EFFECT OF ULTRASOUND ON EXTRACTIONOFPOLYPHENOL FROM THE OLD TEA LEAVES

Phan Van Man[a], **Tran** Anh Vu[a], **Tran** Chi Hai[b]
[a]Faculty of Chemical and Food Technology, Ba Ria Vung Tau University, Viet Nam
[b]Ho Chi Minh City University of Food Industry, Viet Nam
E-mail: pvman.dbv@moet.edu.vn

Abstract
Polyphenol is a main antioxidant in the old tea leaves. These bioactive compounds have been discovered that they link with a risk reduction of cardiovascular cular, cancer and Alzheimer's disease. In this study, the effect of ultrasonic power, extraction time on the concentration and the purity of polyphenol (phenolic compounds) which are extracted from the old tea leaves are investigated. Response of the surface methodology is used to optimize the conditions of ultrasound assisting in the extraction in order to obtain concentration of polyphenol and the highest purity. The results of the experiment show that the optimal ultrasound power and extraction time are respectively 122.48 W/g and 7.84 minutes then the concentration of polyphenol and the polyphenol purity in mass extraction are increased by gradually 2.20 and 1.96 times compared with the control sample without ultrasound on the assisted extraction. Ultrasound treatment significantly improves extraction of the polyphenol's capability, the extraction yield of polyphenol with the assisted ultrasound is increased more 1.13 times than traditional extraction's methods without the assisted ultrasound. Ultrasound treatment can be one of the most effective extraction methods that assist to enhance the extraction of the tea solids, especially the polyphenol.

Keywords: Polyphenol, ultrasound, polyphenol extraction, the old tea leaves, response surface methology.

Submitted: 12.12.2016 Reviewed: 20.03.2017 Accepted: 23.03.2017

1. INTRODUCTION

Tea is the most widely consumed beverage in the world and it is known for the various health benefits. In recent years, tea is used not only as fresh drink but also traditional herb which has many benefits for human health (Saxena et al, 2012).Polyphenols in green tea are believed as excellent free radical scavengers. Green tea which contains various classes of polyphenols, and up to 90% of its polyphenols is composed of flavan-3-ols (tea catechins). (+)-Catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC)asthe major catechins found in green tea leaves(Saxena et al, 2012).

From these benefits, many studies on extraction of polyphenols have been done with different methods. In order to extractthemfrom the green tea, hot water and organic solvent extraction are the most commonly used procedures (Goula, 2013). However, ultrasound has been successfully used for extraction from biological acitive compounds, especially polyphenols in cactus, bluberry and strawberry, but on the old tea leaves, it is still limited. Assisted ultrasound helps to increase the recovery efficiencyof polyphenol compounds. Therefore, in this study, we conducted a survey to assess the effect of ultrasonic to polyphenol compounds that were extracted in the old tea leaves.

2. MATERIAL AND METHODS

Material

Old tea leaves were harvested from Loc Chau, Bao Loc, Lam Dong province, Viet Nam. Tea leaves were inactivated enzyme by steamingfrom 95ºC to 100°C for 2 minutes and dried from 40°C to 50°C for 8 hours. Then they werecrushed and stored in sealed plastic bags to avoid direct sunlight.

Chemical substances

Standards of phenolic acid (Gallic acid, 97.5%) and Folin–Ciocalteu reagentwere purchased from Sigma Aldrich, Vietnam.Methanol (99.6%), Ethanol (99.6%) and Sodium carbonat (99.9%) werepurchased from Merck, Vietnam.

Ultrasonic probe type system

Ultrasonic probe type system (VCX750 ultrasonic processor Sonics, New York, USA) was used for ultrasound treatment of the sample. The ultrasound probe of 19 mm diameter was attached to the ultrasound transducer which has a constant frequency of 20 kHz and maximum amplitude of 58 µm. The amplitude level and time combination range was selected based on preliminary trial and optimized condition was obtained using Design Expert software.

UV-visible spectrophotometer

The total phenolic content was determined using the Folin-Ciocalteu assay. The absorbance at 760 nm was measured using a UV-visible spectrophotometer (UV-2000, Shanghai Analytical Instrument Overall Factory, Shanghai, China).

Experimental methods

Preparation the polyphenol extract

Dried old tea leaves were crushed to 0.5 – 1.0mm, and40g of old tea leaves were used in each assay. The samples were placed into 1000mL flasks. The obtained dried tea leaves were mixed with water at the weight ratio of 1:15 and subsequently used for ultrasound-assisted extraction (UAE). Then the mass extractions were carried outto centrifugation at 3500 rpmin10 minutes.

Effects of ultrasonic power to the content and purity of polyphenol extraction

The mixtures of water and tea were experimented with ultrasonic power at 75 W/g, 93.75 W/g, 112.5 W/gand 131.25W/g respectively,and the control sample untreated with ultrasound. These samples were carried out within 6 minutes. Then the obtained mass was centrifuged at 3500 rpmin 10 minutes.

The totalpolyphenolpurity (PP) in the mash extraction was calculated by the ratio ofthe weight of purified polyphenol in mashextractionand the total solids weight (g).

Polyphenol purity :

$$(PP)= \frac{m_{PP} \times 100}{m_{CKD}} \ (\%) \qquad (1)$$

Whereas:

- m_{PP}: the weight of purified polyphenol in the mass extraction(g).
- m_{CKD}: the total solids weight(g).

Effects of sonication time to the polyphenol content and the purity of polyphenol in mash extraction

In this experiment, the extraction time was changed from from 0 (control samples) to 2, 4, 6, 8, 10 minutes. Ultrasonic power was selected from above mentioned results of experiments.

Optimization of ultrasonic treatment conditions of the old tea leaves mash for maximizing the polyphenol content

The quadratic central composite which was circumscribed response of surface design with 2 factors and 11 levels was used to optimize the conditions of ultrasonic treatment of the old tea leaves mash. The ultrasonic power (W) and time (t) were the input variables while the polyphenol content of the old tea leaves was the output variable (Y). The complete design consisted of 11 experimental points including 4 factorial points, 4 axial points and 3 centre points. The software Modde version 5.0 was used to generate the experimental planning and to process data.

Determination of kinetic parameters of the polyphenol extraction

To determine the extraction rate constant of the antioxidants, the second-order rate law was applied (A. M. Goula, 2013). The general second-order model can be written as:

$$\frac{dC_t}{dt} = k.\,(C_e - C_t)^2 \qquad (2)$$

Where: k is the second-order extraction rate constant (L/g.min), C_e is the extraction capacity (the equilibrium concentration of antioxidants in the extract) (g/L), and C_t is the concentration of the extract at a given extraction time (g/L).

The integrated rate law for a second-order extraction, under the boundary conditions t = 0 to t and C_t = 0 to C_t, can be written as an equation (3) or a linearized equation (4):

$$C_t = \frac{C_e^2.k.t}{1+C_e.k.t} \quad (3)$$

$$\frac{t}{C_t} = \frac{1}{k.C_e^2} + \frac{t}{C_e} \quad (4)$$

The initial extraction rate, v_o(g/L.min), as C_t/t when t approaches 0, can be defined as equation:

$$v_o = k.C_e^2 \quad (5)$$

Analytical methods and statistical analysis

Analytical methods

Total polyphenol content (TP): was evaluated by spectrophotometric method using Folin-Ciocalteu reagent at 765 nm (Charles, 2012). The results were expressed as mg of gallic acid equivalent per litre of extract (mg GAE/L).

Polyphenol purity (PP): The crude polyphenol was dynamically adsorbed on a glass column packed with AB-8 resin. Based on the result of the static AB-8 resin adsorption experiments, the equilibrium time was chosen as 4 h. Distilled water was first used to remove sugars, acids and other water-soluble compounds. Then, the crude PP was eluted with ethanol at a constant flow velocity of 1.5 mL/min, and two bed volumes (BV) of phenolic eluent was collected and concentrated in a rotary evaporator to afford the purified polyphenol.

Statistical analysis

All experiments were performed in triplicate. Means were compared by multiple range tests with $p < 0.05$. Analysis of variance was done to use the software Statgraphics plus centurion XVI.Optimization parameters were used by software Modde 5.0.

3. RESULTS AND DISCUSSION

Effects of ultrasonic power on the extractionand purity of polyphenolfrom the old tea mash extract

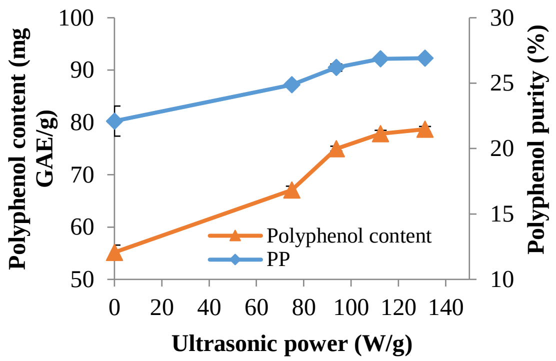


Figure1. Effects of ultrasonic power on the polyphenol content and the purity of polyphenol on the mash extraction

Figure 1 showed that all samples were treated with ultrasound always exhibited higher polyphenol content and purity of polyphenol than the control sample. As the ultrasonic power increased from 25 to 150 W/g, the concentration of total polyphenol in the old tea leaves enhanced 16.63% (from 67.08 to 78.69mg GAE/g) and 7.51% of polyphenol purity (24.88% and 26.90%), respectively. Samples withoutultrasound assistance, the polyphenol content accountedfor 55.17mg GAE/g, polyphenol purity was only22.1%.At the ultrasonic power of112.5 W/g, the polyphenol content achieved maximum, itwas 1.36 timeshigher to compare with the control sample, and 1.16 times higher than that of the sample treated at 75 W/g. It was due to acoustic cavitation generated from ultrasound in solid-liquid extraction. The implosion of cavitation bubbles improved diffusion in the system. In addition, cavitation within the proximity of solid surface promoted surface erosion and particle breakdown. Consequently, mass transfer was improved and the extraction yield was increased (Charles 2012; Mason et al, 2002; Vo et al, 2014; Phanet al, 2012). However, when the ultrasonic power increased to 150W/g, the level of total phenolics of mass extraction was reduced slightly. Mason and Lorimer, (2002) reported that high ultrasonic power could generate hydroxyl radicals that would react with phenolic compounds (Phan et al, 2012; Fernando et al, 2015; Pan et al, 2011).

Effects of ultrasonic time on the leveland purity of polyphenol from the old tea mash extract

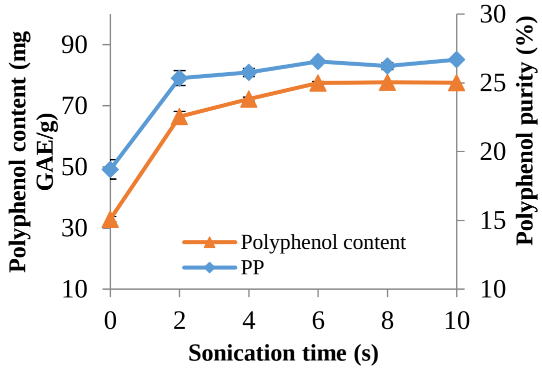


Figure 2. Effects of ultrasonic time on the content and the purity of polyphenol from the mash extraction

The ultrasonic power of 112.5 W/g is used in the experiment. The result wereshown that the control samples were without ultrasonic treatmentultrasonic andthe total polyphenol content was accounted for 32.94 mg GAE/g and 18.71% purity (Figure 2). Using the ultrasonic time of 6 minutes, the total polyphenol content reached at 77.46 mg GAE/g, and 25.75% of polyphenol purity. At 8 minutes, 10 minutes, the polyphenol content andpolyphenol purity of fluid extract have changed slightlybut unsignificantly in6 minutes. Similar observation was mentioned when the sonication of mulberry mash prolonged (Phan et al., 2012).

According to Vo and Le, 2014 when increasing sonication time too long, both concentration and activity of polyphenols in plants will be reduced. Therefore, in this study, the sonication time used for treatment is within 6 minutes. This result is similar study by Phan et al (2012) on extraction phenolic compounds in mulberry tree.

Optimization of the ultrasonic treatment conditions of the old tea leaves mash for maximizing polyphenol content

Experimental planning results were shown in Table 1.

Model quadratic form was determined by quadratic regression as below:

$$Y = b_0 + b_1.W + b_2.t + b_{11}.W^2 + b_{22}.t^2 + b_{12}.W.t \quad (6)$$

Where b_0; b_1; b_2; b_{11}; b_{22}; b_{12} were constant, Y_1;W; t were the total polyphenol content in old tea leaves mash (mgTEAC/g), the ultrasonic power (W/g) and the ultrasonic time (min), respectively.

Table1. Experimental planning and result of total polyphenol content of the old tea leaves from the ultrasound-assisted extraction

No	W (%)	T (s)	Ultrasonic power (%)	Ultrsonic time (s)	Polyphenol content (mg GAE/g solid)
1	-1	-1	93.75	4	67.560
2	1	-1	131.25	4	76.008
3	-1	1	93.75	8	73.857
4	1	1	131.25	8	79.047
5	-1	0	93.75	6	72.536
6	1	0	131.25	6	80.898
7	0	-1	112.5	4	75.273
8	0	1	112.5	8	81.676
9	0	0	112.5	6	78.539
10	0	0	112.5	6	77.069
11	0	0	112.5	6	78.373

Then optimization of the ultrasound-assisted parameters to the polyphenol content was shown in Table 2.

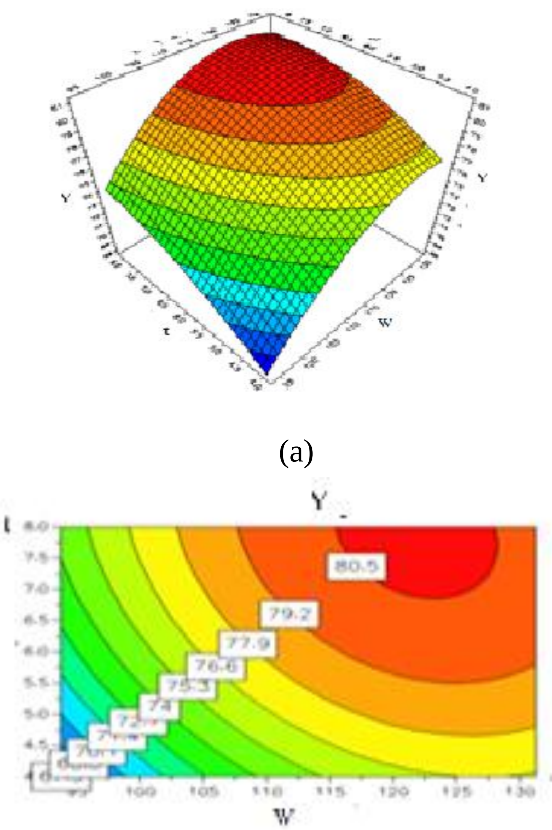
Table2. Multiple regression analysis of the model representing polyphenol content of the old tea leaves extraction

Total phenolics	Coeff.SC	Std.Err	P-value	Conf.int ±
Constant	78,64	0,691	9,96e-10	1,777
W	3,666	0,550	0,00120	1,415
T	2,623	0,550	0,0050	1,415
W*W	-2,897	0,846	0,0188	2,177
t*t	-1,140	0,846	**0,2360**	2,177
W*t	-0,81	0,674	**0,281**	1,732
N = 11	Q^2 = 0.626		Cond. no. =3.0822	
DF = 5	R^2 = 0.945		Y-miss = 0	
	R^2 Adj. =0.890		RSD = 1.3479	
	Conf. lev. = 0.95			

The coefficients of the model were evaluated for significance with a student t-test. The insignificant coefficients were eliminated. The final predictive equation was as follows:

$$Y = 78.64 + 3.666 \times W + 2.623 \times t - 2.897 \times W^2 \quad (7)$$

According to Fernandoet al (2015), The R^2 value of this model was determined to be 0,8–0,9, $Q^2 > 0,5$ which proved that the regression model was significant. The results showed that linear coefficients (W, t) and pure quadratic coefficients (W^2) were significant, but the interaction coefficient (W×t) was not ($P > 0.05$). Experimental results the Q^2 and R^2 value of the model were0.626 and 0.945 respectively that showed regression model to be significant.



(a)

(b)

Figure 3.Tri-dimensional response surface contour plot (a), two-dimensional contour plot (b) showing the effects of ultrasonic power (W) and time (t) on the polyphenol content (Y) of the old tea leaves mash

Our results proved that ultrasonic power and time had significantly positive effects on the polyphenol content in the mash extraction, while their obvious quadratic effects were also observed, but were negative. Surface response graph, obtained by using the fitted model above, is presented in Figure 3. According to the model, the optimal ultrasonic power and time for the old tea leaves mash treatment were 122.48W/g and ultrasonic time was 7.84 minutes, respectively. This value was 5.54% higher than that in the non-optimization experiment. In comparison with the ultrasonic treatment conditions of acerola mash, the ultrasonic power used for old tea leaves mash treatment was higher while the sonication time was nearly similar. It can be suggested that for complete degradation of fruit pulp tissue arerequired different ultrasonic powers for the treatment(Lieu et al, 2010; Liu et al, 2012). In order to verify the accuracy of the model, three independent replicates were carried out for measuring the poplyphenol content in the mash extraction under optimal conditions. The polyphenol content in the ultrasound-assisted extraction was 80.822mg GAE/g, higher than that of the control sample (36.256 GAE/g).

Determizationof kinetic parameters of the ultrasound-assisted extraction from second-order kinetic model

The optimal parameters: ultrasound power was 122.48W/g and ultrasonic time was 7.84 minutes. Meanwhile, polyphenol content waspredicted by the quadratic regressionabout 80.822 mg GAE/g.From the optimal parameters chosen, the kinematic equation was experimented at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 min.

For these experimental data, the extraction rate reciprocal (t/C_t) at various extraction times was presented in Figure 4.

Table 3.Kinetic parameters of extraction with ultrasound assisted extraction and without ultrasound assisted extraction

Sample	Extraction capacity C_e (mg/g)	The initial extraction rate v_o(mg/g.min)	Rate of extraction k (g/mg.min)	R^2
Without ultrasound assisted extraction	75,758±0,01	36,90± 3,95	0,0064±0,007	0,9996
Ultrasound assisted extraction	85,4± 0,41	15,244±1,29	0,0021 ± 0,02	0,9975

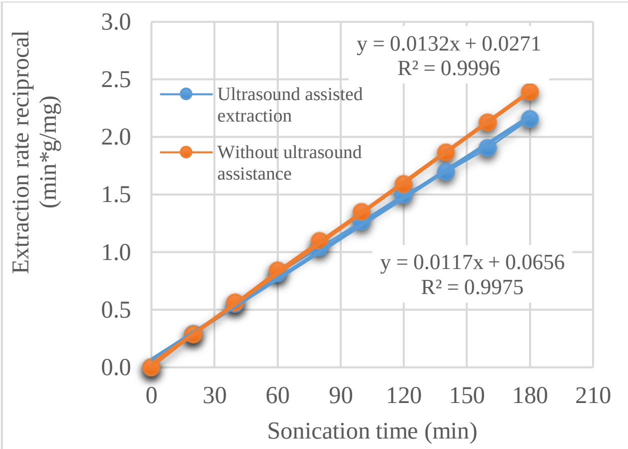


Figure 4. Extraction rate reciprocal (t/C_t) of polyphenol at different extraction times (t) in ultrasound-assisted extraction.

Table 3 shows the kinetic parameters of the polyphenol content in the ultrasound-assisted extraction from the old tea leaves. The values of extraction rate constant k in ultrasound assisted extraction were found approximately 0.0021g/mg.min and 15.244g/mg.min of the initial extraction rate (**v_o**), they were lower than that of the control sample. However, extraction capacity (**C_e**) for polyphenol in ultrasound assisted extraction was 85.4mg/g higher than that of sample withouth ultrasound treatment. Consequently, the ultrasound assisted extraction significantly facilitated the extraction of phenolics from the old tea leaves and highly shortened the extraction time in comparison with the traditional method.

Similar kinetic parameters were recently reported by Khan et al, (2010) and Pan et al, (2011) for polyphenol extraction from orange peel and pomegranate peel, respectively. However, the values and the increase in the kinetic parameters estimated by these authors were much lower than those in our study (Fernando et al, 2015; Khan et al, 2010, Le et al, 2012; Liu et al, 2012; Luque-Rodrıguez et al, 2007). According to Le and Le, (2012), phenolic compounds could be extracted from fruit easier than from peel. In both extraction methods, the coefficient of determination R^2 for both phenolics and anthocyanins was quite high (R^2> 0.99). It can be concluded that the second order kinetic model fitted perfectly the experimental results.

4. CONCLUSION

Ultrasonic treatment of the old tea leaves mash has been shown to be an efficient method for the extraction of juice with high phenoliccontent. Further study will be performed to clarify the effects of sonication variables on the level of important phenolic compounds in the old tea leaves mash. Short extraction time seems to be suitable for the extraction of bioactive compounds in fruit juice. Ultrasound-assisted extraction can be considered as an alternative technique in the extraction.

5. REFERENCES

[1] M. Saxena, J. Saxena, and A. Pradhan, (2012) Flavonoids and phenolic acids as antioxidants in plants and human health,*Int. J. Pharm. Sci. Rev. Res,* vol. 16, pp. 130-134,.

[2] A. M. Goula, Ultrasound-assisted extraction of pomegranate seed oil–Kinetic modeling, (2013).*Journal of Food Engineering,* vol. 117, pp. 492-498.

[3] D. J. Charles, (2012). *Antioxidant properties of spices, herbs and other sources*: Springer Science & Business Media.

[4] Mason T. J. and Lorimer, J. P (2002). Applied sonochemistry,*The uses of power ultrasound in chemistry and processing,* pp. 1-48.

[5] Vo, H. and V. Le, (2014)Optimization of ultrasonic treatment of rose myrtle mash in the extraction of juice with high antioxidant level,*International Food Research Journal,* vol. 21, pp. 2331-2335.

[6] Phan, L. H. N. Nguyen, T. N. T and Le, V. V. M.(2012). Ultrasonic treatment of mulberry (Morus alba) mash in the production of juice with high antioxidant level,*Journal of Science and Technology,* vol. 50, pp. 204-209.

[7] Fernando,C. D.and P. Soysa, 92015). Extraction Kinetics of phytochemicals and antioxidant activity during black tea (Camellia sinensis L.) brewing, *Nutrition journal,* vol. 14, p. 1.

[8] [8]. Pan, Z., Qu, W., Ma, H., Atungulu, G. G. and McHugh, T. H. (2011). Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. *Ultrasonics Sonochemistry 18* (5): 1249 – 1257.

[9] Khan, M. K., Abert-Vian, M., Fabiano-Tixier, A. S., Dangles, O. and Chemat, F. (2010). Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel. *Food Chemistry* 119 (2): 851–858.

[10] Le, H. V. and Le, V. V. M. (2012). Comparison of enzymeassisted and ultrasound-assisted extraction of vitamin C and phenolic compounds from acerola (Malpighia emarginata DC.) fruit. *International Journal of Food Science and Technology 47* (6): 1206–1214.

[11] Li, T.S.C. (2008). Vegetables and Fruits: Nutritional and Therapeutic Values. Boca Raton: CRC Press.

[12] Lieu, L. N. and Le, V. V. M. (2010). Application of ultrasound in grape mash treatment in juice processing. *Ultrasonics Sonochemistry* 17 (1): 273–279.

[13] Liu, G. L., Guo, H. H. and Sun, Y. M. (2012). Optimization of the extraction of anthocyanins from the fruit skin of Rhodomyrtus tomentosa (Ait.)

[14] Hassk and Identification of anthocyanins in the extract using high-performance liquid chromatography - Electrospray Ionization - Mass Spectrometry (HPLC-ESI-MS). *International Journal of Molecular Sciences* 13 (5): 6292-6302.

[15] Luque-Rodrıguez, J. M., Luque de Castro, M. D. and Perez-Juan, P. (2007). Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues. *Bioresource Technology* 98 (14): 2705–2713.