Tomas Bata University in Zlín
Faculty of Technology

THE INFLUENCE OF THE COFFEE ROASTING PROCESS AND COFFEE PREPARATION ON HUMAN PHYSIOLOGY

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ABSTRACT

Caffeine (1, 3, 7-trimethylxanthine) is known as a Central Nervous System (CNS) stimulant. Coffee beans contain between 0.8 and 2.8% caffeine - depending on species and origin. It contributes to 10 - 30% of the bitter taste of coffee brews. The caffeine content in *C. canephora* (Robusta) is about two times that of *C. arabica* (Arabica). The caffeine content of a coffee bean is not significantly changed during coffee roasting. However, the amount of caffeine in coffee brewing substantially varies according to the type of product (Arabica, Robusta, or ratio of Arabica to Robusta in blending), the grinding degree, brewing methods (Espresso, Turkish or Filter coffee) and the serving size (i.e. cup volume). Commercial ground roasted coffees may be available on the market in different roasting degrees, grinding degrees, ratio of coffee species in blending, on all of which depends the quality of the product.

The purpose of this study was to gain more information on the quantitative determination of caffeine content in Vietnamese and Czech ground roasted coffees. The contents and the transfer efficiency of caffeine in the Vietnamese and Czech coffee samples were also compared.

In this study, a rapid, simple and cheap UV/VIS Spectrophotometric Method for the quantification of the caffeine content in commercial coffees was developed and validated. The method involved the Direct Method, Standard Addition Method, Extraction in Chloroform, and the 1^{st} Order Derivative Method.

In the Direct Method, the coffee sample was measured directly by conventional UV/VIS Spectrophotometry. The concentration of caffeine was calculated from the Regression Equation: ($y = 0.0511$, $r^2 = 1$), in which y is the absorbance of the sample at 273.2 nm, and x is the concentration of caffeine in the samples. The results showed that the caffeine contents of the coffee samples were found to be out of documented range.

In the Standard Addition Method, the coffee sample was measured 4 times with a series of increasing volumes of caffeine standard solution, at 273.2 nm. After

measuring the response for a series of standard additions, the concentration of caffeine in the sample was: x = -b/a (µg/mL), when y = ax + b = 0. The results showed that the caffeine contents of the coffee samples were found to be out of documented range.

In the liquid-liquid Extraction by Chloroform process, the coffee sample was extracted in chloroform, over 10 minutes. The extraction was repeated 3 times. The combined extract measured was 276 nm. The concentrations of caffeine were calculated from the Regression Equation: ($y = 0.0489x$, $r^2 = 1$), in which y is the absorbance of the sample at 276.2 nm, and x is the concentration of caffeine in the sample. The results showed that the caffeine contents of the coffee samples were found to be within the documented range.

In the 1st Order Derivative Method, the concentrations of caffeine were calculated from the Regression Equation: ($y = 0.0483x + 0.0107$, $r^2 = 1$), in which y is the peak-to-peak amplitudes of the First Order spectra at the extrema of each sample viz. (Figs. 27-34), and x (µg/mL) is the concentration of caffeine in the samples. The results showed that the caffeine contents of the coffee samples were found to be within the documented range.

The caffeine contents in the coffee samples measured by the Extraction in Chloroform and 1st Order Derivative Method were found to be in agreement with those reported in the literature. The caffeine contents were found to be: In Vietnamese Coffee: (0.48 ± 0.01 – 2.10 ± 0.08%); in Czech Coffee: (2.09 ± 0.10 – 2.12 ± 0.06%). Both methods can be used for the determination of commercial ground roasted coffee.

Keywords: ground roasted coffee, Vietnamese coffee, Czech coffee, caffeine, liquid-liquid extraction, derivative spectrophotometry, UV/VIS.

ABSTRAKT

Kofein (1,3,7-trimethylxanthin) je znám jako stimulant centrální nervové soustavy (CNS). Zrnková káva obsahuje mezi 0,8 až 2,8% kofeinu v závislosti na druhu a původu. Kofein přispívá z 10-30% k hořké chuti kávy. Obsah kofeinu v *C. canephora* (Robusta) je přibližně dvakrát větší než *C. arabica* (Arabica). Obsah kofeinu v kávových zrnech se významně nemění v průběhu pražení. Avšak, množství kofeinu v kávě se významně mění v závislosti na typu produktu (Arabica, Robusta, nebo poměru Arabica a Robusta ve směsi), jemnosti mletí, způsobu přípravy (Espreso, turecká nebo filtrovaná káva) a na objemu šálku kávy. Komerčně prodávaná pražená mletá káva může být dostupná na trhu v různém stupni pražení, různé hrubosti mletí, poměru druhů kávových zrn ve směsi, které určují kvalitu produktu. Například, káva Robusta je mnohem méně kvalitní a aromatická než Arabica, ale Robusta je lacinější; takže Robusta je častěji hlavní součástí výsledných komerčních směsí. Obiloviny a sója jsou dokonce často používány jako ingredience vzhledem k jejich láci. Epidemiologické a experimentální studie ukazují pozitivní efekt konzumace kávy na různé zdravotní problémy, jako jsou psycho-aktivní odezvy (pozornost, náladovost), neurologické podmínky (dětská hyperaktivita, Parkinsonova nemoc), metabolické poruchy (diabetes, žlučové kameny) a funkce jater. Avšak vysoké dávky, mohou u některých vysoce sensitivních individuí vyvolávat negativní projevy, včetně stavů úzkosti, tachykardie a nespavosti. Proto je velice významné znát koncentrace kofeinu v kávě. Přestože kofein zaznamenal zvýšenou pozornost vědecké komunity, je jeho působení velmi komplexní, a jak bylo zdůrazněno výše závisí na mnoha faktorech. Proto je velmi zajímavé stanovit obsah kofeinu v komerčně dostupných vzorcích mleté pražené kávy. Úkolem této studie bylo získání více informací o stanovení obsahu kofeinu ve vzorcích mleté pražené kávy z Vietnamu a České republiky. Navíc byla u obou druhů kávy rovněž porovnána vedle obsahu a účinnost extrakce kofeinu

Klíčová slova: mletá pražená káva, vietnamská káva, česká káva, extrakce v systému kapalina/kapalina, derivační spektrofotometrie, UV/VIS

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LIST OF ABBREVIATIONS

A or ABS	absorbance
CONC	concentration
HPLC	high performance liquid chromatography
RP	reversed phase
SD	standard deviation
UV/VIS	ultraviolet-visible
CE	capillary electrophoresis
GC	gas chromatography
PDA or DAD	photo-diode array
FID	flame ionization detection
FTIR	fourier transform infra-red
FIA-FTIR	flow injection analysis-Fourier transform infrared
CNS	central nervous system

I. LITERATURE REVIEW

1. Introduction

Today, coffee is one of the most consumed beverages in the world mainly due to its stimulating effects, characteristic taste, and richness of coffee aroma which makes it a unique beverage. There has been identified more than 80 species of coffee in the world, but only two main coffee species: *Coffea arabica* (Arabica), which stands for about 60% of the world coffee market, and *Coffea canephora* (Robusta) about 40%. Minor cultivated species include *C. liberica* (Liberica) and *C. excelsa* account for only 1-2% of global production. Arabica and Robusta have a very distinct chemical composition and Arabica coffee is milder, more aromatic and contains less caffeine than Robusta coffee [1]. However, Robusta coffee tree is stronger and more resistant than Arabica tree in various aspects [2]. The different varieties of coffee show differences in the size and shape of the coffee bean but, on average, beans are approximately 9.5 mm long and 7 mm wide. The weight of a parchment seed at 9% moisture content is about 0.15 g for Arabica and 0.16 g for Robusta. The bean sizes are also influenced by environmental conditions and husbandry (nutrition, moisture, care etc.).

Caffeine (1,3,7-trimethylxanthine) is known as a Central Nervous System (CNS) stimulant. Caffeine is a naturally found in the leaves, seeds and/or fruits of at least 63 plant species worldwide. The most commonly known sources of caffeine are coffee and cocoa beans, kola nuts and tea leaves [3,4]. Caffeine is consumed most frequently in beverage such as coffee (71%), soft drinks (16%), and tea (12%) [5]. Coffee beans contain between 0.8 and 2.8% caffeine, depending on species and origin. It contributes to 10-30% of the bitter taste of coffee brews [6]. The caffeine content in *C. canephora* (Robusta) is about two times that of *C. arabica* (Arabica) [7]. Caffeine content of coffee bean is not significantly changed during coffee roasting [2,6]. However, the amount of caffeine in coffee brewing substantially varies according to the type of product (Arabica, Robusta, or ratio of Arabica to Robusta in blending), the grinding degree, brewing methods (Espresso, Turkish or

filter coffee) and the serving size (the volume of the cup) [8,9]. The commercial ground roasted coffee may be available at the market in different roasting degrees, grinding degrees, ratio of coffee species in blending, which is depending on the quality of product. For instances, Robusta coffee tends to produce inferior quality and aroma compared to Arabica coffee, but Robusta coffee is cheaper; as a result, more Robusta coffee are mixed into the blending products. Even corn and soya are also used for blending because of benefit [10]. Based on the data reviewed [11,13] that epidemiological and experimental studies have shown positive effects of regular coffee drinking on various aspects of health, such as psychoactive responses (alertness, mood change), neurological condition (infant hyperactivity, Parkinson's disease), metabolic disorders (diabetes, gallstones), and gonad and liver function, etc. However, high doses may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia [12-22]. Pollack [23] recommended upper limits of caffeine for healthy adults below 300-500 mg daily, pregnant women must stay below 150-200 mg daily and children should stay below 50 mg daily. Amounts exceeding 700 milligrams of caffeine can be dangerous. To know the concentrations of caffeine in coffee brewing is very important. Therefore, it will be interesting to determine caffeine contents in commercial coffee powders. The purpose of this study was to obtain more information on quantitative determination of caffeine contents in Vietnamese and Czech ground roasted coffee. Contents and the transfer efficiency of caffeine in Vietnamese and Czech coffee samples were also compared. Although caffeine has received the most attention from the scientists, it is vastly complex, as stated above it is depending on many factors. The most widely used methods for determination of caffeine in coffee include various analytical techniques such as GC, UV/VIS spectrophotometry, HPLC, Fourier Transform Infrared spectrometry, capillary electrophoresis (CE) [96-116]. A simple, rapid and cheap spectrophotometric method was applied for the quantitative determination of caffeine in commercial coffees.

2. Coffee: Botany, Cultivation, and Distribution

The genus Coffea belongs to the family Rubiaceae. This family comprises many genera including Gardenia, lxora, Cinchona (quinine) and Rubia. They are shrubs or small trees native to tropical and southern Africa and tropical Asia [24]. The coffee plant takes approximately 3 years to develop from seed germination to first flowering and fruit production. A well-managed coffee tree can be productive for up to 80 years or more, but the economic lifespan of a coffee plantation is always less than 30 years [24,25].

Table 1. Coffee species and areas [24]

Species	Area			
	Asia	Africa	America	generally
C.arabica	Yemen, India, Papua New Guinea, Mauritius, Reunion, New Caledonia, Vietnam and Hawaii	Madagascar and also on the West Coast	The high plateaux of the Tropical Americas; mid-altitude regions of South America; the mountainous lands of the Caribbean Islands	at altitudes between 1300 and 2000 m above sea level.
C.canephora	India, Indonesia, Philippines, Malaysia, Thailand, China, etc.	The lowlands of west and central Africa and mid-altitude zones in the East	Humid, tropical regions in the Northeast of Brazil (Conillon), Ecuador, Guyana, Mexico, Trinidad and Tobago, etc.	at altitudes below 1000 m (Low and mid-altitude regions)
C.liberia	Mainly in Malaysia, but also in Indonesia, the Philippines, Vietnam and Thailand	The West coast, Equatorial Africa and Liberia	Guyana and Surinam	Low-altitude region, often coastal
C. dewevrei	Mainly in Vietnam, but also in Indonesia and the Philippines	Central and West Africa, Chad, south Sudan, Madagascar, Mauritania, and others.	Chiefly Puerto Rico	lowland forest habitats

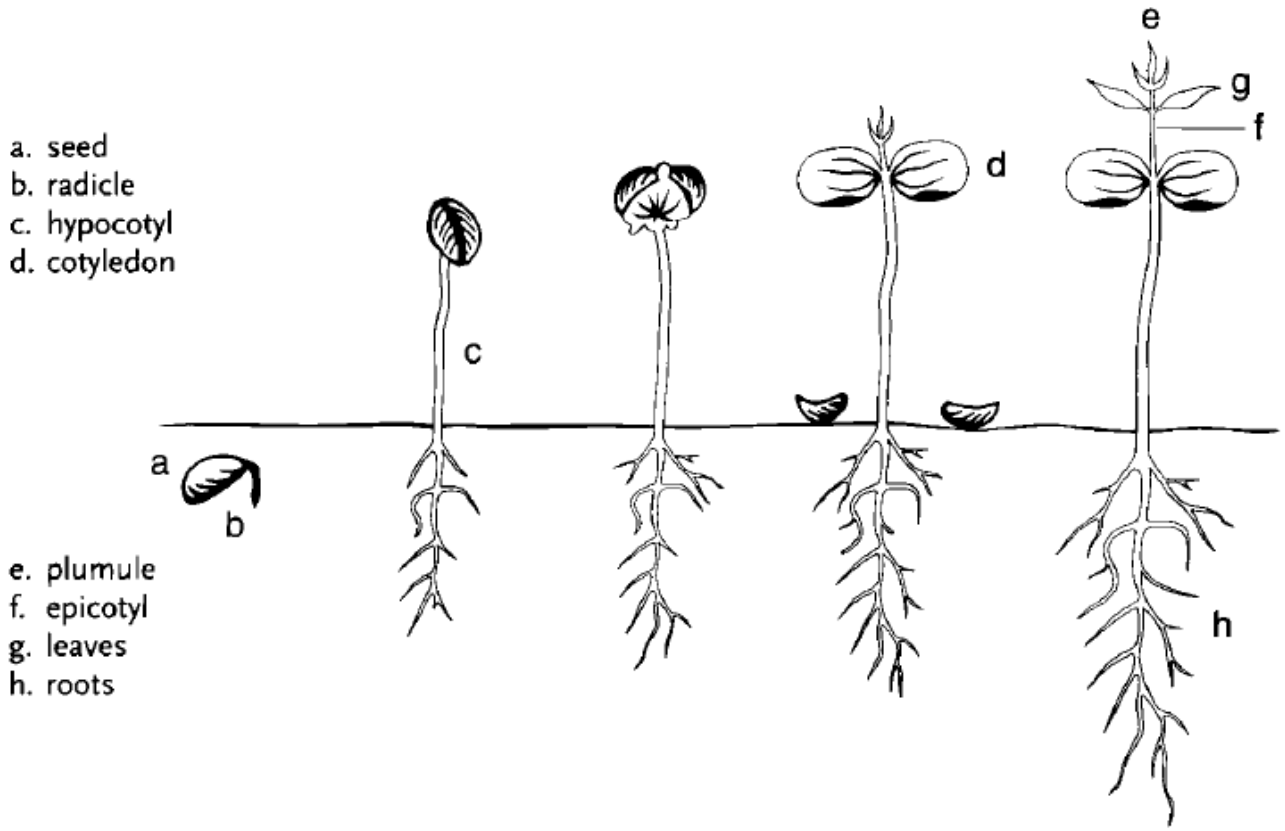

Figure 1. Germination of a coffee plant [24]

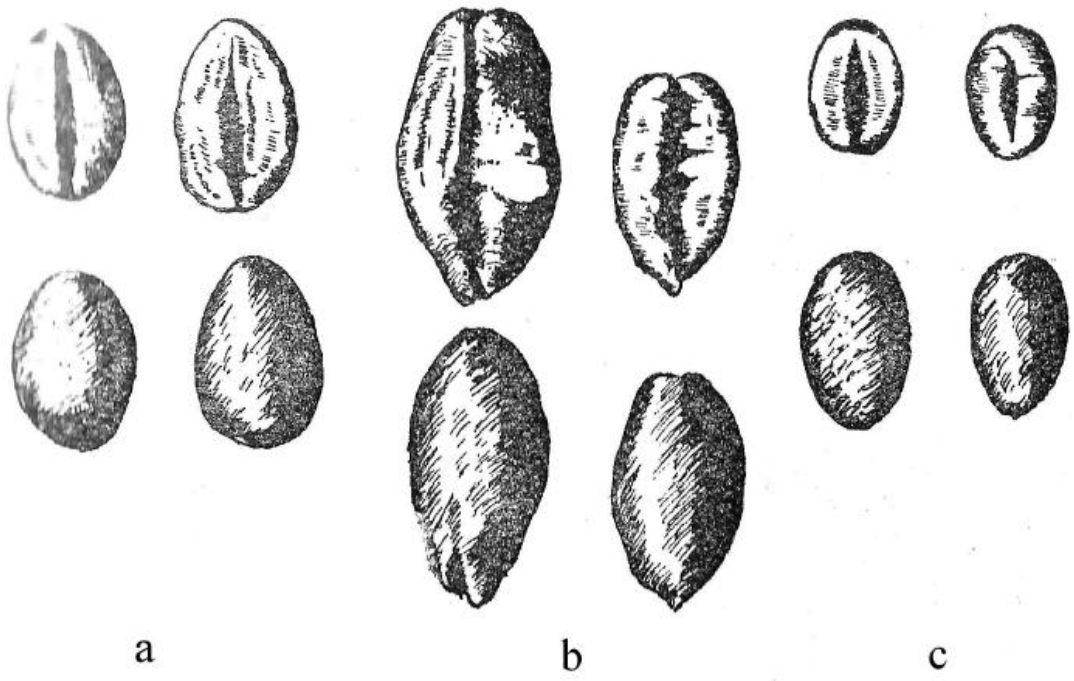

Figure 2. The seed of *C. arabica* (a), *C. liberica* (b) and *C. robusta* (c) [26]

3. Coffee production

Figure 3. The diagram of coffee production (A) green fruits, (B) coffee cherries, (C) green beans, (D) roasted bean (E) ground roasted coffee and (F) cup of coffee

3.1. Green coffee bean

After harvesting, coffee cherries need to be processed for the production of green coffee beans. The production of green beans involves successive removal of the outer most red skin and the pulp of the coffee cherry, followed by removal of the mucilage, parchment covering, and finally, the silver skin surrounding the green coffee bean (endosperm) (Fig. 4).

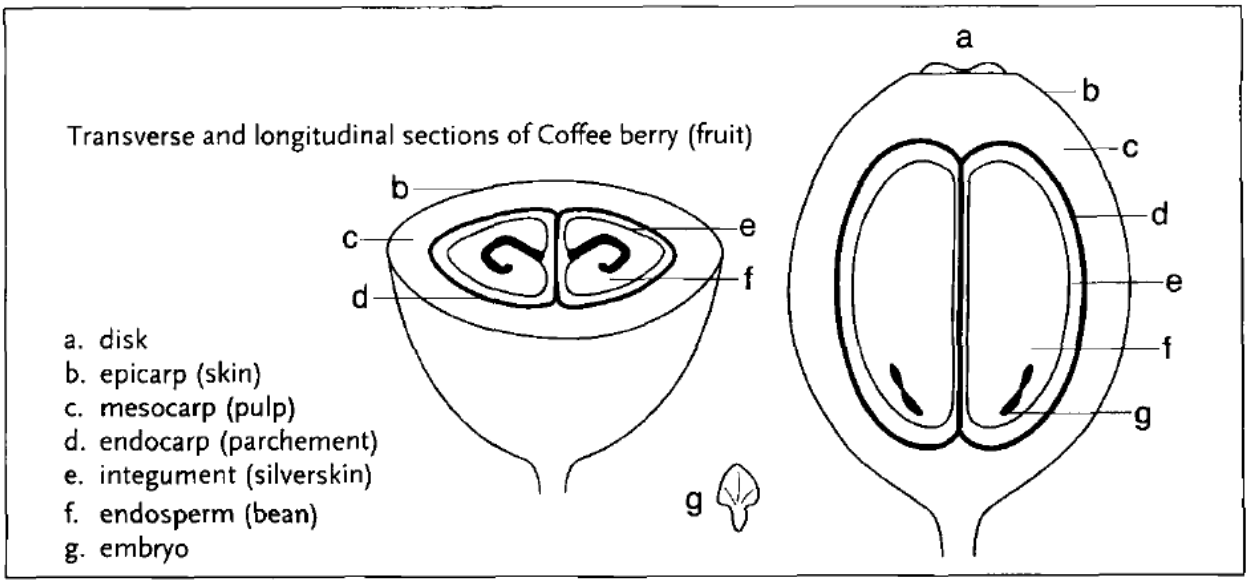

Figure 4. Coffee beans structure [24]

The most common methods for pulp extraction have been dry, semi-dry and wet methods (Fig. 5.). In dry processing whole cherry is dried together (exocarp, mesocarp and endosperm, i.e. pulp, parchment and bean). The whole hull (dried pulp and parchment) is then removed mechanically to obtain green coffee. Dry processed seeds are dried by sun exposure or by air dryers until moisture content is about 10-12% [27]. This method is commonly used in Brazil and Africa, where sun and space are abundant and where stripping is also more common [24]. In wet processing, it is relatively sophisticated and is best carried out with uniformly ripe coffee cherries. As soon as possible they are sorted further in a water-flotation system and sent directly to a pulping machine. Here the cherries have the outermost skin and pulp removed and the mucilage layer is exposed. Removal of the mucilage is brought about in concrete fermentation tanks, where the beans are slurried with water. Fermentation occurs mainly due to lactobacilli and yeasts. Enzymes are produced that dissolve away the mucilage. The beans are washed free from the mucilage and the parchment layer is exposed. Now the beans are dried in the sun or more usually in mechanical driers. The parchment layer and most of the silver skin are removed in a hulling machine. Frequently, wet processing is used in places where coffee is harvested by picking, such as Colombia, Asia and Central America. Semi-drying method is a method combining both dry and wet methods. The method consists of washing and selection of the seeds in flotation tanks without fermentation [7,24,28]. Regarding cup quality, wet method are known to present better quality, less body, higher acidity and more aroma than the dry method [29].

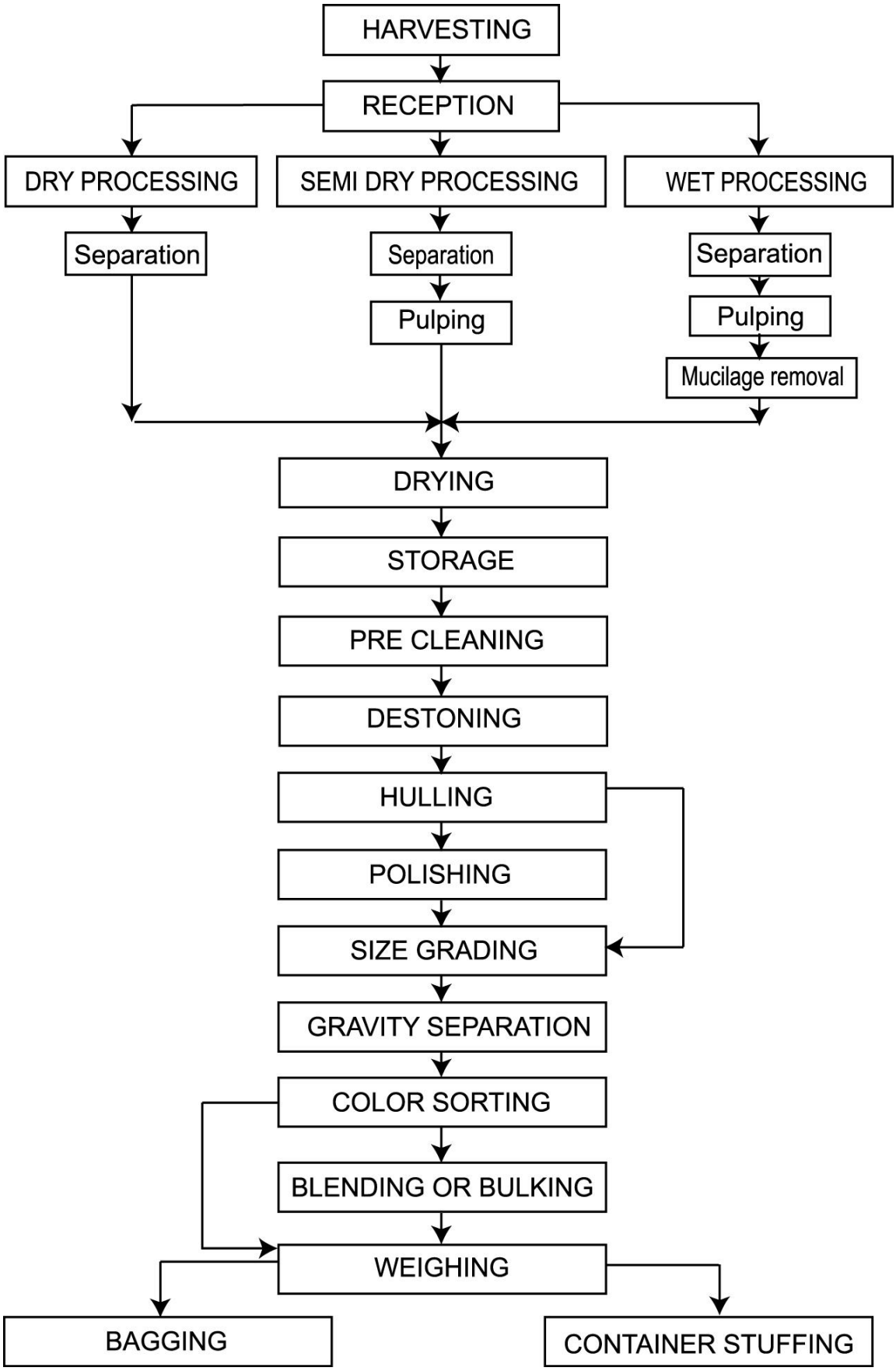

Figure 5. Complete green coffee bean processing [24]

Figure 6. Drying in the sunshine [30]

Drying aims to lower the water content of fresh cherries to a level which allows the preservation of beans (about 11-12%) for long-term storage [24]. Coffee production is seasonal, whereas consumption takes place year round. This also means that producers, exporters, importers and traders alike tend to keep coffee in the expectation of obtaining better prices. The green beans are then ready to undergo roasting. The roasting degrees vary significantly according to the national preferences (light, medium or dark). Optionally, the green beans may be decaffeinated, steam-treated or stored prior to roasting [24].

3.2. Roasting

Roasting is an important step in coffee production for generation of aroma, flavor and color of coffee bean [31]. During the roasting process (commonly at 210-230 °C), the coffee beans composition dramatically changes as a consequence of pyrolysis, caramelization, Maillard and Strecker reactions; as a result, the color of the bean is changing from light brown to almost black, depending on cultural and personal preferences; and the characteristic aroma of roasted coffee is formed, its

volume increases considerably. After roasting, the moisture content of roasted bean is about 1.5% (depending on roasting degree) and the beans have been marketed as roasted beans, ground roasted coffee, instant coffee (powder or granule) or coffee liquid [32].

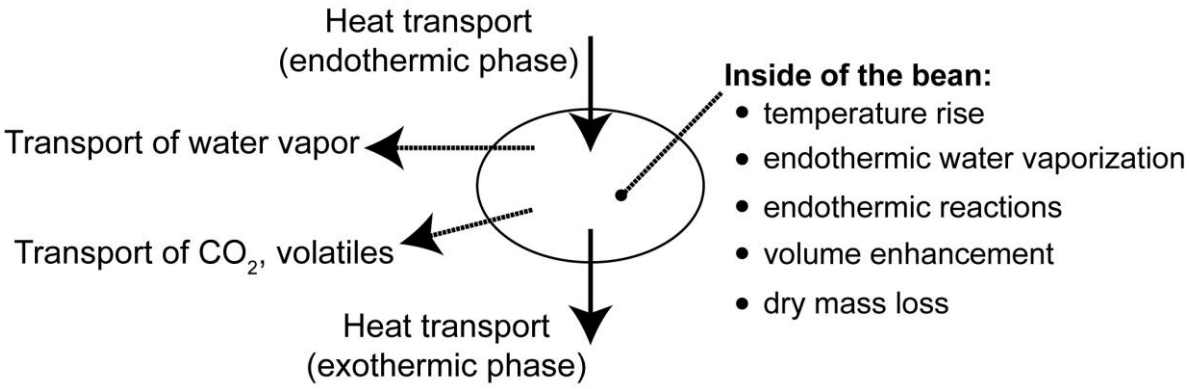

Figure 7. Roasting of coffee beans – main aspects [6]

Figure 8. Roasting degree [33]

Table 2. Coffee roasting styles correlated with approximate value for green coffee bean weight loss, color, and temperature [7]

Roast style	Green bean weight loss (%)	Final color	Final temperature (◦F)
Light city	14	Cinamon	390
City	15	Brown	410
Full city	15.5	Deep brown	
Brazillian	16	Dark brown	
Viennese	17	Very dark	440
Frenche	18	Brown with oil on surface	
Ice	19		
Italian	20	Extremely dark brown to black, shiny oil on surface	465

3.3. Decaffeination

Caffeine is known to have different effects on each person and there are some people that are highly sensitive to it. For these people, decaffeinated coffee is their only choice if they want to enjoy coffee, but without the unpleasant side effects due to stimulant effects and other, still on health concern that caffeine can cause. There are three major groups of the different decaffeination procedures including decaffeination with chemical solvents, water decaffeination, and supercritical carbon dioxide decaffeination. The most common caffeine extraction methods in the coffee industry basically employ an organic solvent such as dichloromethane (CH_2Cl_2), ethylacetate ($CH_3COOCH_2CH_3$), chloroform ($CHCl_3$), associated with the use of water/vapor prior to and after extraction for washing and opening of the pores. The green coffee beans are first steamed at temperature of 20-100 °C for up to 5h until they are swollen to contain 30-40% with water, to make the caffeine available for extraction. Decaffeination of caffeine takes place in static or rotating drum with

water-saturated solvent at temperature 60-105 °C for 2-12h, extracting the caffeine from the beans with a solvent. All residual volatile solvents are removed from the beans by steam stripping at 100-110 °C for 1-4 h to levels usually well below those required by local regulations (<3-10 ppm), according to country and solvent. The USA Food and Drug Administration allows up to 10 ppm in roasted coffee, while the European Union allows up to 3 ppm). Finally, coffee is dried until it reaches its initial moisture content (approximately 10%) at 40-48 °C for 0.5-10 h with hot air or under vacuum. In addition, roasting temperature (commonly 210-230 °C) is high enough to allow volatilization of any remaining amount of solvent in coffee. However, during the process, the coffee beans generally lose their wax surface covering, as well as key flavor components. The Robusta and Arabica coffees that are dry-processed and have the most powerful flavors are usually the types that are decaffeinated. They become milder in the process. Mechanical polishing is used to improve the appearance of decaffeinated green coffee beans if they are not to be roasted immediately. Extra care is required, however, to store these decaffeinated beans since the loss of wax covering as well as caffeine renders them much more susceptible to fungal attack. At the end of the process, caffeine content is usually reduced from 1-2 to 0.02-0.3 g%. It should be noted that, decaffeinated coffee are not caffeine free [34-35,51].

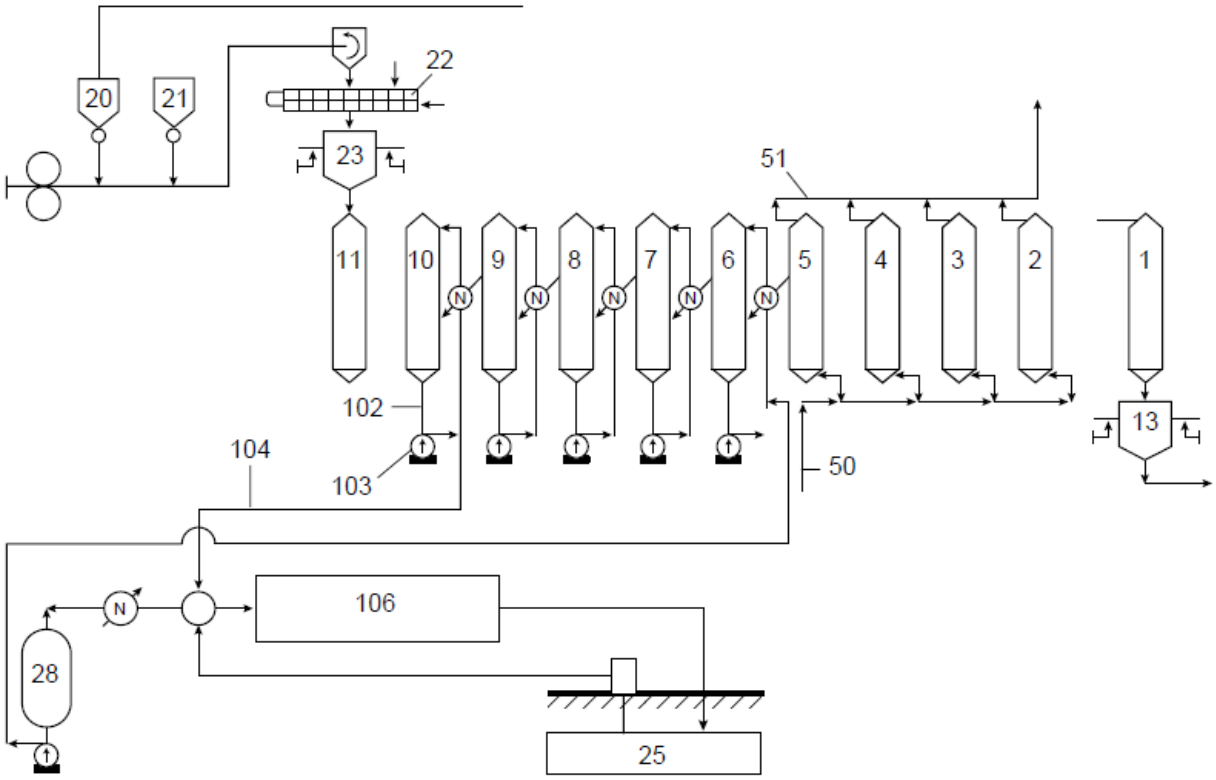

Figure 9. The accelerated decaffeination process [34]

1 vessel with cleaned decaffeinated beans;

2, 3, 4, 5 solvent removal;

6, 7, 8, 9, 10 in decaffeination;

11 fresh beans;

13 discharge lorry;

20, 21 blending bins;

23 mixer for pre-wetting;

25 fresh solvent;

28 solvent saturator;

50 steam;

51 to vacuum source;

104 solvent discharge;

106 solvent recovery system.

3.4. Instant coffee

Instant coffee, also called soluble coffee or coffee powder, is a beverage derived from brewed coffee beans. Instant coffee is commercially prepared by treating ground roasted coffee with hot water and high pressure for extraction of the water soluble material. The soluble material is then dried by either spray volatilize (with

either powdered or granule formation) or freeze drying (to granules only). In the spray drying process, the high temperature under high pressure is used to volatilize the aqueous extract and hot air dehydrates the small drops. Whereas, freeze drying process uses very low temperatures for sublimation of a previously frozen aqueous extract and the direct change from the solid phase to the gas phase. Freeze drying is a widely used method of drying coffee extracts, especially chosen for high quality products.

Advantages of instant coffee include speed of preparation (instant coffee dissolves instantly in hot water), lower shipping weight and volume than beans or ground coffee (to prepare the same amount of beverage), and long shelf life—though instant coffee can also spoil if not kept dry [36].

(a) (b) (c)

Figure 10. Instant coffee of granule (a), powder (b) and liquid (c)

3.5. Grinding

Regard of the coffee brewing method, grinding is one of the steps influencing how the final brew tastes. Grinding is to break down the roasted coffee bean to expose the interior of the bean, to increase the surface area allow water (the extraction agent) to make contact with more coffee when brewing.

The grinding size (fineness/coarseness) of coffee powder is strongly depending on the brewing methods [9,37].

Figure 11. Ground roasted coffee [38]

Table 3. Grind size vs. brewing method chart [38]

Grind Size	**Ideal Brewing Method**
Coarse	Plunger Pot French Press Percolator Vacuum coffee pot
Medium	Drip coffee makers with flat bottom filters (BUNN, Bloomfield, etc.)
Fine	Drip coffee makers with cone shaped filters (KRUPS, Cusinart, etc.) Espresso moka pots
Extra Fine	Espresso machines - pump Espresso machines - steam
Turkish	Ibrik

3.6. Brewing

A coffee beverage is prepared by the intimate contact of water with ground roasted coffee (coffee powder) known as solid/liquid extraction including 2 steps: (1) contact of coffee powder and water to effect mass transfer of soluble compounds to the water, and separation of the resulting coffee solution from the residual solid using filtration. Chemical composition of coffee brew are significantly dependent on brewing methods used including the composition of water, the proportion of coffee to water, the water temperature and length of time coffee is in contact with water and filter material. There are three basic different extraction methodologies: decoction (boiled coffee, Turkish coffee, percolator coffee and vacuum coffee), infusion (filter coffee, Napoletana coffee) or pressure methods (plunger, moka and espresso) [8].

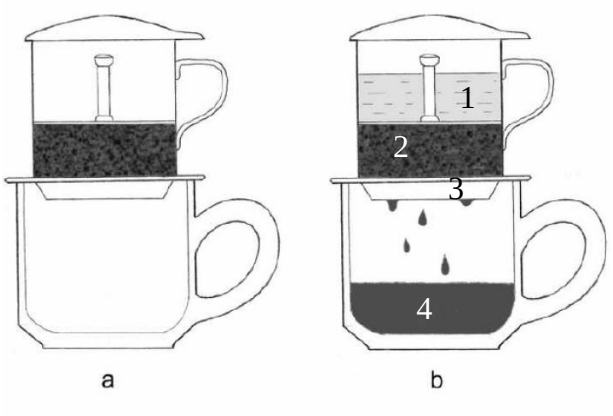

Figure 12. Single-cup filter (Vietnamese style coffee), water (1), coffee powder (2), filter (3) and coffee solution (4)

4. Commercial coffee

The commercial coffee may be available at the market in different forms including green beans; roasted beans varying in color from very light to very dark; ground roasted coffee varying in size of particle; instant powder; instant granule and even coffee liquid. Except for coffee beans, ground roasted coffee, instant coffee and coffee liquid are the blending of many coffee species mainly Robusta and Arabica coffee.

5. Chemical Composition of Coffee Bean

The basic chemical composition of green coffee bean depends mainly on coffee species, soil composition, climate, agricultural practices and storage conditions [2,39].

Table 4. Typical levels of caffeine in common coffee [39]

Coffee	Caffeine content
Drip brewed	135 mg per 240 mL
Percolate	160 mg per 280 mL
Instant	95 – 120 mg per 240 mL
Espresso	30 – 50 mg per 30 mL
Flavored	25 – 100 mg per 240 mL
Brewed decaffeinated (Decaf)	5 – 7 mg per 240 mL
Instant decaffeinated (Decaf)	3 – 4 mg per 240 mL

Table 5. Chemical composition of the non-volatile fraction of green and roasted Arabica and Robusta coffee beans [39]

Constituent (g/100g DM)	Arabica		Robusta	
	Green bean	Roasted bean	Green bean	Roasted bean
Carbohydrates and fibres				
Sucrose	6.0-8.0	4tr-4.2	4.0	1tr-1.6
Reducing sugars	0.1	0.3	0.4	0.3
Polysaccharides (arabinogalactan, Mannan and glucan)	34-44	31-33	48-55	37
Lignin	3.0	3.0	3.0	3.0
Pectins	2.0	2.0	2.0	2.0
Nitrogenous compounds				
Protein	10.0-11.0	7.5-10	11.0-15.0	7.5-10
Free amino acids	0.5	nd	0.8	nd
Caffeine	0.9-1.2	1.1-1.3	1.5-2.5	2.4-2.5
Trigonelline	0.8-2.0	0.2-1.2	0.6-0.7	0.3-0.7
Nicotinic acid		0.016-0.026		0.014-0.025
Lipids				
Coffee oil (triglycerides with unsaponifiables)	16.0	17.0	10.0	11.0
Diterpene esters	0.9	0.9	0.2	0.2
Minerals (40% K and 4% P)	3.0-4.2	4.5	4.4-4.5	4.7
Acids and esters				
Total chlorogenic acids	4.1-7.9	1.9-2.5	6.1-11.3	3.3-3.8
Aliphatic acis	1.0	1.6	1.0	1.6
Quinic acid	0.4	0.8	0.4	1.0
Melanoidins		25	25	25

[a] Content varies with cultivars, cultivation climate, soil, methods of analysis and, critically, roasting degree. tr, trace. nd, not determined.

The non-volatile fraction of green coffee beans mainly consists of water (11-12%), carbohydrates and fibres, proteins, caffeine (0.8-2.8%), phenolic acids (5-8%), trigonelline, lipids, minerals and organic acids.

Caffeine is mainly combined with chlorogenic acids, quinic acid, caffeic acid, trigoneline and sucrose [49]. Caffeine, trigonelline and chlorogenic acids are important compounds that mainly contribute to the flavor, bitterness and bioactivity of coffee beverage after the roasting of coffee beans [8,40-41].

Chlorogenic acids (CGAs)

CGAs are a main class of phenolic compound derived from esterification of trans-cinnamic acids such as caffeic, ferulic and p-coumaric, with quinic acid, about 6-12% of coffee constituents in mass [42-43]. The main subgroups of chlorogenic acid isomers in coffee are the caffeoylquinic acids (CQA), feruloylquinic acids (FQA), dicaffeoylquinic acids (diCQA) and, in smaller amounts, p-couma-roylquinic acids (p-CQA) [40,44-45]. These phenolic compounds not only contribute to the astringency, bitterness and acidity to the coffee beverage, but also may be of potential biopharmacological properties in humans [2,14,46]. Several biopharmacological properties of phenolic compounds are antiviral activity against adenovirus and herpes virus, hepatoprotective activity in injured liver experimental model immune-stimulating activity [47-50]. The levels of CGA in green vary from approximately 7.88 to 14.4% dry matter for *Coffea canephora* (Robusta) and approximately 3.4-4.8% dry matter for *Coffea arabica* [49]. High amount of CGAs in green coffee tends to produce an undesirable flavor because of negative notes of their products of oxidation and degradation prior to and during roasting [51].

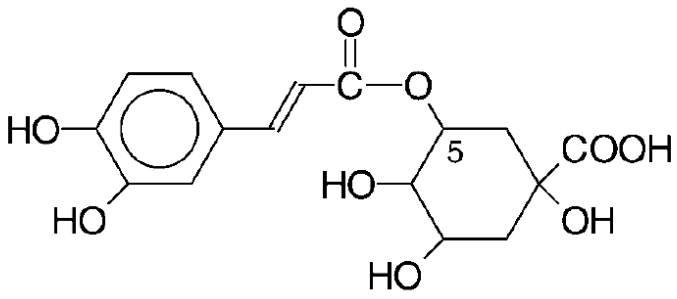

Figure 13. Chemical formula of the chlorogenic acid (5-caffeoyl-quinic acid), the main phenolic compound of coffee [52]

Trigonelline

Trigonelline (0.6-2%) is a pyridine derivative present in green coffee beans, known to contribute indirectly to the formation of desirable and undesirable aroma compounds during roasting [53]. Trigonelline content has been correlated to good cup quality [14]. It contributes to bitterness and a part of the trigonelline is converted to nicotinic acid or niacin, a soluble water vitamin B through demethylation during coffee roasting. Trigonelline also have ant-invasive properties against cancer cells [49,54,56].

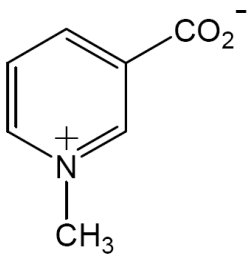

Figure 14. Chemical structure of trigonelline [14]

Caffeine

Caffeine (1,3,7-trimethylxanthine) is an major purine alkaloid of the methylxanthine family known as a central nervous system stimulant through its adenosine antagonist action [8,55]. Caffeine is a naturally found in the leaves, seeds and/or fruits of at least 63 plant species worldwide. The most commonly known sources of caffeine are coffee bean (Coffea *arabica* and *Coffea canephora* var. *robusta*), cocoa beans (*Theobroma cacao*), cola nuts (*Cola acuminata* and *Cola verticillata*) and tea leaves (*Camellia sinensis* vars. *assamica* and *sinensis)* [3,4]. Coffee beans contain between 0.8 and 2.8% caffeine, depending on species and origin , and it contributes to 10 to 30% of the bitter taste of coffee brews [57-58,60]. The caffeine content in *C. canephora* (Robusta) is about two times that of *C. arabica* (Arabica). Caffeine content of coffee bean is not significantly changed during coffee roasting. However, the amount of caffeine in coffee brewing substantially varies according to the type of product (Arabica, Robusta, or ratio of Arabica to Robusta in blending), the grinding degree, brewing methods (Espresso, Turkish or filter coffee) and the

serving size (the volume of the cup) [7,14]. Based on the data reviewed, it can be concluded that low to moderate caffeine intake (300 mg/day or less) is generally associated with improvements in alertness, learning capacity, exercise performance and perhaps mood. In addition, caffeine metabolites, especially 1-methylxantine and 1-methylurate have exhibited antioxidant activity. Antioxidants have been widely linked to a number of potential health benefits including protection against heart disease and cancer. However, high doses may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia [14,59]. A study of Dr. Pollack [23] recommended upper limits for caffeine: healthy adults should consume below 300-500 mg daily, pregnant women must stay below 150-200 mg daily and children should stay below 50 mg daily.

Caffeine metabolites such as theophylline and theobromine have also been identified in coffee.

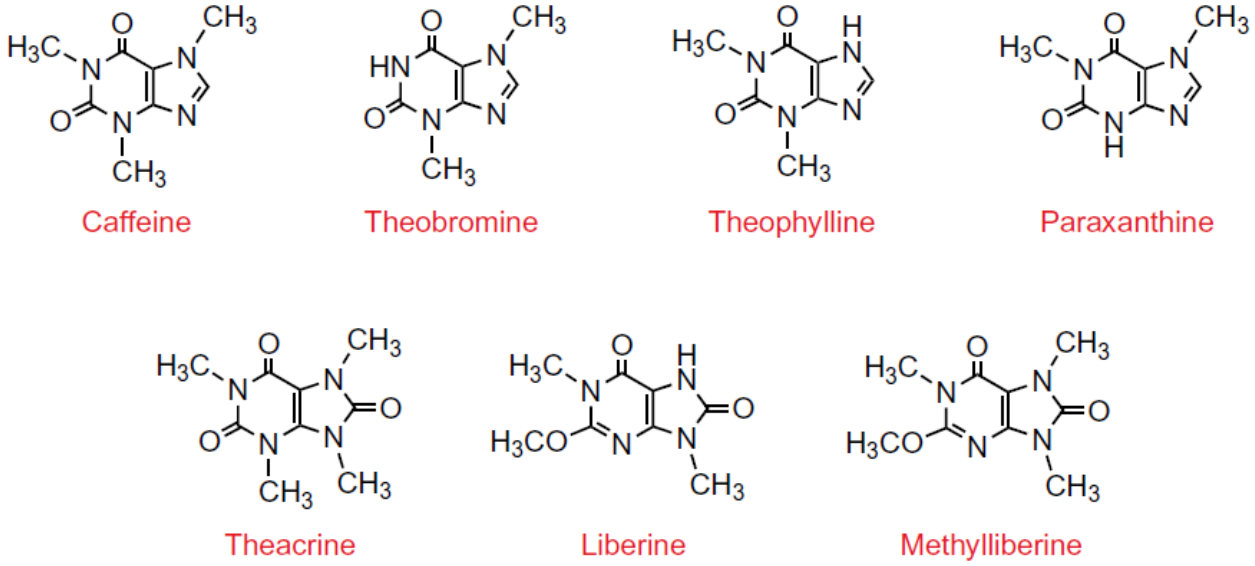

Figure 15. Structures of key alkaloids [55-56]

Table 6. Summaries the properties of caffeine [61]

Systematic name	1,3,7-trimethylxanthine
Chemical formula	$C_8H_{10}N_4O_2$
Molar mass	194.19 g/mol
Density	1.23 g/cm^3, solid
Physical properties	1. Caffeine is a white, odorless powder with a slightly bitter taste. 2. Caffeine is not significantly altered during coffee roasting, but small losses may occur owing to sublimation. The anhydrous form obtained by crystallization from nonaqueous solvents is a crystalline solid that melts at 235 °C to 237 °C. At atmospheric pressure it begins to sublime without decomposition at 120 °C and at 80 °C under high vacuum. 3. Soluble in water, methylene chloric, dichloromethane, ethyl acetate, chloroform. Caffeine is soluble to the extent of 0.6% in water at 0 °C, 2.13% at 25 °C and 66.7% at 100 °C.
Applications	Caffeine is used as a drug on the basis of its effect on respiratory, cardiovascular and the central nervous system. It is included with aspirin in some preparations for treatment of headaches as it decreases cerebral eye blood flow. It is included with ergotamine in some antimirane preparations, the object being to produce a mildly agreeable sense of alertness Caffeine is administered in the treatment of mild respiratory depression caused by central nervous system depressants such as narcotic.

Table 7. Caffeine contents of food products [39]

Food/Beverage	Volume/ weight	Average caffeine content (mg)
Coffee, brewed	150 mL	85
Coffee, instant	150 mL	60
Coffee, brewed, decaffeinated	150 mL	3
Tea, brewed	150 mL	41
Tea, instant	150 mL	28
Regular colas	180 mL	15 – 24
Diet colas	180 mL	13 – 29
Chocolate bar	28 g	20
Dark chocolate, semi sweet	28 g	20
Milk chocolate candies	28g	5

Table 8. The composition of coffee beverage [39]

Constituent	**Content (% dry weight basis)[a]**
Protein[b]	6
Polysaccharides	24
Saccharose	0.8
Monosaccharides	0.4
Lipids	0.8
Volatile acids	1.4
Nonvolatile acids	1.6
Chlorogenic acids	14.8
Caffeine	4.8
Trigonelline	1.6
Nicotinic acid	0.08
Volatile aroma compounds	0.4
Minerals	14
Unidentified constituents	29.4

[a] Arabica coffee, medium roast, 50g/l

[b] Calculated as sum of the amino acids after acid hydrolysis

6. Caffeine and health

6.1. Metabolism of caffeine by humans

Caffeine absorption quickly occurs in the body – caffeine enters the bloodstream through the stomach and small intestine, its effects are felt as soon as 15 minutes after consumption and peak blood levels occurring about 30 minutes after consumption. It is completely absorbed within 45 minutes of ingestion. In healthy liver, caffeine is mostly broken down by the hepatic microsomal enzymatic and xanthine oxidase, which together mediate demethylations and oxidations yielding products including three dimethylxanthines (paraxanthines (70%), theobromine and theophylline (25%)), three monomethyl xanthines, the corresponding trimethyl, dimethyl and monomethyl uric acids, and three uracil derivatives formed by opening of the five membered ring and a small amount of unchanged caffeine which is excreted by urine (5%) [55]. Therefore, the metabolism of caffeine depends on the state of this enzymatic system of the liver. Once caffeine absorbed, it exerts a variety of physiological actions to diverse organs of the body, the main mechanism of action of caffeine is to work as an adenosine receptor antagonist in the brain, typically resulting in inhibitory effects to the central nervous system [63-64]. The effects and mechanisms of caffeine and its metabolites, paraxanthine, theobromine, and theophylline are similar, and they generally occur together.

Caffeine is metabolized in liver into three primary metabolites: paraxanthine (75%), theobromine and theophylline (25%), the corresponding uric acid and uracil compounds (5%) [60] (Figure. 16).

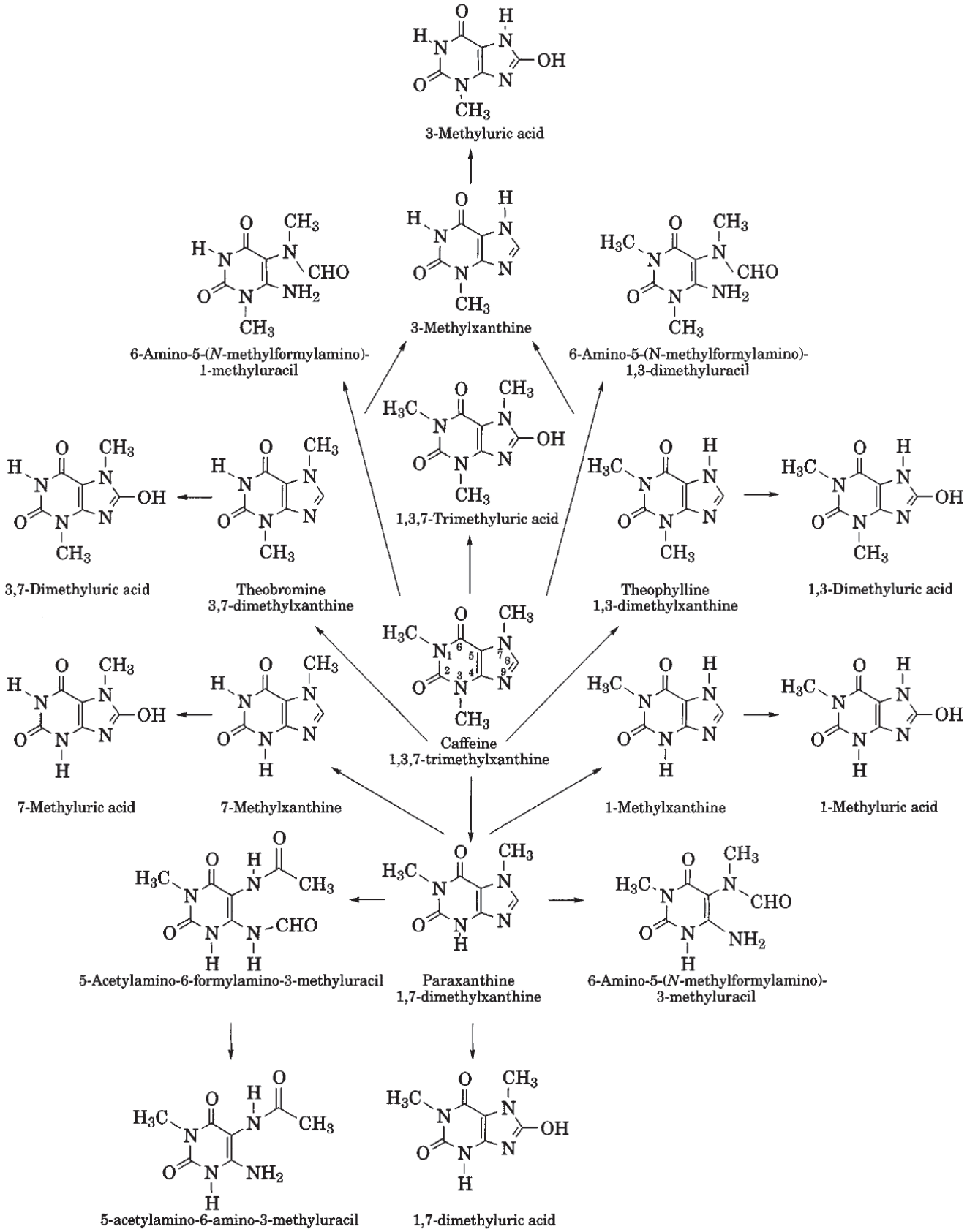

Figure 16. Pathways for human metabolism of caffeine [60]

1. STOMACH

- Stimulates acid production

2. BLOOD

- Transports caffeine to organs

3. HEART

- Higher pulse

4. BRAIN

- Stimulates by replacing the brain's own slow down chemical

Effect: Alertness, concentration.

Constrains blood vessels to the brain

Effect: Helps migraine headache

5. LUNGS

- Relaxes involuntary muscles

Effect: Help asthama

6. MUSCLES

- Stimulates voluntary muscles for higher performance

7. KIDNEYS

- Stimulates urine production

8. INTESTINES

- Relaxes involuntary muscles – such as in colon

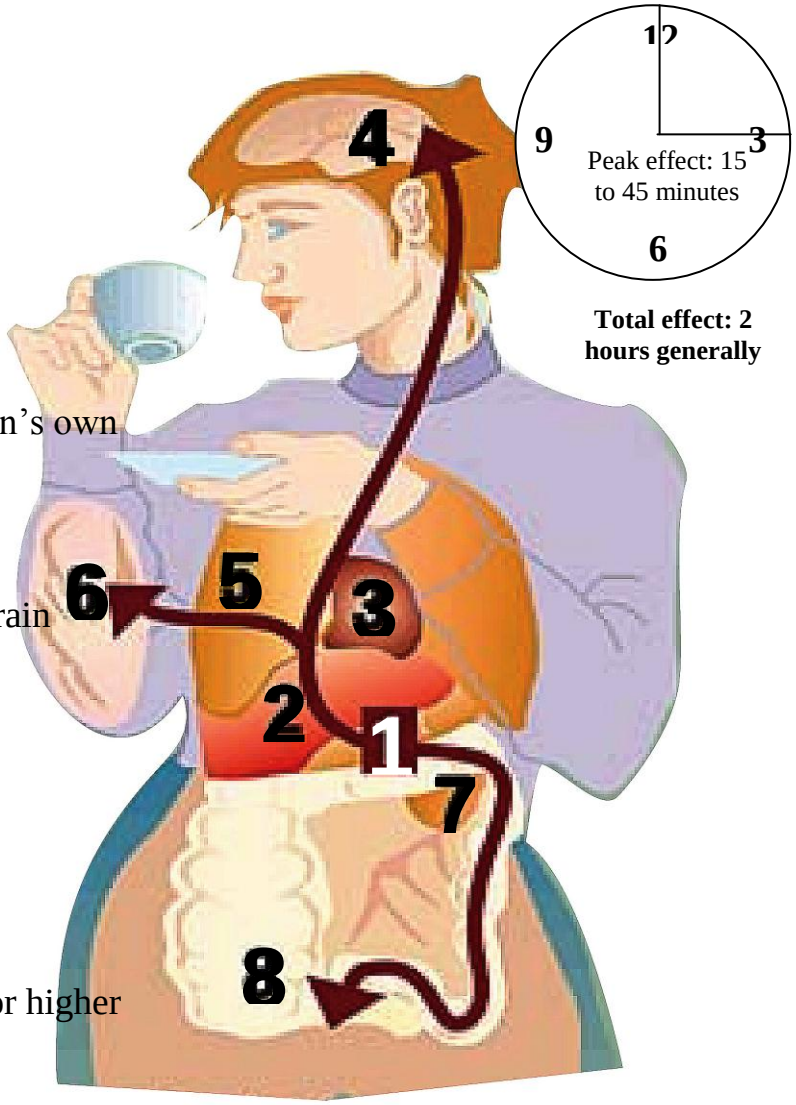

Figure 17. Influence of coffee on human [60,65]

6.2. Caffeine and health

Caffeine present in coffee can cause addiction and mildly stimulates the central nervous and cardiovascular system [66-67]. Many scientists have had an attempt to associate its intake with beneficial or detrimental effects on health. There is little evidence of health risks of caffeine consumption and some evidences of health benefits.

Caffeine consumption can reduce the risk of several chronic diseases, including diabetes [20, 68-76, 80], Parkinson's disease [77], liver disease [78], and colorectal cancer, as well as improve immune function [79], etc. However, there is conflicting evidence.

Many results showed the relation between the caffeine intake of coffee and type-2 diabetics. Keijzers *et al.* [20] showed that caffeine reduces sensitivity to insulin sensitivity in healthy humans. They explained that happened possibly as a result of elevated plasma epinephrine levels, because dipyridamole did not affect glucose uptake, peripheral adenosine receptor antagonism does not appear to contribute to this effect. Recently, Rosengren *et al.* [70] presented their studies that coffee consumption protects against development of diabetes in middle-aged and older Swedish women, although the mechanisms are unclear. A large prospective cohort studies in long-term study on consumption of regular coffee, decaffeinated coffee, and other caffeinated beverages, suggested an inverse relationship between intakes of caffeine and regular coffee and incidence of diabetes in both men and women [71]. Likewise, Tuomilehto *et al.* [72] described their studies on 6974 Finnish men and 7655 women aged 35 to 64 years without history of stroke, coronary heart disease, or diabetes mellitus at baseline, with 175682 person-years of follow-up to determine the relationship between coffee consumption and the incidence of type 2 diabetes mellitus. Their studies revealed unequivocal evidence for an inverse and graded association between coffee consumption and type 2 diabetes mellitus independent of other risk factors for type 2 diabetes mellitus.

Caffeine consumption from coffee and other beverages decreases the risk of Parkinson's Diseases in men and women. Lindsay *et al.* [77] have found that increasing age and educational level were associated with increased risks of incident Alzheimer's disease, while arthritis, regular use of wine consumption, coffee consumption, and regular physical activity were associated with reduced risks.

Some studies suggest that coffee consumption can help reduce the risk of liver cancer, although the reasons for the mechanisms are unknown [80]. However, the epidemiological studies on the carcinogenicity of caffeine as present in coffee have consistently shown that caffeine is not associated with cancer development at several tissue and organ sites [81]. Based on the studies reviewed, Nawrot *et al.* [81] concluded that there are evidences indicate that caffeine, as present in coffee, is not a chemical that causes breast or bowel cancer. However, the association between caffeine and the development of urinary bladder and pancreatic cancer are inconsistent and the data are not conclusive. At other sites (e.g. ovary, stomach, liver) the data are insufficient to conclude that caffeine consumption is related to carcinogenesis.

There has been an attempt to associate caffeine intake with cardiovascular health, especially cardiac arrhythmia, heart rate, serum cholesterol and blood pressure. However, most research results seen to be inconclusive. Loperz-Garcia *et al.* [82] presented their studies that coffee consumption was not associated with an increased risk of coronary heart disease. Based on many studies reviewed, Nawrot *et al.* [81] also concluded that moderated caffeine consumption (400 mg or less, or four or fewer cups of coffee per day) does not adversely affect cardiovascular health. Frost *et al.* [83] analyzed the association between the amount of caffeine consumed daily and the risk of atrial fibrillation, or flutter among 47.949 participants over 7 years in a large Danish study. They found that there was no connection between caffeine consumption and cardiac arrhythmias. Although caffeine can acutely raise heart rate and blood pressure immediately after consumption. Therefore, according to the American Heart Association (AHA)'s policy on caffeine, "whether high caffeine intake increased the risk of coronary heart disease is still under study" [84]. There

has also been an attempt to associate caffeine intake with reproductive health. Based on many studies Leviton *et al.* [85], in his review showed that caffeine does not cause any reproductive health such as delayed conception, miscarriage (both chromosomally normal and aberrant), birth defects, premature birth, and low birth weight. There is only weak evidence that teratogenic (birth defect) can effect in rats administered extremely high levels of caffeine intravenously, which do not necessarily translated to humans and also could never be attained merely by drinking beverages containing caffeine [79,86].

Caffeine have been studied as a cause the risk of osteoporosis as characterized by low bone mineral density and increased susceptibility to fractures because caffeine intake increased the urinary excretion of minerals such as calcium, magnesium [87]. However, calcium excretion is affected by many other dietary constituents such salt, protein etc., [88]. However, Massey *et al.* [88] in their reviews concluded that moderate caffeine intake does not seem to cause a problem with calcium in young adult women, or limiting to three cups of coffee per day (about 300 mg of caffeine) can help prevent hip-bone fractures in older adults [89-91].

In addition, caffeine has been shown to exhibit several biological effects, such as increased fat oxidation and mobilization of glycogen in muscle, increased lipolysis and decreased body fat [92-94].

Therefore, low to moderate caffeine intake is generally associated with improvements in alertness, learning capacity, exercise performance and mood [15]. However, high doses may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia.

The effects of caffeine on humans depend on many factors including the amount of caffeine intake, the weight of body, the age and pregnancy or smoker [81,95]. Some people are more sensitive to the effects of caffeine than others. Here are recommended upper limits for caffeine [62].

- healthy adults should stay below 300-500 milligrams daily
- pregnant women must stay below 150-200 milligrams daily
- children should stay below 50 milligrams daily

- for healthy adults, amounts exceeding 700 milligrams of caffeine can be dangerous.

7. Methods Used on Determination of Caffeine Content in Coffee

7. 1. Spectroscopy Methods

7.1.1. Ultraviolet Spectroscopy

Spectroscopy in the ultraviolet-visible regions (UV/VIS) is the most commonly used method for quantitative analysis of caffeine in coffee because of its simplicity and rapidity. Although spectrophotometry is a fast and simple method, it is not possible to determine caffeine directly in coffees by conventional UV absorption measurement due to the spectral overlapping [96]. Caffeine had to be extracted by organic solvent prior to measurement. Extraction of caffeine from coffee can be realized using a number of different solvents such as benzene, chloroform, trichloroethylene and dichloromethane, which are harmful for health and environment. To demonstrate the error in the determination of caffeine in coffee applying a UV spectrophotometery, Jozef [97] compared the results obtained by UV spectrophotometry with those obtained by HPLC and CE. Purcarea *et al.* [98] reported the method based on the spectrophotometric determination of caffeine in an extract form of a ground roasted coffee using chloroform as an extracting solvent. The levels of caffeine ranged between 1.24 and 1.48% in chloroform and 1.19% and 1.44% in distilled water. Ishler *et al.* [99] determined caffeine contents in green, roasted and soluble coffees using spectrophotometric absorbance at 272 nm. All interfering substances in coffee such as chlorogenic acid, trigonelline were removed by using zinc ferrocyanide or magnesium oxide, respectively. In this study, the amounts of caffeine ranged 0.98-1.00%.

7.1.2. Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR spectroscopy is a rapid and accessible technique, provides a wealth of qualitative and quantitative information on the composition of the samples, and in the last decade it has been a powerful tool to address the direct quantitative analysis of complex samples without prior separation steps. Sigh *et al.* [100] described the

method of FTIR, using an attenuated total reflectance accessory as a sampling system to estimate the amount of caffeine present in common beverages and quantitative determination of caffeine in a regular coffee sample. Coffee samples (powders) were dissolved in hot distilled water and filtered by filter paper and then an aqueous coffee solution was mixed with chloroform. After phase separation, chloroform solution was used for FTIR analysis. The absorbance band at 1655 cm^{-1} was used to estimate caffeine in standards and in the samples. The sensitivity of the technique was 5 ppm. The amount of caffeine per gram of coffee was 8.3 mg/g. The experiment provides a good demonstration of an analytical technique that can be adopted either for biochemistry or analytical chemistry course. Bouhsain *et al.* [101] developed a simple procedure for the fully automatic flow-injection (FI) determination of caffeine in ground coffee through the on-line extraction of caffeine with $CHCl_3$. Extraction was carried out from the solid samples, weighed inside empty solid extraction cartridges and wetted with few drops of an aqueous solution of NH_3, and the on-line determination by FTIR. The extract was injected into the carrier stream of $CHCl_3$ and transported through the measurement cell using a carrier flow of 1.2 mL/min. Analytical measurements were carried out at 1659 cm^{-1}, corrected with a baseline established between 1900 and 830 cm^{-1}, and the corresponding recordings obtained as a function of time. The method provided a limit of detection (LOD) of 9 mg/L caffeine. The caffeine percentage was (1.86-3.91) ± 0.09% (w/w) for commercial coffee. The FI-FTIR measurements provide an accurate methodology but with a clear reduction of the time required for sample preparation, reagent consumption and waste generation. Gallignani *et al.* [102] presented the use a flow injection analysis-Fourier transform infrared spectrometric (FIA-FTIR) method for the determination of caffeine in commercial coffee samples, obtaining a caffeine content ranging from 0.60 to 1.16% (w/w). The method involves the extraction, in batch, of caffeine from solid samples of roasted coffee with hot water (80 °C), on-line re-extraction of caffeine into chloroform and direct analysis of the organic phase by FTIR spectrometry. The measurement criterion selected for the quantification of caffeine was the absolute peak-walley

value (1668-1649 cm^{-1}) of the first order derivate of the absorption spectrum, corresponding to the 1659 cm^{-1} band. The method provides a limit of 0.001% (w/v) caffeine.

7.1.3. Derivative Spectrophotometric Methods

Derivative spectrophotometric methods have been developed for the assay of caffeine in some pharmaceutical preparations [103-105]. They are also supposed to be relative easy, fast and cheap for the determination of caffeine contents of cola, coffee and tea. Alpdogan *et al.* [106] described application of the third order derivative spectrum to quantify caffeine content in instant coffee. In this study, the extreme of 286.7 nm and 265 nm were used for quantitation of caffeine in instant coffee. To verify the results obtained by the derivative spectrophotometric method, the HPLC was used. The samples were analyzed on a C_{18} reversed-phase column using MeOH-H_2O (30:70) isocratic mobile phase, with flow rate 1.5 mL/min. The caffeine contents in both methods were 1.36% and 1.35%, for derivative spectrophotometry and HPLC, respectively. Derivative spectrophotometry can be applied for determination of caffeine in beverages without using pre-separation or background correction procedures. However, it is not reliable for the determination of small concentration of caffeine in samples. Method detection limits (MDLs) were 2 µg/g of caffeine.

7.2. Gas Chromatography

In recent years, gas chromatography (GC) has become the most widely used method in food analysis but has not been applied largely in the determination of caffeine in coffee when compared to HPLC. A GC method for determination of caffeine in 86 home-prepared beverage samples was described by Gilbert *et al.* [107]. The caffeine contents were traditionally quantified by GC-FID via extraction with chloroform solution of n-tetracosane. Flow rates were 250, 24 and 56 mL/min for air, hydrogen and nitrogen, respectively. The amounts of caffeine ranged 102-1170 µg/mL being percolated, drip or filter and instant (regular) coffee infusions; 2-8 µg/mL being decaffeinated coffee infusions. Ling *et al.* [108] compared the

suitability of spectrophotometric, gas chromatographic and high performance liquid chromatographic methods for caffeine determination of mixed coffees in Malaysia. To determine caffeine by gas chromatographic method, GC-FID was used with a Carbowax 30 m x 0.320 mm analytical column, helium flow rate 1.7 mL/min. Oven, injector and detector temperatures were maintained at 190, 193 and 250 °C, respectively. With this condition, caffeine content was found at 1.55 g/100 g depending on the ratio of Robusta in coffee mixtures.

7.3. HPLC Method

HPLC is one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitate the compounds that are present in any sample that can be dissolved in a liquid. The separation of caffeine in coffee was performed commonly by high performance liquid chromatography (HPLC). Successful separation of the individual compounds requires appropriate selection of columns, mobile phases and optimization of the operation conditions. The introduction of reversed-phase (RP) columns has considerably enhanced HPLC separation of caffeine. Columns for the determination of caffeine are almost exclusively composed of a reversed-phase RP C_{18} stationary phases ranging from 125 to 250 mm in length with an internal diameter ranging from 3.9 to 4.6 mm. The reversed-phase columns (RP C_{18}) are widely used for both, isocratic and gradient elution, for analysis of caffeine. Elution systems are usually binary, an aqueous acidified polar solvent such as acetic acid, citric acid and water (solvent A) and a less polar organic solvent such as methanol or acetonitrile (solvent B). Flow rates range from 0.8 to 2.3 mL/min, the most common being 2.3 mL/min. Thermostatically controlled columns are normally held at ambient or slightly above ambient temperatures (25 °C). Injections generally range from 10 μL [109-115].

Caffeine absorbs in the UV region and the most commonly used detector for HPLC is a variable-wavelength UV or UV/VIS detector. UV/VIS detectors includes (photo) diode array detector (DAD or PDA). Photodiode array (PDA) spectrometric detection has become very popular in HPLC of natural antioxidants, as it not only

allows easy selection of suitable detection wavelength providing best sensitivity of determination, but also UV/VIS spectra can be obtained on-line. This is a very useful feature of the PDA detection, in the method described by Franca *et al.* [109], PDA was used for simultaneous determination of caffeine, trigonelline and 5-caffeoyilquinic acid in green coffees. In their studies, ground coffee was extracted with boiling water and filtered prior to injection onto the HPLC column. The HPLC conditions used a Supelco C_{18} column (5 µm, 150 mm x 4.6 mm) and a mobile phase of methanol, water and acetic acid (15:85:1) at a flow rate of 1 mL/min with a photo-diode array detection (PDA) system. This method is accurate, precise, and conserves time. The caffeine content ranged 0.51 ± 0.02 – 0.68 ± 0.07 g/100g. Robert *et al.* [110] described the methods of determination of caffeine content in the regular and decaffeinated coffees brewed by cafeteria using RP-HPLC with UV/VIS detector, mobile phase consisted of H_2O, CH_3OH (70:30) at the flow rate 2.3 mL/min. Fujioka *et al.* [111] compared the caffeine contents among twelve commercial brewed ground roasted coffees (seven regular and five decaffeinated). The samples were analyzed using an Agilent 1100 model HPLC system equipped with a Zorbax Eclipse XDB C-18 5 µm column, with mobile phase of citric acid (A) and methanol (B). The gradient mode was initially set at A/B ratio of 85:15 (0-5min) and then linearly increased to 60:40 (40-80 min). The flow rate was 1.0 mL/min and UV detection at 276 nm. They ranged 10.09 ± 0.68 µg/g to 16.5 ± 0.24 µg/g and 0.34 ± 0.00 µg/g to 0.47 ± 0.01 µg/g for regular and decaffeinated samples, respectively. Recovery (%) was 97.7 ± 0.45%. Nogueira *et al.* [112] presented work on use of HPLC method in determination of caffeine content in instant, ground and decaffeinated coffees. The HPLC conditions used an UV–VIS detector and a mobile phase of water, methanol (60:40), at a flow rate of 0.9 mL/min with detection at 272 nm. The average caffeine content of instant coffee, ground coffee and decaffeinated was identified 32.5 mg/g, 13.5 mg/g and 0.7 mg/g respectively. Wanyika *et al.* [113] determined caffeine content of instant coffee brands found in the Kenyan market using both RP-HPLC and UV/VIS spectrophotometry. In their study, coffee sample was extracted with boiling water

and filtered prior to injection onto the HPLC column. Extracts were analyzed on reverse phase – ODS column, with mobile phase water, acetic acid and methanol (79.9:0.1:20) at a flow rate 1 mL/min. A photodiode array detector was set at 278 nm. In UV/VIS spectrophotometry, caffeine was extracted from coffee sample using a mixture of hydrochloric acid, basic lead acetate solution and sulphuric acid. Caffeine was determined by UV/VIS spectrophotometer at 274 nm using 10 mm quartz cuvette. In this study, higher concentration of caffeine in all the samples (between 717.79 ± 2.68 ppm and 1571.47 ± 2.53 ppm) were realized with the UV/VIS spectrophotometric method compared to HPLC method (between 327.80 ± 2.40 ppm and 684.56 ± 24.35 ppm) indicating that acidified water was a better caffeine extractor than pure water. Andueza *et al.* [114] described a method for determination of caffeine contents in espresso coffee brewed at different temperature (88, 92, 96 and 98 °C) of three types of EC from three roasted coffee samples using HPLC. A reverse phase Hypersil-ODS column (5 µm particle size, 250 mm x 4.6 mm) was used. The mobile phase was acetonitrile, water (15:85) in isocratic condition at a constant flow rate of 2.0 mL/min at 25 °C. Detection was accomplished with a diode array detector, and chromatograms were recorded at 280 nm. The amounts of caffeine ranged from 2.02 ± 0.03 to 2.31 ± 0.10 mg/mL for Arabica; 2.94 ± 0.08 to 3.03 ± 0.05 mg/mL for Robusta Natural blend and 2.90 ± 0.05 to 2.92 ± 0.04 mg/mL for Robusta Torresfacto blend. As we can see, caffeine content in Robusta samples were higher than in Arabica and was not affected by temperature used to brew. Lowor *et al* [115] used HPLC to evaluate the effect of dry method and density of drying on caffeine content of green coffee beans. In this study, the ground green coffee sample was dissolved in distilled water. The extract of caffeine was analyzed by HPLC system with a dual absorbance detector at 280 nm. The chromatographic separation was achieved by a Hypersil ODS C_{18} column (25 cm x 4.6 mm) and compound was isocratically eluted with a mobile phase of methanol, acetic acid and water (20:1:79) at a flow rate of 1 mL/min at 25 °C. With HPLC methods, the disadvantage is the use of expensive equipment and the

demand for more operator attention prevent their application in small industrial laboratories where only a few analyses are performed each day.

7.4. Capillrary Electrophoresis (CE)

As can see, there have not been a large number of publications on the capillary electrophoretic (CE) analysis of caffeine in coffee, although CE provides several advantages, such as extremely low solvent consumption, smaller sample volume requirements, and improved sensitivity. Conte *et al.* [116] determined caffeine content in coffee using CE method with a 50 mM sodium borate buffer at pH 8.5 and UV detection at 254 (a Hg source) and +25 kV (70 μA) applied voltage. Caffeine content was 164 ± 17 mg/355 mL serving.

II. THE OBJECTIVES OF THE STUDY

1. General Objective

- The determination of caffeine in coffee is a difficult analytical problem because of interfering matrices.
- The aim of this study was to develop and validate rapid quantitative UV/VIS spectrophotometric method for the quantification of caffeine that could be applied routinely to commercial coffee products. Results of this study could be used to facilitate selection of the appropriate method in order to obtain satisfactory data on caffeine content in commercial materials.

2. Specific Objective

- Employing UV/VIS for the determination of caffeine content in different ground roasted coffee.
- To compare the caffeine content in different commercial coffee samples.
- To evaluate the content of caffeine and to study their changes during brewing in order to control the negative physiological effects and still keep the desirable attributes of a coffee beverage.
- To describe health benefits of caffeine of coffee brewing.
- To discuss analytical methods on determination of caffeine content in coffee. The experiment will give the ability to measure the concentration of caffeine in a favorite coffee. The consumers want to know how much caffeine is in coffee that they drink every day.

III. MATERIALS AND METHODS

1. Materials and Chemicals

The caffeine was quantified using a UV/VIS spectrophotometry using Lambda 25 system (wavelength range 190 nm to 1100 nm, double monochromator) with UV WinLab v2.85 software and 10 mm quartz cells (all from Perkin-Elmer, Norwalk, USA), balance (BP 210S, d = 0.1 mg, max 210 g), magnetic stirrer, glass filter, beakers, thermometer, 1 cm quartz cuvette, separatory funnel, funnel, distilled water, chloroform ($CHCl_3$) (99%), caffeine standard (>99%), sodium carbonate (Na_2CO_3) were obtained from Sigma-Aldrich (UK).

2. Coffee Samples

The Vietnamese ground roasted coffee samples, including Dak Tin (Ca phe Dak Tin LTD), Di Linh (Cty CP Chè-Cafe Di Linh), Nam Nguyen (Công ty chế biến cà phê Nam Nguyên), Origin (Công ty TNHH một thành viên Tín Nghĩa) and Vinacafe (Công ty cổ phần Vinacafe Biên Hòa) were purchased at Saigon CO.OP, in HoChiMinh, in Vietnam. The Czech ground roasted coffee samples, including Dadák (Dadák s.r.o. Valašské Meziříčí), Jacobs (Kraft Foods CR s.r.o, Czech Republic), Marila 100% Robusta (Mokate Czech s.r.o, Czech Republic), Jihlavanka (Tchibo Praha s.r.o., Jihlava, Czech Republic), Grande 100% Arabica (Grande Ltd., Poland; imported by Kaufland, Czech Republic), Nescafe instant (Nestle Česko s.r.o., Prague, Czech Republic) were purchased at local supermarket in Zlín in the Czech Republic.

3. Preparation of Standards

3.1. Preparation of calibration curve in H_2O

Caffeine stock solution (100 μg/mL) was prepared by dissolving 10 mg of caffeine in 100 mL of water. This solution was diluted to provide the necessary working concentration (10.0-16.0 μg/mL) to obtain standard solutions for the preparation of calibration curves. UV spectrum of 4 caffeine standard solutions (10-16 μg/mL) was

recorded against water over the wavelength range of 190-300 nm, at room temperature.

Table 9. Absorbance of caffeine standards in water

Conc (µg/mL)	0	10	12	14	16
A at 273.2 nm	0	0.514	0.612	0.715	0.817

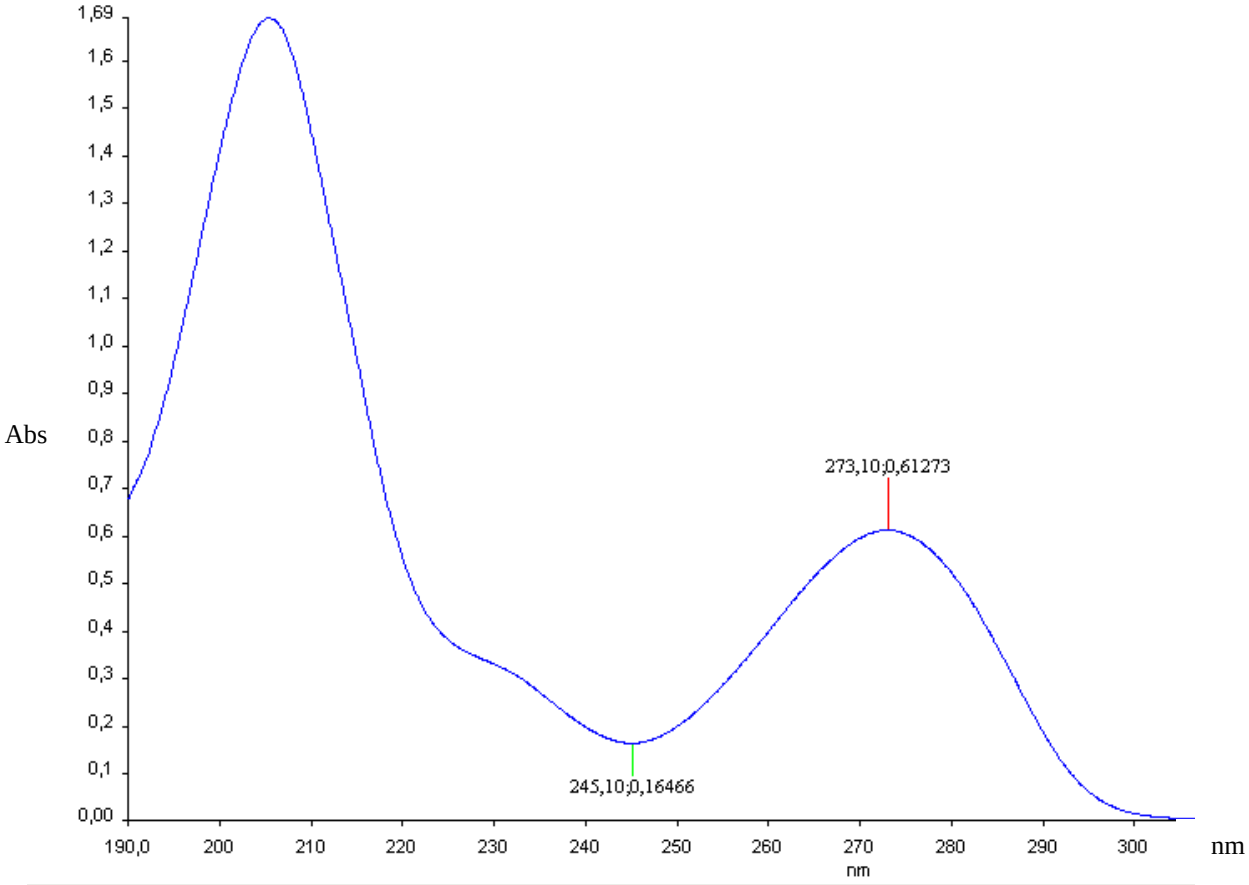

Figure 18. Caffeine standards in water, concentration 12 µg/mL, reference: water

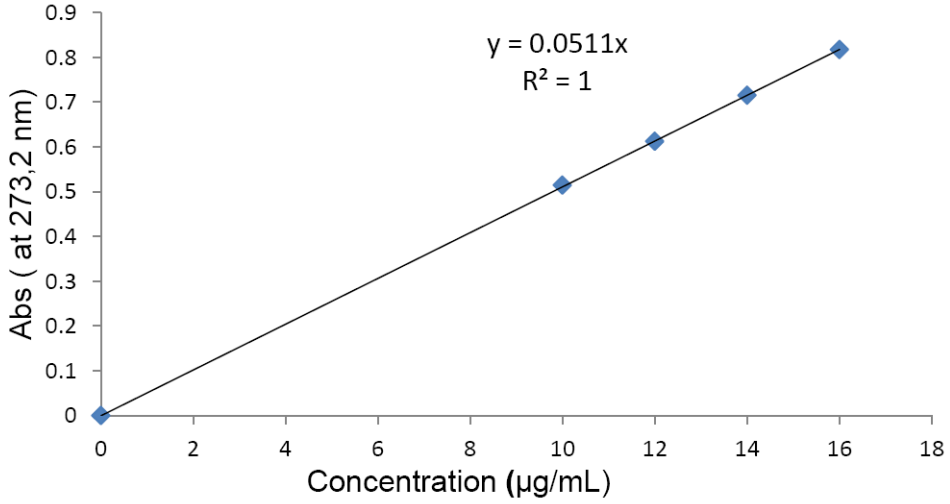

Figure 19. Calibration curve of caffeine standard in H_2O

3.2. Preparation of calibration curve for first order derivative spectrophotometry

The first order derivative absorption spectra of 4 caffeine standard solutions (6.0, 8.0, 10.0 and 12.0 µg/mL) were recorded (see Fig. 1) against distilled water in range of 190 and 300 nm. Peak-to-peak measurements [106,117] of two neighboring peaks of 260.4 nm (minima) and 286.6 nm (maxima) were used for preparation of calibration curve.

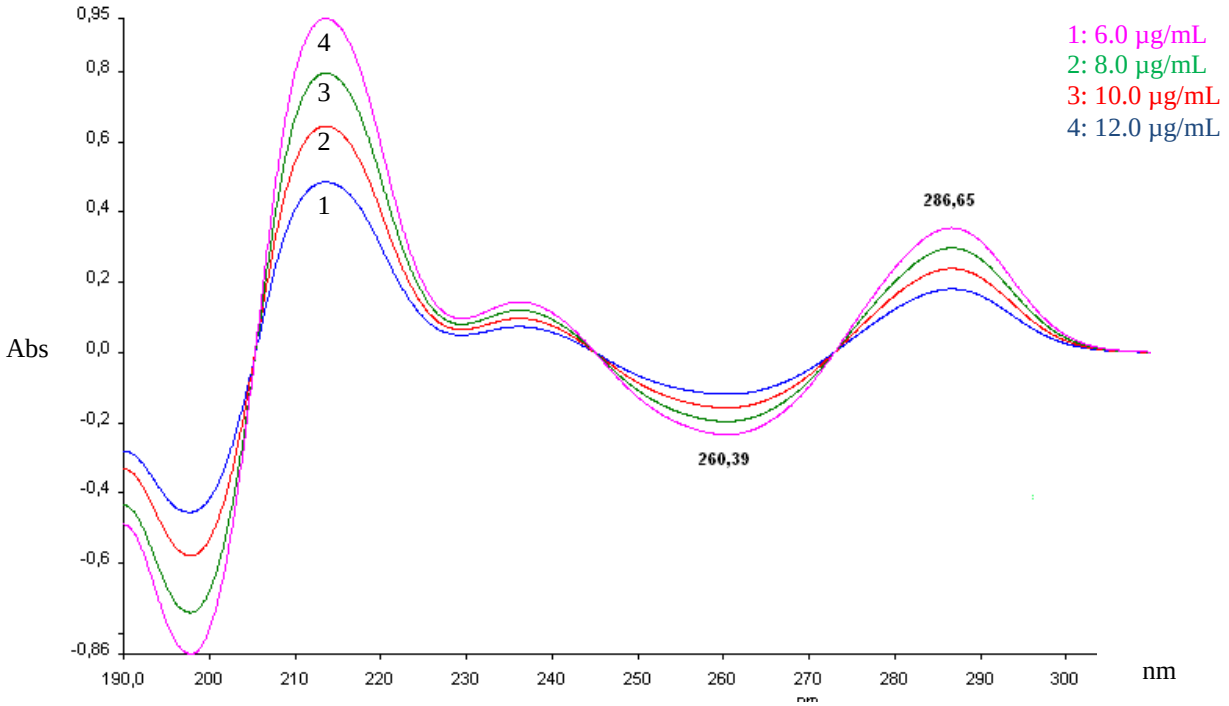

Figure 20. First derivative spectra of caffeine standards (6.0, 8.0, 10.0 and 12.0 µg/mL), reference: water;

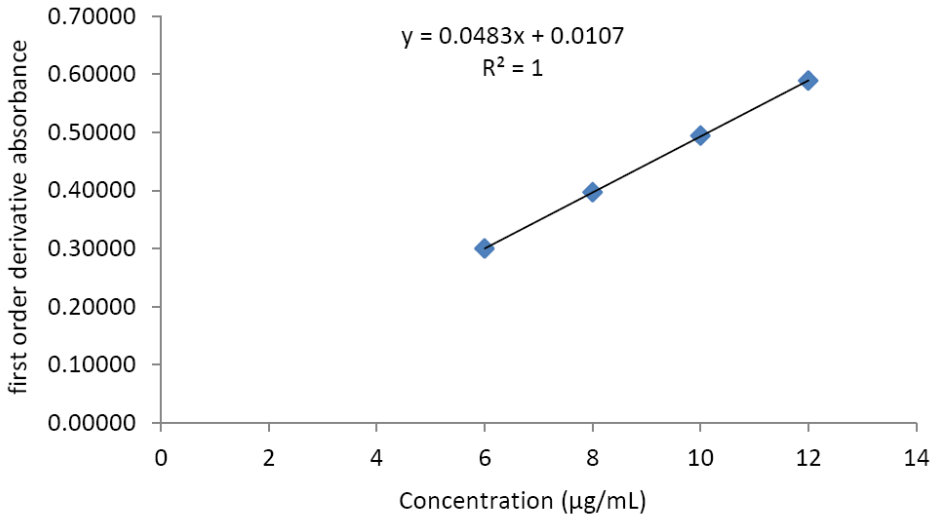

Figure 21. Calibration curve of the 1st derivative absorption spectra of caffeine standards

3.3. Preparation of calibration curve in $CHCl_3$

Standard solutions of caffeine in chloroform (AOAC official method 979.11) were prepared by dissolution of 100 mg of pure caffeine (accurately weighed anhydrous sample) in 100 mL $CHCl_3$ and further dilution of 10 mL aliquot of the solution to 100 mL with $CHCl_3$. Working standard solutions of 10, 20, and 30 µg/mL were prepared by further dilution of 10, 20, and 15 mL aliquots of the solution to 100, 100, and 50 mL with $CHCl_3$, respectively. The absorbance of the working standard solutions and the sample solutions were measured at 276.2 nm against a $CHCl_3$ blank.

Table 10. Absorbance of caffeine standards in chloroform

Conc (µg/mL)	0	10	20	30
At 276.2 nm	0	0.4925	0.9798	1.4652

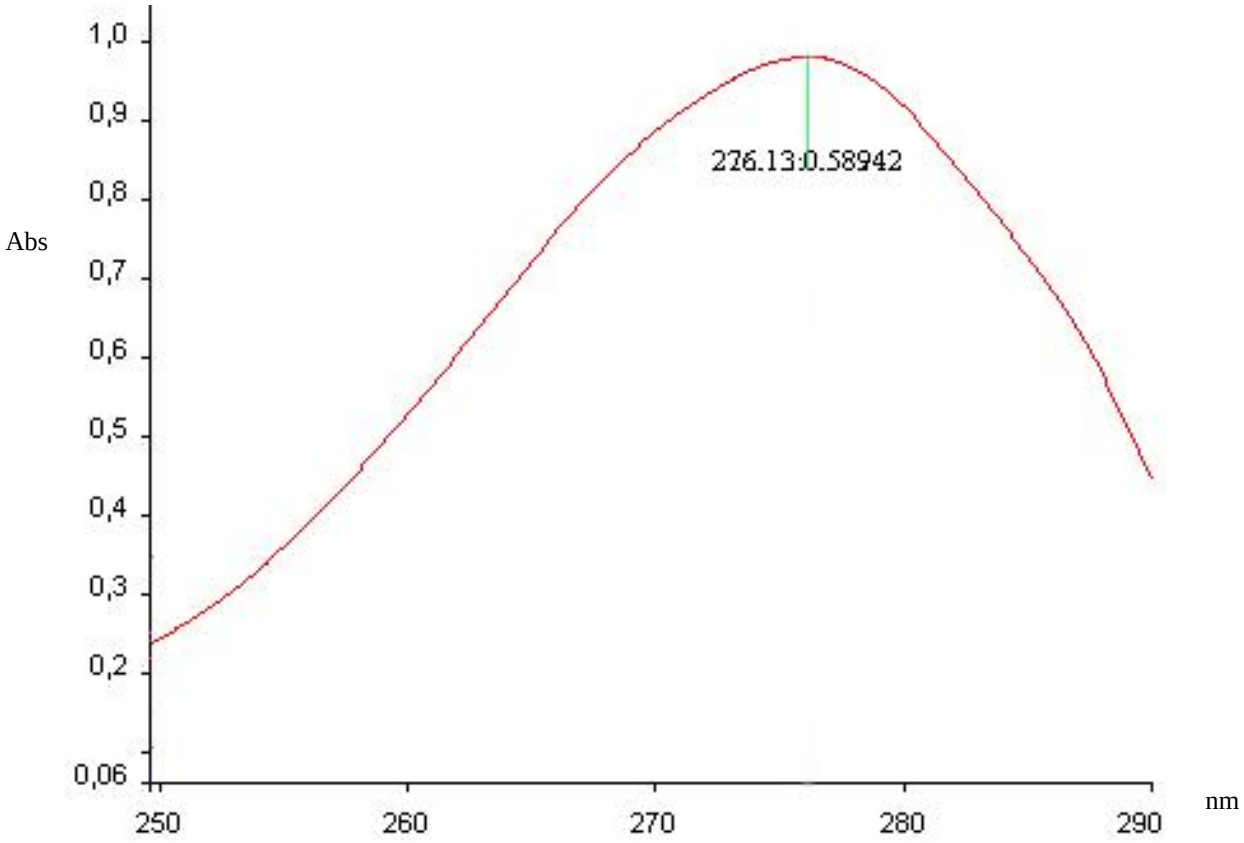

Figure 22. Caffeine standards in $CHCl_3$, concentration 10 µg/mL, reference: chloroform

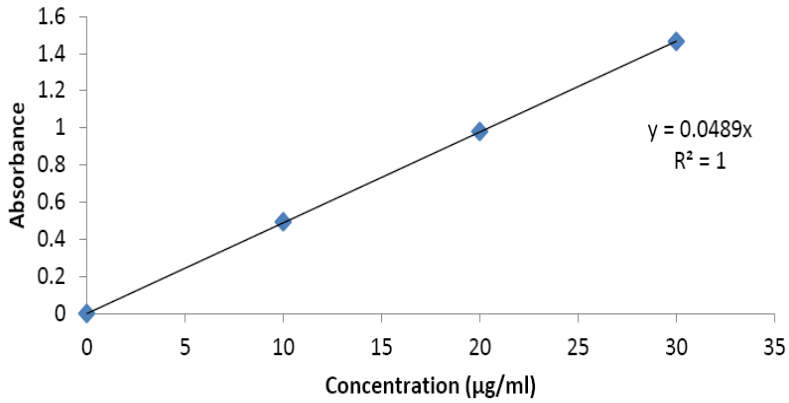

Figure 23. Calibration curves in $CHCl_3$

3.4. Preparation of standard solutions for standard addition method

Caffeine stock solution of 100 µg/mL was prepared by dissolving 10 mg of caffeine in 100 mL of distilled water. The caffeine solution was then diluted to provide the necessary working concentration 16 µg/mL to obtain standard solutions for the preparation of standard addition calibration curves.

10 mL of filtrate (coffee sample) was added to each of four 25 mL volumetric flask. Then a series of increasing volumes (0, 1, 2 and 3 mL) of caffeine standard solution of 16 µg / mL were added. Finally, each flask was made up to the mark with distilled water and mixed well. After measuring the response for a series of standard additions, we get the value of the concentration of caffeine in the sample in 25 mL (x).

$y = ax + b = 0$ (1)

$x = -b/a$ (µg/mL) (2)

Then, concentration of caffeine in coffee sample: $(x*25)/10$ (µg/mL) (3)

Table 11. Standard addition

Volumes added (mL)	Concentration added (µg/mL)
0	0
1	0.64
2	1.28
3	1.92

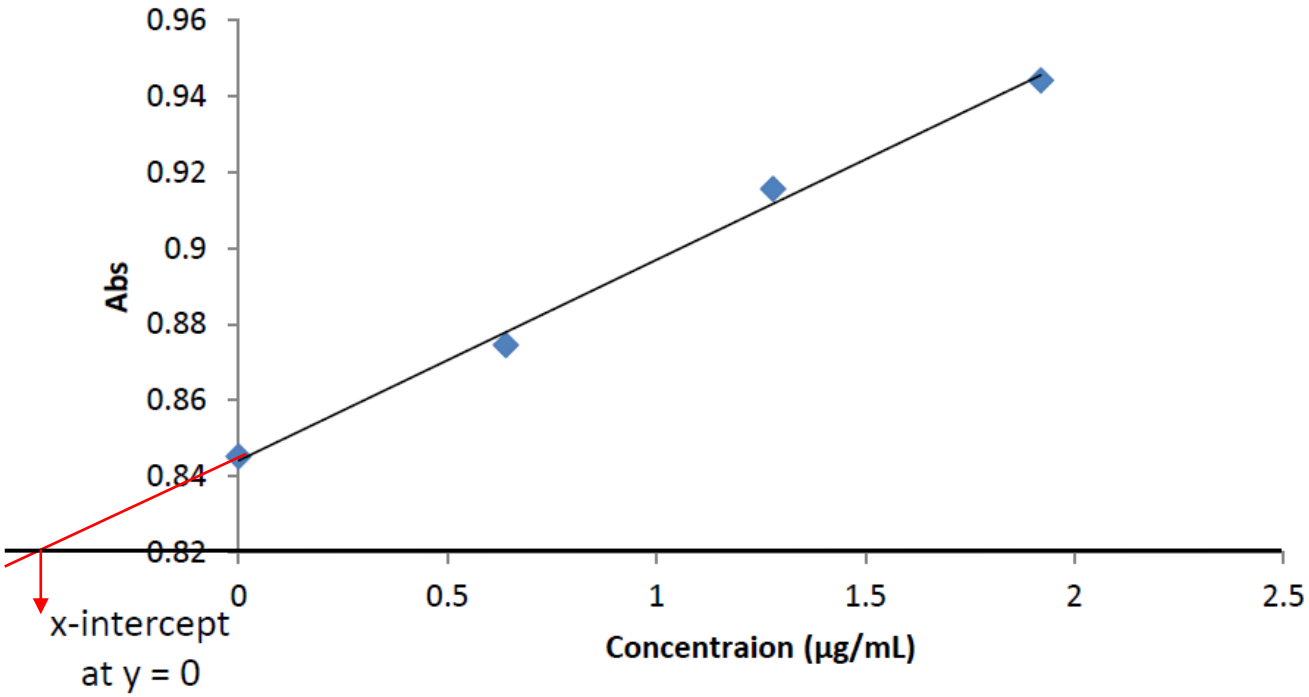

Figure 24. Standard addition method

4. Sample preparation

4.1. Direct conventional UV/VIS spectrophotometry

0.05 g of ground roasted coffee and 50 mL of H_2O (100 °C) was added into the beaker and the mixture was stirred for 1 hour using magnetic stirrer at room temperature. The mixture was filtered by a paper filter. The filter paper and the funnel were rinsed thoroughly with distilled water. The filtrate was collected in 100 mL volumetric flask and diluted to 100 mL by distilled water. The concentrations of caffeine were calculated from the regression equation ($y = 0.0511$, $r^2 = 1$), in which y is absorbance of sample at 273.2 nm and x is concentration of caffeine in the samples. The % caffeine in coffee samples was obtained by the following formula: caffeine content/cake content x 100, (cake content: 0.05 g).

4.2. Derivative spectrophotometry

0.05 g of ground roasted coffee and 50 mL of H_2O (100 °C) was added into the beaker and the mixture was stirred for 1 hour using magnetic stirrer at room temperature. The mixture was filtered by a paper filter. The filter paper and the funnel were rinsed thoroughly with distilled water. The filtrate was collected in 100 mL volumetric flask and diluted to 100 mL by distilled water. The concentrations of

caffeine were calculated from the regression equation ($y = 0.0483x + 0.0107$, $r^2 = 1$) in which y is peak-to-peak amplitudes of the first order spectra at extrema of each sample (Figure 25-34) and x (µg/mL) is concentration of caffeine in samples. The % caffeine in coffee samples was obtained by the following formula: caffeine content/cake content x 100, (cake content: 0.05 g).

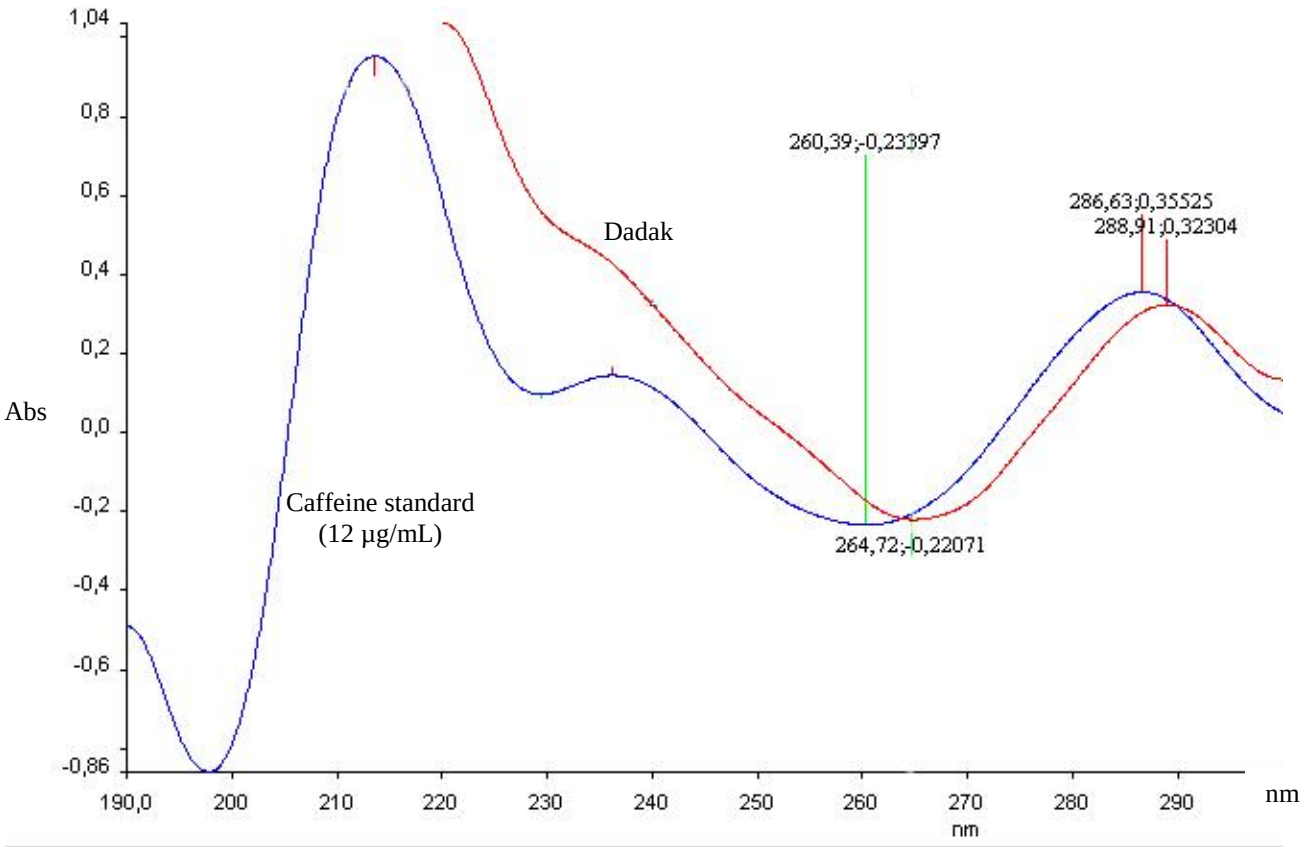

Figure 25. First order derivative spectra of Dadak; and caffeine standard (12 µg/mL), reference: water.

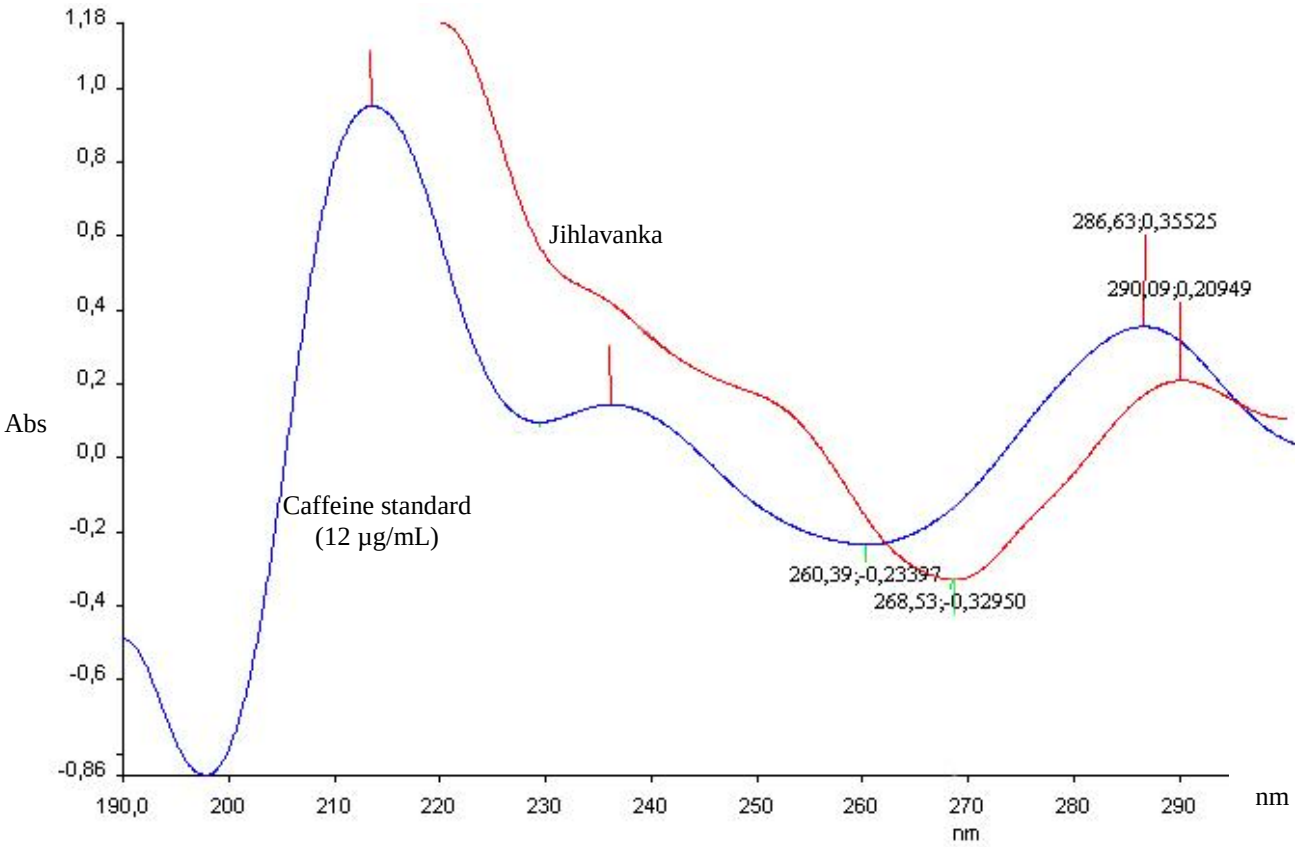

Figure 26. First order derivative spectra of Jihlavanka; and caffeine solution (12 µg/mL), reference: water.

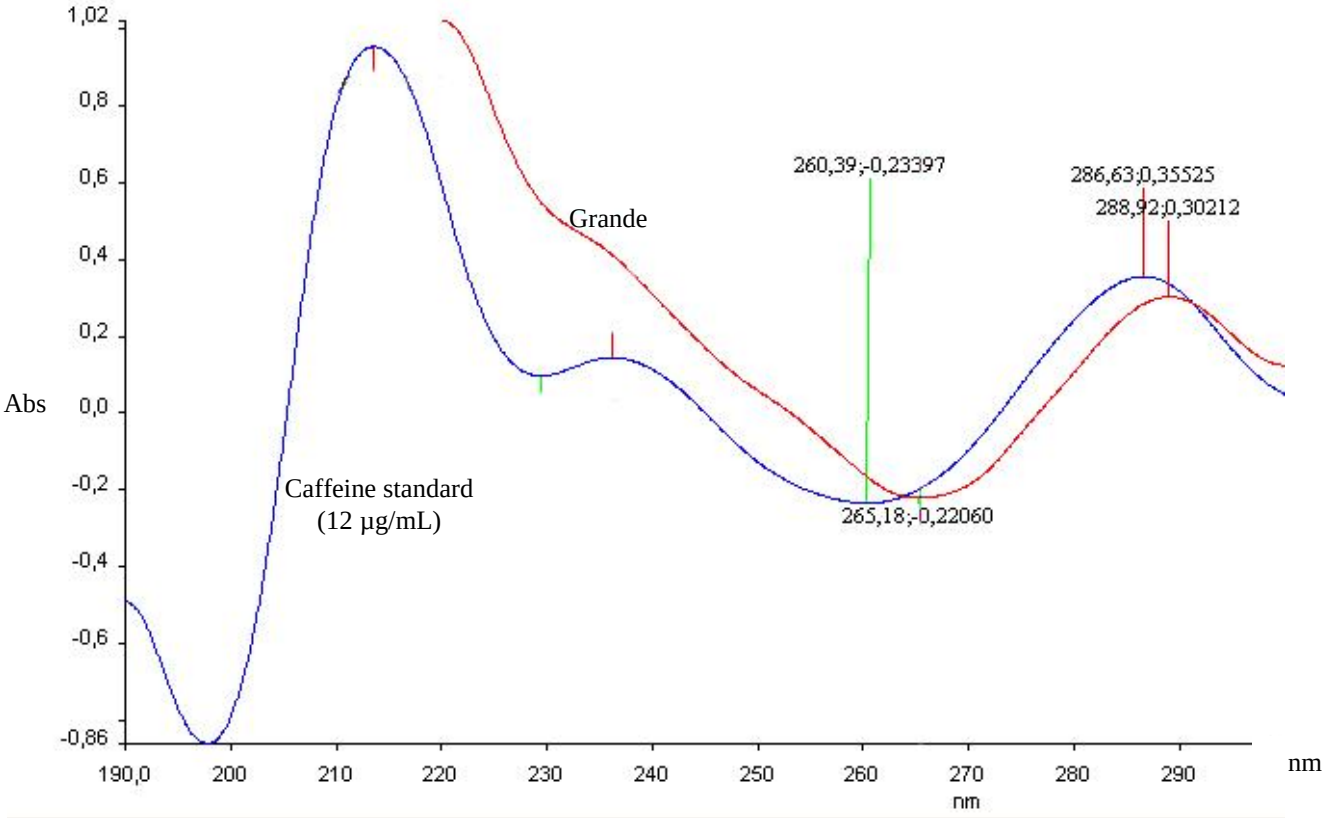

Figure 27. First order derivative spectra of Grande; and caffeine solution (12 µg/mL), reference: water.

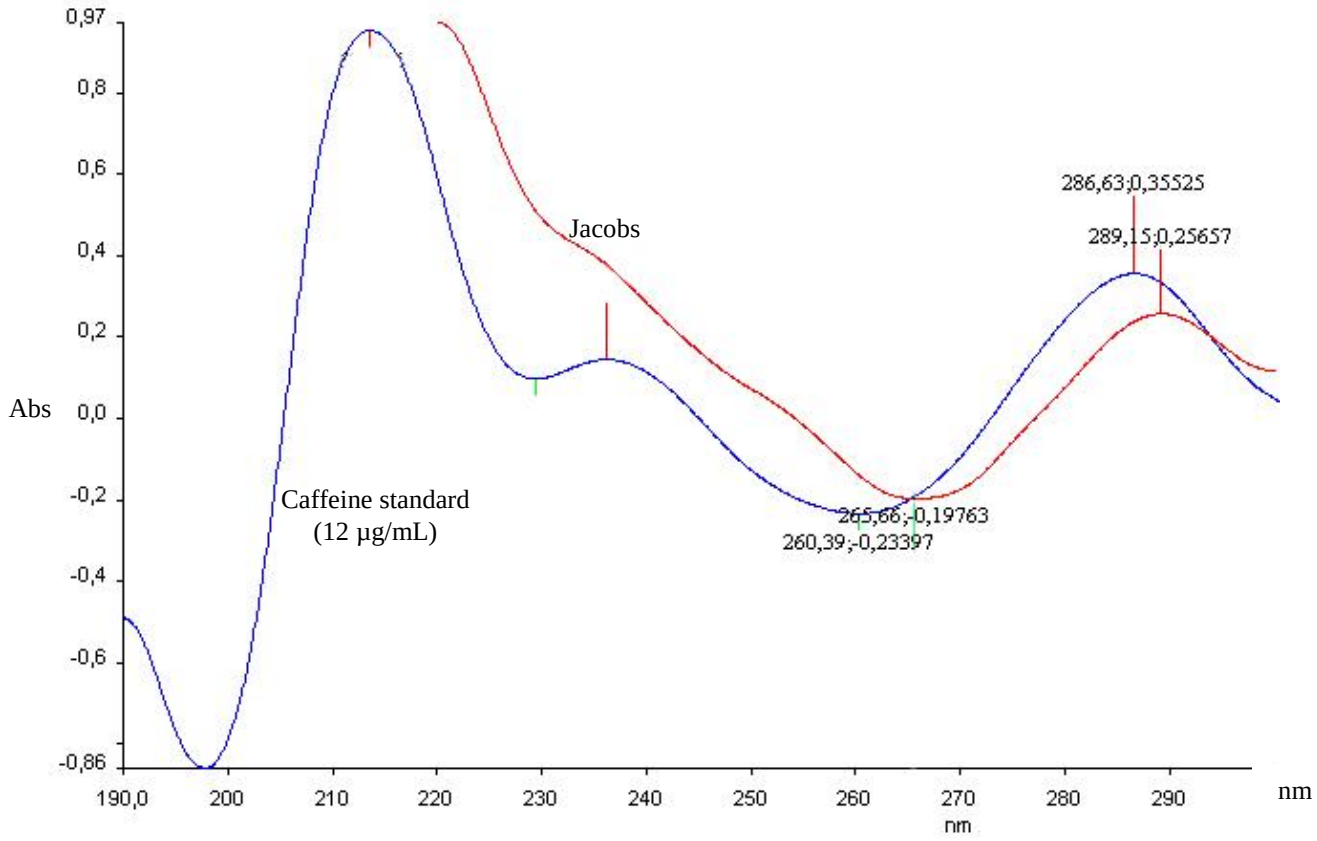

Figure 28. First order derivative spectra of Jacobs; and caffeine solution (12 µg/mL), reference: water.

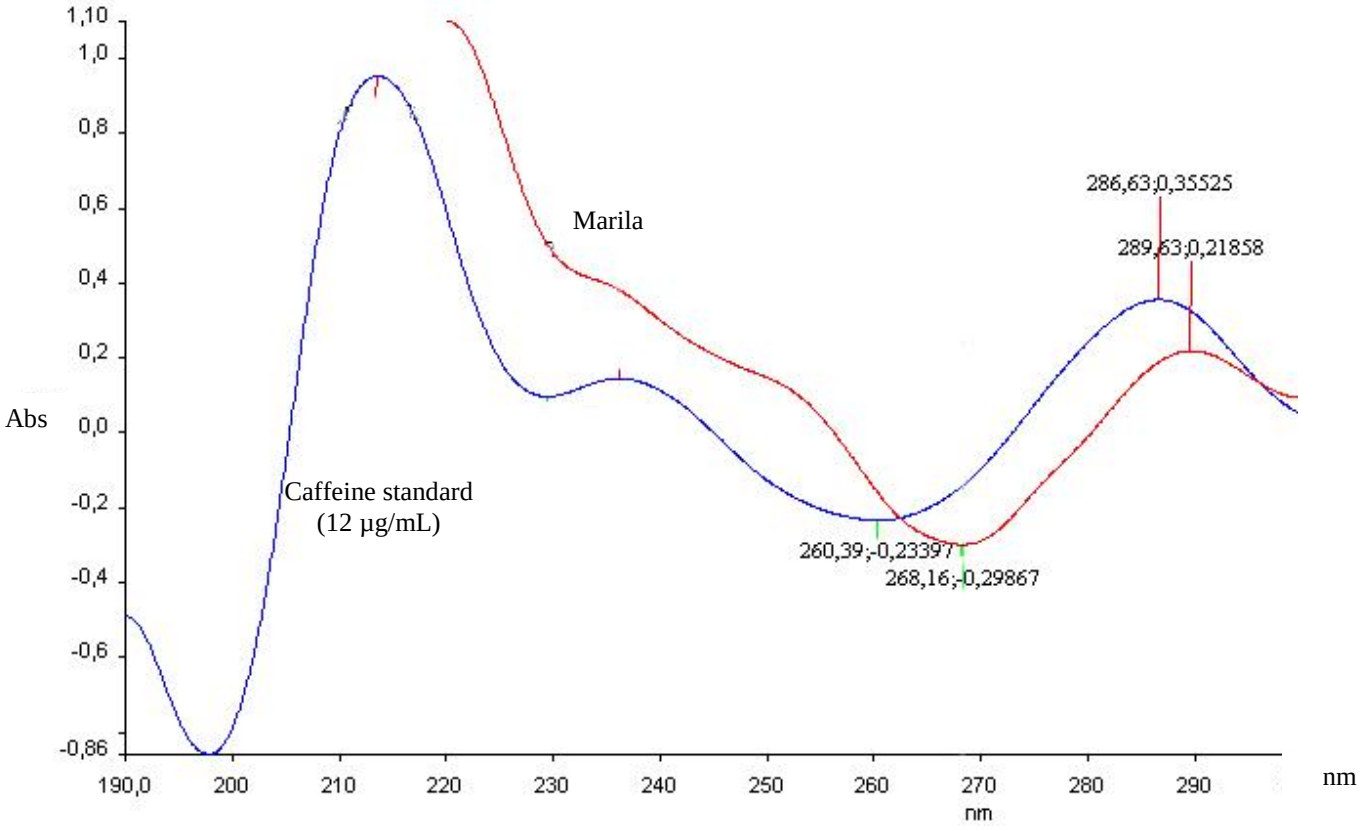

Figure 29. First order derivative spectra of Marila; and caffeine solution (12 µg/mL), reference: water.

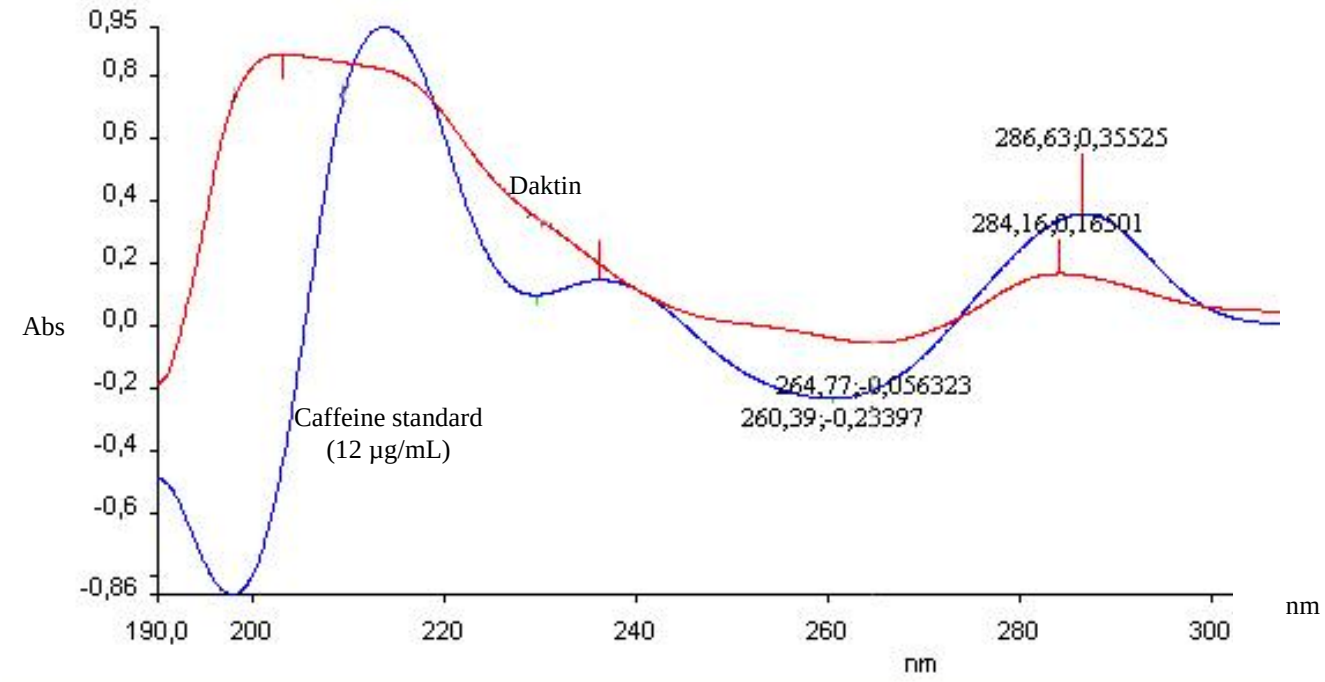

Figure 30. First order derivative spectra of Daktin; and caffeine solution (12 µg/mL), reference: water.

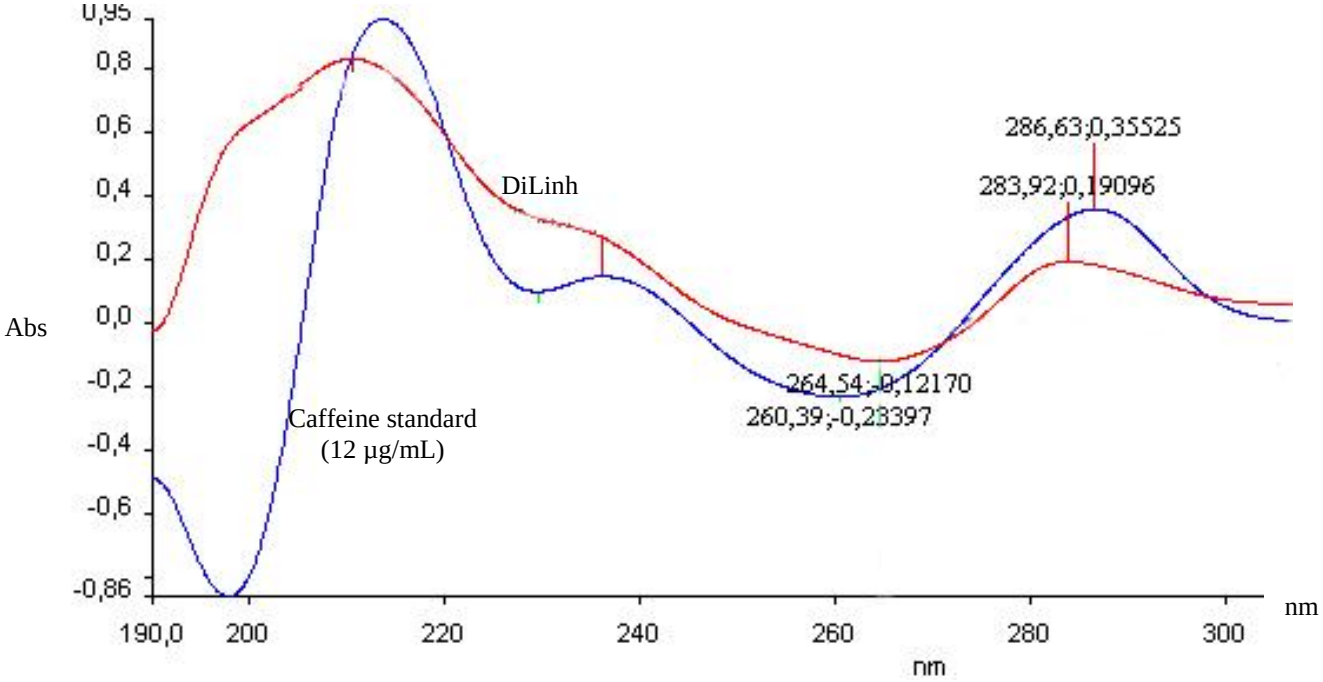

Figure 31. First order derivative spectra of DiLinh; and caffeine solution (12 µg/mL), reference: water.

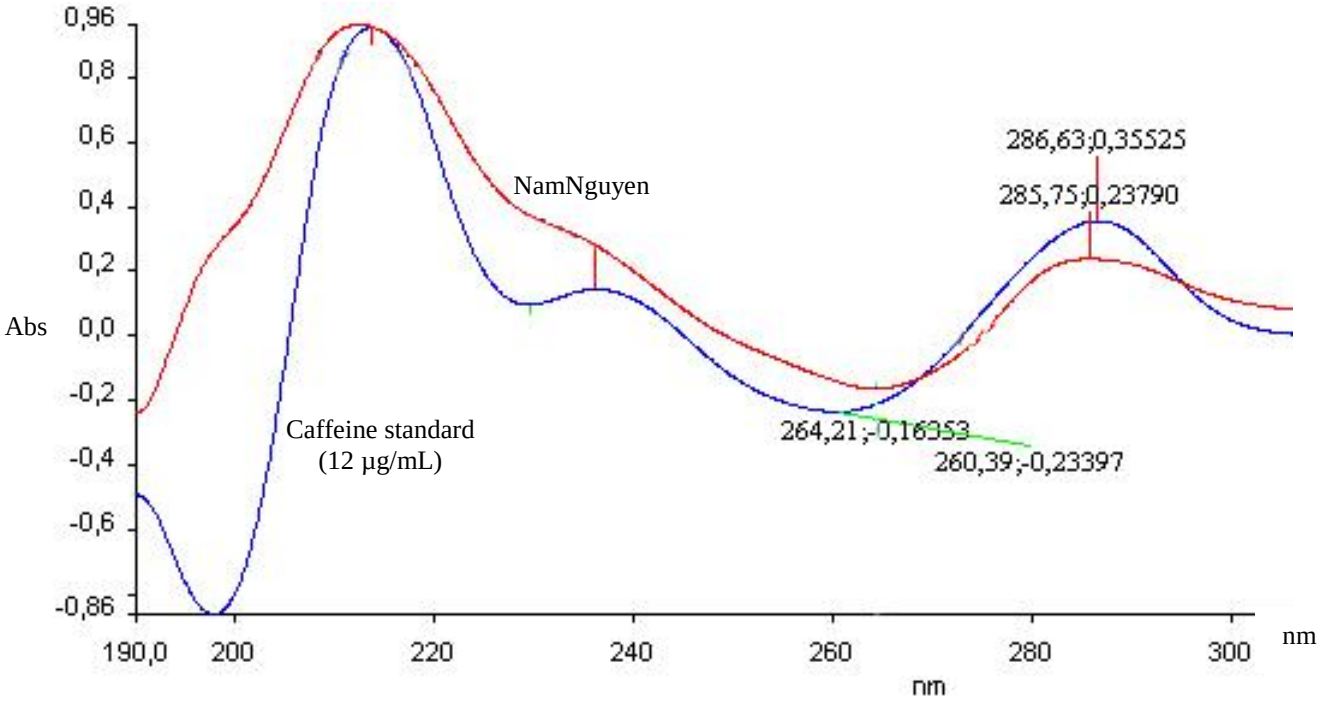

Figure 32. First order derivative spectra of NamNguyen; and caffeine solution (12 µg/mL), reference: water.

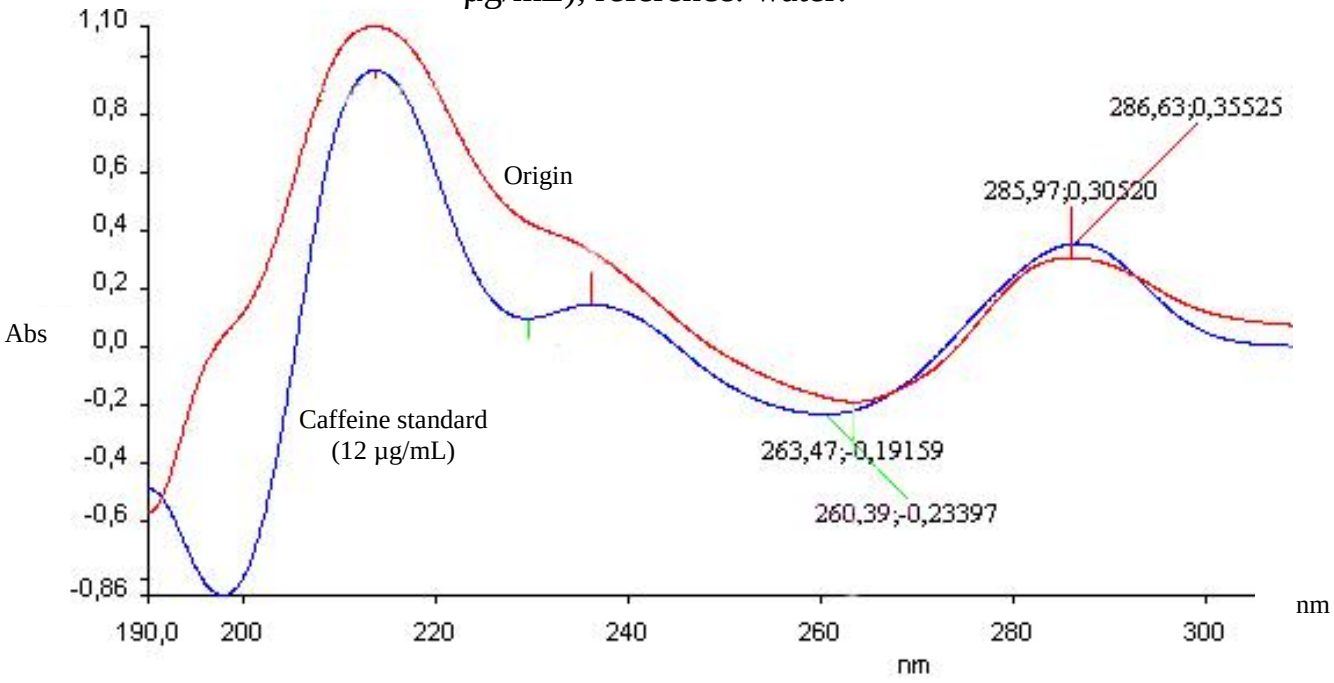

Figure 33. First order derivative spectra of Origin; and caffeine solution (12 µg/mL), reference: water.

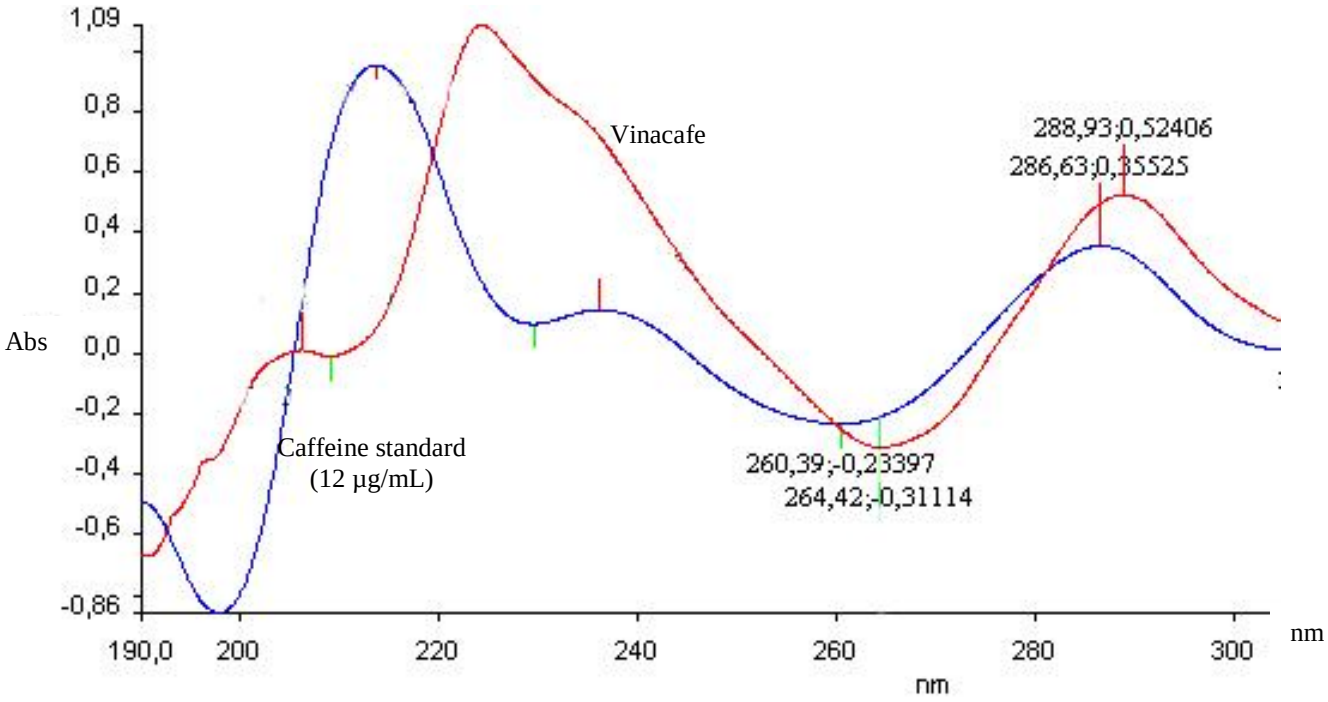

Figure 34. First order derivative spectra of Vinacafe; and caffeine solution (12 µg/mL), reference: water.

4.3. Liquid-liquid extraction by $CHCl_3$

0.05 g of ground roasted coffee and 10 mL of distilled boiling water (100 °C) was poured into the beaker. The mixture was gently stirred for 1 hour using magnetic stirrer to extract caffeine. The mixture was filtered by a paper filter, the filter paper and the funnel were rinsed thoroughly using water. After cooling to room temperature, Na_2CO_3 (0.2 g/10 mL) was added to the filtrate (coffee solution) to remove non-caffeine solids [57], the filtrate was adjusted to 10 mL with distilled water. 10 mL of this solution was mixed with 10 mL $CHCl_3$ for extraction of caffeine from coffee. The mixture was extracted for 10 minutes (venting the funnel after each inversion). The mixture was settled for 30 min and the lower phase (chloroform containing caffeine) was poured into a 50 mL volumetric flask. The extraction was preceded 3 times with 10 mL $CHCl_3$ at each round. The combined extracts collected in the 50 mL volumetric flask were diluted to 50 mL by $CHCl_3$. The caffeine content in coffee solution was quantified using a UV/VIS spectrophotometric procedure described above. The concentrations of caffeine were calculated from the regression equation ($y = 0.0489x$, $r^2 = 1$) in which y is absorbance of sample at 276.2 nm and x

is concentration of caffeine in samples. The % caffeine in coffee samples was obtained by the following formula: caffeine content/cake content x 100, (cake content: 0.05 g).

4.4. Standard addition method

Influence of temperature (80, 90 and 100 °C): 2.0 g of ground roasted coffee was put in a beaker. After that 100 mL of distilled water at 80, 90 and 100 °C was poured into the beaker. After 3 minutes, the mixture was filtered by a paper filter. After cooling, the filtrate was adjusted to 250 mL with distilled water and was ready for analysis.

Influence of water volume (30, 70 and 150 mL): 2.0 g of ground roasted coffee was put in a beaker. After that 30, 70 and 150 mL of distilled water at 90 °C was poured into the beaker. After 3 minutes, the mixture was filtered by paper filter. After cooling, the filtrate was adjusted (diluted) to 250 mL with distilled water and was ready for analysis.

Influence of extraction time (1, 3 and 5 min): 2.0 g of ground roasted coffee was put in a beaker. After that 100 mL of distilled water at 90 °C was poured into the beaker. After 1, 3 and 5 min, the mixture was filtered by paper filter. After cooling, the filtrate was adjusted (diluted) to 250 mL with distilled water and was ready for analysis.

IV. RESULTS AND DISCUSSIONS

Table 12. Caffeine contents of coffee infusions at different volume

Sample (n=3)	**Volume (mL)**	µg/(mL) Mean±SD	Regression equation
DaK Tin			
	30	20.10 ± 0.140	y = 0.0523x + 0.4201
	70	19.64 ± 0.202	y = 0.0515x + 0.4045
	150	21.10 ± 0.450	y = 0.0528x + 0.4363
Di Linh			
	30	31.30 ± 0.410	y = 0.0522x + 0.6533
	70	28.02 ± 0.400	y = 0.0529x + 0.593
	150	31.73 ± 0.240	y = 0.0531x + 0.6739
Nam Nguyen			
	30	43.12 ± 0.222	y = 0.0534x + 0.921
	70	41.52 ± 0.720	y = 0.0523x + 0.8685
	150	40.00 ± 0.300	y = 0.0527x + 0.8396
Origin			
	30	39.73 ± 0.630	y = 0.0524x + 0.8328
	70	40.30 ± 0.674	y = 0.0525x + 0.8456
	150	39.80 ± 0.465	y = 0.052x + 0.8269
Vinacafe			
	30	49.20 ± 0.740	y = 0.049x + 0.9634
	70	49.30 ± 0.590	y = 0.0498x + 0.986
	150	50.10 ± 0.564	y = 0.0506x + 1.0134

All results were evaluated using Origin software 8.5.1.

Table 13. Caffeine contents of coffee infusions at different time

Sample (n=3)	**Time**	μg/(mL)	Regression equation
DaK Tin			
	1	19.40 ± 0.232	y = 0.0518x + 0.4037
	3	20.00 ± 0.430	y = 0.0512x + 0.4109
	5	22.00 ± 0.272	y = 0.0531x + 0.4661
Di Linh			
	1	28.81 ± 0.854	y = 0.0528x + 0.6085
	3	31.00 ± 0.310	y = 0.0521x + 0.645
	5	30.20 ± 0.252	y = 0.0508x + 0.6131
Nam Nguyen			
	1	31.02 ± 0.800	y = 0.0517x + 0.6415
	3	33.23 ± 0.831	y = 0.0516x + 0.6859
	5	35.73 ± 0.420	y = 0.053x + 0.7575
Origin			
	1	35.60 ± 0.440	y = 0.0526x + 0.7482
	3	40.42 ± 0.561	y = 0.0524x + 0.8472
	5	43.30 ± 0.354	y = 0.0522x + 0.9042
Vinacafe			
	1	49.64 ± 0.782	y = 0.0496x + 0.9848
	3	53.00 ± 0.184	y = 0.0475x + 1.0056
	5	54.30 ± 0.470	y = 0.047x + 1.0202

All results were evaluated using Origin software 8.5.1.

Table 14. Caffeine contents of coffee infusions at different temperature

Sample (n=3)	**Temperature**	μg/(mL)	Regression equation
DaK Tin			
	80	20.00 ± 0.360	y = 0.0522x + 0.4158
	90	23.00 ± 0.110	y = 0.0522x + 0.4737
	100	23.80 ± 0.300	y = 0.0521x + 0.4954
Di Linh			
	80	26.00 ± 0.940	y = 0.0521x + 0.536
	90	32.50 ± 0.503	y = 0.0534x + 0.6916
	100	33.00 ± 0.360	y = 0.052x + 0.6988
Nam Nguyen			
	80	32.33 ± 0.080	y = 0.0532x + 0.6879
	90	35.00 ± 0.591	y = 0.0519x + 0.7263
	100	36.04 ± 0.664	y = 0.0532x + 0.7669
Origin			
	80	33.40 ± 0.800	y = 0.0528x + 0.7064
	90	35.00 ± 0.610	y = 0.0537x + 0.7454
	100	36.10 ± 0.170	y = 0.05x + 0.7217
Vinacafe			
	80	51.00 ± 0.700	y = 0.0502x + 1.015
	90	52.00 ± 0.423	y = 0.0532x + 1.0128
	100	53.00 ± 0.300	y = 0.0483x + 1.019

All results were evaluated using Origin software 8.5.1.

Table 15. Caffeine content (mean ± S.D.+ in % w/w; n = 3) in Czech samples and in coffee brewing at 100 °C and for 1 hour

No.	Samples	Caffeine content (%)		
		Direct measurement	**Extraction using $CHCl_3$**	**1st derivative measurement**
1	Dadak	6.51 ± 0.06	2.12 ± 0.06	2.25 ± 0.04
2	Jihlavanka	8.03 ± 0.07	2.12 ± 0.03	2.21 ± 0.04
3	Nescafe Instant [a]	15.00 ± 0.07	3.47 ± 0.08	4.45 ± 0.02
4	Jacobs	6.22 ± 0.02	1.91 ± 0.06	1.98 ± 0.06
5	Marila	7.04 ± 0.10	2.09 ± 0.10	2.20 ± 0.02
	Mean [b]	**6.95 ± 0.06**	**2.06 ± 0.06**	**2.16 ± 0.04**

[a] the values were deleted from the calculation of means due to the different origin of the samples, [b] means calculated from all the values with exception of those for Nescafe Instant; all results were evaluated using Origin software 8.5.1.

Table 16. Caffeine content (mean ± S.D.+ in % w/w; n = 3) in Vietnamese samples and in coffee brewing at 100 °C and for 1 hour

No.	Samples	Caffeine content (%)		
		Direct measurement	**Extraction using $CHCl_3$**	**1st derivative measurement**
1	Dak Tin	2.90 ± 0.05	0.48 ± 0.01	0.75 ± 0.01
2	Di Linh	4.10 ± 0.16	0.78 ± 0.01	1.43 ± 0.01
3	Nam Nguyen	4.50 ± 0.01	1.22 ± 0.02	1.65 ± 0.03
4	Origin	5.55 ± 0.11	1.56 ± 0.02	2.10 ± 0.02
5	Vinacafe	6.62 ± 0.01	2.10 ± 0.08	2.15 ± 0.02
	Mean	**4.73 ± 0.07**	**1.23 ± 0.03**	**1.62 ± 0.02**

All results were evaluated using Origin software 8.5.1.

Table 17. Caffeine content (mean ± S.D.+ in % w/w; n = 3) in Czech samples and in coffee brewing at 100 °C and for 3 minutes

Samples	Extraction using $CHCl_3$		1st derivative measurement			
	Content (%)	Transfer (%)	Content (%)	Transfer (%)	Minima (nm)	Maxima (nm)
Dadák	2.11 ± 0.04	97	2.12 ± 0.01	94	265	289
Jihlavanka	2.20 ± 0.02	96	2.20 ± 0.04	99	268	290
Grande	1.98 ± 0.1	95	2.11± 0.01	100	265	289
Jacobs	1.93 ± 0.02	100	1.83 ± 0.00	92	266	289
Marila	2.10 ± 0.1	99	2.11 ± 0.02	95	268	289

All results were evaluated using Origin software 8.5.1.

Table 18. Caffeine content (mean ± S.D.+ in % w/w; n = 3) in Vietnamese samples and in coffee brewing at 100 °C and for 3 minutes

Samples	Extraction using $CHCl_3$		1st derivative measurement			
	Content (%)	Transfer (%)	Content (%)	Transfer (%)	Minima (nm)	Maxima (nm)
Dak Tin	0.31 ± 0.00	64	0.57 ± 0.00	76	265	284
Di Linh	0.57 ± 0.00	70	1.05 ± 0.04	73	264	285
Nam Nguyen	1.03 ± 0.02	77	1.40 ± 0.01	85	264	287
Origin	1.45 ± 0.01	90	1.80 ± 0.00	86	263	284
Vinacafe	2.10 ± 0.06	100	2.10 ± 0.01	98	265	289

All results were evaluated using Origin software 8.5.1.

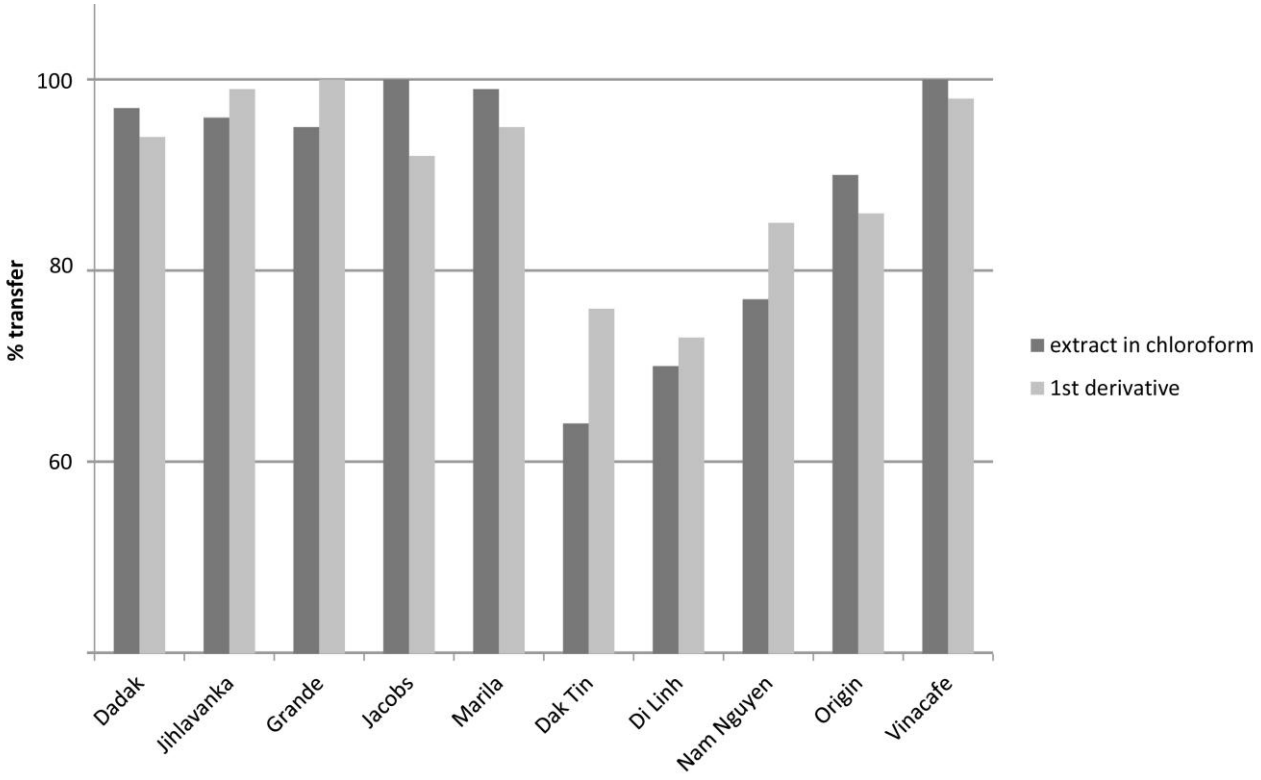

Figure 35. Transfer efficiency to coffee brews at 100 °C and 3 min brewing; measured by the two methods

Table 12, 13 & 14 show the caffeine contents quantified by UV/VIS spectrophotometry by standard addition method. At a glance, it can be observed that caffeine content was dependent on temperature of water used to brew, brewing lengths (see Table 14 & 13) and independent on volume of water (see Table 12). Brewing temperature exerts a predominant effect on yield variability [118]. Contents of caffeine in coffees brewed by 100 °C are significantly higher than those in coffee brewed by 80 °C, in 3 min. Contents of caffeine in coffees brewed in 5 min are significantly higher than those in coffee brewed in 1 min, at 90 °C. The highest amount of caffeine in samples analyzed was found in Vinacafe (54.3 ± 0.47 µg/mL) at 90 °C with 5 min of brewing length, while the lowest was found in Dak Tin (19.4 ± 0.23 µg/mL) at 90 °C with 1 min of brewing length. Among ground roasted coffee samples, Vinacafe was the finest and Dak tin was the coarsest. The values generally agree well with previous published article, Leonard [9] stated that the longer brew time implies longer contact time between water and coffee grounds

leading to more complete caffeine extraction and caffeine content also depended on the extent of grinding. The contents of caffeine in all the coffee brands were found to be more than those in the documented range, an average caffeine concentration of 60 to 85 mg per cup of instant and roasted and ground coffees, respectively [3]. This was due to using addition standard method, which cannot efficiently eliminate the matrix effect of UV absorbing substances.

The results in Table 15 & 16 showed that the caffeine contents (2.9 ± 0.05-15 ± 0.07%) in all coffee samples obtained by conventional UV/VIS spectrophotometry were found to be out of the documented range. The contents of caffeine determined by $CHCl_3$ extraction and by derivative spectrophotometry were found to be within the documented range (0.48 ± 0.01- 2.12 ± 0.06%) in all the coffee samples. There were no significant differences between the results measured by the first order derivative UV/VIS spectrophotometry and by $CHCl_3$ extraction in all the Czech samples, except of the Nescafe instant sample (3.47 ± 0.08 and 4.45 ± 0.02%, respectively) (see Table 15), while there were significant differences in all the Vietnamese samples, except of the Vinacafe sample (2.15 ± 0.08 and 2.1 ± 0.02%, respectively) (see Table 16). This may be explained by a more significant interference of matrices in the Vietnamese samples compared to the Czech ones. The caffeine contents of the Czech coffees were higher than those of the Vietnamese coffees in all samples using the methods. The particle size of ground roasted coffee powders are probably major causes of the difference in caffeine contents between Czech and Vietnamese coffee brands. Previous studies have also shown that caffeine content in coffee was influenced by grinding techniques [9].

Table 17 & 18 showed the contents and transfer efficiency of caffeine in coffee samples brewing at 100 °C and for 3 minutes determined by extracting in $CHCl_3$ and 1^{st} derivative measurement. Once, there were no significant differences between the results measured by the first order derivative UV/VIS spectrophotometry and by the $CHCl_3$ extraction UV/VIS spectrophotometry in all the Czech samples. However, there were significant differences in all the Vietnamese samples (1.2 – 1.8 times), except of the Vinacafe sample (2.10 ± 0.06

and 2.10 ± 0.01%, respectively). The caffeine extractability at 100 °C and 3 min of brewing (commonly used time for brewing coffee) almost reached 100% in Czech coffees, while it was less than 90% in Vietnamese ones (Figure 35). The results showed that the caffeine contents of Grande (100% Arabica) and Marila (100% Robusta) were 1.98 ± 0.01 and 2.10 ± 0.1%, respectively. However, they did not agree with the previous results published, 1.1-1.3% for Arabica and 2.4-2.5% for Robusta [39]. This may be due to deception in blending commercial ground roasted coffee.

V. THE CONTRIBUTION OF THE THESIS TO SCIENCE AND PRACTICE

UV/VIS spectrophotometry is a fast and simple method, available in most of laboratory. To make the result more reliable, the experiments were repeated at least three times and average values were taken.

This study can contribute to a better knowledge of the levels of caffeine in commercial coffee to know the quality of the commercial coffee and also estimate how many cups of coffee we should drink every day. Based on this study, healthy adults can drink 2-3 cups in case of Vietnamese coffee and 1-2 cups in case of Czech coffee per day. This is unlikely to be of any health concern caused by caffeine.

VI. CONCLUSIONS AND RECOMMANDATION

Caffeine content in coffee cannot be determined directly using UV/VIS spectrophotometry due to the matrix effect of UV absorbing substances. This study is mainly concerned with development and validation of UV/VIS spectrophotometry for the determination of caffeine in commercial ground roasted coffee.

This PhD study focuses on determination of caffeine content of ground roasted coffee in the market. Further work could determine caffeine content of samples known species and geographical origin.

Caffeine, trigonelline and chlorogenic acids are important compounds that mainly contribute to the flavor, bitterness and bioactivity of coffee beverage, further work has be done to develop and validate UV/VIS spectrophotometry to determine these components.

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