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Research Article

VALIDATION OF ANALYTICAL METHOD AND INVESTIGATION INTO THE EFFECTS OF BREWING TEMPERATURE ON TOTAL POLYPHENOL CONTENTS IN TEA INFUSIONS PREPARED FROM VIETNAMESE TEA PRODUCTS

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ABSTRACT

Produced from leaves of Camellia sinensis L. *(Kuntz), tea is among the most common beverages worldwide. Tea plants have been grown in nearly 30 countries. In this study, the Folin-Ciocalteu method was applied to determine the total polyphenol contents (referred to milligrams gallic acid mass equivalence or GAE) in tea products (one oolong and three ancient teas, including red, green, and white) based on ISO 14502-1:2005 with some modifications, typically ultrasonic-assisted single extraction using methanol:water (7:3 v/v) as an extraction solvent at 70°C with the extraction ratio of 0.200:10.00 (g:mL). The analytical method was validated using Shimadzu 1800 UV-Vis instrument with favorable linearity of R^2 > 0.995, linear range of 10-70 mg GAE L^{-1}, acceptable repeatability, reproducibility (% RSDs were 0.79 and 1.2 for intra-day and inter-day, respectively), and high recoveries (higher than 98% for spiked samples). The total polyphenol contents (mean values, mg GAE g^{-1} dried weight, in brackets) performed a descending order of white (206.62) ~ green (201.33) > red (167.42) > oolong (139.18) teas due to the variation in the oxidation levels during the fermentation, particularly for red and oolong teas. Higher polyphenol contents released in tea infusions were observed regarding the increasing brewing temperature, except for oolong tea with its specific fluctuation, maybe due to its "wrap-curled" structure, leading to longer brewing durations for polyphenol to be extracted stably and completely. This study contributes to enriching data for Vietnamese tea products in the context of high production and export.*

Keywords: *Camellia sinensis* L., Folin-Ciocalteu, total polyphenol

1. Introduction

Tea trees have a long history of cultivation worldwide, and tea is considered as one of the most popular beverages after water, coffee, and cocoa. According to Chinese mythology, tea plants were discovered thousands of years ago in South-East Asia. Human beings have been drinking tea for more than 5,000 years because of its healthy and medical benefits to

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prevent and treat many diseases, in particular cancer (respiratory, digestive, and urinary) and cardiovascular disorder (Yan, Zhong, Duan, Chen, & Li, 2020). Tea polyphenols also help to prevent mutations in genetic materials, regulate detoxification enzyme activities, and inhibit tumorigenesis. Tea leaves have been used to produce tea infusions, and there are many types of tea products depending on the degree of oxidation processes, such as white tea (young tea leaves or new growth buds, withered, uncured, baked dry), green tea (no oxidized), oolong tea (partially oxidized), black tea or red tea (fully oxidized) (Han, Mihara, Hashimoto, & Fujino, 2014; Schwalfenberg, Genuis, & Rodushkin, 2013). Thanks to the development of analytical chemistry, we now know better various metabolites, in particular catechins, contributing to the antioxidant capacities. The determination of total polyphenol contents or TPCs by UV-Vis spectrophotometry is considered to be a cheap, easy, and effective way to assess the antioxidants among various tea products before proceeding to more modern analytical methods.

Many studies on evaluating the polyphenol contents in various kinds of tea have been published worldwide during the last ten years. Karori, Wachira, Wanyoko, and Ngure (2007) determined the total polyphenol contents in tea products (black, green, oolong, and white teas) and reported those values to range from 11% to over 27% (w/w dried weight-DW), with the lowest and highest recorded in green teas collected in Yinghong (China) and Kenya, respectively. Another study about the determination of the total polyphenol and the antioxidant capacities of tea products performed by Anesini, Ferraro, and Filip (2008) in Argentina showing that the total polyphenol contents in green and black teas were 143.2-210.2 and 84.2-176.2 mg GAE g^{-1} DW, respectively. Yao et al. (2006) in Australia reported the total polyphenol contents of black tea (140-200 mg GAE g^{-1} DW) and green tea (an average value of approximately 250 mg GAE g^{-1} DW), which were higher than several tea types reported by Anesini et al. (2008). The research of Jayasekera, Molan, Garg, and Moughan (2011) also used the Folin-Ciocalteu method to determine the total polyphenol contents in tea products in Sri Lanka, which showed the higher total polyphenol contents in green teas than black ones (148.5-154.7 vs. 86.5-115.2 mg GAE g^{-1} DW). The study conducted by Kerio, Wachira, Wanyoko, and Rotich (2013) reported similar trends in polyphenol contents for green tea (22.0-22.1% w/w DW) and black tea (18.0-18.9% w/w DW).

Although Vietnam is considered as a country with large tea production, limited studies have been published for the identification and evaluation of the polyphenol contents in tea products. One study by Pham, Tong, Nguyen, and Bach (2007) analyzed the total polyphenol contents, total catechins, and DPPH in 30 commercial teas (green, black, and oolong teas) in Ho Chi Minh City. This study showed that the highest polyphenol contents were found in most green tea products (15.06 ± 0.53 mg GAE kg^{-1} DW), followed by oolong and black teas. In general, the polyphenol contents in this study were lower than in several international publications. Another study conducted by Vuong, Nguyen, Golding, and Roach (2011) in Thai Nguyen and Lam Dong Provinces showed that green tea has a remarkably higher total catechin contents than those in oolong and black teas with the values of 70, 34, and 12 mg g^{-1} DW, respectively.

This study employed the Folin-Ciocalteu method as shown in ISO 14502-1:2005 (2005) with some modifications to (i) evaluate the influences of several parameters related to the polyphenol extraction procedure on the extraction efficiency, (ii) validate the analytical method for the determination of total polyphenol contents in tea products, and (iii) apply the validated method to determine the total polyphenol contents in tea products, then (iv) assess the effects of brewing temperature on the polyphenol contents in tea infusions.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid monohydrate (≥ 99%) was purchased from Sigma-Aldrich and used to prepare the stock solution of 1000 mg L^{-1} in methanol. The working standard solutions from 10 to 70 mg GAE L^{-1} were prepared daily prior to use. Methanol (CH_3OH, HPLC grade), sodium carbonate (Na_2CO_3), and Folin-Ciocalteu reagent of 2 N were obtained from Merck, Germany. Deionized water (DI, Milli-Q, Merck, Germany) was used throughout this study.

2.2. Sample collection, pre-treatment, and storage

In this study, we collected four tea samples produced in Vietnam, including three ancient tea samples (white, green, and red) in Suoi Giang, Yen Bai Province (the North) and one oolong tea product in Di Linh, Lam Dong Province (the South). The sampling procedure was based on TCVN 5609:2007 (2007) and QCVN 01-28:2010/BNNPTNT (2010).

Before being analyzed, tea samples undergone a pre-treatment procedure followed by ISO 1572:1980 (1980) and TCVN 9738:2013 (2013) for homogenization purposes. Storage conditions were the temperature of 25 °C, the humidity of 70%, and avoiding direct sunlight.

2.3. Extraction and coloring procedure of total polyphenol contents

The polyphenol extraction was performed according to ISO 14502-1:2005 (2005) with some modifications (Figure 1).

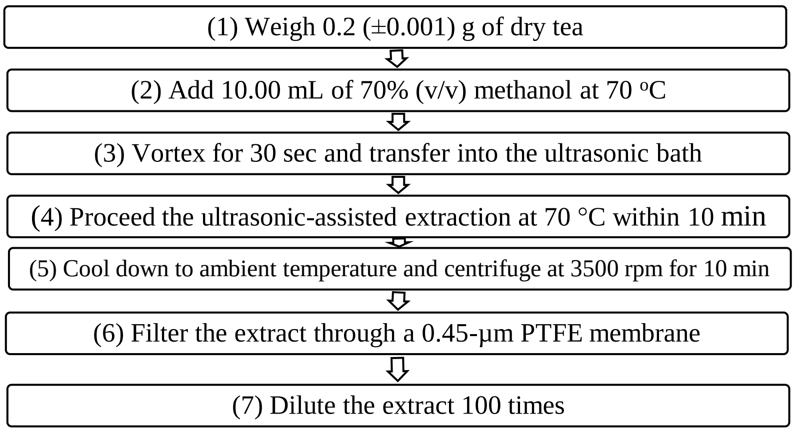


Figure 1. *The polyphenol extraction procedure applying modified ISO 14502-1:2005*

The diluted tea extract was proceeded to the coloring procedure by reacting with the Folin-Ciocalteu reagent described in ISO 14502-1:2005 (2005) (Figure 2).

(1) Pipette 1.00 mL of the diluted extract into a 15-mL PP centrifuge tube

⇩

(2) Add 5.00 mL of 10% (v/v) Folin-Ciocalteu reagent

⇩

(3) Wait for 3-8 min

⇩

(4) Add 4.00 mL 7.5% (w/v) Na_2CO_3

⇩

(5) Wait for another 60 min

⇩

(6) Spectrophotometric measurement at 765 nm

Figure 2. *The coloring procedure with Folin-Ciocalteu reagent*

Several parameters related to the polyphenol extraction efficiency were investigated and considered to figure out the favorable procedure with available laboratory facilities, including (i) extraction temperatures (50-90 °C) without ultrasonic power, (ii) extraction techniques with and without ultrasonic power, and (iii) extraction ratios (material mass per solvent volume, g:mL) and single or multiple extractions (Table 1).

Table 1. *The experiment for evaluating the effects of extraction ratios and multiple extractions*

Experiment	Extraction ratio (g:mL)	Multiple extractions
1	0.200:5.00	double extraction (2 times)
2	0.200:10.00	double extraction (2 times)
3	0.200:10.00	single extraction (1 time)

The analytical method for determining total polyphenol contents in tea products was validated, followed by the requirements as described in Appendix F of AOAC (2016). The evaluated performance characteristics included the calibration curve, limit of detection and quantification (LOD and LOQ) estimation, repeatability/intra-day, reproducibility/inter-day (% RSD_r and % RSD_R), and recovery. The calibration curves were established by plotting the milligrams gallic acid mass equivalence (mg GAE) at various concentrations vs. their UV-Vis absorbance values measured at 765 nm in the format of the linear calibration curve (y = ax + b). The method accuracy and precision were evaluated from the recovery study conducted by samples spiked at levels of 75, 150, and 225 mg GAE g^{-1}. The total polyphenol contents (TPCs) were calculated according to the following equation:

$$\mathrm{TPCs} = \frac{C_x \times DF}{100 \times m}$$

Whereas, C_x: the concentration of total polyphenol calculated from the regression equation (mg GAE L^{-1}); m: weight of used tea sample (g); and DF: dilution factor.

2.4. Effects of brewing temperature on total polyphenol contents in tea infusions

The validated method was then applied to determine the total polyphenol contents in the collected tea samples, then assess the influences of brewing temperature at various values of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100^{o}C on total polyphenol contents in tea infusions when the infusion duration was fixed at 10 minutes for all experimental conditions. The effects of brewing temperatures on total polyphenols were evaluated by comparing the percentages of polyphenols releasing into the tea infusions at each brewing temperature. These percentage values were calculated through the ratios of total polyphenol contents determined in infusions and tea products.

2.5. Statistical analysis

All the analyses were run in triplicate (n = 3) to assure the repeatability among runs. The values of average, standard deviation (SD), relative standard deviation (RSD), and charts were processed by Microsoft Office Excel 2016.

3. Results and discussion

3.1. The polyphenol extraction procedure of tea products

The extraction procedure for the determination of total polyphenol contents in tea products was based on ISO 14502-1:2005 (2005) with some modifications. Various parameters related to the extraction steps were investigated to discover the tea products' optimized extraction procedure under the available laboratory facilities.

3.1.1. Effect of extraction temperature on total polyphenol contents determined in teas

The total polyphenol contents in tea infusions under several extraction temperatures of 50, 60, 70, 80, and 90°C without ultrasonic power were determined (Figure 3) to figure out the influences of temperature on the extraction efficiency.

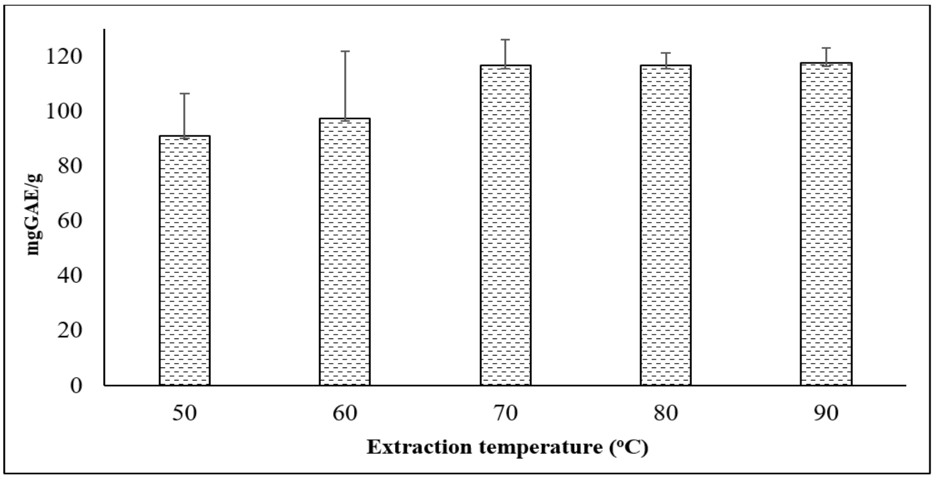


Figure 3. *Effects of temperature during the extraction on the total polyphenol contents*

As shown in Figure 3, the total polyphenol contents increased regarding the rising temperature in the extraction period (the lowest and highest of 90.93 mg GAE g^{-1} at 50^{o}C and 117.50 mg GAE g^{-1} at 90^{o}C, respectively). For the temperatures above 70°C, the total polyphenol contents did not exhibit any remarkable changes (117.06-117.50 mg GAE g^{-1}). Moreover, % RSDs were higher at lower temperatures than higher ones, particularly 50 and 60°C. This is possible because with the same extraction duration (10 minutes), the polyphenols in the sample matrix were not quantitatively extracted at low temperatures, leading to smaller total polyphenol contents determined and poorer repeatability. Therefore, to save energy and as suggested from ISO 14502-1:2005 (2005), we could use the temperature of 70°C as the extraction temperature for further investigations.

3.1.2. Effects of extraction techniques with and without ultrasonic power on total polyphenol contents determined in teas

Among various parameters influencing the extraction efficiency, the energy supporting the extraction is considered an essential and important factor. This study used ultrasonic-assisted extraction within 10 minutes to compare with the conventional method (only water bath at 70°C for 30 minutes without ultrasonic power) as described in ISO 14502-1:2005 (2005). This modification was carried out to reach the optimized extraction efficiency and time saving. The comparison results between the two extraction conditions are shown in Table 2.

Table 2. *The comparison between the water bath and ultrasonic-assisted extractions*

No	Extraction conditions	Replicate	TPCs (mg MAE g^{-1})		RSD (%)
1	Water bath only at 70^{o}C	1	116.20	117.50	1.0
		2	118.17		
		3	118.12		
2	Ultrasonic power at 70^{o}C	1	125.98	126.66	0.81
		2	127.79		
		3	126.21		

TPCs: Total Polyphenol Contents

The analytical results showed that both extraction conditions presented proper repeatability required by Appendix F. AOAC (2016) (%RSD from 0.81 to 1.0%). In the ultrasonic-assisted extraction situation, the TPCs determined were higher than those recorded in the water bath (126.66 vs.117.50 mg GAE g^{-1}) despite the shorter extraction durations. Therefore, ultrasonic-assisted extraction might be an effective way for polyphenol extraction from the tea matrix. This procedure could save the analysis time, particularly in routine analysis. In this study, we applied ultrasonic-assisted extraction for further investigations and method validation.

3.1.3. Effect of extraction ratios and repeated extractions (extraction times) on total polyphenol contents in teas

The variation in the extraction ratios has certain influences on the extraction efficiency in general. In solid-liquid extraction, the ratios between the material and extraction solvent should be selected appropriately to achieve the maximum analyte content in the obtained extracts due to the analyte distribution between the two phases. Additionally, we also reduced the number of extraction cycles from twice (double extraction as in ISO 14502-1:2005 (2005)) to once (single extraction) to save the analytical time and minimize the errors due to many practical steps. The results of comparing the effects of extraction ratios and single or double extraction on the extracts' polyphenol contents are presented in Figure 4.

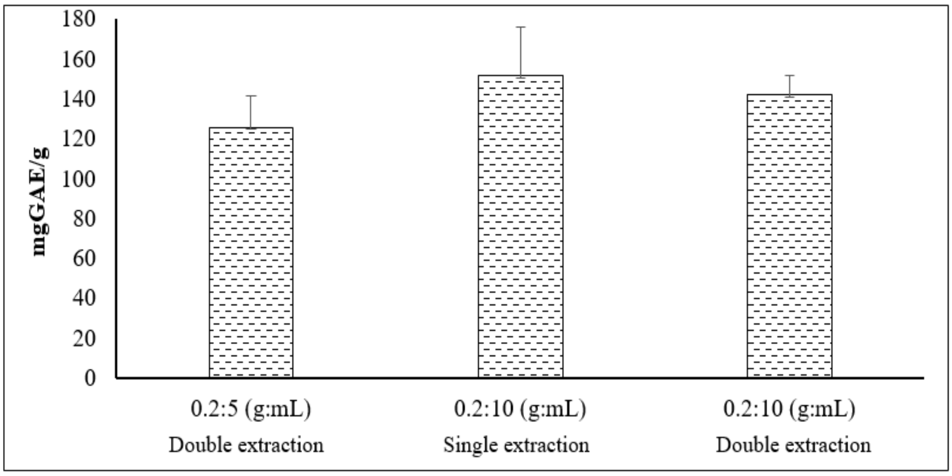


Figure 4. *Effect of extraction ratios and repeated extractions on the total polyphenol contents*

Figure 4 showed that at the extraction ratio of 0.2:5 (g:mL), the total polyphenol contents reached the lowest (125.63 mg GAE g^{-1}) even though the extraction was performed twice. The reason might be that the small extraction solvent volume gave a low distribution coefficient, leading to the analyte's high contents remaining in the material phase. Low coefficients of distribution required more extraction cycles (repeated or multiple extractions) to completely extract the analytes from the sample matrix, resulting in additional time, effort, and chemical consumption. An extraction ratio of 0.2:10 (g:mL) presented the highest total polyphenol contents in the situation of single extraction. At the same extraction ratios, but with double extraction (2 extraction times), the polyphenol contents were lower (142.11 vs. 151.4 mg GAE g^{-1}). Besides, double extraction was not reproducible (%RSD about 1.7%, compared with single extraction with %RSD of only 1.2%). Thus, for further surveys and method validation, we chose the extraction solvent volume of 10.00 mL for 0.200 g of raw material and single extraction to save time and chemicals but still ensure the reliability of the analytical results.

3.2. Validation of method for the determination of total polyphenol contents in tea products

The analytical method for the determination of total polyphenol contents in tea products was validated on Shimadzu 1800 UV-Vis instrument, summarizing in Table 3, including regression equation (y = ax + b), squared correlation coefficient (R^2), the limit of detection and quantification (LOD-LOQ), relative standard deviations for intra- and inter-day, and recoveries for trueness evaluation.

Table 3. *Validation parameters for the determination of total polyphenol contents in teas*

Regression equation	R^2	LOD (mg GAE L^{-1})	LOQ (mg GAE L^{-1})	Intra-day (%RSD_r)	Inter-day (%RSD_R)	Recovery (%)
y=0.0117x+0.0221	0.9995	0.81	2.7	0.79	1.2	98.8-99.4

R^2: squared regression equation correlation coefficient calculated for the linear range of 10-70 mg GAE L^{-1}

The calibration curve of gallic acid was performed in Figure 5. The LOD and LOQ were calculated based on the standard deviation of the response ($S_{y/x}$) of the curve and the slope (a) according to the formula of LOD = $3.3 \times \frac{S_{y/x}}{a}$ and LOQ = $10 \times \frac{S_{y/x}}{a}$.

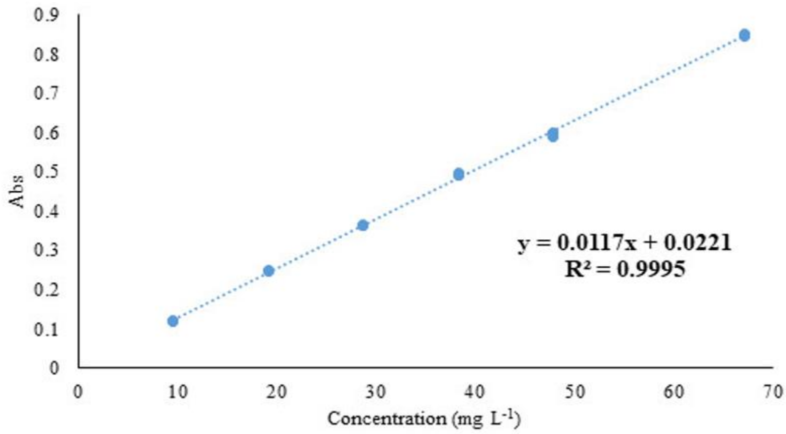


Figure 5. *Calibration curve of gallic acid (10-70 mg GAE L^{-1})*

As can be seen from Table 3 and Figure 5, high squared correlation coefficient values of calibration curve were recorded, $0.995 \leq R^2 \leq 1$, revealing evidence of the goodness of linearity (Appendix F. AOAC 2016).

Relative standard deviation values for both intra-day (%RSD_r) and inter-day (%RSD_R) were acceptable according to Appendix F. AOAC (2016), lower than 5.3 and 8% for %RSD_r and %RSD_R, respectively (analyte concentrations below 0.1%). The obtained recoveries were always higher than 98% for concentrations of 75.0, 150, and 225 mg GAE L^{-1}, acceptable within the concentration ranges of 90-107% (100 ppm-0.1%). The validated analytical method could be applied for daily analysis.

3.3. Variation of total polyphenol contents in tea products

The validated analytical method was applied to determine the total polyphenol contents in three ancient tea products (white, green, and black) and one oolong tea sample (Figure 6).

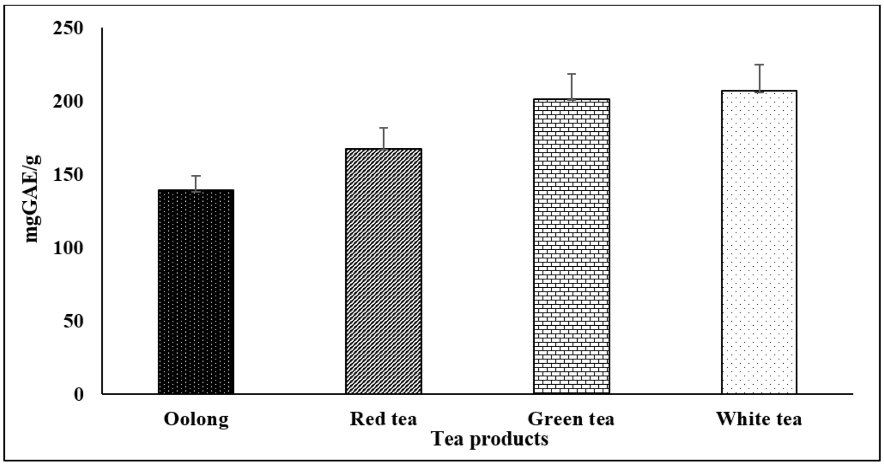


Figure 6. *Variation in total polyphenol contents in collected tea products*

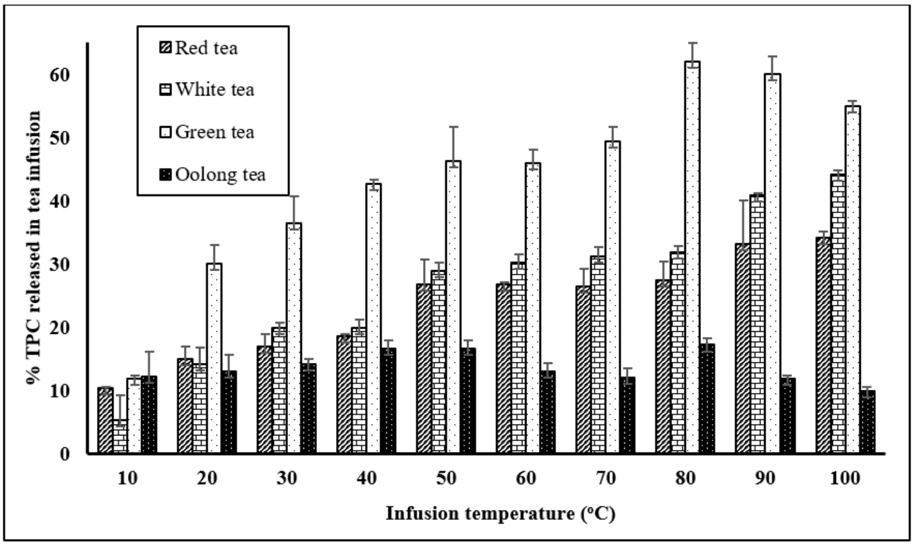
The analytical results in Figure 6 indicated that oolong tea had lower total polyphenol contents than three ancient tea products (139.18 vs. 167.42-206.62 mg GAE g^{-1}). Among the three ancient tea samples, red tea exhibited smaller total polyphenol contents than white and green teas (167.42 vs. 201.33-206.62 mg GAE g^{-1}). The enzymatic oxidation of polyphenols could explain the lower polyphenol contents determined from oolong and red teas during the fermentation of these tea types, in which dimer or polymerization of catechin monomer happened, forming theaflavins and thearubigin, then reducing the catechin contents in red tea, thereby contributing to the decrease in the total polyphenol contents (Turkmen, Sarı, & Velioglu, 2009). It could be seen that oolong tea exhibited smaller polyphenol contents compared to red tea despite the variation in fermentation levels of red (fully fermented) and oolong teas (partially fermented). This phenomenon might be due to the differences in tea species used to produce the collected oolong tea (10-year-old oolong tea trees) and red tea (ancient tea trees grown for hundreds of years).

Although white tea is mainly made from buds and young tea leaves, compared to green tea mainly produced from mature tea leaves, the total polyphenol contents of these two tea types did not differ much (5.29 mg GAE g^{-1}), and the highest content was reported in white tea (206.62 mg GAE g^{-1}). This is possibly the result of the non-selective properties of the Folin-Ciocalteu reagent, which not only reacts with polyphenol species but also with other compounds having similar chemical structure to polyphenols such as ascorbic acid, some proteins, or amino acids, then increasing the analyzed total polyphenol results. Consequently, ancient teas could contain high quantities of these compounds, making the total polyphenol contents determined not to perform much difference between white and green teas. However, for better understanding and conclusions, it is necessary to conduct some more chromatographic analysis.

Compared with several international publications on polyphenol contents in tea products, Zhao et al. (2019) also reported a descending order of total polyphenol contents in various tea types, particularly green tea (205.16 ± 32.02 mg GAE g^{-1}) > yellow tea (192.02 ± 25.36 mg GAE g^{-1}) > oolong tea (108.91 ± 25.59 mg GAE g^{-1}) > black tea (75.66 ± 28.70 mg GAE g^{-1}) > white tea (68.38 ± 12.44 mg GAE g^{-1}). This publication exhibited a similar range of polyphenol in green tea, but nearly three times lower for white tea than our study. Another study conducted by Anesini et al. (2008) determined the total polyphenol contents and antioxidant capacity in commercial tea in Argentina, indicating that the total polyphenol contents in green teas reached the highest value at 210.2 mg GAE g^{-1}, similar to the results obtained in this study. The study of Yao et al. (2006) in Australia also showed the highest total polyphenol content in green tea (250 mg GAE g^{-1}), but higher than the report of Anesini et al. (2008) and this study. The differences could be the tea plant species. In Vietnam, limited publications were found related to the assessment of polyphenols in tea products despite the high quantity of tea production. There was a study carried out by Pham et al. (2007) on the analysis of total polyphenol contents in 30 commercial Vietnamese tea products (green, black, and oolong teas) collected in Ho Chi Minh City. The results of this publication also showed that green tea had the highest polyphenol content (15.06 ± 0.53 mg GAE kg^{-1}), followed by oolong and black teas. These analytical values were remarkably low compared to our existing tea products, which might be due to the variations in tea quality and species.

3.4. Assessment of the total polyphenol contents in tea infusions prepared from various brewing temperature

The temperature of brewing water affected the total polyphenol contents released in the tea infusions; however, there may be tendency differences in the fluctuation of polyphenol contents in tea infusions among various tea types. In this study, we investigated the effects of brewing temperature at the different levels of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100°C. The results were shown in Figure 7.



TPC: Total Polyphenol Content

Figure 7. *Effects of brewing temperature on the total polyphenol contents in tea infusions*

As shown in Figure 7, the brewing temperature had certain effects on the total polyphenol contents in tea infusions of all tea samples. Particularly, with the same tea quantity, brewing duration, and water volume, the higher the brewing temperatures were, the higher the total polyphenol contents in the tea infusions were, reaching the highest value at 80°C (oolong and green teas) and 100 °C (white and black teas). The oolong tea sample showed its specific fluctuation, different from the rest three ancient tea products, in which the polyphenol contents in oolong tea increased gradually from 10 to 50°C (releasing percentage of 12.2-16.7%), decreased again at 60-70°C (12.1-13.0%) and peaked at 80 °C (17.2%) then descended at 100 °C (9.80%). Besides, the releasing percentage of polyphenols in tea infusions for oolong tea (9.80-17.2% TPC extracted) was mostly lower than those of the three ancient tea samples (11.9-62.1%, 5.30-44.1%, and 10.4- 34.2% for green tea, white tea, and black tea, respectively). The reasons might be due to the "wrap-curled" structure of oolong tea after the processing period, making the polyphenols hardly extracted from the oolong tea matrix, or it took longer infusion durations for polyphenol species to be released into the infusions. The same phenomenon was also reported in the study by Tao, Zhou, Zhao, and Wei (2016), showing that polyphenol contents increased gradually regarding the rising number of brewing cycles for oolong teas.

4. Conclusions

This study validated an analytical method to determine the total polyphenol contents in tea products applying the Folin-Ciocalteu method. The ultrasonic-assisted single extraction within 10 minutes resulted in higher efficiency than in water bath double extraction for 30 minutes as shown in ISO 14502-1:2005, which could save time and energy, then suitable for routine analysis. The analytical method was validated on Shimadzu 1800 UV-Vis instrument, performing proper linearity (R^2 = 0.9995) from 10 to 70 mg GAE L^{-1}, acceptable repeatability and reproducibility, and high recoveries. This validated method was employed in four tea products, exhibiting the variations in total polyphenol contents regarding different processing methods and species with the highest polyphenol contents recorded in non-fermented teas as green and white tea samples. The releasing percentage values of polyphenol contents in tea infusions for each brewing temperature were calculated to assess the effects of temperature during the brewing period on polyphenol contents in infusions. We found that the increase in the brewing temperature would increase the polyphenol contents extracted in tea infusion as a general trend, and the highest percentage was observed for green tea (up to 60%), demonstrating the health benefits of tea consumers. Additionally, oolong tea with its specific "wrap-curled" structure possessed its own fluctuation in polyphenol releasing percentage, reaching the highest at 80°C (17.2%) then descending at 100°C (9.80%). However, more tea samples from various processing methods and species need to be collected to obtain more data for the assessment of the polyphenol variations among different products. Besides, the number of brewing cycles should be considered for further study on the brewing conditions for each typical type of tea so that the desired polyphenol contents in tea infusions could be obtained.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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THẨM ĐỊNH PHƯƠNG PHÁP PHÂN TÍCH VÀ ĐÁNH GIÁ ẢNH HƯỞNG CỦA NHIỆT ĐỘ PHA TRÀ ĐẾN HÀM LƯỢNG POLYPHENOL TỔNG TRONG NƯỚC TRÀ CỦA MỘT SỐ SẢN PHẨM TRÀ VIỆT NAM

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TÓM TẮT

Sản xuất từ lá của cây Camellia sinesis L. *(Kuntz), trà được xem là một trong những thức uống phổ biến nhất trên thế giới và cây trà được trồng ở khoảng 30 quốc gia. Trong nghiên cứu này, phương pháp Folin-Ciocalteu được áp dụng để xác định hàm lượng polyphenol tổng (quy về milli đương lượng gam của gallic acid, GAE) trong các mẫu trà thành phẩm (ba mẫu trà cổ thụ bao gồm trà đỏ, trà xanh, trà trắng và một mẫu trà oolong) dựa trên ISO 14502-1:2005 với một số thay đổi, cụ thể là chiết một lần bằng dung môi chiết methanol:nước (7:3, v/v) có sự hỗ trợ của siêu âm ở 70 °C với tỉ lệ chiết 0.200:10.00 (g:mL). Phương pháp phân tích được thẩm định trên thiết bị UV-Vis 1800 của hãng Shimadzu với độ tuyến tính phù hợp (R^2 > 0.995), khoảng tuyến tính 10-70 mg GAE L^{-1}, độ lặp lại và độ tái lặp tốt (%RSD lần lượt là 0.79 và 1.2% cho độ lặp trong ngày và giữa các ngày) và tỉ lệ phục hồi cao (trên 98% cho các mẫu thêm chuẩn). Hàm lượng polyphenol tổng (giá trị trung bình tính bằng mg GAE g^{-1} khối lượng khô trong dấu ngoặc đơn) thể hiện xu hướng giảm dần giữa các mẫu trà, cụ thể trà trắng (206.62) ~ trà xanh (201.33) > trà đỏ (167.42) > trà oolong (139.18) do sự khác biệt về mức độ oxy hoá trong quá trình lên men, đặc biệt đối với trà đỏ và oolong. Hàm lượng polyphenol phóng thích vào nước trà thể hiện xu hướng chung tăng dần khi tăng nhiệt độ pha trà, ngoại trừ mẫu trà oolong có xu hướng riêng, có thể do cấu trúc* "cuộn xoắn" *của loại trà này, dẫn đến thời gian ngâm trà cần phải dài hơn để polyphenol có thể được phóng thích ổn định và hoàn toàn. Nghiên cứu này đóng góp một phần khiêm tốn để làm giàu thêm dữ liệu cho các sản phẩm trà Việt Nam trong bối cảnh lượng trà sản xuất và xuất khẩu cao.*

Từ khoá: *Camellia sinensis* L., Folin-Ciocalteu, polyphenol tổng