

Supplementary Materials

Evaluation of Polyphenol Anthocyanin-Enriched Extracts of Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry for Free Radical Scavenging, Reactive Carbonyl Species Trapping, Anti-Glycation, Anti- β -Amyloid Aggregation, and Microglial Neuroprotective Effects

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HPLC-DAD analyses of phenolics in the berry ACEs

Each berry ACE was analyzed by HPLC-DAD as previously reported by our laboratory for the presence of phenolics (at 280 nm) and anthocyanins (at 520 nm) (Figures S1-S6). Briefly, each berry ACEs (dissolved in DMSO; all at equivalent concentrations of 10 mg/mL) were analyzed on a Luna C18 column (250 × 4.6 mm i.d., 5 μM; Phenomenex) with a flow rate of 0.75 mL/min and injection volume of 20 μL for each sample. A linear gradient solvent system consisting of solvent A (0.1% aqueous trifluoroacetic acid) and solvent B (methanol) at room temperature was used as follows: 0–30 min, 10% to 60% B; 30–35 min, 60% to 100% B; 35–40 min, 100% B; 40–41 min, 100% to 10% B; 41–51 min, 10% B.

Figure S1

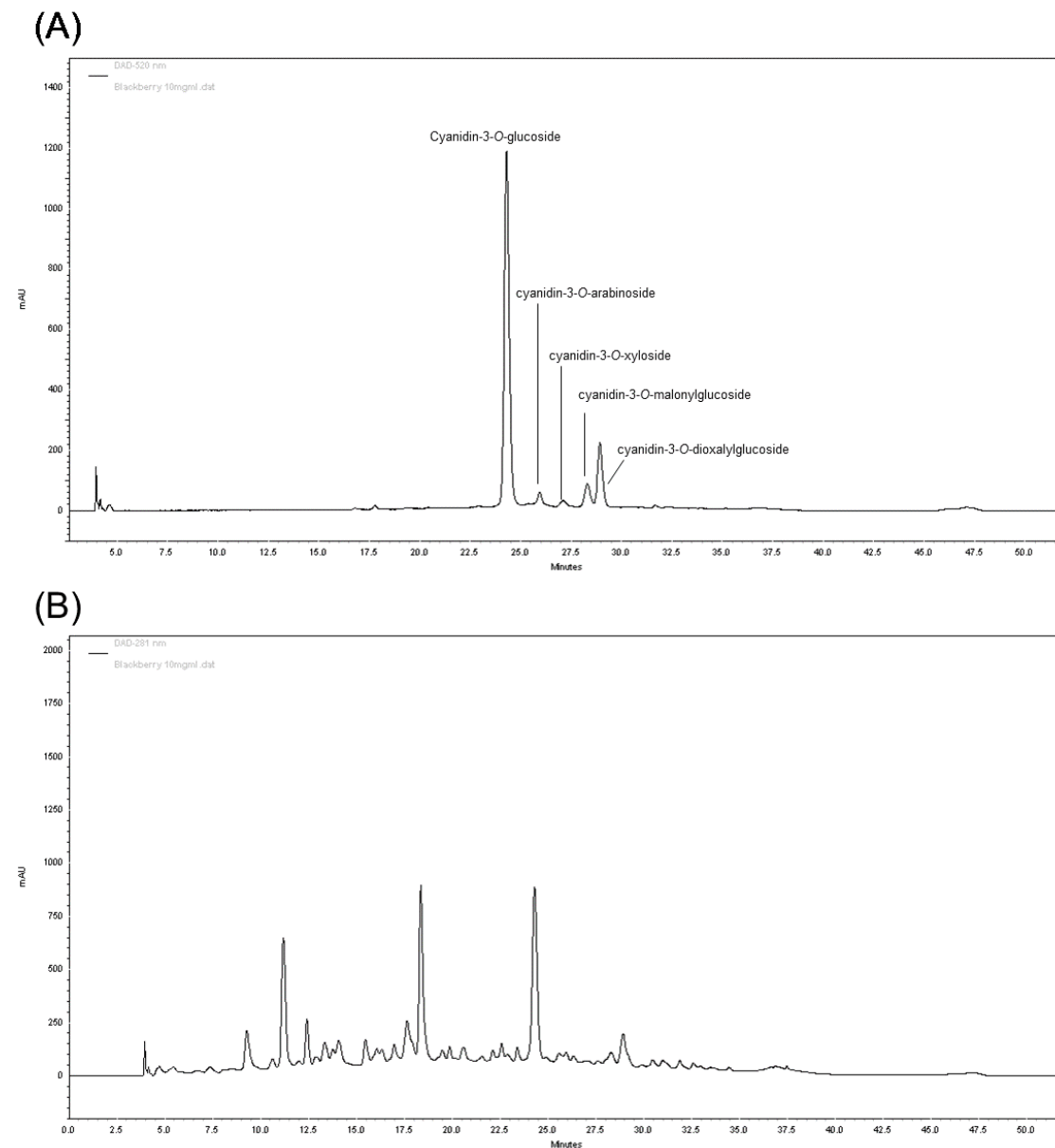


Figure S1. HPLC-DAD chromatogram of blackberry ACE monitored at (A) wavelength of 520 nm showing its major anthocyanins as cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-xyloside, cyanidin-3-*O*-malonylglucoside, and cyanidin-3-*O*-dioxalylglucoside; and (B) wavelength of 280 nm showing the presence of phenolics.

Figure S2

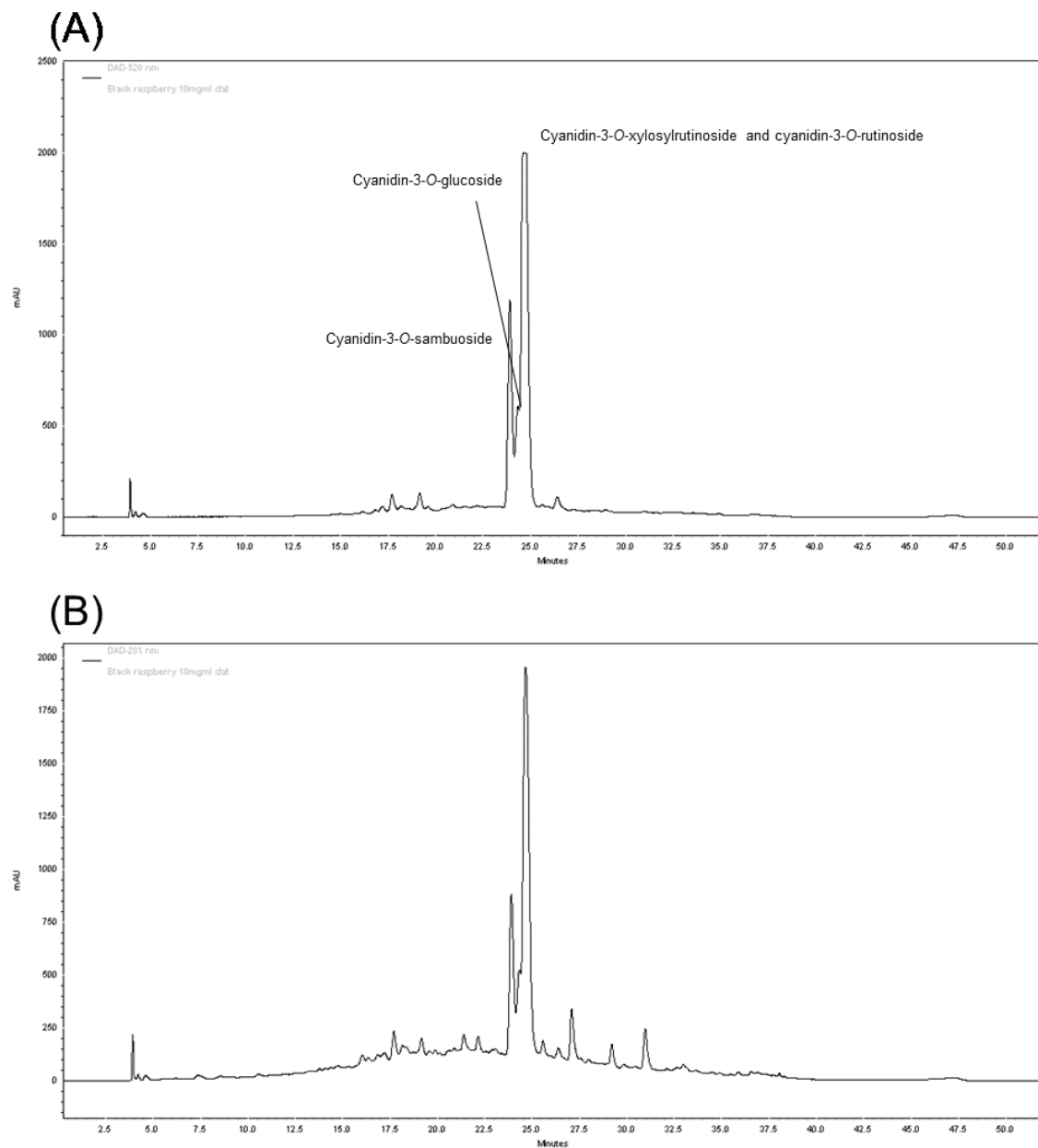


Figure S2. HPLC-DAD chromatogram of black raspberry ACE monitored at (A) wavelength of 520 nm showing its major anthocyanins as cyanidin-3-*O*-sambuoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-xylosylrutinoside, and cyanidin-3-*O*-rutinoside; and (B) wavelength of 280 nm showing the presence of phenolics.

Figure S3

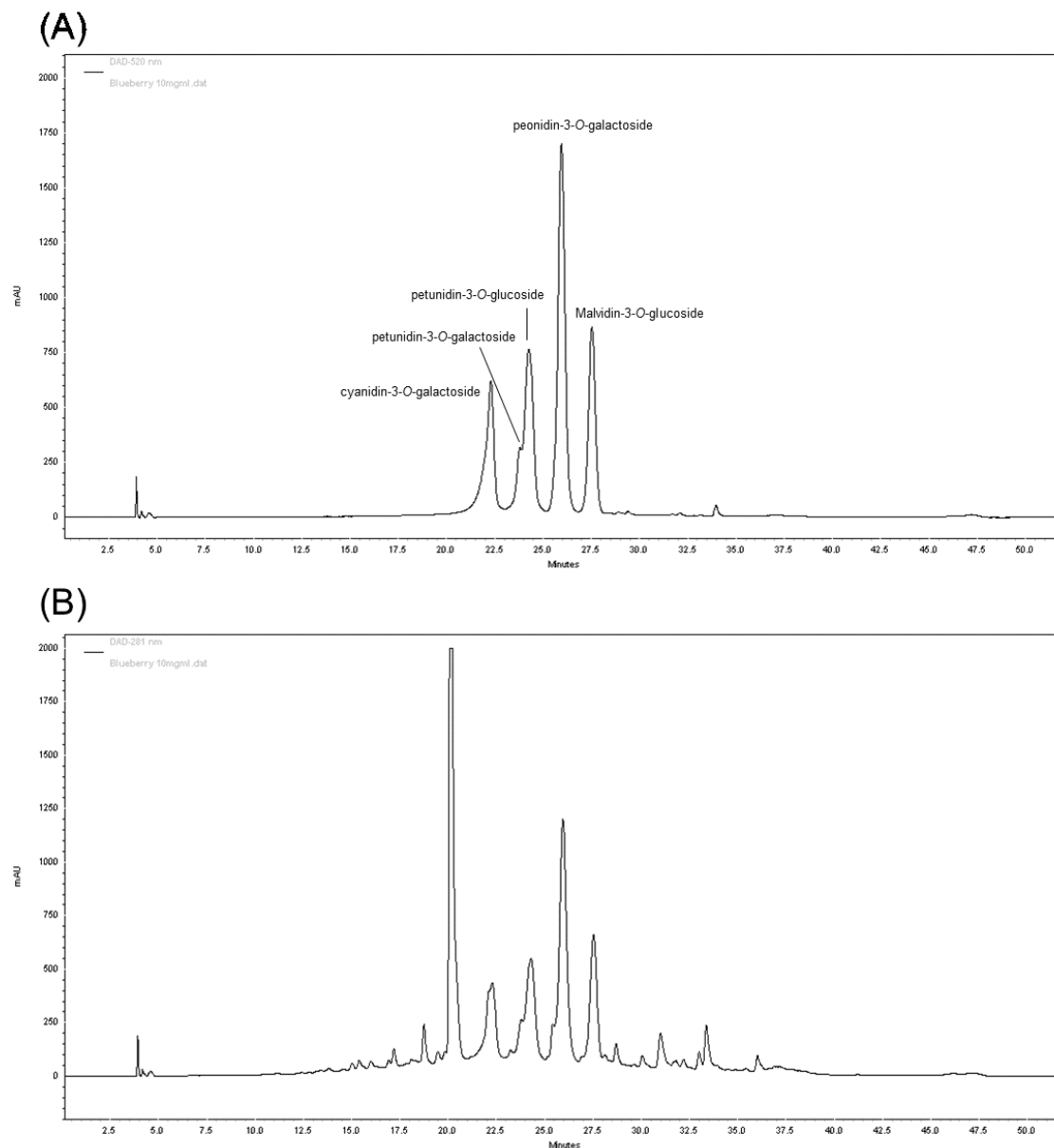


Figure S3. HPLC-DAD chromatogram of blueberry ACE monitored at (A) wavelength of 520 nm showing its major anthocyanins as cyanidin-3-O-galactoside, petunidin-3-O-galactoside, petunidin-3-O-glucoside, peonidin-3-O-galactoside, and malvidin-3-O-glucoside; and (B) wavelength of 280 nm showing the presence of phenolics.

Figure S4

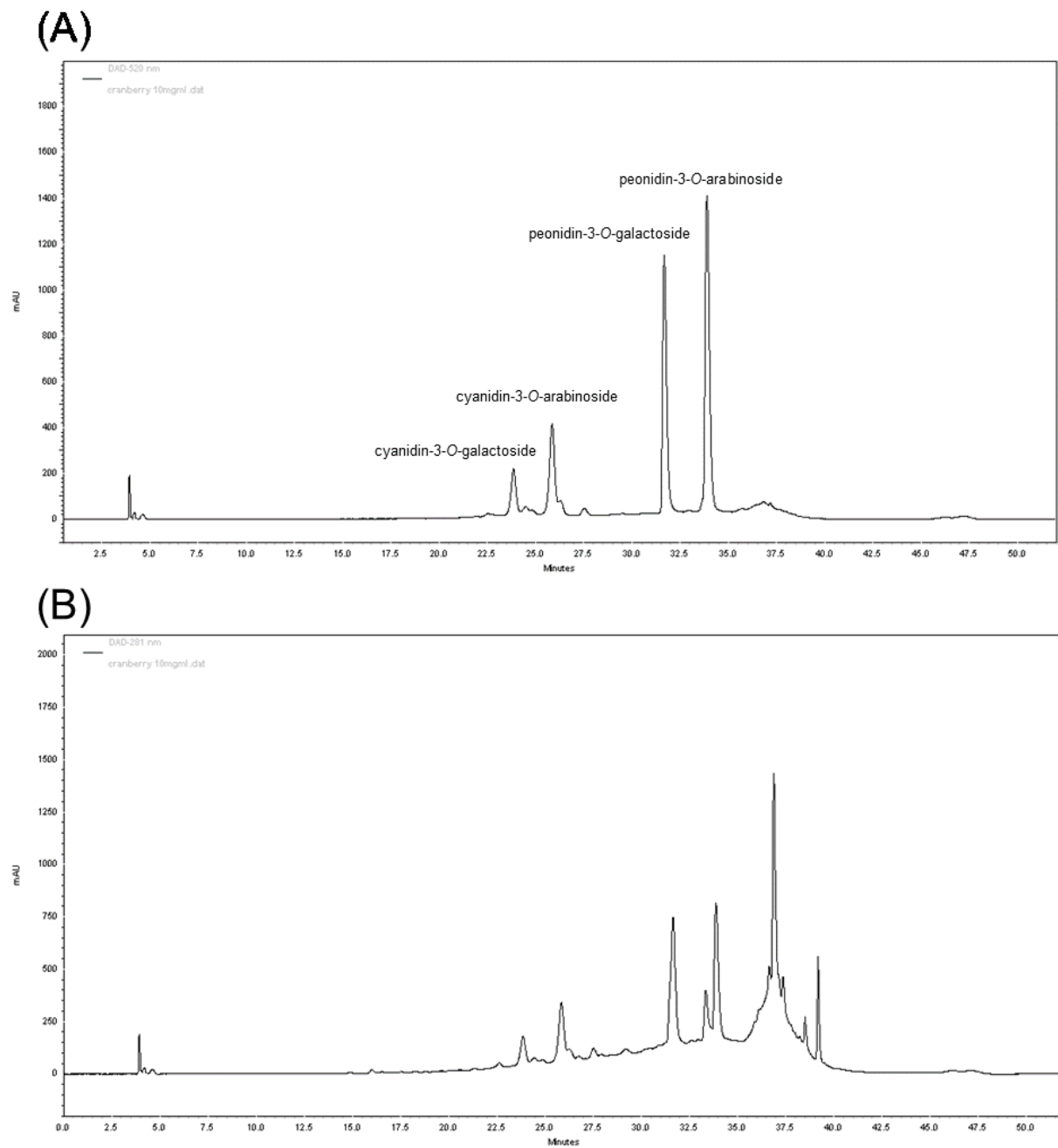


Figure S4. HPLC-DAD chromatogram of cranberry ACE monitored at (A) wavelength of 520 nm showing the identity of its major anthocyanins as cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, peonidin-3-*O*-galactoside, and peonidin-3-*O*-arabinoside; and (B) wavelength of 280 nm showing the presence of phenolics.

Figure S5

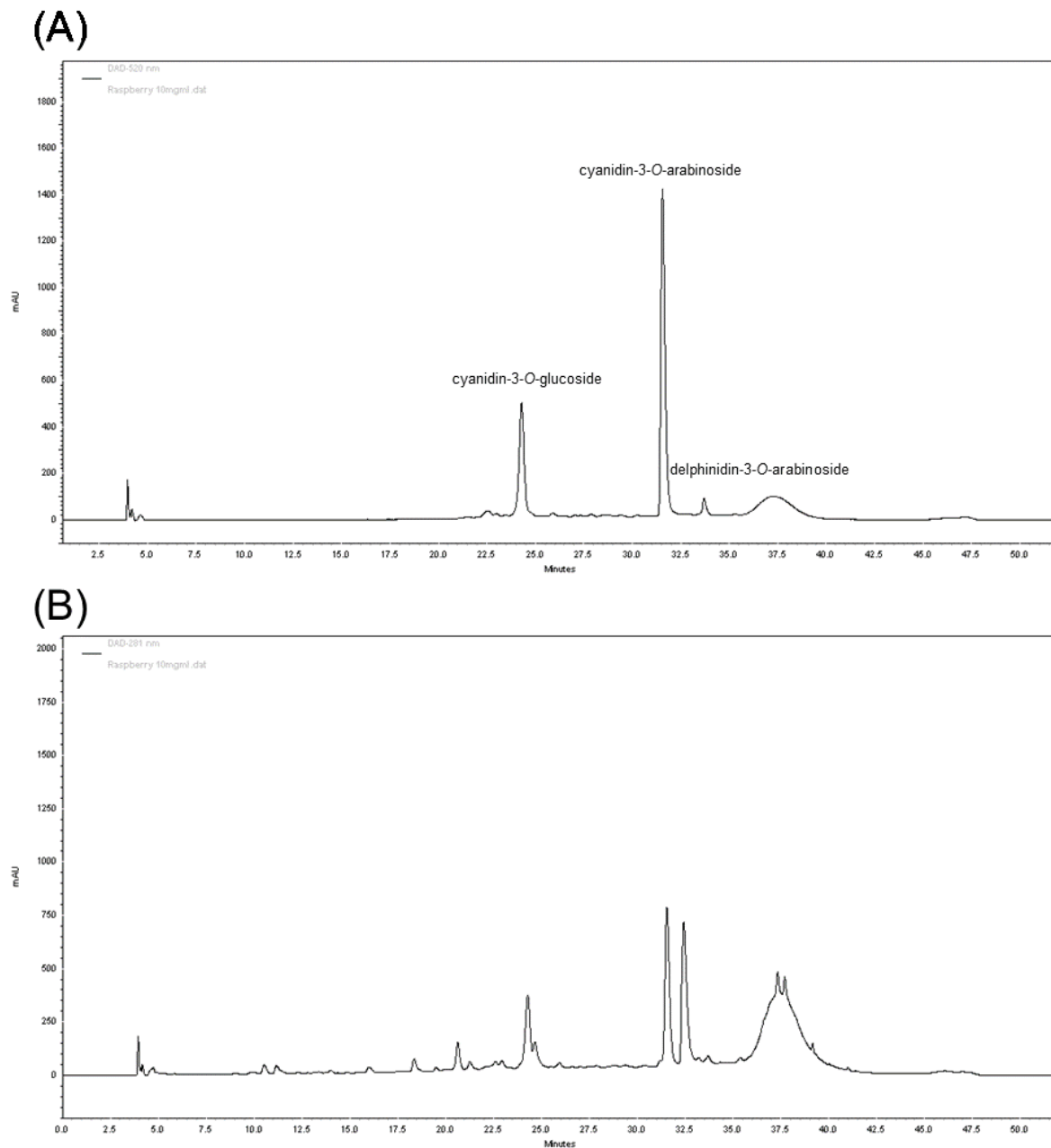


Figure S5. HPLC-DAD chromatogram of red raspberry ACE monitored at (A) wavelength of 520 nm showing its major anthocyanins as cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, delphinidin-3-O-arabinoside; and (B) wavelength of 280 nm showing the presence of phenolics.

Figure S6

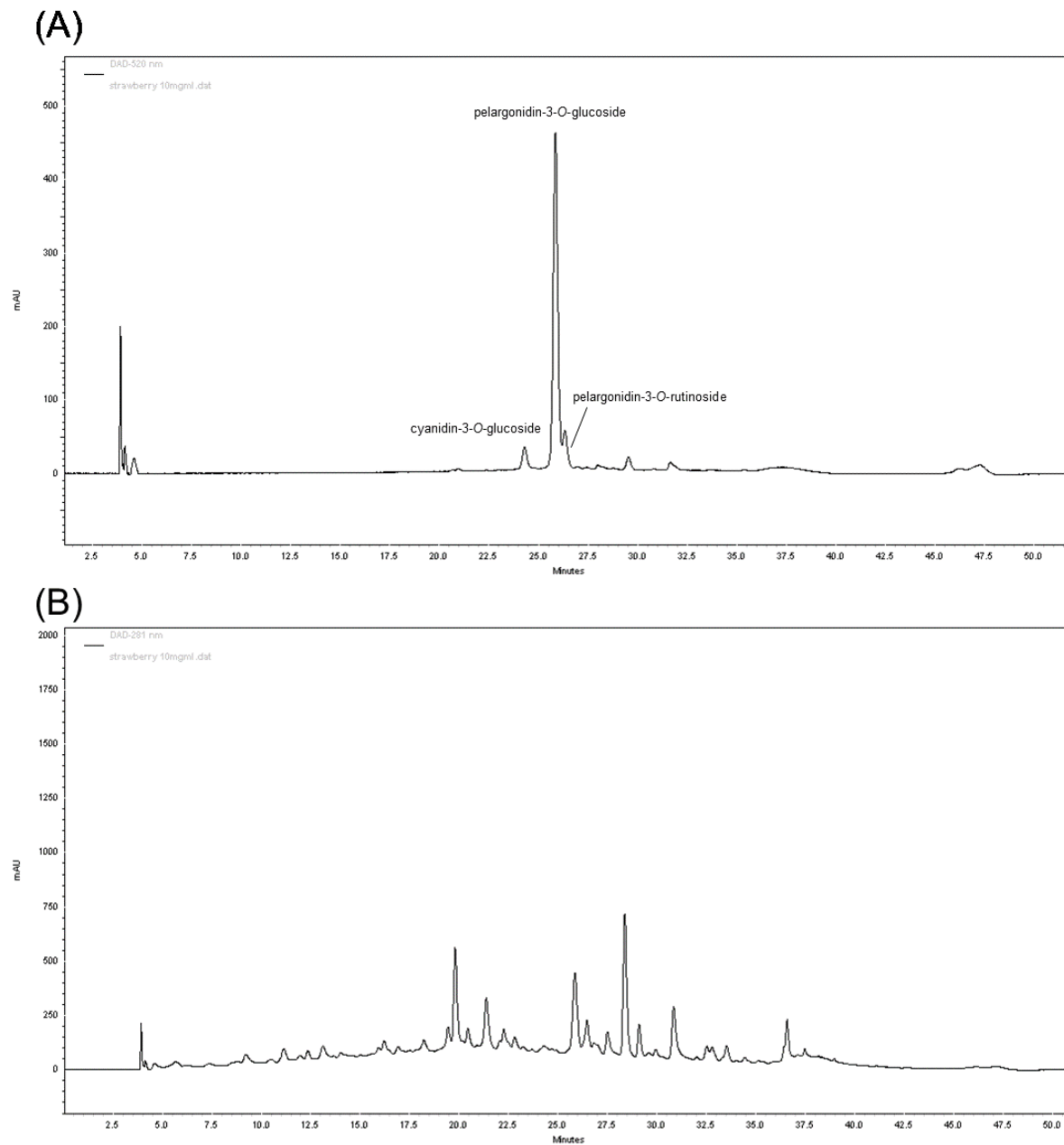


Figure S6. HPLC-DAD chromatogram of red raspberry ACE monitored at (A) wavelength of 520 nm showing its major anthocyanins as cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, and pelargonidin-3-*O*-rutinoside; and (B) wavelength of 280 nm showing the presence of phenolics.

Table S1. Chemical constituents including non-phenolic and phenolic ‘non-anthocyanin’ compounds of blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry.

Berry	Non-phenolic compounds	Phenolic compounds (non-anthocyanins)	Reference
Blackberry	Carbohydrates, organic acids, vitamins, minerals, etc.	Ellagitannins, flavonols, phenolic acids and procyanidins, lignans etc.	[1-4]
Black raspberry	Carbohydrates, organic acids, minerals, folic acid, and β -sitosterol, etc.	Ellagitannins, ellagic acid, flavonols, ferulic acid, etc.	[1, 3-5]
Blueberry	Carbohydrates, organic acids, minerals, vitamins, etc.	Proanthocyanidins, flavonols, catechins, stilbenes, etc.	[1, 3, 4, 6-8]
Cranberry	Carbohydrates, organic acids, vitamins, minerals, etc.	Proanthocyanidins, flavonols, stilbenes, etc.	[1, 3, 4, 9-11]
Red raspberry	Carbohydrates, organic acids, vitamins, minerals, etc.	Ellagitannins, gallotannins, ellagic acid, flavonols, catechins, etc.	[1, 3, 4, 12]
Strawberry	Carbohydrates, organic acids, vitamins, minerals, etc.	Ellagitannins, ellagic acid, flavonols, phenolic acids, etc.	[1, 3, 4]

References:

1. Seeram, N. P.; Adams, L. S.; Zhang, Y.; Lee, R.; Sand, D.; Scheuller, H. S.; Heber, D., Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. *J. Agric. Food Chem.* **2006**, *54*, 9329-9339.
2. Kaume, L.; Howard, L. R.; Devareddy, L., The blackberry fruit: a review on its composition and chemistry, metabolism and bioavailability, and health benefits. *J. Agric. Food Chem.* **2012**, *60*, 5716-5727.

3. Mikulic-Petkovsek, M.; Schmitzer, V.; Slatnar, A.; Stampar, F.; Veberic, R., Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *J. Food Sci.* **2012**, *77*, C1064-1070.
4. Esteban, M. A.; Villanueva, M. J.; Lissarrague, J., Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. *Am. J. Enol. Vitic* **1999**, *50*, 418-434.
5. Tulio, A. Z., Jr.; Reese, R. N.; Wyzgoski, F. J.; Rinaldi, P. L.; Fu, R.; Scheerens, J. C.; Miller, A. R., Cyanidin 3-rutinoside and cyanidin 3-xylosylrutinoside as primary phenolic antioxidants in black raspberry. *J. Agric. Food Chem.* **2008**, *56*, 1880-1888.
6. Sun, X.; Liu, N.; Wu, Z.; Feng, Y.; Meng, X., Anti-tumor activity of a polysaccharide from blueberry. *Molecules* **2015**, *20*, 3841-3853.
7. Morikawa, C.; Saigusa, M., Mineral composition and accumulation of silicon in tissues of blueberry (*Vaccinium corymbosus* cv. Bluecrop) cuttings. *Plant Soil* **2004**, *258*, 1-8.
8. Chen, C.; Li, Y.; Xu, Z., Chemical principles and bioactivities of blueberry. *Acta Pharm. Sinica* **2010**, *45*, 422-429.
9. Sun, J.; Liu, W.; Ma, H.; Marais, J. P.; Khoo, C.; Dain, J. A.; Rowley, D. C.; Seeram, N. P., Effect of cranberry (*Vaccinium macrocarpon*) oligosaccharides on the formation of advanced glycation end-products. *J. Berry Res.* **2016**, *6*, 149-158.
10. Seeram, N. P.; Adams, L. S.; Hardy, M. L.; Heber, D., Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *J. Agric. Food Chem.* **2004**, *52*, 2512-2517.
11. Lacombe, A.; Wu, V. C.; Tyler, S.; Edwards, K., Antimicrobial action of the American cranberry constituents; phenolics, anthocyanins, and organic acids, against *Escherichia coli* O157: H7. *Int. J. Food Microbiol.* **2010**, *139*, 102-107.
12. Mullen, W.; McGinn, J.; Lean, M. E.; MacLean, M. R.; Gardner, P.; Duthie, G. G.; Yokota, T.; Crozier, A., Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *J. Agric. Food Chem.* **2002**, *50*, 5191-5196.