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Optimization of crude polysaccharides extraction from *Hizikia fusiformis* using response surface methodology

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ABSTRACT

A 17-run Box-Behnken design (BBD) was used to optimize the extraction conditions of polysaccharides from *Hizikia fusiformis*. Three factors such as extraction time (h), extraction temperature (°C) and ratio of water to raw material were investigated. The experimental data were fitted to a second-order polynomial equation using multiple regression analysis and also examined using the appropriate statistical methods. The adjusted coefficient of determination (R^2_{Adj}) for the model was 0.9301, and the probability value ($p = 0.0002$) demonstrated a high significance for the regression model. The optimum extraction conditions were found to be: optimized extraction time 2.05 h, extraction temperature 75.45 °C, and ratio of water to raw material 29.89. Under these conditions, the mean extraction yield of polysaccharides was 21.8324%, which was in good agreement with the predicted model value.

1. Introduction

Hizikia fusiformis is one of the most widely consumed seaweeds in China, Japan and Korea. This alga is known to be rich in dietary fiber, and essential minerals, such as calcium, iron and magnesium (Choi, Hwang, Kim, & Nam, 2009; Hwang, Kim, & Nam, 2008). *H. fusiformis* is not only used as a food product, but also has medical applications and has even been suggested as antioxidants (Siriwardhana et al., 2004), anti-coagulants (Kim, Seo, Lee, Cho, & Yang, 1998) and anticancer agent (Han, Cao, Yu, Wang, & Shen, 2009). Polysaccharides are polymeric carbohydrate structures, formed of repeating units joined together by glycosidic bonds. They have widely been investigated because of their chemical properties and biological activities in recent years (Sun, Li, Yan, & Liu, 2010; Wang, Cheng, Mao, Fan, & Wu, 2009). However, little information is available about the polysaccharides from *H. fusiformis*. Aqueous extractions techniques are the most common methods for the extraction of plant polysaccharides (Li, Chen, Wang, Tian, & Zhang, 2009; Wu, Cui, Tang, & Gu, 2007). In order to obtain a high yield of polysaccharides, the extraction process must be optimized by mathematics models (RenJie, 2008; Zhong & Wang, 2010).

In statistics, response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables (Box & Wilson, 1951), and it has successfully been applied in order to optimize the conditions in food and pharmaceutical research (Ibanoglu & Ainsworth, 2004; RenJie, 2008; Varnalis, Brennan, MacDougall, & Gilmour, 2004; Vega, Balaban, Sims, O'Keefe, & Cornell, 1996; Xu et al., 2008). The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response (Sun et al., 2010), and the experiments will be more easily arranged and interpreted using this efficient design (Wu et al., 2007; Yin & Dang, 2008; Zhong & Wang, 2010).

The objective of this study was to investigate the significant variables (extraction time, extraction temperature and ratio of water to raw material) and further to optimize the process for extraction of polysaccharides from *H. fusiformis* using RSM, while employing a three-level, three-variable Box-Behnken design (BBD) (Xu et al., 2008).

2. Experimental

2.1. Reagents

H. fusiformis was from Jungwoo Food (Korea). Ethanol (99.9%) and sulfuric acid was bought from Duksan Pure Chemical Co., Ltd. (Korea). All the other solvents used in the experiment were analytical grade. D-(+)-Glucose (99.5%) and phenol were bought from Sigma–Aldrich (U.S.A.).

2.2. Instrument

Deionized water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and a filter (HA-0.45, Division of Milli-

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Table 1
Independent variables their levels used for BBD.

Variables	Level		
	−1	0	1
Extraction time (X_1) (h)	2	3	4
Extraction temperature (X_2) (°C)	75	85	95
Ratio of water to raw material (X_3)	20	25	30

pore, Waters, U.S.A.) before use. Filter paper (No.5A, 110 mm) was obtained from Hyundai Micro Co., Ltd. (Korea). Micro high centrifuge (H17R+) was from Hanil Science Industrial Co., Ltd. (Korea). UV-VIS-NIR spectroscopy was bought from PerkinElmer (U.S.A.) for analysis of polysaccharides.

2.3. Preparation of crude polysaccharides

In order to remove any substances that affected the color, *H. fusiformis* (100 g) were added into ethanol (100 mL) with 80 °C water bath for 1.5 h. After dried in an oven at 50 °C, each pretreated sample was extracted by water in a designed extraction times, extraction temperatures, and water to raw material ratio. The water extraction solutions were separated from insoluble residue by centrifugation (10,000 rpm for 15 min), and then precipitated by the addition of ethanol. The precipitate was filtered and dried in an oven at 50 °C for 24 h. The dried crude polysaccharides were refluxed three times to remove lipids with acetone and chloroform. The result product was extracted in hot water and then filtered, and the combined filtrate was precipitated using ethanol again. The content of the polysaccharides was measured by *Phenol-Sulfuric acid methods* (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.4. Experimental design

A 17-run BBD was applied to statistically optimize the polysaccharides extraction from *H. fusiformis*. The extraction time, extraction temperature and ratio of water to raw material significantly influenced the yield of the extracted crude polysaccharides. In Table 1, these three factors were designated as X_1, X_2, and X_3 and prescribed into three levels, coded +1, 0, and −1 for high, intermediate and low values, respectively. The three test variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad i = 1, 2, 3 \tag{1}$$

In this equation, x_i is the coded value of the independent variable, X_i is the actual value of the independent variable; X_0 is the actual value of the independent variable at the center point; and ΔX is the step change value of the independent variable. A second-order polynomial model was fitted to correlate the relationship between the independent variables and the response (polysaccharide yield) in order to predict the optimized conditions.

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} A_{ij} X_i X_j \tag{2}$$

In this equation, Y is the dependent variable (yield of the polysaccharides), A_0 is a constant, and A_i, A_{ii} and A_{ij} are coefficients that were estimated by the model. X_i and X_j are the levels of the independent variables that represent the linear, quadratic and cross-product effects of the X_1, X_2 and X_3 factors on the response, respectively. The model evaluated the effects of each independent variable on the response. The experimental design was analyzed and the predicted data were calculated using the Design-Expert software (v.7.1.6, Stat-Ease, Inc, Minneapolis, USA) in order to estimate the response of the independent variables. Subsequently, three additional experiments were conducted to verify the validity of the statistical experimental strategies.

Table 2
Box-Behnken experimental design with the independent variables.

Run	Coded variable levels			Yield of polysaccharide (%)	
	X_1	X_2	X_3	Actual values	Predicted values
1	−1	−1	0	18.28	18.21
2	1	−1	0	15.36	15.17
3	−1	1	0	13.54	14.73
4	1	1	0	12.95	13.01
5	−1	0	−1	11.09	11.32
6	1	0	−1	10.26	10.64
7	−1	0	1	19.88	19.5
8	1	0	1	15.65	15.42
9	0	−1	−1	14.07	13.89
10	0	1	−1	12.20	11.77
11	0	−1	1	20.64	21.07
12	0	1	1	17.39	17.55
13	0	0	0	16.19	15.67
14	0	0	0	15.34	15.67
15	0	0	0	15.32	15.67
16	0	0	0	14.64	15.67
17	−1	−1	0	16.86	18.21

3. Results and discussion

3.1. Model building and statistical analysis

A 17-run BBD with three factors and three levels, including five replicates at the center point, was used to fit a second-order response surface in order to optimize the extraction conditions. The five center point runs were carried out to measure the process stability and inherent variability, and the extraction yields of the polysaccharides from *H. fusiformis* were taken as the response. The design variables in coded units are given in Table 2 along with the predicted and experimental values of the response. Each run was performed in duplicate, and thus, the extraction yields of the polysaccharides were the average of two sets of experiments in Table 2, whereas the predicted values of the responses were obtained from quadratic model fitting techniques by the above-mentioned software.

The predictive equation was obtained by fitting the experimental data to the BBD model in Eq. (3), which represents an empirical relationship between the response (extraction yields) and the tested variables (in coded units):

$$Y = 15.67 - 1.19X_1 - 1.41X_2 + 3.24X_3 - 1.12X_1^2 + 0.73X_2^2 - 0.33X_3^2 + 0.33X_1X_2 - 0.85X_1X_3 - 0.35X_2X_3 \tag{3}$$

The significance of each coefficient was checked using the F-test and the p value (Table 3). The p value was used as a tool to check the significance of each coefficient, and also indicated the interaction strength between each independent variable (Xu et al., 2008). The ANOVA of the quadratic regression model demonstrated that the model was highly significant, as was evident from the F-test with a very low probability value (p = 0.0002). The Model F-value of 24.66 implied that the model was significant, and there was only a 0.02% chance that a “Model *F-Value*” that was this large occurred because of noise. The “Lack of Fit *F-value*” of 0.40 implied the Lack of Fit was not significant relative to the pure error, and there was a 76.09% chance that a “Lack of Fit *F-value*” that was this large could have occurred because of noise. The regression coefficients and the

Table 3
Analysis of variance of the experimental results of the BBD.

Variables	Sum of squares	*DF*	Mean square	*F* value	*p* value Prob. > *F*
Model	122.76	9	13.64	24.66	0.0002[a]
X_1	11.41	1	11.41	20.64	0.0027[a]
X_2	15.86	1	15.86	28.68	0.0011[a]
X_3	83.96	1	83.96	151.80	<0.0001[a]
$X_1 \times X_1$	5.27	1	5.27	9.52	0.0177
$X_2 \times X_2$	2.27	1	2.27	4.11	0.0822
$X_3 \times X_3$	0.46	1	0.46	0.83	0.3936
$X_1 \times X_2$	0.44	1	0.44	0.79	0.4030
$X_1 \times X_3$	2.89	1	2.89	5.23	0.0561
$X_2 \times X_3$	0.48	1	0.48	0.87	0.3832
Residual	3.87	7	0.55	–	–
Lack of fit	0.89	3	0.30	0.40	0.7609
Pure error	2.98	4	0.74	–	–
Correlation total	126.63	16	–	–	–

[a] Means significance (Values of “Prob > *F*” less than 0.0500).

Table 4
Analysis of variance for the fitted quadratic polynomial model of extraction of polysaccharides.

Item	Std. dev.	Mean	C.V.%	Press	R^2	R^2_{Adj}	R^2_{Pred}	Adeq. precision
Value	0.74	15.33	4.85	18.96	0.9694	0.9301	0.8502	18.287

corresponding *p* values are also shown in Table 3. The *p* values of each model, confirmed that the four coefficients ($X_1, X_2, X_3, X_1 \times X_1$) were all significant. Therefore, the extraction time X_1, the extraction temperature X_2 and the ratio of water to raw material X_3 were important factors in the extraction process of the polysaccharides.

The coefficient of determination ($R^2 = 0.9694$), the adjusted coefficient of determination ($R^2_{Adj} = 0.9301$) and the coefficient of variation (*C.V.* = 4.85%) are shown in Table 4. These values indicated that the accuracy and the general availability of the polynomial model were adequate, and the R^2_{Pred} of 0.8502 was in reasonable agreement with the R^2_{Adj}. The “*Adeq. Precision*” measureed the signal to noise ratio, and a ratio of greater than 4 is normally desirable. The “*Adeq. Precision*” of 18.287 indicated that this model could be used to navigate the design space.

3.2. Optimization of the procedure

The response surface curves were plotted to investigate the interactions of the variables and to determine the optimal level of each variable for the maximum response (Li et al., 2009). The optimal values of the selected variables were obtained by solving the regression equation using the Design-Expert software. The 3D response surface and the 2D contour plots were provided as graphical representations of the regression equation (Figs. 1–3). Each contour curve represented an infinitive number of combinations of two test variables while the other factors were fixed at the zero level. Fig. 1(a) and (b) showed the effect of extraction time, extraction temperature and their reciprocal interaction on extraction yield, when ratio of water to raw material was fixed at 25. The polysaccharides yield decreased, when extraction time at the designed range from 2 to 4 h, and there was a quadratic effect on the response yield when the extraction temperature was increased from 75 to 95 °C. As shown in Fig. 2(a) and (b), ratio of water to raw material displayed a positive effect on the response yield when extraction temperature was fixed at 85 °C. There was a linear increase in the yield of polysaccharides with increase in the ratio of water to raw material, but decrease in extraction time. Likewise, Fig. 3(a) and (b) showed that the polysaccharides yield

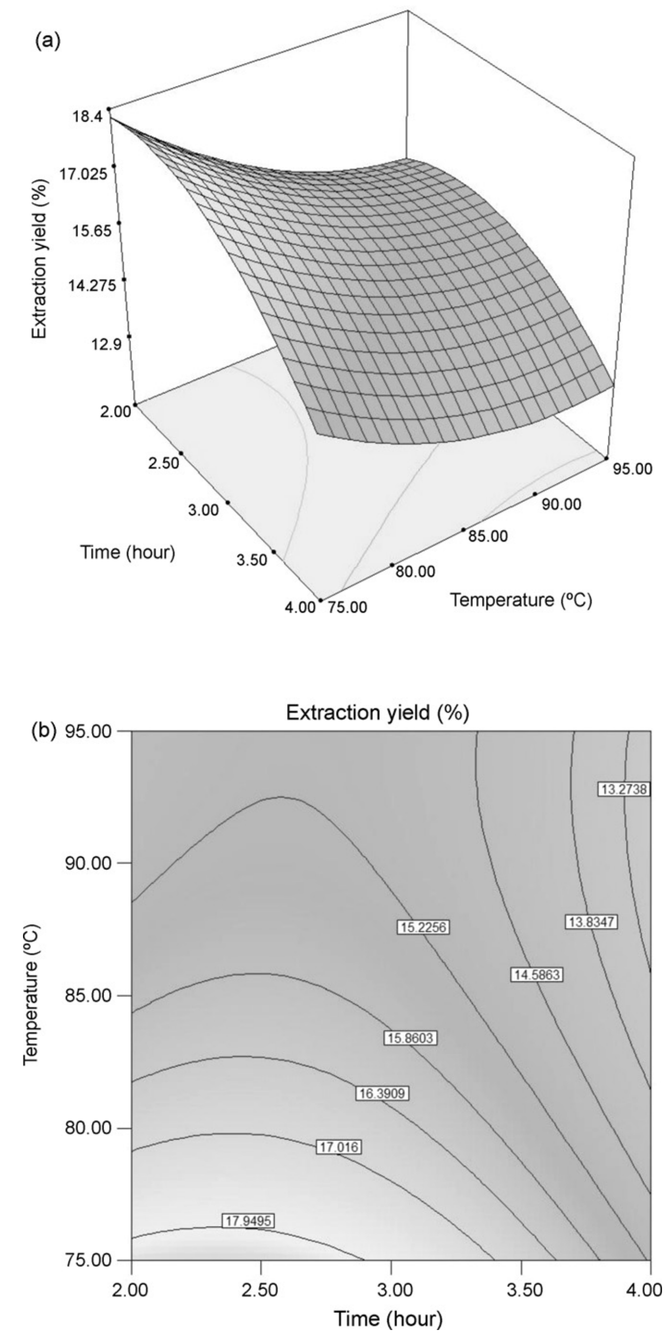

Fig. 1. Effect of extraction time, extraction temperature and their reciprocal interaction on extraction yield (with initial ratio of water to raw material is constant at 25) (a, 3D response surface; b, 2D contour plots).

increased with increase in the ratio of water to raw material, but with decrease in extraction temperature.

The optimum extraction conditions (X_1 = 2.05 h, X_2 = 75.45 °C and X_3 = 29.89) for the polysaccharides extraction yield were estimated using the model equation by solving the regression equation and analyzing the response surface contour plots (Fig. 4). The theoretical polysaccharides extraction yield that was predicted under the above conditions was 22.0686%.

3.3. Validation of the model

The optimum extraction conditions were applied to three independent replicates for the polysaccharides extraction in

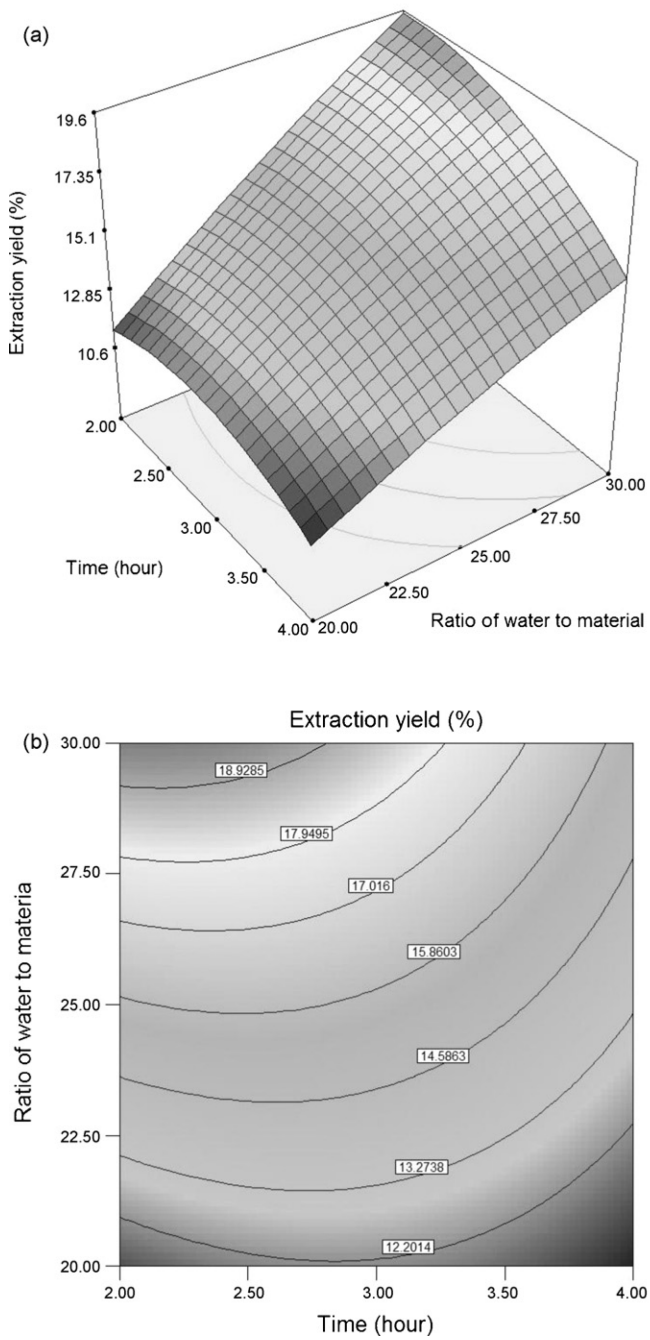

Fig. 2. Effect of extraction time, ratio of water to raw material and their reciprocal interaction on extraction yield (with initial extraction temperature is constant at 85 °C) (a, 3D response surface; b, 2D contour plots).

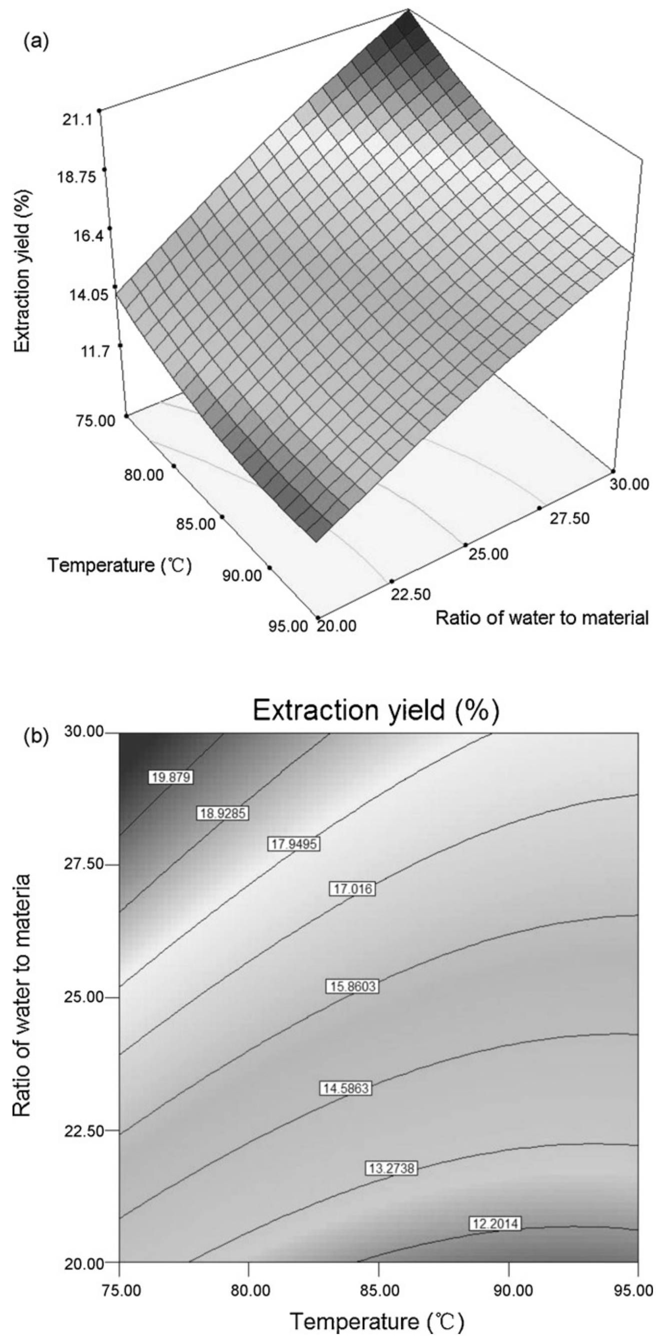

Fig. 3. Effect of extraction temperature, ratio of water to raw material and their reciprocal interaction on extraction yield (with initial extraction time is constant at 3 h) (a, 3D response surface; b, 2D contour plots).

order to verify the prediction from the model. The mean extraction yield for the polysaccharides was 21.8324%, corresponding well to the predicted value of the model equation, which confirmed that the response model was adequate for the optimization.

4. Conclusion

The RSM was confirmed to be a useful tool for the optimization of the polysaccharides extraction from *H. fusiformis*. The coefficient of determination (R^2) for the model was 0.9694, and the probability value ($p = 0.0002$) demonstrated a high significance for the regression model. The optimal conditions (extraction time 2.05 h, extraction temperature 75.45 °C and ratio of water to raw material 29.89) for the polysaccharides extraction yield were estimated using the model equation. Under these conditions, the mean polysaccharides extraction yield was 21.8324%, which corresponded well with the value that was predicted by the model. This study could become the basis for later research according to the optimization predictions of the extraction.

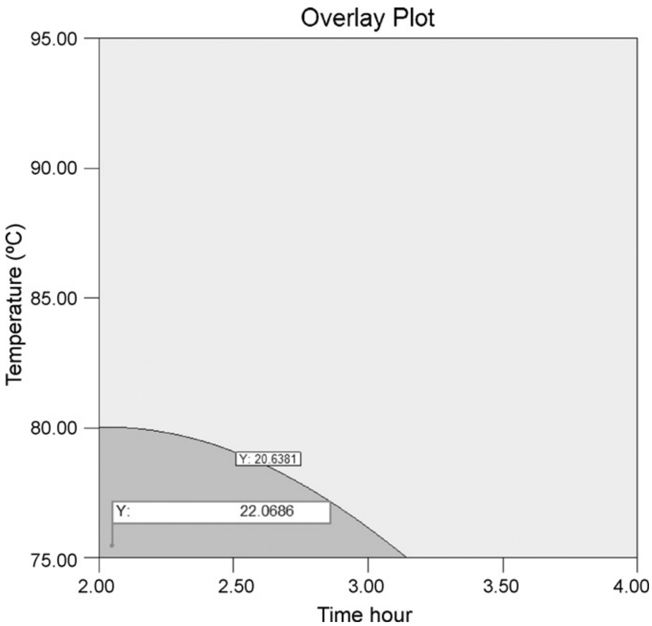

Fig. 4. Predicted optimum values of extraction yield of polysaccharides by the BBD (yield = 22.0686%, when X_1 = 2.05 h, X_2 = 75.45 °C and X_3 = 29.89).

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