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

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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.
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 40°C 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. 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 and stored -20°C. Protein content was determined by micro-Kjeldhal method, AACC. 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. 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:

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%\text{INH} = 1 - \frac{HA \text{ of inhibitor sample}}{HA \text{ of control sample}} \times 100
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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.

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. hazelnut protein had 94.2% ACE inhibitory activity. Moreover, % INH of whey protein concentrate was defined as 88.6%. According to Quist et al. 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\textsubscript{50}. IC\textsubscript{w} 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\textsubscript{50} samples were diluted with 2-100 X diluting factor. IC\textsubscript{w} 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\textsubscript{w} value, which were 0.25 and 0.22 g protein/mL, respectively. Decreasing of IC\textsubscript{w} means more antihypertensive activity. P30 and P60 samples had 5.16 and 5.86 times more IC\textsubscript{w} activity than protein isolate sample, respectively. IC\textsubscript{50} of P30 and P60 were not significantly different ($p=0.72$). According to Aydemir et al., IC\textsubscript{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 of IC\textsubscript{50} of some nuts and cereals. IC\textsubscript{50} of alcalase hydrolyzed peanut protein was determined as 0.548 mg/mL. β-conglycinin and glycmin hydrolysates of soy protein gave an IC\textsubscript{w} values ranging between from 0.126 to 0.340 mg/mL. Moreover, IC\textsubscript{50} of dried and wet corn germ was found between 3.26 to 7.77 mg/mL and IC\textsubscript{50} of whey protein concentrate without hydrolysis was clarified as 0.20 mg/mL.

**CONCLUSION**

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

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**REFERENCES**

SUMMARY

- Hazelnut protein isolate and peptides had more than 95% ACE inhibitory activity.
- Pepsin hydrolysis gave almost 6 times less IC50 values than protein isolates. That means, hydrolysis increased almost 6 times of ACE inhibitory activity.

PICTORIAL ABSTRACT

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