

## Study ability of some yeasts to ethanol production by using dates, apricot and grapes

Fahad K.Y. Al-dulaimi \*, Wijdan I.A. Abd-alwahab \*\*,

Maha E. Jasim \*\*\*

Northern Technical Univ./Institute of Medical Al-Dour  
Samarra Univ./ Education College / Dept. of Biology  
Northern Technical Univ./Institute of Medical Al-Dour

### ABSTRACT

The present research was aimed to comparing and investigate the ability and efficiency of three (3) yeast species; *Saccharomyces cerevisiae*, *Rhodotorulaglutinis* and *Candida kefyr*, to consume sugars in Dates, Apricot and Grapes, then, converting by fermentation to ethanol. The peptone (10g/L), yeast extract (6g/L) malt extract (6g/L) broth were appended with (Dates, Apricot and Grapes) and incubated at 30°C, 120 rpm for 96 hours. The alcoholic production from Dates by *Saccharomyces cerevisiae* 7.0% was significantly higher than of *Rhodotorulaglutinis* 5.0% and *Candida kefyr* 4.5% at 96 hours. While the alcoholic production from Apricot by *Rhodotorulaglutinis* 6.0% was significantly increase than of *Candida kefyr* 5.3% and *Saccharomyces cerevisiae* 5.0% at 96 hours. Moreover, the alcoholic production from Grapes by *Rhodotorulaglutinis* 6.7% was significantly higher than of *Saccharomyces cerevisiae* 6.0% and *Candida kefyr* 5.2% at 96 hours. The utilize of highly adaptable species of yeasts with a varied fruits in sugar sources for endeavor to increase ethanol production may create a singular prospective for large scale industrial applications.

**Keywords:** Ethanol, *Saccharomyces cerevisiae*, *Rhodotorulaglutinis*, *Candida kefyr*, Dates, Apricot, Grapes.

### I. Introduction

Bioethanol has become more important as a sustainable energy source in society, particularly because of produced concerns with utilize of fossil fuels combined with its effect on the environment (Khawetal, 2007). The production of bioethanol that use based on renewable raw materials as agricultural products that include; use of competitive technologies to be suitable for a variant requirements of energy market (Hu & Wen, 2008). The renewable sources for production of ethanol such as sugar cane, dates, Apricot and grapes are affluent in fermentable sugars, therefore, exploiting it as substrate model to production of ethanol. Most studies about dates, Apricot and grapes have reported its potentials as sources of yeasts that isolate for the fermentation industries because it's a nutritional rich medium for growth of yeast species (Zohri, 2000; Yabaya et al., 2016; Chauhan et al., 2016).

Brazil is original country have a large scale in ethanol production as motor fuel through the fermentation of sugar cane molasses by yeasts (Wheals *et al.*, 1999). The yeast of *Saccharomyces cerevisiae* employ as the most respected strain of yeast used to produce bioethanol in the industry (Porro *et al.*, 2009). In a recent study, they are found that other yeasts species like *Rhodotorulaglutinis* and *Candida kefir* were capable for normal growth and metabolism on dates, seeds, cheese whey and other media, in addition to increasing of ethanol amounts (Esabiet *al.*, 2008; Heba *et al.*, 2015). In India utilizing a range of numerous wastes or organic materials like sugarcane, molasses and other simple sugars to produce ethanol with yeast species (Dhaliwa *et al.*, 2011). The aims of this study was determine and investigate ability of three yeast species; *Saccharomyces cerevisiae*, *Rhodotorulaglutinis* and *Candida kefir* to ethanol Production during fermentation.

## II. MATERIALS AND METHODS

### Materials

Dates, Apricot and Grapes were obtained from Local markets in Salah al-din Province, Iraq. The other components of the culture media and the chemical reagents were obtained from Sigma Company.

### Isolation of yeasts

Yeasts were obtained from the Biology Department in College of Science, Tikrit University. This culture was maintained on PDA slant-media at 4°C and sub-cultured monthly.

### Preparation of fermentation juice

Dates, Apricot and Grapes was cleaned and separated seeds, then mixed with a quantity of distilled water and crushed by electric mixer, at the end, the juice was sterilized by autoclave and kept until use.

### Ethanol Production

A loop of each isolate utilized to inoculate a 100ml of autoclaved extract of yeast, peptone, dextrose (YPD) broth in Erlenmeyer flasks (250ml) and incubated for 24 hours at 30°C, 120rpm, subsequently, transferred from each yeast cells suspension 10ml then add to 125ml of broth which composed of 10g/L peptone, 6g/L yeast extract, 6g/L malt extract and 2g/L glucose with adjusted pH 5.0, then add 25ml of Dates, Apricot and Grapes juice to mentioned suspension and introduced into each flask under hood, and later, all flasks incubated at 30°C, 120rpm for 96 hours. After period 24 hour, the samples were collected for measure ethanol production by potassium dichromate method (Caputi *et al.*, 1968; Balasubramanian *et al.*, 2011; Zohri & Mostafa, 2000). Ultraviolet (UV) spectrophotometry and haemocytometer were utilized to determine growth of yeasts. The experiments in present study were performed in triplicate, the results were also calculated as a mean for three replications.

### Statistical Analysis

The data of results were analyzed by using the ANOVA analysis, and utilized the Statistically Analysis System (SAS, 2001). Also, significant differences were evaluated by using Duncan's multiple-range test (Duncan, 1955), and significance level is based on level of probability ( $P < 0.05$ ).

### III.RESULTS AND DISCUSSION

The isolates under study were grown and used in the fermentation of Dates, Apricot and Grape molasses for period 4 days at 30°C. The isolates were active producers of ethanol by utilizing all media for execution of fermentation. The results in table 1 showed ethanol produced by using Dates among the isolates, *Saccharomyces cerevisiae* produced the highest yields of ethanol 7.0 % , it was significantly higher than that of *Rhodotorulaglutinis* 5.0 % and *Candida kefy* strain 4.5 % throughout the 96h into fermented process. This result agreed with Gidado *et al* (2017), they found there was increase in alcohol production through 96 h, also agreed with Izmirlioglu & Demirci (2012), Who used to *Saccharomyces cerevisiae* for ethanol production from waste potato mash.

Table 1: Ethanol production(% w/v) on Dates medium by yeast isolates; *Saccharomyces cerevisiae*, *Rhodotorulaglutinis* and *Candida kefy* through 96 hours.

Time (h) \ Yeast isolates	24 h	28 h	72 h	96 h
<i>Saccharomyces cerevisiae</i>	2.0±0.11 a	3.0±0.10 a	5.0±0.13 a	7.0±0.12 a
<i>Rhodotorulaglutinis</i>	1.5±0.09 b	2.8±0.11 a	4.0±0.11 a	5.0±0.10 b
<i>Candida kefy</i>	1.0±0.09 b	2.0±0.13 b	3.2±0.21 b	4.5±0.11 b

- The values represent mean±S.E.

- Different of letters vertically mean significant difference at the level of significance (P <0.05).

Chtourou *et al* (2012), the report indicated to production of ethanol from Cull Dates by *Candida kefy*. The increase in ethanol production by using dates may be due to its contain in their chemical composition 75% of sugars, that converted to high amount of ethanol, dates which using in production of ethanol may be include of two types of sugars in their chemical composition, starch and sucrose, which are converted into ethanol (Gaily *et al.*, 2012).

While in table 2 observed that the volume of ethanol produced using Apricot by isolates of *Rhodotorulaglutinis* 6.0 % was significantly higher than of *Candida kefy* 5.3 % and *Saccharomyces cerevisiae* strain 5.0 % throughout the 96h into fermented process. The aforementioned results agreed with the results of Esabiet *al* (2008), who were indicated that the production of ethanol of *Rhodotorulaglutinis* by using Ram Horn Peptone, ethanol production in apricot medium may due to the chemical composition of apricot that contains 63% sugars, which makes the yeast grow and converted it to ethanol (Jieni Lian *et al.*, 2010).

Table 2: Ethanol production (% w/v) on Apricot medium by yeast isolates; *Saccharomyces cerevisiae*, *Rhodotorulaglutinis* and *Candida kefir* through 96 hours.

Time (h) \ Yeast isolates	24 h	28 h	72 h	96 h
<i>Saccharomyces cerevisiae</i>	1.5±0.10 b	2.6±0.14 b	4.0±0.23 b	5.0±0.16 b
<i>Rhodotorulaglutinis</i>	2.0±0.11 a	3.0±0.12 a	4.5±0.11 a	6.0±0.22 a
<i>Candida kefir</i>	1.7±0.13 a	3.0±0.22 a	4.0±0.18 b	5.3±0.18 b

- The values represent mean±S.E.

- Different of letters vertically mean significant difference at the level of significance (P <0.05).

Moreover, table 3 showed that the production of ethanol of Grapes by using *Rhodotorulaglutinis* 6.7 % was significantly increase than of *Saccharomyces cerevisiae* 6.0 % and *Candida kefir* strain 5.2 % throughout the 96h into fermented process. This mentioned results agreed with results of Mohammadreza et al (2012), they were refer to produce of ethanol from cheese whey permeate by two yeast strains. The decreases of ethanol produced when increases of sugar in media maybe due to excessive sugar in the fermentation medium which negatively effects on production of ethanol through increases the osmotic pressure between the cell and medium and thus, causing a slow transfer of nutrients into cell, and slow the process of metabolism and convert these substances to ethanol (Jones & Ingledew, 1994).

Table 3: Ethanol production (% w/v) on Grapes medium by yeast isolates; *Saccharomyces cerevisiae*, *Rhodotorulaglutinis* and *Candida kefir* through 96 hours.

Time (h) \ Yeast isolates	24 h	28 h	72 h	96 h
<i>Saccharomyces cerevisiae</i>	1.8±0.11 a	2.8±0.14 a	4.0±0.18 a	6.0±0.19 a
<i>Rhodotorulaglutinis</i>	2.0±0.08 a	3.0±0.20 a	4.3±0.16 a	6.7±0.21 a
<i>Candida kefir</i>	1.6±0.10 a	2.0±0.17 b	3.6±0.22 b	5.2±0.15 b

- The values represent mean±S.E.

- Different of letters vertically mean significant difference at the level of significance (P <0.05).

#### IV.CONCLUSION

This research has indicated that yeasts under study have the ability to grow and capable to producing of ethanol at temperature of room. As a result, the alcohol production from isolates were recorded significantly as high in *Saccharomyces cerevisiae* strain in Dates medium, *Rhodotorulaglutinis* strain in Apricot and Grapes, then *Candida kefyr* strain in Dates and Grapes. The ability of ethanol production should be valuable and beneficial in industrial applications. Also, as a conclusion, this research found that *Saccharomyces cerevisiae* was the most activity and efficiency in production of ethanol on Dates medium in comparison to other isolates on media. This research is suggesting there are various parameters for fermentation which if adjusted that may improve the fermentation and production of ethanol. The probably this study is the first study designed and reported to yeasts fermentation on Dates, Apricot and Grapes by *Saccharomyces cerevisiae*, *Rhodotorulaglutinis* and *Candida kefyr*.

#### REFERENCES

1. Balasubramanian, K., Ambikapathy, V. and Panneerselvam, A. (2011). Studies on ethanol production from spoiled fruits by batch fermentations. *J. Microbiol. Biotechnol. Res*; 1(4): 158-163.
2. Caputi, Jr. A., Uedam, M. and Brown, T. (1968). Spectrophotometric determination of ethanol in wine. *Am. J. Enol. Vitic*; 19(3): 160-165.
3. Chauhan, P., Sharma, S. and Thakur, A. D. (2016). Studies on natural and inoculated alcohol fermentation of wild apricot for vinegar production. *IJBPA*; 5(11): 2918-2930.
4. Chtourou, H., Bouallagui, Z., Sayadi, S. and Dhouib, A. (2012). Bioethanol Production from Cull Dates by *Candida kefyr*. *J. Biobased Mater. Bioenergy*; 6: 588-593.
5. Dhaliwal, S. S., Oberoi, H. S., Sandhu, S. K., Nanda, D., Kumar, D. and Uppal, S. K. (2011). Enhanced ethanol production from sugarcane juice by galactose adaptation of a newly isolated thermotolerant strain of *Pichiakudriavzevii*. *Bioresource Technology*; 102: 5968-5975.
6. Esabi, B. K., Kani, Z., Namudar, I. K., Mesut, T. K. and Hamdullah, K. (2008). Production of (S)-(-)-1-(1'-Naphthyl) Ethanol by *Rhodotorulaglutinis* isolate using Ram Horn Peptone. *Turk. J. Chem*; 32: 685-692.
7. Gaily, M. H., Sulieman, A. K., Zeinelabdeen, M. A., Al-Zahrani, S. M., Atiyeh, H. K. and Abasaed, A. E. (2012). The effects of activation time on the production of fructose and bioethanol from date extract. *African Journal of Biotechnology*; 11(33): 8212-8217.
8. Gidado, R. S., Olatilu, O. A., Etuk-Udo, G. A., Isu-Rosemary, N. and Solomon, B. O. (2017). Ethanol production by alcohol tolerant yeasts using different carbohydrate sources. *Advances in Applied Sciences*; 2(5): 69-74.
9. Heba, M., Kanzy, N. F., Nasri, H. A., El-Shazly, M. and Olfat, S. B. (2015). Optimization of carotenoids production by yeast strains of *Rhodotorula* using salted cheese whey. *Int. J. Curr. Microbiol. App. Sci*; 4(1): 456-469.



10. **Hu,Z., and Wen,Z.** (2008). Enhancing enzymatic digestibility of switch grass by microwave-assisted alkali pretreatment. *Journal of Biochemical Engineering*; 38: 369-378.
11. **Izmirliglu,G. and Demirci,A.** (2012). Ethanol production from waste potato mash by using *Saccharomyces cerevisiae*. *Applied Sciences*; 2: 738-753.
12. **JieniLian,S., Chen,S.,Zhouhong,W.,James,F.,Chun,Z. and Manuel,G.** (2010).Separation, hydrolysis and fermentation of pyrolytic sugars to produce ethanol and lipids. *Bioresource Technology*; 101: 9688-9699.
13. **Jones,A. M. and Ingledew,W. M.** (1994). Fuel alcohol production: Optimization of temperature for efficient very-high-gravity fermentation. *Appl. and Environ. Microbiol*; 60: 1048.
14. **Khaw,T. S., Katakura,Y., Ninomiya,K., Moukamnerd,C., Kondo,A., Ueda,M. and Shioya,S.** (2007). Enhancement of ethanol production by promoting surface contact between starch granules and arming yeast in direct ethanol fermentation. *Journal of Bioscience and Bioengineering*; 103: 95-97.
15. **Mohammadreza,K., Mojtaba,J. and Mohammad,A.** (2012). Comparison of ethanol production from cheese whey permeate by two yeast strains. *Journal of Food Science and Technology*; 49:614-619.
16. **Porro,D. and Branduardi,P.** (2009). Yeast cell factory: fishing for the best one or engineering it? *Microbial CellFactories*; 8: 51.
17. **SAS Version, Statistical Analysis System.** (2001). SAS Institute Inc., Cary, NC. 27512 – 8000, U.S.A.
18. **Wheals, A. E., Basso,L. C., Alvesand,D. M. and Amorim,H. V.** (1999). Fuel ethanol after 25 years. *Trends in Biotechnol*; 17: 482-487.
19. **Yabaya,A., Bobai,M. and Adebayo,L.** (2016). Production of wine from fermentation of vitisvinifera (grape) juice using *Saccharomyces cerevisiae* strain isolated from palm wine. *International Journal of Information Research and Review*; 3(10): 2834-2840.
20. **Zohri,A. A. and Eman M.** (2000). Ethanol production from dates in Saudi Arabia on industrial scale. *Microbiology*; 28(2): 76-81.