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Full Length Research Article

CHARACTERIZATION AND IMPROVEMENT OF THE LEAVENING CAPACITY OF *SACCHAROMYCES CEREVISIAE* ISOLATED FROM NIGERIAN INDIGENOUS ALCOHOLIC BEVERAGES USING A CHEMICAL MUTAGEN

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ABSTRACT
Yeasts are widely distributed in nature and can be isolated from nutrient rich substrates such as fruit and vegetable, fermented foods and beverages. Yeasts have been employed for millions of years in biotechnological applications in the production of bread, beer and wine. *Saccharomyces cerevisiae* is most important species when it comes to practical application in industries. Many developing countries including Nigeria spent billions of dollars annually on importation of active dry yeasts used in their baking and brewery industries. In order to reverse this trend, an attempt was made at improving the leavening capacity of *S. cerevisiae* isolated from locally made traditional alcoholic beverages by mutation induced by N-methyl-N-Nitro-N-Nitrosoguanidine (NTG). The yeast isolates were identified by their morphological properties and biochemical tests using standard methods. The viable counts of yeast isolated from the five alcoholic beverages employed in this study ranged between $1.4x10^7$ and $2.4x10^8$ CFU/mL. The yeast with the lowest percentage of occurrence (1.67%) are *Geotricum candidum*, *Schizosaccharomyces japanicus*, *Sch. pombe*, *Candida intermedia* etc. while *S. cerevisiae* had highest percentage of occurrence of 26.67%. *S. cerevisiae* PWII exhibited the highest dough rising ability among the parent strains, but *S. cerevisiae* M3 had the best dough rising power among the mutants. Physical examinations of the baked revealed that the bread samples produced with mutant strains compared favorably well with ones produced with commercial yeasts in terms of height, weight and volume. On the other hand bread samples produced using parent strains (PT 14 and PWII) had longer shelf life than bread from mutant and commercial strains. Sensory evaluation by taste panelists showed that the ranges of scores obtained are appearance (5.6-7.9), texture (6.3-8.1), taste (5.5-7.0), crumb (6.0-7.1) and overall acceptability (6.2-7.2). It could be concluded from this study that the leavening ability of *S. cerevisiae* could be enhanced by chemical mutagens and therefore could by employed in bread baking.

Keywords: Traditional alcoholic beverages, *Saccharomyces cerevisiae*, baker's yeast, mutation, leavening properties.

INTRODUCTION

Fermented foods and beverages are very important in the diet of African people. These food products are usually produced at household level or at small industrial scale and therefore are often of varying quality and stability (Jespersen, 2002). Indigenous foods which are also called traditional fermented foods are those popular products which have formed part of the diet since early history and can be produced at household level or in cottage industry using relatively simple techniques and equipment (Aidoo *et al*., 2005). Alcoholic beverage can be defined as a drink that contain ethanol and can be classified into beers, wines, spirits and distilled beverages (Savanraj *et al*., 2017). There are different indigenous alcoholic beverages produced and consumed by people of Nigeria and these include, palm wine *burukutu*, *pito* and *agadagidi* (Adesokan *et al*., 2013). Palm wine is commonly consumed in southern Nigeria, Asia and South America and it is obtained from the sap of various types of palm trees (Elijah *et al*., 2010). *Burukutu* is a type of African opaque beer which is usually produced from sorghum, millet and maize and its production involves stages like malting, mashing, straining, souring, boiling and alcoholic fermentation (Atter *et al*., 2014).

Agadadigi is an alcoholic beverage usually produced from overripe bananas and plantains. It is cloudy, effervescent, sweet-sour tasting and is commonly consumed in South West Nigeria (Mogaji *et al*., 2021). The microorganisms associated with these traditional alcoholic beverages are yeasts, lactic acid and acetic acid bacteria. They contribute to the taste, flavor, enhancement of nutritional quality, alcoholic content and shelf life of the beverages (Adesokan *et al*., 2020).

Yeast can be defined as a single-celled fungus which reproduces by budding or fission and sexual states are not formed within or upon fruiting bodies (Rapoport *et al*., 2019; Rodrigues 2020). Fermentation of glucose by yeast results into production of alcohol, lactic acid, carbon dioxide and unique flavor substances (Hittinget *et al*., 2018) and thus can improve the production and quality of fermented foods.

Many industrial processes employ the model yeast *Saccharomyces cerevisiae* which has been used traditionally in the food industry for production of alcoholic beverages like beer, wine and sake, as well as for bread fermentation (Hou *et al*., 2012). Though *S. cerevisiae* and other yeasts are used intensively in biotechnological application and industrial fermentation, there is still significant room for improvement (Steen *et al*., 2014). The strain improvement is the process of improvement and manipulation of microbial strains for the enhancement of metabolic capacities for biotechnological applications (Pathak *et al*., 2015).

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Yeast mutagenesis breeding can be used to alter the genetic structure and function of yeasts and then specific traits in the mutants are screened (Miller, 2018). This is the most basic yeast engineering technology, and it has the advantages of high and rapid mutagenesis rates and mainly includes chemical, physical and biological methods. Chemical methods use alkylating agents, base analogues, hydroxylamine, and other chemicals to induce genetic changes (Lu *et al*., 2021).

It has been reported that bread is one of the most ancient human foods produced by fermentation of microorganisms at an ancient Egyptian bakery at the Giza pyramid area in the year 2577BC (Willey *et al*., 2008). *S. cerevisiae* is the most commonly used species of *Saccharomyces* in bread baking and it has been employed as baker's yeast in production of bread for at least 6000 years (Alcamo, 2001; Bell *et al*., 2001). During fermentation of dough the yeast grows aerobically leading to increased carbon (iv) oxide production and minimum alcohol accumulation (Willey *et al*., 2008). It has been reported that increased production of carbon (iv) oxide increases the dough size thereby giving bread its characteristics of being light and spongy texture (Pattison *et al*., 2001).

Bread is the most common among all baked products of wheat and it is consumed and enjoyed by both children and adults from different socio economic status in Nigeria, therefore leading to high daily demand (Iyang and Asuquo, 2016). However, baking industry is very costly in Nigeria due to the high cost of imported baker's yeast from developed countries such as Europe and America, a process that drains its foreign reserve. The expensive nature of baker's yeast has led to poor production of bread by bakers and making consumption of bread almost out of reach (Yabaya and Jatau, 2009).

As a result of afore mention reasons a number of studies have been conducted to evaluate the potential of indigenous *S. cerevisiae* as baker's yeast. The results obtained clearly demonstrated the potential of local yeast in bread baking compared to the imported baker's yeast (Jahan *et al*., 2007; Yabaya and Jatau, 2009, Karki *et al*., 2017; Gebreskssie *et al*., 2019).
The genome of the organism ultimately controls its metabolism. Although improved fermented engineering design and optimal cultural conditions can quantitatively enhance the microbial products up to a limit. Therefore, genetic improvement of the organism is fundamental to the success of fermentation technology (Petrea and Tofan 2008).

It has been demonstrated that mutation induced in yeast by nitrous acid influences both rate of fermentation process and ethanol quantity produced (Petrea and Tofan, 2008). In another study, mutation was induced in local isolates of *S. cerevisiae* in Saudi Arabia using Diethyl sulfate at varying concentration. It was concluded that all concentration of mutagen caused mutation in the biosynthetic pathway which was observed on the selective media plates (Al-Sum *et al*., 2013). This study was conducted with a view of improving leavening capacity of *S. cerevisiae* isolated locally in Nigeria for possible application in industrial production of baker's yeast.

MATERIALS AND METHODS

Sample collection
The samples of indigenous alcoholic beverages such as palm wine, *burukutu* and *pito* were collected in sterile conical flasks from local producers immediately after production within Ibadan metropolis. The samples of *agadagidi* were obtained from Ile-Ife as described above.

Isolation and identification of yeasts
The samples of each of the indigenous alcoholic beverages were serially diluted and appropriate dilutions were plated onto sterile plates of Potato Dextrose Agar (PDA) containing 30µg/ml streptomycin. The yeast isolates were purified by repeated streaking on PDA and the pure cultures were streaked onto PDA slants, incubated at 25^{o}C for 48 hours (Adesokan, 2013). The morphological, physiological and biochemical properties of the yeast were determined. The parameters determined were cell and colony morphology; pseudomycelium formation, sugar fermentation test, urease test, acid tolerance, acid production from glucose, gelatin hydrolysis, nitrate assimilation etc. The yeasts were then identified by the method of Nouroll *et al* (2013) and Adesokan *et al*., (2020).

Propagation of test isolates
The yeast isolates belonging to the species of *Saccharomyces cerevisiae* were selected and grown in yeast extract peptone dextrose agar slants for 5 days at 30^{o}C. The cells were harvested by pouring little amount of sterile distilled water on the slants and gently scraping with sterile wire loop. The cells were centrifuged at 350 rpm, washed and filtered with sterile fitter paper and funnel. Yeast cells from the agar slants were used to inoculate 150 mL medium yeast extract peptone dextrose (YEPD} broth contained in several 250 mL flasks which were cultivated for 4 days at 30^{o}C. The broth medium was later centrifuged at 2000 rpm for 2 min. and yeast pellets were washed with water and filtered. The yeast cells were then dried in an oven (30^{o}C) to a constant weight and kept in sterile foil paper (Ejiofor *et al*., 1994; Ekunsanmi and Odunfa, 1990).

Assessment of the leavening properties
Cell suspension of the yeast isolates were prepared and 30 mL of each was mixed with 4g of sucrose and 40g of wheat flour to produce a smooth dough and fermented by the cylinder method in a dough leavening test (Staples, 1983). The dough was deposited at the bottom of a 500 mL measuring cylinder whose sides had been smeared with a layer of melted baking fat to prevent the dough from sticking to the sides. The cylinder was incubated at 30^{o}C and the volume of the dough was taken at 0 hour and every 30 minutes for 4 hours (Ejiofor *et al*., 1994).

Improving the dough leavening property
Strains of isolates with varying dough leavening properties were randomly paired according to the criteria of Maraz *et al*. (1978). Isolates harvested at their late logarithmic growth phase were prepared using the procedure of Sakaguchi *et al*. (1980).

Mutation technique
Yeast isolates were grown on YEPD and incubated at 30^{o}C for 3 days. Yeast cells were harvested and centrifuged at 1000 rpm for 10 min at 4^{o}C using Sorvant 50 centrifuge. Cells were washed twice

in buffer (citrate buffer pH 5.5). The washed cells were re-suspended in 4 mL of citrate buffer. Then, 0.2 mL of (0.002g/ml) of N-methyl-N-Nitro-N-Nitrosoguanidine (NTG) was added. The cells were incubated in a water bath at 30°C for 30 minutes. Cells were centrifuged at 1000 rpm for 5 minutes at 4°C and later washed in phosphate buffer pH 7.0. The cell pellets were re-suspended in 10 mL of phosphate buffer. One mL of treated cells were plated out on yeast nitrogen base without amino acid (Difco), supplemented with 2% glucose and 2% agar. The plates were incubated at 30°C for 72 hours. Colonies appearing on the plates within these hours of incubation were picked up and assayed for leavening properties (Matteuzzi *et al*., 1988).

Assessment of the properties of the mutant strains
Mutant strains were grown and their various capabilities to leaven dough were carried out and compared with the parent strains.

Temperature and pH changes during leavening of dough
The temperature of the dough was measured at 0 h, 6 h, 12 h, 24 h and 48 h intervals using a digital thermometer. The pH of the dough was determined at same time intervals using the method of Tansey (1973). Homogenized suspension of the dough was prepared by suspending one gram of the dough into 9 mL sterile distilled water. The pH was determined using pH meter after standardizing with the appropriate buffers.

Baking of bread dough
The straight-dough bread baking method (American Association of Cereal Chemist, 1986) was employed to test the baking properties of the yeast. The dough was prepared by mixing 100 g of flour, 1.8 g dry weight of yeast, 6 g sugar, 3 g fat and 68 mL of water. The resulting batter was allowed to bulk-ferment for 90 min and baked at 220°C for 20 min. Commercial baking yeast was used as control or standard. Loaf volumes were measured 25 min after baking (Ejiofor *et al*., 1994). The bread loaves were allowed to cool for about an hour before physico-chemical analyses were carried out.

Physicochemical properties of bread samples
The loaves were examined in terms of color, texture, crust, shape, structure, cracks as well as crumb and porosity. All these were determined on physical examination. The heights were determined by taking the mean values of three measurements on three different places on the loaves (Sanni *et al*., 1998).

Organoleptic assessment
A sensory evaluation was carried out on the bread within 24 hours of baking. Samples were evaluated by untrained panelists of students in the laboratory (postgraduate and undergraduate students). Samples were assessed using a 9-point hedonic scale of 9 (like extremely) to 1 (dislike extremely) for characteristics such as appearance, taste, crumb and texture (Larmond, 1977).

Determination of the shelf life of bread samples
Shelf life of the loaves was determined by resistance to mould growth. Slices of bread samples were put in sterile transparent plastic bags and stored at room temperature and refrigeration temperature. The shelf life was taken as the storage time until mould growth could be observed by visual observation (Sanni *et al*., 1998).

Statistical analysis
The experiments were conducted in three replicates and data obtained were subjected to statistical analysis using analysis of variance (ANOVA). The means were separated using Duncan Multiple Range Test (Snedecor and Cochran, 1989).

RESULTS
Four different samples of each of the indigenous alcoholic beverages were collected and yeast viable count determined. The ranges of viable counts (CFU/mL) obtained are; agadagidi ($1.4x10^7$ to $2.3x10^7$), burukutu ($4.8x10^7$ to $5.9x10^7$), palm wine ($3.9x10^7$ to $2.4x10^8$) and pito ($2.5x10^7$ to $3.0x10^7$) (table 1). A total of sixty yeasts belonging to different species were recovered from alcoholic beverages under investigation.
The results shows that *S. cerevisiae* had the highest percentage of occurrence (26.67%) while the least percentage of occurrence (1.67%) was exhibited by *Geotricum candidum*, *Schizosaccharomuces pombe*, *Candida* intermedia, *C. tropicalis*, *C. castelli*, *Pichia membrane- faciens* and *Torulaspora delbruckii*. The leavening properties of the parent strains of *S. cerevisiae* before mutation was induced are presented in fig. 1.

The highest leavening capacity of 318 cm^3 was obtained for *S. cerevisiae* BKOT whereas the least leavening capacity of 266 cm^3 was recorded for *S. cerevisiae* AGO4. Furthermore, mutant strain *S. cerevisiae* M_3 had the highest leavening capacity of 565cm^3 and the least value of 535 cm^3 was obtained for the mutant strain *S. cerevisiae* M_5.
The physical examination of the baked bread samples (Plate 1) showed that all were pale brown in color with smooth crust and hard texture with the exception of bread baked with commercial yeast (CY).
The height of the bread samples ranged between 1.0 – 3.5 cm (Figure 3). The weight of the bread from the parent strains ranged from (58.6 – 69.1g) while the mutant strains ranged from (60.6 – 69.8g) and the controls (CY and UL) are 66.9 and 68.1g respectively (Fig. 4).

All the bread volume was found to be in the range of 53.40 cm^3 for M_2 to 232.75cm^3 for CY (Fig. 5).
\Sensory evaluation of the bread samples is presented in table 3 and the results showed that bread samples produced with yeasts from indigenous alcoholic beverages received higher scores then commercial yeast (CY) bread in term of overall acceptability. Bread samples PW11 and PT14 have the highest shelf life of 7 days while bread samples UL, AG15, M_2 and M_3 had the least of 4 days. All samples were able to resist mould growth for between 10 – 13 days at refrigeration temperature.

Table 1: Viable counts of yeast isolates from the traditional fermented alcoholic beverages

Samples	(CFU/ML)			
Agadagidi	1.4×10^7	2.5×10^7	1.8×10^7	2.3×10^7
Burukutu	5.9×10^7	5.2×10^7	4.8×10^7	5.3×10^7
Palm wine	2.4×10^8	4.9×10^7	3.9×10^7	1.3×10^8
Pito	2.5×10^7	2.8×10^7	2.7×10^7	3.0×10^7

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Table 2: Distribution of yeast species isolated from traditional fermented alcoholic beverages

Yeast Species	Burukutu	Pito	Agadagidi	Palm wine	Percentage of occurrence
Candida valida	4	1	-	-	8.33
Saccharomyces cerevisiae	5	3	5	3	26.67
Saccharomyces chevelieri	1	3	1	-	6.67
Pitchiaohmeri	2	3	-	2	11.67
Geotricumcandidum	1	-	-	-	1.67
Saccharomyces uvarum	1	1	-	1	3.33
Schizosaccharomycesjapanicus	1	1	-	1	5.00
Kluyveromycesafricanus	-	2	2	-	6.67
Schizosaccharomycespombe	-	-	1	-	1.67
Candida krusei	-	-	4	-	6.67
Candida intermedia	-	-	1	-	1.67
Kloeckeraapiculata	-	-	1	1	3.33
Rhodetorulagraminis	-	-	3	2	6.67
Candida tropicalis	-	-	-	1	1.67
Candida castelli	-	-	-	1	1.67
Pichiamembranefacrens	-	-	-	1	1.67
Torulaspora, delbruckii	-	-	-	1	1.67
Total	**15**	**14**	**18**	**13**	**60**

DISCUSSION

The yeast viable count obtained for all indigenous alcoholic beverages ranged between 1.4 x 10^7 to 5.9 x 10^7cfu/mL. In a similar study, the yeast viable count obtained from burukutu and fura ranged between 6.8 x 10^3 to 7.0 x 10^3 cfu/mL (Umeh *et al*., 2022). The yeast isolated from this study include *S. cerevisiae*, *S. cheveliari*, *S. uvarum*, *Pichia ohmeri* and so on. *S. cerevisiae* had the highest frequency of occurrence. In a previous study different genera of yeast were isolated from Nigerian indigenous foods and beverages. *S. cerevisiae* was reported to have the highest frequency of occurrence (Adesokan *et al*., 2020; Adesokan *et al*., 2022).

The highest leavening capacity was shown by *S. cerevisiae* BK07 and the least was recorded for *S. cerevisiae* AGO4. In a recent study by Beyene *et al*. (2020) reported that yeast strain AAUTF1 (*Candida humilis*) had the highest dough raising power followed by strain AAUTF5 (*Kazachstania bulderi*) and the least result was recorded for strain AAUTF6 (*C. humilis*). This is a surprising result because, these non *Saccharomyces* yeasts performed better than the *S. cerevisiae* included in the study.

In another study, it was concluded that *S. cerevisiae*, *Saccharomyces species* and *Kluveromyces* demonstrated sufficient potential to be produced commercially after evaluation of some baking parameters (Jahan *et al*., 2007). Moreover, *Saccharomyces cerevisiae* isolated from *burukutu* and palm wine compared favorably with commercial yeast in term of their baking potential (Yabaya and Jatau, 2009).

The results obtained from the bread weight, volume and height showed that the leavening properties of commercial baker's yeast differ from those of the isolated yeast strains. The mutants *S. cerevisiae* M_1, M_2 and M_3 appear not to be improved, the result is in agreement with observation of Petrea and Tofan (2008) when nitrous acid influences both rate of fermentation process and ethanol quantity produced. However, the performance of M4 and M5 was improved and not significantly different from commercial yeast CY. This is in agreement to the report of Obasi *et al*. (2017) when the leavening capacity of a non-*Saccharomyces* yeast was improved by mutation induced by nitrous acid.

The organoleptic properties of bread produced by mutant strains and commercial yeast compared favorably. As a matter of fact, the overall acceptability of the mutant strains M3, M4 and M5 were significantly higher that of CY. The reason for this observation could be that the ability to produce compounds responsible for taste and aroma were enhanced.

The bead samples produced with commercial yeast had shorter shelf life than the wild yeasts and the mutants. The shelf life of bread depends largely on the bread's ability to prevent mould growth and the factors responsible for this are relative humidity of

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the atmosphere surrounding the bread, the type and number of mould contaminants, pH value, the temperature of storage and the season at which the bread was produced (Maraz, 1978). The pH of the bread is to a large extent responsible for antimould activity. However, there are some yeasts that are capable of producing antimicrobial metabolites called killer toxins as reported by Adesokan (2013), and this could also responsible for an extended shelf life of bread.

Conclusion:
It could therefore be concluded that development of yeasts from local sources such as traditional alcoholic beverages for use in bakeries is feasible, but there is still room for further improvement.

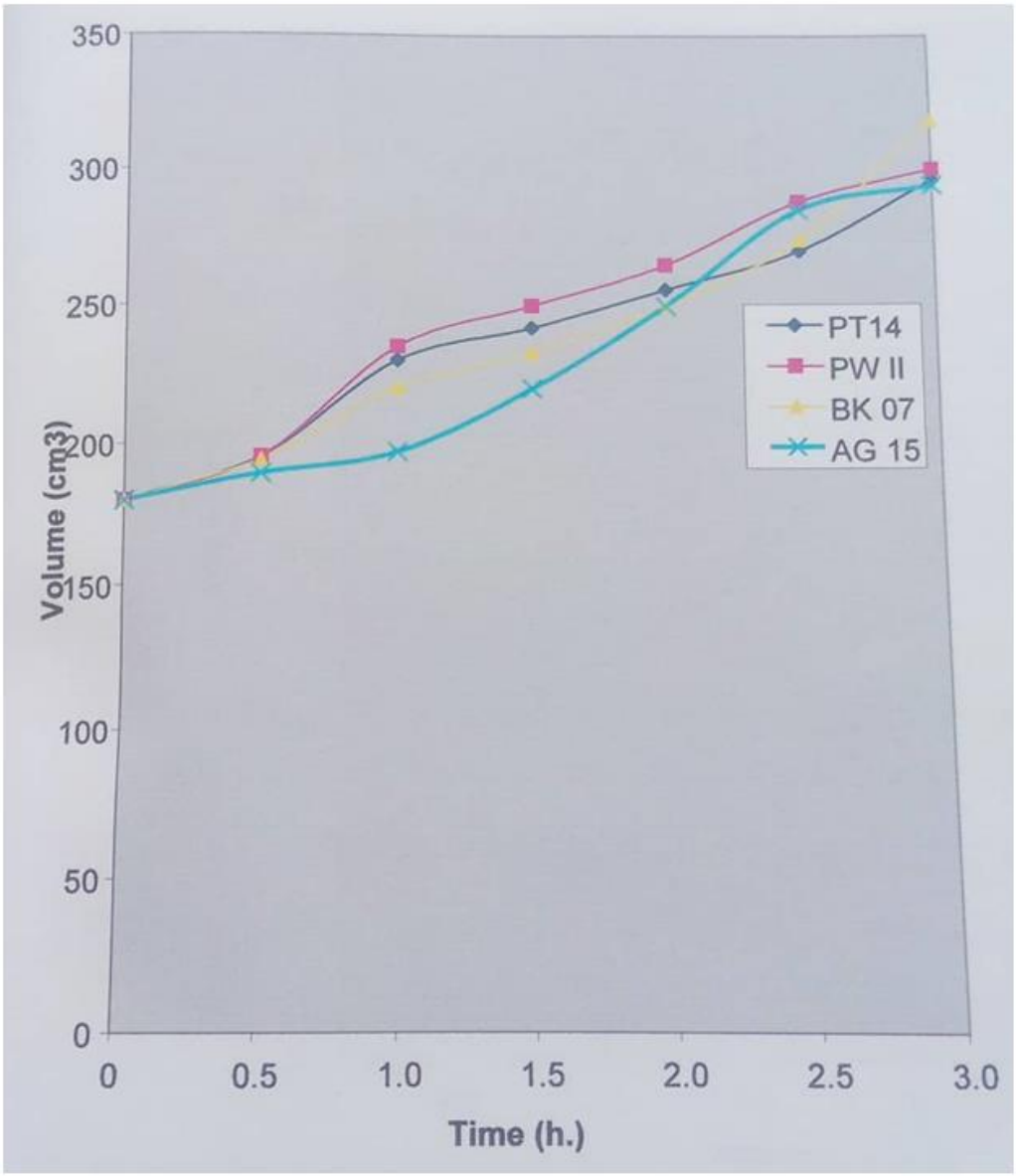

Figure 1: Leavening properties of parent strains.
PT 14 = Dough leavened with *S. cerevisiae* isolated from pito
PW 11 = Dough leavened with *S. cerevisie*isolated from palm wine.
BK 07 = Dough leavened with *S. cerevisiae* isolated from burukutu.
AG 07 = Dough leavened with *S. cerevisiae*isolated from agadagidi

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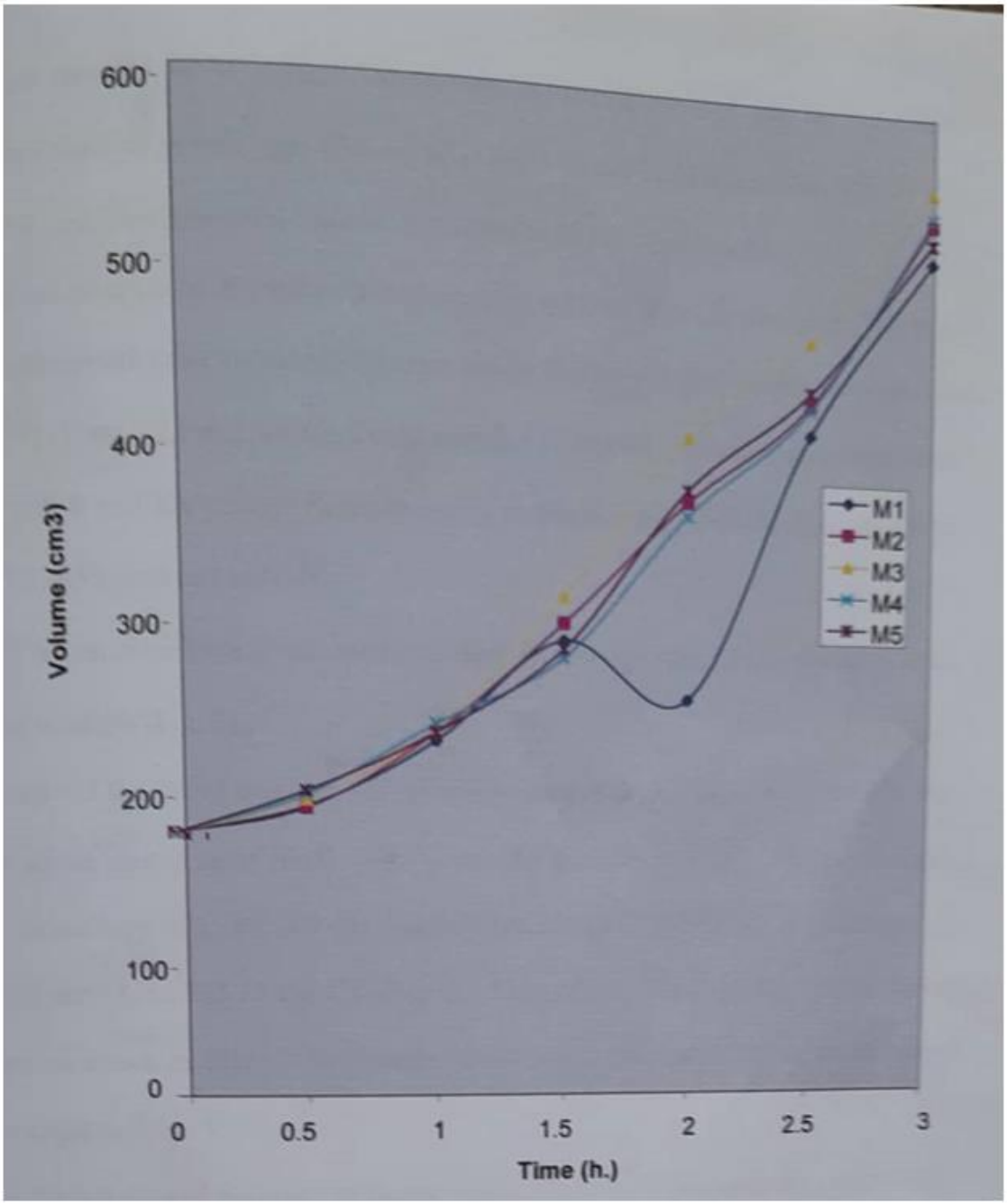

Figure 2: Leavening properties of mutant strains
M1 = Mutant yeast from agadagidi and burukutu
M2 = Mutant yeast from pito
M3 = Mutant yeast from palm wine
M4 = Mutant yeast from agadagidi
M5 = Mutant yeast from burukutu

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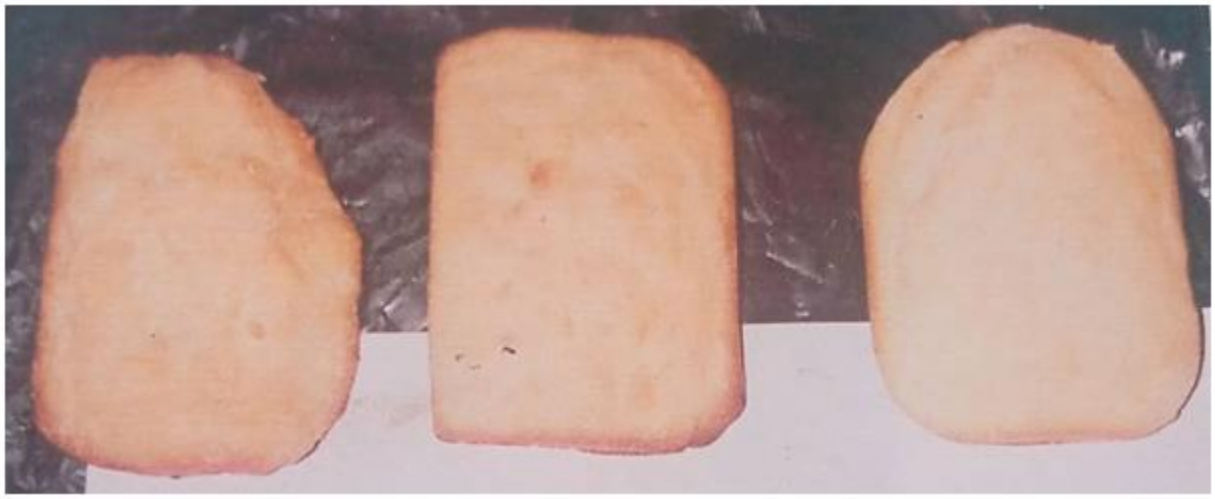
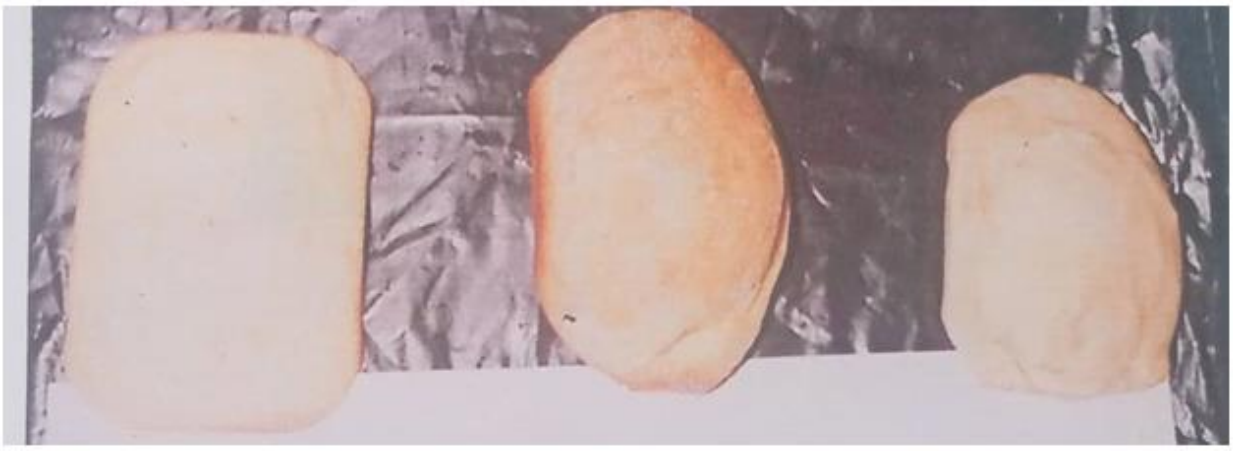


PT 14 **PW 11** **BK7**



AG15 **CY** **UL**

Plate 1: Pan crust of bread and samples
Bread codes:
PT14: Bread from *Pito* yeast.
PW11: Bread from Palm wine yeast
BK07: Bread *burukutu* yeast
AG15: Bread from *agadagidi* yeast
CY: Bread from commercial yeast
UL: Bread without yeast.

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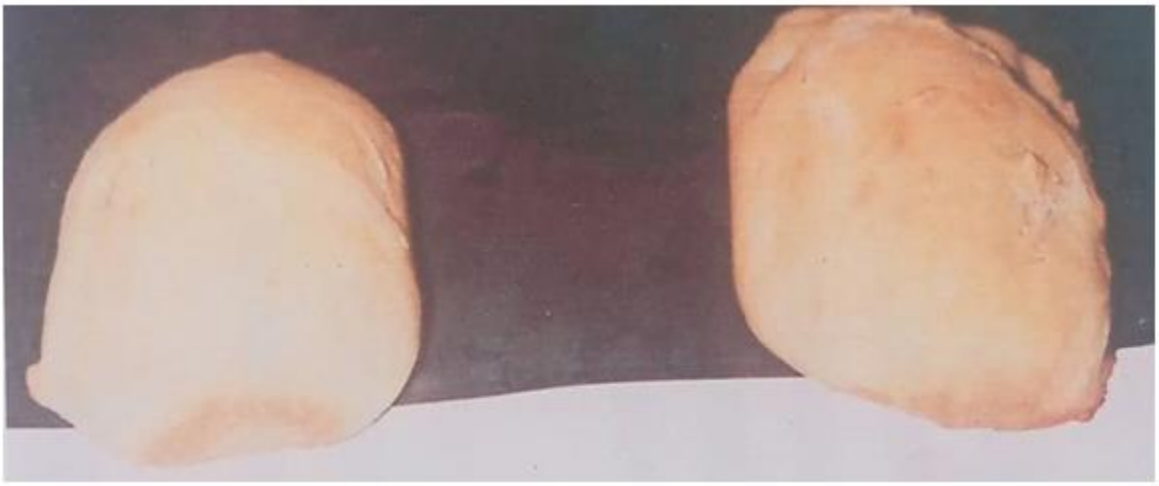
M1 M2 M3



M4 M5

Plate 2: Pan crust of bread and samples
Bread codes:
MI: Bread from mutant yeast from *agadagidi* and *burukutu*.
M2: Bread from mutant yeast of *Pito*
M3: Bread mutant yeast of palm wine
M4: Bread from mutant yeast *agadagidi*
M5: Bread from mutant yeast of *burukutu*.

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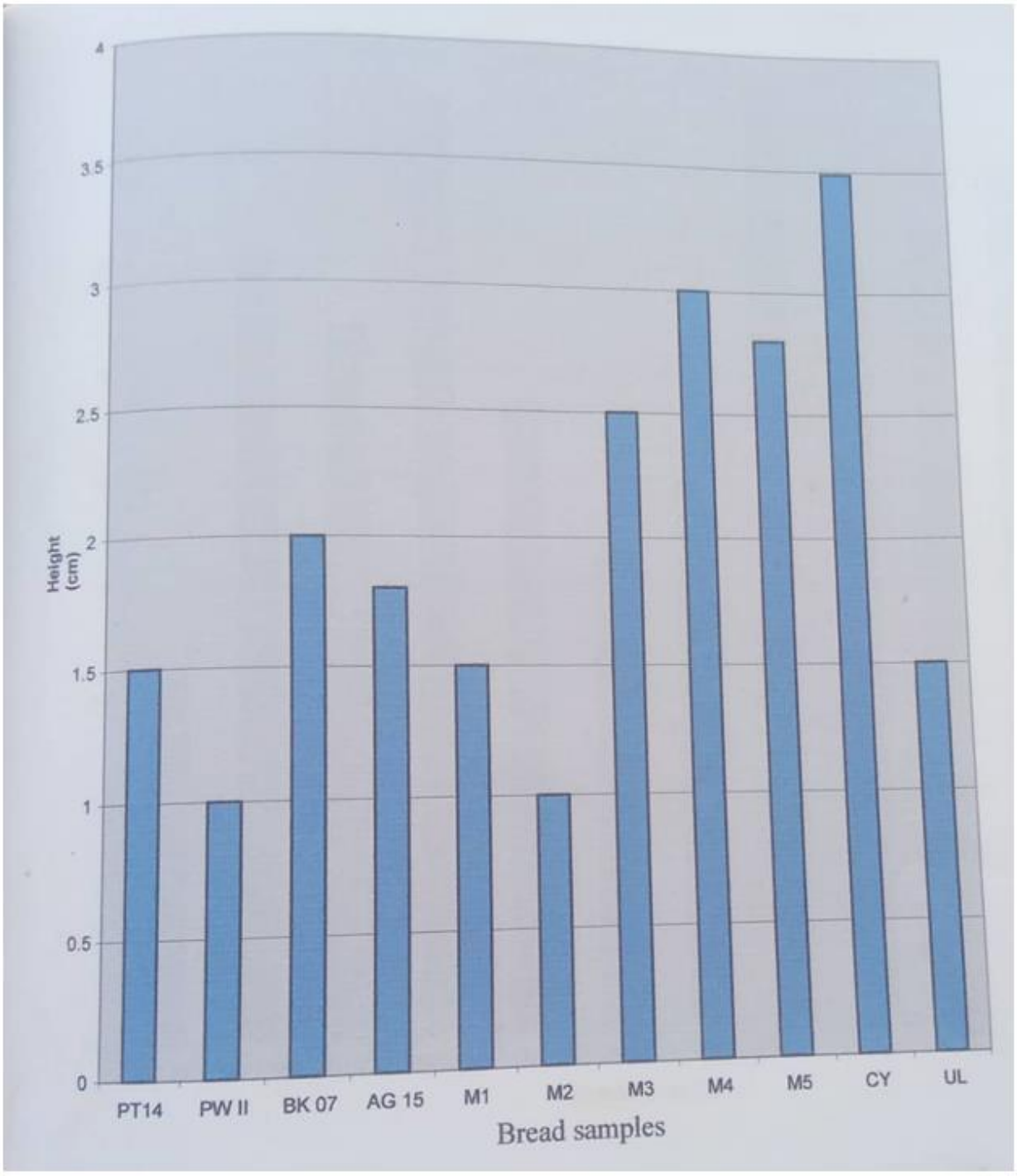

Figure 3: Height of bread samples
PT14: Pito yeast Bread
PWII: Palm wine yeast bread
BK07: B*urukutu* yeast bread
AG15: *Agadagidi* yeast bread
M1: Agadagidi and Burukutu mutant bread
M2: Pito mutant bread
M3: Palm wine mutant bread
M4: *Agadagidi* mutant bread
M5: *Burukutu* mutant bread
CY: Commercial yeast bread
UL: Bread without yeast.

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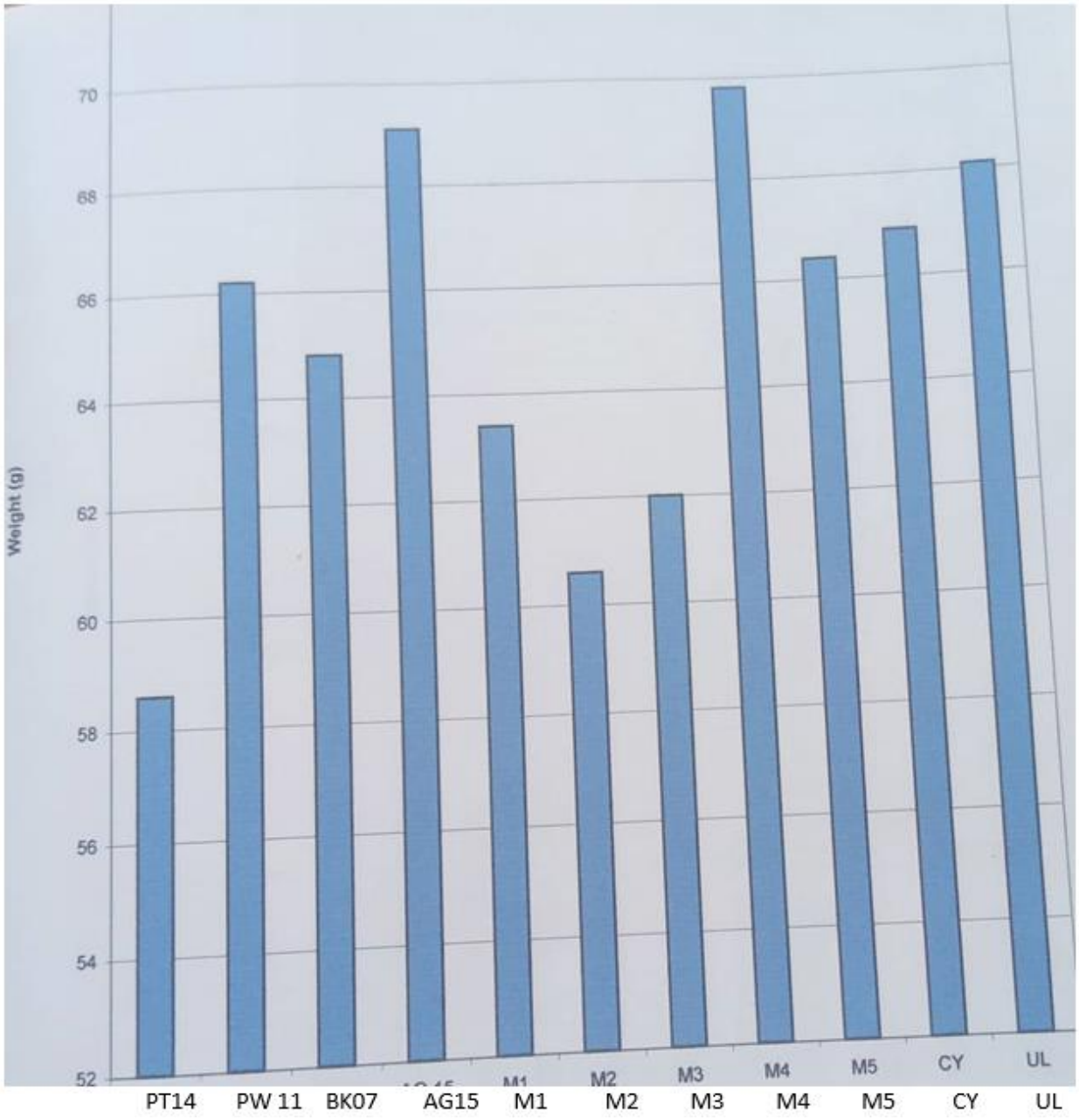

Figure 4: Height of bread samples
PT 14: Pito yeast bread
PWII: Palm wine yeast bread
BK07: *Burukutu* yeast bread
AG15: *Agadagidi* yeast bread
M1: *Agadagidi* and *Burukutu* mutant bread
M2: *Pito* mutant bread
M3: Palm wine mutant bread
M4: *Agadagidi* mutant bread
M5: Burukutu mutant bread
CY: Commercial yeast bread.
UL: Bread without yeast.

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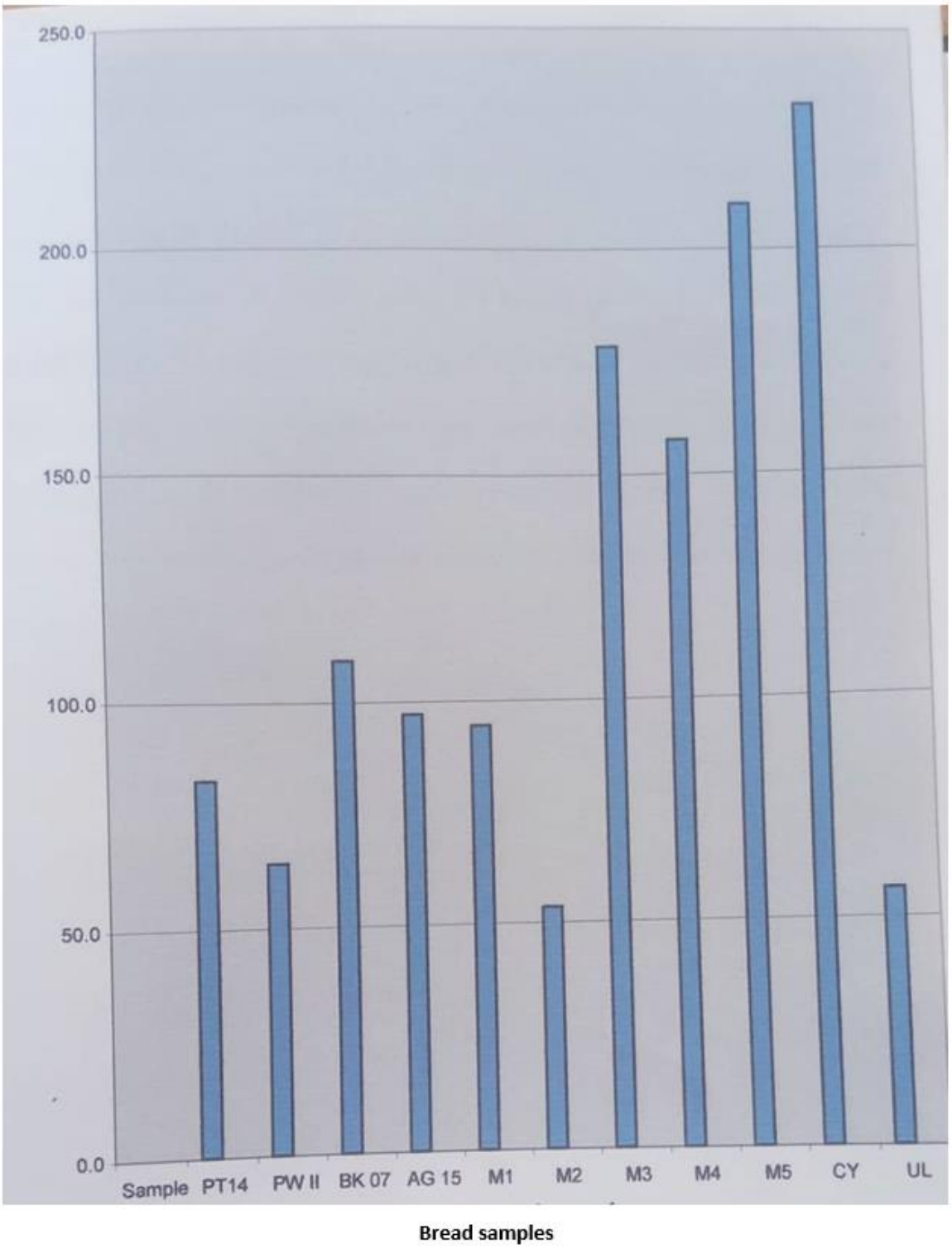

Figure 5: Height of bread samples

PT 14: Pito yeast bread
PWII: Palm wine yeast bread
BK07: *Burukutu* yeast bread
AG15: *Agadagidi* yeast bread
M1: *Agadagidi* and *Burukutu* mutant bread
M2: *Pito* mutant bread
M3: Palm wine mutant bread
M4: *Agadagidi* mutant bread
M5: Burukutu mutant bread
CY: Commercial yeast bread
UL: Bread without yeast.

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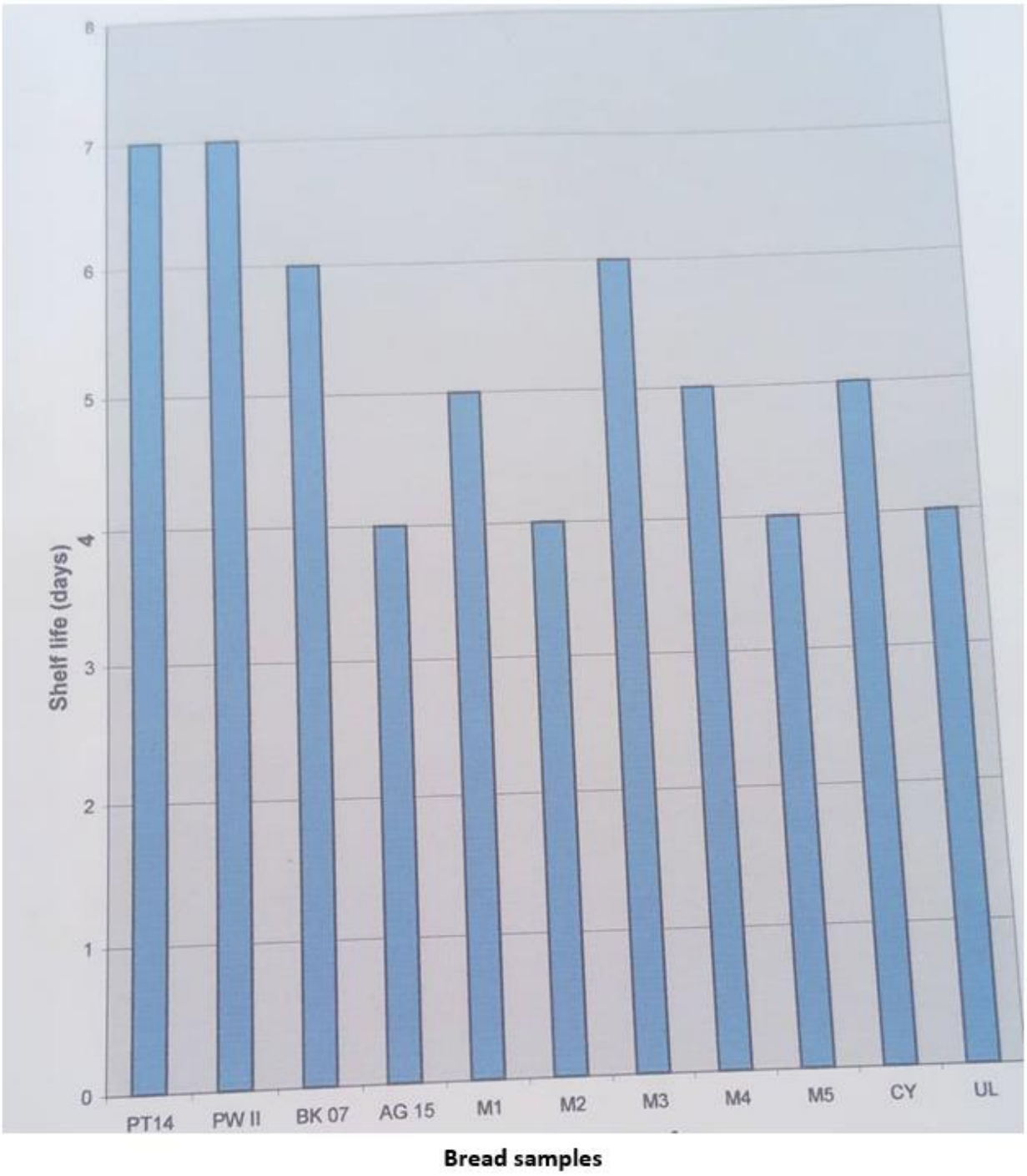

Figure 6: Shelf life of bread samples

PT 14: Pito yeast bread
PWII: Palm wine yeast bread
BK07: *Burukutu* yeast bread
AG15: *Agadagidi* yeast bread
M1: *Agadagidi* and *Burukutu* mutant bread
M2: *Pito* mutant bread
M3: Palm wine mutant bread
M4: *Agadagidi* mutant bread
M5: Burukutu mutant bread
CY: Commercial yeast bread
UL: Bread without yeast.

Table 3: Sensory evaluation of bread samples produced from *S. cerevisiae* isolated from indigenous alcoholic beverages

Species codes	Appearances	Texture	Taste	Crumb	Overall acceptability
PT14	6.9	6.3	6.5	6.0	6.4
PWII	7.4	6.4	6.3	7.1	6.8
BK07	7.9	7.1	6.3	6.6	7.0
AG15	7.5	8.1	6.7	6.5	7.2
M1	6.3	6.5	5.8	7.1	6.4
M2	6.3	6.3	6.2	6.7	6.4
M3	6.3	6.3	7.0	7.0	6.7
M4	7.3	6.6	6.6	7.1	6.9
M5	6.7	6.5	6.6	6.1	6.5
CY	6.9	6.7	5.5	6.6	6.4
UL	5.6	6.4	5.9	6.9	62

Key:
PT 14: *Pito* yeast bread
PWII: Palm wine yeast bread
BK07: *Burukutu* yeast bread
AG15: *Agadagidi* yeast bread
M1: *Agadagidi* and *Burukutu* mutant bread
M2: *Pito* mutant bread
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