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Original Article

INFLUENCE OF PERMANGANATE INDEX IN THE PARAMETERS AS TOTAL PHENOL CONTENT AND TOTAL ANTIOXIDANT ACTIVITY OF EXTRACTS OF *CAMELLIA SINENSIS*

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ABSTRACT

Objective: This study estimated the Permangante Index (I_{KMnO4}) and evaluated its correlation with parameters as total phenols (FT) and total antioxidant activity (TAA) of commercial teas of *Camellia sinensis*, fermented, unfermented and blended with other plants.

Methods: The aqueous extracts were prepared from commercial samples of green and black teas, green tea blended with lemon (*Camellia sinensis* with *Citrus limonium*), green tea blended with mint (*Camellia sinensis* with *Mentha piperita*), green tea blended with peach (*Camellia sinensis* with *Prunus persica*) and green tea blended with orange (*Camellia sinensis* with *Citrus sinensis*). The Permangante index was determined by titrating the tea extract with potassium permanganate solution according to AOAC method (AOAC, 1980) with modifications. The concentration of phenols in the extracts was determined by spectrophotometry according to the standard procedure of Folin-Ciocalteau. The antioxidant activity of the extracts was evaluated using the radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) method. All the determinations were performed in triplicate, and the results presented as mean ± standard deviation (SD). The data were evaluated by univariate statistical analysis (ANOVA), F-test and mean test (Tukey´s test) for comparative results. Pearson's correlation was used to measure the degree of linear correlation and the quantitative variables were from a normal population.

Results: The results for Permanganate Index show a variation ranged from 0.40 to 0.80 mEq/l. The ANOVA analysis to I_{KMnO4} shown there is significant statistically difference at the 5% probability level ($p < 0.05$) between the types of tea as well as the amount of herb used to prepare the infusion. Therefore, it was observed that there significant contrast between tea extracts, the mixture of different plants in blended extracts as well as the tea manufacture process affect the polyphenols tannins content influencing the permanganate index parameter. Studies correlation shown strong correlations ($r > 0.7$) among the parameters. For the *Camellia sinensis* extracts the high correlations between I_{KMnO4} and TAA; FT and TAA demonstrates the suitability of the permanganate index in the evaluation of antioxidant activity.

Conclusion: The results suggest that the Permanganate Index could be used for quality control of tea as an additional parameter.

Keywords: Permanganate Index, *Camellia sinensis*, Tea analysis, Bioactive constituents

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INTRODUCTION

Tea has great economic importance in the area of functional foods to be second most consumed beverages in the world after water. Currently infusions of leaves of *Camellia sinensis* are well recognized because of its effect therapeutics. The health benefits of green tea have been attributed to the high content of polyphenols in the drink. *Camellia sinensis* plants contain large amounts of various phenolic compounds as flavonoids, phenolic acids, tannins and others. The major polyphenols compounds present in the plant are phenolic acids, e. g. hydrocycinnamic acid and tannins. The tannins naturally occurring in tea are condensed tannins, polymeric structures resulting from the polymerization of elementary phenol compounds, polyhydroxyflavan-3-ols known as the catechins. The most common tea catechins are (-) epigallocatechingallate (EGCG), (-) epigallocatechin (EGC), (-) epicatechingallate (EGC), epicatechin (EC) [1]. Phenolic fraction in *Camellia sinensis* commercial extract are also responsible for important quality attributes such as flavour, taste and color [2] and influence the tea industry in relation to market value.

These compounds have been reported to be responsible for the important biological properties of the drink as anti-inflammatory [3] and antibacterial [4] activities, protective effect against various cancer [5-7]; cardiovascular [8] and degenerative diseases [9-10]. In addition, phenolic compound is associated with the high antioxidant activity of the tea [11-12]. The biological activities of these compounds depends on the substituent position on the OH groups of a flavonoid molecule. To tea catechins the reactive structural groups are the pyrogallol groups; the catechol group; the 2,3 double bond in conjugation with a 4-oxo group and a 3-hydroxyl group and additional resonance effective substituents. Therefore, it is more important of measure the catechins total content than to determine each of them individually.

Methods of tannin analysis are based upon precipitation of tannin, formation of colored products with tannin, oxidation of tannin and UV spectroscopy. The AOAC compendium [13] lists the Folin Ciocalteau method for use on alcoholic beverages and permanganate reducing method for alcoholic and non-alcoholic beverages. The latter known as the Permanganate Index has been used to estimation the oxidability polyphenols matter, in terms of the total tannins content, in different drinks as wine [14], cider and juice [15] in a simple and practical way and low cost. Permanganate Index parameter is based on the titration of polyhydric phenols with permanganate ion, in the presence of a selective indicator, to exclude interfering potentially substances (e. g., proteins and reducing sugars) in real samples [13].

Among the characterizing studies of the antioxidant activity of polyphenols tea, many refer to influencing of these compounds in this biological activity [16-17]. However, so far to our knowledge no work has been done about the influencing of the Permanganate Index (I_{KMnO4}) on the antiradical activity shown by the polyphenols and the relationships with total catechin tannin content in the tea samples.

The aim of this report was estimated the Permangante Index (I_{KMnO4}) and evaluated the its correlation with parameters such as total phenols (FT) and total antioxidant activity (TAA) of commercial teas of *Camellia sinensis*, fermented, unfermented and blended with other plants. In case of tea this parameter provide indirect information about processing changes as enzymatic oxidation, thermal

degradation and epimerization which influencing the quality drinking in terms of antioxidants compounds [1].

MATERIALS AND METHODS

Preparing the extracts

The extracts were prepared from commercial samples of green and black teas, green tea blended with lemon (*Camellia sinensis* with *Citrus limonium*), green tea blended with mint (*Camellia sinensis* with *Mentha piperita*), green tea blended with peach (*Camellia sinensis* with *Prunus persica*) and green tea blended with orange (*Camellia sinensis* with *Citrus sinensis*). Initially, for each type of tea, all the content of the individual tea bags within a commercial package were mixed. Then, the extracts were prepared by solid-liquid extraction in deionized water as recommended in the package at known concentrations (10-50 mg/ml). The packages did not list the proportion of each plant type in the blended tea, but that ground parts of the plant like stems, buds and leaves were mixed to the powder of *Camellia sinensis*.

Chemicals and reagents

Samples of tea were obtained from local supermarkets. Amount of the tea samples were dissolved in hot water in triplicate. Folin-Ciocalteau reagent, 1,1-diphenyl-2 picrylhydrazyl (DPPH), potassium permanganate, indigo carmine, sulphuric acid, chlorogenic acid were purchased from Sigma (St. Louis, USA); 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) was supplied by Aldrich (Milwaukee, USA). All aqueous solutions were prepared using water purified a Milli-Q system (Millipore, System).

Determination of permanganate index (I_{KMnO4})

The Permangante Index was determined by titrating the tea extract with potassium permanganate solution according to AOAC method [13] with modifications. In a measuring glass were added 10 ml of tea solution, 1 ml of 0.2% indigo carmine in 10 ml H_2SO_4 1:4. The aqueous solution of $KMnO_4$ at 0.05N was used to titration the mixture and the end-point was carried out until the blue colored solution changed to golden yellow one. This volume was (called as volume A) was used to determine all compounds oxidizable by $KMnO_4$ in the sample. The same evaluation procedure was employed to determine all phenolic compounds that react with $KMnO_4$ (called volume B). In another 10 ml tea aliquot was mixed 1g of activated charcoal, the mixture was shaken for 2 min and then filtrated through a No.1 Whatman paper. After, with the decolorised mixture, the titration was repeated, was added indigo carmine indicator in acid medium and the mixture was titrated against $KMnO_4$ solution until colour changed as earlier. The amount expended by the phenol compounds is the difference in the $KMnO_4$ volume and it was calculated according to relation:

$$IKMnO4 = (Va - Vb) \times N\ KMnO4 \times 10$$

where:

V_a is the volume of $KMnO_4$ solution spent to oxidation of coloring matter;

Vb is the volume of $KMnO_4$ solution spent to oxidation of other reducing substances;

N_{KMNO4} is the normal concentration of $KMnO_4$ solution after previously standardized with oxalic acid.

A blank titration using 5 ml of indigo carmine alone in 200 ml water was also be carried out. All samples were analyzed in triplicate and the results expressed in mEq/l (milli equivalents per litre).

Determination of total phenols content (FT)

The concentration of phenols in the extracts was determined by spectrophotometry according to the standard procedure of Folin-Ciocalteau [18]. In this assay, an aliquot of 50µL of the extract was mixed to 250µL of Folin-Ciocalteau reagent and 750 µL of 20% sodium carbonate. The final volume was adjusted with distilled water. After 2 h at room temperature the color was read at 735 nm in a spectrophotometer UV/Vis Bioespectro model SP-220. The calibration curve was obtained using chlorogenic acid as standard. The total phenols (FT) was expressed in mmol/l per g sample.

Determination of the antioxidant activity total (AAT)

The antioxidant activity of the extracts was evaluated using the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH•) method [19-20]. For this, extracts aliquots with known volume were added to an ethanolic solution with 0.1 mmol/l of DPPH•. In the radical form, the DPPH•has a maximum absorption at 517 nm, but under a reduction by an antioxidant, the stable form (non-radical) of the DPPH• is not absorbed. The decrease in absorbance of the radical solution after 15 min was monitored in a spectrophotometer UV/Vis Bioespectro model SP-220. The 97% Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard. The absorbance values were related to the percentage of antioxidant activity (AA) through the equation:

$$\%\ AA = \frac{Abs_{control} - (Abs_{sample} - Abs_{blank})}{Abs_{control}} \times 100$$

where: $Abs_{control}$ is the initial absorbance of the ethanolic solution of DPPH; Abs_{sample} is the absorbance of the reaction mixture (DPPH+sample) and Abs_{blank} is the absorbance of the blank.

Statistical analysis

All the determinations were performed in triplicate, and the results presented as mean ± standard deviation (SD). The data were evaluated by univariate statistical analysis (ANOVA), F-test and mean test (Tukey´s test) for comparative results. When the F test value was significant, the means were compared by Tukey's test at 5% probability. Differences between the means were considered as significant at p-values under or equal to 0.05. The statistical analyses were carried out using the software BIOSTAT version 5.3.

RESULTS AND DISCUSSION

In the present study, the Permanganate Index was used to estimate polyphenols compounds in tea extracts expressed as catechin tannins content. It was based on oxidation of phenolics compounds by titration with a potassium permanganate standard solution. The test is performed in the presence of an indigo sulfonate organic salt (5,5'-indigo dissulfonic acid sodium salt) which acts as redox indicator and a oxidation restrictor. The potassium permanganate reagent is a very strong oxidizer and thus not very selective. It will not only react with polyphenols but also with many other (organic) reducing agents potentially present in the extract. Thus, it was necessary to perform a second determination by difference, after the separation of tannin content, to avoid these interferents can also be analysed resulting in an overestimation of the polyphenol content. It was carried another separate titration of the sample out using charcoal to remove the coloring matter. In both titration procedures, indigo carmine was used as redox indicator to achieve an accurate end point and also to avoid the oxidation substances other than phenols in samples. The difference between the titration with and without charcoal is Permanganate Index assigned to analysis only the polyphenols content.

In table 1 as can seen the results for Index Permanganate parameter estimated for tea extracts, ranged from 0.40 to 0.80 mEq/l (ranged from 3.15 to 5.85%). *Camellia sinensis* with *Citrus limonium* extracts followed by unfermented *Camellia sinensis* extracts were those with the highest values. Citrus fruits parts, like leaves, are well known to be good source of natural antioxidants [21] thus, Citrus species, when mixed with *Camellia sinensis* extracts can increase the polyphenols content in the samples. Many of the reported studies about the analysis of tannin content involves the I_{KMnO4} parameter determination applied to wine samples. The works about the determination of tea tannin content in *Camellia sinensis* extracts showed mean values of I_{KMnO4} many discrepant i.e., equal 0.18% for green tea [15]; 55.89% and 10.23% for green and black tea respectively [22]. In other previous study the values were 2,65% and 13.36% for green and black tea respectively [23].

The difference in values can be explained considering the tannic phenolic content extracted during the dilution of the plant extracts [24-25]. Diluted infusions of teas may have different and complex phenolic profiles and may present compounds with different kinetic and

reduction behaviors. Associated to this, previous studies with extracts of Camellia sinensis [26-27] pointed out that the method of preparation, infusion time, way of packaging the herb, temperature and the proportion between dry weight of herb and the amount of water used, influence the final quantity of measured tannic compounds.

Analysis of variance (two-way ANOVA) was used to evaluate the influence of the fermentation, mix of plants and concentration of tea used in this study on Index Permanganate parameter. According to the results is observed that there is significant statistically difference at the 5% probability level ($p < 0.05$) between the type of tea as well as the amount of herb used to prepare the infusion. The values for the permanganate index shown Ftabulated were greater than Fcritical and the p values were under 0.01. Therefore, it was observed that there significant contrast between tea extracts. The mixture of different plants in blended extracts as well as the tea manufacture process affect the polyphenols tannins content influencing the permanganate index parameter. It is well known that the tannins content in Camellia sinensis tea varies depending on how the tea is processed [1]. The non-fermented tea (green tea) contains significant quantities for the nonoxidized catechins: (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (EGC) and (-)-catechin gallate (CG) [28]. During the fermentation processes of the extracts of Camellia sinensis, occur oxidation and dimerization of polyphenols, especially catechins, in the presence of polyphenoloxidase and oxygen, with formation of more complex structures, such as theaflavins (TFs), theasinensins and dimerized structures forming the thearubigins (TRs) [29]. These catechins oligomers are presence only in the fermented tea extracts (black tea). As a result, black tea contains a mixture of native unoxidized polyphenols together with oxidized products, whereas green tea contains only unoxidized native polyphenols. Thus, the polyphenols profile in both extracts, fermented and non fermented can influence the analysis. It may suggest that green tea has a greater amount of phenol compounds, which react more readily with potassium permanganate.

The simplest polyphenols content was also estimated by the Folin Ciocalteau method. It was observed a variation ranged from 0.33 to 4.96 mmol/lper g. The highest values were obtained for the fermented extracts (4.96±0.44) and *Camellia sinensis* unfermented (3.54±0.40). For other extracts the phenol content obtained were lower than 2.0 mmol/lper g (data not shown). The Folin Ciocalteau is a redox spectrophotometric method based on the oxide-reducing and chelating abilities of phenolics to react with Folin Ciocalteau oxidizing reagent (mixture of phosphotungstic acid, $H_3PW_{12}O_{40}$ and phosphomolybdic acid, $H_3PWMo_{12}O_{40}$). The variation in the estimation of these values can be justified considering the sample consisting of different herbs. The content of polyphenol in plant is quite variable in distribution and concentration even in the same plant [30]. This is mainly due to environment and genetic factors, cropping system, harvest period and conditions of extraction the plant is submitted, such as difference in the process of manufacture, aging of tea leaves or the differences in climate and soil texture [1].

Table 1: Permanganate index (I_{KMnO4}) of extracts of *Camellia sinensis*

Sample	I_{KMnO4}*(mEq/l)	ANOVA Parameters
Camellia sinensis	0.75±0.14	**Between groups****
Fermented *Camellia sinensis*	0.55±0.40	$F_{tabulated}$ = 2.724
Camellia sinensis+Citrus limonium	0.80±0.05	$F_{critical}$ = 1.747
Camellia sinensis+Mentha piperita	0.53±0.08	**Within groups*****
Camellia sinensis+Prunus persica	0.40±0.20	$F_{tabulated}$ = 26.095
Camellia sinensis+Citrus sinensis	0.50±0.08	$F_{critical}$ = 2.368

*Mean value±standard deviation for three replicates (n = 3); ** type of tea, *** concentration of the extracts.

In order to complement the assessment of the quality of polyphenols tea, it was also assessed the antioxidant activity presented by the extracts against the radical DPPH•. The extracts showed high antioxidant activity (data no shown), the values ranged from 69.70% to 92.50% (values were calculated as means for three replicates). The ability showed by the extracts to scavenge DPPH• decreased in the following order: *Camellia sinen*sis > fermented Camellia sinensis > *Camellia sinensis* with *Citrus limonium* > *Camellia sinensis* with *Mentha piperita* > *Camellia sinensis* with *Prunus persica* > *Camellia sinensis* with *Citrus sinensis*. As previously commented, despite the green and black tea are produced from the same plant, differences in the manufacuring process results that green tea has a catechin content greater than black tea. As a result *Camellia sinensis* extracts show a greater antioxidant potential than fermented *Camellia sinensis* extracts. This is according to the literature, studies have shown that the antioxidant properties demonstrated for *Camellia sinensis* extracts are attributed to catechins as (-) epigallocatechingallate (EGCG), (-) epigallocatechin (EGC) and (-) epicatechingallate (EGC), [23-24]. The three adjacent hydroxyl groups on the B-ring of EGCG, GCG, EGC and GC are more effective in scavenging free radicals than the two adjacent OH groups of ECG, CG and epicatechin (EC) commonly present in fermented *Camellia sinensis* leaves [1].

Correlation among the permangante index (I_{KMnO4}), total phenols content (FT) and total antioxidant activity (TAA)

In order to evaluate the influence of the polyphenols fraction of the teas on the parameters as permanganate index, total phenols content and antioxidant activity, five samples of each tea were analyzed. Table 2 shows the correlation matrix obtained by linear regression plots of the tested extracts in different concentrations (10, 20, 30, 40, 50 mg/ml) and the Pearson correlation coefficient calculated. In general, the samples showed strong correlations ($r > 0.7$) among the parameters. For the *Camellia sinensis* extracts the high correlations between IKMnO4 and TAA; FT and TAA were obtained. These results demonstrate the suitability of the permanganate index and phenol total parameters in the evaluation of antioxidant activity. An individual difference was observed for *Camellia sinensis* with *Prunus persica* extract, a poor correlation between FT and TAA. The exception was also observed for the black tea extract that shown weak correlation between IKMnO4 and FT, r =-0.305; IKMnO4 and TAA, r = 0.219. Black tea is a drink consisting of a mixture of native unoxidized polyphenols together with oxidized products as catechins tannins more complex such as theaflavins and thearubigins. It can be suggest that these compounds do not exhibit good antioxidant activity when compared to unoxidized derivatives compounds.

Table 2: Pearson´s correlation coefficient (r) among permanganate Index (I_{KMnO4}), phenol total (FT) content and antioxidant activity total (AAT) for extracts of *Camellia sinensis*

Sample/Parameter	I_{KMnO4} x FT	I_{KMnO4} x TAA	FT x TAA
Camellia sinensis	0.930	-0.703	-0.897
Fermented *Camellia sinensis*	-0.305	0.219	-0.892
Camellia sinensis+Citrus limonium	0.718	0.640	0.752
Camellia sinensis+Mentha piperita	0.791	0.743	0.965
Camellia sinensis+Prunus persica	0.796	0.862	0.494
Camellia sinensis+Citrus sinensis	0.760	0.793	0.734

CONCLUSION

The study demonstrated the influence of permanganate index in the parameters as total phenol content and total antioxidant activity of extracts of *Camellia sinensis*. According to the obtained results to correlation studies its shown strong correlation (r>0.7) among the parameters. These data demonstrate the suitability of the permanganate index parameter in the evaluation of phenol content and antioxidant activity. The determination of the Permanganate Index for tea samples is relation to tannin fraction that is an important parameter for tea quality in terms of nutritional aspects and biological properties. The results strongly suggest that the Permanganate Index can be used as a useful additional parameter as quality indicator of tea.

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AUTHORS CONTRIBUTION

Lucilene Dornelles Mello: conceived and participated in the writing of the manuscript

Tiele Garcia Tunes: conceived and executed the assays

CONFLICTS OF INTERESTS

Declared none

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