

Effect of Amplitude of Ultrasound-Assisted Solvent Extraction and Extraction Temperature on the Kinetics, Thermodynamics, Antioxidant and Antimicrobial Activity of *Ocimum basilicum* L. Extract

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ABSTRACT

In this study, the effect of three amplitudes of Ultrasound-Assisted Solvent Extraction (UASE) (0, 20 and 40%; 100W, 30 kHz; the 0% treatment serving as control) on kinetics, thermodynamic, rosmarinic acid content, total phenolics, antioxidant activity, and antimicrobial activity of *Ocimum basilicum* L. (basil) extract at different temperatures (25, 35 and 45°C) was evaluated. Increases in ultrasound amplitude and temperature increased yields and biological activities of extracts. The highest rosmarinic acid content, total phenolics, antioxidant and antimicrobial activity were obtained for samples treated with UASE at 40% amplitude and 45°C. The kinetics of extraction were evaluated based on a second order mechanism. Increases of amplitude and temperature significantly increased saturated extraction Capacity (C_s), initial extraction rate (h), and rate constant of extraction (k). The thermodynamic aspects of the extraction process showed that samples treated with UASE at 40% amplitude had higher activation Energy (E_a), frequency factor (A), enthalpy (ΔH^{++}) and entropy (ΔS^{++}) than control. UASE at 40% amplitude and control did not significantly differ in thermodynamic parameters. Results also showed very good linear relationships with high correlation coefficients between E_a and ΔH^{++} and, A and ΔS^{++} . Therefore, ultrasound can affect thermodynamic aspects and kinetics of extraction of basil extract and improve its biological activity.

Keywords: Antimicrobial activity, Antioxidant activity, Basil extract, Extraction kinetics, Extraction thermodynamic, Rosmarinic acid.

INTRODUCTION

Ocimum basilicum L. (Basil), a member of the Lamiaceae family, is used both as an ornamental and culinary herb (Morales and Simon, 1996). The genus *Ocimum* contains between 50 and 150 species of herbs and shrubs found in the tropical regions of Asia, Africa, South America, and the Mediterranean, but usually cultivated in many countries in greenhouses and natural conditions in order to increase the yield and obtain a usual supply of the material (Simon *et al.*, 1999). Basil is a

popular herb in the US, Iran and Mediterranean diets (Javanmardi *et al.*, 2002). Basil has shown antimicrobial and antioxidant activities due to its phenolic compounds. One of the most important phenolic compounds in basil is rosmarinic acid. (Sharafati Chaleshtori *et al.*, 2015). Rosmarinic acid (R-O-caffeoyl-3-4-dihydroxyphenyllactic acid) is one of the most plentiful caffeic acid esters found in species used commonly as culinary herbs such as basil. Rosmarinic acid and its derivatives have been reported to have antioxidant, antibacterial and medicinal properties

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(Petersen and Simmonds, 2003). Therefore, designing a method that increases rosmarinic acid in basil extract is important.

Ultrasound treatment is a food processing method, which possesses various applications when applied alone or in combination with other food processing techniques (Da Porto *et al.*, 2013). This treatment has been reported to be helpful for extraction. Ultrasound treatment is considered to be useful due to its reduced processing time with lower energy consumption and being environmentally friendly (Hashemi *et al.*, 2015; 2016b). The economic importance of plant extracts makes their extraction an important process. Although information on the application of UASE in the extraction of these extracts is both helpful and promising, this new technique is still far from becoming off-the-shelf (Patist and Bates, 2011). In addition, literature is limited, especially for a kinetic and thermodynamic aspect of UASE in the extraction of plant extracts. For instance, there are currently no reports on the kinetic and thermodynamic of the UASE of extracts from basil even though this herb is a well-known source for antioxidant and antimicrobial compounds. A good understanding of kinetics and thermodynamic of basil during extraction can improve our abilities to design extraction methods that maintain the existing extract quality in an extraction system and reduce the appearance of undesirable breakdown products (Hashemi *et al.*, 2015). Therefore, the aims of the present study were to investigate the effect of the amplitude of UASE and extraction temperature on the kinetics, thermodynamic and biological activity of extract and rosmarinic acid from basil.

MATERIAL AND METHODS

Bacterial Strains and Culture Conditions

Escherichia coli O157 H7 ATCC 35150, and *Staphylococcus aureus* ATCC 25923 were obtained from the microbial culture stock of Veterinary School, Shiraz

University. All strains were maintained as 20% glycerol stock at -80°C. Pathogen strains were reactivated in nutrient broth (HiMedia, India) and incubated aerobically at 37°C. All strains were grown in broth medium three times prior to the experiments.

Plant Material and Chemicals

Five kilograms of dried basil leaves (12% moisture content/dry basis) were purchased from the local market in Shiraz city (July, 2015) and stored at -18°C until initiation of the experiments. All chemicals and solvents used in this study were of analytical reagent grade and purchased from Merck and Sigma Chemical Companies.

Extraction Process and Treatments

For UASE, a Hielscher ultrasonic device (UP100H, 100W, 30 kHz) with a titanium sonotrode (tip diameter 10 mm) was used. The effects of three levels of amplitude (0, 20 and 40%) at three temperatures (25, 35 and 45°C) were investigated. The 0% amplitude experiment was the control; basically, ultrasonics was not turned on and the sample-solvent mixture was undisturbed during the process.

Dried basil leaves were ground into powder using a grinder and passed through a standard sieve to select particles smaller than 1.18 mm. Then, ground basil powder was mixed with methanol at a ratio of 1:5 (w/v). Ultrasound treatment was applied to the sample by inserting the probe approximately 5 cm from the top into the sample-solvent suspension in the cell. The ultrasonic treatment was applied for 8 h. To avoid significant solvent evaporation that could potentially occur at long treatment periods and/or at higher amplitudes, a special evaporation-proof cap was used during the ultrasonic extraction process. A water bath was used to maintain the temperature of the extraction medium at 25, 35 and 45°C during the process. Additionally, the temperature of the medium was monitored

every 20 minutes to ensure that there was no temperature overrun. The resulting slurries were filtered under suction through Whatman No. 4 filter paper. This process was repeated twice for the residue, and the filtrates were combined. The supernatants were collected and concentrated under vacuum at 35°C. The remaining crude extracts were stored at -18°C. Each experiment was carried out in triplicate.

Kinetics and Thermodynamic Parameters

The extraction rate can be written as the following Equation (1):

$$\frac{Ct}{t} = \frac{1}{\frac{k(C_s)^2}{2}} + \frac{1}{C_s} \quad (1)$$

Where, k = The second-order extraction rate constant ($L\ g^{-1}\ min^{-1}$); C_s = The extraction capacity (concentration of oil at saturation in $g\ L^{-1}$), C_t = The concentration of oil in the solution at any time ($g\ L^{-1}$), t (min).

Then, when t approaches 0, the initial extraction rate, h , can be written as:

$$h = kC_s^2 \quad (2)$$

By rearrangement of Equation (1), the concentration of oil at any time can be obtained as:

$$Ct = \frac{t}{h} + \frac{t}{C_s} \quad (3)$$

The initial extraction rate, h , the extraction capacity, C_s and the second order extraction constant, k , can be calculated experimentally by plotting t/Ct vs. t (Sayyar *et al.*, 2009).

The effect of temperature on the rates of extraction was evaluated by means of the Arrhenius equation:

$$\log k = \log A - \left(\frac{E_a}{2.303RT} \right) \quad (4)$$

Where, k ($L\ g^{-1}\ min^{-1}$) is the reaction rate constant, R is the molar gas constant ($8.3143\ J\ mol^{-1}\ K^{-1}$), T is the absolute temperature (K), E_a is the activation Energy ($kJ\ mol^{-1}$) and A ($L\ g^{-1}\ min^{-1}$) is the pre-exponential factor.

Enthalpies (ΔH^{++}) and entropies (ΔS^{++}) of activation were determined by regressing $\log k/T$ versus the inverse of Temperature (T , K) via the equation derived from the activated complex theory:

$$\log(k/T) = \log(k_B/h) + (\Delta S^{++}/2.303R) - (\Delta H^{++}/2.303RT), \quad (5)$$

Where, k_B is the Boltzmann constant ($1.380658 \times 10^{-23}\ J\ K^{-1}$, the ratio between R and Avogadro's number, $6.022 \times 10^{23}\ mol^{-1}$) and h is the Planck's constant ($6.6260755 \times 10^{-34}\ Js$). From the slopes and intercepts of the lines, the values of ΔH^{++} and ΔS^{++} were calculated.

HPLC Analysis of Rosmarinic Acid

The HPLC system consisted of a P580 pump (Dionex Co., Sunnyvale, CA), connected to an ASI-100 automated sample injector. A reverse phase C18 column ($5\ \mu m$ particle size, $25\ cm \times 4.6\ mm$) was used. The absorbance at 280 nm was measured by a PDA-100 Photodiode array variable UV/vis detector (Dionex Co.). Mobile phase solution A consisted of 0.1% TFA (trifluoroacetic acid) in water, and absolute acetonitrile was used as solution B. A multistep gradient was used for all separations with an initial injection volume of $15\ \mu L$ and a flow rate of $1\ mL\ min^{-1}$. Rosmarinic acid in each sample was identified using retention time and was further quantified by comparison of the peak area of the standard runs.

Total Phenolic Compounds

Total phenolic content of basil extract was determined using the FolinCiocalteu method (Kahkonen *et al.*, 1999). An appropriately diluted sample ($400\ \mu L$) was placed into a test tube. Diluted Folin-Ciocalteu's reagent ($2,000\ \mu L$) was added and mixed with vortex for 3 minutes. Sodium carbonate solution ($1,600\ \mu L$) was added and incubated in the dark at ambient temperature for 30 minutes. For the preparation of the blank, distilled water ($400\ \mu L$) was used instead of the sample. The absorbance of the samples was determined spectrophotometrically against the blank at 765 nm (UV/Visible Philips Cambridge, UK).



DPPH Assay

The antioxidant activity of basil extract was evaluated by monitoring their ability to quench the stable free radical DPPH using the method described by Choi *et al.* (2002) with brief modifications. Different methanol dilutions of basil extract [15, 45, 65, 85, 105, 125, 155, and 185 ($\mu\text{g mL}^{-1}$)] were mixed with 1.0 mL of a 0.3 mM DPPH methanol solution. Ethanol (1.0 mL) plus basil extract solution was used as a blank. Absorbance was determined at 517 nm after 30 minutes of reaction time at room temperature. The basil extract Concentration providing 50% Inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against basil extract concentration. BHT was used as a control and all tests were carried out in triplicate.

Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) was determined using the method of Benzie and Strain (1996). Briefly, 900 mL FRAP reagent was mixed with 90 mL distilled water then warmed to 37°C in a water bath. The control reading of the reagent was determined at 595 nm. Subsequently, 30 mL of sample solution (100 mg in 10 mL of n-hexane) was added and absorbance was determined at 595 nm against the control solution. A standard curve was prepared using various concentrations (200–2,000 mmol L^{-1}) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The results are expressed in mmol Fe^{2+} per unit mass.

Minimal Inhibitory Concentration (MIC)

To determine MIC, quantitative serial dilutions of ethanol extracts of basil were tested against two foodborne pathogens with brief modification of the method described by Hufford and Clark (1988). Twofold serial

dilutions of extracts were made with MH broth. After adding 20 μL of basil extracts to the first tube containing 1 mL of MH broth, serial transfers were made through to the fourth tube. One drop of Tween 80 (14.5 mg) was added to ethanol extracts followed by vortexing to solubilize the extract. A 0.5 mL aliquot ($5 \times 10^5 \text{ CFU mL}^{-1}$) of test microorganism was added to each tube. A positive control tube contained MH broth and microorganism and a negative control tube contained 10 μL of Streptomycin in MH broth and microorganism was maintained. A further control tube was prepared with broth and basil extract to confirm that broth or basil extracts were not contaminated. Tubes were later incubated at 37°C. The tubes were visually examined for the lowest concentration of extract that showed inhibition of microbial growth after 24 hours. The concentration in the lowest serial dilution of the basil extracts at which growth did not occur on broth was recorded as the MIC.

Statistical Analysis

Two-way ANOVA of data was used by factorial experimental design with two factors: amplitude and temperature. Complete randomized design was used. Significant differences between groups were determined by Duncan's multiple range test. All statistical analyses were performed using the SPSS software (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The kinetics of rosmarinic acid from basil was investigated at various temperatures and ultrasonic amplitudes. The experimental results were analyzed using a second order model by plotting t/C_t versus time. The extraction rate constant (k), the saturation extraction capacity (C_s), the initial extraction rate (h) and the coefficient of

determination (R^2) were determined according to the linear curves as shown in Table 1. The extraction rate constant increased when the amplitude level increased. A similar trend was observed for the saturation extraction capacity and the initial extraction rate. For instance, when changing the amplitude level from 20 to 40% with a constant temperature of 25°C, k and h increased from 0.000498 to 0.000626 $L\ g^{-1}\ min^{-1}$ and 0.009467 to 0.033176 $L\ g^{-1}\ min^{-1}$, respectively. In line with this, Hashemi *et al.* (2015) reported previously in a kinetic study of kolkhoung oil extraction, that by increasing ultrasound amplitude, k , C_s and, h increased. Results also show that the second order extraction parameters (k , C_s and h) increase with temperature in samples treated or untreated with ultrasound and that the changes can be described by the Arrhenius Equation (Table 2). Sayyar *et al.* (2009) found that the extraction temperature has a direct effect on k , C_s and h . By increasing the extraction temperature, the optimum extraction duration can be reduced as reaction happens faster. The final concentration also increases with

temperatures due to the thermodynamic effect of temperature on solubilization of extracts inside the solid. Employing activation energy, which is the minimum energy that must be available to a chemical system with potential reactants to result in a chemical reaction, is an approach to address the dependence of the rate of rosmarinic acid extraction on temperature (Labuza, 1984). E_a of samples treated with 20% ultrasound amplitude was significantly lower than control. Samples treated with 40% ultrasound amplitude had the highest activation energy. A lower E_a means that a higher temperature change is needed to induce a certain change in the rate of reaction (Tan *et al.*, 2001). In other words, the chemical reactions with low activation energies are temperature insensitive and the reaction with high activation energies are temperature sensitive. Consequently, the temperature dependence of rosmarinic acid extraction decreased among the samples treated with 20% ultrasound amplitude in comparison to control and samples treated with 40% ultrasound amplitude. According to the Arrhenius equation, the frequency

Table 1. Kinetic parameters of rosmarinic acid extraction from basil samples.

Parameters Samples	Temperature (°C)	Ultrasound amplitude (%)	K^a ($L\ g^{-1}\ min^{-1}$)	C_s^b ($g\ L^{-1}$)	h^c ($g\ L^{-1}\ min^{-1}$)	R^2	R_{adj}^2	RMSE	D-W Statistic
1	25	-	0.000378 ^g	1.33 ⁱ	0.000668 ^g	0.972	0.965	0.1323	1.83
2	35	-	0.000421 ^f	2.41 ^h	0.002445 ^e	0.953	0.941	0.1714	2.05
3	45	-	0.000488 ^e	3.11 ^g	0.004719 ^c	0.945	0.931	0.1854	1.73
4	25	20	0.000498 ^e	4.36 ^f	0.009467 ^d	0.962	0.952	0.1541	1.96
5	35	20	0.000556 ^d	5.15 ^e	0.014746 ^d	0.974	0.968	0.1275	1.76
6	45	20	0.000612 ^c	6.43 ^d	0.003935 ^f	0.981	0.976	0.1090	2.13
7	25	40	0.000626 ^c	7.28 ^c	0.033176 ^c	0.933	0.916	0.2046	2.01
8	35	40	0.000714 ^b	8.17 ^b	0.047659 ^b	0.946	0.932	0.1837	1.92
9	45	40	0.000836 ^a	9.22 ^a	0.071067 ^a	0.987	0.984	0.0901	2.21

^a Rate constant of extraction; ^b Saturated extraction capacity, ^c Initial extraction rate. All values are means of three determinations with Coefficient of Variations (CV= SD/Mean×100) < 5%. Means within a column with the same superscript letters are not significantly different at $P < 0.05$.



Table 2. Thermodynamic parameters of rosmarinic acid extraction from basil samples.

Samples	Parameters					
	$\log A^a$	E_a^b	R^2	R_{adj}^2	RMSE	D-W Statistic
Control	-1.663 ^b	10052.6 ^b	0.988	0.985	0.0866	2.27
Basil+20%*	-1.876 ^c	8133.98 ^c	0.999	0.999	0.0250	2.22
Basil+40%	-1.207 ^a	11927.18 ^a	0.995	0.994	0.0559	1.99

Samples	Parameters			R^2		
	ΔH^{++c}	ΔS^{++d}	R^2			
Control	7235.95 ^b	-286.14 ^b	0.974	0.968	0.1275	2.08
Basil+20%	6357.07 ^c	-286.91 ^b	0.996	0.995	0.0500	1.79
Basil+40%	9068.4 ^a	-275.94 ^a	0.999	0.999	0.0250	1.72

^a Frequency factor (L g⁻¹ min⁻¹); ^b Activation energy (J mol⁻¹); ^c Enthalpy (J mol⁻¹), d: Entropy (J mol⁻¹ K⁻¹). All values are means of three determinations with Coefficient of Variations (CV= SD/Mean×100) < 6%. Means within a column with the same superscript letters are not significantly different at *P*<0.05.

factor is considered to be the other most important kinetic factor affecting the rate of reaction. This parameter indicated a pattern similar to that of the activation energy for the extraction of rosmarinic acid from basil samples but minor changes in *E_a* values resulted in significant changes in frequency factors (Figure 1-a). This implies a higher contribution for *A* than for *E_a* to the rate of extraction from the basil samples. The loss of rotational freedom in the transition state can lead to low-frequency factors (Cho, 1997). Results also show that entropy of samples treated with 20% ultrasound amplitude was significantly lower than 40% ultrasound amplitude; however, samples treated with 20% ultrasound amplitude were not significantly different from control. It was observed that samples treated with 40 and 20% ultrasound amplitude had the highest and the lowest enthalpy, respectively. The enthalpy change was found to be positive, indicating the endothermic nature of the rosmarinic acid extraction process. Sayyar *et al.* (2009) and Hashemi *et al.* (2015) also found that the extraction process is an endothermic process. Figures 1-b and -C demonstrate very good linear relationships with high correlation coefficients between the *E_a* and ΔH^{++} (*R*²= 0.912) and the *A* and ΔS^{++} (*R*²= 0.936) for the rosmarinic acid extraction from basil samples. Same results were also obtained by Hashemi *et al.* (2015; 2016a) for extraction of oil from kolkhoung kernels

and antioxidant activity of herb extract in vegetable oil. As mentioned above, frequency factor has more impact than activation energy in the rosmarinic acid

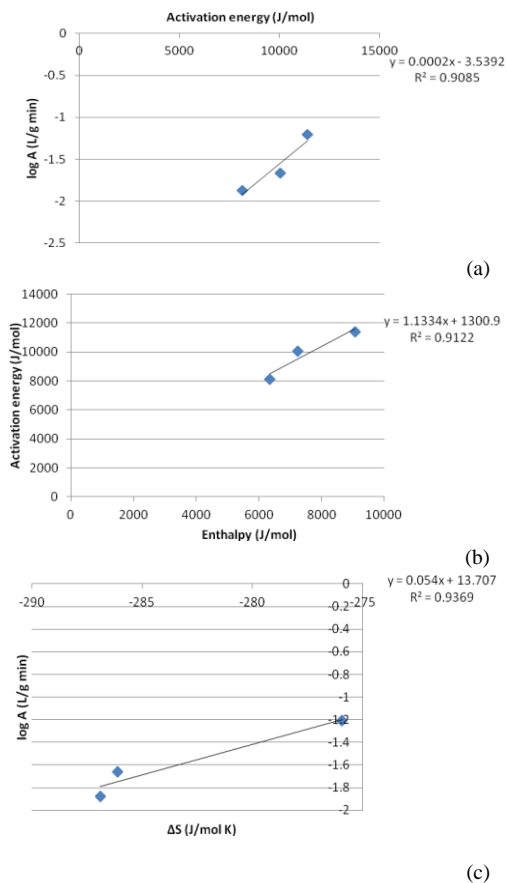


Figure 1. Relationship between frequency factor and activation Energy (*E_a*) (a), enthalpy (ΔH^{++}) and activation Energy (*E_a*) (b), and frequency factor and entropy (ΔS^{++}) (c) for the extraction of rosmarinic acid from basil at 25-45°C.

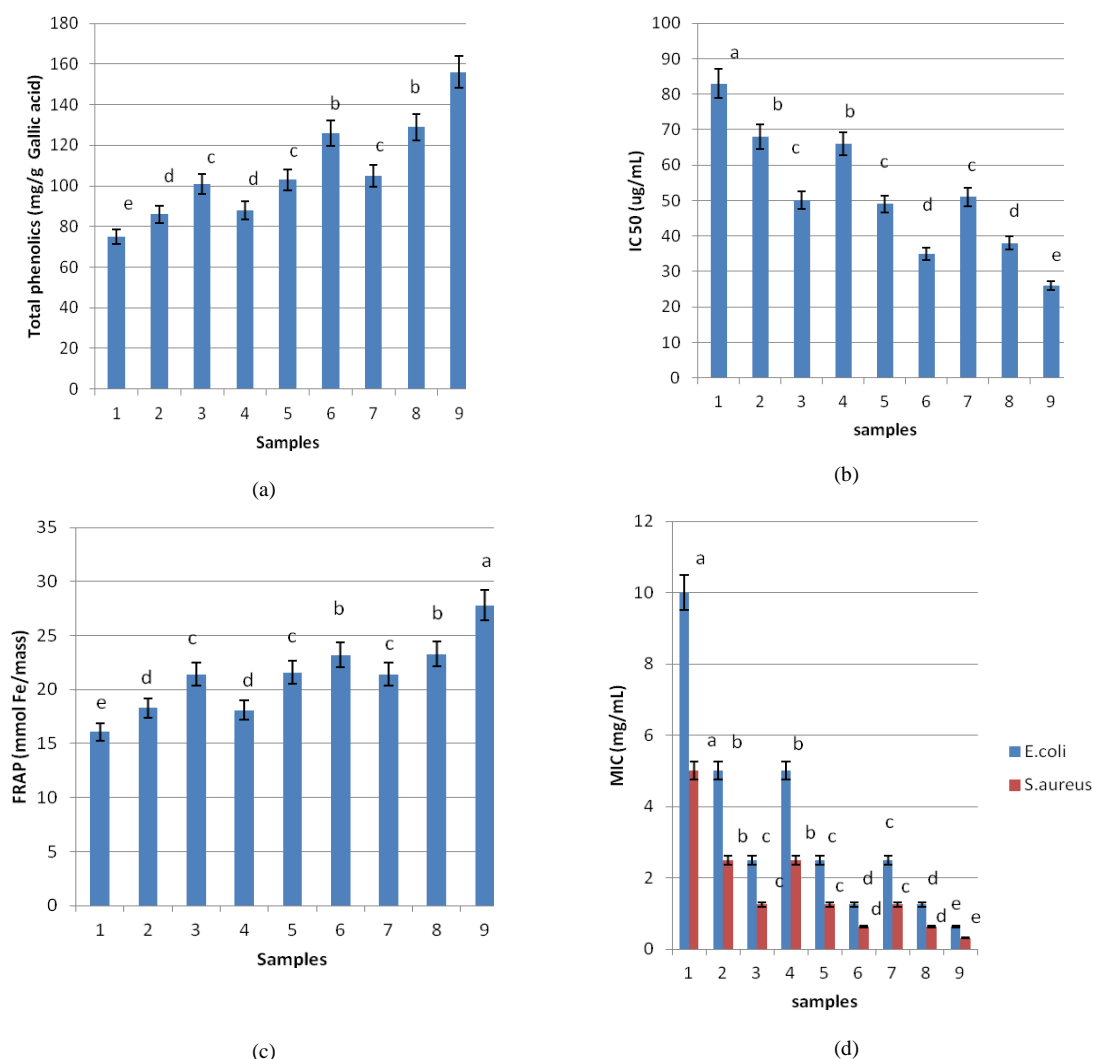


Figure 2. Total phenolics (a); DPPH assay (b); FRAP assay (c), and antimicrobial activity (d) of basil extract samples at different extraction conditions. (1) Extraction at 25°C; (2) Extraction at 35°C; (3) Extraction at 45°C; (4) Extraction at 25°C and 20% amplitude; (5) Extraction at 35°C and 20% amplitude; (6) Extraction at 45°C and 20% amplitude, (7) Extraction at 25°C and 40% amplitude; (8) Extraction at 35°C and 40% amplitude, (9) Extraction at 45°C and 40% amplitude (Mean±SD).

extraction. Thus, application of ultrasound influenced entropy (or A) more than enthalpy (or E_a) in the rates of rosmarinic acid extraction.

The calculation of total phenolic content of methanolic extracts of basil was carried out using the standard curve of gallic acid and presented as mg gallic acid equivalents per gram (Figure 2-a). All samples contained phenolic compounds as they produced the characteristic blue color with Folin-Ciocalteu's reagent. The total phenolic contents in each

sample ranged from 75 to 156 (mg g^{-1}). Both ultrasound and temperature significantly affected total phenolic contents of samples. Samples treated with ultrasound amplitude at 40% and temperature at 45°C had the highest content of phenolic compounds. Ma *et al.* (2009) studied the effects of ultrasonic parameters including extraction time, temperature, and ultrasonic power on the yields of phenolic compounds of citrus peel. They found that the yields of phenolic compounds increased when both temperature



and ultrasonic time were increased, whereas the opposite happened with increasing time at higher temperature to some degree. In the case of 40°C, the decrease in the yields of some phenolic compounds was observed with increased time, while those of other compounds did not significantly change. They also reported that ultrasonic power has a positive effect on the yields of phenolic compounds. Rodrigues and Pinto (2007) reported that high amounts of phenolics can be extracted from coconut shell by ultrasound assisted extraction technology, and that the extraction time was the most important parameter for the process.

The DPPH method is a frequently used method to evaluate the antioxidant activity. On the basis of this assay, the radical scavenging effect of each sample was measured and the results are presented in Figure 2-b. The strongest activity was shown in samples treated with ultrasound amplitude at 40% and temperature at 45°C. Ultrasound and temperature had a positive effect on the antioxidant activity of samples. The FRAP test is a rapid method that measures the ability of antioxidant compounds to reduce Fe^{3+} to Fe^{2+} , as a measure of total antioxidant capacity (Prior and Cao, 1999). Results of FRAP are similar to DPPH assay (Figure 2-c). These results were proportionate to the total phenolic content. The main phenolic in all samples regardless of treatment kind was rosmarinic acid. Rosmarinic acid has been found as the main phenolic in basil by many other researchers (Javanmardi et al., 2002; Nguyen and Niemeyer, 2008). Rosmarinic acid is considered to be responsible for the observed antioxidant activity of nonterpenoid compounds from the Lamiaceae family. It has been found in 110 out of 127 Lamiaceous species (Petersen and Simmonds, 2003). Muñoz-Márquez et al. (2013) found that high amounts of phenolic compounds can be extracted from *Laurus nobilis* by ultrasound-assisted extraction technology, therefore, increase the antioxidant activity of the extract.

The results of the MIC values of basil extracts obtained at 24 hours are presented in Figure 2-d. In general, the Gram-positive

bacteria were more sensitive to the basil extracts than the Gram-negative bacteria. The data demonstrated that the ultrasound amplitude and temperature used in the extraction had a great impact on MIC. For example, when changing the amplitude level from 20 to 40% with a constant temperature of 45°C, MIC values of *E. coli* and *S. aureus* decreased from 1.25 to 0.625 mg mL⁻¹ and 0.625 to 0.312 mg mL⁻¹, respectively. A similar trend was observed for the extraction temperature. This difference in the MIC value is due to differences in the total phenolic content of samples. It was reported that there is a highly positive linear relationship between antibacterial activity, antioxidant activity and total phenolic content in some herbs (Shan et al., 2007). Phenolic compounds of extracts can attack the phospholipid cell membrane, which causes increased permeability and leakage of cytoplasm (Kim et al., 1995). Therefore, application of ultrasound and temperature increase the antimicrobial activity of basil extract.

CONCLUSIONS

The UASE technique was shown to improve the extraction of phenolic compounds and rosmarinic acid from basil. UASE and temperature increased kinetic parameters of rosmarinic acid extraction, antioxidant activity and antimicrobial activity of basil extract. Basil samples treated with ultrasound amplitude at 40% and temperature at 45°C had the highest content of phenolic compounds. Although the application of ultrasound at low amplitude did not affect thermodynamic parameters, ultrasound at high amplitude increased thermodynamic parameters of extraction. A more thorough knowledge of the kinetics of UASE would be beneficial in suggesting improvements that could make this process more attractive commercially and industrially. However, more investigations are needed for measurement of the amount of other biological compounds released during extraction.

ACKNOWLEDGEMENTS

The author would like to express his appreciation to the Fasa University.

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تأثیر دامنه فراصوت و دمای استخراج با حلال بر سرعت استخراج، ترمودینامیک، ویژگی آنتی اکسیدانی و ضد میکروبی عصاره ریحان

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چکیده

در این مطالعه تأثیر سه دامنه متفاوت از فراصوت در استخراج با حلال (۰، ۲۰ و ۴۰ در صد) بر سرعت استخراج، ترمودینامیک، میزان رزمارینیک اسید، میزان ترکیبات فنولیک، ویژگی های ضد میکروبی و آنتی اکسیدانی عصاره ریحان در سه دمای ۲۵، ۳۵ و ۴۵ درجه سانتی گراد بررسی گردید. افزایش دامنه فراصوت و دمای استخراج بازده و فعالیت بیولوژیکی عصاره را افزایش داد. بیشترین میزان رزمارینیک اسید در عصاره، محتوای فنولیک، ویژگی ضد میکروبی و آنتی اکسیدانی برای دامنه ۴۰ درصد و دمای ۴۵ درجه سانتی گراد به دست آمد. سرعت استخراج بر اساس معادله درجه دوم به دست آمد. افزایش در دامنه و دمای استخراج ظرفیت اشباع استخراج، سرعت استخراج اولیه و سرعت استخراج را افزایش داد. اندازه گیری ویژگی های ترمودینامیکی نشان داد نمونه های استخراج شده در دامنه ۴۰ درصد انرژی اکتیواسیون، فاکتور بسامد، آنتالپی و انتروپی بیشتری از نمونه شاهد داشتند. نتایج همچنین نشان داد انرژی اکتیواسیون و آنتالپی و نیز انتروپی و فاکتور بسامد ارتباط خطی با ضریب رگرسیونی بالایی دارند. نتایج این پژوهش نشان داد فراصوت می تواند پارامتر های ترمودینامیکی و سرعت استخراج با حلال ریحان را تغییر دهد و ویژگی های بیولوژیکی آن را افزایش دهد.