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Original Article

Quantification of Caffeine and Chlorogenic Acid in Decaffeinated Arabica Coffee Beans Samples Using HPLC and Evaluation of the effect of Fermentation Time on their Levels: A Study on the Influence of Roasting and Dry Method

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Abstract

Introduction: This study aimed to determine the concentrations of caffeine and chlorogenic acid (CGA) in decaffeinated Arabica coffee beans subjected to different pineapple fermentation durations. **Methods:** Arabica coffee beans were processed using three drying methods (honey, natural, and full wash) and five fermentation durations (12, 24, 36, 48, and 60 hours), employing 80% pineapple flesh as the fermentation medium. **Results:** As the fermentation time increased, caffeine levels decreased, while CGA levels rose, indicating the effectiveness of pineapple fermentation in decaffeinating coffee. Additionally, longer roasting times (50 minutes) resulted in lower caffeine content, but higher CGA levels compared to shorter roasting times (20 minutes). These findings suggest a potential process for producing decaffeinated coffee with higher CGA content. **Conclusion:** Overall, the pineapple fermentation method effectively reduced caffeine and increased the beneficial CGA compounds in Arabica coffee beans, offering an alternative approach to decaffeination. These results have significant implications for the development of nutritionally enhanced decaffeinated coffee products.

Keywords: *Caffeine; Chlorogenic Acid; Coffee; Decaffeination; HPLC*

Introduction

Coffea spp. is considered the foremost commercial crop plant, ranking as the second most valuable global commodity after oil. These tropical plants are renowned for producing roasted and ground seed beverages, which form the universally cherished drink (Czarniecka-Skubina *et al*., 2021; Núñez *et al*., 2021). The most economically significant species are Arabica and Robusta coffee (Das, 2021). Arabica coffee accounts for 60%, while Robusta coffee makes up the remaining 40% (Valencia-Lozano *et al*., 2021). Beverages brewed from coffee are among the most widely consumed drinks, prized for their caffeine content and stimulating effects (Olechno *et al*., 2021).

Coffee contains health-promoting compounds such as chlorogenic acid (CGA), trigonelline, and choline, alongside caffeine (Ciaramelli *et al*., 2019; Lu *et al*., 2020; Seo & Kim, 2019). Due to these chemical compounds, coffee offers a delightful taste, pleasing aroma, stimulating effects, and various health benefits (Ciaramelli *et al*., 2019). Caffeine and CGA are commonly present in coffee as stimulants and antioxidants. It has been documented that unroasted coffee contains higher levels of caffeine and CGA compared to black coffee (Narko *et al*., 2020). However, green coffee bean extract has certain drawbacks, including an unappealing taste, aroma, and the bioactivity of its phytoconstituents (Husni *et al*., 2021). While Robusta beans have higher levels of caffeine and CGA than Arabica beans, they are of lower economic value (Purwoko *et al*., 2022).

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CGA and caffeine are essential components of coffee beans, significantly influencing their flavour and aroma. Additionally, phenols and antioxidants provide considerable health benefits (Awwad *et al*., 2021). The caffeine content in coffee varies between 0% and 17%. Marketing materials often highlight a 50% CGA content as the benchmark for quality in products containing extract from green coffee beans. However, based on this benchmark, only 28% of commercial products meet the standard (Vinson *et al*., 2019). Caffeine, derived from purine nucleotides, typically constitutes 2–5% of the dry weight in tea and 1–2% of the dry weight in coffee (Lin *et al*., 2023). Green coffee beans contain CGA in the range of 2.80–5.42 g/100g and caffeine between 0.85–1.73 g/100g (Sualeh, Tolessa & Mohammed, 2020). Hydroxycinnamates, including CGA are commonly found in various everyday foods and beverages, with a notable presence in certain coffee beverages (Lu *et al*., 2020). CGA, a bioactive compound, exerts its anti-inflammatory effects by regulating the production and release of inflammatory mediators, thus protecting cells and tissues against conditions such as cardio-cerebrovascular diseases and diabetes mellitus (Nemzer *et al*., 2022). CGA is recognised as a polyphenolic compound of significant functionality and abundance in the human diet (Lu *et al*., 2020).

Caffeine, classified as a purine alkaloid, plays a significant role in coffee, tea, and various other beverages. It functions as a central nervous system stimulant and is known to be associated with withdrawal symptoms (Gokulakrishnan *et al*., 2005). Caffeine has been linked to an increase in blood pressure, heightened systemic vascular resistance, adverse effects on endothelial function, and changes in serum lipid concentrations and insulin resistance (Miranda *et al*., 2017). Laboratory studies have suggested a potential correlation between caffeine intake and insomnia, particularly among individuals who exhibit elevated levels of anxiety at the outset (Chaudhary *et al*., 2016).

Decaffeinated beverages are used to counteract the adverse effects of caffeine. Decaffeination is achieved through various techniques, such as solvent extraction, water extraction, and supercritical fluid extraction. Although these methods are non-specific, they are costly and often involve the use of hazardous organic solvents. It is essential to develop a process that incorporates the enzymatic (specific) breakdown of caffeine into non-toxic compounds to address the issues associated with chemically extracting caffeine from food products and treating waste products containing caffeine (Gokulakrishnan *et al*., 2005). CGA, a vital compound found in coffee beans, has been shown to exhibit antiviral properties against various viruses. However, caffeine in coffee beans can lead to undesirable effects such as insomnia, stomach irritation, increased heart rate, and elevated respiration rate. These adverse effects can be mitigated through the decaffeination of Arabica coffee beans, which is achieved by applying dichloromethane treatment, followed by solid-phase extraction using methanol. The decaffeination process that employs organic solvents faces challenges in preserving the flavour and aroma of the coffee. Methylene chloride (DCM) and ethyl acetate (EA) are commonly used as organic solvents in the decaffeination process (Zou *et al*., 2022). Water extraction and supercritical carbon dioxide are alternative methods for decaffeination (Gokulakrishnan *et al*., 2005). Concerns have been raised regarding the use of organic solvents in decaffeination due to the potential presence of solvent residues, which may affect the flavour and pose health risks. Decaffeinated coffee has been observed to have slightly higher antioxidant activity than regular coffee, although in smaller quantities (Asoudeh *et al*., 2022).

Research has been conducted on the use of the bromelain enzyme in the decaffeination process. Bromelain, an enzyme derived from pineapple waste, has been investigated for its potential in decaffeination (Susanti *et al*., 2022). However, the application of pineapple juice in decaffeinating Arabica coffee beans has not been extensively studied. While various decaffeination methods, including solvent-based and water-based techniques, have been thoroughly researched, the specific use of pineapple juice for decaffeination remains an underexplored area. Most studies focus on solvents, supercritical carbon dioxide, or water-based methods for decaffeination (Huamaní-Meléndez & Darros-Barbosa, 2018; Zou *et al*., 2022).

Current studies on the levels of caffeine and CGA in coffee primarily focus on their quantification and the effects of roasting on these levels. However, certain aspects have not been adequately explored. One area of uncertainty is the impact of the decaffeination process on the levels of CGA and caffeine.

Previous research has primarily concentrated on the concentration of CGA and caffeine in prepared coffee (Lu *et al*., 2020). This study employed HPLC with a UV-Vis detector to measure the overall quantities of CGA and caffeine in decaffeinated Arabica coffee beans over varying fermentation durations.

Material and Methods

The materials used in this study comprised Arabica coffee beans sourced from Cigalontang Village, Tasikmalaya Regency, which had been standardised in previous research. For High-Performance Liquid Chromatography (HPLC) analysis, a 1% acetic acid and methanol solution was prepared. Standard caffeine and CGA were employed as reference materials for content analysis.

In this study, three drying methods were utilised: honey (A), natural (B), and full wash (C). The decaffeination of Arabica coffee beans was carried out using a fermentation method involving pineapple flesh. Following this, the decaffeinated coffee beans were analysed for caffeine and CGA levels using HPLC-UV-Vis. The research was conducted at the Pharmaceutical Biology Laboratory of the Health Polytechnic of the Ministry of Health in Tasikmalaya and the Central Laboratory of Padjadjaran University.

Fermentation Media Preparation

The raw material used for the fermentation medium is pineapple flesh (Hariyadi *et al*., 2024). The pineapples are peeled using a knife. The pineapple flesh fermentation medium, derived from the Ananas comosus L. honey pineapple variety, uses an 80% concentration of pineapple. A total of 160 grams of cleaned pineapples is combined with 80 ml of water to ensure even grating of the pineapple, after which the mixture is blended. The resulting blend is then added to 200 grams of coffee beans for each sample.

Sample Preparation and Extraction

The samples used in this study consist of three different drying methods: honey (A), natural (B), and full wash (C). Each sample undergoes 10 different treatments, which include variations in fermentation time (12, 24, 36, 48, and 60 hours), alongside two roasting time variations of 20 and 50 minutes. The fermented pineapple coffee beans are then washed with running water, drained, and dried in an oven at approximately 105°C for about 30 minutes. After drying, the decaffeinated Arabica coffee beans are roasted using a small-scale automatic roasting machine at 180°C, with roasting time variations of 20 and 50 minutes. The samples are subsequently cooled to room temperature. Grinding is then carried out using a grinder machine until the coffee is finely ground, followed by sieving through an 80-mesh sieve (Silitonga *et al*., 2025).

Sample extraction is carried out by modifying the previous research. Coffee samples are extracted using hot water at a temperature of 83°C with a ratio of 1:99 (coffee to solvent ratio). Next, the coffee samples are sonicated for five minutes to achieve uniformity in the coffee solution (ultrasonic bath) the samples of coffee undergo centrifugation for fifteen minutes at a 7900 x g pace utilizing a centrifuge for use in laboratories (MPW-260R). The coffee extract is then filtered by means of a 0.45 μL Millipore filter. Finally, the coffee extract is stored in the freezer at -20 °C till the day of analysis.

HPLC Condition Optimization

The mobile phase used for the analysis of caffeine and CGA levels consists of 1% acetic acid and a 60:40 methanol (MeOH) mixture (Alhaidrai, 2023). The retention time for caffeine was found to be 5.5 minutes, while the retention time for CGA was 4.1 minutes. Prior to use, the mobile phase is degassed through ultrasonic vibration for 20 minutes. Calibration solutions for caffeine and CGA are prepared uniformly, establishing concentrations of 10, 50, 100, 200, 500, and 1000 ppm by appropriately diluting the standard stock solution (1000 ppm) with the mobile phase. The peak areas for each caffeine and CGA concentration are plotted against their respective concentrations, and regression equations are calculated.

Statistical Analysis

Measurements of coffee samples are conducted in pairs. The collected data is subjected to statistical analysis using SPSS software (version 25, Chicago, IL, USA). A one-way ANOVA test is also applied to assess the significance of variations in the levels of CGA and caffeine, as determined through the HPLC-UV-Vis method.

Data processing typically involves editing, coding, data entry, and cleaning. After this initial processing, the data undergoes further analysis. Univariate analysis is used to describe each variable, including age, gender, type of stroke, comorbidities, frequency of attacks, and ability to swallow. To examine the relationship between dependent and independent variables, bivariate analysis, t-tests, and correlation tests are performed.

Result

Coffee Beans' Caffeine and CGA Content Based on a 20-Minute Roast

In this research, coffee samples underwent extraction and analysis to evaluate the concentrations of caffeine and CGA using HPLC-UVVis. The outcomes from the analysis of caffeine and CGA samples of coffee are detailed in Table 1.

Table 1: *Coffee and CGA Levels in Differential Fermentation 20 Minutes*

Coffee Type	Fermentation Time (hours)	Roasting Time (minutes)	% caffeine	% CGA
Honey (A)	12	20	**3.88**	**0.92**
	24		3.83	0.93
	36		3.58	0.94
	48		3.09	0.99
	60		3.08	1.00
Natural (B)	12	20	3.63	0.92
	24		3.42	0.93
	36		3.38	0.94
	48		2.46	0.95
	60		1.09	1.03
Full Wash (C)	12	20	4.05	0.99
	24		3.58	1.02
	36		3.47	1.05
	48		3.21	1.17
	60		0	0.01

Coffee Beans' Caffeine and CGA Content Based on a 50-Minute Roast

Table 2 contains the experimental outcomes of the analysis conducted on coffee samples to determine the presence of caffeine and CGA. The results obtained from this experiment have been documented for reference and further analysis.

Table 2: *Caffeine and CGA Content in Variation Fermentation Time on 50 Minutes*

Coffee Type	**Fermentation Time (hours)**	**Roasting Time (minutes)**	**% caffeine**	**% CGA**
Honey (A)	12	50	1.63	1.09
	24		1.54	1.11
	36		1.26	1.12
	48		1.18	1.16
	60		1.12	1.18
Natural (B)	12	50	1.79	0.71
	24		1.69	1.05
	36		1.15	1.06
	48		1.09	1.10
	60		0.78	1.11
Full Wash (C)	12	50	2.66	1.12
	24		1.82	1.14
	36		1.47	1.16
	48		1.09	1.17
	60		1.08	1.17

Table 3: *Comapartive in Variation Fermentation Time and Roast Time*

Coffee Processing Type		**Full Wash (C)**	**Full Wash (C)**	**Honey (A)**	**Honey (A)**	**Natural (B)**	**Natural (B)**
Roasting Duration (minutes)		20	50	20	50	20	50
	Fermentation Duration (hours)						
Caffeine Content (%)	12	4.05	2.66	3.88	1.63	3.63	1.79
Caffeine Content (%)	24	3.58	1.82	3.83	1.54	3.42	1.69
Caffeine Content (%)	36	3.47	1.47	3.58	1.26	3.38	1.15
Caffeine Content (%)	48	3.21	1.09	3.09	1.18	2.46	1.09
Caffeine Content (%)	60	0	1.08	3.08	1.12	1.09	0.78
CGA Content (%)	12	0.99	1.12	0.92	1.09	0.92	0.71
CGA Content (%)	24	1.02	1.14	0.93	1.11	0.93	1.05
CGA Content (%)	36	1.05	1.16	0.94	1.12	0.94	1.06
CGA Content (%)	48	1.17	1.17	0.99	1.16	0.95	1.1
CGA Content (%)	60	0.01	1.17	1	1.18	1.03	1.11

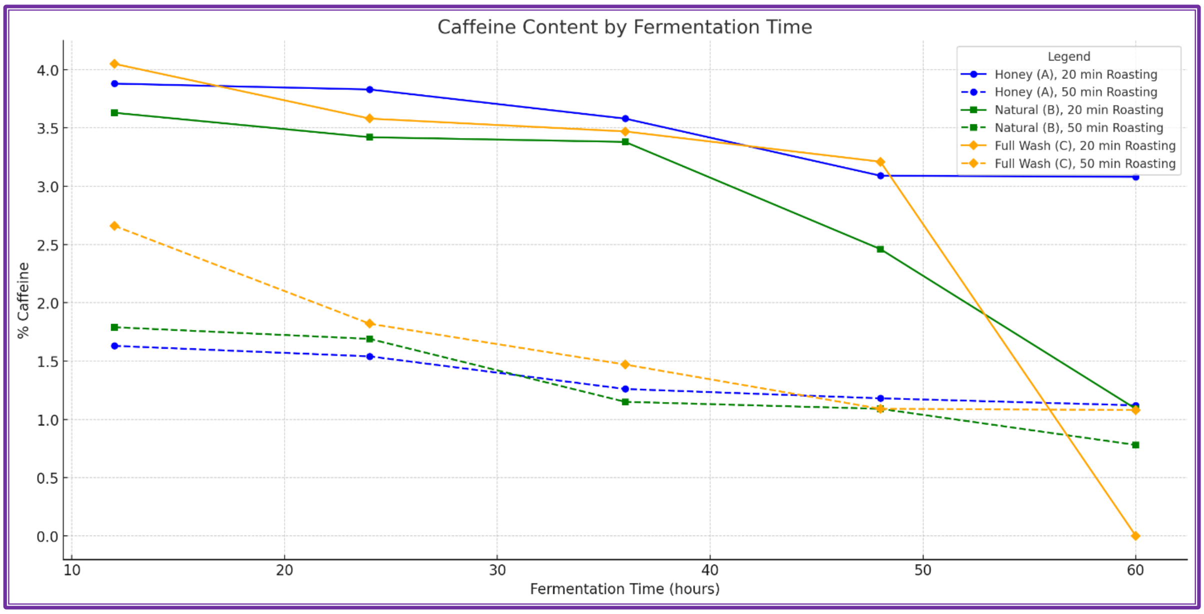

Figure 1: *Caffeine Content by Fermentation Time*

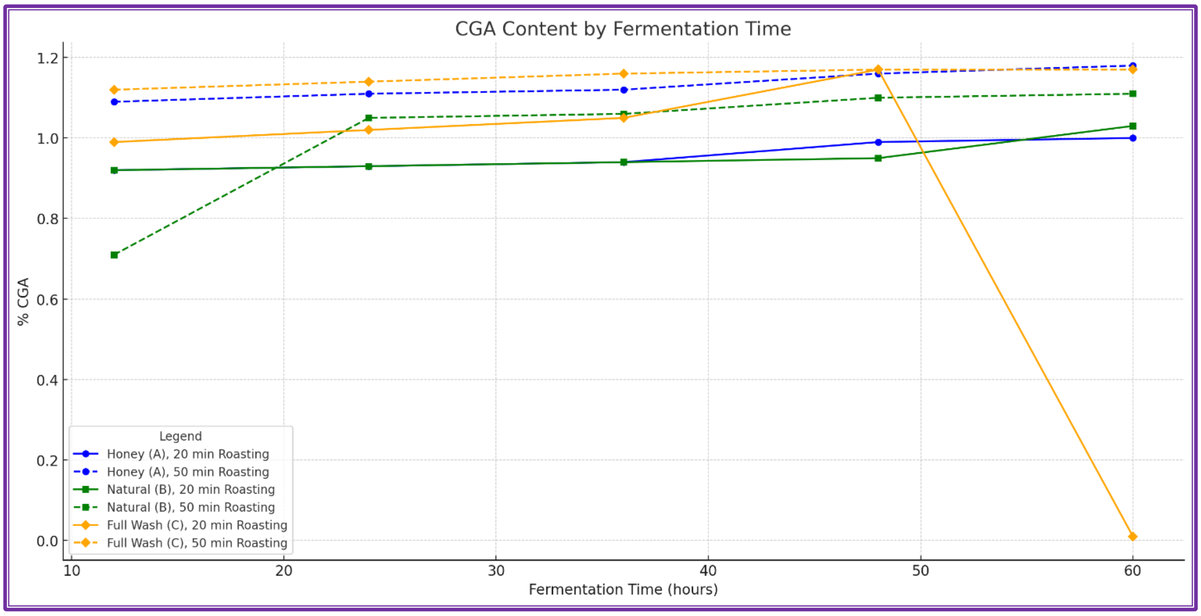

Figure 2: *CGA Content by Fermentation Time*

DISCUSSION

Coffee Beans' Caffeine and CGA Content Based on a 20-Minute Roast

Table 1 provides a comprehensive representation of the analysis outcomes and presents a detailed overview of the results in an organised format for ease of comprehension. The data offer an understanding of the caffeine and CGA content in various types of dried coffee. This information offers interesting perspectives on the complexity and diversity within the world of coffee, highlighting how different strains and roasts can influence caffeine and CGA levels.

The table indicates that caffeine content decreased as fermenting time increased, while CGA content rose with longer fermentation times. Statistical analysis of all coffee samples, employing various drying methods (Honey, Natural, and Full Wash), indicated no significant variation ($p \geq 0.05$) in caffeine concentrations among the samples. Similarly, the analysis of CGA showed no significant variation ($p \geq 0.05$) in CGA concentrations between the coffee samples.

Fermentation can significantly influence the chemical composition of coffee beans (Bastian *et al*., 2021; Junior *et al*., 2021; Febrianto & Zhu, 2023; Galarza & Figueroa, 2022; Tan *et al*., 2023). Investigating the fermentation time of *Ananas comosus* (pineapple) reveals a reduction in caffeine content as the fermentation duration increases. A longer fermentation time may lead to a decrease in caffeine levels due to changes in organic compounds and the fermentation process. Some bacteria and fungi can break down caffeine using enzymes. In these cases, caffeine is the only source of carbon and nitrogen. This process of breaking down involves N-demethylation in steps (Kim *et al*., 2019). The primary application of caffeine's enzymatic breakdown is in the food industry, where specific intermediates, such as theophylline, 7-methylxanthine, and paraxanthine, are produced (Ibrahim *et al*., 2014). Enzymatic caffeine degradation offers several benefits, including the targeted removal of methylxanthines, thereby preserving coffee's distinct flavour and aroma compounds. This method also ensures minimal impact on the taste of food. It is predominantly used in food applications and in the production of specific intermediates such as paraxanthine, 7-methylxanthine, and theophylline (Gokulakrishnan *et al*., 2005; Lin *et al*., 2023). Moreover, the biodegradation of caffeine presents a secure and cost-effective alternative to physical and chemical techniques, making it a more environmentally friendly and economical option (Gummadi *et al*., 2012; Lin *et al*., 2023)

In a research endeavour exploring the fermentation of pineapple by-products, it was revealed that fermented extracts exhibited a 21% elevation in total phenolic compounds (TPC) when contrasted with the control sample, which was devoid of fermentation. This surge in TPC might be ascribed to the optimal growth of Aspergillus xylinum in an acidic environment during the fermentation process. This organism converts glucose into gluconic acid and fructose, which augments organic acid concentration in the medium, consequently enhancing phenolic compounds and antioxidant activity (Rivera *et al*. 2023).

Therefore, the rise in phenolic compounds and, subsequently, antioxidant activity can be ascribed to the action of hydrolytic enzymes on the fruit flesh. This action enhances the accessibility of hydroxy functional groups to phenolic compounds, resulting in increased phenolic groups and improved antioxidant capacity akin to CGA (Rivera *et al*. 2023).

The study observed a decline in caffeine levels and an elevation in CGA content in coffee as fermentation time increased. These outcomes were noted in an investigation into the kinetics and equilibrium chemistry of cold-brew coffee. The study utilised both medium and dark roast coffee obtained from the Kona region of Hawaii, and it was prepared with both medium and coarse grinding grounds (Fuller & Rao, 2017).

Coffee Beans' Caffeine and CGA Content Based on a 50-Minute Roast

After analysing the data presented in Table 2, it can be concluded that there is a correlation between roasting time and the caffeine and CGA contents of the sample. Precisely (same with Table 1), as fermentation time increased, the caffeine content decreased while the CGA content increased. This finding suggests that longer roasting may lead to a higher concentration of beneficial compounds in the final product. These results have significant implications for industries that produce fermented beverages or foods, as they highlight the potential benefits of optimising fermentation conditions to enhance nutritional value and overall quality.

A statistical analysis of all the coffee samples using the three different drying methods (full wash, honey, and natural) revealed no significant difference ($p \geq 0.05$) in the amounts of caffeine found in the samples. The CGA concentration results showed that there was no significant difference ($p \geq 0.05$) in the CGA concentrations found in the coffee samples.

The correlation between the roasting duration and the caffeine and CGA levels in coffee has been examined. The findings reveal a decrease in CGA content with prolonged roasting. In contrast, the caffeine content experiences an increase during roasting, reaching its peak in medium-roasted coffee and declining in dark-roasted coffee. For instance, a study observed a reduction in CGA content with an escalating degree of roasting, accompanied by an increase in caffeine content from green beans to medium-roasted coffee beans, followed by a decrease in dark coffee beans (Awwad *et al*. 2021). However, another study noted a connection between elevated roasting temperatures, a decline in CGA concentrations, and a rise in caffeine concentrations (Fuller & Rao 2017). Similarly, a study found that caffeine content increased gradually with a higher roasting degree, except for Indonesian beans, while the CGA content decreased (Tsai & Jioe, 2021).

Fermentation as an alternative for decaffeination

Using organic solvents in decaffeination may result in a loss of coffee's aroma and flavour if the solvent is not entirely extracted. Methylene chloride and ethyl acetate are frequently employed organic solvents for decaffeination. These solvents can extract a specific quantity of aroma precursors and caffeine, potentially leading to a subdued taste in decaffeinated coffee (Zou *et al*., 2022; Umakanthan & Mathi, 2022). The Swiss Water Process offers an alternative technique where coffee beans undergo immersion in hot water to eliminate caffeine and flavour components. Subsequently, a carbon filter is employed to extract caffeine, and the purified water is reintroduced into the coffee beans to restore their flavour (Umakanthan & Mathi, 2022). Another trending method is utilising supercritical carbon dioxide, which is gaining popularity due to its benefits, including its non-toxic, non-flammable nature and remarkable selectivity (Umakanthan & Mathi, 2022; Zou *et al*., 2022).

The investigation into the enzymatic breakdown of caffeine has been initiated to explore its potential applications in the food industry and the synthesis of specific compounds (Lin *et al*., 2023). Proteases, enzymes capable of protein breakdown, find widespread application in the food and beverage industry, serving various purposes, such as decaffeination. The specific mechanism of decaffeination using proteases may differ based on the particular protease and the process employed.

Throughout the fermentation process, protease enzymes play a crucial role in breaking down the mucilage layer that covers the surface of coffee beans, where caffeine is present in a liberated state. The degradation of the mucilage is significant for the final quality of the coffee, as the degradation profile and microbial by-products generated during fermentation can permeate the coffee beans, contributing to the desired flavour (Krajangsang *et al*., 2022). The primary stages of this process include: (1) Mucilage degradation, where protease enzymes break down the mucilage layer during wet fermentation (Elhalis *et al*., 2023; Krajangsang *et al*., 2022); (2) Dissemination of flavour-affecting components, where the breakdown of the mucilage allows the dispersal of flavour-influencing compounds, such as simple sugars, into the coffee beans, enhancing the aroma and taste of the coffee; and (3) Regulated fermentation, where employing a controlled fermentation method, involving a carefully selected microbial seed culture and appropriate environmental conditions, can enhance the aromatic quality of coffee compared to uncontrolled or spontaneous fermentation (Krajangsang *et al*., 2022).

In figures 1 and 2, the graphs clearly show that the caffeine and chlorogenic acid (CGA) content in coffee undergo significant changes over time during fermentation, influenced by both the processing method and roasting time. The first graph demonstrates that caffeine levels tend to decrease as fermentation time increases, with the most significant decrease occurring in the Full Wash (C) method, particularly after 50–60 hours of fermentation. In contrast, the Honey (A) method remains relatively stable. The second graph shows that CGA levels generally increase with fermentation time, although there is a sharp decline in CGA levels after 60 hours in the Full Wash (C) method. This indicates a complex interaction between the fermentation process, processing method, and roasting time, which collectively influence the chemical profile of the coffee. Differences in processing techniques lead to variations in the final characteristics of caffeine and CGA content.

Based on the data presented in Table 2 and Table 3, it is evident that the caffeine content of coffee decreases as fermentation time increases, with the most significant decrease observed in the Full Wash (C) method, while the Honey (A) method remains more stable. Roasting time also impacts caffeine content, with 50 minutes of roasting resulting in lower caffeine levels compared to 20 minutes of roasting. Conversely, chlorogenic acid (CGA) levels are generally more stable and even tend to increase slightly with longer fermentation times, showing minimal impact from the roasting duration. Overall, these results indicate that fermentation plays a key role in reducing caffeine levels, while CGA is better preserved. Moreover, variations in coffee processing methods also contribute to differences in the resulting chemical composition.

Limitations

- Outline the aspects not covered in the research, such as other coffee bean types (besides Arabica), alternative analytical methods (besides HPLC), or additional factors affecting caffeine and CGA levels.
- Highlight the limited generalisability of the findings, such as their potential inapplicability to other coffee processing methods.

Future Scope

- Suggest directions for future research, such as exploring other coffee varieties, employing additional analytical techniques, or investigating the impact of environmental factors on caffeine and CGA levels.
- Mention potential advancements in fermentation and roasting techniques to improve coffee quality.

Conclusion

Pineapple fermentation media for decaffeination effectively lowers caffeine levels and increases CGA levels. The results of this study are similar to other studies that have been conducted. Bromelain, an enzyme in pineapple waste, has been investigated for its decaffeination potential. Pineapple fermentation may enhance the production of decaffeinated coffee with elevated chlorogenic acid content. Prolonged roasting temperatures reduce caffeine levels while increasing chlorogenic acid concentrations.

Conflict of Interest

Authors have declared no competing of interests.

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