# The effect of *Saccharomyces* strain and fermentation conditions on quality prameters of non-alcoholic beer

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## ABSTRACT

In this study, the effect of several species of fermenting yeast and fermentation conditions (periodic aeration and temperature) on quality parameters of non-alcoholic beer is assessed. Yeast starters with different inoculation percent were added separately into wort with determined gravity. Wort was fermented for 48 h in different temperatures under aerobic condition or periodic aeration (every 12 h). Growth rate, wort gravity and ethanol content were analyzed for 48 hours (12-h interval). Also, 6 trained panelists were asked for sensory evaluation of final product. The highest growth rate and the highest ethanol content were found in treatments with $4\times10^7$ cfu/ml inoculation fermented at 24˚C under periodic aeration and in those fermented under anaerobic conditions, respectively. The highest gravity was observed for treatments with $10^7$ cfu/ml inoculation, periodic aeration and fermentation at 4˚C. The lowest growth rate and ethanol content were observed in treatments with $10^7$ cfu/ml, fermented at 4˚C under anaerobic condition and those fermented under periodic aeration, respectively. In treatments with $4\times10^7$ cfu/ml inoculation, anaerobic condition and fermentation at 4˚C, the lowest gravity was observed. In addition, among yeasts, *Saccharomyces cerevisiae* and *Saccharomyces rouxii* showed the highest and the lowest growth rate, ethanol content and wort gravity, respectively. Additionally, treatments containing *Saccharomyces cerevisiae* resulted in non-alcoholic beer with more satisfactory flavor attributes.

**Key words**: Ethanol; Non-alcoholic beer; Saccharomyces

## INTRODUCTION

Conventional beer is a kind of malt-based beverage containing at least 3-5.5% ethanol, carbone dioxide, inorganic salts and about 800 organic compounds [1, 2]. Beer, corresponding its alcohol content, can be categorized as non-alcoholic, low-alcoholic, classical or normal, and strong. Beer is one of the most popular drinks in the world. Popularity of beer comes from its health benefits as well as its unique sensory properties. However, excessive consumption of alcoholic beer comprises some adverse effects, for instance, on pregnancy, cardiovascular patients, and athletes. Accordingly, the desire for production and consumption of non-alcoholic beer with good sensory properties is increasing [3-6]. There are several methods for production of non-alcoholic beer comprising elimination of fermentation, dilution method, restricted fermentation, and dealcoholization including vacuum distillation and dialysis. Among these methods, restricted fermentation can be conducted in both suspended batch system and immobilized system.

On the other hand, applied methods in suspended batch system include reducing the ratio of fermentable extract to non-fermentable extract, glucose reduction in wort, pressurization during fermentation, use of a particular species and strains of yeasts, wort heating, cold contact/cold fermentation, high temperature mashing, maintenance of malt mash in 172˚F for 30

minutes, and periodic aeration. Moreover, one of the methods used in restricted fermentation is applying a particular species and strains of yeasts. Some genetically modified mutant strains of *Saccharomyces cerevisiae* lack in alcohol dehydrogenase enzyme; and therefore, they are unable to produce ethanol. However, other mutant strains of *Saccharomyces* are capable of producing large amounts of glycerol and sugar alcohols but they produce a minute amount of ethanol [7]. Despite the ability of *Saccharomyces ludwigii* in fermentation of glucose, sucrose and fructose, this yeast is not able to ferment maltose which causes beer to be sweet. Applying this yeast leads to a few amount of ethanol in beer [8, 9]. The aim of this research is to study the effect of *Saccharomyces* and fermentation conditions on the production of non-alcoholic Non-alcoholic beer with good sensory properties.

## MATERIALS AND METHODS

### *Preparation of samples*

Yeast starters (*Saccharomyces cerevisiae* 70424, *Saccharomyces ludwigii* 3447, *Saccharomyces rouxii* 70535 and *Saccharomyces rouxii* 70531) were provided by the DSMZ Company (Braunscheig, Germany). Yeast starters with different inoculation percent ($10^7$ cfu/ml and $4\times10^7$ cfu/ml) were added separately into wort with known gravity. Wort is fermented for 48 h in different temperatures (4˚C, 12˚C and 24˚C) under aerobic condition or periodic aeration, every 12 h.

### *Microbiological and chemical analysis*

Yeast growth in wort was assessed by a spectrophotometer (Hatch, USA) during fermentation using 'optical density' [10, 11]. pH of the samples was measured using a pH meter (Mettler, Schwerzenbach, Switzerland). After cooling and degassing of samples using ultrasound probes, ethanol content and wort gravity were analyzed by a digital beer analyzer (Anton Par, Graz, Austria).

### *Statistical analysis*

Experiments were performed in triplicate and the significant differences among means were analyzed using ANOVA test from Minitab software. The design was a completely randomized one. Graphs were plotted using Excel software.

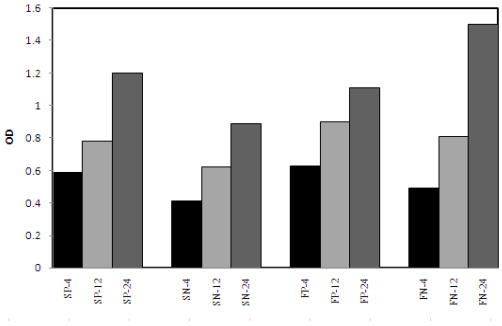
## RESULTS AND DISCUSSION

### *Optical density*

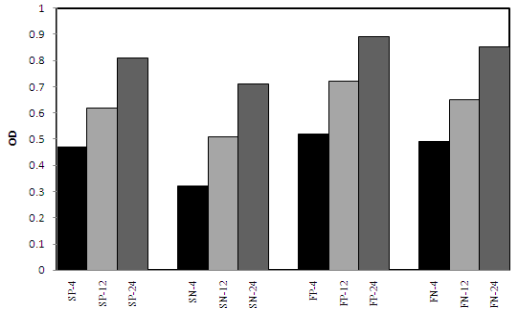
Figure 1 shows the optical density representative of yeast growth rate. The highest and the lowest growth rate during fermentation were for treatments with $4\times10^7$ cfu/ml inoculation, periodic aeration and fermentation at 24˚C (FP-24), and $10^7$ cfu/ml inoculation, anaerobic condition and fermentation at 4˚C (SN-4), respectively. Moreover, by increasing fermentation temperature, the growth rate of all treatments containing four strains of yeasts increased. The highest growth rate in yeasts was observed in h 24-48 of fermentation. Accordingly, this period showed the logarithmic phase of yeast growth. Among yeasts, *S. cerevisiae* showed the highest growth rate and the lowest one was for *S. rouxii* 70531. The maximum optical density of *Saccharomyces rouxii* 70531 was about one-third of *Saccharomyces cerevisiae* 70424. Moreover, *S. rouxii* 70531 has a significantly lower growth rate than strains of the same species.

According to the results related to this research, treatments with periodic aeration showed a higher growth rate with a significant difference than the treatments with anaerobic condition. In anaerobic conditions, glucose resources is used to produce biomass, different organic acids and energy which is higher than alcoholic fermentation [12-14]. Moreover, the existence of aerobic condition through periodic aeration in non-alcoholic beer production significantly led to increase in yeast growth. It should be noted that, an increase in temperature resulted in further growth of yeast cells and causes unfavorable flavor [15].

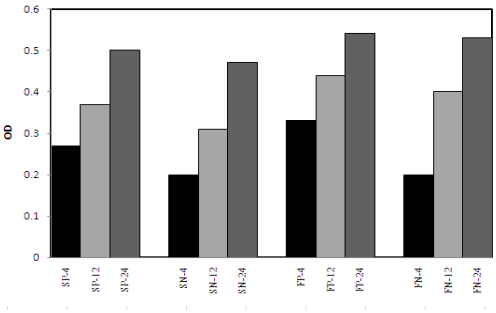
The most significant effect of fermentation temperature during wort fermentation is on the yeast cell metabolism. The optimum temperature for yeast growth should not be more than 30˚C and increasing the temperature has adverse effects on the growth rate. In addition, by increasing the temperature, the oxygen concentration in wort decreases, and thus, the alcoholic fermentation increases. It is worth noting that the low concentration of wort or wort index causes the high solubility of oxygen in it, and therefore, the growth rate would increase.



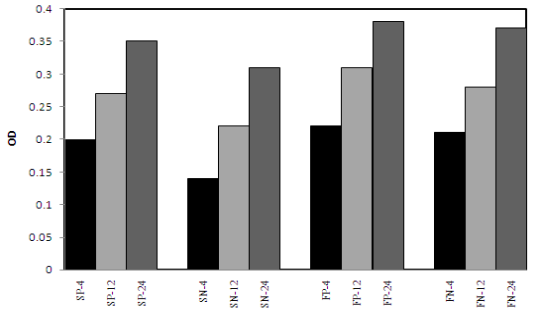

a) *Saccharomyces cerevisiae* 70424




b) *Saccharomyces ludwigii* 3447

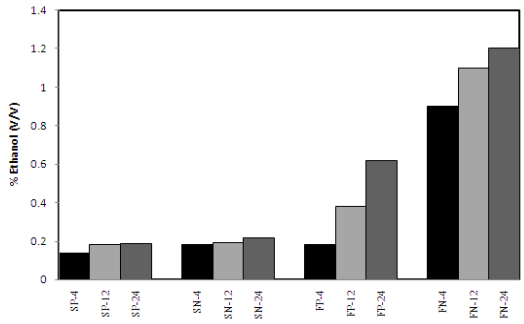



c) *Saccharomuces rouxii* 70535

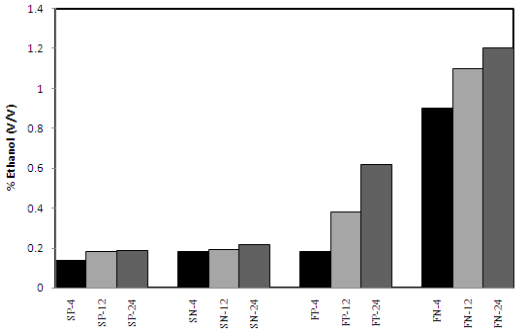



d) *Saccharomyces rouxii* 70531

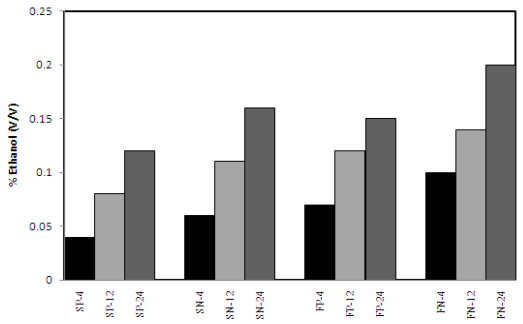
**Figure 1-** The growth index in different situations during fermentation (variables F, S, P, N and numbers show $4\times10^7$ cfu/ml inoculation, $10^7$ cfu/ml inoculation, periodic aeration, anearobic condition and fermentation temperature, respectively)



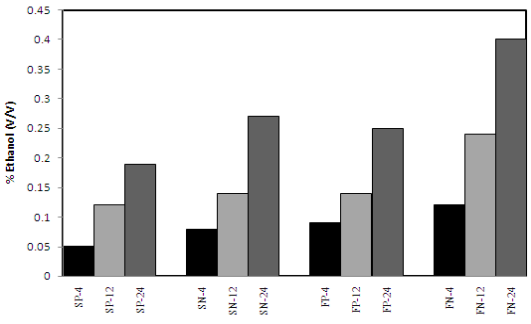

a) *Saccharomyces cerevisiae* 70424




b) *Saccharomyces ludwigii* 3447

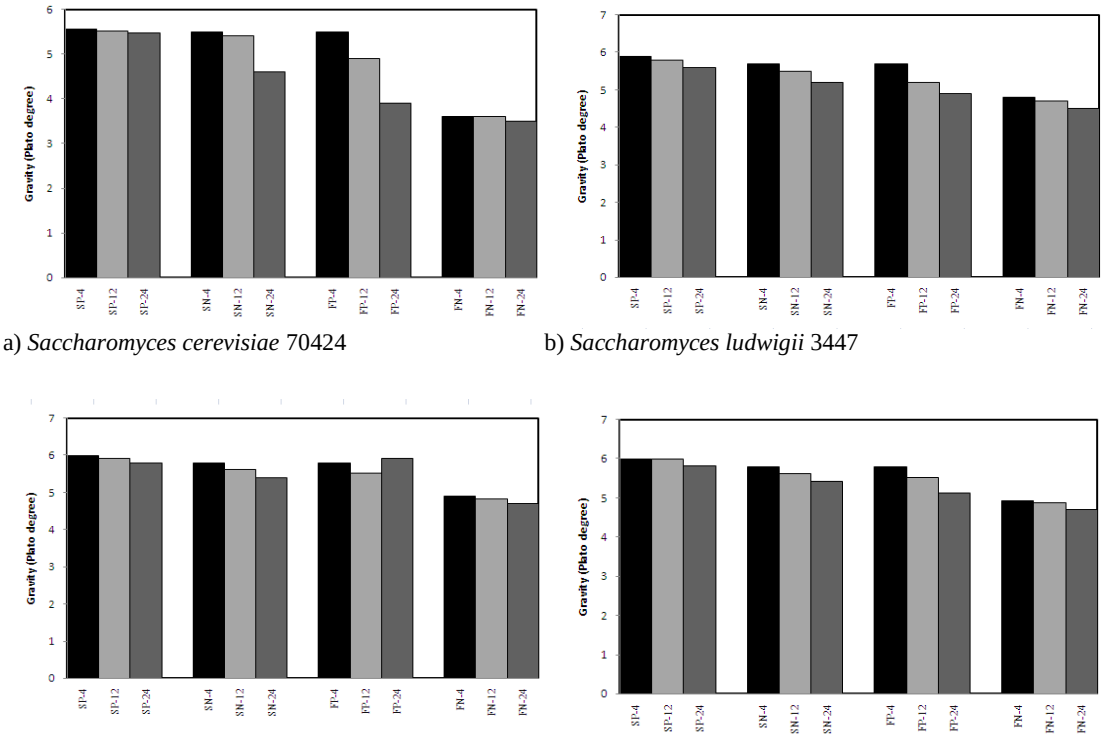



c) *Saccharomyces rouxii* 70535




d) *Saccharomyces rouxii* 70531

**Figure 2-** The ethanol content in different situations during fermentation (variables F, S, P, N and numbers show $4\times10^7$ cfu/ml inoculation, $10^7$ cfu/ml inoculation, periodic aeration, anearobic condition and fermentation temperature, respectively)




a) *Saccharomyces cerevisiae* 70424 b) *Saccharomyces ludwigii* 3447

c) *Saccharomyces rouxii* 70535 d) *Saccharomyces rouxii* 70531

**Figure 3-** The wort gravity in different situations during fermentation (variables F, S, P, N and numbers show $4\times10^7$ cfu/ml inoculation, $10^7$ cfu/ml inoculation, periodic aeration, anaerobic condition and fermentation temperature, respectively)

***Ethanol content***

Figure 2 indicates the procedure of producing ethanol by yeasts. The highest ethanol content was observed for treatments with $4\times10^7$ cfu/ml inoculation rate in anaerobic condition and the lowest one was related to treatments with $10^7$ cfu/ml inoculation rate in periodic aeration. *S. cerevisiae* 70424 showed the highest ethanol content of 1.96% V/V among treatments. The lowest ethanol content (0.04% V/V) was observed in treatments containing *S. rouxii* 70531 with $10^7$ cfu/ml inoculation rate, periodic aeration and fermentation at 4˚C. Treatments with $4\times10^7$ cfu/ml inoculation rate showed higher ethanol content when compared with those with $10^7$ cfu/ml, in all conditions including atmospheric condition and fermentation temperature, throughout the fermentation period. The highest ethanol content was observed in h 24 to 48 of fermentation during logarithmic phase in all treatments. Moreover, ethanol content in treatments containing *S. rouxii* 70531 in maximum amount was half of *S. rouxii* 70535 and one-tenth of *S. cerevisiae* 70424 and ethanol content in maximum and minimum amount was almost half in treatments with *S. ludwigii* when compared to those containing *S. cerevisiae* 70424.

According to the results, in association with various yeasts, comparison of treatments in both anaerobic and periodic aeration conditions showed that type of atmospheric condition had no significant impact on ethanol content in treatments with $10^7$ cfu/ml inoculation rate at the same fermentation temperature ($\rho>0.05$). Ethanol content in all treatments with $4\times10^7$ cfu/ml inoculation rate in all fermentation temperatures was significantly different. Accordingly, the significant difference in ethanol content in low temperatures was higher. According to *S. cerevisiae* 70424 with $4\times10^7$ cfu/ml inoculation, ethanol content in treatments with periodic aeration and anaerobic condition at 4˚C was 0.27 and 1.52 (1.25 difference) and at 24˚C was 1.09 and 1.96 (0.87 difference), respectively. This shows the importance of inoculation percent. At higher fermentation temperatures, especially in $4\times10^7$ cfu/ml inoculation and anaerobic condition, the rate of ethanol production was increased. Compared to anaerobic condition, in treatments fermented at periodic aeration, the increase in fermentation temperature had a significant

effect on the production of ethanol. As an example, in h 48 of fermentation, in treatments with periodic aeration (all the yeasts and $4\times10^7$ cfu/ml inoculation) the ethanol content at 12˚C and 24˚C was 0.53 and 1.03 (0.50 difference), respectively; and in treatments with anaerobic condition was 1.75 and 1.96 (0.21 difference), respectively. This is due to the fact that in anaerobic condition, compared to periodic aeration, the exposure time of yeast cells in anaerobic condition is short; therefore, in periodic aeration, higher fermentation temperature can lead to production of high amounts of ethanol.

It should be noted that, high fermentation temperature (*e.g.*, 12˚C compared to 24˚C) leads to significant decrease in solubility of oxygen in wort [15]. This causes increase of alcoholic fermentation in these temperatures. All the treatments containing *S. rouxii* had ethanol content below 0.5% (V/V) and, therefore, they were suitable for production of non-alcoholic beer. For *S. cerevisiae* 70424 and *S. ludwigii* 3447, except those with $4\times10^7$ cfu/ml inoculation in anaerobic condition (in all fermentation temperatures) and treatments with $4\times10^7$ cfu/ml, periodic aeration and fermentation at 24˚C, the ethanol content in other treatments was below 0.5% (V/V).

***Wort gravity***

Figure 3 shows the wort gravity of four strains of yeasts in treatments. The lowest wort gravity was related to the treatment with $4\times10^7$ cfu/ml, anaerobic condition and fermentation at 24˚C (FN-24). The highest was observed in treatments with $10^7$ cfu/ml inoculation and produced under periodic aeration. Furthermore, treatments with $4\times10^7$ cfu/ml inoculation and anaerobic condition showed lower wort gravity than treatments produced under periodic aeration. Treatments with *S. cerevisiae* 70424 and *S. rouxii* 70531 showed the highest and lowest wort gravity, respectively. Results indicated that wort gravity had a direct correlation with fermentable sugars and on the contrary, a reverse effect on ethanol content [16]. Thus, the treatments produced in anaerobic condition, especially with $4\times10^7$ cfu/ml inoculation, had higher amounts of ethanol compared to those produced in periodic aeration and they showed lower wort gravity. Wort gravity in similar treatments (all variables except fermentation temperature) fermented at 24˚C was lower than the two other fermentation temperatures. This implies more consumption of sugars in anaerobic and aerobic condition and more production of ethanol in anaerobic condition.

***Sensory evaluation***

Two treatments containing *S. cerevisiae* with $4\times10^7$ cfu/ml inoculation, periodic aeration and fermentation at 12˚C (FP-12), and $10^7$ cfu/ml inoculation, anaerobic condition and fermentation at 24˚C (SN-24) showed more satisfactory flavor attributes and overall acceptability. As a result, alcoholic fermentation is more complete in these treatments than the others. Although the ethanol content in both treatments did not exceed 0.5% (V/V). Higher fermentation temperature and oxygen existence caused an inappropriate flavor profile. In addition, all the treatments containing *S. cerevisiae* showed mild worty off-flavor [15].

*S. rouxii* compared with other two yeasts, showed less overall acceptability due to low growth rate and fermentation properties. All the treatments containing *S. ludwigii* showed less maturity in flavor and overall acceptability when compared with those containing *S. cerevisiae*. Furthermore, *S. ludwigii*-containing treatments in higher fermentation temperature (24˚C) exhibited lactic sour attributes due to production of lactic acid [17] and in lower fermentation temperatures (4˚C and 12˚C) showed inappropriate sweet and immature flavor. This can be attributed to the inability of this yeast in fermentation of maltose in wort [18].

## CONCLUSION

This study was undertaken to investigate the suitability of the limited fermentation procedure for producing non-alcoholic beer. Wort with defined gravity was fermented by four strains of yeasts for 48 h in different temperatures under aerobic condition and periodic aeration. Growth rate, wort gravity and ethanol content were analyzed during 48 h of fermentation. Treatments with $4\times10^7$ cfu/ml inoculation, fermented at 24˚C under periodic aeration and those under anaerobic condition showed highest growth rate and ethanol content, respectively. The highest gravity was observed for treatments with $10^7$ cfu/ml inoculation, periodic aeration and fermentation at 4˚C.

Accordingly, treatments with $10^7$ cfu/ml, fermented at 4˚C under anaerobic condition and those at periodic aeration showed the lowest

growth rate and ethanol content, respectively. Moreover, the lowest wort gravity was for treatments with $4\times10^7$ cfu/ml inoculation, fermented at 4˚C and under anaerobic condition. Since the non-alcoholic beer produced by limited fermentation (with *S. cerevisiae*) showed superior taste characteristics, the use of this yeast with fermentation condition of $10^7$ cfu/ml inoculation, periodic aeration and 4˚C (amount of ethanol is less than 0.05%) is recommended. It is worth mentioning that combination of different fragrances can be used for covering the wort-like off flavor caused by *S. rouxii* and *S. ludwigii* for the production of non-alcoholic beer with good sensory properties.

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