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Enhancing the Functional Quality of Liberica Nano-Coffee via Yeast-Lactic Fermentation and Nano-Milling Techniques

Annisa Aurora Kartika[1], Wenny Bekti Sunarharum[1,*], Noor Asnida Asli[2], Eddie Ti Tjih Tan[3], Mohamad Rusop[2], Tunjung Mahatmanto[1], Yuniar Ponco Prananto[4], Durratun Nasihah Mohd Shuhairi[2] and Nur Syazwani Abdul Malek[2]

[1]*Department of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya, Jawa Timur 65145, Indonesia*
[2]*Centre for Functional Materials and Nanotechnology, Institute of Science, Universiti Teknologi MARA, Selangor 40450, Malaysia*
[3]*Food Technology Department, Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah, Negeri Sembilan 72000, Malaysia*
[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Jawa Timur 65145, Indonesia*

([*]Corresponding author's e-mail: wbsunarharum@ub.ac.id)

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Abstract

Coffee is an essential economic commodity, with varieties such as Liberica recognized for their resilience to diverse climates, although it is less popular than Arabica and Robusta. However, its potential remains underutilized, especially in advanced food technology applications. Advances in nanotechnology offer potential improvements in coffee quality by utilizing nano-sized particles to enhance flavor, aroma, and nutrient bioavailability. This study investigates the effects of yeast-lactic acid fermentation processing techniques on the milling process and nanoparticle characteristics in the production of Liberica nano-coffee. Liberica coffee sourced from Banyuwangi, Indonesia, underwent yeast-lactic acid fermentation. After cleaning, sun-drying, and medium-level roasting, the beans were subjected to ball milling at 500 rpm for 60, 120, and 180 min, followed by sonication. The resulting coffee powder was analyzed using Particle Size Analyzer (PSA), Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared Spectroscopy (FT-IR), pH and color measurement, and IC_{50} assessments. PSA results indicate that lactic yeast fermentation led to smaller, more uniform nanoparticles. Physicochemical analysis showed a decrease in pH in fermented (F) coffee (5.07 - 5.13) compared to non-fermented (NF) coffee (5.45 - 5.50). Fermentation also resulted in darker coloration and a reduction in antioxidant capacity as indicated by higher IC_{50} values for F coffee compared to NF coffee, along with alterations in chromatic parameters. The milling process resulted in approximately 10% of the coffee particles being in the nanoscale for both NF and F coffee samples. FESEM revealed rougher surfaces in the milled F coffee sample, with enhanced porosity and flake-like structures. FTIR spectra confirmed biochemical alterations in F coffee, particularly in lipid degradation, organic acid accumulation, and polyphenol breakdown. This study presents a novel focus on *Liberica coffee*, an underexplored species in nanotechnology applications. The combination of fermentation and nanomilling demonstrates potential in enhancing antioxidant-related properties, positioning Liberica nano-coffee as a functional ingredient.

Keywords: Antioxidant activity, Fermentation, Physicochemical properties, Milling process, Nanotechnology, Functional food, *Coffea liberica*

Introduction

Coffee is one of the most economically significant agricultural commodities globally, playing a critical role in the livelihoods of millions of farmers, traders, and processors, particularly in developing countries [1]. Indonesia, as a major coffee-producing nation, cultivates 3 primary species: Arabica (Coffea arabica), Robusta (Coffea canephora), and Liberica (Coffea liberica) [2]. Among these, Liberica coffee remains underutilized, despite its notable adaptability to climate variability and potential to support sustainable agroforestry systems [3]. Its limited market acceptance is primarily due to its perceived inferior cup quality, characterized by a flat and inconsistent flavor profile [4]. However, given its ecological resilience and genetic diversity, Liberica represents a promising candidate for value-added innovation through advanced processing technologies.

One such innovation is the application of nanotechnology, which has gained increasing attention in the food and beverage sector for its ability to enhance the functional, sensory, and bioactive properties of various products. In coffee, nano-sized particles offer improved solubility, extraction efficiency, and potentially enhanced bioavailability of antioxidants and other beneficial compounds [5]. Previous studies have demonstrated the feasibility of producing nano-coffee using mechanical size reduction techniques such as ball milling, often coupled with ultrasonication, to achieve particle sizes within the nanometer range [6,7]. However, most research has focused on Arabica coffee or spent coffee grounds, with limited exploration into the nanoscale processing of Liberica coffee beans.

In parallel, fermentation-based pre-treatment of green coffee beans, particularly using specific microbial strains such as lactic yeast, has been reported to induce biochemical and structural transformations that influence downstream processing performance and end-product quality. Fermentation can modify cell wall composition, reduce mucilage viscosity, and trigger enzymatic degradation, which may influence milling efficiency, surface morphology, and the stability of bioactive compounds [8]. Despite its potential, the impact of such fermentation processes on the physicochemical and microstructural characteristics of nano-coffee, particularly for Liberica species, remains insufficiently understood.

Furthermore, particle size distribution is pivotal in defining key product attributes in coffee, including brew strength, mouthfeel, and antioxidant activity [9-11]. Optimizing milling parameters, such as duration and rotational speed, in combination with pre-processing treatments like fermentation, is essential to achieving target nano-range particles while preserving desirable functional properties. Therefore, the present study aims to investigate the influence of green bean fermentation using lactic yeast and ball milling duration on the physicochemical, microstructural, and functional characteristics of Liberica nano-coffee. By evaluating non-fermented and fermented beans milled at varying durations, this research provides novel insights into optimizing nano-coffee production from an underutilized coffee species. The findings are expected to contribute to developing science-based strategies for enhancing the value chain of Liberica coffee, supporting both product innovation and agroecological sustainability.

Materials and methods

Coffee sample and preparation

Liberica green coffee beans were collected from local farmers and processors in the Ijen Geopark region, Indonesia. The beans underwent a 24-hour fermentation process in an 8-liter fermentation chamber using lactic acid bacteria (LAB) and yeast, following a method described by Sunarharum [12]. Before fermentation, the chamber and lid were sterilized by washing with detergent and rinsing with hot water. Following sterilization, X-ray-irradiated Liberica green coffee beans (at a dose of 7.2 kGy) were combined with the fermentation media in a ratio of 1:3 (w/v), with each batch containing 1.5 kg of beans and 4.5 L of lactic acid and yeast-enriched fermentation media. The beans were submerged and fermented under anaerobic conditions for 24 h. After fermentation, the beans were washed to remove residual media and impurities, then sun-dried in a greenhouse for 5 days until the moisture content was reduced to below 12.5%, following the Indonesian National Standard (SNI) 2907-2008. The dried beans were manually sorted and roasted to a medium level at temperatures ranging from 210 to 220 °C for approximately 15 min. Roasted beans were then ground using a coffee grinder to a fine particle size.

The ground coffee was processed using a ball mill (Retsch PM 200) at a rotational speed of 500 rpm for varying durations (60, 120 and 180 min) to obtain nano-sized coffee particles. The milling was performed using 0.01 mm-diameter balls at 1:3 (w/w) sample-to-ball ratio.

Physicochemical characterization

Color analysis

The color of nano-coffee was analyzed using a modified method based on the procedure described by Kulapichitr [13]. Color parameters, including lightness (L*), redness (a*), and yellowness (b*), were determined according to the CIELAB color system (C.I.E., 1986) with a tristimulus colorimeter (CR-10; Minolta Co., Osaka, Japan). Measurements were performed at 3 different locations on the sample surface.

pH

The pH of the nano-coffee samples was measured using a pH meter (HANNA Instrument, HI-2002-02 edge pH) calibrated according to AOAC guidelines.

Antioxidant potentials IC_{50} (DPPH radical-scavenging activity)

The antioxidant activity of nano-coffee samples was assessed using the DPPH assay, following a modified protocol based on Schouten *et al.* [15]. A series of sample concentrations (50, 25, 20, 15 and 10 mg/mL) was prepared. Each assay solution consisted of 0.5 mL ethanolic DPPH solution (250 µM), 1 mL acetate buffer (100 mM, pH 5.5), 1 mL ethanol, and 10 µL of the sample at the designated concentration. The mixture was incubated in the dark at room temperature for 10 min, after which absorbance was measured at 517 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The decrease in absorbance, indicative of radical scavenging activity, was monitored spectrophotometrically. All measurements were conducted in triplicate, and the results were reported as mean values. The percentage of DPPH inhibition was calculated using the following equation:

$$\%\ \text{inhibition of DPPH} = \frac{(A0-AS)-(TA-AS)}{(A0-AS)} \times 100$$

where A0 represents the absorbance of the DPPH control solution, AS corresponds to the absorbance of methanol (blank), and TA denotes the absorbance of the tested sample. Antioxidant capacity was expressed as IC_{50}, defined as the extract concentration required to inhibit 50% DPPH radicals. IC_{50} values were determined from the linear regression of extract concentration versus inhibition percentage (IA%). Lower IC_{50} indicates greater antioxidant activity.

Particle size analyzer

The nano-coffee samples were sonicated using an ultrasonicator (POWER, Sonic405). During sonication, the samples were mixed with distilled water at a ratio of 1:60 (w/v) and processed for 45 min to ensure uniform dispersion. The particle size distribution was then analyzed using a Malvern Zetasizer Nano Series analyzer, operating at 68 ± 6.8 mV, following a modified method adapted from Buniyamin *et al.* [16].

Microstructure and physical properties

Field emission scanning electron microscope (FESEM)

Surface morphology of the nano-coffee samples was characterized using FESEM (JEOL JSM-7600F) at an accelerating voltage of 5 kV, following a modified protocol adapted from Buniyamin *et al.* [17]. Before analysis, the samples were sputter-coated with a gold alloy using a JEOL JFC-1600 Auto Fine Coater.

Fourier transform infrared (FTIR) spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was conducted to characterize the functional groups present in the nano-coffee samples. The analysis was performed using a Perkin Elmer Spectrum 400 spectrometer equipped with an attenuated total reflection (ATR), operating within a spectral range of 400 - 4,000 cm^{-1}. The procedure followed a modified protocol adapted from Buniyamin *et al.* [17].

Statistical analysis

Statistical analysis was conducted using 1-way analysis of variance (ANOVA) with significance set at $p \leq 0.05$, using Minitab 17.0 statistical software. When significant differences were found ($p < 0.05$), Fisher's least significant difference (LSD) test at $\alpha = 0.05$ was applied to compare.

Results and discussion

Physicochemical analysis of Liberica coffee ground

The physicochemical properties of fermented (F) and non-fermented (NF) nano-coffee and the average across different milling durations (0, 60, 120 and 180 min) are given in **Table 1**.

Table 1 Physicochemical properties of the fermented (F) and non-fermented (NF) nano-coffee in various milling times.

Parameters	pH	L*	a*	b*	IC_{50} (ppm)	Particle size (nm)**
NF0	5.50 ± 0.00	37.53 ± 0.07^{b}	5.13 ± 0.15	9.71 ± 0.19	136.19 ± 1.09^{b}	559.1 ± 96.7^{a}
NF60	5.47 ± 0.03	37.58 ± 0.07^{b}	5.13 ± 0.13	9.75 ± 0.15	132.69 ± 0.83^{c}	358.13 ± 16.1^{b}
NF120	5.45 ± 0.05	37.66 ± 0.09^{b}	5.11 ± 0.14	9.65 ± 0.15	137.51 ± 0.45^{ab}	473.4 ± 68.7^{ab}
NF180	5.45 ± 0.05	38.29 ± 0.16^{a}	5.14 ± 0.13	9.72 ± 0.15	138.27 ± 0.15^{a}	388.3 ± 70.2^{ab}
***p*-value**	0.384	0.000	0.991	0.909	0.000	0.029
F-value	1.16	35.61	0.03	0.18	35.02	5.11
F0	5.10 ± 0.10	36.56 ± 0.14^{b}	4.51 ± 0.08	8.70 ± 0.15	182.57 ± 0.31^{b}	290.3 ± 13.85^{c}
F60	5.13 ± 0.06	36.57 ± 0.14^{b}	4.50 ± 0.13	8.71 ± 0.12	180.54 ± 0.26^{c}	318.57 ± 5^{bc}
F120	5.07 ± 0.12	36.50 ± 0.26^{b}	4.49 ± 0.09	8.63 ± 0.10	183.63 ± 0.45^{ab}	376.6 ± 24.3^{a}
F180	5.10 ± 0.10	37.12 ± 0.12^{a}	4.51 ± 0.06	8.61 ± 0.04	183.82 ± 1.01^{a}	328.5 ± 17.6^{bc}
***p*-value**	0.864	0.007	0.996	0.654	0.000	0.002
F-value	0.24	8.51	0.02	0.556	19.60	13.89
NF Average* **	**5.47 ± 0.04^{a}**	**37.77 ± 0.33^{a}**	**5.13 ± 0.12 ^{a}**	**9.71 ± 0.14^{a}**	**136.16 ± 2,32^{b}**	**444.7 ± 101 ^{a}**
F Average* **	**5.10 ± 0.09^{b}**	**36.68 ± 0.30^{b}**	**4.50 ± 0.08^{b}**	**8.66 ± 0.10^{b}**	**182.64 ± 1.45^{a}**	**328.5 ± 35.5 ^{b}**
***p*-value**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**
F-value	**152.34**	**349.50**	**169.72**	**350.73**	**29,776.22**	**14.14**

- NF, non-fermented nano-coffee; F, fermented nano-coffee; 0, 60, 120 and 180 represented milling time in min.
- Data represented (based on the dry basis) are the means ± SD (n = 3).
- Data represented on NF Average*** and F Average*** (based on the dry basis) are the means of fermentation or non-fermentation nano-coffee over the duration of milling ± SD (n = 12).
- Different superscripts in the same row indicate the significant differences ($p < 0.05$)
- L*, value measuring black (0)/white (100); a*, value measuring green (−)/red (+); b*, value measuring blue (−)/ yellow (+)
- Particle size (nm)** shows the particle size of the majority intensity.

The pH of the nano-coffee samples was significantly influenced by the fermentation treatment, but not by the milling duration. Fermented nano-coffee consistently exhibited lower pH values (5.07 - 5.13) compared to non-fermented nano-coffee (5.45 - 5.50) across all milling times, indicating a statistically significant effect of microbial activity. In contrast, the variation in milling duration had a negligible impact on pH. This consistent reduction in pH in fermented samples is attributed to the accumulation of organic acids, primarily lactic, acetic, and butyric acids, produced by yeast and lactic acid bacteria during fermentation. These acids contribute to releasing hydrogen ions (H^{+}), acidifying the coffee matrix [18]. Lower pH conditions can modify the solubility, structural conformation, and stability of phenolic compounds and antioxidants, potentially affecting their bioavailability and efficacy in health-promoting

applications. Furthermore, pH plays a critical role in determining the sensory attributes of coffee, particularly sourness and freshness, which are crucial for consumer acceptability in specialty beverage formulations and functional product development.

Colorimetric values were significantly influenced by both yeast-lactic acid fermentation and milling. The lightness parameter (L*) was higher in non-fermented nano-coffee samples across all time points, significantly increasing at 180 min (NF180). Conversely, fermented nano-coffee showed consistently lower L* values, indicating darker coloration. These differences may be due to pigment breakdown and the formation of brown melanoidins via Maillard reactions initiated during microbial fermentation [19]. The chromatic values a* and b* (indicative of red and yellow tones, respectively) were also significantly higher in non-fermented nano-coffee. At the same time, fermented coffee displayed a color shift towards green and blue tones. Previous studies have linked such changes to microbial degradation of polyphenolic pigments and modification of aromatic profiles [20,21]. Functionally, color changes impact product appeal and consumer perception and are often used as indirect indicators of biochemical transformations, especially in products marketed as fermented or naturally processed.

The antioxidant capacity, evaluated via IC_{50}, showed that non-fermented nano-coffee exhibited significantly stronger antioxidant activity (132.69 - 138.27 ppm) compared to fermented nano-coffee (180.54 - 183.82 ppm). Among non-fermented nano-coffee samples, 60 min of milling yielded the highest antioxidant activity (lowest IC_{50}), suggesting optimal preservation of bioactives under this condition. The observed decline in antioxidant capacity in fermented nano-coffee samples can be primarily attributed to microbial metabolism and enzymatic degradation of key phenolic compounds, particularly chlorogenic acids, which are known to contribute significantly to the antioxidant potential of coffee. During the fermentation process, lactic acid bacteria and yeast produce hydrolytic enzymes such as polyphenol oxidases and esterases, which catalyze the breakdown of chlorogenic acids into smaller, less bioactive derivatives. This enzymatic activity, coupled with prolonged exposure to oxidative conditions during fermentation, likely leads to the partial loss or transformation of these antioxidants. Moreover, studies have shown that microbial metabolism can redirect phenolic substrates toward pathways that generate organic acids or volatile compounds, reducing the concentration of intact antioxidant compounds [22,23].

Additionally, prolonged dry milling may promote thermal and oxidative degradation of thermolabile compounds, further reducing antioxidant levels [24,25]. A previous study reported similar trends in fermented fruit-based systems [26]. Several strategies may be considered in future studies to mitigate this antioxidant degradation. Optimizing fermentation parameters, such as temperature, duration, and sugar concentration, can enhance antioxidant activity, particularly when using selected microbial starters like *Lactiplantibacillus plantarum* or yeast–lactic acid bacteria cocktails [27]. In addition, proper sample preparation, including storage at –20 °C under a nitrogen atmosphere before milling, can be implemented to minimize the degradation of volatile compounds, chlorogenic acids, and caffeic acid, all of which contribute to the antioxidant properties of coffee [28]. Given the importance of antioxidant activity in health-related claims, such as anti-aging and anti-inflammatory, these findings suggest that non-fermented coffee has greater potential for use in functional food and nutraceutical applications where high antioxidant content is desired.

Particle size analysis showed a significant effect of both fermentation and milling. In non-fermented nano-coffee, particle size was reduced from 559.1 nm (NF0) to 358.13 nm (NF60), followed by moderate variation at longer durations. Fermented nano-coffee samples generally showed smaller particle sizes (290.3 - 376.6 nm), likely due to altered microstructure from fermentation. However, it is notable that only approximately 10% of the particles reached under 100 nm dimensions, indicating that the current dry milling method was insufficient for achieving uniformly nanoscale powders. Achieving smaller particle sizes is critical for increasing surface area, enhancing solubility, and facilitating bioactive delivery in pharmaceutical and cosmeceutical systems. These nanoscale features can improve absorption across biological membranes and promote targeted delivery of health-promoting compounds. Interestingly, particle size in fermented nano-coffee showed slight increases after 120 and 180 min of milling, which may be attributed to

agglomeration phenomena. Due to residual microbial exudates and altered surface hydrophobicity, fermented particles tend to exhibit adhesive surfaces that facilitate bonding and aggregation under high-energy impact conditions [29]. These effects complicate size reduction and reduce process efficiency. In contrast, non-fermented nano-coffee samples followed a more linear particle size reduction trend, suggesting that the absence of microbial modifications leads to more predictable comminution behavior. Agglomeration limits nano-size production and may affect powder flowability and dispersion, which are important considerations in capsule filling, topical formulations, or beverage reconstitution.

Collectively, these findings emphasize that fermentation significantly alters the physicochemical profile of Liberica nano-coffee by reducing pH, darkening color, and decreasing antioxidant activity. Milling primarily influenced lightness (L*), antioxidant capacity, and particle size, with more pronounced effects in non-fermented coffee. The interplay between fermentation and milling determines final particle structure and bioactive integrity, which in turn dictates potential end-use applications. From an application perspective, non-fermented nano-coffee exhibited higher antioxidant capacity, brighter appearance, and more efficient particle size reduction, positioning it as a more suitable candidate for functional product development where clarity, antioxidant load, and dispersion are key, such as in cosmeceutical serums, nano-capsules, or functional instant beverages.

While the findings offer valuable insights, several aspects remain open for further exploration to enhance the scalability and practical application of nano-coffee. These include scalability constraints, a relatively low proportion of particles achieving true nanoscale (< 100 nm), and the absence of sensory and organoleptic evaluations to assess consumer acceptability. Although complete nanoscale dispersion was not achieved, this study provides a valuable baseline for optimizing nano-coffee production. Future studies should explore advanced particle size reduction techniques such as wet media milling, high-pressure homogenization, or cryo-milling to increase nano-yield. Additionally, optimizing microbial consortia and fermentation time could help balance bioactive preservation and biochemical transformation. Investigating particle surface chemistry and colloidal behavior is crucial to enhancing stability and commercial feasibility in health-oriented applications.

Microstructure and physical characterization

Field emission scanning electron microscope (FESEM)

The microstructural micrographs of the fermented and non-fermented coffee beans across various milling durations are shown in **Figure 1**.

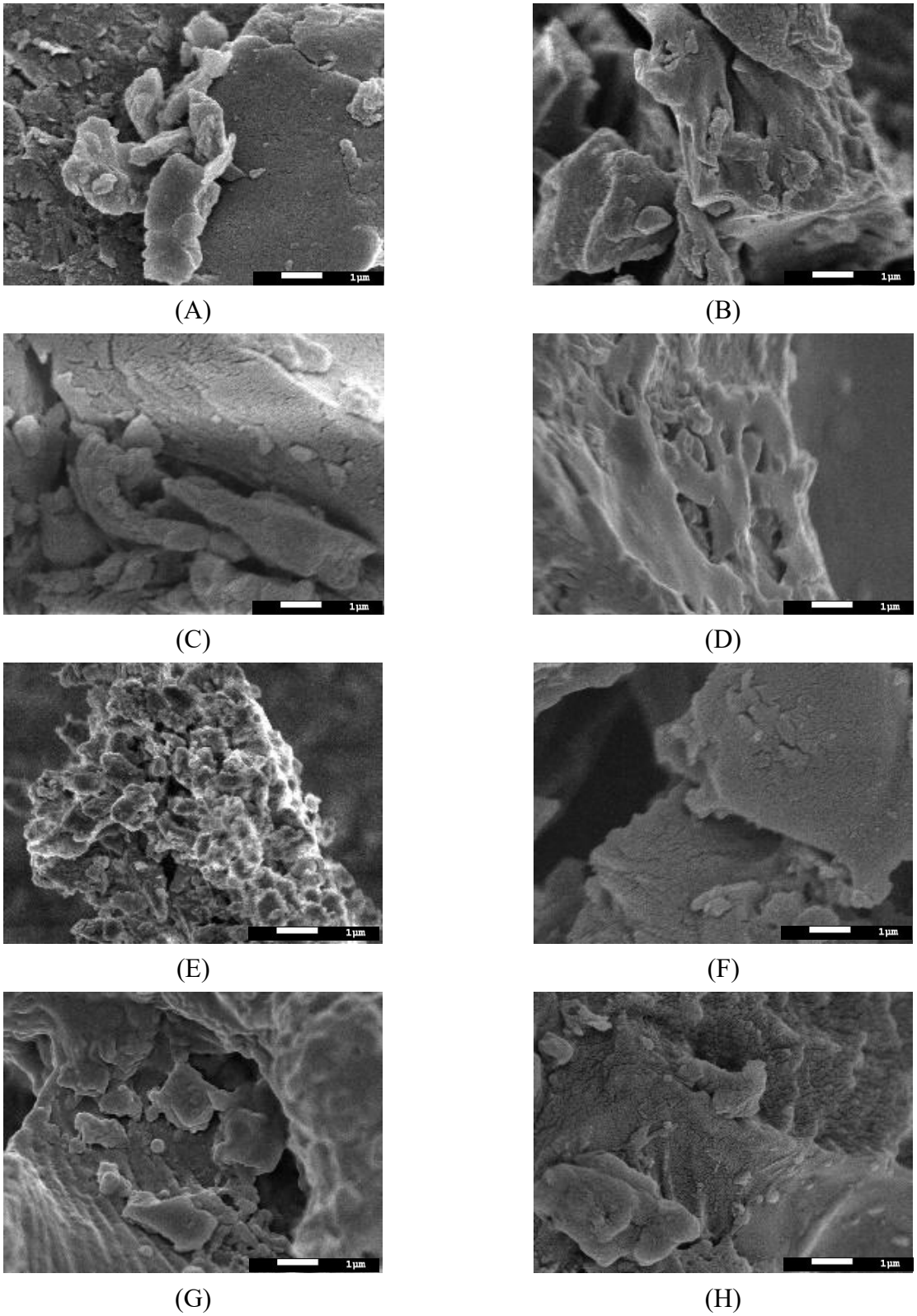


Figure 1 High-resolution FESEM micrographs (15,000× magnification) of *Coffea liberica* powders. (A-D) Non-fermented nano-coffee powders milled for (A) 0 min, (B) 60 min, (C) 120 min, and (D) 180 min. (E) - (H) Fermented nano-coffee powders milled for (E) 0 min, (F) 60 min, (G) 120 min, and (H) 180 min.

Field Emission Scanning Electron Microscopy (FESEM) at 15,000× magnification revealed significant microstructural transformations in *Coffea liberica* subjected to different pre-treatments and durations of ball milling. The comparison between non-fermented and yeast–lactic acid bacteria (LAB) fermented coffee

beans demonstrated distinct morphological patterns, closely linked to the effects of microbial activity and mechanical force on the coffee bean matrix.

Figures 1(A) - 1(D) shows non-fermented coffee samples ball milled for 0, 60, 120, and 180 min, respectively. The micrographs at 0 min (prior to milling) revealed large, compact, and angular particles with well-defined edges and smooth surfaces. These features are typical of an intact and mechanically resilient plant cell wall composed primarily of cellulose, hemicellulose, lignin, and associated proteins. As milling time increased, progressive breakdown of these structures was observed. At 60 min, surface erosion and edge blunting became apparent, signaling the initial disruption of structural integrity. At 120 min, the particles exhibited extensive fragmentation, with layered and irregular morphology. By 180 min, particle structures had visibly collapsed, showing smoother surfaces, reduced porosity, and significant compaction, consistent with the cumulative effects of mechanical stress and energy input. These findings suggest that the non-fermented matrix required prolonged mechanical input for effective comminution due to its rigid, undegraded structure. Conversely, **Figures 1(E) - 1(H)** depicts yeast-lactic acid bacteria (LAB) fermented coffee samples milled for 0, 60, 120, and 180 min, respectively. Fermented samples showed notably different microstructural features even prior to milling. At 0 min, the surfaces were already porous, rough, and loosely packed. This was attributed to microbial enzymatic activity during fermentation, particularly from yeasts and LAB, which produce cellulases, pectinases, and proteases that degrade cell wall components such as cellulose and pectin. These enzymatic actions weaken the structural matrix, leading to surface cracks, increased porosity, and irregular pore distributions. By 60 min of milling, fermented samples exhibited significantly greater fragmentation compared to non-fermented ones. The micrographs at 120 and 180 min revealed highly amorphous, collapsed structures with smoother yet more disrupted surfaces, suggesting deformation and reduced mechanical resistance. The particles appeared more disordered, with micro-fractures and signs of surface melting or fusion, likely due to enhanced susceptibility to the shearing and compressive forces of the milling media.

These morphological changes highlight the critical role of microbial fermentation as an effective pre-treatment that facilitates particle disintegration during ball milling. The weakened cell matrix in fermented coffee allowed for more efficient energy transfer, leading to faster and finer nanoscale fragmentation. Notably, the increased porosity and surface area observed in fermented samples could enhance their physicochemical performance, such as solubility, wettability, and extractability of key bioactive compounds. Fermentation has been reported to induce microstructural breakdown, increase porosity, and create irregular pore distributions due to enzymatic and acidic degradation of cellular architecture [30,31]. These alterations reduce density and increase surface roughness, influencing how coffee particles interact with water and heat during roasting and brewing [32]. Structural changes affect the thermal and moisture absorption behavior of coffee beans, impacting flavor development and aroma release [33,34].

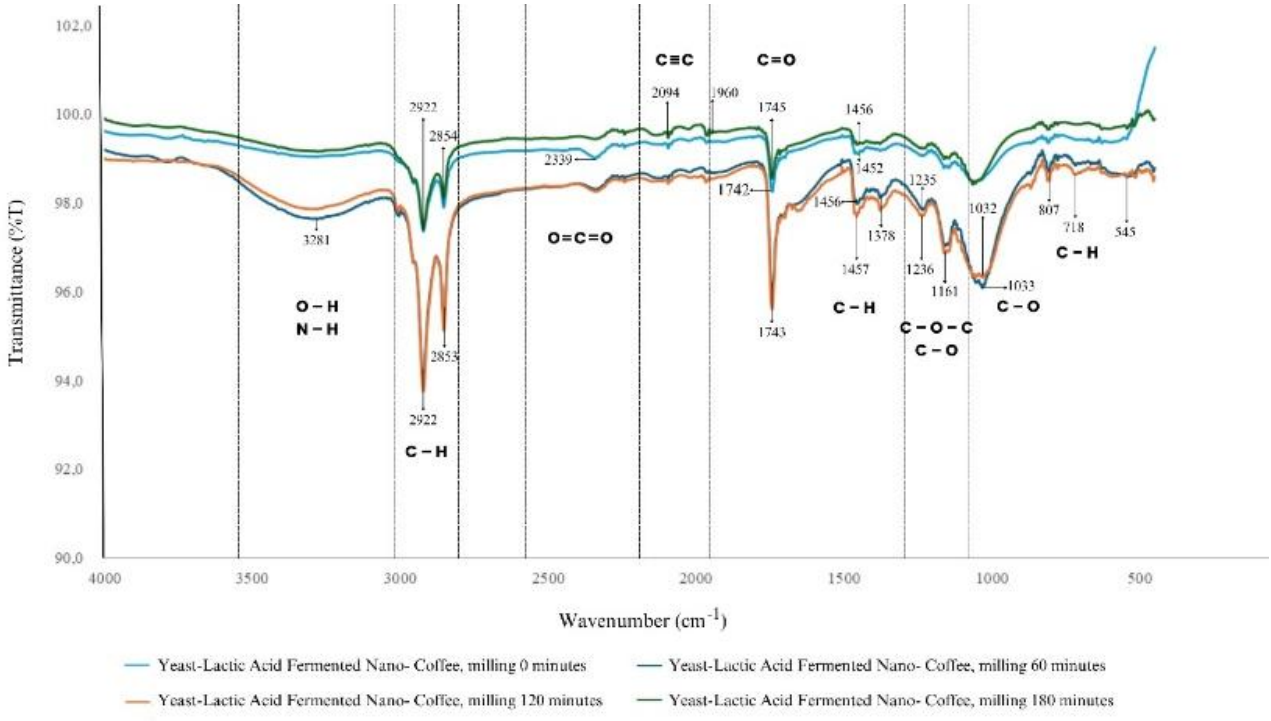
Integrating microbial fermentation and mechanical milling presents a synergistic strategy for producing high-performance nano-coffee powders. Fermentation acts as a biological softening process, reducing structural rigidity and improving the milling efficiency. In contrast, ball milling transforms these preconditioned structures into ultrafine particles with enhanced functionality. The induced structural collapse, enhanced porosity, and amorphization not only promote nanoscale fragmentation but also hold substantial promise for the development of functional foods and advanced delivery systems in pharmaceutical and cosmeceutical fields. This convergence of biotechnological pre-treatment and mechanical processing addresses the increasing demand from both consumers and industries for bioactive-enriched products with improved bioavailability. The observed rise in porosity and surface area in fermented samples may facilitate greater solubility and dispersibility in aqueous environments, enhancing the release and absorption of key compounds such as chlorogenic acids, trigonelline, and caffeine. Besides, caffeine-loaded nano/micro-carriers facilitate controlled delivery in functional food products [35]. Comparable nanostructuring techniques have also been applied to other plant-based food matrices, including fruits, cereals, and roots, further validating the relevance of this

approach beyond coffee. Studies involving quinoa, water chestnut, lotus stem, and horse chestnut have reported that ball milling can generate nanoparticles ranging from 30 to 850 nm, depending on the processing conditions [36]. These structural alterations disrupt starch granules and cell wall polymers, enhancing digestibility and nutrient accessibility [37,38]. These microstructural modifications are essential for formulating functional nano-coffee powders or ready-to-drink beverages for targeted antioxidant or neurostimulant effects.

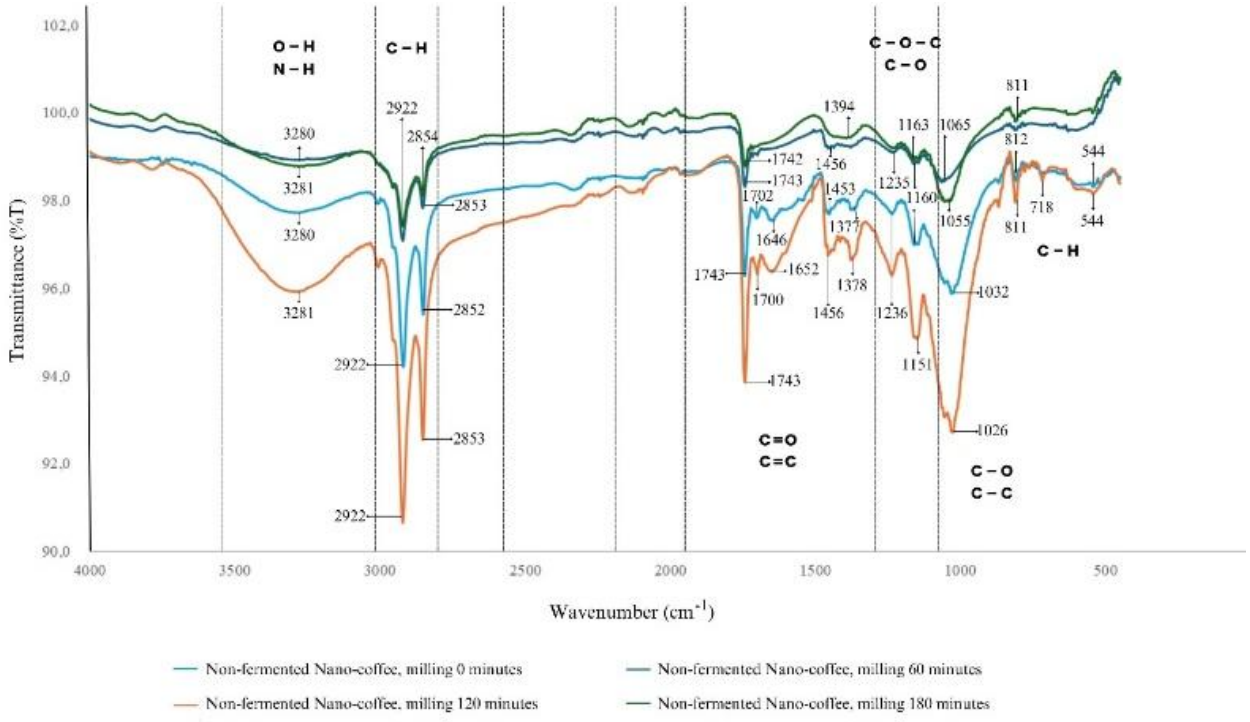
Beyond the food sector, the altered microstructure of nano-coffee particles presents promising applications. In pharmaceutical contexts, nano-sizing plant-derived bioactives enhances dissolution rates, enables controlled release [39], and improves mucosal permeability [40]. The increased surface-to-volume ratio supports efficient encapsulation and stabilization of poorly bioavailable bioactives in drug delivery systems [41]. In cosmeceuticals, fermented nano-coffee extracts, rich in phenolic compounds and exhibiting heightened surface activity, can be utilized in skincare formulations as natural antioxidants, anti-inflammatory agents, or UV-protective ingredients [42,43]. The structural transformations induced by fermentation and milling also align with sustainable processing principles. Fermentation weakens the cellular matrix, reducing the energy demands of subsequent mechanical treatments and lowering the overall carbon footprint of nano-processing. This synergistic approach offers a sustainable strategy for transforming underutilized coffee species such as *Coffea liberica* into high-value, multifunctional nano-ingredients.

FTIR spectroscopy

FTIR spectrometric analysis in the 4,000 - 400 cm^{-1} range was used to identify functional groups. The FTIR spectra of the fermented and non-fermented coffee grinds across various milling durations are shown in **Figure 2**.



(a)



(b)

Figure 2 FTIR spectra of both non-fermented nano-coffee (a) and yeast-lactic acid fermented nano-coffee (b) across varying milling durations.

Generally, the FTIR spectra of yeast-lactic acid fermented coffee and non-fermented coffee samples across varying milling durations (**Figure 2**) reveal chemical modifications resulting from the individual or combined effects of fermentation and mechanical processing. In the 3,000 - 2,800 cm^{-1} region, non-fermented coffee consistently exhibited well-defined aliphatic C-H stretching bands, indicative of intact lipid structures typically associated with long-chain hydrocarbons. In contrast, yeast-lactic acid fermented coffee showed a marked reduction in intensity in this region, suggesting substantial lipid degradation. This degradation is likely attributed to the activity of lipolytic enzymes produced during microbial fermentation, which catalyze the hydrolysis of complex lipids into free fatty acids and esters. Lactic acid bacteria and yeasts are known to secrete such enzymes, facilitating lipid breakdown during fermentation. Supporting this, metagenomic research identified yeast strains belonging to the *Lichtheimia* genus in Liberica coffee cherries and enrichment media, which are capable of converting lignocellulosic material into fermentable sugars and lipids, contributing to enzymatic lipid catabolism [44].

A significant distinction was observed between non-fermented and yeast-lactic acid fermented coffee in the 1,700 - 1,600 cm^{-1} spectral region, which corresponds primarily to carbonyl (C=O) stretching vibrations. The non-fermented coffee exhibited relatively weaker carbonyl absorption, whereas the yeast-lactic acid fermented coffee demonstrated enhanced peak intensity, indicating a greater accumulation of carbonyl-containing compounds. These included organic acids such as lactic and acetic acid, as well as aldehydes, by-products of microbial metabolism during fermentation. This increase is consistent with microbial pathways reported for lactic acid bacteria, wherein sugars and other substrates are metabolized into organic acids [45], thereby modifying the chemical character of the coffee matrix.

Substantial structural differences between non-fermented and yeast-lactic acid fermented coffee samples were apparent in the 1,500 - 1,000 cm^{-1} region, associated with vibrational modes of polyphenols, carbohydrates, and proteins. Non-fermented coffee maintained a relatively stable spectral profile, preserving native constituents such as chlorogenic acids,

polysaccharides, and proteinaceous compounds. Conversely, yeast-lactic acid fermented coffee exhibited altered and attenuated absorption bands in this region, indicative of hydrolysis and partial degradation of macromolecular structures into smaller molecular fragments. These observations highlight the biochemical transformation induced by fermentation, affecting coffee constituents' structural integrity and functional profile.

Milling duration further influenced these chemical changes, as reflected in spectral shifts. Longer milling times intensified molecular disruption due to increased mechanical energy, facilitating the release and transformation of lipids, polyphenols, and organic acids [46,47]. In fermented samples, where microbial activity had already weakened intermolecular bonds [22], mechanical stress amplified degradation, especially in the 1,500 - 1,000 cm^{-1} region, promoting the conversion of complex molecules into simpler, potentially less bioactive forms.

These FTIR observations align with antioxidant activity trends measured via DPPH assays. Non-fermented coffee consistently exhibited stronger antioxidant capacity than its yeast-lactic acid fermented counterpart. This difference is attributed to the greater preservation of structurally complex polyphenols in non-fermented coffee, which are known for their superior free radical-scavenging activity. In contrast, fermentation-induced degradation of polyphenols in yeast-lactic acid fermented coffee diminishes their antioxidant efficacy. Milling duration also plays a critical role: short-duration (60 min) milling improves antioxidant release by increasing surface area and disrupting the cell matrix, thereby enhancing compound accessibility. Prolonged milling (120 - 180 min) in conjunction with yeast-lactic acid fermentation has induced mechanical and thermal degradation of polyphenolic compounds, as evidenced by diminished absorbance in the 1,500 - 1,000 cm^{-1} FTIR region. These results align with findings from rice and fruit studies, where extended milling durations consistently decreased polyphenol content and antioxidant activity [48,49]. Furthermore, microbial transformation of polyphenols during fermentation has been shown to alter their structure and function, supporting the observed reduction in antioxidant efficacy [50,51].

Conclusions

This study demonstrates that both fermentation and milling duration play critical roles in determining the physicochemical and microstructural properties of Liberica nano-coffee. Lactic yeast fermentation significantly altered coffee bean matrix structure, resulting in lower pH, darker color (L*), reduced antioxidant capacity (higher IC_{50}), and more heterogeneous particle behavior during milling. The microstructural analysis revealed increased porosity and surface roughness in fermented coffee, accompanied by biochemical modifications such as lipid degradation and polyphenol breakdown, confirmed by FTIR.

Milling duration affected particle size reduction and lightness, with non-fermented (NF) coffee achieving smaller particle sizes and better preservation of antioxidant activity, particularly at 60 min. In contrast, yeast-lactic acid fermented (F) coffee exhibited signs of agglomeration at prolonged milling times.

While fermentation introduces unique structural transformations beneficial for specific functional applications, it compromises antioxidant retention and is suitable for nanoscale grinding. Non-fermented Liberica coffee, therefore, presents more favorable characteristics for nano-coffee applications requiring fine particle size, lighter color, and higher bioactive compound preservation.

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Declaration of Generative AI in Scientific Writing

The authors note the limited use of generative AI tools (Grammarly and OpenAI's ChatGPT) in the preparation of this manuscript, restricted to language refinement and grammar checking. AI tools did not contribute to the development of ideas, data analysis, or interpretation. The authors accept full responsibility for the originality, accuracy, and conclusions of this manuscript.

CRediT Author Statement

Annisa Aurora Kartika: Validation, Visualization, and Writing – original draft. **Wenny Bekti Sunarharum**: Conceptualization, Methodology, Data curation, Supervision, Validation, Funding acquisition and Writing – review. **Noor Asnida Asli**: Conceptualization, Methodology, Supervision, Funding acquisition and Writing – review. **Eddie Ti Tjih Tan**: Supervision, Funding acquisition, and Writing – review. **Mohamad Rusop**: Methodology, Project administration, Resources. **Tunjung Mahatmanto**: Data curation, Supervision and Validation. **Yuniar Ponco Prananto**: Data curation, Supervision and Validation. **Durratun Nasihah Mohd Shuhairi**: Project administration, Formal analysis. **Nur Syazwani Abdul Malek**: Project administration, Formal analysis.

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