

ANGIOTENSIN-CONVERTING ENZYME INHIBITORY AND ANTIBACTERIAL ACTIVITIES OF LOW MOLECULAR WEIGHT PEPTIDES DERIVED FROM SPENT BREWER'S YEAST

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SUMMARY

According to the World Health Organization (WHO), hypertension is one of the most common chronic diseases. Cardiovascular disease causes 17.5 million deaths each year, in which hypertension is a direct cause to the death of up to 40%. This hypertension is caused by angiotensin-converting enzyme (ACE). ACE inhibitors consider as an effective therapy in hypertension treatment. Many ACE inhibitors are synthesized by chemical pathways to control high blood pressure, however, these drugs often cause side effects. Therefore, in the last two decades, many authors have been interested in studying and producing a variety of ACE inhibitory peptides, which are naturally derived (plants, animals, and microorganisms) that help reduces blood pressure and less cause side effects. On the other hand, biologically active peptides that are resistant to pathogenic bacteria are also of special interest. In this study, we refer to the limited hydrolysis of spent brewer's yeast by proteases to collect peptides with a molecular size of ≤ 10 , 5 and 3 kDa. Peptide fraction with molecular size ≤ 10 kDa have ACE inhibitory activity. The results showed that smaller peptide fractions had higher ACE inhibitory activity. At concentration of 30 $\mu\text{g/mL}$, inhibitory activity of peptides with molecular sizes ≤ 10 , 5, 3 kDa was 16.3, 27.7 and 32.7%, respectively, and the best IC_{50} was 48,85 $\mu\text{g/mL}$. The lowest peptide concentration for completely inhibition bacteria after 24 h of incubation was 30 mg/mL (*V. cholerae*), 35 mg/mL (*E. coli*) and 50 mg/mL (*S. typhi*).

Keywords: ACE inhibitory activity, antibacterial activity, low molecular weight peptide, spent brewer's yeast

INTRODUCTION

Bioactive peptides are supplement peptides which can also affect the physiological function of the body, enhance human health by antioxidant, antimicrobial, anti-hypertension through angiotensin-converting enzyme (ACE) inhibitory activity. Recently, people are very concerned about the need to improve their health as well as their quality of life. Therefore the bioactive peptide-containing functional food industry is growing robustness. This motivation

encourages many scientists to study and discover new forms of peptides for high biological activity (Hasan *et al.*, 2008; Hazem *et al.*, 2011; Mahta *et al.*, 2015). Peptides can be extracted from animals, plants or fermented product by microorganisms or limited hydrolysis of protein originated from different sources by proteases. In addition, spent brewer's yeast is one of the abundant sources containing high protein content, and rich in vitamins, especially B-group vitamins and minerals. There were approximately 84 thousand tons of brewer's yeast

residue recovered according with 4.2 billion beer liters produced in 2018 in Vietnam. In this study, we analyzed the ACE inhibitory activity and antibacterial activity of peptides obtained from spent brewer's yeast hydrolysates.

MATERIALS AND METHODS

Strains and agents

E. coli ATTC 25922, *S. typhi* ATTC 14028 and *V. cholerae* were obtained from IFM Organization 2015 Australia. Angiotensin I converting enzyme (2 units/mg protein), hippuric acid (HA), N-hippuryl-His-Leu hydrate were purchased from Sigma Chemical Co., St. Louis, MO, USA. Xylose lysine deoxycholate (XLD) agar, thiosulfate citrate bile salts sucrose (TCBS) agar, tryptone bile X-glucuronide (TBX) agar, Brain Heart Infusion (BHI) broth were obtained from Merck, Germany.

ACE inhibitory activity determination

ACE enzyme catalyzes the hydrolyzing of HHL (N-hippuryl-His-Leu hydrate) to hippuric acid (HA). If ACE inhibitors such as biological peptides are added, the ACE activity will be reduced and HA content decreased. HHL solution at 1.55mM concentration was prepared in 0.1 M natri borate buffer pH 8.3 containing 1.55 mM NaCl and ACE 0.2 U/ml was added (He Ni *et al.*, 2012). HA amount was quantified by HPLC (C18 column, mobile phase: methanol 60% containing 0.1% TFA, flow rate 0.8ml/min). ACE inhibitory activity was determined by the following formula:

$$\text{ACE inhibitory activity (\%)} = \frac{B - A}{B - C} \times 100\%$$

Where: B is the amount of HA formed by the peptide-free sample

A is the amount of HA formed by the peptide supplement

C is the amount of HA formed by the sample without ACE

Antibacterial activity determination

Agar dish diffusion method

A predefined amount of peptides was added to agar well (about 6mm diameter) on selected medium. Target bacteria strains ($10^5 - 10^6$ CFU/mL) was inoculated (Klančnik *et al.*, 2010). Agar plates were incubated at 37°C for 24 h until the antimicrobial zone was appeared.

Colony count method

Mix peptides solution with target bacteria strain at a density of $10^5 - 10^6$ CFU/mL. Quantification was calculated by number of the colonies on agar plates. Incubation of agar plates was carried out at 37°C for 24 h, then the number of grow colonies was counted. MIC values are defined as the minimum amount of peptide to inhibit microbial growth

RESULTS AND DISCUSSION

Evaluation of ACE inhibitory activities of small size peptides

Evaluation of ACE inhibitory activities of peptides fraction with molecular size ≤ 10 kDa

Spent brewer's yeast were hydrolyzed then centrifuged. Supernatant was collected by using ultrafilter 10 kDa. ACE inhibitory activity was determined according to He Ni method (He Ni *et al.*, 2012). 5 μ l of 0.2 U/mL ACE enzyme and 50 μ L of 1.55mM HHL substrate were used for testing ACE inhibitor activity. Peptides were added to samples except the control sample to get the concentration of 60 μ g/mL, then incubated at 37°C for 1 h. The enzymatic reaction was inactivated by adding 100 μ L TFA 0.1%. Obtained result was shown on Figure 1.

The retention time of HA in the sample was similar to control, pointed in 6.635 min. The peak area of the sample was lower than the control, consequently the HA concentration of the sample was lower than the control, which demonstrated the ACE inhibitory capability of peptides having molecular size ≤ 10 kDa.

Effect of peptides concentration on ACE inhibitory activities

The ACE inhibitory activity of peptide fractions was determined with different concentrations from 30 to 70 µg/mL. The results were shown on Figure 2.

As shown in Figure 2, the peptide concentration was directly proportional to the ACE inhibitory activities. The level of IC₅₀ was determined at 48.85 µg/mL. Recently, most of studies focused on the levels of IC₅₀ of peptides obtained by hydrolyzing various food sources such as milk (Yadav, 2011), egg protein (Majumder, Wu, 2010), flax seeds (Udenigwe, Aluko, 2010), cowpea (Segura *et al.*, 2011), sesame flour (Marrufo – Estrada, 2013). The levels of IC₅₀ were reported from 0.0069 to 7.00 mg/ml. Furthermore, peptides obtained by hydrolyzing spent brewer's yeast also showed the ACE inhibitory capability.

Evaluation of ACE inhibitory capability of peptides having different molecular sizes

ACE inhibitory capability of peptides having molecular size ≤ 3, 5 and 10 kDa were evaluated contemporarily using 30 µg/mL concentration of each peptide with different sizes. Results shown on Figure 3 indicated ACE inhibitory activities of peptides were inversely proportional to their molecular size. Peptide with molecular size < 3 kDa had the highest inhibitory activity which was approximately 2-fold higher than peptide with molecular size <10 kDa). It is probably that peptides having small sizes can bind to ACE better than the larger ones (Hernandaz – Ledesma *et al.*, 2005; Wang *et al.*, 2012).

Antibacterial activity evaluation of peptide fractions

In order to evaluate the bacterial inhibitory activities of the peptides, agar dish diffusion method on selected media was used with three different types of bacteria such as *S. typhi*, *E. coli* and *V. cholerae*. Bacteria were grown in BHI broth. 10⁵ CFU was inoculated onto

corresponding selected agar plates: XLD for *S. typhi*, TBX for *E. coli* and TCBS for *V. cholerae*. After filtration through the cut-off membrane 5 kDa, 100 µL of peptide fractions was added into each agar dishes. Positive control was supplemented with 50 mg/ml of oxytetracycline and negative control was used with distilled water. Then dishes were incubated at 37°C (*S. typhi* and *V. cholerae*) and at 44°C (*E. coli*) in 24 h. The results are shown in Figure 4.

The antibacterial zone were appeared on the agar dishes while bacteria were grown in the negative control plate. This result illustrated peptide size < 5 kDa with bacteria growth inhibition activity.

Evaluation of antibacterial activity of peptides

In order to evaluate antibacterial activity of peptides, 10⁵ CFU of each was cultured on each agar dishes which contained from 10 to 60 mg/mL of peptides. The dishes were incubated at 37°C for *S. typhi* and *V. cholerae* and at 44°C for *E. coli* for 24 h. The results of MIC determination were shown in Figure 5, 6, 7 and Table 1.

S. typhi on the XLD agar (pink, black in center colonies, Figure 5) *E. coli* on TBX (green colonies, Figure 6), *V. cholerae* (yellow colonies, Figure 7).

The results showed that the CFU of three strains significantly decreased after 24h incubation. MIC of peptide to bacteria was different for each species. The lowest peptide concentration for completely inhibiting *V. cholerae*, *E. coli* and *S. typhi* were 30, 35, 50 mg/mL, respectively. These results demonstrated that peptide had the strongest inhibitory ability versus *V. cholerae*, followed by *E. coli*, and finally it was *S. typhi*.

Thus, small peptide hydrolyzed from spent brewer's yeast could inhibit the growth of some micro-organisms causing diseases: *S. typhi* is a bacterium causing typhoid, *V. cholerae* is a bacterium causing cholera, *E. coli* is one of the most frequent causes of many bacterial infections.

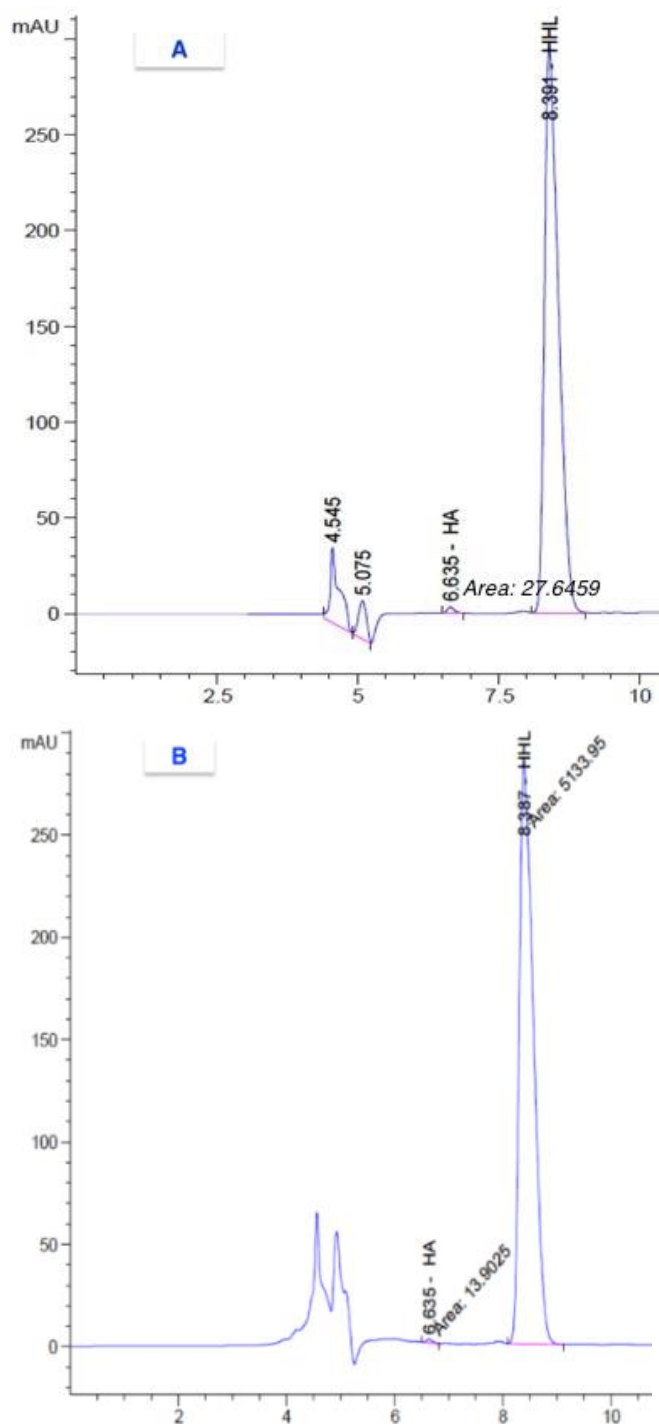


Figure 1. Chromatogram of HA generated from ACE-HHL reaction. A. Control sample (no peptide), B. Sample (peptide, 60µg/ml)

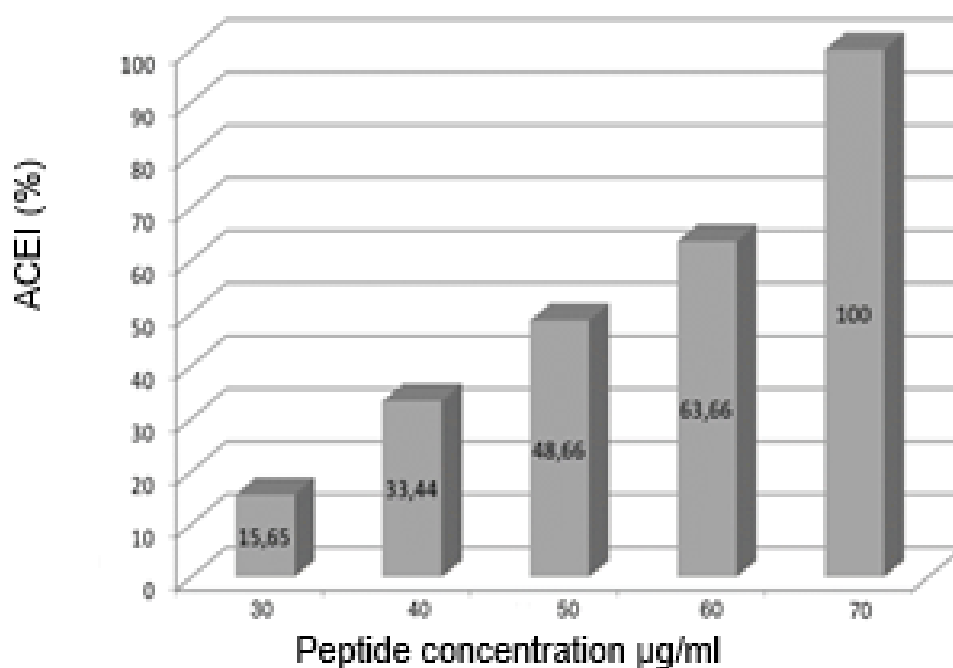


Figure 2. Effect of peptide concentration on ACE inhibitory capability.

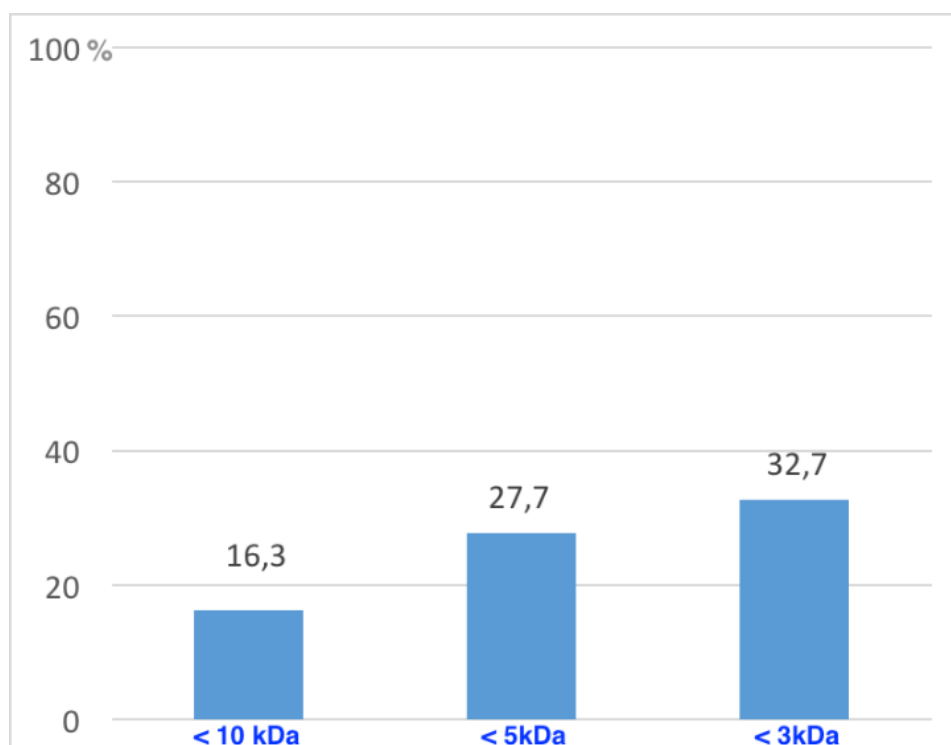


Figure 3. Evaluation of ACE inhibitory activities of peptides having molecular sizes ≤ 10 , 5 and 3 kDa.

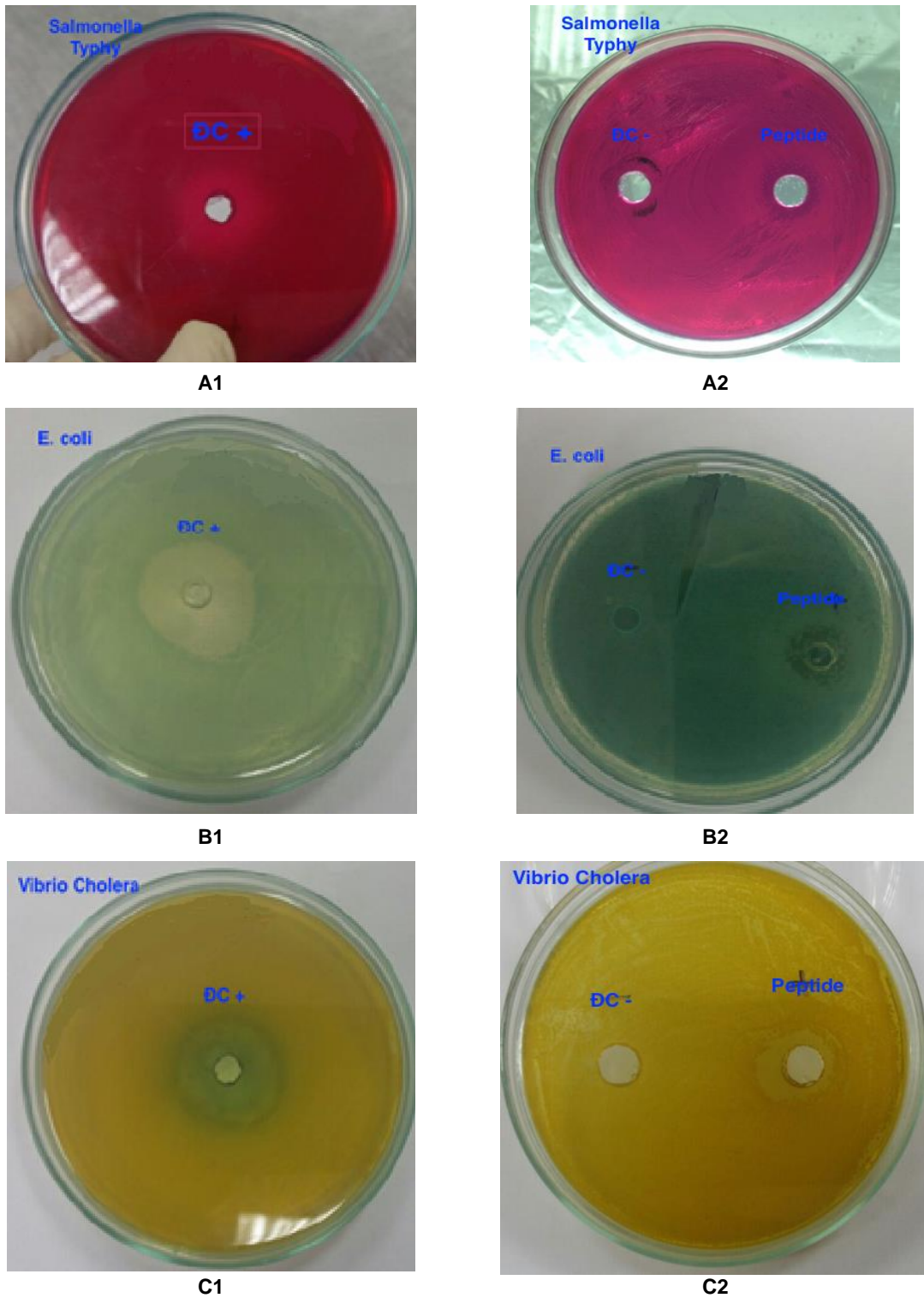


Figure 4. Bacterial inhibitory capability of peptides (negative control: distilled water, positive control: oxytetracycline). A: *S. typhi*; B: *E. Coli*; C: *V. cholera*; A₁, B₁, C₁: positive control: oxytetracycline; A₂, B₂, C₂: negative control: distilled water and peptide

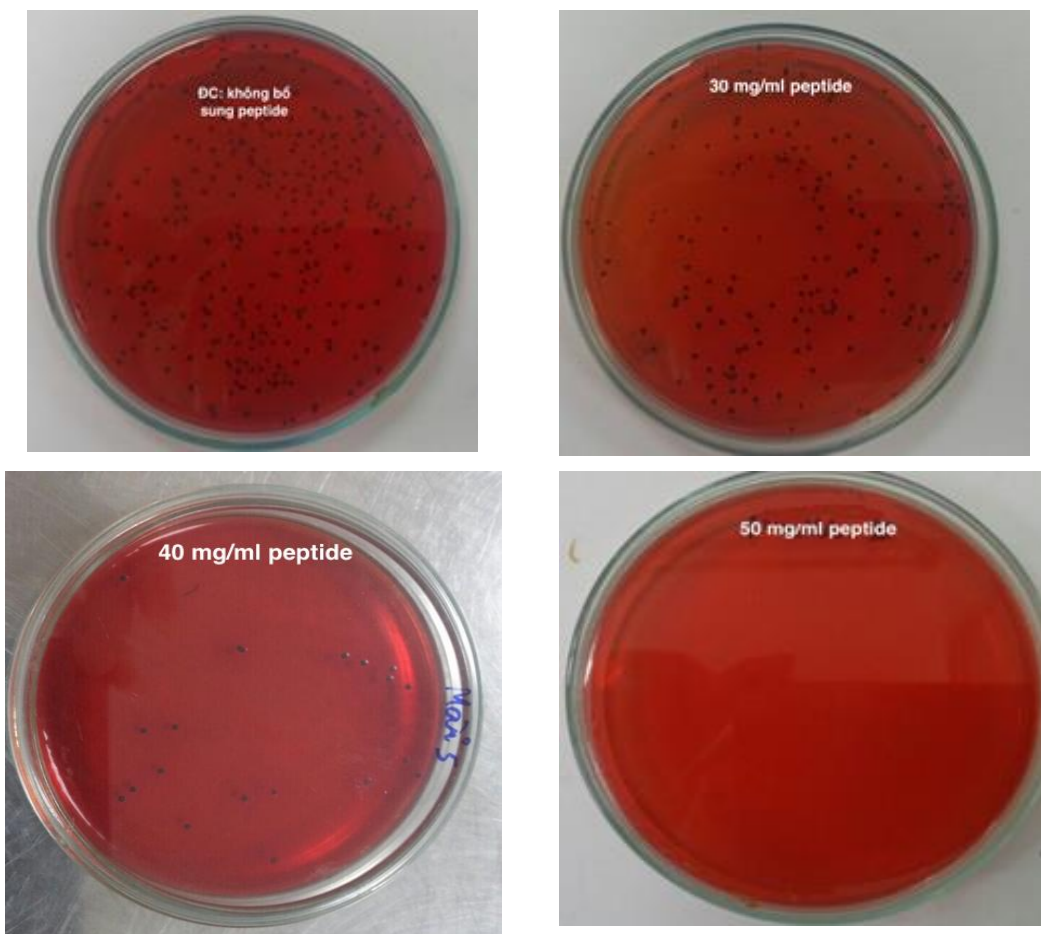
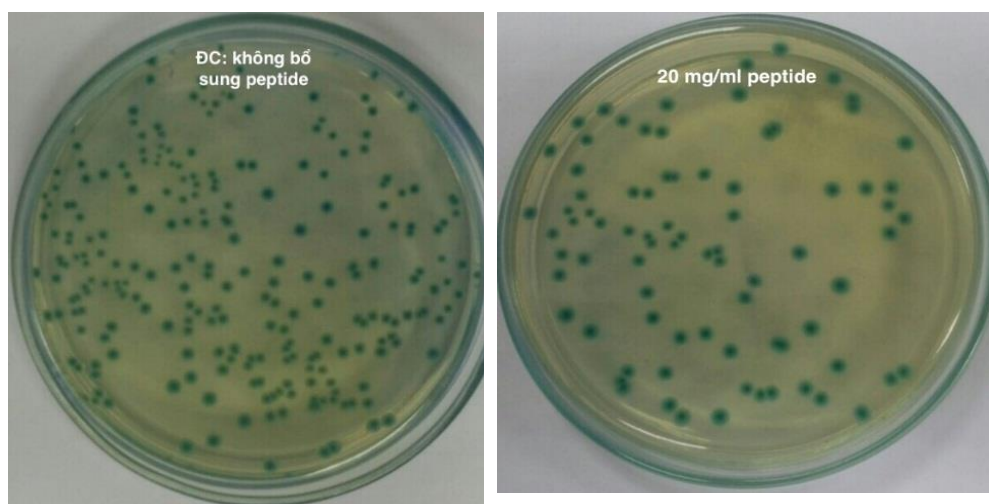


Figure 5. *S. typhi* on XLD media after 24 h of incubation.



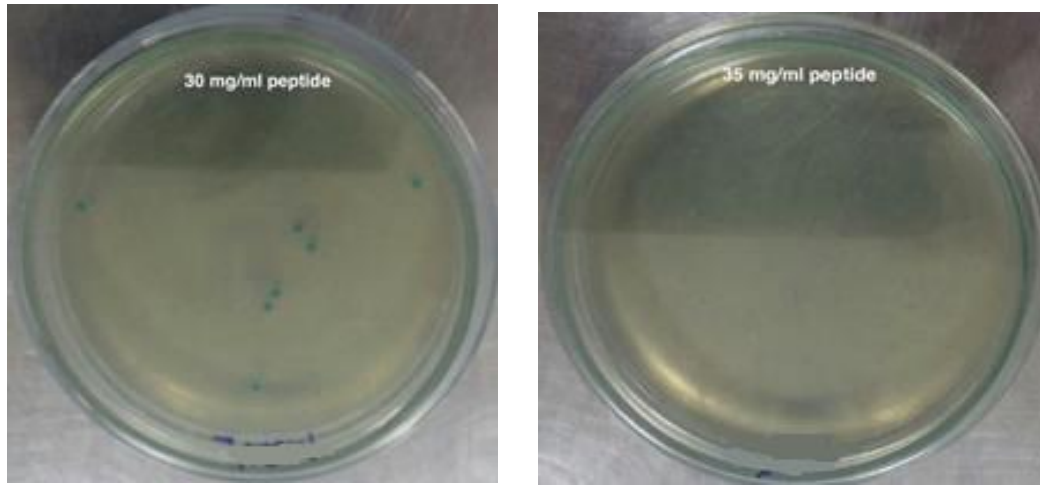


Figure 6. *E. coli* on TBX media after 24 h of incubation.

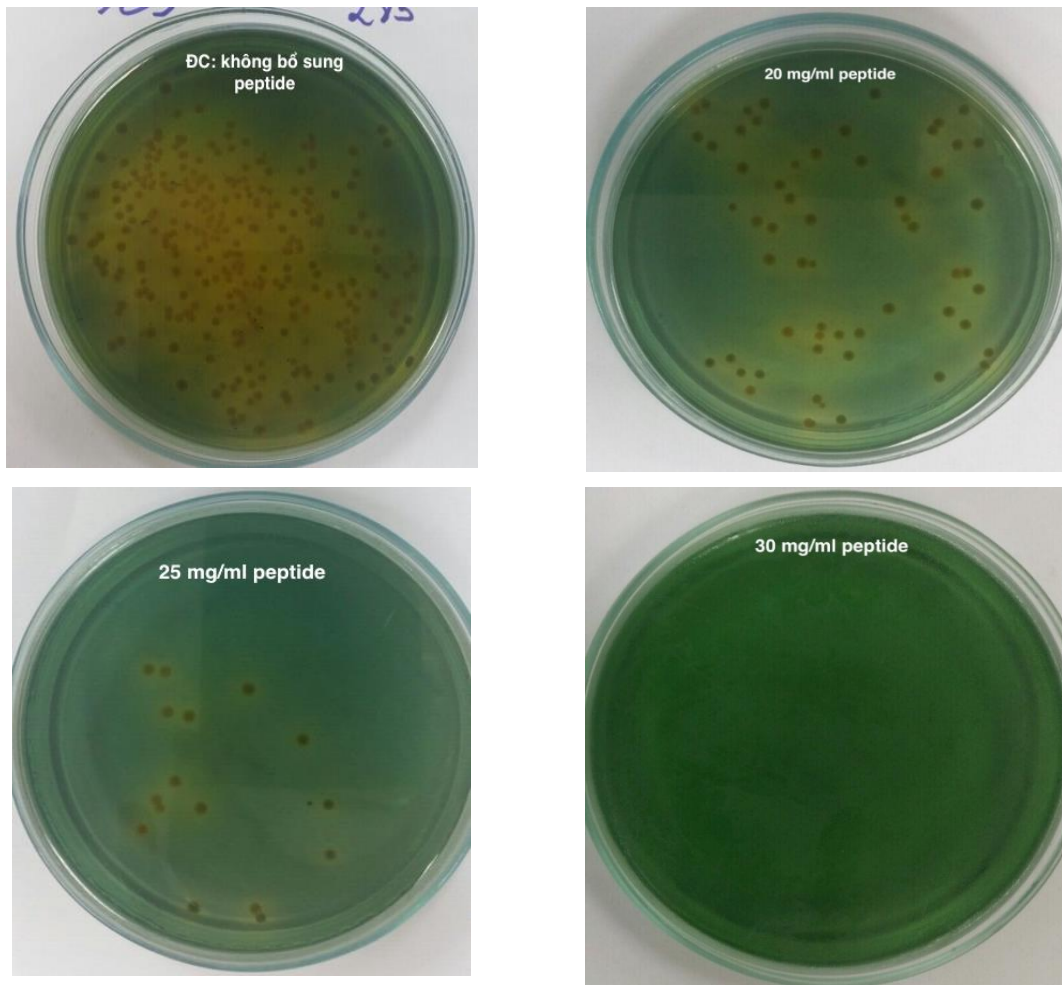


Figure 7. *V. cholerae* on TCBS after 24 h of incubation.

Table 1. MIC of peptides for antibacterial activity against some microorganisms causing diseases.

Bacteria (Appx. 10 ⁵ CFU)	Peptide concentration	CFU after 24h incubation	(MIC)
<i>S. typhi</i>	0 mg/mL	3 x 10 ⁵	50 mg/mL
	30 mg/mL	175	
	40 mg/mL	19	
	50 mg/mL	0	
<i>E. coli</i>	0 mg/mL	2.4 x 10 ⁵	35 mg/mL
	20 mg/mL	79	
	30 mg/mL	7	
	35 mg/mL	0	
<i>V. cholerae</i>	0 mg/mL	2.9 x 10 ⁵	30 mg/mL
	20 mg/mL	58	
	25 mg/mL	17	
	30 mg/mL	0	

CONCLUSION

In this study, the ACE inhibitory activities of peptide fraction having molecular size ≤ 10 kDa obtained from the spent brewer's yeast hydrolyzate were determined. The ACE inhibitory activities of peptide were inversely proportional to their molecular size. IC₅₀ value of peptide fraction <10 kDa was 48.85 μ g/mL. In addition, this peptide fraction could inhibit the growth of some harmful bacteria as *V. cholerae*, *E. coli* and *S. typhi* with the MIC values of 30, and 50 mg/mL, respectively. Peptides obtained by this study could be applied for producing functional food.

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HOẠT TÍNH KÌM HÃM ANGIOTENSIN CONVERTING ENZYME VÀ KHẢ NĂNG KHÁNG KHUẨN CỦA PEPTIDE PHÂN TỬ LƯỢNG THẤP TỪ NẤM MEN BIA

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TÓM TẮT

Theo tổ chức y tế thế giới (WHO), tăng huyết áp là một trong những căn bệnh mãn tính phổ biến nhất. Mỗi năm trong số 17,5 triệu người tử vong do các bệnh tim mạch thì tăng huyết áp là nguyên nhân trực tiếp gây tử vong đến 40%. Sự tăng huyết áp này là do hoạt động của Angiotensin Converting Enzyme (ACE). Kim hãm ACE được coi là một liệu pháp hữu hiệu trong điều trị cao huyết áp. Rất nhiều chất ức chế ACE được tổng hợp bằng con đường hóa học để giảm cao huyết áp, tuy nhiên, các loại thuốc này thường gây ra các tác dụng phụ. Vì vậy, trong khoảng hai thập kỷ gần đây, nhiều tác giả đã quan tâm nghiên cứu và sản xuất nhiều loại peptide ức chế ACE có nguồn gốc từ tự nhiên (thực vật, động vật và vi sinh vật) giúp giảm huyết áp mà ít gây ra tác dụng phụ. Mặt khác, các peptide sinh học có khả năng kháng các vi khuẩn gây bệnh cũng được quan tâm đặc biệt. Trong nghiên cứu này, chúng tôi đề cập đến việc thủy phân giới hạn bã men bia bằng protease để thu peptide có khối lượng phân tử < 10 kDa, 5 kDa và 3 kDa. Các phân đoạn peptide có kích thước phân tử <10 kDa đều có hoạt tính ức chế ACE. Kết quả nghiên cứu cho thấy các phân đoạn peptide có khối lượng phân tử càng nhỏ thì hoạt tính kim hãm ACE càng cao. Với hàm lượng 30 µg/ml, các peptide < 10 kDa, <5 kDa, <3 kDa có hoạt tính kim hãm lần lượt là 16,3%, 27,7%, 32,7% và IC₅₀ tốt nhất là 48,85 µg/ml. Nồng độ peptide thấp nhất có khả năng ức chế hoàn toàn vi khuẩn sau 24 h đối với *V. cholerae* là 30 mg/mL, *E. coli* là 35 mg/mL và *S. typhi* là 50 mg/mL.

Từ khóa: hoạt tính kim hãm ACE, kháng khuẩn, peptide phân tử lượng thấp, nấm men bia