Chapter

A Detail Chemistry of Coffee and Its Analysis

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Abstract

This review article highlights the detailed chemistry of coffee including its components; chemical constituents like carbohydrates, proteins, lipids, and caffeine; aromatic principles; oil and waxes; and minerals and acids. The high extent of caffeine can be found in the coffee plants; hence, in the second part of the study, various analytical methods are designed for the proper identification, separation, optimization, purification, and determination of caffeine present in coffee, tea, and marketed coffee. These analytical methods are appropriated for the separation and quantification of caffeine. The various analytical methods include spectroscopy methods like UV, IR, and NMR spectroscopy; chromatographic methods like paper, TLC, column, HPLC, and gas chromatography; and hyphenated techniques like LC–MS, GC–MS, and GC–MS/MS. This article compares and contrasts the amount of caffeine by various analytical methods.

Keywords: caffeine, spectrophotometer, chromatography, hyphenated techniques, electrochemical methods

1. Introduction

Coffee consists of ripe seeds of *Coffea arabica* Linn., belonging to family Rubiaceae. Coffee extracted from coffee bean is also present in crimson fruits is completely removed, and the spermoderm is removed, occasionally. The seeds of botanical genus *Coffea* may be raw, roasted, whole, or ground. The prepared drink through such coffee seeds is also called as coffee. Among 70 species of coffee, only three are cultivated. 75% of the world's production of coffee is provided by *Coffea arabica*, about 25% by *Coffea canephora*, and less than 1% by *Coffea liberica* and others. Generally, coffee is cultivated at the altitude of 1000–2000 [1]. It is indigenous to Ethiopia, Brazil, India, Vietnam, Mexico, Nepal Guatemala, Indonesia, and Sri Lanka.

2. Chemical constituents

The main constituents of coffee are caffeine, tannin, fixed oil, carbohydrates, and proteins. It contains 2–3% caffeine, 3–5% tannins, 13% proteins, and 10–15% fixed oils. In the seeds, caffeine is present as a salt of chlorogenic acid (CGA). Also it contains oil and wax [2].

The following sections will be discussed in detail after acceptance of this short proposal:

- This article will deal on the types of carbohydrate, protein, lipids, and other chemical constituents in detail.

- This article will review on various analytical methods for the estimation of constituents present in coffee.

Coffee is often used as antioxidants, but more importantly coffee is a good source of chromium and magnesium that assist in controlling blood sugar by ensuring proper usage of insulin.

The main chemical ingredients in coffee beans are given below:

- Caffeine
- Tannin
- Thiamin
- Xanthine
- Spermidine
- Guaiacol
- Citric acid
- Chlorogenic acid
- Acetaldehyde
- Spermine
- Putrescine
- Scopoletin

The carbohydrate content of green and roasted coffee (Santos) was identified and measured. Green coffee contained about 6–7% of sucrose as soluble sugars and low amount of glucose. The soluble sugars of roasted coffee were sucrose, fructose, and glucose. The experiment was also carried out for the isolation of holocellulose fractions of green and roasted coffee.

The holocellulose of green coffee was hydrolyzed by a novel method consisting of anhydrous sulfuric acid and 10% potassium insoluble hydroxide, which was partially solubilized on roasting and results in the following ratio of sugars:

1 L-arabinose/2D-galactose/2D-glucose/6D-mannose. Out of these sugars, the arabinose was easily acid-hydrolyzed. Other coffee constituent analyzed and determined were caffeine, trigonelline, caffeic acid, chlorogenic acid, isochlorogenic acid, and the 10 amino acids. The free amino acids disappeared in roasting. An analytical method was developed for evaluating caffeine on chromatograms [3].

In coffee pulp, condensed tannins are the major phenolic compounds, while in the seeds, phenolic compounds exist primarily as a family of esters formed between hydroxycinnamic acids and quinic acid, collectively recognized as chlorogenic acids (CGA). Green coffee seeds contain up to 14% CGA, which are present in high concentrations and have a greater influence for determining the quality of coffee and play a vital role in the formation of the coffee flavor. The various constituents along with components of coffee are shown in **Table 1**.

Constituent	Components
Soluble carbohydrates	Monosaccharides Fructose, glucose, galactose, arabinose (traces)
Oligosaccharides	Sucrose, raffinose, stachyose
Polysaccharides	Polymers of galactose, mannose, arabinose, glucose Insoluble polysaccharides
Hemicelluloses	Polymers of galactose, arabinose, mannose Cellulose Acids and phenols Volatile acids
Nonvolatile aliphatic acids	Citric acid, malic acid, quinic acid
Chlorogenic acids	Mono-, dicaffeoyl- and feruloylquinic acid Lignin Lipids Wax
Oil	Main fatty acids: N Compounds
Free amino acids	Main amino acids: Glu, Asp, Asp-NH2 Proteins
Caffeine	Traces of theobromine and theophylline Trigonelline Minerals

Table 1.
Constituents along with components of coffee.

3. Carbohydrates

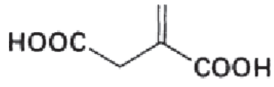
Most of the carbohydrates present, such as cellulose and polysaccharides consisting of mannose, galactose, and arabinose, are insoluble.

4. Lipids

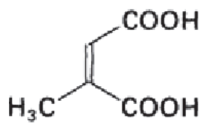
The lipid fraction appears to be very stable, and its composition is given below.
Linoleic acid is the predominant fatty acid, followed by palmitic acid.
Lipid composition.
Triacylglycerols.
Diterpene esters.
Diterpenes.
Triterpene esters.
Triterpenes (sterols).
Unidentified compounds.

5. Acids

The volatile acids include formic acids and acetic acids, while nonvolatile acids include lactic, tartaric, pyruvic, and citric acid. Minor constituents include higher fatty acids and malonic, succinic, glutaric, and malic acids. The degradation products of citric acid are itaconic (I), citraconic (II), and mesaconic acids (III), while fumaric and maleic acids are degraded products of malic acid:



I



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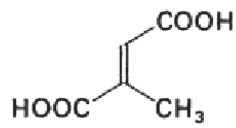

III

Chlorogenic acids are the mainly rich acids of coffee.

6. Trigonelline and nicotinic acid

Green coffee contains trigonelline (N-methylnicotinic acid) up to 0.6% and is 50% decomposed during roasting. The degradants include nicotinic acid, pyridine, 3-methyl pyridine, nicotinic acid, methyl ester, and other compounds.

7. Aromatic principle

The aroma profile of coffee is composed of the following notes: sweet/caramel-like, earthy, sulfurous/roasty, and smoky/phenolic.

8. Minerals

Potassium is major in coffee ash (1.1%), calcium (0.2%), and magnesium (0.2%). The major anions includes phosphate (0.2%) and sulfate (0.1%), along with traces of other elements [4].

9. Caffeine

The best known N compound is caffeine (1,3,7-trimethylxanthine) because of its physiological effects (stimulation of the central nervous system, increased blood circulation, and respiration). It is mildly bitter in taste. 10% of the caffeine and about 6% of the chlorogenic acid are present in a coffee drink. During roasting, the caffeine level in beans is decreased. Synthetic caffeine and caffeine obtained by the decaffeination process are used by the pharmaceutical and soft drink industries. By methylation of xanthine, synthetic caffeine is obtained which is obtained from uric acid and formamide. Medicinally, caffeine is used as a CNS stimulant, usually combined with another therapeutic agent and in analgesic preparations.

Theobromine acts as diuretic and smooth muscle relaxant, but not routinely used. Theophylline is used as smooth muscle relaxant and is frequently dispensed in sustainable formulations to lower the side effects. It is also available as aminophylline (a more soluble preparation containing theophylline with ethylenediamine) and choline theophyllinate (theophylline and choline). The alkaloids may be isolated from natural sources or obtained by total or partial synthesis [5].

The purine alkaloids include caffeine, theobromine, and theophylline as shown in **Figure 1**. They have a limited distribution as alkaloids, but the origins are very close with those of the purine bases like adenine and guanine, fundamental components of nucleosides, nucleotides, and the nucleic acids. Caffeine is mainly consumed in the form of beverages like tea, coffee, and cola and is most widely consumed and socially accepted natural stimulants. Theophylline is much more important as a drug compound because of its muscle relaxant properties, utilized in the relief of bronchial asthma when compared to caffeine, medicinally. The major constituent of cocoa and related chocolate products is theobromine.

Out of four nitrogen atoms, two are supplied by glutamine and a third by aspartic acid. The synthesis of the nucleotides AMP and GMP is by way of IMP and XMP, and the purine alkaloids then branch away via XMP. The loss of phosphate via methylation generates the nucleoside 7-methylxanthosine, which is then released from the sugar moiety. Furthermore, successive methylation on the nitrogen gives caffeine through theobromine, while a different methylation sequence can result in the formation of theophylline (**Table 2**) [6].

AMP = adenosine-5′-monophosphate.

GMP = guanosine-5′-monophosphate.

IMP = inosine-5′-monophosphate.

XMP = xanthosine-5′-monophosphate.

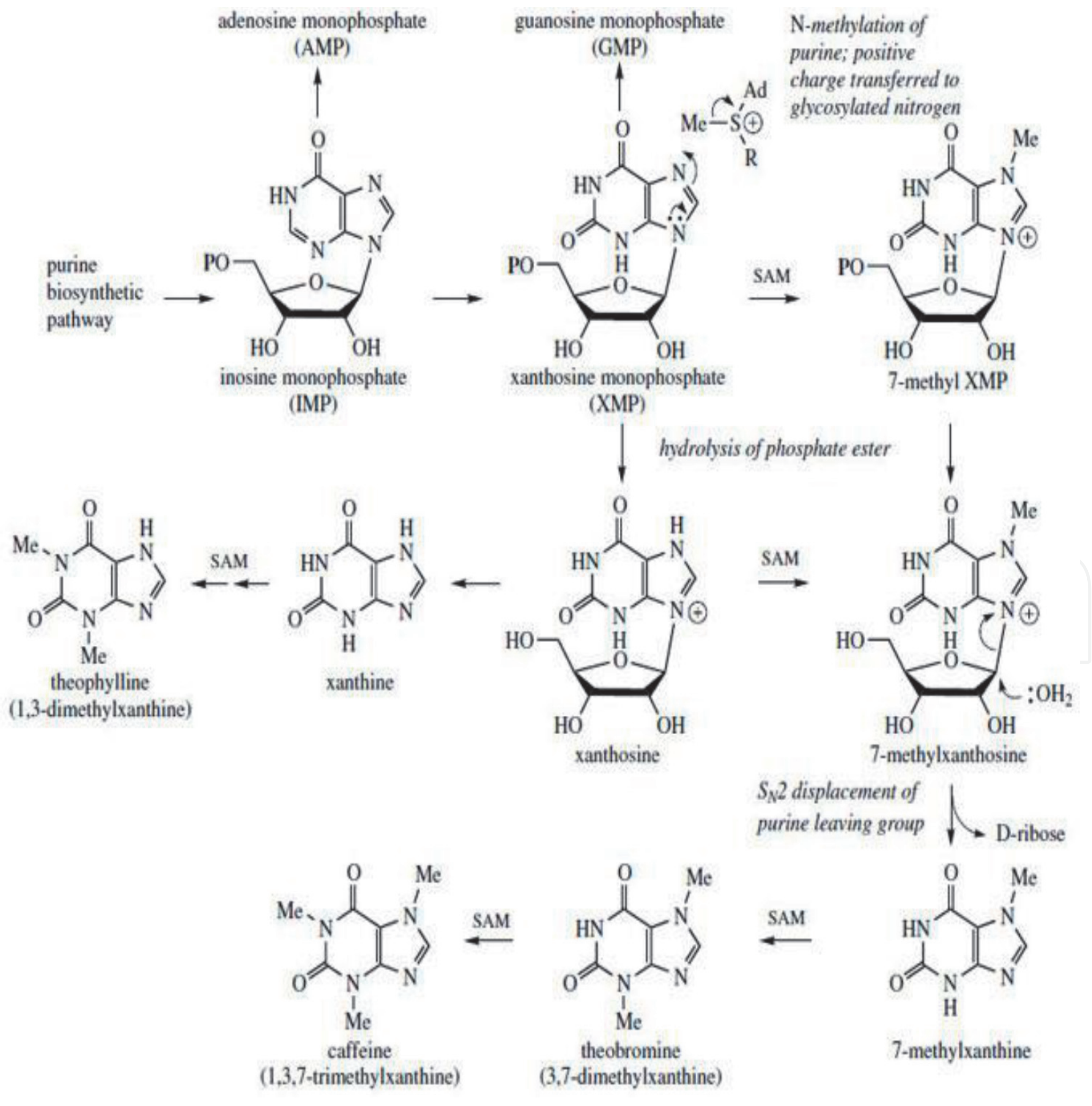

Figure 1.
Chemistry of the purine derivatives.

S.N.	Method	Experiment	Detection	Linearity range	Application	Scientific outcome	Ref.no.
1	UV spectroscopy	Caffeine separated from coffee using paper and TLC and was estimated using spectroscopy	Detection was done at 272 nm	NA	Caffeine from coffee	Good separation	[7]
2.	UV spectroscopy	Caffeine separated from coffee using TLC and was estimated using spectroscopy	Absorbance measured at 274 nm	2–120 μg/ml	Caffeine from tea powder	Good separation	[8]
3.	UV spectroscopy	Method A: simultaneous equation method Method B: isosbestic point method	For method A: absorbance measured at 273 nm For method B: absorbance measured at 259.5 nm	2–32 μg/ml	Tablet containing caffeine and paracetamol	Determination of caffeine in mixture of tablets	[9]
4.	UV spectroscopy	Dual wavelength method	Two wavelengths of 249 and 234 nm were selected for analysis LOD = 0.286 LOQ = 0.863	3–18 μg/ml	Tablet containing caffeine and paracetamol	A new method of determination of caffeine	[10]
5.	HPLC	RP-HPLC comprising C18 column and 24% methanol as mobile phase	UV detector at 272 nm	1–40 ppm	Unroasted coffee and roasted coffee	Unroasted coffee contained 0.89–2.10 (8 samples) Roasted coffee contained 1.03–4.21 (11 samples)	[11]
6.	HPTLC-UV	Silica gel 60F254 as stationary phase and ethyl acetate/methanol (27:3) as mobile phase	UV densitometric remission at 274 nm LOD = 40 ng/zone LOQ = 120 ng/zone	2–14 μg/zone	Caffeine in marketed tea granules	Caffeine in tea samples was found to be 2.145%	[12]
7	HPLC	Zorbax eclipse XDB comprising C8 column as stationary phase and water-tetrahydrofuran-acetonitrile as mobile phase	UV detector at 273 nm LOD = 0.07 LOQ = 0.20	0.2–100 mg/l	Caffeine, theobromine, and theophylline in food, drinks, and herbal products	The recoveries range from 92.00 to 96.8%	[13]

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S.N.	Method	Experiment	Detection	Linearity range	Application	Scientific outcome	Ref.no.
8	HPLC and biosensor method	For HPLC: Shimadzu LC10A fitted with a C18 column as stationary phase and acetonitrile and water (10:90%) as mobile phase set at a flow rate of 1 ml min^{-1}	UV detector set at 273 nm	0.01–0.1%w/v	Commercial coffee samples and cola drinks	0.033–0.072%w/v	[14]
		For biosensor: amperometricbiosensor comprising the biological sensing element, transducer, amplification, and detector systems		0.01–0.1%w/v		0.030–0.076%w/v	
9	HPLC	HPLC with solid phase extraction (SPE) HPLC model: Waters 515, with UV detector (REX, Model pHS-25), Visi TM-1 SPE single-sample processor (Supelco) 50 mM KH2PO4 (pH = 2) Acetonitrile and methanol (40:8:2) was used as solvent as well as mobile phase	Caffeine was extracted from green tea, black tea, and coffee and then characterized by melting point, λ max (UV/vis), IR absorption bands, Rf (TLC), and RT (HPLC) Crude caffeine was purified by solid phase extraction	10–60 ppm	Caffeine in tea, coffee, and soft drinks	Crude black tea, green tea, and coffee contained 7.04%, 4.88%, and 13.7% caffeine, respectively, whereas after purification black tea, green tea, and coffee contained 3.34%, 2.24%, and 5.20% pure caffeine	[15]
10.	HPLC and UV	UV/vis spectrophotometer The molar decadic absorption (MDA) coefficients and transitional dipole moment of pure caffeine in water and dichloromethane (DCM) were obtained at 272 and 274.7 nm	MDA was found to be 1115 and 1010 m^2 mol^{-1}, respectively, in water and DCM Transitional dipole moments of caffeine in water and in dichloromethane are 10.40×10^{-30} and 10.80×10^{-30} C m, respectively	0.90–1.10% for five samples by HPLC	Caffeine in coffee beans	UV/vis spectrophotometer: five independent measurements were 1.1 ± 0.01% for Bench Maji, 1.01 ± 0.04% for Gediyo Yirga Chefe, 1.07 ± 0.02% for Tepi, and 1.19 ± 0.02% for Godere, respectively HPLC: measurements were 1.10% for Bench Maji, 1.10% for Gediyo Yirga Chefe, 1.00% for Gomma Limu, and 0.90% for Besema	[16]

S.N.	Method	Experiment	Detection	Linearity range	Application	Scientific outcome	Ref.no.
11	HPLC with DAD	Stationary phase: RP-HPLC (Spherisorb ODS2 column) Mobile phase: 0.01 M phosphate buffer of pH 4	DAD detector at 265 nm LOD = 0.05 μg/ml	0.05–500 μg/ml	Thermal degradation of caffeine in coffee of Brazil and Ivory Coast	For Brazil: green coffee (g/kg of caffeine), 12.36 ± 0.10; roasted coffee, 16.12 ± 0.05 For Ivory Coast: green coffee (g/kg of caffeine), 20.83 ± 0.22; roasted coffee, 25.55 ± 0.185	[17]
12	HPLC	Stationary phase: RP-HPLC C18 Mobile phase: acetonitrile/water (8:92%)	Detection at wavelength of 245 nm.	Varies with each sample	Caffeine and theobromine in coffee, tea, and instant hot cocoa mixes	Instant tea: 32.4–35.0 mg/cup of caffeine Tea bag: 30.2–67.4 mg/cup, 1.0–7.8 mg/cup of caffeine Instant hot cocoa:46.7–67.6 mg/cup of caffeine Ground coffee: 93.0–163.5 mg/cup of caffeine	[18]
13	LC–MS	For LC stationary phase: Spherisorb S5ODS2, 5 μm Mobile phase: formic acid/ methanol For MS: ESI source with +ve mode	LOD = 11.9 ng/ml LOQ = 39.6 ng/ml	0.05–25.00 μg/mL	Caffeine, trigonelline, nicotinic acid, and sucrose in coffee	Caffeine values ranged from 843.3 to 930.9 mg/100 g coffee in green and roasted Arabica coffee samples	[19]
14	Electrochemical method	Voltammetric method with CH1760D electrochemical working standard Working electrode: lignin modified glassy carbon electrode Auxiliary electrode: platinum coil Reference electrode: Ag/Agcl	LOD = 8.37×10^{-7} LOQ = 2.79×10^{-6}	$6–100 \times 10^{-6}$ mol/L	Caffeine content in Ethiopian coffee samples	10.78, 8.78, 6.35, 5.85 mg/g caffeine in coffee	[20]

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S.N.	Method	Experiment	Detection	Linearity range	Application	Scientific outcome	Ref.no.
15	Electrochemical method	Voltammetric method Working electrode: pencil type graphite carbon electrode Auxiliary electrode: platinum coil Reference electrode: Ag/Agcl electrode	LOD = 9.2 mg/L	0–500 mg/L	Caffeine levels in several tea samples	Caffeine levels in several tea samples yield relative error of 1% in the concentrations	[21]
16	LC–MS/MS	For LC, stationary phase: RP-HPLC C18 Mobile phase: isocratic mobile phase consisting of 0.2% formic acid in distilled water and methanol (80:20, v/v) For MS: spectrometer equipped with an electrospray Ionization mode used to generate positive [M + H] + ions	LLOQ = 5 ng/ml	5–5000 ng/ml	Caffeine and its three primary metabolites in rat plasma		[22]
17	GC-NPD	Stationary phase: capillary fused silica column Mobile phase: carrier gas, helium (1 ml min^{-1})	Detection was made by using nitrogen phosphorus detector LOD = 0.02 μg/ml LOQ = 0.05 μg/ml	0.05–500 μg/ml	Caffeine in teas, coffees, and eight beverages	Caffeine in: Nescafe coffee = 246.8 μg/ml Coffee seed = 267.5 μg/ml Red Bull = 297.9 μg/ml, while other samples contained less caffeine	[23]
18	Infrared spectroscopy	Fourier transform infrared spectroscopy (FT-IR) method	The measurement was done at 1659 cm^{-1} using a baseline established between 1900 and 830 cm^{-1} LOD = 3 mg L^{-1}	NA	Caffeine in roasted coffee samples	Recovery of all samples ranges from 94.4 to 100.1%	[24]

Table 2.
The various analytical methods for the determination of caffeine present in coffee.

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