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Study on the extraction of caffeine from natural sources

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----------ABSTRACT----------

Presently, a variety of methods are employed for the extraction of bioactive compounds from natural sources. These include the use of organic solvents (e.g., methylene chloride), water-based extraction, enzymatic processes, ultrasonic-assisted extraction, microwave-assisted extraction, and extraction utilizing eutectic solvents, such as supercritical carbon dioxide (SC-CO_2). Among these, SC-CO_2 is recognized for its environmental compatibility and high efficiency. However, its application is often limited by the need for sophisticated and costly equipment. Despite their widespread use, these extraction methods are associated with several limitations. In particular, the use of organic solvents raises health and environmental concerns due to the potential presence of residual toxic compounds in final products, such as decaffeinated tea. Furthermore, these techniques can be time-intensive, may result in low extraction yields, and often contribute to environmental pollution through solvent waste and emissions.

KEYWORDS;- C*affeine, Extraction, Ultrasonic, Microwave, Tea.*

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I. INTRODUCTION

Contemporary methods for extracting bioactive compounds such as caffeine from natural sources encompass a range of techniques, including extraction with organic solvents (e.g., methylene chloride), aqueous extraction, enzymatic hydrolysis, ultrasonic-assisted extraction, microwave-assisted extraction, and the use of eutectic solvents such as supercritical carbon dioxide (SC-CO_2). Among these, SC-CO_2 extraction is considered both environmentally friendly and efficient; however, it necessitates advanced and costly equipment, which limits its widespread application.

Despite their effectiveness, these extraction techniques are not without limitations. Organic solvent-based methods, for instance, pose several concerns due to the potential toxicity of residual solvents in the final product, such as decaffeinated tea. Additionally, these processes can be time-consuming, yield suboptimal quantities of the target compound, and contribute to environmental pollution.

Specifically, the conventional approach of caffeine extraction, which involves the use of chlorinated hydrocarbons under atmospheric pressure, demonstrates several drawbacks. Although dichloromethane has been shown to offer high extraction efficiency, it presents health and environmental risks. The possibility of inadequate purification further raises concerns about the safety of products intended for human consumption. Moreover, the use of such organic solvents is associated with high operational costs and the ecological burden of chemical waste disposal.

Extraction Using Dichloromethane (CH_2Cl_2)

The extraction of caffeine using dichloromethane involves a two-step process. Initially, hydrophilic compounds, including caffeine, are extracted from tea leaves using hot water. After cooling the aqueous extract, caffeine is subsequently partitioned into dichloromethane, a water-immiscible organic solvent. This step leverages the differential solubility of caffeine, which is significantly more soluble in dichloromethane (~140 mg/L) than in water (~22 mg/L). Following the extraction, the caffeine content can be quantified by calculating the percentage yield based on the initial mass of tea and the mass of caffeine extracted.

Ultrasonic-Assisted Extraction

Ultrasonic extraction is a highly efficient method for isolating caffeine and other phytochemicals from plant matrices. The application of medium to high-intensity ultrasound enhances the extraction process by

increasing yield and reducing processing time. This method is considered cost-effective and straightforward, offering considerable advantages over conventional extraction techniques.

The primary benefits of ultrasonic-assisted extraction (UAE) include improved extraction efficiency and accelerated kinetics. These effects are attributed to ultrasonic cavitation, which increases the surface contact between the solvent and plant material by disrupting cellular structures. As a result, the permeability of plant tissues is enhanced, facilitating greater mass transfer. UAE is frequently used in combination with organic solvents to further improve extraction yields, especially in solid–liquid extraction processes [1–6].

II. MATERIALS AND METHODS

The present study aimed to determine the caffeine content of natural sources, namely 3 assortments of tea and 2 assortments of Arabica coffee, shown in Table 1 below:

Table 1. Samples of Tea and Coffee Varieties

Sample	Code
Black Indian tea	P_1
Black Russian tea	P_2
Green tea	P_3
Coffee with caffeine	P_4
Decaffeinated coffee	P_5

1. Dichloromethane-Based Extraction

The caffeine extraction procedure using dichloromethane (CH_2Cl_2) involves two main stages:

1.Aqueous Alkaline Extraction: Tea leaves are subjected to hot aqueous extraction in the presence of sodium carbonate (Na_2CO_3), which facilitates the dissolution of hydrophilic compounds, including caffeine. Following extraction, the solution is allowed to cool and is subsequently filtered to remove insoluble residues.

2.Organic Solvent Extraction: The filtered aqueous extract is then subjected to liquid-liquid extraction using dichloromethane, an organic solvent immiscible with water. Caffeine demonstrates a significantly higher solubility in dichloromethane (approximately 140 mg/L) compared to water (approximately 22 mg/L), favoring its selective partitioning into the organic phase. To enhance the purity of the extracted caffeine, residual tannins are precipitated by treatment with anhydrous sodium carbonate, followed by careful phase separation.

2. Ultrasonication-Assisted Solvent Extraction

An alternative extraction approach explored in this study involves a combined method—ultrasonic treatment followed by solvent extraction. Ultrasonication was carried out using a PRO 70 ultrasonic bath operating at a frequency of 40 kHz. The samples were sealed in airtight containers and subjected to ultrasonic treatment for two durations: 5 minutes and 10 minutes, respectively [7–10].

During the 5-minute sonication, the bath temperature increased from 20^0C to 29^0C, while in the 10-minute treatment, the temperature rose from 20^0C to 31^0C. The increase in temperature during sonication contributes to enhanced mass transfer and disruption of cellular structures, facilitating more efficient caffeine release from plant tissues.

3. Sample Characterization

• Moisture Content Determination:

Moisture content was assessed using the oven-drying method to calculate the dry matter basis for caffeine quantification. An IKA Oven 125 Basic-Glass ventilated laboratory oven was used to dry the samples to constant mass at 105^0C.

•Titratable Acidity Determination:

The titratable acidity of the aqueous extracts was measured via standard titrimetric analysis and expressed in milliliters of 0.1 N NaOH per 100 grams of sample.

III. RESULTS AND DISCUSSION

Following the extraction of caffeine by the dichloromethane method from the ultrasonated samples we obtained the results shown in Table 2 and Figure 1.

Table 2. Caffeine concentration of native samples relative to dry matter

Sample	P_1- Black Indian tea	P_2- Black Russian tea	P_3- Green tea	P_4- Coffee with caffeine	P_5- Decaffeinated coffee
Caffeine %	1.0229	0.0736	0.1875	0.3475	0.0476
Dry matter %	92.7775	91.3127	93.6092	95.7052	96.0696
Caff % DM %*	0.9489	0.0672	0.1755	0.3325	0.0457

*Caffeine% referred to dry matter

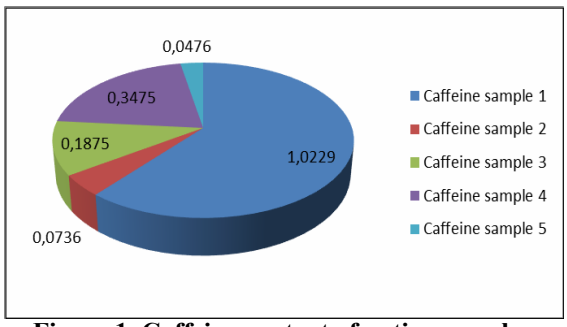

Figure 1. Caffeine content of native samples

As illustrated in Figure 1, the highest caffeine concentration among all tested samples was observed in Indian black tea, with a content of 1.0229%. As expected, the decaffeinated coffee sample still contained a measurable amount of caffeine; however, its caffeine content was approximately seven times lower than that of the regular coffee sample. Among all analyzed samples, Indian black tea exhibited the highest caffeine concentration.

Moisture content was also evaluated in samples subjected to ultrasonication for 5 and 10 minutes. The variation in moisture content across these ultrasonicated samples was within ±0.5%, a difference deemed negligible. Therefore, the caffeine content in these samples was normalized relative to the dry matter of the untreated (native) samples.

Caffeine extraction from the samples ultrasonicated for 5 minutes was subsequently carried out using the dichloromethane method. The resulting data are presented in Table 3 and Figure 2.

Table 3. Caffeine concentration of 5 minute ultrasound samples relative to dry matter

Sample	P_1- Black Indian tea	P_2- Black Russian tea	P_3- Green tea	P_4- Coffee with caffeine	P_5- Decaffeinated coffee
Caffeine %	0.3960	0.3433	0.2918	0.5969	0.1020
Dry matter %	92.7775	91.3127	93.6092	95.7052	96.0696
Caff % DM %*	0.3673	0.3134	0.2731	0.5734	0.0979

*Caffeine% referred to dry matter

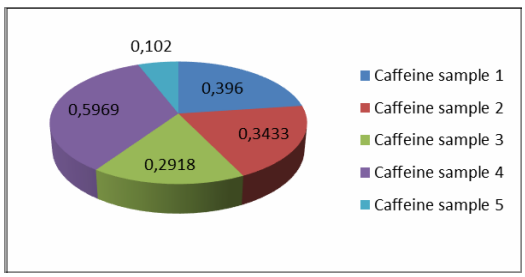

Figure 2. Caffeine content of samples treated with ultrasound for 5 minutes

As shown in Figure 2, a noticeable increase in caffeine content was observed in most samples subjected to 5 minutes of ultrasonication. Among the five samples analyzed, the highest caffeine concentration was obtained from sample P4, followed by the black tea samples, indicating that short-duration ultrasonic treatment enhances extraction efficiency, likely due to improved solvent penetration and cellular disruption.

Subsequent extraction of caffeine from samples ultrasonicated for 10 minutes, using the dichloromethane method, yielded the results presented in Table 4 and Figure 3.

Table 4. Caffeine concentration of 10 minutes sonicated samples relative to dry matter

Sample	P_1- Black Indian tea	P_2- Black Russian tea	P_3- Green tea	P_4- Coffee with caffeine	P_5- Decaffeinated coffee
Caffeine %	0,0949	0,1859	0,0862	0,5398	0,1109
Dry matter %	92,7775	91,3127	93,6092	95,7052	96,0696
Caff % DM %*	0,0880	0,1697	0,0806	0,5185	0,1065

*Caffeine% referred to dry matter

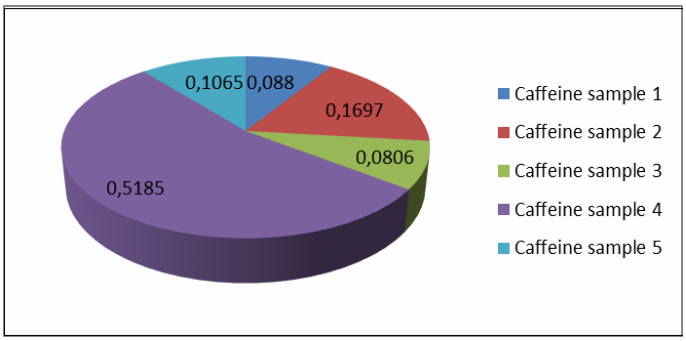

Figure 3. Caffeine content of 10 minutes ultrasonic samples

As illustrated in Figure 3, sample P4 continues to exhibit the highest caffeine concentration (0.5185%) among all tested samples, maintaining its superior extraction efficiency following 10 minutes of ultrasonication. However, a reduction in caffeine yield was observed for Russian black tea and green tea compared to their counterparts ultrasonicated for 5 minutes, suggesting a possible degradation or diminished extraction efficiency with prolonged ultrasonication.

A comprehensive comparison of the caffeine content across all samples studied is presented in Table 5 and Figure 4. Notably, Indian black tea exhibited a decline in caffeine content following ultrasonication, whereas the decaffeinated coffee sample showed a slight increase in caffeine concentration. Overall, ultrasonication for 5 minutes yielded higher caffeine extraction efficiencies across all samples compared to 10-minute treatment.

For both ultrasonication durations, the extracted caffeine content was normalized to the dry matter content, as determined by moisture analysis. The corresponding results are summarized in Table 6 and Figure 5.

Table 5. Caffeine content of native and sonicated samples [%]

Sample	Native sample	Sonicated sample for 5 minutes	Sonicated sample for 10 minutes
P_1	0.9489	0.3673	0.0880
P_2	0.0672	0.3134	0.1697
P_3	0.1755	0.2731	0.0806
P_4	0.3325	0.5734	0.5185
P_5	0.0457	0.0979	0.1065

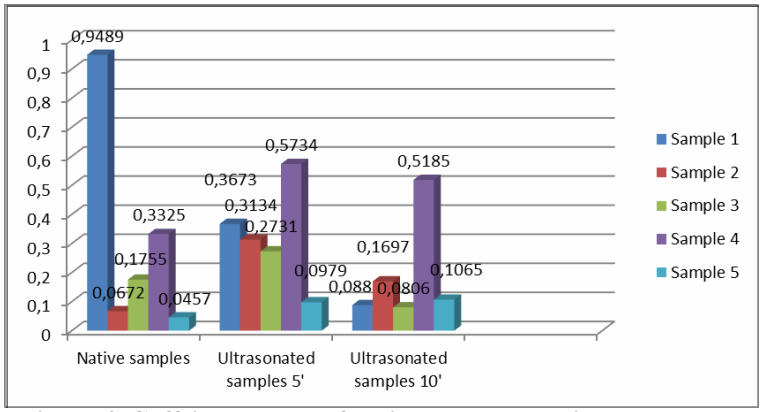

Figure 4. Caffeine content of native and ultrasonicated samples

Table 6. The moisture and dry matter content of samples

Sample	P_1- Black Indian tea	P_2- Black Russian tea	P_3- Green tea	P_4- Coffee with caffeine	P_5- Decaffeinated coffee
Moisture %	7,2225	8,6873	6,3908	4,2948	3,9304
Dry matter %	92,7775	91,3127	93,6092	95,7052	96,0696

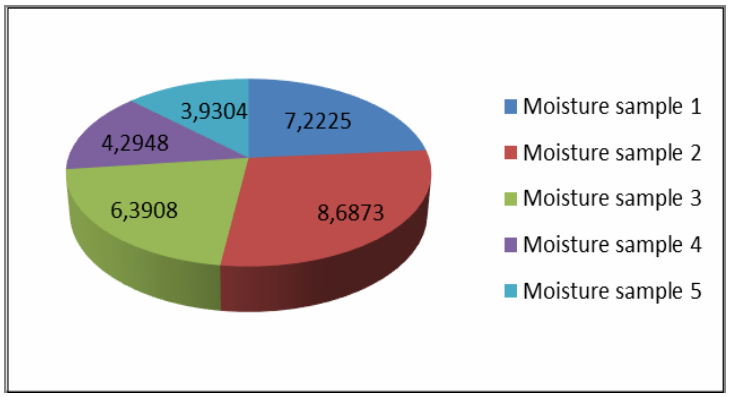

Figure 5. The moisture content of samples

Of the 5 samples (Figure 5), decaffeinated coffee, sample P5 (3.9304%) and green tea (6.3908%) had the lowest moisture content. The acidity of the samples is of interest to the consumer, as it may have gastric repercussions in case of high daily consumption. The titratable acidity of the aqueous extracts is shown in Figure 6.

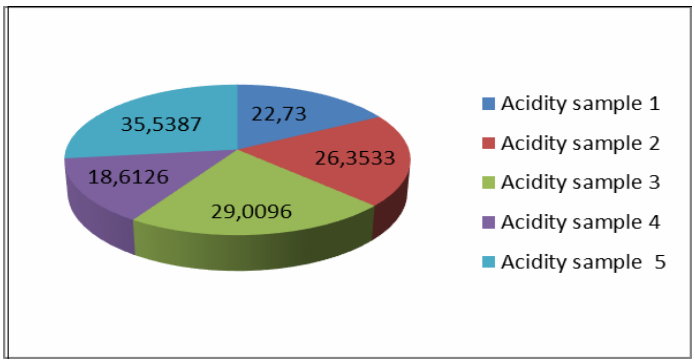

Figure 6. The titratable acidity of native samples

The titratable acidity of the samples analyzed revealed notable differences between tea and coffee varieties. Green tea exhibited the highest titratable acidity among the tea samples, with a value of 29.0096 mL/100 g, compared to 26.3533 mL/100 g for black tea. Among the five samples evaluated (Figure 6), decaffeinated coffee (P5) showed the highest titratable acidity, reaching 35.5387 mL/100 g, which is nearly twice the acidity observed in coffee sample P4 (18.6126 mL/100 g).

The titratable acidity values of samples subjected to 5 minutes of ultrasonication are presented in Figure 7, illustrating the effect of ultrasonic treatment on the acidity profiles of the different matrices.

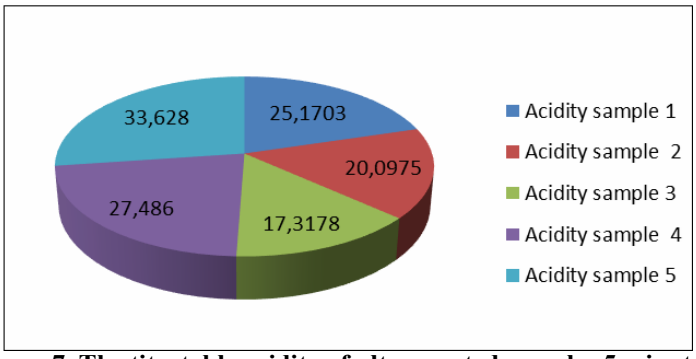

Figure 7. The titratable acidity of ultrasonated samples 5 minutes

As illustrated in Figure 7, the titratable acidity of the samples subjected to 5 minutes of ultrasonication differs from that of the untreated (native) samples, without exhibiting a consistent or linear trend. Specifically, Indian black tea (P1) demonstrated an increase in acidity, while tea samples P2 and P3 showed a decrease. A similar pattern was observed among the coffee samples: sample P4 exhibited an increase in titratable acidity, whereas decaffeinated coffee P5 showed a reduction.

In contrast, Figure 8 indicates that all samples ultrasonicated for 10 minutes displayed a significant increase in titratable acidity compared to both the native samples and those treated for 5 minutes. These results suggest that extended ultrasonication may promote the release of acidic compounds or enhance hydrolytic reactions within the matrix.

A comprehensive comparison of the titratable acidity values for all samples, across all treatments, is presented in Figure 9.

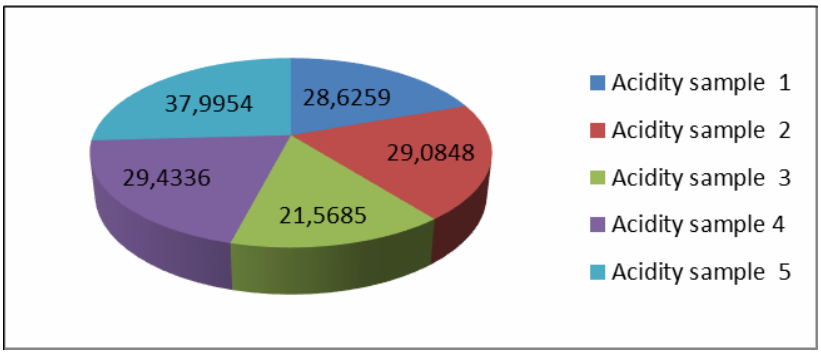

Figure 8. The titratable acidity of ultrasonicated samples 10 minutes

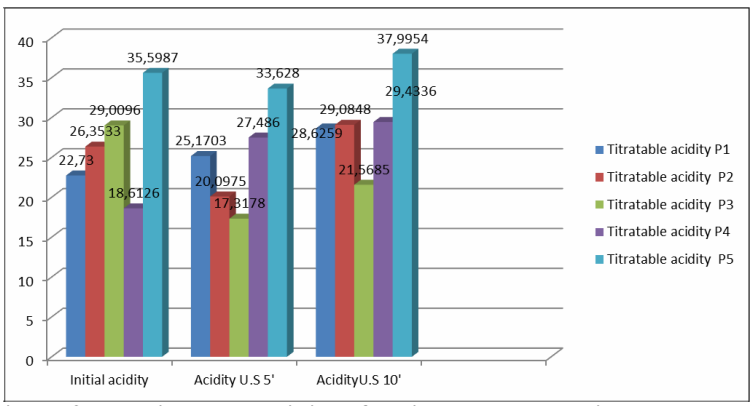

Figure 9. The titratable acidity of native and ultrasonicated samples

IV. CONCLUSION

Extraction using organic solvents remains the most prevalent and cost-effective method for quantifying caffeine content in natural sources. However, the use of highly toxic solvents such as chloroform and methylene chloride has declined due to associated health and environmental risks. In this context, supercritical fluid extraction (SFE) presents a promising alternative, offering several advantages including non-toxicity, solvent-free extracts, and high selectivity for caffeine.

Ultrasound-assisted extraction is an economical and straightforward technique which, when combined with conventional extraction methods, can significantly enhance caffeine yields. This approach is particularly effective for achieving advanced decaffeination of native samples such as coffee and black tea.

Key findings from this study include:

• Among the tested samples, Indian black tea (P1) exhibited the highest caffeine content, approximately three times greater than other samples, while green tea (P3) contained roughly half the caffeine content of native coffee (P4).

• Decaffeinated coffee (P5) showed the lowest caffeine concentration, approximately seven times lower than native coffee (P4). Russian black tea (P2) contained nearly double the caffeine content of the decaffeinated coffee, confirming the effectiveness of the decaffeination process employed by the manufacturer.

• Ultrasonic pretreatment at 40 kHz for 5 minutes increased caffeine extraction by approximately 45% across all samples except Indian black tea, for which caffeine content decreased by about 60%.

• Prolonged ultrasonication (10 minutes at 40 kHz) resulted in a substantial reduction in caffeine yield for most samples, accompanied by a notable increase in titratable acidity. The exception was decaffeinated coffee, where caffeine content after 10 minutes of ultrasonication remained comparable to the 5-minute treatment and doubled relative to the native sample.

These results suggest that ultrasonic treatment parameters should be optimized individually for each botanical source, considering factors such as species, sample type, and prior processing (e.g., drying, fermentation) to maximize caffeine extraction efficiency.

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