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Reduction of Tannins In Black Tea Using Rice Husk Ash: An Innovation to Reduce The Risk of Iron Deficiency Anemia

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Abstract

Background: Iron deficiency anemia may result from inadequate iron intake or hindered iron absorption. Black tea is known to contain tannins, compounds that can interfere with the body's ability to absorb iron. Rice husk ash, which is rich in silica, has the potential to lower the tannin content in black tea. **Objective:** This study aimed to examine the impact of rice husk ash supplementation on the tannin content of black tea. **Methods:** An experimental study was carried out using a completely randomized design involving four treatment groups: control (P0), soaking (P1), boiling followed by soaking (P2), and boiling plus soaking with 15% rice husk ash (P3). The tea samples were prepared in the Culinary Dietetics Laboratory, Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro. Analysis of tannin and phenolic content was performed at Chemix Pratama Laboratory in Yogyakarta using spectrophotometric techniques. The study took place between December 2024 and April 2025. **Results:** The highest tannin concentration was observed in the control group (P0), averaging 9.05% ± 0.09, while the lowest was recorded in the P3 group (treated with rice husk ash), with an average of 4.66% ± 0.14. A statistically significant difference in tannin levels was found between the P2 and P3 groups ($p < 0.05$). Additionally, a very strong positive correlation between tannin and phenolic content was identified ($r = 0.99$; $p < 0.05$). **Conclusion:** The combined treatment of boiling and soaking black tea with 15% rice husk ash effectively reduces tannin content. The most notable decrease in tannin levels was seen in the boiling plus soaking with 15% rice husk ash group, indicating this method's potential in reducing compounds that inhibit iron absorption.

Keywords: black tea, tannins, rice husk ash, anemia, phenolic

INTRODUCTION

One of the major public health issues in Indonesia is iron deficiency anemia, which can affect individuals across all age groups (Kemenkes RI, 2018). Iron deficiency anemia is caused by a prolonged lack of iron (Fe) (Finasari et al, 2023). Groups at higher risk of developing anemia include adolescent girls and pregnant women. During menstruation, iron is needed to form new hemoglobin to replace what is lost, while during pregnancy, iron supports the growth and development of the fetus. This is consistent with data from the 2018 Basic Health Research (Riskesdas), which reported that 32% of females aged 15–24 and 33.7% of pregnant women aged 25–34 was affected by anemia (Kemenkes RI 2019, Pamela et al, 2022).

Black tea (*Camellia sinensis assamica*) belongs to the Theaceae family and is among the most widely consumed beverages in Indonesia (Dzakiyyah et al, 2023). The increasing trend of black tea consumption is one of the reasons it was selected as a sample in this study. Black tea contains polyphenols and flavonoids, which offer health benefits such as antioxidant properties (Sudaryat et al, 2015). However, one of the polyphenols like tannin also acts as an antinutrient. Tannins are polyphenolic compounds with molecular weights ranging from 500 to 3,000 Daltons and are responsible for the astringent taste in tea. Tannins inhibit iron absorption by binding to it before it can be absorbed

by the intestinal mucosa (Riswanda et al, 2017). As a result, consuming tea with meals reduces iron absorption and eventually depletes iron stores in the body (Eyato et al, 2019).

One method to reduce tannin levels involves soaking or boiling, combined with the addition of active compounds from organic materials such as rice husk ash (Soenardjo et al 2017). Previous studies have shown that boiling with rice husk ash and extended soaking time can effectively lower tannin levels in mangrove fruit (*Avicennia marina*). This occurs due to diffusion and osmosis processes. The higher the concentration of ash, the more significant the reduction in tannin content in the mangrove fruit (Soenardjo et al, 2017).

Rice husks are a byproduct of rice milling that offer various benefits, one of which is their use as rice husk ash (Triyana et al, 2017). Typically, rice husk ash is used to loosen soil or as a scouring agent for cleaning cookware. However, rice husk ash also has the potential to act as an absorbent medium due to its high silica content (Triyana et al, 2017). Silica, functioning as activated carbon, can absorb intracellular fluids through three stages: first, carbon granules draw substances out of the material; second, the substances enter the pores of the carbon; and third, the substances are absorbed onto the inner walls of the carbon (Chrissanty et al, 2012). Therefore, an innovation was developed to address the issue of high tannin consumption from tea by creating low-tannin black tea using rice husk ash.

Tannins are recognized inhibitors of non-heme iron absorption because they form insoluble complexes with iron in the gastrointestinal tract. Therefore, reducing tannin content in black tea may improve iron bioavailability and potentially contribute to strategies aimed at reducing the risk of iron deficiency anemia, particularly among vulnerable populations such as adolescent girls and pregnant women.

Therefore, this study aimed to analyze the effect of rice husk ash supplementation on tannin levels in black tea and to evaluate its potential application as an innovative approach to reducing compounds that inhibit iron absorption. We hypothesized that the addition of rice husk ash would significantly reduce tannin content in black tea.

METHODS

Black tea

The preparation of black tea samples was conducted at the Culinary Dietetics Laboratory, Department of Nutrition Science, Faculty of Medicine, Diponegoro University. The analysis of tannin and phenolic content was carried out at the Chemix Pratama Laboratory, Yogyakarta. The study was conducted from December 2024 to April 2025.

Materials

The materials used in this study included fresh tea leaves, water, and rice husk ash. The tea leaves were sourced from the Java Cosmetic and Herbal store, Kendal City. The equipment utilized in the study included basins, saucepans, tablespoons, a digital scale, a stove, strainers, an oven, oven trays, a blender, and tea bags. This research employed an experimental approach using a Completely Randomized Design (CRD). Each treatment group was analyzed in duplicate. Sample preparation began with washing the tea leaves to remove surface impurities. Groups P1, P2, and P3 were subjected to different treatment methods.

Soaking was carried out using water at 25℃ for 0 days (15 hours). This duration was selected based on preliminary tests, which showed spoilage of tea leaves soaked for one day or longer. Soaking increases the moisture content of the tea leaves, thus promoting microbial growth and spoilage (Arizka et al, 2015).

Boiling was performed at 100℃ for 20 minutes to accelerate diffusion and osmosis processes that may reduce tannin content. Rice husk ash was added at a concentration of 15% of the sample's

weight. The 15% concentration of rice husk ash was selected based on previous studies demonstrating the greatest reduction in tannin content at this concentration. In addition, preliminary laboratory observations indicated that higher concentrations did not provide substantial additional reductions while potentially affecting product characteristics.

After undergoing the respective treatments, the black tea leaves were dried in an oven at 60°C for 150 minutes. This drying temperature was selected based on best practices for drying medicinal plant materials (simplicia), which recommend not exceeding 60°C to prevent damage to active compounds (Jumanio et al, 2023). Low-temperature, prolonged drying was chosen to preserve the integrity of the tea leaves and to ensure even drying, which helps reduce moisture content, inhibit microbial growth, and extend shelf life (Anggraiyati et al, 2017). Following the drying process, the samples were ground into a fine powder and packaged into tea bags, with each bag containing 2.4 grams.

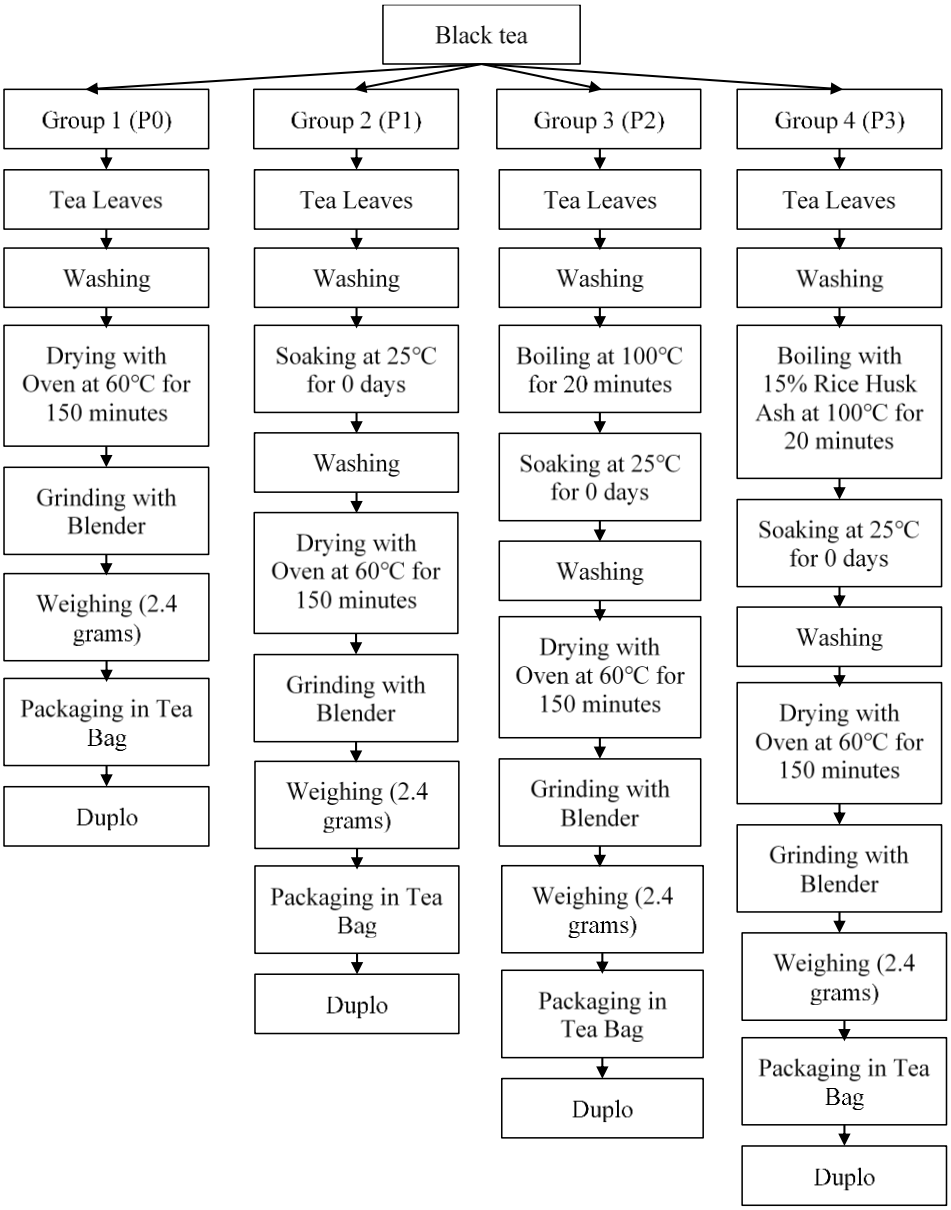

Figure 1. Study Design

Data Collection Techniques

The data collected consisted of tannin content measurements. Four types of samples were tested: (1) untreated black tea leaves (control), (2) black tea subjected to soaking, (3) black tea subjected to both boiling and soaking, and (4) black tea subjected to boiling and soaking with the addition of rice husk ash. Two samples were taken from each treatment group, and each sample was analyzed in duplicate to minimize potential errors.

Statistical Analysis

The data obtained included tannin, phenol, and silica content. To determine the differences in mean tannin levels among the treatment groups, a One-Way Analysis of Variance (ANOVA) was conducted. Pearson correlation analysis was performed to evaluate the relationship between tannin and phenolic content. Prior to ANOVA, data normality was assessed using the Shapiro–Wilk test and homogeneity of variance was evaluated using Levene's test. All assumptions were met before conducting parametric statistical analyses.

RESULTS

Table 1 presents the effects of treatment on tannin and phenol levels across experimental groups. The results demonstrate a clear decreasing trend in both variables from P0 to P3, with the highest values observed in the control group (P0) and the lowest values in P3. This pattern indicates that increasing treatment intensity is associated with a reduction in both tannin and phenolic contents.

Table 1. Effect of Treatment on Tannin and Phenol Levels

Variable	n	P0	P1	P2	P3	p-value
Tannin levels (%)	4	9.05 ± 0.09^{a}	8.85 ± 0.15^{a}	7.27 ± 0.13^{b}	4.66 ± 0.14^{c}	<0.001
Phenol levels (%)	4	7.95 ± 0.04^{a}	7.60 ± 0.34^{a}	6.36 ± 0.17^{b}	4.68 ± 0.14^{c}	<0.001

Values are presented as mean ± standard deviation (SD). Different superscript letters (a, b, c) within the same row indicate significant differences between groups ($p < 0.05$) based on Tukey's HSD post hoc test. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test.

Statistical analysis using one-way ANOVA revealed that the differences among groups were highly significant ($p < 0.001$). Post hoc analysis using Tukey's HSD test showed that groups sharing the same superscript letters were not significantly different ($p > 0.05$), whereas groups with different superscripts differed significantly ($p < 0.05$). Specifically, P0 and P1 were not significantly different, while P2 and P3 showed progressively lower values and differed significantly from the preceding groups.

Table 2 shows the Pearson correlation analysis between tannin and phenol levels in black tea samples. The results indicate that both variables exhibit moderate variability, with tannin levels showing slightly greater dispersion compared to phenol levels. Overall, the descriptive statistics suggest relatively consistent measurements across samples.

Table 2. Pearson correlation between tannin and phenol levels in black tea samples

Variable	Mean ± SD	r	p-value
Tannin levels	7.46 ± 1.82	0.988	<0.001***
Phenol levels	6.64 ± 1.34	—	—

Values are expressed as mean ± standard deviation (SD). r represents the Pearson correlation coefficient. ***$p < 0.001$ indicates a highly significant correlation.

A very strong positive correlation was observed between tannin and phenol levels ($r = 0.988$, $p < 0.001$), indicating a near-perfect linear relationship. This finding suggests that increases in tannin content are closely associated with increases in total phenolic content. These results highlight the

substantial contribution of tannins as a major subclass of polyphenolic compounds in black tea. The strong association further supports the role of tannins as a key determinant of overall phenolic composition in the samples analyzed. From a practical perspective, the substantial reduction in tannin content observed in the rice husk ash treatment group may contribute to improving the nutritional quality of black tea by reducing compounds known to interfere with iron absorption.

DISCUSSION

Tannin is a type of polyphenolic compound with complex hydroxyl groups and exists in various forms. Generally, tannins are classified into two categories: condensed tannins and hydrolyzable tannins (Hersila et al, 2023). Condensed tannins, or proanthocyanidins, are typically derived from flavonoid compounds such as flavan-3,4-diols and catechins. These are polymers of flavonoids classified as phenolic compounds. Hydrolyzable tannins, on the other hand, are tannins that can be hydrolyzed by acids or enzymes to produce ellagic acid and gallic acid (Julianto, 2019). This type is usually found in lower quantities than condensed tannins due to its more toxic nature (Fathurrahman et al, 2018).

Tannins have several beneficial properties and can function as antioxidants, anti-diarrheal agents, and antibacterials. As antioxidants, tannins donate an electron to free radicals, thereby slowing down the activity of these radicals (Safitri et al, 2023). Unpaired electrons cause molecules to become highly reactive (Kusumawati et al, 2024). However, tannins also act as antinutrients by inhibiting iron absorption through binding. Continuous tannin consumption can lead to iron deficiency anemia. One beverage containing tannins is tea, which contains approximately 7–15% tannins (Kusumawati et al, 2024). Tannins are consumed from tea bind to iron, forming insoluble complexes that are difficult for the intestinal mucosa to absorb. This reduction in iron absorption decreases ferritin levels and consequently lowers iron content in the blood (Riswanda et al, 2017). Iron is essential for hemoglobin synthesis and replacing damaged hemoglobin. Therefore, this leads to low hemoglobin levels in the blood and causes iron deficiency anemia. This is supported by a study conducted by Boli on 41 respondents, where 13 individuals (31.7%) who habitually drank tea were at risk of anemia, and 9 of them (69%) were diagnosed with anemia (Boli et al, 2022).

This study on black tea (Camellia sinensis assamica) involved four treatments: control, soaking, boiling and soaking, and boiling with rice husk ash followed by soaking. The highest tannin content was observed in the control group, while the lowest tannin content was found in the group subjected to boiling with 15% rice husk ash and soaking (P3). Tannins in black tea are water-soluble; thus, soaking and boiling treatments facilitate diffusion and osmosis processes that cause tannins to leach from the tea leaves. The solubility of tannins increases with water temperature (Nofita et al, 2021), which explains the significant difference in average tannin content between groups P2 and P3 treated with boiling, while groups P0 and P1 did not show significant differences. Besides temperature, tannin solubility is also affected by the type of solvent used. Previous studies have reported that n-hexane reduces tannin content by 9.45%, ethyl acetate by 15.87%, and methanol by 23.61% (Alwi et al, 2019). Based on the data obtained, boiling contributed to a 17.85% reduction in tannin content.

Rice husk ash was added at 15% of the sample weight. The silica (SiO_2) content of the rice husk ash used in this study was 67.61%. The choice of this concentration was based on previous research using mangrove leaves (Rhizophora mucronata), which showed the lowest tannin content in samples boiled with 15% rice husk ash (Setyaningsih, 2019). The addition of rice husk ash is beneficial for reducing tannin levels in tea due to its silica content, which acts as an adsorbent (Handayani et al, 2014). Silica works by drawing tannins out of the tea and absorbing them onto the inner walls of the silica. The higher the ash content, the lower the tannin levels in the sample (Setyaningsih, 2019). The data obtained indicate that rice husk ash contributed to a 35.9% reduction in tannin content.

The reduction in tannin content may have important nutritional implications because tannins are well known to inhibit non-heme iron absorption. Lower tannin concentrations may improve iron

bioavailability and potentially reduce the risk of iron deficiency anemia among regular tea consumers. This potential benefit is particularly relevant for populations with high iron requirements, including adolescent girls and pregnant women.

The present study demonstrated a significant and progressive reduction in tannin and phenol levels across treatment groups, accompanied by a very strong positive correlation between the two variables. This finding is consistent with the well-established role of tannins as a major subclass of tea polyphenols, which are the dominant bioactive compounds in *Camellia sinensis*. Tea polyphenols comprise a diverse group of compounds, including catechins, flavonoids, and phenolic acids, all of which contribute to the antioxidant capacity and functional properties of tea (Sun et al, 2022). The strong correlation observed in this study indicates that tannin content is a key determinant of total phenolic levels, supporting previous evidence that polyphenolic composition in tea is structurally and functionally interconnected. However, the observed reduction in phenolic content may also indicate a decrease in antioxidant capacity because phenolic compounds are among the primary contributors to the antioxidant properties of tea. Therefore, future studies should evaluate whether reductions in tannin content are accompanied by clinically meaningful changes in antioxidant activity and overall functional quality.

Furthermore, the dose-dependent pattern observed in this study suggests that increasing treatment intensity amplifies the degradation of polyphenolic compounds. This is supported by recent evidence indicating that tea polyphenols exhibit limited stability and bioavailability, which can be affected by processing and environmental factors (Chen et al, 2025). Despite this reduction, tea polyphenols remain biologically significant due to their potent antioxidant, anti-inflammatory, and metabolic regulatory properties (Liczbiński et al, 2022). Therefore, the decrease in tannin and phenol levels observed in this study not only reflects chemical transformations but may also have implications for the functional quality of the final product.

CONCLUSION

The addition of 15% rice husk ash significantly reduced tannin content in black tea, with the greatest reduction observed in the treatment combining boiling, rice husk ash supplementation, and soaking. A very strong positive correlation was observed between tannin and phenolic content, indicating that reductions in tannins were accompanied by reductions in total phenolic compounds. These findings suggest that rice husk ash may be a promising natural adsorbent for producing lower-tannin black tea and potentially improving iron bioavailability. Future studies should evaluate iron absorption, antioxidant activity, and consumer acceptability of the modified tea products.

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Informed Consent Statement: -

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request. The data are not publicly available due to ethical and privacy considerations.

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Conflicts of Interest: The author declares no conflict of interest.

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