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Isolation of α-arbutin from *Xanthomonas* CGMCC 1243 fermentation broth by macroporous resin adsorption chromatography

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ABSTRACT

α-Arbutin is a glycosylated hydroquinone which has inhibitory function against tyrosinase. In this work, a one-step isolation of α-arbutin from *Xanthomonas* CGMCC 1243 fermentation broth by macroporous resin adsorption chromatography was investigated. The research results indicated that S-8 resin offered the best adsorption and desorption capacities for α-arbutin than others and its equilibrium adsorption data were well-fitted to the Freundlich isotherm. In order to optimize the operating parameters for separating α-arbutin, dynamic adsorption and desorption tests on S-8 column chromatography were carried out. Under optimized conditions (adsorption volume of 7 bed volume (BV), mobile phase of 25% (v/v) ethanol solution and elution volume of 3 BV), the purity and recovery of α-arbutin were 97.3% (w/w) and 90.9% (w/w), respectively. The product was identified as α-arbutin by ^{13}C NMR and ^{1}H NMR analysis. Moreover, we scaled up S-8 column from laboratory test (10 cm × 2 cm ID) to large scale (500 cm × 100 cm ID) without diminishing α-arbutin yield. In conclusion, the results in this work provide a one-step and cost-effective method for large-scale production of α-arbutin.

1. Introduction

α-Arbutin, a glycosylated hydroquinone, is commercially used in the cosmetic industry. It has inhibitory function against tyrosinase, a critical enzyme for generating pigments, which leads to the prevention of melanin formation, resulting in a whitening effect on the skin [1]. It was reported that human tyrosinase inhibition of α-arbutin was much more effective than its isomer, β-arbutin, and the whitening effect of α-arbutin was more than 10 times higher than β-arbutin [2,3]. Worldwide demand for α-arbutin is increasing year by year, however whether α-arbutin can compete with β-arbutin is exclusively dependent on its cost in commercial production.

Although β-arbutin is found in various plants including bearberry, wheat, and pear, α-arbutin is mainly produced by enzymatic synthesis. Previously, α-arbutin can be synthesized through amylase from *Bacillus subtilis* [4], sucrose phospholylase from *Leuconostoc mesenteroides* [5], α-glucosidases from *Xunthomonas campestris* and *Saccharomyces cerevisiae* [6–8], and dextransucrase from *L. mesenteroides* [9]. However, there are still several issues that need to be addressed in order to produce α-arbutin biotechnologically within the targeted cost, such as the development of high-performance α-arbutin-producing microorganisms and lowering of the costs of product isolation processes. As far as we know, until now there are few systematic reports on isolation and purification for large-scale production of α-arbutin. Growing attention has been taken to separation of targeted component from other impurities in crude biological samples using polymeric resins for their convenience, low cost, high chemically stability, easy regeneration and adjusted selectivity by modification of surface chemistry and controlling pore structure [10–12].

In our previous experiments, α-arbutin was successfully synthesized by using the α-anomer-selective strain, *Xanthomonas* CGMCC 1243 [13]. The purpose of the present study is to develop a cost-effective method for large-scale isolation of α-arbutin. The adsorption and desorption properties of α-arbutin on different macroporous resins, and a one-step macroporous resin adsorption chromatography method for α-arbutin isolation were investigated. Finally, the chemical structure of the product was confirmed by nuclear magnetic resonance (NMR) analysis. This work is useful for inexpensive and large-scale production of α-arbutin.

2. Materials and methods

2.1. Chemicals and reagents

α-Arbutin and β-arbutin standards were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Methanol

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Table 1
Physical properties of the adsorbents used for α-arbutin isolation.

Name	Polarity	Particle diameter (mm)	Surface area (m^2/g)	Average pore diameter (nm)
NKA-9	Polar	0.3–1.25	250–290	15.5–16.5
NKA-II	Polar	0.3–1.25	160–200	14.5–15.5
S-8	Polar	0.3–1.25	100–120	28.0–30.0
AB-8	Weak-polar	0.3–1.25	480–520	13.0–14.0
HPD100	Non-polar	0.3–1.25	600–630	10.0–12.0
HPD700	Non-polar	0.3–1.25	650–700	8.5–9.0
D3520	Non-polar	0.3–1.25	480–520	8.5–9.0
D4006	Non-polar	0.3–1.25	400–440	6.5–7.5
D4020	Non-polar	0.3–1.25	540–580	10.0–10.5
H103	Non-polar	0.3–1.25	900–1100	8.4–9.4
H107	Non-polar	0.3–1.25	1000–1300	8.5–10.0
X-5	Non-polar	0.3–1.25	500–600	29.0–30.0

(chromatography grade, for high-performance liquid chromatography (HPLC) analysis) was purchased from Beijing Chemical Factory (Beijing, China). Ultra-pure water used for analytical and preparative HPLC was produced by Mili-pore Q System (Millipore, USA). All other chemicals and reagents were analytical grade and purchased from Beijing Chemical Factory (Beijing, China).

2.2. Preparation of sample solution

The fermentation broth of *Xanthomonas* CGMCC 1243 from our laboratory was used in this study (the concentration of α-arbutin was 12.3 g/L) [13]. The sample solution was prepared by centrifuging the fermentation broth at 4200 rpm for 20 min to remove the mycelia and other insoluble impurities. After centrifugation, the supernatant was diluted to 10 g/L of α-arbutin and submitted as sample solution.

2.3. Selection of macroporous resins

Macroporous resins including NKA-9, NKA-II, S-8, AB-8, D3520, D4006, D4020, H103, H107 and X-5 were purchased from Chemical plant of Nankai University (Tianjin, China), while HPD100 and HPD700 from Cangzhou Baoen Chemical Co., Ltd. (Hebei, China). The physical properties of the adsorbents are summarized in the Table 1. The adsorbents needed to be pretreated to remove monomers and porogenic agents trapped inside the pores during the synthesis process prior to use, and then dried at 50 °C under vacuum. Prior to adsorption experiments, pre-weighed amounts of resins were soaked in 95% (v/v) ethanol and washed thoroughly with distilled water.

2.4. Static adsorption and desorption tests

Static adsorption and desorption tests were performed as follows: 1.0 g (dry weight) of pretreated resin was put into three air-tight Erlenmeyer flasks respectively. 15.0 mL sample solution (10 g/L) was added into each flask. The flasks were then shaken at 20 °C with 120 rpm for 12 h. After adsorption equilibrium was reached, the resin was separated from sample solution by filtration and washed by distilled water, then desorbed with 15.0 mL of 25% (v/v) ethanol solution. The flasks were shaken at 20 °C with 120 rpm for 12 h. The adsorption and desorption solutions were analyzed by HPLC. The preliminary selection of the resins was evaluated by their adsorption capacities, desorption capacities and desorption ratios. Then, adsorption kinetics tests, adsorption isotherms (20, 25, and 30 °C), and effects of pH (4.0, 5.0, 6.0, 7.0 and 8.0) of the selected resin were investigated.

2.5. Dynamic adsorption and desorption tests

Dynamic adsorption and desorption tests were carried out on a normal pressure glass chromatographic column (10 cm × 2 cm ID) wet-packed with pretreated S-8 macroporous resin (10 g dry weight) at a BV (bed volume) of 18.9 mL (height of the resin bed was 6.0 cm). Seven BV of sample solution was loaded onto the column and adsorbed at a flow rate of 1.0 BV/h. After adsorption, the column was eluted with distilled water to remove the impurities such as proteins and residual sugars [14]. Then, the α-arbutin rich extract was eluted with 4 BV of 25% (v/v) ethanol solution at a flow rate of 0.5 BV/h and all fractions were carefully collected. At last, 95% (v/v) ethanol was used for resin regeneration. In this way, breakthrough curve, effects of mobile phase compositions (20% (v/v) methanol solution, 25% (v/v) ethanol solution and 45% (v/v) ethyl acetate solution) and elution curve for the selected resin were investigated.

2.6. Analytical methods

The HPLC equipment used was a Shimadzu LC-10ATvp system (Shimadzu, Kyoto, Japan) with two LC-10ATvp solvent delivery units, an SPD-10Avp UV-VIS detector, a CTO-10ASvp column oven (Shimadzu, Kyoto, Japan), a T2000P workstation (Huilong, Beijing, China) and a reversed phase C18 column (250 × 4.6 mm, 5 μm, Diamodsil™).

HPLC conditions of all the samples in this paper were the same. The mobile phase was composed of methanol aqueous solution at a volumetric ratio of 5:95, and run at a flow rate of 1.0 mL/min. Absorbance wavelength was set at 280 nm. Each sample was filtered through 0.45 μm micro-membrane, and 10 μL of the resulting filtrate was loaded into the HPLC system for a single run. Each run of culture experiments and analysis was triplicated. The working calibration curve on α-arbutin standard solution showed good linearity over the range of 0.5–10.0 g/L. The regression line for α-arbutin was $Y = 1{,}000{,}000X + 59{,}668$ ($R^2 = 0.9996$), where Y and X are peak area and the concentration of α-arbutin (g/L), respectively.

The adsorption capacities of the adsorbents were calculated from the following equation:

$$Q_e = \frac{(C_o - C_e)V_i}{W} \quad (1)$$

where Q_e is the adsorption capacity per gram resin at adsorption equilibrium (mg/g resin), C_o and C_e are the initial and equilibrium concentrations of α-arbutin in the solutions, respectively (mg/mL), V_i is the volume of the initial sample solution (mL) and W is the weight of the dry resin (g).

Table 2
Results of adsorption capacities and desorption ratios of different macroporous resins.

	NKA-9	NKA-II	S-8	AB-8	HPD100	HPD700
Adsorption (mg/g resin)	31.5	58.5	115.5	87	70.5	67.5
Desorption ratio (%)	86	95	93	79	80	78

	D3520	D4006	D4020	H103	H107	X-5
Adsorption (mg/g resin)	64.5	61.5	85.5	96	93	112.5
Desorption ratio (%)	85	89	82	92	91	95

The desorption capacities and desorption ratios of the adsorbents after adsorption equilibrium were calculated from the following equations:

$$Q_d = \frac{C_d V_d}{W} \quad (2)$$

$$D = \frac{Q_d}{Q_e} \times 100\% \quad (3)$$

where Q_d is the desorption capacity per gram resin after adsorption equilibrium (mg/g resin), C_d is the concentration of α-arbutin in 25% (v/v) ethanol solution (mg/mL), V_d is the volume of 25% (v/v) ethanol solution (mL) and D is the desorption ratio after adsorption equilibrium (%).

2.7. NMR

The eluate containing α-arbutin was carefully collected and concentrated by rotary evaporator to saturation, then crystallized at 4 °C for 12 h. The purity of the crystals reached 99.5% (w/w) by HPLC analysis. The product was identified by ^{13}C NMR and ^{1}H NMR spectrometry by center of analysis, Beijing University of Chemical Technology (Beijing, China). NMR spectra were performed in D_2O using a Bruker high-resolution AV600NMR spectrometer at 600 MHz (Bruker BioSpin Corporation, Billerica, MA).

3. Results and discussion

3.1. Adsorption capacities on macroporous resins

The preliminary evaluation of the performance of the adsorbents depended on their capacities of adsorption and desorption in the static tests. The adsorption capacities and desorption ratios of α-arbutin on macroporous resins are shown in Table 2. It shows that S-8, H103, H107 and X-5 had higher adsorption capacities for α-arbutin than other macroporous resins. From Table 2, it could also be discovered that desorption ratios of all adsorbents were almost the same. The resins with relatively larger surface areas (H103 and H107) as well as pore diameters around 30 nm (S-8 and X-5) were advantageous to the adsorption of α-arbutin molecule. By comparing the four resins, S-8 and X-5 were selected to further investigate their adsorption behavior towards α-arbutin.

3.2. Adsorption kinetics on selected resins

Adsorption kinetics curves for α-arbutin on the S-8 and X-5 resins are separately described in Fig. 1. It could be seen that adsorption capacities for α-arbutin increased with extension of adsorption time. α-Arbutin was rapidly captured in the initial 1 h, and thereafter a slowdown in adsorption rate was observed. These adsorption reached equilibrium at 2 h and this point could be considered as the equilibrium time for α-arbutin. Compared the data exhibited in Fig. 1, the adsorption capacity of S-8 towards α-arbutin was higher than that of X-5 at any time. In the comprehensive consideration of the desorption ratio, as well as adsorption capacity,

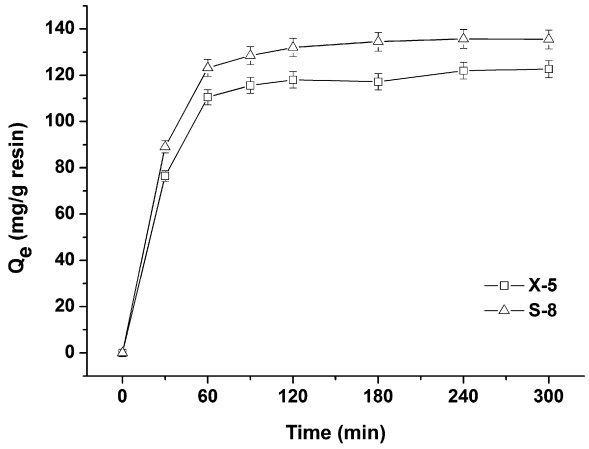

Fig. 1. Adsorption kinetics curves on S-8 and X-5 resins. Adsorption conditions: macroporous resins, 1.0 g; sample solution, 15 mL; rotation speed, 120 rpm; temperature, 20 °C.

S-8 was selected as the most suitable absorbent for the following test.

3.3. Adsorption isotherm

To investigate the adsorption capacity and characterize the adsorption behavior of α-arbutin, sample solutions with various concentrations of α-arbutin (2.5–12.5 g/L) were shaken with S-8 resin at 20, 25 and 30 °C. The results (Fig. 2) indicated that the adsorption capacity of S-8 resin increased with the increment of α-arbutin concentration, and reached the saturation plateau when the initial concentration of α-arbutin was 10 g/L. From Fig. 2, it also shows that the α-arbutin adsorption decreased with increasing temperature at the same initial sample concentration within

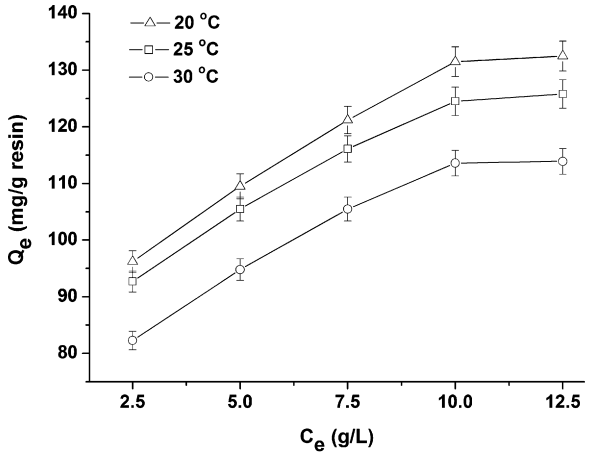

Fig. 2. Adsorption isotherms on S-8 at different temperatures. Adsorption conditions: macroporous resins, 1.0 g; sample solution, 15 mL; rotation speed, 120 rpm; temperature, 20, 25 and 30 °C.

Table 3
Freundlich equations on S-8 resin at different temperatures.

Temperature (°C)	Linearized form of Freundlich equation $\ln Q_e = (1/n) \times \ln C_e + \ln K$	K	$1/n$	R^2
20	$\ln Q_e = 0.21118 \times \ln C_e + 4.36935$	78.99	0.21	0.9817
25	$\ln Q_e = 0.19999 \times \ln C_e + 4.34559$	77.14	0.20	0.9857
30	$\ln Q_e = 0.21522 \times \ln C_e + 4.21451$	67.66	0.22	0.9785

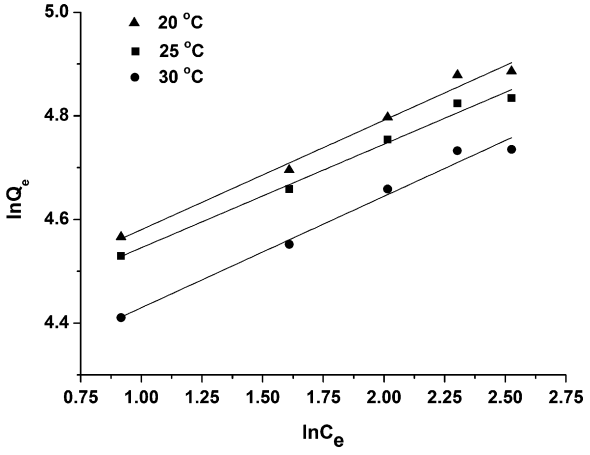

Fig. 3. Linear correlation on S-8 at different temperatures on the basis of the Freundlich model.

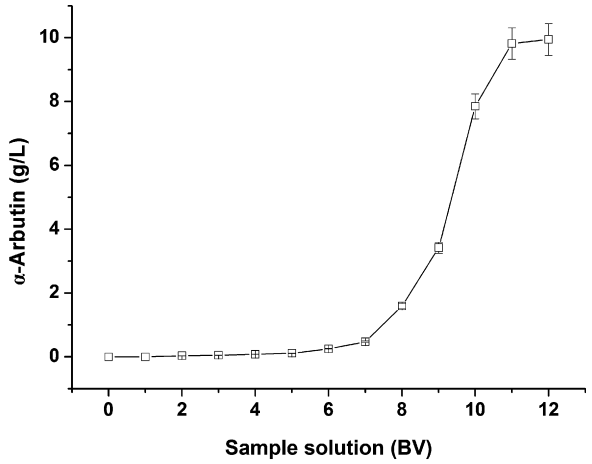

Fig. 4. Breakthrough curve on S-8 column. Adsorption conditions: sample solution, 10 g/L; flow rate, 1 BV/h.

the ranges of temperatures investigated. The reason is that the adsorption of macroporous resin is an exothermic process, and the increase of temperature is negatively related to the adsorption of the resin. Thus, the initial concentration of α-arbutin and temperature of adsorption were selected as 10 g/L and 20 °C, respectively. Under these conditions, adsorption capacity of S-8 resin reached 131.5 mg α-arbutin per gram resin.

In order to describe the interactions between solutes and resin at different temperatures, equilibrium data concerning the adsorption of α-arbutin onto S-8 resin at 20, 25 and 30 °C were further used to fit the Freundlich equation (4) (Fig. 3).

$$Q_e = KC_e^{1/n} \quad (4)$$

where Q_e is adsorption capacity, K is the Freundlich constant that is an indicator of adsorption capacity, and $1/n$ is an empirical constant related to the magnitude of adsorption driving force [15,16]. A linearized form of Eq. (4) can be written as

$$\ln Q_e = (1/n) \times \ln C_e + \ln K \quad (5)$$

The regression equations of Freundlich isotherm at different temperatures are summarized in Table 3. It was obvious that the correlation coefficients of Freundlich equations on S-8 for α-arbutin were rather high. That is to say the Freundlich law is applicable to the adsorption of α-arbutin on selected resin. The correlative parameters of Freundlich adsorption isotherm equations for α-arbutin at different temperatures are also listed in Table 3. Since K values decreased with the increment of temperature, raising temperature was not preferred with regards to the equilibrium of adsorption capacity. As can be seen in Table 3, the $1/n$ values in Freundlich equation were all between 0.1 and 0.5, indicating that α-arbutin could be easily captured by the resin [17]. Thus, S-8 resin was appropriate for the separation of α-arbutin.

This effect of temperature on adsorption observed for S-8 resin could be ascribed to its polarity structure (quaternary ammonium group) which can form hydrogen bonds with α-arbutin [18]. The thermal motion of solute molecules decreases with lowering the temperature, which is beneficial for the hydrogen bonding interactions between the solutes and resin. This result is in accordance with that obtained in the adsorption isotherms test.

3.4. Effects of sample solution pH on adsorption capacity

The adsorption capacity of macroporous resin is mainly through electrostatic force, hydrogen bonding interaction, complexation and size sieving action [19]. The pH value of sample solution affects the affinity between solutes and solutions by influencing the extent of ionization of solutes, thus affecting the adsorption capacity of macroporous resin. Taking into account the stability of α-arbutin that would break down easily in alkali and strong acid conditions [20], different pHs (4.0–8.0) were tested on resin adsorption (results are shown in Table 4). The highest adsorption capacity for α-arbutin was at the pH of 6.0, and then decreased with the increment of pH value. These results indicated that hydrogen bonding might play an important role in the adsorption process on S-8 resin. At a higher pH value, the hydrogen bonding interactions were reduced, because the phenolic hydroxyl groups in α-arbutin dissociated to H^+ and its corresponding anion, thus resulting in a lower adsorption capacity. Consequently, the pH value of sample solution was adjusted to 6.0 in subsequent investigation.

3.5. Dynamic adsorption and desorption tests on S-8 column

The one-step column chromatographic separation of α-arbutin content from *Xanthomonas* CGMCC 1243 fermentation broth was investigated on S-8 resin. The breakthrough curve on S-8 is shown in Fig. 4. At the beginning stages of adsorption, α-arbutin was all adsorbed by the resin. When the adsorption reached 5 BV, a small amount of α-arbutin in the effluent was observed. At 7 BV, the concentration of product in effluent rose to the break point (5% of the

Table 4
Effects of pH on adsorption capacity.

pH value	4	5	6	7	8
Adsorption (mg/g resin)	115.2	121.8	130.5	115.3	89.5

Table 5
Effects of the mobile phase composition on the recovery and purity of α-arbutin.

Mobile phase (v/v)	Purity (%, w/w)	Recovery (%, w/w)
20% methanol solution	96.1	91.2
25% ethanol solution	97.3	90.9
45% ethyl acetate solution	92.3	90.6

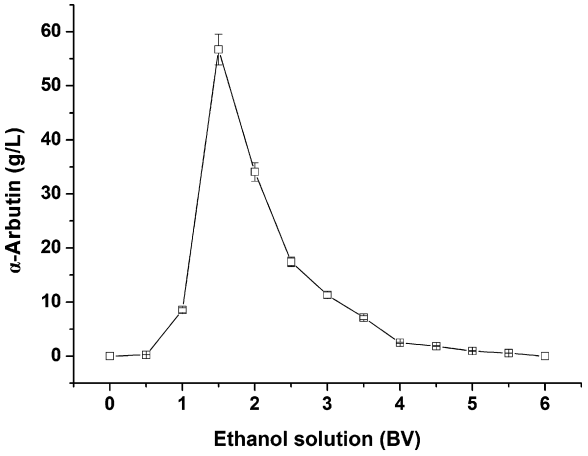

Fig. 5. Elution curve on S-8 column. Desorption conditions: mobile phase, 25% (v/v) ethanol solution; volume, 6 BV; flow rate, 0.5 BV/h.

original concentration). Further increasing the volume of sample solution, product in the effluent rose sharply. Thus, the optimal adsorption volume on S-8 was 7 BV.

The optimum mobile phase compositions were experimentally determined on the basis of the purity and recovery of α-arbutin. Solvent solutions of 20% (v/v) methanol-water, 25% (v/v) ethanol-water and 45% (v/v) ethyl acetate-water were used as mobile phases. The results (Table 5) demonstrated that the composed of methanol-water and ethanol-water were superior to ethyl acetate-water. Taking into account the risk of human health, 25% (v/v) ethanol-water was chosen for mobile phase.

By using ethanol solution as mobile phase, the elution curve on macroporous resin S-8 is shown in Fig. 5. From Fig. 5, the purity and recovery of α-arbutin with the collection from 1 BV to 3 BV were 97.3% (w/w) and 90.9% (w/w), respectively. When applying a somewhat broader collection (1 BV–4 BV), a purity of 95.3% (w/w) was obtained with a recovery of 97.6% (w/w). The reproducibility of adsorption selectivity and separation efficiency of the resin was examined on S-8 column after it has been used for 20 times in our laboratory. And there is no significant change in the purity and recovery of α-arbutin (data not shown). It was testified that the S-8 exhibited highest α-arbutin adsorption selectivity and separation efficiency in the optimal mobile phase compared with previous polymeric adsorbents mentioned above.

Liang et al. [21] reported α-arbutin separation method by using S-8 resin from *Aspergillus oryzae* broth with sample solution pH of 1.0 and adsorption volume of 1/10 BV. The adsorption capacity of resin and recovery of α-arbutin in their research were 0.2 mg/g resin and 77% (w/w) which is lower than our results (131.5 mg/g resin and 90.9% (w/w), respectively). Furthermore, α-arbutin will be decomposed in their strong acid solution (pH = 1.0) [20]. Therefore, the method developed in our experiment is a feasible and cost-effective method for large-scale production of α-arbutin.

3.6. Preparative separation of S-8 resin in α-arbutin isolation

According to the experimental data obtained from above, we successfully scaled up S-8 column from laboratory test (10 cm × 2 cm ID) to large scale (500 cm × 100 cm ID). By analyzing the results of large scale (Table 6), there was no significant difference in the purity and recovery of α-arbutin between laboratory scale and large scale. As a result, large-scale production of α-arbutin was thus successfully achieved.

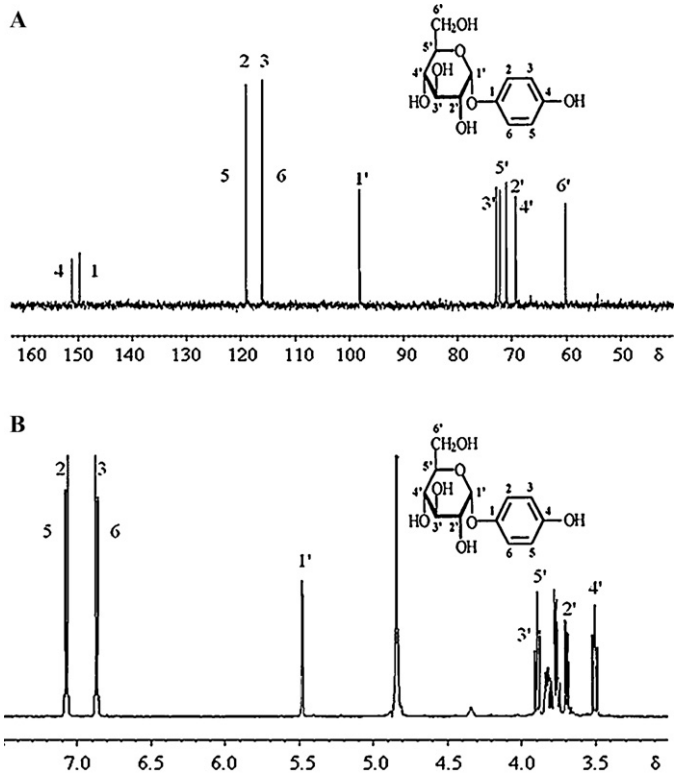

Fig. 6. The ^{13}C NMR spectrum (A) and ^{1}H NMR spectrum (B) of the product. The NMR spectra was performed in D_2O using a Bruker high-resolution AV600NMR spectrometer at 600 MHz.

3.7. Identification of the isolated fraction

The structure of the product was identified by ^{13}C NMR and ^{1}H NMR spectrometry. The spectral analyses of the separated fraction were carried out under the conditions stated in Section 2.7. The 600 MHz NMR spectrum of isolated fraction corresponding to α-arbutin is illustrated in Fig. 6. The data were as follows. ^{13}C NMR (D_2O, 600 MHz) δ (ppm): 151.07 (C-1), 149.75 (C-4), 119.11 (C-2, 6), 116.16 (C-3, 5), 98.22 (C-1′), 71.08 (C-2′), 72.93 (C-3′), 69.30 (C-4′), 72.34 (C-5′) and 60.20 (C-6′). ^{1}H NMR (D_2O, 600 MHz) δ (ppm): 7.081 (2H, d, J = 9.0 Hz, H-2, 6), 6.875 (2H, d, J = 9.0 Hz, H-3, 5), 5.486 (1H, d, J = 3.6 Hz, H-1′) and 4.840–3.490 (6H, m, H-6A, H-6B, H-2′, 3′, 4′, 5′). From these data, a doublet signal at 5.486 ppm (J = 3.6 Hz) was assigned to the anomeric proton of the glucose moiety and showed a smaller coupling constant than that for β-arbutin (5.079 ppm and J = 7.8 Hz), indicating that glycosidic linkage was in an α-configuration. These results confirmed the structure of product as α-arbutin.

Table 6
Preparative separation of S-8 resin in α-arbutin isolation.

Batch	1	2	3	4	5	6
Purity (%, w/w)	96.6	97.5	96.1	95.7	97.1	96.9
Recovery (%, w/w)	91.3	90.2	90.7	93.5	89.9	92.0

4. Conclusions

In this study, a simple method for one-step isolation of α-arbutin from *Xanthomonas* CGMCC 1243 fermentation broth by macroporous resin adsorption chromatography has been successfully developed. In term of representing the best adsorption and desorption capacities for α-arbutin, S-8 resin was selected, and its equilibrium adsorption data were well-fitted to the Freundlich isotherm. Under the optimized conditions obtained from dynamic adsorption and desorption tests, a purity of 97.3% (w/w) was obtained with a recovery of 90.9% (w/w). Moreover, we successfully scaled up S-8 column from laboratory test (10 cm × 2 cm ID) to large scale (500 cm × 100 cm ID) without diminishing the yield of product. Such an easy and inexpensive method is very promising from an industrial perspective for α-arbutin production and would provide reference for other similar isolation systems.

Acknowledgements

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References

[1] M. Funayama, H. Arakawa, R. Yamamoto, Biosci. Biotech. Biochem. 59 (1995) 143.
[2] S. Kazuhisa, N. Takahisa, N. Koji, Chem. Pharm. Bull. 51 (2003) 798.
[3] S. Kazuhisa, N. Takahisa, N. Koji, Biol. Pharm. Bull. 27 (2004) 510.
[4] T. Nishimura, T. Kometani, H. Takii, Y. Terada, S. Okada, J. Ferment. Bioeng. 78 (1994) 31.
[5] S. Kitao, H. Sekine, Biosci. Biotechnol. Biochem. 58 (1994) 38.
[6] J. Kurosu, T. Sato, K. Yoshida, T. Tsugane, S. Shimura, K. Kirimura, K. Kino, S. Usami, J. Biosci. Bioeng. 93 (2002) 328.
[7] T. Sato, N. Hasegawa, J. Saito, S. Umezawa, Y. Honda, K. Kino, K. Kirimura, J Mol. Catal. B: Enzym. 80 (2012) 20.
[8] R. Prodanovic, N. Milosavic, D. Sladic, M. Zlatovic, B. Bozic, T.C. Velickovic, Z. Vujcic, J. Mol. Catal. B: Enzym. 35 (2005) 142.
[9] E.S. Seo, J. Kang, J.H. Lee, G.E. Kin, G.J. Kim, D. Kim, Enzyme Microb. Technol. 45 (2009) 355.
[10] Y. Fu, Y. Zu, W. Liu, T. Efferth, N. Zhang, X. Liu, Y. Kong, J. Chromatogr. A 1137 (2006) 145.
[11] G. Jia, X. Lu, J. Chromatogr. A 1193 (2008) 136.
[12] Y.J. Wang, Y.F. Wu, F. Xue, Z.X. Wu, Y.P. Xue, Y.G. Zheng, Y.C. Shen, J. Chromatogr. B 895 (2012) 146.
[13] C.Q. Liu, S.R. Zhang, P. Zhang, Chin. J. Catal. 27 (2006) 361.
[14] K. Hatano, S. Kikuchi, Y. Nakamura, H. Sakamoto, M. Takigami, Y. Kojima, Bioresour. Technol. 100 (2009) 4697.
[15] M. Scordino, A.D. Mauro, A. Passerini, E. Maccarone, J. Agric. Food Chem. 52 (2004) 1965.
[16] M.W. Jung, K.H. Ahn, Y.H. Lee, K.P. Kim, I.R. Paeng, J.S. Rhee, J.T. Park, K.J. Paeng, J. Chromatogr. A 917 (2001) 87.
[17] R.E. Traybal, Mass Transfer Operation, Mocraw Hill Book Co., Singapore, 1981.
[18] Z.Q. Shi, P.L. Ho, Ion Exc. Adsorpt. 7 (1991) 50.
[19] M. Gao, W. Huang, C.Z. Liu, J. Chromatogr. B 858 (2007) 22.
[20] W.X. Tong, J.X. Li, D.H. Liu, Deterg. Cosmet. 33 (2010) 15.
[21] J.J. Liang, S.X. He, Z.W. Pang, Y.J. Zhou, Mod. Food Sci. Technol. 23 (2007) 66.