

C46 Anthocyanins in Seed Coat of Black Soybean

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Objectives

The purpose of this study is to isolate, characterize and identify the anthocyanin pigment of black soybean seed coat, and to analyze the contents of anthocyanin in several Korean cultivated black soybeans.

Materials and Methods

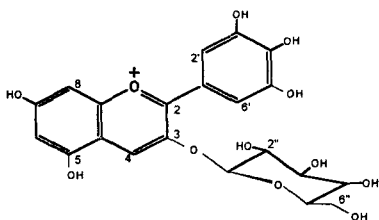
Anthocyanin pigments in black soybean(Ilpumgeomjeongkong) seed coat were extracted with 1% HCl-MeOH solution at 4°C. The crude anthocyanin extract was isolated and purified by Sephadex LH-20 and C₁₈ open column chromatography. The chemical structures of isolated anthocyanins were identified by spectroscopic methods(UV-VIS spectra, TLC, Fab-MS and ¹H·¹³C-NMR) and the contents of anthocyanins were determined by reverse-phase HPLC.

Results and Discussion

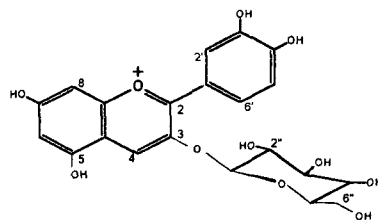
The complete structures of black soybean anthocyanins were elucidated to be Cyanidin-3-O-β-D- glucopyranoside(C3G) and Delphinidin-3-O-β-D- glucopyranoside (D3G) and these anthocyanin pigments were well-known as antioxidant agent.

The contents of C3G and D3G in seed coat of seven cultivated black soybean were ranged 2.9~8.1mg/g and 0~2.8mg/g respectively. Among analyzed seven black soybean cultivars, most black soybeans have C3G and D3G except "Geomjeongkong 1", which contains only C3G.

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Delphinidin-3-o-β -D-glucopyranoside



Cyanidin-3-o-β -D-glucopyranoside

Fig. 1. Chemical structures of isolated anthocyanin in black soybean.

Table 1. Chromatographic and spectral properties of isolated anthocyanins in black soybean seed coat.

Antho cyanin	TLC(Rf × 100) [†]		HPLC Rt.	UV-VIS. Characterization(in 0.5%HCl-50%MeOH)			
	FHW [‡]	BAW [†]		λ max(nm)	Euv/Evis	Eacyl/Evis	E440/Evis
D3G	21	28	10.57	279, 530	69	14	27
C3G	35	38	15.14	281, 522	73	16	32

[‡]FHW : (HCOOH:HCl:H₂O = 1 : 1 : 2), [†]BAW : (n-butanol:HoAc:H₂O = 4 : 1 : 5, upper phase)

[†]Absorbent : cellulose microcrystalline F

Table 2. ¹H-NMR spectral data for isolated anthocyanins in black soybean seed coat (0.5%DCl-CD₃OD at 25°C, 300MHz)

Carbon No.	PPM(δ)	
	D3G	C3G
4	8.94 s	8.99 s
6	6.68 d (J=1.9Hz)	6.69 d (J=2.0Hz)
8	6.91 d (J=1.9Hz)	6.94 d (J=2.0Hz)
2'	7.77 s	8.04 d (J=2.3Hz)
5'	-	7.03 d (J=8.7Hz)
6'	7.77 s	8.26 dd (J=8.8, 2.3Hz)
1''	5.36 d (J=7.7Hz)	5.34 d (J=7.6Hz)
2''	3.72 dd (J= 9.0, 7.5Hz)	3.70 dd (J=9.0, 7.5Hz)
3''	3.55 t (J= 9.0Hz)	3.54 t (J=9.0Hz)
4''	3.48 t (J= 9.0Hz)	3.47 t (J=9.0Hz)
5''	3.62 ddd (J= 8.9, 7.0, 2.3Hz)	3.64 ddd (J=9.0, 7.0, 2.5Hz)
6'' a	3.92 dd (J= 12.0, 1.9Hz)	3.93 dd (J=12.0, 2.0Hz)
6'' b	3.75 dd (J= 12.0, 6.0Hz)	3.74 dd (J=12.0, 6.0Hz)