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Antioxidative mechanisms of tea catechins in chicken meat systems

S.Z. Tang[a], J.P. Kerry[a,*], D. Sheehan[b], D.J. Buckley[a]

[a]*Department of Food Science, Food Technology and Nutrition, University College Cork-National University of Ireland, Cork, Ireland*
[b]*Department of Biochemistry, University College Cork-National University of Ireland, Cork, Ireland*

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Abstract

The effects of dietary tea catechins (TC) supplementation at levels of 50 (TC50), 100 (TC100) 200 (TC200), and 300 (TC300) mg/kg feed on oxidative stability and on protection of α-tocopherol (VE) in long-term frozen stored (−20 °C×12 months) chicken breast and thigh meat were investigated. Dietary TC (TC200) showed inhibiting effects on lipid oxidation equivalent to dietary α-tocopheryl acetate (VEA200) for long-term frozen stored chicken meat. The level of VE in long-term stored frozen chicken meat from TC dietary treatments (TC50, TC100, TC200 and TC300) was significantly ($P<0.05$) higher than that of meat from control diet containing no TC (C). The protective effects of TC against VE-depletion may partly elucidate the antioxidant activity of TC in vivo. The effects of TC on iron-induced docosahexaenoic acid (DHA)-incorporated L-α-phosphatidylcholine (PC) liposome oxidation, and on Fe^{2+}-chelating and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging were also examined. Added TC showed significantly ($P<0.05$) higher antioxidant activity in oxidized DHA-PC liposomes than in controls. In addition to chelating effects on Fe^{2+}, TC showed strong scavenging capacity for the DPPH free radical. The strong free radical-scavenging ability plus the iron-chelating effects of TC provide a plausible mechanism for the antioxidant effects of added TC in the in vitro meat system.

Keywords: Antioxidants; Free radical scavenging; Lipid oxidation; Metal ion chelation; Tea catechins; α-Tocopherol; α-Tocopheryl acetate

1. Introduction

Tea is one of the cheapest and most popular non-alcoholic beverages worldwide (Suganuma et al., 1999). Green tea has received considerable attention for its specific health claims and antioxidant properties because of the presence of functional polyphenols, namely tea catechins (TC). The principal catechins present in green tea are (−)-epicatechins (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG; Graham, 1992). Clinical and experimental studies have shown that TC extracted from green tea are non-toxic and inexpensive anticancer and antioxidant agents (Yamane et al., 1996). TC, especially EGCG and ECG, exerted potent inhibitory effects on the oxidation of low-density lipoprotein in vitro (Miura et al., 2000). Liu, Ma, Zhou, Yang, and Liu (2000) reported a kinetic analysis of the antioxidation process which showed that TC were effective antioxidants against both 2,2′-azobis(2-amidinopropane hydrochloride)-initiated and benzophenone-photosensitized low-density lipoprotein peroxidation. With regard to applications in food products, TC have been reported to be potent antioxidants in pig meat (Shahidi, Zhao, Yang, & Wanasundara, 1992), vegetable oil (Chen & Chan, 1996), fish oil (Wanasundara & Shahidi, 1996), food emulsions (Huang & Frankel, 1997) and animal fat (Wang & Zhao, 1997). The antioxidant capacity of TC in these foods was reported to be equivalent to or greater than α-tocopherol (VE). Effects of dietary TC on oxidative stability of fresh (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2000) and frozen (−20 °C × 3, 6 and 9 months storage) (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2001) chicken meat were also studied and their antioxidant potential for chicken meat was reported to be equivalent to that of dietary α-tocopheryl acetate (VEA).

Mechanistic studies of TC, as cancer inhibitors or preventing agents have been widely reported (Buschman, 1998; Katiyar & Mukhtar, 1996; Yang, Chung, Yang, Chhabra, & lee, 2000). It is generally suggested that TC act as anticancer agents by

* Corresponding author. Tel.: +353-21-490-3798; fax: +353-21-4270213.
E-mail address: joe.kerry@ucc.ie (J.P. Kerry).

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detoxifying cancer-causing substances in vivo or by inducing apoptosis of tumour cells. However, little information is available on the mechanism of TC as antioxidants in food products, in particular, muscle tissue systems. The objectives of this study were to investigate the antioxidative activity of dietary TC in long-term stored chicken meat and added TC in iron-induced oxidized liposomes, and to assess possible mechanisms for TC functioning as antioxidants in the in vivo and in vitro meat systems.

2. Materials and methods

2.1. Chemicals

All general laboratory chemicals (AnalaR grade) were obtained from British Drug House (Poole, Dorset, UK). TC standards including EGCG, EGC, ECG and EC, VE standard, docosahexaenoic acid (DHA), L-α-phosphatidylcholine (PC), and 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA).

TC (KL9790803, purity of 86%), used in the chicken diet, were extracted from green tea (*Camellia sinensis* L. variety *assamica*) by using 30% ethanol solution and supplied by Kinglong Natural Plant Products Industry Ltd, Changsha, Hunan, China. The TC contained 40% of EGCG, 24% of EGC, 12% ECG, and 10% of EC. The remaining 14% is composed of (±)-catechins and caffeine. VEA, used in the chicken diet was obtained from Roche Products Ltd. (Welwyn Garden City, Hertfordshire, UK).

2.2. Animal diets and sampling

Day-old broiler chicks (Cobb 500, $n=200$) were purchased from a local commercial hatchery. Chicks were randomly assigned to six groups. A control group (C) was fed a commercial diet (Tang et al., 2000). The other groups were fed diets supplemented with either 200 mg α-tocopheryl acetate/kg feed (VEA200), or 50 (TC50), 100 (TC100), 200 (TC200) or 300 (TC300) mg TC/kg feed. After 6 weeks, all broilers were slaughtered under commercial conditions. Breast and thigh meat was removed from each carcass upon slaughter. All samples were vacuum packaged using a Webomatic type D463 (Webomatic vacuum packaging system, Werner Bonk, Mausegatt 59, D463, Bochumb, Germany) vacuum packer and stored at −20 °C for 12 months. Breast and thigh meat ($n=6$) from each dietary group, at the time of analysis, was thawed overnight at 4 °C and minced through 5-mm plates (Mainca mincer, Maquinaria Industria, Carnica, Barcelona, Spain). The minced meat was sampled for determination of lipid oxidation levels and VE content.

2.3. Determination of lipid oxidation in meat

Lipid oxidation was measured using the distillation method of Tarladgis, Watts, and Younathan (1960), as modified by Ke, Ackman, Linke, and Nash (1977). Lipid oxidation was expressed as thiobarbituric acid reactive substances (TBARS) numbers in the unit of mg malondialdehyde (MDA) equivalents/kg sample.

2.4. Determination of α-tocopherol in meat

α-Tocopherol (VE) in chicken meat was extracted using the method of Sheehy, Morrissey, and Flynn (1991) and quantified by a high-performance liquid chromatograph (HPLC) method (Sheehy, Morrissey, & Flynn, 1994). The analysis was carried out on a 20 Waters (Model 510) HPLC pump and a Waters (Model 717) Plus autosampler. The column used was a Machery-Nagel Nucleosil C_{18} reverse phase column (250×4 mm i.d., 5 μm particle size). The eluting solvent was methanol: water (97:3) at a flow rate of 2.5 ml/min. Detection took place at a wavelength of 292 nm on a Waters (model 486) UV–visible wavelength detector (Millipore Corporation, Milford, MA, USA). Chromatograms were recorded using the Millennium 2010 Chromatography Manager (Millipore Corporation) performed on a Dell Dimension 466V computer. The VE content of the meat was determined by comparison of peak areas with those obtained for a standard solution of VE.

2.5. Determination of antioxidant activity of tea catechins in a DHA-PC liposome model system

The DHA-PC liposome was prepared using the method of Maerker and Jones (1991) with some modifications. PC (100 mg) and DHA (50 mg) were dissolved and mixed in 3 ml of chloroform in a 10 ml screw-capped glass tube by vortexing. The chloroform was removed under a stream of nitrogen. The coated DHA lipids were hydrated with 8 ml distilled water and allowed to swell for 3 h in a water bath held at 55 °C with vortexing and sonicating every 15 min. After cooling to room temperature, the liposomes were filtered through a 0.45 μm pore hydrophilic cellulose acetate membrane (Lida Manufacturing Corp, 25th Avenue, Kenosha, WI 53143-6615, USA). All operations were performed in an oxygen preventive AtmosBag (Aldrich Chemical Co., Inc. 940 West Saint, Paul Avenue, Milwaukee, Wisconsin 53233, USA) to prevent oxidation.

The ability of TC to inhibit lipid peroxidation of the DHA-PC liposome system was tested using the method of Aruoma, Murcia, Butter, and Halliwell (1993) and lipid oxidation was expressed as TBARS numbers in the unit of nmol MDA equivalents/ml liposome.

Table 1
α-Tocopherol content (μg/g meat) in chicken meat[a]

	C[b]	TC50	TC100	TC200	TC300	VEA200
Breast	1.34±0.31a[c]	1.74±0.08b	1.86±0.05b	1.96±0.13b	2.64±0.24c	4.32±0.42d
Thigh	1.70±0.24a	2.68±0.32b	2.70±0.47b	3.28±0.75c	4.56±0.70d	6.68±0.46e

[a] Chicken meat stored at −20°C for 12 months.
[b] C, control chicken fed with a basal diet containing 20 mg α-tocopheryl acetate (VEA)/kg feed; TC50, a basal diet supplemented with 50 mg tea catechins (TC)/kg feed; TC100, a basal diet supplemented with 100 mg TC/kg feed; TC200, a basal diet supplemented with 200 mg TC/kg feed; TC300, a basal diet supplemented with 300 mg TC/kg feed; VE200, a basal diet supplemented with 200 mg VEA/kg feed.
[c] Values = means ± S.E.M. ($n=4$). Values without common letters in the same row are significant at $P<0.05$.

2.6. Determination of chelating activity of tea catechins on Fe^{2+} ions

The chelating activity of TC on Fe^{2+} was measured using the method of Decker and Welch (1990). One milliliter of TC (200–1000 ppm) was mixed with 3.7 ml of distilled water and then the mixture was reacted with 0.1 ml, 2 mM $FeCl_2$ and 0.2 ml, 5 mM 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1, 2, 4-triazine (ferrozine) for 20 min. The absorbance was read at 562 nm in a Cary 1E UV–Vis Spectrophotometer (Varian Australia Pty. Ltd, 679 Springvale Rd., Mulgrave, Victoria, 317 Australia). One milliliter of distilled water, instead of TC, was used as a control. Chelating activity (%) was calculated as follows:

$$\text{Chelating activity (\%)} = \left(1 - \frac{\text{Absorbance of sample at 562nm}}{\text{Absorbance of control at 562nm}}\right) \times 100$$

2.7. Determination of free radical scavenging effect of tea catechins

The free radical-scavenging activity of TC was measured with DPPH free radical using the method of Yen and Wu (1999) with minor modifications. TC (4 ml of 200–1000 ppm methanol solution) were added to 1 ml, 0.2 mM DPPH in methanol. After reacting for 30 min, the absorbance was read at 517 nm in a Cary 1E UV–Vis Spectrophotometer. Methanol (4 ml) was mixed with 1 ml DPPH and this served as the control. Radical-scavenging activity (%) was calculated as follows:

$$\text{Radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of sample at 517nm}}{\text{Absorbance of control at 517nm}}\right) \times 100$$

2.8. Statistical analysis

All data were subjected to analysis of variance (ANOVA). The statistical significance of the differences between mean values was analyzed by repeated measures and by Tukey's test in the general linear model of SPSS statistical package.

3. Results and discussion

3.1. Effects of dietary tea catechins on oxidative stability of long-term frozen stored meat

The mean TBARS numbers of thigh meat were significantly ($P<0.05$) higher than those of breast meat, with the exception of TC50 (Fig. 1), showing that chicken thigh meat was more susceptible to lipid oxidation than breast meat. In breast and thigh meat, the effects of TC on lipid oxidative stability of frozen (−20 °C×12 months) samples were dose dependent. All TC-treated groups had significantly ($P<0.05$) lower TBARS numbers compared to control groups, with the exception of TC50 for breast meat. Samples from TC300 had significantly ($P<0.05$) lower TBARS numbers than samples from VEA200, regardless of whatever they were breast or thigh meat. TC200 had slightly higher TBARS numbers than VEA200; however, these differences were not statistically significant. The data presented here are in agreement with a previously reported finding by Tang et al. (2000, 2001). These authors showed that dietary TC, at a level of 200 mg/kg feed possessed antioxidative effects equivalent to dietary VEA at the same concentration for fresh and frozen (−20 °C for 3, 6 and 9 months storage) chicken meat.

3.2. Effects of dietary tea catechins on protection of α-tocopherol in meat

The concentrations of VE in long-term frozen stored breast and thigh meat from the control group, fed a basal diet containing 20 mg VEA/kg feed, were 1.34 and 1.70 μg/g meat, respectively (Table 1). Those values of breast and thigh meat for VEA200 (a basal diet plus 200 mg VEA/kg feed) were 4.32 and 6.68 μg/g meat, respectively (three and four times the values of their respective controls). The result obtained is consistent with the data reported by Galvin, Morrissey, and

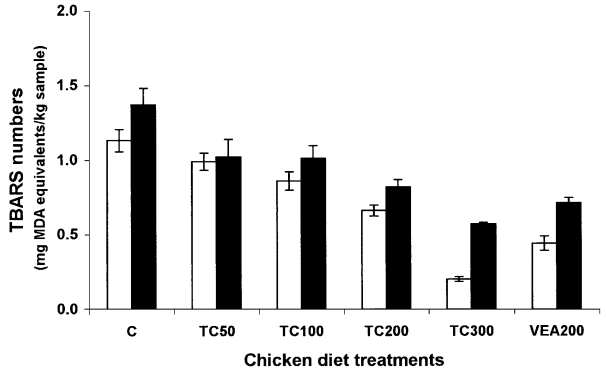

Fig. 1. Effects of dietary tea catechins on lipid oxidation [thiobarbituric acid reactive substances (TBARS) numbers, mg malondialdehyde (MDA) equivalents/kg muscle] of long-term frozen (−20 °C×12 months)-stored chicken breast (□) and thigh (■) meat. C, control sample from chicken fed with basal diet without addition of TC or α-tocopheryl acetate (VEA); TC50, C plus 50 mg TC/kg feed; TC100, C plus 100 mg TC/kg feed; TC200, C plus 200 mg TC/kg feed; TC300, C plus 300 mg TC/kg feed; VEA200, C plus 200 mg VEA/kg feed. Values = means ± S.E.M. ($n = 6$).

Buckley (1998) who showed that dietary VEA increased VE levels in meat and thus improved the oxidative stability of chicken meat during refrigerated and frozen storage.

Meat from all TC-treated groups had significantly ($P < 0.05$) higher levels of VE compared with those from controls (Table 1). The levels of VE present in meat were positively related to the amount of TC supplemented in the diet. TC300 had 2.64 and 4.56 μg VE/g meat for breast and thigh meat, which account for 61 and 68% of VEA200, respectively, indicating that TC could prevent the reduction of VE in meat during storage. Nanjo et al. (1993) reported that dietary TC prevented the destruction of VE in rat plasma and erythrocytes. Roedig-Penman and Gordon (1997) suggested that TC could function, partly, by assisting retention of VE during the storage of food emulsions. Maintenance of an optimum level of VE in meat is an effective approach to inhibition of lipid oxidation during storage (Jensen et al., 1997). Data obtained from this study have shown that dietary TC function as antioxidants in chicken meat and that some of this antioxidant activity may be due to the stabilization, recycling and retaining of VE. However, this hypothesis could only elucidate the antioxidant activity of TC50, TC100 and TC200, which had significantly ($P < 0.05$) lower levels of VE (Table 1) and higher TBARS numbers (Fig. 1) compared to VEA200. The level of VE spared by TC300 was lower compared to VEA200, but VEA200 showed significantly ($P < 0.05$) higher lipid oxidation than TC200. Therefore, TC may contribute antioxidant activities by alternative mechanisms at high concentrations.

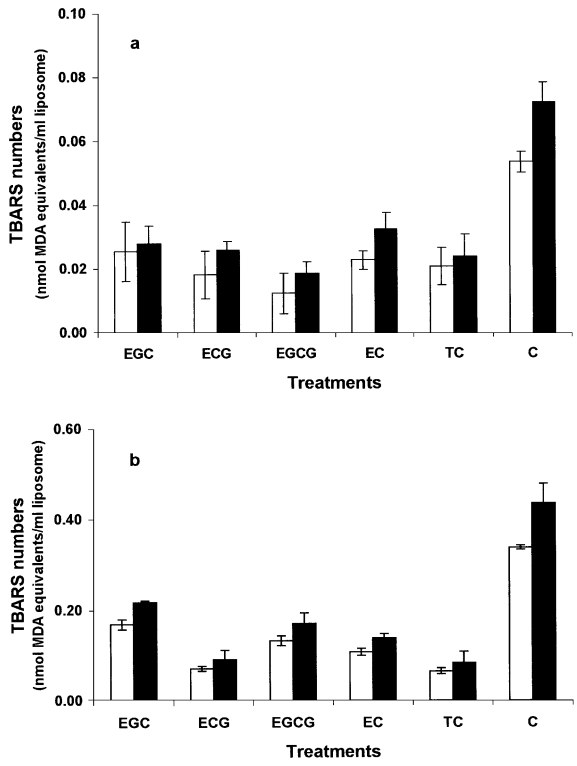

Fig. 2. Effects of tea catechins (TC) on iron-induced lipid oxidation [thiobarbituric acid reactive substances (TBARS) numbers, nmol malondialdehyde (MDA) equivalents/ml liposome] of (a) L-α-phosphatidylcholine (PC)-liposomes and (b) docosahexaenoic acid-(DHA-PC) liposomes incubated at 37 °C for 1 h (□) and 3 h (■). EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, (-)-epigallocatechin gallate; EC, (−)-epicatechin; TC, commercial TC extract containing 86% TC; C, control liposome without addition of TC. Values = means ± S.E.M. ($n = 4$).

3.3. Effects of tea catechins on iron-induced liposome oxidation

The antioxidant effects of standard and commercial TC extract on iron-induced oxidized PC and DHA-PC liposomes are shown in Fig. 2a and b, respectively. TBARS numbers in PC liposome systems were 0.012 (EGCG)–0.053 (control) after oxidizing for 1 h at 37 °C and 0.019 (EGCG)–0.072 (control) nmol MDA/ml liposome after oxidizing for 3 h at 37 °C (Fig. 2a). In the DHA-PC liposome system (Fig. 2b), TBARS numbers were 0.065 (TC)–0.337 (control) after oxidizing for 1 h and 0.084 (TC)–0.435 (control) nmol MDA/ml liposome after oxidizing for 3 h. The TBARS obtained in the DHA-PC liposome system were approximately 10 times that of the PC liposome. TBARS are formed in substantial amounts only from polyunsaturated fatty acids containing three or more double-bonds (Bird & Draper, 1984; Dahele, Hill, & Holman, 1962; Pryor, Stanley, & Blair, 1976). Incorporation of DHA ($C_{22:6}$) in the liposomes is essential to achieve more accurate evaluation of the properties of antioxidants than using PC liposome which contains only stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$).

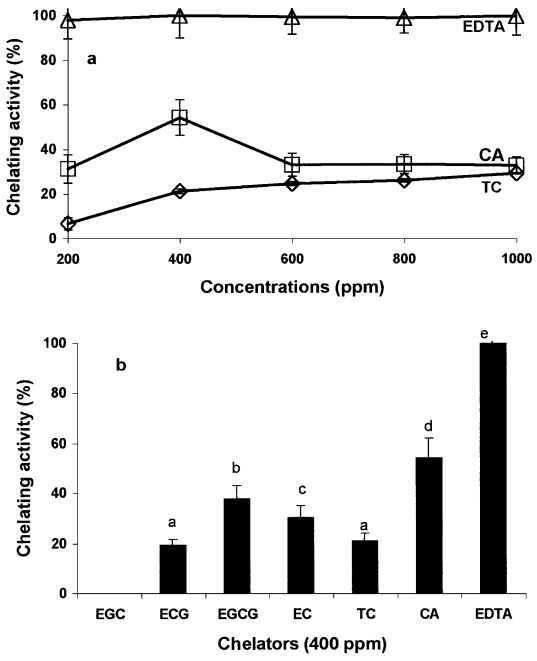

Fig. 3. Fe^{2+} (2 mM)-chelating effects of tea catechins at (a) different concentrations and (b) a concentration of 400 ppm compared with EDTA and citric acid (CA). EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin gallate; EC, (−)-epicatechin; TC, commercial TC extract containing 86% TC; CA; EDTA. Values = means ± S.E.M. ($n = 4$). Error bars with the different letters are significantly at different $P < 0.05$.

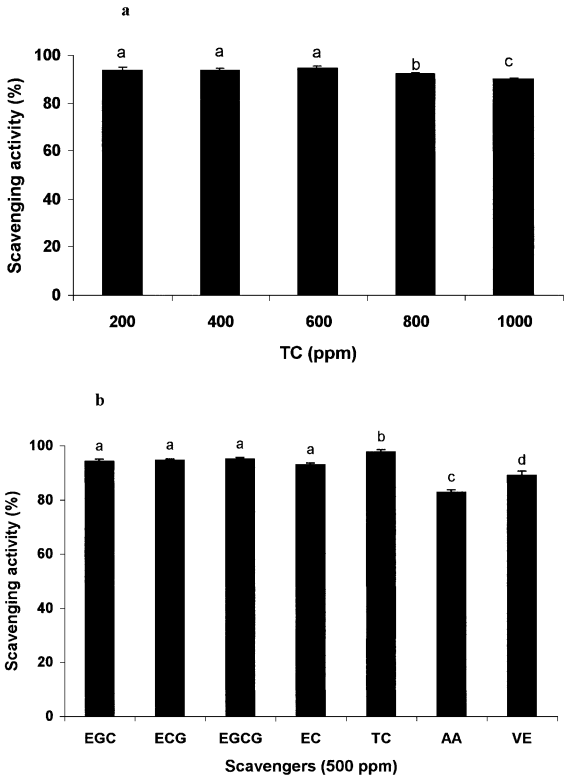

Fig. 4. Effects of tea catechins (TC) at (a) different concentrations and (b) 500 ppm on scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (2 mM). EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin gallate; EC, (−)-epicatechin; TC, commercial TC extract containing 86% TC; AA, L-ascorbic acid; VE, α- tocopherol. Values = means ± S.E.M. ($n = 4$). Error bars with the different letters are significantly at different $P < 0.05$.

TBARS numbers, for all TC treatments were significantly ($P < 0.05$) lower than control samples, regardless of the oxidation period used (Fig. 2a and b). The inhibition of liposome oxidation due to TC decreased in the order: EGCG > TC > ECG = EGC > EC for PC liposome (Fig. 2a) and TC > ECG > EC > EGCG > EGC for DHA-PC liposomes (Fig. 2b). In canola oil, the antioxidant activity of TC decreased in the order: EGC > EGCG > EC > ECG (Chen & Chan, 1996). EGCG > ECG was reported for the long-term stored (30 °C × 30 d) food emulsion system (Roedig-Penman & Gordon, 1997). In a fish muscle model system, the potency of TC in the prevention of oxidation was in the decreasing order: EGCG > ECG > EGC > EC (He & Shahidi, 1997). In soy lecithin liposomes oxidized at 50 °C, the antioxidant activity of TC was in the order: EGCG > EC > EGC > ECG (Huang & Frankel, 1997). Although the antioxidant activities of individual pure standard TC were system-dependent, results from this current study showed clearly that TC with galloyl structures (EGCG, ECG and EGC) are more powerful antioxidants than those of non-galloyl structures.

TBARS numbers, in the oxidized DHA-PC liposomes treated with a commercial TC extract (40% EGCG, 24% EGC, 12% ECG and 10% EC), were the lowest of the samples examined (Fig. 2b), indicating that when individual TC constituents are combined, they have a greater effect on inhibition of lipid oxidation than individual components. As a predominant compound, EGCG plays a most important role in prevention of liposome oxidation.

3.4. Chelating activity of TC on Fe^{2+}

TC had a significantly ($P < 0.05$) weaker chelating activity (6–29%) on Fe^{2+} ions at concentrations of 200–1000 ppm, than citric acid (CA) or EDTA, both strong metal chelators (Fig. 3a). At a concentration of 400 ppm of the individual TC standards (Fig. 3b), EGCG had the highest chelating activity (38%), followed by EC (30%), ECG (19%) and EGC (non-detectable), whereas, EDTA and CA had chelating activities of 100 and 54%, respectively. Record, McInerney and Dreosti (1996) reported that green tea extract, at a concentration of 0.0025%, had significant metal-chelating capacity. However, the result obtained from the current study showed that TC possessed limited chelating effects on Fe^{2+}.

3.5. Free radical scavenging activity of TC

The effects of TC on scavenging free radical DPPH are presented in Fig. 4a and b. The free radical-scavenging activity for commercial TC extract, at concentrations ranging from 200 to 1000 ppm, was over 90% of control values. As the concentration increased from 200 to 600 ppm, the scavenging activities of samples increased, however, these differences were not significant. When the concentrations increased beyond 600 ppm, their scavenging activities decreased significantly ($P < 0.05$; Fig. 4a).

The commercial TC extract and individual TC standards had significantly ($P < 0.05$) higher scavenging activities, with values of over 94%, compared with VE and L-ascorbic acid (AA), with values of 89 and 82%, respectively (Fig. 4b). TC had scavenging activity of 98%, which was significantly ($P < 0.05$) higher than the individual TC standards, showing that individual TC, when combined, had some additive effects on scavenging activity on DPPH. EGCG showed the highest value of scavenging activity, followed by ECG, EGC and EC, but no significant differences were observed among these catechins. Nanjo, Goto, Seto, Suzuki, Sakai, and Hara (1996) reported that TC and their epimers showed 50% DPPH radical-scavenging ability at concentrations ranging from 1 to 3 μM. The authors suggested that the galloyl moiety, attached to flavan-3-ol at position 3, had a strong scavenging ability on the DPPH free radical and also pointed out that the ortho-trihydroxyl group in the B-ring also played a more important role in radical-scavenging than the ortho-dihydroxyl group. Sánchez-Moreno, Larrauri, and Saura-Calixto (1999) reported that VE had a lower activity of DPPH free radical-scavenging than polyphenolic constituents of wines.

4. Conclusion

Dietary TC showed inhibiting effects of lipid oxidation for 1-year frozen-stored chicken meat. TC-treated groups demonstrated significantly ($P < 0.05$) higher levels of VE in their meat than control groups. The inhibition of VE reduction in chicken meat was positively related to levels of TC supplemented in the diet. TC300, in particular, had strong protective effect of VE. The effect of VE-protection, due to dietary TC, may partly contribute to the strong antioxidant activity of TC in vivo.

TC also showed significant antioxidant activity in iron-induced oxidized DHA-PC liposomes. The potency decreased in the order: EGCG > TC > ECG = EGC > EC for PC liposome and TC > ECG > EC > EGCG > EGC for DHA-PC liposome. In addition to some chelating effects on Fe^{2+}, TC possessed strong scavenging capacity for DPPH free radical. The powerful free radical-scavenging activity and some metal chelating effects of TC may be a mechanism for TC as potential antioxidants for chicken meat and fatty foods in vitro.

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