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Leavening Ability of Yeast Isolated from Different Local Fruits in Bakery Product (Keupayaan Menaik Yis yang dipencilkan daripada Buah-Buahan Tempatan dalam Produk Bakeri)

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ABSTRACT

This study focused on the isolation of yeast from a variety of Malaysian local fruits which can potentially be employed as a leavening agent in bakery products. A total of 6 yeast strains (SKS2, SMK9, SDB10, SRB11, SS12, SM16) were isolated from palm kernel pulp (Cocos nucifera L.), longan (Dimocarpus longan spp. malesianus Leenh), soursop (Annona muricata L.), bamboo shoot (Bambusa vulgaris), snake fruit (Salacca zalacca) and mango (Mangifera indica) using an enrichment procedure which can enhance the growth of yeast colonies and eliminat the fruits worm. The isolates were identified as Saccharomyces cerevisiae, through observation of the yeast cells morphology under microscope, temperature tolerance and fermentative capacity test. The leavening ability of the identified yeasts were examined by fermenting dough. Fermentation proofing was carried out at $30\pm 5^{\circ}\text{C}$ with 85% humidity for 120 min. The bread doughs were baked at 180°C for 8 min. Results showed that the yeast strains SRB11, SM16, SS12 and SMK9 were able to leaven the highest specific volume of $3.68\text{ cm}^3/\text{g}$, $3.41\text{ cm}^3/\text{g}$, $3.37\text{ cm}^3/\text{g}$ and $3.23\text{ cm}^3/\text{g}$, respectively. Strain SDB10 and commercial yeast showed less ability with specific volume of $3.02\text{ cm}^3/\text{g}$ and $2.84\text{ cm}^3/\text{g}$, respectively. Thus, the new yeast isolates from local fruits showed much superior with specific volume of more than $3.02\text{ cm}^3/\text{g}$.

Keywords: Fermentation; leavening; local fruits; yeast

ABSTRAK

Fokus kajian ini adalah pemencilan yis daripada pelbagai sumber buah-buahan tempatan di Malaysia yang berpotensi sebagai agen penaik dalam produk bakeri. Sejumlah 6 strain yis (SKS2, SMK9, SDB10, SRB11, SS12, SM16) telah dipencilkan daripada isi rong kelapa sawit Cocos nucifera L.), mata kucing (Dimocarpus longan spp. malesianus Leenh), durian belanda (Annona muricata L.), rebung (Bambusa vulgaris), salak (Salacca zalacca) dan mangga (Mangifera indica), menggunakan kaedah pengayaan yang dapat meningkatkan pertumbuhan koloni yis dan membunuh ulat buah. Pencilan dapat dikenal pasti sebagai Saccharomyces cerevisiae melalui pemerhatian morfologi sel menggunakan mikroskop, ujian toleransi suhu dan ujian keupayaan fermentasi. Semua yis yang dikenalpasti, diuji keupayaannya sebagai agen penaik melalui fermentasi dalam doh roti. Penaikan doh (proofing) dilakukan pada suhu $30\pm 5^{\circ}\text{C}$ selama 120 min pada kelembapan 85%. Doh roti dibakar pada suhu 180°C selama 8 min. Hasil menunjukkan yis dari strain SRB11, SM16 SS12 dan SMK9 berkeupayaan menghasilkan roti dengan isipadu spesifik tertinggi, $3.68\text{ cm}^3/\text{g}$, $3.41\text{ cm}^3/\text{g}$, $3.37\text{ cm}^3/\text{g}$ dan $3.23\text{ cm}^3/\text{g}$. Manakala strain yis SDB10 dan komersial menunjukkan keupayaan yang lebih rendah dengan isipadu spesifik masing-masing adalah $3.02\text{ cm}^3/\text{g}$ dan $2.84\text{ cm}^3/\text{g}$. Ini membuktikan, pencilan yis daripada buah-buahan tempatan menunjukkan ciri-ciri yang lebih baik dengan nilai isipadu spesifik lebih daripada $3.02\text{ cm}^3/\text{g}$.

Kata kunci: Buah-buahan tempatan; fermentasi; penaik; roti; yis

INTRODUCTION

The use of yeast to make bread and alcohol has been recorded for thousands of years. The Babylonians (6000BC) and Egyptians (5000BC) have left written accounts of their production of beer, wines and bread, where all of them warranted the use of yeast (Kevin 2005). Yeast especially *Saccharomyces cerevisiae* is known as sugar-eating fungus and can be found naturally from the surrounding. According to Kurtzman and Fell (1998), fruits, vegetables, drinks and other agricultural products are very important microhabitats for a various of yeast species. A succession of yeast populations in such products involves in a variety of biochemical processes carried out

by yeast to utilize simple sugars present in the agricultural products. Kandasamy (2006) reported that 269 yeasts could easily be found in the Malaysian flora and fauna and Malaysia is also known as one of the world's 12 hot spots for biodiversity that can be sustainably harnessed and exploited for socio-economic gains.

Several investigations have been carried out in different natural and crop-growing environments so as to obtain better knowledge of yeast biodiversity and to define the impact of this on food products. Among the studies that has been reported including a Brazilian sugarcane spirit (plant materials) (Maristela et al. 2006), and fresh orange fruit and juice (Francisco et al. 2002), in crop-growing

environment in Cameroon (Marzia et al. 2008) and on tropical fruits, flowers and leaves (Camotti- Sartori et al. 2005; de Silva et al. 2005; Santos et al. 1996; Trindade et al. 2002).

Yeast especially *S. cerevisiae* strains have been selected for decades for their dough-leavening characteristics. The yeast produces carbon dioxide that results in dough leavening and contributes to the flavor and crumb structure of bread (Francisca et al. 1999). This strain of yeast is very robust and capable of fermenting dough to rise. According to Romano et al. (2008), *S. cerevisiae* is capable of fermenting all sugars present in the dough, for example, glucose, fructose, sucrose and maltose with 8 times faster than *P. membranificiens* which can only ferment glucose.

Burrows (1970) listed four functions of yeast in bread-making: 1) to increase dough volume by evolution of CO₂ during fermentation of the available carbohydrates in the flour, 2) to develop structure and texture in the dough by the stretching due to expansion of gas bubbles, 3) to improve flavor and 4) to add some nutritive values of bread. Most ethanol for human consumption as beer or wine is produced by two common strains yeast, *S. carlsbergensis* and *S. cerevisiae*, which are characterized as bottom and top yeast fermentation respectively.

The leavening agents (yeasts) currently used in Malaysian bakery industries mostly imported from foreign countries such as Australia (Mauripan), France (Saf-instant), Canada (Fermipan) and Turkey (Gold Pakmaya). The presence of yeasts from local fruits is yet to be exploited, especially in bakery products as a leavening agent. Up to date there is no report on the use of yeast isolated from Malaysian local fruits that has potential as a leavening agent in bread making.

Thus the present study was carried out to isolate yeast from local fruits and to examine their dough leavening ability. In addition, we also performed brief physiological tests in order to have better understanding of the yeasts behavior in bread making.

MATERIAL AND METHODS

MATERIALS

Commercial *Saccharomyces cerevisiae* strain was obtained as list (ATCC no. 62418). Samples of fruits around peninsular Malaysia were collected as sources for yeast isolation. The collected samples were placed aseptically in sterile plastic bags and transferred in ice boxes (4°C) and brought to the laboratory for the analysis. Among the selected fruits were palm kernel pulp (*Cocos nucifera* L.), longan (*Dimocarpus longan* spp. *malasianus* Leenh), soursop (*Annona muricata* L.), bamboo shoot (*Bambusa vulgaris*), snake fruit (*Salacca zalacca*) and mango (*Mangifera indica*). The samples then were subjected to the following procedures within 24–36 hours after collection and transfer to the laboratory.

ENRICHMENT PROCEDURES FOR YEAST ISOLATION

The enrichment procedure to detect and isolate fermenting yeast species were carried out by adding 1 mL or 1 g, depending on sample type, into high-sugar medium (grape must, pH 3.2, with sugar added to a final concentration of 27%, w/v). All of the microfermentations were carried out at 25°C in 100-mL Erlenmeyer flasks containing 50 mL pasteurised must. During fermentation (within 10 days), yeast isolation was transferred on WL nutrient agar (Oxoid) at 30°C for 3-5 days. Later the isolated yeast were subcultured in YPD medium (10 g l-1 Bacto Yeast Extract, 10 g l-1 Bacto Peptone and 20 g l-1 glucose) (Oxoid, Basingstoke, UK) added with chloramphenicol to avoid bacterial growth. The plates were incubated at 30°C for 3–8 days. After this, any colonies were counted and selected according to their morphological characteristics (Martini et al. 1996). Representative colonies were picked randomly from the plates and pure cultures were subjected to the next identification procedures.

MICROSCOPE OBSERVATION

A single colony of yeast was mixed in a droplet of sterile distilled water on glass slide and smeared until the smear dry off. The smear was then stained using diluted methylene blue dye, air dried and observed under light microscope at 100× magnification.

MEDIA

The culture medium used in this study was YP (10 g/L yeast extract, 10 g/L peptone, 20 g/L agar) supplemented with different 200 g/L (glucose and sucrose) and 80 ml/L ethanol. The YP medium supplemented with 20 g/L glucose (YPG) was also supplemented with chloramphenicol for strain selection.

TEMPERATURE TOLERANCE TEST

Yeast isolates were plated in YPG medium and incubated at 25, 30, 37 and 45°C for 72 h.

FERMENTATIVE CAPACITY TEST

The fermentative capacity test was carried out using 2 mL Yeast Fermentation Broth (peptone 7.5 g; yeast extract 4.5 g; bromthymol blue) with Durham tube in addition to different carbon sources (1.0 mL sterile carbohydrate solution (glucose, sucrose, fructose and maltose) and incubated at 30°C for 72 h. The changes from green to yellow indicated that yeast using the carbon sources.

CULTIVATION AND DETERMINATION OF LEAVENING ABILITY OF THE YEAST ISOLATES

Different yeasts isolates were inoculated separately in sterilized peptone broth containing 25 (w/v) glucose in 100 mL conical flask and incubated at 30±2°C. The cells were collected by centrifuging the culture at 10,000 rpm for 15 min. The pellet of yeast cells was washed with cold

sterile distilled water after which resuspended in 10 mL sterile distilled water.

Dough containing high protein flour (100%), salt (2%), water (60%) and sugar (5%) was prepared and inoculated with yeast isolate (2.2×10^7 cfu mL⁻¹). Bakers' yeast (pure culture of *S. cerevisiae*) was used as positive control to ferment the dough. Another set of dough without yeast was prepared as a negative control sample. The samples were left to ferment in a proofer at $30 \pm 5^\circ\text{C}$ for about 120 min and baked in an oven at 180°C for 8 min.

LOAF VOLUME

Weight and volume of loaf produced after baking were measured 1 h after removal of loaves from the oven. Loaf volume was determined by the sesame seed displacement method (Mallock & Cook 1930; AACC 1983) and specific volume was measured by dividing the volume with loaf weight.

COLOR MEASUREMENT

Color differences among samples were determined using a Chromameter Minolta (CR-300 Trimulus Color Analyser, Japan). Three values of L, a, and b were measured where L = 100 (white), L = 0 (black); +a = red, - a = green; and +b = yellow, - b = blue.

RESULTS AND DISCUSSION

At present most good baker's yeast used in bakery industries including Small Medium Enterprise (SME) industries in Malaysia was imported from overseas. Malaysia possesses a variety of local fruits and vegetables which can be an important source of yeast species. Until now, we lack of leavening agent (baker's yeast) production which can be potentially marketed in this country. Naumov et al. (2002),

and Antonovics et al. (2003), reported that yeast is common in the natural yeast microflora of sweet botrytized wine. In this study, a total of six sweet and sweet tastes of local fruits were used as a source of yeast isolates. The fruits samples were left at ambient temperature for a certain period to encourage the multiplication of yeast before the isolation took place. Chloramphenicol and grape must (pH 3.2) were supplemented to the media in order to eliminate fruits worm and bacterial contamination.

Result showed that, yeast strains SMK9 and SDB10, have rough morphology of creamy colonies while the other samples including commercial yeast showed a fluffy colonies. The cream colonies were characteristic of as yeast. Several yeast are visualized on surface-grown colonies following colours: cream (*S. cerevisiae*), white (*Geotrichum candidum*), black (*Aureobasidium pullulans*), pink (*Phaffia rhodozyma*), red (*Rhodotorula rubra*), orange (*rhodosporidium* spp.) and yellow (*Cryptococcus laurentii*) (Greame et al. 2005). Irena (2005) reported that the colonies formed by cells of different yeast genera can be smooth, fluffy, rough, and slimy, depending on the ability of the particular yeast to form capsules or other extracellular matrix material, as well as on the capability of the cells to enter different stages of the yeast life cycle for example, mating, sporulation or pseudohyphal growth.

Figure 1 shows the morphology of yeast *S. cerevisiae* using a microscope (100 × magnifications). From the observation the ellipsoid or ovoid shapes known as *S. cerevisiae*. Yeast strain SMK9 showed the capability of budding which indicated the higher growth rate of yeast when compare to other strains including commercial yeast strain. Thus, this was an indicative of active fermentation (Hough et al. 1971). During the fermentation, yeast produces carbon dioxides, ethanol and other secondary metabolites products which contribute to the formulation of flavor and aroma (Thais et al. 2006) while the carbon

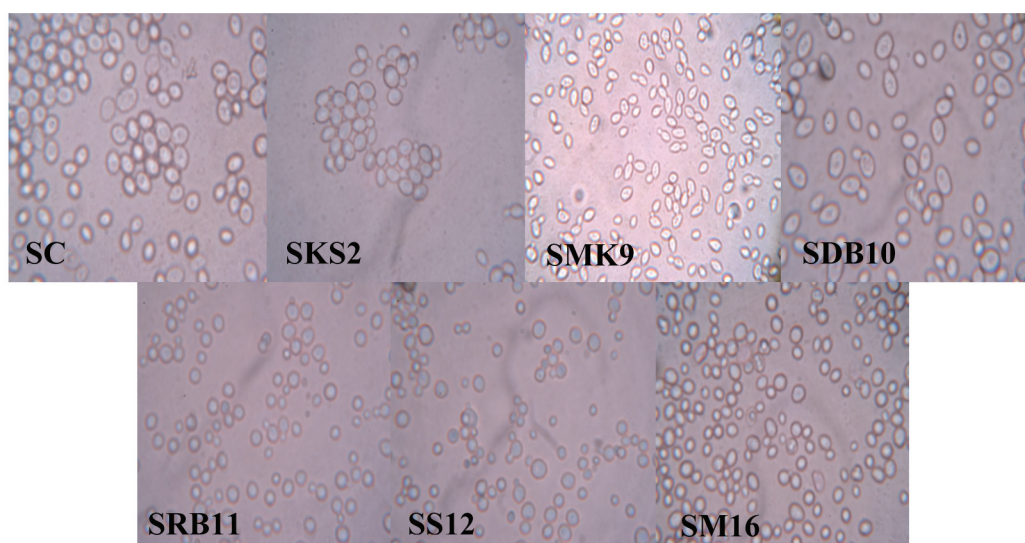


FIGURE 1. The morphology of yeast isolates observed under light microscope at 100× magnification. SC *S. cerevisiae*; SKS2 Palm Kernel; SMK9 Longan; SDB10 Soursop; SRB11 Bamboo shot; SS12 Snake fruit and SM16 Mango

dioxide production reacted as a leavening agent in bread dough.

Temperature and fermentative capacity test were used on the series of yeast's isolates in order to have a better understanding on yeast behavior. The temperature can affect the fermentation process and the metabolism of yeast. Table 1 illustrates the growth and the inhibition of the isolates at different growth temperature. All isolates were able to grow at 37°C. SKS2 showed more resistant at higher temperature compared to other strains. Those yeast strains which were able to survive at high temperature indicated that they may be used in bread making to speed up the proofing process, increased carbon dioxide production and formation of flavor and aroma may be enhanced.

The isolated yeasts were also tested on their ability to ferment glucose, sucrose, maltose and fructose and to produce carbon dioxide (Table 2). Results show that all strains were able to ferment all sugars provided and releasing carbon dioxide gas as observed in durham tube. This could be an important indication of invertase activity and an important feature for strains used in dough or bread making. However, these strains showed different time interval in releasing of carbon dioxide. Strain SMK9, SRB11 and SS12 fermented sugars in less than 30 min (color changes of fermentation broth from green to yellow) while other strains took more than 30 min. This indicated that those strains may initiate fermentation process immediately

after inoculated in bread dough and produce more carbon dioxide, causing dough to rise and contribute to better physico-chemical properties of bread.

The leavening properties of dough fermented with the various yeast isolates from local fruits are showed in Figures 2 and 3. All strains, SRB11, SM16, SS12 and SMK9 showed good fermentation or leavening ability giving the specific volume of 3.68 cm³/g, 3.41 cm³/g, 3.37 cm³/g and 3.23 cm³/g, respectively except for commercial strain (3.02 cm³/g) and SDB10 (2.84 cm³/g). The results indicate that the ability of the yeast isolates is comparable or even better than the commercial yeast to leaven the bread dough. This is supported by the fact that yeasts are capable in fermenting sugars especially glucose, sucrose, fructose and maltose (Table 2). The breakdown of sugars will release carbon dioxide that leavens the dough. Strains SRB11, SM16, SS12 and SMK9 showed the highest leavening ability (Figure 2 & 3) and would be considered as the most active yeasts to ferment bread dough compared to others strains including commercial yeast strain.

Tables 4 and 5 showed the bread crust and crumb color development as different yeast isolate were employed. Table 4 illustrates in significant difference ($p > 0.05$) between SMK9, SDB10, SRB11 and SS12 with the commercial strain. The crust color for bread fermented by SMK9, SRB11 and SS12 as well as commercial yeast was considered darker than SKS2 and SM16 (with lower

TABLE 1. Growth of *S. cerevisiae* at different temperature

Yeast	Temperature (°C)			
	25	30	37	45
SKS2	+++	+++	+++	+++
SMK9	+++	+++	++	-
SDB10	+++	+++	++	-
SRB11	+++	+++	++	-
SS12	+++	+++	+++	-
SM16	+++	+++	+++	-
SC	+++	+++	+++	-

Intensive growth (+++); moderate growth (++); low growth (+); no growth (-); Positive control (SC). SC *S. cerevisiae*; SKS2 Palm Kernel; SMK9 Longan; SDB10 Soursop; SRB11 Bamboo shot; SS12 Snake fruit and SM16 Mango

TABLE 2. Fermentation capacity test of yeast isolates on different carbon sources.

Carbon sources	<i>S. cerevisiae</i>	Isolates					
		SKS2	SMK9	SDB10	SRB11	SS12	SM16
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+

Carbon sources assimilation (+); no assimilation of carbon sources (-). SC *S. cerevisiae*; SKS2 Palm Kernel; SMK9 Longan; SDB10 Soursop; SRB11 Bamboo shot; SS12 Snake fruit and SM16 Mango

L* value). This could be due to progressive reduction of sugar during fermentation releasing carbon dioxide and generating energy thus impaired Maillard Reaction. This statement may be supported by the fact that the higher the dough specific volume by the increase of fermentation by both strain (Figures 2 and 3) which can result in increase carbon dioxide production. For the crumb color (Table 5), there was a significant difference ($p < 0.05$) between

all samples with commercial strain and negative control except for SDB10 and SRB11 strains. For the redness (a^*) and yellowness (b^*) of the crust and crumb showed significant different ($p < 0.05$) between all samples with the negative control. This study showed that different yeast isolates from different local fruits can affect the color development of bread crust and crumb.

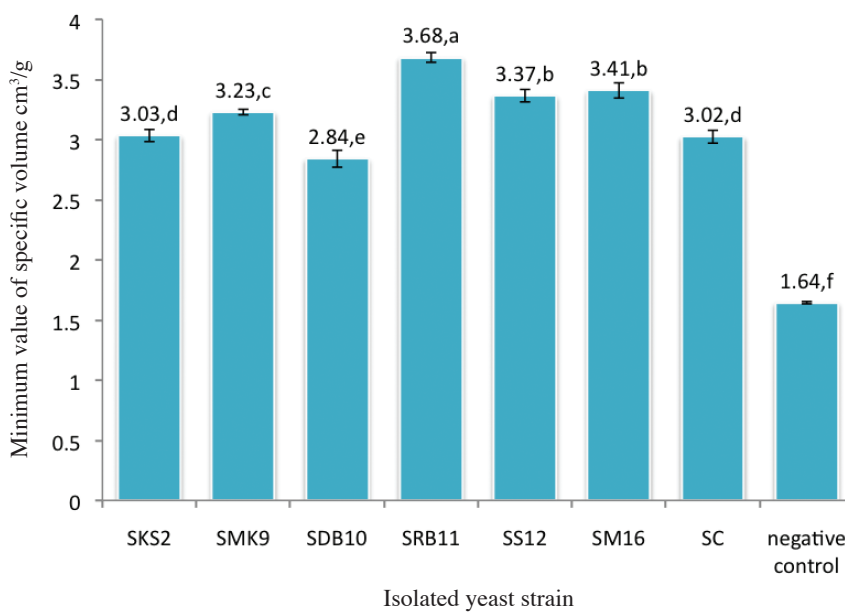


FIGURE 2. Specific volume (cm³/g) of bread using yeast strain isolate observed from different local agri-products. SC *S. cerevisiae*; SKS2 Palm Kernel; SMK9 Longan; SDB10 Soursop; SRB11 Bamboo shot; SS12 Snake fruit and SM16 Mango

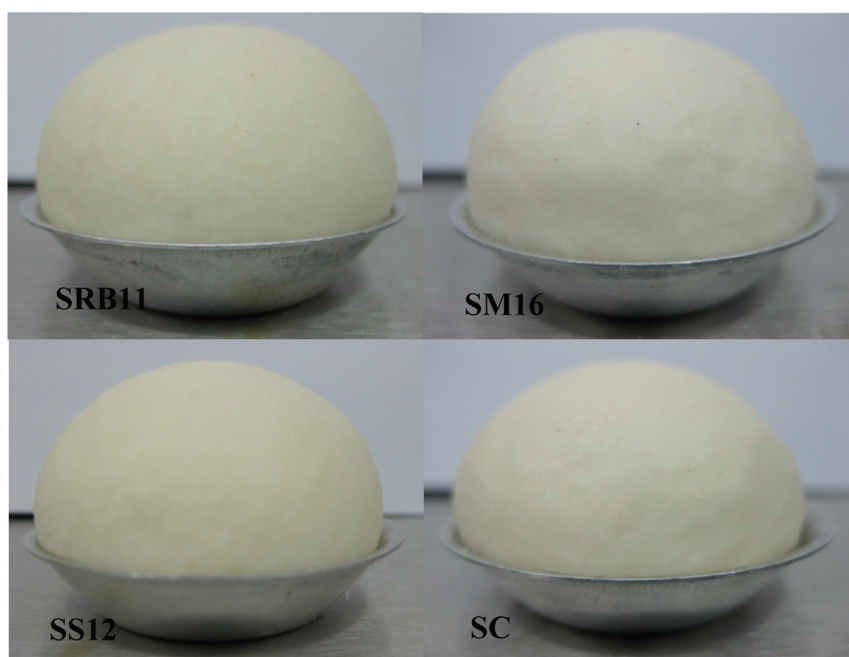


FIGURE 3. Volume of fermented dough by the isolated fruit yeast SRB11 Bamboo shot, SM16 Mango, SS12 Snake fruit and commercial yeast (SC)

TABLE 4. The colour of bread crust developed when different yeasts were used as fermentation agents

Bread Sample	Bread Crust		
	L	a	B
SKS2	68.92±0.45 ^b	8.23±0.26 ^d	29.89±0.27 ^a
SMK9	57.54±1.03 ^c	14.89±0.44 ^a	25.66±0.68 ^c
SDB10	59.46±0.80 ^{cd}	13.62±0.52 ^{bc}	27.10±0.64 ^b
SRB11	57.98±1.53 ^{de}	14.45±0.93 ^{ab}	23.76±0.61 ^d
SS12	57.98±0.61 ^{de}	14.23±0.40 ^{ab}	25.99±0.17 ^c
SM16	60.79±0.60 ^c	12.93±0.23 ^c	27.29±0.43 ^b
SC	58.60±0.53 ^{de}	13.84±0.39 ^b	25.06±0.21 ^c
Negative Control	76.16±0.86 ^a	-0.23±0.12 ^e	13.51±1.19 ^e

L* = lightness, higher values indicate lighter color.

a* = redness. +a = red, - a = green.

b* = yellowness. +b = yellow, - b = blue.

a-e* = mean values with the same letter within a column are not significantly different (p<0.05)

Table 5. The colour of bread crumb developed when different yeasts were used as fermentation agents

Bread Sample	Bread Crumb		
	L	a	b
SKS2	75.58±0.53 ^a	-0.30±0.01 ^c	12.27±0.16 ^a
SMK9	74.89±0.34 ^b	-0.04±0.02 ^b	11.98±0.21 ^{ab}
SDB10	74.03±0.22 ^c	-0.42±0.12 ^c	12.17±0.48 ^a
SRB11	73.09±0.39 ^d	-0.04±0.02 ^b	10.99±0.29 ^c
SS12	71.96±0.33 ^c	-0.14±0.02 ^b	10.95±0.24 ^c
SM16	72.36±0.23 ^c	-0.29±0.07 ^c	11.39±0.33 ^{bc}
SC	73.60±0.28 ^{cd}	-0.66±0.10 ^d	11.27±0.38 ^c
Negative Control	57.22±0.26 ^f	0.23±0.11 ^a	8.35±0.53 ^d

L* = lightness, higher values indicate lighter color.

a* = redness. +a = red, - a = green.

b* = yellowness. +b = yellow, - b = blue.

a-f* = mean values with the same letter within a column are not significantly different (p<0.05)

CONCLUSION

In conclusion, the study reported in this paper indicates that yeast isolated from different local fruits especially the SRB11, SM16, SS12 and SMK9 strains or isolates have potential characteristic as a leavening agent of bread and relatively better than the commercial baker's yeast. The results also confirmed that different sources of isolated yeasts showed different leavening ability of bread and the color development of bread crust and crumb. This may also indicate that different local fruits may provide different environment and source of nutrient for the growth of yeast.

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