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Extraction Condition of Anthocyanin in Black Soybean Seed Coat

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검정콩 종피 함유 안토시아닌의 추출 조건

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Objectives

The anthocyanin of black soybean seed coat is considered as a standard of quality evaluation of black soybean. The purpose of this study was to investigate the most suitable extraction condition and method of anthocyanin in black soybean seed coat.

Materials and Methods

The seed coat of "Geomjeongkong 1(G)" and "Ilpumgeomjeongkong(I)" was used in this study. Six combinations of water and methanol solution containing 1% HCl were used as extraction solvent. The extraction methods were divided into 5 treatments according to extraction temperature or device. Extraction times of 3, 6, 12, 24, 48, 72 and 96hrs. were tested for the optimum extraction. The absorbance at 530nm and Hunter's color value were analyzed by colorimeter(MINOLTA, CM-3500d). Anthocyanin contents were analyzed by reverse phase-HPLC.

Results and Discussions

In the test of extraction solvent, absorbance and Hunter's a value were increased as increasing the concentration of MeOH, but Hunter's L and b value were the exact opposite of absorbance and Hunter's a value. There was no significant difference for anthocyanin content from 1%HCl-H_2O to 1%HCl-80%MeOH. In the aspects of anthocyanin content and HPLC resolution, 1%HCl-20%MeOH solution was the most suitable solvent. In the 5 kinds of extraction method using 1%HCl-20%MeOH solution, the anthocyanin content of room temp. extraction at 72hrs. was the highest among the methods. If the extraction temperature is high or sonication and reflux method is used, anthocyanin content is decreased because of breakdown of anthocyanin. There was no significant difference for extraction time between 6hrs. and 48hrs. However, the most suitable extraction time was 12hrs.

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Table 1. Comparison of absorbance, color value and anthocyanin content of black soybean seed coat extract with different extraction solvent.

Variety	Extraction solvent	Absorbance (@ 530nm)	Hunter value (D65/2°)			Anthocyanin content(mg/g)
			L	a	b	
G	1% HCl-H_2O	0.119	83.56	11.30	2.91	3.99 a†
	1% HCl-20%MeOH	0.151	82.19	14.23	2.58	4.25 a
	1% HCl-40%MeOH	0.159	81.34	14.82	1.96	3.82 a
	1% HCl-60%MeOH	0.190	80.01	17.61	1.16	4.01 a
	1% HCl-80%MeOH	0.168	83.00	15.97	0.22	3.38 ab
	1% HCl-MeOH	0.229	78.67	21.78	-2.33	2.44 b
I	1% HCl-H_2O	0.230	80.21	20.80	3.57	7.33 a
	1% HCl-20%MeOH	0.292	76.39	25.67	2.29	7.61 a
	1% HCl-40%MeOH	0.315	76.45	28.09	0.13	7.55 a
	1% HCl-60%MeOH	0.370	70.43	32.14	-1.76	7.72 a
	1% HCl-80%MeOH	0.343	73.17	31.39	-4.20	6.76 a
	1% HCl-MeOH	0.443	69.09	38.42	-6.06	4.80 b

† Means followed by different letters are significantly different at 5% level of probability by DMRT.

Table 2. Comparison of absorbance, color value and anthocyanin content of 1%HCl-20%MeOH black soybean seed coat extract with different extraction method.

Variety	Extraction method	Absorbance (@ 530nm)	Hunter value (D65/2°)			Anthocyanin content(mg/g)
			L	a	b	
G	Room temp.(25℃, 72hrs.)	0.143	82.19	14.23	2.58	4.25 a†
	Low temp.(4℃, 72hrs.)	0.133	82.53	13.63	1.81	4.08 a
	High temp.(60℃, 72hrs.)	0.058	83.93	2.69	7.91	0.76 d
	Reflux(80℃, 3hrs.)	0.125	83.40	10.79	4.27	2.40 c
	Sonication(25℃, 3hrs.)	0.126	84.85	12.60	2.27	3.38 b
I	Room temp.(25℃, 72hrs.)	0.292	76.42	25.67	2.29	7.61 a
	Low temp.(4℃, 72hrs.)	0.237	77.51	21.66	1.46	6.82 b
	High temp.(60℃, 72hrs.)	0.082	83.46	3.93	9.46	0.65 d
	Reflux(80℃, 3hrs.)	0.245	78.23	20.36	4.22	3.70 c
	Sonication(25℃, 3hrs.)	0.336	75.56	29.10	2.65	6.15 b

† Means followed by different letters are significantly different at 5% level of probability by DMRT

Table 3. Comparison of absorbance, color value and anthocyanin content of 1%HCl-20%MeOH black soybean seed coat extract with different extraction time.

Variety	Extraction time	Absorbance (@ 530nm)	Hunter value (D65/2°)			Anthocyanin content(mg/g)
			L	a	b	
G	3 hrs.	0.105	82.28	10.34	1.53	3.40 b†
	6 hrs.	0.130	83.95	12.62	2.00	3.98 a
	12 hrs.	0.150	85.35	14.23	2.58	4.08 a
	24 hrs.	0.144	82.51	13.65	2.45	4.04 a
	48 hrs.	0.158	80.70	14.91	2.54	4.11 a
	72 hrs.	0.152	84.89	14.42	2.51	4.10 a
	96 hrs.	0.144	83.23	13.69	2.22	4.07 a
I	3 hrs.	0.227	76.40	20.79	1.63	6.38 d
	6 hrs.	0.283	74.74	25.11	1.97	7.85 a
	12 hrs.	0.301	72.69	26.20	2.25	8.06 a
	24 hrs.	0.298	71.17	26.11	2.16	7.80 ab
	48 hrs.	0.293	74.67	25.96	2.28	7.79 ab
	72 hrs.	0.266	73.69	24.81	2.22	7.45 bc
	96 hrs.	0.264	71.37	24.53	2.11	7.11 c

† Means followed by different letters are significantly different at 5% level of probability by DMRT.