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Comparison of Sourdough Bread Made by Using Different Percentages of Sourdough Starter with and without Addition of Instant Dry Yeast

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Abstract: *Sourdough bread is rich in nutrients, antioxidants, and distinctive flavor, contributing health benefits and extended shelf life. Therefore, the objective of this study was to compare the sourdough bread made by using different percentages (100 and 200g) of sourdough starter with and without addition of instant dry yeast (10g). All sourdoughs were fermented at room temperature for 6 hours and baked at 200°C for 25 minutes. The physicochemical analyses were then conducted on both the sourdough and bread. The nutritional profile of sourdough bread was evaluated. Additionally, the microbiological quality and sensory evaluation using a 9-point hedonic scale were also performed. Moreover, the shelf-life of sourdough bread was assessed over five days at room temperature. The results indicated that the fermentation with four conditions significantly reduced pH while increasing titratable acidity and lactic acid bacteria levels, with the values ranging from 4.43 to 5.63, 0.033 to 0.047%, and 6.16 to 9.45 Log CFU/g, respectively. In terms of color, the lightness (L*) decreased, while the redness (a*) and yellowness (b*) increased as more sourdough starter and yeast were added. For nutritional composition of sourdough bread, the carbohydrate, protein, fat, crude fiber and ash content were 38.70±1.14 to 44.74±0.35%, 11.8±50.14 to 12.37±0.09%, 2.75±0.07 to 2.79±0.02%, 1.42±0.14% to 1.7±0.31%, and 1.27±0.32 to 1.67±0.34%, respectively. For the result of sensory evaluation, the sourdough bread with 200g of sourdough starter and 10g of instant dry yeast provided a higher score for overall acceptability. And the moisture content and water activity showed no significant difference between them. For total plate count, and yeast and mold count, four formulated sourdough breads had no significant difference, ranging from 2.17 to 4.02 Log CFU/g, ND to 4.21 Log CFU/g, and ND to 4.03 Log CFU/g, respectively. And the shelf-life of sourdough bread with 200g of sourdough starter and 10g of instant dry yeast was 3 days at room temperature.*

Keywords: Sourdough, Starter culture, Nutritional composition, Organoleptic analysis, Shelf-life

1. INTRODUCTION

Sourdough bread is produced when a sourdough starter is combined with flour and water to make dough. The sour taste recognized in sourdough bread is primarily attributed to the production of organic acids, particularly lactic acid and acetic acid, by lactic acid bacteria during the fermentation process [1]. In recent years, increased consumer awareness of health-related hazards of food chemical preservatives has resulted in a trend toward increased preference for natural preservative-free processed foods [2]. According to Catzeddu [3], sourdough bread also has superior nutritional properties, rich in nutrients, vitamins, and minerals, helps the digestive system, and regulates blood sugar levels. Indeed, an increase in the appreciation for more flavorful and healthy breads over the last decade has raised consumer demand for sourdough breads.

The current renaissance of sourdough as a bioprocessing is encouraged by its beneficial effect on flavor, texture, shelf life, and health-promoting properties [4]. Additionally, the germ and bran of the grains are the sources of the nutritional components found in cereals, including proteins, vitamins, and polyphenol chemicals. Although these fractions are often eliminated during milling to avoid negative effects on the senses and technology, they are a source of fiber and protein that may be helpful in various culinary applications [5,6]. As a result, wheat flour contains 100g of total nutritional composition, including 70g of

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total carbohydrate, protein, lipids, moisture, and dietary fiber, which account for 12g, 11g, 3g, and 4g, respectively.

Currently, Cambodia has many types of bread, with the most popular being the taste of regular and sweet bread. However, the bread with a sour taste was called sourdough bread and was present in some bread stores in Cambodia. According to Reale et al. [7], sourdough-based fermentation is utilized globally to produce a variety of sweet baked items, pizza, crackers, and many traditional forms of bread. Additionally, sourdough bread is still not widely popular in the market, and it's the quality and safety of sourdough bread are still under study and research. Thus, that's why there is a study of sourdough bread production by fermentation. Hence, this study aims to evaluate the investigation of the different amounts of sourdough starter with and without the addition of instant dry yeast.

2. METHODOLOGY

2.1 Materials

Sourdough starter was purchased online. In addition, instant dry yeast (Angel, China), wheat flour (White Swan, Thailand), and other ingredients including sugar (Mitr Phol, Thailand), milk powder (Golden Chef, Thailand), Soybean oil (Simple, Vietnam), Bread improver (Puratos Grand-Place, Vietnam), baking powder (MGarrett, Thailand), fresh milk (Kirisu, Cambodia) and salt were sourced from local markets and supermarkets in Phnom Penh, Cambodia.

2.2 Sourdough starter preparation

One packet of sourdough starter was combined with one tablespoon of flour and one tablespoon of water, stirred, and fermented for 24 hours at 21–26°C. The starter was fed incrementally with flour and water over three to seven days until it was a bubbling, pancake batter-like consistency. For maintenance, the starter was fed daily at room temperature (1:1:2 ratio of starter, water, and flour) or every 5–7 days under refrigerated temperature.

2.3 Sourdough bread preparation

The sourdough bread production process was adapted with slight modifications based on the method outlined by Marco Gobbetti [8], sourdough bread was prepared by mixing a starter with and without instant dry yeast and adding other ingredients. Four formulations were developed with and without instant dry yeast, NYSB1, NYSB2, YSB1, and YSB2 (**Table 1**). After mixing all ingredients, the dough was kneaded, fermented for 4 hours, proofed for 2 hours, and baked at 200°C for 25 minutes. After cooling to room temperature, the bread was ready for further analysis.

Table 1. Four formulations of sourdough bread

Conditions	Sample code			
	NYSB1	NYSB2	YSB1	YSB2
Sourdough starter (g)	100	200	100	200
Instant dry yeast (g)	—	—	10	10

2.4. Characteristics of sourdough starter

2.4.1 Determination of pH value

The pH value of each sample was determined with a digital pH meter (LAQUA F-72-HORIBA, China), according to the AOAC 942.15 [9].

2.4.2 Acidity measurement

Total titratable acidity (TTA) was measured using the AOAC (942.15) volumetric method.

2.4.3 Microbiological analysis

Lactic acid bacteria were counted on *Lactobacillus* MRS Agar according to Merck, Darmstadt, Germany [10]. A 10 g sample of sourdough at 0, 3, and 6 hours of fermentation was put in 90 ml of 0.85% buffered sodium chloride and diluted to 10^{-8}. Then 0.1 mL of dilution was spread on MRS agar plates and incubated at 35-37°C for 24-48 hours.

2.5 Nutritional profile

2.5.1 Determination of protein content

Protein content was tested using the Kjeldahl method and then calculated using a conversion factor of 6.25 based on AOAC method 920.39.

2.5.2 Determination of fat content

Fat content was determined using the Soxhlet extraction method described by Dos et al. [11].

2.5.3 Determination of fiber and ash content

The crude fiber and ash content of the bread samples were determined using AOAC standard methods.

2.5.4 Determination of total carbohydrate

Total carbohydrate was determined using the method described by Yu et al. [12] based on the AOAC method (2004).

The carbohydrate content was calculated by using the following equation:

$$\% \text{ Carbohydrate} = 100 - \% (\text{protein} + \text{fat} + \text{ash}) \quad \text{(Eq. 1)}$$

2.5.5 Determination of total energy

Total energy content was calculated based on the European Parliament and Council Regulation No. 1169/2011 [13], using energy conversion factors of 4 kcal/g for carbohydrates and proteins, and 9 kcal/g for fats. The total energy was determined using the following equation:

$$\text{Total energy} = 4 \text{ carbohydrates} + 4 \text{ protein} + 9 \text{ fat} \quad \text{(Eq. 2)}$$

2.6 Physical analysis of sourdough bread

2.6.1 Determination of moisture content

Moisture content was determined following AOAC methods (2004); 5 g of each sample was cut into small pieces and placed into an aluminum cap. It was dried in an oven at 105°C for 3 hours, then cooled in a desiccator for around 15 minutes.

2.6.2 Determination of water activity

Water activity was measured with an AQUALAB PAWKIT meter (Meter Group, Inc., Pullman, WA, USA). Weigh 2g bread sample was placed in the meter, and results were obtained after 5 minutes at the ambient temperature.

2.6.3 Color measurement

Color was evaluated using a Chroma CR400, Konica-Minolta Ltd., Osaka, Japan, according to the CIELAB color space, which is expressed in CIE L*, a*, and b* values. The instrument was calibrated with a white tile before measuring L*, a*, and b* values were recorded for the crust and crumb of sourdough bread, with triplicate measurements taken. The final results were the average of all measurements.

2.6.4 Spread loaf volume determination

The spread volume of sourdough bread was measured by dividing the diameter (height, width, and length). These dimensions were taken using a caliper tool, and the results were recorded as averages. Measurements were shape of 100 g loaves under three different conditions: after initial proofing, after final proofing, and after baking.

2.7 Sensory evaluation

Sensory evaluation using a 9-point hedonic scale was conducted with 80 panelists to assess acceptability based on appearance, color, pore size, odor, aroma, texture, saltiness, sourness, flavor, and overall preference.

2.8 Statistical analysis

All tests were performed in duplicate, with results presented as mean ± standard deviation. Statistical analysis was done using SPSS (version 25). One-way ANOVA and Tukey's post-hoc test were used to evaluate differences, with significance set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Characteristics of sourdough starter

3.1.1 Physicochemical and microbiological characteristics of sourdough starter

The sourdough starter after fermenting for 8 hours was ready to mix with other ingredients to make sourdough bread. After fermentation for 8 hours, the values of pH and total titratable acid of sourdough starter were 3.61 and 0.10%, respectively. The TTA of sourdough starter increased after fermentation [14]. The result was similar to the study of Ogunsakin et al. [15] and Pei-Ling Chung et al.[16], the pH values ranged from 3.5 to 4.3, total titratable acidity was between 0.0423 to 0.1521%, and lactic acid bacteria were at least 8 Log Colony Forming Unit (CFU)/g. Additionally, lactic acid bacteria provide an acidic environment [17]. In addition, the result of lactic acid bacteria count was 11.41 log CFU/g.

3.2 Physicochemical and microbiological characteristics of sourdough during fermentation

During the fermentation time of the dough, after mixing for 0 hour, and after fermentation for 6 hours. The pH values were decreased after fermentation from 3 h and 6 h for all conditions *($p<0.05$)*. Based on the result, the pH value decreased with fermentation time. The result was in agreement with the study of Bartkiene et al. [18]. While TTA increased from 0.034% to 0.047%. However, in all doughs, a pH drop creates an increase in total titratable acid after fermentation. LAB counts in sourdough can reach up to 9.2 log CFU/g after 6 hours of fermentation, showing high activity and influencing the physical properties of the resulting bread [19]. Lactic acid bacteria (LAB) counts increased in all sourdough conditions during fermentation. All sourdoughs developed LAB counts from log 7.5 to 9.2 log CFU/g sourdough within 24 h [20].

3.3 Nutritional profile of sourdough bread

Table 2 shows the nutritional profile of sourdough bread. The protein contents of NYSB1, YSB1, NYSB2, and YSB2 samples were 11.85%, 11.86%, 12.28% and 12.37%, respectively. The result was in agreement with the report of Ijah et al. [21]. The increase in the percentage of protein content is contributed to by the action of proteolytic enzymes (proteases) and LAB, depending on the fermentation time and pH [22,23].

For carbohydrate content, the mean values were 41.48%, 38.70%, 41.57%, and 44.74% for NYSB1, NYSB2, YSB1, and YSB2, respectively. The result was similar to the study done by Ramita Supasil et al. [24]. Additionally, the carbohydrate content of sourdough bread made from wheat flour was determined through proximate analysis. Sourdough bread samples were analyzed for their moisture, protein, fat, and ash content [25].

The fat content, the mean values were 2.77%, 2.79%, 2.75%, and 2.78%, respectively, for samples NYSB1, NYSB2, YSB1, and YSB2 of sourdough bread. The fat content of sourdough bread remains similar for each condition. Moreover, the fermentation process in sourdough bread is more focused on breaking down starches and improving the bread's texture, flavor, and nutrient profile, not significantly altering the fat content. The result was in agreement with the report of Ramita Supasil. According to Ogunsakin, the fat content of their study was 2.8%.

The fiber contents of NYSB1, NYSB2, YSB1, and YSB2 sample were 1.74 %, 1.79%, 1.42%, and 1.79 %, respectively. The fiber content of all conditions of sourdough bread was within the range of 0.4% to 2.4%, compared to other research [26]. The fiber in sourdough bread comes primarily from the flour used, which is not altered by the fermentation process [27].

The ash content refers to the mineral or inorganic material present in the flour. The ash content of the flour used to make sourdough bread is a critical factor that impacts the development of sour flavor and overall bread quality [28]. Based on the results of this study, the ash contents of the mean values were 1.27%, 1.33%, 1.42%, and 1.67% for samples NYSB1, NYSB2, YSB1, and YSB2, respectively.

Sourdough bread may be easier to digest for some people and contains higher levels of certain vitamins, minerals, and antioxidants compared to other breads. The mean values of the total energy content were 238.65 kcal, 229.05 kcal, 238.74 kcal, and 253.55 kcal for samples NYSB1, NYSB2, YSB1, and YSB2 sourdough bread, respectively. The result was similar to the study by Gobbetti et al. [29]. The highest total energy content in sourdough bread made with lactic acid bacteria and yeast is attributed to the symbiotic growth of these microorganisms, enhancing improved quality characteristics [30].

Table 2. Nutritional profile of freshly baked sourdough bread

Samples	Carbohydrate (%)	Protein (%)^{*ns}	Fat (%)^{*ns}	Fiber (%)	Ash (%)	Total Energy (Kcal)
NYSB1	41.48±1.48^{b}	11.85±0.14	2.77±0.25	1.74±0.46^{a}	1.27±0.32^{c}	238.65±4.21^{c}
NYSB2	38.70±1.14^{c}	12.28±0.34	2.79±0.07	1.79±0.31^{a}	1.33±0.68^{c}	229.05±2.55^{c}
YSB1	41.57±1.08^{b}	11.86±0.31	2.75±0.20	1.42±0.28^{b}	1.42±0.07^{b}	283.74±3.72^{a}
YSB2	44.74±0.35^{a}	12.37±0.09	2.78±0.27	1.79±0.42^{a}	1.67±0.34^{a}	253.55±0.72^{b}

**ns* no significance.

The values are presented as the mean ± standard deviation. In the same column, the values with different superscript letters (a, c) differ significantly from each other *(p<0.05)*.

3.4 Physical characteristics of sourdough bread

3.4.1 Volumes during initial, finish proofing, and after baking of sourdough bread

According to the results of the volume of loaf bread at initial proofing, the dough volume varied between 110-127 cm^3, not very much, and increased slightly changes each condition of sourdough bread. Proofing of dough is indeed a crucial step in achieving voluminous baked goods, as it significantly impacts the quality and characteristics of the final products [31].

3.4.2 Crust and crumb of sourdough bread

There was a significant difference *(p<0.05)* in crumb and crust in terms of all four sourdoughs. Based on the result of the crumb L*, a*, and b* values were higher in NYSB1, NYSB2, and YSB1 compared to YSB2, with YSB2 showing a close to zero a* value. Furthermore, crust color had higher lightness (L*) in the NYSB1, NYSB2, and YSB1, while YSB2 had higher a* values. The result was in agreement with the study of [32].

3.5 Sensory evaluation of sourdough bread

According to the results of this study, YSB2 was the most acceptable. There was no significant difference *(p>0.05)* between the NYSB1, NYSB2, and YSB1 (**Table 3**). Samples NYSB2 and NYSB1 had a lower result in some attributes. YSB2 had the highest pore size, and increasing sourdough levels positively impacted the pore size of the final bread [33]. Overall acceptability is very important for sensory evaluation. All the bread samples had higher overall acceptability, with acceptable scores receiving more than seven. Additionally, condition YSB2 had higher overall acceptability compared with other conditions of sourdough bread. The final product’s texture and sensory attributes are significantly influenced by the sourdough and instant dry yeast, with the fermented dough process [34]

Table 3. Result of sensory evaluation of sourdough bread.

Attributes	NYSB1*ns	NYSB2*ns	YSB1*ns	YSB2*ns
Appearance	6.91±1.2	6.96±0.2	6.95±0.9	7.12±1.6
Texture	7.05±0.2	7.13±0.1	6.73±0.4	7.33±0.3
Color	7.27±0.8	7.3±0.6	6.96±1.9	7.07±1.6
Aroma	6.77±1.2	6.86±0.2	7±0.8	7.08±1.4
Flavor	7.1±0.2	6.86±0.1	7.26±0.4	7.07±0.8
Saltiness	6.83±0.7	6.75±0.6	6.81±1.9	6.87±1.9
Sourness	6.87±1.2	6.75±0.2	7.05±0.8	6.98±1.6
Pore size	6.9±0.2	6.83±0.1	6.88±0.4	7.05±0.8
Overall liking	7.11±0.7	7.1±0.7	7.27±1.9	7.4±1.6

**ns* no significance
The values are presented as the mean ± standard deviation *($p<0.05$).*

3.6 Physicochemical and biological properties of sourdough bread during storage

3.6.1 Moisture content

During the storage period, the moisture content of bread can undergo changes depending on various factors [35]. The moisture content of each condition of sourdough bread shows no significant differences; the mean value changed from 27.86 to 38.67 from day 0 to day 5.The increase in the moisture content of the crust can be attributed to the redistribution of moisture from the crumb to the crust during the storage of bread [36]. The moisture content of the loaf bread changed rapidly as soon as it was removed from the oven, and during the cooling stage [37]. Consequently, this resulted in the variation of vapor pressure between the loaf crust and the internal part of the loaf and led to the migration of moisture from the crumb to the crust [38]. According to the result of this study, the moisture of all bread crumbs was observed to increase from 0 to 5 days. This result is in agreement with Pateras et al. [39], who found a reduction in crumb moisture and an increment in the crust moisture during the storage days.

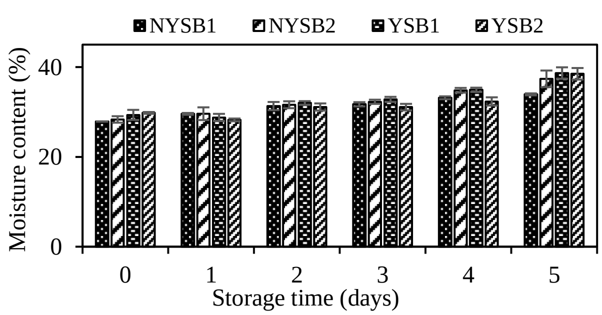

Fig. 1. Moisture content of sourdough bread in different conditions during storage

3.6.2 Water activity

Water activity in bread is indeed a crucial parameter that significantly influences the product's shelf life. Water activity plays a vital role in limiting microbial growth, stabilizing food matrices, and extending the shelf life of various food products, including bakery items like bread [40]. Additionally, it provides information regarding the possibility of microbiological growth on the surface during the storage period [41]. The water activity of sourdough bread with and without adding instant dry yeast, and with the different amounts of 100g and 200g of sourdough starter. From day 0 to day 5, the water activity of each condition of bread increased between 0.063, 0.0875, 0.0953, and 0.954 for NYSB1, NYSB2, YSB1, and YSB2, respectively. The results of this study, the aw values obtained from the present study, were in agreement with those reported by Lazaridou et al. [42], for gluten-free breads, which varied in the range of 0.974 to 0.986 and 0.961–0.979 on days 1 and 3, respectively, of storage.

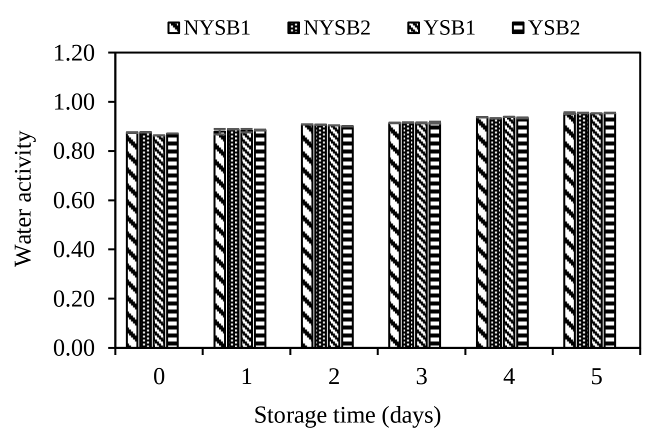

Fig. 2. Water activity with different conditions during storage time.

3.6.3 Total plate count

The total plate count in sourdough bread was determined at intervals from 0, 1, 2, 3, 4, and 5 days. As shown in **Fig. 3**, the results showed that the shelf life of the condition NYSB1 had the initial total plate counts increased from 3.04, 3.07, 3.25, and 4.02 log CFU/g dry weight on days 2, 3, 4, and 5, respectively. While on days 0 and 1, it was not detected in the NYSB1 sample. According to the Cambodian Standards (CS 001:2000), it could be permitted not to be declared the date of minimum durability for the kind of bread products that are normally kept for use for 24 hours only [43]. Additionally, according to the Malaysian Food Law, the total plate count of colonies should be less than 5 log CFU/g in bread. As mentioned above, the critical threshold for yeast and mold is 3 log CFU/g, which did not necessarily occur at the same time; it only requires one of the critical values to be reached for the end of shelf life to be declared. Consumers can reject bread based on texture or visible mold signs [44].

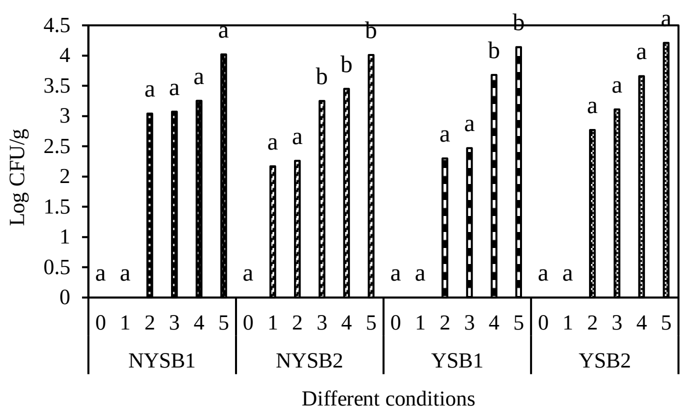

Fig. 3. Total plate count during storage of sourdough bread

3.6.4. Total yeast and mold count

During the storage of bread, the total yeast and mold count can vary based on factors such as temperature, bread type, and storage duration [45]. The microbial analysis of the fresh sample of NYSB1, as shown in **Fig. 4**, revealed that the total yeast and mold count ranged from 2.54 to 3.62 log CFU/g from day 3 to day 5 of the storage period. For the result of condition NYSB2, the mean values range from 2.17 to 3.68 log CFU/g. The results showed that the increased values of total yeast and mold count were found in the YSB1, 2.3 to 4.01 log CFU/g from day 2 to day 5 of storage. The incorporation of sourdough starter with yeast during the preparation of the dough decreased the staling rate of sourdough bread, as determined [46]. The normal microbiological load for baked goods on routine quality-control tests in the baking industry is $\leq 10^3$ CFU/g for molds and yeast. From visual observation of all samples throughout storage, microbial growth was visible on the bread when it was at least 3 log CFU/g.

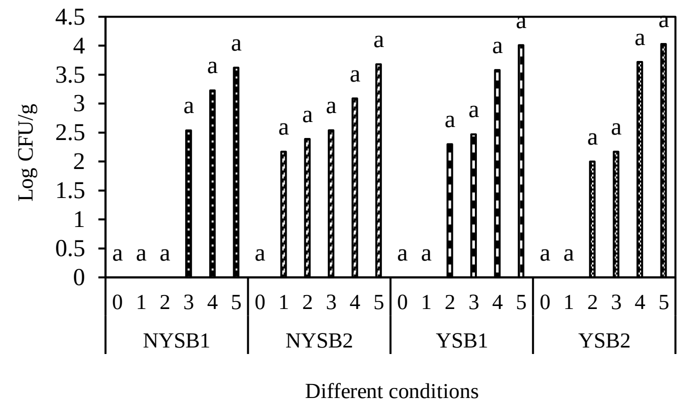

Fig. 4. Total yeast and mold count during storage of sourdough bread

4. CONCLUSIONS

In conclusion, adding sourdough starter with and without instant dry yeast significantly influenced the fermentation and quality of sourdough bread. The best condition used 200g of sourdough starter with addition of instant dry yeast (YSB2), which demonstrated higher nutritional composition and better organoleptic quality when combined with sourdough, leading to greater consumer acceptance. Based on the biological shelf-life study, a complete loss of quality after 4 days, with visible growth of yeast and mold, occurred at ≥ 3 log CFU/g, and the total plate count reached ≥ 5 log CFU/g. For future research, the process could explore more on fermentation conditions such as temperature and the isolation of specific lactic acid bacteria strains as starter cultures for sourdough bread.

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