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^{1}H NMR and multivariate data analysis of the differences of metabolites in five types of dry-cured hams

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ABSTRACT

In order to distinguish the taste styles of dry-cured hams (Jinhua, Xuanwei, Country, Parma and Bama), we established a ^{1}H nuclear magnetic resonance spectroscopy method to identify metabolites. Totally, 33 charged metabolites, including amino acids, organic acids, nucleic acids and their derivatives, sugars, alkaloids and others were identified. The abundant glutamate, lysine, alanine, leucine and lactate could be the major contributors of taste. Total variables were explained by PC1 (67.7%) and PC2 (16.0%) which showed that Parma and Xuanwei styles were close to each other (similar amino acids, peptide, organic acids and alkaloids contents). Bama style showed the highest PC1 and amino acids, organic acids and alkaloids contents. Country style was located on the left-most area of PC1 (the lowest amino acids, organic acids and peptide, but the highest sugars contents). Sensory evaluation revealed that Bama ham had the highest overall taste score, followed by Jinhua, Parma, Xuanwei and American Country ham. We concluded that the proportions and combinations of taste components explained the specific taste instead of any single component. These findings provided a better understanding of different metabolomics among hams.

1. Introduction

Chinese styles of dry-cured hams, including Jinhua and Xuanwei hams, are well received by consumers in China with a long history (Xiao et al., 2010). With the globalization of trade, lots of western styles of dry-cured hams, such as American country, Parma and Bama hams, flow into Chinese market. Chinese consumers find the taste styles differently among domestic and foreign dry-cured hams, but they know little about the reasons. To our knowledge, there is no clear explanation for this phenomenon.

Taste, as an important indicator of dry-cured hams, decides the evaluation of products (Zhao et al., 2005). Studies reported that some non-volatile metabolites were the taste compounds among which free amino acids mainly provided the umami taste of dry-cured hams (Dang, Wang, & Xu, 2008; Laureati et al., 2014). Taste compounds of dry-cured hams included free amino acids, nucleotides, peptides and organic acids etc. (Dang et al., 2008). Zhao et al. (2005) demonstrated that alanine and glycine contributed to sweet taste and that leucine, isoleucine, arginine, valine, phenylalanine, and histidine contributed to bitter taste in Jinhua ham. Sforza et al. (2006) showed aspartic and glutamic acids contributed to umami taste in Parma ham. Dang et al. (2008) claimed that lactate contributed to sour taste because of its high taste activity value in Jinhua ham. Salty taste, sour taste and umami were dominated in Jinhua ham (Dang et al., 2008). Parma ham was significantly sweeter and less salty and dry than San Daniele ham (Laureati et al., 2014). Xuanwei ham had a moderate overall taste profile but a very salty taste (8.7% NaCl of final product) (Huang et al., 2011). American Country ham had a slight bitterness, saltiness, sourness and aftertaste (Pham et al., 2008). Bama ham had a strong aftertaste (Andrés, Cava, Ventanas, Thovar, & Ruiz, 2004). Bitterness, sweetness, saltiness and aftertaste were important factors to influence the acceptability of Bama ham (Ruiz, Garcıa, Muriel, Andrés, & Ventanas, 2002). However, the difference of non-volatile taste metabolites among those dry-cured hams was not clear.

Several diverse metabonomic technologies have been applied to identify non-volatile metabolites of dry-cured hams, such as HPLC (Dang et al., 2008; Hernández-Cázares, Aristoy, & Toldrá, 2011), LC-MS (Martın, Antequera, Ventanas, Benıtez-Donoso, & Córdoba, 2001) and CE-TOF-MS (Sugimoto et al., 2017). However, few studies of NMR-based metabonomics analysis on dry-cured hams have been reported.

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Nuclear magnetic resonance (NMR) spectroscopy has unique advantages with a rapid method for the identification and quantification of metabolites. In the past years, NMR-based metabonomics analysis has been developed well and applied in meat scientific field successfully. Combined with multivariate data analysis, NMR-based metabolomics analysis has potential applications to analyze food components, assess food quality/authenticity and monitor food consumption & diet metabonomics profiling (Bordoni & Capozzi, 2015). Chen, Ye, Chen, Zhan, and Lou (2017) assessed the flavor formation of vinasse eel and analyzed the changes of metabolites, such as sucrose, creatine, organic acids, alcohols, glucose, and creatinine during pickling by NMR spectroscopy. Liu, Pan, Ye, and Cao (2013) studied the influence of ages on the metabolic composition of duck meat by using ^{1}H NMR spectroscopy and showed the changes of lactate and some free amino acids. It was considered to be a superior tool to identify free amino acids, small active peptides, nucleic acids and organic acids systematically (Sacco, Brescia, Buccolieri, & Jambrenghi, 2005; Shintu, Caldarelli, & Franke, 2007). However, few literatures studied the meat components with ^{1}H NMR from the perspective of taste and there was no literature about dry-cured hams. In addition, few researches comparing the differences of metabolites between different types of dry-cured hams were reported with orthogonal projection to latent structure discriminant analysis (OPLS-DA). Finally, there were no reports to distinguish different types of dry-cured hams with ^{1}H NMR and multivariate data analysis.

In the present study, ^{1}H NMR-based metabolomics coupled with multivariate data analysis was used to compare the different metabolomic profiles of 5 types of dry-cured hams systematically using principal components analysis (PCA) and OPLS-DA.

2. Materials and methods

2.1. Sample preparation

Fifty dry-cured hams included 10 Jinhua hams (Jinzi Ham CO., Ltd., Jinhua, China), 10 Xuanwei hams (Xuanwei ham food Co., Ltd., Xuanwei, China), 10 American country hams (Shanghai Hormel Foods Co., Ltd., Shanghai, China), 10 Parma hams (Cim Alimentari Spa, Langhirano, Italy) and 10 Bama hams (Jinzi Ham CO., Ltd., Jinhua, China) were selected in the experiment. The 100 g of the biceps femoris muscles after removing subcutaneous adipose and connective tissue from each ham were taken and cut into strips. Ten strips were sampled at every type ham for replicated analysis. Each strip was cut to small cubes (0.5 cm × 0.5 cm × 0.5 cm), packaged with tinfoil and stored at −80 °C before NMR analysis.

2.2. Metabolites extraction

Metabolites of hams were extracted according to the method of our previous study (Yang et al., 2018). In all cases, 600 μL of methanol/water (2:1, *v*/v) was used to extract ham sample (400 mg) by homogenizing at 12,400 × *g* for 5 min (i.e., 30 s homogenization followed by a 30 s break). The resultant extracts were combined and centrifuged at 12,400 × *g* for 10 min at 4 °C. The supernatants were treated by vacuum to remove the methanol and lyophilized. Every sample was recombined into 600 μL of phosphate buffer (0.15 M K_2HPO_4/NaH_2PO_4, pH 7.55) containing 50% D_2O, 0.01% NaN_3 and 0.001% sodium 3-trimethylsilyl [2, 2, 3, 3-d_4]. The 550 μL supernatants of every extract were collected and transferred into a 5 mm outer diameter NMR tube (Norell, ST500-7; Norell, Inc., Landisville, NJ) for NMR analysis after 10 min centrifugation at 12,400 × *g* at 4 °C.

2.3. NMR analysis

NMR analysis was done according to our previous method (Yang et al., 2018). All of the ^{1}H NMR spectra collection of extracts was finished at 298 K on a Bruker Avance 600 MHz Spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with ultra-low temperature detection probe under the operating condition of 600.13 MHz for ^{1}H.

The metabolite profile of each sample was collected using the standard Bruker pulse sequence NOESYGPPR1D (RD-90°-t1–90°-tm-90°-acquisition), with mixing time (t_m, 100 ms) and a weak irradiation during recycle delay (RD, 2 s) to afford good water signal suppression. A 90° pulse length and parameter t_1 were adjusted to 15 μs and 2 μs, respectively. A total of 32 transients were collected into 32 k data points with a spectral width of 20 ppm. All free induction decays employed an exponential window function with a 1 Hz line broadening factor prior to Fourier transformation.

In order to assign NMR signal, a battery of two-dimensional NMR spectra, including ^{1}H−^{1}H COSY, ^{1}H−^{1}H TOCSY, ^{1}H−^{13}C HSQC, and ^{1}H−^{13}C HMBC, was acquired for selected samples and processed referring to a previously reported method (Dai, Xiao, Liu, Hao, & Tang, 2010).

2.4. Data analysis

After the manual phase and baseline corrections, the ^{1}H NMR spectra (δ 9.0–0.7) were integrated into some regions with equal widths of 0.004 ppm (2.4 Hz). The methanol (δ 3.38–3.35) and residual water (δ 5.0–4.7) signals of the spectral regions were removed. In order to compensate for the entire concentration difference, each bucketed region was normalized to the total sum of the spectral integral. Subsequently, the NMR data sets of normalization were analyzed by multivariate data analysis (the software package SIMCA-P^{+}, version 12.0, Umetrics, Sweden). PCA was carried out using mean-centered NMR data to identify the magistral intrinsic variation and acquire an overview of variation among different groups. The results of PCA were shown as the scores and loading plots. Each point in the former represented an individual sample, while the latter plots presented the magnitude and manners of the NMR signals (thus metabolites) to classification. The orthogonal projection to latent structure with discriminant analysis (OPLS-DA) approach with 7-fold cross-validation and unit-variance scaling (UV) was conducted to further search any intrinsic biochemical dissimilarities between the 5 types of dry-cured hams. The OPLS-DA models were validated by the cross-validated residuals (CV-ANOVA) method. $p < .05$ represented a significant level (CV-ANOVA for significance testing of PLS and OPLS® models). The results of OPLS-DA also were visualized in the scores and coefficient plot. The loading in the coefficient plot showed the altered metabolites correlated to ham types. They were acquired by back-transformation and plotted with a color-coded correlation coefficient for each date point using version 7.1 of MATLAB (MathWorks, Natick, MA). The cold-colored variables had lower significance (positive/negative) than the hot-colored variables in distinguishing between classes. As an absolute cutoff value, $|r| > 0.602$ ($r > 0.602$ and $r < -0.602$) was considered as a statistical significant level ($p < .05$).

For quantitative analysis of 5 types of dry-cured hams, metabolite contents were calculated by summing the peak areas of selected metabolite NMR signals (least overlapping ones) in the normalized NMR data sets in relation to that of internal reference (TSP) with known concentration. The date of metabolite concentration was carried out by the one-way ANOVA procedure (Duncan's Multiple Range Test) of the SAS 8.0 software.

2.5. Sensory evaluation

In order to describe sensory properties of 5 types hams, the sensory profiling method was applied (ISO 13299, 2010). Twenty subjects (10 women and 10 men aged between 25 and 45) were selected from a panelist pool. Panelists were trained and had participated in sensory evaluation of dry-cured hams for two years. The training consisted of a first phase to familiarize the products, followed by a second phase concentrated on the sample evaluation. Subjects were trained over a