Summary

Catechins and catechin derivatives are the most abundant polyphenols in tea. They contribute to the quality of tea and have several prophylactic properties, which include antimutagenic and anticarcinogenic effects. In the tea industry it is important to quantify and control the quality of tea. Chemical analytical methods that are able to separate and quantify the catechins can assist in the Quality Control process.

It is also important to identify all possible mechanisms by which tea polyphenols exert their prophylactic properties. It was thought that one such mechanism might be the inhibition of oxygen radical producing enzymes such as xanthine oxidase. Xanthine oxidase is also important in the occurrence of gout since it forms uric acid from xanthine. Effective inhibition of xanthine oxidase with tea polyphenols may lead to new natural product drugs with less adverse effects than current gout therapy drugs such as colchicine and allopurinol.

The first aim of this research was to develop and implement an analytical method suitable for analyzing tea liquors from green and black tea as well as from fresh tea leaves. The second aim of this research was to evaluate tea catechins as xanthine oxidase inhibitors both *in vitro* and *in vivo*.

Micellar electrokinetic chromatography was deemed the most suitable analytical technique for the separation and quantification of the major compounds influencing the quality of tea. A micellar electrokinetic chromatography method was developed to analyze the five major catechins, caffeine, theanine, gallic acid and ascorbic acid in green, black and bottled iced teas. The pH and the concentration of organic modifier

had the largest influence on the separation of the analytes. The optimal separation conditions used to analyze green and black tea samples were 25 mM phosphate, 100 mM sodium dodecyl sulfate, 6% (v/v) methanol at pH 7.0. For the analysis of iced tea samples the conditions were kept the same, except the methanol concentration was reduced to 5% (v/v). This allowed the added separation of ascorbic acid, which is added to bottled iced tea as fortification.

Seven different tea polyphenols were tested as *in vitro* inhibitors of xanthine oxidase. It was found that (-)-epigallocatechin gallate, the most abundant tea catechin, was one of the more potent inhibitors that were tested. The majority of the tea catechins showed mixed type inhibition. Catechin showed uncompetitive inhibition and (-)-epigallocatechin gallate showed competitive inhibition.

(-)-Epigallocatechin gallate was tested as a xanthine oxidase inhibitor in a rat model. The tests were done in normal and hyperuricemia induced Sprague-Dawley rats. The positive control was allopurinol that is currently used as gout therapeutic drug. A capillary zone electrophoresis method was implemented and used to detect and quantify changes in hypoxanthine, xanthine, uric acid, allopurinol and oxypurinol levels in the serum and urine of treated animals.

Allopurinol prevented the production of uric acid in a dose-dependent manner in both normal and hyperuricemic rats and resulted in the equimolar increase of the xanthine levels. The (-)-epigallocatechin gallate reduced the levels in normal rats slightly, but no xanthine accumulation was observed. This suggests that (-)-epigallocatechin gallate might be a weak uricosuric agent, but did not inhibit xanthine oxidase at the

levels tested. (-)-epigallocatechin gallate also did not inhibit xanthine oxidase activity in hyperuricemic rats. In hyperuricemic rats, low (-)-epigallocatechin gallate levels reduced serum uric acid levels and high (-)-epigallocatechin gallate doses increased the serum uric acid levels. These indifferent results cannot be explained.

The three dimensional molecular structures of the tested inhibitors as well as other synthetic inhibitors from the literature were created. A model is proposed where the polyphenols bind to the active center of the xanthine oxidase enzyme. The polyphenol binds to either the E_{ox} or E_{red} or both forms of the enzyme in the ping-pong catalytic cycle. Inhibitors binding solely to the E_{ox} form show competitive inhibition, while inhibitors binding solely to the E_{red} form show uncompetitive inhibition. When an inhibitor can bind to both forms of the enzyme it exhibits mixed type inhibition. Primary and secondary binding regions are identified on the polyphenol molecules. The primary binding regions mimic the xanthine oxidase substrates and products, causing them to bind to the active center of the enzyme. The secondary binding region enhances the affinity of the inhibitor for the enzyme. It also modulates the selectivity for the E_{ox} and E_{red} forms of the enzyme respectively.

The proposed enzyme inhibition model is a starting point for determining the exact inhibitory mechanism of action that the tea polyphenols have on xanthine oxidase. Molecular modeling and ligand docking studies may confirm the model and also assist in modifying polyphenols into xanthine oxidase inhibitors that are more efficacious *in vivo*.

The analytical capillary electrophoresis method development creates a firm base for future research on other important compounds in tea. capillary electrophoresis may be used to study the polymerized polyphenols (theaflavins, thearubigens and bisflavanols) found in black tea. Very little is known of these compounds' contribution to quality and prophylaxis.

Opsomming

Tee polifenole dra nie net tot die kwaliteit van die tee by nie, maar besit ook gesondheidseienskappe. Katechien verbindings (catechins) is die polifenole wat die volopste in tee voorkom. Analitiese metodes wat die verskillende derivate kan skei en kwantifiseer kan gebruik word in die kwaliteits beheer proses.

Al die meganismes waarmee polifenole hul gesondheids eienskappe manifesteer is nog nie bekend nie. Een moontlike meganisme is die inhibisie van ensieme wat suurstof-radikale vorm. Xantienoksidase is 'n voorbeeld van so 'n ensiem. Xantienoksidase is ook betrokke by die ontstaan van jig aangesien dit uriensuur produseer. Die effektiewe inhibisie van xantienoksidase met tee polifenole kan aanleiding gee tot die tot-standkoming van 'n nuwe reeks natuurlike inhibitore. Die inhibitore kan minder newe-effekte hê as die huidige middels teen jig (allopurinol en kolgisien).

Die eerste doel van die navorsingsprojek was om 'n analitiese metode te ontwikkel wat gebruik kan word om groen- en swarttee ekstrakte te analiseer. Die tweede doel van die navorsing was om katechien verbindings te evalueer as inhibitore van xantienoksidase, beide *in vitro* en *in vivo*.

Misellêre elektrokinetiese chromatografie was as die mees gepaste tegniek geag vir die skeiding en kwantifisering van die verbindings in tee. 'n Misellêre elektrokinetiese chro-matografie metode is ontwikkel om die skeiding van die vyf belangrikste katechien

verbindings, kaffeïen, teanien, gallussuur en askorbiensuur te bewerkstellig in groen-, swart- en gebottelde tee. Die pH van die buffer en die konsentrasie organiese oplosmidel in die buffer het die grootste invloed op die skeiding gehad. Vir die analisering van groen- en swarttee was die optimum kondisies 25 mM fosfaat, 100 mM natrium dodekiel sulfaat, 6% (v/v) metanol by pH 7.0. Gebottelde tee is geanaliseer met dieselfde kondisies, bewalwe vir die metanol konsentrasie wat na 5% (v/v) verminder is. Die verandering verseker dat askorbiensuur ook geanaliseer kan word.

Sewe tee polifenole is *in vitro* as inhibitore van xantienoksidase getoets. Epigallo-katechiegallaat was die beste inhibitor. Die meerderheid van die katechien verbindings het gemengde tipe inhibisie getoon. Katechien was 'n onkompeterende inhibitor en epigallokatechiengallaat 'n kompeterende inhibitor.

'n Rot model was gebruik om epigallokatechiengallaat *in vivo* as xantienoksidase inhibitor te toets. Dit is gedoen in normale rotte sowel as rotte waarin hiperurisemia geïnduseer is. Allopurinol is gebruik as positiewe kontrole. 'n Kapillêre sone elektroforese metode is gebruik om die konsentrasies van hipoxantien, xantien, uriensuur, allopurinol en oksipurinol in rot serum en uriene te bepaal.

Allopurinol het die produksie van uriensuur op 'n dosis afhanklike wyse verlaag in beide die normale en hiperurisemiese rot groepe. Epigallokatechiengallaat het die konsentrasie van uriensuur in normale rotte effens verlaag, maar geen xantien het gevorm nie. Epigallokatechiengallaat inhibeer nie xantienoksidase *in vivo* nie, maar mag dalk 'n swak

urikosuriese (uricosuric) middel wees. 'n Lae epigallokatechiengallaat dosis het die serum konsentrasie van uriensuur in hiperurisemiese rotte verlaag. Hoër epigallokatechiengallaat dosisse het geen effek gehad op die uriensuur konsentrasies nie. Die verskynsel kan nie verklaar word nie.

Drie-dimensionele strukture is gegenereer vir al die inhibitore wat getoets was, sowel as vir sintetiese inhibitore wat vanuit die literatuur verkry was. 'n Model is voorgestel waar die polifenole aan die aktiewe sentrum van xantienoksidase bind. Die polifenol bind aan die E_{ox} of E_{red} vorm van die ensiem in die ping-pong katalitiese siklus. Inhibitore wat aan die E_{ox} vorm bind vertoon kompeterende inhibisie. Inhibitore wat aan die E_{red} vorm bind vertoon onkompeterende inhibisie. As 'n inhibitor aan beide vorme bind vertoon dit gemengde tipe inhibisie. Primêre en sekondêre bindings gebiede is gedefinieer op die polifenol molekule. Die primêre bindings gebied simuleer die xantienoksidase substrate en produkte wat verseker dat die inhibitor aan die aktiewe sentrum bind. Die sekondêre bindings gebiede verhoog die affiniteit van die inhibitor vir die ensiem. Dit moduleer ook die selektiwiteit van die inhibitor vir die twee vorme van die ensiem.

Die voorgestelde model is 'n goeie beginpunt om die presiese meganisme van inhibisie te bepaal. Molekulêre modelering en ligandbinding simulasies kan dalk die model bevestig. Dit kan ook help om beter polifenol gebaseerde inhibitore te ontwikkel. Die analitiese kapillêre elektroforese metodes skep 'n stewige basis waarop toekomstige navorsing kan voortbou. Die gepolimeriseerde katechien verbindings wat in swart tee voorkom kan dalk met die metode geskei en gekwantifiseer word.

References

Albin, M., Grossman, P.D. and Moring, S.E. (1993). Sensitivity enhancement for capillary electrophoresis. *Analytical Chemistry* **65**: 489A-497A.

Angel, P. and Karin, M. (1991). The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochimica et Biophysica Acta* **1072**: 129-157.

Aramendia, M.A., García, I., Lafont, F. and Marinas, J.M. (1995). Determination of isoflavones using capillary electrophresis in combination with electrospray mass spectrometry. *Journal of Chromatography A* **707**: 327-333.

Arce, L., Ríos, A. and Valcárcel, M. (1998). Determination of anti-carcinogenic polyphenols present in green tea using capillary electrophoresis coupled to a flow injection system. *Journal of Chromatography A* **827**: 113-120.

Arts, I.C.W. and Hollman, P.C.H. (1998). Optimization of a quantitative method for the determination of catechins in fruits and legumes. *Journal of Agricultural and Food Chemistry* **46**: 5156-5162.

Atamna, I.Z., Janini, G.M., Muschik, G.M. and Issaq, H.J. (1991). Separation of xanthines and uric acids by capillary zone electrophoresis and micellar electrokinetic capillary electrophoresis. *Journal of Liquid Chromatography* **14**: 427-435.

Aucamp, J., Gaspar, A., Hara, Y. and Apostolides, Z. (1997). Inhibition of xanthine oxidase by catechins from tea (*Camellia sinensis*). *Anticancer Research* **17**: 4381-4386.

Aucamp, J.P., Hara, Y. and Apostolides, Z. (2000). Simultaneous analysis of tea catechins, caffeine, gallic acid, theanine and ascorbic acid by micellar electrokinetic capillary chromatography. *Journal of Chromatography A*. **876**: 235-242

Ayalogu, E.O., Snelling, J., Lewis, D.F.V., Talwar, S., Clifford, M.N. and Ioannides, C. (1995). Induction of hepatic CYP1A2 by the oral adninistration of caffeine to rats: lack of association with the Ah locus. *Biochimica et Biophysica Acta* **1272**: 89-94.

Barroso, M.B. and van de Werken, G. (1999). Determination of green and black tea composition by capillary electrophoresis. *Journal of High Resolution Chromatography* **22**: 225-230.

Barthelman, M., Bair III, W.B., Stickland, K.K., Chen, W., Timmermann, B.N., Valcic, S.l., Dong, Z. and Bowden, G.T. (1998). (-)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity. *Carcinogenesis* **19**: 2201-2204.

Beck, L.H. (1981). Clinical disordes of uric acid metabolism. *Medical Clinics of North America* **65**: 401-411.

Bjergegaard, C., Michaelsen, S., Mortensen, K. and Sørensen, H. (1993). Determination of flavonoids by micellar electrokinetic capillary chromatography. *Journal of Chromatography A* **652**: 477-485.

Blanchard, J. (1981). Evaluation of the relative efficacy of various techniques for deproteinizing plasma samples prior to high-performance liquid chromatograpic analysis. *Journal of Chromatography* **226**: 455-460.

Boone, C.W., Kelloff, G.J. and Malone, W.E. (1990). Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trails: A review. *Cancer Research* **50**: 2-9.

Borris, R.P. (1996). Natural product research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacolgy* **51**: 29-38.

Bu-Abbas, A., Clifford, M.N., Walker, R. and Ioannides, C. (1994 (a)). Marked antimutagenic potential of aqueous reen tea extracts: mechanism of action. *Mutagenesis* **9**: 325-331.

Bu-Abbas, A., Clifford, M.N., Walker, R. and Ioannides, C. (1994 (b)). Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation by green tea. *Carcinogenesis* **15**: 2575-2575.

Bu-Abbas, A., Clifford, M.N., Ioannides, C. and Walker, R. (1995). Stimulation of rat hepatic UDP-glucuronosyl transferase activity following treatment with green tea. *Food and Chemical Toxicity* **33**: 27-30.

Burgi, D.S. and Chien, R.-L. (1991). Optimization in sample stacking for high-performance capillary electrophoresis. *Analytical Chemistry* **63**: 2042-2047.

Chang, W.-S., Lee, Y.-J., Lu, F.-J. and Chiang, H.-C. (1993). Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Research* **13**: 2165-2170.

Chang, W.-S. and Chiang, H.-C. (1995 (a)). Structure-activity relationship of coumarins in xanthine oxidase inhibition. *Anticancer Research* **15**: 1969-1974.

Chang, W.-S., Yan, G.-F. and Chiang, H.-C. (1995 (b)). Inhibitory effects of phenolic carboxylic acid analogues on xanthine oxidase. *Anticancer Research* **15**: 2097-2100.

Chang, W.-S., Wen, P.-C. and Chiang, H.-C. (1995 (c)). Structure-activity relationship of caffeic acid analogues on xanthine oxidase. *Anticancer Research* **15**: 703-708.

Chen, N. and Shigeru, T. (1995 (a)). A quantitative study on the effect of organic modifiers in micellar electrokinetic chromatography. *Electrophoresis* **16**: 2100-2103.

Chen, N., Shigeru, T. and Terumichi, N. (1995 (b)). Effect of organic modifier concentrations on electrokinetic migrations in micellar electrokinetic chromatography. *Electrophoresis* **16**: 1457-1462.

Chen, L., Lee, M.-J., Li, H. and Yang, C.S. (1997). Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metabolism and Disposition* **25**: 1045-1050.

Chen, W., Dong, Z., Valcic, S., Timmermann, B.N. and Bowden, G.T. (1999). Inhibition of ultaviolet B-induced c-fos gene expression and p38 mitogen-activated protein kinase activation by (-)-epigallocatechin gallate in human keratinocyte cell line. *Molecular Carcinogenesis* **24**: 79-84.

Cheng, S.J., Ho, C.T., Lou, H.Z., Bao, Y.D., Jian, Y.Z., Li, M.H., Gao, Y.N., Zhu, G.F., Bai, J.F., Guo, S.P. and Li, X.Q. (1986). A preliminary study on the antimutagenicity of green tea antioxidants. *Acta Biologica Experimentia Sinica* **19**: 427-431.

Chien, R.-L. and Burgi, D.S. (1992). On-column sample concentration using field amplification in CZE. *Analytical Chemistry* **64**: 489A-496A.

Chung, F.-L., Wang, M., Rivenson, A., Iatropoulos, M.J., Reinhardt, J.C., Pittman, B., Ho, C.-T. and Amin, S.G. (1998). Inhibition of lung carcinogenesis by black tea in Fischer rats treated with a tobacco-specific carcinogen: Caffeine as an important constituent. *Cancer Research* **58**: 4096--4101.

Cole and Lehotay (1998). HPLC method for methionine and homocysyeine. *Clinical Chemistry* **44**: 188-190.

Conney, A.H., Wang, Z.-Y., Huang, M.-T., Ho, C.-T. and Yang, C.S. (1992). Inhibitory effect of green tea on tumorigenesis by chemicals and ultraviolet light. *Preventive Medicine* **21**: 361-369.

Conney, A.H., Lu, Y.-P., Lou, Y.-R., Xie, J.-G. and Huang, M.-T. (1999). Inhibitory effect of green and black tea on tumor growth. *Proceedings of the Society of Experimental Biology and Medicine* **220**: 229-233.

Corte, E.D. and Stirpe, F. (1972). The regulation of rat liver xanthine oxidase. Involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme. *Biochemical Journal* **126**: 739-45.

Costantino, L., Albasini, A., Rastelli, G. and Benvenuti, S. (1992). Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Medica* **58**: 342-344.

Cutler, R.G. (1984). Antioxidnts, aging and longevity. Orlando, Academic Press.

D'Arcy, P.F. (1996). Mechanisms of drug interaction. McElnay, J.C., Welling, P.G., Amidon, G.L. and D'Arcy, P.F. Berlin, Springer. **122:** 125-149.

Da Silva, E.L., Piskula, M. and Terao, J. (1998). Enhancement of antioxidative ability of rat plasma by oral administration of (-)-epicatechin. *Free Radical Biology and Medicine* **24**: 1209-1216.

Dalluge, J.J., Nelson, B.C., Thomas, J.B., Welch, M.J. and Sander, L.C. (1997). Capillary liquid chromatography/electrospray mass spectrometry for the separation and detection of catechins in green tea and human plasma. *Rapid Communications in Mass Spectrometry* **11**: 1753-1756.

Das, M., Mukhtar, H., Bik, D.P. and Bickers, D.R. (1987). Inhibition of epidermal xenobiotic metabolism in SENCAR mice by naturally occuring plant phenols. *Cancer Research* **47**: 760-766.

Dashwood, R.H., Xu, M., Herneaz, J.F., Hasaniya, N., Youn, K. and Razzuk, A. (1999). Cancer chemopreventive mechanisms of tea against heterocyclic amine mutagens from cooked meat. *Proceedings of the Society for Experimental Biology and Medicine* **220**: 239-243.

de Simón, F., Estrella, I. and Hernández, T. (1995). Flavonoid separation by capillary electrophoresis. Effect of pH and temperature. *Chromatographia* **41**: 389-392.

Diamond, H.S. and Paolino, J.S. (1973). Evidence for postsecrotory reabsorptive site for uric acid in man. *Journal of Clinical Investigation* **52**: 1491-1499.

Dong, Z., Ma, W.-Y., Huang, C. and Yang, C.S. (1997). Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-)-epigallocatechin gallate, and theaflavins. *Cancer Research* **57**: 4414-4419.

Ekborg-Ott, H.K., Taylor, A. and Armstrong, D.W. (1997). Varietal differences in the total and enantiomeric composition of theanine in tea. *Journal of Agricultural and Food Chemistry* **45**: 353-363.

Ferreres, F., Blázquez, M.A., Gil, M.I. and Tomás-Barberán, F.A. (1994). Separation of honey flavonoids by micellar electrokinetic capillary chromatography. *Journal of Chromatography A* **669**: 268-274.

Gil, M.I., Ferreres, F. and Tomás-Barberán, F.A. (1995). Micellar electrokinetic capillary chromatography of methylated flavone aglycones. *Journal of Liquid Chromatography* **18**: 3007-3019.

Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F. (1985). The pharmacological basis of therapeutics. New York, McGraw-Hill.

Goto, T., Yoshida, Y., Kiso, M. and Nagashima, H. (1996). Simultaneous analysis of individual catechins and caffeine in green tea. *Journal of Chromatography A* **749**: 295-299.

Hagerman, A.E. and Butler, L.G. (1981). The specificity of proanthocyanidin-protein interactions. *The Journal of Biological Chemistry* **256**: 4494-4497.

Haginaka, J., Wakai, J., Yasuda, H. and Nakagawa, T. (1987). Direct serum injection with micellar liquid chromatography: Chromatographic behaviour and recovery of cephalosporins. *Analytical Chemistry* **59**: 2732-2734.

Halliwell, B. and Gutteridge, J.M.C. (1989). Free radicals in biology and medicine. Oxford, Claredon Press.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Communications* **9**: 1-32.

Hara, Y. and Suzuki, T. (1989). Method for treating hypertension. United States Patent. **4**, 840,966.

Hara, Y. and Honda, M. (1990). Inhibition of alpha-amylase by tea polyphenols. *Agicultural and Biological Chemistry* **54**: 1939-1945.

Hille, R. and Stewart, R.C. (1984). The inhibition of xanthine oxidase by 8-bromoxanthine. *The Journal of Biological Chemistry* **259**: 1570-1576.

Hille, R. (1994). The reaction mechanism of oxomolybdenum enzymes. *Biochimica et Biophysica Acta* **1184**: 143-169.

Horie, H., Mukai, T. and Kohata, K. (1997). Simultaneous determination of qualitatvely important components in green tea infusions using capillary electrophoresis. *Journal of Chromatography A* **758**: 332-335.

Horie, H., Yamauchi, Y. and Kohta, K. (1998 (a)). Analysis of organic anions in tea infusions using capillary electrophoresis. *Journal of Chromatography A* **817**: 139-144.

Horie, H. and Kohata, K. (1998 (b)). Application of capillary electrophoresis to tea quality estimation. *Journal of Chromatography A* **802**: 219-223.

Horie, H., Yamasaki, Y., Yamauchi, Y. and Kohata, K. (1999). Improvement of the simultaneous analytical method for the major tea components using capillary electrophoresis. *Tea Research Journal* **87**: 59-65.

Huang, C.Y. (1979). Use of alternative substrates to probe multisubstrate enzyme mechanisms. New York, Academic Press.

Issaq, H.J. (1997). Capillary electrophoresis of natural products. *Electrophoresis* **18**:

Jankum, J., Selman, S.H., Swiercz, R. and Skrzypczak-Jankun, E. (1997). Why drinking green tea could prevent cancer. *Nature* **387**: 134-135.

Kada, T., Kaneko, K., Matsuzaki, T. and Hara, Y. (1985). Detection and and chemical identification of natural bio-antimutagens: A case of green tea factor. *Mutation Research* **150**: 127-132.

Kalckar, H.M. (1947). Differential spectrophotometry of purine compounds by means of specific enzymes. *Journal of Biological Chemistry* **167**: 429-443.

Katiyar, S.K., Rupp, C.O., Korman, N.J., Agarwal, R. and Mukhtar, H. (1995). Inhibition of 12-O-tetradecanoylphorbol-13-acetate and other skin tumor-promoter-caused induction of epidermal interleukin-1a mRNA and protein expression in SENCAR mice by green tea polyphenols. *The Journal of Investigative Dermatology* **105**: 394-398.

Khafif, A.M., Schantz, S.P.M., Al-Rawi, M.M., Edelstein, D.M. and Sacks, P.G. (1998). Green tea regulates cell cycle progression in oral leukoplakia. *Head & Neck* **74**: 528-534.

Khokhar, S., Venema, D., Hollman, P.C.H., Dekker, M. and Jongen, W. (1997). A RP-HPLC method for the determination of tea catechins. *Cancer Letters* **114**: 171-172.

King, E.L. and Altman, C. (1956). A schematic method of deriving the rate laws for enzyme-catalyzed reactions. *The Journal of Physical Chemistry* **60**: 1375-1378.

Koizumi, M., Akao, T., Kadota, S., Kikuchi, T., Okuda, T. and Kobashi, K. (1991). Enzymatic sulfation of polyphenols related to tannins by arylsulfotransferase. *Chemical, Pharmacological Bulletin* **39**: 2638-2643.

Komatsu, Y., Hisanobu, Y. and Suematsu, S. (1991). Stability of functional constituents in canned tea dinks during processing and storage. *International Symposium on Tea Science*, Shizuoka.

Landau, J.M., Wang, Z.-Y., Yang, G.-Y., Ding, W. and Yang, C.S. (1998). Inhibition of spontaneous formation of lung tumors and rhabdomyosacomas in A/J mice by black and green tea. *Carcinogenesis* **19**: 501-507.

Larger, P.J., Jones, A.D. and Dacombe, C. (1998). Separation of tea polyphenols using micellar electrokinetic chromatography with diode array detection. *Journal of Chromatography A* **799**: 309-320.

Lee, C.C., Wu, X., Gibbs, R.A., Muzny, D.M. and Caskey, T. (1988). Generation of cDNA probes directed by amino acid sequence: Cloning of urate oxidase. *Science* **239**: 1288-1291.

Lee, K.J., Heo, G.S., Kim, N.J. and Moon, D.-C. (1992). Separation of theophylline and its analogues by micellar electrokinetic chromatography: application to the determination of theophylline in human plasma. *Journal of Chromatography* **577**: 135-141.

Lee, M.J., Wang, Z.Y., Li, H., Chen, L., Sun, Y., Gobbo, S., Balentine, D.A. and Yang, C.S. (1995). Analysis of plasma and urinary tea polyphenols in human subjecs. *Cancer Epidemioloy, Biomarkers and Prevention* **4**: 393-399.

Li, K.-L. and Sheu, S.-J. (1995). Determination of flavonoids and alkaloids in the scute-coptis herb couple by capillary electrophoresis. *Analytica Chimica Acta* **313**: 113-120.

Liang, H.-R., Sirén, H., Riekkola, M.-L., Vuorela, P., Vuorela, H. and Hiltunen, R. (1996). Optimized separation of pharmacologically active flavonoids from *Epimedium* species by capillary electrophoresis. *Journal of Chromatography A* **74**: 123-129.

Liao, S. and Hiipakka, R.A. (1995). Selective inhibition of steroid 5a-reductase isoenzymes by tea epicatechin-3-gallate and epigallocatechin-3-gallate. *Biochemical and Biophsical Research Communications* **214**: 833-838.

Liu, Y.-M. and Shue, S.-J. (1994). Determination of six major flavonoids in Scutellariae Radix by micellar electrokinetic capillary electrophoresis. *Analytica Chimica Acta* **288**: 221-226.

Maiani, G., Serafini, M., Salucci, M., Azzini, E. and Ferro-Luzzi, A. (1997). Application of a new high-performance liquid chromatographic method for measuring selected polyphenols in human plasma. *Journal of Chromatography B* **692**: 311-317.

Massey, V., Komai, H., Palmer, G. and Elion, G.B. (1970). On the mechanism of Inactivation of xanthine oxidase by allopurinol and other pyrazolo[3,4-d]pyrimidines. *The Journal of Biological Chemistry* **245**: 2837-2844.

McGhie, T.K. (1993). Analysis of sugarcane flavonoids by capillary zone electrophoresis. *Journal of Chromatography A* **634**: 107-112.

Meselhy, M.R., Nakamura, N. and Hattori, M. (1997). Biotransformation of (-)-epicatechin-3-O-gallate by human intestinal bacteria. *Chemical and Pharmaceutical Bulletin* **45**: 888-893.

Michael, J.P. (1997). Quinoline, quinazoline and acridone alkaloids. [Review]. *Natural Products Reports* **14**: 11-20.

Mikkers, F.E.P., Everaerts, F.M. and Verheggen, T.P.E.M. (1979). Concentration distributions in free zone electrophoresis. *Journal of Chromatography* **169**: 11-20.

Morin, P., Archambault, J.C., André, P., Dreux, M. and Gaydou, E. (1997). Separation of hydroxylated and methoxylated flavonoids by micellar electrokinetic capillary chromatography. Determination of analyte partition coefficients between aqueous and sodium dodecyl sulfate micellar phases. *Journal of Chromatography A* **791**: 289-297.

Motojima, K. and Goto, S. (1988). A human genomic sequence highly homologous to the 3'-untranslated region of rat uricase mRNA. *Biochemical, Biophysical Research Communications* **155**: 1266-1270.

Mukhtar, H., Das, M., Khan, W.A., Wang, Z.Y., Bik, D.P. and Bickers, D.R. (1988). Exceptional activity of tannic acid among naturally occuring plant phenols in protecting against 7,12-dimethylbenz(*a*)anthracene-, benzo(*a*)pyrene-, 3-methylcholanthrene- and N-methyl-N-nitrosourea-induced skin tumorigenisis in mice. *Cancer Research* **49**: 5784-5788.

Mukhtar, H., Wang, Z.Y., Katiyar, S.K. and Agarwal, R. (1992). Tea components: Antimutagenic and anticarcinogenic effects. *Preventive Medicine* **21**: 351-360.

Mulder, G.J. and Hagedoorn, A.H. (1974). UDP-glucuronyltransferase and phenol-sulfotransferase *in vivo* and *in vitro*: Conjugation of harmol and harmalol. *Biochemical Pharmacology* **23**: 2101-2109.

Murray, N.J., Williamson, M.P., Lilley, T.H. and Haslam, E. (1994). Study of the interaction between salivary proline-rich proteins and a polyphenol by 1H-NMR spectroscopy. *The European Journal of Biochemistry* **219**: 923-935.

Nagabhushan, M., Amonkar, A.J., Nair, U.J., Santhanam, U., Ammigan, N., D'Souza, A.V. and Bhide, S.V. (1988). Catechin as an antimutagen: its mode of action. *Journal of Cancer Research and Clinical Oncology* **114**: 177-182.

Nakadate, T. (1989). The mechanism of skin tumor promotion caused by phorbol esters: Possible involvement of arachidonic acid cascde/lipoxygenase, protein kinase C and calcium/calmodulin systems. *Japanese Journal of Pharmacology* **49**: 1-9.

Nakagawa, K. and Teruo, M. (1997 (a)). Chemiluminescence-high-performance liquid chromatograpic determination of tea catechin, (-)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma. *Analytical Chemistry* **248**: 41-49.

Nakagawa, K. and Miyazawa, T. (1997 (b)). Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate, in the rat. *Journal of Nutritional Science and Vitaminology* **43**: 679-684.

Nakagawa, K., Okuda, S. and Miyazawa, T. (1997 (c)). Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human

plasma. *Bioscience, Biotechnology and Biochemistry* **6**: 1981-1985.

Nakane, H. and Ono, K. (1990). Differential inhibitory effects of some catechin deivatives on the activities of human immunodeficiency virus reverse transcriptase and cellular deoxyribonucleic and ribonucleic acid polymerases. *Biochemistry* **29**: 2841-2845.

Narisawa, T. and Fukaura, Y. (1993). A very low dose of green tea polyphenols in drinking water prevents N-methyl-N-nitrosourea-induced colon carcinogenesis in F344 rats. *Japanese Journal of Cancer Research* **84**: 1007-1009.

Nelson, B.C., Thomas, J.B., Wise, S.A. and Dalluge, J.J. (1998). The separation of green tea catechins by micellar electrokinetic chromatography. *Journal of Microcolumn Separations* **10**: 671-679.

Ng, C.L., Ong, C.P., Lee, H.K. and Li, S.F.Y. (1992). Systematic optimization of micellar elecrokinetic chromatographic separation of flavonoids. *Chromatographia* **34**: 166-172.

Obanda, M., Owuor, O. and Taylor, S.J. (1997). Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *Journal for Science of Food and Agriculture* **74**: 209-215.

Okamoto, K. and Nishino, T. (1995). Mechanism of inhibition of xanthine oxidase with a new tight binding inhibitor. *The Journal of Biological Chemistry* **270**: 7816-7821.

Okushio, K., Matsumoto, N., Khori, T., Suzuki, M., Nanjo, F. and Hara, Y. (1996). Absortion of tea catechins into rat portal vein. *Biological and Pharmaceutical Bulletin* **19**: 326-329.

Orter, J.M. and Neuhaus, O.W. (1982). Human Biochemistry. St. Louis, The C.V. Mosby Company.

Osada, Y., Tsuchimoto, M., Fukushima, H., Takahashi, K., Kondo, S., Hasegawa, M. and Komoriya, K. (1993). Hypouricemic effect of the novel xanthine oxidse inhibitor, TEI-6720, in rodents. *European Journal of Pharmacology* **241**: 183-188.

Otake, S., Makimura, M., Nishihara, Y. and Hirasawa, M. (1991). Anticaries effects of polyphenolic compounds from Japanese green tea. *Caries Research* **25**: 438-443.

Palmisano, F., Guerrieri, A. and Zambonin, P.G. (1989). Determination of methotrexate in untreated body fluids by micellar liquid chromatography. *Analytical Chemistry* **61**: 946-950.

Perchellet, J. and Perchellet, E.M. (1989). Antioxidants and multistage carcinogenesis. *Free Radical Biology and Medicine* **7**: 377-408.

Pietta, P.G., Mauri, P., Rava, A. and Sabbatini, G. (1991). Application of micellar electrokinetic capillary chromatography to the determionation of flavonoid drugs. *Journal of Chromatography* **549**: 367-373.

Pietta, P.G., Mauri, P.L., Zini, L. and Gardana, C. (1994). Optimization of separation selectivity in capillary electrophoresis of flavonoids. *Journal of Chromatography A* **680**: 175-179.

Pietta, P.G., Simonetti, P., Gardana, C., Brusamolino, A., Morazzoni, P. and Bombardelli, E. (1998). Catechin metabolites after intake of green tea infusions. *Biofactors* **8**: 111-118.

Piskula, M.K. and Terao, J. (1998). Accumulation of (-)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. *Journal of Nutrition* **128**: 1172-1178.

Radi, R., Tan, S., Pradanov, E., Evans, R.A. and Parks, D.A. (1992). Inhibition of xanthine oxidase by uric acid and its influence on superoxide radical production. *Biochimica et Biophysica Acta* **1122**: 178-182.

Rajagopalan, K.V. (1991). Novel aspects of the biochemistry of the molybdenum cofactor. *Advances in Enzymology and Related Areas of Molecular Biology* **64**: 215-290.

Rice-Evans, C. and Miller, N.J. (1994). Total antioxidant status in plasma and body fluids. *Oxygen Radicals in Biological Systems Part D.* Packer, L. New York, Academic Press Inc. **234:** 279-293.

Rice-Evans, C.A., Miller, N.J.l. and Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology & Medicine* **20**: 933-956.

Romão, M.J., Archer, M., Moura, I., José, J.G., LeGall, J., Engh, R., Schneider, M., Hof, P. and Huber, R. (1995). Crystal structure of the xanthine oxidase-related aldehyde oxido-reductase from *D. gigas*. *Science* **270**: 1170-1176.

Romão, M.J. and Huber, R. (1998). Structure and function of the xanthine-oxidase family of molybdenum enzymes. *Structure and Bonding* **90**: 69-95.

Röder, A. and Bächmann, K.J. (1995). Simultaneous determination of organic and inorganic anions in the sub-umol/l range in rain water by capillary zone electro-phoresis. *Journal of Chromatography A* **689**: 305-311.

Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P. and Rice-Evans, C. (1995). Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-

breaking antioxidants. *Archive in Biochemistry and Biophysics* **322**: 339-346.

Sazuka, M., Murakami, S., Isemura, M., Satoh, K. and Nukiwa, T. (1995). Inhibitory effects of green tea infusions on in vitro invasion and in vivo metastasis of mouse lung carcinoma cells. *Cancer Letters* **98**: 27-31.

Sazuka, M., Imazawa, H., Shoji, Y., Mita, T., Hara, Y. and Isemura, M. (1997). Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. *Bioscience, Biotechnology and Biochemistry* **61**: 1504-1506.

Schmutz, A. and Thorman, W. (1994). Factors affecting the determination of drugs and endogenous low molecular mass compounds in human serum by micellar electrokinetic capillary chromatography with direct sample injection. *Electrophoresis* **15**: 51-61.

Seitz, U., Bonn, G., Oefner, P. and Popp, M. (1991). Isotachophoretic analysis of flavonoids and phenolcarboxylic acids of relevance to phytopharmaceutical industry. *Journal of Chromatography* **559**: 499-504.

Seitz, U., Oefner, P.J., Nathakarnkitkoo, l.S., Popp, M. and Bonn, G.K. (1992). Capillary electrophoretic analysis of flavonoids. *Electrophoresis* **13**: 35-38.

Sepaniak, M.J., Swaile, D.F., Powell, C.A. and Cole, R.O. (1990). Capillary electrokinetic separations: Influence of mobile phase composition on performance. *Journal of High Resolution Chromatography* **13**: 679-682.

Seto, R., Nakamura, H., Nanjo, F. and Hara, Y. (1997). Preparation of epimers of tea catechins by heat treatment. *Bioscience, Biotechnology, and Biochemistry* **61**: 1434-1439.

Sheu, S.-J. and Chen, H.-R. (1995). Simultaneous determination of twelve constituents of I-tzu-tang, a Chinese herbal preparation, by high-performance liquid chromatography and capillary electrophoresis. *Journal of Chromatography A* **704**: 141-148.

Shi, S.T., Wang, Z.-Y., Smith, T.J., Hong, J.-Y., W-F., C., Ho, C.-T. and Yang, C.S. (1994). Effects of green tea and black tea on 4-(methylnitroamino)-1-(3-pyridyl-)-1-butanone bioactivation, DNA methylation, and lung tumorigenesis in A/J mice. *Cancer Research* **54**: 4641-4647.

Shihabi, Z.K., Hinsdale, M.E. and Bleyer, A.J. (1995). Xanthine analysis in biological fluids by capillary electrophoresis. *Journal of Chromatography B* **669**: 163-169.

Shihadi, F. and Wanasundra, P.K.J. (1992). Phenolic antioxidants. *Critical Reviews in*

Food Science and Nutrition **32**: 67-103.

Simic, M.G. and Jovanovic, S.V. (1989). Antioxiation mechanisms of uric acid. *Journal of the American Chemical Society* **111**: 5778-5782.

Snyder, L.R. (1978). Classification of the solvent properties of common liquids. *Journal of Chromatographic Science* **16**: 223-234.

Sohn, O.S., Surace, A., Fiala, E.S., Richie Jr., J.P., Colosimo, S., Zang, E. and Weisburger, J.H. (1994). Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. *Xenobiotica* **24**: 119-127.

Springer, R.H., Dimmitt, M.K., Novinson, T., O'Brien, D.E., Robins, R.K., Simon, L.N. and Miller, J.P. (1976). Synthesis and enzymic activity of some novel xanthine oxidase inhibitors. 3-Substituted 5,7-dihydroxypyrazolo[1,5-a]pyrimidines. *Journal of Medicinal Chemistry* **19**: 291-296.

Steele, C.M., Lalies, M. and Ioannides, C. (1985). Inhibition of the mutagenicity of aromatic Amines by the plant flavonoid (+)-catechin. *Cancer Research* **45**: 3573-3577.

Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H. and Fujiki, H. (1998). Wide distribution of [^{3}H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* **19**: 1771-1776.

Terabe, S., Otsuka, K. and Ando, T. (1985). Electrokinetic chromatography with micellar solution and open-tubular capillary. *Analytical Chemistry* **57**: 834-841.

Thomas, M.J. (1992). Urate causes the human polymorphonuclear leukocyte to secrete superoxide. *Free Radical Biology and Medicine* **12**: 89-91.

Thormann, W., Minger, A., Molteni, S., Caslavska, J. and Gebauer, P. (1992). Determination of substituted purines in body fluids by micellar electrokinetic capillary chromatography with direct sample injection. *Journal of Chromatography* **593**: 275-288.

Tsuchiya, H., Sato, M., Kato, H., Okubo, T., Juneja, J.R. and Kim, M. (1997). Simultaneous determination of catechins in human saliva by high-performance liquid chromatography. *Journal of Chromatography B* **703253-258**: 253-258.

Tsuchiya, H., Sato, M., Kato, H., Kureshiro, H. and Takagi, N. (1998). Nanaoscale analysis of pharmacologically active catechins in body fluids by HPLC using borate complex extraction pretreatment. *Talanta* **46**: 717-726.

van Agtmael, M.A., Eggelte, T.A. and van Boxtel, C.J. (1999). Artemisinin drugs in the

treatment of malaria: from medicinal herb to registered medication. [Rview]. *Trends in Pharmacological Science* **20**: 199-205.

van Guldner, C., Janssen, M.J., de Meer, K. and Donker, A.J. (1999). Effect of folic acid and betaine on festing and post-methionine loading plasma homocysteine and methionine. *Journal of Internal Medicine* **245**: 175-183.

VanOrman, B.B., Liversidge, G.G. and McIntire, G.L. (1990). Effects of buffer composition on electroosmotic flow in capillary electrophoresis. *Journal of Microcolumn Separations* **2**: 176-180.

Vinther, A. and Søeberg, H. (1991). Mathematical model describing dispersion in free solution capillary electrophoresis under stacking conditions. *Journal of Chromatography* **559**: 3-26.

Wang, Z.Y., Cheng, S.J., Zhou, Z.C., Athar, M., Khan, W.A., Bickers, D.R. and Mukhtar, M. (1989 (a)). Antimutagenic activity of green tea polyphenols. *Mutation Research* **223**: 273-285.

Wang, Z.Y., Khan, W.A., Bickers, D.R. and Mukhtar, H. (1989 (b)). Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis* **10**: 411-415.

Wang, Z.Y., Zhou, Z.C., Bickers, D.R. and Mukhtar, H. (1990). Inhibition of chemical and photocarcinogenesis in murine skin by green tea polyphenols. *Procceedings of the American Association of Cancer Research* **31**: 945A.

Wanmg, Z.Y., Das, M., Bickers, D.R. and Mukhtar, H. (1988). Interaction of epicatechins derived from green tea with rat hepatic cytochrome P-450. *Drug Metabolism and Disposition* **16**: 98-103.

Watanabe, T., Nishiyama, R., Yamamoto, A., Nagai, S. and Terabe, S. (1998). Simultaneous analysis of individual catechins, caffein, and ascorbic acid in commercial canned green and black teas by micellar electrokinetic chromatography. *Analytical Sciences* **14**: 435-438.

Wauters, P., Eeckhout, Y. and Vaes, G. (1986). Oxidation products are responsible for the resistance to the action of collagenase conferred on collagen by (+)-catechin. *Biochemical Pharmacology* **35**: 2971-2973.

Wayner, D.D.M., Burton, G.W., Ingold, K.W., Barclay, L.R.C. and Locke, S.J. (1987). The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochimica Biophysica Acta* **924**: 408-419.

Wätzig, H., Degenhardt, M. and Kunkel, A. (1998). Strategies for capillary electrophoresis: Method development and validation for pharmaceutical and biological applications. *Electrophoresis* **19**: 2695-2752.

Welsch, C.A., Lachane, P.L. and Wasserman, B.P. (1989). Effects of native and oxidized phenolic compounds on sucrase activity in rat brush border membrane vesicles. *Journal of Nutrition* **119**: 1737-1740.

White, G.J. (1981). Inhibition of oxidative enzymes by anti-allergy drugs. *Agents and Actions* **11**: 503-509.

Wilson, A. and Clifford, M.N. (1992). Tea: Cultivation to consumption. London, Chpman & Hall.

Wiseman, S.A., Balentine, D.A. and Frei, B. (1997). Antioxidants in tea [Review]. *Critical Reviews in Food Science and Nutrition* **37**: 693-704.

Wyngaarden, J.B. (1974). Metabolic defects in primary hyperuricemia and gout. *American Journal of Medicine* **56**: 651-664.

Xu, D.-K., Hua, L., Li, Z.-M. and Chen, H.-Y. (1997). Identification and quantitative determination of uric acid in human urine and plasma by capillary electrophoresis with amperometric detection. *Journal of Chromatography B* **694**: 461-466.

Yang, C.S., Smith, T.J. and Hong, J.-Y. (1994). Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: Opportunities and limitations. *Cancer Research* **54**: 1982s-1986s.

Yang, C.S., Kim, S., Yang, G.-Y., Lee, M.-J., Liao, J., Chung, J.Y. and Ho, C.-T. (1999). Inhibition of carcinogenesis by tea: Bioavaiability of tea polyphenols and mechanisms of action. *Proceedings of the Society of Experimental Biology and Medicine* **220**: 213217.

Yeldandi, A.V., Patel, Y.D., Liao, M., Kao, F.T., Rao, M.S., Reddy, J.K. and Le Beau, M.M. (1992). Localization of the human urate oxidase gene (UOX) to 1p22. *Cytogenetics and Cell Genetics* **61**: 121-122.

Zhang, K. and Das, N.P. (1994). Inhibitory effects of plant polyphenols on rat liver glutathione S-transferase. *Biochemical Pharmacology* **47**: 2063-2068.

Zhu, B.T., Taneja, N., Loder, D.P., Balentine, D.A. and Conney, A.H. (1998). Effects of tea polyphenols and flavonoids on liver microsomal glucuronidation of estradiol and estrone. *Journal of Steroid Biochemistry and Molecular Biology* **64**: 207-215.