Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

The Potential af Robusta Coffee Leaf Flour *(Coffea canephora)* as a Functional Food Ingredient: Antioxidant Activity Analysis

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Received: September 12, 2025. Accepted: November 11, 2025. Published: December 5, 2025

Abstract: Functional food has become a rapidly growing segment in the global food industry, emphasizing the use of natural resources as sources of functional ingredients. One promising candidate for development is Robusta coffee leaf flour. The purpose of this study was to determine the optimal treatment and the effect of drying temperature on the antioxidant activity of Robusta coffee leaf flour. This study employed a one-factor randomized block design (RBD). The treatments consisted of combinations of temperature and time, with six levels: P1T1 = 50°C for 4 hours; P1T2 = 50°C for 5 hours; P1T3 = 50°C for 6 hours; P2T1 = 60°C for 4 hours; P2T2 = 60°C for 5 hours; and P2T3 = 60°C for 6 hours. Each treatment was repeated three times, resulting in a total of 18 experimental units. The data were analyzed using ANOVA and Duncan's multiple range test for significant results. Findings indicated that the highest antioxidant activity was achieved at 60°C for 6 hours (P2T3), with a value of 73.51 ppm. Robusta coffee leaf flour demonstrates strong potential as a functional food ingredient, with optimal antioxidant activity achieved through specific drying conditions. Further research is recommended to investigate its bioactive compound profile and potential applications in food products.

Keywords: Bioactive Compound; Functional Food; Robusta Coffee Leaf Flour.

Introduction

Functional food is a rapidly growing food product worldwide, driven by increasing demand and public awareness of the importance of health. utilization of natural potential as a source of functional food in Indonesia is still relatively minimal [1]. Functional food is not just a source of essential nutrients for the body, and has a protective effect against various health problems, including those caused by free radicals [2].

Functional foods, defined as foods that provide health benefits beyond basic nutrition, have garnered increasing scientific and industrial attention. Despite Indonesia's rich biodiversity, the utilization of local natural resources as functional ingredients remains limited. Coffee leaves are an underutilized agricultural by-product containing various bioactive compounds, such as flavonoids, polyphenols, alkaloids, and saponins, comparable to those in coffee beans. Previous studies have focused largely on coffee beans and beverages, while limited research has optimized the antioxidant potential of coffee leaf flour through controlled drying treatments. Drying temperature and duration are critical parameters affecting the retention and activation of phenolic and flavonoid compounds. Therefore, this study investigates the effect of drying temperature and time on the antioxidant activity, total phenols, and flavonoid contents of Robusta coffee leaf flour, with the goal of identifying optimal processing conditions to support its application as a functional food ingredient [2].

Antioxidant compounds have been shown to reduce the risk of degenerative diseases by binding free radicals in the body [3]. One potential source of antioxidant compounds is coffee leaves. Coffee leaves contain various compounds that are equivalent in benefits to coffee beans, such as flavonoids, alkaloids, saponins, and polyphenols. The phenol content in coffee leaves has the potential to be utilized as a natural fortification ingredient in the innovation of antioxidant-rich food products [4].

Coffee leaf flour is the result of dried and pulverized coffee leaves. This product has a long shelf life and can be used as an additional ingredient in the preparation of cakes, noodles, and other food products. Processing robusta coffee leaves into flour involves several stages, such as sorting, washing, size reduction, drying, and grinding. Drying robusta coffee leaves yields a moisture content of 9.40 ± 1.77%, indicating optimal drying results [5].

Seeing the potential of coffee leaves as a source of antioxidants, it is necessary to study the utilization of robusta coffee leaf flour products that have the best antioxidant activity, phenols, flavonoids, and optimal water content activity.

Research Methods

This research was conducted at the Food Technology Laboratory of the Bali Institute of Technology and Health from February to May 2025. The experiment used a one-factor randomized block design (RBD) with six treatment combinations: 50°C for 4, 5, and 6 hours, and 60°C for 4, 5, and 6 hours, each with three replications (n = 3). The main equipment included a food dehydrator (Getra), an analytical balance, a grinder, an oven, and a spectrophotometer. Old

How to Cite:
N. K. Parwati, I. G. A. Y. Rabani RS, I. A. P. A. Widnyani, A. A. N. D. A. W. Putra, and P. R. Sintyadewi, "The Potential af Robusta Coffee Leaf Flour (Coffea canephora) as a Functional Food Ingredient: Antioxidant Activity Analysis", *J. Pijar.MIPA*, vol. 20, no. 7, pp. 1308–1312, Dec. 2025. https://doi.org/10.29303/jpm.v20i7.10200

Robusta coffee leaves (5th–8th nodes) from Wanagiri Village were sorted, washed, cut into 3 cm pieces, and dried according to treatment. Samples were ground and sieved through an 80-mesh screen to obtain uniform flour. Antioxidant activity was measured using the DPPH method, total phenols by the Folin–Ciocalteu method, and flavonoids using the $AlCl_3$ colorimetric method. All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Data were analyzed using ANOVA, and significant differences ($p \leq 0.05$) were further evaluated using Duncan's Multiple Range Test (DMRT).

Research Procedure

Research design

This type of research is experimental, namely experiments conducted with a randomized block design (RBD) with 1 factor. Combination of temperature and time, drying temperature, namely: 50 ºC, 60 ºC. And the drying times are: 4 hours, 5 hours, and 6 hours. Repeat was done 3 times. so that 18 experimental units were obtained. Data were analyzed with Analysis of Variance (ANOVA). If the ANOVA test results showed significant differences, it was continued with the Duncan test.

Preparation of Robusta Coffee Leaf Flour

Robusta coffee leaves (*Coffea canephora*) are selected from old leaves, which are defined by criteria numbers 5, 6, 7, and 8. Robusta coffee leaves are sorted first and then washed, after which they are reduced to a size of 3 cm to achieve uniformity. Then, they were dried using a food dehydrator for 4 hours, 5 hours, and 6 hours at temperatures of 50 °C and 60 °C. Then ground with a dry food grinder and sieved with an 80-mesh sieve.

Stages of testing

The tests carried out in this study include the antioxidant activity test using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical method, the phenol method with Folin-Ciocalteu reagent, the flavonoids $AlCl_3$ (Aluminium Chloride) method, and the moisture content determination using the AOAC standard method (2005).

Data analysis

The research data on each test were analyzed using the SPSS program, with the first analysis checking the distribution of data or data normality. The results of the normality test were then continued with Analysis of Variance (ANOVA). If the results obtained are significantly different at $p \leq 0.05$, the Duncan test is used to determine the treatment differences.

Results and Discussion

Antioxidant activity

Based on the ANOVA test results, the combination of treatments on robusta coffee leaf flour has a very significant effect on antioxidant activity. Duncan's results showed that the interaction between treatments P1T3 and P2T2 was significantly different, but the treatments P1T1, P1T2, P2T1, and P2T3 were not significantly different. The average antioxidant activity of robusta coffee leaf flour is presented in Table 1.

In Table 1, the highest mean value of antioxidant activity lies in the treatment P1T1d (50(0) °C: 4 hours), which is 77.53 ppm, and the lowest in the code P2T3a (60(0) °C: 6 hours), which is 73.51 ppm. The lower the IC_{50}value, the higher the antioxidant activity. The combination of temperature and time greatly affects the antioxidant bioactive compounds. A drying time that is too short is insufficient to extract the compounds, while a drying time that is too long can cause degradation [6]. A drying temperature that is too low can cause antioxidant compounds to remain bound in plant tissue, so they are not detected optimally [7].

Table 1. Antioxidant activity test results

Code	Average antioxidant activity (ppm)
P1T1	77.53d
P1T2	77.41d
P1T3	74.41b
P2T1	73.72a
P2T2	76.03c
P2T3	73.51a

Notes: *Different notations indicate that the interaction between treatments has a significant effect.

A compound is categorized as a very strong antioxidant if it has an IC_{50} value below 50 ppm, strong if it is in the range of 50-100 ppm, moderate in the range of 100-150 ppm, and weak if the value is between 150-200 ppm [8]. The temperature and duration of drying have a significant influence on the antioxidant activity of natural materials [9]. The results showed that drying at 50°C for 4 hours produced the highest antioxidant activity with an IC_{50} value of 37.68 ppm. Meanwhile, at 60°C for 6 hours, the IC_{50} value obtained was 48.03 ppm. The presence of antioxidant activity in coffee leaf flour products is because coffee leaves contain tannins, alkaloids, flavonoids, saponins, anthraquinones, steroids, and triterpenoids, which are known to have antioxidant properties [10].

Phenol

Based on the results of the ANOVA test, robusta coffee leaf flour has a very significant effect on total phenol ($P < 0.01$). The Duncan test results showed that each treatment combination (P1T2, P1T1, P1T3, P2T1, P2T2, P2T3) had a significant effect on total phenol. The average total phenol of coffee leaf flour can be presented in Table 2.

Table 2 shows that the highest mean value of total phenolics is located in the treatment P2T3 (60 °C, 6 hours) at 5123.07 mgGAE/100gr, while the lowest is in the treatment P1T2 of 5123.07 mgGAE/100gr, while the lowest was in the P1T2 treatment (50º C: 5 hours) of 4027.67 mgGAE/100gr. Testing polyphenol content revealed that the optimal drying temperature (50-60 °C) can maintain phenolic content [11].

Table 2. Phenol test results

Code	Mean Total Phenol (mgGAE/100gr)
P1T1	4054.12f
P1T2	4027.67e
P1T3	4393.57d
P2T1	4623.44c
P2T2	4855.82b
P2T3	5123.07a

Notes: Different notations indicate that the interaction between treatments has a significant effect.

At a drying temperature of 60°C for 4-5 hours, the release of phenolic compounds began to increase because higher temperatures can break down cell walls more effectively. The duration is still insufficient for the maximum release and formation of phenolics. The temperature treatment of 60°C for 6 hours produces the highest phenol content, indicating that this combination of temperature and time is able to optimize phenol extraction and support the formation of new phenolic compounds through chemical reactions such as Maillard reactions [12].

This research aligns with the findings of Faturochman et al., which show that drying at 60°C for 6 hours yields the highest polyphenol content of 4.06 mg GAE/g [13]. Research by Setyowatik et al. showed that drying *Moringa oleifera* leaves at 60°C for 5 hours is one of the most effective treatments for maintaining its bioactive compounds [14]. In this treatment, the level of phenolic compounds produced reached 6,945.121 ppm, which is directly related to the antioxidant activity of 28.373%. Increasing the drying temperature to 60°C increased the total phenol content in gambier leaves [15]. However, excessively high temperatures can cause degradation of phenolic compounds. Therefore, a temperature of 60°C is considered optimal to maintain high phenolic content.

Flavonoids

Based on the ANOVA test conducted, the results show that the formulation of robusta coffee leaf flour has a significant effect on total flavonoids ($P < 0.01$). The Duncan test results showed that the treatment of samples (P1T1, P1T2, P1T3, P2T1, P2T2, and P2T3) had a significant effect. The average total flavonoids of robusta coffee leaf flour are presented in Table 3.

Table 3. Flavonoid test results

Code	Mean total flavonoids mg/100g.
P1T1	2329.88f
P1T2	2402.99e
P1T3	2606.05d
P2T1	2667.00c
P2T2	3057.95b
P2T3	3244.12a

Notes: Different notations indicate that the interaction between treatments has a significant effect.

Table 3 shows that the highest mean value of flavonoid levels was obtained in the P2T3 treatment (60° C, 6 hours) at 3244.12 mg/100g, while the lowest level was found in P1T1 (50° C, 6 hours) amounted to 3244.12 mg/100g, while the lowest level was found in P1T1 (50° C, 4 hours) amounting to 2329.88 mg/100g. This research aligns with the findings of Sari et al. on moringa leaves (Moringa oleifera), which showed that an oven temperature of 50-60 °C is the optimal range to maintain flavonoid content [16].

The results of flavonoid levels obtained at 60 °C for 6 hours were higher than those obtained from treatments of 4 to 5 hours. A shorter heating duration is not enough to optimize the process of releasing flavonoid compounds from plant tissues. Heating time that is too short can cause the release of phenolic compounds and flavonoids that are less than optimal because the plant tissue has not experienced a thorough softening. This softening is important so that bioactive compounds can diffuse out of the cell matrix [17].

The heating process can increase the permeability of cell membranes, thus facilitating the release of phenolic and flavonoid compounds. Based on the findings of this study, the optimal condition for achieving the highest flavonoid content is a combination of 60 °C temperature and a 6-hour heating time, as this time and temperature are sufficient to facilitate tissue softening and maximum compound diffusion without causing excessive degradation [18].

Water Content

Based on the results of the ANOVA test, the combination of treatments on robusta coffee leaf flour products. Flour has no significant effect on moisture content ($P>0.05$). The reason why the formulation of coffee leaf flour products has no significant effect on moisture content is that the difference in formulation numbers between each treatment is close. The average total phenol content of the robusta coffee leaf flour treatment can be presented in Table 4.

Table 4. Water content test results

Code	Average water content (%)
P1T1	5.58
P1T2	5.28
P1T3	5.24
P2T1	5.19
P2T2	5.63
P2T3	5.06

Notes: The same notation indicates that the interaction between treatments has no significant effect.

Table 4 shows that the highest water content of coffee leaf flour is obtained in the treatment with the code P1T1, which has the highest average water content (5.58%), and P2T3 has the lowest water content (5.06%). The best results of the water content test were found in the P2T3 treatment, which had the lowest water content (5.06%). The results of the water content test showed that the sample met the SNI 9228: 2003 standard, which sets the maximum limit of water content at 8%. The best treatment for testing water content in robusta coffee leaf flour is P2T3, which yields a result of 5.06%.

The water content of moringa leaf flour produced by the sun drying method is 6.64% [19]. The moisture content of cemba leaf flour produced by the cabinet dryer method is 7.50% [20]. If a material is in direct contact with heat for an extended period, its moisture content will decrease [21]. The

65°C drying temperature treatment for 5.5 hours yielded the best white oyster mushroom flour, with a yield of 7.34% and a moisture content of 4.30% [22]. The results showed that the water content at 60 °C and 70 °C was 10% in each case, meeting the SNI value of a maximum of 10% [23].

The combination of drying temperature and duration had a significant effect on antioxidant activity, total phenols, and flavonoids ($p < 0.01$). Calibration curves for DPPH ($R^2 = 0.997$), phenol ($R^2 = 0.995$), and flavonoid ($R^2 = 0.994$) assays confirmed analytical accuracy. The highest antioxidant activity was observed at 60°C for 6 hours (P2T3) with $IC_{50} = 48.03$ ppm, categorized as strong antioxidant capacity. Increasing the drying temperature enhanced cell wall rupture and enzyme inactivation, thereby facilitating the release of phenolic and flavonoid compounds. However, excessive heat exposure may cause partial degradation of thermolabile compounds. Phenolic content reached 5123.07 mg GAE/100 g and flavonoids 3244.12 mg/100 g under P2T3, consistent with prior findings on Moringa oleifera and gambier leaves. Water content differences were statistically insignificant ($p > 0.05$), with all samples below the SNI 9228:2003 limit of 8%, ensuring good storage stability. These results demonstrate that controlled drying at moderate temperature and longer duration optimizes bioactive compound retention. Limitations include the absence of sensory and stability testing, which should be addressed in future studies.

Conclusion

Drying temperature and time significantly influenced the antioxidant, phenolic, and flavonoid contents of Robusta coffee leaf flour. The optimal condition (60°C for 6 hours) yielded the strongest antioxidant activity ($IC_{50} = 48.03$ ppm) and the highest total phenolic and flavonoid contents. Robusta coffee leaf flour produced under these conditions shows promise as a natural functional ingredient for applications in teas, bakery, and nutraceutical formulations. Further research on product formulation, sensory properties, and bioavailability is recommended to strengthen its commercial potential.

Author's Contribution

N.K. Parwati: conceptualized the study, designed the methodology. I.G.A.Y. Rabani RS: validated the analytical procedures and refined the manuscript. I.A.P.A. Widnyani: sample preparation. A.A.N.D.A.W. Putra: contributed to data interpretation. P.R. Sintyadewi compiled the literature review.

Acknowledgements

The authors would like to express their deepest gratitude to Mr. I Gede Putu Darma Suyasa, S.Kp., M.Ng., Ph.D., as the Rector of Bali Institute of Technology and Health (ITEKES Bali), for the permission and opportunity given to complete this article. Thank you are also due to Mrs. Ida Ayu Putu Ary Widnyani, S.TP., M.Si., as the first supervisor, and Mr. I Gusti Agung Yogi Rabani RS, S.TP., M.Si., as the second supervisor, for all the directions and guidance given during the process of writing this article.

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