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Exploration of Potential Baker's Yeast from Sugarcane Juice: Optimization and Evaluation

[1]A.N.M. Mamun-Or-Rashid, [1]Biplab Kumar Dash, [2]Md. Nurul Abadin Chowdhury,
[3]Momtaz Fatima Waheed and [4]Md. Kamruzzaman Pramanik
[1]Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh
[2]Food Engineering and Technology, State University of Bangladesh, Dhaka, Bangladesh
[3]Radioisotope Production Division, INST, AERE, Bangladesh Atomic Energy Commission
[4]Microbiology and Industrial Irradiation Division, IFRB, AERE, Bangladesh Atomic Energy Commission

Abstract: The present study was carried out to explore baker's yeasts strains from sugarcane juice to assess its potential in laboratory scale production of breads. Collected juice samples were processed for isolation and identification of yeast strains based on standard cultural, morphological and biochemical characteristics. Among the six isolated strains, four (designated as S1, S2, S5 and S6) were identified as *Saccharomyces cerevisiae* and the rests (designated S3 and S4) were as *S. rouxii*. When assessing their CO_2 production rates as a measure of their baking potential, S6 was found to produce maximum amount of gas (226.67 mm^3 mL^{-1}) in sucrose broth, whereas gas produced by S2, S1 and S5 were relatively insignificant (170, 136.67 and 86.67 mm^3 mL^{-1}, respectively). No strain was found to produce undesirable H_2S gas responsible for off-flavor. Besides, effects of different physicochemical parameters (e.g., pH, temperature, substrate concentration, incubation period, agitation etc.) on the production of yeast cell-mass were studied. Yield of cell mass was indirectly measured by spectrophotometric method at 550 nm. All the test isolates were found to produce maximum cell mass at a pH range of 4.0 to 5.0 in 2 to 4% molasses broth at 30°C after 4 days of incubation. In the laboratory scale production of bread using composite flour, Isolate-S6 formed significant characteristic texture. Considering overall characteristics, Isolate- S6 was found to be satisfactorily potent for baking purpose.

Key words: Baker's yeast, *Saccharomyces cerevisiae*, identification, optimization, respirometer

INTRODUCTION

Now-a-days fast foods became very popular in everywhere of the world, Bangladesh is not an exception. Bread is very common fast food in our daily life prepared by baking a dough of flour and water. Salt, fat and leavening agents such as yeast and baking soda are common ingredients, though bread may contain other ingredients to improve the taste. Baker's yeast (*Saccharomyces cerevisiae*) is the common name for the strains of yeast commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol (Hamelman, 2004; Edwards, 2007). The commercial preparation of baker's yeast consists of dried cells of one or more strains of the fungus *Saccharomyces cerevisiae* (Klieger, 2004). It is not known when yeast was first used to bake bread but bread has been prepared for at least 30,000 years. The first records that show this use came from Ancient Egypt (Legras *et al*., 2007).

Baker's yeast is a high volume low value product with more than 1,00,000 tons being produced per year on a global scale (Madigan *et al*., 2003) and its demand is increasing day by day in Bangladesh. Yeast are unicellular fungi or plant-like microorganisms that exist in water, soil, plants, fruits, foods, air, etc. (Spencer and Spencer, 1997). So, there are numerous possibilities to find out one or more wild *S. cerevisiae* strains which have potentiality as baker's yeast to cultivate commercially to meet the annual demand of our country and thus it can play a vital role in our economy by saving foreign currency. Moreover, baker's yeast are natural in born and produce not only high amount of CO_2 but also several proteins, vitamins, minerals and flavoring agents that aid in the overall taste, color and flavor of bread. The present study was designed from this stand point.

Corresponding Author: Biplab Kumar Dash, Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh
Tel: +880 1717 643 365

MATERIALS AND METHODS

Collection of samples: Sugarcane juice sample was collected in sterilized bottles from local market of Savar and allowed to ferment at room temperature for 2 days.

Isolation and culture media: Modified yeast extract malt extract agar (YMA) with combination of glucose (10 g L^{-1}), yeast extract (3 g L^{-1}), peptone (3 g L^{-1}), malt extract (3 g L^{-1}) and agar (15 g L^{-1}), pH 4.5 was used for isolation of yeasts while inhibiting other microorganisms. 0.1 ml dilutes of fermented sugarcane juice (diluted 10^2, 10^4 and 10^6 fold with autoclaved saline water) were plated onto modified YMA media and incubated for 48 h at 30°C to isolate morphologically well-formed single colonies (Qureshi *et al*., 2007). The yeast isolates were further sub-cultured (streak plated) on the respective medium to obtain pure culture.

Identification: The Gram stained yeast isolates were observed under the microscope (Hundwetzlar, Type-H600/12, Germany) to detect cellular shape and for bud and ascospore formation. Colony morphology was noted with respect to growth vigor, size, shape, margin, elevation, pigmentation, opacity and viscosity (Harrigan and McCance, 1982). Flocculent strains were selected by their capacity to sediment at the bottom of the tube at the end of fermentation as described by Verstrepen *et al*. (2001). The biochemical tests were based on the utilization of carbon and nitrogen sources (Harrigan and McCance, 1982; Vaughan-Martini and Martini, 1993; Sanni and Lonner, 1993), utilization of ethanol as sole carbon source and formation of ascospore (Harrigan and McCance, 1982).

Potency Assessment of identified *S. cerevisiae* for bread production

Production of undesirable compound: The six isolates were stab cultured at Bismuth Sulfite Agar, Kligler Iron Agar (KIA) and Sulfide Indole Motility (SIM) Agar and incubated at 30°C for 30 days and check every day for the capacity to produce H_2S. Butt that exhibit black color are H_2S producers (Jiranek *et al*., 1995).

Measurement of gas production: The production of gas was measured by a special apparatus made by attaching a vial in one end of a silicon adaptor and a 1 mL pipette containing 50 μL mercury at its tips on the other end. The vials were kept in a handmade rack where the pipettes were parallel to ground (to neglect gravitational movement) to confirm the movement of mercury by gas produced by the test microorganisms.

Optimum pH: Yeast strains were cultured in molasses broth of pH 3, 3.5, 4, 4.5, 5 and 5.5 containing 4% (w/v) molasses. Tubes containing 10 mL of these solutions were inoculated with same number of actively grown yeast cells (approximately 3.86×10^5 cells mL^{-1}) and incubated at 30°C for 4 days. Optimum pH for growth and maximum biomass production was detected by measuring optical density at 550 nm using spectrophotometer (Shimadzu A-1075, Japan).

Optimum molasses concentration: Yeast strains cultured in molasses broth of pH 4.5 (previously determined as optimum) containing 2, 4, 8, 12, 16 and 20% (w/v) molasses, were inoculated with same number of actively grown yeast cells (approximately 3.86×10^5 cells mL^{-1}) and incubated at 30°C for 4 days. Optimum molasses concentration for maximum growth and biomass production were detected by measuring optical density at 550 nm using spectrophotometer.

Optimum temperature: Yeast strains cultured in molasses broth of pH 4.5 containing 4% (w/v) molasses (previously determined as optimum), were inoculated with same number of actively grown yeast cells (approximately 3.86×10^5 cells mL^{-1}) and incubated at 25, 30 and 37°C for 4 days. Optimum temperature for growth and maximum biomass production were determined by using spectrophotometer at 550 nm.

Incubation period: Yeast strains cultured in molasses broth of pH 4.5 containing 4% (w/v) molasses, were inoculated with same number of actively grown yeast cells and incubated at 30°C (previously determined as optimum) for 6 days. After incubation of each day, optical density was measured at 550 nm by using a spectrophotometer. The broth was vortex, serially diluted up to 10^6 fold and spread plated to count viable cells (CFU mL^{-1}). After centrifugation pellets were weighted to determine cream cell mass and dried at 105°C in a heat dryer until constant weight to get dry cell mass.

Determination of agitation's effect: Yeast strains cultured in molasses broth of pH 4.5 containing 4% (w/v) molasses, were inoculated with same number of actively grown yeast cells and incubated at 140 rpm and 30°C (previously determined as optimum). After 4 days centrifuged pellets were weighted to determine biomass production.

Preparation of bread from composite flour and cultivated yeast isolates: Bread was prepared with isolate S1 and S6 to observe the baking potency, using conventional method as described by Aboaba and Obakpolor (2010). Baking potency was determined by measuring the percentage of volume increment.

Statistical analysis: The experiments were performed in triplicate. Standard deviations were less than 3% in all experiments.

RESULTS

Six single colonies were selected on the basis of their morphological differences in YMA plates designated as S1, S2, S3, S4, S5 and S6, respectively. Among them, four (designated as S1, S2, S5 and S6) were identified as *Saccharomyces cerevisiae* and two as *Saccharomyces rouxii* by analyzing standard cultural, morphological and biochemical characteristics (Table 1-3).

Four identified *S. cerevisiae* strains were undergone several tests to determine their potency as baker's yeast. None of them found to produce undesirable H_2S. In case of assessment of CO_2 production by the *S. cerevisiae* strains, S6 was found to generate highest amount of gas (226.67 $mm^3 mL^{-1}$) whereas S5 produced about 62% lower CO_2 (Fig. 1).

Molasses broth was found to support better growth of yeast strains than sucrose broth. Highest OD for all the *S. cerevisiae* strains were found in molasses broth likely

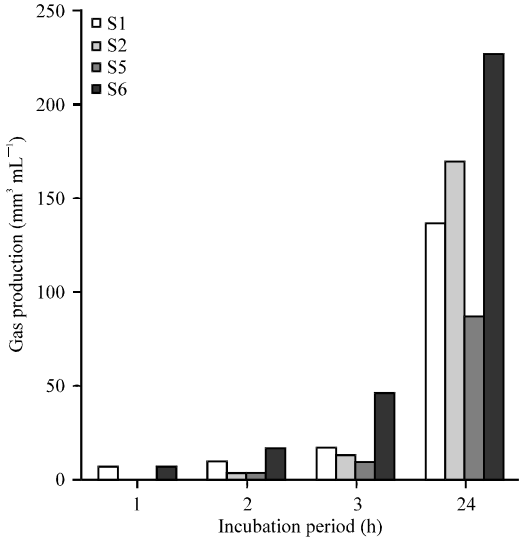

Fig. 1: Gas production by test isolates

Table 1: Cultural (colony) characteristics of isolated yeast strains

Isolates	Shape	Colony color	Opacity	Elevation	Surface	Edge	Consistency	Growth in liquid media
S1	Ci	CW	O	Co	S	L	V	SG
S2	Ci	CW	O	Co	S	L	V	SG
S3	Ci	Cr	O	Um	S	Un	V	ALG
S4	Ci	CW	O	Co	S	E	V	SG
S5	Ci	CW	O	Co	S	L	V	SG
S6	Ci	CW	O	Co	S	L	V	SG

Ci: Circular, Cr: Creamy, CW: Creamy white, O: Opaque, Co: Convex, Um: Umbonate, S: Smooth, E: Entire, L: Lobate, Un: Undulate, V: Viscid, ALG: Ring growth in Air-Liquid-Glass junction, SG: Sediment growth

Table 2: Morphological characteristics of isolated yeast strains

Isolates	Cell shape	Bud	Ascospore
S1	Oval, unicellular	+	-
S2	Oval, unicellular	+	-
S3	Elongated, unicellular	+	-
S4	Spherical, unicellular	+	-
S5	Oval, unicellular	+	-
S6	Oval, unicellular	+	-

'+' indicates present and '-' indicates absent

Table 3: Biochemical characteristics of test yeast isolates

	Isolates					
Parameters	S1	S2	S3	S4	S5	S6
Glucose	+	+	+	+	+	+
Fructose	+	+	w	+	+	w
Sucrose	+	+	w	w	+	+
Maltose	+	+	-	w	+	+
Galactose	+	+	-	w	+	+
Lactose	-	-	-	-	-	-
KNO_3	-	-	-	-	-	-
$(NH_4)_2SO_4$	+	+	+	+	+	+
Urease	-	-	-	-	-	-
Ascospore	-	-	-	-	-	-
Ethanol	+	+	+	+	+	+
Suggested strains	*S. cerevisiae*	*S. cerevisiae*	*S. rouxii*	*S. rouxii*	*S. cerevisiae*	*S. cerevisiae*

"+" indicates highly positive, "-" indicates negative and "w" indicates weak acid and gas production and/or growth

Table 4: OD at 550 nm of culture broth of different pH

		OD after 4 days of incubation					
Isolates	Inoculation OD	At p^H 3.0	At p^H 3.5	At p^H 4.0	At p^H 4.5	At p^H 5.0	At p^H 5.5
S1	0.026	2.323	1.856	2.290	2.451	1.884	2.301
S2	0.012	2.290	1.845	2.280	2.107	2.301	2.045
S5	0.016	2.290	1.892	2.280	2.323	2.165	2.189
S6	0.015	2.232	1.868	2.358	2.197	2.022	2.334

Table 5: OD at 550 nm of culture broth of different molasses concentration

		OD after 4 days of incubation					
Isolates	Inoculation OD	2% molasses	4% molasses	8% molasses	12% molasses	16% molasses	20% molasses
S1	0.026	1.959	2.301	2.232	1.970	1.389	1.601
S2	0.012	2.173	2.165	1.724	1.834	1.649	1.448
S5	0.016	2.181	2.323	2.088	2.069	1.909	1.688
S6	0.015	2.165	2.251	2.135	1.989	1.713	1.519

Table 6: OD after different incubation period

		Optical Density at 550 nm					
Isolates	Inoculation OD	After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	After 6 days
S1	0.026	1.497	1.922	2.011	2.451	2.232	2.591
S2	0.012	1.557	1.954	2.121	2.358	2.189	2.323
S5	0.016	1.612	2.016	2.135	2.422	2.334	2.498
S6	0.015	1.632	1.864	2.232	2.436	2.358	2.370

Table 7: Cream cell mass mL^{-1} after different incubation period

		Cream cell mass mL^{-1} (mg)					
Isolates	Inoculation CFU mL^{-1}	After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	After 6 days
S1	7.8×10^4	11.90	17.68	20.78	27.22	25.80	33.14
S2	8×10^4	14.04	20.46	22.82	27.00	25.28	27.96
S5	8.5×10^4	14.48	20.87	22.66	29.12	26.08	25.00
S6	8.3×10^4	12.78	18.60	27.32	29.68	27.24	26.20

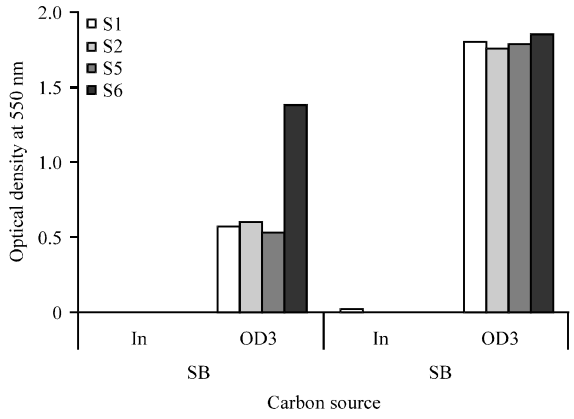

Fig. 2: Growth in different carbon sources, all the test isolates showed much better growth in molasses broth than sucrose broth ('In' for Inoculation, 'OD3' for Optical Density after 3 days, 'SB' for Sucrose Broth and 'MB' for Molasses Broth)

1.81, 1.771, 1.803 and 1.864 for S1, S2, S5 and S6, respectively (Fig. 2) which were comparatively very low in sucrose broth (0.583, 0.607, 0.548 and 1.393, respectively). *S. cerevisiae* S1 strain showed the maximum cell density (2.451) at pH 4.5. The growth of other test isolates were somehow similar and found to be 2.301, 2.323 and 2.358 for S2, S5 and S6 in pH 4.5, 5.0, 4.5 and 4.0, respectively (Table 4). Very good growth patterns of yeast isolates were observed for *S. cerevisiae* strains at 2 to 4% of molasses broth. The best cell density was achieved for S5 strain (2.321) at 4% molasses broth. Among others, S5 had better cell density (2.173) at 2% of molasses broth (Table 5). Optimum temperature for growth was found 30°C for all the *S. cerevisiae* strains. It was found that 25 and 37°C suppress cellular growth significantly (Fig. 3). In case of optimization of incubation period, highest OD were found 2.591 and 2.498 for S1 and S5, respectively after 6 days of incubation at 30°C (Table 6). Cream cell mass mL^{-1} (Table 7) was 33.14 and 27.96 mg for S1 and S2, respectively after 6 days and 29.12 and 29.68 mg for S5 and S6, respectively after 4 days of incubation. Dry cell mass mL^{-1} (Table 8) was 4.94 and 4.16 mg for S1 and S2, respectively after 6 days and 4.20 and 4.54 mg for S5 and S6, respectively after 4 days of incubation. However, maximum CFU mL^{-1} (Table 9) was found 6.76×10^9, 5.28×10^9, 1.528×10^{10} and 7.68×10^9 for isolates S1, S2, S5 and S6, respectively after 4 days of incubation. All the test isolates showed much better production in shake culture than non-shake culture. S1, S2, S5 and S6 produced highest biomass of 84.2, 72.6, 81.4 and 68.8 mg mL^{-1} in shake culture whereas it was 27.22, 27, 29.12 and 29.68 mg mL^{-1}, respectively in non-shake condition (Fig. 4).

Table 8: Dry cell mass mL^{-1} after different incubation period

Isolates	Inoculation CFU mL^{-1}	Dry cell mass mL^{-1} (mg) After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	After 6 days
S1	7.8×10^4	0.96	2.08	2.34	3.84	3.52	4.94
S2	8×10^4	1.46	2.70	2.90	3.80	3.40	4.16
S5	8.5×10^4	1.48	2.86	3.04	4.20	3.52	4.04
S6	8.3×10^4	1.46	1.98	3.54	4.54	3.68	3.50

Table 9: CFU mL^{-1} after different incubation period

Isolates	Inoculation CFU mL^{-1}	CFU mL^{-1} After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	After 6 days
S1	7.8×10^4	5.664×10^8	4.9×10^8	9×10^8	6.76×10^9	6.08×10^7	1.6×10^7
S2	8×10^4	1.71×10^7	2.64×10^9	3.2×10^9	5.28×10^9	2.8×10^7	3.0464×10^7
S5	8.5×10^4	1.94×10^7	1.55×10^9	6.5×10^9	1.528×10^{10}	9.92×10^7	3.136×10^7
S6	8.3×10^4	1.99×10^7	1.45×10^9	3.4×10^9	7.68×10^9	7.84×10^7	3.36×10^7

Table 10: Leavening profiles of dough using *S. cerevisiae* S1 and S6 strain

Samples	Fresh S1	Fresh S6
Amount (g)		
Yeast	2.4	2.4
Dough	356	356
Incubation		
Temperature	Room	Room
Time	30 min	30 min
Increased dough volume (%)	102	111
Baking		
Temperature	180°C	180°C
Period	1.5 h	1.5 h
Increased bread volume (%)	118	128
Color	Characteristics	Characteristics
Texture	Bad	Bad
Taste	Poor	Poor
Flavor	Pleasant	Pleasant
Mouth feeling	Poor	Poor

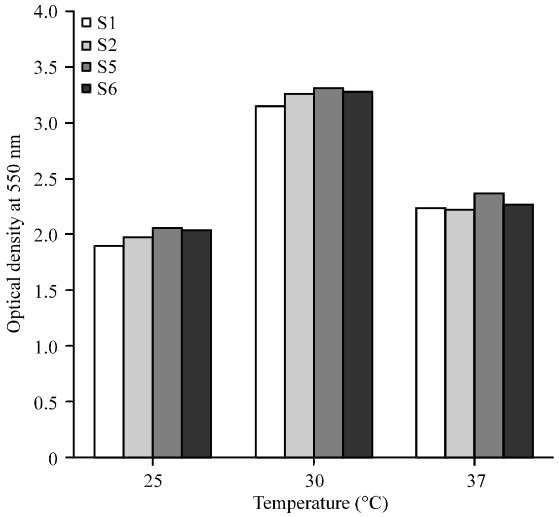

Fig. 3: OD at 550 nm of culture broth incubated at different temperature

The strain S6 showed comparatively good baking potential than S1. S6 was relatively good in producing flavor and color of bread baked at 180°C for 1.5 h although bread texture, taste and mouth feeling were not uncourageous (Table 10).

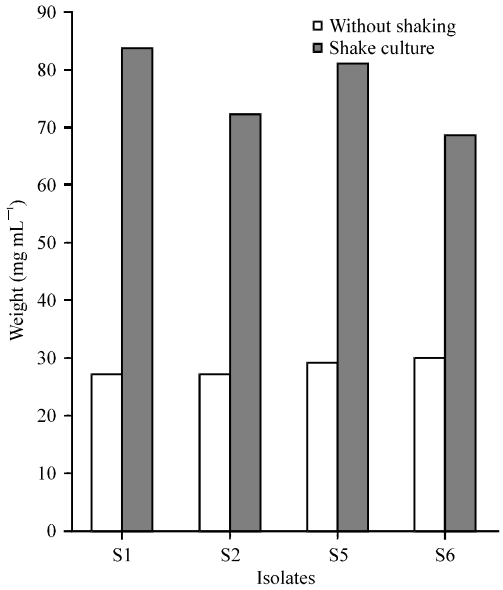

Fig. 4: Effect of shaking on biomass production by test isolates. Biomass was produced much better in shake culture than non-shake culture by all the test isolates

DISCUSSION

Successful commercial production of baker's yeast depends on the availability of cheap suitable carbon source, good yeast strain, easily manageable fermentation process for maximum viable biomass production and the process development for preservation. Beside this the production efficiency of a strain depends on many factors such as nature, composition and concentration of substrate, temperature, pH, inoculums size, incubation period etc. (Supanwong *et al*., 1983). Selection of optimum cultural and fermentation conditions with maximum viable biomass production and later gas production at a

moderate rate is mandatory for natural yeast isolates to be used as an effective commercial baker's yeast.

Baker's yeast act as a natural leavener, or rising agent of baked bread by producing CO_2 gas during the time of dough incubation. In our experiment, *S. cerevisiae* S6 strain was found to be the highest gas producer among four isolates. H_2S is an undesirable compound associated with an off-flavor and unpleasant taste that must be absent in processed foods (Ribeiro and Horii, 1999; Vicente *et al*., 2006). Production of H_2S by test isolates was checked and no strain found to produce H_2S.

The cost of baker's yeast production depends mostly on the medium carbon source used. Molasses containing about 60% sucrose (Patrascu *et al*., 2009) was found to be better carbon source than pure sucrose by measuring the max cell biomass yielded in the respective culture media. It may be due to the presence of some important growth promoting substances likely vitamins, minerals and other natural molecules in molasses broth that enhance yeast growth significantly. This finding indicates the use of molasses as an effective cheap and widely available carbon source for baker's yeast production. Qureshi *et al.* (2007) reported low pH to be helpful for inhibiting the growth of undesirable microorganisms in large scale fermentation. All the test *Saccharomyces cerevisiae* strains grown well at a pH range of 4.0 to 5.0 that is suitable to resists contamination by unwanted microbes.

Cream cell mass and dry cell mass were maximum for S1 and S2 after 6 days and S5 and S6 after 4 days of incubation. However, all the test isolates produced maximum CFU mL^{-1} after 4 days of incubation and falls down drastically after 4 days. This might be due to accumulation of waste products, high cell concentration and depletion of nutrients that cause rapid demise of yeast cell after given time period. As maximum cell density was found before the death phase begins, fed-batch or continuous culture could be used effectively for the production of baker's yeast instead of batch culture that initiates ethanol production. Four days of incubation seemed to be suitable/optimum period of incubation when highest number of viable cell with lowest cell weight was obtained.

Optimization of relevant growth parameters is important to obtain maximum production of yeast cell biomass. The biomass and viable cell production rate by *S. cerevisiae* S6 was significantly higher than the other yeast strains. Aeration and agitation are two important parameters influenced the production of cell mass reported by Ivanova *et al.* (2001). In all the test, *S. cerevisiae* stains showed two to three fold better biomass production in shake than non-shake culture. This might be happened as shaking ensures the availability of nutrients and oxygen for all the cells and thus confirms maximum biomass production within minimum period of time (Narang and Satyanarayana, 2001).

Aboaba and Obakpolor (2010) found increased dough volume with increased agitation time but only to a limited extent as longer period (40 min) caused a drastic drop in dough volume. The period for mixing therefore is important as it influences the size and quality of bread that enhances the ability of the dough to trap more air. About 111% increase in bread size was observed after mixing *S. cerevisiae* S6 strain followed by incubating the dough for 30 min. Baking at 180°C for 1.5 h found to increase the bread volume to about 128%. Although, the color and flavor of baked bread were somehow better, the texture, taste and mouth feeling were poor.

CONCLUSION

The present study found *S. cerevisiae* strain S6 as potent candidate to be used as baker's yeast. Although in some instances it showed insignificant activity that can be overcome by adding some chemical food additives when producing bread. Further research should be carried out to modify *S. cerevisiae* strain S6 genetic level by radiation, chemical mutagen or genetic incorporation to overproduce the enzymes responsible for breaking down dough sugar into ethanol and carbon-dioxide and thereby increasing its bread making efficiency.

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