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# PRODUCING VINEGAR FROM UNMARKETABLE DATES OF THREE LIBYAN CULTIVARS USING DOUBLE STAGE FERMENTATION METHOD

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***Abstract***

*This work investigated producing vinegar from unmarketable 'Taleese', 'Athwi' and 'Hellawi' Libyan date cultivars. Sugar was extracted from 'Athwi' and 'Talees' dates by soaking in distilled water at 1:3 (w/w) for 10 hours and pressing while 'Hellawi' dates were mixed with distilled water at the similar ratio, heated at 80°C and agitated for 2 hours and pressed. Total soluble solids (TSS) of solutions obtained from the three cultivars were 16.5, 17.4 and 25.0 °Brix, respectively. Solutions were adjusted to 15.5°Brix and 8 litre of solution of each cultivar were fermented in 15L setup equipped with an airlock. Baker's yeast was added and solutions were incubated at room temperature averaging 22°C. The process lasted 9 days, afterward aerobic acetification was carried out by adding virgin date vinegar at 10% (v/v) and solutions were kept under same conditions. Weekly measurements of titratable acidity (TA) and alcohol percentage were made until alcohol content dropped below 1%. The process lasted 60 days for 'Taleese' and 'Athwi', while 'Hellawi' took additional 11 days. Both fermentations were described by linear relations ($R^2$>0.97). In the anaerobic reaction, cultivars were significantly different in their alcohol percentages and TSS but were similar in acidity and pH. In the aerobic fermentation, no significant differences in TA, pH but significant different in alcohol residues and TSS were recorded. Moreover, sensory evaluation of the three kinds of vinegar was made on acidity, taste, and acceptance; again no significant differences were recorded. Color analysis in Hue angle, Chroma and L* were also made, vinegar of the three cultivars were significantly different; 'Hellawi' vinegar was much darker than 'Athwi' and 'Talees'. Quantification of acetic acid showed its contents above 5% (v/v), representing nearly 85% of total acids. The study demonstrated the potential of producing good quality vinegar from unmarketable Libyan dates.*

***Key words*****:** *vinegar, unmarketable dates, fermentation, alcohol, acetic acid*

## INTRODUCTION

Vinegar is one of the most ancient beverages produced by fermentation. It is a food additive and preservative, also a medical and dietetic remedy [5]. It is primarily produced from carbohydrate and sugar based agriculture products, while seed carbohydrates are normally hydrolyzed for yeast fermentation, sugars of most fruits are directly fermented. In general, vinegar is produced by two means; traditional (surface), known in France as Orleans method, and the industrial submerged methods [7]. The first is a slow process but requires less investment and vinegar quality is believed to be high in quality and value [16], [11].

Economy-wise, vinegar is not a major industry comparing with other commodities produced by fermentations. Yet, there are several companies specialized in vinegar production under several varieties and brands. In Europe alone, vinegar market value in 2002 was estimated at nearly €268.7 million [24]. Unlike distilled (white vinegar) which is produced by acetic oxidation of ethanol, natural vinegar is produced by single and double stage fermentation of wide range of agricultural products such as apples, berries, guava, banana, kiwi, apricot, figs, dates, cereals, cashew fruit and many others [23]. In the first stage, sugars are converted anaerobically to ethanol by yeast, while in the second an aerobic acetous fermentation of ethanol into acetic acid is made by acetic acid bacteria belonging to the Acetobacteriaceae

family [12].

Dates are rich in sugars and with great advantages over other fresh commodities; they can be handled dry; giving benefits in transportation and storage until fermentation process is initiated. Despite that, date vinegar is not a wide spread commodity. Most production operations are limited to traditional and family small businesses, in addition to weakness in marketing and technical assistance to vinegar producers. In Mediterranean and Middle East countries, low-quality dates are generally used in vinegar production [4]. Typically, it is a spontaneous single stage fermentation process in which alcohol and acetification processes take place simultaneously within the same enclosure. However, the process is believed to be time-consuming, susceptible to reaction rendering and vinegar may contain high alcohol residues due to several uncontrollable factors [11].

Libya is an important date producing country; in fact, it attains the tenth place among the world top producers [13]. Moreover, it has a climatic advantage over many other date producers, varying from the Mediterranean in the northern part to hyper-arid Saharan proper far south, offering suitable conditions for producing soft dates in the coastal area, semidry in the middle and dry dates in far south oases [8]. Nearly 400 cultivars are reported nationwide, yet about 95 are commercially important [21]. Although most date production takes in the south, dates are transported to the northern populated region, however insignificant quantities are exported. Unfortunately, postharvest infrastructures are rather weak and therefore losses are high, mostly due to over-drying, insect and disease infestation [9]. Utilization of low-quality dates is almost absent, nevertheless, small quantities are used in date syrup (Debbis), date paste, while large volumes are abandoned or used as animal feed.

In Libyan, date vinegar is produced in small operations carried out by farmers and homemade hobbyist; mainly for their own uses, and date vinegar is not a commonly sold commodity. However, people of Wahat, Jufra, Fezzan and Ghadames oases describe their traditional method as mixing dates with water in a clay made enclosure called ('Tass') at ratios between 1:1 and 1:3 (w/w), some table salt is added, 'Tass' mouth is closed with fabric, and kept in a warm place for about 45 days. The process is generally carried out in autumn when warm conditions prevail. In fact, it is an inherited process through generations, and the product is known as the traditional date vinegar. However, prior to carrying out this work, samples were collected from Gadames and Jufra, and analyzed for titratable acidity and alcohol residues at the postharvest laboratory (PL), Faculty of Agriculture, Tripoli University, results showed low titratable acidity (<2.5%) and alcohol residues as high as 4.41% (unpublished data). It is apparent that local traditional date vinegar is very much doubtful, especially in alcohol residue content. It is quite evident that using single stage spontaneous fermentation process and local methods were mostly responsible for high alcohol residues and low acidity observed in the samples.

Studies on date vinegar in Libya are scarce, the only investigation reported producing vinegar from local dates was published [9], the work was carried out at our laboratory and dealt with producing vinegar from low-quality dry date cultivar 'Tasfer'. The study recommended extracting sugars and using other cultivars. Therefore, the current work investigated the potential of using double stage fermentation method for producing vinegar from low-quality dates of three Libyan date cultivars, 'Talees', 'Athwi' and 'Hellawi'. Extraction of sugar solution its efficiency and its properties were targeted, fermentation performance and reactions behavior are also investigated. Also, quality evaluation of vinegar produced from the three cultivars was made.

## MATERIALS AND METHODS

### Data

'Talees' and 'Athwi' dates were collected from a public market located at about 150km south west of Tripoli. Apparently, they were transported from Fezzan region in an unrefrigerated pickup, fairly dry, handled in

jute bags and sold in bulk. Moreover, they were unsorted, unclean mixed with debris, soil, damaged fruits, and pits. In such state, they are considered as cull; commonly sold for making date syrup (Debbis) and date paste. Samples were transported to the PL, they were sorted, infested and damaged units were eliminated, washed with water, dried and kept in a walk-in cold room at 0˚C (±0.5) until experimental procedures were carried out. Moisture content of the two dry cultivars was determined using the standard oven method. Samples were dried in an oven at 105 ˚C for 24 hours and water contents were determined on the wet basis at 13.6% and 14.3% for 'Talees' and 'Athwi' dates, respectively. On the other hand, 'Hellawi' dates at 'Rutab' stage previously had been collected from an orchard near Tripoli and were kept in a freezer at -18 ˚C.

**Sugar Extraction**

'Talees' and 'Athwi' dates were taken from the cold room, given time to warm up, weighed 3kg of each cultivar, mixed with 9 liters of distilled water and kept at room temperature for 10 hours. Afterwards, were mechanically blended until a thick solution was formed, then press filtered and clear solutions were collected. Volumes of solution were determined and their total soluble solids (TSS) were also measured using handheld digital refractometer (Model PAL-α, ATAGO Co, Ltd, Tokyo, Japan). TSS of 'Talees' and 'Athwi' solutions were 18 and 15.5˚Brix, respectively. For 'Hellawi' dates, since it is a soft cultivar, dates were removed from the freezer, thawed and mixed with distilled water at the similar ratio, heated at 80˚C with continuous agitation for 2 hours, then pressed, TSS was determined at 25 ˚Brix.

**Anaerobic Fermentation**

For obtaining comparable results, solutions of the three cultivars were adjusted to similar TSS (15.5 ˚Brix ±0.5). A setup consisted of a 15-liter plastic bottle equipped with an airtight lid was used as a fermenter. For securing airtightness, a brass tire valve was installed on the lid. A plastic pipe was mounted between the valve from one end and a vessel filled with water from the other, serving as an airlock. Moreover, for assuring anaerobic conditions headspace air constituents of oxygen and carbon dioxide were measured using portable $CO_2$-$O_2$ gas analyzer (Model CANAL120 $O_2$ & $CO_2$ Gas Analyzer, EMCO Packaging Systems Ltd, Kent, CT14 0BD, UK). 8 liter of solution of each cultivar were filled in the fermenter, an inoculum of commercial baker's yeast was prepared by adding 200mg/L of dry yeast to 200 ml of warm solutions from each cultivar, stirred well, incubated at 27˚C for 20 minutes and added. The three fermenters were periodically checked until $CO_2$ bubbling stopped; giving a clear sign of the anaerobic reaction end. Afterwards, the three fermenters were opened and aerated; samples were taken for analyzing alcohol content, titratable acidity (TA), pH and TSS. Measurements were made in triplicates.

**Acetification Process**

Virgin date vinegar (unsterilized) was added to the fermented content at 10%, fermenter opening was covered with a fabric and kept at previous stage conditions. Samples were weekly withdrawn and analyzed for titratable acidity, alcohol content and pH using procedures reported in our previous investigation [9].

**Acetic acid content**

Acetic acid percentages in vinegar samples were determined using high-performance liquid chromatography HPLC system (PerkinElmer, 200). The HPLC is equipped with Supelco C18 column, 5µm, 250m, 4.6 mm. Also, it is equipped with a Diode Array Detector (DAD), an auto sampler, and Total Chroma Software. Analysis conditions used were similar to conditions reported by [22]. Settings used in the analysis were mobile phase A: 10mM $KH_2PO_4$, pH 2.4 achieved with Phosphoric acid and mobile phase B: Acetonitrile (CAN), both phases resulted into solvent isocratic 80:20 percentage. Flow rate was sat at 1.5 ml.min-1, oven temperature was kept at 30˚C, maximum $\lambda$ = 210 nm, samples were fed at 20µL, and analysis time was sat at 15minutes. Prior to feeding the three vinegar samples, a high purity acetic acid (99.9%) supplied by Food and Drug Control Center (FDCC) was used as a standard. A 0.1% solution was prepared and injected under the

analysis conditions, its peak area and eluting time were used subsequently in identifying acetic acid peaks of vinegar samples.

**Color analysis**

Color was analyzed using handheld Tristimulus reflectance colorimeter (Minolta CR 400, Minolta Corp., New Jersey, USA). (Lab color spaces), with (L*) indicates lightness, (a*) for chromaticity from green (-) to red (+), while (b*) represents chromaticity from blue (-) to yellow (+). Measurements were made in triplicates.

**Sensory Evaluation**

Qualitative descriptive sensory analysis (QDSA) was conducted using 7 untrained taste panel randomly selected from employees and staff of the Department of Agricultural Engineering, Faculty of Agriculture, Tripoli University. Samples were put in coded cups and introduced independently to panelist, he/she was asked to take a teaspoon of each type, taste it and mark down his/her assessment to sample acidity, taste, and acceptability on a 100mm scale line representing 0-100% in each category. Freshwater was provided for rinsing between samples, and panelist marked down their assessment in the three categories. For acidity, the line ranged from weak to very strong, taste from unpleasant to pleasant, and acceptability from unacceptable to acceptable. The evaluation form is widely used in QDSA analysis, and similarly used by [12], [19].

**Statistical Analysis**

Data analysis was performed using analysis of variance (one way ANOVA), the significance level was selected at (0.05) and analyses were made using Microsoft Excel 2010. Tukey Kramer HSD post hoc test was carried for pairwise comparison by programming formulas on an Excel data sheet.

## RESULTS AND DISCUSSIONS

**Sugar Extraction**

TSS of extracted sugary solution (juice) from 'Talees', 'Athwi', and 'Hellawi' dates were 15.5, 18, and 25 ˚Brix, respectively. Juice volume obtained from the two dry cultivars, 'Talees' and 'Athwi' were 8,320 and 8,460ml, respectively. For 'Hellawi' cultivar, however, collected juice was 10,100ml and its TSS was 25 ˚Brix. Fruits were in the 'Rutab' stage, at high moisture content 84% (w.b) and rich in sugar (TSS > 50%). Extraction for 'Hellawi' was made by heating and agitation, leading to extracting most sugars. Fruit high moisture content and extraction method used may explain juice volume increase and high sugar content obtained. On the other hand, the two dry cultivars 'Talees' and 'Athwi' had low moisture content 13.6% and 14.3% and accordingly collected juice was lower in volume. Certainly, some sugar remained in date tissue after pressing; however, it was measured at 8 ˚Brix in fruit residues of the two cultivars. Taking into consideration that some moisture remained in fruits after pressing, thus efficiencies of recovering moisture added to dates were, 92 and 94% for the 'Talees' and 'Athwi', respectively. Nonetheless, using fruit juice is more efficient than fermenting fruits, easier to handle and faster in the anaerobic fermentation [9]. Though, mixing ratio 1:3 gave TSS around 15 ˚Brix was quite convincing, giving good agreement with literature [6], in their extraction of sugars of some date cultivars. Moreover, TSS content near 15 % (w/v) was used in producing vinegar from Iraqi 'Zahdi' dates [14]. Additionally, TSS content between 10 and 22 ˚Brix was investigated [7], which indicated that TSS was very much inversely related to the anaerobic fermentation time; higher TSS content extended fermentation time. Furthermore, it has been suggested that for achieving good ethanol yield and subsequently vinegar yield in traditional vinegar-making methods, TSS content must be not less than 14 ˚Brix [10].

**Performance of Anaerobic Fermentation**

The anaerobic fermentation process lasted for 9 days at 22˚C average room temperature, the reaction was considered completed once $CO_2$ bubbling in the airlocks stopped. Samples were withdrawn and analyzed for TSS expressed as ˚Brix, TA, ethanol percentage and pH. Table 1 shows ANOVA results for alcohol content, TSS, and pH. As can be observed, there were no significant differences among the three cultivars in pH and accordingly the acidity at ($p < 0.05$) level.

Table 1. ANOVA for anaerobic fermentation yield properties

| Source | SS | d.f | MS | F | P |
|---|---|---|---|---|---|
| *Alcohol* | | | | | |
| Between groups | 2.31 | 2 | 1.07 | 8.22 | 0.019 |
| Within groups | 0.78 | 6 | 0.13 | | |
| Total | 2.91 | 8 | | | |
| *TSS* | | | | | |
| Between groups | 2.94 | 2 | 0.147 | 441.3 | 0.00 |
| Within groups | 0.02 | 6 | 0.00 | | |
| Total | 2.96 | 8 | | | |
| *pH* | | | | | |
| Between groups | 0.03 | 2 | 0.01 | 1.76 | 0.251 |
| Within groups | 0.05 | 6 | 0.01 | | |
| Total | 0.08 | 8 | | | |

Source: Results of the own experiments.

However, alcohol content (percentage v/v) showed significant difference among the three cultivars, therefore, post hoc Tukey Kramer HSD test was carried out. As it can be observed in Figure 1, 'Hellawi' produced the highest alcohol content (5.94±0.61) followed by 'Athwi' (5.56±0.066), though the two were not significantly different at ($p < 0.05$). However, 'Athwi' was not different from 'Talees' (4.80±0.13). This meant that 'Hellawi' was the highest in sugar conversion to alcohol and 'Talees' was lowest.

TSS measured as ˚Brix also showed significantly different among the three cultivars (Figure 1), 'Hellawi' (8.03±0.058), 'Talees' (6.77±0.058) and 'Athwi' (6.53±0.058). Despite using similar TSS, variations in remaining TSS were recorded at the end of the anaerobic fermentation. Thus, sugar conversion to alcohol percentages was calculated at 52, 44 and 42% for 'Hellawi', 'Talees', and 'Athwi', respectively. Such results showed variations among the three cultivars. Indeed, it is related to cultivar properties, that may affected yeast ability in converting sugar to ethanol. However, efficiencies reported here are in a fair agreement with values reported in the literature, [12], [14]. Factors such as nutrient availability, enzymes, and substrate pH are very much cultivar related.

The anaerobic fermentation lasted for 9 days for the three cultivars, while in our previous study on 'Tasfert' dates; it lasted for two weeks due to using whole fruit [9].

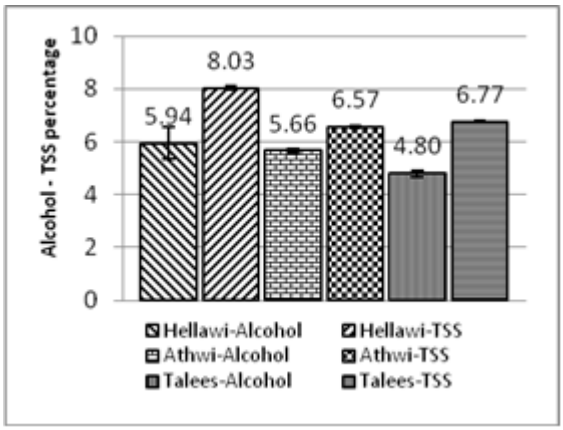


Fig. 1. Alcohol and TTS content after anaerobic fermentation

Source: Results of the own experiments.

Nonetheless, length of the anaerobic fermentation of vinegar in traditional and spontaneous methods is always a matter of contradiction; while some references reported an extended period for up to three weeks [12], others reported 15 days [15], and with substrate adjustments and controlled conditions, it can be as fast as 96 hours [14]. In general, TSS, pH, temperature, and yeast nutrient are the key factors in the anaerobic fermentation lasted for 9 days for the three cultivars, while in our previous study on 'Tasfert' dates; it lasted for two weeks due to using whole fruit [9].

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Nonetheless, length of the anaerobic fermentation of vinegar in traditional and spontaneous methods is always a matter of contradiction; while some references reported an extended period for up to three weeks [12], others reported 15 days [15], and with substrate adjustments and controlled conditions, it can be as fast as 96 hours [14]. In general, TSS, pH, temperature, and yeast nutrient are the key factors in the anaerobic process; they may affect the reaction either independently or in their interactions [3].

**Performance of aerobic fermentation**

TA and ethanol content (AC) are generally considered very important in the aerobic reaction, therefore they were measured weekly until AC dropped below 1%, the

upper limit for alcohol content sat be the Libyan Vinegar Specification (LNS 823: 2015). However, Table 2 shows ANOVA results for properties of the vinegar produced after natural acetification process that lasted 8 weeks for 'Athwi' and 'Talees' an additional 11 days for 'Hellawi'. Mean TA for the three cultivars were (6.72±0.15), (6.15±0.115) and (6.61±0.56) for 'Hellawi', 'Athwi' and 'Talees', respectively. As can be observed, cultivars were insignificantly different in their TA at ($p < 0.05$) level. However, for alcohol content (residues), significant differences among the three cultivars were noted, similar to the anaerobic stage. Post hoc Tukey Kramer HSD test showed that 'Hellawi' cultivar (0.87±0.105) was similar to 'Talees' (0.673±0.11), while 'Athwi' (0.442±0.128) was different from 'Hellawi' while similar to 'Talees'. Additionally, TSS content after acetification exhibited differences among the three cultivars, results showed 'Hellawi' cultivar (7.53±0.58) was significantly higher than 'Talees' (6.87±0.58) and 'Athwi' came lower at (6.13±0.058). Nevertheless, studies on comparing date cultivars in vinegar making are very scarce. In fact, virtually all reviewed literature had used one cultivar [11], [15], [14], [1].

Table 2. ANOVA Table for final vinegar properties

| Source | SS | d.f | MS | F | P |
|---|---|---|---|---|---|
| *Titratable acidity* | | | | | |
| Between groups | 0.36 | 2 | 0.18 | 1.65 | 0.27 |
| Within groups | 0.65 | 6 | 0.11 | | |
| Total | 1.01 | 8 | | | |
| *Alcohol residues* | | | | | |
| Between groups | 0.28 | 2 | 0.14 | 10.4 | 0.011 |
| Within groups | 0.08 | 6 | 0.01 | | |
| Total | 0.35 | 8 | | | |
| *TSS* | | | | | |
| Between groups | 2.94 | 2 | 0.147 | 441.3 | 0.000 |
| Within groups | 0.02 | 6 | 0.00 | | |
| Total | 2.96 | 8 | | | |

Source: Results of the own experiments.

However, regardless of alcohol content as the primary middle stage between sugar and acetic acid, TSS around 15 ˚Brix produced acetic acid above 6% (v/v) with efficiency range 40–50%. It is important to mention that 'Talees' cultivar gave alcohol content lower than 'Athwi', but gave higher TA. This gives quite clear evidence that cultivar properties may be affected conversion efficiency of alcohol to vinegar.

Theoretically, ethanol conversion to acetic acid is at 1:1.304 ratio [14], thus final acetic acid yield for the three cultivars was in quite realistic range. In fact, the actual conversion efficiency of ethanol to vinegar of 'Athwi' was 100%, for 'Talees' was 83% and 'Hellawi' conversion efficiency was above that. Conversion efficiencies of 'Athwi' and 'Talees' were in good agreement with the finding of [14], for 'Hellawi' however; this may be linked to either conversion of sugar to alcohol that might occur during the acetification process. It could happen similar to reactions of one stage spontaneous fermentation processes [11]. Nonetheless, taking into account the uncontrolled fermentation conditions in addition to cultivar variations, results obtained from the current study were quite realistic.

Figure 2 shows the performance of the acetification process as accumulative titratable acidity (TA) (% v/v) and alcohol depletion (alcohol concentration) (% v/v), respectively. Acidity linearly increased throughout the acetification process. Acidity and acetification time were in quite good agreement with ref. [20] in their investigation of producing vinegar from coconut sap. Moreover, acetic acid accumulation was fairly linear for the three cultivars; it may be due to fermentation conditions, such as low temperature and natural aeration of the acetified material. Comparing such reaction behavior with acetification curve for Iraqi 'Khastawi' cultivar [15], similarities were observed between the starting point and the 32$^{nd}$ day of their aerobic reaction. Furthermore, a similar linear trend was presented [2] in their study on producing mango vinegar; both acetic acid accumulation and alcohol depletion were fairly linear but sharper than that recorded in the current study.

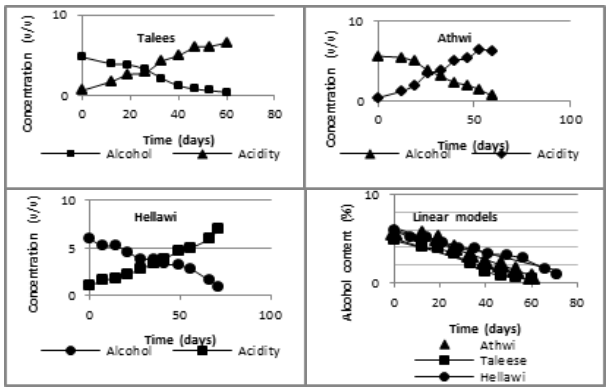


Fig. 2. Acetic acid accumulation and ethanol depletion
Source: Results of the own experiments.

Titratable acidity and alcohol depletion were fitted to linear models, as can be seen in Figure 2 (bottom right), yet linear relations and their $R^2$ are presented in Table 3. As can be observed, both acetic acid and alcohol depletion followed linear relations with $R^2$ values above 0.96. Acidity increase and ethanol depletion followed fairly linear in flatten relations for the three cultivars, flattening were mainly due to the slow ethanol conversion. Nonetheless, small variations among the three cultivars were noticed, such as the extra time taken for 'Hellawi' to drop alcohol content below 1%, once again it may be related to cultivar properties.

Table 3. Linear models of the acetic acid accumulation and alcohol depletion versus time (t)

| Cultivar | Model | $R^2$ |
|---|---|---|
| *Alcohol content (AC) (%)* | | |
| 'Talees' | AC = 5.001 - 0.081t | 0.960 |
| 'Athwi' | AC = 6.211 - 0.090t | 0.971 |
| 'Hellawi' | AC = 5.932 - 0.064t | 0.967 |
| *Titratable acidity (TA) (%)* | | |
| 'Talees' | TA = 0.592 + 0.105t | 0.98 |
| 'Athwi' | TA = 0.317 + 0.108t | 0.97 |
| 'Hellawi' | TA = 0.715 + 0.080t | 0.98 |

Source: Results of the own experiments.

**Vinegar quality assessment**

*Quantification of acetic acid*

Figure 3 shows the acetic acid peak of 'Hellawi' vinegar sample analyzed by the HPLC system. Peak appeared at 2.17 minutes, yet peaks of other vinegar samples and standard are similar in time and shape. Peak area of the acetic acid standard injected at 0.1mM was used for converting areas acetic acid peaks eluted from injected vinegar samples to percentages. Acetic acid percentages for 'Hellawi', 'Athwi' and 'Talees' were calculated at 5.70, 5.36 and 5.70, respectively. On the other hand, titratable acidities were 6.71, 6.25 and 6.61 for 'Hellawi', 'Athwi' and 'Talees', respectively. This gives percentages of acetic acid from the total titratable acidity at 85%, 86, and 86% for the three cultivars vinegar, respectively, indicating other organic acids (undetermined) in the sample at nearly 1.5% (v/v). Nonetheless, it is quite anticipated that other organic acids exist in the fermentation [17] the presence of several organic acids other than acetic acid in wide range of vinegar produced from agricultural products was reported. Organic acids such as succinic acid, malic acid, tartaric acid, lactic acid, and citric acid were reported in a small percentage of less than 2% in several types of vinegars including grapes and apples.

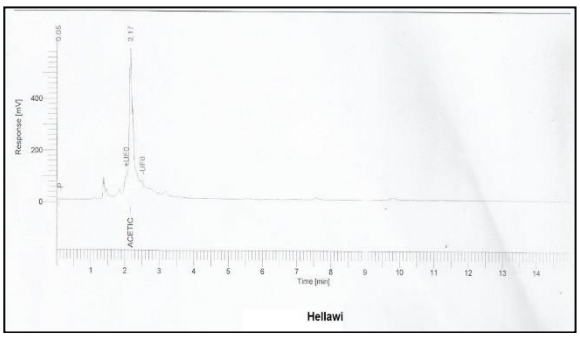


Fig. 3. Acetic acid peak in HAPLC analysis in Hellawi sample
Source: Results of the own experiments.

Thus, a good agreement of our work with the literature can be clearly observed. However, the scope of this work was to investigate the potential of the process and to perceive whether differences in reaction performance and quality attributes among investigated cultivars do exist. Thus quantifying acetic acid in vinegar of the three cultivars was mainly intended to confirm the acetic acid is dominant acid in the three types of vinegar.

*Sensory evaluation*

Figure 4 shows the graphical representation of the sensory results in acidity, taste, and acceptance. ANOVA results showed no significant differences among vinegar of the three cultivars in the three tested attributes.

Substantial variations were observed, this may be linked to the untrained panel. However, similar to analytical results, differences in mean acidity among the three cultivar kinds of vinegar can be clearly observed (first three columns in Figure 4).

Nonetheless, wide variations can be observed in the judgment of the panel to the tasted samples (error bars = standard deviations), it is rather normally occurring phenomena due to variations related to differences in human likeness and acceptance, and accordingly judgment to food items.

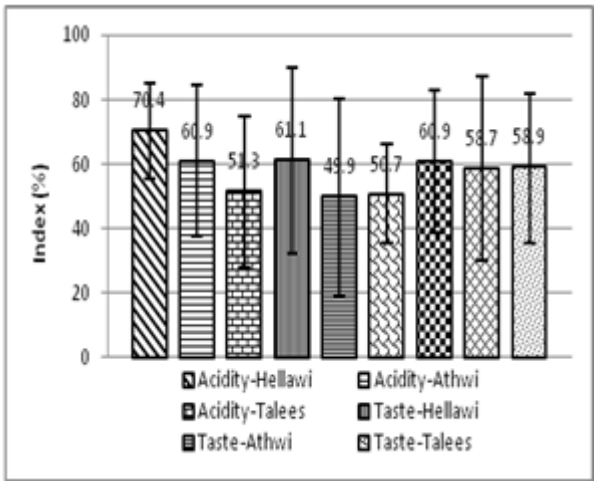


Fig. 4. Sensory analysis results
Source: Results of the own experiments.

However, quite similar observations were reported [12], in his sensory analysis to apple vinegar produced from several cultivars. Despite that, sensory evaluation gave a good indication of similarities between vinegar of the three cultivars, most important in acidity and acceptance, as it can be observed means were in quite close range.

*Color analysis*

Measured color attributes (L*, a*, and b*) were used in calculating two important color qualities, Hue angle and Chroma. Hue angle was calculated as Hu = tan-1 (b*/a*), while Chroma was calculated as $Cr = [(a^{*2}+b^{*2})^{1/2}]$, while L* presented the degree of lightness or darkness similar to the method was applied [18]. Nonetheless, Hue is an angle on the color circle (360˚). Generally, the circle is divided into four portions; 0-90˚ embodies red-purple Hues, 90-180˚ for yellow Hue, 180-270˚ for bluish-green, and 270-360˚ for blue Hue. Chroma, however, is the intensity of the Hue itself, while L* is the color lightness-darkness. Every three particular combinations give specific three-dimensional point within HunterLab color ball. Table 3 shows Tukey results for the three color qualities of vinegar, clearly, significantly different among the three cultivars exist. 'Hellawi' with lower Hue angle, Chroma, and L* had the darkest color, 'Athwi' and 'Talees', were significantly different among each other but much lighter than 'Hellawi'. Nonetheless, Hue of the three cultivars fell in the first quarter of the color circle, 'Hellawi' had relatively dark reddish color, while the other two were quite lighter towards yellowish. Generally, color is very much related to fruit pigments, indeed cultivars are quite different in this regard.

Table 4. Color attributes means (means with the same letter raw-wise are insignificant at ($p<0.05$)

| Color attribute | 'Hellawi' | 'Athwi' | 'Talees' |
|---|---|---|---|
| Hue Angle | $26.9^{a}$ | $39.55^{b}$ | $42.04^{c}$ |
| Chroma | $3.57^{a}$ | $13.32^{b}$ | $13.20^{b}$ |
| L* | $15.88^{a}$ | $21.05^{b}$ | $19.23^{c}$ |

Means with the same letter are no significantly different at 0.05 level
Source: Results of the own experiments.

## CONCLUSIONS

Double stage fermentation method was applied for making vinegar from three Libyan date cultivars, 'Hellawi', 'Athwi' and 'Talees'. Sugars were extracted from dates and similar TSSs were used in the process. The anaerobic process lasted for 9 days, 'Hellawi' produced the highest alcohol percentage and the highest TSS remained after fermentation, followed by 'Athwi', while 'Talees' came last. The aerobic process lasted 8 weeks for 'Athwi and 'Talees' an extra 11 days for 'Hellawi', alcohol contents were converted to acetic acid with high efficiencies. No significant difference in acidity among the three cultivars, however, they exhibited differences in residual alcohol and TSS. Also, acetic acid accumulation and alcohol depletion followed linear models with $R^2$ above 96. HPLC analysis showed that

acetic acid is dominant in the three types of vinegar at about 85%. Color and sensory analyses were also carried out on kinds of vinegar. The difference in color among vinegar of the three cultivars were recorded, 'Hellawi' vinegar has the darkest color, while 'Athwi' and 'Talees' kinds of vinegar were rather lighter. Sensory evaluation in acidity, taste, and acceptance showed insignificant difference among kinds of vinegar of the three cultivars. The study demonstrated the potential of producing vinegar from the three investigated cultivars at acceptable quality.

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