



Study on the adsorption kinetics of L-citrulline from the pseudo aqueous solution of watermelon rind on ion exchange resin

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ABSTRACT

This study aimed to extract L-citrulline from the pseudo aqueous solution of watermelon rind through the ion exchange process using Purolite® C100 resin. The adsorption kinetics of L-citrulline onto the cation exchange resins was investigated under different experimental conditions including pH, potassium concentration and L-citrulline concentration of initial solution. The UV spectrophotometer was used to determine L-citrulline content at 490 nm before and after adsorption. The initial sorption rate, pseudo-first order, pseudo-second order and Weber - Morris rate constants for different initial concentrations were evaluated and discussed. For the kinetic analysis, the pseudo-second order was suitable to describe the L-citrulline adsorption. Purolite® C100 resin has an saturated adsorption capacity of 79.128 ± 0.523 mg/g and 138.366 ± 0.749 mg/g with the initial L-citrulline concentrations of 600 and 1,200 mg/L, respectively.

1. Introduction

L-citrulline is a non-essential amino acid found abundantly in watermelon (*Citrullus vulgaris*), and is a potent endogenous precursor of L-arginine, which is a substrate for NO (nitric oxide) synthase (NOS) [1,2]. L-citrulline exhibits good health effects such as blood pressure and cardiovascular disease reduction [3], cardio protection [4], sexual stamina and erectile functions [5]. L-citrulline can also improve athletic performance and relieves muscle soreness [6-8]. In industry, L-citrulline could be produced by extraction from natural plants, chemical synthesis, fermentation,

and enzymatic synthesis [9]. The enzymatic synthesis of L-citrulline using arginine deiminases from microorganisms such as *Pseudomonas putida* [9] and *Bacillus subtilis* [10] with the conversion rate reached more than 90% but the biocatalytic process often was heterogeneous and lacked of operational stability of certain enzymes when utilized in industrial scale [10,11], while chemical synthesis is less efficient because of harsh reaction conditions, tedious separation procedures and non-environmental reagents such as using a rigorous alcoholic and/or acidic solvents (HCl 6 M) at high temperatures (145 °C) within 4 hr for extracting L-citrulline from watermelon rind [12]. Therefore, it is

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necessary to have a simple and less chemical consumption process for L-citrulline from watermelon rind, which is a potential source for L-citrulline. Because watermelon juice has been reported containing high levels of L-citrulline (3.9 to 28.5 mg/g dry weight (dwt) [12]); whereas watermelon rind accounting for 30-35% total weight of fruit [13] that contains higher amount of L-citrulline (24.7 mg/g dwt) than fruit flesh (16.7 mg/g dwt) [12].

Ion exchange chromatography has been utilized widely in the industrial separation and purification of amino acids due to their amphoteric behavior. These low cost and reusable adsorbed resins show selective adsorption capacity based on their differential charges and chemical stability [14]. Luo et al. (2010) investigated the adsorption behavior and mechanisms of L-tryptophan (L-trp) on HZ-001 and JK006 resins [14]. Kim et al. (1995) also investigated the intraparticle transport of amino acids, phenylalanine and tyrosine through a macroreticular cation exchange resin, Amberlite 200, with a finite batch adsorber [15]. Some kinds of strong cation exchange resins including sulfonated copolymers of styrene and divinylbenzene (Dowex 50W or Dowex 50) may be used to separate L-citrulline from watermelon juice [16]. Previous studies have been conducted to demonstrate that a cation resin can attach to an amino acid such as L-citrulline at pH above the amino acid's isoelectric point (pI) 5.9 [17] where the compound reveals negative charges; whereas at pH below pI, the resin might release L-citrulline because of its positive charges [16,18]. However, the presence of D-glucose and K⁺ ions within the watermelon rind solution [19] might influence the efficiency adsorption of L-citrulline on cation resin.

This study focused on investigating adsorption kinetics of L-citrulline from the pseudo aqueous solution of watermelon rind on ion exchange resin. Then the equilibrium and kinetics of L-citrulline adsorption were investigated to understand the adsorption behavior of L-citrulline. In order to eliminate the effect of charged components within the solution on L-citrulline adsorption capacity, watermelon rind pseudo aqueous solution was made of distilled water, L-citrulline, D-glucose and potassium at different concentrations. The effect of initial L-citrulline concentration and contact time on the adsorption capacity together with adsorption mechanism will be discussed. Because the solid-liquid adsorption behaviors were inferred through the equilibrium isotherm, adsorption kinetics and thermodynamics [14,20-24], it is necessary to investigate these values to design and optimize the ion exchange

process for large-scale purification of L-citrulline from natural watermelon rind solution.

2. Experimental

Watermelon rind extraction

Hac My Nhan (TN010) watermelon was acquired from local market (Long An province, Viet Nam), preserved at room temperature. The fruit was washed, removed the dark green skin and the flesh. The collected white rind was pressed by a kitchen juice compressor for the crude juice. The residue continued to be coarsely filtered with a cloth, a filter pore size of 1 µm and pressed again. The combined juice was then vacuum-filtered to remove remaining residues. The filtrate was kept at -20°C [25] for adsorption and chemical analysis. The chemical compositions of watermelon rind extraction were analysed to prepare pseudo aqueous solution of watermelon rind (Table 1).

Table 1: Chemical compositions of watermelon rind extraction in wet weight basis

Chemical composition	Unit	Value
Moisture	%	93.71 ± 0.07
Protein	%	0.55 ± 0.02
Total sugar	%	7.10 ± 0.10
Reduced sugar	%	5.98 ± 0.03
Ash	%	3.16 ± 0.05
L-citrulline	mg/L	1,000.15 ± 4.95
pH	-	5.21 ± 0.02

Pseudo aqueous solution of watermelon rind preparation

1,000 mg L-citrulline was diluted in 1,000 mL distilled water, 60 g D-glucose was added before pH was adjusted to pH 3 with HCl 0.1 N solution [16].

Ion exchange resin preparation

The cation exchange resin purolite® C100 with sulfonic acid SO₃H group from An Ecolab Company, USA; particle size 300 – 1200 µm was used in this work [26]. Prior to use, the resin was washed with HCl 5% solution for one hour and deionized water until all chloride ions were removed [26,27]. The prepared resin is kept in distilled water for further experiment. If the resins were dry, swelling with saturated NaCl solution for 4 hours [28]. Any solution with a turbidity greater than 10 NTU (Nephelometric Turbidity Unit) would cause the plastic particles to accumulate dirt very quickly [20].

Chemicals: L-citrulline ($\geq 99\%$, Acros, USA), D-glucose ($\geq 98\%$, Xinglong chemicals, China), diacetyl monoxime (DAMO) ($>99\%$, Sigma Aldrich, Germany), potassium chlorua ($\geq 98\%$, Xinglong chemicals, China), distilled water were used.

Determination of Citrulline

The watermelon rind extraction was decolorized with 10 g activated carbon, then 1 ml of the filtered solution was diluted with 7 ml distilled water. After that, 1 ml of the diluted solution was added to 4 ml distilled water, 2 ml of sulphuric acid:phosphoric acid (3:1 in volume), 0.25 ml 30 g/L diacetyl monoxime in order. Then, the solution was then heated in a 100°C water bath for 30 min. The final samples were measured with UV spectrophotometer at 490 nm. The citrulline content was calculated according to a calibration curve of an external standard citrulline. The data was the mean of three repeats [29].

Batch experiments

Effect resin dose, pH and K^+ concentrations on L-citrulline adsorption capacity and adsorption efficiency

The resin amounts were taken 1 g/L, 2 g/L, 3 g/L and 5 g/L. HCl 0.1 N was added to adjust pH of the pseudo aqueous solution of watermelon rind from 2 to 5. The different KCl concentrations of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L were applied. Each experiment was added to 100 mL pseudo aqueous solution of watermelon rind, stirred at 200 rpm for 24 hours at room temperature.

The adsorption capacity (AC) was calculated by eq. 1 and eq. 2. The adsorption efficiency (AE) was calculated by eq. 3.

$$q_t = \frac{(C_0 - C_t) \cdot V}{m} \text{ (Eq. 1); } q_e = \frac{(C_0 - C_e) \cdot V}{m} \text{ (Eq. 2).}$$

$$H(\%) = \frac{C_0 - C_t}{C_0} \cdot 100\% \text{ (Eq. 3),}$$

where q_t , q_e are adsorption capacity at time t and at the equilibrium, respectively; C_0 , C_e , C_t (mg/L) are the concentrations of solutes at the initial, equilibrium and given time t , respectively; V (l) is the solution volume and m (g) is the amount of resin.

Adsorption kinetics

Pseudo first order model (PFO), Pseudo second order (PSO) model, Intra-particle diffusion (IP) model [30] were applied to identify the dynamics of the adsorption process. The adsorption kinetics were determined by

following: 100 mL pseudo aqueous solution of watermelon rind containing 600 - 1,200 mg/L L-citrulline, 60 g/L D-glucose were added to 5 g resin/L at different time intervals (from 30 to 300 min). A pH value of 3 was maintained throughout the experiment by adding HCl 0.1 N.

Data analysis

Data analysis was done by ANOVA using Statgraphics 18; all experiments were triplicated and mean values were compared by LSD (Least Significant Difference) with confident coefficient $p \leq 0.05$.

3. Results and discussion

Effect of resin dose

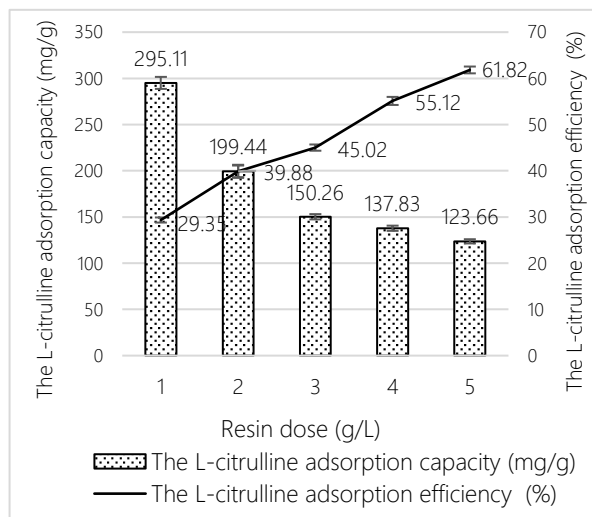


Fig 1: The L-citrulline adsorption capacity and efficiency at different resin doses

The effect of the resin amount on L-citrulline adsorption was investigated; and results displayed in Figure 1. The binding efficiency of L-citrulline raised from 29.35% to 61.82% corresponding to the increased of resin amount (from 1 to 5 g). It might relevant to the larger surface area and higher adsorption sites of increasing adsorbent dosage for a given initial solute concentration [14]. Whereas, the adsorption capacity gradually decreased nearly 2.4 times (from 295.11 to 123.66 mg/g, accordingly) because of the adsorption sites remain unsaturated while the number of available sorption sites increases by an increase in resin amount [22]. Due to the limited dosage range of adsorbents that has been tested, the loading capacity determined might be not the highest. Therefore, the resin/solution ratio 5 g/L was chosen as a constant condition to conduct following experiments.

Effect of pseudo solutions pH

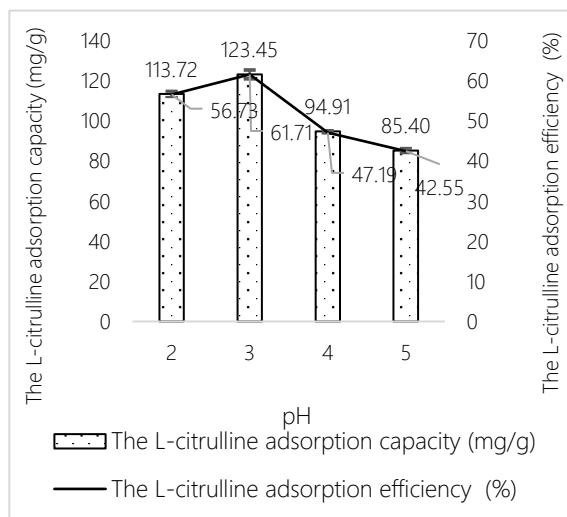


Fig 2: The L-citrulline adsorption capacity and efficiency at different model solutions pH

Figure 2 showed the influence of different pseudo solution's pH (from 2 to 5) on the L-citrulline adsorption capacity; as can be seen, both adsorption capacity and efficiency increased (from 113.72 to 123.45 mg/L and from 56.73% to 61.71%, respectively) at high acidic pH of 2 to 3. Then both of capacity and efficiency decreased to 85.40 mg/l and 42.55% at declined pH from 4 and 5, which are close the isoelectric point of L-citrulline ($pI = 5.9$). This could related to the charged forms of L-citrulline at low pH (acidic pH) [18]. As pH value of solution moved away from the pI isoelectric point of L-citrulline, the percentage of positive or negative citrulline ions increased. Therefore, Cit^+ content decreased at pH 4.0 - 5.0 and the ion exchange process was less efficient at this time. However, at pH 2.0, the molar fraction of Cit^+ have increased quite far from the isoelectric point 5.9, but the amount of hydrogen ions in the initial solutions were so high to compete strongly to hydrogen ions of resins [15], leading to a decrease in the L-citrulline adsorption efficiency. These results were similar to the adsorption of L-tryptophan (pI 5.9) on strong cation exchange resins HZ-001 and JK006 [14]. Luio (2011) analysed the effect of pH (1.0, 3.0 and 5.0) on adsorption capacity and reported that at pH 1.0 the binding capacity was lower than that at pH 3.0. Fish (2012) also suggested that pH at 3.0 was optimal for the cation exchanged adsorption of L-citrulline [16]. Therefore, pH 3.0 was chosen for the next adsorption experiments.

Effect of K^+ concentration

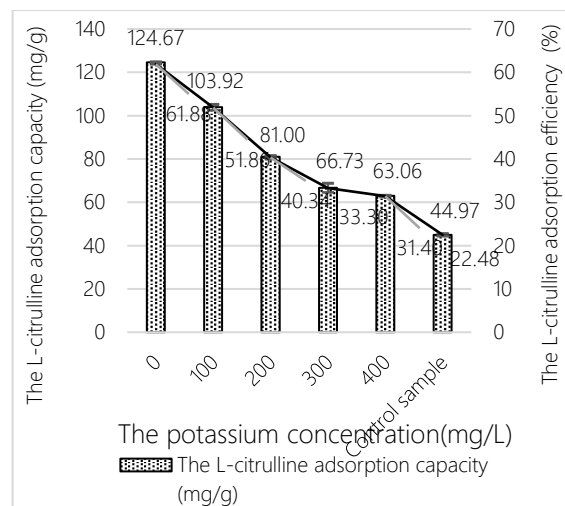
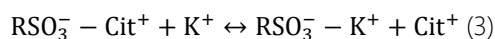
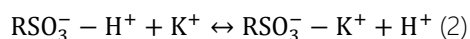
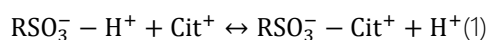


Fig 3: The L-citrulline adsorption capacity and adsorption efficiency in different potassium concentrations vs. control sample

The influence of K^+ concentration on the adsorption capacity of L-citrulline was investigated because this ion was one of the main mineral found in the watermelon rind [19]. Figure 3 showed the effect of K^+ concentration ranged from 0 mg/L to 400 mg/L on the adsorption of L-citrulline on H^+ resin. Interestingly, the maximum adsorption ability (61.88% and 124.67 mg/g) was found in the solution without K^+ (0 mg/L K^+), and that capacity decreased to 63.06 mg/g according to the increase of K^+ concentration (from 0 to 400 mg/L). Noticably, when K^+ concentrations ranged from 0 to 300 mg/L, the adsorption capacity reduced nearly two times (124.67 vs. 66.73 mg/g), whereas at higher K^+ concentration of 300 and 400 mg/L, the adsorption capabilities were not much different (66.73 vs. 63.06 mg/g). This might correlate to the competition between the L-citrulline charged form and potassium ions attaching to negative charges of the resin as described in reaction (1), (2), (3):



At high potassium concentrations, the attachment of K^+ to binding sites of the resin almost reached the equilibrium point, that caused the small changes in binding capacity of citrulline. Guo et al. (2013) [31] found similar results within Pb^{2+} adsorption experiments using cation exchange method; in which Mg^{2+} was the interfered ion. These results indicated that K^+ might hamper the binding of L-citrulline on the resin. Therefore, the use of watermelon rind solution to investigate the kinetic models is not effective.

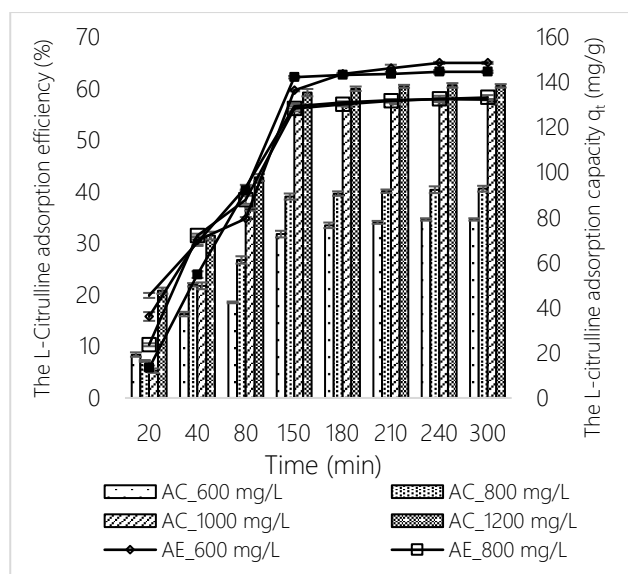
Effect of initial L-citrulline concentration and contact time

Fig 4: The L-citrulline adsorption capacity (q_t) and adsorption efficiency according to the contact times and different L-citrulline initial concentrations

Figure 4 displayed the adsorption capacity and efficiency according to of L-citrulline concentrations (600 – 1,200 mg/L) and contact time. The results confirmed that the adsorption ability was affected by initial concentrations and reaction time. The adsorption capacity increased sharply (from 72.64 to 135.34 mg/g) when the concentration of L-citrulline changed from 600 mg/L to 1,200 mg/L within 150 minutes reaction; then the adsorption declined and reached equilibrium after 300 minutes (Figure 4).

Meanwhile, adsorption efficiency achieved after 150 minutes was $62.85 \pm 0.19 \%$, $56.91 \pm 0.32 \%$, $62.63 \pm 0.23 \%$, $57.31 \pm 0.21 \%$ and $48.99 \pm 0.07 \%$ corresponding to the initial concentration of L-Citrulline from 600 to 1,200 mg/L (Fig. 4). The increased absorbate concentration was found to enhance adsorption kinetics due to the gradient concentration, which caused the citrulline ions to move faster to the active sites on resins surface, and that extent the interaction between citrulline the exchanged material [21]. Beside, the limitation of cross links on the resins and the reduction of the resins active sites at equilibrium might lower the adsorption rate after 180 mins reaction [20].

Adsorption kinetics

Table 2: Adsorption kinetic parameters of L-citrulline at different initial L-citrulline concentrations on cation resin

Contrentation (mg/L)		600	800	1,000	1,200
q_e experimental (mg/g)		79.13	92.75	131.45	138.37
pseudo first order	R^2	0.925	0.923	0.918	0.926
	q_e	104.12	103.10	185.94	137.09
	k_1	0.019	0.019	0.022	0.019
pseudo second order	R^2	0.975	0.962	0.978	0.994
	q_e	108.70	126.58	175.44	166.67
	$k_2 \times 10^{-5}$	8.5	6.2	3.2	3.6
Weber -Morris	R^2	0.969	0.940	0.941	0.954
	k_i	5.05	6.08	8.51	9.95

Table 2 showed that the pseudo-second-order model simulated better the adsorption kinetic of L-citrulline on the resin than the first-order model (R^2 average of 97% vs. 92%, respectively). The best model fit in this model indicated that the rate-limiting step of the adsorption was the electrostatic interaction between L-citrulline and the sulfonic group (SO_3^-) covalently bonded in the ion exchange matrix [20]. Therefore, the adsorption rate depended on the adsorption efficiency, not because of the adsorbent concentration. However, the values of k_2 was not constant and varied from 2.6×10^{-5} to 1.9×10^{-4} g/mg min. This was similar to the adsorption kinetics of nitrate ions on ion exchange resins reported by Hekmatzadeh et al. (2013) [23], in which the parameters of the pseudo-first order and pseudo second order adsorption models were purely empirical.

Moreover, the maintained ability of the apparent adsorption rate constants had also been previously studied by Azizian (2004) [32] and explained that the apparent rate constant of the quadratic kinetic equation was the combination of the adsorption and the desorption rates as well as experimental conditions; therefore, it was not an intrinsic reaction rate constant of the process. In fact, the mass transfer of substrates was often characterized by film diffusion, or intraparticle diffusion, or both [21]. In contrast, neither the pseudo-first order or pseudo-second order adsorption kinetic models could account for the diffusion of the substrate into the resins [30]; therefore, in order to describe the subsequent process after the initial stage, Weber-Morris model was applied and result was shown in Table 2.

In the solid-liquid adsorption system, there were two types of solute transportation including external mass

transfer and intra-particle diffusion [21]. It was important to decide which step was the rate-limiting step for the entire ion-exchange process. The adsorption of Cit^+ on Purolite® C100 resins in the initial contact time from 0 to $6.32 \text{ min}^{0.5}$ showed a rise in capacity which could be attributed to the external ion-exchange process on the interface, or be controlled by external diffusion. Whereas during the end of the adsorption process (from 6.32 to about $15 \text{ min}^{0.5}$), a lighter increase in adsorption capacity was seen. It could be explained by the gradual ion-exchange of intra-particle diffusion, which was the control kinetic which made the adsorption reach equilibrium. As the intra-particle diffusion rate constant, k_i increased with the initial L-citrulline concentrations, which gave a positive relationship between diffusion rate and initial concentration based on the correlation coefficient ($R^2 > 0.94$). Similar phenomena were found in the Cu(II) and Pb^{2+} adsorption process on ion exchange resins [21] [31], or the adsorption and desorption kinetics of ectoine by ion exchange resins [20].

4. Conclusion

This research indicated that the Purolite® C100 resin was an effective adsorbent for Cit^+ ions from aqueous pseudo solutions of watermelon rind. The adsorption process depended on the adsorbent dosage and initial adsorbate concentration, pH and the presence of K^+ in the solution. Pseudo-second-order and Weber-Morris kinetics models explained better the adsorption kinetics of L-citrulline toward the Purolite® C100 resin than pseudo-first-order model, in which two stages of the adsorption were controlled by intra-particle diffusion.

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