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# Investigation of the Effect of Heating Duration in Gambier (*Uncaria gambir* Roxb.) Processing on Its Phytochemical Composition and the Resulting Characteristics of a Gambier-based Acne Balm Product

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***Abstract.*** *Gambier (Uncaria gambir Roxb.) contains potent bioactive compounds with strong natural antimicrobial properties, making it a promising ingredient for acne treatment. However, its application in topical formulations remains limited due to a lack of understanding regarding the impact of processing conditions, particularly heating, on its functional efficacy. This study investigated the effect of heating duration on the characteristics of an acne treatment balm formulated with black cube gambier. Experiments using a completely randomized design (CRD) were carried out for 0, 30, 60, 90, and 120 minutes of heating durations to evaluate the balm's physical, chemical, and microbial properties. The results demonstrate that heating duration significantly influenced key parameters, including homogeneity, spreadability, melting point, irritation potential, texture, color, pH, total phenol content, and antibacterial activity ($p < 0.05$). The most optimal results were obtained at 120 minutes, yielding a balm with superior homogeneity, spreadability (4.47 cm), melting point (50°C), pH (7.44), total phenol content (54.28%), and diameter of the inhibition zone (16.35 mm). FTIR analysis confirmed an increase in phenolic compound stability, indicating enhanced antibacterial potential. These findings suggest that extended heating duration improves the stability and performance of gambier-based formulations. The optimized acne balm formulation highlights the potential of locally sourced gambier as a natural, eco-friendly acne treatment. Further research is recommended to evaluate product shelf-life, consumer acceptability, and industrial scalability.*
***Keywords:*** *gambier; black cube gambier; acne treatment balm; heating duration; acne.*

**Type of the Paper:** Regular Article.

## 1. Introduction

West Sumatra is recognized for its rich agricultural potential, particularly in commodities that contain bioactive compounds beneficial for health and industry. One of the region's most prominent agricultural products is gambier (*Uncaria gambir Roxb.*), an industrial crop with significant economic value [1,2]. Gambier cultivation is especially concentrated in Lima Puluh Kota Regency, the contributor of approximately 90% of Indonesia's total gambier production [1]. This highlights gambier's strategic role for both local farmers and the broader agro-industry sector.

Gambier has traditionally been used in a variety of industries, including leather tanning, dye production, and traditional medicine [3]. Its diverse applications are attributed to its rich chemical composition, particularly its high content of catechin and tannin. Catechin is a flavonoid


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compound known for its antioxidant, antibacterial, and anti-inflammatory properties, while tannin is widely utilized for its astringent characteristics. Interestingly, catechin and tannin levels in gambier are inversely related, where higher catechin content is typically associated with lower tannin concentrations [4].

In the pharmaceutical and cosmetic industries, catechin has gained considerable attention for its role in skincare formulations. Catechin's antioxidant properties allow it to neutralize free radicals, which helps prevent collagen and elastin degradation—key factors in skin aging [5]. Furthermore, catechin has been shown to act as a natural sunscreen by absorbing UV radiation and protecting skin cell DNA from damage [6]. Its antibacterial properties are particularly valuable in combating *Propionibacterium acnes*, a major contributor to acne vulgaris [7].

Acne vulgaris is a common skin condition in Southeast Asia, affecting 40–80% of adolescents and young adults [8]. In Indonesia, it ranks among the top three most prevalent dermatological issues, particularly in teenagers aged 14–19 years. As a result, there is growing demand for effective, safe, and natural skincare solutions to address this condition. Gambier-based personal care products such as facial masks, scrubs, and solid shampoos have shown promising antibacterial effects, supporting gambier's potential in acne treatment formulations [1,3,5].

Recent studies suggest that thermal treatment can enhance the bioactive properties of gambier. Heating gambier has been found to increase its catechin concentration by reducing impurities and improving the stability of phenolic compounds [9]. These phenolic compounds play a crucial role in disrupting the functioning of bacterial cell membranes, ultimately enhancing antibacterial activity [10]. By optimizing heating duration, it may be possible to improve the effectiveness of gambier extracts in skincare products.

The growing demand for natural and eco-friendly skincare products has intensified interest in plant-derived ingredients with therapeutic potential. Gambier (*Uncaria gambir* Roxb.), a traditional botanical resource native to West Sumatra, is rich in bioactive compounds known for their antimicrobial and antioxidant properties, making it a promising candidate for topical acne treatment. Despite its potential, the utilization of gambier in modern cosmetic formulations remains limited, particularly due to the lack of research on how processing parameters, such as thermal treatment, affect its functional properties. This study aimed to investigate the effect of heating duration on the physical, chemical, and antimicrobial characteristics of an acne treatment balm formulated with black cube gambier. The objective was to evaluate how different heating durations would influence the stability and efficacy of phenolic compounds within the formulation. By optimizing the heating process, the research sought to advance the development of effective, natural acne treatments based on locally sourced gambier, thereby enhancing its value in the health and cosmetic industries.

## 2. Materials and Methods

### *2.1. Time and place*

This research was conducted at multiple laboratories within the Faculty of Agricultural Technology, Andalas University, Padang. These included the Laboratory of Agricultural Product Biochemistry and Food Nutrition, the Laboratory of Engineering Technology and Agricultural Product Processing, the Laboratory of Microbiology and Biotechnology of Agricultural Products, and the Central Laboratory of Andalas University. The study was carried out from November 2024 to June 2025.

### *2.2. Materials and equipment*

The materials used in this study included raw gambier sourced from local farmers in Lima Puluh Kota Regency, stearic acid, glycerin, triethanolamine (TEA), propylene glycol, and virgin coconut oil (VCO) for balm formulation. For microbiological analysis, 0.5 McFarland solution, Mueller–Hinton agar, physiological saline, plate count agar (PCA), and distilled water were utilized.

The equipment employed included beakers, spatulas, a hotplate, measuring cylinders, an analytical balance, a stove, containers, trays, Petri dishes, a Mettler Toledo FiveEasy pH meter, a HunterLab ColorFlex EZ spectrophotometer, a digital thermometer, sieves, test tubes, glass plates, sterile paper discs, a magnetic stir bar, calipers, Erlenmeyer flasks, micropipettes with tips, a Bunsen burner, aluminum foil, cotton, cotton swabs, and an oven.

### *2.3. Research Design*

This research employed a Completely Randomized Design (CRD) with five treatments and three replications. Observational data for each parameter were analyzed using Analysis of Variance (ANOVA), and significant differences between treatments were further examined using Duncan's New Multiple Range Test (DMRT) at a 5% significance level. The treatments applied in this study included Treatment A (no heating), Treatment B (heating for 30 minutes), Treatment C (heating for 60 minutes), Treatment D (heating for 90 minutes), and Treatment E (heating for 120 minutes). The formulations employed in this study are displayed in Table 1.

**Table 1.** Acne Balm Formulations

| No | Material | Treatment (g) | | | | | Function |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | |
| 1 | Black cube gambier | 1 | 1 | 1 | 1 | 1 | Active substance |
| 2 | Stearic acid | 5 | 5 | 5 | 5 | 5 | Basic/emulsifier |
| 3 | VCO | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | Moisturizer |
| 4 | TEA | 4 | 4 | 4 | 4 | 4 | Basic/emulsifier |
| 5 | Glycerin | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | Solvent |
| 6 | Propylene glycol | 4 | 4 | 4 | 4 | 4 | Humectant |

The preparation of black cube gambier began by weighing and crushing gambier into smaller pieces, followed by soaking it in water at a 1:2 ratio at a temperature of approximately 70°C. The mixture was stirred until homogeneous and filtered to remove impurities. The resulting solution was then cooked at 70°C for varying durations of 0, 30, 60, 90, and 120 minutes, with constant stirring during these periods until the mixture became thickened. The thickened mixture was poured into a baking dish, left overnight, and subsequently dried under sunlight. The dried gambier, known as black cube gambier, was ground into a fine brown powder using a blender.

The balm formulation involved preparing two separate phases: oil phase (comprising stearic acid and VCO) and water phase (containing propylene glycol, TEA, and glycerin). Both phases were heated separately at 70°C and constantly stirred. Upon reaching homogeneity, the phases were combined, added with the black cube gambier powder, and stirred for 15–20 minutes. The mixture was then poured into containers and allowed to solidify for 30 minutes before being stored for further testing.

*2.4. Analysis of balm quality*

The formulated balm underwent a series of evaluations to assess its quality and performance. Homogeneity was tested by spreading the balm between two glass slides to observe the presence of coarse grains or phase separation. A spreadability test was conducted by placing a fixed amount of balm between two glass plates under a standard weight and measuring the diameter of the circular area formed after five minutes. The melting point was determined by gradually heating 1 g of sample until complete melting occurred. Potential for skin irritation was evaluated using an open patch applied to panelists' forearms, with informed consent from the panelists and institutional ethical approval. Organoleptic properties, including color, aroma, and stickiness, were assessed through a hedonic test involving 20 semi-trained panelists. FTIR analysis was carried out to identify the functional groups contained in the black cube gambier sample. For pH testing, 1 g of balm was dissolved in 10 mL of distilled water, and then the pH was measured using a pH meter. Color attributes (L*, a*, and b*) were analyzed using a HunterLab spectrophotometer. Total phenol content was quantified using the Folin–Ciocalteu method, with absorbance measured at 725 nm. Microbial safety was assessed through total plate count using PCA medium incubated at 37°C for 24–48 hours. Antibacterial activity against *Propionibacterium acnes* was measured using the disc diffusion method, with inhibition zones recorded post-incubation [11-15]. All measurements were conducted in triplicate (n = 3) to ensure accuracy and reproducibility. This comprehensive procedure ensured systematic evaluation and quality control of the formulated acne treatment balm.

## 3. Results and Discussion

*3.1. Analysis of balm quality*

Table 2 presents the color characteristics of black cube gambier subjected to various heating durations (0, 30, 60, 90, and 120 minutes), highlighting changes in lightness (L*), red–green intensity (a*), yellow–blue intensity (b*), and hue. The data show a decreasing trend in L* values with prolonged heating, where the initial sample (0 minutes) recorded the highest lightness (60.11 ± 1.15), which gradually decreased to 47.77 ± 10.95 after 120 minutes, indicating a darkening effect. The a* values showed minor fluctuations, with the highest value (7.95 ± 2.13) observed at 120 minutes, suggesting a slight increase in red intensity. Conversely, the b* values steadily declined from 19.10 ± 0.15 to 16.00 ± 1.79 over the heating period, reflecting reduced yellowness. Despite these changes, the hue values remained relatively stable, ranging between 1.11 ± 0.15 and 1.25 ± 0.09, maintaining a consistent red–purple color across all treatments. As shown in Table 2, the coefficient of variation (CV) was 2%, indicating minimal variability and reinforcing the reliability of the data. Overall, while prolonged heating influenced lightness and color intensity, the distinct red–purple hue characteristic of black cube gambier remained unchanged. The results in Table 2 demonstrate that heating duration significantly affected the color characteristics of black cube gambier. Among the three CIELAB parameters—L* (lightness), a* (red–green axis), and b* (yellow–blue axis)— L* experienced the most prominent and consistent change in values. A clear decreasing trend was observed, with L* dropping from 60.11 ± 1.15 at 0 minutes to 47.77 ± 10.95 after 120 minutes of heating. This suggests that prolonged thermal exposure led to visible darkening, likely due to the Maillard reaction, caramelization, or phenolic oxidation commonly seen in heat-processed plant materials. The a* values, representing the red–green axis, showed minor yet noticeable increases, especially at 120 minutes (7.95 ± 2.13), which could indicate mild enhancement in red pigments, possibly due to pigment concentration or structural changes in anthocyanin-like compounds under heat. However, the variability in a* was less consistent across treatments compared to L*. The b* values, associated with the yellow–blue axis, also decreased gradually, reflecting a reduction in yellowness. This decline may be attributed to thermal degradation of flavonoid or carotenoid compounds, which typically contribute to yellow coloration in plant extracts. Despite changes in L*, a*, and b* values, the hue remained relatively stable, ranging between 1.11 and 1.25, indicating that the core color tone of black cube gambier—classified as red–purple—was preserved regardless of the heating duration. Based on the magnitude and consistency of the changes, L* (lightness) emerged as the most relevant and sensitive indicator for assessing the thermal impact on gambier color. It provided a clear and quantifiable representation of browning or darkening effects, which are critical for visual quality evaluation in both raw material standardization and formulation development. In contrast, a* and b* served as supplementary indicators that provided insight into more subtle chromatic shifts, but were less dominant in defining the overall visual perception of black cube gambier during heating.

**Table 2**. Black cube gambier color test results

| Treatment | L* (Average ± SD) | a* (Average ± SD) | b* (Average ± SD) | °Hue (Average ± SD) | Color |
|---|---|---|---|---|---|
| A (0 minutes) | 60.11 ± 1.15[a] | 6.56 ± 0.53[a] | 19.10 ± 0.15[a] | 1.24 ± 0.02[a] | Red–Purple |
| B (30 minutes) | 57.26 ± 5.42[b] | 6.67 ±1.05[a] | 18.32 ± 0.23[b] | 1.22 ± 0.05[b] | Red–Purple |
| C (60 minutes) | 58.09 ± 7.70[c] | 5.86 ± 1.89[b] | 17.61 ± 0.68[b] | 1.25 ± 0.09[c] | Red–Purple |
| D (90 minutes) | 57.17 ± 5.42[d] | 6.39 ± 1.34[a] | 18.06 ± 0.49[b] | 1.23 ± 0.06[d] | Red–Purple |
| E (120 minutes) | 47.77±10.95[e] | 7.95 ± 2.13[c] | 16.00 ± 1.79[c] | 1.11 ± 0.15[e] | Red–Purple |

The FTIR spectra depicted in Fig. 1 illustrate the effect of heating duration on the chemical structure of black cube gambier over the wavelength range of 1000 to 4000 nm. Each curve represents a sample subjected to different heating times (0, 30, 60, 90, and 120 minutes). The absorbance values decreased progressively as heating time increased, indicating structural or compositional changes. The sample heated for 0 minutes showed the highest absorbance, suggesting higher concentrations of functional groups such as hydroxyl (–OH) or carbonyl (C=O) that typically contribute to strong absorption bands. As heating time increased, the absorbance decreased gradually, which may be attributed to the thermal degradation or evaporation of volatile compounds, particularly moisture or low-molecular-weight organic components. This degradation is common in plant-derived materials when subjected to a prolonged heat exposure [16,17]. The relatively stable pattern in the overall spectral shape across all samples indicates that while some structural changes occurred, the fundamental molecular framework of black cube gambier remained intact. The decreasing absorbance trend, especially in regions commonly associated with O–H stretching (around 3200–3600 nm) and C–H stretching (around 2800–3000 nm), suggests that prolonged heating may have reduced the intensity of these functional groups. This reduction could result from moisture loss, breakdown of heat-sensitive organic compounds, or slight carbonization effects [18]. Overall, the FTIR results align with the observed color changes presented in Table 2, where prolonged heating led to darkening and increased redness. The combined evidence suggests that while heating altered the physical appearance and some chemical characteristics of black cube gambier, the material retained key structural stability even after 120 minutes of exposure.

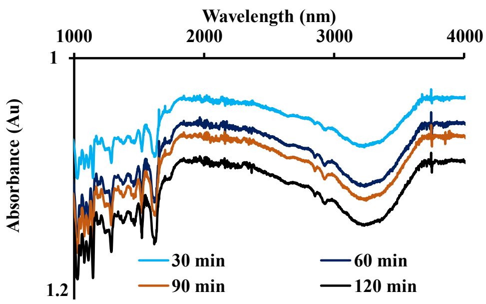


**Fig. 1.** FTIR spectra of black cube gambier based on heating duration

The parameters of the balm are presented in Table 3. The evaluation of balm preparations subjected to various heating durations (0, 30, 60, 90, and 120 minutes) provided comprehensive insights into their homogeneity, spreadability, thermal stability, and potential for skin irritation. The results highlight the impact of heating on the physical and functional properties of the balm formulations.

**Table 3**. Results of application testing of the black cube gambier balm

| Treatment | Homogeneity | Spreadability (cm) | Thermal stability (°C) | Potential for skin irritation |
|---|---|---|---|---|
| A (heating for 0 minutes) | (-) | 3.03 ± 0.15 | 50 | (-) |
| B (heating for 30 minutes) | (+) | 3.43 ± 0.21 | 50 | (-) |
| C (heating for 60 minutes) | (+) | 3.90 ± 0.17 | 50 | (-) |
| D (heating for 90 minutes) | (+) | 4.10 ± 0.10 | 50 | (-) |
| E (heating for 120 minutes) | (+) | 4.47 ± 0.47 | 50 | (-) |

The homogeneity test results, presented in Table 3, reveal that the unheated balm (0 minutes of heating) exhibited non-homogeneous characteristics, with visible coarse granules observed in the preparation. This non-homogeneous texture may be attributed to incomplete mixing or phase separation during the formulation process. In contrast, all heated samples (30–120 minutes of heating) demonstrated improved homogeneity, with no visible granules detected. This suggests that heating promoted better integration of the ingredients, likely by enhancing the melting and uniform dispersion of key components, such as waxes, oils, and active substances. Improved homogeneity is crucial in ensuring consistent texture, stability, and effectiveness in topical applications. Furthermore, the results illustrate the effect of heating on the physical properties of the balm. The spreadability increased progressively with longer heating times, ranging from 3.03 ± 0.15 cm in the unheated sample to 4.47 ± 0.47 cm in the sample heated for 120 minutes. Statistical analysis indicated significant differences between treatments, with samples heated for longer durations showing enhanced spreadability. This improvement may be linked to changes in the viscosity and texture of the balm, where prolonged heating likely facilitated better dissolution and distribution of the oil phase, resulting in a smoother, more pliable consistency [19]. Enhanced spreadability is advantageous for balm applications, as it allows for easier application, improved absorption, and more uniform coverage on the skin. The melting point test results indicate no variation in the thermal stability of the balm across treatments, with all samples maintaining a melting point of 50°C. This consistency suggests that the heating process did not significantly alter the structural integrity of the formulation's key components, such as waxes and stabilizers [20]. Maintaining a stable melting point is essential to ensure that the balm remains solid at room temperature yet melts readily upon contact with the skin. The irritation test results demonstrate that none of the samples induced irritation, with all treatments scoring 0 on the irritation index.

This outcome confirms that heating neither introduced skin irritants nor degraded the formulation in a way that could compromise its safety. The absence of irritation across all treatments highlights the suitability of the balm for topical use, even after prolonged heating. Overall, the findings suggest that heating the balm formulation enhanced its homogeneity and spreadability without affecting its melting point or causing irritation. The improved texture and ease of application resulting from heating are desirable characteristics in balm products, contributing to better user experience and product performance. These results highlight the potential for controlled heating as an effective strategy to improve the quality of balm formulations.

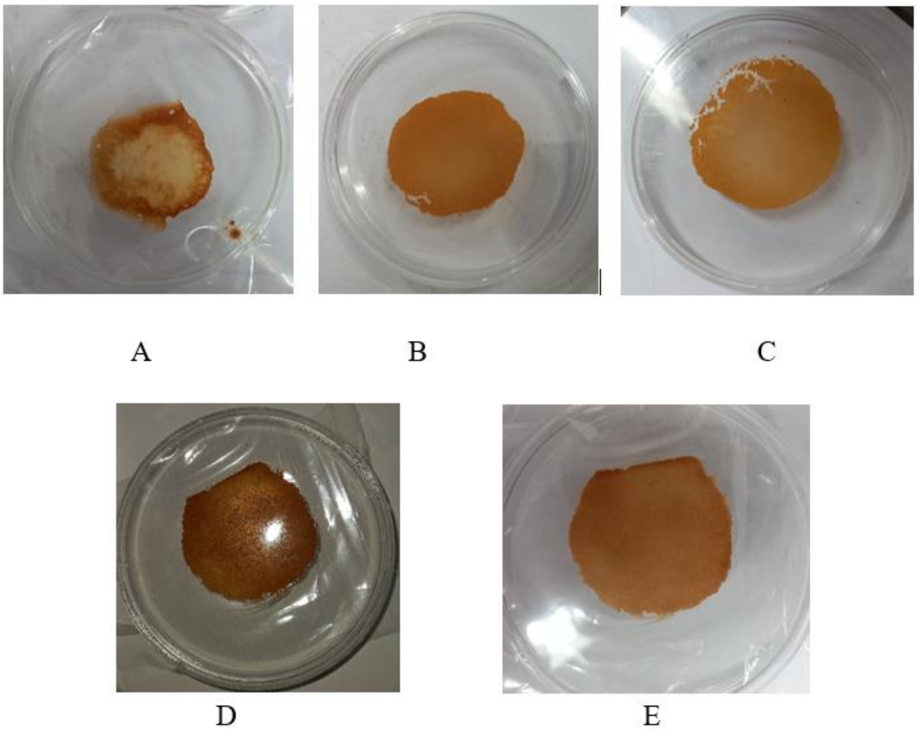


**Fig. 2.** The appearance of balm samples with different heating durations: **(A)** unheated (0 min) – coarse texture; **(B)** 30 min – smoother; **(C)** 60 min – refined texture; **(D)** 90 min – uniform and glossy; **(E)** 120 min – smooth and even.

Fig. 2 shows the appearance of black cube gambier balm samples from the perspective of application quality parameters. The improved homogeneity across heated samples was consistent with the explanation that heating could promote better integration of ingredients by enhancing the melting and uniform dispersion of waxes, oils, and active substances. The progression in texture improvements aligned with the reported increase in spreadability, ranging from 3.03 ± 0.15 cm in the unheated sample to 4.47 ± 0.47 cm after 120 minutes of heating. This enhanced spreadability suggests improved pliability and smoother application properties. Importantly, while heating improved texture and spreadability, the melting point remained stable at 50°C across all treatments, confirming the structural stability of key formulation components. Additionally, the irritation test results confirm that none of the samples induced skin irritation, reinforcing the safety of the balm formulation even after prolonged heating. Therefore, the visual evidence in the figure supports the findings that controlled heating effectively enhanced the homogeneity and spreadability of the balm without compromising its thermal stability or safety.

**Table 4.** Results of chemical quality parameters of black cube Gambier balm

| Treatments | pH | Total Phenol (mg GAE) |
|---|---|---|
| A (heating for 0 minutes) | 7.17 ± 0.03[a] | 47.30 ± 0.78[a] |
| B (heating for 30 minutes) | 7.25 ± 0.01[b] | 48.41 ± 0.59[a] |
| C (heating for 60 minutes) | 7.31 ± 0.01[c] | 47.97 ± 0.78[a] |
| D (heating for 90 minutes) | 7.34 ± 0.02[c] | 49.11 ± 0.51[b] |
| E (heating for 120 minutes) | 7.44 ± 0.02[d] | 54.28 ± 0.78[c] |

Moreover, the results presented in Table 4 and Table 5 highlight the influence of heating duration on the chemical and microbial quality of the balm preparation, respectively. From the perspective of chemical quality, the pH values show a gradual increase from 7.17 (0 minutes of heating) to 7.44 (120 minutes of heating), suggesting that prolonged heating altered the chemical composition of the balm. This pH shift can be attributed to the breakdown of acidic components or the formation of alkaline by-products [21]. Since pH stability is crucial for product consistency, prolonged heating may improve the balm's stability and compatibility with skin conditions that favor a neutral to slightly alkaline pH range. The slight increase in pH also aligns with potential chemical transformations that may influence the product's preservation and efficacy.

The phenolic content data reveal a positive correlation between heating duration and phenolic compound concentration, increasing from 47.30 mg GAE to 54.28 mg GAE with prolonged heating. This trend indicates that heating enhanced the extraction or release of phenolic compounds, which are known for their antioxidant and antimicrobial properties [22]. These bioactive components play a vital role in improving the therapeutic benefits and shelf-life of the balm [23]. However, the sharp rise in total phenolic content after 90 to 120 minutes of heating suggests that prolonged thermal exposure may unlock additional bound phenolics from the formulation's botanical ingredients.

With regard to microbial quality, Table 5 highlights that heating effectively reduced microbial counts in the balm. The initial decrease in total plate numbers from $6.0 \times 10^5$ CFU/g (0 minutes) to $2.0 \times 10^5$ CFU/g (30 minutes) demonstrates effective microbial inactivation. However, the spike observed at 60 minutes ($1.2 \times 10^6$ CFU/g) may indicate potential contamination or the survival of heat-resistant strains. Extended heating for 90 to 120 minutes mitigated this risk, reducing microbial counts to $5.2 \times 10^5$ and $3.5 \times 10^5$ CFU/g, respectively. These results suggest that heating for at least 90 minutes is essential to achieve microbial safety without compromising the balm's quality.

**Table 5**. Results of microbiology quality parameters of the black cube gambier balm

| Treatment | Total Plate Numbers (CFU/g) | Inhibition zone (mm) |
|---|---|---|
| A (heating for 0 minute) | $6.0 \times 10^{5a}$ | $11.57 \pm 0.15^{a}$ |
| B (heating for 30 minutes) | $2.0 \times 10^{5b}$ | $12.82 \pm 0.07^{b}$ |
| C (heating for 60 minutes) | $1.2 \times 10^{6c}$ | $13.83 \pm 0.66^{bc}$ |
| D (heating for 90 minutes) | $5.2 \times 10^{5d}$ | $14.87 \pm 0.35^{c}$ |
| E (heating for 120 minutes) | $3.5 \times 10^{5e}$ | $16.35 \pm 0.81^{d}$ |

Table 5 also illustrates consistent improvement in antibacterial activity, as reflected in the increasing inhibition zone diameter from 11.57 mm to 16.35 mm. This enhancement aligned with the increase in phenolic content and the improved microbial control, as presented in earlier tables. Phenolic compounds are known to disrupt bacterial cell membranes, and their elevated concentrations likely strengthen the balm's antibacterial properties [24-25]. Thus, heating not only boosts the chemical profile of the balm but also enhance its functional properties by reinforcing its antimicrobial effectiveness.

Overall, the combined data from Table 4 to Table 5 demonstrate that prolonged heating significantly influenced both the chemical stability and microbial quality of the balm. Heating for 90 to 120 minutes appears to offer the optimal balance, promoting higher phenolic content, improved antibacterial performance, and enhanced microbial safety. These findings provide valuable insights for formulating balms with improved stability, efficacy, and overall product quality.

*3.2. Organoleptic test*

Table 6 indicates the organoleptic data of black cube gambier. The organoleptic test evaluated consumer acceptance of balm characteristics using human senses, such as sight, touch, and smell. This test was conducted on the acne balm prepared with black cube gambier heated for 0, 30, 60, 90, and 120 minutes using a hedonic scale (1 = strongly disliked, 5 = strongly liked). Observations focused on texture, scent, and color, involving 25 semi-trained panelists. The texture test measured panelists' preference for balm texture, with results ranging from 3.32 to 3.92. The lowest scores were found in Treatments A (0 minutes of heating) and D (90 minutes of heating), while the highest were found in Treatments B (30 minutes of heating) and E (120 minutes of heating). A good balm has a semi-dense texture that is smooth and easy to apply. Balms generally feel denser and oilier than creams due to higher oil and wax content, while creams have a balanced oil–water ratio, making them lighter and more absorbent. The scent test measured panelists' preference for the balm's scent, with results ranging from 3.00 to 3.48. The highest score was found in Treatment B (30 minutes of heating), and the lowest scores were found in Treatments A (0 minutes of heating) and D (90 minutes of heating). The balm had a mild scent, derived only from black cube gambier, without fragrance addition to minimize irritation risks, as fragrances often contain alcohol that can trigger irritation. The color test assessed panelists' preference for the balm's color, with results ranging from 3.08 to 3.96. The highest score was found in Treatment C (60 minutes of heating) and the lowest was found in Treatment A (0 minutes of heating). The balm's color evolved from yellowish-brown (0 minutes of heating) to dark brown (heating for 30 minutes and longer). Longer heating intensified the color due to the red–purple base of black cube gambier. Overall, the results suggest that heating black cube gambier for 30 to 60 minutes produces a balm with improved texture, scent, and color characteristics preferred by panelists, making these conditions optimal for balm formulation.

**Table 6**. Results of the organoleptic test of the black cube gambier balm

| Treatment | Texture | Flavor | Color |
|---|---|---|---|
| A (heating for 0 minutes) | 3.32 ± 0.80 | 3.00 ± 0.76 | 3.08 ± 0.81 |
| B (heating for 30 minutes) | 3.92 ± 0.81 | 3.48 ± 0.96 | 3.80 ± 1.00 |
| C (heating for 60 minutes) | 3.84 ± 0.47 | 3.24 ± 0.72 | 3.96 ± 0.89 |
| D (heating for 90 minutes) | 3.32 ± 0.75 | 3.00 ± 0.91 | 3.12 ± 1.13 |
| E (heating for 120 minutes) | 3.92 ± 0.47 | 3.32 ± 1.03 | 3.60 ± 1.00 |

Note: Values are presented as means ± standard deviations. No statistically significant differences ($p > 0.05$) were observed among treatments for texture, flavor, and color based on the hedonic test results.

Moreover, while the balm formulations heated for 30 to 60 minutes exhibited improved sensory characteristics—such as texture, scent, and color—preferred by panelists, these conditions were not optimal in terms of bioactive content and antimicrobial performance. The most favorable chemical and microbiological results were achieved at 120 minutes of heating, as indicated by the highest total phenol content (54.28%) and the largest antibacterial inhibition zone against *Propionibacterium acnes* (16.35 mm). Additionally, the 120-minute treatment produced a balm with optimal pH (7.44), good spreadability (4.47 cm), and stable homogeneity, without causing skin irritation. These findings highlight that although moderate heating (30–60 minutes) enhanced organoleptic appeal, prolonged heating (up to 120 minutes) was more effective for maximizing the functional bioactivity and microbial safety of the formulation. Therefore, for acne treatment applications prioritizing therapeutic efficacy, a 120-minute heating duration is recommended for gambier balm production. This balance between sensory quality and bioactive potency supports the potential of black cube gambier as a natural, high-performance ingredient in eco-friendly acne care products.

## 4. Conclusions

Based on the analysis conducted, it is concluded that heating duration significantly influenced several characteristics of the acne balm at a 5% significance level, including homogeneity, dispersion, melting point, irritation potential, texture, color, pH, total phenolic content, and antibacterial activity, but had no significant effect on the balm's scent, the color of the raw material, and the total plate count. Prolonged heating resulted in the darkening of the color of black cube gambier and increased the phenolic content, as confirmed by the IR spectral analysis. The optimal treatment identified was Treatment E (120 minutes of heating), which exhibited desirable characteristics, such as an homogeneous texture, a dispersion power of 4.47 cm, a melting point of 50°C, no irritation effects, a texture score of 3.92, a color score of 3.60, a pH value of 7.44, a total phenolic content of 54.28, and antibacterial activity with a clear zone diameter of 16.35 mm. FTIR analysis confirmed the presence of functional groups linked to phenolic compounds, supporting the increase in phenolic content.

## Abbreviations

| | |
|---|---|
| ANOVA | Analysis of Variance |
| CRD | Completely Randomized Design |
| DMRT | Duncan's New Multiple Range Test |
| FTIR | Fourier Transform Infrared |
| IR | Infrared |
| PCA | Plate count agar |
| pH | Potential of hydrogen (degree of acidity/basicity) |
| TEA | Triethanolamine |
| VCO | Virgin coconut oil |

## Data availability statement

Data will be made available on request.

## CRediT authorship contribution statement

**Rilvi Muhammad Tio**: writing – original draft, investigation. **Najla Rosyadah**: writing & project administration. **Novizar**: formal analysis. **Rini**: data curation. **Tuty Anggraini**: data curation. **Umar Ahmed**: writing – review & editing. **Cesar Welya Refdi**: supervision. **Daimon Syukri**: supervision, validation.

## Declaration of Competing Interest

The authors declare that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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