Enhancing Efficiency of Enzymatic-Assisted Extraction Method for Evaluating Bioactive Compound Analysis in Mulberry: An Optimization Approach

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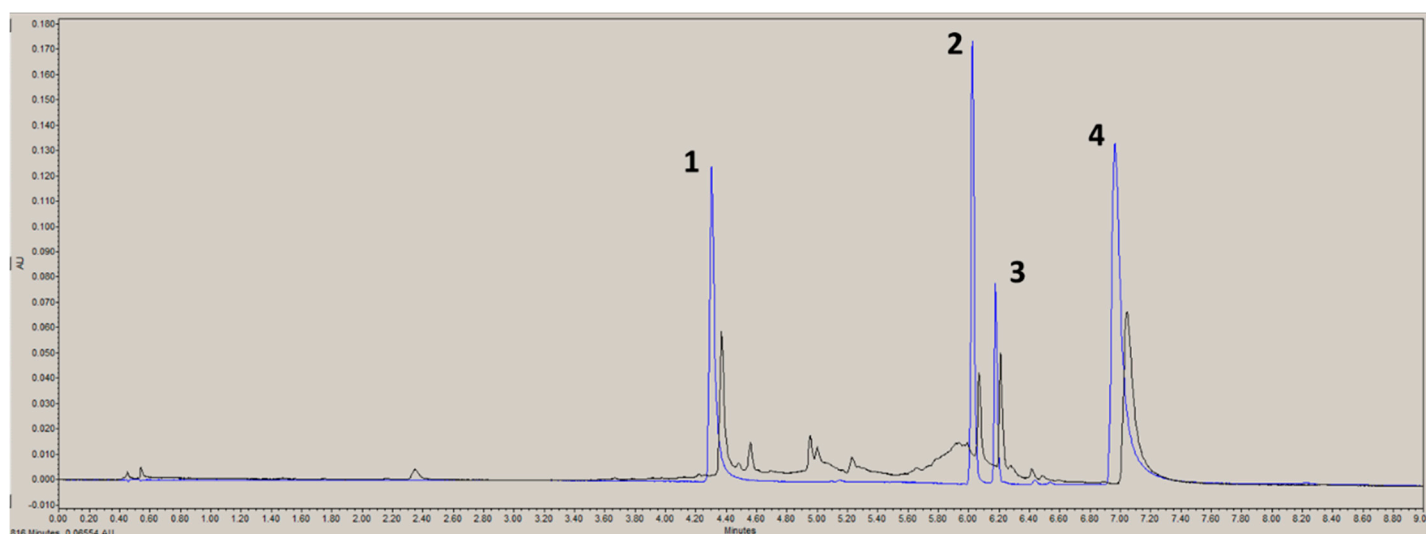
Figure S1. UV Chromatogram at 320 nm. In blue, a mixture of standards; in black, mulberry extract. 1. Chlorogenic acid, 2. Vanillic acid, 3. Rutin, 4. Resveratrol.
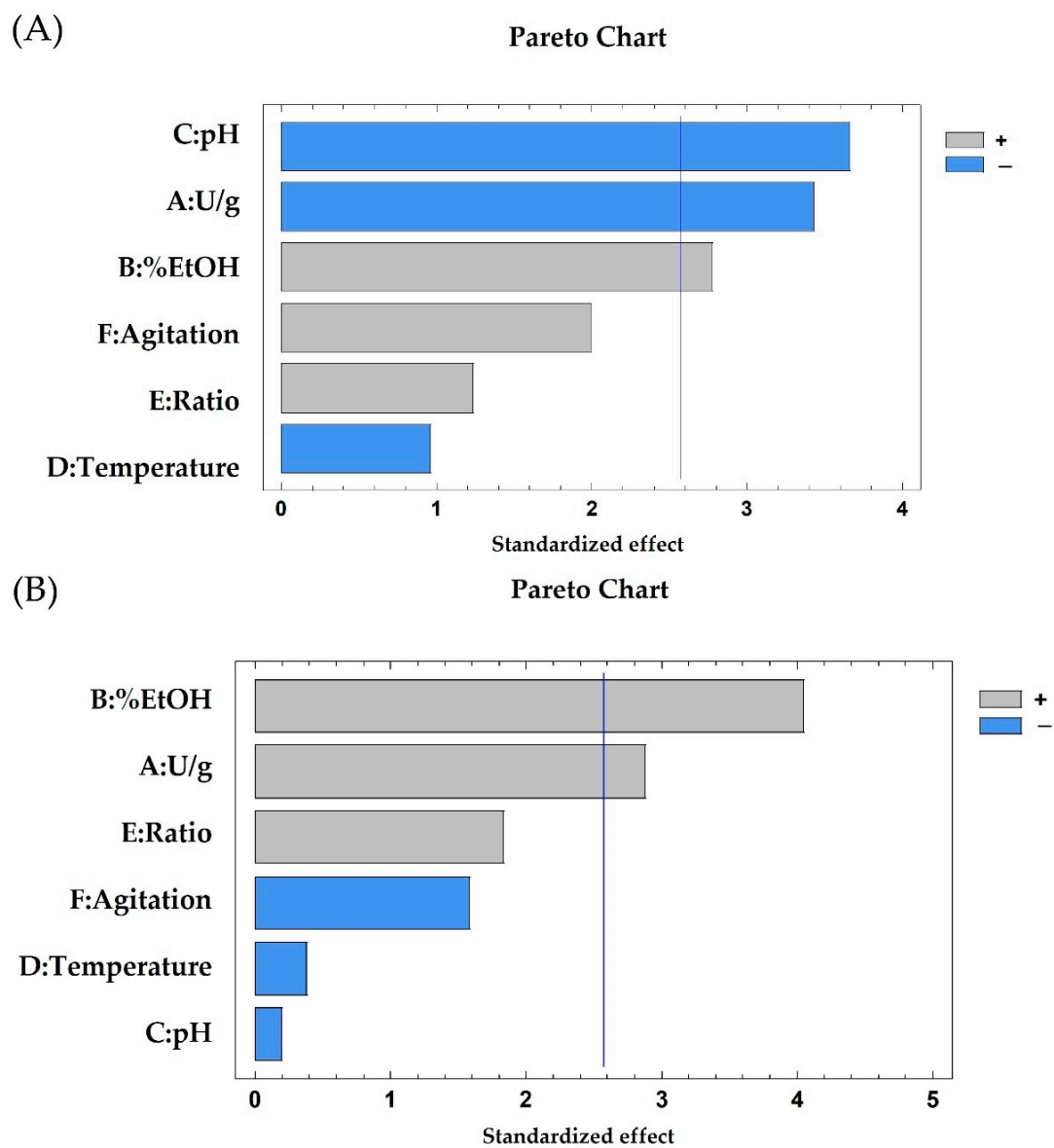
(A)

Pareto Chart

(B)

Pareto Chart

Figure S2. Pareto Chart for (A) anthocyanins (mg/100 g) and (B) phenolic compounds (mg/100 g) according to the analysis for PB.

Table S1. Experimental extraction conditions and total anthocyanins and phenolic compounds concentration (mg/ 100 g) obtained and adjusted to the 12 Plackett–Burman tests with six variables.

Experiment	% EtOH	pH	U/g	Temp (°C)	Ratio (g/15 mL)	Agitation (rpm)	Total Anthocyanins (mg/100 g)			Total Phenolic Compounds (mg/100 g)		
							Observed	Adjusted	% Error	Observed	Adjusted	% Error
1	10	6	1000	40	0.1	50	4.27	4.30	0.82	605.35	595.98	1.57
2	10	4	1000	40	0.2	200	25.79	28.06	8.11	645.01	627.32	2.82
3	60	6	1000	40	0.2	200	137.22	140.13	2.08	704.20	714.98	1.51
4	60	6	100	60	0.1	200	140.77	126.78	11.03	649.85	607.20	7.02
5	10	6	100	60	0.2	50	4.06	4.14	1.87	563.39	542.39	3.87
6	10	6	1000	60	0.1	200	6.28	6.06	3.66	492.74	539.02	8.58
7	60	4	1000	60	0.1	50	142.23	137.80	3.22	667.92	703.34	5.04
8	60	4	1000	60	0.2	50	132.47	136.90	3.24	780.85	745.43	4.75
9	10	4	100	60	0.2	200	69.68	73.81	5.59	544.26	561.63	3.09
10	60	4	100	40	0.1	200	114.80	119.69	4.09	667.51	653.41	2.16
11	10	4	100	40	0.1	50	79.25	77.96	1.66	532.09	546.50	2.64
12	60	6	100	40	0.2	50	112.93	119.11	5.19	630.30	676.26	6.80

Table S2. Experimental extraction conditions and total anthocyanins and phenolic compounds concentration (mg/100 g) obtained and adjusted to the BBD-RSM.

Experiment	pH	% EtOH	U/g	Total Anthocyanins (mg/100 g)			Total Phenolic Compounds (mg/100 g)		
				Observed	Adjusted	Error (%)	Observed	Adjusted	Error (%)
1	5	60	300	118.81	108.23	9.78	905.12	860.90	5.14
2	5	40	550	23.93	23.54	1.65	604.28	576.66	4.79
3	4	80	300	120.44	109.36	10.13	784.56	805.82	2.64
4	5	80	550	102.81	117.77	12.70	615.15	620.23	0.82
5	4	60	550	85.49	91.61	6.69	779.41	753.07	3.50
6	6	60	50	77.79	71.67	8.54	797.99	776.73	2.74
7	5	40	50	65.02	70.07	7.20	554.25	547.88	1.16
8	6	60	550	97.18	100.57	3.38	755.56	760.90	0.70
9	6	80	300	91.90	93.55	1.76	622.02	560.90	10.90
10	5	60	300	102.54	108.23	5.25	639.18	645.55	0.99
11	5	60	300	103.34	108.23	4.52	800.21	801.49	0.16
12	4	40	300	7.45	7.80	4.44	534.28	561.90	4.92
13	5	80	50	14.88	15.35	3.06	93.89	88.81	5.72
14	4	60	50	96.10	92.71	3.66	685.69	684.40	0.19
15	6	40	300	18.45	19.53	5.52	512.39	538.73	4.89

Table S3. Values obtained from the ANOVA analysis of anthocyanins and phenolic compounds of mulberry by BBD-RSM.

Source	Anthocyanins			Phenolic Compounds		
	Sum of Squares	F-Value	*p*-Value	Sum of Squares	F-Value	*p*-Value
A:U/g (mL)	8.27E+12	2.65	0.1642	9.16E+12	1.49	0.2763
B:%EtOH	2.82E+13	9.05	0.0298	4.54E+11	0.07	0.7964
C:pH	5.69E+12	1.83	0.2343	2.60E+11	0.04	0.8451
AA	1.51E+12	0.49	0.5171	5.13E+12	0.84	0.4027
AB	7.22E+12	2.32	0.1883	1.02E+13	1.66	0.2545
AC	1.61E+12	0.52	0.5039	9.47E+11	0.15	0.7107
BB	4.23E+12	1.36	0.2963	3.41E+13	5.56	0.0649
BC	1.29E+13	4.14	0.0974	1.07E+12	0.17	0.6943
CC	2.99E+11	0.10	0.7690	2.32E+12	0.38	0.5653
Total Error	1.56E+13			6.14E+12		