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Suitability of Producing Vinegar from Date Juice

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Abstract. Substandard dates were used as a cheap source for production of date juice. The dates were mixed with soft water and heated at 80°C for 30 min. to automatically separate the kernals. The remaining seedless dates solution was mixed well, decanted and clarified by microfiltrated. The total soluble solids concentration in the produced date's juice was nearly 20° Brix . The total sugars concentration in this juice was 18%. This amount of sugars was converted into ethanol by using *Saccharamyces cerevisiae* or *S. bayanus* at 30°C and pH 3.5 in special submerged fermentors. *S. bayanus* was more active than *S. cerevisiae*, where it completed the fermentation process in 72 hours while *S. cerevisiae* completed the process in 96 hours. Also, the average fermentation rate by *S. bayanus* was 2.33° Brix consumed per 12 hours compare with 1.75° Brix for *S. cerevisiae*. At the end of fermentation, ethanol produced was 9.161% (50.89% of total sugars in used juice) and 9.104% (50.58% of total sugar in used juice) by using *S. bayanus* and *S. cerevisiae*, respectively. Fermented date juice was subjected to aerobic submerged acetification by using *Acetobacter aceti* at 29 ±1°C in special acetator. The reaction is highly exothermic and requires cooling. A temperature controller maintains a constant temperature of 29±1°C. The average acetification rate was 0.167% acetic acid produced per two hours and the process was completed in 46 hours. At the end of vinegar production cycle, the acetic acid concentration reached 10.05% while the alcohol concentration decreased to 0.506%. The acetification efficiency was 74.88% of the theoretical value. The produced vinegar was treated with 0.1% food grade Bentonite, diluted to exactly 5% concentration by using soft water, microfiltrated and bottled under complete sterilized conditions.

Keywords: Vinegar, Fermentation, Acetifiaction, Dates, Juice, Yeast *Acetobacter*.

Introduction

Dates are considered a staple crop in Suadi Arabia, especially in the Eastern Province, where they supply high-energy food, basic livestock feed and raw material for several home applications. Al-Hassa is one of the most important date producing districts in the kingdom of Saudi Arabia [1] .

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Sawaya *et al.* [2] determined the chemical composition of twenty five date cultivars grown in Saudi Arabia and found that the concentrations of protein, fat, fiber and ash ranged from 1.84 - 2.88%, 0.11- 0.32%, 2.04 - 3.82% and 1.47 - 2.96% , respectively, in the tamer stage. Also, they found that the concentrations of Na , K, Ca, Mg, P, Fe, Cu and Zn fluctuated between 18 - 42 mg, 566 - 1223 mg , 14 - 76 mg, 37-58 mg, 35-109 mg, 1.05-2.44 mg, 0.26-1.27 mg and 0.19-0.87 mg/100g dates in tamer stage, respectively. Mustafa *et al.* [3] recorded that the concentration of reduced sugar (glucose + fructose), sucrose, lipid, pectin and protein varied from 67.5 - 75.9%, 0.4-4.7%, 0.204-0.985% , 2.4-3.9% and 0.523-1.791%, respectively, in the tamer stage of 20 different date varieties collected from four regions (Al-Madina Almunawara, Al-Hassa, Riyadh, and Gatif) of Saudi Arabia. The presence of these compounds in dates makes it suitable for the growth and productivity of the yeast and bacteria used in vinegar production.

Vinegars are the result of an alcoholic fermentation of starchy or saccharine materials followed by an acetification of the fermented liquore. It can be manufactured from almost any product capable of yielding alcohol by fermentation. Fruits such as apples, grapes, pears, peaches, dates, figs, oranges; hydrolyzed starchy materials and wine may serve as raw materials. Thus, in France, Italy, Spain and Greece, wine vinegar is popular but in England most of the vinegar made comes from malt. In U.S.A., cider vinegar is popular but in Canada, spirit vinegar produced from ethanol is the major product [4].

In Al-Hassa Food Industries Company (Date Processing Plants), Al-Hassa, Saudi Arabia, the surplus dates, which are soft and of low quality, are used in the production of vinegar. Production of venigar from dates was previously studied on the laboratories scale by some authors [5-7]. This paper describes the experimental studies on the production of vinegar on industrial scale from the surplus date juices by using symbiotic fermentation by using *S. bayanus* or *S. cerevisiae* for fermenting the sugars in date juice into ethanol and then using *Acetobacter aceti* to oxidize ethanol into acetic acid.

Materials and Methods

Date samples: Date samples used in this study were the substandard and surplus dates which are soft and of low quality dates. These dates were a mixture of all date cultivars grown in Al-Hassa region (Saudi Arabia) such as Khalas, Raziz, Shabibi and Shashi.

Preparation of date juice: Date samples were cleaned and mixed with soft water in special machine. In this machine, the date - water mixture was heated at 80°C for 30 minutes and then the kernals were separated automatically. The mixed seedless dates solution was transferred into special three tanks (the capacity of each is 2000 liters). In these tanks, seedless dates solution was mixed well automatically and allowed to stand at room temperature. Afterwards, the date extract was decanted by special decanter, further clarified and then microfiltrated. The total soluble solids concentration (Brix) of the date extract (juice) was adjusted to 20°.

Preparation of yeast inocula: For each fermentor, 500 gm of yeast (*S. cerevisiae or S. bayamus*) spores were mixed well with 170 liters of microfiltrated date juice in special small tank under sterilized conditions and 250 gm of sucrose were added with continous stirring for 30 minutes at 30°C. Afterwards, this inoculum transferred automatically into the fermentor.

Alcoholic batch fermentation: Volumes of 1700 liters of the clarified and microfiltrated date juice with 20° Brix (18% total sugars concentration) were transferred into each of six special fermentors (the capacity of each is 2000 liters). The pH was adjusted at 3.5 by using citric acid. Fermentation temperature was 30°C. Three fermentors were inoculated with the prepared inoculum suspension (170 liter for each fermentor) of *Saccharomyces cerevisiae* while the other three fermentors were inoculated with *S. bayamus*. All fermentors were subjected to a very well stirring. The fermentation was continued until completed.

At the end of fermentation, sugar concentration in fermented date juice was tested by using Glukotest (strips for determination of sugar concentration in urine, manufactured by Boehringer Mannheim Tamer Co. Ltd, Saudi Arabia; under Liceonce from Boehringer Mannheim GmbH, Germany). The results revealed that the sugar concentration was less than 2.8 mmol/L.

Semicontinous Acetification (Vinegar production): The fermented date juice was microfiltrated and transferred into a storage tank. Bionyl 100 (0.1%) and Bionyl 60 (0.06%) produced by Chansard Fermentation 87 rue Gorge De Loup - 69009, Lyon, France were added into this juice as nutrients for acetic acid bacteria and stirring well. Volumes of 1400 liters of this prepared juice were transferred into special acetator (their capacity is 6000 liters). About 2800 liters of microfiltrated soft water were added until the alcohol concentration reached 4.1%. The acidity was adjused to 6.54% by using synthetic acetic acid (food grade). Frings (0.1%) was added (as bacterial food for acetator produced by Heinrich Frings GmbH & Co. KG - Jonas - Cohn – Str. 9 - D - 53115, Bonn, Germany) into the acetator and then inoculated with a selected isolate of *Acetobacter aceti*. The alcohol was oxidized to acetic acid by the bacterial culture. The reaction was highly exothermic and requires cooling. A temperature controller maintains a constant temperature of 29 $\pm$ 1°C. The acetator was extremely well and evenly aerated, and was provided with a mechanical foam destroyer. Acetification proceeded until the alcohol concentration dropped to nearly 0.5%. As soon as this value was reached, nearly one third of the produced vinegar (1400 liters) was discharged and solwly replaced by the same volume of the previously prepared mash which had 9-10 % alcohol and less than 0.5% acetic acid from the storage tank. Recycling the acetification took place and so on. Acetification must be run 24 hours per day. The discharged acetic acid solution (vinegar with 10.05% concentration) was treated with 0.1% Bentonite (food grade) and lefted 24 hours, and then diluted to exactly 5% concentration by using soft water, microfiltrated and bottled under complete sterilized conditions.

Microorganisms: *Saccharomyces cerevisiae* and *S. bayamus* were obtained from Laboratoire Oenotechnique, Zone industrielle BP 94-34800, Clermont L'herault, France. *Acetobacter aceti* strain was obtained from IMECA Co. BP94-34800, Clermont L'herault, France.

Analytical methods: Total soluble solids expressed as Brix° values were determined with Euromex hand Reflactometer RF 232 (Holland). pH values were measured by Microprocessor pH - mV meter pH 526. Titratable acidity was measured by titrating the samples with 0.5 N NaOH [8]. Alcohol contents were estimated by bichromate method as the following: 5 ml of sample to be analysed and 20ml of 0.5 N NaOH were transferred into a boiler and distillated the mixture until approximately 10 ml had been evaporated from the boiler. The evaporated gas was collected in a collecting flask , containing exactly 30 ml of 0.2 N potassium bichromate solution and 10 ml of concentrated sulphuric acid. If the sample contains alcohol, the colour of the solution in collecting flask turns from orange to dark green. The content of the collecting flask was transfered into 250 ml Erlenmeyer flask with addition of 100 ml distilled water, 10 ml 0.12 N potassium iodide and 3-5 drops of 5% starch solution and then titrated against 0.1 N sodium thiosulfate solution. Alcohol content (%volume) in the sample = (bichromate volume x 2- thiosulfate volume) x 0.0289. This analytical method is very sensitive to the alcohol concentration ranged from 0 to 1.5% only. If the alcohol concentration in the sample is more than 1.5%, it must be diluted to suitable concentration. This method for alcohol estimation was adopted from IMECA Co. (which supplied and installed the vinegar production line in the Company's Date Processing Plant) BP 94-3800, Clermont L'herault, France.

Results and discussion

Ethanol production

The results shown in Tables (1and 2) represent the fermentation of 18% sugars date juice by *Saccharomyces cerevisiae* and *S. bayamus* at 30°C and pH 3.5. The results revealed that *S. bayamus* was more active than *S. cerevisiae*, where *S. bayamus* completed the fermentation process in 72 hours (Table 2) while *S. cerevisiae* completed it in 96 hours (Table 1). Also, The average fermentation rate by *S. bayamus* was 2.33° Brix consumed per 12 hours compare with 1.75° Brix for *S. cerevisiae*. At the end of fermentation, ethanol produced was 9.161% (50.89% of total sugars in used juice) and 9.104% (50.58% of total sugars in used juice) by using *S. bayamus* and *S. cerevisiae*, respectively (Tables 1 and 2). This result is nearly similar to those recorded in many studies [4,7 & 9]. Prescott and Dunn [4] reported that each 100 parts of sucrose gave rise to 105.4 parts of invert sugar, which in turn by fermentation yielded 51.1 parts of ethanol. Al-Talibi *et al.* [9] found that the alcohol yield by *S. cerevisiae* fermented 20% pure sugar solution or 25% sugars Iraqi date juice was reached 9.96%. Mehaia and Cheryan [7] found that the ethanol produced by *S. cerevisiae* was 48.27% and 47.00% of the total sugar concentration when used 9.8% and 13.83% sugars date juice in batch fermentation, respectively. Also they reported that the productivity was ranged from 0.28 to 0.4% ethanol production per one hour in batch fermentation using the two sugar concentrations (9.8% and 13.83%) date juice. This is better than those recorded in our study where we observed that the productivites ranged from 0.578 to 1.156% and 0.723

to 1.734% ethanol produced per 12 hours by *S. cerevisiae* and *S. bayamus*, respectively (Tables, 1and 2).

Table 1. Fermentation of the prepared date juice containing 18%sugars to ethanol by *Saccharomyces cerevisiae* at 30°C in submerged fermentors

Time (hour)	Brix° (M±SD) *	Rate of fermentation (B°/12hours)**	Density (M±SD)	Alcohol(%) (M±SD)	Productivites ****
0	20 ± 0.5	-	1.083 ± 0.015	NDa**	---
12	19 ± 0.7	1.0	1.072 ± 0.011	ND	---
24	17.5 ± 0.7	1.5	1.059 ± 0.013	3.179 ± 0.058	---
36	16 ± 0.5	1.5	1.048 ± 0.013	3.757 ± 0.058	0.578
48	14 ± 0.5	2.0	1.030 ± 0.012	4.913 ± 0.072	1.156
60	12 ± 0.5	2.0	1.016 ± 0.006	5.925 ± 0.043	1.012
72	10 ± 0.4	2.0	1.008 ± 0.008	6.936 ± 0.043	1.011
84	8 ± 0.4	2.0	1.000 ± 0.005	8.092 ± 0.058	1.156
96	6 ± 0.5	2.0	0.996 ± 0.005	9.017 ± 0.072	0.925
108	6 ± 0.3	0.0	0.995 ± 0.003	9.104 ± 0.014	0.087
120	6 ± 0.3	0.0	0.995 ± 0.002	9.104 ± 0.014	-

* M ± SD = The mean values ± standard deviation based on three replicates.
** B° /12 hours = The mean value of Brix° consumed per 12 hours, the average was 1.75° Brix.
*** ND = Not detected .
**** Productivites = The mean values of alcohol % produced per each 12 hours .

Table 2. Fermentation of the prepared date juice containing 18% sugars to ethanol by *Saccharomyces bayamus* at 30°C in submerged fermontors

Time (hour)	Brix° (M±SD) *	Rate of fermentation (B°/12hours)**	Density (M±SD)	Alcohol(%) (M±SD)	Productivites ****
0	20 ± 0.5	-	1.083 ± 0.015	ND***	---
12	18 ± 0.7	2.0	1.063 ± 0.010	ND	---
24	15.5 ± 0.5	2.5	1.040 ± 0.013	3.757 ± 0.058	---
36	13 ± 0.5	2.5	1.027 ± 0.015	5.491 ± 0.087	1.734
48	10.5 ± 0.6	2.5	1.012 ± 0.012	6.936 ± 0.087	1.445
60	8 ± 0.4	2.5	1.002 ± 0.008	8.381 ± 0.072	1.445
72	6 ± 0.3	2.0	0.996 ± 0.004	9.104 ± 0.058	0.723
84	6 ± 0.3	0.0	0.995 ± 0.003	9.161 ± 0.014	0.057
96	6 ± 0.3	0.0	0.995 ± 0.002	9.161 ± 0.014	---

* M ±SD = The mean values ± standard deviation based on three replicates.
** B° /12 hours = The mean value of Brix° consumed per 12 hours, the average was 2.33° Brix.
*** ND = Not detected.
**** Productivites = The mean values of alcohol % produced per each 12 hours.

On the other hand, karuwanna *et al.* [10] fermented juice (with 20° Brix) prepared from pineapple canning waste by using *S. cerevisiae* at pH 3.5-4.0 and required for completing the fermentation a longer period than those recorded in this study by each of *S. cerevisiae* or *S. bayamus*. They reported that the period for the complete fermentation

was 15 days with about 10% ethanol production. This is probably due to the difference in the juice sources . these results revealed that the date juice is more suitable than pineapple juice for growth and productivity of the yeast strain .

Vinegar production

The prepared mash was subjected to aerobic submerged acetification and at the beginning of acetification cycle, the alcohol concentration was 4.1% and the acidity was 6.54%. The bacterial inoculum required 4 hours for adaptation on this medium and 42 hours to complete the acetification. The average acetification rate was 0.167% acetic acid per two hours (Table 3). The acetification period and rate of acetification which obtained in this study were better than those recorded in previous studies [10 and 11]. Karuwanna *et al.* [10] used two methods (stationary and shaking at 100 rev/min at room temperature) for acetification of wines produced from pineaple canning waste juice had 10% ethanol and recorded the production of 6-7% acetic acid after 4 days (the production rate was 0.125-0.146% /2hours) by shaking method and the same amount of acid after 5-6 days (the production rate was 0.097 - 0.1% /2 hours) by stationary method. Tewari *et al.* [11] found that *Acetobacter aceti* NCIM- 2094 produced 4.5% acetic acid after 69 days acetification of sugar cane juice (the production rate was 0.0054% /2 hours) under stationary conditions. These results indicated that, the mash prepared from date juice is quite suitable medium than those prepared from pineapple or sugar cane juice for acetification.

Table 3. Production of vinegar from the mash containing 4.1% alcohol and 6.54% acetic acid by using *Acetobacter acet*: at 29±1°C in submerged acetator

Time (hour)	Alcohol (%)	Acidity (%)	*RA (%/2hours)
0	4.100	6.54	-
2	4.100	6.54	0.00
4	4.100	6.54	0.00
6	4.046	6.60	0.06
8	3.902	6.69	0.09
10	3.757	6.84	0.15
12	3.613	7.02	0.18
14	3.468	7.17	0.15
16	3.266	7.35	0.18
18	3.092	7.50	0.15
20	2.948	7.65	0.15
22	2.803	7.80	0.15
24	2.717	7.95	0.15
26	2.543	8.13	0.18
28	2.370	8.40	0.27
30	2.081	8.67	0.27
32	1.850	8.94	0.27
34	1.445	9.24	0.30
36	1.156	9.51	0.27
38	0.867	9.81	0.30
40	0.636	9.93	0.12
42	0.535	9.99	0.06
44	0.512	10.02	0.03
46	0.506	10.05	0.03

*RA(% / 2 hours) = Rate of acetification (precent of acetic acid produced per two hours).

On the other hand, Mehaia and Cheryan [7] reported that the acetification rate of a batch fermentation by *Acetobacter aceti* in fermented date juice containing 4.7% ethanol was 0.5 g L^{-1} h^{-1} (=0.1% / 2 hours) while it of continuous acetification of the fermented date juice in the membrane recycle bioreactor at a dilution rate of 0.06 / hour was 2.8 g L^{-1} h^{-1} (= 0.56% / 2 hours).

At the end of acetification cycle, the acetic acid concentration reached 10.05% while the alcohol concentration decreased to 0.506%. This means that the amount of acetic acid produced was 10.05 - 6.54 = 3.51% while the amount of alcohol consumed was 4.1-0.506 = 3.594%. The yield of acetic acid was 97.66 ml from each 100 ml ethanol. Therefore, the acetification efficiency was 74.88% of the theoretical value (where, theoretically each 180 parts of reduced sugar must give 92 parts of ethanol, which must give 120 parts of acetic acid [4]). This decrease in acetic acid production could be due to loss of ethanol and acetic acid by evaporation, utilization of the acid by the bacteria and overoxidation of acetic acid. .This result is similar to those recorded in many other studies [4-7 and 12]. Prescott and Dunn [4] reported that 50 to 55 parts of acetic acid may be obtained from 100 parts of sugar (nearly 74.996 to 82.496% of the theoretical values) under favorable fermentation conditions. Gupta *et al.* [5] converted the alcohol in wine produced from palm sap to vinegar by submerged fermentation with *Acetobacter* sp. and reported that the yield of vinegar after 96 hours of acetification was 77.4%. Grewal *et al.* [12] found that the acetification efficiency of *Acetobacter aceti* was 83.9% when used apple wine and 84.7% when used grape wine as mash. Grewal and Tewari [6] subjected the wine produced from plums, which had 10.26 – 10.37% alcohol, to acetification using *A. aceti* and found the acetification efficiency was 81.5%. Mehaia and Cheryan [7] found that the acetification efficiency of a batch acetification by *A. aceti* of fermented date juice containing 4.7% ethanol was about 65% of the theoretical value; while it of continuous acetification of the fermented date juice in the membrane recyle bioreactor at a dilution rate of 0.06/hour was about 76% of the theoretical value.

Conclusion

The result obtained in this study revealed that the substandard and surplus dates are a very good substrate for ethanol and vinegar production by fermentation. The strain used of *S. bayamus* is more active than *S. cerevisiae* in ethanol fermentation of the date juice. Fermentation process was completed in 72 hours and converted 50.89% of the total sugars in date juice to ethanol. The strain used of *Acetobacter aceti* completed the acetification cycle through 46 hours and converted 97.66% of ethanol in the fermented juice to acetic acid with an average of acetification rate was 0.167% acetic acid produced per two hours. Acetification efficieny reached 74.88% of the theoretical value. Therefore, each 100 parts of total sugars in a date juice yield 50.89 parts of ethanol by using *S. bayamus* in submerged fermentor and each 100 ml of the produced ethanol yield 97.66 ml of acetic acid by using *A. aceti* in submerged acetator under the conditions used in this study. The concentration of vinegar produced by this method was more than 10%, it diluted to produce the commercial natural vinegar with 5% concentration.

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جدوى إنتاج الخل من عصير التمر

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ملخص البحث. تم في هذا البحث استخدام تمور الدرجة الثالثة والتي لا تصلح للتعبئة أو عمل العجائن أو الدبس في تصنيع عصير التمر حيث كانت تمزج بالماء النقي وتسخن عند درجة ٨٠م لمدة ٣٠ دقيقة في ماكينة خاصة ثم يفصل منها النوى أوتوماتيكياً ثم يمزج سائل التمر الناتج والخالي من النوى في تنكات خاصة ثم يرسب ويرشح وينقي العصير الناتج والذي يكون تركيز المواد الصلبة الذائبة به في حدود ٢٠ بركس وتركيز السكريات حوالي ١٨٪.

هذه الكمية من السكريات يتم تحويلها إلى كحول اثيلي عن طريق عملية تخمر في مخمرات خاصة ذات مستوى عالي جداً من التقنية عند درجة حرارة ٣٠م وأس هيدروجيني ٣.٥ باستخدام نوعين من الخمائر هما *Saccharamyces 'cerevisiae '.S. bayamus*. أثبت البحث أن خميرة *S.bayamus* أكثر كفاءة من خميرة *S.cerevisiae* في إتمام عملية التخمر حيث أن الأولى أنهت العملية في ٧٢ ساعة بينما الثانية احتاجت إلى ٩٦ ساعة لإنهاء العملية. كذلك كان معدل التخمر بالخميرة الأولى أفضل منه بالثانية. بعد انتهاء عملية التخمر كان تركيز الكحول الإثيلي باستخدام *S.bayamus* هو ٩.١٦١٪ (٥٠.٨٩٪ من تركيز السكريات) أما تركيزه باستخدام *S.cerevisiae* كان ٩.١٠٤٪ (٥٠.٥٨٪ من تركيز السكريات).

هذا وقد خضع العصير المتخمر الناتج لعملية أكسدة هوائية بواسطة عزله من بكتريا *Acetobacter aceti* عند ١+٢٩م في مخمر خاص لإنتاج الخل ذو تقنية عالية جداً . كان متوسط معدل عملية الأكسدة وإنتاج الخل هو ٠.١٦٧٪ حمض خليك ناتج كل ساعتان. وقد تم إنهاء عملية الأكسدة في ٤٦ ساعة. بعد إنهاء عملية الأكسدة وصل تركيز حمض الخليك إلى ١٠.٠٥٪ وتركيز الكحول إلى ٠.٥٠٦٪ وبذلك تصل كفاءة عملية التحول حوالي ٧٤.٨٨٪ من المعدل النظري المعروف. يتم معالجة الخل الناتج بمادة البنتونيت (تركيز ٠,١٪) ثم يخفف الى تركيز ٥٪ خل ثم يرشح ويتم تعبئته تحت ظروف تعقيم .