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Preparative purification of polyphenols from sweet potato (*Ipomoea batatas* L.) leaves by AB-8 macroporous resins

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ABSTRACT

In this study, the adsorption properties of AB-8 macroporous resin for sweet potato leaf polyphenols was investigated. The adsorption mechanism was elaborated by the Langmuir and Freundlich equations, and the purification parameters were optimised by adsorption and desorption tests. The constituents and their contents of the purified products were analysed, and the antioxidant activities were determined. The results showed that the optimal processing parameters were as follows: an initial polyphenol concentration of 2.0 mg chlorogenic acid equivalent (CAE)/ml, pH 3.0, an ethanol desorption solution concentration of 70% (v/v) and a flow rate for feeding and elution of 1 BV/h. The purified products mainly contained eight phenolic constituents and the contents of three di-caffeoylquinic acids were relatively higher than the other constituents. The purified products possessed strong antioxidant activities. In conclusion, purification by AB-8 macroporous resin was highly efficient, economic and environmentally friendly and has a great industrial production potential.

1. Introduction

Polyphenols are secondary plant metabolites and are important determinants of the sensory and nutritional qualities of fruits, vegetables and other plants. Owing to their high antioxidant capacity, polyphenols may have possible beneficial implications on human health, such as in the treatment and prevention of cancer, cardiovascular disease and other pathologies, and have been an important research area in recent years (Ignat, Volf, & Popa, 2011; Quideau, Deffieux, Douat, & Pouysegu, 2011).

Sweet potato leaves are the aboveground parts of the sweet potato (*Ipomoea batatas* L.) and have a high nutritional and functional value (Islam et al., 2002). Sweet potato leaves can be harvested several times a year, but 95–98% are discarded during the harvesting period, while the remaining 2–5% are used as animal food, leading to a huge waste of resources (Hue, Boyce, & Somasundram, 2012). In our previous study, we found that the total polyphenol content of 40 sweet potato leaves cultivars was 3–12% dry weight (Sun, Mu, Xi, Zhang, & Chen, 2014), which was two to three times greater than that in some common vegetables (e.g. spinach, kale) (Karna et al., 2011; Xu et al., 2010). Sweet potato leaf polyphenols have strong antioxidant capacities, e.g. free radical scavenging, metal chelating and lipid peroxidation inhibition activities (Huang, Chu, Juang, & Wang, 2010; Kurata, Adachi, Yamakawa, & Yoshimoto, 2007; Kurata, Yahara, Yamakawa, & Yoshimoto, 2011). However, the crude polyphenol extracts of sweet potato leaves always contain chlorophyll, proteins, polysaccharides and other impurities, which limit the application of sweet potato leaf polyphenols. Therefore, an efficient purification method is needed to obtain sweet potato leaf polyphenols with a high purity.

The purification methods for plant polyphenols are mainly organic solvent extraction, membrane separation methods and supercritical fluid extraction (Dai & Mumper, 2010; Farías, Rostagno & Meireles, 2013; Nawaz, Shi, Mittal, & Kakuda, 2006; Turkmen, Sari, & Velioglu, 2006). However, these methods have some disadvantages, such as long production cycles or high cost, which make them unsuitable for use on an industrial scale. Macroporous resins are durable polar, non-polar or slightly hydrophilic polymers with high adsorption capacities for organic molecules (Fu et al., 2006). They can selectively adsorb the targeted constituents from aqueous and non-aqueous systems through electrostatic force, hydrogen bonding interactions, complexation and size sieving (Gao, Huang, & Liu, 2007). Therefore, macroporous resins have been widely used in the separation and purification of biologically active substances due to their physicochemical stability, high adsorption selectivity and easy recycling (Wan et al., 2014). AB-8 macroporous resins are weak polar resins and have been widely used in the purification of plant polyphenols because of their

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appropriate surface area and nuclear pore size (Gao, Yu, Yue, & Quek, 2013; Zhao et al., 2013). However, there is only limited information on the purification of sweet potato leaf polyphenols by AB-8 macroporous resin.

In this study, the adsorption properties of AB-8 macroporous resins for sweet potato leaf polyphenols was investigated, the adsorption mechanism was elaborated by analysing the adsorption isotherms with Langmuir and Freundlich equations and the purification parameters were optimised by static and dynamic adsorption and desorption tests. The constituents and contents of the purified products were analysed by HPLC and the antioxidant activities were determined by photochemiluminescence assay and oxygen radical absorbance capacity assays. The objective of this study was to provide data for the utilisation of AB-8 macroporous resins in the purification of sweet potato leaf polyphenols.

2. Materials and methods

2.1. Materials

Two sweet potato leaf cultivars, Yuzi No. 7 and Ximeng No. 1, were collected in August 2012 from the Sweet Potato Research Institute in Xuzhou, Jiangsu Province, China. The 10–15 cm long leaf parts at the tips were selected. The fresh sweet potato leaves were washed, drained, freeze-dried and powdered with a grinder. After the samples were screened by a 40 mesh sieve, the leaf powder was packed into aluminium foil bags and stored at 4 °C for further use.

The AB-8 macroporous resins were purchased from Soledad Technology Ltd. (Beijing, China) and their physical and chemical properties are summarised as follows: they are weakly polar, have a surface area of 480–520 m^2/g, a moisture content of 60–70% and an average pore diameter of 130–140 A. The resins were pretreated according to Sun, Guo, Fu, Li, and Li (2013). Briefly, the resins were soaked with four times (w/v) 95% (v/v) ethanol for 24 h, washed thoroughly with distilled water until the water clarified and then soaked with four times (w/v) 2 mol/l HCl and 2 mol/l NaOH solution successively for 4 h. They were then thoroughly washed in distilled water until the washing fluid became neutral. The resins were then filtered to remove the water before use.

2.2. Reagents

Folin–Ciocalteu reagent, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), ascorbic acid, Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid), caffeic acid (CA) and chromatography grade acetonitrile and methanol were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). The HPLC grade caffeoylquinic acids (CQA) standards (3-CQA, 4-CQA, 5-CQA, 3,4-CQA, 3,5-CQA, 4,5-CQA and 3,4,5-CQA) were purchased from AMRESCO Biotechnology Co., Ltd. (Solon, OH, USA). Tea polyphenols (TP) and grape seed polyphenols (GSP) were purchased from Yihe Biotechnology Co., Ltd., Xi'an, China. Sodium fluorescein, sodium hydroxide, phosphate and other analytical grade reagents were purchased from Beijing Chemical Reagents Co., Beijing, China.

2.3. Extraction of polyphenols from sweet potato leaves

The sweet potato leaf powder (10 g) was extracted with 70% (v/v) ethanol solution (200 ml) for 30 min at 50 °C using ultrasonic (59 kHz) assistance. After the solution was centrifuged at 8711 g for 10 min, the residue was re-extracted twice with 70% ethanol as described above. The supernatants were combined and concentrated in a rotary evaporator to obtain the crude polyphenol extraction.

2.4. Total polyphenol contents

The total phenolic content (TPC) was measured using the Folin–Ciocalteu method described by Yoshimoto et al. (2002) with slight modification. Briefly, a 0.5 ml sample solution was mixed with 1.0 ml of Folin–Ciocalteu reagent (ten times dilution) and allowed to react at 30 °C for 30 min. Then 2.0 ml of saturated Na_2CO_3 (10% w/v) was added and kept at 30 °C for 30 min. The absorbance was measured at 736 nm using a UV-3010 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). A calibration curve for the chlorogenic acid standards (at concentrations of 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml) was prepared. The linear regression equation was $y = 8.7671x + 0.0068$ and $R^2 = 0.9994$. The TPC was expressed as mg chlorogenic acid equivalent per ml of sample solution (mg CAE/ml).

2.5. Static adsorption and desorption test

2.5.1. Adsorption and desorption kinetics

Exactly 2 g of the pretreated AB-8 macroporous resin was put into a 250 ml triangular flask and 50 ml of crude polyphenols solution (TPC, 1.0 mg CAE/ml) was added. The flask was shaken on an immersion oscillator (130 r/min) at 25 °C for 24 h to reach the adsorption equilibrium. The TPC of the solution after adsorption was determined using the Folin–Ciocalteu method. The adsorption capacities were calculated using formula (1). After the adsorption equilibrium had been reached, the resins were washed twice using 50 ml distilled water and then desorbed by 50 ml 70% (v/v) ethanol solution in a flask, which was shaken on an immersion oscillator (130 r/min) at 25 °C for 24 h. The TPC of the desorption solution was then determined. The desorption ratios were calculated using formula (2).

$$Q_t = V_0(C_0 - C_t)/M \quad (1)$$

where Q_t is the adsorption capacity of the resin at time t (mg/g); C_0 and C_t are the TPCs of the sample solution at beginning and at time t, respectively (mg CAE/ml); V_0 is the initial volume of the solution added to the flask (ml) and M is the weight of the resin (g).

$$D = 100\ C_d V_d/[V_0(C_0 - C_e)] \quad (2)$$

where D is the desorption ratio (%); C_d is the TPC of the desorption solution (mg CAE/ml); V_d is the desorption solution volume (ml); C_e is the equilibrium TPC in the sample solution and C_0 and V_0 are the same as those defined in formula (1).

2.5.2. Adsorption isotherms

Crude polyphenol solutions with TPCs of 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mg CAE/ml were prepared. Exactly 50 ml of each solution was adsorbed by 2 g of resin at 30 °C, 35 °C and 40 °C using the method described in Section 2.5.1. The TPCs (i.e. C_e) of the solutions were determined after adsorption equilibrium had occurred and the equilibrium adsorption capacities (i.e. Q_e) had been calculated using formula (1). The adsorption isotherms were described by C_e and Q_e.

2.5.3. The effects of different parameters on the adsorption amount and the desorption ratio of AB-8 resin

The crude polyphenol solutions were diluted with distilled water to different TPCs (0.2, 1.0, 1.5, 2.0, and 2.5 mg CAE/ml). About 50 ml of each solution was adsorbed by 2 g of resin as described in Section 2.5.1 and the TPC of the solution was determined when adsorption had finished. The adsorption amounts at different sample concentrations were calculated to detect the effects of sample concentration on the adsorption capacities of AB-8 resin. The crude polyphenol solutions (TPC, 2.0 mg CAE/ml) were prepared and each of them was adjusted to a different pH

value (2.0, 3.0, 5.0, 7.0 and 8.0) using 1.0 mol/l HCl and 1.0 mol/l NaOH solutions. Then 50 ml of each solution was adsorbed by 2 g resin as described above and TPC of the solution was determined when adsorption had finished. The adsorption amounts were calculated in order to investigate the relationship between adsorption capacities and sample pH values.

After reaching equilibration, the resins were washed twice with 50 ml distilled water to remove impurities and each of the 2 g resins were desorbed as described in 2.4.1 by 50 ml ethanol solution at different concentrations [30%, 50%, 70%, 90% and 100% (v/v)]. The desorption ratios were calculated in order to investigate the relationship between desorption ratio and ethanol concentration.

2.6. Dynamic adsorption and desorption

2.6.1. Effects of flow rate on dynamic adsorption and desorption

Dynamic adsorption and desorption experiments were carried out in a glass column (1 cm × 10 cm) wet-packed with pretreated AB-8 resin. The bed volume (BV) of the resins was 10 ml (equal to 5 g resin). Then 50 ml sample solutions (TPC, 2.0 mg CAE/ml, pH 3.0) were allowed to flow through the glass column at different flow rates (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 BV/h). The TPCs of the effluent solution were determined and the resin adsorption amounts were calculated in order to investigate the relationship between adsorption capacities and flow speed. After the adsorption equilibrium had been reached, the column was first washed with 100 ml distilled water at a flow rate of 1.0 BV/h and then eluted by 50 ml 70% (v/v) ethanol solution at different flow rates (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 BV/h). After desorption, the TPC of the effluent solution was determined and the desorption ratio of the resin was calculated so that the effects of elution speed on the desorption ratio of the resin could be determined.

2.6.2. Dynamic handling capacity of AB-8 resin under optimal conditions

Exactly 100 ml sample solution (TPC, 2.0 mg CAE/ml, pH 3.0) was allowed to flow through the glass column at a flow rate 1.0 BV/h. The bed volume was 10 ml. The eluted solution was collected after every 5 ml of solution had flowed through and the TPC was determined. The resin was defined as being at adsorption equilibrium when the TPC of the eluted solution was a tenth of the initial sample solution and the total volume of eluted solution was the dynamic handling capacity of the resin. After the adsorption equilibrium had been determined, the column was first washed with 100 ml distilled water and then eluted by 70% (v/v) ethanol solution at a flow rate of 1.0 BV/h. The eluted solution was collected after every 5 ml of solution had flowed through and the TPC was determined. The resin was defined as reaching desorption equilibrium when the TPC of the eluted solution was at its lowest level and did not substantially change.

2.7. Preparative purification under optimal conditions

Yuzi No. 7 and Ximeng No. 1 crude polyphenol solutions were dynamically adsorbed and desorbed by an AB-8 macroporous resin column. The eluted solution was collected and concentrated in a rotary evaporator at 45 °C to remove the ethanol, and then freeze-dried. The purified products were weighed and dissolved in distilled water to make sample solutions. The TPCs of the sample solutions were determined and the polyphenol purification level was calculated using the following formula (3):

$$P = 100\ CV/M \tag{3}$$

where P is the polyphenol purification level (%); C is the TPC of the sample solution (mg CAE/ml); V is the sample solution volume (ml) and M is the sample weight (mg).

2.8. HPLC analysis

Qualitative and quantitative analyses of the purified sweet potato leaf polyphenol products were carried out by an Agilent1200 series HPLC, which consisted of a Model G1322A degasser, a Model G1311A quat pump, a Model G1329A auto injector, a Model G1316 column oven and a Model C1315D diode array detector. The separation was completed using a ZORBAX Eclips Plus C18 (4.6 × 150 mm, 5 μm). The mobile phase consisted of ultrapure water containing 0.5% (v/v) phosphoric acid (A) and acetonitrile (B). The elution was performed with the following linear gradient: 0–15 min: 20–65% B, 15–15.1 min: 65–80% B and 15.1–20 min: 80% B. The flow rate was 1 ml/min, the injection volume was 20 μl, the column oven temperature was 40 °C and the constituents were detected at 326 nm.

The standards were accurately weighed and dissolved in methanol to prepare a stock solution (1 mg/ml) and stored in a refrigerator until needed. Each of the standard stock solutions were diluted to 50 μg/ml with 80% (v/v) methanol. Mixed standard solutions (0.5, 1.0, 5.0, 10.0 and 50.0 μg/ml) were also prepared so that a corresponding standard curve could be created. Each peak in the mixed standard chromatogram was identified by comparing the retention time with the single standard chromatogram. All solutions were filtered through a 0.45 μm membrane and analysed as described above.

The purified products of the sweet potato leaf polyphenols from the two cultivars (Yuzi No. 7 and Ximeng No. 1) were accurately weighed and then dissolved in 80% (v/v) methanol to prepare a sample solution (200 μg/ml), which was then analysed as described above.

2.9. Antioxidant activity analysis

2.9.1. Photochemiluminescence assay

The antioxidant activity of the purified products was determined in triplicate using an automated photo chemiluminescent (PCL) system (Photochem, Analytik Jena AG, Germany), which measures the capacity of a sample to quench superoxide anion radicals (O_2^-). This system is based on a controlled photochemical generation of radicals, part of which is quenched by antioxidants present in the sample. The remaining radicals in the sample are quantified by a sensitive chemiluminescence-detection method as reported by Cofrades et al. (2011). Briefly, 20 μl sample solution at different concentrations (5, 10 and 20 μg/ml) was used as the antioxidant capacity in the water soluble components kit during antioxidant capacity determination. Ascorbic acid was used as the standard. The results were expressed as μg ascorbic acid equivalents per ml sample solution (μg ACE/ml).

2.9.2. Oxygen radical absorbance capacity assay

Oxygen radical absorbance capacity (ORAC) assay was carried out following the procedure established by Prior et al. (2003) with slight modification. All samples and reagents in this experiment were dissolved and diluted with phosphate buffer (0.075 M, pH 7.4). Briefly, 20 μl sample solutions at different concentrations (5, 10, and 20 μg/ml) were added to 20 μl phosphate buffer and then mixed with 20 μl 63 nmol/l sodium fluorescein solution in a clear 96-well microplate and incubated at 37 °C for 15 min. Then, 140 μl 18.28 mmol/l AAPH solution was rapidly added to the well. After vigorous shaking, the microplate was placed in the multifunctional microplate reader (Hidex Ltd. Co., Finland). The system was set in fluorescence mode and the fluorescence intensity of each well was read 60 times at 2 min intervals. The excitation and emission filter wavelengths were set at 485 nm and 535 nm and the detection temperature was 37 °C.

The fluorescence intensity of each sample was determined without the effect of AAPH (i.e. the AAPH solution was replaced by the same amount of phosphate buffer) in order to calculate the relative fluorescence intensity using formula (4). The relative fluorescence intensity was used to calculate the area under the curve (AUC) by the approximate integration method shown in formula (5). The ORAC values were expressed by the net area under the curve (netAUC) between the samples and the blank, as shown in formula (6). A calibration curve for the trolox standards (at concentrations of 5, 10, 20, 40 and 60 μg/ml) was prepared. The linear regression equation was $y = 0.8898x + 2.5805$ and $R^2 = 0.9929$. The ORAC values of the samples were expressed as μg trolox equivalent per ml of sample solution (μg TE/ml).

$$F_i = f_i(+\text{AAPH})/f_i(-\text{AAPH}) \quad (4)$$

$$\text{AUC} = \Delta t \times (F_0 + F_1 + \ldots + F_n) - F_1 - F_n \quad (5)$$

$$\text{netAUC} = \text{AUC}_{\text{sample}} - \text{AUC}_{\text{blank}} \quad (6)$$

where $f_{i(+\text{AAPH})}$ is the fluorescence intensity of the reaction solution containing the AAPH solution; $f_{i(-\text{AAPH})}$ is the fluorescence intensity of the reaction solution without AAPH; F_i is the relative fluorescence intensity of the reaction solution; AUC is the area under the curve; Δt is the interval time and the value of Δt in this study was 2; $\text{AUC}_{\text{sample}}$ and $\text{AUC}_{\text{blank}}$ are the AUCs of the sample and the blank, respectively and netAUC is the net area under the curve between the sample and the blank.

2.10. Statistical analysis

All the experiments were run in triplicate. The experimental data were subjected to analysis of variance (ANOVA) and the differences between means were evaluated by Duncan's Multiple Range Test with $p < 0.05$ considered to be a statistically significant difference. Data analysis was performed using SAS 9.2 and Origin 8.0 data analysis software.

3. Results and discussion

3.1. Static adsorption and desorption

3.1.1. Static adsorption and desorption kinetics

The adsorption and desorption kinetics of AB-8 macroporous resins on sweet potato leaf polyphenols are shown in Fig. 1(A). The sweet potato leaf polyphenols were adsorbed rapidly by AB-8 macroporous resins. Within 1 h, the adsorption level had increased sharply. After 3 h, the adsorption level did not show any further significant changes, which suggested that the adsorption equilibrium occurred at 3 h when the adsorption amount was 20.06 mg CAE/g. As shown by the desorption curve, the polyphenols absorbed into the resin were desorbed effectively by 70% (v/v) ethanol solution and the desorption ratio increased rapidly during the initial stage (within 1 h). After 2 h, the desorption ratio did not change significantly and equilibrium occurred when the desorption ratio was 82.51%.

3.1.2. Adsorption isotherms

The adsorption properties of macroporous resins vary for different targets and this is decided by the macroporous resin adsorption mechanism. Analysis of the adsorption mechanism of resins can enable researchers to obtain a better understanding of the whole adsorption process, which means that they can better control the process and get a desirable adsorption result (Zhang, Liu, Chen, Li, & Zhao, 2014). Adsorption isotherms can indicate the qualitative interaction between the adsorbent and the adsorbate. Adsorption isotherms, together with some adsorption equations, can elaborate the accumulation pattern of targets onto the resin surface at a constant temperature. The adsorption process and mechanism of a resin can be inferred from the adsorption isotherm, which improves control of the adsorption programme (Kammerer, Boschet, Kammerer, & Carle, 2011).

The Langmuir and Freundlich equations are frequently used to describe adsorption isotherms because of their relative simplicity and reasonable accuracy (Lin, Zhao, Dong, Yang, & Zhao, 2012). The Langmuir isotherm is usually used when there is an ideal monolayer adsorption on a homogeneous surface (Saeed, Iqbal, & Höll, 2009). The Freundlich isotherm is usually suitable for non-ideal adsorption on heterogeneous surfaces and it assumes that there are a large number and many different types of available sites acting simultaneously, each with a different free energy of sorption (García, González, & Isasi, 2009).

The models are expressed by the following equations:

Langmuir equation: $Q_e = Q_m K_L C_e/(1 + K_L C_e)$

Where Q_e is the amount of total polyphenols absorbed per unit mass of resin, mg CAE/g; C_e is the equilibrium concentration of total polyphenols in solution (mg CAE/ml); Q_m is the theoretical maximum adsorption capacity (mg CAE/g) and K_L is a constant related to the free energy of adsorption.

Freundlich equation: $Q_e = K_F C_e^{1/n}$

Where Q_e is the amount of total polyphenols absorbed per unit mass of resin (mg CAE/g); K_F is the Freundlich constant, which represents adsorption capacity; $1/n$ is an empirical constant indicating

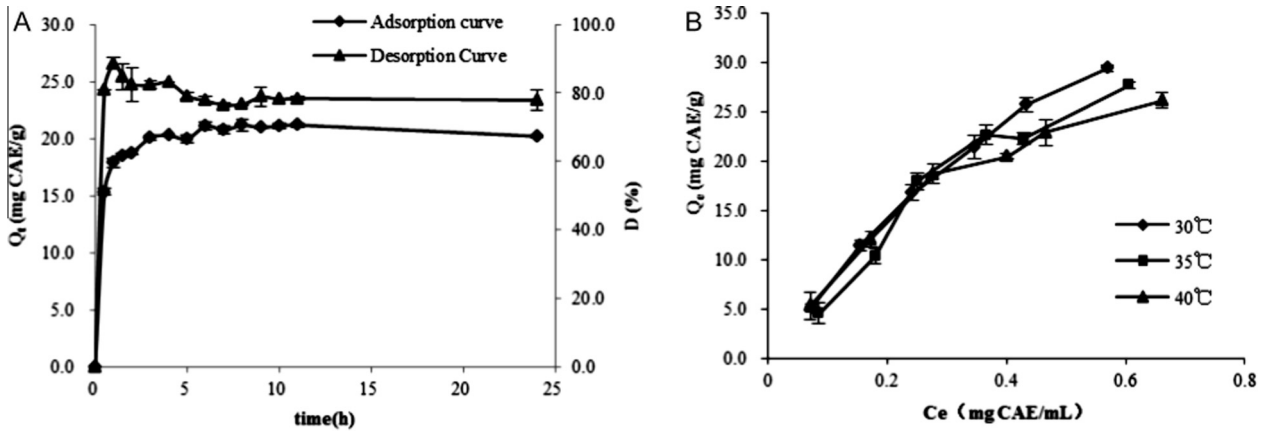

Fig. 1. Adsorption and desorption properties of AB-8 resin. (A) Static adsorption and desorption curve. (B) Adsorption isotherms of AB-8 resin (Q_e was the adsorption capacity of resin at time t, mg CAE/g resin; D was the desorption ratio, %; Q_e was the equilibrium adsorption capacity, mg CAE/g resin).

the adsorption intensity of the system and C_e is the equilibrium concentration of total polyphenols in solution (mg CAE/ml).

The equilibrium adsorption isotherm for total polyphenols on AB-8 resin was measured at 30 °C, 35 °C and 40 °C. Fig. 1(B) shows that the adsorption capacity rose as the equilibrium concentration of total polyphenols increased, which confirmed the excellent adsorption of sweet potato leaf polyphenols to AB-8 resin. Two model parameters and coefficients of determination (R^2) are listed in Table 1. The Langmuir equation described the adsorption behaviour of polyphenols on AB-8 resin better than the Freundlich equation since the related coefficient of the Langmuir equation was higher than that of the Freundlich equation at all the temperatures selected. Q_m in the Langmuir equation is the theoretical maximum adsorption capacity and the higher the Q_m value, the better the adsorption of the resin. Table 1 shows that, the Q_m value decreased as the temperature increased and a similar result was found by Wu, Li, Ming, and Zhao (2010). This was presumably due to the swelling of resin and the increasing kinetic energies of the polyphenol molecules at higher temperatures (Sun et al., 2013). The result also suggested that the adsorption process was an exothermic reaction, namely, a relatively low temperature facilitated sorption of polyphenols onto AB-8 resin. In the Freundlich model, it is relatively easy for a sorbent to adsorb solute when $1/n$ is less than 1. In Table 1, all $1/n$ values were less than 1, which indicated that AB-8 resin was good at adsorbing sweet potato leaf polyphenols.

3.1.3. Effect of initial sample TPC on the adsorption capacity of AB-8 resin

The effect of initial TPC on static adsorption is shown in Fig. 2(A). The adsorption capacity rose as the original TPC increased if the original TPC was less than 2.0 mg CAE/ml. The highest adsorption capacity was observed when the original TPC was 2.0 mg CAE/ml. However, the adsorption capacity decreased if the original TPC was higher than 2.0 mg CAE/ml. When the sample concentration was low, the adsorption capacity increased as the TPC rose because the number of active sites related to the polyphenols increased. However, with further TPC increases, more impurities were adsorbed on the AB-8 resin, resulting in competition for active sites between the polyphenols and the impurities (Sun et al., 2013), which led to a slight drop in adsorption capacity.

3.1.4. Effect of sample solution pH values on the adsorption capacity of AB-8 resin

The pH value of the sample solution is very important for the adsorption properties of resins because the pH value determines the extent to which polyphenols ionise, thereby affecting the adsorption of polyphenols onto the resin surface from solution. It also influences the physical interaction between polyphenols and the adsorptive sites of the adsorbent (Liu, Shan, & Wang, 2009). The effect of pH value on the adsorption of AB-8 resin is shown in Fig. 2(B). The adsorption capacity of AB-8 resin decreased as the pH value rose, especially when the pH value was higher than 6.0. Our result was similar to Wang et al. (2013) who found that the adsorption capacity of a resin declined as the pH value of a pomegranate polyphenol solution rose. The phenolic molecules were slightly polar and acidic, which made them sensitive to pH value. In the low pH value solution, the polyphenols exist as molecules that are easily identified and adsorbed by the resin. In contrast, in relatively high pH solutions, the polyphenols might exist as ions due to the ionisation reaction, which are more difficult to adsorb (Wang et al., 2013). It has been reported that plant polyphenols were relatively stable in solution when the pH ranged between 3 and 5 (Zhang, Yang, Zhao, & Liu, 2008). As the adsorption capacities did not change significantly at pH 2.0 and 3.0, pH 3.0 was chosen as the optimum pH value of the sample solution.

Table 1
Langmuir and Freundlich parameters of sweet potato leaf polyphenols on AB-8 resin.

Temperature (°C)		30	35	40
Langmuir equation	Q_m	74.4464	64.8734	43.2006
	K_L	1.1784	1.2893	2.3838
	R^2	0.9961	0.9451	0.9834
Freundlich equation	K_F	46.5142	42.1401	34.9736
	$1/n$	0.7480	0.7300	0.5882
	R^2	0.9856	0.9185	0.9512

3.1.5. Effect of ethanol concentration on the desorption ratio of AB-8 resin

As shown in Fig. 2(C), the desorption ratio increased as the ethanol concentration rose from 30% (v/v) to 70% (v/v) and the highest desorption ratio of 90.9% was observed when the ethanol concentration was 70% (v/v). The desorption ratio did not significantly change when the ethanol concentration continued to increase and similar results were found by Yin et al. (2010) and He and Xia (2008). Polyphenols cannot dissolve in low concentrations of ethanol, whereas some impurities are desorbed at high ethanol concentrations (Sun et al., 2013). Therefore, 70% (v/v) ethanol solution was selected as the optimum ethanol concentration.

3.2. Dynamic adsorption and desorption

3.2.1. Effect of flow and elution speed on the adsorption capacity and the desorption ratio of AB-8 resin

Liquid flow rate affected the reaction between solute and resin and further affected the adsorption capacity and desorption ratio of the resin (Liu et al., 2009). Fig. 2(D) shows that the adsorption capacity stayed at a high level when flow speed was lower than 1 BV/h, but decreased when the speed was higher than 1 BV/h. This might suggest that some of the polyphenols leaked out without being adsorbed by the resin due to the high flow speed (Fu et al., 2006). Therefore, 1.0 BV/h was selected as the optimal flow speed because the adsorption capacity did not show any significant differences at speeds of 0.5 and 1.0 BV/h and the production efficiency was too low when the flow speed was lower than 1.0 BV/h, Similar regulation was observed between elution speed and the desorption ratio. The desorption ratio was higher than 90% when the elution speed was less than 1.5 BV/h. However, the desorption ratio decreased when the elution speed was higher than 1.5 BV/h, which indicated that polyphenols could be desorbed more completely at low elution speeds. The possible reason for this was that ethanol may be entering the resin micropores, which meant that polyphenols could be dissolved and eluted more thoroughly at low elution speeds (Jia & Lu, 2008). The desorption ratios at elution speeds of 0.5, 1.0 and 1.5 BV/h were not significantly different, so 1.0 BV/h was selected as the optimal elution speed.

3.2.2. Dynamic adsorption and desorption properties under optimum conditions

A sample solution (TPC, 2.0 mg CAE/ml, pH 3.0) was injected into the AB-8 resin column at a speed of 1.0 BV/h, and with a bed volume of 10 ml. The dynamic adsorption curve is shown in Fig. 2(E). The TPC of the effluent solution was less than 0.15 mg CAE/ml when the injection volume was less than 3 BV, which indicated that polyphenol leakage stayed at a low level. When the injection volume was 5 BV, the TPC of the effluent solution was 0.2 mg CAE/ml, which was a tenth of the initial concentration. This indicated that the dynamic adsorption equilibrium had been reached. The 5 BV sample solution of sweet potato leaf polyphenols could be dynamically adsorbed by AB-8 resin and the adsorption capacity reached 26.8 mg CAE/g. The saturated resin was eluted

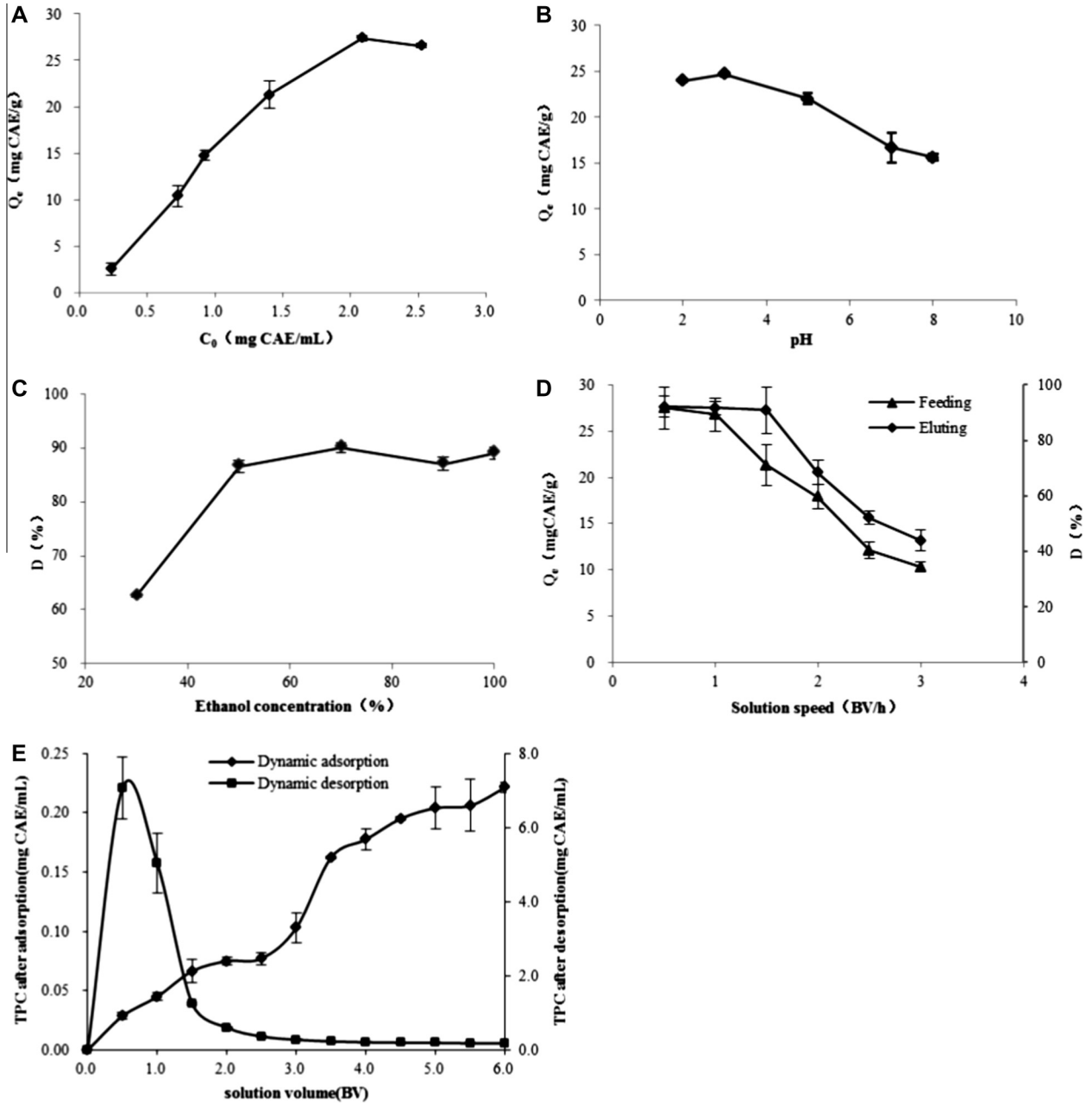

Fig. 2. Factors affect the adsorption and desorption properties of AB-8 resin (A) Effect of sample concentration on adsorption capacity of AB-8 resin. (B) Effect of sample pH value on adsorption capacity of AB-8 resin. (C) Effect of ethanol concentration on desorption ratio of AB-8 resin. (D) Adsorption and desorption ratios of AB-8 resin at different flow rates. (E) Dynamic adsorption and desorption properties at the optimal parameters (C_0 was the initial TPC of sample solution, mg CAE/mL; Q_e and D was the same as described in Fig. 1.).

by 70% (v/v) ethanol solution at 1.0 BV/h. Fig. 2(E) shows the elution peak did not possess a tail, which was in agreement with Zhang et al. (2008). The desorbed polyphenols were mainly concentrated in the 0–2.0 BV effluent solutions, which suggested that most of polyphenols were desorbed by low concentration ethanol solutions. The desorption equilibrium occurred when the resin was eluted by 3 BV ethanol solution with a desorption ratio of 90.9%.

3.3. Qualitative and quantitative analysis of sweet potato leaf polyphenols

After the samples were purified under optimal conditions, 5.56 ± 0.47 and 6.03 ± 0.61 g of purified products were obtained from 100 g Yuzi No. 7 and Ximeng No. 1 sweet potato leaf powder, respectively, with polyphenol purifications of 87.33 ± 1.53% and 82.67 ± 4.51%, respectively.

The identification results for the purified polyphenols by HPLC are shown in Fig. 3 and Table 2. Sweet potato leaf polyphenols consisted mainly of seven caffeoylquinic acids and a small amount of caffeic acid, which was similar to several previous reports (Islam et al., 2002; Jung, Lee, Kozukue, Levin, & Friedman, 2011; Padda & Picha, 2008). Three di-caffeoylquinic acid contents were relatively higher in Yuzi No. 7 than in Ximeng No. 1. The 3,5-CQA (31.39 ± 0.26% DW) content was highest. The content of 3,4, 5-CQA was 2.64 ± 0.03% DW in Yuzi No. 7, which was lower than the di-caffeoylquinic acids, but higher than 3-CQA, 4-CQA and 5-CQA, while the 3-CQA content (0.87 ± 0.00% DW) was the lowest

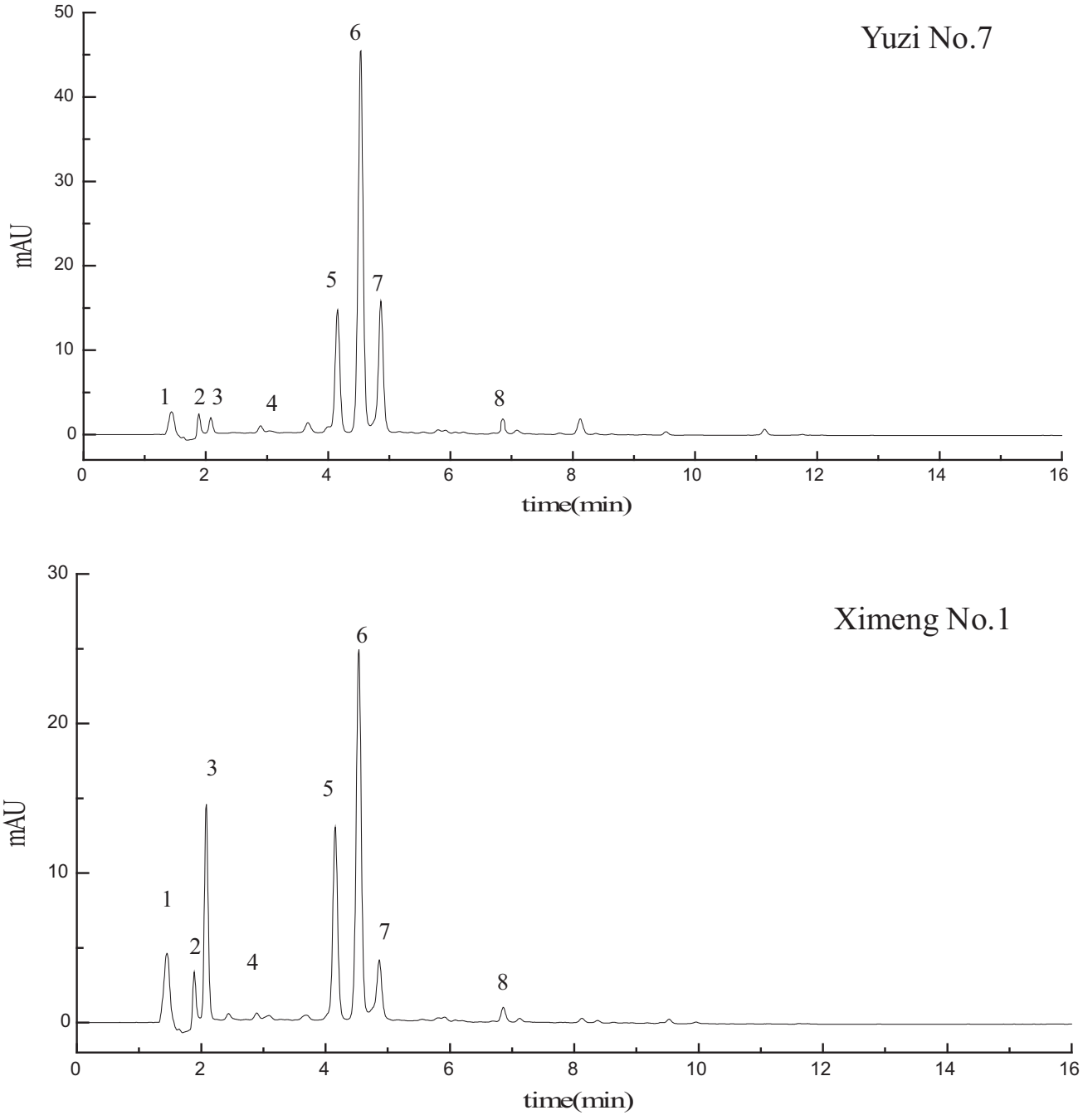

Fig. 3. The HPLC chromatography of sweet potato leaves polyphenols A: Yuzi No. 7, B: Simon No. 1. Peak 1: 5-CQA, peak 2: 3-CQA, peak 3: 4-CQA, peak 4: CA, peak 5: 4,5-CQA, peak 6: 3,5-CQA, peak 7: 3,4-CQA, peak 8: 3,4,5-CQA.

Table 2
The constituents of polyphenols purified from two sweet potato leaf cultivars (Yuzi No. 7 and Ximeng No. 1).

Peak No.	Retention time (min)	Identification	Standard curve	R^2	Yuzi No. 7		Simon No. 1	
					Peak area	Content (%, DW)	Peak area	Content (%, DW)
1	1.47	5-CQA	$y = 11.372x - 0.4279$	0.9962	55.55 ± 0.92	2.46 ± 0.03	130.85 ± 7.85	5.77 ± 0.25
2	1.91	3-CQA	$y = 9.9086x + 0.2857$	1.0000	17.45 ± 0.07	0.87 ± 0.00	34.65 ± 0.64	1.73 ± 0.02
3	2.10	4-CQA	$y = 25.894x - 17.128$	0.9988	35.90 ± 0.57	1.02 ± 0.01	277.35 ± 0.92	5.69 ± 0.01
4	2.92	CA	$y = 28.183x - 1.2114$	1.0000	3.80 ± 0.14	0.36 ± 0.01	384.90 ± 0.99	0.22 ± 0.01
5	4.16	4,5-CQA	$y = 9.2077x - 7.244$	0.9987	378.7 ± 57.00	20.96 ± 0.27	683.15 ± 3.13	21.29 ± 0.04
6	4.54	3,5-CQA	$y = 18.056x - 18.405$	0.9981	1115.10 ± 13.29	31.39 ± 0.26	132.80 ± 0.14	19.45 ± 0.06
7	4.88	3,4-CQA	$y = 15.353x - 12.021$	0.9987	388.30 ± 4.67	13.04 ± 0.11	31.95 ± 0.21	4.72 ± 0.00
8	6.87	3,4,5-CQA	$y = 6.2184x - 5.1579$	0.9949	27.70 ± 0.57	2.64 ± 0.03	1.95 ± 0.07	2.98 ± 0.01
Sum						72.74 ± 0.99		61.86 ± 0.25

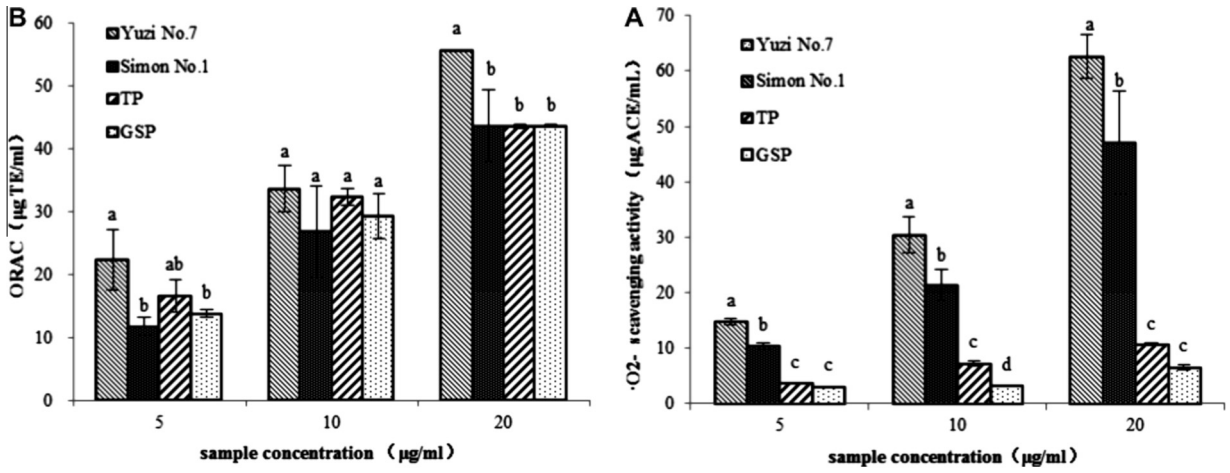

Fig. 4. Antioxidant activities of purified sweet potato leaf polyphenols. (A) $\cdot O_2^-$ scavenging activity of samples determined by PCL method. (B) Oxygen radical absorbance capacity of samples determined by ORAC method.

among the caffeoylquinic acids. The 4,5-CQA content (21.29 ± 0.04% DW) was highest in Ximeng No. 1, followed by 3,5-CQA (19.45 ± 0.06% DW). The 5-CQA (5.77 ± 0.25% DW) and 4-CQA (5.69 ± 0.01% DW) contents in Ximeng No. 1 were higher than in Yuzi No. 7. The total content of these eight phenolic constituents in Yuzi No. 7 was 72.74 ± 0.99%, which was lower than the TPC (87.33 ± 1.53%) determined by the FC method. This result also occurred with Ximeng No. 1. It is possible that the detection range of the HPLC was different from the FC method. Specifically, some unknown phenolic constituents, indicated by some small absorption peaks in the HPLC chromatograms, were not identified by the HPLC method, but were detected by the FC method.

3.4. Antioxidant activity of sweet potato polyphenols

The antioxidant activity of sweet potato leaf polyphenols was measured by the PCL and ORAC methods. Tea polyphenols (TP) and grape seed polyphenols (GSP) were chosen as the positive controls. Sweet potato leaf polyphenols showed strong concentration-dependent scavenging activity against O_2^- radicals [Fig. 4(A)]. The O_2^- radical scavenging activities of Yuzi No. 7 and Ximeng No. 1 were higher than TP and GSP at all concentrations selected. When the sample concentration was 20 μg/ml, the O_2^- radical scavenging activity of Yuzi No. 7 was 62.61 ± 4.02 μg ACE/ml, which was 3.1, 5.9 and 9.6 times higher than ascorbic acid, TP and GSP, respectively; at the same sample concentration. The activity of Ximeng No. 1 was 47.06 ± 9.03 μg ACE/ml, which was 2.4, 4.4 and 7.2 times higher than ascorbic acid, TP and GSP, respectively. Fig. 4(B) shows that the oxygen radical absorbance capacity of sweet potato leaf polyphenols increased as the sample concentration rose. At 5 and 10 μg/ml, the sweet potato leaf polyphenol activities were not significantly different to TP and GSP. When the concentration of sweet potato leaf polyphenols was 20 μg/ml, the activity of Yuzi No. 7 was 55.78 ± 0.05 μg TE/ml, which was significantly higher than the other samples, and was 2.8, 1.3 and 1.3 times higher than trolox, TP and GSP, respectively. The activity of Ximeng No. 1 was 43.72 ± 5.72 μg TE/ml, which was 2.2 times higher than trolox, but was not significantly different from TP and GSP.

Sweet potato leaf polyphenols possess strong antioxidant activities *in vitro* and these activities have a close relationship with their constituents. It has been reported that the radical scavenging activity of caffeoylquinic acids had a positive correlation with the amount of coffee acyls in molecules. Iwai, Kishimoto, Kakino, Mochida, and Fujita (2004) found that di-caffeoylquinic acid activities were 2.0 times higher than mono-caffeoylquinic acids and 1.0–1.8 times higher than ascorbic acid, which was in accordance with this study's results. Phenolic antioxidant activity is not only affected by the number of hydroxyl groups, but also by the activity of electron donators and the electron donating activity of caffeic acid is higher than catechin (main constituent of TP) (Medina, Gallardo, González, Lois, & Hedges, 2007). This agrees with the results from this study, which showed that the antioxidant activities of sweet potato leaf polyphenols were higher than TP.

4. Conclusions

The adsorption and desorption properties of AB-8 macroporous resin for sweet potato leaf polyphenols was investigated and the processing parameters were optimised. The 5 BV sample solution can be dynamically adsorbed by AB-8 resin column and the resin column at adsorption equilibrium can be desorbed thoroughly by a 2 BV ethanol solution. More than 5 g of purified polyphenols was obtained from 100 g sweet potato leaf powder under optimal conditions. The purified polyphenols mainly consisted of caffeoylquinic acids, especially three types of di-caffeoylquinic acid, and possess strong O_2^- radical scavenging activities and oxygen radical absorbance capacities.

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