Determination of Caffeine, the Active Ingredient in Different Coffee Drinks and its Characterization by FTIR/ATR and TGA/DTA

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Abstract— **In this article a special attention has been given to the use of FTIR/ATR, a unique accessoryof Fourier Transform Infra-red Spectroscopy. This technique uses attenuatedtotal reflectance accessory(ATR) as a sampling system for determinationand quantification ofcaffeine contentin coffeesamples at the wavenumber(cm^{-1})of the pure caffeine spectrum selected between (1600-1700 cm^{-1}) where any possible traces ofchloroform does not show any absorption[1]. Attenuated Total Reflectance accessory represented a simple technique that requires a very little samplepreparation [2]. In order to reach a meaningful approach of sample characterization, Thermal Gravimetric Analysis (TGA) hasbeen included in the research to identify the thermal properties of coffee samples selected for the study[3].The targeted coffee samples represented the coffee samples consumed,particularly: Arabian coffee blend home-made, roasted coffee blend home-made,instant coffee bought from the market and pure caffeine analytical reagent bought from SigmaChemicals.**

Index Terms— **FTIR/ATR,TGA/DTA, Arabian Coffee blend, roasted coffee blend, Instant Coffee,caffeine.**

I. INTRODUCTION

Caffeine or1,3,7-trimethylxanthineexists as white powdered alkaloid with an intenselybitter taste. Caffeine acts as stimulant for the central nervous system(CNS)having an instantaneous effect on restoring arousness in both humans and experimental animals. Caffeine formula is $C_6H_{10}N_4O_2$. Molecular weight 194.19 g/mol. Melting point 237^oC. Boiling point 178^oC(sublimation temperature).Density 1.05g/cm^3.pKa 10.4 at 40^oC. Caffeine, a well-known name, with some possible impacton human health, for that reason, a lot of interest has been directed towards more investigation on it as other components a represent in it which have controversial health effects [4-9].Caffeine is a methylamine whose Primary biological effect is the antagonism of adenosine receptor [4].The instant physiological consequences of caffeine intake include, increased blood pressure, increased serum fatty acids, increase dplasmacatecholamine levels, increase durinationandincreasedgastric acid secretion. Caffeine has pharmacological uses as a cardiac and respiratory stimulantanddiuretic in small doses which is present in an averagecup of coffee(100-200mg).

High concentration of caffeine canlead to addiction, ulcer, breast cancer, coronary diseases, myocardialinfarction [4-5] and death if the lethal dose is reached around10 to 15 g. However moderate use of coffee has littleeffects if it is not usedin association with smokingasit is a habit that happens so often[4,5,6,7,8].The article shades lighton the samples usedin the research. The coffee samples wereselected to represent the mostly used qualities, namely :1-Arabian coffee blend which consistsofgreen coffee partially roasted to a yellow color then powdered with little cardamom,a spicemostly used in Arabian peninsulaas coffee additive.Alsopeople in Arabian societieseat dates while drinking coffee.Cardamom spiceisknown for treatingdigestive problemsincluding heart-burn, intestinal spasm, intestinal spasm, intestinalgas, constipation,liver,gallbladder complaints,commoncold, loss of appetite.Some people use this spice as astimulant for urinary problems (ref- WebMD)2/Roasted coffee blend homepreparedby roasting coffee beans completely to a black product then powdered with dry ginger. Ginger is known to have curative benefits. Some of the curatives have been discovered by researchersin the treatments of cancer to migraines.A study was conducted at the university of Michigan Comprehensive Cancer Center found that ginger induces cell death in ovarian cancer cellsto which it was applied. Cinnamon is some time sadded with powderedgingeror cloves to rousted coffee asspices mixture additive. Cinnamon is known to haveananti-ulcercurative effectonulcers possibly caused by the acidityoforganicacids present in coffee. Cloves oil, an activeingredient of cloves is known totreat many health disordersincluding head-ache, indigestion, cough, blood impurities. Cloves is mostlyusedin dental care and asan antiseptic, antiviral cure.The spices additives to roasted coffeeand Arabian coffee have been used formany centuries in Arabian, African and Asiancountries[9]3/ Instant coffee of commercial origin has been bought from the market for comparison with the other coffee samples with no details on the preparation recipe.4/ Pure Caffeine has been included as a reference sample.

II. EXPERIMENTAL

A. Apparatus

FTIR Nicolet 6700 equipped with Attenuated Total Reflectance accessory designed for use with a diamond crystal (200-30000 cm^{-1}) to measure the reflectance of solid and liquid samples. The spectra can be transformed by recording the absorbance as a function of wavelength. The concentrations can be calculated from absorbance measurements using a calibration curve. The interferogram is equipped with KBr beam splitter and DTGS/KBr detector.

Conditions of measurements: wavenumbers 400-4000 cm^{-1}, number of scans 32,resolution 4 cm^{-1}.

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III. METHODS

A. *Preparation of samples*

Solid samples

Arabian coffee blend:partially rousted beans was blended with Cardamom spicein the ratio of (100:1) and stored at room temperature for analysis.Roasted coffee blend:Completely roasted coffee beans was blended with Ginger and Cloves spicesin the same ratio and storedfor analysis. Instant coffee:roasted coffeeof commercial origin.Pure caffeine used as a standard sample.Aqueous extracts of thecoffee samples wereprepared by boiling (1g) of each in about 70mlof distilled water for ten minutes,cooled,completed to(100ml) in volumetric flasks and filtered through Whatman filter paper No. 1.The extracts represented almost the concentrationof coffee consumed in a 100 ml cup of coffee. Caffeine content of each was measured byFTIR/ATR.

B. *Standard Caffeine*

(1g) Caffeine in 100 ml Chloroform.

Procedures

Preparation of standard caffeine

Standard solutions of caffeine containing 0, 2,4,6,8 and 10 mg in chloroform were pipette separately in 100 mg dry KBr. The standard samples were mixed, crushed and dried under N_2gasto removetraces chloroform.

Preparation of coffee samples:

Solid coffee samples finely powdered were used directly for analysis.

FTIR/ATR data collection for the standard samples andcoffee samples under investigationwere collected after collecting the backgroundspectrum. Solid samples of standard and coffeewere layered on the crystal.Each interferogram of caffeine films was rationed against the background interferogram and the result was collected in absorbance.

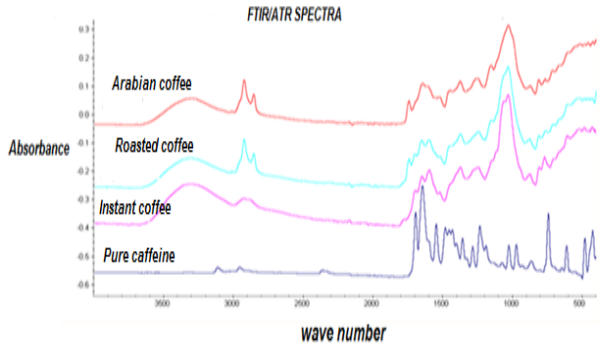
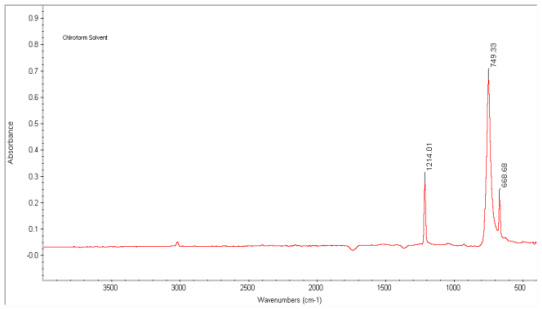


Fig 1a. FTIR/ATR spectra of solid samples ofpure caffeineand coffee samples:from top to bottom, Arabian coffeeblend, roastedcoffee blend,Instant coffee and caffeine standard respectively.

Fig 1 b- below is chloroform spectrum, shows no interferingabsorptionbetween 1600-1700 cm^{-1}.



Figures2a,2b,2c,2d below show the spectra of coffee samples and caffeine with theirrespective wavenumbers.

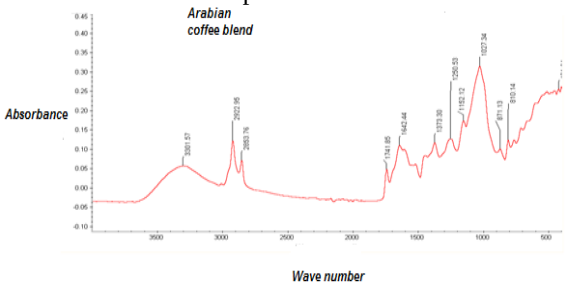
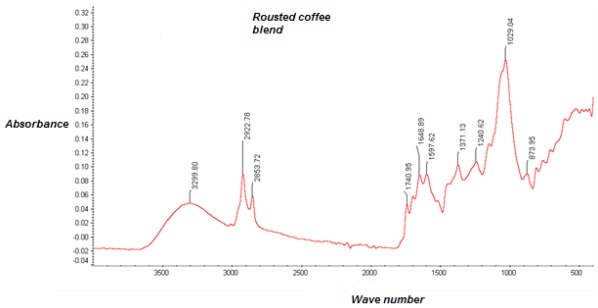


Figure (2a)



Figures (2b)

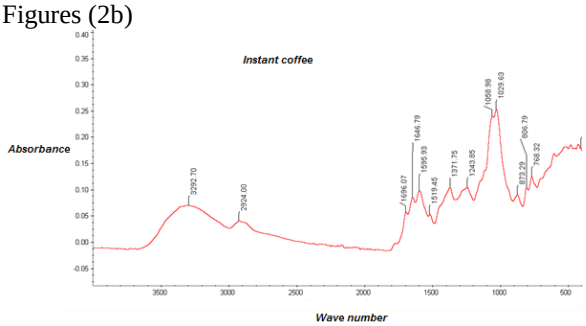


Figure (2c)

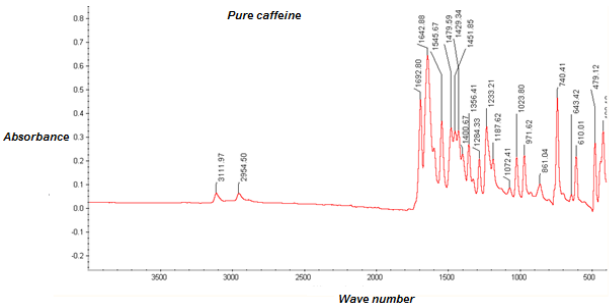
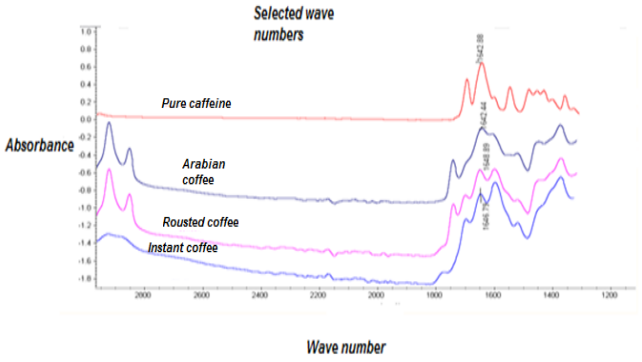


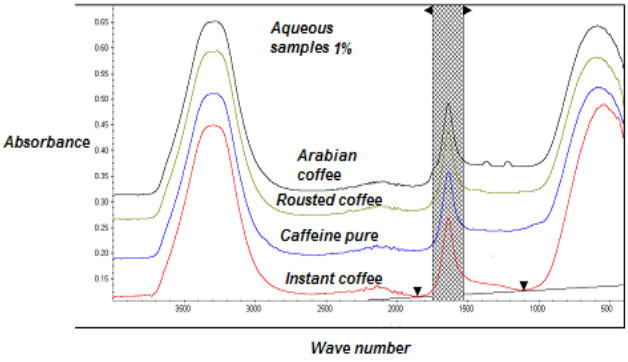
Figure (2d)

The spectra below showthe wavenumber selected from pure caffeineas a standardwavenumber for quantification of caffeine content in coffee samples. The slight shift from thewavenumber 1642.44 $cm^{-1}$of caffeine wasdue tothepreparation process of coffee exacts, e.g.roasting

procedure and temperature. Arabian coffee wavenumber 1642.44 cm^{-1}.Roasted coffee 1648.69 cm^{-1}.Instant coffee1646.79 cm^{-1}.



Aqueous samples (1%) in distilled waterfor coffee samplesand caffeine were introducedin the text to represent approximately the coffee concentration consumed in(100ml) extract.The overlay of spectra below, figure 3,shows 1% aqueous extracts of Arabian coffee, roasted coffee,pure caffeineand instant coffee.



The measured pH of extracts were: 4.5, 5.0, 5.0 and 5.1 for Arabian coffee, roastedcoffee, caffeine and instant coffee respectively.

TGA experiments were run on coffee samples under investigation and pure caffeine to study the thermal properties of each ,using Mettler Toledo TGA-DSC 1.Weights of crushed, dry samples under 10mgwere weighed in 70 ml alumina crucibles and placed on the precisionbalance. The samples were heated in aconstant heating rate mode from ambientto 700°Cat 10°C/min. The acquired thermograms demonstrate the effect of heat flow on samples.

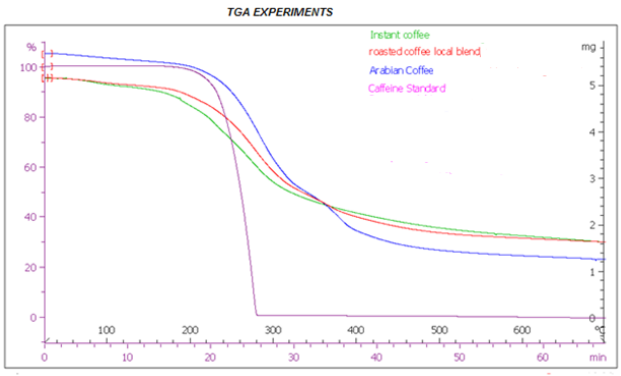


Figure-4- above demonstrates the thermograms of the samples with percentages loss of massescaused by heat flow through coffee samples.

A set of TGA experiments were also performed to show the loss of masses due to volatiles from ambient to200°C at the same rate (10°C/min).

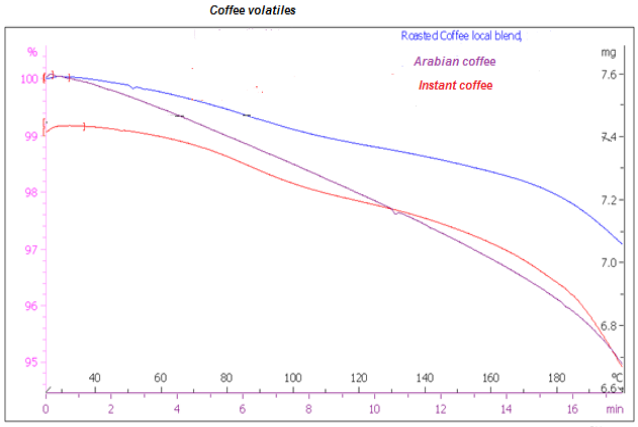


Fig 5 above :shows in the thermograms the percentage loss in mass in each sample after heating from ambient temperature to 200°C.

TGA /DTA

TGA measures changes caused by mass loss. Differential Thermal Analysis (DTA), first derivative of TGAhave been introduced in the text.The material under study and an inert reference are made to undergo identical thermal behavior. Any temperature difference between sample and reference is plotted against temperature. DTA phenomena causing changes in heat/temperature. Physical adsorption (exothermic), desorption (endothermic), vaporization (endothermic, sublimation) [10] Combining the two techniques (TGA/DTA)gives a comprehensive behavior of coffeesamples and caffeine and characterize thermal stability and sample purity [11]. The thermograms in figures 6, a, b, c, d. show a combination of the two techniques.

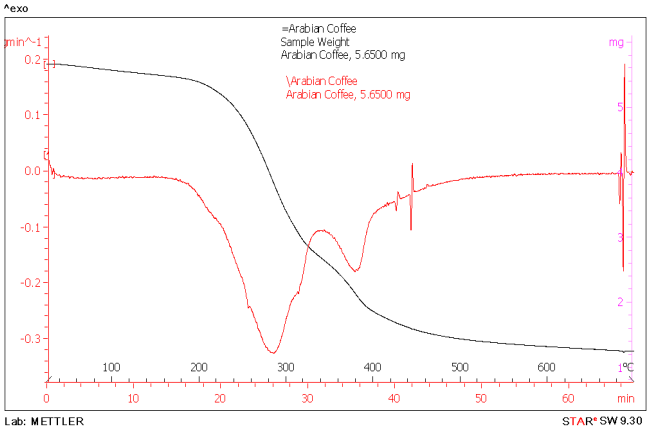


Figure (6 a) showsTGA/DTA thermogram of Arabian coffee.

Sample	Absorbance	Caffeine%
Arabian coffee	0.26	5.050
Roasted coffee	0.38	7.053
Instant coffee	0.44	8.636

Figure 6 b, showsTGA/DTA thermogram of instant coffee.

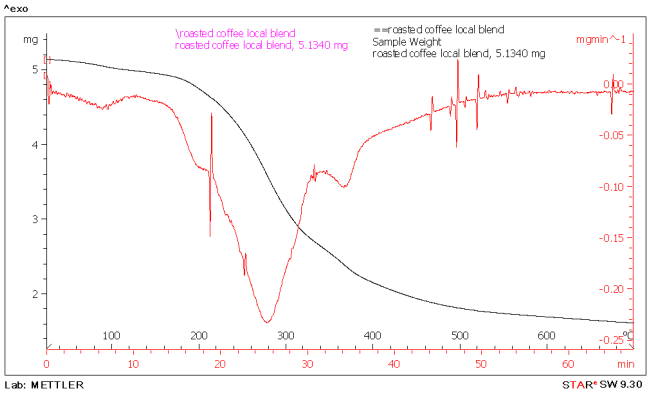


Fig 6c shows TGA/DTAthermogramof roasted coffee.

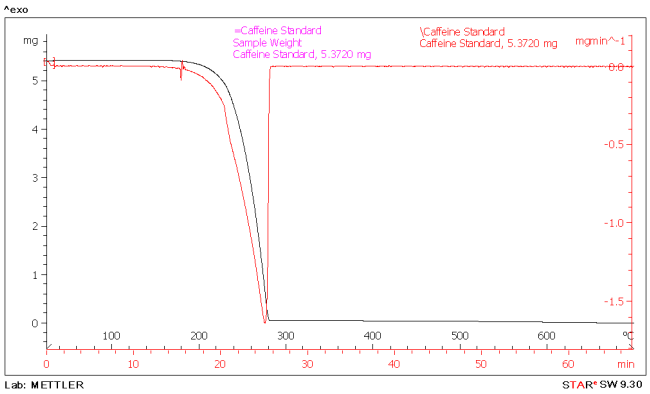


Figure.6d showsTGA/DTA thermogramof pure caffeine.

Results and discussion

A standard curve of absorbance vs % caffeine in KBr is shown in Fig.7.Table 1 shows % caffeine in dry coffee samples.

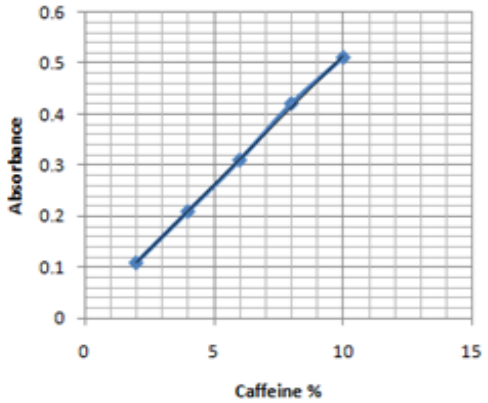


Figure (7)

A standard curve ofaqueous caffeine was prepared by diluting 1%caffeine a hundred timesin distilled water and extracting the aqueous caffeine contents of20,40,60,80,100µlin 0.5ml chloroform then diluting the extracts in 100mg KBr, grinding the mixture andevaporating chloroformbefore taking the FTIR/ATR spectra.A standard curve of absorbance vs ppmcaffeine wasdrawn for calculating the caffeine contents in aqueous coffee samples. Volumes of 100ulfrom aqueouscoffee samples were each extracted in 0.5ml chloroform, then diluted in 100mg KBr for FTIR/ATR analysis. The standard concentrations of caffeine were 20,40,60,80 and 100ppm respectively.The concentrations of caffeinein coffee samples in ppm was obtained by multiplying theresultsfrom the curve by a factor of ten.

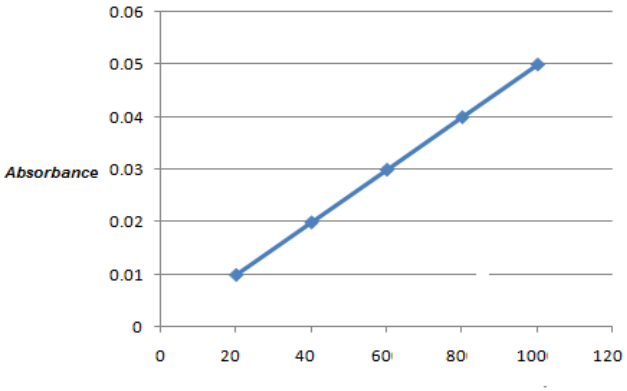


Figure (8)

Figure 8 shows the absorbance of standard aqueous caffeine concentrations in ppm.

Table 2 shows the concentrations in ppm of 1% aqueous coffee samples analyzed.

Coffee sample	Absorbance	Concentration of caffeine in ppm
Arabian coffee	0.016	32.3
Roasted coffee	0.022	45.2
Instant coffee	o.340	68.3

The quantification of coffee samples analyzed indiated that the average mass of caffeinecontent consumed in a 100ml coffee cup, was around32.3,45.2 and 68.3 milligramsforArabian coffee,roasted coffee and instant coffee respectively.

Table 3 shows loss in mass of coffee samples because of heat flow in TGA thermograms.

Coffee sample	% loss in mass at200°C	%loss in mass at 700°C
Instant coffee	8.63	68.62
Roasted coffee	6.17	68.70
Arabian coffee	5.05	78.09

The rate in mass loss at 200 ℃ is mostly pronouncedandclosely relatedto caffeine because of sublimation.The mass loss at 200℃ was high inInstant coffee and low in Arabian coffee because of roasting temperature and time.In Arabian coffee moderate roasting temperature reservedcaffeinein the beans and permittedslow release of it to the surrounding compared withother samples.Mass loss at 700 ° C gave the highest percentage to Arabian coffee.TheDTA studyof coffee samples illustrated on the themograms indicatedthat thecaffeine content of Arabian coffee sample(Fig.6 a), gave a clear themal groupthatagreed withthat of standard caffeine.The other thermal group that followed wasrelated to spice additives.The same result was shown in roasted coffeeThe result of Instant coffee DTA illustrated the presence of a distorted caffeine peak withminor peaks in the surrounding which belongedto impurities.

IV. CONCLUSION

Fourier transform infrared spectroscopy has proved to be a good techniquefor quantifying a wide range of materials especially phamaceutial productswhen it is used in combination with Attenuated Total Reflectance Accssory.TGA/DTA a combined technique in thermal analysis measures the physicalchangesand chemical changes that occur in characterizing the samples to showthermalstability and material purity. The purity is compared with pure references.The aim of the research has been focused on coffee samples containing spices toknow their effect on caffeine.The results showed the absence of any chemical effect on the caffeine content of coffee samples as exihibited in the FTIR/ATR spectra.The purity of caffeine in Arabian coffee and roasted was maintained in thethermogram whereas the Instant coffee showed signs of impuity in the therogram.Finally, the presence of spices in Arabian coffee and roasted home made coffeeoffers curative benefits and preventive medication as presented in the introductionwhich is good for the health of coffee consumers.

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