Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Zhao, Liyan , Chen, Guitang , Zhao, Guanghua and Hu, Xiaosong(2009) 'Optimization of Microwave-Assisted Extraction of Astaxanthin from Haematococcus Pluvialis by Response Surface Methodology and Antioxidant Activities of the Extracts', Separation Science and Technology, 44: 1, 243 — 262
To link to this Article: DOI: 10.1080/01496390802282321
URL: http://dx.doi.org/10.1080/01496390802282321

Separation Science and Technology, 44: 243–262, 2009

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390802282321

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Optimization of Microwave-Assisted Extraction of Astaxanthin from Haematococcus Pluvialis by Response Surface Methodology and Antioxidant Activities of the Extracts

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Abstract: Microwave-assisted extraction (MAE) was applied for the extraction of astaxanthin from *Haematococcus pluvialis* and response surface methodology (RSM) was used to optimize extraction parameters to the content of astaxanthin. Four independent variables such as microwave power (W), extraction time (sec), solvent volume (mL), and the number of extraction were optimized in this paper. The optimal conditions were determined and tri-dimensional response surfaces were plotted from the mathematical models. The F-test and *p*-value indicated that microwave power, extraction time, the number of extraction, and their quadratic had a highly significant effect on the response value ($p < 0.01$), then the solvent volume and the interaction effects of microwave power and the number of extraction also displayed significant effect ($p < 0.05$). Considering the extraction efficiency, the optimized conditions of MAE were as follows: microwave power was 141 W, extraction time 83 sec, solvent volume 9.8 mL, the number of extraction four times. About 594 ± 3.02 μg astaxanthin was extracted from *Haematococcus pluvialis* the dried powders (100 mg) under the optimal conditions, and it close to the predicted contents (592 μg). The antioxidant activities of the extracts obtained under optimal conditions were analyzed, and the results showed that the

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extracts presented strong ability of inhibiting the peroxidantion of linoleic acid, exhibited strong radical-scavenging properties against the DPPH, as well as strong reducing power.

Keywords: Antioxidant activities, astaxanthin, haematococcus pluvialis, microwave-assisted extraction (MAE), response surface methodology

INTRODUCTION

The principal natural sources of astaxanthin are the *Phaffia rhodozyma* (1) and the green alga *Haematococcus pluvialis* (2). The content of astaxanthin in *P. rhodozyma* is low ($0.4\,mg\,g^{-1}$ dry cell), while that in *Haematococcus pluvialis* is very high (about $10–30\,mg\,g^{-1}$ dry cell) (3). Interestingly, astaxanthin is predominately found as esters in *Haematococcus pluvialis* (4). The algal astaxanthin is composed of its monoester (70–80% of total astaxanthin) and its diester (20–30%) with higher fatty acid, in which $C_{18:1}$ is the main ester (2,4,5).

Extraction is widely used for the separation of these biologically active carotenoids from various plant, bacteria, or animal sources. The processes of conventional liquid extraction such as stirring extraction and Soxhlet extraction for solids and semi-solids materials are generally time-consuming and laborious. Moreover, large volumes of organic solvent are required, which can lead to sample contamination, "losses" due to volatilization during concentration steps, and environmental pollution from solvent waste. In contrast, microwave-assisted extraction (MAE) is known as one of the green technologies. MAE carries the advantage of being a simple device, and has a wide area of application, high extraction efficiency, good reproducibility, and low consumption of organic solvents and time, as well as low environmental pollution. It is based upon the selective and rapid localized heating of moisture in the sample by microwaves. Due to the localized heating, pressure builds up within the cells of the sample, leading to a fast transfer of the compounds from the cells into the extracting solvent, usually transparent to microwaves, then not heated by them. Additionally, by using closed vessels the extraction can be performed at elevated temperatures accelerating the mass transfer of target compounds from the sample matrix. In recent years, this technique has been widely used for the extraction of natural products (6,7,8) including carotenoids (9,10) as well as in the extraction of effective constituents in medicines (11).

For extraction of carotenoids from marine materials, many recent studies have been carried out to investigate the effect of operating conditions and to find optimal conditions for the process (12,13,14). Studies on the extraction of astaxanthin from *Haematococcus pluvialis* with

supercritical carbon dioxide have been reported (15,16,17). It was found in these previous investigations that the total amount of astaxanthin in the extract and its concentration in the extract were influenced by the extraction pressure and temperature. But there are no reports on the extraction of astaxanthin by MAE, and considering the advantage of microwave extraction, MAE was adopted to the extraction of astaxanthin from *Haematococcus pluvialis* in our research.

Carotenoids are naturally occurring pigments that provide the yellow, orange, and red colors of fruit, vegetables, plants, and marine animals. These colors are a result of the presence of conjugated bonds that are responsible for their light absorption as well scavenging free radicals activity (18). According to epidemiological studies, carotenoids play an important role in the prevention of cancer, cataracts, and aging diseases such as heart disease. There also exits an inverse relationship between the consumption of foods containing carotenoids and the risk of lung, intestinal, skin, and bladder cancer (19,20). The antioxidant activity of astaxanthin was 10 times stronger than that of other carotenoids, namely, zeaxanthin, lutein, canthaxanthin, and β-carotene (21). *Haematococcus pluvialis* is a good source of astaxanthin and has the highest astaxanthin content in all the natural resourses (www.aquasearch.com.). Much research on the pure antioxidant activity and physiological function of astaxanthin have been carried out, but little attention was paid to the direct extracts from *Haematococcus pluvialis*, so the antioxidant activities of the extracts without purification was studied in this paper.

MATERIALS AND METHODS

Materials

The powder of *Haematococcus pluvialis* was purchased from Jingzhou Natural Astaxanthin Co. (Jingzhou, China), *trans*-Astaxanthin was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all other chemicals and solvents were of analytical grade from Beijing Chemicals Co. (Beijing, China). The process of MAE was performed in experimental microwave equipment (Model NJL07-3, Jiequan microwave equipment Co., Ltd., Nanjing, China) at a frequency of 2450 MHz and its schematic diagram is shown in Fig. 1.

Experimental Design

RSM was applied to determine the working conditions of microwave equipment for the extraction of astaxanthin from *Haematococcus*

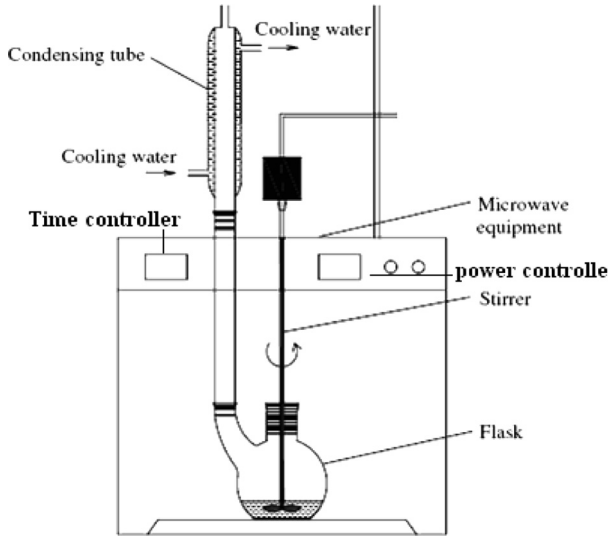

Figure 1. Schematic diagram of microwave equipment.

pluvialis. The effect of independent variables X_1 (microwave power, W), X_2 (extraction time, min), X_3 (solvent volume, mL) and X_4 (the number of extraction, times) at three variation levels (in Table 1) in the extraction process, is shown in Table 2. The choice of levels of every parameter is based on single factor experiments (data not given). The correspondence between the coded and uncoded values can be obtained using the following formula:

$$x_i = \frac{(X_i - X_i^0)}{\Delta X_i} \tag{1}$$

Table 1. Independent variable of the process and their corresponding levels

	Symbol		Levels		
Independent variable	Uncodede	Coded	−1	0	1
Power of microwave (w)	X_1	x_1	100	150	200
Extraction time (s)	X_2	x_2	40	70	100
Solvent volume (mL)/200mg alge powder	X_3	x_3	6	8	10
The number of extraction times	X_4	x_4	2	3	4

Table 2. Experimental data and the observed responses value with different combinations of every parameter used in the CCRD

Run	Coded variable levels				Astaxanthin content[a] (μg/100 mg dry alga powder)
	x_1	x_2	x_3	x_4	
1	−1	−1	−1	−1	523.55
2	−1	−1	−1	1	571.01
3	−1	−1	1	−1	533.52
4	−1	−1	1	1	576.32
5	−1	1	−1	−1	540.26
6	−1	1	−1	1	580.02
7	−1	1	1	−1	568.92
8	−1	1	1	1	583.22
9	1	−1	−1	−1	557.98
10	1	−1	−1	1	578.68
11	1	−1	1	−1	565.73
12	1	−1	1	1	572.99
13	1	1	−1	−1	550.26
14	1	1	−1	1	578.61
15	1	1	1	−1	568.95
16	1	1	1	1	580.22
17	−2	0	0	0	527.60
18	2	0	0	0	565.85
19	0	−2	0	0	529.43
20	0	2	0	0	560.23
21	0	0	−2	0	565.43
22	0	0	2	0	578.07
23	0	0	0	−2	518.19
24	0	0	0	2	589.14
25	0	0	0	0	582.22
26	0	0	0	0	577.78
27	0	0	0	0	580.18
28	0	0	0	0	578.53
29	0	0	0	0	582.13
30	0	0	0	0	580.31
31	0	0	0	0	581.30

[a]Date expressed in above table is the mean of triplicate analyses. And the unit of data in the last column is micrograms of astaxanthin equivalents per 100 mg dried powder of *Haematococcus pluvialis*.

Where x_i is the coded value, X_i is the corresponding actual value, X_i^0 is the actual value in the center of the domain, and ΔX_i is the increment of X_i corresponding to 1 unit of $x_i \cdot x_1$ (coded value of microwave power), x_2

(coded value of the number of extraction), x_3 (coded value of solvent volume), x_4 (coded value of the number of extraction) were given by Eqs. (2)–(5):

$$x_1 = \frac{(X_1 - 150)}{50} \tag{2}$$

$$x_2 = \frac{(X_2 - 70)}{30} \tag{3}$$

$$x_3 = \frac{(X_3 - 8)}{2} \tag{4}$$

$$x_4 = \frac{(X_4 - 3)}{1} \tag{5}$$

The complete design consisted of 31 experimental points including seven replications of the center points, and the triplicates were performed at all design points in randomized order.

Extraction of Astaxanthin from Haematococcus Pluvialis using MAE

Different extractions were carried out by microwave heating 200 mg of dried *Haematococcus pluvialis* powder in different volume of mixed reagent of ethanol and ethyl acetate (2:1, v/v), the selection of the extraction solvent was decided by comparing some usual reagent (detailed experiment was not stated in this paper). The power of the microwave equipment can be modulated from 0 W to 700 W, the timing range can be selected from 0 minute to 999 minutes by the time the controller and the speed of the stirrer can be adjusted. the extraction process was shown as Fig. 1. After extraction by MAE, the mixture was then separated by centrifugation at 10 000 × g for 15 min, and the supernatant was collected. The collected supernatant was diluted with the mixed reagent and was used to determine the astaxanthin content.

Determination of Astaxanthin Content of the Extract

The concentration of astaxanthin in the extract was analyzed using a spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). For the maximal absorption of astaxanthin in various solvents are different, the absorption spectrum of astaxanthin in the

mixture solvent of ethanol and ethyl acetate was measured between 400 nm and 600 nm and the maximal absorption was obtained (480 nm). Then the MAE extracts of astaxanthin was determined at the wavelength of 480 nm following the method adapted from Choi et al. (22).

Measurement of the Antioxidant Activities

The collected extracts were condensed by a vacuum rotary evaporator (Shanghai Shenshun Biotech. Co., Ltd., Shanghai, China) at 40°C. After condensation, the extracts were blown to dryness under a stream of nitrogen, and the residue was stored at −18°C and was used for analyzing the antioxidant activities.

Inhibition to Linoleic Acid Peroxidation

The FTC method was adapted from the method of Osawa and Namiki (23) with slight modification. 1.0 mL linoleic acid (2.5% v/v) in 99.5% (w/v) ethanol and 2.0 mL of 0.05 M phosphate buffer (pH7.0) were mixed in a test tube (volume, 10 mL), and then 2.0 mL of samples dissolved in 99.5% (w/v) ethanol were added. The final concentration of extracts of *Haematococcus pluvialis* was 0.05, 0.1, 0.2, 0.3, 0.4 mg mL^{-1}, respectively. Distilled water and BHT (butylated hydroxyanisole) were used as control and standard antioxidant. Then the mixtures were kept in a screw-cap container in the dark at 60°C to accelerate oxidation. At regular intervals (24 h), 0.1 mL of the reaction mixture was withdrawn. Then 9.7 mL of 75% (v/v) ethanol, 0.1 mL of 30% (w/v) ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid was added to the reaction mixture. After precisely 3 min, the absorbance of the colored solution was measured at 500 nm.

Thiobarbituric Acid Reactants (TBARS) Test. The TBARS method was adapted from the method of Kikuazki and Nakatani (24). One day after the absorbance of the control reached the maximum value of the FTC method, One milliliter of the reaction mixture was added to 2.0 mL of 20% (w/v) trichloroacetic acid (TCA) and 2.0 mL of 0.67% thiobarbituric acid (TBA) solution. The mixture was then placed in a water bath at 100°C for 10 min. After cooling, it was centrifuged at $3000 \times g$ for 20 min and the absorbance of the supernatant was then measured at 532 nm.

The inhibition percentage is expressed as $[(A_{control} - A_{sample})/A_{control}] \times 100\%$, where $A_{control}$ and A_{sample}, are the absorbance of the control and sample, respectively.

Measurement of the DPPH Radical-Scavenging Activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable DPPH free radical (25,26). A volume of 3.0 mL of each sample was added to 2.0 mL of 0.1 mM DPPH in ethanol. The mixture was slightly shaken for 30 min at room temperature in darkness, and the absorbance of the resulting solution was measured at 517 nm. A lower absorbance represented a higher DPPH scavenging activity. The scavenging percentage was calculated as $[(A_{control} - A_{sample})/A_{control}] \times 100$, where $A_{control}$ is the absorbance at 517 nm of 0.1 mM DPPH and A_{sample} is the absorbance at 517 nm of 0.1 mM DPPH with sample at different concentrations.

Test for Reducing Power

The reducing power of the extracts was tested according to the method of Oyaizu (27). Each sample of 2.0 mL was added to 2.0 mL of 0.2 M phosphate buffer (pH 6.6) and 2.0 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then 2.5 mL of 10% (w/v) TCA was added to the reaction mixture, which was then centrifuged at $1500 \times g$ for 10 min. The supernatant (2.0 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride in a test tube. After a 10 min reaction, the absorbance of the resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used to compare the reducing power.

Data Analysis

The design of experiments procedure of SAS (SAS version 9.00, SAS Institute Inc., USA) was used to design the central composite rotatable design (CCRD) and analyze the experimental data. Statistical analysis was conducted with SPSS 10.0 software (version 10.0, SPSS Inc., USA).

RESULTS AND DISCUSSION

Optimization of MAE Condition of Astaxanthin Extracts from Haematococcus Pluvialis

The standard curve of astaxanthin in the mixed reagent of ethanol and ethyl acetate was obtained ($y = 0.2253x - 0.0251$, $r^2 = 0.9993$), and the content of astaxanthin was calculated on the equation.

A regression analysis (in Table 3) was carried out to fit the mathematical models to the experimental data aiming at an optimal region for the responses studied. The significance of each coefficient was determined using the *F*-test and *p*-value in Table 3. The result suggested that the change of microwave power, extraction time, the number of extraction and their quadratic had a highly significant effect on the response value ($p < 0.01$) and the solvent volume and the interaction effects of microwave power and the number of extraction also displayed significant effect ($p < 0.05$).

The corresponding variables would be more significant if the absolute *F*-value becomes greater and the *p*-value becomes smaller (28). Some

Table 3. Estimated regression model of relationship between response variables (astaxanthin content) and independent variables (x_1, x_2, x_3, x_4)

Variables	DF	SS	MS	*F*-value	*P*-value[a]
x_1	1	976.6504	976.6504	14.7416	0.0014^+
x_2	1	729.0833	729.0833	11.0048	0.0044^+
x_3	1	374.3020	374.3020	5.6497	0.0302^+
x_4	1	5215.6020	5215.6020	78.7245	0.0001^+
x_1^2	1	1289.4170	1289.4170	19.4625	0.0004^+
x_1x_2	1	266.9956	266.9956	4.0300	0.0619^+
x_1x_3	1	38.3780	38.3780	0.5793	0.45767
x_1x_4	1	368.0642	368.0642	5.5556	0.0315^+
x_2^2	1	1477.7750	1477.7750	22.3056	0.0002^+
x_2x_3	1	75.7770	75.7770	1.1438	0.30072
x_2x_4	1	37.6382	37.6382	0.5681	0.4619
x_3^2	1	6.0180	6.0180	0.0908	0.7670
x_3x_4	1	229.8256	229.8256	3.4690	0.0810^+
x_4^2	1	709.1859	709.1859	10.7045	0.0048^+
Model	14	11243.7800	803.1271	12.1224	0.0001
Error	16	1016.6790	66.2513		
Total	30	12303.8000			

[a]The effects marked "+" were used in optimizing the response.

insignificant terms, such as, x_1x_3, x_2x_3, x_2x_4, x_3^2 were neglected, but considering the p-value of x_1x_2 and x_3x_4 is comparatively smaller and the predictive model with good fit, the x_1x_2 and x_3x_4 had been used in the predictive model. The predicted model can be described by the following equation in terms of coded values:

$$\begin{aligned} Y_1 = {} & 579.8815 + 6.379167x_1 + 5.511667x_2 + 3.949167x_3 \\ & + 14.74167x_4 - 6.666197x_1^2 \\ & - 4.085x_1x_2 - 4.79625x_1x_4 - 7.139947x_2^2 \\ & - 3.79x_3x_4 - 4.931197x_4^2 \end{aligned} \tag{6}$$

The coefficient of determination (R^2) of the predicted model was 0.9010, suggesting a good fit, the predicted model seemed to reasonably represent the observed values.

In order to make it more directly express the effects of the processing parameters, we took Eqs. (2)–(5) into Eq. (6) and obtained Eq. (7) as followed:

$$\begin{aligned} Y_1 = {} & 227.3709 + 1.405935X_1 + 1.702881X_2 + 7.659583X_3 \\ & + 73.8776X_4 - 0.002666X_1^2 \\ & - 0.002723X_1X_2 - 0.095925X_1X_4 - 0.007933X_2^2 \\ & - 1.895X_3X_4 - 4.931197X_4^2 \end{aligned} \tag{7}$$

The suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. The experimental extraction content of astaxanthin was found to be in agreement with the predicted one (in Table 4).

Table 4. Predicted and experimental extraction content of astaxanthin at optimum conditions

Optimum conditions		Predicted yield	Experimental yield[a]
Power of microwave (W)	141		
Extraction time (sec)	83		
Solvent volume (mL)/200 mg alga powder	9.8	591.30 mg	594.03 ± 3.02 mg
Extraction time	4		

[a]Mean ± standard deviation of triplicate determinations.

Analysis of Response Surface

The regression model Eq. (7) allowed the prediction of the effects of the four parameters on the extraction content of astaxanthin (Y_1) from *Haematococcus pluvialis*. The relationship between independent and dependent variables is illustrated in the tri-dimensional representation of the response surfaces (Fig. 2–4).

The response surface of the effect of the microwave power and extraction time on Y_1 was shown in Fig. 2. The microwave power and extraction time displayed a similar quadratic effect on Y_1 when the solvent volume and the number of extraction were kept constant (solvent volume = 8 mL, the number of extraction = 4), and Y_1 increased with the increase of microwave power and extraction time at first, then decreased when the two parameters increased to some degree. The results illustrated that higher power and longer extraction time were not suitable for astaxanthin extraction. Higher power can lead to temperature increasing, which had an impact on the stability of astaxanthin and may disrupt the structure of astaxanthin (29).

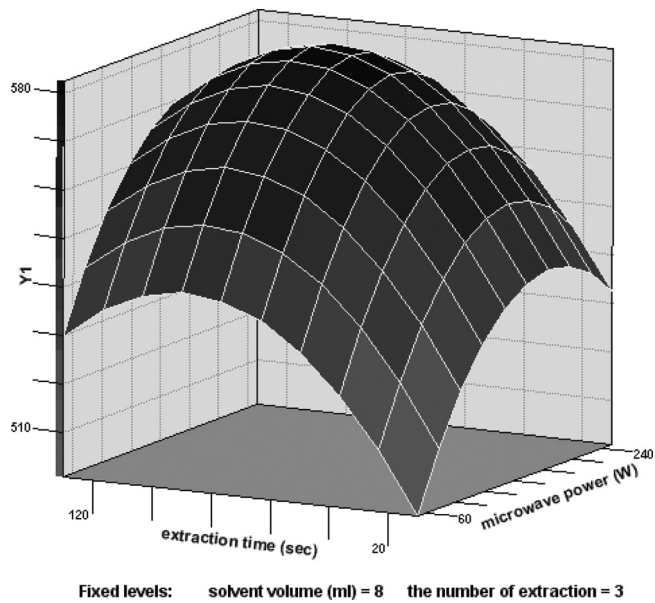

Figure 2. Response surface for the effects of microwave power and extraction time at constant solvent volume (8 mL) and the number of extraction (3 times) on extraction content of astaxanthin from dried *Haematococcus pluvialis*.

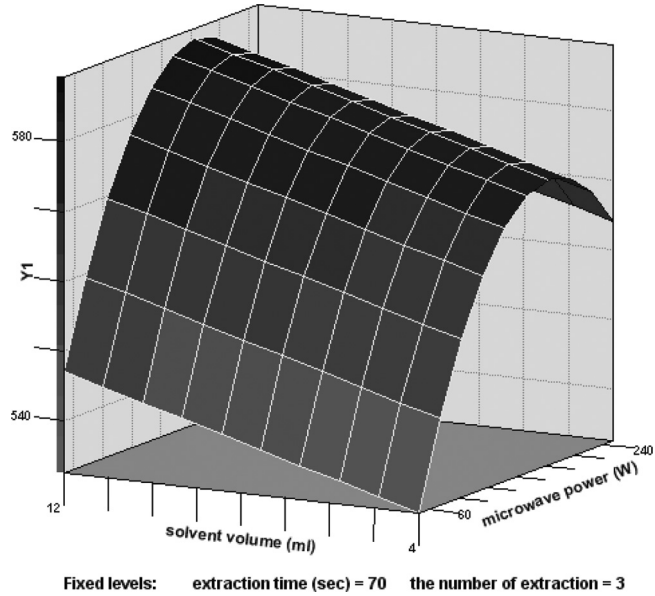

Figure 3. Response surface for the effects of microwave power and solvent volume at constant extraction time (70 sec) and the number of extraction (3 times) on extraction content of astaxanthin from dried *Haematococcus pluvialis*.

Figure 3 depicts the response surface of the effects of microwave power and solvent volume on the Y_1. The solvent volume demonstrated a linear increase on the response in our experimental scope from 4 mL to 12 mL when the extraction time and the number of extraction were kept constant. The effect of microwave power on the surface displayed a linear increase when power ranged from 60 W to 140 W, but it showed a quadratic effect when the microwave power was higher than 140 W, which agreed with the analysis of Table 3.

The slight tortuose surface in Fig. 4 showed the interaction effects of the microwave power and the number of the extraction which agreed with the analysis of Table 3. Y_1 increased with the number of extraction when the microwave power was less than 140 W while two different change trends were observed. It indicated that the more the number of extraction the less the astaxanthin extraction content at higher power.

In this study, the optimal MAE conditions were obtained from response surface analysis as follows: microwave power was 141.15 W, extraction time 83.42 sec, solvent volume 9.81 mL, the number of extraction 4.15 times, then considering the efficiency and the feasibility of the experiment, the optimal conditions were fixed as: microwave power

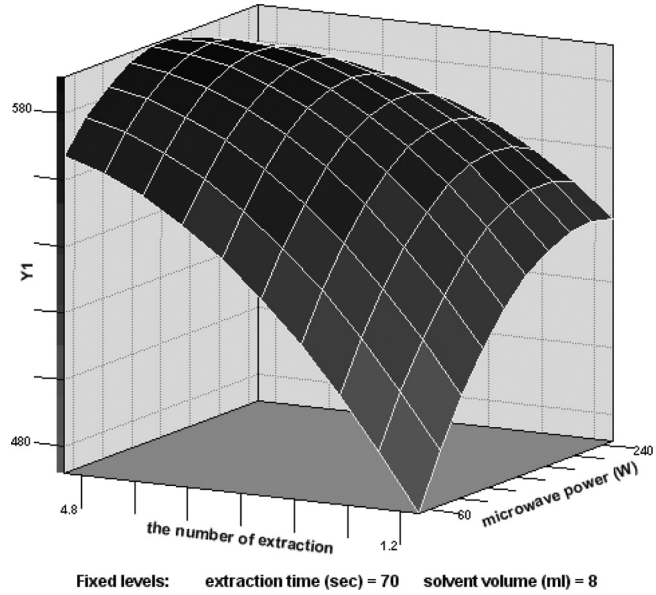

Figure 4. Response surface for the effects of microwave power and the number of extraction at constant extraction time (70 sec) and solvent volume (8 mL) on extraction content of astaxanthin from dried *Haematococcus pluvialis*.

was 141 W, extraction time 83 sec, solvent volume 9.8 mL, the number of extraction 4 times.

The Antioxidant Activities of the Extracts

Inhibition to Linoleic acid Peroxidation

Lipids involve a variety of categories, which constitute the principal structural material of living cells. Unsaturated fatty acid, one of lipids, that produce a large number of free radicals and lipid peroxide (LOOH) during the reaction of lipid peroxidation. The free radicals includes radical of linoleic acid ($L^{\cdot}$), alkyl radical ($LO^{\cdot}$), lipid peroxide free radical ($LOO^{\cdot}$), and these free radicals and LOOH damage the living cells. Therefore, inhibition to the peroxidation of lipid is of biological significance.

The FTC method was used to evaluate the antioxidant activity of the sample at the initial stage of lipid peroxidation. At acidic condition, Fe^{2+} can be oxidated to Fe^{3+} by peroxides, and Fe^{3+} forms a red complex with thiocyanate, the complex has maximal absorbance at (480–515) nm, so

Abs_{500} is generally used to show the inhibition ability to lipid peroxidation. High absorbance is an indication of high concentrations of formed peroxides. The TBARS method is a common method to evaluate the oxidation of lipids. The results of these two methods showed a similar trend on the inhibition ability to lipid peroxidation.

The peroxides of linoleic acid accelerated notably when incubated at 60°C (Control), and reached the highest concentration on the third day (Fig. 5 Insert). The peroxides were unstable and they gradually decomposed into secondary metabolites (30), so their content was decreased on the fourth day. Compared with the control, the extracts and BHT ($0.2\,mg\,mL^{-1}$) had an obvious antioxidant effect on linoleic acid. After five days, the antioxidant activities were still strong and stable. But the antioxidant ability of the extracts were lower than BHT for BHT was pure but the extracts was a compound. In order to obtain the optimal dosage of the extracts to inhibit linoleic acid peroxidation, the antioxidant effect of the extracts at different concentration was determined. As shown in Fig. 5 and Fig. 6, the antioxidant effect increased with the concentration in the test concentration range from 0.05 to $0.4\,mg\,mL^{-1}$. When the concentration is $0.05\,mg\,mL^{-1}$, the inhibition percentage is

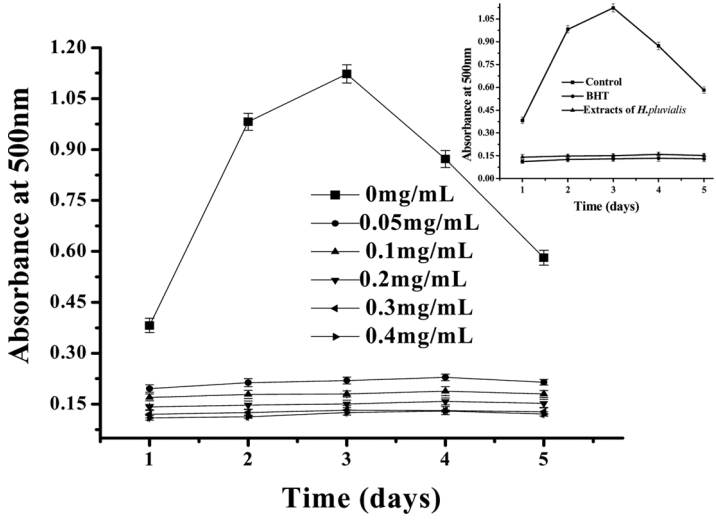

Figure 5. Antioxidant effects of different concentrations of extracts of H. pluvialis on linoleic acid emulsion in the FTC method. The error bars indicate the standard deviation of 3 replicates. Inset: Antioxidant effects of extracts of *H. pluvialis* and BHT ($0.2\,mg\,mL^{-1}$) on linoleic acid emulsion in the FTC method. The error bars indicate the standard deviation of 3 replicates.

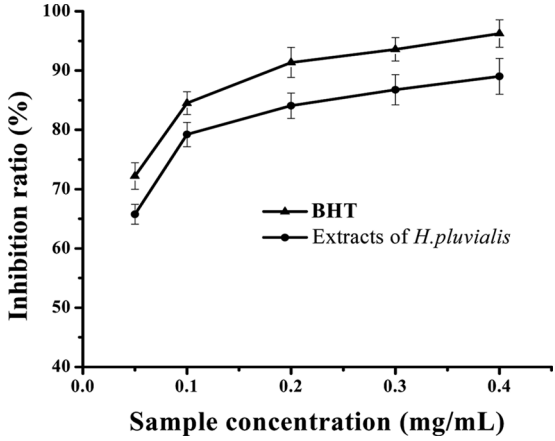

Figure 6. Antioxidant effects of extracts of H. pluvialis and BHT on linoleic acid emulsion in the TBARS method. The error bars indicate the standard deviation of 3 replicates.

above 60% and the concentration is up to 0.4 mg mL^{-1}, the inhibition percentage is about 90% (Fig. 6).

DPPH Radical-Scavenging Activity

Free radical scavenging is one of generally accepted mechanisms against lipid oxidation, and DPPH as a stable free radical compound, which shows maximum absorbance at 517 nm in ethanol, has been widely used to test the free radical-scavenging ability of various samples (26,31). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (32). When DPPH encounters a hydrogen-donating substance, the radical would be scavenged and the absorbance is reduced (33).

Results indicated that BHT and the extracts have the ability of scavenging DPPH radical and the scavenging ability increased with increasing concentration in our used concentration range (Fig. 7). At the concentration of 0.05 mg mL^{-1}, the extracts showed very low scavenging effect on the DPPH radical, but the scavenging percentage increased to 39.25% with the concentration is up to 0.2 mg mL^{-1}, and when the concentration is 0.3 mg mL^{-1}, the scavenging percentage increased to 77.42%. This revealed that the extracts possibly contained substances that are hydrogen donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. It indicates that the extracts of *Haematococcus pluvialis* are also an excellent DPPH scavenger.

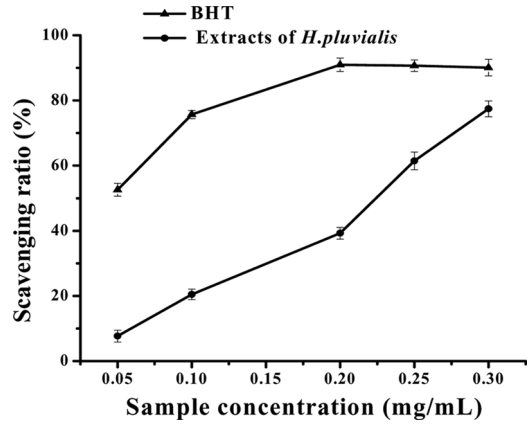

Figure 7. Scavenging effects of different concentration of extracts of H. pluvialis and BHT on DPPH radicals. The error bars indicate the standard deviation of 3 replicates.

Reducing Power

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (34,35). The measurement of reducing power may directly reflect the production condition of the electron donor. As shown in Fig. 8, the reducing power of ascorbic acid,

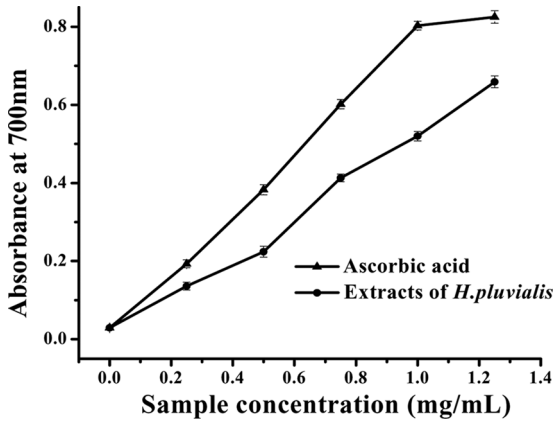

Figure 8. Reducing power of extracts of H. pluvialis and ascorbic acid. The error bars indicate the standard deviation of 3 replicates.

which is a well-recognized reducing agent, increased quickly at the concentration from 0.25 mg mL^{-1} to 1.25 mg mL^{-1}. The reducing power of the extracts increased rapidly with the increasing concentration. This indicates that extracts from *Haematococcus pluvialis* could act as an antioxidant as a good electron donor.

CONCLUSION

RSM was effective for estimating the effect of four independent variables. The optimal conditions of MAE were obtained as follows: microwave power was 141 W, extraction time 83 sec, solvent volume 9.8 mL, the number of extraction four times. Under theses conditions about 594 ± 3.02 μg astaxanthin was extracted from *Haematococcus pluvialis* the dried powders (100 mg) and it was close to the predicted contents. Moreover, a conventional stirring extraction was also carried out in our study (detailed process and relative data not given in this paper) and 583 ± 4.09 μg astaxanthin was obtained from the dried alga powders (100 mg) under it's optimal conditions (extraction time 6 h, solvent volume 12 mL, the number of extraction 2). Compared with the conventional stirring extraction, the application of MAE in the extraction astaxanthin from *Haematococcus pluvialis* increased the content of astaxanthin and dramatically reduced the extraction time.

In addition, the results of antioxidant activities of the extracts obtained under optimal conditions showed that the extracts presented strong ability of inhibition of the peroxidantion of linoleic acid, exhibited strong radical-scavenging properties against the DPPH, as well as strong reducing power. So MAE can be used for the theoretical precondition for development of *Haematococcus pluvialis* function products.

ACKNOWLEDGEMENT

This project was supported by Program for New Century Excellent Talents in University (NCET-04-0134), and the National Natural Science Foundation of China (30570181).

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