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Oil Extraction and Evaluation from Yellow Horn Using a Microwave-Assisted Aqueous Saline Process

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Abstract: This study investigates an aqueous salt process (ASP) combined with microwave-assisted extraction (MAE) for the seed oil extraction from yellow horn (*Xanthoceras sorbifolium* Bunge). The NaCl concentration in the oil extraction process affected the oil extraction yield. Box–Behnken design (BBD) and response surface methodology (RSM) were used to optimize the extraction process. The optimal operating parameters were: 24 g/L NaCl, 300 W microwave power, 4:1 water to material ratio, an 80 min extraction time, and 45 °C extraction temperature. The chemical composition of the extracted seed oil was analyzed using gas chromatography–mass spectrometry (GC-MS). This extraction technique for yellow horn seed oil provided high throughput and high-quality oil. The present research offers a kind of green extraction method for edible oil in the food industry.

Keywords: yellow horn; aqueous salt process; Box–Behnken design; antioxidant activities; fatty acids

1. Introduction

Yellow horn (*Xanthoceras sorbifolium*) is a woody perennial shrub in the soapberry family, Sapindaceae. It is native to northwestern China, where it is well-adapted to cold, drought, salt, and starvation [1]. Yellow horn can live well below −40 °C except on saline–alkali soils or waterlogged fields. It is an important oil crop in China because of its abundant seed oil content (55–65%) [2]. The high content of linoleic acid is favorable for medicinal and nutritional application due to its cardioprotective, antidiabetic, and antimicrobial properties [3]. In addition, the kernel contains nutritionally valuable substances such as carbohydrates, fats, proteins, steroids, terpenoids, coumarins, flavonoids, organic acids, anthraquinones, and other compounds [4].

There are several industrial extraction methods (Table 1) for yellow horn oil including the expeller pressing method, solvent extraction, supercritical extraction, and water generation method. These methods have limited application because the setup costs are high, and the use of organic solvents contaminates the environment and is harmful to human health [2,5]. Furthermore, the defatted oilseed kernels, which contain nutritional and healthy constituents, have to be discarded as useless residues after oil extraction. For these reasons, a green and economical extraction method is needed [1]. An aqueous salt process (ASP) is a simple and green demulsification technology of water-in-oil emulsions. The demulsification efficiency may reach 100% in a very short time under microwave radiation. Microwave assisted extraction (MAE) is an accepted alternative to conventional extraction techniques, whereby microwave irradiation generates an electromagnetic field to accelerate the movement of molecules during the extraction process [6]. A methodology that combines these two techniques (ASP–MAE) may provide a viable alternative to the current methods.

Table 1. Major differences between the four extraction methods for yellow horn seed oil.

Oil Extraction Method	Oil Yield	Time (h)	Temperature (°C)	Cost
Expeller pressing method	57.25%	1–2	room temperature	Low
Solvent extraction	60.43%	5–10	70	High
Supercritical extraction	56.47%	2.5–3	50	High
Water generation method	58.74%	1–2	40–60	Low

Demand for efficient and green oil extraction techniques has increased [7], so the potential for using microwave and salt effects in the aqueous extraction in this research is just meeting the demand. The objective of this research is to optimize MAE under varying salt conditions of seed oil, which might provide valuable data for the economic process, green design, and pilot-scale, and the salt effects will also be evaluated.

This study aims to develop an ASP-MAE method for extracting oil from yellow horn seeds. The main factors affecting MAE efficiency are temperature, duration, and solvent [8]. Hence, our extraction variables included extraction temperature, the water to material ratio, and the extraction time. Response surface methodology (RSM) is an ideal tool for process optimization. Hence, we used it to optimize the extraction process. Furthermore, we analyzed the fatty acid composition to investigate the quality of the extracted seed oil using gas chromatography–mass spectrometry (GC-MS).

2. Results and Discussion

2.1. Optimisation of ASP-MAE

2.1.1. Effect of Single Factors on Oil Yield

Materials were extracted by MAE at 100–500 W and oil was separated, meanwhile the other invariant extraction parameters were the water to material ratio of 4:1 (v/w), NaCl concentration of 25 g/L, and extraction duration of 60 min. The extraction yield of oil was positively correlated with increasing irradiation power, reaching $84.11 \pm 3.21\%$ at 300 W (Figure 1a).

The ratio of water to material is an important factor that can influence the extraction efficiency. The effect of the ratio of water to material on oil yield is shown in Figure 1b. The ratios of water to material were set at 2:1, 3:1, 4:1, 5:1, 6:1 (v/w), with the other extraction parameters constant: 300 W microwave power, 25 g/L NaCl, 45 °C extraction for 60 min. Oil yields increased with increasing ratio of water to material. The extraction yield reached its peak value ($83.97 \pm 2.41\%$) when the ratio of water to material was 4:1. When the ratios of water to material were higher than 4:1, the oil extraction yield decrease gradually, which may be due to the decrease of oil–water separation effect caused by the high ratio of water to material.

The concentration of NaCl is an important factor that can influence the extraction efficiency. The effect of NaCl concentration on oil yield is shown in Figure 1c. NaCl concentrations were set at 20, 25, 30, 35, and 40 g/L with the other extraction parameters constant: 300 W microwave power, 4:1 (v/w) ratio of water to material, 45 °C extraction for 60 min. The extraction yield of oil was positively correlated with increasing NaCl concentration. The maximum yield reached $84.53 \pm 2.13\%$ with 25 g/L NaCl. This is mainly due to the presence of inorganic salts; on the one hand break down of the electric double layer of emulsion interface increased the density difference between oil and water two phase; and on the other hand, inorganic salt increased the polarity difference of the oil and water, the solubility of the oil in water is reduced, enhancing salt-assisted effect for demulsification under microwave radiation [9].

The effect of extraction time on the yield of oil was studied with the extraction power of 300 W, and the other conditions were fixed at a water to material ratio of 4:1 (v/w) and extraction temperature 45 °C. Extraction times from 20 to 100 min had a positive linear effect on the oil yield (Figure 1d). Longer extraction times may have induced oil degradation, which will reduce yields.

The effect of extraction temperature on oil yields was studied with the extraction power of 300 W, and the other conditions were fixed at a water to material ratio of 4:1 (v/w), and extraction time 80 min. The extraction temperature had a significant linear effect on the oil yield from 35 to 55 °C, with a maximum yield ($83.10 \pm 3.305\%$) at 55 °C; after this point, oil yield started to decline (Figure 1e).

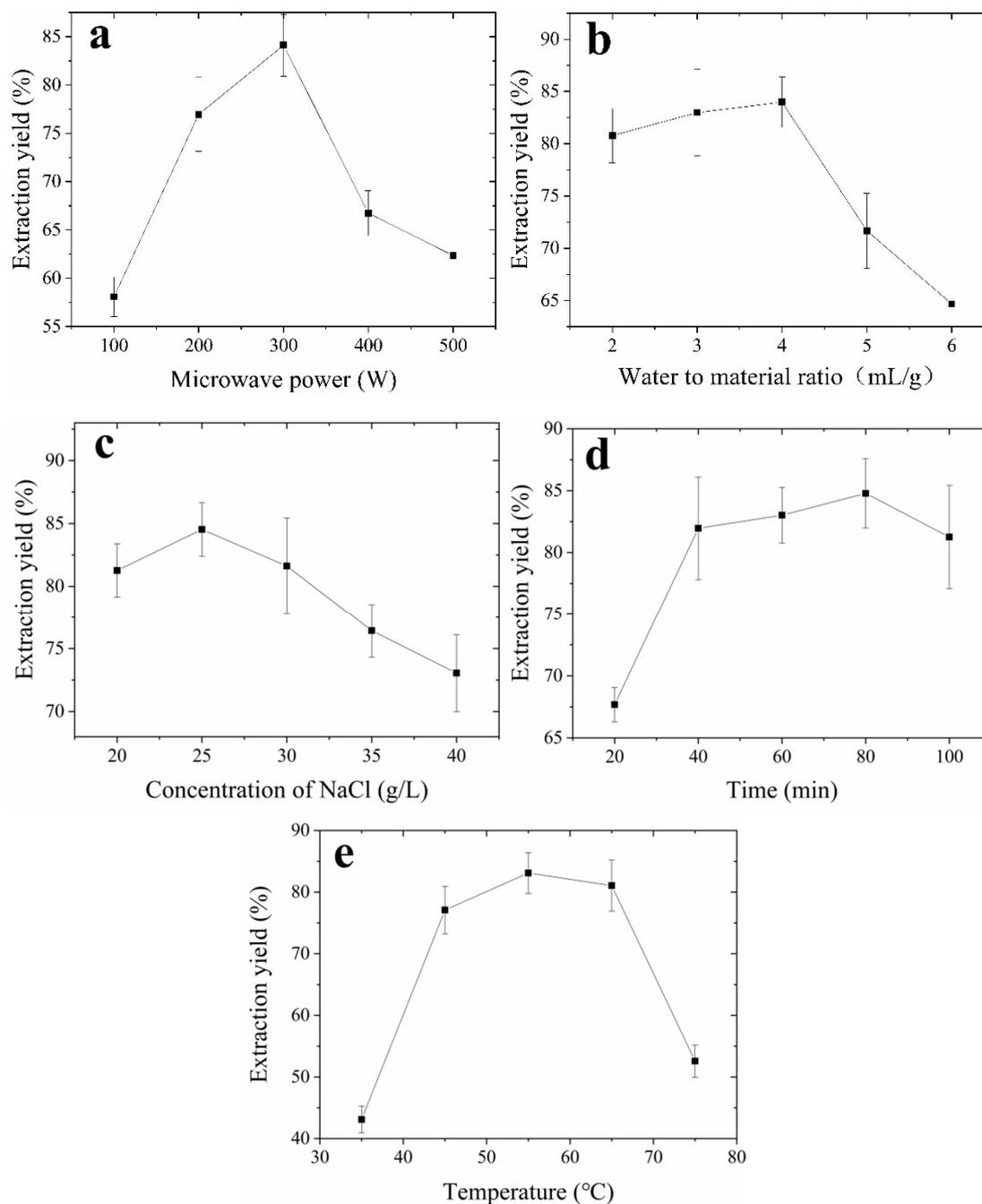


Figure 1. The effect of microwave power, water to material ratio, NaCl concentration, extraction time, and extraction temperature on oil extraction. (a) water to material ratio of 1:4 (w/v), 25 g/L NaCl, 60 min extraction at 45 °C; (b) 300 W microwave, 25 g/L NaCl, 60 min extraction at 45 °C; (c) 300 W microwave, water to material ratio of 1:4 (w/v), 60 min extraction at 45 °C; (d) 300 W microwave, water to material ratio of 1:4 (w/v), 25 g/L NaCl, 45 °C extraction; (e) 300 W microwave, water to material ratio of 1:4 (w/v), 25 g/L NaCl, 60 min extraction. Data are means \pm SD (n = 3).

2.1.2. Statistical Analysis and Model Fitting

To highlight the most influential factors and possible interactions in this study, a BBD model was designed. Thus, the ratio of material to solvent, microwave power, and extraction time and temperature were included in the model. There were 29 permutations of the four individual parameters that entered into the BBD, according to the factorial design shown in Table 2.

Table 2. Operating parameters and the experimental and predicted values for oil extraction yields for the different experimental treatments.

Run	Microwave Power (W)	Water to Material Ratio	NaCl (g/L)	Time (min)	Extraction Yield (%)	Predicted Yield (%)
1	300	3:1	25	60	73.67 ± 2.77	74.36
2	300	3:1	30	80	71.04 ± 3.02	71.46
3	300	4:1	25	80	86.73 ± 2.89	86.37
4	200	4:1	30	80	71.34 ± 3.45	69.21
5	300	4:1	30	60	69.64 ± 3.30	69.77
6	200	3:1	25	80	69.67 ± 3.19	70.39
7	300	4:1	20	100	73.17 ± 2.67	73.60
8	400	4:1	25	60	68.64 ± 2.83	68.25
9	300	5:1	25	100	77.89 ± 2.92	76.76
10	300	4:1	25	80	85.88 ± 3.25	86.37
11	300	4:1	30	100	71.77 ± 3.33	71.90
12	300	4:1	25	80	86.54 ± 3.74	86.37
13	300	3:1	25	100	70.71 ± 3.56	71.31
14	200	5:1	25	80	73.47 ± 3.38	75.55
15	200	4:1	25	60	71.41 ± 2.83	71.57
16	300	5:1	20	80	77.73 ± 2.80	77.18
17	400	4:1	30	80	70.48 ± 3.43	71.13
18	300	4:1	25	80	84.97 ± 4.09	86.37
19	400	5:1	25	80	73.23±2.65	73.07
20	300	4:1	20	60	74.79 ± 1.91	75.23
21	300	3:1	20	80	78.98 ± 3.42	78.05
22	300	5:1	25	60	74.23 ± 2.36	73.20
23	400	4:1	25	100	72.65 ± 3.02	72.36
24	300	4:1	25	80	87.75 ± 1.64	86.37
25	400	3:1	25	80	75.45 ± 2.37	73.94
26	200	4:1	20	80	75.25 ± 1.29	74.17
27	200	4:1	25	100	67.71 ± 1.06	67.97
28	300	5:1	30	80	75.82 ± 2.99	76.62
29	400	4:1	20	80	71.63 ± 1.78	73.32

Table 3 depicts the ANOVA data for the fitted model. The *P*-value of <0.0001 indicates that the model was significant and the lack-of-fit 0.2523 showed was not significant. The ANOVA for oil extraction yields produced a correlation coefficient (R^2) of 0.9724 with the calculated model, which is in close agreement with the experimental results. In addition, a lack-of-fit statistics was used to test the adequacy of the model, high significant levels for these ($p < 0.001$) were obtained by statistical analysis. The results suggest that the model can well explain for the prediction of oil extraction from this method. The response and test variables are related according to the following second-order polynomial equation:

$$Y = 86.37 + 0.27X_1 + 1.07X_2 - 1.79X_3 + 0.13X_4 - 1.51X_1X_2 + 0.69X_1X_3 + 1.93X_1X_4 + 1.51X_2X_3 + 1.66X_2X_4 + 0.94X_3X_4 - 8.50X_1^2 - 4.63X_2^2 - 5.91X_3^2 - 7.83X_4^2 \quad (1)$$

where X_1 is microwave power (W), X_2 is the water to material ratio (ml/g), X_3 is NaCl (%), X_4 is time (min), and Y is yield of extraction (%).

Table 3. ANOVA of quadratic model for the compositions of yellow horn oil.

Source	Sum of Squares	df	Mean Square	F-Value	P-Value Prob > F	
Model	925.6534	14	66.1181	35.1998	<0.0001	Significant
X ₁	0.8694	1	0.8694	0.4629	0.5074	
X ₂	13.7602	1	13.7602	7.3256	0.0170	
X ₃	38.3776	1	38.3776	20.4314	0.0005	
X ₄	0.1925	1	0.1925	0.1025	0.7536	
X ₁ X ₂	9.0601	1	9.0601	4.8234	0.0454	
X ₁ X ₃	1.9044	1	1.9044	1.0139	0.3311	
X ₁ X ₄	14.8610	1	14.8610	7.9117	0.0138	
X ₂ X ₃	9.0902	1	9.0902	4.8394	0.0451	
X ₂ X ₄	10.9561	1	10.9561	5.8328	0.0300	
X ₃ X ₄	3.5156	1	3.5156	1.8716	0.1928	
X ₁ ²	468.9611	1	468.9611	249.6642	<0.0001	
X ₂ ²	139.2204	1	139.2204	74.1178	<0.0001	
X ₃ ²	226.8738	1	226.8738	120.7824	<0.0001	
X ₄ ²	398.0942	1	398.0942	211.9363	<0.0001	
Residual	26.2971	14	1.8784			
Lack of Fit	22.0342	10	2.2034	2.0675	0.2523	Not significant
Pure Error	4.2629	4	1.0657			
Cor Total	951.9505	28				

2.1.3. Response Surface Analysis

To investigate the interactive effects of operational parameters on the yield of oil extraction, the three-dimensional profiles of multiple non-linear regression models and the two-dimensional contour generated by the model are illustrated in Figure 2. Two variables are depicted in a 3D surface plot while the other two variables are kept constant at zero level. The shapes of the contour plots, circular or elliptical, indicate whether mutual interactions between the variables are significant or not [10].

For oil yield, the interactions between microwave power (X₁) and NaCl concentration (X₃), and NaCl concentration (X₃), and extraction time (X₄) were not evident due to lack of or only weak functional relationship between the two variables in the break emulsification (Figure 2b,f).

Oil yield increased gradually with increasing microwave power and water to material ratio (Figure 2a) to a threshold level beyond which oil yield slightly decreased.

Figure 2c shows the response surface plot at various microwave powers and extraction times. Oil yield was higher at longer extraction times. However, the yield decreased with the increasing of extraction time. It indicated that the maximum extraction yield of oil could be achieved. This result indicated that extraction time had a different extent of influence on extraction yield in different microwave power.

Figure 2d shows the effects of water to material ratio and NaCl concentration on oil yield. As the water to material ratio and NaCl concentration increased, oil yields increased sharply from 20 to 25 g/mL NaCl but declined at higher concentrations.

Oil yield increased gradually with increasing water to material ratios and extraction times (Figure 2e) up to a threshold level, beyond which oil yield slightly decreased.

According to the RSM test results, the optional conditions of ASP-MAE process for oil extraction were 24 g/L NaCl, 300 W microwave power, 4:1 water to material ratio, and an 80 min extraction time. To validate the adequacy of the model equations, a verification experiment was carried out under the optimal conditions identified above. The model predicted a maximum response of 86.55%. The mean value of 84.62 ± 0.51% (n = 5) from the physical experiments verifies the validity of the extraction model. These findings confirm that the model is adequate for estimating the optimal combination of variables.

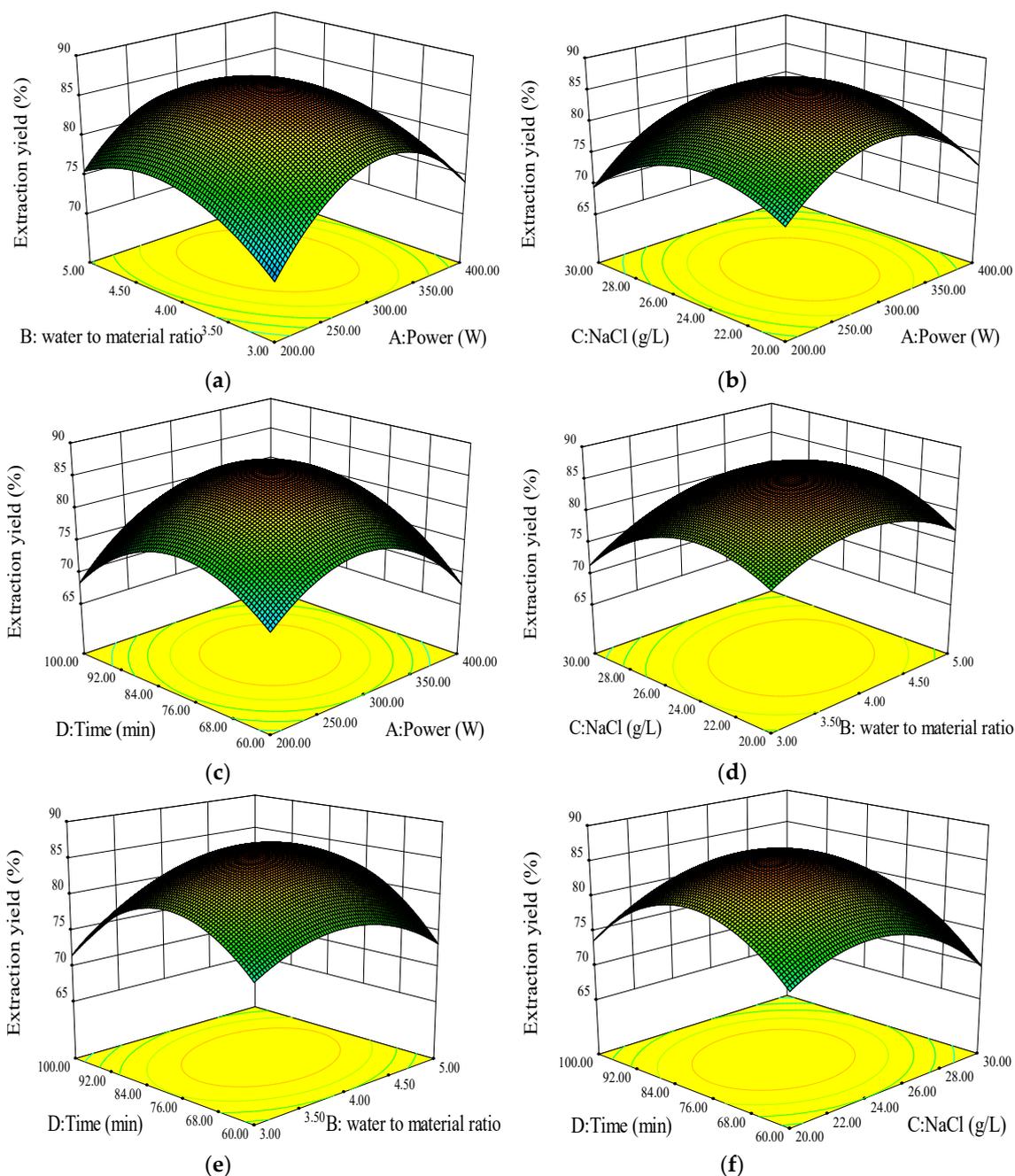


Figure 2. Tri-dimensional response surface showing the experimental factors and their mutual interactions on oil extraction. (a) microwave power and ratio of water to material, (b) microwave power and NaCl concentration, (c) microwave power and time, (d) ratio of water to material and NaCl concentration, (e) ratio of water to material and time, and (f) NaCl concentration and time.

2.2. Antioxidant Activity

2.2.1. Scavenging Activity of DPPH Radicals

The DPPH free-radical-scavenging effect of yellow horn oil is depicted in Figure 3a. It is an organic nitrogen radical with visible, ultraviolet absorption at 517 nm, and its color fades upon reduction [11]. As the concentration increased from 0.2 to 1.2 mg/mL, the DPPH radical-scavenging activity rose with increasing concentration of the extracted oils (Figure 3a). Beyond 1 mg/mL, the increasing became less

obvious and the overall DPPH radical-scavenging activity was as strong as BHT. The results indicate that yellow horn oil had a noticeable effect on scavenging DPPH free radicals.

2.2.2. Reducing Power Assay

In this assay, the ability of the sample to reduce Fe (III) to Fe (II) was determined and compared with BHT. The reducing power increased with increasing sample concentration (Figure 3b). The yellow horn oil showed higher reducing ability (absorbance of 0.4 at 700 nm) that was similar with the BHT. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Studies have revealed that the potential antioxidant function of plant oils is mainly played by polyunsaturated fatty acids (PUFAs), tocopherols, and other components [12]. The relative contents of PUFAs (9,12-octadecadienoic acid) in the yellow horn oil extracted by ASP-MAE was $47.35\% \pm 3.79\%$ in this study (Table 4). Moreover, current literature [13] indicates that yellow horn oil contains α -, γ -, and δ -tocopherols, of which γ -tocopherol is the major tocopherol, and the total tocopherol content is 83.28–106.27 mg/100 g for various extraction methods. The antioxidant activity of tocopherols is mainly attributed to their ability to donate hydrogen atoms to free radicals, thus inhibiting lipid oxidation.

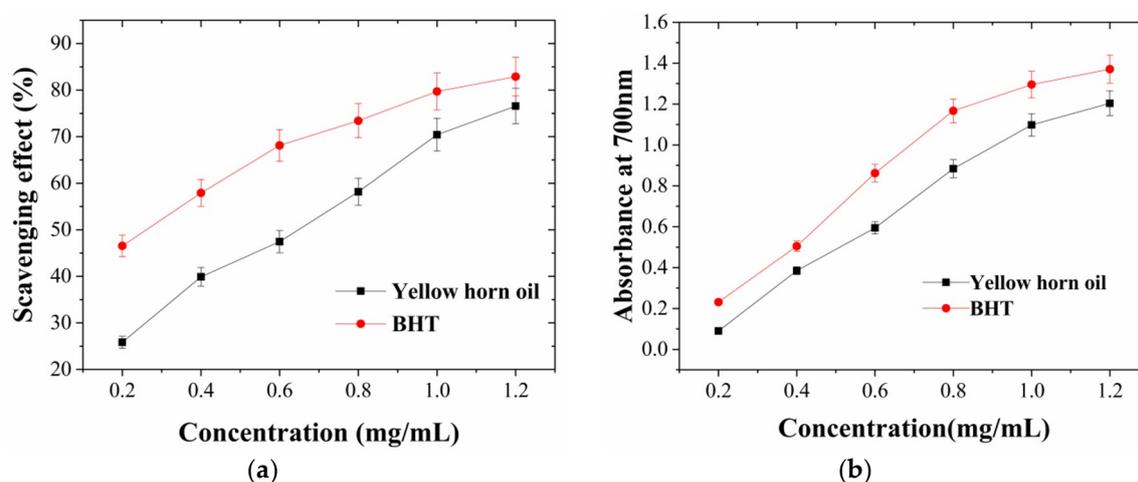


Figure 3. Activity of yellow horn oil and butylated hydroxytoluene (BHT) at different concentrations. (a) scavenging activity, (b) of reducing power activity. Data are means \pm SD ($n = 3$).

2.3. Chemical Composition of the Extracted Seed Oil

The fatty acid profiles extracted from yellow horn oil via ASP-MAE or SE were analyzed using GC-MS (Table 4), with no differences observed between the extraction methods. The contents of monounsaturated fatty acids and polyunsaturated fatty acids in seed oil by ASP-MAE were slightly higher [14]. We conclude that yellow horn oil obtained by ASP-MAE is of excellent quality.

Table 4. Fatty acid profiles and relative contents of yellow horn oil by ASP-MAE and SE.

No.	Component	Molecular Formula	Relative Content (%)	
			ASP-MAE	SE
1	Hexadecanoic acid	$C_{16}H_{32}O_2$	3.40 ± 0.26	4.41 ± 0.37
2	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	47.35 ± 3.79	45.02 ± 4.05
3	9-Octadecenoic acid	$C_{18}H_{34}O_2$	27.25 ± 2.13	30.02 ± 3.71
4	Octadecanoic acid	$C_{18}H_{36}O_2$	1.51 ± 0.11	1.63 ± 0.15
5	15-Tetracosenoic acid	$C_{24}H_{46}O_2$	1.99 ± 0.17	1.72 ± 0.13
6	11-Eicosenoic acid	$C_{20}H_{38}O_2$	6.82 ± 0.76	6.31 ± 0.84
7	13-Docosenoic acid	$C_{22}H_{42}O_2$	11.23 ± 0.87	10.15 ± 1.27
8	Docosanoic acid	$C_{22}H_{44}O_2$	0.46 ± 0.04	0.73 ± 0.06

3. Experimental

3.1. Materials and Chemicals

Seeds of yellow horn were collected in the summer of 2017 from Gansu province, China, and identified by Prof. Xuelin Chen, College of Life Science, Northwest Normal University, Lanzhou, China. The seed capsules were manually cracked to release the seeds. The collected seeds were milled in a pulping machine (Langong 110, Kaifeng, China) prior to oil extraction.

Sodium chloride of analytical grade was purchased from Fuchen Chemical Reagents Factory (Tianjin, China). 1,1-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical or HPLC grades.

3.2. Oil Extraction by ASP-MAE Method

Microwave extraction under varying salt levels was carried out using a microwave device with power settings ranging from 100 to 800 W (NJC 03-2, 2450 MHz, Nanjing Jiequan microwave equipment Co. Ltd., Nanjing, China). The microwave was equipped with a power sensor, a temperature sensor, a temperature controller and cooling system, and a special two necks round-bottomed flask. The amount of NaCl required for a certain concentration was added to ultra-pure water followed by 20 g of seed pulp and extraction solvent of a specified volume in an extraction flask. The MAE device was set to the preliminary conditions for extraction temperature, microwave power, and extraction time. After the scheduled time, the mixture obtained was transferred to a centrifuge tube and centrifuged for 10 min at 9000 rpm. The upper oil phase was collected and the other was used for subsequent research. The amount of extracted oil was gravimetrically analyzed, and the yield expressed as the percent ratio of the mass of extracted oil to the mass of oil for Soxhlet extraction is as follows:

$$\text{Extraction yield of seed oil(\%)} = \left(\frac{\text{mass of extracted oil(g)}}{\text{mass of extracted oil for Soxhlet extraction(g)}} \right) \times 100\% \quad (2)$$

3.3. Soxhlet Extraction of Seed Oil

An optimized Soxhlet extraction method (SE) was performed for the comparison with the ASP-MAE extraction [15]. Twenty grams of milled yellow horn seed kernels were extracted with petroleum ether (60–90 °C) in a Soxhlet extractor by heat reflux at 75 °C for 10 h. The extract was filtered, and petroleum ether in the filtrate removed at 40 °C under reduced pressure using a rotary evaporator. The extracted oil was weighed to calculate the extraction yield.

3.4. Box-Behnken Design (BBD)

Box-Behnken statistical design was used to statistically optimize the parameters of extraction conditions and to evaluate the main effects [16], interaction effects, and quadratic effects of the influencing factors on the seed oil yield (Table 5). The BBD identified strong effects of microwave power (W), the water to material ratio (mL/g), NaCl concentration, and extraction time on oil yield, and were used as the tested variables in a 29-group experiment. As shown in Table 1, the four factors selected for this study were designated as X_1 , X_2 , X_3 , and X_4 and prescribed three levels, coded 1, 0, and -1 for high, intermediate, and low value, respectively. All experiments were performed in triplicate, with the averages for seed oil yield taken as a response. To predict the optimal point, a second-order polynomial model was fitted to correlate the relationship between independent variables and response. Test variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (3)$$

where x_i is the coded value of an independent variable; X_i is the actual value of an independent variable; X_0 is the actual value of an independent variable at the center point; ΔX is the step change value of an independent variable. For the three factors, the equation is:

$$Y = A_0 + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j \quad (4)$$

where Y is the response variable (yield of seed oil in real values); A_0 , A_i , A_{ii} , A_{ij} are the regression coefficients of variables for intercept, linear, quadratic, and interaction terms respectively; and X_i and X_j are the independent variables ($i \neq j$). The variables of each factor were transferred to a scale between -1 and 1 for the appraisals, while the dependent variable was the oil extraction yield. According to the analysis of variance, the effect and regression coefficients of individual linear, quadratic, and interaction terms were determined. The regression coefficients were then used to make a statistical calculation to generate dimensional and contour maps from the regression models.

Table 5. Independent variables and their levels used in the response surface design.

Independent Variables	Symbol		Factor Level		
	Coded	Uncoded	-1	0	1
Microwave power (W)	x_1	X_1	200	300	400
Water to material ratio (mL/g)	x_2	X_2	3:1	4:1	5:1
NaCl (g/L)	x_3	X_3	20	25	30
Time (min)	x_4	X_4	60	80	100

3.5. Evaluation of Physicochemical Properties

3.5.1. Gas Chromatography-Mass Spectrometry (GS-MS) Analysis

The reference standard for the preparation of fatty acid methyl esters was the ester exchange method which is part of the Chinese national standard GB/T17376-2008.

A GC-MS analysis was performed using a gas chromatography/mass spectrometer (Thermo electron, Milan, Italy) equipped with an HP-5 silica capillary column (30 m \times 0.25 mm \times 0.25 μ m, model HP6820, Hewlett-Packard, Palo Alto, CA, USA). The column temperature was initially set to 160 $^{\circ}$ C (held for 3 min), then increased to 210 $^{\circ}$ C at 2 $^{\circ}$ C/min (held for 1 min) and to 250 $^{\circ}$ C at 5 $^{\circ}$ C/min (held for 1 min). The mass spectrometer was operated in positive ion mode with ionization energy of 70 eV. Injector and detector temperatures and the ion source temperature were 250 $^{\circ}$ C. Helium was used as a carrier gas, and the split ratio was 50:1. The retention indices and mass spectra, provided by the GC-MS controlling system, of the oil components were compared with the database of National Institute of Standards and Technology (NIST, 3.0).

3.5.2. DPPH Radical-Scavenging Assay

The DPPH radical-scavenging effect of the extracts and essential oil was estimated using the method described by Brand-Williams, et al. [17] with some modifications. Briefly, 0.1 mL of extract or essential oil solution was mixed with 2 mL of DPPH solution with an absorbance at 517 nm. The mixture was incubated for 30 min at 23 $^{\circ}$ C. The absorbance was then measured at 517 nm. BHT was used as the reference compound. The DPPH radical-scavenging activity (%) was calculated from the following equation:

$$\text{Scavenging effect (\%)} = \frac{A_0 - (A_s - A_x)}{A_0} \times 100\% \quad (5)$$

where A_0 is the absorbance of DPPH solution without a sample, A_s is the absorbance of the test sample mixed with DPPH solution, and A_x is the absorbance of the sample without DPPH solution.

3.5.3. Reducing Power Assay

The reducing power of the yellow horn oil samples was determined using the method of Zeng et al. [18]. Accordingly, 1 mL of yellow horn oil sample (2–20 mg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferric cyanide solution (1%). The resulting mixture was incubated at 50 °C for 20 min and then cooled rapidly. To this mixture, 2.5 mL of trichloroacetic acid solution (10%) was added, mixed well, and then centrifuged for 10 min at 3000 rpm. The upper layer of the solution (2.5 mL) was diluted with distilled water (2.5 mL), and 0.5 mL of ferric chloride solution (0.1%) was added and mixed. The absorbance of the mixture was measured at 700 nm. A higher absorbance indicated a higher reducing power. BHT was used as the reference compound.

3.6. Statistical Analysis

The SPSS 17.0 software package was used to analyze the experimental data. *P*-values of less than 0.05 were considered statistically significant. All statistical analyses were performed with Origin 8.0 (Microcal Software Inc., Northampton, MA, USA).

4. Conclusions

The study identified that ASP–MAE is an efficient, environmentally friendly, and easy procedure for oil extraction, and the optional parameters of the extraction process were 24 g/L NaCl, 300 W microwave power, 4:1 water to material ratio, and an 80 min extraction time; high-quality oil from yellow horn can be obtained under these extraction conditions. The antioxidant showed that yellow horn oil had a noticeable effect on scavenging DPPH free radicals and reducing capacity indicator of its potential antioxidant activity.

In all, the analyzed results of antioxidant activities and chemical composition demonstrate that yellow horn oil obtained from this emerging method can be used as a high-quality edible oil for the food industry in the future.

Author Contributions: J.Z. and Z.Y. conceived and designed the experiments. Z.Y., J.G. and Y.H. performed the experiments. Y.H. and Z.Y. wrote the manuscript. Y.H. and F.W. revised the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Li, J.; Zu, Y.G.; Luo, M.; Gu, C.B.; Zhao, C.J.; Efferth, T.; Fu, Y.J. Aqueous enzymatic process assisted by microwave extraction of oil from yellow horn (*Xanthoceras sorbifolia* Bunge.) seed kernels and its quality evaluation. *Food Chem.* **2013**, *138*, 2152–2158. [[CrossRef](#)] [[PubMed](#)]
2. Zhang, S.; Zu, Y.G.; Fu, Y.J.; Luo, M.; Liu, W.; Li, J.; Thomas, E. Supercritical carbon dioxide extraction of seed oil from yellow horn (*Xanthoceras sorbifolia* Bunge.) and its anti-oxidant activity. *Bioresour. Technol.* **2010**, *101*, 2537–2544. [[CrossRef](#)] [[PubMed](#)]
3. Szentmihályi, K.; Vinkler, P.; Lakatos, B.; Illés, V.; Then, M. Rose hip (*Rosacina*, L.) oil obtained from waste hip seeds by different extraction methods. *Bioresour. Technol.* **2002**, *82*, 195–201. [[CrossRef](#)]
4. Li, Z.L.; Li, X.; Zhang, P. Research progress in the chemical constituents and pharmacological activities of *Xanthoceras sorbifolia* Bunge. *J. Shenyang Pharm. Univ.* **2004**, *21*, 472–475.
5. Deng, H.; Sun, J.; Zhang, Y.; He, L. GC-MS analysis of oils from *Xanthoceras sorbifolia* Bunge seeds with different extraction methods. *Food Sci.* **2007**, *28*, 354–357.
6. Eskilsson, C.S.; Björklund, E. Analytical-scale microwave-assisted extraction. *J. Chromatogr. A* **2000**, *902*, 227–250. [[CrossRef](#)]

7. Passos, C.P.; Yilmaz, S.; Silva, C.M.; Coimbra, M.A. Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail. *Food Chem.* **2009**, *115*, 48–53. [[CrossRef](#)]
8. Pan, X.J.; Niu, G.G.; Liu, H.Z. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process.* **2003**, *42*, 129–133. [[CrossRef](#)]
9. Xia, L.X.; Cao, G.Y.; Lu, S.W. Influence of inorganic salts on demulsification of emulsions. *Acta Petrolei Sin.* **2003**, *19*, 94–97.
10. Muralidhar, R.; Chirumamila, R.; Marchant, R.; Nigam, P. A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochem. Eng. J.* **2001**, *9*, 17–23. [[CrossRef](#)]
11. Deng, H.; Tian, Z.; Fan, X.; Guo, Y.; Qiu, N. Antioxidation activity in vitro of cold pressed *Xanthoceras sorbifolia* Bunge kernel oil. *China Oils Fats* **2012**, *37*, 28–32.
12. Jiao, J.; Li, Z.G.; Gai, Q.Y.; Li, X.J.; Wei, F.Y.; Fu, Y.J.; Ma, W. Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. *Food Chem.* **2014**, *147*, 17–24. [[CrossRef](#)] [[PubMed](#)]
13. Gu, L.B.; Zhang, G.J.; Du, L.; Du, J.; Qi, K.; Zhu, X.L.; Zhang, X.Y.; Jiang, Z.H. Comparative study on the extraction of *Xanthoceras sorbifolia* Bunge (yellow horn) seed oil using subcritical n-butane, supercritical CO₂, and the Soxhlet method. *LWT* **2019**, *111*, 548–554. [[CrossRef](#)]
14. Yu, H.Y.; Fan, S.Q.; Bi, Q.X.; Wang, S.X.; Hu, X.Y.; Chen, M.Y.; Wang, L.B. Seed morphology, oil content and fatty acid composition variability assessment in yellow horn (*Xanthoceras sorbifolium* Bunge) germplasm for optimum biodiesel production. *Ind. Crop Prod.* **2017**, *97*, 425–430. [[CrossRef](#)]
15. Liang, J.Y.; Yin, Z.X.; Zhao, B.T.; Song, S.; Gao, Q.Y.; Yao, J.; Zhang, J. Extraction of xanthocera seeds oil by the method of ultrasound and aqueous enzymatic and fatty acid evaluation. *Sci. Technol. Food Ind.* **2013**, *34*, 254–259.
16. Zhao, B.T.; Zhang, J.; Guo, X.; Wang, J.L. Microwave-assisted extraction, chemical characterization of polysaccharides from *Lilium davidii* var. unicolor Salisb and its antioxidant activities evaluation. *Food Hydrocoll.* **2013**, *31*, 346–356. [[CrossRef](#)]
17. Brand-Williams, W.; Cuvelier, M.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT* **1995**, *28*, 25–30. [[CrossRef](#)]
18. Zeng, L.B.; Zhang, Z.R.; Luo, Z.H.; Zhu, J.X. Antioxidant activity and chemical constituents of essential oil and extracts of *Rhizoma homalomenae*. *Food Chem.* **2011**, *125*, 456–463. [[CrossRef](#)]

Sample Availability: Samples of the compounds are not available from the authors.



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