

Preparation and Some Properties of Protein Hydrolysate from Broiler Esophagus

Manee Vittayanont¹⁺, Preecha Tang¹ and Pijittra Sumpuchanee¹

¹Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Thailand

Abstract. The production of protein hydrolysate from esophagus of broiler from poultry processing waste was studied. The esophagus is composed of 13.91% protein, 82.77% moisture, 0.85% fat, 2.15% carbohydrate and 0.32% ash. The pigment and fat removal by washing for 15 minutes with 0.3, 0.5 and 0.7% sodium bicarbonate (NaHCO₃) solution compared with water (control) showed that when the NaHCO₃ concentration increased the redness (a*) and fat content of chicken esophagus decreased. The redness of sample washed by 0.3 and 0.5% NaHCO₃ was not significantly different while a significant (p<0.05) higher fat reduction was observed at 0.5% NaHCO₃. Therefore washing with 0.5% NaHCO₃ solution was adopted as raw material preparation for hydrolysis study using Alcalase and Papain at 55 and 65°C for 1 h. At the same concentration (0.1-0.9% w/w) Papain exhibited higher efficiency than Alcalase. Alcalase of 0.3% was selected to study the effect of hydrolysis time on degree of hydrolysis (DH). The results suggested that digestion for 17, 50 and 85 min resulted in the protein hydrolysis with DH of 6, 14 and 22% respectively. The compositions of all three DH level hydrolysates were 78.75-86.85% protein, 6.46-7.45% moisture, 0.76-0.84% fat and 3.16-3.99% ash. The higher DH caused the higher solubility but lower emulsifying activity index, emulsion stability index, foaming capacity and foaming stability of protein hydrolysate from broiler esophagus.

Keywords: Protein hydrolysate, Esophagus, Degree of hydrolysis, Emulsifying property, Foaming property

1. Introduction

Poultry meat processing is one of the biggest industries of Thailand. Each day, tons of viscera are produced as waste and discarded or used as animal feed. Esophagus is a part of this waste containing muscle and connective tissue which can be used as a raw material for production of protein hydrolysate. This is not only for the valorisation of industrial by-products but also for the broader variety of the hydrolysate products produced from different sources and production conditions. There are abundant source of proteins such as porcine, bovine, cattle, poultry and marine processing by-products. However, the industrial use of non-mammalian species is growing in importance as they are free from religious constrain and neurodegenerative disease (encephalopathy). Enzyme-hydrolysed protein plays an important role in various products and applications. Degree of hydrolysis (DH) is an important index influenced by types and condition of the enzymes used and known to govern most properties of the hydrolysate. This work aimed to study washing of esophagus using different concentration of NaHCO₃ and suitable condition for preparation of hydrolysates having different DH levels. The composition and some properties of the hydrolysate were also determined to evaluate their potential application in food industry.

2. Materials and Methods

⁺ Corresponding author. Tel.: + 6674286339; fax: +6674558866
E-mail address: manee.v@psu.ac.th

Materials: Esophagus samples were obtained from broiler slaughter house of Bangkok produce merchandising public Co., Ltd Thailand. They were trimmed off fat tissue and washed thoroughly with running tap water before stored at -20 °C until used. Alcalase 2.4L (E.C.3.4.21.62) and Papain (EC 3.4.22.2) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemical for analyses were analytical grade. Food grade NaHCO₃ was used in washing step.

2.1. Effect of NaHCO₃ Concentration on Fat Content and Color of Esophagus

Frozen broiler esophagus was thawed overnight in refrigerator before washing by stirring in 10x volume of 0, 0.3, 0.5 and 0.7% sodium bicarbonate (NaHCO₃) solutions for 15 minutes at room temperature. The meat samples obtained after centrifuge for 30 min at 5000 rpm were then determined for their fat content [1]) and color (L* a* and b*) using Hunter Lab system.

2.2. Hydrolysis of Proteins from Washed Esophagus Samples

Effect of concentration of Alcalase and Papain on hydrolysis yield was investigated by digesting washed sample, previously chopped using a blender, for 1 h with in 5x volume of solution containing 0.1-0.9% Alcalase pH 8 at 55°C or Papain pH 7 at 65°C. After hydrolysis, enzymes were inactivated at 85°C for 10 min. The supernatants obtained from centrifugation at 10000 rpm for 30 min were freeze dried, weighed and determined for their protein content by biuret assay [2]. Protein and weight yield were calculated based on protein content and weight of initial raw material, respectively.

To study effect of hydrolysis time on protein yield and degree of hydrolysis (DH), the type and concentration of enzyme selected from previous study was used to hydrolyse esophagus samples for 15, 30, 45, 60, 120 and 240 min. After enzyme inactivation and centrifugation, DH of supernatants obtained from different hydrolysis time were determined using TNBS assay [3].

2.3. Properties of Protein Hydrolysates

Protein hydrolysates were prepared by using selected enzyme concentration and time that gave 3 levels of DH: 5-10, 10-20 and 20-25. The freeze dried hydrolysates were analyzed for the content of protein, moisture, fat, and ash using official method [1], protein solubility [4], emulsifying activity index (EAI) and Emulsion stability index (ESI) following methods used by Pearce and Kinsella [5], Foaming capacity (FC) and Foaming stability (FS) following Sathe and Salunkhe [6].

2.4. Statistical Analysis

All experiments were carried out in triplicate. The obtained data were analyzed using one way analysis of variance (ANOVA). Means were compared by Duncan multiple range test with mean square error at 5% probability (SPSS 10.0 for Windows statistical software).

3. Results and Discussion

3.1. Effect of Washing on Fat Content and Color of Esophagus

The chemical compositions of the broiler esophagus were 82.77 ± 0.19% moisture, 1.85 ± 0.01% fat, 13.91 ± 0.15% protein and 0.32 ± 0.01% ash. The dry weight basis of protein was 80.73% indicating high potential as protein source for hydrolysate preparation. After washing with 0-0.7% NaHCO₃, the lightness (L*) and blueness (b*) of the samples slightly increased while the redness (a*) decreased with increasing of NaHCO₃ concentration (Table I). This may due to the increasing of the washing solution pH (from 6.85 to 9.89) that enhanced solubilization of heme pigments.

A significant reduction of fat content of the washed sample was observed (Fig. 1). The major defatting effect is more likely to be the density and polarity difference between fat and water [7]. Higher amount of fat was leashed out from sample washed with water compared to those of unwashed one while less fat was removed from sample washed with higher NaHCO₃ concentration than those of lower NaHCO₃ concentration.

Since the a* value which is a represent of heme pigment in samples washed by 0.3 and 0.5% NaHCO₃ was not significantly different (p ≥ 0.05) while fat reduction was significantly higher by using 0.5% than 0.3% NaHCO₃. Therefore washing with 0.5% NaHCO₃ solution was adopted as raw material preparation for hydrolysis study in further step.

Table I: L* a* b* value of esophagus samples washed with different concentration of NaHCO₃

NaHCO ₃ (%)	L*	a*	b*
unwashed	69.84 ± 0.03 ^a	4.63 ± 0.31 ^d	16.23 ± 0.05 ^a
0.0	72.12 ± 0.93 ^b	3.16 ± 0.23 ^c	14.89 ± 0.61 ^b
0.3	72.38 ± 0.60 ^b	2.81 ± 0.16 ^b	17.29 ± 0.79 ^{bc}
0.5	71.52 ± 0.63 ^b	2.94 ± 0.21 ^{bc}	18.29 ± 0.48 ^c
0.7	71.71 ± 1.28 ^b	2.46 ± 0.06 ^a	18.48 ± 0.97 ^c

Values are mean ± standard deviation from triplicate determination.

^{a-d} In a column, mean values followed by the same superscript are not significantly different (P ≥ 0.05).

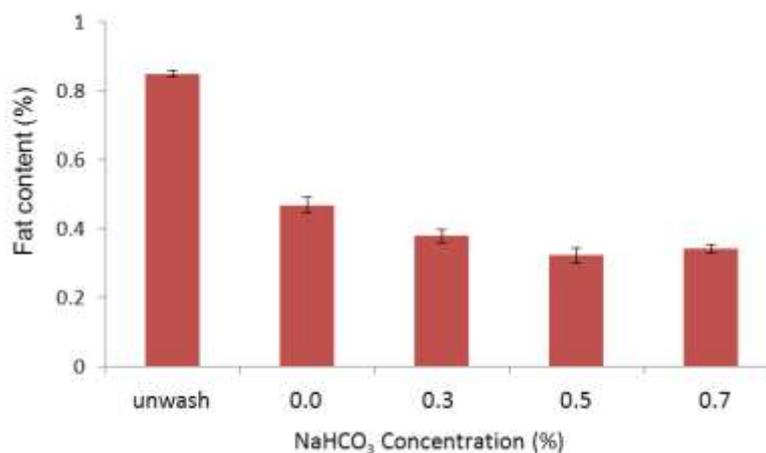


Fig. 1: Fat content of broiler esophagus before (unwash) and after washing with 0.0, 0.3, 0.5 and 0.7% NaHCO₃ solution

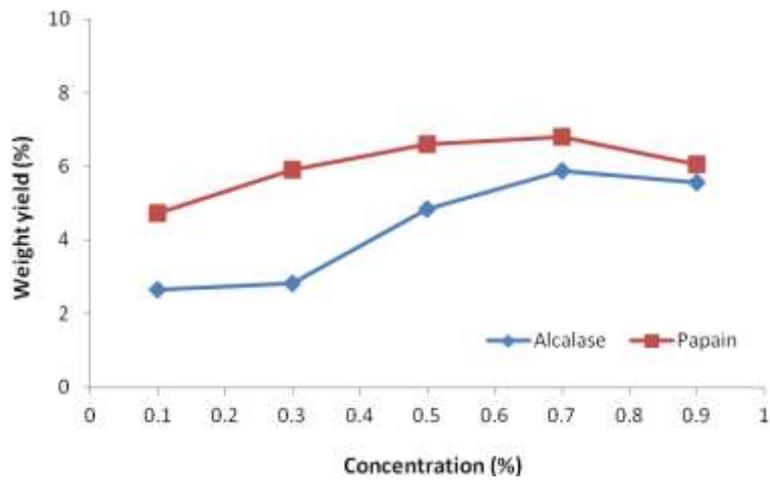
3.2. Hydrolysis of Proteins from Washed Esophagus Samples

Fig. 2A and Fig. 2B showed weight and protein yields from the hydrolysis of broiler esophagus using Alcalase and Papain at their optimum condition. As the concentration of enzymes increased the hydrolysis yields increased with higher efficiency of Papain than that of Alcalase at the same concentration. Alcalase is an endo-peptidase while Papain is an endo-exopeptidase enzyme which capable of simultaneously digesting at the end and within peptide, hence exhibiting faster activity. As Papain showed relatively high activity within concentration range of study, therefore 0.3% of Alcalase was chosen to ease the controlling of DH by varying hydrolysis time. The results in Fig. 3 showed that DH of hydrolysates increased rapidly over the first two hours of hydrolysis similar to those previously reported in production of protein hydrolysates from fish waste [8] and [9]. The experiment was then carried on by choosing time periods (15, 50, 85 minutes) for preparation of hydrolysates with 5-10, 10-20 and 20-25 DH levels.

3.3. Composition and Properties of Three Different Level Dh Protein Hydrolysates

The DH and compositions of freeze-dried hydrolysates prepared from broiler esophagus by digesting with 0.3% Alcalase at pH8, 55°C for 17, 50 and 85 minutes were shown in Table II. The hydrolysate products were comprised of 79-87% protein and less than one percent of fat content.

The solubility of proteins is very important character that governs most functional properties of protein hydrolysate [10] and [11]. The protein solubility of hydrolysates increased from 90.59 ± 2.16 to 96.68 ± 1.67 when their DH increased from 6 to 22 (Table III). The high DH hydrolysate consists of smaller molecule protein and higher hydrophilic groups leading to higher solubility [11]. The change of foaming and emulsifying properties of the hydrolysate as affected by DH was monitored. The results showed that emulsifying activity index (EAI), emulsion stability index (ESI), foaming capacity and foaming stability of the hydrolysates significantly decreased (P<0.05) as the DH increased. Hydrolysis may reduce size and non-amphiphilic property of peptides to the level that alter their ability to firmly align at the interface of colloidal particles of emulsion or foam.



B

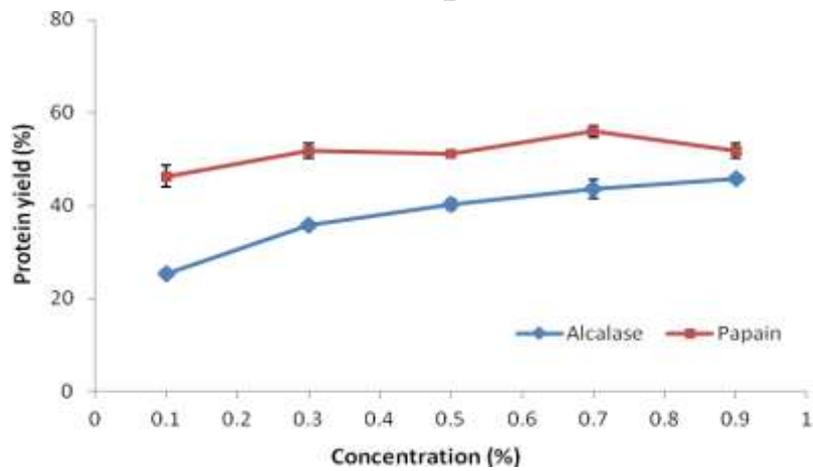


Fig. 2: Weight (A) and protein (B) yields obtained from hydrolysis of broiler esophagus using 0.1-0.9% Alcalase and Papain for 1 hour

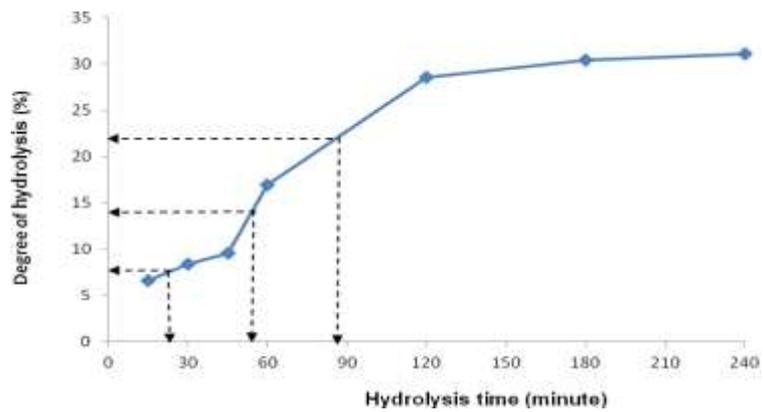


Fig. 3: Degree of hydrolysis (DH) of products hydrolyzed from broiler esophagus using 0.3% Alcalase for different period of time

In this work, digestion of the intact protein in esophagus sample simultaneously acted for both extraction and fragmentation of proteins. Therefore to obtain the high production yield, the higher DH is a prerequisite. In this case, foaming and emulsifying properties might not be a focused functionality. Bioactive properties which have been reported in the high DH products should be further studied.

Table II: Proximate composition (%) of protein hydrolysates with different degree hydrolysis (DH)

Hydrolysis time (min)	DH	Protein	Fat	Moisture	Ash
17	5.96	78.75 ± 0.42 ^a	0.84 ± 0.01 ^c	6.46 ± 0.36 ^a	3.16 ± 0.05 ^a
50	14.09	83.01 ± 0.24 ^b	0.76 ± 0.01 ^a	6.50 ± 0.40 ^a	3.98 ± 0.01 ^b
85	21.94	86.85 ± 0.37 ^c	0.81 ± 0.01 ^b	7.45 ± 0.36 ^b	3.99 ± 0.07 ^b

Values are mean ± standard deviation from triplicate determination.

^{a-c} In a column, mean values followed by different superscript are significantly different (P < 0.05).

Table III: Protein solubility, foaming and emulsifying properties of different degree of hydrolysis (DH) products

DH	Solubility (%)	Foaming properties		Emulsifying properties	
		FC (%)	FS (%)	EAI(m ² /g)	ESI (min)
6	90.59 ± 2.16 ^a	25 ± 0.0 ^c	11.67 ± 1.44 ^b	21.40 ± 0.25 ^b	29.51 ± 0.33 ^c
14	92.34 ± 2.59 ^a	15.83 ± 1.44 ^b	4.17 ± 1.44 ^a	16.86 ± 0.16 ^a	19.47 ± 0.07 ^b
22	96.68 ± 1.67 ^b	5.83 ± 1.44 ^a	2.5 ± 0.0 ^a	16.67 ± 0.02 ^a	15.26 ± 0.05 ^a

Values are mean ± standard deviation from triplicate determination.

^{a-c} In a column, mean values followed by different superscript are significantly different (P < 0.05).

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