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Effect of high pressure treatment on metabolite profile of marinated meat in soy sauce

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ABSTRACT

Marinated meat in soy sauce was produced using hind leg by washing, rubbing salt, marinating with soy sauce and spices, and air dry-ripening for 15 d. The effect of high pressure (HP) (150 and 300 MPa for 15 min) on the metabolite profiles of products was characterized using ^{1}H NMR and multivariate data analysis. The results showed that the metabonome was dominated by 26 metabolites, including amino acids, sugars, organic acids, nucleic aides and their derivatives. PC1 and PC2 explained a total of 75.4 and 11.9% of variables, respectively. HP treatments increased most of the metabolites, especially PC1, glutamate, sugars, nucleotides, anserine, lactate and creatine compared to the control. The increase of metabolites under HP was not dependent on pressure level except for alanine, lactate, acetate, formate, fumarate, glucose and 5′-IMP. These findings demonstrated that HP treatment at 150 MPa was economical to improve the taste of marinated meat in soy sauce.

1. Introduction

Marinated meat in soy sauce, a popular traditional cured meat product in China, is produced using hind leg meat or belly meat by washing, rubbing salt, marinating with soy sauce and spices, and air dry-ripening. Owing to the unique flavor and attractive red ocher, it is one of four different typical sub-groups cured meat product and is popular with consumers in many south regions of China (Zeng et al., 2016). Its consumption has displayed a significant increase in the last two decades and the quality demand correspondingly increased, especially its sensory characteristics, which greatly affect consumers' acceptability (Verbeke, Van Oeckel, Warnants, Viaene, & Boucqué, 1999). Taste, one of the major sensory qualities of cured meat product, is closely related to its biochemical composition (i.e., metabolite profile), such low molecular weight metabolites as sugars, amino acids, dipeptides and nucleotides in particular (Jung et al., 2010; Liu, Xu, & Zhou, 2007). The modification of metabolite profile of meat product could influence its taste.

High pressure (HP) treatment, a non-thermal technology, is being increasingly used in the meat industry to extend the shelf life of product and improve its quality characteristics (Wang et al., 2013). The impact of HP treatment on various quality properties of meat has been the subject of numerous researches, but with respect to taste, it was rarely investigated. HP may affect the taste development of meat. HP treatments at 100 and 300 MPa increased the overall autolysis of raw meat and led to a higher content of free amino acids (Ohmori, Shigehisa, Taji, & Hayashi, 1991). Whereas Suzuki et al. (1994) found that the contents of amino acids and peptides in meat were scarcely affected by pressure (100–400 MPa). Campus, Flores, Martinez, and Toldrá (2008) reported that pressure treatments (300–400 MPa) could stabilize the concentration of free amino acid in dry-cured loin during storage due to the decrease of aminopeptidases activity. Mori et al. (2007) found that the inosinic monophosphate (5′-IMP, one of the constituents responsible for "umami" taste) content in rabbit muscles was immediately increased by a HP treatment of 300 MPa but had almost no change under lower pressures. No study, however, has been done to determine the influence of HP treatment on the taste quality of marinated meat in soy sauce.

Metabonomics has become possible as a result of recent technological breakthroughs in small molecule separation and identification (Liu et al., 2017). Nuclear magnetic resonance spectroscopy (NMR), as a metabolomics technology, has been employed in low molecular weight metabolites analysis. Compared with available official analytical procedures, the NMR-based approach not only simplifies sample preparation but reduces the time required for analysis (Zanardi et al., 2015). Furthermore, the utilization of high-resolution NMR spectroscopy to gain metabolite profile has made great progress in the last decade. It has been considered as a superior tool for reliable and high-throughput metabonomics analysis (Graham, Amigues, Migaud, & Browne, 2009; Graham et al., 2010). Especially, NMR spectroscopy has been performed to acquire metabolite profiles of meat samples. Researchers have made a lot of efforts to determine the

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variations that occurred during ageing of meat by ^{1}H NMR-based metabonomics (Graham et al., 2010). Castejón, García-Segura, Escudero, Herrera, and Cambero (2015) characterized changes in the chemical components of beef meat during storage by ^{1}H NMR. Liu, Pan, Ye, and Cao (2013) used ^{1}H NMR spectroscopy to analyze the relationship between the age and quality of duck meat. In addition, a metabolic insight was obtained on the formation of characteristic flavor of vinasse pike eel through ^{1}H NMR spectroscopy (Chen, Ye, Chen, Zhan, & Lou, 2017). These reports demonstrated that the relationship between metabolite profiling and meat quality could be revealed by an NMR-based metabonomics strategy.

In order to acquire a better understanding of the effect of HP treatment (150 and 300 MPa) on the characteristic taste of marinated meat in soy sauce, it is essential to comprehensively analyze the metabonomic variation during pressurization. Therefore, in the present study, we applied ^{1}H NMR-based metabonomics together with multivariate data analysis to analyze the metabolite profiles of marinated meat in soy sauce at different pressure treatments.

2. Materials and methods

2.1. Preparation and pressurization of marinated meat in soy sauce samples

A total of 30 raw hind leg muscle pieces with an average weight of approximately 0.8–1 kg from Duroc × Landrance crossbreed pigs weighting 80–100 kg were purchased from a local processing plant. The experimental pigs were slaughtered in a commercial abattoir. The hind leg muscles of the carcass were sampled and cut into strips (about 6 cm × 4 cm × 20 cm) after removing subcutaneous fat and connective tissue. Then, the raw strips were washed, rubbed 3% sodium chloride and air-dried for 1 day. Thereafter, the strips were marinated in brine (including 15% soy sauce, 1.5% white wine, 1% pepper, 5% sugar, 0.4% five-spice powder) at 4 °C for 7 days; 2–3 turnovers were given. After marinating, each strip was put in individual polythene bags (oxygen permeability of 40–50 cm^3/m^2/day at 20 °C) with 30 mL brine and vacuum-packed immediately. The vacuum-packed samples were divided into three groups, 10 samples for each treatment. One group was not pressurized and served as a control; the remaining samples were treated at 150 and 300 MPa for 15 min, respectively, at 20 °C in a high-pressure equipment (Stansted Fluid Power Ltd., Harlow, England), using water as the pressure-transmitting fluid. This pressure vessel had a 2 L capacity; the maximum operating pressure of the machine was 900 MPa. The rate of pressure increase was about 100 MPa/min; releasing time was just a few seconds to minimize adiabatic heating. Pressure treatment time excluded pressure come-up and releasing time. After treatment, the samples removed vacuum-packed bags were air-dried and ripened for 15 days according to the following temperature and humidity procedures: 40% relative humidity (RH), 15 °C under constant temperature and humidity. The ripening samples were cut to small cubes (about 0.5 cm × 0.5 cm × 0.5 cm), packaged with tinfoil and stored at −80 °C prior to NMR analysis. Each treatment was carried out in ten replicates; one sample from an individual hind leg muscle equated to one replicate.

2.2. Metabolites extraction

In all cases, each marinated meat in soy sauce sample (400 mg) was extracted twice with 600 μL of methanol/water (2:1, v/v) via homogenization for 2 min and discontinuous ultrasonication on wet ice for 10 min (i.e., 2 s ultrasonication followed by a 2 s break). The resultant extracts were combined. After centrifugation for 10 min at 12, 000 rpm and 4 °C, the methanol was removed in vacuum and the supernatants were lyophilized. Each sample was reconstituted into 600 μL phosphate buffer (0.1 M K_2HPO_4/NaH_2PO_4, pH 7.4) containing 0.1% NaN_3, and 0.005% sodium 3-trimethylsilyl [2, 2, 3, 3-d_4] propionate (TSP) prepared with 99.9% D_2O. After 10 min centrifugation at 12, 000 rpm at 4 °C, 550 μL supernatant of each extract was transferred into a 5 mm outer diameter NMR tube (Norell, ST500-7; Norell, Inc., Landisville, NJ) for NMR analysis.

2.3. NMR analysis

^{1}H NMR spectra of the marinated meat in soy sauce extracts were performed at 298 K on a Bruker Avance 600 MHz Spectrometer (operating at 600.13 MHz for 1H) equipped with an inverse detection probe (Bruker Biospin, Rheinstetten, Germany). For each sample, a standard one-dimensional pulse sequence (RD-90°-t_1-90°-t_m-90°-acquisition) was applied to achieve marinated meat in soy sauce metabolite profiles with a weak irradiation during recycle delay (RD, 2 s) and mixing time (t_m, 100 ms) to suppress the water signal. A 90° pulse length was set to about 10 μs and t_1 was adjusted to 3 μs. A total of 32 transients were collected into 32 k data points with a spectral width of 20 ppm. All free induction decays (FIDs) were subjected to an exponential window function with a line broadening factor of 1 Hz prior to Fourier transformation (FT).

For NMR signal assignment purposes, a series of two-dimensional NMR spectra were acquired for selected samples and processed according to a previously reported method, including ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{1}H total correlation spectroscopy (TOCSY), ^{1}H–^{13}C heteronuclear single quantum correlation (HSQC), and ^{1}H–^{13}C heteronuclear multiple bond correlation spectra (HMBC) (Dai, Xiao, Liu, Hao, & Tang, 2010).

2.4. Sensory evaluation

Marinated meat in soy sauce samples were placed in a steamer and steamed for approximately 18 min (the final core temperature was 70–75 °C). The trained panel for the sensory analysis consisted of 5 males and 5 females (age 25–45). The assessment was scored on a 9-point scale. Each meat sample was tasted by at least 5 panel members. Drinking water was provided to cleanse the mouth cavity between testing each sample. The test samples were awarded points on the basis of sweetness, saltiness, umami, bitterness, sourness mouthfulness and continuity. All the panellists had extensive experience in tasting and had made a consensus on scoring beforehand for sweetness, saltiness, umami, bitterness, sourness mouthfulness and continuity.

2.5. Data analysis

The ^{1}H NMR spectra (δ 9.0–0.7) were integrated into regions with equal width of 0.004 ppm (2.4 Hz) after phase and baseline corrections. The spectral regions containing the residual water (δ 5.23–4.7) and methanol signals (δ 3.4–3.32) were removed. Each bucketed region was normalized to the total sum of the spectral integral to offset for the entire concentration difference. Thereafter, the normalized NMR data sets were analyzed by multivariate data analysis (the software package SIMCA-P^+, version 12.0, Umetrics, Sweden). Principal component analysis (PCA) was carried out using mean-centered scaling, and the results were showed as the scores and loading plots; each point in the former presented an individual sample, while the latter presented the magnitude and manners of the NMR signals (thus metabolites) to classification. The orthogonal projection to latent structure with discriminant analysis (OPLS-DA) method with 7-fold cross-validation and unit-variance scaling was employed to further analyze any intrinsic biochemical dissimilarities between the different processing stages. All of OPLS-DA models were validated by ANOVA of the cross-validated residuals (CV-ANOVA) approach with $p < 0.05$ as significant level (Eriksson, Trygg, & Wold, 2008). The results were also visualized in the scores and coefficient plot. The loading in the coefficient plot showed the changed metabolites related to processing, and were gained by back-transformation and were color-coded with absolute value of correlation coefficients using version 7.1 of MATLAB (MathWorks, Natick,