Food Chemistry 192 (2016) 886–892

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evolution of metabolomics profile of crab paste during fermentation

Daian Chen, Yangfang Ye *, Juanjuan Chen *, Xiaojun Yan

School of Marine Sciences, Ningbo University, Ningbo 315211, Zhejiang, China

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 2 June 2015
Accepted 22 July 2015
Available online 23 July 2015

Keywords:
Metabolite profiling
Crab paste
Fermentation
Nuclear magnetic resonance

ABSTRACT

Crab paste is regularly consumed by people in the coastal area of China. The fermentation time plays a key role on the quality of crab paste. Here, we investigated the dynamic evolution of metabolite profile of crab paste during fermentation by combined use of NMR spectroscopy and multivariate data analysis. Our results showed that crab paste quality was significantly affected by fermentation. The quality change was manifested in the decline of lactate, betaine, taurine, trimethylamine-N-oxide, trigonelline, inosine, adenosine diphosphate, and 2-pyridinemethanol, and in the fluctuation of a range of amino acids as well as in the accumulation of glutamate, sucrose, formate, acetate, trimethylamine, and hypoxanthine. Trimethylamine production and its increased level with fermentation could be considered as a freshness index of crab paste. These results contribute to quality assessment of crab paste and confirm the metabolomics technique as a useful tool to provide important information on the crab paste quality.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Crab paste is a salt and liquor saturated product of fresh swimming crab, *Portunus trituberculatus*, well-known and sought-after in the coastal area of China (Ma, Xu, & Yang, 2008). It is produced by directly mixing crab meat with ingredients including salt, sugar, monosodium glutamate, and liquor under air. Since no sterilization is usually carried out throughout the whole processing of crab paste, the diverse microflora can easily develop during the fermentation of crab meat. The bacteria which have been found in crab paste include *Staphylococcus*, *Arthrobacter*, *Sphingobacterium*, *Bacillus*, *Psychrobacter*, *Agrobacterium*, *Salinivibrio*, *Vibrio*, *Moraxella*, *Micrococcus*, *Kocuria*, *Corynebacterium*, and *Rhodococcus* species (Huang et al., 2011; Ma et al., 2008; Zhang et al., 2011). Although it is the microbial fermentation that generates the crab paste with the characteristic flavor, taste, and nutritional composition, the spoilage of crab paste can be caused in some cases. Obviously, eating spoiled crab paste creates health risks. Furthermore, evidence suggests that the bacterial metabolites may induce the disease (Caldwell et al., 2009; Ray, Martinez, Berkowitz, Caldwell, & Caldwell, 2014). Thus, detailed metabolite compositions are important for its quality and safety. In fact, our previous NMR study has already reported the grade-related metabolite compositions of crab paste (Ye, Zhang, Tang, & Yan, 2012). Nevertheless, little information is available regarding the evolution of metabolite profile of crab paste during fermentation, which might be closely related to the quality and freshness of crab paste.

Since nuclear magnetic resonance (NMR)-based metabolomics analysis is capable of simultaneously detecting all ^{1}H containing metabolites with concentrations above tens of micro-molar level, it has been widely and successfully applied in understanding the changes of metabolic profiles of fermented food such as Cheonggukjang (Choi, Yoon, Kim, & Kwon, 2007), soy sauce (Ko, Ahn, van den Berg, Lee, & Hong, 2009), and set-yoghurt (Settachaimongkon et al., 2014). This technique is also powerful for evaluating the quality of fish such as gilt-head bream (*Sparus aurata*) (Picone et al., 2011), mullet roes (Piras, Scano, Locci, Sanna, & Marincola, 2014), and Atlantic salmon fillets (Shumilina, Ciampa, Capozzi, Rustad, & Dikiy, 2015). It is particularly interesting to note that the metabolomics approach has also been used in differentiating of small-molecule metabolic profiles between two grades of crab paste (Ye et al., 2012) and revealing the metabolic response of crab to elevated level of carbon dioxide (Hammer, Pedersen, & Størseth, 2012).

In this study, we systematically analyzed time-dependent metabolite changes in aqueous extracts of crab paste during fermentation using ^{1}H NMR-based metabolomics coupled with multivariate data analysis. Our aim is to reveal the evolution of biochemical compositions of crab paste related to the quality and freshness of crab paste.

* Corresponding authors.
E-mail addresses: yeyangfang@nbu.edu.cn (Y. Ye), chenjuanjuan@nbu.edu.cn (J. Chen).

http://dx.doi.org/10.1016/j.foodchem.2015.07.098
0308-8146/© 2015 Elsevier Ltd. All rights reserved.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, dipotassium hydrogen phosphate trihydrate and sodium dihydrogen phosphate dihydrate (all in analytical grade) were purchased from Sinopharm chemical Co., Ltd. (Shanghai, China). Sodium 3-trimethylsilyl [2,2,3,3-$^2D_4$] propionate (TSP) and Deuterated water (D_2O, 99.9% in D) were purchased from Cambridge Isotope Laboratories. (Miami, FL, USA). Phosphate buffer (K_2HPO_4/NaH_2PO_4, 0.1 M, pH 7.4) was prepared in H_2O containing 90% D_2O to provide an NMR field lock and 0.005% TSP (w/v) as an internal standard (Xiao, Dai, Liu, Wang, & Tang, 2008).

2.2. Sample treatment

Eight alive *P. trituberculatus* (about 200 g) and ingredients including salt, sugar, monosodium glutamate, and liquor were purchased from a supermarket at Ningbo city in Zhejiang province, southeast China. The crabs were kept on the ice and brought into the laboratory in one hour. The crabs were all cleaned with tap water and then cut into pieces, respectively. To make crab paste, each group of crab meat was mixed thoroughly with ingredients including 2% salt, 4% sugar, 1% monosodium glutamate, and 1% liquor and then kept at 4 °C. The crab paste samples were collected at 0 day prior to fermentation and 1, 3, 5, and 7 days after fermentation. All samples were then snap-frozen in liquid nitrogen and stored at −80 °C until metabolomics analysis.

2.3. Metabolite extraction of crab paste

Each crab paste sample (0.5 g) was extracted twice with 600 μL of ice-cold methanol/H_2O (2:1 v/v) solution via discontinuous ultrasonication on wet ice for 150 cycles with each cycle consisting of 2 s sonication and 2 s break. After 10 min centrifugation at 12,000 rpm and 4 °C (Eppendorf centrifuge, International Equipment Company, Dunstable, U.K.), the methanol was removed *in vacuo* and the supernatants were lyophilized. Each sample extract was reconstituted into 600 μL of phosphate buffer. Following 10 min centrifugation at 12,000 rpm and 4 °C, 550 μL of the supernatant from each extract was transferred into a 5 mm NMR tube for NMR analysis.

2.4. NMR analysis

All NMR analyses for crab paste extracts were conducted on a Bruker Avance III 400 MHz spectrometer (Bruker Biospin, Germany). ^{1}H NMR spectra were acquired at 298 K using a NOESYGPPR1D pulse sequence with an inverse probe. The water signal was suppressed with weak continuous wave irradiation during a 2 s relaxation delay and a 100 ms mixing time. Meanwhile, a 90° pulse length was adjusted to approximately 10 μs. Sixty-four transients were collected into 32 k data points for each spectrum with a spectral width of 20 ppm. For metabolite signal assignment purposes, five two-dimensional (2D) NMR spectra were acquired at 298 K for selected samples and processed with similar parameters described previously (Aue, Bartholdi, & Ernst, 1976a, 1976b; Braunschweiler & Ernst, 1983). These 2D spectra included ^{1}H *J*-resolved spectroscopy, ^{1}H–^{1}H correlation spectroscopy, ^{1}H–^{1}H total correlation spectroscopy, ^{1}H–^{13}C heteronuclear single quantum coherence, and ^{1}H–^{13}C heteronuclear multiple bond correlation spectra.

2.5. NMR data processing and multivariate data analysis

One dimensional ^{1}H NMR spectra were multiplied by an exponential function with a line broadening factor of 1 Hz and zero-filled to 128 k prior to Fourier transformation. These spectra were phase- and baseline-corrected manually using TOPSPIN software (Bruker Biospin, Germany) with chemical shift referenced to the TSP signal as δ 0.00.

For multivariate data analysis, the region δ 0.7–9.3 of each one dimensional ^{1}H NMR spectrum was uniformly bucketed into bins with a 2.0 Hz width. The regions δ 4.7–5.1 and δ 3.35–3.37 were removed to eliminate solvent signals. These binned data were further normalized to the total sum of all integrals for each spectrum to compensate for the overall concentration differences prior to the multivariate data analysis. Principal component analysis (PCA) was carried out using the mean-centered data whereas the orthogonal projection to latent structure discriminant analysis (OPLS-DA) was conducted from the unit-variance scaled data with the software package SIMCA-P$^+$ (V12.0, Umetrics, Sweden). For OPLS-DA, 7-fold cross validation was used with NMR data as the *X*-matrix and the group information as the *Y*-matrix. The quality of all OPLS-DA models were evaluated with R^2X and Q^2 values, respectively indicating the explained variations and model predictabilities, and further validated for their robustness with a cross validation-analysis of variance (CV-ANOVA) approach with $p < 0.05$ as significant level (Eriksson, Trygg, & Wold, 2008). The coefficient plots for OPLS-DA models were generated from the back-transformed data (Cloarec et al., 2005) with an in-house developed MATLAB script (MATLAB 7.1, Mathworks Inc., USA). A cutoff value of 0.666 was used in this study and the metabolites with absolute value of *r* above 0.666 were considered to be statistically significant ($p < 0.05$).

3. Results and discussion

3.1. NMR spectra for crab paste extracts

^{1}H NMR spectroscopy was performed first to investigate the metabolic profiling of aqueous extracts of crab paste to obtain a detailed overview of the metabolome of crab paste. Fig. 1 shows the representative ^{1}H NMR spectra of crab paste extracts obtained from aqueous methanol solvent. Twenty-eight metabolites were readily assignable unambiguously according to our previously reported findings (Ye et al., 2012) and published results (Fan, 1996) and further confirmed with a series of 2D NMR spectra. These crab paste metabolites included 13 amino acids (isoleucine, leucine, valine, alanine, methionine, glutamate, lysine, arginine, glycine, tyrosine, phenylalanine, histidine, and tryptophan), six organic acids (lactate, acetate, succinate, taurine, fumarate, and formate), five metabolites of purines and pyrimidines (hypoxanthine, inosine, adenosine diphosphate (ADP), 2-pyridinemethanol, and trigonelline), three organic bases (betaine, trimethylamine (TMA), trimethylamine-N-oxide (TMAO)), and sucrose (Table 1).

Inspection of the spectra from different fermentation time points of crab paste indicates that differed from each other mainly in terms of metabolite concentration. However, a particular attention has been paid in the signal of TMA which was only observed in the crab paste extracts on day 5 and day 7, whereas not in the samples from day 0 to day 3. TMA is a basic aliphatic tertiary amine and its content is strongly correlated to the freshness of fish such as cod (Burt, Gibson, Jason, & Sanders, 1976; Gill, 1990). Fish freshness lowers with the increasing concentration of TMA (Zhao et al., 2002). Hence TMA concentration is one of the most important indicator for the assessment of quality in many marine fish (Botta, Lauder, & Jewer, 1984; Heising, van Boekel, & Dekker, 2014;

Download English Version:

https://daneshyari.com/en/article/7590470

Download Persian Version:

https://daneshyari.com/article/7590470

Daneshyari.com