Journal of Kerbala University , Vol. 11 No.3 Scientific . 2013

Vinegar Production from Low Cost Iraqi Dates

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Abstract

Low cost 'Khastawi' dates was fermented at 30 ºC for 48 days through spontaneous (without artificial inoculation) and simultaneous unique cycle process according to the overall traditional procedure used in Iraq. The levels of ethanol, acetic acid and total sugar during the production of vinegar were determined by chromatographic, titrimetric and refractometric methods, respectively.

The spontaneous fermentation of the clarified juice produced 4.02% (w/v) of acetic acid and 0.176% (w/v) ethanol after 33 days of fermentation. The total soluble sugar of the juice was reduced from 17.80 to 6.44 (w/v), which means that only 63.28% of the total sugars present at the medium were utilized by the spontaneous fermentation processes. These results indicate that 2 kg of low quality dates can yield 5 L of 4% (w/v) acid, which is economically valuable.

The industrially manufactured vinegars (except Al Badawi from Iraq) presented minimum acidity of 4.00%, value that is demanded by most international legislation, whereas all artisanally manufactured vinegars, showed lower acid percentage (0.90-3.24% w/v). On the other hand, the majority of both types had alcohol contents between 0.001 and 2.877%. Based on the concept of *Al Istihlak* (assimilation or consumption) most halal certifying bodies accept small amounts of inherent alcohol.

Keywords: Dates; Spontaneous fermentation; Gas chromatography; Ethanol; Acetic acid.

المستخلص

تم اختيار تمر خستاوي منخفض السعر وجرى تحضير عصيره بنسبة كيلوغرامين من التمر إلى خمسة ألتار من الماء وترك ليتخمر طبيعيا في درجة 30°C لمدة 48 يوما بعد إضافة خل قديم إليه وفقا للعادات المتوارثة في العراق.

جرى متابعة مستوى الكحول الاثيلي وحامض ألخليك والسكر الكلي باستخدام طرق الكروماتوغرافيا والمعايرة وقياسات معامل الانكسار على التوالي حيث بينت النتائج تكون حامض ألخليك بنسبة 4.02% والكحول الاثيلي بنسبة 0.176% وانخفاض المجموع الكلي للسكريات من 17.80 إلى 6.44% بعد مرور 33 يوما. تدل هذه النسب على أن من الممكن إنتاج خمسة ألتار من الخل باستخدام كيلوغرامين من تمور رديئة النوعية وهي عملية مجدية من الناحية الاقتصادية ويمكن تحسين الجدوى بشكل كبير باستخدام آليات تخمير سريعة.

من جهة أخرى لوحظ أن جميع أنواع الخل التجاري الموجودة في الأسواق (باستثناء خل البدوي العراقي) تحتوي على مالا يقل عن 4.00% من الحامض وهي نسبة مقبولة عالميا. أما العينات المحضرة محليا والتي تتوفر في محلات بيع المخللات فان نسب الحموضة فيها تتراوح بين 0.90 و3.24% وهي نسب واطئة غير مقبولة عالميا. وقد تبين أيضا أن سبعة وعشرين عينة من الخل التجاري تحتوي على مقادير ضئيلة من الايثانول تتراوح بين 0.001 و 2.877%, وفي هذا الصدد اجمع فقهاء المسلمين (بناء على مفهومي الاستحالة والاستهلاك) على إن تخلف مقادير صغيرة من الايثانول في الخل المحضر طبيعيا مسموح به طالما لا تسبب تأثيرا مسكرا.

Introduction

Iraq has historically been one of the major date-producing countries in the world. In the 1980s and mid-1990s, Iraq was consistently among the top five date-producing countries in the world and often ranked number one in terms of production by volume. Into the 1990s, Iraq had 22 million date palms planted over 120,000 hectares. According to the most up-to-date information available, in 2001 the top five governorates in terms of date production by volume are Babil (Babylon), Karbala, Diyala, Baghdad and Basrah[(1)].

The “Date Strategy Report” indicated that 14.3% of the total production is wasted and 35.7% of the date crop (the lowest-quality dates) are sold as animal feed to the dairies and sheep herders[(1)]

Because of the high concentration of sugars in the date[2], it is important to develop new and more attractive uses of these sugars (especially low quality dates) to produce ethanol and natural vinegar at low cost and in as short a time as possible **.**

Vinegar may be defined as a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic fermentation. The vinegar bacteria are members of the genus *Acetobacter* and characterized by their ability to convert ethyl alcohol (C_2H_5OH) into acetic acid (CH_3CO_2H) by oxidation. Vinegar can be produced from various raw materials like fruit juices, distilled alcohol, wine, and any kind of alcoholic solution by several major production techniques such as the Orleans process, generator process and submerged acidification process[3].

Generally, all vinegar products are solutions containing mainly acetic acid which has been reported to possess physiological effects; including antihypertensive properties, enhancement of glycogen repletion in liver and muscle, reduction of serum cholesterol and triacylglycerols[4,5].

Unlike distilled and artificial vinegars, fruit vinegar contains other nutrients such as carbohydrates, amino acids and peptides, vitamins and minerals and non-nutrient substances, such as carotenoids, phenolic compounds and some other pigments. The composition of fruit vinegars can vary due to the different sources of raw materials they are derived from[6].

Recently intensive results on the fermentation of guava, banana, onion, kiwi, orange, cajá (*spondias mombin* L.), cashew[7], strawberry and persimmon surpluses[8], Sweet sorghum[9], Mango[10, 11] and sugarcane[12] have been reported. In contrast, there is a general lack of information concerning the composition of date vinegar in the scientific literature[13], although traditional vinegar produced from fermented dates is very popular in Iraq and many other Mediterranean countries. Only limited overviews from Malaysia[14], Algeria[15] and Egypt[2] are available.

The purpose of this work is to evaluate the concentration of ethanol, acetic acid and total sugar in spontaneously fermented date's juice compared with thirty one commercial and artisanally manufactured vinegars currently available in Babil-Iraq.

Materials and Methods

Materials

Mature khustawi dates (the leading soft date in Iraq), were selected from the local markets in Babil-Iraq. All damaged and spoiled ones were eliminated, especially those in which the process of fermentation had already started. The dates were washed by submerging them in chlorine water for about 30 minutes in order to remove dirt and microorganisms, after that washed with running water to remove the residual chlorine.

Thirty one samples of commercial vinegar were collected in 2012 from local markets in Babil-Iraq and subsequent chemical analyses were conducted. Of the 31 vinegars tested, seventeen samples are industrially manufactured and fourteen are homemade dates vinegars (artisanally manufactured and unrecorded) supplied by pickle sellers. The industrially manufactured vinegars had their source from Iraq, Syria, Saudi Arabia, Lebanon, Turkey, USA and Jordan. Each sample was filtered and centrifuged at 4,000 rpm for 20 min in Janetzki centrifuge before the analysis. Three samples of each brand were analyzed. All chemicals and solvents used were analytical grade and purchased from Sigma Chemical Co., Ltd (USA) and Sigma Aldrich Co., Ltd (Germany).

Production of Date Vinegar

Samples with seeds were pitted, crushed and blended with distilled water in a ratio of 2:5 (w/w sample to water) to form a suspension. The suspension was filtered through a piece of Muslin cloth, aliquoted (2.00 L) into 2.25 L plastic bottles, capped and incubated at 30 ºC. The date juice has been submitted to spontaneous (without artificial inoculation) and simultaneous fermentation in unique cycle for 40-50 days according to the overall traditional procedure used in Iraq. Non-pasteurized old vinegar 5.00% (w/v), provided by a vinegar industry located in the city of Babil was added at the start of fermentation.

Determination of ethanol, acetic acid and total sugars in vinegars

At appropriate time intervals, samples from the homemade fermenter were centrifuged at 4000 rpm for 20 min. The supernatant was used to determine the total acidity, pH, total sugars and ethanol. Total acidity was evaluated by titration with standardized solution of

0.1 M sodium hydroxide, using phenolphthalein as indicator and the results were expressed as acetic acid content. The acidity of vinegar is mainly due to the presence of acetic acid and smaller amounts of other acids come from raw materials or are generated by the fermentation[16]. The pH values of all the vinegars were determined through a pH-meter (Model 240, WTW) previously calibrated with buffers at pH 4 and 7.

The total sugar contents were evaluated during the whole fermentation process with Abbe refractometer (Novex, 98.490, Holland)[7].

Ethanol determination in vinegar was carried out by a Shimadzu-2010 gas chromatograph[17] equipped with flame ionization detector, an electronic processor/integrator and Zebron column (ZB-FFAP, length 30 m, I.D. 0.25 mm, film thickness 0.25 μm). The operational temperatures for the injector, detector and column were 120 ºC, 120 ºC and 90 ºC respectively. Ultra-pure nitrogen carrier gas with a flow velocity of 2.4 mL/min and sample volumes of 1μL injected into split mod (1:20) were utilized. All determinations were executed by the internal standard method[18]. Ethanol 10 g/L was mixed with 10 g/L butanol in various ratios (ethanol:butanol = 15:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, and 1:15). A linear regression line was generated with the GC peak area ratio of ethanol to butanol against the concentration ratio of ethanol to butanol. Ethanol content in date vinegars was calculated using the slop of the regression line. ANOVA analysis was performed on the data using SPSS version 17.0 for windows. Significance was accepted at $P< 0.05$.

Results and Discussion

The reliability of Gas Chromatography

Figure 1 shows the chromatogram of ethanol and butanol as an internal standard under isothermal temperature program at 90°C. Both peaks are sharp and well resolved. The calibration curves between ratios of ethanol peak area per butanol peak area and concentrations of ethanol showed a good linear correlation with $\mathbf{r^2} = 0.998$ with a detection limit of 0.001% w/v.

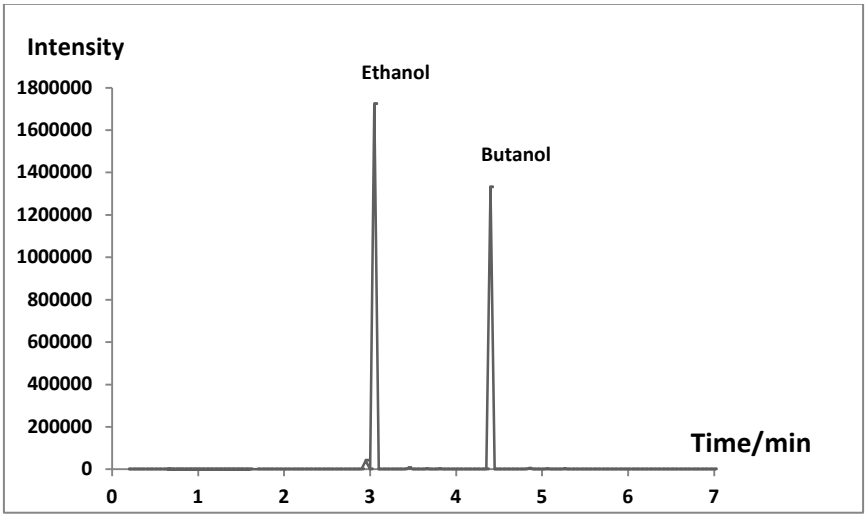

Figure1. Gas chromatographic peaks of ethanol and butanol as the internal standard, obtained from a sample of vinegar contained 1.770% (w/v) ethanol.

Spontaneous fermentation of date juice

Figure 2 demonstrates the course of variation of ethanol and production of acetic acid as functions of fermentation time. The alcoholic fermentation curve indicated that the highest rate of ethanol production occurred after 15 days of fermentation. Ethanol is mainly transformed into

vinegar with a small amount converted into esters during storage, giving the vinegar the characteristic flavor of the raw material utilized in the acidification process.

The profile of this kinetic curve shows that the maximum concentration of acetic acid produced {4.02% (w/v)} was reached at about 33 days of fermentation. After this time, the production of vinegar started decreasing probably due to the oxidation of acetic acid by oxygen present in the fermentation medium when the concentration of alcohol was very low. In general the spontaneous fermentation of vinegar by the simple batch process is generally slow and requires 4 to 5 weeks for a complete fermentation.

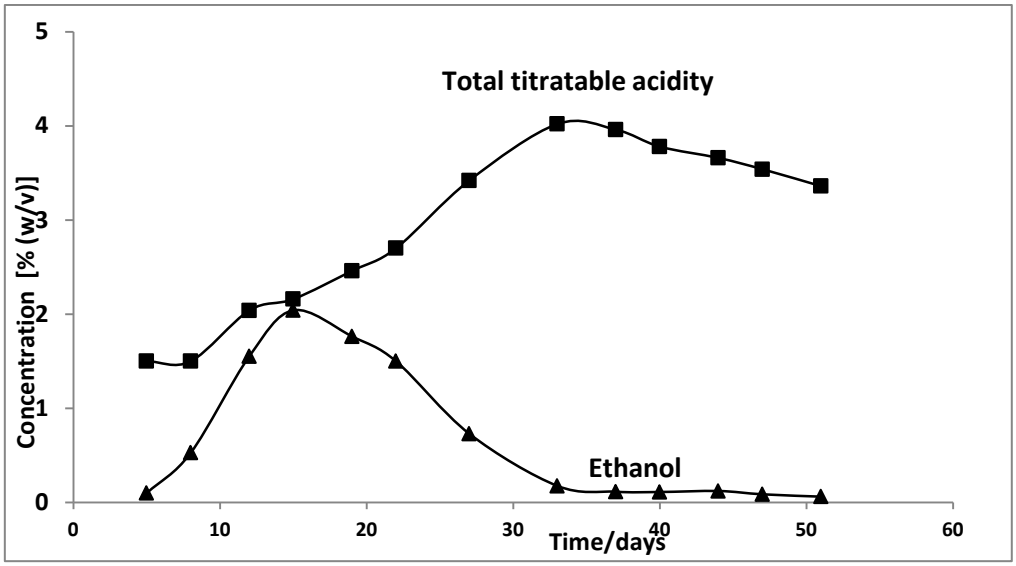

Figure 2: Vinegar production kinetics for acetic fermentation of Iraqi dates at 30 ºC

Fermentation yield calculation

All sugars in dates consist of a mixture of sucrose (C_{12} H_{22} 0_{11}), glucose (C_6 H_{12} 0_6) and fructose (C_6 H_{12} 0_6) of which the latter two are the derivations of sucrose after inversion. Total sugars (at the tamr stage) on a dry weight basis for the more known varieties in Iraq are around 73.8% [13]**.** Generally, most if not all sucrose could be inverted into glucose and fructose by the enzyme invertase at the stage at which they are consumed**[13].** During the alcoholic fermentation of sugars with the utilization of yeast, ethanol and carbon dioxide are the main products obtained in equimolar proportions. This mechanism was quantified for the first time by Gay Lussac, where 100 g of glucose yields 51.1 g of ethanol and 48.9 g of carbon dioxide. The theoretical yield of 51.1% in weight is known as Gay Lussac coefficient, and is a basic data for efficiency conversion. The acetic fermentation efficiency was calculated from the stoichiometry of the conversion reaction of ethanol to acetic acid, where 1 g ethanol yields 1.304 g acetic acid[19]**.** Based on these calculations, 100 g of glucose theoretically yields 66.63 g of acetic acid.

In the present work, initially date's juice had 17.80% (w/v) total sugars concentration, which is suitable for yeast cells thrive. After 33 days, the concentration of sugars, determined at the end of the juice fermentation was reduced to 6.44% (w/v). This means that only 63.28% of the total sugars (11.36 % (w/v)) present at the medium were utilized by the spontaneous fermentation processes. Theoretically the converted sugar should yield 7.51% (w/v) acetic acid. Practically the highest concentration reached in the present work, was only 4.02% (w/v) acetic acid, which means that the yield obtained from spontaneous fermentation was rather low (53.53%). However, the acetic fermentation yield of five replicates of the experiment indicates that approximately 5 L of vinegar with 4% acetic acid (w/v) were obtained from 2 kg of low quality Khustawi dates which means that the process was still economic.

Commercial Vinegars

Table 1 shows the physiochemical parameters of 17 industrially manufactured and 14 artisanal manufactured vinegars currently consumed in Babil-Iraq. In all the vinegars samples analyzed, acetic acid was the most abundant component (1.20-6.36% w/v) followed by ethanol concentration (0.000-2.877% (w/v).

The industrially manufactured vinegars (except Al Badawi from Iraq) presented minimum acidity of 4.00%, value that is demanded by the American legislations and confirmed in the labels of 90% of the samples[20]. In contrast all artisanally manufactured vinegars, showed low acidic percentage (0.90-3.24%) which is not permitted in several countries such as USA and Brazil[7,20].

Unfortunately, artisanally manufactured vinegar is subject to different falsifications, such as adding water, salts, organic acids and even mineral acids resulting in health problems and medical complications (to be published). Sulphuric, nitric and hydrochloric acids are used to give a false strength and burned sugar and ether to give color and flavor.

Finally, 27 commercial vinegars had alcohol contents between 0.001 and 2.877% w/v. The highest concentration was detected in Mayas (2.877% w/v) and Sham gardens (2.526% w/v), whereas the lowest ethanol concentration (≤ 0.001% w/v) was recorded in 8 samples probably due to their artificial origin.

Muslim scholars generally agree that when khamr is added to the vinegar during production, then such vinegar will be haram while if the minute amount of alcohol in vinegar is not due to alcohol added as an ingredient, but is the remnant after the natural process of transformation, then the vinegar will be halal[21,22]. Permissibility of small amount of alcohol in vinegar is based on the concept of *Al Istihlak* (assimilation or consumption), that is, if a small amount of a prohibited substance mixed with a dominant permissible substance and the prohibited substance loses all its attributes such as taste, color and smell, this substance loses the qualifications of being impure[14, 23]. In the light of these directives our samples are considered to be halal and pure.

Table 1: Total titratable acidity, Ethanol and total sugar in commercial vinegars from different sources.

Name of Vinegar	Type	Source	Total titratable Acidity% (w/v)	Concentration of ethanol % (w/v)	Total sugar content (w/v)
Commercially available vinegars (industrially manufactured and taxed)					
Albaldawi	Dates	Iraq	2.85	0.003	2.26
Albaldawi	Artificial	Iraq	4.56	0.002	2.47
Hello	Artificial	Jordan	4.08	0.001	2.05
Mayas	Ginger	Syria	5.04	2.877	6.42
Sham gardens	Grapes	Syria	4.63	0.501	4.90
Sham gardens	Dates	Syria	5.26	2.526	8.23
Durra	Date	Syria	4.22	1.072	7.32
Al Walaem	Garlic	Syria	4.98	0.001	3.51
Hamra	White grape	Lebanon	6.18	0.000	2.33
Hamra	Red grape	Lebanon	5.76	0.000	2.19
Jabal	Artificial	Lebanon	5.34	0.000	2.19
Alwadi alakhdar	Grapes	Lebanon	5.64	0.177	4.13
Baider	Sugarcan	K.S.A.	6.36	0.000	2.40
Zer	Apple	Turkey	5.17	0.091	4.62
Kemal Kukrer	Grape	Turkey	5.11	0.002	4.13
American garden	Artificial	U.S.A	6.12	0.1 64	2.47
American garden	Grapes	U.S.A	5.40	0.176	3.23
Artisinally manufactured and unrecorded vinegars (supplied by pickle sellers in Babil-Iraq)					
Maithem Al Tamar	Dates	Iraq	1.56	0.108	3.08
Abu Tahseen	Dates	Iraq	1.92	0.046	3.93
Dur Al Najaf	Dates	Iraq	3.00	0.007	1.29
Al Baraka	Dates	Iraq	1.86	1.129	1.01
Al Qaria Al Asria	Dates	Iraq	1.44	0.772	3.51
Ahbab Al Karar	Dates	Iraq	1.20	0.006	2.82
Al Ikhlas	Dates	Iraq	2.16	0.008	1.85
Al Khadra	Dates	Iraq	2.64	0.001	1.33
Al Kaabi	Dates	Iraq	1.26	0.021	2.61
Al Khairat	Dates	Iraq	0.90	0.097	2.19
Sharara	Dates	Iraq	1.86	0.063	4.07
Haydar Al Shemary	Dates	Iraq	3.24	0.001	0.88
Al Nahrawan	Dates	Iraq	2.70	0.047	1.43
Al Najafi	Dates	Iraq	2.88	0.134	2.12

* All averages derived from three readings with a maximum standard deviation of 5%

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