

Article

Collagen-based bioactive bromelain hydrolysate from salt-cured cod skin

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Raw data and full statistical analysis of Box Behnken experimental design.

Data matrix

Run	Factors [†]			Responses [‡]		
	X _A	X _B	X _C	[Protein]	ORAC	iACE
1	5.6	25	270	2.8077	312.9679	64.0155
2	7.2	25	150	3.1316	267.1010	65.2356
3	4	25	150	2.4045	391.0358	70.9775
4	5.6	25	30	2.8438	319.6965	68.8579
5	5.6	25	270	2.1844	246.5149	73.6071
6	7.2	25	150	2.8059	225.7193	84.5224
7	4	25	150	2.4903	623.9773	75.5033
8	5.6	25	30	2.4322	211.2518	86.2897
9	5.6	25	270	3.0150	288.4817	71.6761
10	7.2	25	150	2.7458	178.6970	79.6776
11	4	25	150	2.3690	590.2320	87.5724
12	5.6	25	30	2.5668	253.8846	83.4549
13	4	35	270	3.4717	507.8452	41.3232
14	5.6	35	150	4.3183	251.1170	38.8276
15	5.6	35	150	4.2678	172.6473	49.7438
16	4	35	30	4.3668	455.3834	37.2216
17	7.2	35	30	5.5767	216.4834	37.8524
18	7.2	35	270	5.5772	152.2511	36.2591
19	5.6	35	150	4.9796	216.8595	38.9254
20	4	35	270	3.3964	531.1823	51.5090
21	5.6	35	150	4.9226	196.3015	40.8878
22	5.6	35	150	4.2015	203.3489	48.1914
23	4	35	30	3.3010	522.6383	43.3766
24	7.2	35	30	4.6158	211.2193	49.5350
25	7.2	35	270	5.6915	157.5332	41.8385
26	5.6	35	150	4.7918	200.2970	45.8980
27	4	35	270	3.3893	539.0283	50.4565
28	5.6	35	150	4.8557	257.0052	42.3945

Supplementary Materials

29	5.6	35	150	4.8894	257.4112	43.7063
30	4	35	30	3.5354	499.7016	50.9770
31	7.2	35	30	4.6200	193.6275	49.7366
32	7.2	35	270	4.9859	147.3719	45.7310
33	5.6	35	150	4.3216	192.2897	50.0022
34	5.6	45	30	4.0766	184.8559	32.2266
35	5.6	45	270	3.6976	241.6388	44.5442
36	7.2	45	150	4.5862	212.5449	39.9314
37	4	45	150	3.9920	311.8249	48.6797
38	5.6	45	30	4.6160	194.3948	42.9110
39	5.6	45	270	4.5578	222.5939	48.9965
40	7.2	45	150	4.6048	208.0790	48.1362
41	4	45	150	4.0828	479.2639	51.6984
42	5.6	45	30	4.4294	206.4739	41.7037
43	5.6	45	270	3.7670	134.3875	53.0597
44	7.2	45	150	4.0202	219.2664	52.7398
45	4	45	150	3.3177	513.1803	56.6459

[†] Factors: pH (X_A); temperature (X_B) expressed in °C; time (X_C) expressed in min.

[‡] Responses: protein concentration expressed in mg/mL; ORAC expressed in $\mu\text{mol TE/g protein}$; iACE expressed in % inhibition/(0,15 mg/mL protein).

1. Factorial analysis of experiments for protein concentration

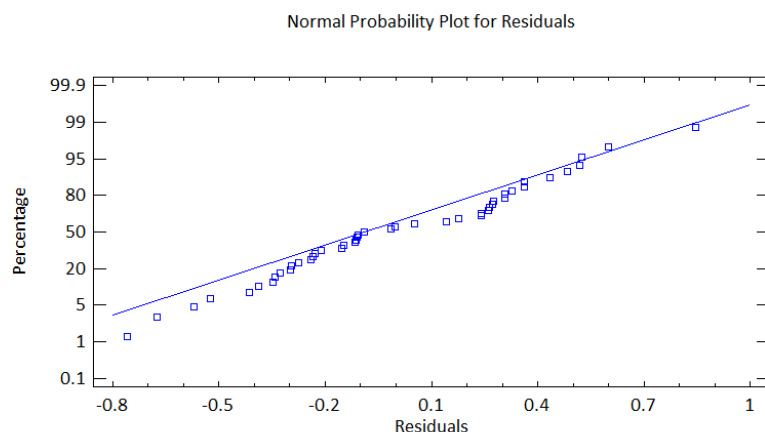
1.1. Initial model

Estimated effects for protein concentration (mg/mL)

<i>Effect</i>	<i>Estimated</i>	<i>Std. Error</i>	<i>V.I.F.</i>
Mean	4.61648	0.119632	
X_A	1.07039	0.146519	1.0
X_B	1.49592	0.146519	1.0
X_C	-0.0365833	0.146519	1.0
X_A^2	-0.276128	0.21567	1.01111
$X_A X_B$	0.0665333	0.207209	1.0
$X_A X_C$	0.397983	0.207209	1.0
X_B^2	-2.19836	0.21567	1.01111
$X_B X_C$	-0.21065	0.207209	1.0
X_C^2	-0.202211	0.21567	1.01111

Standard errors based on total error with 32 d.f.

Supplementary Materials



Analysis of Variance for protein concentration

Source	Sum of squares	DF	Mean Square	F	P
X _A	6.87443	1	6.87443	53.37	0.0000
X _B	13.4267	1	13.4267	104.24	0.0000
X _C	0.00803004	1	0.00803004	0.06	0.8044
X _A ²	0.211144	1	0.211144	1.64	0.2096
X _A X _B	0.0132801	1	0.0132801	0.10	0.7502
X _A X _C	0.475172	1	0.475172	3.69	0.0637
X _B ²	13.3831	1	13.3831	103.90	0.0000
X _B X _C	0.13312	1	0.13312	1.03	0.3170
X _C ²	0.113232	1	0.113232	0.88	0.3555
Lack of fit	1.79215	3	0.597384	4.64	0.0084
Total Error	4.12181	32	0.128807		
Total (corr.)	40.2616	44			

R² = 85.3112 %

R²_{adj} = 81.534 %

Standard error of the estimate = 0.358896

Mean absolute error = 0.310503

Durbin-Watson = 1.51607 (P = 0.0601)

Lag 1 residual autocorrelation = 0.23682

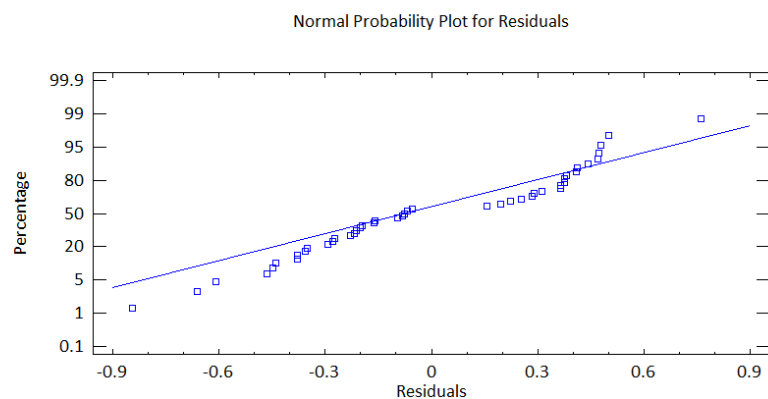
1.2. Recalculated model

Estimated effects for protein concentration (mg/mL)

Effect	Estimated	Std. Error	V.I.F.
Mean	4.47981	0.0783176	
X _A	1.07039	0.146519	1.0
X _B	1.49592	0.146519	1.0
X _A X _C	0.397983	0.207209	1.0
X _B ²	-2.16419	0.214482	1.0

Standard errors based on total error with 32 d.f.

Supplementary Materials



Analysis of Variance for protein concentration

Source	Sum of squares	DF	Mean Square	F	P
X _A	6.87443	1	6.87443	53.37	0.0000
X _B	13.4267	1	13.4267	104.24	0.0000
X _A X _C	0.475172	1	0.475172	3.69	0.0637
X _B ²	13.1145	1	13.1145	101.82	0.0000
Lack of fit	2.24896	8	0.28112	2.18	0.0561
Total Error	4.12181	32	0.128807		
Total (corr.)	40.2616	44			

$R^2 = 84.1766\%$

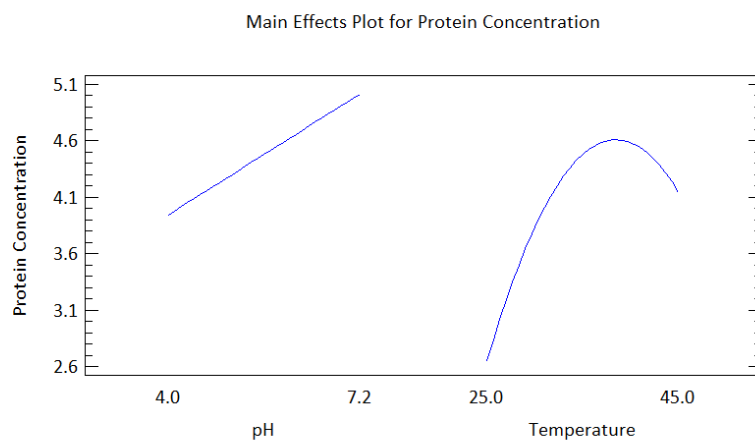
$R^2_{adj} = 82.5942\%$

Standard error of the estimate = 0.358896

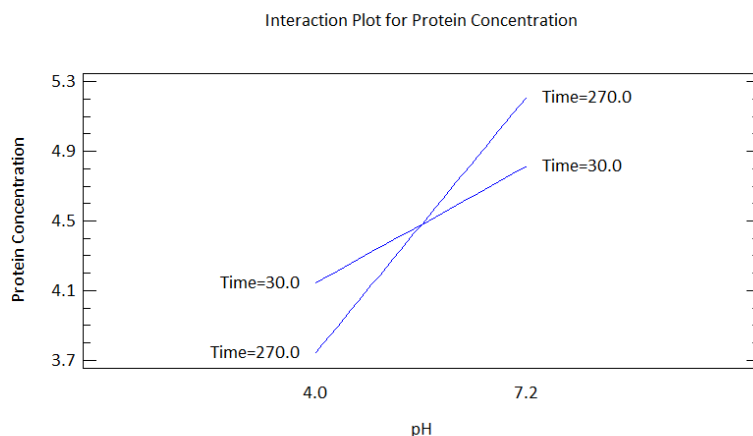
Mean absolute error = 0.334313

Durbin-Watson = 1.70281 ($P = 0.1152$)

Lag 1 residual autocorrelation = 0.139911



Supplementary Materials



Regression coefficients for protein concentration

<i>Coefficient</i>	<i>Estimated</i>
β_0	-12.3963
β_A	0.179035
β_B	0.832264
$\beta_{A,C}$	-0.00580392
$\beta_{B,B}$	0.00103641

Correlation Matrix for Estimated Effects

	(1)	(2)	(3)	(4)	(5)
(1) Mean	1.0000	0.0000	0.0000	0.0000	-0.7303
(2) X_A	0.0000	1.0000	0.0000	0.0000	0.0000
(3) X_B	0.0000	0.0000	1.0000	0.0000	0.0000
(4) $X_A X_C$	0.0000	0.0000	0.0000	1.0000	0.0000
(5) X_B^2	-0.7303	0.0000	0.0000	0.0000	1.0000

Estimated Results for protein concentration

<i>Run</i>	<i>Observed values</i>	<i>Adjusted values</i>	<i>Std. Error</i>
1	2.8077	2.64975	0.373551
2	3.1316	3.18495	0.380667
3	2.4045	2.11455	0.380667
4	2.8438	2.64975	0.373551
5	2.1844	2.64975	0.373551
6	2.8059	3.18495	0.380667
7	2.4903	2.11455	0.380667
8	2.4322	2.64975	0.373551
9	3.0150	2.64975	0.373551
10	2.7458	3.18495	0.380667
11	2.3690	2.11455	0.380667
12	2.5668	2.64975	0.373551
13	3.4717	3.74562	0.388640
14	4.3183	4.47981	0.367342
15	4.2678	4.47981	0.367342
16	4.3668	4.14361	0.388640
17	5.5767	4.81601	0.388640
18	5.5772	5.21400	0.388640
19	4.9796	4.47981	0.367342
20	3.3964	3.74562	0.388640
21	4.9226	4.47981	0.367342
22	4.2015	4.47981	0.367342

Supplementary Materials

23	3.3010	4.14361	0.388640
24	4.6158	4.81601	0.388640
25	5.6915	5.21400	0.388640
26	4.7918	4.47981	0.367342
27	3.3893	3.74562	0.388640
28	4.8557	4.47981	0.367342
29	4.8894	4.47981	0.367342
30	3.5354	4.14361	0.388640
31	4.6200	4.81601	0.388640
32	4.9859	5.21400	0.388640
33	4.3216	4.47981	0.367342
34	4.0766	4.14567	0.373551
35	3.6976	4.14567	0.373551
36	4.5862	4.68087	0.380667
37	3.9920	3.61048	0.380667
38	4.6160	4.14567	0.373551
39	4.5578	4.14567	0.373551
40	4.6048	4.68087	0.380667
41	4.0828	3.61048	0.380667
42	4.4294	4.14567	0.373551
43	3.7670	4.14567	0.373551
44	4.0202	4.68087	0.380667
45	3.3177	3.61048	0.380667

Response optimisation

Goal: maximise protein content

Optimal value = 5.34325 mg/mL

<i>Factor</i>	<i>Low</i>	<i>High</i>	<i>Optimal</i>
X _A	4.0	7.2	7.2
X _B	25.0	45.0	38.4554
X _C	30.0	270.0	270.0

2. Factorial analysis of experiments for ORAC

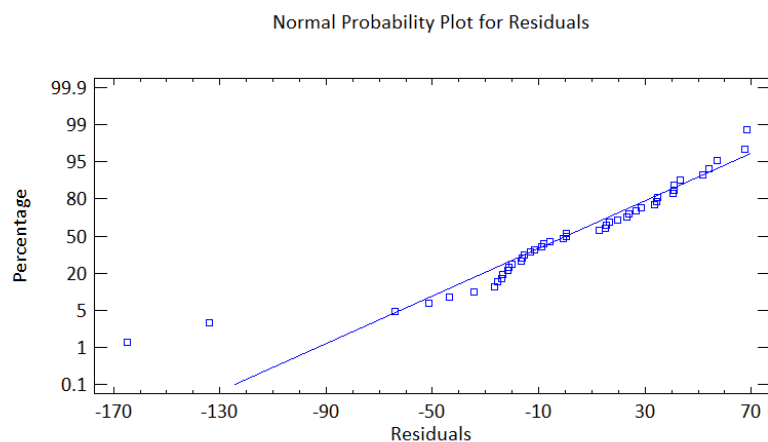
2.1. Initial model

Estimated effects for ORAC (μmol TE/g protein)

<i>Effect</i>	<i>Estimated</i>	<i>Std. Error</i>	<i>V.I.F.</i>
Mean	216.364	17.1788	
X _A	-297.95	21.0397	1.0
X _B	-65.088	21.0397	1.0
X _C	1.01547	21.0397	1.0
X _A ²	245.14	30.9696	1.01111
X _A X _B	44.8915	29.7546	1.0
X _A X _C	-44.0844	29.7546	1.0
X _B ²	25.6191	30.9696	1.01111
X _B X _C	-8.37267	29.7546	1.0
X _C ²	11.1763	30.9696	1.01111

Standard errors based on total error with 32 d.f.

Supplementary Materials



Analysis of Variance for ORAC

Source	Sum of squares	DF	Mean Square	F	P
X _A	532645.	1	532645.	200.54	0.0000
X _B	25418.7	1	25418.7	9.57	0.0041
X _C	6.18714	1	6.18714	0.00	0.9618
X _A ²	166413.	1	166413.	62.65	0.0000
X _A X _B	6045.74	1	6045.74	2.28	0.1412
X _A X _C	5830.31	1	5830.31	2.20	0.1482
X _B ²	1817.55	1	1817.55	0.68	0.4142
X _B X _C	210.305	1	210.305	0.08	0.7802
X _C ²	345.907	1	345.907	0.13	0.7206
Lack of fit	7365.27	3	2455.09	0.92	0.4402
Total Error	84992.5	32	2656.01		
Total (corr.)	829241.	44			

R² = 88.8624 %

R²_{adj} = 85.9984 %

Standard error of the estimate = 51.5365

Mean absolute error = 33.356

Durbin-Watson = 2.38906 (*P* = 0.8930)

Lag 1 residual autocorrelation = -0.228248

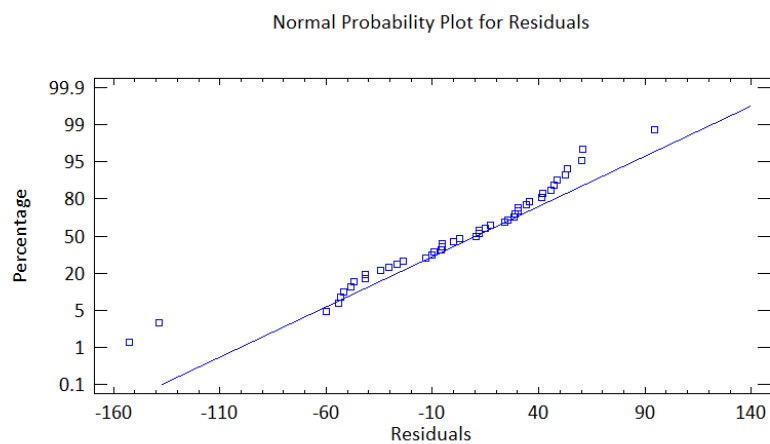
2.2.Recalculated model

Estimated effects for ORAC (μmol TE/g protein)

Effect	Estimated	Std. Error	V.I.F.
Mean	226.877	11.0227	
X _A	-297.95	20.6216	1.0
X _B	-65.088	20.6216	1.0
X _A ²	242.511	30.187	1.0

Standard errors based on total error with 36 d.f.

Supplementary Materials



Analysis of Variance for ORAC

Source	Sum of squares	DF	Mean Square	F	P
X _A	532645.	1	532645.	208.76	0.0000
X _B	25418.7	1	25418.7	9.96	0.0032
X _A ²	164673.	1	164673.	64.54	0.0000
Lack of fit	14649.5	5	2929.9	1.15	0.3533
Total Error	91854.4	36	2551.51		
Total (corr.)	829241.	44			

R² = 87.1565 %

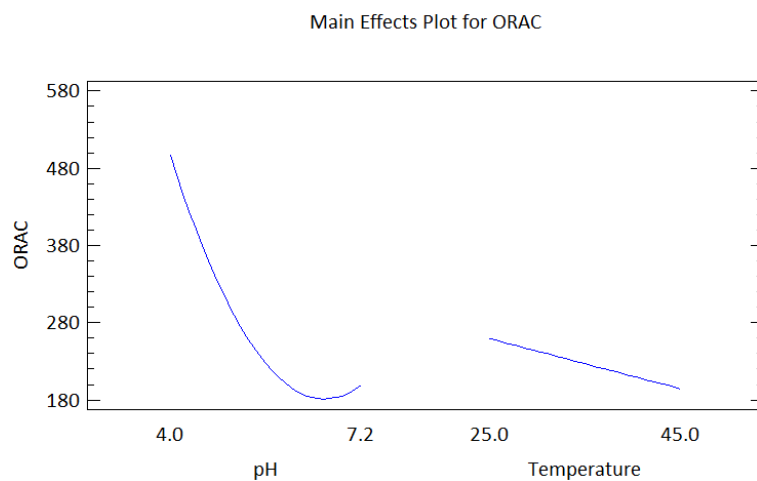
R²_{adj} = 86.2167 %

Standard error of the estimate = 50.5125

Mean absolute error = 37.9319

Durbin-Watson = 2.42138 (*P* = 0.9031)

Lag 1 residual autocorrelation = -0.235249



Regression coefficients for ORAC

Coefficient	Estimated
β_0	2347.58
β_A	-623.603
β_B	-3.2544

Supplementary Materials

$\beta_{A,A}$

47.3655

Correlation Matrix for Estimated Effects

		(1)	(2)	(3)	(4)
(1)	Mean	1.0000	0.0000	0.0000	-0.7303
(2)	X_A	0.0000	1.0000	0.0000	0.0000
(3)	X_B	0.0000	0.0000	1.0000	0.0000
(4)	X_A^2	-0.7303	0.0000	0.0000	1.0000

Estimated Results for ORAC

Run	Observed values	Adjusted values	Std. Error
1	312.968	259.421	52.7193
2	267.101	231.702	53.5766
3	391.036	529.652	53.5766
4	319.697	259.421	52.7193
5	246.515	259.421	52.7193
6	225.719	231.702	53.5766
7	623.977	529.652	53.5766
8	211.252	259.421	52.7193
9	288.482	259.421	52.7193
10	178.697	231.702	53.5766
11	590.232	529.652	53.5766
12	253.885	259.421	52.7193
13	507.845	497.108	52.5751
14	251.117	226.877	51.7012
15	172.647	226.877	51.7012
16	455.383	497.108	52.5751
17	216.483	199.158	52.5751
18	152.251	199.158	52.5751
19	216.86	226.877	51.7012
20	531.182	497.108	52.5751
21	196.302	226.877	51.7012
22	203.349	226.877	51.7012
23	522.638	497.108	52.5751
24	211.219	199.158	52.5751
25	157.533	199.158	52.5751
26	200.297	226.877	51.7012
27	539.028	497.108	52.5751
28	257.005	226.877	51.7012
29	257.411	226.877	51.7012
30	499.702	497.108	52.5751
31	193.628	199.158	52.5751
32	147.372	199.158	52.5751
33	192.29	226.877	51.7012
34	184.856	194.333	52.7193
35	241.639	194.333	52.7193
36	212.545	166.614	53.5766
37	311.825	464.564	53.5766
38	194.395	194.333	52.7193
39	222.594	194.333	52.7193
40	208.079	166.614	53.5766
41	479.264	464.564	53.5766
42	206.474	194.333	52.7193
43	134.387	194.333	52.7193
44	219.266	166.614	53.5766
45	513.18	464.564	53.5766

Response optimisation

Goal: maximise ORAC

Optimal value = 529.652 $\mu\text{mol TE/g protein}$

Supplementary Materials

<i>Factor</i>	<i>Low</i>	<i>High</i>	<i>Optimal</i>
X _A	4.0	7.2	4.0
X _B	25.0	45.0	25.0
X _C	30.0	270.0	150.0

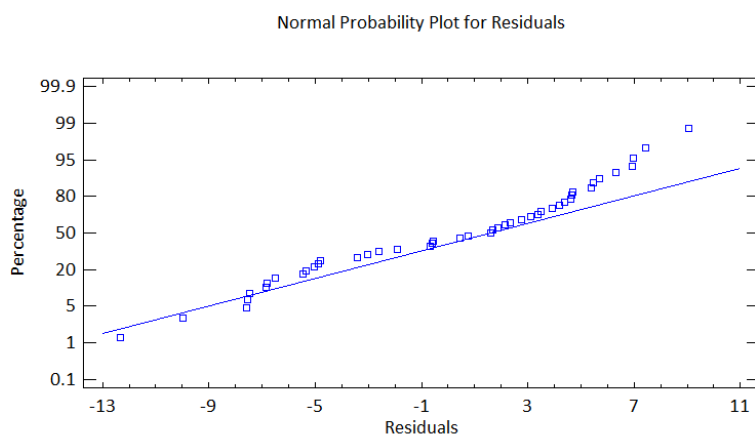
3. Factorial analysis of experiments for iACE

3.1. Initial model

Estimated effects for iACE (% inhibition/(0,15 mg/mL protein))

<i>Effect</i>	<i>Estimated</i>	<i>Std. Error</i>	<i>V.I.F.</i>
Mean	44.2863	2.08018	
X _A	-2.89546	2.54769	1.0
X _B	-29.1764	2.54769	1.0
X _C	-0.0938833	2.54769	1.0
X _A ²	4.52982	3.75009	1.01111
X _A X _B	-1.93317	3.60297	1.0
X _A X _C	-4.16815	3.60297	1.0
X _B ²	33.7842	3.75009	1.01111
X _B X _C	9.84382	3.60297	1.0
X _C ²	-3.79973	3.75009	1.01111

Standard errors based on total error with 32 d.f.



Analysis of Variance for iACE

<i>Source</i>	<i>Sum of squares</i>	<i>DF</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
X _A	50.3021	1	50.3021	1.29	0.2642
X _B	5107.58	1	5107.58	131.15	0.0000
X _C	0.0528845	1	0.0528845	0.00	0.9708
X _A ²	56.8225	1	56.8225	1.46	0.2359
X _A X _B	11.2114	1	11.2114	0.29	0.5953
X _A X _C	52.1204	1	52.1204	1.34	0.2559
X _B ²	3160.73	1	3160.73	81.16	0.0000
X _B X _C	290.702	1	290.702	7.46	0.0102
X _C ²	39.9821	1	39.9821	1.03	0.3185
Lack of fit	16.8998	3	5.63327	0.14	0.9324
Total Error	1246.22	32	38.9443		
Total (corr.)	10065.8	44			

R² = 87.4514 %

R²_{adj} = 84.2247 %

Standard error of the estimate = 6.24053

Mean absolute error = 4.5921

Durbin-Watson = 1.2655 (P = 0.0080)

Supplementary Materials

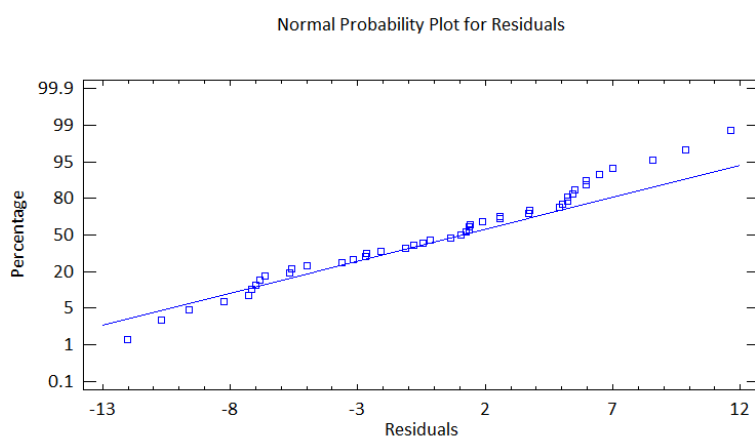
Lag 1 residual autocorrelation = 0.34637

3.2.Recalculated model

Estimated effects for iACE (% inhibition/(0,15 mg/mL protein))

<i>Effect</i>	<i>Estimated</i>	<i>Std. Error</i>	<i>V.I.F.</i>
Mean	44.4949	1.30679	
X _B	-29.1764	2.44478	1.0
X _B ²	33.7321	3.57879	1.0
X _B X _C	9.84382	3.45744	1.0

Standard errors based on total error with 38 d.f.



Analysis of Variance for iACE

<i>Source</i>	<i>Sum of squares</i>	<i>DF</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
X _B	5107.58	1	5107.58	142.42	0.0000
X _B ²	3185.99	1	3185.99	88.84	0.0000
X _B X _C	290.702	1	290.702	8.11	0.0071
Lack of fit	118.819	3	39.6064	1.10	0.3593
Total Error	1362.74	38	35.8616		
Total (corr.)	10065.8	44			

R² = 85.2813 %

R²_{adj} = 84.2043 %

Standard error of the estimate = 5.98846

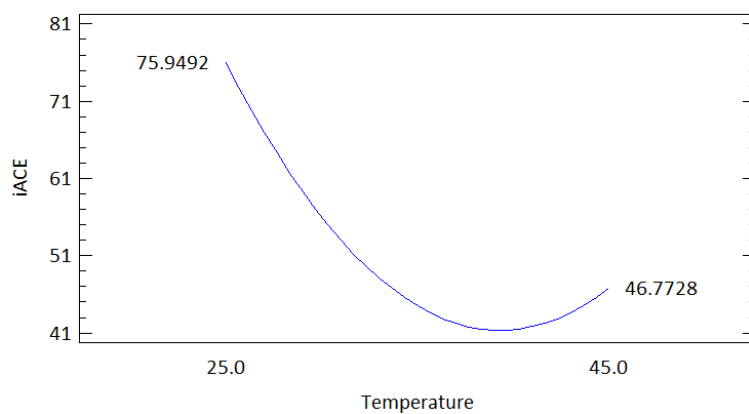
Mean absolute error = 4.81994

Durbin-Watson = 1.36357 (*P* = 0.0069)

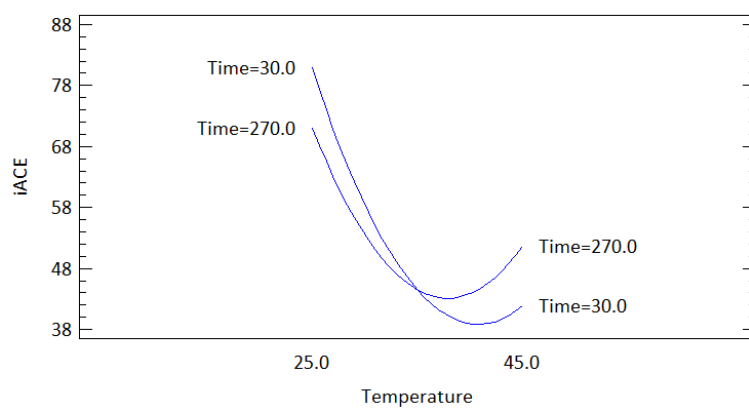
Lag 1 residual autocorrelation = 0.268724

Supplementary Materials

Main Effects Plot for iACE



Interaction Plot for iACE



Regression coefficients for iACE

	<i>Coefficient</i>	<i>Estimated</i>
	β_0	323.696
	β_B	-13.8803
	β_C	-0.143556
	$\beta_{B,B}$	0.16866
	$\beta_{B,C}$	0.00410159

Correlation Matrix for Estimated Effects

		(1)	(2)	(3)	(4)
(1)	Mean	1.0000	0.0000	-0.7303	0.0000
(2)	X_B	0.0000	1.0000	0.0000	0.0000
(3)	X_B^2	-0.7303	0.0000	1.0000	0.0000
(4)	$X_B X_C$	0.0000	0.0000	0.0000	1.0000

Estimated Results for iACE

<i>Run</i>	<i>Observed values</i>	<i>Adjusted values</i>	<i>Std. Error</i>
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Supplementary Materials

1	64.0155	71.0273	6.46827
2	65.2356	75.9492	6.23298
3	70.9775	75.9492	6.23298
4	68.8579	80.8711	6.46827
5	73.6071	71.0273	6.46827
6	84.5224	75.9492	6.23298
7	75.5033	75.9492	6.23298
8	86.2897	80.8711	6.46827
9	71.6761	71.0273	6.46827
10	79.6776	75.9492	6.23298
11	87.5724	75.9492	6.23298
12	83.4549	80.8711	6.46827
13	41.3232	44.4949	6.12938
14	38.8276	44.4949	6.12938
15	49.7438	44.4949	6.12938
16	37.2216	44.4949	6.12938
17	37.8524	44.4949	6.12938
18	36.2591	44.4949	6.12938
19	38.9254	44.4949	6.12938
20	51.5090	44.4949	6.12938
21	40.8878	44.4949	6.12938
22	48.1914	44.4949	6.12938
23	43.3766	44.4949	6.12938
24	49.5350	44.4949	6.12938
25	41.8385	44.4949	6.12938
26	45.8980	44.4949	6.12938
27	50.4565	44.4949	6.12938
28	42.3945	44.4949	6.12938
29	43.7063	44.4949	6.12938
30	50.9770	44.4949	6.12938
31	49.7366	44.4949	6.12938
32	45.7310	44.4949	6.12938
33	50.0022	44.4949	6.12938
34	32.2266	41.8508	6.46827
35	44.5442	51.6947	6.46827
36	39.9314	46.7728	6.23298
37	48.6797	46.7728	6.23298
38	42.9110	41.8508	6.46827
39	48.9965	51.6947	6.46827
40	48.1362	46.7728	6.23298
41	51.6984	46.7728	6.23298
42	41.7037	41.8508	6.46827
43	53.0597	51.6947	6.46827
44	52.7398	46.7728	6.23298
45	56.6459	46.7728	6.23298

Response optimisation

Goal: maximise iACE

Optimal value = 80.8711 % inhibition/(0,15 mg/mL protein)

<i>Factor</i>	<i>Low</i>	<i>High</i>	<i>Optimal</i>
X _A	4.0	7.2	5.6
X _B	25.0	45.0	25.0
X _C	30.0	270.0	30.0

4. Multiple Response Optimisation

Responses:

Protein concentration (mg/mL)

ORAC (μmol TE/g protein)

iACE (% inhibition/(0,15 mg/mL protein))

Supplementary Materials

<i>Response</i>	<i>Lower observed</i>	<i>Higher observed</i>
Protein concentration	2.1844	5.6915
ORAC	134.387	623.977
iACE	32.2266	87.5724

<i>Response</i>	<i>Desirability</i>		<i>Goal</i>	<i>Weight</i>	<i>Impact</i>
	<i>Low</i>	<i>High</i>			
Protein concentration	2.1844	5.6915	Maximise	1.0	3.0
ORAC	134.387	623.977	Maximise	1.0	3.0
iACE	32.2266	87.5724	Maximise	1.0	3.0

<i>Run</i>	<i>Protein concentration</i>	<i>ORAC</i>	<i>iACE</i>	<i>Desirability</i>	
				<i>Expected</i>	<i>Observed</i>
1	2.8077	312.968	64.0155	0.287471	0.333924
2	3.1316	267.101	65.2356	0.355156	0.352134
3	2.4045	391.036	70.9775	0.000000	0.284528
4	2.8438	319.697	68.8579	0.309974	0.361142
5	2.1844	246.515	73.6071	0.287471	0.000000
6	2.8059	225.719	84.5224	0.355156	0.314936
7	2.4903	623.977	75.5033	0.000000	0.408570
8	2.4322	211.252	86.2897	0.309974	0.221286
9	3.0150	288.482	71.6761	0.287471	0.375940
10	2.7458	178.697	79.6776	0.355156	0.231589
11	2.3690	590.232	87.5724	0.000000	0.365951
12	2.5668	253.885	83.4549	0.309974	0.290965
13	3.4717	507.845	41.3232	0.418137	0.358354
14	4.3183	251.117	38.8276	0.301503	0.258643
15	4.2678	172.647	49.7438	0.301503	0.244929
16	4.3668	455.383	37.2216	0.451015	0.332686
17	5.5767	216.483	37.8524	0.280225	0.254514
18	5.5772	152.251	36.2591	0.293694	0.137009
19	4.9796	216.860	38.9254	0.301503	0.253290
20	3.3964	531.182	51.5090	0.418137	0.460385
21	4.9226	196.302	40.8878	0.301503	0.249071
22	4.2015	203.349	48.1914	0.301503	0.285898
23	3.3010	522.638	43.3766	0.451015	0.370516
24	4.6158	211.219	49.5350	0.280225	0.324039
25	5.6915	157.533	41.8385	0.293694	0.201739
26	4.7918	200.297	45.8980	0.301503	0.291319
27	3.3893	539.028	50.4565	0.418137	0.453921
28	4.8557	257.005	42.3945	0.301503	0.327251
29	4.8894	257.411	43.7063	0.301503	0.342563
30	3.5354	499.702	50.9770	0.451015	0.460069
31	4.6200	193.628	49.7366	0.280225	0.298457
32	4.9859	147.372	45.7310	0.293694	0.172907
33	4.3216	192.290	50.0022	0.301503	0.284993
34	4.0766	184.856	32.2266	0.228350	0.000000
35	3.6976	241.639	44.5442	0.288792	0.276050
36	4.5862	212.545	39.9314	0.230928	0.247819
37	3.9920	311.825	48.6797	0.416157	0.381514
38	4.6160	194.395	42.9110	0.228350	0.254094
39	4.5578	222.594	48.9965	0.288792	0.333053
40	4.6048	208.079	48.1362	0.230928	0.310242
41	4.0828	479.264	51.6984	0.416157	0.511915
42	4.4294	206.474	41.7037	0.22835	0.252713
43	3.7670	134.387	53.0597	0.288792	0.005577
44	4.0202	219.266	52.7398	0.230928	0.322799
45	3.3177	513.180	56.6459	0.416157	0.479592

Supplementary Materials

Desirability optimisation

Optimal value = 0.516368

<i>Factor</i>	<i>Low</i>	<i>High</i>	<i>Optimal</i>
pH	4.0	7.2	4.0
Temperature	25.0	45.0	29.8106
Time	30.0	270.0	30.0008

<i>Response</i>	<i>Optimal</i>
Protein concentration	3.46406
ORAC	513.996
iACE	59.1614