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Impact of Postharvest Processing on the Metabolite Profile of Arabica Green Coffee Beans

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Abstract

Postharvest techniques greatly affect the composition and quality of green coffee beans by altering some primary and secondary metabolites. This study aimed to assess the impact of three postharvest processing techniques (natural, honey, and fully washed) on the phenolic compounds, antioxidant activity, caffeine, protein, and color in Arabica green coffee beans from Solok Regency, Indonesia. Spectrophotometric and colorimetric analyses were performed. Data were analyzed using Duncan's multiple range test, multivariate PCA, and heatmap clustering. Results showed that honey process had the highest polyphenol content of 3918.61 ± 349.85 mg GAE/g, whereas beans that were fully washed had the highest antioxidant activity (1.41 ± 0.21%) and caffeine content (1.41 ± 0.21%). Natural processing yielded the highest protein content (24.26 ± 0.40%). There were significant differences in the hue angle that were related to some of the biochemical differences among the treatments. This research emphasizes the importance of postharvest processing in defining the metabolite profile and quality features of green coffee beans. These findings can assist in formulating the processing steps to be used in refining coffee and maximizing its health-related advantages.

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1. Introduction

Coffee has unparalleled economic value worldwide, and Arabica coffee (Coffea arabica) accounts for nearly 60% of its production because of its rich aroma and taste (ICO 2022). In Indonesia, Arabica coffee is grown in highland areas and is crucial to the income of smallholder farmers, as its economic value lies in the Simalungun Highlands of North Sumatera (Saragih and Situmorang, 2018). In addition to genetic and environmental factors, postharvest processing steps play a crucial role in determining the quality of green coffee beans, which directly influences the roasting outcomes (Joët et al., 2010; De Bruyn et al., 2017).

Three post-harvest coffee-processing methods are commonly practiced by farmers: natural (dry), honey (semi-washed), and fully washed. Each of these methods has a distinctive set of fermentation and drying steps, which greatly affect the creation and retention of vital chemical components, such as caffeine, polyphenols, proteins, and antioxidants (Worku et al., 2018; Kowalska et al., 2023; Cortés-

Macías et al., 2022). In addition to being crucial for taste development, these elements also support the health value and economic price of coffee (Daglia et al., 2000).

A research gap remains concerning coffee varieties in Indonesia and how different post-harvest processing methods alter the metabolite profiles of green Arabica beans. Joët et al. (2010) and Toci & Farah (2008) focused on roasted coffee beans and sensory attributes without linking the biochemical composition of green coffee beans to the roasting process. This gap in research creates a gap in scientific evidence for farmers and processors to choose a processing technique based on chemical quality rather than on tradition or market trends.

Using natural, honey, and fully washed methods, this study aimed to analyze and compare the metabolite profiles of Arabica green beans to fill this research gap. This study aimed to assess the impact of three postharvest processing techniques (natural, honey, and fully washed) on the phenolic compounds, antioxidant activity, caffeine content, protein levels, and physical attributes of Arabica green coffee beans from Solok Regency, Indonesia. Principal Component Analysis and heatmap clustering, both multivariate analysis techniques, were used to evaluate the polyphenol profiles for each processing method to identify and differentiate the patterns.

This study offers a novel approach by shifting the focus from roasted coffee quality to the biochemical characteristics of green coffee beans at the pre-roasting stage, which are currently underrepresented in the literature. By combining metabolite profiling with objective color analysis and multivariate statistics, this study provides a scientific foundation for early stage quality evaluation. These findings are expected to assist farmers, processors, and cooperatives in making informed decisions regarding post-harvest techniques, ultimately improving product consistency, traceability, and competitiveness in both local and international markets.

2. Material and Methods

2.1 Simple Preparation

Arabica coffee cherries were harvested and processed in the Solok Regency using three post-harvest methods: natural, honey (semi-washed), and fully washed. Green bean samples from each post-harvest method were collected in triplicates. All samples were dried, sorted, and stored in airtight containers at room temperature prior to analysis.

2.2 Parameter Analysis

2.2.1 Colour Measurement

The color of the green bean samples was measured using a spectrocolorimeter. Each sample from the three post-harvest methods was measured three times. The color values (L*, a *, b *) and hue angle (h*) were recorded. The hue angle was calculated using the following equation:

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$$h = tan^{-1}\frac{b^*}{a^*} \quad (1)$$

2.2.2 Total Polyphenol Content (based on ISO 14502-1)

The green bean extract (10 mg) was diluted with distilled water to a concentration of 1000 mg/L. One milliliter of this solution was mixed with 1 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3, vortexed, and incubated in the dark for 1 h. The absorbance was measured at 765 nm using UV-Vis spectrophotometry. Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalents per gram (mg GAE/g).

2.2.3 Caffeine Content (modified from Rehman and Ashraf, 2017)

Two grams of green bean sample was boiled in 100 mL of distilled water for 5 min. The filtrate was added with 2 g $CaCO_3$ and reheated, followed by three sequential extractions using 15 mL of chloroform. The lower organic layer was collected and evaporated using a rotary evaporator. The residue was diluted to 100 mL with chloroform, and the caffeine content was determined using a UV-Vis spectrophotometer at 275 nm. Caffeine content was calculated using the following equation:

$$KF = \frac{M \times V \times FP}{w} \quad (2)$$

$$\%Kafein = \frac{KF}{1000\,mg} \quad (3)$$

Where: M = measured concentration (mg/L, V = total extract volume (mL), FP = dilution factor, w = sample weight (g)

2.2.4 Protein Content

The protein content was determined using the Kjeldahl method according to ISO 5983-1:1997 (ISO, 1997). Green bean samples (0.5 g) were digested with 15 mL of concentrated sulfuric acid and 1 g of a selenium catalyst mixture using the Kjeldahl method. After 3 hours of digestion until the solution became clear, it was cooled and diluted to 100 mL. Ten milliliters of the digest was distilled with 30 mL of 50% NaOH solution, and the released ammonia was trapped in a 3% boric acid solution containing mixed indicators. The distillate was titrated with 0.02 N HCl until the endpoint color changed from green to reddish-purple.

2.2.5 Antioxidant Activity (modified from Huang et al., 2005)

Ten milligrams of Green bean sample were extracted with 10 mL methanol to make a 1000 ppm solution, followed by vortexing and ultrasonication. Two milliliters of the extract were mixed with 1 mL of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm. The percentage of DPPH radical-scavenging activity was calculated as follows:

$$\%\ \text{Inhibition} = \frac{(Abscontrol - AbsSample)}{Abscontrol}\ x\ 100\% \quad (4)$$

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2.2.6 Preprocessing

Quantitative metabolite data were normalized and auto-scaled (mean-centered and divided by the standard deviation of each variable) to standardize the variance across features. All analyses were conducted using MetaboAnalyst 5.0 software.

Principal Component Analysis (PCA). PCA was performed on autoscaled data to reduce dimensionality and summarize the covariation among metabolites. This method projects samples onto orthogonal principal components that capture the maximum variance in the dataset, enabling the visualization of global patterns and potential group separation while mitigating noise. Score plots (PC1–PC2) were generated to visualize sample clustering, and loading plots were inspected to identify metabolites with high contributions to principal components. PCA computations were performed using MetaboAnalyst 5.0, using its default singular value decomposition (SVD) implementation.

2.2.7 Heatmaps and hierarchical Clustering

Heatmaps were created from the auto-scaled data using hierarchical clustering applied to both samples and metabolites to visualize the relative abundance patterns and co-variation of metabolites. Dendrograms were created using Ward's linkage and Euclidean distance, and each value is shown as z-scores to highlight features that were upregulated or downregulated across groups. This method identifies sample groups with similar metabolic profiles and metabolite modules with comparable behaviors. MetaboAnalyst 5.0 was used to create heat maps and clustering, applying the default color mapping and scaling to features (rows).

3. Results and Discussion

3.1 Color Variations in Green Coffe Beans Due to Postharvest Processing

The color properties of green coffee beans varied significantly depending on the post-harvest process (Table 1). The hue values ranged from 81.41 to 87.39, indicating that the beans fell within the yellowish to pale green spectrum on the color wheel. According to the standard hue angle classification, values between 60–120° represent yellow to green hues (Pathare et al., 2013), and the typical hue range of green coffee beans is between 70–90°, which corresponds to a pale yellowish-green appearance (Damayanti, Warsito, & Wulandari, 2021).

Table 1. Color characteristics of green coffee beans from different post-harvest processes.

Postharvest Processing	L* (Lightness)	a* (Red-Green)	b* (Yellow-Blue)	Hue Angle (°)	Duncan Group
Fully Washed	41.44 ± 0.67	0.51 ± 0.06	11.21 ± 0.24	87.39 ± 0.29	a
Natural	40.45 ± 0.30	1.94 ± 0.58	12.86 ± 0.36	81.41 ± 0.55	b
Honey	40.72 ± 0.82	1.14 ± 0.13	11.56 ± 0.93	84.35 ± 0.67	c

Note: The sam row with different superscripts means differ significantly(P<0.01)

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Fully washed beans exhibited the highest hue angle (87.39 ± 0.29), which was closest to the ideal greenish tone often associated with high-quality Arabica green beans. In contrast, the naturally processed beans showed the lowest hue value (81.41 ± 0.55), indicating a shift towards a more yellowish tint. Honey-processed beans presented intermediate hue values (84.35 ± 0.67), suggesting moderate pigment transformation during the mucilage fermentation and drying.

The L* values, which represent lightness, were relatively consistent across treatments, ranging from 40.45 to 41.44, indicating minor variations in brightness. However, the a* and b* values revealed subtle but significant differences in chromaticity. The a* component (red-green axis) was notably higher in natural-processed beans (1.94 ± 0.58), suggesting a shift toward redder tones, possibly due to increased enzymatic browning during sun-drying with intact mucilage (De Bruyn et al., 2017). Meanwhile, the b* values (yellow-blue axis) were highest in the natural process (12.86 ± 0.36), indicating an increase in yellowish coloration compared to the fully washed and honey processes.

Although visual differences may be negligible to the naked eye, statistical analysis (Duncan's multiple range test) confirmed significant differences ($p < 0.05$) among the treatments. This implies that postharvest methods can influence the colorimetric profile of green beans, which may serve as an early, non-destructive indicator of the processing type and potential quality classification.

3.2 Polyphenol Content in Green Coffe Bean Under Different Poshharvest Process

Polyphenols are secondary metabolites with antioxidant properties and potential health benefits. In coffee, they contribute not only to bioactivity but also to flavor precursors and browning reactions during roasting (Clifford, 1999; Cruz et al., 2018). Among these, chlorogenic acids are the most abundant in green beans and serve as strong radical scavengers (Farah & Donangelo, 2006).

The polyphenol content of green beans is strongly affected by postharvest processing methods (Table 2). Honey-processed beans had the highest polyphenol concentration at 3918.61 ± 349.85 mg GAE/g, followed by natural beans at 2830.54 ± 359.62 mg GAE/g and fully washed beans at 2756.43 ± 278 mg GAE/g. These differences matched the clustering seen in the heatmap analysis (Figure 2). This suggests that mucilage during honey processing may help preserve or improve polyphenolic compounds during drying. Earlier studies have reported similar results, where less washing and longer mucilage contact maintained higher levels of bioactive compounds (Joët et al., 2010; De Bruyn et al., 2017).

Tabel 2. Total polyphenol content of green coffee beans subjected to different post-harvest processes.

Postharvest Process	Total Polyphenol (mg GAE/g)	Duncan Group
Natural	2756.43 ± 278	a
Honey	2830.54 ± 359.62	b
Fully Washed	3918.61 ± 349.85	c

Note: The same row with different superscripts means differ significantly (P<0.01)

The elevated polyphenol levels in honey-processed beans may be attributed to the semi-fermentation that occurs during drying, which activates enzymatic transformations in phenolic pathways (Varady et al., 2022). This finding highlights the importance of mucilage retention and drying conditions in modulating the chemical profile of green beans prior to roasting.

3.3 Caffeine Content

Caffeine is the most well-known alkaloid in coffee beans. It is well known for its sensory and physiological properties. The specific variety, geographic area, and postharvest method affect biochemical changes and the retention of varietals (Joët et al., 2010; Bastian et al., 2021). The postharvest process is a determining factor because it determines biochemical changes and the retention of the variables.

In this study, we observed differences in caffeine content among the three post-harvest processing methods (Table 3). Fully washed beans had the highest caffeine concentration (1.41 ± 0.21%), followed by naturally processed (0.82 ± 0.13%) and honey-processed beans (0.75 ± 0.30%). The variation in caffeine concentration and content was statistically significant at the 95% confidence level using Duncan's test ($p < 0.05$).

The higher caffeine content in fully washed beans may arise from the improved leaching of mucilage and the presence of specific microbial processes operating during the fermentation period, which may be related to the internal diffusion and retention of alkaloids (De Bruyn et al., 2017). The honey process retains the majority of mucilage and has limited fermentation, which could allow for dilution or inconsistency in caffeine content during drying. There are also inherent differences in the moisture dynamics and microbial loads in the different processing methods, which could influence the caffeine concentration (for instance, changes in enzymatic activity or cellular breakdown may be seen without influencing the stages of caffeine content degradation) (Zhang et al., 2019).

These results correspond with previous studies indicating that wet (fully washed) processing contributes to maintaining alkaloid content under certain conditions due to the efficient removal of the outer layer and enhanced diffusion gradients (Bytof et al., 2005). Importantly, all values reported in this study reflect the composition of unroasted green beans, providing a baseline for understanding compositional differences before roasting and brew preparation.

Table 3. Caffeine Content in Green Beans with Different Methods of Postharvest Processing.

Postharvest Processing	Caffeine (%)	Duncan Analysis
Fully Washed	1.41 ± 0.21	a
Natural	0.82 ± 0.13	b
Honey	0.75 ± 0.30	c

Note: The same row with different superscripts means differ significantly (P<0.01)

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3.4 Protein Content

Primary metabolites are essential compounds required for the growth, development, and reproduction of living organisms. Primary metabolites, such as amino acids, sugars, nucleotides, lipids, and proteins, directly participate in metabolic pathways, such as glycolysis, the tricarboxylic acid (TCA) cycle, and the Calvin cycle (Salam et al., 2023). In addition to being structural and enzymatic, proteins also serve as storage or reservoirs for nitrogen and precursors of secondary metabolites, including phenolics, alkaloids, and terpenoids (Fritz et al., 2006).

Proteins, which are macromolecules derived from amino acids, contribute significantly to plant metabolism, development, and defense (Clarke & Macrae, 1985). Proteins enhance the nutritional value of coffee beans and are precursors to significant amounts of volatile aroma compounds that are released during thermal processing. These proteins undergo heat-induced Maillard reactions with reducing sugars to produce a wide array of volatiles, including pyrazines, furans, and aldehydes, which potentially affect the flavor and aroma of brewed coffee through direct and indirect means (Clarke & Macrae, 1985; Buffo & Cardelli-Freire, 2004). Furthermore, cultivars, environmental conditions, and postharvest processing can modify protein levels and composition. This shows that the role of proteins is not only that of nutrients, but also of key drivers of cup quality in Coffea arabica and Coffea canephora (Buffo & Cardelli-Freire, 2004; Semedo Tavares & Silveira, 2022).

There were noticeable differences in the protein content of green beans among the three postharvest processing methods, as shown in Table 5. The natural process had the highest protein content (24.26 ± 0.40%), followed by fully washed (22.66 ± 0.23%) and honey-processed beans (22.57 ± 0.22%). Duncan's test statistical analysis showed the differences were significant ($p < 0.05$) at 95% confidence. The higher protein content observed in the naturally processed beans could be attributed to the absence of mechanical and/or microbial intervention during drying, which minimizes chemical changes and preserves cellular aspects and protein storage. For the fully washed and honey processes, varying levels of fermentation are likely responsible for some level of hydrolysis or transformation of the structural components of proteins. Microbial proteolytic enzymes and/or enzymatic activities instigated during fermentation have the potential to change protein structures into peptides and free amino acids, possibly lowering the amount of total measured protein in its intact structural form (De Bruyn et al., 2017).

Additionally, exposure to environmental factors during natural sun drying could limit microbial proteolytic activity, thereby stabilizing the protein potential remains more stable. These results demonstrate the intricacy of the biochemical processes occurring during postharvest processing and their effect on the retention of primary metabolites from coffee beans, increasing our knowledge of protein dynamics. Although it is exciting from a nutritional consideration, it also becomes rather interesting when considering the further transformation of these primary metabolites into secondary metabolites, particularly when considering the maturation of the coffee beans and the fermentation

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Table 4. Protein content of green beans processed using different methods.

Postharvest Processing	Protein (%)	Duncan Analysis
Natural	24.26 ± 0.40	b
Fully Washed	22.66 ± 0.23	a
Honey	22.57 ± 0.22	c

Note: The same row with different superscripts means differ significantly (P<0.01)

3.5 Antioxidant activity of green coffee beans as influenced by fermentation intensity

Antioxidant activity demonstrates how coffee constituents, particularly phenolic compounds, can neutralize reactive oxygen species (ROS). While polyphenols play a major role in antioxidant behavior, total antioxidant capacity also reflects the combined effects of other compounds, such as caffeine, trigonelline, and various organic acids (Jeszka-Skowron et al., 2016; Górecki & Hallmann, 2020).

Unlike the pattern observed for polyphenol content, the antioxidant activity was highest in fully washed green beans (1.41 ± 0.21 %). Natural beans followed at 0.82 ± 0.13%, and honey-processed beans were at 0.75 ± 0.30% (Table 3). This indicates that fermentation, especially during wet processing, may improve the antioxidant potential of green beans by transforming precursor compounds or releasing bound antioxidants (Jeszka-Skowron et al., 2016; Mestanza et al., 2023).

Fermentation during wet processing can break down complex phenolics and create smaller and more active metabolites. This process can enhance the antioxidant properties, even when the total polyphenol content is low. Additionally, microbial activity during washing may produce specific metabolites with greater antioxidant activity (De Bruyn et al., 2017). The inverse relationship between polyphenol content and antioxidant activity observed in this study highlights the complexity of post-harvest biochemical interactions. This suggests that optimizing both fermentation conditions and drying methods is important for maintaining and boosting the beneficial properties of green coffee beans.

Tabel 5. Antioxidants in green beans during postharvest processing.

Postharvest Process	Antioxidant Activity (%)	Duncan Group
Natural	66,54 ± 3,34	a
Honey	67,93 ± 6,12	b
Fully Washed	69,71 ± 7,04	c

Note: The same row with different superscripts means differ significantly (P<0.01)

3.6 Metabolite profile of Arabica green coffee bean

PCA analysis helped identify differences in the phytochemical profiles of green bean samples exposed to three post-harvest processes: natural, honey, and fully washed (Figure 1). The resultant score plot revealed a clear separation of the coffee samples into clusters for each post-harvest method. This suggests that each green bean sample, in this case, underwent distinct metabolism due to the processes used, supporting previous claims that postharvest practices greatly impact the concentration of metabolites (Putri et al., 2019; Lee et al., 2022).

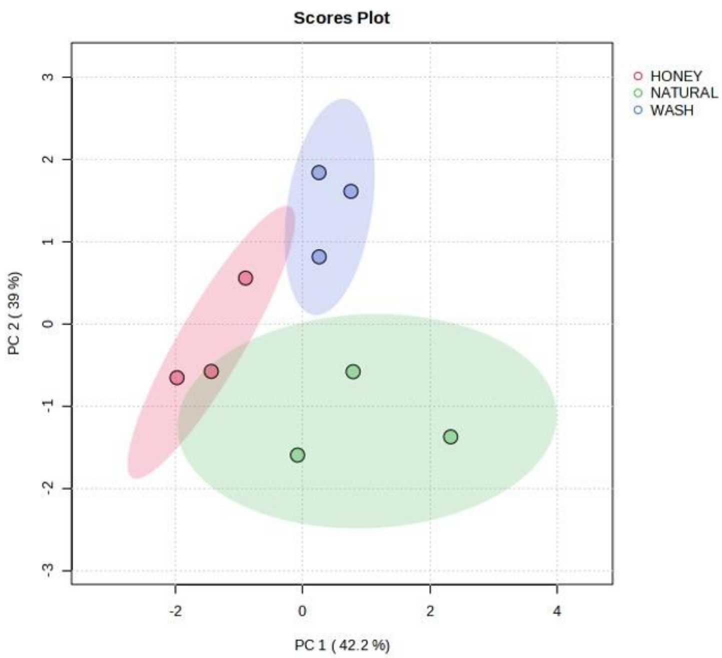

Figure 1. PCA score plot.

Specifically, fully washed samples were clearly separated from both honey and naturally processed samples along the first principal component (PC1), indicating different chemical profiles for each. Conversely, honey and natural samples appeared to be more closely related within the PCA space, suggesting overlapping phytochemical features likely stemming from comparable mucilage retention and fermentation environments (Silva et al. 2022; Várady et al. 2022). PC1 and PC2 explained 42.2% of the total variance, and R^2X and Q^2 were 0.869 and 0.866, respectively, suggesting a strong model fit with high exploratory power (Zainal et al., 2022; Aurum et al., 2023).

Although PCA yielded discriminative visualizations, it is critical to emphasize that PCA alone does not provide sufficient coffee classification or authentication based on the processing method, as there is no supervised predictive power (Trygg & Wold, 2002; Van den Berg et al., 2006). To validate the groupings, hierarchical cluster analysis (HCA) based on the selected discriminant metabolites was performed (Figure 2). The dendrogram supported the clustering pattern from PCA, revealing the robustness of metabolite-based differentiation, with samples forming three main groups corresponding to their post-harvest treatments.

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Specific chemical indicators associated with each postharvest process were revealed by metabolite profiles. Green beans from natural processing showed a higher abundance of certain nitrogen-related compounds, possibly reflecting more nitrogen assimilation during drying on raised beds or increased enzymatic activity during the sun exposure drying phase (Happyana et al., 2024; Bytof et al., 2005). In contrast, fully washed beans show higher concentrations of caffeine, a purine alkaloid produced during the early stages of bean development, which is more abundantly retained when mucilage is shed during wet fermentation (Ashihara et al., 2011; Chan et al., 2024). Honey-processed beans have higher polyphenol concentrations, which counterbalance and stabilize green coffee (Yulianti et al., 2023).

However, antioxidant metabolites, including some of the more prominent chlorogenic acids, were found at similar levels across all processing methods. This indicates that the antioxidant potential is a conserved characteristic of green Arabica beans (Cwikova et al., 2022). These results illustrate the effect of postharvest processing on the metabolic profile of green coffee beans and strengthen the hypothesis that metabolomic profiling can serve as a quality control tool and for the verification of authenticity in coffee.

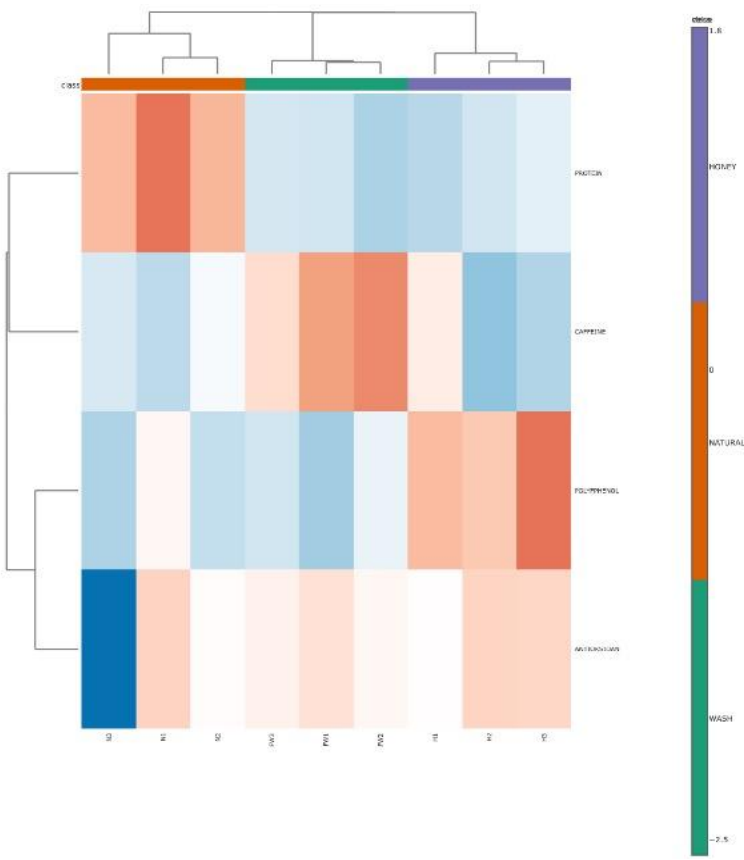

Figure 2. Heat map of the metabolite profiles of green coffee beans.

4. Conclusion

Postharvest processing methods strongly influence the metabolite profiles and quality attributes of Arabica green coffee beans. Natural processing preserved the highest protein content, honey processing enhanced polyphenol accumulation, and fully washed beans exhibited the highest antioxidant activity and caffeine concentration. These differences were also reflected in the bean color parameters, indicating a link between biochemical and physical traits. Overall, postharvest techniques are decisive factors in shaping the nutritional, functional, and sensory potential of green coffee beans. These findings provide valuable insights for optimizing processing strategies to improve coffee quality and health-related benefits. Importantly, these results can guide the coffee industry in selecting and refining processing techniques to achieve targeted quality specifications and meet market demands.

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