

Functional and Microbial Analysis of Non-Alcoholic Wine Formulated from Flowers of *Clitoria ternatea*

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Abstract

Flower based wine is a fermented alcoholic beverage formulated from a wide array of commonly available flowers. *Clitoria ternatea* flower possesses a varied range of pharmacological properties and therefore considering all the nutritional advantages, an attempt was made by the researcher to formulate a flower-based wine from *Clitoria* and to analyze its various quality aspects. Non-alcoholic Wine was formulated using two different variation of sugar such as refined sugar (Sample 1) and palm sugar (Sample 2). Both the samples were subjected to Physicochemical, functional and microbial analysis and compared with the control sample (grape wine). The results of the study revealed that both the samples exhibited desirable physicochemical properties. The ethanol content of sample 1 and sample 2 was found to be 0.167 % and 0.254 % respectively. The yeast count of sample 1 was found to be 280×10^2 CFU/ml, whereas the yeast content of sample 2 was 320×10^2 CFU/ml. The antioxidant activity was found to be higher in sample 1 (73.09%) and lower in sample 2 (68.96%). The phytochemical analysis showed the presence of flavonoids in both the samples. The study findings conclude that the formulated flower-based wine contains significant nutritional benefits and was also found to be well acceptable based on sensory evaluation.

Key words: *Clitoria ternatea*, Non-alcoholic wine, Flower-based wine, Fermented beverage, Yeast (*Saccharomyces cerevisiae*)

The word "fermentation" is derived from the Latin word "fermentum," and it describes a procedure that makes use of the development and metabolic processes of microbes found in plant or animal matter (1). As defined by Maicas (2) "Fermentation is a process of central metabolism in which an organism converts a carbohydrate, such as starch or sugar, into an alcohol or an acid." Fermentation is a process that breaks down the complex form of organic substances into simpler compounds with the help of microbes in the presence of sugar or carbohydrates. The process is carried out from ancient times as a part of preservation of food products (2). Wine is an alcoholic beverage that is produced from naturally fermented fruit, vegetables and flowers (3). *Saccharomyces cerevisiae* is the common yeast culture used for the conversion of sugars into alcohol compounds and organic acids and it is typically required for the fermentation of raw ingredients to formulate wine (4). Alcoholic fermentations are the most frequently used type of fermentation employed in wine production. It is scientifically proven that the moderate consumption of wine can improve the longevity of life and has a probability in reducing the risk of chronic diseases. In the past many researchers have documented the positive health effects of consumption of wine in lowering risk of cardiovascular disease and cancer (5). There are findings that suggest that consumption of low alcoholic

wine can prevent the probability of breast cancer and bowel cancer (6). Regular consumption of wine is also associated with decreased blood pressure and probability of reducing myocardial infarctions in men of age above 65 years (7).

In general, wine making retains all the essential micronutrients and phytochemicals of the raw ingredients used when compared to other processing techniques. Furthermore, regular consumption of wine provides various positive health benefits as many beneficial compounds such as vitamins, tartaric acids, phytochemicals, antioxidants and Glucose Tolerance Factor (GTF) are synthesized by yeast during fermentation and released into fermentation medium (8). The flowers are a good source of nutrients and phytochemicals and are also highly perishable which demands preservation and processing strategies to formulate value added products incorporating locally available flowers. Previous literature work has documented that the edible flowers possess excellent antioxidants properties than that of fruits (9). In addition to its antioxidant activity, edible followers also contain phytochemicals, flavonoids, phenolic compounds, tannins and anthocyanins (10). Butterfly pea, also identified as *Clitoria ternatea*, is an annual herbaceous plant belonging to the Fabaceae family and commonly available in Tamil Nādu. The flowers of butterfly pea possess vibrant blue colour and the

alcoholic extracts of the roots, leaves, and flowers of *Clitoria ternatea* have a variety of pharmacological effects, including anti-leprosy, anti-inflammatory, anti-helminthic, immunomodulatory, anti-asthmatic, antidepressant, anticonvulsant, analgesic, antipyretic, antifungal, proteolytic, and antihyperlipidemic properties (11).

The alcoholic content of the wine can also cause harmful health issues if consumed excessively. Low-alcoholic beverages may be seen as a healthier approach in minimizing the negative health effects of alcohol (6). Soft drinks, which are primarily consumed along with dairy products, have long-term negative effects on health, particularly in children, which can lead to obesity, diabetes, and other conditions (12). Traditionally wine is made up of grapes and other fruits. But an attempt was made by the research to utilise unconventional raw ingredients such as flowers to formulate health-promoting, non-alcoholic fermented wine which can be consumed by all age groups. Since the selected blue pea flowers are locally available with great medicinal properties, the formulated wines will also be cost effective with medicinal properties.

MATERIALS AND METHODS

The formulated *Clitoria ternatea* flower wine was analysed for its quality aspects such as physicochemical properties, functional properties, microbial properties and

Ethanol concentration. This study entitled “Formulation and Quality Evaluation of Non-alcoholic *Clitoria ternatea* Wine” has been approved by the Independent Human Ethics Committee (IHEC) dated: 12/10/2022 (Protocol no. SDNBVC/HSC/IHEC/2021/29) and was conducted in the Department of Home science - Food science, Nutrition and Dietetics, Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women, Chromepet, Chennai – 44.

Procurement of ingredients

The *Clitoria ternatea* flowers were collected from a home garden located in Porur, Chennai. The other raw ingredients such as refined sugar and Palm sugar were purchased from a local supermarket in Chennai. Pure culture of Yeast strain (*Saccharomyces cerevisiae* - NCIM 3090) was obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh and cultured in Sasaam Biological Food Lab Testing Services, Chennai.

Formulation of *Clitoria ternatea* wine

The non-alcoholic flower-based wine was formulated as per the method suggested by (13) with slight modifications. The control sample was formulated with grapes with addition of refined sugar. Two variations of wine were prepared using two different types of sugar (Refined sugar and Palm sugar). Table 1 elucidates the different variations of Ingredients used for formulation of Control and sample wine.

Table 1 Different variations of ingredients used for formulation of wine

S. No	Ingredients	Control (g) Grape Wine	Sample I (g)	Sample II (g)
1	<i>Clitoria ternatea</i> (g)	--	40	40
2	Water (mL)	900	900	900
3	Sugar (g)	50	50	50
4	Yeast (%)	10	10	10
5	Grapes (g)	40	--	--

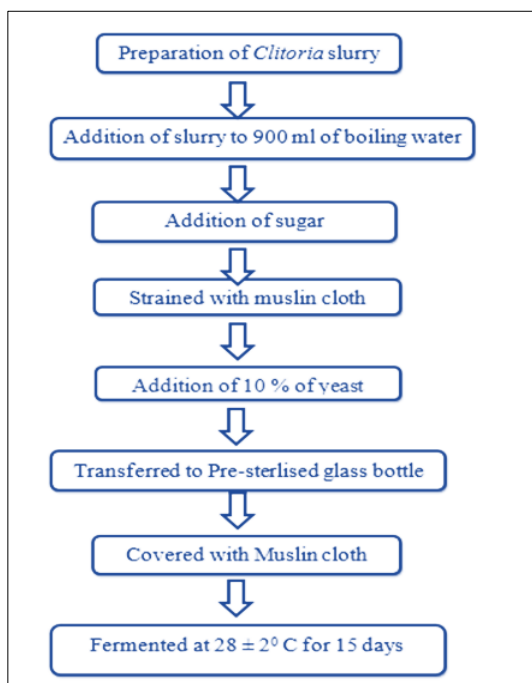


Fig 1 Preparation of *Clitoria ternatea* wine

Preparation of *Clitoria ternatea* wine

Clitoria ternatea flower slurry was added to the boiling water at 60°C. The solution was allowed to boil for 15-20 minutes and then 50 g of palm sugar or refined sugar was added to the boiling solution. Then the solution is completely strained through muslin cloth to obtain a clear solution. The yeast strain

(*Saccharomyces cerevisiae*) was cultured in the medium at a temperature of 20-30 °C for two days. Once the solution was cooled to room temperature, 10 % of cultured yeast was added and inoculated. The obtained solution was then transferred to the pre - sterilised glass bottle and mixed well. The bottle was covered with muslin cloth and allowed for 15 days of fermentation at 28 ± 2°C. The detailed procedure followed for preparation of wine is depicted in the flowchart below:

Quality analysis of formulated *Clitoria ternatea* wine

The Physicochemical properties, Functional Properties and microbial quality of formulated *Clitoria ternatea* wine were analysed using standard procedure. The physicochemical properties such as Total titratable acidity, pH, Viscosity, Total sugar, and Ethanol concentration were analysed as per the method suggested by (14), whereas Functional properties of the formulated wine such as Total phenolic content, Total Flavonoid content, Antioxidant activity and qualitative determination of Phytochemicals were evaluated as per the methods suggested by (15), (16) and (17). The formulated flower-based wine with both varieties of sugar was subjected to microbial examination such as Total bacterial count and Yeast and Mould count using standard procedure suggested by (18) on intervals of 1,5, 10 days.

Statistical analysis

The raw data obtained will be coded, classified and tabulated using SPSS software. The raw data obtained by analysis of *Clitoria ternatea* wine were subjected to the following statistical analysis such as Mean, Standard Deviation, and ANOVA.

RESULTS AND DISCUSSION

Physicochemical analysis of formulated wine

The Physicochemical properties of the formulated wine prepared using refined sugar and Palm sugar were analysed for pH, colour, total titratable acidity, total soluble solids, total sugar measurement, viscosity and ethanol content. The results of the Physico chemical properties of the formulated wine were furnished in the (Table 2).

The total soluble solids were analysed for the Sample I and Sample II. The total soluble solids for the Sample I was found to be 9.85 % whereas the total soluble solids of sample II were found to be 7.85%. Both the samples contain high total soluble solid content when compared to control samples and also exhibited statistically significant differences. The total soluble solids in the sample I was found to be higher than the

value of sample II. The higher levels of total soluble solids may be due to increased acidity as reported by (19). The sapota wine formulated by (20) also obtained similar results with a total soluble solid value ranging from 8.77-27.41% on different treatments. The total titratable acidity was analysed for the both samples where the value of sample I was found to be 7.11 g/l and Sample II possessed a total titratable acidity value of 7.21 g/l. It was evident from the results that the sample II had a greater titratable acidity than the sample I prepared with refined sugar. According to previous research findings, prolonged fermentation could produce low acid wine (8 g/L) with high titratable acidity and a lower pH value (21). Red wine formulated by (22) also possessed a similar titratable acidity value (7.23 g/l) which was comparable to the values of the current investigation. The titratable acidity analysed in other wines were found to be between a ranges of 2.7 to 9.2 g/l (23).

Table 2 Physicochemical properties of the *Clitoria ternatea* wine

S. No	Properties	Control (Grape wine)	Sample I Refined sugar	Sample II Palm sugar	p-value
1	Total soluble solids (%)	6 ±0.02	9.85 ± 0.2	7.85 ± 0.1	<i>p</i> <0.01
2	Total titratable acidity (g/l)	5.03±0.03	7.11 ± 0.2	7.21 ± 0.1	<i>p</i> <0.01
3	Viscosity (cP)	1.4± 0.05	1.667 ± 0.05	2.885 ± 0.05	<i>p</i> <0.01
4	pH	3.5± 0.1	2.6 ± 0.05	2.4 ± 0.03	<i>p</i> <0.01
5	Total sugar	21.23± 0.03	8.61 ± 0.03	8.012 ± 0.04	<i>p</i> <0.01
6	Ethanol Concentration	2.9 ± 0.2	0.167±0.001	0.254±0.001	<i>p</i> <0.01

Values are represented as Mean and Standard Deviation

All the values are Mean of triplicate determination ± Standard Deviation

NS- Not significant, S* - Significance at 5% level, and S***- Significance at 1% level

The viscosity value of sample I and sample II were found to be 1.667 cP and 2.885 cP respectively. It was discovered that sample II exhibited higher viscosity value than both the control and sample I formulated with refined sugar. According to a study by (24), the presence of tannins can be linked to greater wine viscosity. The developed wine was also found to have similar viscosity values reported by (24) in Shiraz wine (2.435 cP) and in Chardonnay wines (1.794 cP).

The pH of sample I was determined to be 2.6, while sample II's pH was found to be 2.4. In general, due to alcoholic fermentation, wine should possess acidic pH. Both the samples constitute less pH value when compared to the control sample. When analysed statistically all the three values exhibited statistically significant differences. The disparity in the results could be attributed due to difference in the ingredients used, fermentation duration and types of strain. A similar pH value (3.0) was also reported in wine made from various fruits documented by (18).

The total sugar measurement of the formulated wine for both the sample I, sample II and control were analysed. The total sugar measurement of sample I, sample II and control was found to be 8.61 %, 8.012 % and 21.23 % respectively. The total sugar content of the sample I and sample II were found to be very less when compared to the control sample and exhibited a statistically significant difference at *p*<0.01. The difference in the total sugar content between the samples and the control could be due to the presence of natural fruit sugar in the control

sample. Previous research work by (20) who formulated sapota wine also documented a similar total sugar value (8.65%).

The ethanol content of the formulated flower-based wine was analysed. The alcoholic content of sample I and sample II was found to be 0.167% and 0.254% respectively, whereas the ethanol content of the control wine was found to be 2.9%. Very low ethanol values of both the samples clearly indicate that the formulated flower-based wine belongs to the Non-alcoholic category of wine (25). A study done by (18) also obtained an identical ethanol value of 0.93% in Lime based wine.

Functional properties of *Clitoria ternatea* wine

The formulated *Clitoria ternatea* based wine was analysed for various functional properties such as Total phenolic content, Total flavonoid content, Antioxidant activity and phytochemical content.

Total phenol content

The total phenolic content of the formulated wine can also be affected due to the varying methods of fermentation. The capacity of phenolic and flavonoid compounds to give hydrogen atoms to free radicals enables them to operate as potent antioxidants and deactivate free radicals. They also have perfect structural qualities for scavenging free radicals. The total phenol content of the formulated wine was found and the results are furnished in the below table.

Table 3 Total phenolic content of the *Clitoria ternatea* wine

S. No.	Concentration (µg/ml)	Sample I	Sample II	Standard (Gallic acid) GAE/g
1	100	0.092	0.075	0.634
2	200	0.106	0.096	1.321
3	400	0.125	0.123	2.246

The total phenolic content of the Sample I was found to be 0.123 GAE/g whereas the total phenolic content of the

sample II was 0.125 GAE/g at the concentration of 400µg/ml. The total phenolic content of the formulated wine can be

directly correlated with its antioxidant activities (26). Comparatively wine prepared by (27) was found to possess similar total phenolic content with values ranging from 0.05-3.0 GAE/g.

Total flavonoid content

Table 4 Total flavonoid content of the *Clitoria ternatea* wine

S. No.	Concentration (µg/ml)	Sample I	Sample II	Standard (Quercetin) QE/g
1	100	0.056	0.065	1.462
2	200	0.093	0.096	2.167
3	400	0.125	0.135	3.478

The total flavonoids content of the sample I and sample II was found to be 0.125 QE/g and 0.135 QE/g respectively at the concentration of 400 µg/ml. A study done by (28) and his co-workers analysed the total flavonoid content of the Ethiopian alcoholic beverages with a similar value (0.06-8.09 QE/g) was reported. The similar value of total flavonoid content was also observed in Borde (Ethiopian fermented beverage) with the value of 0.90 QE/g as reported by (28).

Qualitative analysis of phytochemicals in wine

The phytochemicals are naturally found in the flowers of *Clitoria ternatea* and therefore the presences of phytochemicals are determined in both the sample I and sample II. The phytochemicals determined are carbohydrates, protein, starch. Flavonoids, tannins, saponins, anthocyanins, alkaloids, cardiac glycosides, coumarins, glycosides, phenols, terpenoids, steroids, quinones and glycosides. The results of Phytochemicals determination are represented in Table 5.

Table 5 Qualitative analysis of phytochemicals in wine

S. No.	Phytocompounds	Sample-1	Sample-2
1	Acid	-	-
2	Alkaloids	-	-
3	Anthocyanin/Betacyanin	-	-
4	Carbohydrates	+	+
5	Cardiac Glycosides	-	-
6	Coumarins	-	-
7	Flavonoids	+	+
8	Glycosides	-	-
9	Phenols	-	-
10	Proteins	-	+
11	Quinones	-	-
12	Saponins	-	+
13	Starch	+	+
14	steroids	-	-
15	Tannins	+	+
16	Terpenoids	-	-

+ Presence of Compound; - Absence of the Compound

The phytochemicals present in the formulated wine samples are Flavonoids and Tannins in both the wine samples. Apart from these two phytochemicals, the presence of starch and Carbohydrates was noticed in both the samples. Sample II specifically contains Proteins and Saponins which could be attributed to the addition of Palm sugar (29). A study by (30) also indicated the presence of saponins in palm fruit extract. The presence of Flavonoids and other phytochemicals in both the samples represents the presence of an array of phytochemicals in the flower extracts (30). Another study has also reported a high level of flavonoids content in flower extract rather than the leaf extracts (31). so, it can be concluded that the

Flavonoids are secondary metabolites that have antioxidant activity. The total flavonoids content of the flower wine is determined by the quantity and location of free OH groups that determine their potency. The total flavonoid content of the both the sample I and II of the formulated wine are represented in the (Table 4).

flowers in general possess excellent phytochemical composition and antioxidant properties thereby making them a potential raw ingredient to formulate wine and other fermented beverages. The presence of tannins in the formulated wine samples also indicates their presence in the flower which was reported in the study by (31). The presence of carbohydrates can be attributed due to the addition of sugar in both samples as sugars are rich sources of carbohydrates (32).

Antioxidant activity of formulated wine

The antioxidant activity of both the sample I and sample II were determined by DPPH radical scavenging assay and the results are exhibited in the (Table 6).

Table 6 Antioxidant activity of the *Clitoria ternatea* wine

Concentration(µg/ml)	Sample I	Sample II
100	48.48%	12.36%
200	55.27%	20.96%
400	85.09%	33.09%

The antioxidant activity of the formulated samples of wine were analysed for DPPH activity as the flower extracts generally possess excellent antioxidant activity due to its phytochemical content. The results obtained for the formulated flower wine for the sample II at concentration of 400 µg/ml was found to be 33.09% which is comparatively lesser than the antioxidant activity of sample I at the concentration of 400 µg/ml which was found to be 85.09 %. The findings revealed that antioxidant activity of the Sample I was found to be two-fold higher than the antioxidant activity of Sample II. The results conclude that the addition of refined sugar had a positive effect on growth of yeast which in turn would have increased the antioxidant activity of the wine. The value of the camellia japonica-based wine done by (13) observed a similar antioxidant value ($73.16 \pm 2.9\%$).

Microbial analysis of *Clitoria ternatea* wine

Total bacterial count

The TBC of the formulated wine was analysed for both the sample and represented in (Table 7).

Table 7 Total bacterial count of the *Clitoria ternatea* wine

S. No.	Sample	Total bacterial count
1	Sample I	11×10^3 CFU/ml
2	Sample II	13×10^3 CFU/ml

The TBC of the sample I was found to be 11×10^3 CFU/ml whereas the TBC of the sample II was found to be 13×10^3 CFU/ml. The study done by (33) in formulation of mixed fruit wine reported the total bacterial count as 5.5×10^3 CFU/ml which was comparatively less than the results obtained in this current investigation. Nevertheless, the obtained values were microbially safe for human consumption.

Yeast and mould count

The formulated wine was analysed for its yeast count on three consecutive days (1st day, 5th day and 10th day). The yeast

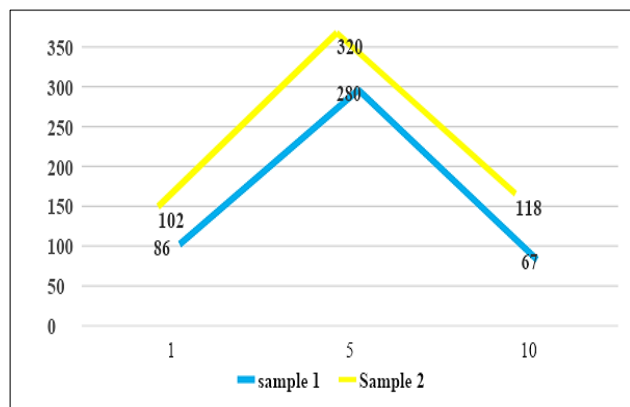


Fig 2 Yeast and Mould Count of Sample 1 and Sample 2 at 10^2 dilution

The results concludes that in both the samples the yeast count has increased initially and reached the peak value during 5th day of fermentation and decreased gradually on the 10th day of storage. The yeast count of both the samples on 5th day was found possess maximum value of 120×10^3 CFU/ml and 98×10^3 CFU/ml respectively. It can be concluded from the study that the sample I wine formulated with refined sugar supported excellent growth of yeast when compared to sample II formulated with palm sugar. Therefore it was clearly evident from the research findings that refined sugar suits better to formulate wine rather than other varieties of sugar. Similarly, a study done by (18) observed yeast count of orange wine for consecutive days and showed study decline in the yeast count with inclining days.

CONCLUSION

This paper studied the possibility of vinification of *Clitoria ternatea* flower extract. Numerous research works indicates that the consumption of wine in moderate quantities has the ability to reduce the probability of cardiovascular disease, reduce platelet aggregation, and reduce the probability of Alzheimer's disease. *Clitoria ternatea* flowers are commonly available throughout the year and can be considered

and mould count for the sample I and sample II were assessed in intervals as per the method suggested by (18). The pictorial representation of yeast is depicted in (Fig 2-3).

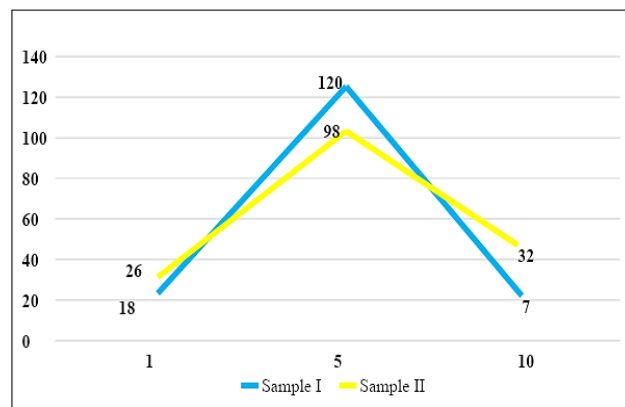


Fig 3 Yeast and Mould count of Sample 1 and Sample 2 at 10^3 dilution

as potential raw ingredients to formulate non - alcoholic wine. The research findings of the study concludes that the formulated *Clitoria ternatea*-based wine with refined sugar possessed satisfactory physicochemical properties with a significant amount of phenols and antioxidant activity when compared to wine formulated with palm sugar. The wine developed with Palm sugar contains high levels of yeast and mould count. The non-alcoholic wine can serve as an excellent substitute for alcoholic beverages and carbonated drinks. Consumption of fermented beverages can be considered an ideal replacement for highly processed beverages. The non-alcoholic wine is high in cost due to the processing involved in dealcoholizing the wine to reduce the alcohol content and is highly recommended which naturally overcomes the negative outcomes caused by the consumption of alcoholic wine. The flowers of *Clitoria ternatea* are highly perishable and contain pharmacological properties. Considering the Pharmacological properties of the flowers and to preserve their functional properties an attempt was made to develop wine from flower. The formulated wine exhibited excellent source of Phenol, Flavonoid and antioxidant activity. Thus, formulation of *Clitoria ternatea* wine can be beneficial to society as they are highly cost efficient. The *Clitoria ternatea* can be used in formulation of wine adding value to the flower.

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