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RESEARCH ARTICLE

Characterization of caffeine crystals obtained from the extraction process of green robusta coffee beans

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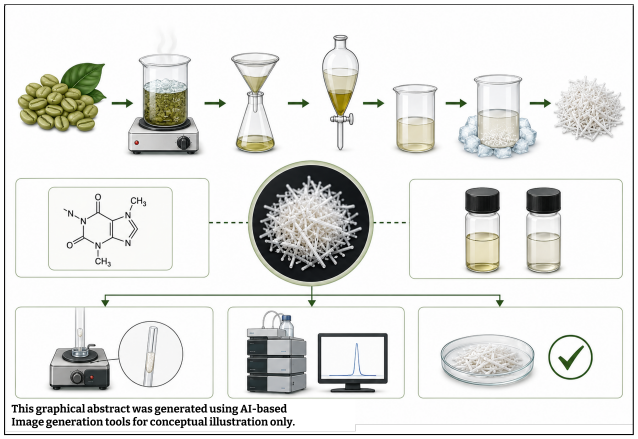

OBJECTIVES Robusta coffee contains a higher caffeine content, ranging from 1.6–2.4%, nearly twice as much as Arabica coffee, which contains only 0.9–1.2%. Caffeine content is widely utilized in various fields, such as pharmaceuticals and the food and beverage industry. This study aims to investigate the effects of temperature and extraction time on the solid–liquid extraction of green coffee beans using water as a solvent, followed by the isolation of caffeine crystals using two different solvents and the characterization of the resulting caffeine crystals. METHODS Chloroform and dichloromethane were employed as solvents for the isolation of caffeine from the coffee extract through liquid–liquid extraction in a batch system. The caffeine content in the extract was quantified using UV–Visible spectrophotometry. Furthermore, the purity of the isolated caffeine was evaluated by High-Performance Liquid Chromatography (HPLC) based on chromatographic peak analysis, while its identity and purity were further confirmed by comparing the measured melting point with that of standard caffeine. RESULTS Solid–liquid extraction followed by liquid–liquid extraction achieved the maximum caffeine yield at 97 °C after 60 minutes of extraction, yielding 2.18 mg/g with chloroform and 1.38 mg/g with dichloromethane. The reported caffeine concentrations (mg/g) are expressed as the mass of caffeine per gram of dry coffee feed. CONCLUSIONS Melting point determination and HPLC retention time analysis indicated that the caffeine crystals isolated via liquid–liquid extraction using dichloromethane exhibited higher purity, as evidenced by their closer agreement with standard caffeine values, namely a melting point of 236 °C and a retention time of 2.06 minutes.

KEYWORDS Caffeine; chloroform; dichloromethane; green coffee beans; extraction

1. INTRODUCTION

Coffee is one of the most economically valuable plantation commodities among cultivated crops (Azizah et al. 2019). Among the various coffee species, Robusta coffee is known to contain a significantly higher caffeine content (1.6–2.4%) compared to Arabica coffee, which typically contains only 0.9–1.2% (Clarke and Macrae 1987; Azizah et al. 2019). Caffeine is widely utilized not only as a dietary component but also as an active ingredient in both prescription and over-the-counter medications for the treatment of headaches, colds, and allergies. Notably, the presence of caffeine has been reported to enhance the analgesic effectiveness of these medications (Reddy et al. 2024). Caffeine finds extensive applications as a flavor enhancer in beverages and as an analgesic adjuvant in pharmaceutical formulations, frequently combined with aspirin, acetaminophen, or ergotamine (Chaugule et al. 2019). Comparative phytochemical analyses reveal significant differences in caffeine content between coffee species: raw Robusta beans contain approximately 2.2% caffeine, whereas Arabica beans contain about 1.2% (Aditya et al. 2015).

The use of green coffee beans as a raw material in this study is of particular significance due to their distinct physicochemical characteristics compared to roasted beans. Unlike roasted coffee, which undergoes extensive thermal degradation during processing, green coffee beans retain

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their native composition of bioactive compounds, including chlorogenic acids, proteins, and other thermally sensitive constituents. This preservation of the original matrix may influence both the mass transfer behavior during extraction and the stability of caffeine in the extract. Moreover, the absence of roasting-induced structural and chemical changes allows for a more controlled evaluation of extraction parameters, thereby providing a clearer understanding of the intrinsic factors governing caffeine release and separation.

Given its widespread utilization and economic significance, the demand for high-purity caffeine continues to increase, particularly in the pharmaceutical and food industries. Consequently, the development of efficient and sustainable caffeine extraction methods has become an important area of research. Conventional extraction techniques often involve the use of organic solvents, which may pose environmental and health concerns, as well as limitations in selectivity and efficiency. Therefore, there is a growing need to explore alternative extraction approaches that are not only effective but also environmentally friendly. In this context, Robusta coffee, with its higher caffeine content, presents a promising raw material for optimizing caffeine extraction processes and improving overall yield and quality.

Caffeine can be separated from coffee beans through an extraction process. One common method for caffeine separation from coffee beans is solid-liquid extraction using water as the solvent (Widagdyo et al. 2017). The factors influencing the solid-liquid extraction process are temperature and extraction time. Increasing the solvent temperature enhances its capacity to extract caffeine content from coffee. Similarly, extraction duration affects the concentration of dissolved compounds, color intensity, and aroma (Rahayuningsih 2014; Zarwinda and Sartika 2019). Previous studies have explored the influence of these parameters on caffeine extraction. For instance, Zarwinda and Sartika (2019) reported that the maximum caffeine content (0.181 mg/g) in Arabica coffee was achieved at 100°C with an extraction time of 1 hour. Their findings also indicated that both temperature and extraction time significantly affect caffeine yield, with increasing values improving extraction efficiency up to an optimum point. However, prolonged extraction beyond the optimum condition may lead to the degradation of compounds such as chlorogenic acid, potentially reducing caffeine purity. In addition, comparative studies have shown that Robusta coffee contains higher caffeine levels than Arabica, both in beans and by-products, reinforcing its potential as a superior raw material for caffeine extraction (Caracostea et al. 2021; Hurniati 2023).

Despite these advances, most existing studies primarily focus on individual process variables or are limited to specific coffee types, particularly Arabica. Comprehensive investigations evaluating the combined effects of temperature and extraction time on Robusta coffee using water as a solvent remain limited. Furthermore, insufficient attention has been given to the trade-off between maximizing caffeine yield and preserving product quality, especially in relation to the degradation of other bioactive compounds during extraction.

Following the solid–liquid extraction stage, caffeine is typically further separated from the aqueous extract through liquid–liquid extraction using organic solvents. The efficiency of this process is strongly influenced by several parameters, including solvent type, temperature, extraction time, pH, and solvent-to-feed ratio (Rahayuningsih 2014; Zarwinda and Sartika 2019). From a physicochemical perspective, caffeine exhibits solubility in both polar and semi-polar solvents; however, its polarity is closer to that of organic solvents, resulting in higher solubility in such media compared to water. This behavior is supported by the dielectric constant values of common solvents, where water (80 at 20°C) is significantly more polar than dichloromethane (8.9) and chloroform (4.8) (Marthia 2021). Since caffeine's polarity is closer to that of organic solvents, it exhibits greater solubility in organic solvents than in water (Wilantari 2018). Consequently, the selection of an appropriate organic solvent plays a crucial role in enhancing caffeine recovery during the separation process.

Several studies have explored the effectiveness of different solvents in liquid–liquid extraction. For example, Marthia (2021) employed Microwave-Assisted Extraction (MAE) followed by liquid–liquid extraction to compare chloroform and dichloromethane for caffeine isolation from Roasted Robusta coffee. The results showed that caffeine crystal yields ranged from 0.47% to 0.79% using chloroform and from 0.48% to 0.86% using dichloromethane. These findings indicate that dichloromethane provides slightly higher extraction efficiency, suggesting its greater suitability as a solvent for caffeine recovery.

Based on the background described above, this study is entitled "Characterization of Caffeine Crystals from the Extraction Process of Green Robusta Coffee Beans." Many studies have investigated caffeine extraction from Robusta coffee; however, most of them focus on roasted beans. In addition, liquid–liquid extraction in previous studies is usually carried out using only one type of solvent, with limited discussion on how different solvents affect extraction efficiency. Therefore, there are still research gaps, especially in determining the optimal extraction conditions for unroasted Robusta beans (green coffee beans) and in evaluating the effectiveness of different solvents in liquid–liquid extraction.

Therefore, this study aims to investigate the effects of temperature and extraction time during solid–liquid extraction of green Robusta coffee beans on caffeine content in the extract solution. Furthermore, this study evaluates the influence of two different solvent types on the efficiency of the subsequent liquid–liquid extraction process. Through this approach, the study seeks to provide a more comprehensive understanding of caffeine extraction and separation, contributing to the development of more efficient and effective extraction methods.

2. RESEARCH METHODOLOGY

2.1 Materials

Green Robusta coffee beans used as the raw material in this study were obtained from Temanggung, Central Java, Indonesia. The chemicals used in the extraction and analysis processes included distilled water (aquadest), 0.1 N hydrochloric acid (HCl), chloroform (98%, CAS 67-66-3, technical grade), dichloromethane (98%, CAS 75-09-2, technical grade), and lead(II) acetate [$Pb(CH_3COO)_2$] (CAS 6080-56-4, Merck). All chemicals were used as received without further purification.

2.2 Research procedures

This study was conducted at the Food Technology Laboratory, Unit Processes Laboratory, and Analytical Instrumentation Laboratory of Politeknik Negeri Bandung. The research consisted of three main stages: solid–liquid extraction of green coffee beans, isolation of caffeine, and product characterization.

2.2.1 Solid–liquid extraction

The solid–liquid extraction of green Robusta coffee beans was initiated with sample preparation, in which the green beans were ground using a grinder obtain a uniform particle size of 0.125 mm to increase the particle surface area and enhance mass transfer. Distilled water was used as the extraction solvent and heated to the desired temperature prior to extraction. This process was carried out by mixing 50 g known mass of coffee powder with distilled water at 500 mL. The extraction process was carried out in a three-neck flask equipped with a reflux condenser to prevent solvent loss. Continuous stirring using a magnetic stirrer was applied throughout the extraction to improve contact between the solid and liquid phases.

After extraction, the mixture was filtered using a Büchner funnel to separate the liquid extract from the solid residue. Pb acetate was then added to the filtrate to precipitate co-extracted impurities. The extraction conditions yielding the highest caffeine content were identified and subsequently used for the caffeine isolation process.

2.2.2 Caffeine isolation

Caffeine was isolated from the aqueous extract using liquid–liquid extraction in a separatory funnel. The extract obtained under optimal extraction conditions was mixed with an organic solvent, namely chloroform or dichloromethane. The mixture was allowed to stand until two immiscible liquid layers were formed. The organic phase containing caffeine was collected and concentrated by evaporating the solvent using a hot plate, resulting in crude caffeine crystals. To improve the purity of the obtained product, a recrystallization step was performed.

2.2.3 Product characterization

The obtained caffeine crystals were characterized in terms of purity and content. Purity analysis was conducted through melting point determination and High-Performance Liquid Chromatography (HPLC) based on chromatographic peak analysis. The caffeine content was determined using UV–Visible spectrophotometry at a wavelength of 271.6 nm.

The caffeine content was calculated using Equation 1.

$$\text{Caffeine content (mg/g)} = \frac{C \times V \times F_p}{m} \quad (1)$$

where C represents the caffeine concentration (mg/L) from UV-Visible Spectrophotometry, V is the total sample volume (L), F_p is the dilution factor (90×), and m is the mass of the sample (g). This equation expresses the caffeine content as the mass of caffeine per gram of dry sample, allowing for consistent comparison across different experimental.

The product yield was calculated using Equation 2.

$$\text{Yield (\%)} = \frac{m_1}{m_2} \times 100\% \quad (2)$$

where m_1 represents the mass of the product obtained (g), and m_2 denotes the mass of the feed used (g). This equation expresses the yield as the percentage of product recovered relative to the initial feed mass, providing an indicator of the overall efficiency of the extraction and isolation processes.

2.2.4 Experimental design

The study employed both fixed and variable parameters. The fixed variables included the use of green Robusta coffee beans as the raw material, a solvent volume of 500 mL (water-to-coffee ratio of 10:1, v/w), and the application of the reflux extraction method. The independent variables consisted of extraction temperature (70, 80, 90, and 97 °C), extraction time (30, 45, 60, 75, and 90 minutes), and the type of solvent used in the liquid–liquid extraction process (chloroform and dichloromethane).

2.3 Instrument

Subsequent characterization of the obtained caffeine crystals included purity assessment through melting point determination and high-performance liquid chromatography (HPLC), as well as quantitative analysis using UV–Vis spectrophotometry. The melting point was determined using a digital melting point apparatus to evaluate the purity of the isolated crystals. HPLC analysis was performed using a reversed-phase system equipped with a C18 column (e.g., 250 mm × 4.6 mm, 5 µm particle size), with a suitable mobile phase consisting of water and an organic modifier (e.g., methanol or acetonitrile) under isocratic conditions. The detection was carried out using a UV detector at an appropriate wavelength (e.g., 272–275 nm) specific for caffeine. Meanwhile, UV–Vis spectrophotometric analysis was conducted using a double-beam spectrophotometer at the maximum absorbance wavelength of caffeine to determine its concentration in the extract solution. All measurements were performed under controlled conditions to ensure accuracy and reproducibility of the analytical results.

3. RESULTS AND DISCUSSION

Based on the results presented in Figure 1, both chloroform and dichloromethane exhibit a similar increasing trend in caffeine content at each temperature point. This proportional increase observed in both solvent systems indicates that the amount of caffeine obtained is primarily governed by the extraction conditions, particularly temperature (and correspondingly extraction time when considered in the process). The consistent trend suggests that the extraction mechanism is dominated by the solid–liquid extraction stage rather than the type of solvent used in the subsequent separation step. The highest caffeine content was achieved at 97 °C, reaching 1.39 mg/g for chloroform and 2.14 mg/g for dichloromethane.

The increase in caffeine content obtained in this study is primarily influenced by the temperature applied during the solid–liquid extraction process. An increase in temperature enhances the kinetic energy of molecules, thereby accelerat-

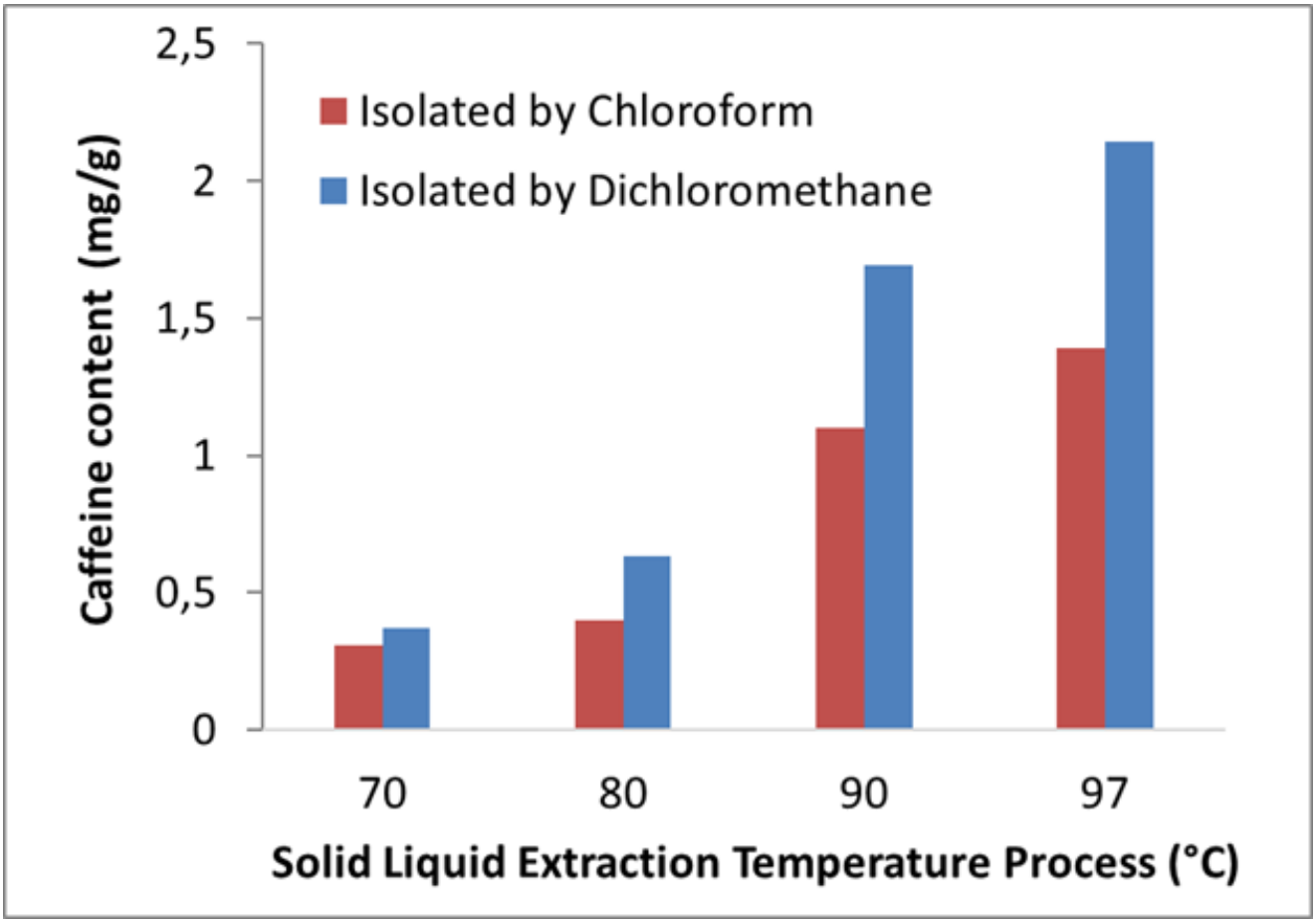

FIGURE 1. Effect of temperature on caffeine content extracted using chloroform and dichloromethane.

ing the diffusion of caffeine from the solid coffee matrix into the aqueous solvent. In addition, higher temperatures improve the solubility of caffeine and increase the diffusion coefficient, which collectively enhances the overall mass transfer rate. As a result, higher extraction temperatures lead to greater amounts of caffeine being transferred into the liquid phase until an optimal condition is reached.

Meanwhile, the use of chloroform and dichloromethane in this study does not affect the amount of caffeine extracted from the coffee beans but rather plays a role in the isolation stage of caffeine from the liquid extract. These organic solvents are used to separate caffeine that has already been dissolved in the aqueous phase through liquid–liquid extraction. Therefore, the differences in caffeine content obtained using the two solvents reflect variations in the efficiency of the separation and recovery processes, rather than the amount of caffeine initially extracted during the process.

Elevated temperatures induce molecular expansion within the coffee matrix, as reflected by increased intermolecular spacing (Putri and Ulfin 2015). This structural alteration enhances the diffusivity of the aqueous solvent and promotes mass transfer through two synergistic mechanisms: (1) improved solvent penetration into the expanded and more porous matrix, and (2) facilitated release of caffeine from intracellular structures. As the temperature increases, the integrity of complex interactions binding caffeine within the cellular matrix is progressively weakened, initiating the dissolution process through thermal cleavage of these interactions. Consequently, higher temperatures accelerate the kinetics of bond dissociation, allowing caffeine to be more readily liberated (Amin et al. 2020). Once released, caffeine molecules exhibit reduced effective molecular constraints and increased mobility, which enhances their ability to diffuse through cell walls and migrate into the surrounding solvent. This combination of enhanced diffusivity, improved solubility, and accelerated mass transfer ultimately leads to a more efficient extraction process at elevated temperatures.

While temperature significantly enhances caffeine extraction, the duration of extraction also contributes to the overall yield. Increased extraction time allows sufficient interaction between the solvent and the coffee matrix, facilitating the gradual diffusion of caffeine into the liquid phase until equilibrium conditions are approached. The caffeine content was determined from extracts obtained via solid–liquid extraction under reflux conditions at a constant temperature of 97 °C, while systematically varying the extraction time (30, 45, 60, 75, and 90 minutes) to investigate its effect on caffeine recovery. Subsequently, two organic solvents, chloroform and dichloromethane, were utilized for comparative evaluation in the liquid–liquid extraction stage. This methodology allowed for a comprehensive assessment of the influence of extraction time as well as the efficiency of solvent-based caffeine isolation. The experimental results are illustrated in Figure 2.

Based on the results presented in Figure 2, extraction time has a significant influence on the caffeine content obtained from green coffee bean extracts. The data show a con-

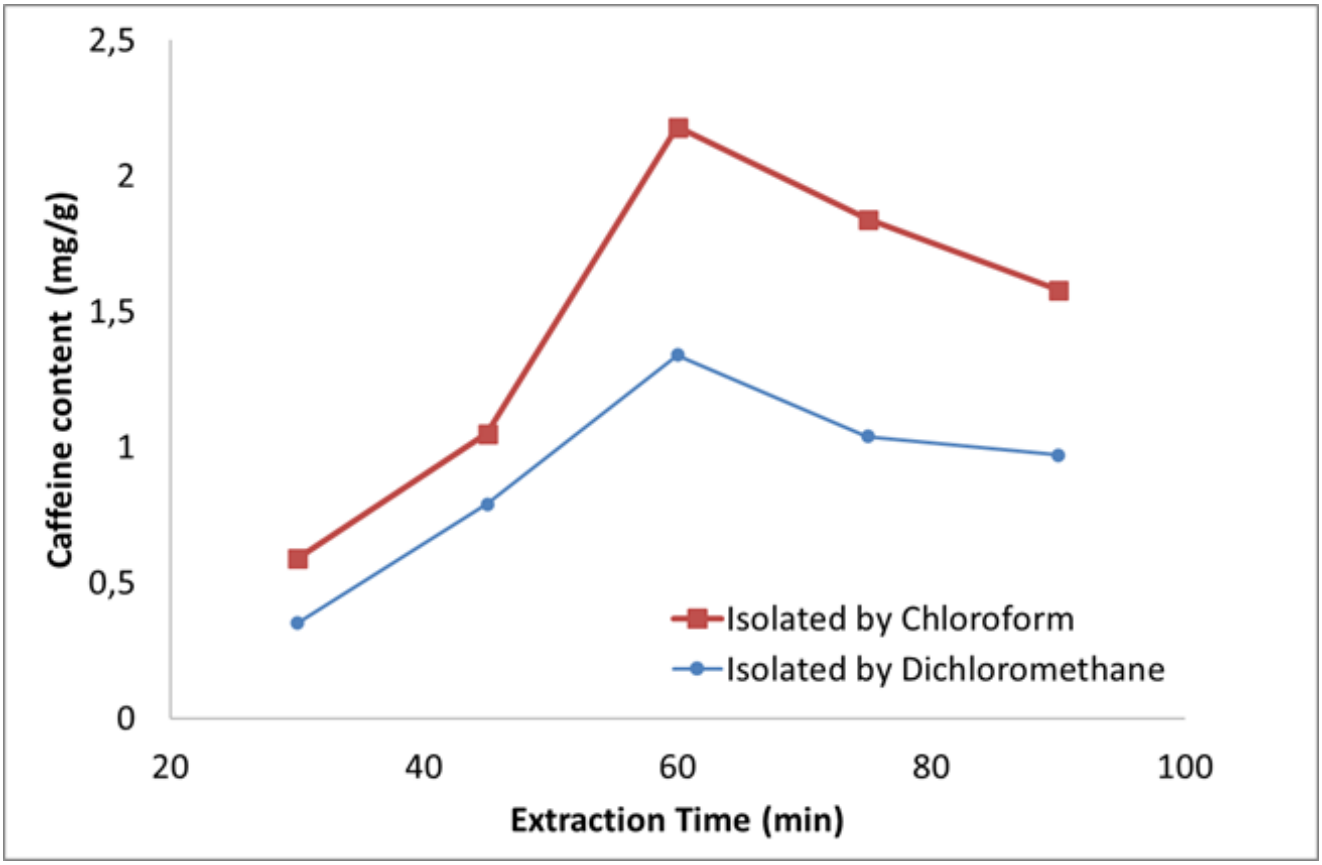

FIGURE 2. Effect of extraction time on caffeine content extracted using chloroform and dichloromethane.

sistent increasing trend in caffeine concentration for both chloroform and dichloromethane systems as the extraction time increases from 30 to 60 minutes. At 30 minutes, the caffeine content is relatively low, reaching approximately 0.59 mg/g for chloroform and 0.35 mg/g for dichloromethane, indicating that the extraction process is still in its initial stage and mass transfer from the solid matrix to the solvent is not yet optimal.

As the extraction time increases to 45 and 60 minutes, a substantial rise in caffeine content is observed, with maximum values achieved at 60 minutes, namely 2.18 mg/g for chloroform and 1.34 mg/g for dichloromethane. This behavior can be attributed to enhanced solvent–solid interaction and prolonged contact time, which allow more caffeine to diffuse from the coffee matrix into the aqueous phase. During this stage, the concentration gradient between the solid and liquid phases remains sufficiently high, promoting efficient mass transfer and increasing extraction yield. This phenomenon is consistent with classical mass transfer theory, where extended contact time enhances solute diffusion until equilibrium is approached (Cussler 2009; Treybal 1980).

However, beyond the optimal extraction time of 60 minutes, a decline in caffeine content is observed for both solvent systems. At 75 and 90 minutes, the caffeine concentration decreases gradually, suggesting that the system may have approached equilibrium, where further extraction becomes limited. In addition, prolonged exposure to elevated temperatures may lead to partial degradation or transformation of caffeine, although caffeine is relatively thermally stable under moderate conditions (Belitz and Grosch 1999). Alternatively, the decrease may also be attributed to re-association or interactions with other co-extracted compounds, which can reduce the measurable free caffeine concentration in the solution. Similar trends have been reported in the extraction of bioactive compounds, where excessive extraction time leads to reduced yield due to equilibrium limitations and compound instability (Azmir et al. 2013).

Importantly, both chloroform and dichloromethane exhibit similar extraction trends across all time variations, indicating that the primary factor influencing caffeine recovery is the extraction time during the solid–liquid extraction process. The difference in absolute values between the two solvents is associated with their role in the liquid–liquid extraction stage, particularly in terms of separation efficiency and recovery of caffeine from the aqueous phase.

Overall, the results demonstrate that an extraction time of 60 minutes represents the optimal condition under the studied parameters, providing the highest caffeine yield while avoiding the negative effects associated with prolonged extraction.

Significant emulsion formation was observed when dichloromethane was used during the liquid–liquid extraction stage. Upon contact between dichloromethane and the aqueous coffee extract, microdroplet dispersions formed at the interface and gradually developed into a stable emulsion, which hindered phase disengagement and reduced caffeine recovery efficiency.

This emulsion behavior is likely associated with co-extracted lipophilic compounds originating from green coffee beans. As reported by Dong et al. (2021), elevated extraction temperature can promote extraction of coffee oil and other amphiphilic constituents. These compounds may ac-

TABLE 1. Melting point of caffeine crystals from liquid-liquid extraction process with solvent variation.

Material	Melting Point (°C)
Caffeine (Anhydrouse) as the standard	235-238
Caffeine extracted by Chloroform	230
Caffeine extracted by Dichloromethane	236

cumulate at the water–dichloromethane interface, lower interfacial tension, and stabilize emulsion droplets.

Although co-extracted oil was also expected to be present in the chloroform system, different phase behavior was observed. In the chloroform extraction, two distinct liquid layers formed rapidly without persistent emulsion. This suggests that the difference was not due to the absence of oil in the chloroform system, but rather due to different solvent–solute and interfacial interactions.

The contrasting behavior may be related to physicochemical properties of the solvents. Dichloromethane possesses a dielectric constant of approximately 8.9, whereas chloroform has a lower value of about 4.8, compared with water (≈80 at 20 °C), indicating different polarity relationships and intermolecular interactions with the aqueous phase. In addition, literature reports differences in interfacial tension and solvation behavior between these solvent systems, which can affect emulsion stability and phase separation.

Thus, the higher caffeine recovery obtained with chloroform is attributed not only to extraction capability, but also to improved phase disengagement and reduced emulsion stabilization during liquid–liquid extraction.

3.1 Caffeine characterization

The purity and identity of the isolated caffeine crystals were evaluated through melting point determination and High-Performance Liquid Chromatography (HPLC) analysis. These complementary techniques provide both physical and chromatographic evidence to assess the quality of the obtained product.

Caffeine was isolated from the extract via liquid-liquid extraction (LLE), a process leveraging differential solubility of compounds between two immiscible liquid phases. In LLE, solute partitioning occurs according to distribution coefficients (Abriyani et al. 2022). Melting point analysis was conducted by heating the solid sample in a capillary tube immersed in a paraffin oil bath and recording the temperature at which the phase transition occurred. As presented in Table 1, the standard anhydrous caffeine exhibited a melting point range of 235–238 °C, which is consistent with literature values. The caffeine crystals obtained using dichloromethane showed a melting point of 236 °C, which falls precisely within the standard range, indicating a high degree of purity. In contrast, the caffeine crystals isolated using chloroform exhibited a lower melting point of 230 °C, suggesting the presence of impurities.

The observed deviation in melting point can be explained by the well-established principle that impurities disrupt the crystal lattice structure, leading to a reduction in melting temperature and, in many cases, a broader melting range (Cahyono and Suzery 2018). Therefore, the closer agreement of the dichloromethane-extracted caffeine with the standard melting point indicates that this solvent produces a purer crystalline product. Meanwhile, the lower melting point observed in chloroform-extracted samples suggests that residual impurities, possibly co-extracted compounds, remain within the crystal structure.

Further characterization was performed using HPLC to qualitatively assess purity based on retention time analysis. As shown in Table 2, the retention time of the standard caffeine was approximately 2.03 minutes. The caffeine crystals isolated using dichloromethane exhibited a retention time of 2.06 minutes, showing only a minimal deviation of 0.03 minutes from the standard. In contrast, the chloroform-isolated caffeine displayed a retention time of 2.15 minutes, corresponding to a larger deviation of 0.12 minutes.

Retention time in HPLC is highly sensitive to the chemical composition and purity of the analyte. A small deviation from the standard retention time indicates that the compound is chemically similar and contains minimal impurities, whereas larger deviations may suggest the presence of co-eluting or interfering substances (Sheline et al. 2010). The greater shift observed in the chloroform-isolated sample implies that impurities are still present, affecting the interaction between the analyte and the stationary phase and consequently altering the elution behavior.

Overall, both melting point and HPLC analyses consistently indicate that caffeine crystals obtained using dichloromethane exhibit higher purity compared to those obtained using chloroform. This finding highlights the importance of solvent selection in the liquid–liquid extraction stage, not only in terms of recovery but also in determining the final product quality. The superior performance of dichloromethane in producing purer caffeine crystals may be attributed to its more selective solvation behavior, which reduces the co-extraction of impurities and facilitates cleaner phase separation.

TABLE 2. Characterization results using HPLC.

Material	Retention Time (Min)
Caffeine (Anhydrouse) as the standard	2.03
Caffeine extracted by Chloroform	2.15
Caffeine extracted by Dichloromethane	2.06

4. CONCLUSIONS

Based on the analytical results and discussion presented above, the following conclusions can be drawn: The optimal temperature for obtaining the highest caffeine content in the solid-liquid extraction process was determined to be 97°C, yielding caffeine concentrations of 2.14 mg/g using chloroform and 1.39 mg/g using dichloromethane in the subsequent liquid-liquid extraction. While elevated temperatures enhance caffeine solubility in water, excessively high temperatures may lead to degradation of other compounds such as chlorogenic acid. Regarding extraction duration, the highest caffeine content was achieved at 60 minutes of solid-liquid extraction, producing concentrations of 2.18 mg/g with chloroform and 1.34 mg/g with dichloromethane in the liquid-liquid extraction step. Prolonged extraction beyond 60 minutes resulted in decreased caffeine content due to thermal degradation. Comparative analysis revealed that while chloroform yielded higher caffeine quantities (2.18 mg/g) in liquid-liquid extraction, dichloromethane-produced crystals demonstrated superior purity characteristics. This was evidenced by a melting point of 236°C and an HPLC retention time of 2.06 minutes, closely approximating the pure caffeine standard value of 2.03 minutes, suggesting fewer impurities in dichloromethane-extracted samples compared to those obtained with chloroform.

5. ACKNOWLEDGEMENTS

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