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## Standardization of herbal blended wine for pilot-scale production and storability in Bael fruit, *Aegle marmelos* (L.) Correa

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### Abstract

Underutilized fruits, such as Bael, *Aegle marmelos* (L.) Correa possesses immense potential for value addition due to their nutritional and medicinal properties. This study explores the development and evaluation of herbal blended wines using Bael fruit as a substrate, with an emphasis on incorporating *Centella asiatica* (L.) Urban leaves extract and *Chrysopogon zizanioides* (L.) Roberty (Vetiver) root extract to enhance sensory and functional qualities. The research was conducted at the University of Agricultural Science, Dharwad, during 2023-2024, employing nine treatments with varying concentrations (2, 4, 6, and 8%) of herbal blends (*C. asiatica* and *C. zizanioides*) along with an untreated control. The results revealed that among the different herbal blends, T<sub>6</sub> (Bael fruit juice + 2% *C. zizanioides* root extract) emerged as the optimal formulation, achieving significantly high (9.09%) alcohol content, low reducing sugar (6.62 mg/ml), and recorded the highest sensory evaluation scores, including appearance (4.5), aroma (4.4), and overall acceptability (4.3) on a 5-point hedonic scale. The efficient herbal blend (2% vetiver) was further selected for parameter optimization (inoculum size, pH, and temperature) for pilot-scale production of Bael blended wine. Vetiver blended Bael juice at pH-4.5, added with 3% inoculum at 30°C temperature, was found to be ideal for large-scale production. The resulting optimum wine parameters were used for pilot-scale production of Bael blended for a storability study, and wine quality parameters were observed to improve over a 90-day storage period. These findings demonstrate the commercial viability of Bael-based herbal wines, aligning with consumer preferences for innovative, sustainable, and health-oriented beverages. The research contributes to the diversification of the beverage industry while promoting the utilization of underexploited fruits and traditional herbs.

### 1. Introduction

Herbal blended wines are unique, combining traditional wine making techniques with the infusion of medicinal herbs, roots, or other botanicals to create distinctive flavors and potential health benefits. This innovative approach offers an exciting opportunity to explore the synergy between wine and herbal medicine. Herbal blended wines are crafted using herbs rich in tannins, polyphenols, and titratable acidity, resulting in extracts that contain an abundance of esters and aldehydes, which offer both nourishing and therapeutic qualities (Vaishali, 2018; Ahmed *et al.*, 2017). Bael (*A. marmelos*) is an underutilized fruit species, belonging to the family Rutaceae. Fruits are available in seasonal surpluses during specific parts of the year and are highly perishable (Kumar *et al.*, 2011; Mali *et al.*, 2020). Fully ripe fruits reported to contain bioactive compounds such as carotenoids (2.5-3.5 mg/100 g), phenolics (120-150 mg/100 g), alkaloids (0.5-1.0% of dry weight), pectin (2.0-3.0% of dry weight), tannins (0.30-0.55 mg/ml), coumarins (0.1-0.2%), flavonoids (10-15 mg/100 g), and terpenoids (0.5-1.0%) and traditionally used to treat chronic diarrhoea, diabetes, hypertension, and stomach disorders

(Maity *et al.*, 2009; Tiwari *et al.*, 2018; Yadav *et al.*, 2018). Hence, processing, especially wine making, can play a crucial role in the conservation and better utilization of fruits, allowing for the surplus to be utilized during the off-season (Singh and Kaur, 2009). Therefore, value addition can be a potential remedy for the preservation of Bael fruits, primarily through fermentation techniques.

In the present study, two important herbs, abundantly available in the Uttara Kannada District, *C. asiatica* (Indian penny wort leaves) and *C. zizanioides* (Vetiver roots), were selected for blending due to their medicinal and aromatic properties. *C. asiatica* is used as a *Medhya* drug for the treatment of memory-related disorders, epilepsy, and Alzheimer's disease, and enhances cognition (Singh *et al.*, 2008; Brinkhaus *et al.*, 2000). The use of *C. asiatica* in food and beverages has increased over the years, basically due to its health benefits, anti-inflammatory activity, and memory-enhancing property (Subathra *et al.*, 2005; Ullah *et al.*, 2009; Pitella *et al.*, 2009; Kimura *et al.*, 2008). Likewise, Vetiver, *C. zizanioides* is a perennial aromatic grass characterized by the production of dense biomass and growth of a large, strong, fibrous root system (Sharma, 2024a). Medicinally, roots possess cooling properties used in aromatherapy, relieving stress and treating rheumatism, paralysis, arthritis, and gouty joints (Lunz and Stappen, 2021; Sharma, 2024b). The acceptance of wine is primarily influenced by its aroma and taste. Herbs and spices play a crucial role in the creation of alcoholic beverages, serving as sources of antioxidants, preservatives, and flavour enhancers. Wine production merges individual creativity with cutting-edge technology,

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embodying both art and science (Thalisa *et al.*, 2012). With this background, the investigation was planned to study the influence of various concentrations of herbal blends (*C. asiatica* and *C. zizanioides*) in Bael wine, including optimization of fermentation parameters and pilot-scale production of herbal blended wine in Bael.

## 2. Materials and Methods

The study was conducted at the Department of Food Safety and Quality Assurance Laboratory, College of Community Science, Dharwad, and at the College of Forestry, Sirsi, University of Agricultural Science, Dharwad, during the year 2023-2024.

### 2.1 Standardization of herbal blending

#### 2.1.1 Sample collection and extraction

Fully ripened, disease-free Bael fruits and herbs (*Centella asiatica* leaves and *Chrysopogon zizanioides* roots) were collected from the farmer's fields of the Uttara Kannada District. The plant (*Aegle marmelos*) sample was authenticated by Dr. Vinayak Upadhyya, Plant Taxonomist, University of Agricultural Sciences, Dharwad, India, and was given an identification number, RMRC-1049. Bael fruit pulp

was scooped and blended with an equal weight of water (1:1 w/v ratio), and the juice was then filtered through a cheesecloth.

The known weight of freshly harvested *C. asiatica* leaves was washed and ground with 1:1 (leaves: water ratio) distilled water, and the juice was strained with cheesecloth. Similarly, the known weight of dried *C. zizanioides* root was boiled in distilled water (1:2 ratio) for 20 min and strained through cheesecloth.

#### 2.1.2 Preparation of inoculum

The yeast strain *Saccharomyces cerevisiae* MTCC 178 was obtained from the Microbial Type Culture Collection (MTCC), Dharwad, and it was maintained on Malt Extract-Glucose-Yeast Extract-Peptone (MGYP) agar slants under refrigeration. A loopful of the yeast culture was transferred to 6 ml of MGYP broth and incubated at 37°C for 24 h to obtain a fresh culture. Following incubation, 1 ml of this broth was added to 99 ml of a 1% sterilized sucrose solution and incubated for 3 h before inoculation.

#### 2.1.3 Fermentation of samples

The experiment was laid out in a completely randomized design with nine treatments and three replications as follows:

T <sub>1</sub>	Control - 100% fruit juice
T <sub>2</sub>	Fruit pulp + 2% <i>C. asiatica</i> extract (aqueous extract of 2 g of fresh <i>C. asiatica</i> leaves blended with 98 g of fruit pulp)
T <sub>3</sub>	Fruit pulp + 4% <i>C. asiatica</i> extract (aqueous extract of 4 g of fresh <i>C. asiatica</i> leaves blended with 96 g of fruit pulp)
T <sub>4</sub>	Fruit pulp + 6% <i>C. asiatica</i> extract (aqueous extract of 6 g of fresh <i>C. asiatica</i> leaves blended with 94 g of fruit pulp)
T <sub>5</sub>	Fruit pulp + 8% <i>C. asiatica</i> extract (aqueous extract of 8 g of fresh <i>C. asiatica</i> leaves blended with 92 g of fruit pulp)
T <sub>6</sub>	Fruit pulp + 2% <i>C. zizanioides</i> extract (aqueous extract of 2 g of dry <i>C. zizanioides</i> roots blended with 98 g of fruit pulp)
T <sub>7</sub>	Fruit pulp + 4% <i>C. zizanioides</i> extract (aqueous extract of 4 g of dry <i>C. zizanioides</i> roots blended with 96 g of fruit pulp)
T <sub>8</sub>	Fruit pulp + 6% <i>C. zizanioides</i> extract (aqueous extract of 6 g of dry <i>C. zizanioides</i> roots blended with 94 g of fruit pulp)
T <sub>9</sub>	Fruit pulp + 8% <i>C. zizanioides</i> extract (aqueous extract of 8 g of dry <i>C. zizanioides</i> roots blended with 92 g of fruit pulp)

Thus, herbal blended fruit juice was added uniformly with sugar up to 24°B and 150 ppm potassium metabisulfite (KMS) to inhibit the growth of unwanted microorganisms.

Further, the juice was added with yeast inoculum that had a concentration of 10<sup>6</sup> colony-forming units per ml (CFU/ml), and was set aside for fermentation. Initially, it was incubated under aerobic conditions for 24 h, and subsequently, anaerobic conditions were established by sealing the fermentation flasks with rubber stoppers, equipped with nylon tubes to capture carbon dioxide. The incubation continued until CO<sub>2</sub> production ceased.

### 2.2 Optimization of fermentation parameters in selected blended wine

Based on the alcohol percent, reducing sugar content, and sensory evaluation results, the most acceptable herbal blend combination was selected for parameter optimization. Three levels of inoculum size (1, 2, and 3%), pH (3.5, 4.5, and 5.5), and incubation temperature (25, 30, and 35°C) were imposed along with an untreated control to evaluate the best wine parameters for fermentation of Bael wine. The pH of fruit juice was adjusted to various levels by the addition of saturated citric acid or calcium carbonate solutions.

### 2.3 Pilot-scale production of herbal blended Bael wine

The optimal fermentation parameters were a herbal blend combination of fruit wine, with refined fermentation parameters, which was further analysed for storage stability at intervals of T<sub>1</sub>(30 days), T<sub>2</sub>(45 days), T<sub>3</sub>(60 days), T<sub>4</sub>(75 days), and T<sub>5</sub>(90 days) post-fermentation.

### 2.4 Biochemical evaluation

**Alcohol percent:** The ethanol content in the wine sample was estimated using the colorimetric method as described by Cauputi *et al.* (1968) and Lehninger *et al.* (2008) and expressed in per cent.

**Reducing sugar content:** The reducing sugar was estimated by the 3, 5-dinitro salicylic acid method (Miller, 1959) and expressed in mg/ml.

**pH:** pH of the wine was determined with a digital pH meter.

**Total soluble solids (TSS):** The TSS of blended wine was determined using an ERMA hand refractometer having a range of 0-32°B, and the readings were expressed as °B.

**Titrateable acidity:** It was estimated by using the titration method expressed in terms of citric acid (Ranganna, 1986).

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times 0.0064}{\text{The volume of the aliquot taken}} \times 100$$

**Total phenolic content (TPC):** It was estimated using the Folin-Ciocalteu method, as described by Singleton and Rossi (1965), using gallic acid standard and expressed as (mg/ml).

**Tannins (mg/ml):** Tannin estimation will be made as per the procedure cited in the Handbook of Analysis and Quality Control (Ranganna, 1986) by using Folin denins reagent and tannic acid standard.

**Total sugars and reducing sugars (%):** Lane and Eyon's titration method was followed as suggested by Ranganna (1986). The titre value was used for calculations of total sugars and reducing sugars, and expressed in %.

$$\text{Total reducing sugars (\%)} = \frac{\text{Factor (0.052)} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{Weight of sample}}$$

## 2.5 Sensory evaluation

The prepared wine was subjected to sensory evaluation using a 5-point Hedonic scale, and grading based on the quality parameters, such as colour, appearance, taste, flavour, aroma, and mouth feel, was analysed by a panel of 10 judges on a 5-point Hedonic scale (Amerine *et al.*, 1972).

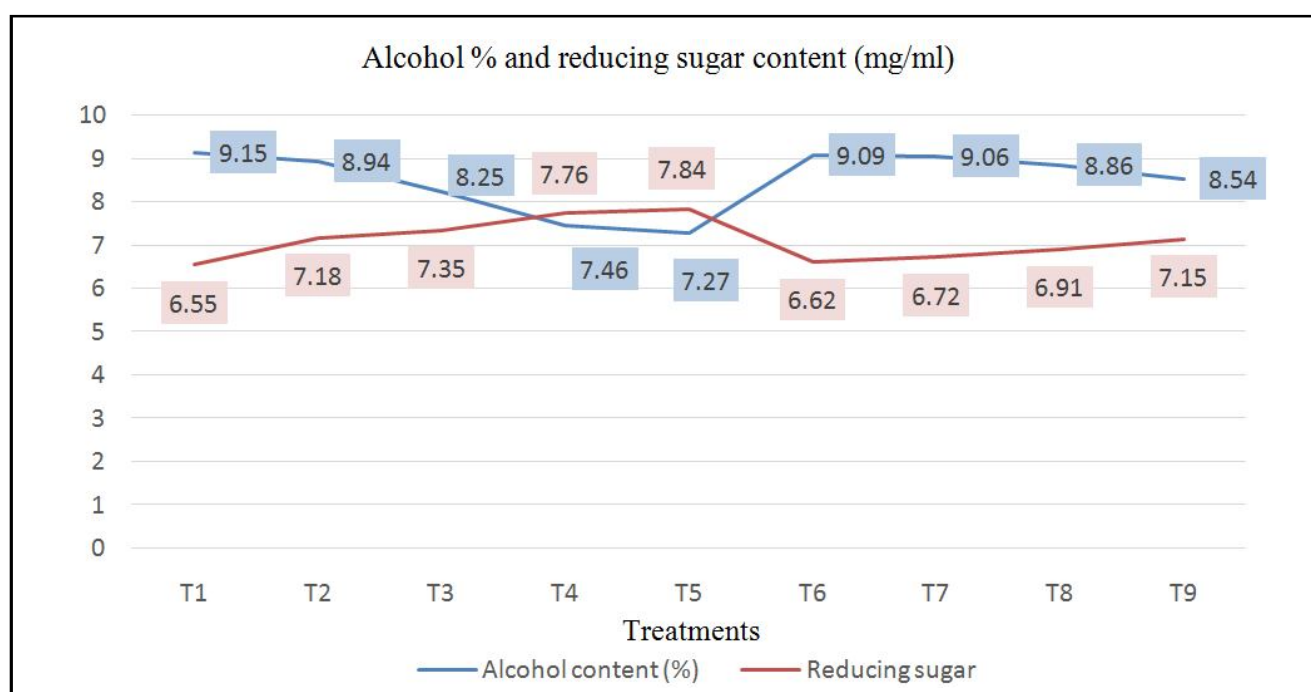
## 2.6 Statistical analysis

The results obtained were analysed statistically using a completely randomized design as described by Khan and Khanum (2004).

## 3. Results

### 3.1 Influence of various herbal blends on the quality of Bael wine

The results of the effect of different concentrations of herbal blends on alcohol percentage and reducing sugar content are depicted in Figure 1.



**Figure 1: Influence of different concentrations of herbal blends on alcohol percentage and reducing sugar content in Bael wine.**

Among the different herbal blends, Bael juice with 2% *C. zizanioides* root ( $T_6$ ) recorded a significantly high alcohol content (9.09%), followed by  $T_7$  (Bael juice with 4% *C. zizanioides* root), which was comparable to the control (9.15%). Even though, the lowest reducing sugar content (6.55 mg/ml) was observed in the control, significantly lower reducing sugar content (6.62 mg/ml) was noted in  $T_6$  (2% *C. zizanioides* roots), followed by  $T_7$  (4% *C. zizanioides* roots blend, 6.72 mg/ml). As the alcohol percentage increased, the reducing sugar levels decreased gradually during the fermentation process of Bael fruit juice.

### 3.2 Influence of herbal blends on sensory parameters of Bael wine

The sensory evaluation of the herbal blended Bael wine was conducted using the 5-point Hedonic scale, focusing on appearance,

colour, aroma, acidity, sweetness, body, astringency, and overall acceptability (Table 1). Among all the herbal blends,  $T_6$  (2% *C. zizanioides* roots blend) consistently received the highest scores across most sensory attributes, particularly for appearance (4.5), colour (4.4), aroma (4.4), body (4.3), and overall acceptability (4.3). Thus, *C. zizanioides* root extract, particularly at lower concentrations (2-4%), positively influences the sensory qualities of Bael wine.

Based on the quality parameters (alcohol and reducing sugar content) and sensory evaluation results, the best herbal blend combination for Bael wine was selected for parameter optimization. Among the different blends,  $T_6$  (2% *C. zizanioides* root), which scored maximum alcohol (9.09%) and the least reducing sugar content (6.62 mg/ml) with the highest overall acceptability (4.3), was selected for parameter optimization in Bael wine.

**Table 1: Sensory evaluation of herbal blended Bael (*A. marmelos*) wine**

Treatments	Appearance	colour	Aroma	Acidity	Sweetness	Body	Astringency	Overall acceptability
<b>Hedonic scale</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>
T <sub>1</sub> : Control	3.9	4.3	3.5	3.8	3.7	3.9	3.5	3.9
T <sub>2</sub> : Bael pulp + 2% <i>C. asiatica</i> extract	3.5	3.8	3.3	3.5	3.1	3.2	3.7	3.7
T <sub>3</sub> : Bael pulp + 4% <i>C. asiatica</i>	3.3	3.3	3.2	3.3	3.0	3.3	3.3	3.3
T <sub>4</sub> : Bael pulp + 6% <i>C. asiatica</i>	3.6	3.4	3	3.4	3.2	3.3	3.3	3.7
T <sub>5</sub> : Bael pulp + 8% <i>C. asiatica</i>	3.8	3.8	2.9	3.5	3.3	3.6	3.1	3.5
T <sub>6</sub> : Bael pulp + 2% <i>C. zizanioides</i> root extract	4.5	4.4	4.4	3.9	3.8	4.3	3.8	4.3
T <sub>7</sub> : Bael pulp + 4% <i>C. zizanioides</i>	4.3	4.0	4	3.5	3.7	3.8	4.0	3.9
T <sub>8</sub> : Bael pulp + 6% <i>C. zizanioides</i>	3.8	3.7	3.7	3.5	3.4	3.7	3.5	3.8
T <sub>9</sub> : Bael pulp + 8% <i>C. zizanioides</i>	3.7	3.5	2.9	3.3	3.1	3.5	3.2	3.6
Commercial red wine (Control 2)	4	4.5	4	4	4	4	3.5	4.5

Scale: Like extremely - 5, Like very much - 4.5, Like moderately - 4, Like slightly - 3.5, Neither like nor dislike - 3, Dislike slightly - 2.5, Dislike moderately - 2, Dislike very much - 1.5, Dislike extremely - 1.

### 3.3 Influence of inoculum size, pH and temperature on quality of Bael blended wine (2% *C. zizanioides*)

Quality of Bael blended wine (2% *C. zizanioides* root extract) as influenced by various fermentation parameters, viz., inoculum size,

pH, and temperature, is presented in Table 2. Among the different levels of inoculum concentration, the highest alcohol content (9.63%) and the lowest reducing sugar (6.05 mg/ml) were observed at 3% inoculation.

**Table 2: Influence of different inoculum size, pH, and temperature on alcohol and reducing sugar content of herbal blended Bael wine**

Parameters	Treatments	Alcohol content (%)	Reducing sugar content (mg/ml)
<b>Inoculum size</b>	T <sub>1</sub> : Control	6.41	9.54
	T <sub>2</sub> : 1%	9.07	6.67
	T <sub>3</sub> : 2%	9.38	6.39
	T <sub>4</sub> : 3%	9.63	6.05
	SE <sub><math>\bar{x}</math></sub> ( $\pm$ )	0.0201	0.0207
	CD (1%)	0.0866	0.0894
	F test	**	**
<b>pH</b>	T <sub>1</sub> : Control	9.62	6.03
	T <sub>2</sub> : 3.5	8.46	7.21
	T <sub>3</sub> : 4.5	9.66	6.01
	T <sub>4</sub> : 5.5	8.31	7.33
	SE <sub><math>\bar{x}</math></sub> ( $\pm$ )	0.0129	0.0116
	CD (1%)	0.0558	0.0503
	F test	**	**
<b>Temperature</b>	T <sub>1</sub> : Control	9.68	6.01
	T <sub>2</sub> : 25°C	7.90	8.05
	T <sub>3</sub> : 30°C	9.71	6.00
	T <sub>4</sub> : 35°C	8.12	7.95
	SE <sub><math>\bar{x}</math></sub> ( $\pm$ )	0.0067	0.0076
	CD (1%)	0.0289	0.0327
	F test	**	**

\*\* Significant at 1% level

Likewise, maximum alcohol content (9.66%) was detected at pH 4.5, as compared to higher and lower levels of acidity (Table 2). Furthermore, the lowest reducing sugar concentration (6.01 mg/ml) was also observed at the same pH (4.5). A slightly acidic environment is optimal for yeast fermentation, promoting efficient sugar conversion and high alcohol production. In contrast, the lowest alcohol content was observed at pH 5.5 (8.31%), indicating that higher pH levels may inhibit yeast activity and reduce fermentation efficiency. Among the different temperature levels, the highest alcohol (9.71%) and the lowest reducing sugar (6.00 mg/ml) were observed at 30°C. On the other hand, both lower (25°C) and higher temperatures (35°C) were recorded to have significantly lower alcohol content.

### 3.4 Sensory parameters as influenced by inoculum size, pH, and temperature in Bael blended wine

The sensory evaluation of herbal blended Bael wine, as influenced by various inoculum sizes, pH levels, and temperatures, was conducted using a 5-point Hedonic scale and is presented in Table 3. The highest scores across all sensory parameters like appearance (4.2), colour (4.4), aroma (4.5), acidity (4.0), sweetness (4.3), body (4.3), astringency (4.2), and overall acceptability (4.3) were recorded in T<sub>4</sub> (3% inoculum size) and T<sub>3</sub> (pH 4.5, temperature 30°C), reflecting optimum fermentation conditions. The sensory evaluation indicated that a combination of 3% inoculum size, pH 4.5, and a temperature of 30°C was remarked to be optimum for producing herbal blended Bael wine with the best sensory characteristics. This optimized blend produced wines with superior appearance, colour, aroma, acidity, sweetness, body, astringency, and overall acceptability, making them excellent candidates for consumer preference and pilot-scale production.

### 3.5 Influence of storage period on the quality of herbal blended wine in Bael

A pilot-scale production of herbal blended (2% *C. zizanioides* root extract) Bael wine was produced with optimized wine parameters, and various quality traits were studied over 90 days of storage. Influence of storage period on quality of herbal blended Bael wine, such as pH, TSS content, titratable acidity, alcohol, tannins, total phenols, sugars, and reducing sugar content, is displayed in Table 4. During the aging process, there was a gradual decrease in pH of the Bael wine, indicating an increase in acidity over time (4.25 at 15 days and 3.89 at 90 days). The total soluble solids (TSS) also showed a declining trend (9.50-8.43°B) over time. In the present study, the reduction in pH is correlated with an increase in titratable acidity from 0.78-1.00%, confirming ongoing organic acid production during storage. During storage, yeast cells can produce organic acids, resulting in an increase in titratable acidity over time. These results are consistent with earlier findings by Kulkarni *et al.* (1980), who investigated the changes in the chemical composition of mango wine over 12 months of storage and found that titratable acidity increased significantly (from 0.65 to 1.20%) and pH decreased (3.8-3.0) during storage.

The alcohol content increased slightly (from 9.79-10.00%) during the 90-day maturation period. On the contrary, the tannin content in the herbal blended Bael wine decreased (0.35 to 0.31 mg/ml) during storage, reflecting a softening of the wine due to tannin degradation. The total phenolic content in the Bael wine decreased slightly over time (from 0.51 to 0.48 mg/ml). The total sugars (6.33 to 6.11%) and reducing sugars (4.40 to 3.51%) levels decreased significantly over 90 days of Bael wine storage.

**Table 3: Sensory evaluation of herbal blended Bael wine as influenced by various concentrations of inoculum size, pH level and temperatures**

Parameters	Treatments	Appearance	Colour	Aroma	Acidity	Sweetness	Body	Astringency	Overall acceptability
<b>Hedonic scale</b>		<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>
<b>Inoculum size</b>	T <sub>1</sub> : Control	3	3.1	1.5	2.5	2.2	1.5	3	<b>2</b>
	T <sub>2</sub> : 1%	3.8	3.9	4	3.5	3.8	3.6	3.7	3.8
	T <sub>3</sub> : 2%	3.9	4.1	4.2	3.8	3.9	3.7	3.9	4
	T <sub>4</sub> : 3%	4.3	4.4	4.5	3.9	4.1	4.3	4.2	4.3
<b>pH</b>	T <sub>1</sub> : Control	3.8	3.8	3.5	3.5	3.3	3.6	3.1	3.8
	T <sub>2</sub> : 3.5	3.5	4	4.2	3.9	3.8	4.3	3.8	3.5
	T <sub>3</sub> : 4.5	4.2	4.4	4.5	3.9	4.3	4.3	3.8	4.2
	T <sub>4</sub> : 5.5	4.0	3.7	3.7	3.5	3.4	3.7	3.2	3.8
<b>Temperature</b>	T <sub>1</sub> : Control	3.7	3.8	3.5	3.5	3.3	3.5	3.2	3.8
	T <sub>2</sub> : 25°C	3.5	3.5	3.3	3	3	3.2	3	3
	T <sub>3</sub> : 30°C	4.1	4.4	4.5	4	4.3	4.3	4.1	4.1
	T <sub>4</sub> : 35°C	3.6	3.5	3.8	3.4	3.1	3.3	3.1	3.2
<b>Commercial red wine</b>		4.5	4.5	4	4.2	4.5	4.5	4.5	4.5

**Scale:** Like extremely - 5, Like very much - 4.5, Like moderately - 4, Like slightly - 3.5, Neither like nor dislike - 3, Dislike slightly - 2.5, Dislike moderately - 2, Dislike very much - 1.5, Dislike extremely - 1.

**Table 4: Results of quality analysis of herbal blended Bael wine as influenced by storage period**

Treatment	Storage period (Days)	pH	(TSS°B)	Titratable acidity (%)	Alcohol (%)	Tannins (mg/ml)	TPC (mg/ml)	Total sugars (%)	Reducing sugar (%)
T <sub>1</sub>	15 days	4.25	9.50	0.78	9.79	0.35	0.51	6.33	4.40
T <sub>2</sub>	30 days	4.17	9.10	0.83	9.85	0.34	0.50	6.29	4.10
T <sub>3</sub>	45 days	4.06	8.70	0.90	9.89	0.33	0.49	6.19	3.90
T <sub>4</sub>	60 days	3.95	8.50	0.95	9.96	0.32	0.48	6.15	3.60
T <sub>5</sub>	90 days	3.89	8.43	1.00	10.00	0.31	0.48	6.11	3.51
SE <sub><math>\bar{x}</math></sub> ( $\pm$ )	0.0121	0.0113	0.0116	0.0093	0.0010	0.0010	0.0065	0.0137	
CD (1%)	0.0543	0.0504	0.0522	0.0417	0.0044	0.0044	0.0291	0.0616	
F test	**	**	**	**	**	**	**	**	**

NOTE:\*\* Significant at 1% level

#### 4. Discussion

In the present investigation, the quality of the Bael blended wine was significantly enhanced by herbal blending (2% *C. zizanioides* roots) in terms of higher alcohol content and lower reducing sugar levels. During the fermentation process, as the reducing sugar decreased, the alcohol percentage increased in the wine and vice versa. Singh and Kaur (2009) reported that higher concentrations of herbal extracts could reduce fermentation efficiency, causing an increase in residual sugar levels due to altered sugar metabolism. Bely *et al.* (2008) also observed that enhanced sugar utilization and alcohol yield at moderate concentrations (0.5-2%) of herbal blending. Conversely, increasing the concentrations of *C. asiatica* leaves and *C. zizanioides* root extract blending resulted in a significant decline in alcohol content and an increase in reducing sugar content. This might be attributed to the inhibition of yeast metabolism due to the antimicrobial properties of herbs at higher concentrations (Jagtap *et al.*, 2009; Sharma, 2024a; Samson *et al.*, 2017). Similarly, all the sensory attributes were optimum in a low concentration (2-4%) of *C. zizanioides* extract blended wine in the study. In general, *C. zizanioides* roots and essential oils were infused into food and beverages to induce aroma (Sharma, 2024a). A similar study was carried out on the evaluation of Bael vermouthe with 2-5% spice levels (Chauhan *et al.*, 2016), where they reported 2% spice blending recorded optimum sensory parameters compared to higher spice levels in Bael wine (3.5-5.0%). Blending of higher concentrations of *C. asiatica* leaves and *C. zizanioides* root extract in Bael wine reported lower scores in sensory evaluation, and this might be due to the over expression of herbal taste in the wine. A similar trend was observed in pomegranate blended wine, where herbal extracts adversely influenced the taste and aroma of the wine at higher concentrations (Paul and Sahu, 2014).

Taking the best herbal blending (2% *C. zizanioides* root extract), Bael wine making, 3% inoculum level, 30°C temperature, and 4.5 pH levels were found to be optimum during the parameter standardization experiments. This might be due to the balance between yeast cell density and the availability of sugars. Possibly, a moderate inoculum level that led to efficient sugar utilization and resulted in higher alcohol (Bely *et al.* 2008; Panesar *et al.*, 2009). Togarepi *et al.* (2012) observed that excessively high inoculum concentrations could result in substrate limitation, where increased yeast biomass does not enhance alcohol production, and Fleet (2003)

emphasized the importance of optimizing yeast inoculum for efficient fermentation. It was reported that a pH around 4.5 is ideal for maximizing alcohol production in fruit wines (Mathewson *et al.*, 1980; Paul and Sahu, 2014), and maintaining this pH level supports optimal yeast metabolism, resulting in minimal residual sugars (Singh *et al.*, 1998). Typically, cooler conditions inhibit yeast metabolism, and high temperatures lead to the denaturation of enzymes involved in fermentation, thereby reducing alcohol production (Singh *et al.*, 1998; Usansa, 2003; Veloso *et al.*, 2019). Sharma *et al.* (2013) evaluated various temperatures (20-35°C) for jackfruit wine production and concluded that 30°C optimized yeast metabolism with the highest alcohol content (10.5%). Alike, Wang (2009), reported optimal fermentation and sugar conversion at 30°C in fruit wines with higher alcohol (10.2%). All the sensory parameters were also satisfactory in the same treatments. Similar studies on various fruit (litchi, jackfruit, and carambola) wines parameter optimization resulted in the best sensory attributes at 30°C temperature with 3% inoculum levels (Singh and Kaur, 2009; Paul and Sahu, 2014; Sharma *et al.*, 2013). It emphasized the importance of balanced inoculum size, pH, and temperature in enhancing the sensory elements of wines and ensuring a positive consumer experience.

The pilot-scale production and storage stability of herbal blended Bael wine demonstrate that wines undergo significant changes in their physicochemical properties over time. The gradual increase in alcohol content, decrease in pH, TSS, and tannin content, and the reduction in phenol and sugar levels highlight the ongoing fermentation and maturation processes, which are essential for developing the desired sensory and quality attributes of the wine. Nandagopal and Nair (2013) described a similar trend in ginger-amlam wine pH reduction (3.79 to 3.56) over 24 days of storage. A study on the impact of storage time (30-180 days) showed a reduction in TSS content (12-7.7°B) of wine after 180 days (Mendes-Ferreira *et al.*, 2004). A previous study on the storage of wine for 12 months showed a similar increase in alcohol content from 11.2 to 12% (Romano *et al.*, 2006). Vignault *et al.* (2019) evaluated the changes in alcohol content (13.5 to 14.5%) over 24 months of wine storage. A gradual decrease in tannin content (0.28 to 0.20 mg/ml) was reported during ageing of red wine (Vignault *et al.*, 2020). Katalinic *et al.* (2004) reported a significant reduction in phenolic composition (0.34 to 0.26 mg/ml) of red wine during 24 months of ageing. Likewise, Attri *et al.* (1994) found a gradual decline in total phenols (0.12 to 0.08 mg/ml) in sand pear vermouthe during 12 months of storage. The

total sugars and reducing sugars (levels decreased significantly over 90 days of Bael wine storage. It was observed that a substantial utilization of sugar occurs during the yeast fermentation process of winemaking (Fleet, 2003). Further research could explore the long-term storage effects and potential adjustments in winemaking techniques to optimize the balance between acidity, sweetness, and astringency, thereby producing wines with enhanced sensory attributes and health benefits.

## 5. Conclusion

The study reveals that Bael fruit can be effectively used for producing herbal-infused wine. Among various combinations tested, a 2% *C. zizanioides* extract blend demonstrated the best results in terms of quality and sensory parameters. The production of herbal blended Bael wine was successfully accomplished by implementing optimised fermentation parameters (3% inoculum level, pH of 4.5, and temperature of 30°C) for pilot-scale production. The study also indicated that the storage stability of herbal blended Bael wine demonstrated a gradual improvement of the physicochemical properties of wine over a period of 90 days. Hence, the sensory appeal and storage stability highlight the potential of *C. zizanioides* (Vetiver) blended Bael wine for commercial viability, catering to consumer preferences for innovative and sustainable beverages.

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## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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