eISBN 978-967-16145-1-8

Konvensyen Kebangsaan Kejuruteraan Pertanian Dan Makanan 2019
21 Mac 2019, Wisma Tani, Kementerian Pertanian Malaysia, Putrajaya

MSAE2019-PFE26

Ultrasonic Pretreatment Prior to Soxhlet Extraction for Essential Oil from Basil Leaves (*Ocimum Basilicum L.*)

M. Nuramanina[1], H. Muhammad Hazwan[2,*] and C.M. Hasfalina[3]
[1]Department of Biological and Agricultural Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
*Corresponding author. Tel.: +603-89466422, Email: hazwanhamzah@upm.edu.my

Abstract
The extraction of oil from basil leaves was investigated by using conventional Soxhlet extraction method and ultrasonic as a pre-treatment prior to Soxhlet extraction. The effects of two operating parameters on the oil extraction namely solvent to solid ratio and reaction time were investigated to optimize the processing conditions of conventional Soxhlet extraction for achieving maximum oil yield using Response Surface Methodology (RSM). The optimum conditions were found at 5 hours, and a solvent to solid ratio of 1:33. The oil recovery from ultrasonic pretreatment prior to Soxhlet extraction under the same optimum parameters was compared. The yield of oil from samples underwent ultrasonic as a pretreatment was 4.14% while the conventional Soxhlet method yielded 6.24%. This finding is consistent with the evidence of Scanning Electron Microscopy (SEM) images showed that the treated samples were flakier and porous than untreated samples. Overall, this study has shown that treated samples via ultrasonic is incapable to increase the oil yield percentage compared to the conventional Soxhlet extraction method.

Keywords: basil, ultrasonic, Soxhlet, essential oil, optimization

Introduction

Basil, one of the most popular herbs over 150 different species growing in the world can be observed growing in tropical regions of Asia, Africa, Central and South America (Zaree et al., 2014). The most common species grown *O. africanum* Lour. (syn. *O.* x *citriodorum* Vis.), O. *americanum* L. (syn. *O. canum* Sims.), *O. basilicum* L., O. *gratissimum* L., *O. minimum* L., and *O. tenuiflorum* L. (syn. *O. sanctum* L.) (Carović-Stanko et al., 2010). Sweet basil (*O. basilicum L.*) is an aromatic herb belonging to the *Lamiaceae* family (Ladwani et al., 2018). The essential oil from *O. basilicum* L. is shown to have an economic value for cosmetic, cookery, and pharmaceutical purposes (Srivastava et al., 2014).

Essential oils are obtained by various extraction methods depending on the nature of the plant, the stability of the chemical components and the specification of the targeted product. Conventional extraction technologies such as cold expression, solvent extraction and distillation have been used. Various promising or green extraction technologies such as supercritical fluids and microwave-assisted extraction also available and capable of producing products with the same or improved characteristics (Stratakos and Koidis, 2016). All extraction techniques have limitations and understanding the advantages or disadvantages are crucial in order to help in selection of proper methods (NN, 2015).

Pre-treatments have become a great interest for enhancing and accelerating the extraction process. Other than manipulating the extraction parameters and conditions, treatment can be applied to increase the extraction yield and improve the parameters condition. One of the pretreatment is indirect ultrasonic. The sample for indirect ultrasonic treatment process is carried out first before being extracted and not simultaneously in between the two processes.

Dharmendra and Pandey (2010) defined ultrasonic as the acoustics wave above the human hearing range (the audio frequency limit) of 20 kHz whereby the wavelength of wave induced particle vibrations in elastic medium such as a liquid or a solid. The suitability of ultrasonic pre-treatment on essential oil extraction has been described by Nora and Borges, (2017), that the cavitation bubbles in relation to structural rupture facilitates the mass transfer of solvent from the continuous phase into plant cells, thus enhance the essential oil yield.

Soxhlet extraction, an example of solid-liquid extraction transfers the target analytes from the solid to appropriate organic solvents to ensure that the extraction solvent maintains continual contact with the sample during the extraction process (Daso and Okonkwo, 2015). In this study, indirect ultrasonic treatment is used prior to Soxhlet extraction. The aim of this work is to study on ultrasonic pretreatment prior to Soxhlet extraction as an improved method for basil essential oil. The objectives of this work are summarized as follows: (i) To verify the optimize extraction conditions of basil essential oil using Response Surface Methodology (RSM); (ii) To compare the amount of oil yield extracted between with and without pretreatment prior to Soxhlet extraction; and (iii) To identify the morphological structure differences between with and without pretreatment prior to Soxhlet extraction.

Materials and methods

Sample preparation

Basil leaves was purchased from Tesco IOI City Mall Putrajaya. The basil leaves sample was grown and packed by Monoluxury Sdn. Bhd., Hydroponic Unit, Genting Highlands, 69000 Pahang, Malaysia. The leaves are dried in oven for 2 hours until constant weight was achieved. The dried leaves were crushed to powder by using pestle and mortar and stored in resealable bag at room temperature. The flowchart of methodology was presented in Figure 1.

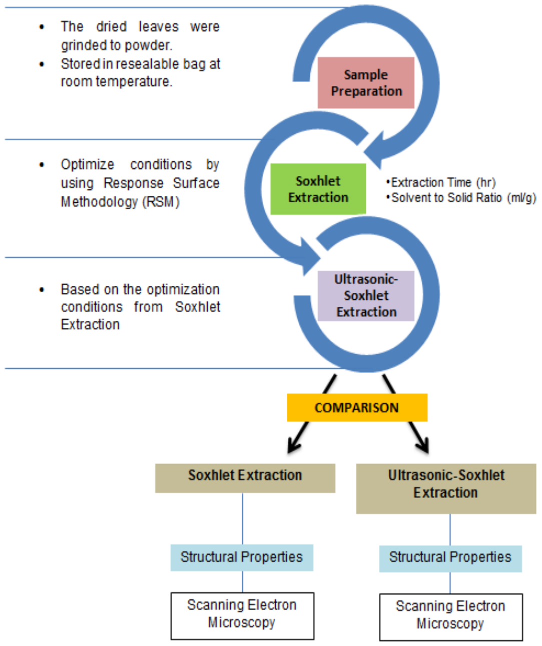


Figure 1: Flow chart of methodology.

Experimental design of Soxhlet extraction

Extraction parameters i.e. solvent to solid ratio and extraction time influencing extraction yield were investigated at three levels (-1, 0 and 1) using randomized central composite design as expressed in Table 1.

Table 1: Selected variables and coded level

Variables	Symbol	Coded levels		
		-1	0	1
Time	A	4	5	6
Solvent to solid ratio	B	10:1	20:1	30:1

A second degree polynomial equation derived from RSM was used. Data were analyzed by Analysis of Variance (ANOVA) to determine the lack of fit and the effects of quadratic and interaction variables on basil oil extraction. Data analyses and RSM were performed with Design Expert software program (Version 10.0.6.0; Stat-Ease, Inc., Minneapolis, MN, USA).

Extraction of basil essential oil

The effect of two parameters which are reaction time and solvent to solid ratio were investigated on the extraction operating conditions for basil essential oil. Five grams (5g) of basil leaves were extracted by using hexane as solvent. The reaction time was varied between 4, 5, and 6 hours. 100, 150 and 200 ml of solvent volume were used to determine the extraction yield giving out the solvent to solid ratio of 10:1, 20:1 and 30:1 respectively. The extraction process was carried out by using Soxhlet apparatus. Finally, the solvent was separated from the oil using Rotavapor EYELA running at 60°C temperature and speed of 4. The oil which was remained in the sample flask was weighed after the process was completed.

Ultrasonic

The ultrasonication of crushed basil leaves sample was conducted using Ultrasonic Cleaner Elmasonic S30H. Before the sample was sonicated, the water in the bath was first sonicated for five minutes for degassing purpose. After that, the sample was immersed with hexane inside a 250ml beaker and covered with aluminium coil. The beaker with its content was then suspended in the water bath of the ultrasonic cleaner. The sample was sonicated for 30 minutes with constant frequency at 37kHz, 280W. After ultrasonication, the samples underwent Soxhlet extraction using optimized conditions. The result was then compared to the oil yield obtained from basil sample extracted at the same optimized parameters without ultrasonic pretreatment.

Scanning Electron Microspcopy (SEM)

SEM Hitachi S-3400N was used at operating voltage of 15 kV. Two types of basil leaves sample namely treated and untreated sample images were digitally recorded in high resolution topograhic images at 1000x magnification.

Results and discussion

RSM of Soxhlet Extraction

The detail of the outcome of experimental design is shown in Table 2. The response ranges from 0.02 g to 0.28 g depending on the condition of experiments. These results can be fitted into a second order polymonial equation of coded units as given in Equation 1.

$$\text{Oil yield (Y)} = 0.30 + (-0.01A) + 0.07B + 0.03AB + (-0.04A^2) + (-0.09B^2) \quad (1)$$

ANOVA is carried out for the response in order to test the significant of the suitability as presented in Table 3.

Table 2: Central composite design for optimization of basil essential oil using Soxhlet extraction.

Run	Time (Hour)	Solvent to solid ratio (ml/g)	Response: Oil yield (g)
1	6	40	0.24
2	5	44.1	0.20
3	5	15.9	0.02
4	5	30	0.33
5	6.4	30	0.22
6	3.6	30	0.23
7	6	20	0.05
8	5	30	0.26
9	5	30	0.31
10	4	40	0.22
11	4	20	0.14
12	5	30	0.29
13	5	30	0.28

Table 3: ANOVA for model.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F
Model	0.10	5	0.02	42.12	<0.0001
A	1.09 x10^{-3}	1	1.09 x10^{-3}	2.22	0.18
B	0.03	1	0.03	69.68	<0.0001
AB	2.60 x10^{-3}	1	2.60 x10^{-3}	5.28	0.06
A^2	9.80 x10^{-3}	1	9.80 x10^{-3}	19.90	0.003
Residual	3.45 x10^{-3}	7	4.93 x10^{-4}		
Lack of fit	7.06 x10^{-4}	3	2.35 x10^{-4}	0.34	0.80
Cor total	0.11	12			
R-squared	0.97				
Adj R-squared	0.95				

A= Time, B = Solvent to solid ratio

The model p-value (<0.0001) indicates that the model was statistically significant as the probability >F is less than 0.05. The smaller the p-value, the higher significance the results (Zareen et al., 2014). Based on ANOVA, the results were obtained, the effects of experimental factors on oil yield, time and solvent to solid ratio; corresponding three dimensional responses surface plot was shown in Figure 2. The values of the axis in Figure 2 are real values. Overall, the model terms suggested variables with significance influence on oil yield was solvent to solid ratio (B). However, the time effect had lesser effect on the oil yield. The model was significant at 95% confidence level. The coefficient of determination (R^2) of the model was 0.97, indicating that 97% of the experimental oil yield values matched the model predicted values. The lack of fit also measures the significant of the model. The lack of fit *F*-value of 0.34 is not significant as the *p*-value is >0.05. The non-significance lack-of-fit proved that the model was valid for the present work (Mourabet et al., 2017).

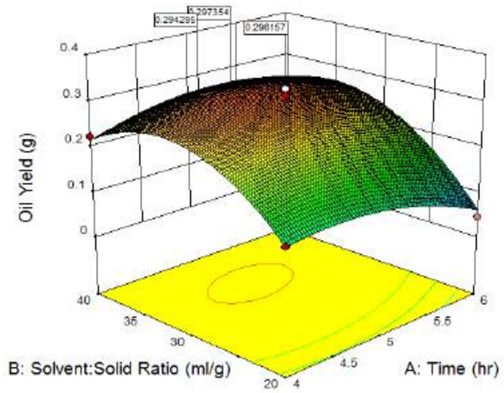


Figure 2. Combined effect of solvent to solid ratio and time on oil yield.

Figure 3 showed the contour plot of solvent to solid ratio and time on oil yield at optimum condition. The optimum conditions for oil yield were 4 hours 54 minutes extraction time and a solvent to solid ratio of 1:33.44. The predicted and experimental values for oil yield were obtained as 0.307 g and 0.312 g, respectively. A comparison between the experimental and predicted results indicates that the error was less than 1.6%. From the results, it was concluded that the developed model could accurately predict the oil yield. For convenience purposes, the optimum conditions were slightly modified to an extraction time of 5 hours, and a solvent to solid ratio of 1:33.

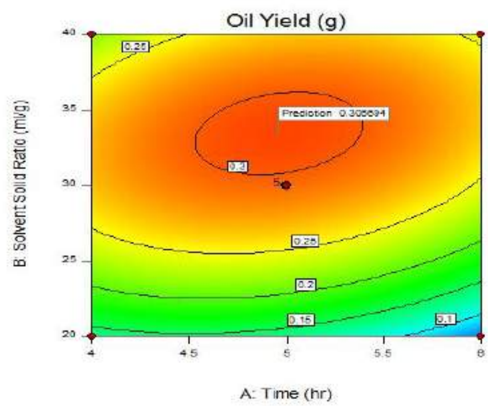


Figure 3. Contour plot of solvent to solid ratio and time on oil yield at optimum condition.

Comparison of Soxhlet extraction and ultrasonic as pretreatment prior to Soxhlet extraction

The comparison of extracted oil yield between Soxhlet extraction (untreated sample-SE) and ultrasonic as pretreatment prior to Soxhlet extraction (treated sample-USE) at optimum extraction time and solvent to solid ratio is shown in Figure 4.

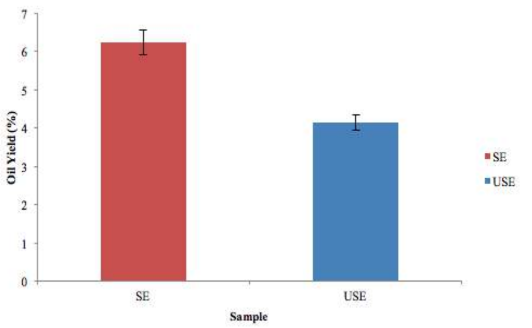
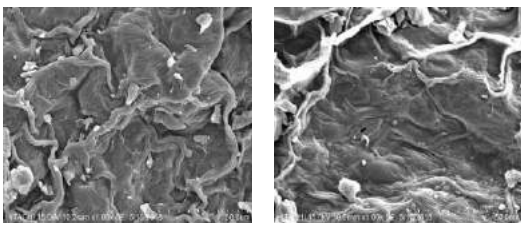


Figure 4. Comparison of treated (SE) and untreated samples (USE) on oil yield.

The maximum yield from untreated sample (6.24%) was obtained at 5 hours extraction time with 1:33 solvent to solid ratio meanwhile the treated sample producing minimum yield (4.14%) at the same condition. In this study, there was no evidence that ultrasonication has an influence on oil extraction for basil leaves. This result is somewhat counterintuitive. Overall, these results indicated that the ultrasonic pre-treatment did not increase the oil yield. Lou et al. (2010) stated that when the ultrasonic power was too high, the oil might be degraded by free radicals, thus the oil yield decreased. An extreme or longer exposure of sonication time on crushed basil leaves in this study had negative impact on degradation of phenolic compounds, therefore reduced the cavitation and decreased the extraction efficiency (Giacometti et al., 2018).

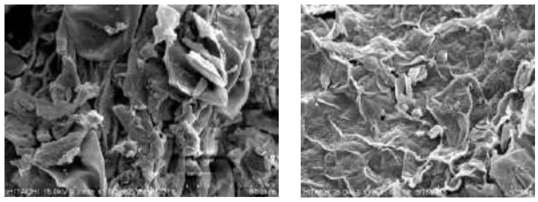
Figure 5 (a) and (b) showed the SEM images that indicated the morphological differences before and after ultrasonic was carried out, respectively. No evidence was found for structural changes between the samples before and after ultrasonic pretreatment.



(a) (b)

Figure 5. Scanning electron microscope (SEM) images of basil leaves cells: (a) before extraction (b) after sonication at 30 minutes, 37 kHz.

When comparison was made between samples after Soxhlet extraction for treated and untreated samples, the cell wall disruption made the untreated sample (Figure 6 (a)) became more porous and flaky compared to treated sample (Figure 6 (b)) after Soxhlet extraction. Overall, the results of SEM images correlated with the oil yield.



(a) (b)

Figure 6. SEM images of basil leaves: (a) treated (b) untreated samples after Soxhlet extraction.

Conclusion

This study has identified ultrasonic as a pretreatment prior to Soxhlet extraction made no significant difference to oil extraction. A greater focus on power or exposure time for ultrasonic as a pretreatment could produce interesting findings that account more for extraction efficiency.

Conclusion

The authors would like to thank staff of Department of Biological and Agricultural Engineering for administrative and technical support.

References

Carović-Stanko, K., Liber, Z., Besendorfer, V., Javornik, B., Bohanec, B., Kolak, I., and Satovic, Z. (2010). Genetic relations among basil taxa (*Ocimum* L.) based on molecular markers, nuclear DNA content, and chromosome number. *Plant Systematics and Evolution*, *285*(1), 13–22.

Daso, A. P., and Okonkwo, O. J. (2015). Conventional Extraction Techniques: Soxhlet and Liquid-liquid Extractions and Evaporation. In Jared Anderson, A. Berthod, V. Pino, and A. M. Stalcup (Eds.), *Analytical Separation Science* (1st ed., pp. 1437–1468). Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

Dharmendra, and Pandey, S. (2010). Ultrasonics: A Technique of Material Characterization. In D. Dissanayake (Ed.), *Acoustic Waves* (pp. 397–420). London, United Kingdom: InTechOpen.

Giacometti, J., Žauhar, G., and Žuvić, M. (2018). Optimization of ultrasonic-assisted extraction of major phenolic compounds from olive leaves (*Olea europaea* L.) using Response Surface Methodology. *Foods*, *7*(9), 149.

Ladwani, A. M., Hameed, A. E., and Amal ALadwani, C. M. (2018). Chemical composition of *Ocimum basilicum* L. essential oil from different regions in the Kingdom of Saudi Arabia by using Gas chromatography mass spectrometer. *Journal of Medicinal Plants Studies*, *6*(1), 14–19.

Lou, Z., Wang, H., Zhang, M., and Wang, Z. (2010).

Improved extraction of oil from chickpea under ultrasound in a dynamic system. *Journal of Food Engineering*, *98*(1), 13–18.

Mourabet, M., El Rhilassi, A., El Boujaady, H., Bennani-Ziatni, M., and Taitai, A. (2017). Use of response surface methodology for optimization of fluoride adsorption in an aqueous solution by Brushite. *Arabian Journal of Chemistry*, *10*, S3292–S3302.

NN, A. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, *4*(3), 3–8.

Nora, F. M. D., and Borges, C. D. (2017). Ultrasound pretreatment as an alternative to improve essential oils extraction. *Food Technology*, *47*(9), 1–9.

Srivastava, H. C., Shukla, P., Tripathi, S., and Shanker, B. (2014). Antioxidant and antimicrobial activities of sweet basil oils. *International Journal of Pharmaceutical Sciences and Research*, *5*(1), 279-285.

Stratakos, A. C., and Koidis, A. (2016). Methods for Extracting Essential Oils. In V. R. Preedy (Ed.), *Essential Oils in Food Preservation, Flavor and Safety* (pp. 31–38). Oxford, United Kingdom: Academic Press.

Zareen, A., Gardezi, D. A., Naeemullah, M., Masood, M. S., and Tahira, R. (2014). Studies screening of antibacterial potential of Siam queen , Holy basil and Italian basil essential oils. *Journal of Medicinal Plants Studies*, *2*(2), 63–68.