Journal of Food and Nutrition Sciences
2015; 3(1-2): 106-110
Published online January 28, 2015 (http://www.sciencepublishinggroup.com/j/jfns)
doi: 10.11648/j.jfns.s.2015030102.30
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Optimizing the extraction conditions of phenolic compounds from fresh tea shoot

Nguyen Ngoc Tram[1, *], Phan Phuoc Hien[2], Huynh Ngoc Oanh[1]

[1]Faculty of Chemistry, Department of Biochemistry, University of Technology HCMC, Vietnam
[2]Faculty of Food and Life Sciences, University of Hung Vuong, HCMC, Vietnam

Email address:
nguyenngoctram14@gmail.com (Nguyen N. T.), ngocoanh_huynh@yahoo.com (Huynh N. O.), pphien@gmail.com (Phan P. H.)

To cite this article:
Nguyen Ngoc Tram, Phan Phuoc Hien, Huynh Ngoc Oanh. Optimizing the Extraction Conditions of Phenolic Compounds from Fresh Tea Shoot. *Journal of Food and Nutrition Sciences.* Special Issue: Food Processing and Food Quality. Vol. 3, No. 1-2, 2015, pp. 106-110.
doi: 10.11648/j.jfns.s.2015030102.30

Abstract: In the traditional method, the single factor models can be analysed easily but the multifactorial models cannot. To avoid these limitations, the Design Expert 8.0 software was used to analysis the complex multifactorial models. In this study, four factors: assisted microwave time (10 – 20 seconds), water-to-tea ratio (1:40 – 1:80 (w/v)), extraction time (30 – 50 minutes) and temperature (77 – 87^{o}C) were conducted and total polyphenol content was considered indicators for assessment. The preliminary results showed that this model is significant and can be applied for the large scale.

Keywords: Assisted Microwave Time, Extraction Time and Temperature, Multifactorial, Optimize, Water-To-Tea ratio, Total Polyphenol Content

1. Introduction

Tea, *Camellia sinensis L.,* is a rich source of compounds with bioactive properties, such as antioxidant and antimicrobial activities [1, 2]. It is well known for its content in polyphenols including catechins, flavanols, flavanones, phenolic acids, glycosides and the aglycons of plant pigments [3, 4]. Tea polyphenols have important applications in food industry and medicine for daily use. Tea polyphenols have a stronger antioxidative activity than butylated hydroxyanisole, butylated hydroxytoluene and DL-α-tocopherol; and the toxicity of tea polyphenols is lower than butylated hydroxyanisole, butylated hydroxytoluene and DL-α-tocopherol [5, 6]. Extraction of tea bioactive compounds was abundantly investigated in the last decades focusing mainly on conventional solvent extraction (methanol, ethanol, ethyl acetate, and acetone) [1, 3, 5, 7]. However, water was known as the best solvent to extract tea bioactive compounds because of its dielectric constant [3, 4, 8, 9].

Microwave cooking is also increasingly used as a time-saving technology but it induces severe changes in the lipids, such as hydrolysis and oxidative reactions that lead to quality and nutritional losses [2]. According to Ricardo Malheiro et al, white and green tea aqueous extracts were used to increase olive oil stability under microwave cooking because of their wealthy antioxidants [2]. It indicated that the tea antioxidants were endurable under microwave heating. Moreover, the effect of enzyme inactivation by microwave heating time was determined on the quality of tea. Catechin - one of the most important antioxidants, took part in the enzymic oxidation in the presence of polyphenol oxidase and peroxidase, and led to the production of tea pigments, theaflavins, thearubigins [10, 11]. These enzymes were responsible for browning and quality deterioration in mate tea leaves. The measured color parameters showed that after microwave treatment, mate tea leaves showed a more intense green color [12]. And the preservation qualities of green tea harvested were greatly enhanced by microwave heating [13].

In the recent reports have been published on application of microwave heating for extraction of organic compounds from green, Oolong and black tea [8, 9, 12, 14, 15]. However, they were focused on extracting tea bioactive compounds by using microwave as an extraction method. In this study, microwave treatment method was used to inactivate enzymes in fresh tea leaves. Furthermore, a response surface methodology was applied to optimize the extraction conditions of phenolic compounds in fresh tea leaves.

2. Materials and Methods

2.1. Materials

The green tea - raw material for Oolong tea production, which was made from the leaves of *Camellia sinensis* L. in April, 2014, was kindly provided by Cau Tre Tea Factory (Lam Dong province, Vietnam). In due to protect tea polyphenol compounds, 5g fresh tea shoots were inactivated enzyme by using microwave heating in the high-pressure reactor 1100W (Microwave Sharp 33L R-399VN (S) 1100W) for 10 – 20 seconds before using.

2.2. Determination of Total Polyphenols of Tea Samples

For determination of total polyphenols 1 g of tea powder was added to different volumes of water (40 – 80 ml) in a 100 ml glass bottle and boiled in a water bath at 77 - 87°C for 30 - 50 minutes. Then, the solution was filtered, cooled to room temperature. It was diluted to 100 ml with distilled water after that.

The tartrate solution was prepared by dissolving 1g of $FeSO_4$ and 5g of $KNaC_4H_4O_6$ in distilled water, and the volume is made up to 1000 ml. The phosphate buffer solution consists of 85% (v/v) of Na_2HPO_4 solution and 15% (v/v) of KH_2PO_4 solution.

In according to Yao et al (2006), one milliliter of tea solution, 4 ml of distilled water and 5 ml of tartrate solution were taken in a volumetric flask and then diluted to 25 ml with phosphate buffer solution. The mixture absorbance was measured at 540 nm using spectrophotometer. The tea samples were analyzed in duplicate for total polyphenols [16].

$$Total\ polyphenol\ (\%) = \frac{3.914 \times E \times V_o \times 100}{1000 \times V_1 \times w}$$

where

E: absorbance reading

V_o: total volume of the solution (250 ml)

V_1: the volume used for the measurement (1 ml)

W: dry weight of the tea sample

2.3. Antioxidant Activity Determination

Antioxidant activities of the tea extracts were tested and compared by measuring the ability of the extracts to scavenge the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) in vitro. The method was based on the description of tea researchers [4, 7, 16, 17]. Details of the method were as follows.

0.1 ml the tea extracts was added to 2.9 ml of DPPH solution (2µg DDPH was diluted to 100ml with 80% methanol) and 0.5 ml of 80% methanol solution. The solutions were mixed using a vortex (Model ZX3, VELP, Italy) and the mixture was then incubated for 30 minutes in darkness at room temperature, after which the absorbance was measured at the wavelength of 517 nm using methanol as a reference. The values of percent inhibition (PI) can be calculated using the following equation:

$$PI\ (\%) = \frac{A_{control} - A_{samples}}{A_{control}} \times 100$$

In which, A_{sample} and $A_{control}$ were absorbance of test sample and absorbance of the DPPH reference, respectively.

2.4. Experimental Design and Statistical Analysis

The analysis of variance of the quality parameters and chemical composition data was analyzed by the Statgraphics Centurion XV software. The significance of differences at a 5% level between averages was determined by one-way ANOVA using T-test.

In the present study, the effects of three independent variables: microwave time, extraction temperature, extraction time and the ratio of water to raw materials (V/w) on the extraction of tea polyphenols were investigated. Version 8.0.7.1 of Stat-Ease Design Expert (Stat-Ease Inc., Minneapolis, MN, USA) was used for the experimental design and regression analysis of experimental data. The adequacy of the model was determined by evaluating the lack-of-fit, the coefficient of determination (R^2) and the F-test value obtained from the analysis of variance (ANOVA) that was generated. The Student's t-test permitted the checking of the statistical significance of the regression coefficient, and the Fisher's F-test determined the second-order model equation at a probability level ($P \leq 0.05$). In order to visualize the relationships between the responses and the independent variables and also to deduce the optimum conditions, the fitted quadratic polynomial equation was expressed as both response surface and contour plots.

3. Results and Discussion

3.1. Impact of Single Factors on the Extraction yield of Total Polyphenol Content

3.1.1. Impact of Different Microwave Time

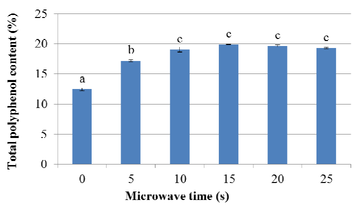

Figure 1. Impact of microwave time on the extraction of total polyphenol content. Water-to-tea ratio 50:1 (ml/g), temperature: 100°C, extraction time: 30 minutes.

3.1.2. Impact of Different Temperatures

Temperature plays an important role in the extraction yield of bioactive components from plant materials into solvents. High extraction temperatures usually increase extraction yield [10]. In this study, the extraction process was investigated from 70 to 100°C, at 30 minutes, 50:1 ml/g for the water-to-tea ratio was used.

The impact of different temperatures was showed in Figure 2. The yield of polyphenols increased steadily while the extraction temperature increased from 70 to 90°C, peaked at 90°C and maintained at 100°C (P_{value} (90°C, 100°C) = 0.0655 > 0.05) (Anova: Single factor).

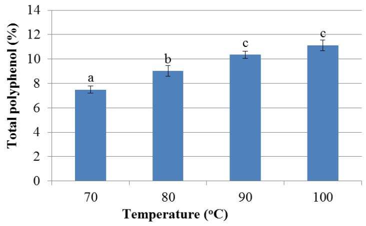

Figure 2. Impact of extraction temperature on the extraction of total polyphenol content. Microwave time: 0 second, water-to-tea ratio 50:1 (ml/g), extraction time: 30 minutes.

3.1.3. Impact of Different Water-To-Tea Ratios

The ratio of solvent-to-plant materials also influences the extraction yield of bioactivity from plants. In general, the higher the ratio of solvent to plant material was, the higher the extraction yield was [8]. However, the concentration of tea soluble dry matter will be reduced. Thus the optimum ratio was determined by extracting green tea at 100°C for 30 min with the water-to-tea ratio ranging from 30:1 to 80:1 mL/g.

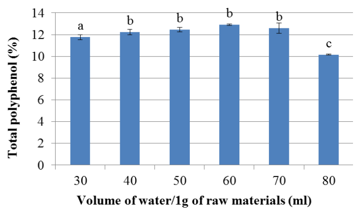

Figure 3. Impact of volume of water/1g of tea powder on the extraction of total polyphenol content. Microwave time: 0 second, temperature: 100°C, extraction time: 30 minutes

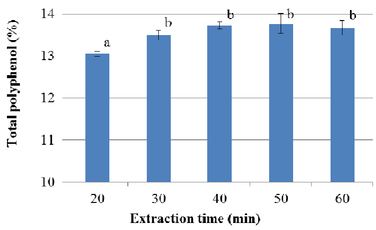

Figure 4. Impact of extraction time on the extraction of total polyphenol content. Microwave time: 0 second, water-to-tea ratio 50:1 (ml/g), temperature: 100°C.

The impact of different water-to-tea ratios was showed in Figure 3. It increased lightly from 30 to 70ml, rapidly decreased at 80ml water-to-tea ratios. Although, it had a lightly increase between 40 and 70ml water-to-tea ratio, they were not noticeable differences (P_{value} > 0.05).

3.1.4. Impact of Different Extraction Time

The impact of different extraction time was similar to temperature and water-to-tea ratio. The concentrations of the polyphenols rose from 20 to 30min and remain stable after that.

3.2. Analysis of Experimental Data and Prediction of Performance

The influence of individual factors on tea-based esterquat production and their performance at optimum condition using Taguchi approach were analyzed by software (Design Expert Version 8.0.7.1). In this study, there were four factors with their ranges: microwave time (10-20s), temperature (77–87°C), water-to-tea ratios (40 – 80 ml/g), extraction time (30 – 50min) were investigated. The contents of tea polyphenols were considered as response values.

3.2.1. Tea Polyphenols

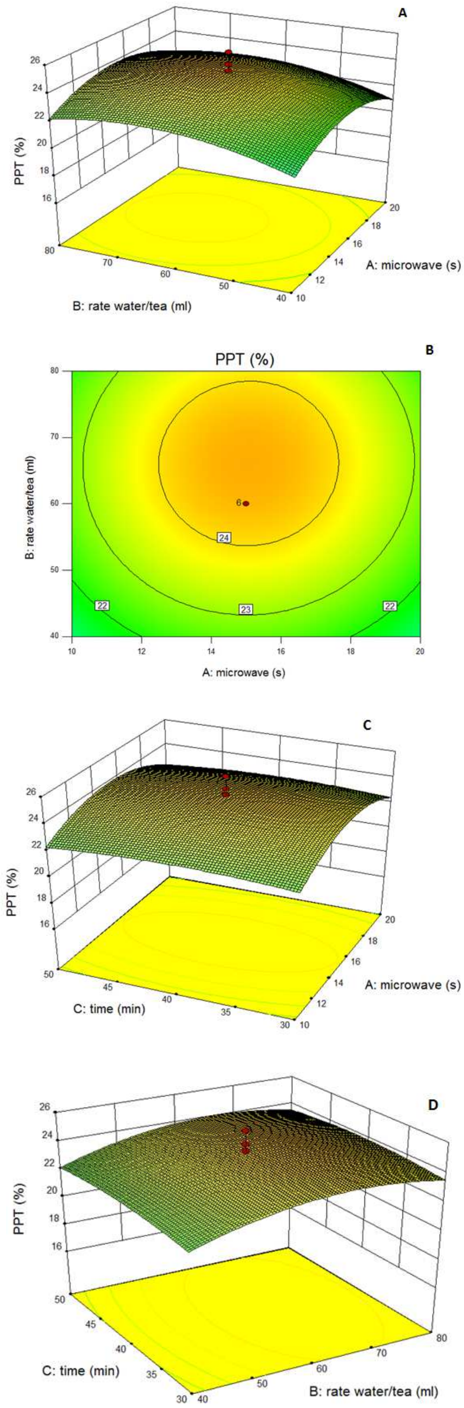

Figure 5. Response surface plots showing the effects of water-to-tea ratio and microwave time (A,B), extraction time and microwave time (C), extraction temperature and water-to-tea ratio (D) on the yield of tea polyphenols (%)

Mathematical models were developed to obtain a better understanding of the nature of the true relationship between the input variables and the output variables of the system under study [1]. This approximate formula could be used as a proxy for the full-blown simulation itself in order to get at least a rough idea of what would happen for a large number of input-parameter combinations. The data obtained were fitted to second-order polynomial equation, and the predictive equation for tea polyphenols was given as

Total polyphenol content (%) = $24.32 + 0.034A + 0.66B - 0.012C + 0.22D + 0.034AB - 7.376\text{x}10^{-3}AC - 1.05AD + 0.025BC + 0.18BD - 0.18CD - 1.57A^2 - 1.09B^2 - 0.36C^2 - 0.32D^2$

The significance and adequacy of the model was tested. The applicability of the model was adequate ($p<0.0001$). The F-value (11.52), R^2 (0,9149) and P-value of lack-of-fit (0.6376) implied the lack-of-fit was not significant relative to the pure error. It indicated that the model equation was adequate for prediction under any combination of values of the variables.

The effects of water-to-tea ratio, microwave time, extraction temperature and time on the yield of total polyphenol content were graphically presented as a 3-D response surface plot in Figure 5. The microwave time and water-to-tea ratio were more effective than the extraction time and temperature.

3.2.2. DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity was seen as one indicator of extraction efficiency. It was doubted that microwave heating time could reduce the quality of polyphenol compounds. The high extraction yield of the polyphenol content was not meant that its antioxidant capacity would increase. Figure 6 showed some of experimental verifications and their desirability. Some of the random experiments (number 5, 6, 8, 9, 11, 14) was conducted. The results showed in Table 1.

Number	microwave*	rate water/tea*	time*	tempurature*	Desirability	
1	20.00	80.00	50.00	87.00	1.000	Selected
2	20.00	80.00	30.00	87.00	1.000	
3	20.00	80.00	30.00	77.00	1.000	
4	20.00	40.00	50.00	77.00	1.000	
5	15.00	60.00	40.00	82.00	1.000	
6	20.00	40.00	30.00	87.00	1.000	
7	10.00	40.00	50.00	77.00	1.000	
8	10.00	40.00	50.00	87.00	1.000	
9	10.00	80.00	30.00	87.00	1.000	
10	10.00	80.00	50.00	87.00	1.000	
11	10.00	40.00	30.00	87.00	1.000	
12	20.00	80.00	50.00	77.00	1.000	
13	20.00	40.00	30.00	77.00	1.000	
14	20.00	40.00	50.00	87.00	1.000	
15	10.00	80.00	50.00	77.00	1.000	

Figure 6. *Some of experimental verifications*

In Table 1, the differences between theoretical and actual total polyphenol content did not exceed 5% and their antioxidant capacities were different. Compared between number 6 and 14, the total polyphenol content and antioxidant capacity of number 14 were higher than number 6 in the same conditions, which was also found in number 9 and 11. The ratio of water-to-tea was a strong influence on the yield of polyphenols extraction and antioxidant capacity. However, the impacts of the extraction time on the yield of polyphenols extraction and antioxidant capacity were not noticeable. It was similar to the extraction temperature (Table 1).

Table 1. *Some of experimental verifications and their results*

Number	Actual total polyphenol content (%)	Theoretical total polyphenol content (%)	Alpha (%)	DPPH (%)
5	20.68 ± 0.37	20,89	1.01	52.46 ± 3.60
6	20.99 ± 0,13	19,92	0.16	53.14 ± 0.57
8	21,63 ± 0,50	22,87	1.34	37.41 ± 3.27
9	22,10 ± 0,84	19,54	3.27	43.69 ± 1.33
11	23,97 ± 0,35	21,63	0.19	36.66 ± 0.57
14	20,84 ± 0,23	19,08	2.40	55.33 ± 1.04

4. Conclusion

The impact of extraction conditions on the yield of polyphenol extracted from fresh tea leaves was conducted. The results showed that the microwave treatment time and water-to-tea ratio had major significant impacts on the yield of polyphenols extracted. In particularly, The microwave time and ratio of water-to-tea were strong influences on the yield of polyphenols extraction and antioxidant capacity. Plus, the yield of polyphenols and antioxidant capacity reached at 15 seconds and 60:1 ml/g, respectively. The impacts of the extraction time and temperature were not almost significant.

Acknowledgments

We gratefully acknowledge the financial support from *Cau Tre Export Goods Processing Joint Stock Company*. We also extend special thanks to Cau Tre Tea Factory for kindly providing tea material.

References

[1] J. Prakash Maran; S. Manikandan; B. Priya; P. Gurumoorthi, "Box-Behnken Design Based Multi-Response Analysis and Optimization of Supercritical Carbon Dioxide Extraction of Bioactive Flavonoid Compounds from Tea (Camellia Sinensisl.) Leaves," *Journal of Food Science and Technology,* pp. 1-13, 2013.

[2] Ricardo Malheiro; Susana Casal; Hugo Lamas; Albino Bento; José Alberto Pereira, "Can tea extracts protect extra virgin olive oil from oxidation during microwave heating?," *Food Research International,* vol. 48, pp. 148–154, 2012.

[3] G. Spigno; D.M. De Faveri, "Microwave-assisted extraction of tea phenols: A phenomenological study," *Journal of Food Engineering,* vol. 93, pp. 210–217, 2009.

[4] Ezzohra Nkhili; Valerie Tomao; Hakima El Hajji; Es-Seddik El Boustani; Farid Chemat; Olivier Dangles, "Microwave-assisted water extraction of green tea polyphenols," *Phytochemical Analysis,* vol. 20, pp. 408–415, 2009.

[5] Xuejun Pan; Guoguang Niu; Huizhou Liu, "Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves," *Chemical Engineering and Processing,* vol. 42, pp. 129-133, 2003.

[6] W.J. Chen; S.Q. Wan., "Research progress on polyphenols of tea," *Natural Production Research and Development,* vol. 6, pp. 74-80, 1994.

[7] Surasak Hemwimon; Prasert Pavasant; Artiwan Shotipruk, "Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda Citrifolia*," *Separation and Purification Technology,* vol. 54, pp. 44–50, 2007.

[8] Vuong QV; Stathopoulos CE; Golding JB; Nguyen MH; Roach PD, "Optimum conditions for the water extraction of L-theanine from green tea," *Journal of Separation Science,* vol. 34, pp. 2468-2474, 2011.

[9] Shuntaro Tsubaki; Hiroyuki Iida; Masahiro Sakamoto; Jun-Ichi Azuma, "Microwave heating of tea residue yields polysaccharides, polyphenols, and plant biopolyester," *Journal of Agricultural and Food Chemistry,* vol. 56, pp. 11293–11299, 2008.

[10] G. W. SANDERSON, "Changes in the level of polyphenol oxidase activity in tea flush on storage after plucking," *Journal of the Science of Food and Agriculture,* vol. 15, pp. 634-639, 1964.

[11] S. N. Stephen Thanaraj, "Influence of Polyphenol Oxidase Activity and Polyphenol Content of Tea Shoot on Quality of Black Tea," *Journal of the Science of Food and Agriculture,* vol. 51, pp. 57-69, 1990.

[12] Giovana Cristina Ceni; Eliana Maria Baldissera; Maristela dos Santos Primo; Octávio Augusto Ceva Antunes; Cláudio Dariva; José Vladimir de Oliveira; Débora de Oliveira, "Influence of application of microwave energy on quality parameters of mate tea leaves (Ilex Paraguariensis st. Hil.)," *Food Technology and Biotechnology,* vol. 47, pp. 221–226, 2009.

[13] Yuanyuan Huang; Jianchun Sheng; Fangmei Yang; Qiu Hui Hu, "Effect of enzyme inactivation by microwave and oven heating on preservation quality of green tea," *Journal of Food Engineering,* vol. 78, pp. 687–692, 2007.

[14] Hui Wang; Ligang Chen; Yang Xu; Qinglei Zeng; Xiaopan Zhang; Qi Zhao; Lan Ding, "Dynamic microwave-assisted extraction coupled online with clean-up for determination of caffeine in tea," *LWT - Food Science and Technology,* vol. 44, pp. 1490-1495, 2011.

[15] Juane Donga; Xihan Mab; Zhuorui Fu; Ying Guo, "Effects of microwave drying on the contents of functional constituents of eucommia ulmoides flower tea," *Industrial Crops and Products,* vol. 34, pp. 1102– 1110, 2011.

[16] L.H. Yao; Y.M. Jiang; N. Caffin; B.D Arcy; N. Datta; X. Liu; R. Singanusong; Y. Xu, "Phenolic compounds in tea from Australian supermarkets," *Food Chemistry,* vol. 96, pp. 614-620, 2006.

[17] Afify Ael-M; El-Beltagi HS; El-Salam SM; Omran AA, "Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties," *Asian Pacific Journal of Tropical Biomedicine,* vol. 2, pp. 203-209, 2012.

[18] Francis Muigai Ngure; John K. Wanyoko; Symon M. Mahungu; Anakalo A. Shitandi; Biswas A. K.; Biswas A. K.; Sarka, "Catechins depletion patterns in relation to theaflavin and thearubigins formation," *Food Chemistry,* vol. 115, pp. 8-14, 2009.