ELSEVIER

Food Chemistry 84 (2004) 383–388

Food Chemistry

www.elsevier.com/locate/foodchem

Does caffeine bind to metal ions?

Sevgi Kolaylı*, Miraç Ocak, Murat Küçük, Rıza Abbasoğlu

Karadeniz Technical University, Faculty of Arts and Sciences, Department of Chemistry Trabzon, Trabzon, Turkey

Received 7 March 2003; accepted 6 May 2003

Abstract

The complex formation capacity of caffeine, a highly-consumed tea and coffee component, was determined for Ca, Mg, Fe, Zn, Pb, Mn, Co and Cr metal ions. The binding constants of metal ion–caffeine complexes for the metals chosen were determined spectrophotometrically. The results were compared with the known stability constants of metal ion–EDTA complexes, EDTA being known for its high metal binding capacity. Furthermore, iron chelating activity of caffeine, using the ferrozine reference method, was studied and compared with that of EDTA. The results showed very little complex formation capacity of caffeine with binding constants of 29.6, 22.4, 59, 396, 55, 9.3, 83 and 592 M^{-1} for Ca, Mg, Fe, Zn, Pb, Mn, Co and Cr metal ions, respectively, in contrast to that of EDTA. The iron chelating activity of caffeine was also found to be 6%, which was considered to be quite low compared with EDTA.

Keywords: Caffeine; EDTA; Ferrozine; Iron chelating activity; Metal ions; Complex formation; Binding constant

1. Introduction

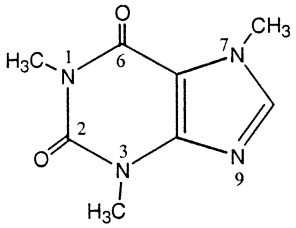
Caffeine, 1,3,-trimethylxanthine, a purine alkaloid, is a key component of many popular drinks, mainly tea and coffee, but most phytochemists know little about its biochemistry and molecular biology (Ashihara & Crozier, 2001). Green tea leaves contain 10–30% (w/w) tea polyphenols and 2–4% (w/w) caffeine and all are soluble in water. A cup of tea or coffee typically contains approximately 30–175 mg caffeine. While many reports are available about antioxidant and anticancer properties and health benefits of tea, there is also a strong belief that the people consuming high amounts of caffeine tend to carry a higher risk of developing bone problems, including osteoporosis, as well as problems in metal absorption, excretion and reabsorption processes in intestines and in kidney (Borse, Jagan Mohan Rao, Nagalakshmi, & Krishnamurthy, 2002; Chen & Whitford, 1999; Massey, 2001; Pan, Guaguang, & Liu, 2003), and iron deficiency anemia (Hallberg & Rossander, 1982). Although some epidemiological studies show a negative relationship between consumption of caffeine-containing beverages and intestinal mineral absorption (Heaney, 2002; Huang, Yang, Hsieh, & Liu, 2002; Rapuri, Gallagher, Kinyamu, & Ryschon, 2001; Yazdani, Gottschalk, Ide, & Nakamoto, 2002), and urinary and intestinal mineral secretion (Heaney & Recker, 1982), many others suggest no correlation between caffeine consumption and metal absorption in intestines and kidneys (Conlisk, Deborah, & Gakuska, 2000; Grainge, Coupland, Cliffe, Chilvers, & Hosking, 1998; Nafisi, Slamboo, Mohajerani, & Omidi, 2002) and bone gain in adolescents (Lloyd, Rollings, Kieselhorst, Eggli, & Mauger, 1998). Hegarty, May, and Khaw (2000) found a positive relationship between tea drinking and bone mineral density measurements in older women. The issue of whether or not caffeine consumption is harmful for humans still remains controversial.

Calcium, magnesium, iron, zinc, manganese, cobalt and chromium are essential elements for many organisms. They are involved in a variety of biological reactions (Turcot, Stintzi, Xu, & Raymond, 2000; Voet & Voet, 1995) and their deficiencies result in many medical problems (Prohaska & Brokate, 2001; Salgueiro, Zubillage, Lysionek, Sarabia, Caro, & Paoli, 2000). Caffeine (Structure 1) has been suspected to bind many metal ions and alter their balance in the human body (Chen & Whitford, 1999; Riesselmann, Rosenbaum, Roscher, &

* Corresponding author. Tel.: +90-462-377-24-87; fax: +90-462-325-31-96.

E-mail address: skolayli@ktu.edu.tr (S. Kolaylı).

0308-8146/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00244-9



Structure 1. Caffeine.

Scheineder, 1999). The interactions between caffeine and metal ions can be through its oxygen and nitrogen atoms. Because of the blockage on N1, N3 and N7 atoms by methyl groups, caffeine probably binds to metal ions through its O2 and O6 atoms. Indeed our previous theoretical study showed that caffeine would bind to metal ions through its O2 and O6 atoms in the gaseous phase (Abbasoğlu, Küçük, Kolaylı, & Ocak, 2002). Therefore, we aimed at finding the extent of in vitro metal ion interactions with caffeine in aqueous solutions using EDTA for comparison. This study was partly presented at an international meeting (Ocak, Kolaylı, Küçük, & Abbasoğlu, 2002).

2. Materials and methods

Caffeine was purchased from Fluka and ferrozine, 3-(2-pyridyl)-5,6-bis(2-5-furylsulfonic acid-1,2,4-triazine, was purchased from Sigma. Ethylenediaminetetraacetic acid disodium salt (EDTA), and Ca(II), Mg(II), Fe(II), Pb(II) and Zn(II) standard solutions were obtained from Merck. All of the solutions were prepared with deionized distilled water.

The stability constants of caffeine–metal ion complexes were determined according to the method used by Nafisi et al. (2002) with a slight modification. The solutions of Ca (II), Mg (II), Fe(II), Pb(II), Zn(II), Co(II) and Mn(II) of 0.5 mM concentration were prepared by diluting the standard metal ion solutions in deionized distilled water. 5 ml, from each appropriately diluted metal ion solution, were added separately into 5 ml caffeine solutions to attain the desired metal ion concentrations of 0.001, 0.01, 0.1, and 1.0 mM with a final caffeine concentration of 0.0005% w/v, remaining the same throughout all of the experiments. Following vortexing and incubation for 10 min at room temperature, absorbances were measured at 273 nm with a Unicam UV2 UV/vis spectrometer. The absorbance of a caffeine solution of the same concentration containing no metal ion was also measured.

Iron chelating activity of caffeine was determined by the method of Dinis, Madeira, and Almeida (1994). This is based on the measurement of absorbance at 593 nm resulting from a colourful complex of ferrozine with the iron that remains unbound in the solution containing EDTA or caffeine. A 0.2 ml sample solution of varying concentration, 1.2 ml deionized distilled water and 0.2 ml 0.2 mM $FeCl_2.4H_2O$ solution were mixed and vortexed immediately. 0.4 ml 1 mM ferrozine was added to the reaction mixture and change in colour was monitored at 593 nm with a Unicam UV2 UV/vis spectrometer after a 10 min incubation period at room temperature. EDTA was used for comparison.

The values presented in this report are the means of triplicate measurements.

3. Results

The binding constants were calculated using the data obtained from the UV spectrophotometric method as reported (Klotz & Hunston, 1971; Purcell, Neault, & Tajmir-Riahi, 2000). The equilibrium between caffeine and a metal ion can be represented by the following reaction:

$$\text{Caffeine} + \text{Metal ion} \leftrightarrow \text{Caffeine} : \text{Metal ion}$$

The binding constant of caffeine with a metal ion is defined as in Eq. (1):

$$k = \frac{[\text{caffeine} : \text{metal ion}]}{[\text{caffeine}][\text{metal ion}]} \tag{1}$$

In order to determine the binding constants of caffeine for the metal ions studied, Klotz plots were utilized. In order to obtain these plots, Eq. (2) was used.

$$\frac{1}{c} = \frac{1}{n.a.k}\frac{1}{L} + \frac{1}{n.a} \tag{2}$$

where c is the concentration of metal ion–caffeine complex, n is the number of metal binding sites on caffeine, a is caffeine concentration, L is total metal ion concentration and k is binding constant for metal ion–caffeine complex. The absorbance of metal ion–caffeine complex (A_c) can be used instead of c by multiplying it with the inverse of the extinction coefficient of caffeine, $1/\varepsilon$, at that wavelength (Bobrovnik, 2003), by which Eq. (3) was obtained.

$$\frac{1}{A_c\frac{1}{\varepsilon}} = \frac{1}{n.a.k}\frac{1}{L} + \frac{1}{n.a} \tag{3}$$

By placing $1/A_c$ on one side of the equation, Eq. (4) was obtained and used to calculate binding constants by plotting $1/A_c$ against $1/L$.

$$\frac{1}{A_c} = \frac{1}{\varepsilon.n.a.k}\frac{1}{L} + \frac{1}{\varepsilon.n.a} \tag{4}$$

where $A_c = A - A_o$, A and A_o representing the absorbances of metal ion–caffeine complex and caffeine alone, respectively.

The plots of 1/(absorbance of complexed ion) vs. 1/(total metal ion concentration)—$1/A\text{-}A_o$ vs. $1/L$—are shown in Figs. 1–8 for metal ion–caffeine complexation. The graphs show a linear relationship for all of the metal ions. The binding constants (k: M^{-1}) were calculated as the ratio of the intercept on the vertical axis to the slope [Eq. (5)].

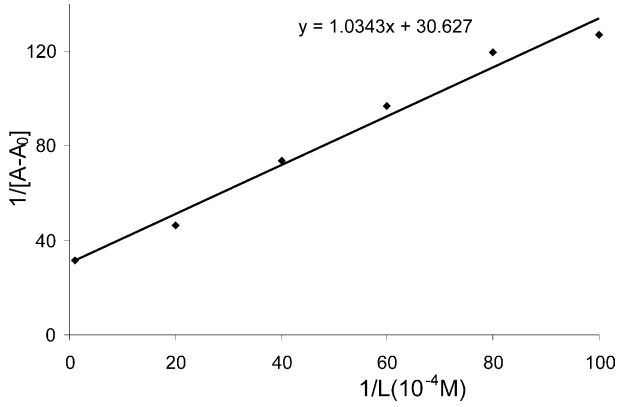


Fig. 1. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Ca^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

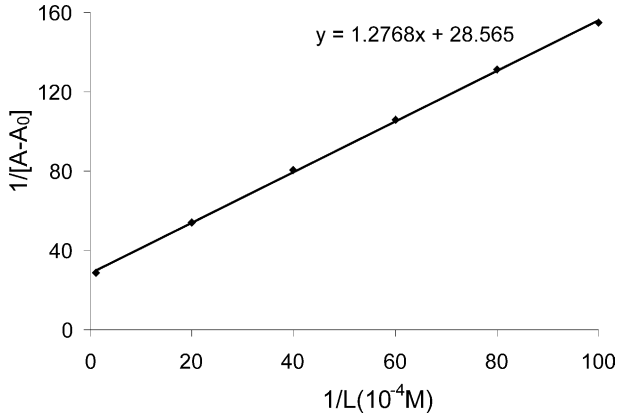


Fig. 2. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Mg^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

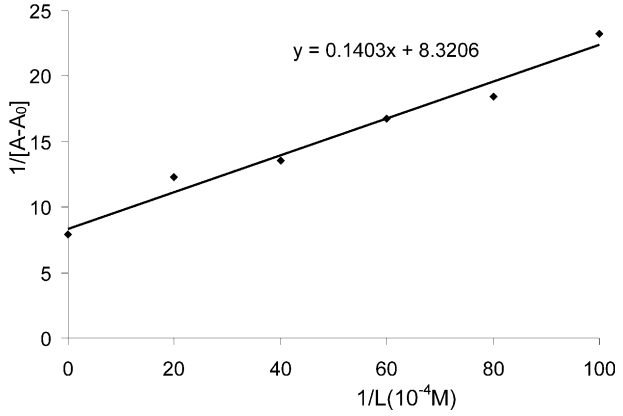


Fig. 3. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Fe^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

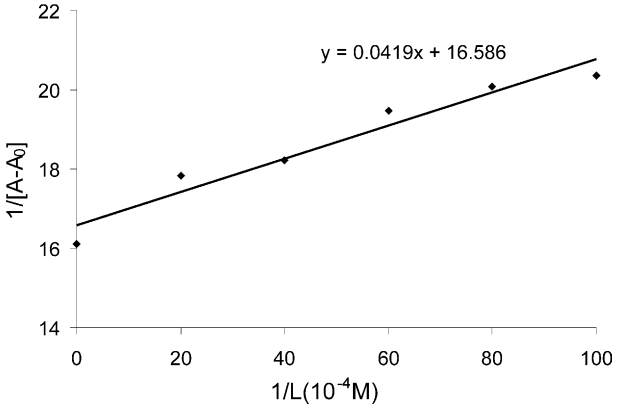


Fig. 4. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Zn^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

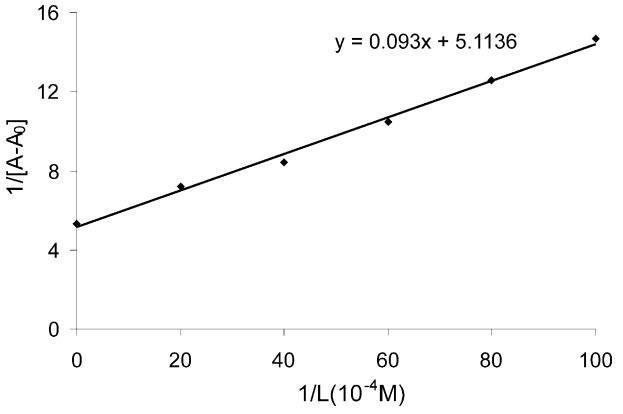


Fig. 5. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Pb^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

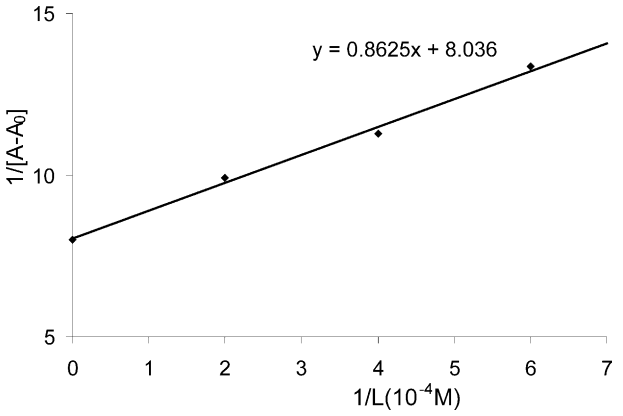


Fig. 6. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Mn^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

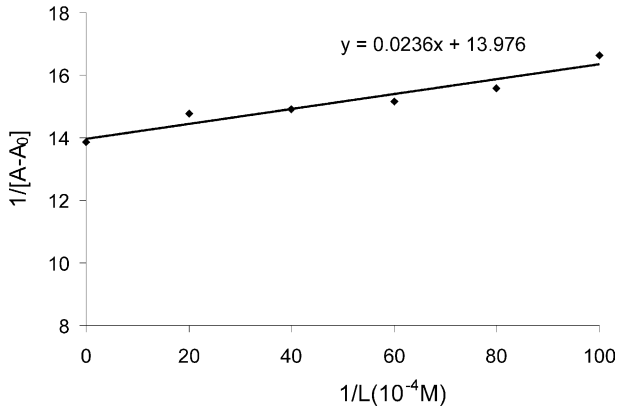


Fig. 7. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Cr^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

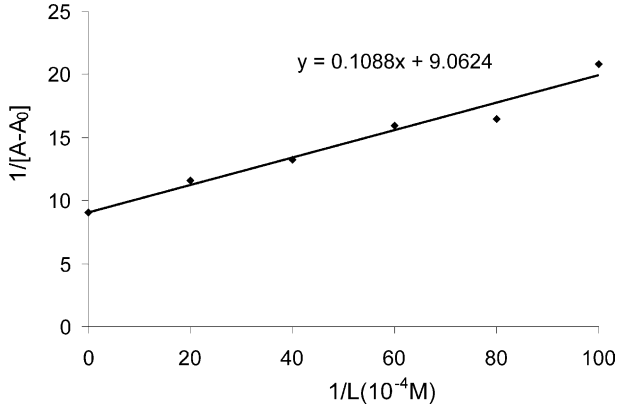


Fig. 8. The plot of $1/(A-A_0)$ vs. $(1/L)$ for caffeine–Co^{+2} interaction. A_0 is the initial absorbance of caffeine at 273 nm and A is the recorded absorbance at varying ion concentration.

$$k = \frac{\text{intercept on ordinate axis}}{\text{slope}} = \frac{\frac{1}{\varepsilon na}}{\frac{1}{\varepsilon nak}} \quad (5)$$

In order to calculate the concentrations of complexed metal ions, the absorbance of complexed caffeine was subtracted from that of uncomplexed caffeine at 273 nm. The concentrations of free metal ions could be calculated by subtracting that of complexed metal ion from the total metal ion concentration used in the experiments. The calculated binding constants of metal ion–caffeine complexes and the binding constants for metal ion–EDTA complexes are presented in Table 1.

The binding constants of metals with EDTA were orginally reported by Christian (1994) and presented in Table 1 for comparison. The binding constants of caffeine with all the metal ions studied appear to be very low when compared to the binding constants of EDTA. When the binding capacities of EDTA and caffeine in terms of k values were compared, no significant correlation was found with respect to the type of metal ions, $r^2 = 0.06$.

Table 1
Binding constants for the metal ion–caffeine and EDTA complexes

Element	Caffeine	EDTA[a]
Calcium	29.6	5.01×10^{10}
Magnesium	22.4	4.90×10^{8}
Iron (Fe^{2+})	59	2.14×10^{14}
Zinc	396	3.16×10^{16}
Chromium	592	–
Lead	55	1.10×10^{18}
Cobalt	83	2.04×10^{16}
Manganese	9.3	1.10×10^{14}

[a] The data were obtained from literature (Christian, 1994).

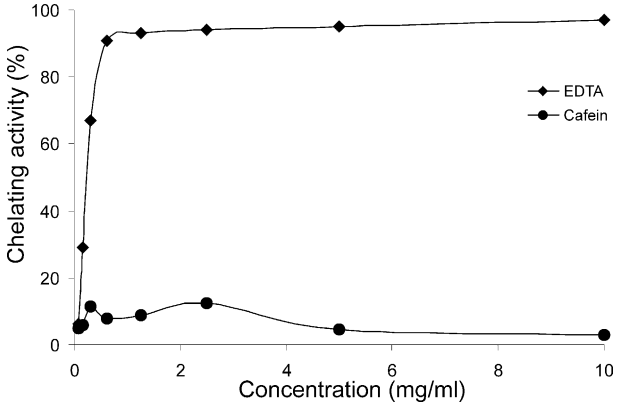


Fig. 9. Chelating activity of various concentrations of caffeine and EDTA. Each value is the average of tree determinations.

Iron chelating activities of caffeine and EDTA were calculated according to Eq. (6).

$$\%\ \text{Chelating activity} = \frac{A_0 - A}{A_0} \quad (6)$$

where A is the absorbance in the presence of ligand while A_0 is the absorbance with no ligand present.

The Fe(II) binding capacities of the two ligands were compared in terms of iron chelating activity (Dinis et al., 1994). While EDTA showed a concentration-dependent activity, from 99% metal binding at 1–10 mg/ml EDTA concentrations to less then 5% at low concentrations, caffeine showed a low but steady chelating activity with around 6% binding at all caffeine concentrations (Fig. 9).

4. Discussion

Caffeine, a well known alkaloid, is taken daily with beverages and known to have effects on energetic arousal, to stimulate the autonomic nervous system and to increase alertness (Quinlan, Lane, Moore, Aspen, Rycroft, & O'Brien, 2000). There is a belief among the public that those who drink too much tea or coffee are

prone to have mineral deficiency problems (Conlisk et al. 2000; Horie, Nesumi, Tomomi, & Kohata, 2000). Previous studies on this subject appear to be more of the in vivo and epidemiological kind. Chen and Whitford (1999) investigated mineral metabolism for calcium, fluorine and phosphorus in rats given caffeine at various doses, and reported that they found no significant change in mineral balance. Similarly, Prystai, Prystai, Constance, Kies, Judy and Driskell (1999) reported that no change was observed in the balance of Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+} and Zn^{2+} ions in young adults who regularly took tea and decaffeinated tea. Conlisk et al. (2000) studied the effect of drinking beverages with caffeine on bone mineral density (BMD) on 77 healthy women, using decaffeinated beverages as control and reported that no change had occurred in BMD of the subjects. Yeh, Aloia, Semla, and Chen (1986) gave caffeine to rats by injection in daily doses of 25 and 100 mg/kg and compared the results with the control group. The urinary excretion of phosphorus was increased in both caffeine groups and the group injected with 100 mg/kg caffeine daily showed a significant reduction in phosphorus balance. Barger-Lux and Heaney (1995) reported that caffeine intake reduced the calcium balance slightly in 39–69-year-old women but the effect was due to the reductions in intake and absorption. Nasifi et al. (2002) investigated the binding constants of caffeine and theophylline with Ca^{2+} and Mg^{2+} and reported that both alkaloids form a very weak complex with Ca^{2+} and Mg^{2+} ions.

In the current study, we found that the eight metal ions investigated, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+} and Cr^{2+}, formed complexes with caffeine in varying capacities but these were very weak in strength when compared to EDTA (Table 1). EDTA shows a 10^{10}-fold higher metal binding activity compared to caffeine. Nafisi et al. (2002) reported the binding constants of Ca^{2+} and Mg^{2+} with caffeine to be 29.8 and 22.4 M^{-1}, respectively. The results of our experiments and theirs were quite similar.

5. Conclusions

The current study clearly showed that caffeine does not bind to Ca, Mg, Fe, Zn, Pb, Mn, Co and Cr metal ions in significant extent to alter their functions in the human body. The interactions between caffeine and the metal ions studied were very low and this fact was proved earlier with quantum mechanical investigations (Abbasoğlu et al., 2002). The results of this study suggest that caffeine does not change the metal ion balance in the human body directly by binding metal ions. Instead, there may be other mechanisms which are induced by caffeine, resulting in undesired consequences of caffeine consumption, including osteoporosis.

Acknowledgements

This research was supported by the Research Fund of Karadeniz Technical University, (Project No: 21.111.002. 9).

References

Abbasoğlu, R., Küçük, M., Kolaylı, S., & Ocak M. (2002). Theoretical investigation of Mg^{2+} binding to caffeine and theophylline. In *Proceedings of the 1st Int. Congress on the Chemistry of Natural Products*, Trabzon, Türkiye.

Ashihara, H., & Crozier, A. (2001). Caffeine: a well known but little mentioned compound in plant science. *Trends in Plant Science*, *6*(9), 407–413.

Barger-Lux, M. J., & Heaney, R. P. (1995). Caffeine and calcium economy revisited. *Osteoporosis International*, *5*, 97–102.

Bobrovnik, S. A. (2003). Ligand-receptor interactions: a new method for determining the binding parameters. *Journal of Biochemical and Biophysical Methods*, *55*, 71–86.

Borse, B. B., Jagan Mohan Rao, L., Nagalakshmi, S., & Krishnamurthy, N. (2002). Fingerprint of black teas from India: identification of the regio-specific characteristics. *Food Chemistry*, *79*, 419–424.

Chen, X., & Whitford, G. M. (1999). Effects of caffeine on fluoride, calcium and phosphorus metabolism and calcified tissues in the rat. *Archives of Oral Biology*, *44*, 33–39.

Christian, G. D. (1994). *Analytical chemistry* (5th ed.). New York: John Wiley & Sons.

Conlisk, A. J., Deborah, A., & Gakuska, M. P. H. (2000). Is caffeine associated with bone mineral density in young adult women? *Preventive Medicine*, *31*, 562–568.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, *315*, 161–169.

Grainge, M. J., Coupland, C. A. C., Cliffe, S. J., Chilvers, C. E. D., & Hosking, D. J. (1998). Cigarette smoking, alcohol and caffeine consumtion, and bone mineral density in postmenopausal women. *Osteoporosis International*, *8*(4), 355–363.

Hallberg, L., & Rossander, L. (1982). Effect of different drinks on the absorption of non-heme iron from composite meals. *Human Nutrition. Applied Nutrition*, *36*(2), 116–123.

Heaney, R. P., & Recker, R. R. (1982). Effects of nitrogen, phosphorus and caffeine on calcium balance in women. *The Journal of Laboratory and Clinical Medicine*, *99*(1), 46–55.

Heaney, R. P. (2002). Effects of caffeine on bone and the calcium economy. *Food and Chemical Toxicology*, *40*(9), 1263–1270.

Hegarty, V. M., May, H. M., & Khaw, K. T. (2000). Tea drinking and bone mineral density in older women. *American Journal of Clinical Nutrition*, *71*(4), 1003–1007.

Horie, H., Nesumi, A., Tomomi, U., & Kohata, K. (2002). Rapid determination of caffeine in tea leaves. *Journal of Chromatography*, *942*, 271–273.

Huang, T. H., Yang, R. S., Hsieh, S. S., & Liu, S. H. (2002). Effects of caffeine and exercise on the development of bone: a densitometric and histomorphometric study in young Wistar rats. *Bone*, *30*(1), 293–299.

Klotz, I. M., & Hunston, D. L. (1971). Properties of graphical representations of multiple classes of binding sites. *Biochemistry*, *10*, 3065–3069.

Lloyd, T., Rollings, N. J., Kieselhorst, K., Eggli, D. F., & Mauger, E. (1998). Dietary caffeine intake is not correlated with adolescent bone gain. *Journal of the American College of Nutrition*, *17*(5), 454–457.

Massey, L. K. (2001). Is caffeine a risk factor for bone loss in the elderly. *American Journal of Clinical Nutrition*, *74*, 569–570.

Nafisi, S., Slamboo, D. S., Mohajerani, N., & Omidi, A. (2002). A comparative study of caffeine and teophylline binding to Mg(II) and Ca(II) ions: studies by FTIR and UV spectroscopic methods. *Journal of Molecular Structure*, *608*, 1–7.

Ocak, M., Kolaylı, S., Küçük, M., Abbasoğlu, R. (2002). A comparative study of caffeine binding to some metal ions. Proceedings *1st Int. Congress on the Chemistry of Natural Products* (p. 94) Trabzon, Türkiye.

Pan, X., Guaguang, N., & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing*, *42*(2), 129–133.

Prohaska, J. R., & Brokate, B. (2001). Lower copper, zinc-superoxide dismutase protein but not mRNA in organs of copper-deficient rats. *Archives of Biochemistry and Biophysics*, *393*(1), 170–176.

Prystai, E., Prystai, M. S., Constance, V., Kies, P. D., Judy, A., & Driskell, P. D. (1999). Calcium, copper, iron, magnesium and zinc utilization of humans as affected by consumption of black, decaffeinated black and green teas. *Nutrition Research*, *19*(2), 167–177.

Purcell, M., Neault, J. F., & Tajmir-Riahi, H. A. (2000). Interaction of taxol with human serum albumin. *Biochimica et Biophysica Acta*, *1478*, 61–68.

Quinlan, P. T., Lane, J., Moore, K. L., Aspen, J., Rycroft, J. A., & O'Brien, D. C. (2000). The acute physiological and mood effects of tea and coffee: the role of caffeine level. *Pharmacology Biochemistry and Behavior*, *66*(1), 19–28.

Rapuri, P. B., Gallagher, J. C., Kinyamu, H. K., & Ryschon, K. L. (2001). Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes. *American Journal of Clinical Nutrition*, *74*, 694–700.

Riesselmann, B., Rosenbaum, F., Roscher, S., & Scheineder, V. (1999). Fatal caffeine intoxication. *Forensic Science International*, *103*, 549–552.

Salgueiro, M. J., Zubillage, B. M., Lysionek, A., Sarabia, I., Caro, R., & Paoli, T. (2000). Zinc as an essential micronutrient. *Nutrition Research*, *20*(5), 737–755.

Turcot, I., Stintzi, A., Xu, J., & Raymond, K. N. (2000). Fast biological iron chelators: kinetics of iron removal from human diferric transferrin by multidentate hydroxypridonates. *Journal of Biological Inorganic Chemistry*, *5*, 634–641.

Voet, D., & Voet, J. G. (1995). *Biochemistry* (2nd ed.). New-York: John Wiley & Sons.

Yazdani, M., Gottschalk, S., Ide, K., & Nakamoto, T. (2002). Association of different zinc concentrations combined with a fixed caffeine dose on plasma and tissue caffeine and zinc levels in the rat. *Biological Trace Elements Research*, *87*, 183–190.

Yeh, J. K., Aloia, J. F., Semla, H. M., & Chen, S. Y. (1986). Influence of injected caffeine on the metabolism of calcium and the retention and excretion of sodium, potassium, phosphorus, magnesium, zinc and copper in rats. *Journal of Nutrition*, *116*, 273–280.