ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORY POTENTIAL OF HAZELNUT PROTEIN AND TRYPSIN HYDROLYSATES

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Abstract

Plant proteins and enzymatically produced bioactive peptides have attracted due to their health-promoting potential and minimizing disease risk in functional foods. In this study angiotensin-converting enzyme (ACE) inhibitory effect of hazelnut protein and its trypsin hydrolysates for 60 and 120 minutes at 37°C were examined. ACE inhibition percent of protein isolate and hydrolysates were about 80 % and 90 % respectively. The IC_{50} concentrations of trypsin hydrolysates were 5.06, 0.59, and 0.57 mg protein/ml respectively. Hazelnut protein and hydrolysates have reasonable inhibition in comparison to corn, gluten, soy, legume proteins and their hydrolysates.

Key words: *hazelnut, bioactive peptides, angiotensin-converting enzyme (ace)*

1. INTRODUCTION

Turkey, with 70% of total world production, is the most important hazelnut producer (Seyhan et al., 2007). Hazelnut has rich bioactive nutrition content. Especially its monounsaturated fatty acids, antioxidant, vitamin E, phenolic and protein content makes hazelnut as special on human diet (Alasalvar, 2003). The protein content of hazelnut changes between 17.4 and 20.8% (Köksal et al., 2006). This high protein intent especially makes hazelnut important for protein hydrolysis works. Functional properties of enzymatically digested food protein can be improved. One of the foremost functional properties of peptides is antihypertensive effect. This property is related to inhibition of Angiotensin converting enzyme (ACE) that converts angiotensin I into highly potent vasoconstrictor angiotensin II.

The aim of this work is to examine Angiotensin converting enzyme (ACE) inhibition effect of enzymatically hydrolyzed hazelnut protein.

2. MATERIALS AND METHODS

2.1. Materials

Unshelled hazelnut was purchased from local market in Mersin.

2.2. Chemicals and Reagents

Sodium Hydroxide (No: 106467) and Boric acid (No:100165), were purchased from Merck (KGaA, Germany). Bromocresol Green ACS reagent (No: 114359), trypsin (sigma), Sodium di-Hydrogen Phosphate (NO:106342), Phosphoric acid (No:345245), Methyl Red acid-base indicator (No:32654), N-Hippuryl-His-Leu hydrate powder (No: H1635), Hippuric acid (No:112003) and Angiotensin Converting Enzyme from rabbit lung (ACE- A6778; 0.25unit) were purchased from Sigma-Aldrich (USA)

2.3. Hazelnut Protein Concentrate

Unshelled hazelnut was first grinded by grinder (IKA-Werke M20, Almanya). Grinded hazelnut was defatted by petroleum ether for 5 hours using soxhlet device (Gerhardt, Sox 412, Germany). After defatting, petroleum ether is removed in drying incubator at 30^0C for 2 hours.

In order to concentrate of protein, alkaline extraction and isoelectric precipitation method is used (Alukoand Monu, 2003). Figure 1 shows flowchart of production of hazelnut protein concentrate.

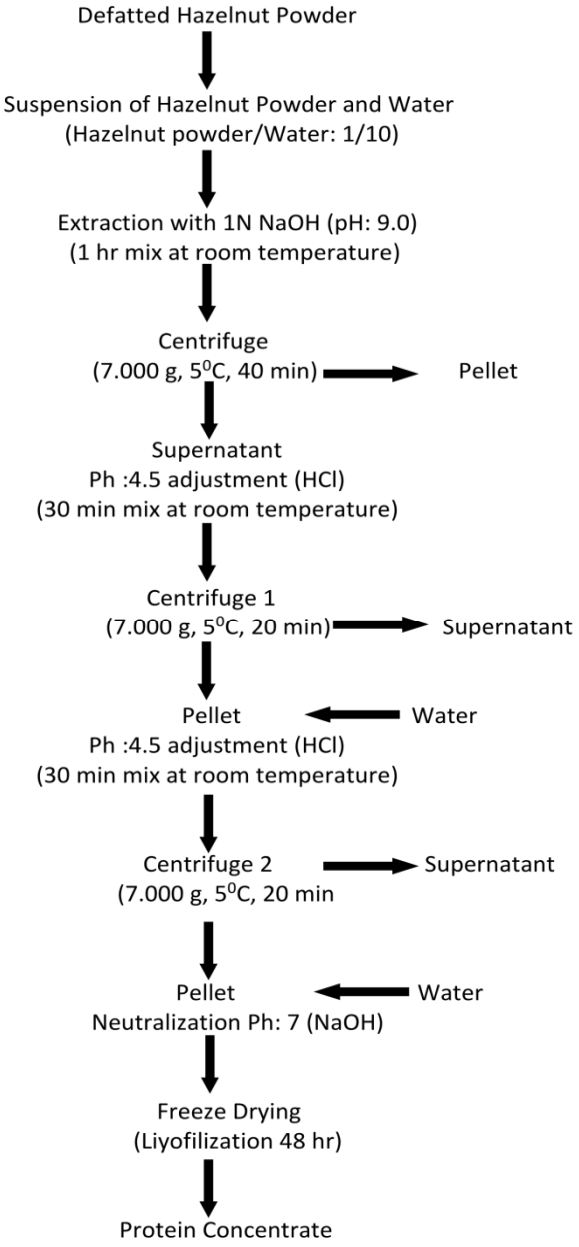

Figure 1. Production of Protein Concentrate

2.4. Hydrolysis of Protein

Trypsin enzymatic hydrolysis of protein was carried out according to Kong et al. [2006]. Before hydrolysis, hazelnut protein isolate was mixed with pH7.5 buffer (NaH_2PO_4. $2H_2O$ – H_3PO_4) for 30 minutes. Trypsin enzyme was added with 1:20 (w:w) enzyme/substrate ratio at 37°C for 120 minutes. During the hydrolysis samples were taken at several time periods. The first sample was taken just before adding the enzyme. Hydrolysis was finished after 120 minutes. The samples were taken at 0 minutes, 60 minutes and 120 minutes. Samples were heated to 80°C for enzyme inactivation. Then hydrolysates were centrifuged at 6000g for 6 minutes and stored at -20 °C for ACE inhibition analyses.

2.5. ACE Inhibitory Activity

Hydrolysates were analyzed for ACE inhibitory effect according to Wu et al. (2006) with some modifications. The principle of experiment is to catalyze N-hippuryl-l-histidyl-l-leucine (HHL) by ACE and inhibition of this reaction by protein hydrolysates.

Total volume was adjusted to 140µL with 100µL of 2mM HHL, 20 µL of different concentration of protein hydrolysates and 20 µL of 2mU of ACE. All reagents were prepared with 100mM of borate buffer. ACE was added to HHL and hydrolysate after incubated in 37°C for10min. Reaction was terminated by adding 85 µl of 1M HCl after 1 hr. of hydrolysis at 37°C in water bath. All samples were filtered with through 0.45µm syringe filter before loading to HPLC.

2.6. High- Performance Liquid Chromatography (HPLC)

A Shimadzu 20 AD (Japan) HPLC system with Inertsil ODS 4 column and diode array detector was used. 10 µL of sample was eluted with 0.05% TFA in water and (B) 0.05% TFA in acetonitrile at 1ml/min flow rate. Elution was gradient for 10min from 5 to 60% of acetonitrile. After maintained 2 min, acetonitrile ratio was reduced to 5% in 1 minute and held for 4 minutes at that ratio. Total elution time was 17 minutes. HA was detected at 228nm. HA standard curve was made up between 0.02mM to 1mM concentrations of HA using NaCl-borate buffer at pH 8.3. Control sample was prepared without protein inhibitor to understand total HA production. Percent inhibition was calculated as following equation where $M_{Control}$ was HA concentration of control sample and M_{Sample} was HA concentration of samples with protein inhibitor.

$$\% \text{ Inhibition} = (M_{Control} - M_{Sample}) / M_{Control} \times 100$$

In order to understand the inhibitor concentration for 50% ACE inhibition (IC_{50}), protein hydrolysates were diluted with 2 to 150X dilution factor and IC_{50}value is calculated graphically.

3. RESULTS AND DISCUSSIONS

The Percent of ACE Inhibition (INH%) value of non-hydrolyzed sample at time 0was observed around 75% During trypsin hydrolysis the INH % values increased to around to 90% and remained stable (Figure 2).

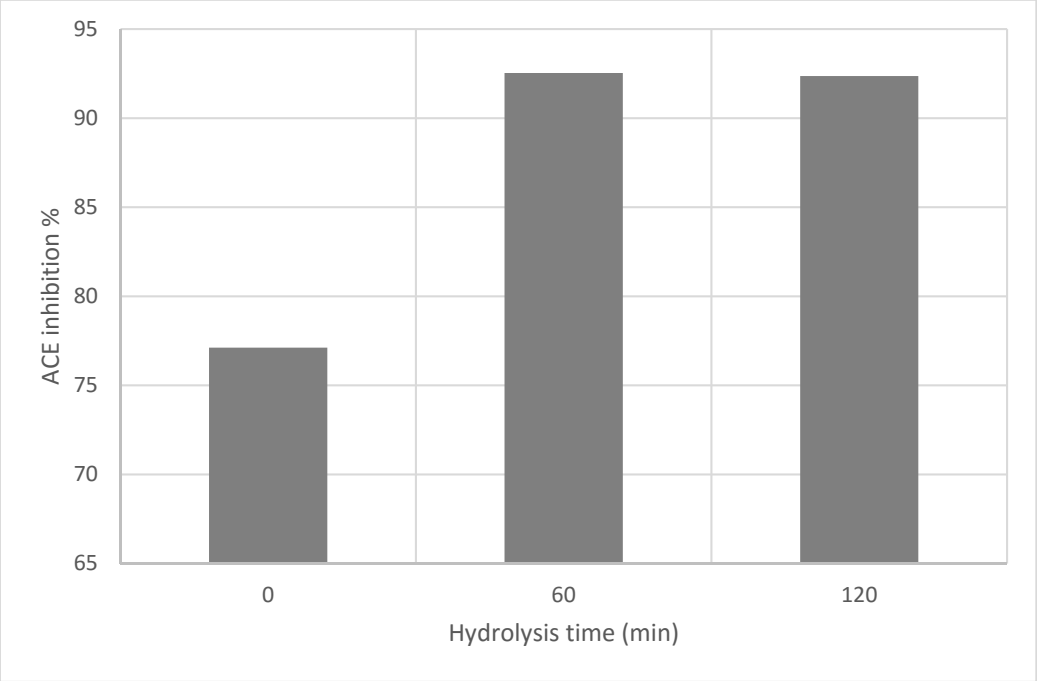

Figure 2. ACE Inhibition percent during Trypsin Hydrolysis time

According to results, there were appreciable differences between hydrolyzed and non-hydrolyzed hazelnut protein concentrates on ACE activity. However, after 1 hr. of hydrolyses, INH %values remains stable. Although there was not too much study on hazelnut inhibitory effect on ACE, Aydemir et al. (2014) claimed that 70-80% of INH % value of non-hydrolysed hazelnut protein sample at pH 7.5 was because of the resistant peptide part of hazelnut. In a similar point of view, Otte et al. (2007) shoved that 60% of ACE inhibitory activity before hydrolysis of milk protein.

The hazelnut protein concentration that inhibits 50% of ACE activity value (IC_{50} value) was generally used to compare with different food proteins. At this work, protein concentration was diluted between 2 and 150X dilution factor to understand IC_{50} values. The protein content vs INH % graphs of samples that were taken 0 min, 60 min and 120 of hydrolysis were drawn at Figure 3, Figure 4 and Figure 5 respectively.

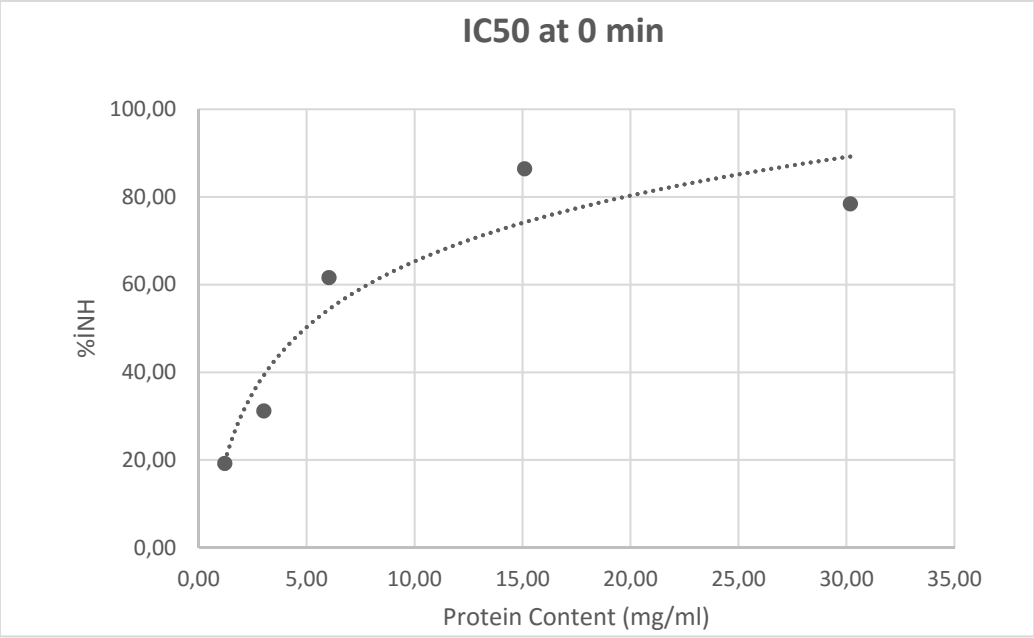

Figure 3. ACE inhibition IC_{50}concentration of non-hydrolyzed hazelnut protein (t:0min)

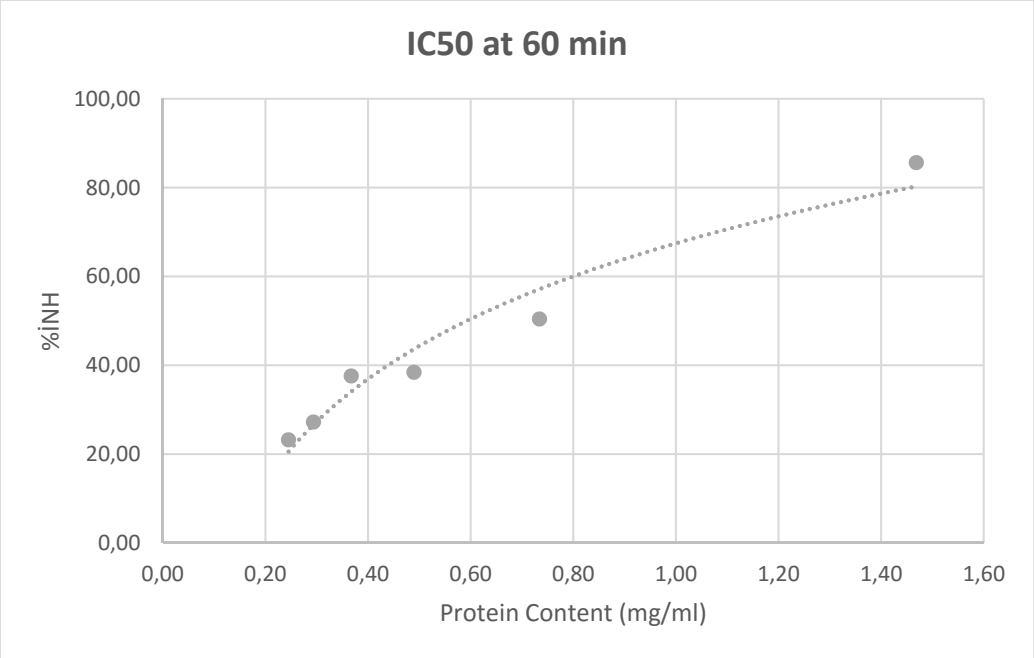

Figure 4. ACE inhibition IC_{50}concentration of 60 min hydrolyzed hazelnut protein

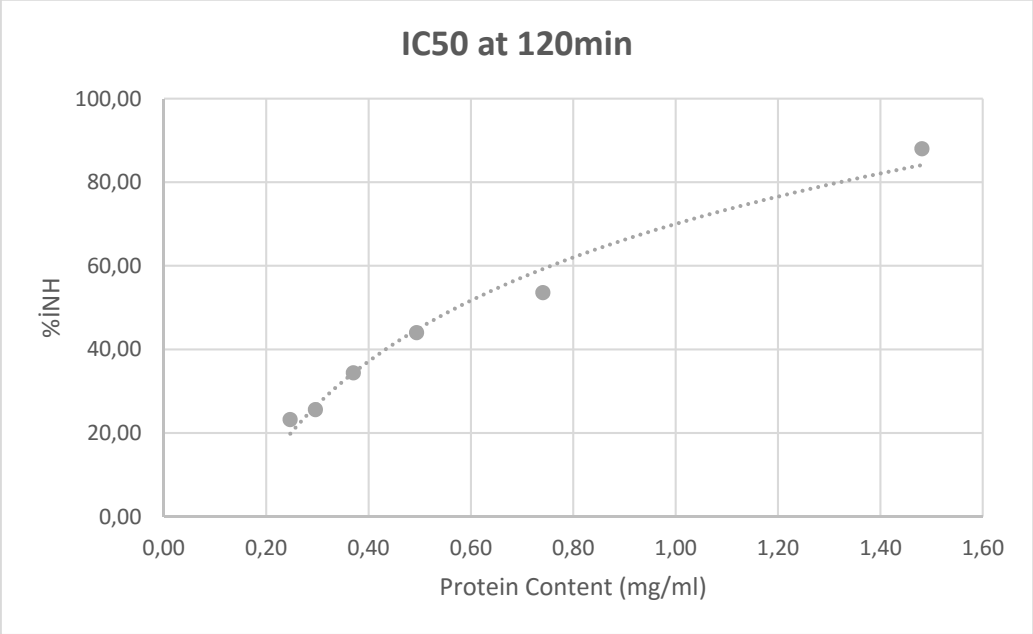

Figure 5. ACE inhibition IC_{50}concentration of 120 min hydrolyzed hazelnut protein

The IC50 values of 0, 60 and 120 minutes samples of trypsin hydrolysis were 5.06 mg protein/ml, 0.59 mg protein/ml and 0.57 mg protein/ml respectively. Changes of IC_{50}values was given at Figure 6.

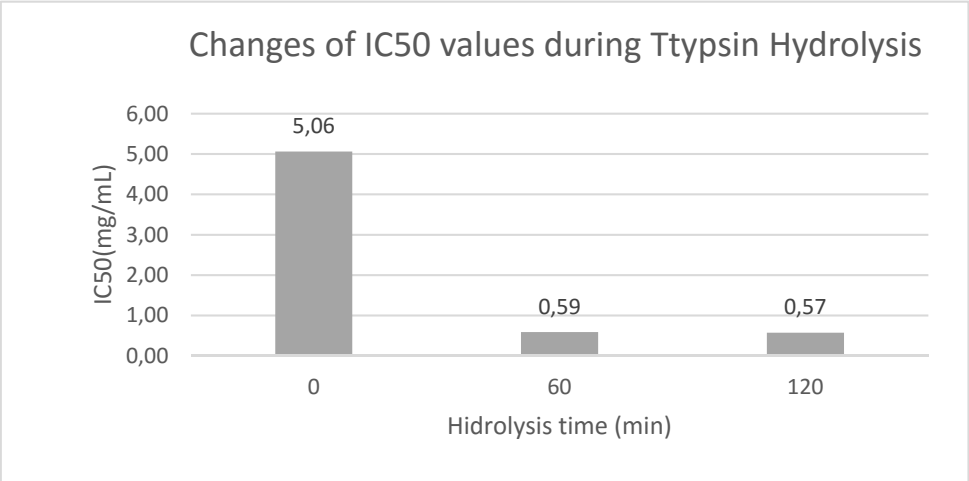

Figure 6. IC50 of Pepsin and Trypsin Hydrolysates

Figure 6 shows that IC50 of 60 min decreases 5.06 to 0.59 mg protein/ml, which was almost 8.5 fold lower than that of non-hydrolyzed samples. This means that after 60 min of hydrolysis antihypertensive activity of protein increases around 8.5 times. The IC50 of 60 min and 120 min samples gives almost same IC_{50} values. The present study has proved compatible $IC_{50}$with several food protein sources. IC_{50} of soy which hydrolyzed with pepsin were measured as 0.34 (Wu and Ding, 2002). IC50 of corn gluten, hydrolyzed with alfa amylase were 0.18 (Kim et al., 2004) and IC50 of whey that was hydrolyzed with corolase pp enzyme has 0,88 mg protein/ml of IC50 value (Van der Ven et al., 2002).

4. CONCLUSION

Results from present work show that trypsin hydrolysates of hazelnut protein show almost 8.5 times more anti-hypertensive effect than non-hydrolyzed hazelnut protein. This study can be repeated with some other enzymes in order to understand the different enzymes on ACE inhibition.

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