



Comparative Effects of Hand- and Machine-Whisked Matcha Preparation on Antioxidant and Anti-Inflammatory Activities in Macrophage Cell Models

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Abstract

Matcha is rich in antioxidants, which contribute to enhanced fat metabolism, anti-inflammatory activity, and immune system support. The key bioactive compounds found in matcha are flavonoids known as catechins, which possess potent antioxidant properties. These compounds help delay cellular degeneration and reduce the risk of various diseases. Currently, matcha preparation can be classified into two main methods: (1) the hand-whisking method using a traditional bamboo whisk (chasen), and (2) the machine-whisking method, such as using an electric milk frother, which is more convenient and time-efficient. This study aimed to compare the effects of hand-whisking and machine-whisking methods on the antioxidant and anti-inflammatory activities of matcha at the cellular level. A 100% premium-grade matcha product (2 g) was mixed with 100 g of water at 80°C. The matcha was prepared manually using a bamboo whisk (chasen). The whisking motion followed an "M-shaped" pattern at a frequency of approximately two strokes per second for 1 min. The prepared matcha from both methods was then tested in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells at concentrations of 3.12%, 6.25%, and 12.5%. Antioxidant activity was evaluated by measuring intracellular reactive oxygen species (ROS), while anti-inflammatory activity was assessed by determining cytokine levels, including TNF- α , IL-6, and IL-8. The results demonstrated that machine-whisked matcha significantly reduced ROS production compared with hand-whisked matcha ($p < 0.05$). Furthermore, machine-whisked matcha significantly decreased cellular cytokine levels more effectively than hand-whisked matcha ($p < 0.05$), particularly for IL-8 and TNF- α . In conclusion, machine-whisking preparation enhances the antioxidant and anti-inflammatory activities of matcha in LPS-induced macrophage cells more effectively than the traditional hand-whisking method.



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Abbreviations

DCFH-DA	2',7'-Dichlorofluorescein Diacetate
DMEM	Dulbecco's Modified Eagle's Medium
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
HRP	Horseradish Peroxidase
IL-6	Interleukin-6
IL-8	Interleukin-8
LPS	Lipopolysaccharide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
PBS	Phosphate-Buffered Saline
ROS	Reactive Oxygen Species
TMB	Tetramethylbenzidine
TNF- α	Tumor Necrosis Factor-alpha

Introduction

Free radicals are highly reactive molecules or particles characterized by an imbalance in electron configuration, which renders them prone to rapid interactions with cellular components. Such reactivity can disrupt normal cellular function and induce structural damage, ultimately contributing to cellular degeneration and the aging process. Moreover, elevated levels of free radicals are strongly linked to chronic inflammatory responses, which are characterized by increased secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin (IL)-6, and IL-8. Sustained inflammation contributes to the pathogenesis of numerous non-communicable diseases (NCDs), including diabetes, cardiovascular diseases, and various types of cancer.¹

The human body possesses intrinsic defense mechanisms to neutralize or inhibit free radicals through antioxidant systems derived from both endogenous processes and dietary intake. Endogenous antioxidants involve essential nutrients such as selenium, zinc, and the amino acid cysteine, which play critical roles in reducing oxidative stress. In addition, exogenous antioxidants obtained from food sources, including vitamins C, E, and A, as well as various phytochemicals, contribute substantially to maintaining redox balance. Among these, catechins derived from green tea have been widely recognized for their potent antioxidant properties.² Matcha, a finely powdered form of green tea, has gained widespread popularity due to its unique processing method and high concentration of

bioactive compounds. Unlike conventional green tea infusions, matcha allows for the direct consumption of whole tea leaves in powdered form, enhancing the intake of functional compounds. It is commonly used in beverages and incorporated into various food products such as milk-based drinks, ice cream, and confectionery items. Importantly, matcha is rich in antioxidants and has been associated with multiple health benefits, including enhanced fat metabolism, detoxification, anti-inflammatory effects, and immune system support.^{3,4} The primary bioactive compounds in matcha are flavonoids known as catechins, particularly epigallocatechin gallate (EGCG), which plays a significant role in scavenging free radicals, delaying cellular aging, and reducing the risk of chronic diseases.⁵⁻⁷ However, the biological activity of matcha may not depend solely on its chemical composition, as different preparation methods could influence the release, dispersion, and bioavailability of these bioactive compounds, thereby affecting its antioxidant and anti-inflammatory properties.

The preparation of matcha beverages involves dispersing matcha powder uniformly in hot water to achieve a homogeneous mixture with a characteristic fine foam layer while preserving its bioactive components. Currently, two primary preparation methods are employed: (1) the traditional hand-whisking method using a bamboo whisk (chasen), and (2) the machine-whisking method using an electric frother, which offers greater convenience and efficiency.⁴ Previous studies have indicated that preparation techniques can influence the physicochemical properties of matcha, particularly

particle dispersion, which is closely related to the release of antioxidant compounds.^{8,9} Finely dispersed matcha particles facilitate the release of bioactive compounds, leading to higher *in vitro* antioxidant capacity and potentially improved bioavailability.¹⁰ Furthermore, factors such as water temperature, whisking time, and particle size have been shown to significantly affect antioxidant activity, with reported ferric reducing antioxidant power (FRAP) values ranging from 5,767.30 to 6,129.53 $\mu\text{M Fe(II)}/\text{dm}^3$ and DPPH radical scavenging activity between 12.08% and 41.24%.¹¹ Notably, higher antioxidant activity has been observed when matcha is prepared at temperatures between 60 and 80°C.¹² In particular, whisking methods may alter particle dispersion efficiency, foam formation, and the extraction of catechins and other bioactive compounds, which could subsequently influence their biological activity at the cellular level.^{13,14}

Despite growing interest in matcha preparation techniques, existing studies have mainly focused on physicochemical properties and chemical antioxidant assays, while the biological effects of different whisking methods at the cellular level remain poorly understood. Specifically, it is still unclear whether differences in particle dispersion and bioactive compound release between hand-whisking and machine-whisking methods lead to distinct effects on oxidative stress and inflammatory responses in immune cells. To address this gap, the present study systematically compares traditional hand-whisking and machine-whisking methods using a macrophage cell model. By linking preparation techniques with functional cellular outcomes, this study provides novel mechanistic insights into how whisking methods may influence the health-promoting properties of matcha beyond conventional chemical analyses. These findings contribute to a more evidence-based understanding of matcha preparation and may provide practical implications for consumers and the functional food industry.

Materials and Methods

Materials

Dulbecco's Modified Eagle's Medium (DMEM) and penicillin–streptomycin were purchased from Invitrogen (Invitrogen Ltd., Paisley, UK). MTT reagent, lipopolysaccharide (LPS), and 2',7'-dichlorofluorescein diacetate (DCFH-DA) were obtained from Sigma-Aldrich (Dorset, UK).

Enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioLegend (San Diego, CA, USA). All cell culture plasticware was obtained from Corning Inc. (Corning, NY, USA).

Sample Selection and Preparation

Premium-grade 100% matcha powder was selected using a non-probability sampling approach, specifically purposive sampling, based on the highest sales ranking from online retail platforms. In addition, matcha powder derived from the Ujihikari cultivar and sourced from Uji, Kyoto, Japan, was selected for this study. The product was chosen based on its origin and cultivar, as Uji matcha is widely recognized for its high quality and rich catechin content. Matcha samples were prepared by dissolving 2 g of matcha powder in 100 g of water at 80°C.¹² Two preparation methods were applied: (1) Hand-whisking, using a traditional bamboo whisk (*chasen*). The whisking motion followed an “M-shaped” pattern at a frequency of approximately two strokes per second for 1 min to ensure consistent preparation conditions; and (2) Machine-whisking, using an electric matcha frother (MatchaLabo, 3 V, high-speed mini motor, approximately 10,000 rpm) for 1 min. Each preparation was performed in triplicate. The resulting matcha samples were transferred into amber bottles, wrapped with aluminum foil to minimize light exposure, and stored in an ice-containing insulated container prior to laboratory analysis. For cellular assays, the experimental design included a negative control group (untreated cells without LPS stimulation), a positive control group treated with LPS alone (100 ng/mL), and a sample-only control group treated with matcha samples without LPS stimulation.

Cell Culture

Murine macrophage cells (RAW 264.7) obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) were used as the experimental model. Cells were cultured in complete Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin–streptomycin. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Cell Viability Assay

The potential cytotoxic effects of matcha samples were assessed using the MTT assay as previously

study.¹⁵ Matcha prepared by both whisking techniques was diluted in DMEM to obtain final concentrations of 3.12%, 6.25%, 12.5%, 25%, 50%, and 100%. RAW 264.7 macrophages were seeded into 48-well plates at a density of 0.2×10^6 cells per well and cultured for 24 h. Following incubation, the medium was discarded, and the cells were rinsed once with DMEM prior to exposure to the designated concentrations of matcha samples for an additional 24 h. Cells maintained in DMEM without matcha treatment served as the control group. Any concentration that produced a statistically significant decrease in cell viability relative to the control group ($p < 0.05$) was considered cytotoxic and was therefore excluded from subsequent analyses.

LPS-Induced Inflammation Model and Treatment

For antioxidant and anti-inflammatory assays, RAW 264.7 cells were seeded in black 96-well plates (5×10^4 cells/well) for ROS measurement and 48-well plates (0.2×10^6 cells/well) for cytokine analysis. After 24 h incubation, the culture medium was removed, and cells were washed with DMEM. Cells were then pre-treated with non-cytotoxic concentrations of matcha samples for 1 h. The experimental design included a negative control group (untreated cells without LPS stimulation) and a positive control group treated with LPS alone, and a sample-only control group treated with matcha samples without LPS stimulation. Inflammation was induced by adding LPS at a concentration of 100 ng/mL, followed by incubation at 37°C for 24 h. After treatment, both culture media and cells were collected for further analysis.

Measurement of Intracellular ROS

Intracellular reactive oxygen species (ROS) production was determined using the DCFH-DA fluorescence assay with minor modifications based on a previously published method.¹⁶ After treatment, cells were exposed to 10 μ M 2',7'-dichlorofluorescein diacetate (DCFH-DA) dissolved in 0.1 M phosphate-buffered saline (PBS, pH 7.4) and incubated at 37 °C for 30 min in the dark. Subsequently, the cells were rinsed twice with PBS (0.1 M, pH 7.4) to eliminate residual dye, followed by the addition of fresh PBS to each well. Fluorescence signals generated from intracellular oxidation of DCFH-DA were recorded using a microplate reader at excitation and emission wavelengths of 480 and 530 nm, respectively. The resulting ROS values were normalized to the

untreated control and expressed as percentages. These measurements were used to assess cellular oxidative stress and to determine the antioxidant effects of the matcha preparations.

Pro-inflammatory Cytokine Analysis

The concentrations of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-8, were determined using commercially available ELISA kits following the manufacturer's protocol. In brief, 96-well microplates were pre-coated with capture antibodies and incubated overnight. Non-specific binding sites were subsequently blocked prior to the addition of standards and appropriately diluted samples. After the washing steps, biotinylated detection antibodies were applied, followed by incubation with avidin-horseradish peroxidase (HRP). Signal development was performed using tetramethylbenzidine (TMB) substrate, and the reaction was terminated with stop solution. Optical density was recorded at 450 nm using a microplate reader. Cytokine levels were quantified from calibration curves generated with serially diluted standards and reported as pg/mL. The obtained cytokine profiles were used to assess the anti-inflammatory potential of the matcha preparations.

Statistical Analysis

All experimental data were collected and expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Scheffé's post hoc test to determine significant differences between groups. A p -value of < 0.05 was considered statistically significant.

Results

Effects of Matcha on Macrophage Cell Viability

The effects of matcha prepared by hand-whisking (Chasen) and machine-whisking (EF) on the viability of RAW 264.7 macrophage cells were evaluated after 24 h of treatment. As shown in Figure 1, Chasen and EF matcha at concentrations of 1.56%, 3.12%, 6.25%, and 12.5% did not significantly affect cell viability, with viability values of $100.50 \pm 0.71\%$, $100.30 \pm 1.27\%$, $99.96 \pm 1.16\%$, and $98.79 \pm 2.46\%$ for Chasen, and $101.69 \pm 1.26\%$, $100.14 \pm 2.00\%$, $99.20 \pm 2.27\%$, and $97.80 \pm 2.88\%$ for EF, respectively. However, concentrations above 12.5% significantly reduced cell viability compared with the control group ($p < 0.05$). At concentrations

of 25%, 50%, and 100%, cell viability decreased to $86.66 \pm 2.27\%$, $68.53 \pm 5.34\%$, and $54.24 \pm 4.62\%$ for Chasen, and $82.62 \pm 3.28\%$, $67.96 \pm 3.34\%$, and $52.17 \pm 4.96\%$ for EF, respectively. These findings indicate that matcha at concentrations exceeding

12.5% exhibits cytotoxic effects on macrophage cells. Therefore, 12.5% was defined as the maximum non-cytotoxic concentration and was selected for subsequent experiments investigating antioxidant and anti-inflammatory activities.

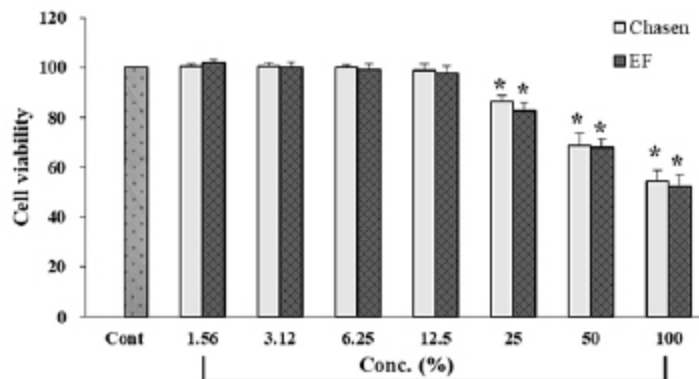


Fig. 1: Viability of RAW 264.7 macrophages after 24 h exposure to matcha prepared by traditional hand-whisking (Chasen) or electric frothing (EF). Matcha samples were tested at concentrations of 3.12%, 6.25%, 12.5%, 25%, 50%, and 100%. Values are presented as mean $\pm$ SD from three independent experiments, each conducted in triplicate ($n = 3$). * $p < 0.05$ compared with the 0.5% DMSO control.

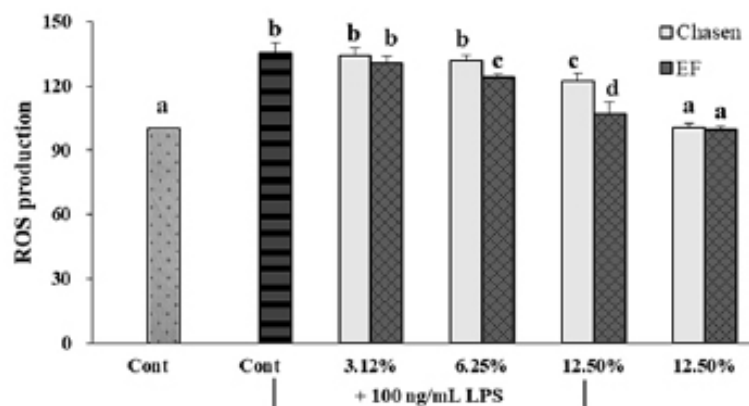


Fig. 2. Intracellular ROS production in LPS-stimulated RAW 264.7 macrophages following treatment with matcha prepared by traditional hand-whisking (Chasen) or electric frothing (EF). Cells were pretreated with matcha at concentrations of 3.12%, 6.25%, and 12.5% for 1 h prior to exposure to LPS (100 ng/mL) for 24 h. Results are presented as mean $\pm$ SD from three independent experiments, each performed in triplicate ($n = 3$). Different letters (a–d) indicate significant differences among treatment groups ($p < 0.05$).

Effects of Matcha on Antioxidant Activity in LPS-Induced Macrophages

Stimulation of RAW 264.7 macrophages with LPS (100 ng/mL) for 24 h resulted in a significant

increase in intracellular ROS levels compared with the control group ($p < 0.05$), as illustrated in Figure 2, confirming the successful induction of oxidative stress. Pre-treatment with both Chasen and EF

matcha significantly attenuated ROS production compared with the LPS control group. A dose-dependent reduction in ROS levels was observed across concentrations of 3.12%, 6.25%, and 12.5%. Notably, EF matcha demonstrated a significantly greater reduction in ROS levels than Chasen matcha at concentrations of 6.25% and 12.5% ($p < 0.05$). In addition, treatment with matcha alone (without LPS stimulation) did not significantly alter ROS levels compared with the control group, indicating that matcha does not induce oxidative stress under normal conditions. These results suggest that both preparation methods confer antioxidant effects in LPS-induced macrophages, with machine-whisked matcha exhibiting superior antioxidant activity.

Effects of Matcha on Anti-inflammatory Activity in LPS-Induced Macrophages

LPS stimulation (100 ng/mL, 24 h) significantly increased the production of pro-inflammatory cytokines, including IL-6, IL-8, and TNF- α , compared with the control group ($p < 0.05$), as shown in Figure 3. Cell viability under these conditions remained

comparable to the control group (Figure 3A–3D), indicating that LPS effectively induced inflammation without causing cytotoxicity. Pre-treatment with both Chasen and EF matcha significantly reduced the levels of IL-6, IL-8, and TNF- α compared with the LPS control group. This reduction followed a dose-dependent pattern, with progressive decreases observed at concentrations of 3.12%, 6.25%, and 12.5%. Comparative analysis revealed that EF matcha exerted a significantly stronger inhibitory effect on pro-inflammatory cytokine production than Chasen matcha ($p < 0.05$), particularly for IL-8 and TNF- α across multiple concentrations. Furthermore, no significant differences in cell viability were observed between matcha-treated groups and the control group, indicating that both matcha preparations were non-cytotoxic under the experimental conditions. These findings demonstrate that both Chasen and EF matcha possess anti-inflammatory properties in LPS-induced macrophages by suppressing key pro-inflammatory cytokines, with machine-whisked matcha showing greater efficacy.

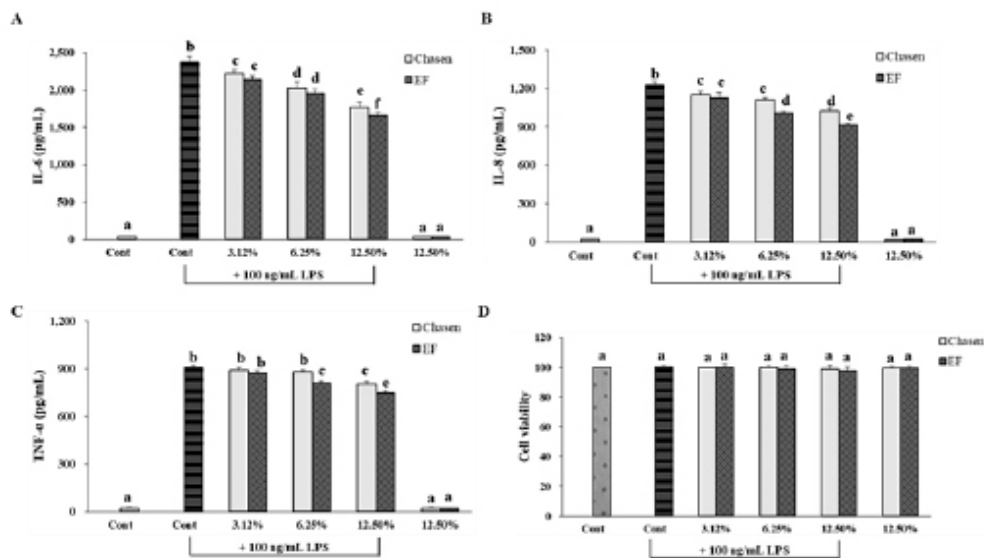


Fig. 3. Pro-inflammatory cytokine production and cell viability of LPS-stimulated RAW 264.7 macrophages following treatment with matcha prepared by traditional hand-whisking (Chasen) or electric frothing (EF). Cells were pretreated with matcha at concentrations of 3.12%, 6.25%, and 12.5% for 1 h before stimulation with LPS (100 ng/mL) for 24 h. The levels of (A) IL-6, (B) IL-8, and (C) TNF- α were determined, while (D) cell viability was assessed after treatment. Values are expressed as mean $\pm$ SD from three independent experiments, each performed in triplicate ($n = 3$). Different letters (a–d) denote statistically significant differences among groups ($p < 0.05$).

Discussion

The present study demonstrates that matcha prepared using a machine-whisking method exhibits superior antioxidant and anti-inflammatory activities compared with the traditional hand-whisking method. This effect may be associated with the higher mechanical energy generated during machine-whisking, which could promote more efficient particle dispersion and potentially enhance the release of catechins and other polyphenols into the aqueous phase.¹⁷⁻¹⁹ These findings are consistent with previous studies indicating that particle size and dispersion significantly influence the bioavailability and functional properties of matcha. Maeda-Yamamoto *et al.* reported that finely dispersed matcha particles facilitate the release of bioactive compounds, leading to increase *in vitro* antioxidant capacity and potentially enhanced absorption *in vivo*.¹⁰ Similarly, Zaiter *et al.*²⁰⁻²² demonstrated that smaller green tea particle sizes (100–180 µm), particularly when processed under high-speed conditions (6,000 rpm), resulted in higher catechin content and antioxidant activity. In the present study, the greater reduction in intracellular ROS observed in the machine-whisking group compared with the hand-whisking group may support these previous findings and suggest that preparation techniques can influence the biological activity of matcha at the cellular level.

The enhanced antioxidant activity observed in the present study is reflected by the significant reduction in intracellular ROS levels. Antioxidants present in matcha, particularly catechins, are known to effectively scavenge ROS and mitigate oxidative stress.^{11,23,24} The observed reduction in ROS levels in the present study was accompanied by decreased levels of pro-inflammatory cytokines, including TNF-α and IL-8, which is consistent with previous reports describing the anti-inflammatory effects of green tea catechins.²⁵⁻²⁹ Zhang *et al.*²⁷ reported that green tea polyphenols can attenuate oxidative stress associated with bacterial infection and intestinal dysfunction. In addition, El-Beshbishy demonstrated that EGCG can inhibit ROS generation and reduce TNF-α production,²⁸ while Kim *et al.*²⁹ showed that green tea catechins have been shown to attenuate inflammatory cytokine production in both *in vitro* and *in vivo* systems. The underlying effects may be mediated through the regulation of signaling cascades, including NF-κB and MAPK; however,

these pathways were beyond the scope of the current investigation and should be explored in future studies.

In contrast, the traditional hand-whisking method using a bamboo whisk (chasen) relies heavily on manual technique, including whisking speed, force, and consistency. Although this method can effectively suspend matcha particles and generate foam, variability in whisking conditions may lead to less consistent particle dispersion. This variability may partly explain the comparatively lower antioxidant and anti-inflammatory activities observed in the hand-whisking group. Although direct molecular comparisons between hand-whisking and machine-whisking methods remain limited, existing evidence on tea preparation processes supports the importance of mechanical force and particle dispersion in influencing extraction efficiency and functional properties of tea-derived bioactive compounds.¹⁸⁻²²

Although the present findings are encouraging, several limitations should be considered. The experiments were performed in an *in vitro* macrophage model, which cannot fully replicate the complexity of human physiology, including interactions among tissues, organs, and metabolic processes.³⁰ Furthermore, the inflammatory response induced by LPS mainly reflects acute inflammatory events, despite the recognized involvement of LPS-mediated signaling pathways in the progression of chronic inflammatory disorders.^{31,32} Collectively, the results suggest that preparation techniques can influence the functional characteristics of matcha, with electric whisking showing greater potential to enhance its antioxidant and anti-inflammatory effects under the conditions evaluated in this study.

Conclusion

Differences in matcha preparation methods were found to significantly affect its biological functions. Matcha prepared using an electric whisk exhibited enhanced protective effects against oxidative stress and inflammation, as evidenced by the suppression of intracellular ROS generation and pro-inflammatory cytokine production (TNF-α, IL-6, and IL-8) in LPS-challenged macrophages when compared with traditionally hand-whisked matcha. These findings suggest that enhanced particle dispersion and increased release of bioactive compounds,

particularly catechins and polyphenols, contribute to the improved functional properties observed with machine-whisking. The results highlight the importance of preparation techniques in maximizing the health-promoting potential of matcha and provide practical implications for both consumers and the food industry.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable

Author Contributions

- **Kanitkul Netbute:** Conceptualization, Methodology, Investigation, Data Collection, Formal Analysis, Visualization, Writing – Original Draft.
- **Kritmongkhon Kamonsuwan:** Validation, Writing – Review & Editing.
- **Akkarach Bumrungpert:** Supervision, Project Administration, Resources, Writing – Review & Editing.

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