LEAVENING ABILITY OF SOME WILD YEASTS AND THE MUTANT SPECIES ISOLATED FROM FERMENTED ORANGE JUICE IN BAKERY PRODUCT (BREAD)

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Abstract: The characterization of the indigenous yeast flora was carried out using analytical profile index techniques (API AUX 20C kit), physiological characterization and genetic modification by mutation (nitrous acid and ultra violet irradiation treatment). The physiological characterization involved the testing of several parameters, which include sugar fermentative ability that is growth of yeast in 50% glucose, 3% and 10% sodium, elevated temperatures of 30, 35, 37 and 42ºC. The isolates for dough leavening ability of bread were identified as *Kodamaea ohmeri, Candida norvegensis, Candida zeylanoides, Geotrichum capitatum* and *Rhodotorula minuta.* The leavening capacities of wild strains, nitrous acid-induced mutants and the control commercial baker's yeast ranged from (75-275 ml), (84-275 ml) at 48 h and (98-280 ml) at 180 min and (98-130 ml) at 48 h for the control sample, respectively. Based on the physiological attributes and mutagenicity of the wild yeast strains for dough leavening, the result obtained showed that the mutants of sweetened dough (nitrous acid) had a better leavening capacity than that of the unsweetened dough, while for the ultraviolet irradiated mutants unsweetened dough had a better leavening capacity than that of sweetened dough. However, some species of the wild yeast strains, *Kodamaea ohmeri, Candida norvegensis* and *Rhodotorula minuta* had an outstanding dough leavening capacity both in sweetened and unsweetened dough when compared with the commercial baker's yeast. Thus, this study has enabled the acquisition of data on the basic physiological and mutagenic pattern of yeasts required for industrial production of higher dough leavening capacity with regard to their use in bakery products.

Keywords: Baker's yeast, dough fermentation, mutants, fermented orange juice, wild yeasts

Introduction

The use of yeast to make bread and alcohol has been recorded for thousands of years. The Babylonians 6000 BC and Egyptians 5000 BC have left written account of their production of beer, wines and bread, where all of them warranted the use of yeast (Kevin, 2005; Mararuf *et al*., 2011). Yeast especially *Saccharom cycescerevisiae* is known as sugar-eating fungus and can be found naturally from the surrounding. Kurtzman and Fell (1998) stated that fruits, vegetables, drinks and other agricultural products are very important microhabitats for various yeast species. Succession of yeast populations in such products involves variety of biochemical processes carried out by yeast to utilize simple sugars present in the agricultural products.

Several studies have been carried out in different natural and crop-growing environments to obtain better knowledge of yeast biodiversity and to define the impact of this on food products such as fresh orange fruits and juices (Francisco *et al*., 2002), and in crop-growing environment in Cameroon (Marzia *et al*., 2008). There are different sources for isolation of yeast species however, their presence, were reported mostly from the acidic foods. Among them, citrus juice (Arias *et al*., 2002; Obasi *et al*., 2014), dahi (Savova and Nikolova, 2002) and sugarcane juice are considered to be the best. Citrus juices are acidic beverages (pH3-4) with high sugar content (15ºBrix) and typical yeast species found in citrus juice are *Saccharomyces exugus* and *Saccharomyces ludwigii.* Other species include *Candida parapsilosis, Candida Stellata, Torulaspora delbrukii, Geotrichum capitatum, Kodamaea ohmeri, Zygosaccharomyces rouxii* and the species from the genus *Rhodotorula*, *Pichia, Hansenispora* and *Metschickowia* (Hatcher *et al*., 2002; Obasi *et al*., 2014).

In bread-making, fermentation by yeasts is of primary importance for its leavening function and possible contribution to the production of desirable flavor compounds. France and Wragg (1998) listed four functions of yeast in bread-making: (1) to increase dough volume by evolution of carbon oxide during fermentation of 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 to bread.

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 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 other indigenous strains such as *P. membranificiens* which can only ferment glucose.

Today's baker's yeast is used for bread manufacturing throughout the world at industrial scale. With the improvement of bread industry, the use of starter culture increased tremendously. Development of improved starter organisms for fermentation of citrus fruits in low utilization environment, and places where the production of citrus concentrates is low or non-existence is required. Studies have shown that microorganisms mutated with physical and chemical treatments exhibited higher-yielding, faster growing, tolerant of less oxygen, and able to use a more concentrated medium (Wang *et al*., 1980).

The purpose of any leavening is to produce the gas that makes bread to rise during fermentation. Yeast does this by

feeding on sugars in flour, and expelling carbon dioxide in the process. This carbon dioxide from yeast fills thousands of balloon-like bubbles in the dough .Once the bread is baked, that is, what gives the loaf its airy texture (Ali *et al*., 2012). At present, the leavening agents (yeasts) currently used in Nigerian bakery industries are mostly imported. The presence of yeast from citrus fruits (orange juice) is yet to be exploited, especially in bakery products as leavening agent. There is no report on the use of yeast isolated from Nigerian citrus fruits that has potential as a leavening agent in bread making .So there is a dire need to explore the potential of indigenous strains other than *S. cerevisiae* (baker's yeast) in order to meet the national requirements and to save the foreign exchange.

Thus, the present study was carried out to determine the leavening ability of wild yeast and genetically manipulated yeast isolated from fermented orange juice. In addition, a brief physiological test was performed in order to have a better understanding of the yeast behaviour in bread making.

Materials and Methods

Sample collection

The wild yeasts strains of *Candida norvegensis, Candida zeylanoides, Geotrichum capitatum, Kodamae ohmeri, Geotrichum capitatum,* and *Rhodotorula minuta* used in this study were obtained from fermented single- strength orange juice from sound and defective orange fruit. The techniques of Teramoto *et al*. (2005) were used in isolation of the organisms. This involved using a medium containing potato dextrose agar (Oxoid), 3.5% (w/v), streptomycin 1.0% (w/v), and sodium propionate, 0.5% (w/v). A 2-stage modification mutation technique was carried out on the yeast species by the use of nitrous acid and ultra violet irradiation at 254 nm according to Ado (2004). These mutants were used in dough leavening because of its high fermentative capacity.

The dough leavening process

The test was carried out in two phases: First, sweetened and second unsweetened dough leavening test. The method used was as described by Ricon *et al. (*2001) and Brookline (2004).The test material consisted of 80% (w/v) wheat flour, 16% (w/v) sucrose, 1% (w/v) sodium chloride (salt), 1% (w/v) vegetable oil, and 2.0% commercial bakers instant dry yeast. All were purchased from a local market in Zaria, Kaduna State. The laboratory harvested cream yeast 2% (w/v) (wild and modified) siphoned from the medium was used for the fermentation of the dough. Thirty percent (30%) and 40% (v/v) sterile distilled water was used for mixing the sweetened and unsweetened dough respectively. The ingredients (wheat flour, sugar, salt, vegetable oil and yeast) were mixed and the dough kneaded until it was smooth. Then it was deposited at the bottom of 250 ml sterile measuring cylinders whose sides had been smeared with a layer of vegetable oil to prevent the dough from sticking to the sides of the cylinders. The cylinders were labeled, sealed with foil paper and incubated at 30^{o}C for 3, 24 and 48 h, respectively. The volumes of the dough were recorded at zero hour and thereafter for every 30 min for 3, 24 and 48 h (180, 1440 and 2880 min), respectively. The fermentation time was extended from 3 - 48 h because the laboratory yeast did not leaven the dough at 3 h interval as was observed in the control strain (commercial baker's yeast). The unsweetened dough was prepared as stated above except that sucrose was not added. The dough leavened with baker's yeast served as the control. Proof heights of each of the fermented dough are taken as the difference between the final dough volume and the initial reading taken at zero minute.

Fermentative ability of the yeast strains

The following parameters were used to characterize the yeast for attributes important in dough-leavening capacity:

Growth of yeast in 50% glucose test for yeasts

The yeast isolates were grown on a basal agar medium of 50% (w/v) glucose, 1.0% (w/v) yeast extract (Oxoid), 0.75% (w/v) peptone (LABM) and 2% (w/v) agar (Oxoid), incubated at room temperature (26– 28^{o}C) and examined for growth within four to seven days.

Growth of yeast at elevated temperatures

Yeast strains were streaked on potatoes dextrose agar plates and incubated at 30, 35, 37 and 42^{o}C, respectively. Observation for growth was made after 48-72 h of incubation.

Growth of yeast in 3 and 10% sodium chloride

The medium that was used to observe growth on sodium chloride (NaCl) was composed of 3% and 10% (w/v) sodium chloride, 3.0% PDA, 1.0% (w/v) yeast extract (Oxoid), 0.75% (w/v) peptone and 2.0% (w/v) agar (Oxoid) The sterile medium was dispensed into sterile petri dishes and allowed to set. Yeast strains were streaked on agar surface and examined for growth after 4-7 days of incubation (26 – 28^{o}C).

Propagation of yeast species for use in dough leavening

The yeast isolate was propagated using the procedure of Ameh and Umaru (2000) with some modifications. This involved the use of UV irradiated and nitrous acid treated mutants of yeast. The propagation was carried out in 250 ml Erlenmeyer flasks each containing 150 ml basal medium composed of glucose (2% (w/v); yeasts extract (Oxoid) (0.5% (w/v); peptone (LABM) 1% (w/v); ammonium tetraoxosulphate VI $(NH_4)_2SO_4$), 0.1% (w/v) and magnesium tetraoxosulphate VI ($MgSO_4$) 0.1% (w/v). The pH of the medium was adjusted to 5.6 using few drops of 1.0N HCl prior to autoclaving. The sterilised medium was inoculated with 3.0 X 10^9 cells/ml wild and modified yeasts strains and incubated on a shaker at an agitation rate of 150 rpm at 30^{o}C for 7 days. The cells were harvested by centrifugation and rinsed twice with sterile distilled water. The cream yeast obtained after decanting supernatant was stored in refrigerator for approximately 24 h before use.

Statistical analyses

Data obtained from the study were subjected to statistical analyse using statistical package social sciences (SPSS) version 20. The results of dough leavening test was analysed using ANOVA and paired T-test to determine the mean performance between the baker's yeast strain, the wild strains and the mutants of nitrous acid-induced and UV light irradiation on sweetened and the unsweetened dough. All tests were conducted at 5% significant level.

Results and Discussion

Fermentative capacities and temperature profile

Table 1 shows the result of the yeast strains most of which exhibited high fermentation rates and releasing carbon dioxide gas as observed in Durham tube. The gas (CO_2) produced during baking is responsible for the increase in volume and porosity of the bread. Hence, a good baker's yeast is expected to liberate gas from sugars. Majority of the yeast strains isolated from the study were found to produce gas vigorously (Table 1). This could be an important indication of invertase activity and an important feature for strains used in dough or bread making. This result is in agreement with the report of Ma'aruf *et al*. (2011).

The temperature can affect the fermentation process and the metabolism of yeast. Table 1 illustrates the growth rate of the isolates at the different growth temperatures. The ability of all the yeast strains to survive at high temperatures 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. This result is in agreement with the report of other workers (Oke and Ijebor, 1997; Ma'aruf *et al*., 2011).

Growth in 3 and 10% NaCl

Since a pinch of sodium chloride forms one of the basic ingredients of bread, it is expected that baker's yeast should tolerate as much as 3% NaCl (Bell *et al.*, 2001). It is however, inhibited by a large quantity as much as 10% depending on the type of yeast. All the yeasts tested had positive growth in 3% NaCl but most of the yeasts were inhibited in 10% (Table 1).

Growth in 3% ethanol

The strains tested were able to grow in 3% ethanol, but evaporated during baking. It is expected that the growth of baker's yeast should not be inhibited by the presence of 3% ethanol. Since the yeast strains isolated in this study possess this important characteristic, they can be used for baking (Table 1).

Dough leavening capacity of the wild, modified and baker's yeast strains for sweetened and unsweetened dough

The isolated yeast strains having proved to possess markers peculiar to baker's yeast were tested for their fermentative ability in dough leavening both for sweetened and unsweetened types. The results showing the leavening capacities of the yeasts strains of wild, mutant (nitrous acid) and the control commercial baker's yeast are presented in Figs. 1 and 2, respectively. The proof heights of the sweetened dough fermented with the wild yeasts ranged from (108 - 220 ml), (27 - 193 ml), nitrous acid induced mutants (45 – 185 ml) and the commercial baker's yeast (32 – 182 ml) (Figs. 2 and 4). The rate of fermentation for the yeast strains of both wild and nitrous acid induced showed a uniform pattern since all of them showed lagging periods in the course of the 180 min and subsequent rise from 24-48 h of incubation periods (Figs. 1 and 3). The baker's yeast used as the control also showed a lagging period and subsequent rise within the 180 minutes and declined from 24-48 h of incubation periods (Fig. 4). The leavening capacities of wild strains, nitrous acid induced mutants and the control commercial baker's yeast (*Saccharomyces cerevisiae*) measured as increase in dough volume or leavening abilities ranged from (75 – 275 ml) (84 – 275 ml) at 48 h and (98 – 280 ml) at 180 min and (98 – 130 ml) at 48 h for the control sample respectively (Figs. 1 and 3). The commercial baker's yeast in both sweetened and unsweetened dough fermented the dough faster than the wild and the mutants from zero to 180 minutes. The wild type and mutants were slower and fermented the dough from zero - 48 h. The baker's yeast showed a better leavening activity at 180 min and declined from 24 - 48 h in leavening activity from 280 – 130 ml. The wild and mutant strains also exhibited a good performance of leavening from 24 - 48 h and the best result was obtained at 48 h of incubation periods (Figs. 1 and 2).

The wild type and mutants of yeast obtained in this study showed a slower rate of dough fermentation when compared with the control, a commercial baker's yeast (*Saccharomyces cerevisiae*), within 180 min of incubation (Figs. 1 and 3). From 24 - 48 h, however, a fast dough fermentation period occurred. This could be attributed to the lag phase of growth, size of inoculum and rate of carbon (iv) oxide production by the different yeast strains used. Studies have shown that yeast produces carbon (iv) oxide from the sugar present in the dough and the more yeast available for the sugar, the more carbon (iv) oxide produced which in turns make the dough fermentation faster and vice versa (Plyer, 1988; Ana *et al*., 2001; Rao and Asoke, 2006). Moreover, the baker's yeast used as the control also contains fast active ingredients as against the cream yeast which was used in this study.

The sweetened or sugar dough showed a better leavening capacity than the unsweetened dough for almost all the wild type, nitrous acid induced mutants and the control, the commercial baker's yeast (Figs. 2 and 4). However, there was a remarkable improvement in the leavening capacity of wild type *Rhodotorula minuta* on unsweetened or plain or lean dough (Fig. 5). The addition of sucrose in sweetened dough provided additional source of energy and nutrient, thereby producing more carbon (iv) oxide from fermentation of the sugar and the rising of the dough. Also an important trait that influences the baking performance of yeast in sweet dough fermentation is the level of invertase activity (Evans, 1990). This enzyme catalyzes the hydrolysis of sucrose into glucose and fructose as rapidly as possible, increasing osmotic pressure, thereby enhancing the leavening of the dough fermentation. It is also noteworthy that the isolate of the wild type *Kodamaea ohmeri* showed a higher leavening ability when compared with the control (the commercial baker's yeast, *Saccharomyces cerevisiae*) in both sweetened and unsweetened dough (Figs. 4 and 5). This report is in agreement with the studies of other workers, where they reported yeasts such as *Issatchenkia orientalis*, *Pichiamembranaefaciens* and *Torulaspora delbrueckii* showed higher dough leavening abilities than *Saccharomyces cerevisiae* (Almeida and Pais, 1996; Harnandez-Lopez *et al*., 2003).

Table 1: Performance of yeast strains with respect to the markers

Yeast strains	Fermentative ability	Growth in		Growth in	Growth at elevated temperatures				Dough Leavening Ability	
		3% NaCl	10% NaCl	50% glucose	30ºC	35ºC	37ºC	42ºC	Sweetened	Unsweetened
Instant dried yeast (1DY) (*S. cerevisiae*)	**	+	–	+	+	+	+	–	+	+
C. norvegensis	*	+	+	+	+	+	+	+	+	+
C. zeylanoides	**	+	–	+	+	+	+	+	+	+
G. capitatum	*	+	–	–	+	+	+	+	+	+
K. ohmeri	**	+	–	+	+	+	+	+	+	+
R. minuta	*	+	+	+	+	+	+	+	+	+

** = Fermentation with gas production; * = Fermentation without gas production; + = Positive result; – = Negative result

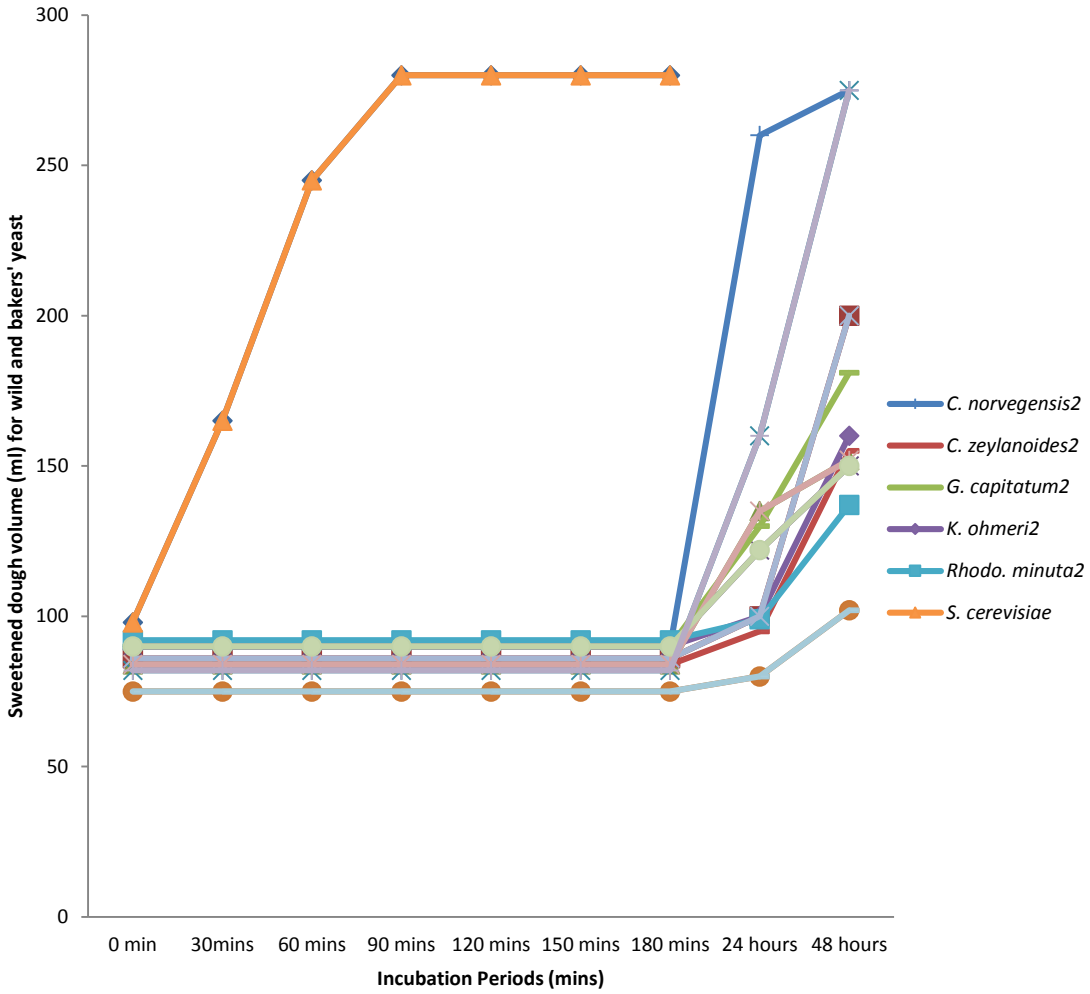

Fig. 1: Leavening profiles of wild strains and baker's yeast sweetened dough

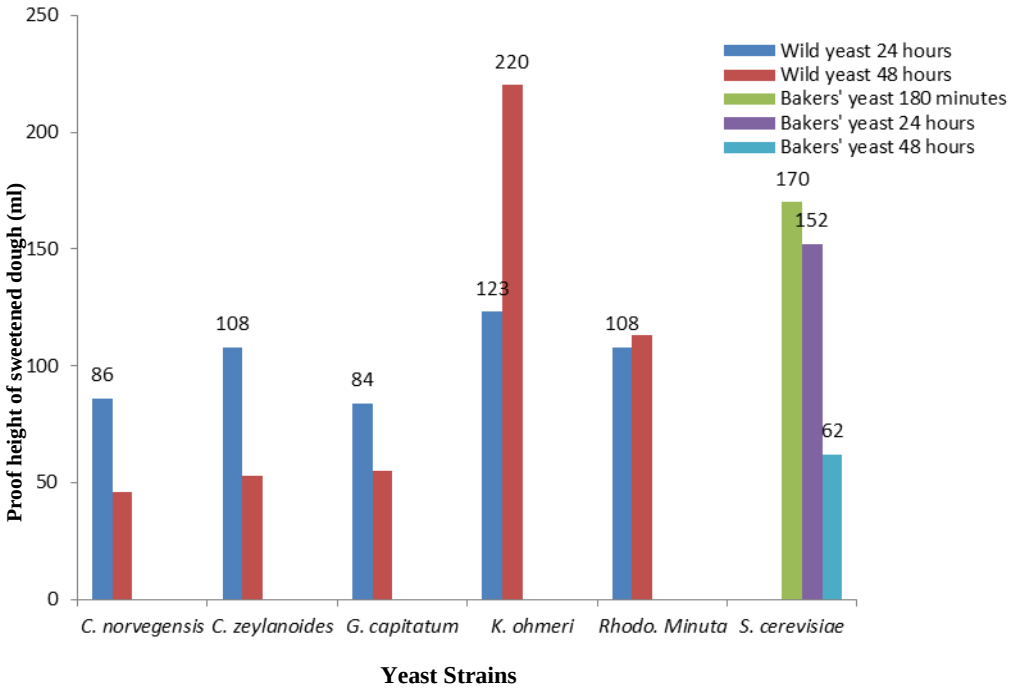

Fig. 2: Proof heights of wild strains and baker's yeast sweetened dough

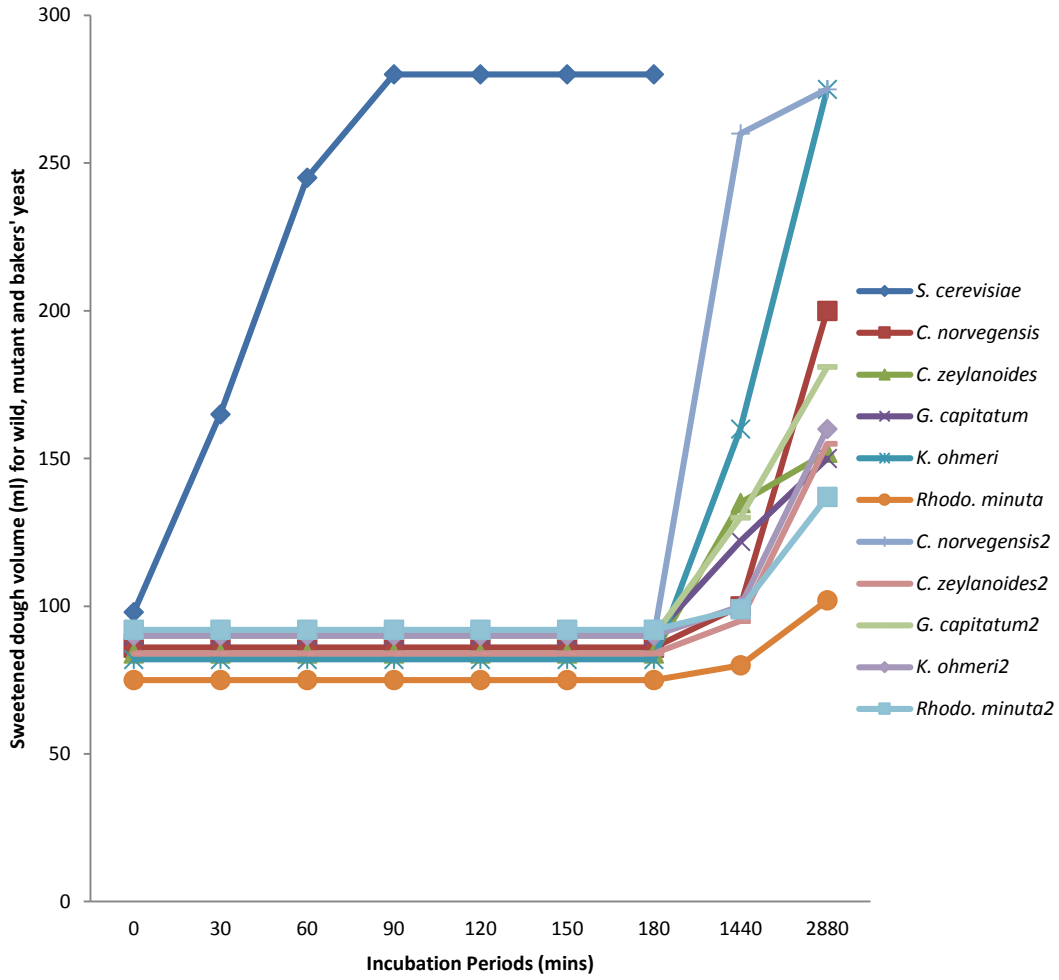

Fig. 3: Leavening profiles of wild, mutant (nitrous acid) treatment and baker's yeast in sweetened dough

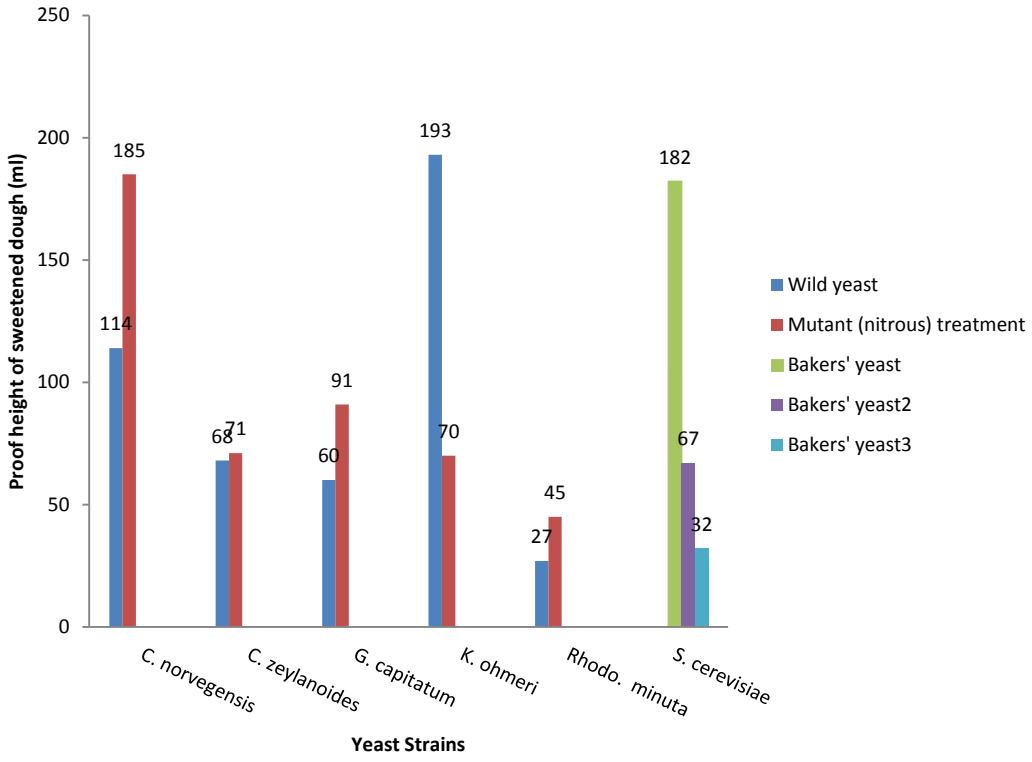

Fig. 4: Proof heights of sweetened dough fermented with wild strains, mutant (nitrous acid) treatment and baker's yeast

Unsweetened dough leavening

The proof heights for the unsweetened dough fermented with the wild yeasts which ranged from (46 – 145 ml), nitrous acid induced mutants (50 – 80 ml) and the control baker's commercial yeast (62 – 170 ml) (Fig. 5). The leavening abilities of the yeasts in the fermentation of the unsweetened dough for wild and nitrous acid-induced mutants showed a uniform pattern since all of them exhibited a lagging in the course of 180 min and subsequent rise from 24 - 48 h (1440 – 2880 min) of incubation periods (Fig. 6). The baker's yeast, the control strain, also showed a lagging period and subsequent rise within 180 min and declined from 24 - 48 h of incubation periods.

The leavening abilities ranged from (71 – 220 ml) for unsweetened wild type of yeasts, nitrous acid-induced mutants, (70 – 150 ml) and the baker's yeast ranged from (78 – 248 ml) (Figs. 6 and 7a). From the results obtained, *Kodamaea ohmeri* is taken as the best among the wild strains in terms of leavening activity in both sweetened and the unsweetened dough (Figs. 4, 5 and 7b). Most of the wild yeast strains used for sweetened dough showed a better leavening performance when mutated (nitrous acid). The strain of *Candida norvegensis* showed the best in leavening activity for the sweetened dough (Fig. 4). However, it was observed that the mutation caused a reduction in the leavening activity of the wild type of *Kodamaea ohmeri* for sweetened dough and an increase in *Rhodotorula minuta* for the unsweetened dough (Figs. 4 and 5).

In dough without addition of sugar (lean or plain), the principal fermentable sugar for yeast is maltose, liberated from the starch of the flour by amylases (Oda and Ouchi, 1990; Higgins *et al*., 1999). The leavening ability of sponge dough is closely related to maltose fermentability (Blazquez *et al*., 1993; Higgins *et al*., 1999). It is generally accepted that a good baker's yeast should be able to rapidly ferment maltose, because if bread is made without added sugar, the "existing" free sugars are completely fermented within the first hour, leaving only the starch-derived maltose to sustain

The result also revealed that for the unsweetened dough, majority of the wild yeast strains had a better leavening capacity than the mutants of nitrous acid (Figs. 7a and 7b). The wild strains and mutants from nitrous acid treated sweetened dough at incubation period of 48 hours showed a better leavening capacity than the commercial baker's yeast (Figs. 1, 2, and 3). The commercial baker's yeast which was the control, showed the best leavening capacity at 180 min and poor leavening capacity at 48 h for both sweetened and unsweetened dough (Figs. 1 and 7b). The sweetened dough had a better leavening capacity than the unsweetened dough in wild, nitrous acid-induced mutants and baker's yeast (Fig. 7b). However, there was a remarkable improvement in leavening capacities of the yeast strains of *Kodamaea ohmeri*and *Rhodotorula minuta* (wild strains) in the unsweetened dough when compared with their performance in sweetened dough (Figs. 4 and 7b).

The results for the dough leavening test statistically showed that there were no significant differences in the mean performance between the baker's yeast and the wild strains in the sweetened and the unsweetened dough (P>0.05). The mutants of nitrous acid and UV light when compared with the wild type statistically, their mean performances varied between the different species. Some species showed significant differences at P> 0.05 at different time intervals 24 and 48 h for sweetened and unsweetened dough, respectively.

fermentation. The result obtained in this study on unsweetened dough fermentation showed an improved leavening capacity both in the wild type and mutant strains (Fig. 5) and is consistent with the report of other workers (Oda and Ouchi, 1990; Higgins *et al*., 1999). The most desirable property for mutants of industrial strains is genetic stability and physiological reproducibility (Oda and Ouchi, 1990). The mutants isolated in this study were stable and their fermentative capacities were in many cases improved with regards to wild type. The mutants with the best properties (UV irradiation) were *Candida zeylanoides* and *Geotrichum capitatum*. These had a lower leavening capacity in their wild state but showed an increase in their leavening activities in lean or unsweetened dough (Figs. 8 and 9). The mutant of *Candida norvegensis* (nitrous acid treatment) also showed an improved fermentative capacity, that is, higher leavening ability on sweetened dough than the wild type and control (commercial baker's yeast, *Saccharomyces cerevisiae*) (Fig. 4). This finding is in agreement with the report of other studies (Oda and Ouchi, 1991; Randez-Gil *et al*., 1995).

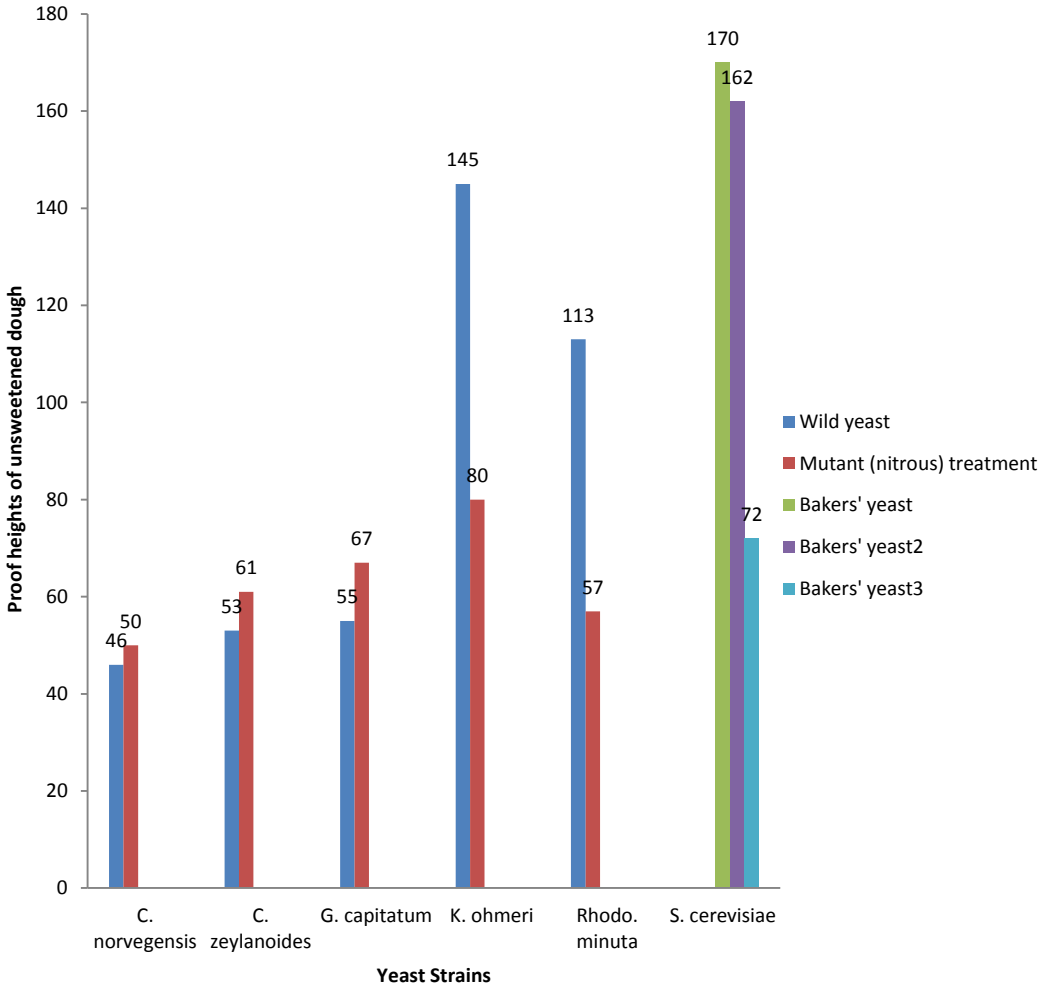

Fig. 5: Proof heights of unsweetened dough fermented with wild strains, mutant (nitrous acid) treatment and baker's yeast

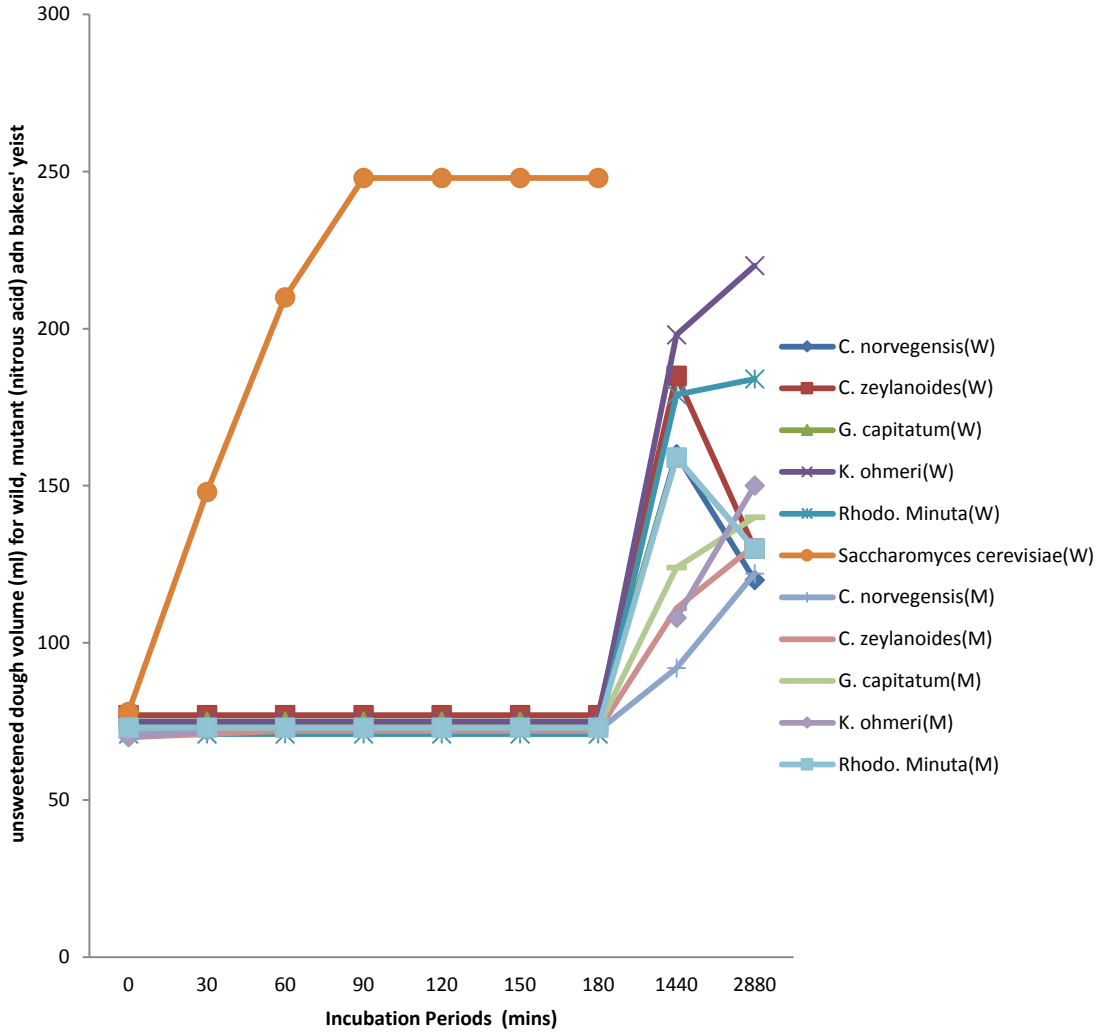

Fig. 6: Leavening profile of unsweetened dough fermented with wild strains, mutant (nitrous acid) treatment and baker's yeast

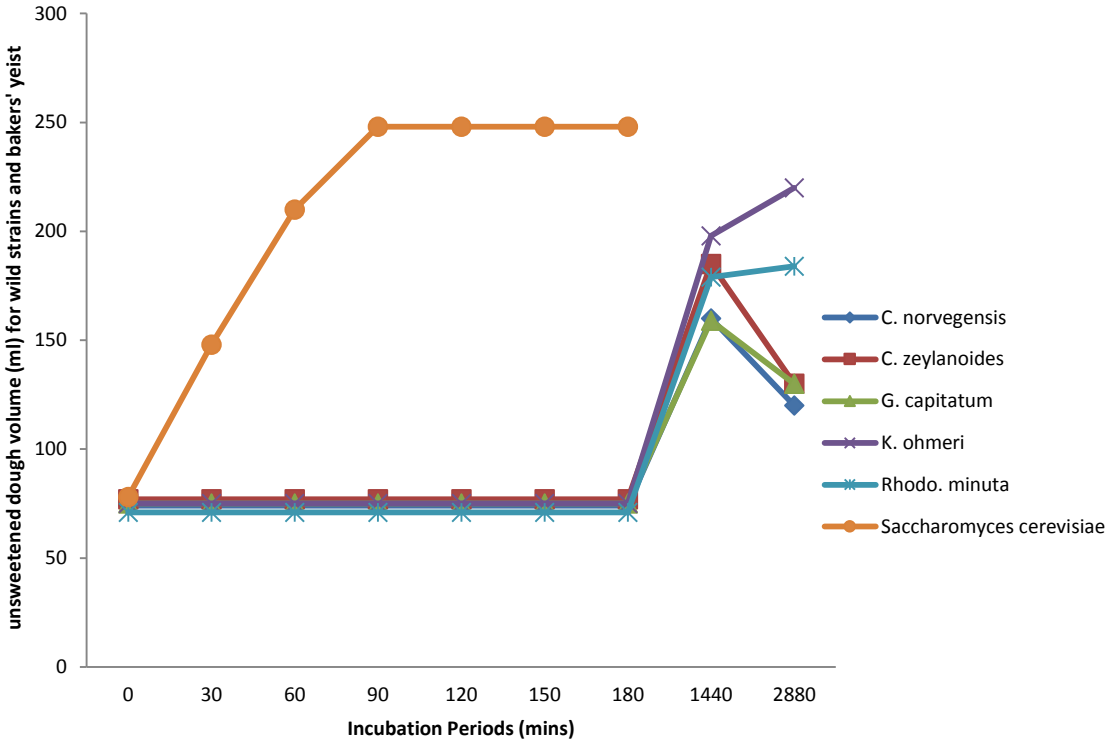

Fig. 7a: Leavening profile of unsweetened dough fermented with wild yeast and baker's yeast

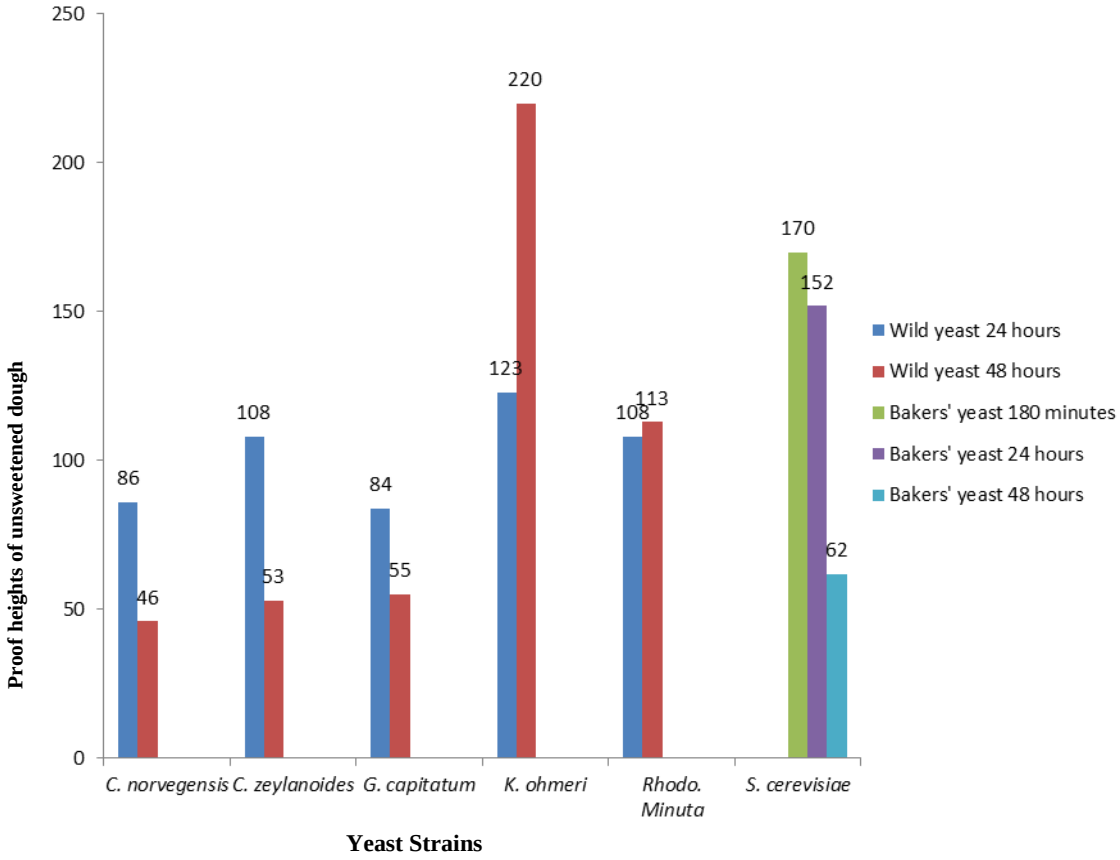

Fig. 7b: Proof heights of unsweetened dough fermented with wild yeast and baker's yeast

Ultra violet (UV) light-irradiated yeast strains used for sweetened and unsweetened dough test

The test was carried out as described in sweetened and unsweetened dough for wild and nitrous acid mutants. They only differed in the UV light exposure time intervals of 4, 6, 10, 15, 20, 25 and 30 min. The results were recorded as presented in Tables 2 and 3. The dough leavening capacities of the UV- light-irradiated yeast strains (mutants) in relation to time intervals (min) ranged from 4 min (60 – 104 ml), 6 min (65 – 112 ml), 10 min (60 – 108 ml), 15 min (66 – 86 ml), 20 min (68 – 116 ml), 25 min (70 – 150 ml) and 30 min (70 – 94 ml) (Table 3) for the sweetened.

The results for unsweetened dough ranged from 4 min (69 – 175 ml), 6 min (70 – 185 ml), 10 min (70 – 195 ml), 15 min (71 – 230 ml), 20 min (70– 203 ml), 25 min (70 – 170 ml) and 30 min (70 – 186 ml) (Table 3). The above dough leavening activities were observed with various isolates obtained from the study namely: *Candida norvegensis*, *Candida zeylanoides*, *Geotrichum capitatum*, *Kodamaea ohmeri* and *Rhodotorula minuta* for both sweetened and unsweetened dough. Considering the result obtained from the sweetened dough, it was observed that at 4 minutes, the mutant that gave the best leavening activity was *Candida norvegensis* (25 ml) and the least was *Candida zeylaniodes* (2 ml) (Fig. 8). At 6 and 10 min, *Kodamaea ohemri* and *Candida norvegensis* mutants gave the leavening activities of 46 and 20 ml (Fig. 8). The mutant strain of *Kodamaea ohmeri*, at 15, 20 and 25 min intervals gave the best leavening activities of 12, 36 and 25 ml (Fig. 8). At 30 min, *Candida norvegensis* gave the best leavening activity of 24 ml (Fig. 4.28 m). However, combining the leavening capacities of sweetened dough from UV mutation times from 4 - 30 min intervals showed that the least mutants in terms of leavening capacity is the yeast strain *Candida zeylanoides* (Fig. 8).

The unsweetened dough leavening capacities of the different UV-light-induced mutants at various time intervals showed the following leavening activities with the highest and the least respectively as follows; at 4 min, *Kodamaea ohmeri* (105 ml), the least (50 ml) for *Geotrichum capitatum* and *Rhodotorula minuta* (Fig. 9). At 6 min, *Candida norvegensis* (110 ml), the least, *Rhodotorula minuta* (40 ml) (Fig. 9). At 10 min, *Candida zeylanoides* (125 ml), the least *Candida norvegensis* (10 ml) (Fig. 9). At 15 min, *Kodamaea ohmeri* (154 ml), the least *Candida norvegensis* (15 ml) (Fig. 9). At 20 min, *Geotrichum capitatum* (130 ml), the least *Candida norvegensis* (20 ml) (Fig. 9). At 25 min, *Geotrichum capitatum* (100 ml), the least, *Candida norvegensis* (08 ml) (Fig. 9). At 30 min, *Geotrichum capitatum* (113 ml), the least *Candida zeylanoides* (80 ml) (Fig. 9). From the result, it was observed that the unsweetened dough favoured better leavening capacities with the various mutants than the sweetened dough at various time intervals (Figs. 8 and 9). For the sweetened dough, the mutants with the highest leavening activities were *Candida norvegensis* at 4 min (25 ml). *Kodamaea ohmeri*, at 6 min (46 ml), 20 (36 ml) and at 25 (60 ml), followed by *Geotrichum capitatum* at 6 min (37 ml) and at 30 min *Candida norvegensis* (24 ml). But for the unsweetened dough, the mutants with the best leavening performance at 4, 6, 10, 15 min were *Kodamaea ohmeri*, 4(105 ml), 6(106 ml), 10(118 ml), 15(154 ml), *Geotrichum capitatum*, 10(118 ml), at 6 and 10 minutes *Candida norvegensis* (110 and 125 ml), respectively. This is followed by *Geotrichum capitatum* at 20 min (130 ml), 25 min (100 ml), 30 min (113 ml) and at 20 min *Rhodotorula minuta* (105 ml). The result of the UV-light-induced mutants showed a better leavening ability in the lean or unsweetened dough when compared with the sweetened dough fermented with the wild type. This observation was made in the wild type yeasts and are stated as follows: *Rhodotorula minuta* (27- 105 ml), *Candida zeylanoides* (68 – 125 ml) and *Geotrichum capitatum* (60 – 130 ml) which had a lower leavening capacity at 10 and 20 min mutation time intervals respectively (Figs. 8 and 9). This result is agreement with the report of (Oda and Ouchi, 1990) in terms of genetic stability and reproducibility for yeast required in industries for the production of desired dough leavening capacity in bread making.

Table 2: Assessment of leavening capacities of mutant yeast strains with UV- light at different time intervals in sweetened dough

Yeast strain	Dough volume (ml) at Different Incubation Times (min)									
	Time interval (min)	0 min	30 min	60 min	90 min	120 min	150 min	180 min	24 h	48 h
Candida norvegensis	4	60	65	75	75	75	75	75	75	85
	6	65	70	70	70	70	70	70	70	80
	10	60	70	70	70	70	80	80	80	80
	15	76	76	76	76	76	76	76	78	80
	20	70	70	70	70	70	75	75	75	80
	25	70	70	70	70	70	75	75	75	75
	30	70	74	74	74	74	84	84	84	94
Rhodotorula minuta	4	73	73	73	73	73	73	73	77	80
	6	73	73	73	73	73	73	73	78	89
	10	73	73	73	73	73	73	73	76	82
	15	73	73	73	73	73	73	73	77	78
	20	73	73	73	73	73	73	73	75	76
	25	74	74	74	74	74	74	74	76	82
	30	77	77	77	77	77	77	77	86	88
Geotrichum capitatum	4	65	65	67	67	67	67	67	70	80
	6	75	75	77	77	77	77	77	84	112
	10	91	91	91	91	91	91	91	95	108
	15	66	66	66	66	68	68	68	68	74
	20	68	68	69	69	69	69	69	74	77
	25	76	76	76	76	76	76	76	82	96
	30	72	72	73	73	73	73	73	75	80
Kodamaea ohmeri	4	80	80	80	80	80	80	80	84	104
	6	84	84	84	84	84	84	84	90	130
	10	90	90	90	90	90	90	90	90	104
	15	74	74	74	74	74	74	74	76	86
	20	80	80	80	80	80	80	80	80	116
	25	90	90	90	90	90	90	90	94	150
	30	90	90	90	90	90	90	90	92	92
Candida zeylanoides	4	76	76	76	76	76	76	76	77	78
	6	75	75	75	75	75	75	75	77	78
	10	76	76	76	76	76	76	76	77	80
	15	77	77	77	77	77	77	77	77	79
	20	73	73	73	73	73	73	73	73	74
	25	82	82	82	82	82	82	82	82	83
	30	79	79	79	79	79	79	79	79	80

Table 3: Assessment of leavening capacities of mutant yeast strains with UV- light at different time intervals in unsweetened dough

Yeast strain	Dough volume (ml) at Different Incubation Times (min)									
	Time interval (min)	0 min	30 min	60 min	90 min	120 min	150 min	180 min	24 h	48 h
Candida norvegensis	4	70	70	70	70	70	70	70	108	138
	6	75	75	75	75	75	75	75	118	185
	10	75	75	75	75	75	75	75	85	85
	15	75	75	75	75	75	75	75	90	90
	20	75	75	75	75	75	75	75	95	95
	25	72	72	72	72	72	72	72	80	80
	30	72	72	72	72	72	72	72	118	118
Candida zeylanoides	4	72	72	72	72	72	72	72	146	166
	6	72	72	72	72	72	72	72	100	116
	10	70	70	70	70	70	70	70	140	195
	15	74	74	74	74	74	74	74	118	149
	20	74	74	74	74	74	74	74	99	114
	25	74	74	74	74	74	74	74	98	120
	30	75	75	75	75	75	75	75	100	155
Geotrichum capitatum	4	69	69	69	69	69	69	69	102	129
	6	73	73	73	73	73	73	73	102	119
	10	72	72	72	72	72	72	72	185	190
	15	71	71	71	71	71	71	71	114	168
	20	73	73	73	73	73	73	73	191	203
	25	70	70	70	70	70	70	70	135	170
	30	73	73	73	73	73	73	73	161	186
Kodamaea ohmeri	4	70	70	70	70	70	70	70	135	175
	6	76	76	76	76	76	76	76	146	182
	10	76	76	76	76	76	76	76	157	194
	15	76	76	76	76	76	76	76	186	230
	20	70	70	70	70	70	70	70	125	170
	25	70	70	70	70	70	70	70	75	100
	30	70	70	70	70	70	70	70	100	125
Rhodotorula minuta	4	70	70	70	70	70	70	70	90	120
	6	70	70	70	70	70	70	70	88	110
	10	70	70	70	70	70	70	70	200	150
	15	75	75	75	75	75	75	75	220	170
	20	75	75	75	75	75	75	75	190	180
	25	75	75	75	75	75	75	75	100	120
	30	78	78	78	78	78	78	78	180	140

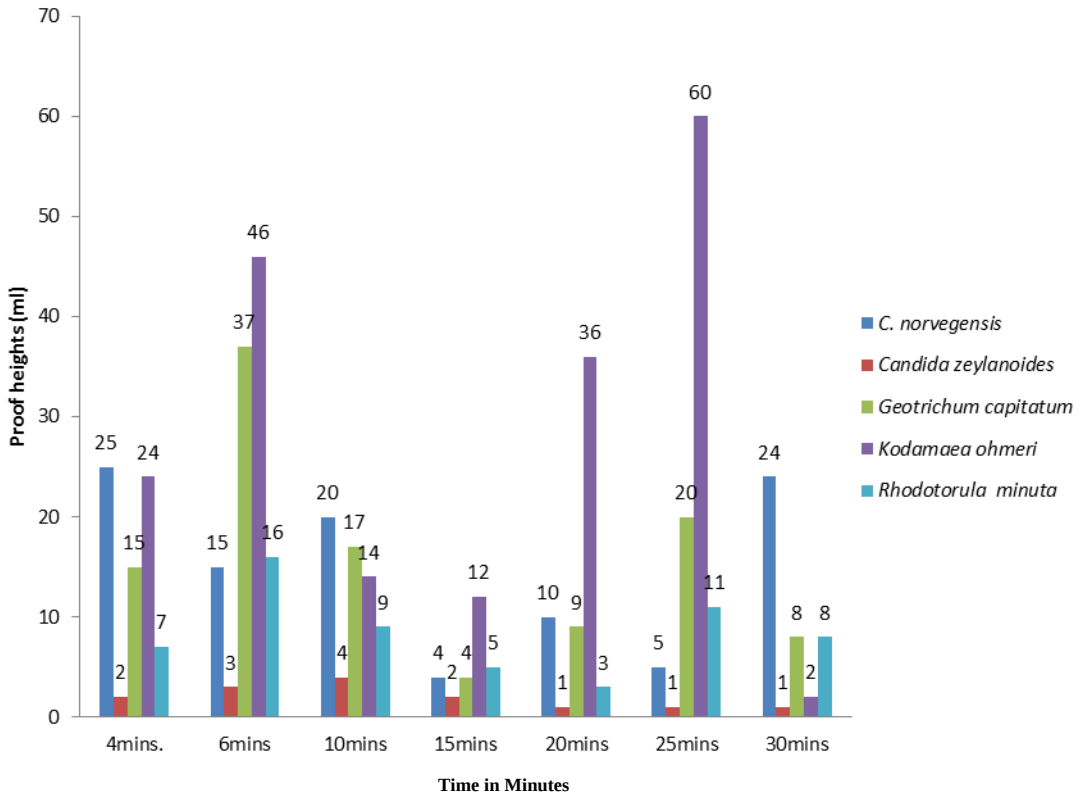

Fig. 8: Proof heights of UV mutants 4 to 30 min of sweetened dough

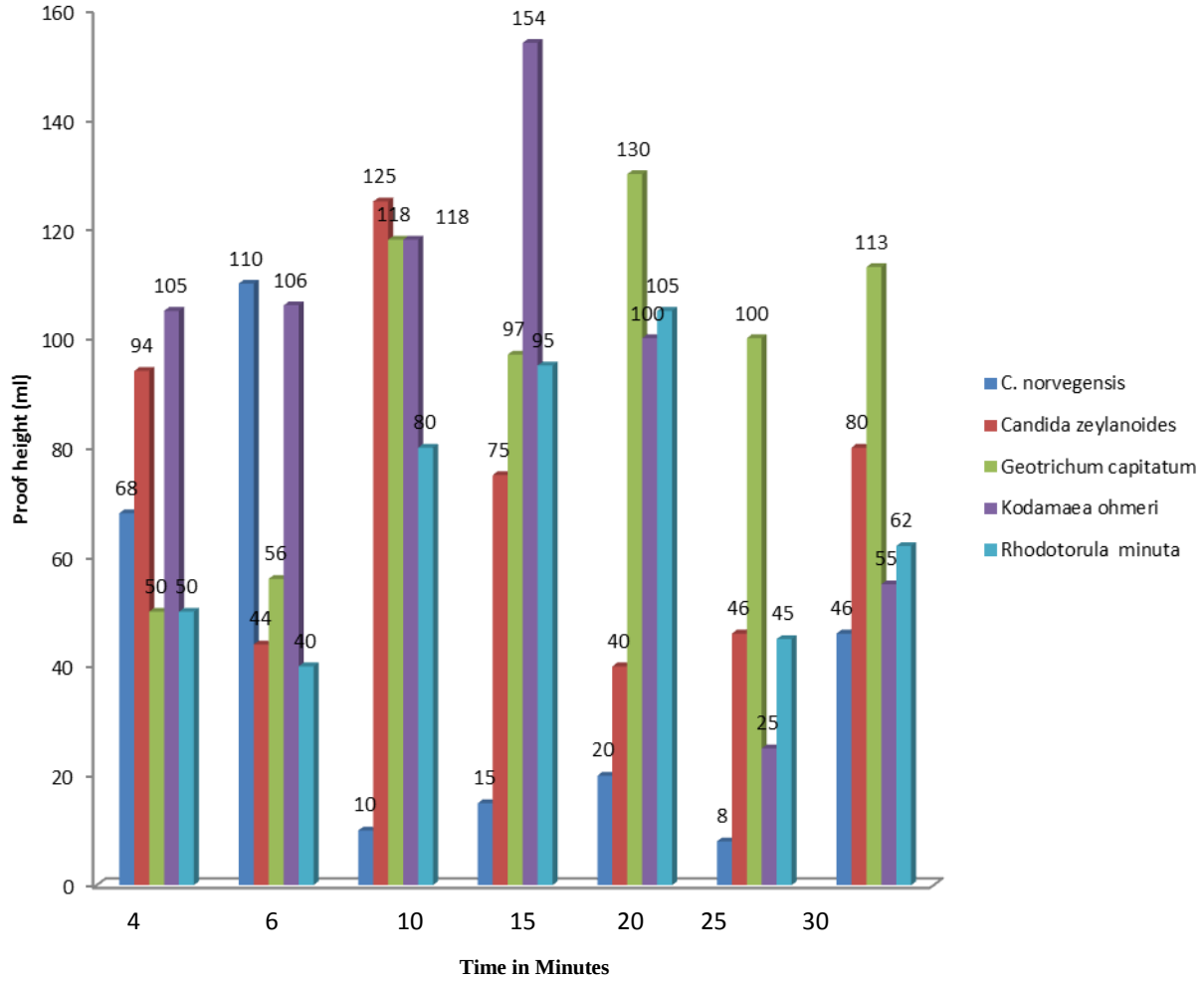

Fig. 9: Proof heights of UV mutants 4 to 30 min of unsweetened dough

Conclusion

The wild and mutant strains of yeast obtained in this study have sufficient potential to be produced commercially, because their fermentative abilities on sweetened dough and unsweetened dough used in bread production were greatly improved. The strains of yeast obtained, both wild and its mutant matched baker's yeast in their ability to utilize maltose and showed a better leavening capability than the commercial baker's yeast. Osmotolerance was widely distributed in the wild, mutant and baking strains. Therefore, it is very feasible to produce leavened bread using wild and mutant yeasts obtained from fermented orange juice as the sole leavening agent for optimum loaf height and volume. This is possible as most of the strains efficiently produced carbon (iv) oxide in their respective dough types.

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