OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM ELAEGNUS ANGUSTIFOLIA USING RESPONSE SURFACE METHODOLOGY

[1]AKRAM ARIANFAR, [2]MARYAM SARDARODIYAN, [3]MASOOMEH MEHRABAN SANG ATASH

[1]Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
[2]Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, Iran
[3]Food Science and Technology Research Institute, ACECR Mashhad Branch, Mashhad, Iran
E-mail: a_aria_1443@yahoo.com

Abstract- In this investigation optimization of flavonoid extraction process frome methanolic (80%) extract (v/v) of elaegnus angustifolia through Response Surface Methodology by according to Lamaison and Carnat is performrd for optimizing the optimizing the process three factor (time, temperature and solution to solid ratio) were investigated each factor was repeated three times (5,20,35 min), temperature (30,45,60 ° C), and solution to solid ratio (20,35,50 ml/g). The experiment was conducted based on Box-Behnken design with three factor and three levels comprising a total of 17 tests. During optimization tests in an experiment with extraction conditions as (time:31/13 min, temperature:46/79 ·C and solution to solid ratio: 32/71 ml/g) the higest flavonoid 32/21 mg quercetin (standard of Flavonoid compound) per g of dry material compound was recorded. In addition to Flavonoid test, beta-carotene / linoleic test was also carried out for investigating antioxidant activity. In beta-carotene / linoleic test methanolic extract with 24/65 mg E.Q/g had the highest antioxidant activity.Considering the results and graphs temperature was indetified as the most effective factor.

Keywords- Elaegnus Angustifolia, Flavonoids, Antioxidant, Extraction, Ultrasound

I. INTRODUCTION

Free radicals are compounds with unpaired electrons, and they could be generated during the normal body metabolism or from the ingestion of foreign chemicals and pollutants. Because of their reactive nature, free radicals could interact with and cause oxidative damage on different cellular compartments, including cell membranes and DNA. [21,22]. Medicinal plants are especially rich in various secondary metabolites, including but not limited to polyphenols and flavonoids, which are capable of eliminating free radicals [18]. Antioxidants counter act free radicals and prevent the damage by crumbling oxidants, preventing chain reactions or preventing the activation of oxygen to highly reactive products [10]. Along the past two decades, several methods such as the ABTS (2,2-azinobis-(3-ethylbenzobis-(3-ethylbenzothiazoline- 6-sulfonate))) radical cation assay [20], the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay [23], the ferric reducing antioxidant power total reducing capacity [9] have been proposed for assessment of total antioxidant capacity. (FRAP) assay [6], oxygen radical absorbance capacity (ORAC) assay [1] and the electrochemical estimation of total reducing capacity [9] have been proposed for assessment of total antioxidant capacity.

The extraction of bioactive compounds under ultrasound irradiation (20–100 kHz) is one of the upcoming extraction techniques that can offer shorter operation times, simplified manipulation, reduced solvent consumption and temperature and lower energy input. The main advantages of ultrasound-assisted extraction over conventional Soxhlet extraction are: (a) cavitation increases the polarity of the system and increases the extraction efficiency, (b) allows the extraction of thermolabile analytes and (c) operating time is invariably shorter. Disadvantages of ultrasound-assisted extraction with respect to conventional Soxhlet extraction are: (a) the solvent cannot be renewed during process, so its efficiency is a function of its partitioning coefficient, (b) the need for filtering and rinsing after extraction increases solvent consumption and risk of losses or contamination of the extract and (c) Soxhelt extraction is usually more reproducible [7].:(a) the equipment is much simpler, so the overall cost of the extraction is much lower and (b) allows the extraction of wide variety of compound with different polarity. However, SFE surpasses ultrasound-assisted extraction in following respects: (a) the SFE technique is simpler and faster, (b) supercritical CO2 is not environmentally hazardous and (c) usually, SFE methods are more precise [7].

Ultrasound-assisted extraction provides advantages over microwave-assisted extraction as follows: (a) it is sometimes faster and (b) in many cases, the ultrasonic procedure is simpler. Disadvantages of ultrasound-assisted extraction when compared to microwave-assisted extraction are: (a) it is usually less robust and (b) particle size is a critical factor in ultrasound-assisted applications [7].

The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave[13]. Because most

of the available energy is transferred to the accelerating microjet, rather than the bubble wall itself, this microjet can reach velocities of hundreds of meters per second [11]. Response surface methodology (RSM) has been used to optimize food processing operations in recent years [14]. In this study, UAE parameters such as extraction temperature, extraction time and solution to solid ratio of ultrasound were optimized using response surface methodology (RSM), by employing a Box–Behnken design to maximize extraction of antioxidant polyphenolic compounds from Elaegnus angustifolia.

II. MATERIALS AND METHODS

Plant material

Elaegnus angustifolia were purchased from local markets. The purchased samples were milled into uniform dry powder by rondo and stored at 4 ˚C.

Chemicals

Quercetin and aluminium chloride were purchased from Sigma Chemical Co. (St., Louis, USA). All other organic solvents used in the study were analytical grade.

Ultrasound-assisted extraction:

The process of polyphenols and antioxidants extraction from Elaegnus angustifolia by ultrasonic was performed in an ultrasonic bath RK103H (BANDELIN SONOREX, Germany) with a maximum capacity of 4 L (35 KHZ, 140 W). Elaegnus angustifolia powder (1g) was sonicated in the solvent (20,35,50 ml) for different times at required temperature. After the extraction, the Elaegnus angustifolia extract centrifuged at 10000 rpm for 30 min. The extracts were concentrated by rotary evaporation at 40˚C under vacuum to dryness and the yield of extraction was determined.

Determination of total flavonoids:

The flavonoid content of each extract was determined according to Lamaison and Carnat, using a method based on the formation of a flavonoid-aluminium complex [3]. Different concentrations of quercetin (Sigma,USA) were used to make the calibration curve. One ml of each diluted sample was mixed with 1 ml of 2% AlCl3 methanol solution. Following incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using a UV-Vis spectrophotometer. The flavonoid contents of extracts were expressed as quercetin equivalents (QE)[2]. Quercetin was purchased from Sigma, USA.

β-Carotene–linoleic acid bleaching inhibition

The ability of the extract to inhibit the bleaching of the b-carotene–linoleic acid emulsion was determined using a modification of the method described by Koleva, van Beek, Linssen, de Groot, and Evstatieva (2002) [8]. In brief, 0.2 mg b-carotene dissolved in 1ml chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were transferred into a round-bottom flask. Once the chloroform had been removed under nitrogen, 50 ml distilled H_2O was added and the resulting mixture was vigorous stirred for 30 min. Thereafter, 5ml aliquots of this emulsion were transferred to tubes containing either 200 ml of dissolved extract (1 mg/ ml) or 200 ml of positive controls (1 mg/ml). After mixing, the absorbance (Abs_0) at 470 nm was recorded. The remaining samples were placed in a water bath at 50 1C for a period of 2 h. Thereafter, the absorbance of each sample was remeasured at 470nm (Abs_{120}). The data (n = 2) are presented as antioxidant activity % (AA%) values, calculated using :

$AA\% = [1- (Ab_0\text{sample} – Ab_{120}\text{sample}) / (Ab_0\text{control-} Ab_{120}\text{control}) \times 100]$

Experimental design and statistical analysis

A three-variable, three-level Box–Behnken design (BBD) was applied to optimizing the extraction condition in order to obtain the high oil recovery from the autoclaved almond powder [2].. The three independent variables set were extraction time (min, X1), extraction temperature (˚C, X2), and solvent/sample ratio (ml/g, X3), and each variable set at the three levels. A total of 17 experiments were designed (Table 1). Each experiment was performed in triplicate and the average oil recovery (%) was taken as the response, Y. Regression analysis was performed for the experiment data and was fitted into the empirical second order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta iXi + \sum_{i=1}^{3} \beta ii X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Where, β_0, βi, βii and βij are regression coefficients in the intercept, linear, quadratic, and interaction terms, respectively; Xi and Xj are the independent variables. A software Design-Expert 6.0.1 Trial (State-Ease, Inc., Minneapolis MN, USA) was used to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was checked by an F-test.

III. RESULTS AND DISCUSSION

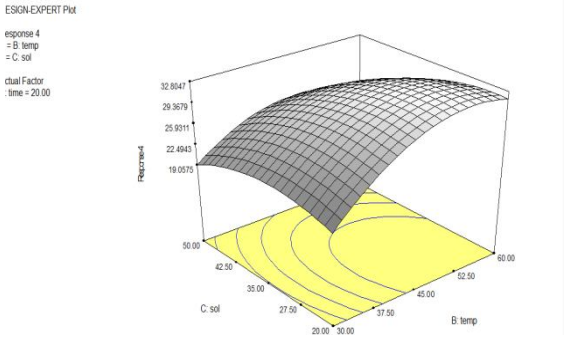
Optimization of ultrasonic assisted extraction

The extraction parameters were optimized using BBD combined with response surface methodology. The coded values, experimental and predicted values are given in Table 1 respectively. Among the 17 experiments including 5 replicates , the greatest total flavonoids content (32/21 mg quercetin /g dried plant) extraction temperature: 46/79 ˚C, extraction

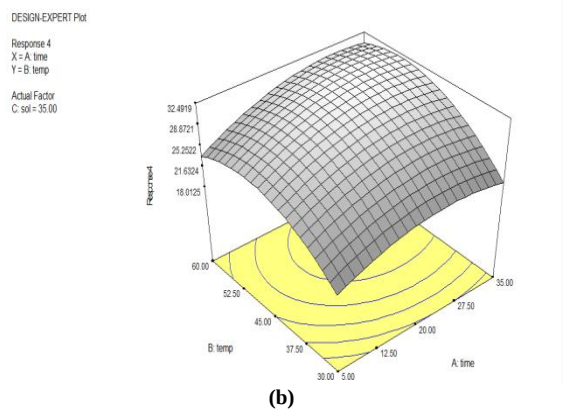
time: 31/13 min and solution to solid ratio: 32/71 ml/g (Fig1). Fitting of the data with various models and subsequent the analysis of variance (ANOVA) showed that total flavonoids content was most suitably described with quadratic polynomial model. The quadratic polynomial model was highly significant and sufficient to represent the actual relationship between the response and significant parameters with very low p-value (<0.0001 for flavonoids) from the ANOVA. The Model F-value of 32.95 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Lack of Fit F-value" of 6.04 implies there is a 5.75% chance that a "Lack of Fit F- value" this large could occur due to noise (Table 1).

Source	Flavonoids (R^2 = 0.9769)				
	DF	SS	MS	F_{value}	p_{value}
Model	3	177.17	59.06	42.17	0.0001
Lack of fit	3	8.03	2.68	6.04	0.0575
Pure error	4	1.77	0.44		

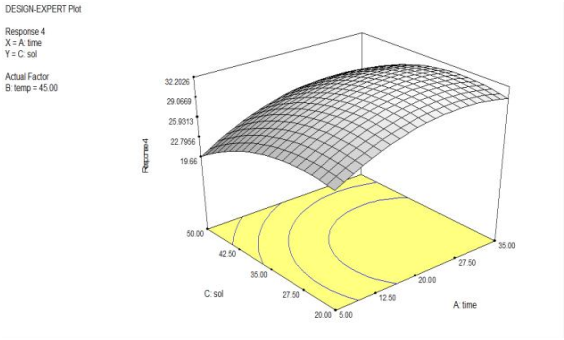
DF, degree of freedom; SS, sum of squares; MS, mean square



(a)



(b)



(c)

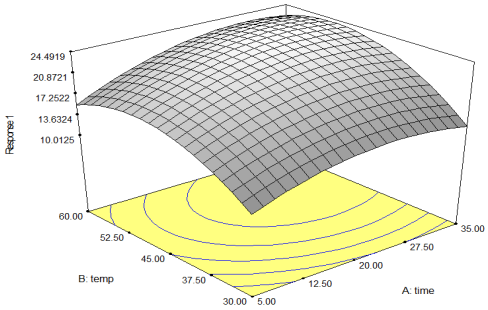
Fig 1 . Responses surface plots for the effect of (a) time/temperature, (b) temperature / solution to solid ratio, (c) time/ solution to solid ratio on total flavonoids

The Model F-value of 32.95 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Lack of Fit F-value" of 6.04 implies there is a 5.75% chance that a "Lack of Fit F- value" this large could occur due to noise (Table 2).

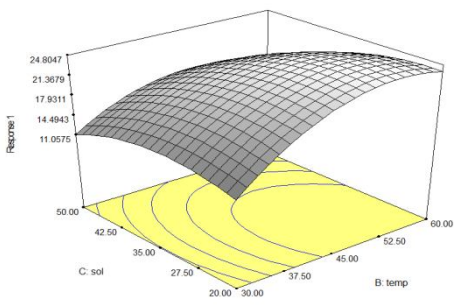
Table 2 - Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of extraction parameters.

Source	Beta –carotene/linoleic (R^2 = 0.9769)				
	DF	SS	MS	F_{value}	p_{value}
Model	3	177.17	59.06	42.17	0.0001
Lack of fit	3	8.03	2.68	6.04	0.0575
Pure error	4	1.77	0.44		

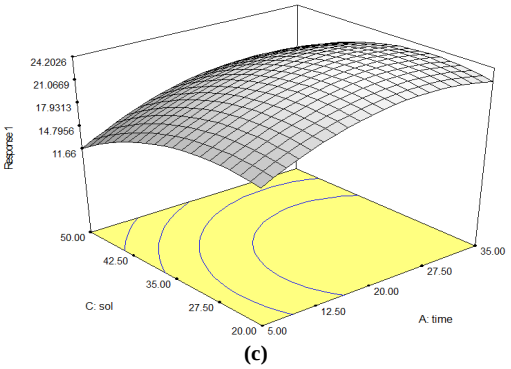
DF, degree of freedom; SS, sum of squares; MS, mean square



(a)



(b)



(c)

Fig 2 . Responses surface plots for the effect of (a) time/temperature, (b) temperature / solution to solid ratio, (c) time/ solution to solid ratio on total beta –carotene/linoleic

CONCLUSIONS

In the present paper, the ultrasound-assisted Flavonoid extraction of Elaegnus angustifolia almond powder was performed with a three-variable, three-level Box–Behnken design (BBD) based on the RSM. The experiment results showed that the extraction temperature was the major contributing factor to the Flavonoid extraction. It was revealed that the ultrasonic and autoclaving pretreatment did not affect the composition of the almond, but the ultrasonic cavitating energy could cause structure breakage of the almond powder, and greatly reduced the extraction time. Elaegnus angustifolia is one of the most abundant co-products produced in the rice milling industry. Elaegnus angustifolia could be a good commercial source of Glycolipid, phospholipid, phosphatidylinositol, Phosphatidylcholine, Oxalic, Tartaric, Malick Lactic, Citric, Ascorbic acid and they can be separated and concentrated through extraction processbe separated and concentrated through extraction process [16].The extract can be used as substitute of synthetic antioxidants for food products, stabilization of fats, frying oils and fried products [16], stabilization of cookies under ambient storage [17], stabilization and development of functional foods such as low-heat whole milk powder [16]. In this study, ultrasonic technology was used for extraction of the polyphenols and antioxidants from Elaegnus angustifolia. Response surface methodology (RSM) was used to optimize the experimental variables such as solution to solid ratio (ml/g), extraction temperature(°C) and extract time (min). Therefore, ultrasound-assisted extraction of antioxidants from Elaegnus angustifolia is an environment-friendly for the preparation of extracts rich in natural antioxidants aimed at replacing synthetic antioxidants.

REFERENCES

[1]. B. Ou, M. Hampsch-Woodill, J. Flanagan, E.K. Deemer, R.L. Prior, D. Huang, J. Agric. Food Chem. 50 (2002) 2772–2777.

[2]. Box, G. E. P., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. Journal of the Royal Statistical Society: Series B, 13, 1–45.

[3]. Chahar MK, Sharma N, Dobhal MP, Joshi YC (2011). Flavonoids: a versatile source of anticancer drugs. Pharmacogn. Rev., 5: 1-12.

[4]. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. (2006). Antioxidant activity of some algerian medicinal plants extract containing phenolic compounds . Food Chem., 97, 654- 660.

[5]. Gescher A, Pastorino U, Plummer SM, Manson MM (1998). Suppression of tumour development by substances derived from the diet—mechanisms and clinical implications. Br. J. Clin. Pharmacol., 45: 1-12.

[6]. I.F.F. Benzie, J.J. Strain, Anal. Biochem. 239 (1996) 70–76

[7]. J.L. Luque-Garcı´ a, M.D. Luque de Castro, Trends Anal. Chem. 22 (2003) 41–47.

[8]. Koleva, I. I., van Beek, T. A., Linssen, J. P. H., de Groot, A., &Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochemical Analysis, 13, 8–17.

[9]. K.Z. Brainina, A.V. Ivanova, E.N. Sharafutdinova, E.L. Lozovskaya, E.I. Shkarina, Talanta 71 (2007) 13–18.

[10]. L.A. Sayre, G. Perry, M.A. Smith, Chem. Res. Toxicol. 21 (2008) 172–188.

[11]. L. Wolloch, J. Kost, J. Control. Release 148 (2010) 204–211.

[12]. M.K. Khan, M. Abert-Vian, A.S. Fabiano-Tixier, O. Dangles, F. Chemat, Food Chem. 119 (2010) 851–858.

[13]. M. Vinatoru, Ultrason. Sonochem. 8 (2001) 303–313.

[14]. M.B. Hossain, C. Barry-Rayan, A.B. Martin-Diana, N.P. Brunton, Food Chem. 126 (2011) 339–346.

[15]. M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar, L.A. Escaleira, Talanta 76 (2008) 965–977.

[16]. M.J. Lerma-Garcia, J.M. Herrero-Martinez, E.F. Simo-Alfonso, C.R.B. Mendoca,

[17]. G. Ramis-Ramos, Composition, industrial processing and application of rice bran–oryzanol, Food Chem. 115 (2009) 389–404.

[18]. M.I. Bhanger, S. Iqbal, F. Anwar, M. Imran, M. Akhtar, M. Zia-ul-Haq, Antioxidant potential of rice bran extracts and its effects on stabilization of cookies under ambient storage, Int. J. Food Sci. Technol. 43 (2008) 779–786.

[19]. Ren W, Qiao Z, Wang H, Zhu L, Zhang L (2003). Flavonoids: promising anticancer agents. Med. Res. Rev., 23: 519-534.

[20]. R.P. Singh, K.N. Chidambara Murthy, G.K. Jayaprakasha, J. Agric. Food Chem. 50 (2002) 81–86.

[21]. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radical Biol. Med. 26 (1999) 1231–1237.

[22]. Sugamura K, Keaney JJF (2011). Reactive oxygen species in cardiovascular disease. Free Radic. Biol. Med., 51: 978-992.

[23]. Uttara B, Singh AV, Zamboni P, Mahajan RT (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol., 7: 65-74.

[24]. W. Brand-Williams, M.E. Cuvelier, C. Berset, LWT – Food Sci. Technol. 28 (1995) 25–30.

[25]. Z. Pan, W. Qu, H. Ma, G.G. Atungulu, T.H. McHugh, Ultrason. Sonochem. 18(2011) 1249–1257.

★★★