Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 10 (3): 114-125, 2015
ISSN 1816-4935 / DOI: 10.3923/jm.2015.114.125

Enhancement of Bio-Ethanol Production from Date Molasses by Non-Conventional Yeasts

[1]Saad A. Alamri, [1,2]Mohamed Hashem, [1]Sulaiman A. Alrumman and [1]Malak S.A. Al-Qahtani

[1]Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia
[2]Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt

Corresponding Author: Mohamed Hashem, Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia Tel: +96672417625 Fax: +96672418205

ABSTRACT

This study aimed to enhance the bio-ethanol production from date molasses as a cheap and renewable resource by local species of non-*Saccharomyces* yeasts by optimization the production's conditions. *Hanseniaspora guilliermondii* KKUY-0036 and *H. uvarum* KKUY-0078 were used based on their ability to ferment the date molasses efficiently. They were identified by the sequencing of D1/D2 domain of the 26S rRNA gene. Their identity was confirmed by comparing the obtained sequence with similar sequences allocated in the GenBank. To enhance the ethanol productivity, temperature, pH, fermentation period, molasses concentration and addition of some elements were optimized. Results revealed that the two yeast species exhibited their maximum productivity of ethanol at 30°C when they were grown on 20-25% of the date molasses after 96-120 h of incubation. The highest ethanol concentration was achieved in weak acidic medium (pH 4-6). Addition of zinc, magnesium and manganese induced the production of ethanol by the two yeasts and the optimum concentrations were 0.6, 0.2-0.3 and 0.03 g L^{-1}, respectively. The study introduces both *H. guilliermondii* KKUY-0036 and *H. uvarum* KKUY-0078 as new ethanol-producers that ferment date molasses efficiently and will greatly reduce the biofuel production cost.

Key words: Biofuel, date molasses, *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, zinc, magnesium, manganese

INTRODUCTION

Over recent years, the world is paying more attention to find new alternative energies instead of petroleum for fear of the running out of oil. Alternative energies include solar, electrical and organic fuel (ethanol). Other reasons the stimulated the use of these alternatives by millions of people include the fact that they are environment-friendly and do not cause air pollution. Among these fuels, bio-ethanol is a good candidate, because it is a clean product derived from natural resources that could reduce the emissions into biosphere (Hansen *et al*., 2005; Hashem *et al*., 2013). Currently, the production of bio-ethanol on commercial scale depends up on sugar-cane and molasses or glucose from starchy crops including corn, wheat and cassava. Therefore, expanding the production from other sugar-containing materials is needed (Lim *et al*., 2013). To reduce the ethanol production cost, inexpensive lignocellulosic materials such as agricultural and industrial residues are investigated to be utilized as new resources (Schell *et al*., 2004; Prasad *et al*., 2007; Erdei *et al*., 2010).

Cost reduction for pretreatment and bioconversion processes is a key objective necessary to the successful placement of a bio-ethanol industry (Nagle *et al*., 2002). Enhancement of fuel production industry by the conventional fermentation is necessary to substitute the petrochemical production (Koutinas *et al*., 2004).

From the microbiological point of view, the best microorganism for fermentation process should have a rapid and efficient way to convert the available carbon sources to ethanol and tolerate the high concentration of ethanol and other inhibitory contents in the hydrolyzate (Sonderegger *et al*., 2004). *Saccharomyces cerevisiae* does not ferment pentoses, such as D-xylose and L-arabinose that are abundantly found in lignocellulosic raw materials. So, many laboratories try to modify *S. cerevisiae* strains to ferment pentose sugars (Karhumaa *et al*., 2006; Guimaraes *et al*., 2010).

Consequently, this study aims to produce bio-ethanol from date palm's molasses which is an inexpensive fermentable alternative source available in high quantities in the Kingdom of Saudi Arabia and Middle East region. In addition, ways to enhance the ethanol production by optimizing the cultural conditions will be studied.

MATERIALS AND METHODS

Microorganisms: *Hanseniaspora guilliermondii* KKUY 0036 and *Hanseniaspora uvarum* KKUY-0078, were isolated from naturally fermented date molasses on YMPGA (yeast extract-malt extract-peptone-glucose-agar) (Kurtzman and Fell, 1998). For the purpose of this document, *Hanseniaspora guilliermondii* KKUY 0036 and *Hanseniaspora uvarum* KKUY-0078 will be referred to as *H. guilliermondii* and *H. uvarum*, respectively. These species were identified via the sequencing of D1/D2 domain of the 26S rDNA region. The genomic DNA of these yeasts was extracted according to the method described by Hesham *et al*. (2006). The D1/D2 domain of the 26S rDNA region was amplified using the primers NL1 (5'-GCATATCAA TAAG CGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGT TTCAAGACGG-3') (Kurtzman and Robnett, 1998). Purification and sequencing of D1/D2 fragments were carried out at Macrogen Company, Korea. The obtained sequences were compared with known 26S rDNA sequences in the GenBank database to detect their homology scores. The sequences of 26S rRNA gene of both *H. guilliermondii* and *H. uvarum* have been deposited in GenBank databases under Accession Numbers: JQ690237 and JQ690236, respectively.

Fermentation substrate: Date molasses was obtained from Durrah Dates Factory www.durrahdates.com", Riyadh, Kingdom of Saudi Arabia. The date molasses is composed of protein, ash, sugar, pectin, tannin and its pH ranges from 4.3-5.2. Physical and chemical characteristic of the date molasses are listed in Table 1.

Table 1: Chemical structure and physical properties of date molasses

Contents	For each 100 mL
Moisture (%)	13-16
Protein (%)	1.0-1.3
Ash (%)	1.4-1.8
Total sugar (%)	75 6
Fat	Not detectable
Fiber	Not detectable
Pectin (%)	0.1-0.2
Tannin (%)	0.2-0.3
pH	4.3-5.2
TDS (%)	76-78

Ethanol estimation: Ethanol was estimated enzymatically using the ethanol estimation kit (K620-100) provided by BioVision company. The procedures provided with the product were employed. Briefly, the method depends on the presence of alcohol oxidase which oxidizes ethanol to produce H_2O_2 and then reacts with the probe to generate color (λmax = 570 nm). A standard curve was constructed using the standard ethanol provided within the kit. The amount of ethanol in a given sample was determined based on the standard curve after measuring its absorbance at 570 nm. The ethanol concentration was calculated as g L^{-1}.

Optimization of ethanol production conditions: To maximize the ethanol production from the date molasses by *H. guilliermondii* and *H. uvarum*, different cultural conditions were optimized. The inoculum of each species was prepared by growing it in YMPG liquid medium for 48 h at 25°C and 150 rpm. Two hundred milliliter bottles containing 100 mL of sterilized diluted date molasses (25%) were inoculated with 5 mL of desired yeast species (10^8 cells mL^{-1}). The inoculated bottles were plugged with sterilized rubber plugs and incubated at 150 rpm for 72 h in a rotatory incubator. The other conditions, including temperature, pH, fermentation period and the addition of some metals were conducted as described in the following paragraphs. All treatments were studied in triplicate and arranged in a complete randomized design and the mean and standard error were calculated. At the end of the incubation time, 5 mL of the fermented medium was withdrawn into the glass tube and was centrifuged at 10000 rpm for 15 min. Then, 1 mL of the supernatant was taken to determine the ethanol concentration.

Effect of fermentation period: The selected two yeast species (*H. guilliermondii* and *H. uvarum*) were grown on diluted date molasses by 25% with distilled water (v/v) and incubated for different fermentation periods (24, 48, 72, 96, 120, 144 and 168 h) at 25°C. The percentage of ethanol yield was calculated.

Effect of temperature: The two yeast species were grown on date molasses medium, that was diluted by 25% and incubated for various temperatures (20, 25, 30, 35 and 40°C). *H. guilliermondii* was incubated for 96 h and *H. uvarum* was incubated for 120 h under the different temperatures. Then, the percentage of ethanol yield was estimated.

Effect of pH value: The effect of different pH (4.0, 5.0, 6.0 and 7.0) on ethanol production from diluted date molasses (25%) by the selected two yeasts was evaluated at 30°C. The fermentation process was stopped after 96 and 120 h for *H. guilliermondii* and *H. uvarum*, respectively and the percentage of ethanol yield was estimated. The pH was adjusted using 1N HCl or 0.1N NaOH.

Effect of molasses concentration: The selected two yeast species were grown on different concentrations of molasses medium (10, 15, 20, 25 and 30%) at 30°C. The pH was adjusted before inoculation at 4.0 for *H. guilliermondii* and incubated for 96 h. *H. uvarum* was incubated for 120 h at pH 6.0. The yield of ethanol (%) was calculated at the end of the fermentation period for each set of concentrations.

Effect of zinc concentration: Effect of different doses of zinc (0, 0.2, 0.4, 0.6 and 0.8 g L^{-1}) on bio-ethanol production by the selected yeast species was studied. *H. guilliermondii* was grown in 25% (v/v) of date molasses and the initial pH was adjusted at 4.0 for 96 h at 30°C. *H. uvarum* was grown in 20% (v/v) of molasses dilution with initial pH 6.0 for 120 h under the same conditions at 30°C. The percentage of ethanol was calculated at the end of the fermentation period.

Effect of magnesium concentration: Effect of different magnesium concentrations (0, 0.1, 0.2, 0.3 and 0.4 g L^{-1}) on bio-ethanol production by the selected yeast species was studied. *H. guilliermondii* was grown on 25% (v/v) of molasses dilution with initial pH 4.0 for 96 h at 30°C. *H. uvarum* was grown on 20% (v/v) molasses dilution with initial pH 6.0 for 120 h at 30°C. The percentage of ethanol yield was measured at the end of the fermentation period for each magnesium concentration.

Effect of different concentration of manganese: Effect of different concentration of manganese (0, 0.01, 0.02, 0.03 and 0.04 g L^{-1}) on bio-ethanol production by the selected yeast species was studied. *H. guilliermondii* was grown on 25% (v/v) of molasses dilution with initial pH 4.0 for 96 h at 30°C. *H. uvarum* was grown in 20% (v/v) of molasses dilution with initial pH 6.0 for 120 h at 30°C. The percentage of ethanol was calculated at the end of the fermentation period.

Statistical analysis: All data statistically analyzed using SPSS, ANOVA. The mean were compared by Duncan's multiple range tests at $p<0.05$.

RESULTS

Effect of fermentation period: Figure 1 shows that the ethanol concentration, produced by *H. guilliermondii* and *H. uvarum* from the date molasses, increased gradually by increasing the fermentation period to reach its maximum after 96 and 120 h of fermentation, respectively. Then it began to decrease with further extension of fermentation time. *H. guilliermondii* produced the maximum amount of ethanol after 96 h, however, *H. uvarum* produced its maximum after 120 h.

Effect of temperature: The selected yeast species were screened for ethanol production at various temperatures of fermentation (20, 25, 30, 35 and 40°C) from date molasses (25%, v/v). Results of Fig. 2 clearly show that the maximum production of ethanol by the two stains was achieved at 30°C. *H. guilliermondii* produced the highest quantity of ethanol as 6.115%, however *H. uvarum* produced 5.59%. At 25°C, the produced ethanol by the two yeasts was 5.29 and 5.12%, respectively. Below 25°C or above 30°C the production of ethanol by both yeast species was significantly reduced.

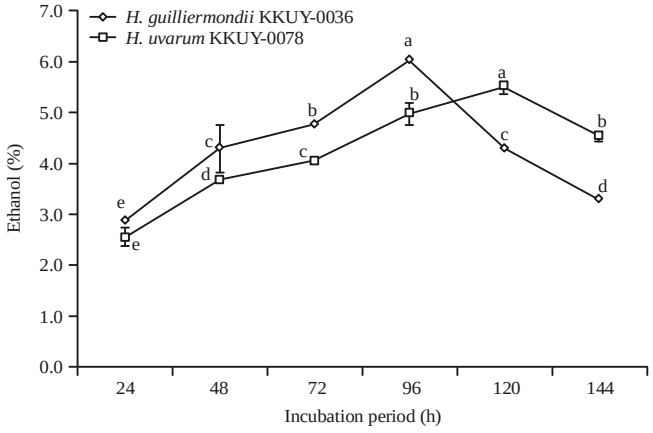

Fig. 1: Effect of fermentation period on ethanol production by *Hanseniaspora guilliermondii* and *H. uvarum* grown on 25% of date molasses. Marks of the same line followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

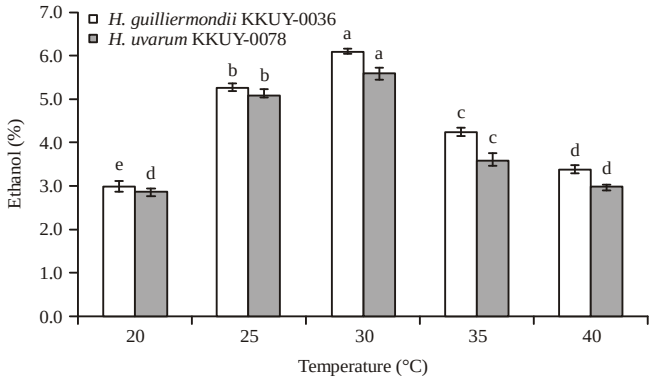

Fig. 2: Effect of temperature on ethanol production by *H. guilliermondii* and *H. uvarum* grown on 25% of date molasses. Columns in the same color followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

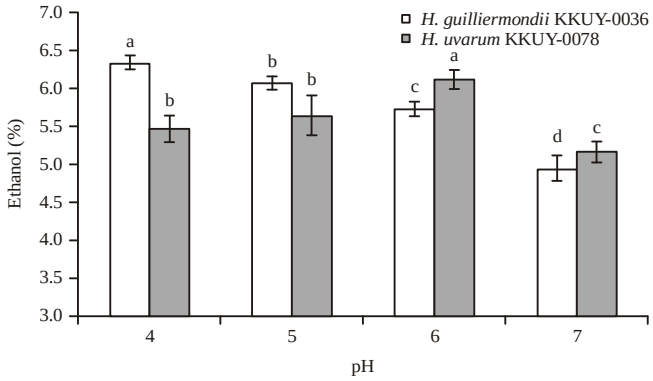

Fig. 3: Effect of different level of pH on ethanol production by *H. guilliermondii* and *H. uvarum* grown on 25% of date molasses. Columns in the same color followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

Effect of hydrogen ion concentration (pH): Figure 3 shows that the highest concentration of ethanol formed by *H. guilliermondii* was produced when the fermentation process started at pH 4.0. It produced 6.48% at this pH; however, the production was reduced to 6.07% when the pH increased to 5.0. The production was declined with the increase in pH values, where it was 5.73 and 4.93% at pH 6.0 and 7.0, respectively. The results prove that the optimum pH value of ethanol production by this yeast species was 4.0. On the contrary, *H. uvarum* tended to produce the maximum ethanol concentration (6.11%) at very weak acidic medium, pH 6.0. Below or above this pH value the ethanol productivity was significantly reduced (Fig. 3).

Effect of molasses concentration: Figure 4 shows that the highest concentration of ethanol produced by species *H. guilliermondii* was 6.56% from 25% of the molasses at 30°C after 96 h of fermentation period. Production of ethanol by this yeast species was significantly reduced in either low concentration (15-20%) or high concentration (30%). These results approve that 25% is the optimum concentration of date molasses for ethanol production by *H. guilliermondii. H. uvarum* produced 6.5% ethanol when the concentration of date molasses was 20% after 120 h of

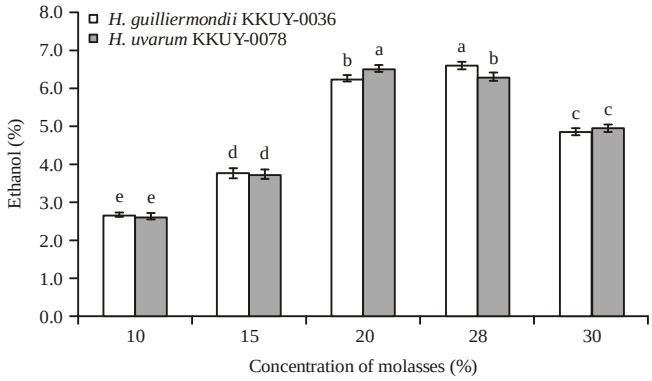

Fig. 4: Effect of different concentration of molasses on ethanol production by *H. guilliermondii* and *H. uvarum*. Columns in the same color followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

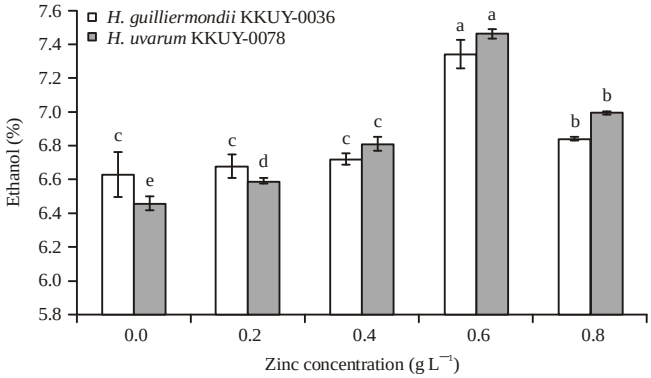

Fig. 5: Effect of different level of zinc on ethanol production by *H. guilliermondii* and *H. uvarum* at 30°C. Columns in the same color followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

fermentation period. Higher or lower concentration of the date molasses had a significant negative effect on the production of ethanol. From these results, 20% of molasses showed to be the optimum concentration for ethanol production by *H. uvarum* (Fig. 4).

Effect of different concentration of zinc: Figure 5 reveals that the ethanol production by both yeast species increased by increasing the level of zinc up to 0.6 g L^{-1}, however, above this level the production declined. *H. guilliermondii* produced the maximum concentration of ethanol as 7.3%. While, *H. uvarum* produced 7.46% at 0.6 g L^{-1} of zinc. Above this concentration of Zn, the productivity decreased to 6.84 and 6.99% by both strains, respectively. This indicates that 0.6 g L^{-1} of Zn is the optimum concentration for ethanol production by the tested yeast strains.

Effect of different concentration of magnesium: Ethanol production by the two yeasts grown in different concentrations of Mg is shown in Fig. 6. Data show that 0.2 g L^{-1} of this metal induced the maximum production of ethanol by *H. guilliermondii* (7.02%) and above this concentration, the productivity was not significantly affected. On the other hand, *H. uvarum* produced the maximum

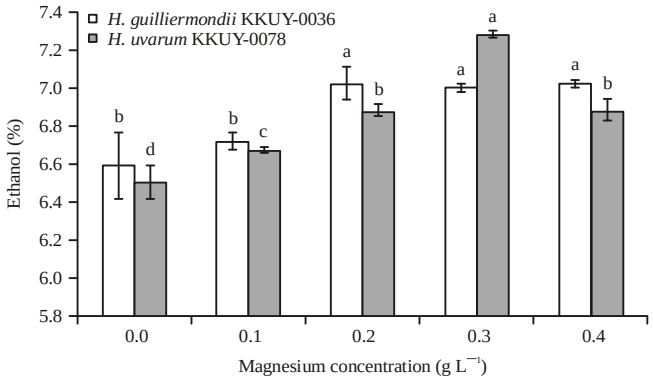

Fig. 6: Effect of different levels of magnesium on ethanol production by *H. guilliermondii* and *H. uvarum* at 30°C. Columns in the same color followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

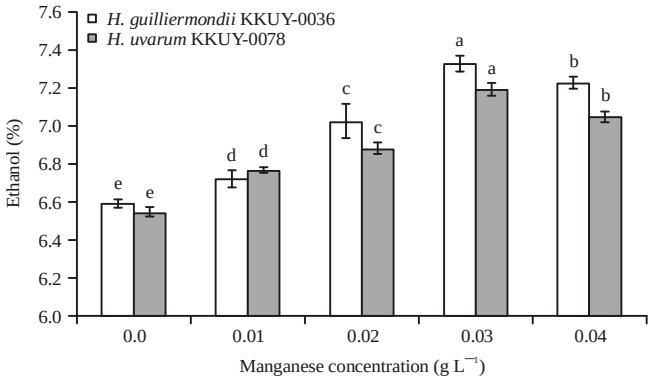

Fig. 7: Effect of different levels of manganese on ethanol production by *H. guilliermondii* and *H. uvarum* at 30°C. Columns in the same color followed by the same letter are not significant at $p<0.05$ and bars represent the standard error

yield of ethanol (7.28%) at 0.3 g L^{-1} of mg but the highest concentration of the metal decreased the production to 6.88%. Therefore, the optimum concentration of magnesium for ethanol production by these yeast species is greater than 0.3 g L^{-1}.

Effect of different concentration of manganese: The production of ethanol by both *H. guilliermondii* and *H. uvarum* was stimulated by the addition of low concentrations of manganese up to 0.03 g L^{-1}; however, the higher concentration resulted in an inhibitory effect on the production process. *H. guilliermondii* produced a relatively higher quantity of ethanol (7.32%) than *H. uvarum* (7.19%) at 0.03 g L^{-1} of the metal (Fig. 7). Based on these results, 0.03 g L^{-1} of Mn is likely to be the most appropriate concentration for ethanol production by the two yeasts species.

DISCUSSION

Using of yeast in the industry, especially for bio-fuel production, is extending rapidly and will require a wider range of yeast species than those that have so far been used. Therefore, this study introduces two new yeast species as new producers of bioenergy from an inexpensive renewable

resource. We used date molasses as a fermentation substrate for production of bio-ethanol. This substrate is an attractive by-product due to its richness in fermentable sugars. Date molasses is a cheap and abundant agro-industrial material in Kingdom of Saudi Arabia. Therefore, it is a good economic candidate substance for ethanol production via fermentation. In addition, it does not require any physical or chemical pretreatments. It also contains a substantial level of nutrients that are required for the growth of microorganisms (Myhara *et al*., 1999).

Hanseniaspora spp. (apiculate wine yeasts) have become an object of interest as they are frequently found in fermentation processes. In this context, Caridi and Ramondino (1999) tested about 20 *Hanseniaspora* spp. for their ability to ferment a must and they found that many species of *Hanseniaspora* produced high quantities of ethanol. Present results revealed that the ethanol level increased gradually by increasing the fermentation period to reach the maximum after 96-120 h of fermentation and then dramatically decreased. These finding is so beneficial because the fermentation period is an important factor from the economic point of view in ethanol production on an industrial scale. Results were better than those obtained by Zohri and Mostafa (2000), who found that the periods for complete the fermentation process of date juice with 13.5% sugar concentration by *Saccharomyces cerevisiae* and *S. bayamus* were 120 and 132 h, respectively. Controversially, Karuwanna *et al*. (1987) reported that fermentation of the juice prepared from pineapple canning waste with 20 Brix (nearly 18% sugar) by using *S. cerevisiae* required very long time (15 days) for completing the fermentation process.

The results of the present study showed that the maximum production of ethanol by the two yeasts was achieved at 30°C. *H. guilliermondii* produced the highest quantity of ethanol 6.11%; however, *H. uvarum* produced 5.59%. The results confirm that these yeast species are mesophylls that perform their maximum fermentation at moderate temperature. According to Fleet and Heard (1993), temperature of fermentation is the most important factor that affects the production of ethanol. It has a direct effect on the biochemical reactions of yeast. In addition, temperature is known to affect yeast metabolism (Lafon-Lafourcade, 1983). The enzyme activities are expected to be low at low temperature. These findings are in agreement with those of Reddy and Reddy (2006), who reported that the fermentation temperature up to 30°C should be considered. The optimal temperature for maximum productivity of ethanol was at 32°C, however, when the temperature increased, alcohol yield was reduced as a result of cell death (Edgardo *et al*., 2008). Therefore, it is necessary to select the optimum temperature at which yeast species can ferment the sugars from date molasses material. The increase in temperature to 35-40°C showed a negative effect on fermentation process, resulting in lower productions. In agreement with our results, many findings approved that temperature above 37°C is harmful for ethanol production (Skotnicki *et al*., 1981; Diez and Yokoya, 1996).

When we applied different initial pH (4-7) of the fermentation media, we found that the highest ethanol concentrations was produced by *H. guilliermondii* at pH 4.0 while *H. uvarum* produced its highest ethanol production at initial pH 6.0. The highest ethanol quantity obtained by *H. guilliermondii* and *H. uvarum* was 6.48 and 6.11%, respectively. It was observed that these species preferred the weak acidic pH to perform their optimum enzyme system for ethanol production. The pH has a significant effect on fermentation process due to its direct effect on yeast growth, fermentation rate and formation of different by-product. Therefore, maintenance of pH is principal in fermentation processes (Pramanik, 2003). This author reported that the maximum ethanol concentration by *Saccharomyces cerevisiae* was achieved with pH 4.25- 5.0. The activity of the yeast species at pH 3.75 was too low, because this pH was too low to activate the enzymes to

react. The ethanol production increased at higher pH at the beginning, but the maximum ethanol yield was less than that was obtained with pH 4.25. The low ethanol yield and sugar conversion at higher pH values could be due to the formation of undesired products like glycerol, organic acids etc., instead of ethanol. Limtong *et al*. (2007) reported that the maximum ethanol production by *K. marxianus* DMKU 3-1042 from sugar cane juice (22% sugar) at 37°C was 8.7% (77.5% of theoretical yield) when pH was 5.0.

The highest concentration of ethanol was obtained from 25% of date molasses by for *H. guilliermondii*; however, *H. uvarum* produced the highest ethanol concentration from 20% of the molasses. At high molasses concentrations 30%, there was a significant decrease in the ethanol production by both yeasts. This could be because high substrate concentrations are inhibitory to the fermentation process by yeasts due to osmotic stress. For many decades, Prescott and Dunn (1959) reported that the high sugar concentration reacts adversely on the yeast growth or the alcohol produced may inhibit the action of the yeast strain, with the consequence that the fermentation time is prolonged and some of the sugar is not properly utilized. In agreement with this concept, our results revealed that the growth of yeast and ethanol production was greatly retarded in fermentation medium with high concentration of molasses. Reddy and Reddy (2006) reported that sugar utilization was decreased by increasing the sugar concentration that resulted in reduction of ethanol yield. The reduction in ethanol yield may be due to many reasons including releasing of other compounds like glycerol or acetic acid. Also, the intracellular ethanol (which may be increased by increasing ethanol production at high sugar concentration) cause toxicity on yeast and the nutrient may be deficient at the end of fermentation process (Sols *et al*., 1971). Similar results were recorded previously by Pratt *et al*. (2003), who observed that fermentation of concentrated worts have a negative impact on the performance of yeast due to the higher osmotic pressure.

The importance of some microelements for the yeast growth as well as the production of ethanol was reported (Jones and Gadd, 1990; Stehlik-Tomas *et al*., 1997). The available microelements in molasses that could be metabolized by yeast cells is very low because of these metal ions are binding to the organic carriers. So, we studied the effect of Zn, Mg and Mn on the production of ethanol by the two yeasts. The data obtained from our experiments revealed that 0.6 g L^{-1} of Zn was the optimum concentration for ethanol production by the two yeasts. Zinc in a biological form "Zn^{2+}" is important as catalytic cofactor of many enzymes, especially alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase and several carboxy-peptidases. It also plays a structural role in enzymes and many non-catalytic proteins (Berg *et al*., 2002). It has been pointed out that availability of Zn^{2+} in nutrient medium in 5-15 μM enhanced the growth of yeast and increased the ethanol production (Jones and Gadd, 1990). Zinc deficiency suppressed the yeast growth and reduced the fermentation activity. However, high concentration of zinc ions may be toxic because it affects the permeability of cell membranes to potassium that decreases the yeast growth and the fermentation activity (Liu *et al*., 1997).

In case of Mg, 0.2 g L^{-1} was the optimum concentration for *H. guilliermondii*, however 0.3 g L^{-1} of this metal was the optimum concentration for *H. uvarum* that induced the maximum production of ethanol. The highest concentration of this metal decreased the ethanol production. It is seemed that the optimum concentration of magnesium for ethanol by these yeasts doesn't exceed than 0.3 g L^{-1}. Sugar metabolism by yeasts is affected by magnesium concentration in growth media. Magnesium is among many micro-metal ions that are needed in milli-molar for optimal growth of yeast and fermentation (Birch *et al*., 2003). In addition, magnesium is involved in different

functions of yeast physiology such as cell division, mitochondrial function, respiro-fermentative metabolism and responses to environmental stress (Birch and Walker, 2000). Owades (1991) and Gawande *et al*. (1998) found Mg^{2+} to be essential for enzyme production and consequently alcohol released.

The production of ethanol by both *H. guilliermondii* and *H. uvarum* was stimulated by the addition of low concentrations of Mn up to 0.03 g L^{-1}; however, the higher concentration exposed an inhibitory effect of the production process. Manganese is essential as trace element (2-10 μmole) for the growth of yeast cells (Jones and Gadd, 1990). Entrance of Mn^{2+} into yeast cells could be passive and driven by the concentration gradient (Kihn *et al*., 1988) or energy-dependent, stimulated by glucose (Blackwell *et al*., 1998).

CONCLUSION

The present study provides important information about the production of bio-ethanol from inexpensive renewable resource, date molasses which is abundant in many countries. This source does not require any pretreatment which reduces the production cost. In addition, the results revealed that the non-*Saccharomyces* yeasts, *H. guilliermondii* and *H. uvarum* could play an important role in ethanol production from sugar-containing agro-wastes. The result also defined the required conditions including temperature, pH, molasses concentration, fermentation period and the optimal concentration of the microelements (Zn, Mg and Mn) for the production of maximum concentration of ethanol by the tested yeasts. We recommend scaling up our new results on semi-industrial and industrial scales to make the bio-ethanol is a good competitive as a fuel.

REFERENCES

Berg, J.M., J.L. Tymoczko and L. Stryer, 2002. Biochemistry. 5th Edn., WH Freeman and Company, New York, ISBN-13: 978-0716746843, pp: 465-687.

Birch, R.M. and G.M. Walker, 2000. Influence of magnesium ions on heat shock and ethanol stress responses of *Saccharomyces cerevisiae*. Enzyme Microb. Technol., 26: 678-687.

Birch, R.M., M. Ciani and G.M. Walker, 2003. Magnesium, calcium and fermentative metabolism in wine yeasts. J. Wine Res., 14: 3-15.

Blackwell, K.J., J.M. Tobin and S.V. Avery, 1998. Manganese toxicity towards *Saccharomyces cerevisiae*: Dependence on intracellular and extracellular magnesium concentrations. Applied Microbiol. Biotechnol., 49: 751-757.

Caridi, A. and D. Ramondino, 1999. Biodiversita fenotipica in ceppi di hanseniaspora di origine enologica. Enotecnico, 45: 71-74.

Diez, J.C. and F. Yokoya, 1996. Fermentacaoalcoo lica de mosto de melaco de cana-de-acucar por processo descontinuo utilizando *Zymomonas mobilis*. Arq. Biol. Tecnol., 32: 419-426.

Edgardo, A., P. Carolina, R. Manuel, F. Juanita and J. Baeza, 2008. Selection of thermotolerant yeast strains *Saccharomyces cerevisiae* for bioethanol production. Enzyme Microb. Technol., 43: 120-123.

Erdei, B., Z. Barta, B. Sipos, K. Reczey, M. Galbe and G. Zacchi, 2010. Ethanol production from mixtures of wheat straw and wheat meal. Biotechnol. Biofuels, Vol. 3.

Fleet, G.H. and G.M. Heard, 1993. Yeasts: Growth during Fermentation. In: Wine Microbiology and Biotechnology, Fleet, G.M. (Ed.). Harwood Academic Publishers, Chur, Switzerland, pp: 27-54.

Gawande, B.N., R.K. Singh, A.K. Chauhan, A. Goel and A.Y. Patkar, 1998. Optimization of cyclomaltodextrin glucanotransferase production from *Bacillus firmus*. Enzyme Microbial. Technol., 22: 288-291.

Guimaraes, P.M.R., J.A. Teixeira and L. Domingues, 2010. Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnol. Adv., 28: 375-384.

Hansen, A.C., Q. Zhang and P.W.L. Lyne, 2005. Ethanol-diesel fuel blends-a review. Bioresour. Technol., 96: 277-285.

Hashem, M., R. Abel-Basset and E.H. Ali, 2013. Recycling the rice straw into biofuel Ethanol by *Saccharomyces cerevisiae* and *Pichia guilliermondii*. J. Agric. Sci. Technol., 15: 709-721.

Hesham, A.E., Z. Wang, Y. Zhang, J. Zhang, W. Lv and M. Yang, 2006. Isolation and identification of a yeast strain capable of degrading four and five ring aromatic hydrocarbons. Ann. Mcrobiol., 56: 109-112.

Jones, R.P. and G.M. Gadd, 1990. Ionic nutrition of yeast-physiological mechanisms involved and implications for biotechnology. Enzyme Microb. Technol., 12: 402-418.

Karhumaa, K., B. Wiedemann, B. Hahn-Hagerdal, E. Boles and M.F. Gorwa-Grauslund, 2006. Co-utilization of L-arabinose and D-xylose by laboratory and industrial *Saccharomyces cerevisiae* strains. Microbial Cell Factories, Vol. 5. 10.1186/1475-2859-5-18

Karuwanna, P., M. Boonyaratanakornkit, C. Wongkhalaung and P. Faungfupong 1987. Wine vinegar from skins and cores of pineapple. Food, 17: 137-145.

Kihn, J.C., C.M. Dassargues and M.M. Mestdagh, 1988. Preliminary ESR study of Mn(II) retention by the yeast *Saccharomyces*. Can. J. Microbiol., 34: 1230-1234.

Koutinas, A.A., R. Wang and C. Webb, 2004. Restructuring upstream bioprocessing: Technological and economical aspects for production of a generic microbial feedstock from wheat. Biotechnol. Bioeng., 85: 524-538.

Kurtzman, C. and J.W. Fell, 1998. The Yeast-A Taxonomic Study. 4th Edn., Elsevier, Amsterdam, ISBN: 9780080542690, Pages: 1076.

Kurtzman, C.P. and C.J. Robnett, 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek, 73: 331-371.

Lafon-Lafourcade, S., 1983. Wine and Brandy. In: Biotechnology: Food and Feed Production with Microorganisms, Rehm, H.J. and G. Reed (Eds.). VCH Publishing Company, New York, pp: 81-163.

Lim, S.H., D. Ibrahim and I.C. Omar, 2013. Effect of physical parameters on second generation bio-ethanol production from oil palm frond by *Saccharomyces cerevisiae*. BioResources, 8: 969-980.

Limtong, S., C. Sringiew and W. Yongmanitchai, 2007. Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*. Bioresour. Technol., 98: 3367-3374.

Liu, F.X., F. Supek, N. Nelson and V.C. Culotta, 1997. Negative control of heavy metal uptake by the *Saccharomyces cerevisiae* BSD2 gene. J. Biol. Chem., 272: 11763-11769.

Myhara, R.M., J. Karkala and M.S. Taylor, 1999. The composition of maturing Omani dates. J. Sci. Food Agric., 79: 1345-1350.

Nagle, N.J., R.T. Elander, M.M. Newman, B.T. Rohrback, R.O. Ruiz and R.W. Torget, 2002. Efficacy of a hot washing process for pretreated yellow poplar to enhance bioethanol production. Biotechnol. Prog., 18: 734-738.

Owades, J.L., 1991. Method of making salt-free sauerkraut. United States Patent US5064662. http://www.freepatentsonline.com/5064662.html

Pramanik, K., 2003. Parametric studies on batch alcohol fermentation using *Saccharomyces* yeast extracted from toddy. J. Chin. Inst. Chem. Eng., 34: 487-492.

Prasad, S., A. Singh and H.C. Joshi, 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour. Conserv. Recycl., 50: 1-39.

Pratt, P.L., J.H. Bryce and G.G. Stewart, 2003. The effects of osmotic pressure and ethanol on yeast viability and morphology. J. Inst. Brewing, 109: 218-228.

Prescott, S.C. and C.G. Dunn, 1959. Industrial Microbiology. 3rd Edn., McGraw-Hill Book Co., New York, USA., Pages: 945.

Reddy, L.V.A. and O.V.S. Reddy, 2006. Rapid and enhanced production of ethanol in very high gravity (VHG) sugar fermentation by *Saccharomyces cerevisiae*: Role of finger millet (*Eleusine coracana* L.) flour. Process Biochem., 41: 726-729.

Schell, D.J., C.J. Riley, N. Dowe, J. Farmer and K.N. Ibsen *et al*., 2004. A bioethanol process development unit: Initial operating experiences and results with a corn fiber feedstock. Bioresour. Technol., 91: 179-188.

Skotnicki, M.L., K.J. Lee, D.E. Tribe and P.L. Rogers, 1981. Comparison of ethanol production by different *Zymomonas* strains. Applied Environ. Microbiol., 41: 889-893.

Sols, A., C. Gancedo and G. DelaFuente, 1971. Energy-Yielding Metabolism in Yeasts. In: The Yeasts, Rose, A.H. and J.S. Harrison (Eds.). Vol. 2, Academic Press, New York, pp: 271-307.

Sonderegger, M., M. Jeppsson, C. Larsson, M.F. Gorwa-Grauslund and E. Boles *et al*., 2004. Fermentation performance of engineered and evolved xylose-fermenting *Saccharomyces cerevisiae* strains. Biotechnol. Bioeng., 87: 90-98.

Stehlik-Tomas, V., S. Grba and V. Runjic-Peric, 1997. Zinc uptake by *Saccharomyces cerevisiae* and its impact on alcohol fermentation. Chem. Biochem. Eng. Q., 11: 147-151.

Zohri, A.A. and E. Mostafa, 2000. Ethanol production from dates in Saudi Arabia on industrial scale. Microbiology, 28: 76-81.