

CHARACTERIZATION OF GENETIC VARIABILITY PARAMETERS RELATED TO LEAVENING ABILITY IN SACCHAROMYCES CEREVISIAE

Mervat I. Kamal

Department of Genetics, Faculty of Agriculture, Mansoura University, Egypt.
60 El Gomhoureya St., EL Mansoura, EL Dakahleya Governorate, Egypt.
Corresponding authors E. mail: dr_mervat@mans.edu.eg; Tel: 002-01008665560

<https://doi.org/10.35410/IJAEB.2023.5870>

ABSTRACT

Environmental variance is not dependent on the genotype and therefore subject to change. It is governed by some degree of genetic control. This study aimed to characterize genetic variability parameters and factors influencing on leavening ability of baker's yeast to determine the factors governing fermentation performance. To do so, three parental strains of *Saccharomyces cerevisiae* and two hybrid genotypes were used. Genetic variability parameters for the weight of fermented bread dough were assessed over the time of fermentation. The biomass weight of yeast strains and their hybrids was shown as follows: H1> H2> P1> P3> P2. This indicated more viability of hybrid yeast cells leading to generating high quantities of carbon dioxide. Hybrid genotypes exhibited heterogeneity at 8 g sucrose leading to evolution toward sugar stress. Heterosis obtained reflected the optimum range of divergence in gene expression in changing environments. Fermentation performance appeared phenotypic coefficient of variance (PCV) slightly higher than the correspondence genotypic coefficient of variance (GCV). This indicated the influence of environmental factors on fermentation activity. So, fermentation performance exhibited low heritability coupled with low and moderate genetic advance as a percent of the mean. This arises from the low values of genotypic variance. So, the fermentation performance was governed by non-additive gene action. The heterosis tool will be of great value for improving this trait followed by a selection of good quality genotypes. These, therefore, superior hybrid genotypes have a potential value in bread bread-making industry.

Keywords: Genetic Variability Parameters, Heritability, Genetic Advance, Heterogeneity, Leavening Ability, Survivability, *Saccharomyces Cerevisiae*, Recombinant Genomes.

1. INTRODUCTION

Saccharomyces cerevisiae is routinely used in the bread-making industry because it combines several advantages including fermentation power and production of desirable flavors. The bread-making industry occupies an important place in the food sector industries. Hybridization between yeast strains gives rise to offspring carrying an admixture of both parental genomes. The dominance of yeast in industrial fermentations limits the diversity of the end products. Here there is a growing interest in yeast genotypes that can help to generate the complexity of desirable traits in bread bread-making industry. The bread industry occupies an important place in the food sector industries. Hybridization between yeast strains gives rise to offspring carrying an admixture of both parental genomes. The budding cells of diploid *Saccharomyces cerevisiae* can express varied differentiation states during nutrient starvation. Thus, these states take place in response to starvation of one or more nutrients. This leads the yeast cells may leave the mitotic cycle to enter the meiotic cycle and form sporulation with

haploid spores (Tomova *et al.* 2019). Meiosis and sporulation in *Saccharomyces cerevisiae* is a good-studied example in genetics. The deprivation of nitrogen and fermentable carbon sources leads the diploid cells to enter meiosis to be generated asci containing four haploid spores (Kassir *et al.* 2003). If adverse conditions happen during meiosis as heat or limitation of the carbon source, then the yeast cells respond to these conditions by producing more asci containing two spores instead of four haploid genomes (Okamoto and Lino 1981). The cells of baker's yeast are heterozygous for the locus of mating type (Hartwell 1974). Baker's yeast, *Saccharomyces cerevisiae* was used in bread making industry (Newberry *et al.* 2002). The yeast cells metabolize flour sugars to release carbon dioxide and ethanol. Carbon dioxide (CO₂) released increased dough volume via the injection of air bubbles through the dough matrix (Romano *et al.* 2007). The bread volume provided a quantitative measure of leavening activity (Rathnayake *et al.* 2018). The elasticity of the bread core depends on the quality and quantity of gluten protein contained flour (Munteanu *et al.* 2019). Hybridization techniques based on mating between different genotypes of yeast have been successfully used for the isolation of hybrid genotypes (Spencer *et al.* 1985). Some recombinants obtained from hybridization had a much greater leavening ability of dough prepared from wheat flour than either the original strains of commercial baker's yeast (Wongkhalaung *et al.* 2004). Hybridization between *Saccharomyces cerevisiae* with cry-resistant mater strain produced a recombinant genotype with improved leavening performance in low and high-sugar wheat flour dough, in addition to freeze-tolerant ability (Wongkhalaung *et al.* 2004).

In natural populations, quantitative traits that vary continuously are often shown to be under stabilizing selection (Kingsolver *et al.* 2001). Genotypic variation in quantitative traits determined the amount of nature in evolutionary genetics. Furthermore, stabilizing selection showed the least residual environmental variability given genotypic value (Wagner *et al.* 1997). The distribution of environmental deviations is independent of genetic effects for that the phenotypic variance among individuals of the same genotype was the same for all genotypes (Falconer and Mackay 1996). Environmental variance including all variations of genetic sources embraces external conditions (e. g. nutritional, climatic factors, diseases) and internal conditions (e. g. developmental noise). Environmental variance is assumed to be free of such genetic components (Zhang *et al.* 2004). The mutant genes caused direct change in both mean and variance (Gibson and Dworkin 2004). The environmental variance is determined by the genotype can influence the variance and the mean of quantitative traits. Thus varies as a function of the genetic structure and sensitivity of phenotype to environmental conditions (Hill and Zhang 2004).

Maintenance of environmental variance was modeled by assuming some modifier loci may differ from the loci determining the mean effects but directly controlling the phenotypic variance (Wagner *et al.* 1997). The relative performances of different genotypes vary between different environments indicating the existence of genotype by environment interaction. This interaction provided a major challenge to understanding the genetic control of variability. The biometrical study of this interaction was important not only from genetic and evolutionary points of view but also concerning agricultural produce in general and plant breeding in particular (Breese 1969). Variability is the occurrence of differences among individuals due to variations in their genetic structure and/ or the environment in which they live (Falconer and Mackay 1996). For effective selection, the magnitude of variation in the populations is necessary (Yagdi 2009).

The choice of promising genotypes from diverse genetic bases is one of the techniques for improving the fermentation activity in yeast (**Mulugeta et al. 2013**).

Development of high fermenting yeast genotypes requires a knowledge of the existing genetic variations for fermentation and sugar utilization. The observed variability in yeast is a combined estimate of genetic and environmental causes. However, the assessment of heritability alone does not provide knowledge about the expected genetic gain in the next generation. Therefore, it must be considered in conjunction with the estimates of genetic advance as a percentage of the mean. This means the change in mean estimates between generations (**Wani and Khan 2006**). Hybridization in yeast has been used to generate genetic variability and has been successfully utilized to improve the fermentation of yeasts. This study shows that induced recombinants in yeast are a potential tool to be employed for yeast improvement.

Genetic-environment interactions are of great interest in evaluating the stability of breeding yeast genotypes under different environmental conditions. The reliability of yeast genotype performance across different environmental conditions can be a significant consideration in inducing new recombinant genotypes in yeast. Yeast geneticists are primarily concerned with high fermentation and stable genotypes as much as possible since new genotype development is a time-consuming endeavor. Successfully developed new yeast genotypes should have a stable performance and broad adaptation over a wide range of environments in addition to high fermentation performance. Evaluating the genotype's stability of fermentation performance and the range of adaptation has become increasingly important for improving yeast genotype programs. Hence, if genotypes are being selected for a large group of environmental conditions, therefore stability and the mean of fermentation performance across all the environments are more important than fermentation in specific environments (**Piepho1996**). The phenotypic variance among the yeast colonies of the same genotype is the same for all genotypes (**Zhang et al. 2004**). Environmental variance is all variations of nongenetic origin due to external environmental factors such as nutritional and climatic conditions. **Francis and Kannenberg (1978)** used the environmental variance, as well as, the coefficient of variation to define stable genotype.

Variability is the occurrence of differences among the cells resulting from hybridization due to differences in their genetic composition or to the environmental effects (**Falconer and Mackay 1996**). For efficient selection, information about the nature and magnitude of variability in the population of yeast cells is necessary (**Yagdi 2009**). The choice of better promising genotypes from diverse genetic bases, as well as, their subsequent utilization for hybridization is one of the techniques for improving yeast fermentation capabilities (**Mulugeta et al. 2013**). Assessment of genetic parameters such as phenotypic and the genotypic coefficient of variations (PCV, GCV), heritability (h^2), as well as, genetic advance (GA) for different economic traits in yeast are important in designing an effective producing technique (**Kozgar2014**). The genotypic coefficient of variation estimates the range of genetic variations shown in yeast populations. The GCV alone cannot measure the amount of variation that is heritable (**Wani 2011**). Information about heritability is essential for indicating the extent of transmissibility of genes-related traits into future generations, as well as, it is important for selection-based improvement. Assessment of heritability alone does not indicate information about the expected genetic gain in future generations. It must be joined with estimates of genetic advance, the alteration in mean value between generations (**2011**). Hence the present investigation was undertaken to evaluate genetic

variability parameters and heritability of recombinant genotypes with their commercial parental lines for bread-leavening ability.

2. MATERIALS AND METHODS

Stains and growth conditions

Three diploid strains of *Saccharomyces cerevisiae* were isolated from different commercial samples in the market, as well as two hybrids resulted from two hybridizations between the parental strains were used in this study (**Table 1**). These strains were described before by **Kamal (2023)**. Yeast strains and their hybrid genotypes were grown in the complete medium yeast extract peptone glucose medium (YEPG). It consists of 1% yeast extract, 2% bacto-peptone, and 2% glucose according to **Tomova et al. (2019)**. The strains were stored on slopes from the same medium in a refrigerator, at 4 °C.

Table 1. Yeast strains and their hybrids were used in this study.

Sample code	Sample	Source
P ₁	Pakmaya	Pak Gida Uretim Ve Pazarlama A. S., Made in Turkey
P ₂	Holw El-Sham	Holw El-Sham Company for Food Industries and Agriculture investment (S. A. E), 6 October City, Egypt
P ₃	Dreem	Dreem Mashreq Foods (S. A. E) New Borg El-Arab City, Alexandria, Egypt.
H ₁	Hybridization	P ₁ x P ₃
H ₂	Hybridization	P ₁ x P ₂

Prepared yeast cell suspension

Each genotype of yeast strains and their hybrids was grown in 250 ml YEPG medium, pH 6.0 at 30 °C in 500 ml Erlenmeyer flasks. The flasks were undergone shaking (160 rpm) at 30 °C for 72 hours. The cultures were centrifuged to isolate yeast cells in the stationary phase. The cells were weighted, washed twice with distilled water and then resuspended in 210 ml tap water to prepare yeast cell suspension to be used in preparing the dough (**Almeida and Pais 1996**).

Dough manufactured

The following ingredients were mixed manually as well to prepare bread dough as follows; 325 g wheat flour, 3.5 g salt, 210 ml yeast cell suspension, in addition to the following sucrose concentrations, 0, 2, 4, 6 and 8 g sucrose to each weight of bread dough.

The dynamic of fermentation power

After baking bread dough, each sample was cut into three symmetrical cores. The cores were weighted and directly transferred into a 500 ml baker without water. The dough was immediately fermented in an incubator at 40 °C for 15 minutes. The dynamics of fermentation were evaluated during the fermentation period by weighting the dough at zero time, as well as, every 5 minutes. Dough size and weight were significantly influenced by the carbon dioxide released by the yeast cells in bread dough (**Kasaie et al. 2017**).

Testing the viability of cells with methylene blue stain

The viability of dried yeast cells was defined as the percentage of survived cells in the whole population. This was assayed using a stain-based technique through methylene blue stain which provide rapidly the objective results. Cell viability was important to estimate the physiological state of yeast cells after drying which influenced dough fermentation. The mode of action of methylene blue depends on the properties of the yeast cell membrane. The survived cells can reduce the dye color of methylene blue and are still colorless, while dead cells are unable to do this and therefore stained blue. This technique distinguishes between alive or dead cells and surviving cells in the whole population (**Mirek and Tecza 2014**).

Survivability of cells

The survivability of yeast cells was measured as colony-forming cells per gram of dried yeast cells. Dissolving one gram of dry yeast cells in nine ml tryptophan salt buffer and then culturing in yeast extract peptone glucose (YEPG) medium solidified with 2% agar. Dilutions were made if necessary. The petri dishes were incubated at 30 °C for 24 hours. Obtained data were expressed as the percentage of yeast cell survivability (**Kasaie et al. 2017**).

Viability assays

The accepted level of survival was ranged between 60-80%. The values of viability were calculated according to **Luarasi et al. (2016)** as follows;

$$\text{Viability percentage} = \frac{\text{Total counted cells} - \text{Total counted of dead cells}}{\text{Total counted cells}}$$

Cell concentration

The cells were grown in 250 ml YEPG medium prepared in 500 ml Erlenmeyer flasks. The flasks were undergone shaking (160 rpm) at 30 °C for 72 hours. Then, cultures were centrifuged to isolate the biomass of cells which weighted according to **Luarasi et al. (2016)**.

Homogeneity assessment

The degree of homogeneity between yeast hybrids and their parental strains was estimated based on the coefficient of variation for the weight of bread dough after fermentation according to

Gomez and Gomez (1984).

Statistical analysis

All experiments conducted in this study are in triplicates. The data were subjected to the analysis of variance (ANOVA) according to **Steel and Torie (1960)** to be used as the variance components for the assessment of genetic parameters. In addition, factorial analysis was used according to **Steel and Torie (1960)** to investigate the effects of multiple variables on fermentation power. This is because factorial analysis enables geneticists to examine the main effects of each variable and their interaction on the fermentation performance. This analysis provides a more comprehensive understanding of the interrelationship between variables. This technique can gain deeper insight into the effect of each variable on the fermentation power. Interactions between variables reflected how the effects of one variable may differ across the levels of another variable. These interactions may be additive (no interaction), synergistic (enhances the effect) or antagonistic (diminishes the effect).

The phenotypic and genotypic variance were estimated according to **Singh and Chaudhary (1985)**. In addition, heritability in a broad sense and genetic advance were estimated according to **Allard (1999)**. The estimated parameters included the phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV) and expected genetic advance (GA). The expected genetic advance was expressed as a percentage of the mean value with an assumed 1% selection intensity by the formula of **Allard (1960)** as stated by **Khan (1979)**.

3. RESULTS AND DISCUSSION

Viability of yeast cells

The results of three samples for survivability of commercial yeast are presented in **Table 2**. The weight of yeast cells among three samples of yeast is different between them. The weight of the P₁ genotype is higher than others and the P₂ genotype has the lowest. The weight of hybrid yeast cells is higher than the parental strains. Therefore, hybrid yeast cells have a higher amount of live cells, leading to higher gas production than their parents. The hybrid H₁ genotype showed a biomass yield of 1.77. This means that the biomass yield by hybrid yeast cells of the H₁ genotype was increased above the mid-parents by 177%. Meanwhile, the hybrid cells of the H₂ genotype produced a biomass yield reached 1.01. This means that the biomass yield by H₂ genotype was increased above the mid-parent with 101%. Therefore, heterosis in biomass yield reached 177% and 101% for the hybrid yeast cells of the H₁ and H₂ genotypes, respectively. This leading hybrid yeast cells had higher carbon dioxide production in the fermentation medium than their parents. This reflected that hybrid yeast cells had a high number of colony-forming units per gram of yeast cell suspension that led to higher fermentation performance than their parental strains. Biomass weight was H₁> H₂> P₁>P₃> P₂. So, more biomass weight means more viability of yeast cells and bioactivity which leads to more gas production power during fermentation.

Table 2. Number and weight of live yeast cells and their hydrolysis in yeast suspension.

Genotypes	Weight of biomass (g)	Yield of biomass	Viability	Number of colonies / 0.1 ml yeast cell suspension
P ₁	1.80	1.00	0.93	532
P ₃	1.56	1.00	1.16	444
MP	1.68	1.00	1.04	488
H ₁	4.66	2.77		
Heterosis	1.77	1.77		
P ₁	1.80	1.00	0.93	532
P ₂	1.32	1.00	0.78	110
MP	1.56	1.00	0.85	321
H ₂	3.14	2.01		
Heterosis	1.01	1.01		
F – test	NS	**	NS	**
LSD 0.05	4.18	0.54	2.77	219.89
LSD 0.01	6.95	0.92	4.60	365.16

NS, ** : Not significant and significance at 0.01 probability level, respectively.

The viability of dried yeast when analyzed using a stain-based method (with methylene blue) provides the following results: $P_3 > P_1 > P_2$. Therefore, cell viability is required for assessing the physiological state of yeast cells after drying. The physiological state has an important influence on fermenting bread dough and dough growth. The mechanism of action of methylene blue depends on the properties of the cell membrane. Thus, living cells can reduce methylene blue and remain colorless, meanwhile, dead cells are unable to do this and therefore stained blue. This technique enables microbial geneticists to observe a single yeast cell, making a distinction between alive or dead cells and viable cells, as well as, assessing the percentage of these two categories among the whole population (**Mirek and Tecza 2014**). Thus, dough prepared with a high number of alive cells was strongly influenced by this amount of cells and its ability to retain the gas which led the dough volume to expand.

It has been found that cell survival was $P_1 > P_3 > P_2$. Therefore, colony counting of three genotypes showed that the number of alive cells is different between the three genotypes. The genotype P_1 had a higher number of yeast cells than others and the P_2 genotype had the lowest. This indicated that the yeast genotype has a higher amount of live cells and may have higher gas production power than other genotypes. So, more live yeast cells means better fermentation performance leading to the highest volume and height of bread dough. These results agreed with **Kasaie et al. (2017)**, who found a positive direct correlation between yeast survivability, amount of cell frequency unit per mg, yeast gas production power, as well as, volume and height of fermented bread dough. According to the results obtained herein, hybrid yeast cells are the best genotypes for the bread dough-making industry, because of their higher biomass yield that leads to high ability of gas production. Among all parental genotypes P_3 is the better genotype because it has higher biomass yield than others. This result is due to its highest bioactivity which had a direct correlation with gas production activity. This is in harmony with **Kasaie et al. (2017)**, who decided that a greater number of live yeast cells means high viability and bioactivity that leads to high quantities of gas production power. The results obtained herein also agreed with **Munteanu et al. (2019)**, who found that bread dough prepared with yeast cells from Dr. Oetker and Pakmaya recorded increases in dough height reached to 321.43%, respective 226.32% versus the initial height of dough. Meanwhile, the low viability of yeast cells from the other parental genotypes may delay fermentation power. Therefore, the highest decrease in fermented bread weight was recorded by hybrid yeast cells due to a larger quantity of CO_2 , which led to an increase in the volume of bubbles existing in the dough, where the weight declined.

Looking at the baking weight loss, it might be said that hybrid yeast cells, as well as, the P_3 genotype are the most appropriate in fermentation performance. Therefore, bread prepared with these genotypes requires a shorter fermentation time compared with doughs prepared with other genotypes of yeast cells. Thus, bread dough fermented with these superior yeast genotypes had the highest porosity value leading dough to be sufficiently fermented. Therefore, bread doughs showed higher losses in weight when using hybrid yeast cells in the fermentation process. This is because of high gas production leading the volume of the dough to rise and reduce in density and weight. This agrees with **Munteanu et al. (2019)**, who demonstrated that the fermentation process of bread dough is a major step in obtaining good quality bread with high volume, texture, and taste of final bread product. The same authors also found that the fermentation speed of dough is strongly influenced by the yeast genotype and dough fermentation condition. Meanwhile, **Kasaie et al. (2017)** reported that one of the most significant discussions in bread dough fermentation is the survivability and bioactivity of yeast cells. The

same authors found that yeast sample (A) had the highest number of green cells and yeast sample (D) had the lowest number of green cells, stained with fluorescence diacetate (FDA). In microbial genetic tests, **Kasaie et al.(2017)** found that the number of viable yeast cell (A) genotypes was highest and lowest in yeast (D) genotype. In gasography yeast (A) produced the highest gas productivity and yeast D produced the lowest amount of CO₂. In addition, bread dough fermented with the A genotype had the highest volume and height, whereas bread fermented with the D genotype had the lowest volume and height. Therefore, the high viability of yeast cells leads to more CO₂ production which leads bread dough to high volume and height.

Homogeneity of hybrids

The degree of homogeneity was assessed depending on the coefficient of variability (**Table 3**), which was used to determine the magnitude of variations within every genotype. Hybrid H₁ genotype showed values lower than the check of mid-parent values at all fermentation times under the effect of 0.0 and 4 g sucrose in the fermentation medium, indicating high homogeneity at these concentrations. This is because the H₁ genotype recorded a coefficient of variability close to or lower than the check value.

Table 3. Coefficient of variance for the weight of bread dough fermented with yeast strains and their hybrids under sucrose stress.

Genotypes	Sucrose concentrations (g/ 325 g wheat flour)/ fermentation time											
	0.00				2				4			
	0	5	10	15	0	5	10	15	0	5	10	15
P ₁	0.17	0.15	0.15	0.15	0.12	0.17	0.17	0.17	0.10	0.10	0.10	0.10
P ₃	0.04	0.04	0.10	0.04	0.01	0.01	0.01	0.01	0.06	0.06	0.06	0.06
MP	0.11	0.19	0.13	0.09	0.06	0.09	0.09	0.09	0.08	0.08	0.08	0.08
H ₁	0.04	0.04	0.08	0.04	0.23	0.20	0.20	0.20	0.07	0.07	0.07	0.07
P ₁	0.17	0.15	0.15	0.15	0.12	0.17	0.17	0.17	0.10	0.10	0.10	0.10
P ₂	0.06	0.06	0.06	0.06	0.12	0.07	0.07	0.05	0.07	0.07	0.05	0.07
MP	0.12	0.11	0.11	0.11	0.12	0.12	0.12	0.11	0.09	0.09	0.09	0.09
H ₂	0.12	0.12	0.12	0.12	0.05	0.05	0.15	0.05	0.19	0.02	0.02	0.02

Table 3. Continued.

Genotypes	Sucrose concentrations (g/ 325 g wheat flour)/ fermentation time							
	6				8			
	0	5	10	15	0	5	10	15
P ₁	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.05
P ₃	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
MP	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
H ₁	0.05	0.05	0.05	0.05	0.11	0.11	0.11	0.11
P ₁	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.05
P ₂	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
MP	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04
H ₂	0.02	0.02	0.02	0.02	0.25	0.24	0.14	0.09

At the remaining sucrose concentrations including 2, 6, and 8 g sucrose, the H₁ genotype recorded higher values than that in check of the mid-parent, indicating high heterogeneity. Furthermore, the H₂ genotype recorded a coefficient of variability higher than the check at all fermentation times under the effect of 0.0 and 8 g sucrose in the fermentation medium, indicating high heterogeneity. In addition, the hybrid H₂ genotype appeared the same trend after 10 minutes of fermentation time under the effect of 2 g sucrose in the fermentation medium. Furthermore, the H₂ genotype recorded coefficient of variability values lower than the mid parent under the effect of 2, 4 and 6 g sucrose in the fermentation medium, indicating high homogeneity over these concentrations. These results agreed with **El-Morsy et al.(2021)**, who reported that seven genotypes of tomato showed a coefficient of variance lower than that in the check cultivar, indicating high homogeneity, whereas genotypes recorded their values higher than that of the check indicating high heterogeneity in this trait.

Generally, the degree of homogeneity differed for each genotype among the different sucrose concentrations for the same trait, as well as, from genotype to another at the same concentration of sucrose. These results agreed with **Ahmed et al.(2017)**, who found that the F₁ hybrid in tomatoes recorded high heterogeneity for most traits, whereas 14 selected genotypes are enough homogenous since they recorded a coefficient of variance near or lower than those of the two check varieties. The results are also in harmony with **Islam et al.(2011)**, who selected some lines of tomato from the F₂, F₃, and F₄ generations which became higher inhomogeneity after the F₆ generation, then could be considered as new lines. The heterogeneity obtained by H₁ and H₂ hybrids at 8 g sucrose concentration indicated that yeast hybrids adapted to new brewing conditions, which facilitated hybrids to tolerate sucrose stress, leading to the evolution of these genotypes. This suggests a link between hybrid genotypes and tolerance to sucrose stress (**Krogerus et al.2017**). The high level of heterozygous variants is the outcome of presenting two chromosome sets divergent, one from each parental strain.

The allele heterozygosity will be proportional to genetic divergence between the parents, which is detrimental to the viability of hybrids in changing environments as seen at higher sucrose concentrations (8 g). Therefore, hybridization played an important role in the evolution of many yeast species likely adaptation to new niches. This represents the optimum range of divergence in gene expression of successful hybrid offspring (**Gabaldón2020**). The results obtained in this study are consistent with numerous studies that described *Saccharomyces cerevisiae* hybrid as a suitable leavening agent for the preparation of baked products (**Asyikeen et al. 2013**). The results showed that yeast hybrids were able to assimilate the higher concentration (8 g) of sucrose. In general, *Saccharomyces cerevisiae* ferments the sugars present in the dough in the following order: glucose, sucrose, fructose, as well as, lastly maltose (**Oda and Ouchi 1990**). Flour from wheat contains mostly starch, maltose, sucrose, fructose, glucose, as well as, other oligosaccharides (**Randez-Gil et al. 2013**). Meanwhile, 2 g sucrose added to the flour as an ingredient in the preparation of dough improved leavening performance. Overall, the leavening properties of hybrid yeast cells which appeared heterogeneity at 8 g sucrose classify them as probable active starters in dough fermentation. This attribute hybrids has an important technological value. Therefore, an assessment of sucrose tolerance is needed to classify better source-tolerance hybrid genotypes than the parental strains. This agrees with **Zhou et al.(2017)**, who found that yeasts isolated from high osmotic foods were more resistant to 6% NaCl than commercial baker's yeast. However, a high dose of sucrose exerts severe osmotic stress on yeast cells resulting in cell damage and reduced fermentation ability (**Struyf et al. 2017**). To avoid this,

yeast cells should have the appropriate resistance tools toward sucrose stress. This parameter affects cell viability and influences the behavior of yeast cells during fermentation. Therefore, hybrid genotypes that appeared more heterogeneous than their parents had the best performance as a leavening agent in dough proofing and stress tolerance if compared with the parental strains.

Genetic variability parameters

As shown from the results tabularized in **Table 4**, the phenotypic coefficient of variance (PCV) was greater than the genotypic coefficient of variance (GCV) values across different concentrations of sucrose at all fermentation times. The differences between PCV and GCV were medium. The PCV and GCV values exceeded 20% at all sucrose concentrations, except for at 6 g sucrose. According to **Deshmukh et al. (1986)** PCV and GCV above 20% are regarded as high. When these values range between 10 and 20% they are regarded as medium. If these values are below 10% they are regarded as low.

Table 4. Genetic variability parameters influenced the weight of fermented bread dough over fermentation time under sucrose stress.

Genetic parameters	5 min					10 min				
	0	2	4	6	8	0	2	4	6	8
σ^2G	23.99	41.83	21.47	2.54	60.78	29.89	41.75	64.88	2.52	24.17
σ^2E	72.08	125.51	64.46	13.10	182.37	97.70	125.26	66.45	13.07	99.56
σ^2P	96.07	167.34	85.93	15.64	243.15	127.59	167.01	131.39	15.59	48.17
PCV %	17.76	55.59	39.83	17.04	67.02	48.64	55.55	49.34	17.02	48.17
GCV %	21.05	27.79	19.91	6.86	33.51	23.54	27.77	34.67	6.48	21.29
ECV %	75.01	27.80	19.92	10.10	33.51	25.10	27.78	14.67	10.18	26.88
H %	24.97	24.99	24.98	16.24	24.99	23.42	24.99	49.37	16.16	19.53
EGA	6.49	8.25	6.13	1.68	9.95	6.90	8.24	15.02	1.67	78.68
GAM %	5.99	7.61	5.66	1.56	9.19	6.39	7.61	13.91	1.55	73.78

Table 4. Continued.

Genetic parameters	15 min					Mean				
	0	2	4	6	8	0	2	4	6	8
σ^2G	23.98	41.74	64.40	2.55	99.95	25.95	41.77	50.25	2.54	61.63
σ^2E	72.03	125.23	64.41	13.18	23.48	80.60	125.33	65.11	13.11	101.80
σ^2P	96.01	166.97	128.81	15.73	123.43	106.55	167.10	115.37	15.65	138.25
PCV %	42.14	55.55	48.79	17.10	48.08	36.18	55.56	45.98	17.05	54.42
GCV %	21.06	27.77	34.50	6.88	20.97	21.88	27.78	29.69	6.86	25.26
ECV %	21.08	27.78	14.29	10.22	27.11	40.40	27.79	16.29	10.17	29.17
H %	24.97	24.99	99.98	16.21	19.02	24.45	24.99	58.11	16.20	21.18
EGA	6.24	8.55	29.86	1.68	5.60	6.54	8.35	17.00	1.68	31.41
GAM %	5.77	7.90	27.59	1.56	5.24	6.05	7.71	15.72	1.56	29.40

σ^2G , genotypic variance; σ^2E , environmental variance; σ^2P , phenotypic variance; PCV (%), phenotypic coefficient of variation in percent; GCV (%), genotypic coefficient of variation in percent; ECV (%), environmental coefficient of variation in percent; H (%), heritability in broad

sense in percent; EGA, expected genetic advance; GAM (%), genetic advance as percent of mean at 5% selection intensity.

Based on this classification PCV and GCV obtained in this study were regarded as high values. The mean environmental coefficient of variance was relatively greater than the genotypic coefficient of variance at 0, 2, 6, and 8 g sucrose concentration. This indicated the greater share of environmental variance in the total variability of fermentation. Meanwhile, the mean of the genotypic coefficient of variance was relatively greater than the environmental coefficient of variance under the effect of 4 g sucrose. This indicated the greater share of genotypic variance in the total variability under the effect of 4 g sucrose in the fermentation medium.

The small difference obtained between PCV and GCV under the effect of 4 g sucrose reflected that the environmental effect was small for the expression of fermentation genes under this concentration of sucrose. Meanwhile, the mean of ECV was greater than GCV across the other concentrations of sucrose. The mean of differences between ECV and GCV showed considerable low variability. This indicates that little opportunity for improvement in fermentation power through selection alone. Therefore, the improvement of fermentation performance in yeast needs to induce new recombinants through hybridization followed by a selection of superior genotypes. These results agree with **Chand et al.(2008)**, who found that day-to-maturity in barley appeared considerable variability, this indicated little opportunity for improvement through selection. The genetic variability parameters were worked out in this study to understand which of the variations were obtained due to genetic or environmental factors. However, fermentation performance revealed PCV and GCV above 20% across all sucrose concentrations, except for at 6 g sucrose. These are classified as high values according to **Deshmukh et al.(1986)**. Therefore, fermentation performance was partially governed by additive gene action. So, there is a scope for improvement in fermentation performance through hybridization followed by selection for further improving the yeast genotypes program. This agrees with **Wani and Khan (2006)**, who reported that high phenotypic and genotypic variance indicate better chances for selection. The environmental coefficient of variance exceeds 20% under the effect of 0, 2 and 8 g sucrose. This indicates the higher influence of environmental factors than genetic makeup in the expression of fermentation genes. This is in harmony with **Amir et al. (2015)**, who decided that the larger difference between GCV and PCV is due to the higher influence of environmental factors on that trait. In addition, **Nechifor et al. (2011)** suggested that the presence of a relatively large difference between GCV and PCV was attributed to environmental factors. Therefore, genetic variability is a prerequisite to the selection of better genotypes in any organism.

Thousands of years of human selection have led to the loss of potentially important allelic variation. Strategies aimed to increase genetic variations in the populations can improve the efficiency of improving techniques. Variation can be assessed phenotypically and genotypically. Genotypic analysis allows precise assessment of DNA variation. Meanwhile, phenotypic analysis provides important results on the expression of important traits and their heritability. In general, high coefficient of variations reflected that there is a scope of selection for improving these traits. Lower values in the coefficient of variability indicated that the population needs to create variability either by hybridization or induced mutations followed by selection as seen in this study (**Tiwari et al. 2011**). The magnitude of PCV was found to be slightly higher than the respective GCV. This might be a result of the influence of environmental factors on the fermentation process. This agrees with **Idris and Mohamad(2013)**, who found small differences

between GCV and PCV for plant height and panicle length in some rice genotypes. Thus, hybridization followed by selection based on fermentation performance would be effective in bringing out considerable genetic improvement. Besides, there were considerable differences between PCV and GCV. This indicates a greater influence of environmental factors on the phenotypic performance of fermentation. Therefore, selection alone would be ineffective for genetic improvement of fermentation expression genes.

Heritability

According to **Johnson et al.(1955)**, heritability was classified as low (below 30%), high (above 60%) and medium (30-60%). Considering this delineation, lower heritability values were observed across the effect of 0, 2, 6 and 8 g sucrose. Meanwhile, medium heritability was observed under the effect of 4 g sucrose in the fermentation medium. Low heritability values in a broad sense indicated that fermentation performance is highly influenced by environmental factors. Therefore, yeast geneticists must use hybridization followed by selecting superior genotypes based on fermentation performance. The lower heritability values limit the scope of improvement through selection. This is in harmony with **Dursun (2007)**, who also reported low broad-sense heritability for grain yield in *Phaseolus vulgaris*. The disparity in this study could be because heritability is important for improvement not only the fermentation performance but also for the population of yeast cells and the environment to which the genotypes are subjected. Therefore, its ultimate value depends on the magnitude of all variance components. Thus, the concept of heritability demonstrates whether differences obtained among genotypes arose as a result of differences in the genetic makeup or due to environmental factors. If the heritability of a trait is very high it reaches 80% or more. Then selection for such a trait could be easy. This is because there was a close correspondence between the genotype and the phenotype due to the small contribution of the environmental factors to the phenotype (**Singh 2001**). Therefore, fermentation performance exhibited low heritability in this study indicating the higher effect of environmental factors on the phenotypic expression of this trait. This leading selection alone would be ineffective in the improvement of the fermentation process but it must be before hybridization. **Johnson et al. (1955)** categorized genetic advance as a percent of mean as low (0-10%), medium (10-20%) and high (20% and above). In this study, low heritability was coupled with low genetic advance across the effect of 0, 2, and 6 g sucrose. Moderate heritability (58.11%) was coupled with moderate genetic advance (15.72%) under the effect of 4 g sucrose. In addition, low heritability (21.18%) was coupled with high genetic advance (29.40%) under the effect of 8 g sucrose. In this respect, **Johnson et al. (1955)** suggested that heritability values along with genetic advancement are more helpful than heritability value alone in predicting the resultant influence of selecting the best genotypes. Genotypic coefficient of variation, heritability and expected genetic advance showed considerable effects of environmental factors on fermentation expression genes. Therefore, the improvement of the fermentation process must be subjected to hybridization at first to increase genetic variations in the population. This is because this trait would not have a high selection value to be improved through selection alone but it must be before hybridization.

The low estimate of genetic advance as a percent of the mean arises from low estimates of genotypic variance and heritability. This is in line with **Eid(2009)**, who found low heritability coupled with low genetic advance for plant height and number of grains per spike in wheat, indicating slow progress of improvement through selection. The trait possessing low heritability

coupled with high genetic advance as seen at 4 g sucrose indicates the presence of non-additive gene action in controlling this trait. Thus, the selection procedure will not be effective for screening the desirable trait of fermentation performance. This agreed with **Sardana et al.(2007)**, who suggested that high heritability values might not necessarily lead to increased genetic gain without sufficient genetic variations existing in the population. Therefore, heritability in conjunction with genetic advancement would present a more reliable selection value (**Johnson et al. 1955**). Low estimates of genetic advance as a percent of the mean obtained in this study indicate that fermentation performance was governed by non-additive gene action and the heterosis procedure will be useful for improving this trait. Similar findings were obtained by **Hoque (2013)** for the number of fertile tillers per panicle in rice. Therefore, hybridization followed by selection based on the fermentation performance of hybrid genotypes could increase the mean performance of the selected progenies (**Ejara et al. 2018**).

Interestingly, baker's yeast used in fermenting bread dough was subjected to various environmental stress conditions. This could provide useful information about its ability to carry out fermentation under these conditions as impaired yeast. These stresses include osmotic and ethanol stress (**Phaff and Starmer 1987**). In this respect, **Pataro et al.(2000)** suggested that most *Saccharomyces cerevisiae* strains isolated from conventional fermentation were physiologically adapted to extreme conditions. Yeast cells used in bread-making produced ethanol as a secondary metabolite. A suitable concentration of ethanol is needed to achieve the preferred flavor in bread making. High concentrations of ethanol are toxic to yeast cells via inhibiting cell growth due to the destruction of cell membranes (**Smit et al. 1992**). Salt as one of the dough ingredients is another stress to baker's yeast. It modifies flavor and controls the rate of fermentation and enzyme activity. With salt gluten holds more water and carbon dioxide leading the dough to expand without tearing (**Snodgrass 2004**). Therefore, bread making industry was influenced not only by the yeast genotypes but also by environmental factors as seen from the genetic variability parameters assessed in this study. Thus, the dynamics of fermentation and intensity of CO₂ are influenced by flour properties, dough ingredients, as well as, enzymes in the dough (**Sluimer2005**).

Temperatures showed significant effects on the dough volume and the end product of bread. The appreciable level of leaving was at 37 °C. This temperature is required for optimal enzyme activity (**Aboaba and Obakpolor 2010**). The same authors found that dough volume increased as fermentation time increased but only to a limited extent because a longer time of fermentation (40 min) caused a drastic drop in volume. The agitation enhances the ability of the dough to acquire more air which influences the size and quality of the bread. **Istudor et al. (2020)** found that there was a correspondence between CO₂ values and the obtained volume of bread dough. The level of dough fermentation is correspondence with the proving temperature and the quantities of CO₂ released during a specific time. The same authors found that the quantities of CO₂ released by yeast cells after 37°C de-development curve do not have a linear pattern. Therefore, doubling the volume of dough is considered a criterion to stop the fermentation step to start the baking step(**Chevallier et al. 2012**). Furthermore, **Hackenberg et al. (2017)** found that the enzymatic activity in dough is influenced by grinding. This influences the hydrolysis of fermentable carbohydrates, dough rheology and dough viscosity. They could influence the resulting bread quality. Grinding starch granules resulted in better hydrolysis by amylolytic enzymes leading to an increase in the substrate concentration for yeast (**Hackenberg et al. 2017**).

Ethanol concentration was not the sole source causing stress in yeast cells during fermentation. The concentration of CO₂ dissolved in the fermentation medium seems to play an important role in the stress of yeast because of trehalose synthesis (**Guadalupe-Daqui et al. 2023**). Yeast cells can synthesize trehalose in response to physiological stressors like temperature and high concentration of ethanol (**Wang et al. 2014**). Therefore, this study provides an understanding of how environmental factors influence on fermentation process. Thus, this investigation significantly shows the link between fermentation attributes factors typically the yeast genotypes, as well as, environmental conditions in the bread location industry. The latter is the main significant approach in fermentation performance based on the health of yeast genotypes. It is interesting to note that the genetic variability parameters reflected that fermentation performance was influenced not only by the yeast genotypes but also mainly by environmental factors.

Factors influenced fermentation power.

As shown from the results tabulated in **Table 5**, the genotypes of baker's yeast showed a highly significant effect on fermentation activity at 0, 4, 6 and 8 g sucrose concentrations. Meanwhile, the fermentation times, as well as, the fermentation times by genotypes interaction achieved insignificant effect on fermentation performance overall sucrose stress. This indicated that the bioactivity of yeast genotypes is likely linked to the increase of yeast cells in suspension which leads to gas production power. Therefore, yeast vitality had a direct correlation with gas production ability. So, more vitality of yeast cells leads to more bioactivity and gas powering. Therefore, heterosis in yeast confers a competitive advantage in changing environments by facilitating transgressive phenotypes in fermentation power. This is known to be a driver of yeast evolution and adaptation (**Steensels et al. 2021**). Thus, heterosis in yeast is especially important because of the many fermentation stages in which rapid adaptation may be advantageous. Therefore, hybrid yeast cells resulting from the large genetic distances between parental strains are especially important to confer a competitive advantage over changing environments. A total comparison of factors affecting fermentation power indicated that yeast genotypes are the best factor affecting on bread dough-making industry rather than fermentation time and the interaction between both factors. So, more live yeast cells means more vitality and bioactivity which leads to more carbon dioxide production power.

Table 5. Sources of variance affecting the weight of fermented bread dough at different sucrose stress.

Sources of variation	DF	Sum and mean squares at different sucrose concentrations								
		0			2			4		
		SS	MS	F	SS	MS	F	SS	MS	F
Replications	2	4.33	2.16	0.08 ^{NS}	0.81	0.41	0.03	18.03	0.02	0.001 ^{NS}
Genotypes	4	702.43	175.60	6.83**	300.78	75.19	6.55**	712.56	0.96	0.04 ^{NS}
Fermentation times	2	0.14	0.07	0.003 ^{NS}	0.03	0.02	0.001	0.04	0.02	0.001 ^{NS}
Genotypes x Fermentation times	8	0.13	0.02	0.001 ^{NS}	0.21	0.03	0.002	0.22	0.03	0.001 ^{NS}
Error	28	720.08	25.70		321.67	11.48		738.90	26.37	
Total	44	1427.11			623.5			1469.75		

Table 5. Continued.

Sources of variation	DF	Sum and mean squares at different sucrose concentrations					
		6			8		
		SS	MS	F	SS	MS	F
Replications	2	22.99	11.49	1.69	1.86	0.93	0.03 ^{NS}
Genotypes	4	172.12	43.03	6.33**	790.34	197.58	6.93**
Fermentation times	2	19.36	9.68	1.42	0.02	0.01	0.00 ^{NS}
Genotypes x Fermentation times	8	19.47	2.43	0.36	0.21	0.01	0.00 ^{NS}
Error	28	190.67	6.80		789.12	28.50	
Total	44	424.61			1581.55		

SS, MS, F: Sum squares, mean squares, calculated F, respectively.

** : Significance at 0.01 probability level.

NS: Not significant.

Reduced the weight of fermented bread dough confirmed a direct link between yeast genotypes, cell survivability, yeast gas production, height and volume of bread. These results agreed with Kasaie et al. (2017), who found that yeast genotype A produced the highest amount of CO₂ if compared with yeast genotype D which produced the lowest amount of CO₂. Therefore, bread fermented with A genotype had the highest volume and height. In contrast, bread fermented with the D genotype had the lowest volume and height. The same authors also concluded that the high survivability and bioactivity of yeast cells lead to increased CO₂ production that increased the volume and height of bread. The results are also in line with Krogerus et al. (2015), who found that interspecific hybrids in *Saccharomyces cerevisiae* with *Saccharomyces eubayanus* inherited beneficial properties from both parents. These hybrids showed apparent heterosis because they are fermenting faster and producing beer with higher alcohol content than their parents.

As shown from the results presented in Table 6, sucrose concentrations appeared significant effect on fermentation performance by all yeast strains and their hybrids. Meanwhile, the fermentation times and sucrose concentrations by fermentation times interaction achieved insignificant effect on fermentation power.

Table 6. Sources of variance affecting the weight of fermented bread dough by different yeast genotypes.

Sources of variation	DF	Sum and mean squares of different yeast genotypes								
		P1			P3			H1		
		SS	MS	F	SS	MS	F	SS	MS	F
Replications	2	5.00	2.50	0.06 ^{NS}	20.09	10.04	1.48 ^{NS}	1.48	0.74	0.04 ^{NS}
Sucrose concentrations	4	1104.06	122.73	3.05*	175.56	43.88	6.47**	498.36	124.59	6.61**
Fermentation times	2	0.366	0.024	0.001 ^{NS}	0.025	0.01	0.002 ^{NS}	0.01	0.001	0.00 ^{NS}
Sucrose concentrations x Fermentation times	8	227.57	28.44	0.7 ^{NS}	0.065	0.01	0.001 ^{NS}	0.2	0.001	0.001 ^{NS}
Error	28	1125.62	40.20		190	6.78		527.45	18.83	
Total	44	2462.61	193.89		385.73	60.72		1027.5	144.16	

Table 6. Continued.

Sources of variation	DF	Sum and mean squares of different yeast genotypes					
		P2			H2		
		SS	MS	F	SS	MS	F
Replications	2	159.99	32.50	2.46 ^{NS}	2.29	0.76	0.02 ^{NS}
Sucrose concentrations	4	880.79	220.15	6.77**	928.77	232.19	5.76*
Fermentation times	2	0.09	0.04	0.001 ^{NS}	35.99	17.99	0.45 ^{NS}
Sucrose concentrations x Fermentation times	8	0.26	0.03	0.001 ^{NS}	217.38	27.17	0.67 ^{NS}
Error	28	910.10	32.50		1127.33	40.26	
Total	44	1951.23	285.22		2311.76	318.37	

SS, MS, F: Sum squares, mean squares, calculated F, respectively.

*, **: Significance at 0.05 and 0.01 probability levels, respectively. NS: Not significant.

These results agreed with **Luarasi et al. (2016)**, who decided that yeast cell membrane is affected by the stresses that occur through the brewing process, especially during storage. In the sweet dough used in this study, the ability of yeast genotypes to ferment dough under sucrose stress is of crucial industrial importance. The assimilation of sucrose varied among the genotypes. This indicated some metabolic diversity related to the genetic makeup of baker's yeast that can be harnessed in industrial applications. It can be seen that the genotypes that tolerated sucrose are very active in invertase production. Because the invertase inverts sucrose into glucose and fructose during fermentation. Then, the content of reducing sugars decreases because of their intense consumption by the yeast cells. This leads to the conclusion that the hybrid genotypes have a high fermentation rate.

The leavening ability of bread dough is a result of CO₂ released by fermenting genotypes which is usually *Saccharomyces cerevisiae* (**Plyer 1973**). Because they are converted fermentable sugars in bread dough into carbon dioxide. This caused the dough to expand or rise in volume as the CO₂ formed bubbles in the dough. When the dough is baked the bubbles remaining to be giving the product a spongy texture. Dough ingredients also influence fermentation power because they are usually agitated by mixing to create air bubbles. The dough volume increased as agitation time increased only to a limited value because a longer time of leavening (40 min) caused a drastic drop in volume. The agitation enhances the ability of the dough to acquire more air. The period of bread dough mixing is significant as it influences bread size and quality (**Aboaba and Obakpolor 2010**). Sweetbreads consist of high sugar concentrations reached in some recipes to 40% sucrose per flour weight (**Takagi and Shima 2015**). The high dose of sucrose in bread dough exerts severe osmotic stress on *Saccharomyces cerevisiae* resulting in cell damage and reduced the activity of fermentation (**Struyf et al. 2017**). To avoid harmful stress, *Saccharomyces cerevisiae* genotypes should have the appropriate resistance mechanisms which may be better in hybrid yeast cells. Therefore, hybridization in yeast followed by selection was preferred for obtaining the appropriate resistance mechanism with optimal growth. This leads to the highest tolerance genotypes toward sucrose stress. The survival of hybrid yeast cells under sucrose stress indicates that these genotypes can handle leavening ability over a wide range of sucrose. This could have a positive impact on increasing CO₂ production leading the dough volume to rise and the development of new flavors.

Sweet dough is a selective stress on the propagation of hybrid yeast cells. Therefore, the resistance of hybrid yeast genotypes to high sucrose stress is essential to select the most competitive starter to be used in sweet dough fermentation. So, hybrid yeast cells possess values considered desirable in bread-making industry. This agrees with **Almeida and Pais (1996)**, who found that half of yeast strains isolated from corn and rye bread dough produced between 400 and 500 ml of CO₂ in three hours per 100 g of dough. According to the results obtained by **Istudor et al. (2020)** about the graphics between temperature and CO₂ production by compact yeast the CO₂ development curves are linear. Taking into this consideration the same authors found that there is a correspondence between carbon dioxide values and the obtained volume of fermented bread dough. The increase in CO₂ released by yeast cells is very much influenced by the characteristics of flour used in preparing the dough, as well as, the quality and quantity of yeast genotype (**Istudor et al. 2020**). The temperature of the dough at the starting point of fermentation is also important. Therefore, high-quality yeast genotypes will result in a high concentration of CO₂ during fermentation time. Therefore, **Wongkhalaung and Boonyaratankornkit (2007)** found that hybrid genotypes of baker's yeast

achieved better maltose fermentation than their parental strains. Maltose is the main fermentable sugar in flour ingredient. **Oura et al.(1982)**decided that lower invertase activity genotypes were considered beneficial for fermentation power in low and high-sugar-containing bread doughs. Interestingly, **Wongkhalaung and Boonyaratanakornkit (2007)** found that the hybrid genotype of baker's yeast improved leavening activity with distinctively higher scores in sweetbread dough. It was evident that hybrid genotypes of yeast cells have high potential in leavening activity to be used in bread bread-making industry as a result of their CO₂ release leading to better bread quality if compared with their parents. Therefore, yeast genotypes showed a significant effect on the weight of fermented bread dough in terms of total gas production when applied in bread bread-making industry. Thus, hybrid yeast cells appeared to be suitable for bread making industry, especially with sweet bread dough (**Wongkhalaung and Boonyaratanakornkit 2007**)

Varying concentrations of sucrose were added to different doughs to improve the product flavor and as a nutrient source for yeast cells (**Nagodawithana and Trivedi 1990**). If sucrose concentration is high as in sweet dough yeast cells severe osmotic stress. This is because sucrose damages cellular components leading to reduced fermentation power(**Verstrepen et al. 2004**). Indeed, yeast cells exposed to hyperosmotic pressure exhibited rapid cell dehydration that limits cell growth and CO₂-generated capacity (**Randez-Gil et al. 2003**). As a consequence, the final volume of baked products was decreased (**Hernandez-Lopez et al. 2003**). Therefore, baker's yeast needs to induce new recombinant genotypes through hybridization or mutation adapted to the hyperosmotic stress in the doughs.

High sugar levels in dough prolong the time of fermentation that the yeast cells need to start producing CO₂ at acceptable rates. **Aslankoohi et al.(2015)**demonstrated that yeast mutants produced higher rates of osmolyte glycerol during overexpression of *GPD1* had taken a shorter fermentation time than the wild type. The same authors also demonstrated that the total quantities of carbon dioxide released by baker's yeast in dough containing 18% sugar is much lower than that in dough containing 6% sugar due to prolonging the time that the yeast cells need to release their maximal fermentation rate. Indeed, yeast cells need to acquire osmo tolerance via induction of stress protein expression, as well as, changes in their membrane composition (**Shima and Takagi 2009**). When osmotic pressure is sensed, then yeast cells can accumulate glycerol and trehalose (**Shima and Takagi 2009**). The synthesis of trehalose is catalyzed by the glucose-dependent-trehalose synthase protein complex which is encoded by four genes (**Jules et al. 2004**). Proline accumulation also confers tolerance to high sucrose stress (**Sasano et al. 2012**). Therefore, most strategies aimed to increase the osmo tolerance in baker's yeast are based upon improved accumulation of intracellular products such as glycerol, trehalose, or proline.

The time of fermentation can affect the rate of CO₂ released by yeast cells leading to a decrease in the weight and density of fermented bread dough during bread dough fermentation. Indeed, increasing fermentation time can increase dough maltose levels and the damaged starch content, as well as, amylase activity in the flour (**Potus et al. 1994**). If sugars are limited, maltose serves as a nutrient for yeast cells during fermentation. The effect of fermentation time on generated CO₂ is also dependent upon the genotype of yeast. The results obtained herein agreed with **Sahlström et al. (2004)**, who found that short fermentation time was needed to reach maximal fermentation rates with compressed yeast. Meanwhile, instant dry yeast needed prolonged time to allow full rehydration of yeast cells to increase the fermentation rate.

At the onset time of fermentation, gene expression changes drastically. Furthermore, genes involved in glycerol synthesis are up-regulated at the beginning times of fermentation. This indicated that the osmotic response is activated. This reflected that yeast cells in bread dough suffer from severe osmotic pressure. Thus, induction of glycerol synthesis is necessary for optimal leavening (**Aslankoohi et al. 2015**). In that middle fermentation phase, the genes involved in the amino acids and vitamin metabolism, such as riboflavin and thiamin synthesis are up-regulated. At the end of fermentation time, the cells suffer from nutrient depletion. Then, the pathways related to starvation and stress responses are induced (**Aslankoohi et al. 2013**). Hydrolysis of sucrose to glucose and fructose increases osmotic pressure on yeast cells (**Evans 1990**). Osmo tolerance is a factor determining the performance of yeast genotypes in high sugar environment. Thus, **Myers et al. (1997)** found that invertase is the most important determinant of fermentation activity in sweet doughs. The same authors stated that there was a strong correlation between the performance of yeast cells in high-sugar media and their capacity to produce and retain glycerol intracellular. It was shown that all yeast genotypes utilized respective sugars as sucrose used in this study also produced CO₂. The carbon dioxide generated during the fermentation process is prominent as a leavening agent of dough which leads the dough volume to expand and reduces its weight. The assimilation of sucrose varied among the yeast genotypes which indicated some metabolic diversity in yeast genotypes. Therefore, yeast genotypes have a significant impact on increasing the size of dough and reducing its weight, where it converts fermentable sugars in the dough into CO₂. It is interesting to note that the release of CO₂ corresponded with decreasing the weight of fermented bread dough.

The dynamics and intensity of carbon dioxide released by yeast cells influenced by the weight of fermented bread dough are affected by sucrose concentrations, yeast genotypes, fermentation time, etc. These factors are interdependent. The fermentation process takes place if there are optimal conditions regarding yeast genotypes, dough ingredients, and fermentation time. Thus, the proving time under favorable conditions allows for efficient action of yeast genotypes and enzymes in the dough (**Sluimer 2005**). Sufficient proofing time results in products with increased volume and high crumb structure. Whereas, excessive proofing can lead to sticky doughs with reduced viscosity that are difficult to handle. Furthermore, excessive proofing and fermentation times also can produce reduced costs for the bakers (**Spinelli et al. 2008**). Thus, the traits necessary for good control of proofing are the proofing time, fermentation time, temperature and relative humidity. The optimum proofing time can vary between 15 and 60 minutes, depending on dough weight, dough loaf, the quality genotype of yeast, the quantity of yeast, fermentation temperature and relative humidity. If the bread-making industry applied a multi-phase technological process, the proofing time would be greater than that in the direct technological process (**Burluc 2007**). To obtain optimal genotype performance for leavening dough, it is important to harvest yeast cells in the early stationary phase (**Rezaei et al. 2014**). Thus, optical density (OD) must be measured before harvesting yeast cells to find the best OD for cell harvest before being used in dough inoculation. Next, tested the weight of fermented bread dough which reflected CO₂ production if the weight was greatly reduced. Specifically, the good fermentation performance of dough with hybrid yeast genotypes resulted in a highly reduced weight of fermented bread dough if compared to bread fermented with the reference yeast strain. This is because the size of fermented bread dough was expanded.

Bread dough fermentation is an important process in bread bread-making industry. Yeast metabolizers flour sugars into CO₂ gas. The gas was diffused in the air nuclei formed in dough

during the mixing of dough ingredients. The final CO₂ volume can be over 70% of the loaf volume. This leading the weight of fermented bread dough to reduced (Scanlon and Zghal 2001). All phases of bread bread-making industry can influence gas cell size distribution (Bloksma 1990). Chiotellis and Campbell (2003) found that increasing the fermentation temperature increases the final bubble size. This is partly due not only to an increased rate of CO₂ production but also as a result of decreased gas solubility. Frozen dough was used for controlling the dough expansion as shown by Le-Bail *et al.* (2010). A rapid freezing rate results in better preservation of the final bread dough volume. A longer fermentation time could allow for reaching larger volumes of dough at lower fermentation temperatures. The fermentation needed to stop when the volume of dough was doubled to start the baking step. Chevallier *et al.* (2012) decided that the increase in dough hydration increased the expansion rate. This may result from the reduced surface tension of liquid film (less water concentrated) that surrounds the gas bubbles which leads the dough volume to expand during fermentation.

Besides, the fermentation temperature had a very significant impact on the rate of fermentation. Li *et al.* (2014) found that the mechanical forces used for grinding the dough led starch granules to break down into smaller particulates. This increased their surface area and thus the hydration rate. High levels of mechanical grinding leading flour starch can be better hydrolyzed by amylolytic enzymes, in contrast to native starch which is resistant to digestion via enzymatic reaction (Dhital *et al.* 2010). It must be assumed that bread dough volume increases was associated with the decreasing in their weight due to increasing gas formation. The weight of fermented bread dough was reduced due to enough fermentable mono- and disaccharides with available yeast genotype which led to increasing gas formation. This in turn seems that the enzymatic activity of hydrolyzed flour starch is influenced by grinding. This influences the hydrolysis of fermentable carbohydrates and dough rheology, which in turn influences the resulting bread quality (Hackenberg *et al.* 2017). Maltose was produced by starch hydrolysis due to amylase, a starch-degrading enzyme in flour. The amount of mono and disaccharides in flour reached 4mg/g of flour: Sucrose is the most abundant reaching more than 50% of the total soluble sugars (Hutkins 2006). Sucrose is converted immediately to glucose and fructose by yeast invertase (Sahlström *et al.* 2003).

Maltose is constantly releasing new glucose and maltose in flour starch and glucose is the preferred sugar. Maltose is the main disaccharide formed under the action of flour amylase. When glucose and fructose are finished, maltose concentration begins to decrease due to hydrolysis by amylase. The yeast cells do not have the necessary enzymatic equipment for degrading maltose (Gabriela and Daniela 2010). The content of reducing sugars decreases due to their consumption by yeast cells. The quantity of sugars through the fermentation time varies with the yeast genotypes used in ascending order. The yeast genotype with a high fermentation rate revealed a high decrease in bread dough weight which no longer contains glucose (Gabriela and Daniela 2010). The same authors found that the maximum height of dough was correlated with the quantity of CO₂ released by yeast cells. From this point of view, the decrease in bread dough weight was related to the quantities of CO₂ released during fermentation time, which corresponds to glucose fermentation. Therefore, the active genotype of yeast presents the most intensive fermentation activity. Because it adapts most easily to sucrose stress. Carbon dioxide released by yeast cells transforms the dense mass of dough to a specific volume and flavor. No bubbles are created during the fermentation time. The volume of bubbles already presented in the dough can only be increased due to CO₂ production which leads to increasing the dough volume.

Looking at the weight of baking loss, it might be said that yeast genotypes were affected by this trait because of CO₂ production by yeast cells. Therefore, the hybrid yeast cells are the most appropriate in terms of bread bread-making industry.

However, genetic diversity was important in *Saccharomyces cerevisiae* to select high-quality genotypes in fermentation performance. HO encodes endonuclease that causes DNA double-strand breaks in the locus and determines sex type. This already performs a sex change and allows the cell to mate with another cell of the opposite mating type. Hybrids are produced in baker's yeast by using drug resistance markers and the HO technique. CRISPR/Cas9 was used to force DNA double-strand breaks in the mating type locus to increase the genetic diversity in baker's yeast and produce their hybrids(Krogerus *et al.* 2021). Hybridization in *Saccharomyces cerevisiae* bears several advantages in brewing to isolate transgressive phenotypes that increase fermentation performance or stress tolerance (Fu *et al.* 2015). Create hybrids in baker's yeast vary in their specificity to target genetic or phenotypic results. Efforts were made to induce yeast hybrids in brewing broadly to increase the variability of fermentation performance in yeast. These added depth to the complexity of fermentation performance and advanced fermentation science based on genetic knowledge. During industrial application, yeast spend their life in active growth and metabolism of sugars into ethanol, flavor molecules and carbon dioxide to prepare fermented foods for human consumption(Winans2022). Therefore, yeast genotypes showed a significant impact on the fermentation of bread dough. As shown from the results one of the most significant impacts on bread dough fermentation is the genotypes of yeast strains. Therefore, this study highlights the impact of genomic diversity in *Saccharomyces cerevisiae* on fermentation performance. Thus, increasing research investigating the hybrid nature of modern industrial yeast must be understood to be recognized in fermentation powering.

Baker's yeast has been a key ingredient in bread bread-making industry for at least 6,000 years. The success of genotypes in releasing CO₂ is an important index in the yeast industry (Ahi *et al.* 2010). The fermenting power of yeast genotypes is usually determined by measuring the weight of fermented bread dough which reflects the gas production by each genotype of yeast cells. This agrees with Kasaie *et al.* (2017), who found that there was a direct correlation between gas production and yeast vitality. So, more viability of yeast cells led to more bioactivity and gas powering.CO₂ generated throughout the fermentation process is a passive final product of yeast metabolism (Guadalupe-Daqui *et al.* 2023). The increased CO₂ caused deleterious effects on yeast cells as a consequence of the fermentation process. Chen and Gutmanis(1976)found significant inhibition in yeast growth if the concentration of CO₂ above 0.65 g/L dissolved in the media. The same authors found a 20% reduction in fermentation power if CO₂ concentration reached 1.1 g/L. Guadalupe-Daqui *et al.* (2023) found that each gram of glucose produced 0.49 g ethanol and 0.47 gCO₂ during fermentation. Therefore, CO₂ released during fermentation time was calculated based on sugar consumption over time.

Concluding remarks

This study focused on comparison between *Saccharomyces cerevisiae* strains for their survivability, as well as, between yeast strains and their hybrids for bioactivity in bread dough fermentation. In addition, it measures genetic variability parameters influenced by fermentation power. The viability of dried yeast after being stained with methylene blue provides the following results: P₃> P₁> P₂. At the same time, the biomass weight of the P₁ genotype was higher than other parental strains. Biomass yield by hybrid yeast cells of H₁ and H₂ genotypes

was increased by 177% and 101% over the mid-parent, respectively. These results lead hybrid yeast cells to achieve the highest bioactivity to produce higher quantities of CO₂ in the fermentation medium. Because yeast viability had a direct correlation with gas production ability. So, more vitality of yeast cells leads to more fermentation performance and gas powering. Fermentation tests showed a positive direct association between yeast survivability, and gas production power, decreased the density and weight of fermented bread dough, as well as, increased its height and volume. The biomass weight of yeast cells and their hybrids was as follows: H₁> H₂> P₁> P₃> P₂. Therefore, hybrid yeast cells are the most appropriate for fermentation power. So, they require a shorter fermentation time than their parental genotypes. High heterogeneity was obtained by the H₁ genotype under the effect of 2, 6, and 8 g sucrose. Meanwhile, the H₂ genotype recorded high heterogeneity under the effect of 0.0 and 8 g sucrose in the fermentation medium. Both H₁ and H₂ genotypes contributed to their heterogeneity at 8 g sucrose. This indicated their adaptation to tolerate sucrose stress. So, this is the outcome of presenting two chromosome sets divergent in the hybrid cells, one from each parent. This is detrimental to the viability of hybrid yeast cells in changing environments. The differences between phenotypic and genotypic coefficient of variances were greater enough. This indicated a greater contribution of environmental variance in the total variability of fermentation performance. This leading to the improvement of fermentation power has a little opportunity through selection alone but it must be before hybridization. This indicates a higher influence of environmental factors than genetic makeup in the expression of leavening ability genes. Thus, the population of yeast cells needs to create variability either by hybridization or induced mutation followed by selection. Fermentation performance exhibited low heritability coupled with low or moderate genetic advance as a percent of the mean. This indicated higher effect of environmental factors on the phenotypic expression of fermentation activity. Therefore, fermentation power was governed by non-additive gene action and the heterosis technique has great value for improving this trait. The temperature of the bread dough is also important at the fermentation starting point. So, using a softer dough with a better-quality of yeast genotype will result in a higher concentration of CO₂ released during the fermentation process. In conclusion, hybrid genotype properties make them candidates of potential value for bread bread-baking industry. Hybridization between highly divergent genotypic strains followed by selection was needed to improve the performance of leavening ability in baker's yeast.

Conflict of interest statement

The author declares that this manuscript was done in the absence of any commercial or financial relationships that could be conducted as a potential conflict of interest.

Authors contribution

Not applicable because this manuscript is a single author.

Ethical approval

This study does not indicate any human or animal testing or feeding.

Funding

This study was carried out at my own expense without any funds from any foundation.

Acknowledgments

The author was grateful to here Foundation, Faculty of Agriculture, Mansoura University, as well as, to Mansoura University, Egypt for their logistic support.

REFERENCES

- Aboaba OO, Obakpolor EA. 2010.** The leavening ability of baker's yeast on dough prepared with composite flour (wheat/cassava). *African Journal of Food Science*. 4 (6): 325 – 329.
- Ahi M, Hatamipour M and Goodarzi A. 2010.** Optimization of leavening activity of baker's yeast during the spray-drying process. *Dry Technology*. 28 (4): 490-494.
- Ahmed MF, Hamza HA, Ibrahim IA, Nower AA and Alansary M. 2017.** Developing new Egyptian local lines of tomato (*Solanumlycopersicum* l.). *Menoufia J. Plant Prod*. 2 (1): 1-10.
- Allard RW. 1960.** Principles of Plant Breeding. John Wiley, New York. p 485.
- Allard RW. 1999.** Principles of plant breeding. Wiley, New York.
- Almeida M J, Pais C. 1996.** Leavening ability and freeze tolerance of yeasts isolated from traditional corn and rye bread doughs. *Applied And Environmental Microbiology*. 62 (12): 4401-4404.
- Amir BW, Mohd AB and Saba M. 2015.** Morphological characterization as indices for yield and yield components collection in common bean (*Phaseolus Vulgaris* L.) in J and K. *J. Glob. Bio. Sci*. 4 (9): 3391-3394
- Aslankoohi E, Rezaei MN, Vervoort Y, Courtin CM and Verstrepen KJ. 2015.** Glycerol production by fermenting yeast cells is essential for optimal bread dough fermentation. *PloS One* 10 (3): 1-13.
- Aslankoohi E, Zhu B, Rezaei MN, Voordeckers K, De Maeyer D, Marchal K, Dornez E, Courtin CM and Verstrepen KJ. 2013.** Dynamics of the *Saccharomyces cerevisiae* transcriptome during bread dough fermentation. *Appl. Environ. Microbiol*. 79: 7325-7333.
- Asyikeen ZN, Ma'aruf AG, Sahilah AM, Khan AM and Aida WW. 2013.** A new source of *Saccharomyces cerevisiae* as a leavening agent in bread making. *Int. Food Res. J*. 20 (2): 967 - 973.
- Bloksma AH. 1990.** Rheology of the breadmaking process. *Cereal Food World*. 35: 228-236.
- Breese EL. 1969.** The measurement and significance of genotype-environment interaction in grasses. *Heredity*. 24: 27-44.
- Burluc RM. 2007.** Technology and quality control in bread bread-making industry. (Tehnologia și controlul calității în industria panificației). Galati.
- Chand N, Vishwakarma SR, Verma OP and Manoj K. 2008.** The worth of genetic parameters to sort out new elite barley lines over heterogeneous environments. *Journal of Pharmacognosy and Phytochemistry* 8 (3): 332-334.
- Chen S and Gutmanis F. 1976.** Carbon dioxide inhibition of yeast growth in biomass production. *Biotechnology and Bioengineering*. 18 (10): 1455-1462.
- Chevallier S, Zúñiga R and Le-Bail A. 2012.** Assessment of bread dough expansion during fermentation. *Food Bioprocess Technol*. 5: 609-617.
- Chiotellis E and Campbell GM. 2003.** Proving of bread dough I. modeling the evolution of the bubble size distribution. *Trans. I. Chem. E*. 81 (Part C): 194-206.
- Deshmukh S N, Basu MS and Reddy PS. 1986.** Genetic variability, character association and path coefficient analysis of quantitative traits in Virginia bunch varieties of groundnut. *Indian Journal of Agricultural Science* 56 (1): 816-821.
- Dhital S, Shrestha AK and Gidley MJ. 2010.** Effect of cryo-milling on starches: functionality and digestibility. *Food Hydrocolloids*. 24 (2-3): 152-163.

- Dursun A. 2007.** Variability, heritability and correlation studies in bean (*Phaseolus vulgaris* L.) genotypes. World Journal of Agricultural Sciences 5: 12-16.
- Eid MH. 2009.** Estimation of heritability and genetic advance of yield traits in wheat (*Triticumaestivum* L.) under drought conditions. International Journal of Genetics and Molecular Biology 1 (7): 115-120.
- Ejara E, Mohammed Wand Amsalu B. 2018.** Genetic variability, heritability and expected genetic advance of yield and yield-related traits in common bean genotypes (*Phaseolus vulgaris* L.) at Abaya and Yabello, Southern Ethiopia. African Journal of Biotechnology 17 (31): 973-980.
- El-Morsy AE, El-Kassas AI, Kansouh AM and Ibraheem MM. 2021.** Selection and breeding of new lines of tomato (*SolanumLycopersicon* L.) resistance to tomato yellow leaf curl virus. Sinai Journal of Applied Sciences. 10 (2): 99-106.
- Evans I. 1990.** Yeast strains for baking: recent developments. In: Spencer J, Spencer D. editors. Yeast technology. Berlin: Springer Verlag. 13-54.
- Falconer DS, Mackay TFC. 1996.** Introduction to Quantitative Genetics, 4thedn. Longmans Green, Harlow, Essex, UK.
- Francis TR, Kannenberg LW. 1978.** Yield stability studies in short-season maize. A descriptive method for grouping genotypes. Can. J. Plant Sci. 58: 1029-1034.
- Fu D, Xiao M, Hayward A, Jiang G, Zhu L, Zhou Q, Li J and Zhang M. 2015.** What is crop heterosis: New insights into an old topic. J. Appl. Genet. 56: 1-13.
- Gabaldón T. 2020.** Hybridization and the origin of new yeast lineages. FEMS Yeast Research. 20 (5): 1 – 8.
- Gabriela CG and Daniela V. 2010.** The influence of different forms of bakery yeast *Saccharomyces cerevisiae* type strain on the concentration of individual sugars and their utilization during fermentation. Romanian Biotechnological Letters. 15: 5417-5422.
- Gibson G, Dworkin I. 2004.** Uncovering cryptic genetic variation. Nature Reviews Genetics. 5: 681-691.
- Gomez KA, Gomez AA. 1984.** Statistical procedures for agricultural research. 2nd ed. Chichester, UK: Wiley.
- Guadalupe-Daqui M, Goodrich-Schneider RM, Sarnoski PJ, Carriglio JC, Sims CA, Pearson BJ and MacIntosh AJ. 2023.** The effect of CO₂ concentration on yeast fermentation: rates, metabolic products, and yeast stress indicators. Journal of Industrial Microbiology and Biotechnology. 50: (1): 1-9.
- Hackenberg S, Verheyen C, Jekle M and Becker T. 2017.** Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. Eur. Food Res. Technol. 243: 287-296.
- Hartwell LH. 1974.** *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. 38: 164-198.
- Hernandez-Lopez M, Prieto J and Randez-Gil F. 2003.** Osmotolerance and leavening ability in sweet and frozen sweet dough. Comparative analysis between *Torulasporadelbrueckii* and *Saccharomyces cerevisiae* baker's yeast strains. Antonie Leeuwenhoek 84: 125-34.
- Hill WG, Zhang X. 2004.** Maintaining genetic variation in fitness. Adaptation and Fitness in Animal Populations. 59-81.
- Hoque A. 2013.** Morpho-physiological and molecular characterization of rice (*Oryza sativa* L.) advanced breeding lines for earliness. Doctoral dissertation presented to Sokoine University of Agriculture, Morogoro, Tanzania.

- Hutkins RW. 2006.** Bread fermentation; in microbiology and technology of fermented foods. Ed. By Blackwell Publishing. 261-299.
- IdrisAE, Mohamed KA. 2013.** Estimation of genetic variability and correlation for grain yield components in rice (*Oryza sativa* L.). Global Journal of Plant Eco. physiolog. 3 (1): 1-6.
- IslamMT, Ara MI, Hossain MA, Sen AK and DuttaRK. 2011.** Identification of tomato genotypes for salt tolerance. Int. J. Sustain. Crop Prod. 6 (1): 17-21.
- Istudor A, Voicu G, Muscalu G and Tudor P. 2020.** Evaluation of carbon dioxide released by bread dough during the proving stage. E3S Web of Conferences. 180: 1-7.
- Johnson HW, Robinson HF and Comstock RE. 1955.** Estimates of genetic and environmental variability in soybeans. Agronomy Journal.47 (7): 314-318.
- Jules M, Guillou V, Francois J and Parrou J. 2004.** Two distinct pathways for trehalose assimilation in the yeast *Saccharomyces cerevisiae*. Appl Environ. Microbiol. 70: 2771-2778.
- Kamal Mervat I. 2023.** Screening heterosis in hybrid yeast cells for their leavening properties under osmotic stress. International Journal of Agriculture, Environment and BioResearch.
- Kasaie Z, Rad AH, Kargozari Mand Oskouie MJ. 2017.** Evaluation of survivability and bioactivity of *Saccharomyces cerevisiae* in bread dough. Scientific Study & Research Chemistry & Chemical Engineering; Biotechnology; Food Industry. 18 (3): 249-257.
- Kassir Y, Adir N, Boger-Nadjar E, Raviv NG, Rubin-Bejerano I, Sagee S and Shenhar G. 2003.** Transcriptional regulation of meiosis in budding yeast. Int. Rev. Cytol. 224: 111–171.
- Khan IA .1979.** Induced quantitative variability in mungbean (*Phaseolus aureus R oxb.*). The Journal of Cytology & Genetics. 14: 142-145.
- Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P and Beerli P. 2001.** The strength of phenotypic selection in natural populations. The American Naturalist. 157 (3): 244-261.
- Kozgar M I. 2014.** Mutation breeding in chickpea: perspectives and prospects for food security. De Gruyter Open, Berlin, Germany.
- Krogerus K, Magalhães F, Vidgren V and Gibson B. 2015.** New lager yeast strains generated by interspecific hybridization. J. Ind. Microbiol. Biotechnol. 42: 769 - 778.
- Krogerus, K., F. Magalhães, V. Vidgren and B. Gibson. 2017.** Novel brewing yeast hybrids: Creation and application. Appl. Microbiol. Biotechnol. 101: 65-78.
- KrogerusK, Fletcher E, Rettberg N, Gibson B and Preiss R. 2021.** Efficient breeding of industrial brewing yeast strains using CRISPR/Cas9-aided mating-type switching. Appl. Microbiol. Biotechnol. 105: 8359 - 8376.
- Kwolek-Mirek Mand Zadrag-TeczaR. 2014.** Comparison of methods used for assessing the viability and vitality of yeast cells. FEMS Yeast Research 14 (7): 1068-1079.
- Le-BailA, NicolitchCand VuillodC. 2010.** Fermented frozen dough: impact of pre-fermentation time and freezing rate for a pre-fermented frozen dough on the final volume of the bread. Food and Bioprocess Technology. 3: 197-203.
- Li E, DhitalS and Hasjim J. 2014.** Effects of grain milling on starch structures and flour/starch properties. Starch. 66 (1-2): 15-27.
- Luarasi L, Troja R and Pinguli L. 2016.** The relationship between yeast viability and concentration in the fermentation process of wort for beer production. European Journal of Biotechnology and Genetic Engineering. 3 (1): 83-86.

- Mirek MK, Tecza RZ. 2014.** Comparison of methods used for assessing the viability and vitality of yeast cells. *FEMS Yeast Res.* 14(7):1068-79.
- Mulugeta A, Hussein M and HabtamuZ. 2013.** Inheritance of primary yield component traits of common beans (*Phaseolus vulgaris* L.): number of seeds per pod and 1000 seeds weight in an 8x8 diallel cross population. *International Journal of Agricultural and Biosystems Engineering* 7 (1): 84-88.
- Munteanu G, VoicuG, FerdeşM, ŞtefanE, Constantin G, Tudor P. 2019.** Dynamics of the fermentation process of bread dough prepared with different types of yeast. *Scientific Study & Research.* 20 (4): 575-584.
- Myers D, Lawlor D and Attfield P. 1997.** Influence of invertase activity and glycerol synthesis and retention on fermentation of media with a high sugar concentration by *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 63: 145-150.
- Nagodawithana, TW, TrivediNB. 1990.** Yeast selection for baking. In: Panchal CJ, editor. *Yeast strain selection.* New York, N.Y., U.S.A.: Marcel Dekker, Inc. 139-84.
- Nechifor B, FilimonR and Szilagyil. 2011.** Genetic variability, heritability and expected genetic advance as indices for yield and yield components selection in common bean (*Phaseolus vulgaris* L.). *Scientific Papers, UASVM Bucharest, Series A.* 1222-5339.
- Newberry M, Phan-Thien N, Larroque O, Tanner R and Larsen N. 2002.** Dynamic and elongation rheology of yeasted bread doughs. *Cereal Chem.*, 79: 874 – 879.
- Oda Yand Ouchi K. 1990.** Hybridization of bakers' yeast by rare-mating method to improve leavening ability in dough. *Enzyme Microb. Technol.* 12(12): 989-993.
- Okamoto S, Iino T. 1981.** Selective abortion of two non-sister nuclei in a developing ascus of the *hfd-1* mutant in *Saccharomyces cerevisiae*. *Genetics.* 99: 197-209.
- Oura E, Suomalainen H and Viskari R. 1982.** Breadmaking. In A.H. Rose (ed.). *Economic Microbiology.* Academic Press. 7: 86-141.
- Pataro C, Guerra JB, Petrillo-Peixoto ML, Mendonça LC, Linardi VR and Rosa CA. 2000.** Yeast communities and genetic polymorphism of *Saccharomyces cerevisiae* strains associated with artisanal fermentation in Brazil. *J. Appl. Microbiol.* 89 (1): 24-31.
- Phaff HJ, Starmer WT. 1987.** *The Yeasts, A Taxonomic Study.* 3rd Edition. Elsevier. Amsterdam.
- Piepho H. 1996.** A Montecarlo test for variance homogeneity in linear models. *Biometrical Journal.* 38 (4): 461-473.
- Plyer EJ. 1973.** *Baking science and technology.* Siebel Publication Co., Chicago. 171-201.
- Potus J, Poiffat A and Drapon R. 1994.** Influence of dough-making conditions on the concentration of individual sugars and their utilization during fermentation. *Cereal. Chem.* 71: 505-508.
- Randez-Gil F, Córcoles-Sáez I and Prieto JA. 2013.** Genetic and phenotypic characteristics of baker's yeast: relevance to baking. *Annu. Rev. Food Sci. Technol.* 4: 191-214.
- Randez-Gil F, Aguilera J, Codon A, Rincon AM, Estruch F and Prieto JA. 2003.** Baker's yeast: challenges and future prospects. In: de Winde JH, editor. *Functional genetics of industrial yeasts.* Berling, Heidelberg: Springer-Verlag. 57-97.
- Rathnayake HA, Navaratne SB and Navaratne CM. 2018.** Porous crumb structure of leavened baked products. *International Journal of Food Science.* 2: 1-15.
- Rezaei MN, Dornez E, Jacobs P, Parsi A, Verstrepn KJ and Courtin CM. 2014.** Harvesting yeast (*Saccharomyces cerevisiae*) at different physiological phases significantly affects its functionality in bread dough fermentation. *Food Microbiol.* 39: 108-15.

- Romano A, Toraldo G, Cavella S and Masi P. 2007.** Description of leavening of bread dough with mathematical modeling. *Journal of Food Engineering*. 83: 142-148.
- Sahlström S, Park W and Shelton D. 2004.** Description of leavening of bread dough with mathematical modelling. *Journal of Food Engineering*. 83 (2): 143-148.
- Sahlström S, Park W and Shelton DR. 2003.** Factors influencing yeast fermentation and the effect of LMW sugars and yeast fermentation on hearth bread quality. *Cereal Chemistry*. 81 (3): 328 - 335.
- Sardana S, Mahajan RK, Gautam NK and Ram B. 2007.** Genetic variability in pea (*Pisum sativum* L.) germplasm for utilization. *SABRAO Journal of Breeding and Genetics* 39 (1): 31-42.
- Sasano Y, Haitani Y, Hashida K, Ohtsu I, Shima J and Takagi H. 2012.** Simultaneous accumulation of proline and trehalose in industrial baker's yeast enhances fermentation ability in frozen dough. *J. Biosci. Bioeng.* 113 (5): 592-595.
- Scanlon MG, Zghal MC. 2001.** Bread properties and crumb structure. *FRI*. 34: 841-864.
- Shima J, Takagi H. 2009.** Stress-tolerance of baker's-yeast (*Saccharomyces cerevisiae*) cells: stress-protective molecules and genes involved in stress tolerance. *Biotechnol. Appl. Biochem.* 53: 155 - 64.
- Sinelli N, Casiraghi E and Downey G. 2008.** Studies on proofing of yeasted bread dough using near- and mid-infrared spectroscopy. *J Agric Food Chem*. 56: 922-931.
- Singh BD. 2001.** Plant breeding: Principles and methods. Kalyani Publishers, New Delhi, India.
- Singh RK, Chaudhary BD. 1985.** Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India. 215-218.
- Sluimer P. 2005.** Principles of breadmaking: functionality of raw materials and process steps. St. Paul: The American Association of Cereal Chemists Inc.
- Smit G, Straver MH, Lugtenberg BJ and Kijne JW. 1992.** Flocculence of *Saccharomyces cerevisiae* cells are induced by nutrient limitation, with cell surface hydrophobicity as a major determinant. *Appl. Environ. Microbiol.* 58: 3709-3714.
- Snodgrass ME. 2004.** Encyclopedia of kitchen history. New York.
- Spencer JFT, Bizeau C, Reynolds N and Spencer DM. 1985.** The use of mitochondrial mutants in hybridization of industrial yeast strains. VI Characterization of the hybrid, *Saccharomyces diastaticus* x *Saccharomyces rouxii*; obtained by protoplast fusion; and its behavior in simulated dough-raising tests. *Current Genetics*, 9: 649 - 652.
- Steel RG, Torrie JH. 1960.** Principles and procedures of statistics. The Biological Sciences. McGraw Hill. New York. 187 - 287.
- Steensels J, Gallone B and Verstrepen KJ. 2021.** Interspecific hybridization as a driver of fungal evolution and adaptation. *Nat. Rev. Microbiol.* 19: 485-500.
- Struyf N, Maelen EV, Hemdane S, Verspreet J, Verstrepen KJ and Courtin CM. 2017.** Bread dough and baker's yeast: An uplifting synergy. *Comprehensive Reviews in Food Science and Food Safety*. 16 (5): 850-867.
- Takagi H, Shima J. 2015.** Stress tolerance of baker's yeast during bread-making processes. In Takagi H, Kitagaki H (eds.), *Stress Biology of Yeasts and Fungi: Applications for Industrial Brewing and Fermentation*. Springer Japan. Tokyo. 23-42.
- Tiwari DK, Pandey P, Tripathi S, Giri SP and Dwivedi JL. 2011.** Studies on genetic variability for yield components in rice (*Oryza sativa* L.). 3: 76-81.

- Tomova AA, Kujumdzieva AV and Petrova VY. 2019.** Carbon source influences *Saccharomyces cerevisiae* yeast cell survival strategies: quiescence or sporulation. *Biotechnology & Biotechnological Equipment*. 33 (1): 1464-1470.
- Verstrepen KJ, Iserentant D, Malcorps P, Derdelinckx G, Van Dijck P, Winderickx J and Pretorius IS. 2004.** Glucose and sucrose: hazardous fast-food for industrial yeast? *Trends in Biotechnology* 22 (10): 531-537.
- Wagner M, Pierce M and Winter E. 1997.** The CDK-activating kinase CAK1 can dosage suppress sporulation defects of *smk1* MAP kinase mutants and is required for spore wall morphogenesis in *Saccharomyces cerevisiae*. *EMBO J*. 16: 1305-1317.
- Wang J, Yu H, Weng X, Xie W, Xu C, Li X, Xiao J and Zhang Q. 2014.** An expression quantitative trait loci-guided co-expression analysis for constructing a regulatory network using a rice recombinant inbred line population. *J. Exp. Bot.* 65 (4): 1069-1079.
- Wani AA. 2011.** Spectrum and frequency of macromutations induced in chickpea (*Cicer arietinum* L.). *Turkish Journal of Biology* 35 (2): 221-231.
- Wani MR, Khan S. 2006.** Estimates of genetic variability in mutated populations and the scope of selection for yield attributes in *Vignaradiata*(L.) Wilczek. *Egyptian British Biological Society (EBB Soc)*, 8: 1-6.
- Winans MJ. 2022.** Yeast hybrids in brewing. *Fermentation*. 8 (87): 1 - 12.
- Wongkhalaung C, Boonyaratanakornkit M. 2007.** Characterization of new baker's yeast strains and their leavening ability in bread dough. *Kasetsart J. (Nat. Sci.)* 41: 751-763.
- Wongkhalaung C, Nakatomi Y and Takano H. 2004.** Hybridization and selection of *Saccharomyces cerevisiae* strains from industrial baker's yeasts. *Kasetsart J. (Nat. Sci.)*, 38: 255 - 266.
- Yagdi K. 2009.** Path coefficient analysis of some yield components in durum wheat (*Triticum durum* Desf.). *Pakistan Journal of Botany* 41 (2): 745-751.
- Zhang X, Wang J and Hill WG. 2004.** Redistribution of gene frequency and changes of genetic variation following a bottleneck in population size. *Genetics Society of America*. 167: 1475-1492.
- Zhou N, Schifferdecker AJ, Gamero A, Compagno C, Boekhout T, Piškur J and Knecht W. 2017.** Kazachstaniagamospora and Wickerhamomyces uzbekicus: Two alternative baker's yeasts in the modern bakery. *Int. J. Food Microbiol.* 250: 45-58.