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The Effect of Saccharomyces Strains and Fermentation Condition on the pH, Foam Property and CO_2 Concentration of Non-alcoholic Beer (Ma-al-shaeer)

Sohrabvandi, S.[1], *Malganji, S.H.*[2], *Razavi, S.H.*[3*], *Mousavi, S.M.*[3]

1- Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2- Department of Food Science and Technology, Faculty of Food Science and Technology, Islamic Azad University, Khorasgan (Isfahan) Branch, Isfahan, Iran.
3- Department of Food Science, Engineering & Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.

Abstract

This study aims to determine the effect of fermentation condition and *Saccharomyces strains* on the pH, foam property and CO_2 concentration of non-alcoholic beer (Ma-al-shaeer). For this, the beer samples were inoculated with four different species of Saccharomyces (*Saccharomyces rouxii* 70531, *S. rouxii* 70535, *S. ludwigii* 3447 and *S. cerevisiae* 70424) and fermented for 48h in both aerobic and periodic aeration at three different temperatures. Then their pH, CO_2 concentration and foam property were analyzed in 12h intervals during 48h fermentation. The results shows that the treatments with 4×10^7 $CFU.ml^{-1}$ and periodic aeration at 24˚C showed the greatest decrease in pH, and the treatments with 10^7 $CFU.ml^{-1}$ and aerobic-periodic aeration at 4˚C showed the lowest decrease in pH. The highest and lowest amounts of CO_2 and foam property were obtained in the treatments with 4×10^7 $CFU.ml^{-1}$ inoculation, aerobic condition, and the treatments with 10^7 $CFU.ml^{-1}$, periodic aeration, respectively. These results further demonstrated that the highest drop in pH, and the highest ability of producing CO_2 and foam were for *S. cerevisiae* 70424, and the lowest belonged to *S. rouxii* 70531. The overall outcome of the study points to the fact that physico-chemical properties of Ma-al-shaeer is important from the consumers' point of view. Therefore, *S. cerevisiae* with 4×10^7 $CFU.ml^{-1}$ inoculation and aerobic condition at 4˚C has promising potential for producing Ma-al-shaeer with good physicochemical properties.

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Correspondence to
Seyed Hadi Razavi,
Department of Food Science, Engineering & Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.
P.O. Box: 31587-77871.
Email: srazavi@ut.ac.ir

1. Introduction

Ma-al-shaeer (MAS) is a kind of carbonated beverage (non-alcoholic) based on malt, which contains inorganic salts, hops and CO_2 gas (carbonic acid forms), as well as several organic compounds with health benefits [1, 2]. There are some ideal properties of MAS, which would benefit human health such as increasing HDL cholesterol and preventing cardiovascular disease, and inhibitory effect on the growth of cancer cells and osteoporosis [3-6]. It has been determined that organic acids in MAS result in the reduction of uric acid [7, 8]. Furthermore, high concentration of proteins and vitamin B increases the nutritional value of MAS [9].

MAS has some physical properties including CO_2 retention and foam stability, and some chemical properties such as acidity, pH and different chemicals. Moreover, this beverage consists of gas (CO_2) and liquid phases. Polypeptides, glycoproteins, peptides, poly phenols and dextrin, which are categorized as colloidal, are responsible for production of CO_2 and foam head. Glycoproteins contain a polar sector that entraps CO_2 bubbles, and a non-polar sector, which creating local high viscosity, which is effective in foam production. It has been indicated that the type of used malt determines the type of glycoproteins, and the latter, stabilizes foam. Dextrin, polypeptide fragments and some gums play an important role in foam stability [10]. In spite of their ability in extracting protein fragments from malt, these fragments do not have fermentability. Therefore, their ability of foam production is improved by adding glycerol or yeasts, which produce high amount of alcohol sugars. Concentration of 1.5-4% of glycerol has been reported as a sufficient amount for achieving optimum foam body, and desirable flavor [11]. pH of MAS differs from 3.9 to 4.5. Water soluble materials in malt and pH of water will affect wort pH, which is about 5-6. Moreover, organic acids produced by yeasts and carbonic acid gas reduce the pH of the solution. On the other hand, some minerals consumed by yeasts and autolysis of yeast cells have resulted in an increase in pH [10].

Due to the important role of physicochemical properties of MAS in consumer acceptance, the objective of this study was to determine the effect of Saccharomyces strains and fermentation condition on some physic-chemical properties of MAS.

2. Materials and Methods

2.1 Sample preparation

Saccharomyces (S.) cerevisiae 70424, *S. ludwigii* 3447, *S. rouxii* 70535 and *S. rouxii* 70531 were supplied by the DSMZ Company (Braunscheig, Germany). Yeasts were inoculated with two different inoculation levels (10^7 CFU.ml^{-1} and 4×10^7 CFU.ml^{-1}) to the wort. Then the wort was fermented for 48h under aerobic and periodic aeration (12h interval) at 4˚C, 12˚C and 24˚C.

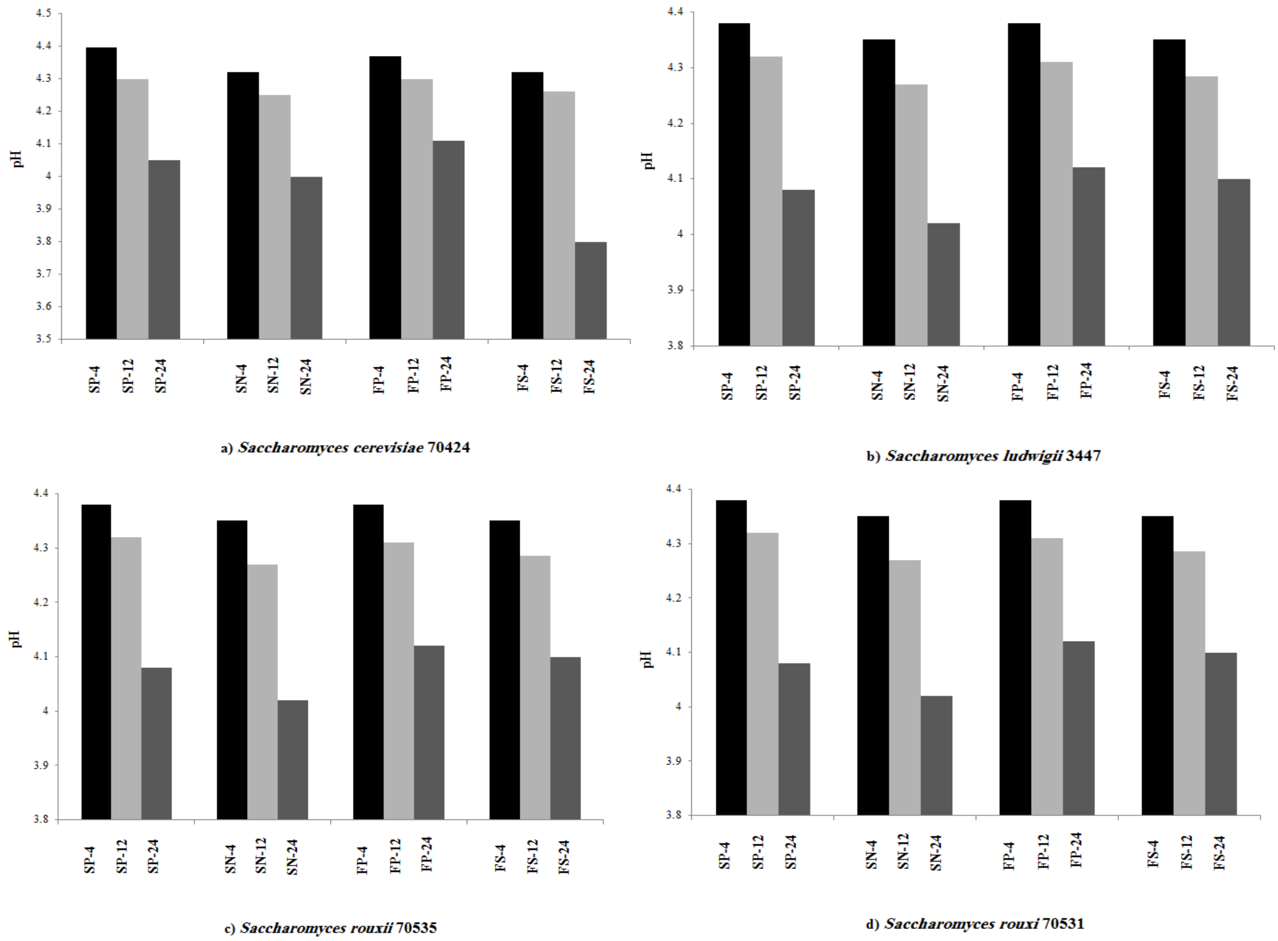

Figure 1. pH drop during the fermentation (variables F, S, P and N and numbers show 4×10^7 CFU.ml^{-1} inoculation, 10^7 CFU.ml^{-1} inoculation, periodic aeration, anaerobic condition, and fermentation temperature, respectively).

2.2 Chemical analysis

A digital pH meter (Mettler, MA 235, Switzerland) was used for pH measurement. CO_2 concentration was measured by a pressure gauge (TG, Italy). Head space of the samples was calculated using pressure temperature table related to CO_2 at a constant temperature. In order to measure the foam property, the samples were poured into cylinder and their foam property (percentage of foam) was calculated by the following equation:

(foam • head height/total height)×100= foam% (ml)

2.3 Statistical analysis

The experiments were carried out in triplicate. The results were analyzed by ANOVA Software (SAS 9.1 software Institute Inc., Cary, NC, USA), and expressed as mean±standard deviation.

3. Results and discussion

Figure 1 shows the changes in pH during fermentation. The highest drop in pH was mentioned for FP-24 and FN-24 (4×10^7 CFU.ml^{-1}, aerobic-periodic aeration, 24˚C). During the fermentation, the pH of treatments with aerobic and periodic aeration did not show a significant difference. According to FP-4 and FN-4, due to low temperature of fermentation during the yeast metabolism, different types of organic acids are produced. Also nitrogen compounds are consumed by the yeasts in order to grow. Moreover, CO_2 is produced during the anaerobic fermentation. Thus, these factors led to a pH drop during the fermentation [10]. In the anaerobic metabolism of yeasts, carboxylic acids are produced by incomplete three carboxylic acids and in the aerobic metabolism, carbohydrates are converts to energy, water and CO_2 [11]. It was reported that, *S. rouxii* 70531 showed the lowest drop in pH, which is due to lower ethanol production of this yeast in comparison to other yeasts during both aerobic and periodic aeration metabolisms. High speed of ethanol production and aerobic yeast metabolism causes a pH drop.

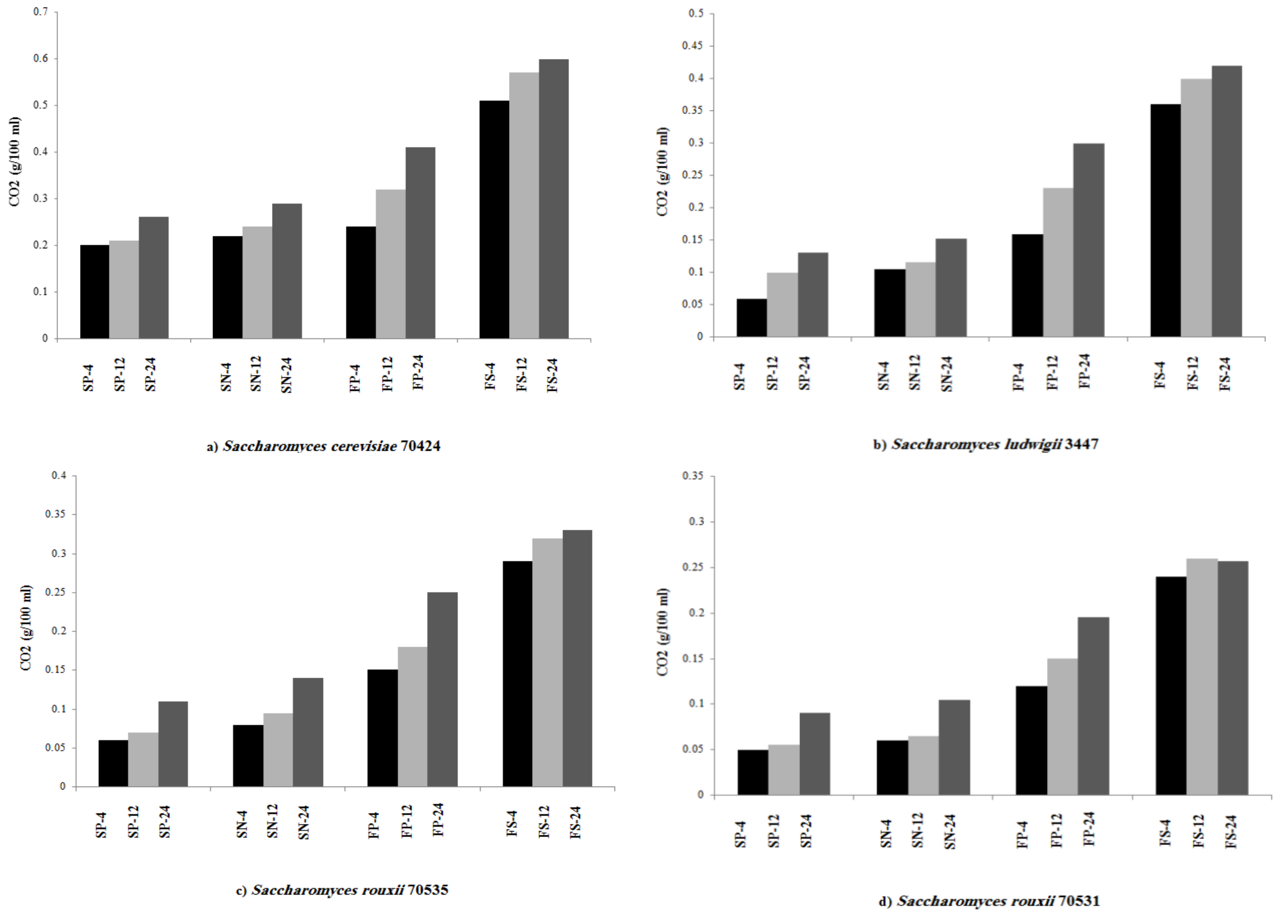

Figure 2. CO_2 concentration during the fermentation (variables F, S, P and N and numbers show 4×10^7 CFU.ml^{-1} inoculation, 10^7 CFU.ml^{-1} inoculation, periodic aeration, anaerobic condition and fermentation temperature, respectively).

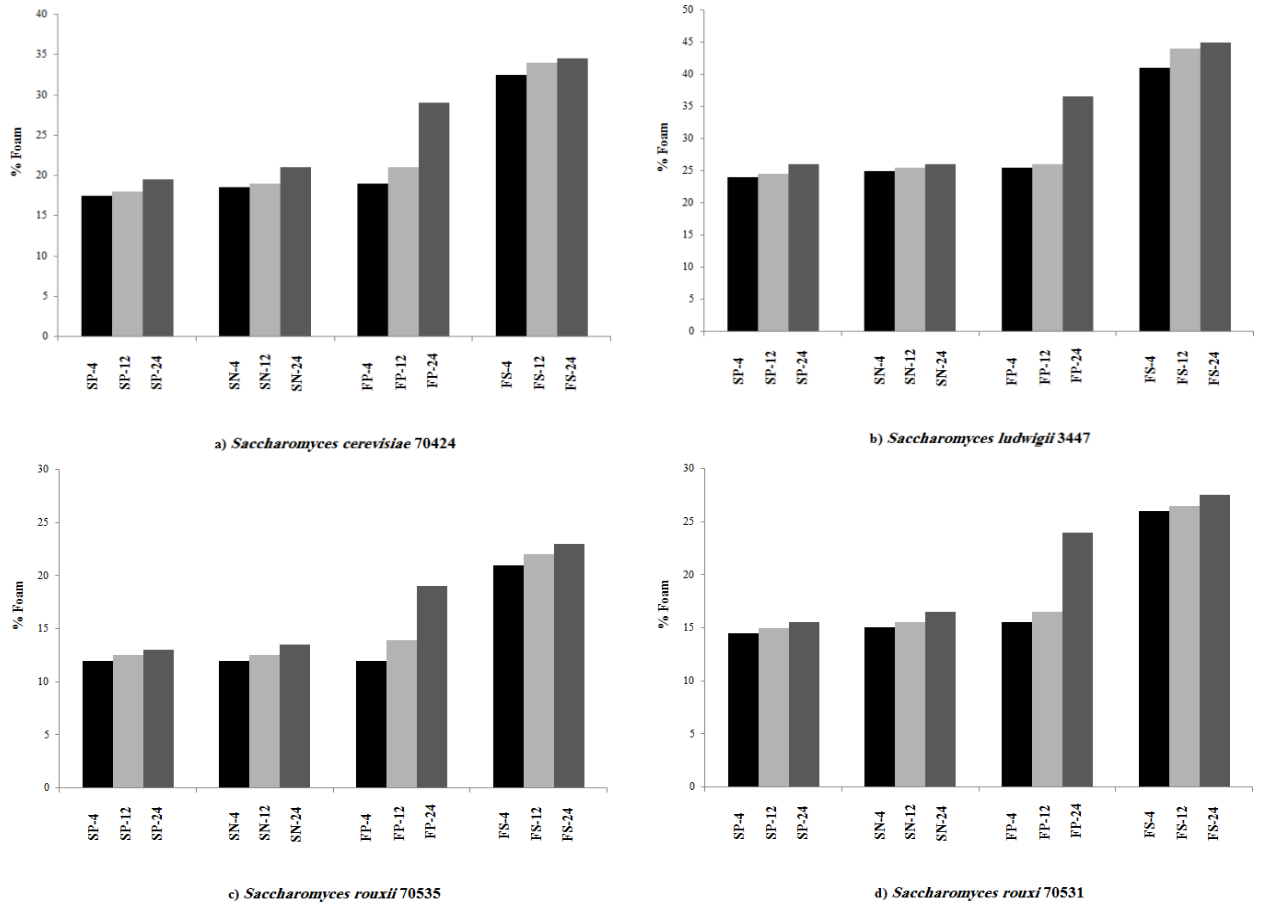

Figure 3. Foam property during the fermentation (variables F, S, P and N and numbers show 4×10^7 CFU.ml^{-1} inoculation, 10^7 CFU.ml^{-1} inoculation, periodic aeration, anaerobic condition and fermentation temperature, respectively).

Figure 2 and 3 show the CO_2 concentration and foam property of treatments. The highest and lowest concentrations of CO_2 and foam property were obtained in the treatments with 4×10^7 CFU.ml^{-1} in aerobic condition (FN24), and the treatments with 10^7 CFU.ml^{-1} in periodic aeration (SP4), respectively.

Fermentation temperature did not show significant difference. *S. cerevisiae* 70424 and *S. rouxii* 70531 showed the highest and the lowest ability in producing CO_2 and foam, respectively.

Based on the results, the amount of ethanol has a reinforcing effect on the treatments, CO_2 and foam property. This effect takes place with protein denaturation and appropriate foaming agents in the presence of ethanol [10, 12]. However, in the treatments with high amount of ethanol, CO_2 is produced in greater amounts because this gas is the product of alcoholic fermentation [13, 14]. FN-24 and SP-4 showed the highest and lowest amount of CO_2 and foam, respectively. The worth noting point is the effect of ethanol on foam production and synchronization of ethanol production with CO_2, which is produced during the alcoholic fermentation.

4. Conclusions

Four species of Saccharomyces were inoculated into MAS, and the samples were fermented for 48h under both aerobic and periodic aerations at three different temperatures. pH, foam property and CO_2 concentration were analyzed during the fermentation. The results of this research suggest that the physical properties of MAS such as foam stability and production of CO_2 gas play an important role in the consumers' acceptability. Therefore, production of MAS by *S. cerevisiae* with 4×10^7 CFU.ml^{-1} inoculation, fermented at 4˚C under anaerobic condition, is suggested.

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