

Supplementary material

Brewers Spent Grain Diluted Acid Thermal Pretreatment Optimisation and Enzymatic Hydrolysis of Whole Slurry and Resuspended Pellet

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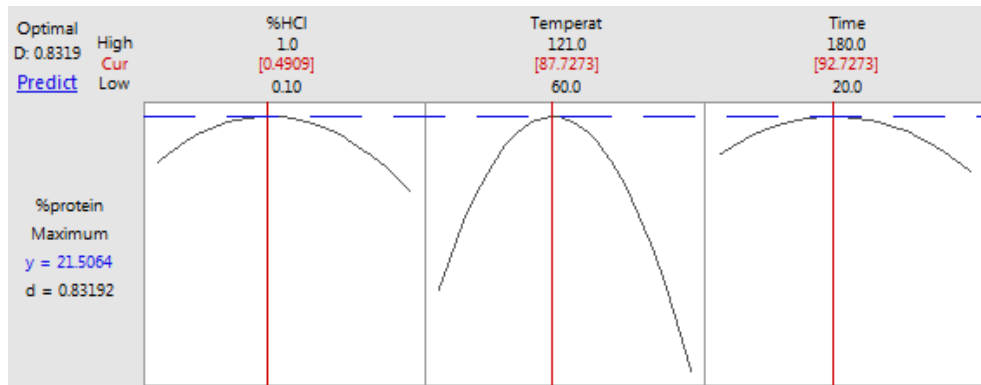
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Supplementary Table 1 45 combinations given by the software for the Box Behnken design optimisation

RunOrder	[HCl] (% v/v)	Temperature (°C)	Time (min)
1	0.55	60	20
2	1	60	100
3	0.55	90.5	100
4	0.1	121	100
5	0.55	90.5	100
6	0.55	60	20
7	0.55	121	180
8	0.55	90.5	100
9	1	60	100
10	0.55	90.5	100
11	0.55	90.5	100
12	1	90.5	180
13	1	90.5	20
14	0.55	90.5	100
15	0.55	60	180
16	0.1	60	100
17	0.55	121	20
18	0.55	121	20
19	0.55	121	180
20	1	90.5	180
21	1	60	100
22	1	121	100
23	1	90.5	180
24	0.1	60	100
25	1	121	100
26	0.55	90.5	100
27	0.1	90.5	20
28	0.1	90.5	180
29	0.1	121	100
30	0.1	90.5	20
31	0.55	121	20
32	0.1	60	100
33	0.55	121	180
34	0.55	90.5	100
35	1	90.5	20
36	0.55	90.5	100
37	0.55	60	20
38	0.1	90.5	180
39	1	121	100
40	0.1	121	100
41	0.1	90.5	20
42	0.55	60	180
43	1	90.5	20
44	0.1	90.5	180
45	0.55	60	180

a) Condition 1: Optimisation of maximum protein in the pellet.



Response Optimization: %protein

Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
%protein	Maximum	13.8889	23.0455		1	1

Solution

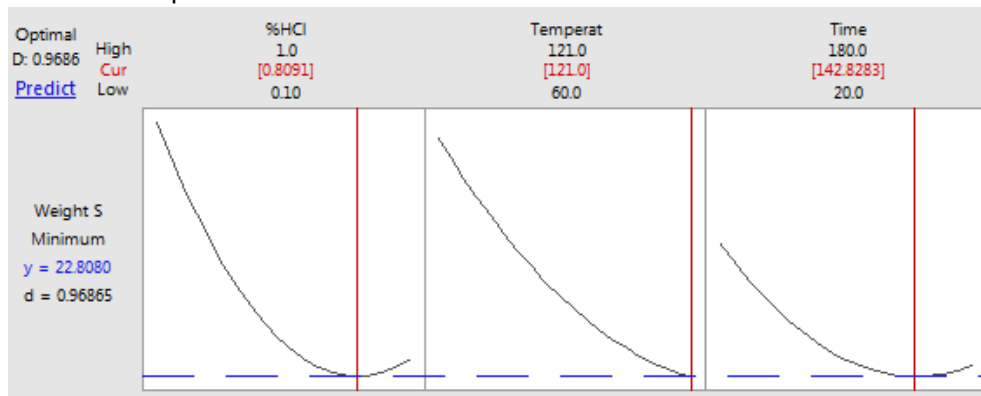
Solution	%HCl	Temperature	Time	%protein Fit	Composite Desirability
1	0.490909	87.7273	92.7273	21.5064	0.831919

Multiple Response Prediction

Variable	Setting
%HCl	0.490909
Temperature	87.7273
Time	92.7273

Response	Fit	SE Fit	95% CI	95% PI
%protein	21.506	0.462	(20.569, 22.444)	(18.514, 24.499)

b) Condition 2: Optimisation of minimum fraction of solids



Response Optimization: Weight Solid

Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
Weight Solid	Minimum		21.3	69.4	1	1

Solution

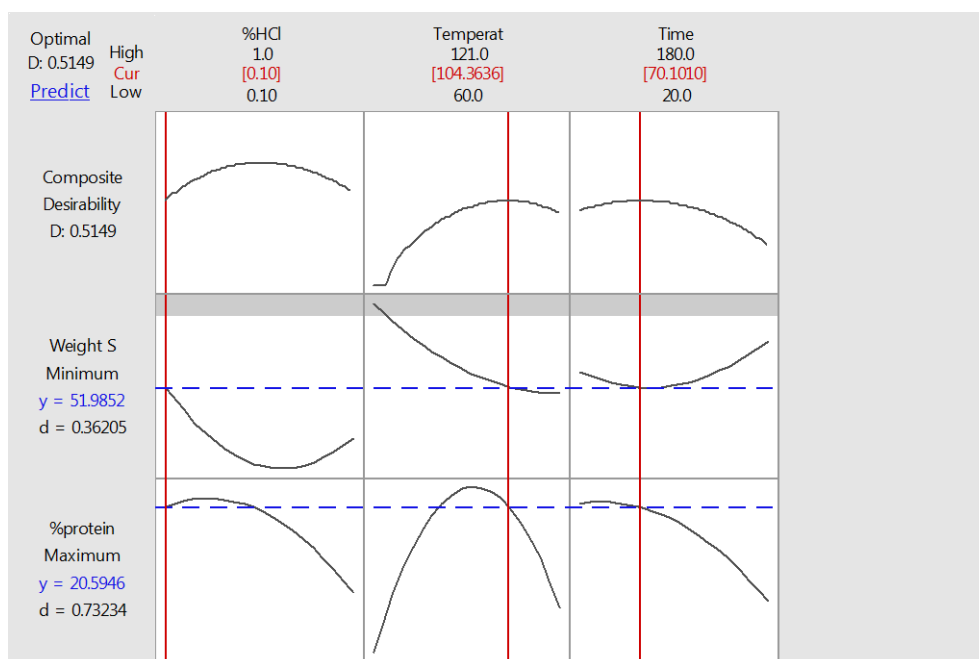
Solution	%HCl	Temperature	Time	Weight Solid Fit	Composite Desirability
1	0.809091	121	142.828	22.8080	0.968649

Multiple Response Prediction

Variable	Setting
%HCl	0.809091
Temperature	121
Time	142.828

Response	Fit	SE Fit	95% CI	95% PI
Weight Solid	22.81	2.87	(16.99, 28.62)	(7.76, 37.86)

c) Condition 3: Optimisation of maximum of protein in pellet and lowest solid residue keeping [HCl] at 0.10% (v/v)



Response Optimization: Weight Solid, %protein

Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
Weight Solid	Minimum		21.3000	69.4	1	1
%protein	Maximum	13.8889	23.0455		1	1

Variable Ranges

Variable	Values
%HCl	0.1
Temperature	(60, 121)
Time	(20, 180)

Solution

Solution	%HCl	Temperature	Time	Weight Solid	%protein	Composite
				Fit	Fit	Desirability
1	0.1	104.364	70.1010	51.9852	20.5946	0.514924

Multiple Response Prediction

Variable	Setting
%HCl	0.1
Temperature	104.364
Time	70.1010

Response	Fit	SE Fit	95% CI	95% PI
Weight Solid	51.99	2.65	(46.61, 57.36)	(37.10, 66.87)
%protein	20.595	0.542	(19.494, 21.695)	(17.547, 23.642)

Supplementary Figure 1 Optimal conditions obtained using the response surface methodology. **a)** Condition 1: Optimisation of maximum protein in the pellet; **b)** Condition 2: optimisation of minimum fraction of solids; and **c)** Condition 3: Optimisation of maximum of protein content in the pellet with the lowest solid residue while keeping [HCl] at 0.10% (v/v)

Supplementary Table 2 ANOVA Response Surface Regression: %protein versus %HCl, Temperature, Time

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	150.840	16.760	8.55	0.000
Linear	3	16.399	5.466	2.79	0.055
%HCl	1	1.513	1.513	0.77	0.385
Temperature	1	14.607	14.607	7.46	0.010
Time	1	0.279	0.279	0.14	0.708
Square	3	111.940	37.313	19.04	0.000
%HCl*%HCl	1	8.162	8.162	4.17	0.049
Temperature*Temperature	1	105.879	105.879	54.04	0.000
Time*Time	1	5.118	5.118	2.61	0.115
2-Way Interaction	3	22.501	7.500	3.83	0.018
%HCl*Temperature	1	11.663	11.663	5.95	0.020
%HCl*Time	1	4.524	4.524	2.31	0.138
Temperature*Time	1	6.315	6.315	3.22	0.081
Error	35	68.576	1.959		
Lack-of-Fit	3	48.305	16.102	25.42	0.000
Pure Error	32	20.271	0.633		
Total	44	219.417			
Model Summary					
	S	R-sp	R-sp(adj)	R-sq(pred)	
	1.39976	68.75%	60.71%	46.87%	

Supplementary Table 3 ANOVA Response Surface Regression: Weight Solid versus %HCl, Temperature, Time

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	9929.7	1103.30	23.61	0.000
Linear	3	6582.7	2194.22	46.96	0.000
%HCl	1	1464.8	1464.84	31.35	0.000
Temperature	1	4659.3	4659.31	99.71	0.000
Time	1	458.5	458.50	9.81	0.003
Square	3	2502.9	834.31	17.85	0.000
%HCl*%HCl	1	1887.3	1887.31	40.39	0.000
Temperature*Temperature	1	342.6	342.60	7.33	0.010
Time*Time	1	572.1	572.06	12.24	0.001
2-Way Interaction	3	844.1	281.36	6.02	0.002
%HCl*Temperature	1	119.1	119.07	2.55	0.119
%HCl*Time	1	713.0	713.02	15.26	0.000
Temperature*Time	1	12.0	12.00	0.26	0.616
Error	35	1635.5	46.73		
Lack-of-Fit	3	1061.5	353.83	19.73	0.000
Pure Error	32	574.0	17.94		
Total	44	11565.1			
Model Summary					
	S	R-sp	R-sp(adj)	R-sq(pred)	
	6.83579	85.86%	82.22%	76.88%	

Supplementary Table 4 ANOVA Response Surface Regression: %protein versus 0.10 %HCl, Temperature, Time

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	150.840	16.760	8.55	0.000
Linear	3	16.399	5.466	2.79	0.055
%HCl	1	1.513	1.513	0.77	0.385
Temperature	1	14.607	14.607	7.46	0.010
Time	1	0.279	0.279	0.14	0.708
Square	3	111.940	37.313	19.04	0.000
%HCl*%HCl	1	8.162	8.162	4.17	0.049
Temperature*Temperature	1	105.879	105.879	54.04	0.000
Time*Time	1	5.118	5.118	2.61	0.115
2-Way Interaction	3	22.501	7.500	3.83	0.018
%HCl*Temperature	1	11.663	11.663	5.95	0.020
%HCl*Time	1	4.524	4.524	2.31	0.138
Temperature*Time	1	6.315	6.315	3.22	0.081
Error	35	68.576	1.959		
Lack-of-Fit	3	48.305	16.102	25.42	
Pure Error	32	20.271	0.633		
Total	44	219.417			
Model Summary					
	S	R-sp	R-sp(adj)	R-sq(pred)	
	1.39976	68.75%	60.71%	46.87%	