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Research paper

Preparation of a novel chitosan-based biosorbent cross-linked with phenethylamine for adsorption of aromatic amino acids

Wen Jiang, Yang Yang, Renyou Tao, Xiaoqin Jiang, Xiaohua Zhou*, Zhiming Zhou*

Department of Chemical Engineering, School of Chemistry and Chemical Engineering, Chongqing University, Chongqing 401331, PR China

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ABSTRACT

Rice protein was successively hydrolyzed by rice protein hydrolysis special enzyme, chymotrypsin and carboxypeptidases A. Then a novel chitosan-based biosorbent cross-linked with phenethylamine (PMCCR) was synthesized and firstly used for adsorption of aromatic amino acids (AAA) from rice protein enzymatic hydrolysates (RPEH). The characterizations of PMCCR indicated that phenethylamine was successfully cross-linked with chitosan, and the particle size of PMCCR was 500–1000 μm with pore diameter of 50–100 μm. Furthermore, PMCCR showed an efficient adsorption property to AAA in RPEH, reaching equilibrium at 80 min of 28.54 mg/g, and the adsorption data could be well fitted with Freundlich isotherm model. The amino acids analysis of RPEH after adsorption (RPEHA) showed that AAA in RPEH was basically adsorbed by PMCCR, with a Fisher's ratio of 21.2. Overall, this novel chitosan-based biosorbent cross-linked with phenethylamine might be highly promising to facilitate efficient adsorption of aromatic amino acids and prepare protein enzymatic hydrolysates with a high Fischer's ratio.

1. Introduction

Enzymatic hydrolysis is a common method used to improve functional and nutritional qualities of food proteins. Especially the enzymatic hydrolysates with extensive degree of hydrolysis which have attracted considerable attention in recent years since it act as main constituents of geriatric products, high-energy supplements, enteral and parenteral solutions, and hypoallergenic foods (Mobarhan & Trumbore, 1991). Protein enzymatic hydrolysates present several advantages as constituents of medical diets, one of them is that the intestine adsorption of it is more effective than either free amino acids or intact protein (Silk et al., 1979). The percentage of branched-chain amino acids (BCAA) to aromatic amino acids (AAA) in protein enzymatic hydrolysates containing peptides referred to as Fischer's ratio. And protein enzymatic hydrolysates with a Fischer's ratio higher than 20 are used in specific medical diets (Fischer, 1990) for the treatment of patients with liver diseases, including hepatic encephalopathy in order to avoid the adverse effects of AAA (Hemeth, Steindl, Ferenci, Roth, & H. Ortnalg, 1998; Kawamura-Yasui et al., 1999;).

However, the preparation of protein enzymatic hydrolysates with such a highly Fischer's ratio is not a simple task, and demands the use of a number of proteases for producing a well-defined digestion pattern. Also, it is of interest to use high nutritional value proteins as starting material for preparing this type of protein enzymatic hydrolysates.

Rice protein (RP), a major plant protein, has drawn considerable attention owing to its physiological effects (Burris et al., 2010; Yang, Chen, Xu, Nie, & Yang, 2013). Rice proteins are normally regarded as hypoallergenic (Helm & Burks, 1996) and have been considered to have a higher biological value and protein digestibility compared to other cereals (i.e., wheat, corn, barley, millet and sorghum) (Young & Pellett, 1994). Rice protein enzymatic hydrolysates (RPEH) had been prepared using soluble proteases. After this work, the rice protein enzymatic hydrolysates (RPEH) held a number of aromatic amino acids (AAA). In order to prepare rice protein enzymatic hydrolysates (RPEH) with Fischer's ratio higher than 20, the essential of this type of enzymatic hydrolysates is the separation of AAA. The known strategies of the separation of AAA include physical, biological and chemical methods. AAA can be separated by adsorption on to carbon on account of their high affinity for this adsorbent, and can be displaced by a number of compounds with even greater tenacity for carbon such as benzyl alcohol (David & Arne, 1951). It was also reported by Philip Zakaria et al., that AAA were separated by electrokinetic capillary chromatography utilising temperature variations coupled with the use of sulphated-β-cyclodextrin as a pseudo stationary phase (Zakaria, Macka, & Haddad, 2004).

Meanwhile, an increasing number of attentions were drawn on

* Corresponding authors at: PO Box 94 55, Daxuecheng South Road, Shapingba District, Department of Chemical Engineering, School of Chemistry and Chemical Engineering, Chongqing University, Chongqing, 401331, PR China.
E-mail addresses: zhou65306590@cqu.edu.cn (X. Zhou), 1211092726@qq.com (Z. Zhou).

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biosorbent owning to its low cost. Besides, it was easy to get effective biosorbent by grafting special chemical groups, which was the source of different functional groups such as amine, hydroxyl, sulfonate, carboxyl and phosphate (Niderkorn, Morgavi, Pujos, Tissandier, & Boudra, 2007;Yuan et al., 2014; Zhao, Jia, Yu, & Sun, 2011). Herein, we reported a novel biosorbent to adsorb AAA from RPEH.

Chitosan, a cheap and environmentally friendly nature polymer, has attracted a considerable attention as effective biosorbent owning to its low cost compared with activated carbon. What's more, its high contents of functional groups, such as amino and hydroxyl, reveal high adsorption potential (Tran et al., 2015; Wang, Peng, Yang, Liu, & Hu, 2011). However, chitosan could be soluble in acid media which limited its applications (Guo, Su, Liao, Ding, & Sun, 2017; Zhou, Liu, & Liu, 2009). It was found that the cross-linked chitosan polymers can enhance the resistance to acid media, as well as improving its adsorption capability, which conquered its limits in practical application (Vakili et al., 2014; Zhao et al., 2015). Kinds of reagents, including glutaraldehyde, epichlorohydrin, ethylene glycol diglycidyl ether and tripolyphosphate, had been used to synthesize cross-linked chitosan polymers and to enhance its stability in acidic media (Huang, Yang, Zhang, & Shi, 2009; Martinez et al., 2007; Sankararamakrishnan & Sanghi, 2006). It was also reported that cross-linked chitosan complex showed an excellent adsorption capability (Zhao et al., 2015). In this context, cross-linking and insertion of new functional groups have been performed.

It was found that many compounds had planar ring structures, such as benzene, pyrrole, furan, etc. A conjugated system with the average distribution of electron cloud formed in these structures, which led to the formation of hydrophobic interaction (Lo et al., 2008; Shi et al., 2010; Zhang, Shan, & Gao, 2011). When these functional groups in the adsorbent got close to the adsorbate with the same ring structure, the hydrophobic interaction formed which could improve the adsorption selectivity towards target objects (Zhou, Zhao, & Zhao, 2011).

Meanwhile, phenethylamine was a natural monoamine alkaloid and a trace amine, and the phenethylamine skeleton was composed of an aromatic ring with a side chain of two carbons ending by an amine group (Gaël et al., 2015). Therefore, a novel chitosan-based biosorbent cross-linked with phenethylamine could be used to adsorb AAA from RPEH. The cross-linking reaction would consume a large portion of amino groups, and the aromatic ring was cross-linked on chitosan to introduce the novel functional group. The novel functional group in the chitosan-based biosorbent would form hydrophobic interaction with the planar ring of AAA which could realize selective adsorption towards RPEH.

In this study, rice proteins with an extensive degree of hydrolysis have been generated using rice protein hydrolysis special enzyme, chymotrypsin and carboxypeptidases A, successively. However, the resulting hydrolysates still have a number of AAA. In this context, a novel chitosan-based biosorbent was synthesized and used to adsorb AAA from RPEH. Furthermore, the characteristics of the novel chitosan-based biosorbent and the adsorption capacity were executed in detail. The isotherm model was also explored. Our study provided a novel chitosan-based biosorbent for adsorption of compounds with aromatic ring and preparation of protein enzymatic hydrolysates with a Fischer's ratio higher than 20.

2. Materials and methods

2.1. Materials

Rice protein (protein content 85%) was purchased from Golden Agriculture Biotech Co. Ltd. in Jiangxi, China. Rice protein hydrolysis special enzyme (200 U/mg), was purchased from Nanning Doing-higher Biotechnology Co. Ltd. in Guangxi, China. Ascorbic acid, phenylalanine, tyrosine, tryptophan, aspartic acid, arginine, leucine, Bovine serum albumin (BSA), were purchased from Qiude Biotechnology Co. in Shanghai, China. All the reagents above were of biological grade. Chitosan with the deacetylation degree of 90.32% was purchased from Yuhuan Biochemical Co. Ltd. in Zhejiang, China. Phenylethylamine, glutaraldehyde 50% (w/w) solution in water, liquid paraffin, Tween-80, calcium carbonate, ethylene glycol diglycidyl ether and other chemicals were purchased from Sichuan Huatian science and techonlogy industrial Co. in Sichuan, China, and all of them were of analytical grade.

2.2. Hydrolysis of rice protein

Rice protein (10 g) was firstly poured into 90 mL distilled water, then the pH was adjusted to 8.0 with sodium hydroxide solution, 1% (w/w) rice protein hydrolysis special enzyme was added into the above solution and stirred for 2 h at 60 °C. Thereafter, 0.20% (w/w) chymotrypsin was added and reacted 2 h. Then 3% (w/w) carboxypeptidase A was added and continued to react 2 h at 60 °C. After the reaction finished the solution was treated in boiling water for 10 min and later cooled to room temperature, then centrifuged with the speed of 5000 RPM for 10 min. Thereafter, the supernatant was ultrafiltered by ultrafiltration through the 10,000 molecular weight ultrafiltration membrane under 0.2 MPa, the filtrate was collected to obtain rice protein enzymatic hydrolysates (RPEH).

2.3. Measurement of rice protein hydrolysis degree

The hydrolysis degree (HD) of rice protein means the percentage of the number of peptide bonds broken to the total number of bonds in per unit weight. pH-stat method (Adler-Nissen, 1979) was used and the hydrolysis degree (HD) was calculated as follows:

$$HD = \frac{C_b V_b}{\alpha h_{tot} M_p} \times 100\%$$

Where C_b is the concentration of the base used; V_b is the volume of the base used; α is the average degree of dissociation of the α-NH_2 groups; M_p is the mass of rice protein (Shih & Daigle, 2000); h_{tot} is the total number of peptide bonds in the protein substrate (7.40 meq/g rice protein) (Li et al., 2012). α is calculated as:

$$\alpha = \frac{10^{pH-pK_a}}{1 + 10^{pH-pK_a}}$$

Where pH is the value during the enzyme hydrolysis; pK_a is the average pK_a of α-NH_3^+.

2.4. Optimization of rice protein hydrolysis

To achieve the best hydrolysis degree of rice protein, reaction time, temperature, pH and mass ratio of enzyme to rice protein should be optimized. Since the optimum reaction time, temperature and pH of protease in protein hydrolysis reaction was set as 2 h, 60 °C and 8.0, other independent variables were investigated, including the mass ratio of rice protein hydrolysis special enzyme to rice protein (0.25%, 0.50%, 1.00%, 1.50%, 2.00%, 2.50%, 3.00%), the mass ratio of chymotrypsin to rice protein (0.05%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%), the mass ratio of carboxypeptidase A to rice protein (1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%).

2.5. Preparation of chitosan-based biosorbent crosslinked with phenethylamine (PMCCR)

Phenethylamine (0.75 g) was poured in 100 mL 0.72% (v/v) hydrochloric acid to prepare 0.75% (w/v) phenethylamine solution. Thereafter 1 g chitosan was added into the above solution, 9.3 mL 5% (w/v) glutaraldehyde was then added and stirred at 35 °C for 0.5 h. After the reaction completed, 0.5 g calcium carbonate and 1 g ethylene glycol diglycidyl ether were added to the above reaction solution. Then