Rapid Selection of Polyphenol-rich Tea Trees (Camellia sinensis L.) Employing a Colorimetric Method

Heo, Chang-Mi
Division of Environment Forest Science, Gyeongsang National University (Institute of Agriculture of Life Science)

Kim, Yong-Duck
Institute of Hadong Green Tea | Division of Environment Forest Science, Gyeongsang National University (Institute of Agriculture of Life Science)

Oh, Taek-Keun
Science for Bioproduction Environment, Faculty of Agriculture, Kyushu University

Min, Ji-Yun
Sancheong Oriental Medicinal Herb Institute

他

https://doi.org/10.5109/25207

出版情報：九州大学大学院農学研究院紀要. 57 (2), pp.467-471, 2012-09-20. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **57** (2), 467–471 (2012)

Rapid Selection of Polyphenol–rich Tea Trees (*Camellia sinensis* L.) Employing a Colorimetric Method

Chang–Mi HEO[1,†], Yong–Duck KIM[2,†], Taek–Keun OH[3,†], Ji–Yun MIN[4], Jung–Gyu HWANG[2], Mi–Jin JEONG[1], Hyun–Jin SONG[1], Hak–Gon KIM[1], Seon–Jeong SIM[1], Myung–Suk CHOI[1], Chandrakant S. KARIGAR[5], Hyun–Shik MOON[1,*], Seung–Joo LEE[6] and Yoshiyuki SHINOGI

Laboratory of Irrigation and Water Management, Division of Bioproduction Environmental Sciences, Department of Agro–Ervironmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
(*Received April 27, 2012 and accepted May 10, 2012*)

An efficient and rapid colorimetric method was established for the selection of tea tree lines rich in polyphenol from 160 tea tree lines. The efficient selection method was made possible by the adoption of the Folin–Ciocalteu (FC) colorimetric method for qualitative detection of the polyphenols. The polyphenol composition varied widely among the randomly sampled 160 tea tree lines. The tea tree lines were classified into three groups on the basis of color intensity obtained after treatment of tea extracts with FC reagent. The polyphenol content of the three groups was quantified and validated by UV spectrophotometric analysis. Among various tea lines, H–23 tea line exhibited the highest amounts of polyphenol that was 2.89–fold higher than those found in low polyphenol producing tea tree (H–9). Hence FC colorimetric method can become a reliable selection method for easy and rapid selection of tea population rich in polyphenol in a tea tree breeding programme.

Keywords: *Camellia sinensis*, Folin–Ciocalteu colorimetric method, polyphenol, selection

INTRODUCTION

Tea tree (*Camellia sinensis* L.) extract is consumed by a very large population worldwide, and its health effects are an important topic for scientific investigation (Chung *et al*., 1998). Green tea consists of polysaccharides, flavonoids, vitamins B, C, E, caffeine, catechin compounds and fluoride (Kyung and Yinzhe, 2006). Specially, the green tea polyphenols has been emerging as one major category of natural products that is important to human health. Polyphenol compounds are good antioxidants, are effective in preventing cardiovascular, inflammatory, coronary heart diseases and can also be used as chemopreventing agents for cancer (Frankel *et al*., 1993; Yang and Wang, 1993; Rice *et al*., 1996; Chen *et al*., 1998; Otsuka *et al*., 1998; Yang *et al*., 1998; Mukhtar and Ahmad, 1999, 2000; Khan and Mukhtar, 2007). In general, green tea polyphenol compounds, commonly known as catechins, caffeine and tannin. It is caused by with like this positive effect and polyphenols the interest regard in is augmented.

Conventional tea tree breeding is well established, though time consuming and labor intensive due to its perennial nature and long gestation period (Mondal *et al*., 2004). Additionally, tea breeding has been slow due to lack of reliable selection criteria (Islam *et al*., 2005). Particularly, the studies concerning the selection and breeding of tea tree containing valuable plant secondary metabolites are scanty. Therefore, the development of methods for the rapid selection of polyphenol– rich tea trees is an important strategy for breeding new cultivars. In earlier studies, bioactive compounds from various plant sources have been determined employing several methods like, high performance liquid chromatography (Ryoyasu *et al*., 1999), and gas–chromatography or capillary electrophoresis (Mikkers *et al*., 1979; Choi *et al*., 2005). However such methods are cumbersome due to the involved complicated procedures. Hence, reliable analytical methods suitable for rapid selection of tea–tree rich in polyphenol are required. In this study, we report an efficient method for the selection of polyphenol–rich individual tea plants among Hadong tea tree population. Also, also report the other bioactive compounds found in polyphenol–rich individual tea trees.

MATERIALS AND METHODS

Plant materials

The samples of tea trees used in this experiment were collected from specimen plantation in the Hadong region,

[1] Division of Environment Forest Science, Gyeongsang National University (Institute of Agriculture of Life Science), Jinju 660–701, South Korea
[2] Institute of Hadong Green Tea, Hadong 667–804, South Korea
[3] Science for Bioproduction Environment, Faculty of Agriculture, Kyushu University, Hakozaki 6–10–1, Higashi–ku, Fukuoka city 812–8581, Japan
[4] Sancheong Oriental Medicinal Herb Institute, Sancheong 666–831, South Korea
[5] Department of Biochemistry, Bangalore University, Bangalore 560001, India
[6] Department of Biosystem Machinery Engineering, Chungnam National University, Daejeon 305–764, South Korea
[†] These two authors contributed equally to this work and should be considered co–first authors
* Corresponding author (E–mail: hsmoon@gnu.ac.kr)

South Korea in May 2007. Folin–Ciocalteu's reagent and polyphenol standard for quantitative analysis were purchased from Sigma–Aldrich (USA). All other solvents and chemicals used analytical grade. To screen of high polyphenol containing tea tree, 160 different tea tree lines were randomly chosen for investigation from the region. After sampling, the leaves and stems were stored at –70°C until further use.

Preparation of extracts from tea tree leaves

The tea tree leaves (500 mg) were homogenized and extracted with 8 mL of distilled water in water bath (ANALAB KSB–201) maintained at 80°C for 30 min. After cooling at room temperature, the extracts were made up to 10 mL with distilled water and filtered (filter paper No 2, Advantec) and subjected to UV spectrophotometric analysis. The resulting filtrate was further extracted twice with 10 mL of ethyl acetate and evaporated under vacuum. The dry residue obtained was dissolved in 500 μL of ethyl acetate, filtered through a pre–filter (Ψ 0.2 μm, Supelco) and subjected to colorimetric and HPLC analysis.

Establishment of colorimetric method

Total phenolic contents were analyzed by the Folin–Ciocalteu (FC) colorimetric method using gallic acid as a standard. Extract sample of 0.1 mL was added with 0.1 mL FC reagent. After 3 min, 0.1 mL of 10% sodium carbonate solution was added with mixing. The mixture was diluted to 2 mL with distilled water. After incubation for 60 min at room temperature and the absorbance was measured at 760 nm using a spectrophotometer. Total polyphenolic content was expressed as gallic acid equivalents in mg 100 g^{-1} sample.

Quantitative analysis of caffeine and catechins in selected tea tree lines

The quantitative analysis of catechins in selected tea tree lines was carried out by HPLC analysis. HPLC analysis of samples was conducted as described previously (Kim *et al.*, 2007). A filtrate sample was introduced into a HPLC system (Dionex, USA) equipped with TSK gel ODS–80Ts (10 μm, 4.5×250 mm, Tosoh) column and UV detector (Dionex, Ultimate 3000). The solvents were (A) 0.2% phosphoric acid, containing H_2O and (B) acetonitrile. The elution system was as follows: 0–7 min, 85:15 of B; 7–15 min, 100:0 of B; 15–20 min, 85:15 of B. The flow rate was 1 mL min^{-1}, the injection volume was 10 μL, and the column over was set at 40°C. The eluent was monitored at 280 nm. After the injection of 20 μL of the tea extracts, the column was operated with a flow rate of 0.5 mL min^{-1}. Quantitative analysis of catechins was achieved by co–chromatogram of the standards and samples and by comparison of the retention times. The samples for HPLC were selected from the primary screening of tea trees employing FC colorimetric method.

RESULTS AND DISCUSSION

Establishment of colorimetric assay

The color obtained from extracts of tea leaves and standard gallic acid increased correspondingly in commensurate with polyphenol concentrations. The results showed a high correlation coefficient (extract : r = 0.980, gallic acid : r = 0.984) to that of the obtained values from measurements using the UV spectrophotometer and colorimetric methods (Fig. 1). The development of dark blue color of the mixture was taken as a positive test for the presence of polyphenol in the tea extract (Fig. 2). The color intensity of the tea extract increased proportionally with the FC reagent mixture. The correlation between the polyphenol contents determined with FC

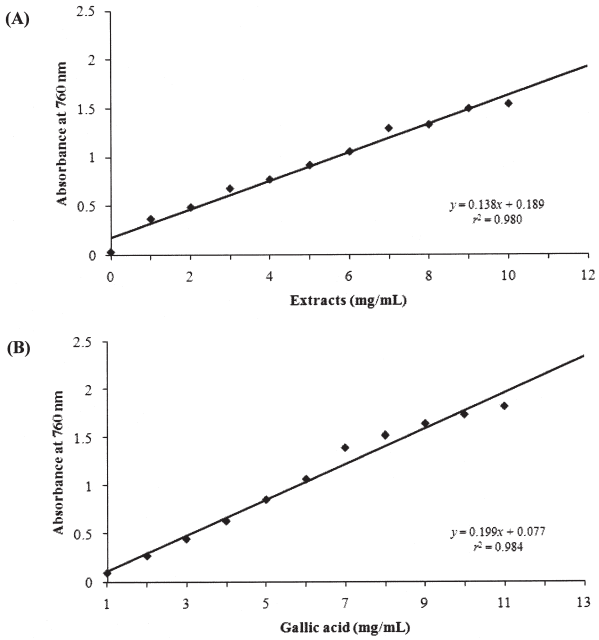

Fig. 1. (A) Determination of polyphenols from tea extracts; (B) Gallic acid standard curve by UV spectrophotometric analysis.

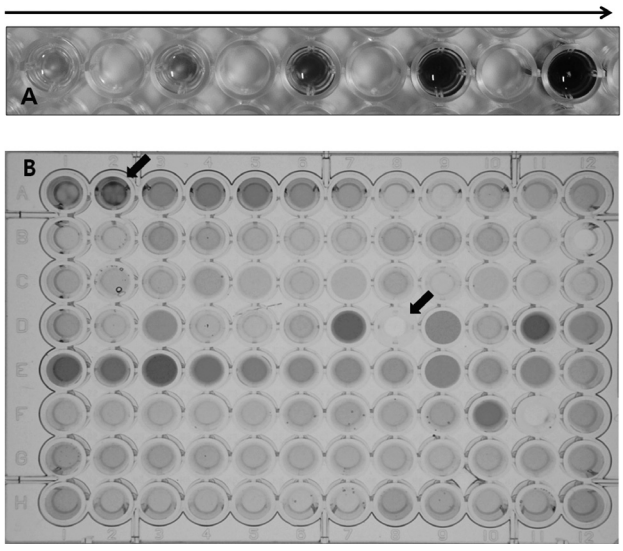

Fig. 2. Screening of tea trees for polyphenol levels by FC colorimetric method. (A) Standard gallic acid; (B) Tea extract.

colorimetric method were compared with UV spectrometric method using gallic acid as standard compound. The FC colorimetric reagent reacts with phenolic compounds (Singleton *et al*., 1999), and has been used for many years to quantify total phenolics in natural products (Geroge *et al*., 2005). However, application this method for tea tree breeding has not been reported.

Selection of polyphenols–rich tea tree lines by colorimetric method

The polyphenols in tea tree lines were screened according to a previously established FC colorimetric method for phenol determination. The reagent was not only helpful in the detection of polyphenols but also formed concentration dependent patterns for extracts from different tea tree lines (Fig. 3). This result suggests that the FC colorimetric method is of practical value for the efficient selection of polyphenols–rich tea tree lines. The mean polyphenol content among 160 individual trees was noted to be 499 mg g^{-1} FW.

Five individual lines of tea trees were selected and grouped as polyphenols–rich, moderate, and poor tea trees. Thereafter, their polyphenol composition was quantified by UV spectrophotometer analysis. Tea trees that were rich in polyphenols contained 518 to 548 mg 100 g^{-1} FW, while low polyphenol contents in polyphenol–poor tea tree lines had 188 to 227 mg 100 g^{-1} FW. The contents of polyphenol in tea tree line (HR–23) a rich polyphenol–rich group was 548 mg 100 g^{-1} FW and polyphenol poor tea tree line (HP–9) was 188 mg 100 g^{-1} FW. Among them, the HR–23 line had the highest contents of polyphenol, which was 3.5–fold higher than those of the lowest polyphenol–producing tea tree (HP–9). The mean polyphenol content among high producing group (5 lines) was 532 mg g^{-1}, moderate polyphenol producing group (5 lines) was 374 mg 100 g^{-1} and polyphenol content in low polyphenol group (5 lines) showed 205 mg 100 g–1 fresh weight (Fig. 4).

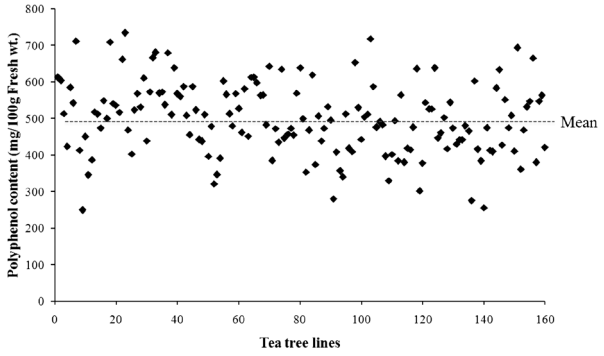

Fig. 3. Variation of total phenols in 160 Hadong tea trees.

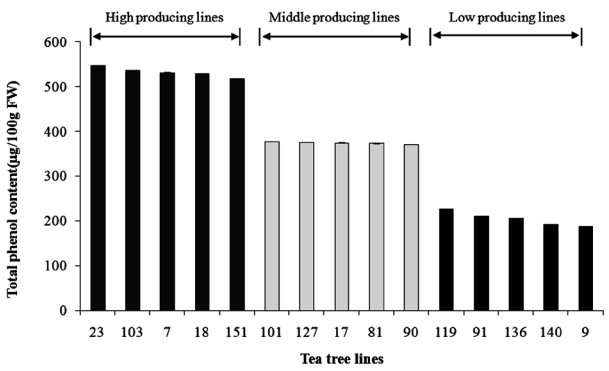

Fig. 4. Comparative levels of polyphenols (high, moderate and poor) in selected tea tree lines.

Correlationship of polyphenols, catechin and caffeine in selected polyphenol rich tea trees

Caffeine contents did not correlate significantly with the polyphenol lines in selected tea tree lines (Fig. 5). The high level of caffeine content was observed in polyphenol–poor lines, but polyphenol–rich tea lines were low in caffeine. On the basis of the catechin levels, the FCG contents not the EC contents, were higher similar with the polyphenol levels (Fig. 6). In general the polyphenol–rich tea tree lines produced high level of catechins.

This study thus established an efficient selection of tea trees rich in polyphenol through FC colorimetric method. These results indicate that the FC method is a reliable method for screening and selection of high polyphenol containing tea trees. A wide variation in polyphenol contents was observed, which ranged from was 189 to 548 mg g^{-1} fresh weight among the Hadong tea tree population. This result suggests that colorimetric methods would be of handy help in selection of individuals among Hadong tea tree population based on the polyphenols. The wild type tea trees that grow indigenously in the Hadong region have wide genetic variation. These were introduced to the Korean mainland before millennium and not bred. Thus, tree cultivars of this region are valuable genetic resources for breeding new tea cultivars. The objectives for breeding of tea tree have been changing from high yield to high quality then to diverse objectives such as high quality, high efficiency, high functional components and high tolerance to stress (Chen *et al*., 2007). The patterns of intra specific variation in composition and concentration of secondary metabolites among plants from various geographic regions

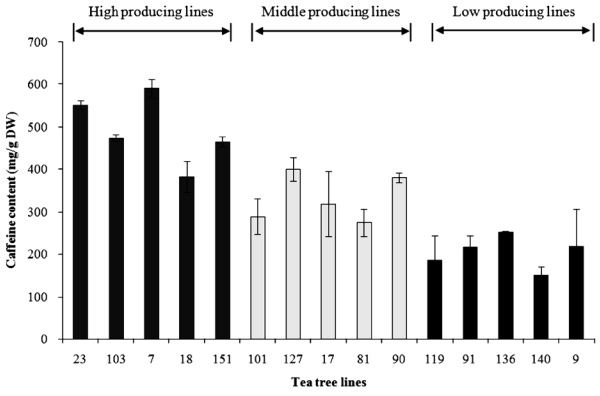

Fig. 5. Determination of caffeine in selected tea tree lines by HPLC. The bars represent the standard error of mean (n=3).

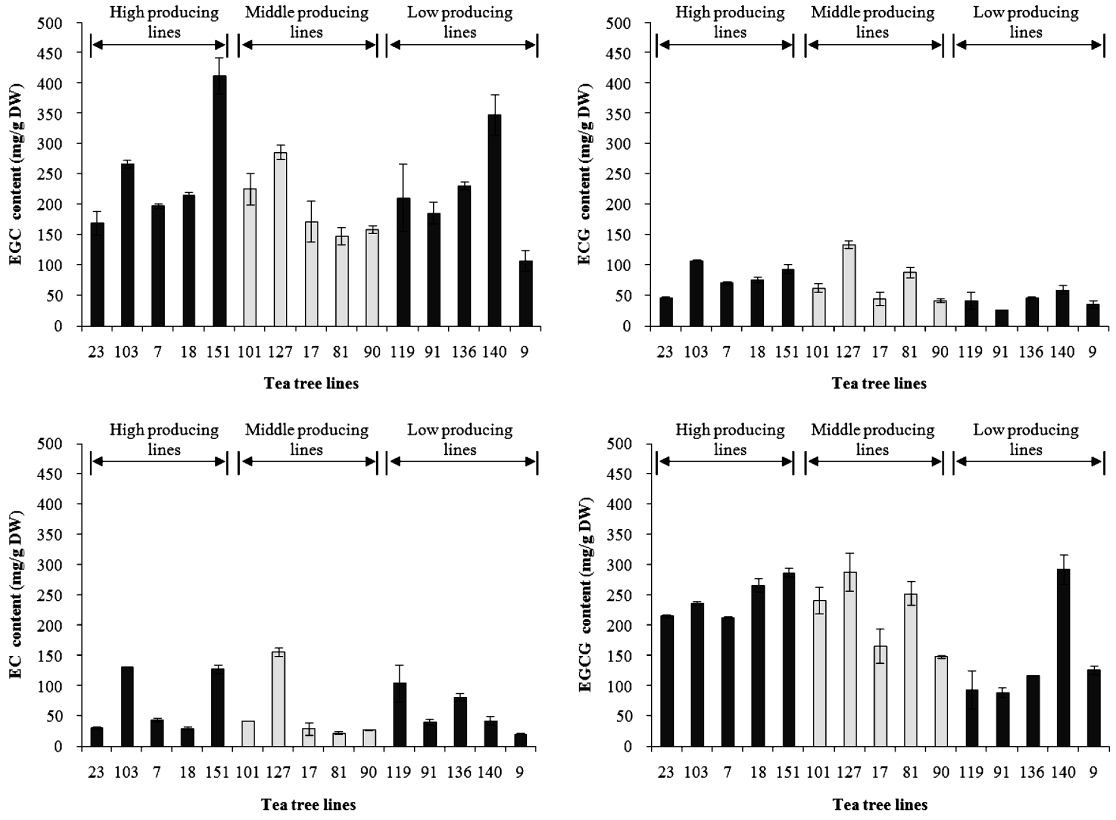

Fig. 6. Estimation of catechins in the extracts selected tea tree lines by HPLC. The bars represent the standard error of mean (n=3).

or habitats have been well documented for terrestrial plants (Gershenzon and Groteau, 1991).

Traditional selection or breeding methods for tea tree have relied on a combination of morphological characteristics, which are somewhat empirical and slow, and laborious to assess (Owuor and obanda, 2007). Conventional tea tree breeding takes an average of 20–25 years to breed a tea variety from mating to registration, valuable tea tree breeding needs to develop efficient selection method. The advancing tea tree breeding techniques like colorimetric selection methods will benefit the current and future tea tree breeding programs. Thus through classic and modern breeding methods, a new tea tree species can be developed effectively using genetically diverse wild–type Hadong tea trees.

CONCLUSIONS

This study elucidated an efficient selection procedure to obtain polyphenol–rich tea trees by an colorimetric method. Polyphenol–rich tea trees had increased levels compared to polyphenol–poor trees of 2.89–fold for polyphenol. And, among the selected tea tree, caffeine and catechins content analysis showed that the kind of significance. This research is expected that the selection period of superior tea tree variety can be shortened. And it is thought that its competitiveness can be externally secured by selecting and breeding tea tree with high biological active compounds. However, screening for individual trees with high biological active compounds among tea tree population has not been reported. Therefore the development of colorimetric method for selection of high biological active compounds containing tea trees is an important strategy for breeding new and beneficial cultivars.

ACKNOWLEDGMENT

This work was supported by the Institute of Hadong Green Tea.

REFERENCES

Chen L., Z. X. Zhou and Y. J. Yang 2007 Genetic improvement and breeding of tea plant (*Camellia sinensis*) in China: from individual selection to hybridization and molecular breeding. *Euphytica*, **154**: 239–248

Choi O. K., Y. S. Chung and H. Y. Chung 2005 Capillary electrophoretic method for the determination of (+)–catechin, (–)–epicatechin in grape seed ethanol extract. *Korean Society of Food Science and Technology*, **37**: 513–518 (in Korean)

Chung K. T., T. Y. Wong, C. I. Wei, Y. W. Huang and Y. Lin 1998 Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition*, **38**: 421–464

Frankel E. N., J. Kanner, J. B. German, E. Parks and J. E. Kinsella 1993 Inhibition of oxidation of human low–density lipoprotain by phenolic substance in red wine. *The Lancet*, **341**: 454–457

George S., P. Brat, P. Alter and M. J. Amiot 2005 Rapid determination of polyphenols and vitamin C in plant–derived products. *J. Agricultural and Food Chemistry*, **53**: 1370–1373

Gershenzon J. and R. Croteau 1991 Terpenoids. *In* "Herbivores:

their Interactions with secondary plant metabolites", Vol. I, ed. by G. A. Rosenthal and M. R. Berenbaum, Academic press Inc., San Diego, pp. 165–219

Islam G. M. R., M. Iqbal, K. G. Quddus and M. Y. Ali 2005 Present status and future needs of tea industry in Bangladesh. *Proceedings of the Pakistan Academic of Sciences*, **42**: 305–314

Khan N. and H. Mukhtar 2007 Tea polyphenols for health promotion. *Life Sciences*, **81**: 519–533

Kim Y. D., J. Y. Min, C. S. Karigar, G. W. Cheong, J. W. Kim and M. S. Choi 2007 Rapid screening and selection of low–caffeine–contentaining tea (*Camellia sinensis*) trees by a colorimetric method. *Plant Breeding*, **126**: 634–637

Kyung H. R. and J. Yinzhe 2006 Recovery of catechin compounds from Korean tea by solvent extraction. *Bioresource Technology*, **97**: 790–793

Mikkers F. E. P., F. M. Everaerts and T. P. E. M. Verheggen 1979 High–performance zone electrophoresis. *Chromatographia*, **169**: 11–20

Mondal, T. K., A. Bhattacharya, M. Laxmikumaran and P. S. Ahuja. 2004. Recent advances of tea (*Camellia sinensis*) biotechnology. *Plant Cell Tissue Organ Culture*, **76**: 195–254

Mukhtar H. and N. Ahmad 1999 Cancer chemoprevention: future holds in multiple agents. *Toxicology and Applied Pharmacology*, **158**: 207–210

Mukhtar H. and N. Ahmad 2000 Tea polyphenol: prevention of cancer and optimizing health. *The American J. Clinical Nutrition*, **71**: 1698–1702

Otsuka T., T. Ogo, T. Eto, Y. Asano, M. Suganuma and Y. Niho 1998 Growth inhibition of leukemic cells by (–)epigallocatechin gallate, the main constituent of green tea. *Life Sciences*, **63**: 1397–1403

Owuor P. O and M. Obanda 2007 The use of fresh tea (*Camellia sinensis*) leaf flavan–3–ol composition in predicting plain black tea quality potential. *Food Chemistry*, **100**: 873–884

Rice, E. C. A., N. J. Miller and G. Paganga. 1996. Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, **20**: 933–956

Ryoyasu, S. and T. Yoshiyuki. 1999. HPLC analysis of catechins in various kinds of green teas produced in Japan and abroad. *J. Japanese Society for Food Science and Technology*, **46**: 138–147

Singleton V. L., R. Orthofer and R. M. Lamuela–Raventos 1999 Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. *Methods in Enzymology*, **299**: 152–178

Yang G. Y., J. Liao, Y. Kim, E. J. Yurkow and C. S. Yang 1998 Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis*, **19**: 611–616

Yang C. S. and Z. Y. Wang 1993 Tea and cancer: review. *J. National Cancer Institute*, **85**: 1038–1049