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ORIGINAL PAPER

Microwave-assisted D-pinitol extraction from carob: application of Box–Behnken design

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Abstract

This study investigates the effects of temperature (50–80 °C), time (10–60 min), carob-to-solvent ratio (2–8 g/40 mL), and solvent concentration (0–50 ethanol/water%, v/v) on microwave-assisted extraction of D-pinitol compound from carob fruit with optimization using Box–Behnken design. The optimal conditions for maximum D-pinitol yield (64.16 g/kg dry sample) include 50 °C temperature, 5.6 g carob/40 mL solvent, water used as a solvent, and an extraction time of 10 min. The fitness of the model was determined by ANOVA analysis with a high coefficient ($R^2 = 0.9057$). Extraction was performed under optimum conditions for model validation, and 63.89 g/kg dry sample of D-pinitol extraction was achieved. In addition, the effects of the same variables on the total phenolic (TP) content of MEA extraction of carob were also investigated. Extraction efficiency of 426 g/kg was obtained under the optimum conditions determined for TP (80 °C, 8 g of carob, 50% ethanol, and 10 min), but it was observed that TP content decreased to 49.7 g/kg under optimum conditions determined for D-pinitol. Sucrose, glucose, and fructose sugar contents of the extract were determined only for optimum conditions for D-pinitol, and the fructose content was found to be the lowest. Determining the D-pinitol, TP, and sugar contents of the extract in optimum condition confirms the direct usability of the extract, because only water is used as a solvent. Conventional extraction method was carried out at 50 °C for control purposes, yielding 42.83 g/kg of D-pinitol, thereby supporting the effect of the microwave on extraction.

Keywords D-Pinitol · Microwave-assisted extraction green chemistry · Total phenolics · Sugar contents

Introduction

The carob tree (*Ceratonia siliqua* L.) mainly grows in mild and dry places with unproductive soils in Mediterranean countries including Greece and Turkey [1]. The fruit of the carob tree has a variety of usages in the food industry, such as gum, syrup, powder, biofertilizer, ethanol, mannitol, lactic acid, and citric acid [2, 3]. D-Pinitol (1D-3-O-methyl-chiro-inositol), an important bioactive compound, is obtained from carob, which has significant biological and medical activities, is a kind of functional cyclitol, and is also very soluble in water [3–5]. This biodegradable, sugar-like, non-toxic compound is used as a food supplement because of its many functional properties [6, 7]. Many studies have shown that D-pinitol has positive effects on human health. This insulin-like compound regulates the blood sugar level in patients with Type-II diabetes by increasing insulin sensitivity [7, 8]. Some of the other beneficial effects of D-pinitol are the protection of susceptible organs such as the liver and kidney [9], decreasing the cholesterol levels and cardiovascular risk factors [8], and anti-inflammatory action [10]. D-Pinitol also has some biocontrol effects such as reducing worm moth growth and mosquito proliferation and stimulating butterfly oviposition [11, 12]. It is also an effective agent for controlling powdery mildew [4, 7].

D-Pinitol can be extracted and purified from plants such as soybean and carob trees by using the solvent extraction technique. Both the conventional, Soxhlet extraction, and the non-conventional extraction techniques, supercritical fluid extraction and ultrasound-assisted extraction, can be employed to extract D-pinitol [11–13].

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Phenolic substances contain one or more hydroxyl groups in the aromatic ring. It is known that the fruits have rich phenolic content. Recent scientific investigations showed that carob pods' extracts possess strong antioxidant activity [14–16]. The phenolic content of the product increases the antioxidant property as well as changes its flavor and color [17].

Many studies have shown that the non-conventional extraction techniques are clean, fast, solvent-saving, selective, and environmentally friendly methods for the extraction of bioactive compounds from plant material. These methods also have an improved extraction efficiency compared to conventional methods [18–22]. Microwave-assisted extraction (MAE), one of the non-conventional extraction techniques, has been applied as a method of extracting substances from different plant raw materials [23–26]. MAE has many advantages such as improved extraction efficiency, rapid energy transfer, effective heating, lower solvent consumption, reduced processing time, and low operating costs [23, 26]. Ruiz-Aceituna et al. [26] investigated the extraction of inositols from artichoke external bracts by MAE and pressurized liquid extraction (PLE) methods using water as the solvent. MAE method at 60 °C for 3 min and 0.3 g of sample, in 10 mL ultrapure water, allowed them to extract slightly higher concentrations of inositol than PLE at 75 °C for 26.7 min. They also showed that MAE was less time-consuming than PLE. Ru et al. [27] applied different methods for the extraction of flavonoids from pomelo peel and showed that MAE is a potential alternative to conventional extraction techniques due to lesser process time and amount of solvents used. Currently, MAE has gained attention in green technology to extract valuable natural compounds from plant matrices using green solutions only at laboratory scale; however, there are a few reports of industrial-scale applications and investments by some companies (e.g., Crodarom) [28–30].

In this study, water was used as a solvent. As a polar compound, the dielectric constant of water is 80 and because of which, water absorbs microwave energy strongly and heats up fast. Other advantages of using water as a solvent are that it is environmentally friendly, inexpensive, and readily available. Therefore, we used water as a solvent to extract D-pinitol from carob. Also, certain percentage of ethanol was also added to examine the effect of solvent concentration. The effects of temperature, time, carob-to-solvent ratio, and solvent concentration on microwave-assisted D-pinitol extraction and total phenolic content were investigated using the response surface method (RSM) with optimum working conditions. Sugar contents of extract were examined when the highest D-pinitol extraction efficiency was achieved.

Experimental

Materials

Carob fruit grown in the Mersin region was ground without seeds and stored at 4 °C for further use. Moisture content was gravimetrically determined (TS 1280, 2009). D-Pinitol (95%), Folin–Ciocalteu's phenol reagent, gallic acid, and fructose were purchased from Sigma-Aldrich (USA). Sucrose, glucose (99%), and ethanol were purchased from Merck (Germany). Ultrapure water (18 MΩ cm at 25 °C) was provided using a Millipore Milli-Q Advantage A10.

Microwave-assisted extraction

MAEs were carried out using a closed vessel microwave extraction system (Milestone, USA) equipped with a temperature control system and a magnetic stirrer. The power of MAE was fixed at 300 W that would allow us to work in the desired temperature range and minimize the time needed to obtain the set temperature. Carob and solvent (40 mL) were charged into a polytetrafluoroethylene (PTFE)-lined extraction vessel. Then, the vessel was closed, heated to the desired temperature for 5 min, and held at the desired temperature for a certain time period according to the experimental design. During the extraction, the temperature was monitored in a single (control) vessel with the help of a sensor. Continuous stirring during the extraction resulted in homogeneous heating. After the extraction, the solid residue and the liquid phase were separated by filtration. Each experiment was repeated three times and averaged. The analysis was carried out after the samples were filtered through a syringe filter (PTFE 0.45 µm, Sartorius).

Statistical design

Four independent variables were selected to examine the effects on D-pinitol extraction yield (Y_1) and total phenolic (TP) content (Y_2): temperature (x_1, °C), time (x_2, min), carob-to-solvent ratio (x_3, g/40 mL), and solvent concentration (x_4, ethanol/water%, v/v). Effects of these independent variables on D-pinitol and total phenolic were determined using the RSM and Box–Behnken design (BBD) (Design Expert 8, Germany). The low, medium, and high levels of each factor were coded as −1, 0, and +1, respectively. Experimental range levels of the independent variables are given in Table 1.

The correlation of response and independent variables can be represented by linear or quadratic models (Eq. 1):

$$Y = \beta_0 + \sum_i \beta_i x_i + \sum_i \beta_{ii} x_i^2 + \sum_i \beta_{ij} x_i x_j, \quad (1)$$

where Y symbolizes the approximation response, x_i depicts the coded independent variable effect, $x_i x_j$ demonstrate the

Table 1 Levels and codes of the independent variables in the BBD

Variables	Factors	Range and level		
		−1	0	+1
Temperature (°C)	x_1	50	65	80
Time (min)	x_2	10	35	60
Solvent concentration (EtOH/water%, vv)	x_3	0	25	50
Carob-to-solvent ratio (g/40 mL)	x_4	2	5	8

interaction effect, and x_i^2 represents the square effect. β_i, β_{ii}, and β_{ij} show the linear, square, and interaction coefficients, respectively. Finally, β_0 represents the constant coefficient [31, 32].

Classical hot water extraction

After dissolving 5.6 g of carob in 40 mL of deionized water, it was kept in a hot water bath at 50 °C for 10 and 20 min. The sample was filtered and HPLC analysis was performed.

Determination of D-pinitol and sugars by HPLC

For the quantitative analysis, five standard solutions ranging from 1 to 10 g/L concentration were prepared from the stock solution of D-pinitol. The stock solution contained a sugar mixture of 100 g/L sucrose, 10 g/L glucose, and 10 g/L fructose. Calibration range for fructose and glucose was 0.1–10 g/L, while for sucrose it was 1–100 g/L. Analyses of D-pinitol and sugars were carried out an HPLC (Agilent 1200 Series, USA) equipped with a CARBOSep CHO 87P (7.8 × 300 mm, Transgenomic, Nebraska, USA) column and a refractive index detector (RID) [4]. When the column temperature was 80 °C, Milli-Q ultrapure water was used as a mobile phase with a flow rate of 0.5 mL/min. The injection volume was 20 µL. The extract was directly analyzed in the case, where 2 g carob was used, and was diluted 1:2 and 1:5, respectively, when 5 and 8 g carobs were used.

Determination of total phenolic content

Total phenolic contents of the extracts were analyzed by Folin–Ciocalteu's method. The calibration curve was prepared from 25 to 800 mg/L by selecting gallic acid as standard. 1 mL Folin–Ciocalteu's phenol reagent and 1 mL of diluted (1:9) sample solution were mixed and stored in dark for 5 min. Then, 2 mL of sodium carbonate (20%, w/h) was added to this. This mixture was shaken and adjusted to 6 mL, by adding 2 mL of ultrapure water. After 30 min, the absorbance was measured using UV–visible spectrophotometer (Shimadzu, Japan) at 714 nm. The concentration of total phenolic content was expressed as milligram of gallic acid equivalents [33, 34].

Results and discussion

A 4-factor and 3-level BDD with actual/coded values and results of the microwave-assisted extraction of D-pinitol and TP are shown in Table 2. The highest and lowest experimental D-pinitol yields' dry weights were obtained as 64.42 and 40.44 g/kg, respectively. The values of the extract in terms of TP content varied between 67.87 and 343.11 g/kg dry weight.

Evaluation of ANOVA

Statistical analysis of the results was done based on the analysis of variance (ANOVA). Table 3 shows the results of the employed quadratic models. The F values of the models for D-pinitol and TP were obtained as 20.29 and 15.15, respectively. There is only 0.01% chance that the F values could occur due to noise. The fact that the F values of the obtained models were higher than the tabulated ones ($F_{0.05,\, df,\, (n-(df+1))} = 2.423$) is a proof of the model fit. Other indicators of the model fit were high R^2 (adjusted R^2) coefficients, obtained as 0.9057 (0.8611) and 0.8777 (0.8197) for D-pinitol and TP, respectively.

The fit model equations for D-pinitol yield (Eq. 2) and TP content (Eq. 3) are as follows:

$$\text{D-Pinitol}\left(\frac{\text{g}}{\text{kg}}\right) = +57.53 - 1.91x_1 + 0.052x_2 - 1.42x_3 + 3.93x_4 + 1.94x_1x_2 + 3.06x_1x_3 + 1.82x_3x_4 - 1.78x_3^2 - 6.99x_4^2 \quad (2)$$

$$\text{TP}\left(\frac{\text{g}}{\text{kg}}\right) = +149.48 + 41.88x_1 + 0.31x_2 + 42.74x_3 + 47.13x_4 - 39.26x_1x_2 + 22.13x_1x_3 + 21.73x_1x_4 - 20.04x_3^2 + 82.07x_4^2. \quad (3)$$

The fact that the difference between R^2 and adjusted R^2 values is less than 0.2 indicates that the proposed equations are sufficient to evaluate the relationship between process-independent variables and responses.

The effects of variables on the results from the first and second order and interaction with other variables are shown in the Pareto graph in Fig. 1. It seems that for the maximum extraction of D-pinitol and TP, the most effective variable was the carob-to-solvent ratio (x_4). Actually, the quadratic (x_4^2) effect of this variable has been more effective than the first-order effect (x_4) (Fig. 1). When all of the independent

Table 2 Box–Behnken design for microwave-assisted extraction of carob and observed response of the D-pinitol and total phenolic contents

Run	Factors				Responses (dry weight)	
	Temperature (°C)	Time (min)	Solvent concentration (EtOH/water%)	Carob-to-solvent ratio (g/40 mL)	D-Pinitol (g/kg)	TP (g/kg)
	x_1	x_2	x_3	x_4		
1	65 (0)	35 (0)	0 (−1)	8 (+1)	50.75	210.30
2	65 (0)	10 (−1)	25 (0)	2 (−1)	45.82	176.39
3	80 (+1)	35 (0)	25 (0)	8 (+1)	51.37	343.11
4	65 (0)	35 (0)	25 (0)	5 (0)	56.67	120.93
5	80 (+1)	35 (0)	50 (+1)	5 (0)	56.32	220.00
6	65 (0)	60 (+1)	25 (0)	2 (−1)	49.27	187.27
7	65 (0)	10 (−1)	0 (−1)	5 (0)	58.18	67.87
8	50 (−1)	35 (0)	0 (−1)	5 (0)	64.42	109.44
9	65 (0)	35 (0)	50 (+1)	2 (−1)	40.44	275.00
10	80 (+1)	10 (−1)	25 (0)	5 (0)	54.55	278.43
11	80 (+1)	60 (+1)	25 (0)	5 (0)	58.72	171.02
12	65 (0)	35 (0)	0 (−1)	2 (−1)	47.00	95.61
13	50 (−1)	35 (0)	25 (0)	2 (−1)	48.90	137.50
14	50 (−1)	10 (−1)	25 (0)	5 (0)	59.58	97.78
15	65 (0)	35 (0)	50 (+1)	8 (+1)	51.47	311.69
16	65 (0)	35 (0)	25 (0)	5 (0)	56.63	159.35
17	65 (0)	60 (+1)	0 (−1)	5 (0)	55.14	87.41
18	65 (0)	10 (−1)	50 (+1)	5 (0)	55.59	111.02
19	80 (+1)	35 (0)	25 (0)	2 (−1)	44.69	194.68
20	65 (0)	60 (+1)	25 (0)	8 (+1)	56.70	278.82
21	65 (0)	60 (+1)	50 (+1)	5 (0)	53.19	152.32
22	80 (+1)	35 (0)	0 (−1)	5 (0)	53.56	113.70
23	65 (0)	35 (0)	25 (0)	5 (0)	59.05	129.54
24	50 (−1)	35 (0)	50 (+1)	5 (0)	54.95	127.22
25	65 (0)	10 (−1)	25 (0)	8 (+1)	54.69	289.01
26	50 (−1)	35 (0)	25 (0)	8 (+1)	58.25	199.02
27	65 (0)	35 (0)	25 (0)	5 (0)	53.91	145.28
28	65 (0)	35 (0)	25 (0)	5 (0)	57.25	142.13
29	50 (−1)	60 (+1)	25 (0)	5 (+1)	56.01	147.41

variables had positive effects on TP yield, temperature (x_1) and the solvent concentration (x_3) showed a negative effect on D-pinitol extraction yield (Eqs. 2, 3).

D-Pinitol and total phenolic content of carob

Figure 2a–c demonstrates the 3D plots displaying the effect and interaction of independent variables on the D-pinitol yield. The interaction effects of temperature and extraction time at fixed carob-to-solvent ratio (5.6 g/40 mL) and solvent concentration (0% ethanol/water) are shown in Fig. 2a. The interaction between temperature and time (x_1x_2) gave a positive effect (Eq. 2) on the results and found to be insignificant due to P value (>0.05) in Table 2. When the linear effects of these terms were examined, the extraction time was also found to be insignificant ($P=0.9260$, Table 3). But, it was seen here that temperature was an important parameter due to F value with a significant P value in Table 3. Figure 2a shows that lower temperature and shorter extraction time were more effective for a better D-pinitol yield. For instance, increasing temperature from 50 °C up to 80 °C decreased D-pinitol yields, respectively, from 64 to 50 g/kg in a 10-min extraction treatment. Chen et al. [4] studied the effect of temperature (50–90 °C), time (40–120 min), and dilution rate (1:10–1:30 w/v) on D-pinitol extraction from vegetable soybean leaves by RSM and determined the optimum conditions as temperature of 65.5 °C, extraction time of 86.8 min, and dilution rate of 1:10 w/v. Chafer et al. [35] similarly reported that the solubility of D-pinitol in supercritical (SC)-CO_2 extraction diminished when the temperature was increased. This result was explained by the decrease in SC-CO_2 density and solvent power due to temperature increase. Tetik and

Table 3 ANOVA results of quadratic models for D-pinitol and total phenolic by BBD

Source	*df*	D-Pinitol (g/kg)			Total phenolic (g/L)		
		Sum of squares	*F* value	*P* value prob > *F*	Sum of squares	*F* value	*P* value prob > *F*
Model	9	660.42	20.29	< 0.0001	1.33×10^5	15.15	< 0.0001
x_1: Temperature	1	43.70	12.08	0.0025	21,048	21.57	0.0002
x_2: Time	1	0.03	0.009	0.9260	1.17	0.0012	0.9727
x_3: Solvent concentration	1	24.34	6.73	0.0178	21,924	22.47	0.0001
x_4: Carob-to-solvent ratio	1	184.95	51.13	< 0.0001	26,649	27.31	< 0.0001
x_1x_2	1	14.98	4.14	0.0561	6165	6.32	0.0211
x_1x_3	1	37.39	10.34	0.0046	1958	2.01	0.1727
x_1x_4	1	–	–	–	1888	1.94	0.1803
x_3x_4	1	13.25	3.66	0.0708	–	–	–
x_3^2	1	21.88	6.05	0.0237	2771	2.84	0.1083
x_4^2	1	337.03	93.17	< 0.0001	46,484	47.64	< 0.0001
Residual	19	68.73			18,539		
Lack of fit	15	55.11	1.08	0.5253	17,661	5.36	0.0582
Pure error	4	13.61			878		
Cor total	28	729.15			1.52×10^5		

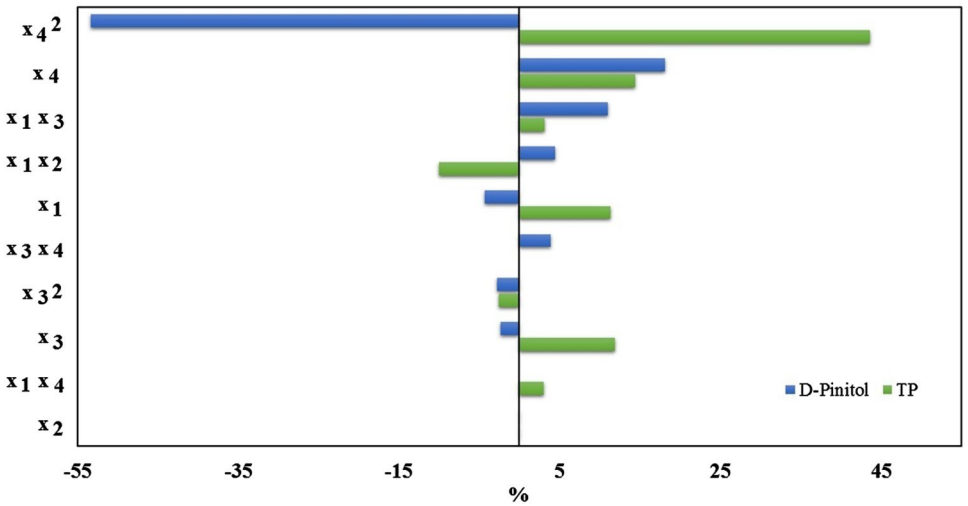

Fig. 1 Graphical Pareto analysis for D-pinitol yield and total phenolic (TP) content

Yüksel [13] examined the temperature effect (30–50 °C) in ultrasound-assisted extraction of D-pinitol from carob and obtained the highest D-pinitol concentration at 50 °C. In the aforementioned studies, using the similar temperatures, despite different extraction methods, suggests that the elevation of the temperature causes degradation of the extracted D-pinitol compound.

Water and water–ethanol mixtures were also tested as solvents in the microwave-assisted extraction of D-pinitol from carob. Figure 2b represents the interaction effect between temperature and solvent concentration. Maximum D-pinitol extraction yield was obtained using 100% water (0% EtOH/water) as a solvent. Solvent concentration is an effective and significant ($P < 0.05$) parameter, but has shown a negative effect (Eq. 2) on the results. D-Pinitol extraction yield was 64 g/kg with 0% ethanol, and in case of using 25% and 50% ethanol the yield was 62 g/kg and 56 g/kg at 50 °C, respectively. It can be thought that the percentage of ethanol in the solvent concentration increases and the polarity of the solvent mixture decreases, which results in reduced D-pinitol extraction efficiency. The ability of a solvent to absorb and convert microwave energy into heat depends in part on the dissipation factor (tan δ). This factor is equal to the ratio between the dielectric constant (ε') and the dielectric loss factor (ε''), which represents the conversion of electromagnetic energy to heat [36]. The values of dielectric constant and dielectric loss factor for water and ethanol are 78.3, 1.69 and 24.3, 1.87, respectively. The dissipation factor values ($\times 10^{-4}$) for water and ethanol are 1570 and 2500, respectively. As

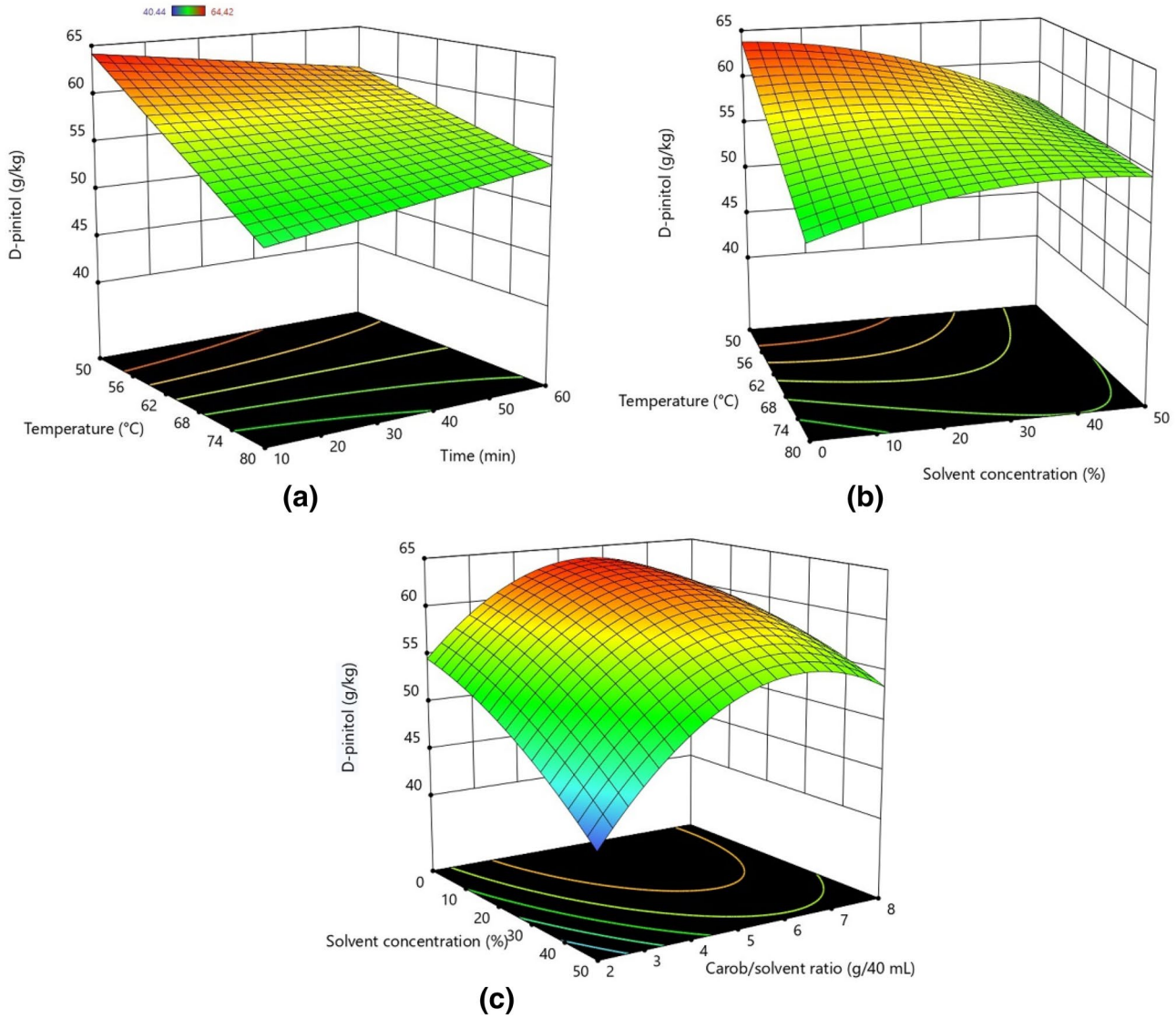

Fig. 2 Interaction effects of **a** temperature and time (5.6 g carob/40 mL solvent, 0% EtOH/water), **b** temperature and solvent concentration (5.6 g carob/40 mL solvent, 10 min), and **c** solvent concentration and carob-to-solvent ratio (50 °C, 15 min) on D-pinitol yield

a result, the negative effect of the amount of ethanol in the solvent can also be explained by the rise in ambient temperature.

The interaction effect of solvent concentration and carob-to-solvent ratio at 15 min and 50 °C is shown in Fig. 2c. The interactive effect of these two factors (x_3x_4) is not very meaningful ($P > 0.05$) and effective on the model which can also be seen from Eq. 2 and the F value in the ANOVA analysis. The effect of the carob-to-solvent ratio on the yield of D-pinitol was found to be more quadratic ($P < 0.0001$). As can be seen, the yield increased with increasing the amount of carob from 2 to 5.6 g, but showed a tendency to decrease over this amount. If the amounts of carob used were 2, 5.6, and 8 g, the yields obtained were 55, 64, and 59 g/kg at 50 °C, respectively. In case the amount of carob used increases, the mass transfer rate slows down due to the volume of the solvent, which was kept the same. Especially in MAE, if the amount of solvent is kept the same, the increase in the amount of feed (solid) prevents sufficient stirring of the solvent by microwaves and at the same time the recovery is reduced due to excessive swelling of the solid [36, 37].

Desirability function and numerical optimization methods were used to optimize variables by targeting the maximum extraction yield of D-pinitol. The optimum conditions for the highest D-pinitol yield were determined as temperature of 50 °C, carob-to-solvent ratio of 5.6 g/40 mL, solvent concentration of 100% water, and extraction time of 10 min. Validation experiments carried out under optimum conditions yield 63.89 ± 0.89 g/kg D-pinitol that is consistent with the estimated value of the model (64.16 ± 1.90 g/

kg). D-pinitol extraction from carob was not previously performed by the MAE method, and it is known that D-pinitol content varies depending on the origin of the carob used [3]. However, the results obtained by different methods can be compared with the results obtained in this study. Tetik and Yüksel [13] obtained 11.98 g/L (47.92 g/kg) D-pinitol yield using 25 g carob (fruit/water ratio 1:4) by the ultrasound-assisted extraction method. Turhan [3] determined the highest D-pinitol concentration as 9.23 g/L when the extraction conditions were a temperature of 90 °C, dilution rate of 1:4, and extraction time of 120 min. Baumgartner et al. [38] extracted roasted carob with five times their weight of water at 50 °C for 2 h. Then, the extract was fermented with dried yeast at 30 °C for 7 days and obtained D-pinitol yield in the range of 5–7.5%.

Figure 3a–c demonstrates the 3D plots displaying the effect and interaction of independent variables on the total phenolic (TP) content. The interactive effect of treatment time and temperature (x_1x_2) on the TP content is shown in Fig. 3a at fixed carob-to-solvent ratio (8 g/40 mL) and solvent concentration (50% EtOH/water). The interactive effect of these two terms is significant according to the *P* value, and its contribution to the model is negative (Eq. 3) and important. Increasing temperature from 50 up to 80 °C raised TP content from 176 to 426 g/kg under fixed extraction time of 10 min, solvent concentration of 50% EtOH/water, and carob-to-solvent ratio of 8 g/40 mL. Temperature is an important parameter in the MAE, like all extraction techniques, and increases the yield. Increasing the temperature increases the solvent's ability to dissolve the target

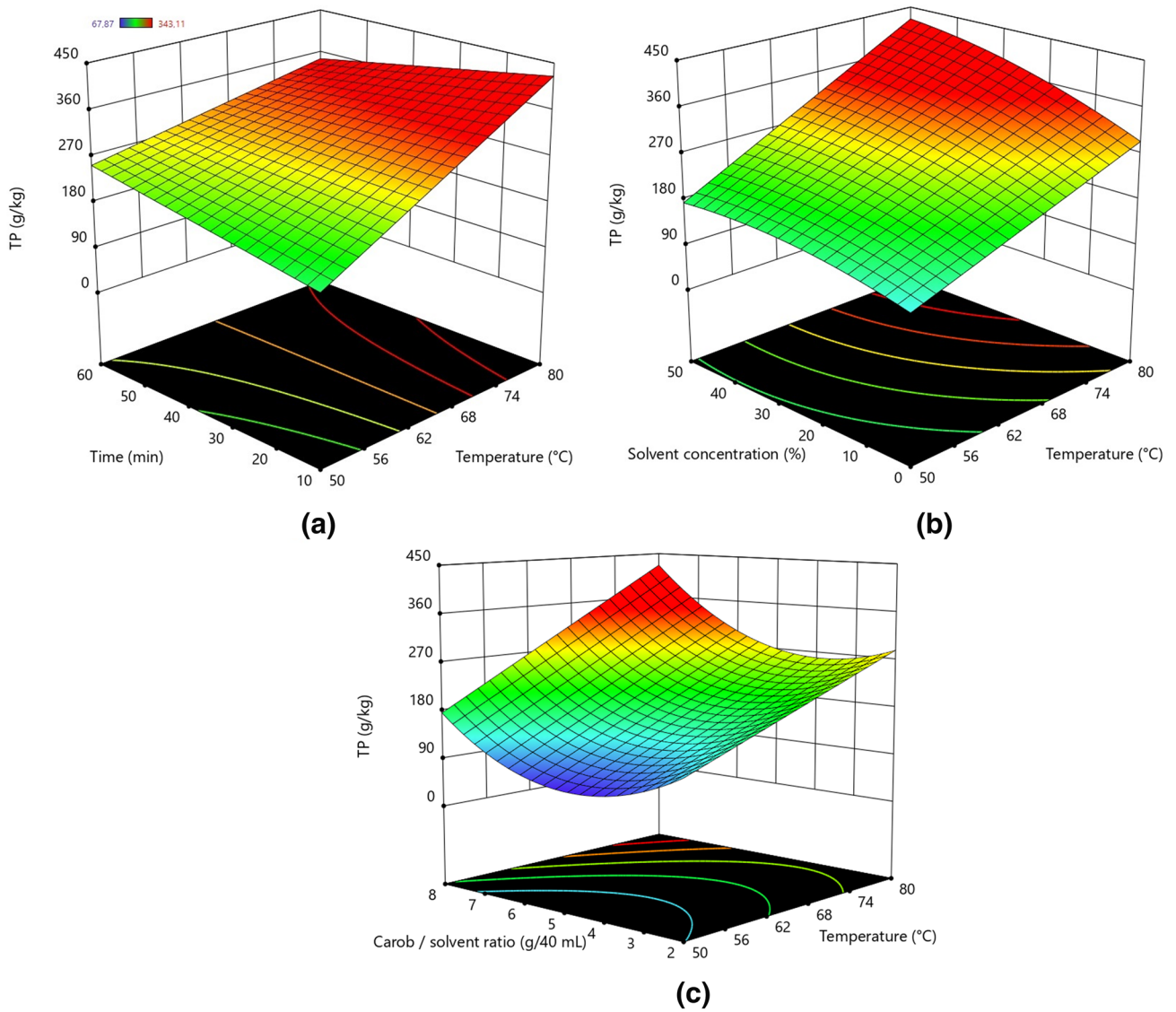

Fig. 3 Interaction effects of **a** temperature and time (8 g carob/40 mL solvent, 50% EtOH/water), **b** temperature and solvent concentration (8 g carob/40 mL solvent, 10 min), and **c** temperature and carob-to-solvent ratio (50% EtOH/water, 10 min) on TP yield

compounds. This is due to a decrease in surface tension and solvent viscosity, which increases the wetting of the sample and the penetration of the solvent into the matrix [36]. The extraction time did not affect the amount of TP much at low temperatures, but an adverse was observed as the temperature increased. To reach higher TP yield at 80 °C, the extraction time should be kept lower. Increasing the time may cause thermal decomposition of some compounds. This negative effect of time on yield has also been reported in the MAE processes of flavonoids [37] and triterpenoid saponins [39].

The effects of temperature and solvent concentration on the TP yield are shown in Fig. 3b at fixed carob-to-solvent ratio (8 g/40 mL) and extraction time (10 min). It is clearly illustrated that increasing the percentage of ethanol in the solvent concentration resulted in an increase in the TP content at high temperature. When the solvent concentrations were 0%, 25%, and 50%, the determined TP values at 60 °C were 189 g/kg, 244 g/kg, and 259 g/kg, respectively. Similar results have been reported by Huma et al. [40]. They used the MAE method for extracting phenolic compounds from carob kibbles and achieved more efficient recovery using ethanol and water mixture. The optimum conditions obtained by RSM are as follows: microwave power of 367 W, solvent concentration (ethanol/water) 44%, and sample-to-solvent ratio of 50 mL/g [40].

Table 3 shows that the effect of carob-to-solvent ratio (x_4) on the TP content was high due to F value and both linear and quadratic effects were significant ($P < 0.0001$). The increase, especially beyond the 5 g/40 mL in the carob-to-solvent ratio, in the extraction led to an increase in the TP content of the extract (Fig. 3c). While the carob-to-solvent ratio is low, the decomposition of the extracted compounds may occur due to the higher solvent volume and heated zone. Increased carob amount while the solvent volume remains constant may have reduced the possibility of heat exposure and degradation. The maximum amount of TP as 426 g/kg was obtained at working conditions where the time was kept to a minimum when the solvent concentration, the carob-to-solvent ratio, and temperature were kept at the maximum level.

In this study, since the main objective is the maximum D-pinitol extraction efficiency, the TP value extracted under these conditions is important and measured as 49.7 g/kg.

Sugar content of extract

It has been reported that D-pinitol is non-toxic and can be used in diabetic nutrition by reducing glucose levels in the blood and it has many medical functions [3–6]. Turhan [3] compared D-pinitol contents obtained from wild with cultivated types of carob beans using the ULTRA-TURRAX macerator. He showed that there was a positive correlation between glucose and D-pinitol for both carob types [3]. The obtained extract in this study can be used directly; therefore, besides the D-pinitol and TP contents, the three sugar contents in the extract at optimum condition were quantitatively analyzed. Sucrose, fructose, and glucose were found as 342.4 g/kg, 116.2 g/kg, and 52.9 g/kg, respectively, in the extract.

Comparison of MAE versus conventional extraction

In this study, to compare the microwave method with the conventional method, experiments were conducted at determined optimum conditions using both methods. The extraction conditions were carob-to-solvent ratio of 5.6 g/40 mL, solvent concentration of 100% water, extraction temperature of 50 °C, and extraction time of 10 min. The extraction yield achieved by the conventional method was 42.83 g/kg. When the extraction time was extended to 20 min, no significant increase in D-pinitol yield was observed. On the other hand, the extraction yield achieved by the MAE was significantly higher, being 63.89 g/kg. In short, MAE is more efficient than the conventional extraction method for the extraction of D-pinitol.

Conclusion

Microwave-assisted extraction of D-pinitol from carob pods, growing naturally in Mersin city, was carried out. The yield of D-pinitol and total phenolic content in dry weight were optimized by using RSM based on Box–Behnken design, which ensures the estimation of each parameter of the obtained quadratic model. The optimum conditions for D-pinitol yield as 63.89 g/kg were determined as 50 °C for 5.6 g/40 mL of carob-to-solvent ratio, with water used as a solvent and 10 min extraction time. Compared to the D-pinitol value (42.83 g/kg) obtained by the conventional solid water extraction method, it can be said that the MAE method is very effective. In the case of total phenolic (421 g/kg), the optimum condition was obtained at 10 min of time, with 50% solvent concentration of ethanol, at 80 °C temperature, and with 8 g/40 mL of carob-to-solvent ratio. However, 49.7 g/kg of TP was obtained under the optimum conditions for D-pinitol. At the same time, glucose, fructose, and sucrose contents of the extracts were determined and glucose was found to be at the lowest amount (52.9 g/kg) in these three sugar structures. The main purpose of this study was considered to be the highest D-pinitol yield. However, it can be recommended in conditions where high yields can be achieved both for D-pinitol and for TP. For example, the recommended conditions for 57 g/kg D-pinitol and 260 g/kg TP yield were as follows: 40 °C of temperature, 6 g/40 mL of carob-to-solvent ratio, 40% of solvent concentration, and

40 min of extraction time. As a result, the D-pinitol extraction was easily achieved with the microwave-assisted extraction method in which water was used as a solvent in a short time and at a low temperature.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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