

## Biochemical and Organoleptic Study of the Mahua Flower and Mahua Flower Wine.

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**Abstract:** Mahua (*Madhuca indica*) is a large, deciduous tree native to the northern and central parts of India. Tribal populations across the country value the flowers of this tree as a source of food. The mahua flowers are one of the most underutilized nutrient sources due to their extremely rare consumption among the Indian populace. The flowers are nutritionally balanced as a rich source of carbohydrates, proteins, dietary fibers in addition to minerals like iron and calcium. The most interesting attribute of the mahua flowers is their long shelf life after drying. In the dried state these flowers maintain the integrity of nutrients, thus significantly reducing nutrient losses upon storage. Due to all these reasons, exploration of the nutritional profile of the mahua flowers contributes a new perspective to biochemical research.

In this research, a detailed biochemical study was carried out to evaluate the nutritional profile of the mahua flowers. Subsequently, a wine was prepared using the mahua flowers. The developed wine was then subjected to various physicochemical techniques to assess its aging profile. In addition, a sensory evaluation exercise using the developed mahua wine was carried out to ascertain its organoleptic acceptability amongst trained panelists. Future prospects include isolation and characterization of alkaloids in mahua flowers and novel product development using mahua flowers as the core ingredient.

**Keywords:** biochemistry, fermentation, mahua wine, nutrition, oenology, sensory evaluation.

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### I. Introduction

Food is the basis of life. It fulfills the need of nourishment and enriches our well-being. Humans as psychosomatic beings associate their emotions with food. Thus, since ancient times, there was investment of significant amount of time and resources in planning, procuring and processing of food for its consumption. Age-old recipes formulated around incorporation of seasonal produce for the supply of adequate nutrients are the prerequisite at mealtimes. Mealtimes with family members aimed to increase bonding and strengthen relations within and across families. Festivities and religious occasions also have a strong association and are almost synonymous with specific foods. Thus, food for us humans is always viewed as a medium to enhance the physical, social, emotional and spiritual quotients of all individuals in a family and or a community.

The advent of modern technology and innovation dawned the era of the Industrial age. Rampant progress in all arenas ensured a higher quality of life accompanied by a hectic lifestyle. This has reduced the time invested in the procurement and consumption of foods. Meeting work deadlines and targets has left very little time for consumption of food. As palates have become global, local recipes are not usually preferred. Thus, favorable novel food options nowadays include on-the-go alternatives of international recipes. These products offer the utmost advantage of consumer convenience but on the flipside lack quality nutrition.

Thus, the need of the time is to reinvent traditional recipes promising consumer convenience. For this reason, the focus is also shifting towards the development of novel products using unexplored ingredients and nutritional sources. The main objective of this research is to highlight the nutritional superiority of the mahua flower. The findings thus obtained served as the basis of the product development of mahua flower wine.

### II. Materials And Methods

The study on Mahua (*Madhuca indica*) flowers was carried out in the following manner:

#### 2.1 Procurement of samples

Locally procured dried Mahua flowers procured from the market were used for analysis.

#### 2.2 Processing of samples for analysis

- For estimation of proteins, 1g of the sample was homogenized in a mortar pestle with 10ml of phosphate buffer (pH 7.0). 1 ml of the above sample solutions was then diluted to 100 ml with distilled water.
- For estimation of carbohydrates, 100mg of the dried sample was hydrolyzed for 3 hours with 5ml of 2.5 N HCl. The excess acidity of the sample was then neutralized using Na<sub>2</sub>CO<sub>3</sub> powder until there was no effervescence observed. The resultant solution was then diluted to 50ml with distilled water.

- c) For estimation of minerals in the sample, 5g of the sample was dissolved in 100ml distilled water and placed in a shaker overnight. The supernatant subsequently obtained was used for analysis.

### **2.3. Analysis of Proximate Principles**

This section of the study involved using standardized AOAC certified biochemical methods for estimation of various nutrients in Mahua flowers. The following proximate principles were estimated from Mahua flowers:

#### **2.3.1 Macronutrients**

- a) Estimation of carbohydrates by Anthrone method.
- b) Estimation of proteins by Folin-Lowry method.
- c) Estimation of dietary fibers by Ashing method.

#### **2.3.2 Micronutrients**

- a) Estimation of iron by Wong's method.
- b) Estimation of phosphorus by Fiske-Subbarow method.
- c) Estimation of sodium by Flame Photometry.
- d) Estimation of potassium by Flame Photometry.
- e) Estimation of calcium by Flame Photometry.

### **2.4. Preparation of Mahua Flower wine**

Winemaking is one of the most traditional and the finest form of bioprocess technology. It is one of the earliest forms of preservation, wherein the nutritional qualities of a foodstuff are concentrated and retained during the process. The major principle behind this method involved the fermentation of mahua flowers with Baker's yeast in the presence of a sugar-rich environment.

The process of winemaking is detailed as follows:

- a. A slurry was prepared by crushing 500g of good quality mahua flowers with 250ml of water. The resulting slurry was then filtered using a 60-80mm mesh sieve.
- b. 750gm of sugar was dissolved in 500ml of water heated to 60°C. The resultant solution was allowed to cool to room temperature.
- c. 50gm of baker's yeast was dissolved carefully in 150ml of water warmed to 50°C to yield the yeast solution.
- d. The Mahua flower slurry was then transferred into a huge, wide-mouthed jar (2-2.5 Liters capacity, made up of glass, ceramic or plastic with a fitting lid)
- e. Subsequently, sugar solution was added to the flower slurry in the jar and mixed, followed by gradual addition of the yeast solution and mixed again.
- f. Care must be taken to keep the lid loose because there will be intense gas formation in the first week of fermentation. The pH will be maintained at around 5.0.
- g. The jar containing the wine preparation must be kept undisturbed for a month, taking care to swirl the contents of the flask daily.
- h. After a month, the contents of the jar are filtered to yield a clear solution.
- i. The wine thus obtained was subjected to regular clarification procedures to obtain a clear wine.
- j. The wine was stored in glass bottles for ageing.

### **2.5. Clarification of Mahua Flower wine**

After the process of fermentation is complete, a huge amount of particles sediment, which reduces the organoleptic acceptability and flavor profile of the prepared wine. Thus a key aspect of processing wine is its clarification. The process of clarification ensures removal of sediments and other fine particles to increase the clarity in appearance thus elevating the value of the prepared wine. Key processes in wine clarification include:

#### **2.5.1 Siphoning**

This ensures thorough removal of sediments. The general procedure of siphoning carried out for clarifying mahua wine was as follows:

- 1. An empty container is placed 50cm below the original wine fermentation container. One end of the siphon tube was submerged so that the sediment tap was about halfway down into the wine.
- 2. The siphon tube was then attached to the container using the clip provided.
- 3. One end of the siphon tube was sucked until the wine flowed past the bottom of the original fermentation container, after which the siphon tube was quickly placed into the second empty container. The wine flows from the first to the second container.
- 4. As the wine level drops in the original container, the rigid siphon tube was lowered further into the wine by gently pushing the siphon tube through the clip. This process is performed cautiously until maximum transfer of wine (without any sediments) occurred from the fermentation vessel to the other container.

#### **2.5.2 Clarification of Mahua wine using gelatin**

Even after siphoning, mahua wine showed presence of suspended particles imparting a haze to it. This was attributed to the presence of the tannin-iron complex in the wine. For efficient haze removal, the mahua wine was clarified

using a gelatin solution (made by dissolving 0.1g unflavored gelatin in 2.5ml warm water). This was then added after cooling to 1 L of the prepared wine.

Settling of haze using gelatin offered the following advantages:

- Efficient removal of natural haze.
- Reasonably rapid method of sedimentation.
- Wine remains reasonably clear and stable after sedimentation.
- Maintenance of flavor integrity.

After clarification, the wine was racked within 2 weeks.

### **2.6 Assessment of Mahua Flower wine**

The prepared wine was then assessed using various physicochemical methods that served as quality indicators for the given wine. These tests also aided in understanding the biochemical changes in the wine during the ageing process. The tests carried out were as follows:

#### **2.6.1 Estimation of Titrable acidity**

The titrable acidity is one of the most important quality indicators of wine. Acidity in mahua wine is essential to balance the sweetness that arises due to the carbohydrates and sugars in mahua flowers. Estimation of titrable acidity aids in estimating the tartness imparted to the wine. Also, acids produced during the course of fermentation act as a preservative and aid in prolonging the shelf life of the wine.

In this technique, 10ml of mahua wine was titrated against 0.1 N NaOH using phenolphthalein indicator. End point is indicated by a color change from golden to deep purple. Hence, regular estimation of the wine's acidity levels helps to optimize process parameters, and thus serves as the most important quality check during fermentation.

#### **2.6.2 Estimation of alcohol content**

The alcohol content of the given wine sample is an indicator of successful fermentation and ageing. Apart from alcohol, other volatile metabolites can be produced during the course of fermentation. The process of distillation determined the alcohol content of mahua wine. The distillation unit was maintained between 75-80 °C, and the evolving fractions were collected and their volumes were measured. The fractions were analyzed by measuring their boiling point and also by subjecting them to various confirmatory chemical tests.

#### **2.6.3 Estimation of pH**

pH is defined as the negative logarithm of hydrogen ion concentration. The active acidity of wine influences the flavor by imparting sourness. Monitoring pH levels is important to prevent the wine from turning into vinegar. The estimation of pH of mahua wine was done by dipping the electrode of a standardized pH meter into 20 ml of the wine.

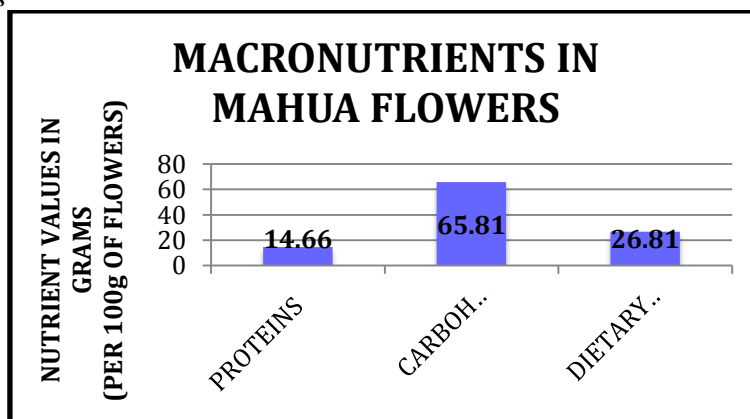
### **2.7 Sensory evaluation**

Sensory evaluation is a scientific discipline, which measures human responses to the composition of food and drink. Sensory evaluation is based on the assessment of the organoleptic acceptability of the developed products. Sensory evaluation involves assessment of various attributes of the product that influences its acceptability and marketability.

Expert panelists who understood the intricacies of the intricate relationship between flavors and the human senses performed sensory evaluation of mahua wine. The 9 expert panelists answered a detailed questionnaire focused on both non-invasive and invasive parameters. The non-invasive parameters evaluated the general awareness of desirable product attributes related to wine. In the invasive part of the questionnaire, panelists compared the sophistication of flavor between commercially available grape wine (Sample A) and mahua wine (Sample B). The data obtained from the questionnaires was analyzed biostatistically analyzed to ascertain the organoleptic acceptability of mahua wine.

## **III. Results**

### **3.1 Proximate Analysis**



*Figure 1: Proximate analysis of Macronutrients in Mahua flowers*

Mahua flowers are a rich source of carbohydrates, proteins and dietary fibers. Thus, mahua flower serves to be a balanced source of energy. The rich carbohydrate content makes mahua flowers a good substrate for wine and sugar syrup production. Protein in mahua flowers is a complete protein, rich in all the essential amino acids. Mahua flowers are rich in dietary fibers. Assessment of dietary fiber values aids in the detection of adulteration in mahua flowers. Any adulterant used to increase bulk will cause an exceptional increase in dietary fiber values. This analysis could thus serve as a quality indicator for mahua flowers.

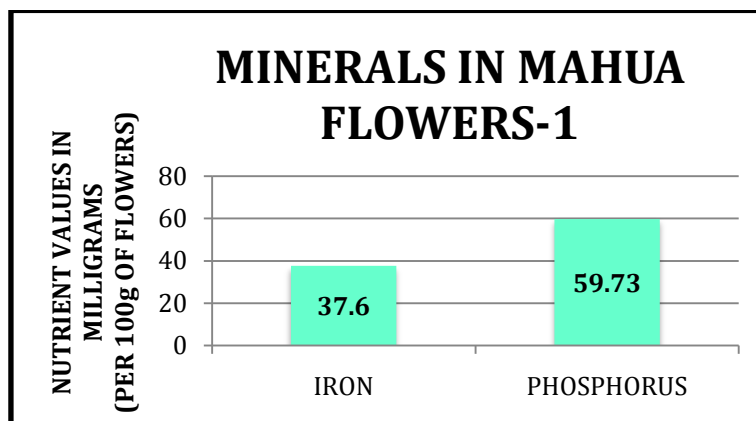


Figure 2: Proximate analysis of Minerals in Mahua flowers

Mahua flowers are also a rich source of iron and phosphorus. Thus it promises to be a natural food source that meets the daily requirements of iron and thus aid in the prevention of iron deficiency anemia. Mahua flowers are also a rich source of sodium, and a moderate source of potassium. In addition, mahua flowers are an abundant source of calcium (Figure 3). The rich calcium and phosphorus content in mahua flowers makes it a food product that ensures optimal maintenance of bones and teeth. Mahua flowers can thus aid in preventing bone decalcification during ageing and prevention of osteoporosis. Thus, consumption of mahua flowers ensures delivery of balanced nutrition.

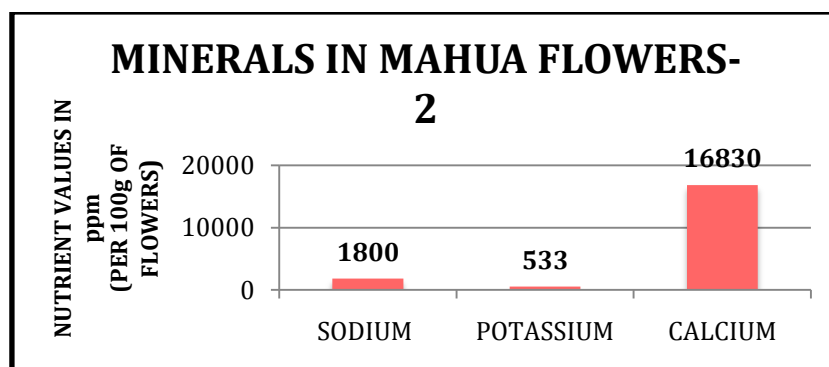


Figure 3: Proximate analysis of Minerals in Mahua flowers

### 3.2 Assessment of Mahua flower wine

#### 3.2.1 Titrable acidity of mahua wine

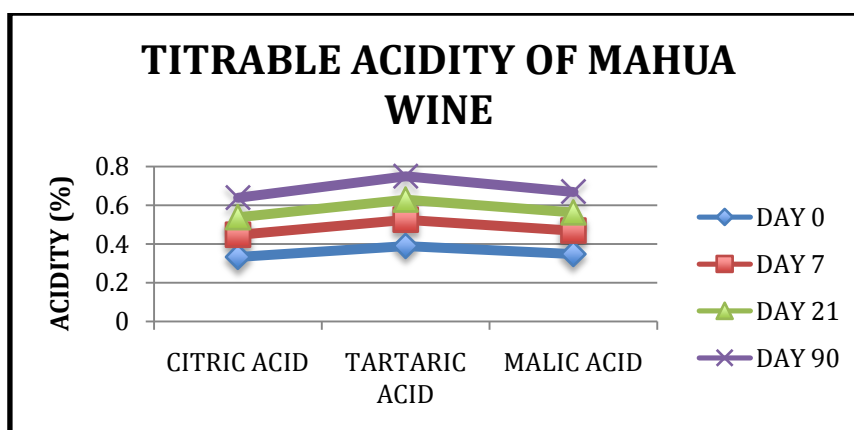


Figure 4: Variations in the titrable acidity of mahua wine with time.

During the fermentation of mahua wine, the concentration of citric, malic and tartaric acids rise during the course of fermentation (around day 21). The relative proportions of the acids remain the same, and the acidity increases during the course of fermentation. The titrable acidity of mahua wine remains restricted in the range of 0.4-0.8% during fermentation and ageing, which is considered to be optimal for most wines.

### 3.2.2 Estimation of pH of mahua wine

The pH of mahua wine was maintained in the range of 3.8 to 4.8 during the course of fermentation and ageing. The pH of mahua wine was higher at the initial stages of fermentation, drops to around 3.8 due to acid production. During ageing, the pH of mahua wine then stabilizes to a value of 4.42, as seen in Figure 5 below.

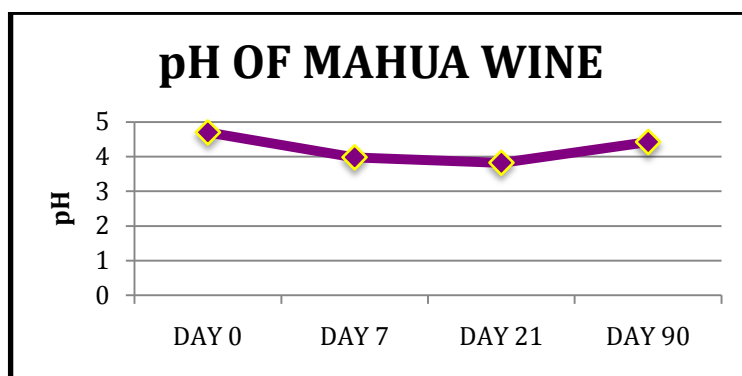


Figure 5: Variations in pH of mahua wine with time.

### 3.2.3 Alcohol distillation

Fractions collected during alcohol distillation were obtained as seen in Table 1:

Amount of collected sample	Collected at	Test	Observation	Inference
6.0 ml	75-80°C	Collected fraction+ Sodium metal	Effervescence due to evolution of hydrogen gas	Ethyl Alcohol confirmed
17.0 ml	90°C	2.0ml distilled fraction + 2.0 ml distilled water+ 2ml 2N NaOH+ 2ml dilute iodine	Yellow precipitate	Methyl ketone confirmed.

Thus, ethanol and methyl ketone were the major volatiles isolated from mahua wine.

### 3.3 Sensory Evaluation

The results of organoleptic analysis of commercially available grape wine (Sample A) with mahua wine (Sample B) were as follows:

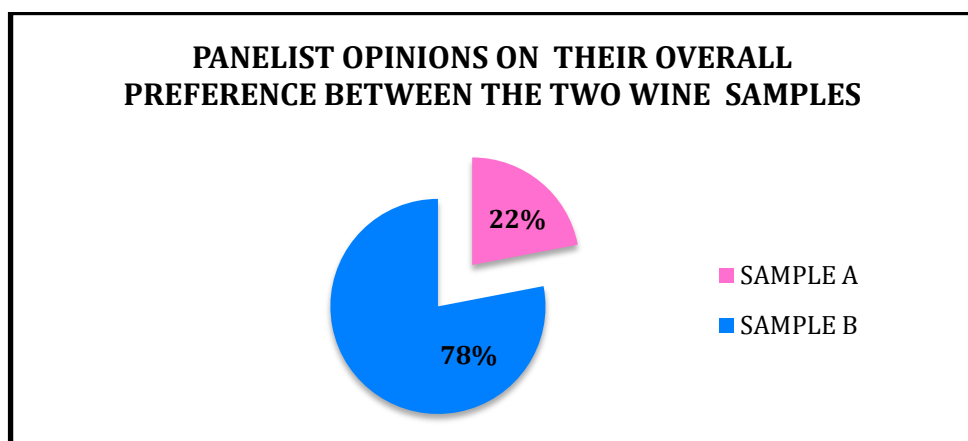


Figure 6: Panelist opinions on their overall preference between grape wine and mahua wine. 78% of panelists preferred mahua wine to commercial grape wine.

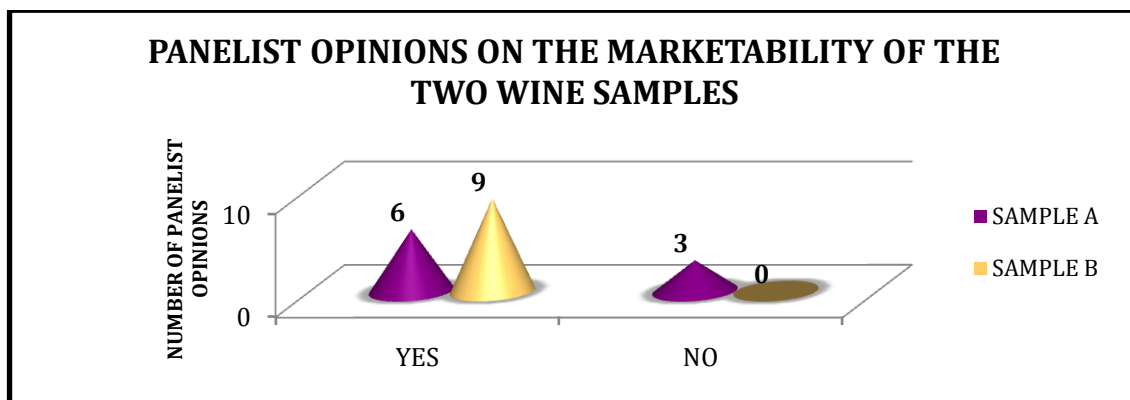


Figure 7: Panelist opinions on the marketing potential of grape wine versus mahua wine. 100% of panelists agreed that mahua wine could be marketed commercially.

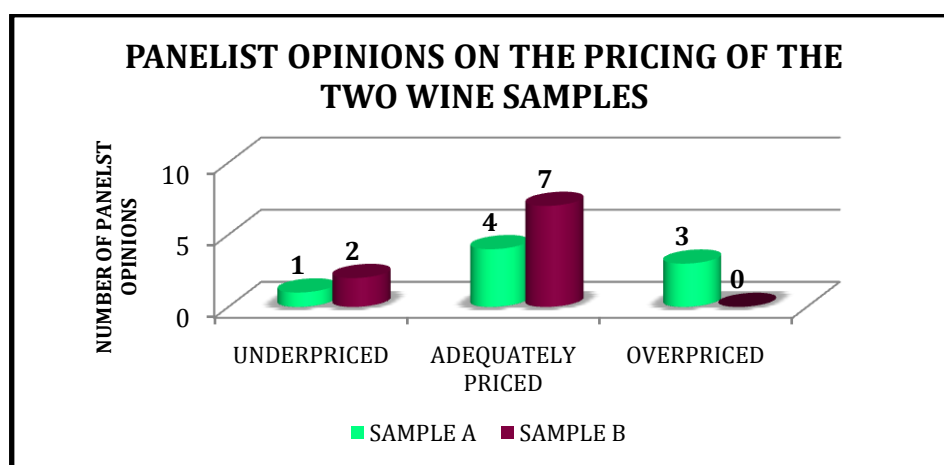


Figure 8: Panelist opinions on the pricing of grape wine versus mahua wine. 78% panelists agreed that mahua wine is adequately priced.

Thus, it is evident from the above analysis that mahua wine is not only organoleptically acceptable, but also commercially marketable as a novel product idea. The studies also showed that the pricing is appropriate. Thus, mahua wine exhibited high value for money (VFM) that further accentuates its marketability.

#### IV. Conclusion

The results show that mahua flowers are one of the most balanced nutritional sources. Another commendable attribute of mahua flowers is their nutritional stability during preservation and processing. Mahua flowers thus show promise as the versatile ingredient that can be consumed in various recipes. Hence, mahua flowers can be processed and marketed in the form of various recipes for maximum utilization of their health benefits by the general population. Consumption of mahua flowers is thus recommended for optimizing the biochemistry of the individual.

Mahua wine incorporates all the nutritional benefits of mahua flowers during the course of fermentation and ageing. The chemical analysis of mahua wine reveals its superior oenological stability. It thus shows promise as a novel product that can be mass marketed delivering the advantage of optimal nutrition, high value for money and elevated organoleptic acceptability.

In addition, mass production of mahua flower products would create abundant employment opportunities to indigenous tribal communities. The mass manufacture of mahua flower products could add a new impetus to the ever-expanding food and nutraceutical industry in India. This in turn, could contribute to the make in India revolution and further strengthens the Indian economy.

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