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Treatments of Chicken Feather Waste

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Abstract – Feather waste is a potential renewable source to recover valuable products because it is being a rich source of keratin proteins and amino acids. It can used to make feather meal, fertilizer and yarn sizing agent. Various treatments have been used to recover the protein from chicken feathers as the keratinous feathers cannot be easily degraded due to its tough structure. This paper reviews the existing treatment methods used to hydrolyze chicken feathers. The treatment methods for feather hydrolysis such as physical, chemical, biological and combined treatments as well as their advantages and challenges are highlighted. The effects of these treatments on feather hydrolysis are complex and vary in regards to the performance of feather hydrolysis and product yielded. Hence, it is important to choose an appropriate treatment method since the type of treatment applied affects the product yielded qualitatively and quantitatively. In addition, the economic assessment and environmental impact of the choice of treatment should be considered also.

Keywords: Chicken feather, Hydrolysis, Keratin, Soluble protein, Treatments

Introduction

Chicken is one of the most popular protein sources for majority of the population. Malaysia has been reported to be one of the highest poultry meat consumption countries in the world (Abdul Ghani, 2014; Elizabeth, 2015). According to United State Department of Agriculture reports (2015), Malaysian broiler meat domestic consumption has increased throughout 10 years. In 2015, it has reached approximately 1500 MT. Millions tons of chicken feathers are generated every year as by-product in the world- wide poultry industries as the consumption of poultry meat increases (Ali *et al.*, 2011). Disposal of the feather waste without proper management and treatment results in landscape degradation and local disturbance such as odour, flies and rodents near the poultry farms, water and soil pollution (Gerber *et al.*, 2007). Conventionally, feather waste is disposed through landfilling or combustion. However, these methods may bring adverse effects such as high operating costs and high energy consumption which result in loss of natural resources and extreme environmental implications (Mehta *et al.*, 2014). Alternative way to manage the feather wastes is by recycling or converting these wastes into useful products. For example, feathers can be recycled to make feather meal. This is because feather is made up of more than 90% of protein, namely keratin and it contains high levels of cysteine, glycine, arginine, and phenylalanine (Kumar *et al.*, 2012). Feathers can also be used to make fertilizers as feathers contain about 15% nitrogen. Hence, they can be utilized as slow nitrogen releasing fertilizers in the greenhouse and nursery industries (Saber *et al.*, 2010). Feathers can be used to make yarn sizing agent which is a protective layer added on to the surface of textile yarns to improve the weaving performance as well tensile strength and abrasion resistance of the yarns (Yang and Reddy, 2013). Reddy *et al.* (2014) reported that the sizing agent made by feather keratin can replace polyvinyl alcohol (PVA) on polyester/cotton blends and polyester. Nowadays, many treatments have been developed to treat feather waste in order to add value to them which include production of amino acids, production of more digestible protein hydrolysate and/or obtaining a

protein hydrolysate which can be easily modified to another valuable product. This paper aims to overview the type of chicken feather treatments available as well as their pros and cons in recovering of valuable products from chicken feather waste.

Physical and Chemical Characteristics of Chicken Feather

Chicken feathers play important roles in insulatory, locomotory and structural (Onifade *et al*., 1998). Chicken feathers constitute 10% of total chicken weight and contain approximately 91% protein (keratin), 1% lipids, and 8% water (Thyagarajan *et al*., 2013). According to Débora (2013), chicken feathers have a porous internal structure, crystalline segments and hollow and cylindrical microstructure which is in nano- and micro- scale. They also have a density as low as 0.8 g/cm^3 (Débora, 2013). In addition, the amino acids of keratin interact between themselves, through hydrogen and disulphide (-S-S-) covalent bonds that give the material mechanical resistance and impermeability to water, as well as insoluble in organic solvents. They are flexible and non-abrasive. The lipids, which have fatty acid groups in their structure, may confer the keratin low solubility in water and thermal insulation. The presence of barbs and barbules in the main structure (rachis) offer a unique structural interaction with other fibers and better adherence with various types of resins (Reddy and Yang, 2007; Hernandez and Santos, 2012). In addition, chicken feathers are cheap and available abundantly as renewable source for protein fibers (Reddy and Yang, 2007).

Keratin

Keratin is a structural and insoluble supercoiled protein which is mechanically resistant and recalcitrant to degradation by common proteolytic enzymes such as pepsin, trypsin and papain (Brandelli, 2008). This is because keratin has tightly packed molecular structure which is stabilized by cross-linking of disulphide bridges, hydrogen bonds or hydrophobic interactions (Brandelli, 2008). Keratins comprises a tight packing of supercoiled long polypeptide chains with a molecular weight of approximately 10 kDa (Schmidt and Barone, 2004; Coward-Kelly *et al*., 2006).

Keratins can be grouped into two types which are α-keratin and also β-keratin (Voet and Voet, 1995). The α-keratin is mainly found in the mammal tissues such as claw, horn, hair and nail. This keratin is packed in the α-helix configuration in its polypeptide. In contrast, ß-keratin's structure is rich with ß-plate sheets. This keratin is the major component of bird's flaw, feather and beak. It can also be found in reptile's shell, claw, and scale (Meyers *et al*., 2008). ß-keratin has higher rigidity compared to α-keratin due to its higher cystine content and thus greater present of disulphide (S-S) bonds which link adjacent keratin protein (Voet and Voet, 1995; Saravanan, 2012).

The secondary structure of feather has been discussed many years ago. Scientists predicted the secondary structure of feather based on its primary structure (Hernández and Santos, 2012). A summary of the reported keratin types in chicken feather is shown in Table 1.

Table 1: Keratin secondary structure found in chicken feather.

Feather parts	Proposed Secondary structure	References
Barb	α-keratin type	Reddy and Yang, 2007
	Slightly more α-helix over sheet structure	Saravanan, 2012
Rachis	78% of β-sheet, 18% of helical from twisted sheet and remaining of turn and other arrangement	Schor and Krimm, 1961
Quill	Much more sheet than α-helix	Saravanan, 2012
Non-specified	9.38% α-helix, 47.19% β-sheet, 32.25% β-turn and 11.18% random	Sun *et al*., 2009
	41% α-helix, 38% β-sheet and 21% random	Fraser *et al*., 1971

Amino acid composition

The amino acid composition of chicken feathers is presented in Table 2. The content of amino acid in the feathers depends on the breed, food and environment (Schmidt, 1998; Hernández *et al*., 2005). Besides, Fisher *et al*. (1980) reported that methionine, threonine, isoleucine and valine contents of feather changed with the bird age. As the bird age increased, methionine content decreased while threonine, isoleucine and valine contents increased. Feathers generally have high cysteine content along with high concentrations of serine, proline, and acidic amino acids, they are deficient in some essential amino acids, like methionine and histidine (Forgács, 2012). Keratin protein consists both hydrophilic and hydrophobic amino acids, but 41% of them are hydrophilic (Chinta *et al*., 2013). Hence, the chicken feathers possess both hydrophobic and hygroscopic character. Serine is the most abundant amino acid and each of the serine residue consists of -OH group which can help chicken feathers to absorb moisture from the air. Chicken feather fibers and quill have similar content of moisture which was around 6% (Saravanan, 2012). Feather also consists of high content of cystine which has -SH groups and leads the formation of disulphide bonds. The high content of cystine makes the feather keratin stable by forming network structure by joining adjacent polypeptides by disulphide cross-links (Saravanan, 2012).

Table 2: Main amino acids present in chicken feather and their concentrations.

Amino acid composition (%)		**References**			
		(Gupta *et al*., 2011)	(Saravanan, 2012)	(Forgacs, 2012)	
Essential	Histidine	0.02	-	0.23	0.28
	Isoleucine	4.93	3.32	3.94	2.08
	Leucine	7.48	2.62	5.69	5.60
	Lysine	0.57	-	1.54	2.09
	Methionine	0.03	1.02	0.71	0.28
	Phenylalanine	4.11	0.86	3.46	1.99
	Threonine	4.11	4.00	3.45	7.58
	Valine	7.24	1.61	5.30	3.70
Non-essential	Alanine	3.66	3.44	2.88	2.56
	Arginine	6.57	4.30	6.76	6.07
	Asparagine	-	4.00	-	-
	Aspartic acid	4.76	6.00	4.18	4.55
	Cystine	2.11	8.85	-	-
	Cysteine	-	-	6.58	5.78
	Glutamine	-	7.62	-	-
	Glutamic acid	9.18	-	8.22	10.80
	Glycine	7.57	-	5.18	0.47
	Proline	1.01	12.00	7.39	4.00
	Serine	13.57	16.00	8.73	6.92
	Tyrosine	1.85	1.00	-	-

Chicken Feather Hydrolysis Treatments

Physical treatment

Generally, the physical treatment can be divided into mechanical and thermal treatments. Mechanical treatment for chicken feathers involves milling and grinding to reduce the particle size of the substrate. This results in a larger specific surface area of the feathers. The size reduction of chicken feather may ease the feather hydrolysis but it is best to combine with other treatments. However, these methods need high energy consumption (Nasir and Tinia, 2015). Hence, it is expensive and possibly not favored in a full-scale process. Ultrasound is another mechanical method that can be used to disintegrate and destruct the feather biomass. The efficiency of the ultrasound treatment is affected by

the frequency, the time, the energy level, and the characteristics of the substrate (Anna, 2013). However, it is worth noting that the yield produced by this method alone is low. It is better to combine with other treatments. Eslahi *et al*. (2014) reported that the combination of enzyme hydrolysis and ultrasonic method has potential in production of nanoparticles from chicken feather. This method has advantages over other methods such as mechanical milling due to the reduction in energy consumption, cost and environmental pollution. Moreover, it is also able to conserve main characteristics of creatine existing in feather without changing its properties or microstructure. Thermal treatments such as autoclave, pressure cooking and steam heating, using heat energy to change the structure of the protein in feather by breaking the bonds. The solubility of the feather is thus improved and hence can enhance the performance of feather hydrolysis (Hii *et al*., 2014). However, these methods consumed high energy. Method that employ irradiation or high-energy electronic beams such as microwave energy, is also one of the physical treatments that can disrupt the cell structure and increase the accessible surface area of the chicken feather. Microwave irradiation works through 'in-core' volumetric heating which results in rapid increase in temperature which penetrates into the interior of the substrate (Jingyang *et al*., 2015). However, structural destruction may occur due to the formation of intensive vapour in the material (Kratchanova *et al*., 2004). This method is fast and easily handle but it is not efficient in feather hydrolysis.

Chemical treatment

The chemical methods for chicken feather hydrolysis are mostly employing strong acid and alkali to achieve chemical cleavage of the disulphide bonds and hence to extract soluble keratins (Wei *et al*., 2012). Hydrolysis with strong acid or base is nonspecific (Mohammed *et al*., 2009). Theoretically, these chemicals attacked all peptide bonds and complete broke them down into low chain length peptides and amino acids (Mohammed *et al*., 2009). One of the advantages of acid hydrolysis as compared with base hydrolysis is that acid will not destroy the optical activity of the amino acids (Haurowitz, 1955). However, acid hydrolysis destroys tryptophan and partially destroys cystine, serine, and threonine. Moreover, asparagine and glutamine are converted to their acidic form (Haurowitz, 1955). Study of Stiborova *et al*. (2016) showed that approximately $85.9 \pm 0.5\%$ of chicken feathers were hydrolyzed by 0.6% KOH within 24 hours at 70 °C. Around 326.9 ± 45.4 mg L^{-1} of free amino acids were produced and only approximately 12.8% of them were essential. At the same time, the study also showed that the feather hydrolysis efficiency by other acid with similar molarity and same condition was very low. On the other hand, Kim *et al.* (2002) achieved around 78.83 ± 1.85% of feather in 50 mL of 1.0 M NaOH in 24 hours at 37 °C. Alkaline was efficient in feather hydrolysis but the reagent may degrade quality of protein and hence produce poor quality feather meal. Prolonged processing time and high concentrations of alkaline reduced amino acid digestibility of feather meal and amino acid production (Kim *et al*., 2002). Oxidizing agents such as bromine, permanganate and hydrogen oxide were also used in breaking the disulfide bonds and hence protein extraction but its yield was very slow (Forgacs, 2012). In contrast, the reducing agents such as sodium sulfide solution, potassium cyanide and thiglycolate acted very quickly and dissolved keratin only at alkaline reaction (Gupta *et al*., 2011). Gupta *et al*. (2011) used sodium sulphide, potassium cyanide and thioglycolic acid to hydrolyze chicken feather at temperature of 30°C and pH 10-13 for six hours and obtained approximately 53%, 29.6% and 8.8% of the total mass of soluble protein, respectively. However, these chemicals used in chemical methods, such as sulfites, thiols, 1,4-dithiothreitol (DTT) or peroxides, are harmful, often toxic, and difficult to handle (Wei *et al*., 2012).

Biological treatment

Biological treatment can be divided into two categories which are microbial treatment and enzymatic treatment. Microbial keratinolysis treatment employs microorganisms that produce keratinase enzyme to break the rigid and strongly cross-linked keratin structure in feathers (Tiwary and Gupta, 2012). Up to date, many keratinolytic microbes have been isolated. Most of them are fungi, bacteria and actinomyces. Some of the commonly found keratinolytic fungi are known as dermatophytes, which can cause skin diseases. Dermatophytes have been frequently studied for the medical importance. These fungi showed less industrial interest since they are pathogenic to human. However, *Aspergillus niger*, a filamentous fungus, is one of the most important industrial microorganisms that produce a variety of enzymes. Study reported on the keratinase production by *A. niger* strains (Lopes *et al*.,

2011). Kanchana and Mesta (2013) also reported that *Aspergillus* sp. FK1 degraded the chicken feather completely within 96 hours under conditions of pH 7.0 and 30°C. Other than that, many bacteria have been found to produce keratinases. Among them, *Bacillus* spp. appeared as the prominent keratinase producer. Several strains of *Bacillus* such as *Bacillus licheniformis, Bacillus amiloliquefaciens,, Bacillus subtilis, Bacillus pumillus* and *Bacillus cereus* have been identified as keratinolytic bacteria (Adıguzel *et al*., 2009; Cai and Zheng, 2009; Matikevičienė *et al*., 2009; Fakhfakh *et al.*, 2011; Tiwary and Gupta, 2012). *Bacillus cereus* KB043 was able to degrade feather in six days achieving around 78.16 ± 0.4% hydrolysis and producing 1.2 ± 0.02 mg mL^{-1} of soluble protein as well as 20.63 ± 0.4 µg mL^{-1} of cysteine (Nagal and Jain, 2010). Gram negative bacteria such as *Chryseobacteriums* sp., *Burkholderia* sp. and *Pseudomonas* sp. are also the predominant keratinolytic bacteria isolated from the decomposing feather sites (Riffel and Brandelli, 2002). According to Stiborava *et al*. (2016), around 70 to 93% of feather were hydrolyzed by *Pseudomonas* sp. P5 within five days. At the same time, around 301.2 ± 31.2 mg L^{-1} of free amino acid and 6.2 ± 0.2 g L^{-1} soluble peptides were produced. Approximately 48.2% of the produced amino acids were essential amino acids.

Meanwhile, the enzymatic treatment involves degradation of feather by semi-purified or purified extracellular keratin hydrolyzing enzyme (keratinase). Biological treatment is strongly affected by several factors such as medium compositions, pH, temperature, and incubation time (Sivakumar *et al*., 2012). Most of the keratinases acted optimally under alkaline conditions (pH 7.0-9.0) (Gupta and Ramnani, 2006). For instant, purified alkaline β-keratinase from ethyl methyl sulphonate (EMS)-induced mutant *Brevibacillus* sp. strain AS-S10-II could hydrolyze 78-82% of feather within 48 hours at pH 9.0-10.0 (Mukherjee *et al*., 2011). Moreover, the crude enzyme extracted from *Alternaria tenuissima* K2 and *Aspergillus nidulans* K7 could degrade 71% and 76.5% of feather respectively within 24 hours at pH 8.5 (Saber *et al*., 2010). Keratinases with extremely alkalophilic pH characteristic (pH 11.0) was reported too (Gupta and Ramnani, 2006). For example, the keratinase produced by *Bacillus subtilis* performed maximum keratinase activity at 40°C and pH 11 (Mousavi *et al*., 2013). Different kinds of microorganisms produce different types of keratinase at different conditions. Generally, biological treatment could produce keratin hydrolysates containing soluble proteins and reduce loss in essential amino acids (Mokrejs *et al*., 2010). Moreover, the biological treatment is also more environmental friendly compared to others treatments as the conditions applied in biological treament are often milder and there is less by-products produced. On the other hand, there is drawback in using direct bacterial degradation which is the partial consumption of amino acids and peptides by the bacteria (Stiborova *et al*., 2016). Moreover, biological treatment has low reaction rate and low yield, hence limited its application in industrial scale. The higher cost of enzymes themselves, with a long production cycle, has become a limitation of this treatment.

Combined treatments

Several studies showed that combined treatment could improve the feather hydrolysis. Combined treatment can be divided into two categories which are single-stage combined treatment and two-stage combined treatment. Both of them involve the combination of at least two treatments from physical, chemical or biological treatments. Single- stage combined treatment usually involves the combination of physical and chemical treatments which are conducted simultaneously. Hydrothermal and microwave-chemical treatments are examples of single-stage combined treatment. They can hydrolyze feather in a short duration but these combined treatments were costly as they usually consumed high amount of energy and employed expensive equipment (Weidele, 2009). Keratinous feathers are more robust than cellulosic wastes containing carbohydrates (Barone *et al*., 2006). Hydrothermal treatments hydrolyzed feather at high temperature or pressure with addition of diluted acid like hydrochloric acid or alkaline like sodium hydroxide (Guillermo *et al*., 2006). Coward-Kelly *et al.* (2006) reported that around 80% of soluble protein could be recovered by treating feather with 0.1 g of $Ca(OH)_2$ at 150°C within 25 minutes. However, this treatment methods resulted in severe degradation of keratin with reduction of molecular weight and loss of mechanical properties (Barone *et al*., 2006). The high temperature under increased pressure might cause the losses in nutritively significant essential amino acids of keratin hydrolysate (Mokrejs *et al*., 2010). Hydrothermal has been reported to have destroyed some amino acids such as methionine, lysine and tryptophan (Mehta *et al*.,

2014). On the other hand, microwave- chemical hydrolysis involves microwave heating with low chemical concentration to break the hydrogen and sulphide bonds of chicken feathers. This combination of treatments on feathers might produce high content of essential amino acids, such as leucine and valine, as well as non-essential amino acids, like serine, glycine, and tyrosine (Lee *et al*., 2016). It is simple and fast in hydrolyzing chicken feather as well as efficient in breaking the disulphide bonds and solubilizing feather keratins.

Two- stage combined treatment is an extension from the single-stage combined treatment in which feather was hydrolysed in two stages. The initial stage sometime is referred as pretreatment. Pretreatment is a crucial process to alter the structure of the keratinolytic biomass by breaking the disulphide bonds in the structure so that subsequent hydrolysis of keratin can be achieved more rapidly with greater yields. The pretreatment usually applied is physical treatment, chemical treatment or their combination. Then, the pretreated feathers will undergo second stage of treatment which is usually the biological treatment. Mild condition of pretreatment was applied to open up the structure of chicken feather in order to facilitate the attack of enzyme to the feather. For instant, the feathers were initially boiled for 10-20 minutes, and the boiled feathers were subsequently treated with dimeric keratinase from *Bacillus licheniformis* ER-15 (Tiwary and Gupta, 2012). This resulted in more than 90% of feather degradation and production of feather meal that showed 73% *in-vitro* digestibility. Moreover, Forgács (2012) showed that feathers pretreated at 120°C for 10 minutes and then treated with Savinase, a commercial keratinase, led to 94% of feather degradation as well as methane yields of 0.21–0.27 Nm^3 /kg VS. In this case, the feathers were pretreated in order to enhance the biogas production. Laba and Szczekała (2013) reported that the feathers subjected to autoclaving with 10 mM sodium sulfite could also enhance the activity of crude keratinase extracts of *Bacillus cereus* B5esz by 160% and resulted in 86.3% feather hydrolysis as well as the production of amino acids such as leucine, valine, glutamate, glycine, serine and cysteine. Mokrejs *et al.* (2010) also indicated that chicken feathers treated with two-stage alkaline-enzymatic hydrolysis achieved high efficiency under mild reaction condition and was more economically feasible. Basically, two-stage combined treatment can solve the problem of severe protein degradation and loss of significant amino acids that happened in the single-stage combined treatment that usually involved harsh treatment conditions. Moreover, this two-stage combined treatment is also efficient in feather hydrolysis. However, the overall cost for the combined treatment would be higher as compared to other single treatments since combined treatment involves more units of treatment. Table 3 summarizes the pros and cons of all treatments on chicken feather hydrolysis.

Table 3: Pros and cons of various feather hydrolysis treatments.

Treatments	Pros	Cons
Physical treatment	Can reduce particle sizes	Low yield
	No pollution risk	High energy consumption
		Expensive
Chemical treatment	Fast reaction	Loss of essential amino acids
	High yield	Have pollution risk
Biological treatment	Environment friendly	Time consuming
	No destruction of essential amino acid	Low reaction rate
Single- stage combined treatment	Fast	Destruction of essential amino acid
	High yield	
Two- stage combined treatment	Fast	More units of treatment
	High yield	High cost
	No destruction of essential amino acid	

Conclusion

Chicken feather can be an outstanding source of valuable protein with probable developments due to its interesting characteristics. Several chicken feather treatments were compared based on their process descriptions, hydrolysis performance followed by the advantages and disadvantages of each

process. It is important to choose an appropriate treatment to hydrolyze feather and break its tough structure to release amino acids and small peptides. The choice depends very much on the ultimate objective of the biomass treatment and the target final product since different treatment results in different product yielded. Moreover, the choice of treatment method should not only be based on its potential yield but also on other important parameters such as its economic assessment and environmental impact so that the product obtained from the feather hydrolysis can add value to the poultry industry and may be other industries as well.

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