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Brief Communication

Evaluation of secondary metabolites, nutraceutical potential and amino acid profile of fresh dates (*Phoenix dactylifera*) alcoholic beverage

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Abstract
The present study comprehensively investigated the fermentation process of fresh dates pulp to alcoholic beverage, analyzing various aspects including physicochemical properties, nutraceutical potential, amino acid composition, color properties, sensory evaluation, and microbial load. Over the fermentation period, the alcohol content of the beverage reached 8.79%, suggesting it is an off-dry wine. Total Soluble Solids (TSS) decreased from an initial 24.17°Brix to 5°Brix. The pH level dropped from 7 to 3.9, and the titratable acidity increased from 0.15 to 0.22%. The nutraceutical potential of the alcoholic beverage displayed dynamic transformations, with total phenolic content (TPC) decreasing from 23 to 9 mg GAE mL^{-1}, while total flavonoid content (TFC) increased significantly ($p < 0.05$). Amino acid analysis revealed distinct concentrations, with alcoholic beverage exhibiting higher levels of essential amino acids due to fermentation. Color analysis showed a shift towards reddish hues, and a progression towards a more yellowish color during fermentation. Sensory evaluation indicated a non-significant difference ($p > 0.05$) between the alcoholic beverage and juice in terms of color, but the alcoholic beverage scored higher in appearance, flavor, texture, taste, mouthfeel, and overall acceptability. Microbial load increased from an initial 5–6 log CFU mL^{-1} to 8–9 log CFU mL^{-1} after 5 days, indicating the occurrence of alcoholic fermentation. Kinetics study was also performed for different quality parameters. This is the first study provides a comprehensive understanding of the multifaceted changes occurring during the fermentation process, offering valuable insights for winemakers and researchers.

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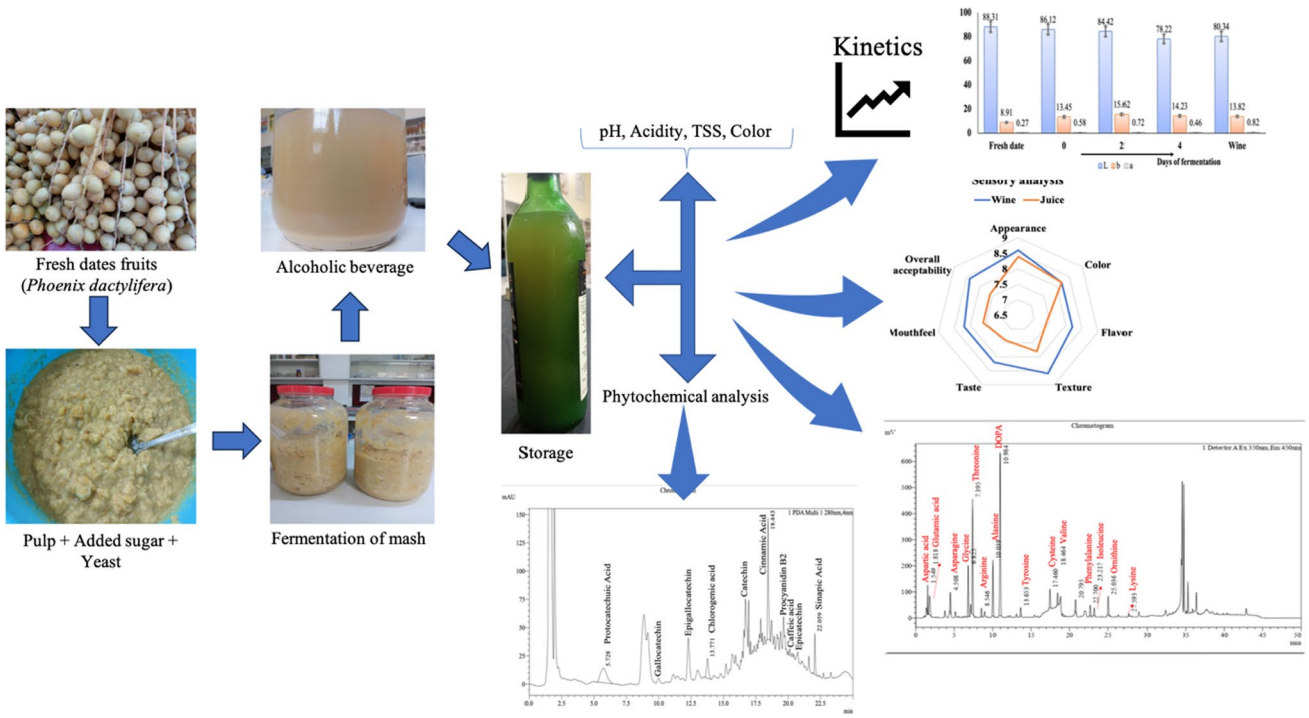
Graphical Abstract



Highlights

1. First study to report the alcoholic beverage making process using fresh dates fruits.
2. Nutraceutical potential experiences substantial shifts, providing insights into the potential health benefits.
3. Alcoholic beverage exhibits higher levels of amino acid, phenolic acid and metabolites due to the process.
4. Kinetics study was also performed for different quality parameters.

Keywords Secondary metabolites · Alcoholic beverage · Value-added product · Amino-acid · *Phoenix dactylifera*

1 Introduction

Wine, an alcoholic beverage derived from the fermentation of grapes or other fruits and plants, is categorized as a fruit wine when produced from undistilled fermented beverages [1]. Fruit wines, including those from fresh dates, have been recognized for their nutritional content and moderate stimulant properties [2]. The fermentation process enhances the nutritional profile of the product, as amino acids and yeast nutrients released during fermentation contribute to increased nutritional value compared to fresh juice [2]. Fermentation is a globally employed economical processing method, widely utilized in the production of traditional fermented foods. This method involves the use of microbial and raw inoculants, leading to various biochemical changes that improve nutritional quality, extend shelf life, and enhance sensory characteristics [3–5].

Fresh dates pulp is known for its nutritional richness, including vitamins, minerals, and antioxidant and antimicrobial properties. The pulp's composition, as highlighted by the USDA, indicates significant amounts of carbohydrates, sugars, calcium, iron, magnesium, phosphorus, sodium, and potassium, making it a valuable raw material for value-added products [6]. Health benefits associated with fresh dates include anti-inflammatory and antioxidant potential, as demonstrated

in a study using methanolic and water extracts [2]. Dates, being rich in phenolic compounds, exhibit anti-fungal, anti-bacterial, and anti-viral qualities, attributed to antioxidants such as ascorbate, carotenoids, selenium, and others [7]. The antioxidant properties of dates are crucial in protecting against oxidative damage caused by diseases, pests, and lymphocyte phagocytosis [8]. Fresh dates are recognized as a primary source of natural antioxidants, contributing to their health-promoting attributes [2, 9].

In the context of fermentation, *Saccharomyces cerevisiae* plays a pivotal role, particularly in the production of beer, ales, sakes, and wines. Hybrids of *S. cerevisiae* with other *Saccharomyces* species, such as *S. mikatae, S. kudriavzevii,* and *S. uvarum*, are also utilized in certain fermented beverages. The vigorous fermentation capacity of *S. cerevisiae*, especially in the presence of oxygen, helps overcome microbial competition by rapidly consuming sugar resources and producing ethanol. The ability of *S. cerevisiae* to utilize accumulated ethanol for aerobic respiration further contributes to its dominance in the fermentation process [10–12].

The fermentation process in wine production can be manipulated to create sweet wines by interrupting the process before complete sugar conversion [13]. Furthermore, the study explores the utilization of exotic fruits like passion fruit for wine production, emphasizing the potential for creating diverse fruit wines. Additionally, the development of value-added products, such as beetroot wine, utilizing different strains of *S. cerevisiae*, aims to enhance the use of beetroot, minimize post-harvest losses, and identify the optimal fermenting strains for high-quality wine production [14]. By investigating physicochemical compounds, inoculum sizes, and total soluble solids, the study provides insights into the complex interplay of factors that influence the quality of beetroot wine. This comprehensive approach highlights the scientific exploration of fermentation processes and their application in producing a wide variety of high-quality fruit wines, including sweet wines.

To forecast changes throughout processing, kinetic modelling helps by providing fundamental kinetic information about the reaction rate in a food system as a function of experimental variables [15]. For the degradation of quality parameters in fruits and vegetables, the employment of zero-order, first-order, and fractional conversion first-order kinetic models is helpful [15]. There is no published information on the kinetics of fresh dates (*Phoenix dactylifera*) alcoholic beverage. The main objective of the present study is to solve the problem of higher yield, short harvesting time, and storage via making value-added products from fresh fruits and providing a long-term stable market for fresh dates with the improvement of economic benefits for farmers. Additionally, the resulting fresh dates alcoholic beverage has an appealing color, with no particles or sediment present, a subtle aroma of fresh dates, and a refined alcohol odor. It also has a well-balanced taste between sourness and sweetness, and a harmonious blend of alcohol aroma and flavor, making it enjoyable to drink. The nutrient and minerals from the product may have health benefits, such as acting as antioxidants to help fight off certain ailments.

2 Materials and methods

2.1 Raw materials

The fresh dates were obtained from the local market of Dehradun (30.316496, 78.032188), Uttarakhand, India. All the chemical and reagents used in the present study were of analytical grade and procured from Merck-Sigma, India. The standards of amino acids were sourced from Toronto Research Chemicals Inc. (North York, Canada). All standards exhibited a purity exceeding 98%. Phosphate-buffered saline (PBS) was acquired from Merck-Sigma, India. Methanol and formic acid were both of HPLC grade.

2.2 Starter culture and maintenance media

The freeze-dried *S. cerevisiae* strain used in the present study for wine making was procured from the National Collection of Dairy Culture (NCDC), NDRI, Karnal, Haryana (India) and revived as per Singh et al. [9].

2.3 Inoculum preparation

One inoculation loop (6 log CFU mL^{-1}) of *S. cerevisiae* NDRI strain colonies was transferred in 100 mL of clarified date juice for the inoculum preparation. The total soluble solids (TSS) was kept constant at 24°Brix. After that, the inoculated

juice was incubated at 25 ± 0.2 °C. According to Buxaderas and Lopez [16], the presence of bubbles and overripened odor indicated that the inoculum preparation was complete.

2.4 Preparation of must for fermentation and fermentation process

The date samples (7 kg) were sorted and washed using potable water (Fig. 1). Subsequently, the fruit blanched in hot water at 100 °C for 2–3 min followed by cooling in cold water for 5–10 min before being pulped using a pulper (Phillips, Netherlands). The pulp was then homogenized using a laboratory blender (Bajaj, India), and the resulting pulp was collected and stored at −20 °C for further analysis. Next, the pulp was transferred to a 500 mL sterile conical flask and diluted with distilled water. To maintain the initial TSS at 24°Brix, 0.120 kg of sugar was added to the date pulp, followed by vigorous stirring. Potassium metabisulphite is added to must at a concentration of 0.05% (w/v) before inoculating with yeast culture to ensure optimal conditions for fermentation and wine quality. This compound serves multiple crucial roles: it acts as an antimicrobial agent, effectively inhibiting the growth of unwanted bacteria and wild yeasts that could spoil the must. Additionally, it has antioxidant properties, preventing the oxidation of the juice, which can lead to undesirable flavors and spoilage. By preserving the color and flavor of the must, potassium metabisulphite helps to prevent oxidative browning and other oxidative reactions that can negatively impact the final product. Therefore, its addition is essential for maintaining the integrity and quality of the wine during the fermentation process [17]. The batch fermentation took place in sterile 2 L glass jar flasks, with each flask containing 1000 mL of date pulp. A 25 mL inoculum of the yeast strain (6 log CFU mL^{-1}) was added to the must, which were then incubated at 25 °C for 12 days. The fermentation process was conducted in three biological replicates to ensure the reliability and reproducibility of the results. All analyses, including measurements of Total Soluble Solids (TSS), pH, titratable acidity, and sensory evaluations, were performed on each of these replicates to ensure the accuracy and

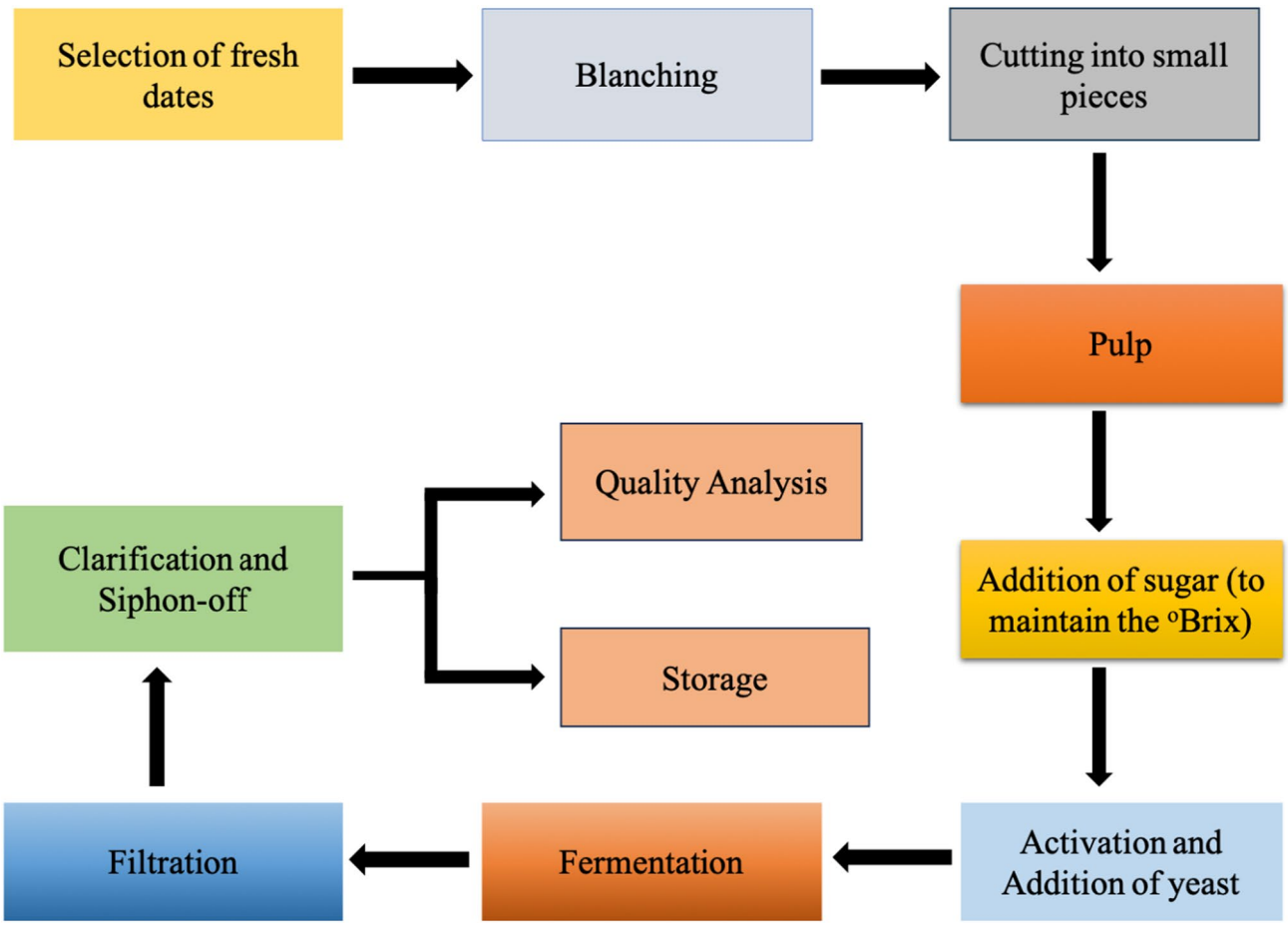


Fig. 1 Process of making date alcoholic beverage

consistency of our findings. After fermentation, the fermented juice was filtered through a 3-layered muslin cloth and clarified using bentonite (at a concentration of 0.05%). Filtration through cheese cloth was done after 5 days of fermentation. The sample was siphoned into the sieve containing four layers of muslin cloth, removing residues, and collecting the filtrates for further physiochemical analysis.

2.5 Physicochemical properties of fresh fruit juice and alcoholic beverage

The AOAC official procedures (21st edition) [18], including AOAC 942.15, AOAC 981.12, and AOAC 967.21, were used to determine the TSS, TA, and pH of the sample, respectively. The color properties of the alcoholic beverage were determined using the pre-calibrated Color flex (Hunter Associates Laboratory Inc., Reston, VA, USA), where L (lightness), a (redness), b (yellowness) values were estimated [19].

The alcohol content of date beverage during fermentation days was performed using hydrometer (BEER-40, SSU Other Lab Supplies, India). The initial specific gravity of the sample was measured using a hydrometer calibrated for the specific range of the beverage. This initial reading served as a baseline before fermentation began. After fermentation was complete and the beverage reached stability, the final specific gravity was measured again with the same hydrometer. The difference between the initial and final SG readings was used to calculate the alcohol content by volume (ABV) using a formula where 131.25 was the constant multiplier.

2.6 Spectroscopic determination of nutraceuticals

2.6.1 Extract preparation and determination of nutraceutical properties

According to Gupta et al. [20], methanolic extract was made (solvent: water, 80:20, v/v) for determining the bioactive compound. The mixture was centrifuged and filtered using Whatman no. 1 filter paper (0.42 µM). For subsequent analysis, the filtered extract was kept in a dark amber colored bottle at 4 °C. The total phenolic content (TPC) of alcoholic beverage was calculated where extract (1 mL) was mixed with 0.25 mL of Folin-Ciocalteu reagent (1:2 diluted), maintained at 40 °C for 30 min and then 1 mL of sodium bicarbonate solution (20%) was added followed by incubation at 40 °C for 60 min. The intensity of developed color was measured using UV–Vis spectrophotometer (Cary 60, Agilent UNICO Products and Instruments Inc., Shanghai, China) and expressed as the Gallic acid equivalent (GAE) mL^{-1} of sample [20]. TFC was estimated as per Zhishen et al. [21], where extract (2.5 mL) was mixed with 5% sodium nitrate solution (150 µL) followed by the addition of 10% aluminum chloride (150 µL). The mixture was vortexed and 1 mL of NaOH was added after 5 min. Then, the absorbance of the solution was measured at 510 nm against distilled water using UV–Vis spectrophotometer. The result was expressed in terms of quercetin equivalent (QE) mL^{-1}.

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was conducted following the procedure described by Lee et al. [22]. To prepare the working solution, 7 mM ABTS solution and 2.4 mM potassium persulfate solution were mixed in equal proportions and allowed to react for 14 h in the dark at room temperature. The resulting solution was diluted by combining 1 mL of the ABTS solution with 60 mL of methanol to achieve an absorbance of 0.707 ± 0.01 at 734 nm. A fresh ABTS solution was prepared for each assay. During the assay, 1 mL of the extract was mixed with 3 mL of the ABTS solution, and the absorbance was measured at 734 nm after 7 min. The ABTS scavenging capacity of the extract was compared to the ascorbic acid, and the percentage inhibition was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100 \quad (1)$$

2.7 Amino acid analysis

Amino acid analysis of juice and alcoholic beverage samples was conducted using an Agilent 1290 series UHPLC system (Agilent Technologies, Germany) coupled with an Agilent 6460 triple-quadrupole mass spectrometer featuring an electrospray ionization (ESI) source (Agilent Technologies, Wilmington, DE, USA). Reverse phase chromatographic separation was performed using an Agilent Zorbax SB-C18 column (3.0 mm × 150 mm, 5 µm). The buffer flow rate, consisting of methanol

(A) and water (B, containing 0.2% formic acid and 0.02% heptafluorobutyric acid), was maintained at 0.4 mL min^{-1} in a gradient elution setup. Injection of sample was 2 µL, and the column temperature was set to 50 °C. The system pressure was kept constant at an average of 64 bar throughout the analysis. Data acquisition was carried out in multiple reaction monitoring (MRM) mode in the positive mode. MS parameters were optimized with a drying gas temperature of 325 °C, drying gas flow of 10 L min^{-1}, sheath gas temperature of 350 °C, sheath gas flow of 12 L min^{-1}, and a capillary voltage of 5000 V. Under these conditions, the UHPLC-MS/MS analysis was completed within 13.5 min [23].

2.8 Determination of phenolic profile

The alcoholic beverage sample for phenolic identification was prepared and quantified according to Magiera and Zaręba [24] using an Agilent 1290 series UHPLC system (Agilent Technologies, Germany).

2.9 Metabolomics via HR-LCMS

The metabolomics profile of fruit and alcoholic beverage was characterized using HR-LC-MS (HRLCMS-QTOF-Agilent Technologies, USA) as per the method of Jha and Sit [25]. Separations were performed on a ZORBAX Eclipse Plus column (C-18 150 × 2.1 mm, 5 µm). Formic acid (0.1%) in Milli-Q water (A) and acetonitrile (B) were the mobile phases. The injection volume was 20 µL, the flow rate was 0.300 mL min^{-1}, and the monitoring wavelength was 217 nm. A gradient solvent system with the following solvent concentrations was used: 1 min, 95% A and 5% B; 2–25 min, 100% B; 25–30 min, 100% B; 30–31 min, 95% A and 5% B and 31–35 min, 95% A and 5% B. An ESI probe was used in the MS analysis of the LC effluent. Negative ion mass spectra were generated by using the Bruker microTOF-Q system with the capillary temperature set to 300 °C and the ESI spray voltage set to 4 kV. The retention duration, ESI MS, and UV max of phytoconstituent peaks were compared to published literature data to identify them.

2.10 Sensory analysis

The sensory quality of alcoholic beverage was assessed by 80 semi-trained panellists (age group 25–40 years) of Graphic Era (Deemed to be University), Uttaranchal University, and Swami Rama Himalayan University, India. Using a 9-point hedonic scale, the alcoholic beverage was assessed for sensory qualities such appearance, mouthfeel, colour, aroma, flavour, and overall acceptability. The participants gave their permission and acknowledged that the findings would be impartial. They were also informed about the non-toxicity of the alcoholic beverage. Each assessment was conducted to minimise any health hazards to the participants [1]. Also, we confirm that written consent was obtained from all participants for their voluntary participation in the sensory analysis of the wine. Furthermore, we ensured compliance with the institutional guidelines governing studies involving human subjects (sensory analysis).

2.11 Statistical analysis

Analytical determination for the juice and alcoholic beverage were carried out in triplicates and standard deviations were noted. One-way ANOVA was used to analyse the data in order to identify any significant differences. Ducan's Multiple Range Test (DMRT) ($p < 0.05$) was used to analyze the significant difference between the mean values of samples using IBM SPSS Statistics, version 20.0. Armonk, NY: IBM corporation.

The model parameters for all equations were estimated by non-linear least squares regression using Microsoft Excel Solver (Microsoft office, USA). Coefficient of determination, R^2 was taken to determine goodness of fit (Eq. 2) along with an analysis of residuals [15].

$$R^2 = 1 - \frac{SSE}{SSTO} \tag{2}$$

where,

SSTO is the total sum square errors $\sum_{i=1}^{N} (y_{Exp,i} - y_{Avg,Exp})^2$ and SSE the sum squared errors, $\sum_{i=1}^{N} (y_{Exp,i} - y_{Pred,i})^2$

The adequacy of the model fittings was indicated by root mean squared error (RMSE) as in Eq. (3) [15].

$$RMSE = \sqrt{\sum_{i=1}^{N} \frac{\left(y_{pred,i} - y_{exp,i}\right)^2}{N_t - N_p}} \quad (3)$$

where, $y_{exp,i}$ is the ith experimental observation,$y_{pred,i}$ is the ith model predictions, N_t is the number of experimental data points and N_p is the estimated model parameters. Higher R^2 value and lower RMSE value indicated that the model was best fitted [15].

3 Results and discussion

3.1 Physicochemical properties of juice and alcoholic beverage

The results of the fermentation process over different days reveal significant changes in key parameters that play a crucial role in the quality and characteristics of the resulting wine (Table 1). The sweetness of wine is directly related to the concentration of residual sugars present in the liquid, which is measured by TSS. At the start of fermentation, the TSS of the must was set at 24.17 ± 0.61°Brix. As fermentation progresses, the yeast converts these sugars into alcohol, resulting in a decrease in TSS. By the 2nd day, the TSS drops significantly to 15.60 ± 0.01°Brix, showing a reduction in sweetness. This downward trend continues with the TSS reaching 8.40 ± 0.02°Brix on the 4th day and further down to 5.00 ± 0.13°Brix by the 5th day. This marked decrease in TSS indicates that much of the sugar has been fermented, leading to a less sweet wine. Therefore, alcoholic beverage with a TSS of 5.00°Brix is significantly less sweet compared to its initial state, potentially resulting in a dry wine, depending on the final desired sweetness level. Initially, must had a pH level of 7 and titratable acidity at 0.15% citric acid. As the fermentation progressed to the 2nd day, there was a significant decrease in pH to 5.6 and a slight rise in titratable acidity to 0.16%. By the 4th day, pH further dropped to 4.2, and titratable acidity reached 0.19% citric acid. TSS estimation marked the initial phase in sucrose utilization by ethanolic yeasts, involving its complete hydrolysis into glucose and fructose by an extracellular invertase [26]. On the other hand, increased acidity observed towards the end of fermentation may be attributed to continued yeast activity, wherein remaining sugars are further utilized and converted into ethanol and CO_2 [26].

The alcohol content of date alcohol beverage was monitored throughout the fermentation process, revealing significant changes in specific gravity indicative of ethanol production. Initially, the specific gravity of the must was measured at 1.095, a high value typical of a must rich in fermentable sugars, essential for achieving substantial alcohol content. As fermentation progressed, the specific gravity decreased, reaching a final value of 1.028, which corresponds to an alcohol by volume (ABV) of 8.79%. This decrease in specific gravity reflects the yeast's consumption of sugars and subsequent conversion into ethanol and carbon dioxide. Given the TSS of 5°Brix in the final product, the classification of the date wine becomes more nuanced. This level of residual sugar content aligns more closely with the characteristics of an off-dry wine. Off-dry wines are characterized by a slight sweetness, resulting from residual sugars that are not fully fermented into alcohol. TSS of 5°Brix in the date wine suggests a modest amount of residual sugar, which provides a subtle sweetness without dominating the overall flavor profile. This aligns with the typical profile of off-dry wines, which strike a balance between sweetness and acidity.

The changes in TSS during fermentation days as represented in Table 2 can be best described by zero-order kinetics ($R^2 = 0.898$ and RMSE = 0.2229 with $K_o = 0.1967$) than first order ($R^2 = 0.842$ and RMSE = 0.0868 with $K_1 = 0.0479$) as for zero

Table 1 Physicochemical and phytochemical properties of fresh dates alcoholic beverage

Days of fermentation	TSS (°Brix)	pH	Titratable acidity (% citric acid)	TPC (mg GAE/mL)	TFC (mg QE/mL)	ABTS (% inhibition)
0	24.17 ± 0.61^{a}	7.00 ± 0.11^{a}	0.15 ± 0.00^{a}	26.20 ± 0.23^{a}	8.50 ± 0.14^{d}	91.71 ± 02.13^{a}
2nd	15.60 ± 0.01^{b}	5.60 ± 0.03^{b}	0.16 ± 0.00^{a}	24.82 ± 0.73^{a}	54.12 ± 1.01^{c}	95.41 ± 1.09^{a}
4th	8.40 ± 0.02^{c}	4.20 ± 0.01^{c}	0.19 ± 0.01^{a}	11.77 ± 0.11^{b}	156.78 ± 3.02^{b}	94.28 ± 1.26^{a}
Wine (5th)	5.00 ± 0.13^{d}	3.90 ± 0.01^{d}	0.22 ± 0.03^{a}	9.67 ± 0.21^{c}	314.12 ± 2.13^{a}	93.56 ± 0.77^{a}

Value reported as Mean ± SD of three replications. Means followed by different capital letter superscripts within a row are significantly different ($p < 0.05$)

Table 2 Kinetics of the changes in chemical properties of alcoholic beverage based on fermentation days

Parameters	Zero order			First Order		
	K_0	R^2	RMSE	K_1	R^2	RMSE
TSS (°Brix)	0.1967	0.898	0.2229	0.0479	0.842	0.0868
pH	−0.3750	0.584	0.7961	−0.0790	0.757	0.0869
Titratable acidity (% Citric acid)	0.0073	0.951	0.0061	0.0401	0.928	0.0490
TPC (mg GAE/ mL)	−1.8815	0.736	3.8264	−0.1178	0.817	0.1215
TFC (mg QE/ mL)	31.1713	0.984	14.7774	0.3628	0.712	7.4151
ABTS radical scavenging activity (% inhibition)	0.3015	0.771	1.7895	0.0032	0.782	0.0196

order kinetics the higher R^2 and lower RMSE values was observed. Again, the both rate constant was found to be positive which implies positive impact of fermentation days on TSS. The change in pH during fermentation days was best fitted with first-order kinetics (Table 2). The slope value (K_1) was found negative which indicated decreased value of pH with increase fermentation days.

The final stage, represented by the alcoholic beverage at day 5, exhibited a TSS of 5 °Brix, a pH of 3.9, and titratable acidity peaking at 0.22%. The study by Soibam et al. [26] investigated the production of wine from sugarcane and beet juice, focusing on fermentation conditions and preparation methods applicable to date wine production. Sugarcane juice, adjusted to 45°Brix with added sugar, was blended with beet extract at concentrations ranging from 40 to 70%. Each blend was divided into pasteurized and unpasteurized portions, with pH adjustment to 4.5 using citric acid before pasteurization. The optimal results were observed in the non-pasteurized blend of sugarcane and beet juice at a 50% concentration, achieving a TSS of 24.4°Brix, fermented at 26 °C with a pH range of 3.45–3.70. This blend yielded wine with 9.4% alcohol content, 7.7°Brix TSS, 0.3% titrable acidity, and 1.19% total reducing sugar, noted for its favorable flavor, color, and overall acceptability. In comparison, the present study on date alcoholic beverages at day 5 exhibited a TSS of 5°Brix, a pH of 3.9, and peaked at 0.22% titratable acidity. These parameters suggest a different fermentation profile and potentially different sensory attributes compared to the sugarcane and beet juice blend. The lower TSS and higher pH in the date beverage indicate a different sugar composition and acidity level, which can influence flavor development and overall product acceptance. The findings from both studies highlight the importance of fermentation conditions and substrate composition in determining the quality attributes of alcoholic beverages. While the sugarcane and beet juice blend aimed for higher alcohol content and acidity balance, the date beverage exhibited lower TSS and slightly higher pH, suggesting a potentially lighter body and different flavor profile. Understanding these differences is crucial for optimizing fermentation processes tailored to each specific substrate, thereby ensuring consistent quality and desired sensory characteristics in both beet and date wines.

Haliu and Mekonnen [27] investigated fermentation conditions for beetroot wine using approximately 2.5 kg of prepared beetroot fruits to extract fresh juice. They employed four distinct treatments: F-1 included 0.25 kg of sugar, 15 mL of citric acid, 2.25 L of water, 1.25 L of beetroot juice, and 0.00017 kg of yeast under anaerobic conditions. F-2 combined 1.25 L of beetroot juice with 0.25 kg of sugar, 15 mL of citric acid, and 2.25 L of water, omitting yeast under anaerobic conditions. F-3 used 1.25 L of beetroot juice with 10 mL of citric acid, 2 L of water, and 0.0017 kg of yeast under aerobic fermentation. F-4 utilized 1.25 L of beetroot juice with 10 mL of citric acid, 2 L of water, and no yeast, also under aerobic conditions. Their study reported titratable acidity levels of 0.62–0.73% and pH levels ranging from 2.89 to 3.53 across treatments for beetroot wine. Acidity is crucial in wine production, influencing freshness, brightness, and structural balance. Higher acidity (lower pH) in wines often enhances crispness and tartness, elevating perceived quality with a lively palate sensation. Beetroot wine, with moderate titratable acidity (0.62–0.73%), mirrors the balanced profiles found in many white wines that emphasize fruit flavors and freshness. In contrast, date beverages typically exhibit higher pH levels (around 3.5–4.5) and lower acidity (0.1–0.3%), resulting in a smoother, less tart profile favored by those preferring sweeter drinks derived from natural date sugars. These acidity and pH levels significantly shape sensory attributes in beetroot wine, guiding producers in crafting well-balanced, flavorful products that meet consumer expectations.

pH of the alcoholic beverage was notably affected by different strains and inoculum sizes [9]. The maintenance of increased acidity levels aids in preserving the quality of the product by restricting the growth of harmful bacteria. Succinic acid, a significant non-volatile acid, contributes to the increased acidity observed in alcoholic beverage during the fermentation process [28]. These results underline the dynamic nature of the fermentation process, showcasing how TSS, pH, and titratable acidity evolve over time. The importance of these changes lies in their direct impact on the

sensory characteristics and overall profile of the alcoholic beverage. TSS levels are indicative of sugar content, influencing sweetness and alcohol content, while pH affects the acidity and stability [29, 30]. Titratable acidity, involving citric acid, contributes to the overall taste and balance of the wine [31–33]. Understanding and controlling these parameters during fermentation are vital for winemakers to produce wines with desired flavors, aromas, and structural attributes.

3.2 Microbial load

The initial microbial count ranged from 5 to 6 log CFU mL^{-1} and exhibited an observable increase during the fermentation period, reaching 8–9 log CFU mL^{-1} after 5 days of storage. The observed trend in the microbial growth curve throughout fermentation underscored the occurrence of alcoholic fermentation, marked by the gradual consumption of sugar and simultaneous production of alcohol. Correlating the microbial load results with the physicochemical properties of the wine, it was evident that after fermentation, sugar, TSS, and alcohol levels surpassed those of the control, indicating the dynamic impact of microbial activity on these key parameters [9]. The microbial load in wine is intricately connected to various aspects of the fermentation process and the resulting quality of the product. Maintaining an appropriate and well-managed microbial balance is essential for achieving the desired sensory characteristics, stability, and overall excellence in wine production.

3.3 Nutraceutical potential

The present study investigated the changes in TPC, TFC, and ABTS antioxidant activity during the fermentation of alcoholic beverage, spanning from the initial stage (0 Day) to the completion of the process (Table 1). The phenolic content found in alcoholic beverage encompasses a diverse array of chemical compounds, such as phenolic acids, flavanols, dihydroflavanols, anthocyanins, monomeric flavanols (catechins), polymeric flavanols (proanthocyanidins), and quercetin. These compounds collectively influence the color, mouthfeel, and taste of the wine [34, 35]. TPC exhibited a notable reduction, suggesting dynamic transformations in phenolic compounds during fermentation (from 23 to 9 mg GAE mL^{-1}). Winemaking exerted a notable impact on the levels of phenols and antioxidant activity. Initially, date juice exhibited the highest levels of phenols and antioxidant activity. However, these levels underwent a significant decrease during the fermentation process, which was followed by a gradual stabilization during the ageing process [1]. When compared to other sources, the development of polymeric pigments and consequently the higher phenolic content of fruits or vegetables may be attributed to the beet's deeper color, which is indicative of its higher anthocyanin content [9].

With a general chemical structure of a 15-carbon skeleton made up of two phenyl rings (A and B) and one heterocyclic ring (C), flavonoids are a large family of phytochemicals with pharmacological and biological activities that include antiviral, antidiabetic, and antioxidative effects [36, 37]. In contrast, the TFC demonstrated a substantial increase, reaching its highest level in the final alcoholic beverage. This elevation in flavonoid content may positively contribute to the wine's sensory attributes and potential health benefits [1]. The quercetin level in condensed juice was 1.98 mg L^{-1}, and it increased by around 50% via fermentation in msalais wine, contrary to previous study that the quercetin content did not change significantly throughout the ageing stage [1]. Because quercetin is less soluble in water during alcoholic fermentation than anthocyanins, increasing the quantity of ethanol throughout the process can help extract more quercetin [38, 39]. Huo et al. [1] observed a rise in flavones, kaempferol, quercetin, flavanols, and derivatives of quercetin. Various bioactive compounds, such as polyphenols, undergo transformations throughout the brewing process. Numerous studies have highlighted the antioxidant richness of wine, attributed to polyphenols that can inhibit free-radical chain reactions and bind with metal ions [40, 41]. The antioxidant capacity of foods is closely linked to their phenolic content [42]. Zhu et al. [43] documented the total phenolic content in Msalais wines, ranging from 16.12 to 58.75 mg L^{-1} [43]. Phenolic compounds and their antioxidant properties in wine originate primarily from grape berries during fermentation, aging, and storage processes.

It is commonly recognised that the human body produces reactive oxidants, which may be toxic in a variety of ways and cause a wide range of chronic disease including cancer, atherosclerosis, mutations, and cardiovascular disorders [44]. Assays measuring antioxidant capacity are required in order to provide regulatory requirements for food quality and health claims [45]. Numerous studies have demonstrated that phenolic chemicals are crucial to wine's antioxidant property [46]. The ABTS antioxidant activity remained relatively stable throughout the fermentation process (91–93%), indicating the wine's capacity to maintain its antioxidant properties. These findings shed light on the intricate interplay between phenolic compounds and antioxidant activity during alcoholic beverage fermentation, providing valuable insights for both wine enthusiasts and researchers exploring the health implications of wine consumption [47]. Further

investigations into specific phenolic compounds responsible for these changes would enhance our understanding of the complex relationship between wine composition, quality, and potential health benefits.

For kinetics study, the changes in TFC during fermentation days as represented in Table 2 can be best described by zero-order kinetics ($R^2 = 0.984$ and RMSE = 14.7774 with $K_o = 31.1713$) than first order ($R^2 = 0.712$) as for zero order kinetics the higher R^2 was observed. Again, the both rate constant were found to be positive which implies positive impact of fermentation days on TFC. But for TPC and ABTS activity fermentation days kinetics was best fitted with first-order kinetics (Table 2). The slope value (K_1) for TPC was found negative which indicated decreased value of TPC with increase in fermentation days.

3.4 Amino acid

The amino acid analysis reveals distinct concentrations in fresh date juice and alcoholic beverage (Fig. 2). In fresh date juice, notable values include glutamic acid (0.887), threonine (0.766), ornithine (0.231), lysine (0.028), and DOPA (1.482). Conversely, some amino acids such as serine, histidine, amino butyric acid, methionine, tryptophan, and leucine were not detected. In alcoholic beverage, the amino acid profile is characterized by a higher concentration of glutamic acid (4.492), along with notable amounts of histidine (0.035), threonine (0.735), DOPA (1.445), lysine (0.012), and ornithine (0.698). The presence of histidine in alcoholic beverage, absent in fresh date juice, underscores the impact of fermentation on amino acid development. These increased values compared to fresh juice signify the fermentation impact, and these results provide valuable insights into the specific amino acid compositions of each beverage, contributing to our understanding of their nutritional distinctions [48]. Wine, being a fermented product, demonstrates elevated levels of specific essential amino acids compared to fresh date juice. For instance, histidine and threonine are detected in alcoholic beverage but not in fresh date juice, suggesting potential nutritional advantages. Additionally, amino acids like isoleucine and leucine are more abundant in alcoholic beverage, enhancing its nutritional value. This discrepancy is attributed to the fermentation process in wine production, wherein yeast and microorganisms modify and synthesize amino acids, resulting in a diverse amino acid profile. In contrast, fresh date juice, while rich in certain amino acids, may lack the diversity and concentrations found in alcoholic beverage due to the absence of fermentation processes. However, the nutritional significance of fresh date juice lies in its natural, unaltered amino acid composition, contributing to a balanced diet. In summary, while alcoholic beverage offers a broader spectrum of essential amino acids due to fermentation, fresh date juice serves as a natural and unprocessed source of these vital nutrients. Hernández-Orte et al. [49] analyzed the levels of 20 amino acids and ammonium present in must and wine derived from the Airen variety in the La Mancha Designation of Origin region (Spain). Arginine and proline were found to be the predominant amino acids in the must, with concentrations of 214.6 mg L^{-1} and 122.05 mg L^{-1}, respectively. Conversely, methionine and glycine exhibited the lowest concentrations, measuring 1.52 mg L^{-1} and 2.54 mg L^{-1}, respectively. In the alcoholic beverage, proline and arginine continued to display the highest concentrations at 94 mg L^{-1} and 33.77 mg L^{-1}, respectively, while isoleucine and leucine were among the lowest with concentrations of 0.46 mg L^{-1} and 0.90 mg L^{-1}, respectively.

In the present study, the fermentation period was shorter (5 days); therefore, the yeast might not have fully consumed all the available nutrients in the must, including amino acids. During a typical fermentation process, yeast prioritizes consuming sugars for energy and growth but also requires amino acids and other nutrients to function effectively. In a shortened fermentation period, the yeast may not fully ferment all the sugars, resulting in a alcoholic beverage with residual sweetness and potentially incomplete amino acid consumption.

Residual amino acids left in the alcoholic beverage can influence its flavor profile and stability, and may also serve as nutrients for spoilage microorganisms. Therefore, a shorter fermentation period can result in incomplete consumption of amino acids by the yeast, impacting the fermentation process, the final composition of the alcoholic beverage, and its sensory characteristics. Proper fermentation management is crucial to ensure that yeast has sufficient time to utilize both sugars and amino acids effectively, resulting in a well-balanced and stable alcoholic beverage.

3.5 Phenolic compounds identified via UHPLC

Phenolic compounds are another bioactive compounds known for its versatile nutraceutical properties. In addition to their potent antioxidant properties, phenolic compounds are crucial to the flavour and stability of wine [50]. The majority of the anthocyanins and phenolic compounds found in wine come from fruits; however, a variety of conditions, such as pH, yeast, storage temperature and duration, and light exposure during the fermentation process, can impact these

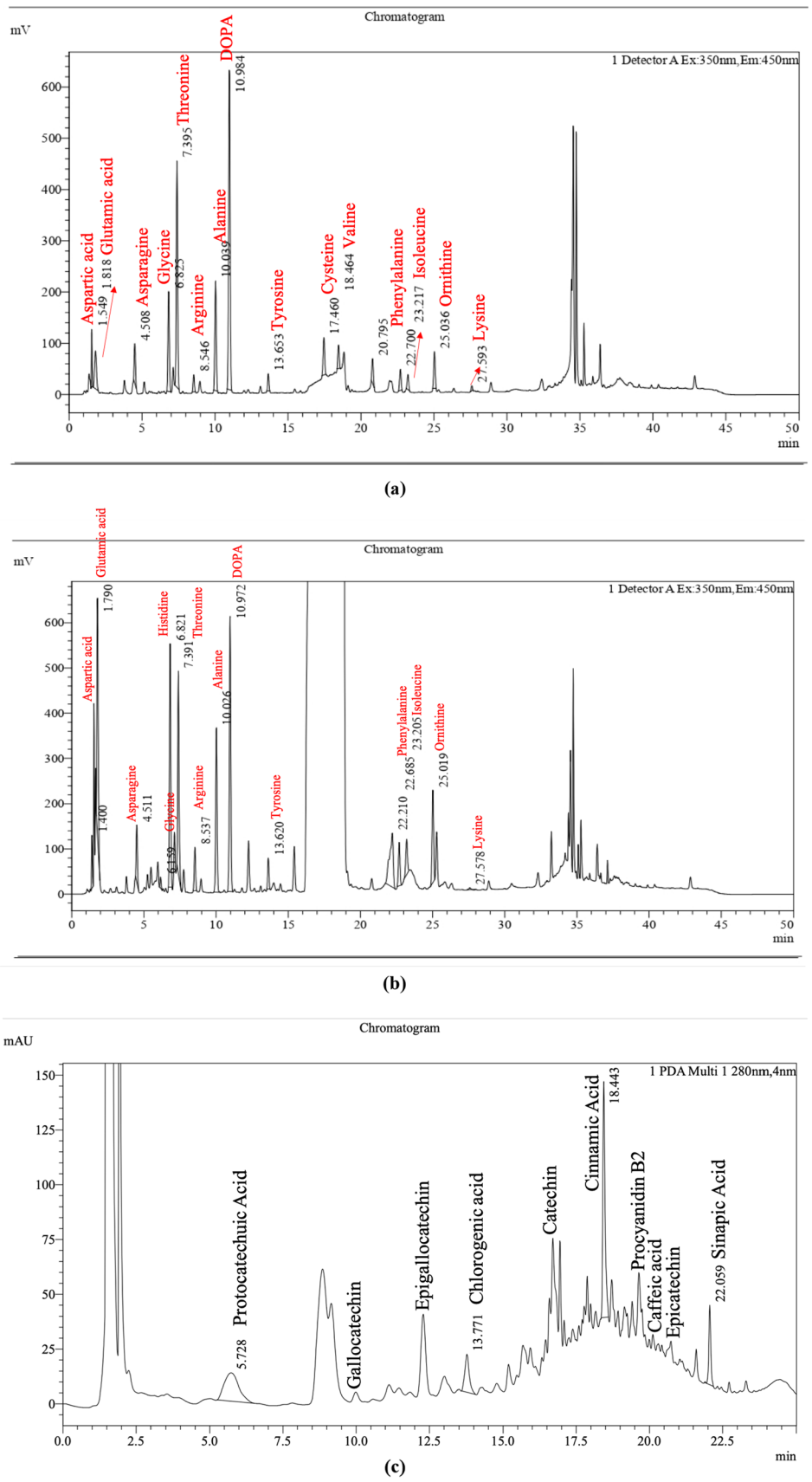


Fig. 2 Amino acid profile **a** Fresh dates juice **b** Alcoholic beverage; **c** Phenolic acid determined via UP-HPLC

compounds' stability and composition [51, 52]. Strong light will break down the phenolic acids and anthocyanins in alcoholic beverage. Color stability of alcoholic beverage, taste quality, nutritional value, and consumption can all be impacted by changes in the composition and concentrations of anthocyanins and phenolic compounds [53, 54]. Figure 2c presents the various phenolic compounds identified in the alcoholic beverage. It was noticed that the alcoholic beverage contains several important phenolic compounds such as protocatechuic acid, gallocatechin, epigallocatechin, chlorogenic acid, catechin, cinnamic acid, procyandin B2, caffeic acid, epicatechin and sinapic acid. It was reported that sinapic acid has various beneficial effects on human health such as anti-inflammatory, antioxidant, antibacterial and anticancer activities [55, 56]. Protocatechuic, caffeic, *p*-coumaric and ellagic acids were found in all investigated samples, while gallic, chlorogenic and gentisic acids were found in almost all samples. Previous studies have reported the significant increase of phenolic acids during wine fermentation. Yue et al. [50] demonstrated that addition of mannoprotein in wine during storage had the opposite effect, adding MP prior to fermentation greatly increased the wine's TPC, total anthocyanin content (TTC), total tannin content (TTC), total flavonoid content, and total flavanol content.

3.6 Identification of metabolites in juice and alcoholic beverage via HR-LCMS

The identified compounds in both fruit and alcoholic beverage samples revealed a diverse array of bioactive components, each with potential health attributes (Table 3). Among the compounds identified in the alcoholic beverage samples, notable findings include isoquercetin, which is known for its antioxidant properties and potential cardiovascular benefits, and rutin, which has been associated with anti-inflammatory and anti-cancer effects [57–59]. Additionally, compounds such as luteolin and apigenin hexoside are flavonoids with reported anti-inflammatory and neuroprotective properties. Ferulic acid, found in alcoholic beverage samples, possesses antioxidant and anti-inflammatory properties, potentially contributing to overall health benefits [60]. Glutamic acid isomer, leucine isomer, and isorhamnetin-3-*O*-glucoside are among the identified compounds known for their roles in metabolism and cellular function [61]. In contrast, compounds detected in fruit samples, such as caffeic acid hexoside and *p*-coumaric acid, are associated with antioxidant and anti-inflammatory activities, while catechin and quercetin are flavonoids with recognized cardiovascular benefits [1]. These findings underscore the potential health-promoting properties of both fruit and alcoholic beverage, highlighting the importance of dietary inclusion of such bioactive compounds for overall well-being.

3.7 Color properties

The color analysis of the alcoholic beverage at different stages, represented by L, a, and b values, provides valuable information about the quality and acceptability of the final product. The L value, which represents the lightness or darkness of the color, decreased from the fresh date (88.31) to 0 days (86.12), 2 days (84.42), and 4 days (78.22), indicating a gradual darkening of the alcoholic beverage over the fermentation period (Fig. 3a). This change in L value may be attributed to the accumulation of pigments or other compounds during the fermentation process. The a value, representing the green to red spectrum, increased from the fresh date (0.27) to 0 days (0.58), 2 days (0.72), and 4 days (0.46). This suggests a shift towards a more reddish hue in the alcoholic beverage as fermentation progressed, possibly influenced by chemical changes in the composition. The b* value, indicating the blue to yellow spectrum, showed an increase from the fresh date (8.91) to 0 days (13.45), 2 days (15.62), and 4 days (14.23). This suggests a progression towards a more yellowish color in the alcoholic beverage, which could be linked to the transformation of compounds during fermentation [50]. Finally, the overall color of the alcoholic beverage at the end of the fermentation period reflects a balance between lightness, redness, and yellowness. The acceptability of the color is subjective and varies among consumers. However, it is common for consumers to associate certain color characteristics with specific wine varieties and styles [1]. For example, a balanced and rich color, as indicated by the L, a, and b values of the final alcoholic beverage, may contribute positively to the perceived quality and acceptability of the product [9]. Winemakers often aim to achieve a visually appealing color that aligns with the expectations of their target market.

3.8 Sensory evaluation

The sensory evaluation results for both alcoholic beverage and juice provide valuable insights into the perceived attributes by panellists. In terms of appearance, the alcoholic beverage scored 8.6 ± 0.13, slightly higher than the juice at 8.4 ± 0.34, indicating a favorable visual impression for the alcoholic beverage (Fig. 3b). Similarly, both the

Table 3 Compounds identified in fresh dates alcoholic beverage via HR-LCMS

S. No	Fruit juice	Alcoholic beverage
Characterization of determined compounds using ES + mode		
1	Apigenin pentosyl hexoside	Pterolactam
2	Gluconic acid	Miraxanthin-I
3	Caffeic acid hexoside	Phentermine
4	p-Coumaroyl hexose	Leteolin hexoside
5	O-dicaffeoyl shikimic acid	Apigenin
6	Isoquercetin	Quercetin rhamnosyl dihexoside
7	Dihydrokaempferol hexoside	Hydroxy octadecadienoic acid
8	Isoquercetin sulfate	Pinenol-O-glucoside
9	Chrysoeriol	Trihydroxy-octadecenoic acid
10	Rutin	Gluconic acid
11	Protocatechuic acid hexoside	Isoquercetin
12	(5-Heptyl-6-methyloctahydroindolizin-8-yl)methanol	Dihydrokaempferol hexoside
13	Eriodictyol xyloyldeoxy hexose	Isoquercetin sulfate
14	Luteolin hexoside	Chrysoeriol
15	O-dicaffeoyl shikimic acid	Rutin
16	Luteolin	Protocatechuic acid hexoside
17	Caffeic acid hexoside	Trihydroxy-octadecenoic acid
18	Ferulic acid	Syringic acid
19	Glutamic acid isomer	p-coumaric acid
20	Leucine isomer	t-Piceatannol
21	Isorhamnetin-3-O-glucoside	Vanillic acid
22	Guanosine	Catechin
23	Leteolin hexoside	Kaempferol
24	Apigenin	Delphindin
25	Quercetin rhamnosyl dihexoside	Malvidin
26	Hydroxy octadecadienoic acid	Peonidin-3-O-glucoside
27	Pinenol-O-glucoside	Quercetin
28	Trihydroxy-octadecenoic acid	Myricetin
Characterization of determined compounds using ES- mode		
1	Isoflavone	L-Glyceric acid
2	Asparagine	Quinic acid
3	Glutamine	7,9-Dimethyluric acid
4	1,5-diaminopentyl) phosphonic acid isomer 1	beta-D-Glc-(1- > 4)-alpha-L-Rha-(1- > 3)-beta-D-Glc
5	1,5-diaminopentyl) phosphonic acid isomer 2	3,5-Dihydroxyphenyl 1-O-(6-O-galloyl-beta-D-glucopyranoside)
6	Malic acid	3-b-Galactopyranosyl glucose
7	Succinic acid	L-Ribulose
8	Pyroglutamic acid isomer 2	Glutamine
9	Protocatechuic acid	2-Dehydro-3-deoxy-D-glucarate
10	Quinic acid	Altersolanol A
11	7,9-Dimethyluric acid	Trigoneoside XIIb
12	beta-D-Glc-(1- > 4)-alpha-L-Rha-(1- > 3)-beta-D-Glc	Cortisone
13	Glucoconvallasaponin B	9S,12S,13S-trihydroxy-10E-octadecenoic acid
14	11S-HpODE	Malic acid
15	Remikiren	Succinic acid
16	Hexazinone	Pyroglutamic acid isomer 2

Table 3 (continued)

S. No	Fruit juice	Alcoholic beverage
17	Graecunin G	Protocatechuic acid
18	Glucoconvallasaponin B	Quinic acid

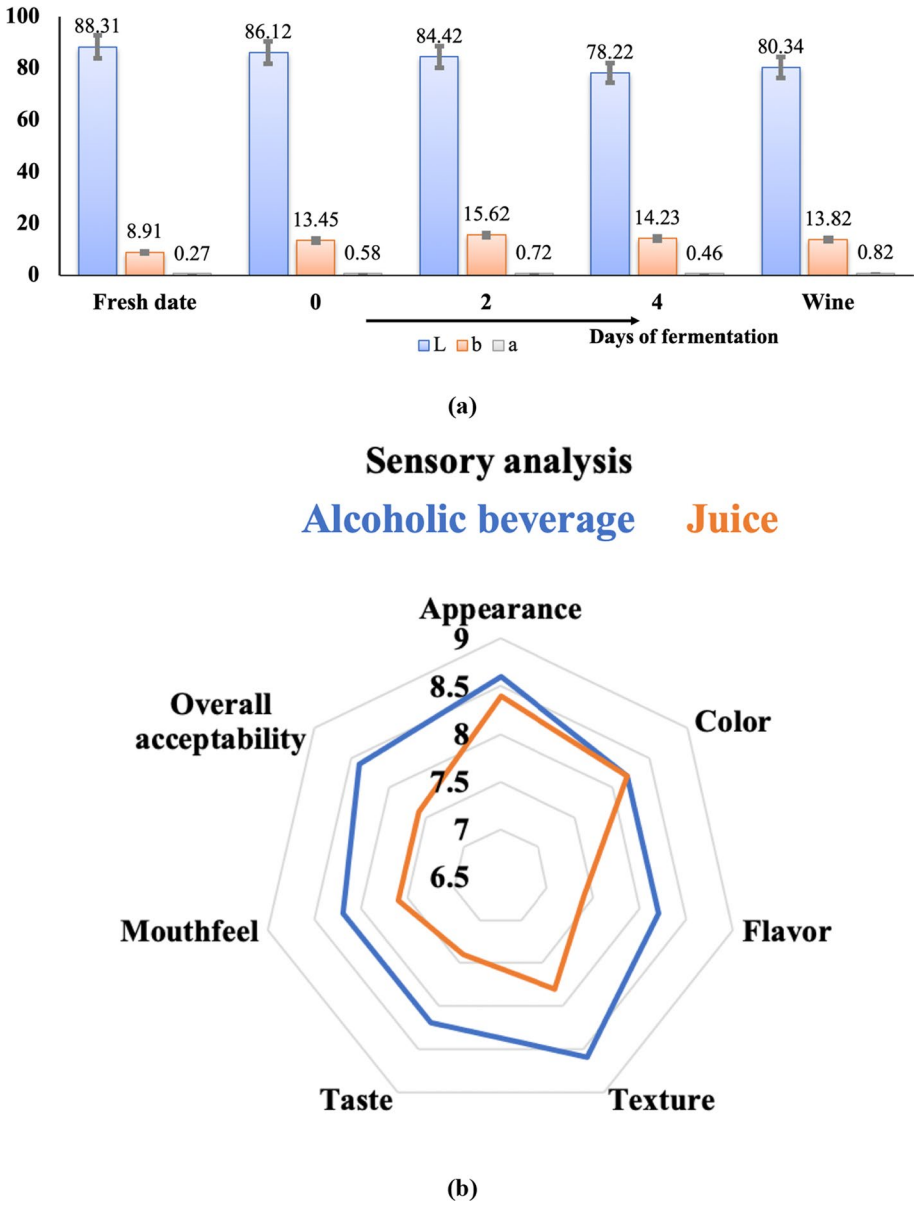


Fig. 3 **a** Color values of fresh dates juice and Alcoholic beverage; **b** Sensory analysis of fresh dates juice and Alcoholic beverage

alcoholic beverage and juice did not have any significant difference ($p < 0.05$) in color, suggesting that the coloration of both beverages was well-received by the evaluators. Moving on to flavor, the v scored 8.2 ± 0.19, outpacing the juice which obtained a score of 7.4 ± 0.10, indicating a more preferred flavor profile in the alcoholic beverage. In terms of texture, the alcoholic beverage again garnered a higher score of 8.6 ± 0.12 compared to the juice at 7.8 ± 0.09, suggesting a more satisfying textural experience in the alcoholic beverage. The taste attribute also favored the alcoholic beverage with a score of 8.2 ± 0.17, while the juice scored slightly lower at 7.4 ± 0.15. The same trend continued for mouthfeel, where the alcoholic beverage achieved a score of 8.2 ± 0.18, surpassing the juice at 7.6 ± 0.13. Finally, in the overall acceptability category, the wine received a higher score of 8.4 ± 0.19 compared to the juice at 7.6 ± 0.18,

indicating a greater level of satisfaction with the overall sensory experience of the alcoholic beverage. These results reflect the subjective preferences of the panelists, indicating a slight preference for the alcoholic beverage over the juice in terms of appearance, flavor, texture, taste, mouthfeel, and overall acceptability. However, due to the small difference in averaged values of juice and alcoholic beverage, it is important to interpret these findings cautiously before attributing a significantly superior sensory profile to the alcoholic beverage. Previous studies have noted the wine's perceptible sweetness, though not to the extent found in sweet wines. The pleasant aroma observed in the wines may be attributed to their higher alcohol content, as reported by Clemente-Jimenez [62].

In conclusion, the evolution of key parameters such as TSS, pH, and titratable acidity throughout the fermentation period demonstrates the intricate transformations occurring in the chemical composition of the fermenting liquid. The observed microbial load during the fermentation period, indicative of alcoholic fermentation, contributes to the understanding of the interplay between microbial activity and key parameters crucial for achieving desired sensory characteristics and stability. The observed reduction in TPC, while TFC showed a substantial increase, indicating the complex transformations in phenolic compounds. These changes contribute to the sensory attributes and potential health benefits of the alcoholic beverage. The maintenance of ABTS antioxidant activity throughout fermentation emphasizes the ability of alcoholic beverage to preserve its antioxidant properties, offering potential health advantages associated with the consumption of antioxidant-rich beverages. Amino acid analysis revealed distinct concentrations in both fresh date juice and alcoholic beverage, with alcoholic beverage exhibiting higher levels of specific essential amino acids due to the shorter fermentation period. The balance between lightness, redness, and yellowness contributes to the perceived quality and acceptability of the alcoholic beverage. The sensory evaluation results further highlighting the subjective preferences of the panellists that need to be further explore in future. Future research could focus on optimizing fermentation conditions and yeast strains to enhance sensory attributes and phenolic transformations by investigating microbial dynamics during fermentation. Additionally, understanding consumer preferences through sensory evaluations could inform targeted marketing and product development.

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Data availability Data will be available upon request.

Declarations

Ethics approval and consent to participate Ethical approval was obtained from the Department of Food Science & Technology, GEU. Additionally, proper approval was obtained from the Institutional Biosafety Committee of GEU. The committee confirmed the non-toxic nature of the sample before sensory analysis. Furthermore, written consent was obtained from the participants for their willingness to participate in the analysis.

Consent for publication Not applicable.

Competing interests No competing interest with the present submission. Authors declares they don't have any competing interests with present submission.

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