Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

Comparative evaluation of commercial Douchi by different molds: biogenic amines, non-volatile and volatile compounds

Aijun Li[a], Gang Yang[a,b], Zhirong Wang[a,b], Shenglan Liao[a], Muying Du[a,b,c,d], Jun Song[e], Jianquan Kan[a,b,c,d,*]

[a] College of Food Science, Southwest University, Beibei, Chongqing 400715, China
[b] Chinese-Hungarian Cooperative Research Centre for Food Science, Chongqing 400715, China
[c] Laboratory of Quality & Safety Risk Assessment for Agri-products on Storage and Preservation (Chongqing), Ministry of Agriculture and Rural Affairs of the People's Republic of China, Chongqing 400715, China
[d] Chongqing Key Laboratory of Speciality Food Co-Built by Sichuan and Chongqing, Chongqing 400715, China
[e] Shu Xiang Douchi Food Research Institute Limited Company, Chongqing 402160, China

ARTICLE INFO

Article history:
Received 28 April 2022
Received in revised form 22 June 2022
Accepted 15 July 2022
Available Online 1 June 2023

Keywords:
Douchi
Starting strains
Non-volatile compounds
Volatile compounds
Sensory evaluation

ABSTRACT

To provide new insights into the development and utilization of Douchi artificial starters, three common strains (*Aspergillus oryzae*, *Mucor racemosus*, and *Rhizopus oligosporus*) were used to study their influence on the fermentation of Douchi. The results showed that the biogenic amine contents of the three types of Douchi were all within the safe range and far lower than those of traditional fermented Douchi. *Aspergillus*-type Douchi produced more free amino acids than the other two types of Douchi, and its umami taste was more prominent in sensory evaluation ($P < 0.01$), while *Mucor*-type and *Rhizopus*-type Douchi produced more esters and pyrazines, making the aroma, sauce, and Douchi flavor more abundant. According to the Pearson and PLS analyses results, sweetness was significantly negatively correlated with phenylalanine, cysteine, and acetic acid ($P < 0.05$), bitterness was significantly negatively correlated with malic acid ($P < 0.05$), the sour taste was significantly positively correlated with citric acid and most free amino acids ($P < 0.05$), while astringency was significantly negatively correlated with glucose ($P < 0.001$). Thirteen volatile compounds such as furfuryl alcohol, phenethyl alcohol, and benzaldehyde caused the flavor difference of three types of Douchi. This study provides theoretical basis for the selection of starting strains for commercial Douchi production.

© 2024 Beijing Academy of Food Sciences. Publishing services by Tsinghua University Press.
This is an open access article under the CC BY-NC-ND license
(http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Douchi is a traditional soybean product produced via fermentation by various groups of microorganisms and is widely favored by consumers due to its enriched flavor, unique taste, and high nutrient levels[1]. Based on the type of microorganism used, Douchi can be divided into four types, *Aspergillus*-type (e.g., Liuyang Douchi, Yangjiang Douchi, etc.), *Mucor*-type (e.g., Yongchuan Douchi), *Rhizopus*-type (e.g., tempeh), and *Bacterial*-type (e.g., natto)[2]. Mold can secrete abundant hydrolases, which can remarkably change the nutritional structure of soybean products and are extensively employed in the industry to decomposes, flatulence factors in soybean products, such as raffinose and stachyose, reduce levels of antinutrients, including phytic acid and trypsin inhibitor, increase soybean digestibility, inhibit bacterial spoilage, and promote product quality[3,4]. Koji-making and post-fermentation are the two most important stages of Douchi production. The principal function of koji fermentation is the induction and supply of hydrolytic enzymes, which play important roles in the post-fermentation process, degrading soybean proteins, starches, fibers, and other macromolecules into free amino acids, organic acids, soluble sugars, and other molecules to endow Douchi with abundant flavor and high nutrition value[5].

* Corresponding author at: College of Food Science, Southwest University, Chongqing 400715, China.
E-mail address: kanjianquan@163.com (J.Q. Kan)
Peer review under responsibility of Tsinghua University Press.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2022.9250037
2213-4530/© 2024 Beijing Academy of Food Sciences. Publishing services by Tsinghua University Press. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Although Douchi produced by traditional fermentation using natural starter in the koji-making process is typically richer in flavor than that produced by commercial fermentation[5], the post-fermentation period is quite long, often up to 10 months[6], Furthermore, for traditional fermentation, the fungal community is determined by the production environment, making product quality uneven[2]. According to a three-year study (2018−2020)[7], traditional fermentation methods are susceptible to contamination with harmful microorganisms like *Aspergillus flavus* and *Aspergillus parasiticus*, both of which produce a highly toxic secondary metabolism, aflatoxin, which can cause food safety issues and threaten human health. Therefore, commercial fermentation has been widely suggested for Douchi production due to its safety, stability, and minimal-time consumption (usually only one month)[8].

As has been established, the choice of artificial starter contributes considerably to the quality of commercial fermented soybean products. For example, in terms of flavor formation, He et al.[9] found that the levels of organic acids and fatty acids in *Mucor*-type Douchi were significantly higher than those in *Aspergillus*-type Douchi during the koji-making stage, meaning that *Mucor*-type Douchi may be sourer. Meanwhile, umami taste, a characteristic of fermented soybean products, was shown to be highly associated with glutamine levels in *Penicillium*-type soybean paste, but with aspartic acid and asparagine levels in *Aspergillus*-type soybean paste[10]. In addition, previous studies have demonstrated a close relationship between the choice of artificial starters and the presence of harmful factors in many fermented products. In particular, the excessive intake of biogenic amines (BAs), a class of nitrogen-containing organic compounds has been linked to potential toxic effects, such as nausea, vomiting, and dizziness[11]. Our previous study[12] demonstrated that both *Mucor*-type and *Aspergillus*-type Douchi contains ornithine and tyrosine decarboxylase, and the BA levels of *Mucor*-type Douchi were lower than those of *Aspergillus*-type Douchi. Therefore, the choice of starting strains is crucial to Douchi. However, previous research, including our earlier study, focused solely on the one-sided influence of different starting strains on fermented soybean products, with little information available regarding the comprehensive evaluation of Douchi metabolites, taste, flavor, and sensory characteristics by starting strain. The results of one-sided information can not provide reliable starting strain selection for commercial Douchi, and it is also insufficient for the development of artificial starters.

Therefore, this study aimed to comprehensively explore the effects of different molds (*Aspergillus oryzae*, *Mucor racemosus*, and *Rhizopus oryzae*) on the safety, non-volatile compounds, volatile compounds, and sensory of Douchi. Four critical control points (mixing and cooling, koji-making, mixing, post-fermentation) in the fermentation process were selected for comparison. Pearson and PLS analyses were used to explore the contribution components of flavor and taste. The insights provided by this work provide a basis for the selection of starting strains for the production of safe and high-quality commercial Douchi, thus giving a new idea for the development of mixed commercial starter.

2. Material and methods

2.1 Material and chemicals

Soybean and flour were purchased from the Yonghui supermarket (Chongqing, China). All reference standards were at least 98% purity. BAs (putrescine, cadaverine, tryptamine, phenylethylamine, spermine, spermine, tyramine, and histamine) were purchased from Sigma-Aldrich (St. Louis, USA). Soluble sugars (fructose, glucose, maltose, and sucrose) and organic acids (oxalic, tartaric, malic, lactic, acetic, citric, and succinic acids) were purchased from Solarbio (Shanghai, China). Food-grade of sensory reagents (citric acid, sucrose, quinine hydrochloride, sodium chloride, sodium glutamate, and alum), gas chromatography (GC)-grade reagents (2-methyl-butanal, ethyl caproate, γ-nonyl lactone, 2-phenyl ethanol, and p-Methoxybenzaldehyde), high-performance liquid chromatography (HPLC)-grade reagents (acetonitrile and methanol), analytical reagent (AR)-grade reagents (diammonium hydrogen phosphate, phosphoric acid, and sulfosalicylic acid) were purchased from Macklin (Shanghai, China).

2.2 Preparation of raw materials

2.2.1 Preparation of starting culture strains

The pure culture strains used in this study, including *A. oryzae* 2339, *M. racemosus* 40714, and *R. oligosporus* 3152, were purchased from the China Center of Industrial Culture Collection (Beijing, China). Each strain was grown in potato dextrose agar (PDA) at 30 °C for 3 days. Then, spore suspension was generated by addition of 1 mL sterile water after which samples were incubated in a bran medium at 30 °C for 3 days.

2.2.2 Preparation of Douchi

The process of Douchi preparation was performed in detail as described in our previous research, with further optimization[12] (Supplementary Fig. A.1). 5% (*m*/*m*) steamed flour and soybeans were mixed evenly to make koji, and then 8% salt, and 10% rice wine were added. The duration of koji-making is determined by protease activity, as represented by the ability of a sample to convert soybean proteins into small molecules with active ingredients or taste contributions[13], the important factors affecting the quality of koji. Therefore, the time of koji-making of *Aspergillus*-type and *Rhizopus*-type were 64 h, while that of *Mucor*-type was 120 h. The three types of Douchi were cultured until amino acid nitrogen reached stability in the post-fermentation (Supplementary Fig. A.2), so *Aspergillus*-type, *Mucor*-type, and *Rhizopus*-type Douchi were cultured for 22, 27 and 32 days, respectively. The mixtures were then packed into sealed bags and cultured in an incubator at 45 °C[14]. During the koji-making stage, samples were collected regularly at 8 h. During the rapid post-fermentation stage, the degree of change in amino acid nitrogen gradually decreased, so samples were collected at the following time points: 0, 2, 4, 7, 10, 14, 18, 22, 27, and 32 days. Among them, four critical control points (after mixing and cooling, S1; after koji-making, S2; after mixing, S3; after post-fermentation, S4) in the

fermentation process were selected to determine the level of biogenic amines, soluble sugars, and organic acid in the three types of Douchi. All samples were stored at −20 °C until further testing.

2.3 Determination of biogenic amines (BAs)

Qualitative and quantitative analyses of BAs were performed using HPLC (1260, Agilent, USA). The determination was performed as described by Tan et al.[12] using a Zorbax Eclipse XDB C_{18} (250 mm × 4.6 mm, 5 μm) with a UV detector.

2.4 Determination of non-volatile compounds

2.4.1 Neutral protease and amino acid nitrogen

Neutral protease was assessed using the standard method described by GB/T 23527-2009[15]. For each sample, 1 mL diluent was added to a test tube, preheated for 2 min in a water bath at 40 °C, and treated with 1 mL casein before being heated for precisely 10 min. Two mL 0.4 mol/L trichloroacetic acid was added to stop the reaction, after which samples were incubated for additional 20 min in a water bath. The samples were then filtered to remove residual protein, and 1 mL filtrate was transferred to a fresh test tube and combined with 5 mL 0.4 mol/L sodium carbonate. One mL diluted Folin reagent was added with shaking, and samples were incubated at 40 °C for 20 min, followed by measurement at 680 nm. For blank control, one test tube was processed as described above, but with the addition of 2 mL 0.4 mol/L trichloroacetic acid to inactivate the enzyme before addition of casein. Data were obtained by subtracting blank group value from test group values. The experiments were repeated five times, and average values were calculated.

Amino acid nitrogen levels were measured using the formalin titration method[16]. Briefly, the mixed supernatant of the treated sample (10 mL) was added to 60 mL water and titrated to pH 8.2 with 0.05 mol/L NaOH. Then, 10 mL 360 g/L formaldehyde was added to the solution and titrated to pH 9.2 with 0.05 mol/L NaOH. The level of amino nitrogen was obtained based on the consumed volume of 0.05 mol/L NaOH in increasing the pH value from 8.2 to 9.2.

2.4.2 Organic acids and soluble sugars

Organic acids were measured using HPLC (1260, Agilent, USA) with SB-Aq (250 mm × 4.6 mm, 5 μm) and a DAD detector according to the method described in our previous study[17]. Soluble sugars were determined using a Polaris NH_2 (250 mm × 4.6 mm, 5 μm) with a RID detector; about 20 μL of each sample was injected, separation was carried out using a mobile phase of 70% acetonitrile-water, a flow rate of 0.4 mL/min, and column and detector temperatures of 35 °C.

2.4.3 Free amino acid

The free amino acid composition of Douchi samples was measured as described by Suo et al.[18] with slight modifications. For each sample, ground Douchi (200 mg) was weighed, put into a 10 mL plastic tube and vortex-mixed with 2 mL of 4% sulfosalicylic acid solution. After an incubation at 4 °C for 15 h, the mixture was centrifuged at 8 000 r/min for 10 min. The supernatant was filtered through a 0.22-μm aqueous filter, and the filtrate was analyzed using an automatic amino acid analyzer (L-8900, Hitachi, Japan).

2.5 Determination of volatile compounds

The extraction of volatile compounds was manually performed using a headspace-solid phase microextraction (HS-SPME) sampler equipped with 50/30 μm fused silica DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA). GC-mass spectrometry (GC-MS) was used to analyze volatile compounds in three types of Douchi as described by Lu et al.[19] with minor modifications. Each sample was sealed and balanced at 60 °C for 20 min, and then extracted by exposing the fiber to the headspace for 30 min. The extraction fiber was then placed into the GC-MS apparatus (GP201010200, Shimadzu Corporation, Japan) equipped with a DB-5MS column (30 m × 0.25 mm, 0.25 μm, Agilent, USA) in splitless mode. The oven temperature was maintained at 40 °C for 5 min, increased at 5 °C/min to 100 °C, maintained for 5 min, increased at 8 °C/min to 180 °C, maintained for 5 min, increased at 10 °C/min to 250 °C, and maintained for 2 min. Helium served as the carrier gas (1 mL/min).The key MS operating parameters were as follows: the detector temperature was 230 °C; the ion source temperature was 250 °C; the ionization voltage was 70 eV (electron impact ionization), and the scan window during acquisition was 35−500 *m/z*. In addition, a mixture of n-alkane standards (from C_7 to C_{40}) was injected for external retention index calibration. Volatile compounds were identified by comparing their mass spectra to the NIST17 database. *p*-Methoxybenzaldehyde was used as an internal standard for the quantitative calculation of volatile compound levels in three types of Douchi.

2.6 Sensory evaluation

Descriptive sensory analysis (DA) was used to characterize the flavor and taste attributes of Douchi. The panelists (5 men and 7 women, aged 20−28) assessed the sensory characteristics, and then, 12 training sessions were performed within four weeks. The sensory characteristics measured for Douchi included sour, sweet, bitter, salty, umami, astringent, aftertaste, soy sauce aroma, Douchi aroma, fermentation, ester, sweet aroma, and aromatic. As previously reported[20], the reference materials were as follows: sour (0.2 g/L citric acid), sweet (10.0 g/L sucrose), bitter (0.1 g/L quinine hydrochloride), salty (2.5 g/L sodium chloride), umami (2.5 g/L sodium glutamate), astringent (1.0 g/L of alum), aftertaste, soy sauce aroma (commercial soy sauce), Douchi aroma (commercial Douchi), fermentation (10.5 g/L 2-methyl-butanal), ester (15.0 g/L ethyl caproate), sweet aroma (15.0 g/L γ-nonyl lactone) and aromatic (12.5 g/L 2-phenyl ethanol). The samples of three types of Douchi were weighed and placed into a clean opaque plastic cup at room temperature. The samples were presented using three-digit random coding. Each sensory analyst selected one grain with toothpick and held it in their mouth for 30 s to perform taste sensory evaluation. For flavor sensory analysis, sensory personnel first sniffed the reference material and memorized its flavor, and then sniffed the samples with their eyes closed. Each sample was sniffed no more than three times, and the sniffing time was no more than 5 s. A scale of 0−10 with 0.5 increments, where 0 represented none and 10 represented extremely high, was applied to each flavor and taste to provide a measure of intensity.

2.7 Statistical analysis

All measurements were performed at least in triplicate, and data are expressed as mean ± SD. Statistical analyses were carried out with SPSS 24.0 (SPSS Inc, Chicago, IL, USA), using one-way analysis of variance (ANOVA) and the least significant difference (LSD) test with $P < 0.05$ indicating significance. Partial least squares (PLS) and partial least squares discrimination analysis (PLS-DA) were performed using SIMCA 14.1 (Airsense Analytics GmbH).

3. Results and discussion

3.1 Analysis of species and content changes of BAs during fermentation

BAs are primarily formed by enzymatic decarboxylation of amino acids and reductive amination of ketones and aldehydes[21], and have attracted wide concern as a potential hazard factor in fermented soybean products[22]. To evaluate the safety of three types of Douchi, four critical control points were selected to determine the composition and changes of BAs. Tryptamine, histamine, and tyramine were not detected in all groups, but putrescine, cadaverine, spermidine, and spermine were detected in the S1 stage, with total BA levels of 54.02 mg/kg (Table 1). During the fermentation process, the BA content of *Mucor*-type and *Rhizopus*-type Douchi decreased continuously, and the total BA content was 6.28 and 22.57 mg/kg in the S4 stages, respectively (Fig. 1A), which was significantly lower than that in S1 stage. However, the total BA content increased by 3.39-fold for *Aspergillus*-type Douchi in the S2 stage, but decreased by 50.23% in the S4 stage, after which samples were dominated by phenylethylamine and putrescine.

In the S1 stage, a certain level of BAs was detected, mainly spermidine, indicating that the materials affected the BA content. Spermidine itself has no adverse effects on health, but it may react with nitrite to form carcinogenic nitrosamines, which are easily absorbed by the gastrointestinal tract and can inhibit the catabolism of toxic amines such as Tyramine and Tryptamine to increase toxicity[23]. Interestingly, the spermidine content in the S4 stage was detected as 0.33−5.99 mg/kg, potentially due to the degradation of spermidine by microbial metabolism during the fermentation process[24]. In previous reports[25], it was shown that the BA content of traditional fermented Douchi was 43.0−2 067.56 mg/kg, while the BA content of the final product in this study was 6.28−94.65 mg/kg, which was much lower than that of traditional fermented Douchi. Therefore, commercial fermentation can effectively reduce BA content. In this study, pure strain was used to make koji, which reduced the pollution of exotic microorganisms, thus reduced the levels of BA compared with traditional fermentation. In addition, according to the study of Benkerroum[26], BAs were controlled by reducing ripening times, thus reducing the amount of proteolytic activity to limit the availability of precursor amino acids. Commercial fermentation adopted in this study has achieved the effect of limiting BAs levels by obtaining products with the shortest fermentation time. The BA content of *Aspergillus*-type Douchi was higher than those of the other two types of Douchi due to the differences in the free amino acid content of the BA precursor, consistent with our previous research results[12]. This study detected the presence of phenethylamine, which may be related to the different process conditions, as the addition of wheat flour may provide more synthetic BA precursors[27]. Previous reports[28] showed that a total food amine content of more than 1 000 mg/kg may be harmful to health. The United States Food and Drug Administration (USFDA) guidelines suggest a threshold of 50 mg/kg for histamine in seafood[29]. This study did not detect histamine, and the total BA content was far below the recommended limit, indicating that the three types of Douchi are relatively safe.

3.2 Analysis of non-volatile compounds

3.2.1 Soluble sugars

The presence of soluble sugars makes Douchi have a kind of eating back to sweet taste. During the fermentation process, starch is hydrolyzed to produce glucose and fructose, resulting in a decrease in

Table 1
The content of biogenic amines in Douchi with different molds.

Group	Sample	Biogenic amines (mg/kg)								
		Putrescine	Cadaverine	Tryptamine	Phenethylamine	Spermidine	Spermine	Histamine	Tyramine	Total biogenic amines
Aspergillus-type	S1	7.59 ± 0.27^{Ad}	2.59 ± 0.04^{Ab}	ND	ND	34.61 ± 3.07^{Aa}	9.24 ± 1.97^{Aa}	ND	ND	54.02
	S2	83.69 ± 0.76^{Aa}	5.36 ± 0.56^{Aab}	ND	123.55 ± 6.13^{Aa}	21.56 ± 0.43^{Ab}	2.96 ± 0.04^{Ab}	ND	ND	237.12
	S3	67.21 ± 1.95^{Ab}	5.86 ± 0.13^{Aa}	ND	108.77 ± 3.48^{Aa}	8.32 ± 0.41^{Ac}	ND	ND	ND	190.16
	S4	40.37 ± 0.46^{Ac}	3.48 ± 0.68^{Aab}	ND	44.80 ± 0.63^{Ab}	5.99 ± 0.58^{Ac}	ND	ND	ND	94.65
Mucor-type	S1	7.59 ± 0.27^{Ab}	2.59 ± 0.04^{Aa}	ND	ND	34.61 ± 3.07^{Aa}	9.24 ± 1.97^{Aa}	ND	ND	54.02
	S2	26.27 ± 2.55^{Ca}	ND	ND	ND	7.60 ± 0.01^{Bb}	ND	ND	ND	33.87
	S3	22.53 ± 0.87^{Ca}	ND	ND	ND	1.50 ± 0.25^{Bb}	ND	ND	ND	24.03
	S4	0.83 ± 0.22^{Cc}	ND	ND	4.29 ± 0.11^{Ba}	1.16 ± 0.16^{Bb}	ND	ND	ND	6.28
Rhizopus-type	S1	7.59 ± 0.27^{Ac}	2.59 ± 0.04^{Ab}	ND	ND	34.61 ± 3.07^{Aa}	9.24 ± 1.97^{Aa}	ND	ND	54.02
	S2	55.24 ± 4.08^{Ba}	2.46 ± 0.02^{Bb}	ND	ND	1.91 ± 0.08^{Cb}	ND	ND	ND	59.61
	S3	42.87 ± 2.96^{Bb}	5.33 ± 0.08^{Ba}	ND	ND	1.96 ± 0.77^{Bb}	ND	ND	ND	50.16
	S4	13.28 ± 0.18^{Bc}	2.15 ± 0.94^{Bb}	ND	6.82 ± 0.79^{Ba}	0.33 ± 0.01^{Bb}	ND	ND	ND	22.57

S1: After mixing and cooking stage; S2: After koji-making stage; S3: After mixing stage; S4: After post-fermentation. Data are expressed as mean ± SD ($n = 3$). ND: not detected. Means with different lower case letters with in the same column for one group stand for significant differences ($P < 0.05$). Means with different upper case letters with in the same column for the same fermentation time point among different groups stand for significant differences ($P < 0.05$).

sucrose and an increase in glucose[5]. No maltose was detected during the fermentation process (Supplementary Table A.1). Three kinds of soluble sugars, sucrose, fructose, and glucose, were detected in the S1 stage, with a total soluble sugar content of 51.69 mg/g, and the sucrose content was (32.23 ± 0.15) mg/g due to the flour and soybean starch. In the S2 stage, starch was hydrolyzed into glucose by the action of glycosidase, leading to a decrease in sucrose content and a rapid increase in glucose content (Fig. 1B). Sucrose was not detected in *Aspergillus*-type and *Mucor*-type Douchi in the S2 stage, at the same time, the glucose content in the three types of Douchi increased from (8.82 ± 2.52) mg/g in the S1 stage to (124.39 ± 6.61)–(153.15 ± 3.67) mg/g in the S4 stage, at least partially due to the addition of rice wine in the S3 stage. In addition, in the post-fermentation stage, soluble sugar is consumed by the Maillard reaction and microorganism metabolism, resulting in the decrease of glucose in *Aspergillus*-type Douchi[30]. In the finish products, the soluble sugar contents of three types of Douchi were all found to be dominated by glucose, with total soluble sugar levels of *Aspergillus*-type, *Mucor*-type, and *Rhizopus*-type Douchi were 126.11, 166.19, and 131.42 mg/g, respectively, which were significantly different ($P < 0.05$). Based on the study of Han[31], both glucose and fructose were detected in the doenjang fermentation, and the final product was dominated by fructose, which was inconsistent with the results in this study and this may be due to the difference in fermentation process, resulting in higher glucose content in the final product.

3.2.2 Organic acid

Furthermore, organic acids not only provide a sour taste, which gives a mellow taste to the Douchi, but also are the main factors inhibiting the growth of pathogens and microorganisms that cause spoilage, helping prolong the shelf life of fermented products[32]. During the fermentation process, organic acids are continuously produced, and ester formation was consumed to a certain extent during the post-fermentation stage. In the S1 stage, tartaric, acetic, and citric acid were detected, and the total organic acid content was 3.30 mg/g (Supplementary file Table A.2). As shown in Fig. 1C, the organic acid content of three types of Douchi first increased, and then decreased, primarily driven by changes in citric, lactic, and acetic acid. The addition of rice wine in the S3 stage increased the contents of lactic acid, malic acid, and succinic acid. Lactic acid was the main organic acid present in the final products of three types of Douchi, and there was no significant difference ($P > 0.05$) between its content in the three types of Douchi, with levels falling between (16.17 ± 0.41)–(17.38 ± 0.28) mg/g. In the S2 stage, the acetic acid content of *Mucor*-type and *Rhizopus*-type Douchi increased rapidly by 2.50- and 6.65-fold, respectively, while the acetic acid content of *Aspergillus*-type Douchi had no significant ($P > 0.05$) change. However, in the S4 stage, the acetic acid levels of *Mucor*-type and *Rhizopus*-type Douchi decreased significantly ($P < 0.05$), while the acetic acid content of *Aspergillus*-type Douchi increased. In the final product, the levels of acetic and succinic acids were significantly different ($P < 0.05$) between the three types of Douchi. *Aspergillus*-type Douchi had a higher acetic acid content ((9.18 ± 0.25) mg/g), while succinic acid ((8.44 ± 0.04) mg/g) was most abundant in *Rhizopus*-type Douchi. From the study of Liu[30], acetic acid and lactic acid were common organic acids in fermented soybean products, showing consistency with this study, suggesting that heterolactic acid fermentation is the major process during fermentation. According to previous studies[30,31], the acetic acid content is related to the presence of bacterial communities such as *Tetragenococcus*, *Pediococcus*, *Weissella*, and *Enterococcus*, while succinic acid content is positively correlated with *Lactobacillus*. Due to the different environments associated with molds, different bacterial communities were present in the three types of Douchi, leading to differences in organic acid distribution. *Aspergillus*-type Douchi contained more acetic acid, making it sourer, simultaneously, the presence of succinic acid provides an umami taste for *Rhizopus*-type Douchi[33].

3.2.3 Free amino acid content

Free amino acids and their nitrogen-containing degradation products are important flavor compounds and precursors in fermented foods and are an important index to assess the flavor of a fermented

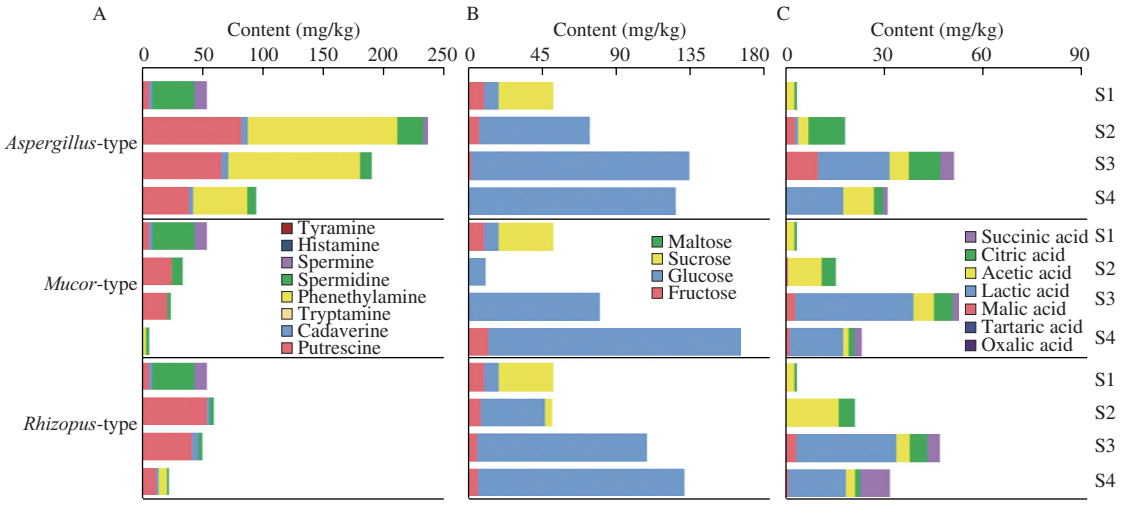


Fig. 1 Changes of non-volatile compounds at four key points in the process of fermenting Douchi with different molds (A): biogenic amine; (B): soluble sugar; (C): organic acid; *: $P < 0.05$; **: $P < 0.01$; S1: After mixing and cooling; S2: After koji-making; S3: After mixing-stage; S4: After post-fermentation.

product[34]. According to the contribution of free amino acid to the taste, it can be divided by taste into umami, sweet, bitter, sour, and tasteless categories[35]. Glutamic acid, leucine, and arginine were the most abundant free amino acids in the three types of Douchi. There were extremely significant differences ($P < 0.001$) in free amino acid levels among them (Table 2). The content of free amino acids in *Aspergillus*-type Douchi was significantly higher than those of the other two types of Douchi ($P < 0.01$) (Table 2), consistent with the results of our previous study[12], related to the higher protein activity of *Aspergillus*-type Douchi in the S2 stage (Supplementary Fig. A.2). Comparing the proportion of different taste amino acids in total free amino acids, umami amino acids of *Aspergillus*-type and *Mucor*-type Douchi represented 18.01% and 18.20% of the total free amino acids, respectively, which were significantly higher than that of *Rhizopus*-type Douchi ($P < 0.05$), at the same time, the percentage of sweet amino acids of *Aspergillus*-type Douchi was 24.47%, which was significantly higher than those of the other two types of Douchi, implying more prominent umami and sweet taste. Notably, bitter amino acids of *Rhizopus*-type Douchi represented 62.29% of the total free amino acids, indicating a more bitter taste. The higher protease capacity of *Aspergillus*-type Douchi did not increase the proportion of bitter free amino acids in total free amino acids, in contrast, *Aspergillus*-type Douchi accumulated more umami and sweet free amino acids in the fermentation process, therefore, the consumption of bitter free amino acids may be related to the formation of melanoid in Maillard reaction[36].

Table 2
Free amino acid content of Douchi with different molds.

Taste	FAA	Free amino acid (mg/kg)		
		Aspergillus-type	*Mucor*-type	*Rhizopus*-type
Umami (U)	Asp***	3 068.85 ± 22.08^a	1 679.54 ± 5.06^b	712.40 ± 8.50^c
	Glu**	4 275.76 ± 30.24^a	2 849.52 ± 7.23^c	2945.32 ± 31.21^b
	U/TFAA (%)	18.01 ± 0.06^a	18.20 ± 0.03^a	16.87 ± 0.26^b
Sweet (S)	Thr***	2 105.13 ± 18.72^a	1 102.36 ± 1.26^b	951.81 ± 10.12^c
	Ser***	2 192.82 ± 16.63^a	1 118.95 ± 1.92^b	747.33 ± 9.16^c
	Gly***	1 287.79 ± 12.92^a	585.13 ± 0.98^b	372.63 ± 4.38^c
	Ala***	2 516.78 ± 19.47^a	1 450.68 ± 2.32^b	1 295.09 ± 14.35^c
	Pro***	1 878.18 ± 22.63^a	1 257.65 ± 3.66^b	1 189.73 ± 16.30^c
	S/TFAA (%)	24.47 ± 0.04^a	22.16 ± 0.02^b	21.02 ± 0.31^c
Bitter (B)	His***	1 081.25 ± 12.07^a	665.64 ± 7.83^b	399.41 ± 6.84^c
	Ile***	2 440.65 ± 20.98^a	1 373.26 ± 3.44^b	1 264.59 ± 14.52^c
	Leu***	4 500.18 ± 31.82^a	2 790.66 ± 6.81^b	2 629.62 ± 3.63^c
	Val***	2 099.14 ± 15.18^a	1 312.76 ± 1.82^b	1 215.45 ± 13.82^c
	Tyr***	2 959.11 ± 54.55^a	1 989.58 ± 3.63^b	1 883.43 ± 18.48^c
	Arg***	3 921.94 ± 29.30^a	2 195.82 ± 4.65^b	1 911.00 ± 23.11^c
	Met***	726.58 ± 5.75^a	517.46 ± 0.98^b	457.38 ± 3.96^c
	Lys***	2 346.73 ± 1.60^a	1 472.11 ± 1.38^b	1 336.76 ± 0.85^c
	Phe**	3 303.51 ± 19.85^a	2 475.15 ± 9.50^c	2 572.92 ± 30.65^b
	B/TFAA (%)	57.32 ± 0.02^c	59.45 ± 0.05^b	62.29 ± 0.01^a
Tasteless (T)	Cys**	85.57 ± 8.71^a	44.65 ± 5.89^c	49.78 ± 13.87^b
	T/TFAA (%)	0.21 ± 0.01^b	0.18 ± 0.01^c	0.23 ± 0.01^a
Total-FAA (TFAA)		40 789.98	24 880.91	22 088.62

Data are expressed as mean ± SD ($n = 3$); ND: not detected; **: $P < 0.01$; ***: $P < 0.001$. Means with different lower case letters with in the same free amino acid of different groups for significant differences ($P < 0.05$). Asp: aspartic acid; Glu: glutamate; Thr: threonine; Ser: serine; Gly: glycine; Ala: alanine; Pro: proline; His: histidine; Ile: isoleucine; Leu: Leucine. Val: valine; Tyr: tyrosine; Arg: arginine; Met: methionine; Phe: phenylalanine; Cys: cysteine; Lys: lysine.

3.3 Analysis of volatile compounds

Volatile compounds are believed to be produced by the Maillard reaction, Strecker degradation, oxidative thermal degradation of fatty acids and microbial catabolism[34]. Volatile compound content of Douchi with different molds were shown in Tables 3. In this study, a total of 67 volatile substances were detected (Fig. 2A), including 11 alcohols, 5 acids, 11 aldehydes, 9 alkenes, 14 esters, 4 pyrazines, and 13 other substances. Of these, 26 volatile compounds were common among all three types of Douchi, and 9, 7, and 9 volatile compounds were unique to *Aspergillus*-type, *Mucor*-type, and *Rhizopus*-type, respectively. Generally, alcohols are formed during fermentation, and their synthesis is linked to amino acid metabolism and sugar metabolism of yeast. 1-octen-3-ol, maltol, and phenethyl alcohol were detected in the three types of Douchi, described as mushroom-like, flower, and caramel-like aroma, respectively[37,38]. 2,3-Butanediol and 3-methyl-1-butanol are the characteristic flavors substances of the fermented product, to endow Douchi fruit with sweet aroma[39]. The contents of ethanol and 3-methyl-1-butanol in *Mucor*-type Douchi were (13.51 ± 1.29) and (10.78 ± 0.29) μg/g, respectively, and the content of 2,3-butanediol in *Rhizopus*-type Douchi was (4.70 ± 0.34) μg/g, but they were not detected in *Aspergillus*-type Douchi.

Aldehydes are mainly produced by lipid oxidation and amino acid degradation, and exhibit lower thresholds, so they exert more impact on the flavor[40]. Benzaldehyde exhibits the smell of bitter almonds, which is not desired. There was a significant difference ($P < 0.05$) in its content among the three types of Douchi, and the content of *Mucor*-type Douchi was the highest ((3.65 ± 0.16) μg/g). *Aspergillus*-type Douchi contains more 2-methyl butyraldehyde ((9.17 ± 0.61) μg/g), which gives a malty flavor[38]. Aldehydes can be converted into acid by aldehyde dehydrogenase. The content of acetic acid in *Aspergillus*-type Douchi was up to (5.43 ± 0.13) μg/g, while it was not detected in *Mucor*-type and *Rhizopus*-type Douchi.

As a typical source of aroma in fermented products, the variety and content of esters have a major impact on the flavor of Douchi[41]. Among the esters detected, the types and contents of esters in *Mucor*-type Douchi were more abundant. The content of ethyl acetate in *Mucor*-type Douchi was up to (9.92 ± 0.52) μg/g, similar to that of cheese and yogurt. In addition, as the unique pyrazines of *Rhizopus*-type Douchi, 2,3-dimethyl pyrazine, 2,3,5-trimethyl pyrazine, and tetramethyl pyrazine have been reported to contribute to nutty flavor[37].

To assess the differences in flavor between the three types of Douchi, volatile profiles were analyzed using partial least squares discrimination analysis (PLS-DA). The Score Plot (Fig. 2B) showed that the three types of Douchi exhibited good parallelism, and the variance explanation rate of this model was $R_X^2 = 0.736$ (R_X^2 (1) = 0.4, R_X^2 (2) = 0.336). PLS-DA can be used to successfully separate three types of Douchi based on GC-MS analysis data. *Mucor*-type Douchi had the highest score on P1, indicating that *Mucor*-type Douchi contributed substantially to P1. While *Rhizopus*-type Douchi exhibited a positive load on P2, *Aspergillus*-type Douchi exhibited a negative load, indicating a major difference between *Rhizopus*-type Douchi and *Aspergillus*-type Douchi on P2.

3.4 Analysis of sensory evaluation

The descriptive sensory analysis results indicated that all three

Table 3
Volatile compound content of Douchi with different molds.

Compounds	RI	RT (min)	CAS	Relatively Content (μg/g)		
				Aspergillus-type	*Mucor*-type	*Rhizopus*-type
Alcohols						
ethanol	463	1.525	64-17-5	ND	13.51 ± 1.29[a]	3.31 ± 0.14[b]
3-methyl-1-butanol	697	3.767	123-51-3	ND	10.78 ± 0.29[a]	2.13 ± 0.25[b]
1-heptyn-3-ol	877	3.824	7383-19-9	0.15 ± 0.02[a]	ND	ND
2,3-butanediol	743	5.455	24347-58-8	ND	0.32 ± 0.11[b]	4.70 ± 0.34[a]
furfuryl alcohol	885	7.716	98-0-0	0.43 ± 0.04[b]	2.02 ± 0.70[a]	ND
methionol	912	12.375	505-10-2	ND	0.40 ± 0.01[a]	0.33 ± 0.03[b]
1-octen-3-ol	969	12.486	3391-86-4	1.17 ± 0.04[b]	2.02 ± 0.22[a]	0.84 ± 0.08[b]
benzyl alcohol	1 036	14.365	100-51-6	ND	0.17 ± 0.03[b]	0.67 ± 0.03[a]
linalool	1 082	16.568	78-70-6	0.68 ± 0.08[a]	0.75 ± 0.02[a]	0.46 ± 0.01[b]
maltol	1 063	16.769	118-71-8	0.32 ± 0.05[b]	0.20 ± 0.02[b]	0.53 ± 0.07[a]
phenethyl alcohol	1 136	16.933	60-12-8	0.27 ± 0.06[c]	3.70 ± 0.60[a]	1.53 ± 0.05[b]
Acids						
acetic acid	576	2.263	64-19-7	5.43 ± 0.13[a]	ND	ND
isobutyric acid	711	4.465	79-31-2	0.11 ± 0.00[b]	ND	0.41 ± 0.07[a]
2-methylbutyric acid	811	8.22	116-53-0	0.32 ± 0.03[b]	0.43 ± 0.09[b]	0.96 ± 0.02[a]
4-oxopimelic acid	1 566	8.434	502-50-1	ND	0.21 ± 0.07[b]	1.65 ± 0.20[a]
isovaleric acid	811	8.586	503-74-2	ND	ND	0.41 ± 0.07[a]
Aldehydes						
2-methylbutyraldehyde	643	2.531	96-17-3	9.17 ± 0.61[a]	7.73 ± 0.89[a]	1.85 ± 0.24[b]
3-furaldehyde	831	6.783	498-60-2	0.88 ± 0.12[a]	ND	ND
3-(methylthio)propionaldehyde	858	9.647	3268-49-3	0.31 ± 0.02[a]	0.39 ± 0.06[a]	0.31 ± 0.02[a]
benzaldehyde	982	11.675	100-52-7	1.74 ± 0.11[c]	3.65 ± 0.16[a]	2.57 ± 0.19[b]
phenylacetaldehyde	1 081	14.624	122-78-1	1.81 ± 0.18[a]	2.04 ± 0.08[a]	1.53 ± 0.17[b]
(*E*)-2-octenal	1 013	15.153	2548-87-0	0.94 ± 0.08[a]	ND	ND
phenylglyoxal	1 217	15.642	1074-12-0	0.29 ± 0.03[a]	0.27 ± 0.05[a]	ND
5-methyl-2-thiophene carboxaldehyde	1 072	17.149	13679-70-4	ND	0.29 ± 0.11[a]	ND
2-phenyl-2-butenal	1 265	23.695	4411-89-6	0.20 ± 0.02[b]	0.33 ± 0.02[a]	0.29 ± 0.00[a]
4-methyl-2-phenyl-2-pentenal	1 400	26.844	26643-91-4	0.19 ± 0.03[a]	ND	ND
cocal	1 499	29.527	21834-92-4	0.31 ± 0.06[a]	0.07 ± 0.00[b]	0.06 ± 0.00[b]
Alkenes						
3-thujene	902	10.346	2867-05-2	0.21 ± 0.00[ab]	0.60 ± 0.20[a]	0.10 ± 0.01[b]
α-pinene	948	10.565	7785-70-8	0.09 ± 0.00[a]	0.22 ± 0.07[a]	ND
sabenene	897	12.079	3387-41-5	0.83 ± 0.01[a]	0.75 ± 0.48[a]	0.54 ± 0.06[a]
myrcene	958	12.759	123-35-3	1.09 ± 0.10[b]	2.03 ± 0.13[a]	1.08 ± 0.15[b]
2-carene	948	13.676	554-61-0	0.23 ± 0.03[b]	0.46 ± 0.06[a]	0.17 ± 0.01[b]
3,7,7-trimethyl-1,3,5-cycloheptatriene	1 010	13.955	3479-89-8	ND	ND	0.20 ± 0.00[a]
γ-terpinene	998	15.113	99-85-4	ND	0.51 ± 0.09[a]	0.20 ± 0.00[b]
trans-anethole	1 190	24.418	4180-23-8	0.13 ± 0.01[a]	0.12 ± 0.02[a]	0.10 ± 0.01[a]
l-caryophyllene	1 494	28.152	87-44-5	ND	ND	0.25 ± 0.02[a]
Esters						
ethyl acetate	586	2.085	141-78-6	ND	9.92 ± 0.52[a]	ND
ethyl butyrate	785	5.673	105-54-4	ND	0.86 ± 0.07[a]	ND
ethyl lactate	848	6.033	97-64-3	ND	0.39 ± 0.26[a]	ND
ethyl 2-methylbutyrate	820	7.483	7452-79-1	ND	0.33 ± 0.12[b]	0.66 ± 0.05[a]
isoamyl acetate	820	8.56	123-92-2	ND	0.45 ± 0.12[a]	ND
4-hydroxyphenyl phosphonic acid	987	12.643	33795-18-5	ND	ND	0.80 ± 0.06[a]
ethyl caproate	984	13.141	123-66-0	ND	2.15 ± 0.15[a]	ND
ethyl benzoate	1 160	19.031	93-89-0	0.11 ± 0.00[b]	0.31 ± 0.04[a]	0.17 ± 0.02[b]
ethyl phenylacetate	1 259	22.585	101-97-3	0.07 ± 0.00[c]	0.16 ± 0.00[b]	0.20 ± 0.00[a]
methyl *cis*-7-hexadecenoate	1 886	38.407	56875-67-3	0.16 ± 0.01[c]	0.55 ± 0.02[a]	0.24 ± 0.00[b]
methyl hexadecanoate	1 878	38.898	112-39-0	0.11 ± 0.01[b]	0.21 ± 0.00[a]	0.12 ± 0.00[b]
ethyl palmitate	1 978	40.199	628-97-7	0.32 ± 0.02[b]	0.84 ± 0.09[a]	0.31 ± 0.03[b]
methyl octadeca-9,12-dienoate	2 292	42.606	2566-97-4	0.24 ± 0.01[b]	0.55 ± 0.05[a]	0.14 ± 0.02[b]
(*Z*)-ethyl heptadec-9-enoate	2 085	42.688	1089325-29-0	0.15 ± 0.01[b]	0.35 ± 0.03[a]	0.14 ± 0.02[b]

Table 3 (*Continued*)

Compounds	RI	RT (min)	CAS	Relatively Content (μg/g)		
				Aspergillus-type	*Mucor*-type	*Rhizopus*-type
Pyrazine						
2,5-dimethyl pyrazine	894	9.872	123-32-0	ND	0.85 ± 0.04[a]	ND
2,3-dimethyl pyrazine	894	10.06	5910-89-4	ND	ND	0.19 ± 0.00[a]
2,3,5-trimethyl pyrazine	1 008	13.144	14667-55-1	ND	ND	1.98 ± 0.13[a]
tetramethyl pyrazine	1 121	15.991	1124-11-4	ND	ND	2.27 ± 0.14[a]
Others						
ammonium acetate	630	2.328	631-61-8	1.30 ± 0.08[b]	ND	4.36 ± 0.02[a]
3-hydroxy-2-butanone	717	3.14	513-86-0	ND	3.46 ± 0.09[a]	2.34 ± 0.29[b]
methyl *N*-hydroxybenzenecarboximidate	1 301	9.781	0-0-0	0.61 ± 0.16[a]	0.55 ± 0.04[a]	ND
2-pentadecyl-1,3-dioxepane	2 270	12.279	41563-29-5	ND	0.65 ± 0.10[a]	0.93 ± 0.15[a]
1-octen-3-one	943	12.318	4312-99-6	1.54 ± 0.19[a]	ND	ND
decane	1 015	13.157	124-18-5	0.29 ± 0.02[a]	ND	ND
2-cyclohexyl-4,5-dimethyl-1,3-dioxolane	1 321	13.547	74793-11-6	ND	0.09 ± 0.05[ab]	0.14 ± 0.00[a]
1,2-bis(1-methylethenyl)- (1*R*,2*R*)-rel-cyclobutane	934	14.119	19465-02-2	2.71 ± 0.57[b]	6.78 ± 0.90[a]	2.59 ± 0.22[b]
2-acetyl pyrrole	1 035	15.357	1072-83-9	0.19 ± 0.03[a]	ND	ND
2-chloro-octane	957	15.64	628-61-5	ND	ND	0.30 ± 0.02[a]
guaiacol	1 090	16.021	90-05-1	0.36 ± 0.05[b]	1.84 ± 0.26[a]	ND
1,3-bis(1,1-dimethylethyl)benzene	1 334	22.848	1014-60-4	0.22 ± 0.04[a]	0.23 ± 0.08[a]	0.26 ± 0.03[a]
4-hydroxy-3-methoxystyrene	1 293	25.234	7786-61-0	0.06 ± 0.00[b]	0.30 ± 0.03[a]	0.08 ± 0.01[b]

RI: retention index; RT: retention time. Data are expressed as mean ± SD (n = 3). ND: not detected. Means with different lower case letters with in the same volatile substance of different groups for significant differences ($P < 0.05$).

types of Douchi were rich in umami, saltiness, and aftertaste. The taste intensity of *Aspergillus*-type was the highest, and there was a significant difference in umami intensity ($P < 0.01$), and sour intensity ($P < 0.05$) (Fig. 2C). It can be explained by the fact that *Aspergillus*-type Douchi is rich in acetic acid and umami amino acids (glutamic acid and aspartic acid) (Fig. 1C and Table 2). In terms of flavor, soy sauce aroma, Douchi aroma, and aromatic flavor were noted for three types of Douchi. there were no significant differences in flavor between the three types of Douchi ($P > 0.05$) (Fig. 2D), but it can be seen that the evaluation scores of *Mucor*-type and *Rhizopus*-type Douchi were higher than those of *Aspergillus*-type Douchi in the aromatic and sweet aroma. *Mucor*-type Douchi contains more esters and alcohols, endowing it with aromatic and fruity flavors. 2,3,5-trimethyl pyrazine and tetramethyl pyrazine were present in *Rhizopus*-type Douchi and were considered to be the main sources of soy sauce and Douchi aroma[33]. Conversely, the excessive fermentation of *Aspergillus*-type Douchi increased the levels of acetic acid and aldehydes, which are associated with beany and unpleasant flavors[39],

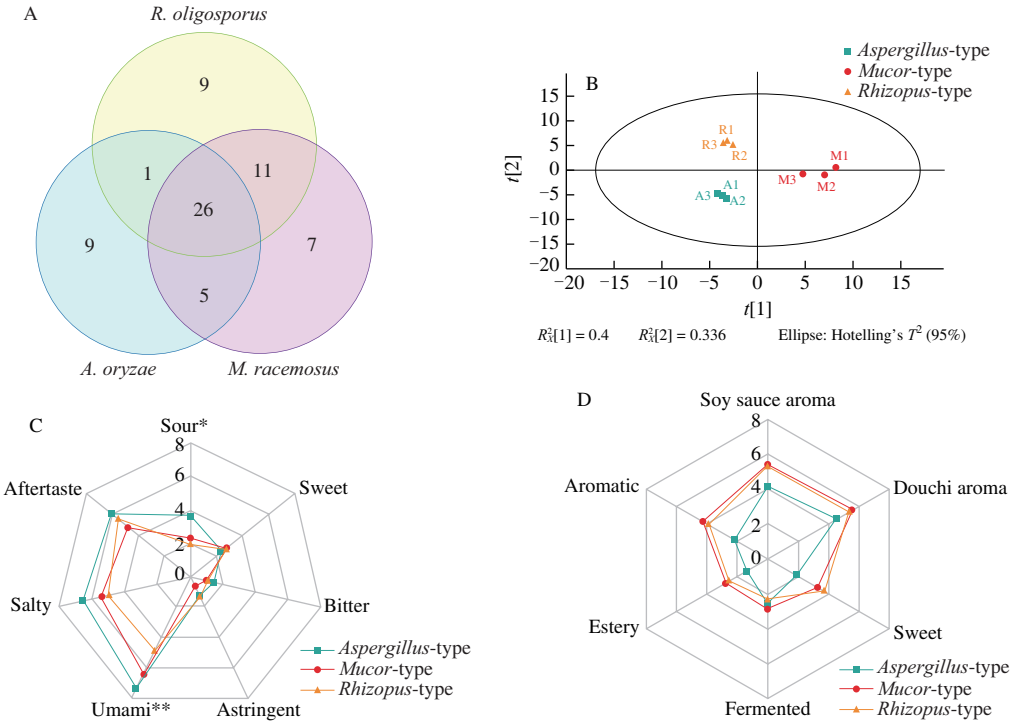


Fig. 2 Volatile compounds and sensory evaluation of Douchi with different molds (A): Venn diagram obtained from GC-MS results; (B): The Score Scatter Plot obtained by PLS-DA analysis; (C): taste sensory evaluation; (D): flavor sensory evaluation. * $P < 0.05$; ** $P < 0.01$.

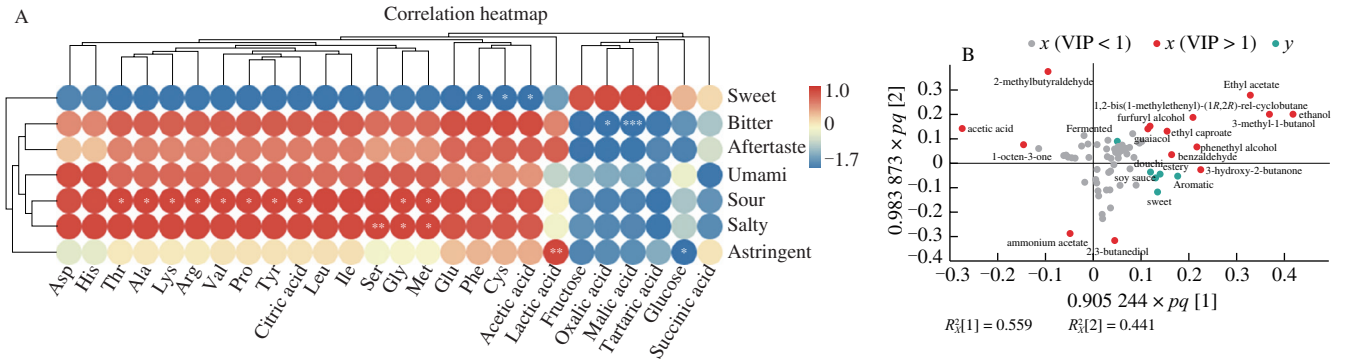


Fig. 3 Correlation between non-volatile and volatile compounds and sensory evaluation (A): Heatmap obtained by Pearson analysis combining taste evaluation and non-volatile compounds; (B): The Loading Scatter Plot obtained by PLS analysis combined with flavor sensory evaluation and volatile compounds (x: volatile components; y: flavor characteristics); $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

leading to flavor deficiencies. From the study of Allwood[42], *A. oryzae* produced an enzyme called glutaminase that converts glutamine into glutamic acid during koji-making, which was particularly important for providing the unique umami taste of Douchi, so *A. oryzae* was considered very suitable for fermentation. However, there were many aromatic substances were metabolized due to overexpression of hydrolase by *A. oryzae*, which produced some unpleasant flavors. Therefore, although *Mucor*-type and *Rhizopus*-type Douchi have relatively low protease activity so that the taste is not prominent, they retain more aromatic substances and have attractive flavor. In conclusion, in order to enrich the taste and flavor of Douchi, it is not enough to pursue high protease activity, and muti-strain fermentation mode dominated by starting strains may be a potential means of improvement.

3.5 Correlation analysis between non-volatile compounds, volatile compounds, and sensory evaluation

To explore the contribution of flavor and taste, Pearson and PLS were used to analyze the correlation between non-volatile compounds, volatile compounds, and sensory evaluation (Fig. 3). The results showed that sweetness was significantly negatively correlated with phenylalanine, cysteine, and acetic acid ($P < 0.05$), bitterness was significantly negatively correlated with oxalic acid and malic acid, and sour taste was significantly positively correlated with citric acid, threonine, alanine, lysine, arginine, valine, proline, tyrosine, glycine, and methionine ($P < 0.001$). The salty taste was significantly positively correlated with glycine and methionine, and extremely significantly positively correlated with serine ($P < 0.01$), and the astringent taste was significantly positively correlated with lactic acid ($P < 0.01$), and significantly negatively correlated with glucose ($P < 0.05$). The PLS-DA analysis method was used to analyze correlations between flavor sensory evaluation results and volatile compound levels (Fig. 3B). First, a total of 13 VIP > 1 species were screened out as substances with large contribution rates to sample classification in the principal component region[43]. Furfuryl alcohol, phenethyl alcohol, benzaldehyde, ethyl caproate, 3-hydroxy-2-butanone, 1,2-bis (1-methyl phenyl)-(1*R*,2*R*)-rel-cyclobutane, and guaiacol were associated with aromatic, estery, and sweet flavors, indicating strong correlations with flavor presentation.

4. Conclusion

This study showed that commercial fermented Douchi has a significantly low level of BA in Douchi, and the BA content of three types of Douchi is within the safe level. *A. oryzae* has a strong protein decomposition ability, which makes its free amino acid content higher and its umami taste more prominent, but this excessive fermentation produces unpleasant flavor substances such as acids and aldehydes. On the contrary, although *Mucor*-type Douchi and *Rhizopus*-type Douchi are insufficient in taste, their moderate fermentation makes them produce more ester and pyrazine aromatic substances. The selection of starting strain determines the microbial composition of artificially mixed starter, by revealing the advantages and disadvantages of different molds, more targeted addition of flavor or taste promoting strains was performed to make up for the defects of single strain fermentation. It is worth exploring to make up for the disadvantage of single strain fermentation by adding exogenous microorganisms, which will be the focus of our future.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was financially supported by Special key project of technological innovation and application development in Yongchuan District, Chongqing (2021yc-cxfz20002) and the special funds of central government for guiding local science and technology development, and the funds for the platform projects of professional technology innovation (CSTC2018ZYCXPT0006).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2022.9250037.

References

[1] M. Jayachandran, B. Xu, An insight into the health benefits of fermented soy products, Food Chem. 271 (2019) 362-371. https://doi.org/10.1016/j.foodchem.2018.07.158.

[2] L. Liu, X. Chen, L. Hao, et al., Traditional fermented soybean products: Processing, flavor formation, nutritional and biological activities, Crit. Rev. Food Sci. Nutr. 62 (2020) 1-19. https://doi.org/10.1080/10408398.2020.1848792.

[3] J. Cai, J. Feng, Z. Ni, et al., An update on the nutritional, functional, sensory characteristics of soy products, and applications of new processing strategies. Trends Food Sci. Technol. 112 (2021) 676-689. https://doi.org/10.1016/j.tifs.2021.04.039.

[4] X. Pi, Y. Sun, G. Fu, et al., Effect of processing on soybean allergens and their allergenicity, Trends Food Sci. Technol. 118 (2021) 316-327. https://doi.org/10.1016/j.tifs.2021.10.006.

[5] Q. Chen, Y. Xu, P. Wu, et al., Aroma impact compounds in Liuyang douchi, a Chinese traditional fermented soya bean product, Int. J. Food Sci. Technol. 46 (2011) 1823-1829. https://doi.org/10.1111/j.1365-2621.2011.02687.x.

[6] Y. Zhang, T. Zeng, H. Wang, et al., Correlation between the quality and microbial community of natural-type and artificial-type Yongchuan Douchi, LWT-Food Sci. Technol. 140 (2021) 110788. https://doi.org/10.1016/j.lwt.2020.110788.

[7] S.Y. Lee, S.Y. Woo, F. Tian, et al., Contamination characteristics and risk assessment of aflatoxins in homemade soybean paste, a traditional fermented soybean food, in South Korea, J. Hazard. Mater. 424 (2022) 127576. https://doi.org/10.1016/j.jhazmat.2021.127576.

[8] H.S. Park, S.C. Jun, K.H. Han, et al., Diversity, application, and synthetic biology of industrially important *Aspergillus* fungi, Adv. Appl. Microbiol. 100 (2017) 161-202. https://doi.org/10.1016/bs.aambs.2017.03.001.

[9] B. He, H. Li, Z. Hu, et al., Difference in microbial community and taste compounds between *Mucor*-type and *Aspergillus*-type Douchi during koji-making, Food Res. Int. 121 (2019) 136-143. https://doi.org/10.1016/j.foodres.2019.03.031.

[10] X. Sun, G. Lyu, Y. Luan, et al., Metabolomic study of the soybean pastes fermented by the single species *Penicillium glabrum* GQ1-3 and *Aspergillus oryzae* HGPA20, Food Chem. 295 (2019) 622-629. https://doi.org/10.1016/j.foodchem.2019.05.162.

[11] R. Guan, Z. Liu, J. Zhang, et al., Investigation of biogenic amines in sufu (furu): A Chinese traditional fermented soybean food product, Food Control. 31 (2013) 345-352. https://doi.org/10.1016/j.foodcont.2012.10.033.

[12] Y. Tan, R. Zhang, G. Chen, et al., Effect of different starter cultures on the control of biogenic amines and quality change of douchi by rapid fermentation, LWT-Food Sci. Technol. 109 (2019) 395-405. https://doi.org/10.1016/j.lwt.2019.04.041.

[13] K. Ito, A. Matsuyama, Koji molds for Japanese soy sauce brewing: Characteristics and key enzymes, J. Fungi. 7 (2021) 658. https://doi.org/10.3390/jof7080658.

[14] D. Wang, Z. Wang, X. Chen, et al., Study on the kinetics of amino nitrogen production and quality changes by *Aspergillus*-type Douchi at different post-fermentation temperatures, Food Ferment. Ind. 09 (2021) 91-99. https://doi.org/10.13995/j.cnki.11-1802/ts.026975.

[15] GB/T 23527-2009, Protease preparations determination of protease activity. (2009).

[16] L. Wang, Y. Cheng, L. Yin, et al., Changes during processing and sodium chloride supplementation on the physical and chemical properties of douchi. Int. J. Food Prop. 13 (2010) 131-141. https://doi.org/10.1080/10942910802256149.

[17] X. J. Ye, J. Zheng, H. Y. Suo, et al., Determination of six organic acids in Yongchuan Douchi, a Chinese traditional fermented soybean product, by HPLC, Food Sci. 35 (2014) 114–118 (In Chinese). https://doi.org/10.7506/spkx1002-6630-201420023.

[18] H. Suo, X. Zhao, Y. Qian, et al., Changes in total sugar and amino acids, and formation of taste compounds in Yongchuan douchi during fermentation, Food Sci. 36 (2015) 100–104 (in Chinese). https://doi.org/10.7506/spkx1002-6630-201521020.

[19] Y. Lu, Y. Wang, G. Zhao, et al., Identification of aroma compounds in Zhuhoujiang, a fermented soybean paste in Guangdong China. LWT-Food Sci. Technol. 142 (2021) 111057. https://doi.org/10.1016/j.lwt.2021.111057.

[20] X. Gao, J. Zhang, J. M. Regenstein, et al., Characterization of taste and aroma compounds in Tianyou, a traditional fermented wheat flour condiment, Food Res. Int. 106 (2018) 156-163. https://doi.org/10.1016/j.foodres.2017.12.063.

[21] K. B. Biji, C. N. Ravishankar, R. Venkateswarlu, et al., Biogenic amines in seafood: a review. Int. J. Food Sci. Technol. 53 (2016) 2210-2218. https://doi.org/10.1007/s13197-016-2224-x.

[22] L. Li, L. Ruan, A. Ji, et al., Biogenic amines analysis and microbial contribution in traditional fermented food of Douchi, Sci. Rep. 8 (2018) 12567. https://doi.org/10.1038/s41598-018-30456-z.

[23] P. Kalac, P. Krausova, A review of dietary polyamines: formation, implications for growth and health and occurrence in foods, Food Chem. 90 (2005) 219-230. https://doi.org/10.1016/j.foodchem.2004.03.044.

[24] L. Manfroi, P.H. A. Silva, L. A. Rizzon, et al., Influence of alcoholic and malolactic starter cultures on bioactive amines in Merlot wines, Food Chem. 116 (2009) 208-213. https://doi.org/10.1016/j.foodchem.2009.02.034

[25] J. Yang, X. Ding, Y. Qin, et al., Safety assessment of the biogenic amines in fermented soya beans and fermented bean curd, J. Agric. Food Chem. 62 (2014) 7947-7954. https://doi.org/10.1021/jf501772s.

[26] N. Benkerroum, Biogenic amines in dairy products: Origin, incidence, and control means, Compr. Rev. Food. Sci. Food Saf. 15(2016) 801-826. https://doi.org/10.1111/1541-4337.12212.

[27] S. Akan, Ö. Özdestan-Ocak, Influence of grape seed extract on suppression of biogenic amine accumulation, chemical and color traits of wet tarhana during fermentation, Food Biosci. 42 (2021) 101065. https://doi.org/10.1016/j.fbio.2021.101065.

[28] M.H.S. Santos, Biogenic amines: Their importance in foods, Int. J. Food Microbiol. 29 (1996) 213-231. https://doi.org/10.1016/0168-1605(95)00032-1.

[29] U.S. Food & Drug, Fish and fishery products hazards and controls guide (2021).

[30] X. Liu, M. Qian, Y. Shen, et al., An high-throughput sequencing approach to the preliminary analysis of bacterial communities associated with changes in amino acid nitrogen, organic acid and reducing sugar contents during soy sauce fermentation. Food Chem. 349 (2021) 129131. https://doi.org/10.1016/j.foodchem.2021.129131.

[31] D.M. Han, B.H. Chun, H.M. Kim, et al., Characterization and correlation of microbial communities and metabolite and volatile compounds in doenjang fermentation, Food Res. Int. 148 (2021) 110645. https://doi.org/10.1016/j.foodres.2021.110645.

[32] J. Cui, P. Xia, L. Zhang, et al., A novel fermented soybean, inoculated with selected *Bacillus*, *Lactobacillus* and *Hansenula* strains, showed strong antioxidant and anti-fatigue potential activity, Food Chem. 333 (2020) 127527. https://doi.org/10.1016/j.foodchem.2020.127527.

[33] F. Birk, F. F. Brescia, M. A. Fraatz, et al., Aroma active alkylated pyrazines are produced by *Basfia succiniciproducens* as by-products of succinic acid production, Flavour Frag. J. 36 (2021) 605-612. https://doi.org/10.1002/ffj.3674.

[34] C. Luan, M. Zhang, S. Devahastin, et al., Effect of two-step fermentation with lactic acid bacteria and *Saccharomyces cerevisiae* on key chemical properties, molecular structure and flavor characteristics of horseradish sauce, LWT-Food Sci. Technol. 147 (2021) 111637. https://doi.org/10.1016/j.lwt.2021.111637.

[35] T.C. Merlo, J.M. Lorenzo, E. Saldaña, et al., Relationship between volatile organic compounds, free amino acids, and sensory profile of smoked bacon, Meat Sci. 181 (2021) 108596. https://doi.org/10.1016/j.meatsci.2021.108596.

[36] X. Zhou, H. Cui, Q. Zhang, et al., Taste improvement of Maillard reaction intermediates derived from enzymatic hydrolysates of pea protein, Food Res. Int. 140 (2021) 109985. https://doi.org/10.1016/j.foodres.2020.109985.

[37] C. Diez-Simon, C. Eichelsheim, R. Mumm, et al., Chemical and sensory characteristics of soy sauce: a review. J. Agric. Food Chem. 68 (2020) 11612-11630. https://doi.org/10.1021/acs.jafc.0c04274.

[38] S. Yu, X. Huang, L. Wang, et al., Characterization of selected Chinese soybean paste based on flavor profiles using HS-SPME-GC/MS, E-nose and E-tongue combined with chemometrics, Food Chem. 375 (2022) 131840. https://doi.org/10.1016/j.foodchem.2021.131840.

[39] C. Yi, Y. Li, H. Zhu, et al., Effect of *Lactobacillus plantarum* fermentation on the volatile flavors of mung beans, LWT-Food Sci. Technol. 146 (2021) 111434. https://doi.org/10.1016/j.lwt.2021.111434.

[40] Y. Wang, H. Zhou, S. Ding, et al., Changes in free amino acids of fermented minced peppers during natural and inoculated fermentation process based on HPLC-MS/MS. J. Food Sci. 85 (2020) 2803-2811. https://doi.org/10.1111/1750-3841.15363.

[41] Y. Xu, J. Zhao, X. Liu, et al., Flavor mystery of Chinese traditional fermented baijiu: The great contribution of ester compounds, Food Chem. 369 (2022) 130920. https://doi.org/10.1016/j.foodchem.2021.130920.

[42] J. G. Allwood, L. T. Wakeling, D. C. Bean, Fermentation and the microbial community of Japanese koji and miso: A review, J. Food Sci. 86 (2021) 2194-2207. https://doi.org/10.1111/1750-3841.15773.

[43] Q. Chen, M. Zhang, M. Chen, et al., Influence of *Eurotium cristatum* and *Aspergillus niger* individual and collaborative inoculation on volatile profile in liquid-state fermentation of instant dark teas, Food Chem. 350 (2021) 129234. https://doi.org/10.1016/j.foodchem.2021.129234.