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Research Article
Characteristics of Broiler Feather Protein Concentrate Prepared Under Different Production Processes

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Abstract

Background and Objective: Broiler feather waste (BFW) resulting from poultry slaughter houses (PSHs) is considered a serious solid waste problem. To deal with this problem, the waste can be hydrolyzed to create broiler feather protein concentrate (BFPC), which has the potential to serve as feed material for non-ruminant animals. This study aimed to evaluate the in vitro protein digestibility (In-VtPD), yield and chemical composition (CC) of Broiler feather protein concentrate (BFPC) prepared under different conditions. **Methodology:** This study involved four types of processes and used a combination of chemical and physical processes. The chemical processes used 1 M HCl (10 and 20% (v/v) and 1 M NaOH [10 and 20% (w/v)], while the physical processes used a high pressure heating system. The treatment consisted of four conditions including P_0 (control /without hydrolysis), P_1 [(20% (w/v) 1 M NaOH+autoclaving (21 Psi)], P_2 [(20% (v/v) 1 M HCl+autoclaving (21 Psi)] and P_3 [10% (v/v) 1 M NaOH+10% (w/v) 1 M HCl+autoclaving (21 Psi)]. A completely randomized design (CRD) with 5 replications was used and the data obtained were evaluated using analysis of variance (ANOVA). **Results:** The results showed that different hydrolysis conditions had significant effects ($p<0.01$) on the In-VtPD, yield, Cc and pH of BFPC but had no significant effect ($p>0.05$) on the protein content. The hydrolysis process using 20% (w/v) 1 M NaOH+autoclaving (21 Psi) (P1) significantly increased the In-VtPD of BFPC (59.53 ± 0.7%). The combination of chemical and physical processes was able to hydrolyze the components of keratin protein in feather waste. The resulting yield was not different from that of the other treatments (P2 and P3). The hydrolysis process uses a chemical and physical combination to maintain a stable protein composition but the digestibility level of the protein increases. The amino acid profiles of BFPC before and after the hydrolysis process did not differ significantly. The amino acid component in BFPC is dominated by glutamic acid. In the composition of the constituent substances BFPC is dominated by K_2O and CaO compounds. **Conclusion:** The hydrolysis process that uses a combination of chemical [20% (w/v) 1 M NaOH] and physical processes (autoclaving) (P1) is the best hydrolysis treatment. Therefore, this treatment is recommended as the best process to produce feather flour concentrate.

Key words: Broiler feather waste, digestibility, hydrolysis, feather meal, animal feed

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Broiler feather waste (BFW) resulting from poultry slaughterhouses (PSHs) has been regarded as a problematic solid waste. To reduce waste production, an effort is needed to utilize the waste. One of the efforts made is processing into animal feed. This study is very important as one of the solutions in reducing the production of livestock waste, especially feather. Declining waste production will improve environmental quality and improve human health status. Broiler feather are rich in keratin protein[1-2], which is an abundant polymer in the world[3-5]. The waste rapidly decays, leading to undesirable effects on the environment[6]. At present, livestock by-products have been widely used to meet human needs. Livestock by-products that have been used such as skin, bones and feathers[7-9]. The feather waste contains various components that can accelerate the process of decomposition, such as feces, blood, fat and soil. Each feather is histologically composed primarily of keratin proteins. Keratin structure has activity in the process of digestion and this structure can be degraded by the keratinase enzyme[10]. Increasing the uses of BFW could enable the presence of BFW in the environment to be reduced. Therefore, this current study aimed to reduce BFW through a process and also use of BFW as an alternative protein source for livestock.

The use of chicken feather waste for the production of a protein source has been widely studied. However, this waste has some disadvantageous properties such as low digestibility, which is associated with a large number of disulfide bonds (S-S) present in keratin. The major component of feathers is keratin, which contains a high sulfur content in its amino acids[11]. This type of keratin is different from the keratin comprising wool, nails and horns. Feather waste can also be an alternative source of protein especially for monogastric animals[12], as well as a source of nutrients for plants[13].

Broiler feather protein concentrate (BFPC) with higher digestibility could be prepared from BFW. The BFPC is considered as a good protein source in feed formulation and produced through some physical and chemical treatments. The physical treatment including the provision of pressure in a certain time provides different characteristics[14]. The BFPC product was identified as capable of improving the digestibility of protein components in feathers that can be utilized as feed ingredients of alternative protein sources. BFPC is a processed product produced through several processes to improve the digestibility of both ruminants and non-ruminants[15]. This study aimed to evaluate the *In vitro* protein digestibility (In-VtPD), yield and chemical composition (CC) of BFPC produced under different conditions.

MATERIALS AND METHODS

Preparation of broiler feather samples: BFW was obtained from PSHs in Daya district, Makassar City, South Sulawesi, Indonesia. BFW samples (1000 g) were prepared for all treatments. The feathers were washed and dried at 60°C for 15 h. For hydrolysis treatment, 1 M HCl solution [10 and 20% (v/v)] (Merck, USA) and 1 M NaOH [10 and 20% (v/v)] were used.

Preparation and analysis of BFPC: The feather sample (50 g) was soaked in the hydrolysis solution (P_1, P_2, or P_3) for 4 h, then heated autoclave (Olla Autoclave Electrica All American 75×) at a pressure of 21 Psi (1.24 atm) for 10 h. The hydrolyzed feather was dried using oven (Memmert) at 60°C for 24 h and ground using electrical blender (Sanyo) to produce BFPC powder. For *In vitro* protein digestibility (In-VtPD), the procedure was performed according to a previous study which imitated the digestive function of livestock[16]. In this study, In-VtPD of BFPC was performed using pepsin method. BFPC sample (1 g) was placed in a porcelain cup to determine the dry matter and the organic material. Each cup was added 25 mL of pepsin acid solution (Merck), incubated in a shaking incubator for 72 h at 50°C. Filtration was performed using crucible no 2. The crucible was dried overnight at 103°C and furnace at 520°C for 3 h. In-VtPD value was calculated by the following equation:

$$\text{In-VtPD} = 100\%\text{-DII} (\%)$$

$$\text{Dry ingested ingredients (DII\%)} = \text{B-C/A}\times 100\%$$

where, A is sample weight, B is crucible weight after oven and C is empty crucible weight. The yield was calculated using a following equation[17]:

$$\text{Yield} = \text{A/B}\times 100\%$$

where, A is weight of BFPC (g), B is weight of BFW (g). For chemical composition, proximate analysis including water content Wc (%), fat content Fac (%), fiber content Fic (%) and ash content Ac (%) was performed[18]. pH meter was used to determine pH[18].

Experimental design and statistical analysis: A completely randomized design (CRD) consisting of 4 treatments and 5 replications was used. The treatments included P_0 (control/without hydrolysis), P_1 [20% (w/v) 1 M NaOH+autoclaving (21 Psi)], P_2 [20% (v/v) 1 M HCl+autoclaving (21 Psi)] and P_3 [10% (v/v) 1 M NaOH+10%

(w/v) 1 M HCl+autoclaving (21 Psi)]. Data were analyzed using One Way analysis of variance (ANOVA) using the SPSS (Version 15.0) statistical program. Significant difference between means were determine using Duncan's multiple range test (DMRT) at 5%[19].

RESULTS AND DISCUSSION

***In vitro* protein digestibility (In-VtPD):** Digestibility represents the number of substances that can be absorbed by the gastrointestinal tract. The digestibility rate is strongly related to the quality of the feed ingredients. Fig. 1(a) displays the In-VtPD of BFPC prepared under various treatments. The results showed that the hydrolysis conditions significantly affected ($p<0.01$) the In-VtPD of BFPC. The P_1 treatment resulted in the highest In-VtPD (59.53±0.7%), which was much higher than that of P_0 (21.38±0.54%), P_2 (42.81±1.01%) and P_3 (45.92±0.39%). Physical treatment using autoclaving combined with NaOH can improve the In-VtPD value of BFPC compared to the control (19.20%, n = 3). The soaking process of BFW in NaOH solution enables to weaken chemical bonds in the feather. Thus, the use of heating process can improve performance of BFPC digestibility. Hydrolysis is considered as the technique to degrade sulfur bonds in cystine. In-VtPD of BFW under treatment of hydrolysis using NaOH and heating process was 59.53±0.57% (n = 3), which was lower than that of using the enzymatic hydrolysis (68-70%)[20].

Yield: Figure 1(b) presents the yield of BFPC prepared under various conditions. The results showed that the application of different processes had significant effects ($p<0.01$) on the yield of BFPC. Hydrolysis treatment using NaOH, HCl or their combination tended to reduce yield of BFPC. The yield of BFPC under the treatment of P_0 (90.8±1.23%) was higher than that of P_1 (83.7±1.94%), P_2 (84.5±0.74%) and P_3 (80.3±1.13%). Decreased yield indicates that the production process is less efficient[21].

Water content: The water content (Wc) of a food product determines its quality and storability. Therefore, the determination of Wc is very important and aids in the understanding of the proper process and distribution methods. Fig. 2(a) displays the Wc of BFPC prepared under different conditions. The results showed that the treatments strongly affected ($p<0.01$%) the Wc. The P_2 treatment resulted in the lowest Wc (4.11±0.31%) when compared with P_1 (5.83±0.53%), P_3 (4.67±0.58%) and P_0 (5.21±0.56%). This finding may be due to the presence of the OH^- group in NaOH used as the hydrolyzing agent. When NaOH is dissolved in water, exothermic reactions occur that releases heat into the air. The accurate determination of Wc in animal feed materials is very important for the feed industry. Most of the analytical methods used to determine the clear Wc of feed ingredients are empirical. Such methods involve estimating the amount of water evaporation and weight lost during the drying process (oven drying method)[22].

Protein content: The results indicated that different conditions had no significant effects on the protein content (PC) of BFPC [Fig. 2(b)]. This result indicates that the hydrolysis process had no impact on changes in the protein content of BFPC. This result also indicates that the hydrolysis process using a combination of chemical and physical processes was effective in BFPC production. The PC value of BFPC is similar to that of hair meal from Bali cattle[23]. Feather meal is an excellent source of amino acids with a predominant supply of cysteine[24].

The PC of BFPC ranged from 89.07±6.04-91.77±3.69% (P_1, P_2 and P_3, respectively; n = 5), which was not different from

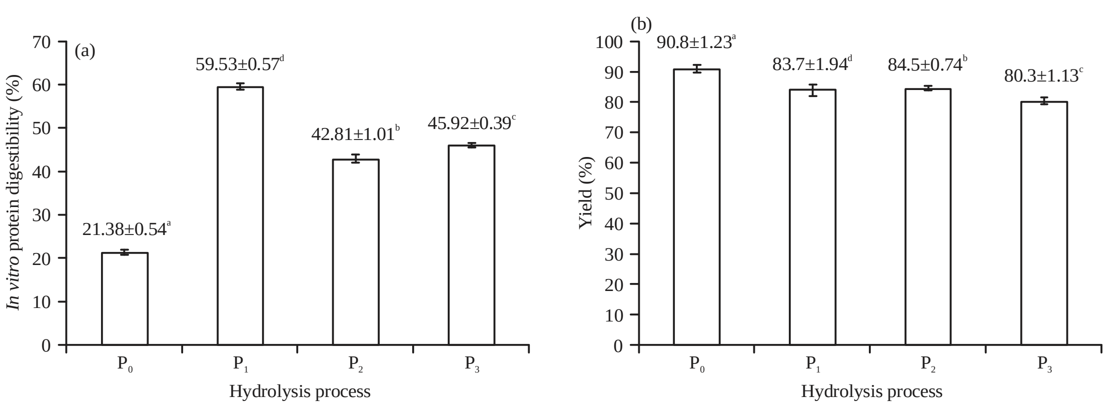

Fig. 1(a-b): *In vitro* protein digestibility (In-VtPD) and Yield of BFPC prepared under different hydrolysis processes

P_0 (control/without hydrolysis), P_1 (20% (w/v) 1 M NaOH+autoclaving (21 Psi), P_2 (20% (v/v) 1 M HCl+autoclaving (21 Psi) and P_3 (10% (v/v) 1 M NaOH+10% (w/v) 1 M HCl+autoclaving (21 Psi)). Different superscripts in each treatment showed very significant differences ($p<0.01$)

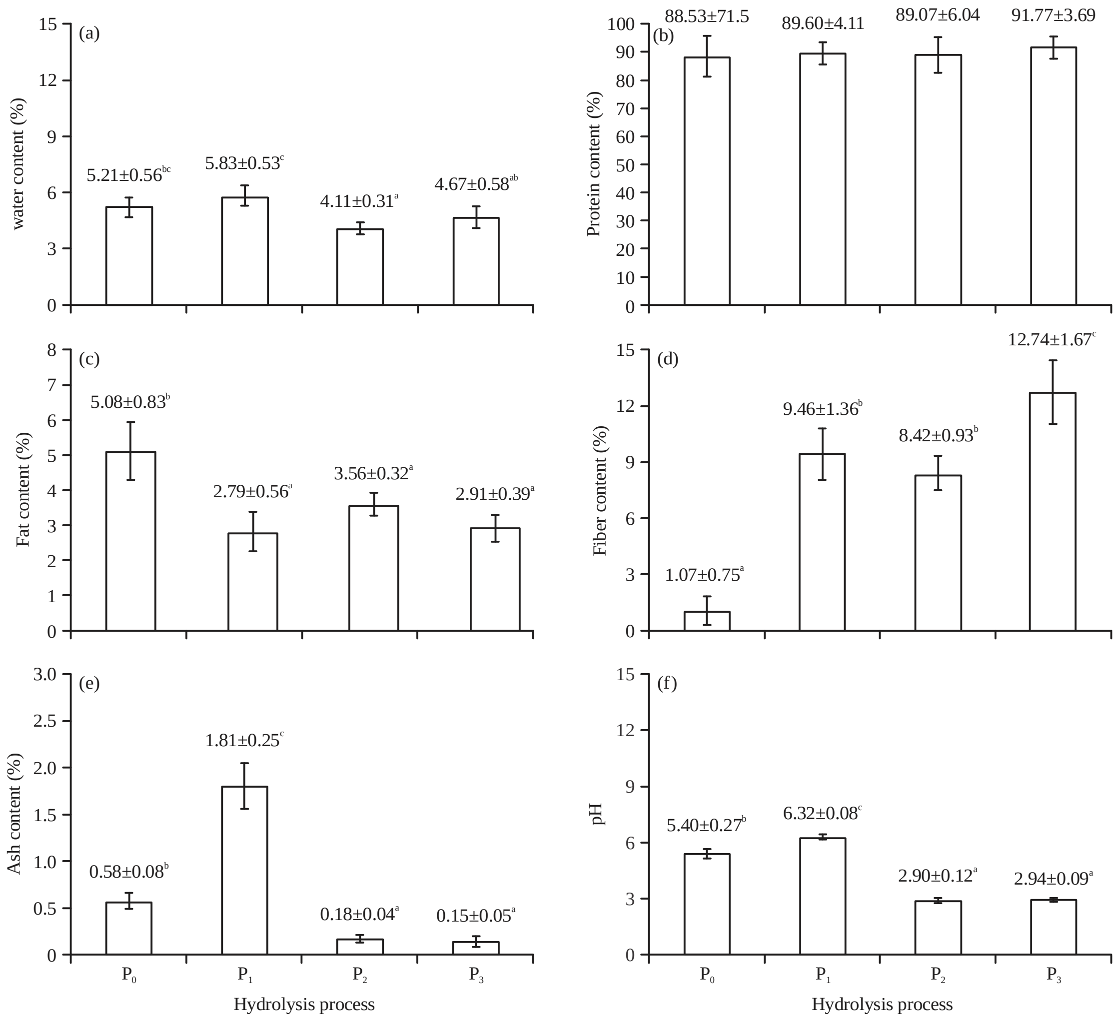

Fig. 2(a-f): Water, protein, fat, fiber, ash and pH content of BFPC prepared under different hydrolysis processes
P_0 (control/without hydrolysis), P_1 [20% (w/v) 1 M NaOH+autoclaving (21 Psi)], P_2 [20% (v/v) 1 M HCl+autoclaving (21 Psi)] and P_3 [10% (v/v) 1 M NaOH+10% (w/v) 1 M HCl+autoclaving (21 Psi)]. Different superscripts in each treatment showed very significant differences (p<0.01)

that in the control (88.53±7.15%)[25]. Feathers from livestock have potential as a source of protein in livestock feed[26]. Feather meal added with hydrogen peroxide (5% w/w) can increase the degradability of in situ proteins but not change the dry matter intake, average weight, feed efficiency and improved carcass characteristics[27]. The poultry not only requires amount of protein but also requires the intake of carbohydrates to meet energy needs for the body[28]. The use of NaOH in the hydrolysis process could affect the feather protein structure. NaOH is a type of alkaline solution enabling to alter the salt bridge on the protein structure. The denaturation process can occur due to the change of ions in the protein structure of the feather.

Fat content: Figure 2(c) indicates that different hydrolysis conditions strongly affected (p<0.01) the fat content (FC) of BFPC. The application of P_1, P_2 and P_3 significantly reduced the FC by 2.79±0.56, 3.56±0.32 and 2.91±0.39%, respectively. These FC values were lower than that in P_0 (5.08±0.83%). The process of fat dissolution was caused by the high temperature heating treatments and pressure using an autoclave. The average FC of BFPC was higher than that of feather meal in another study[29]. Fiber in acid detergent fiber (ADF) and neutral detergent fiber (NDF) is regarded as difficult to digest but it is directly involved in the nutrient digestion process[30]. Keratin is a fiber protein that forms hair, feathers and nails and is rich in cysteine and cystine. Feather meal from poultry can be used as a protein source feed of 8% in pigs without affecting the growth performance or carcass characteristics[26]. The protein content of poultry meal reached 63.46% which was used as pig feed[31]. The NaOH compound is a chemical compound having alkaline properties resulting in a higher pH. Some protease can hydrolyze the feather waste at pH 5.5-7.0[32].

Fiber content: Figure 2(d) shows that fiber content of BFPC is significantly affected by different hydrolysis conditions ($p<0.01$). The treatments (P_1, P_2, P_3) resulted in higher fiber content ranging from 8.42±0.93-12.74%±1.67) than P_0 (1.07%±0.75). Similarly, this finding was also in accordance with a result observed in Bali cattle hair (0.002 - 0.01%)[23]. Fiber compound in BFPC was regarded soluble, thus it was applicable for a feed ingredient. Fiber in ADF and NDF form is difficult to digest. ADF and NDF were directly involved in the nutrient digestion process[30].

Ash content: Figure 2(e) demonstrates that different hydrolysis conditions significantly affected ($p<0.01$) ash content of BFPC, ranging from 0.15±0.05-1.81±0.25%. P_1 resulted in the highest ash content (1.81±0.25%). The results also indicated that average ash content of BFPC was lower than that of the concentrate of cattle hair[23]. The hydrolysis using NaOH could change minerals in BFPC into carbon-carbon bonds in the arrangement of amino acids cysteine to form keratin.

pH value: Figure 2(f) shows that different hydrolysis conditions significantly affect pH of BFPC ($p<0.01$). P_1 resulted in the highest pH value (6.32±0.08) compared to P_0 (5.40±0.27), P_2 (2.90±0.12) and P_3 (2.94±0.09). This presumably reflects the use of NaOH as a hydrolyzing agent.

Amino acid profile: Amino acids are protein-forming molecules. The main protein that makes up feathers in poultry is keratin. The amino acid test results showed that the amino acid profiles before and after hydrolysis were not significantly different (Fig. 3). This result shows that the hydrolysis process did not substantially change the amino acid composition of the BFPC. This finding means that the level of damage to amino acid components during the hydrolysis process can be minimized. Based on Fig. 3, it can be seen that the amino acid type of glutamic acid has the highest composition. Glutamic acid in the amino acid structure has the composition of each (14.87 and 14.46%) for BFPC without hydrolysis and with hydrolysis. Next is leucine amino acids (10.68 and 9.77%). The results of these amino acid profiles did not differ significantly with the results of previous studies. The highest levels of amino acids were glutamic acid (14.46%) and leucine (9.77%)[33]. Amino acid glutamic acid is one type of amino acid that can give a savory flavor to a product. This property strongly supports this product as an alternative to animal feed ingredients.

Composite compounds of BFPC: The test results on the components of the compounds that make up the BFPC show that, K_2O and CaO compounds are the most dominant components in the BFPC structure (Fig. 4 and Table 1). The oxide component formed comes from the material used in the hydrolysis process. Besides that, the SO_3 compound was also found in the BFPC but the value was very small. The components that make up SO_3 are sulfur (S) elements which are the main elements that make up keratin proteins. Sulfur is a major component in keratin protein formation (>90%). This component is rich in hydrophobic residues and cysteine amino acids. This component has a cross link in the form of disulfide bonds[34]. Disulfide bond is a determinant of strength in feathers[35-37].

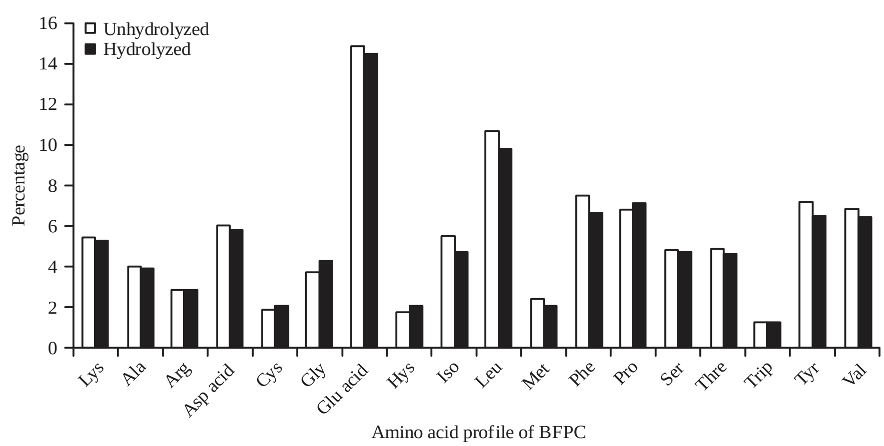

Fig. 3: Amino acid profile of BFPC un hydrolyzed and hydrolyzed process

Lys: Lysin, Ala: Alanine, Arg: Arginine, Asp Acid: Aspartate Acid, Cys: Cysteine, Gly: Glycine, Glu Acid: Glutamic Acid, Hys: Histidine, Iso: Isoleucine, Leu: Leucine, Met: Methionine, Phe: Phenylalanine, Pro: Proline, Ser: Serine, Thre: Threonine, Trip: Tryptophan, Tyr: Tyrosine and Val: Valine

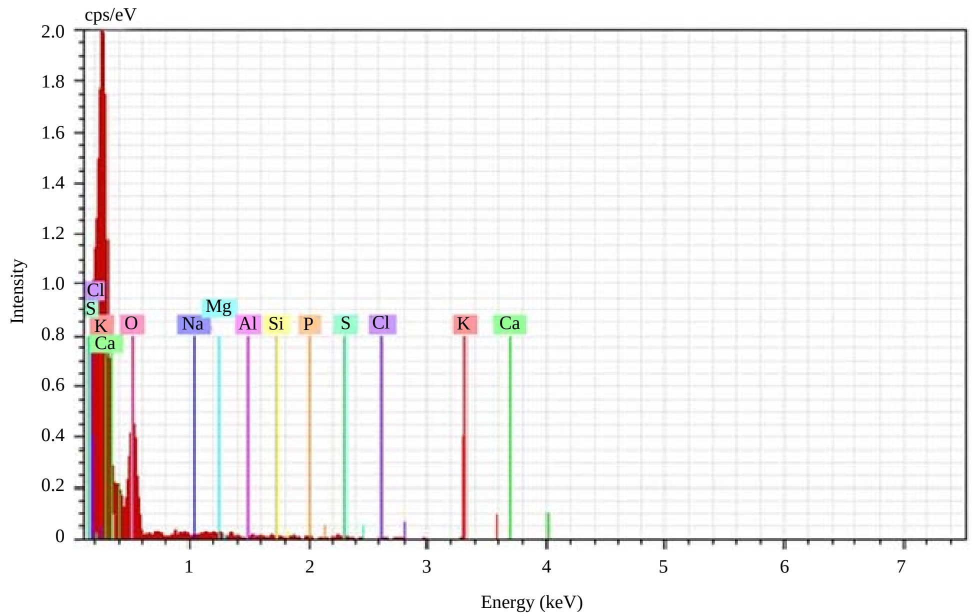

Fig. 4: Appearance of dispersion results spectrogram showing the compounds that compose from BFPC
Cl: Chlorine, K: Potassium, Ca: Calcium, O: Oxygen, Na: Sodium, Mg: Magnesium, Al: Aluminum, Si: Silicon, P: Phosphorus and S: Sulfur

Table 1: Composite compounds of BFPC after hydrolysis process using energy-dispersive x-ray spectroscopy (EDS) (VEGA 3 TESCAN)

Elements	Unn. C (wt.%)	Norm. C (wt.%)	C. Atom (at.%)	C. Compound norm	Comp. C (wt.%)	Error (3 Sigma)
Potassium	86.47	70.44	55.47	K_2O	84.85	43.91
Calcium	12.92	10.52	8.08	CaO	14.72	10.35
Sodium	0.03	0.03	0.04	Na_2O	0.04	0.08
Magnesium	0.04	0.03	0.04	MgO	0.05	0.08
Aluminium	0.03	0.02	0.03	Al_2O_3	0.04	0.08
Silicon	0.04	0.03	0.03	SiO_2	0.06	0.08
Phosphorus	0.04	0.03	0.03	P_2O_5	0.07	0.08
Sulfur	0.07	0.05	0.05	SO_3	0.13	0.08
Chlorine	0.04	0.03	0.03		0.03	0.08
Oxygen	23.10	18.82	36.21		0.00	16.79
Total	122.77	100.00	100.00			

The hydrolysis process was needed to improve the quality and digestibility of feather waste. Combination of chemical and physical process can be applied to hydrolyze broiler feather waste (BFW) into broiler feather protein concentrate (BFPC) as an alternative animal feed ingredient. The use of 20% (w/v) 1 M (NaOH) concentration is recommended for application in the process of hydrolysis of feather waste.

CONCLUSION

The application of different hydrolysis processes results in different attributes of BFPC. In this study, different hydrolysis conditions significantly affected the In-VtPD, yield, water content, fat content, fiber content, ash content and pH but not the protein content of BFPC. The hydrolysis process using 20% (w/v) 1 M NaOH (P_1) demonstrated optimum protein digestibility with more varied chemical compositions.

SIGNIFICANCE STATEMENT

This study discover that the process of hydrolysis using chemical compounds (NaOH) combined with high-pressure heating (21 Psi) is very effective in increasing digestibility of feather meal from broilers. This study is very helpful for the feed industry as a source of scientific information in the production process of feather concentrate by utilizing poultry slaughterhouse waste. The very low digestibility of broiler feather waste due to keratin disulfide bonds can be increased by a combination of hydrolysis processes. This combination of processes can also maintain protein and amino acid levels as in fresh conditions.

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