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The impact of temperature and ultrasound on the polyphenols extraction and antioxidant activity of Mozafati, Sayer, and Kabkab date byproduct varieties

Azin Khosravi Largani[1], Seyed Hadi Razavi*[2]

1- PhD Student, Department of Food Science & Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, Iran

2-*Corresponding author: Professor, Department of Food Science & Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.

ABSTRACT

In this study, the effect of ultrasound and temperature at three different levels—30, 50, and 70 °C on the amount of phenolic compounds and antioxidant properties of the extract obtained from the byproducts of three date byproducts—Kabkab, Mozafati, and Sayer were evaluated. In order to conduct a comprehensive assessment of antioxidant activities, three methods including DPPH and H_2O_2 radical scavenging activities, and ion chelating ability were employed. The Pierson analysis was used to examine the correlation between polyphenolic compounds and antioxidant activities. According to the results, the use of ultrasound treatment enhanced the amount of extracted polyphenols and flavonoids in all date varieties. Furthermore, the polyphenol and flavonoid contents were found to increase up to 50°C during extraction, but decreased, subsequently. The highest amount of polyphenols and flavonoids was achieved using the ultrasound treatment in 50° C in Sayer variety, 4.64 ± 0.07 mg Gallic acid/g and 0.326 ± 0.0112 mg quercetin/g, respectively. DPPH and H_2O_2 radical scavenging activities were increased after ultrasound treatment at 50°C but then decreased ($P<0.05$). Maximum H_2O_2 scavenging activity was observed in Mozafati variety. The iron chelating ability decreased after extraction with ultrasound treatment at 30° C and then increased significantly ($P<0.05$) until reached the maximum amount of 40.433 ± 0.802% in Sayer variety. Results showed a strong correlation between polyphenols and flavonoids with antioxidant activities. Therefore, the antioxidant properties of the tested date byproducts are likely to derive from their polyphenolic content, making them an economical source of these bioactive compounds.

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*Corresponding Author E-Mail:
ahmadpedram@yahoo.com

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1- Introduction

Nowadays, many chemical additives are used in the preparation of most food, pharmaceutical, cosmetic and health products. The importance of these additives is such that without using them, it becomes almost impossible to produce and consume many food items and products. One of the most important food additives are flavoring compounds that play an important role in creating taste and overall acceptance by consumers. On the other hand, the concerns caused by the consumption of synthetic compounds in terms of health and safety issues, as well as restrictions on the permissible limits of consumption, have encouraged food consumers to use natural products in food [1]. One of the valuable sources for natural flavor production are the effective compounds of medicinal plant extracts. Rasak is a medicinal plant of the rose order[1] and dark*Cannabis*[2] and the genus Homolus[3] Is. More than 95% of the hops produced in the world (148,603 tons with a cultivated area of 91,881 hectares [2]) are used as flavoring in the soft drink industry, especially beer production. In Iran, this plant grows in the form of a car in the northern regions of Iran (Gorgan, Chalus, Tankabon, Rasht, Lahijan and Astara), which does not meet the needs of the malting industry, and the country is dependent on imports for its supply. According to customs statistics in 2019, 22,835 kilograms of hop extract and juice (in the form of pellets and pellets) worth about 24 billion Rials were imported from Germany, Russia and Switzerland [3]. According to the country's development plan regarding the increase in per capita consumption of malt beer (from 5 liters to 10 liters and as an alternative to carbonated drinks) and the growth of this industry and the possibility of exporting products to foreign markets (Turkey, Iraq, Afghanistan, Armenia, Azerbaijan, Turkmenistan, Kuwait), optimal extraction of the extract of this medicinal plant seems necessary.

The hop plant contains various bioactive compounds such as phenolic, flavonoid and resin compounds (soft and hard resins). Among the mentioned materials, resin compounds are the most important compounds. Soft resins are divided into two categories: alpha and beta bitter acids and unspecified compounds (most of the components are beta acids). Alpha acids and their derivatives including Humulon compounds[4], Comulon[5] and adhomolone[6]Adperihomolone[7], prehomolon and posthomolon[8] Are. Beta acids and their derivatives, in addition to cumulone, adhomolone and analogues, have lupolone[9]are also [4]. The amount of these compounds is different in different varieties and also in different parts of the plant (flowers, stems and leaves) and they are affected by weather conditions, cultivation conditions, type of variety and also post-harvest conditions (storage and drying) [5]. Most of the female flowers of the plant (female cones) are used to prepare the extract due to the presence of different amounts of compounds that create a bitter taste, and the aerial parts (stem and leaves) are considered as side products; While these organs also have significant amounts of bioactive compounds that can be extracted by choosing the appropriate method [6].

Qualitative and quantitative studies on phenolic and bioactive compounds are highly dependent on the appropriate extraction method. Therefore, many efforts have been made to find

[1]- *Rosales*
[2]- *Cannabaceae*
[3]- Humulus

[4]- humulone
[5]- cohumulone
[6] -humulone
[7]- Adprehumulone
[8]- Prehumulone
[9]- Posthumulone

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new technologies without causing environmental pollution. Ultrasound, subcritical water, supercritical carbon dioxide, high hydrostatic pressure and strong pulsed electric fields are among the latest extraction methods that are used as pre-processing operations depending on the type of raw material and the compounds present in it [7]. Among the new methods mentioned, extraction with the help of ultrasound is a very simple and efficient method that improves the quality of extracted extract, and in the last few years, it has received more attention in the food industry [12,11,10,9,8].

The extraction efficiency of bioactive compounds varies with the type and polarity of the solvent, the ratio of substance to solvent, time, temperature, and the number of times of extraction. Solvents of ethanol, methanol, methylene chloride, ethylacetate, stane, hexane and their mixture with water have been used for solid-liquid extraction of active compounds from hop plant [13]. Bartmanska et al. (2018) used ethylene chloride, acetone, ethylacetate and methanol solvents to recover flavonoids from the edible parts of the hop plant and reported that the efficiency of extracting effective compounds with methanol was higher than other solvents [14]. Musicovich et al. (2019) extracted phenolic compounds from freshly harvested leaves of hop plant with different methyl, ethyl and isopropyl alcohol solvents and with the help of sonication process. The test conditions included different extraction times (15, 30, and 60 minutes) and different solvent dilutions (40, 70, and pure) with constant sonication power (40 kHz) at room temperature. The results showed that methanol solvent without dilution is the best solvent for extracting phenolic compounds with the help of sonication process [15]. But due to endangering human health by solvent residue in the final product, the use of safe solvents such as water, ethanol and carbon dioxide has increased. Ethanol, as a polar solvent, is suitable for extracting a wide range of bioactive compounds. Almeida et al. (2020) optimized the extraction conditions of phytochemical and antioxidant compounds from Brazilian hop flowers using a rotating compound design. Based on the results of regression analysis, the ratio of liquid to solid had the greatest effect on the extraction of phenolic compounds, while temperature and ethanol concentration had less effects. Under the optimal extraction conditions (49% ethanol solvent with a temperature of 52 degrees Celsius and a liquid-to-solid ratio of 34 ml/g), the extraction rate of total phenolic compounds was 33.93 mg of gallic acid/g. Also, the results showed that Brazilian hops have more phenolic compounds with higher antioxidant power than American hops [16].

The extraction efficiency and characteristics of extracts prepared with the help of sonication process depend on the sample preparation method (particle size and storage conditions) and extraction factors (temperature, time and sonication intensity). Therefore, optimization with the aim of increasing the feasibility and efficiency of extraction without increasing the costs is very important. Response surface method[10]is a set of mathematical and statistical techniques that are used to develop and optimize processes where the desired response is affected by a number of variables and has been used in numerous researches to optimize the process of extracting bioactive compounds from different sources. But in order to achieve the best operational conditions with the help of ultrasound process for extracting bioactive compounds from the aerial parts of hop plant, more study and development of more suitable models is needed.

[10]-RSM:Response surface method

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Therefore, considering the importance of the optimal use of medicinal plants, localization and application of valuable extracts obtained from them and preventing foreign currency outflow, as well as the lack of documented research in this regard, the present study aims to optimize the extraction of bioactive compounds from hops with the help of the sonication process. Sounding was done under the influence of temperature, time and intensity.

2- Materials and methods

2-1- Materials

The medicinal plant hops (including the aerial parts of the stem and leaves and without the cone flowers) was obtained from the local market in Mashhad. Then, by a laboratory mill (IKA, model A11 basic analytical mill, made in Germany), the powder and its particle size were adjusted using a sieve with a mesh of 50 micrometers, and until the tests were performed, they were packed in zipped plastic bags and stored in cold, dry conditions and away from light.Other materials used in this research include 96% ethanol, Folinciocalcio reagent,TPTZ[11]،[12] DPPH, gallic acid, methanol, sodium carbonate, bivalent iron sulfate, sodium chloride, sodium acetate, potassium chloride, concentrated hydrochloric acid and other required chemicals from Merck, Sigma-Aldrich, Charlo and Caldon companies with analytical grade[13] were purchased

2-2- Extraction by immersion method (maceration)

For this purpose, the mixture of plant powder and aqueous-alcoholic solvent (80% ethanol-20% water, ratio of 1:10 (weight/volume) dry material to solvent) for 24 hours in a dark place and at room temperature with a magnetic stirrer (RCT Basic model, IKA, made in Germany) was mixed. Then the solution was filtered under vacuum and the extraction process continued on the remaining materials on the filter for another 24 hours. The filtered solutions obtained from the first and second stage of extraction were mixed together and concentrated with a rotary evaporator under vacuum (Laborota 4000 efficient model, made in Germany) under a temperature of 45 degrees Celsius until complete dehydration. .

2-3- Extraction of the extract with the help of sonication process

Extraction of effective compounds was done using 80% ethanol solvent and with the help of sonication process. For this purpose, first, the hops plant was mixed with solvent at a ratio of 1 to 10 weight by volume and sonicated in time (5, 10, and 15 minutes), temperature (25, 35, and 45 degrees Celsius) and different intensities (20, 60, and 100 percent). . The conditions of each test, including temperature, time, and intensity of sounding, were considered based on the levels of variables predicted in the Benken box design according to Table 1. To apply ultrasound waves, use an ultrasonic device made by Helscher, Germany, model UP 400S[14] With 400 watts power and H type probe$_7$ It was made of titanium with a diameter of 7 mm and a length of 100 mm. Then the extract extraction was continued for 48 hours in a dark environment with vigorous stirring. The solution was filtered under vacuum and concentrated with a rotary evaporator under vacuum (Laborota 4000 efficient model, made in Germany) under a temperature of 45 degrees Celsius until complete dehydration.

[11] - 2, 4, 6-tris (2-pyridyl)-s-triazine

[12] - 2, 2-diphenyl-1-picrylhydrazyl

[13] -Analytical grade

[14] - Heilscher, Germany – UP400S

Table 1- Box-Behnken design of three variable in the extraction of bioactive compounds by assisting ultrasound

Run	Ultrasonic exposure time (min, X_1)	Ultrasonic Extraction temperature (°C, X_2)	Ultrasonic Amplitude (%, X_3)
1	15	25	60
2	10	25	100
3	15	45	60
4	5	35	20
5	10	25	20
6	10	35	60
7	10	35	60
8	10	45	20
9	10	45	100
10	15	35	100
11	5	35	100
12	5	25	60
13	15	35	20
14	10	35	60
15	5	45	60

2-4- Extraction efficiency

Extraction efficiency by calculating the weight of concentrated extract without solvent (In_2(relative to the initial weight of the plant)In_1) and was calculated and reported using equation 1 [17].

$$Extraction\ efficiency = Y$$

Relationship 1)

5-2- Measurement of total phenolic compounds

The total phenolic content of the extracted extracts was measured with Folinciocalcio reagent. The content of total phenol was expressed as gallic acid equivalent in mg per 100 grams of plant dry weight. For this purpose, to 100 microliters of the sample extract (diluted with methanol 1:10 volume/volume), 6 milliliters of double distilled water and 500 microliters of Folin Ciocalcho reagent were added, after keeping for 8 minutes and 8 seconds at room temperature, 5/ 1 ml of sodium carbonate (20% by weight/volume) was added. The mixed extract was kept at 40°C for 30 minutes and then its absorbance was read at 765 nm. The amount of total phenolic compounds in the sample was determined from the standard curve. The standard curve was obtained by plotting the absorption data of gallic acid at a wavelength of 765 nm in concentrations of 100 to 950 ppm. The results were reported based on milligrams of gallic acid per 100 grams of dry sample using the equation fitted to the standard curve.]18[.

5-2- Measuring the ability to inhibit free radicalsDPPH

First, 0.006% free radical solutionDPPH It was prepared in methanol. Then, one milliliter of the above solution was added to the test tubes containing one milliliter of methanolic sample solution with different concentrations (depending on the free radical inhibitory power). After stirring, the test tubes were kept in a dark place for one hour, and then their absorbance was read at a wavelength of 512 nm against a control.]19[.The percentage of free radical inhibition was calculated according to relation 2.

$$A\% = \frac{A_c - A_s}{A_c} \times 100$$

relationship 2)

where in:%A Percentage of free radical inhibitionDPPH ،A_C Attracting witnesses andA_S Sample absorption.

6-2- The regenerative power of ironFRAP[15])

First, a solution containing 100 milligrams of the sample was prepared in 2 milliliters of methanol, and 30 microliters of it was mixed with 900 microliters of the solution.FRAP And 90 microliters of distilled water was mixed in the

[15]- *Ferric reducing* antioxidant potential

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test tube. After the vortex, the test tube was placed in a banmari and after its temperature reached 37 degrees Celsius, the absorption value was read against the control at a wavelength of 595 nm. amount of ironII Using the equation fitted to the standard curve (absorption value of iron sulfate standard solutions II with concentrations of 200 to 2000 micromoles per liter at a wavelength of 595 nm)]20[.

7-2- Determining the amount of alpha and beta acids

To measure the amount of alpha and beta acids, 250 mg of the extract was mixed with 5 ml of methanol for half an hour. After filtering, 50 µl of the filtered solution was made up to 25 ml with 1 M methanolic solution. The absorption of methanolic solution with a spectrophotometer at three wavelengths of 275, 325 and 355 nm against the reference sample and the amount of alpha and beta acids and bitter compounds were calculated using the following relationships.

$A355=31.8C\alpha+46.0C\beta+1.0Cc$ Relationship 3)

$A325=38.1C\alpha+33.1C\beta+1.5Cc$ Relationship 4)

$A275=9.0C\alpha+3.7C\beta+3.1Cc$ Relationship 5)

that in these relationshipsA، Cα, Cβ and Cc respectivelyThe amount of absorption, alpha acids, beta acids and bitter partial compounds, which are in grams per liter [21].

2-8- Test plan and statistical analysis

In order to investigate the effects of variables on the extraction of bioactive compounds from the hop plant with the help of sonication process, Benken's box design was used. This design includes three variables (Sounding time(X_1), process temperature (X_2) and ultrasound intensity (X_3)) was in three levels and three repetitions. The resulting treatments are listed in Table 1. From Designexpert software version 11[16] It was used to design the experiment, analyze the results, show the relationship of each of the dependent variables in the regression model with the independent variables and draw a diagram of their levels and optimization. In order to evaluate the correctness of the fitted models, the test of the lack of fit, the coefficient of variation, the values of the explanatory coefficient, the adjusted explanatory coefficient and the probability model were determined. Also, optimization with the aim of obtaining the extract with the highest amount of bioactive compounds and antioxidant activity using the models obtained from examining the behavior of variables on the properties of the extracted extracts (extract extraction efficiency, the amount of total phenolic compounds, the ability to inhibit free radicalsDPPH, the reducing power of iron and the amount of alpha and beta acids) were done. Then, the properties of the extracts were measured under optimal conditions. In order to confirm the correctness of the models, predicted and experimental results with the control sample with the softwareMStatC and based on Duncan's test at the level of 5% (<0.05).p) were compared.

3. Results and Discussion

3-1- Determining the right model

In order to determine the experimental model for predicting the response, polynomial equations including linear, two-factorial (interactive), 2nd and 3rd degree were fitted to the data obtained from the response surface method. Then these models were subjected to statistical analysis (Table 2). It should be noted that it is a statistically appropriate model that the lack of fit test[17] It is not significant and has the highest explanatory coefficient and modified explanatory coefficient

[16]- Design Expert 11

[17] -Lack of fit

be Variance analysis (Table 3) and regression were used to check the consistency of the proposed models and to check the statistical significance of the model variables. Based on the indexPThe significance of models and equations was investigated. In total, the results show that the used models can be used to estimate the optimal extraction conditions from hops.

Table 2- Model selection for dependent (response) variable

	Extraction Yield (%)		**TPC (mg/100 g)**		**DPPH$_{Sc}$ (%)**		**FRAP (µmol/l)**		**a acids (mg/l)**		**β acids (mg/l)**	
Source	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F
Mean	3764.85		9916000		17621.36		1806000		228.41		148.00	
Linear	16.77	0.21	133200	0.077	1465.85	0.19	17358.66	0.09	43.45	0.343	11.147	0.19
Polynomial	9.03	0.47	70347.60	0.191	1559.99	0.08	3914.55	0.68	91.31	0.015	17.48	0.005
Quadratic	**22.78**	**0.01**	**82609.90**	**0.008**	**1049.93**	**0.03**	**18293.16**	**0.004**	**31.67**	**0.017**	**4.28**	**0.008**
Cubic	2.93	0.14	10148.05	0.056	170.60	0.39	997.11	0.541	4.49	0.313	0.536	0.034
Residue	0.31		398.60		67.00		680.67		1.28		0.012	
Total	3816.67		10210000		21934.74		1848000		400.60		181.77	

TPC: total polyphenolic compounds
DPPH sc: DPPH radical scavenging power
FRAP: ferric reducing/antioxidant power

Table 3- Analysis of variance for Quadratic modelon responses

	Extraction Yield (%) (mg/l)		**TPC (mg/100 g)**		**FRAP (µmol/l)**		**DPPH $_{Sc}$ (%)**		**a acids (mg/l)**		**β acids (mg/l)**	
Source	Sum of squares	Pb > F	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F	Sum of squares	Pb> F
Model	33.22	0.0000	166.43	0.003	4075.78	0.011	39566.37	0.005	28620	0.004	48.58	0.02
X_1	1.33	0.017	5.71	0.07	317.93	0.049	8.66	0.88	1874.80	0.389	5.41	0.03
X_2	2.88	0.0	4.14	0.12	1120.20	0.005	14999.90	0.001	11310	0.0007	11.33	0.008

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X_3	7.26	0.0005	33.59	0.003	27.72	0.48	2350.10	0.045	18221.09	0.0323	0.03	0.85
X_1X_2	1.26	0.0019	12.85	0.020	1141.74	0.004	59.70	0.69	11255.72	0.069	3.42	0.07
X_1X_3	16.21	< 0.0001	2.29	0.217	397.05	0.034	69.16	0.67	2285.89	0.346	3.20	0.07
X_2X_3	0.005	0.8290	76.16	0.00005	21.20	0.54	3785.69	0.02	56806.02	0.003	2.40	0.11
X_1^2	0.675	0.0560	0.04	0.864	89.76	0.22	2043.31	0.06	19421.25	0.0289	2.29	0.12
X_2^2	0.22	0.2101	12.11	0.023	722.32	0.01	169.15	0.51	35537.04	0.009	19.32	0.002
X_3^2	3.19	0.0003	21.25	0.0078	361.42	0.04	14049.45	0.0008	24080.46	0.019	0.001	0.92
Residual	0.548		5.77		237.60		1677.78		10546.66		3.24	
Lack of Fit	0.536	0.0034	4.49	0.313	170.60	0.392	997.11	0.54	10148.05	0.056	2.93	0.14
Pure Error	0.012		1.28		67.00		680.67		398.60		0.31	
Cor Total	33.77		172.20		4313.37		41244.15		296700		51.82	

X_1: Ultrasonic exposure time (min,), X_2: Ultrasonic extraction temperature (°C,), X_3: Ultrasonic Amplitude (%,)

2-3- The effect of extraction parameters on extract extraction efficiency

The results of the present study indicate a significant effect Linear parameters of ultrasonic time, tempProcessAnd the second-order parameter of ultrasonication time on extraction efficiency was (Table 3), (0.05).p). With increasing sonication time, extract extraction efficiency increased; while with increasing temperatureProcessAt first, the extraction efficiency of the extract decreased and then increased strongly (Figure 1).

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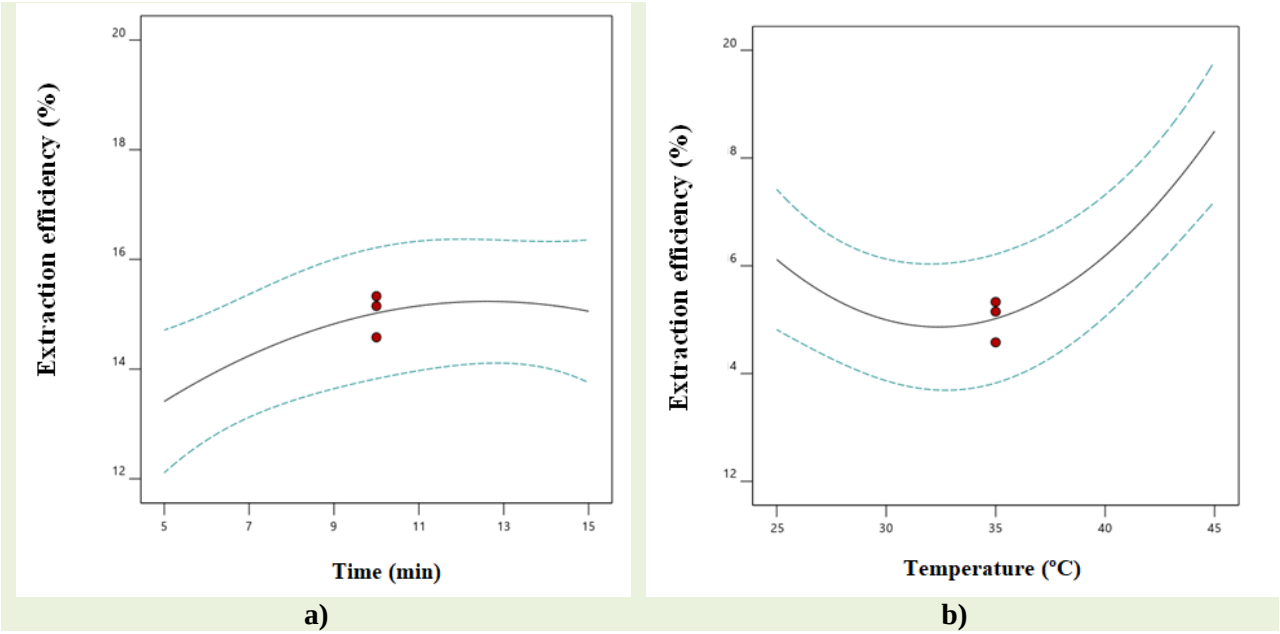

Fig 1- The effect of a) ultrasound exposure time and b) ultrasonic extraction temperature on the extraction effiviency (%) of hops extract

The increase in extract extraction can be attributed to the various effects of ultrasound waves such as cavitation, emulsification, diffusion and damage to the tissue, which help to extract the desired components from raw materials [9]. Castanda-Valbona et al. (2021) stated that the use of ultrasound leads to an increase in extract extraction from the skin and seeds of the mango plant [22]. The results of this section were consistent with the results of Ezadi et al. (2022), who stated that the extraction efficiency of sugar beet leaves increases with the increase in ultrasonication time [23]. Increasing the temperature and sonication time by increasing the penetration coefficient of the solvent and increasing the duration of the solidification, increase the extraction efficiency of the extract [24]. However, it should be noted that high process temperatures (more than 80 degrees Celsius) reduce extraction efficiency by destroying bioactive compounds. The final model presented in Table 4 (Model 1) indicates the greater influence of the quadratic variable of temperatureProcessIt was based on the extraction efficiency of the extract.

Table 4- Designed equation models for dependent variables.

Number	Dependent variable	Equation	R^2	R^2-adj	cv
1	**Extraction efficiency (%)**	$y= +15.02 + 0.822_1+1.19\ X_2– 0.057\ X_3 – 0.92\ X_1^2 + 0.89\ X_2^2 +0.77X_3^2 – 0.79\ X_1X_2 + 2.29X_1X_3 + 0.04\ X_2X_3$	0.937	0.825	5.08
2	**TPC (mg/100g)**	$y= +756.34 + 15.31_1– 118.91\ X_2– 47.72\ X_3 – 72.53\ X_1^2 + 98.11\ X_2^2 + 80.76\ X_3^2 +53.05X_1X_2 + 23.91X_1X_3 – 116.17\ X_2X_3$	0.964	0.900	5.65

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3	**DPPH sc (%)**	$y= +18.91 +6.30_1–11.83 X_2– 1.86 X_3 +4.93 X_1^2 + 13.99 X_2^2 +9.89X_3^2 +16.89 X_1X_2 – 9.96 X_1X_3 +2.30 X_2X_3$	0.945	0.846	20.11
4	**FRAP (µmol/l)**	$y= +294.62 – 1.04_1– 43.30 X_2+17.14 X_3 + 23.52X_1^2 + 6.77 X_2^2 – 67.95X_3^2 – 3.86X_1X_2 +4.16X_1X_3 +30.76 X_2X_3$	0.959	0.886	5.28
5	**α acids (mg/L)**	$y= +1.71 – 0.845X_1+ 0.719 X_2+2.05 X_3 – 0.10X_1^2 + 1.81 X_2^2 – 2.40X_3^2 – 1.79X_1X_2 -0.757X_1X_3 +4.36 X_2X_3$	0.966	0.906	27.53
6	**β acids (mg/l)**	$y= +2.74 – 0.41_1+0.60 X_2+0.952 X_3 –0.428X_1^2 + 0.245 X_2^2 +0.929 X_3^2 – 0.56X_1X_2 – 2.01X_1X_3 +0.038 X_2X_3$	0.983	0.954	10.55

3-3- The effect of extraction parameters on the amount of total phenolic compounds

The results showed that, like extract extraction efficiency, the best model for fitting the amount of phenolic compounds was the quadratic polynomial model (Table 2). The results of the analysis of variance table showed that the linear parameters of sonication time, the interaction of time and sonication temperature, as well as the interaction of time with sonication intensity had no significant effect on the amount of phenolic compounds in the extract, but other parameters were significant on this feature at the 5% level. As the temperature increasesProcess,The amount of total phenolic compounds in the samples decreased; However, with the increase in sound intensity at any temperature, the amount of these compounds increased. Also, with increasing the sonication time, the amount of these compounds increased to a small amount at first and then decreased (Figb 2). The reason for the increase in total phenolic compounds is the intensification of mass transfer caused by the collapse of cavitation bubbles near the cell wall, which causes better contact between the solvent and plant material. Also, during the collapse of the cavitation bubbles, a rapid flow of ultrasound waves is produced, which acts as a micropump and forces the solvent into the cell to dissolve the effective compounds.]25[.In fact, as a result of the propagation of sound waves in the solid-liquid phase, a contraction and expansion cycle is created in the environment, which causes the formation of bubbles, and these bubbles grow and finally collapse. This action causes the oscillation of solid and liquid particles and they gain speed under the action of ultrasound, as a result, the soluble materials quickly diffuse from the solid phase to the solvent. In addition, other effects such as emulsification, diffusion and tissue damage also help to increase the extraction of desired components from raw materials. Also, the effect of the solvent during long contact time causes the extraction of impure compounds[26 and 15].Nejatirad et al. (2020) inInvestigating the effect of different pretreatments on the extraction of bioactive compounds with antioxidant activity from Velik fruit, stated that the use of ultrasound leads to an increase in the total phenolic compounds of the extract]27[. The reason for the decrease of phenolic compounds with increasing temperature can also be attributed to the destruction of these compounds due to high temperatures. From the F numbers as well as model number 2 (Table 4), it can be stated that the linear parameter of the process temperature had the greatest effect on the amount of total phenol in the extracts.

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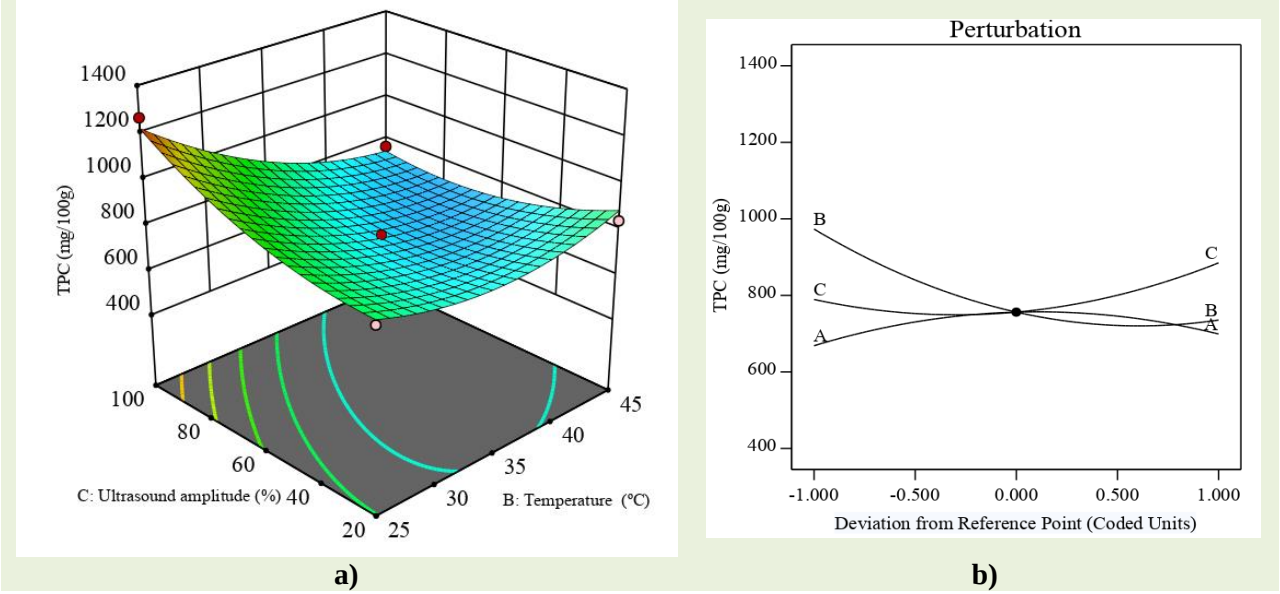

a) **b)**

Fig 2- Response surfaces of the total phenolic compound (TPC, mg/100g) of ultrasound- assisted hops extracts as a function of (a) ultrasonic extraction temperature with ultrasonic amplitude and b) perturbation graph

3-3- The effect of extraction parameters on the ability to inhibit free radicalsDPPH

To fit the data from free radical scavenging abilityDPPH, the quadratic polynomial model was selected as the best model (Table 2). Also, the results showed that the linear parameter of ultrasound time, temperatureProcessand the interaction effect of ultrasound time with temperatureProcess, ultrasound time with ultrasound intensity and quadratic parameters of temperatureProcessand ultrasound intensity on the level of this feature were significant at the 5% level (Table 3).Figure a 3 showed that with the increase in sonication time, the DPPH free radical scavenging ability increased, while this characteristic decreased with the increase in process temperature, and Figure b3 also indicated that in the initial times of the process, with the increase in sonication intensity, the free radical scavenging ability increased. DPPH increased, but during most of the process, with increasing ultrasound intensity, probably due to the degradation of bioactive compounds (phenolic compounds and resinous compounds), the ability to inhibit DPPH free radicals decreased.The cause of the increase in radical absorption activity with increasing ultrasound time can be attributed to the phenomenon of cavitation, which is actually due to the propagation of sound waves in the solid-liquid phase, contraction and expansion cycles are created in the environment, which causes the formation of bubbles that continue to grow and Eventually they disintegrate. This action causes solid and liquid particles to oscillate and they gain speed under the action of ultrasound. As a result, the solutes quickly diffuse from the solid phase to the solvent. This causes the extraction of more effective compounds and ultimately increases the antioxidant activity]28[. The reason for the decrease in the ability to inhibit DPPH free radicals with increasing temperature can also be attributed to the increase in solvent vapor pressure, solvent surface tension, reduction in cavitation phenomenon, and the destruction of phenolic compounds in the extract.]29[. The results of this section were consistent with the findings of Maqsoodlou and Esmailzadeh Kanari (2020), who investigated the effect of ultrasound treatment (bath and prop) on the antioxidant properties of oregano extract.]30[. From the F numbers as well as model number 3 (Table 4), it can be stated that the greatest effect on the ability to inhibit DPPH free radicals was related to the interaction

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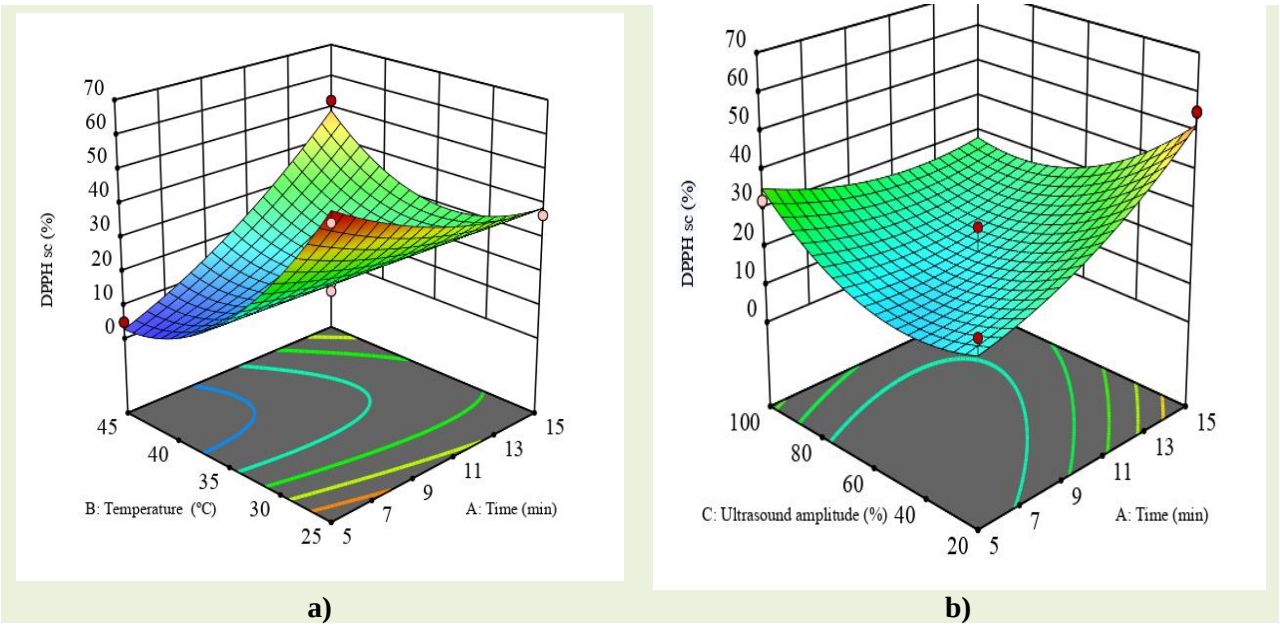
parameter of sonication time with process temperature.



a) **b)**

Fig 2- Response surfaces of the DPPH radical scavenging power (DPPH sc, %) of ultrasound- assisted hops extracts as a function of (a) ultrasonic extraction temperature with ultrasonic exposure time and b) ultrasonic amplitude with ultrasonic exposure time

4-3- The effect of extraction parameters on iron reduction power

The reductive power of extracts shows their ability to reduce trivalent iron and convert it into divalent iron. The presence of antioxidants (regenerators) in the extract, which are electron donors, causes the regeneration of ferricyanide complexes and their conversion into ferrous form, which changes the yellow color of the solution to different degrees of green and blue depending on the reductiveness of the tested extracts.]31[. According to the results given in Table 2, it can be stated that the best model for fitting the data obtained from the reductive power of iron was the quadratic polynomial model ($p = 0.004$). The variance analysis table of the data (Table 3) also indicated that all the studied linear parameters, except the linear parameter of sonication time, had a significant effect on the reductive power of iron at the level of 5%, and among the interaction parameters, only the interaction of process temperature with The intensity of ultrasound on the reductive power of the extracts was significant at the level of 5%. As can be seen from Figure 4, with the increase in process temperature, the reductive power of the extracts decreased; however, with the increase in the sonication intensity, the reductive power of the extracts first decreased and then increased. The increase and decrease of iron reduction power is related to the increase or decrease of the phenolic compounds of the extracts]32[. Ilghami et al. (2015) also investigated the effect of ultrasound on the extraction of antioxidant compounds from sugar beet and stated that with the increase in the intensity of ultrasound, the iron reduction power of the extract increases.]33[. According to the numbers and also the coefficients of the models given (model no. 4) in Table 3, it can be said that the greatest effect on the reductive power of iron was related to the second-order parameter of sounding intensity.

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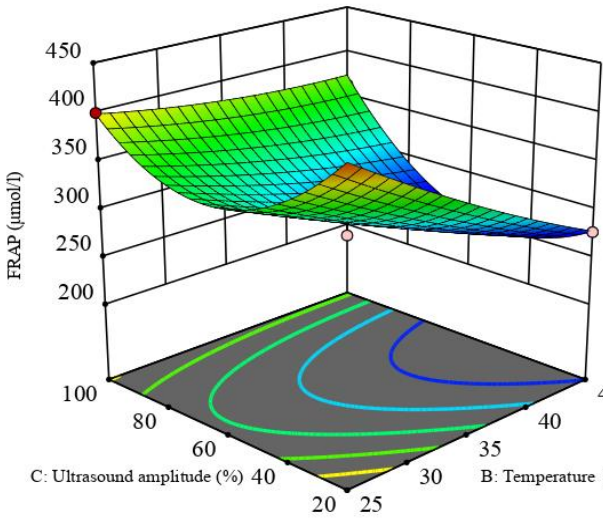

Fig 4- Response surfaces of the ferric reducing/antioxidant power (FRAP, µmol/l) of ultrasound- assisted hops extracts as a function of ultrasonic amplitude with ultrasonic extraction temperature

5-3- The effect of extraction parameters on alpha acids

As mentioned earlier in the introduction, hop medicinal plant has soft resinous compounds that create a bitter taste (alpha and beta acids) that have soothing, antioxidant, apoptosis-inducing, tumor suppressing, inflammation-reducing, and antimicrobial effects.[34]. As can be seen from Table 1, the best model for fitting the data obtained from the amount of alpha acids in hop extract was the second degree polynomial model. The results of the analysis of variance also revealed that among the studied linear parameters, only the ultrasound intensity was significant on the amount of alpha acids in the extract at the 5% level, and the interaction parameters of the ultrasound time with the ultrasound intensity, as well as the quadratic parameter of the ultrasound time, were significant at the 5% level. They were not meaningful. The findings showed that the amount of alpha acids increased with the increase in sonication intensity and process temperature in the time and high temperatures studied, but with the increase of sonication time, the amount of these compounds decreased (Figure 5). Carbone et al. (2020) also showed that the use ofProcess Ultrasonication leads to the increase of bitter compounds in the extract, which was in line with the results of this section]35[. Aniol et al. (2008) attributed the increase in alpha acids (lupolone) of hop extract when using ultrasound to the increase in polarity of the solvent, which extracted these compounds more.]36[. According to the coefficients of the models given (model no. 5) in Table 3, it can be stated that the greatest effect on the amount of alpha acids was related to the interaction parameter of process temperature with sonication intensity.

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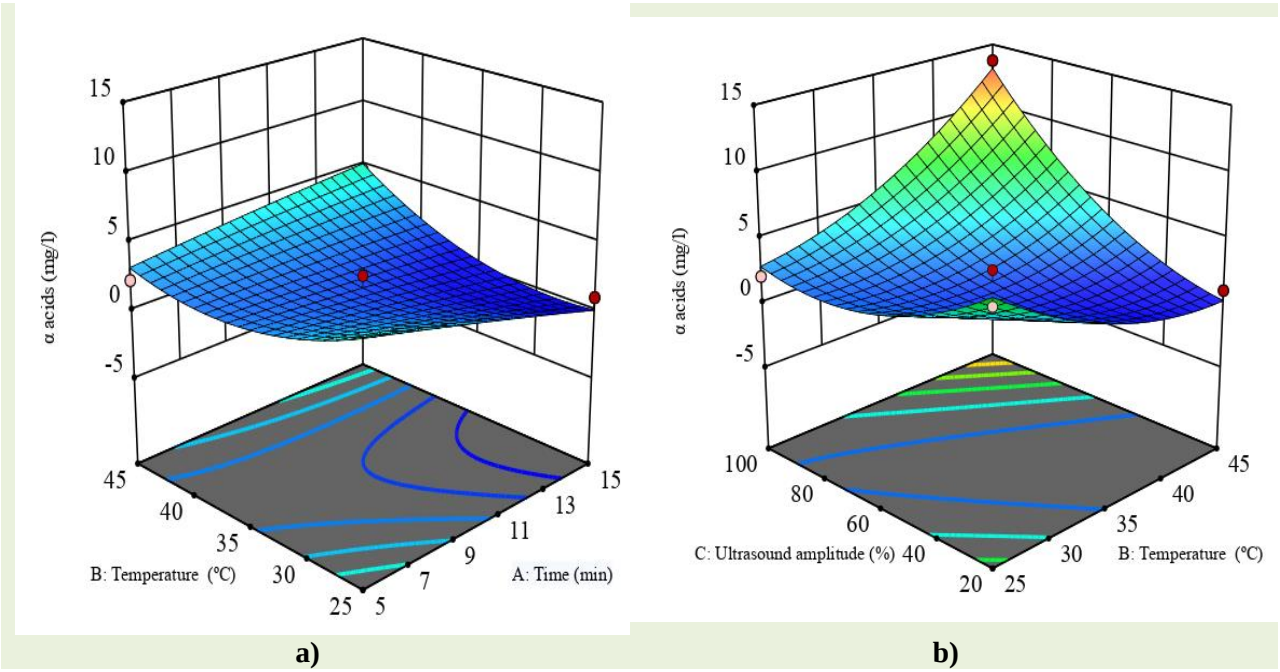
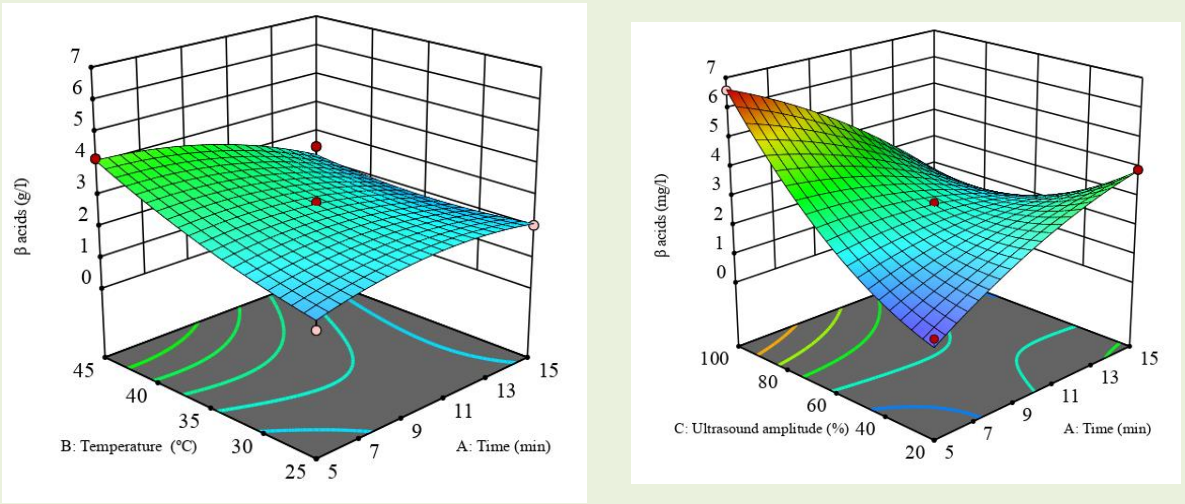


a) **b)**

Fig 5- Response surfaces of the **α-acids** (mg/l) of ultrasound- assisted hops extracts as a function of (a) ultrasonic extraction temperature with ultrasonic exposure time and b) ultrasonic amplitude with ultrasonic extraction temperature

6-3- The effect of independent parameters studied on beta acids

As can be seen from Table 2, the best model for fitting the data obtained from the amount of beta acids in hop extract was the quadratic polynomial model. On the other hand, Table 3 also indicated that all the studied linear parameters on the amount of beta acids in the extract were significant at the level of 5% and among the interaction parameters of temperatureProcessWith the intensity of sounding on this feature, it was not significant at the 5% level. The results showed that in the early timesProcessUltrasonic extraction with increasing temperatureProcessAlso, the intensity of sounding of beta acids increased, but at the end timesProcessWith the increase of these two parameters, the amount of these compounds decreased (Figure 6).The reason for the increase of this compound can be attributed to the formation of their disintegrating bubbles, which causes more of this compound to be released into the extract.]26[. The results of this section with the results of Carbon et al (2020) was in agreement]35[. According to the coefficients of the models given (model no. 6) in Table 3, it can be said that the greatest effect on the amount of hemolon was related to the parameter of the interaction of time with sounding intensity.



a) **b)**

Fig 6- Response surfaces of the **β-acids** (mg/l) of ultrasound- assisted hops extracts as a function of (a) ultrasonic extraction temperature with ultrasonic exposure time and b) ultrasonic amplitude with ultrasonic exposure time

7-3- process optimization Extraction of hop plant extract

Optimum conditions for hop extract extraction were determined by using the process of voicing the responses through numerical and graphical optimization of the software. Maximizing the extraction efficiency, the amount of total phenolic compounds, compounds that produce bitter taste, and antioxidant activity were considered as the desired goals of the experiments in the statistical analysis, and thus the conditions for performing the optimal process that were obtained from the response surface method using the utility function It has been obtained. the results showed thatProcessExtracting the extract from the hop plant at a point with a time of 12.73 minutes, a temperature of 25 degrees Celsius and an ultrasound intensity of 100% with a desirability factor of 0.778 results in the extractionThe extract has the highest efficiency and antioxidant activity. To ensure the correctness of the conditions, the experiment was repeated in optimal conditions and the obtained answers were shown in Table 5. The lack of significant difference between the models and experimental observations proves the efficiency of the models well (<0.05).P).

Table 5- Predicted and experimental values of the responses at optimum conditions for ultrasound-assisted and conventional extraction methods.

Extraction method	Extraction efficiency (%)	TPC (mg/100 g)	DPPH $_{Sc}$ (%)	FRAP (µmol/l)	a acids (mg/l)	β acids (mg/l)
Solvent extraction						
Experimental values[1]	13.67±0.76b	790.64±45.41b	24.90±2.19b	340.300±14.09b	0.78±0.05b	2.11±0.15b
Ultrasound-assisted extraction (UAE)						
Optimized values[2]	16.54a	1191.75a	40.69a		1.01a	3.09a
Experimental values[1]	15.78±0.15a	1147.49±50.48a	39.90±0.185a	409.87a 400.58±12.18a	0.89±0.042a	3.01±0.18a

[1] Mean ± standard deviation of triplicate determinations from experiments

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[2] Predicted using response surface quadratic model
* The data within a column with the same letters are not significantly different at $p < 0.05$.

4- General conclusion

In this research, the extraction conditions were optimized with the help of the voicing process with the response level statistical method based on the Baxbenken scheme. The obtained results confirmed the appropriateness of the selected statistical method for modeling the extraction conditions of bioactive compounds from hops. The proposed models had high explanatory coefficient values and a meaningless test of poor fit, which showed the effectiveness of the presented models in predicting the evaluated parameters. In optimal extraction conditions, with the help of ultrasonic process, the extract was obtained with a much higher efficiency and antioxidant activity compared to the conventional method (maceration). By using these models, in addition to setting the extraction conditions, the desired properties can be predicted and modified according to the conditions used in the extraction.

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مقاله علمی_پژوهشی

بهینه‌سازی فرآیند استخراج ترکیبات موثره گیاه رازک (*Humulus lupulus*) با کمک فراصوت

مهدی کاملان[۱]، احمد پدرام‌نیا[۲]، پروین شرایعی[۳]، امیرحسین الهامی‌راد[۲]، محمدرضا سعیدی اصل[۲]

۱- دانشجوی دکترای گروه علوم و صنایع غذایی، واحد سبزوار، دانشگاه آزاد اسلامی، سبزوار، ایران

۲- گروه علوم وصنایع غذایی، واحد سبزوار، دانشگاه آزاداسلامی، سبزوار، ایران

۳- بخش تحقیقات فنی و مهندسی کشاورزی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان خراسان رضوی، سازمان تحقیقات، آموزش و ترویج کشاورزی، مشهد، ایران.

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* مسئول مکاتبات:

ahmadpedram@yahoo.com

چکیده

پژوهش حاضر با هدف استفاده بهینه از گیاه دارویی رازک به عنوان عامل طعم دهنده (طعم تلخ) نوشیدنی ماءالشعیر و استخراج بهینه ترکیبات زیست فعال آن انجام پذیرفت. به منظور بهینه‌سازی راندمان استخراج، میزان ترکیبات فنلی، میزان ترکیبات ایجاد کننده طعم تلخ (آلفا و بتا اسیدها) و فعالیت آنتی‌اکسیدانی (مهار رادیکال‌های آزاد DPPH، قدرت احیاکنندگی آهن) با کمک فرآیند صوت‌دهی از طرح باکس‌بنکن استفاده شد. زمان صوت‌دهی (۵، ۱۵ و۲۵ دقیقه)، دمای استخراج (۲۵، ۳۵ و ۴۵ درجه سلسیوس) و شدت‌های مختلف صوت‌دهی (۲۰، ۶۰ و ۱۰۰ درصد) به عنوان متغیرهای استخراج ترکیبات زیست فعال در نظر گرفته شدند. نتایج نشان داد که با افزایش زمان و شدت صوت‌دهی راندمان استخراج، میزان ترکیبات زیست فعال و قدرت آنتی‌اکسیدانی عصاره افزایش یافت؛ در حالیکه با افزایش دما میزان استخراج و فعالیت آنتی‌اکسیدانی عصاره‌ها کاهش یافت. بر اساس مدل‌های حاصل، در شرایط بهینه استخراج با کمک فرآیند فراصوت (زمان استخراج ۱۲/۷۳ دقیقه، دمای استخراج ۲۵ درجه سلسیوس و شدت صوت‌دهی ۱۰۰ درصد) راندمان استخراج (۱۶/۵درصد)، میزان ترکیبات فنلی (۱۱۹۱/۷۵ میلی‌گرم بر ۱۰۰ گرم)، میزان ترکیبات ایجاد کننده طعم تلخ (الفا و بتا اسیدها به ترتیب ۱/۰۱ و ۳/۰۹ میلی‌گرم بر لیتر) و فعالیت آنتی‌اکسیدانی (بر اساس گیرندگی رادیکال آزاد DPPH و قدرت احیاء کنندگی آهن به ترتیب ۴۰/۷۰ درصد و ۴۰۹/۸۷ میکرومول بر لیتر) بالاتری نسبت به روش استخراج مرسوم (ماسراسیون، شاهد) بدست آمد. استخراج در نقطه بهینه نتایج بدست آمده را تایید نمود.