



Effect of pH on the Radical Quenching Capacity of Tea Infusions Using the ABTS^{•+} Assay

Yuk Man Chan¹, Nga Kwan Cheng¹, Poonam Singh Nigam¹
and Richard Owusu-Apenten^{1*}

¹Faculty of Life and Health Sciences, School of Biomedical Sciences, Ulster University, Cromore Road, BT51 8DX, Coleraine, UK.

Authors' contributions

This work was carried out in collaboration between all authors. Author ROA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors YMC and NKC managed the literature searches, performed the spectroscopy analysis. Authors ROA and PSN managed the experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/27235

Editor(s):

(1) Martin Koller, University of Graz, Research Management and Service, c/o Institute of Chemistry, Austria.

Reviewers:

(1) Gokhan Zengin, Selcuk University, Turkey.

(2) Anonymous, National Institute of Fundamental Studies, Sri Lanka.

Complete Peer review History: <http://sciencedomain.org/review-history/15117>

Short Research Article

Received 25th May 2016
Accepted 16th June 2016
Published 22nd June 2016

ABSTRACT

Aims: The aims of this study were to assess the impact of pH on the free radical quenching activity of tea infusions using a modified 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay and three antioxidant compounds as reference.

Study Design: *In-vitro* method.

Place and Duration of Study: Faculty of Life and Health Science, School of Biomedical Sciences, Ulster University, UK. From Sept 2014 and May 2016.

Methodology: Free radical quenching capacity of tea (Earl grey, black tea, Ceylon tea, & green tea) infusions were investigated using persulfate activated ABTS with acetate buffer (pH 4.5) or phosphate buffer saline (pH 7.0) as solvent. Tests were performed using 96-well microplates, 20 μ l of sample and 280 μ l of ABTS reagent, and calibrated using ascorbic acid, trolox or gallic acid as reference antioxidants.

Results: Gallic acid free radical quenching was pH dependent and unsuitable as reference. The free radical quenching capacity of trolox and ascorbic acid was not significantly different at pH 4.5

*Corresponding author: E-mail: r.owusu-apenten@ulster.ac.uk;

and pH 7.0. The radical quenching capacity of tea infusions expressed as Trolox Equivalent Antioxidant Capacity (TEAC) or Ascorbic Acid Equivalent Antioxidant Capacity (AAEAC) was greater by 50-300% at pH 7 compared to pH 4.5.

Conclusion: The modified ABTS assay is suitable for examining the influence of pH on free radical quenching ability of tea samples. Gallic acid was not a suitable reference compound. The radical quenching capacity of tea infusions increases with rising pH.

Keywords: Radical quenching; ABTS; tea; gallic acid; ascorbic acid; TEAC value.

1. INTRODUCTION

The role of different reference compounds for standardizing antioxidant assays has not been explored in detail [1]. The first international congress on antioxidant methods recommended the "TEAC assay" for collaborative study [2]. The radical quenching assay, which uses 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and persulfate radical initiator [3], is sensitive for lipophilic and hydrophobic antioxidants over a wide pH-range [4-10]. Recent investigations highlighted the effect of pH on ATBS assay kinetics [11,12].

Consumption of tea increases plasma antioxidant capacity [13-15]. There is increasing interest also in the possible health benefits of tea extracts [16]. Currently, the evaluation of tea antioxidant capacity remains problematic due to chemical ionizations and the varying pH levels observed for the human digestive tract and also plasma [17,18]. The extraction efficiency and stability of antioxidants are also influenced by pH [19].

Free radical quenching capacity is frequently expressed as the Trolox Equivalent Antioxidant Capacity (TEAC) as measured using the ABTS assay. In a recent publication, trolox was demonstrated to be a more suitable reference compared to gallic acid for the FRAP assay of tea infusions at different pH values [20]. The aims of the present study were to examine the effect of pH on the free radical quenching activity of tea infusions using the ABTS assay. Four tea varieties were examined with trolox, ascorbic acid or gallic acid as reference antioxidants. Radical quenching capacity was evaluated at pH 7.0 and pH 4.5 and described in terms of TEAC, Ascorbic Acid Equivalent Antioxidant Capacity (AAEAC) or Gallic Acid Equivalent Antioxidant Capacity (GAEAC). The results indicate that the radical quenching capacity was increased for tea infusions at pH 7.0 compared to pH 4.5. The size of the pH effect on free radical quenching capacity varied with the antioxidant reference adopted. Such findings were confirmed using

values for the IC_{50} , which is the concentration of tea required for 50% inhibition of ABTS radicals, independent of any particular reference antioxidant.

2. MATERIALS AND METHODS

2.1 Materials

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), methanol, trolox, gallic acid, ascorbic acid and most reagents were purchased from Sigma Aldrich Ltd. Four of tea bag brands were purchased from local supermarkets as follows: green tea (ASDA), Ceylon tea (Marks & Spencer), English black tea (Knightsbridge), Earl grey (Twinning's).

2.2 ABTS Reagent Formulations at pH 7.0 and pH 4.5

Free radical quenching was evaluated using the ABTS persulfate assay described previously with modification [21]. Briefly, ABTS stock solution (0.53mM) was prepared with phosphate buffer saline (pH 7) or sodium acetate (0.1M, pH 4.5) buffer. Sodium persulfate (7.4 mM) was prepared in the corresponding buffer and an ABTS working solution was prepared by adding 10ml persulfate solution and 90 ml ABTS stock solution and incubating in the dark for 16 hrs. The resulting solution was diluted with buffer so that the initial absorbance at 734 nm ($A_{734.1}$) was 0.85 (~55 μ M) as measured in a standard colorimeter (Ultrospec 2000 UV/VIS Spectrophotometer; Pharmacia Biotech).

2.3 Tea Infusions and Antioxidant Standard

Tea infusions were prepared by soaking pre-weighed tea bags with boiling water (100 ml) for 10 minutes and allowing cooling to room temperature. Samples were centrifuged ($\times 11,000$ RPM for 5 min) using a microcentrifuge and analyzed as described below.

2.4 Microplate ABTS Assay for Tea Infusions

Exactly 20 μl of reference compounds (trolox, ascorbic acid or gallic acid 0-1 mM) was added to 96-well microplates followed with 280 μl of ABTS solution. The mixtures were agitated briefly, stored in the dark for 30 minutes and absorbance was read at 734 nm using a microplate reader. Tea infusions were pre-diluted with deionized water (250-200 fold) and analyzed similarly.

2.5 Data Analysis and Statistical Analysis

2.5.1 Calibration parameters for the ABTS assay

The ABTS colorimetric analysis conforms to Beer's Law, (eq. 1), where ΔA equal $A_{734\perp}$ minus the absorbance of the ABTS solution with added test sample ($\Delta A_{734(S)}$); C = concentration of test compound, and ϵ ($\text{M}^{-1} \text{cm}^{-1}$) is the molar absorptivity when the *test compound* reacts with excess ABTS.

$$\Delta A = \epsilon I C \quad (1)$$

Calibrations graphs (ΔA versus C) were fitted with linear trend-lines ($Y = x \cdot \text{GRAD}$) and values for GRAD and standard errors ($\text{GRAD} \pm \text{SEM}$) were determined using 16 data points collected from triplicate experiments.

Calibration parameters such as the minimum detectable concentration for antioxidant (MDC) were determined; $\text{MDC} = 3\text{SD}_0/\text{GRAD}$ where SD_0 = standard deviation for colorimetric analysis for a reagent blank. Values for GRAD equal to the apparent molar absorptivity (ϵ_{\perp}) for microplate analysis were subjected to pathlength correction as described previously [22]. In brief, ϵ ($\text{M}^{-1} \text{cm}^{-1}$) = $\text{GRAD}/\text{microplate pathlength}$ (0.73 cm). The stoichiometry (n) showing the number of ABTS radicals transformed per molecule of antioxidant, was determined using the molar absorptivity for $\text{ABTS}^{\bullet+}$ ($1.5 \times 10^4 \text{M}^{-1} \text{cm}^{-1}$) [3] and the relation, $n = \epsilon$ ($\text{M}^{-1} \text{cm}^{-1}$)/ $1.5 \times 10^4 \text{M}^{-1} \text{cm}^{-1}$. All routine data manipulation was conducted in Microsoft excel.

2.5.2 ABTS radical quenching referenced to trolox and other standards

The radical quenching capacity (RQC) of tea infusions was calculated from eq. (2) below;

$$\text{RQC} = \frac{\Delta A}{\text{GRAD}} * A_v * \frac{V_{\text{ex}}}{S_v} * \text{DF} * \frac{\text{FW}}{W} * 10^5 \quad (2)$$

where, A_v = microplate assay volume ($300 \times 10^{-6}\text{l}$), V_{ex} = tea extract volume ($1 \times 10^{-2}\text{l}$), S_v = tea infusion sip volume assayed ($20 \times 10^{-6}\text{l}$), DF = dilution factor for sample (1 if undiluted), FW = formula weight of the reference antioxidant (g/mole), W = dry weight of tea sample. With trolox as reference, the units for RQC are mg-Trolox Equivalent Antioxidant Capacity per 100 g dry weight of sample (mg-TEAC/100 g). By replacing the terms for GRAD and FW in eq. (2) using the corresponding calibration parameters for ascorbic acid and gallic acid the radical quenching capacity was expressed as Ascorbic Acid Equivalent Antioxidant Capacity (AAEAC) or Gallic Acid Equivalent Antioxidant Capacity (GAEAC), respectively.

2.5.3 Radical quenching referenced to ABTS inhibitory concentration (IC_{50})

Values for the IC_{50} , which is concentration of test compound that inhibits 50% of ABTS, were determined based on the percent ABTS inhibition (%INH) defined in (eq. 3).

$$\% \text{INH} = (\Delta A / A_{734\perp}) * 100 \quad (3)$$

Combining eq. (3) and eq. (1) gives eq. (4) and eq. (5).

$$\% \text{INH} = (\epsilon_{\perp} C / A_{734\perp}) * 100 \quad (4)$$

$$\% \text{INH} = m \cdot C \quad (5)$$

It may be expected from eq. (5) that a graph %INH versus concentration (C) produces a straight-line graph with the gradient (m) and IC_{50} was calculated from eq. (6).

$$\text{IC}_{50} = 50 / m \quad (6)$$

3. RESULTS

3.1 ABTS Assay Calibration Using Three Reference Compounds

Table 1 show calibration parameters for the ABTS assay using trolox, gallic acid or ascorbic acid as reference antioxidants. The graphs of antioxidant concentration (mol/l) plotted versus ΔA produced straight-lines with regression coefficients (R^2) of 0.96-0.99. Tables 1 also lists the "true" molar absorptivity values obtained by

adjusting microplate values to match output from a 1-cm spectrophotometer, allowing for a pathlength of 0.73 cm [22]. The reaction stoichiometry (n) indicated that one molecule of antioxidant could quench 1-10 moles of ABTS radical (Table 1). Moreover, the response with gallic acid was sensitive to pH whilst trolox and ascorbic acid were less affected. All assays had 30-minute duration and so kinetic effects were not considered.

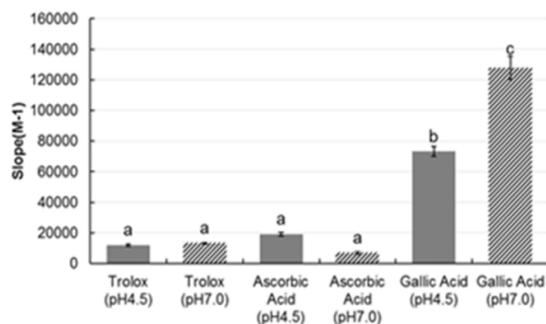


Fig. 1. ABTS calibration slopes using trolox, ascorbic acid, or gallic acid as standards at pH 4.5 and pH 7.0

Analyses were performed using 96-microplates, with 20 μ l of sample and 280 μ l of ABTS solution and absorbance at 734 nm. Solvents were acetate buffer (pH 4.5) or phosphate buffer saline (pH 7.0)

From Fig. 1 and Table 1, rising solvent pH increased the reaction stoichiometry for gallic acid from ~6 to 11 molecules of ABTS. In addition, the molar absorptivity change arising from gallic acid reaction with ABTS radical increased from 9.1×10^4 ($M^{-1} cm^{-1}$) to 16.0×10^4 ($M^{-1} cm^{-1}$). By contrast, the molar absorptivity with ascorbic acid or trolox were not significantly different ($P = 0.05$) as a function of pH. On the basis of molar absorptivity value, the ranking of free radical quenching capacity was, gallic acid (pH 7.0) > gallic acid (pH 4.5) > trolox = ascorbic acid (pH 4.5 & pH 7.0).

3.2 Radical Quenching by Tea Infusions Referenced to Standard Compounds

Fig. 2 shows the ABTS radical quenching activity for Earl Grey, black tea, green tea, and Ceylon tea infusions expressed as mg-GAEAC/100 g (Fig. 2a), mg-AAEAC/100 g (Fig. 2b) or mg-TEAC/100 g (Fig. 2c) of dried sample. Based on values for GAEAC the free radical quenching capacity for three tea infusions (green tea, Ceylon tea, and black tea) were slightly greater at pH4.5 compared to pH7 or unaffected by pH

(Earl grey). By contrast values TEAC or AAEAC for tea infusions were higher at pH7.0 compared to pH4.5 (Figs. 2b and 2c).

3.3 Radical Quenching Referenced to IC₅₀ Value for Tea Infusions

Fig. 3 shows values for IC₅₀ ranged from $8(\pm 0.6)$ μ g/ml for the green tea (pH 7) rising to $24 (\pm 1.2)$ μ g/ml for Earl gray tea infusion (pH 4.5). All tea infusions showed *decreased* IC₅₀ (increasing radical quenching) at pH 7 compared to pH 4.5. These results are not dependent on any particular reference antioxidant.

4. DISCUSSION

4.1 Effect of Reference Compounds and pH for Calibration

Trolox and ascorbic acid produced pH-stable radical quenching activity and their calibration characteristics were not statistically different over pH 4.5 to pH 7.0. In contrast, gallic acid showed pH-sensitive radical quenching capacity with ABTS^{•+}. Such results are consistent with past reports showing that rising pH increases polyphenol hydroxyl group ionization, facilitates electron transfer and increases antioxidant capacity [20]. The current results agree also with suggestions for adopting ascorbic acid as a reference standard for the ABTS assay under some circumstance [1]. ABTS radical quenching was reportedly unchanged from pH 3-pH 6 with each molecule of ascorbic acid scavenging two ABTS molecules [7]; the current results showed an average ABTS: ascorbic acid stoichiometry of 1:1 in agreement with other results [21].

4.2 Effect of pH on the Free Radical Quenching Capacity of Tea Samples

The radical quenching capacity for tea samples showed clear differences at pH 7.0 and pH 4.5 where trolox or ascorbic acid were used as the reference antioxidant. Since, free radical quenching is ultimately a reflection of electron-transfer ability, the tendency for tea components to quench ABTS radical should decrease at low pH with increasing H⁺ ion concentration. Interestingly, antioxidant capacity was sometimes found to be elevated with decreasing pH due to enhanced extractability and stability of plant components [23]. Other investigators employed trolox as a reference compound in order to compare results for ABTS, DPPH and FRAP assays [24].

Tea antioxidant capacity was demonstrably higher at pH 7.0 compared to pH 4.5, using values for IC₅₀ which were not dependent on using trolox, ascorbic acid or gallic acid as a reference. However, it can be anticipated that the IC₅₀ depend on the initial concentration of ABTS free radical. From eq. 4- eq. 6, it may be noted

that, $m = 100\epsilon_{1} / A_{734\perp}$ and consequently the IC₅₀ increases if one uses higher initial concentrations of ABTS (e.g. $A_{734\perp}/\epsilon_{1}$). This observation highlights that need to adopt well-defined ABTS concentrations during free radical quenching analysis.

Table 1. Calibration parameters for the ABTS assay at pH 4.5 and pH 7.0

pH 4.5	R ²	MCD	ϵ (M ⁻¹ cm ⁻¹)	CV (%)	n
Gallic acid	0.9956	5.5E-07	91080	3.3	6.1
Ascorbic acid	0.9676	6.2E-07	24170	3.3	1.6
Trolox	0.9993	7.3E-07	17500	0.5	1.2
pH 7.0	R ²	MCD	ϵ (M ⁻¹ cm ⁻¹ *)	CV (%)	n
Gallic acid	0.9914	2.9E-07	160428	6	10.7
Ascorbic acid	0.9898	1.6E-05	10903	5.8	0.7
Trolox	0.9971	4.5E-06	17166	3.9	1.1

Notes: Analysis were performed in 96-well microplates, ϵ (M⁻¹ cm⁻¹) = molar absorptivity normalized for 1-cm pathlength instrument with a microplate pathlength = 0.76 cm, reaction stoichiometry (n) = moles of ABTS quenched per mole of reference antioxidant, MDC = minimum detectable concentration of test compound, CV (%) is the within-assay coefficient of variation (n = 16 data points)

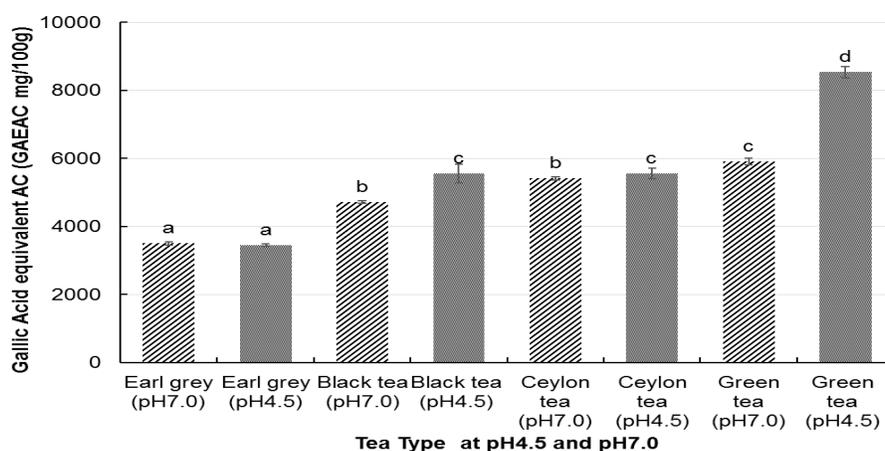


Fig. 2a. Free radical quenching activity of tea infusions at pH 4.5 and pH7.0
Data is referenced to gallic acid as standard (see text for details)

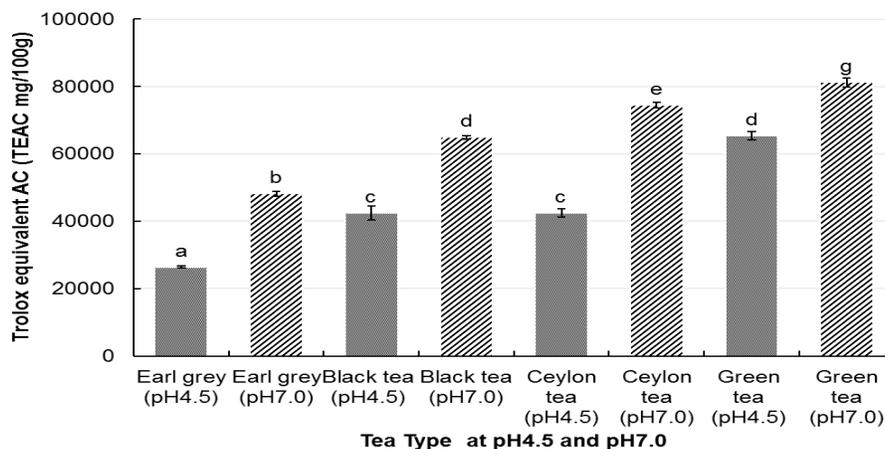


Fig. 2b. Free radical quenching activity of tea infusions at pH 4.5 and pH7.0
Data is referenced to trolox as standard (see text for details)

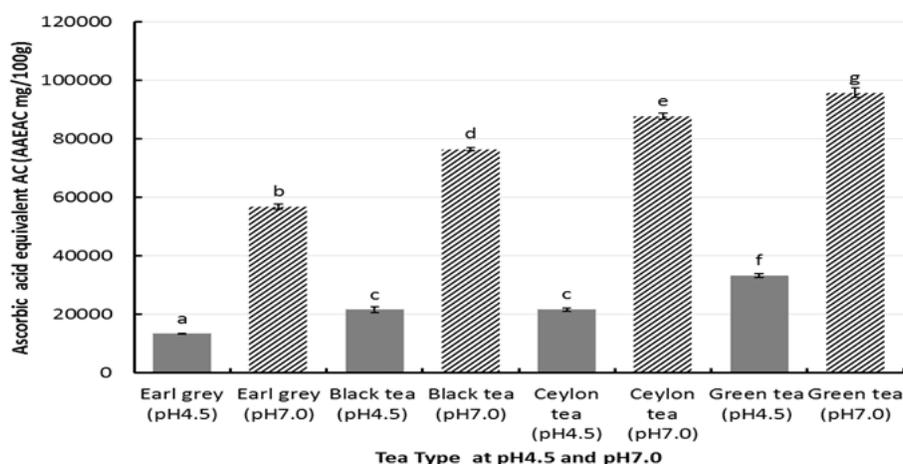


Fig. 2c. Free radical quenching activity of tea infusions at pH 4.5 and pH 7.0
Data is referenced to ascorbic acid as standard (see text for details)

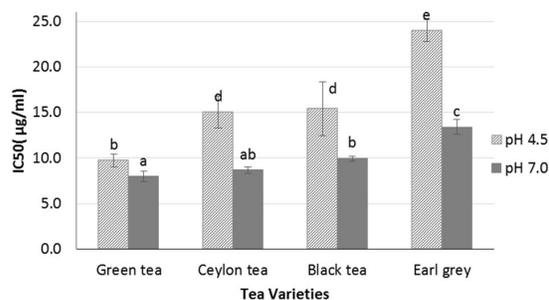


Fig. 3. Free radical inhibitory activity of tea infusions at pH 4.5 and pH 7.0

Values are expressed as the 50% inhibitory concentration of tea infusion (IC50 µg/ml). Bars with different letters are significantly different ($P = 0.05$)

In agreement with current results, previous studies showed that the radical quenching activity of hydroxyflavones were increased due to the dissociation of hydroxyl groups at high pH [4]. The antioxidant capacity of anthocyanins were increased at neutral pH compared to acidic pH [5,6]. Caffeic acid showed increased radical quenching capacity with increases of pH but the kinetics slowed at high pH [8].

5. CONCLUSION

The free radical quenching capacity for tea infusions increases with pH but the magnitude observed depends on choice of antioxidant reference compound. Trolox or ascorbic acid are more suitable as reference compound for the ABTS assay as compared to gallic acid, which is itself pH-sensitive. As a future recommendation,

assays for total antioxidant capacity should be checked for potential confounding responses which may arise from adopting a single internal reference compound.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agric Food Chem.* 2002;50(13):3713-7. PMID: 12059148.
- Finley JW. Introduction: White papers from the first international congress on antioxidant methods. *J Agric Food Chem.* 2005;53(10):4288-4289.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* 1999;26(9-10):1231-7. PMID: 10381194.
- Lemańska K, Szymusiak H, Tyrakowska B, Zieliński R, Soffers AE, Rietjens IM. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radic Biol Med.* 2001;31(7):869-81. PMID: 11585705.
- Matsumoto H, Nakamura Y, Hirayama M, Yoshiki Y, Okubo K. Antioxidant activity of black currant anthocyanin aglycons

- and their glycosides measured by chemiluminescence in a neutral pH region and in human plasma. *J Agric Food Chem.* 2002;50(18):5034-7. PMID: 12188603.
6. Nielsen IL, Haren GR, Magnussen EL, Dragsted LO, Rasmussen SE. Quantification of anthocyanins in commercial black currant juices by simple high-performance liquid chromatography. Investigation of their pH stability and antioxidative potency. *J Agric Food Chem.* 2003;51(20):5861-6. PMID: 13129285.
 7. Takebayashi J, Tai A, Yamamoto I, pH-dependent long-term radical scavenging activity of AA-2G and 6-Octa-AA-2G against 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation. *Biol Pharm Bull.* 2003;26(9):1368-70.
 8. Amorati R, Pedulli GF, Cabrini L, Zambonin L, Landi L. Solvent and pH effects on the antioxidant activity of caffeic and other phenolic acids. *J Agric Food Chem.* 2006;54(8):2932-7. PMID: 16608211.
 9. Dawidowicz AL, Olszowy M. Antioxidant properties of BHT estimated by ABTS assay in systems differing in pH or metal ion or water concentration. *Eur Food Res Technol.* 2011;232(5):837-842.
 10. Ferri M, Gianotti A, Tassoni A. Optimization of assay conditions for the determination of antioxidant capacity and polyphenols in cereal food components. *J. Food Comp. Anal.* 2013;30(2):94-101.
 11. Zheng L, Zhao M, Xiao C, Zhao Q, Su G. Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: Importance of controlling reaction pH and time. *Food Chem.* 2016;192:288-294. PMID: 26304349.
 12. Ozgen M, Reese RN, Tulio AZ Jr, Scheerens JC, Miller AR. Modified 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of Selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *J Agric Food Chem.* 2006;54(4):1151-7. PMID: 16478230.
 13. Benzie IF, Szeto YT, Strain JJ, Tomlinson B. Consumption of green tea causes rapid increase in plasma antioxidant power in humans. *Nutr Cancer.* 1999;34(1):83-7. PMID: 10453446.
 14. Langley-Evans SC. Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *Int J Food Sci Nutr.* 2000;51(5):309-315.
 15. Leenen R, Roodenburg AJ, Tijburg LB, Wiseman SA. A single dose of tea with or without milk increases plasma antioxidant activity in humans. *Eur J Clin Nutr.* 2000;54(1):87-92. PMID: 10694777.
 16. Johnson R, Bryant S, Huntley AL. Green tea and green tea catechin extracts: An overview of the clinical evidence. *Maturitas.* 2012;73(4):280-287. PMID: 22986087
 17. Chen GL, Hu K, Zhong NJ, Guo J, Gong YS, Deng XT, Huang YS, Chu DK, Gao YQ. Antioxidant capacities and total polyphenol content of nine commercially available tea juices measured by an in vitro digestion model. *Eur Food Res. Technol.* 2013;236(2):303-310.
 18. Henning SM, Zhang Y, Rontoyanni VG, Huang J, Lee RP, Trang A, Nuernberger G, Heber D. Variability in the antioxidant activity of dietary supplements from pomegranate, milk thistle, green tea, grape seed, goji, and acai: Effects of in vitro digestion. *J Agric Food Chem.* 2014; 62(19):4313-21. PMID: 24745654.
 19. Li N, Taylor LS, Ferruzzi MG, Mauer LJ. Kinetic study of catechin stability: Effects of pH, concentration, and temperature. *J Agric Food Chem.* 2012;60(51):12531-9. PMID: 23205662.
 20. Wong CW, Cheung WS, Lau YY, Bolanos de la Torre AAS, Owusu-Apenten R. A frap assay at pH 7 unveils extra antioxidant activity from green, black, white and rooibos tea but not apple tea. *F Nutr Repr.* 2015;1(1):16-23.
 21. Walker RB, Everette JD. Comparative reaction rates of various antioxidants with ABTS radical cation. *J Agric Food Chem.* 2009;57(4):1156-61. PMID: 19199590.
 22. Bolanos de la Torre AA, Henderson T, Nigam PS, Owusu-Apenten RK. A universally calibrated microplate ferric reducing antioxidant power (FRAP) assay for foods and applications to Manuka honey. *Food Chem.* 2015;174:119-23. PMID: 25529660.

23. Liyana-Pathirana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J Agric Food Chem. 2005;53(7):2433-40. PMID: 15796575.
24. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. 2006;19(6-7):669-675.

© 2016 Chan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

*The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/15117>*