Effect of different yeasts on lean bread quality

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Abstract

The most widely known natural bread yeasts are Shirakamikodama yeast (S) and Hoshino natural yeast leaven (H). However, few studies have investigated the characteristics of these yeasts and their effects on bread quality. We previously reported on the quality and taste characteristics of bread made using these two yeasts. In this study, we compared the quality and bread-making characteristics of lean bread prepared using S and H, with instant dry yeast serving as the control. We evaluated the weight, volume, water content, color, texture, aromatic compound content, and structure of the prepared bread. Furthermore, to assess the flavor characteristics and favorability of the resulting bread, sensory evaluations were performed. S bread had the smallest volume. Regarding texture, S bread was the hardest, least cohesive, and most adhesive. In the visual evaluation, S bread showed small alveolar cells extremely thick alveolar cell walls, and poor elongation of the gluten strands. Moreover, the alveolar cell walls appeared to be severely damaged, exposing numerous starch granules, both large and small, and spheres that are believed to be fat. The score was 3.2 in the 7-point scale palatability sensory evaluation, placing it in the “somewhat unfavorable” range. These results suggest that S is not a highly suitable yeast for lean bread preparation. H bread had the highest volume. Regarding texture, H bread was the softest and most cohesive. It is speculated that this result is because of the presence of amylases ostensibly produced by kōji mold in H, which breaks down the starch in the dough, thereby providing a continuous sugar and nutrient supply to the yeast. In H bread, the alveolar cell walls contained greater numbers of small pores and were thinner than in the other two bread types. Additionally, cracks were observed in the alveolar cell walls. It is believed that these cracks may be caused by proteases derived from the kōji mold in H acting on the cell membranes, gluten network, and cell walls. In the overall palatability sensory evaluation, H bread received the highest overall palatability score (4.3) among the three bread types, placing it in the “neither” range, suggesting that the bread can be preferred. These results suggest that H can be a suitable leaven for lean bread preparation.

1. Introduction

Increasing health consciousness has led to a growing interest in so-called "lean bread" that does not contain added fats, oils, or sugar [1]. Lean bread is made using only the following four main bread ingredients: flour, water, salt, and yeast. French bread and hard rolls are typical examples of lean bread [2]. As the flour and fermentation products largely determine the flavor of lean bread [3], fermentation starters are frequently used. In the bread market, fermentation starters are commonly referred to as "natural yeasts" [4]. In Japan, Shirakamikodama yeast and Hoshino natural leaven are the two most widely known commercial natural yeasts. Shirakamikodama yeast is a wild yeast that was isolated from leaf litter in the Shirakami Mountains by the Akita Research Institute for Food and Brewing in 1997 [5]. This yeast does not need to be activated and can be used for bread making without processing in granular form. Hoshino natural leaven is a starter made by collecting yeast attached to grains; culturing this yeast with wheat, kōji, and water; and then subsequently drying it to make a powder [6]. This yeast must be activated before it can be used for bread preparation [7]. These widely used commercially available natural yeasts are easy to use and lack the instability of home-made starters. However, few studies have investigated the characteristics of these yeasts and their effects on bread quality [8-14].

We previously reported on the dynamic viscoelasticity of dough, fermentation performance, and the quality and taste characteristics of bread made using these two yeasts compared with bread made using instant dry yeast [13]. In dough fermentation tests, the addition of sugar significantly affected the dough made using Shirakamikodama yeast, suggesting that fermentation time can be shortened for sugar-added bread. Sugar-free dough made using Hoshino natural leaven exhibited good expansion, and the finished bread was rated as soft, less cohesive, and sweet in sensory evaluations. From these results, we inferred that amylase and protease present in Hoshino natural leaven affect bread quality [13]. We isolated yeast from both products to exclude the influence of components other than yeast. We reported on the identification, viable counts, and carbon dioxide production of the isolated yeasts as well as the physical properties and taste characteristics of bread prepared using the equivalent amounts of viable yeast [14]. All three yeasts were identified as strains of Saccharomyces cerevisiae. Hoshino natural leaven had a substantially lower viable yeast count per gram (approximately 1/10,000) than instant dry yeast and Shirakamikodama yeast. Although Hoshino natural leaven typically takes longer to ferment [6, 8, 13], carbon dioxide generation and bread-baking tests indicated that bread of similar quality to that made using the other two yeasts can be produced with similar fermentation times when an equivalent amount of viable yeast is used [14].

We here aimed to investigate the suitability of Shirakamikodama yeast and Hoshino natural leaven for lean bread production.

2. Materials and Methods

(1) Materials

Yeasts used in this study were Shirakamikodama yeast (Shirakamikodama yeast [dry]; Akita Jujo Chemicals Co., Akita, Japan; hereinafter designated as "S") and Hoshino natural leaven (Hoshino-koubo Co., Ltd., Tokyo, Japan; hereinafter designated as "H"), with instant dry yeast (Super Kameriya Dry Yeast; Nisshin Seifun Welna Inc., Tokyo, Japan; hereinafter designated as "D") serving as the control.

In the case of H, activated leaven was prepared from the granular product ("HN") [7], and the resulting liquid leaven ("HL") was used in the experiments.

The ingredients for the bread included semi-strong flour (LYS D'OR; Nisshin Seifun Welna Inc.), table salt (The Salt Industry Center of Japan, Tokyo, Japan), and distilled water. Bread samples made using each yeast were designated as D, S, and H bread.

(2) Bread recipes and preparation methods

The quantities of bread ingredients used are shown in **Table 1.** The formulation was based on the recipe for French bread provided in the instruction manual included with the Home Bakery bread maker [15] and Takeya [16].

D and S were added in granular form at a rate of 2% the amount of flour (5 g). S was prepared according to Shimosaka [10, 11] as follows: 15 g of distilled water (three

Table 1 Bread formula (g)

Ingredients	Sample		
	D	S	H
Semi-strong flour [1]	250	250	250
Yeast	5 (Granules)	5 (Granules)	23.4 [2] (Liquid)
Salt	5	5	5
Water	162.5	162.5	144.1 [3]

D : Bread prepared with instant dry yeast
S : Bread prepared with Shirakamikodama yeast
H : Bread prepared with Hoshino natural leaven
1) 10.7% protein content,"LYS D'OR", Nisshin Seifun Welna Inc..
2) The weight of 23.4 g of HL (Liquid) is equivalent to 5 g of HN (granules).
3) The moisture content of HL (Liquid) is 78.6%. Therefore, The weight of water (162.5 g) was subtracted by the weight of HL water (18.4 g).

times the amount of yeast) was apportioned from the total amount of distilled water to be used and heated to 35°C; S was dissolved in this water, allowed to sit for 5 min, and added to the other ingredients. For H, HN (granular) was activated by incubating at 28°C for 24 h (VS-404WH; Versos, Hiroshima, Japan). The moisture content of the resulting HL (liquid) was measured using an infrared moisture analyzer (FD-600; Kett Electric Laboratory Co., Ltd., Tokyo, Japan). The moisture content at 110°C for 80 min as measured by the author (n = 3) was 78.6% [13]. Therefore, 23.4 g of activated leaven, which is equivalent to 5 g of HN (granular), was used in the bread-making test. The 18.4 g of moisture contained in the HL was subtracted from the amount of water added during preparation.

Bread loaves were baked using a Home Bakery bread maker (SD-BH1001; Panasonic Corporation, Tokyo, Japan) using the sandwich bread program (4 h for all processes) for D and S and the natural yeast bread program (7 h for all processes) for H. For the sandwich bread program, the time for each operation was as follows: first kneading, 20 min,; resting, 36 min; second kneading, 13 min; rising, 133 min; and baking, 38 min. For the natural yeast bread program, the times for the first kneading, first rising, second kneading, second rising, and baking were 20, 182, 10, 170, and 38 min, respectively [13].

Yeasts were added to the dough as follows: D was added immediately following the first kneading according to the timing for dry yeast dosing specified in the instruction manual [15]. S and HL were added before the first kneading.

(3) Measurement methods

1) Volume, weight, specific volume, and baking loss

After baking, the bread was cooled for 1 h at 25°C. After confirming that the bread had reached 25°C, the bread was wrapped using a food-grade plastic wrap and stored in a zippered polyethylene bag. Before being subjected to measurement, the pieces of bread were kept for 12 h at 25°C. A laser volumeter (Selnac-Win VM2100; Astex, Osaka, Japan) was used for measuring the volume and weight of the bread. Specific volume was calculated by dividing the volume of the bread by its weight [17]. Baking loss was calculated using the following equation: baking loss = (weight of dough before baking − weight of bread after baking)/weight of dough before baking × 100 [17]. The mean and standard deviation were calculated for five replicates of each bread type.

2) Moisture content

Moisture content was measured using 5 g subsamples of crumbs taken from the bread stored in the manner described in 1). The infrared moisture analyzer was set to 110°C for 80 min, and the moisture content was determined after confirming that a constant weight had been reached. The mean and standard deviation were calculated for five replicate subsamples prepared for each bread type.

3) Color

The color of the top crust and the crumb of bread stored in the manner described in 1) was analyzed using a spectrophotometer (CM-700d; Konica Minolta, Tokyo, Japan). Lightness (L^*) and chromaticity (a^* and b^*) were measured, and the color difference (ΔE^*) was calculated using the following equation: $\Delta E^* = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$ [18]. The color of the top crust was measured at five points: the center and each of the four corners. For the crumb analysis, the pieces of bread were cut lengthwise, and one point near the center of each of the two cut surfaces was measured. The mean and standard deviation were calculated for five replicates of each bread type.

4) Texture

The texture of 2.0 × 2.0 × 2.5 cm (L × W × H) subsamples taken from near the crumb center of the bread stored in the manner described in 1) was analyzed using a creep meter (RE2-33005C; Yamaden Co., Ltd., Tokyo, Japan). To obtain the mean and standard deviation, three subsamples from each of the five replicates for each bread type (total, 15) were measured. The following were the measurement conditions: load cell, 20 N; cylindrical plunger diameter, 8 mm; measurement strain rate, 90%; and measurement speed, 10 mm/s [13, 19, 20].

5) Aromatic components

The flavor of the bread stored in the manner described in 1) was analyzed by the Taste & Aroma Strategic Research Institute using gas chromatography/mass spectrometry (GC/MS) (ISQ QD TRACE1310; Thermo Fisher Scientific Japan Inc., Tokyo, Japan). The flavor components of each bread type were collected using a method involving headspace solid-phase microextraction (SPME) [21,22] and compared. Crumb subsamples (1 g) were placed in vials into which SPME fibers (DVB/Car/PDMS, 10 mm; Agilent Technologies Japan Co., Ltd., Tokyo, Japan) were inserted. After shaking and heating the flavor components at 100°C for 30 min, the fibers were used for GC/MS with a TG-WAX MS column (60 m × 0.25 mm i.d. × 0.25 μm film thickness; Thermo Fisher Scientific Japan Inc.). The following were the measurement conditions: carrier gas (He) in constant pressure mode (250 kPa), split ratio of 1:5, with temperature increasing from 40 to 250°C at 5°C/min.

6) Visual evaluation of bread structure

For visual evaluation of the bread structure, thin sections of crumb (1.0 × 1.0 × 0.2 cm; L × W × H) were obtained from near the crumb center of the bread stored in the manner described in 1,) using single-use microtome blades (High-Profile Disposable Blades 818; Leica Microsystems GmbH, Wetzlar, Germany). After confirming orientation, crumb samples were affixed to the sample stage as is for observing alveolar cell size, small pores [23, 24], and gluten strands [25]. Using a tabletop scanning electron microscope (Miniscope TM3030Plus; Hitachi High-Tech Corporation, Tokyo, Japan), visual evaluations were performed at a 10-kV acceleration voltage and 40× magnification. The long diameters of cells were measured using image analysis and measurement software (WinROOF 2015; Mitani Corporation, Tokyo, Japan). Longshort axis ratios were calculated by dividing the vertical length of cells by their horizontal length.

Double-fixed samples were used for the visual evaluation of the alveolar cell walls [23, 24]. Similar samples were first fixed by immersing the samples in 2.5% glutaraldehyde (TAAB; Nissin EM Co., Ltd., Tokyo, Japan) and 0.1-M phosphate buffer solution (pH, 7.4; Mutoh Pure Chemicals Co., Ltd., Tokyo, Japan) at 5°C for 2 h. After washing, samples were fixed a second time by immersing them in a 1% osmium tetraoxide solution (TAAB, Nissin EM Co., Ltd.) at 5°C for 2 h and dehydrating in an ethanol series [19, 25]. Specimens were dried and Au-Pd coated (MSP-1S magnetron sputtering system; Vacuum Device Co., Ltd., Ibaraki, Japan). Samples were observed using the aforementioned tabletop scanning electron microscope at a 10-kV acceleration voltage at 40× and 1,000× magnifications.

7) Sensory evaluation

Sensory evaluation was performed using 4.5 × 3.5 × 1.5 cm (L × W × H) subsamples of the bread stored in the manner described in 1), with the crust removed. Analytic sensory and preference sensory evaluations were conducted using a 7-point scale. Analytic sensory evaluation involved the following eight items: smoothness, crumb color, flavor, softness, elasticity, moistness, sweetness, and saltiness, with scores ranging from 1 (“very weak,” “coarse,” or “thin”) to 7 (“very strong,” “fine,” or “thick”), with 4 being “neither.” Palatability evaluation comprised the following two items: flavor preference and overall evaluation, with scores ranging from 1 (“very unfavorable”) to 7 (“very favorable”), with 4 being “neither.”

The evaluation panel comprised 20 female students and faculty members of the university, with ages ranging from 20 to 60 years. Sensory evaluation was performed only by individuals who provided consent after receiving an explanation about the purpose of the study. Individuals with allergies to wheat flour were excluded. The Tokyo Kasei University Graduate School Ethics Committee approved the study protocol (approval number: R2-7).

(4) Statistical analyses

Measurement data were tabulated using statistical processing software (IBM SPSS Statistics Ver. 24; International Business Machines Corporation, Armonk, NY, USA). One-way analysis of variance with the bread type as the explanatory variable was used to test for significant differences. For sensory evaluation, two-way analysis of variance was performed with the bread type and panelist as explanatory variables. Significant differences were further analyzed using Tukey’s multiple comparison test. The significance level was set at 5% ($p < 0.05$).

3. Experiment results

(1) External/longitudinal sectional appearance, volume, weight, specific volume, moisture content, and baking loss

Longitudinal sectional views, volume, weight, specific volume, and moisture content of the pieces of bread are presented in **Table 2**. The crusts of D and H bread appeared slightly more reddish than that of S bread. Browning of the crust is because of caramelization and the Maillard reaction, both of which involve sugar [26]. As no sugar was added to any of the bread, the overall color was whitish.

Visual evaluation of bread longitudinal sections revealed the presence of large cells in D and H bread, with a non-uniform distribution of different-sized cells. Alveolar cell sizes in D bread varied widely. The alveolar cells in S bread were small with little variation in size, and appeared to be fine-grained.

H bread had the highest volume and specific volume, with D bread being intermediate, and S bread being significantly smaller ($p < 0.05$). The specific volume

Table 2 Longitudinal sectional views, volume, weight, specific volume, moisture content of bread prepared with different yeasts

Sample	D	S	H
Longitudinal section			
Volume (cm^3)	1665.7 ± 16.3 [b]	979.1 ± 13.7 [c]	1850.3 ± 64.9 [a]
Weight (g)	378.5 ± 3.8 [b]	388.8 ± 2.1 [a]	375.3 ± 4.0 [b]
Specific volume (cm^3/g)	4.40 ± 0.08 [b]	2.52 ± 0.05 [c]	4.93 ± 0.21 [a]
Moisture content (%)	49.3 ± 0.1 [a]	48.3 ± 0.2 [c]	48.9 ± 0.2 [b]

D : Bread prepared with instant dry yeast
S : Bread prepared with Shirakamikodama yeast
H : Bread prepared with Hoshino natural leaven
1) $n = 5$
2) Values: Average ± SD
3) a-c : Significant differences ($p < 0.05$) are indicated by different letters.

Table 3 Color of bread prepared with different yeasts

Part	Sample	Hunter value			ΔE^*	
		L^*	a^*	b^*	Difference with D	Difference with S
Top of crust	D	75.62 ± 3.18	1.25 ± 0.56 [a]	20.76 ± 0.75	-	-
	S	77.78 ± 0.90	-1.00 ± 0.16 [b]	18.47 ± 0.87	3.87	-
	H	73.99 ± 2.86	1.66 ± 1.12 [a]	20.65 ± 1.81	1.69	5.12
Crumb	D	69.31 ± 4.21 [b]	-1.58 ± 0.07	9.28 ± 2.78 [b]	-	-
	S	76.03 ± 1.01 [a]	-1.51 ± 0.05	14.15 ± 0.48 [a]	8.30	-
	H	70.88 ± 3.34 [b]	-1.22 ± 1.11	8.47 ± 5.09 [b]	1.81	7.66

D : Bread prepared with instant dry yeast
S : Bread prepared with Shirakamikodama yeast
H : Bread prepared with Hoshino natural leaven
1) $n = 5$
2) Values: Average ± SD
3) a, b : Significant differences ($p < 0.05$) are indicated by different letters.

of S bread did not meet the standard for English bread of 4–4.5 [2]. We previously reported that in dough fermentation tests, the volumes of S dough with and without added sugar substantially differed [13]. After 120 min, the volume of S dough with no added sugar was only approximately 60% that of S dough with added sugar and two-thirds that of D dough with no added sugar prepared under similar conditions [13]. These results indicate that the expansion of the S dough in the dough without added sugar is low [26]. We believe that this is the reason for the low volume and specific volume of S bread in the present study. In the same dough fermentation study [13], the H dough with no added sugar continued to rise up to 210 min; we speculated that this finding was because H contains kōji mold (*Aspergillus oryzae*), which produces amylases [6, 27, 28, 29] that break down starch in the dough and provide a continuous sugar supply for the yeast [6, 30, 31]. Moreover, kōji mold produces proteases [32, 33] that are believed to act on gluten, which forms the skeletal structure of bread [23, 33]. This incidence is believed to increase the extensibility of the gluten and the rise of H bread [23].

S bread was significantly heavier than D and H bread ($p < 0.05$). D bread had the highest moisture content, whereas S bread had the lowest ($p < 0.05$). The moisture content of H bread was intermediate to those of the other two bread types ($p < 0.05$).

Baking loss is a simple method for monitoring internal doneness [3]. The baking losses for D, S, and H bread were 10.41% ± 0.89%, 7.99% ± 0.51%, and 11.16% ± 0.94%, respectively. S bread had a significantly lower baking loss than the other two bread types ($p < 0.05$).

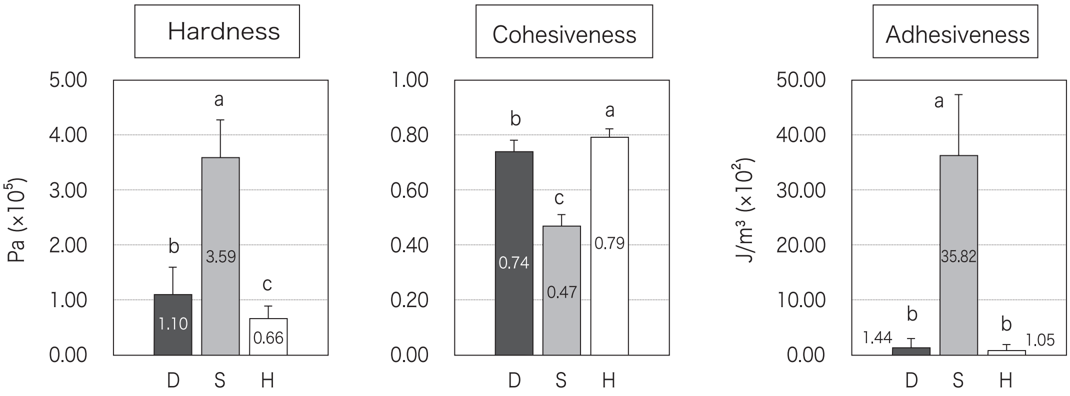
(2) Color

Bread color analyses results are shown in **Table 3**. Positive a^* values indicate the degree of redness, whereas negative values indicate the degree of greenness. Positive b^* values indicate the degree of yellowness, whereas negative values indicate the degree of blueness [34].

For the top crust, the three bread types showed no significant differences in L^* values (brightness). D and H bread had a stronger reddish color and significantly higher a^* values than S bread. The three bread types showed no differences in b^* values.

Although the top crust of H bread had a slightly lower L^* value and higher a^* value than that of the other two bread types, the differences were not statistically significant. This finding is likely because of the breakdown of starch into dextrin by amylases produced by kōji mold in H, which contributed to browning reactions [35].

Regarding crumb color, no differences were observed in the L^* values of D and H bread, which were significantly lower than that of S bread. The three bread types showed no differences in a^* values, whereas S bread had higher b^* values than D and H bread. S bread had higher L^* and b^* values and was lighter and yellower than D and H bread. Owing to the shadow effect of alveolar cell walls, greater alveolar cell sizes of the crumb result in darker colors [34]. Both the visual evaluation and measurement of alveolar cell major axis indicated that S bread had



D : Bread prepared with instant dry yeast
S : Bread prepared with Shirakamikodama yeast
H : Bread prepared with Hoshino natural leaven
1) $n = 15$
2) Values: Average ± SD
3) a-c : Significant differences ($p < 0.05$) are indicated by different letters

Fig.1 Texture of bread crumbs prepared with different yeasts

smaller alveolar cells. From this finding, it was inferred that the lighter color of S bread was because of the lesser influence of alveolar cell wall shadows.

Regarding top crust color, the color difference (⊿E*) between D and S bread (3.87) was "appreciable" (3.0–6.0) [18]; that between D and H bread (1.69) was "noticeable" (1.5–3.0); and that between S and H bread (5.12) was "appreciable" (3.0–6.0) [18].

Regarding crumb color, the color differences (⊿E*) between D and S bread (8.30) and between S and H bread (7.66) were "substantial" (6.0–12.0) [18], whereas that between D and H bread (1.81) was "noticeable" (1.5–3.0).

Differences in the top crust and crumb colors of the pieces of bread made using different yeasts were substantial enough to be discriminatory.

(3) Texture

The hardness, cohesiveness, and adhesiveness of the different bread types are depicted in **Fig. 1**. S bread had the highest hardness value, followed by D and H bread ($p < 0.05$). Considering that bread softness increases with increasing specific volume [36], it is believed that compared with the other two bread types, the hardness of S bread was influenced by its low specific volume. The fact that H bread had the lowest hardness corresponds to the bread having the highest specific volume. Moreover, previous studies reported that the amylases produced by the yeasts in H have a softening effect on the bread [30, 37]. The results of the present study are also consistent with these effects.

H bread had the highest cohesiveness, whereas S bread had the lowest ($p < 0.05$). S bread had substantially higher adhesiveness than D and H bread ($p < 0.05$).

(4) Flavor components

Overall, 66 volatile flavor components were detected in the three bread types. The main flavor components are listed in **Table 4**. The most frequently detected compound in all three bread types was ethanol. Considering its high volatility, it is unlikely that ethanol substantially contributes to bread aroma; it is believed that other less-volatile alcohols present in lower quantities contribute to bread aroma [38, 39, 40]. The second and third most common compounds detected in all three bread types—3-methylbutan-1-ol and 2-mehtylpropan-1-ol—are produced by the amino-carbonyl reaction [40, 41], and both are highly volatile alcohols [39].

The flavor components detected only in S bread—e.g., hexan-1-ol, 2-methylbutyl 2-methylbutanoate, 3,7-dimethylocta-1,6-dien-3-ol, and oct-1-en-3-ol—are associated with flowers and fruits. This finding is consistent with descriptions in the literature of bread made using S having a "vibrant and sweet aroma" [5]. 3-Hydroxybutan-2-one, which was detected only in

Table 4 Main flavor components of bread

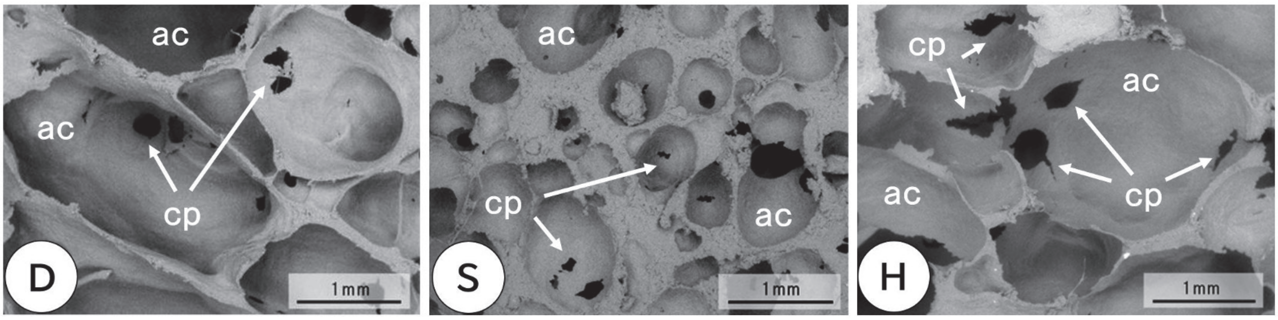
Flavor components	Sample			Odor description
	D	S	H	
ethanol	$1.4×10^8$	$0.9×10^8$	$1.1×10^8$	alcohol, flowers, ripe apples, sweet
3-methylbutan-1-ol	$2.7×10^7$	$2.9×10^7$	$1.0×10^7$	banana, cocoa, floral, fusel, nail polish
2-methylpropan-1-ol	$6.5×10^6$	$4.1×10^6$	$1.7×10^6$	apple, cocoa, fusel, malt
2-methylpropanoic acid	$6.6×10^6$	0	0	burnt, butter, cheese, sweat
ethyl octanoate	$3.7×10^6$	$0.5×10^6$	$8.1×10^6$	brandy, burnt, grapes, nuts, pears
3-hydroxybutan-2-one	0	0	$1.5×10^7$	butter, cream, peppers, sweat
benzaldehyde	0	0	$2.5×10^6$	almond kernels, burnt sugar, cherries, malt, roasted pepper
pentan-1-ol	$0.4×10^6$	$5.2×10^6$	$0.6×10^6$	balsamic, fruit, green, medicine, yeast
3-methylbutanoic acid	$1.9×10^6$	$5.1×10^6$	0	cheese, feces, putrid fruit, sweat
2-pentylfuran	$1.9×10^6$	$1.6×10^6$	$1.4×10^6$	butter, flowers, fruits, mung beans
(E)-oct-2-enal	$1.5×10^6$	0	$0.6×10^6$	fat, fish oil, green, nutty, sweet
nonanal	$1.3×10^6$	$1.0×10^6$	$0.6×10^6$	citrus, fat, green, paint, stimulating
(Z)-non-3-en-1-ol	0	$8.3×10^5$	$2.1×10^5$	flowers, green, stimulating
acetic acid	0	0	$6.7×10^5$	acetic acid, vinegar, stimulating
hexan-1-ol	0	$7.1×10^5$	0	flower, fruit, green, herb, tree
alpha-muurolene	0	0	$5.8×10^5$	wood
hexanal	0	0	$4.5×10^5$	fresh, fruit, green, grass
ethyl nonanoate	$2.7×10^5$	$0.6×10^5$	$2.5×10^5$	fruit
2-methylbutyl 2-methylbutanoate	0	$2.5×10^5$	0	apples, berries, rum
3-ethoxypropan-1-ol	0	0	$1.9×10^5$	fruit
non-2-en-1-ol	0	0	$1.9×10^5$	green
decanal	$1.8×10^5$	$1.0×10^5$	$1.9×10^5$	flower, fried, orange peel, tallow
octan-1-ol	0	$1.5×10^5$	$0.5×10^5$	fat, jasmine, lemon, metal, walnut
3,7-dimethylocta-1,6-dien-3-ol	0	$1.5×10^5$	0	bergamot, coriander, grape, lavender, rose
oct-1-en-3-ol	0	$1.4×10^5$	0	soil, fat, flowers, mold, mushrooms
propan-1-ol	$1.3×10^5$	0	$0.3×10^5$	candy, plastic, spicy, ripe fruit
acetaldehyde	0	0	$9.5×10^4$	ethereal, floral, fruit, green apple, sweet

1) The analysis of flavor components was commissioned to Taste & Aroma Strategic Research Institute.
2) Numbers represent peak area values.

H bread, is a ketone and carbonyl compound with an aroma reminiscent of a sweet creamy butter [42]. This compound was the second most commonly detected compound in H bread following ethanol. Benzaldehyde, alpha-muurolene, hexanal, and non-2-en-1-ol, which are aromatic components associated with fruits and wood, are other compounds detected in H bread. Additionally, acetaldehyde [42], which is known as the sweet aroma of cooked rice and freshly brewed coffee, and acetic acid [43], which is the source of the flavor of sourdough bread, were detected only in H bread. This finding is likely one of the reasons why bread made using H has a rich and flavorful aroma [7, 8].

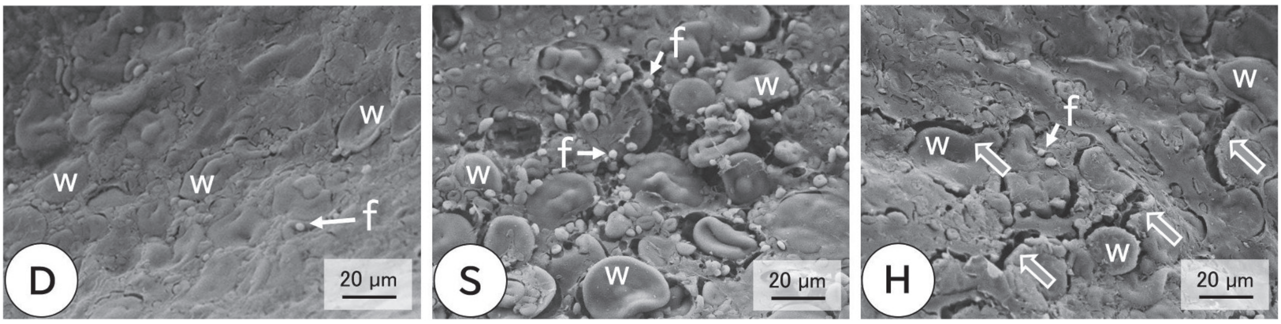
(5) Visual evaluation of bread structure

The structure of different bread types (40×) is shown in **Fig. 2**. In D and H bread, the dough stretched during baking, thereby resulting in large alveolar cell formation. The gluten strands [25] surrounding the alveolar cells were elongated and formed continuously; the alveolar cell walls were thin with a smooth surface. Examination of alveolar cell wall longitudinal sections in these two bread types indicated sharply cut surfaces. In D bread, the alveolar cell walls stretched to encapsulate the gas evolved by fermentation [44], which contributed to the bread's large volume. In H bread, the alveolar cell walls contained greater numbers of small pores and were thinner than in the other two bread types. Proteases produced by



D : Bread prepared with instant dry yeast ac : Air cell
S : Bread prepared with Shirakamikodama yeast cp : Cell wall perforations
H : Bread prepared with Hoshino natural leaven
1) Samples were cut into 1.0 × 1.0 × 0.2 cm (L × W × H) pieces from the center of the bread crumb and placed on the table to examine the torn surface.

Fig.2 Scanning electron micrograph of bread prepared with different yeast



D : Bread prepared with instant dry yeast w: Large starch granule
S : Bread prepared with Shirakamikodama yeast f: A sphere that is believed to be fat
H : Bread prepared with Hoshino natural leaven ⇒: Cracks that appeared in sample H
1) Samples were cut into 1.0 × 1.0 × 0.2 cm (L × W × H) pieces from the center of the bread crumb and were fixed using glutaraldehyde and osmium tetraoxide solution. For SEM (Miniscope TM3030Plus), the samples were mounted onto SEM stubs and were sputter coated with Au-Pd.

Fig.3 Scanning electron micrograph of gluten membranes

kōji mold in H leaven may have caused these cell wall perforations, thereby enabling greater gluten network stretching [23, 44, 45]. This incidence is likely the reason that H bread had the largest volume. Moreover, these small pores and the thinness of the alveolar cell walls may have contributed to the bread's lower hardness values [46]. In S bread, the alveolar cells were small, the alveolar cell walls were extremely thick, and the gluten strands exhibited poor elongation. Examination of alveolar cell wall longitudinal sections revealed coarsely cut surfaces, suggesting that collapse was underway. The alveolar cell walls were not as smooth as those in D and H bread. The fact that in previous dough fermentation tests [13], S bread with no added sugar expanded the least among the three bread types suggests that only a small amount of gas was retained in the alveolar cells. Regarding S bread prepared in this study, we believe that this prevented gluten network elongation during baking, thereby resulting in thicker cell walls.

The following were the mean alveolar cell major axes for the different bread types: 1.27 ± 0.69, 0.57 ± 0.50, and 1.36 ± 0.51 mm for D, S, and H bread, respectively. The major axes of alveolar cells in D and H bread were not different from each other but were significantly greater than those in S bread ($p < 0.05$). The mean long-to-short axis ratios of alveolar cells in D and H bread were 1.37 ± 0.58 and 1.33 ± 0.69, respectively, indicating an elliptical shape; both were greater than that for S bread ($p < 0.05$). No difference was observed between D and H bread. The mean long-to-short axis ratio of alveolar cells in S bread was 1.18 ± 0.24, indicating a round shape, which may have contributed to S bread's low volume.

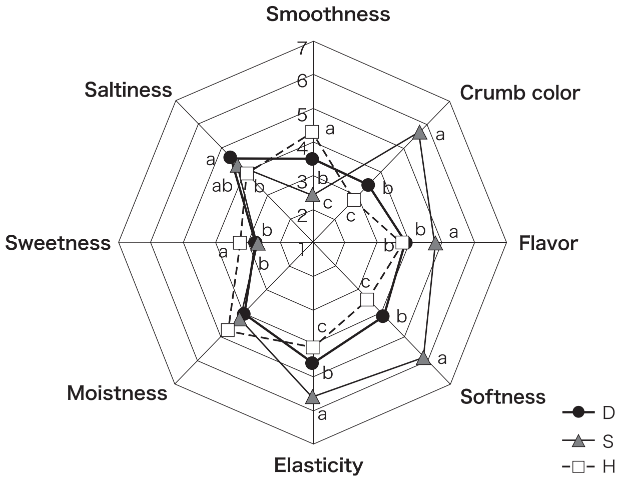
The alveolar cell wall structure (1,000×) of the different bread types is shown in **Fig. 3**. Visual evaluation of the alveolar cell walls in D bread revealed an overall smooth elongation of gluten membranes [25,47], with large starch granules (w) being incorporated into the membranes. Gluten membranes provide the main skeletal support in expanded bread. When bread dough is heated to 74°C or higher, gluten membranes denature, starch granules swell, and the two interact as they transform into a firm semi-solid structure [26, 48]. Cell walls with good extensibility increase the dough's ability to retain the gas evolved in the initial stages of baking, which increases oven spring and bread volume [3]. The state of the alveolar cell walls in D bread showed good gluten membrane extensibility, which is consistent with the bread's large volume.

In S bread, substantial damage to alveolar cell walls and the presence of large and small starch granules and particles that are believed to be fat (f) exposed on the surfaces of the gluten membranes were observed. The fact that in previous dough fermentation tests [13], S bread with no added sugar expanded the least among the three bread types suggests that the amount of gas retained in alveolar cells is low. Accordingly, we believe that the gluten membranes, which did not stretch during baking, remained thick and inflexible, thereby causing the starch granules and fat particles to fall into the alveolar cells and become exposed during sample preparation for optical microscopic observation [19].

In H bread, although we observed gluten membrane elongation cracks in the alveolar cell walls were also observed. In some instances, starch granules appeared to emerge from such cracks. We believe that the cracks resulted from proteases derived from kōji mold in H leaven acting on the alveolar cell wall. The cracks and perforations of the alveolar cell walls reduced internal pressure, thereby preventing shrinkage following baking [49] and resulting in H bread having the largest volume and the softest texture.

(6) Sensory evaluation

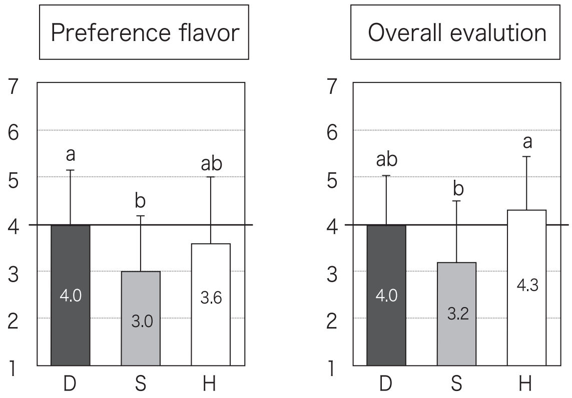
Results of the 7-point scale analytic sensory evaluation are shown in **Fig. 4**. Regarding crumb color, S bread was scored as being the darkest, and H bread as the lightest ($p < 0.05$). The crumb color of D bread was intermediate to that of the other two bread types. Color analysis revealed that the crumb of S bread was



D : Bread prepared with instant dry yeast
S : Bread prepared with Shirakamikodama yeast
H : Bread prepared with Hoshino natural leaven
1) $n = 20$
2) Analysis evaluation
1: Very weak, coarse, or thin
4: Neither
7: Very strong, fine, or thick
3) a-c : Significant differences ($p < 0.05$) are indicated by different letters.
4) Samples were cut into 4.5 × 3.5 × 1.5 cm (L × W × H) pieces from the center of the bread crumb.

Fig.4 Analytic sensory evaluation of bread prepared with different yeasts using 7-point scale

strongly yellow, which is consistent with the results of sensory evaluation. S bread was evaluated as being more flavorful than D or H bread ($p < 0.05$), whereas no difference was observed between D and H bread. S bread was judged as being the hardest, and H bread as the softest, with D bread being intermediate to the other two bread types ($p < 0.05$). These results were consistent with the hardness values obtained in the texture evaluation. Visual evaluation revealed that the alveolar cell walls of H bread were thin and contained small perforations, which may have contributed to the bread's low hardness scores. Furthermore, the proteases derived from kōji mold in H leaven may have been responsible for cleaving the peptide chains of gluten, thereby resulting in a better mouth feel [27, 30, 32, 33, 50]. Amylases produced by kōji mold are also believed to soften the bread [28, 29, 35], which appears to be consistent with the results of the present study. S bread was evaluated as being the most elastic, and H bread as the least elastic, with D bread being intermediate to the other two bread types ($p < 0.05$). Texture measurements showed that S



D : Bread prepared with instant dry yeast
S : Bread prepared with Shirakamikodama yeast
H : Bread prepared with Hoshino natural leaven
1) $n = 20$
2) Values: Average ± SD
3) Palatability evaluation
1: Very unfavorable
4: Neither (line)
7: Very favorable
4) a, b : Significant differences ($p < 0.05$) are indicated by different letters.
5) Samples were cut into 4.5 × 3.5 × 1.5 cm (L × W × H) pieces from the center of the bread crumb.

Fig.5 Palatability sensory evaluation of bread prepared with different yeasts using 7-point scale

bread had the highest hardness and adhesiveness values. Visual evaluation revealed extremely thick alveolar cell walls of S bread, which may have contributed to its high elasticity [3]. H bread was perceived as being the sweetest ($p < 0.05$), with no significant difference being observed between S and D bread. The damaged starch in the dough was broken down by amylases derived from kōji mold into more soluble dextrin and maltose [32, 33, 35], thereby resulting in the bread being evaluated as sweet. D bread was evaluated as being the saltiest, and H bread as the least salty ($p < 0.05$). The saltiness of S bread was intermediate but did not significantly differ from that of the other two bread types. S bread was evaluated as having the coarsest texture, with D and H bread having progressively finer textures. The three bread types showed no differences in moistness.

Palatability sensory evaluation results are shown in **Fig. 5**. In the palatability sensory evaluation, overall palatability was scored using a 7-point scale. Considering that unique flavor components were detected using GC/MS in S and H bread, we decided to also perform a flavor preference evaluation (7-point scale). The most preferred bread was D bread, whereas S bread was the least ($p < 0.05$). Preference for H bread was intermediate; however, the preference for H bread was not statistically different from those of the other two bread types. GC/MS analysis revealed the presence of pentan-1-ol, oct-1-en-3-ol, and other compounds with medicinal or earthy tones in S bread, which may have affected the low ratings for S bread. S bread underwent lower baking loss than the other two bread types ($p < 0.05$). Consequently, there may have been less volatilization of flavor components during baking, resulting in some lingering aromas from the raw ingredients (wheat flour and starch) [26]. This result is consistent with the strong “flavor” score received in the analytic sensory evaluation. H bread received the highest overall palatability score of 4.3 ± 1.3, whereas S bread received the lowest score of 3.2 ± 1.5, placing it in the “somewhat unfavorable” range. The overall palatability score for H bread was intermediate (4.0 ± 1.2) but was not significantly different from those of H and S bread. Overall palatability scores for both H and D bread were above 4.0, placing them in the “neither” range, suggesting that these pieces of bread can be preferred.

4. Conclusions

We compared the quality and flavor characteristics of lean bread prepared using S and H, with D serving as the control.

Bread prepared using S was markedly smaller in volume than the other two bread types. The specific volume did not meet the standard for English bread of 4–4.5 [2]. These results are consistent with previously reported dough fermentation test results [13]. The top crust and crumb colors of the three bread types were sufficiently different to enable discrimination based on color. Regarding texture, S bread was the hardest, least cohesive, and most adhesive. Its flavor profile included components associated with flowers and fruits. Regarding crumb structure, the alveolar cells were small and had extremely thick cell walls, and the gluten strands showed poor elongation. Moreover, the alveolar cell walls appeared to be severely damaged, exposing numerous starch granules, both large and small, and spheres that are believed to be fat. In the analytic sensory evaluation, the bread was judged as having dark color, strong flavor, and high elasticity. In the palatability sensory evaluation, S bread received low flavor preference scores. The overall palatability score was 3.2 ± 1.5, placing it in the "somewhat unfavorable" range. These results suggest that S is not a highly suitable leaven for lean bread preparation.

Bread made using H had the highest volume and highest specific volume. It is speculated [13] that this finding is because of the presence of amylases [6, 27, 28, 29] ostensibly produced by kōji mold in H, which breaks down the starch in the dough, thereby providing a continuous sugar and nutrient supply to the yeast [6, 30, 31]. Additionally, kōji mold produces proteases [32, 33] that are believed to act on gluten, which forms the skeletal structure of the bread [23, 33]. We speculate that this incidence increased the extensibility of the gluten and the degree of expansion caused by H [23]. Regarding texture, H bread was the softest and most cohesive. The flavor profile included components reminiscent of butter and those adding fruity and woody notes. Regarding crumb structure, the alveolar cell walls contained numerous cracks, with starch granules appearing to emerge from some of these cracks. It is likely that these cracks are caused by proteases derived from kōji mold in H acting on the cell membranes, gluten network, and cell walls [23, 44, 45, 46]. In the analytic sensory evaluation, the bread was the softest, least elastic, and sweetest. In the palatability sensory evaluation, H bread received the highest overall palatability score (4.3 ± 1.3) among the three bread types, placing it in the "neither" range, suggesting that the bread can be preferred.

French bread, which is a representative lean bread, usually contains malt as one of its ingredients [16]. Malt contains α- and β-amylases, which are activated when barley germinates, as well various maltoses, which are produced by these amylases during germination [51]. French bread is made without adding any sugars. Adding malt causes the α-amylase in the malt to break down the starch in the wheat flour into maltose, thereby promoting fermentation by providing a nutrient source for the yeast. H contains amylases derived from kōji mold, enabling the preparation of high-quality no-sugar-added bread without adding malt. These results suggest that H can be a suitable leaven for lean bread preparation.

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酵母の違いがリーンなパンの品質に与える影響

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Key Words: 白神こだま酵母，ホシノ天然酵母パン種，パン，発酵，テクスチャー特性，官能評価，走査型電子顕微鏡

要旨

天然酵母パンには，広く知られている白神こだま酵母（以下，S），ホシノ天然酵母パン種（以下，H）等があるが，酵母の特性や製パンへの影響についての研究は少ない。これら酵母を用いた食パンの品質および食味特性については既に報告した。本研究では，リーンなパンの品質および食味特性を比較・検討し，無糖パンに与える酵母の影響を明らかにすることを目的にした。対照酵母はインスタントドライイースト（以下，D）とした。体積・重量・比容積・水分含有率・テクスチャー・色度・香気成分を測定し，組織構造を観察した。また，官能評価による食味特性を検討した。S 試料は，体積が小さく，かたく，凝集性の値が低かった。組織観察では，S 試料の気泡のサイズは小さく，気泡壁が極めて厚く，グルテンストランドの伸長は悪かった。また，気泡壁面の著しい損傷が観察され，大小のでんぷん粒と，脂肪と思われる球体が多く露出していた。7 段階評点法による嗜好型官能評価の総合評価において，S 試料の評点は 3.2 で，「やや好ましくない」という評価であった。リーンなパンにおいて，S は適性が低い可能性があることが示唆された。H 試料はパンの体積が最も大きく，やわらかく，凝集性の値が高かった。H は材料に麹を含むため，麹菌が生産したと考えられるアミラーゼがドウ中のでんぷんを分解することにより糖が供給され続け，酵母の栄養源となったと推察された。組織観察では，H 試料は気泡膜に小孔が多く，気泡壁の厚さが薄かった。また，気泡壁面に複数の亀裂が生じており，H の材料に含まれる麹由来のプロテアーゼが，気泡膜・グルテンネットワーク・気泡壁面に作用したためと推察された。嗜好型官能評価では，H 試料の総合評価は 4.3 で 3 試料内で最も高く，好まれる可能性がある。H はリーンなパンへの適性があることが示唆された。

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