PAPER

IMPROVING BREAD QUALITY USING CO-CULTURES OF *SACCHAROMYCES CEREVISIAE*, *TORULASPORA DELBRUECKII* JK08, AND *PICHIA ANOMALA* JK04

AGUNG WAHYONO[1], SAE-BYUK LEE[1], WOO-WON KANG[2] and HEUI-DONG PARK*[1]

[1]School of Food Science and Biotechnology, Kyungpook National University, Republic of Korea

[2]Department of Food & Food-service Industry, Kyungpook National University, Republic of Korea

*Corresponding author: hpark@knu.ac.kr

ABSTRACT

Co-cultured *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* JK08, and *Pichia anomala* JK04 were used as a leavening agent. The leavening ability, crumb structure, texture profile, crumb aroma, and sensory properties of bread were evaluated. The leavening ability of the co-cultures tested was lower than for *S. cerevisiae* alone. Leavening containing a co-cultures produced bread crust and crumb that were slightly yellow in colour and bright. Generally, co-cultured bread produced a larger diversity and higher abundance of volatile organic compounds. Superior colour properties, favorable aroma, and decent textural and structural features resulted in higher sensorial ratings for co-cultured bread. We suggest the use of co-culture as leavening agents for improved bread quality.

Keywords: bread quality, co-culture, *Pichia anomala*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*

1. INTRODUCTION

Bread is a food that not only has a long history, but also a long future. Over 12,000 years ago, the first bread was probably developed by deliberate experimentation using wheat flour and water. Product development and process innovation in bread making is still an active field. Consumer interest in bringing unique and alternative breads to the table is driving the production of a wide variety of breads (CAUVAIN, 2012; MONDAL and DATTA, 2008). A multitude of studies conducted over the 20th century focused on improving bread quality and have explored the immense terrain of recipes (GARDNER *et al*., 2002; ROSELL *et al*., 2009; MOVAHED *et al*., 2012; NILUFER-ERDIL *et al*., 2012), process innovations (KARAOĞLU *et al*., 2006; BOSMANS *et al*., 2013) and the use of novel microorganisms as leavening agents (CABALLERO *et al*., 1995; PLESSAS *et al*., 2005; CHOI and CHOI, 2009; MO and SUNG, 2014). Although the experience of bread quality is highly personal, it may be described as the sum of the sensory pleasures associated with flavour, taste, texture, and appearance (CAUVAIN, 2012).

The technological role of yeast in bread making has been well established. The main role of yeast is to produce gas by degrading the sugars available in the flour or that are added to the recipe. The gas produces air bubbles that are contained by the stretchy gluten proteins in the flour, which causes the dough to rise and produces an aerated structure in the resulting bakery product. During fermentation, the yeast produces abundant alcohols and other volatile compounds that impart unique tastes and flavours to the bread (STEAR, 1990; GÉLINAS, 2009; ALI *et al*., 2012; PACHECO *et al*., 2012).

Over the last two decades, there has been great interest in using new strains of baker's yeast that produce particular aromas, anti-molding properties, or desirable nutritional characteristics to fulfill the needs of the baking industry (GÉLINAS, 2009). Furthermore, the use of co-cultures as leavening agents has been reported to confer favorable effects in baking products. Examples include the use of *Lactobacillus plantarum* or *Pediococcus cerevisiae*, combined with *Saccharomyces cerevisiae*, as a co-culture for improving bread quality and delaying staling (ELHARIRY *et al*., 2011); employing lactic acid bacteria and *S. cerevisiae* as a starter to enhance the nutritional content and shelf life of cassava-wheat bread (OGUNBANWO *et al*., 2008); the use of *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus* mixed with the lactose-fermenting yeast *Kluyveromyces marxianus* to improve bread quality and increase shelf-life (PLESSAS *et al*., 2008); and the use of starter culture combinations of *Lactobacillus fermentum*, *S. cerevisiae*, and *Candida krusei* to enhance aroma in Ghanaian maize dough fermentation (ANNAN *et al*., 2003).

Torulaspora delbrueckii and *Pichia anomala* have unique traits that improve the quality of bakery products. As reported by HERNANDEZ-LOPEZ *et al*. (2003), *T. delbrueckii* strains IGC5321 and IGC5323 exhibited higher leavening ability and CO_2 production than *S. cerevisiae* after exposure to hyperosmotic and freeze-thaw stress. MO and SUNG (2014) reported that *P. anomala* SKM-T enhanced flavour properties and extended shelf life. The use of *T. delbrueckii, P. anomala*, and *S. cerevisiae* as co-cultured leavening agents has not yet been reported. Here, we investigated the effects of co-cultured *S. cerevisiae*, *T. delbrueckii* JK08, and *P. anomala* JK04 as a leavening agent on bread quality. Leavening ability, structural crumb features, texture profile, crumb aroma, and sensory properties of bread were evaluated.

2. MATERIALS AND METHODS

2.1. Microorganism strains and bread ingredients

T. delbrueckii JK08 (TD) and *P. anomala* JK04 (PA) were isolated from Korean traditional starter (*Nuruk*), and then identified and collected at the Institute of Fermentation Biotechnology, Kyungpook National University, Daegu, South Korea. The strains were sub-cultured in yeast extract-peptone-dextrose (YPD, Difco, Le Pont de Claix, France) agar (Oxoid, Hampshire, United Kingdom) and stored at 2-4°C. *S. cerevisiae* (SC) was isolated from compressed instant baker's yeast (saf-instant S.I., Lesaffre, Marcq, France). Commercial wheat flour (Beksul, CheilJedang, Seoul, South Korea) contained 13.93% protein and 0.47% ash, with a pH of 4.35. Sugar and salt were purchased from a supermarket in Sangju, South Korea.

2.2. Preparation of cultures

Preparation of cultures was described previously (WAHYONO *et al*., 2015). The strains were sub-cultured in 5% YPD broth and cultivated in a rotary shaker (JSSI-300C, JS Research, Gongju, South Korea) at 30°C for 48 h, with shaking at 180 rpm. The yeast cells were collected by centrifugation (Hanil Supra 22K, Hanil, Incheon, South Korea) at 4000 × g for 10 min at 4°C. The supernatant was discarded, and the pellet was re-suspended in distilled water, vortexed thoroughly, and stored at 4 °C. Cell density was measured using a hemocytometer (Neubauer chamber, Celeromics, Cambridge, United Kingdom). To measure cell density, 10^{-1} to 10^{-4} dilutions of culture were prepared; 10 µl of diluted culture was loaded into the hemocytometer chamber. The chamber was observed at 100× magnification using a binocular microscope (CX31RTSF, Olympus, Tokyo, Japan). Cells in four different regions of the chamber were counted. Cell density was calculated using the following formula:

$$c = \frac{N}{n \cdot d} \times 10\,000 \qquad (1)$$

where c is the concentration (expressed as number of cells per millilitre), N is the number of cells, n is number of squares counted, and d is the dilution factor. Number 10 000 represents a constant converter to millilitre.

2.3. Leavening ability

The leavening ability of co-cultures was measured as described previously (WAHYONO *et al*., 2015). Bread dough containing 20 g flour, 20 ml water, and 4 × 108 yeast cells per ml of water was mixed thoroughly in a 100-ml graduated cylinder and then incubated at 30°C for 210 min. This was done in triplicate and each sample was observed every 30 min. The maximum leavening rate (ml/h) was calculated from the highest volume reached in 210 min divided by the time the highest volume was first recorded.

2.4. Bread-making procedure

Baking was carried out in a bread maker (National SD-BT102, Panasonic, Osaka, Japan) using the 4-h standard bread-making setting. The basic recipe consisted of wheat flour, salt, sugar, and drinkable water. A single culture SC and co-cultures of *S. cerevisiae* + *T.*

delbrueckii JK08 (SCTD), *S. cerevisiae* + *P. anomala* JK04 (SCPA), and *S. cerevisiae* + *T. delbrueckii* JK08 + *P. anomala* JK04 (SCTDPA) were used as leavening agents. The working ratio for co-cultures of *S. cerevisiae* + *T. delbrueckii* JK08 (SCTD) and *S. cerevisiae* + *P. anomala* JK04 (SCPA) were 50:50, respectively. The co-culture ratio for *S. cerevisiae* + *T. delbrueckii* + *P. anomala* JK04 (SCTDPA) was 30:35:35. In the bread dough, the SC cell density was adjusted to approximately 5×10^8 cells per ml of water added to the dough. For TD and PA, the cell densities were approximately 10^9 cells per ml of water added. Dough compositions are given in Table 1. The bread maker protocol included a first mixing for 20 min, resting for 25 min, and a second mixing for 10 min, followed by a first fermentation at 35°C for 50 min. A third mixing was performed for 3 min, followed by a second fermentation at 35°C for 40 min, and then a fourth mixing for 2 min. Proofing was done at 40°C for 50 min and finally, loaves were baked at 210°C for 40 min. Baking was performed in triplicate. After baking, the bread loaves were tempered at ambient temperature (28-30°C) before analysis.

2.5. Moisture content, specific volume, and bread yield efficiency

The moisture content of the bread crumb was determined by the oven-drying method (CZUCHAJOWSKA *et al*., 1989). Specific volume was determined by the seed displacement method (AACC 10-05, 2000). Bread yield efficiency was calculated as described by MOVAHED *et al*. (2012) using the following formula:

$$P_2 = \left(\frac{W_3}{W_2}\right) \times 100 \qquad (2)$$

where P2 is bread yield efficiency, W3 is bread weight and W2 is flour weight.

2.6. Chromaticity of bread crust and crumb

The crust and crumb colour were measured using the CR-400 Chroma Meter (Konica-Minolta, Tokyo, Japan); *L* (lightness), *a* (redness), and *b* (yellowness) values (Hunter colour) were measured for six regions of bread crust and crumb. The whiteness index (*WI*) was calculated according to HSU (2003) and LIN *et al*. (2009).

2.7. Texture profile analysis

Texture profile analysis (TPA) was carried out in triplicate for two slices of bread. TPA was performed using a texture analyzer (CT3 4500, Brookfield, Middleboro, USA). Bread samples were sliced to approximately 25-mm thickness. Hardness, cohesiveness, springiness, and chewiness of the center of the bread slices were measured (BLANDINO *et al*., 2013). The settings and conditions were carried out as described previously by ULZIIJARGAL *et al*. (2013), with some modifications: the acrylic cylindrical probe had a 38.1 mm diameter (TA4/1000), the pretest speed was 2 mm/s, the test speed was 2 mm/s, the post-test speed was 2 mm/s, the distance was 10 mm (40% compression), and the trigger load was 50 g.

2.8. Bread crumb image analyses

The structural features of the bread crumb were analyzed using ImageJ software (1.47v, National Institutes of Health, Bethesda, MD, USA). Structural features included bread cell

density, mean cell area, and the fraction of cell area to total area. Bread crumb images were captured using a scanner (Epson Perfection V370 Photo, Epson, Japan) at a resolution of 800 × 800 dpi. Images were calibrated to reflect actual size using a known scale, were cropped to 60 × 60 mm, filtered using a bandpass filter, and converted into binary images using the *convert to mask* feature for differentiating between the cell and non-cell area. Before analyzing particles (cells), the particle size was set from 0.01 mm^2 to infinity and the circularity was set from 0 to 1. This particle size corresponds to a particle diameter of 0.1 mm, which can be resolved by the human eye (PONGJARUVAT *et al*., 2014).

2.9. Analysis of volatile compounds in bread crumbs

Volatile compounds were analyzed as described previously by PLESSAS *et al*. (2008), using gas chromatography and mass spectrometry (7890A GC-MS; Agilent, Santa Clara, CA, USA) with a flame ionization detector (FID). The separation was performed with a DB-WAX column (60 m × 250 μm × ϕ 0.25 mm) (Waters, Milford, MA, US). The detector was an Agilent 5975C Inert XL MSD with Triple-Axis Detector. Helium was used as a carrier gas with a constant flow of 1 ml/min. Using solid-phase microextraction technique (SPME), 1 g of each bread sample was put into a 20-ml vial accessible to the SPME needle through the vial septum. Then, the vial was submerged in a water bath at 60°C and the SPME fiber (50/30 μm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was exposed to the headspace of the vial for 60 min. When the extraction process was finished, the SPME fiber was inserted into the injector port (set at 280°C) of the gas chromatograph (GC) for thermal desorption of volatile compounds for 5 min in splitless mode. The GC temperature program was set as follows: 35°C for 5 min, increased by 5°C/min to 50°C (held for 5 min), increased by 5.5°C/min to 230°C (held for 5 min). Volatile compound identification was based on comparison of GC retention times and peak areas with spectral data from the Wiley9Nist 0.8 library (Wiley9Nist 0.8 library, mass spectral search program, version 5.0, USA).

2.10. Sensory evaluation

Bread samples were prepared from a freshly baked loaf (6-8 h after baking). The bread samples were cut about 50 × 20 × 25 mm and served on a small paper plate. Sensory evaluations were conducted by 15 semi-trained consumers who were students and professors at Kyungpook National University, South Korea. The sensory attributes tested included appearance, colour, flavour, mouthfeel, and overall acceptability. The sensory attribute scale used for assessing the bread was as follows: 1, extremely dislike; 4, neither like nor dislike; and 7, extremely like (ULZIIJARGAL *et al*., 2013).

2.11. Statistical analysis

To examine statistical significance, the data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at the $p < 0.05$ level of significance. The correlation among structural features and textural profiles of bread crumb was analyzed using a 2-tailed Pearson correlation at $p < 0.05$. The analysis was carried out using SPSS for windows (ver. 19, IBM, New York, New York, USA). The graphs were constructed using Microsoft Excel (2007v, Microsoft, Redmond, Washington, USA).

3. RESULTS AND DISCUSSION

3.1. Leavening ability

The leavening ability of co-cultures was compared to that of single cultures (SC) in lean dough containing wheat flour and water (Fig. 1). The leavening rates were significantly different among the cultures tested ($p < 0.05$). For SC, the dough was greatly leavened after only 30 min of incubation, but for co-cultures, the dough was greatly leavened after 60 min of incubation. The leavening rates for SC, SCTD, SCPA, and SCTDPA were 55.4, 41.6, 36.5, and 31.6 ml/h, respectively. In a previous study, we found that the leavening rate for single cultures of *T. delbrueckii* JK08 and *P. anomala* JK04 were 8.67 and 2.29 ml/h, respectively. The lower performance of *T. delbrueckii* JK08 and *P. anomala* JK04 may be due to slower growth relative to *S. cerevisiae* (WAHYONO *et al*., 2015). BELY *et al*. (2008) also reported lower performance for *T. delbrueckii* 27828 and *T. delbrueckii* 31703 in terms of fermentation rate in comparison to *S. cerevisiae*. On the contrary, HERNANDEZ-LOPEZ *et al*. (2003) reported that *T. delbrueckii* strains IGC5321 and IGC5323 exhibited higher leavening ability and CO_2 production than *S. cerevisiae* after exposure to hyperosmotic and freeze-thaw stress. We demonstrated that incorporating TD and PA with SC produced a co-culture with greatly improved leavening ability for a longer leavening period (> 120 min). ELHARIRY *et al*. (2011) revealed that incorporating *S. cerevisiae* and *L. plantarum* in a sourdough system delivered favorable effects such as improving the leavening ability, as well as the sensory and physical properties of the bread.

Table 1: List of ingredients used in bread making.

Ingredients	Quantity	Yeast addition (total cells)[a]		
		S. cerevisiae	***T. delbrueckii*JK08**	***P. anomala*JK04**
Wheat flour (g)	280			
Sugar (g)	16.8			
Salt (g)	5.6			
Water (ml)	200			
Leavening agent;				
SC		1×10^{11}	-	-
SCTD		5×10^{10}	1×10^{11}	-
SCPA		5×10^{10}	-	1×10^{11}
SCTDPA		3×10^{10}	7×10^{10}	7×10^{10}

[a]Calculated according to the yeast concentration and ratio determined in the bread-making procedures in the materials and methods.

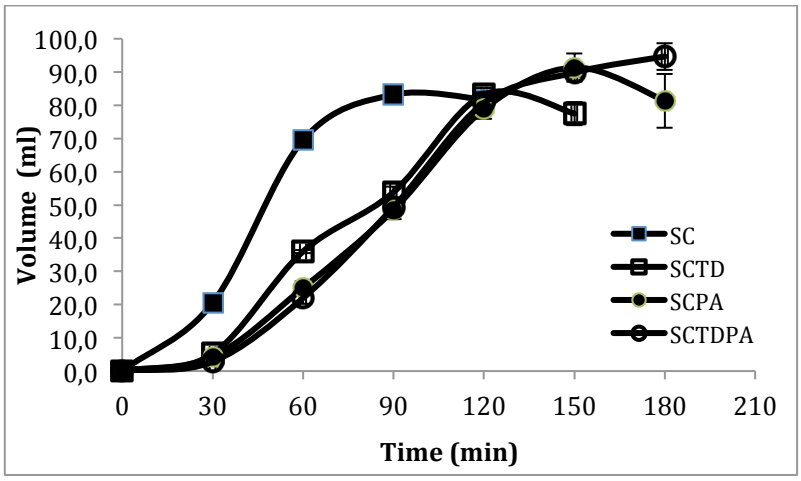

Figure 1: Leavening rates for co-cultures or a single culture in lean dough. Results are means±SD of triplicate.

3.2. Physical properties

The co-cultures did not affect the moisture content or bread yield, but significantly affected the bread-specific volume ($p < 0.05$, Table 2). The specific volumes of breads produced with the cultures tested were in the range of 4.01-4.37 cm^3/g. In comparison to breads leavened with co-cultures, the bread leavened with SC produced the greatest specific volume (4.37 cm^3/g), which is consistent with the observation that SC exhibited the highest leavening activity (55.4 ml/h). We previously reported that lower leavening abilities for *T. delbrueckii* JK08 and *P. anomala* JK04 produced significantly lower specific volumes in comparison to *S. cerevisiae* (WAHYONO *et al*., 2015). These results strongly suggest a connection between the yeast's leavening ability and the specific volume of the resulting bread.

No significant differences were observed in bread-specific volume for the co-cultures tested. Even though there were significant differences in leavening rates for the co-cultures tested, the time period for fermentation in the bread-making process was not long enough for optimum leavening activity for SCTD, SCPA, SCTDPA (120, 150, and 180 min, respectively). However, the specific volume of breads produced by co-cultures is well within the range of standard bread, with specific volumes that ranged from 3.5 to 6 cm^3/g (ULZIIJARGAL *et al*., 2013).

The co-cultures significantly affected the chromaticity of the bread crust (Table 3). SCTD-leavened bread showed the greatest *L*, *b*, and *WI* values. The *L* value for SCTD was significantly greater than that for SC or SCPA. The *b* value for SCTD was only significantly greater than that for SC. The *WI* value for SCTD was significantly higher than those for the other cultures tested. SCTDPA produced the highest *a* value, which was significantly greater than that of SC or SCTD. In contrast, SC-leavened bread produced the lowest *L*, *a*, *b*, and *WI* values. These results were consistent with previous work that demonstrated greater *L* and *b* values for bread crust when *T. delbrueckii* JK08 was used as a leavening agent (WAHYONO *et al*., 2015). For crumb colour, the co-cultures significantly affected the *L* and *b* values, but not the *a* and *WI* values (Table 3). SCPA produced the greatest *L* value, which was significantly greater than that of SC. It also produced the greatest *b* value, which was significantly higher than that of SC or SCTD. The greatest *L* and *b* values of bread crumb arose when *P. anomala* JK04 was used as a leavening agent, while lower *L* and

b values (darker colour) were produced when *S. cerevisiae* was used (WAHYONO *et al*., 2015). In short, TD produced greater lightness and unsaturated yellowish colour in the bread crust, and PA produced similarly a coloured bread crumb. On the other hand, SC produced a darker and saturated yellowish colour in bread crust and crumb. HERNANDEZ-LOPEZ *et al*. (2003) reported that commercial baker's yeast (*S. cerevisiae*) exhibited greater maltase and invertase activity than *T. delbrueckii* IGC5321. Consequently, SC produced more reactive saccharides, which may contribute to darker colour formation. The saccharides and nitrogen-containing substances involved in the browning reaction create the dark-coloured pigment melanoidin, which confers a darker colour to the bread crust and crumb (STEAR, 1990).

Table 2: Physical properties of bread leavened with co-cultures or a single culture[a].

Yeast	Crumb moisture content (%)	Specific volume (cm^3/g)	Bread yield efficiency (%)
SC	46.33 ± 0.50^{A}	4.37 ± 0.15^{A}	147.97 ± 0.75^{A}
SCTD	46.58 ± 0.59^{A}	4.08 ± 0.05^{B}	148.86 ± 1.00^{A}
SCPA	46.83 ± 0.35^{A}	4.15 ± 0.01^{B}	147.85 ± 0.21^{A}
SCTDPA	46.61 ± 0.16^{A}	4.01 ± 0.09^{B}	148.13 ± 0.75^{A}

[a]Means with the same superscript letter in a column are not significantly different at the level $p < 0.05$.

Table 3: Chromaticity of bread crust and crumb leavened with co-cultures or a single culture[a].

Yeast	Crust colour				Crumb colour			
	L	*a*	*b*	Whiteness index (*WI*)	*L*	*a*	*b*	Whiteness index (*WI*)
SC	38.22 ± 1.22^{C}	6.26 ± 0.72^{C}	15.85 ± 0.97^{B}	35.90 ± 0.88^{B}	56.85 ± 2.88^{B}	-2.52 ± 0.11^{A}	8.53 ± 0.78^{C}	55.93 ± 2.76^{A}
SCTD	41.63 ± 0.93^{A}	6.92 ± 0.67^{BC}	17.77 ± 0.24^{A}	38.59 ± 1.00^{A}	59.08 ± 0.11^{AB}	-2.56 ± 0.05^{A}	9.25 ± 0.38^{BC}	57.97 ± 0.03^{A}
SCPA	39.39 ± 0.34^{BC}	7.79 ± 0.26^{AB}	17.00 ± 0.28^{A}	36.57 ± 0.35^{B}	60.22 ± 0.75^{A}	-2.42 ± 0.02^{A}	10.34 ± 0.13^{A}	58.82 ± 0.71^{A}
SCTDPA	40.12 ± 0.83^{AB}	8.23 ± 0.22^{A}	17.66 ± 0.46^{A}	37.02 ± 0.64^{B}	58.37 ± 1.55^{AB}	-2.43 ± 0.10^{A}	9.58 ± 0.47^{AB}	57.21 ± 1.40^{A}

[a]Means with the same superscript letter in a column are not significantly different at the level $p < 0.05$.

3.3. Structural features of bread crumb

The structural parameters of bread crumb are expected to influence its mechanical behavior. By using image analysis, the structural features of bread crumb can be quantified (ZGHAL *et al*., 2002). Hence, we carried out an image analysis of bread crumb and the results are presented in Table 4. The digital binary images of bread crumb from which the structural features could be extracted are shown in Fig. 2. The use of co-cultures significantly affected the cell density and the mean cell area of bread crumb, but not the fraction of cell area to total area. The cell density of bread crumb leavened with SCTD (58.89 1/cm) was the greatest among cultures tested, and was significantly greater than that leavened with SC (50.04 1/cm). Inversely, the mean cell area of bread crumb leavened with SC (0.90 mm^2) was the largest, significantly larger than that leavened with SCTD (0.73 mm^2). These results suggest that as mean cell area increases, cell density decreases. The

large mean cell area for SC-leavened bread is consistent with its high specific volume. In other words, bread leavened with SC was more porous than bread produced using co-cultures, probably because of superior leavening ability and higher CO_2 production (WAHYONO *et al*., 2015). Alternatively, PONGJARUVAT *et al*. (2014) reported that high specific volume is tightly correlated with cell density and cell area fraction.

We performed correlation analysis for structural features and mechanical parameters (TPA) of bread crumb. We found that the cell density, mean cell area, and fraction of cell area to total area were correlated with cohesiveness, but not hardness, springiness, or chewiness (Table 5). Structural features were not strongly correlated with overall bread quality, which is more strongly affected by attributes such as odor and appearance (LAMPIGNANO *et al*., 2013).

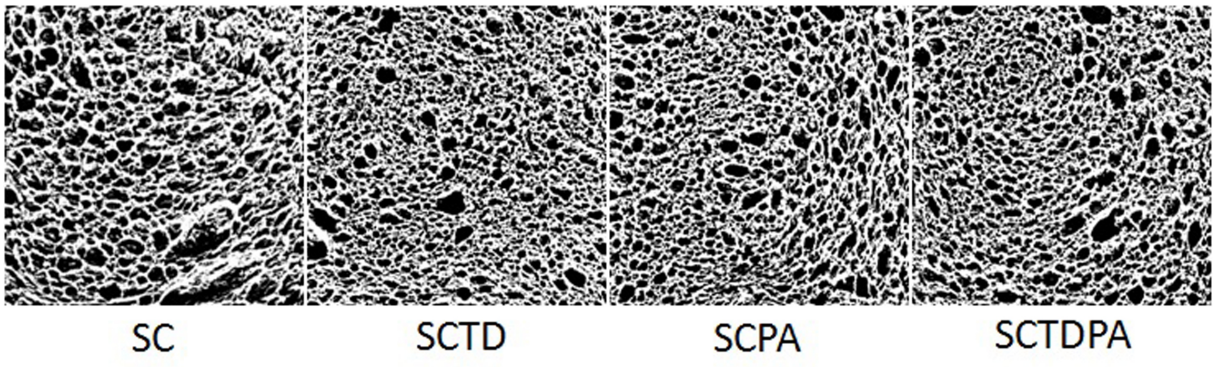

Figure 2: The digital binary images of breads crumb leavened with co-cultures or a single culture.

Table 4: Structural features of bread crumb leavened with co-cultures or a single culture quantified by image processing[a].

Yeast	Cell density (1/cm^2)	Mean cell area (mm^2)	Fraction of cell area to total area (%)
SC	50.04±5.59^{B}	0.90±0.10^{A}	44.87±0.40^{A}
SCTD	58.89±2.92^{A}	0.73±0.03^{B}	42.83±2.06^{A}
SCPA	52.55±3.37^{AB}	0.85±0.09^{AB}	44.34±1.87^{A}
SCTDPA	53.38±3.59^{AB}	0.84±0.04^{AB}	44.57±1.03^{A}

[a]Means with the same superscript letter in a column are not significantly different at the level $p < 0.05$.

Table 5: Pearson correlation of structural features and textural profiles of bread crumb.

	Hardness	Springiness	Cohesiveness	Chewiness	Cell density	Mean cell area
Springiness	0.360					
Cohesiveness	-0.485	-0.391				
Chewiness	0.981**	0.353	-0.319			
Cell density	0.450	0.365	-0.633*	0.380		
Mean cell area	-0.435	-0.432	0.732**	-0.340	-0.950**	
Cell fraction	-0.175	-0.326	0.658*	-0.043	-0.273	0.541

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

3.4. Texture profile analyses

TPA was used to evaluate the textural properties of bread crumb (Fig. 3). The co-cultures altered hardness, chewiness and cohesiveness, but these changes were insignificant, except for cohesiveness. SCTD-leavened bread was of lower cohesiveness than bread leavened with other cultures (Fig. 3), consistent with our previous study, demonstrating that *T. delbrueckii* JK08 produces bread crumb with lower cohesiveness (WAHYONO *et al*., 2015). As stated earlier, the cohesiveness of bread crumb was the only parameter that correlated with its structural features. According to SCANLON and ZGHAL (2001), the crumb textural properties were largely determined by the bread crumb structural features. Fine and uniformly-sized cells produce a softer texture. Here we have demonstrated that a greater mean cell size conferred greater cohesiveness and vice versa. However, this result should be further evaluated in light of previous work that established that crumb cohesiveness is controlled by moisture content and the strength of networks surrounding the cell pore (CAUVAIN, 2004). We have shown that the use of co-cultures produced bread of quality and textural properties comparable to bread leavened using a single culture.

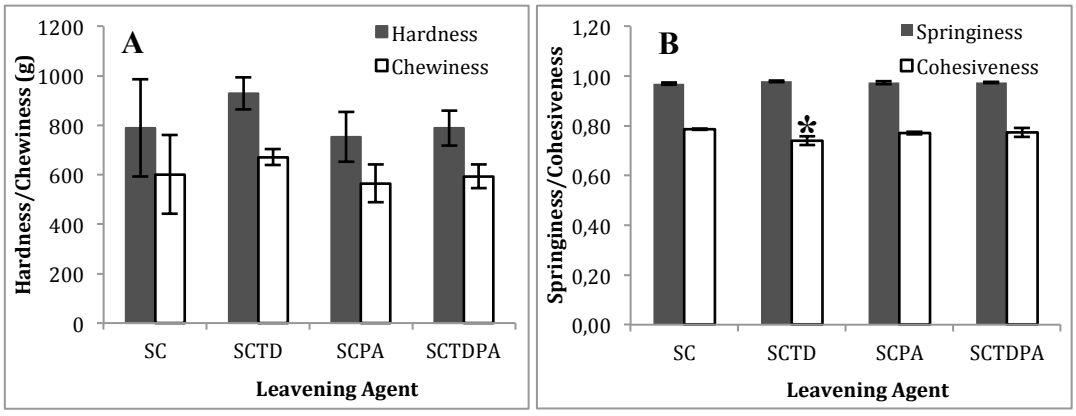

Figure 3: Texture profiles of bread leavened with co-cultures or a single culture.
(A) Hardness and chewiness of bread crumb. (B) Springiness and cohesiveness of bread crumb. Results are means ± SD of triplicate.

(*) Significantly different at the level $p < 0.05$.

3.5. Volatile compounds of bread crumb

A total of 54 volatile compounds were identified in the bread crumb leavened with SCTDPA, whereas 50, 47, and 49 volatile compounds were identified in the bread crumb leavened with SC, SCTD, and SCPA, respectively (Table 6). SCTDPA-leavened bread not only produced more unique volatile compounds, but in greater abundance, as indicated by the greater peak area. In most cases, the bread leavened by co-cultures produced more volatile compounds than that of a single culture.

The volatile compounds were predominately alcohols, aldehydes and esters. Isobutyl alcohol (i-BuOH), isoamyl alcohol (i-AmOH), and phenethyl alcohol (PEA) were the predominant alcohol groups. i-BuOH levels were highest in SCTDPA bread (20,169) and the lowest in SC bread (1,986). Inversely, i-AmOH levels were the highest in SCPA bread (11,168) and lowest in SCTDPA bread (6,265). As reported by KIM *et al*. (2013), *P. anomala* Y197-13 produced high i-AmOH levels and conferred a banana flavour that significantly affected the flavour and taste of turbid rice wine. WATANABE *et al*. (1990) reported that bread containing high levels of i-AmOH was less favorable than bread containing high levels of i-BuOH. The bread leavened with *P. anomala* SKM-T exhibited a higher PEA content and was preferred over *S. cerevisiae* (MO and SUNG, 2014) due to the favorable honey and flower odor of PEA (JENSEN *et al*., 2011).

The predominant aldehydes in bread crumb were n-hexanal, furfural, and benzaldehyde. The bread leavened with the co-cultures containing PA (SCPA and SCTDPA) produced the highest amounts of these compounds. n-hexanal was the most abundant aldehyde and conferred a green flavour. The second most abundant was benzaldehyde which produces an almond odor (BIRCH *et al*., 2013a). Fulfural is characterized by a burnt and sweet, caramel-like, odor (PROST *et al*., 2012). The other volatile compounds that contributed to either favorable (n-octanal, n-decanal) or unfavorable (n-heptenal) odors were comparable among all cultures tested.

Esters typically have pleasant, fruity, or sweet odors (BIRCH *et al*., 2013b). In most cases, SCTDPA leavened bread contained a greater abundance and higher diversity of ester compounds. Compounds including isoamyl acetate (fruity), ethyl caproate (fruity, wine, apple, banana, brandy), ethyl octanoate (fatty, fruity), ethyl decanoate, and ethyldodecanoate were enhanced in the bread leavened with co-cultures containing PA (SCPA and SCTDPA). SC and TD enhanced particular compounds such as ethyl acetate and methyl salicylate, respectively. In alcoholic beverages, ethyl acetate can produce unfavorable sensory qualities. Mixed cultures of *S. cerevisiae* and *P. anomala* (mutant type) produced higher ethyl acetate-hydrolyzing esterase activities. This enzyme is crucial in the formation of acetate ester, which delivers superior flavour (KURITA, 2008).

The bread leavened by co-cultures was obviously superior to that leavened by a single culture with regard to the volatile compound content. These compounds were produced mainly from the metabolism of yeasts during dough fermentation and flour lipid oxidation (BIRCH *et al*., 2013b). These processes are influenced by the availability of free, reactive amino acids, sugars, alcohols, enzyme activity, and the degree of polymerization and hydration of substrates due to mixing to baking (STEAR, 1990). SADOUDI *et al*. (2012) reported that the use of co-cultures altered the production of volatile compounds in wine, because co-culture interactions influenced the entire metabolic pathway.

Table 6: Volatile compounds contained in the bread crumb leavened with co-cultures or a single culture.

No	Group	RT	Compound	Flavour Description	Peak Area*			
					SC	SCTD	SCPA	SCTDPA
1	Acids	30.40	Acetic acid	Acid, pungent [a]	1,973	1,838	2,483	4,043
2		33.28	Isobutyric acid		339	275	287	408
3		35.61	2-Methylbutanoic acid	Sweaty [e]	680	603	514	939
4		40.00	2-Methylpropanoic acid	Sweat, butter[a]	81	59	83	176
5	Alcohols	18.63	Isobutyl alcohol		1,986	7,494	13,946	20,169
6		23.30	Isoamyl alcohol	Banana [f]	8,011	8,174	11,168	6,265
7		26.30	2-Ethyl-1-decanol		ND	ND	ND	301
8		27.69	2-Methyl-3-pentanol		146	180	67	88
9		27.90	1-Hexanol	Flower [a]	465	610	527	674
10		28.14	2-Nonanol		153	159	ND	87
11		28.63	3-Ethoxy-1-propanol	Fruity [a]	87	236	ND	116
12		32.76	1-Dodecanol		228	163	280	684
13		35.42	2-Furanmethanol		309	552	615	1,857
14		40.86	Phenethyl alcohol	Honey, Flower [a]	9,506	12,292	8,391	12,643
15	Aldehydes	18.01	n-Hexanal	Green [d]	5,369	19,874	37,144	71,305
16		22.38	n-Heptanal	Fatty, rancid [d]	233	262	528	302
17		25.99	n-Octanal	Citrus [d]	207	263	333	495
18		27.11	2-Heptenal		600	1,086	1,594	2,335
19		30.88	Furfural		1,646	2,068	2,764	7,529
20		31.79	n-Decanal	Citrus [d]	151	230	237	379
21		32.56	Benzaldehyde	Almond [d]	3,507	5,475	6,389	8,374
22		33.64	5-Methyl-2-furfural		132	47	131	529
23		38.80	2,4-Decadienal	Fatty, waxy [b]	38	94	103	224
24	Alkenes	8.96	2,4-Dimethyl-1-heptene		62	ND	ND	396
25		29.86	3-Ethyl-2-methyl-1,3-hexadiene		36	58	83	120
26	Benzenes	15.92	Methyl benzene		269	330	551	413
27		24.95	Ethenylbenzene		762	134	8,026	6,265
28		29.97	1,3-bis(1,1-dimethylethyl)benzene		381	563	85	1,503
29	Esters	9.10	Ethyl acetate	Pineapple [a]	1,688	120	635	5,590
30		14.66	Isobutyl acetate	Ethereal, fermented	ND	ND	ND	134

				odor [b]				
31		19.87	Isoamyl acetate	Fruity [e]	305	666	5,290	7,923
32		24.16	Ethyl caproate	Fruity, wine, apple, banana, brandy [c]	305	51	4,641	3,903
33		25.43	n-Hexyl acetate		37	34	124	256
34		30.10	Ethyl octanoate	Fatty, Fruity [a]	625	796	4,646	12,307
35		30.75	Amyl caproate		7	ND	84	351
36		34.96	Ethyl decanoate		204	171	4,066	9,553
37		36.12	Ethyl-9-decenoate		ND	ND	104	1,658
38		38.27	Methyl salicylate		220	1,686	316	1,876
39		38.91	2-Phenethyl acetate	Roasty[e]	235	101	209	868
40		39.22	Ethyldodecanoate		336	281	1,069	1,407
41	Furans	24.06	2-Pentylfuran	Floral, fruity [d]	309	246	887	566
42		31.99	2-Acetylfuran		282	339	450	1,243
43	Ketones	12.73	2,3-Butanedione	Buttery, caramel [d]	353	592	460	542
44		22.29	2-Heptanone		ND	ND	58	67
45		25.89	3-Hydroxy-2-butanone	Butterscotch [d]	867	744	922	1,149
46		26.37	1-Octen-3-one	Mushroom [g]	48	263	419	765
47	Phenols	40.61	Butylatedhydroxytoluene		1,585	2,788	4,507	2,683
48	Pyrazines	25.33	Methylpyrazine		185	179	228	546
49		27.33	2,6-Dimethylpyrazine	Hazelnut [e]	68	73	219	314
50		27.49	Ethylpyrazine		58	95	95	223
51	Terpenes	37.04	(-)-.beta.-Bisabolene		220	ND	341	222
52	Others	28.90	Dimethyl trisulfite		51	120	217	263
53		37.64	Naphthalene		79	143	446	288
54		41.42	1,4-Methanobenzocyclodecene		75	139	265	275

*the values of volatile compounds calculated from the peak area divided by 1000.
ND, not detected
[a]. JENSEN *et al*. (2011); [b]. MO and SUNG (2014); [c]. DAIGLE *et al*. (1999); [d]. BIRCH *et al*. (2013a); [e]. PROST *et al*. (2012); [f]. KIM *et al*. (2013); [g]. BIRCH *et al*. (2013b)

3.6. Sensory properties

The average results of the sensory evaluation of appearance, colour, flavour, mouthfeel, and overall acceptability are shown in Fig. 4. In most cases, the co-cultures slightly enhanced all the sensory attributes, except for appearance. All attributes produced satisfactory scores in the range of 4.73–5.57 out of a total 7 points. On average, the co-

cultures produced marked improvement over the single culture, which scored in the range of 4.07-5.71. SCTDPA leavened bread was superior in overall acceptability (5.57), which is attributable to higher ratings in flavour (5.27) and mouthfeel (5.30). High flavour ratings are probably due to the high abundance of favorable volatile compounds (Table 6). SCTD leavened bread had a superior colour rating (5.53). These results demonstrate that incorporating SC, TD, and PA as leavening agents conferred beneficial characteristics to bread. SC contributed to improving bread appearance through greater leavening ability, and TD and PA contributed to enhanced flavour, colour, and mouthfeel.

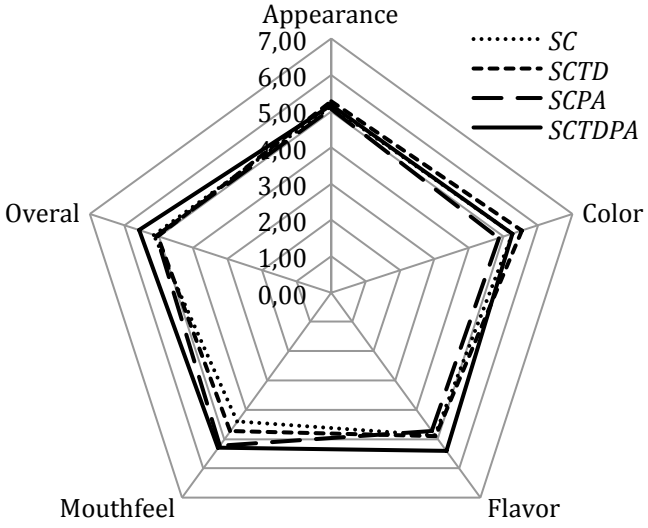

Figure 4. Radar plot of the sensory properties of bread leavened with co-cultures or a single culture. Result reflects the means of scores from 15 semi-trained panelists.

4. CONCLUSIONS

We have shown that the use of mixed cultures of *S. cerevisiae*, *T. delbrueckii* JK08, and *P. anomala* JK04 enhanced bread quality. The bread leavened by the co-cultures produced textural and structural properties comparable to single cultures of S. *cerevisiae*. The co-cultured bread had a superior aroma and enhanced sensorial qualities. Thus, the use of co-cultures as leavening agents has great promise in fulfilling the consumer need for unique and high-quality bread.

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