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Purification Of Angiotensin-Converting Enzyme Inhibition Peptides From Bovine Bone (#475)

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Introduction

Hypertension is a serious threat to human health causing many diseases including cardiovascular disease, kidney failure, arteriosclerosis, and stroke. Renin-angiotensin- aldosterone system (RAS) and kallikrein-kinin system (KKS) are the two major hormone systems that regulate blood pressure [1]. The relationship between the RAS system and the KKS system is mutually antagonistic. Angiotensin I-converting enzyme (ACE) plays a central physiological role in the RAS and KKS systems [2]. At present, hypertensive patients control high blood pressure by taking potent synthetic ACE inhibitors such as captopril, quinapril and lisinopril [3]. However, these potent synthetic ACE inhibitors can cause many side effects including coughing, rashes, elevated blood potassium levels and headaches [4]. Thus, it is great significant to explore stable ACE inhibitors without side effects. It has been reported that some peptides released from natural proteins have ACE inhibitory activity without the side-effects [5]. Therefore, the aim of our research was to investigate the optimal enzymatic hydrolysis conditions to generate the bovine bone hydrolysate with the highest ACE inhibiting activity.

Methods

Decalcified bone powder was mixed with deionized water and the pH of the solution was adjusted to 2.0 using 2 M HCl. The homogenate was then hydrolyzed by the addition of pepsin with stirring at 37 °C for 4 h, 6 h or 8 h. The enzyme was then inactivated for 10 min using boiling water bath. The cooled slurries were centrifuged and the supernatant (BBH) was freeze-dried before storage at -80 °C. Response surface methodology (RSM) was used to determine the optimal enzymatic process parameters for obtaining the bovine bone hydrolysate with maximum ACE inhibition rate. All experiments were repeated separately at 3 times.

The ACE inhibitory activity was measured by HPLC [6]. Briefly, 40 μL of samples were mixed with 200 μL of 5 mM HHL and the mixture was pre-incubated at 37 °C for 5 min. Typically, six concentrations were used for each sample. The 20 μL of 0.1 U/mL ACE was then added to the mixture and incubated for 40 min at 37 °C. Finally, the reaction was stopped with 250 μL of 1 M HCl. The control was boric acid buffer instead of samples. The aqueous solution was analyzed by HPLC after membrane filtration. All experiments were repeated separately at 3 times. The Duncan's Multiple Range Test was used to calculate significant differences.

%ACE inhibition=$[A_0-A_1]/A_0\times100$ (A_0 is the peak area of control and A_1 is the peak area of sample).

Results

Multivariate quadratic equation regression analysis was performed on the experiment data using Design Expert 8.0.6 software to obtain the required mathematical model. The model fitted to the second-order polynomial is $Y=74.88+1.95A+2.85B+2.71C+0.27AB+0.77AC-1.38BC-6.12A^2-2.01B^2-3.25C^2$ (A: enzymatic hydrolysis time, B: substrate concentration, and C: E/M ratio). In order to intuitively express the influence of three factors on the ACE inhibition rate of bovine bone hydrolysate, the response surface and contour map of the three factors were plotted (Fig. 1). It can be observed that the P value of the regression model was 0.0004 ($P < 0.01$), indicating that the model reached a significant level (Table 1). The lack of fit analysis of the regression model was not significant ($P = 0.1179$), indicating that the selected model was suitable. The regression coefficient and the correction coefficient of the model were 0.9618 and 0.9127 indicating that the model has good fitting degree and the experimental error is small. The results showed that the optimal enzymatic hydrolysis time was 6.38 h, the substrate concentration was 7.23 g/100 mL and the E/M ratio was 0.91%. Under these conditions, the theoretical ACE inhibition rate of the bovine bone hydrolysate could reach 76.36%.

Conclusion

Considering the convenience of operation, the enzymatic hydrolysis process parameters were partially modified: the enzymatic hydrolysis time was 6.5 h, the substrate concentration was 7.5 g/100 mL and the enzyme bottom ratio was 0.9%. The average ACE inhibition rate of bovine bone hydrolysate was 78.01% which is consistent with the theoretical value. Therefore, the optimized extraction process parameters based on the response surface method are both accurate and reliable.

Notes

Source	Sum of squares	df	Mean square	F value	P-value Prob>F	Significance level
Model	403.03	9	44.78	19.60	0.0004	**
A	30.54	1	30.54	13.36	0.0081	**
B	64.75	1	64.75	28.33	0.0011	**
C	58.92	1	58.92	25.78	0.0014	**
AB	0.29	1	0.29	0.13	0.7338	
AC	2.40	1	2.40	1.05	0.3393	
BC	7.59	1	7.59	3.32	0.1112	
A^2	157.73	1	157.73	69.02	< 0.0001	**
B^2	16.98	1	16.98	7.43	0.0295	*
C^2	44.35	1	44.35	19.41	0.0031	**
Residual	16.00	7	2.29			
Lack of fit	11.79	3	3.93	3.73	0.1179	
Pure error	4.21	4	1.05			
Cor total	419.02	16				

Table 1. ANOVA for response surface quadratic model analysis of variance

Note: (1) A: enzymatic hydrolysis time, B: substrate concentration, and C: E/M ratio; (2) *P<0.01* is extremely significant, denoted by **; *P<0.05* is significant, denoted by *; *P>0.05* is not significant; (3) R^2=0.9618, R_{Adj}^2=0.9127.

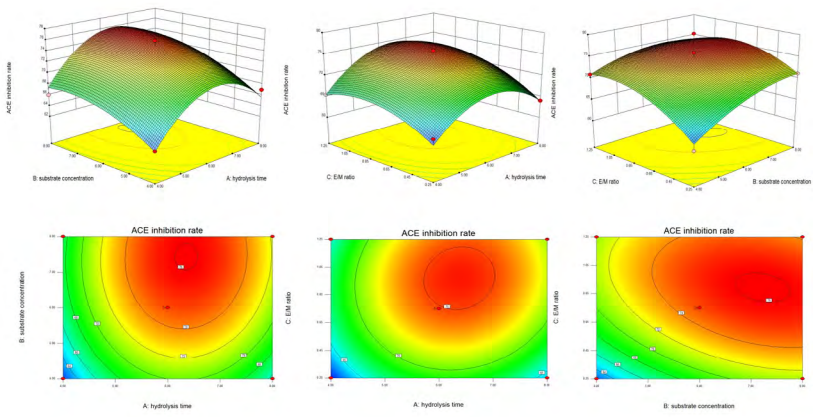

Fig. 1

Three-dimensional response surface and contour plots

Notes