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Recommended Citation
DOI: 10.6119/JMST-014-0623-1
Available at: https://jmstt.ntou.edu.tw/journal/vol23/iss5/29

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INVESTIGATION ON INTERMITTENT MICROWAVE-ASSISTED EXTRACTION OF SULFATED POLYSACCHARIDES FROM *PORPHYRA DENTATA*

Shao-Chi Wu and Yeu-Pyng Lin

Key words: microwave-assisted extraction, intermittency, sulfated polysaccharides, response surface methodology, *Porphyra dentata*.

**ABSTRACT**

This study investigated the intermittent microwave-assisted extraction (MAE) of sulfated polysaccharides from *Porphyra* (*P.*) *dentata* Kjellman, 1897. The experiment consisted of adjusting 10% to 90% ethanol solutions (v/v) used as extraction solvents, and then applied intermittent MAE to extract *P. dentata* solutions. These different extract solutions were then further evaluated for their sulfated polysaccharides contents (µg/mL). The optimal conditions of the sulfated polysaccharides content using intermittent MAE with 3, 4, and 5 cycle treatment were studied using response surface methodology. This study was performed using a three-level, three-factor design and aimed to determine the optimal combination of ethanol concentration (10~90%, v/v, *X*₁), microwave power (200~400 W, *X*₂), and intermittency of the microwave field (0~1, *X*₃) for obtaining the optimal results in extracting sulfated polysaccharides from *P. dentata*. The response variable of the sulfated polysaccharides content (µg/mL) was significantly affected by the ethanol concentration (*X*₁), microwave power (*X*₂), and intermittency (*X*₃) at 5% level of significance. The optimal treatment was established by adjusting the ethanol concentration to 44.4%, followed by using a 200 W microwave with an intermittency of 0.75 at 4 cycles for this study. Verification tests indicated that the second order polynomial model generated was adequate. The optimal extraction condition was provided and the validation proved that the results were acceptable.

I. INTRODUCTION

Seaweeds are the rich source of sulfated polysaccharides and some of which have become valuable additives in the food industry because of their favorable rheological properties as gelling and thickening agents (e.g., carrageenan). In addition, sulfated polysaccharides are known to possess a number of biological activities including anti-oxidation, anti-coagulant, anti-tumor, anti-virus, immuno-modulatory, and immuno-inflammatory effects as well as specific activities against kidney, liver, and urinary system disorders, and may be relevant in nutraceutical/functional food, cosmetic/cosmeceutical, and pharmaceutical applications (Ale et al., 2011; Jiao et al., 2011). Sulfated polysaccharides are found in three main divisions of marine algal groups, i.e. Rhodophyta, Phaeophyta, and Chlorophyta (Costa et al., 2010). Among them, Phaeophyta has caught most attention because its polysaccharides possess higher sulfated contents than the other two (de Jesus Raposo et al., 2013). However, the researches on the sulfated polysaccharides from *Porphyra* (Rhodophyta) have been rare and they deserve to be explored (Silva et al., 2012). Porphyran, one of the main constituents of the *Porphyra* species, is a linear sulfated polysaccharide comprised of a hot-water soluble portion of the cell wall and an intracellular matrix. It consists of D-galactose, 3,6-anhydro-L-galactose, 6-O-methyl-D-galactose, and L-galactose-6-sulphate. The *Porphyra* species are edible red algae abundantly growing in East and Southeast Asia (Jiang et al., 2012), and *P. dentata* (Bangiaceae) is widely distributed in East-Asian countries and abounds in Penghu, Taiwan (Lee and Yoon, 2006). In Taiwan, *P. dentata* of great economical values and its bioactive substances have been widely applied. Recently, polyphenols extracted from *P. dentata* by microwave-assisted extraction (MAE) have been studied (Lin et al., 2011; 2013). In this study, the method of intermittent MAE of sulfated polysaccharides was used and it is a new technology worthy of extensive research.

MAE has been reported as a novel method for the extraction of bioactive compounds. It utilizes microwave energy to cause molecular movement and rotation in liquids by means of
permanent or induced dipoles (Sun et al., 2007). When a biological material with suitable dielectric properties, e.g. a plant material and solvent for extraction, is placed in a microwave field, the molecules would try to align with the oscillating electromagnetic field, either by distortion or distribution of the electron cloud within the molecule or by physical rotation of the molecular dipoles, both leading to rapid heating of the solvent and the sample matrix (Dandekar and Gaikar, 2002). MAE has the advantages over conventional extraction techniques due to its improved efficiency, reduced extraction time, rapid volumetric heating of the absorbing medium, low solvent consumption, higher selectivity of target molecules, and a high potential for automation (Krishnaswamy et al., 2013). For heat-sensitive bio-products, high heat applied to the surface has always resulted in quality problems. Unlike the conventional heating practice, the intermittent heating process employs time-varying heat supplies and could match the kinetics of the extraction of the material. In order to avoid overheating and to ensure the efficient application of the energy, the intermittent heating mode could be employed in MAE of bio-products since both heat and mass transfer could be well balanced (King and Lin, 2009; Lin et al., 2011; 2013).

Response surface methodology (RSM) is a statistical procedure frequently used in optimization studies. It uses quantitative data based on an appropriate experimental design to determine the optimal condition while simultaneously solving multivariate problems (Chen et al., 2012). Several authors have used RSM in their optimization studies for MAE of polysaccharides from *Cathelasma ventricosum* (Zhao et al., 2012), *Cyclocarya paliurus* (Batal.) Iljinskaja (Xie et al., 2010), and *Lilium davidii* var. *unicolor* Salisb (Zhao et al., 2013).

In our previous study, we focused on the effect of MAE of polyphenols on the scavenging of free radicals and the ferrous chelating abilities of *Lilium davidii* polyphenols on the scavenging of free radicals and the ferrous chelating abilities of *Cyclocarya paliurus*

2. Intermittent Microwave-Assisted Extraction (Intermittent MAE)

Jumah et al. (1996) defined the intermittency ratio, $\alpha$, as the fraction of the cycle time during which spouting gas is supplied for drying. The MAE was performed using a modified method according to Soysal et al. (2009). A microwave accelerated reaction system (MARS5, CEM Corporation, Matthews, North Carolina, U.S.A.) was equipped with solvent extraction. The operation frequency of the magnetron was 2,450 MHz. The test suspensions used were 0.5 g *P. dentata* powder particles mixed into 50 mL water-ethanol solutions with water-ethanol ratios of 90:10, 50:50, and 10:90 (v/v) to make 1.0% solutions, and the pH was then adjusted to 7. Solutions were radiated at regular intervals in the microwave system. The intermittency ($\alpha$) was defined as follows (Jumah et al., 1996; King and Lin, 2009):

$$\alpha = \frac{\tau_{on}}{\tau_{on} + \tau_{off}}$$

where $\tau_{on}$ and $\tau_{off}$ are the ‘on’ and ‘off’ periods of the microwave field, respectively. One cycle time ($\tau_{on} + \tau_{off}$) is set to be 60 sec. When $\alpha = 0$, the suspension stands for 10 min at room temperature (25°C) without MAE as a blank. When $\alpha = 0.5$, it means that $\tau_{on} = 30$ sec and $\tau_{off} = 30$ sec. When $\alpha = 1.0$, it means that $\tau_{on} = 60$ sec and $\tau_{off} = 0$ sec. The latter conditions were conducted for 3, 4, and 5 cycles, respectively and temperature was kept below 80°C. The extracts were allowed to cool down to room temperature, and then filtered and stored in a refrigerator at -20°C to determine the sulfated group contents of polysaccharides from *P. dentata*.

3. Quantification of the Sulfated Group Contents

1) Reagents

- BaCl$_2$ buffer: 2 M acetic acid 10 mL, 5 µM BaCl$_2$ 2 mL, and 20 µM NaHCO$_3$ 8 mL were quantified to 100 mL by absolute ethanol; Na-rhodizonate solution: 5 mg Na-rhodizonate and 100 mg L-ascorbic acid were dissolved in 20 mL distilled water. The volume was quantified to 100 mL by absolute ethanol. These reagents were light sensitive and protected from light. A standard curve was established using 2-12 µg/mL Na$_2$SO$_4$ as the standard (Dodgson, 1961).

2) Sample Treatment

For all test samples, the total sugar content was adjusted to 10 mg/mL. Then 0.5 mL 1 N HCl was added to 0.5 mL sample solution and boiled for 1 h. All solvents were removed by means of vacuum concentration at 60–65°C and 0.5 mL de-ionized water was then added (Dodgson, 1961).

3) Determination of the Sulfated Group Content

Two mL absolute ethanol was added to 0.5 mL sample solution, and the insoluble portion was removed by centrifugation at 2,500 × g. One mL BaCl$_2$ buffer was then added,
followed by 1.5 mL Na-rhodizonate solution. The reaction was carried out in the dark for 10 min and \( A_{320} \) was measured within 30 min (Dodgson, 1961).

### 4. Experimental Design

The sulfated group content of the polysaccharides of *P. dentata* was investigated as the response variable. A three-level-three-parameter experimental design reported by King and Lin (2009) was used to evaluate the optimal treatment condition. The experimental error was estimated by performing the experimental procedure and measuring the center point three times.

Three mathematical functions of \( f_k \) were assumed to exist for \( \eta_k \) as follows:

\[
\eta_k = f_k(\varepsilon_1, \varepsilon_2, \varepsilon_3)
\]

where \( \varepsilon_1 \) is the ethanol concentration, \( \varepsilon_2 \) is the microwave power, and \( \varepsilon_3 \) is the intermittency of the microwave field. A second order polynomial was used to express the function \( f_k \) as follows:

\[
\eta_k = \beta_{0k} + \sum_{i=1}^{3} \beta_{i0}X_i + \sum_{i=1}^{3} \beta_{ik}X_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij}X_iX_j
\]

where \( \beta_{0k}, \beta_{i0}, \beta_{ik}, \beta_{ij} \) are regression coefficients and \( X_i \) represents the coded independent variables of \( \varepsilon_1, \varepsilon_2, \) and \( \varepsilon_3. \)

The values of the independent variables were coded within the range of -1 and +1, and the original independent variables, \( X_i, \) have been normalized by the following equation:

\[
X_i = \frac{2}{I_i} (\varepsilon_i - \bar{\varepsilon}_i)
\]

where \( \varepsilon_i \) is the current value of the variable, \( \bar{\varepsilon}_i \) is the mean arithmetic value of the largest and the smallest value of the set, and \( I_i \) is the greatest difference between those extremes.

### 5. Statistical Analysis

Contour and surface plots were determined using Sigmaplot software (Scientific Graph System, version 7.00, SPSS Inc., 2001. U.S.A.). Analysis of the ANOVA table and the estimation of the response in the model were conducted using the PROC RSREG procedure of the SAS program, and the validity of the model was evaluated (SAS, version 8.1, SAS Inc., 1999. U.S.A.).

### III. RESULTS AND DISCUSSION

#### 1. Effect of the Parameters

The experiments were performed according to a design with three variables and three levels for each variable (Box and Behnken, 1960). The independent variables were the ethanol concentration \( (X_1) \), the microwave power \( (X_2) \), and the intermittency of the microwave field \( (X_3) \). The experimental design of the coded and actual levels is shown in Table 1. The experimental design and the response surface analysis data of this study are shown in Table 2. The experiments were performed in random order in order to study the relationships between the dependent variable, which was the sulfated polysaccharides content extracted from *P. dentata*, and the independent variables \( X_1, X_2, \) and \( X_3, \) which included the ethanol concentration, the microwave power, and the intermittency of the microwave field. Analysis of the variance was performed to determine the lack-of-fit and the significance of the linear, quadratic, and cross-product effects of the independent variables on the quality attribute (Table 3). The lack-of-fit test is a measure of the failure of a model to represent data in the experimental domain at points which are not included in the regression (Montgomery, 1984). The analysis of the lack-of-fit was performed for the dependent variable, and the result was not significant. In the case of the model, a high coefficient of the determination value \( (R^2 > 0.98) \) was also obtained for the significant response surface model, i.e. the sulfated content of the polysaccharides extracted from *P. dentata*, indicating a

### Table 1. Process variables and their levels in the three variables-three levels response surface design.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Symbols</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol concentration</td>
<td>( X_1 )</td>
<td>( \varepsilon_1 )</td>
</tr>
<tr>
<td>Microwave power level</td>
<td>( X_2 )</td>
<td>( \varepsilon_2 )</td>
</tr>
<tr>
<td>Intermittency of microwave field</td>
<td>( X_3 )</td>
<td>( \varepsilon_3 )</td>
</tr>
</tbody>
</table>

#### Table 2. The experimental design and data for the response surface analysis.

<table>
<thead>
<tr>
<th>Treatment numbera</th>
<th>( X_1 )</th>
<th>( X_2 )</th>
<th>( X_3 )</th>
<th>Sulfated polysaccharides content (( \mu g/mL ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>60.4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>58.0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>61.6</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>70.6</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>61.6</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>49.4</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>62.0</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>56.9</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>74.5</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>68.1</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>77.8</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>68.1</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>75.9</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>77.0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>75.5</td>
</tr>
</tbody>
</table>

\( a \) The experimental runs were performed in random order.
high proportion of the variability could be explained. The model developed in this study was proven to be adequate.

An analysis of variance was conducted to assess the significant effect of each independent variable on the response and to analyze whether the response was significantly affected by the variables. As shown in Table 3, ethanol concentration \((X_1)\) and intermittency \((X_3)\) significantly affected the sulfated polysaccharides content at the 0.1% level. Besides, the microwave power \((X_2)\) also significantly affected the sulfated polysaccharides content at the 5% level. Table 4 shows the regression coefficients for the second-order polynomial model of the sulfated polysaccharides content and the model was used to predict the value of optimal condition. Contour plots were generated using significant parameters for the response. Since the response of sulfated polysaccharides content was significantly affected by three independent variables, i.e. the ethanol concentration, the microwave power, and the intermittency, these three variables were used to determine the optimal treatment condition. The response surface plot and contour plot were used for optimization.

### 2. Optimization Studies and Verification of the Models

Response surface methodology is an appropriate method widely used in optimization studies. The optimum condition could be determined by finding the maximum value of the response variable. In this study, the change of the maximum value of the sulfated polysaccharides at 3, 4, and 5 extract cycling treatments from 200 to 400 W at an interval of 100 W were created, and at 4 cycle, a maximum value point of the sulfated polysaccharides content was located, i.e. 80.09 µg/mL, forming the basis for the optimum treatment (Fig. 1). Fig. 2 shows the point of the optimum treatment condition by adjusting the ethanol concentration to 44.4% \((X_1 = -0.14)\) and the intermittency of 0.75 \((X_3 = 0.5)\) with a 200 W microwave power \((X_2 = -1)\) at 4 cycles.

According to the response surface plot of the sulfated polysaccharides content (µg/mL), the optimum operating condition was determined with the maximum sulfated polysaccharides content 80.09 µg/mL. The optimum treatment was established with an intermittency of 0.75 at 4 cycles at a 200 W microwave power by the operation controlling the ethanol concentration 44.4% \((X_1 = -0.14)\) and the intermittency of 0.75 \((X_3 = 0.5)\) with a 200 W microwave power \((X_2 = -1)\) at 4 cycles.

The verification test was performed at the optimum condition, i.e. \(X_1 = -0.14, X_2 = -1,\) and \(X_3 = 0.5\) to determine the adequacy of the second order polynomial model and the result is shown in Table 5. The experimental value was the average of three replications and the difference between the experimental and the predicted values was found to be insignificant by the \(t\)-test at a 5% level of significance, indicating that the second order polynomial model obtained was adequate. The optimum treatment condition was recommended and validation results were adequate and acceptable, demonstrating that the second order polynomial model generated for the response was appropriate.

### 3. Efficiency of Microwave Treatment

Zhao et al. (2013) studied the effects of four parameters, i.e. extraction time, microwave power, extraction temperature, and the ratio of solids to water, on MAE of LPMAE (polysaccharides from *Lilium davidii* var. *unicolor* Salish) and optimized, using the Box-and-Behnken design (BBD) with a quadratic regression model built by RSM. Our results showed that when the ratio of solids to water increased, the microwave extraction efficiency of LPMAE also increased. However, when the solids decreased and the water increased, the microwave

### Table 3. Analysis of variance for response variables.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>3 cycle</th>
<th>4 cycle</th>
<th>5 cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>9</td>
<td>1036.96***</td>
<td>1085.90**</td>
<td>1035.42***</td>
</tr>
<tr>
<td>Linear</td>
<td>3</td>
<td>210.56***</td>
<td>219.02***</td>
<td>200.01**</td>
</tr>
<tr>
<td>Quadratic</td>
<td>3</td>
<td>778.59***</td>
<td>833.75***</td>
<td>779.16***</td>
</tr>
<tr>
<td>Cross product</td>
<td>3</td>
<td>47.82**</td>
<td>33.13**</td>
<td>56.25*</td>
</tr>
<tr>
<td>Residual</td>
<td>5</td>
<td>6.42</td>
<td>3.93</td>
<td>11.38</td>
</tr>
<tr>
<td>Lack-of-fit</td>
<td>3</td>
<td>5.89NS</td>
<td>2.97NS</td>
<td>8.21NS</td>
</tr>
<tr>
<td>Pure error</td>
<td>2</td>
<td>0.53</td>
<td>0.96</td>
<td>3.17</td>
</tr>
</tbody>
</table>

### Table 4. Regression coefficients of the second order polynomial for three response variables.

<table>
<thead>
<tr>
<th>Coefficient (^a)</th>
<th>Sulfated polysaccharides content (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta_0)</td>
<td>75.93***</td>
</tr>
<tr>
<td>(\beta_1)</td>
<td>-2.71***</td>
</tr>
<tr>
<td>(\beta_2)</td>
<td>-1.24*</td>
</tr>
<tr>
<td>(\beta_3)</td>
<td>4.18***</td>
</tr>
<tr>
<td>(\beta_{11})</td>
<td>-13.97***</td>
</tr>
<tr>
<td>(\beta_{21})</td>
<td>2.85**</td>
</tr>
<tr>
<td>(\beta_{22})</td>
<td>0.68</td>
</tr>
<tr>
<td>(\beta_{31})</td>
<td>1.78*</td>
</tr>
<tr>
<td>(\beta_{32})</td>
<td>-0.83</td>
</tr>
<tr>
<td>(\beta_{33})</td>
<td>-4.49***</td>
</tr>
</tbody>
</table>

\(^a\) These are coefficients of Equation (3), and the numbers 1 to 3 in the subscripts refer to ethanol concentration, microwave power level, and intermittency of microwave field, respectively.

*Significant at 5% level; **Significant at 1% level; ***Significant at 0.1% level.

* DF: Degree of freedom. NS: Not significant.
power increased and the LPMAE slightly decreased. This study also showed that the lower the microwave power the higher the efficiency of sulfated polysaccharides extraction when the ratio of solids to liquid of continuous phase was constant.

The difference between our study and that of Zhao et al. (2013) is that they extracted LPMAE by putting Lilium davidii var. unicolor powder in 3,000 mL of liquid under different MAE conditions and using an extraction time of 30–90 min. However, sulfated polysaccharides was extracted with 50 mL solvent and an extraction time of 0–60 sec in our research, and energy, time, and space were successfully saved. The specific heats of ethanol and water are 0.618 and 1.000 cal/g, respectively, and the dielectric constants of ethanol and water are 74.0, 52.6, and 29.2, respectively, showing that moderate polarity is more suitable for the MAE of sulfated polysaccharides.

### Table 5. Predicted and observed values for the response variable at the optimum value condition (4 cycle).

<table>
<thead>
<tr>
<th>Coordinate (X₁, X₂, X₃)</th>
<th>Predicted value</th>
<th>Observed value (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-0.14, -1, 0.5)</td>
<td>80.09</td>
<td>79.91 ± 1.20NS</td>
</tr>
</tbody>
</table>

NS: Not significant for t-test at 5% level significance.

Results are of three replicates.

### 4. Effect of Intermittent Microwave Treatment

In the present research, results of intermittent MAE showed that there are advantages in extraction efficiency, microwave power, and that the application of intermittency of microwave field is superior to hot water extraction, ultrasonic assisted extraction, and Soxhlet extraction (Afoakwa et al., 2012). Intermittent MAE is also superior to the supercritical fluid extraction because of its easy operation and low cost (Afoakwa et al., 2012). The intermittent application of microwave energy has been proven to be an excellent alternative method which avoids uneven heating and improves the quality of the product as well as the energy utilization by allowing a redistribution of the temperature and moisture profiles within the product during off times due to the thermal diffusion (Soysal et al., 2009). Therefore, the intermittent technology possesses the advantages of energy conservation and a shorter extraction time of the extraction of bioactive substances. The stress effect of continuous extraction makes it inferior to intermittent extraction because the latter promotes osmosis and cell lysis of the bioactive substances. King and Lin (2009) used the intermittent heat supply mode and found that it was beneficial for the FIR (far-infrared) drying operation in the falling rate period, while the internal diffusion of heat and moisture controlled the overall drying rate. At the t(off) periods of the intermittent FIR heating process, little or no heat was supplied for drying, and the moisture and heat diffused within the material and the surface temperature of the product would not exceed the value which might cause thermal damage to the product. As a result, a better rehydration ratio was obtained. Besides, the intermittent MAE in this study also confirmed that intermittent MAE is better than continuous MAE, i.e. intermittency α = 1. Besides, the cold water extraction of sulfated polysaccharides was 68.1 μg/mL in 50% ethanol at pH 7, and the same solvent condition used in MAE at 200 W was 78.1 μg/mL, increasing only 10 μg/mL. However, from the point of view of extraction time, MAE of sulfated polysaccharides showed significant saving compared to the cold water extraction method.

### IV. CONCLUSIONS

The aim of this study was to improve the effect of the ethanolic extraction treatment of Porphyra dentata with in-
termittent MAE, and to find the optimal processing condition. A satisfactory prediction equation was developed using response surface methodology for the optimization of intermittent MAE of sulfated polysaccharides from Porphyra dentata. The ethanol concentration and the intermittency significantly affected the sulfated polysaccharides content at the 0.1% level, and microwave power significantly affected the sulfated polysaccharides content at the 5% level. Optimal conditions were established by adjusting the ethanol concentration to 44.4% with 200 W microwave cycling with an intermittency of 0.75 at 4 cycles.

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