

## Microbial Analysis of Traditional Starter Culture (Jiaotou) and Its Influence on the Quality of Chinese Steamed Bread

DING Chang-he<sup>1</sup>, QI Guang-ce<sup>1</sup>, ZHANG Jian-hua<sup>2</sup>, CHEN Fu-sheng<sup>1</sup>, LI Li-te<sup>1</sup>

(1. College of Grain and Food, Henan University of Technology, Zhengzhou 450052, China 2. Department of Food Science and Engineering, College of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 201101, China)

**Abstract:** Chinese steamed bread (CSB) is a kind of Chinese traditional fermented staple food. It is traditionally fermented by Jiaotou, which is the starter culture of CSB. This study was to determine the microbial characteristics of Jiaotou and its influence on CSB quality. In this paper, lactic acid bacteria (LAB) and yeasts counts in 12 Jiaotou samples were enumerated and separated by plate method. The isolated strains were identified by BioMerieux Vitek-32 system. The qualities of Jiaotou fermented CSB and that of commercially produced ones were compared by sensory analysis and texture profile analysis (TPA). The average LAB counts of Jiaotou were 8.1 lg CFU/g, while the average yeasts counts were slightly lower (7.7 lg CFU/g). The yeasts counts of dry Jiaotou (8.2 lg CFU/g) were significantly higher than wet Jiaotou (7.2 lg CFU/g,  $p < 0.05$ ). The isolated yeasts were identified as *Saccharomyces cerevisiae*, and three strains of bacteria were identified as *Bacillus cereus*, *Brevibacillus brevis* and *Acinetobacter lwoffii*, respectively. During dough fermentation, Jiaotou made the dough pH go down with the LAB but the yeasts counts increase. With better interior structure but inferior odor, the general sensory scores of Jiaotou fermented CSB were not significantly different to those of commercial ones. The TPA results indicated that Jiaotou fermented CSB was significantly superior to some kind of commercial CSB in hardness, gumminess, chewiness, cohesiveness and adhesiveness ( $p < 0.05$ ). In general, Jiaotou fermented CSB showed better eating quality indicated by parameters of TPA.

**Key words:** Chinese steamed bread; starter; microflora; sensory analysis; TPA; quality

## 传统起子(酵头)的微生物分析及其对馒头品质的影响

丁长河<sup>1</sup>, 戚光册<sup>1</sup>, 张建华<sup>2</sup>, 陈复生<sup>1</sup>, 李里特<sup>1</sup>

(1. 河南工业大学粮油食品学院, 河南 郑州 450052

2. 上海交通大学农业与生物学院食品科学与工程系, 上海 201101)

**摘 要:** 馒头是一种中国主要的发酵食品。传统方法制作馒头是用酵头发酵的, 酵头也叫起子、面肥。本文主要研究酵头的微生物组成以及对馒头品质的影响。采用平板划线法对 12 个酵头进行乳酸菌、酵母菌计数和分离。分离得到的菌种用生物梅里埃的 Vitek-32 系统进行自动鉴定。通过感官评价和 (TPA) 质地剖面分析分析来比较酵头发酵馒头和工厂化生产馒头的品质差别。12 个酵头的乳酸菌计数的均数为 8.1 lg CFU/g, 酵母菌计数的均数为 7.7 lg CFU/g。干燥的酵头的酵母菌计数 (8.2 lg CFU/g) 显著高于湿酵头 (7.2 lg CFU/g,  $p < 0.05$ )。分离得到的 3 株酵母都为 *Saccharomyces cerevisiae* (啤酒酵母), 3 株细菌分别为 *Bacillus cereus* (蜡样芽胞杆菌), *Acinetobacter lwoffii* (鲁氏不动杆菌), *Brevibacillus brevis* (短小芽胞杆菌)。在酵头发酵馒头面团过程中, 随时间延长 pH 降低, 酵母菌和乳酸菌计数增加。与工厂化生产的馒头比较, 酵头发酵馒头有较好的内部结构, 相对较差的气味, 而在感官评价的总分上与工厂化生产的馒头没有显著的差别。TPA 分析的结果表明酵头发酵馒头在以下几个指标上显著高于其中一种工厂化生产的馒头 ( $p < 0.05$ ): 硬度、胶性, 咀嚼性, 凝聚性, 粘附性。总的来说, TPA 分析结果表明酵头发酵馒头有着较好的食用品质。

**关键词:** 馒头; 酵头; 微生物; 感官评价; TPA; 品质

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作者简介: 丁长河 (1968-), 男, 副教授, 博士, 主要从事发酵食品、低聚糖等方面的研究。

As a kind of traditional fermented food based on wheat flour, Chinese steamed bread (CSB) has been produced for thousands years. It has been the staple food of the people in northern China, and a kind of popular cake-like products in southern area.

Ingredients of CSB are usually including flour, water and yeast, and it is made ready to eat by steaming the fermented dough. However, the CSB is traditionally fermented by Jiaotou, which is a starter culture that was still widely used in northern Chinese countryside today. Traditional starter culture (Jiaotou) could be made from wheat flour, wheat bran, corn flour, or rice flour by solid fermentation or by submerged fermentation.

In recent years, commercial dry yeast products were used instead of Jiaotou for the industrial production of CSB. Comparing with Jiaotou, the advantages of commercial yeast products would include more rapid dough fermentation, less acid produced and purer flavor, but the disadvantages include shortage of fermentation flavor, poor sensory evaluation score and poor re-steaming ability. Jiaotou were usually produced in the natural environment, the microorganism compositions were complicated. It was considered that the main mixed microflora inherent in it were yeasts and LAB<sup>[1]</sup>. However, little was exactly known about microorganism composition in the traditional starter cultures of CSB and it might be very important to affect the quality of CSB.

This study was undertaken to enumerate, isolate and identify the microflora in Jiaotou samples, and the quality of Jiaotou fermented CSB were compared with that of commercial CSB by sensory evaluation and TPA.

## 1 Materials and Methods

### 1.1 Sample collection

12 samples of Jiaotou were collected from nearby areas of Nanyang and Shangqiu, Henan, China. The half of 12 Jiaotou samples was dry, water content less than 8%; while the other were wet, water content 50%~60%. The samples were stored at 0~4°C before analysis.

### 1.2 Microorganisms enumeration and isolation

0.2 g Jiaotou was mixed with 1.8 ml sterile physiological saline made to  $10^{-1}$  dilution. Further tenfold serial dilutions, ranging from  $10^{-2}$  to  $10^{-6}$ , were prepared with the same method and the microbial counts were determined according to the pour plate method of Harrigan and McCance<sup>[2]</sup>.

The counts of yeasts were determined with PDA, acidified to pH 5.5 with hydrochloric acid and incubated at

28°C for 2 days. Counts of LAB were determined using MRS agar and the reducing agent (L-cysteine hydrochloride) was added into the medium (1%, W/V) incubated anaerobically at 38°C for 2 days. The cycloheximide (0.1%, W/V) was added in MRS to inhibit yeast growth.

Representative colonies were isolated from MRS and PDA Plates, and then purified by streak plating using the same medium. Representative yeasts colonies on PDA were examined by microscope, purified by successive streaking on PDA and stored on slants at 4°C for further identification. Same isolation steps were done to examine representative bacteria.

### 1.3 Identification of microorganisms

The yeasts were primarily classified by colony characteristics (pigmentation and shape). Identification of the yeasts isolates on species level was done with the Vitek-32 (BioMérieux, France) system of carbohydrate assimilation profiles. Bacteria were also identified to species level by Vitek-32 system.

### 1.4 Changes of the microbial counts and pH values during dough fermentation by Jiaotou

Dough making and fermentation were done as follows: 1 g dry Jiaotou, 50 g distilled water and 100 g flour were mixed to 2 equal dough by hands, then fermented for 7 h at 35°C with 85% relative moisture in incubator. The microbial counts of LAB and yeasts during dough fermentation were determined by the methods described above.

5 g fermented dough and 5 ml neutral distilled water (pH 7.0) were mixed 5 min to homogenization with a laboratory blender, and then the pH value of the blended slurry was measured with a Hanna pH meter (model 211).

### 1.5 Sensory and texture profile analysis of Jiaotou fermented CSB by and commercial CSB

Based on the traditional method, Jiaotou fermented CSB was made as follows: 140 g flour, 95 ml distilled water and 1 g Jiaotou were mixed to paste, and then fermented for 12 h at 25°C with 85% relative moisture. After 12 h fermentation, 60 g flour was added, while four equal weight doughs with smooth hemispherical surface were made by hands. Then the doughs were fermented for 2 h at the above conditions. The CSB were made by steaming the fermented dough for 20 min.

Two kinds of commercial yeast were used to be fermented into CSB, Dangju™ and Furong™, were purchased in the markets of Zhengzhou, Henan, China.

### 1.6 Sensory valuation

For each consumer preference test, 5 panelists were recruited to rate the flavor, texture and overall attributes of the CSB. Three samples were evaluated per day and each sample was evaluated three times over an one week period, and the evaluation was completed in 7 days.

The CSBs were scored subjectively for the following quality parameters: interior structure, elasticity, stickiness, cohesiveness and odor as the five main sensory parameters of CSB with eating quality<sup>[3-5]</sup>. The scales of attributes were set as follows: elasticity 0~10, cohesiveness 0~10, stickiness 0~10, interior structure 0~15, and odor 0~5.

### 1.7 Texture profile analysis

The Texture Analyzer TA-XT2i (Stable Micro System, UK) was used to test the TPA with the Texture Expert Software<sup>[6]</sup>. A 15 mm thick slice of CSB was made by special tools for testing. Texture analysis was carried out on the slices 30 min after steaming. Texture parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess chewiness and resilience were tested.

### 1.8 Analysis of data

The value was the mean of three determinations. The data were analyzed by students t-test and significance was accepted at  $p < 0.05$ <sup>[7]</sup>.

## 2 Results and Discussions

### 2.1 Enumeration of microorganisms

Table 1 Average counts of LAB and yeasts in wet and dry Jiaotou samples

		lg counts (lg CFU/g)	
		Dry Jiaotou	Wet Jiaotou
Ranges of counts for all samples		n=6	n=6
L A B	Dry Jiaotou 7.4~9.0	8.4(0.55)	7.8(0.35)
	Wet Jiaotou 7.5~8.4		
Yeasts	Dry Jiaotou 7.1~9.2	8.2 <sup>a</sup> (0.77)	7.2 <sup>a</sup> (0.45)
	Wet Jiaotou 6.7~7.8		

Note: Standard deviation in parentheses, 6 samples each of dry and wet Jiaotou. Means with the same letter are significantly different ( $p < 0.05$ ).

Table 1 showed the average counts of LAB and yeasts in wet and dry Jiaotou samples. There was no significant difference in LAB between wet and dry ones. However, the dry had significantly higher numbers of yeasts counts compared to wet Jiaotou ( $p < 0.05$ ). This was probably because the water content of two kinds of Jiaotou samples differed. It seemed that the water content did not play significant role in LAB counts comparing to yeasts in Jiaotou. And there was no significant difference between in the number LAB and

yeasts in same Jiaotou samples. This indicated that there were no dominant microorganisms in Jiaotou. Dough fermentation of CSB by Jiaotou might be resulted by associations of LAB and yeasts. They might potentially contribute to the characteristics of CSB. Associations of LAB and yeasts have been found to be responsible for the spontaneous fermentation of many cereal-based foods<sup>[8-10]</sup>. Interaction between LAB and yeasts were found in some other fermented food<sup>[11]</sup>. However, interaction between yeasts and LAB in the dough of CSB was of little information published. Although the fermented CSBs were regarded as predominantly yeast fermentations, the cooccurrence of yeasts and LAB has led to the suggestion that interactions might occur and influence product characteristics and qualities.

Such interaction might stimulate or inhibit the growth of either one, or both, of the co-cultured strains. The co-cultured organisms might compete for growth nutrients or produce metabolic products that would inhibit each other's growth. LAB might produce acid that inhibited the growth of yeasts. Furthermore, mutual influence of the microorganisms on each other's metabolism might lead to different profiles of important flavor compounds in CSB.

### 2.2 Identification of microorganisms

On the basis of carbohydrate fermentation and assimilation, 4 bacteria isolated and 3 yeasts isolated from the 12 traditional Jiaotou samples were identified to species level. Three bacteria were identified as *Bacillus cereus*, *Brevibacillus brevis*, *Acinetobacter lwoffii*. The source of *Bacillus cereus*, *Brevibacillus brevis*, *Acinetobacter lwoffii* in Jiaotou was assumed to be casual contamination from the environment. However, this has not been proved yet in any scientific researches.

The species of isolated yeasts were identified as *Saccharomyces cerevisiae*, which was widely used in brewing and genetics researches. Carbohydrates were fermented by yeasts to produce carbon dioxide and a little ethanol. The carbon dioxide formed gas bubbles as contribution to the interior structure of CSB, similar to the interior structure of bread.

### 2.3 Changes in microbial number and pH values during fermentation of CSB dough by Jiaotou

Fig. 1 showed the changes in microbial growth during dough fermentation by Jiaotou. Within the first and last 3h of the fermentation, there was an increase in LAB counts by about 0.8 lg cycles to 7.2 and 8.0, respectively. Between 3 and 5 h, the counts of LAB were fluctuated from 7.2 to 6.8, then

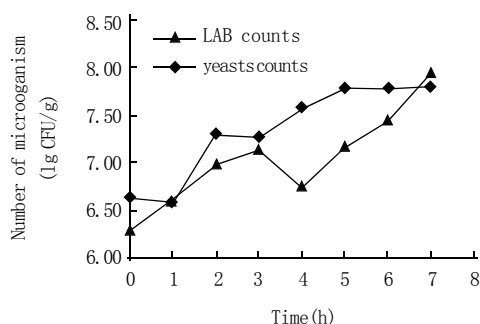


Fig.1 Changes of microbial numbers during dough fermentation by Jiaotou

back to 7.2, with no increase as a whole in this period. The yeast counts steadily increased at first 5 h, about 1.2 lg cycles from 6.6 to 7.8. At last 2 h, the yeasts counts remained the same. The yeasts counts were higher than LAB except the last hour, especially in 3 to 6 h.

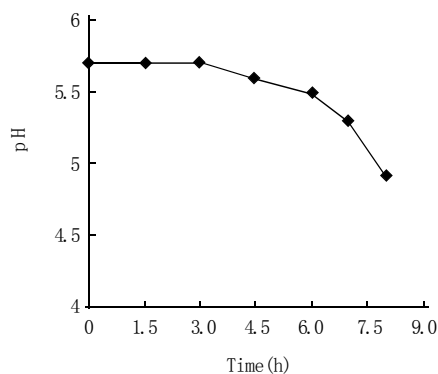


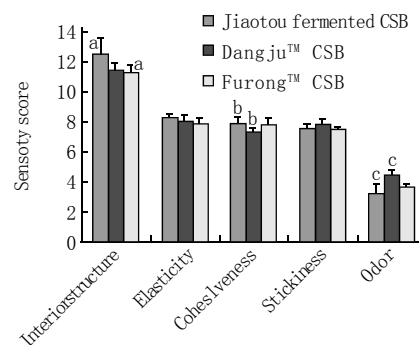
Fig.2 pH value changes during dough fermentation by Jiaotou

Fig.2 showed the pH value changes during dough fermentation by Jiaotou. During fermentation, the pH value remained unchanged in the first 3 h. Between 3 and 6 h, the pH decreased gradually from pH5.7 to pH5.5. At the last 2 h, the pH value decreased sharply to pH4.9. The LAB and yeast counts changed at the last 2 h were the reason of the pH value decrease observed. Despite the relatively low level of LAB in the fermented dough by Jiaotou, these organisms were likely to be significant in flavor development in CSB. After 5 h fermentation, the pH gradually declined, especially for the last 2 h, while the yeast counts kept un-fluctuated other than the increase in the preceding 5 h. This reduction in pH value as a result of the organic acids (e.g. lactic acid) production by LAB and yeasts was most likely to have reflected the cause of suppression of yeasts population in the dough. The CSB fermented by Jiaotou might be sourer than yeast, with the extension of dough fermentation time. Comparing to Jiaotou, commercial yeast had shorter dough fermenta-

tion time (usually no more than 2 h), while dough pH values remained almost unchanged during the fermentation (the data was not shown).

The microbial counts changes shown in Fig.1 suggested that in all the stages of dough fermentation, yeasts were predominant microorganisms than LAB except in the latestages.

2.4 Quality comparison between Jiaotou fermented CSB and commercial yeast fermented CSB by sensory analysis and TPA



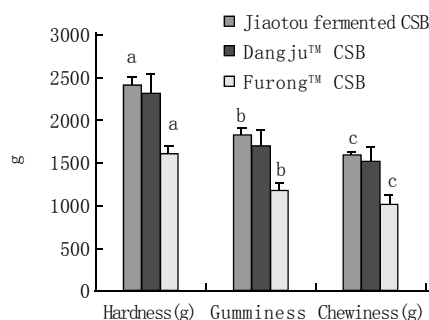
Means (bar values) with the same letter are significantly different ( $p < 0.05$ ). Higher scores mean better eating quality. CSB of better eating quality should be more elastic, more cohesive and less sticky when eaten<sup>[8]</sup>.

Fig.3 Quality comparison between Jiaotou fermented CSB and two commercial CSB by sensory analysis

Fig.3 showed the quality comparison between Jiaotou fermented CSB and commercial yeast fermented CSB by sensory analysis. The interior structure of Jiaotou fermented CSB was superior to that of the two commercial ones, but only significantly different to that of Furong™ CSB ( $p < 0.05$ ). As to the cohesiveness, the Jiaotou fermented CSB was also superior to the other two samples, but the Dangju™ CSB was significantly different ( $p < 0.05$ ). The elasticity of Jiaotou fermented CSB was superior to that of commercial CSB, but not significantly. There were no significantly difference in stickiness between two kinds of CSB. The odor of Jiaotou fermented CSB was inferior to that of two commercial ones. The odor score SD (standard deviation) of Jiaotou fermented CSB was higher than that of two commercial CSB. This indicated that consumer's odor preference to Jiaotou fermented CSB varied. It was considered that the special odor was formed during dough fermentation by the microorganisms from the starter culture Jiaotou. The general sensory evaluation indicated that the quality between two kinds of CSB was not significantly different.

Sensory evaluation was sometimes limited by the pref-

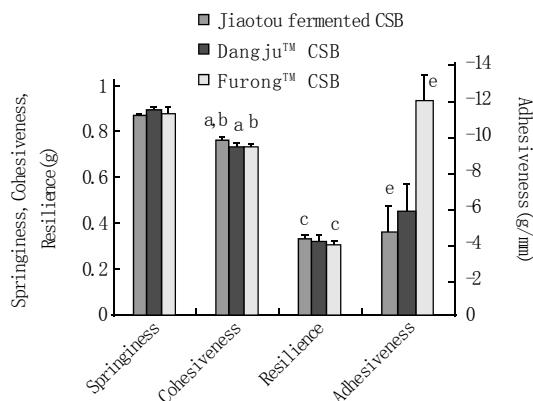
erence of different panelists. Thus TPA was used to meet the shortage of sensory method.



Means (bar values) with the same letter are significantly different ( $p < 0.05$ ). Higher chewiness, hardness and gumminess mean better eating quality.

Fig.4 Comparison of hardness, gumminess and chewiness between Jiaotou fermented CSB and commercial yeast fermented CSB by the Texture Analyzer

Fig. 4 illustrated the comparison of hardness, gumminess and chewiness between Jiaotou fermented CSB and commercial yeast fermented CSB by the Texture Analyzer. As shown in Fig. 4, the hardness, gumminess and chewiness of Jiaotou fermented CSB were significantly higher than that of Furong™ CSB ( $p < 0.05$ ), but not significantly different to Dangju™ CBS.



Means (bar values) with the same letter are significantly different ( $p < 0.05$ ). Higher scores of springiness, cohesiveness, resilience and adhesiveness mean better eating quality.

Fig.5 Comparison of springiness, cohesiveness, resilience and adhesiveness between Jiaotou fermented CSB and commercial yeast fermented CSB by the Texture Analyzer

Fig. 5 illustrated the comparison result of springiness, cohesiveness, resilience and adhesiveness of Jiaotou fermented CSB and commercial yeast fermented CSB by the Texture Analyzer. As shown in Fig. 5, the cohesiveness of Jiaotou fermented CSB was significantly higher than that of the two commercial CSB ( $p < 0.05$ ). The chewiness and cohesiveness by texture analysis could be corresponding to

the cohesiveness of the sensory evaluation. The resilience of Jiaotou fermented CSB was higher than the other two commercial CSB, especially significantly different to that of Furong™ CSB ( $p < 0.05$ ). The springiness of Jiaotou fermented CSB was lower than that of two commercial CSB, but not significantly different. The springiness of TPA was similar to elasticity by sensory analysis. As shown in Fig. 5, the adhesiveness of Jiaotou fermented CSB was worse than that of two commercial CSB, but only significantly different to that of Furong™ CSB. The adhesiveness was corresponding to stickiness of the sensory evaluation. Lower adhesiveness mean better eating quality to CSB<sup>[11]</sup>.

The results of TPA indicated that for Jiaotou fermented CSB, the chewiness and cohesiveness were higher and the adhesiveness was lower, which meant Jiaotou fermented CSB had better eating quality than commercial yeast fermented ones.

The comparison between two kinds of CSB indicated that Jiaotou fermented CSB was significantly superior to Furong™ CSB in most parameters ( $p < 0.05$ ). Jiaotou fermented CSB was only significantly higher in cohesiveness than Dangju™ CBS ( $p < 0.05$ ). As for other parameters, there were no significant difference between Dangju™ CBS and Jiaotou fermented ones.

As for two analysis methods (sensory evaluation and TPA), most of parameters analysis were coincident to each other.

### 3 Conclusion

In the 12 Jiaotou samples, the average LAB counts of Jiaotou were 8.1 lg CFU/g, while the average yeasts counts were slightly lower (7.7 lg CFU/g). The yeasts counts of dry Jiaotou samples (8.2 lg CFU/g) were significantly higher than those of wet Jiaotou (7.2 lg CFU/g,  $p < 0.05$ ). Three isolated strains of yeasts were all identified as *Saccharomyces cerevisiae* and three typical strains of bacteria were identified as *Bacillus cereus*, *Brevibacillus brevis* and *Acinetobacter lwoffii*.

During the dough fermentation, the yeasts counts were slightly higher than LAB in most of the fermentation time, except the last hours. During the dough fermentation by Jiaotou, the LAB counts increased from 6.3 to 8.0 lg CFU/g, while the yeasts counts increased from 6.6 to 7.8 lg CFU/g. The pH value decreased from pH5.7 to pH4.9 dough fermentation by Jiaotou, especially sharply decreased in the last several hours, with the increasing of LAB and yeasts counts.

Comparing to the commercial yeast fermented ones,

## Analysis of White Particles in Xuanwei Ham

WANG Xing-hong, JIANG Dong-fu, MA Ping, PENG Qian  
(Institute of Microbiology, Yunnan University, Kunming 650091, China)

**Abstract:** Xuanwei Ham is a famous ham in China with some white particle spots often appeared in ham, mostly in ham muscle. However, examined by microbiological methods and observed by light microscope, there are neither microbes nor parasites in the white particle spots. Proteins in particles would decompose significantly, and two main proteins with molecular weights of respectively 51000 and 13200 were detected by gradient SDS-PAGE in particles. The content of tyrosine in white particles was eight times higher than that in normal ham muscle, which indicated that the particles might be decomposed protein, tyrosine and some other component. All the contents of inorganic elements analyzed are less than those in ham muscle, and the chondroitin sulfate occurred in white particle implies that pathological disease might occur in muscle at the particle position during live stage of pig. Inoculating yeast on ham during salting can prevent the formation of particles effectively.

**Key words:** Xuanwei Ham; white particles; tyrosine; component

## 宣威火腿白点的分析

王兴红, 江东福, 马 萍, 彭 谦  
(云南大学微生物研究所, 云南 昆明 650091)

**摘 要:** 宣威火腿是中国三大著名火腿之一, 常常有白点在火腿中出现, 影响产品外观。通过微生物学方法和

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作者简介: 王兴红(1966-), 男, 副研究员, 博士, 主要从事发酵食品及食品微生物的研究。

Jiaotou fermented CSB had higher sensory scores in interior structure and cohesiveness, but had lower sensory scores in odor and stickiness. The general sensory scores of two groups were not significantly different. The TPA results indicated that Jiaotou fermented CSB was significantly superior to Furong™ CSB in hardness, gumminess, chewiness, cohesiveness and adhesiveness ( $p < 0.05$ ) respectively, but only significantly better to Dangju™ CBS in cohesiveness ( $p < 0.05$ ). In general, Jiaotou fermented CSB had better eating quality indicated by parameters of TPA, while its odor preference varied.

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