Research Article

Angiotensin-Converting Enzyme (ACE) Inhibitory Effects of Hazelnut Protein Hydrolysate Prepared Using Pepsin

Evren Caglar Eroglu[1*], Salih Aksay[2]

[1]Alata Horticultural Research Institute, Department of Postharvest Physiology, Mersin, TURKEY.
[2]University of Mersin, Department of Food Engineering, Mersin, TURKEY.

ABSTRACT

Objective / Purpose: The aim of this study is to find out the effect of protein isolate and pepsin hydrolysates of hazelnut protein on Angiotensin-Converting Enzyme (ACE) inhibition. **Material and Methods**: Unshelled hazelnuts (*Corylus avellana* L.) was firstly grinded and defatted by petroleum ether. Alkaline extraction and isoelectric precipitation method was used in order to obtain protein isolate. Neutralized protein suspension was freeze dried before analysis and stored at -18°C. Pepsin hydrolysis was conducted at pH 2 with 1:20 (w:w) enzyme/substrate ratio at 37°C for 60 min. Hydrolysis was stopped by heating samples to 85°C for enzyme inactivation and hydrolysates were analyzed for ACE inhibitory effect. 100 μL of 2 mM HHL, 20 μL of hydrolysate and 20 μL of 2 mU of ACE were incubated in 37°C for 60 min. Reaction was terminated by adding 85 μl of 1 M HCl. Shimadzu HPLC system with C18 column and DAD was used for ACE inhibition analysis. **Results and Discussion**: Hazelnut protein isolate and peptides have over 95% ACE inhibition. On the other hand, IC50 values of samples taken at 0, 30 and 60 minutes of pepsin hydrolysis were 1.29 mg protein/ml, 0.25 mg protein/mL and 0.22 mg protein/mL, respectively. **Conclusion:** Results shows that hazelnut protein isolate and peptides have reasonable ACE inhibition properties. Pepsin hydrolysates of hazelnut isolates gives almost 6 times more anti-hypertensive activity than hazelnut protein isolate.

Key words: Hazelnut, Pepsin Hydrolysis, ACE Inhibition, Anti-Hypertensive.

INTRODUCTION

Hypertension is of primary important disease all over the world.[1-5] Change of life style and pace of life trigger number of patients day by day.[6] However, increasing of cost of illness as well as awakening divert people and governments to find alternative way.[7] Functional foods have always been good alternative treatment.[8-12] Although there are numerous studies on functional properties of animal and plant-derived bioactive proteins, plant-derived protein studies become prominent on the ground that they are cheap and readily available.[13,14] In order to increase bioactive properties of proteins, they are usually digested to peptides. Peptides, by enzymatic hydrolysis or other digesting methods, can be obtained and purified for functional properties.

In this study, defatted and purified hazelnut isolates and pepsin hydrolyzed peptides were investigated for angiotensin converting enzyme inhibitory activity.

MATERIAL AND METHODS

Hazelnut (*Corylus avellana* L.) was purchased from local market (Turkey). Hippuric acid (HA), N-Hippuryl-His-Leu hydrate powder (HHL), pepsin from porcine gastric mucosa and Angiotensin Converting Enzyme (ACE) from rabbit lung (0.25 unit) were purchased from Sigma-Aldrich. The other chemicals were of analytical grade.

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Correspondence:
Evren Caglar Eroglu,
Alata Horticultural Research Institute, Department of Postharvest Physiology, Mersin, TURKEY.
Phone no: 0090-5055482936
Email Id : evcager@gmail.com

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Defatting of Hazelnut Flour

100 gr of hazelnut fruits with shell were hydrated with twice as much as water for 24 h. In order to separate shell, rehydrated fruits were dried in an oven at 40ºC for a night and grinded using a grinder. In order to remove fat, ether was added to ground meal and mixed using magnetic stirrer under fume hood for 8 h. Meal was filtered using coarse paper under vacuum. This procedure was repeated 2 times. Excess ether was removed by drying in oven at 40°C.

Preparation of Protein Isolate and Hydrolysis

Protein isolate was prepared according to modified methods of Siddeeg *et al.*[15] Method was based on isoelectric point precipitation. Defatted hazelnut meal was extracted in 1:8 (hazelnut meal/water) in pH 9 (prepared by 1 N NaOH) for 1 h. and centrifuged at 9,000 *g* for 15 min. This procedure was repeated 2 times more to collect all protein. For isoelectric point precipitation, pH was adjusted to 4.5 with 1N HCl and entire protein was precipitated for 30 min. Supernatant was extracted. And pellet was neutralized using 1N NaOH. Hazelnut isolate was freeze dried for and stored -20°C. Protein content was determined by micro-Kjeldhal method, AACC.[16]

Pepsin hydrolysis of hazelnut isolate was carried at pH 2 at 37°C for 60 min. Enzyme/substrate ratio was 1:20. Samples were taken at 0 min (hazelnut protein isolate), 30 min (P30) and 60 min (P60) of hydrolysis. All samples were aliquoted and stored at -20°C until analyzed.

ACE Inhibitory Activity

ACE Inhibitory activity was determined by some modifications of method described by Wu and Ding.[17] ACE catalyzes normally whole HHL to form hippuric acid (HA) in case of being non-inhibitor (called as control sample). ACE inhibitory activity of hazelnut protein was determined after adding protein isolate or peptides (called as inhibitor sample) and monitored for differences between inhibitor samples and control samples using HPLC.

20 µL of 2.0 mU ACE, 20 µL hazelnut protein hydrolysate and 100 µL of a 2.0 mM HHL were mixed and incubated for 1 h. Enzymatic activity was stopped by adding 85 µL of 1M HCl. It is assumed that control samples produce 100% of HA. Percent of inhibitory activity of protein (% INH) was calculated as follows:

%INH = 1- (HA of inhibitor sample / HA of control sample) × 100

HA analysis was carried out by Shimadzu HPLC with PDA detector. Mobile phase were 0.05% acetonitrile (A) and water (B). First 10 min solvent A was increased linearly from 5 to 60%. After 2 min isocratic elution, it was decreased to 5% acetonitrile. Elution was finished after 4 min at this level. Flow rate was 1 ml/min.

IC_{50} value, concentration of protein isolate to inhibit 50% of ACE, was calculated by diluting samples with 5-100X dilution factor.

Table 1: Percent of ACE Inhibitory Activity (%INH) of Hazelnut Protein Isolate and Peptides

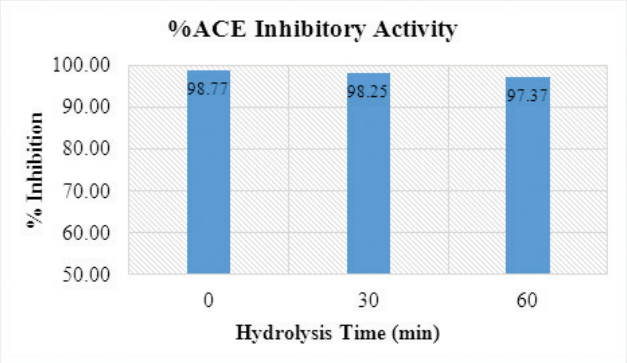
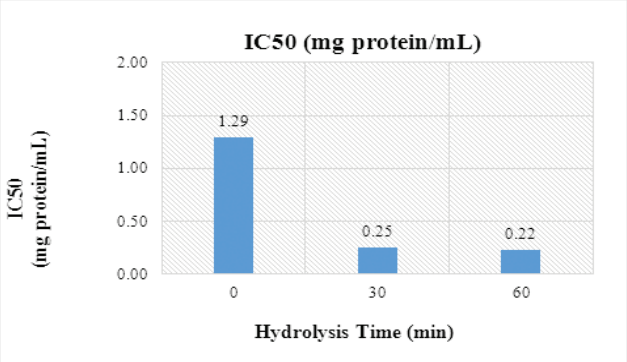


Table 2: IC50 values of Hazelnut Protein Isolate and Peptides



Statistical analysis

All results were analyzed by one-way analysis of variance (ANOVA) using SPSS. Mean values were analyzed Tukey's test ($p < 0.05$).

RESULTS & DISCUSSION

In this study, antihypertensive activity (ACE inhibition activity) of hazelnut isolate and hazelnut peptides were determined. Table 1 shows ACE inhibitory activity of hazelnut protein isolate sample (taken at the beginning of hydrolysates) and 2 different hazelnut peptides samples (P30 and P60).

According to results, it was realized that samples inhibited more than 95% of ACE. There were a slight decreasing of % INH throughout hydrolysis. Although %INH

values of protein isolate and P30 samples were not statistically different (p=0.25), that of protein isolate sample and P60 were significantly different (p=0.02). % INH of hazelnut protein isolate was comparable with previous studies. According to Siddeeg *et al.*[18] hazelnut protein had 94.2% ACE inhibitory activity. Moreover, % INH of whey protein concentrate was defined as 88.6%.[19] According to Quist *et al.*[20] ACE inhibitory activity of peanut protein increased from 66% to 97% after alcalase hydrolysis.

In order to compare the results with literature, it was necessary to calculate the amount of protein that inhibits 50% of ACE. This amount of protein is called as IC_{50}. IC_{50} values of isolate and peptides were given Table 2. Although all samples resulted in more than 95% of ACE inhibition, their protein contents were averagely 28.12 g protein/mL. In order to define IC_{50}, samples were diluted with 2-100 X diluting factor. IC_{50} values of control sample was determined as 1.29 g protein/mL. P30 and P60 samples had almost one sixth of control sample's IC_{50} value, which were 0.25 and 0.22 g protein/mL, respectively. Decreasing of IC_{50} means more antihypertensive activity. P30 and P60 samples had 5.16 and 5.86 times more IC_{50} activity than protein isolate sample, respectively. IC_{50} of P30 and P60 were not significantly different (p=0.72). According to Aydemir *et al.*,[18] IC_{50} of hazelnut protein isolate was found 1.0 mg protein/mL. This value was comparable with present study. Despite limited studies of hazelnut protein, there were lots of studies on IC_{50} of some nuts and cereals. IC_{50} of alcalase hydrolyzed peanut protein was determined as 0.548 mg/mL.[21] β-conglycinin and glycinin hydrolysates of soy protein gave an IC_{50} values ranging between from 0.126 to 0.340 mg/mL.[22] Moreover, IC_{50} of dried and wet corn germ was found between 3.26 to 7.77 mg/mL.[23] and IC_{50} of whey protein concentrate without hydrolysis was clarified as 0.20 mg/mL.[19]

CONCLUSION

ACE inhibition capacity of defatted and purified hazelnut protein isolate can be good alternative to synthetic and some natural ACE inhibitors. Although IC_{50} of hazelnut isolate has a remarkable IC_{50} value, peptides of hydrolyzed hazelnut protein can result by far the best IC_{50}.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

None

ABBREVIATION USED

HA: Hippuric acid; HHL: N-Hippuryl-His-Leu hydrate powder; ACE: Angiotensin Converting Enzyme; P0: Sample taken at the beginning of hydrolysis (Hazelnut isolate); P30: Samples taken at 30 min of hydrolysis; P60: Samples taken at 60 min of hydrolysis; %INH: Percent of protein inhibitory activity; IC_{50}: Concentration of protein isolate to inhibit 50% of ACE.

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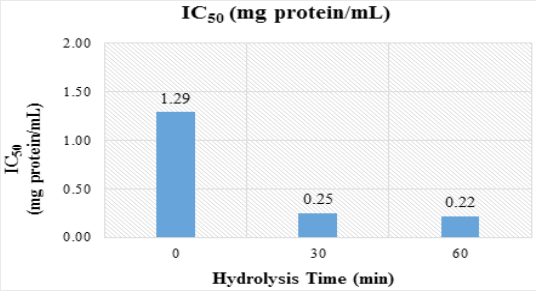
PICTORIAL ABSTRACT



SUMMARY

- Hazelnut protein isolate and peptides had more than % 95 ACE inhibitory activity.
- Pepsin hydrolysis gave almost 6 times less IC_{50} values than protein isolates. That means, hydrolysis increased almost 6 times of ACE inhibitory activity.

ABOUT AUTHORS

Evren Caglar Eroglu: Working as Research Specialist at ALATA Horticultural Research Institute, Ministry of Food, Agriculture and Livestock, Mersin, Turkey. His research subjects are biotechnology, food chemistry; esp. enzyme technology, protein purification and identification; microbiology; post-harvest studies and Functional Food Researches.

Salih Aksay: Working as Assistant Professor at Department of Food Engineering and Head of Vocational School of Silifke, University of Mersin, Turkey. He has various research publications in National and International Journals.

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