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SPECTROPHOTOMETRIC METHODS FOR DETERMINING TROLOX EQUIVALENT ANTIOXIDANT CAPACITIES OF TEA THROUGH DIFFERENT *In Vitro* ASSAYS

(Kaedah Spektrofotometrik bagi Penentuan Trolox Kapasiti Antioksida Teh Melalui Pelbagai Ujian *In Vitro*)

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Abstract

Tea (*Camellia sinensis*) is among the most popular non-alcoholic beverages consumed daily in the world due to its unique taste and flavor. Tea leaves and their products have obtained significant attention because of their high antioxidant capacities. In this study, the analytical methods based on the molecular absorption spectrophotometric principle were evaluated for determining the Trolox equivalent antioxidant capacities (TEACs), including 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity, ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) in several commercial dried tea products originated from Lam Dong Province (Vietnam). Double liquid extraction in a water bath at 70 ℃ (10 min for each extraction cycle) was employed to obtain the extracts for further colorimetric reactions. The results demonstrate low limits of detection and quantification (12.3-37.3 µmol TE/L, 9.9-30.1 µmol TE/L, and 11.8-35.7 µmol TE/L for ABTS, FRAP, and CUPRAC, respectively), wide working ranges (100-700 µmol TE/L), favorable repeatability (RSD_r of 1.03-1.52%), reproducibility (RSD_R of 1.26-1.55%), and acceptable recoveries (98.0-101%) according to Appendix F of AOAC (2016). The Shewhart charts were also constructed based on the UV-Vis 1800 Shimadzu (Japan) from 21 separate working days to control the accuracy and precision of the analytical results. For tea products in Lam Dong, the TEACs of all samples in the descending order were determined as green tea > oolong tea > black tea, which could be mainly due to the differences in the oxidation levels during the fermentation. In addition, strong correlation was recorded for pairs of FRAP-CUPRAC (R^2 = 0.8579), ABTS-FRAP (R^2 = 0.8453), and ABTS-CUPRAC (R^2 = 0.710).

Keywords: tea, antioxidant capacities, 2,2'-Azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid radical scavenging activity, ferric reducing antioxidant power, cupric reducing antioxidant capacity

Abstrak

Teh (*Camellia sinensis*) merupakan minuman tak beralkohol popular yang diambil setiap hari di seluruh dunia kerana keunikkan rasanya. Daun teh dan produknya mendapat perhatian penting kerana mempunyai kapasiti antioksida yang tinggi. Dalam kajian

ini, kaedah analisis berasaskan prinsip spektrofotometrik serapan molekul telah dinilai bagi penentuan kapasiti antioksida setara Trolox (TEACs), termasuk cerakin pemerangkapan radikal 2,2'-Azino-bis(3-etilbenzothiazolin-6-asid sulfonik) (ABTS), kuasa antioksida penurunan ferik (FRAP) dan kapasiti antioksida penurunan kuprik (CUPRAC) di dalam pelbagai produk teh komersial berasal dari wilayah Lam Dong (Vietnam). Pengekstrakan cecair cecair di dalam rendaman air pada suhu 70 °C (10 min bagi setiap kitaran pengekstrakan) telah dibangunkan bagi tujuan memperolehi hasil ekstrak sebelum tindak balas kolorimetrik. Hasil menunjukkan had pengesanan dan pengkuantitian rendah (12.3-37.3 µmol TE/L, 9.9-30.1 µmol TE/L,dan 11.8-35.7 µmol TE/L masing-masing bagi ABTS, FRAP, dan CUPRAC), julat kelinearan (100-700 µmol TE/L), kebolehulangan (RSD_r of 1.03-1.52%), kebolehasilan semula (RSD_R of 1.26-1.55%), dan perolehan semula (98.0-101%) berdasarkan Appendix F AOAC (2016). Carta Shewhart telah dibangunkan berdasarkan UV-Vis 1800 Shimadzu (Jepun) dari 21 hari bekerja berbeza bagi mengawal ketepatan dan kejituan hasil analisis. Bagi produk teh Lam Dong, nilai TEACs semua sampel di susun tertib menurun iaitu teh hijau > teh oolong > teh hitam, disebabkan oleh perbezaan tahap pengoksidaan semasa penapaian. Tambahan lagi, hubungkait yang kuat direkodkan bagi pasangan FRAP-CUPRAC (R^2 = 0.8579), ABTS-FRAP (R^2 = 0.8453), dan ABTS-CUPRAC (R^2 = 0.710).

Kata kunci: teh, keupayaan antioksida, ,2'-Azino-bis(3-etilbenzothiazolin-6-asid sulfonik), kuasa antioksida penurunan ferik, kapasiti antioksida penurunan kuprik

Introduction

Antioxidants are known as any substances capable of slowing or preventing the oxidation of easily oxidized components [1]. In addition, they have been commonly used in food production to limit the deterioration in food quality and maintain nutritional values, in which the antioxidants can slow lipid oxidation by inhibiting the initial free radical formation or preventing the generation of free radicals [2]. Plants and food products containing high contents of antioxidants perform great biological benefits since they are reported to have the ability to protect the animals' and humans' bodies against oxidizing agents, such as reactive oxygen, nitrogen, and chlorine species [3]. Dietary antioxidants generally belong to the family of phenolic and polyphenolic compounds. The phenolic compounds occurring in foods are in the phenylpropanoid (C6-C3) family and are also the derivatives of cinnamic acid. The condensation of C6–C3 compounds through the participation of malonyl coenzyme A leads to the formation of chalcones, which can then cyclize under acidic conditions to produce flavonoids and isoflavonoids. The antioxidant activity of flavonoids is significantly dependent on the number and position of hydroxyl groups in the molecule structures [2]. In tea leaves and their products, the antioxidant activity is mainly due to the combination of aromatic rings and hydroxyl groups incorporating their chemical structures, thereby binding and neutralizing lipid free radicals basically by these hydroxyl groups. The antioxidant compounds in tea, including catechins, inhibit the formation of lipid peroxides by binding lipid alkoxyl radicals. Such activity depends on the structure of the molecules, and the number and position of the hydroxyl groups in the molecule. Specifically, the –OH groups at the 3'-, 4'-, and 5'- positions in the B-ring of flavonoids would increase the value of the measured antioxidant capacities. Sample matrices containing the vast majority of simple phenolic compounds with only one hydroxyl group exhibit lower antioxidant activity [4, 5]. The catechin compounds in green tea also exhibit antioxidant capacities through the inhibition of pro-oxidant enzymes and the formation of antioxidant enzymes. The antioxidant capacities generally show a general trend as follows: green tea > oolong tea > black tea [2].

Food contains macronutrients such as carbohydrates, proteins, and lipids as well as trace elements such as minerals and vitamins. These components with the potential to interact with antioxidant compounds are present at various concentrations in the sample matrices and performed different activity behaviors. Therefore, the antioxidant capacities of food products obtained from a single chemical reaction mechanism do not provide adequate information due to the diversity of the chemical compositions in different samples, which may influence the results [2, 6, 7]. Moreover, there have been no officially published standardized methods for determining the antioxidant capacities of any food in general, and tea products in specific. Thus, there are discrepancies in the extraction and analytical methods presented in different papers. The performance of the analytical method in determining the antioxidant capacities based on different mechanisms of chemical

reaction in a specific food product should be evaluated to be applied in routine analysis and research activities. The determination of antioxidant capacities based on molecular absorption spectrophotometry is considered as one of the most effective approaches to assess and compare the general information about the antioxidant activity in various foods and drinks. Particularly, the changes in antioxidant capacities can occur when foods undergo processing and storage periods [2, 7, 8]. However, the antioxidant activity should be interpreted specifically based on the chemical nature of the applied methods and the chemical compositions of the analyzed samples. The chemical nature of the method used is determined based on the types of reagents used and the reaction mechanisms between the agents and the antioxidant components present in the sample matrices. The antioxidant activity was evaluated by various methods, both in vitro and/or in vivo. Based on the chemical nature of the in vitro process, the methods for the determination of antioxidant activity can be classified into different mechanisms such as single electron transfer (SET), hydrogen atom transfer (hydrogen atom transfer-HAT), and association mechanisms. The single-electron transfer methods are known as the most popular method, which is based on free radical scavenging reaction, ferric reducing antioxidant power (FRAP) [7], 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical neutralizing/scavenging activity [9], 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical neutralizing/scavenging activity [5], cupric-reducing antioxidant capacity (CUPRAC) [7]. For tea leaves and their products, most of the published studies have focused on 1 and/or 2 determination assays to assess antioxidant capacities. The combination of two or more assays assists to perform a more adequate reflection of the antioxidant activity behavior [10, 11].

The objectives of this current study are to evaluate the performance of the analytical method for determining the Trolox equivalent antioxidant capacities (TEACs) via three different in vitro assays, namely ABTS, FRAP, and CUPRAC in dried tea products. The evaluation included the estimation of the limit of detection and quantification (LOD and LOQ), the establishment of calibration curves, the assessment of repeatability (intra-day precision) and reproducibility (inter-day precision), method accuracy through the recovery test, and the construction of the quality control charts. Then, the analytical methods were applied for the determination and assessment of TEACs of several commercial dried tea products belonging to different types of green, oolong, and black teas originating from Lam Dong Province, one of the prominent tea plantation regions located in the South of the Vietnam.

Materials and Methods

Sampling and pretreatment

To evaluate the analytical methods for determining the Trolox equivalent antioxidant capacities (TEACs) based on the three different assays of ABTS, FRAP, and CUPRAC in dried tea products. The commercial black tea product, called "investigated sample", was used to carry out the analytical method evaluation. Then, the evaluated methods were applied to 15 commercial dried tea products collected from the local supermarkets in Ho Chi Minh City (Vietnam). The tea products belong to three different types of green, oolong, and black tea, representing the non-fermented, partially fermented, and fully fermented tea types, respectively. As displayed on the tea product labels, these products originated from Lam Dong Province, one of the prominent tea plantation regions in the southern part of Vietnam. The tea products were packed in non-permeable bags (250 g per bag) and sealed under a vacuum. The purchased samples were transferred to the laboratory in Ho Chi Minh City and stored at −10 ℃ until pre-treatment and further use. The tea samples underwent a pre-treatment procedure [12] which involved homogenization in the for determination of TEACs. The homogenized tea samples were put in non-permeable bags sealed under a vacuum, stored at 25 ℃ with 70% humidity, away from direct sunlight.

Extracts were obtained from the well-homogenized tea products via the extraction procedure performed in ISO 14502-1:2005 [13] with some modifications. Shortly, 0.2 ($\pm$0.001) g of dried tea product was subjected to solid-liquid extraction using the 7:3 v/v mixture of methanol ($\geq$ 99.9%, Merck, Germany) and deionized water (MeOH:DIW) as the extraction solvent. The extraction was carried out in duplicate within 20 min (i.e., 10.00 mL solvent and 10 min for each extraction

cycle) at 70 ℃ in a water bath. All extracts were collected and transferred to a 50-mL volumetric flask. The extraction solvent with a ratio of MeOH:DIW (7:3 v/v) was used to generate the calibration mark. The extracts were then filtered through a 0.45 µm PTFE membrane, and further diluted 20 times by DIW before being subjected to colorimetric assays and spectrophotometric measurement on the UV-Vis 1800 Shimadzu (Japan).

ABTS assay

For the ABTS free radical scavenging activity, the reagent of 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS (≥ 98%, Merck, Germany) was used and the assay was carried out according to the method reported by Abdullahi et al. [14]. The ABTS radical cation was generated through the reaction between aqueous stock solutions of ABTS and $K_2S_2O_8$ for 12 to 16 hours in the dark at the ambient temperature to reach the final concentrations of 7.0 mmol/L and 2.45 mmol/L, respectively. Before use, the ABTS stock solution was diluted using acetate buffer solution of pH = 4.5 (0.3 mol/L) to obtain an absorbance of 0.74 ± 0.03 at 734 nm (working ABTS solution). In the presence of Trolox as the standard or an antioxidant in the sample matrices that are capable of giving hydrogen, the nitrogen atoms can quench the hydrogen atom in the ABTS reagent, resulting in the decolorization of the reaction solutions [15, 16]. In this current study, 200 µL of the diluted tea extract was mixed with 4000 µL of working ABTS solution. The mixture was shaken gently and protected from direct light for 7 minutes. The absorbance was measured at 734 nm for quantification purposes.

FRAP assay

The ferric reducing antioxidant power or FRAP was utilized to evaluate the reducing ability of the antioxidants present in dried tea products. The FRAP procedure was carried out according to the publication of Pellegrini et al. [7], which is based on the reduction reaction of Fe^{3+}–TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine, ≥ 98%, Merck, Germany) with antioxidant compounds in the sample matrices, forming Fe^{2+}–TPTZ. Briefly, a volume of 100 µL of the diluted tea extract was reacted with 3900 µL of working FRAP solution, comprising 300 mmol/L sodium acetate-acetic acid buffer of pH = 3.6, 10 mmol/L TPTZ solution, and 20 mmol/L ferric chloride solution at a volume ratio of 10:1:1. The binding of Fe^{2+} to the ligands from the reduction reaction creates a color of deeply intense navy blue, which absorption values at 593 nm were measured to determine the concentrations of antioxidants that correlates with the amount of iron reduced [7].

CUPRAC assay

For the analysis of cupric reducing antioxidant capacity or CUPRAC of tea products, the tea extract was mixed with $CuCl_2$ and neocuproine (Nc, ≥ 98%, Merck, Germany) working solutions, in which Cu(II) was reduced to Cu(I) through the action of electron-donating antioxidants. The procedure was briefly described as follows: 4.00 mL of the diluted tea extract was mixed with 1.00 mL of 10^{-2} mol/L $CuCl_2$; 1.00 mL of 7.5×10^{-3} mol/L neocuproine was diluted in ethanol; and 1.00 mL ammonium acetate buffer (pH = 7.0) was prepared by dissolving 19.27 g ammonium acetate in 250 mL DIW. After 1 hour of reaction, the absorbance was measured at 450 nm for the quantification of antioxidants that wer present in the tea products [7].

Analytical method evaluation

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%, Merck, Germany) was used as the standard for all criteria of interest. The quantification was carried out according to the calibration curves, which were established based on the linear relationship (y = ax + b) between the analyte concentrations (µmol TE/L) and the respective absorption. The calibration curves of FRAP and CUPRAC (reducing power) were constructed based on the linear relationship between the Trolox concentrations and the absorption signal of each standard solution after subtracting the signal of the blank solution ($Abs_{std} - Abs_{blk}$). For ABTS (radical scavenging activity), the calibration curve was built up according to the linear relationship between the Trolox concentrations and the values derived from the subtraction of absorbances (Abs) between the blank solution (blk) as the control sample and the signal of each standard (std) point ($Abs_{blk} - Abs_{std}$). The bias values for each standard point of the calibration curves

(calculated in percentage) were calculated by the equation 1 [17]:

$$\text{bias } (\%) = \frac{C_{cal.} - C_{std}}{C_{std}} \times 100 \quad (1)$$

where $C_{cal.}$ and C_{std} stand for the concentration of the standard solutions calculated from the established calibration curves and from the practical preparation (concentration values used to establish the calibration curves), respectively.

The limit of detection (LOD) and quantification (LOQ) of the analytical methods was estimated by simultaneously analyzing 11 separate blank solutions (containing only the extraction solvents and colorimetric reagents). Next, the estimated average concentrations ($\bar{x}$) and their standard deviation value (SD) were calculated to apply in the following relationships [18]:

$$LOD = \bar{x} + 3SD \text{ and } LOQ = 3LOD \quad (2)$$

The black tea (as the "investigated sample") was used for the repeatability assessment (intra-day precision) within one day (six replicates, n = 6) and reproducibility assessment (inter-day precision) for three separate days (six replicates per day) through the calculation of relative standard deviations, RSD_r and RSD_R, respectively, at the significance level of 0.05. In addition, the standard solution was spiked into this sample at three different levels ($0.5C_x$, $1C_x$, and $1.5C_x$, where C_x is the estimated analyte concentration in the investigated sample for the recovery test to evaluate the method accuracy.

The Shewhart charts were constructed for quality control by analyzing the investigated sample in successive runs along with "warning" and "action" limits employed to detect the arisen problems in different measurements on the UV-Vis 1800 Shimadzu (Japan) [18].

The evaluated analytical methods for determining ABTS, FRAP, and CUPRAC were applied to the collected dried tea products (performed in dried weight). All the analytical data were carried out in triplicate and analyzed by Microsoft Office Excel 2016 (version 16.54, Microsoft 365 Subscription), expressed as mean value ± standard deviation (SD). For potential correlation assessment of the measurements, the correlation analysis was performed using the statistical package software of SPSS (version 23).

Results and discussion

Calibration curves

Trolox, a derivative of vitamin E, is the most commonly used chemical standard for quantifying the antioxidant capacities from different sample matrices, including the reducing power, e.g., FRAP and CUPRAC, and free radical scavenging ability, e.g., ABTS. In addition, Trolox is soluble in polar solvents [19], thus compatible with the extraction solvent used in this study, i.e., 70% v/v MeOH in DIW.

For the analysis of ABTS, FRAP, and CUPRAC in dried tea products, the calibration curves were established for quantification purposes. Table 1 shows the parameters of the established calibration curves, which performed the goodness of linearity ($R^2 > 0.995$) according to the Appendix F of AOAC (2016) [20]. The working ranges for all TEAC detection methods were wide (from 100 to 700 µmol TE/L), which was suitable for different tea types with varying analyte concentrations, prepared through the same sample preparation procedure and dilution factor. The bias values obtained from all standard points were from 0.010 to 14.2% (Table 1), which are in agreement with the recommended value (< 15%) published in "Method validation for chemical and microbiological analysis" published by Vietnam National Institute for Food Control [17].

Table 1. Parameters of the calibration curves

	ABTS	FRAP	CUPRAC
Working range (μmol TE/L)	100-700	100-700	100-700
Slope (a)	0.0009	0.0011	0.0011
Intercept (b)	−0.0111	0.0113	0.0127
R^2	0.9985	0.9982	0.9979
Bias range (%)	0.20-10.5	0.010-14.2	0.42-9.6

Limit of detection and quantification

The results in Table 2 indicate that the LODs and LOQs of ABTS, FRAP, and CUPRAC (LOD-LOQ) were low (in the range from 9.9 to 37.3 μmol TE/L) compared to the remarkably high concentrations of antioxidants (up to thousands of μmol TE/g dried weight) in tea products due to the rich phenolic contents [21-25]. Therefore, the obtained LOD and LOQ values were suitable for tea matrices, and it is not necessary to lower these values for analytical method development.

Table 2. LODs, LOQs, intra-day precision (RSD_r), inter-day precision (RSD_R), and recoveries

Criteria	LOD (μmol TE/L)	LOQ (μmol TE/L)	RSD_r (%)	RSD_R (%)	Recovery (%)		
					$0.5C_x$	$1C_x$	$1.5C_x$
ABTS	12.3	37.3	1.37	1.48	99.3-99.6	98.7-99.8	99.7-101
FRAP	9.9	30.1	1.03	1.26	98.7-98.9	99.6-100	99.1-99.9
CUPRAC	11.8	35.7	1.52	1.55	98.0-100	98.5-99.5	98.9-99.6

Repeatability and reproducibility

In this current study, the precision (intra- and inter-day) of the analytical methods for TEACs was evaluated through the repeatability and reproducibility tests. The results in Table 2 indicate the favorable repeatability (RSD_r from 1.03 to 1.52%) regarding the Appendix F of AOAC (2016) [20]. The calculation of one-way ANOVA was carried out to evaluate the reproducibility in three separate working days (6 replicates/day) at the confidence level of 95%. The results of RSD_R ranged from 1.26 to 1.55%, consistent with the requirements of AOAC (2016) [20] for the analyte concentrations of 10-100% in Appendix F.

Recovery test

In this study, the accuracy of the analytical methods for ABTS, FRAP, and CUPRAC was assessed by the recovery test. Typically, the Trolox standard solution was spiked into the investigated sample in different concentrations, i.e., $0.5C_x$, $1C_x$, and $1.5C_x$, where C_x is the estimated analyte concentration of the corresponding investigated sample. The results in Table 2 demonstrate the high recoveries (98.0-101% for all spiked samples), favorable to the requirements of AOAC (2016) [20] for method validation in Appendix F. The analytical methods can be applied for the quantification of the antioxidant capacities of dried tea products with proper precision and accuracy. The extraction was performed once to obtain the sample liquids for the measurement of ABTS, FRAP, and CUPRAC, as well as for better comparison and assessment. Moreover, the analytical methods are simple, inexpensive, and suitable for both research and routine analysis of tea products and potentially, tea-related matrices.

Shewhart quality control chart

To control the sensitivity and stability of the analytical system, the Shewhart quality control charts were constructed based on the analysis of the investigated sample (black tea) within 21 days. The black tea product, representing the fully fermented tea type, was used to generate the quality control chart to ensure consistent results under proper preservation conditions during the analysis period. In this situation, the investigated black tea sample plays the role of quality control or QC sample. Figure 1 demonstrates the Shewhart charts for ABTS, FRAP, and CUPRAC, including the mean concentration (C_{mean}) and warning values listed as upper warning limit

(UWL), lower warning limit (LWL), upper action limit (UAL), and lower action limit (LAL). These limits were calculated following the principles of ± 2SD and ± 3SD (SD: standard deviations of three replicates within one day) for warning and action limits, respectively. For each working day, the QC sample can be analyzed to examine the stability and robustness of the measurement system, in which the analyzed results of the QC sample should be between the LWL and UWL to ensure the accuracy of the analytical results [18]. However, during the analytical period, there could be a different emerging situation that needs to be solved based on the QC results. Typically, the analysis should be temporarily stopped and the obtained results and the measurement system need to be carefully checked if one of the following situations happens: (i) any QC results that are beyond the range of LAL-UAL, (ii) the values of more than two in three continuous QC samples are beyond the range of LWL-UWL, (iii) the QC results tend to be located at the same side of the mean line, which it is not acceptable if more than nine continuous points are located at the same side (above or under the mean line), and (iv) the QC results has the tendency to increase or decrease, which it is not acceptable if there are six continuous points tend to go up or down [26]. The results in Figure 1 indicate that the measurement system was stable, i.e., all the values of UAL, UWL, LWL, and LAL were close to C_{mean}, the obtained results are in the range of LWL-UWL and none of the nine continuous points were located at the same side of the mean line. Therefore, the evaluated analytical method meets the proper stability, and robustness for long-term analysis and could be applied in the analysis of dried tea products both for research activities and routine approaches. Moreover, tea products belonging to other types and different geographical origins could be analyzed for their TEACs by employing the evaluated analytical methods due to the favorable adaptability of the operating conditions for diverse tea matrices.

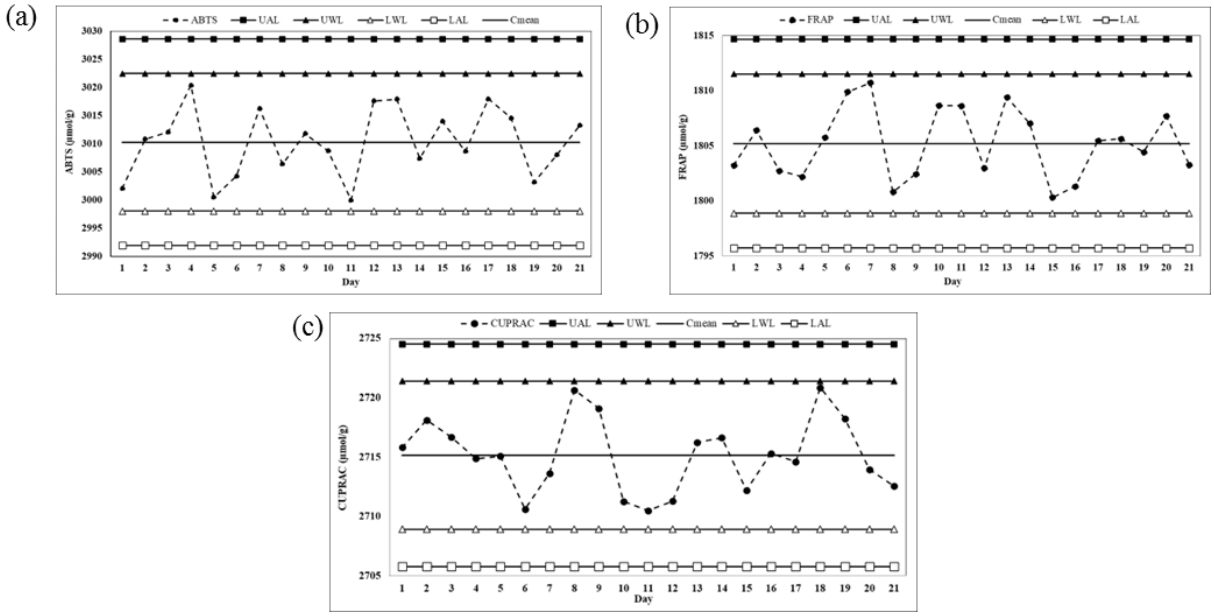

Figure 1. Quality control charts of (a) ABTS, (b) FRAP, and (c) CUPRAC

Application of the evaluated analytical methods for dried tea products

The evaluated analytical methods were applied to determine ABTS, FRAP, and CUPRAC in several commercial dried tea products originating from Lam Dong Province (Vietnam). The results in Figure 2 indicate the TEACs of the tea products in descending order: green tea > oolong tea > black tea. For ABTS, Green-05 and Black-05 exhibited the highest (4006 ± 31 μmol TE/g) and lowest values (3593 ± 41 μmol TE/g), respectively. A similar observation for ABTS in dried tea products was published in Carloni et al. [23], which

reported the highest ABTS values for green tea samples, followed by white and black tea products. Another study by Qian et al. [27] employed a liquid chromatography system coupled with a reaction compartment containing the ABTS reagents after the column, which also demonstrated an ABTS radical scavenging activity of different tea products in descending order: green tea > oolong tea > black tea. Generally, green tea products exhibit significant antioxidant capacities due to the high contents of phenolic and flavonoid compounds [2, 28-30]. The antioxidant capacities of black and dark tea products may be decreased by the activity of polyphenol oxidase enzymes and bacteria during the fermentation period, which could lead to the degradation of simple catechins and other phenolic compounds, as well as formation of more complicated dimers and/or polymers [31-33]. These large molecular compounds could be less reactive with the ABTS reagents, leading to lower ABTS readings [8, 27]. Moreover, the oxidation of catechins to produce the theaflavins during fermentation may also contribute to the intense color and characteristic flavor of oolong and black tea [34, 35].

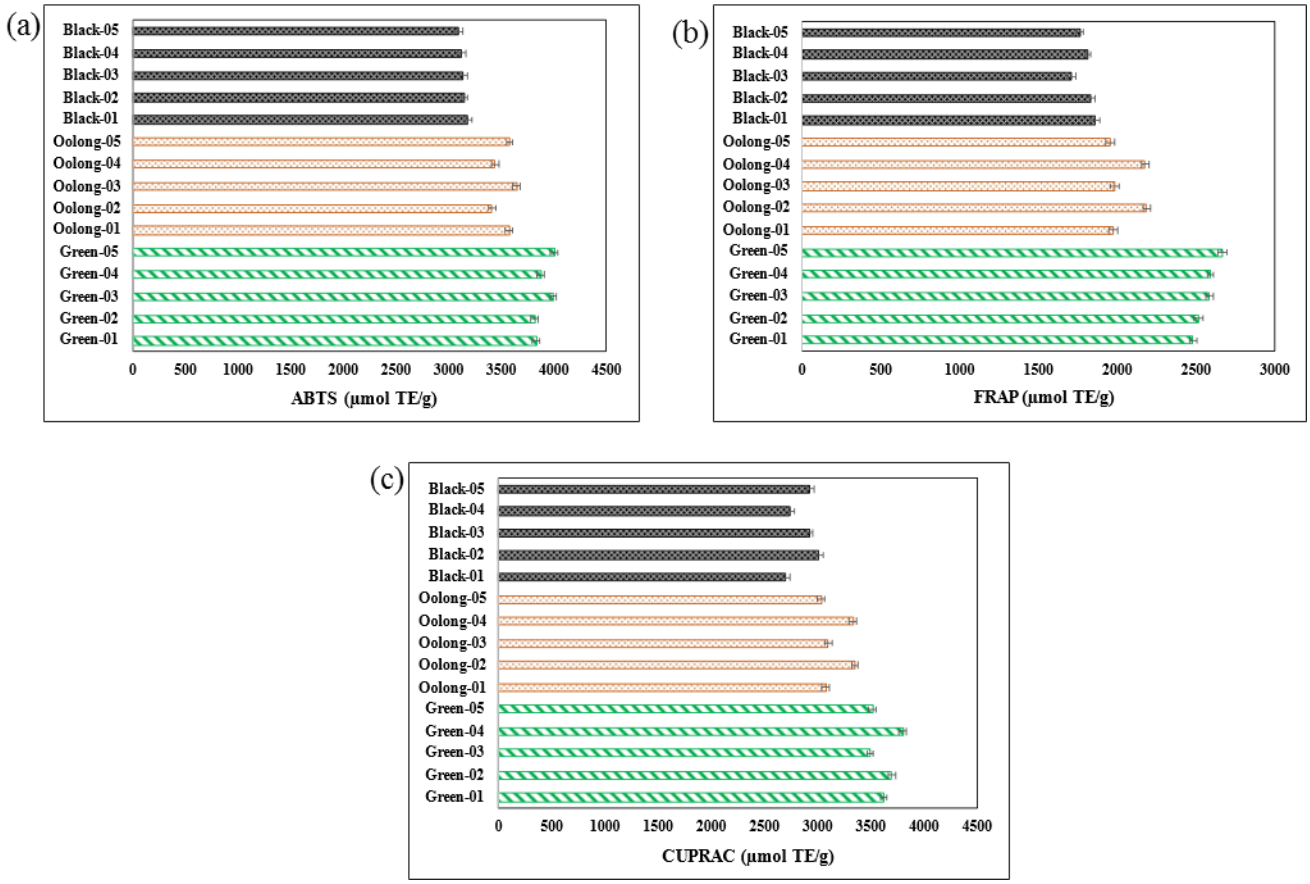

Figure 2. Trolox equivalent antioxidant capacities: (a) ATBS, (b) FRAP, and (c) CUPRAC

The TEACs of the dried tea products in CUPRAC analysis were higher than that of FRAP (2701 ± 35 to 3805 ± 35 μmol TE/g *vs.* 1708 ± 26 to 2668 ± 29 μmol TE/g). However, among the three types of tea, both FRAP and CUPRAC produced results that were similar to that of ABTS. This was supported by the strong correlation obtained (Figure 3) (R^2 = 0.8579, 0.8453, and 0.710 for pairs of FRAP-CUPRAC, ABTS-FRAP, and ABTS-CUPRAC, respectively). Similar results were observed in the publications of Zhao et al. [8] and Lee et al. [36]. As mentioned before, oxidation during fermentation could change the chemical compositions of tea, especially compounds with antioxidant properties, through decomposition, dimerization, and

polymerization, forming complex compounds with larger molecular weight [31-33]. The formation of these new compounds is likely to degrade the reactivity by the transition metal reduction mechanism. This was supported by the FRAP and CUPRAC values of the tea products in descending order green tea > oolong tea > black tea (FRAP: 2479 ± 24 to 2668 ± 29 µmol TE/g > 1956 ± 25 to 2187 ± 21 µmol TE/g > 1708 ± 26 to 1860 ± 27 µmol TE/g, respectively, and CUPRAC: 3494 ± 34 to 3805 ± 35 µmol TE/g > 3033 ± 40 to 3363 ± 31 µmol TE/g > 2701 ± 35 to 3017 ± 32 µmol TE/g, respectively), which also indicates the oxidation during the fermentation has certain effects on the TEACs by the transition metal reduction mechanisms [8, 22].

Generally, the variabilities in TEACs between samples of the same species or different species can also be contributed by the variations in raw materials, i.e., fresh tea leaves, due to the different plantation of origin, soil type, altitude, cultivation condition, quality of tea leaves, processing procedure, preservation method, and growing condition of tea plants [22, 37-39].

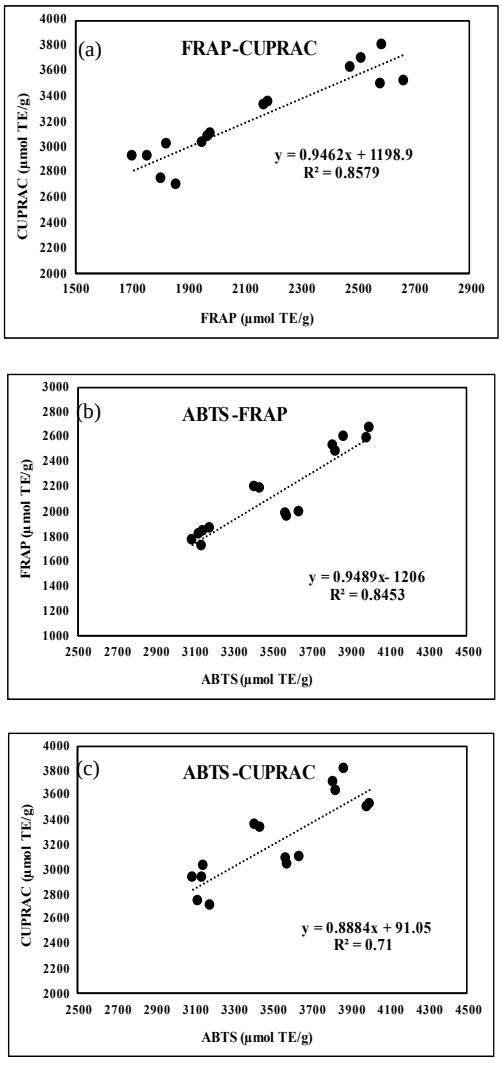

Figure 3. Correlation between (a) FRAP and CUPRAC, (b) ABTS and FRAP, and (c) ABTS and CUPRAC

Conclusion

This study evaluated the molecular absorption spectrophotometric methods for the determination of Trolox equivalent antioxidant capacities (TEACs) through different chemical reactions or in vitro assays. The reactions investigated for determination of TEACs were classified into two mechanisms: radical scavenging activity of ABTS and transition metal reduction via FRAP and CUPRAC. The performance analysis of the analytical method demonstrated low LODs and LOQs compared to the concentrations of antioxidants in dried tea products. The working ranges were wide (from 100 to 700 µmol TE/L), which is compatible with different types of tea matrices prepare through the same sample preparation procedure and dilution factor. The established calibration curves demonstrated the goodness of linearity ($R^2 > 0.995$). The repeatability, reproducibility, and recovery were favorable according to Appendix F of AOAC (2016). The Shewhart charts were constructed to control the analytical results achieved from the UV-Vis 1800 spectrophotometer (Shimadzu, Japan) on different working days. The analytical methods were then applied to several purchased dried tea products, i.e., green, oolong, and black teas, which demonstrated the variabilities in TEACs of the three tea types according to the differences in the processing period. The discrepancies in the result could be due to the increasing oxidation levels from green to oolong and black teas during the fermentation. This study contributes to food areas in terms of the determination and assessment of antioxidant capacities in tea and other tea-related products.

To broaden this study and better assess the tea products, more dried tea samples from various tea plantations should be collected. Additionally, the chemical profiles could be analyzed to adequately support the determined antioxidant capacity behaviors of different tea samples. The changes in TEACs due to the processing demonstrate the potential use of these TEACs as the contributing variables for grouping or discrimination of tea products, particularly in the case of samples in large quantity.

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