

- - -

2010/08/09

2011/04/11

(60:40)

:

---

# **Determination of Caffeine and Theobromine in Six different Brands of Tea Infused by Different Methods Using HPLC Method**

**S. Almalla; M. Aljoubbeh and K. Hussien**

Department of Chemistry, Faculty of Sciences, Damascus University, Syria.

Received 09/08//2010

Accepted 11/04/2011

## **ABSTRACT**

This paper presents a developed HPLC method for the determination of caffeine and theobromine in different black and green tea infusion samples, using different infusion methods, such as infusion with and without boiling, repeated and continuous infusion. A mixture of double-distilled water and methanol (60:40 v/v), was used as a mobile phase. In this condition, the retention time for theobromine was three minutes and for caffeine five minutes.

**Key word:** Tea, Infusion, Caffeine, Theobromine, HPLC.

**-1**

127

1542

( 5.08 2.54)

.[1]

:[2]

( ) Oolong

( )

.( )

:

( )

[3]

.[4]

[6,5]

:

[13]

[12]

[11-7]

.[14]

Xanthine

.[16,15] (1)

[1, 3, 7- trimethyle- 1H - purine -2, 6 (3H, 7H) - dione]

[3, 7-dihydro- dimethyl- 1H- purine- 2, 6-dione]

[20]

[19-17]

.[22]

[21]

( 400 )

[25,24]

[23]

.[25]

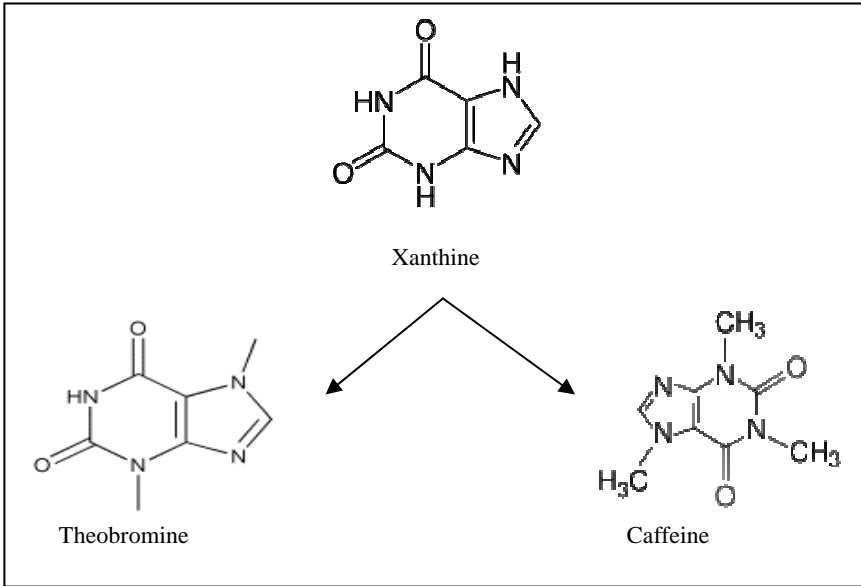
( / 175 )

.[26]

.[27]

/ 1000

[28]



(1)

[29]

		1996	Meyer	
2002	[30]	(10:90)	pH=3.5	
=5.2	)	(75:25)		Franeta
	Brannstrom		[31] (	pH
	(40:60)	(pH=2.4	/ 0.05)	
% 0.1	Srdienovic	2008	[32]	
	[33]	(10:90)		

-2

:

.1

.2

.3

-3

B1,G1,B2,G2,B3,G3) G

B

.(

(40:60)

/ 1

(Kromasil, 25x0.46) C<sub>18</sub>

273

UV

-4

JASCO 980

-

(JASCO, PU-908)

(JASCO, UV-970) UV

.(Fluka)

-

.(Fluka)

-

.(ShamLab)

-

.(G)

(B)

-

-5

:

-A

° 100

50

0.5

:

.i

.(

10

)

.ii

327

100 : **-B**  
 50 0.5 °  
 :

.i  
 .( 5 ) .ii

5 : **-C**  
 50 1

: **-D**

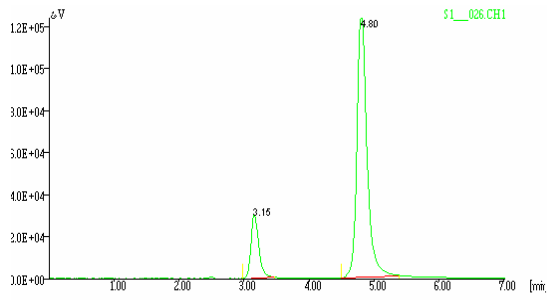
0.5 50 °  
 82 ° 75 °

**-6**

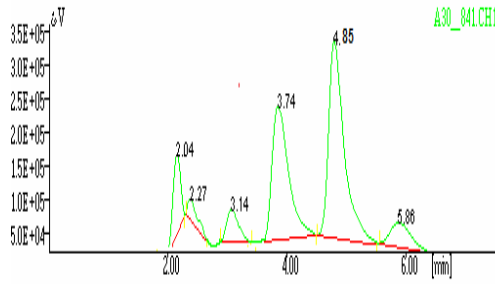
/ (200-10)  
 / (50-1) / 1000  
 . / 100

UV / 1 ) ( 40) ( 60)  
 2002 Brannstrom  
 (Kromasil, 25×0.46) C<sub>18</sub> ( 273)  
 (2)

R<sup>2</sup> .( 0.999) ( 0.996)



A



B

(A)

(2)

(B)

-7

537

.A

:

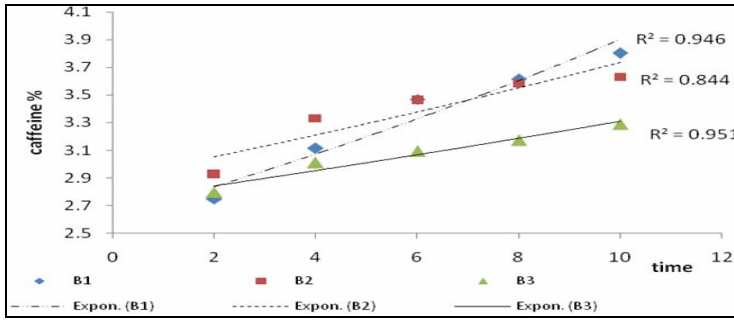
( 50 / 0.5 )

.(3 )

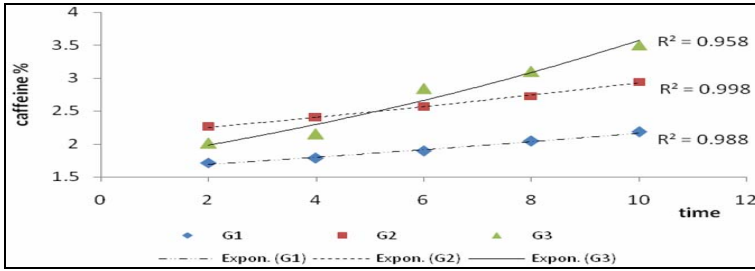
:

4 0.5

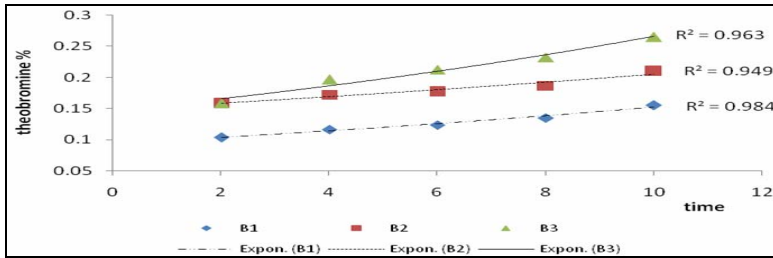
.(2,1 )



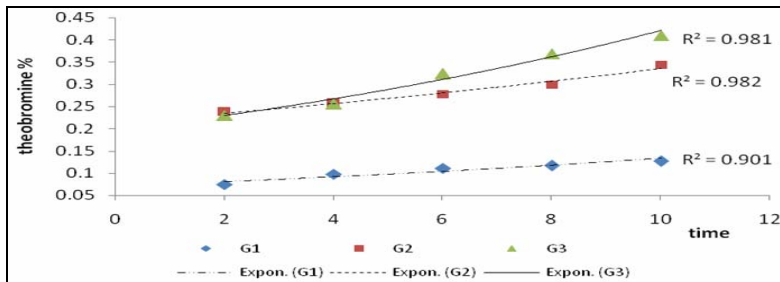
.A



.B



.C



.D

(A,B,C,D) (3)



(ppm)

(1)

()						
4	3	2	1	0.5		
1052.841±36.121	856.985±42.463	551.783±21.437	364.253±17.672	170.717±8.363	Xi+ SD	Tea
3.431	4.955	3.885	4.839	4.899	RSD%	B1
1145.545±53.776	948.197±39.371	634.515±16.258	434.481±19.758	193.986±7.359	Xi+ SD	Tea
4.694	4.152	2.562	4.545	3.793	RSD%	G1
2015.792±58.545	1602.135±45.461	1178.922±42.972	657.342±16.554	365.971±11.767	Xi+ SD	Tea
2.853	2.837	3.645	2.518	3.215	RSD%	B2
1558.879±61.591	1277.297±54.245	941.121±42.534	495.227±21.216	262.818±12.676	Xi+ SD	Tea
4.336	4.247	4.519	4.284	4.823	RSD%	G2
1569.495±46.359	1295.185±59.217	957.789±46.473	489.981±9.867	264.111±10.676	Xi+ SD	Tea
2.954	4.572	4.852	2.014	4.042	RSD%	B3
1830.292±52.135	1557.948±33.965	979.984±37.245	528.128±21.862	300.327±5.608	Xi+ SD	Tea
2.848	2.181	3.801	4.139	1.867	RSD%	G3

(ppm)

(2)

()						
4	3	2	1	0.5		
41.214±1.044	32.731±1.604	20.521±0.976	14.443±0.677	7.159±0.319	Xi+ SD	Tea
2.534	4.891	4.749	4.687	4.451	RSD%	B1
83.513±3.253	68.132±3.442	48.157±2.307	25.451±0.858	12.121±0.547	Xi+ SD	Tea
3.895	5.053	4.791	3.371	4.513	RSD%	G1
120.118±3.769	74.858±2.819	59.962±2.562	33.175±1.156	18.268±0.471	Xi+ SD	Tea
3.138	3.765	4.273	3.484	2.578	RSD%	B2
146.275±6.205	119.881±5.135	85.221±3.741	43.518±2.147	22.783±0.661	Xi+ SD	Tea
4.242	4.283	4.388	4.933	2.901	RSD%	G2
75.628±2.856	63.233±2.761	49.331±0.525	29.648±1.473	17.622±0.409	Xi+ SD	Tea
3.776	4.365	1.065	4.967	2.319	RSD%	B3
269.808±9.947	208.753±7.793	135.411±2.307	65.117±2.295	38.778±1.254	Xi+ SD	Tea
3.687	3.733	1.704	3.524	3.234	RSD%	G3

: .B  
 : -  
 ( 50 / 0.5)  
 .(4 )

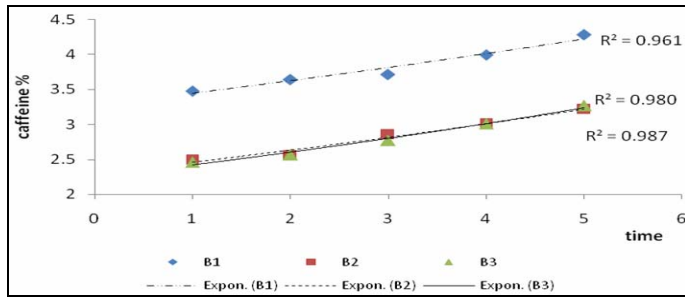
4 0.5  
 (4,3 )

: .C  
 ) ( 50 / 1) ( (5 )

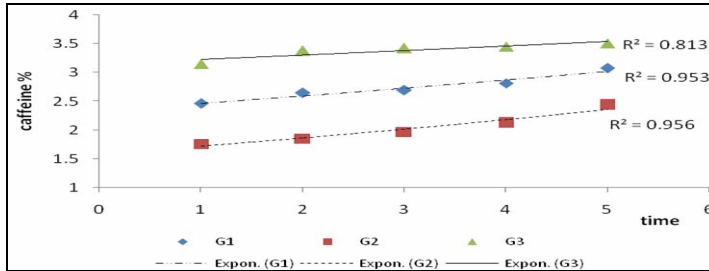
: .D

0.5 50  
 . (180 120 60 30 5)

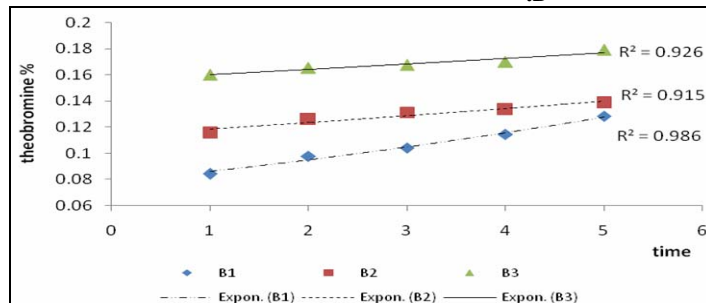
.(6 )° (82-75)



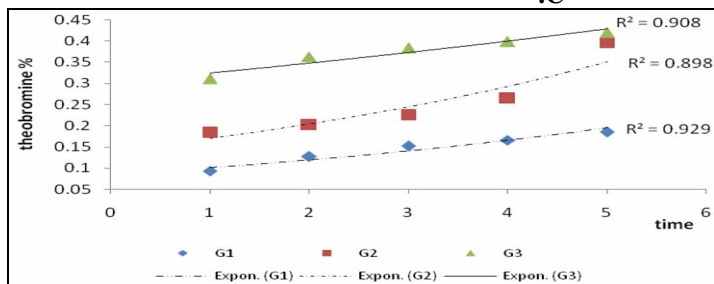
.A



.B



.C



.D

:(A,B,C,D) (4)

(ppm)

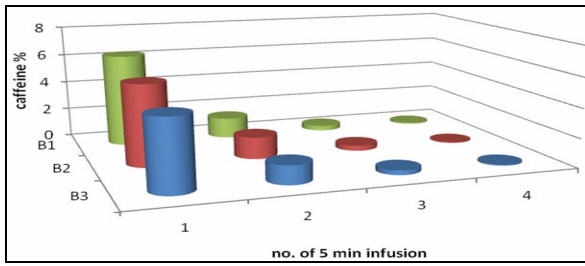
(3)

()						
4	3	2	1	0.5		
2011.121±38.939	1636.422±35.633	1118.097±49.636	612.893±23.851	287.555±8.373	<b>Xi+ SD</b>	<b>Tea</b>
1.936	2.177	4.439	3.892	2.912	<b>RSD%</b>	<b>B1</b>
1224.338±42.199	902.622±35.754	659.083±31.033	356.238±17.601	180.177±7.464	<b>Xi+ SD</b>	<b>Tea</b>
3.446	3.961	4.708	4.941	4.142	<b>RSD%</b>	<b>G1</b>
1548.563±67.725	1264.489±47.179	930.788±23.655	491.133±16.073	243.58±9.827	<b>Xi+ SD</b>	<b>Tea</b>
4.373	3.731	2.541	3.273	4.034	<b>RSD%</b>	<b>B2</b>
1114.085±17.088	881.128±6.858	613.728±16.879	341.712±16.884	172.757±6.142	<b>Xi+ SD</b>	<b>Tea</b>
0.615	1.621	2.751	4.941	3.555	<b>RSD%</b>	<b>G2</b>
1550.695±38.272	1304.892±57.631	981.142±28.368	501.552±19.047	273.555±10.771	<b>Xi+ SD</b>	<b>Tea</b>
2.468	4.416	2.891	3.797	3.937	<b>RSD%</b>	<b>B3</b>
1472.193±35.922	1154.118±43.674	872.602±38.691	460.128±18.638	219.189±9.979	<b>Xi+ SD</b>	<b>Tea</b>
2.442	3.784	4.434	4.049	4.554	<b>RSD%</b>	<b>G3</b>

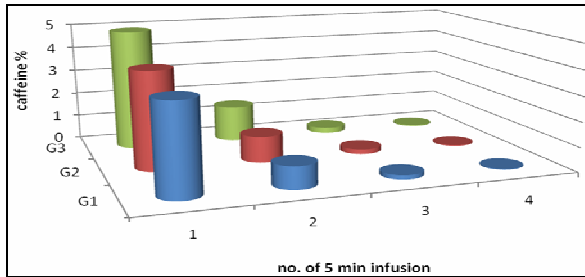
(ppm)

(4)

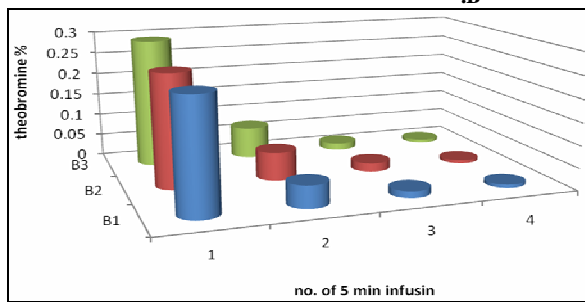
()						
4	3	2	1	0.5		
61.175±2.245	43.611±1.254	38.128±1.178	20.971±0.808	10.713±0.458	<b>Xi+ SD</b>	<b>Tea</b>
3.669	2.876	3.089	3.856	4.276	<b>RSD%</b>	<b>B1</b>
60.853±1.765	44.655±2.115	32.068±1.432	17.153±0.788	8.743±0.344	<b>Xi+ SD</b>	<b>Tea</b>
2.901	4.736	4.466	4.595	3.934	<b>RSD%</b>	<b>G1</b>
72.205±2.541	50.877±1.133	44.665±2.147	24.776±0.887	11.833±0.331	<b>Xi+ SD</b>	<b>Tea</b>
3.518	2.227	4.807	3.579	2.797	<b>RSD%</b>	<b>B2</b>
113.122±3.286	79.822±3.755	60.203±2.114	34.955±1.224	16.562±0.712	<b>Xi+ SD</b>	<b>Tea</b>
2.905	4.704	3.511	3.502	4.299	<b>RSD%</b>	<b>G2</b>
91.758±4.566	76.255±3.279	58.247±0.792	31.047±0.731	17.383±0.376	<b>Xi+ SD</b>	<b>Tea</b>
4.976	4.301	1.361	2.353	2.163	<b>RSD%</b>	<b>B3</b>
224.563±10.366	180.433±7.359	128.218±4.261	66.756±1.697	43.678±1.294	<b>Xi+ SD</b>	<b>Tea</b>
4.616	4.078	3.323	2.542	2.962	<b>RSD%</b>	<b>G3</b>



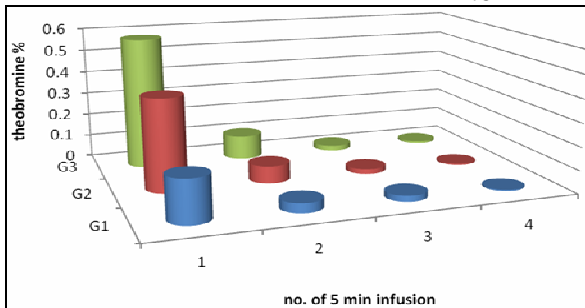
.A



.B

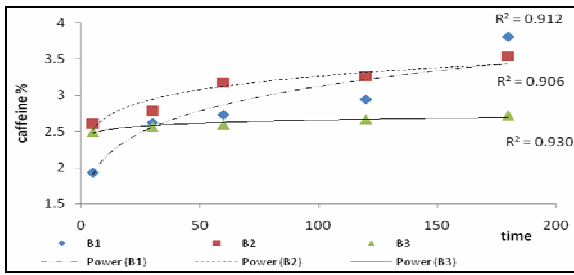


.C

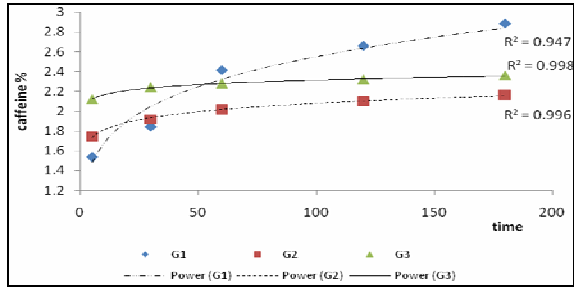


.D

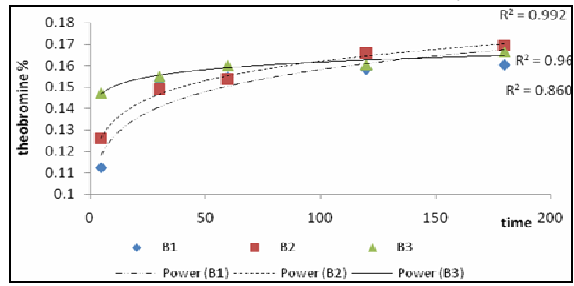
:(A,B,C,D) (5)



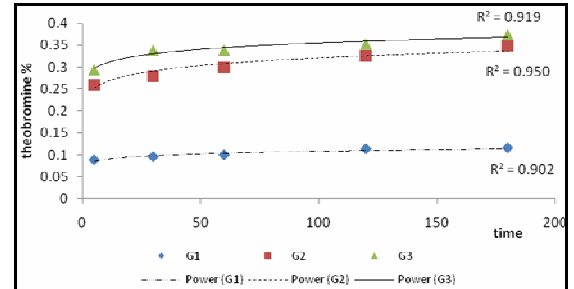
**.A**



**.B**



**.C**



**.D**

(A,B,C,D) (6)

-8

Y=a e<sup>bx</sup> (3 ) :

(% 3.802) B1

(% 3.288) B3

G3

(% 3.506)

G1

(% 2.181)

Fernandez %4

(%108.4 )

[34] 2002

%4.8

%3.8

%2.2

%2.8

%1.4

2003

Lopez-Martinez

. [33]

/ 11.7

/ 17.41

(% 0.265) B3

B1

(% 0.155)

(% 0.411) G3

(%0.127) G1

(%155 )

2010

Wanyika

Sasini tea, Chai mara moja tea, Kericho gold tea

[36] %1.8, %7.36, %2.37

2009

Komes

. [37] %2.35

%2.79

Khanchi

[38] 2006

12.32

0.45

2002

/ 0.34

Bispo

/ 19.60

/

/

217

. [39]

/

12

(1 )  
 0.995 0.956 R<sup>2</sup> Y=aX  
 0.999 0.939

(4 ) :  
 Y=a e<sup>bX</sup>

3.269) B3 (% 4.279) B1  
 (% 3.217) B2  
 (%3.551)  
 G2 (%2.435) G3 (%)  
 (%3.076) G1

(% 0.179) B3  
 B1 (% 0.128)  
 B2 (% 0.139)  
 G3 (% 0.421)  
 (% 0.395) (% 0.185) G1  
 G2

(2 )  
 0.992 0.956 R<sup>2</sup> Y=aX  
 0.995 0.941

100 1 X Y ° 100  
 Y=aX<sup>b</sup>  
 .(5 ) b a

(% 6.336) B1 (% 0.085)  
 78



(% 4.824) B3  
 .( 64 ) (% 0.075)  
 G3  
 121 ) (% 0.041) (% 4.922)  
 3.639) G1 ( (% 0.031) (%  
 (. 121 )  
 .G3 G1  
 (% 0.292) B3  
 ( 43 ) ( / 0.007)  
 (% 0.252) B1  
 .( 34 ) (%0.007)  
 (% 0.577) G3  
 ( 71 ) (% 0.008)  
 (% 0.185) G1  
 .( 36 ) (% 0.005)  
 / 1  
 :  
 Y=a X<sup>b</sup> (6 )  
 B1  
 (% 3.808) 5 (% 1.926)  
 (% 2.721 % 2.494 ) B3  
 (% 10  
 .(% 3.543 % 2.601 ) %36 B2  
 G1  
 (% 2.888) 5 (% 1.538)  
 % 1.746 ) G2 B1  
 %10 %25 (% 2.161  
 .(% 2.361 % 2.123 ) G3  
 .(B2)% 0.169 (B1)% 0.161

---

B2  
(% 0.169) 5 (% 0.126)  
%30  
%40 (% 0.161 % 0.113 ) B1  
(% 0.166 % 0.147 ) B3 %10  
5 (% 0.294) G3  
%25 (% 0.374)  
0.113 ) G1  
%30 (% 0.161 %  
B2  
(% 0.349) 5 (% 0.259)

**-9**

**.1**

**.2**

**.3**

**.4**

**.5**

**.6**

.i

B1

G1  
G3

.G1

.ii

B1

.G1  
G3

.G1

.iii

B1

.G1

G3

.G1

.iv

B2

.G1

G3

.G1

4

0.5

.7

. %500

.8

.9

**-10**

B1

100/ 1

400

---

## REFERENCES

1. Tea glossary URL, 2007.
2. Yamamoto, T. Juneja, L. Chu, D. and Kim, M. (1997). Chemistry and Applications of Green Tea. *CRC Press, Boca Raton, New York*. ISBN 0-84, 3-4006-3, 4.
3. Engelhardt, U. (2010). Chemistry of Tea. *Comprehensive Natural Products II*, V. 3, pp. 999-1032.
4. Ody, P., Complete Guide to Medicinal Herbs. *Dorling Kindersley Publishing*. New York, 48. ISBN 0-7894-6785-2.
5. Tijburg, L. Mattern, T. Folts, J. Weisgerber, U. and Katan, M. (1997). Tea flavonoids and cardiovascular diseases. *J. Food Sci. Nutr*, V.37, pp. 771-785.
6. Arab, L. and Liebeskind, D. (2010). Tea, flavonoids and stroke in man and mouse. *Archives of Biochemistry and Biophysics*, V.501 (1), pp. 31- 36.
7. Atoui, A. Mansouri, A. Boskou, G. and Kefalas, P. (2005). Tea and herbal infusions: Their antioxidant activity and phenolic profile . *Food Chemistry*, V. 89, pp. 27-36.
8. Sagara, Y. Miyata, Y. Nomata, K. Hayashi, T. and Kanetake, H. (2010). Green tea polyphenol suppresses tumor invasion and angiogenesis in N-butyl-(4-hydroxybutyl) nitrosamine-induced bladder cancer. *Cancer Epidemiology*. V. 34 (3), pp. 350-354.
9. Lambert, J. and Yang, C. (2003). Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutation Research*. V. 523-524, pp. 201- 208.
10. Dai, Q. Shu, X. Li, H. Yang, Shrubsole, G. Cai, H. Ji, B. Wen, W. Franke, A. Gao, Y. and Zheng, W. (2010). Is Green Tea Drinking Associated With a Later Onset of Breast Cancer? *Annals of Epidemiology*. V. 20(1), pp.74- 81.
11. Johnson, J. Bailey, H. and Mukhtar H. (2010). Green tea polyphenols for prostate cancer chemoprevention: A translational perspective. *Phytomedicine*. V. 17(1), pp. 3- 13.
12. Moro, C. and Basile, G. (2000). Obesity and medicinal plants. *Fitoterapia*. V. 71, pp. 73-82.
13. Emekli-Alturfan, E. Yarat, A. and Akyuz, S. (2009). Fluoride levels in various black tea, herbal and fruit infusions consumed in Turkey. *Food and Chemical Toxicology*. V. 47 (7), pp. 1495-1498
14. Westerterp-Plantenga, M. (2010). Green tea catechins, caffeine and body-weight regulation. *Physiology & Behavior*. V. 100(1), pp. 42- 46.
15. Is Caffeine a Health Hazard? By Ben Best. (2003) . .16

17. Nehlig, A. Daval, J. and Debry, G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Research Reviews*. V. 17 (2), pp. 139-170.
18. Caleb, K. (2005). Effects of theobromine should be considered in future studies. *American Journal of Clinical Nutrition*. V. 82 (2), pp. 486-8.
19. Glade, M. (2010). Caffeine-Not just a stimulant. *Nutrition*. V. 26 (10), pp. 932- 938.
20. Einother, S. Martens, V. Rycroft, J. and De Bruin, E. (2010). L-Theanine and caffeine improve task switching but not intersensory attention or subjective alertness. *Appetite*. V. 54 (2), pp. 406- 409.
21. Greenberg, J. Dunbar, C. Schnoll, R. Kokolis, S. Kokolis, R. and Kassotis, J. (2007). Caffeinated beverage intake and the risk of heart disease mortality in the elderly: a prospective analysis. *American Journal of Clinical Nutrition*. V. 85 (2), pp. 392-398.
22. Morelli, M. and Simola N. (2010). Can dietary substances protect against Parkinson's disease? The case of caffeine. *Experimental Neurology*. V. 225 (2), pp. 246- 249.
23. Temple, J. Dewey, A. and Briatico, N. (2010). Effects of Acute Caffeine Administration on Adolescents. *Experimental and Clinical Psychopharmacology*. V. 18 (6), pp. 510-520
24. Caffeine-related disorders., 2006. *Encyclopedia of Mental Disorders*.
25. Pohler, H. (2010). Caffeine Intoxication and Addiction. *The Journal for Nurse Practitioners*. V. 6 (1), pp.49-52.
26. Malisoff, M. (1943). Dictionary of Bio-Chemistry and Related Subjects. *Philosophical Library*. pp. (311, 530, 573). ISBN B0006AQ0NU.
27. Polk, I. (1997). All about Asthma: Stop Suffering and Start Living. *New York: Insight Books*. pp. 100. ISBN 0306455692.
28. Rambali, B. Van An del, I. Schenk, E. Wolterink, G. Werken van de, G. Stevenson, H. and Vleeming W. (2002). Effects of theobromine should be considered in future studies. *The National Institute for Public Health and the Environment (Netherlands)*.
29. Alpdogan, G. Karabina, K. and Sungur, S. (2000). Derivative spectrophotometric determination of caffeine in some beverages. *Turk J Chem*. V. 26, pp. 295-302.
30. Meyer, A. Ngiruwonsanga, T. and Henze G. (1996). Determination of adenine, caffeine, theophylline and theobromine by HPLC with amperometric detection. *Anal Chem*. V. 356, pp. 284-287.
31. Franeta, J. Agbaba, D. Eric, S. Pavkov, S. Aleksic, M. and Vladimirov, S. (2002). HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets. *Farmaco*. V. 57(9), pp.709-713.
32. Brannstorm, M. and Edenteg, K. (2002). Determination of caffeine in Vietnamese coffee using HPLC. UMEÅ UNIVERSITY, SWEDEN, MINOR FIELD STUDY.
33. Srdjenovic, B. Djordjevic-Milic, V. Grujic, N. Injac, R. and Lepojevic, Z., 2008. Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. *Chromatographic Science*. V. 46 (2), pp. 144-149.

- 
- ...
34. Fernandez, P. Pablos, F. Martin, M. and Gonzalez, G. (2002). Study of catechin and xanthine tea profiles as geographical tracers. *Agriculture and Food Chemistry*. V. 50, pp. 1833-1839.
  35. Lopez-Martinez, L. Lopez-Alba, P. G. Garcia-Campos, R. and Rodrigues, L. (2003). Simultaneous determination of methylxanthines in coffees and teas by UV-Vis spectrophotometry and partial least squares. *Analytical Chimica Acta*. V. 493(1), pp. 83-94.
  36. Wanyika, H. Gatebe, E. Gitu, L. Ngumba, E. and Maritim, C. (2010). Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. *African Journal of Food Science*. V. 4(4), pp. 353-385.
  37. Komes, D. Horzic, D. Belscak, A. KovacevicGanic, K. and Baljak, A. (2009). Determination of Caffeine Content in Tea and Maté Tea by using Different Methods. *Czech J.Food Sci*. V. 27, pp. 213-216.
  38. Khanchi, A. Mahani, M. Hajihosseini, M. Maragheh, M. Chaloosi, M. and Bani, F. (2007). Simultaneous spectrophotometric determination of caffeine and theobromine in Iranian tea by artificial neural networks and its comparison with PLS. *Food Chemistry*. V. 103 (3), pp. 1062-1068.
  39. Bispo, M. Velloso, M. Pinhero, H. Deoliveira, R. Reis, J. and Deandrade, J. (2002). Simultaneous Determination of Caffeine, Theobromine, and Theophylline by High-Performance Liquid Chromatography. *Journal of Chromatographic Science*. V. 40, pp. 45-48.