



By-product Utilization of Industrially Underutilized Cocoa Mucilage through Wine Fermentation

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Authors' contributions

This work was carried out in collaboration among all authors. Author HSUH designed the study, wrote the protocol and wrote the first draft of the manuscript. Author PMV managed the analysis of the study. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/acri/2025/v25i51183>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/134609>

Original Research Article

Received: 15/02/2025

Accepted: 19/04/2025

Published: 23/04/2025

ABSTRACT

Cocoa (*Theobroma cacao* L.), native to the Amazon basin, was introduced to India in the early 20th century and is now classified as a plantation crop, like coffee, tea, and rubber. Its beans are widely used in chocolates, beverages, and confectionery products. As a plantation crop, proper processing and preservation are essential to ensure product availability. Cocoa pulp is the sweet, white, nutrient-rich mucilage surrounding the beans inside the pod. It contains sugars (glucose, fructose, sucrose), organic acids (mainly citric acid), vitamins (notably ascorbic acid), and minerals

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Cite as: U. H., Hyderali Shihabudheen, Prince M. V., Sreelakshmi P., Sreeja R., Lilia Baby, and Rajesh G. K. 2025. "By-Product Utilization of Industrially Underutilized Cocoa Mucilage through Wine Fermentation". Archives of Current Research International 25 (5):1-9. <https://doi.org/10.9734/acri/2025/v25i51183>.

such as potassium. Though vital for natural fermentation during cocoa processing, this pulp is often discarded as waste. However, when collected hygienically, it can be used to produce value-added health beverages, like low-alcohol, nutrient-rich wine, offering farmers better resource utilization and income. This study focused on the basic physicochemical properties of cocoa mucilage and wine during fermentation. Parameters such as total soluble solids (TSS), pH, acidity, antioxidant activity (DPPH), total phenolic content (TPC), ascorbic acid, reducing sugars, and color were analyzed. These properties help in understanding the biochemical changes occurring during wine fermentation. The physicochemical properties of cocoa mucilage wine like Total Phenolic Content (TPC), Total Soluble Solids (TSS), Reducing sugar and DPPH scavenging activity tends to increase compared to that of cocoa mucilage. The values of TPC are 107.03 mgGAE/ml and 258 mgGAE/ml for mucilage and wine respectively as well as that of DPPH scavenging activity are 34.3% and 41.72% for mucilage and wine respectively. Whereas pH, acidity and ascorbic acid showed a decreasing trend for wine compared to that of its mucilage. The result of pH is 3.97 and 3.66 for mucilage and wine respectively. These properties highlight its significant potential as a nutrient rich health drink from industrially underutilized cocoa mucilage. Therefore, it can be concluded that cocoa bean mucilage can be used for alcoholic beverage fermentation, contributing to diversifying processing products and increasing the application potentials for cocoa.

Keywords: Cocoa mucilage; cocoa wine; fermentation; physiochemical properties.

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a highly valued but underutilized tropical tree crop native to Central and South America. It belongs to the family Malvaceae. Commonly known as cacao, cocoa, or chocolate tree, it is widely appreciated for its seeds, which are processed into cocoa powder and chocolate products. Cocoa is rich in phytochemicals, particularly flavonoids, as well as essential minerals and vitamins, contributing to its significant therapeutic potential. Often referred to as a "Food of the Gods," cocoa has demonstrated a range of pharmacological effects in animal models, including antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antihypertensive, and antidiabetic properties (Rusconi, M. and Conti, 2010). Studies exploring the use of cocoa in managing chronic diseases such as diabetes and cardiovascular disorders have shown promising outcomes (Gonzalez et al., 2022; Sharma, 2024; Singh et al., 2022).

India's favourable tropical climate offers significant potential for cocoa cultivation. In India, Kerala was the leading state in promoting cocoa cultivation. At present, the global production and consumption of cocoa is around 27 lakh MT, compared to this, India's production is meager i.e. 10,000 MT (DCCD, 2021). The leading cocoa-producing state is Kerala, followed by Andhra Pradesh, Tamil Nadu, and Karnataka. Cocoa cultivation in India is often integrated with coconut and areca nut plantations, making it a popular crop among smallholder farmers. Prominent cocoa varieties grown in India include

VTK 1, CCRP 1, CCRP 2, CCRP 3, and hybrids developed through research initiatives by institutions like CPCRI and the Cocoa Research Centre. From the humid coastal regions of the Western Ghats to parts of the Eastern Ghats, cocoa is cultivated in diverse agro-climatic zones. Notably, cocoa plants can also tolerate partial shade and moderately acidic soils, making them adaptable to mixed cropping systems and sustainable farming practices (Nair et al., 2017; Maney et al., 2022).

Cocoa pulp mainly consists of water, sugars (sucrose, fructose, glucose), and organic acids, with citrate being the most dominant. It also contains small amounts of malic, tartaric, and oxalic acids. Potassium is the primary mineral, and ascorbic acid accounts for 97% of its vitamin content (Pettipher, 1986). During chocolate processing, cocoa beans and pulp are fermented, and the nutrient-rich mucilage is usually discarded. However, if collected hygienically, this mucilage can be used to produce value-added health drinks like low-alcohol, nutrient-rich wine, helping farmers increase utilization and earn better returns.

Wine production, or vinification, uses biotechnology to convert fruit juice into alcohol, typically with *Saccharomyces cerevisiae* yeast. Cocoa pulp juice has been successfully used to make products like vinegar, beer, and jelly. Fermenting the mucilage not only creates value-added products but also improves bean quality by reducing acidity. The process involves separating the pulp, fermenting it with

Saccharomyces cerevisiae, and evaluating the resulting wine's quality (Ngangonum et al., 2022).

Wine aging, or reductive aging, involves chemical changes that occur after bottling without oxygen. In cocoa pulp wine, aroma and flavor are key to its quality and market value. Aging enhances these traits by altering phenolic compounds, which affect taste, aroma, and color. These changes involve reactions like anthocyanin breakdown, tannin interactions, oxidation, and polymerization (Hatice & Ezgi, 2017).

The objective of the study is to examine the physicochemical properties of cocoa mucilage and wine, with the aim of improving quality and enhancing the post-harvest handling of cocoa fruit and mucilage.

2. MATERIALS AND METHODS

2.1 Sample Collection

Cocoa pods were procured from local farmers of Idukki district of Kerala during the month of November, 2022. The following flow chart (Fig. 1) shows the preparation of wine from cocoa mucilage.

2.2 Preparation of Cocoa Mucilage

To break the pods, a cocoa pod breaker was employed. The extracted beans were then

processed using a cocoa pulp extractor, to remove the desired amount of pulp and facilitate proper fermentation. The extractor features two concentric cylinders, with the inner one containing uniform holes and rotating at a fixed RPM using a motor and variable frequency drive (VFD). The pulp extraction rate depends on the inner cylinder's speed, with 20% extraction achieved at 25 RPM. Physicochemical properties of the extracted cocoa mucilage such as pH, total soluble solids (TSS), titratable acidity, ascorbic acid content, color, antioxidant activity (DPPH), reducing sugar, and total phenolic content (TPC) were analysed using standard methods.

2.3 Setup for Anaerobic Fermentation

Approximately 1 litre of filtered cocoa mucilage drippings was mixed with 1.5 litres of water and about 1 litre of sucrose solution. *Saccharomyces cerevisiae* strains from a commercial yeast culture were then added to the mixture. A 2% concentration of commercially dried yeast was used as the starter culture. The Fermentation experiments were carried out in ceramic vats of 5 L capacity. The ceramic vats were tied with muslin cloth and stored in a dark room. The fermentation temperature for cocoa wine production was approximately 25 30°C and stirring was performed during the initial seven days of fermentation. The vat was stirred once in a day at regular time intervals in clockwise and anticlockwise direction for seven days. After seven days, the vats were tied tightly and kept without agitation up to 21st day of storage.

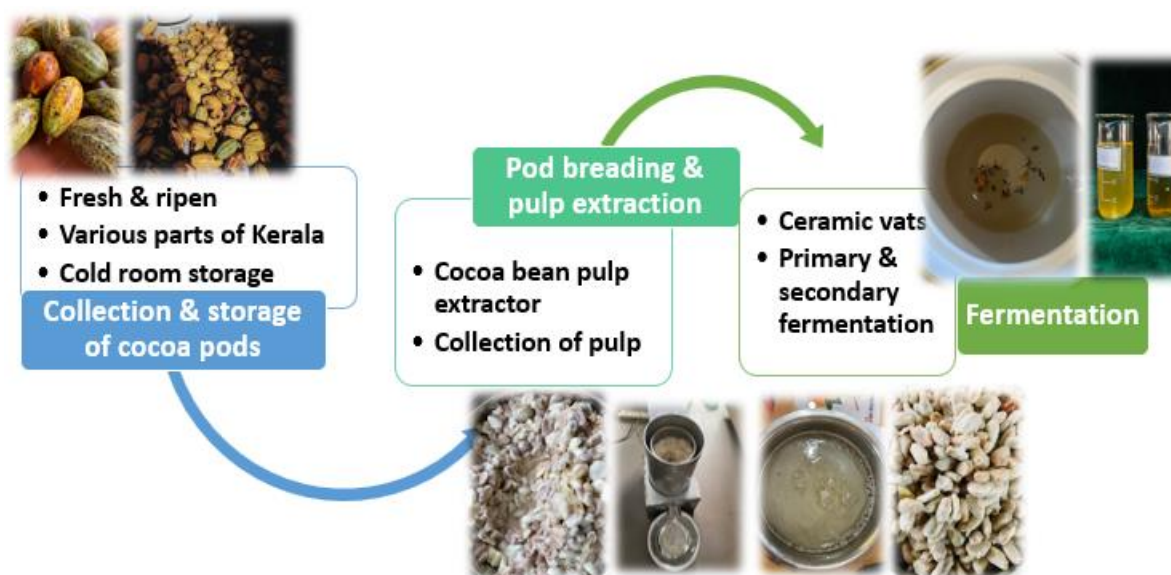


Fig. 1. Preparation of cocoa mucilage wine

At 21st day, the suspended solid particles were removed with filter under aseptic conditions. The wine was stored for 21 days more and then tested for quality parameters.

2.4 Physicochemical Properties

Physicochemical properties are important in the area of nutritional aspects, quality, storage and overall acceptability of the product. Physicochemical properties of the extracted cocoa mucilage and prepared cocoa mucilage wine such as pH, total soluble solids (TSS), titratable acidity, ascorbic acid content, color, antioxidant activity (DPPH), reducing sugar, and total phenolic content (TPC) were analysed using standard methods.

2.4.1 TSS

A hand refractometer (Lead series, ERMA, Japan) was used to assess the total soluble solid (TSS) of freshly extracted cocoa mucilage and wine. Total soluble solids were measured at 20°C using it and the results were expressed as °Brix. The device operates on the basis of the sample's refractive index. Using a cleaned dropper, two to three drops of the sample were put onto a prism plate. The sample's °Brix reading was then acquired, and the amount of TSS was expressed appropriately (Joy et al., 2015).

2.4.2 pH

The pH of the fruit sample was determined using a digital pH meter of model (M/s. Systronics; Model MK VI). One gram of fruit juice was diluted to 10ml with water respectively. The pH was analysed with buffer (pH 4.7). Triplicate was taken from each sample (AOAC, 2000).

2.4.3 Acidity

A 10% sample was prepared by diluting 10 ml of juice to 100 ml with distilled water in a volumetric flask. Using phenolphthalein as an indicator, 10 ml of this 10% sample was titrated against 0.1 N NaOH, which had been standardized using normal oxalic acid. The endpoint was identified by a color change from colorless to pale pink (Rekha et al., 2012). The total acidity, expressed as a percentage of tartaric acid was calculated using the formula provided in the equation.

$$\text{Acidity (\%)} = \frac{\text{Normality of alkali} \times \text{Titre volume} \times \text{Equivalent weight of tartaric acid} \times 100}{\text{Weight of sample} \times \text{volume made up}}$$

2.4.4 Total phenolic content

The total phenolic content was determined using the colorimetric method described by Gao et al., (2019), with the Folin-Ciocalteu reagent and gallic acid as the standard. It is measured with the Shimadzu UV 1800 Spectrophotometer. To 1 ml of appropriately diluted sample, 5 ml of Folin-Ciocalteu reagent solution was added, mixed thoroughly and incubated for 5 minutes. Subsequently, 4 ml of 7.5% (m/v) Na₂CO₃ solution was added, mixed well and the mixture was kept in the dark at room temperature for 1 hour. The absorbance was measured at 760 nm and the total phenolic content was calculated and expressed as milligrams of gallic acid equivalents per millilitre of sample.

2.4.5 Antioxidant capacity (DPPH)

The antioxidant activity was evaluated using the DPPH free radical scavenging assay as described by Hayat et al., (2011). Various concentrations of sample were placed in clean test tubes and 2 ml of DPPH solution was added to each. The tubes were incubated in the dark for 30 minutes and the absorbance was measured at 517 nm using spectrophotometer (Shimadzu UV 1800). Control absorbance was also measured alongside the samples. The scavenging activity was calculated using the formula provided in equation.

$$\text{DPPH Scavenging activity (\%)} = \left(\frac{A-B}{A} \right) \times 100$$

where A is control absorbance, and B is the absorbance of DPPH and substrate.

2.4.6 Ascorbic acid

The volumetric titration method was used to determine ascorbic acid content. In a conical flask, 5 ml of standard ascorbic acid solution (100 µg/ml) containing 10 ml of 4% oxalic acid was titrated against 2,6-dichlorophenol indophenol dye. The endpoint was indicated by the appearance and persistence of a pink color. The volume of dye consumed (V₁ ml) was equivalent to the amount of ascorbic acid. Similarly, 5 ml of the sample (prepared by dissolving 5 g of sample in 100 ml of 4% oxalic acid) was added to a conical flask along with 10 ml of 4% oxalic acid and titrated against the dye (V₂ ml). The ascorbic acid content was calculated using the formula provided by Rekha et al., (2012).

$$\text{Ascorbic acid} \left(\frac{\text{mg}}{100 \text{ ml}} \right) = \left(0.5 \frac{\text{mg}}{V_1 \text{ ml}} \right) \times \left(\frac{V_2}{15 \text{ ml}} \right) \times \left(\frac{100 \text{ ml}}{\text{Weight of sample}} \right) \times 100$$

2.4.7 Reducing sugar

The reducing sugar content was determined using a modified DNS method (Khatri, 2020). To prepare the DNSA reagent, 1 g of 3,5-dinitrosalicylic acid (DNSA) was dissolved in 80 ml of 0.5 N NaOH at 45°C, along with 30 g of sodium-potassium tartrate. The solution was brought to a final volume of 100 ml with distilled water after cooling to room temperature. A 0.1 ml sample was diluted with 10 ml of distilled water and 0.5 ml of this diluted sample was transferred to a test tube containing 2.5 ml of distilled water. Then 3 ml of DNSA reagent was added and the mixture was heated in a boiling water bath for 5 minutes. The absorbance was measured at 540 nm using a spectrophotometer (Shimadzu UV 1800). The reducing sugar content was calculated from a standard calibration curve of D-glucose, and the results were expressed in terms of percentage of reducing sugar.

2.4.8 Color

A significant visual quality factor for the majority of food products is colour. Using a Lovibond tintometer (wavelength range of 380 - 780 nm) the colour values (L^* , a^* and b^*) of cocoa mucilage and wine were determined. In the procedure, cocoa mucilage and wine were taken and placed in the sample port. The parameters, lightness (L^*), red to green (a^*), yellow to blue (b^*) were obtained from the instrument (Sasikumar et al., 2024).

2.5 Quality Analysis of Cocoa Mucilage Wine

2.5.1 Alcohol content

The alcohol percentage was determined using the dichromate spectrophotometric method (Miller, 1959). A 3 ml sample was transferred to a 100 ml distillation flask, diluted with 30 ml of distilled water and heated in a distillation unit at 70–80°C for 20 minutes. The distillate was collected in a 50 ml flask containing 25 ml of potassium dichromate solution. This mixture was then placed in a water bath maintained at 60°C for 20 minutes. After cooling to room temperature, the volume was adjusted to 50 ml with distilled water and the absorbance was measured at 600 nm using a spectrophotometer

(Shimadzu UV 1800). The total alcohol content (%) was calculated using a calibration curve with ethanol as the standard.

3. RESULTS AND DISCUSSION

The physicochemical properties of a fruit determine its ideal use, whether for direct consumption, processing, or technological applications ultimately affecting the quality of the final product (Najman et al., 2023). The physicochemical properties of cocoa mucilage and cocoa mucilage wine, such as total soluble solids (TSS), pH, acidity, total phenolic content (TPC), antioxidant activity (DPPH), ascorbic acid, reducing sugar and colour, were analysed according to standard protocols.

3.1 Physicochemical Properties

The TSS content of prepared wine is 21.5°Brix which is higher compared to TSS of mucilage (17.7°Brix). TSS was increased in the wine because sucrose solution was added for the purpose of fermentation which provided the medium for activation of yeast.

The pH of prepared wine was obtained as 3.66 which is slightly lower than the pH of mucilage (3.97). The preferred wine pH is around 3.6 and the better pH for yeast and lactic acid bacteria is around 4.5. However, spoilage bacteria can also grow well at pH 4.5. But spoilage bacteria do not grow well below pH 3.6. Wine yeasts and some lactic acid bacteria can still metabolize in a pH range of 3.3–3.6. The low pH can prolong the fermentation process due to slow growth of microorganisms involved (Jacobson, 2006).

The titrable acidity of wine was found to be 0.0381 g/ml, which is lower than the value for mucilage (0.3 g/ml). Throughout the fermentation process, no consistent pattern in the change in titrable acidity was observed. However, it was found that the titratable acidity of the must had reduced by the end of fermentation (Kasture and Kadam, 2018).

The total phenolic content of the wine was measured at 258 mg GAE/mL, significantly higher than the TPC of the pulp, which was 107.03 mg GAE/mL. Several processes contribute to the phenolic transformations in wine during and after production. These include anthocyanin degradation, tannin interactions with proteins and polysaccharides, procyanidin cation formation, oxidation and polymerization reactions

of procyanidins, anthocyanin copigments synthesis, reactions of anthocyanins with compounds containing polarized double bonds and condensation reactions between anthocyanins and tannins (Hatice & Ezgi, 2017).

The antioxidant scavenging activity (DPPH) of wine showed a higher value (41.72%) compared to that of cocoa mucilage (34.3%). Red wines exhibit stronger antioxidant activity. Polymeric phenolic compounds are responsible for half of the total scavenging radical activity of red wine (as measured by the DPPH and ABTS techniques). The order of reactivity for the remaining 50% is as follows: phenolic acids and flavanols are the next most active, followed by anthocyanins and flavan-3-ol. (Fernandez et al., 2004).

The vitamin C value for wine was obtained as 0.016 mg/100 ml. This is lower compared to vitamin C content of mucilage (1.01 mg/100 ml). All wine varieties showed a drop in L-ascorbic acid level following the conclusion of the fermentation process. Other publications claim

that several percent of L-ascorbic acid were lost during the alcoholic fermentation process (Czyżowska et al., 2015). The loss of L-ascorbic acid could have been caused by a number of circumstances.

The reducing sugar content was determined to be 21.42%, indicating an increase compared to the 10.41% observed in mucilage. The typicality of the cultivars and the winery's winemaking process are closely related to the higher reducing sugar concentration of wine. Additionally, the fluctuation in reducing sugar content and other physicochemical qualities is influenced by other factors such soil type, grape sanitary conditions, climate, weather and wine management (Neto et al., 2015).

The cocoa mucilage wine had colour characteristics: L* value of 68.25, a* value of 2.02 and b* value of 11.03. Higher L* value indicates lighter colour of wine. The color of the wine is one crucial factor that influences consumer preference. a* & b* values showed an increase and L* value decreased as the wine fermentation progress.

Table 1. Physico-chemical characteristics of cocoa mucilage

Quality parameters	Result
Total phenolic content (mgGAE/ml)	107.03±0.12
Total soluble solids (°Brix)	17.7±0.2
pH	3.97
Titration acidity (g/ml tartaric acid)	0.3±0.05
Vitamin C (mg/100ml)	1.01±0.04
Antioxidant scavenging activity (DPPH) (%)	34.3±0.1
Reducing sugar (%)	10.41±0.2
Color	
L*	72.25±0.04
a*	2.0±0.03
b*	9.3±0.03

Table 2. Physico-chemical characteristics of freshly prepared cocoa mucilage wine

Quality parameters	Result
Total phenolic content (mgGAE/ml)	258±0.2
Total soluble solids (°Brix)	21.5°±0.12
pH	3.66±0.01
Titration acidity (g/ml tartaric acid)	0.0381±0.07
Vitamin C (mg/100ml)	0.016±0.02
Antioxidant scavenging activity (DPPH) (%)	41.72±0.09
Reducing sugar (%)	21.42±0.1
Alcohol content (%)	7±0.4
Color	
L*	68.25±0.03
a*	2.02±0.02
b*	11.03±0.03

3.2 Quality Analysis of Cocoa Mucilage Wine

Alcohol content of the wine was found to be 7.4%. Alcohol content increases as fermentation continues. It is due to the steeped decrease of TSS during fermentation. This is attributed to high fermentability of wine, due to the availability of high amount of sugar (Thungbeni et al., 2020). Cocoa mucilage contains less sugar compared to wine because additional sucrose is typically added during wine fermentation. This added sugar causes an initial increase in the sugar content of wine, serving to activate the yeast and initiate alcoholic fermentation. Over time, as the wine ages, the sugar content decreases as it is consumed during the fermentation process. It was observed that alcohol generally had a greater impact on taste and mouthfeel characteristics than fragrance descriptors (King et al., 2013).

In addition to the amount of alcohol, other factors that affect the final quality after fermentation include pH, acidity, antioxidant activity (DPPH), TPC, TSS, reducing sugar, ascorbic acid concentration, and colour. In the section 3.1, it was discussed how these characteristics changed throughout the wine fermentation process and their importance.

4. CONCLUSION

To improve food processing methods and create novel cocoa-based products, it is essential to research the physicochemical characteristics of cocoa. Enhancing processing efficiency, storage, and handling requires consideration of parameters such as total soluble solids (TSS), pH, acidity, antioxidant activity (DPPH), total phenolic content (TPC), ascorbic acid, reducing sugars, and colour. This information helps to increase overall processing effectiveness, reduce waste, and produce high-quality products. These characteristics have a big impact on shelf life and product preservation, and they also affect customer appeal. Cocoa quality can be raised and post-harvest handling losses can be greatly decreased by improving its by-product utilization through wine fermentation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

Author acknowledges the work which mentioned in this manuscript was done under the Kerala Agricultural University and All India Coordinated Research Project on Post-Harvest Engineering and Technology (AICRP on PHET, Tavanur, Kerala).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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