Preliminary Evaluation of Halal Protein Hydrolysate Production in Indonesia

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Abstract. Protein hydrolysate is widely used in industry, for example as a substrate for microbial fermentation. With respect to the halal certification of the final fermentation products, the halal status of all media components, including protein hydrolysate, needs to be clarified as well. Indonesia has abundant protein-rich natural resources as well as protein-rich industrial by-products that have not been utilized optimally. Industrial production of halal protein hydrolysate has been overlooked. This research explored the potential of using protein-rich industrial/agricultural by-products, such as cassava leaves, soybean waste (tofu and soy sauce dregs), cow waste (bones and cow skin), fish waste (fish bones and skin), chicken waste (chicken feet and skin), cheese whey, and corn steep liquor, to be processed using halal and green processes to produce halal protein hydrolysate. The best combination of raw material and protease was obtained by simulating the breaking of the peptide bond of the raw material by a protease to determine the effectiveness of the protein hydrolysis process of each combination. Further simulations were carried out using the Analytical Hierarchy Process (AHP) method, to consider the availability/accessibility of raw materials, the protein content of the raw materials, the cleavage of peptide bonds by the enzyme, the price of the enzymes, and the ease of processing which included pre-treatment of raw materials.

1. Introduction

Protein hydrolysate is one of the essential raw materials in the development of fermentation products and biotechnology. Currently, protein hydrolysate is widely used in the food and pharmaceutical industries to produce flavorings, drugs, and supplements. Protein hydrolysate is also used in the microbial growth media for Nitrogen source [1]. In its application, the halalness of a protein hydrolysate is important to be considered, especially in Indonesia, where the majority of the population is Muslim. The critical points for the halalness of protein hydrolysate are in the raw material and the hydrolyzing enzyme (protease) used, as well as the potential of cross-contamination with non-halal materials during the production process. To ensure that, the whole production process, from raw materials to the packaging of the end products, needs to be carefully considered. Despite the big market for protein hydrolysate in Indonesia, a commercial scale protein hydrolysate industry has not been established in Indonesia until now. According to data from Statistics Indonesia, in 2018 Indonesia imported 4,014,254 kg of protein hydrolysate with a value of 20,025,992 US $ [2].

On the other hand, Indonesia has abundant protein-rich industrial/agricultural by-products which have not been utilized optimally including cassava leaves, the waste from soybean derivate products (such as tofu and soy sauce), the waste from slaughtering house, the waste from fish canning industry, cheese whey, and corn steep liquor. Food processing mostly leads to waste or by-products such as
industrial cheese (85-90%), corn starch (41-43%), beef slaughter (40-52%), poultry slaughter (31-38%), and fish’ filleting, salting, preserving, and smoking (50-75%) [3].

One of the methods used to produce protein hydrolysate is through the enzymatic hydrolysis process using proteases. Proteases that can be used to produce protein hydrolysate include papain, bromelain, ficin, rennin, pepsin, and thermolysin. Literature review shows that papain can be used to hydrolyze yellowtail fish (Caesio cuning) waste [4], bromelain can be used to hydrolyze nyamplung seeds (Calophyllum inophyllum) [5], pepsin can be used to hydrolyze cow and pork skin [6].

This study aimed to evaluate the potential of protein hydrolysate production in Indonesia by determining the best combination of protein-rich industrial/agricultural by-products as the raw materials and proteases for the development of industrial-scale protein hydrolysate and further evaluate the potential of this process to be industrialized commercially.

2. Methodology

This research was generally divided into several stages including analyzing the amino acid composition of raw materials, determining the peptide bond cleavage site by proteases, simulating the protein hydrolysis process, determining the optimum protein hydrolysate production process, that was the combination of raw material and protease, using the Analytical Hierarchy Process (AHP) method; and analyzing of the economics. Secondary data from various literature were used throughout the research.

Information regarding the amino acid composition of each raw material was obtained from various literature by adjusting the data of amino acid composition units that have been collected to grams of amino acids per 100 grams of protein. When more than one piece of literature was found, the average value was used.

The determination of the specific cleavage site of protease was carried out by gathering information on the preferred amino acids for cleaving the peptide bond by a protease from various references. Due to the broad specificity of the action of some proteases, the preferential amino acid used in the study was the amino acid mentioned in all literature.

The protein hydrolysis process was simulated by utilizing the raw material amino acid composition data and the specific cleavage site of each protease, to estimate the efficiency of the hydrolysis process. The degree of hydrolysis was calculated by summing up the amino acid content of the raw material which was the site for cleaving the peptide bonds by proteases for each variation of the raw material.

Determination of optimum protein hydrolysate production process, which was the combination of raw material and protease used in the process, was further conducted using the AHP method. Information related to the required data was obtained from various literature such as books, journal articles, proceedings, publications from institutions, news media, and personal information from reliable sources.

The economic added value of the selected raw material-protease combination from the AHP method was then analyzed by determining the difference between the commercial protein hydrolysate price and the raw material price used to produce the protein hydrolysate in the same amount.

3. Results and Discussion

3.1. Amino Acid Composition of Various Protein-Rich Industrial/Agricultural Byproducts

Eleven types of protein-rich industrial/agricultural byproducts were evaluated as the potential raw materials for the protein hydrolysate production. They were cassava leaves, tofu waste, soy sauce solid waste, the waste from fish canning industry: fish bones and fish skin; the waste from beef and poultry slaughtering house: beef bones, cow skin, chicken feet, chicken skin; cheese whey, and corn steep liquor. Each of these raw materials has a unique amino acid composition which can affect the effectiveness of the protein hydrolysis process in the production of protein hydrolysate. The amino acid compositions of these materials are summarized in Table 1.
Table 1. Amino acid composition of raw materials (grams of amino acids per 100 grams of protein).

| Amino acid | Cassava leaves<sup>a</sup> | Tofu waste<sup>b</sup> | Soy sauce solid waste<sup>a</sup> | Fish bones<sup>c</sup> | Fish skin<sup>c</sup> | Beef bones<sup>c</sup> | Cow skin<sup>c</sup> | Chicken feet<sup>d</sup> | Chicken skin<sup>d</sup> | Cheese whey<sup>d</sup> | Corn steep liquor<sup>d</sup> |
|------------|--------------------------|-----------------------|-------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Alanine (Ala) | 5.39 | 4.28 | 0.68 | 7.28 | 10.38 | 8.52 | 8.18 | 2.39 | 8.32 | 4.20 | 10.17 |
| Arginine (Arg) | 5.01 | 7.62 | 0.40 | 7.80 | 7.54 | 7.67 | 7.46 | 3.89 | 7.57 | 2.05 | 12.62 |
| Asparagine (Asn) | N/A | N/A | N/A | 6.64 | 7.12 | N/A | N/A | N/A | N/A | N/A | N/A |
| Aspartic Acid (Asp) | 9.27 | 10.42 | 2.54 | 8.09 | 5.76 | 4.78 | 4.87 | 8.14 | 5.91 | 9.31 | 4.16 |
| Cysteine (Cys) | 1.04 | 1.41 | N/A | 1.57 | N/A | 0.13 | N/A | N/A | N/A | 1.51 | 3.22 |
| Glutamic Acid (Glu) | 11.69 | 18.97 | 3.23 | 11.56 | 10.14 | 9.77 | 8.93 | 10.63 | 9.59 | 17.92 | 11.97 |
| Glutamine (Gln) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Glycine (Gly) | 7.09 | 4.17 | 0.32 | 15.15 | 21.52 | 22.23 | 20.60 | 15.20 | 20.26 | 1.64 | 4.56 |
| Histidine (His) | 2.22 | 2.76 | N/A | 2.05 | 0.88 | 0.98 | 0.72 | 4.97 | 0.74 | 1.27 | 19.37 |
| Hydroxyproline (Hyp) | N/A | N/A | N/A | 4.81 | 5.68 | 11.51 | 9.80 | 11.10 | 11.36 | N/A | 0.23 |
| Isoleucine (Ile) | 4.73 | 4.99 | 4.03 | 2.72 | 1.40 | 1.46 | 1.38 | 5.56 | 1.48 | 2.89 | 2.11 |
| Leucine (Leu) | 8.49 | 7.96 | 7.08 | 4.88 | 2.58 | 3.08 | 2.89 | 9.46 | 3.25 | 8.90 | 6.21 |
| Lysine (Lys) | 5.93 | 6.54 | 6.16 | 5.25 | 3.82 | 3.38 | 2.76 | 5.84 | 3.21 | 6.52 | 1.54 |
| Methionine (Met) | 1.90 | 1.26 | 0.53 | 2.46 | 2.25 | 0.89 | 0.47 | 4.27 | 1.12 | 1.95 | 3.39 |
| Phenylalanine (Phe) | 5.59 | 5.30 | 5.09 | 2.92 | 2.51 | 2.25 | 1.96 | 4.99 | 2.76 | 3.35 | 1.91 |
| Proline (Pro) | 4.83 | 4.83 | 0.82 | 7.65 | 12.04 | 12.03 | 21.23 | 3.32 | 15.12 | 6.76 | 7.86 |
| Serine (Ser) | 4.62 | 5.19 | N/A | 5.00 | 3.06 | 3.22 | 3.11 | N/A | 2.67 | 5.52 | 1.42 |
| Threonine (Thr) | 4.43 | 3.99 | 4.50 | 3.55 | 2.84 | 2.10 | 1.84 | N/A | 2.70 | 5.28 | 2.17 |
| Tryptophan (Top) | 2.31 | 1.37 | N/A | 0.47 | N/A | N/A | N/A | N/A | N/A | 2.05 | 0.11 |
| Tyrosine (Tyr) | 4.06 | 3.93 | 3.35 | 2.22 | 0.90 | 0.75 | 0.60 | 5.53 | 0.82 | 2.97 | 1.65 |
| Valine (Val) | 5.65 | 4.79 | 6.13 | 3.50 | 2.06 | 2.32 | 1.90 | 4.69 | 2.07 | 3.32 | 4.44 |

Resource: *processed [7, 8]; *processed [9, 10, 11, 12, 13]; *processed [14]; *processed [15, 16]; *processed [17, 18]; *processed [17, 19, 20]; *processed [21]; *processed [22]; *processed [23, 24]; *processed [25, 26].

Table 1 shows that the amino acid composition varies with the sources (Table 1). Cheese whey is dominated by glutamic acid. Corn steep liquor is dominated by the amino acid histidine. The amino acid composition of cassava leaves is the average of amino acid compositions of the very young, young, and mature cassava leaves [7,8]. It is dominated by glutamic acid, aspartic acid, leucine, and glycine (Table 1).

The amino acid composition of tofu waste is very similar to those of soybeans, dominated by glutamic acid, aspartic acid, and leucine (Table 1). Although soy sauce is produced from soybeans, the different amino acid composition is found in soy sauce solid waste. The latter is dominated by leucine, lysine, and valine (Table 1). According to Fishanda [27], the lysine and methionine content of the soybean decrease during the fermentation process for soy sauce production. Tryptophan is also denatured in this process.

Wastes from animal body parts including fish bones and skins, beef bones and cow skins, as well as chicken feet and skin are dominated by collagen fiber so that they contain lots of glycine, proline, and hydroxyproline. Fish bones and skin are both dominated by the amino acid glycine, but the glycine content in fish skin is higher than fish bones. Fish skin also contains high proline, glutamic acid, and alanine, while in fish bones the highest amino acids after glycine are glutamic acid, aspartic acid, and arginine. Beef bones and fish skin are both dominated by proline and glycine, but the proline content of cow skin is higher than that of beef bones, while the glycine content of both is relatively similar. Chicken feet and skin are dominated by the amino acid glycine, but the content in chicken skin is higher, as is the case with fish bones and fish skin.

3.2. Specific Cleavage Site of Protease
Each protease has its own specificity, as well as different cleavage strengths. For example, papain cleaves strongly in phenylalanine while weak in cysteine [28]. Therefore, in this study, an analysis of
specific cleavage of proteases was carried out from several data to obtain strong cleavage for each protease. The specific cleavage sites of each protease are summarized in Table 2.

| Protease   | Cleavage site                     |
|------------|-----------------------------------|
| Papain     | arginine, phenylalanine, lysine    |
| Bromelain  | alanine, glycine, lysine, tyrosine |
| Ficin      | glycine, lysine, serine, tyrosine  |
| Rennin     | alanine, phenylalanine, tyrosine   |
| Pepsin     | phenylalanine, leucine, tyrosine   |
| Thermolysin| alanine, phenylalanine, isoleucine, leucine, methionine, valine |

Plant-derived proteases have very broad specificity compared to other sources. Papain has the tendency to cleave peptide bonds in alkaline amino acids such as arginine, lysine, and phenylalanine. Bromelain typically cleaves peptide bonds in alanine, lysine, glycine, and tyrosine. Ficin has several preferential amino acid similarities with bromelain, those are lysine, glycine, tyrosine, and serine. On the other hand, animal protease has a narrow specificity. Rennin cleaves peptide bonds in alanine, phenylalanine, and tyrosine. Pepsin, another animal protease, cleaves peptide bonds with a specificity similar to rennins in hydrophobic amino acids such as leucine, phenylalanine, and tyrosine. Thermolysin is a microbial protease, produced by *Bacillus sp.* Thermolysin cleaves peptide bonds at isoleucine, leucine, valine, alanine, methionine, and phenylalanine. The information regarding the cleavage sites of each protease is used to simulate the hydrolysis process.

3.3. Hydrolysis Process Simulation

The efficiency of the hydrolysis process is indicated by the degree of hydrolysis, which is the proportion of cleaved peptide bonds in a protein hydrolysate [44]. A high degree of hydrolysis indicates a more proportion of cleaved peptide bonds, thus the higher efficiency of the hydrolysis process. Based on the amino acid composition of the raw materials (Table 1) and the specificity of the protease (Table 2), the maximum/theoretical degree of hydrolysis was simulated. The results are presented in Table 3.

| Protease  | Cassava leaves | Tofu waste | Soy sauce solid waste | Fish bones | Fish skin | Beef bones | Cow skin | Chicken feet | Chicken skin | Cheese whey | Corn steep liquor |
|-----------|----------------|------------|-----------------------|------------|-----------|------------|----------|--------------|--------------|-------------|-----------------|
| Papain    | 16.5           | 19.5       | 11.6                  | 16.0       | 13.9      | 13.3       | 12.2     | 14.7         | 13.5         | 11.9        | 16.1            |
| Bromelain | 22.5           | 18.9       | 10.5                  | 29.9       | 36.6      | 34.9       | 32.1     | 29.0         | 32.6         | 15.3        | 17.9            |
| Ficin     | 21.7           | 19.8       | 9.8                   | 27.6       | 29.3      | 29.6       | 27.1     | 26.6         | 27.0         | 16.7        | 9.2             |
| Rennin    | 15.0           | 13.5       | 9.0                   | 12.4       | 13.8      | 11.5       | 10.7     | 12.9         | 11.9         | 10.5        | 13.7            |
| Pepsin    | 18.1           | 17.2       | 15.4                  | 10.0       | 6.0       | 6.1        | 5.5      | 20.0         | 6.8          | 15.2        | 9.8             |
| Thermolysin| 31.8           | 28.6       | 23.5                  | 23.8       | 21.2      | 18.5       | 16.8     | 31.4         | 19.0         | 24.6        | 28.2            |

Table 3 shows that all raw materials can be hydrolyzed by all types of proteases in spite of the different efficiency. Each of the raw material-protease combinations results in a unique degree of hydrolysis due to differences in the amino acid composition and the specificity of the protease.
Cassava leaves, tofu waste, and soy sauce solid waste were most efficiently hydrolyzed by thermolysin giving the degree of hydrolysate of 31.7, 28.6, and 23.5% correspondingly. The cleavage of peptide bonds in these three raw materials occurs mostly in leucine.

Fish bones, fish skins, beef bones, cow skin, and chicken skin are most efficiently hydrolyzed by using bromelain or ficin. This is due to their high content of glycine, which is one of the cleavage sites of bromelain and ficin. Hydrolysis of chicken feet by thermolysin, bromelain, and ficin proteases were relatively high giving the degree of hydrolysis of 31.4, 29.0, and 26.6%, respectively.

The amino acid composition of leucine, phenylalanine, isoleucine, valine, and methionine in chicken feet is as such that it provides high efficiency of thermolysin hydrolysis (Table 2). The alanine content in chicken feet is much smaller than in other animal-derived waste (Table 2) thereby its degree of hydrolysis by bromelain is also lower than the five other animal-derived waste discussed previously.

Thermolysin also gives the highest degree of hydrolysis for cheese whey and corn steep liquor (Table 3). This is related to the high content of leucine in cheese whey and the high content of alanine in corn steep liquor (Table 1), whereas leucine and alanine are the specific cleaving site of thermolysin (Table 2).

3.4. Determination of the Optimum Protein Hydrolysate Production Process

The AHP method was used to obtain the optimum protein hydrolysate production process, which was the combination of raw material and protease used in the process, was further conducted using the AHP method. Between the five criteria used in the decision-making process, the protein content of the raw materials (Table 4) and the protein hydrolysis process were considered to be the most important as they directly influenced the yield and the quality of the product. High protein content of the raw material would give high product yield. The efficient hydrolysis process, which is the optimum combination of raw material and type of peptone used, would lead to high degree of hydrolysis (Table 3) or good protein hydrolysate quality.

The availability of raw materials is an important factor for the sustainability of the protein hydrolysate industry. Two sets of important data were considered here: the productivity of the raw materials (Table 4), that was the availability of this raw material in Indonesia, and the resource capacity (Table 4), which indicated the accessibility of the raw materials. As an example, the national scale availability of tofu waste was 1,062,284 ton/year however, a protein hydrolysate industry can only process the waste from the nearby tofu industries, which was around 3 ton/month. The availability (productivity and accessibility) of raw materials has a lower level of importance than the two previously described criteria because basically the protein hydrolysate industry can be set up on a small scale. Nonetheless, the high availability of raw materials is important for scaling up the industry.

The ease of processing criterion had a lower level of importance. Even though the ease of processing, for example, the types and number of related pretreatment processes, would affect the overall production costs, it impacts less than the accessibility of the raw materials and the sustainability of the industry.

Of least important was the price of protease. Despite the small amount of protease that was used in the process, the price of enzyme/protease commonly significantly contribute to the production cost. The price of protease varies a lot: bromelain, papain, and ficin are relatively cheap, whereas rennin and thermolysin may reach 367,000 US $ (per 100 grams of protease).

The price of raw materials was not considered here because all evaluated raw materials were industrial by-products and the development process would provide an added value, supporting the circular economy. Based on these considerations, the weight of importance of all the criteria is presented in Table 5.

Table 4. Protein composition, productivity, accessibility, and the pretreatment process of various type of raw materials.

| Raw material | Protein content of raw | Productivity (ton/year) | Resource capacity (ton/month) | Required Pre-treatment Process |
|--------------|------------------------|--------------------------|-------------------------------|-------------------------------|
|              |                        |                          |                               |                               |
Prior elimination of some raw materials and protease was carried out to minimize the alternatives evaluated using the AHP process. Cassava leaves, tofu waste, and cheese whey were eliminated due to their limited accessibility. Soy sauce solid waste, corn steep liquor, and chicken skin were also eliminated because these three raw materials have the lowest initial protein content compared to other raw materials. For the protease, pepsin was eliminated because it was mostly obtained from a non-halal source. Further, rennin and thermolysin were also eliminated due to their extremely high price. This prior elimination resulted in 5 potential raw materials: fish bones, fish skins, beef bones, cow skin and chicken feet, and 3 proteases: papain, bromelain, and ficin. In total, there were 15 alternatives that were considered for the AHP process.

| Table 5. Weight of importance between criteria. |
|-----------------------------------------------|
| **Criterion** | **Weight** |
| Raw materials’ availability | 0.183 |
| Raw materials’ protein content | 0.319 |
| Degree of hydrolysis | 0.319 |
| Protease’s price | 0.069 |
| Processing’s ease level | 0.110 |

Table 6. The value of alternative process’ final weight.
The use of different proteases affects the protein hydrolysis efficiency, thus careful consideration should be given in choosing the raw materials-protease combination. By considering the availability/accessibility of raw materials, the protein content of raw materials, the hydrolysis efficiency, the enzyme price, and the ease of processing which included pretreatment of raw materials; beef bone and bromelain protease was chosen to be the optimum raw material and protease combination for the production of protein hydrolysate.
production of protein hydrolysate in Indonesia. Other potential alternatives are cow skin-bromelain and fish skin-bromelain. These processes would increase the added value of the raw materials, food industrial waste, by 1.8 – 17.7 times and would provide an interesting example for the circular economy.

References
[1] Naegeli C 1880 Klasse Akad. Wiss Meunchen 10 277
[2] Statistics Indonesia (Accessed at http://www.bps.go.id/)
[3] de las Fuentes L, Sanders B, Lorenzo A and Alber S 2004 Awarenet: Agro-Food Wastes Minimisation and Reduction Network In: Protein Byproducts: Transformation from Environmental Burden into Value-added Product ed. Dhillon G ( Canada: Elsevier Inc) 2016 p 5
[4] Bernadeta P A and Silalahi I H 2012 J. Kim. Khatulistiwa 1 26–30
[5] Restiani R 2016 J. Biota 1 103–10
[6] Vidal A R, Cechin C F, Cansian R L, Mello R O, Schmidt M M, Demiate I M, Kempka A P and Dornelles R C P 2018 Ciênc. Rural 48 1–9
[7] Fasuyi A O and Aletor V A 2005 Pak. J. Nutr. 4 43–9
[8] Ravindran G and Ravindran 1988 Food Chem. 27 299–309
[9] Kuiken K A, Lyman C M, Bradford M, Trant M and Dieterich S 1949 J. Biol. Chem. 177 29–36
[10] Cavins J F, Kwolek W F, Inglott G E and Cowan J C 1972 JAOAC 55 686–91
[11] Yamazaki K I, Takao S and Yamamoto K 1990 U.S. Patent No. 4,940,662
[12] Liu K S 1997 Chemistry and nutritional value of soybean components. In: Mateos-Aparicio I, Redondo Cuenc a A, Villanueva-Suárez M J and Zapata-Revilla M A 2008 Nutr. Hosp. 23 305–312
[13] Li S, Zhu D, Li K, Yang Y, Lei Z and Zhang Z 2013 ISRN Ind. Eng. 2013 1–6
[14] Susanti S 2006 Buana Sains 6 59–66
[15] Bechtel P J, Bland J M, Watson M A, Lea J M and Bett K L 2019 Food Sci. Nutr. 7 1396–1405
[16] Toppe J, Albrectsen S, Hope B and Aksen 2007 Comp. Biochem. Phys. 146 395–401
[17] Gauza-włodarczyk M, Kubisz L and Włodarczyk D 2017 Int. J. Biol. Macromol. 104 987–91
[18] Gunawan F, Suptijah P and Uju 2017 JPHPI 20 568–581
[19] Eastoe J E 1955 Biochem. J. 61 589–600
[20] Masirah 2018 Proc. Seminar Nasional Kelautan Dan Perikanan (Swiss-Berlin, Tunjungan-Surabaya 05 September 2018) vol IV p 285–292
[21] Aykın-Dincer E, Koç A and Erbaş M 2017 Poult. Sci. 96 4124–31
[22] Araujo I B S, Bezerra T K A, Nascimento E S, Gadelha C A A, Santi-Gadelha T and Madruga M S 2018 Food Sci. Tech. 2061 167–73
[23] Yasmin A, Butt M S, Sameen A and Shahid M 2013 Pak. J. Nutr. 12 455–9
[24] Paskaš S, Miočinović J, Savić M, Ješić G, Rašeta M and Becskei Z 2019 Mac. Vet. Rev. 42 151–61
[25] Hofer A A, Hauer S, Kroll P and Herwig C 2018 Process Biochem. 70 20–8
[26] Nebraska Corn Board 2005 Corn Processing Co-Products Manual (Lincoln: University of Nebraska-Lincoln) p 6
[27] Fishanda R R 2019 Thesis, Universitas Pasundan (Accessed at http://repository.unpas.ac.id/)
[28] Belitz H, Grosch W and Schieberle P 2009 Food Chem. 4th revised (Heidelberg: Springer) p 78
[29] Tapal A and Tiku P K 2019 Enzymes in Food Biotechnology chapter 27 (Myysuru: Elsevier Inc.) p 471–81
[30] Menard R, Khouri H E, Plouffe C, Dupras R, Ripoll D, Vernet T, Ressier D, Laliberte F, Thomas D Y and Storer A C 1990 Biochemistry 29 6706–13
[31] Hou Y, Wu Z, Dai Z, Wang G and Wu G 2017 J. Anim. Sci. Biotechnol. 8 24
[32] Biocon 2016 Product data sheet: Bromelain purified enzymatic preparation (Accessed on 22 July 2020 at http://biocon.es/)
[33] Buyukyavuz A 2014 Thesis, Clemson University (Accessed at https://tigerprints.clemson.edu/)
[34] Yang Y, Shen D, Long Y, Xie Z and Zheng H 2017 Sci. Rep. 7 1–8
[35] Rawlings N D, Barrett A J, Thomas P D, Huang X, Bateman A and Finn R D 2018 Nucleic Acids Res. 46 D624–32
[36] Jiang T, Chen L J, Xue L and Chen L S 2007 J. Dairy Sci. 90, 3126–33
[37] Simpson B K 2000 Digestive proteinases from marine animals. In Seafood Enzymes: Utilization and Influence on Postharvest Seafood Quality, ed N. F. Haard and B.K. Simpson (New York: Marcel Dekker) pp 531–40
[38] Palashoff M H 2008 Doctoral dissertation, Northeastern University (Accessed at https://repository.library.northeastern.edu/)
[39] Dunn B M 2001 Curr. Protoc. Protein Sci 25 21.3.1–6
[40] Keil B 1992 Specificity of proteolysis (Heidelberg: Springer) pp 43–228
[41] Dua A and Desai S S 2013 Journal of Histology 2013 1–5
[42] Promega 2018 (Accessed on 18 July 2020 at https://worldwide.promega.com/)
[43] Cold Spring Harb Protoc 2007 (Accessed on 31 July 2020 at http://cshprotocols.cshlp.org/)
[44] Rutherfurd 2010 J. AOAC Int. 93 1515–22 ALmasyhr, Yuniarti H, Luciasari E, and Muhilal1996 J. Nutr and Food Res. 19 p 1–8
[45] [Kementerian Perindustrian Republik Indonesia](https://kemenperin.go.id) (Accessed at https://kemenperin.go.id)
[46] Bsisn.com (Accessed on 5 August 2020 at http://ms.bsisn.com/)
[47] Puger A W, Suasta I M, Astawa P A and Budaarsa K 2015 JPTHP 6 1–5
[48] Almasyhuri, Yuniarti H, Luciasari E, and Fathoni R M 2017 JPTHP 6 1–5
[49] Beritagar.id (Accessed on 5 August 2020 at http://beritagar.id/)
[50] Hadinoto S and Idrus S 2018 Majalah BIAM Kemenperin 51–57 (Accessed at ejournal.kemenperin.go.id/bpbiam)
[51] Rahaldo P 2012 Thesis, Institut Pertanian Bogor (Accessed at https://repository.ipb.ac.id/)
[52] Dewantara B F, Hamdani M D I, Sulastrti S and Adhianto K 2017 JIPT 15 35–40
[53] Ulupi N, Nuraini H, Parulian J and Kusuma S Q 2018 JIPTHP 6 1–5
[54] Fathoni R M 2017 JTHP 6 5–8 (Accessed at http://journal.unpad.ac.id/ejournal/)
[55] P.T Tereos Indonesia 2020 Private communication
[56] Sukmawati D 2011 Japerti Universitas Udayana 1143-1148
[57] Tribun News Medan (Accessed on 18 August 2020 at https://medan.tribunnews.com/)
[58] PD Dharma Jaya (Accessed on 18 August 2020 at https://dharmajaya.co.id/)
[59] Republika (Accessed on 18 August 2020 at https://republika.co.id/)
[60] Dhillon G S (ed) 2016 Protein Byproducts: Transformation from Environmental Burden into Value-added Product. (Canada: Elsevier Inc.) chapter 9.3 p 171
[61] Yeoh C 1976 Phytochemistry 15 1597–99
[62] Takeshi N, Yasuhiro T, Norihisa K and Nobutaka S 2012 Food and Nutr. Sci. 03 1121
[63] Nurilmala M, Wahyu M and Wiratmaja H 2006 JTHP 9 22–33
[64] Astiana I, Nurjanah and Nurhayati T 2016 JHPHI 19 170–81
[65] Septimus S 1961 Anatomy of Domestic Animal. Mc. (New York: Graw Hill)
[66] Sabtu B, Djojowidagdo S and Triatmojo S 2000 J. Agric. Sci. 13 211–24
[67] Mayasaroh I, Rusmana D and Wiradimadja R 2012 JTHP 1 3–4
[68] Kafri I, Cherry J A, Jones D E and Siegel P B 1985 Poult. Sci. 64 2143–49
[69] Djuric M, Caric M, Milanovic S, Tekic M and Panic M 2004 Eur. Food Res. Technol. 219 321–28
[70] Nisa M, Khan M A, Sarwar M, Lee W S, Ki K S, Ahn B S and Kim H S 2006 Asian-Australas J. Anim. Sci. 19 1610–16
[71] Murdiana A and Saidin S 2001 J. Food Nutr. Res. 24 33–7
[72] Malaka R 2018 Thesis Universitas Hassanudin (Accessed at https://digilib.unhas.ac.id/)
[73] Russo J M, Watson S A and Heiman V 1960 Poult. Sci. 39 1408–12
[74] Liputan 6 News (Accessed on 18 August 2020 at https://liputan6.com/)
[75] Agromaret (Accessed on 18 August 2020 at https://agromaret.com/)