

Supplementary Information

2. File structure

- Sheet 1: Proanthocyanidin absorption curve
- Sheet 2: Proanthocyanidin standard curve
- Sheet 3: Effect of light on the stability of proanthocyanidins in sample solution
- Sheet 4: Single Factor Experiments
- Sheet 5: RSM Design Matrix
- Sheet 6: Validation Experiments
- Sheet 7: Sample Measurements
- Sheet 8: precision, repeatability and spike recovery

2. Sheet descriptions and mapping to the manuscript

- Sheet 1 (Proanthocyanidin absorption curve): Corresponds to [Fig. 1. Absorption spectra of proanthocyanidin standards at different concentrations].
- Sheet 2 (Proanthocyanidin standard curve): Corresponds to [Fig. 2. Proanthocyanidin standard curve].
- Sheet 3 (Effect of light on the stability of proanthocyanidins in sample solution): Corresponds to [Fig. 3. Effect of light on the stability of proanthocyanidins in sample solution].
- Sheet 4 (Single Factor Experiments): Predicted and observed values under the optimized conditions. Corresponds to [Fig. 4. Results of the extraction process of Proanthocyanidins from Purple Rice: (a) Effect of ultrasound time on the extraction rate; (b) Effect of ethanol concentration; (c) Effect of water bath temperature; and (d) Effect of water bath time.].
- Sheet 5 (RSM Design Matrix): Corresponds to [Table 1/2/4].
- Sheet 6 (Validation Experiments): Corresponds to five replicate model validation.
- Sheet 7 (Sample Measurements): Corresponds to [Table 3 Proanthocyanidin content of purple rice from different origins (n = 9)].
- Sheet 8 (precision, repeatability and spike recovery): Corresponds to Method validation

Sheet 1: Proanthocyanidin absorption curve

λ (nm)	Abs.				
	10	20	30	40	50
200	0.074	0.135	0.199	0.257	0.307
201	0.085	0.161	0.207	0.27	0.317

202	0.081	0.155	0.21	0.261	0.311
203	0.038	0.133	0.17	0.24	0.28
204	0.099	0.172	0.216	0.278	0.319
205	0.099	0.17	0.216	0.28	0.323
206	0.101	0.175	0.219	0.277	0.335
207	0.089	0.159	0.213	0.267	0.31
208	0.094	0.166	0.213	0.275	0.329
209	0.091	0.147	0.203	0.269	0.316
210	0.101	0.161	0.215	0.286	0.338
211	0.083	0.153	0.221	0.259	0.335
212	0.07	0.153	0.212	0.269	0.347
213	0.083	0.153	0.212	0.28	0.347
214	0.09	0.153	0.221	0.28	0.347
215	0.09	0.153	0.221	0.29	0.347
216	0.096	0.153	0.221	0.301	0.347
217	0.083	0.153	0.221	0.301	0.347
218	0.083	0.162	0.221	0.301	0.347
219	0.096	0.162	0.221	0.301	0.347
220	0.096	0.166	0.212	0.284	0.369
221	0.091	0.181	0.229	0.307	0.357
222	0.092	0.21	0.258	0.336	0.386
223	0.084	0.172	0.229	0.307	0.357
224	0.092	0.183	0.229	0.313	0.361
225	0.096	0.186	0.221	0.301	0.347
226	0.096	0.186	0.221	0.301	0.359
227	0.096	0.186	0.221	0.301	0.384
228	0.096	0.186	0.221	0.301	0.372
229	0.096	0.186	0.221	0.301	0.384
230	0.096	0.186	0.221	0.301	0.397
231	0.096	0.184	0.236	0.307	0.39
232	0.1	0.188	0.245	0.322	0.389
233	0.102	0.181	0.251	0.316	0.404
234	0.092	0.171	0.233	0.316	0.394
235	0.107	0.202	0.263	0.334	0.409
236	0.094	0.185	0.242	0.338	0.39
237	0.099	0.19	0.247	0.334	0.395
238	0.126	0.209	0.285	0.353	0.433
239	0.124	0.485	0.268	0.354	0.41
240	0.119	0.22	0.283	0.358	0.429
241	0.118	0.237	0.301	0.382	0.466
242	0.145	0.271	0.333	0.426	0.536
243	0.166	0.318	0.387	0.486	0.631
247	0.151	0.269	0.342	0.486	0.652
249	0.129	0.203	0.367	0.419	0.553
250	0.119	0.212	0.387	0.508	0.39
255	0.093	0.176	0.232	0.317	0.393
256	0.093	0.174	0.23	0.316	0.391
260	0.098	0.187	0.25	0.344	0.421
261	0.1	0.193	0.258	0.356	0.432
265	0.116	0.227	0.306	0.421	0.508
270	0.139	0.278	0.379	0.521	0.628
271	0.143	0.289	0.393	0.541	0.653
272	0.147	0.299	0.407	0.56	0.677

273	0.152	0.309	0.422	0.579	0.701
274	0.156	0.318	0.433	0.595	0.72
275	0.158	0.325	0.443	0.608	0.737
276	0.162	0.332	0.453	0.622	0.753
277	0.164	0.337	0.46	0.631	0.765
278	0.165	0.34	0.465	0.638	0.773
279	0.166	0.342	0.468	0.642	0.779
280	0.167	0.343	0.469	0.643	0.78
281	0.166	0.341	0.467	0.639	0.775
282	0.163	0.336	0.46	0.63	0.764
283	0.155	0.329	0.45	0.616	0.748
284	0.15	0.32	0.437	0.599	0.727
285	0.145	0.31	0.423	0.578	0.701
290	0.112	0.226	0.303	0.413	0.502
310	0.055	0.065	0.092	0.122	0.148
320	0.033	0.045	0.066	0.087	0.104
340	0.025	0.028	0.047	0.058	0.073
350	0.023	0.025	0.043	0.054	0.066
370	0.02	0.023	0.037	0.045	0.058
380	0.018	0.02	0.034	0.041	0.053
400	0.017	0.019	0.032	0.036	0.05

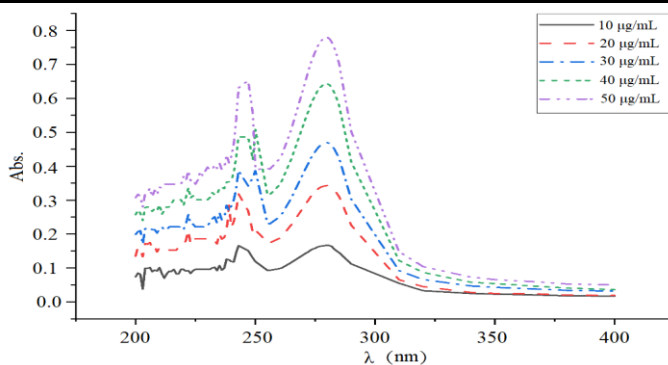


Fig. 1. Absorption spectra of proanthocyanidin standards at different concentrations.

Sheet 2: Proanthocyanidin standard curve

Concentration (µg/mL)	Absorbance
10	0.159
20	0.303
30	0.45
40	0.593
50	0.735

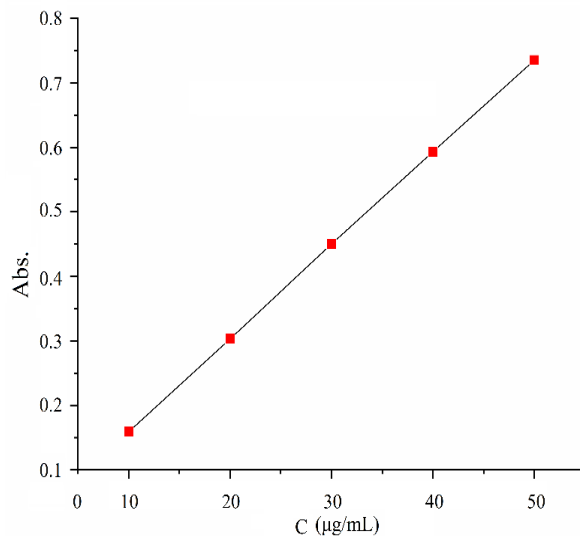


Fig. 2. Proanthocyanidin standard curve.

Sheet 3: Effect of light on the stability of proanthocyanidins in sample solution

Time(h)	Abs.		
	darkness	light-proof	direct sunlight
0	0.446	0.458	0.453
1	0.445	0.454	0.444
2	0.443	0.447	0.424
4	0.44	0.432	0.396
6	0.434	0.418	0.38
8	0.429	0.401	0.365
10	0.428	0.395	0.342

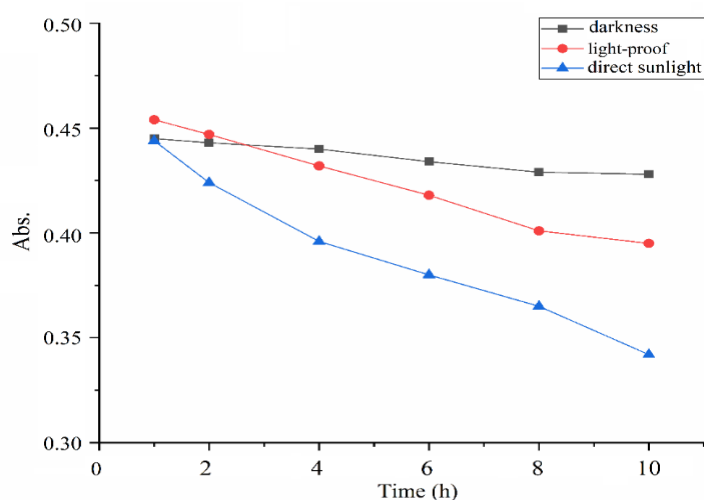


Fig. 3. Effect of light on the stability of proanthocyanidins in sample solution

Sheet 4: Single Factor Experiments

$$y=0.0146x+0.0073$$

	ultrasound time (min)	Abs.	c (µg/mL)	The rate of extraction (µg/g)	SD
	10	0.389	26.14	2614	
	10	0.392	26.35	2635	
	10	0.395	26.55	2655	
	Average	0.392	26.35	2635	
	20	0.429	28.91	2891	
	20	0.430	28.96	2896	
	20	0.425	28.59	2859	
	Average	0.428	28.82	2882	
	30	0.470	31.70	3170	
	30	0.468	31.57	3157	
	30	0.465	31.38	3138	
	Average	0.468	31.55	3155	
	40	0.433	29.14	2914	
	40	0.432	29.07	2907	
	40	0.429	28.85	2885	

Average	40	0.431	29.02	2902	15.1
	50	0.408	27.44	2744	
	50	0.405	27.24	2724	
	40	0.402	27.04	2704	
Average	40	0.405	27.24	2724	20.0

	The concentration of ethanol (%)	Abs.	c (µg/mL)	The rate of extraction (µg/g)	SD
	22	0.142	9.25	925	
	22	0.153	10.00	1000	
	22	0.154	10.06	1006	
Average	22	0.150	9.77	977	45.1
	27	0.176	11.58	1158	
	27	0.185	12.14	1214	
	27	0.191	12.58	1258	
Average	27	0.184	12.10	1210	50.1
	32	0.218	14.45	1445	
	32	0.227	15.08	1508	
	32	0.235	15.62	1562	
Average	32	0.227	15.05	1505	58.6
	37	0.525	35.43	3543	
	37	0.537	36.29	3629	
	37	0.540	36.52	3652	
Average	37	0.534	36.08	3608	57.5
	42	0.338	22.64	2264	
	42	0.346	23.20	2320	
	42	0.351	23.55	2355	
Average	42	0.345	23.13	2313	45.9

	The temperature of water bath (°C)	Abs.	c (µg/mL)	The rate of extraction (µg/g)	SD
	50	0.486	32.79	3279	
	50	0.488	32.95	3295	
	50	0.478	32.21	3221	
Average	50	0.484	32.65	3265	38.9
	60	0.495	33.42	3342	
	60	0.498	33.60	3360	
	60	0.489	33.00	3300	
Average	60	0.494	33.34	3334	30.8
	70	0.462	31.17	3117	
	70	0.466	31.42	3142	
	70	0.455	30.65	3065	
Average	70	0.461	31.08	3108	39.3
	80	0.367	24.64	2464	

	80	0.369	24.79	2479	
	80	0.362	24.28	2428	
Average	80	0.366	24.57	2457	26.2
	90	0.348	23.32	2332	
	90	0.349	23.41	2341	
	90	0.341	22.87	2287	
Average	90	0.346	23.20	2320	28.9

	The time of water bath (min)			The rate of extraction (µg/g)	SD
	20	0.189	12.43	1243	
	20	0.186	12.26	1226	
	20	0.174	11.40	1140	
Average	20	0.183	12.03	1203	55.2
	40	0.232	15.39	1539	
	40	0.231	15.31	1531	
	40	0.218	14.45	1445	
Average	40	0.227	15.05	1505	52.1
	60	0.273	18.20	1820	
	60	0.272	18.14	1814	
	60	0.256	17.03	1703	
Average	60	0.267	17.79	1779	65.9
	80	0.562	38.00	3800	
	80	0.559	37.76	3776	
	80	0.547	36.98	3698	
Average	80	0.556	37.58	3758	53.3
	100	0.468	31.55	3155	
	100	0.467	31.50	3150	
	100	0.454	30.58	3058	
Average	100	0.463	31.21	3121	54.6

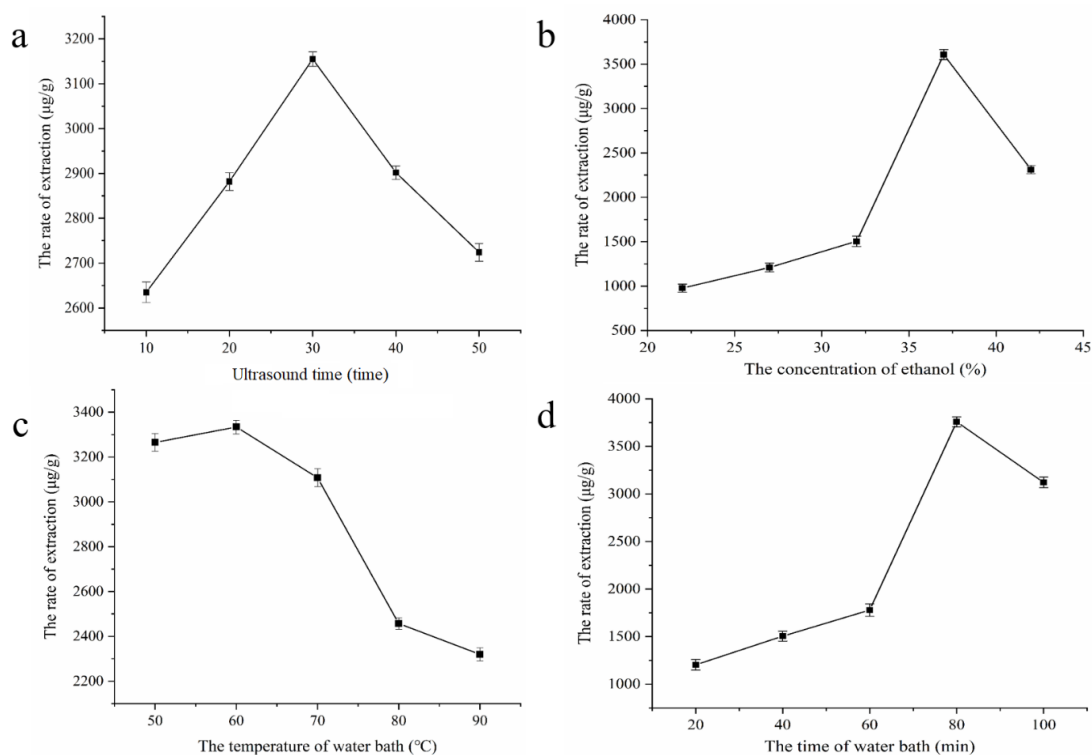


Fig. 4. Results of the extraction process of Proanthocyanidins from Purple Rice: (a) Effect of ultrasound time on the extraction rate; (b) Effect of ethanol concentration; (c) Effect of water bath temperature; and (d) Effect of water bath time.

Sheet 5: RSM Design Matrix

Table 1. Experimental design matrix and corresponding response values for the response surface methodology

Experimental groups	A: ultrasound time (min)	B: ethanol concentration (%)	C: water bath temperature (°C)	D: water bath time (min)	Abs.	Extraction rate (μg/g)
1	0	0	-1	1	0.092	2,020.27
2	1	1	0	0	0.097	1,669.59
3	0	1	0	1	0.101	1,587.40
4	-1	0	1	0	0.312	628.49
5	1	-1	0	0	0.323	491.51
6	1	0	0	-1	0.136	765.48
7	-1	1	0	0	0.376	513.42
8	0	0	0	0	0.245	2,327.12
9	0	-1	1	0	0.113	590.14
10	0	1	1	0	0.147	2,277.81
11	-1	0	0	-1	0.257	579.18
12	-1	-1	0	0	0.328	464.11
13	0	0	0	0	0.296	2,431.23
14	0	1	-1	0	0.312	1,669.59
15	-1	0	-1	0	0.115	1,669.59
16	1	0	0	1	0.423	1,757.26

17	0	0	0	0	0.312	2,579.18
18	1	0	1	0	0.334	1,083.29
19	1	0	-1	0	0.122	1,790.14
20	0	-1	0	-1	0.205	475.07
21	0	-1	0	1	0.094	743.56
22	-1	0	0	1	0.143	1,368.22
23	0	0	0	0	0.143	2,781.92
24	0	0	1	-1	0.297	705.21
25	0	0	-1	-1	0.478	1,729.86
26	0	1	0	-1	0.505	743.56
27	0	0	0	0	0.585	2,727.12
28	0	0	1	1	0.432	1,302.47
29	0	-1	-1	0	0.451	1,581.92

Table 2. Analysis of variance for the quadratic response surface model. Note: $p > 0.05$: not significant; $p < 0.05$: significant different; $p < 0.01$: extremely significant different.

Sources of variance	Sum of Squares	Degrees of Freedom	Mean Square	<i>F</i> -value	<i>p</i> -value	Significance
Model	1.50E+07	14	1.07E+06	15.68	< 0.0001	***
<i>A</i>	4.54E+05	1	4.54E+05	6.65	0.0218	*
<i>B</i>	1.41E+06	1	1.41E+06	20.68	0.0005	**
<i>C</i>	1.25E+06	1	1.25E+06	18.32	0.0008	**
<i>D</i>	1.19E+06	1	1.19E+06	17.45	0.0009	**
<i>AB</i>	3.19E+05	1	3.19E+05	4.67	0.0486	
<i>AC</i>	27930.77	1	27930.77	0.41	0.5327	
<i>AD</i>	10275.88	1	10275.88	0.15	0.7038	
<i>BC</i>	6.40E+05	1	6.40E+05	9.38	0.0084	
<i>BD</i>	82756.91	1	82756.91	1.21	0.2894	
<i>CD</i>	23539.23	1	23539.23	0.34	0.5664	
<i>A</i> ²	4.82E+06	1	4.82E+06	70.69	< 0.0001	***
<i>B</i> ²	4.79E+06	1	4.79E+06	70.13	< 0.0001	***
<i>C</i> ²	7.02E+05	1	7.02E+05	10.28	0.0063	**
<i>D</i> ²	3.53E+06	1	3.53E+06	51.73	< 0.0001	***
Residual	9.56E+05	14	68248.32			
Lack of Fit	8.08E+05	10	80755	2.18	0.2349	
Pure error	1.48E+05	4	36981.63			
Total variation	1.59E+07	28				
<i>R</i> ²	0.94	Adj <i>R</i> ²	0.8801	Pr <i>R</i> ²	0.6936	

Table 4. Independent variables and their coded levels used in the response surface design.

Symbol	Independent variable (unit)	Coded level −1	Coded level 0	Coded level +1
<i>A</i>	Ultrasound time (min)	10	30	50

B	Ethanol concentration (% v/v)	22	32	42
C	Water bath temperature (°C)	50	70	90
D	Water bath time (min)	20	60	100

Sheet 6: Validation Experiments

$$y=0.0146x+0.0073$$

NO.	Abs.	The rate of extraction	Mean Extraction Rate (Within Group)	Mean Extraction Rate (Outside Group)	RSD(%)	SD
		(μg/g)	(μg/g)	(μg/g)		
1	0.388	2,606.58	2628.49	2701.92	2.1	57
	0.393	2,639.45				
	0.393	2,639.45				
2	0.404	2,716.16	2738.08			
	0.410	2,760.00				
	0.407	2,738.08				
3	0.403	2,710.68	2697.90			
	0.401	2,694.25				
	0.400	2,688.77				
4	0.393	2,639.45	2666.85			
	0.397	2,672.33				
	0.400	2,688.77				
5	0.413	2,781.92	2776.44			
	0.411	2,765.48				
	0.414	2,787.40				

Sheet 7: Sample Measurements

Origin	NO	No. of repetitions/Abs.		
		1	2	3
Loudi, Hunan	1	0.397	0.400	0.393
	2	0.389	0.392	0.391
	3	0.402	0.406	0.403
Hanzhong, Shaanxi	1	0.289	0.285	0.287
	2	0.301	0.301	0.301
	3	0.289	0.289	0.290
Harbin, Heilongjiang	1	0.340	0.340	0.340
	2	0.361	0.357	0.360
	3	0.351	0.346	0.347
Pu'er, Yunnan	1	0.214	0.211	0.213
	2	0.221	0.224	0.223
	3	0.217	0.215	0.215

Qiannan, Guizhou	1	0.292	0.289	0.296
	2	0.288	0.291	0.290
	3	0.278	0.274	0.279
Shaoyang, Hunan	1	0.264	0.265	0.257
	2	0.253	0.255	0.251
	3	0.252	0.249	0.250

$$y=0.0146x+0.0073$$

Origin	NO	No. of repetitions			Mean content (μg/g)	RSD (%)	SD
		1	2	3			
Loudi, Hunan	1	2669.18	2689.73	2641.78	2,669.18	1.6	41
	2	2614.38	2634.93	2628.08			
	3	2703.42	2730.82	2710.27			
Hanzhong, Shaanxi	1	1929.45	1902.05	1915.75	1,953.04	2.3	45
	2	2011.64	2011.64	2011.64			
	3	1929.45	1929.45	1936.30			
Harbin, Heilongjiang	1	2278.77	2278.77	2278.77	2,341.17	2.5	59
	2	2422.60	2395.21	2415.75			
	3	2354.11	2319.86	2326.71			
Pu'er, Yunnan	1	1415.75	1395.21	1408.90	1,436.20	2.2	31
	2	1463.70	1483.29	1477.40			
	3	1436.30	1422.60	1422.60			
Qiannan, Guizhou	1	1950.00	1929.45	1977.40	1,911.19	2.7	51
	2	1922.60	1943.15	1936.30			
	3	1854.11	1826.71	1860.96			
Shaoyang, Hunan	1	1758.22	1765.07	1710.27	1,697.34	2.4	40
	2	1682.88	1696.58	1669.18			
	3	1676.03	1655.48	1662.33			

Table 3 Proanthocyanidin content of purple rice from different origins ($n = 9$)

Origin	Mean±SD (μg/g)	RSD (%)
Loudi, Hunan	2669.18 ± 41	1.6
Hanzhong, Shaanxi	1953.04 ± 45	2.3
Harbin, Heilongjiang	2341.17 ± 59	2.5
Pu'er, Yunnan	1436.20 ± 31	2.2
Qiannan, Guizhou	1911.19 ± 51	2.7
Shaoyang, Hunan	1697.34 ± 40	2.4

Sheet 8: precision, repeatability and spike recovery

precise experimental results

Standard	No. of repetitions						RSD%
	1	2	3	4	5	6	

Abs.	0.447	0.453	0.448	0.452	0.448	0.449	0.54
repeatability experimental results							
Sample	No. of repetitions						
	1	2	3	4	5	6	RSD%
Abs.	0.454	0.471	0.468	0.473	0.469	0.476	1.64

Supplementary Table S1. Spike recoveries of proanthocyanidin from plant matrix at low, medium, and high concentration levels ($n = 3$).

sample weight	endogenous mass	mass of standard added	spiked sample	spiked recovery	mean recovery	RSD
(g)	(μg)	(μg)	(μg)	(%)	(%)	(%)
0.0238	63.56		115.6	101.9		
0.0238	63.56	51.11	117.2	104.9		
0.0239	63.82		117.3	104.9		
0.0232	61.96		125.1	101.3		
0.0233	62.22	62.39	126	102.6	100.8	2.0
0.0231	61.69		124.9	100.8		
0.0245	65.43		144	99.7		
0.0246	65.69	78.55	144.4	100.1		
0.0245	65.43		144.2	99.9		