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SCREENING HETEROSIS IN HYBRID YEAST CELLS FOR THEIR LEAVENING PROPERTIES UNDER OSMOTIC STRESS

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ABSTRACT

Bread-making industry occupies an important place in the food sector. This industry is in full process of expansion and automation for obtaining good quality and cost-efficient products. Hybridization in yeast gives rise to offspring carrying an admixture of both parental genotypes which display unique traits that may drive adaptation to changing environments. This study aimed to evaluate the fermentation performance of hybrid yeast cells under sucrose stress, as well as, to elucidate the role of fermentation time in decreasing the weight of fermented bread dough. Two hybrids originated from two hybridizations between three diverged strains of Saccharomyces cerevisiae were used in this study. Hybrids were characterized concerning the parental strains in a wort fermentation of bread wheat containing four sucrose concentrations. Hybrid genotypes have apparent hybrid vigor for reducing the weight of fermented bread dough over time than their parents. This indicated faster fermentation and producing higher quantities of CO2 leading to a decrease in the weight of fermented bread dough under sucrose stress up to 6 g /325 g wheat flour. Results apparent a strong positive correlation in the association between the decrease in fermented bread dough weight and fermentation time. Both variables vary in the same direction because they are dependent on each other. Results suggest that hybrid yeast genotypes are suitable for bread bread-making industry because of their unique properties in fermentation ability and producing high carbon dioxide leading the weight of fermentation bread dough to decrease over time.

Keywords: Saccharomyces Cerevisiae, Heterosis, Leavening Ability, Bread Dough Weight, Sucrose Stress, Correlation Coefficient, Regression Analysis.

1. INTRODUCTION

Yeasts have been used for the production of fermented foods and beverages for centuries as bread, wine, and beer **(Steensels and Verstrepen 2014)**. In ancient times, food fermentation processes were spontaneous processes. In the late 19th century, spontaneous fermentations were gradually replaced with controlled methodology. Then, pure cultures of yeast genotypes were used as starter agents because they yielded increased fermentation speed, consistency, and good quality. The predominant yeast used for controlled fermentation is *Saccharomyces cerevisiae*. These strains combine several desirable traits such as efficient fermentation of high-sugar media, producing desirable flavors, absence of toxin released, high ethanol production, and tolerance **(Piskur *et al*. 2006)**. Nowadays, only a relatively lower number of genetically related and highly domesticated strains *of Saccharomyces cerevisiae* are being used in bread bread-making industry. Where much of the potential natural diversity in yeast was still unexplored **(Gallone *et***

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***al*. 2016)**. The use of a relatively homogenous group of *Saccharomyces cerevisiae* for bread dough leavening has improved the quality and overall speed of the fermentation process. This also limited the sensorial complexity of the fermented end products **(Daenen *et al*. 2008)**. The production of sourdough is one notable exception, where several microbial genetic diversity consists of yeasts and bacteria coexisting during the fermentation process. These resulted in unique sensorial features of the resulting bread **(De Vuyst *et al*. 2014)**. Bread yeast is only used to provide the necessary carbon dioxide gas for leavening. Meanwhile, flavor compounds produced through fermentation would not contribute to the flavor profile of bread wheat dough due to evaporation during baking. Recently, several studies have shown that yeast-derived compounds increasingly contribute to the flavor profile of bread crumbs **(Birch *et al*. 2013)**.

The discovery of yeast to leaven bread centuries ago led to the development of the bakery industry. Bakers used brewer's yeast till about 8-1000 years ago because of its performance in fermentation. Isolation of *Saccharomyces cerevisiae* strains possessing the desired traits that brought revolutionary changes in the bakery industry. Baker's yeast is commonly isolated from sugary fermented foods and alcoholic beverages such as palm wine and Borututu beer **(Amri *et al.* 1982)**. It can ferment sugars and produce ethanol and CO_2 under anaerobic conditions. It can grow rapidly on sugar and produce high cells under aerobic conditions. Suitable genotypes of yeast are used in the industrial production of beers, wines, bread, fuel alcohol, biomass of yeast cells, as well as, byproducts from these. Yeasts used for bread bread-making industry are usually carefully selected genotypes of *Saccharomyces cerevisiae*. It converts fermentable sugars and some starch-containing flour by enzymatic action into CO_2 and alcohol. The CO_2 caused the dough volume to rise and reduce weight because the density of fermented dough was reduced **(Ayanru 1989)**.

Information on genetics and molecular biology has accumulated on *Saccharomyces cerevisiae* making this organism the best-characterized eukaryotic system today. It could be used for the leavening of bread dough for bread bread-making industry. Some genotypes of yeast showed tolerance to high sucrose and ethanol concentrations. An acceptable wine could be produced from the fruits with palm wine yeasts **(Ezeronye and Okerentugba 2001)**. The production of fermentation metabolites in bread dough can be influenced by several factors such as dough ingredients, dough fermentation environment, yeast pre-growth-growth conditions, as well as, the genetic makeup of yeast strains **(Rezaei *et al.* 2015)**. Therefore, the selection of appropriate yeast genotypes is required for several applications such as frozen dough to optimize the production of CO_2 rate. Bread dough is a complex fermentation matrix because it contains several ingredients that influence the rate of fermentation by yeast cells. The important factor is the availability of fermentable sugars in the dough **(Codina and Voica 2010)**. These sugars are variable in different flour varieties. These depend on the level of carbohydrates present in flour, as well as, the activity of yeast-associated enzymes that need to degrade carbohydrates into fermentable sugars through the fermentation process **(Struyf *et al*. 2017)**.

Besides wheat flour's endogenous sugars, the addition of sugars such as sucrose is often supplemented. In sweet dough, up to 30% sucrose was added resulting in high osmotic pressure. The osmotic stress can strongly influence the fermentation performance in dough **(Sasano *et al*. 2012)**. Next to sugar ingredients, the fermentation condition as storage temperature plays a crucial role in CO_2 production by yeast cells during the fermentation process. Frozen doughs, on

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the other hand, are stored for longer times at temperatures below 0.0 ^{0}C. The low temperature generally decreases dough leavening ability due to loss of yeast cell viability which leads to a higher decrease in final bread quality **(Ribotta *et al.* 2001)**. Therefore, many microbial geneticists have focused on the genetic modification or selection of efficient yeast genotypes to improve their leavening ability in different dough systems and conditions. The strategies used for enhancing yeast genotypes are very diverse. The available techniques related to improving yeast genotypes toward fermentation activity are increasing every year **(Struyf *et al.* 2017)**. Sucrose was added to dough ingredients at varying levels for improvement of flavor, as well as, a nutrient source for yeast cells. If sucrose in dough is very high as in sweet dough's that contain sucrose levels up to 30%.Then yeast cells have severe osmotic stress. Because the high concentrations of sucrose damage cellular components and decrease their fermentation activity **(Verstrepen *et al.* 2004)**. The exposure of yeast cells to hyperosmotic pressure leads to rapid cell dehydration, as well as, limits cell growth and CO_2 production power. As a consequence, the fermentation rate and final volume of baked products are decreased**(Hernandez-Lopez *et al.* 2003)**. Therefore, yeast strains need to genetically adapt the hyperosmotic pressure in the dough fermentation medium to produce CO_2 at acceptable rates **(Aslankoohi *et al.* 2015)**. Bread dough fermentation is a unit operation in bread making. Yeast transforms flour sugars into carbon dioxide gas. The final gas volume in the breed can be over 70% of the loaf volume **(Scanlon and Zghal 2001)**. During fermentation, no new bubbles were generated, but only the volume of bubbles can be increased through the production leading to increasing the dough volume and decreasing in their weight. To increase the dough volume the fermentation temperature is between 25-30 ^{0}C. The rate of fermentation is closely related to the availability of glucose and the ability to hydrolyze maltose by flour amylolytic enzymes on starch **(Bordei *et al.* 2000)**. Breweries try to asses yeast quality genotype by measuring the viability of yeast cells within a population.

Testing the viability of yeast cells depends on three ways; loss of replication capability, cell damage, and loss of metabolic pathways **(White *et al.* 2008)**. Breed production depends on a controlled fermentation by a good genotypic of yeast. The quality of *Saccharomyces cerevisiae* genotypes depends on the formation of the yeast plasma membrane. This membrane was affected by the stresses that occur during the fermentation process, especially during storage. The number of viable cells in the population and the total concentration of yeast slurry are good indicators of the performance of fermentation. Historically, these traits were measured using methylene blue to stain the yeast population. Staining with methylene as an autoxidisable dye when enters the cytoplasm of living cells results in oxidation to the colourless leuco-form **(Luarasi *et al.* 2016)**. Staining methods were used to differentiate between survived and non-survived cells. In the methylene blue technique, when the dye was mixed with a yeast sample, the viable cells reduced the stain, and the dead cells stained blue **(Boyd 2003)**. Viability can also be assayed by direct observation using a microscope to determine the viable yeast cell number. The “spin” method is also used traditionally to assess the viability of yeast cells. The spin technique involves centrifugation of yeast slurry and then measuring the subsequent pellet volume. This assay assumed a constant cell size **(Priest 2003)**.

Normal *Saccharomyces cerevisiae* genome has 16 distinct chromosomes (n = 16). These chromosomes were ranged in length between 230 to 1532 kilobases. Yeast chromosomes were protective at both ends with telomeres. Mutations induced accidentally during chromosome fusion trials affected cell tolerance to the new genome organization **(Goffeau *et al.* 1996)**.

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Overall, cell growth, size, and shape seem to be buffered throughout the series of fusions **(Pryde 1999)**. Yeast cells lack the genetic variability that the geneticists need to select efficient recombination through the generations. Therefore, if the cells were obligated to be starved of nutrients, these cells would undergo meiosis to develop haploid spores **(Herskowitz 1988)**. The genetic material during meiosis is exchanged between the paired chromosomes in a process called recombination **(Shao *et al.* 2018)**. Diploids *Saccharomyces cerevisiae* budding cells express various differentiation states if nutrients are limited leading to distinct biological functions. The different states take place in response to different environmental conditions **(Honigberg 2016)**. In response to external stress such as a starvation of one or more nutrients, the yeast cells may leave the mitotic cycle to enter one or another specific state, one of them sporulation with haploid spores, switching to pseudohyphal growth generating elongated chains of cells, entering a stationary phase where their age undergo programmed cell death **(Neiman 2011)**. This investigation focuses on the comparison between parental strains of *Saccharomyces cerevisiae* and their hybrids for the leavening ability of the bread wheat fermentation process. This study also aimed to analyze regression and correlation between decreasing bread dough weight and fermentation time.

2. MATERIALS AND METHODS

Strains and media

The wild-type diploid strains of *Saccharomyces cerevisiae* isolated from different samples of dry yeast obtained from the local market in Egypt were used in this study **(Table 1)**. They are grown in a standard yeast extract peptone glucose medium (YEPG) which contains 1% yeast extract,2% bacto-peptone, and 2% glucose **(Tomova *et al.* 2019)**. Plates were incubated at 30^0C for 72 hours. Representative colonies were purified by restreaking on YEPG medium containing 0.05 mg/ml chloramphenicol, in addition to gentamicin to inhibit bacterial growth. The isolates were stored on slopes of the same medium. Yeast isolates were identified by standard morphological and physiological methods depending on leavening activity **(Fardi and Faubion 1990)**.

Isolation of yeast strains

Yeast strains were isolated from dry yeast samples using the standard serial technique. Briefly, 0.1 gram of dry yeast was aseptically suspended in 99.9 ml of sterile water and mixed for 15 minutes at room temperature. Serial dilutions were prepared and then 0.1 ml from each was spread on YEPG agar plates containing 0.05 mg/ml chloramphenicol, as well as gentamicin to suppress bacterial growth. The plates were incubated at 30 ^{0}C for 72 hours. Single colonies were isolated and examined microscopically. Colonies showed the same morphology of yeast cells were selected and streaked on YEPD agar slants **(Aouine *et al.* 2021)**. Pre – sporulation medium was used to stimulate the parental yeast strains to be sporulated according to **Bahler *et al*. (1994)**. Meanwhile, the sporulation medium consisted of yeast extract 0.5 %, peptone 0.5 %, and sodium acetate 2% according to **Sherman *et al.*(1982)**. A sporulation medium was used for the induction of sporulation in which the sexual phase takes place through the development of asci which takes a long period reaching 30 days.

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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	Dream	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

Microscopical examination

For the microscopical test, a thin smear was prepared according to **Frazier and Westhoff (1995)** by spreading a loop full of the cells on the clean slide with a water drop. The loop full of cells was spread to make a thin film which turned to air dried and then stained with methylene blue to be observed with a light microscope using x10 and x40 objective lenses.

The parental yeast strains were genetically marked with some antifungal agents. These include Trefflucan (*Trf*), Flucoral (*Flu*), and Fungican (*Fun*), which are used with a concentration of 150 mg/10 ml distilled water. Gyano - Daktarin (*Gyn*) was used with 20 mg/10 ml distilled water. Benefits (*Ben*) was used with 250 mg/10 ml distilled water. Itracon (Itraconazole) (*Itr*) was used with 100 mg/10 ml distilled water. After the parental strains were genetically marked, the parental strain (P_1) took the following genotype, $Trf^{-}\ Flu^{-}\ Gyn^{+}\ Ben^{+}\ Fun^{-} Itr^{-}$. Meanwhile, the parental strain (P_2) was taken in the following genotype, $Trf^{-} Flu^{-} Gyn^{-} Ben^{+}\ Fun^{-}\ Itr^{+}$. In addition, the parental strain(P_3) was taken in the following genotype, $Trf^{-} Flu^{-}\ Gyn^{-} Ben^{-} Fun^{-} Itr^{+}$.

Hybridization procedure

The parental strains were grown in liquid YEPG for 24 h at 30° C. One ml of yeast cell suspension from each of the parental strains was inoculated in the pre-sporulation medium. The mated cells that carried the opposite genetic markers were grown in the pre-sporulation medium for 24 hours and then transferred to one loop to be spread on the sporulation medium. The mated cells were served on a sporulation medium for one month at 30° C until the small single colonies appeared. These colonies were tested microscopically for the development of asci. If asci were formed then the single colonies were picked up to be grown on a YEPG medium containing the selectable markers of antifungal agents. The hybrids were only grown on a selective medium. The hybrid H_1 resulted from the mating between (P_1) $Ben^{+}\ Itr^{-}$ x (P_3) $Ben^{-}\ Itr^{+}$ that grown on a selective medium containing the antifungal agents, *Ben* and *Itr*.

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Meanwhile, the hybrid H_2 resulted from the mating between (P_1) *Gyn* $^+$ *Itr* $^-$ x (P_2) *Gyn* $^-$ *Itr* $^+$. The resulting hybrid was grown on a selective medium containing the antifungal agents *Gyn* and *Itr*. Hybrid isolates were tested for the leavening ability of bread dough.

Dough manufactured and leavening ability assays.

Strains were shaking (160 rpm) that were grown in 500-ml Erlenmeyer flasks containing 250 ml YEPG medium, pH 6.0, at 30 ^{0}C. Wheat flour was used for preparing dough. Cells were harvested by centrifugation at stationary phase, washed twice, weighted for final biomass, and then resuspended in 210 ml water to prepare cell suspension used for prepared dough. The following ingredients were mixed manually to form the dough though 8 minutes;325 g wheat flour, 3.5 g salt, and 210 ml of yeast cell suspension at stationary phase, in addition to the following concentrations of sucrose;0, 2, 4, and 8 gram to each weight of bread dough. The dough was immediately fermented at 40^{0}C for 15 minutes. The leavening activity of the yeast strains and their hybrids was determined by recording the dough weight every five minutes without placing the dough in water as a medium of fermentation **(Almeida and Pais 1996)**.

Genodynamics of the fermentation process

The dynamics of the fermentation process of dough were evaluated during the fermentation period. The weight of the dough was recorded at zero minutes of introducing the dough into the baker without tap water. Analysis was done on the qualitative indices of the dough fermented by different genotypes of yeast. The qualitative grades of dough are highly affected by the size of carbon dioxide formed in bread dough and the ability of strains to retain the gas. After baking the samples of bread, they are weighted and then cut into three symmetrical cores. The cores were directly transferred into 300 ml baker without water to measure the weight of fermented cores which were affected by CO_2 released from each yeast genotype. The weight of cores was recorded every five minutes for 15 minutes for each genotype of yeast cells according to **Kasaie *et al*. (2017)**.

Heterosis

Hybris yeast cells are chimeric genomes harboring two divergent sets of chromosomes within a single nucleus **(Gabaldón 2020)**. Heterosis in the leavening ability of hybrid yeast cells was estimated from the performance of the F_1 hybrid about the mid-parent according to **Shull (1908)**. Heterosis leads to a superior fitness of the F_1 hybrid for desirable traits. Heterosis increased heterozygosity in F_1 hybrid yeast cells which might buffer the effect of deleterious alleles or induce positive interactions between the parental alleles. This provides an F_1 hybrid better for adaptation than the parental populations **(Dobzhansky 1950)**.

Regression analysis

Linear regression is used to study the linear relationship between the dependent variable (Y) weight of fermented bread dough and the independent variable (x) fermentation time. The linear regression technique describes the dependent variable with a straight line that is defined by equation Y = a + bx. Where a is the Y-intersect of the line, as well as, b is its slope. The regression line enables researchers to predict the value of response variable Y from that in the independent variable x according to **Schneider *et al*. (2010)**.

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Correlation analysis

If two variables vary in such a way that movements in one are accompanied by movements in the other, then these variables are said to be correlated. Correlation is a statistical tool used to assess the relationship between two variables fermented bread dough weight and fermentation time. The correlation coefficient was used to estimate the degree and direction of the relationship between two variables according to **Moore *et al*. (2013)**.

Statistical analysis

The data obtained in this study were collected from the experiments conducted in triplicates. They were subjected to the analysis of variance (ANOVA) to determine the significance of differences among the results. In addition, the least significant difference (LSD) was calculated to compare between two means according to **Steel and Torie (1960)**.

3. RESULTS AND DISCUSSION

Heterosis in fermentation power

As shown from the results presented **in Table 2**, fermentation activity was increased over time by the hybrid H_2 genotype. This is because of negative hybrid vigor which increased from – 0.060 after five minutes of fermentation to – 0.074 after 15 minutes of fermentation time. The negative hybrid vigor was preferin this study because the weight of bread dough fermented by the hybrid genotype was decreased about the mid parent. Breed dough weight influenced by the H2 genotype was reduced over time because of CO_2 generated during fermentation time based on sugar consumption from wheat flour.

Table 2. Weight of bread dough without sucrose ingredient which affected by yeast strains and their hybrids during different fermentation times.

Genotypes		Fermentation time(minutes)				Decrease in bread weight %
		0.00	5	10	15	
P1		108.34	108.28	108.23	108.21	0.12
P3		107.93	107.89	106.42	107.83	0.09
MP		108.135	108.085	107.325	108.02	0.10
H1		108.32	108.20	108.17	108.15	0.16
Hybrid vigor %		0.00	+ 0.016	+ 0.787	+ 0.120	
P1		108.34	108.28	108.235	108.21	0.12
P2		108.33	108.31	108.24	108.21	0.11
MP		108.335	108.295	108.23	108.21	0.15
H2		108.33	108.23	108.21	108.13	0.18
Hybrid vigor %		0.000	- 0.060	- 0.018	- 0.074	
F – test		**	NS	NS	NS	
LSD	0.05	28.22	15.98	18.61	15.98	
	0.01	41.06	23.26	27.07	23.24	

**: Significance at 0.01 probability level. NS: Not significant.

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The fermentation power obtained by the H_2genotype reflected the amount of CO_2 generated in bread dough leading to a decrease in the dough weight and achieving negative hybrid vigor. The increased concentration of CO_2 released by the H_2 genotype in the fermentation medium is the hypothesized main cause of decreasing the weight of fermented bread dough leading to negative hybrid vigor. Overall, the decrease in the weight of fermented bread dough ranged between 0.09 (P_3) to 0.18% (H_2). Both hybridsH_1 and H_2genotypes achieved higher values in decreasing the weight of fermented bread dough which reached 0.16 and 0.18%, respectively. The increase in CO_2 production by the hybrid yeast cells is likely linked to the reduction in fermented bread dough weight during fermentation. Thus, fermenting with hybrid genotypes leads to a faster fermentation process with more sugar consumption from the wheat flour. These results agreed with **Guadalupe-Daqui *et al*.(2023)**, who reported that the increase in fermentation products was linked to the increase in the amount of fermentable sugars consumed by yeast cells during fermentation. The same authors also found that the higher consumption of sugars also led to the formation of additional cells and other metabolic products based on **Balling's formula (1865)**. Therefore, increased CO_2 production during fermentation by hybrid yeast cells could have a significant impact on the bread industry, as it could potentially improve fermentation efficiency without the need to add sucrose. Also, similar to other technologies, increased CO_2 production by hybrid yeast cells could mean significant savings in the time and energy needed for to fermentation process. Therefore, fermentation under hybrid yeast cells increased the formation of CO_2 over time leading the weight of fermented bread dough to be decreased. These results are in harmony with **Shen *et al*.(2003)**, who have shown that higher CO_2 concentrations resulted from the fermentation process leading to reduced concentrations of volatile organic compounds (VOCs). These results combined with previous findings which showed a clear inverse relationship between CO_2 production and the weight of fermented bread dough. Therefore, this study provides a better understanding of how negative hybrid vigor was preferred in fermentation bioactivity based on CO_2 production which leads to a decrease in the weight of fermented bread dough to optimize this process in an industrial scale of bread manufacture.

The percent of hybrid vigor under the effect of 2-gram sucrose in the fermentation medium during dough fermentation is shown in **Table** 3. It can be seen that negative hybrid vigor was obtained by the H_1 genotype which ranged between – 0.04 after five minutes to 0.00 after 15 minutes of fermentation time. This indicated that the negative hybrid vigor for decreasing the weight of bread dough was gradually reduced with increasing fermentation time. The same trend was also obtained by hybrid H_2 genotype but at 15 minutes of fermentation course. These results reflected the bioactivity of yeast hybrids which are more tolerant to sucrose stress if compared with their parents. This is due to secreted active invertase by yeast hybrids, which inverts the sucrose-supplemented flour into glucose and fructose during the kneading phase **(Gabriela and Daniela 2010)**. Therefore, the quantity of CO_2 released by different yeast genotypes during fermentation leads to a decreased weight of fermented bread dough. This weight varied between yeast genotypes, but these are higher in the hybrid genotypes. Likewise, the dough prepared using hybrid genotypes is the only one that does not need longer fermentation time.

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Table 3. The weight of bread dough supplemented with 2 grams of sucrose is affected by yeast strains and their hybrids during different fermentation times.

Genotypes		Fermentation time(minutes)				Decrease in bread weight %
		0.00	5	10	15	
P_1		108.33	108.28	108.24	108.19	0.120
P_3		108.33	108.30	108.27	108.24	0.080
MP		108.33	108.29	108.25	108.21	0.100
H_1		108.34	108.25	108.23	108.21	0.120
Hybrid vigor %		0.00	- 0.04	- 0.02	0.00	
P_1		108.33	108.28	108.24	108.19	0.130
P_2		108.33	108.22	108.18	108.14	0.180
MP		108.33	108.25	108.21	108.16	0.155
H_2		108.30	108.29	108.26	108.12	0.170
Hybrid vigor %		0.00	+ 0.04	+ 0.05	- 0.04	
F - test		*	*	*	*	
LSD	0.05	21.09	21.09	21.07	18.60	
	0.01	30.83	30.68	30.65	30.65	

*: Significance at 0.05 probability level.

This leads to the conclusion that hybrid yeast cells were more active in fermentation power for a high fermentation rate. The fermentation activity of the yeast cells expressed through the emission of CO_2 shows that this activity is more intense for the dough prepared with hybrid yeast cells. The hybrid yeast cells registered high negative heterosis by the H_1genotype after five minutes of fermentation, whereas the H_2genotype showed the same trend after 15 minutes of fermentation time. These results indicated that the negative hybrid vigor for the maximum decreasing weight of fermented bread dough was related to the quantity of CO_2 released by hybrid yeast cells. From this point of view, the quantities of carbon dioxide released by hybrid yeast cells correspond with the volume and decrease the weight of bread dough. Therefore, the results indicate that hybrid yeast cells present the most intensive fermentation activity than the parental yeast strains. Thus, the hybrid yeast cells adapt most easily to the fermentation of 2 g sucrose. These results agreed with **Hutkins (2006)**, who decided that the fermentation intensity depends on the form of yeast, as well as, on the availability of fermentable sugars in the flour including maltose released from starch hydrolysis. Therefore, yeast is responsible for the leavening of dough, in addition to the formation of desired sensorial traits **(Cukier *et al*. 2012)**. The characteristics structure and volume of yeast fermented products depend on carbon dioxide released by yeast cells. The success of bread bread-making technological process depends on the formation of CO_2 which leads to decreasing the weight of fermented bread dough. Today, the activity of yeast cells in fermenting dough is usually determined either by plate count method by measuring CO_2 produced by yeast cells or by decreasing the weight of fermented bread dough, which is influenced by the quantities of CO_2 released by yeast cells. The weight decrease of fermented bread dough is attributed to the quantities of gas production, whereas the volume of fermenting dough was increased.

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As shown from the results recorded in **Table 4**, concerning the effect of 4 g sucrose on the fermentation process, the hybrid H_1 genotype appeared negative hybrid vigor for decreasing the weight of fermented bread dough over time of the fermentation course. This hybrid vigor ranged between – 0.009 after 10 minutes to – 0.041% after five minutes of fermentation time. Meanwhile, the hybrid H_2 genotype revealed negative hybrid vigor (- 0.060) after 15 minutes of fermentation time. These results still reflect the bioactivity of hybrid genotypes about their parents for producing higher quantities CO_2leading to a decrease in the weight of fermented bread dough, as well as, increased dough volume.

Table 4. The weight of bread dough supplemented with 4 gram sucrose which affected by yeast strains and their hybrids during different fermentation times.

Genotypes	Fermentation time(minutes)				Decrease in bread weight %
	0.00	5	10	15	
P_1	108.33	108.28	108.23	108.21	0.110
P_3	108.34	108.31	108.27	108.25	0.080
MP	108.34	108.30	108.25	108.23	0.095
H_1	108.34	108.25	108.24	108.21	0.120
Hybrid vigor %	0.00	- 0.041	- 0.009	- 0.018	
P_1	108.33	108.28	108.23	108.21	0.110
P_2	108.33	108.32	106.64	108.22	0.100
MP	108.33	108.30	107.44	108.22	0.105
H_2	108.34	108.30	108.28	108.15	0.180
Hybrid vigor %	0.00	0.00	+ 0.786	- 0.060	
F - test	NS	NS	NS	NS	
LSD 0.05	144.14	15.12	15.34	15.11	
LSD 0.01	209.70	21.99	22.33	21.98	

NS: Not significant.

Hybrid genotypes produced the lowest weight of fermented bread dough due to high quantities of CO_2 released by their cells. This indicated that the hybrid yeast cells have a higher amount of live cells leading to higher gas production power than the parental strains. The high number of hybrid colony-forming units per one ml yeast cell suspension led to high performance in fermentation power. So, more live hybrid yeast cells means more viability and bioactivity that leads to higher quantities of gas production which contributes to decreasing the weight of fermented bread dough. Therefore, hybrid yeast cells lead to the highest volume and height of bread, as well as, decreasing the weight of bread dough. These results showed interrelationship between the quantities of gas production and decreasing the weight of fermented bread dough. This is in harmony with **Kasaie *et al*.(2017)**, who found that there was a positive direct correlation between yeast survivability, amount of cell frequency unit, yeast gas production, and height and volume of bread. According to these results, hybrid yeast cells are the best samples for the bread dough-making industry, because of their high number of live cells in the fermentation medium, high bioactivity, high ability in gas production, and efficiency in decreasing the weight of fermented bread dough. Among all yeast genotypes, the gas production rate was increased over time by H_1 hybrid, as well as, H_2 hybrid after 15 minutes of fermentation time. This result is due to the higher bioactivity of the H_2 genotype in the fermentation medium

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after 15 minutes of fermentation time. The decrease in fermented bread dough weight had a direct association with the gas production ability of yeast strains and their hybrids. Therefore, the high gas-powering ability of yeast genotypes leads to a decrease in the weight of fermented bread dough.

As shown from the results presented in **Table 5**, hybrid H_1 is still expressed in negative hybrid vigor for decreasing the weight of fermented bread dough at all fermentation times. In contrast, hybrid H_2 appeared positive hybrid vigor under the same effect of sucrose stress (6 g/325-gram wheat flour). The results indicated that the amount of CO_2 generated by hybrid H_1 in the fermentation medium was higher than that produced by the parental strains.

Table 5. The weight of bread dough supplemented with 6-gram sucrose is affected by yeast strains and their hybrids during different fermentation times.

Genotypes		Fermentation time(minutes)				Decrease in bread weight %
		0.00	5	10	15	
P_1		108.33	108.22	108.15	108.09	0.220
P_3		108.33	108.29	108.25	108.23	0.090
MP		108.33	108.25	108.20	108.16	0.155
H_1		105.33	105.25	105.21	105.14	0.180
Hybrid vigor %		0.00	- 2.76	- 2.79	- 2.79	
P_1		108.33	108.22	108.15	108.09	0.220
P_2		108.33	108.25	108.23	108.18	0.140
MP		108.33	108.23	108.19	108.13	0.180
H_2		108.33	108.31	108.30	108.21	0.110
Hybrid vigor %		0.00	+ 0.074	+ 0.102	+ 0.074	
F - test		NS	NS	NS	NS	
LSD	0.05	6.77	6.81	6.81	6.83	
	0.01	9.85	9.91	9.91	9.94	

NS: Not significant.

The overall CO_2 generated by the H_1 genotype throughout the fermentation leads to a decrease in the weight of fermented bread dough, as well as, leading the dough volume to rise. The reduced weight of fermented bread dough is the hypothesized main influence of CO_2 production by the H_1genotype. Throughout this fermentation course under the effect of 6 g sucrose, the results indicated that H_1 genotypes were tolerant to 6 g sucrose concentration in the fermentation medium, where the high number of yeast cells under sucrose stress generated a high concentration of CO_2. These results are in agreement with **Guadalupe-Daqui and Macintosh (2019)**, who found that the increased number of cells in suspension was reported to ferment under vacuum pressure. The results reflected a faster fermentation process by hybrid H_1 genotype. This is linked to the increase in the amount of generated CO_2, as well as, the decrease in the weight of fermented bread dough. Therefore, higher quantities of CO_2 released led to a decrease in the weight of fermented bread dough. This mainly due to the higher sugar consumption also led to the formation of additional viable cells which increased generated CO_2 and other metabolic products based on Balling's equation **(Balling 1865)**. Increased CO_2 production by hybrid H_1 during fermentation could have a significant impact on the weight of

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fermented bread dough. CO_2 production could have potentially improved fermentation efficiency without the need to add or modify the capacity of existing fermenters. The results reflected the interrelationship between CO_2 production by H_1 hybrid leading to a decrease in the weight of fermented bread dough and yeast viability. This study focused on the weight of fermented bread dough which reflects fermentation power, where the hybrid yeast cells released high quantities of CO_2 leading the dough volume to expand and reduce in their weight. For all genotypes tested in this study, gas production by hybrid H_1genotype was increased over time leading to negative hybrid vigor obtained for decreasing the weight of fermented bread dough. The negative hybrid vigor obtained by the H_1 genotype means more hybrid cells in the fermentation medium with more viability and bioactivity. This leads to more gas production power influenced to decrease the weight of fermented bread dough. So, all tests showed a direct association between yeast cell survivability, gas production power, height, and volume of bread, as well as, the decrease in the weight of fermented bread dough.

According to the results tabularized in **Table 6**, hybrid yeast cells were sensitive to 8 g sucrose concentration in the fermentation medium, where they appeared positive hybrid vigor for decreasing the weight of fermented bread dough. Under sucrose stress, only H_2 hybrid cells appeared negative hybrid vigor after five minutes of fermentation times. The concentration of 8 g sucrose hurts carbon dioxide and ethanol production which are responsible for dough leavening ability during the fermentation phase and the oven rise. Sucrose was commonly added to dough ingredients with different levels for the improvement of bread flavor and as carbon for yeast cells. Then, if the sucrose concentration in dough was high, as in sweet dough that contains sucrose up to 30%, the yeast cells had severe osmotic stress. This damaged cellular components and reduced the fermentation power of yeast cells**(Sasano *et al.* 2012)**. Indeed, exposure of yeast cells to hyperosmotic stress leads to rapid cell hydration, as well as, limits cell growth and CO_2 production capability. As a consequence of hyperosmotic stress, the fermentation rate and final volume of the bread product are reduced, and the weight of fermented products is not decreased enough.

Table 6. The weight of bread dough supplemented with 8-gram sucrose is affected by yeast strains and their hybrids during different fermentation times.

Genotypes	Fermentation time(minutes)				Decrease in bread weight %
	0.00	5	10	15	
P_1	108.33	108.21	108.11	108.00	0.30
P_3	108.32	108.28	108.25	108.23	0.08
MP	108.32	108.24	108.18	108.11	0.19
H_1	108.33	108.25	108.18	108.16	0.16
Hybrid vigor %	0.00	+ 0.009	0.00	+ 0.046	
P_1	108.33	108.21	108.11	108.00	0.30
P_2	108.34	108.32	107.31	108.25	0.08
MP	108.33	108.26	107.71	108.12	0.19
H_2	108.33	108.15	108.30	108.15	0.17
Hybrid vigor %	0.00	- 0.102	+ 0.922	+ 0.028	
F – test	NS	NS	*	NS	
LSD 0.05	25.43	25.42	18.78	18.82	

	0.01	36.99	36.99	27.33	27.38

NS = Not significant. * : Significance at 0.05 probability level.

Hybrid yeast cells may need to adapt to the hyperosmotic pressure in dough because they are not producing CO_2 at acceptable rates under these conditions. This indicated that high sucrose levels in dough prolong the time that the hybrid yeast cells need to achieve their maximal fermentation power. Therefore, yeast cells need to acquire tolerance to stress by the induction of stress protein expression**(Shima and Takagi 2009)**. Glycerol and trehalose were accumulated in yeast cells when osmotic pressure was sensed **(Shima and Takagi 2009).** Thus, high-sucrose tolerant yeast show a high expression of proteins required for the production of glycerol and trehalose, if sucrose levels reach 5 to 40%. Also, proline accumulation confers tolerance to high sucrose stress **(Sasano *et al*. 2012)**. Improved the accumulation of intracellular trehalose, proline or glycerol increased the Osmo tolerance of yeast cells **(Sasano *et al*. 2012)**. Decreasing bread dough weight during fermentation is very often used as a criterion for gas production. **Chiotellis and Campbell (2003)** reported that increasing the temperature during fermentation increases the growth of bubbles inside the dough, not only due to the increased rate of CO_2 production but also as a result of decreased gas solubility.

Heterosis seen in yeast cells up to 6 g sucrose in the fermentation medium is not only important for speciation and adaptation of yeast cells but also for an intrinsic economic value. Since heterosis was discovered in our food chain from crop production to cattle breeding **(Shull 1908)**. Heterosis in *Saccharomyces* yeasts was first reported by **Lindgren *et al.*(1953)**when they obtained F_1 hybrid had an advantage in the fermentation of maltose. Nowadays heterosis is a more general view because of the fitness advantage of the F_1 hybrid if compared to one or both parents **(Zörgö *et al*. 2012)**. The results obtained in this study are in line with **Wongkhalaung and Boonyaratanakornkit (2007)**, who found that hybrid yeast strain proved to be highly promising in leavening ability with all types of bread dough in terms of total CO_2 production. The results are also in harmony with **Krogerus *et al.*(2015)**, who found that hybrid genotypes in yeast successfully displayed fermentation rates higher than both parental strains. The same authors decided that not only do the hybrid genotypes ferment faster but also they are produced with higher alcohol content. Therefore, the hybrid yeast genotypes exhibited improved fermentation rates if compared with the parental strains. Some differences obtained between hybrid yeast cells in fermentation performance could be a result of meiotic segregation during spore formation or differential inheritance of mitochondrial DNA, as mitochondrial DNA is inherited from only one parent **(Krogerus *et al*. 2015)**. Therefore, hybrids resulting in novel lineages that have chimeric genomes exhibit unique phenotypic traits that are not necessarily intermediate between both parents. Thus, **Gabaldón (2020)** reported that different yeast hybrids in the *Saccharomycotina* were discovered many of them have industrial applications or clinical relevance.

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Correlation and regression analysis

As shown from the results presented in **Figures 1, 2, 3, 4 and 5**, the regression coefficient of the interrelationship between the decrease in fermented bread weight affected by the P_1 genotype at zero sucrose concentration ranged between 0.006 (at 4 g sucrose) to 0.019 (at 8 g sucrose). These results mean that the highest reduction in fermented bread dough was achieved at 8 g sucrose, which released higher values of CO_2 leading to high decreases in bread dough weight. This indicated that the weight of fermented bread dough was decreased by 0.019 with each additional unit of fermentation time. The correlation coefficient between both variables ranged between 0.982 (at 4 g sucrose) to 1.0 (at 8 g sucrose). The highest correlation coefficient was obtained at 8g sucrose. Therefore, the coefficient of determination is 1.0, which means that 100% of the variance in bread dough was attributed to the time of fermentation. The results reflected that both variables vary in the same direction. This means that if the time of fermentation is increasing, the decrease in fermented bread dough is also increasing. Then the correlation between both variables is said to be positive correlation. This means that the changes in two variables are in the same direction. The results also indicated that the parental strain P_1 genotype was tolerant to 8 g sucrose in the fermentation medium. These results agreed with **Moore *et al*. (2013)**, who demonstrated that if the correlation between two variables is 0.7, then the coefficient of determination is 0.49, therefore approximately 50% of the variance in the response variable can be due to the explanatory variable.

According to the results diagrammatic in **Figures 6, 7, 8, 9, and 10**, the regression coefficient between the decrease in fermented bread dough affected by P_2 genotype and fermentation times ranged between - 0.082 (at 4 g sucrose)to 0.009 (at 0 g sucrose). These results mean that the higher decrease in bread dough was achieved at zero sucrose concentration in the fermentation medium. This indicated that the parental strain P_2 genotype was intolerant to the different concentrations of sucrose. The weight of fermented bread dough was decreased by 0.009 g with each additional unit of fermentation time without any addition of sucrose. The correlation coefficient between both variables ranged between - 0.546 (at 4 g sucrose)to 0.982 (at 0.0sucrose). This means that at zero sucrose concentration the coefficient of determination was 0.9643, therefore96.43% of the variance in decreasing bread dough weight was attributed to fermentation time. The remaining variance of 3.67% remains unexplained and is attributed to other factors that are not present in the regression formula as yeast genotype. CO_2production etc. The results indicated that the P_2 genotype was bioactive at the beginning time of fermentation under the effect of 4 g sucrose in the fermentation medium. If the time of fermentation increased under sucrose stress with 4 gin the fermentation medium the bioactivity of P_2 genotype was decreased.

Regarding the results presented in **Figures11, 12, 13, 14 and 15**, the regression coefficient between the decrease in fermented bread dough by P_3 genotype and fermentation times ranged between 0.004 under the effect of 8 g sucrose in the fermentation medium to 0.005 at all other sucrose concentrations. This indicated that sucrose addition did not stimulate the power of fermentation. The correlation coefficient between both variables ranged between 0.032 (at zero sucrose stress) to 1.00 at 8 g sucrose in the fermentation medium. This means that the coefficient of determination for this relationship is 1.00. Then 100% of the variance in decreasing bread weight at 8 g sucrose is due to the time of fermentation. This agrees with **Schneider *et al*.(2010)**, who reported that if the coefficient of determination between height and

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weight is 0.785, then 78.5% of the variance in weight is due to height, the remaining 21.5% is attributed to individual variation as eating habits, exercise, sex or age, that were not taken into account of the analysis.

As shown from the results presented in **Figures 16, 17,18, 19, and 20**, the regression coefficient between the decrease in fermented bread weight by the hybrid genotype H_1 and fermentation time ranged between 0.004 (at 2 and 4 g sucrose) to 0.010 (at 6 g sucrose). This means that the highest decrease in the weight of fermented bread dough was achieved at 6 g sucrose in the fermentation medium. Furthermore, the weight of fermented bread dough decreases by 0.010 gwith each additional unit of fermentation time using 6 g sucrose in the fermentation medium of the H_1 genotype. On the other hand, the correlation coefficient between both variables ranged between 0.952 (at 8 g sucrose) to 1.00 (at 2 g sucrose). The coefficient of determination was equal to 1.0 at 2 g sucrose. This means that 100% of the variance in decreasing bread through weight is due to fermentation time. The results indicated that the hybrid H_1genotype was tolerant to high sucrose stress.

Therefore, correlation analysis helps microbial geneticists in deriving precisely the degree and the direction between fermentation power and the times of fermentation. In most cases, the time of fermentation leads to reduce the weight of fermented bread dough. Thus, predicting the weight of fermented bread dough based on regression analysis at different fermentation times will be more reliable and near to reality. Today's yeast industry has many activities which are mainly dependent on yeast genotypes. Hybrid genotypes help yeast geneticists resolve many problems in the yeast industry because of the heterosis phenomenon in the yeast chimeric genome. Heterosis in yeast confers a competitive advantage in changing environments which drives yeast to evaluation and adaptation. This is especially significant for yeast through many stages of fermentation in which rapid adaptation may be advantageous **(Steensels *et al*. 2021)**. The results obtained herein are in harmony with **Winans (2022)**, who found that interspecific hybrid in yeast produces the lion's share, in volume, of the global beer productivity. In addition, the same author found that the composition of genetic material transferred through hybridization and retained in yeast hybrids impart significant fermentation traits and phenotypes as efficient fermentation of maltose and maltotriose. In the same criteria, **Walther *et al*.(2014)** found that the Frohberg hybrids containing more *Saccharomyces cerevisiae* genome conferring higher ethanol production, differing ester profiles and higher variabilities. Therefore, investigation into yeast hybrids supports bolstering the fermentation capacity of *Saccharomyces cerevisiae* because of hybrid vigor incorporation of a positive phenotype.

According to the results diagrammatic in **Figures 21, 22, 23, 24 and 25**, the regression coefficient obtained by the hybrid genotype H_2 ranged between - 0.17 (at 8 g sucrose) to 0.016 (at 2 g sucrose). This means that the highest decrease in the weight of fermented bread dough occurred under the effect of 2 g sucrose in the fermentation medium. The regression coefficient of 0.016 at 2 g sucrose means that fermented bread dough was decreased by 0.016 g with each additional unit of fermentation time. The correlation coefficient between the decrease in fermented bread dough weight and fermentation times ranged between 0.00 at 8 g sucrose to 0.952 at zero sucrose concentration. The highest correlation (0.952) between both variables was obtained at zero sucrose concentration. Then, the coefficient of determination is 0.9063. This means that at zero sucrose concentration, 90.63% of the variance in decreasing bread dough

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weight was attributed to fermentation times. The rest variance 9.37% remains unexplained and is attributed to other factors that are not present in the formula of regression. Zero correlation obtained at 8 g sucrose means that plotted points are scattered over the graph haphazardly as shown in Figure 25. This indicates that there is no correlation or zero correlation between both variables under the effect of 8 g sucrose stress. Then regression line is perpendicular as seen in Figure 25**.** In the perfect positive correlation as seen in some Figures diagrammatic in this study, the points will form a straight line falling from the lower left-hand corner to the upper right-hand corner. Meanwhile, the plotted points in the high degree of positive correlation fall in a narrow hand, with a rising tendency from the lower left-hand corner to the upper right-hand corner. Furthermore, the points with a low degree of positive correlation are widely scattered over the diagram which are rising from the left-hand corner to the upper right-hand corner. Thus, the correlation coefficient measures a linear relationship between the decrease in bread dough weight and fermentation time. This indicates the amount of variation in one variable accounted for by the other variable. Meanwhile, regression analysis establishes a functional relationship between the decrease in bread dough weight and fermentation time. This leads this relationship to be used to predict future projections in the baker's industry. Thus, regression analysis is a powerful tool used to predict the weight of fermented bread dough at different fermentation times with each genotype of *Saccharomyces cerevisiae*. Usually, the variables are labeled as response variables as the weight of fermented bread dough or independent of the fermentation time. The independent variable also called an input or driver factor has an impact on the dependent variable called an outcome. Therefore, regression analysis can be used for predicting the weight of fermented bread dough based on its relationship with the times of fermentation. **Ali and Naylor (2010)** presented the relationship between the two variables using scatter plots, which were commonly used graphs for the results displayed in regression analysis. In a scatter plot, the clustering of dots denoted the strength of interrelationship, whereas the direction indicates the nature of the relationship among variables which was shown positive between the decrease in bread dough weight increases due to an increase in fermentation time. This means that if the fermentation times increase, this results in an increase in the reduction of bread dough weight. Therefore, the slope of the regression line depends on the correlation between these variables. Thus, the correlation coefficient helps to identify how good a variable is at predicting the other variable. Therefore, regression analysis is an important technique in microbial genetics data. It enables microbial geneticists to identify the relationship between two variables, as seen between the fermentation times and increasing the reduction of fermented bread dough weight used in this study. Both variables appeared a positive correlation and positive regression among all genotypes, which are discussed in this study in detail. For example, microbial geneticists would like to know the weight of fermented bread dough after different fermentation times. To evaluate whether the likelihood of different yeast genotypes having high performance in fermentation power is influenced by the weight of bread dough. Baker's workers have attempted to determine the quality genotype available in the market by measuring fermentation power, which is critical for consistent fermentation performance. To inoculate the correct genotype to be used in the baker's industry, microbial geneticists must measure accurately yeast cell number and yeast viability**(Thornton 2002)**.

The results obtained in this study reflected that the P_1 genotype expressed fermentation activity at 8 g sucrose (b = 0.019) for reducing the weight of fermented bread dough with 0.019 g

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with each unit of fermentation time. Meanwhile, the P2 genotype was intolerant to sucrose because of the higher rate (b = 0.009) in decreasing the weight of fermented bread dough at zero sucrose concentration. Furthermore, the P_3 genotype did not show a higher rate of decreasing the weight of fermented bread at any sucrose concentration. The hybrid H_1 genotype appeared high fermentation power at 6 g sucrose (b = 0.010) which led to a decrease in the weight of fermented bread dough because of high quantities of CO_2 released in bread dough. On the other hand, the hybrid H_2 genotype revealed high fermentation power (b = 0.016) at 2 g sucrose in the fermentation medium. This is because one of the parents of this hybrid is the P_1 genotype which achieved the higher fermentation power (b = 0.019) at 8 gsucrose in the fermentation medium. Therefore, the H_2 hybrid had acquired fermentation activity from the P_1 genotype as one of his parents. This needs to increase attenuation about the parental strains used in hybridization experiments, as a valuable tool for yeast hybrid development. This enables the combination and enhancement of traits from both parental strains**(Krogerus *et al.* 2017)**. Hybridization results in yeast cells with high chromosome numbers, influencing gene dosage, as well as, gene expression during cellular processes and partially explains the outperformance over a diploid strain from the same genotype **(Krogerus *et al.* 2017)**. Similarly, the Advanced Industrial Science and Technology Center in the Biomedical Research Institute in Japan designed a protocol focused on avoiding the heterozygosity losses of yeast cells. A series of isolating the mating types α - and a - type cells from the mixed cell populations to undergo continued cross-breeding **(Fukuda *et al.* 2016)**.In addition, CRISPR/Cas 9 has been utilized to force double-stranded breaks in mating type locus to increase the diversity in industrial yeast strains and their hybrids **(Krogerus *et al.* 2021)**.

In the coming years, industrial yeast genotypes will be designed by utilizing these protocols with molecular toolkits. Hybridization in yeast cells bears several advantages in fermentation power as increased bread dough fermentation through CO_2 released, shifting fermentation temperatures toward traditional inhibitory conditions, and increased ethanolic fermentation performance. Targets of yeast hybridization may include increased production of yeast longevity molecules such as trehalose. The techniques to create yeast hybrids vary in their specificity to target genetic or phenotypic results. However, efforts conducted in yeast hybridization led to an increase in the biodiversity in fermentation activity, adding depth to the complexity of fermentation profiles, as well as advanced fermentation science knowledge. There are many yeast genotypes favored for the production of quality fermented foods and beverages, their status may be strains or hybrids. By increasing yeast products in the markets, the yeast genotypes of tomorrow will focus on metabolic specifics, that will be driven by scientific innovation in microbial genetics laboratories today **(Winans 2022)**.

The results obtained in this study are in harmony with **Schober *et al.*(2018)**, who decided that the correlation measure of an association between variables that is the change of one variable is associated with a change in another variable, either in the same direction (named positive) or in the opposite direction (named negative). The bread industry includes a series of aeration phases in which bubbles are incorporated during mixing and inflated with CO_2 gas during proofing. This gas results from converting the fermentable sugars in the dough into carbon dioxide and ethanol alcohol as the main products. This gas leads them to increase the dough in volume and decrease their weight when fermentation time increases. This is the most apparent physical change related to fermentation activity in the dough **(Hutkins2006)**.

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Therefore, wheat flour is most commonly used in the bread industry due to the quality of its protein content, called gluten, which forms a viscoelastic matrix, which responsible for the dough behavior during the manufacture stages and gas retention during proofing **(Istudor *et al*. 2018)**. The yeast can ferment sugars in the dough to produce ethanol and carbon dioxide under anaerobic conditions in the dough. It can grow rapidly on sugar to produce high yields of cells each contributing informing ethanol and CO_2. The enzymatic reaction of *Saccharomyces cerevisiae* converts fermentable sugars and some starch-containing the dough into ethanol and carbon dioxide which causes the dough to rise and weight decrease **(Amendola and Rees 2002)**.

The assimilation of sucrose varied among yeast genotypes which indicated some metabolic diversity that can be harnessed in industrial application. Carbon dioxide productivity which leads to a decrease in the weight of bread dough, as shown in this study, is an important factor for bread making **(Amendola and Rees 2002)**. Therefore, *Saccharomyces cerevisiae* is used in bread baking as a leavening agent because it converts the dough's fermentable sugars into CO_2. This leads the dough to expand in their volume and decrease in their weight as CO_2 forms pockets or bubbles. If the dough is baked, the pockets remain, giving the baked product a soft and spongy texture. This agrees with **Okagbue(1988)**, who reported that yeast fermented sugars such as glucose and fructose, the breakdown release CO_2that causes the dough volume to rise. The highest leavening activity recorded in this study is the parental strain P_1 genotype and its hybrid-released H_2 genotype. These are the best biological wheat dough leavens obtained in this study because they can perform better fermentation power than other yeast genotypes used in this study. This is in line with **Somiari and Udoh (1993)**, who found that *Saccharomyces* species isolated from palm wine were observed to be better in leavening wheat dough. Thus, yeast strains that release high levels of carbon dioxide can be used in other processes, where this gas can be trapped for commercial purposes. In bread manufacture, wheat flour contains naturally different sucrose of fermentable sugars. These include glucose, fructose, sucrose, and maltose, in addition to any other fermentable sugar as sucrose added by the baker as seen in this study. These can reach 25% w/w in some. In sweet dough, the activity of yeast genotypes to ferment bread dough under these conditions of high osmotic stress is of crucial industrial importance. Ethanol tolerance is a unique property that makes yeast genotypes exploitable for industrial applications.

In this study, as indicated before, some of the yeast genotypes P_1 and H_2 were able to express their fermentation activity under stressful conditions of sucrose. Therefore, *Saccharomyces cerevisiae* genotypes were needed to physiologically adapt to extreme conditions before transferring to the industrial sector. A suitable concentration of ethanol is needed in bread manufacturing to show the preferred flavor. However, a high concentration of ethanol is reported to be toxic against the used cells because of inhabiting the cells to grow due to the destruction of cell membranes **(Smit *et al*. 1992)**. The results obtained in this study agreed with **Krogerous *et al*.(2017)**, who reported that yeast hybrids can display unique traits that may drive adaptation to new niches, suggesting a higher fitness under changing environments and potential to colonies in new niches. However, hybrids can inherit certain combinations of traits from their parental strains, or even novel traits. This may be beneficial for their survival or adaptation to new environments. This phenomenon is known as heterosis or hybrid vigor. The allele heterozygosity will be proportional to the genetic divergence of hybrids between their parents **(Krogerous *et al*. 2017)**. The results are also in line with **Gabaldón (2020)**, who decided that yeast hybrids have

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chimeric genomes leading to exhibit unique phenotypic traits that may enable them to grow in new environments. Many hybrids discovered among yeast in the *Saccharomycotina* have industrial or clinical relevance. Therefore, hybridization seems to play an important role in the evaluation of *Saccharomycotina* and other fungal clades.

The results are also in agreement with **Spencer *et al*. (1985)**, who reported that some segregants obtained from fusion hybrids in yeast had much greater dough-raising capability in simulation bread dough and sweet dough than either of the commercial baker's yeast or the original genotype. The data clearly show that fermented bread dough decreases if the fermentation time increases. This is an indicator for the quantities of carbon dioxide released by yeast cells which leads the dough volume to rise, as well as, the loaf weight to decrease. In this context, hybrid genotypes had already the best fermentation performance. Therefore, the production of CO_2 by yeast cells increases dough volume and decreases their weight because of CO_2 production which is incorporated in air bubbles through the dough matrix **(Romano *et al*. 1998)**. Other secondary metabolites such as amino acids, aldehydes and esters produced through the fermentation time contribute to the sensory profile of the final product **(Gélinas2012)**. Thus, *S. cerevisiae* is considered as safe for health and has been generally recognized as safe (GRAS) by the US Food and Drug Administration.

The results are also consistent with numerous investigations that describe *Saccharomyces cerevisiae* as a suitable leavening agent for the production of baked products **(Asyikeen *et al*. 2013)**. Furthermore, **Aouine *et al*.(2021)** used 2 g sucrose as an ingredient in the dough preparation, which improved leavening performance. Overall, the leavening properties of hybrid yeast genotypes classify them as probable active starters in dough fermentation. This attribute has an important technological value in the yeast industry. Wheat flour contains mostly starch, maltose, sucrose, glucose, fructose and other oligosaccharides **(Vaisey and Unrau 1964)**. **Henry and Saini (1989)** decided that the most important carbohydrates in flour affecting the loaf volume are glucose, fructose and sucrose. Which glucose is the preferred sugar for *Saccharomyces cerevisiae*? Sucrose is converted into glucose and fructose due to yeast invertase **(Sahlström *et al*. 2003)**. The results agreed with **Gabriela and Daniela (2010)**, who found that the maximum height of dough was correlated with the quantity of carbon dioxide released. In this study, the highest decrease in the weight of bread dough was registered by P_1 and H_2 genotypes. This indicated that these strains produced high quantities of CO_2 leading the dough volume to rise and the weight to decrease. This is due to the bioactivity of these genotypes in the fermentation medium. From this point of view the quantities of CO_2 released during fermentation time, lead to a decrease in the weight of bread dough, as well as, to increase in the volume of fermented loaf. Therefore, **Kasaie *et al*. (2017)** found that the CO_2 production rate increased over time. Therefore, the increase in CO_2 concentration in the dough which leads the fermented bread dough weight to decrease over time is very much influenced by the quality of yeast genotype. Thus, the use of a high-quality yeast genotype will result in a higher concentration of CO_2 during the fermentation process. The carbon dioxide production rate is related to the metabolism of yeast cells. Therefore, the development of dough is strongly dependent on the yeast genotype and the fermentation time.

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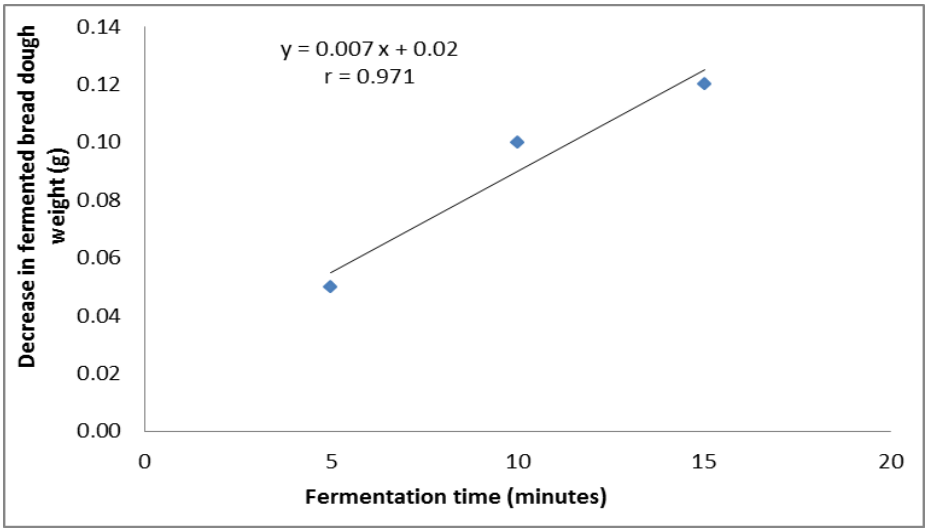

Figure 1. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_1 genotype at different fermentation times using zero sucrose concentration.

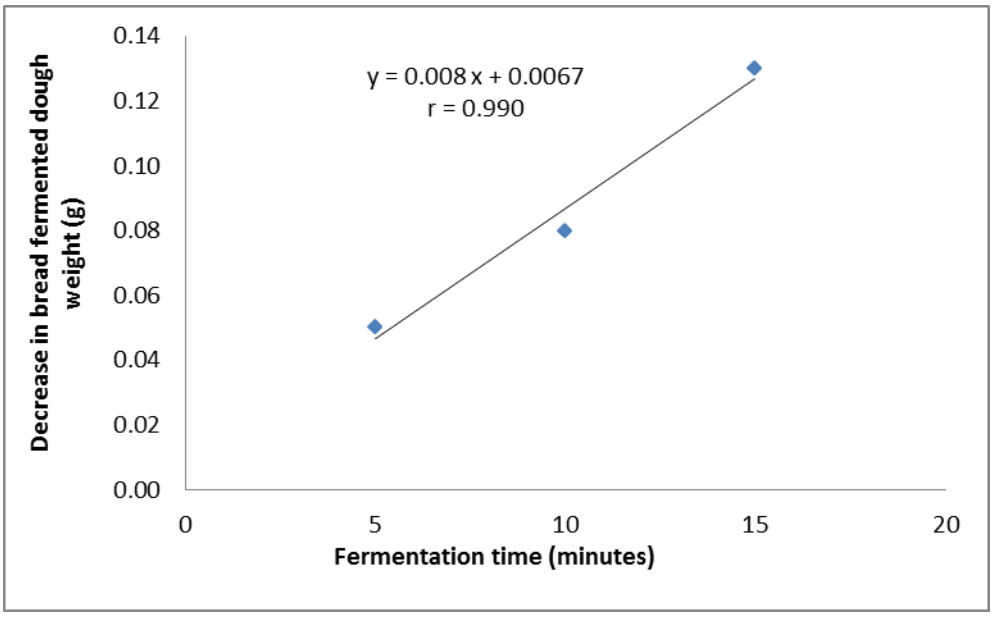

Figure 2. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_1 genotype at different fermentation times using 2 g sucrose concentration.

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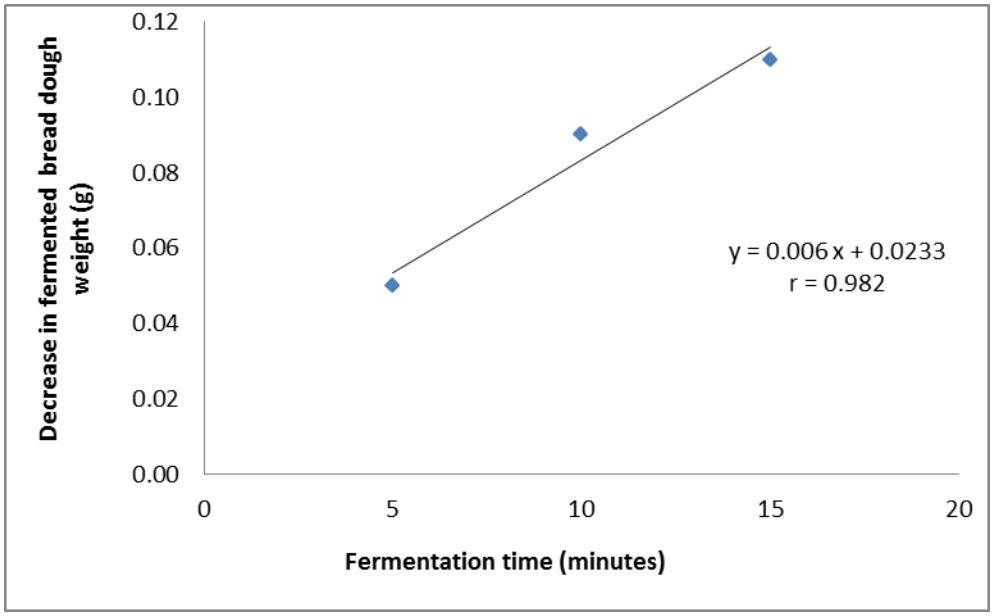

Figure 3. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_1 genotype at different fermentation times using 4 g sucrose concentration.

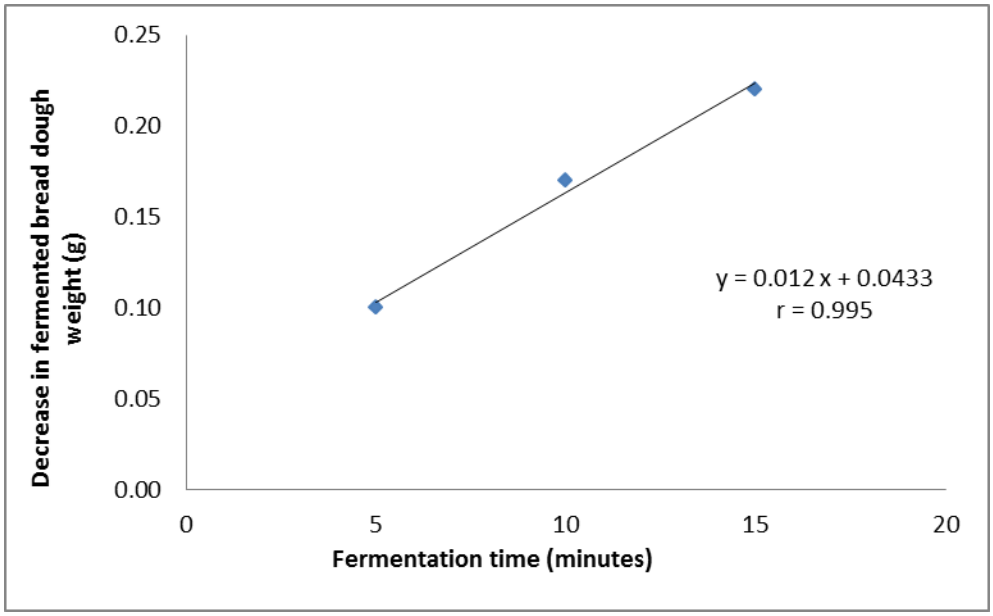

Figure 4. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_1 genotype at different fermentation times using 6 g sucrose concentration.

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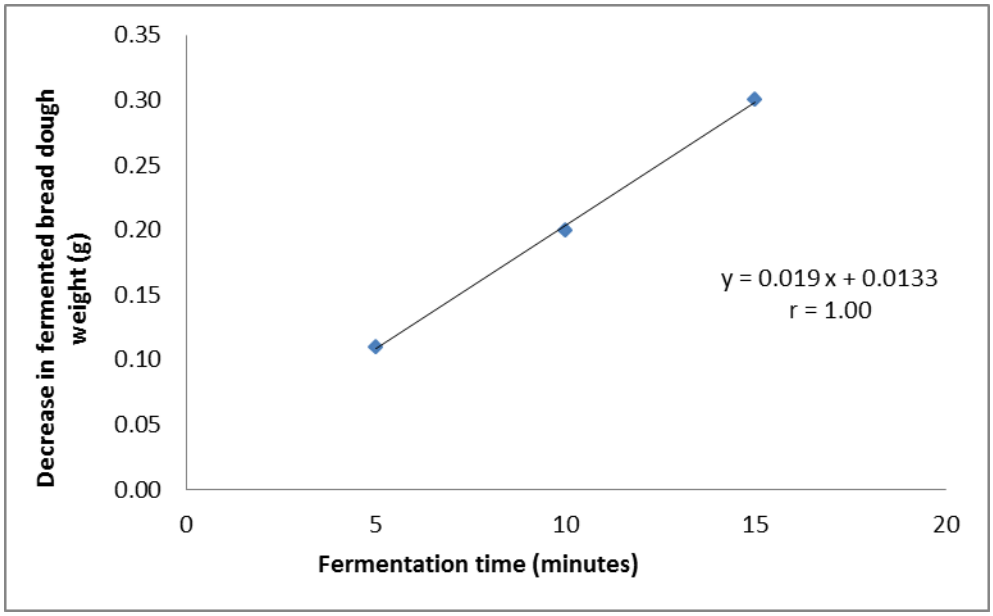

Figure 5. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_1 genotype at different fermentation times using 8 g sucrose concentration.

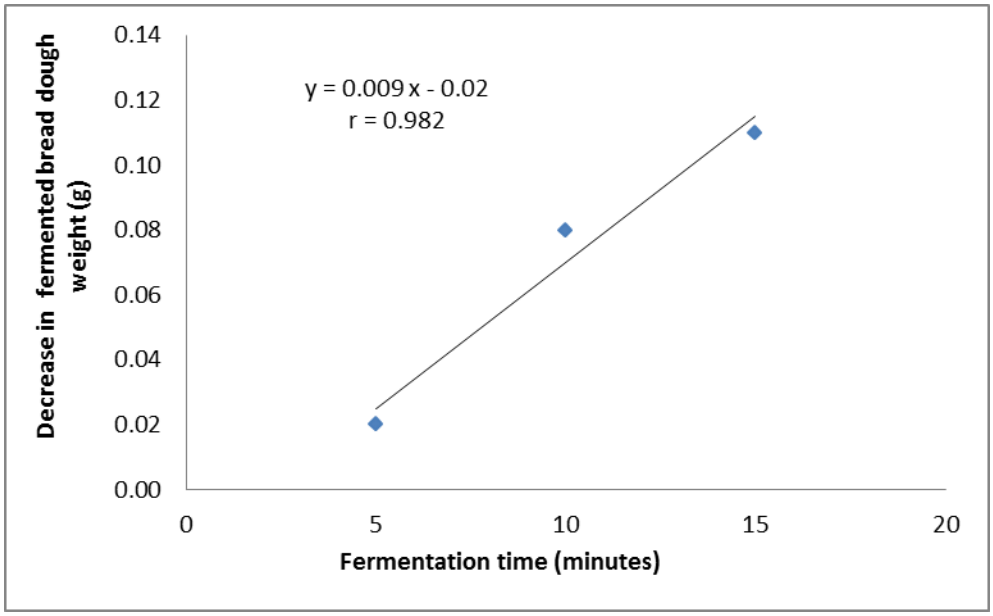

Figure 6. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_2 genotype at different fermentation times using zero sucrose concentration.

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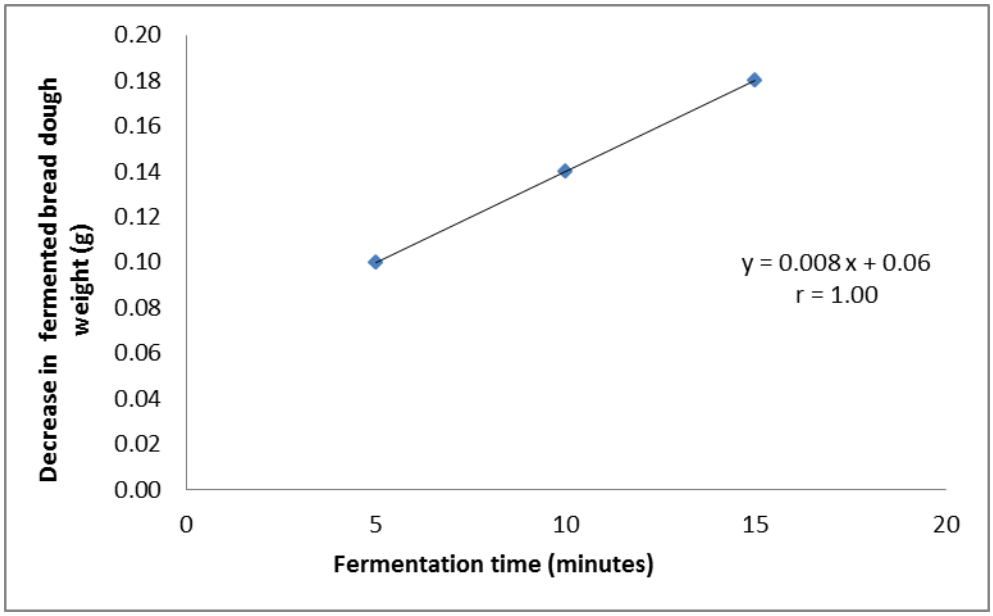

Figure 7. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_2 genotype at different fermentation times using 2 g sucrose concentration.

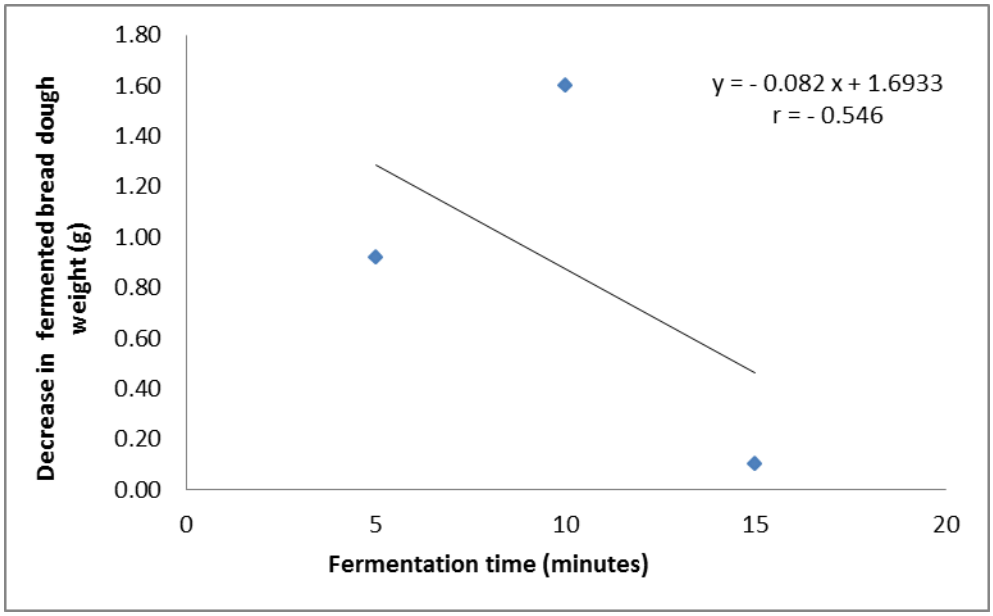

Figure 8. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_2 genotype at different fermentation times using 4 g sucrose concentration.

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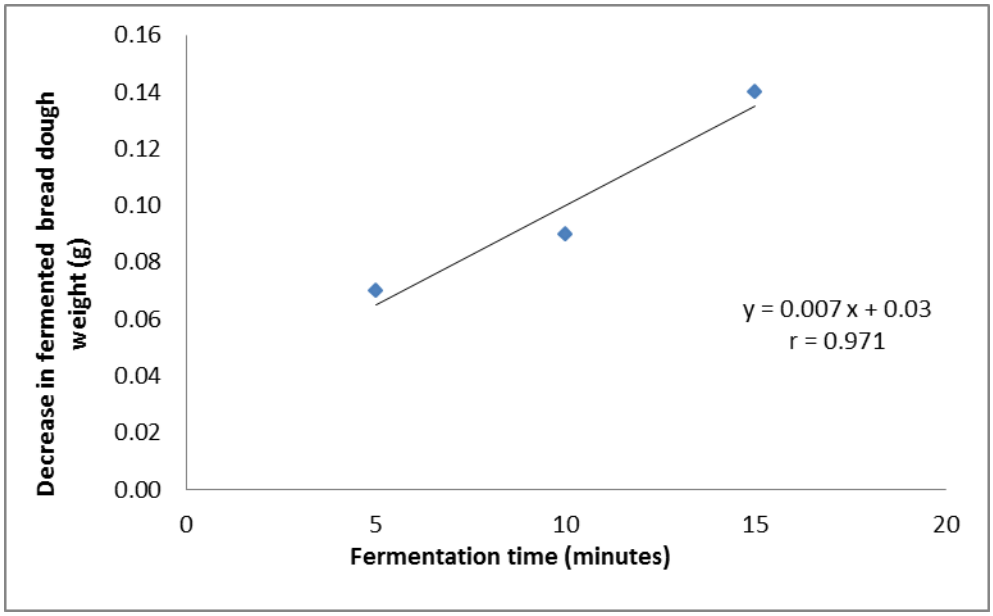

Figure 9. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_2 genotype at different fermentation times using 6 g sucrose concentration.

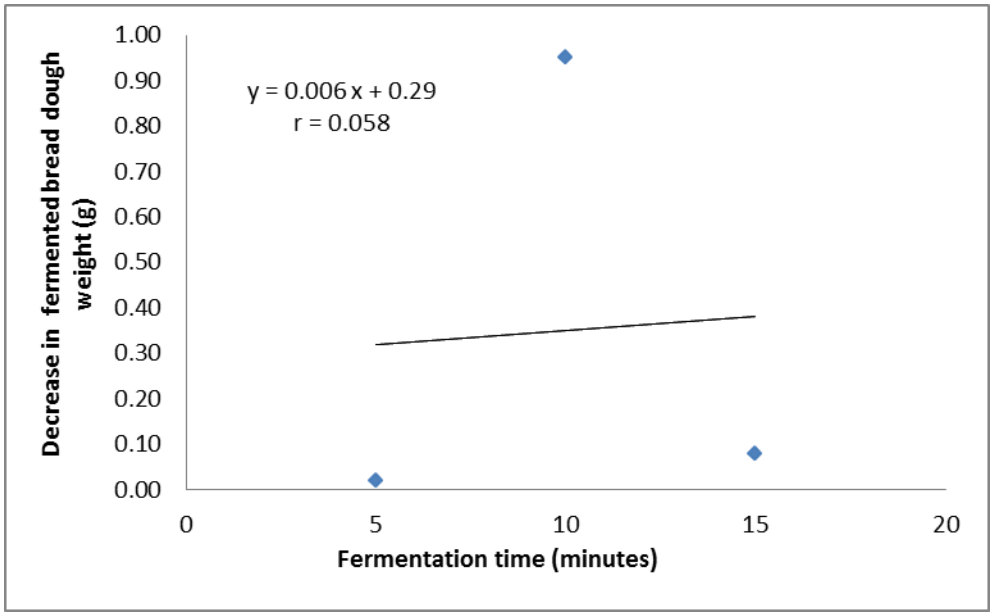

Figure 10. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_2 genotype at different fermentation times using 8 g sucrose concentration.

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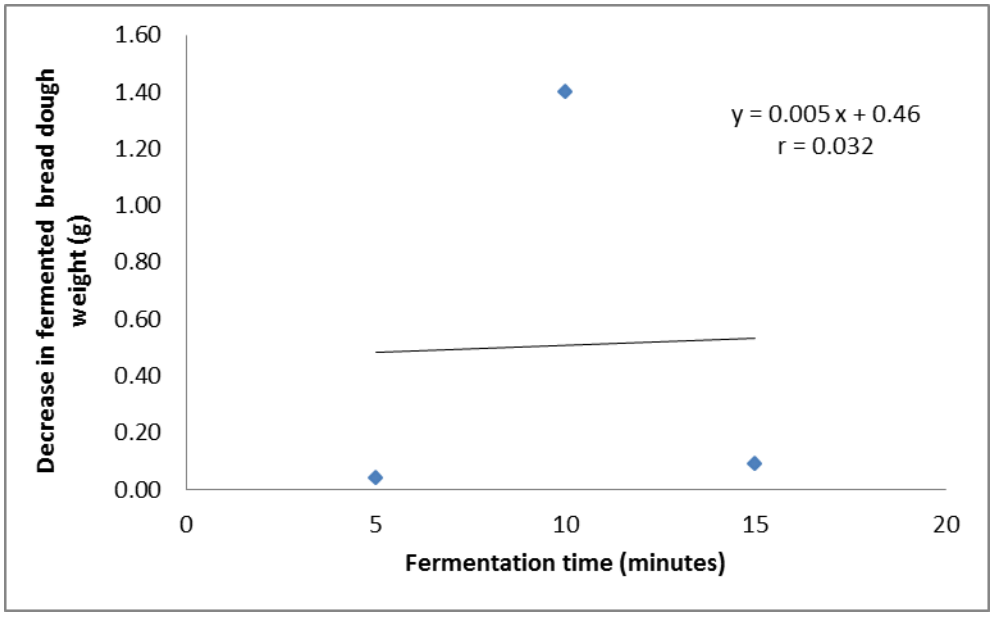

Figure 11. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_3 genotype at different fermentation times using zero sucrose concentration.

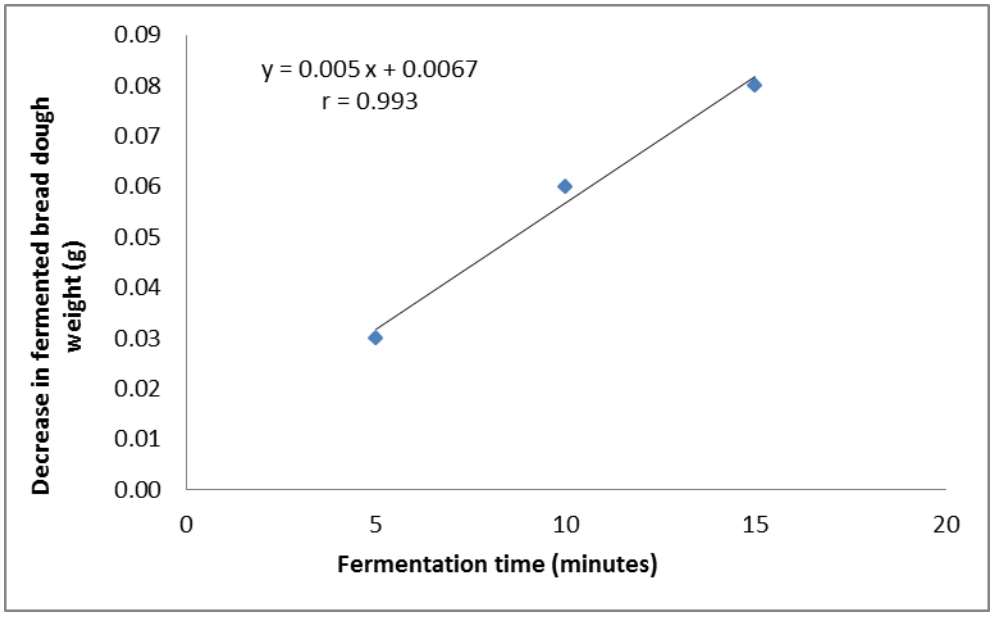

Figure 12. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_3 genotype at different fermentation times using 2 g sucrose concentration.

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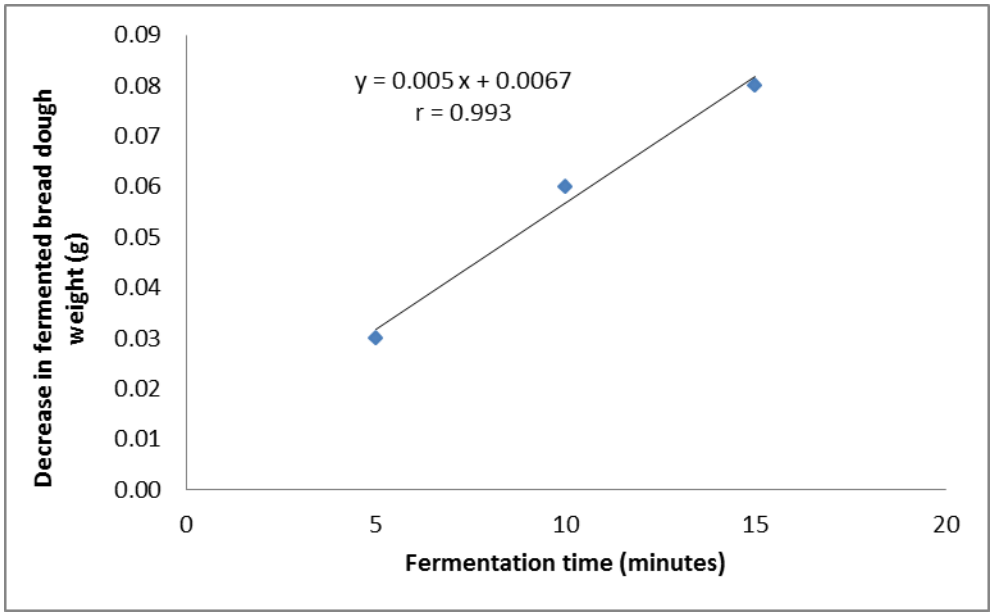

Figure 13. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_3 genotype at different fermentation times using 4 g sucrose concentration.

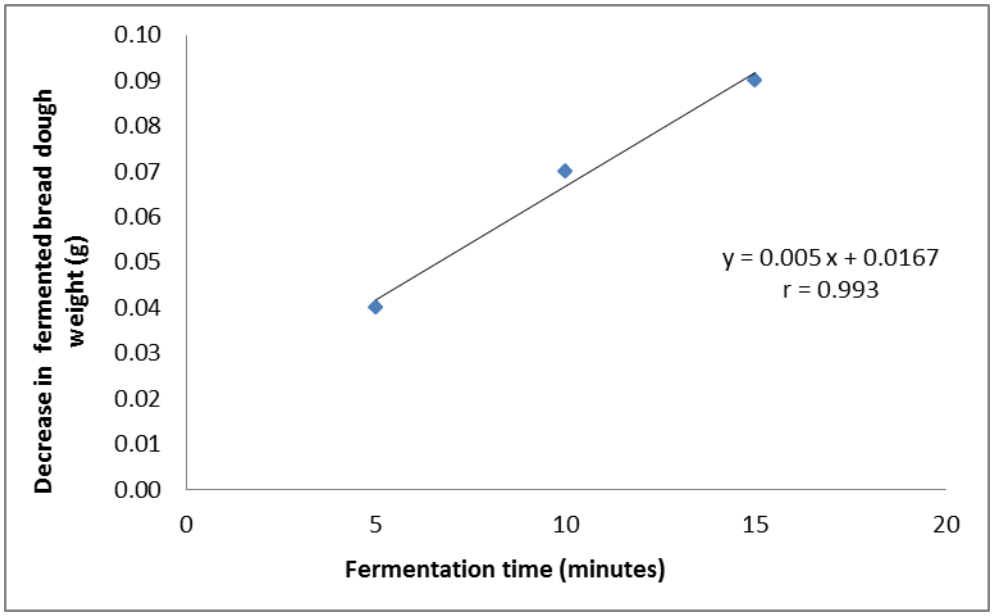

Figure 14. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_3 genotype at different fermentation times using 6 g sucrose concentration.

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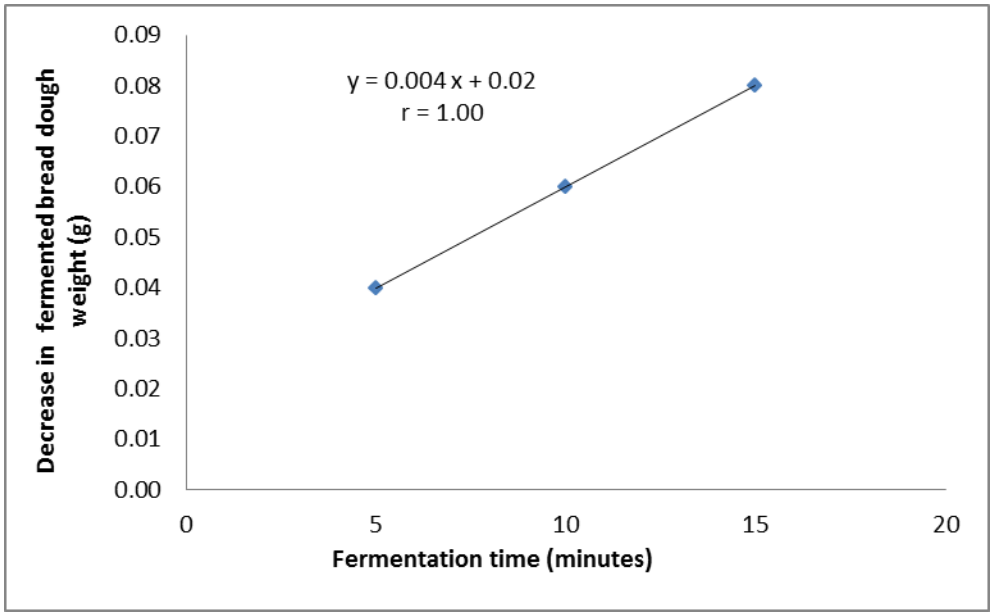

Figure 15. Regression line of relative decrease in fermented bread dough weight affected by the parental strain P_3 genotype at different fermentation times using 8 g sucrose concentration.

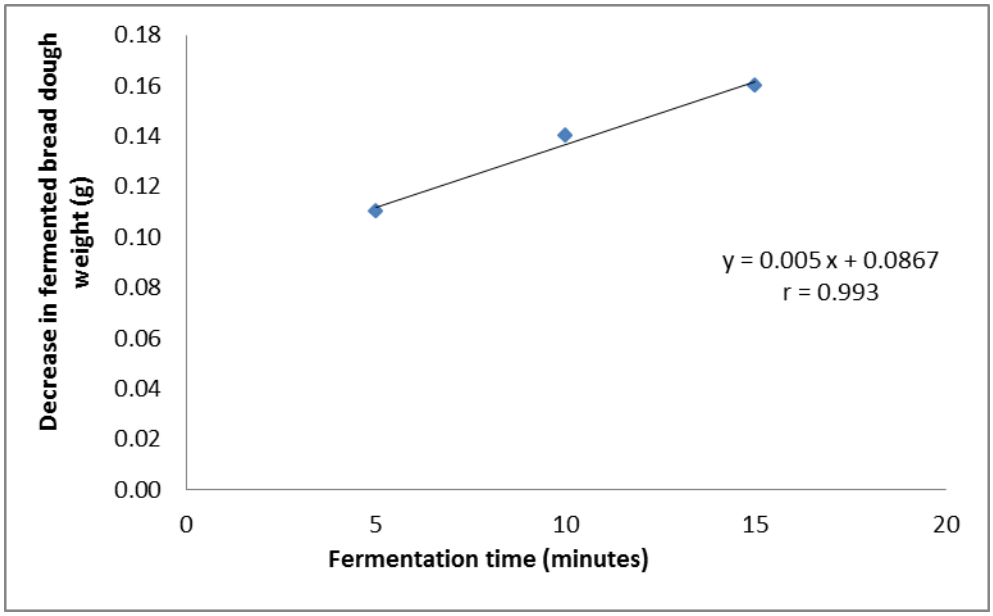

Figure 16. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_1) resulted from the mating between P_1 x P_3 at different fermentation times using zero sucrose concentration.

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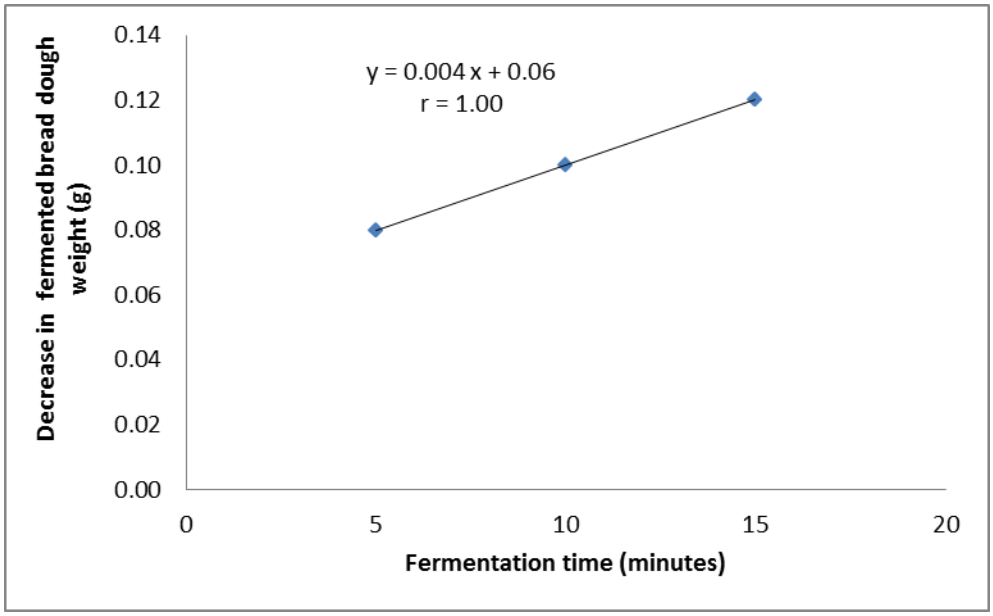

Figure 17. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_1) resulted from the mating between P_1 x P_3 at different fermentation times using 2 g sucrose concentration.

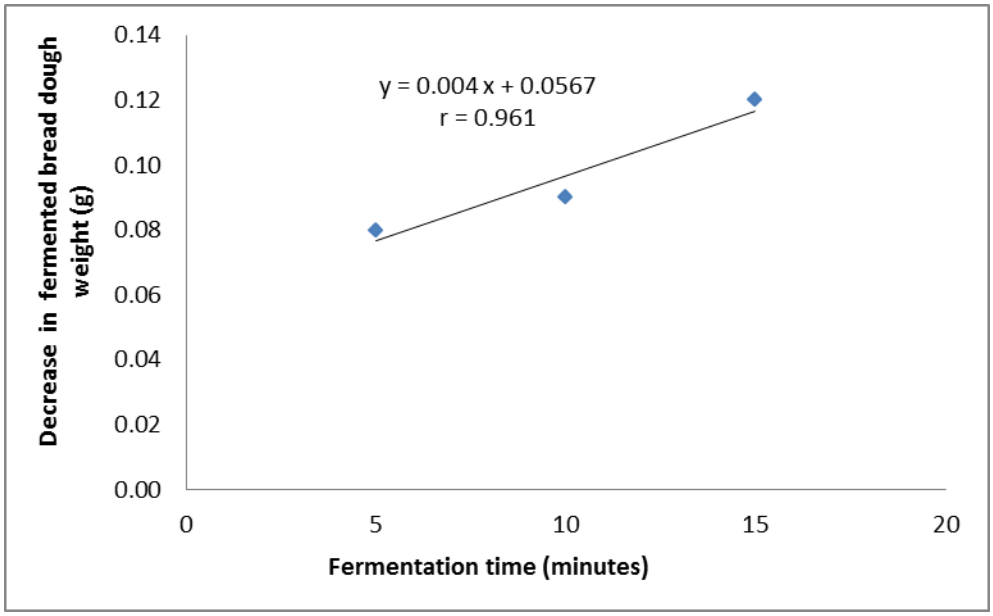

Figure 18. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_1) resulted from the mating between P_1 x P_3 at different fermentation times using 4 g sucrose concentration.

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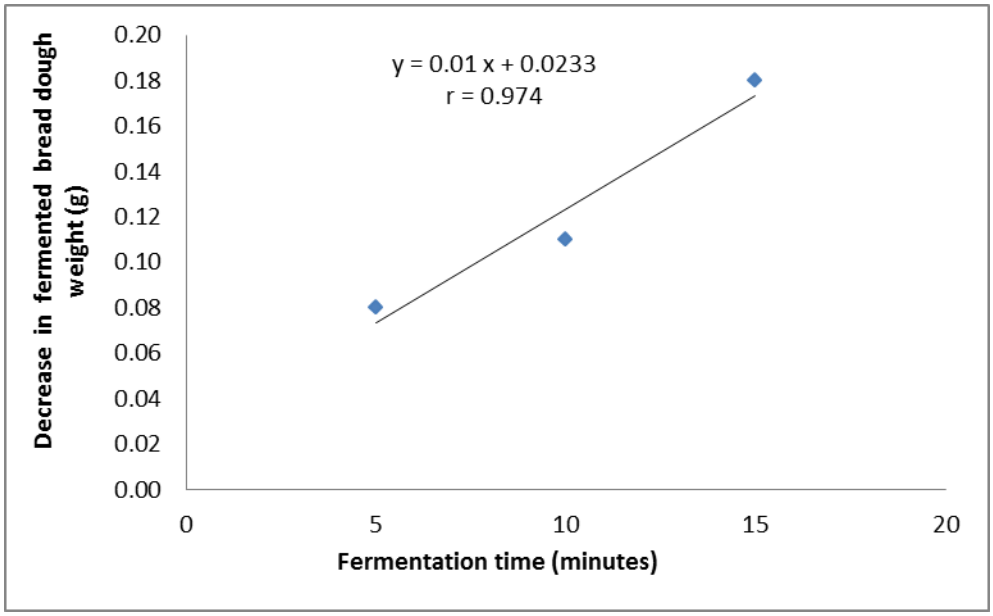

Figure 19. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_1) resulted from the mating between P_1 x P_3 at different fermentation times using 6 g sucrose concentration.

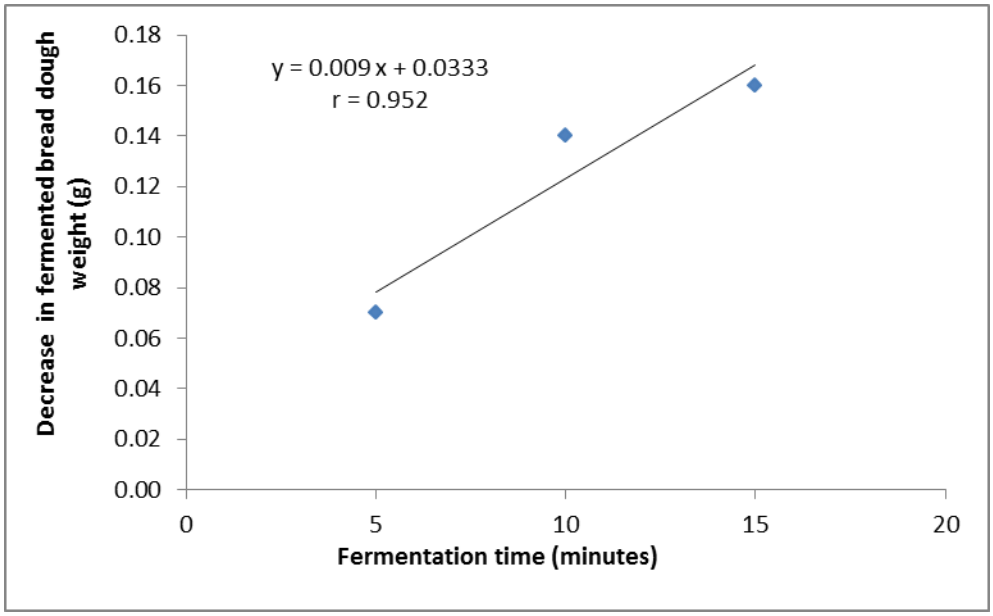

Figure 20. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_1) resulted from the mating between P_1 x P_3 at different fermentation times using 8 g sucrose concentration.

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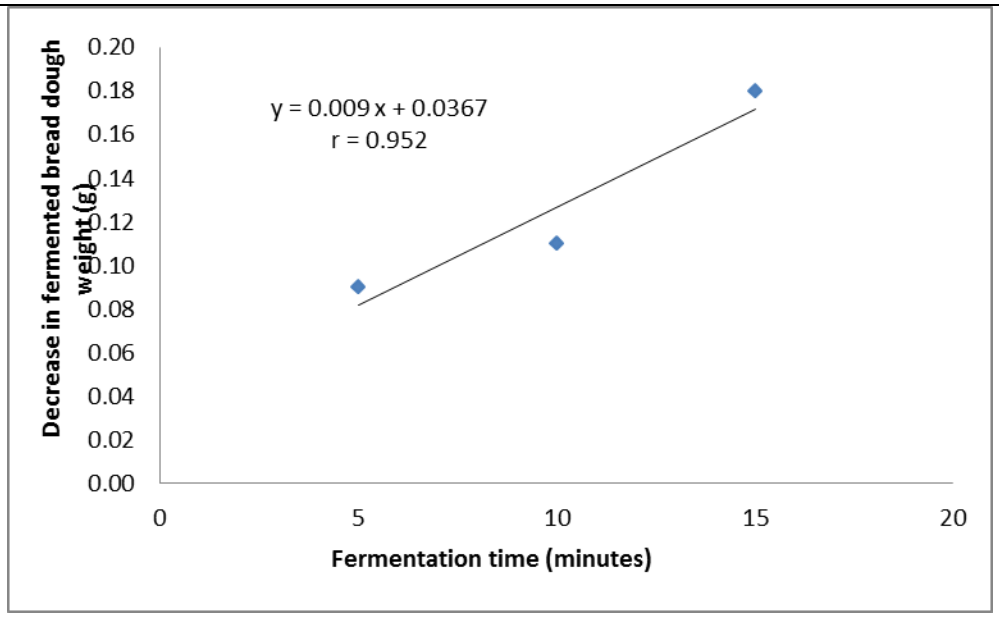

Figure 21. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_2) resulted from the mating between P_1 x P_2 at different fermentation times using zero sucrose concentration.

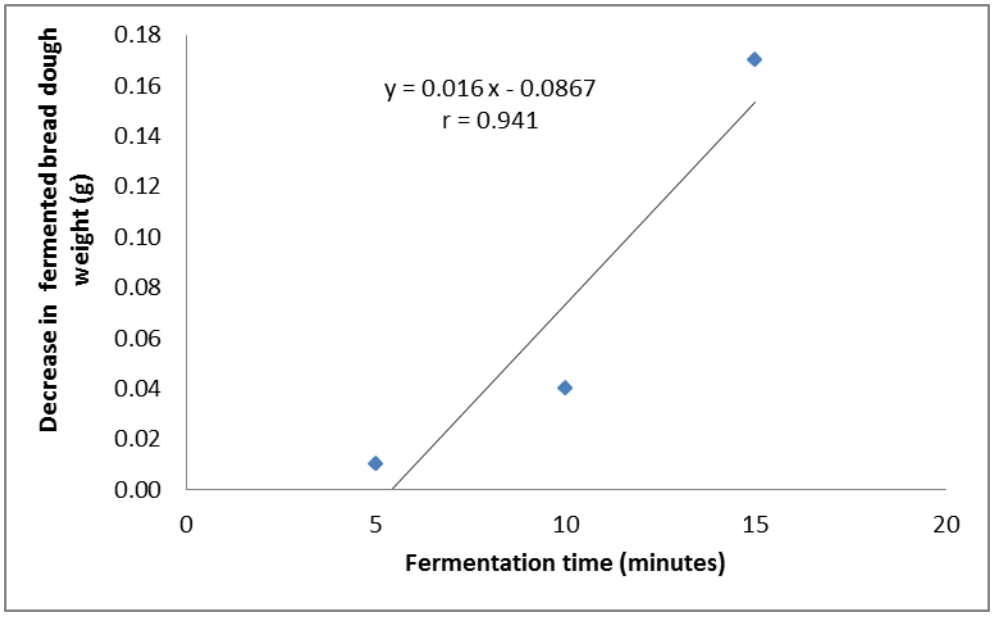

Figure 22. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_2) resulted from the mating between P_1 x P_2 at different fermentation times using 2 g sucrose concentration.

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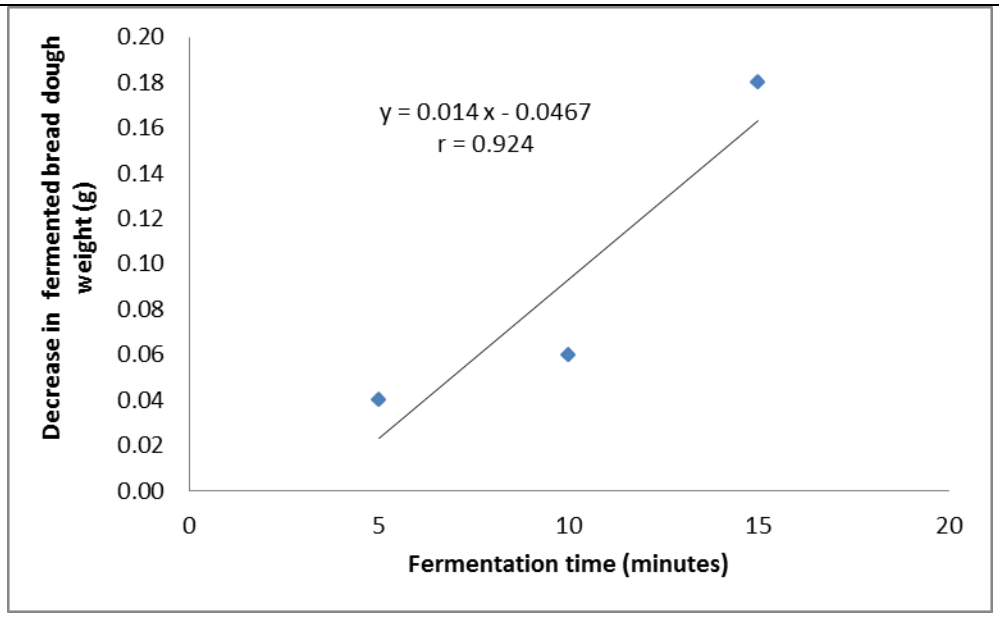

Figure 23. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_2) resulted from the mating between P_1 x P_2 at different fermentation times using 4 g sucrose concentration.

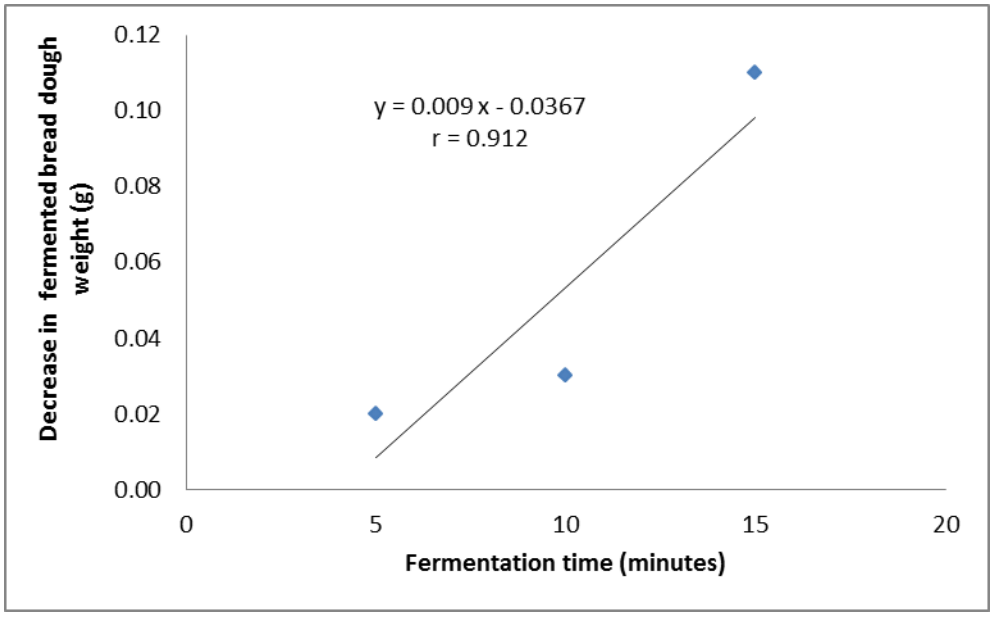

Figure 24. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_2) resulted from the mating between P_1 x P_2 at different fermentation times using 6 g sucrose concentration.

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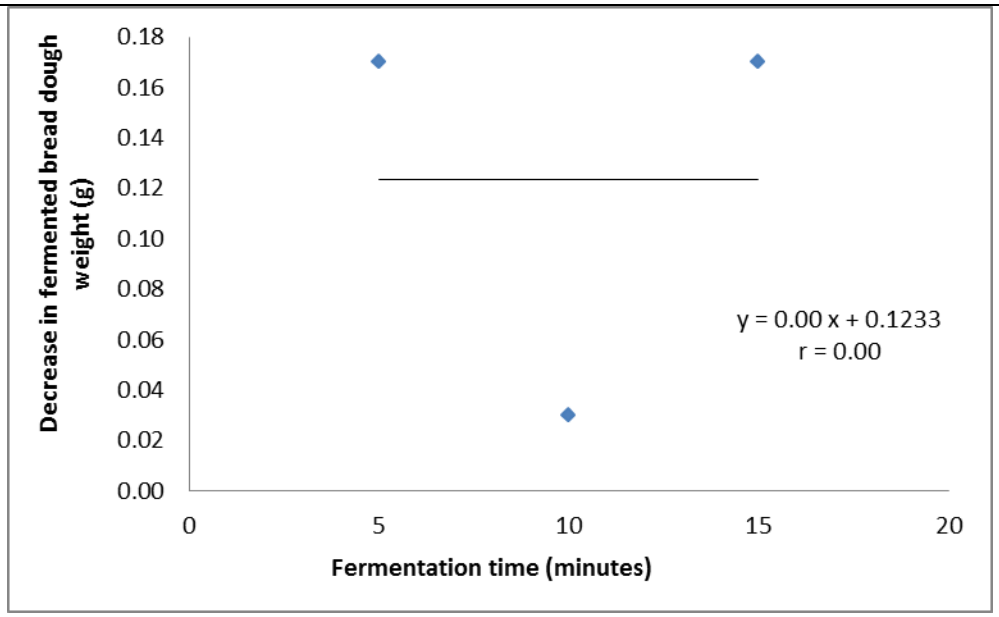

Figure 25. Regression line of the relative decrease in fermented bread dough weight affected by hybrid genotype (H_2) resulted from the mating between P_1 x P_2 at different fermentation times using 8 g sucrose concentration.

In conclusion, hybrid yeast cells can form new lineages that further diversity in the population. Hybridization represents an important tool in genomic and phenotypic diversity. It played a pivotal role in the evaluation of *Saccharomyces cerevisiae* in the performance of fermentation under sucrose stress. Thus, hybrid yeast cells proved to be a reliable source of industrial properties of bread making. This is because of high viability, leavening ability, CO_2 production, and osmotic tolerance. It can be seen that there was a positive strong correlation between the decrease in bread dough weight and fermentation times. Hybrid genotypes possess high performance in leavening activity under sucrose stress in the fermentation medium up to 6 g sucrose/ 325 g wheat flour. This is because of the high rate of CO_2 production by hybrid yeast cells. This implies a reduction of time needed for fermenting bread dough, as well as, the importation of hybrids for bakeries. Therefore, hybrid genotypes must be taken in bread bread-making industry to obtain bread of good quality.

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

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