ISOLATION of a AMYLASE INHIBITORS from MUNGBEAN and SOYBEAN and INHIBITORY EFFECT on HUMAN SALIVARY and PORCINE PANCREATIC AMYLASE

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Abstract
Penghambat alfa amilase adalah salah satu komponen dalam suplemen makanan yang telah lama digunakan untuk terapi kegemukan karena penghambat amilase mempengaruhi metabolisme karbohidrat dalam sistem pencernaan. Sejumlah peneliti melaporkan terdapat dua grup penghambat amilase, yaitu protein dan non protein. Penghambat protein dilaporkan terdapat dalam kelompok kacang-kacangan dan biji-bijian. Tujuan penelitian ini adalah untuk mengisolasi penghambat protein yang terdapat dalam kacang hijau dan kedele. Kacang hijau dan kedele merupakan kacang-kacangan yang penting dalam makanan popular di Indonesia. Penghambat protein diendapkan dengan konsentrasi bertingkat garam ammonium sulfat [(NH)4SO4] dari 30-70%. Penghambat protein diuji terhadap amilase saliva manusia (HSA) dan amilase pankreas babi (PPA), serta kestabilannya terhadap pemanasan pada 100oC selama 30 menit. Hasil penelitian menunjukkan bahwa semua endapan jenuh dari semua konsentrasi amonium sulfat yang diuji menghambat PPA, tetapi tidak semua endapan jenuh tersebut dapat menghambat HSA. Hanya semua endapan jenuh (NH)4SO4 dari kacang hijau yang dapat menghambat HSA, dan penghambatan tertinggi terhadap HSA adalah endapan jenuh (NH)4SO4 50%. Endapan jenuh (NH)4SO4 40 % dari kedele putih dan endapan jenuh (NH)4SO4 60% dari kedele hitam dengan masing-masing penghambatan 98.67; 26.86 and 27.63%. Endapan jenuh (NH)4SO4 60-70% dari kacang hijau, 50% dari kedele putih dan 50% dari kedele hitam menghambat PPA 100%. Pemanasan penghambat pada 100oC selama 30 menit hampir tidak mempengaruhi penghambatannya terhadap PPA. Profil protein juga diamati menggunakan analisis SDS/PAGE.

Kata kunci: penghambat alfa amilase, kacang hijau, kedele

1. INTRODUCTION

Obesity leads development of cardiovascular diseases, diabetes and other chronic health problems has become a world problem including in Indonesia. The varying therapy currently available to control excess body weight include pharmacological preparations and dietary supplements intended to control energy absorbance and create weight loss, many of them based on plant products. Particular attention has focused on the so-called “starch-blockers”; these supplements contain high levels of α–amylase inhibitor. α-amylase inhibitor interferes with the breakdown of complex carbohydrates; therefore it may promote weight loss by reducing, or at least slowing the digestion of starch by allowing them to pass undigested into the lower gastrointestinal tract (Boniglia *et al*, 2008). Proteinaceous α-amylase inhibitors are found in microorganisms, plants and animals. In plants, proteinaceous inhibitors are mainly present in cereals such as wheat (*Triticum aestivum),* barley *(Hordeum vulgareum)*, sorghum *(Sorghum bicolor)*, rye *(Secale cereale)* and rice (*Oryza sativa)* but also in leguminosae such as pigeon pea *(Cajanus cajan)*, cowpea (*Vigna unguiculata)* and bean *(Phaseolus vulgaris)* (Franco *et al*, 2002). Protein concentrate of *Phaseolus vulgaris* has been reported to be commercially available and claimed as “starch blockers” (Boniglia et al, 2008; Barret &

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Udani, 2011). Some reports showed that commercial *Phaseolus vulgaris* extract containing supplements were proven to reduce body weight of the tested subjects (Celleno *et al*, 2007; Preuss *et a*l, 2007a, Preuss *et al*, 2007b; Boniglia *et al*, 2008; Barret & Udani, 2011).

The objective of this study was to isolate α amylase inhibitor that might be presence in some common legumes in Indonesian popular food such as mungbean and soybean.

2. MATERIALS AND METHODS

Mungbean (*Phaseolus radiatus),* yellow soybean *(Glysin Max),* black soybean *(Glysin Max),* Porcine pancreatic amylase (Sigma), Human Salivary Amylase (Sigma), protein low molecular weight marker (Amersham).

Preparation of crude inhibitor solution. Extraction of protein inhibitor from the legumes was carried out according to Burgos-Henandez et al (1999). Fractional precipitation with Ammonium sulfate was carried out at concentration from 30 to 70% saturation according to Harris & Angel (1989). The precipitated protein recovered by centrifugation in each steps (5000g at 30 min). The protein was suspended in 0.50mM Tris-Cl buffer pH 8.0.

Inhibitory assay: All crude extract and $(NH_4)_2SO_4$ fraction (fresh and heated @ 100^oC, 30 minutes) were tested for inhibitory activity by alkaline 3,5 dinitrosalicylic acid (DNS) procedure of Bernfeld (1955). One enzyme unit is defined as the enzyme activity that liberates 1 mg of maltose equivalents under the condition of the assay. One inhibitor unit is the amount of inhibitory extract which inhibits one unit of enzyme activity.

Electrophoresis:

Electrophoresis of inhibitor extracts was carried out in 12% polyacrylamide gels under denaturing conditions according to the procedure described by Copland (1994)

3. RESULTS AND DISCUSION

Inhibitory effect and protein profile of $(NH_4)_2SO_4$ precipitates:

All concentration of $(NH_4)_2SO_4$ precipitates of all samples inhibited Porcine pancreatic amylase but only $(NH_4)_2SO_4$ precipitates of mungbean inhibitated Human Salivary Amylase (Figure 1, 3, and 5).

Based on Figure 1 the highest inhibition of mungbean sample on PPA was in 60% saturated $(NH_4)_2SO_4$ precipitates (100% inhibition) followed by 70%, 40% and 30% saturated $(NH_4)_2SO_4$ precipitates. The SDS/PAGE (Figure 2) showed that the protein profiles of the all lanes were very similar except protein bands of was 24.35kD only present in lane well 60 and 70. The protein band of 21.62kD was in the all wells but the band was more distinct in the well 60 and 70. This indicated that the protein bands of 24.35 and 21.62kD might be the protein which inhibited PPA since the protein bands were unclear in the well 30, 40 and 60. The highest inhibition of the mungbean samples on Human Salivary Amylase (HSA) was the 50% saturated $(NH_4)_2SO_4$ precipitates. To confirm which protein bands inhibit PPA and HSA further purification has to be done. Study on mungbean inhibition on PPA and HSA have not been found so far. Most studies concerning the α-amylase inhibition were done using *Phaseolus vulgaris* extract. The study on mungbean protein on α-amylase inhibition were done by Kokiladevi *et al* (2005) and Wissesing *et al* (2010), however the experiments were on α-amylase inhibition of *Callosobruchus maculatus* an insect that damage mungbean seeds.

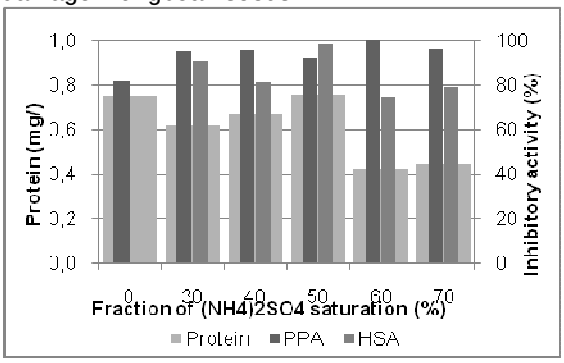

Figure 1. Mungbean inhibition on Human Salivary (HSA) and Porcine Pancreatic Amylase (PPA)

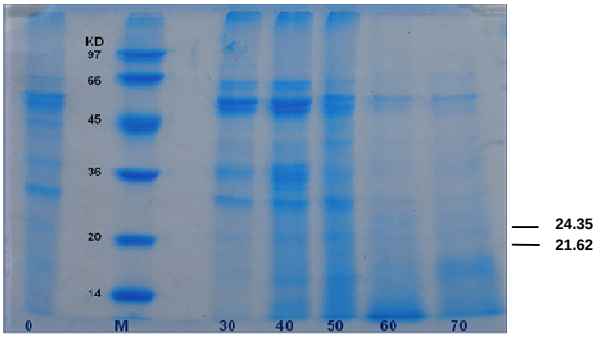

Figure 2. SDS/PAGE of 0, 30, 40, 50, 60, 70% saturated $(NH_4)_2$ SO_4 precipitate of mungbean. (0,30,40,50, 60, 70) and protein marker (M).

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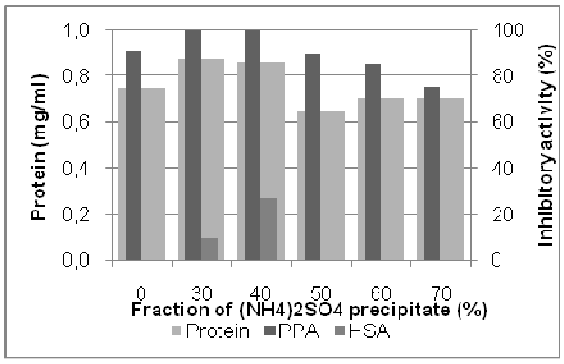

Figure 3. Yellow soybean inhibition on Human Salivary (HSA) & Porcine Pancreatic Amylase (PPA)

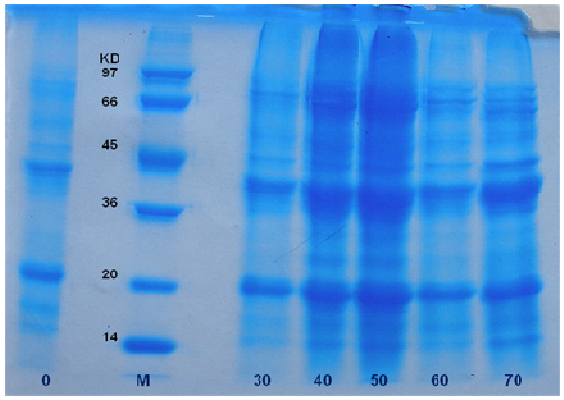

Figure 4. SDS/PAGE of 0, 30, 40, 50, 60, 70% saturated $(NH_4)_2$ SO_4 precipitate of yellow soybean (line 0, 30, 40, 50 60 and 70) protein marker (M).

30 and 40% saturated $(NH_4)_2$ SO_4 precipitates of Yellow soybean samples completely inhibited PPA but none of the samples inhibited HSA (Figure 3). Almost all the protein band presence in all precipitate samples with different intensity, therefore it was difficult to recognize which band might be the inhibitor.

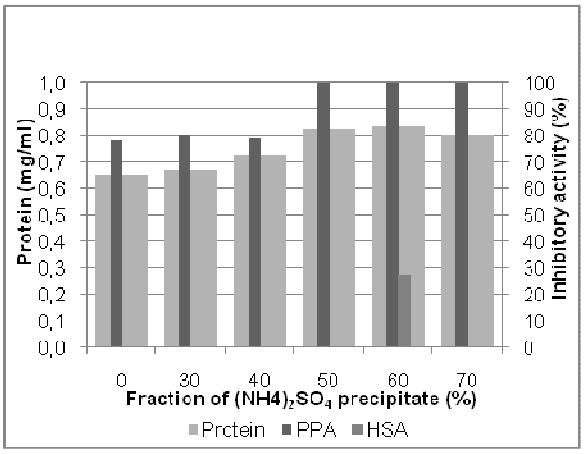

Figure 5. Black soybean inhibition on Human salivary (HSA) & Porcine pancreatic Amylase (PPA)

50, 60 and 70% saturated $(NH_4)_2$ SO_4 precipitates of black bean samples completely inhibited PPA but none of the black bean sample inhibited HSA (Figure 5). Based on Fig 6, the protein bands with approximate molecular weight of 76.98; 65.16; 34.59; 14.30 and 13.60 kD were only present in those precipitates. This indicated the inhibitors might be one of those proteins. In order to find the inhibitor the precipitates have to be purified further. Other experiments conducted by McCue *et al* (2005) and Maiti and Majumdar (2012) reported that soybean seeds contain α amylase inhibitor that inhibited PPA.

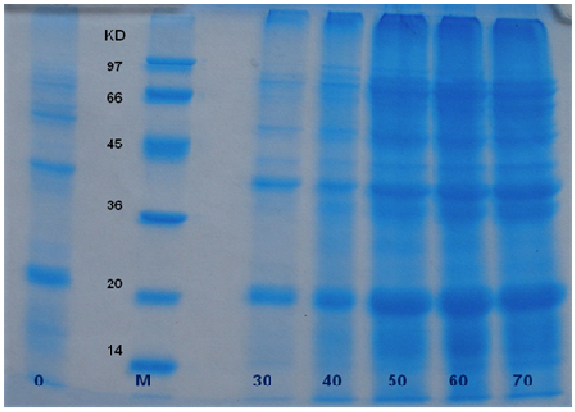

Figure 6. SDS/PAGE of 0, 30, 40, 50, 60, 70% saturated $(NH_4)_2$ SO_4 precipitate of black soybean (0, 30, 40, 50, 60, 70) & protein marker (M).

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Heat Stability of Amylase Inhibitors

All the mungbean samples heated at 100ºC for 30 minutes did not affect the inhibition activity (Figure 7). Heating fresh, 30 and 40% saturated $(NH_4)_2 SO_4$ precipitate of yellow beans samples decreased inhibition activity, but slightly increased the 50, 60 and 70% saturated $(NH_4)_2 SO_4$ precipitate samples (Figure 8). Heating the black bean samples did not affect their inhibition activity (Figure 9).

Heat stability study of α amylase inhibitor from wheat and rey protein extracts was done by Hernandez et al (1999). The inhibitors were heated in water bath at 70ºC for 90 minutes. The experiment showed that the inhibitor extracted from rey was more stable than that of the inhibitor extracted from wheat. The rey inhibitor was highly stable during 90 minutes heating treatment at 70ºC, whereas the heat stability of wheat inhibitor was dropped after 15 minutes of heating.

It seems that mungbean, white as well as black soybean inhibitors were more stable as compared to rey and wheat inhibitors.

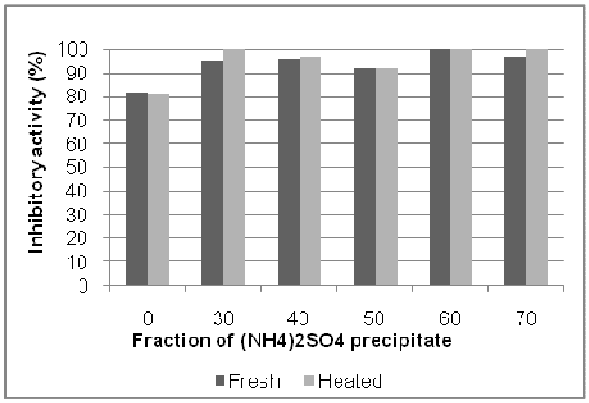

Figure 7. Effect of heating @100°C for 30 minutes on inhibitory activity of mungbean

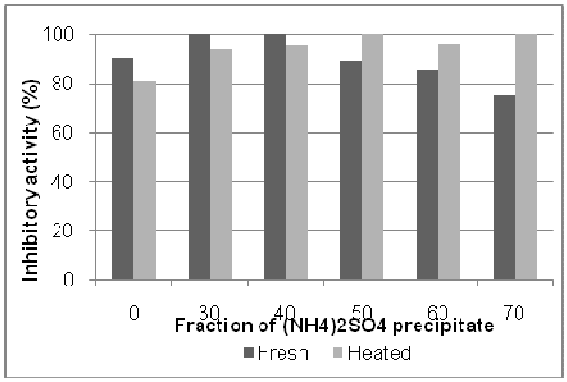

Figure 8. Effect of heating @100°C for 30 minutes on inhibitory activity of yellow soybean

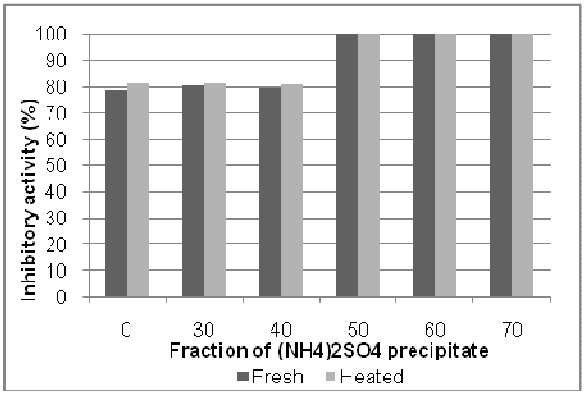

Figure 9. Effect of heating @100°C for 30 minutes on inhibitory activity of black soybean

4. CONCLUSIONS

Mungbean, yellow and black soybean contain proteins that inhibit Porcine Pancreatic Amylase but only mungbean protein inhibits Human Salivary Amylase. Heating the protein at 100ºC for 30 minutes hardly affect the inhibitory activity.

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