Characteristics of different brewer's yeast strains used for non-alcoholic beverage fermentation in media containing different fermentable sugars

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Abstract

Fermentation characteristics of four strains of brewer's yeast (*Saccharomyces cerevisiae* strain 70424, *S. rouxii* strain 2535, *S. rouxii* strain 2531 and *Saccharomyces ludwigii* strain 3447) in Yeast Mold-broth containing four different fermentable sugars (glucose, fructose, maltose, or sucrose) were studied. The aim was to consider the suitability of different strain/sugar treatments for the production of non-alcoholic beer as well as to devise treatments resulting in greatest growth rate of yeast cells. Experimental parameters were yeast cell growth, ethanol production, pH drop, and changes in fermenting media attenuation (°PI), during a 48 h fermentation period. Fermentation was performed at 24°C using periodic aeration practice. For *S. cerevisiae*, the greatest growth rate was achieved in presence of sucrose. The maximum and minimum ethanol contents at the end of fermentation were related to sucrose- (0.94% V/V) and glucose-containing (0.4% V/V) treatments, respectively. In the case of *S. ludwigii*, fructose stimulated the highest growth rate and the maximum and minimum ethanol contents at the end of fermentation were observed in sucrose- (0.49%), and maltose-containing (0.04%) treatments, respectively. For *S. rouxii* 2535, highest growth rate was observed in the presence of fructose/glucose. The maximum and minimum ethanol contents belonged to the fructose/glucose- (~ 0.40) and maltose/sucrose-containg (~ 0.01%) treatments, respectively. In the case of *S. rouxii* 2531, glucose and to lesser extent, fructose led to the highest growth rate and the maximum and minimum ethanol contents were observed in glucose (0.01%) and maltose/sucrose (0.00%) treatments, respectively. Applying different strains of *Saccharomyces* in presence of different types of sugars caused various fermentation characteristics especially with regard to growth rate and ethanol production.

Keywords: Beer; Brewer's yeast; Ethanol; Saccharomyces; Sugar

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INTRODUCTION

Beer is a universally popular beverage and is supposedly the oldest known beverage. It is produced through fermentation of starches, mainly derived from cereal grains (Hardwick *et al*., 1995; Arnold, 2005; Nelson, 2005). The most common ingredients of beer are malted barley, hops and brewer's yeast (Hardwick *et al*., 1995). Therefore, brewer's yeast is a principal element in beer production. The normal beer usually comprises three types of beer from the alcohol content viewpoint; namely, low-strength (~ 2-3%), medium strength (~ 5%) and high-strength (~ 6-12%) (Hardwick *et al*., 1995; Lewis and Younger, 1995). Most beers produced worldwide have alcohol content of 3-6% (V/V) in range (Bamforth, 2002; Hardwick *et al*., 1995). In recent years, there has been an increased market share for low-alcohol beer (<2.5% alcohol content) and non-alcoholic beer (<0.5% alcohol content). The low alco-

hol beer is demanded in many parts of the world and the non-alcoholic one is much demanded in Islamic countries (Hardwick, *et al*., 1995; Lewis and Younger, 1995; Sohrabvandi, 2009a).

There are several methods for the production of low-alcohol and non-alcoholic beers. Alcohol removal or dealcoholization is a common practice used for industrial production of beers of these types. During recent years, a new method called "restricted alcoholic fermentation" has come to attention. In this procedure, production of alcohol during the brewing process is reduced from the very beginning. This may be achieved either by using yeast that can only partially ferment wort, or by repressing or interrupting fermentation with different compositional and/or process factors (Caluwrerts, 1995; Dziondziak, 1989a; Sohrabvandi, 2009a). The biggest advantage of this method compared to current dealcoholization methods is that with the limited alcoholic fermentation, there will be no need for going through difficulties of the delacoholization practice. Meanwhile, the product usually receives more organoleptic acceptance than the dealcoholized beer, especially with regard to flavor (Caluwrerts, 1995). In the course of low-alcohol and non-alcoholic brewing, strain of the brewer's yeasts, as well as the predominant fermentable sugar in the yeast growth medium is among the most important factors affecting the performance of alcohol production. Thus, the present study was aimed at investigating the fermentation characteristics of four strains of brewer's *S.* (i.e., *S. cerevisiae* DSM 70424, *S. rouxii* DSM 2535, *S. rouxii* DSM 2531 and *S. ludwigii* DSM 3447) in YM-broth medium containing different fermentable sugars naturally available in wort (glucose, fructose, maltose, or sucrose) in order to consider the suitability of treatments (strains/sugars) for production of non-alcoholic beer as well as to devise treatments resulting in greatest growth rate of yeast cells. The latter would help to develop optimized culture media for multiplication, transformation and maintenance of the mentioned yeast starters in research and industrial laboratories.

MATERIALS AND METHODS

Yeast starter cultures: *S. cerevisiae* DSM 70424, *S. rouxii* DSM 2535, *S. rouxii* DSM 2531 and *S. ludwigii* DSM 3447 were supplied by DSMZ (Braunschweig, Germany) in freeze-dried and slant forms. These cultures are commonly used to produce low- and non-alcoholic beer. The starters were propagated in YM-broth (Merck, Darmstadt, Germany) followed by cultivation on YM-agar. The cultures were kept in refrigerator (5°C) until used. Subcultures were made every 2 weeks in order for starter renewal using YM-agar.

Experimental analysis: Trends in growth of yeast starter cultures (optical density) during the fermentation was measured using spectrophotometer at 600 nm (Van Iersel *et al.*, 1999), in 12 h intervals. Ethanol content and fermenting media attenuation (°Pl) in YM-broth culture media were analyzed using Digital Beer Analyzer (Anton Par, Austria) (Sohrabvandi *et al*., 2009b). The pH of fermenting media was measured using a pH meter (MA 235, Mettler, and Switzerland).

Sample preparation: Definite amounts of yeast colonies (from *S. cerevisiae* DSM 70424, *S. rouxii* DSM 2531, *S. rouxii* DSM 2531, or *S. ludwigii* DSM 3447) grown on YM-agar were harvested and inoculated into YM-broth media containing 1.0% different sugars (glucose, fructose, maltose, or sucrose), 0.3% yeast extract, 0.3% grinded malt extract, 0.5% peptone and 1 liter distillated water. Fermentation process was carried out at 24°C for 48 h, involving periodic aeration practice in order to perform restricted fermentation procedure (Sohrabvandi, 2009a). Fermenting media were subjected to aerobic conditions every 12 h. The experimental parameters including optical density of the fermenting media (representing growth trend of yeast cells), ethanol production, pH drop and media attenuation (°Pl) were measured during 48 h of fermentation, at 12 h intervals.

Statistical analysis: Experiments were performed in triplicate, and the significance of differences among means in a completely randomized design was analyzed using ANOVA test in Minitab software (University of Arkansas, Fayetteville, USA, Version 13, 2002).

RESULTS

Effects of fermentable sugar type on growth trends of four *Saccharomyces* strains: Effects of fermentable sugar type on growth trends of the four strains is shown in Figure 1. As shown in Figure 1A, the greatest and the lowest growth rates were observed in sucrose and glucose treatments, respectively ($p<0.05$). Maltose and glucose treatments showed the lowest growth rate at above mentioned fermentation

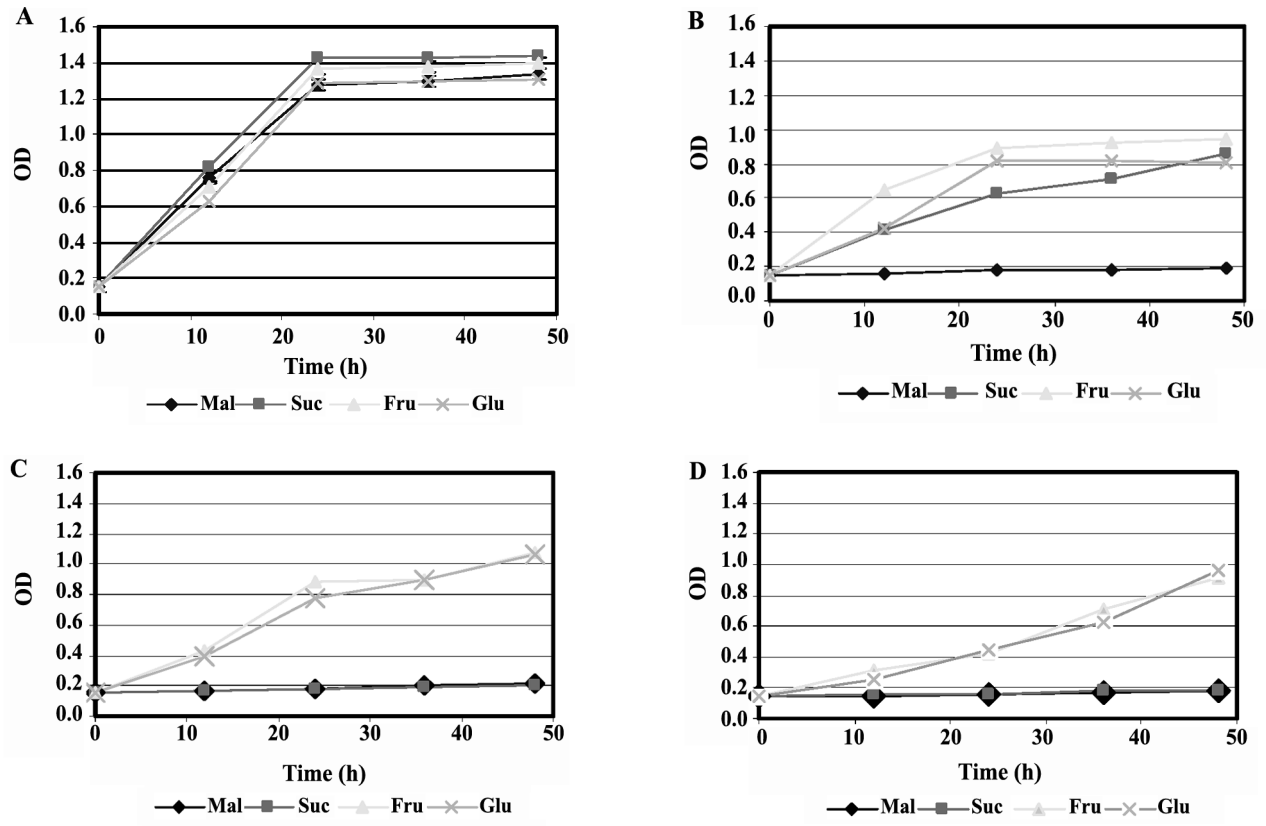

Figure 1. Growth trends of A: *S. cerevisiae* DSM 70424, B: *S. ludwigii* DSM 70447, C: *S. rouxii* DSM 2535, and D: *S. rouxii* DSM 2531 in medium containing different fermentable sugars. *Glu = glucose, Fru = fructose, Mal = maltose, Suc = sucrose.

period. In the case of *S. ludwigii* DSM 3447, the greatest and the lowest growth rates were observed in fructose and maltose treatments, respectively (p<0.05) (Fig. 1B). Therefore, *S. ludwigii* DSM 3447t was capable of utilizing fructose more than other sugars. The trends were similar in principle for *S. rouxii* DSM 2531 and 2535, as also inferable from Table 1 and parts C and D in Figures 1-4. Glucose and fructose treatments, with no significant difference (p>0.05), rendered the greatest growth rates of *S. rouxii* DSM 2531 and 2535 compared to other treatments (p<0.05). In contrast, sucrose and maltose treatments exhibited

Table 1. Sugars causing maximum and minimum growth as well as maximum and minimum ethanol contents at the end of fermentation period with different yeasts.

Yeast type	Sugar(s) caused maximum growth	Sugar(s) caused minimum growth	Sugar(s) caused maximum ethanol at the end of fermentation	Sugar(s) caused minimum ethanol at the end of fermentation
S. cerevisiae DSM 70424	Suc	Glu	Suc	Glu
S. ludwigii DSM 70447	Fru	Mal	Suc	Mal
S. rouxii DSM 2535	Fru / Glu	Mal / Suc	Fru / Glu	Mal / Suc
S. rouxii DSM 2531	Fru / Glu	Mal / Suc	Fru / Glu	Mal / Suc

*Glu. = glucose, Fru. – fructose, Mal. = maltose, Suc. = sucrose.

the lowest growth rates (Figs. 1C and 1D).

Effects of fermentable sugar type on pH trends of four *Saccharomyces* strains: For *S. cerevisiae* DSM 70424, sucrose led to the highest pH drop rate, whilst the lowest pH drop rate was observed for glucose (Fig. 2A). At about 24 h of fermentation, when the growth curve of yeast cells reached the turning point, the pH value took the lowest level (Figs. 1 and 2A). From that time onwards, the growth and pH values did not change significantly. For *S. ludwigii* 3447, it was observed that fructose treatment, which resulted in the greatest growth rate of *S. ludwigii* 3447, also led to the highest pH drop rate. The same result could be seen for maltose, leading to the lowest pH drop rate (Fig. 2B). It is implied in Figures 2C and 2D that fructose and glucose treatments which caused the greatest growth rates of *S. rouxii* DSM 2535 and 2535 (Figs. 1C and 1D), led to the highest pH drop rates throughout the fermentation period, significantly higher than the two other treatments.

Effects of fermentable sugars on ethanol production in *Saccharomyces* strains: For *S. cerevisiae* DSM 70424 (Fig. 3A), sucrose treatment resulted in the greatest amount of ethanol production throughout the fermentation period ($p<0.05$). The lowest ethanol amounts within the mentioned period were related to the glucose treatment. The maximum amount of ethanol at the end of fermentation period belonged to sucrose treatment (0.94% V/V), whilst the minimum amount was related to glucose treatment (0.4% V/V). These amounts of ethanol correspond to the ethanol levels found in low-alcohol beer and non-alcoholic beer, respectively.

Glucose and sucrose treatments resulted in the highest ethanol yields for *S. ludwigii* 3447 (Fig. 3B). Meanwhile, the differences among them was not significant. As shown in the figure, maltose treatment displayed the lowest amounts of ethanol within the fermentation period. For this yeast, the maximum amount of ethanol at the end of fermentation period belonged to sucrose treatment (0.49% V/V), whilst the minimum amount was related to maltose treatment (0.04% V/V). These amounts of ethanol correspond to the non-alcoholic beer.

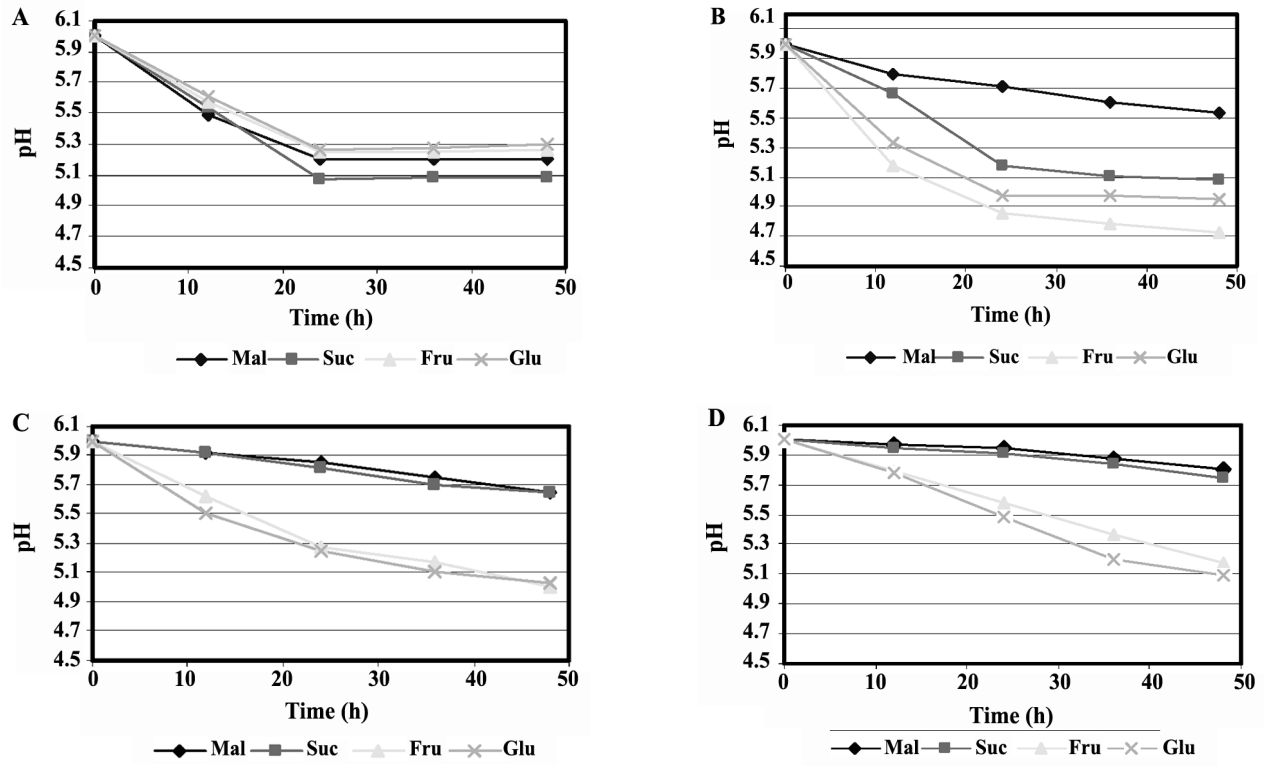

Figure 2. pH drop trends of A: *S. cerevisiae* DSM 70424, B: *S. ludwigii* DSM 70447, C: *S. rouxii* DSM 2535, and D: *S. rouxii* DSM 2531 in medium containing different fermentable sugars. Glu = glucose, Fru = fructose, Mal = maltose, Suc = sucrose.

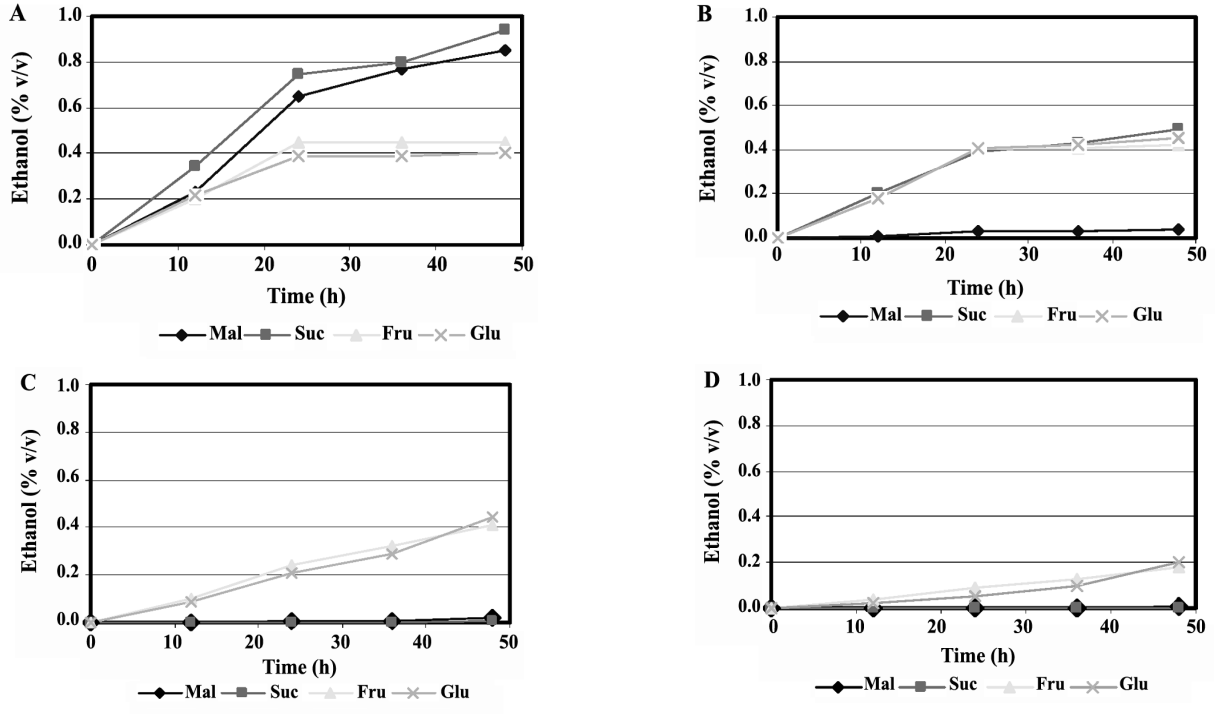

Figure 3. Ethanol production trends of A: *S. cerevisiae* DSM 70424, B: *S. ludwigii* DSM 70447, C: *S. Rouxii* DSM 2535, and D: *S. rouxii* DSM 2531 in medium containing different fermentable sugars. *Glu = glucose, Fru = fructose, Mal = maltose, Suc = sucrose.

The highest amounts of ethanol for *S. rouxii* DSM 2535 and 2535 were obtained from treatments that had the greatest growth rates and the highest pH drop rates, namely, glucose and fructose treatments (Figs. 3C and 3D). In the rest of the treatments, production of ethanol was not noticeable, and their ethanol production curves approximately coincided (Figs. 3C and 3D). The highest and the lowest amounts of ethanol in *S. rouxii* DSM 2535 and *S. rouxii* DSM 2531 treatments were 0.40%-0.01% and 0.20%-0.00%, respectively.

Effects of fermentable sugars on attenuation trends of four *Saccharomyces* strains: For *S. cerevisiae* DSM 70424, sucrose treatment resulting in the greatest growth and ethanol production rates (Figs. 1A and 3A) showed the lowest medium attenuation (p<0.05) (Fig. 4A). In glucose and fructose treatments from about h 24 onwards, the attenuation curve reached the plateau state (Fig. 4A). It could be inferred from Figures 1A and 4B for *S. ludwigii* 3447 that glucose and fructose resulted in the greatest growth rates, led to the lowest media attenuation (p<0.05). Also, Figure 4B shows that maltose treatment, changes in attenuation of media were not significant (p>0.05). Also the media attenuation in disaccharide treatments remained unchanged (Figs. 4C and D).

DISCUSSION

Sucrose assimilation into the yeast cells is dependent on the production of invertase. During the fermentation, sucrose is hydrolyzed by yeast invertase that is secreted into the periplasmic space or to some extent, into the fermenting medium. The resultant fructose and glucose are assimilated simultaneously (Briggs *et al*., 2004; Lewis and Younger, 1995). In the presence of sufficient glucose concentrations (e.g., 1%), the activity of high affinity hexose carriers located in the yeast cell membranes is repressed (Ozcan *et al*., 1997; Does and Bisson, 1989). This explains why in thisstudy, glucose treatments (i.e., 1%) possess noticeably lower growth rate and ethanol production rate compared to sucrose treatment. In sucrose treatment, sucrose molecules are gradually broken down into glucose and fructose, mainly in periplasm, and immediately assimilate into the cells. Therefore, the concentration of glucose is not sufficiently high to repress

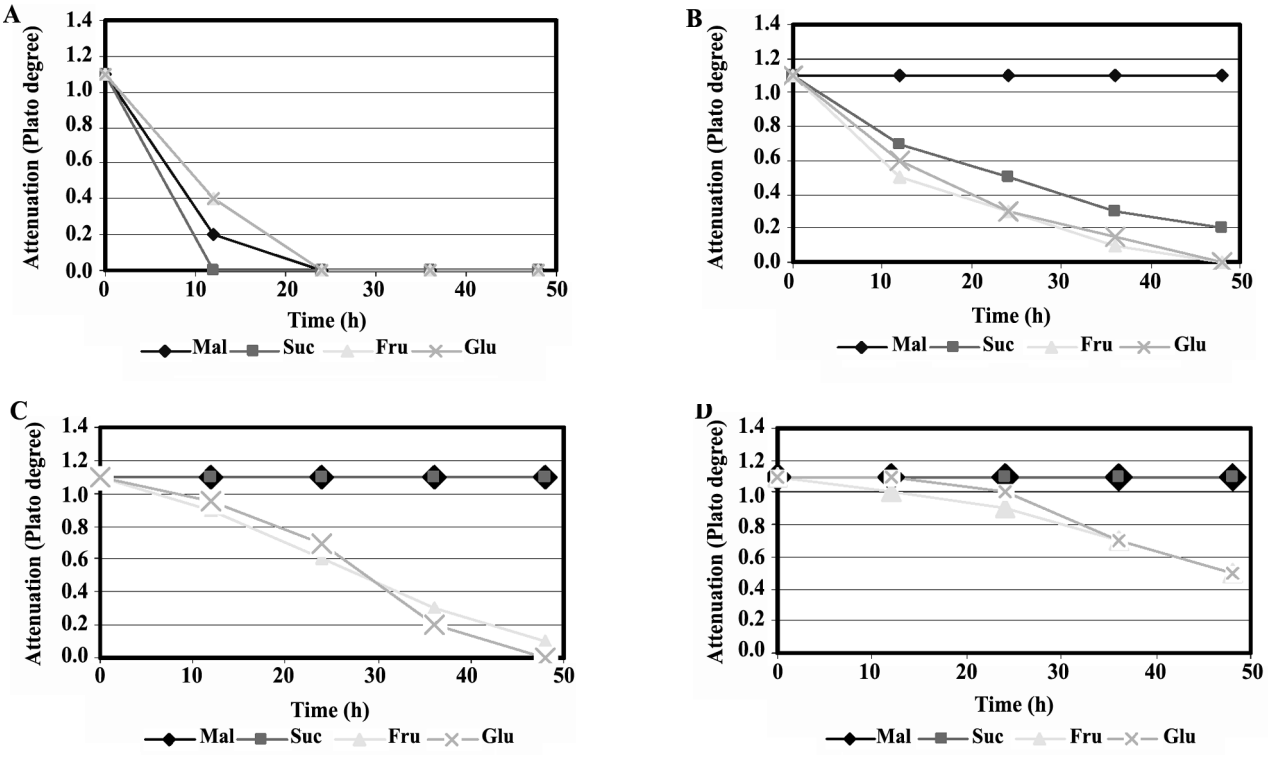

Figure 4. Attentuation trends of A: *S. cerevisiae* DSM 70424, B: *S. ludwigii* DSM 70447, C: *S. Rouxi*i DSM 2535, and D: *S. rouxii* DSM 2531 in medium containing different fermentable sugars. * Glu = glucose, Fru = fructose, Mal = maltose, Suc = sucrose.

mentioned hexose carriers (Fig. 1A).

Maltose, the most abundant sugar that naturally occurs in wort, is not significantly assimilated and utilized by ale yeast strains, till the concentration of sucrose, glucose and fructose in fermenting medium considerably fall down (Briggs *et al*., 2004). In this study, as no other sugar was present along with maltose in the maltose treatment, the sugar was utilized by yeast cells from the very beginning stages of fermentation (Fig. 1). As inferred from Figure 1, independent of sugar type, *S. cerevisiae* cells in all treatments reached the turning point in their growth curve in 24 hours after the start of fermentation. This fact reveals that the type of sugar did not considerably change the state of yeast cell growth phases during the fermentation time, but affected the growth rate.

S. ludwigii DSM 3447 was capable of utilizing fructose more than other sugars. This characteristic could be regarded as an advantage for the production of non-alcoholic beer, as maltose is the most abundant sugar in wort (Briggs *et al*., 2004) and therefore fermentation would be limited from the early stages after depletion of other sugars. Our observations are in consistence with previous reports indicating that *S. ludwigii* spp. is not capable of utilizing maltose (Huige *et al*., 1990). According to Figure 1, growth curves of *S. ludwigii* in fructose and glucose treatments reached a plateau state at about 24 h of fermentation, and remained statistically unchanged afterwards ($p>0.05$). By contrast, the growth curve relevant to sucrose treatment increased continuously and significantly ($p<0.05$) towards the end of fermentation. This phenomenon can be attributed to the considerably greater growth rates of yeast cells in fructose and glucose treatments compared to sucrose treatment that caused sooner inactivation of these cells during fermentation.

It is evident that *S. rouxii* DSM 2535 and 2535 utilize monosaccharides (glucose and fructose) noticeably greater than disaccharides (sucrose and maltose) (Sohrabvandi *et al*., 2009c). In other words, the yeast does not display appreciable maltase and invertase activities (Figs. 1C and D). The reason why the greatest growth rate was reached in parallel with attaining the lowest pH value level (Figs. 1A and 2A) is evident: Yeast cells produce various types of organic acids along their growth and activity. The carbon dioxide produced during this period can be also effectual in this regard (Hardwick, 1995). As mentioned previously, the fact that the treatment with greatest growth rate renders also the greatest pH drop rate is established (Fig. 2B).

In the presence of sufficient oxygen, the yeast cells completely oxidize the resultant pyrovate to carbon dioxide and water while producing energy for other metabolic processes. In the absence of oxygen, in alcoholic fermentation, pyrovate is converted to ethanol and carbon dioxide primarily through acetaldehyde pathway (Munroe, 1994). Therefore, reasonably, higher yeast growth rate (greater biomass production) is in contrary to ethanol production. Under aerobic conditions, because the unsaturated fatty acids are synthesized and accumulated in yeast cell membrane in order to provide enough flexibility for their growth, aldehydes are not reduced to ethanol, but are consumed to produce the fatty acids (Figs. 1A and 2A) (Dziondziak, 1989 a, b).

Maltose treatment displayed the lowest amounts of ethanol within the fermentation period. It is evident that the growth of *S. ludwigii* DSM 3447, in contrast to *S. cerevisiae* DSM 70424, was not repressed by presence of sufficient glucose contents. Considering that glucose, fructose, and maltose treatments (but not the sucrose one) did not show significant growth from about 24 h of fermentation onwards, as was similarly observed in ethanol production trend, it could be concluded that since *S. ludwigii* DSM 3447 cells did not grow significantly, they have not produced considerable ethanol either (Figs. 1B and 3B). This specification is in contrary to what was observed for *S. cerevisiae* DSM 70424: the cells in presence of sucrose and maltose although did not grow significantly, produced ethanol towards the end of fermentation (Figs. 1A and 3 A).

For *S. cerevisiae* DSM 70424, sucrose-containing treatment that resulted in the greatest growth and ethanol production rates, showed the lowest attenuation ($p<0.05$) (Fig. 4A). This is due to the parameter of attenuation being proportional to the sugar amount in the medium (Odibo *et al*., 2002); and the lowest amount of sugar was in sucrose treatment, which displayed the greatest growth rate. According to Figures 4B, C and D, for the treatments containing other yeasts, until 24 h, fructose and from that time onwards, fructose or glucose or both rendered the lowest attenuation. It could be inferred from Figures 1B and 2B when compared to Figure 4 that glucose and fructose treatments which resulted in the greatest growth rates, have led to the lowest attenuation ($p<0.05$); the reason was discussed above. In maltose treatment, changes in attenuation of the medium were not significant ($p>0.05$).

CONCLUSIONS

This study revealed that applying different strains of *Saccharomyces* in presence of different types of sugars caused various fermentation characteristics especially with regard to growth rate and ethanol production. *S. rouxii* DSM 2531 was recognized as an adequate species for production of non-alcoholic beer; as it was unable to utilize maltose (the most abundant sugar in wort) and the ethanol levels in all treatments did not exceed 0.20% (V/V). Generally, *S. cerevisiae* DSM 70424 exhibited the greatest growth and ethanol production rate compared to other species, especially in presence of sucrose. Glucose displayed repressing impact on growth and activity of this yeast. Fructose was realized as the best growth and ethanol production enhancer for the rest of yeast strains. Glucose rendered the same results only for the two strains of *S. rouxii*. The latter strains were unable to utilize disaccharides. *S. ludwigii* DSM 3447 was unable to utilize maltose.

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