REBRAPA
Brazilian Journal of Food Research

Brazilian Journal of Food Research
ISSN: 2448-3184

https://periodicos.utfpr.edu.br/rebrapa

A handy-assay procedure to measure simultaneously the polyphenol content and the antioxidant capacity in teas using the Folin Ciocalteu reagent

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ABSTRACT

Folin Ciocalteu reagent (FCR) is commonly used for the quantification of total polyphenol content (TPC) in plants and theirs derived materials. Tea is one of the most consumed beverages in the world and widely used in developing countries in folk medicine. Tea contains several compounds (especially polyphenols) which are believed to have antioxidant capacity. In the present study, a handy-assay procedure to measure simultaneously the TPC and the antioxidant capacity in teas using the FCR was developed. A spectrophotometric procedure using the FCR was undertaken to evaluate simultaneously the TPC and the antioxidant capacity, expressed in Trolox equivalents antioxidant capacity (TEAC), of 32 samples of 7 different types of teas. For comparison purposes, the antioxidant activity of the same samples was determined using the CUPRAC (cupric reducing antioxidant capacity) reagent. Finally, the antioxidant capacity in a cup of tea (200 mL) obtained with the FCR and expressed in ascorbic acid equivalents (AAEC) was performed. The TEAC values obtained with FCR presented a good positive correlation with the CUPRAC method (r^2 = 0.853), suggesting that both reagents can be used to quantify the antioxidant capacity. The TEAC and CUPRAC values also showed good agreement with the TPC (r^2 = 0.969 and r^2 = 0.809, respectively) indicating that the antioxidant capacity should be due to the presence of polyphenols. The results obtained and the calculation strategy used may be an easier way to present the antioxidant capacity values to the final consumer who is most commonly unfamiliar with this important concept.

KEYWORDS: spectrophotometry; Folin Ciocalteu reagent; antioxidant capacity; ascorbic acid equivalents; tea bags.

INTRODUCTION

The Folin Ciocalteu reagent (FCR), a mixture of phosphomolybdate and phosphotungstate, is widely used in alkaline solution for *in vitro* spectrophotometric determination of various reducing compounds. In these analytical procedures (generally called the FC methods) a solution containing certain reducing agent (the analyte) is mixed with an amount of FCR and then sodium carbonate solution is added to alkalize the mixture. After some waiting time (usually 30 minutes) at room temperature (23 °C), the solution acquires a quite stable blue color which can be achived more rapidly under heating at 40 °C despite the fact that loss of color is more pronounced over time (SINGLETON; ORTHOFER; LAMUELA-RAVENTOS, 1999). The absorbance values recorded between 715 and 760 nm are then related to the analyte (reducing agent) concentration.

Historically, the FCR was first proposed by Otto Folin and Vintila Ciocalteu (1927) for the determination of tyrosine (which containing a single phenol group) and tryptophan (without phenolic groups) in protein samples and then used by other authors in several foodstuffs' samples (MCFARLANE; FULMER, 1930). In the same decade studies on the determination of some aminoacids (tyrosine, tryptophan, cystine and histidine) in lake water samples and for quantification of phenol in pasteurized milk were carried out (KAY; GRAHAM, 1935; KUISEL, 1935).

Latterly, Schild and Enders (1936) mentioned that the FCR was not specific for tryptophan determination since it was reduced by a large number of substances which promote the appearance of the same blue coloration.

Those findings led to further studies using the FCR reagent in the quantification of other reducing species.

In fact, some years later Balls and Arana (1941) quantified the vanillin contents in vanilla beans extracts. Their results showed that different phenolic substances (other than vanillin and coumarin) contributed to the flavor and aroma of those products. Those results were then reported as "phenol values".

Heintze (1964) pointed out that polyphenols present in plants and foods could be quantified with the so-called "Folin-Ciocalteu method" using chlorogenic acid as a polyphenol standard.

After a period of one year Singleton and Rossi (1965) investigated the spectral properties of various phenolic compounds using the Folin-Denis reagent and FCR. Their conclusions suggested that the latter would be more appropriate for the quantification of tannins in spirits and wine samples.

As a matter of in the late 60's the total phenolic composition of red wine samples was measured using FCR, finding much better results when compared with the then extensively used "permanganate index" (RIBEREAU-GAYON; SARTORE, 1970).

Afterwards, Peri and Pompei (1971) using a procedure with several precipitation steps quantified different phenolic groups (condensed tannins, hydrolysable tannin, non-tannin flavans and simple phenolics) in vegetable extracts using the FCR.

Since then, the FCR has been used for the quantification of polyphenols in several samples of vegetable-based matters such as aqueous extracts of medicinal

plants, wines, beers, fruit juices and teas (ATOUI *et al*., 2005; DU TOIT; VOLSTEEDT; APOSTOLIDES, 2001; GORJANOVIĆ *et al*., 2012; IVANOVA *et al*., 2005; LEE *et al*., 2011; NAKAMURA; COICHEV; MOYA, 2012; PEKAL *et al*., 2012; WU *et al*., 2015). As the identification of individual phenolic compounds is not possible with the FCR without other analytical steps, it is more usual to quantify the total polyphenol content (TPC) in those samples expressing the obtained value in equivalents of a standard phenolic compound (e.g., tannic, gallic, pyrogallic, chlorogenic or ferulic acids and catechin) (ROBBINS, 2003; SINGLETON; ORTHOFER; LAMUELA-RAVENTOS, 1999).

Tea is one of the most consumed beverages in the world and widely used in folk medicine especially in developing countries where traditional medicine is not always easily accessible.

The natural constituents present in the tea leaves are considered to be responsible for bringing various benefits for human health. Moderate and regular ingestion of teas has been linked to reduced levels of cholesterol, blood pressure, reduction of the risk of coronary heart disease and even certain types of cancer (AHMED-BELKACEM *et al*., 2005; FUJIKI; SUGANUMA, 2012; JUNG *et al*., 2008; YANG *et al*., 2011; WANG *et al*., 2000; WENZEL *et al*., 2000).

A retrospective study carried with 13,842 individuals in Taiwan proved that the daily consumption of tea in controlled amounts reduces the risk of obtaining kidney stones, thus encouraging tea consumption in that country (CHEN *et al*, 2018). In a study with 20,643 participants China LI and colleagues found that drinking tea was beneficial to women's bone health. (LI *et al,* 2019).

A recent review evaluated the relationship of green tea consumption with some types of cancer and cardiometabolic diseases. For endometrial, lung, oral, ovarian and non-Hodgkin's lymphoma cancer) positive results were observed while for other cancer types they were null or inconclusive results. Although this review does not show positive results for all types of cancer, it mentions that tea intake can be considered beneficial to human health (ABE; INOUE, 2021).

In fact, infusions of *Camellia sinensis* L. Kuntze, for instance, presented antibacterial activity and other health benefits that were associated with the high content of polyphenols and, consequently, the antioxidant capacity (CHAN *et al*., 2011).

In reality, it is well known that due to their antioxidant properties polyphenols can interrupt some chain reactions caused by reactive oxygen species, such as $O_2^{\bullet -}$, HO•, ROO•, and reactive nitrogen species, such as NO•, $ONOO^-$ and (ALIPÁZAGA; MOYA; COICHEV, 2021; DE SOUZA; MOYA, 2014) protect the human body against the so-called oxidative stress (HUSSAIN *et al*., 2016).

Hence, the interest in the quantification of polyphenols and antioxidant capacity in teas has increased over the years since polyphenols are an important exogenous source of antioxidants. However, the antioxidant compounds present in teas (mainly polyphenols) responsible for antioxidant capacity are chemically different from each other, which effectively hinders their identification and individual determination in routine analyzes. In this context, simple methods that can simultaneously quantify the total polyphenolic content and also the total antioxidant capacity are definitely wanted and eventually welcome (TABART *et al*., 2009).

In the present work the FCR procedure was undertaken to evaluate simultaneously the TPC and the total antioxidant capacity in 32 samples of 7 different types of tea herbs (*Baccharis genistelloides, Camellia sinensis* L. Kuntze, *Cymbopogon citratus* Stapf, *Ilex paraguariensis* St. Hil., *Matricaria recutita* L., *Mentha piperita* L., *Peumus boldus*) from 11 brands commercially available in the local market (Santo Sandré city, state of São Paulo).

For comparison purposes, the total antioxidant capacity values obtained with the FCR were compared with values obtained with the cupric reducing antioxidant capacity (CUPRAC reagent) method, which is based on reduction of Cu(II) to Cu(I) in a solution containing neocuproine (APAK *et al*., 2004).

The results presented in this study allow inferring that the FCR procedure and the calculation strategy adopted here can help in the choice of which tea to be consumed or even to be used as a routine trial in quality control.

MATERIALS AND METHODS

EQUIPMENTS

Absorbance measurements were performed on the HPUV 8453 (Agilent Technologies, USA) spectrophotometer using a glass cuvette (1.00 cm optical path).

REAGENTS AND SOLUTIONS

Reverse osmosis water (Quimis Q842-210, Brazil) was used to prepare all solutions, except when another solvent is indicated.

The Folin-Ciocalteu reagent (FCR) was prepared by dissolving 10 g of sodium tungstate dihydrate ($Na_2WO_4.2H_2O$, ≥ 99.0%, FW 329.85 g mol^{-1}, Sigma-Aldrich, USA), 2.0 g of phosphomolybdic acid hydrate ($H_3[P(Mo_3O_{10})_4]\times H_2O$, ≥ 99.99%, FW 1825.25 g mol^{-1}, Sigma-Aldrich, USA) and 5 mL of phosphoric acid (H_3PO_4, 85 %, FW 98.00 g mol^{-1}, Merck, Germany) in 75 mL of water. This mixture was heated under reflux (at 100°C) for 2 hours and after reaching room temperature was transferred to a 100.0 mL volumetric flask and its volume fulfilled with water. The FC reagent was maintained at 8° C and in the case of turning into a greenish color it was regenerated by mixing (without heating) with a few drops of liquid bromine (Br_2, ≥ 98%, FW 159.8 g mol^{-1}, J. T. Baker, USA), until re-establishing the yellow color (Brazilian Pharmacopoeia, 2010).

A 10.0 % sodium carbonate solution (Na_2CO_3, ≥ 99.5%, FW 105.99 g mol^{-1}, Sigma-Aldrich, USA) was prepared by dissolving 10 g of the salt in water in a 100.0 mL volumetric flask.

A 0.94 mg mL^{-1} (5.0×10^{-3} mol L^{-1}) gallic acid ($C_7H_6O_5.H_2O$, ≥ 98%, FW 188.13 g mol^{-1}, Carlo Erba, France) stock solution was prepared daily by dissolving 0.094 g in water in a 100.0 mL volumetric flask. The stock solution was further diluted in water to give a 0.094 mg mL^{-1} (5.0×10^{-4} mol L^{-1}) standard working solution.

A 2.5 mg mL^{-1} (1.0×10^{-2} mol L^{-1}) Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, $C_{14}H_{18}O_4$, 97%, FW 250.29 g mol^{-1}, Sigma-Aldrich, USA) stock solution was prepared daily by dissolving 0.1251 g in water in

a 50.0 mL volumetric flask. This stock solution was further diluted in water to give a 0.10 mg mL^{-1} (4.0×10^{-4} mol L^{-1}) working solution.

A 1.76 mg mL^{-1} (1.0×10^{-2} mol L^{-1}) ascorbic acid ($C_6H_8O_6$, 99.7%, FW 176.12 g mol^{-1}, Merck, Germany) stock solution was prepared daily by dissolving 0.1761 g in 100.0 mL volumetric flask. A 0.088 mg mL^{-1} (5.0×10^{-4} mol L^{-1}) working solution was prepared by dilution of the stock solution.

CUPRAC reagent was prepared as described elsewhere procedure (APAK *et al*., 2004; APAK *et al*., 2006; LEE *et al*., 2011; NAKAMURA; COICHEV; MOYA, 2012).

PREPARATION OF TEA SAMPLES

In this study only tea bags (different herbs of various brands) purchased from local marketplaces were included. All samples were dry and within the expiration date established by the manufacturer.

Extraction of the water-soluble compounds from dried material was performed using the same procedure described in Brazilian Pharmacopoeia for preparation of aqueous extracts of medicinal plants (Brazilian Pharmacopoeia, 2010). Briefly, 0.300 g of dry material was transferred to a 100 mL becker containing 50.0 mL of water which was maintained in a water-bath (30 min; 65-70°C). After cooling, the mixture was transferred to a 100.0 mL volumetric flask and its volume fulfilled with water. This mixture was then filtered through quantitative filter paper (Nalgon, 3552, Germany) and if necessary diluted before analysis.

QUANTIFICATION OF THE TOTAL POLYPHENOL CONTENT

A calibration curve using gallic acid (GA) was obtained by mixing aliquots (100.0-400.0 µL) of a 0.094 mg mL^{-1} (5.0×10^{-4} mol L^{-1}) GA standard solution with 200 µL of FCR in a 5.0 mL volumetric flask and completed with 10% Na_2CO_3 solution providing GA final concentration from $(1.9\text{-}7.5)\times10^{-3}$ mg mL^{-1} ($(1.0\text{-}4.0)\times10^{-5}$ mol L^{-1}). With the calibration curve (A_{715nm} *vs* C_{GA}, being C_{GA} the concentration of GA in mg mL^{-1}) the linear equation $A_{715nm} = a + b \times C_{GA}$ was obtained, where *a* and *b* are the values of the linear and angular coefficients, respectively.

The standard multiple addition method was used in all analyzes as follows: aliquots of freshly prepared tea samples ranging from 150-1000 ?L (depending on the type of tea) were transferred to 5.0 mL volumetric flasks containing 200 ?L of the FCR which were filled up with 10% Na_2CO_3 solution. In four out of five volumetric flasks aliquots (100-400 µL) of 0.094 mg mL-1 (5.0×10^{-4} mol L^{-1}) GA solution were added and the volume was completed with the same 10% Na_2CO_3 solution (HARRIS, 2005; MARINO *et al*., 2009; SANTO; NUNEZ; MOYA, 2013).

In both curves (calibration and multiple standard additions with tea samples) the absorbance measurements were recorded at 715 nm (A_{715nm}) after 30 minutes using water as the reference solution (blank). All measurements were made in triplicate. The TPC values were expressed in mg GA/g dry material (DM) as shown in Table 1 and can be more easily calculated using the Equation 1 where *a* and *b* are the linear and angular coefficients values of the linear equation of the standard multiple addition calibration curve, respectively, *fd* is the dilution factor (see *Preparation of tea samples*) and *V* is the volume (mL) of the tea sample. 313500 is

a numerical constant that includes the volumes of 100.0 mL (see *Preparation of tea samples*) and 5.0 mL (see *Quantification of the total polyphenol content*), the molar mass of gallic acid (188.13 g mol^{-1}), the mass of dry material (0.300 g) and the conversion of *g* to *mg* (1000).

TPC (mg GA/g DM) = (a x fd x 31350)/(b x V) (Equation 1)

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY IN TROLOX® EQUIVALENTS

A calibration curve with Trolox® was obtained as performed with GA and can be described by the linear equation $A_{715nm} = a + b \times C_{Trolox®}$ (where $C_{Trolox®}$ is in mg mL^{-1}). Interpolating the A_{715nm} value obtained with the tea sample using with FCR (without GA addition) into the above equation a corresponding concentration in Trolox® (mg mL^{-1}) is obtained. Considering the tea aliquot used and the dilution required, the mass of Trolox® in 100 mL of tea is found. The results of the total antioxidant capacity in Trolox® equivalents (TEAC) were expressed in µmol Trolox®/g DM (Table 1) and can be also found using the Equation 2, in which A_{715nm} is the absorbance value at 715 nm of the diluted tea obtained with FCR. *a* and *b* are the linear and angular coefficients of the calibration curve with Trolox®, respectively, *fd* is the dilution factor and *V* is the volume (mL) of the tea sample. 6659 is a numerical constant that incorporates the volumes of 100.0 mL (see *Preparation of tea samples*) and 5.0 mL (see *Quantification of the total polyphenol content*), the molar mass of Trolox® (250.29 g mol^{-1}), the mass of dry material (0.300 g) and the conversion of *g* to *mg* (1000).

µmol Trolox®/g DM = $[(A_{715\ nm} - a) \times fd \times 6659]/(b \times V)$ (Equation 2)

CALCULATION OF THE ANTIOXIDANT CAPACITY IN A TEA CUP EXPRESSED IN ASCORBIC ACID EQUIVALENTS

A calibration curve with ascorbic acid (AA) was obtained as performed with Trolox® and can be described by the linear equation $A_{715nm} = a + b \times C_{AA}$ (where C_{AA} is in mg mL^{-1}). Interpolating the A_{715nm} value obtained with the tea sample using with FCR into the above equation a corresponding concentration in AA (in mg mL^{-1}) is calculated. Considering the tea aliquot used and the dilution required the mass of AA in 200 mL (regular cup of the tea) is found. The ascorbic acid equivalents (AAEC) values were expressed in mg AA/200 mL tea (Table 1) and can be also found using the equation (3), in which A_{715nm} is the absorbance value at 715 nm of the tea obtained with FCR. *a* and *b* are the linear and angular coefficients of the calibration curve with AA, respectively, *fd* is the dilution factor, *V* is the volume (mL) of the tea sample and 1000 is a numerical constant that incorporates the volume of 5.0 mL (see *Quantification of the total polyphenol content*) and the regular volume of a cup of tea (200 mL).

mg AA/200 mL of tea = $[(A_{715\ nm} - a) \times fd \times 1000]/(b \times V)$ (Equation 3)

DETERMINATION OF ANTIOXIDANT CAPACITY WITH CUPRAC REAGENT.

This procedure (APAK *et al*., 2004; APAK *et al*., 2006) was performed as described in previous works with minor modifications, notably in the replacement of nitrate; chloride; or copper (II) sulphate by a solution of copper(II) perchlorate previously synthesized and standardized (LEE *et al*., 2011; NAKAMURA; COICHEV; MOYA, 2012).

RESULTS AND DISCUSSION

Several studies are found in the literature dealing with the determination of total polyphenol content (TPC) and the antioxidant capacity of Brazilian medicinal plant species (BLAINSKI; LOPES; MELLO, 2013; BRIGHENTE *et al*., 2007; HABERMANN *et al*., 2016; MANOEL; MOYA, 2015; SANTANA; NUNEZ; MOYA, 2015). Most of them investigated a unique part of the plant species (leaves, stem, seeds, etc.). There are not many studies about herbs as they are found by consumers in supermarkets (sachets) so a study of this nature is worthy of investigation. In the present study only samples commercially available in teas bags, used as infusion, were analyzed.

Commercial teas consumed by the population are normally prepared by using one sachet bag (~ 2.0 g) per cup (150 - 225 mL) (ATOUI *et al*., 2005; CHAN *et al*., 2011; DU TOIT; VOLSTEEDT; APOSTOLIDES, 2001; KARORI *et al*., 2007; KIM *et al*., 2011; PEKAL; DROZDZ; PYRZYNSKA, 2012; TEJERO *et al*., 2014). On the other hand, the procedures used for infusion preparation used for scientific studies change greatly (particularly regarding time and temperature), which effectively interfere in the compound's extraction. It has been mentioned that five minutes using hot water is a handicap to extract the antioxidant compounds from tea (CAMPANELLA; BONANNI; TOMASSETTI, 2003).

TOTAL POLYPHENOL CONTENT

The TPC results found in the tea samples analyzed varied from type to type as it would expect (Table 1). Although the establishment of a TPC-based classification of each type requires a greater number of samples to avoid misleading conclusions, tea samples used in the present study can be divided into two mainly groups: i) *Cymbopogon citratus* Stapf*, Matricaria recutita* L., *Baccharis genistelloides* (11.5-23.1 mg GA/g DM) and ii) *Mentha piperita* L., *Peumus boldus, Ilex paraguariensis* St. Hil., *Camellia sinensis* L. Kuntze (56.9-133 mg GA/g DM).

It is noted that *Matricaria recutita* L. type has the lowest TPC value (11.5 - 23.0 mg GAg DM) while *Camellia sinensis* L. Kuntze provided the largest TPC values (69.5 - 133 mg GA/g DM).

Regarding only *Camellia sinensis* L. Kuntze group the TPC ranking is: black (82 ± 17) mg AG/g DM < green (103 ± 22) mg AG/g DM < white (128 ± 4) mg AG/g DM. It can be explained by considering the degree of fermentation to which the leaves were submitted that affected their polyphenolic components (BALENTINE; WISEMAN; BOUWENS, 1997). In fact, in white tea the young leaves are partially affected by steaming. On the other hand, in green tea the leaves are more affected by steaming and in the black tea the fermentation process is the most intense. In

addition, it was pointed out that different manufacturing processes of the teas can also affect their characteristics (WAN; LI; ZHANG, 2008).

TOTAL ANTIOXIDANT CAPACITY

Trolox® (a water-soluble vitamin E analogue) was chosen to express the antioxidant capacity of the teas analyzed with FCR since it is used as a standard antioxidant compound in the $ABTS^{\bullet+}$ method, a widely used procedure for the quantification of antioxidant capacity (RE *et al*., 1999, SAHIN, 2013, WOJDYLO; OSZMIAŃSKI; CZEMERYS, 2007). Besides, Trolox® has a high sensitivity (represented by the value of the angular coefficient, *'b'*, of the calibration curve) and very good reproducibility with FCR (*'b'* = (18.5 ± 0.9) L cm^{-1} mg^{-1}; n = 12; CV = 5.3 %; linear range (1.0-3.8)×10^{-2} mg mL^{-1}).

Table 1 shows the TEAC results (mol Trolox®/g DM) of 32 samples of 7 types of teas (*Baccharis genistelloides, Camellia sinensis* L. Kuntze*, Cymbopogon citratus* Stapf, *Ilex paraguariensis* St. Hil., *Matricaria recutita* L., *Mentha piperita* L., *Peumus boldus*) analyzed (11 different trademarks). Although *in vitro* TEAC values obtained can not be closely related to bioavailability of the compounds responsible for *in vivo* antioxidant capacity, a TEAC ranking of the analyzed samples can be established: *Matricaria recutita L.* < *Cymbopogon citratus* Stapf. < *Baccharis genistelloides* < *Mentha piperita* L < *Peumus boldus* < *Ilex paraguariensis* St. Hil. < black < green < white (*Camellia sinensis* L. Kuntze).

Figure 1 shows the comparison of the TPC values with the TEAC results for all analyzed samples. High positive correlation (TPC *vs.* TEAC, adjusted r^2 = 0.969) was verified between the antioxidant capacity and the polyphenolic content. Furthermore, it was verified that the group with lower polyphenol content, 11.5 - 23.1 mg GA/g DM, presents a lower correlation with TEAC (TPC *vs.* TEAC, adjusted r^2 = 0.472) than the group with the higher polyphenol content, 56.9 - 133 mg GA/g DM, (TPC *vs.* TEAC, adjusted r^2 = 0.912) which can, at first, be attributed to the phenolic compounds. Although polyphenols seem to be the compounds responsible for the antioxidant capacity of these samples, specific assays for other compounds should be performed if a phytochemical screnning is required.

Considering only the three teas of the same plant species (*Camellia sinensis* L. Kuntze) the TEAC average value (mol Trolox®/g DM) of the analyzed samples follows the order: black (907 ± 156) < green (1193 ± 235) < white (1586 ± 73), (TPC *vs.* TEAC, adjusted r^2 = 0.968), proving that the steaming process suffered by the leaves affected the antioxidant capacity of the tea (Figure 1, inserted). Considering all analyzed samples, the antioxidant capacity values obtained with the CUPRAC reagent showed good correlation with the TEAC (CUPRAC vs. TEAC vs. adjusted r^2 = 0.853) and with the TPC values as well (CUPRAC vs TPC *vs.* adjusted r^2 = 0.809). It confirms that the FCR can effectively be used to quantify the antioxidant capacity of tea samples.

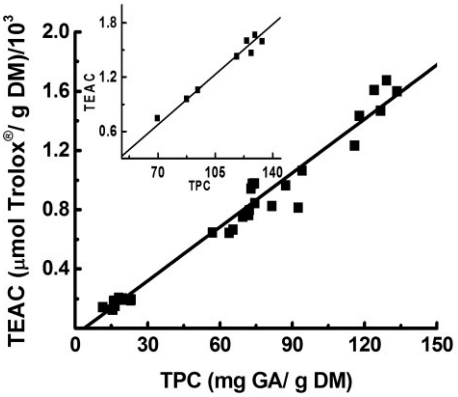

Figure 1. Values obtained for total polyphenol content (TPC, mg GA/ g DM) and Trolox® equivalents antioxidant capacity (TEAC, µmol Trolox/ g DM) of 32 samples of 7 different types of teas analyzed, $r^2 = 0.969$. Inserted figure: Values obtained for TPC (mg GA/ g DM) and the TEAC values (µmol Trolox/ g DM) for *Camellia sinensis* L. Kuntze group ($r^2 = 0.968$).

It is also possible to express the antioxidant capacity of all analyzed teas in 200 mL of infusion, taking this volume as regular cup of tea (Table 1). For this, ascorbic acid (AA) currently the most widely used vitamin supplement worldwide can be used as a standard despite the little (or none) amount of AA in these samples, and the results expressed as AA. In fact, AA also showed a high sensitivity and reproducibility with FCR (b = (55.5 ± 3.5) L cm^{-1} mg^{-1}, n = 11, CV = 6.3 % for a linear range (1.8-14)×10^{-3} mg mL^{-1}) and besides being less costly than Trolox®/g.

The CUPRAC reagent can also be used for this purpose. A calibration curve with CUPRAC method obtained with AA and can be described by the linear equation $A_{454nm} = a + b \times C_{AA}$ (where C_{AA} is the ascorbic acid concentration in mg mL^{-1}). AA resulted a high reproducibility with CUPRAC reagent (CV = 4.7 %) for twelve curves in a linear range (1.76-1.23) mg mL^{-1} but with less sensitivity (b = (0.0883 ± 0.0042) L cm^{-1} mg^{-1}.

Obviously, the AA/cup of tea values presented in Table 1 show the same good correlation with TPC as those found with TEAC. In any case, it may be a good alternative to present the antioxidant capacity values of teas to the consumers who are commonly unfamiliar with this concept and. In this context, Table 1 can be used as an easy guide for choosing which tea to intake.

Table 1. Total polyphenol content and antioxidant capacity of 32 commercial tea bags obtained with FCR and CUPRAC reagents.

Sample	Scientific name	Used part[a]	TPC[b]	Antioxidant capacity		AA/cup of tea[e]
				TEAC[c]	CUPRAC[d]	
1	*Baccharis genistelloides*	Leaves	23.1 ± 0.6	308 ± 14	16 ±2.4	15.9 ± 0.7
2	*Baccharis genistelloides*	Leaves	23.1 ± 1.3	196 ± 11	8.0 ± 0.7	10.6 ± 0.5
3	*Baccharis genistelloides*	Leaves	18.0 ± 0.8	205 ± 8	9.0 ± 0.7	10.6 ± 0.4
1	*Camellia sinensis* L. - green	Leaves and stalks	87.3 ± 2.3	964 ± 11	60 ± 9.0	50.0 ± 0.6
2	*Camellia sinensis* L. - green	Leaves and shoots	118 ± 6	1433 ± 30	51 ± 8.2	73.3 ± 1.5
1	*Camellia sinensis* L. - black	Leaves and stalks	69.5 ± 1.4	751 ± 51	45 ± 4.7	39.9 ± 2.6
2	*Camellia sinensis* L. - black	Leaves	94.1 ± 2.9	1063 ± 22	60 ± 7.8	54.9 ± 1.1
1	*Camellia sinensis* L. - white	Leaves and shoots	124 ± 4	1607 ± 83	71 ±11	83.8 ± 4.2
2	*Camellia sinensis* L. - white	Leaves and shoots	129 ± 3	1671 ± 26	68 ±10	85.4 ± 1.3
3	*Camellia sinensis* L. - white	Leaves	133 ± 6	1598 ± 39	69 ± 12	83.9 ± 1.9
4	*Camellia sinensis* L. - white	Leaves and shoots	127 ± 7	1469 ± 25	48 ±5.8	75.3 ± 1.2
1	*Cymbopogon citratus* Stapf	Leaves	19.5 ± 0.1	199 ± 7	23 ± 2.1	10.6 ± 0.4
3	*Cymbopogon citratus* Stapf	Leaves and flower heads	16.0 ± 0.9	179 ± 17	8.0 ±0.9	9.5 ± 0.8
1	*Ilex paraguariensis* St. Hil.	Leaves and stalks	116 ± 1	1234 ± 5	55 ± 6.6	64.1 ± 0.2
2	*Ilex paraguariensis* St. Hil.	Leaves and stalks	73.4 ± 1.4	974 ± 18	51 ± 7.1	50.3 ± 0.9
3	*Ilex paraguariensis* St. Hil.	Leaves and stalks	81.6 ± 1.7	824 ± 24	25 ± 4.0	43.6 ± 1.2
1	*Matricaria recutita* L.	Flower heads	16.1 ± 0.4	186 ± 5	3.0 ± 0.5	9.7 ± 0.2
2	*Matricaria recutita* L.	Flower heads	15.5 ± 0.4	125 ± 9	5.0 ± 0.6	7.0 ± 0.4
3	*Matricaria recutita* L.	Flower heads	23.0 ± 0.8	188 ± 12	5.0 ± 0.7	10.4 ± 0.6
4	*Matricaria recutita* L.	Flowers and stalks	16.2 ± 0.5	165 ± 4.0	5.0 ± 0.5	8.7 ± 0.2
5	*Matricaria recutita* L.	Flowers	16.5 ± 0.2	151 ± 8	6.0 ± 0.5	8.1 ± 0.4
6	*Matricaria recutita* L.	Flower heads	19.4 ± 0.2	193 ± 14	4.0 ± 0.3	10.2 ± 0.7
7	*Matricaria recutita* L.	Flowers	11.5 ± 0.1	143 ± 1	5.0 ± 0.7	7.4 ± 0.1
1	*Mentha piperita* L.	Leaves and branches	56.9 ± 2.4	644 ± 10	23 ± 2.5	33.5 ± 0.5
2	*Mentha piperita* L.	Leaves and branches	74.3 ± 1.4	977 ± 42	34 ± 4.1	50.9 ± 2.1
3	*Mentha piperita* L.	Leaves and branches	65.4 ± 2.2	665 ± 14	13 ± 1.8	35.0 ± 0.7
4	*Mentha piperita* L.	Leaves	63.8 ± 1.9	643 ± 12	25 ± 2.8	34.1 ± 0.6
1	*Peumus boldus*	Leaves	74.5 ± 1.2	844 ± 22	21 ± 1.1	44 ± 1.0
2	*Peumus boldus*	Leaves	92.5 ± 2.6	812 ± 48	26 ± 2.3	44.5 ± 0.8
3	*Peumus boldus*	Leaves	72.9 ± 4.0	942 ± 38	35 ± 3.9	48.4 ± 1.9
4	*Peumus boldus*	Leaves	72.0 ± 4.7	764 ± 56	26 ± 2.8	39.4 ± 2.8
5	*Peumus boldus*	Leaves	72.2 ± 0.7	797 ± 25	26 ± 3.1	40.9 ± 1.3

[a]As reported by the supplier. [b]Total polyphenol content expressed in mg GA/g dry material. [c]Trolox® equivalents antioxidant capacity expressed in µmol Trolox/g dry material. [d]Cupric reducing antioxidant capacity expressed in 10^3 g dry material/mg AA. [e]expressed as mg AA/200 mL (cup of tea).

CONCLUSIONS

In the present study a large number of commercially available teas were evaluated in order to simultaneously determine the polyphenol content and antioxidant capacity using the Folin Ciocalteu reagent. These results were compared and showed excellent correlation with each other, indicating that the polyphenols should be responsible for the antioxidant capacity in these samples. In addition, the results of antioxidant capacity obtained with FCR were well correlated with the values obtained with the CUPRAC reagent, confirming that the former can effectively be used to quantify the antioxidant capacity in teas.

All the analyzed samples presented some antioxidant capacity so possible health benefits can be obtained if these teas are regularly consumed. The antioxidant compounds present in these infusions are chemically different which make it difficult to adopt a single method to evaluate this important parameter. In this context, the FCR procedures and calculation strategies adopted here can serve as an easy guide to choose which tea to be intake or even as a routine test to be used in quality control.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support from Brazilian agencies FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

Metodologia simplificada para avaliação simultânea do teor de polifenóis e capacidade antioxidante em chás comerciais usando o reagente de Folin Ciocalteu

RESUMO

PALAVRAS-CHAVE: .

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Recebido: 06 jun. 2018.
Aprovado: 29 out. 2021.
DOI: 10.3895/rebrapa.v11n3.8378
Como citar:
MARCELINO, C. P. et al. A handy-assay procedure to measure simultaneously the polyphenol content and the antioxidant capacity in teas using the Folin Ciocalteu reagent. **Brazilian Journal of Food Research,** Campo Mourão, v. 11 n. 3, p. 54-70, jul./set. 2020. Disponível em: https://periodicos.utfpr.edu.br/rebrapa

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