EFFECTS OF DEGREE OF HYDROLYSIS ON ANTIOXIDATIVE ACTIVITIES OF EDIBLE BIRD’S NEST PROTEIN HYDROLYSATE

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Introduction
A number of studies has shown that most cardiovascular diseases are associated with oxidative stress, leading to an increased production of free radical species. The oxidative stress would damage lipid and protein that resulted in cell death and tissue injury damage (Kehrer 1993). Thus, ingestion of antioxidant supplements or food containing antioxidants may reduce oxidative damage in human (Lin and Yen 1999). Bioactive peptides could be produced by enzymatic hydrolysis of food proteins and shown to has antioxidant activities against the peroxidation of lipids or fatty acids. Edible Bird's Nest (EBN) is a valuable functional food and is believed to have a therapeutic function in herbal medicine that has been used for hundreds of years in China. Potential bioactive peptides in protein rich EBN might possess anti-oxidative which could overcome most cardiovascular diseases. Alcalase had been used in this study because it is nonspecific, had the highest protein recovery and the lowest lipid content than those made using Papain and Neutrase (Addler-Nielsen 1986). In the process of enzymatic hydrolysis, factors such as pH, time, enzyme to substrate level and temperature will influence enzymatic activity (See et al 2011). In this study, different hydrolysis time had been used to investigate antioxidant activities of EBN hydrolysates.

Material and Method
Preparation of EBN protein hydrolysate
Raw EBN was soaked in a water using 1/100 ratio before incubated at 4°C for 16 hours. After that, the sample was boiled at 100°C for 30 minutes and then cooled at room temperature before adjusting to suitable pH for hydrolysis.

Enzymatic hydrolysis of EBN protein hydrolysate
Alcalase (pH 8, 60°C) was used in this experiment. The enzymes were added to substrate at ratio 1:100. The resulting hydrolysates were heated in boiling water for 5 min to inactivate the enzymes, and then centrifuged at 4°C and 4,000 rpm for 10 min. The supernatant was filtered by Whatman No.1 and the filtrate was freeze dried for storage until further analysis.

Degree of hydrolysis (%DH)
The degree of hydrolysis of EBN protein hydrolysates were calculated using pH-stat method as described by Addler-Nielsen (1986).
Determination of the antioxidative activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured as described by Wu et al. (2003) with a slight modification. ABTS radical-scavenging activity of EBN protein hydrolysates was determined as described by Najafian et al. (2013). The ability of the EBN protein hydrolysates to reduce iron (III) was measured according to the method of Oyaizu (1988).

Statistical analysis

All experiments were performed in triplicate. The significance value (p<0.05) between samples was determined using Duncan Multiple Test and the program used was SAS.

Results and Discussion

According to Table 1, the DH values increased with increasing time. At a 90 min hydrolysis time, the DH values of EBN protein hydrolysates gave 82.7% ± 1.86 which were significantly higher compared to other time (p<0.05). However, DH value exhibit decreasing trend when hydrolysis time was continued from 90 min to 120 min. Najafian (2013) also reported the slight decrease in the activity of patin fish protein hydrolysates when the time was increased. It could be due to the rate of enzyme activity which had decreased. Adding more substrate does not give much significant effect to the enzyme activity, thus decreasing the DH value.

Table 1: Antioxidant activities of EBN protein hydrolysates at different DH values.

<table>
<thead>
<tr>
<th>Hydrolysis time (min)</th>
<th>DH (%)</th>
<th>DPPH Radical Scavenging activity (%)</th>
<th>ABTS Radical scavenging activity (%)</th>
<th>Reducing power (Abs at 700nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>71.2 ± 1.84^b</td>
<td>68.1481 ± 0.014</td>
<td>59.0686 ± 0.0006</td>
<td>0.0375 ± 0.0007</td>
</tr>
<tr>
<td>60</td>
<td>72.0 ± 3.72^b</td>
<td>67.9259 ± 0.001</td>
<td>58.4412 ± 0.0020</td>
<td>0.0535 ± 0.0021</td>
</tr>
<tr>
<td>90</td>
<td>82.7 ± 1.86^a</td>
<td>68.4444 ± 0.001</td>
<td>65.9314 ± 0.0045</td>
<td>0.0665 ± 0.0148</td>
</tr>
<tr>
<td>120</td>
<td>73.5 ± 1.90^b</td>
<td>65.5556 ± 0.001</td>
<td>63.7745 ± 0.0025</td>
<td>0.0625 ± 0.007</td>
</tr>
</tbody>
</table>

^a-d Same letter at different row indicates no significant difference (p > 0.05).
*Mean ± SD for 3 sample/treatment

Antioxidants can terminate or retard the oxidation process by interacting with free radicals and forming stable species. Table 1 showed that at 30, 60 and 90 min, there were no significant difference for DPPH radical scavenging activity (p>0.05). This indicated that scavenging activity for EBN protein hydrolysate had already occurred even at the low DH (71.2 ± 1.84^b). This result was similar with Klompong et al (2007) which reported that yellow stripe trevally mince fish prepared using alcalase at low DH, also gave the highest antioxidative activity.

For the ABTS radical scavenging activity, the result was quite similar with DPPH value, reaching a maximum of 65.9314% ± 0.0045 for a DH value of 82.7% ± 1.86 but in ABTS case, the highest DH value gave the highest ABTS radical scavenging activity. High
ABTS radical-scavenging activities indicate that the antioxidative compounds are mostly hydrophilic (Klompong et al. 2007). Enzymatic hydrolysis potentially affects the molecular size and hydrophobicity, as well as polar and ionizable groups of protein hydrolysates (Mutilangi et al. 1996). The smaller peptides from myofibrillar proteins are expected to have proportionally more polar residues, with the ability to form hydrogen bonds with water and augment solubility (Gbogouri et al., 2004). As a consequence, hydrolysates with smaller peptides, i.e. higher DH, were more soluble and therefore resulting in more antioxidative activity as shown in the ABTS result.

In the reducing power assays, the antioxidants present in the protein hydrolysates convert the oxidised form of iron (Fe$^{3+}$) in ferric chloride to the ferrous form (Fe$^{2+}$). As showed in Table 1, all hydrolysates with different DH had some degree of electron donation capacity. The reducing power of EBN protein hydrolysates increased significantly when the DH increased (p<0.05).

**Conclusion**

EBN protein hydrolysates prepared using alcalase showed the antioxidative activities as measured by DPPH, ABTS and Reducing power assay at 90 min of hydrolysis time resulted in the highest degree of hydrolysis (82.7 ± 1.86).

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**References**


