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RESEARCH ARTICLE

Effect of Water Bath Heating on Tannin Content and Quality Improvement Potential of Gambier (*Uncaria gambir* Roxb.)

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Abstract: Gambir (*Uncaria gambir* Roxb.) is a source of natural phenolic compounds rich in catechins and tannins and has potential as a sustainable industrial raw material. During heating, phenolic compounds may undergo structural changes, including possible oxidation and polymerization, which can affect gambier quality beyond its catechin content. This study evaluated the effect of indirect heating duration using a water bath on the measured tannin content and phenolic characteristics of gambier. Four heating durations were evaluated: 0, 120, 240, and 360 minutes. The samples were analyzed for color, tannin content, total phenolic content, and functional groups using Fourier Transform Infrared (FTIR) spectroscopy. Data were analyzed using Analysis of Variance (ANOVA) at the 5% significance level. Heating duration significantly affected the characteristics of gambier. Increasing the heating duration resulted in darker and redder gambier powder, accompanied by changes in measured tannin and total phenolic contents. Tannin content increased from 23.89% in the control to 35.89% after 240 minutes of heating, then decreased to 28.17% after 360 minutes. Total phenolic content showed a similar pattern, reaching the highest value of 255.21 mg GAE/g after 240 minutes. FTIR analysis showed the presence of major phenolic functional groups, including O–H, aromatic C = C, C–O, and C–O–C, which remained detectable after heating. Based on the measured parameters, indirect heating using a water bath for 240 minutes was the most effective condition for obtaining the highest measured tannin content while maintaining the characteristic phenolic functional groups of gambier. The findings provide a basis for further development and standardization of gambier as a tannin-rich raw material for potential green industrial applications.

Keywords: Condensed Tannin, Phenolic Compounds, Catechin Polymerization, Green Industry, Natural Biomaterials

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Introduction

Tannins are secondary metabolites belonging to the polyphenol group and are widely used in the food, pharmaceutical, cosmetics, and biomaterial industries because of their ability to interact with proteins, polysaccharides, and metal ions. Based on their chemical structure, tannins are generally classified into hydrolyzable and condensed tannins, with condensed tannins showing relatively high stability toward heat and enzymatic degradation [1]. These characteristics make tannins valuable natural compounds for developing functional and industrial materials from renewable and environmentally friendly resources.

One important source of natural tannins is gambir (*Uncaria gambir* Roxb.), a major commodity of West Sumatra that has long been utilized as a source of phenolic compounds. Gambier contains considerable amounts of catechins and tannins, with catechin contents ranging from 7–33% and tannin contents from 22–55%, depending on the raw material and processing conditions [2]. Catechin is an important phenolic component of gambier and can act as a precursor for condensed tannin formation through oxidation and polymerization during thermal processing. Consequently, changes in catechin and tannin contents during processing can substantially influence the chemical characteristics and potential applications of gambier.

The transformation of catechins into tannins is particularly relevant for gambier subjected to heating. Thermal processing can promote oxidation and polymerization of catechins, resulting in the formation of more complex tannin structures, accompanied by changes in color and phenolic composition. Therefore, gambier quality after thermal processing cannot be evaluated solely based on catechin content; the formation and accumulation of tannins should also be considered. Understanding how processing conditions influence tannin formation is thus important for developing a more consistent gambier raw material with controlled chemical characteristics.

Standardization of tannin formation is particularly important because gambier-derived tannins have potential applications as natural alternatives to synthetic materials in several sectors, including natural adhesives, leather tanning, functional foods, cosmetics, and biomaterials. Improving and controlling tannin content could therefore increase the added value of gambier and support its utilization as a renewable raw material for green industrial applications. In this context, a controlled thermal process may provide a relatively simple approach for modifying and standardizing the phenolic characteristics of gambier before its use in downstream applications.

Previous studies have demonstrated that heating conditions can influence the transformation of phenolic compounds in gambier. [3] reported that heating temperature and duration affected color and phenolic characteristics, with increased heating intensity promoting oxidation and polymerization of catechins. Similarly, [4] demonstrated that heat treatment influenced condensed tannin formation in gambier through catechin polymerization. However, these studies do not establish a specific heating duration under controlled indirect-heating conditions that can be used to optimize and standardize tannin formation.

The heating method itself is also an important consideration. Direct heating may result in less uniform heat transfer and localized overheating, which can accelerate uncontrolled oxidation and degradation of phenolic compounds. In contrast, indirect heating using a water bath provides heat through a surrounding water medium and allows the sample to be exposed to a more controlled and relatively uniform thermal environment. Such a system may provide better control over the balance between phenolic transformation and thermal degradation. Previous research by [4] showed that indirect heating produced greater condensed tannin formation than the other heating methods evaluated. Nevertheless, that study focused on comparing heating methods rather than determining the effect of different heating durations under a fixed indirect-heating condition.

Therefore, a specific research gap remains regarding the optimization of heating duration under controlled water-bath conditions for increasing and standardizing tannin content in gambier. In particular, it is necessary to determine whether prolonged indirect heating continuously increases tannin formation or whether an optimum duration exists beyond which degradation of phenolic compounds occurs. The novelty of the present study lies in evaluating a defined duration gradient of indirect heating at a controlled temperature of 70°C (0, 120, 240, and 360 min) and relating the resulting changes in tannin content to color, total phenolic content, and FTIR characteristics. This approach provides a process-oriented assessment for identifying an appropriate heating duration rather than merely comparing different heating methods.

Accordingly, this study aimed to evaluate the effect of indirect heating duration using a water bath on the tannin content and phenolic characteristics of gambier (*Uncaria gambir* Roxb.). The study also aimed to identify the heating duration that provides the most favorable balance between tannin formation and preservation of phenolic characteristics. The resulting optimized condition is expected to provide a practical basis for producing gambier with more consistent tannin characteristics and to support its further development as a standardized natural raw material for green industrial applications, including natural adhesives, leather tanning, cosmetics, functional food ingredients, and biomaterials.

Research Methodology

Materials and Equipment

The main material used in this study was raw gambier (*Uncaria gambir* Roxb.) obtained from local farmers in Lima Puluh Kota Regency, West Sumatra, Indonesia. The chemicals used included distilled water, 70% acetone, butanol–HCl reagent, $FeCl_3$, 1 N HCl, 1 N NaOH, and 1 N NaCl. The equipment used included a water bath, analytical balance, HunterLab ColorFlex EZ spectrophotometer, UV–Vis spectrophotometer, oven, food dehydrator, mortar, blender, 60-mesh sieve, FTIR spectrometer, and other laboratory glassware.

Sample Preparation

Raw gambier was first crushed using a mortar to obtain a powder and then dissolved in distilled water at a ratio of 1:10 (w/v). The mixture was homogenized and filtered through a fine sieve to remove impurities carried over from the raw material. The resulting filtrate was collected and dried using a food dehydrator at 70 °C for 24 h to obtain dried gambier. The dried material was subsequently ground and passed through a 60-mesh sieve to obtain a uniform sample before the heating treatment.

Heating Treatment

Heating was conducted using an indirect heating system with a water bath maintained at 70 °C. The prepared gambier sample was placed in a glass beaker and heated in the water bath, allowing heat to be transferred gradually and uniformly through the surrounding water.

Four heating-duration treatments were applied: 0 min (control, A), 120 min (B), 240 min (C), and 360 min (D). The control treatment was not subjected to water-bath heating, whereas the other treatments were heated for their respective treatment durations under the same temperature conditions. After completion of the designated heating period, the gambier filtrate was dried again using a food dehydrator at 70°C for 24 h. The dried samples were then ground and sieved through a 60-mesh sieve before further analysis.

Research Design

The study used a Completely Randomized Design (CRD) with one factor, namely water-bath heating duration. The treatments consisted of no heating (0 min), heating for 120 min, 240 min, and 360 min. Each treatment was repeated three times. The observed parameters included color characteristics, tannin content, total phenolic content, and functional group characteristics determined using Fourier Transform Infrared (FTIR) spectroscopy. The quantitative data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level. When the analysis showed a significant treatment effect, Duncan's New Multiple Range Test (DNMRT) was performed at the 5% significance level to determine differences among treatment means.

Gambier Characteristics Analysis

Gambier characteristics were analyzed based on color, tannin content, total phenols, and Fourier Transform Infrared (FTIR) spectroscopy [5, 6].

Color Analysis

Color characteristics were analyzed using a HunterLab ColorFlex EZ spectrophotometer with L*, a*, and b* parameters. The L* value represents lightness, while a* and b* represent the red–green and yellow blue components, respectively. The Browning Index (BI) was calculated from the L*, a*, and b* values to determine the intensity of browning associated with the heating process.

Tannin Content Analysis

Tannin content was determined using UV–Vis spectrophotometry. A 2 mg sample of gambier powder was dissolved in 70% acetone and subsequently reacted with butanol–HCl and ferric-HCl reagents. The mixture was homogenized using

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vortexing and ultrasonication for 15 min before absorbance measurements were performed. The absorbance was measured using a UV–Vis spectrophotometer over a wavelength range of 200–500 nm, and the tannin content was determined based on the resulting spectrophotometric response.

Total Phenolic Content Analysis

Total phenolic content was determined using the Folin Ciocalteu method. The sample was reacted with Folin Ciocalteu reagent, and the absorbance was measured using a UV–Vis spectrophotometer. Gallic acid was used as the reference standard. The total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg GAE/g).

Fourier Transform Infrared Spectroscopy Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify changes in the functional groups of gambier before and after heating. FTIR spectra were recorded over the wavenumber range of 4000–400 cm^{-1}. The spectra obtained from the different heating treatments were compared to identify changes in characteristic functional groups associated with phenolic compounds.

Results and Discussion

This study used a heating temperature of 70°C based on previous research showing that this temperature is the most effective condition for maintaining and improving the quality of gambier's phenolic compounds. A study by [7] reported that heating at 70°C resulted in the best phytochemical characteristics of gambier, including the stability of active compounds and improved product quality. Therefore, 70°C was chosen as the operating condition in this study to ensure optimal oxidation and polymerization of catechins without causing excessive thermal degradation. Using this optimized temperature, this study then focused on evaluating the effect of indirect heating duration using a water bath on tannin formation and phenolic characteristics of gambier. This approach allows for the assessment of time as a critical variable in the transformation of catechins into condensed tannins at a temperature that has been proven optimal.

Color Analysis

The results of gambier powder color testing for various indirect heating durations using a water bath are presented in Table 1. Color is an important parameter in this study because changes in gambier color are related to changes in phenolic compounds during the heating process. Oxidation and polymerization of catechins into tannins cause the color of gambier to darken and become reddish, so color observation can be used as an early indication of tannin formation [8].

Table 1: Color analysis of treated Gambier under different durations

Treatments	L*	a*	b*	°Hue	Color interpretation
A (0 minutes)	56.04±0.005	7.67±0.012	18.27±0.008	67.23±0.04	*Reddish yellow*
B (120 minutes)	49.62±0.012	8.65 ±0.036	17.32±0.008	63.46±0.08	*Reddish yellow*
C (240 minutes)	39.74±0.005	14.31±1.193	17.13±0.012	50.13±0.40	*Red*
D (360 minutes)	31.43±0.005	11.45±0.024	10. 84±0.05	43.43±0.05	*Red*
CV: 0.27 %					

According to Table 1, the L* value decreased from 56.04 in the unheated treatment to 31.43 after 360 minutes of heating. The decrease in the L* value indicates that the longer the heating time. the darker the gambier color. This is suspected to be caused by the oxidation and polymerization of phenolic compounds during the heating process. The a* values for all treatments were positive. This indicates the presence of a red color component. The a* value tended to increase with heating time. This indicates that the red color became more dominant due to the formation of polymerized tannin compounds. Conversely, the b* value decreased from 18.27 to 10.84. This indicates a reduction in the yellow color component during the heating process. The °Hue values in Table 1 indicate that the unheated treatment produced a reddish-yellow color, whereas the heated treatments showed a red color. Overall, the decrease in L and °Hue values, together with changes in a and b*, indicates that increasing the heating duration resulted in darker and redder gambier powder. These changes are consistent with alterations in the phenolic characteristics of gambier during heating.

Tannin Content Analysis

The changes in gambier color during indirect heating were accompanied by changes in tannin content. As shown in Table 2, analysis of variance (ANOVA) at the 5% significance level indicated that heating duration significantly affected the tannin content of gambier powder. Tannin content ranged from 23.89 to 35.89%, with the unheated treatment showing the lowest value (23.89%). Tannin content increased to 32.65% after 120 minutes and reached the highest value of 35.89% after 240 minutes of heating. However, the tannin content decreased to 28.17% after 360 minutes.

The increase in tannin content up to 240 minutes was accompanied by the darker and redder color characteristics observed in the preceding color analysis (Table 1). This pattern is consistent with changes in phenolic compounds during heating, including the possible oxidation and polymerization of catechin-derived compounds into condensed tannins. [3] reported that increasing heating duration can increase tannin content to an optimum level, while [9] reported that heating can promote condensation and polymerization reactions of phenolic compounds, resulting in more complex tannin structures. However, because individual catechins were not directly quantified in the present study, the observed increase in tannin content should not be interpreted as direct confirmation of catechin polymerization.

The decrease in tannin content after 360 minutes suggests that prolonged heating no longer favored an increase in the measured tannin content. Further oxidation or degradation of phenolic compounds during extended thermal exposure may have contributed to this decrease. [10] reported that tannins are sensitive to prolonged thermal exposure, while [11] reported that excessive heating duration can reduce phenolic compound content. The very dark color observed after 360 minutes was also consistent with substantial changes in the phenolic characteristics of the sample. However, the present study did not determine extraction yield or mass recovery; therefore, the possible contribution of concentration effects associated with moisture loss cannot be completely excluded. The decrease at 360 minutes should consequently be interpreted as a reduction in measured tannin content rather than definitive evidence of a specific degradation mechanism.

Overall, the 240-minute treatment produced the highest measured tannin content (35.89%) under the conditions evaluated. This result indicates that extending the heating duration beyond 240 minutes did not provide an additional increase in tannin content and may adversely affect the measured phenolic characteristics of gambier.

Table 2: Tannin content of treated Gambier under different heating durations

Treatments	Tannin Content (%)	
A (0 minutes)	23.89±0.532	a
D (360 minutes)	28.17±1.929	b
B (120 minutes)	32.65±0.780	c
C (240 minutes)	35.89±0.192	d
CV = 0.38 %		

Total Phenol Analysis

The darkening of gambier powder and the increase in tannin content observed during the previous heating treatment were accompanied by changes in total phenolic content. Therefore, total phenolic content was analyzed to evaluate the changes in measurable phenolic compounds during indirect heating. The results are presented in Table 3.

Table 3: Phenolic content of treated Gambier under different heating durations

Treatments	Total phenolic mg GAE/g	
A (0 minutes)	225.81±2.41	a
D (360 minutes)	232.64±6.39	a
B (120 minutes)	241.19±2.47	b
C (240 minutes)	255.21±0.49	c
CV = 1,25 %		

Based on analysis of variance (ANOVA) at the 5% significance level, heating duration significantly affected the total phenolic content of gambier powder. As shown in Table 3, total phenolic content ranged from 225.81 to 255.21 mg GAE/g.

The unheated treatment had the lowest value of 225.81 mg GAE/g, which increased to 241.19 mg GAE/g after 120 minutes and reached the highest value of 255.21 mg GAE/g after 240 minutes of heating. However, total phenolic content decreased to 232.64 mg GAE/g after 360 minutes.

The increase in total phenolic content up to 240 minutes was consistent with the changes observed in color and tannin content. At this treatment duration, the gambier powder showed darker and redder characteristics and the highest measured tannin content. This pattern indicates that heating duration influenced the measurable phenolic characteristics of gambier. As reported by [9], heating can enhance the release of phenolic compounds into the extraction medium and increase total phenolic content until an optimum condition is reached.

The highest total phenolic content at 240 minutes suggests that the applied indirect-heating condition was favorable for maintaining or increasing the measurable phenolic compounds while producing the highest measured tannin content. However, because individual phenolic compounds were not quantified in the present study, the increase in total phenolic content cannot be attributed specifically to the release or transformation of catechins. In addition, extraction yield and mass recovery were not determined; therefore, possible concentration effects associated with moisture loss cannot be completely excluded.

At 360 minutes, the decrease in total phenolic content to 232.64 mg GAE/g was accompanied by a decrease in tannin content. The reduction may be associated with further oxidation or degradation of phenolic compounds during prolonged heating. Nevertheless, the present data do not allow a specific molecular degradation mechanism to be established. Overall, the results indicate that extending the heating duration beyond 240 minutes did not further increase the measured total phenolic content and was associated with a decline in both total phenolic and tannin contents.

FTIR Analysis

FTIR analysis was conducted to confirm the chemical changes that occur in gambier powder due to indirect heating using a water bath. Particularly those related to phenolic compounds and tannin formation. This test was performed on samples with varying heating times. The results are presented in Figure 1. Based on the FTIR spectrum, in the wavenumber region of 3200–3600 cm⁻¹. There is an absorption peak around 3230 cm⁻¹. This indicates the presence of hydroxyl groups (-OH). These groups are O–H stretching groups associated with hydrogen bonds in phenolic and polyphenolic compounds such as catechins and tannins [12]. The absorption peak at 2910 cm⁻¹ indicates the presence of a C–H stretching group from the aliphatic group $-CH_2-$. This is related to the carbon structure of flavonoid compounds. Furthermore, the peak at 1608 cm⁻¹ indicates the presence of an aromatic C = C group. This indicates the presence of conjugated aromatic rings typical of flavonoids and condensed tannins in gambier [13]. At 1280 cm⁻¹. a C–O group was detected. associated with a phenolic or aromatic ether bond. The peak at 1018 cm⁻¹ indicates a C–O–C group. This is characteristic of the flavonoid structure in gambier [14]. Meanwhile. the weak peak at 2328 cm⁻¹ is suspected to originate from atmospheric CO_2 interference during the measurement and therefore is not interpreted as the main group of the sample.

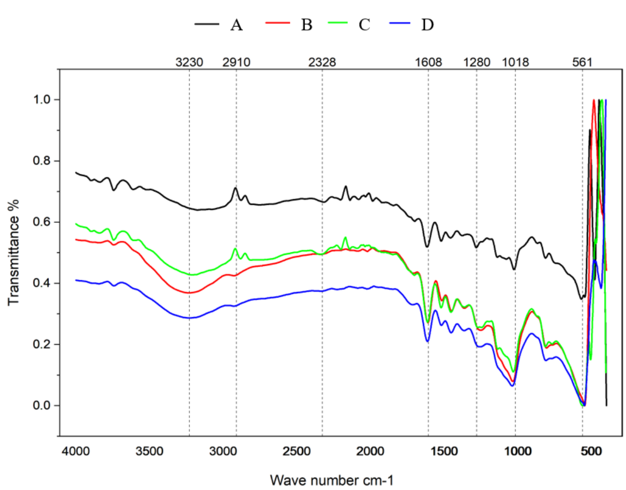

Fig. 1: FTIR Spectrum of treated Gambier under different heating durations

These FTIR results support the observed changes in the phenolic characteristics of gambier during indirect heating. The presence of O–H, aromatic C = C, C–O, and C–O–C groups indicates that phenolic compounds remained the major components after heating. Changes in the spectral characteristics at 1608 cm^{-1}, associated with aromatic C = C stretching, and 1018 cm^{-1}, associated with C–O–C stretching, were observed among the different heating treatments. These changes were consistent with the quantitative variation in tannin content (Table 2), which increased from 23.89% in the control to 32.65% after 120 minutes and reached the highest value of 35.89% after 240 minutes, before decreasing to 28.17% after 360 minutes. The fingerprint region below 1000 cm^{-1} also showed differences among treatments, reflecting changes in the overall molecular characteristics of the heated gambier samples. However, because this region contains overlapping absorption bands, the observed changes cannot be assigned to a specific molecular transformation without further structural analysis. The decrease in tannin and total phenolic contents after 360 minutes may be associated with further oxidation and degradation of phenolic compounds during prolonged heating. Nevertheless, the main phenolic functional groups remained detectable in the FTIR spectra, indicating that prolonged heating affected the amount and stability of measurable phenolic compounds rather than completely eliminating the major phenolic structures. Overall, 240 minutes of indirect heating provided the highest measured tannin content while maintaining the characteristic phenolic functional groups of gambier. However, FTIR analysis alone cannot directly confirm the conversion of individual catechins into condensed tannins.

From an industrial perspective, the 240-minute treatment provides a potentially useful condition for preparing gambier with a higher measured tannin content and relatively consistent phenolic characteristics. Tannin-rich gambier could potentially be further developed as a renewable phenolic resource for natural adhesives, where tannins can interact with polymeric materials and contribute to adhesive bonding, or for vegetable leather tanning, where tannins can interact with collagen. The treated gambier may also be considered for applications in cosmetics, functional materials, and other biomaterial products requiring plant-derived phenolic compounds. However, these applications require further formulation and functional-performance testing. Therefore, the present study provides a processing condition for producing a tannin-rich gambier raw material rather than direct evidence of its performance in a specific commercial product.

However, several aspects require further investigation to strengthen the understanding and practical optimization of the thermal treatment. The present study did not directly quantify individual catechin compounds; therefore, HPLC-DAD or LC-MS/MS analysis would be required to determine the specific changes in catechins during heating. Further studies should also evaluate the kinetics of tannin formation and phenolic degradation using a broader range of heating times and temperatures to establish reaction-rate parameters and improve predictive process control. In addition, although the 240-minute treatment produced the highest measured tannin content, its functional performance in specific commercial applications was not evaluated in the present study. Further work should therefore assess the performance of the standardized gambier material in relevant formulations, such as natural adhesives, leather tanning systems, or active packaging materials.

Conclusion

Indirect heating using a water bath affected the physical and chemical characteristics of gambier (*Uncaria gambir* Roxb.) powder. Increasing the heating duration resulted in darker and redder gambier powder, accompanied by changes in tannin and total phenolic contents. Tannin content increased from 23.89% in the control to 35.89% after 240 minutes of heating, while total phenolic content increased from 225.81 to 255.21 mg GAE/g. However, prolonged heating for 360 minutes resulted in decreases in both tannin and total phenolic contents, indicating that extended heating may adversely affect the measured phenolic characteristics. FTIR analysis showed the presence of characteristic phenolic functional groups, including O–H, aromatic C = C, C–O, and C–O–C, in gambier after heating. The observed changes in tannin content, total phenolic content, color, and FTIR characteristics were consistent with phenolic transformation during heating. However, the present study did not directly quantify individual catechins; therefore, the observed changes cannot be considered direct confirmation of catechin polymerization. Further studies using HPLC or LC–MS are needed to characterize changes in individual catechins during heating. Based on the measured parameters, indirect heating using a water bath for 240 minutes was the most effective treatment for obtaining the highest measured tannin content while maintaining the characteristic phenolic functional groups of gambier. This controlled heating condition provides a practical basis for improving the consistency of gambier as a natural tannin-rich raw material for potential applications in food, pharmaceutical, cosmetic, adhesive, leather, and biomaterial industries.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Author's Contributions

Daimon Syukri: Wrote the manuscript and conceptual.
Bastian Nova and Santosa: Data curation.
Rina Yenrin: Research Supervisor and conceptual.
Muhammad Sulthan and Syalki Habib Akbar: Lab Work.
Efrina: Administration.
Diana Silvy: Farmer Coordination.

Ethics

Ethical approval was not required for this study as it did not involve human participants or animals.

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