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Chicken feather valorization by thermal alkaline pretreatment followed by enzymatic hydrolysis for protein-rich hydrolysate production

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ABSTRACT

A huge amount of feathers is generated as a waste every year. Feathers can be a protein source if it is treated with an appropriate method. The present study investigates feasibility of autoclave alkaline and microwave alkaline pretreatments to be combined with enzymatic treatment for feather solubilization and protein production. Hydrolysis of chicken feather by autoclave alkaline pretreatment followed by an enzymatic method (AAS) or microwave alkaline pretreatment followed by an enzymatic method (MAS) was optimized by response surface methodology. Various NaOH concentrations for autoclave alkaline pretreatment (0.01–0.1 M) and microwave-alkaline pretreatment (0.01–0.05 M) were applied. The holding time for both pretreatments ranged from 1 to 10 min. The pretreated feathers were subjected to enzymatic hydrolysis using a commercial enzyme prior to analysis of protein content, feather solubilization, functional groups, and elemental composition (carbon, hydrogen, nitrogen and sulfur) of the treated feathers. The results revealed that both autoclave alkaline pretreatment and microwave alkaline pretreatment under optimized conditions of 0.068 M NaOH, 2 min holding time, 105 °C and 450 W, 0.05 M NaOH for 10 min, respectively, enhanced the subsequent Savinase hydrolysis of chicken feathers to achieve more than 80% degradation and more than 70% protein recovery. Fourier transform infrared spectroscopy results showed that both thermal-alkaline pretreatments weakened the structure of the feather. Reduction of carbon, nitrogen, and sulfur occurred in both thermal-alkaline pretreatments of feathers indicating degradation of the feather as well as protein release. Thermal-alkaline pretreatment may be a promising method for enhancing the enzymatic hydrolysis of chicken feathers and for producing a protein-rich hydrolysate.

1. Introduction

The poultry industry is an important source of meat protein. According to a United States Department of Agriculture Report (2017), Malaysian poultry meat consumption has increased throughout the past 11 years. In 2017, it increased by ~2.45% and reached 1632 million tons (MT). The amount of poultry feather wastes generated by the poultry processing plants in Malaysia was estimated to approach 43 thousand tons in 2016 according to the broiler output reported by the Department of Statistics Malaysia (2017). Some of this feather waste is reused to produce dusters or some decorative products and high-end bedding such as pillows and mattresses. Nonetheless, most of the feathers end up in dumps, landfills, and incinerators. These handling methods may cause contamination of the environment by generating greenhouse gases (Acda, 2010). Besides, these methods have also been restricted owing to a lack of lands (Acda, 2010) and require high expenses (Tonkova et al., 2009). Efforts have been made to seek alternative applications of chicken feather such as feather meal (Kumar Mukesh et al., 2012), bio-fertilizers (Saber et al., 2010), biofuel (Abdoli et al., 2014), and thermoplastic (Ullah et al., 2011). Feather was also suggested to be used for biomedical applications especially wound healing (Wang et al., 2017).

Raw feathers have to undergo appropriate treatment prior to the above-mentioned applications. Feathers are difficult to hydrolyze or degrade because they consist of 90% of keratin proteins that are tightly packed either in α-helices or β-sheets into keratin supercoiled polypeptides (Mokrejs et al., 2011). These polypeptides

Abbreviations: MAS, microwave alkaline pretreatment combined with Savinase hydrolysis; AAS, autoclave alkaline pretreatment combined with Savinase hydrolysis; MT, million tons.

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are stabilized by hydrogen bonds and hydrophobic interactions and hence possess high mechanical and thermal stability in final keratin (Brandelli, 2008). Various treatments have been developed to treat feather waste, and these treatments can be categorized into three groups, i.e., physical, chemical, and biological approaches. Each treatment has pros and cons in terms of feather degradation as well as protein and amino acid recovery. In a physical treatment, feathers are degraded at a high temperature and/or pressure, which leads to excess denaturation of certain amino acid sequences and require a large amount of energy. According to Desrosier and Savoie (1991), excessive heating of whey (contains protein), at 121 °C, for 83 min, significantly destroys cystine, lysine, and arginine. At the same time, the amounts of lysine, proline, aspartic acid, glutamic acid, threonine, alanine, glycine, and serine are also affected. Nevertheless, this procedure can be completed within a short period and is easy to handle. Next, the chemical treatment may be performed by simply applying a strong acid or alkali to degrade the feathers. Nevertheless, this approach might also lead to some losses of essential amino acids. Biological treatment is an environmentally friendly method that involves keratinase-producing microorganisms or keratinase alone to break down the rigid bonds in feathers. On the other hand, this method is time consuming. Considering the pros and cons of each treatment, the development of an effective approach that combines 2 or more methods may be another option to compensate for the disadvantages of the above treatment methods while retaining quality of the recovered products like soluble proteins and amino acids.

Lee et al. (2016) treated chicken feather with combined thermal and chemical methods, (microwave NaOH treatment), in 100 ml of 0.5 M NaOH, at 800 W for 10 min. Approximately 26.74 mg/ml protein and 69.4 mg/g amino acids were recovered. Łaba and Szczekała (2013) reported that feathers autoclaved with 10 mM sodium sulfite can also enhance the activity of crude keratinase extracts of *Bacillus cereus* B5esz by 160% and result in 86.3% feather hydrolysis as well as release of amino acids such as leucine, valine, glutamate, glycine, serine, and cysteine. Amino acids like glutamine and asparagine in feather degrade under high-temperature and high-pressure conditions (Wu, 2013). According to Taira (1973), 1.1 g/16 (g N) of arginine and 2.0 g/16 (g N) of lysine in soybean are lost after treatment at 120 °C for 30 min or at 160 °C for 10 min, respectively. Nonetheless, there are no changes in the amino acid profile when the soybean is treated at 100 °C for 5 min. This finding indicates that the heating temperature, incubation time, and pressure involved in this thermal pretreatment can be optimized to reduce the protein losses. Although a strong alkali may lead to losses of certain essential amino acids, treating a protein sample under milder conditions may improve feather degradation. Stiborova et al. (2016) reported that approximately 85.9% ± 0.5% of chicken feathers are hydrolyzed and 10.25 mg/g free amino acids can be recovered by treating feathers with 0.107 M KOH at 70 °C for 24 h. Based on the above findings, it can be hypothesized that the rigid structure of chicken feathers can be weakened by a thermal-chemical method as a pretreatment, under appropriate conditions to improve the protein and amino acid recovery. On the other hand, microwave heating is also a possible relevant method because it has a high heating rate, and no direct contact between a heating source and the target material, saves energy, is easy to control for a heating process, and reduces equipment size and waste (Jones et al., 2002). Binod et al. (2012) reported that a 0.665 g/g sugar yield can be obtained from sugarcane bagasse by pretreatment with microwaves and NaOH (at 1% NaOH, and 600 W for 10 min) in combination with a commercial cellulase digestion for 72 h at 50 °C and 120 rpm. Nevertheless, there is no report on the use of microwaving with an alkali as a pretreatment for chicken feathers. Hence, the feasibility of autoclave alkaline and microwave alkaline pretreatments to be combined with enzymatic treatment for feather solubilization and protein recovery was examined here.

2. Materials and methods

2.1. Materials

Chicken feathers were supplied by Kerabat Processing House (Pedas) Sdn. Bhd. Negeri Sembilan, Malaysia (2.5646° N, 102.0450° E). The alkali used in thermal-alkaline pretreatment was sodium hydroxide (NaOH), which was purchased from UNIVAR, Australia. Savinase® Ultra 16L from Novozymes, Denmark, was the commercial enzyme used in enzymatic treatment.

2.2. Chicken feather preparation

The feathers were cleaned according to a previously published method (Ayutthaya et al., 2015) with modifications. Prior to all the experiments, the chicken feathers were washed thoroughly with tap water. The feathers were soaked in the 50% (v/v) ethanol for 2 days for defatting. The solvent was replaced every day. Finally, the feathers were washed with distilled water several times to eliminate the solvent residues. The washed feathers were dried at 50 °C for 2 days. The dried chicken feathers were stored at room temperature and ready for use.

2.3. Autoclave-alkaline pretreatment followed by Savinase hydrolysis (AAS)

The AAS was a 2-stage treatment in which an autoclave and NaOH acted as pretreatment and then the pretreated feather was subjected to the enzymatic treatment. The autoclave-NaOH pretreatment was carried out in an autoclave, model Tomy-ES315. In all cases, the pretreatment involved 2 g of feather and 100 ml of a NaOH solution with the concentration ranging from 0.01 to 0.10 M at 105 °C, a method of Lee et al. (2016) with modifications. After pretreatment, the samples were centrifuged at 8944*g* for 10 min. The supernatant was discarded and the remaining pretreated feather was washed with water several times to eliminate the residual chemicals and then was dried at 50 °C for a day and thus was ready for subsequent enzymatic treatment.

The enzyme employed in this experiment was a commercial protease, Savinase® Ultra 16L, in solution that can catalyze the hydrolysis of peptide bonds in a protein under alkaline conditions at pH ranging from 8 to 12. According to Eslahi et al. (2013), enzymatic treatment was applied to 1 g of pretreated feather supplemented with 25 ml of sodium borate buffer (50 mM, pH 8.5). The pH of the mixture was adjusted to 9.0 with 2 M NaOH. Then, a 2.6% (v/v) solution of Savinase was added. The mixture was incubated at 55 °C at the agitation speed of 200 rpm for 4 h. After that, trichloroacetic acid was added to deactivate the enzyme. The mixture was centrifuged at 8944*g* for 10 min. Protein content of the supernatant was analyzed. Insoluble feathers were washed with distilled water and centrifuged again to eliminate residual chemicals and dried at 50 °C until constant weight. The solubilization percentage of the feather was calculated.

2.4. Microwave-alkaline pretreatment followed by Savinase hydrolysis (MAS)

The microwave-NaOH pretreatment was carried out in a domestic microwave oven (Samsung, ME86V-BBH) with the operating frequency of 2450 MHz. In all cases, the pretreatment involved 2 g of feather and 100 ml of a NaOH solution at 450 W, method of Lee et al. (2016) with modifications. The enzyme used