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The effect of drying process temperature and milling duration on total flavonoid levels in dry green tea (Camellia sinensis) powder

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Abstract

This study investigated the effect of drying process temperature and milling duration on the total flavonoid levels of dry green tea powder. It employed an experimental approach, subjecting green tea leaves to varying temperatures (40°C, 80°C, and 100°C) and milling durations (0, 1, 3, 5, and 8 hours), followed by nanoparticle production using high-energy ball milling. All data were analyzed using a two-way analysis of variance (ANOVA) test with SPSS version 28. The results demonstrated that drying temperature and milling duration significantly influenced flavonoid levels ($p = 0.000$), with a drying temperature of 40°C and a milling duration of 3 hours generating the optimal flavonoid concentration, beyond which flavonoid levels declined. SEM and PSA findings confirmed that particle size reduction significantly influences flavonoid availability. Therefore, controlled processing conditions could maintain green tea's antioxidant properties. These results suggest that the food and pharmaceutical industries of green tea-based products could optimize drying and milling conditions to improve the bioavailability of flavonoids. Future research could explore additional variables, such as humidity and storage conditions, to enhance flavonoid preservation and product quality.

Keywords: Dry milling, Flavonoids, Green tea, Milling duration, Temperature.

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1. Introduction

Tea (Camellia sinensis) is one of the most familiar natural products consumed daily globally. Tea has active compounds that act as antioxidants, including polyphenols, alkaloids, amino acids, polysaccharides, and volatile components [1, 2]. It usually grows in the tropics at altitudes of 200 to 2,000 meters above sea level [3]. Indonesia is one of the major tea producers in the world. Daily tea, when consumed as a beverage, is reported to have various health benefits

and properties. One of the most popular types of tea is green tea, which possesses antioxidant properties [4, 5]. The antioxidant properties of green tea are derived from polyphenol compounds [6], mainly from the flavonoid group [7, 8]. Catechins are natural flavan-3-ols known as 'flavonols', a type of polyphenolic compound belonging to the flavonoid family [9]. Specifically, the types of green tea catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [1, 10].

Catechins from green tea have antioxidant benefits [4, 11, 12], inhibit adipocyte differentiation, regulate microRNA suppressor tumors, and inhibit IkB [13-15]. Although the toxic side effects of green tea are relatively small, it must be used with caution during pregnancy, in children, and among the elderly population [1]. Overconsumption of green tea has many disadvantages that can cause catechin levels to reach extremely low circulation, including low absorption, low pharmacokinetics and bioavailability, and first-pass metabolism [16, 17]. The percentage of catechins that can be absorbed by the intestine after drinking tea is small. Therefore, this condition affects the levels that can reach circulation in the blood and tissues. To achieve a therapeutic effect, EGCG is required to be in a relatively large dose, but consuming green tea in massive quantities may also cause toxic effects [18, 19].

Overcoming this issue can be addressed in various ways, including through the application of nanotechnology for dry milling methods. Moreover, nanostructure-based medicine delivery systems are rapidly evolving technologies to improve the bioavailability of medicines. In many studies, nanoparticles have been proven to be more effective than conventional forms in achieving specific targets in organs or tissues [20, 21]. Small-sized nanoparticles can conduct high penetration, thereby increasing the efficacy of medicines and minimizing the toxic effects of therapeutic agents [1, 22-24]. Therefore, this research aimed to investigate the effect of the drying process temperature and milling duration on the total flavonoid levels of dry green tea powder.

2. Materials and Methods

2.1. Research design

This research followed an experimental procedure using green tea leaves exposed to varying temperatures (40°C, 80°C, and 100°C) and milling durations (0, 1, 3, 5, and 8 hours). A total of 72 samples were used.

2.2. Green tea processing

Green tea nanoparticle powder was produced using fresh tea leaves as the basic ingredients in the dry milling method. The dry milling process used a microwave with temperature variations of 40°C, 80°C, and 100°C for 4 minutes, followed by drying in the oven at 60°C for 2 hours. The variations in the milling duration were 0, 60, 180, 300, and 480 minutes, followed by a subsequent milling process.

2.3. Making of nanoparticle powder samples

Further crushing was performed using high-energy ball mill machines. Milling time varied from 0, 120, 180, 300 to 480 minutes, with a running time of 5 minutes, an off time of 60 seconds, a powder-to-ball ratio of 1:5 (9:45 grams), and ball sizes of 0.5, 1, and 3 mm in a ratio of 2:2:5 (10:10:25 grams).

2.4. Green tea nanoparticle analysis

The process of green tea nanoparticle synthesis and characterization using Scanning Electron Microscopy (SEM) and Particle Size Analyzer (PSA) was performed at the Nano Center Indonesia and the Science and Technology Research Center Laboratory. These processes were performed on dry matter. The next step involved characterization using two methods:

1) SEM (ZEISS Sigma®, Germany), to analyze the morphological characteristics of the solid sample;
2) PSA characterization, to measure particle size distribution. Approximately 0.25 mg of the samples were placed in a cuvette, then aqua pro injection was added up to 2.5 mg. Thereafter, the cuvette was inserted into the PSA tool holder. Finally, the tea leaf powder sample was analyzed using SEM (ZEISS Sigma®, Germany).

2.5. Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

The total flavonoid levels in green tea powder samples were determined through quantitative analysis of total flavonoid compounds using the LC-MS method. This technique combines the separation capabilities of liquid chromatography with the detection and characterization capabilities of mass spectrometry [25]. It has high sensitivity and selectivity [26, 27] that allow for a detailed analysis and an accurate identification of compounds in complex mixtures [28]. In this study, a quercetin standard solution with concentrations of 6, 8, 10, 12, and 14 parts per million (ppm) was used to determine the total flavonoid content in the sample.

2.6. Qualitative Analysis of the Total Flavonoid Content in Green Tea Powder

2.6.1. Calculation of the Quercetin Maximum Wavelength (λmax)

It was done by running a quercetin solution in the wavelength range of 400-450 nanometers (nm). The finding showed that the maximum wavelength of the standard quartz was 435 nm. It was used to measure the uptake of green tea powder samples.

2.6.2. Making A Standard Quartz Curve

After weighing, 25 milligrams (mg) of the standard quercetin solution were dissolved in 25 milliliters (mL) of ethanol. The stock solution was pipetted (1 mL) and diluted to 10 mL with ethanol to obtain a concentration of 100 ppm. A standard quercetin solution (100 ppm) was then prepared into several concentrations: 6 ppm, 8 ppm, 10 ppm, 12 ppm, and 14 ppm. Approximately 1 mL of each standard quercetin solution concentration was pipetted. Then, 1 mL of aluminum chloride ($AlCl_3$) 2% and 120 mL of potassium acetate (CH_3CO_2K) were added. The sample was incubated for 1 hour at room temperature. The absorbance was determined using the UV-Vis spectrophotometry method (Spectrophotometers UV-Vis®, Hitachi High-Tech) at a maximum wavelength of 435 nm.

2.6.3. Calculation of Total Flavonoid Levels in Green Tea Powder

After weighing, 15 mg of green tea powder was dissolved in 10 mL of ethanol, resulting in a final solution of 1,500 ppm. Approximately 1 mL of the solution was pipetted, then 1 mL of 2% $AlCl_3$ solution (Sigma-Aldrich®, USA) and 1 mL of 120 mm CH_3CO_2K (Sigma-Aldrich®, USA) were added. The samples were incubated for 1 hour at room temperature. Their absorbance was determined using the UV-Vis spectrophotometry method (Spectrophotometers UV-Vis®, Hitachi High-Tech) at a maximum wavelength of 435 nm. The samples were prepared in three replications for each analysis, and the average absorbance value was calculated.

2.7. Statistical Analysis

All data was analyzed using a two-way analysis of variance (ANOVA) test with the SPSS version 28 to determine the effect of drying temperature and milling duration on the total flavonoid levels of the green tea powder samples.

3. Results

3.1. Findings of Drying Stage of Fresh Tea in Microwave For 4 Minutes with Varying Temperatures

After going through the microwave drying process for 4 minutes at three different temperatures – 40°C, 80°C, and 100°C – the dry leaves became brownish, as shown in Figure 1.

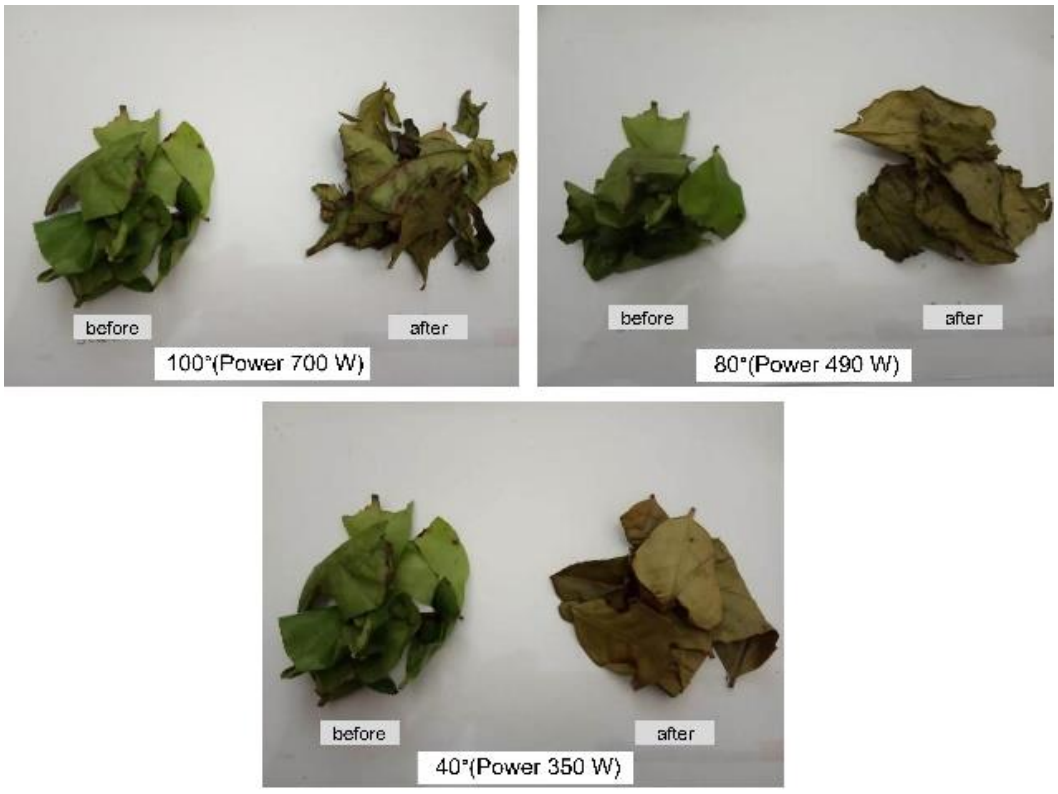


Figure 1.
The tea leaves before and after drying in different drying temperatures.

The color loss was mainly caused by the loss of Magnesium (Mg^{2+}) from chlorophylls [29-31]. However, their forms were not notably different. There was no striking difference among samples resulting from processing at 40°C, 80°C, and 100°C. The structure of tea cells was uniform and better maintained by microwave drying with or without vacuum [32].

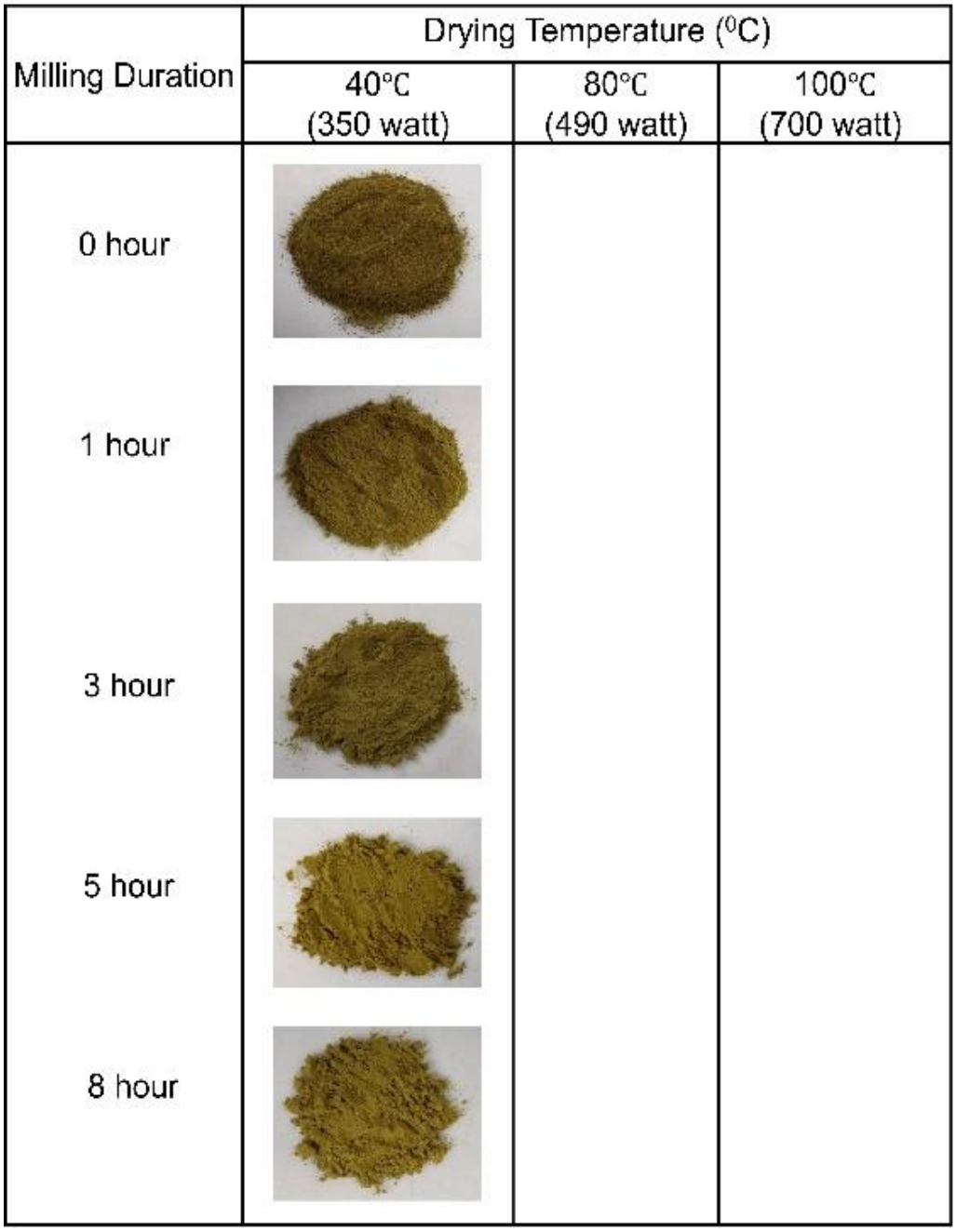


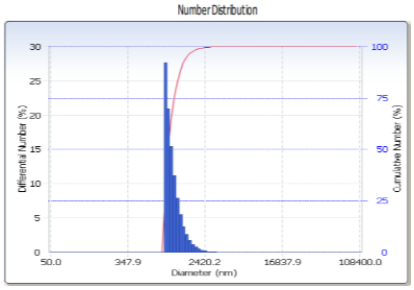
Figure 2.
Milling findings in different drying temperatures (40℃, 80℃, 100℃).

Figure 2 shows the dry green tea leaves after milling with variations in milling times (0, 1, 3, 5, and 8 hours). Changes in their color gradation look different among milling times; the longer the powder milling duration, the lighter the resulting powder color [2].

3.2. Characterization of Green Tea Particles' Dry Milling Powder Samples

The findings of PSA indicated that the average particle sizes of green tea powder across different drying temperatures were above 1,000 nm. The smallest particle size, 1,082.7 nm, was observed at 40°C (Figure 3). The particle sizes obtained from different drying temperatures were statistically different (P = 0.000), which means there was a statistically notable difference in the particle size of green tea powder from different drying processes.

a



Distribution Results (Contin)

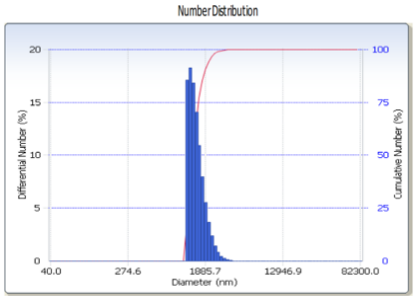
Peak	Diameter (nm)	Std. Dev
1	1.082.7	265.0
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	1.082.7	265.0

Residual : 1.961 e-002 (O.K)

Cumulants Results
Diameter (d) : 3684.5 (nm)
Polydispersity Index (P.I) : 0.667
Diffusion Const. (D) : 1.339e-009 (cm^2/sec)

Measurement Condition
Temperature : 25.0 (°C)
Diluent Name : WATER
Refractive Index : 1.3328
Viscosity : 0.8852 (cP)
Scattering Intensity : 8825 (cps)

b



Distribution Results (Contin)

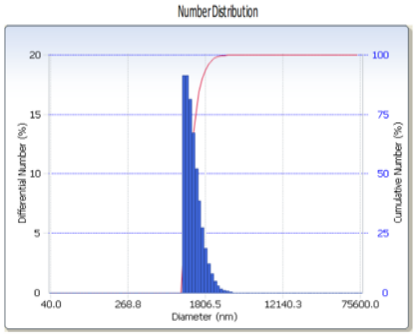
Peak	Diameter (nm)	Std. Dev
1	1.513.4	328.3
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	1.513.4	328.3

Residual : 2.232 e-002 (O.K)

Cumulants Results
Diameter (d) : 2291.0 (nm)
Polydispersity Index (P.I) : 0.583
Diffusion Const. (D) : 1.649e-009 (cm^2/sec)

Measurement Condition
Temperature : 25.0 (°C)
Diluent Name : WATER
Refractive Index : 1.3328
Viscosity : 0.8852 (cP)
Scattering Intensity : 9237 (cps)

c



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev
1	1.351.6	309.4
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	1.351.6	309.4

Residual : 1.591 e-002 (O.K)

Cumulants Results
Diameter (d) : 2879.5 (nm)
Polydispersity Index (P.I) : 0.531
Diffusion Const. (D) : 1.713e-009 (cm^2/sec)

Measurement Condition
Temperature : 25.0 (°C)
Diluent Name : WATER
Refractive Index : 1.3328
Viscosity : 0.8852 (cP)
Scattering Intensity : 13883 (cps)

Figure 3.
PSA analysis findings of samples with different temperatures: (a) 40°C, (b) 80°C, and (c) 100°C.

3.3. SEM Analysis Findings of Green Tea Powder Sample

The findings of microscopic analysis by SEM at different milling durations are presented as particle-size images in Table 1 and Figure 4.

Table 1.
Findings of SEM analysis.

Measuring Method	Milling Time Parameters			
	60 minutes	180 minutes	300 minutes	480 minutes
Grain size (μm)	12.810 ± 7.866	10.450 ± 5.957	5.182 ± 2.307	4.287 ± 2.805

As presented in Table 1, the particle size resulting from the milling process with different time durations (60, 180, 300, and 480 minutes) was greater than 1.000 μm in all cases and showed a statistically significant difference (P = 0.000). It means there was a statistically notable difference in particle size from the variations in milling duration.

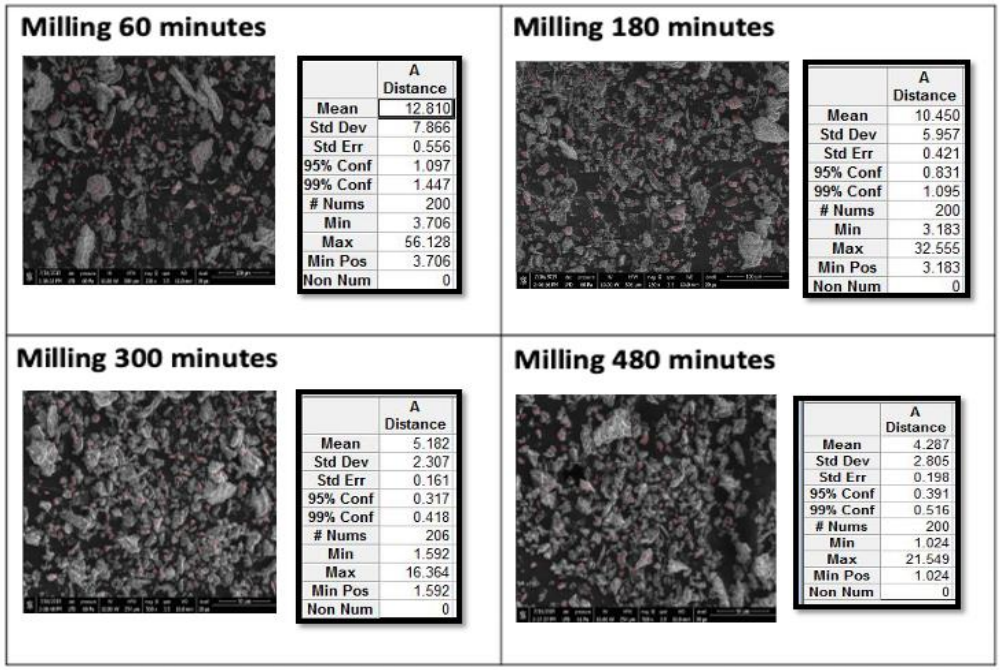


Figure 4.
The SEM analysis finding of green tea powder samples from different milling durations (60, 180, 300, and 480 min)

A picture of particle size was obtained from microscopic analysis by SEM at different milling durations. Figure 4A shows that a milling duration of 60 minutes resulted in a mean particle size of 12.810 ± 7.866 ɥm. Besides, Figure 4B shows that a milling duration of 180 min resulted in a mean particle size of 10.450 ± 5.957 ɥm. Furthermore, Figure 4C shows that a milling duration of 300 minutes resulted in a mean particle size of 5.182 ± 2.307 ɥm. Lastly, Figure 4D shows that a milling duration of 480 minutes resulted in a mean particle size of 4.287 ± 2.805 ɥm.

3.4. Analysis of Total Flavonoid Content of Green Tea Powder

Total flavonoid compounds were tested by quantitative analysis using the liquid chromatography-mass spectrometry (LC-MS) method to determine the total flavonoid levels contained in green tea powder samples [33, 34]. In this study, a standard solution with a series of concentrations of 6, 8, 10, 12, and 14 ppm was used to quantify the total flavonoid content in the sample. A series of standard concentrations was applied to generate a standard curve equation for qualification. Standard curves are first created by repeatedly testing the concentration series to obtain a linear equation, which was then used to calculate the percentage of the flavonoid content. The use of quercetin as a standard solution was selected because it is a flavonoid that is separated from the flavonol group, with a keto group in C-4 and a hydroxyl group in the C-3 or C-5 atom neighboring flavones and flavonoids. The maximum wavelength absorption was measured within a range of 400-450 nm. The results of this study showed that the maximum absorption wavelength of quercetin was 435 nm. Sample absorption was measured using the maximum wavelength. Table 2 presents the average of the measured total flavonoid levels in green tea powder samples.

Table 2.
The average levels of total flavonoids (µg/g) in green tea powder processed with different drying temperatures and milling durations

Drying Temperature	Milling duration				
	0 hour	**1 hours**	**3 hours**	**5 hours**	**8 hours**
40^0C	5579.19 ±71.04	5200.45 11.25	±6375.10 ±105.4	6106.27 ±20.25	5997.62 ±63.9
80^0C	4568.68 ±22.55	5101.32 10.58	±5374.23 ±11.45	5197.02 ±24.17	5514.73 ±35.34
100^0C	5338.25 ±149.69	4880.27 30.27	±5471.20 ±44.62	5213.96±146.52	4948.45 ±34.32

As shown in Figure 5, drying at 40°C produced the highest total flavonoid levels compared to drying at 80°C and 100°C. In terms of the milling duration, the milling process for 3 h produced the highest levels of flavonoids. The findings of statistical analysis showed that the drying temperature and the milling duration separately and together had a statistically notable influence on flavonoid levels of green tea powder with a significance value of 0.000, respectively.

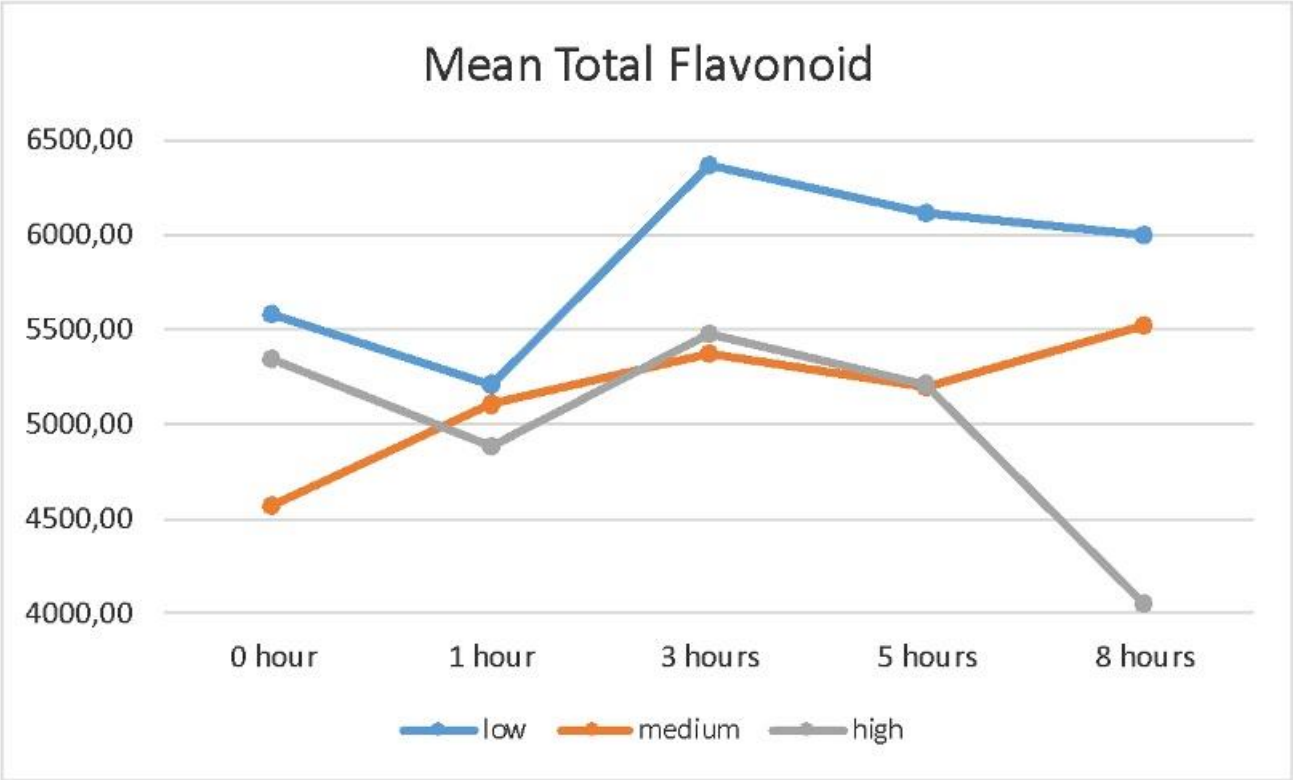


Figure 5.
The average of total flavonoid levels in green tea powder samples processed with different drying temperatures (low = 40°C, medium = 80°C, high = 100°C) and milling durations.

4. Discussion

The findings of this study show that processing temperature is notably related to the total flavonoid content (Table 2 and Figure 5). This is in line with other studies that room temperature is the most efficient temperature for the extraction process of tea leaves, where the process at room temperature acquires a high total phenolic and flavonoid content [16, 35]. Another study that extracted tea at cold (25°C) and hot (80°C) temperatures found that the total flavonoid content was higher after being processed at 80°C than at the other temperature [22, 36]. Different from the findings of Jin, et al. [37] which compared antioxidant activity and the content of green tea catechins processed at 60°C and 90°C, this study found that the most efficient processing to obtain the highest catechin levels was at 95°C [37]. The study by Eneighe, et al. [16] also reported that leaf temperature and size affect antioxidant activity. In this study, the maximum extraction process efficiency was obtained at 90°C in 120 minutes, so extracts from milling-processed leaves were obtained at the highest levels [16, 20].

Overall, processing temperature and particle size are important factors that affect the level of flavonoids (Tables 1-2). Several factors may cause variations in research findings regarding the optimum temperature for processing green tea to acquire the highest levels of flavonoids. One such factor is the types of tea leaf samples used. Different types of tea leaves or leaves from different regions can exhibit different characteristics and content of active compounds. In terms of milling time (Table 2 and Figure 5), this study found that 3 hours of milling produced the highest levels of flavonoids. This may be related to particle size manufactured by the milling process. Tea leaves through the milling process have increased the efficiency of the extraction process compared to those without milling [16, 38].

5. Conclusion

This study demonstrated that there is a relationship between process drying temperature and milling durations, which has a statistically notable effect on flavonoid levels of green tea powder. A drying temperature of 40°C led to the highest flavonoid retention compared to 80°C and 100°C. Additionally, milling for 3 hours resulted in the highest flavonoid levels. Statistical analysis confirmed that both drying temperature and milling duration had a statistically significant impact on flavonoid content ($P = 0.000$). These findings, therefore, suggest the optimization of processing conditions to maximize flavonoid retention in green tea powder, which may improve its potential health benefits. Future research could explore additional processing variables and their interactions to improve green tea production techniques.

References

[1] T. Zhao, C. Li, S. Wang, and X. Song, "Green tea (Camellia sinensis): A review of its phytochemistry, pharmacology, and toxicology," *Molecules*, vol. 27, no. 12, p. 3909, 2022. https://doi.org/10.3390/molecules27123909

[2] Z. Zhao *et al.*, "Influence and optimization of long-time superfine grinding on the physicochemical features of green tea powder," *Journal of Food Composition and Analysis*, vol. 117, p. 105124, 2023. https://doi.org/10.1016/j.jfca.2022.105124

[3] S. L. Jayasinghe and L. Kumar, "Climate change may imperil tea production in the four major tea producers according to climate prediction models," *Agronomy*, vol. 10, no. 10, p. 1536, 2020. https://doi.org/10.3390/agronomy10101536
[4] C. Musial, A. Kuban-Jankowska, and M. Gorska-Ponikowska, "Beneficial properties of green tea catechins," *International Journal of Molecular Sciences*, vol. 21, no. 5, p. 1744, 2020. https://doi.org/10.3390/ijms21051744
[5] A. P. Reverberi, M. Vocciante, M. Salerno, M. Ferretti, and B. Fabiano, "Green synthesis of silver nanoparticles by low-energy wet bead milling of metal spheres," *Materials*, vol. 13, no. 1, p. 63, 2019. https://doi.org/10.3390/ma13010063
[6] J. Bae, N. Kim, Y. Shin, S.-Y. Kim, and Y.-J. Kim, "Activity of catechins and their applications," *Biomedical Dermatology*, vol. 4, pp. 1-10, 2020. https://doi.org/10.1186/s41702-020-0057-8
[7] S. A. Banse *et al.*, "Antioxidants green tea extract and nordihydroguaiaretic acid confer species and strain-specific lifespan and health effects in Caenorhabditis nematodes," *GeroScience*, vol. 46, no. 2, pp. 2239-2251, 2024. https://doi.org/10.1007/s11357-023-00978-0
[8] Z.-Y. Cai *et al.*, "Bioavailability of tea catechins and its improvement," *Molecules*, vol. 23, no. 9, p. 2346, 2018. https://doi.org/10.3390/molecules23092346
[9] S. R. Mita, P. Husni, N. A. Putriana, R. Maharani, R. P. Hendrawan, and D. A. Dewi, "A recent update on the potential use of catechins in cosmeceuticals," *Cosmetics*, vol. 11, no. 1, p. 23, 2024. https://doi.org/10.3390/cosmetics11010023
[10] M. Farhan, "Green tea catechins: Nature's way of preventing and treating cancer," *International Journal of Molecular Sciences*, vol. 23, no. 18, p. 10713, 2022. https://doi.org/10.3390/ijms231810713
[11] R. Ahmad *et al.*, "The effect of natural antioxidants, pH, and green solvents upon catechins stability during ultrasonic extraction from green tea leaves (Camellia sinensis)," *Ultrasonics Sonochemistry*, vol. 94, p. 106337, 2023. https://doi.org/10.1016/j.ultsonch.2023.106337
[12] B. R. Meyer, H. M. White, J. D. McCormack, and E. D. Niemeyer, "Catechin composition, phenolic content, and antioxidant properties of commercially-available bagged, gunpowder, and matcha green teas," *Plant Foods for Human Nutrition*, vol. 78, no. 4, pp. 662-669, 2023. https://doi.org/10.1007/s11130-023-01121-2
[13] O. L. Pop *et al.*, "Polyphenols—ensured accessibility from food to the human metabolism by chemical and biotechnological treatments," *Antioxidants*, vol. 12, no. 4, p. 865, 2023. https://doi.org/10.3390/antiox12040865
[14] L. S. Ramírez-Aristizabal, A. Ortíz, M. F. Restrepo-Aristizabal, and J. F. Salinas-Villada, "Comparative study of the antioxidant capacity in green tea by extraction at different temperatures of four brands sold in Colombia," *Vitae*, vol. 24, no. 2, pp. 132-145, 2017. https://doi.org/10.17533/udea.vitae.v24n2a06
[15] S. F. Markhali, "Roles of drying, size reduction, and blanching in sustainable extraction of phenolics from olive leaves," *Processes*, vol. 9, no. 9, p. 1662, 2021. https://doi.org/10.3390/pr9091662
[16] S. A. Eneighe, F. B. Dzelagha, and D. B. Nde, "Production of an herbal green tea from ambang (Xymalos monospora) leaves: Influence of drying method and temperature on the drying kinetics and tea quality," *Journal of Food Science and Technology*, vol. 57, pp. 3381-3389, 2020. https://doi.org/10.1007/s13197-020-04371-z
[17] G. Wang, J. Wang, and M. R. Momeni, "Epigallocatechin-3-gallate and its nanoformulation in cervical cancer therapy: the role of genes, MicroRNA and DNA methylation patterns," *Cancer Cell International*, vol. 23, no. 1, p. 335, 2023. https://doi.org/10.1186/s12935-023-03161-9
[18] Y. Peristiowati and Z. Kusnul, "Synthesis and characterization of green tea paste nanoparticles based on wet milling," *Journal of Advanced Pharmaceutical Technology & Research*, vol. 11, no. 2, pp. 86-88, 2020. https://doi.org/10.4103/japtr.JAPTR_148_19
[19] F. Safarzadeh Markhali, "Roles of drying, size reduction, and blanching in sustainable extraction of phenolics from olive leaves," *Processes*, vol. 9, no. 9, p. 1662, 2021. https://doi.org/10.3390/pr9091662
[20] K. Dua *et al.*, "Increasing complexity and interactions of oxidative stress in chronic respiratory diseases: An emerging need for novel drug delivery systems," *Chemico-Biological Interactions*, vol. 299, pp. 168-178, 2019. https://doi.org/10.1016/j.cbi.2018.12.009.
[21] A. Yusuf, A. R. Z. Almotairy, H. Henidi, O. Y. Alshehri, and M. S. Aldughaim, "Nanoparticles as drug delivery systems: A review of the implication of nanoparticles' physicochemical properties on responses in biological systems," *Polymers*, vol. 15, no. 7, p. 1596, 2023. https://doi.org/10.3390/polym15071596.
[22] H.-Y. Liu *et al.*, "Phenolic content, main flavonoids, and antioxidant capacity of instant sweet tea (Lithocarpus litseifolius [Hance] Chun) prepared with different raw materials and drying methods," *Foods*, vol. 10, no. 8, p. 1930, 2021. https://doi.org/10.3390/foods10081930
[23] C.-Y. Hsu *et al.*, "An overview of nanoparticles in drug delivery: Properties and applications," *South African Journal of Chemical Engineering*, vol. 46, pp. 233-270, 2023. https://doi.org/10.1016/j.sajce.2023.08.009
[24] X. Cheng, Q. Xie, and Y. Sun, "Advances in nanomaterial-based targeted drug delivery systems," *Frontiers in Bioengineering and Biotechnology*, vol. 11, p. 1177151, 2023. https://doi.org/10.3389/fbioe.2023.1177151
[25] C. Y. J. Ng, N. P. Y. Lai, W. M. Ng, K. T. H. Siah, R.-Y. Gan, and L. L. Zhong, "Chemical structures, extraction and analysis technologies, and bioactivities of edible fungal polysaccharides from Poria cocos: An updated review," *International Journal of Biological Macromolecules*, vol. 261, p. 129555, 2024. https://doi.org/10.1016/j.ijbiomac.2024.129555
[26] S. N. Thomas, D. French, P. J. Jannetto, B. A. Rappold, and W. A. Clarke, "Liquid chromatography–tandem mass spectrometry for clinical diagnostics," *Nature Reviews Methods Primers*, vol. 2, no. 1, p. 96, 2022. https://doi.org/10.1038/s43586-022-00175-x
[27] K. R. Dewi *et al.*, "Advances and key considerations of liquid chromatography–mass spectrometry for porcine authentication in halal analysis," *Journal of Analytical Science and Technology*, vol. 14, no. 1, p. 13, 2023. https://doi.org/10.1186/s40543-023-00376-3
[28] J. A. Broadbent, M. R. Condina, and M. L. Colgrave, "Quantitative mass spectrometry-based analysis of proteins related to cattle and their products–Focus on cows' milk beta-casein proteoforms," *Methods*, vol. 186, pp. 112-118, 2021. https://doi.org/10.1016/j.ymeth.2020.09.011
[29] H. Wang *et al.*, "The effect of microwave radiation on the green color loss of green tea powder," *Foods*, vol. 11, no. 16, p. 2540, 2022. https://doi.org/10.3390/foods11162540

[30] K. Singh *et al.*, "Manipulation of the phenolic quality of assam green tea through thermal regulation and utilization of microwave and ultrasonic extraction techniques," *Horticulturae*, vol. 8, no. 4, p. 338, 2022. https://doi.org/10.3390/horticulturae8040338

[31] V. E. Shofi and B. K. Betari, "Effect shading intensity on color, chemical composition, and sensory evaluation of green tea (Camelia sinensis var Assamica)," *Journal of the Saudi Society of Agricultural Sciences*, vol. 22, no. 7, pp. 407-412, 2023. https://doi.org/10.1016/j.jssas.2023.03.006

[32] L. X. Lin XiangYang *et al.*, "Effect of drying technologies on quality of green tea," *International Agricultural Engineering Journal*, vol. 19, no. 3, pp. 30–37, 2010.

[33] C.-S. Seo and M.-Y. Lee, "Hplc–pda and lc–ms/ms analysis for the simultaneous quantification of the 14 marker components in sojadodamgangki-tang," *Applied Sciences*, vol. 10, no. 8, p. 2804, 2020. https://doi.org/10.3390/app10082804

[34] M.-F. Sun, C.-L. Jiang, Y.-S. Kong, J.-L. Luo, P. Yin, and G.-Y. Guo, "Recent advances in analytical methods for determination of polyphenols in tea: A comprehensive review," *Foods*, vol. 11, no. 10, p. 1425, 2022. https://doi.org/10.3390/foods11101425

[35] S. Raza *et al.*, "Enhancing the antimicrobial activity of silver nanoparticles against ESKAPE bacteria and emerging fungal pathogens by using tea extracts," *Nanoscale Advances*, vol. 5, no. 21, pp. 5786-5798, 2023. https://doi.org/10.1039/d3na00220a

[36] B. J. Murokore *et al.*, "Effect of extraction period on total phenolics, total flavonoids, and antioxidant capacity of Ugandan Camellia sinensis (L) kuntze, black primary grades and green tea," *Journal of Food Quality*, vol. 2023, no. 1, p. 3504280, 2023. https://doi.org/10.1155/2023/3504280

[37] Y. Jin *et al.*, "Comprehensive investigation of the effects of brewing conditions in sample preparation of green tea infusions," *Molecules*, vol. 24, no. 9, p. 1735, 2019. https://doi.org/10.3390/molecules24091735

[38] W. Aditama, F. Y. Sitepu, and R. Saputra, "Relationship between physical condition of house environment and the incidence of pulmonary tuberculosis, Aceh, Indonesia," *International Journal of Science and Healthcare Research*, vol. 4, no. 1, pp. 227-231, 2019.