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Review Article

An Overview of Techniques for Extracting Caffeine from Coffee for Quantification

Kasahun Wale*, Bealu Girma

Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia

Email address:

kasahun322@mail.com (Kasahun Wale)

*Corresponding author

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Abstract: Coffee is one of the most important agricultural commodities. Of the coffee alkaloids, caffeine is the most common component. Caffeine in coffee affects the central nervous system, heart muscle, respiratory system, and stomach secretion, impacting health and defining the quality of the beverage. However, excessive caffeine consumption has health hazards such as worsening heart disease and raising blood pressure. Thus, the level of Caffeine in coffee determines its quality. The objective of this paper is to compare different extraction methods for caffeine. Several traditional (e g., conventional heat reflux extraction, fusion) and modern (e g., soxhlet extraction, Microwave Assisted extraction, MAE) methods have been optimized & reported to extract Caffeine from coffee before chromatographic and spectroscopic analysis. Conventional Extraction methods are time-consuming, offer lower recovery yields, and use more solvent, whereas modern Extraction methods are faster, more efficient, and give the maximum recovery yield. Microwave Assisted Extraction (MAE) has better Caffeine Extraction efficiency with similar time, and it uses less solvent. But it uses a power supply, in addition to time, temperature, and solvent, whereas with Conventional heat reflux extraction, there is no need for a power supply. On the other hand, water Extraction is better for being economically and environmentally friendly (non-toxic and easily available) as well as greater dielectric constant and polarity than alcohol. For Caffeine quantification, hyphenated methods such as HPLC and electro-analytical methods are preferable. Modern extraction methods are better for their efficiency, time, and volume of solvent required, while modern quantification methods are better for their accuracy and precision.

Keywords: Coffee, Caffeine, Extraction, HPLC, Quantification

1. Introduction

The story of coffee was beginnings in Ethiopia. The country is the original home of the coffee plant; coffee Arabica, which still grows wild in the forest of the highlands of Ethiopia [2]. Coffee is one of the most important agricultural products in international trade. It is a commodity of great economic, social and environmental importance to coffee cultivating countries, particularly for developing countries, like Ethiopia, which generates 60% of its total export earnings [2, 9, 13].

The two main species of coffee exploited in the world are Coffea arabica and Coffea canephora (robusta). They account for as large as 99% of the world's coffee, of which 75% is Coffea arabica and 24% Coffea canephora (robusta) types [8, 11, 18]. Other less cultivated species of Coffea include Coffea liberica, Coffea abeakutyae, Coffea dewevrei, Coffea congensis, etc. Different species of Coffea genus have very diverse appearances and behaviours [19]. Coffee is one of the most traded commodities and one of the world's most popular drinks due to its distinct flavour and sensory features. Coffee beans are a good source of caffeine, which is the most widely, consumed alkaloid in the world, second only to oil in terms of global commodity trading. Coffee is one of the most popular drinks in the world today. It includes about 700 components that contribute to its fragrant and distinct flavour. The most important Coffea species are Coffea Arabica and Coffea conephora, which account for 60-40% of global production [3]. The World coffee consumption has progressively increasing, due to its appreciated taste, aroma, and beneficial effects on health and its refreshing and stimulating effect. Few of the significant bioactivities documented are antioxidant activity, anticarcinogenic activity, antimutagenic activity etc. [1, 2]. As a functional food with antioxidant properties, coffee reduces the incidence of cancer, diabetes, and liver disease, protects against Parkinson's disease, and reduces mortality risk [8]. Furthermore, consumption of caffeinated and decaffeinated coffee was associated with a lower risk of T2D [4].

Ethiopia is the first largest coffee producer in Africa and ranks fifth in the world, next to Brazil, Vietnam, Colombia, and Indonesia. In other words, Ethiopians contributed about 4.2% of the total world coffee production [16].

Coffee Alkaloids

Alkaloids such as Caffeine (1, 3, 7-trimethylxanthine), theobromine (3, 7-dimethylxanthine), and theophylline (1, 3-dimethylxanthine) are natural alkaloids found in tea leaves, coffee, and cacao seeds, as well as the food and beverages prepared from these ingredients. They are found in a variety of pharmaceutical goods and medications because of their ability to stimulate the central nervous system, generate gastrointestinal secretions, and serve as a diuretic. These alkaloids have also been studied for their antioxidant effects [14]. Form different types of alkaloids; Caffeine is a major constituent of coffee components. In this review we will focus on methods of Extraction and quantification of Caffeine from coffee.

Caffeine chemical formula Its chemical formula is $C_8H_{10}N_4O_2$; (1, 3, 7-trimethylxanthine) is a naturally occurring chemical found in the leaves, seeds, or fruits of over 63 plant species around the world. The coffee bean, which is the seed of the coffee plant, is the world's principal source of caffeine. Green coffee beans from Coffea arabica have between 0.7 and 1.6% caffeine, while green coffee beans from Coffea robusta contain between 1.5 and 4.0% caffeine. It is obtained from a variety of sources, the most frequent of which are coffee, tea, and soft drinks. It was found in coffee, tea, cocoa, chocolate, soft drinks, energy drinks, and medications on a daily basis. Caffeine works as a central nervous system stimulant, increasing alertness, decreasing sleep, improving short-term memory, and increasing the efficacy of certain medications [20].

Caffeine activates the central nervous system, heart muscle, lungs, and stomach secretion. The daily recommended dose is 200 mg. A deadly dose of 10 g is comparable to around 100 cups of coffee. Caffeine use has been linked to an increased incidence of miscarriage in recent epidemiological research. Caffeine in high amounts has been linked to a variety of problems affecting the central nervous system, cardiovascular system, increased stomach secretion, and poor liver function. This drug has the potential to cause addiction and anxiety. Caffeine is considered one of the prohibited nervous system stimulants by a lot of athletic organizations since it improves performance while decreasing weariness. However, because it is part of a typical daily diet, a urine concentration level of 12 g/L is permissible. Many cola-based enterprises employ this addition by stating that caffeine improves the aroma, despite the fact that at such a high concentration, only a small fraction of customers (about 8%) benefit from it [14]. Caffeine is under the class of compounds called alkaloids by organic chemists. Alkaloids are basic nitrogen-containing chemicals found in plants. They usually have a bitter taste and are frequently physiologically active in humans. Caffeine is a mild stimulant and diuretic that is regularly utilized in patent medications for the stimulating effect to prevent sleepiness. It is also the most widely used psychoactive a chemical and can be a modest stimulant of the central nervous system. Caffeine can be contained in a variety of foods that individuals consume. Caffeine is a stimulant. It is a diuretic that stimulates the heart, breathing, and the central nervous system. Its use can result in anxiety, sleeplessness, and headaches. It has an actual addicting substance.

Caffeine extracted in this manner can then be transformed into objects we enjoy in our daily lives, such as sodas, cosmetics, and pharmaceutical products. Caffeine is primarily consumed by diet pills and cosmetic industries. It can also be used to make body wash, soap, lip balm, facial scrub, and a variety of other goods, including caffeine lipstick. Caffeine use has been linked to health risks such as worsening heart disease and raising blood pressure [7, 18].

2. Types of Extractions

Extraction is an important process for determining and characterizing different compounds from different matrixes. It can either be traditional (conventional) or new (modern). For numerous years, traditional procedures such as maceration, boiling, soaking, and Soxhlet have been employed. Still they are inefficient because they are time-consuming, offer lower recovery yields, and use more solvents. To address the shortcomings of conventional methods, new (modern) extraction techniques such as Microwave-assisted Extraction (MAE), Solid phase Extraction (SPE), Ultrasound-assisted Extraction (UAE), Accelerated solvent Extraction (ASE), and Supercritical fluid Extraction (SFE) are being used. In addition, when compared to traditional approaches, all unconventional methods are faster, more efficient, and give the maximum recovery yield. The optimal extraction method is chosen based on the highest recovery, with the least amount of chemicals degradation due to multiple process steps [6]. Extraction processes are maceration, infusion, Digestion, decoction, Percolation, Soxhlet Extraction, fermentation, counter-current extraction (CCE), Ultrasound Extraction and Supercritical Fluid Extraction (SFE). This review is mainly focused on the various features of the Soxhlet Extraction technique [12].

Extraction of Caffeine

Solvents such as chloroform, methyl chloride, ethanol, acetone, and ethyl acetate are commonly employed for solvent extraction of caffeine. For this reason, a number of approaches can be used such as Soxhlet Extraction, Fusion, Heat Reflux extraction, Ultrasound-Assisted Extraction & Microwave-Assisted Extraction.

2.1. Soxhlet Extraction

Soxhlet extraction is also known as the hot continuous extraction process the main advantage of this method is complete Extraction in minimum amount of solvent. Extraction solvent is taken in the round bottom flask and heated by using heating source like heating mantle. The heating temperature is built on the solvent employed to Extraction [12].

2.2. Experimental Method for Caffeine Extraction by Soxhlet Extraction

Green coffee beans were crushed, and introduced to the extraction thimble. 100 ml of dichloromethane was placed in the flask. The Extraction lasted for 30 minutes, after which the organic solution was left to cool to room temperature and filtered. The filtrate was distilled in vacuum at a bath temperature of 60°C in order to remove the solvent used for the Extraction. The distillation residue was taken up in 6 ml of chloroform. The obtained solution was diluted to a fixed volume and subjected to HPLC analysis.

2.3. Water Extraction by Boiling (Fusion)

Boiling the coffee power using hot water has been reported as one alternative method of caffeine Extraction. Usually, up to 20 g of coffee was used to extract in 100 mL of boiling water for about 10 min. In some cases, the water Extract is directly injected to HPLC, whereas, for HPLC and other techniques, Caffeine was re-extracted from the aqueous solution into dichloromethane under alkaline conditions. Such pre-treatment stage is used to selectively Extract Caffeine in the organic phase, while leaving other constituents such as chlorogenic acid, trigonelline and others in the aqueous phase [6].

This technique seems simple, but it needs several volumes of organic solvent for the clean-up stage. Secondly, it might be susceptible to analyte loss during the liquid-liquid extraction phase. This study also compared the result obtained by Soxhlet extraction of the sample. It was reported that a significantly higher concentration of Caffeine was extracted in case of hot water infusion (1.6 mg/mL) as compared to Soxhlet extraction (0.212 mg/L) [6].

2.4. Microwave Assisted Extraction

Microwave-assisted Extraction (MAE) is a process that separates target compounds from number of matrices by coupling microwave energy with a solvent. In contrast to conventional extracts, the highly localized temperature and pressure can trigger selective migration of target molecules from the material at a faster rate, resulting in enhanced extracts. MAE can be greatly decreased both time and solvent use compared to the conventional method. In Microwave-assisted Extraction; solvent, time, temperature, and microwave power (Wattage) on Extraction have great impact on the yield of Caffeine.

Table 1 shows effects of the solvent, time of MAE radiation, temperature, microwave power (Wattage) on% Yield of MAE Extract and% yield of Caffeine.

Table 1. *Yields of MAE extracts of Caffeine.*

Exp. Set	Variable parameter	Constant parameters	Variables	% Yield caffeine
			MeOH	3.44 ± 0.07^{b} (38.10)
	Solvent	Time (5 min), Temp (50 C) Wattage (800 W)	EtOH	3.05 ± 0.07^{a} (34.08)
			H_2O	7.25 ± 0.07^{c} (40.06)
2	For aqueous extracts			
			2 min	3.75 ± 0.07^{a} (21.87)
2a	Time	Temperature (50°C), Wattage (800 W)	5 min	4.6 ± 0.14^{c} (26.29)
			10 min	4.4 ± 0.14^{b} (25.81
			30°C	6.68 ± 0.07^{b} (38.06)
2b	Temp	Time (5 min) Wattage (800 W)	50°C	7.25 ± 0.07^{b} (40.17)
			70°C	4.75 ± 0.07^{a} (26.99)
			90°C	4.65 ± 0.07^{a} (27.76)
			400W	3.7 ± 0.14^{a} (24.03)
2c	Power supply	Time (5 min) Temperature (50°C)	600W	4.25 ± 0.07^{b} (23.94)
			800 W	7.25 ± 0.07^{c} (39.81)

[17].

When water was employed as a solvent, the Extraction yield was maximum (40.06%), when compared to the other solvents, which were in the range of 34.08 - 38.10%. The reason could be water has to have a greater dielectric constant and polarity than alcohol, which aids in microwave absorption. The above result indicates the amounts of extracts varied by changing Extraction time. Caffeine value is higher at 5 minute Extraction time. Thus the optimum time was 5 minutes.

The temperature of MAE has an effect on extraction efficiency. The above table shows the experimental results at various temperatures (30, 50, 70, and 90°C). At of 50°C, the entire Extraction efficiency is at its maximum intensity peak. At the same temperature, Caffeine in microwave-assisted Extracts is at its highest. The above table indicates that the Extraction temperature is optimum at 50°C.

It was noted that Extraction power was also affected the extraction efficiency. As the Extraction power increased, the yield of Caffeine was also increased. As per the report, 800 W yielded better efficiency as compared to 400 W and 600 W. Furthermore, the authors did not show the maximum power limit that gives the maximum yield.

Instead, from the report, it was not an increase in yield with an increase in microwave power.

In general, water as a solvent and higher Microwave power is the better choice for good Extraction yield. In doing so, 5 min and 50°C are optimum conditions. Table 2, compares the conventional extraction (refluxing) and MAE using water as a solvent.

Table 2. *Comparative analyses between conventional heat reflux extraction and MAE.*

Exp. Set	Method of Extraction	Parameters	% of Caffeine
I	Conventional heat reflux Extraction	Time (5min), Temp. (50°C), Sample: solvent (1: 4)	3.05 ± 0.21^a
II	Microwave-assisted Extraction	Time 5min), Temp. 50°C, Wattage (800W), Sample: solvent (1: 4)	7.25 ± 0.07^b

[17].

The optimal MAE extraction time, temperature, and wattage were determined to be 5 minutes, 50 degrees Celsius, and 800 watts.

Table 2 compares the extraction efficiency of and caffeine using the standard heat reflux method vs MAE under ideal conditions.

MAE yields were found to be higher for water than for alcoholic solvents such as ethanol and methanol. This could be owing to differences in dielectric constants of the liquids.

The comparative findings (Table 2) clearly reveal that MAE produces much higher and better extraction yields than the standard heat reflux method for caffeine extraction under optimal extraction circumstances [17].

3. Determination of Caffeine from Coffee

For determination of caffeine content, different methods of quantification have been developed. UV/Visible spectrophotometer, near infrared ray spectroscopy (NIRS), high performance liquid chromatography (HPLC), gas chromatography coupled with mass chromatography (GC-MS), proton nuclear magnetic resonance (H-NMR), electroanalytical technques such as Cyclic voltametry (CV), Pulse voltammetry (PV) are some of the quantification methods.

Table 3. *Different analytical methods Caffeine quantification.*

Methods	Type of sample	Range of caffeine concentration (%w/w)	References
UV/VIS	Green coffee	0.60 - 1.20	[10]
FTIR-ART	Green coffee	1.465- 1.629	[20]
HPLC-UV	Green coffee	0.87 - 1.38	[5]
NIR spectroscopy	Green coffee	1.44 - 1.68	[20]
Fluorescence spectroscopy	Green coffee	1.434 – 1.530	[20]
Electro analytical method	Green coffee	0.089 – 0.164	[15]

Them most common quantification methods for caffeine are spectroscopic methods and HPLC. The conventional late quantification methods are UV/Vis method and IR methods, whereas the hyphenated methods are HPLC, GC/MS and Electro analytical methods. The drawbacks of NIR spectroscopy is that it predict but not record the accurate result. But its advantage is it can analyze more than 100 samples per a day. In view of analytical chemistry, its accuracy is low and not advisable. But it may be important for those who analyzes bulky samples and for breeders.

From the above table, the quantification of Caffeine is from green coffee. It is better to determination of caffeine from roasted coffee.

4. Industrial Applications of Caffeine

Here are a few examples: Caffeine is primarily consumed by diet pills and cosmetics firms. It's used in body wash, soap, lip balm, facial scrub, and a variety of other items, including caffeine lipstick. When paired with pain drugs, it is also used to treat minor headaches and to prevent and manage migraines after epidural anesthesia. Caffeine is also used to help with weight loss and type 2 diabetes treatments. In dermatitis, caffeine creams are applied to the skin to relieve redness and itching [18].

5. Conclusion and Recommendation

From this paper it can be concluded that conventional methods of Extractions are most commonly inefficient. because they are time-consuming, offer lower recovery yields, and use more solvents. Therefore unconventional (Modern) methods are better. In other way the effects of variables that contribute in Extraction are solvents, duration of extraction time, temperature and power supply have an impact on efficiency of Caffeine. From thus solvent types and duration of extraction time have greatest effect on of Caffeine, however temperature and power supply have little effect on of% Caffeine.

In other hands the quantification methods, the hyphenated methods like HPLC and electrochemical methods like CV and DPV are preferred for quantification of caffeine.

Generally from the findings, we recommend that unconventional (modern) extraction methods are better for their efficiency, time, and amount of solvent they need and quantification methods for their better accuracy, precision, detection limits.

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