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Modification and validation of Folin-Ciocalteu assay for faster and safer analysis of total phenolic content in food samples

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ABSTRACT

Folin-Ciocalteu (F-C) is a routine assay in several laboratories around the world and has been widely employed for total phenolic content (TPC) quantification from food samples. F-C assay was modified to obtain a system reaction safer, as well as decrease the use of reagents, energy expenditure and time-consuming. After evaluating alternatives solvents and supports, and the effect of the binomial time/temperature, the modified F-C assay was as follows: 40% ethanol as solvent, 20 minutes at room temperature (25±2°C) using 50% F-C reagent and 5% sodium carbonate solution. The modified F-C assay was linear in 2.5-50.0 µg/mL range to gallic acid. Limit of detection and limit of quantification were 0.195 and 0.591 µg/mL, respectively. Intra-day and inter-day precision (relative standard deviation (RSD) 0.06-3.28%) and accuracy (93.28-104.28%) were also demonstrated. The assay was robust for F-C reagent (45-55%) and sodium carbonate (4.5-5.5%) changes. The modified assay was employed to analyse food samples containing phenolic compounds and the results corroborated with conventional assay. The modified F-C assay demonstrated to be reproducible, robust, fast, easy, inexpensive, safe and reliable for quantify phenolic compounds in food samples. The employment of ethanol in F-C assay decreases the environmental impact and, consequently, makes the analysis safer than conventional F-C assay. Furthermore, the modified F-C assay is conducted under milder conditions (time/temperature), which is particularly helpful for numerous analyses. Thus, the modified and validated F-C assay can be used as a routine assay in quality control and chemical profiling for natural product extracts and foods.

KEYWORDS: bioactive compounds; extraction; food analysis; food control; food quality; phenolic compounds.

INTRODUCTION

Phenolic compounds are the secondary metabolites found in all plant organs. They are widespread constituents of plant foods and beverages, and therefore they are an important part of the human diet (DAI & MUMPER, 2010). Chemically, phenolic compounds possess at least one aromatic ring containing one or more hydroxyl substituents and may be associated with carbohydrates, lipids, organic acids, amines and cell wall components. The structure of phenolic compounds ranges from simple molecules (phenolic acids) up to polymers (proanthocyanidins) (IGNAT *et* al., 2011). In general, phenolic compounds are excellent antioxidant and antimicrobial agents and have been described as food preservatives, as well as the consumption of fruits rich in (poly)phenols can prevent, slow or reverse the development of diseases, such as cancer, cardiovascular diseases and neurological disorders (VALDÉS *et al.*, 2015).

The interest in phenolic compounds from foods has progressively increased and spectrophotometric analytical methods, such as Folin-Ciocalteu (F-C) assay, have become a routine in a number of laboratories around the world due to their simplicity and low cost. The F-C assay is widely used for measuring total phenolic content (TPC) and provides simple and fast screening of TPC from food samples (DAI & MUMPER, 2010; IGNAT *et al.*, 2011; KHODDAMI *et al.*, 2013).

The F-C assay is a colorimetric method based on the reduction of Folin-Ciocalteu reagent (a mixture of sodium molybdate, sodium tungstate, and other reagents) through single electron transfer from phenol to complexed Mo (VI) **(**Mo (VI) + e → Mo (V)**)**. In alkaline medium (pH ~10 adjusted by a sodium carbonate solution), the dissociation of a phenolic proton leads to a phenolate anion that is able to reduce the F-C reagent. The result of the reaction between phenolic compounds and the F-C reagent is the development of blue complex, which presents wavelength of maximum absorption close to 760 nm (EVERETTE *et al.*, 2010; HUANG *et al.*, 2005; SÁNCHEZ-RANGEL *et al.*, 2013).

The F-C reagent is significantly reactive toward other food compounds besides phenols, such as vitamins, amino acids, proteins, carbohydrates, organic acids, inorganic ions and metal complexes (EVERETTE *et al.*, 2010). However, different methodologies have been proposed to improve the specificity of the F-C assay (CASTRO-ALVES & CORDENUNSI, 2015; SÁNCHEZ-RANGEL *et al.*, 2013). Likewise, different modifications were suggested over the past years, for example, to eliminate interferences due to fine solids formation (CICCO *et al.*, 2009; CICCO & LATTANZIO, 2011) and to decrease the time-consuming of the F-C assay (AINSWORTH & GILLESPIE, 2007; MAGALHÃES *et al.*, 2010). Furthermore, the effect of the basification step on results was demonstrated (CHEN *et al.*, 2015).

Certain limitations of the conventional F-C assay were overcome. However, there are still two main problems: i) the employment of toxic solvent (methanol) and ii) the heating of the reaction medium. In this way, we evaluated the use of alternative solvents (water and ethanol) and supports (glass or polypropylene microtube) and the effect of time/temperature on the development of the blue complex. The modified F-C assay was validated according to guidelines established by the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH Guidelines, 2017). In this approach, the aim of this work was to improve the F-C assay to obtain a system

reaction safer, as well as decrease the reagents, energy and time-consuming of conventional F-C assay.

MATERIAL AND METHODS

APPARATUS

All of the spectrophotometric measurements were performed with a UV-Vis spectrophotometer (Beckman, model DU600, CA, USA).

CHEMICALS AND REAGENTS

All chemicals were analytical-reagent grade and the water was ultrapure (18 MΩ cm^{-1}) obtained from a Milli-Q water purification system (Millipore, Bedford, USA). The chemicals included methanol, ethanol, Folin-Ciocalteu reagent (Dinâmica, Diadema, Brazil), anhydrous sodium carbonate (Synth, Diadema, Brazil), and gallic acid with purity > 95% (Sigma-Aldrich, St. Louis, MO, USA).

GALLIC ACID STOCK SOLUTIONS PREPARATION

Gallic acid was chosen as standard phenol because of its abundance in food matrices (DANESHFAR *et al*., 2008). Gallic acid stock solutions (100 µg/mL) were prepared by dissolving 2.5 mg of the standard in ultrapure water, 40% methanol (v/v) or 40% ethanol (v/v) in a 25 mL volumetric flask.

EFFECT OF THE SOLVENT ON THE FOLIN-CIOCALTEU ASSAY

For assessing the effect of the solvent on the F-C assay, three solvents (ultrapure water, 40% methanol (v/v) and 40% ethanol (v/v)) were used to prepare the gallic acid standard solutions. The influence of the solvent on the development of the blue complex was performed through measuring of absorbance at 760 nm (maximum wavelength absorption), related to mixtures containing 100 µL of 10, 30 and 50 µg/mL gallic acid, 100 µL of 50% F-C reagent (v/v), and 800 µL of 5% sodium carbonate (w/v), after 20 minutes of incubation at 40 °C using a water bath shaker. Sealed glass test tubes were used to perform the reactions. The absorbance values obtained for each studied solvent were evaluated statistically by analysis of variance using one-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$).

EFFECT OF THE REACTION SUPPORTS ON THE FOLIN-CIOCALTEU ASSAY

Glass test tubes and polypropylene microtubes were used to evaluate the effect of the reaction supports on the F-C assay. The influence of the reaction support on the development of the blue complex was performed through measuring the absorbance at 760 nm, related to mixtures containing 100 µL of 10, 30 and 50 µg/mL gallic acid prepared in 40% ethanol (v/v), 100 µL of 50% F-C

reagent (v/v), and 800 µL of 5% sodium carbonate (w/v), after 20 minutes of incubation at 40 °C. The statistical significance of differences between the reaction supports was evaluated by the Student's independent-samples t test ($p \leq 0.05$).

DETERMINATION OF THE OPTIMUM REACTION TIME AND TEMPERATURE

In order to establish the lower reaction time at which the development of the blue complex can be considered maximum, the time-behaviour of absorbance curve at 760 nm, related to a mixture containing 100 µL of 30 µg/mL gallic acid prepared in 40% ethanol (v/v), 100 µL of 50% F-C reagent (v/v), and 800 µL of 5% sodium carbonate (w/v), was monitored in a period of 60 minutes at 25, 30 and 40 °C. The absorbance values of the reaction temperatures for each reaction time were observed and evaluated statistically by analysis of variance using one-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$).

ANALYTICAL ASSAY VALIDATION

The TPC assay was modified and optimized based on F-C assay given by Ainsworth and Gillespie (2007), Cicco *et al.* (2009) and Cicco and Lattanzio (2011). The analytical assay was conducted as follows: 100 µL of properly diluted samples, calibration solutions or blank were pipetted into separate polypropylene microtubes. Then 100 µL of 50% F-C reagent (v/v) and 800 µL of 5% sodium carbonate (w/v) were added to each. The material was mixed and incubated at room temperature for 20 minutes. The absorbance was measured at 760 nm against a blank on a UV-Vis spectrophotometer. 40% ethanol (v/v) was used for samples, calibration solutions and blank preparation. The 50% F-C reagent (v/v) and 5% sodium carbonate (w/v) was prepared in deionized water.

The validation of the analytical assay was conducted on the basis on the guidelines established by the ICH (International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use) (ICH Guidelines, 2017). For assessing the validation parameters, the assays were performed according to the experimental conditions previously mentioned.

For the linearity study, an eleven-point calibration curve was constructed using different concentrations of gallic acid stock solutions (range of 2.5-50.0 µg/mL). These ranges let to cover absorbance values up to 1. The absorbance at 760 nm was plotted versus the gallic acid concentrations to produce a calibration curve. The verification of the outliers' absence was made by Grubbs' test while data homoscedasticity was checked by Cochran's C test. Linear regression analysis was performed by analysis of variance (ANOVA) to check the statistical significance of regression equation and linearity deviation. All measurements were carried out as three genuine replicates.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the Equations 1 and 2.

$$LOD\ (\mu g/mL) = \frac{3.3\sigma}{S} \quad \text{(Equation 1)}$$

$$LOQ\ (\mu g/mL) = \frac{10\sigma}{S} \quad \text{(Equation 2)}$$

Where σ is the standard deviation of the blank and S is the slope of the calibration curve.

The modified F-C assay precision was determined by repeatability (intra-day) and reproducibility (inter-day) assays. The repeatability was assessed using three concentrations of gallic acid (10, 30 and 50 µg/mL) analysed three times within the same day, whereas the reproducibility was assessed using the above mentioned concentrations of gallic acid analysed on three successive days. Precision was expressed as relative standard deviation (RSD) of three replicates. A RSD over 5% were considered unacceptable.

The robustness of the modified F-C assay was determined by analysing the effect of the sodium carbonate (Na_2CO_3) (4.5, 5.0 and 5.5% (w/v)) and F-C reagent (45, 50 and 55% (v/v)) concentrations on the stability of the reaction using three concentrations of gallic acid (10, 30 and 50 µg/mL). All measurements were performed in three replicates and the results were expressed as relative standard deviation (RSD). A RSD over 5% were considered unacceptable.

The accuracy of the modified F-C assay was evaluated using different food samples spiked with gallic acid solution (10 µg/mL). Accuracy was measured by means of the recovery percentage, which was calculated from Equation 3.

$$Recovery\ (\%) = \frac{A_{Spiked\ Sample} - A_{Standard}}{A_{Sample}} \times 100 \qquad \text{(Equation 3)}$$

Where $A_{Spiked\ Sample}$ = absorbance of the sample after addition of the standard (10 µg/mL gallic acid); $A_{Standard}$ = absorbance of the standard solution (10 µg/mL gallic acid); and A_{Sample} = absorbance of the sample without addition of the standard.

All measurements were performed in three replicates. The assay was considered accurate if the recovery percentages were between 85% and 115%.

APPLICATION OF THE MODIFIED FOLIN-CIOCALTEU ASSAY TO FOOD EXTRACTS

In order to demonstrate the applicability of the modified F-C assay, the TPC quantification in common food sample-derived extracts was carried out.

The food samples (apple, banana, grape, pineapple, cabbage, lettuce, carrot, tomato, broccoli, soybean, peanut, flaxseed, bean and popcorn) used for the assay validation were purchased from the local market of Campinas (São Paulo, Brazil). The food samples were washed with tap water to remove surface dirt. The edible parts of food samples (10 g) were extracted in 100 mL of 50% ethanol (v/v) with the aid of Ultra-Turrax Homogenizer at 11,000 rpm for 30 seconds (PEREIRA *et al.*, 2017). Only the grains and seeds were previously immersed overnight in 50% ethanol (v/v) at 5 °C. Once the extraction was completed, the supernatant was separated from the insoluble solids by centrifugation at 3,801 *g* for 11 minutes at 5 °C (Hettich Zentrifugen, model Rotanta 460R, Germany). The supernatant was used for the TPC analysis.

The TPC determination in the food samples by the modified F-C assay was performed according to the experimental conditions previously mentioned (see Section Analytical Assay Validation). The absorbance values were interpolated in the calibration curve to calculate the TPC. The results were expressed as mg gallic acid equivalents per 100 g of fruit fresh weight (g GAE/100 g fw). TPC was also

determined by means of conventional F-C assay (ROESLER *et al.*, 2007). All measurements were carried out in triplicate.

STATISTICAL ANALYSIS

All statistical analyses were performed at a significance level of 5% ($p \leq 0.05$) using STATISTICA software (Statsoft, Oklahoma, USA) version 12.0. The data are reported as mean values with standard deviation of three measurements.

RESULTS AND DISCUSSION

OPTIMIZATION OF REACTION CONDITIONS

Solvent used to prepare the standard solutions and samples, reaction support, and reaction time were considered and analysed in order to establish the optimal reaction conditions to the present analytical assay.

The solvent affects the performance of F-C assay due to phenolic compounds solubility and fine solids formation. Table 1 presents the effect of the solvents on the F-C assay. The use of methanol and ethanol as solvent did not affect the absorbance, whereas water decreased the response. In the low gallic acid concentration (10 µg/mL) the absorbance was statistically equal to all solvents tested. However, when the standard concentration increased (30-50 µg/mL) the response decreased only to water. Phenolics are compounds of low polarity and therefore, present higher solubility in methanol (76.2 polarity) and ethanol (65.4 polarity) than water (100 polarity) (SMALLWOOD, 1996). For example, the relative solubility of gallic acid in the solvents was found as methanol > ethanol > water > ethyl acetate (DANESHFAR *et al*., 2008). According to the above-mentioned comments, the ethanol can be employed as substitute of methanol in F-C assay. Methanol has similar physical properties to ethanol; however, it is toxic and therefore, ethanol is preferred solvent in most applications (KERTON & MARRIOTT, 2013). The employment of ethanol in F-C assay decreases the environmental impact, furthermore, it makes the analysis safer than conventional F-C assay.

Table 1. Effect of the solvents on the Folin-Ciocalteu assay.

Gallic acid concentration (µg/mL)	Absorbance at 760 nm		
	Water	40% Ethanol	40% Methanol
10	$0.1438^{A} \pm 0.0035$	$0.1473^{A} \pm 0.0041$	$0.1476^{A} \pm 0.0047$
30	$0.4091^{B} \pm 0.0060$	$0.4322^{A} \pm 0.0027$	$0.4292^{A} \pm 0.0051$
50	$0.6636^{B} \pm 0.0048$	$0.7063^{A} \pm 0.0090$	$0.7079^{A} \pm 0.0057$

NOTE: Data are presented as mean with standard deviation of three measurements (n=3). Values on the same line indicated with the same capital letter do not differ according to Tukey's test ($p>0.05$).

Fine solids can be formed throughout the F-C assay due to alcohol and sodium carbonate concentrations in the final mixture and this affects the TPC results. The formation of particles is delayed and slowed down with decreasing alcohol concentration and can be further prevented when a carbonate solution at 5% (w/v) is used (CICCO & LATTANZIO, 2011), which justify the use of alcohol solutions at 40% (v/v) in the present study.

For reaction supports, there was no statistically significant difference ($p>0.05$) between glass test tubes and polypropylene microtubes (Table 2). The use of microtubes presents the advantage to make the assay faster and safer than conventional F-C assay because glass test tubes require careful handling.

Table 2. Effect of the reaction supports on the Folin-Ciocalteu assay.

Gallic acid concentration (µg/mL)	Absorbance at 760 nm		Student's t test	
	Glass test tubes	Polypropylene microtubes	*t* value	*p*-value
10	0.1473 ± 0.0041	0.1467 ± 0.0057	0.1573	0.8827^{ns}
30	0.4322 ± 0.0027	0.4342 ± 0.0070	-0.4618	0.6682^{ns}
50	0.7063 ± 0.0090	0.7063 ± 0.0105	0.0042	0.9969^{ns}

NOTE: Data are presented as mean with standard deviation of three measurements (n=3). ns: not significant according to Student's t test ($p>0.05$).

In order to find optimal reaction time, 30 µg/mL gallic acid solutions were tested with the modified F-C assay. The absorbance of the blue complex was monitored in a period of 60 minutes at 25, 30 and 40 °C (Figure 1). These data showed that the F-C reaction was stable during the period analysed for all studied temperatures, since after 20 minutes the absorbance increased less than 5% of the value at 5 minutes, and did not decrease between 20 and 60 minutes. Furthermore, it could be observed that the reaction temperature did not affect the formation rate and stability of blue complex, once the mean absorbance values of the reaction temperatures for each reaction time were statistically equal ($p>0.05$). These results suggested the optimum reaction condition was 20 minutes at room temperature. The optimal reaction time of 20 minutes (at room temperature) of the modified F-C assay was lower than the protocol time established by other conventional F-C assays available: 120 minutes (AINSWORTH & GILLESPIE, 2007; BOBO-GARCÍA *et al.*, 2015).

ASSAY VALIDATION

The linearity of standard calibration curve was evaluated by linear regression analysis. The standard calibration curve was calculated by the least squares regression method to calculate the calibration equation and the determination coefficient (R^2). Figure 2 shows the standard calibration curve of the modified F-C assay. A linear correlation was found between absorbance of the blue complex at 760 nm and concentration of gallic acid in the range 2.5-50.0 µg/mL. The determination coefficient obtained from the linear regression was 0.9997, indicating excellent linear correlation between the data. Furthermore, the linear regression of the gallic acid calibration curve were statistically analysed and the

results obtained are shown in Table 3. No significant outliers were detected in any of the groups of absorbance for each concentration level of gallic acid by Grubbs' test. Data were homoscedastic by Cochran's C test, indicating that data variances were homogeneous. The regression equation was statistically significant and it did not exhibit linearity deviation by analysis of variance (ANOVA) in the range evaluated. The slope and intercept of linear regression were statistically different from zero. These data indicates a good fit of regression model. Therefore, the obtained regression equation can be satisfactorily used to estimate the TPC in unknown samples.

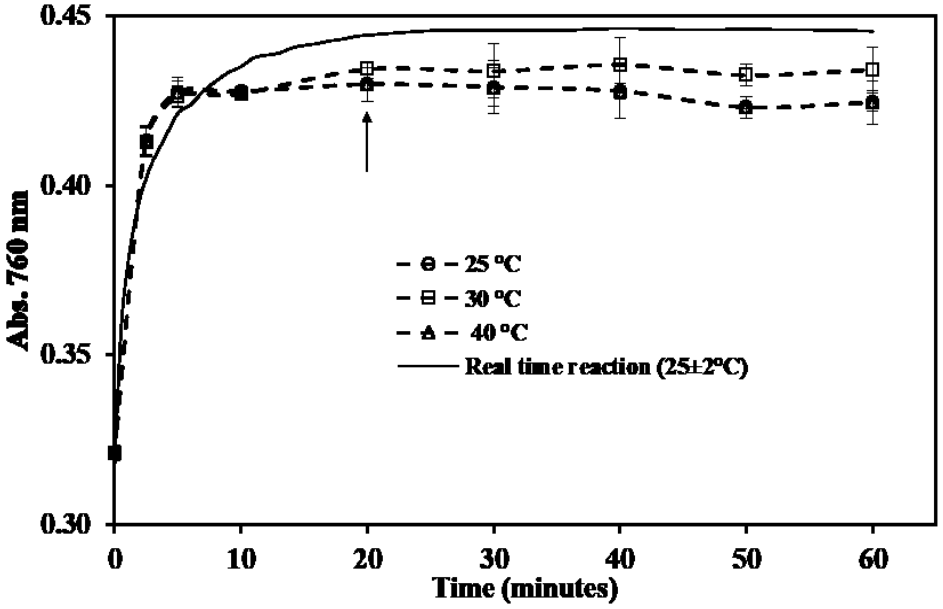

Figure 1 - Time required to complete the Folin-Ciocalteu reaction at different temperatures. The absorbance (Abs.), data referred to mixtures contain 30 µg/mL gallic acid, represent means of triplicates (n=3).

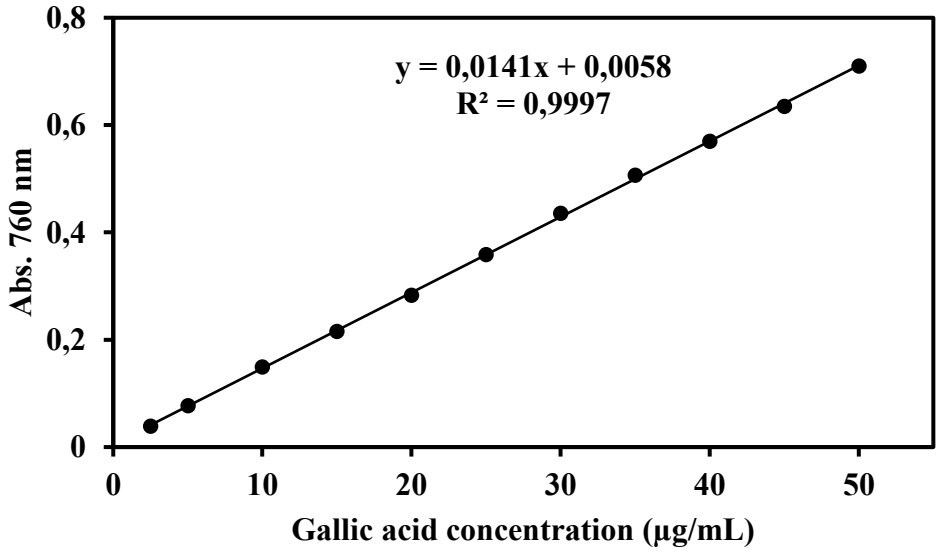

Figure 2 - Standard calibration curve for modified Folin-Ciocalteu Micro-assay. Markers correspond to the means of triplicates (*n*=3).

From the slope of the calibration curve, LOD and LOQ were established. The LOD and LOQ for the modified F-C assay were 0.195 and 0.591 µg GAE/mL, respectively (Table 3). The LOD and LOQ found herein were lower than the values obtained in other TPC assays available in the literature (MITIC *et al.*, 2014; STOICESCU *et al.*, 2012), which indicates that the actual modified F-C assay shows high sensitivity. The modified spectrophotometric assay was even more sensitive than microplate assays (BOBO-GARCÍA *et al.*, 2015; PUEYO & CALVO, 2009).

Table 3. Statistical data for the linear regression of the gallic acid calibration curve for modified Folin-Ciocalteu assay.

Parameter	Gallic acid calibration curve
Linear range (µg/mL)	2.5-50.0
Slope (SE)[a]	0.0141 (0.00007)
t value	197.26
p-value	<0.0001
Intercept (SE)[a]	0.0058 (0.00212)
t value	2.73
p-value	0.0123
Determination coefficient (R^2)	0.9997
Linear Regression (Analysis of variance)	
F value	38,912.28
p-value	<0.0001
Lack of fit (Linearity deviation test)	
F value	1.44
p-value	0.2295
Cochran's C test (Homoscedasticity test)	
C value	0.33
p-value	0.2099
Grubbs' test (Outlier test)	No significant outliers were detected in any of the groups
LOD[a] (µg/mL)	0.195
LOQ[a] (µg/mL)	0.591

SE: standard error; LOD: limit of detection; LOQ: limit of quantitation.

The precision of the modified F-C assay was evaluated by measuring repeatability (intra-day) and reproducibility (inter-day) of the measurement for three replicates at three different concentrations and expressed in terms of RSD. As can be seen in Table 4, the RSD values were ≤0.88% and ≤3.28% for repeatability and reproducibility, respectively. These results show that the proposed assay is adequately precise.

Table 4. Intra-day (repeatability) and inter-day (reproducibility) precision of the modified Folin-Ciocalteu assay.

Gallic acid concentration (µg/mL)	Intra-day precision (*n*=3)		Inter-day precision (3 days)	
	Absorbance at 760 nm	RSD (%)	Absorbance at 760 nm	RSD (%)
10	0.1493 ± 0.0010	0.68	0.1500 ± 0.0049	3.28
30	0.4313 ± 0.0038	0.88	0.4302 ± 0.0020	0.47
50	0.7095 ± 0.0004	0.06	0.7088 ± 0.0004	0.06

NOTE: Data are presented as mean with standard deviation of three measurements (*n*=3). RSD: relative standard deviation.

The robustness of the modified F-C assay was examined by evaluating the influence of small variations in the assay variables (sodium carbonate and F-C reagent concentrations) on its analytical performance and expressed in terms of RSD. The results presented in Table 5 revealed that small variations in the sodium carbonate (4.5-5.5%, w/v) or F-C reagent (45-55%, v/v) concentrations did not induce considerable changes in absorbance values at 760 nm, in which the RSD values were ≤2.95% and ≤3.48% for sodium carbonate and F-C reagent concentrations, respectively. The proposed assay is sufficiently robust under the described experimental conditions.

Table 5. Effect of the sodium carbonate (Na_2CO_3) and Folin-Ciocalteu reagent concentrations on the robustness of the modified Folin-Ciocalteu assay.

Gallic acid concentration (µg/mL)	Sodium carbonate (Na_2CO_3) concentration (4.5-5.5%, w/v)		Folin-Ciocalteu reagent concentration (45-55%, v/v)	
	Absorbance at 760 nm	RSD (%)	Absorbance at 760 nm	RSD (%)
10	0.1441 ± 0.0042	2.95	0.1432 ± 0.0050	3.48
30	0.4265 ± 0.0018	0.42	0.4283 ± 0.0048	1.11
50	0.7067 ± 0.0050	0.71	0.7099 ± 0.0029	0.40

NOTE: Data are presented as mean with standard deviation of three measurements (*n*=3). RSD: relative standard deviation.

The accuracy of the modified F-C assay was determined by means of recovery test. Different food samples were spiked with gallic acid solution (10 µg/mL) and then analysed using the proposed assay. The results presented in Table 6 show good recovery percentages (93.28-104.28%) and small RSD values (≤4.02%), which indicates excellent accuracy of the proposed assay to TPC quantification in food samples.

APPLICATION OF THE MODIFIED FOLIN-CIOCALTEU ASSAY TO FOOD SAMPLES

In order to demonstrate the performance of the present assay, real food samples were submitted to quantification process. The results were compared with those obtained from conventional F-C assay (Table 6). In this study, the RSD values and error percentages were used as an indicator of the suitability of the modified F-C assay. TPC results obtained by means of modified F-C assay demonstrated to be reproducible and comparable with those from conventional F-C assay, once RSD values and error percentages between the assays were ≤6.85%

and ≤9.23%, respectively (Table 6). Taking into consideration that conventional assays are not necessarily absolute nor accurate, RSD values and error percentages lower than 10% are quite satisfactory. In addition, the modified F-C assay is safer and has low environmental impact than the conventional F-C assay because the described procedure does not need heating, requires less organic solvent, employs GRAS solvent (ethanol) and generates less wastes (reduction in the reaction volume). Although the LC-MS/MS methods and others more advanced techniques are more selective and sensitive, the F-C assay is considered more convenient for routine analyses to be fast and low cost. Moreover, the proposed F-C assay herein showed advantages, such as the shorter analysis time (20 minutes of reaction), easier procedure (requires less manipulation) and lower cost (employment of cheaper reagents), besides the afore-mentioned advantages. Therefore, the usefulness of proposed assay is evident in routine natural product and food chemistry studies.

Table 6. Accuracy of the modified Folin-Ciocalteu assay by recovery test and TPC in food samples using conventional and modified Folin-Ciocalteu assays.

Sample	Accuracy		TPC (mg GAE/100 g fw)			
	Recovery (%)[a]	RSD (%)[b]	Conventional assay	Modified assay	RSD (%)	Error (%)
Apple	101.40 ± 1.80	1.07	79.90 ± 2.36	77.72 ± 0.28	1.95	-2.72
Banana	100.55 ± 1.74	1.73	34.12 ± 0.16	33.47 ± 0.23	1.35	-1.89
Grape	97.56 ± 0.09	0.09	47.82 ± 0.59	47.20 ± 0.08	0.92	-1.30
Pineapple	97.74 ± 0.81	0.83	30.05 ± 0.37	27.28 ± 0.62	6.85	-9.23
Cabbage	98.21 ± 0.02	0.02	217.21 ± 4.42	216.41 ± 6.64	0.26	-0.37
Lettuce	99.91 ± 0.76	0.76	41.06 ± 0.31	41.49 ± 1.04	0.72	1.03
Carrot	104.28 ± 0.88	0.84	20.44 ± 0.87	20.04 ± 0.20	1.40	-1.96
Tomato	99.51 ± 0.99	1.00	19.87 ± 0.66	18.83 ±0.01	3.81	-5.25
Broccoli	98.33 ± 1.09	1.11	113.21 ± 1.88	110.93 ± 1.00	1.44	-2.02
Soybean	99.89 ± 1.16	1.16	129.23 ± 1.19	132.73 ± 0.92	1.87	2.68
Peanut	93.28 ± 3.75	4.02	304.40 ± 4.81	303.74 ± 2.25	0.15	-0.22
Flaxseed	98.52 ± 1.90	1.93	266.32 ± 1.93	249.31 ± 5.53	4.67	-6.39
Bean	96.49 ± 0.51	0.53	112.28 ± 1.99	111.93 ± 1.84	0.22	-0.31
Popcorn	96.08 ± 0.46	0.47	58.22 ± 0.15	54.77 ± 0.25	4.32	-5.93
Overall	98.70 ± 2.62	2.65				

NOTE: Data are presented as mean with standard deviation of three measurements (*n*=3).
RSD: relative standard deviation.

CONCLUSION

A spectrophotometric assay was modified and validated for the TPC determination using F-C reagent. This spectrophotometric assay demonstrated to be precise, robust and accurate using a simple, inexpensive, sensitive, easy, and fast procedure. Furthermore, the modified F-C assay involved simple and safe experimental conditions; once it is free from extreme experimental conditions such as heating and time-consuming reaction, beyond to employ GRAS solvent (ethanol). The modified F-C assay also exhibited comparable analytical performance with conventional assay for TPC quantification and proved to provide adequate analytical results on food extracts as representative real samples. Therefore, the proposed assay can be used as a routine assay in quality control and chemical profiling for natural product extracts and foods.

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Modificação e validação do ensaio de Folin-Ciocalteu para análise mais rápida e segura do conteúdo total de compostos fenólicos em alimentos

RESUMO

O Folin-Ciocalteu (F-C) é um ensaio de rotina em vários laboratórios do mundo e tem sido amplamente empregado para a quantificação do conteúdo total de compostos fenólicos (TPC) a partir de alimentos. No presente estudo, o ensaio de F-C foi modificado para obter um sistema reacional mais seguro, bem como diminuir o uso de reagentes, o gasto de energia e o tempo de análise. Após avaliar o uso alternativo de solventes e suportes para a reação, e o efeito do binômio tempo/temperatura, o ensaio F-C modificado foi definido como segue: etanol 40% (v/v) como solvente, tempo de reação de 20 minutos a temperatura ambiente (25±2 °C) empregando reagente F-C 50% (v/v) e solução de carbonato de sódio 5% (p/v). O ensaio de F-C modificado foi linear no intervalo de 2,5-50,0 µg/mL de ácido gálico. O limite de detecção e limite de quantificação apresentaram valores de 0,195 e 0,591 µg/mL, respectivamente. A repetitividade, reprodutibilidade (desvio padrão relativo (RSD) 0,06-3,28%) e acurácia (93,28-104,28%) do método modificado também foram demonstradas. O ensaio foi robusto para as alterações na concentração do reagente F-C (45-55%) e carbonato de sódio (4,5-5,5%). O ensaio modificado foi empregado para analisar amostras de alimentos contendo compostos fenólicos e os resultados corroboraram com o ensaio convencional. O ensaio F-C modificado demonstrou ser reprodutível, robusto, rápido, fácil, barato, seguro e confiável para quantificar compostos fenólicos em alimentos. O emprego do etanol no ensaio F-C diminui o impacto ambiental e, consequentemente, torna a análise mais segura do que o ensaio convencional. Além disso, o ensaio F-C modificado é conduzido em condições mais brandas (tempo/temperatura), o que é particularmente útil para um grande volume de análises. Assim, o ensaio F-C modificado e validado pode ser usado como ensaio de rotina no controle de qualidade e no perfil químico de extratos de produtos naturais e alimentos.

PALAVRAS-CHAVE: compostos bioativos; extração; análise de alimentos; controle de qualidade; compostos fenólicos.

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