

Analysis of Amino Acids and Biogenic Amines during the Fermentation of *Jianghong* Yulu

Qi Ningli

Chinese Academy of Tropical Agricultural Sciences
Center for Food Quality Supervision and Testing
Ministry of Agriculture
Zhanjiang, China
e-mail: qiqi120518@163.com

Liu Yangyang

Chinese Academy of Tropical Agricultural Sciences
Agricultural Product Processing Research Institute
Zhanjiang, China
e-mail: hws082319@163.com

Gong Xiao

Chinese Academy of Tropical Agricultural Sciences
Agricultural Product Processing Research Institute
Zhanjiang, China
e-mail: yiyesuifeng@126.com

Zhu Yiting

College of food science and technology
Huazhong Agricultural University
Wuhan, China
e-mail: zhuyiting_1990@163.com

Yuan Xiaoli

Chinese Academy of Tropical Agricultural Sciences
South Subtropical Crops Research Institute
Zhanjiang, China
e-mail: 42839221@qq.com

Abstract—1. **Objective:** *Jianghong* yulu is a traditional seasoning produced through the salting and fermentation of fresh Harengula Zunasi Bleeker in Suixi, South of Guangdong in China. The manufacturing process is quite complex, including two stages, namely brining and spontaneous fermentation. In this work, the physicochemical properties were studied. 2. **Methods:** Amino acids and biogenic amines as the most important precursors of flavor development in *Jianghong* yulu were analyzed using chromatographic methods. 3. **Results:** One-way ANOVA analysis showed that the type and content of amino acids has undergone significant changes during the natural process. 4. **Conclusion:** The results are effective for better understanding the flavor formation and quality controlling of traditional Chinese fermented foods.

Keywords—*Jianghong* yulu; flavor development; amino acids; biogenic amines; quality controlling

I. INTRODUCTION

Yulu, also known as ‘nampla’ in Thailand, ‘nucmam’ in Vietnam, ‘patis’ in the Philippines, and ‘shottsuru’ in Japan, is a traditional seasoning produced through the fermentation of salted fish or shrimp by naturally occurring bacteria and fungi at high salt content 20%-30%, with a fermentation period in excess of 3 months or more, and has been consumed as an important condiment in various cuisines in Southeast and East Asia (Fukui et al., 2012; Park et al., 2001).

Jianghong yulu, traditionally made from Harengula Zunasi Bleeker and sea salt in a sealing terrine, is one of the most representative and characteristic seafood seasoning in Guangdong.

It is necessary to investigate the microbial communities and physicochemical properties in the view of commercial importance. Until now, *Achromobacter*, *Salimicrobium*, *Kocuria*, *Pseudoalteromonas*, *Bacillus*, *Micrococcus*, *Vibrio*, *Staphylococcus*, *Streptococcus*, *Tetragenococcus*, and *Holomonas* were identified and/or isolated from spontaneous fermentation of salted seafood (Jiang et al., 2013). More recently, yulu has been produced commercially by local firms with some slightly different procedures. At the same time, it has received much more attention due to its potential carcinogenicity with relatively high biogenic amines content, whose formation is usually related to the microbial metabolic process (Zaman et al., 2009; Zhai et al., 2012). However, the chemical composition of yulu is different among countries, and its quality is mainly affected by the ingredients used, which could be different varieties of fish, the microflora involved in the environment, and fermentation process. However, no study has been carried out to investigate the flavor development of *Jianghong* yulu, and physiochemical properties have not been studied.

Amino acids are the most important nitrogen compounds in yulu, and have important functions in both nutrition and health, they are key precursors for syntheses of hormones and low-molecular weight nitrogenous substances with each having enormous biological importance. They are also precursors of some volatile compounds, such as higher alcohols, aldehydes, esters and ketonic acids, which make up the majority of aroma (Wang et al., 2014).

Biogenic amines (BAs) are basic nitrogenous compounds of low molecular weight with aliphatic or aromatic structure that are mainly formed through

decarboxylation of amino acids. Most of them have strong physiological effects and play an important biological role as sources of nitrogen and precursors for the synthesis of hormones, alkaloids, nucleic acids, and proteins. Small amounts of BAs are generally bio-synthesized in plant and animal cells, and larger quantities are found as a consequence of microbial metabolism in a wide range of fermented foods. BAs partially contribute to food aroma during food processing. It is important to note, however, it is also potential precursors for the formation of carcinogenic N-nitroso compounds. When excessive amount of BAs was ingested with food, the human body generally showed distinctive toxic effects, such as headache, nausea, palpitations, blood pressure changes, respiratory disorder, and even anaphylactic shock (Stadler et al., 2008).

For these reasons, amino acids and biogenic amines are important for the flavor development of *Jianghong yulu*. In this work, they were analyzed throughout the fermentation process, which will provide more effective information for quality control.

II. Materials and methods

A. Preparation of *Jianghong yulu* and sampling

Jianghong yulu is usually produced during February. In this study, Harengula Zunasi Bleeker, caught off the *Jianghong* gulf located in South of China in Guangdong province, were kept in ice on board and transported to Jianghong town within 3 h after catch. Immediately used for yulu fermentation.

Each fish sample was mixed with solar salt at a ratio 7:3. Each mixture (5 kg) was then packed in a 6-L glass jar (17-cm dia; 27-cm height) and occupied about 90% of total jar volume. Each jar was covered with a glass plate. Fermentation was carried out at ambient temperature (28 °C to 32 °C) for 52 wk and occupied about 90% of total jar volume.

During the naturally fermentation, samples of *Jianghong yulu* prepared in the same year using traditional homemade method were collected. Samples (30 to 40 g) were taken at each time interval and centrifuged at 8000 rpm (PK 121R, ALC Intl. Srl, Cologno Monzese, Italy) at 4 °C for 10 min.

B. Amino acid profiles of *Jianghong yulu* mash

In the amino acid analysis, the lyophilized *Jianghong yulu* mash was hydrolyzed in 6 M HCl for 24 h. Amino acids were quantified using the Hitachi L8800 amino acid analyzer (Hitachi High-Technologies Co., Tokyo, Japan) employing sodium citrate buffers as step gradients with the cation exchange postcolumn ninhydrin derivatization method. The data were described as grams of amino acid per 100 g of sample (Chou et al., 2015).

C. Biogenic amines of *Jianghong yulu* mash

Five grams of food samples was homogenized in a plastic beaker with 0.4 M HClO₄ for 5 min. The slurry was centrifuged at 4 °C for 10 min at 1800 × g and then filtered through a filter paper. The residue was extracted twice, the supernatants were combined and the volume was set to 25 mL with HClO₄ (Šimat & Dalgaard, 2011).

Derivatization of BAs was prepared according to the method of Ben-Gigley (Ben-Gigley et al., 1998). Briefly,

one milliliter of the extract was spiked with 100 µL of internal standard solution (1,7-heptanediamine) and mixed in a plastic test tube with 200 µL of 2.0 M sodium hydroxide, 300 µL of saturated sodium bicarbonate, and 2.0 mL of dansyl chloride solution (10 mg/mL dansyl chloride in acetone). After thoroughly overtaking, the test tube was incubated for 45 min in a water bath at 40 °C with continuous shaking in darkness. Subsequently, 100 µL of 25% ammonium hydroxide was added to each sample for removing residual dansyl chloride. The mixture was complemented to 5.0 mL with acetonitrile after the resulting solution was left for 30 min at room temperature. Finally, the supernatant was filtered through membrane glass fiber filters (0.22 µm, Millipore membrane). The quantitative analysis of amines was separated on UPC₂ methond with a HSS C₁₈ SB column (3.0 × 100 mm, 1.7 µm, Waters, USA) as reported by Gong with some modifications (Gong et al., 2014). The elution gradient (eluent A, CO₂; eluent B, n-hexane:isopropanol:ammonium hydroxide = 70:30:0.25, v/v/v) was: started at 2% B; increased via linear gradient to 30% B at 5 min, and 2% B at 7.2 min. The back pressure was set at 1880 psi. The flow rate was kept at 2.0 ml/min, column temperature at 50 °C, detection wavelength at 254 nm with compensation from 300 to 500 nm, and the injection volume was 1.0 µL. Data processing was performed using Empower 3.0 software.

D. Statistical analysis

Statistical analysis was performed with SPSS 17.0 software (SPSS Inc, Chicago). All data were expressed as mean of at least three determinations for each sample, n = number of samples.

III. RESULTS

A. Qualitative analysis of amino acids

Fig. 1 shows the typical chromatographic profile of amino acid in *Jianghong yulu* mash at 570 and 440 nm. It suggested that the amino acid was efficiency separated, which allowed perfect identification and quantification of amino acids in yulu mash.

During the fermentation process (from stage 1 to 2), the hydrolysis of protein occurs under the action of microorganisms and enzymes, and significant increase in the free amino acids free was observed except glutamate (Table 1). However, the proportion of different amino acids is different after fermentation, with a range of increase from 14 fold (lysine) to 2.01 fold (cystine) were found, which is closely related to changes and raw materials, fermentation conditions and the environment in the microflora and resulting to complex flavors of the yulu.

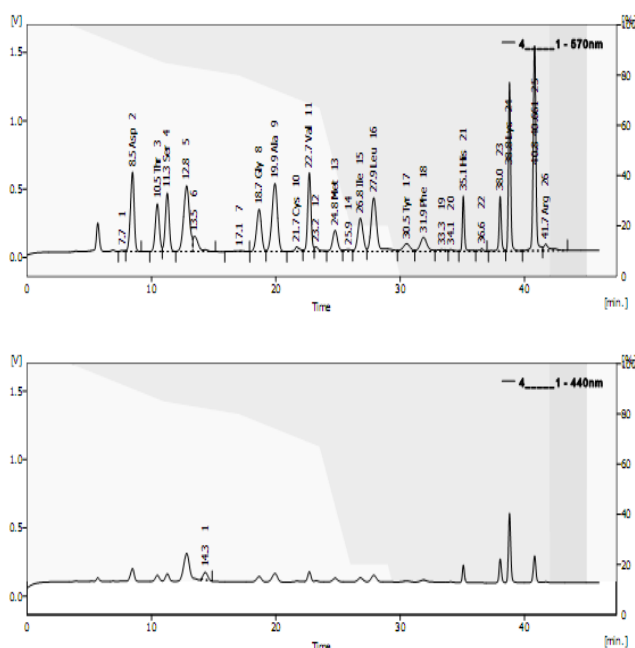


Figure 1. Chromatograms of amino acid in *Jianghong yulu* mash.

Amino acids are important to maintain human life activities substance, which not only has a variety of physiological functions, but most amino acids and their salts have sweet, bitter or sour flavor. Glutamic acid, aspartic acid, phenylalanine, alanine, glycine, and tyrosine can exhibit special taste called umami amino acids. Glutamate is a natural amino acid in the flavor of the strongest material, followed by aspartic acid and its sodium salt. In South of China, traditional fermented yulu products could play an important role in improving food flavor and nutritional reinforcement of our diet.

TABLE 1. AMINO ACIDS CONTENTS (MG 100 G⁻¹) IN THE SELECTED *JIANGHONG YULU* SAMPLES.

| Amino acids | Fermentation periods (n = 3) | |
|---------------|------------------------------|----------------|
| | Stage 1 | Stage 2 |
| Aspartic acid | 1.23 ± 0.04 | 11.61 ± 0.12 a |
| Threonine | 0.54 ± 0.01 | 6.55 ± 0.04 a |
| Serine | 0.48 ± 0.02 | 6.66 ± 0.04 a |
| Glycine | 0.58 ± 0.01 | 4.39 ± 0.09 a |
| Alanine | 0.65 ± 0.03 | 9.37 ± 0.24 a |
| Cystine | 0.59 ± 0.06 | 1.19 ± 0.12 a |
| Valine | 0.92 ± 0.11 | 7.76 ± 0.10 a |
| Methionine | 0.43 ± 0.08 | 4.59 ± 0.10 a |
| Isoleucine | 0.55 ± 0.04 | 6.57 ± 0.08 a |
| Leucine | 0.93 ± 0.05 | 12.10 ± 0.16 a |
| Tyrosine | 0.45 ± 0.03 | 3.06 ± 0.09 a |
| Phenylalanine | 0.54 ± 0.02 | 4.77 ± 0.21 a |
| Histidine | 0.79 ± 0.04 | 4.72 ± 0.06 a |
| Lysine | 1.00 ± 0.03 | 14.77 ± 0.22 a |
| Arginine | 0.71 ± 0.02 | 1.90 ± 0.06 a |
| Glutamine | 1.65 ± 0.01 | - |

a. "a" indicate significant differences
b. "--", not detected.

B. Qualitative analysis of biogenic amines

Fig. 2 shows the typical chromatographic profile of the mixture of standard solutions of the eight BAs and internal standard (IS). It suggested that the derivatization used in this work allowed perfect identification and quantification of BAs. Analysis of food samples takes 10 min for a full column-balancing.

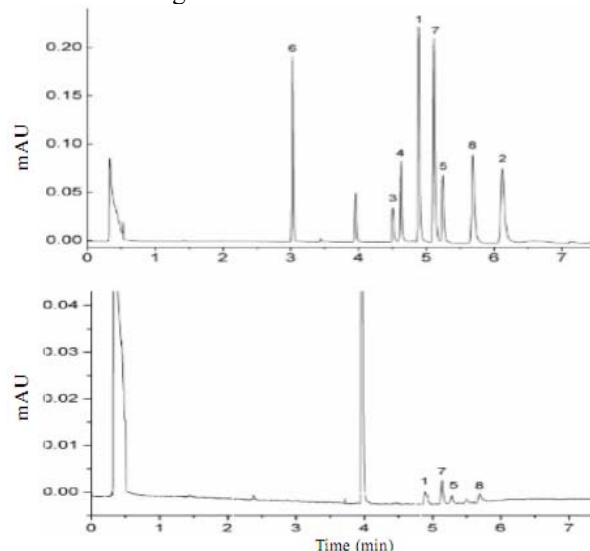


Figure 2. Separation of standard mixture and yulu sample of biogenic amines as dansylamides. Peaks numbering: 1-spermine, 2-spermidine, 3-tyramine, 4-tryptamine, 5-putrescine, 6-phenylethylamine, 7-cadaverine, 8-histamine, and IS-1,7-heptanediamine.

The most important BAs found in the selected foods are histamine, cadaverine, phenylalanine, and putrescine was the major biogenic amines in *Jianghong Yulu*, and their contents significantly increased from stage 1 to 2, with the range of 5.26 to 32.64 mg/kg (Table 2). However, tyramine was not detected after fermentation. It suggested that cadaverine and putrescine provides a reliable indicator of the degree of food freshness and spoilage, and histamine can be regarded as poisoning indicator. The results indicated the changes of good hygienic quality with negligible contents from toxicological point of view, the composition and content of eight biogenic amines in the selected samples are varied among different process periods and batches for evaluated.

TABLE 2. BIOGENIC AMINES CONTENTS (MG 1000 G⁻¹) IN THE SELECTED *JIANGHONG YULU* SAMPLES.

| Biogenic amines | Fermentation periods (n = 3) | |
|------------------|------------------------------|----------------|
| | Stage 1 | Stage 2 |
| Spermine | 0.83 ± 0.04 | 17.25 ± 0.67 a |
| Tyramine | 2.11 ± 1.18 | - |
| Putrescine | 1.58 ± 0.02 | 20.75 ± 0.28 a |
| Cadaverine | 0.71 ± 0.12 | 32.64 ± 0.86 a |
| Histamine | 1.67 ± 0.24 | 23.43 ± 2.89 a |
| phenylethylamine | 6.94 ± 0.98 | 8.19 ± 0.12 a |
| tyramine | 6.41 ± 1.90 | 7.76 ± 0.10 a |
| spermidine | - | 5.26 ± 0.93 a |
| Phenylalanine | 6.02 ± 1.48 a | 11.60 ± 2.41 a |

Although the average content for each amine is within the level limit that may elicit direct adverse reactions,

consumers should be aware of the potential synergistic effect among different amines and limit their consumption at daily diet. Therefore, the analysis of BAs is necessary in order to evaluate their toxicological risk. Anyway, the information obtained in this work will also be useful for quality control of traditional Chinese fermented foods in future.

IV. Discussion

Yulu is one major type of traditional fermented foods in China. In recent years, many national enterprises take local specialty as a development point. In this research those enterprises can have a greater awareness and understanding of local products, and it can also help to popularize traditional foods and further develop local foods. The formation of biogenic amines and changes in the amino acids are closely related.

Intervention in the fermentation process is essential, the desired effect can be achieved by controlling the degree of hydrolysis of amino acids to suppress the generation of biogenic amines, which is important for the protection of *Jianghong* yulu and has a practical meaning.

ACKNOWLEDGMENT

This work was financially supported by grants from the Fundamental Scientific Research Funds for Chinese Academy of Tropical Agricultural Sciences (Project No. 1630062015021), and we thanks Dai Qingpeng for the supply of *Jianghong* yulu samples.

REFERENCES

- [1] C. H. Chou, C. W. Liu, D. J. Yang, Y. H. S. Wu, Y. C. Chen. 2015. "Amino acid, mineral, and polyphenolic profiles of black vinegar, and its lipid lowering and antioxidant effects in vivo," *Food Chem*, vol. 168, pp. 63-69. July 2015.
- [2] Y. Fukui, M. Yoshida, K. i. Shozen, Y. Funatsu, T. Takano, H. Oikawa, Y. Yano, M. Satomi. "Bacterial communities in fish sauce mash using culture-dependent and-independent methods," *J Gen Appl Microbiol*, vol. 58, pp. 273-281. Apr 2012.
- [3] J. N. Park, Y. Fukumoto, E. Fujita, T. Tanaka, T. Washio, S. Otsuka, T. Shimizu, K. Watanabe, H. Abe. "Chemical composition of fish sauces produced in Southeast and East Asian countries," *J Food Compos Anal*, vol. 14, pp. 113-125. Apr 2001.
- [4] M. Z. Zaman, A. Abdulamir, F. A. Bakar, J. Selamat, J. Bakar. "Microbiological, physicochemical and health impact of high level of biogenic amines in fish sauce," *American Journal of Applied Sciences*, vol. 6, pp. 1199-1211. 2009
- [5] H. Zhai, X. Yang, L. Li, G. Xia, J. Cen, H. Huang, S. Hao. "Biogenic amines in commercial fish and fish products sold in southern China," *Food Control*, vol. 25, pp. 303-308. May 2012.
- [6] V. Šimat, and P. Dalgaard. "Use of small diameter column particles to enhance HPLC determination of histamine and other biogenic amines in seafood," *LWT-Food Sci Technol*, vol. 44, pp. 399-406. Aug 2011.
- [7] B. Ben-Gigley, J. M. Vieites Baptista de Sousa, T. G. Villa and V. Barros. "Changes in biogenic amines and microbiological analysis in Albacore (*Thunnus alalunga*) muscle during frozen storage," *Journal of Food Protection*, vol. 61, pp. 608-615. May 1998.
- [8] X. Gong, N. L. Qi, X. X. Wang, L. J. Lin, J. H. Li. "Ultra-performance convergence chromatography (UPC²) method for the analysis of biogenic amines in fermented foods," *Food Chem*, vol. 162, pp. 172-175. Nov 2014.
- [9] W. Jiang, Y. Xu, C. S. Li, X. Y. Dong and D. F. Wang. "Biogenic amines in commercially produced Yulu, a Chinese fermented fish sauce," *Food Addit & Contm B*, vpl. 7, pp. 1-5. Jul 2014
- [10] Y. Q. Wang, D. Q. Ye, B. Q. Zhu, G. F. Wu, C. Q. Duan. "Rapid HPLC analysis of amino acids and biogenic amines in wines during fermentation and evaluation of matrix effect," *Food Chem*, vol. 16, pp. 6-15. Nov 2014.
- [11] Kalač, P., & Krausová, P. "A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods," *Food Chem*, vol. 90, pp. 219-230. Mar 2005.
- [12] R. H. Stadler and D. R. Lineback. "Process-induced food toxicants: occurrence, formation, mitigation, and health risks," 1st ed. New Jersey: John Wiley & Sons. 2008