Antioxidant Effect and Functional Properties of Hydrolysates Derived from Egg–White Protein

Dae-Yeon Cho1, Kyungae Jo2, So Young Cho2, Jin Man Kim3, Kwangsei Lim4, Hyung Joo Suh2,5,*, and Sejong Oh*

Division of Animal Science, Chonnam National University, Gwangju 500-757, Korea
1Comimax Co. Ltd., Seoul 139-860, Korea
2Department of Food and Nutrition, Korea University, Seoul 136-703, Korea
3Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea
4Dairy Food R&D Center, Maeil Dairies Co., Ltd. Pyungtaek 451-861, Korea
5BK21PLUS Program in Embodiment: Health-Society Interaction, Department of Public Health Science, Graduate School, Korea University, Seoul 136-703, Korea

Abstract

This study utilized commercially available proteolytic enzymes to prepare egg-white protein hydrolysates (EPHs) with different degrees of hydrolysis. The antioxidant effect and functionalities of the resultant products were then investigated. Treatment with Neutrase yielded the most α-amino groups (6.52 mg/mL). Alcalase, Flavourzyme, Protamex, and Ficin showed similar degrees of α-amino group liberation (3.19-3.62 mg/mL). Neutrase treatment also resulted in the highest degree of hydrolysis (23.4%). Alcalase and Ficin treatment resulted in similar degrees of hydrolysis. All hydrolysates, except for the Flavourzyme hydrolysate, had greater radical scavenging activity than the control. The Neutrase hydrolysate showed the highest 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity (IC50 = 3.6 mg/mL). Therefore, Neutrase was identified as the optimal enzyme for hydrolyzing egg-white protein to yield antioxidant peptides. During Neutrase hydrolysis, the reaction rate was rapid over the first 4 h, and then subsequently declined. The IC50 value was lowest after the first hour (2.99 mg/mL). The emulsifying activity index (EAI) of EPH treated with Neutrase decreased, as the pH decreased. The EPH foaming capacity was maximal at pH 3.6, and decreased at an alkaline pH. Digestion resulted in significantly higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ABTS radical scavenging activity. The active peptides released from egg-white protein showed antioxidative activities on ABTS and DHHP radical. Thus, this approach may be useful for the preparation of potent antioxidant products.

Key words: egg-white protein, hydrolysate, Neutrase, radical scavenging activity, functionality

Introduction

Hen eggs are a traditional food used in many basic and formulated preparations and have excellent nutritive value. The egg has a significant reserve of highly digestible proteins, lipids, vitamins, and minerals as well as other molecules with health-promoting properties. Moreover, the egg contains molecules that can be exploited for biotechnological purposes (Anton et al., 2006). Over the last decade, numerous studies have characterized the biophysiological functions of egg components and have identified novel biologically active substances (Mine, 2007). As such, eggs have been recognized as a source of biologically active substances with significant therapeutic potential.

The application of egg in food preparation depends primarily on its protein properties. Many attempts have been made to develop chemical or enzymatic modifications that alter functional characteristics of egg white protein (Kato et al., 1989; Matsudomi et al., 1991). The proteolytic enzyme such as papain is capable of breaking down larger molecules of protein into smaller constituents. Papain has been used to prepare resynthesized protein hyd-
Sakanaka et al. (2004) found that the egg-yolk protein hydrolysates, when compared with its original protein or amino acids mixture, showed stronger antioxidant activity in a linoleic acid oxidation system. Peng et al. (2009) used alcalase to hydrolyze whey protein isolate, obtaining a hydrolysate fraction that possessed strong scavenging activities on DPPH, hydroxyl and superoxide radicals. Similarly, Li et al. (2008) reported that chickpea hydrolysates possess a scavenging ability on superoxide anions. What is more, protein hydrolysates present nutritional and functional properties beside their antioxidant activity (Chen et al., 2012; Xie et al., 2008). These food-derived antioxidants are considered to be safe and free of side effects, which may be occurred in the synthetic antioxidants.

Preparative process for bioactive peptides potentially influences the molecular size, hydrophobicity and polar groups of the hydrolysate (Adler-Nissen, 1979; Kristinsson and Rasco, 2000). These alterations in hydrolysate characteristics directly affect the functional properties, physical activities, and the uses as food ingredients (Kristinsson and Rasco, 2000). Hydrolysates from different protein sources, such as whey, soy (Pena-Ramos and Xiong, 2003), egg-yolk, prawn (Suetsumu, 2000), tuna cooking juice (Jao and Ko, 2002), yellowfin sole frame (Jun et al., 2004), and capelin (Amarowicz and Shahidi, 1997), have been known to possess antioxidant activity. Levels and compositions of free amino acids and peptides were reported to determine the antioxidant activities of protein hydrolysates (Wu et al., 2003). In addition, a recent study reported the antioxidant activity of peptides which are produced from crude egg white by pepsin treatment (Davalos et al., 2004). Nevertheless, information on functional properties and antioxidant activity of peptides produced from enzyme hydrolysis is still limited to be understood.

### Materials and Methods

#### Chemicals and enzymes

t-Leucine, 2,4,6-trinitrobenzenesulphonic acid (TNBS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (USA).

Alcalase, Neutrase, Flavourzyme, and Protamex were purchased from Novozymes (Bagsvaerd, Denmark). Collupulin was purchased from DSM Corp. (Heerlen, Netherlands). Papaya and Ficin were purchased from Sigma Co. (USA). The characteristics of each enzyme are summarized in Table 1. All chemicals were of analytical grade.

#### Preparation of EPH and in vitro digestion

Egg-white powder (Edentown FnB, Korea) (10 g) was mixed with 100 mL deionized water and pH adjusted to 6.0 with 0.1 N NaOH. The suspension was preincubated at 45°C for 20 min prior to enzymatic hydrolysis and then hydrolyzed for 12 h. The hydrolysis conditions are reported in Table 1. After hydrolysis, the enzymes were inactivated by boiling for 15 min. The hydrolysates were centrifuged in a refrigerated centrifuge (Beckman model J2-21, Beckman Coulter, INC., USA) at 2,800 g for 20 min, and the supernatants were lyophilized (TFD, Ilshin, Korea) and stored in a desiccator before further use. Non-enzymatic hydrolysis was used as control.

To mimic in vivo digestion process, an in vitro digestion model system using enzymes similar to those in the upper gastrointestinal digestive tract of humans was used.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Optimum condition</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temperature (°C)</td>
<td>pH</td>
</tr>
<tr>
<td>Alcalase</td>
<td>Bacillus sp.</td>
<td>50-60</td>
<td>8.0-9.0</td>
</tr>
<tr>
<td>Neutrase</td>
<td>B. amyloliquefaciens</td>
<td>45</td>
<td>6.0-7.0</td>
</tr>
<tr>
<td>Protamex</td>
<td>Bacillus sp.</td>
<td>35-60</td>
<td>5.5-7.5</td>
</tr>
<tr>
<td>Flavourzyme</td>
<td>Aspergillus sp.</td>
<td>45-50</td>
<td>5.0-7.0</td>
</tr>
<tr>
<td>Collupulin</td>
<td>Caruca papaya</td>
<td>50-70</td>
<td>5.0-7.5</td>
</tr>
<tr>
<td>Ficin</td>
<td>Ficus carica</td>
<td>45-55</td>
<td>5.0-6.0</td>
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