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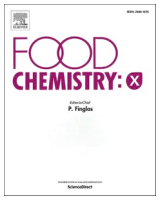

Metabolomic analysis, extraction, purification and stability of the anthocyanins from colored potatoes

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ABSTRACT

Colored potatoes have many health benefits because they are rich in anthocyanins. However, the constituent and property of anthocyanins in colored potatoes have not been systematically studied yet. Herein, metabolomic analysis was carried out to investigate the chemical composition of anthocyanins in the four different colored potatoes. After that, the extract and purification conditions, and the stability of the anthocyanins were further studied. The results indicated that the four colored potatoes contained abundant of polyphenols, flavonoids, and anthocyanins. Cyanidin, delphinidin, and malvidin were identified as the major anthocyanidins in purple potatoes, whereas red potatoes were mainly consisted of pelargonidin and its derivatives. 84.47 mg C3GE/100 g DW of anthocyanins was obtained at the optimal conditions, which could be effectively purified macroporous resin of D101. Moreover, the anthocyanins were sensitive to pH, temperature, light, redox agents, and divalent or trivalent metal ions, but stable to sugars and univalent metal ions.

1. Introduction

Potato (*Solanum tuberosum* L.) is widely consumed as one of the most important staple foods worldwide. Until now, >4000 cultivars of potatoes have been reported, colored potatoes are one of the most preferred cultivars among people due to the rich in anthocyanins. Previous study demonstrated that the content of total phenolic compounds, particularly anthocyanins, in red and purple-fleshed potatoes presented about three times higher than that of white and yellow-fleshed potatoes (Sampaio et al., 2021). Further analysis showed that the total anthocyanins content among the seven cultivars of colored potatoes ranged from 478.3 mg/100 g to 886.2 mg/100 g, and the main anthocyanins in red and purple varieties were acylated pelargonidin glycosides and acylated petunidin glycosides, respectively (Sampaio et al., 2021). Earlier paper identified eleven different compounds in colored potatoes and the principal anthocyanin was petunidin 3-*O*-coumaroylrutinoside-5-*O*-glucoside (Alcalde-Eon, Saavedra, De Pascual-Teresa, & Rivas-Gonzalo, 2004). Moreover, acylated glucosides of pelargonidin were broadly existed in red and purple-fleshed potatoes, while acylated glucosides of malvidin, petunidin, peonidin, and delphinidin were additionally presented in purple ones (Lachman et al., 2012). However, to the best of our knowledge, the composition of anthocyanins in colored potatoes are still not fully investigated, especially by the metabolomic characterization.

Colored potatoes have gathered much more attention of the scientists mainly due to their rich in anthocyanins. Various emerging technologies, such as enzymatic-assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, deep eutectic solvent extraction, were developed to extract anthocyanins from food sources (Tena & Asuero, 2022). Several parameters, including extraction solvent, temperature, and time, have been reported to obviously affect the yield of anthocyanins (Silva, Costa, Calhau, Morais, & Pintado, 2017). For the extraction solvent, acidified ethanol or methanol was considered as the most efficient extractant to obtain anthocyanins from foods, which could also enhance their stability (Ongkowijoyo, Luna-Vital, & Gonzalez De Mejia, 2018). However, these solvents for anthocyanins extraction are not selective. Besides anthocyanins, the extracts also contain large amounts of other compounds, including sugars, proteins, organic acids, etc. (Silva et al., 2017). Purification of anthocyanins to remove the interferents is the necessary step to obtain high quality product. Macroporous resins are the widely used materials for anthocyanins purification due to their efficient, cheap, easy, and reproducible properties (Tan et al., 2022). Previous study found that the purity of the anthocyanins could be increased 8.5 times higher than that of the crude extract by macroporous resins (Chen et al., 2016). Similar

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results were observed in the purification of anthocyanins from Roselle (Yang et al., 2022) and purple cabbage (Paul, Dutta, Kundu, & Saha, 2023). Besides purification, another property of anthocyanins needs attention is stability, which is influenced by many factors, including pH, temperature, light, redox agents, metal ions, and sugars, etc. (Enaru, Drețcanu, Pop, Stănilă, & Diaconeasa, 2021).

As mentioned above, anthocyanins are the valuable components in the colored potatoes, but the composition, extract condition, purification, and stability of anthocyanins in colored potatoes are still not fully investigated. Herein, metabolomic analysis was employed to comparatively study the chemical composition of anthocyanins in the four different cultivars of colored potatoes. After that, the extraction condition of anthocyanins was optimized by response surface method and the purification process was also investigated. Finally, the stability of anthocyanins influenced by pH, temperature, light, redox agents, metal ions, and sugars was examined. This study will provide much more details for the further utilization of the anthocyanins in colored potatoes.

2. Materials and methods

2.1. Materials and reagents

Four different cultivars of colored potatoes, including W2, W8, R69, and R16–5 are cultivated by Professor Qin Chen, Northwest A & F University. Gallic acid is purchased from Sinopharm Group (Shanghai, China). Rutin, Trolox, α-amylase (50 U/mg), cellulase (400 U/mg), pectinase (50,000 U/g) are purchased from Yuanye Biotechnology (Shanghai, China). Three different types of macroporous resins, D101, AB-8, and HPD100 are obtained from Xi'an Lanshen Special resin Co. (Shannxi, China), Anhui Samsung resin Co. (Anhui, China), and Xi'an Hanyu Resin Technology Co. (Shannxi, China), respectively.

2.2. Sample preparation and extraction

The fresh colored potatoes were washed, peeled and cut into slices before freeze-drying. After that, the potatoes were ground and passed the sieve (80 mesh) for the further use (Fig. S1). The extraction processing was performed by the description of Tan et al. (2022) with some modifications. Briefly, 70% (*v*/v) acid-ethanol (hydrochloric acid, pH 2.5) and 70% (v/v) acid-methanol (hydrochloric acid, pH 2.5) were employed to prepare the extracts from colored potatoes and the extraction conditions were as follows: solid-liquid ratio, 1:20 (*w*/*v*); ultrasonic power, 350 W; ultrasonic time, 30 min. The extracts were filtered by the filter paper, and the residues were extracted two more times. Finally, the filtrate was merged and concentrated by the rotary evaporator (RE 52-86 A, Shanghai Yarong Biochemical Equipment Co.), and then the concentrated extracts were freeze dried and stored at −20 °C for use.

2.3. Measurement of the basic nutritional compositions

The basic nutritional compositions, including moisture content, protein, fat and ash were measured by the national standards of China (GB 5009.3–2016, GB 5009.5–2016, GB 5009.6–2016, and GB 5009.4–2016, respectively). The content of starch was determined by the Boxbio Starch Kit according to the instruction (Boxibio, Beijing, China).

2.4. Determination of total phenols, flavonoids, and anthocyanins

The content of total phenols and flavonoids was measured by our previous descriptions (He et al., 2023). The content of total anthocyanins was determined via pH-differential method (Fu et al., 2021). Briefly, the extracts were resolved in the phosphate buffer solution with two pH values (pH = 1.0 and pH = 4.5, respectively). After standing in dark for 30 min, the maximum absorbance (λ_{max}) and the absorbance at 700 nm ($\lambda_{700\ nm}$) were recorded respectively. Cyanidin-3-*O*-glucoside (C3G) was used as standard and the results were expressed as mg C3G equivalent per 100 g of dry weight of extract (mg C3GE/100 g DW). The content of total anthocyanins was calculated as the following equations:

$$A = (A_{\lambda max} - A_{700\ nm})_{pH\ 1.0} - (A_{\lambda max} - A_{700\ nm})_{pH\ 4.5} \quad (1)$$

$$\text{Anthocyanins content (mg C3GE/100 g DW)} = \frac{A \times V \times DF \times M}{\varepsilon \times m \times L} \times 100 \quad (2)$$

where $A_{\lambda max}$ is the absorbance at the maximum absorption wavelength; $A_{700\ nm}$ is the absorbance at 700 nm; *V* is the volume of the solution; *DF* is the dilution ratio; *M* is the relative molecular weight of cyanidin-3-*O*-glucoside (449.2 g/mol); ε is the extinction coefficient of cyanidin-3-*O*-glucoside (29,600 L/mol/cm); *m* is the weight of the samples; L is the optical length (1 cm).

2.5. Metabolomic analysis

The metabolomic analysis of extracts from colored potatoes was performed by ultra-performance liquid chromatography (UPLC) (ExionLC™ AD) coupled to an electrospray ionization tandem mass spectrometer (MS/MS) (QTRAP®6500+). The extracts were redissolved in 50% (*v*/v) methanol (containing 0.1% hydrochloric acid) and filtrated by 0.22 μM of filter membrane for UPLC-MS/MS analysis. The conditions of the UPLC: ACQUITYBEH C18 chromatographic column (2.1 mm × 100 mm, 1.7 μm); the mobile phases were acidified ultra-pure water (0.1% formic acid) (A phase) and acidified methanol (0.1% formic acid) (B phase); the elution progress: 0–6 min, 5% B; 6–12 min, 50% B; 12–14 min, 95% B; 14–16 min 5% B; the flow rate is 0.35 mL/min; column temperature, 40 °C; injection volume, 2 μL. The conditions of the MS: the temperature of electrospray ionization, 550 °C; MS voltage, 5500 V; cationic mode. Additionally, the qualitative and quantitative analysis was performed through the Metware Database (established by the standard samples) and multiple reaction monitoring mode (MRM) of triple quadrupole mass spectrometry, respectively.

2.6. Optimization of extraction process for anthocyanins

2.6.1. Single-factor experiments

The cultivar of W8 was chose to perform the optimization of extraction process due to its highest content of anthocyanins. Different enzymes, including α-amylase, cellulase, and pectinase were selected for the enzyme-based extraction of anthocyanins (Swer, Chauhan, Paul, & Mukhim, 2016). Briefly, 20% (*w*/w) of the three enzymes were added into the powder of the colored potatoes, and 70% (*v*/v) ethanol was used to extract according to the procedures of 2.2. After that, different doses of α-amylase (10%, 15%, 20%, 25%, and 30%) were investigated for the extraction efficiency of anthocyanins. Extract temperature (30 °C, 40 °C, 50 °C, 60 °C, 70 °C), extract time (10 min, 30 min, 50 min, 70 min, and 90 min) and ethanol concentration (50%, 60%, 70%, 80%, 90%, v/v) were selected as the single factors to determine their effects on the total anthocynins of colored potatoes.

2.6.2. Response surface optimization

Box-Behnken design (BBD) was used to perform the response surface optimization. Briefly, extract temperature (A), extract time (B) and ethanol concentration (C) were used as experimental factors, and the yield of anthocyanins (Y) was determined as the response value to conduct a response surface optimization experiments with three factors and three levels (Table S1).

2.7. Purification of anthocyanins

The anthocyanins of colored potatoes (W8) was extracted by the optimal conditions and concentrated at 50 °C by rotary evaporator. The total anthocyanins of the concentrate was determined (c_0). Three different macroporous resins, D101, HPD100, and AB-8, were soaked with 95% (*v*/v) ethanol for 24 h, and washed by distilled water. After that, the macroporous resins were soaked with 5% (v/v) HCl and 5% (v/v) NaOH orderly for 5 h, respectively, following washed with distilled water to neutral.

For macroporous resins screening test, the drained resins (0.7 g) were mixed with 40 mL anthocyanins concentrate (pH 2.5), respectively, and shaken (150 r/min) for 24 h. Then, the filtrate were collected to determine the content of anthocyanins (c_1). Extra 75% (v/v) ethanol (40 mL, pH 2.5) was added to each resin and shaken for another 24 h. After that, the content of anthocyanins (c_2) in the filtrate was measured. The adsorption and desorption rate were calculated as follows, respectively:

$$\text{Adsorption rate}\ (\%) = 100 \times ((c_0 - c_1)/c_0).$$

$$\text{Desorption rate}(\%) = 100 \times (c_2/c_0 - c_1).$$

where c_0 is the total anthocyanins of the concentrate (mg C3GE/L); c_1 is the anthocyanins in the first filtrate (mg C3GE/L) and c_2 is the anthocyanins in the second filtrate (mg C3GE/L).

To investigate the static adsorption characteristics of D101, the pretreated and drained resin (0.7 g) was mixed with 40 mL anthocyanins concentrate (pH 2.5) and shaken at 150 r/min. The content of anthocyanins was measured at different times to calculate the adsorption capacity (mg C3GF/g) and draw static adsorption curve (Hoang, Nguyen, Dong, & Le, 2023). Meanwhile, pseudo-first-order model, pseudo-second-order model, and particle diffusion model were employed to further analyze the static adsorption of D101(Wang & Guo, 2020). The following equations were used to build the models:

For pseudo-first order kinetic model:

$$ln(q_e - q_t) = lnq_e - k_1 t$$

For pseudo-second order kinetic model:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\,2}} + \frac{t}{q_e}$$

For particle diffusion model:

$$q_t = k_p t^{1/2} + n$$

where k_1 is the pseudo-first-order constant (min^{-1}), k_2 is the second-order rate constant (g· C3GE mg^{-1}·min^{-1}), k_p is diffusion reaction rate constant of particles, q_t is the anthocyanin adsorption capacity of the resin (mg C3GE/g), q_e is the anthocyanin adsorption capacity of resin at equilibrium (mg C3GE/g), n is the diffusion constant of the reaction boundary layer (mg C3GE/g).

To detect the leakage curve, the resin of D101 was wet-loaded and the anthocyanins concentrate (pH 2.5) was added at a constant flow rate (1 mL/min). Then, the effluent was collected every 0.5 bed volume (BV) and the concentration of anthocyanins (c_V, mg C3GE/L) were determined. The leakage curve was calculated as:

$$\text{Leakage curve}\ (\%) = 100 \times c_V/c_0.$$

For elution curve analysis, the pretreated and drained resin (0.7 g) was mixed with 40 mL anthocyanins concentrate (pH 2.5) and shaken at 150 r/min. After that, 75% (*v*/v) ethanol (pH 2.5) was employed to elute (1 mL/min) and the effluent was collected every 0.5 BV to determine the anthocyanins. The elution curve was created by elution volume (BV) vs anthocyanins content (mg C3EG/L).

2.8. Stability analysis of anthocyanins

2.8.1. Effect of different pH on the stability of anthocyanins

The color of the anthocyanins (0.5 mg/mL) was recorded after re-dissolving in 70% (v/v) ethanol with different pH (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0), and the values of L*, a*, and b* of the anthocyanins were determined by colorimeter (Ci7600, USA) (Tang, He, & Fan, 2023). Meanwhile, the spectra from 400 nm to 700 nm were screened.

To evaluate the effect of pH on the thermal stability of anthocyanins, 0.5 mg/mL of the anthocyanins was prepared and incubated at 70 °C. After that, the concentration of anthocyanins was measured at 0, 1, 2, 3, 4, 5, 7, 9, and 11 h with different pH (3.0, 5.0, 7.0). Additionally, thermal degradation kinetics analysis was performed to further investigate the effect of pH on the thermal stability of anthocyanins as follows:

$$1n\frac{c_t}{c_0} = -kt$$

where c_t is the concentration of anthocyanins at different time (mg C3GE/L); c_0 is the total anthocyanins of the concentrate (mg C3GE/L); k is the rate constant (h^{-1}) and t is the time (h).

2.8.2. Effect of temperature, light, redox agents (H_2O_2, Na_2SO_3), carbohydrates (glucose and sucrose) on the stability of anthocynins

The purified anthocyanins were re-dissolved in 70% (*v*/v) ethanol to prepare a concentration of 0.5 mg/mL. The solution of the purified anthocyanins (pH 2.5, 0.5 mg/mL) was kept at 4 °C, 25 °C, and 37 °C in dark, and the absorbance ($\lambda = 530$ nm and $\lambda = 700$ nm) was recorded every 2 days. Similarly, two groups of purified anthocyanins (pH 2.5, 0.5 mg/mL) were prepared, one was stored in dark and the other was placed under natural light, and the absorbance ($\lambda = 530$ nm and $\lambda = 700$ nm) was recorded every 2 days (Enaru et al., 2021).

4.9 mL of the purified anthocyanins (pH 2.5, 0.5 mg/mL) was mixed with H_2O_2 (0.1 mL) to reach the final concentrations were 0%, 0.1%, 0.2%, 0.3%. Similarly, 5.4 mL of the purified anthocyanins (pH 2.5, 0.5 mg/mL) was mixed with Na_2SO_3 (0.6 mL) to reach the final concentrations were 0, 0.025 mg/mL, 0.05 mg/mL, 0.01 mg/mL. After that, the absorbance was measured at 5 min, 10 min, 20 min, 40 min, 60 min, and 120 min, respectively (Cavalcanti, Santos, & Meireles, 2011).

The solution of the purified anthocyanins (pH 2.5, 0.5 mg/mL, 4.0 mL) was added to glucose (1.0 mL) or sucrose (1.0 mL) with the final concentrations of 0 g/mL, 0.01 g/mL, 0.025 g/mL, 0.05 g/mL, and 0.1 g/mL, respectively (Zhou, Wang, Zhang, & Zhao, 2018). The mixture was stored at room temperature in dark and the absorbance was recorded everyday. The anthocyanins retention rates were calculated.

2.9. Effect of different metal ions on the stability of anthocynins

The solution of the purified anthocyanins (pH 2.5, 0.5 mg/mL, 8.8 mL) was mixed with 0.2 mL of metal ions, including Na^+ (NaCl), K^+ (KCl), Zn^{2+} ($ZnSO_4$•$7H_2O$), Cu^{2+} ($CuCl_2$•$2H_2O$), Fe^{2+} ($FeSO_4$•$7H_2O$), and the final concentration of these metal ions were 0 mmol/L, 1 mmol/L, 5 mmol/L, and 10 mmol/L. The mixture was stored at room temperature in dark and the absorbance was measured every 2 days for the calculation of retention rates. For Fe^{3+} ($FeCl_3$•$6H_2O$) treatment, the absorbance was scanned after 2 days in the same conditions (Enaru et al., 2021).

2.10. Statistical analysis

The results were expressed as mean ± standard deviations ($n = 3$) and ANOVA analysis was employed for the comparison between different colored potatoes. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was used by SIMCA software 14. $P < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. The basic nutritional compositions, total phenols, flavonoids, and anthocyanins in colored potatoes

>4000 cultivars of potatoes have been reported, but the exact number of colored potatoes are still difficult to calculate as most of them are planted in remote regions (Bvenura, Witbooi, & Kambizi, 2022a). The dominant colored potatoes across the world are variations of red, purple, yellow and blue. Presently, four different cultivars of colored potatoes (W2, W8, R16–5, R69) with purple and red colors were selected to extract and identify the anthocyanins (Fig. S2). Firstly, we measured the basic nutritional compositions of colored potatoes and the results were presented in Table S2. Generally, the moisture content in the purple potatoes (W2 and W8) was lower than that of red potatoes (R69 and R16–5), but the former contained higher starch. Meanwhile, the cultivar of W8 possessed less protein and ash than those of the other three samples. However, the fat among these colored potatoes showed no significance. Secondly, we determined the total phenols, flavonoids, and anthocyanins in the colored potatoes. As shown in Fig. S2, 70% (*v*/v) ethanol exhibited higher efficiency than 70% (v/v) methanol for the extraction of total phenols, total flavonoids, and anthocyanins from

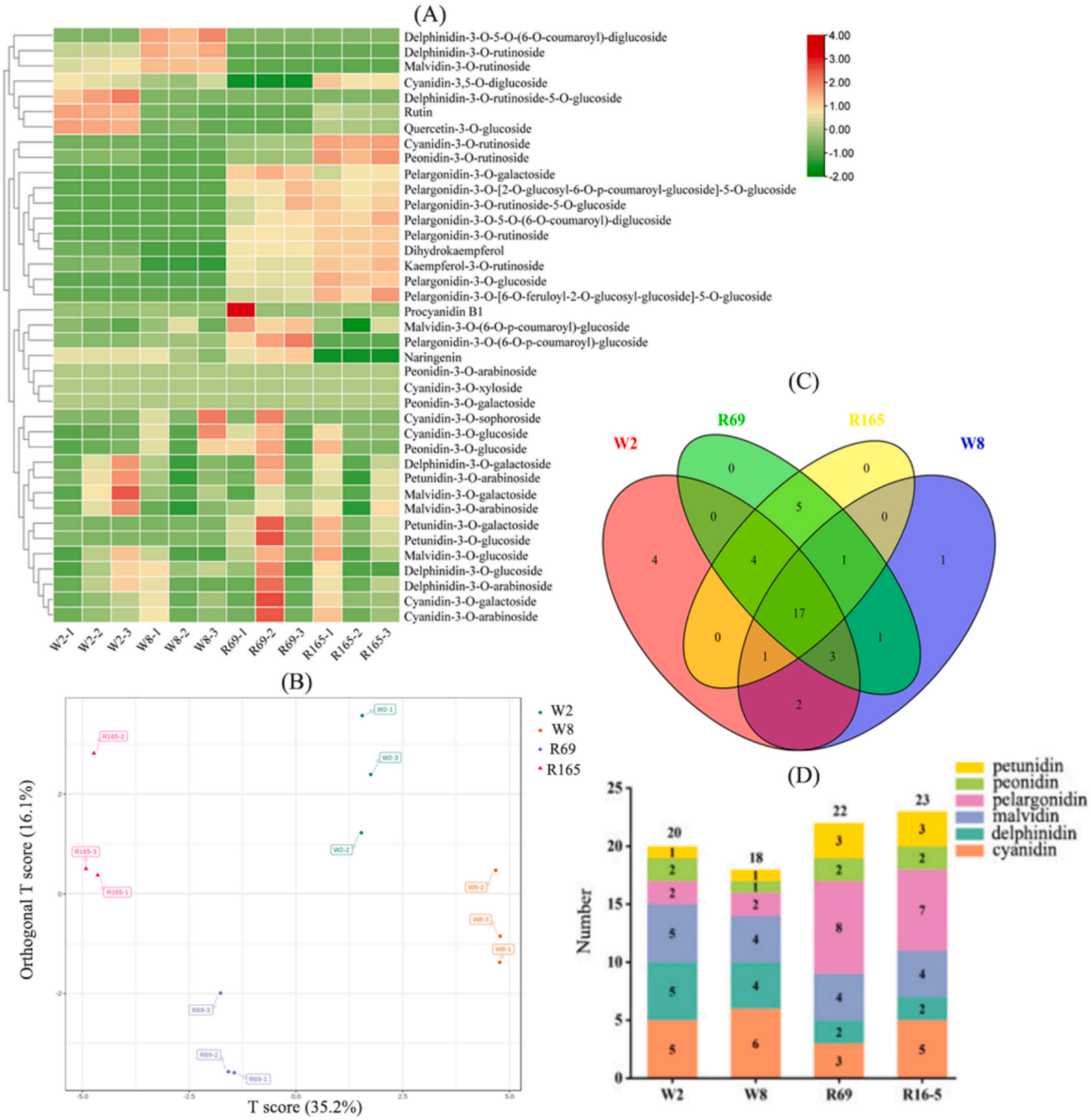

Fig. 1. Metabolomic analysis of anthocyanins. (A) the heatmap of the identified anthocyanins from the three cultivars of colored potatoes; (B) the OPLS-DA score; (C) the Venn diagram; (D) the compositional classification.

colored potatoes. Meanwhile, the total phenols and total flavonoids in the cultivar of W2 were much higher than those of W8, R69, and R16–5. For the concerned anthocyanins, the maximum absorption wavelength in each extract (70% (v/v) ethanol) was near 530 nm. Interestingly, the total anthocyanins in W8 had the highest content than the other three samples, which up to 72.924 ± 0.924 mg/100 g in methanol and 72.172 ± 0.889 mg/100 g in ethanol, respectively (Fig. S2). These results were consistent with the previous study that the amount of anthocyanins in the colored potatoes ranged from 0.220 g/kg to 0.648 g/kg fresh weight and the average anthocyanins content of the samples was 0.311 g/kg fresh weight (Jansen & Flamme, 2006). Similarly, Lachman et al. (2009) discovered that the content of total anthocyanins varied between 0.7 mg/100 g FW and 74.3 mg/100 g FW in 15 red and purple-fleshed potato cultivars (Lachman et al., 2009). Therefore, colored potatoes are the good source of dietary anthocyanins.

Table 1
Metabolomic analysis of chemical composition of anthocyanins from colored potatoes.

Anthocyanins	Type	Molecular formula	Q1 (DA)	Q2 (DA)	Ion mode	Content (μg/g)			
						W2	W8	R69	R16–5
Cyanidin-3-O-glucoside	cyanidin	$C_{21}H_{21}O_{11}$	449.10	287.10	$[M]^+$	0.054 ± 0.038	0.692 ± 0.249	0.576 ± 0.160	0.166 ± 0.004
Cyanidin-3-O-sophoroside	cyanidin	$C_{27}H_{31}O_{16}$	611.20	287.15	$[M]^+$	ND	0.005 ± 0.002	ND	ND
Cyanidin-3-O-galactoside	cyanidin	$C_{21}H_{21}O_{11}$	449.10	287.10	$[M]^+$	0.035 ± 0.001	0.037 ± 0.015	ND	0.033 ± 0.022
Cyanidin-3-O-rutinoside	cyanidin	$C_{27}H_{31}O_{15}$	595.17	287.10	$[M]^+$	0.066 ± 0.002	0.039 ± 0.017	0.260 ± 0.028	1.122 ± 0.033
Cyanidin-3,5-O-diglucoside	cyanidin	$C_{27}H_{31}O_{16}$	611.20	287.10	$[M]^+$	0.009 ± 0.001	0.006 ± 0.001	ND	0.010 ± 0.001
Cyanidin-3-O-arabinoside	cyanidin	$C_{20}H_{19}O_{10}$	419.10	287.10	$[M]^+$	0.049 ± 0.038	0.012 ± 0.008	ND	0.016 ± 0.010
Delphinidin-3-O-galactoside	delphinidin	$C_{21}H_{21}O_{12}$	465.10	303.10	$[M]^+$	0.188 ± 0.075	0.036 ± 0.009	0.017 ± 0.001	0.045 ± 0.025
Delphinidin-3-O-rutinoside-5-O-glucoside	delphinidin	$C_{33}H_{41}O_{21}$	773.21	303.10	$[M]^+$	0.007 ± 0.002	ND	ND	ND
Delphinidin-3-O-glucoside	delphinidin	$C_{21}H_{21}O_{12}$	465.10	303.10	$[M]^+$	0.111 ± 0.064	0.030 ± 0.011	0.043 ± 0.026	ND
Delphinidin-3-O-rutinoside	delphinidin	$C_{27}H_{31}O_{16}$	611.10	303.10	$[M]^+$	0.164 ± 0.057	0.264 ± 0.018	ND	ND
Delphinidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	delphinidin	$C_{36}H_{37}O_{19}$	773.19	303.10	$[M]^+$	ND	0.013 ± 0.001	ND	ND
Delphinidin-3-O-arabinoside	delphinidin	$C_{20}H_{19}O_{11}$	435.50	303.10	$[M]^+$	0.074 ± 0.039	ND	ND	0.023 ± 0.005
Malvidin-3-O-(6-O-p-coumaroyl)-glucoside	malvidin	$C_{32}H_{31}O_{14}$	639.17	331.10	$[M]^+$	1.007 ± 0.017	1.031 ± 0.034	1.128 ± 0.020	1.013 ± 0.055
Malvidin-3-O-rutinoside	malvidin	$C_{29}H_{35}O_{16}$	639.06	331.10	$[M]^+$	0.058 ± 0.006	0.081 ± 0.002	ND	ND
Malvidin-3-O-glucoside	malvidin	$C_{23}H_{25}O_{12}$	493.10	331.10	$[M]^+$	0.125 ± 0.063	ND	0.037 ± 0.022	0.050 ± 0.021
Malvidin-3-O-galactoside	malvidin	$C_{23}H_{25}O_{12}$	493.20	331.10	$[M]^+$	0.168 ± 0.046	0.029 ± 0.001	0.036 ± 0.014	0.046 ± 0.003
Malvidin-3-O-arabinoside	malvidin	$C_{22}H_{23}O_{11}$	463.30	331.06	$[M]^+$	0.064 ± 0.043	0.014 ± 0.001	0.031 ± 0.007	0.044 ± 0.001
Pelargonidin-3-O-galactoside	pelargonidin	$C_{21}H_{21}O_{10}$	433.20	271.10	$[M]^+$	ND	ND	0.355 ± 0.031	0.244 ± 0.037
Pelargonidin-3-O-glucoside	pelargonidin	$C_{21}H_{21}O_{10}$	433.20	271.10	$[M]^+$	ND	ND	0.097 ± 0.002	0.150 ± 0.012
Pelargonidin-3-O-[2-O-glucosyl-6-O-p-coumaroyl-glucoside]-5-O-glucoside	pelargonidin	$C_{42}H_{47}O_{22}$	903.26	271.10	$[M]^+$	ND	ND	0.085 ± 0.010	0.081 ± 0.004
Pelargonidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	pelargonidin	$C_{36}H_{37}O_{17}$	741.20	271.10	$[M]^+$	ND	ND	0.093 ± 0.015	0.124 ± 0.012
Pelargonidin-3-O-rutinoside-5-O-glucoside	pelargonidin	$C_{33}H_{41}O_{19}$	741.22	271.10	$[M]^+$	ND	ND	0.087 ± 0.023	0.100 ± 0.007
Pelargonidin-3-O-rutinoside	pelargonidin	$C_{27}H_{31}O_{14}$	579.06	271.10	$[M]^+$	0.011 ± 0.002	0.016 ± 0.003	22.990 ± 1.426	43.032 ± 1.411
Pelargonidin-3-O-[6-O-feruloyl-2-O-glucosyl-glucoside]-5-O-glucoside	pelargonidin	$C_{43}H_{49}O_{23}$	933.27	271.10	$[M]^+$	ND	ND	0.008 ± 0.001	0.014 ± 0.002
Pelargonidin-3-O-(6-O-p-coumaroyl)-glucoside	pelargonidin	$C_{30}H_{27}O_{12}$	579.15	271.10	$[M]^+$	0.059 ± 0.015	0.074 ± 0.006	0.291 ± 0.063	ND
Peonidin-3-O-rutinoside	peonidin	$C_{28}H_{33}O_{15}$	609.50	301.10	$[M]^+$	0.027 ± 0.011	ND	0.034 ± 0.001	0.127 ± 0.010
Peonidin-3-O-glucoside	peonidin	$C_{22}H_{23}O_{11}$	463.30	301.10	$[M]^+$	0.145 ± 0.138	0.033 ± 0.005	0.044 ± 0.006	0.007 ± 0.001
Petunidin-3-O-galactoside	petunidin	$C_{22}H_{23}O_{12}$	479.10	317.10	$[M]^+$	ND	ND	0.201 ± 0.105	0.148 ± 0.045
Petunidin-3-O-arabinoside	petunidin	$C_{21}H_{21}O_{11}$	449.10	317.06	$[M]^+$	0.190 ± 0.139	0.052 ± 0.008	0.067 ± 0.052	0.094 ± 0.001
Petunidin-3-O-glucoside	petunidin	$C_{22}H_{23}O_{12}$	479.10	317.10	$[M]^+$	ND	ND	0.041 ± 0.023	0.029 ± 0.010

ND, not detected.

3.2. Metabolomic analysis of anthocyanins

Presently, the metabolomic profiling of anthocyanins from the four different colored potatoes was identified by UPLC-MS/MS analysis in positive ion mode. As shown in Fig. 1 and Table 1, a total of 30 anthocyanins were identified in the colored potatoes and 17 of them were shared by the four samples (Fig. 1A and Fig. 1C). Exactly, 20, 18, 22, and 23 anthocyanins were identified in the cultivar of W2, W8, R69, and R16–5, respectively (Fig. 1D). All the six types of anthocyanidins, including delphinidin, malvidin, cyanidin, peonidin, pelargonidin, and petunidin were detected in the four cultivars. The main anthocyanidins in the purple potatoes were cyanidin, delphinidin, and malvidin, which totally occupied 15/20 and 14/18 of the anthocyanins in W2 and W8, respectively. However, pelargonidin was the main type of anthocyanidins in the red potatoes, followed by malvidin, cyanidin, and petunidin (Fig. 1D). These results indicated that the anthocyanins between purple and red potatoes existed significant differences, which were also exhibited by the score plots of OPLS-DA (Fig. 1B). Quantitative analysis revealed that the content of pelargonidin-3-*O*-rutinoside was most abundant in R69 and R16–5, up to 22.990 ± 1.746 μg/g and 43.033 ± 1.728 μg/g, respectively, followed by malvidin-3-*O*-glucoside, which was evenly distributed among the four cultivars with the content >1.000 μg/g. Sampaio et al. (2021) identified the anthocyanins in aqueous extracts from seven colored potato varieties and found that acylated glycosides or pelargonidin and petunidin aglycones were the main anthocyanins forms in the red and purple potatoes, respectively (Sampaio et al., 2021). Moreover, seven pelargonidin derivatives were further identified in the red-fleshed varieties, but in the purple-fleshed varieties, seven petunidin derivatives and one acylated peonidin glycoside were found as the major anthocyanins (Sampaio et al., 2021). Similarly, another study reported that anthocyanins that were responsible for purple or red coloration of potato were mainly petunidin derivatives and pelargonidin derivatives, respectively (Oertel et al., 2017). In agree with our study, pelargonidin and its derivatives were the major anthocyanins in the red potatoes (R69 and R16–5), while the derivatives of cyanidin, delphinidin, and malvidin were mainly discovered in the purple cultivars (W2 and W8). The anthocyanins of colored potatoes attracted most phytochemical studies due the various functional activities, such as antioxidant, anticancer, anti-inflammation, antiallergic, antimutagenic, antibacterial, and antiviral activity, etc. (Bvenura, Witbooi, & Kambizi, 2022b; Ezekiel, Singh, Sharma, & Kaur, 2013). These results indicate that the colored potatoes are rich in different types of anthocyanins, which can be used as the good material to develop functional foods.

3.3. Optimization of extraction process

3.3.1. Effect of enzymes on the total athocyanins

Three enzymes (α-amylase, cullulase, and pectinase) were employed to evaluate their effects on the yield of total anthocyanins from colored potatoes (W8). As shown in Fig. S3A—B, cellulase revealed no significant activity to release the anthocyanins, but α-amylase and α-amylase could obviously increase the content of total anthocyanins in the 70% ethanol extract of W8, among which α-amylase showed the better efficiency. Therefore, we checked the addition of α-amylase on the extraction of anthocyanins. When the amount of α-amylase increased from 10% to 20%, the yield of total anthocyanins significantly increased from 70.257 ± 1.377 mg C3GE/100 g to 78.740 ± 1.456 mg C3GE/100 g. However, the content of anthocyanins no longer raised obviously when the addition of α-amylase exceeded 20%. Hence 20% of α-amylase was used in the later experiments. Enzyme-assisted extraction was widely used to obtain anthocyanins from different plant materials. Four enzymes, including α-amylase, cellulase, pectinase, and protease were utilized to extract anthocyanins from *Prunus nepalensis* L. and the results indicated that cellulase treatment obviously increased the yield of anthocyanins, followed by pectinase (Swer et al., 2016). Meanwhile, α-amylase treated group revealed the best output at the concentration of 20% (Swer et al., 2016). Similarly, these enzymes were used to assisted-extract anthocyanins from eggplant (*Solanum melongena* L.) peel, *Lycium ruthenicum* Murr. and black rice, and all of which significantly increased the yield of anthocyanins (Amulya & Ul Islam, 2023; Shen et al., 2020; Yi et al., 2021). Enzyme treatment could break the cell wall and release the target compounds, including anthocyanins from the plant materials. Thus, enzyme-assisted extraction exhibited various advantages, such as higher efficiency, lower solvent consumption, and environmentally friendly, which is one of the most effective and practical methods to extract anthocyanins greenly (Amulya & Ul Islam, 2023).

3.3.2. Effect of extract temperature, time, and ethanol concentration on the total anthocyanins

As shown in Fig. S3C-E, the yield of total anthocyanins linearly raised with the increase of extract temperature from 30 °C to 50 °C. But this tendency was reversed when the temperature continuing to rise as high temperature could destroy anthocyanins (Zhang, Fan, Khan, Yan, & Beta, 2020). When the extract time increased from 10 min to 30 min, the yield of anthocyanins increased to 70.379 ± 1.186 mg C3GE/100 g to 81.739 ± 0.754 mg C3GE/100 g (Fig. S3D). However, further increase in extract time decreased the yield after 50 min, which was probably due to the mechanical or chemical destruction with longer extract time (José Aliaño González, Carrera, Barbero, & Palma, 2022). Similarly, the total anthocyanins yield increased with the increasing of ethanol concentration, which reached the maximum of 80.551 ± 2.167 mg C3GE/100 g with 80% (*v*/v) ethanol (Fig. S3D). Therefore, temperature, 50 °C; time, 30 min and concentration of ethanol, 80% were selected as the central values for the response surface optimization.

3.3.3. Response surface optimization

A three-factor and three-level Box-Behnken design was used to optimize the enzyme-assisted extraction conditions of anthocyanins from colored potatoes. As shown in Table S2, a total of 17 randomly experiments were designed and performed, and the yield of total anthocyanins was conducted as the response value (*Y*), which ranged from 64.07 mg C3GE/100 g DW to 85.33 mg C3GE/100 g DW. The results were analyzed by ANOVA to carry out quadratic regression analysis, and the regression equation was obtained as follows:

$$Y = 83.72 - 3.78A + 4.01B + 2.16C - 0.1250AB + 1.98\,AC + 0.6975BC - 5.16A^2 - 4.36B^2 - 6.06C^2.$$

where *Y* is the yield of total anthocyanins, A, B, and C represent ethanol concentration, extract time and extract temperature, respectively. As seen in Table S3, the values of F and *p* were 42.39 and < 0.0001, indicating that this model was significant. Meanwhile, all the three factors showed obvious influence on the yield of total anthocyanins, among which extract time revealed the greatest impact, followed by ethanol concentration and extract temperature. Additionally, the quadratic terms A^2, B^2, C^2 and the interactive term AC exhibited significant differences ($p < 0.05$), but there were no significance ($p > 0.05$) between the interactions of A and B, or B and C. These results were also observed in Fig. S4, indicating that the interaction between ethanol concentration and extract temperature showed the greatest impact on the yield of total anthocyanins from colored potatoes. Proper heating could increase the yield of anthocyanins via softening the plant tissue or enhancing the solvent penetration. But the higher extract temperature might cause anthocyanins degradation and reduce the yield (Shen et al., 2020). If the extract time is too long, the anthocyanins will be further destroyed. Therefore, the optimal conditions for extracting anthocyanins from colored potatoes were as follows: ethanol concentration, 75.43%; extract time, 39.96 min; extract temperature, 51.80 °C, and the predicted yield of total anthocyanins was 85.41 mg C3GE/100 g DW. According to the optimal conditions, the verification experiments were performed to obtained the yield of total anthocyanins was 84.47 mg

C3GE/100 g DW, which was close to the predicted value, suggesting that this model could successfully optimized the extract conditions of anthocyanins from colored potatoes (Zhang et al., 2020). Presently, various extraction methods were developed to effectively extract anthocyanins from plant materials, such as ultrasound assisted extraction, supercritical carbon dioxide extraction, and deep eutectic solvent extraction, etc. (Li, Zhang, Wang, Feng, & Han, 2023). Nevertheless, acid-solvent extraction is widely used in industrial production due to the convenient operation and simple equipment (Tan et al., 2022). Herein, we optimized the extract conditions of anthocyanins from colored potatoes, which could provide the guidance for the preparation of anthocyanins in practical applications.

3.4. Purification of anthocyanins

3.4.1. The adsorption and desorption rates of three different macroporous resins

Macroporous resins are the widely used materials for anthocyanins purification. Adsorption and desorption capacities of the macroporous resins are the two most critical properties to indicate the purification performance. Presently, AB-8 showed the highest adsorption rate than that of D101 and HPD100, which might be related to its weak polarity (Fig. 2A). However, high adsorption rate caused the lowest desorption rate of AB-8 (Fig. 2A). Comparatively, D101 exhibited the best ratio of desorption rate vs adsorption rate, indicating that D101 was more suitable for purification of anthocyanins from colored potatoes. Surface area, pore diameter and surface polarity are the three most important parameters that affect the purification performance of macroporous resins (Yang et al., 2022). Although AB-8 revealed the best adsorption capacity, the desorption rate was too low to limit its usage for anthocyanins purification in the present study. With the similar surface area of AB-8, D101 displayed significant desorption capability, which was selected to purify anthocyanins from colored potatoes in the further experiments.

3.4.2. The static adsorption test

As shown in Fig. 2B, the static adsorption capacity of D101 increased rapidly from 0 min to 100 min, and then gradually slowed down until 540 min, which finally reached the equilibrium. Many models have built to calculate the adsorption kinetics of resin according to different theoretical grounds (Wang & Guo, 2020). Pseudo-first-order model is one of the widely applied method to assume the adsorption kinetics, which was used to further analyze the static adsorption of D101 in the present study. As seen in Table S4, the R^2 values of pseudo-first-order model and pseudo-second-order model were 0.8772 and 0.9802, respectively, indicating that pseudo-second-order model could better describe the adsorption process of D101 for anthocyanins from colored potatoes. The equilibrium adsorption capacity, simulated by the pseudo-second-order model, was 10.76 mg C3GE/g (Table S4). However, particle diffusion model could not be applied to the whole adsorption process, and three steps were divided according to the values of k_p ($0.4516 > 0.1786 > 0.0206$), representing the diffusion rate from fast to slow (Wang & Guo, 2020).

3.4.3. Analysis of leakage curve and elution curve

As shown in Fig. 2C, the leakage rate was rapidly increased with the increase of anthocyanins loading. When the anthocyanins loading quantity exceeded 8 BV, the leakage rate of anthocyanins was >5%,

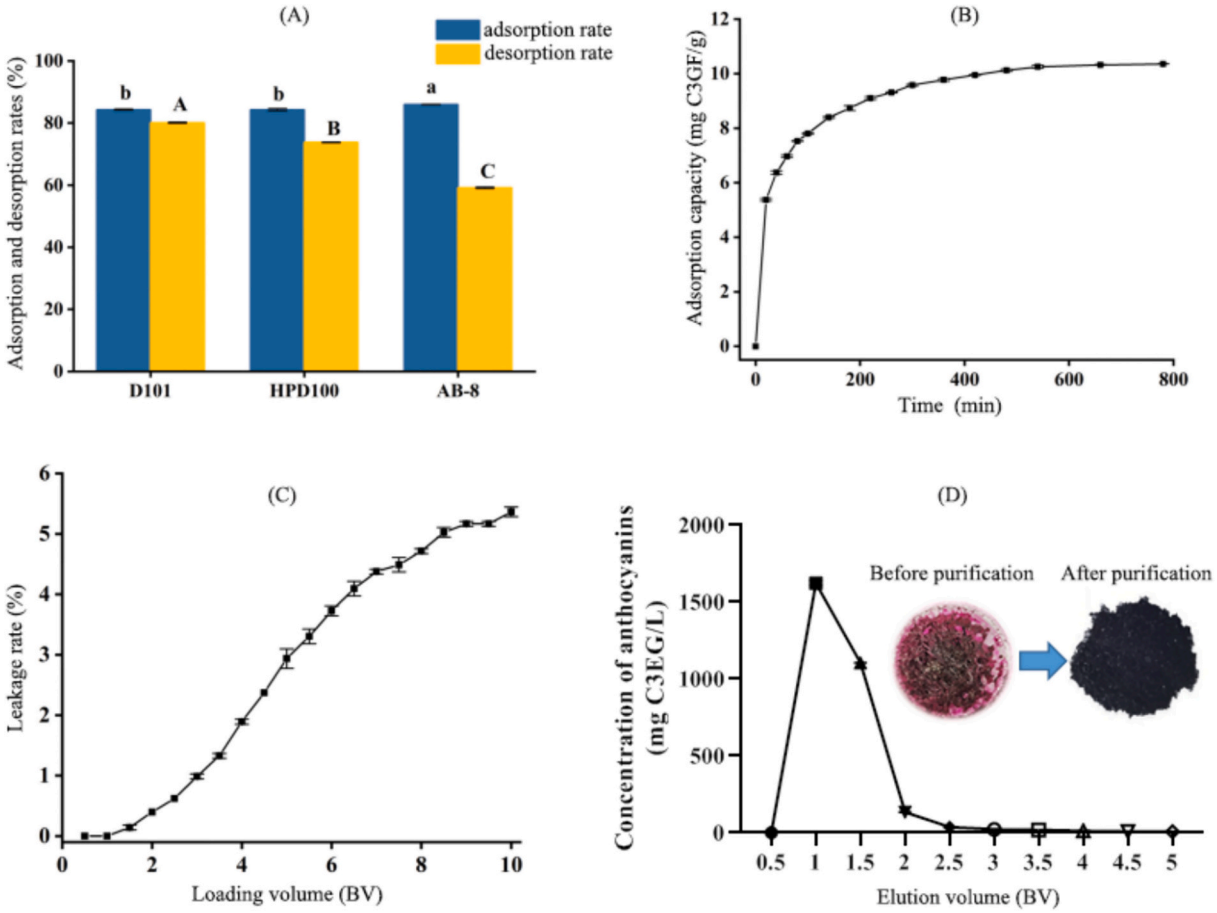

Fig. 2. Purification of anthocyanins. (A) the adsorption and desorption rates of macroporous resins; (B) the static adsorption curve; (C) the leakage curve; (D) the elution curve (the illustration is the photo of anthocyanins before and after purification).

indicating that the adsorption capacity of the resin reached saturation (Belwal, Li, Yanqun, Cravotto, & Luo, 2020). Therefore, 8 BV was selected as the loading quantity of anthocyanins. The elution curve of D101 was presented in Fig. 2D, which revealed that the elution peak of D101 was relatively narrow and the concentration of anthocyanins in the elution reached the highest concentration by using 1 BV of 75% (*v*/v) ethanol (pH 2.5). After washing by 3 BV, the content of anthocyanins in the solution approached 0 (Fig. 2D). Moreover, the illustration in Fig. 2D showed that the anthocyanins from colored potatoes changed from a purple viscous solid to a black powder after purification by D101. Meanwhile, the color value increased from 0.70 to 21.78 and the purity of the anthocyanins reached 10.49% via purification. During the extraction process of anthocyanins, many other components, such as sugar, protein, amino acids, etc., are also extracted, which decrease the purity of anthocyanins and also may influence the stability of anthocyanins in the storage step (Heinonen et al., 2016). Macroporous resin assisted purification is an effective industrial technique to purify anthocyanins (Paul et al., 2023). Herein, the resin of D101 was successfully selected to purify the anthocyanins from colored potatoes. D101 has been widely used in the food industry for isolation and purification, indicating that this resin can be easily employed to produce purified anthocyanins (Hoang et al., 2023). Therefore, the results of the present study provided more details for the further practical application of D101 in the anthocyanins purification.

3.5. Stability analysis of anthocyanins

3.5.1. Effect of pH on the stability of anthocyanins

pH is one of the most important factors to affect the stability of anthocyanins due to their ionic nature of the molecular structure (Tajner-Czopek, Rytel, Kita, Sokół-Łętowska, & Kucharska, 2023). As seen in Fig. 3A, the color of the anthocyanins was red at the pH 1.0, which became colorless with the increase of pH. When the pH was neutral (pH 7.0) or slightly alkaline (pH 8.0), the color of anthocyanins gradually revealed purple. The values of L*, a*, b* were determined by the Colorimeter (Ci7600, X-Rite, Shanghai). As shown in Table S5, the values of L* increased firstly, and then decreased, representing that the brightness of anthocyanins became much more brighter with the pH increasing from 1.0 to 5.0, but converted to darker with the further increase of pH. The values of a* were much more bigger when the pH was lower, indicating that the color of anthocyanins preferred red in the acidic solution (Table S5). However, there was no obvious regular changes of the values of b*, but which exhibited the minimum value when the pH reached to 8 (Table S5). These results were consistent with Fig. 3A. In the extreme acidic environment, anthocyanins existed as the form of falvylium cation, which was easily soluble in water to appear red (Khoo, Azlan, Tang, & Lim, 2017). However, with the increase of pH (pH 5.0–6.0), the formation of carbinol pseudobase and chalcone leaded to colorless. Additionally, alkaline environment may degrade anthocyanins, especially influence the stability of the ring B of anthocyanins, causing significant color changes (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Different pH also affected the maximum adsorption wavelength of anthocyanins. At pH 1.0, the λ_{max} of anthocyanins was 523 nm with the absorbance of 2.001, but which was markedly reduced when the pH increased to 5.0. Interestingly, the absorbance restored to increase with the further rise of pH, and the λ_{max} also had undergone a red shift, which was moved to 578 nm with pH of 8.0 (Fig. 3B). Food is a complex system with different pH, and the present study indicated that anthocyanins from colored potatoes were more suitable for use in acidic foods.

The thermostability of anthocyanins at 70 °C with three different pH (3.0, 5.0, and 7.0) was investigated and the result was shown in Fig. 3C. Anthocyanins from colored potatoes revealed much better thermostability at pH 3.0 than that of pH 5.0 and 7.0, which still reserved 66.74% after 11 h at 70 °C. Comparatively, almost no anthocyanins could be detected at pH 7.0 under the same conditions. From the thermal degradation kinetics of Fig. 3D, we found that anthocyanins happened a quickly degradation with the increase of pH at 70 °C and the half-life $t_{1/2}$

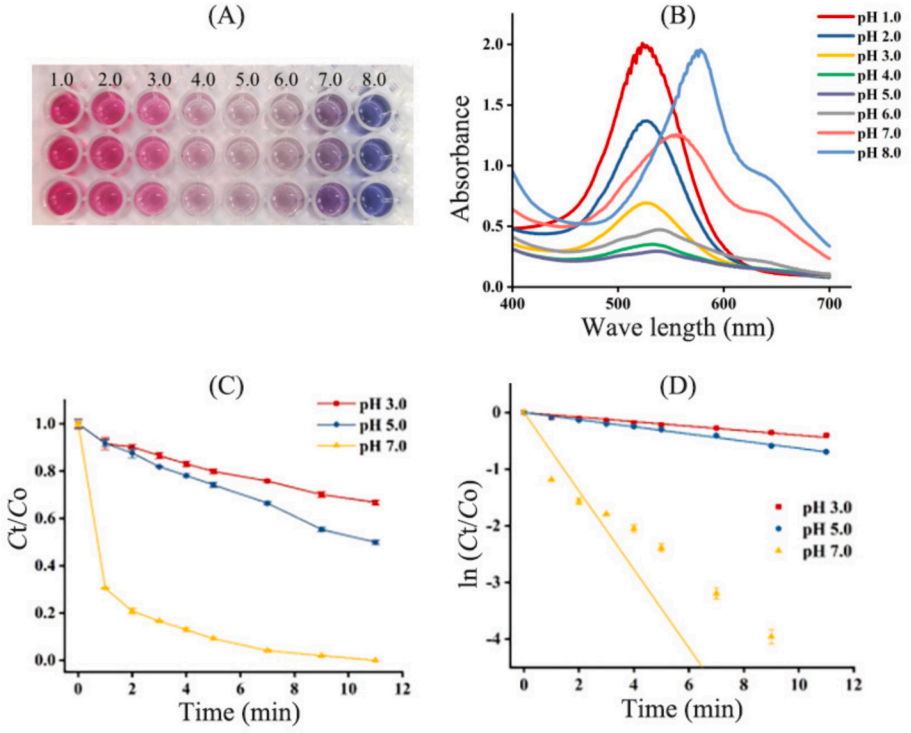

Fig. 3. Effect of pH on the stability of anthocyanins from colored potato. (A) The color of anthocynins at different pH; (B) The absorbance of anthocynins from 400 nm to 700 nm at different pH; (C) The thermostability of anthocyanins at 70 °C and (D) Thermal degradation kinetics of anthocyanins from colored potato at different pH.

of the degradation of anthocyanins decreased from 17.2855 h at pH 3.0 to 1.0006 h at pH 7.0. These phenomenon was also observed by Liu et al. (2018) and Hou, Qin, Zhang, Cui, and Ren (2013), who discovered that the anthocyanins from blueberry or black rice exhibited much more strong thermostability at low pH (Hou et al., 2013; Liu et al., 2018). The thermostability of the anthocyanins is closely related to their structures, such as acylation can enhance the stability. However, food processing is always accompanied by high temperature treatment, and how to increase the thermostability of the anthocyanins remains a challenge for the future.

3.5.2. *Effect of temperature, light, redox agents, and sugars on the stability of anthocyanins*

As known to all, heat treatment is one of the most widely used methods during food processing. But high temperature always destroy the nutrients of food, including anthocyanins (Enaru et al., 2021). Herein, we found that the stability of anthocyanins from colored potatoes was obviously decreased with the increase of temperature from 4 °C to 37 °C during 10 days' storage (Fig. 4A). High temperature may cause the degradation of pigment via multiple mechanisms, such as glycosylation, cleavage, polymerization, etc. (Rodriguez-Amaya, 2019). How to maintain the stability of anthocyanins in heating processing is still an unresolved issue. Effect of light on the stability of anthocyanins was shown in Fig. 4B, the anthocyanins retention rate was 82.73% under dark conditions on the 10th day, compared to which was only 57.73% under natural light, indicating that illumination could destroy anthocyanins. This phenomenon was also confirmed by the anthocyanins from grape or berries (West & Mauer, 2013). The aromatic acyl group of anthocyanins has the capacity to absorb light energy, which can break the conjugated double bond in the anthocyanins and lead to

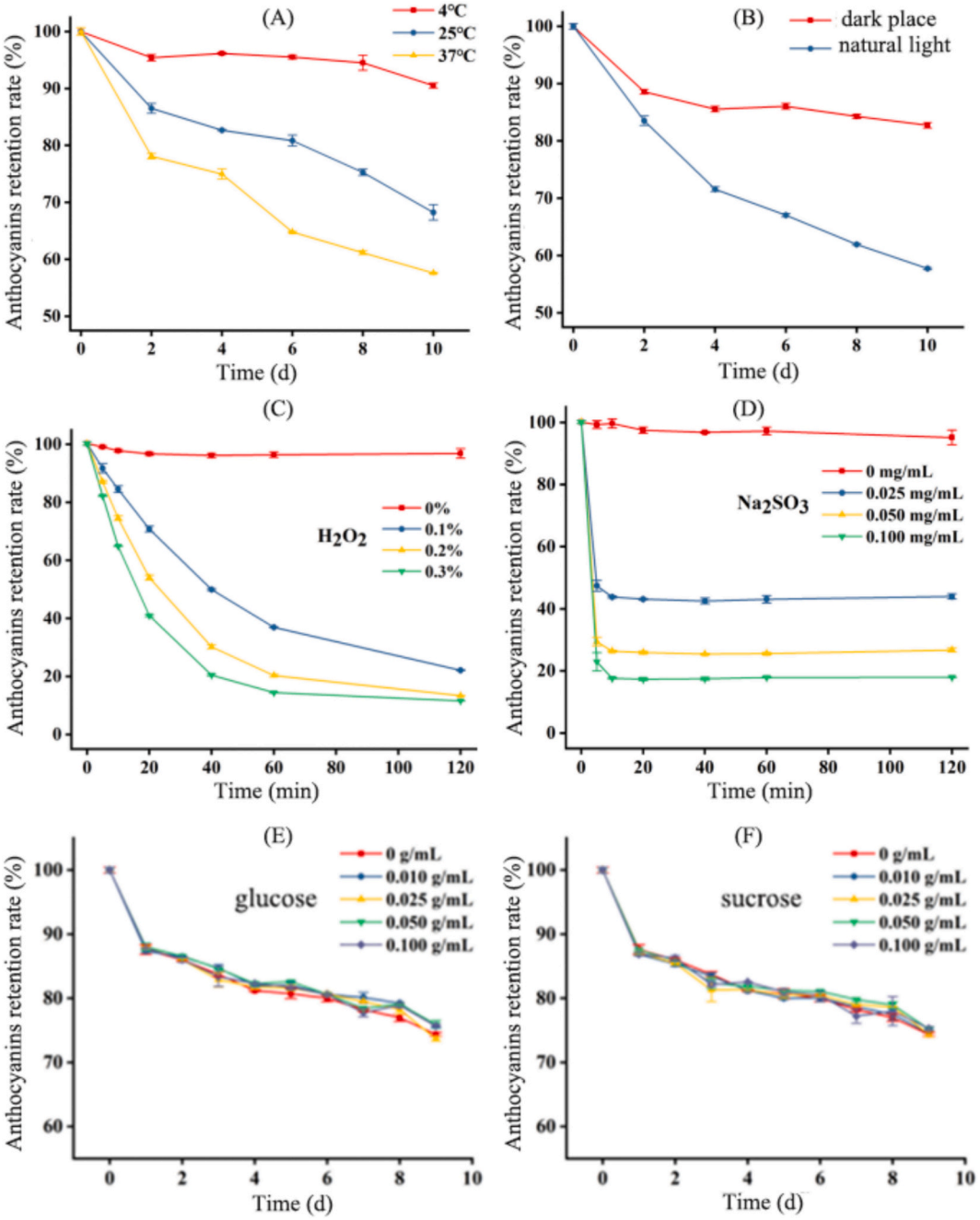

Fig. 4. Effect of temperature (A), light (B), H_2O_2 (C), Na_2SO_3 (D), glucose (E), and sucrose (F) on the stability of anthocyanins from colored potato.

photodegradation (Li et al., 2023). These results demonstrated that the anthocyanins from colored potatoes are sensitive to temperature and light, indicating that low temperature and opaque package materials were the priority selection during colored potato processing.

Figure 4C-D revealed the effect of redox agents, including H_2O_2 and Na_2SO_3, on the stability of anthocyanins. With the increase of the two redox agents, the retention rate of anthocyanins were markedly reduced until to 120 min (Fig. 4C-D). Comparatively, Na_2SO_3 showed much more degradability for anthocyanins than that of H_2O_2, as the retention sharply decreased with in 10 min (Fig. 4C-D). Previous report demonstrated that sulfites and sulfates could cause the loss of anthocyanin pigment via forming colorless sulfur derivative structures, which is consistent with the present observation (Cavalcanti et al., 2011). These results implied that redox agents (especially reducing agents) were not suitable for use in the processing of colored potatoes.

Effects of monosaccharides (glucose) and disaccharides (sucrose) on the stability of anthocyanins were presented in Fig. 4E-F. The retention rate of anthocyanins was gradually decreased with different concentration of glucose or sucrose during the 9 days' storage. However, there were no significant differences between different treated groups, indicating that anthocyanins were stable in the sugar solutions. Similar to our results, Zhou et al. (2018) found that the degradation of anthocyanins could be inhibited with the supplement of 5% to 20% sucrose (Zhou et al., 2018). Comfortingly, glucose or sucrose can be used in the processing of colored potatoes for their ineffective to the anthocyanins.

3.5.3. *Effect of different metal ions on the stability of anthocyanins*

Effect of different metal ions, including Na^+, K^+, Zn^{2+}, Cu^{2+}, Fe^{2+} and Fe^{3+} on the stability of anthocyanins were presented in Fig. 5. After supplement with Na^+ and K^+, the retention rate of anthocyanins was

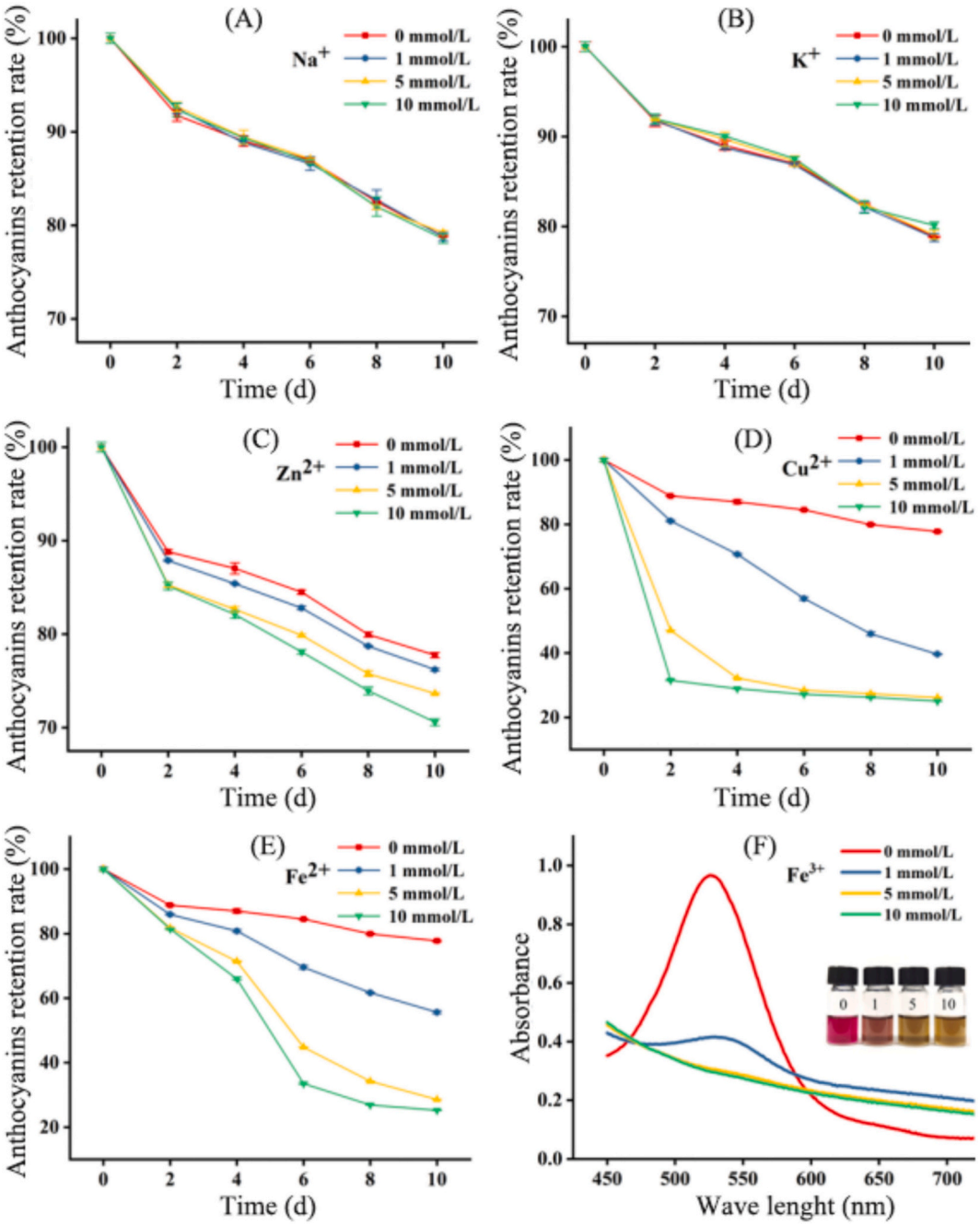

Fig. 5. Effect of different metal ions on the stability of anthocyanins from colored potato. (A) Na^+; (B) K^+; (C) Zn^{2+}; (D) Cu^{2+}; (E) Fe^{2+}; (F) Fe^{3+}.

gradually reduced with the increase of the storage time, which revealed no significant differences between the different groups, indicating that Na^+ and K^+ had little effect on the stability of anthocyanins of colored potatoes (Fig. 5A-B). However, co-treatment with different concentration of Zn^{2+}, Cu^{2+} and Fe^{2+} obviously decreased the retention rate of anthocyanins during the 10 days' storage (Fig. 5C-E). Among the three metal ions, Cu^{2+} showed the greatest adverse effect on the stability of anthocyanins, followed by Fe^{2+} and Zn^{2+}. Furthermore, the color of the anthocyanins solution immediately undergone the visible changes after mixed with different concentration of Fe^{3+} (the illustration of Fig. 5F). Meanwhile, the maximum absorption peak around 530 nm of anthocyanins was weakened by Fe^{3+}, suggesting that Fe^{3+} might rapidly react with the chromophores of anthocyanins (Fig. 5F).

Similar results were observed by addition of Fe^{2+} to a solution of anthocyanin, which caused the quick development of a large low-energy visible ban feature of the mixture (Fenger, Moloney, Robbins, Collins, & Dangles, 2019). The interaction between metal ions to anthocyanins had fully elucidated that the catechol or pyrogallol at B-ring of anthocyanins could simultaneously capture the two protons from metallic ions to form anthocyanins-metal ions-complexes via π-π stacking networks (Chen et al., 2022; José Aliaño González et al., 2022). Thus, Na^+ and K^+, which only supplying one proton, might not interact with anthocyanins, therefore, revealed no significant effect on the stability of anthocyanins (Fig. 5A-B). Co-pigmentation with metallic ions in mildly acidic to neutral librium might be an effective strategy to stabilize anthocyanins. But the addition of metal ions, such as Sn^{2+}, Fe^{3+}, Al^{3+}, and Ca^{2+}, obviously promoted the degradation of red cabbage anthocyanin during thermal treatment, storage, and encapsulation (Ratanapoompinyo, Nguyen, Devkota, & Shrestha, 2017). Consistent with our present results, addition of Zn^{2+}, Cu^{2+} and Fe^{2+} obviously reduced the storage stability of anthocyanins from colored potatoes in a concentration-dependent manner (Fig. 5C-E). Various metal ions may involved during the food processing, packaging and storage. Hence, it is important to avoid contact with metal ions when the colore potatoes are used as the materials for food processing.

4. Conclusion

Presently, we compared the content of basic nutritional compositions, total phenols, flavonoids, as well as anthocyanins in the four different colored potatoes and found that the cultivar of W8 had the highest content of total anthocyanins. Nextly, metabolomic analysis discovered that cyanidin, delphinidin, malvidin, and their derivatives were the major anthocyanins in purple potatoes, whereas red potatoes were mainly composed of pelargonidin and its derivatives. The results of response surface optimization revealed that a total of 84.47 mg C3GE/100 g DW anthocyanins was obtained at the optimal conditions. Three types of macroporous resins, including D101, HPD100, and AB-8, were screened to purify the anthocyanins and D101 presented the better efficiency, which could increase the color value from 0.70 to 21.78 after purification. Stability analysis indicated that anthocyanins from colored potatoes were sensitive to pH, temperature, light, redox agents (H_2O_2 and Na_2SO_3), and divalent or trivalent metal ions (Zn^{2+}, Cu^{2+}, Fe^{2+} and Fe^{3+}), but stable to sugars (glucose and sucrose) and univalent metal ions (Na^+ and K^+).

In conclusion, the present study found that cyanidin, delphinidin, and malvidin were the major anthocyanins in purple potatoes, whereas red potatoes were mainly consisted of pelargonidin and its derivatives. 84.47 mg C3GE/100 g DW of anthocyanins was obtained at the optimal conditions and the macroporous resin of D101 could effectively purify the anthocyanins. Furthermore, anthocyanins from colored potatoes were sensitive to pH, temperature, light, redox agents, as well as divalent or trivalent metal ions, but stable to sugars and univalent metal ions. However, the purity of the anthocyanins in the present study is still low, which can be further isolated by other method to improve their purity. Anthocyanins have many health benefits, such as antioxidant, anti-inflammatory, anticancer, anti-diabetic, and anti-bacterial activities, and these biological activity of the anthocyanins from colored potatoes will be investigated in the future.

CRediT authorship contribution statement

Lin Han: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Ruijie Li:** Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Xiying Jin:** Writing – original draft, Software, Methodology, Formal analysis. **Yixin Li:** Writing – original draft, Methodology, Investigation, Formal analysis. **Qin Chen:** Resources. **Caian He:** Writing – review & editing, Writing – original draft, Validation. **Min Wang:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present paper.

Data availability

Data will be available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101423.

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