

The Effects of Acidic and Alkaline Hydrolysis Process on some Physical and Chemical Properties of Broiler Chicken Feathers

Research Article

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Received on: 26 Oct 2018 Revised on: 21 Dec 2018 Accepted on: 30 Dec 2018 Online Published on: Sep 2019

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ABSTRACT

The objective of this study was to evaluate the physical and chemical properties of the broiler feather concentrate (BFC) in different hydrolysis processes. The raw materials in the form of broiler feather waste (BFW) were hydrolyzed into broiler feather concentrate (BFC) products in the form of feather meal. A total of 1000 g of dry feather samples were used in this study. The study was designed up to 4 treatments with 5 repetitions. Each treatment unit requires 50 g of dry feather samples. The repetition of the sample was the number of times the hydrolysis process. The sample was hydrolyzed using NaOH or HCl. The treatments were applied, namely: H-1) without hydrolysis; H-2) 1M-NaOH 20% (b/v); H-3) 1M-HCl 20% (v/v); H-4) 1M-NaOH 10% (b/v) + 1M-HCl 10% (v/v) (the treatment in H-4 was carried out sequentially). The results showed that the application of chemical processes to produce amino acid profiles of BFW was similar to BFC. The microstructure of the broiler feather differs in each chemical process. The composition of the BFC products was dominated by the compounds of SO₃, SiO₂, and Al₂O₃. An application of the chemical hydrolysis process to the BFC increases the value of the water and the ash content, but it decreases the fat and fiber content. The process of chemical hydrolysis using the H-2 treatment 1M-NaOH (20% (b/v) is the best hydrolysis process compared to other treatments.

KEY WORDS broiler, chemical, concentrate, feather, hydrolysis.

INTRODUCTION

There is a growing increase in the production of feather waste in the world arising from the poultry industry sector. Chicken feather is one waste that has a large number of keratin protein components (Darah *et al.* 2013). Keratin is classified into a biopolymer. This biopolymer has enormous potential in the world (Amieva *et al.* 2014; Cao *et al.* 2012). Feather has a series of disulfide (S-S) bonds that makeup keratin proteins. This bond is a strong bond so the feathers have a very low digestibility (Pruekvimolphan *et al.* 2011). Van-Heughten and Van-Kempen (2002) have conducted a series of studies by processing poultry feathers as pig feed. The results of the study concluded that the use of 8% poultry feather meal in pigs did not affect on the growth performance and carcass quality. Liu *et al.* (2014) have also used poultry feather meal which is capable of replacing 63.46% of protein from pig feed needs. A series of studies have been conducted by Campos *et al.* (2017) to evaluate the effect of using hydrolyzed feather flour to replace fish meal in feed, growth performance and muscle fatty acid composition of European seabass (*Dicentrarchus labrax*). Results were further evaluated for 18 weeks. The results obtained showed that 12.5% of feather flour was able to replace 76% of protein from fish meal without damaging feed intake, growth, immune response or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in the muscle.

Gang *et al.* (2013) developed a second biotechnology process using *Bacillus licheniformis S6* as a simple and economical procedure but significantly increased the nutritional value of feather flour. Compared to feather and concentrate raw materials, feather meal products fermented with *Bacillus licheniformis S6* enzymatically have a significant increase on the *in vitro* digestion, oligopeptides and soluble proteins. The use of enzymatic method is one of the other methods that can be developed in the process of feather hydrolysis.

Feather is one of the feed ingredients in poultry. Keratin is a type of protein that makes up the composition of feather. Feathers can be hydrolyzed using heat steam. Keratin of protein is rich in digestible cystine amino acids up to 75%. Keratin has major amino acids such as glycine, serine, threonine, arginine, isoleucine, leucine, phenylalanine and valine. Feather meal is a good source of protein and can be used to replace other protein sources, especially in monogastric animals. Amino acid profiles in feather meal are similar to fishmeal (Sarmwatanakul and Bamrongtum, 2000). The substitution of fish meal with feather meal in feed does not reduce performance, carcass or cause death in broiler chickens. The use of feather meal can reduce the use of feed costs. Feather meal can be replace 100% of fish meal in feed intake without showing a negative effect on the broiler performance (Hasni et al. 2014).

Feathers in poultry comprise about 10% of the total weight of bird (Lasekan et al. 2013). Feather waste has been used as a source of protein and is also one of the most important and inexpensive elements in the production of keratinase enzymes (Balakumar et al. 2013). Various ways have been done to improve the quality of feather waste as animal feed. One is to employ use the role of microorganisms through the fermentation process (Reddy et al. 2017). In edible parts obtained after processing of chicken account for approximately one-third of the chicken body weight. On this amount, certainly, it produces millions of more tons of chicken feather waste in each year throughout the world (Daroit et al. 2011). The poultry slaughtering industry is one of the feather-waste producers (Darah et al. 2013). Keratin from feather wastes has been developed in to feed ingredients as well as bioplastic ingredients. It is based on the nature of the chicken feathers that are biodegradable and non-burning hydrophilic (Ramakrishnan et al. 2018). The utilization of livestock wastes, especially feather waste, has not been done optimally. An increase in livestock slaughter has an impact on the amount of waste produced. Increased chicken meat production encourages the increased production of feather waste (Zhang et al. 2014a; Cedrola et al. 2012).

The feathers consist of 50-70 g/kg of alive livestock weight and contain 900 g/kg of crude protein in particular feathers originate from adult chickens (Zhang *et al.* 2014b). In addition, the use of animal by-products as a source of animal protein, the use of plant products has also started to develop lately. An example of the development of vegetable products is the use of green concentrated Indigofera plants (Hutapea *et al.* 2018).

Utilization of by-product livestock such as feathers has been widely applied, using both physical and chemical treatment. The enzyme used is a keratinolytic enzyme produced by the bacteria *Flavobacterium* sp. combined with the heating process at the initial stage (Riffel and Brandelli, 2002). Physical and chemical treatments are expensive. They are also potentially damaging nutrient components such as amino acids (Queiroga *et al.* 2012). In addition, the use of other by-products in the form of cattle skin has also been developed as a raw material of gelatin. The production process also uses chemical processes using Ca(OH)₂ (Said *et al.* 2011; Said *et al.* 2015).

Broiler feather waste (BFW) is one of the livestock wastes which can be produced into broiler feather concentrate (BFC). To produce the BFC from the BFW, it is required special treatment, for example hydrolysis process.

Chemical hydrolysis process is a process which is considered to be better than other processes (Said *et al.* 2018). In the process of chemical hydrolysis, the use of chemicals must be appropriate, because it can affect the livestock and the environment. Therefore, it is necessary to combine the right formula. The objective of this study was to evaluate the chemical and nutritive properties of broiler's feather concentrate produced through different chemical processes.

MATERIALS AND METHODS

The samples of the study were using broiler feather waste (BFW) obtain from poultry slaughterhouse (PSH) of Daya village, Makassar City, South Sulawesi, Indonesia. BFW is a waste coming from the process of removing broiler feathers (defeathering) without other waste from PSH. Supporting materials include distilled water aquadest, 1 M-NaOH; 1 M-HCl. The amino acid profile was analyzed using near infrared (NIR) (NIRFlex Solids Buchi), microstructure and morphology of broiler feather (SEM-Tescan Vega 3SB), composition of broiler feather (EDS-Tescan Vega 3SB), oven (Memmert), grinder (Hitachi), analytical scales (Sartorius), beaker glass (pyrex), erlen mayer (pyrex), measuring glass (pyrex) and the volume pipette (pyrex).

Preparation of samples

A total of 3 kg of wet feather waste samples were taken from the waste disposal of chicken slaughterhouse at Daya, Makassar city, South Sulawesi province, Indonesia. The feather waste was selected from base on fresh feather waste category (one day after slaughtered of poultry). Sources of the feather raw materials were obtained from broilers chicken with mixed sexing, 45 day slaughter age, strain Cobb. The samples were put into high density polyethylene (HDPE) plastic bags and then taken to the laboratory for further preparation. The samples were washed with running water to remove any residual dirt, blood and fat. Later on, the samples were dried in an oven at 60 °C for 60 mins (Said et al. 2018). The dried feather samples were prepared to be treated using chemical treatment. (the process of drying feather waste decreased the feather weight about 50% after the oven process).

Implementation of research

The flow chart of the overall stages of the research implementation was presented in Figure 1. A total of 1000 g of dry feather waste samples were used in the treatment. Each treatment unit used 50 g of dry feather samples. A total of 20 treatment units were applied, consisting of 4 treatments with 5 replications) (20 treatment units×50 g= 1000 g).

A total of 4 treatment stages of the hydrolysis process were applied, namely (1) H-1 (without hydrolysis process as control); (2) H-2 (1 M-NaOH, 20% (w/v); (3) H-3 (1 M-HCl, 20% (v/v) and (4) (1 M-NaOH, 10% (w/v) and (1M-HCl, 10% (v/v). Each hydrolysis process was carried out for 4 days at room temperature (H-2 and H-3 treatment), while in H-4 treatment, each hydrolysis process stages was carried out for 2 days. The hydrolysis process in H-4 treatment was carried out in 2 stages. After the hydrolysis processes, the samples were proceeded with washing with running water for 1 minute. The dry feather was then ground using a grinder and then tested.

The parameters observed in this study included: (1) amino acid profiles (Escuredo et al. 2014), (2) microstructure and morphology of the BFW and the BFC (Ramakrishnan et al. 2018), (3) element composition of the BFW and the BFC (Mishra et al. 2017), (3) proximate analysis of the BFC (water, fat, fiber and ash content) The experiment was (AOAC, 2005). conducted experimentally based on the completely randomized design pattern which is unidirectional 4 treatment and 5 repetitions. The data were analyzed using ANOVA. The treatment showed a significant effect, then tested with Duncan's multiple range test at 5% level (Steel and Torrie, 1991).



Figure 1 Flow chart of the implementation of hydrolysis process on broiler feather using different stage

RESULTS AND DISCUSSION

Amino acid profile

Broilers generally produce approximately 37% of byproducts from the activities of poultry slaughter houses. This amount can no longer be consumed directly by humans (Meeker and Hamilton, 2006). One of these products is the feather. Feathers from broilers are one of the broiler by-products of in the poultry slaughterhouses (Azmi *et al.* 2018). The main constituent of feathers is protein. Based on the amount of protein, feathers consist of 80-90% keratin type proteins (Mazotto *et al.* 2017).

Amino acids are one of the compounds needed by the livestock body. Amino acids are useful as the main source of protein formation in the body. Approximately 75% of amino acids are used for protein synthesis. Feather concentrate is rich in essential amino acid compounds (Newsome *et al.* 2014). The amino acid profile of the BFW with and without hydrolysis process was presented in Figures 2 and 3.

Based on Figure 2, it shows that the amino acid profile of the BFW with and without the hydrolysis process has a similar trend. This suggests that the hydrolysis process does not significantly affect the amino acid composition. The highest percentage of amino acid in BFW is amino acid glutamate acid (15.85%), leucine (Leu) (11.77%) and phenylalanine (Phe) (8.85%). The lowest percentage is the amino acid histidine (His) (0.94%). The amino acid concentration is lower than the fermentation feather concentrate used in other research: glutamate acid (84.6%), then leucine (66.9%) and phenylalanine (42.0%) (Pan *et al.* 2016).

The other results reported that the fermented feather concentrate contains high oligopeptide compounds and some important amino acids such as histidine (His) and lysine (Lys) (Cedrola *et al.* 2012; Grazziotin *et al.* 2006).

The amino acid composition in the feather meal has a similarity to the amino acid composition in the fish meal. The results of the feather meal analysis of broiler amino acids were: lysine (5.28%); arginine (2.91%); glycine (4.28%); histidine (2.04%); isoleucine (4.70%); methionine (2.11%); phenylalanine (6.67%) and valine (6.44%). On the other hand, the amino acid profile of fishmeal consists of: lysine (8.83 mg/100 g); arginine (4.56 mg/100 g; glycine (5.82 mg/100 g); histidine (1.84 mg/100 g); isoleucine (4.21 mg/100 g); methionine (1.39 mg/100 g); phenylalanine (4.79 mg/100 g) and valine (5.24 mg/100 g) (Prado *et al.* 2016).

Amino acids profile in feather meal is not very different from blood meal, but in general, the amino acid levels are higher in certain types.

The results of the research are related to the amino acid profile of the blood meal obtained data, namely: lysine (7.313%); arginine (3.801%); glycine (3.787%); histidine (5.703%); isoleucine (0.835%); methionine (0.458%); phenylalanine (6.219%) and valine (6.483%) (Wandita *et al.* 2018).

This is what causes the feather meal has great potential to be developed as animal feed ingredients (Arunlertaree and Moolthongnoi, 2008). Increased hydrolysis process due to the chemical reaction can cause the decrease of protein solubility. It also occurs on the protein amino acid (Coward-Kelly *et al.* 2006). Amino acid metabolism affects the body. Protein hydrolysis is the process of breaking down the peptide bonds of proteins into smaller components, such as peptides and amino acids. The process of hydrolysis of peptide bonds will cause protein denaturation which is characterized by a change in the form of a decrease in solubility. This is due to the appearance of secondary structural changes in the peptide chain that which is followed by a reduced molecular weight of proteins or polypeptides. In addition, the hydrolysis process causes damage to the globular structures. The denaturing process can occur due to the influence of chemical compounds such as acids or bases.

Broiler feather concentrate (BFC) is broiler feather waste (BFW) that has undergone a hydrolysis process, using both acids and bases. The hydrolysis process is a process of breaking down molecules into simpler compounds with the help of water molecules. Protein hydrolysis is the process of breaking down the peptide bond of the protein into a simpler molecule. The process of hydrolysis on the peptide bond will cause changes in the protein. The use of alkaline solutions (NaOH) can loosen the bonds of the protein component in the feather (Kim and Patterson, 2000). These changes cause an increase in solubility due to the increase in the content of NH_3^+ and COO⁻ as well as the reduction in the molecular weight of proteins or polypeptides. In addition, this is because of the damage to the globular structure of proteins. The hydrolysis process can occur due to the influence of chemical compounds (acids and bases), heat and enzymatically. Proteins consist of groups of amino acids. Similarly, keratin protein consists of several types of amino acids, the typical amino acids are dominated by disulfide compounds (Riffel and Brandelli, 2006). Hydrolysis with acids and bases can affect the structure and profile of amino acids.

Microstructure and morphological of broiler's feather

The appearance of microstructure and morphology of broiler's feather using scanning electron microscope (SEM) was presented in Figure 4. This parameter aims to provide a comparative micrograph of broiler feathers which with and without hydrolysis. Feather is the by-product of broilers composed of a number of keratin proteins.

Based on Figure 3, it can be seen that the structure of the feather is different in the two images. Figure 4(a) shows that the structure of the feather is still arranged regularly, while in Figure 4(b) describes that the structure of the feather changes significantly. The feather structure is stretched and irregular.



Figure 2 Profile of essential amino acids of broiler's feather without (BFW) (H-1 treatment) and after hydrolyzed (BFC) (H-2 treatment) (Lys: lysine; His: histidine; Iso: isoleucine; Leu: leucine; Met: methionine; Phe: phenylalanine; Thre: threonine; Trip: tryptophane and Val: valine)



Figure 3 Profile of non essential amino acids of broiler's feather without (BFW) (H-1 treatment) and after hydrolyzed (BFC) (H-2 treatment) (Ala: alanine; Arg: arginine; Asp Acid: aspartate acid; Cys: cysteine; Gly: glycine; Glu Acid: glutamine acid; Pro: proline; Ser: serine and Tyr: tyrosine)

This change is one of the phenomena of protein denaturation process caused by the hydrolysis process. The peptide bond structure changes the shape so that the structure of the feathers also changes.

The micrograph in the (Figure 4a) is still visible, while the tissue morphology of the BFC in Figure 4b is irregular. This indication suggests that there is a process of denaturation of the keratin protein components which make up the tissue in the hair. This denaturation process occurs because there are active side reactions on the keratin amino acids with chemical compounds used in the reaction process. The chemical process is able to loosen the polypeptide and disulfide bonds and dissolve the protective layer of wax found on the feather, making it fragile and easily ground (Rahayu and Bata, 2014). Chicken feathers have continuous tissue structure. Chicken feathers also have long and short shapes naturally.

Increasing the surface area of the feather will increase efficiency (Fathima and Balasubramanian, 2006). Feathers consist of three parts: rachis (primary), barbs (secondary) and barbules (tertiary). The length of rachis ranges from 1 to 15 mm while barbs and barbules are (1-45 mm) and (1-800 mm) respectively (Tesfaye et al. 2017a). The density level of chicken feathers and fractions ranges from 0.44-0.91 g/cm³. This value is correlated but is lower than the density values in animals and plants such as wool (1.31 g/cm³), silk (1.27 g/cm³), jute (1.3 g/cm³), coir (1.2 g/cm³) and cotton (1.5-1.6 g/cm³) (Hearle and Morton, 2008). The keratin structure which composes the feathers is nonburning hydrophilic and biodegradable. Therefore, the keratin is also widely considered to be a bioplastic-making agent through a chemical process (Ramakrishnan et al. 2018).

The composition of compounds in BFW and BFC

In general, the illustration of the compounds composition composing the BWF and the BFC is shown in Figure 4. The illustration is generated using energy dispersive spectroscopy (EDS).

Based on Figure 6 and Table 1, it is seen that the composition of the largest compound in the FBW products is dominated by SO₃ (44.89 wt. %). Furthermore, the SiO₂ compounds (27.75 wt. %) and Al₂O₃ (12.80 wt. %) also predominate. A small fraction contains K_2O compounds (4.43 wt. %) and $P_2O_5\%$ (3.60 wt. %). When compared with the FBW composition data on Figure 5, it is seen that the component is still dominated by K_2O compounds (71.14 wt. %) and CaO (28.59 wt. %). The SO₃ compound has a very low component (0.12 wt. %). The SO₃ compounds dominate the composition of FBC. This is due to a hydrolysis process involving a chemical compound (NaOH).

The main component of SO₃ is sulfur, formed by disulfide bond (S-S). Feather composed of structural protein components of keratin (>90%), rich in hydrophobic residues and cysteine, has crosslinked the disulfide bonds (Dou *et al.* 2015). Disulfide bond formation increases strength on feathers (Garrido *et al.* 2018).

Water

Water content is one indicator of the feasibility of feed ingredient. Water content becomes one of the ingredients in feed formulation. The water content in foodstuffs greatly affects the quality and saving of the food. Therefore, the determination of water content of food is very important for the processing, and distribution process gets the right handling. The data of water content of the BFC which is produced from several different chemical process methods were presented in Table 2.

The data results of ANOVA in Table 2 show that the hydrolysis process applied to the production process of the feather concentrate has a significant effect (P<0.05) on the water content of BFC. The hydrolysis process using H-2 (20% (w/v) of NaOH 0.1 M) in the actual production process improved the digestion concentration of feather $(8.49\% \pm 0.24^{\text{b}})$ compared to H-1 treatment $(7.53\% \pm 0.44^{\text{a}})$ with H-3 $(7.81\%\pm0.27^{a})$ with H-4 $(7.50\%\pm0.47^{a})$. In general, the water content varies from 7.50% to 8.49%. The ability of chicken feathers to absorb water from the environment has important implications for the processing, storage, transport, and durability of composite materials. The increase in water content may disrupt the process or binding of the end product and increase product weight and product damage due to microorganism contamination (Munawar et al. 2007; Jones et al. 1998). The OH⁻ group of the NaOH compound which is used as a hydrolysis agent affects the water content in the product. The hydrolysis process causes the exothermic reaction of NaOH into the water and frees the heat into the air. The water concentration of feather concentrate was lower than the feather concentrate product which resulted from the previous research which was 12.33% (Tesfaye et al. 2017a). Not much information is available about changes caused by the different moisture content of hydrolyzed feather flour. Papadopoulos et al. (1986) reporting that with humidity levels of 50, 55, 60, 65 and 70% and processing times of 30, 40, 50, 60 and 70 minutes, at constant pressure 436 kPa and a temperature of 146 °C indicate a linear positive effect on the amino acids sulfur, cystine and methionine. He also stated that most essential amino acids and all non-essential foods are lower in foods that contain more water than those that are processed with lower water content.

Crude fat

Fat content was measured using the soxhlet method. The comparison of the fat content in the BFC on different chemical hydrolysis process was presented in Table 2.

The data (Table 2) of ANOVA results showed that the difference in the process of hydrolysis has a significant effect (P<0.05) to the value of the fat content of the BFC. Fat content is a collection of some fat derivatives triglycerides, waxes, terpenes, steroids, pigments, esters, aldehydes and other lipids. The fat content of flour products varies from 2.42 to 3.33%. The fat content of BFC obtained is still lower than the fat content of feather meal obtained by Adejumo *et al.* (2016) (4.54-5.83%). The lower value of fat content was caused by the production process using the cooking process combined with pressure. It can be cause the fat molecules to be dissolve, so that the fat content becomes low.



Figure 4 Micrograph of feather of broiler before and after hydrolysis process using scanning electron microscope (SEM) (VEGA 3 TESCAN) 4(a): without hydrolysis/BFW (H-1 treatment) (Magnification 500x); 4(b): after hydrolysis (H-2 treatment) (Magnification 500x) The arrows indicate changes in the structure of the feather tissue due to the influence of the hydrolysis process The feather tissue looks 3(a) normal and 3(b) stretch An arrow indicates a different structure

Table 1 Composite compounds of BF without (BFW) (H-1 treatment) and after hydrolysis (BFC) (H-2 treatment) process using energy-dispersive x-ray spectroscopy (EDS)

Without hydrolysis (BFW) (H-1 treatment) (n=3)			After hydrolysis (BFC) (H-2 treatment) (n=3)		
Element composition	C. compound	C. atom (At. %)	Element composition	C. compound	C. atom (At. %)
Sulfur	SO_3	0.05	Aluminium	Al_2O_3	5.28
Chlorine	-	0.03	Silikon	SiO ₂	9.71
Potassium	K_2O	45.84	Phosphorus	P_2O_5	1.07
Calcium	CaO	15.47	Sulfur	SO_3	11.78
Oxygen	-	38.56	Chlorine	-	3.87
Copper	-	0.02	Potassium	K_2O	1.98
Iron	FeO	0.03	Oxygen	-	66.33
Total		100.00	Total		100.00

BFW: broiler feather waste; BFC: broiler feather concentrate and C: composite.

Table 2 The chemical analysis results of without (BFW) (H-1 treatment) and after hydrolysis (BFC) (H-2 treatment)¹

D	Treatment groups (n=5)					
Parameters	H-1 (BFW)	H-2 (BFC)	H-3 (BFC)	H-4 (BFC)		
Water (%)	7.53±0.44 ^a	$8.49{\pm}0.24^{b}$	$7.81{\pm}0.27^{a}$	$7.50{\pm}0.47^{a}$		
Crude fat (%)	3.33±0.77°	2.53±0.41 ^{ab}	3.17±0.53 ^{bc}	$2.42{\pm}0.34^{a}$		
Dietary fiber (%)	0.54±0.19 ^a	0.48±0.13 ^a	$0.45{\pm}0.07^{a}$	$0.79{\pm}0.17^{b}$		
Crude ash (%)	0.51 ± 0.06^{b}	3.34±0.34°	$0.21{\pm}0.05^{a}$	$0.14{\pm}0.04^{a}$		
¹ H 1: without hydrolysis: H 2: 1M N2OH 20% (w/y): H 3: 1 M HCl 20% (y/y) and H 4: 1 M N2OH 10% (w/y) + 1M HCl 10% (y/y)						

¹H-1: without hydrolysis; H-2: IM-NaOH, 20% (w/v); H-3: 1 M-HCl, 20% (v/v) and H-4: 1 M-NaOH, 10% (w/v) + 1M-HCl, 10% (v/v).

BFW: broiler feather waste and BFC: broiler feather concentrate.

The means within the same row with at least one common letter, do not have significant difference (P > 0.05).

In his research, using immersion methods in distilled water, wood ash and NaOH were then combined with soaking in boiling water at temperature of 150 °C for 1 hour. The process of heating without pressure has not been able to melt fat of feather, so that the fat content is still high. The fat content of feather meal has been reported by El-Boushy et al. (1990) with a value of 2.5%. This result is not much different from the fat content of feather meal produced by chemical methods. In contrast to the results of previous studies, Tesfaye et al. (2017b) have reported the results of fat content of feather waste which is 0.83%. Feather waste is not only used as feed ingredients, but also has the potential to be used in a variety of textile, plastic, cosmetic, pharmaceutical, biomedical and bioenergy industries. Fats on the feather concentrate in accordance with the needs of fats in animal feed. The recommended fat content in feather processing is below 2% (Bateup and Warner, 1986). Therefore, of course, the advanced process is still needed. The results of the study have been reported by Bertsch and Coello (2005) related to the fat content of feather meal. The fat content from 3 types of feather meal products has been compared, namely untreated feather (UF), fermented feather meal (FFM) and commercial feather meal (CFM) of 2.13%; 5.15% and 9.8%. The higher value differences in CFM may be caused by a mixture of other biomass such as viscera, blood or subcatounos fat. A study result has been reported by Latshaw et al. (1994) related to the crude fat content test results in 5 types of feather meal products which are produced using different commercial hydrolysis process equipment.

A total of 5 types of processes have been applied, namely 3 processes using a steam pressure hydrolysis system continuously (207, 310 and 414 kPa) and 1 process using a batch hydrolysis system (283 kPa). Test results are compared with control (without process). The fat content of each product is for controlless process (7 g/kg), 207 kPa (27 g/kg), 310 kPa (24 g/kg) and 414 kPa (44 g/kg) and 283 kPa (18 g/kg).

Dietary fiber

The dietary fiber content is a mixture of most vegetable origin substances which are obtained as a defined digestive residue. The results of the dietary fiber content analysis on the different chemical hydrolysis process were presented in Table 2 (AACC, 2000).

The results of ANOVA show that the difference in the process method of hydrolysis has a significant effect (P<0.05) on dietary fiber content. The dietary fiber of BFC was produced through different chemical processes obtained in the range of 0.45-0.79%. This result is lower than the results of the dietary fiber level reported by Tesfaye *et al.* (2017b) which is 2.15%.

The results of the analysis can differ from one product to another. This is very much related to the production process. Fiber is a non-nutritional substance, there are two types of fiber, namely dietary fiber and crude fiber. The main role of fiber in food is in its ability to bind water, cellulose and pectin. Crude fiber is a part of food that cannot be hydrolyzed by strong acids or bases, chemicals used to determine the levels of crude fiber, namely sulfuric acid (1.25% H_2SO_4) and sodium hydroxide (3.25% NaOH).

The composition comprises a number of cellulose and cell wall substances and is determined using acetic acid, nitrate, and trichloro-acetic acid (AACC, 2000). A series of studies conducted by Latshaw et al. (1994) in the production process of feather meal using different commercial hydrolysis process equipment. The results of dietary fiber tests on 5 types of feather meal products produced have been reported. Five types of hydrolysis processes have been applied, namely 3 processes using a steam pressure hydrolysis system continuously (207, 310 and 414 kPa) and 1 process using a batch hydrolysis system (283 kPa). Test results are compared with control (without process). The dietary fiber levels for without process/control were (12 g/kg), 207 kPa (7 g/kg), 310 kPa (10 g/kg) and 414 kPa (8 g/kg) and 283 kPa (11 g/kg). Raw feather is very difficult to digest by livestock. The production process can be carried out using pressures from 207-690 kPa, 6-60 minutes with humidity of 60-70%, will break down their keratin ingredients, produce hydrolyzed feather meal that has 70% digestible crude protein. Processing by continuous heating, time, pressure, humidity and agitation affect the nutritional value of hydrolyzed feather flour. Some additions, such as synthetic amino acids, methionine, lysine, fish meal, dry whey powder during processing will increase the nutritional value of this product. Hydrolyzed feather meal can be added up to 6% of rations for broilers, 7% for layers and 5% for turkeys in balanced feed without harmful effects (El-Boushy et al. 1990). As many as 3 types of crude fiber content of feather meal products have been compared, namely untreated feather (UF), fermented feather meal (FFM) and commercial feather meal (CFM) of 0.65%; 0.42% and 1.11% (Bertsch and Coello, 2005). These results are similar to dietary fiber of BFC produced using different hydrolysis techniques (0.45-0.79%).

Ash

Ash content is a mixture of inorganic components or minerals contained in a foodstuff. Ash content is a mixture of inorganic components or minerals contained in a foodstuff. The description of the ash content on the BFC as shown in Table 2. Based on the data in Table 2, it can be seen that the difference in the chemical hydrolysis process affects the ash content.



Figure 5 Spectrogram of constituent element components and composition of compounds in broiler's feather without hydrolized/BFW (H-1 treatment) using energy-dispersive x-ray spectroscopy (EDS); (n=3)



Figure 6 Spectrogram of constituent element components and composition of compounds in broiler's feather after hydrolized/BFC (H-2 treatment) using energy-dispersive x-ray spectroscopy (EDS); (n=3)

Foodstuffs comprise 96% of inorganic materials and water, while the remainder is mineral elements. Ash content varies between treatments (0.14-3.34%). The results of this test are lower when compared with the value of ash content in feather meal as a result of research by Adejumo *et al.* (2016) (3.19-9.04%). The results of this test are likely caused by differences in the production process. This study only used boiling water at 150 °C for 1 hour in the presoaked process. In addition, the NaOH concentration used was lower (0.3 M) than used to hydrolyze of BFW.

The applied treatment has not been able to hydrolyze the feather component, so that the dry matter content which is converted to ash produces a lower value.

The H-2 treatment yielded the highest value of the ash content compared to the others. The value of this ash content is not much different from the fermented feather meal made by the others (Tesfaye *et al.* 2017a) (1.49%) and 0.57% (Mazotto *et al.* 2017). The results of the study have been reported by Bertsch and Coello (2005) related to the ash content of feather meal products. The ash content of 3

types of feather meal products has been compared, namely untreated feather (UF), fermented feather meal (FFM) and commercial feather meal (CFM), each 0.95%; 16.7% and 4.85%. This difference may be caused by differences in the processes and sources of raw materials. This result is similar to the results obtained in BFC (0.14-3.34%). Latshaw *et al.* (1994) have reported the results of research on 5 types of feather meal products using different commercial hydrolysis process equipment. Researchers have compared 5 types of processes, namely three processes were carried out using the pressure system of continuous hydrolysis (207, 310 and 414 kPa) and one process using a batch hydrolysis system (283 kPa).

Subsequent results are compared with control (without process). The ash content of the product is then compared. The treatment process produced ash content for without process (control (2 g/kg), 207 kPa (19 g/kg), 310 kPa (20 g/kg) and 414 kPa (23 g/kg) and 283 kPa (19 g/kg)).

CONCLUSION

The applications of chemical process on the production of feather waste produce the BFW amino acid profile which is similar to the BFC. The application of different chemical hydrolysis processes to the BFW affects the amino acid profile of the BFC products. The appearance of the feather microstructure differs in the application of treatment (control and chemical). The different stages of the hydrolysis process using chemical compounds have a direct effect on the tissue structure in broiler feather waste (BFW). The process of hydrolysis actually stretches the tissues that make up the structure of the broiler feathers concentrate (BFC). BFC product composition is dominated by SO₃, SiO₂ and Al₂O₃ compounds. The difference in the composition of feathers is influenced by the different hydrolysis processes. Different hydrolysis causes changes in composition especially those related to certain compounds. Application of chemical hydrolysis process at the BFW increases the value of water and ash content in the BFC, but on the contrary, it lowers the fat and fiber content. Test results related to water, crude fat, dietary fiber and ash show different values of each hydrolysis process applied, but these values still have similarities. Value differences are strongly influenced by the process methods that have been applied. The hydrolysis process using H-2 treatment (20% (w/v) of 1 M-NaOH is the best chemical hydrolysis process compared to other treatments.

ACKNOWLEDGEMENT

The team of authors would like to thank the Ministry of Research and Technology of Higher Education of the Republic of Indonesia, Rector of Hasanuddin University and Institute for Research and Community Service (IRCS), Hasanuddin University for the funding facilities to Excellence Applied Research for University Scheme (Skim Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT)) (Contract No. 005/ADD/SP2H/LT/DPRM/VIII/2017).

REFERENCES

- AACC. (2000). Approved Methods of the American Association of Cereal Chemists. American Association of Cereal Chemists, Saint Paul, Minnesota, USA.
- Adejumo I.O., Adetunji C.O., Ogundipe K. and Osademe S.N. (2016). Chemical composition and amino acid profile of differently processed feather meal. J. Agric. Sci. 61(3), 237-246.
- Amieva E.J.C., Velasco-Santos C., Martínez-Hernández A.L., Rivera-Armenta J.L. and Mendoza-Martínez A.M. (2014). Composites from chicken feathers quill and recycled polypropylene. J. Compos. Mater. 49(3), 275-283.
- AOAC. (2005). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.
- Arunlertaree C. and Moolthongnoi C. (2008). The use of fermented feather meal for replacement fish meal in the diet of oreochromis niloticus. *Environ. Nat. Resour. J.* 6(1), 13-24.
- Azmi N.A., Idris A. and Yuso N.S.M. (2018). Ultrasonic technology for value added products from feather keratin. *Ultrason. Sonochem.* 47, 99-107.
- Balakumar S., Mahesh N., Arunkumar M., Sivakumar R. and Hemambujavalli V. (2013). Optimization of keratinase production by keratinolytic organisms under submerged fermentation. *Int. J. PharmTech Res.* 5(3), 1294-1300.
- Bateup B.O. and Warner J.J. (1986). Selective scouring of dirt from greasy wool part I: A strategy to improve dirt recovery from scouring liquor effluent. *Text. Res. J.* **56(8)**, 489-496.
- Bertsch A. and Coello N. (2005). A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. *Bioresour. Technol.* 96(15), 1703-1708.
- Campos I., Matos E., Marques A. and Valente L.M.P. (2017). Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture*. 476, 152-159.
- Cao Z.J., Lu D., Luo L.S., Deng Y.X., Bian Y.G., Zhang X.Q. and Zhou M.H. (2012). Composition analysis and application of degradation products of whole feathers through a large scale of fermentation. *Environ. Sci. Pollut. Res. Int.* **19**, 2690-2696.
- Cedrola S.M.L., de Melo A.C.N., Mazotto A.M., Lins U., Zingali R.B., Rosado A.S., Peixoto R.S. and Vermelho A.B. (2012). Keratinases and sulfide from *Bacillus subtilis* SLC to recycle feather waste. *World J. Microbiol. Biotechnol.* 28, 1259-1269.
- Coward-Kelly G., Chang V.S., Agbogbo F.K. and Holtzapple M.T. (2006). Lime treatment of keratinous materials for the generation of highly digestible animal feed: 1. Chicken feathers. *Bioresour. Technol.* **97**, 1337-1343.
- Darah I., Nurdiyana A., Nurulhusna S., Jain K. and Lim S. (2013). Microsporum fulvum IBRL SD3: As novel isolate for chicken feathers degradation. *Appl. Biochem. Biotechnol.* 171(7), 1900-1910.

- Daroit D.J., Corrêa A.P.F. and Brandelli A. (2011). Production of keratinolytic proteases through bioconversion of feather meal by the Amazonian bacterium *Bacillus* sp. P45. *Int. Biodeterior. Biodegrad.* 65, 45-51.
- Dou Y., Huang X., Zhang B., He M., Yin G. and Cui Y. (2015). Preparation and characterization of a dialdehyde starch crosslinked feather keratin film for food packaging applications. *RSC Adv.* 5, 27168-27174.
- El-Boushy A.R., Poel A.F.B.V. and Walraven O.E.D. (1990). Feather meal-a biological waste: Its processing and utilization as a feedstuff for poultry. *Biol. Wastes.* **32(1)**, 39-74.
- Escuredo O., Martín M.I.G., Moncada G.W., Fischer S. and Hierro J.M.H. (2014). Amino acid profile of the quinoa (*Chenopodium quinoa Willd.*) using near infrared spectroscopy and chemometric techniques. J. Cereal Sci. 60(1), 67-74.
- Fathima M. and Balasubramanian A. (2006). Effect of plant growth regulators on the quality of bast fibres in *Abelmoschus* esculentus Moench. Acta Bot. Croat. **65(1)**, 101-112.
- Gang G., Jie C., Jun-gao W., Qiu-xia H. and Ke-chun L. (2013). A two-step biotechnological process for improving nutrition value of feather meal by *Bacillus licheniformis S6. J. Northeast Agric. Univ.* **20**(3), 71-77.
- Garrido T., Leceta I., de-la Caba K. and Guerrero P. (2018). Chicken feathers as a natural source of sulphur to develop sustainable protein films with enhanced properties. *Interl J. Biol. Macromol.* **106**, 523-531.
- Grazziotin A., Pimentel F.A., de-Jong E.V. and Brandelli A. (2006). Nutritional improvement of feather protein by treatment with microbial keratinase. *Anim. Feed Sci. Technol.* **126**, 135-144.
- Hasni M.S., Sahito H.A., Memon M.A., Sanjrani M.I., Gopang M.A. and Soomro N.A. (2014). Effect of feeding various levels of feather meal as a replacement of fish meal on the growth of broiler. *Int. J. Agric. Innov. Res.* 3(2), 505-511.
- Hearle J.W. and Morton W.E. (2008). Physical Properties of Textile Fibres. Horwood Publishing, Cambridge, United Kingdom.
- Hutapea P.S., Abdullah L., Karti P.D.M.H. and Anas I. (2018). Improvement of *Indigofera zollingeriana* production and methionine content through inoculation of nitrogen-fixing bacteria. *Trop. Anim. Sci. J.* **11(1)**, 37-45.
- Jones L., Riven D. and Tucker D. (1998). Handbook of Fiber Chemistry. Marcel Dekker, Inc., New York, USA.
- Kim W.K. and Patterson P.H. (2000). Nutritional value of enzyme or sodium hydroxide-treated feathers from dead hens. *J. Poult. Sci.* **79**, 528-534.
- Lasekan A.F., Bakar A. and Hashim D. (2013). Potential of chicken byproduct a source of useful biological resources. *Waste Manage*. 33, 552-565.
- Latshaw J.D., Musharaf N. and Retrum R. (1994). Processing of feather meal to maximize its nutritional value for poultry. *Anim. Feed Sci. Technol.* **47**(3), 179-188.
- Liu J., Bai C., Yan L., Chen S., Zhang Y. and Yang H. (2014). Nutritional value evaluation of *Saccharum spontaneum* germplasm resources. *Agric. Sci. Technol.* **15**, 1981-1986.
- Mazotto A.M., Ascheri J.L.R., Godoy R.L.O., Damaso M.C.T., Couri S. and Vermelho A.B. (2017). Production of feather protein hydrolyzed by *B. subtilis* AMR and its application in a

blend with cornmeal by extrusion. *LWT Food Sci. Technol.* **84**, 701-709.

- Meeker D. and Hamilton C. (2006). An overview of the rendering industry. Pp. 1-16 in Essentials of Rendering: All About the Animal By-product Industry. D.L. Meeker, Ed. National Renderers Association Inc., Arlington, Virginia.
- Mishra R.K., Zachariah A.K. and Thomas S. (2017). Microscopy Methods in Nanomaterials Characterization. Elsevier, Elsevier, Amsterdam, Netherlands.
- Munawar S.S., Umemura K. and Kawai S. (2007). Characterisation of the morphological, physical, and mechanical properties of seven non-wood plant fibre bundles. *J. Wood Sci.* **53(2)**, 108-113.
- Newsome S.D., Wolf N., Peters J. and Fogel M.L. (2014). Amino acid δ^{13} C analysis shows flexibility in the routing of dietary protein and lipids to the tissue of an omnivore. *Integr. Comp. Biol.* **54**(5), 890-902.
- Pan L., Ma X.K., Wang H.L., Xu X., Zeng Z.K., Tian Q.Y., Zhao P.F., Zhang S., Yang Z.Y. and Piao X.S. (2016). Enzymatic feather meal as an alternative animal protein source in diets for nursery pigs. *Anim. Feed Sci. Technol.* **212**, 112-121.
- Papadopoulos M.C., E-Boushy A.R., Roodbeen A.E. and Ketelaars E.H. (1986). Effect of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal. *Anim. Feed Sci. Technol.* 14, 279-290.
- Prado J.P.S., Cavalheiro J.M.O., Silva J.A., Cavalheiro T.B. and Silva F.V.G. (2016). Amino acid profile and percent composition of meal and feeds used in shrimp farming. *Gaia Sci.* **10(4)**, 347-360.
- Pruekvimolphan S. and Grummer R.R. (2011). Lactation responses to sulfur-containing amino acids from feather meal or rumen-protected methionine. J. Dairy Sci. 84(11), 2515-2522.
- Queiroga A.C., Pintado M.E. and Malcata F.X. (2012). Potential use of wool associated *Bacillus* species for biodegradation of keratinous materials. *Int. Biodeterior. Biodegrad.* **70**, 60-65.
- Rahayu S. and Bata M. (2014). Quality of chicken feather processed in different conditions. *Anim. Prod.* **16(3)**, 170-175.
- Ramakrishnan N., Sharma S., Gupta A. and Alashwal B.Y. (2018). Keratin based bioplastic film from chicken feathers and its characterization. *Int. J. Biol. Macromol.* **111**, 352-358.
- Reddy R.M., Reddy S.K., Chouhan R.Y., Bee H. and Reddy G. (2017). Effective feather degradation and keratinase production by *Bacillus pumilus* GRK for its application as bio-detergent additive. *Bioresour. Technol.* 243, 254-263.
- Riffel A. and Brandelli A. (2002). Isolation and characterization of a feather degrading bacterium from the poultry processing industry. *J. Ind. Microbiol. Biotechnol.* **29**, 255-258.
- Riffel A. and Brandelli A. (2006). Keratinolytic bacteria isolated from feather waste. *Brazilian J. Microbiol.* **37(3)**, 395-399.
- Said M.I, Triatmojo S., Erwanto Y. and Fudholi A. (2011). Gelatin properties of goat skin produced by calcium hydroxide as curing material. *Media peternakan. J. Anim. Sci. Technol.* 34(3), 184-189.
- Said M.I., Abustam E., Wahab A.W., Sartini and Hifizah A. (2015). Chemical characteristics of collagen extract from scapula of Bali cattle (Os scapula) produced using different extractant. *Pakistan J. Nutr.* **14(3)**, 174-179.

- Said M.I., Abustam E., Pakiding W., Mide M.Z. and Umar A. (2018). Characteristics of broiler feather protein concentrate (BPFC) prepared under different production processes. *Int. J. Poult. Sci.* **17(10)**, 507-514.
- Sarmwatanakul A. and Bamrongtum B. (2000). Aquarium Fish Nutrition. Ornament Fish Research and Public Aquarium. Bangkok, Thailand.
- Steel R.G.D. and Torrie J.H. (1991). Principle and Procedure of Statistics. McGraw-Hill Book Company, New York.
- Tesfaye T., Sithole B., Ramjugernath D. and Chunilall V. (2017a). Valorisation of chicken feathers: Characterisation of chemical properties. J. Clean. Prod. 149, 349-365.
- Tesfaye T., Sithole B., Ramjugernath D. and Chunilall V. (2017b). Valorisation of chicken feathers: Characterisation of chemical properties. *Waste Manage*. **68**, 626-635.
- Van-Heughten E. and Van-Kempen T.A. (2002). Growth performance, carcass characteristics, nutrient digestibility and fecal odorous compounds in growing-finishing pigs fed diets containing hydrolyzed feather meal. J. Anim. Sci. 80, 171-8.

- Wandita T.G., Joshi N., Nam I.S., Yang S.H., Park H.S. and Hwang S.G. (2018). Supplementation of purified amino acid derived from animal blood on immune response and growth performance of broiler chicken. *Trop. Anim. Sci. J.* 41(2), 108-113.
- Zhang Y., Yang R. and Zhao W. (2014a). Improving digestibility of feather meal by steam flash explosion. J. Agric. Food Chem. 62, 2745-2751.
- Zhang Z., Xu L., Liu W., Yang Y., Du Z. and Zhou Z. (2014b). Effects of partially replacing dietary soybean meal or cottonseed meal with completely hydrolyzed feather meal (defatted rice bran as the carrier) on production, cytokines, adhesive gut bacteria, and disease resistance in hybrid tilapia (*Oreochromis niloticus* \mathcal{Q} ×*Oreochromis aureus* \mathcal{J}). *Fish Shell. Immunol.* **41**, 517-525.