Hydrochloric Acid-Catalyzed Hydrothermal Pretreatment of Brown Seaweed Residues for Enhancing Biofuel Production

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The hydrochloric acid-catalyzed hydrothermal pretreatment of brown seaweed residues (*Laminaria japonica*) was optimized with respect to three operating factors: temperature, time, and the concentration of HCl, using response surface methodology (RSM). In order to confirm the significance of the quadratic model, an analysis of variance was performed, which resulted in an F-value of 11.09. Therefore, the regression model was highly significant. Additionally, the pareto chart was used to contrast the absolute values of the standardized effects. Response surface and contour plots were used to illustrate a surface with a maximum. The perturbation plot showed the sensitivity of the reducing sugars yield to the independent factors. Under the reaction conditions of 142 °C, 9 °C, 18.6 min, and 0.1 N HCl concentration, the experimental yield of 113.0 mg/g and the predicted yield of 107.5 mg/g were obtained.

Keywords: Hydrothermal acid pretreatment; Reducing sugars yield; Laminaria japonica; Response Surface Methodology; Central Composite Design

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INTRODUCTION

Seaweed or marine macroalgae is classified into three major groups: red seaweed (Rhodophyceae), brown seaweed (Phaeophyceae), and green seaweed (Chlorophyceae). Brown seaweeds consist of 34 to 73% carbohydrates, 3 to 24% proteins, 0.2 to 5% lipids, and 12 to 46% minerals by dry weight (Matanjun *et al*. 2009; Kim *et al*. 2011; Hong *et al*. 2014; Lee *et al*. 2014; Manns *et al*. 2014). The main carbohydrates found in brown seaweed are alginate, mannitol, fucoidan, laminarin, and cellulose. Alginates, a linear copolymer of β-1,4-D-mannuronic acid (M) and α-1,4-L-guluronic acid (G) (Ravanal *et al*. 2017), can be depolymerized by acid hydrolysis (Moen 1997). Mannitol, sugar alcohol formed by reduction of mannose (Adams *et al*. 2011), is a water-soluble and easily available carbohydrate (Horn 2000). Fucoidan, mostly constituted of sulphated L-fucose, is readily extracted from brown seaweed using acids (Black 1954). Laminarin, a polysaccharide of glucose, can also easily be extracted from seaweed. Since the brown seaweeds lack lignin and contain low amounts of cellulose, they are a simpler feedstock for biological treatment than land plants (Horn 2000; Kraan 2013; Obata *et al*. 2016). Polysaccharides from seaweed can be converted to monosaccharides (*i.e.*, arabinose, galactose, glucose, xylose, fucose, and mannitol) through pretreatment that are classified as physical, chemical, physicochemical, and biological treatment (Enquist-Newman *et al*. 2014; Kostas *et al*. 2016; Sharma and Horn 2016; Offei *et al*. 2018).

Pretreatment methods such as ball milling (Schultz-Jensen *et al.* 2013), acid treatments (with hydrochloric acid, sulfuric acid, sulfuric acid and hot water) (Schultz-Jensen *et al.* 2013; Abd-Rahim *et al.* 2014; Yazdani *et al.* 2015), alkaline treatments (with liquid ammonia, sodium hydroxide) (Adams *et al.* 2011; Kim *et al.* 2011), and physicochemical treatments (Wang and Wan 2009; Park *et al.* 2013; Schultz-Jensen *et al.* 2013; Choi *et al.* 2016) have been used during hydrolysis of seaweed for bioethanol production.

Enzymatic hydrolysis with Viscozyme L/Novozyme 188, Meicelase, cellulase/cellobiase, Celluclast 1.5L/Novozyme 188, and cellulase have also been studied (Ge *et al.* 2011; Tan and Lee 2014; Puspawati *et al.* 2015; Yazdani *et al.* 2015). Several studies have carried out the combined treatments to maximize the yield (Meinita *et al.* 2012, 2013; Hargreaves *et al.* 2013; Kim *et al.* 2013; Mutripah *et al.* 2014). Dilute acid hydrolysis is the most extensively used treatment in seaweed bioethanol, since it is considered faster, easier, and cheaper than other treatments (Ho *et al.* 2013; Chirapart *et al.* 2014).

Moreover, some studies reported that the dilute acid hydrolysis is more economically efficient in comparison to the use of enzymes and other hydrolysis methods (Lukajtis *et al.* 2018; Offei *et al.* 2018). Yet, it has major drawbacks such as degradation of sugars to by-products (*i.e.* furfural, 5-HMF, phenol, acetate, and formic acid) that can inhibit fermentation. Furan aldehydes (*i.e.* furfural and 5-HMF) originate from the dehydration of pentose and hexose simple sugars, respectively. Therefore, it is important to develop cheap and optimal methods for dilute acid pretreatment or to avoid dilute acid pretreatment. For this purpose, some studies optimized dilute acid concentration to control furan aldehydes production (Jung *et al.* 2011; Chaudhary *et al.* 2012; Kupiainen *et al.* 2014), and various dilute acid hydrolysis conditions have also been studied to obtain the maximum yield of reducing sugars for seaweed bioethanol production (Meinita *et al.* 2012, 2013; Chirapart *et al.* 2014; Mutripah *et al.* 2014; Kostas *et al.* 2016).

This experiment was conducted to determine the optimum conditions of hydrothermal pretreatment of brown seaweed residues (*Laminaria japonica*) for maximizing the reducing sugars yield (*RSy*). In order to design the experimental procedure, generate a model, evaluate the significance of independent variables (temperature, time, and HCl concentration), and optimize a response (the yield of reducing sugars) influenced by three variables, RSM was used.

EXPERIMENTAL

Materials

The carbohydrate, protein, lipid, and ash contents of previously reported *Laminaria spp.* are listed in Table 1. *Laminaria japonica* (*L. japonica*) was purchased from the local market of Wando, Korea. It was washed manually using tap water to remove the dirt. *L. japonica* was dried for 3 days at 80 °C in a hot-air oven (OF-22, Jeio Tech, Daejoen, Korea), mechanically reduced to a particle size (< 2 mm) (HMF-600, Hanil Electric, Seoul, Korea), and stored away from direct sunlight and moisture until needed. Hydrochloric acid (HCl), 35% (Junsei, CAS No. 7647-01-0, Tokyo, Japan) was used in the hydrothermal pretreatment as a chemical catalyst.

Table 1. Chemical Composition of Dried *Laminaria spp.*

Seaweed	Species	Carbohydrate	Protein	Lipid	Ash
		(% Dry Weight)			
Laminaria spp.	*L. japonica*[1)]	59.7	9.4	2.4	28.5
	L. japonica[2)]	51.9	14.8	1.8	31.5
	L. japonica[3)]	51.5	8.4	1.3	38.8
	L. digitata[4)]	64.2	3.1	1.0	11.9
	L. digitata[5)]	77.4	4.0	0.5	18.1
	Mean±SD	**60.9±10.7**	**7.9±4.7**	**1.4±0.7**	**25.8±10.7**

1) Jung *et al*. 2011; 2) Kim *et al*. 2011; 3) Hong *et al*. 2014; 4) Manns *et al*. 2014; 5) Chades *et al*. 2018

Methods

Hydrochloric acid-catalyzed hydrothermal pretreatment

The hydrothermal pretreatment of *Laminaria japonica* was carried out in a 100 mL polytetrafluoroethelene-lined reaction vessel (Hydrothermal Reactor, HR-8200, Hanwoul Engineering Inc., Gyeonggi-do, Korea), into which 1 g of dried *L. japonica* powder (DLP) and 30 mL of 0.016 to 0.184 N HCl acid were introduced. The vessel was mounted within the hydrothermal reactor. The process of hydrothermal acid hydrolysis was carried out at 116.4 to 183.6 °C for 11.6 to 28.4 min. Next, the hydrolysate was cooled to room temperature, then neutralized with sodium hydroxide (1 N NaOH). The hydrolysate obtained by hydrothermal pretreatment was centrifuged at 4500 rpm for 15 min (HSR-4S, Hanil Scientific Inc., Gyeonggi-do, Korea) and filtered by at 0.45 μm filter-paper. The reducing sugars (*RS*) were determined by the dinitro salicylic acid method (DNS), which was carried out in duplicate. After a centrifugal filtration of the specimen, it was diluted with distilled water. Next, 3 mL of the DNS reagent was added to 1 mL of the diluted specimen, and it was reacted at 90 °C for 5 min. The specimen was then diluted with 20 mL of distilled water and the absorbance (UV-Vis Spectrophotometer-1650, Shimadzu corp., Kyoto, Japan) was measured at 550 nm (Miller 1959).

Design and analysis of experiment

L. japonica was acid-hydrolyzed at different operating conditions according to the three independent variables. Table 2 shows the input variables for a given coding level. Statistical analysis was performed using Design-Expert (Version 11, Stat-Ease Inc., MN, USA), and the remaining calculations were performed using Microsoft Excel.

Table 2. Input Variables for Hydrothermal Acid Hydrolysis

Variable	Symbol	Coding level				
		$-\alpha$	-1	0	+1	$+\alpha$
Temperature, (℃)	x_1	116.4	130	150	170	183.6
Time, (min)	x_2	11.6	15	20	25	28.4
HCl Concentration, (N)	x_3	0.02	0.05	0.1	0.15	0.18

Independent variables were determined from the results of applying the central composite design for the optimization of the hydrothermal acid hydrolysis. The number of experiments, *N*, was calculated by Eq. 1,

$$N = 2^k + 2 \times k + n = 2^3 + 2 \times 3 + 6 = 20 \quad (1)$$

where *k* is the number of factors. Hence, the number of experiments for 2^k factorial points, $2 \cdot k$ axial points and *n* central points ($n \geq 1$) can be calculated as Eq. 1. There are 8 factorial points, 6 axial points, and 6 central points, which yielded a total of 20 experiments. A model to predict *RS*y over the experimental region is

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \hat{\beta}_3 x_3 + \hat{\beta}_{12} x_1 x_2 + \hat{\beta}_{13} x_1 x_3 + \hat{\beta}_{23} x_2 x_3 + \hat{\beta}_{11} x_1^2 + \hat{\beta}_{22} x_2^2 + \hat{\beta}_{33} x_3^2 \quad (2)$$

where $\hat{y}$ is the yield of reducing sugars (*RSy*, mg/g), the intercept $\hat{\beta}_0$ is the average of all responses in the twenty runs in the design, and $\hat{\beta}$'s are regression coefficients that can be obtained from the effect estimates. Also, x_1, x_2, and x_3 are the process parameters.

Optimization

The polynomial model proposed was used for the optimization of the hydrothermal pretreatment process and statistically validated using the ANOVA test. In order to display the standardized effect for each independent variable, the Pareto chart was used. Response surface plots (surface and contour plots) were used to explain the relationship between the response and operating conditions. Also, a perturbation plot was used to show the effect of all the factors on a single plot. Finally, the reliability of the proposed model was verified by performing experiments under optimum conditions.

RESULTS AND DISCUSSION

Experimental Model Validation

The relationships between the response and three independent factors, temperature, time, and HCl concentration, were studied. The quadratic model expressed as coded factors is given by following Eq. 3.

$$y = 103.67 - 28.45x_1 + 0.061x_2 + 11.55x_3 + 12.20x_1x_2 + 11.28x_1x_3 - 8.38x_2x_3 - 65.81x_1^2 - 10.46x_2^2 - 42.46x_3^2 \quad (3)$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. Therefore, the plus and minus signs (+ and −) in front of the terms indicate a synergistic effect and an antagonistic effect, respectively.

Statistical Evaluation

Analysis of variance (ANOVA) was carried out to determine the statistical significance and the significant terms of the quadratic model. The results are listed in Table 3. The model F-value of 11.09 implies the model was significant (> 99.96%). The obtained value of the determination coefficient ($R^2 = 0.9089$) indicated a good correlation between the yield calculated based on Eq. 4 and the actual reducing sugars yield within the investigated range of variables. When $R^2 \geq 0.9$, the model is considered very appropriate (Kim 2017). The lower the coefficient of variation, the lesser the level of dispersion around the mean. A coefficient of variation (C.V. = 16.37 %) is considered a good degree of accuracy and quadratic precision of the experimental values (Rosner 2006; Ryan and

Morgan 2007). Therefore, it was concluded that the proposed model was adequate to predict the yield of *RS* within the experimental region. P-values (≤ 0.05) indicate model terms are significant. In this case, the quadratic term x_1^2 was highly significant (> 99.99%), and that the linear term x_1, and the term x_3^2 were also significant (> 95%), whereas other cubic terms are not significant. F-value ($x_1 = 25.91$) of temperature had a significant effect, whereas the cubic effect of temperature and time ($x_1x_2 = 148.78$) was more specific than the other two cubic terms relative to yield of *RS*. The temperature was the most important factor in this study.

Table 3. Analysis of Variance for the Second Order Model

Source	DF	Sum of Squares	Mean Square	*F*-Value	*p*-Value Prob > *F*
Model	9	15047.84	1671.98	11.09	0.0004*
x_1	1	3907.93	3907.93	25.91	0.0005*
x_2	1	0.0180	0.0180	0.0001	0.9915
x_3	1	643.73	643.73	4.27	0.0657
x_1x_2	1	148.78	148.78	0.9865	0.3440
x_1x_3	1	127.20	127.20	0.8434	0.3800
x_2x_3	1	70.21	70.21	0.4655	0.5105
x_1^2	1	7801.78	7801.78	51.73	0.0001*
x_2^2	1	197.22	197.22	1.31	0.2795
x_3^2	1	3247.86	3247.86	21.54	0.0009*

$R^2 = 0.9089$; Adjusted $R^2 = 0.8269$; Coefficient of variation (C.V.) = 16.37 %
* Significant at $P \leq 0.05$.

A pareto chart in Fig. 1 shows the standardized effects (positive or negative) for linear, quadratic, and cubic effects with the three independent variables. Significance level (p=0.05) is shown. As mentioned above, temperature (x_1) and quadratic terms of (x_1^2) and (x_3^2) had a statistically significant result on the yield of reducing sugars.

Optimization of Hydrochloric Acid-catalyzed Hydrothermal Pretreatment

A total of 20 experiments were planned according to the central composite design (CCD). Table 4 summarizes combinations of the three variables (temperature, time, and HCl concentration (C_{HCl})) along with the experimental values.

Figure 2 shows the response surface graphically, where *y* (*RSy*) is plotted versus the levels of two factors when the other factor is kept constant. In order to help visualize the shape of a response surface, the contour plots of the response surface were drawn as shown in Fig. 2. By representing a contour plot for response surface analysis, one can usually distinguish the form of the surface and find the optimum with a reasonable level of precision (Montgomery 2012; Llano 2018). As shown in Fig. 2a, the *RSy* increased with increasing temperature; however, when the optimal temperature (150 °C) was reached, a significant decrease in reducing sugars yield was observed if the temperature was further increased.

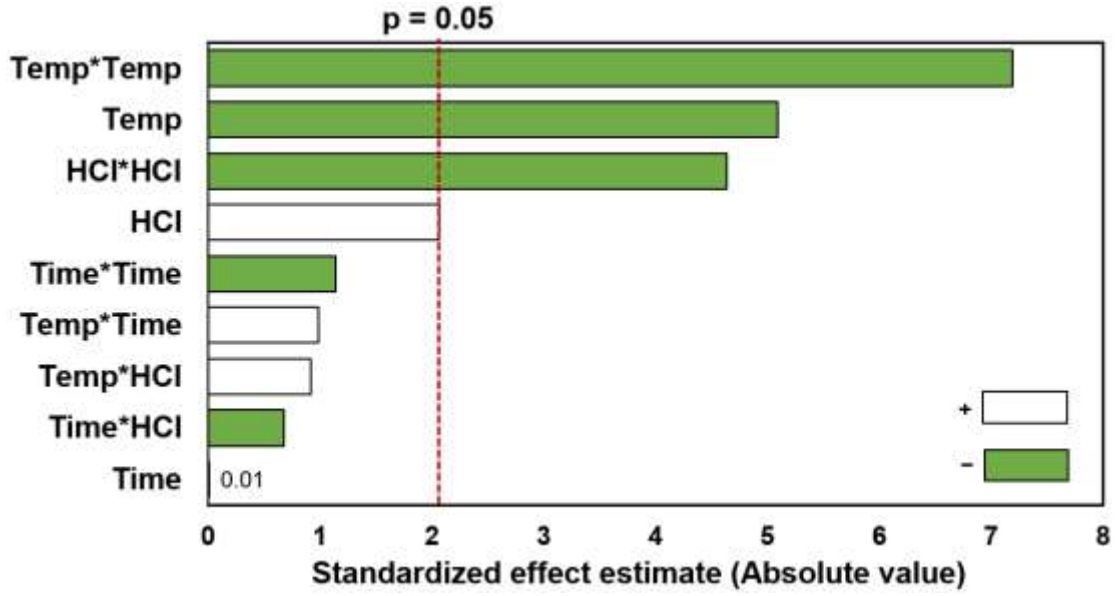

Fig. 1. Pareto chart contrasting absolute values of the standardized effects

Table 4. Central Composite Design (CCD) for Hydrothermal Acid Hydrolysis

No.	Temperature (℃)	Time (min)	C_{HCl} (N)	*RSy* (mg/$g_{biomass}$)
1	-1	-1	-1	92.7
2	1	-1	-1	21.0
3	-1	1	-1	82.9
4	1	1	-1	41.3
5	-1	-1	1	90.3
6	1	-1	1	47.4
7	-1	1	1	81.5
8	1	1	1	43.0
9	-1.682	0	0	47.5
10	1.682	0	0	25.9
11	0	-1.682	0	91.1
12	0	1.682	0	93.0
13	0	0	-1.682	39.4
14	0	0	1.682	80.7
15	0	0	0	104.6
16	0	0	0	107.0
17	0	0	0	106.3
18	0	0	0	105.5
19	0	0	0	101.2
20	0	0	0	97.8

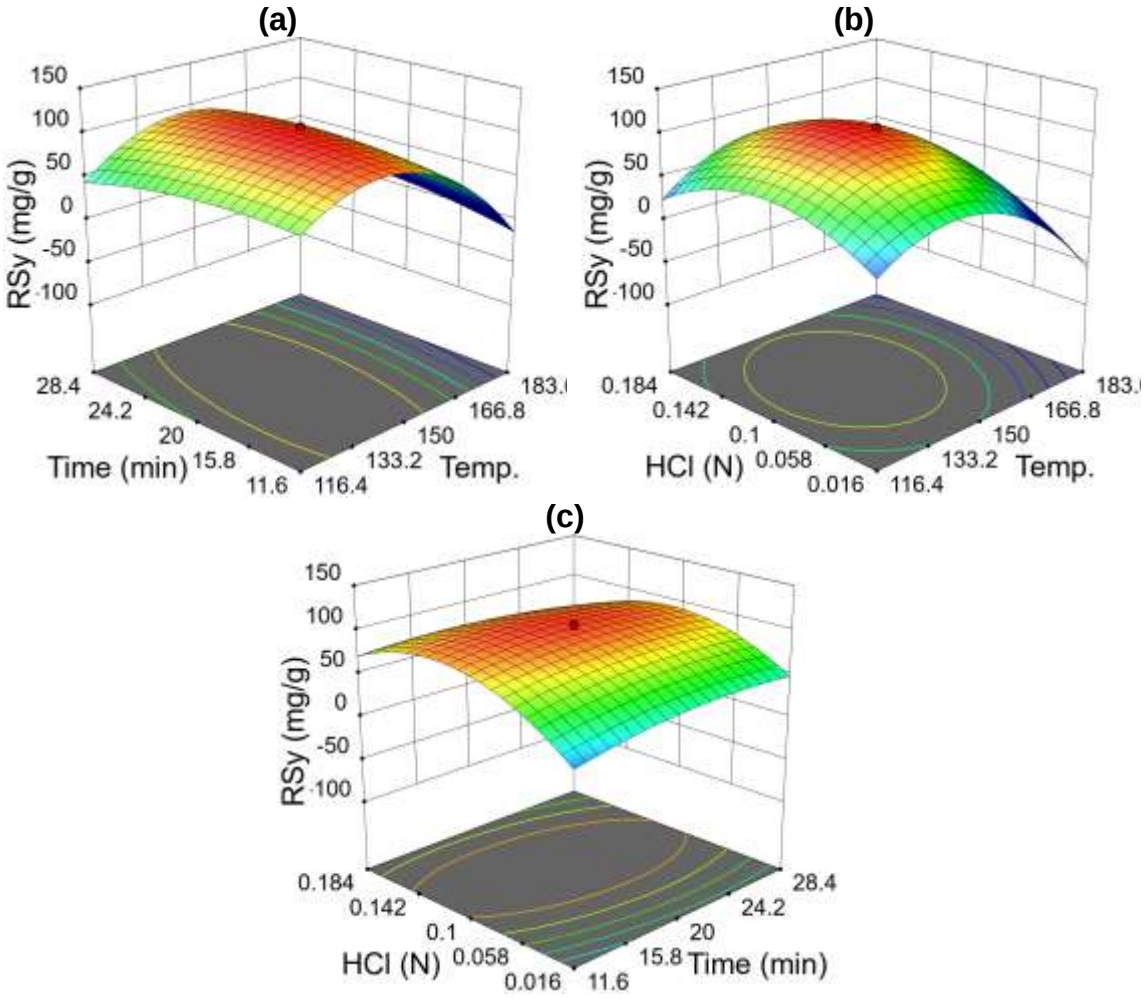

Fig. 2. Response surface and contour plots illustrating a surface with a maximum

For temperatures in the range of 140 to 160 °C, and for HCl concentration of hydrothermal acid hydrolysis (0.1 N), the highest reducing sugars yield was obtained. An increase in HCl concentration over 0.1 N resulted in a decrease in hydrolysis efficiency (Fig. 2a). As shown in Fig. 2c, based on the course of the response surface, it can be stated that for the change in HCl concentration, a local maximum around 0.1 N was observed. Obtained results confirmed that 0.1 N HCl concentration was an adequate concentration of acidic catalyst chosen as the optimal value. Out of the three plots shown in Fig. 2, temperature was the most important factor in this study. This was because the temperature was a significant factor in the hydrochloric acid-catalyzed hydrothermal pretreatment.

The optimum combination of the findings included 142.9 ℃ of temperature, 18.6 minutes, and 0.1 N of HCl concentration; these parameters produced the predicted yield of 107.5 mg/g. To assay this optimized condition, the test was conducted. The results showed the experimental yield of 113.0 mg/g, which is well matched with the model's prediction.

As provided in Fig. 3, the perturbation plot shows the sensitivity of the *RSy* to the independent factors (temperature, time, and HCl concentration). The yield of reducing sugars (mg/g) was most sensitive to the change in temperature (A).

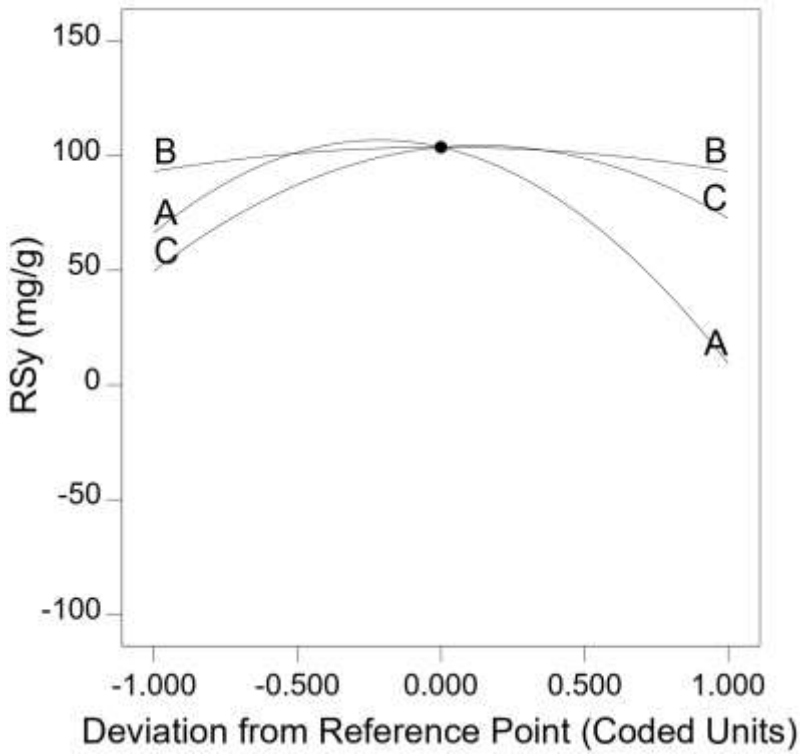

Fig. 3. Perturbation plot showing the effect of actual factors on the *RSy*

CONCLUSIONS

The optimum experimental conditions for the yield of reducing sugars from *Laminaria japonica* residues were evaluated. A central composite design (CCD) of response surface methodology (RSM) was used to determine the optimization of the operational factors for maximizing reducing sugars yield.

1. The optimum experimental conditions found using RSM were: 142.9 °C, 18.6 minutes, and 0.1 N of HCl concentration. Under these conditions, the experimental yield of 113.0 mg/g and the predicted yield of 107.5 mg/g were obtained.
2. The temperature was the most important factor in hydrochloric acid-catalyzed hydrothermal pretreatment.

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