Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 99 (2006) 835–841
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods

Nihal Turkmen, Ferda Sari, Y. Sedat Velioglu *

Ankara University, Faculty of Engineering, Department of Food Engineering, 06110-Diskapi, Ankara, Turkey

Received 1 March 2005; received in revised form 28 July 2005; accepted 29 August 2005

Abstract

Effect of the use of water and different organic solvents such as acetone, *N,N*-dimethylformamide (DMF), ethanol or methanol at various concentrations on the total polyphenol content and antioxidant activity was studied for the black tea and mate tea. Polyphenol contents of extracts were determined using ferrous tartrate (method # 1) and Folin–Ciocalteu (method # 2) assays. For black tea, 50% DMF extract showed the highest polyphenol content of 131.9 mg/g and 99.8 mg GAE/g by method # 1 and method # 2, respectively. For mate tea, 50% acetone showed the highest polyphenol content of 132.5 mg/g and 120.4 mg GAE/g by method # 1 and method # 2, respectively. Fifty percent ethanol extract from mate tea and 50% acetone from black tea had the greatest antioxidant activity. The results showed that solvent with different polarity had significant effect on polyphenol content and antioxidant activity. A high correlation between polyphenol content and antioxidant activity of tea extracts was observed.

Keywords: Black tea; Mate tea; Phenolics; Extraction; Determination; Antioxidant activity

1. Introduction

Tea is the most widely consumed beverage worldwide and has become an important agricultural product (Benzie & Szeto, 1999; Lin, Juan, Chen, Liang, & Lin, 1996). Recent experimental studies have recognized that tea exhibits a significant health protecting activity due to its high polyphenol content (Manzocco, Anese, & Nicoli, 1998). Tea polyphenols are the most significant group of tea components and have a wide range of pharmaceutical properties including antioxidative, anticarcinogenic and antiarteriosclerotic (Atoui, Mansouri, Boskou, & Kefalas, 2005; Dufresne & Farnworth, 2001; Filip & Ferraro, 2003; Wang & Helliwell, 2001).

Different solvent systems have been used for extraction of polyphenols from plant materials (Chavan, Shahidi, & Naczk, 2001). Extraction yield is dependent on the solvent and method of extraction (Goli, Barzegar, & Sahari, 2004). The extraction method must enable complete extraction of the compounds of interest and must avoid their chemical modification (Zuo, Chen, & Deng, 2002). Water, aqueous mixtures of ethanol, methanol and acetone are commonly used to extract plants (Sun & Ho, 2005). Researchers usually use boiling water for the extraction of polyphenolics from green, black and mate teas (Filip & Ferraro, 2003; Khokhar & Magnusdottír, 2002; Larger, Jones, & Dacombe, 1998; Lee & Ong, 2000; Liang, Lu, Zhang, Wu, & Wu, 2003; Obanda, Owuor, & Mang'oka, 2001; Pomilio, Trajtemberg, & Vitale, 2002). Also, aqueous methanol, acetone and ethanol (Martínez, Pelotto, & Basualdo, 1997; Wang & Helliwell, 2001), absolute methanol (Yao et al., 2004), and absolute ethanol (Opie, Robertson, & Clifford, 1990) have been used for this purpose. However, so far use of dimethylformamide (DMF) for extraction of polyphenols has not been reported. Absolute methanol

* Corresponding author. Tel.: +90 3125 961 166; fax: +90 312 317 87 11.
E-mail address: velioglu@eng.ankara.edu.tr (Y.S. Velioglu).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.08.034

for tea polyphenolics (Yao et al., 2004) and aqueous acetone for extraction of wheat total phenolics (Zhou & Yu, 2004) were found to be more effective than water. Wang and Helliwell (2001) reported that aqueous ethanol was superior to aqueous methanol and acetone for extraction of the flavonoids from tea. However, Khokhar and Magnusdottír (2002) found water to be the best solvent for extracting tea catechins compared with 80% methanol and 70% ethanol. Also, in the extraction of polyphenol a single extraction compared to multiple extraction procedure is not sufficient (Zuo et al., 2002).

It can be concluded that it is not clear which solvent system is more effective for extracting total phenolics of tea and evaluating the antioxidant activity. On the other hand, little is known about polyphenol content and antioxidant activity of mate tea. Thus, the objective of this research was to investigate the effect of different extracting solvents on total polyphenol and antioxidant activity of both black and mate tea. The organic solvent systems with different polarities included absolute methanol, ethanol, acetone and DMF and their aqueous solutions at different concentrations. In addition, in this study two spectrophotometric methods based on different reactions were tested for determination of polyphenols. One is the Folin–Ciocalteu method widely used in routine analysis (Atoui et al., 2005; Wright, Mphangwe, Nyirenda, & Apostolides, 2000) and other is the method (Caffin, D'Arcy, Yao, & Rintoul, 2004; Li, Wang, Ma, & Zhang, 2005; Liang et al., 2003) which has been used especially for analysis of tea polyphenols.

2. Materials and methods

2.1. Plant materials

Black tea (*Camellia sinensis* L.) and black mate tea (*Ilex paraguariensis*) samples were purchased from local markets in Ankara-Turkey and Sydney-Australia, respectively. Tea samples were ground to pass a 1 mm screen and stored at +4 °C before experiments.

2.2. Chemicals

N,N-dimethylformamide, ethanol and methanol were either analytical or HPLC grade from Fluka (BioChemica-Fluka Cheme GmbH Buchs-Switzerland) and acetone was from Aldrich (St. Louis, MO, USA). Folin–Ciocalteu's reagent and other chemicals were from Merck (Darmstadt-Germany). DPPH (2,2-diphenyl-1-picryhydrazyl) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were analytical grade and from Merck.

2.3. Extraction of tea polyphenols

Ground tea sample (0.2 g) was extracted with distilled water or organic solvents. For water extraction, black and mate tea were infused with 10 ml freshly boiled distilled water for 10 min in a thermos flask. The infusion was filtered through Whatman No. 1 and rapidly cooled under tap water.

For organic solvent extraction, three different concentrations (50%, 80% and 100%) of acetone, DMF, ethanol and methanol were used. Our preliminary experiments showed that DMF extraction of tea resulted in higher polyphenol content and better chromatographic separation compared to ethanol and acetone extractions. Although, at present, this is not the subject of this study DMF was included to test the possibility to be an alternative solvent to other common solvents for further studies. Ground tea sample (0.2 g) was extracted with 2 ml of solvent for 1 h on a horizontal shaker. The mixture was centrifuged at 8500*g* for 10 min and subsequently decanted. The residue was re-extracted twice more for 2 h and the extraction procedure was repeated twice more for 3 h as explained above. The five supernatants were combined and stored at −18 °C until analyzed. Each solvent extraction was carried out in triplicate.

2.4. Determination of polyphenol content

2.4.1. Ferrous tartrate method (method # 1)

Content of tea polyphenols (TP) in tea extracts was determined by the spectrophotometric method described by Liang et al. (2003) and Li et al. (2005). One milliliter of tea extract was transferred into a 25 ml volumetric flask to react with 5 ml dyeing solution (1 g ferrous sulfate and 5 g potassium sodium tartrate tetrahydrate dissolved in 1000 ml distilled water), 4 ml distilled water and 15 ml buffer (0.067 M potassium phosphate, pH 7.5). Several minutes were required for color development. Absorbance readings were made at 540 nm by a Shimadzu UV-VIS 1601 spectrophotometer, using a blank solution prepared with distilled water replacing the tea extract.

The content of TP was calculated by the following equation:

$$\mathrm{TP(mg/g)} = 2A * 1.957 * (L_1/L_2 * M),$$

where L_1 is the total volume of extract solution in ml; L_2 is the volume of the extract solution used for analysis in ml; M is the mass of tea leaves in mg; A is the absorbance at 540 nm; 1.957: constant, meaning that when absorbance at 540 nm was 0.5 under the earlier conditions, the concentration of TP was 1.957 mg/ml.

2.4.2. Folin–Ciocalteu method (method # 2)

The amount of total phenolics was determined using the Folin–Ciocalteu method (Obanda & Owuor, 1997). A calibration curve of gallic acid (ranging from 0.005 to 0.05 mg/ml) was prepared and the results, determined from regression equation of the calibration curve ($y = 62.94x - 0.67$, $R^2 = 0.99$), were expressed as mg gallic acid equivalents per gramme of the sample. In this

method, 1 ml of tea extract diluted 10–75 times with deionized water (to obtain absorbance in the range of the prepared calibration curve) was mixed with 1 ml of 3-fold-diluted Folin–Ciocalteu phenol reagent. Two milliliter of 35% sodium carbonate solution is added to the mixture, shaken thoroughly and diluted to 6 ml by adding 2 ml of water. The mixture is allowed to stand for 30 min and blue color formed is measured at 700 nm using a spectrophotometer.

2.5. Determination of antioxidant activity by the DPPH radical scavenging method

The antioxidant activity of tea samples was measured by using the DPPH assay (Atoui et al., 2005; Katalinić, Milos, Modun, Musić, & Boban, 2004). Fifty microliter of tea extract diluted 15-fold with distilled water (directly, 5- and 10-fold dilution in additional assays) was mixed with an aliquot of 1950 μl of 6×10^{-5} M DPPH radical in methanol. Distilled water was used as a control instead of extract. The reaction mixture was vortex-mixed and let to stand at 25 °C in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer using methanol as a blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation (Yen & Duh, 1994):

$$\mathrm{AA}(\%) = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100.$$

2.6. Statistical analysis

All data were expressed as means ± standard errors of triplicate measurements and analysed by SPSS for Windows (ver. 10.1). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to test any significant differences between solvents used. Statistical comparisons between variables (e.g., polyphenol with method # 1–polyphenol with method # 2, antioxidant activity of black tea–antioxidant activity of mate tea and polyphenol of black tea–polyphenol of mate tea) were performed with Student's *t*-test. Differences were considered significant at $p < 0.05$. Correlations between variables were established by regression analysis.

3. Results and discussion

3.1. Polyphenol content

The polyphenol contents of black tea and mate tea were examined and presented in Table 1. For black tea, polyphenol contents determined by method # 1 and method # 2 ranged from 2.1 to 131.9 mg/g and from 1.8 to 99.8 mg GAE/g, respectively. In the case of mate tea, polyphenol content ranged from 3.6 to 132.5 mg/g and from 2.6 to 120.4 mg GAE/g, respectively. For black tea, there was a significant difference between polyphenol contents obtained with two methods (Table 2). However, for mate tea two methods gave similar polyphenol values and there was no significant difference ($p > 0.05$) between polyphenol

Table 1
Effect of different solvents on polyphenol content and antioxidant activity of tea extracts

Solvent	Black tea			Mate tea		
	Polyphenol content (mg/g)		Antioxidant activity (%)[b]	Polyphenol content (mg/g)		Antioxidant activity (%)
	Method # 1[a]	Method # 2		Method # 1	Method # 2	
Water	33.3 ± 1.67	30.5 ± 0.62	29.1 ± 0.68	65.0 ± 0.46	64.2 ± 1.36	61.2 ± 0.89
Acetone						
50%	130.6 ± 1.32[b]	92.4 ± 0.83[c]	83.1 ± 0.22[c]	132.5 ± 1.87[c]	120.4 ± 1.49[c]	93.7 ± 0.18[a]
80%	130.2 ± 0.53[b]	87.2 ± 1.21[b]	80.4 ± 0.97[b]	128.5 ± 0.74[b]	113.4 ± 1.00[b]	94.2 ± 0.30[a]
100%	2.1 ± 0.28[a]	1.8 ± 0.09[a]	1.2 ± 0.16[a]	3.6 ± 0.24[a]	2.6 ± 0.44[a]	nd
DMF						
50%	131.9 ± 0.59[c]	99.8 ± 1.24[b]	82.5 ± 0.94[c]	117.9 ± 1.58[b]	108.8 ± 2.28[b]	91.1 ± 0.42[b]
80%	127.6 ± 0.46[b]	96.8 ± 1.02[b]	78.8 ± 0.78[b]	120.7 ± 0.92[b]	113.4 ± 1.09[b]	92.4 ± 0.66[b]
100%	52.4 ± 0.52[a]	35.6 ± 0.34[a]	39.0 ± 0.44[a]	58.0 ± 0.17[a]	54.4 ± 0.42[a]	51.7 ± 0.94[a]
Ethanol						
50%	104.3 ± 0.41[c]	74.0 ± 0.94[c]	68.7 ± 1.93[b]	121.1 ± 1.37[c]	106.1 ± 3.24[c]	94.3 ± 0.53[c]
80%	77.3 ± 0.60[b]	53.7 ± 0.91[b]	49.6 ± 1.04[a]	85.8 ± 2.97[b]	83.5 ± 2.66[b]	78.7 ± 1.13[b]
100%	2.8 ± 0.40[a]	2.1 ± 0.09[a]	nd[c]	5.9 ± 0.13[a]	4.8 ± 0.06[a]	4.7 ± 0.75[a]
Methanol						
50%	82.3 ± 1.82[c]	62.6 ± 0.62[c]	53.8 ± 1.21[c]	100.6 ± 0.51[c]	96.6 ± 2.63[c]	89.6 ± 0.17[c]
80%	77.0 ± 0.11[b]	56.0 ± 1.70[b]	47.1 ± 0.65[b]	94.2 ± 0.28[b]	85.6 ± 1.60[b]	82.0 ± 1.20[b]
100%	23.5 ± 0.85[a]	13.5 ± 0.72[a]	11.0 ± 0.50[a]	45.8 ± 0.62[a]	35.5 ± 0.36[a]	40.0 ± 1.73[a]

[a] Data are expressed as means ± SE of triplicate experiments. For each organic solvent, values in the same column bearing different letters are significantly different at $p < 0.05$.
[b] Samples were diluted 15-fold for antioxidant activity determination.
[c] nd = not detected.

Table 2
Results of Student's *t*-test significance for polyphenol content and antioxidant activity between variables

Parameter	Variables	*t*-value[a]	Degree of significance
Polyphenol content	Black tea wm.[c] # 1v. black tea wm. # 2	2.21	0.03[b]
	Mate tea wm. # 1 v. mate tea wm. # 2	0.74	0.461
	Black tea wm. # 1 v. mate tea wm. # 1	0.78	0.436
	Black tea wm. # 2 v. mate tea wm. # 2	2.59	0.012[b]
Antioxidant activity	Black tea v. mate tea	2.70	0.008[b]

[a] The obtained *t*-value was compared with t_{critic} ($p < 0.05$) = 1.99.
[b] $p < 0.05$.
[c] wm. = with the method, v. = versus.

contents of the extracts analyzed by two mentioned methods. It is clear that the difference between the results obtained with two methods depended on the tea analyzed. Higher polyphenol values with method # 1 compared to those with method # 2 in black tea extracts might be due to interfering non-phenolic compounds. For both methods, polyphenol contents of tea extracts were strongly dependent on the solvents at different concentrations used as shown in Table 1.

Excluding DMF extract from mate tea, all extracts prepared with 50% solvents contained highest level of polyphenol measured by both methods and followed by those with 80% and 100% solvents, respectively. The lowest amounts of polyphenol were obtained with 100% acetone and 100% ethanol, respectively. For black tea, among the solvents tested, the highest level of polyphenol determined by method # 1 was achieved by using 50% DMF, closely followed by 50% acetone, 80% acetone and 80% DMF, respectively. The obtained polyphenol amounts with using other solvents were lower. But the order of four extracts with highest value of polyphenol content by method # 2 was as follows: 50% DMF > 80% DMF > 50% acetone > 80% acetone.

In the case of mate tea, the order of polyphenol content of extracts was slightly different from that of black tea. The highest amount of polyphenol measured by method # 1 was found in 50% acetone extract, closely followed by 80% acetone, 50% ethanol and 80% DMF, respectively. Similarly, the highest polyphenol content by method # 2 was found in 50% acetone extract. But this was closely followed by 80% acetone, 80% DMF and 50% DMF, respectively. Our results clearly showed that higher content of polyphenols was obtained with an increase in polarity of the solvent used (Table 1). Chavan et al. (2001) reported that aqueous acetone (70%) with or without acid was more efficient than absolute acetone for recovery of a maximum amount of condensed tannins from different peas. Zhou and Yu (2004) reported that among solvents tested, 50% acetone extracts contained greatest level of total phenolics from wheat and ethanol was the least effective solvent, which is in agreement with our results. In the study carried out by Yu, Ahmedna, and Goktepe (2005), 80% ethanol and 80% methanol were found more effecient than water for extracting total phenolics from peanut skin, which also agrees with the results from this study. The results indicated that the order of increasing amount of polyphenol content with both methods was almost similar in black tea and mate tea, showing good correlation (Fig. 1).

According to the results obtained with method # 1, there was no significant difference between polyphenol content of black and mate tea ($p > 0.05$) but with method # 2 significant difference was observed (Table 2), which agrees with Mello, Alves, Macedo, and Kubota (2004).

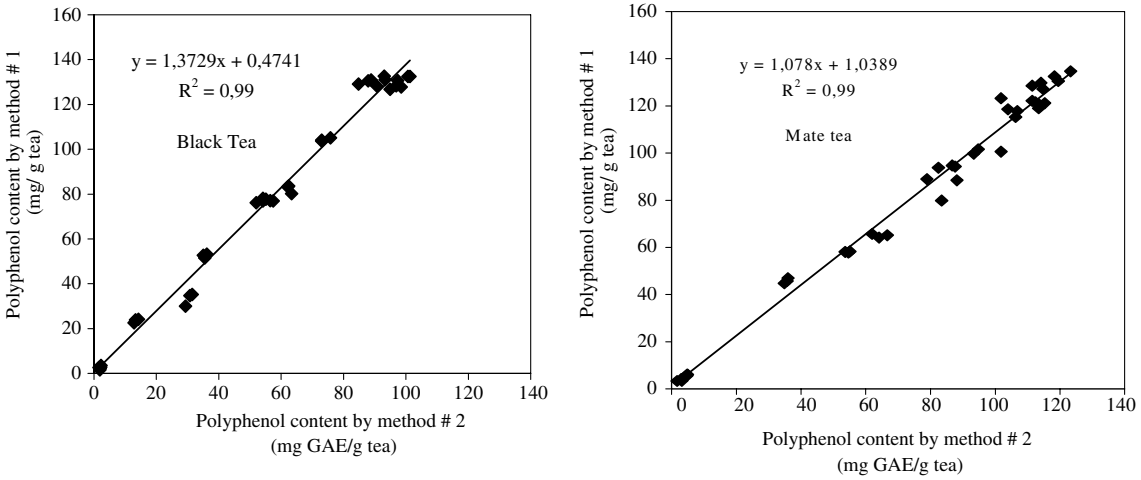

Fig. 1. Correlation between polyphenol content by method # 1 and method # 2.

3.2. Antioxidant activity

Solvents used for polyphenol extraction had significant effects on DPPH scavenging capacity determination for black and mate tea extracts (Table 1). DPPH method has been widely used in antioxidant activity studies of plant extracts (Canadanovic-Brunet, Djilas, & Cetkovic, 2005; Pinelo, Rubilar, Sineiro, & Nunez, 2004; Sun & Ho, 2005). The method is based on the reduction of alcoholic DPPH solutions at 517 nm in the presence of an hydrogen donating antioxidant (Koleva, Van Beek, Linssen, De Groot, & Evstatieva, 2002) and polyphenols have been reported to be potent hydrogen donators to the DPPH radical (Von Gadow, Joubert, & Hansmann, 1997) because of their ideal structural chemistry (Rice-Evans, Miller, & Paganga, 1997). Regardless of tea types, extracts with concentrations of 50% and 80% of solvents used exhibited considerably higher DPPH radical scavenging activity than those with their respective absolute ones and this trend was similar to that observed for content of polyphenol. Among black tea extracts, the order of high antioxidant activity was 50% acetone > 50% DMF > 80% acetone > 80% DMF. These extracts had also higher polyphenol content. The rest had lower activity, but absolute ethanol extract exhibited activity only in additional assays without dilution (Table 3). In the case of mate tea, among solvents tested the highest antioxidant activity was observed in 50% ethanol extracts, closely followed by 80% acetone, 50% acetone and 80% DMF extracts, respectively. Others had lower activity, but absolute acetone extract showed activity only without dilution (Table 3). As observed in black tea, extracts with higher antioxidant activity also had higher polyphenol content. It can be concluded that the extracts obtained using high polarity solvents were considerably

Table 3
Antioxidant activity of absolute acetone and ethanol extracts from tea

Extracts	Antioxidant activity (%)		
	Dilution factor		
	1/10	1/5	No dilution
Ethanol extracts from black tea	nd[a]	nd	32.0 ± 2.42
Acetone extracts from mate tea	nd	nd	23.6 ± 1.20

[a] nd = not detected.

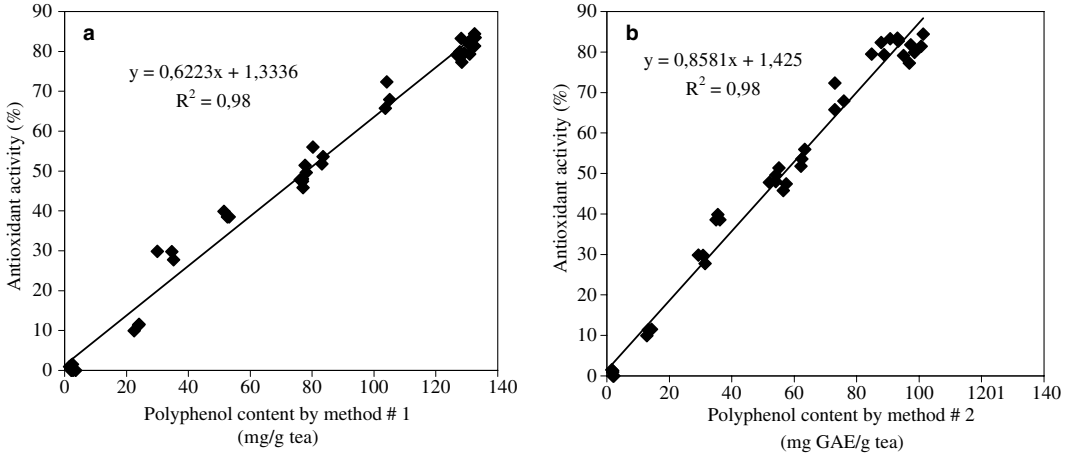

Fig. 2. Correlation between polyphenol content antioxidant activity for black tea: (a) method #1; (b) method #2.

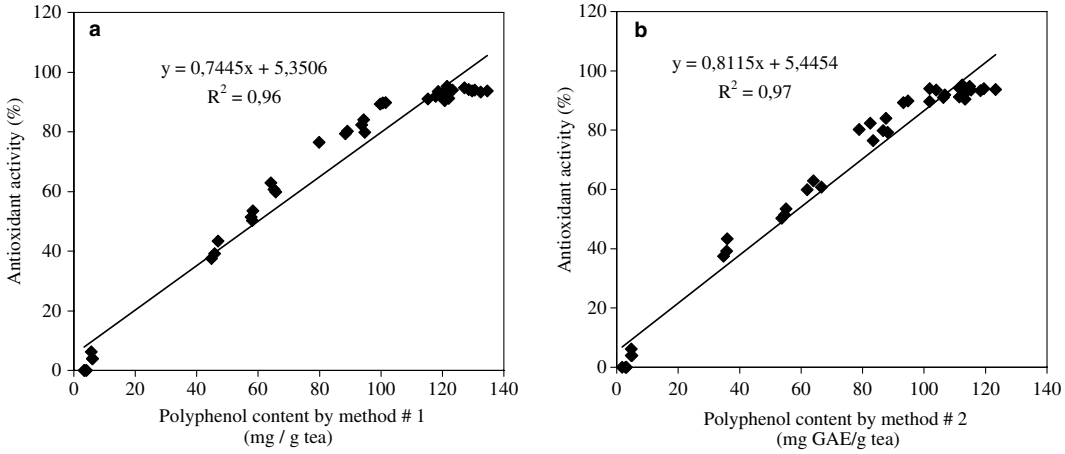

Fig. 3. Correlation between polyphenol content antioxidant activity for mate tea: (a) method #1; (b) method #2.

more effective radical scavengers than those using less polarity solvents, indicating that antioxidant or active compounds of different polarity could be present in black and mate tea. Change in solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and influences the antioxidant activity estimation (Zhou & Yu, 2004). As seen in Figs. 2 and 3, the content of polyphenols determined by both methods in the extracts of black and mate tea correlates with their antioxidant activity, confirming that polyphenols are likely to contribute to the radical scavenging activity of these plant extracts (Miliauskas, Venskutonis, & van Beek, 2004). This result is agreement with Mello et al. (2004), who observed correlation between total phenol content and antioxidant activity was very good for black tea ($R^2 = 0.989$) and mate tea ($R^2 = 0.986$). Similar results have also been reported for different plants by various studies (Katalinić et al., 2004; Maksimović, Malenčić, & Kovačević, 2005; Miliauskas et al., 2004; Yu et al., 2005).

Antioxidant activity in mate tea extracts was substantially higher compared with those of black tea and significant difference was found between them (Table 2). This can be the result of higher polyphenol content by method #2 of mate tea extracts and may also be due to differences in their polyphenol composition. Mate tea contains more of the simple phenols and flavonoids, while black tea contains more complex components called theaflavins and thearubigins as a result of oxidation of simple phenolics in green tea leaves (Mello et al., 2004). The most important of phenolic compounds in mate tea are known as caffeoylquinic acids (Mazzafera, 1997; Pomilio et al., 2002) which include chlorogenic acid with high antioxidant activity (Clouatre, 2004; Filip & Ferraro, 2003).

4. Conclusions

Extracting solvent significantly affected total polyphenol content and antioxidant activity of tea extracts. Rankings in the polyphenol content of extracts varied depending on the concentration of solvent, the method used and tea plant. Regardless of the method used, the most efficient solvents for polyphenol extraction were 50% DMF and 50% acetone for black and mate tea, respectively. In both cases, polyphenol content of absolute acetone extracts was the lowest. In this study, DMF which has not been used for polyphenol extraction was proven to be as efficient as commonly used solvents such as acetone, even more, for this purpose. 50% acetone and 50% ethanol extract from black and mate tea showed the highest antioxidant activity, respectively. A good correlation was obtained between antioxidant properties of tea extracts and their total polyphenol content and the extracts of mate tea possessed higher radical scavenging ability than those of black tea. Higher antioxidant activities of mate tea extracts, except for water extracts, compared to those of black tea seem to be consistent with their higher polyphenol contents determined by method #2. Therefore, it is recommended that method #2 for determination of polyphenol will be more appropriate than method # 1 in future studies.

Acknowledgments

The authors thank Ankara University Scientific Research Projects (BAP) for financial support and Mrs. Seda Can (Sydney-Australia) for providing black mate tea.

References

Atoui, A. K., Mansouri, A., Boskou, G., & Kefalas, P. (2005). Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry, 89*, 27–36.

Benzie, I. F. F., & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 47*, 633–636.

Caffin, N., D'Arcy, B., Yao, L., Rintoul, G., (2004). *Developing an index of quality for Australian tea.* RIRDC Publication No. 04/033. Queensland-Australia: Australian Government Rural Industries Research and Development Corporation.

Canadanovic-Brunet, J. M., Djilas, S. M., & Cetkovic, G. S. (2005). Free-radical scavenging activity of wormwood (*Artemisia absinthium*) extracts. *Journal of the Science of Food and Agriculture, 85*, 265–272.

Chavan, U. D., Shahidi, F., & Naczk, M. (2001). Extraction of condensed tannins from beach pea (*Lathyrus maritimus L.*) as affected by different solvents. *Food Chemistry, 75*, 509–512.

Clouatre, D. (2004). Green mate for energy, weight control and more. *Total Health, 26*, 52–54.

Dufresne, C. J., & Farnworth, E. R. (2001). A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry, 12*, 404–421.

Filip, R., & Ferraro, G. E. (2003). Researching on new species of "Mate": *llex brevicuspis*. *European Journal of Nutrition, 42*, 50–54.

Goli, A. H., Barzegar, M., & Sahari, M. A. (2004). Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chemistry, 92*, 521–525.

Katalinić, V., Milos, M., Modun, D., Musić, I., & Boban, M. (2004). Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chemistry, 80*, 593–600.

Khokhar, S., & Magnusdottír, S. G. M. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry, 50*, 565–570.

Koleva, I. I., Van Beek, T. A., Linssen, J. P. H., De Groot, A., & Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis, 13*, 8–17.

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

Lee, B. L., & Ong, C. N. (2000). Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis. *Journal of Chromatography A, 881*, 439–447.

Li, P., Wang, Y., Ma, R., & Zhang, X. (2005). Separation of tea polyphenol from *green tea leaves* by a combined CATUFM-adsorption resin process. *Journal of Food Engineering, 67*, 253–260.

Liang, Y., Lu, J., Zhang, L., Wu, S., & Wu, Y. (2003). Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. *Food Chemistry, 80*, 283–290.

Lin, Y., Juan, I., Chen, Y., Liang, Y., & Lin, J. (1996). Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *Journal of Agricultural and Food Chemistry, 44*, 1387–1394.

Maksimović, Z., Malenčić, D., & Kovačević, N. (2005). Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. *Bioresource Technology, 96*, 873–877.

Manzocco, L., Anese, M., & Nicoli, M. C. (1998). Antioxidant properties of tea extracts as affected by processing. *Lebennsmittel-Wissenschaft und-Technologie, 31*, 694–698.

Martínez, M. A. D. P., Pelotto, J. P., & Basualdo, N. (1997). Distribition of flavonoid aglycones in *llex* species (Aquifoliaceae). *Biochemical Systematics and Ecology, 25*, 619–622.

Mazzafera, P. (1997). Mate drinking: Caffeine and phenolic acid intake. *Food Chemistry, 60*, 67–71.

Mello, L. D., Alves, A. A., Macedo, D. V., & Kubota, L. T. (2004). Peroxidase-based biosensor as a tool for a fast evaluation of antioxidant capacity of tea. *Food Chemistry, 92*, 515–519.

Miliauskas, G., Venskutonis, P. R., & van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry, 85*, 231–237.

Obanda, M., & Owuor, P. O. (1997). Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *Journal of the Science of Food and Agriculture, 74*, 209–215.

Obanda, M., Owuor, P. O., & Mang'oka, R. (2001). Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chemistry, 75*, 395–404.

Opie, S. C., Robertson, A., & Clifford, M. N. (1990). Black tea thearubigins – their HPLC separation and preparation during in vitro oxidation. *Journal of the Science of Food and Agriculture, 50*, 547–561.

Pinelo, M., Rubilar, M., Sineiro, J., & Nunez, M. J. (2004). Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). *Food Chemistry, 85*, 267–273.

Pomilio, A. B., Trajtemberg, S., & Vitale, A. A. (2002). High-performance capillary electrophoresis analysis of *mate* infusions prepared from stems and leaves of *llex paraguariensis* using automated micellar electrokinetic capillary chromatography. *Phytochemical Analysis, 13*, 235–241.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Sun, T., & Ho, C. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry, 90*, 743–749.

Von Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chemistry, 60*, 73–77.

Wang, H., & Helliwell, K. (2001). Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. *Food Research International, 34*, 223–227.

Wright, L. P., Mphangwe, N. I., Nyirenda, H. E., & Apostolides, Z. (2000). Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinesis* for predicting the quality of the black tea produced in Central and Southern Africa. *Journal of the Science of Food and Agriculture, 80*, 1823–1830.

Yao, L., Jiang, Y., Datta, N., Singanusong, R., Liu, X., Duan, J., et al. (2004). HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. *Food Chemistry, 84*, 253–263.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *Journal of Agricultural and Food Chemistry, 42*, 629–632.

Yu, J., Ahmedna, M., & Goktepe, I. (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chemistry, 90*, 199–206.

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebennsmittel-Wissenschaft und-Technologie, 37*, 717–721.

Zuo, Y., Chen, H., & Deng, Y. (2002). Simultaneous determination of catechins, caffeine and gallic acids in geen, oolong, black and pu-erh teas using HPLC with a photodiode array detector. *Talanta, 57*, 307–316.