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Stability of Blueberry Anthocyanin, Anthocyanidin and Pyranoanthocyanidin Pigments and Their Inhibitory Effects and Mechanisms in Human Cervical Cancer HeLa Cells

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Method S1. Experimental details

HPLC analysis

HPLC analysis of anthocyanin, anthocyanidin and pyranoanthocyanidin pigments was performed on a Shimadzu LC-30AD HPLC system (Shimadzu Co., Kyoto, Japan) coupled with a PDA detector (SPD-M20A, Shimadzu). Chromatographic separation was carried out on a Waters Symmetry Shield C18 column (150 mm ×4.6 mm, 5 µm). Detection was recorded from 240 to 700 nm. The mobile phase consisted of 100% methanol as solvent A and 3% (v/v) formic acid in water as solvent B. The flow rate was set at 0.5 mL/min. The compound separation was performed using linear gradient elution: 0–5 min, 85% B; 5–10 min, 85–80% B; 10–25 min, 80–75% B; 25–30 min, 75% B; 30–40 min, 75–30% B; 40–45 min, 30% B; 45–55 min, 30–85% B; 55–60 min, 85% B. The samples were dissolved in 3% formic acid, and the injection volume was 10 µL. Prior to injection, all samples were filtered through 0.22 µm polytetrafluoroethylene (PTFE) syringe filters. The profiles of anthocyanins, anthocyanidins and pyranoanthocyanidins pigments were first identified according to the retention time and UV-Vis spectra. Second, the composition and quantitative analysis of anthocyanin and anthocyanidin extracts were carried out at 530 nm using a calibration curve obtained by analyzing different concentrations of cyanidin-3-O-glucoside and cyanidin standards, respectively. Since there are no commercial standards of pyranoanthocyanidins available at present, the pyranoanthocyanidin standard (pyranocyanidin) can be prepared through the reaction of the standard of cyanidin chloride and acetone according to the preparation and purification method of the pyranoanthocyanidin sample described above. Thus, the composition and quantitative analysis of pyranoanthocyanidins were conducted at 480 nm using a calibration curve by analyzing different concentrations of pyranoanthocyanidin standard. All the analyses were performed in triplicate.

HPLC-MS analysis

The HPLC-PDA-ESI-MS/MS analysis of anthocyanin, anthocyanidin and pyranoanthocyanidin pigments was performed using a Waters Alliance 2695 Separation Module (Waters, Milford, MA, USA) equipped with a Triple Quadrupole Mass spectrometer system (Waters). A Waters Symmetry Shield C18 column (150 × 4.6 mm, 5 µm) was used in the HPLC analysis and operated at 25 °C. The flow rate was 0.5 mL/min, with mobile phases A and B composed of 100% methanol and 0.3% (v/v) formic in water, respectively. The elution gradient conditions were the same as those described above. The MS conditions were as follows: positive ion mode; gas (N2) temperature, 450 °C; gas (N2) flow rate, 18 L/min; nebulizer pressure, 206.9 kPa; spray voltage, 4.5 kV; and capillary voltage, 200 V. Mass spectra were recorded from m/z 100 to 1,000. At the beginning of each set of measurements, the ESI-MS system was tuned using the standards of three anthocyan pigments. Data acquisition was performed using the Mass Lynx software 4.1 (Waters).

Table S1. Spectroscopic and chromatographic data of anthocyanidin and pyranoanthocyanidin pigments.

Peak no.	t_R (min)	λvis-max（nm）	$[M]^+$ (m/z)	Tentative identification	Chemical formula
1	39.6	534	303.0	Delphinidin	$C_{15}H_{11}O_7$
2	41.9	529	287.2	Cyanidin	$C_{15}H_{11}O_6$
3	42.7	538	317.0	Petunidin	$C_{16}H_{13}O_7$
4	43.9	530	300.5	Peonidin	$C_{16}H_{13}O_6$
5	44.2	539	331.2	Malvidin	$C_{17}H_{15}O_7$
a	43.9	486	341.2	Methyl pyranodelphinidin	$C_{18}H_{13}O_7$
b	44.8	477	325.2	Methyl pyranocyanidin	$C_{18}H_{13}O_6$
c	45.2	486	355.3	Methyl pyranopetunidin	$C_{19}H_{15}O_7$
d	45.9	486	340.2	Methyl pyranopeonidin	$C_{19}H_{15}O_6$
e	46.1	477	369.3	Methyl pyranomalvidin	$C_{20}H_{17}O_7$

Notes, the peak no. responds to the numbers in **Fig. 1.**

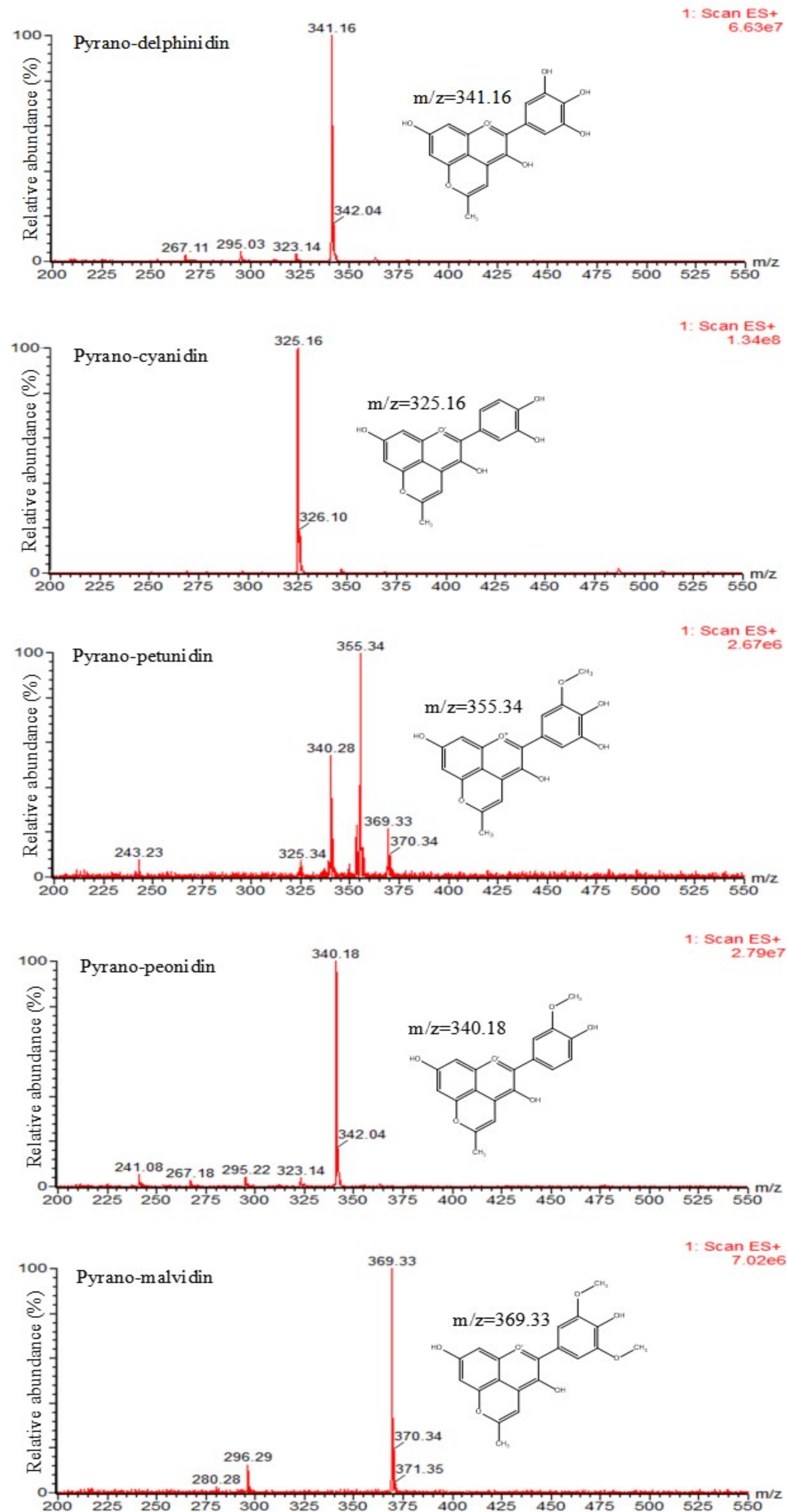

Fig. S1. HPLC-PDA-ESI-MS/MS analysis on the chemical structures of pyranoanthocyanidin pigments.

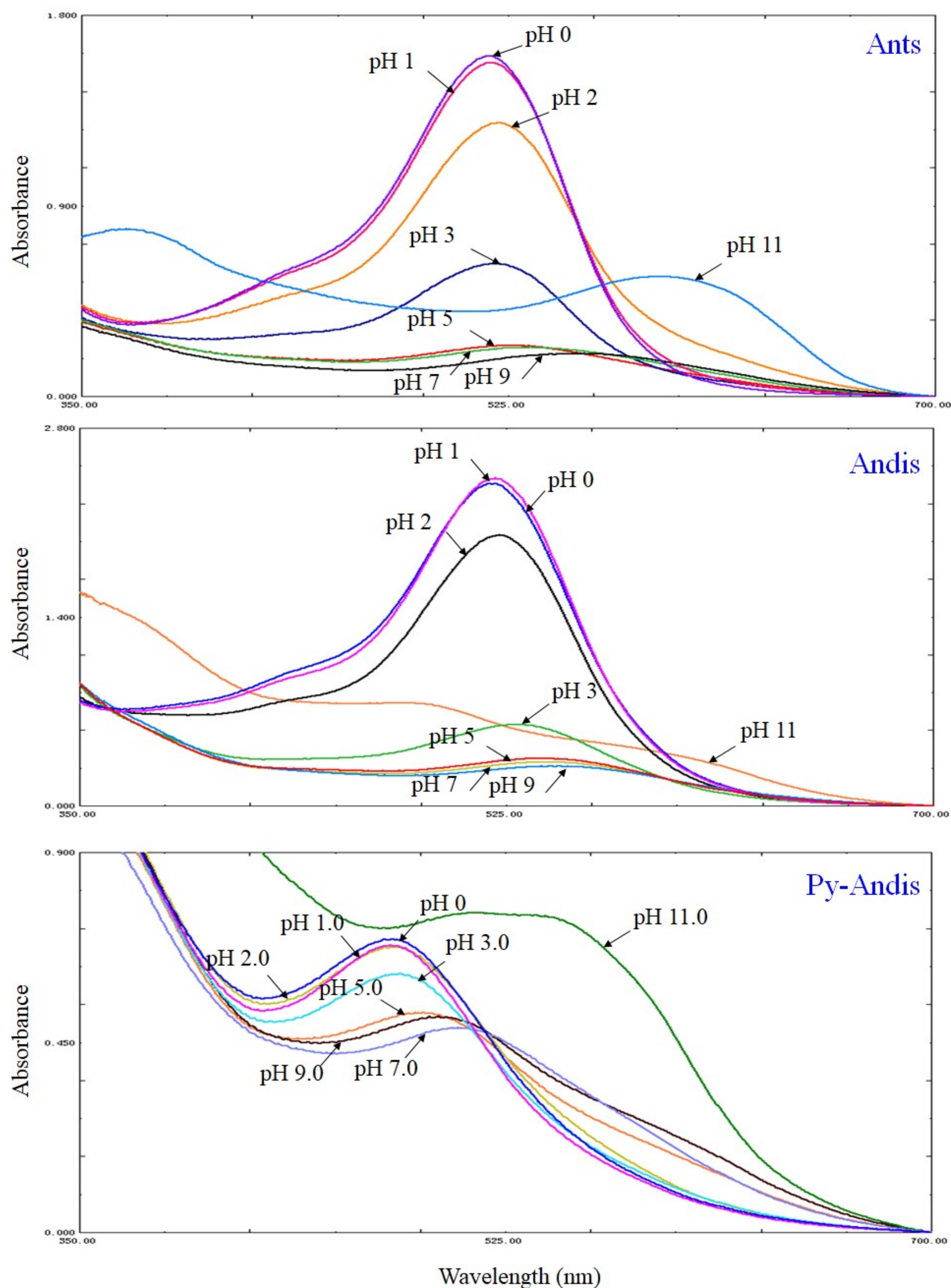

Fig. S2. UV–Visible Absorption spectra of three anthocyan pigments in aqueous solution at different pH values (0.0–11.0). The UV-visible spectra absorbance maxima of each sample were measured after 2 h of storage in the dark at room temperature (25 °C). Abbreviations: Ants, anthocyanins; Andis, anthocyanidins; Py-Andis, pyranoanthocyanidins.

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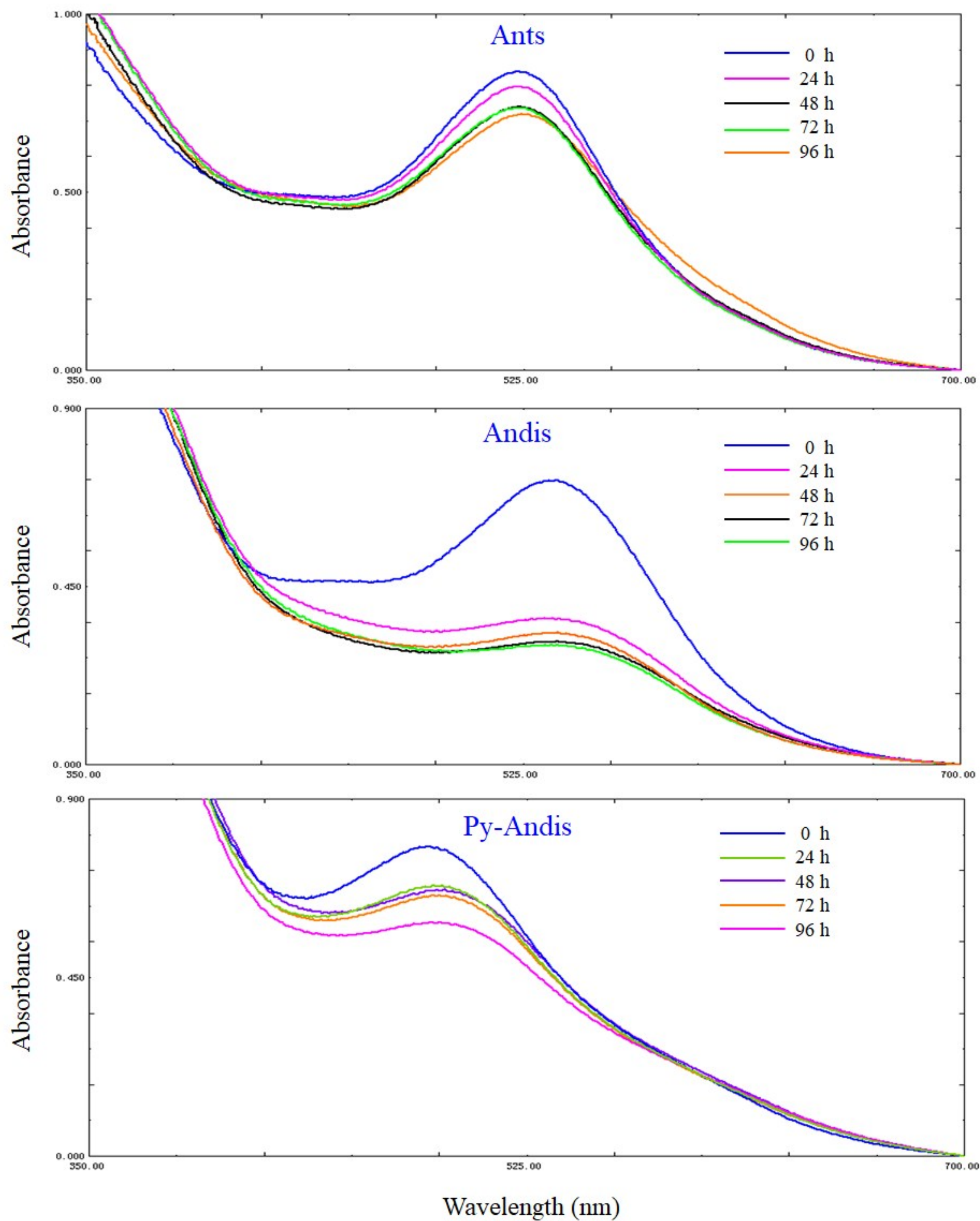

Fig. S3. UV–Visible Absorption spectra of three anthocyan pigments in aqueous solution at 25 °C with the changing time. Abbreviations: Ants, anthocyanins; Andis, anthocyanidins; Py-Andis, pyranoanthocyanidins.

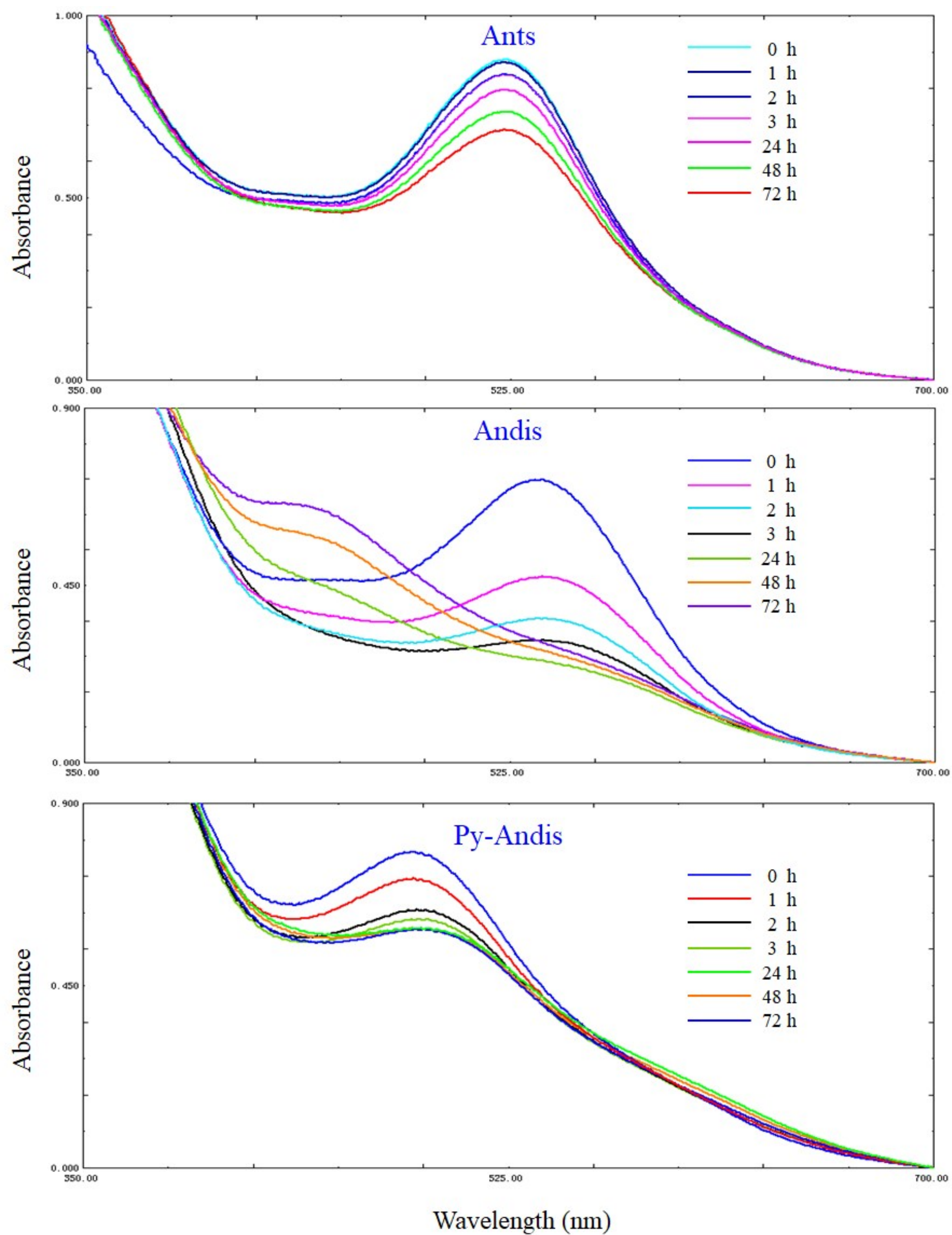

Fig. S4. UV–Visible Absorption spectra of three anthocyan pigments in aqueous solution at 40 °C with the changing time. Abbreviations: Ants, anthocyanins; Andis, anthocyanidins; Py-Andis, pyranoanthocyanidins.

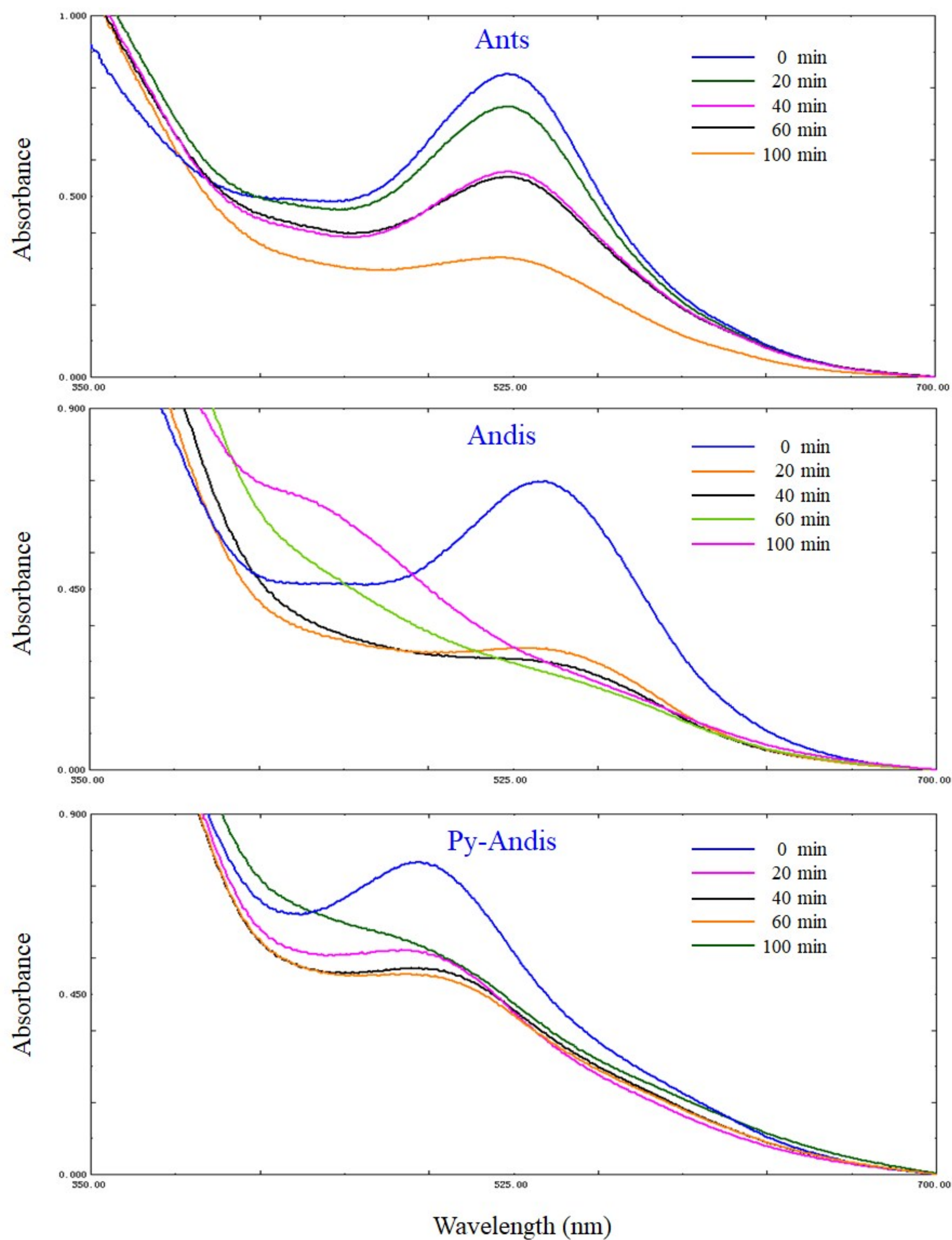

Fig. S5. UV–Visible Absorption spectra of three anthocyan pigments in aqueous solution at 80 °C with the changing time. Abbreviations: Ants, anthocyanins; Andis, anthocyanidins; Py-Andis, pyranoanthocyanidins.