

Supplementary Material

Optimisation of microwave-assisted extraction process of bioactive compounds and antioxidant activity from cocoa pod husk (*Theobroma cacao* L.)

Esteban Jurado-Beizaga,^a Erick Alvarez-Yanamango,^{*bc} Oscar Herrera-Calderon^a and Alfredo Ibañez^d

^a Department of Pharmacology, Bromatology and Toxicology, Faculty of Pharmacy and Biochemistry, National University of San Marcos, Lima 15001, Peru.

^b Agroindustrial Technologies and Processes Research Group (ITEPA), Pontifical Catholic University of Peru, Lima 15001, Peru

^c Engineering Department, Pontifical Catholic University of Peru, Lima 15001, Peru

^d Institute for Omics Sciences and Applied Biotechnology (ICOPA PUPC), Pontifical Catholic University of Peru, Lima 15001, Peru

*erick.alvarez@pucp.edu.pe

Table S1 ANOVA results of the total phenolic content (TPC)

	Sum of squares	df	Mean sum of square	F - ratio	p - value	Significance
Model	1.84	9	0.2046	62.73	< 0.0001	significant
A: microwave power	1.29	1	1.29	396.44	< 0.0001	significant
B: extraction time	0.0282	1	0.0282	8.64	0.0054	
C: liquid/solid ratio	0.1902	1	0.1902	58.34	< 0.0001	significant
AB	0.1551	1	0.1551	47.56	< 0.0001	significant
AC	0.0115	1	0.0115	3.52	0.0677	
BC	0.0001	1	0.0001	0.0230	0.8802	
A ²	0.1300	1	0.1300	39.87	< 0.0001	significant
B ²	0.0006	1	0.0006	0.1809	0.6728	
C ²	0.0195	1	0.0195	5.99	0.0187	
Lack of fit	0.0989	3	0.0330	35.94	< 0.0001	significant
Pure error	0.0348	38	0.0009			
R ² = 0.9323						
Adj. R ² = 0.9174						

Table S2 ANOVA results of the total saponin content (TSC)

	Sum of squares	df	Mean sum of square	F - ratio	p - value	Significance
Model	0.3765	9	0.0418	134.82	< 0.0001	significant
A: microwave power	0.1302	1	0.1302	419.64	< 0.0001	significant
B: extraction time	0.0152	1	0.0152	49.08	< 0.0001	
C: liquid/solid ratio	0.0889	1	0.0889	282.62	< 0.0001	significant
AB	0.0007	1	0.0007	2.18	0.1437	
AC	0.0293	1	0.0293	94.56	< 0.0001	significant
BC	0.0464	1	0.0464	149.56	< 0.0001	significant
A ²	0.0454	1	0.0454	146.37	< 0.0001	significant
B ²	0.0034	1	0.0034	10.99	0.0019	
C ²	0.0104	1	0.0104	33.52	< 0.0001	significant
Lack of fit	0.0022	3	0.0007	2.66	0.0619	
Pure error	0.0105	38	0.0083			
R ² = 0.6819						
Adj. R ² = 0.6616						

Table S3 ANOVA results of the DPPH radical scavenging capacity (DRSC)

	Sum of squares	df	Mean sum of square	F - ratio	p - value	Significance
Model	831.55	9	92.39	10.12	< 0.0001	significant
A: microwave power	432.31	1	432.31	47.36	< 0.0001	significant
B: extraction time	12.47	1	12.47	1.37	0.2492	
C: liquid/solid ratio	29.10	1	29.10	3.19	0.0816	
AB	0.5142	1	0.5142	0.0563	0.8136	
AC	39.06	1	39.06	4.28	0.0449	
BC	73.21	1	73.21	8.02	0.0079	
A ²	0.4581	1	0.4581	0.0502	0.8239	
B ²	1.80	1	1.80	0.1977	0.6589	
C ²	236.79	1	236.79	25.94	< 0.0001	significant
Lack of fit	267.88	3	89.29	31.90	< 0.0001	significant
Pure error	106.36	38	2.80			
R ² = 0.6896						
Adj. R ² = 0.6215						

Table S4 ANOVA results of the ABTS radical scavenging capacity (ARSC)

	Sum of squares	df	Mean sum of square	F - ratio	p - value	Significance
Model	841.29	9	93.47	17.39	< 0.0001	significant
A: microwave power	136.80	1	136.80	25.45	< 0.0001	significant
B: extraction time	55.33	1	55.33	10.29	0.0026	
C: liquid/solid ratio	96.43	1	96.43	17.94	0.0001	
AB	3.81	1	3.81	0.7094	0.4045	
AC	12.56	1	12.56	2.34	0.1341	
BC	0.9633	1	0.9633	0.1792	0.6743	
A ²	15.96	1	15.96	2.97	0.0924	
B ²	484.80	1	484.80	90.17	< 0.0001	significant
C ²	39.27	1	39.27	7.30	0.0100	
Lack of fit	125.51	3	41.84	16.75	< 0.0001	significant
Pure error	94.91	38	2.50			
R ² = 0.7924						
Adj. R ² = 0.7468						

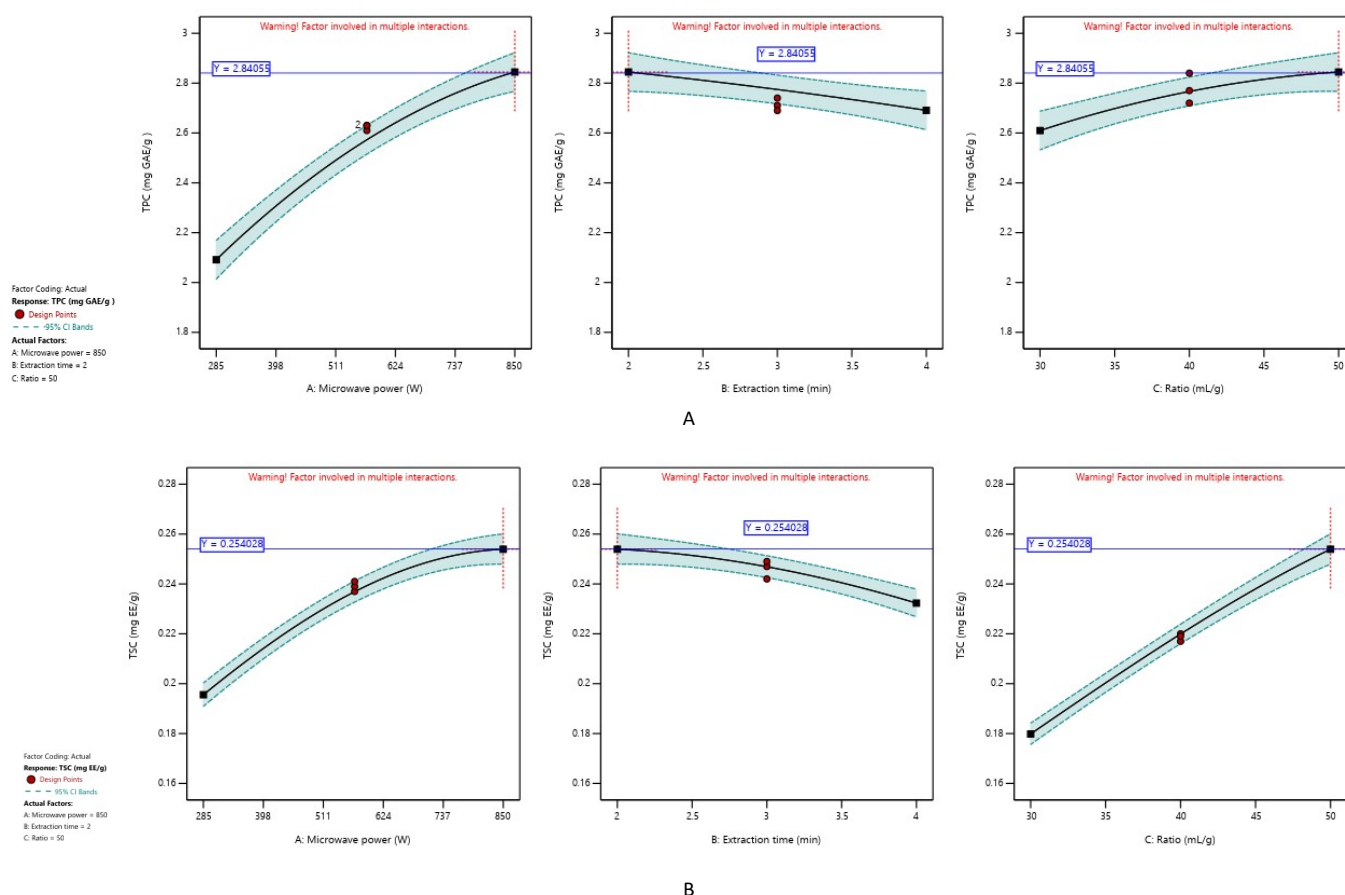
Table S5 ANOVA results of the ferric reducing antioxidant power (FRAP)

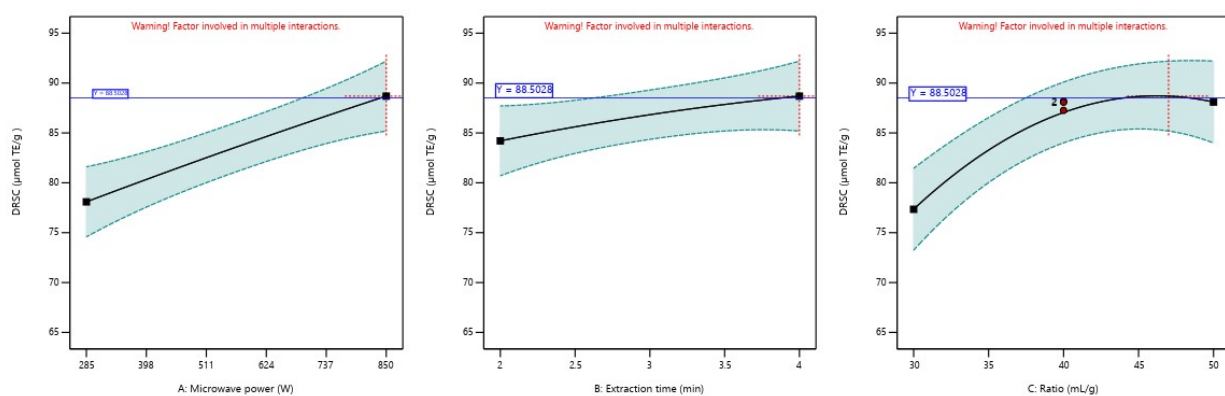
	Sum of squares	df	Mean sum of square	F - ratio	p - value	Significance
Model	1386.37	9	154.04	26.38	< 0.0001	significant
A: microwave power	49.22	1	49.22	8.43	0.0059	
B: extraction time	543.86	1	543.86	93.12	< 0.0001	significant
C: liquid/solid ratio	60.42	1	60.42	10.34	0.0025	
AB	78.09	1	78.09	13.37	0.0007	
AC	89.82	1	89.82	15.38	0.0003	
BC	299.50	1	299.50	51.28	< 0.0001	significant
A ²	31.92	1	31.92	5.47	0.0244	
B ²	76.16	1	76.16	13.04	0.0008	
C ²	159.01	1	159.01	27.23	< 0.0001	significant
Lack of fit	170.37	3	56.79	31.24	< 0.0001	significant
Pure error	69.08	38	1.82			
R ² = 0.8527						
Adj. R ² = 0.8204						

Table S6 Regression coefficients and significance of Box-Behnken models^a

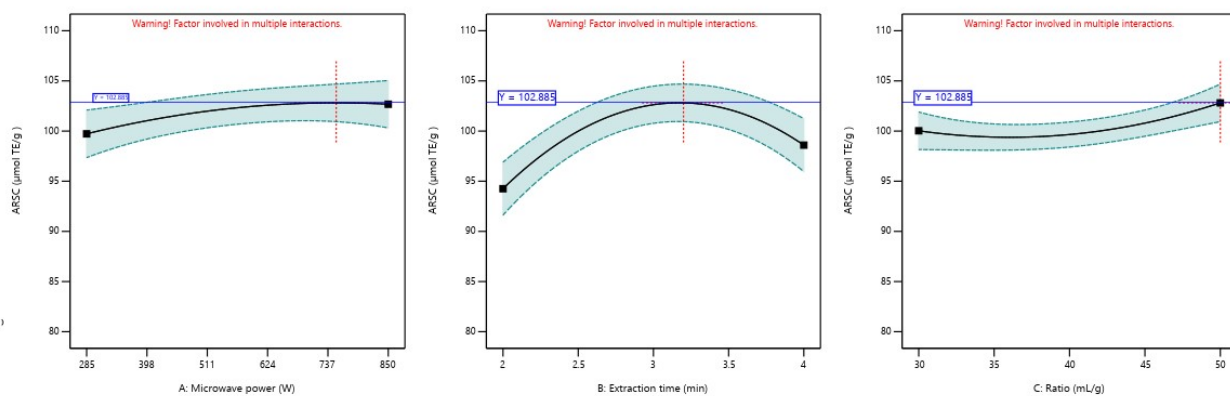
	Intercept	A	B	C	AB	AC	BC	A ²	B ²	C ²
TPC	2.56329	0.232083*	0.034253*	0.0890298*	-0.113679*	0.0309354	0.0025	-0.101454*	-	-0.0393333*
<i>p</i> -values	-	< 0.0001*	0.0054*	< 0.0001*	< 0.0001*	0.0677	0.8802	< 0.0001*	0.00683333	0.0187*
TSC	-1.49425	0.0736544*	0.0251894*	0.0608731*	-	0.0494438*	-	-	-	-0.0286955*
<i>p</i> -values	-	< 0.0001*	< 0.0001*	< 0.0001*	0.00751059	< 0.0001*	0.0621843*	0.0599663*	0.0164283*	< 0.0001*
DRSC	82.8618	4.24417*	0.720916	1.10118	-0.206998	1.80421*	2.47*	-0.190456	-0.378	-4.32967*
<i>p</i> -values	-	< 0.0001*	0.2492	0.0816	0.8136	0.0449*	0.0071*	0.8239	0.6589	< 0.0001*
ARSC	98.4443	2.3875*	1.51834*	2.00453*	0.563732	-1.02312	0.283333	-1.12413	-6.19517*	1.76317*
<i>p</i> -values	-	< 0.0001*	0.0026*	0.0001*	0.4045	0.1341	0.6743	0.0924	< 0.0001*	0.0100*
FRAP	88.4699	1.43208*	4.76045*	1.58664*	-2.55087*	2.73584*	-4.99583*	1.5898*	-2.4555*	-3.548*
<i>p</i> -values	-	0.0059*	< 0.0001*	0.0025*	0.0007*	0.0003*	< 0.0001*	0.0244*	0.0008*	< 0.0001*

^aThe *p* - value results are expressed as follows: *p* < 0.05, *. Abbreviations: A, microwave power; B, extraction time; C, Liquid/solid ratio; TPC, total phenol content; TSC, total saponin content; DRSC, DPPH radical scavenging capacity; ARSC, ABTS radical scavenging capacity; FRAP, ferric reducing antioxidant power.

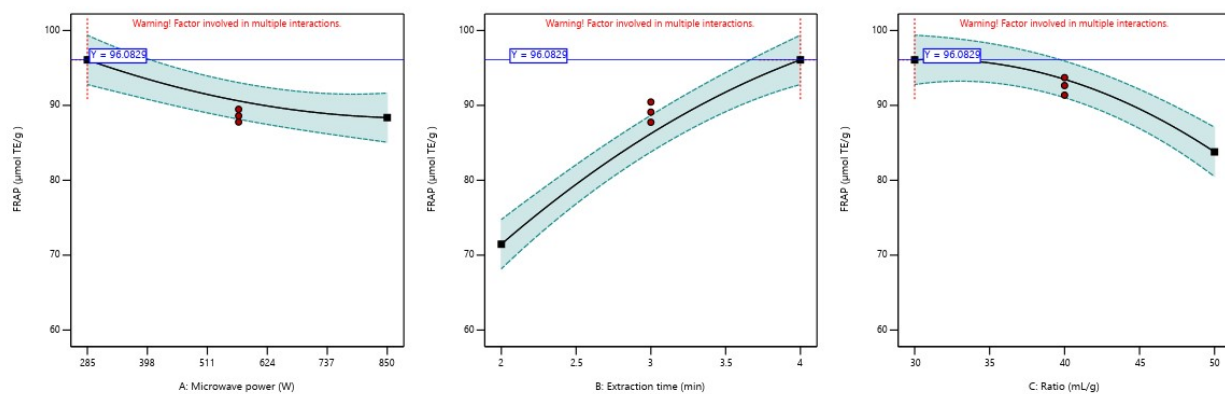




C



D



E

Fig. S1. Interaction curves of parameters of MAE (microwave power, extraction time and liquid/solid ratio) of (A) Total Phenolic Content (TPC), (B) Total Saponin Content (TSC), (C) DPPH Radical Scavenging Capacity (DRSC), (D) ABTS Radical Scavenging Capacity (ARSC) and (E) Ferric Reducing Antioxidant Power (FRAP)