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Interactive Effects of Growing Environment and Fermentation on Polyphenol Content, Antioxidant Activity, and Sensory Acceptance of Black Tea

Nunuk Helilusiatiningsih; Titik Irawati, Rysma Putri Aditama

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Interactive Effects of Growing Environment and Fermentation on Polyphenol Content, Antioxidant Activity, and Sensory Acceptance of Black Tea.

Efeitos Interativos do Ambiente de Cultivo e Fermentação sobre o Conteúdo de Polifenóis, Atividade Antioxidante e Aceitação Sensorial do Chá Preto.

Efeitos Interativos do Ambiente de Cultivo e Fermentação no Conteúdo de Polifenóis, Atividade Antioxidante e Aceitação Sensorial do Chá Preto.

Nunuk Helilusiatiningsih (Conceptualization) [1] https://orcid.org/0009-0008-7588-7546; **Faculty of Agriculture, Universitas Islam Kadiri, Indonesia.**

Rysma Putri Aditama (Formal analysis) [2] https://orcid.org/0009-0005-6631-1623; **Faculty of Agriculture, Universitas Islam Kadiri, Indonesia.**

Titik Irawati (Project administration) [3] https://orcid.org/0009-0007-7964-0332 **Faculty of Agriculture, Universitas Islam Kadiri, Indonesia.**

***Corresponding Author:** Nunuk Helilusiatiingsih, Universitas Islam Kadiri, Faculty of Agriculture, Department of Agrotechnology, Sersan Suharmaji Roat 38, Manisrenggo, Kediri, Indonesia, e-mail: nunukhelilusi@gmail.com

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Abstract

Polyphenols are major bioactive compounds responsible for tea's antioxidant properties. This study evaluated the interactive effects of tea-growing location and fermentation duration on polyphenol content, antioxidant activity, and sensory acceptance of black tea produced in East Java, Indonesia. We used a factorial completely randomized design with two growing locations (Wonosari, Malang, and Sirah Kencong, Blitar) and four fermentation durations (0, 60, 90, and 120 min), with three replications. Total polyphenol content, antioxidant activity determined by the DPPH radical scavenging assay, and sensory acceptance of appearance, color, aroma, and taste were analyzed. The interaction between growing location and fermentation duration significantly affected ($p < 0.05$) polyphenol content, antioxidant activity, and sensory acceptance. The highest values came from tea leaves grown in Wonosari and fermented for 120 min, with a total polyphenol content of 7.25 mg GAE g^{-1} and antioxidant activity of 91.42%, along with the highest consumer acceptance scores. Under the same fermentation duration, black tea from Sirah Kencong exhibited lower polyphenol content (6.64 mg GAE g^{-1}) and antioxidant activity (80.48%). These findings show that both growing environment and fermentation duration influence the chemical and sensory characteristics of black tea, with tea from Wonosari fermented for 120 min exhibiting the most desirable quality attributes.

Keywords: black tea; polyphenol content; antioxidant activity; fermentation; sensory acceptance; growing environment

Highlights

- Growing environment and fermentation duration significantly affected black tea quality.
- Tea from Wonosari fermented for 120 min showed the highest antioxidant activity.
- The highest polyphenol content (7.25 mg GAE g^{-1}) was obtained after 120 min fermentation.
- Sensory acceptance of black tea depended on the interaction between location and fermentation.
- Pre-harvest and post-harvest factors jointly influenced the chemical and sensory properties of black tea.

1. Introduction

Black tea (Camellia sinensis L.) is among the most widely consumed beverages globally and is an important plantation crop in many tea-producing nations, including Indonesia. Besides being a popular drink, black tea is known for its rich content of bioactive compounds that may offer health benefits. Polyphenols are the primary compounds responsible for its antioxidant properties, as they can neutralize reactive oxygen species and mitigate oxidative damage. Consuming tea polyphenols regularly has been linked to a lower risk of various chronic diseases, such as cardiovascular problems, diabetes, neurodegenerative conditions, and some types of cancer (X. Wang et al., 2022; Weisburger & Chung, 2002).

Beyond pre-harvest factors, post-harvest processing plays a crucial role in determining black tea's final quality. Fermentation stands out as the most vital stage in black tea production, as it facilitates enzymatic oxidation of catechins into theaflavins, thearubigins, and other polymerized phenolics. These oxidation products directly influence black tea's color, aroma, taste, and antioxidant properties. Recent metabolomic research reveals that fermentation causes dynamic shifts in both volatile and non-volatile metabolites, greatly impacting the tea's flavor and bioactive compounds. It controls the oxidative conversion of tea polyphenols and the development of aroma-active substances, which collectively define the quality of black tea (Abudureheman et al., 2022; Roginsky & Alegria, 2005).

Theaflavins are considered important indicators of quality in fermented compounds because they give black tea its bright orange-red color, appealing taste, and antioxidant properties (Chen et al., 2024; Ntezimana et al., 2021). They are formed through enzymatic oxidation and condensation of catechins, playing a crucial role in determining both the sensory characteristics and commercial worth of the tea. Recent studies have shown that fermentation conditions significantly impact the production and buildup of theaflavins and thearubigins, thereby influencing black tea's functional and sensory qualities (Qu et al., 2026; S. Xu et al., 2024). Consequently, carefully optimizing fermentation time is vital to preserve beneficial phenolic compounds while developing desirable sensory features.

While many studies have explored how the growing environment affects tea phytochemicals and how fermentation impacts black tea quality, these factors are usually studied independently. (Ahmed et al., 2019). Teshome (2019). Existing evidence confirms that environmental conditions affect the biochemical composition of fresh tea shoots, whereas fermentation governs the transformation of these compounds during processing. However, information regarding the interaction between growing environment and fermentation duration remains limited. Specifically, there is limited knowledge about how tea leaves from different agroecological zones react to different fermentation times, and how these factors affect polyphenol levels, antioxidant activity, and sensory appeal of black tea (Le et al., 2021). This knowledge gap is especially relevant for Indonesian tea-growing regions, where plantations vary substantially in environmental conditions.

Based on our knowledge, no prior research has systematically examined how growing environment and fermentation duration together affect polyphenol levels, antioxidant activity, and sensory acceptance of black tea in Indonesian tea-growing conditions. Therefore, this study provides new insights into how differences in growing environment influence tea leaves' response to fermentation and, consequently, the chemical and sensory characteristics of black tea.

This study aimed to explore the interaction between growing environment and fermentation duration on the polyphenol content, antioxidant activity, and sensory acceptance of black tea produced in East Java, Indonesia.

2. Materials and methods

The research took place between August and December 2025. Fresh tea shoots were collected from two tea-growing locations in East Java, Indonesia, namely Wonosari Tea Estate, Malang (L1), and Sirah Kencong Tea Estate, Blitar (L2).

2.1 Experimental design

A completely randomized factorial design was used, involving two factors: tea-growing location and fermentation time. The locations studied were Wonosari Tea Estate in Malang (L1) and Sirah Kencong Tea Estate in Blitar (L2). Fermentation times included four levels: 0, 60, 90, and 120 minutes. Each treatment was repeated three times, yielding 24 experimental units. We measured total polyphenol content and antioxidant activity through quantitative analysis, and assessed sensory acceptance with a hedonic test involving 15 untrained panelists..

2.2 Chemicals and Reagents

Fresh tea shoots are raw materials for black tea. The chemicals used included ethanol, DPPH, Folin–Ciocalteu reagent, sodium carbonate, gallic acid, distilled water, and filter paper. All reagents were analytical grade.

2.3 Black Tea Processing

Fresh tea shoots obtained from Wonosari Tea Estate, Malang, and Sirah Kencong Tea Estate, Blitar, were harvested in the morning and sorted to obtain uniform samples. The leaves were withered for 8–12 h until the moisture content reached approximately 68%, then rolled to disrupt leaf tissue and initiate enzymatic oxidation. The fermentation process was carefully conducted for 0, 60, 90, or 120 minutes at a comfortable room temperature of 18–25 °C, with humidity levels staying above 90%. Afterward, the leaves were gently dried in a hot-air oven set between 100 and 120 °C, until their moisture content dropped to between 2.8% and 3.4%. Once dried, the black tea samples were thoughtfully packed into airtight, plastic-lined paper bags, labeled clearly, and stored carefully until it was time for further chemical and sensory analyses.

2.4 Determination of Antioxidant Activity

The antioxidant activity of black tea samples was evaluated based on their capacity to neutralize DPPH free radicals, following the method of Syahara et al. (2024). Ethanol-based tea extracts (95% v/v) were combined with DPPH reagent and incubated under controlled conditions. Afterward, absorbance readings were taken at 517 nm with a UV–Vis spectrophotometer. The radical scavenging activity was quantified as a percentage of inhibition, determined by comparing the absorbance values to those of the control solution.

2.5 Sensory Evaluation

Consumer acceptance of black tea samples was evaluated using a hedonic preference test conducted with 15 non-trained panelists. The sensory attributes assessed included dry tea appearance, liquor color, aroma, and taste. Each attribute received a score on a five-point hedonic scale, with 1 representing strong dislike and 5 indicating strong liking.. The average scores from all panelists were used to determine consumer acceptance for each treatment.

2.6 Statistical Analysis

The experiment followed a 2 × 4 factorial completely randomized design comprising two tea-growing locations (Wonosari and Sirah Kencong) and four fermentation durations (0, 60, 90, and 120 min), resulting in eight treatment combinations with three independent replications. We analyzed data on total polyphenol content, antioxidant activity, and sensory acceptance using factorial ANOVA to evaluate main and interaction effects. Significant treatment differences were identified using Duncan's test at $\alpha = 0.05$. Analyses were performed with IBM SPSS Statistics v26.0.

3. Results and discussion

3.1 The Antioxidant Activity

The antioxidant activity of black tea was significantly influenced by the interaction between the growing environment and fermentation duration ($p < 0.05$).. As shown in Table 1, antioxidant activity ranged from 71.58 to 91.42%. The highest antioxidant activity was observed in treatment L1F3 (Wonosari, 120 min fermentation), reaching 91.42%, whereas the lowest value was recorded in treatment L2F0 (Sirah Kencong, no fermentation), at 71.58%. In both growing locations, antioxidant activity tended to increase with longer fermentation. As shown in Table 1, Antioxidant activity (%) of black tea produced under different growing environments and fermentation durations.

Table 1. Percentage of Antioxidant Activity (%) in Black Tea Cultivated in Various Environments and with Different Fermentation Times

Growing Location	Antioxidant Activity (%)

Growing	0 Min	60 Min	90 Min	120 Min
Wonosari	80.92 ± 0.35 d	86.72 ± 0.28 c	88.66 ± 0.31 b	91.42 ± 0.24 a
Sirah Kencong	71.58 ± 0.28 g	74.41 ± 0.30 f	77.70 ± 0.41 e	81.48 ± 0.37 d
DMRT	0.04			

Footnote:

Data are reported as means obtained from three independent replications together with their corresponding standard deviations. Mean separation was conducted with Duncan’s multiple range test. Treatment means marked by different superscript letters were considered significantly different at $p < 0.05$..

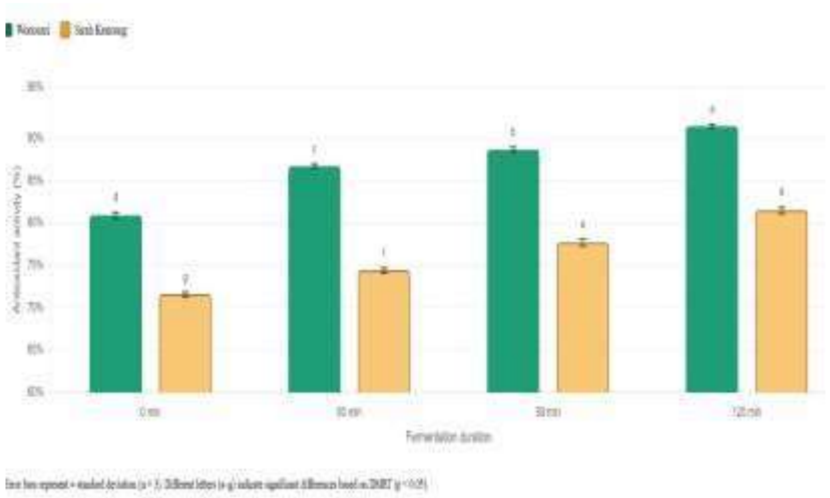

Figure 1. Grouped bar chart showing antioxidant activity (%) of black tea from Wonosari and Sirah Kencong at 0, 60, 90, and 120 minutes of fermentation duration

The higher antioxidant activity observed in tea samples collected from Wonosari may be associated with differences in agroclimatic conditions, including altitude, temperature, relative humidity, and soil characteristics. Furthermore, extended fermentation enhances enzymatic oxidation of catechins and leads to the formation of theaflavins and thearubigins, compounds that bolster black tea's antioxidant qualities..

3.2 The Polyphenol Content

The interaction between growing environment and fermentation duration significantly influenced the total polyphenol content of black tea; see Table 2 and Figure 2. The polyphenol content varied significantly among treatment combinations, ranging from 4.87 to 7.25 mg GAE g^{-1} dry weight.

Table 2. Total polyphenol content (mg GAE g^{-1} DW) of black tea as affected by growing environment and fermentation duration

Components	polyphenol content			
	0 Min	60 Min	90 Min	120 Min
Wonosari	4.92 ± 0.08 f	5.62 ± 0.10 e	6.97 ± 0.12 b	7.25 ± 0.09 a
Sirah Kencong	4.87 ± 0.07 f	5.28 ± 0.09 e	6.15 ± 0.11 d	6.64 ± 0.10 c
DMRT	0.04			

Values are shown as mean ± SD (n=3). Different letters indicate significant differences per Duncan's test (DMRT) at $p < 0.05$.

Figure 2. Polyphenol content based on fermentation time

The interaction between growing environment and fermentation duration significantly influenced the total polyphenol content of black tea ($p < 0.05$). As presented in Table 2, the polyphenol content varied significantly among treatment combinations, ranging from 4.87 to 7.25 mg GAE g^{-1} dry weight. The highest value was obtained from tea leaves grown in Wonosari and fermented for 120 min, whereas the lowest polyphenol content was recorded in tea from Sirah Kencong without fermentation. In general, longer fermentation resulted in higher polyphenol contents in both growing environments, with Wonosari samples showing higher values than Sirah Kencong samples at each fermentation level (L. Xu & Wang, 2025). Leaves often contain higher phenolic concentrations because they are directly exposed to environmental stresses such as ultraviolet radiation and oxidative stress. However, these results indicate that the seeds exhibited the highest antioxidant activity despite having a lower total phenolic content. This implies that antioxidant activity is influenced not only by phenolic concentration but also by chemical structure and interactions among various metabolites.. Recent studies show that flavonoids in seeds play an important role in protecting embryonic tissues from oxidative damage and contribute significantly to antioxidant capacity (de Oliveira et al., 2024). Furthermore, the synergistic interactions between phenolic compounds, flavonoids, and other phytochemicals can boost the antioxidant capacity of plant extracts (Ji et al., 2024; Rifai et al., 2020). Consequently, the potent antioxidant activity seen in pokak eggplant seeds may be linked to their high flavonoid levels and the presence of particular antioxidant compounds.

3.3 The Sensory acceptance

3.3.1 Steady shear properties

Sensory acceptance of black tea samples was evaluated using a five-point hedonic scale based on four attributes, namely appearance, infusion color, aroma, and taste. The mean preference scores obtained from 15 panelists are presented in Table 3. Black tea showed higher sensory acceptance (Figure 3).

Table 3. Sensory acceptance ratings of black tea

Sample	Appearance	Infusion color	Aroma	Taste
L1F0	3.60 ± SD	3.65 ± SD	3.58 ± SD	3.90 ± SD
L1F1	4.03 ± SD	4.17 ± SD	4.15 ± SD	4.27 ± SD
L1F2	4.37 ± SD	4.32 ± SD	4.20 ± SD	4.37 ± SD
L1F3	4.60 ± SD	4.67 ± SD	4.35 ± SD	4.47 ± SD
L2F0	3.57 ± SD	3.57 ± SD	3.37 ± SD	3.80 ± SD
L2F1	4.05 ± SD	4.15 ± SD	4.03 ± SD	4.22 ± SD
L2F2	4.10 ± SD	4.22 ± SD	4.13 ± SD	4.30 ± SD
L2F3	4.33 ± SD	4.27 ± SD	4.21 ± SD	4.33 ± SD

Table 3 presents sensory acceptance scores for appearance, infusion color, aroma, and taste. Mean scores ranged from 3.37 to 4.67, indicating that panelists generally accepted all black tea samples. The highest scores across all sensory attributes were observed for tea from Wonosari fermented for 120 min (L1F3), whereas the lowest scores were recorded for tea from Sirah Kencong without fermentation (L2F0). Overall, longer fermentation improved consumer preference for appearance, color, aroma, and taste, regardless of growing environment.

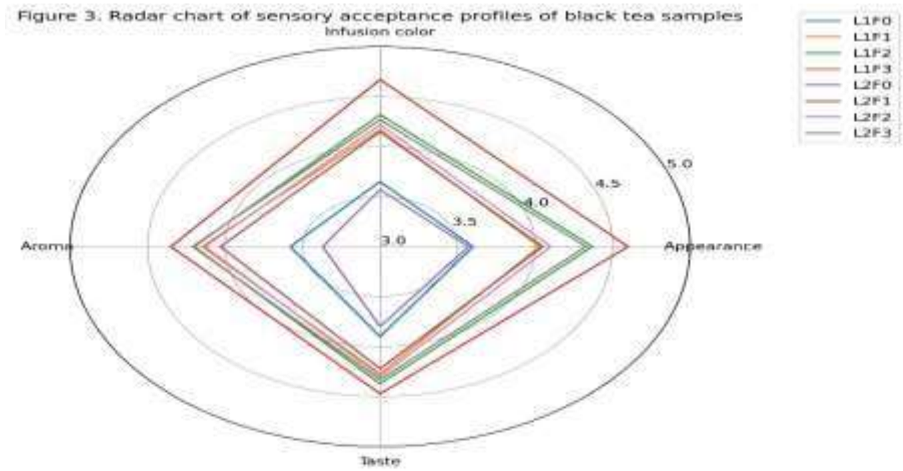

Figure 3. Sensory Acceptance Profiles of Black Tea Samples.

3.4 Discussion

The present study demonstrated that both growing environment and fermentation duration significantly affected the polyphenol content, antioxidant activity, and sensory acceptance of black tea. Tea leaves gathered from Wonosari and fermented for 120 minutes exhibited the highest levels of polyphenols and antioxidant activity, suggesting that both environmental factors and processing methods together affect the chemical quality of black tea. Previous studies reported similar observations, showing that black tea quality is strongly associated with both agroclimatic conditions and fermentation practices (Bakhshi et al., 2025; Ren et al., 2025).

The increase in antioxidant activity and polyphenol content with increasing fermentation time may be attributed to enzymatic oxidation reactions occurring during black tea manufacture. During fermentation, catechins are converted into theaflavins and thearubigins, which enhance both the antioxidant qualities and the distinctive color and flavor of black tea. Fermentation therefore represents a critical stage in determining the product's final quality. Lee et al. (2002) reported similar findings, showing that prolonged fermentation promotes the formation of flavor-active compounds and enhances the antioxidant potential of black tea.

Differences between Wonosari and Sirah Kencong tea samples likely reflect variations in growing conditions, including altitude, temperature, relative humidity, rainfall, and soil characteristics. Environmental factors impact photosynthesis and the production of secondary metabolites, which in turn influence the levels of polyphenols and other bioactive compounds in tea leaves. Previous studies has also highlighted the significance of agroclimatic conditions in shaping the chemical composition and antioxidant capacity of tea. (Vinci et al., 2022; W. Wang et al., 2023).

Sensory evaluation showed that black tea made from Wonosari and fermented for 120 minutes received the highest scores for appearance, infusion color, aroma, and taste. This positive feedback might be due to the formation of theaflavins and thearubigins during fermentation, which give the tea its reddish hue, characteristic aroma, and balanced astringency. Similar findings have been reported in previous studies, where the right fermentation time improved the sensory quality and popularity of black tea (Fathonah Sadek & Melyana, 2023; Moreira et al., 2024; D. Wang et al., 2024).

Overall, the results show that the combination of growing environment and fermentation time significantly influences the chemical and sensory qualities of black tea. Among the treatment combinations evaluated, tea leaves grown in Wonosari and fermented for 120 min produced the most desirable quality attributes, suggesting that optimization of both agronomic conditions and fermentation processes is essential for producing black tea with superior antioxidant properties and consumer acceptance.

4. Conclusion

The present study demonstrated that black tea quality is jointly influenced by growing environment and fermentation duration. Tea leaves cultivated in Wonosari, Malang, and fermented for 120 min produced the most desirable chemical and sensory characteristics. These findings provide new evidence regarding the interactive contribution of agroclimatic conditions and processing parameters to black tea quality. The results may serve as a scientific basis for optimizing black tea production and supporting the development of value-added tea products in Indonesia. Further studies are needed to characterize individual catechins, theaflavins, thearubigins, and volatile compounds associated with consumer acceptance.

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Data Availability Statement

All data produced or examined in this study are included in this published article.

The conflict of interests

This research is conducted without any conflicts of interest.

The Committee Approval Statement

The research involves human beings. The Ethics Committee of the institution responsible for the research approved the manuscript.

Authors' contribution statement

Nunuk Helilusiatiningsih contributions (Conceptualization; Visualization; Writing – original draft https://orcid.org/0009-0008-7588-7546; Writing – review & editing). Rysma Putri Aditama contributions (Formal analysis; Funding acquisition; Investigation) https://orcid.org/0009-0005-6631-1623; Titik Irawati contributions (Methodology; Project administration; Resources; Software; Supervision) https://orcid.org/0009-0007-7964-0332

The statement on the use of Artificial Intelligence

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) solely as a language-support tool for improving English grammar, clarity, readability, and textual organization. The authors have thoroughly reviewed, edited, and verified all AI-assisted content and assume full responsibility for the accuracy, originality, interpretation of the research data, and the final content of the manuscript. Artificial intelligence was not employed to generate, manipulate, or analyze the experimental data, nor to determine the study results or conclusions.

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