Supporting Information

Separation and identification of anthocyanins extracted from blueberry wine lees and the pigment binding properties toward *β*-glucosidase

Qian Wu,[†] Yang Zhang,[§] Hu Tang,[ǀ] Yashu Chen,[§] Bijun Xie,[§] Chao Wang,[*,†] Zhida Sun,[*,§]

[†]Hubei Collaborative Innovation Center for Industrial Fermentation, Research Center of Food Fermentation Engineering and Technology of Hubei, Hubei University of Technology, Wuhan, Hubei 430068, China.

[§]Natural Product Laboratory, Department of Food Science and Technology, Huazhong AgriculturalUniversity, Wuhan, Hubei 430070, People's Republic of China

[ǀ]Department of Product Processing and Nutriology, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Hubei Key Laboratory of Lipid Chemistry and Nutrition, Ministry of Agriculture Key Laboratory of Oil Crops Biology, Wuhan 430062, China

Tel: +86-27-87-28-3201; Fax: +86-27-87-28-2966.

Contents:

Supporting Information Figure S1: Diagrammatic structures of anthocyanins isolated from blubeberry wine lees

Supporting Information Figure S2: Elution renderings of anthocyanins from blubeberry wine lees using Sephadex LH-20 chromatographic column

Supporting Information Figure S3: Elution renderings of anthocyanins from blubeberry wine lees using semi-preparative HPLC method

Supporting Information Figure S4: The Stern-Volmer plots for the quenching of *β*-glucosidase by Mv-3-hex (a) and Mv-3-ace-hex (b) at different temperatures (pH 7.4, λ_{ex}=280 nm, λ_{em}=340 nm)

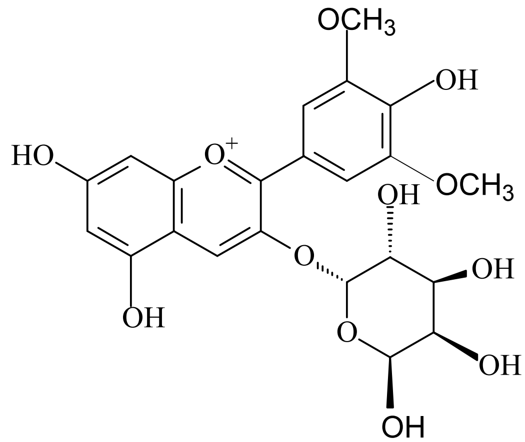
Supporting Information Figure S5: The synchronous fluorescence spectra of *β*-glucosidase in the presence of Mv-3-hex (a: Δλ=15 nm, b: Δλ=60 nm) and Mv-3-ace-hex (c: Δλ=15 nm, d: Δλ=60 nm). C(*β*-glucosidase)=1 mg/mL, C(Mv-3-hex)=0.0 (a), 1.0 (b), 2.0 (c), 3.0 (d), 4.0 (e), 5.0 (f), 6.0 (g), 7.0 (h), 8.0 (i), 9.0×10^{-5}mol/L (j), C(Mv-3-ace-hex)=0.0 (a), 4.0 (b), 8.0 (c), 12.0 (d), 16.0 (e), 20.0 (f), 24.0 (g), 28.0 (h), 32.0 (i), 36.0×10^{-6}mol/L (j).

Supporting Information Table S1. Total contents of phenolics and types of anthocyanin in the blueberry fruits, blueberry wine lees and wine extracts.

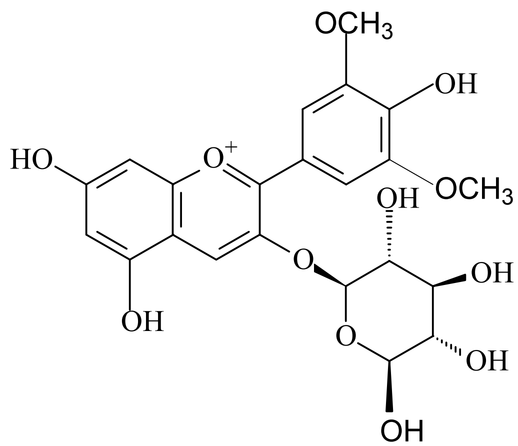
Supporting Information Table S2. The quenching constants of the interaction between *β*-glucosidase and anthocyanins.

Supporting Information Table S3. Binding constants and thermodynamic parameters of β-glucosidase-anthocyanins at different temperatures.

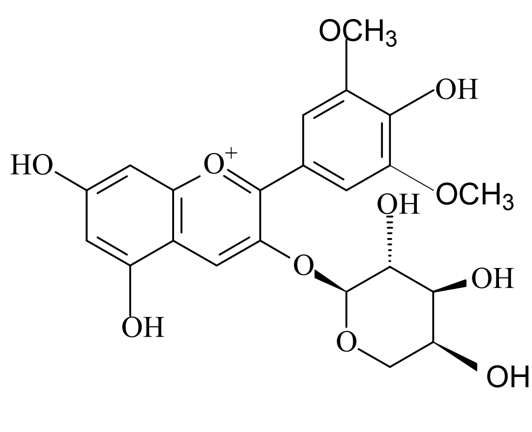
Supporting Information Table S4. Effect of Mv-3-hex and Mv-3-ace-hex on secondary structural contents of β-glucosidase.



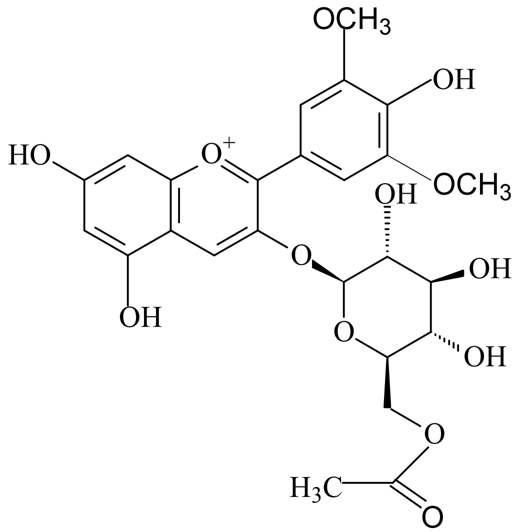
malvidin-3-galactoside



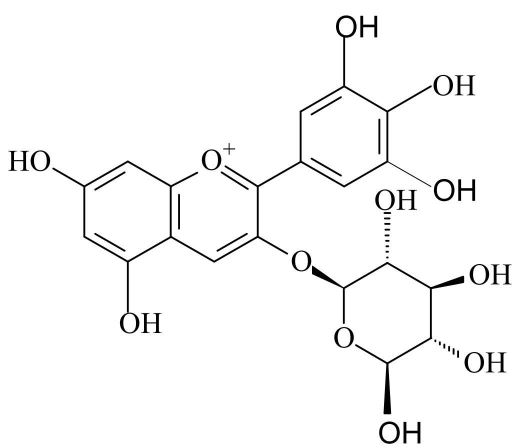
malvidin-3-glucoside



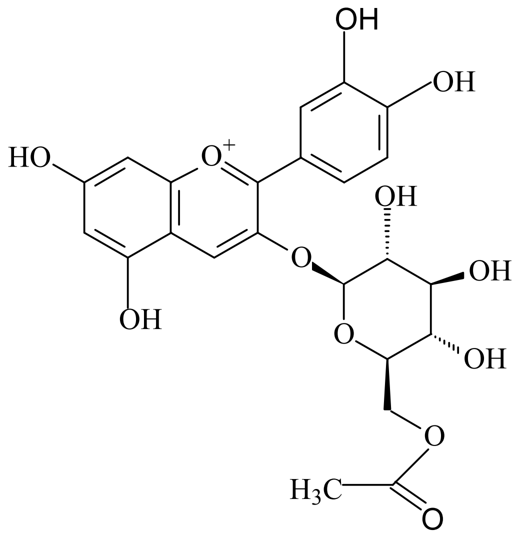
malvidin-3-arabinoside



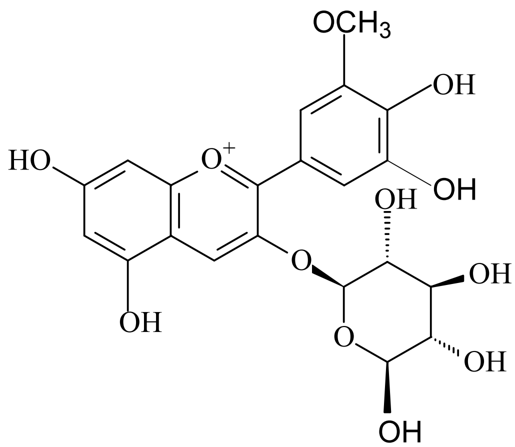
malvidin-3-acetyl-glucoside



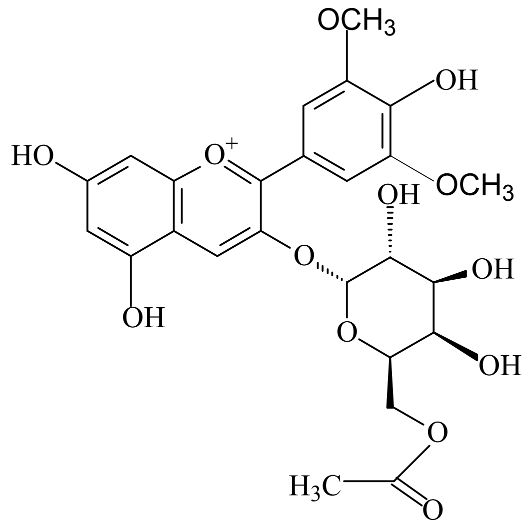
delphinidin-3-glucoside



cyanidin-3-acetyl-glucoside

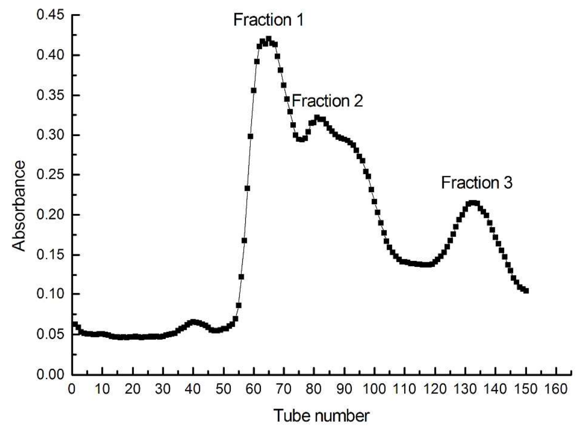


petunidin-3-glucoside

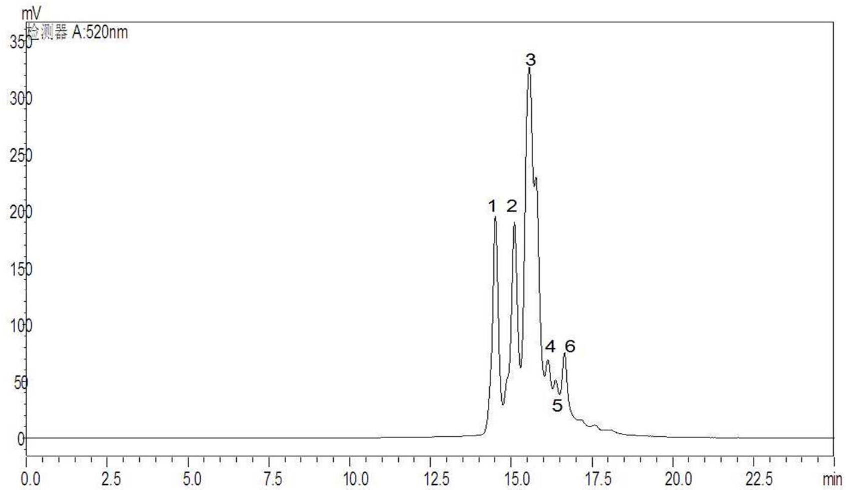


malvidin-3-acetyl-galactoside

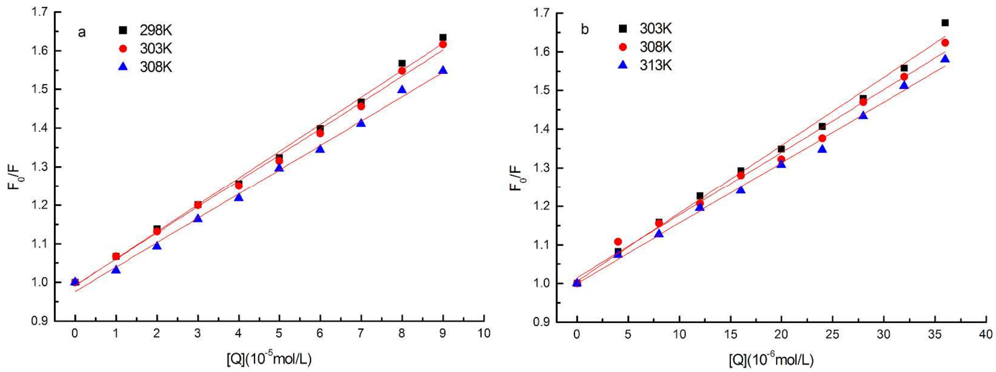
Supporting Information Figure S1. Diagrammatic structures of anthocyanins isolated from blubeberry wine lees



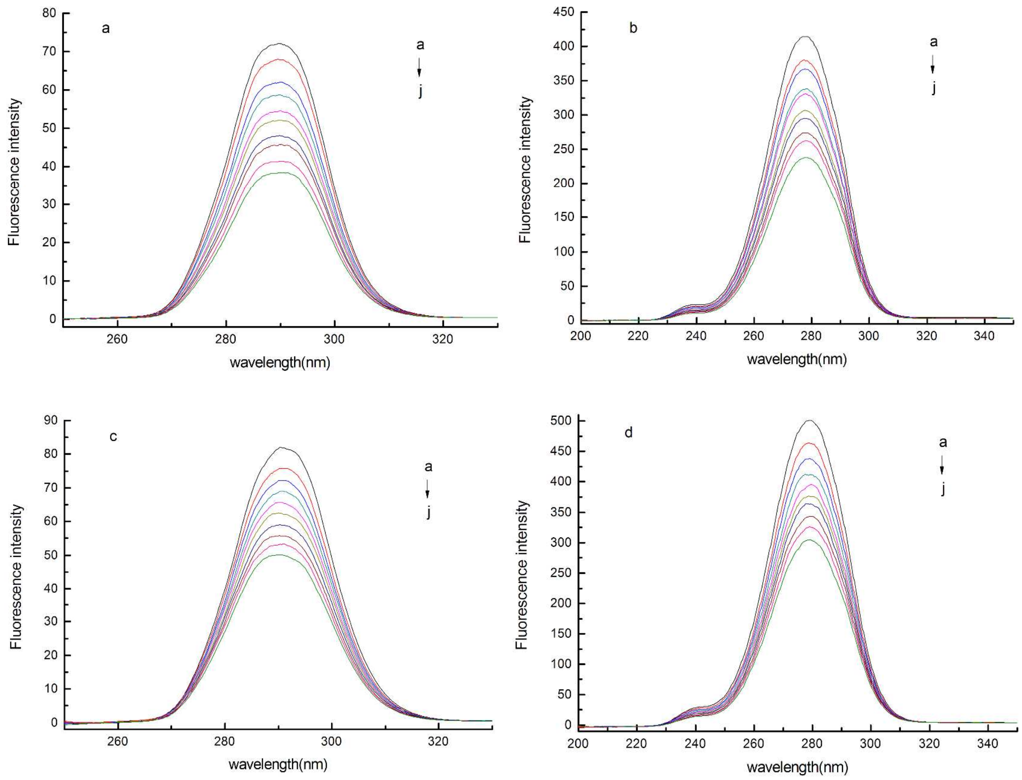
Supporting Information Figure S2. Elution renderings of anthocyanins from blubeberry wine lees using Sephadex LH-20 chromatographic column



Supporting Information Figure S3. Elution renderings of anthocyanins from blubeberry wine lees using semi-preparative HPLC method



Supporting Information Figure S4. The Stern-Volmer plots for the quenching of *β*-glucosidase by Mv-3-hex (a) and Mv-3-ace-hex (b) at different temperatures (pH 7.4, λ_{ex}=280 nm, λ_{em}=340 nm)



Supporting Information Figure S5. The synchronous fluorescence spectra of β-glucosidase in the presence of Mv-3-hex (a: $\Delta\lambda$=15 nm, b: $\Delta\lambda$=60 nm) and Mv-3-ace-hex (c: $\Delta\lambda$=15 nm, d: $\Delta\lambda$=60 nm). C(β-glucosidase)=1 mg/mL, C(Mv-3-hex)=0.0 (a), 1.0 (b), 2.0 (c), 3.0 (d), 4.0 (e), 5.0 (f), 6.0 (g), 7.0 (h), 8.0 (i), 9.0×10^{-5}mol/L (j), C(Mv-3-ace-hex)=0.0 (a), 4.0 (b), 8.0 (c), 12.0 (d), 16.0 (e), 20.0 (f), 24.0 (g), 28.0 (h), 32.0 (i), 36.0×10^{-6}mol/L (j)

Supporting Information Table S1. Total Contents of Phenolics and Types of Anthocyanin in the Blueberry Fruits, Blueberry Wine Lees and Wine Extracts

	total contents of phenolics(mg/g)	contents of anthocyanins(mg/g)	types of anthocyanins
blueberry	508.89±21.87[c]	210.52±5.74[c]	delphinidin-3-galactoside
			cyanidin-3-galactoside
			cyanidin-3-glucoside
			petunidin-3-galactoside
			petunidin-3-glucoside
			malvidin-3-galactoside
			peonidin-3-glucoside
			malvidin-3-glucoside
			malvidin-3-arabinoside
blueberry wine lees	436.67±6.80[b]	151.12±0.88[b]	delphinidin-3-galactoside
			delphinidin-3-glucoside
			petunidin-3-galactoside
			petunidin-3-glucoside
			malvidin-3-galactoside
			malvidin-3-glucoside

			malvidin-3-arabinoside
			cyanidin-3-acetyl-glucoside
			cyanidin-3-acetyl-rhamnoside
			delphinidin-3-acetyl-arabinoside
blueberry wine	292.22 ± 3.93^{a}	8.93 ± 0.14^{a}	delphinidin-3-galactoside
			delphinidin-3-glucoside
			cyanidin-3-galactoside
			petunidin-3-galactoside
			peonidin-3-glucoside
			malvidin-3-galactoside
			malvidin-3-glucoside
			malvidin-3-arabinoside
			petunidin-3-acetyl-rhamnoside

[a]Different letters in each column represents there was a significant difference. (p<0.05)

Supporting Information Table S2. The Quenching Constants of the Interaction between β-Glucosidase and Anthocyanins

compound	T (K)	K_{sv} (10^4L/mol)	K_q (10^{12}L/mol/s)	R^2
Mv-3-hex	298	0.698	0.698	0.9963
	303	0.677	0.677	0.9967
	308	0.631	0.631	0.9961
Mv-3-ace-hex	303	1.76	1.76	0.9936
	308	1.63	1.63	0.9919
	313	1.57	1.57	0.9951

Supporting Information Table S3. Binding Constants and Thermodynamic Parameters of *β*-Glucosidase-Anthocyanins at Different Temperatures

compound	T (K)	K_a (10^3L/mol)	n	R^2	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol/K)
Mv-3-hex	298	7.42	1.0096	0.9978	-14.92	-22.07	24.00
	303	6.75	1.0026	0.9979		-22.19	24.00
	308	87.66	1.2781	0.9928		-29.15	46.19
Mv-3-ace-hex	303	7.66	0.9207	0.9966	-6.83	-22.53	51.81
	308	7.33	0.9220	0.9899		-22.79	51.81
	313	7.93	0.9369	0.9941		-23.36	52.81

Supporting Information Table S4. Effect of Mv-3-hex and Mv-3-ace-hex on Secondary Structural Contents of β-Glucosidase

Content of secondary structure (%)	β-glucosidae mg/mL	Mv-3-hex-β-glucosidase μmol/L		Mv-3-ace-hex-β-glucosidase μmol/L	
	1	10	20	10	20
α-helix	17.67	19.80	17.40	17.40	17.57
β-sheet	56.07	55.67	55.93	61.10	59.10
β-turn	2.47	2.60	2.47	0.23	1.13
random	23.80	21.87	24.23	21.30	22.17