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ORIGINAL RESEARCH PAPER

EVALUATION OF SURVIVABILITY AND BIOACTIVITY OF *SACCHAROMYCES CEREVISIAE* IN BREAD DOUGH

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ABSTRACT: One of the most significant current discussions in dough fermentation is survivability and bioactivity of *Saccharomyces cerevisiae*. In this study, four samples of instant dry yeast (all the yeast strains are active dry type) were evaluated by epifluorescence light microscopy using 0.2 % fluorescein diacetate. Microbial tests were carried out in order to count the number of viable cells yeast. Gasograph used to measuring CO_2 produced during fermentation. Bread volume and height were assessed. Yeast (A) had the highest number of green cells (178 ± 7) due to its more bioactivity as compared to types B, C and D ($P<0.05$) and yeast (D) had the lowest number of green yeast cells (48 ± 8). In microbial tests, the number of viable yeast in yeast (A) was highest (15×10^{10} cfu·mg^{-1}) and in yeast (D) was the lowest (9×10^{10} cfu·mg^{-1}). In gasography yeast (A) produced the highest amount of CO_2 (163 ± 2 mL CO_2/3h) and in yeast (D) produced the lowest amount of CO_2 (140 ± 2 mL CO_2/3h). Bread (A) (that made by yeast A) had the highest volume (132 ± 1.0 cm^3) and height (4.8 ± 0.3 cm) and bread (D) (that made by yeast D) had the lowest volume (102 ± 6cm^3) and height (3.7 ± 0.2 cm). High survivability and bioactivity of baker's yeast leads to more CO_2 production that leads to high volume and height in bread.

Keywords: *Baker's yeast, Bioactivity, Epifluorescence light microscopy, Gasography, Survivability*

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INTRODUCTION

Yeasts are one of the most widely used groups of microorganisms in food fermentation. *Saccharomyces cerevisiae* has been used in bread manufacturing for at least 6000 years. It has been a key ingredient in bread making [1]. It is responsible for the leavening of the dough, as well as for the formation of desired sensorial characteristics [2]. Leavening activity by carbon dioxide (CO_2) is an important quality index in the bakery's yeast industry [3]. The success of the technological process in bread making is conditioned by the formation of gas in the final hours of the technological process [4]. The characteristic structure and volume of yeast-fermented products depend on the production of CO_2 by the yeast [5]. Today, the activity of yeast cells in dough is usually determined either by measuring the gas production of the yeast or by the microbial plate count method. However, this method has the disadvantage that a large amount of sample is needed, and it is very laborious. It cannot be used for studying the state of yeast cells in dough at the time of freezing or the activity of yeast cells in different parts of the dough [6]. For sample two methods frequently used to evaluate the fermenting power of bakery yeast, one of them measures the time required for dough to attain a given volume and the other measures the volume of the dough for a given proof time [4 – 9]. The latter attribute is most conveniently determined by measuring the volume increase of fermenting dough, whereas gas production can be estimated by any of several available procedures such as the oven rise recorder method, alveography method or pressure meter methods. A Gasography system offers 12-channel mechanical gas measurement device for this purpose. Nowadays the Gasograph has been computerized. Because of the extreme importance of yeast bioactivity, it has attracted the attention of scientists and their efforts come in two main parts: the first part reviews the amount of carbon dioxide produced by the yeast and the other is to examine the yeast viability. Measurement of the gas production amount was done by assessing the volume and pressure of the produced gas [6, 10 – 13]. Also, microscopic techniques and microbiologically tests have been used for observation of yeast viability. In these tests researchers used special media cultures and methods of microscopic counting. A Few articles have been published on yeast viability. The viability of yeast plays an important role in industrial microbiology. Traditional methods of assessing viability based on counting colony forming unit (CFU) typically take between 24 and 48 hours, and are too slow for practical uses in bakeries [13]. The researchers developed a new method that, based on fluorescence microscopy, is able to detect active yeast cells in cryosections of wheat dough. The sections were stained with 4', 6-diamidino-2-phenylindole (DAPI) and counterstained with Evans blue. The active yeast cells in the sections appeared brilliant yellow and were readily distinguished from the red dough matrix. The dead cells allowed penetration of the Evans blue through the cell membrane, which interfered with the DAPI staining and caused the dead cells to blend into the red environment. Several staining methods in both fluorescent and non-fluorescent are available for determining the viability of yeast cells, but there is no absolute method. Several staining methods, both fluorescent and nonfluorescent, are available for determining the viability of yeast cells, but there is no absolute method. Existing methods can be grouped into three categories according to what is measured: loss of replication, cell damage (vital staining), and loss of metabolic activity. For the most part, the staining methods are very specific to the target in question. Most research

has been carried out with baker's yeast. The fluorescence techniques are of interest because they provide high sensitivity. Fluorescence studies have mainly been carried out with pure suspensions of yeast cells, which eliminate the problems of interfering background [8].
This study focuses on comparison of different methods for evaluating survivability and bioactivity of *Saccharomyces cerevisiae* in bulk bread dough that never have been compared together before and to assess the correlation between fermentation activity and survivability of bakery's yeast in dough. The novelty of this study is combination of evaluation survivability and bioactivity of *Saccharomyces cerevisiae* in bulk bread dough, using carbon dioxide volume measurement, epifluorescence light microscopy and colony counting. The aid of this investigation is to allow researchers to select the best way to evaluate the fermentation activity of baker's yeast.

MATERIAL AND METHODS

All the reagents used were obtained from Merck Company and were used without any further purification. Four samples of instant dry yeast were purchased from the local market and companies of Iran, including Fariman (Mashhad, Iran), Razavy (Mashhad, Iran), Golmaye (Tabriz, Iran) and Dezmaye (Ahvaz, Iran) and named A, B, C and D (Table 1). Flour was obtained from the Athar Company (Maragheh, Iran).

Table1. *List of yeast strains used in this study*

Collection number	Source
A	Fariman company, Iran
B	Razavy company, Iran
C	Golmaye company, Iran
D	Dezmaye company, Iran

The prepared yeast solution had a solid content of 10 % and initial leavening activity of 122 mL = 0.15 g dry yeast.

Preparation of dough

0.15 g of dry yeast was mixed with 20 g flour and then 15 mL distillated water at 30 degrees Celsius (30 °C) was added, and then mixed together. After mixing, immediately a small piece of dough (5 g) was gently rounded for Gasography method.

Measurement of gas production activity of yeasts in dough (gasography method)

According to Pieghambardoust *et al.* [12], a direct measurement of dough volume as a result of the CO_2 production during proofing was carried out using a simple measuring device. Figure 1 illustrates the scheme of the measurement apparatus. Immediately after processing, a small piece of dough (5 g) was gently rounded and placed inside a flask, which is submerged in a water bath at a constant temperature. The flask is connected via tubing to an inverted graded cylinder (250 mL) to collect the produced CO_2. To avoid any CO_2 dissolution in water, the water in the beaker was acidified to a *p*H of 2.5 (by adding sulfuric acid). The temperature of water bath was kept constant at 40 °C to

mimic the dough proofing process. The amount of gas produced was reported as cubic centimeters for 5 g dough. The gas volume was recorded for four samples of yeasts every minute during the total testing time (180 min).

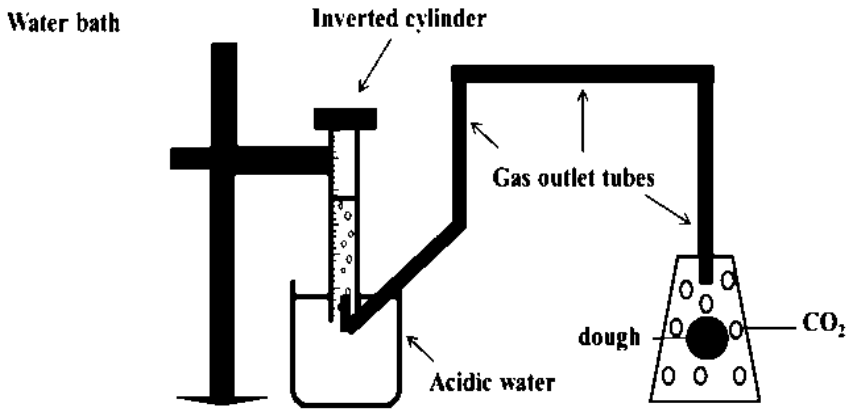


Figure 1. *Schematic presentation of the setup to measure proofing dough volume*

Preparation of suspensions

One gram of yeast powder was dissolved in 100 mL distilled water, then diluted to 10^{-6} and used for determination of living yeasts.

Determination of living yeasts

Slide samples of yeast suspension stained with fluorescein diacetate (FDA) were observed using an Epifluorescence microscope (EFLM) (Olympus bx51 microscope) with a 100-W Hg vapor arc lamp as a light source. The suspension of yeast cells was filtered through a membrane, and the yeast cells collected on the filter were stained and the number of active yeast cells was counted. Cells in a 10 mm^2 area were counted. Real color images were acquired using a two-stage Peltier cooled, Charge-Coupled Device (CCD) camera (Nikon, Eclipse, Evolution MP) with a spectral range of 290 - 1000 nm. The dynamic range of RGB was 14 bits. Staining was performed by the addition of 0.02 % FDA per 1 mL of sample and held for 10 min at room temperature in the dark.

Measurements of yeast cell survivability

Yeast survivability is determined as colony-forming units per gram of dry matter. After dissolving 1 g of dry yeast in 9 mL of tryptophan salt buffer and culturing in glucose agar media, incubation was done at 30 °C for 24 h. Obtained results were expressed as percentage of yeast viability.

Baking test

With the aim of studying the correlation between yeast viability results with bread volume and height, a handling bread-making test was carried out. Due to the evaluation of baker's yeast gas power ability, we did not use any reagents that increase or decrease

gas power of yeasts in baking test. The loaves were prepared using the following ingredients: 530 g of flour, 280 mL of water, 1.5 % yeast solution and 5 g of sodium chloride. Fermentation was carried out at 30 °C in 85 min with a relative humidity of 80 %. Cooking time was 25 min at 200 °C. Volume and height were recorded in all loaves.

Height and volume measuring

We used rapeseed displacement method for volume measuring in bread. For height measuring after the cut, bread height is calculated by a measuring instrument.

Statistics

Statistical analyses were performed using Cohort software (798 Lighthouse Ave. PMB 320) for Windows (Costat Anova version 6.311). Leavening ability, baking and viability were analyzed by variance analysis (ANOVA) using Duncan's multiple range tests. The experimental design included three trials for each experimental condition.

RESULTS AND DISCUSSION

The comparison of gas production activity in yeasts

The results with 4 samples of yeasts (A, B, C and D) as obtained on gasograph were presented in Figure 2.

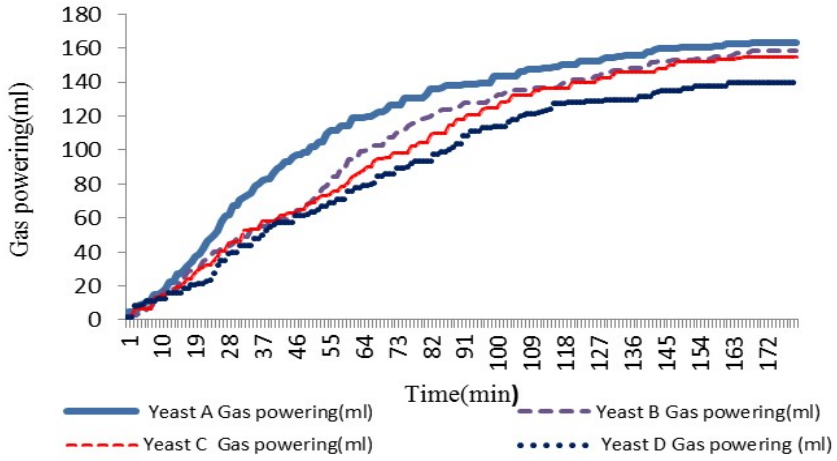
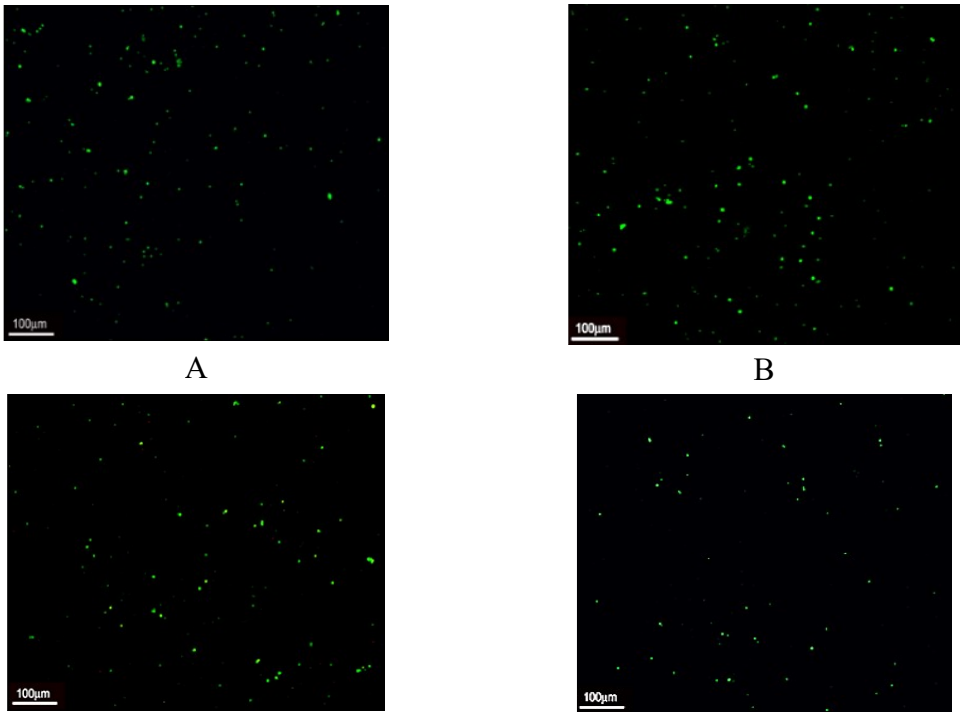


Figure 2. *Gas production activity of yeast samples (A, B, C and D) in bread dough*

As shown in this figure, for all yeasts, gas production rate increased over time, but in yeast A this more than in other samples. Results are due to yeast bioactivity in the fermentation medium. The comparison of gas production activity of yeasts showed that yeast gas production power was: A>B>C>D. So, the yeast sample A (163 ± 2 mL CO_2/3h) was the best sample for bread dough making industry.

Comparing of yeasts vitality

The images obtained from EFLM (Figure 3) confirmed gasography test. Yeast A had more living cells than others. Green cells show live yeasts in these images. Yeast vitality had a direct correlation with the gas production ability of them. Comparison of the vitality of yeasts showed that yeast vitality was: A>B>C>D. So more vitality of yeast (more cells that fluoresced green in Epifluorescence light microscopy test) lead to more bioactivity and gas powering.



A B C D

Figure 3. *Live yeasts in yeast-water suspension shown in EFLM after staining with FDA. Magnification, x 10*

Counting of live yeasts

Counting the number of live yeast was performed and the results were shown in Table 1. Live yeasts obtained by EFLM, done in five replicates at microscopic fields of 650 × 850 microns were counted. Results showed that the high number of green cells after staining with FDA lead to the high gas powering ability of yeasts. Counting of live yeasts showed that live yeast number was: A>B>C>D.

Table1. *Number of live yeasts in yeasts suspension at field of 650 x 850 microns*

Yeast	Colony forming units/mg dry matter[a]
A	15×10^{10}
B	14.6×10^{10}
C	12×10^{10}
D	10×10^{10}

[a] Results represent the means of duplicate measurements

Comparing of yeasts survivability

Colony counting of samples showed that number of cfu·mg^{-1} yeasts is different between them. Yeast A has a higher number of cfu·mg^{-1} than others and yeast D has the lowest. Gasography and EFLM tests confirmed this result (Table 2). In normal conditions if cfu·mg^{-1} in dry matter of yeast is higher than others, that yeast has higher amount of live cells, and had higher gas production power than other yeasts. Microbial test showed that a high number of colony forming units per mg dry matter of yeasts lead to high performance of them. Colony counting of samples was: A>B>C>D. So, more number of live yeast means more viability and bioactivity that leads to more gas production power.

Table2. *Comparing of cfu·mg^{-1} dry matter with same ratio of live yeast cells*

Yeast	Counting of live cells[a]
A	178 ± 7
B	163 ± 8
C	78 ± 9
D	48 ± 8

[a] Results represent the means of duplicate measurements

Comparing breads height and volumes

Baking test results also confirmed the previous tests' results. Yeast A use lead to the highest volume and height of breads while yeast D lead to the lowest volume and height of breads. This result showed a positive direct correlation between yeast survivability, amount of cfu·mg^{-1}, yeast gas production power and height and volume of breads. Height and volume of breads were: A> B>C>D and are compared in Table 3.

Table3. *Breads height and volume*

Yeast	Bread height average [cm]	Bread volume average [cm^3][b]
A	4.7 ± 0.3	132 ± 10
B	4.6 ± 0.3	127 ± 6
C	3.8 ± 0.2	112 ± 5
D	3.7 ± 0.2	101 ± 6

[b] Results represent the means of duplicate measurements

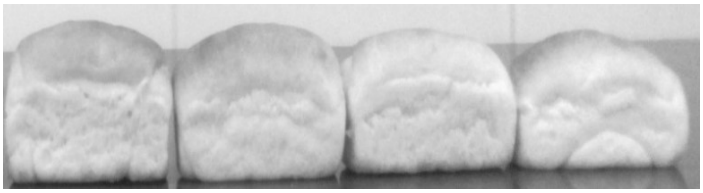


Figure 4. *Breads height and volume*

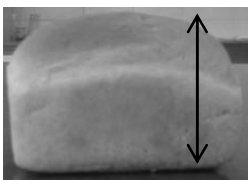


Figure 5. *Bread height measuring method*

Total comparison of gasography, microbial tests (cfu·mg^{-1}), EFLM test (microscopy staining) and baking test are shown in Table 4. All of the tests showed the same result. According to the results of these tests the yeast sample A was the best sample for the bread dough making industry because of its high bioactivity, high number of live cells in fermentation medium and high ability to gas production.

Table4. *Total comparison of gasography, microbial tests, EFLM test and volume and height of breads*

Yeast	Gas powering [mL]	Cfu·mg^{-1} dm	Volume [cm^3]	Height [cm]	Cell counting [EFLM]
A	163 ± 2	15×10^{10}	132 ± 10	4.7 ± 0.3	178 ± 7
B	159 ± 1.6	14.6×10^{10}	127 ± 6	4.6 ± 0.3	163 ± 8
C	154 ± 2	12×10^{10}	112 ± 5	3.8 ± 0.2	78 ± 9
D	140 ± 2	10×10^{10}	101 ± 6	3.7 ± 0.2	48 ± 8

A factorial analysis was calculated to value the significance level between the two factors and their interaction (Table 5).

Table5. *Variance and significance (P<0.001) for different parameters of yeasts*

	Leavening Aactivity	Viability	Baking
Yeast type (A)	43583.1***	236.3***	80.7***
Fermentation hour (B)	7763.9***	352.4***	2131.2***
A × B	691.7***	4.2***	30.3***
Error	48.5***	0.8***	2.5***

*** Significant level of 0.001

Pearson's simple correlation

All of tests have very high positive correlation together in significant level of 0.01 (Table 6).

Table 6. *Pearson's simple correlation between all of the tests*

Test name	Gasography	Microbial test	Epifluorescence light microscopy
Gasography			
Microbial test	0.97**		
Epifluorescence light microscopy	0.95**	0.97**	
Bread volume	0.97**	0.97**	0.96**

**Significant level of 0.01

CONCLUSION

This study focused on comparison of different methods for evaluating survivability and bioactivity of *Saccharomyces cerevisiae* in bulk bread dough. For all samples of yeast gas production rate was increased over time, but in yeast A this was more than other samples. This result is due to its highest bioactivity in the fermentation medium. The images obtained from EFLM confirmed gasography test results. Yeast vitality had a direct correlation with the gas production ability of them. So more vitality of yeast (more cells that fluoresced green in Epifluorescence light microscopy test) lead to more bioactivity and gas powering. Results showed that the high number of green cells after staining with FDA lead to the high gas powering ability of yeasts. In normal conditions

if cfu·mg^{-1} in dry matter of yeast is higher than others, that yeast has higher amount of live cells, and had higher gas production power than other yeasts. Microbial test showed that a high number of colony forming units per mg dry matter of yeasts lead to high performance of them. More number of live yeast means more viability and bioactivity that leads to more gas production power. Baking test results also confirmed the previous tests results. So, all of the tests showed a positive direct correlation between yeast survivability, amount of cfu·mg^{-1}, yeast gas production power and height and volume of breads.

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