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Optimization of Phenolic Compounds and Antioxidant Activity of Ultrasound Assisted Extraction of Red Beet

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ABSTRACT

The red beet (*Beta vulgaris* L.) root is a significant botanical raw material with proven positive effects on the human body. This plant contains a substantial number of antioxidants, vitamins, fiber, and natural pigments. Additionally, it is rich in phenolic compounds with antioxidant properties. This study investigated the influence of pH (3-5), temperature (15-25 °C), and extraction time (10-20 minutes) on the concentration of soluble solids, phenolic compounds, and antioxidant activity of the red beet root extract obtained. The extraction process was performed using water: ethanol (1:1 ratio) mixture. Ultrasonic waves with an intensity of 75% and a frequency of 37 kHz were employed to facilitate the extraction process. Design Expert software and response surface optimization method were used for analysis. The results indicated that an increase in pH and extraction time led to higher concentrations of total soluble solids, phenols, and antioxidant activity. Moreover, elevating the temperature up to 20°C increased these responses, but temperatures exceeding 20°C resulted in a decline in these values. The experimental results obtained at optimal point suggested by the software did not show a significant difference compared to the predicted results by the model ($p < 0.05$).

1- Introduction

Red beet (*Beta vulgaris* L.) belongs to the Chenopodiacea family, which is primarily found along the coasts of the Atlantic Ocean and the Mediterranean [1]. Sugar beets contain betalains which are associated with cationic antioxidants. These compounds are water-soluble nitrogenous compounds found in cellular extracts, classified into two groups: red betacyanins and yellow betaxanthins. Betalains possess antioxidant properties and the ability to neutralize free radicals. The antioxidant property of betacyanins is attributed to their free phenolic groups. Furthermore, the betalamic acid ring amino group acts as a hydrogen donor in betacyanins and betaxanthins [2]. Among the diverse plant species, red beetroot stands out as one of the ten plants with the highest antioxidant potential, attributed to the structural characteristics of betalains and phenolic compounds[3]. Phenolic compounds include phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols, and flavonoids[4]. Phenolic compounds, along with other antioxidants found in fruits and vegetables, can neutralize free radicals, playing a crucial role in preventing specific diseases[5].

The extraction process of phenolic compounds and antioxidants is influenced by the chemical nature of the compounds, the employed extraction method, the duration and conditions of storage, and interfering substances. Several factors affect the solvent-based extraction process, including the solvent type, pH, temperature, extraction stages, solvent-to-solid ratio, and the size of solid matrix particles[6]. Antioxidant compounds are commonly obtained from plant sources through solid–liquid extraction using organic solvents and conventional methods. However, techniques that reduce extraction time, energy consumption, and solvent volume such as ohmic heating, ultrasonication, microwave-assisted extraction, and high-pressure extraction have attracted increasing attention due to their greater alignment with green extraction principles [7-9].

Ultrasonication, due to cavitation phenomena, results in the disruption of solid matrix cell walls, solvent penetration, mass transfer, and consequently, an increase in extraction rate[3, 10, 11].

Da Silva et al. (2018) investigated the ultrasonic-assisted extraction of red beetroot to assess the process variables (temperature, time, and solvent-to-water ratio) effects on the obtained extract using a Box-Behnken experimental design. They aimed to determine optimal extraction conditions. The optimal extraction conditions for betacyanins and betaxanthins were found to be a temperature of 52 and 37 °C, a time of 90 minutes, and the use of a 25% ethanol solution in water, respectively. The extract obtained through ultrasonic extraction exhibited higher phenolic content and antioxidant activity compared to extracts obtained through conventional methods[3]. Kushwaha et al. (2018) extracted betalains, phenolic compounds, and antioxidants from beetroot using various experimental variables such as solid-to-liquid ratio, temperature, time, and pH, employing response surface optimization. The results indicated that the

optimal conditions for the extraction of betalains and other phytochemicals were a solid-to-liquid ratio of 1:15, a temperature of 50.04°C, a time of 10 minutes, and a pH of 2.50 [12].

Considering the affordability of red beetroot cultivation in Iran and the richness of red beetroot extract in phenolic compounds and other antioxidants, this study aimed to investigate the effect of process parameters, including temperature, time, and pH, on the concentration of soluble solids, phenolic content, and antioxidant capacity of the extracted red beetroot extract. Furthermore, ultrasonic waves with an intensity of 75% and a frequency of 37 kHz were utilized to facilitate the extraction process in this study.

2- Materials and Methods

2.1 Materials

The used materials included red beetroots purchased from the local market in Isfahan, phenol (Merck, Germany), Folin-Ciocalteu reagent (Merck, Germany), sodium carbonate (Merck, Germany), gallic acid (Sigma, India), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, India).

2.2 Preparation of red beetroot

To prepare plant material, red beetroots were washed with water to remove impurities. After peeling and grating, the samples were air-dried in an oven at 30 °C for 48 hours. Subsequently, the dried beetroot powder was milled, and the preparation for juice extraction was carried out. For the extraction, the obtained powder (10 g) was mixed with 200 mL of water: ethanol solvent at a 1:1 ratio. The mixture underwent ultrasonication for 10, 15, and 20 minutes at three different temperatures (15, 20, and 25 °C) and three pH levels (3, 4, and 5). Ultrasonication was performed using a probe with a diameter of 7 mm, an intensity of 75%, and a frequency of 37 kHz. The residue was then separated using Whatman No 1 filter paper and dried in an oven at 35 °C. The resulting powder was used for subsequent analyses [13].

2.3 Methods

2.3.1 Measurement of soluble solids content

Before drying the beetroot extracts, the amount of soluble solids content was measured using a refractometer. The results were reported in degrees Brix[14].

2.3.2 Measurement of phenolic compounds in the extract

The folin-Ciocalteu method was employed to measure phenolic compounds. Hence, 1 mL of the obtained extract was mixed with 0.5, 1, and 7.5 mL of Folin-Ciocalteu solution, 20% sodium carbonate, and distilled water, respectively. The mixture was vortexed for a few seconds. After 30 minutes of incubation at room temperature, the absorbance of the samples was read at 760 nm. The results were expressed in mg (gallic acid). mL^{-1} (extract) [15]. To determine the phenolic compound content in the red beetroot extract, its standard curve should be established based on gallic acid (Figure 1).

Fig (1) Standard graph for phenolic compounds according to gallic acid equivalent (mg/ml)

2.3.3 Measurement of free radical scavenging activity

The antioxidant activity of the extract was measured using the DPPH free radical scavenging method. Accordingly, 0.1 mL of the extract was added to 3.9 mL of DPPH solution (concentration: 0.060 mMol) and vortexed for 30 seconds. The resulting mixture was left in the dark for 30 minutes to complete the reaction. Subsequently, the absorbance at 515 nm was read. Notably, the control sample was the same methanolic DPPH solution without adding the extract. Ultimately, the percentage of free radical scavenging was reported based on the calculation formula and presented as a percentage[16].

Eq. (1)

$$\text{Antioxidant activity percentage} = Aa - \{\frac{100}{Aa} * (Ab - Ac)\}$$

Where Aa is the adsorption of DPPH solution without sample, Ab is the adsorption of the solution containing DPPH and sample, and Ac is the absorption of the control solution without sample.

2.4 Statistical population and data analysis method

For the statistical analysis of the data, as mentioned in the study by Lovarestagh et al. (2024), Design Expert software version 7 and the response surface optimization method were employed for data analysis. Historical data design in 29 standard run was used to analyze the data. The studied variables level and used treatments in the experiment are presented in Tables 1 and 2, respectively. After optimizing the conditions based on the obtained optimal points for maximum level of responses in the process, the extraction was performed again. The factors investigated for the optimal formulations were outlined in Table 3. A comparison between the optimal response and the predicted response by the software was conducted using the Student's t-test ($p < 0.05$). To minimize errors, all experiments were conducted in three replicates[17].

Table (1) The real and coded values of independent variables of process

Table (2) Experimental design of independent variables of red beet extraction

Table (3) Process parameter levels for the optimal point

3-Results and Discussion

The response surface methodology is a set of statistical techniques used to optimize processes influenced by several variables affecting the desired response. Design Expert program was used to determine the best-proposed model among various existing models (including mean, cubic, quadratic, two-factor interaction (2FI), and linear). Accordingly, the model with significant differences in the sum of squares and non-significant lack of fit was selected as the best model. Accordingly, results revealed that the quadratic model was significant for all measured responses in this study.

3.1 Total soluble solids (TSS)

The proposed model based on actual experimental data, was a quadratic polynomial equation which refers to (A) pH, (B) temperature, and (C) time.

Eq. (2)

$$TSS = 12.60 - 0.30A + 0.66B - 1.81C + 0.04AB + 0.05AC + 3.26 \times 10^{-3}BC + 1.26 \times 10^{-3}A^2 - 0.02B^2 + 0.06C^2$$

(R^2=0.91)

Table 4 presents the analysis of variance (ANOVA) results for evaluating the response parameter of the total soluble solids in red beet extract concerning the three-dimensional model with 2FIs. The pH and time parameters significantly influenced the response of total soluble solids, while temperature had no effect. The P-values for the 2FIs of AC, BC, and AB were not significant. The lack of fit value was insignificant ($p > 0.05$). In other words, the model fits the experimental data. The coefficient of determination or explanation (R^2) value was 0.9050, indicating a high agreement between the model results and experimental results. The adjusted R^2 in this model was 0.86, indicating that only 0.14% of the total variables were not justified by the unadjusted model. The predicted R^2 in this model was also 0.72, which did not have a good agreement with the adjusted R^2.

Table (4) Analysis of variance of total soluble solids

Figure 2(a) Shows the effect of pH on TSS while the other parameters are considered intermediate (temperature: 20°C and time: 15 minutes). According to ANOVA results, increased pH has led to a significant enhancement in the TSS content. Besides, Figure 2(b) displays the effect of temperature on TSS while the other parameters are considered intermediate (pH: 4 and time: 15 minutes). Increasing the temperature to 20°C resulted in a significant rise in the TSS content, but beyond 20°C, its value decreased.

Furthermore, Figure (2) represents the TSS as a response to the parameter of time, while pH and temperature were considered intermediate (pH: 4 and temperature: 20°C). As observed, an increase in time led to a significant increase in the total soluble solids.

Fig (2)The amount of soluble solids in terms of parameters (A) pH, (B) Temperature and (C) Time

Figure 3(a) illustrates the 2FI of temperature and time on the TSS content at a constant pH of 3. As the temperature increases up to 20 °C, the TSS content increases. However, beyond 20 °C, there is a decrease in TSS. Simultaneously, an increase in extraction time results in an overall increase in the mentioned compound content. Moreover, the 2FI of temperature and time at a constant pH of 4 is depicted in Figure 3(b). The maximum amount of TSS was observed at 20 °C and an extraction time of 20 minutes. Similarly, Figure 3(c) illustrates the 2FI of temperature and time at a constant pH of 5. An increase in temperature up to 20 °C, along with an extension of the extraction time to 20 minutes, leads to enhanced TSS content. However, beyond 20 °C, an increase in temperature results in a reduction in these compounds content.

Fig (3) Interaction of temperature and time on soluble solids at pH 3, 4 and 5

Figure 4(a) demonstrates the 2FI of pH and time on the TSS content at a constant

temperature of 15 °C. Accordingly, an increase in both pH and time leads to an overall upsurge in the TSS content. Besides, Figure 4(b) depicts the 2FI of pH and time at a constant temperature of 20 °C. The increased pH and time resulted in a noticeable increase in the TSS content at a constant temperature of 20 °C. The 2FI of pH and time on the TSS content at a constant temperature of 25 °C is shown in Figure 4(c). As observed, the highest TSS level corresponded to a pH of 5 and a time of 20 minutes.

Fig (4)Interaction of pH and time on soluble solids at temperatures 15, 20 and 25

Furthermore, Figure 5(a) represents the 2FI of pH and temperature on the TSS content at a constant time of 10 minutes. Increasing pH from 3 to 5 and raising the temperature from 15 to 20 °C lead to a higher TSS content. However, beyond 20 °C, a decrease in these compounds content was observed. In Figure 5(b), the 2FI of pH and temperature on TSS content at a constant time of 15 minutes is displayed. Accordingly, enhanced pH has led to a higher TSS content. This Figure shows an ascending trend from a temperature of 15 to 20 °C at a constant time of 15 minutes, but beyond 20 °C, a descending trend was observed. The 2FI of pH and temperature on the TSS content at a constant time of 20 minutes is depicted in Figure 5(c). Accordingly, with enhancing pH value to 5 and temperature to 20 °C, the TSS content increases, but beyond 20 °C, it undergoes a reduction.

Fig (5) Interaction of pH and temperature on soluble solids at 10, 15 and 20 min

3-2 Total phenolic compounds

Table 5 presents the ANOVA results for evaluating the response parameter of total phenolic content in red beet regarding the quadratic model with 2FIs. Accordingly, pH, time, and temperature parameters significantly influenced the total phenolic content in red beet. The 2FIs of pH and temperature, as well as pH and time, did not have a significant impact on the model. Quadratic terms A^2, B^2, and C^2 had F-values less than 0.05, indicating a significant effect on the model. The lack of fit in this model was not significant ($p > 0.05$), indicating a satisfactory fit of the model to the experimental data. In other words, the model successfully fitted the experimental data. The R^2 was 0.99, indicating a close match between the model results and the experimental results. The adjusted R^2 in this model was 0.98, suggesting that only 2% of the total variables were not justified by the model. The predicted R^2 in this model was 0.97, consistent with the obtained adjusted R^2. The final proposed model, based on actual experimental data, was presented as a second-order polynomial equation in which total phenolic content (TPC) was considered the response variable, and pH (A), temperature (B), and time (C) were taken as the independent variables.

Eq. (3)

$$phenol = -300.46 + 82.07A + 32.93B + 4.92C + 0.18AB - 0.25AC - 0.04BC - 6.04A^2 - 0.78B^2 - 0.04C^2$$

(R^2=0.99)

Table (5)Analysis of variance of total phenolic compound

The total phenolic content as a function of the pH parameter is shown in Figure 6(a). Other parameters are considered

intermediate (temperature: 20 °C and time: 15 minutes). As observed, the pH parameter significantly influenced the total phenolic content in red beet. Furthermore, Figure 6(b) illustrates the effect of the temperature parameter on the total phenolic content (pH parameter: 4, and time: 15 minutes), showing a significant effect of increasing temperature up to 20 °C but a decrease beyond 20 °C. Figure 6(c) also displays the total phenolic content in terms of the time parameter (pH parameter set to 4, and temperature set to 20 °C). According to ANOVA results, increased time had a significant effect on the total phenolic content.

Fig (6)The amount of total phenolic compounds in terms of parameters (A) pH, (B) Temperature and (C) Time

Figure 7(a) illustrates the 2FI of temperature and time on the total phenolic content at a constant pH of 3. The highest total phenolic content was observed at a temperature of 20 °C, while the lowest amount was recorded at temperatures of 15 and 25 °C. Increasing the extraction time also resulted in an upward trend in the total phenolic content. Furthermore, the 2FI of temperature and time on the total phenolic content at a constant pH of 4 is shown (Figure 7(b). Accordingly, the enhanced time and temperature up to 20 °C has led to higher total phenolic content, but beyond 20 °C, there was a decrease. Figure 7(c) displays the 2FI of temperature and time at a constant pH of 5. With an increase in time, the total phenolic content also increased, with the highest amount obtained at a temperature of 20 °C.

Fig (7) Interaction of temperature and time on total phenolic compounds at pH 3, 4 and 5

Figure 8(a) depicts the 2FI of pH and time on the total phenolic content at a constant temperature of 15 °C. As observed, an increase in pH and duration resulted in the total phenolic content enhancement. The 2FI of pH and time on the total phenolic content at a constant temperature of 20 °C (Figure 8b) also demonstrates that these two factors at higher levels led to an increase in the total phenolic content. Figure 8(c) depicts the 2FI of pH and time on the total phenolic content at a constant temperature of 25 °C. Increasing the pH value from 3 to 5 and extending the time from 10 to 20 minutes led to an increase in the total phenol content.

Fig (8) Interaction of pH and time on total phenolic compounds at temperatures 15, 20 and 25°C

Figure 9(a) shows the 2FI of pH and temperature on the total phenolic content at a constant time of 10 minutes. As observed, an increase in pH and temperature up to 20 °C improved the total phenolic content. Figure 9(b) elucidates the 2FI of pH and temperature on the total phenolic content at a constant time of 15 minutes, displaying that an increase in pH and temperature up to 20 °C led to a higher total phenolic content. Besides, Figure 9(c) displays the 2FI of pH and temperature on the total phenolic content at a constant time of 20 minutes, revealing that the highest total phenolic content was observed at a pH equal to 5 and a temperature of 20 °C.

Fig (9)Interaction of pH and temperature on total phenolic compounds at 10, 15 and 20 min

The extraction of phenolic compounds from plant matrices is influenced by

various factors such as their chemical nature, extraction method, particle size, extraction time, storage conditions, and interfering substances. These compounds may contain different hydroxyl groups that can be associated with sugars, acids, or alkyl groups. Consequently, the polarity of phenolic compounds varies significantly, making the optimization of a single method for the effective extraction of phenolic compounds challenging. Therefore, optimizing the extraction process is crucial for the accurate evaluation of phenolic compounds from various food matrices [18]. Acidic conditions in the extraction of phenolic compounds act through various mechanisms. The first method involves enhancing the stability of phenolic compounds such as anthocyanins. The second technique involves dissolving phenolic compounds through hydrolysis mechanisms, initially present as part of polymers or bonded to cell wall components. Finally, the third one involves the degradation of cell walls, facilitating the solubility and release of phenolic compounds from plant materials [12]. Results indicated that an increase in temperature up to 20 °C caused the higher phenolic compounds production, while temperatures above 20 °C resulted in a reduction. The initial increase in phenolic compounds with temperature can be attributed to the softening of plant tissues and disruption of interactions between phenolic compounds and proteins or carbohydrates, thus improving the rate of release [19]. However, it is noteworthy that an excessive increase in extraction temperature may lead to the degradation of previously mobilized phenolic compounds or even the decomposition of residual phenols in the plant matrix. Additionally, high temperatures may reduce solvent levels through evaporation, increasing extraction costs from an industrial perspective[18].Time consistently increases the amount of extracted compounds since sufficient time is required to create cavities in the beet cell membranes, facilitating easier extraction. Therefore, in this study, an extended extraction time resulted in a higher extraction yield.

3.3 Free radical scavenging activity

The proposed model, based on actual experimental data, for predicting the level of free radical scavenging activity is given by the following quadratic equation:

Eq. (4)

$$DPPH = -7.88 + 1.15A + 1.74B - 0.88C + 0.03AB + 0.08AC + 7.80 \times 10^{-3}BC - 0.18A^2 - 0.04B^2 + 0.04C^2$$

(R^2=0.99)

Table (6) presents the ANOVA results to evaluate the antioxidant parameter of red beet regarding the quadratic model with 2FIs. Accordingly, parameters such as pH, time, and temperature significantly influenced the level of free radical scavenging activity in red beets. The lack of fit for this model was not significant ($p > 0.05$). In other words, the model adequately fits the experimental data. Besides, R^2 was 0.99, indicating a high alignment of the model results with the experimental ones. The adjusted R^2 in this model was 0.99, demonstrating that only 1% of the total variables were left unexplained by the model. The predicted R^2 in this model was 0.98, consistent with the adjusted R^2.

Table (6) Analysis of variance of free radical inhibition

Figure 10(a) depicts the level of free radical scavenging activity as a function of the pH parameter. Other parameters were considered at an intermediate level (temperature: 20 °C, and time: 15 minutes). According to the figure and ANOVA results, the pH parameter led to a significant increase in free radical scavenging activity. Figure 10 (b) shows the response of the free radical scavenging activity to the temperature parameter, with other parameters at an intermediate level (pH: 4, and time:15 minutes). An increase in temperature up to 20 °C resulted in a significant increase in free radical scavenging activity, but beyond 20 °C, the antioxidant activity decreased. Additionally, Figure 10 (c) displays the response of free radical scavenging activity to the time parameter, with other parameters at an intermediate level (pH: 4, and temperature: 20 °C). An increase in time from 10 to 20 minutes led to a significant increase in free radical scavenging activity.

Fig (10) The amount of free radical inhibition in terms of parameters (A) pH, (B) Temperature and (C) Time

Figure.11 (a) illustrates the 2FI of temperature and time on the level of free radical scavenging activity at a constant pH of 3. Accordingly, increasing the time from 10 to 20 minutes and the temperature from 15 to 25 °C led to a higher free radical scavenging activity. Figure. 11 (b) displays the 2FI of temperature and time at a constant pH of 4, indicating an ascending trend in free radical scavenging activity with a rise in temperature up to 20 °C, followed by a descending trend beyond 20 °C. The 2FI of temperature and time at a constant pH of 5 is presented in Figure 11 (c), demonstrating that enhancing time and temperature up to 20 °C improved the free radical scavenging activity.

Fig (11) Interaction of temperature and time on free radical inhibition at pH 3, 4 and 5

Furthermore, the 2FI of pH and time on the free radicals scavenging at a constant temperature of 15 °C is depicted in Figure 12 (a). At higher pH and time values, this parameter is also enhanced. Similarly, 2FI of pH and time on the free radicals scavenging at a constant temperature of 20 °C is illustrated in Figure 12 (b), showing an increased scavenging ability at higher pH and time values.

Figure 12 (c) demonstrates 2FI of pH and time on the same parameter at a constant temperature of 25 °C, with the highest scavenging observed at 20 minutes and pH 5.

Fig (12) Interaction of pH and time on free radical inhibition at temperatures 15, 20 and 25ºC

Figure 13 (a) displays 2FI of pH and temperature on the free radicals scavenging at a constant time of 10 minutes. Accordingly, enhancing pH value from 3 to 5, and temperature from 15 to 20 °C has led to higher free radicals scavenging. Besides, the 2FI of pH and temperature on the free radicals scavenging at a constant time of 15 minutes was presented in Figure 13 (b). Accordingly, an increase in pH resulted in the higher free radicals scavenging. While higher temperatures up to 20 °C enhanced the scavenging, beyond 20 °C, the scavenging decreases with temperature rise. In Figure 13 (c), the 2FI of pH and temperature on the free radicals scavenging at a constant time of 20 minutes is shown, with the highest value

observed at pH 5 and a temperature of 20 °C.

Fig (13)Interaction of pH and temperature on free radical inhibition at 10, 15and 20 min

Our results indicated that enhanced time significantly affected antioxidant activity and a temperature of 20°C improved antioxidant activity. Chirinos et al (2007) also observed a significant increase in antioxidant activity with prolonged extraction time. Furthermore, improved antioxidant activities were observed at higher pH as antioxidants exhibit higher activity in neutral and basic pH conditions [6]. Brunet et al (2011) documented a significant correlation between the content of phenolic compounds and betacyanins in sugar beet roots with antioxidant activity. The antioxidant activity of phenolic compounds depends on their molecular structure, such as the availability of phenolic hydrogen and the stability of phenoxy radicals formed by hydrogen donation [5]. Therefore, based on the results obtained, it can be claimed that increased pH and time can result in higher phenolic compounds and, consequently, an increase in antioxidant activity. In this regard, Alousman et al (2009) found a high correlation between the phenolic compounds content and antioxidant activity [20].

3-4. Model Validation

The average soluble solids, total phenols, and free radical scavenging under optimal conditions are shown in Tables 7. A comparison between the predicted and experimental reaction values at the proposed optimum point, performed using Student's t-test (at a significance level of 5%), and showed a very good correlation between the results.

Table (7)Predicted soluble solids, phenolic compounds and Free radical scavenging activity and experimental data obtained at optimum point

4-Conclusion

Our results demonstrated that increased sample pH can enhance soluble solids content, phenolic compounds, and antioxidant activity. In addition, extending the extraction time can lead to an increase in these responses, as there is sufficient time to create cavities in the cell membranes of the sugar beet and facilitate the extraction process. In addition, an increase in temperature up to 20 °C increases the values of total soluble solids, phenolic compounds, and antioxidant activity. However, these values decrease at temperatures above 20 °C.

5-References

[1] Y. Cai, M. Sun, and H. Corke, "Antioxidant activity of betalains from plants of the Amaranthaceae," *Journal of agricultural and food chemistry,* vol. 51, no. 8, pp. 2288-2294, 2003.

[2] J. Czapski, K. Mikołajczyk, and M. Kaczmarek, "Relationship between antioxidant capacity of red beet juice and contents of its betalain pigments," *Polish journal of food and nutrition sciences,* vol. 59, no. 2, 2009.

[3] H. Righi Pessoa da Silva, C. da Silva, and B. C. Bolanho, "Ultrasonic-assisted extraction of betalains from red beet (Beta vulgaris L.)," *Journal of food process engineering,* vol. 41, no. 6, p. e12833, 2018.

[4] Q. D. Do *et al.*, "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica," *Journal of food and drug analysis,* vol. 22, no. 3, pp. 296-302, 2014.

[5] J. M. Čanadanović-Brunet *et al.*, "Antioxidant and antimicrobial activities of beet root pomace extracts," *Czech journal of food sciences,* vol. 29, no. 6, p. 575, 2011.

[6] R. Chirinos, H. Rogez, D. Campos, R. Pedreschi, and Y. Larondelle, "Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers," *Separation and Purification Technology,* vol. 55, no. 2, pp. 217.٢٠٠٧ ,٢٢٥-
[7] M. Keshani and R. Hajian, "Effect of immersion ohmic heating on thawing rate and properties of frozen tuna fish," 2020.
[8] N. Z. Maryam Keshani, Leila Lakzadeh,Mohammad Goli, "Optimizing the foaming capacity and stability of extracted sesame flour protein using ohmic
[9] method," presented at the Iran's 5th conference on Protein & Peptide Science, University of Tehran, 2024, 2024.
[10] M. Keshani, N. Zamindar, and R. Hajian, "Physicochemical properties of frozen tuna fish as affected by immersion ohmic thawing and conventional thawing," *Food Science and Technology International,* vol. 28, no. 8, pp. 728-734, 2022.
[11] F. Barati, N. Zamindar, and S. Rafiaei, "The Study of Kinetics of Polyphenol Oxidase Inactivation in Carrot Juice by Ohmic Heating," *Iranian journal of food science and industry,* vol. 21, no. 153, 2024.
[12] S. Rafiaei and N. Zamindar, "Investigating the effect of ohmic and water bath heating on surviving of Escherichia coli," *Journal of food science and technology (Iran),* vol. 2 ,٢no. 158, pp. 50-62, 2025.
[13]]R. Kushwaha, V. Kumar G. Vyas, and J. Kaur, "Optimization of different variable for eco-friendly extraction of betalains and phytochemicals from beetroot pomace," *Waste and Biomass Valorization,* vol. 9, no. 9, pp. 1485-1.٢٠١٨ ,٤٩٤
[14] D. R. De Sotillo, M. Hadley, and E. Holm, "Phenolics in aqueous potato peel extract: extraction, identification and degradation," *Journal of Food Science,* vol. 59, no. 3, pp. 649-651, 1994.
[15] A. o. O. A. Chemists and A. o. O. A. Chemists, *Official methods of analysis of the Association of Official Analytical Chemists*. Association of Official Analytical Chemists., 1920.
[16] C.-C. Chang, M.-H. Yang, H.-M. Wen, and J.-C. Chern, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *Journal of food and drug analysis,* vol. 10, no. 3, 2002.
[17] K. M. Herbach, F. C. Stintzing, and R. Carle, "Betalain stability and degradation—structural and chromatic aspects," *Journal of food science,* vol. 71, no. 4, pp. R41-R50, 2006.
[18] M. Lavarestagh, N. Zamindar, and Y. Esmaeili, "Optimization of Pigment Extraction from Red Beet," *Optimization,* vol. 20, no. 4, pp. 381-393, 2024.
[19] A. Mokrani and K. Madani, "Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit," *Separation and purification Technology,* vol. 162, pp. 68-76, 2016.
[20] G. J. Swamy, A. Sangamithra, and V. Chandrasekar, "Response surface modeling and process optimization of aqueous extraction of natural pigments from Beta vulgaris using Box–Behnken design of experiments," *Dyes and Pigments,* vol. 111, pp. 64-74, 2014.
[21] M. Alothman, R. Bhat, and A. Karim, "Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents," *Food chemistry,* vol. 115, no. 3, pp. 785-788, 2009.

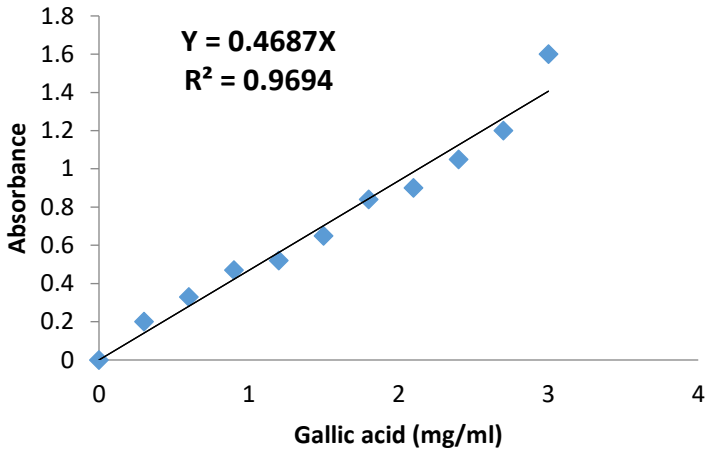
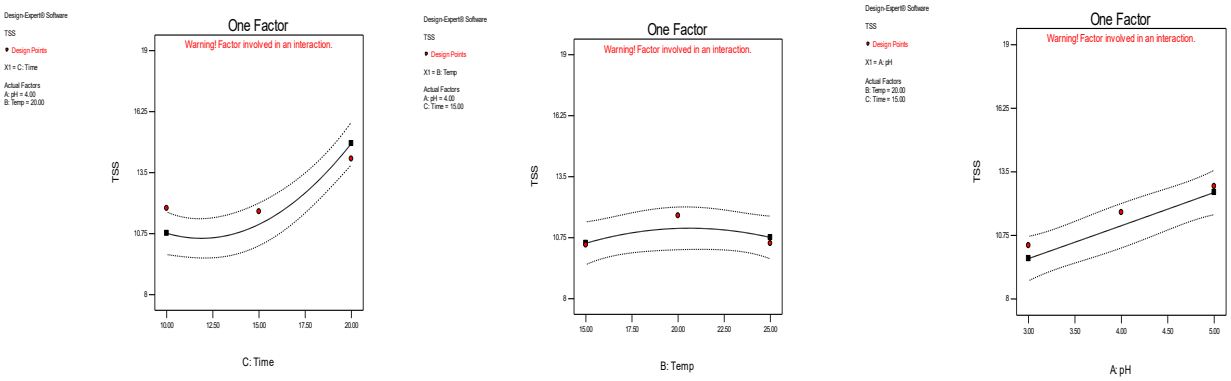


Fig (1) Standard graph for phenolic compounds according to gallic acid equivalent (mg/ml)



(C) (B) (A)

Fig (2)The amount of soluble solids in terms of parameters (A) pH, (B) Temperature (C) Time

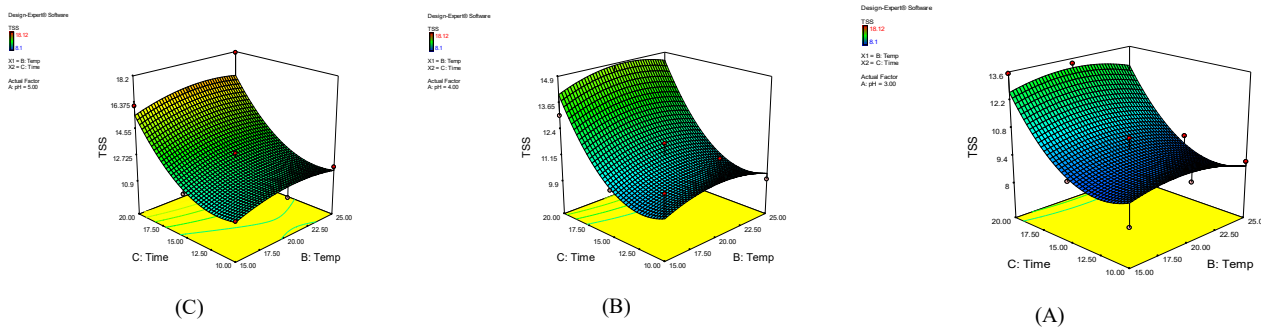


Fig (3) Interaction of temperature and time on soluble solids constant pH 3, 4 and 5

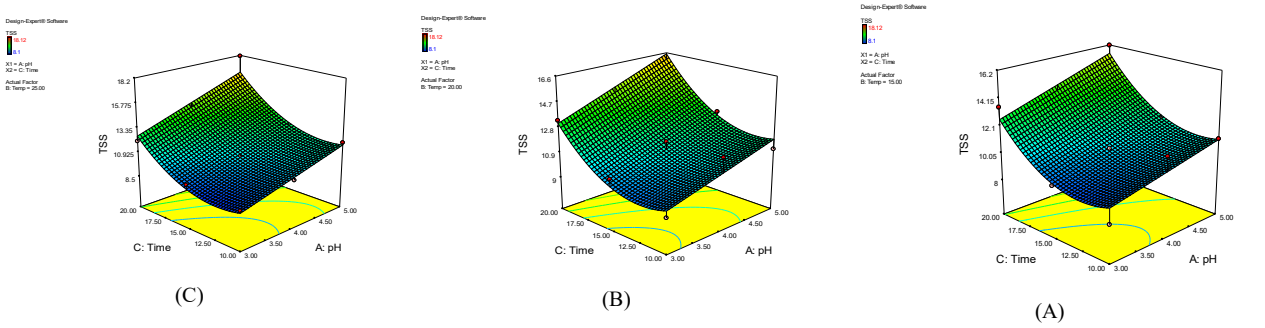
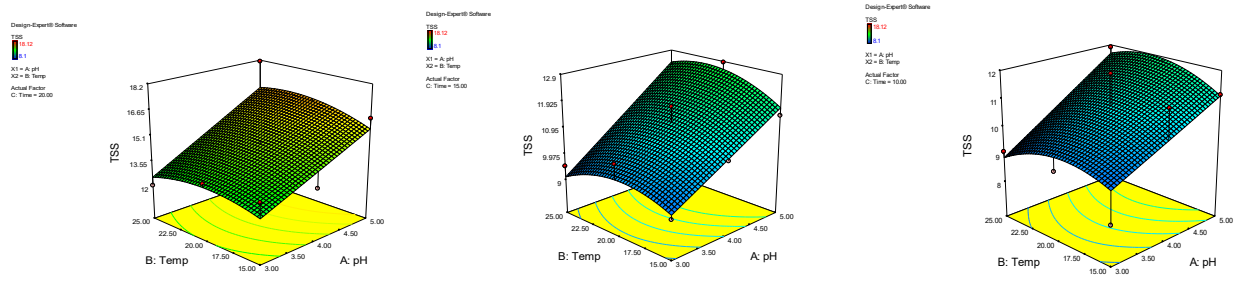
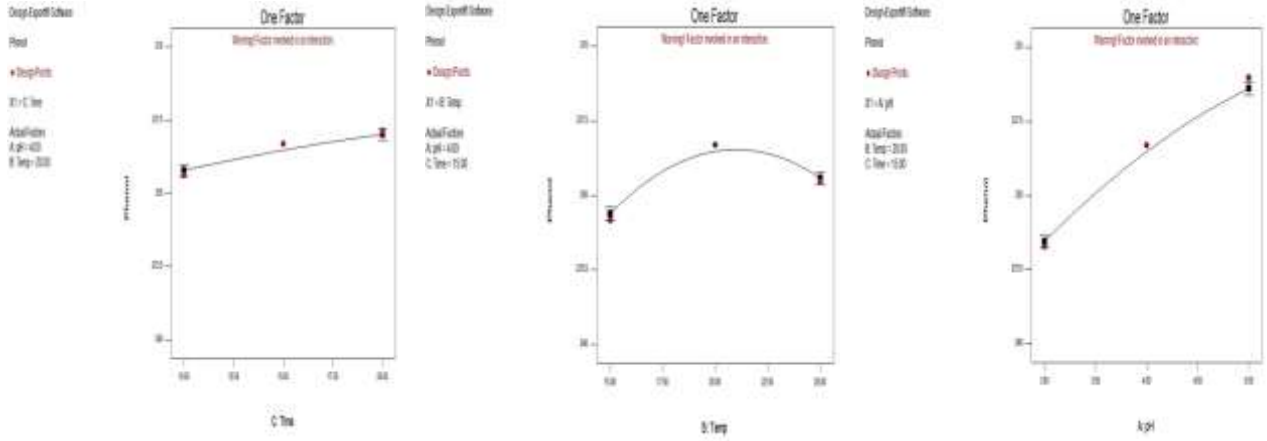


Fig (4) Interaction of pH and time on soluble solids constant temperatures 15 , 20 and 25



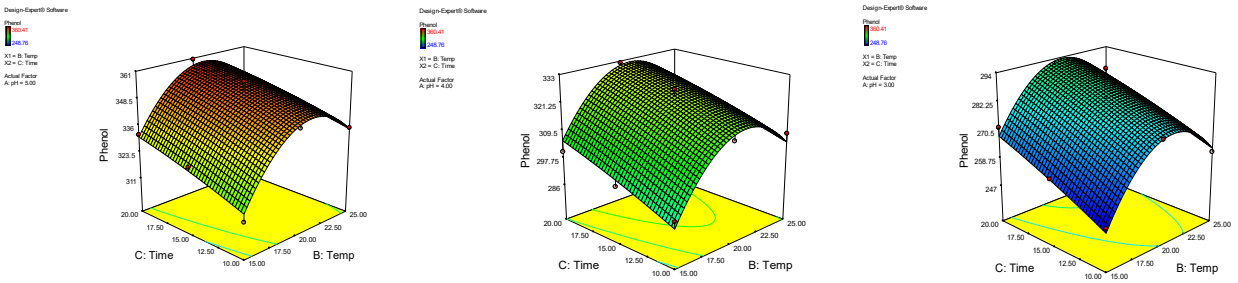
(C) (ب) (الف)

Fig(5) Interaction of pH and temperature on soluble solids constant times 10, 15and 20



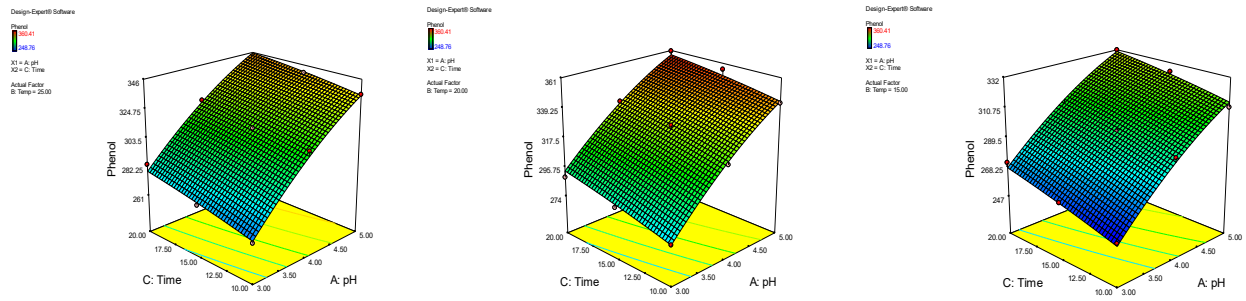
(C) (B) (A)

Fig (6) The amount of total phenol in terms of parameters (A) pH (B) Temperature (C) Time



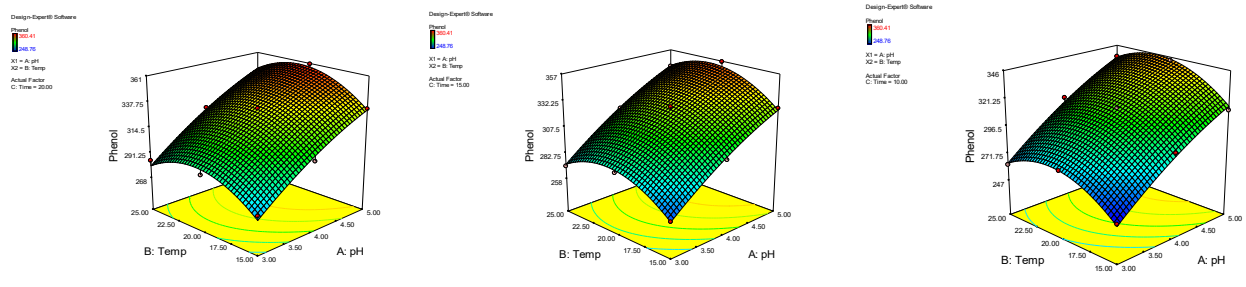
(C) (B) (A)

Fig(7) Interaction of temperature and time on total phenol constant pH 3, 4 and 5



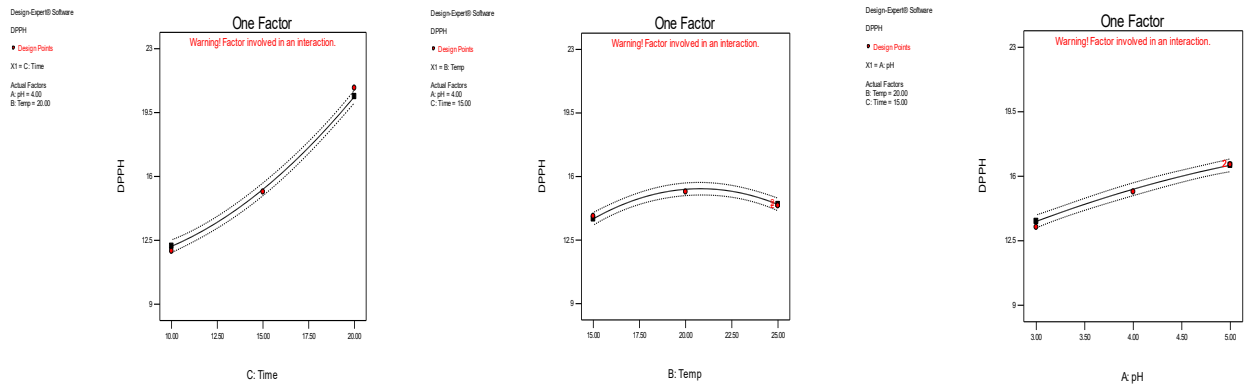
(C) (B) (A)

Fig (8) Interaction of pH and time on total phenol constant temperatures 15, 20 and 25



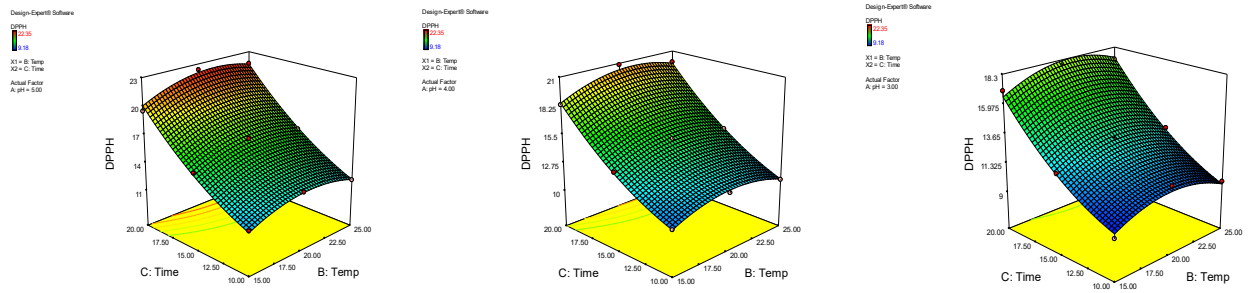
(ج) (ب) (الف)

Fig (9) Interaction of pH and temperature on total phenol constant times 10, 15and 20



(C) (B) (A)

Fig (10) The amount of free radical inhibition in terms of parameters (A) pH (B) Temperature (C) Time



(C) (B) (A)

Fig (11) Interaction of temperature and time on free radical inhibition constant pH 3, 4 and 5

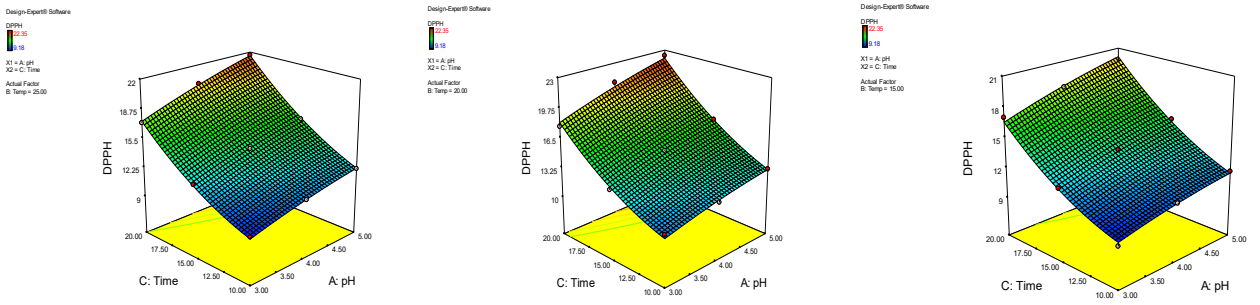


Fig (12) Interaction of pH and time on free radical inhibition constant temperatures 15 , 20 and 25

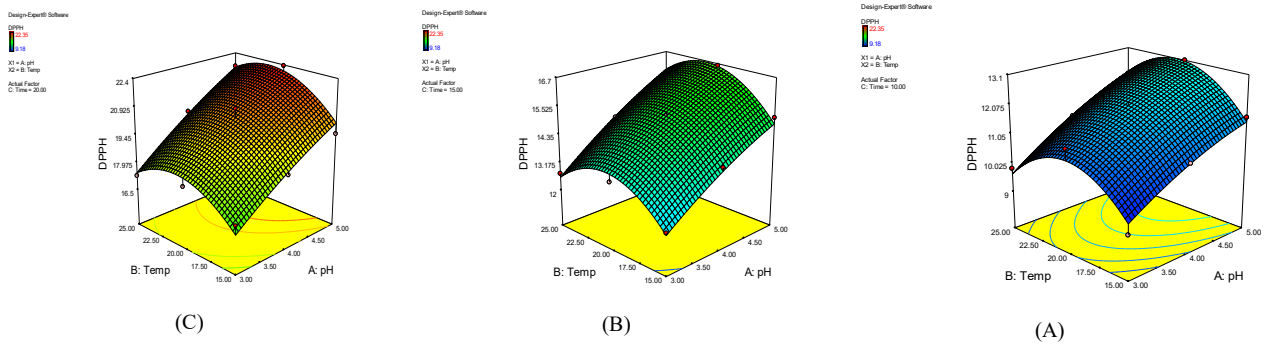


Fig (13)Interaction of pH and temperature on free radical inhibition constant times 10, 15and 20

Table (1) Display process independent variables and their values

Levels of the independent variables examined in this study			
	Levels		
Independent Variables	−1	0	+1
pH	3	4	5
Temperature (°C)	15	20	25
Time (min)	10	15	20

Table (2) Treatments for extraction of red beet

Treatment	pH	Temperature (°C)	Time (min)	Treatment	pH	Temperature (°C)	Time (min)
1	3	15	10	16	4	25	10
2	3	15	15	17	4	25	15
3	3	15	20	18	4	25	20
4	3	20	10	19	5	15	10
5	3	20	15	20	5	15	15

6	3	20	20	21	5	15	20
7	3	25	10	22	5	20	10
8	3	25	15	23	5	20	15
9	3	25	20	24	5	20	20
10	4	15	10	25	5	25	10
11	4	15	15	26	5	25	15
12	4	15	20	27	5	25	20
13	4	20	10	28	4	20	15
14	4	20	15	29	4	20	15
15	4	20	20				

Table (3) Process parameter levels for the optimal points

Optimized Levels of Process Parameters				
No.	**pH**	**Temperature (°C)**	**Time (min)**	**Desirability**
1	5.00	21.328	20	0.937

Table (4) Analysis of variance soluble solids

Source	Sum of Squares	df	Mean Square	F Value	p-value	Significance
Model	130.83	9	14.54	20.15	< 0.0001	significant
A-pH	36.84	1	36.84	51.05	< 0.0001	
B-T	0.3068	1	0.3068	0.4252	0.5222	
C-time	73.69	1	73.69	102.13	< 0.0001	
AB	0.6440	1	0.6440	0.8926	0.3566	
AC	0.8587	1	0.8587	1.19	0.2890	
BC	0.0800	1	0.0800	0.1109	0.7427	
A^2	0.0000	1	0.0000	0.0000	0.9970	
B^2	1.98	1	1.98	2.74	0.1142	
C^2	17.37	1	17.37	24.07	< 0.0001	
Residual	13.71	19	0.7215			
Lack of Fit	13.45	17	0.7912	6.10	0.1499	not significant
Pure Error	0.2595	2	0.1297			
Cor Total	144.54	28				

Table (5) Analysis of variance of total phenolic compounds

Source	Sum of Squares	df	Mean Square	F Value	p-value	Significance
Model	25698.84	9	2855.43	248.91	< 0.0001	significant
A-pH	20296.42	1	20296.42	1769.23	< 0.0001	
B-T	1070.15	1	1070.15	93.28	< 0.0001	
C-time	1155.20	1	1155.20	100.70	< 0.0001	
AB	10.30	1	10.30	0.8982	0.3552	
AC	19.97	1	19.97	1.74	0.2027	
BC	18.11	1	18.11	1.58	0.2242	
A^2	242.71	1	242.71	21.16	0.0002	
B^2	2559.88	1	2559.88	223.14	< 0.0001	
C^2	7.90	1	7.90	0.6890	0.4168	
Residual	217.97	19	11.47			
Lack of Fit	215.91	17	12.70	12.33	0.0775	not significant
Pure Error	2.06	2	1.03			
Cor Total	25916.80	28				

Table (6) Analysis of variance of free radical inhibition response

Source	Sum of Squares	df	Mean Square	F Value	p-value	Significance
Model	365.43	9	40.60	403.30	< 0.0001	significant
A-pH	41.80	1	41.80	415.18	< 0.0001	
B-T	2.80	1	2.80	27.82	< 0.0001	
C-time	303.07	1	303.07	3010.27	< 0.0001	
AB	0.2883	1	0.2883	2.86	0.1069	
AC	1.94	1	1.94	19.23	0.0003	
BC	0.4563	1	0.4563	4.53	0.0466	
A^2	0.2192	1	0.2192	2.18	0.1564	
B^2	9.41	1	9.41	93.50	< 0.0001	
C^2	6.96	1	6.96	69.17	< 0.0001	
Residual	1.91	19	0.1007			
Lack of Fit	1.89	17	0.1113	11.13	0.0855	not significant
Pure Error	0.0200	2	0.0100			
Cor Total	367.34	28				

Table (7)Predicted soluble solids, phenolic compounds and free radical scavenging activity and laboratory results obtained at optimum point

Optimal levels of process parameters				Total soluble solids (%)	Total phenolic compounds (µg GA/ml)	DPPH (%)
	Temperature (ºC)	Time (min)	pH			
Predicted Values	21.328	20.00	5.00	16.602 [a]	358.310 [a]	22.18 [a]
Experimental Values (Mean ± SD)	21.328	20.00	5.00	16.613±0.027[a]	359.10 ± 0.78[a]	21.995±0.30[a]

The non-common letters in each row indicate a significant difference based on the T-Test at the 5% significance level.

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مقاله علمی_پژوهشی

بهینه‌سازی شرایط استخراج ترکیبات فنولی و آنتی‌اکسیدانی از چغندر قرمز توسط اولتراسونیک

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چکیده

ریشه چغندر قرمز (*Beta vulgaris rubra*) یک ماده خام مهم گیاهی است که اثر مثبت اثبات شده در بدن انسان دارد. ریشه چغندر حاوی مقدار زیادی آنتی اکسیدان، ویتامین‌ها، فیبر و رنگ های طبیعی است. ریشه چغندر قرمز همچنین غنی از ترکیبات فنولی است که خواص آنتی-اکسیدانی دارند. در این تحقیق اثر متغیرهای pH (۵–۳)، دما (۱۵–۲۵ درجه سانتی گراد) و زمان استخراج (۱۰–۲۰ دقیقه) بر میزان مواد جامد محلول، ترکیبات فنولی و فعالیت آنتی‌اکسیدانی عصاره حاصل از چغندر قرمز بررسی شد. استخراج از چغندر قرمز با استفاده از مخلوط آب:اتانول با نسبت ۱:۱ انجام شد. از امواج اولتراسوند با شدت ۷۵ درصد و فرکانس ۳۷kHz برای تسهیل فرآیند استخراج استفاده گردید. داده ها با استفاده از نرم افزار دیزاین اکسپرت و روش بهینه سازی سطح پاسخ آنالیز شدند. نتایج نشان داد که با افزایش pH و زمان استخراج مقادیر مواد جامد محلول کل، فنول‌ها و فعالیت آنتی اکسیدانی نیز افزایش می‌یابد. همچنین افزایش دما تا ۲۰ درجه سانتی‌گراد مقادیر این پاسخ‌ها را افزایش داد اما دماهای بالاتر از ۲۰ درجه سانتی‌گراد باعث کاهش در این مقادیر شد. نتایج تجربی به دست آمده در نقطه بهینه پیشنهادی توسط نرم افزار با نتایج پیش‌بینی شده توسط مدل تفاوت معنی‌داری نداشت ($p < 0/05$).