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# Apple spontaneous fermentation sourdough: Microbial dynamics and optimization for enhanced bread quality

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**Abstract:** This study explored using apple-derived spontaneous fermentation sourdough to replace commercial cultures in bread production, analyzing its microbial composition and optimizing process parameters. Microbial profiling via 16S and 26S rDNA sequencing identified Lactobacillus plantarum as the dominant lactic acid bacteria. SFS addition levels (0–30%) and proofing times (60–180 minutes) were varied to assess impacts on bread quality. Results revealed that shorter proofing initially increased specific volume, which stabilized over time, while hardness decreased and then increased. Similarly, lower apple-derived spontaneous fermentation sourdough percentages enhanced volume, but higher doses reduced it, with hardness showing a comparable trend. Lower apple-derived spontaneous fermentation sourdough has improved nutritional properties (free amino acids) and flavor (volatile compounds). The findings demonstrate lower apple-derived spontaneous fermentation sourdough's potential to enhance bread quality, offering natural alternatives to commercial starters by balancing fermentation parameters to optimize texture, volume, shelf-Keywordslife, and sensory attributes, highlighting its role in sustainable, artisanal bread production.

**Key words:** spontaneous fermentation sourdough; microbial community structure; isolation and identification; baking properties; volatile flavor compounds

## 1. Introduction

Naturally fermented sourdough is a natural mixed starter obtained by natural fermentation of grains, fruits and vegetables or other substrates with water and active microorganisms (mainly lactic acid bacteria andyeast flora) . The expression "natural yeast" is often used in the baking industry, and naturally fermented sourdough is a typical natural yeast system (Islam and Islam, 2024). It is one of the oldest biological technologies in China and even in the world to ferment dough products by using naturally fermented sourdough as starter. As early as the Zhou Dynasty, Chinese people mastered the technology of making steamed bread using "wine noodles", where "wine noodles" are the earliest recorded naturally fermented sour dough; during the Song Dynasty (AD960 ~ 1279),"fermented flour fermentation method" appeared to produce steamed bread. "Fermented flour" continues to develop intothe main form of naturally fermented sourdough in China at present Zhang and He, 2012). In western countries, however, the natural sourdough technology is mainly used in bread. Foreign technology of naturally fermented sourdough bread beganin Egypt in 1500 BC and hasa history of more than 5000 years. With the development of social civilization, people in North America and central Europe gradually mastered the use of natural fermentationtechniques to produce wheat, rye bread, etc. (Lau et al., 2021). Today, 30%of bread production in Italy is still done by natural fermentationsourdough. Up to now, natural sourdough technology still exists firmly in the bread making industry in western countries with its unique advantages and has been studied extensively Hernández-Figueroa et al., 2024). With the rise and popularity of bread products in China, domestic research on the production of bread using natural fermentation sourdough technology has also begun. Since its invention in the 1860s, commercial yeast has been rapidly popularized in the bakery industry due to its long storage time, quick and easy use, and strong controllability (Akamine et al., 2023). But at the same time, the common commercial yeast bread became the shackles of its development because of its single flavor, rough taste and heavy yeast flavor. The diversified demand of consumers for food quality has brought back the natural sourdough technology that endows bread products with natural health, rich nutrition and good quality (Islam and Islam, 2024; Chavan and Chavan, 2011) Raisins are often used as the best source of cellulose and natural sugar added to bread to enhance the nutritional value and taste of bread products.

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There are few reports on the application of raisin fermentation products in food Abdollahzadeh et al., 2024).Apple is the main fruit consumed in many countries in the world, which has the advantages of strong ecological adaptability, high nutritional value and storage resistance. In addition to being rich in vitamins and minerals, apples are an important dietary source of phenols and have considerable antioxidant properties (Arnold and Gramza-Michalowska, 2024).Apples are mainly used in food production to produce apple juice. Apple fermentation products are mainly used in the production of apple drinks, apple vinegar and apple cider, and less in bread (Tsoupras et al., 2025). Gary et al. used fermented apples forbread production to improve the gas holding properties of bread Gary et al., 2003). Fermentation substrate has a great influence on the quantity of yeast, but a small influence on the species, mainly Saccharomyces cerevisiae; different fermentation substrates have a small influence on the quantity of lactic acid bacteria, but a large influence on the species.

This study (1) aimed develop production methods for apple-based spontaneous sourdoughs by monitoring sugar/acid metabolism to determine optimal fermentation endpoints; (2) isolate and characterize lactic acid bacteria to clarify their roles in enhancing bread quality; (3) evaluate the superior baking performance and nutritional benefits (e.g., free amino acids) of sourdough bread compared to commercial yeast bread; and (4) analyze flavor enhancement and storage advantages imparted by spontaneous sourdough, elucidating mechanisms behind delayed staling and improved sensory properties. The work underscores sourdough's potential as a natural, multifunctional alternative to industrial starters.

## 2. Materials and Methods

### 2.1 Experimental Materials and Equipment

#### 2.1.1 Experimental materials and reagents

Wheat Flour: COFCO Flour Industry Pengtai Co., Ltd.; Instant Dry Yeast: Guangdong Meishan Mali Yeast Co., Ltd.; Shortening: Donghai Grain and Oil Industry (Zhangjiagang) Co., Ltd.; Sugar, Salt, Apple, Honey: Provided by Fujian Anmai Biotechnology Co., Ltd.

Glucose, fructose, maltose, lactose, sucrose, lactic acid, acetic acid, perchloric acid, sodium hydroxide, sodium chloride,peptone, beef extract, yeast extract, potassium hydrogen sulfate, anhydrous sodium acetate, ammonium citrate, $MgSO_4 \cdot 7H_2O$,$MnSO_4 \cdot 4H_2O$, Tween 80, agar powder: Sinopharm Chemical Reagent Co., Ltd.; actinomycin: Shanghai BaomanBiotechnology Co., Ltd.; bacterial genomeDNA extraction kit, yeast genomeDNA extraction kit: Tiangen Biochemical Technology (Beijing) Co., Ltd.; primers and enzyme preparations: Shanghai Sunny Technology Co., Ltd. Lactic acid bacteria culture medium (MRS): yeast extract5g, beef extract10g, glucose20g, peptone10g, Ammonium citrate 2 g, anhydrous sodium acetate 5 g, dipotassium hydrogen sulfate 2 g, $MgSO_4 \cdot 7H_2O$ 0.58 g, $MnSO_4 \cdot 4H_2O$ 0.25 g, 1 mL Tween 80, 1000 mLdistilled water, pH6.2-6.6, sterilized at 115 ℃° for 20 min, and20g agar powder was added to the solid medium.

#### 2.1.2 Main equipment

JY20002electronic balance: Shanghai Liangping Instrument Co., Ltd.; mixer, incubator, electric oven, microtome:Xinmai Machinery Co., Ltd.; CT3texture analyzer: BrookfieldCompany; constant temperature and humidity incubator: ShanghaiBoxun Industrial Co., Ltd. Medical Equipment Factory; FE20laboratorypH meter: Switzerland Toledo Company;Waters244high performance liquid chromatograph:Agilent Technology;XSP-10Celectron microscope: Shanghai Biaimu Optical Instrument Manufacturing Co., Ltd.;SHY-2Awater bath constant temperature oscillator: Jiangsu Jincheng Guosheng Experimental Instrument Factory;DYY-8Celectrophoresis instrument: Beijing City Liuyi Instrument Factory; 5427R desktop high speed refrigeration centrifuge, Mastercycler nexus X1 PCR instrument, Eppendorf BioPhotometer D30 nucleic acid protein analyzer:Eppendorf (China) Co., Ltd.

### 2.2 Experimental method

#### 2.2.1. Preparation of naturally fermented sourdough

The selection of fruit substrate in naturally fermented sourdough follows the following principles: wide source and easy storage; high sugar content suitable for microbial growth; low seasonality, and season has little effect on the final quality of the product. In the preliminary experiment, apple was chosen as fermentation substrates, bubbles were generated evenly and repeatability was good, so that was finally selected as fermentation substrates to prepare naturally fermented sourdough.

Weigh100g apples, wash them with warm boiled water, cut them into pieces and pour them into sterilized wide-mouth glass bottles, add10g honey and 200 g sterilized distilled watercooled to 30℃° respectively, seal them with cotton plugs, and put the glass bottles into constant temperature of 30℃° in a wet box, culture for 5-7days to obtain stable product, and filtering with sterile gauze to obtain fermentation broth.

### 2.3 Determination of pH offermentation broth

During the natural fermentation of apples until the fermentation broth in 2.2.1 is obtained, the pH value of the fermentation broth on d 0, d 1, d 3, d 5 and d 7is directly measured by pH meter.

#### 2.3.1 Determination of soluble sugar in fermentation broth

Soluble sugar content of fermentation broth was determined at 0,1,3,5 and 7d. According to the extraction method of Zhang Qing , dilute the solutionto100mL, centrifuge at4000r/minfor10min, and collect the supernatant. The supernatant was assayed by HPLC (High Performance Liquid Chromatography). Among them, glucose, fructose, lactose, maltose and sucrose were used as standard samples.

#### 2.3.2 Determination of Organic Acids in Fermentation Broth

The organic acid content of fermentation broth was determined. Add 5 mL of 1 mol/L $HClO_4$to 10 mL of fermentation broth, mix well, centrifugeat 5000r/min for15 minat 4C°, add distilled water to make 25 mL of supernatant, precipitate in ice bath, microfilter the supernatant (0.45μm), and thenperformHPLC analysis. Determine the contents of lactic acid and acetic acid in the fermentation broth of the sample with lactic acid and acetic acid as standard samples.

#### 2.3.3 Determination of basic properties of naturally fermented sourdough

##### 2.3.3.1. sample preparation

Take apple naturally fermented sourdough finally obtained.

##### 2.3.3.2 Determination of pH and total acidityof naturally fermented sourdough

The pH and total acidity of apple naturally fermented sourdough were determined.
According toAACC method(2000) 02-52, respectively take10 g ofsample into conical flask, add90 mL of distilled water, magnetically stir for30 min, stand for10 min, and determinepH. Titrate with0.1 mol/L NaOH and stir topH 8.6,the volume of NaOH consumed is the total acidity, repeat the operation at least three times for each sample[47].

### 2.4 Counting of lactic acid bacteria in naturally fermented sourdough

Ten (10) g apple sourdoughwere mixed with90 mL ata concentration of 0.85%. The sterile saline solution was mixed and diluted ten-fold (Wu et al., 2011). Lactic acid bacteria count: MRSsolid plate coating method. Take seven dilution gradients of10-1-10-7, apply100 μL, incubate at 38C° for48 h, and count.

### 2.5 Identification of lactic acid bacteria and yeasts in naturally fermented sourdough

#### 2.5.1 Isolation, purification and preservation of lactic acid bacteria from naturally fermented sourdough

**a**.Preparation of bacterial suspension
Mix 10 g of apple sourdough with 90mLof sterile saline shake well. Dilute to 10-8 in steps.
**b**.Isolation, purification and preservation of lactic acid bacteria
The isolation, purification and preservation steps of lactic acid bacteria are shown in Figure 1.

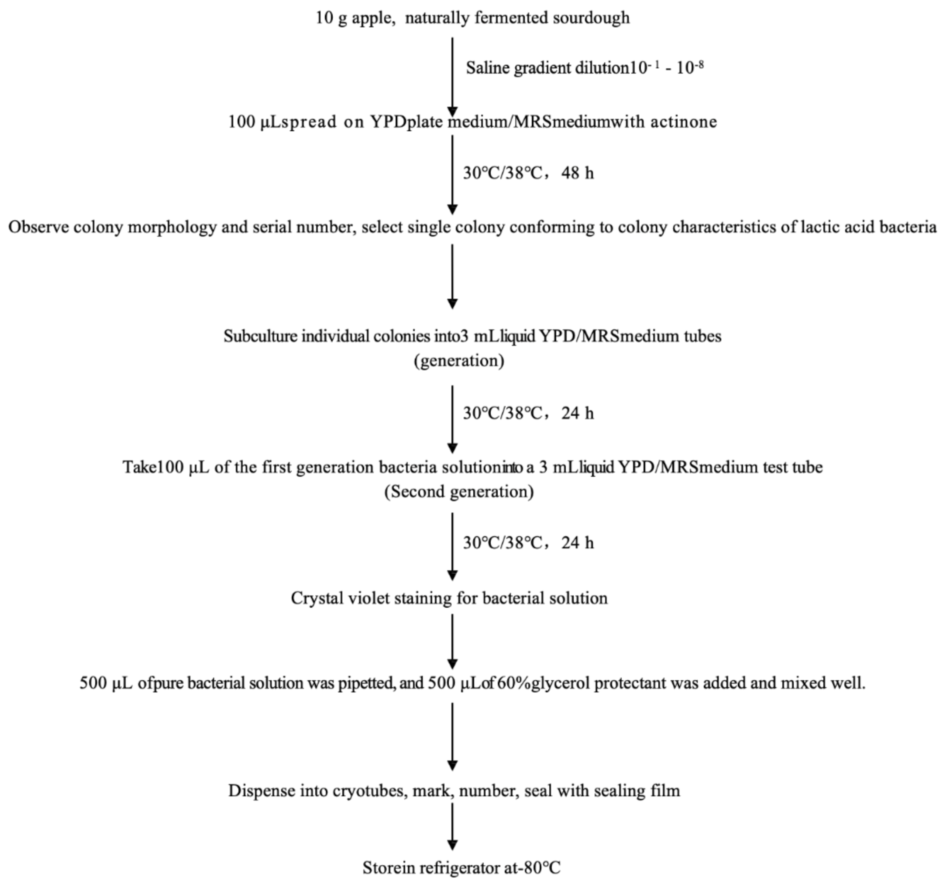


**Fig. 2.** Procedure for isolation, purification and conservation of strains

### 2.6 Identification of lactic acid bacteria isolates from naturally fermented sourdough

#### a.DNAExtraction and Purity Test ofIsolated Strains

DNA of isolated strains was extracted using bacterial genomic DNAextraction kit and yeast genomic DNAextraction kit. Take1 μL yeast and lactic acid bacteriaDNA stock solution, and detect OD ratio and concentration of DNA extracted by micro-ultraviolet spectrophotometer. After sequencing of amplified products, sequence splicing was carried out by DNAstar software, and the obtained base sequence was compared with NCBI database for Blasthomology, and the sequence of known species with the closest homology was found (Wu et al. 2011).

### 2.7 Preparation of naturally fermented sourdough bread

Naturally fermented sourdough bread has slow microbial action, so the method of secondary fermentation is used to make bread. Process: Mix seed dough components, pre-ferment at 30 C° for 4.5h, add dry ingredients and water and stir slowly, add shortening and stir quickly for 3 min until gluten network structure is formed, stirring slowly for 1 min, coating and standing for 10 min at room temperature.
Process: dry materials mixed evenly, add water slowly stir into dough, add shortening and stir quickly for 3minuntil gluten network structure is formed, stir slowly for1min, cover and stand for 10 min at room temperature. Divide the dough into 90 g pieces, and roll them into round shapes. Naturally fermented sourdough bread was proofed for 4 h, 6 h and 8 h respectively in proofer (30C°, relative humidity85%). Common yeast bread was proofed for 90 min in proofer (38C°, relative humidity 85%). The proofed dough was put into oven (170 C°, 210C°) andbaked for 25 min.

#### 2.7.1 Determination of baking characteristics of naturally fermented sourdough bread

#### 2.7.2 Determination of specific volume of bread

After thebread is cooled for2h, the volume is determined by rapeseed displacement method (Liu, 2014), and themass and specific volume are determined by electronic balance (mL/g).

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### 2.7.3 Determination of bread hardness

The hardness of the cooled bread was measured by texture analyzer with different additive amounts and different proofing times. Cut the bread into uniform slices with a thickness of 12.0 mm, and take the middle two slices to measure the hardness of the bread core. Parameter settings: test type TPA, probe model P/36, induction force 5g, pre-test rate1.0mm/s, test rate3.0mm/s. The post-test velocity is 3.0 mm/s, the compression degree is50%, and the interval between two compressions is 1s.

### 2.7.4 Analysis of free amino acids in naturally fermented sourdough bread

Free amino acids of two kinds of sourdough bread (apple) fermented naturally by the best technology were determined and analyzed, and common yeast bread was used as a control. Take 1 g of dried bread sample, add 15mL trichloroacetic acid,10000 Centrifuge at r/minfor 10min, filter with filter paper, transfer supernatant to 25mL volumetric flask, centrifuge at 10000r/min for 10min after constant volume, and analyze free amino acids by HPLC after pre-column derivatization of o-phthalaldehyde. ODSHypersil column (250mm×4.6 mm×5μm) was used.The column temperature was40℃°. The mobile phase was 20 mmol/L sodium acetate and 1:2 (vol/volume) of methanol-acetonitrile at a flow rate of 1mL/min (Zhang, 2014).

### 2.7.5 Determination of volatile flavor compounds in naturally fermented sourdough bread

Volatile flavor compounds of two kinds of sourdough bread (apple) fermented naturally by the best technology were determined and analyzed, and common yeast bread was used as control.

### 2.7.6 Headspace Solid Phase Microextraction(SPME)of Volatile Flavor Components

Divide the bread intopieces of5 mm×5 mm×3 mm, place them ina 15 mL SPMEsample bottle, andthen insert the extraction head from the top of the sample bottleina constant temperature water bath at 60℃°. After headspace extraction for40 min, inject the sample[1].

### 2.8 GC-MS analysis

Chromatographic conditions: DB-5MScapillary column (60 m×0.32 mm, 1 μm)chromatographic column; carrier gas is He; heatingprogram: constant40℃°,1minafter6℃°/minto160℃°, then10℃°/minto250℃°, keep10min; 1.2mL/min constant flow for 2 min, thensplit at a rate of 10 mL/min and a split ratio of 12:1.
Mass spectrum conditions: electron ionization, injection hole temperature250℃°, electron energy70eV, ion source temperature200℃°,emission current200 μA. The acquisition mode is full scan, and the acquisition mass range ism/z 33-495 [1].

### 2.8.1 Qualitative and quantitative analysis of volatile flavor compounds

GC-MSpeaks were matched with Wiley Library and NIST Library by computer and manual retrieval, and the identification results were set as matching degree and purity greater than800. Compound quantification: Calculate the relative percentage content according to peak area normalization method.

### 2.9 Analysis of storage characteristics of naturally fermented sourdough bread

### 2.9.1 Determination of texture parameters of bread during storage

The bread was sealed and stored at 4℃°. The texture parameters of bread stored at 0,1,3,5 and 7 days were determined by texture analyzer.

### 2.9.2 Determination of moisture migration during bread storage

The bread was sealed and stored at 4℃° for accelerated aging. The retrogradation enthalpy of amylopectin was determined by DSC at 0,1,3,5and 7daysafter storage. Weigh 10mg of sample, temperature rise rate is5℃°/min, scanrange is30-110℃° (Zhang et al., 2014).

### 2.10Data analysis and processing

Excel 2007 andSPSS 19.0 software were used for statistical analysis of the data, and analysis of variance (ANONA) was used to analyze significance at alpha< 0.05 level. DesignExpert 8.0 was used to design and analyze response surface experiments.

## 3. Results and discussion

### 3.1 Metabolization of substances in fermentation broth during fermentation

The preparation of apple fermentation broth is the main step in making two kinds of naturally fermented sourdough. During the incubation period, acid flavor and ethanol smell appear in the fermentation broth, accompanied by bubble formation. In order to determine the end-point of fermentation, this part explored the metabolic behavior of microorganisms in fermentation broth.

### 3.2 pH change of fermentation broth

It was observed that at 0d, the initial pH value had little difference, and with the increase of fermentation time, the pH value decreased to different degrees, and the decrease was most obvious at 1-3d. At the later stage of fermentation (5-7d), the pH value of fermentation broth remained basically unchanged due to high acidity inhibiting the growth of microorganisms.

Lactic acid bacteria brought in by fruits mainly exist in the natural fermentation liquid of fruits. Lactic acid bacteria usually carry out metabolic reactions in an anaerobic or facultative anaerobic manner [77]. In the process of fermentation, organic acids such as acetic acid, lactic acid and propionic acid produced [78]impart acidic flavor to the fermentation broth, and at the same time, various acids produced inevitably cause the pH of the fermentation brothtodrop. The type, activity and quantity of lactic acid bacteria have different effects on the pH value of fermentation broth (Ma et al. 2013), and the reason for the significant difference in pH value of two fermentation broths may be due to the different initial raw materials selected (apples) resulting indifferent lactic acid bacteria generated during natural fermentation. Yeast had little effect on pH of fermentation broth.

### 3.3 Changes of Organic Acids in Fermentation Broth

The main microorganisms in apple fermentation broth were lactic acid bacteria and yeast. Lactic acid bacteria can pass throughEMP and HMP two metabolic pathways (Kang and Liu 2012), its main products are lactic acid and acetic acid. Therefore, two organic acids, lactic acid and acetic acid, are selected as standard samples for determination of organic acids in two fermentation solutions in this study. The concentration of organic acids in samples is determined by comparing the concentration of standard samples with high performance liquid chromatography(HPLC)..

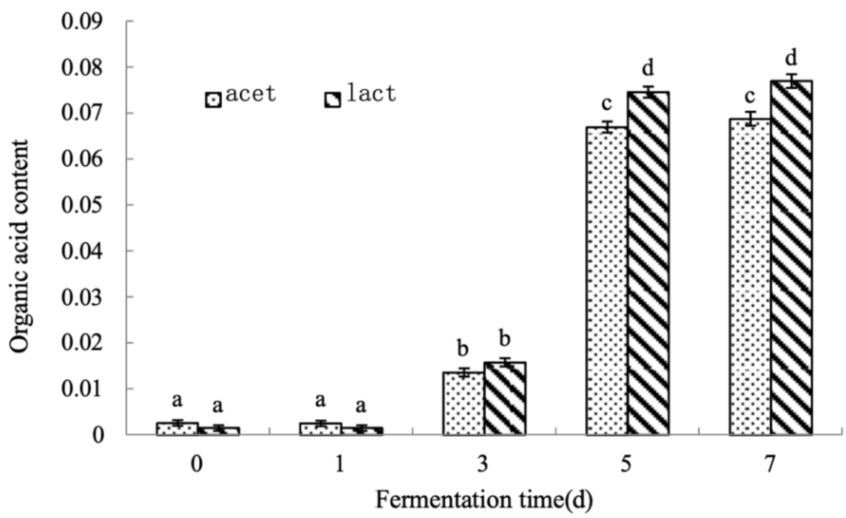


**Fig. 3-5**. The changes of organic acids content in apple broth during the fermentation process

As can be seen from Figures 3-4and3-5, in the two fermentation solutions, at 0d, natural fermentation has not yet started, andthe acetic acid and lactic acid contents in the fermentation solution are extremely low, which may be introduced by apples. With the fermentation time increasing, lactic acid bacteria fermentation acid production, the content of two kinds of organic acids in the fermentation broth gradually increased,the organic acid increased to the maximum within 3-5 days, and within5-7days, with the consumption of carbohydrates completely, the organic acid content increased slowly, basically unchanged.

According tothe experimental results of pH, soluble sugar and organic acid, the pH of fermentation broth remained basically unchanged at7d, the main soluble sugar was consumed, and the content of organic acid did not increase, the metabolic activities in the fermentation brothhad reached a relatively stable state, indicating that the microorganisms in the fermentation broth were also in dynamic equilibrium, so 7 d was selectedas the fermentation endpoint of apple fermentation broth. After fermentation for 7 days, the fermented liquid is added to high-gluten flour and cultured for 5-6 hours to obtain naturally fermented sourdough.

### 3.4 Basic properties of naturally fermented sourdough

The value of T T A refers to the total acid content of naturally leavened sourdough, while pH indicates the amount of strong acid produced by microorganisms in naturally leavened sourdough[81]. Table 3 - 1 shows the pH and TA values and the number of lactic acid bacteria and yeast contained in apple sourdough. From Table 3 -1, it can be seen that the pH values of the two naturally fermented sourdoughs are 5.08 and 3.88, respectively, and the corresponding T T A is 7.70 mL and 12.32 mL. The pH of apple naturally fermented acid dough is lower, the acidity is stronger, and the corresponding total acid content is also higher. The pHandT T A results are close to those of traditional sourdough in some regions of China [82]. The study of Zh ang[39]showed that pH and TA of naturally fermented sourdough collected from western Inner Mongolia were limited to a narrow range of 3.32 - 3.82 and 11.2 - 12.9 mL, respectively. The main reason for the differences between naturally fermented sourdough is the number and type of microorganisms in naturally fermented sourdough in different regions[82], which will produce differences in acid production capacity.

**Table 3-1** The pH、TTA, and result of counting lactic acid bacteria and yeast in two kinds of spontaneous fermentation sourdough

| | pHvalue | TTA（mL） | LAB（log CFU/g） | Yeast（log CFU/g） |
|---|---|---|---|---|
| **Apple naturally fermented sourdough** | 3.88±0.04 | 12.32±0.23 | 7.92±0.13 | 5.84±0.17 |

The counts of lactic acid bacteria and yeasts in Table 3-1 show that the average number of lactic acid bacteria apple sourdough was7.92logCFU/g. According to literature reports [36], when the ratio of lactic acid bacteria to yeast in the naturally fermented sourdough system is100:1, the microorganisms in the system reach a relatively stable state of existence. The colony count results in this study showed that the ratio of lactic acid bacteria apple sourdough was close to100:1, that is, both reached a stable state. More lactic acid bacteria in apple sourdough corresponded to lowerpHvalues. Compared with other authors [83,84] whocounted10 8 - 10 9CFU/g of lactic acid bacteriaand10 6 - 10 7CFU/g of yeast for naturally fermented sourdough, the numbers of lactic acid bacteria and yeast in this study were lower, probably due to the introduction of fruit substrate.

### 3.5 Isolation and identification of lactic acid bacteria from naturally fermented sourdough

#### 3.5.1 Isolation and identification of lactic acid bacteria from naturally fermented sourdough

MRSsolid medium was used to isolate lactic acid bacteria in this study.The colonies of lactic acid bacteria are usually white, smooth and moist, with flat edges, colony diameters of0.5-1.5 mm, and Gram-positive by microscopic examination[85]. In this study, 12 strains were isolated from apple sourdough.

**Table 3-2.** The identification result of LAB based on 16S rDNA sequencing

| strain number | identification results | homology |
|---|---|---|
| AL1 | *Lactobacillus plantarum* | 99% |
| AL2 | *Lactobacillus plantarum* | 100% |
| AL3 | *Lactobacillus plantarum* | 100% |
| AL4 | *Lactobacillus plantarum* | 100% |
| AL5 | *Lactobacillus plantarum* | 100% |
| AL6 | *Lactobacillus plantarum* | 100% |
| AL7 | *Lactobacillus plantarum* | 100% |
| AL8 | *Lactobacillus plantarum* | 100% |

| | | |
|---|---|---|
| AL9 | *Lactobacillus plantarum* | 100% |
| AL10 | *Lactobacillus plantarum* | 99% |
| AL11 | *Lactobacillus plantarum* | 100% |
| AL12 | *Lactobacillus plantarum* | 100% |

The strain could be identified as species if the homology exceeded 99%. The identification results of lactic acid bacteria 16S rDNA in two kinds of naturally fermented sourdoughare shown in Table3-2. All of the above strains have99%or100% homology, so they can beidentified as species.
All strains were identified as Lactobacillus plantarum. It is also facultative heterofermentative lactic acid bacteria with strong acid-producing ability. Its abundant existence also explains the low pH value of apple naturally fermented sour dough.

## 3.6 Effect of naturally fermented sourdough on baking characteristics of bread

Two technological parameters of sourdough addition and fermentation time of apple sourdough bread were selected and determined as10%, 20%, 30%, 40% and 4,6,8h respectively.

### 3.6.1 Effect of naturally fermented sourdough on bread specific volume

The results are shown in Figure 3-8. Different combinations of natural sourdough addition and proofing time have different effects on the specific volume of bread. The bread specific volume increased firstly and then kept constant with the increase of proofing time when the additive content was constant, and increased firstly and then decreased with the increase of additive content when the proofing time was constant.

The content of yeasts in naturally fermented sourdough system determines the ability of bread to produce gas, whilelactic acid bacteria in naturally fermented sourdough system can play an acidification role, and the type and degree of acidification will also have different effects on bread specific volume.Therefore, different combinations of these two important process parameters will have different effects on bread specific volume. Thedifference of specific volume between 4 h and 6 h islarger, but the difference between6 hand8 his smaller. When the additive content was40%, the specific volume decreased probably due to excessive acidification. Under moderate acidity conditions, the specific volume of bread can reach a maximum.[92]The proper amount of acid will activate the egg white enzyme in wheat flour, jointly degrade gluten to release water, improve the water absorption rate of starch and polysaccharide, the activity of other enzymes and the gas holding capacity of gluten network structure, while too high acidity and too strong protein hydrolysis will weaken gluten and reduce bread specific volume [93]. At the same time, the symbiosis between lactic acid bacteria and yeast will increase the gas production of yeast[50]. There was a positive correlation between bread specific volume and bread quality.

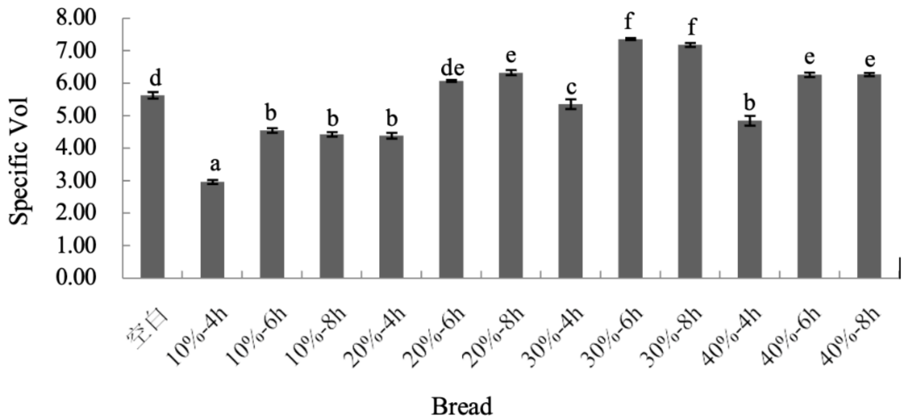


**Fig. 3-8** Specific volume of different breads Apple spontaneous fermentation sourdough bread

## 3.7 Effect of naturally fermented sourdough on bread hardness

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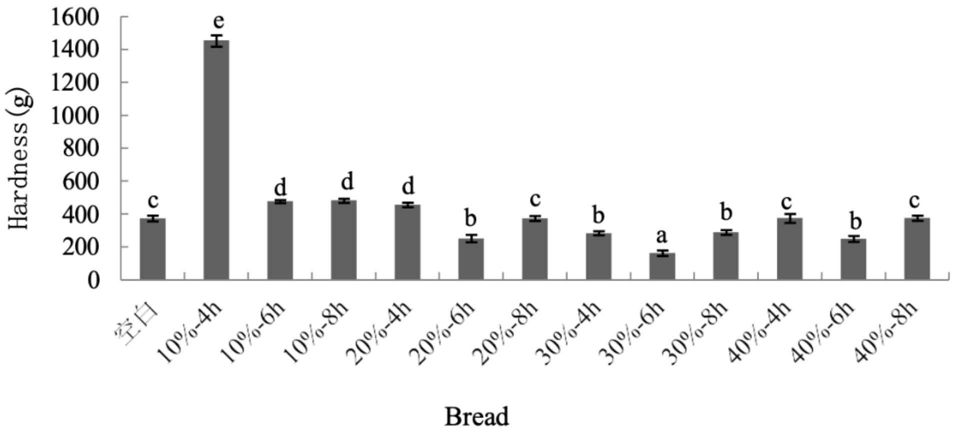
Common yeast bread was used as the blank control group. The experimental results areshown in Figure3-9. Hardness is one of the most important textural parameters to characterize bread quality. Different combinations of naturally leavened sourdough addition and proofing time will affect the hardness of bread. The hardness of the two kinds of bread reached themaximum at 10%-4h, which was due to the small amount of yeast added,the short time of bread proofing, the insufficient gas production of dough, and the incomplete proofing, resulting in dense bread tissue and high hardness. With the increase of the amount of yeast added and the proofing time, the hardness of bread decreased in different degrees. Thehardness of bread decreased firstly and then increased with the time of proofing when the amount of bread added was fixed, and decreased firstly and then increased with the amount of bread added when the time of proofing was fixed.

There was a negative correlation between bread hardness and bread quality. The above experimental results show that proper addition amount and proofing time are beneficial to reduce bread hardness and improve bread quality, while excessive addition amount leads to large acid production of lactic acid bacteria, excessive acidification of bread dough, resulting in increased bread hardness, which is consistent with Zhang Qing's research results [67]. As shown in Figure3-9, for grapes For dry sourdough bread and apple sourdough bread, the hardness minimum appeared in 30%-6h group.




### 3.8 Effect of naturally fermented sourdough on nutritional properties of bread- free amino acids

Amino acids not only affect the flavor and color of bread, but also as the basic component unit of protein, amino acids have nutritional support, immune regulation, anti-tumor and other nutritional effects[97]. Both naturally fermented sourdough breads contained18 amino acids and tryptophan, which was not present in regular yeast bread. Tryptophan is an essential amino acid that can only be synthesized by plants or microorganisms.[98] Tryptophan can promotesleep, relieve migraine headaches, and relieve anxiety and stress. The contents of 11 kinds of amino acids and 6 kinds ofessential amino acids in bread were increased after adding apple naturally fermented sour dough. Afterfermentation, the total amino acids increased, and the proportion of essential amino acids in total amino acids increased from14.15%to35.02%and28.79% respectively.

The increase in free amino acid content in naturally fermented sourdough bread is mainly due to protein degradation.[47] Lactobacillus plantarum and Lactobacillus sake in the naturally fermented sour dough system ferment to produce acid, which stimulates the protease activity in flour, and the protease hydrolyzes protein[9], thus releasing free amino acids and small peptides [8], resulting in an increase in free amino acid content. Since yeast fermentation reduces the free amino acid content[100], we can assume that the use of naturally fermented sourdough instead of yeast fermentation can strengthen the amino acids in bread and make it more nutritious.

| Compounds | Plain Yeast Bread | Apple Naturally fermented bread |
|---|---|---|
| glutamic acid | 3.43533×10-2 | 2.28895×10-2 |
| aspartic acid | 5.52572×10-2 | 4.42513×10-2 |
| serine | 7.29053×10-4 | 3.60440×10-4 |
| histidine | 1.45920×10-3 | 1.41331×10-3 |
| glycine | 7.16986×10-3 | 8.40532×10-3 |
| threonine | 4.76751×10-3 | 6.33333×10-3 |
| arginine | 8.87450×10-3 | 1.03350×10-2 |
| alanine | 1.66298×10-2 | 1.46599×10-2 |
| tyrosine | 1.95145×10-3 | 3.02735×10-3 |
| cysteine | 2.88976×10-5 | 6.59661×10-5 |
| valine | 6.44574×10-3 | 9.86554×10-3 |
| methionine | 9.24600×10-4 | 6.41383×10-4 |
| tryptophan | - | 1.35858×10-2 |
| leucine | 2.24189×10-3 | 7.11628×10-3 |
| lysine | 4.23283×10-3 | 1.84217×10-3 |
| proline | 1.75837×10-2 | 2.04498×10-2 |

### 3.9 Characteristics of Volatile Flavor Compounds in Naturally Fermented Apple sourdough Bread

Flavor of bread products is an important factor affecting bread acceptability. The metabolic activities of lactic acid bacteria and yeast coexisting in naturally fermented sourdough system endows naturally fermented sourdough bread with special flavor characteristics. The metabolic products of yeast and lactic acid bacteria become the chemical components that determine this special flavor. The flavor substances produced by naturally fermented sour dough in bread are mainly divided into ketones, aldehydes, esters and alcohols produced by biochemical action during fermentation and organic acids produced by lactic acid bacteria fermentation.[55]

GC-MS technique was used to determine and analyze the flavor compound characteristics of common yeast bread, raisin naturally fermented sourdough bread and apple naturally fermented sourdough bread. The GC-MS spectra of flavor compounds of three kinds of breadare shown in Appendix A-4. After sourdough fermentation, the peak positions and peak areas in the spectra were different, andthe types and relative contents of volatile flavor substances in bread were changed. From Table3-5, we cansee that 99% of the three kinds of bread were detected.

Volatile flavor compounds in the three breads were classified intoeightcategories as shown in Tables3 - 6. Alcohol content is the highest, and natural fermentation sour dough makes its content further increase, among which ethanol, phenylethanol and n-hexanol increase more, phenethyl alcohol can give bread rose flavor, and n-hexanol has grassy flavor[101]. The content of acids in naturally fermented sourdough breadis also increased, especially acetic acid, which is the signature flavor in the bread core and is produced byheterotypic fermentation of lactic acid bacteria in naturally fermented sourdough system[1 02]. Aldehydes decreased and estersincreased as a result of the interaction between acids produced by lactic acid bacteria and alcohols produced by Saccharomyces cerevisiae in the naturally fermented sourdough system, which introduced ethyl linoleate and isoamyl acetate into the bread, which gave the bread a creamy and banana flavor, respectively[1 01]. Hydrocarbon compounds with high threshold have little effect on flavor[103]. Aromaticcompounds increased after sourdough fermentation, as did heterocyclic and nitrogen-containing compounds. In addition, the flavor differences between the two naturally fermented sourdoughbreads are likely due to differences in their internal microflora structure.[104]Thedifference in microorganisms between raisin sourdough and apple sourdough resulted in different flavor compound profiles of bread products.

| Compounds | kind | Peak area/$10^7$ | kind | Peak area/$10^7$ |
|---|---|---|---|---|
| alcohol | 11 | 1616.67 | 14 | 2274.32 |
| acid | 9 | 195.41 | 8 | 431.41 |
| aldehyde | 11 | 319.73 | 12 | 208.80 |
| ester | 12 | 111.23 | 16 | 204.12 |
| ketone | 7 | 181.24 | 6 | 377.29 |
| hydrocarbon | 4 | 43.98 | 4 | 73.37 |
| aromatic compounds | 3 | 150.75 | 5 | 174.05 |
| Heterocycles and nitrogen-containing compounds | 3 | 66.78 | 3 | 256.67 |

## 4. Conclusion

This study demonstrates that incorporating naturally leavened sourdough—derived from apples—significantly enhances the nutritional, flavor, and storage properties of bread. Raisin sourdough notably elevated the content of essential amino acids, increasing the proportion of essential amino acids. Furthermore, sourdough bread exhibited superior other bread characteristics. These findings underscore the potential of natural sourdough as a multifunctional leavening agent, offering a sustainable strategy to produce nutrient-dense, flavorful, and shelf-stable bread while reducing reliance on commercial additives. Future applications could optimize sourdough microbiota to tailor amino acid profiles and flavor outcomes for artisanal baking innovations.

**Credit statement**

Xuanyu Xing designed, performed experiments and analyzed data.

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