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Full Length Research Paper

Microwave-assisted extraction and antioxidant activity of total phenolic compounds from pomegranate peel

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Pomegranate peel is the main waste fraction of pomegranate fruits, which is an excellent source of phenolic compounds. In this study, microwave-assisted extraction was employed to extract the phenolic compounds from pomegranate peel with water. By using response surface methodology, the effects of microwave output power, extraction time, and solid-liquid ratio on total phenolic yield were investigated and the optimal conditions were determined as follows: microwave output power 600 w, extraction time 60 s, and solid-liquid ratio 20. The average experimental phenolic yield under the optimum conditions was found to be 210.36 ± 2.85 mg GAE/g, which agree with the predicted value of 214.46 mg GAE/g. Different antioxidant assays were utilized to evaluate antioxidant activity of the obtained extract. It was found the extract was an effective scavenger in quenching 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals with IC_{50} of 14.53 µg/ml. A linear correlation between concentration of the extract and reducing power was observed with a coefficient of r^2 =0.9992. In Rancimat test, the antioxidant performance of the extract was inferior to that of tertiary butylhydroquinone, which could be attributed to its poor solubility in oil.

Key words: Pomegranate peel, microwave-assisted extraction, response surface methodology, antioxidant.

INTRODUCTION

Pomegranates (*Punica granatum*) have been used extensively in the folk medicine of many cultures. Pomegranate peel is the main waste fraction of pomegranate fruits, which had been widely studied because they contain numerous biologically active compounds including natural antioxidants such as phenolic acids and flavonoids (Li et al., 2006; Singh et al., 2002). Phenolic compounds have attracted more and more attention for their antioxidant behavior and beneficial health-promoting effects in chronic and degenerative diseases (Rice-Evans et al., 1996; Kim and Lee, 2004; Lodovici et al., 2001).

Microwave-assisted extraction (MAE), a relatively novel extracting approach using a microwave applicator as an energy source, has received increasing attention. Compared with conventional methods, MAE has many merits with shorter time, less solvent, higher extraction rate, and superior products quality at lower cost (Zheng et al., 2009). However, limited information has been published on the use of microwave technology for the extraction of total phenolic compounds from pomegranate peel with water.

The objective of this study was to employ response surface methodology to study the effects of microwave output power, extraction time, and solid-liquid ratio on total phenolic yield, and to determine the optimum parameters to maximize total phenolic yield. The antioxidant activity of the obtained extract under the optimum condition was evaluated by DPPH radical scavenging assay, reducing power and Rancimat test.

MATERIALS AND METHODS

Materials and chemicals

Ripened pomegranates were obtained from Huili, Yunnan Province, China. The peel and pulp were separated manually. The fresh peels collected were freeze-dried (Alpha 1-4, Christ, Germany) and

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Table 1. Variables and their levels for central composite design.

Variable	Symbol	Coded variable level		
		-1	0	1
Microwave output power (%)	X_1	20	60	100
Extraction time (s)	X_2	10	35	60
Liquid-solid ratio	X_3	10	25	40

Table 2. Central composite design arrangement and results.

Experiments	Coded Levels			Phenolic Yield Y(mg GAE/g)
	X_1	X_2	X_3	
1	20	10	10	195.73
2	60	35	40	214.78
3	100	10	40	189.38
4	20	35	25	195.15
5	20	60	10	199.19
6	60	10	25	209.01
7	20	60	40	192.84
8	100	35	25	212.47
9	100	60	40	225.17
10	60	35	25	209.58
11	100	60	10	202.66
12	60	35	10	206.7
13	60	35	25	215.94
14	60	60	25	210.74
15	20	10	40	189.95
16	100	10	10	200.92
17	60	35	25	207.85

powdered for this study. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteu's phenol reagent were purchased from Sigma. Gallic acid was the product of BBI. Other chemical were of analytical grade.

Microwave-assisted extraction

The microwave-assisted extraction was performed using a microwave reactor (WBFY-201, Gongyi Yuhua Instrument Co., Ltd) with emission frequency of 2450 MHz and maximum output power 750 W. Samples of 1 g of the dried powder were extracted with different volumes of distilled water at different output powers for different times and then filtered under vacuum. The filtrate was diluted to 100 ml for determining the total phenolic content.

Total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu method (Chun et al., 2005). Briefly, 0.2 ml of the extract was added to a 25 ml volumetric flask, and additional distilled water was added to make a final volume of 10 ml. A reagent blank was prepared using distilled water. Folin-Ciocalteu phenol reagent (0.5 ml) was added to the mixture and shaken vigorously. After 5 min, 5 ml of 5% Na_2CO_3 solution was added with mixing. The solution was immediately diluted to 25 ml with distilled water and mixed thoroughly and then allowed to stand for 90 min. After that, the absorbance was measured at 750 nm versus the prepared blank. The total phenolic content of the sample was expressed as gallic acid equivalents (GAE) milligrams/ml.

Experimental design

One response was measured: Total phenolic yield (Y), defined as the ratio of total phenolic in the extract to total amount of raw material expressed as GAE milligrams per gram of raw material. Each of variables to be optimized was coded at 3 levels: -1, 0, and 1. Table 1 showed the variables, their symbols and levels. The selection of variable levels was based on our preliminary study.

A central composite design (CCD), shown on Table 2, was arranged to allow for fitting of a second-order model. The CCD combined the vertices of a hypercube whose coordinates are given by the 2^n factorial design with the "star" points.

The star points were added to the factorial design to provide for estimation of curvature of the model. Six replicates (run 10, 13 and 17) at the center of the design were used to allow for estimation of "pure error" sum of squares. Experiments were randomized in order to minimize the effects of unexplained variability in the observed response due to extraneous factors. The model proposed for the response (Y) was:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j$$

Where β_0 was the value of the fitted response at the center point of the design, which is point (0, 0, 0). β_0, β_i, β_{ii}, and β_{ij} were the constant, linear, quadratic and cross-product regression terms, respectively.

Preparation of the extract from pomegranate peel

The powdered pomegranate peel (5 g) were extracted with 100 ml of distilled water at 80% output power (600 W) for 60 s and then filtered under vacuum. The filtrate was collected and freeze-dried (Alpha 1-4, Christ, Germany), about 2.42 g of the extract was gained for the following antioxidant assays.

DPPH radical scavenging assay

DPPH radical scavenging assay was done according to a published method (Sun and Ho, 2005). Briefly, 2 ml of DPPH solution (0.2 mmol/L in ethanol) was incubated with different concentrations of the extract, TBHQ. The reaction mixture was shaken and incubated in the dark for 30 min, at room temperature. And the absorbance was read at 517 nm against ethanol. Controls containing ethanol instead of the antioxidant solution, and blanks containing ethanol instead of DPPH solution were also made. The inhibition of the DPPH radical by the samples was calculated according to the following formula:

$$\text{DPPH scavenging activity} = \frac{\text{Abs. of Control} - (\text{Abs. of sample} - \text{Abs. of blank})}{\text{Abs of Control}}$$

And the percentage of DPPH radical scavenging activity was plotted against the sample concentration to obtain the IC_{50}, defined as the concentration of sample necessary to cause 50% inhibition.

Reducing power assay

The reducing power of the sample was determined according to a published method (Strivastava et al., 2006). 0.5 ml of the extract in ethanol was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1 %). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min.

The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of reaction mixture indicated reducing power.

Rancimat test

The antioxidant activities of the samples were measured on a 743 Rancimat analyzer (Switzerland) according to a published method (Proestos et al., 2006). The samples of lard oil (3 g) containing 0.02 % of the extract and BHT were subjected to oxidation at 110°C (air flow 20 L/h). Induction periods, IP (h), were recorded automatically.

The protection factors (PF) were calculated according to the following formula:

$$PF = IP_{sample} / IP_{control}.$$

Statistical analysis

The data obtained in this study were expressed as the mean of three replicate determinations and standard deviation (SD). Statistical comparisons were made with student's test. P values of < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Diagnostic checking of the fitted model

When many factors and interactions affect desired responses, response surface methodology (RSM) is an effective tool for optimizing the process. The basic principle behind response surface methodology (RSM) analysis is to relate the observed value (dependent variables) to process parameters (independent variables) using statistical methods, yielding a multivariate regression equation, often of second-order. RSM takes interactions into consideration and optimizes the process parameters to reasonable range, with the advantage of less the number of replicates and the total time required to perform the experiments (Lee et al., 2006). RSM uses an experimental design such as the central composite design (CCD) to fit a model by least squares technique. If the proposed model is adequate, as revealed by the diagnostic checking provided by an analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response surface and locate the optimum (Rustom et al., 1991). Multiple regression analysis of the experimental data yielded the following second-order polynomial stepwise equation:

$$Y=193.29771+0.51593xX_1-0.071414\times X_2-0.31156\times X_3+3.8975\times 10^{-3}\times X_1X_2+4.8125\times 10^{-3}\times X_1X_3+0.011160\times X_2X_3-5.23592\times 10^{-3}\times X_1^2-3.69994\times 10^{-3}\times X_2^2-6.43318\times 10^{-3}\times X_3^2$$

The result of ANOVA was shown on Table 3. The model F-value of 3.84 implied the model was significant. There was only a 4.48% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicated model terms were significant. Values of "Prob > F" greater than 0.1000 indicate the model terms were not significant. The coefficient of determination (R-Squared) is the proportion of variability in the data explained or accounted for by the model. The "R-Squared" of 0.8317 was desirable.

Effects of extraction variables on total phenolic yield

Three-dimensional response surfaces presented in Figures 1 to 2 for the independent variables (microwave output power, extraction time, and solid-liquid ratio) were obtained by keeping another variable constant, which indicated the changes in total phenolic yield under different MAE conditions.

Table 3. ANOVA for the fitted model.

Source	Sum of squares	d_f	Mean square	F value	Prob>F
Model	1341.76	9	149.08	3.84	0.0448
X_1	333.39	1	333.39	8.59	0.022
X_2	208.03	1	208.03	5.36	0.0537
X_3	4.79	1	4.79	0.12	0.7357
X_1X_2	121.52	1	121.52	3.13	0.1201
X_1X_3	66.70	1	66.70	1.72	0.2312
X_2X_3	140.11	1	140.11	3.61	0.0991
X_1^2	188.03	1	188.03	4.85	0.0636
X_2^2	14.33	1	14.33	0.37	0.5626
X_3^2	5.61	1	5.61	0.14	0.7149
Residual	271.57	7	38.80		
Cor total	1613.33	16			

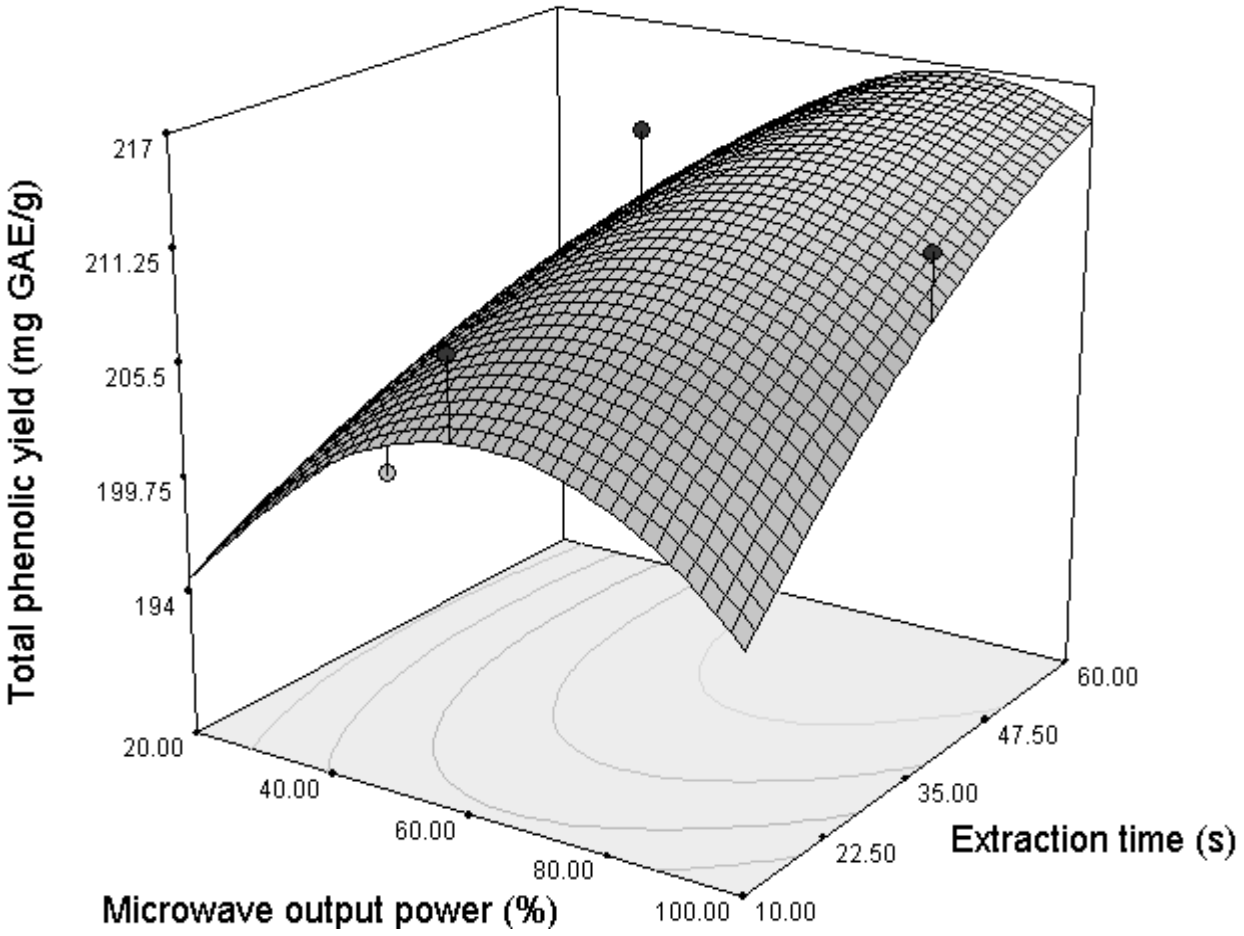

Figure 1. Response surface for the effect of microwave output power and extraction time on total phenolic yield.

The effect of microwave output power and extraction time on total phenolic yield was shown in Figure 1. Another factor, liquid-solid ratio, was set at 25, respectively. It was concluded that total phenolic yield had a positive linear relationship with extraction time. The total phenolic yield depended upon microwave output power, which resulted in a curvilinear increase until microwave output power 80%, and then to decreased in the total

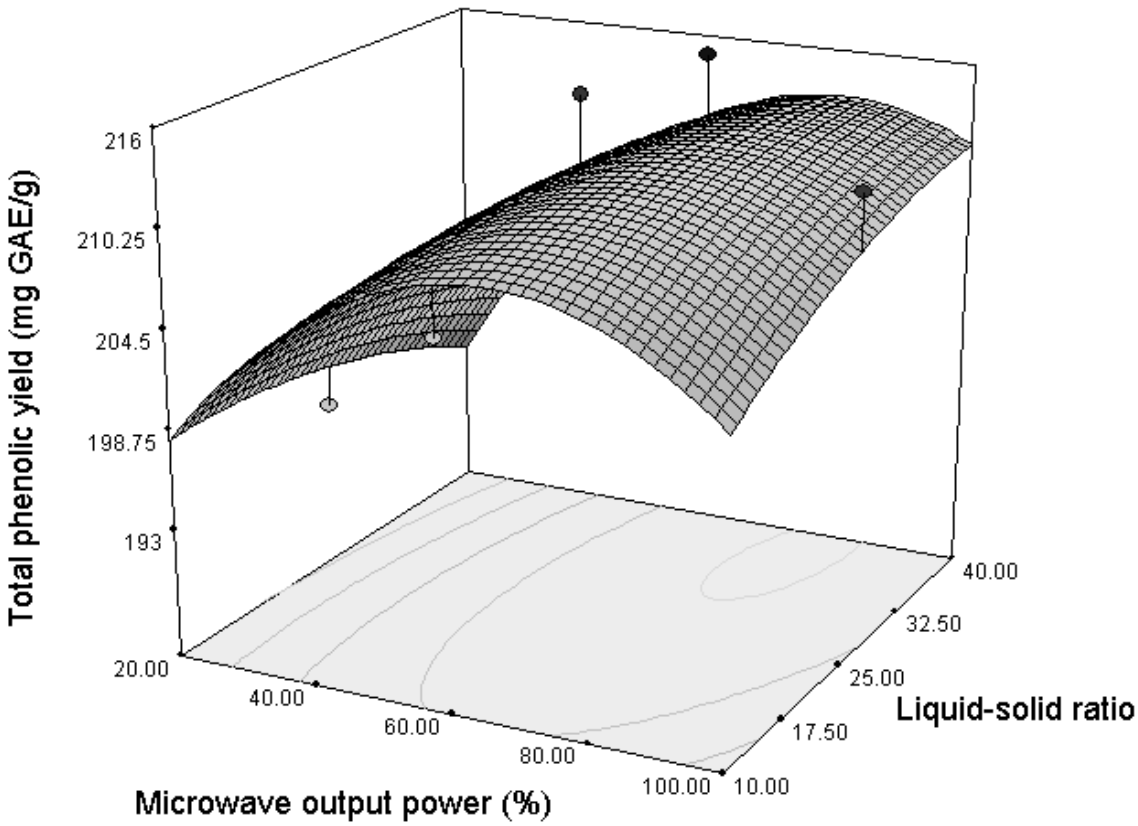

Figure 2. Response surface for the effect of microwave output power and liquid-solid ratio on total phenolic yield.

phenolic yield. Figure 2 demonstrated the effect of microwave output power and solid-liquid ratio on total phenolic yield at a constant extraction time of 35 s. It was found the effect of liquid-solid ratio on total phenolic yield was no significant.

Optimization of microwave-assisted extraction

According to the desired goals, each factor and response was chosen to optimize MAE process conditions are shown in Table 4. The optimum conditions were obtained by running the program of central composite design. The optimum conditions for independent variables and the predicted values of the responses also are presented as follows: extraction time 60 s, 80% microwave output power (600 w), liquid-solid ratio 20. The estimated values for total phenolic yield, 214.46 mg GAE/g was obtained at those conditions. A verification experiment at the optimum condition, consisting of 3 runs, was performed and the practical yield of 210.36 ± 2.85 mg GAE/g was obtained.

DPPH radical scavenging activity

The DPPH radical is a stable organic free radical with adsorption band at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow (Sánchez-Moreno, 2002). Because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations, it was used for evaluating the antioxidant potential of the extract and TBHQ. In this study, a high DPPH radical scavenging activity was observed in both the extract and TBHQ in a concentration manner (Figure 3). The DPPH radical scavenging activity of the extract (IC_{50}, 14.53 µg/ml) was slightly lower than that of TBHQ (IC_{50}, 6.87 µg/ml), which should attribute to the high phenolic content.

Reducing power

The reducing power of the extract, which may sever as a significant reflection of antioxidant activity, was determined using a modified Fe (III) to Fe (II) reduction assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of samples. The presence of antioxidants in the samples causes the reduction of the Fe^{3+}/Ferricyanide complex to the ferrous form. Therefore,

Table 4. The optimum condition of total phenolic extraction.

Microwave output power (%)	Extraction time (s)	Liquid-solid ratio	Predicted yield (mg GAE/g)	Desirability (%)
80	60	20	214.46	0.837

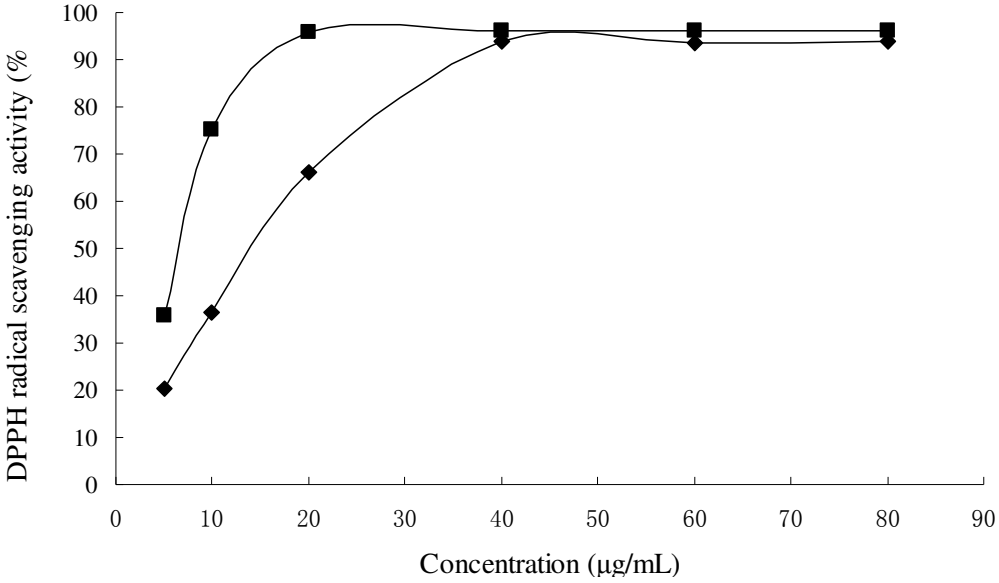

Figure 3. DPPH radical scavenging activity of the extract (-♦-) and TBHQ (-■-).

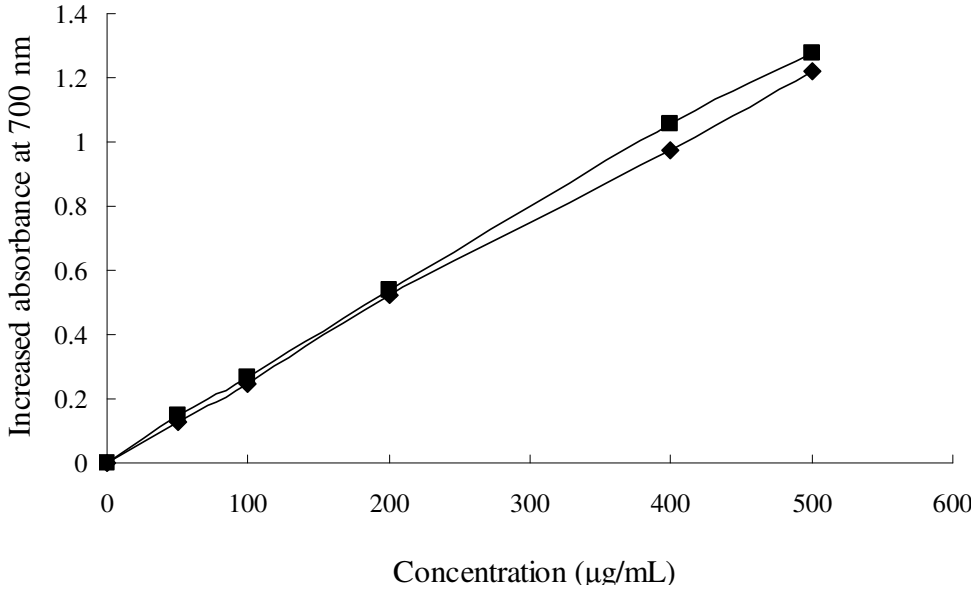

Figure 4. Reducing power of the extract (-♦-) and TBHQ (-■-).

the Fe^{2+} can be monitored by measurement of the formation of Perl's Prussian blue at 700 nm (Zou et al., 2004). Figure 4 showed the reducing power of the extract and TBHQ. Both the samples showed some degree of

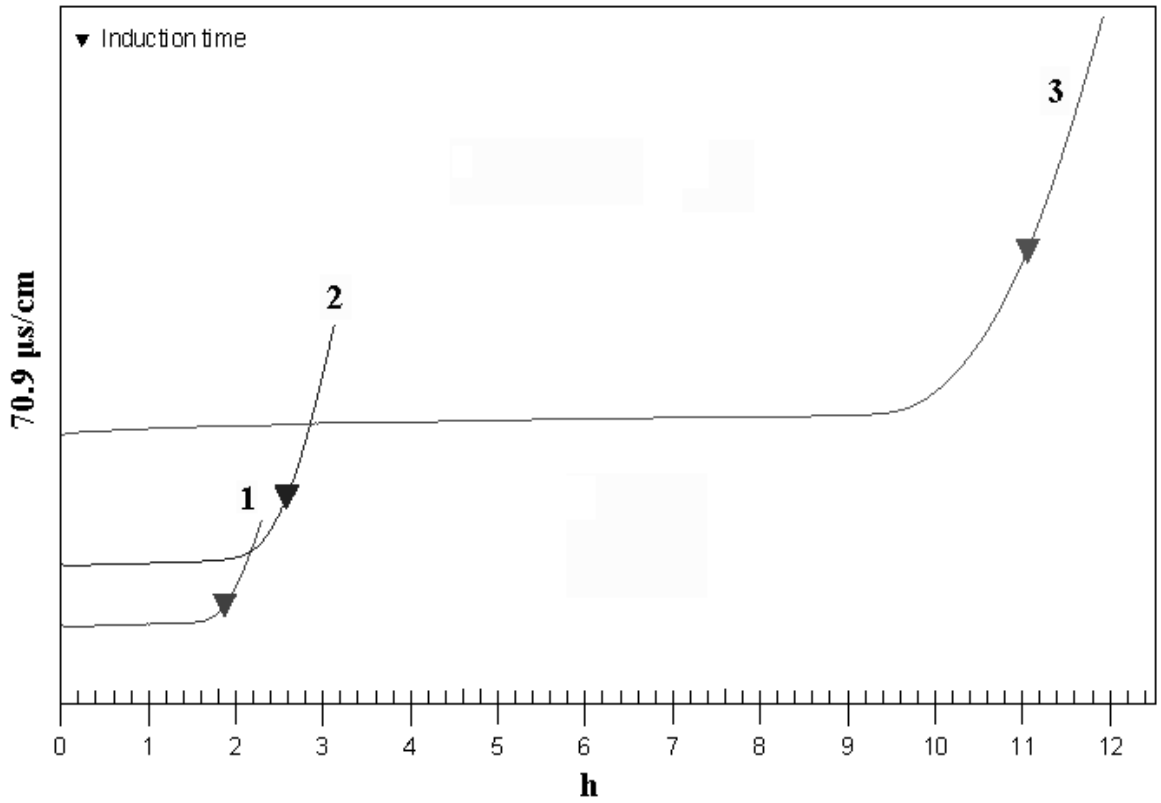

Figure 5. Antioxidant activity of control (1), extract (2), TBHQ (3) in Rancimat test.

reducing power. And the reducing power of TBHQ was lightly superior to that of the extract. In this study, the reducing power of the samples linearly increased with increasing concentration and the correlative coefficient (r^2) of the extract and TBHQ was 0.9992 and 0.9993, respectively.

Antioxidant activity in Rancimat test

In Rancimat method, the sample is exposed to a stream of air at temperatures from 50 to 220°C. The volatile oxidation products (chiefly formic acid) are transferred to the measuring vessel by the air stream and absorbed there in the measuring solution (distilled water). When the conductivity of this measuring solution is recorded continuously, an oxidation curve is obtained whose point of inflection is known as the induction time; this provides a good characteristic value for the oxidation stability. As shown in Figure 5, the induction times of control, the extract and BHT were 1.87, 2.58 and 11.06 h, respectively. The performance of the extract with PF of 1.38 was inferior to that of TBHQ with 5.91. The main phenolic compound in pomegranate peel was tannin, which could not solve in oil. As a result, the solubility of the extract in oil was very low, which contributed to its low antioxidant activity in oil.

Conclusion

Optimum of microwave-assisted extraction of total phenolic compounds from pomegranate peel with water could be achieved by extracting 1 part of pomegranate peel with 20 parts water at the microwave output power of 600 W for 60 s. Such conditions resulted in the total phenolic yield of 210.36 ± 2.85 mg GAE/g. In the hydrophilic system, the antioxidant activity of the obtained extract could compare with that of TBHQ. The extract from pomegranate peel maybe becomes a new source of natural antioxidant and health food with great commercial interest in the food and phyto-pharmaceutical market.

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