Flavor Analysis and Strain Exploitation of Fermented Rice Noodles

Di Chen[1], Zhengbiao Gu[1,2,3], Zhaofeng Li[1,2,3], Yan Hong[1,2,3], Li Cheng[1,2,3], Caiming Li[1,2,3*]

[1] School of Food Science and Technology, Jiangnan University, Wuxi, 214122, China

[2] The State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, 214122, China

[3] Collabrative Innovation Center for Food Safety and Quality Control, Jiangnan University, Wuxi,214122, China

Abstract: Fermented rice noodles is popular for its unique flavor, but the study on flavor is lacking, especially the screening of corresponding strains. For the first time, the relationship of microorganism and flavor was unveiled, based on the research of a typical fermented rice noodles from Guilin in China. Flavors were dissected by HPLC-MS, GC-MS, GC-MS-O (Gas chromatography-mass and spectrometry-olfactometry) analyses, while microbial community was investigated by amplicon sequencing and metagenomics, afterwards microorganisms and flavors were connected via correlation analysis and function annotation, hen strains could be explored based on the analyses. Ethanol, 1-butanol,3-methyl-, 2-butanone, 3-hydroxy-, acetic acid and phenylethanol were discovered to be key flavors, biogenic amines were not abundant enough to have bad effect on flavor, but putrescine, tyramine and histamine produced in fermentation needed supervision for safety. Analysis indicated that fermentation was dominated by lactic acid bacteria. Further analysis revealed that Lactobacillus, Acetobacter and Lactococcus were primary creators for these key flavors. In particular, Lactobacillus showed great potential in flavor forming. Additionally, later experiment suggested that samples fermented by L. fermentum and L. plantarum (both belong to Lactobacillus) possessed great flavors, when L. fermentum also showed the ability to inhibit mold and reduce biogenic amine content.

Keywords: Food Science; Rice Noodles; Fermentation; High Throughput Sequencing; Flavor

Introduction

Fermented food plays an important role in people's daily diet because of its long shelf life and rich nutrition and flavor. There are more than 5000 fermented foods in the world, covering staple foods, snacks and beverages[1]. Fermented rice flour is a traditional fermented food in China, which is made from fermented japonica rice. Fermentation will reduce the protein and fat content of rice raw materials and improve the quality of rice.

The purity and crystallinity of 55 rice starch[2,3] therefore, the gelatinization temperature of the raw materials increases, the peak viscosity decreases[3] and the rice flour shows more good taste and color[2,4,5]. However, the flavor of fermented rice noodles has always been a neglected problem. In fact, spontaneous fermentation often produces unpleasant odor due to contamination of miscellaneous bacteria, and mold is easy to breed during rice fermentation. These two problems increase the energy consumption required for raw material cleaning and bring food safety risks, thus hindering the industrial production and promotion of fermented rice flour. In addition, the composition of natural fermentation microorganisms is complex and diverse, and there are many influencing factors, so it is

---

doi: 10.18686/fsr.v2i1.1318

difficult to effectively control. Fermentation is one of the effective ways to realize rice flour fermentation control, and can also achieve the effect of optimizing product texture[6], but strains that can solve various problems in the fermentation process at the same time need to be excavated.

Clarifying the microbial fermentation mechanism is helpful for the directional screening of strains and the effective control of fermentation[7]. However, the natural environment is rich and varied in microflora, so whether it is traditional culture method (separation, culture, identification) or non-traditional analysis method commonly used at present (PCR-DGGE, Rep-PCR, RADP) is difficult to reveal the microbial community structure[8,9] accurately. Moreover, in fermented foods does not necessarily play an important role of dominant bacteria, but functional bacteria[10]. Therefore, more advanced analytical methods are needed to study microorganisms in fermented foods.

High-throughput sequencing can carry out microbial analysis from the gene level, avoiding the limitation of culture method, thus realizing more systematic and comprehensive microbial research. Currently, the commonly used high-throughput sequencing methods include amplicon sequencing and metagenome analysis[7,11]. For example, Wolfe and others[12] used 16S amplicon sequencing and metagenome analysis to study 137 from 10 cities. Site cheese, and pointed out that water is the main factor affecting fermentation. Combining high-throughput sequencing with various statistical methods can screen and develop microorganisms with application potential. For example, the combination of amplicon sequencing and correlation analysis can realize the correlation between species and environmental factors, thus finding the core microorganism[7] of fermentation. Meanwhile, the functional gene[12] possessed by microorganisms can be directly analyzed through functional annotation of metagenome.

In this paper, a kind of Guilin fermented rice flour with good flavor is taken as the research object, and HPLC-MS, GC-MS, GC-MS-O (gas chromatography y-mass and specificity-olfactometry), amplicon sequencing, metagenome and other analysis methods are combined with statistical analysis to clarify the role of microorganisms in flavor formation and screen out strains suitable for rice flour fermentation. This study can provide theoretical guidance for the fermentation control of rice flour and lay a foundation for the large-scale production of fermented rice flour.

1. Materials and methods

1.1 High-throughput sequencing

Amplicon sequencing samples were collected from Guilin Rice Noodle Culture Center. Amplicon sequencing (16S and ITS) collected solid-state fermentation. For the samples of 0 h (0h group), 24 h (24 h group), 48 h (48h group) and 72 h (72 h group), since the raw materials need to be soaked for 24 h before solid state fermentation, the 0 h group refers to the samples soaked for 24 h, and each group is set with three parallel ones. Metagenomic library is constructed by using 72 h group of mixed samples (mixing 3 samples of 72 h group evenly and taking 10 g for DNA extraction) as samples. Aseptic operation was adopted in the collection process. the collected samples were packed in aseptic self-sealing bags and quenched in liquid nitrogen for 10 min, then transported to the laboratory and sequencing company (Shanghai arca biotechnology co., ltd.) in dry ice for subsequent experiments.

The content analysis of biological and flavor substances shows whether there is a synchronous change. When there is a positive and significant correlation between the two, it is believed that microorganisms in actual fermentation may be associated with flavor substances[13]. Gene function analysis integrates species annotation and function annotation information of metagenome. In the analysis process, the spliced genes are attributed to the corresponding KEGG map through function annotation, and then relevant genes are collected in a specific metabolic path. Finally, the owner (i.e. [14] of these genes is found through species annotation. Gene contribution is the percentage of related genes of a species in the total number of functional genes in the library in the statistics of certain functional genes. The greater the gene contribution, the greater the potential of the species in the function. Genetic contribution analysis sample information: NaF: naturally fermented sample in laboratory; PrF: the sample fermented by Guilin yeast head (5%) is added.

1.2 Flavor analysis

Flavor analysis is carried out according to method[15] and actual parameters are slightly changed according to laboratory conditions.

The flavor of samples was analyzed by gas chromatography-mass spectrometry (689N-5975, Agilent) equipped with olfactory detector (ODP). Half of the column flow enters the mass spectrometry detector and half enters the olfaction detector. Choose 2 names (1 male 1 female, accept in advance. The evaluators who are familiar with the flavor of rice noodles will carry out GC-O analysis. The Osme method is used to evaluate the flavor contribution. During the test, the flavor intensity perceived by the assessor is divided into 4 grades: 0 means no smell, 1 means extremely weak smell, 2 means moderate smell intensity, 3 means extremely high smell intensity. Record retention time, flavor intensity and flavor description accurately. GC condition: the column is DB-Wax column (60 m×0.32 mm×0.25 μm, Agilent). THe temperature of the injection port is 250 ℃ , the carrier gas is he, the flow rate is 1.8 mL/min, the column temperature is: the initial temperature is 50 ℃ , then the temperature is raised to 230 ℃ at 5 ℃ /min, and the temperature is kept at 5 min. MS condition is the same as 1.2.2.

1.3 Determination of biogenic amine content

The content of biogenic amine is determined by HPLC-MS, and the determination scheme refers to GB 5009.208-2016[16].

1.4 Sensory evaluation

Sensory evaluation was conducted according to GB/T 10220-2012[17] method, and the preference degree of sample flavor was evaluated by scoring method. Scoring system: 5 points, 5 points (like it very much), 4 points (like it), 3 points (don't like it, don't hate it), 2 points (hate it), 1 point (hate it very much). The total number of evaluators is 20 and the male-female ratio is 1:1. The samples were packed into 10 g/ bags with 4 self-sealing bags, and the samples were coded with random three digits. The codes were changed during each inspection, and the secondary order of samples was randomly distributed. Accurate statistical results and variance analysis were carried out to check whether there were differences in flavor preferences among the samples.

2. Results and discussions

2.1 Flavor composition and contribution of fermented rice noodles

According to reports, the decrease in alcohol content (mainly ethanol) is caused by metabolism of specific microorganisms, for example, acetic acid bacteria can oxidize ethanol to acetic acid[19].

Biogenic amine is also one of the components that make up fermented flavor, and biogenic amine has serious potential food safety hazards. Excessive intake of biogenic amine leads to adverse reactions[20]. Therefore, for the sake of flavor and safety, the experiment has determined it. First of all, in terms of food safety, the detection of biogenic amines in food has not received sufficient attention. At present, only a few countries and organizations have issued relevant limit standards. For example, the limit standards for histamine in fish products are as follows: FDA and EFSA are 50 mg/kg, South Korea is 200 mg/kg, and China is 200-400 mg/kg[21]. A total of 7 biogenic amines, including putrescine, tyramine, histamine, cadaverine, phenethylamine, spermidine and spermine, were detected in the sample. It is noteworthy that the concentrations of putrescine, tyramine and histamine reached 59.92, 52.02 and 32.37 mg/kg.ww (wet weight, moisture content: 42%), respectively. Regubalan[22] detected the same level of putrescine and tyramine in fermented rice cakes, but did not detect histamine.

According to literature reports, fermented rice products have a wide variety of flavor components including alcohols, acids, esters, aldehydes, ketones, alkanes, etc. For example, high contents of ethanol and phenylethanol[23] were detected in fermented rice cakes, and alcohols were also pointed out as the main flavor component[24] in the sour dough. Guilin rice noodles also contain high levels of alcohols, especially through fermentation with laboratory samples (**Figure 1-b**) comparison, it can be found that high content of ethanol, isoamyl alcohol and phenylethanol are the characteristics of Guilin sample (**Figure 1**-c). The 3 flavor substances are all typical flavor substances in white spirit and have pleasant odor, while ethanol has typical alcoholic aroma.

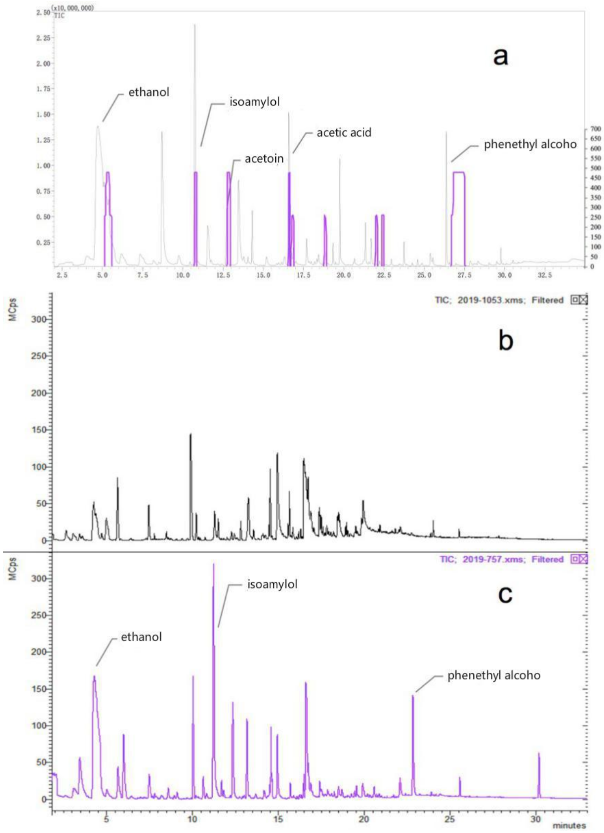

Figure 1. GC-MS-O and GC-MS spectra of fermentation flavor

2.2 Analysis of microbial composition in fermentation process

Microbial composition analysis during fermentation is based on amplicon sequencing. **Figure 2-a** and **2-b** show the species distribution of microorganisms at the genus level. Bacteria including Weiss, Lactococcus, Pediococcus pentosaceus, Leuconostoc, Acetobacter and Lactobacillus occupy a high proportion, of which Weiss occupies a high proportion (≥ 27.03%)), Lactococcus (48 h group has the highest content: 23.03%)), Pediococcus pentosaceus (72 h group has the highest content: 17.42%)), Acetobacter (72 h group has the highest content). The highest abundance was found in 4.78% and lactobacillus (48 h group had the highest content: 13.58%). on the contrary, the abundance of candida albicans (0 h group had the highest content: 16.76%) was higher in the soaking stage. Leuconostoc is more competitive in the environment with high water content, which is consistent with the report by Bruyn[25]. Finally, the abundance of Weiss and Acetobacter in the 72 h group increased significantly ($p < 0.05$). Among fungi, Aspergillus has always occupied an absolute advantage (≥ 26.65%). When the fermentation transits to the solid phase, a small proportion of Trichosporon is detected, and the number of unknown strains is relatively increased.

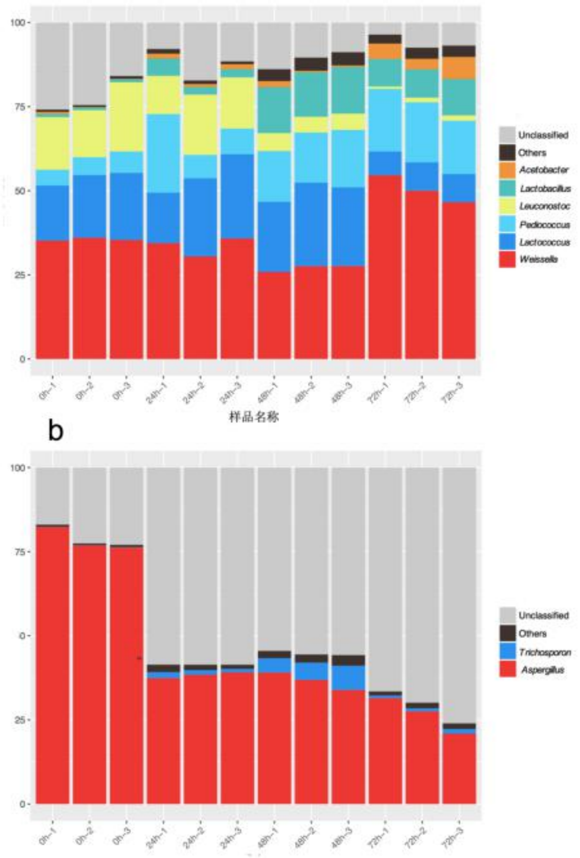

Figure 2. Microbial composition (atgene level) a: microbial composition of bacteria at genus level; B: composition of fungi at genus level.

2.3 Analysis of microbial flavor formation function

Compared with amplicon sequencing, metagenome analysis is aimed at total DNA, so more accurate information[26] on species, metabolism and function of microbial communities can be obtained. Some reports have pointed out that lactobacillus has the function of producing amine[27]. As for tyramine (K01593) and histamine (K01590, K01593), no species with abundance greater than 1.00% have been detected to have related synthetic genes.

The data show that the sample contains a high proportion of Weiss bacteria, but there is little information related to it in functional analysis. Weissbrodt has been detected in many fermented foods, such as soy sauce, white wine, kimchi, lobster sauce, sausage, etc. On the last day of fermentation, the content of Weissbrodt increased, accompanied by the complication of flavor components. Therefore, it is presumed that Weissbrodt plays a certain role in the flavor maturation of fermented rice flour. In addition, high levels of Aspergillus detected in the sample may play an important role in egg white and starch degradation[28].

It is common and beneficial to human health[29] and has the ability to oxidize ethanol and produce organic acids, which is crucial in the formation of fermented flavor[30]. It shows that lactobacillus is the one with the largest gene contribution in ethanol and phenethyl alcohol among the newly added species of PrF, and it even occupies the first place in the gene contribution of phenethyl alcohol, which shows that lactobacillus is probably related to the superior flavor of Guilin rice flour.

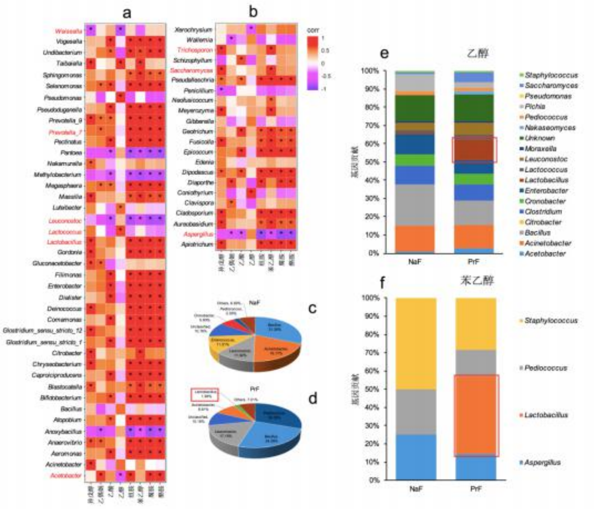

Figure 3. Function analysis for flavora: bacteria and flavor correlation analysis; B: Correlation Analysis of Fungi and Flavors.

The preliminary analysis results confirmed the application potential of Lactobacillus in flavor formation. Therefore, the experiment further explored the lactobacillus. Application effect of bacteria in rice flour fermentation. Four Lactobacillus strains were isolated from Guilin samples and constructed based on 16S rDNA sequences. The phylogenetic tree established showed stable genetic relationship (**Figure 4-a**). the four strains were identified as lactobacillus brevis gl1, lactobacillus casei gl2, lactobacillus fermentum gl3 and lactobacillus plantarum gl4. Microscopically, Lactobacillus brevis and Lactobacillus curvatus are both long rods, Lactobacillus casei is mostly curved, Lactobacillus plantarum and Lactobacillus fermentum are short rods (**Figure 4**-b). The growth curve shows that the logarithmic growth periods of Lactobacillus brevis, Lactobacillus casei, Lactobacillus fermentum and Lactobacillus plantarum are 2 to 18 h, 2 to 16 h, 2 to 8 h and 2 to 12 h, respectively (**Figure 4**-c).

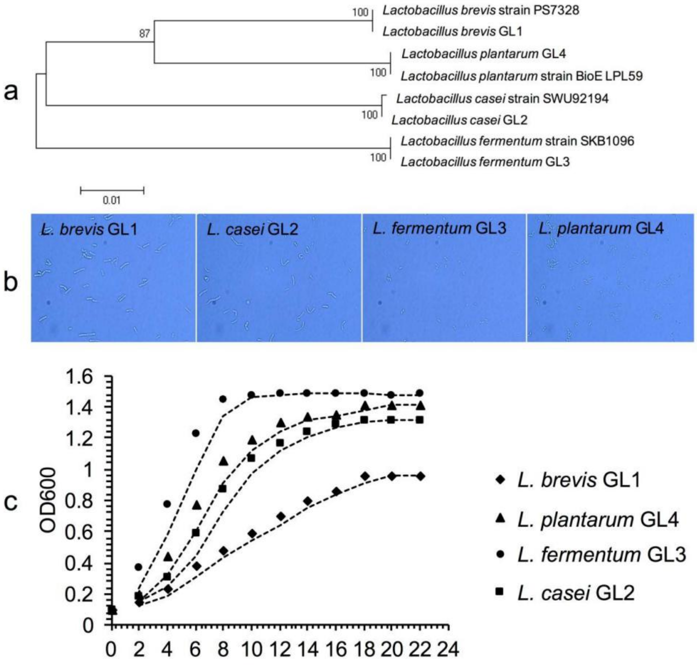

Figure 4. Physical information of strainsa: lactobacillus phylogenetic tree; B: microscopic morphology of lactobacillus; C: growth curve of lactobacillus.

Inoculate the bacterial liquid cultured to logarithmic growth phase with the proportion of 1% (v/w) into the raw materials for intensified fermentation, and the score of flavor preference of the needle is shown in figure 5-a.

the flavor preference of lactobacillus intensified fermentation samples is higher than that of natural fermentation, especially lactobacillus plantarum (L. plantarum) and lactobacillus fermentum (L. fermentum), and the sensory score is significantly different from that of natural fermentation （ $p< 0.05$). In addition, lactobacillus plantarum and lactobacillus fermentum show different mold inhibition characteristics. as shown in **Figure 5-b**, the samples of natural fermentation and lactobacillus plantarum intensified fermentation show mold exceeding the standard one day after fermentation[31], visible mould spots will appear after two days. On the contrary, Lactobacillus fermentum shows good mould inhibition characteristics. No mould exceeding the standard occurred in the fermentation samples during the 6 days. Figures 5-c, 5-d and 5-e show the determination results of volatile flavor substances in the three groups of samples. From the figure, it can be seen that the flavor composition ratio of lactobacillus fortified fermentation samples is from. However, the fermentation is simple, mainly including ethanol, isoamyl alcohol, acetic acid, phenethyl alcohol and other important flavor substances mentioned above, which makes the enhanced fermentation flavor more stable and less prone to generate peculiar smell, and reflects the potential of Lactobacillus plantarum in flavor formation (which also confirms the above analysis); Higher contents of ethanol and acetic acid were detected in the samples subjected to intensified fermentation by Lactobacillus fermentum, which is presumed to be the reason why intensified fermentation by Lactobacillus fermentum can inhibit mold growth[32].

3. Conclusion

Based on the flavor and microbial properties of Guilin fermented rice flour with good flavor, this paper explored the relationship between wind flavor and microbes in fermented rice flour. Ethanol, isoamyl alcohol, acetoin, acetic acid and phenylethanol are the key flavor components, among which high content of ethanol, isoamyl alcohol and phenylethanol are the characteristics of Guilin fermented rice flour samples. In addition, putrescine, tyramine and histamine are potential safety hazards of fermented rice flour. Bacteria including Weiss bacteria, Lactococcus lactis, Pediococcus pentosaceus, Leuconostoc, Acetobacter, Lactobacilli and Aspergillus-dominated fungi are dominant microorganisms in fermentation. Further research shows that Lactobacillus, Lactococcus lactis and Acetobacter play a role in the production of the above key flavor substances, especially Lactobacillus, which has superior application potential in the flavor formation of rice noodles. Among them, Lactobacillus plantarum has the best flavor forming ability, and Lactobacillus fermentum is an ideal rice flour fermentation strain because it can not only impart good flavor to the product, but also inhibit the growth of mold in raw materials and reduce the content of biogenic amine. Therefore, further optimization of the intensified fermentation process of Lactobacillus fermentum is of positive significance to the industrialization of rice flour fermentation. The above results not only provide theoretical guidance for the production of fermented rice flour, but also provide reference for other fermented foods. At the same time. The screening strategy of functional strains and the application of metagenomic analysis in the food field are expanded.

References

1. Tamang J P. Fermented Foods and Beverages of the World. New York: CRC Press, 2010.
2. Lu Z H, Cao W, Peng H H, *et al*. Effect of fermentation metabolites on rheological and sensory properties of fermented rice noodles. Journal of the Science of Food and Agriculture, 2008,88(12): 2134-2141.
3. Lu Z H, Cao W, Peng H H, *et al*. The effects of natural fermentation on the physical properties of rice flour and the rheological characteristics of rice noodles. International Journal of Food Science and Technology, 2005,40(9): 985-992.
4. Lu Z H, Li L L, Cao W, *et al*. Influence of natural fermentation on physico-chemical characteristics of rice noodles. International Journal of Food Science and Technology, 2003,38(5): 505-510.
5. Seo M J, Kang B W, Park J U, *et al*. Noodle Development and Its Quality Characteristics Using Fermented White and Brown Rice. Journal of Life Science, 2012,22(10): 1378-1383.
6. Min Weihong. Study on the mechanism of lactic acid bacteria fermentation to improve the edible quality of rice flour. Beijing: China Agricultural University, 2003.
7. Francesca De Filippis, Eugenio Parente, Danilo Ercolini. Recent Past, Present,and Future of the Food Microbiome. Annual Review of Food Science

and Technology, 2018,9: 589-608.

8. Li Yun, the changes of bacterial phase during the production of fermented rice flour and the influence of fermentation on the quality of rice flour. Beijing: China Agricultural University, 2015.
9. Garcia-Armisen T, Papalexandratou Z, Hendryckx H, *et al*. Diversity of the total bacterial community associated with Ghanaian and Brazilian cocoa bean fermentation samples as revealed by a 16 S rRNA ge ne clone library. Applied Microbiology and Biotechnology, 2010. 87(6): 2281-92.
10. Wang S L, Wu Q, Nie Y, *et al*. Construction of synthetic microbiota for reproducible flavor metabolism in Chinese light aroma type liquor produced by solid-state fermentation. Food Microbiology, 2019,85(10): e03090-18.
11. Zhao Z, Mu T, Sun H. Microbial characterization of five Chinese traditional sourdoughs by high-throughput sequencing and their impact on the quality of potato steamed bread. Food Chemistry, 2019. 274: 710-717.
12. Wolfe B E, Button J E, Santarelli, *et al*. Cheese Rind Communities Provide Tractable Systems for In Situ and In Vitro Studies of Microbial Diversity. Cell, 2014,158(2): 422-433.
13. Zhang J C, Yang Y C, Deng J, *et al*. Dynamic profile of the microbiota during coconut water pre-fermentation for Nata de coco production. LWT - Food Science and Technology, 2017,81: 87-93.
14. Lu Z M, Liu N, Wang L J, *et al*. Elucidating and Regulating the Acetoin Production Role of Microbial Functional Groups in Multispecies Acetic Acid Fermentation. Applied and environmental microbiology, 2016,82(19): 5860-8.
15. Jiang Wenguang. Study on Free Aroma Compounds in Grape of Tonglongzhu from Yantai Production Area. Wuxi: Jiangnan University, 2008.
16. National Health and Family Planning Commission of the People's Republic of China ,GB 5009.208-2016, Determination of Biogenic Amines in National Food Safety Standards,. Beijing : China Standards Publishing House, 2016.
17. National Health and Family Planning Commission of the People's Republic of China, GB/T 10220-2012. General Review of Sensory Analysis Methodology, Beijing: National Standards Publishing House, 2012.
18. Li Z Y, Feng C X, Luo X G, *et al*. Revealing the influence of microbiota on the quality of Pu-erh tea during fermentation process by shotgun metagenomic and metabolomic analysis. Food Microbiology, 2018,76: 405-415.
19. Solieri L,Giudici P. Yeasts associated to Traditional Balsamic Vinegar: Ecological and technological features. International Journal of Food Microbiology, 2008,125(1): 36-45.
20. Shalaby A R. Significance of biogenic amines to food safety and huaman healthy. Food Research International, 1996,29(7): 675-690.
21. Park, Y K, Lee JH, Mah JH. Occurrence and reduction of biogenic amines in traditional Asian fermented soybean foods: A review. Food Chemistry, 2019,278: 1-9.
22. Regubalan B, Ananthanarayan L. Investigation of biogenic amines content in fermented idli batter during storage. Journal of Food Science and Technology, 2019,56(4): 1775-1784.
23. Xiong Qing, Fan Lu, Bao Fang *et al*. Flavor formation and characteristic analysis of rice cake. Food Science, 2011.32 (24): 232-236.
24. Liu N, Chen X, Sun Y *et al*. GC- MS Analysis of the Flavor of Traditional Sour Dough Steamed Bread and the Effect of Adding Edible Alkali on Its Flavor. Food Industry Science and Technology ,2014,16: 76-81.
25. Bruyn F D, Zhang S Y, Pothakos V, *et al*. Exploring the Impacts of Postharvest Processing on the Microbiota and Metabolite Profiles during Green Coffee Bean Production. Applied and environmental microbiology, 2017,83(1).
26. Lyu C Y, Chen C Y, Ge F, *et al*. A preliminary metagenomic study of puer tea during pile fermentation. Journal of Food Agriculture and Environment, 2013,93(13): 3165-74.
27. Park S E, Seo S H, Kim E J, *et al*. Changes of microbial community and metabolite in kimchi inoculated with different microbial community starters. Food Chemistry, 2019,274: 558-565.
28. Li Q, Fang F, Du G. Application of weissbrodt in fermented food. Food and Fermentation Industry, 2017,43(10): 241-246.
29. Tamang, J P, Watanabe K, Holzapfel W H. Review: Diversity of Microorganisms in Global Fermented Foods and Beverages. Front Microbiol, 2016,7: 377.
30. Tao Y, Wang X, Li X Z *et al*. The functional potential and active populations of the pit mud microbiome for 360 the production of Chinese strong-flavour liquor. Microbiol Biotechnology, 2017,10(6): 1603-1615.
31. National Health and Family Planning Commission of the People's Republic of China, GB 7099-2015, National Food Safety Standard Pastries and Bread. Beijing : China Standards Publishing House, 2016.
32. Cui L, Guo W. Bacteriostatic substances produced by lactic acid bacteria and their mechanism of action. Journal of Food Safety and Quality Testing, 2018, 9 (11):2578-2584.