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EFFECT OF THE DRIED LIMES (*CITRUS AURANTIFOLIA*) ON THE CAFFEINE KINETIC EXTRACTION IN BLACK TEA LEAVES (KINETIC AND THERMODYNAMIC STUDY)

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ABSTRACT. The kinetic and thermodynamic parameters were studied for the extraction of caffeine in black tea leaves and tea leaves containing antioxidant dried limes (*Citrus aurantifolia*). Caffeine is widely used in human consumption and is present in any liquid suitable for drinking except water. However, the health and environmental demerits make it significant to reduce caffeine to much less concentration or decaffeination. Therefore, the effect of the dried antioxidant *Citrus aurantifolia* on caffeine concentration followed by pseudo-first-order reaction, kinetic parameters (pseudo-first-order rate constant (k), equilibrium absorbance (A_∞)), and thermodynamic activation parameters, the activation energy (E_a), change in enthalpy of activation ($\Delta H^\#$), the change in entropy of activation ($\Delta S^\#$) and change in activation Gibbs free energy ($\Delta G^\#$) was calculated at four different temperatures 30, 60, 80, and 100 °C. Using the spectroscopic method the activation energy of the caffeine concentration in free black tea leaves solution (E_a = 13.32 kJmol^{-1}) was higher than the black tea leaves mixed with lime solution (E_a = 9.89 kJmol^{-1}), the higher collision of molecules A-factor (1.96 min^{-1}), and large correlation factor (R^2 = 0.95). The thermodynamic activation parameters $\Delta H^\#$, $\Delta S^\#$, and $\Delta G^\#$ demonstrate less heat absorption, irreversible, and more spontaneous interaction of lime with caffeine.

KEY WORDS: Caffeine, Tea leaves, *Citrus aurantifolia*, Thermodynamic parameters, Kinetic

INTRODUCTION

Tea is an ancient drink; it has been consumed as a beverage for almost 2,000 years ago starting in China. It is the most widely consumed beverage after water. Tea is a product of the *Camellia Sinensis* plant and it is usually divided into three main groups: green, oolong, and black. The leaves of all three are rolled, dried, and packaged. Although traditionally served hot, the western world has increasingly consumed iced tea as a popular beverage [1].

Tea contains many chemical compounds and ingredients such as polyphenol, fluoride, and caffeine, several of which are believed to affect the human body and health. The most essential ingredients in tea are caffeine, which belongs to a family of naturally occurring components known as xanthine. The xanthines which come from plants are possibly the oldest known stimulants. The most potent xanthine is 1,3,7-trimethylxanthine, also known as caffeine, its ability to increase alertness, put off sleep, and help to improve attention in the study done by Bolton and Null and Jacobson and Kulling [2, 3].

Decaffeination of tea is an essential industrial process because many consumers prefer to avoid caffeine wholly or partially due to its stimulant effects and others, still health concerns. However, they were noted that decaffeinated tea is not caffeine-free. In addition, caffeine has a slightly bitter flavor. The decaffeination process uses the solvent, which extracts caffeine from tea. The most available solvents used to extract caffeine, such as benzene, chloroform, dichloromethane, ethyl acetate, etc. Dichloromethane is often used in place of chloroform and also used to deactivate a high proportion of conventional teas. It is also a relatively non-toxic solvent [4, 5].

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One of the most essential fruits crop worldwide is *Citrus aurantifolia*. Much of the orange (peel and pulp) is dried and exported around the world. It is easier to haul and manage and can be stored year-round. It has a higher nutritive value than fresh pulp [6, 7]. The drying process is usually done at the fruit processing site to protect it from transportation costs [8]. Dried pulp is often pelleted, which nearly doubles its bulk density (up to 300 kg/m^3), improves handling efficiency, storage bins, and self-feeders decrease binding and reduce dustiness [6]. It is hygroscopic and needs to be dry when stored and kept dry during storage [9].

Dried limes (also known as black limes or limu-omani) usually added an aromatic, sour-citrus flavor to Middle Eastern and Persian dishes [10]. Whole limes are boiled in a salt brine before being dried in the sun, creating a musky, grinding them in coffee and tea leaves. It also contains many vitamins and minerals, assists the body's growth, and boosts your immune system, acts as an antioxidant to protect the body from diseases and illnesses [11]. Caffeine is removed from teas with a special ultrasound method with high-frequency, and liquid-liquid extraction and quantitated by gas chromatography with nitrogen-phosphorus detection [12, 13].

This study aimed to show the effect of temperature and time on the extraction of caffeine in black tea leaves and black tea leaves containing a powder of dry *Citrus aurantifolia*.

EXPERIMENTAL

Sample preparation

A mixture of three commercial black tea samples (Cihan tea, Ahmed tea and Alghazaleen tea, all from Sri-Lanka) were obtained from supermarkets in Erbil-Kurdistan-Iraq.

Fresh oranges (*Citrus sinensis* var. Valencia) are obtained from a local market and washed with deionized water. Hesperidin and naringin are given the bitter taste of orange peel. These flavonoids are water-soluble.

For the debittering of the solution, sodium bicarbonate was used by preparing of a solution 2 g $NaHCO_3$ in a 100 g solution of lime boiled at 95 ºC for 20 min. The samples are cooled, then left to dry in the desert sun until all of the juice has dehydrated and the lime powder was obtained it was dark brown in color [14].

Equipment

UV-visible spectrometer (Spectroscan 80D instrument spectrophotometer with serial no.: 18-1884-01-0113) and UV-spectroscan software wavelength range 190 nm to 1100 nm for measuring spectra of caffeine were used. Filter halther disposable filter holder 0.2 µm (Schleicher and Schuell) and dichloromethane (assay: 99.0% GCC Analyst, UK) were used.

Caffeine kinetic extraction

A set of tea samples at 30, 60, 80, and 100 °C were dissolved in hot water and stirred for 30 min then 20 mL of dichloromethane was added to the tea solution stirred for 10 min. The solubility of caffeine in dichloromethane is 140 mg/mL, the two phases were separated by the separated funnel. The absorbance (A) of the dichloromethane solution which contains most of the caffeine was measured in four extraction samples, at temperatures 30, 60, 80, and 100 °C. Then, directly the UV spectra were taken. The total caffeine intake in tea samples was found by the spectroscopy from combinations of the four extractions. The caffeine content of the 5th extract was determined to be insignificant.

While for the tea leaves with dried lime, a set of the mixture of 25 mg of tea leaves and 12.5 mg of dried limes were dissolved in hot water for different times ranging between (3-120) min and stirred for 30 min, and the procedures were repeated for the four temperatures (30, 60, 80 and 100 °C).

Data analysis

The kinetic extraction rate constant (k) and equilibrium absorbance (A_∞) were calculated using linear regression in Microsoft Excel according to the first-order Equation (2) [15].

$$A_t = A_\infty(1 - \exp(-kt)) \quad (1)$$

$$\ln(A_\infty - A_t) - lnA_\infty = -kt \quad (2)$$

where (A_∞-A_t): is the absorbance of caffeine extracted at any time, k value at each temperature was from the slope of the linear plots of ln(A_∞-A_t) against time (t).
Arrhenius equation,

$$Lnk = LnA - E_a/RT \quad (3)$$

where E_a is activation energy $J.mole^{-1}$, R is gas constant =8.314 $J.mole^{-1}.K^{-1}$, T is the temperature, and (A) is a pre-exponential factor (Arrhenius factor) (has the unit of k) and k: is the rate constant (min^{-1})

The thermodynamic data change in enthalpy of activation ($\Delta H^{\#}$), the change in entropy of activation ($\Delta S^{\#}$), and the change in activation Gibbs free energy ($\Delta G^{\#}$) can be obtained using Equations (4-7) [16]:

$$\Delta H^{\#} = E_a - RT \quad (4)$$

$$A = (ek_bT/h)e^{\Delta S^{\#}/R} \quad (5)$$

$$\Delta S^{\#} = R(LnA - Ln\left(\frac{ek_bT}{h}\right)) \quad (6)$$

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \quad (7)$$

where k_b is the Boltzmann constant = $1.3806*10^{-23}$ J K^{-1} and h is the Plank constant = $6.626*10^{-34}$ Js.

RESULTS AND DISCUSSION

Determination of caffeine in black tea leaves

Many analytical methods have been used for the extraction of caffeine including HPLC, GC, and spectrophotometry. Spectrophotometry is a fast and simple method where pure caffeine was first dissolved in water and then extracted using dichloromethane as a solvent because UV-Visible spectrometer cannot be used directly to determine caffeine content in black tea leaves due to the matrix effect of UV absorbing substances and caffeine spectra interface with other compounds in black tea leaves [17].

The wavelength of caffeine is between 244 nm to 300 nm as published by Tadelech and Gholap [18]. Figure 1 shows the caffeine's spectrum absorbs at λ_{max} = 275 nm and uses dichloromethane as a blank.

The UV-Visible spectrum of the extracted caffeine at different temperatures (30, 60, 80, 100 °C) was determined using dichloromethane as a blank (Figure 2 a,b), and the fifth extraction, the content of caffeine was found negligible. These results agree with the result obtained by Tadelech and Gholap [17]. However, this investigation shows a higher caffeine concentration extracted during the first extraction as the absorbance for black tea leaves and black tea leaves containing lime are equal to 1.716 and 1.185, respectively.

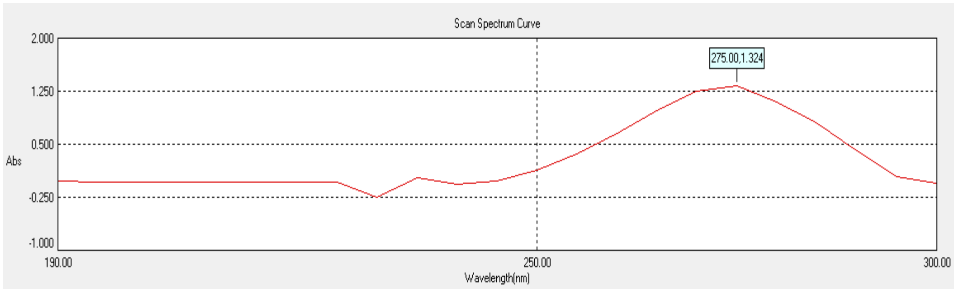
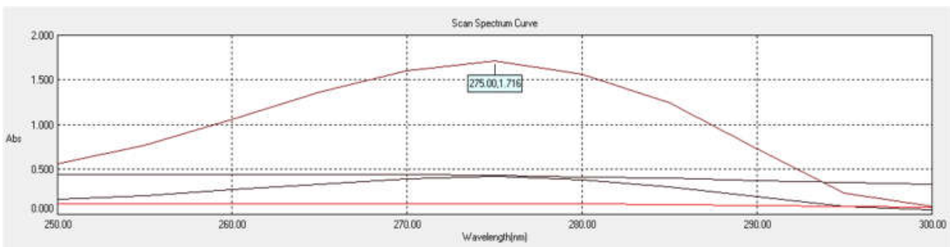
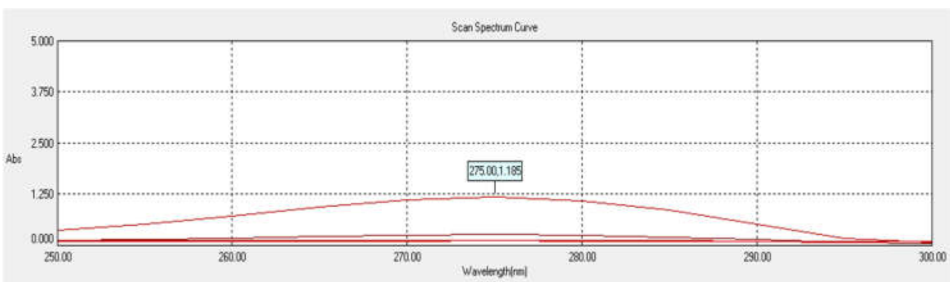


Figure 1. UV-Vis spectrum for caffeine using dichloromethane as a blank.



(a)



(b)

Figure 2. The overlapped spectra of caffeine in (a) black tea leaves and (b) black tea leaves containing lime for different rounds of extraction.

From Figures 2(a) and (b), the difference in absorbance of the first extraction round for caffeine, in tea leaves = 1.716 and in black tea leaves containing lime = 1.185 indicating that some caffeine can be interacted with the organic structure of the lime, leading to decreasing concentration of caffeine extraction.

Extraction kinetics

The variation of caffeine absorbance with time (min) found that the extraction of the caffeine from both black tea leaves and black tea leaves mixed with lime followed pseudo-first-order kinetics. Figure 3 shows the absorbance changes and explains very clearly how absorbance changes over time with temperatures (30, 60, 80, 100 °C), this means more amounts of caffeine in black tea leaves and black tea leaves with lime were extracted with time as the temperature increase.

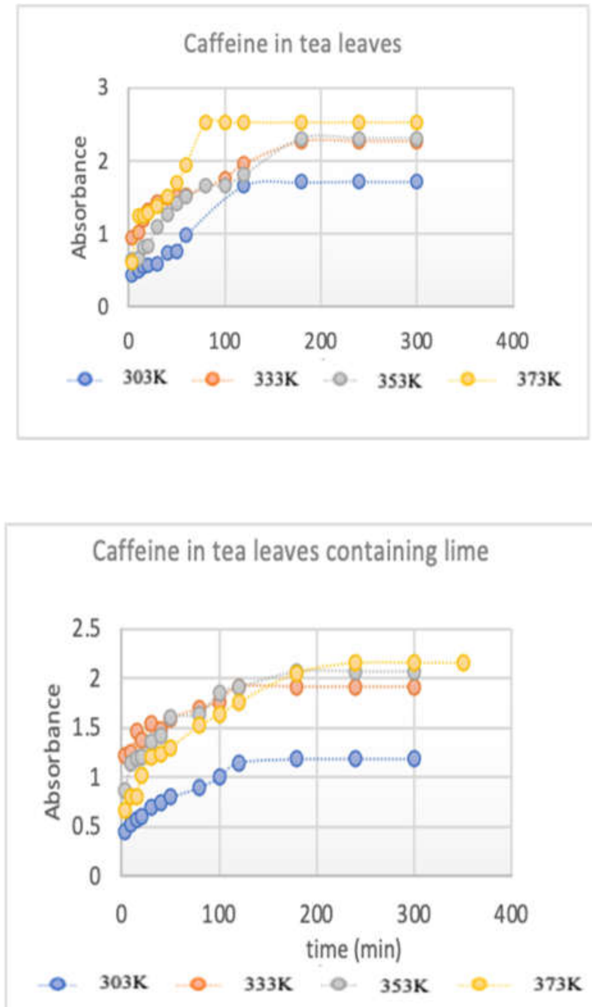
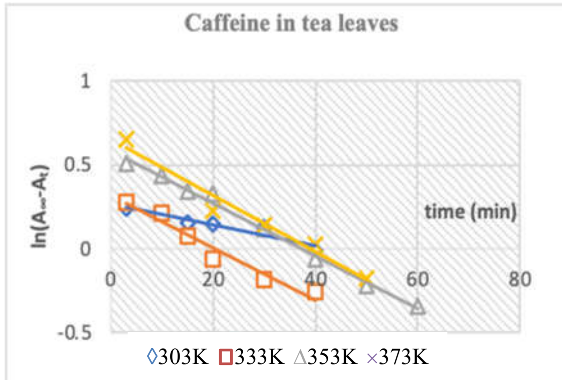


Figure 3. Kinetic extraction of the caffeine in black tea leaves and black tea leaves containing lime at four different temperatures.

In Figure 4, the assumption of pseudo-first-order reaction was fully proved and was always linear; typical plots show an excellent fit to Equation (2). Moreover, in all cases, the correlation coefficient (R^2) values are close to unity, showing good data fit the first-order equation.



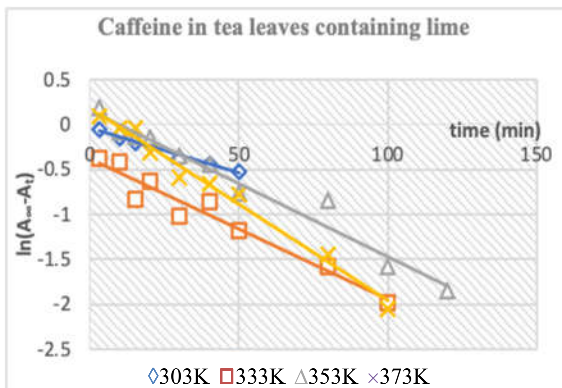


Figure 4. First-order plot for the caffeine in black tea leaves and black tea leaves containing lime at four different temperatures.

Table 1. Pseudo first-order rate constant (k) and equilibrium absorbance (A_∞) of caffeine in tea leaves and tea leaves containing lime at four different temperatures (303, 333, 353, and 373 ºC).

Temperature (K)	Caffeine in tea leaves		Caffeine in tea leaves Containing lime	
	k (min^{-1})	A_∞	k (min^{-1})	A_∞
303	0.0062	1.716	0.0098	1.185
333	0.0155	2.263	0.0156	1.913
353	0.0157	2.308	0.0162	2.060
373	0.0167	2.525	0.0214	2.154

Table 1 contains the kinetic parameters of the caffeine extraction in both black tea leaves and black tea leaves containing lime at four different temperatures, the pseudo-first-order rate constant (rate of extraction) (k) of caffeine in black tea leaves increased from 0.0062 to 0.0167 min^{-1} with increased temperature from 303 to 373 K, but for caffeine in black tea leaves containing lime has a higher rate of extraction from 0.0098 to 0.0214 min^{-1} and the equilibrium absorbance (A_∞) of the caffeine in black tea leaves increased from 1.716 to 2.525 with increased temperature, but have lower A_∞ from 1.185 to 2.154 for black tea leaves containing lime, which means low caffeine concentration obtained as compared with caffeine concentration that extracted in black tea leaves without lime indicating that some caffeine will interact with the organic structure of the lime.

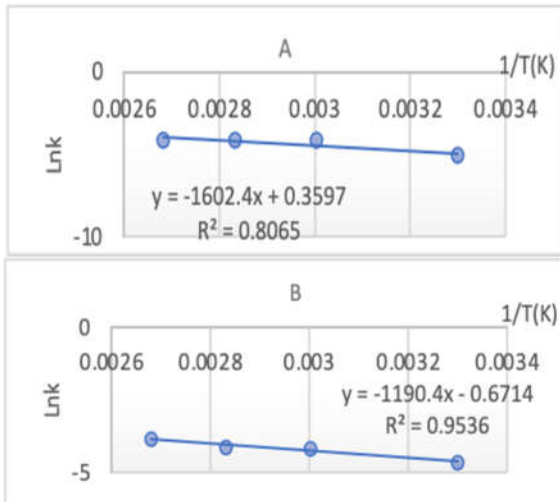


Figure 5. Arrhenius plot for caffeine concentration in (A) black tea leaves and (B) black tea leaves with dried lime powder.

Table 2. Arrhenius and thermodynamic activation parameters for caffeine concentration in black tea leaves and black tea leaves with lime powder.

Caffeine	Temp. (K)	k (min^{-1})	E_a ($kJ.mol^{-1}$)	R^2	A-factor (min^{-1})	Enthalpy change $\Delta H^\#$ ($kJ.K^{-1}$ mol^{-1})	Entropy change $\Delta S^\#$ ($kJ.K^{-1}$ mol^{-1})	Gibs free energy $\Delta G^\#$ ($kJ.mol^{-1}$)
Black tea leaves	303	0.0062	13.32	0.81	1.43	13.07	1.08	-19.30
	333	0.0155				12.82	1.07	-51.57
	353	0.0157				12.66	1.07	-73.01
	373	0.0167				12.49	1.07	-94.40
Black tea leaves with lime	303	0.0098	9.89	0.95	1.96	9.65	1.08	-22.80
	333	0.0156				9.40	1.08	-55.15
	353	0.0162				9.23	1.07	-76.64
	373	0.0214				9.07	1.07	-98.09

The rate constants at four different temperatures were plotted against 1/T and the activation energy (E_a) was calculated from the slope of the Arrhenius plot (as shown in Figure 5) which shows a good straight line with the slope of ($-E_a/R$) as in the equation (3). All the thermodynamic parameters are calculated and displayed in Table 2 obtained enthalpy $\Delta H^\#$ values heat absorbed interaction, and the rate of successful collisions is interpreted by the value of the Arrhenius factor are more with higher activation energy, and more correlation coefficient indicating the significant interaction of caffeine with lime structure. The entropy of activation gives a measure of the inherent probability of the transition state as molecules vary widely in their conformational stability, and Gibbs free energy $\Delta G^\#$ increase with increasing temperature indicates a more spontaneous process. In the literature, Araújo and the coworkers obtained the negative value for $\Delta G^{\#}$ for the soluble solids extraction from green coffee beans at higher temperatures and showed that the process was spontaneous and reasonable so the extraction increased with increasing temperature as $\Delta G^{\#}$ became more negative [19].

CONCLUSION

The result of the kinetic and thermodynamic study of the extraction of caffeine in commercial black tea leaves investigated that as the temperature increase more caffeine will extract and the dried lime will decrease the caffeine concentration and from the values of activation energy (E_a), change in enthalpy of activation ($\Delta H^{\#}$), the change in entropy of activation ($\Delta S^{\#}$) and change in activation Gibbs free energy ($\Delta G^{\#}$) indicating the faster, endothermic, irreversible and spontaneous process. It was suggested to use dried lime as an effective decaffeinated natural compound for whom which preferred to avoid caffeine in black tea.

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