclear single-quantum correlation spectroscopy (HSQC) were as follows: spectral width, 6 000 Hz for ^{1}H, 20 742 Hz for ^{13}C; number of t_2 data points, 2 048; number of t_1 data points, 512; number of scans, 256; acquisition time, 0.341 s; and delay time, 2.0 s. The acquisition parameters of ^{1}H−^{13}C constant-time heteronuclear multiple-bond correlation spectroscopy (CT-HMBC)[21] were as follows: spectral width, 6 000 Hz for ^{1}H, 28 902 Hz for ^{13}C; number of t_2 data points, 2 048; number of t_1 data points, 256; number of scans, 416; acquisition time, 0.341 s; and delay time, 2.0 s.

NMR Data Processing and Multivariate Analysis. Five cultivars of 11 mango fruits, for a total of 55 fruits, were used in the multivariate analysis. All ^{1}H NMR spectra were manually phased, baseline corrected, and referenced to DSS at 0.00 ppm (in nonselective excitation experiments) or histidine (C2H) at 8.64 ppm (in selective excitation experiments, pH 4.20). All of the 1D NMR spectra used for the multivariate analysis are without any spectral problem. For nonselective excitation, each ^{1}H NMR spectrum was divided into regions having an equal bin size of 0.01 ppm in the range from 0.1 to 10.0 ppm (excluding the region around the water signal, 4.5−6.0 ppm), and the regions within each bin were integrated. For selective excitation, each ^{1}H NMR spectrum was divided into regions having an equal bin size of 0.01 ppm in the range from 6.3 to 10.0 ppm, and the area within each bin was integrated.

These NMR data were imported into SIMCA-P+ version 11.0 for multivariate analysis. Unsupervised principal component analysis (PCA) was carried out to classify different mango cultivars and to evaluate the multivariate analysis data using selective ^{1}H NMR spectra.

NMR Signal Assignment of Mango Juice. The signals observed in 1D and 2D NMR spectra were compared with the data given in several online databases for metabolomics, such as BMRB,[22] HMDB,[23] and MMCD,[24] and in papers on mango juice,[25−27] and several candidate components were picked out. Finally, signal assignment was accomplished by spiking authentic standard compounds (special grade; Wako Pure Chemical Industries, Ltd., Osaka, Japan, or Nacalai Tesque Inc., Tokyo, Japan) into the mango juice and comparing the spectra of the resulting mixtures with those of plain mango juice.

RESULTS AND DISCUSSION

^{1}H NMR Spectra of Mango Juice and Signal Assignment. Figure 1a shows the nonselective ^{1}H NMR spectrum of the mango juice (cultivar Nam Doku Mai). The spectrum is dominated by the sucrose, fructose, and glucose, which are observed in the range of 3.0−5.5 ppm. In the high-field region (0.5−3.0 ppm), many peaks that were thought to be due to amino acids or organic acids were observed. In contrast, only a few peaks were observed in the low-field region (6.0−10.0 ppm). To assign these signals, we performed various 2D NMR experiments (DQF-COSY, TOCSY, NOESY, ^{1}H−^{13}C HSQC, ^{1}H−^{13}C CT-HMBC). As a result, various minor components [amino acids (isoleucine, leucine, valine, alanine, arginine, glutamine, and γ-aminobutyric acid (GABA)), organic acids (quinic acid, shikimic acid, malic acid, and citric acid), fucose,

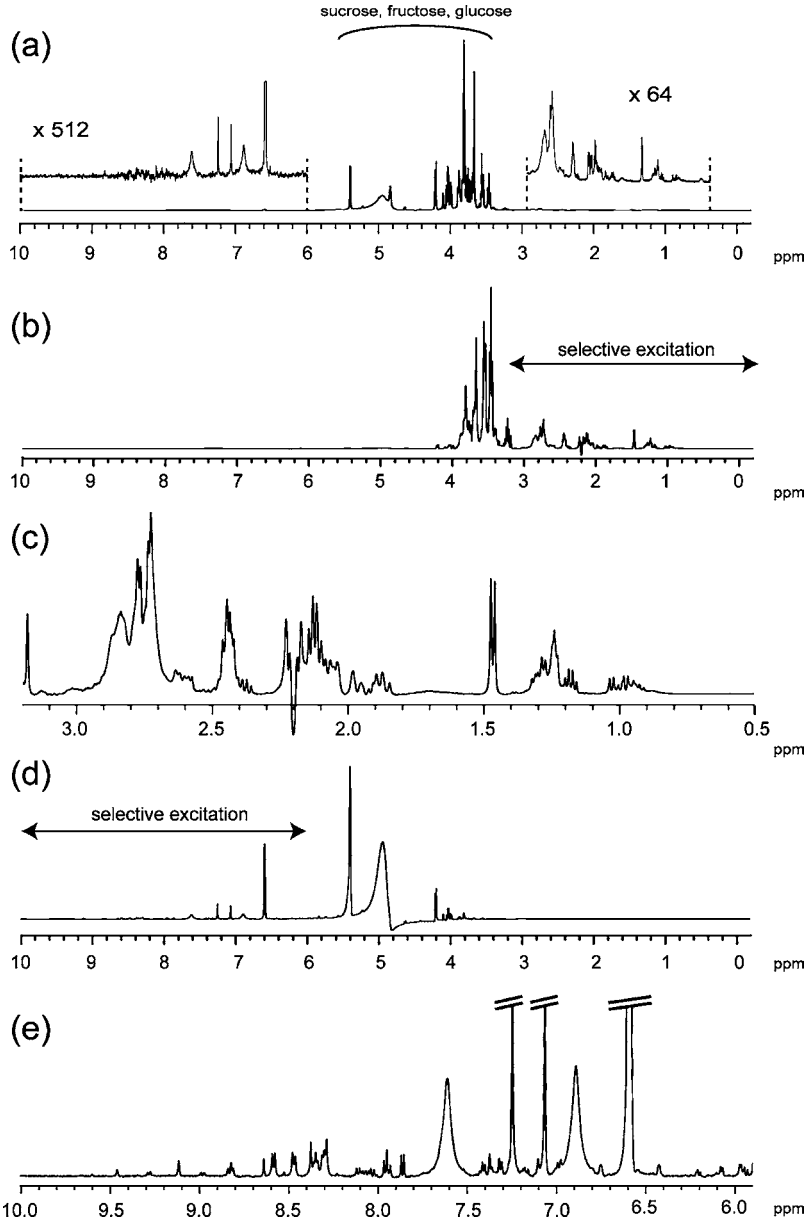

Figure 1. ^{1}H NMR signals of the mango juice (cultivar Nam Dok Mai). All the ^{1}H NMR spectra in this figure were obtained from the same sample. (a) Full nonselective ^{1}H NMR spectrum with a vertical expansion, (b) full selective ^{1}H NMR spectrum (range of excitation = −0.2 to 3.1 ppm), (c) expansion of the selective ^{1}H NMR spectrum from 0.5 to 3.2 ppm as observed by the selective excitation method, (d) full selective ^{1}H NMR spectrum (range of excitation = 6.0−10.5 ppm), and (e) expansion of the selective ^{1}H NMR spectrum from 5.9 to 10.0 ppm as observed by the selective excitation method.

ethanol, and choline] were identified in the high-field region (Figure 2a, Table 1).

Much as for the nonselective ^{1}H NMR spectrum, only a few peaks were observed in the nonselective 2D NMR spectrum in the low-field region. One of the reasons for the observation of few signals in the low-field region was considered to be the problem of dynamic range caused by the strong signals of the sugars. Namely, when most of the range is used to digitize the strong signals, the weak signals may be lost or distorted. To remove the dominant signals from sugars and improve the problem of the dynamic range, we used band-selective excitation and measured the spectra in the low-field region (6.0−10.0 ppm). As a result, the receiver gain increased from 18 dB to the maximum gain of 60 dB, and many minor component signals that were not detected with the nonselective experiments were newly detected (Figure 1, parts d and e). The results indicate that band-selective excitation can remove the strong signals and allows an increase in the receiver gain.[16] For the high-field region (0.0−3.1 ppm), band-selective excitation also allowed an increase of the receiver gain (18−42 dB). Although the signal-to-noise (S/N) ratio of the signals was improved, new peaks were not observed. The signal intensities of the high-field region were much higher (>10 times higher) than those of the low-field region, as shown in Figure 1a. Therefore, the signals in the high-field region were digitized with nonselective excitation in the mango juice. Thus, band-selective excitation may allow detection of the minor components without any solvent extraction or separation and improves the S/N ratio of the signals, which helps to reduce the experimental time.

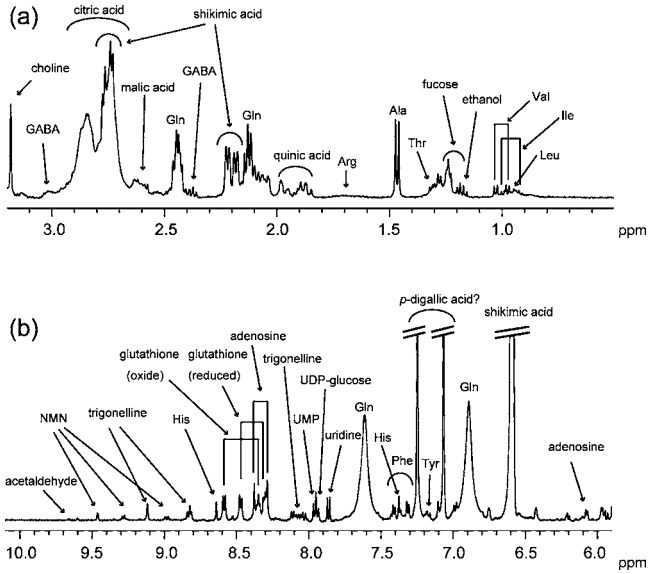

Figure 2. Assignment of ^{1}H NMR signals of the mango juice (cultivar Nam Dok Mai). (a) Expansion of the nonselective ^{1}H NMR spectrum from 0.5 to 3.2 ppm and (b) expansion of the selective ^{1}H NMR spectrum from 5.9 to 10.1 ppm as observed by the selective excitation method.

In addition, the suppression method is also effective for detecting minor components in food nondestructively. It was recently shown that simultaneous suppression of eight frequencies in the NMR spectrum is possible.[28] It was considered that the two methods have different advantages. The suppression method can measure several regions in one experiment. For example, in alcoholic beverages, the suppression of water and ethanol signals provides the spectra of minor components in the high- and low-field regions simultaneously. In contrast, band-selective excitation requires more than two experiments. Furthermore, band-selective excitation is a weak method for the measurement of narrow regions between strong signals. On the other hand, band-selective excitation is a simple and easy method for the measurement of a single specific region (e.g., measurement of the low-field region only). Moreover, band-selective excitation has another advantage for 2D NMR spectroscopy. For example, in alcoholic beverages, the suppression method may also suppress the minor component signals overlapped with ethanol, causing loss of the information on the minor components. In addition, the suppression method may lose cross-peaks between ethanol and minor components in the NOESY spectra. In contrast, band-selective excitation, especially F_2-selective NOESY, can provide these cross-peaks. For these reasons, it may be important to use the most suitable method depending on the situation.

Signal Assignment of Minor Components Using F_2-Selective 2D NMR Spectroscopy. Figure 3a shows the F_2-selective TOCSY spectrum of the mango juice. A high-quality selective 2D TOCSY spectrum of minor components was obtained, and the signals overlapping in the 1D NMR spectrum were separated. Using these F_2-selective 2D NMR spectra (TOCSY, DQF-COSY, and NOESY), the signal assignment of minor components was efficiently carried out (Table 1). For example, the cross-peaks at F_1/F_2 = 8.08/8.83 and 9.12/8.83 ppm may have been due to a nicotinic acid derivative, and the cross-peaks at F_1/F_2 = 8.29/8.99, 9.28/8.99, and 9.46/9.28 ppm were suggested to be due to a nicotinamide derivative (Figure 3b). Finally, these signals were assigned to trigonelline and nicotinamide mononucleotide (NMN) by spiking experiments. The two singlet peaks at 7.07 and 7.25 ppm were not assigned with these F_2-selective 2D NMR spectra. However, the CT-HMBC

Table 1. ^{1}H NMR Chemical Shifts of the Mango Juice Metabolites (Cultivar Nam Dok Mai, 10% D_2O, pH 4.3) Identified by Authentic Standards and Using 500 MHz 1D and 2D NMR Spectra

compound	chemical shift (ppm)
acetaldehyde	2.24 (CH_3), 9.66 (CHO)
adenosine	4.29 (C4′H), 4.43 (C3′H), 4.78 (C2′H), 6.08 (C1′H), 8.29 (C8H), 8.38 (C2H)
alanine	1.47 (βCH_3), 3.78 (αCH), 7.72 (αNH_2)
γ-aminobutyric acid (GABA)	1.91 (βCH_2), 2.37(αCH_2), 3.02 (γCH_2), 7.60(γNH_2)
arginine	1.69 (γCH_3), 1.90 (βCH_2), 3.23(δCH_2), 3.75 (αCH), 7.25 (δNH)
choline	3.18 ($N(CH_3)_3$)
citric acid	2.74 (CH_2), 2.85 (CH_2)
ethanol	1.17 (CH_3), 3.65 (CH_2)
β-fructofuranose	3.54 ($C1H_2$), 3.58 ($C1H_2$), 3.66 ($C6H_2$), 3.78 ($C6H_2$), 3.81 (C5H), 4.10 (C3H), 4.10 (C4H)
β-fructopyranose	3.55 ($C1H_2$), 3.69 ($C6H_2$), 3.70($C1H_2$), 3.78 (C3H), 3.89 (C4H), 3.99 (C5H), 4.01 ($C6H_2$)
fucose	1.19 ($\alpha C6H_3$), 1.23 ($\beta C6H_3$), 3.79 (βC5H), 4.19 (αC5H)
α-glucose	3.40 (C4H), 3.52 (C2H), 3.70 (C3H), 3.75 ($C6H_2$), 3.82 (C5H), 3.83 ($C6H_2$), 5.22 (C1H)
β-glucose	3.23 (C2H), 3.39 (C4H), 3.45 (C5H), 3.48 (C3H), 3.71 ($C6H_2$), 3.89 ($C6H_2$), 4.63 (C1H)
glutamine	2.13 (βCH_2), 2.45(γCH_2), 3.77 (αCH), 6.89 (NH_2–CO–), 7.61 (NH_2–CO–)
glutathione (oxidized)	2.97 (Cys, CH_2), 3.30 (Cys, CH_2), 3.81 (Gly, CH_2), 4.75 (Cys, CH), 8.35 (Gly, NH), 8.59 (Cys, NH)
glutathione (reduced)	2.95 (Cys, CH_2), 3.82 (Gly, CH_2), 4.57 (Cys, CH), 8.30 (Gly, NH), 8.47 (Cys, NH),
histidine	7.38 (C4H, ring), 8.64 (C2H, ring)
isoleucine	0.93 (δCH_3), 1.00 ($\gamma' CH_3$), 1.24 (γCH_2), 1.46 (γCH_2), 1.97 (βCH), 3.67 (αCH)
leucine	0.95 (δCH_3), 1.70 (γCH, βCH_2)
malic acid	2.62 (CH_2), 2.81 (CH_2), 4.38 (CH)
methanol	3.35 (CH_3)
myo-inositol	3.26 (C5H), 3.52 (C1H, C3H), 3.61 (C4H, C6H), 4.06 (C2H)
nicotineamide mononucleotide (NMN)	8.29 (C5H), 8.99 (C6H), 9.28(C4H), 9.46 (C2H)
phenylalanine	7.31 (C2H, C6H, ring), 7.36 (C4H, ring), 7.40 (C3H, C5H, ring)
quinic acid	1.87 ($C2H_2$), 1.97 ($C6H_2$), 2.06 ($C6H_2$), 2.07 ($C2H_2$), 3.55 (C4H), 4.01 (C3H), 4.14 (C5H)
shikimic acid	2.20 ($C6H_2$), 2.76 ($C6H_2$), 3.74 (C4H), 4.00 (C5H), 4.42 (C3H), 6.59 (C2H)
sucrose	3.46 (Glu C4H), 3.54 (Glu C2H), 3.67 (Fru $C1H_2$), 3.75 (Glu C3H), 3.81 (Glu $C6H_2$), 3.81 (Fru $C6H_2$), 3.83 (Glu C5H), 3.87 (Fru C5H), 4.04 (Fru C4H), 4.20 (Fru C3H), 5.39 (Glu C1H)
threonine	1.32 (γCH_3), 3.58 (αCH), 4.26 (βCH)
trigonelline	8.08 (C5H), 8.83 (C4H, C6H), 9.12 (C2H)
tyrosine	6.89 (C3H, C5H, ring), 7.18 (C2H, C6H, ring)
UDP-glucose	5.94 (C5H, ring), 7.93 (C6H, ring)
uridine	5.90 (C5H, ring), 7.86 (C6H, ring)
uridine mono phosphate (UMP)	5.93 (C5H, ring), 7.94 (C6H, ring)
valine	0.98 ($\gamma' CH_3$), 1.03 (γCH_3), 2.26 (βCH_2), 3.60 (αCH)

dx.doi.org/10.1021/jf2041438 | J. Agric.Food Chem. 2012, 60, 1158–1166

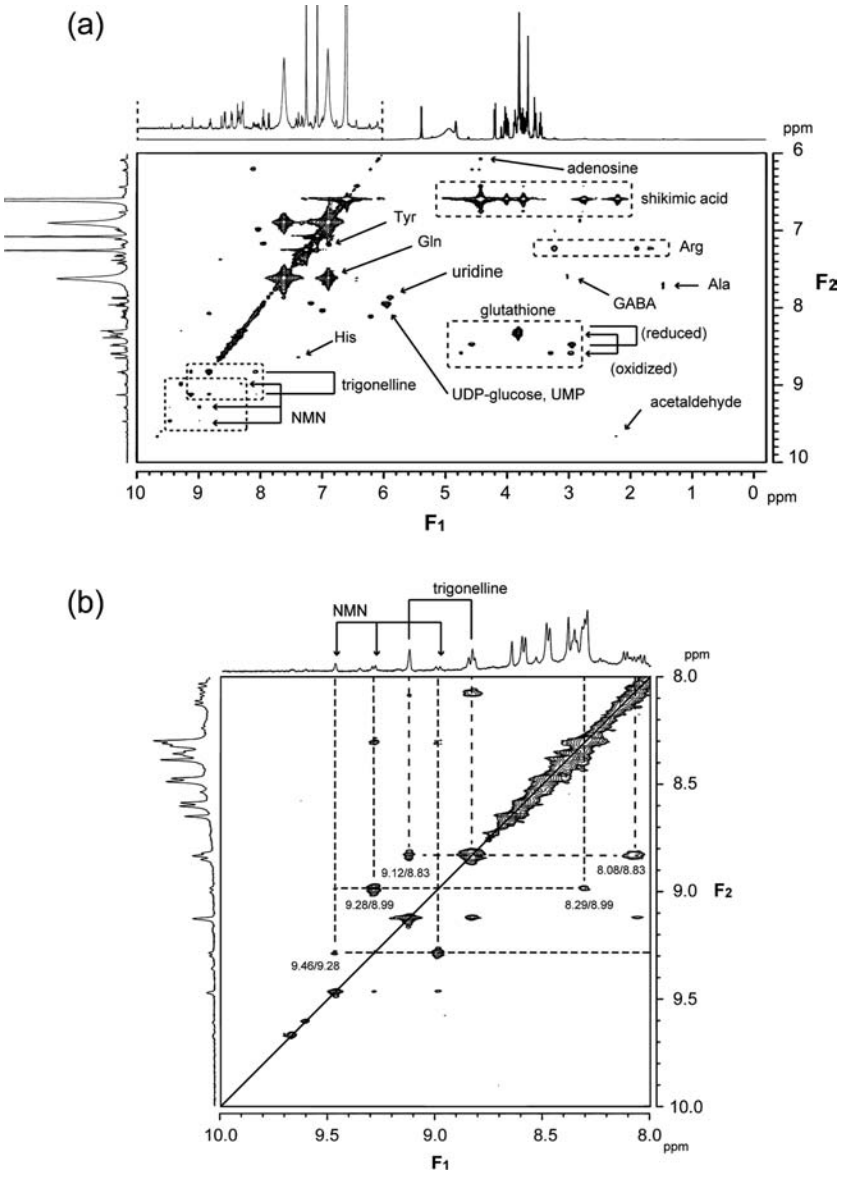

Figure 3. (a) Expansion of the F_2-selective TOCSY spectrum (F_1 = −0.2 ti 10.0 ppm, F_2 = 6.0−10.0 ppm) and (b) expansion of the F_2-selective TOCSY spectrum (F_1 = 8.0−10.0 ppm, F_2 = 8.0−10.0 ppm) of the mango juice (cultivar Nam Dok Mai, 10% D_2O, pH 4.3). The range of excitation and the number of scans were 6.0−10.5 ppm and 16, respectively.

spectrum indicated that these peaks may arise from a galloyl group; therefore, these peaks were presumed to arise from *p*-digallic acid by referring to the former reports.[29,30]

In the F_2-selective 2D NMR spectra, amine protons were observed with high sensitivity (Figure 2b, 3a). Because amine protons are exchangeable, they are difficult to detect in a deuterated solvent. However, under sufficient light water conditions (e.g., the addition of only 10% heavy water for the field lock), amine proton signals may be observed at approximately 7−9 ppm. The two broader singlets at 6.89 and 7.61 ppm were assigned to the amine protons (NH_2−CO−) of glutamine. These signals had no cross-peak in the F_2-selective TOCSY and DQF-COSY spectra (except the cross-peaks between 6.89 and 7.61 ppm in the F_2-selective TOCSY), but the F_2-selective NOESY spectrum showed cross-peaks at 2.45 ppm, which implied it may be glutamine. Its presence was also confirmed by spiking experiments. Amine protons (NH_2−CO−) of glutamine and asparagine had similar chemical shifts in the ^{1}H NMR spectra, but glutamine and asparagine were clearly distinguished by the F_2-selective NOESY spectra. The signal of δNH of arginine was detected at 7.25 ppm. The cross-peaks of the F_2-selective TOCSY spectrum at 7.25/1.69, 7.25/1.90, and 7.25/3.23 ppm suggested that it may be due to an arginine, and subsequent addition of the authentic standard indicated that these cross-peaks indeed arose from arginine. The cross-peak at 7.25/3.23 ppm of the F_2-selective DQF-COSY spectrum indicated that the signal at 7.25 ppm arose from δNH of arginine. GABA and alanine were detected at 7.60 and 7.72 ppm, respectively. These signals were weak and broad, and they were difficult to detect in the 1D NMR spectra due to the overlap with the broader signal of glutamine at 7.61 ppm. However, the F_2-selective TOCSY spectrum was able to detect these weak signals. Amine protons of reduced glutathione (Gly NH = 8.30 ppm; Cys NH = 8.47 ppm) and oxidized glutathione (Gly NH = 8.35 ppm; Cys NH = 8.59 ppm) were detected. The signals of the reduced glutathione at 8.47 ppm

and the oxidized glutathione at 8.59 ppm were sharp and not overlapped in the 1D NMR spectra (Figure 2b), and it was thought that these peaks may be available for measuring the amount of glutathione or the ratio of reduced/oxidized glutathione in the mango juice nondestructively. Since Gómez et al. reported that the concentration of glutathione increased during the ripening process or cold storage in tomato fruit,[31] the band-selective excitation technique may be useful for quality control to evaluate glutathione levels rapidly or exactly. Although only limited chemical shift data on amine protons are available in the existing databases and literature, the assignment of amine protons was facilitated by F_2-selective 2D NMR spectra. Figure 4 shows a comparison of the F_1- and F_2-selective

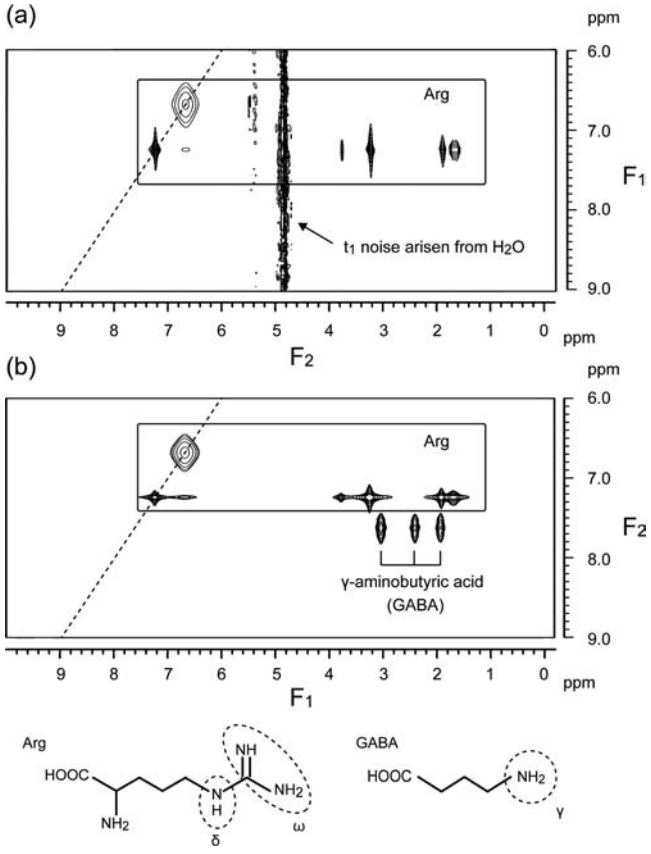

Figure 4. Comparison of (a) F_1- and (b) F_2-selective TOCSY spectra of arginine (100 mM) and γ-aminobutyric acid (100 mM).

TOCSY spectra of the aqueous solution containing 100 mM each of arginine and GABA. These spectra were measured with the same sample and the same receiver gain value. Cross-peaks arising from the arginine (δNH and ωNH) were detected in both the F_1- and F_2-selective TOCSY spectra, but the F_2-selective TOCSY provided the more sensitive spectrum. In contrast, cross-peaks due to GABA were detected only in the F_2-selective TOCSY spectrum. These results demonstrated that F_2-selective TOCSY was superior to conventional F_1-selective TOCSY for detecting amine protons in a complex mixture.

In addition, threonine was newly identified at 1.32 ppm with the F_2-selective TOCSY spectrum of the high-field region (data not shown).

Multivariate Analysis Using Band-Selective Excitation. We explored the metabolic profiling of five mango cultivars (Awin, Carabao, Keitt, Kent, and Nam Dok Mai) using four different regions: a nonselective, full spectra region (0.0−10.0 ppm); a nonselective, high-field region (0.0−3.1 ppm); a nonselective, low-field region (6.3−10.0 ppm); and a selective, low-field region (6.3−10.0 ppm). Because the high-field region spectra obtained by nonselective and selective excitation showed the same spectrum pattern, PCA with high-field region spectra was performed with the spectra obtained by nonselective excitation.

Figure 5 shows the band-selective low-field regions of ^{1}H NMR spectra of mango juices from the five cultivars.

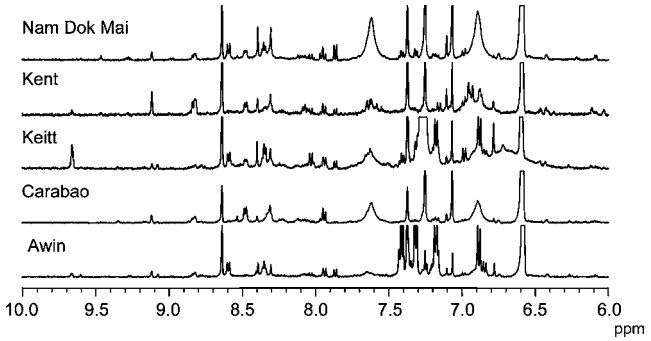

Figure 5. Low-field region of the band-selective ^{1}H NMR spectra of mango juices of five cultivars.

Although a few signals were observed in the nonselective, low-field region of the spectra, the band-selective, low-field region of the spectra exhibited definite differences among mango cultivars. From this result, it was expected that the band-selective, low-field region of the spectra may be available for the discrimination of mango cultivars. To assess the usefulness of the band-selective, low-field region of the spectra, we performed an unsupervised PCA and compared the results with the score plots obtained by nonselective and band-selective spectra. Figure 6 shows the PCA score plots using the four different regions. The PCA score plot using nonselective spectra could not separate the mango cultivars because of the complex overlap (Figure 6, parts a−c). In contrast, the PCA score plot using the band-selective, low-field region of the spectra provided a good discriminant model of the five mango cultivars (Figure 6d). Although Carabao and Nam Dok Mai overlapped in the score plot with PC1 and PC2 (Figure 6d), the use of PC3 separated these two cultivars (Figure 7a). The loading plot of PC1 (Figure 7b) indicated that the amounts of arginine, histidine, and acetaldehyde were higher in the cultivar Keitt, whereas the amount of shikimic acid was lower than the others. Although histidine was added to the mango juices as a chemical shift reference, the mango juices contain histidine originally (Figure 2b). Therefore, in the ^{1}H NMR spectra, the signal intensity of histidine varied according to the original amount in the mango juices. In fact, the signal intensity of histidine was obviously strong in the cultivar Keitt. Figure 7c shows the loading plot of PC2. The amount of phenylalanine was found to be higher in the cultivar Awin, and the amounts of glutamine and shikimic acid were higher in the cultivars Carabao and Nam Dok Mai. The cultivars Carabao and Nam Dok Mai were separated along the PC3 axis. The loading plot of PC3 indicated that the amount of trigonelline was higher in the cultivars Carabao and Kent (data not shown). These results suggested that PCA with the low-field region spectra

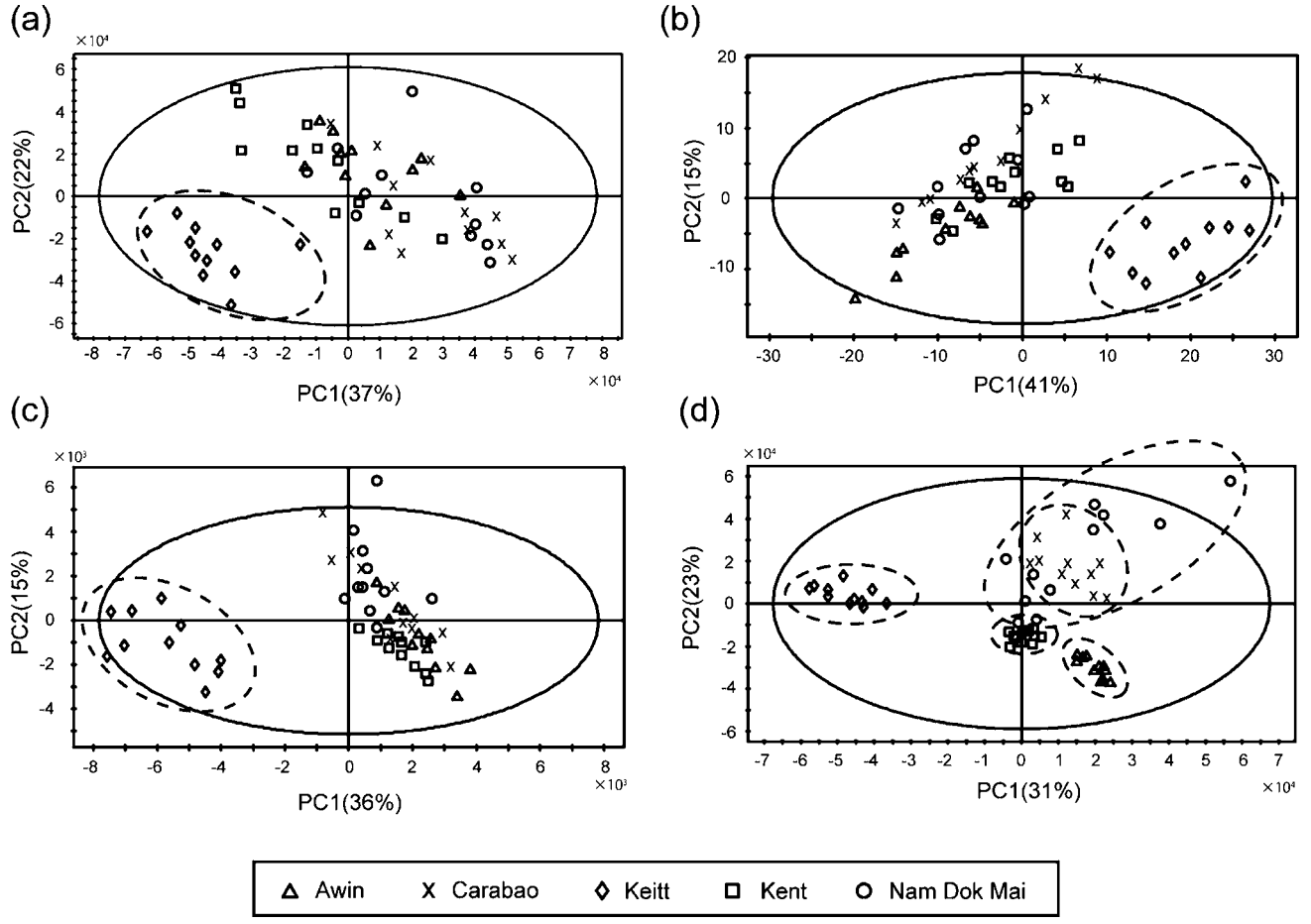

Figure 6. PCA score plots derived from the ^{1}H NMR spectra of the mango juices using PC1 and PC2: (a) nonselective full spectra (0.0−10.0 ppm), (b) nonselective, high-field region (0.0−3.1 ppm), (c) nonselective, low-field region (6.3−10.0 ppm), and (d) selective, low-field region (6.3−10.0 ppm).

obtained by selective excitation may be useful for the discrimination of mango cultivars. The PCA loading plot and score plot may be improved by preprocessing of raw NMR spectra (such as spectral alignment and normalization). In addition, it was thought that the PCA using both band-selective, low-field and nonselective, high-field regions of spectra may separate Carabao and Nam DokMai more clearly, but the score plots were not improved, and the obtained score plots were similar to that obtained using low-field region spectra only.

For the PCA with nonselective spectra, it was difficult to classify the mango cultivars except Keitt, because the plot of different cultivars overlapped (Figure 6, parts a−c). For the nonselective full spectra, the mango juices of all the cultivars were dominated by sugars; therefore, it was inferred that differences in sugar concentrations in the individual fruits strongly affected the PCA score plot and caused the complex overlap. To improve the PCA score plot, we attempted to perform PCA on weighted NMR spectra (weighted range = 3.2−4.4 ppm), but the score plot was not improved for our samples. The obtained score plots were similar to that obtained using the high-field region. Because the signals from various amino acids and other organic acids appear in the high-field region, the high-field region can be considered informative for metabolic profiling. However, the nonselective, high-field region of spectra did not provide a good discriminant model in the study. Because the contents of organic acids and amino acids change along the ripening process,[24,25] this complex overlap may have been caused by the nonuniform ripening condition of the samples. In addition, according to the loading plot, the amount of ethanol was higher in the cultivar Keitt. The content of ethanol was probably increased by fermentation during ripening (we confirmed that the sample composition had not changed during sample storage at −20 °C); therefore, it seemed that the high-content of ethanol of the Keitt cultivar may have been due to not only the cultivar difference but also the ripening conditions. These results suggest that the PCA using band-selective, low-field region spectra may have robustness for individual differences or a ripening condition difference and may be favorable for practical use.

In conclusion, we performed a discriminant analysis of mango juices using band-selective 1D and 2D NMR spectra. These spectra allowed identification of various minor components in the mango juice nondestructively. The low-field region contained several signals from exchangeable amine protons, and these signals were efficiently assigned by F_2-selective 2D NMR spectra. A combination of unsupervised PCA with the low-field region of ^{1}H NMR spectra obtained by band-selective excitation provided a good discriminant model of the mango cultivars. The signal assignment obtained by the F_2-selective 2D NMR spectra led to easy interpretation of the loading plot, and it was found that the amounts of arginine, histidine, phenylalanine, glutamine, shikimic acid, and trigonelline were important for classification of the five mango cultivars. The band-selective excitation method, which allows the analysis

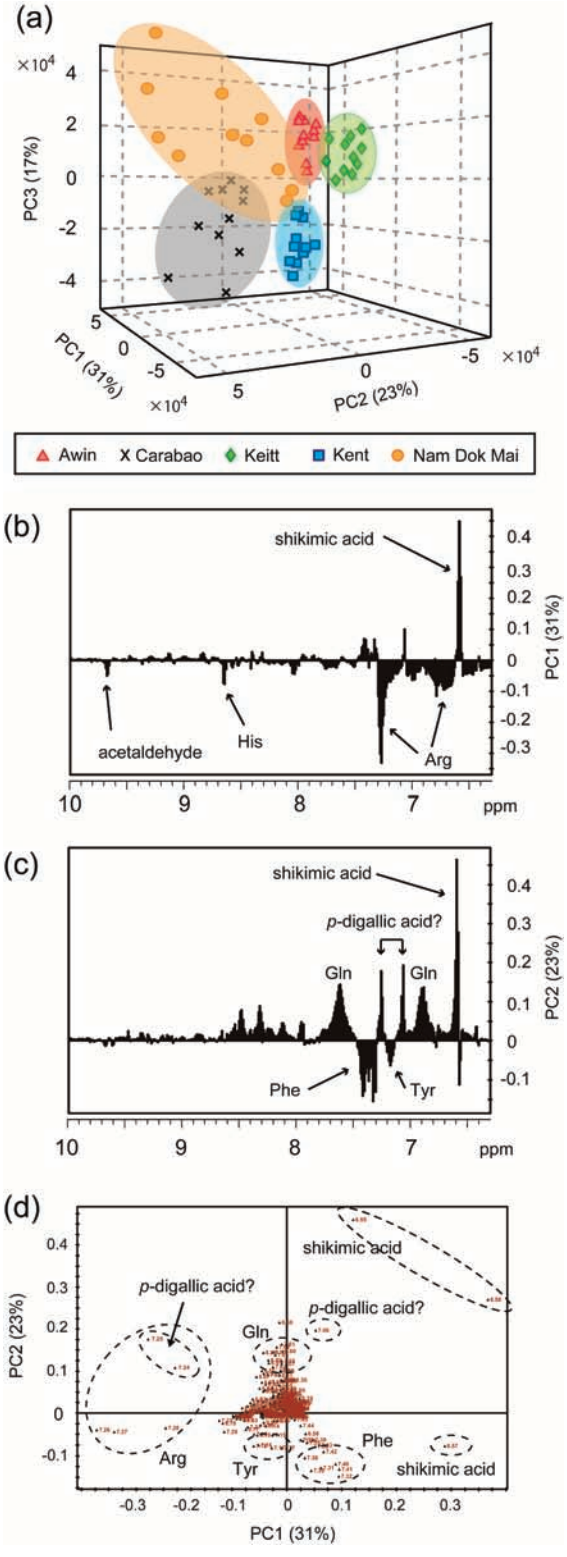

Figure 7. (a) PCA score plots derived from the ^{1}H NMR spectra of the mango juices from five cultivars in the selective, low-field region (6.3−10.0 ppm) using PC1, PC2, and PC3. Loading plot of (b) PC1 and (c) PC2. (d) Loading scatter plot using PC1 and PC2. All the ellipses on the PCA scatter plot are drawn by hand to surround each cultivar of mango juice.

of minor components without solvent extraction or separation, is expected to become more important for NMR-based metabolic analysis.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +81 3 5841 5165. Fax: +81 3 5841 8023. E-mail: amtanok@mail.ecc.u-tokyo.ac.jp.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Mafra, I.; Ferreira, I. M. P. L. V. O.; Oliveira, M. B.P. P. Food authentication by PCR-based methods. *Eur. Food Res. Technol.* **2008**, *227*, 649−665.

(2) Benincasa, C.; Lewis, J.; Sindona, G.; Tagarelli, A. The use of multi element profiling to differentiate between cow and buffalo milk. *Food Chem.* **2008**, *110*, 257−262.

(3) Jamin, E.; Gonzalez, J.; Remaud, G.; Naulet, N.; Martin, G. G. Detection of exogenous sugars or organic acids addition in Pineapple juices and concentrates by ^{13}C IRMS Analysis. *J. Agric. Food Chem.* **1997**, *45*, 3961−3967.

(4) Defernez, M.; Kemsley, E. K.; Wilson, R. H. Use of Infrared Spectroscopy and Chemometrics for the Authentication of Fruit Purees. *J. Agric. Food Chem.* **1995**, *43*, 109−113.

(5) Reid, L. M.; O'Donnell, C. P.; Downey, G. Potential of SPME-GC and Chemometrics to detect adulteration of soft fruit purees. *J. Agric. Food Chem.* **2004**, *52*, 421−427.

(6) Cynkar, W.; Dambergs, R.; Smith, P.; Cozzolino, D. Classification of Tempranillo wines according to geographic origin: Combination of mass spectrometry based electronic nose and chemometrics. *Anal. Chim. Acta* **2010**, *660*, 227−231.

(7) Cevallos-Cevallos, J. M.; Reyes-De-Corcuera, J. I.; Etxeberria, E.; Danyluk, M. D.; Rodrick, G. E. Metabolic analysis in food science: A review. *Trends Food Sci. Tech.* **2009**, *20*, 557−566.

(8) Wei, F. F.; Furihata, K.; Hu, F. Y.; Miyakawa, T.; Tanokura, M. Complex mixture analysis of organic compounds in green coffee bean extract by two-dimensional NMR spectroscopy. *Magn. Reson. Chem.* **2010**, *48*, 857−865.

(9) Ali, K.; Maltese, F.; Zyprian, E.; Rex, M.; Choi, Y. H.; Verpoorte, R. NMR metabolic fingerprinting based identification of grapevine metabolites associated with downy mildew resistance. *J. Agric. Food Chem.* **2009**, *57*, 9599−9606.

(10) Sundekilde, U. K.; Frederiksen, P. D.; Clausen, M. R.; Larsen, L. B.; Bertram, H. C. Relationship between the metabolite profile and technological properties of bovine milk from two dairy breeds elucidated by NMR-based Metabolomics. *J. Agric. Food Chem.* **2011**, *59*, 7360−7367.

(11) Sacchi, R.; Mannina, L.; Fiordiponti, P.; Paolillo, L.; Patumi, M.; Segre, A. Characterizationof Italian extra virgin olive oils using ^{1}H NMR spectroscopy. *J. Agric. Food Chem.* **1998**, *46*, 3947−3951.

(12) Beretta, G.; Caneva, E.; Regazzoni, L.; Bakhtyari, N. G.; Facino, R. M. A solid-phase extraction procedure coupled to ^{1}H NMR, with chemometric analysis, to seek reliable markers of the botanical origin of honey. *Anal. Chim. Acta* **2008**, *620*, 176−182.

(13) Schievano, E.; Peggion, E.; Mammi, S. ^{1}H nuclear magnetic resonance spectra of chloroform extracts of honey for chemometrics determination of its botanical origin. *J. Agric. Food Chem.* **2010**, *58*, 57−65.

(14) Bertelli, D.; Lolli, M.; Papotti, G.; Bortolotti, L.; Serra, G.; Plessi, M. Detection of honey adulteration by sugar syrups using one-dimensional and two-dimensional high-resolution nuclear magnetic resonance. *J. Agric. Food Chem.* **2010**, *58*, 8495−8501.

(15) Sandusky, P.; Raftery, D. Use of selective TOCSY NMR experiments for quantifying minor components in complex mixtures: Application to the metabonomics of amino acids in honey. *Anal. Chem.* **2005**, *77*, 2455−2463.

(16) Rastrelli, F.; Schievano, E.; Bagno, A.; Mammi, S. NMR quantification of trace components in complex matrices by band-selective excitation with adiabatic pulses. *Magn. Reson. Chem.* **2009**, *47*, 868−872.

(17) Kessler, H.; Mronga, S.; Gemmecker, G. Multi-dimensional NMR experiments using selective pulses. *Magn. Reson. Chem.* **1991**, *29*, 527−557.
(18) Koda, M.; Furihata, K.; Wei, F. F.; Miyakawa, T.; Tanokura, M. F_2-selective two-dimensional NMR spectroscopy for the analysis of minor components in foods. *Magn. Reson. Chem.* **2011**, *49*, 710−716.
(19) Hwang, T.; Shaka, A. J. Water suppression that works. Excitation sculpting using arbitrary waveforms and pulsed field gradient. *J. Magn. Reson., Ser. A* **1995**, *112*, 275−279.
(20) Geen, H.; Freeman, R. Band-selective radiofrequency pulses. *J. Magn. Reson.* **1991**, *93*, 93−141.
(21) Furihata, K.; Seto, H. Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett.* **1998**, *39*, 7337−7340.
(22) Ulrich, E. L.; Akutsu, H.; Doreleijers, J. F.; Harano, Y.; Ioannidis, Y. E.; Lin, J.; Livny, M.; Mading, S.; Maziuk, D.; Miller, Z.; Nakatani, E.; Schulte, C. F.; Tolmie, D. E.; Kent Wenger, R.; Yao, H.; Markley, J. L. BioMagResBank. *Nucleic Acids Res.* **2008**, *36*, D402−408.
(23) Wishart, D. S.; Knox, C.; Guo, A. C.; Eisner, R.; Young, N.; Gautam, B.; Hau, D. D.; Psychogios, N.; Dong, E.; Bouatra, S.; Mandal, R.; Sinelnikov, I.; Xia, J.; Jia, L.; Cruz, J. A.; Lim, E.; Sobsey, C. A.; Shrivastava, S.; Huang, P.; Liu, P.; Fang, L.; Peng, J.; Fradette, R.; Cheng, D.; Tzur, D.; Clements, M.; Lewis, A.; Souza, A. D.; Zuniga, A.; Dawe, M.; Xiong, Y.; Clive, D.; Greiner, R.; Nazyrova, A.; Shaykhutdinov, R.; Li, L.; Vogel, H. J.; Forsythe, I. HMDB: A knowledgebase for the human metabolome. *Nucleic Acids Res.* **2009**, *37*, D603−D610.
(24) Cui, Q.; Lewis, I. A.; Hegeman, A. D.; Anderson, M. E.; Li, J.; Schulte, C. F.; Westler, W. M.; Eghbalnia, H. R.; Sussman, M. R.; Markley, J. L. Metabolite identification via the Madison Metabolomics Consortium Database. *Nat. Biotechnol.* **2008**, *26*, 162−164.
(25) Gil, A. M.; Duarte, I. F.; Delgadillo, I.; Colquhoun, I. J.; Casuscelli, F.; Humpfer, E.; Spraul, M. Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.* **2000**, *48*, 1524−1536.
(26) Duaret, I. F.; Goodfellow, B. J.; Gil, A. M. Characterization of mango juice by high-resolution NMR, hyphenated NMR, and diffusion-ordered spectroscopy. *Spectrosc. Lett.* **2005**, *38*, 319−342.
(27) Duarte, I. F.; Delgadillo, I.; Gil, A. M. Study of natural mango juice spoilage and microbial contamination with *Penicillium expansum* by high resolution ^{1}H NMR spectroscopy. *Food Chem.* **2006**, *96*, 313−324.
(28) Monakhova, Y. B.; Schäfer, H; Humpfer, E.; Spraul, M.; Kuballa1, T.; Lachenmeier, D. W. Application of automated eightfold suppression of water and ethanol signals in ^{1}H NMR to provide sensitivity for analyzing alcoholic beverages. *Magn. Reson. Chem.* **2011**, *49*, 734−739.
(29) Masibo, M.; He, Q. Major mango polyphenols and their potential significance to human health. *Compr. Rev. Food Sci. Food Saf.* **2008**, *7*, 309−319.
(30) Verzele, M.; Delahaye, P.; Dijck, J. V. Digallic acid. *Bull. Soc. Chim. Belg.* **1983**, *92*, 181−186.
(31) Gómez, P.; Ferrer, M. Á.; Fernández-Trujillo, J. P.; Calderón, A.; Artés, F.; Egea-Cortines, M. Weiss, Structural changes, chemical composition and antioxidant activity of cherry tomato fruits (cv. Micro-Tom) stored under optimal and chilling conditions. *J. Sci. Food Agric.* **2009**, *89*, 1543−1551.