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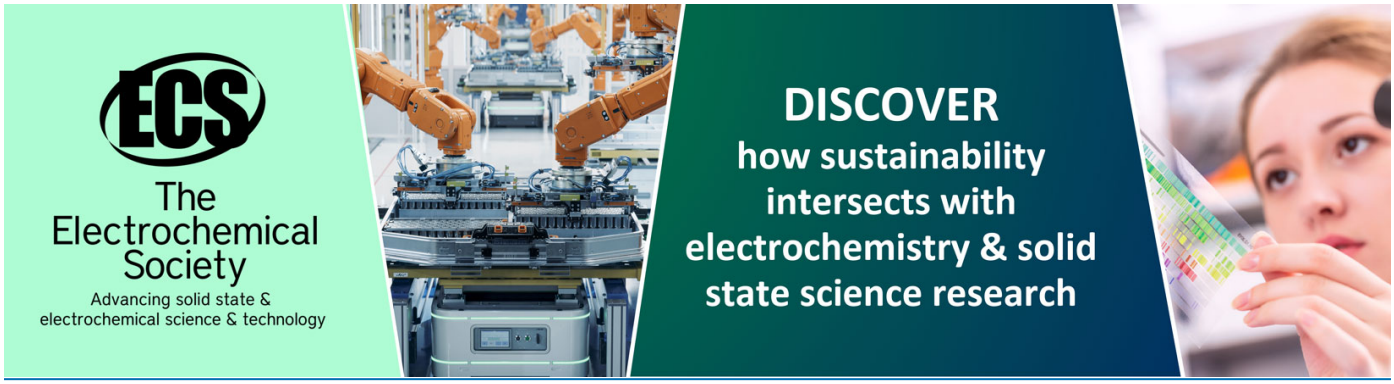

Microwave-assisted Degradation of Poultry Feather to Synthesize Protein-based Surfactant

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Abstract. Poultry feather is hydrolyzed into peptides and amino acids in sub-critical water without any acid or alkali as catalyst under microwave-assisted heating to produce protein-based surfactant. The optimal hydrolysis condition is as follows: temperature 200 °C, time 60 min, and the yield of peptides and amino acids 84.31%. The critical micelle concentration (CMC) and surface tension of the product of peptides and amino acids are 2.6 g/L and 48.2 mN/m. In order to increase lipophilicity, subsequently anionic protein-based surfactant was synthesized by acylation reaction between the hydrolysis product of peptides and amino acidsand oleoyl chloride. The CMC and surface tension decreased greatly with linkage with oleoyl. The synthesized anionic surfactant with weight ratios of 10% (wt.%) hydrolysis liquid/oleoyl chloride of 3:1, 4.5:1 and 6:1 are 0.5, 0.7 and 0.8 g/L and corresponding surface tension are 30.1, 34.0 and 35.7mN/m.

Keywords: Poultry feather; Sub-critical water hydrolysis; Acylation reaction; protein-based surfactant; Critical micelle concentration.

1. Introduction

Because of nontoxicity, excellent biocompatibility and environmental compatibility, bio-surfactants have broad application prospects and attract increasing attention[1]. Proteins are composed of amino acids which have hydrophilic amine and carboxyl groups by amide linkage. Thus, the hydrolysis product of peptides and amino acids from protein has similar structural characteristics and can be used directly or improved as surfactant[2]. Up to date, as an important category of bio-surfactant, peptide- and amino acid-based surfactant is used in many fields such as cosmetics[3], textile, agriculture[4], medicine and so on.

The poultry industry produces a great amount of feathers each year, whose disposal causes an environmental problem[5]. Whereas, poultry feature is rich in protein with keratin content above 70%. Therefore, from both an economic and environmental point of view, it is important to recycle the keratin from feather with eco-friendly method. Formerly, we studied extraction of keratin from feather with ionic liquid [6]. Up to date, the hydrolysis of feather into high-value product is an important strategy to recycle feather and it draws extensive attention. Conventionally, catalytic hydrolysis with acids, alkalis [7] or enzymes [8] is the primary method for the hydrolysis. The acid or alkali catalytic hydrolysis is not eco-friendly and results in complicated downstream separation. The enzymatic hydrolysis is very slow owing to the strong self- interaction of keratin molecules. Moreover, the enzyme is usually very expensive and susceptible to activity loss.

As an eco-friendly and high-efficient way, we studied direct microwave-hydrothermal approach to produce amino acid [9]and photoluminescent carbon dots[10]. Owing to the complexity of feather, the

high cost for separation of purified amino acid is a critical disadvantage for industrial application. Thus, we investigated to produce protein-based surfactant with hydrolysate which is mainly composed of low molecular weight peptides and amino acids.

2. Experimental

2.1. Materials

Duck feather is a typical poultry feather and the sample of duck feather was provided by Xinyi Hanling Biological Engineering Co., Ltd. (Xuzhou, China). It was washed with water, dried and cut into small pieces for use. C, N and H elemental analysis of the feather were as follows: C (70.05%), N (12.19%) and H (11.82%). The protein content was calculated as N×6.25, and thus it could be estimated about 76%. NaOH, oleoyl chloride and ninhydrin were purchased from Sinopharm Chemical Reagent Co. LTD (Shanghai, China). All the reagents were of analytical grade and used as received without further purification.

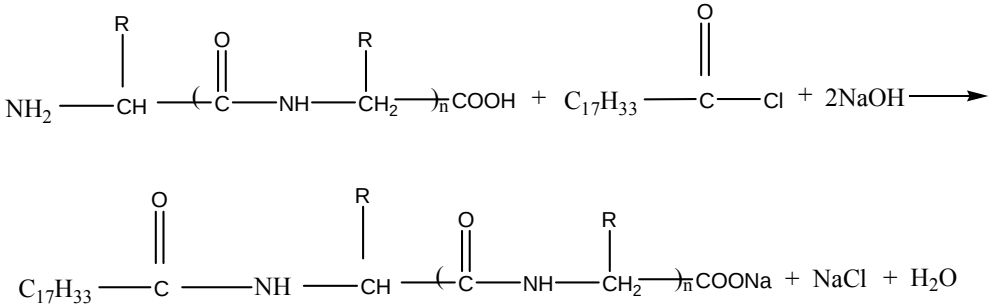
2.2. Hydrolysis of Feather

The hydrolysis reactor is the same described formerly with microwave-assisted heating [3]. The 4g duck feather and 300 ml water were put into 500 mL Teflon-line autoclave and heated with the maximum power of 1200W microwave radiation to a fixed temperature for some time. The hydrolysis temperature ranged from 160 to 220 °C and time from 30 to 90 min were studied.

After hydrolysis, the sample was poured out, and then the residual solid and liquid were separated by vacuum filtering through a filter paper (2.5 mm) and a microporous membrane (0.22 mm). The suspension was removed by the dialysis against Milli-Q water with a cellulose ester membrane bag (Mw=3500) to obtain low molecular weight peptides and amino acids for synthesis of surfactant.

2.3. Synthesis of Anionic Protein-based Surfactant

As for the seperatedlow molecular weight peptides and amino acids, first it was adjusted to 10% (wt.%) peptides and amino acids solution. Then the solution was reacted with oleoyl chloride through acylation reaction as the following chemical equation to synthesize more lipophilic group surfactant.



The different weight ratios of 10% (wt.%) solution/oleoyl chloride were reacted at 75 °C, pH 10 for 3 hours to obtain protein-based surfactant according to the above reaction.

The mixture after reation was cool to about 5 °C, and then the yellowish-brown viscous liquid was seperated. The yellowish-brown liquid was dissolved in deionized water and was cool to 5 °C to obtain yellowish-brown liquid again. The process was repeated about 3 times and the yellowish-brown liquid was the synthesized protein-based surfactant.

3. Result and Discussion

3.1. Hydrolysis Temperature and Time

The yield of the low molecular weight peptides and amino acids was used to evaluate synthesis efficiency and optimize reaction conditions and it is defined as $M_t/M_0 \times 100\%$, where M_0stands for the initial weight of feather and $M_t$refers to the mass of obtained low molecular weight peptides and amino acids in liquid phase after hydrolysis.

Intuitively, reaction temperature and time were critical to the hydrolysis of feather. Because the hydrolysis of feather was very low at the temperature below 160 °C without catalyst and degradation of the produced peptides and amino acids was fast at the temperature above 220 °C, the temperature range of 160-220 °C was studied.

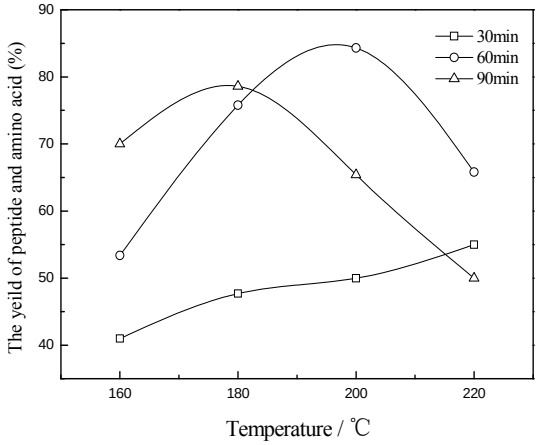


Figure 1. The change of yield of peptides and amino acids with temperature at different times.

Figure 1showed the yield of obtained low molecular weight peptides and amino acids from hydrolysis of feather with different temperature and reaction time. With increasing temperature, the hydrolysis of feather would increase, but with different reaction time, the yield of peptides and amino acids has different change. As for the shortest reaction time of 30 min, the yield increases with increasing temperature. The result indicates that the hydrolysis of keratin should not be completed at the reaction time of 30 min. But as for the longer reaction time of 60 and 90 min, the yield show transition change. As for the time of 60 min, the yield attains the maximum of 84.3 % at the temperature of 200 °C and it decreases to 65.8 % at the higher temperature of 220 °C. The decrease should result from the decomposition of the produced peptides and amino acids at the temperature of 220 °C.

Similarly, the longest reaction time of 90 min, the transition temperature is 200 °C and it indicates that with more time there is more decomposition of the produced peptides and amino acids. Thus, with the consideration of the efficiency of hydrolysis and decomposition of the produced peptides and amino acids, the appropriate temperature and reaction time should be 200 °C and 60 min.

3.2. Acylation Product

The different anionic protein-based surfactants with different 10% (wt.%) solution/oleoyl chloride of 3:1, 4.5:1 and 6:1 were measured with the micro-Kjeldahl method to determinethe total nitrogen content and it was given in Table 1.

Table 1. The total nitrogen content of obtained anionic protein-based surfactants.

Ratio of liquid/oleoyl chloride	3:1	4.5:1	6:1
Total nitrogen conten	12.6	13.7	14.5

The total nitrogen content of the peptides and amino acids before reaction was about 15.3% and with the increase of oleoyl chloride, the content decreased. The result indicated that the increase of oleoyl chloride should be remain in the synthesized surfactants and thus, the excess oleoyl chloride in the reaction will no doubt remain in the product. Therefore, in order to know the suitable amount of oleoyl chloride that the almost all free amino group of the peptides and amino acids can finish and no excess oleoyl chloride. The residual amino group of the product were determined by ninhydrin colorimetric method and it was given in Table 2.

Table 2. The free amino group of obtained anionic protein-based surfactants.

Ratio of liquid/oleoyl chloride	3:1	4.5:1	6:1
Free residual amino group (%)	5.7	6.1	23.6

With increasing amount of oleoyl chloride from 1:4.5 to 1:3, the free residual amino group was almost the same. Whereas, with decreasing amount of oleoyl chloride from 1:4.5 to 1:6, the free residual amino group had greatly increase from 6.1 to 23.6%. The result indicates that amout of oleoyl chloride 1:3 should be excess and 1:6 was insufficient to the acylation reaction. Therefore, the suitable amount of oleoyl chloride from the acylation readtion should be 4.5: 1 of 10% (wt.%) solution/oleoyl chloride.

3.3. Surface Tension and CMC

As for the important parameter of surfactant, the critical micelle concentration (CMC) should be studied. According to the curve of the surface tension of aqueous solution versus the corresponding logarithm of concentrations, the surfactant value can be determined. First the CMC and the surface tension of obtained low molecular weight peptides and amino acids is determined to be 2.6 g/L and 48.2mN/m.

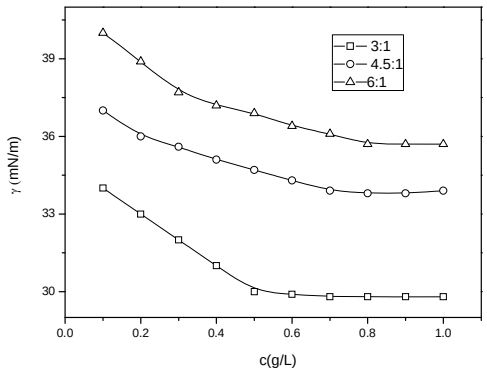


Figure 2. The curve of surface tension γ and the obtained surfactant with different weight ratio of 10% (wt.%) solution/oleoyl chloride.

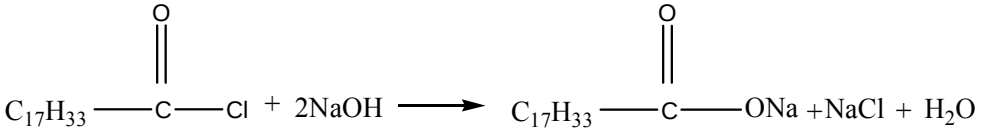
Figure 2 gives the surface tension of synthesized surfactant after acylation reaction versus the corresponding concentrations. The CMC is determined similar with the above method. Table 3 shows pH, tension surface and CMC of different ratio of oleyl chloride reacted with 10% (wt.%) solution of low molecular weight peptides and amino acids.

Table 3. The pH, CMC and corresponding surface tension of the synthesized surfactant with different weight ratio of 10% (wt.%) solution/oleoyl chloride.

Ratio of liquid/oleoyl chloride	3:1	4.5:1	6:1
pH	11.2	10.6	10.5
CMC (g/L)	0.5	0.7	0.8
Surface tension (mN/m)	30.1	34.0	35.7

It is obvious that the CMC value of synthesized surfactant after acylation reaction is much lower than the unreacted peptides and amino acids. The result is accorded with that the increasing amounts of lipophilic group should result in the decrease of CMC value.

As for the different content of oleoyl chloride, with increasing content of oleoyl chloride, the excess oleoyl chloride also can be hydroylzed as following equation to produce sodium oleate.The safety and degradability of proteined-based surfactant should be worse with increasing sodium oleate.



It is known that the CMC of the by-product sodium oleate was about 0.4 g/L, the value is less than the acylationanionic protein-based surfactant. As for the CMC 0.5 g/L of obtained proteined-based surfactant of 3:1 weight ratio of 10% (wt.%) solution/oleoyl chloride indicated if the by-product was

existed, the content should be little.
With increasing content of peptides and amino acids, the excess of oleoyl chloride should decrease. Therefore, there is the less by-product sodium oleate of 4.5: 1 weight ratio of 10% (wt.%) solution/oleoyl chloride than 3: 1 and the increase of CMC is consistent with the decrease of sodium oleate. With the increasing content of peptides and amino acids further, there is more free residual amino group and the oleoyl chloride is no doubt insufficient, and thus the CMC and surface tension of the protein-based surfactant increase. Therefore, the synthesized surfactant resulted from 4.5: 1 weight ratio of 10% (wt.%) solution/oleoyl chloride should be appropriate to practical application.

4. Conclusion
Under microwave-assisted sub-critical conditions, poultry feather can be hydrolyzed into peptides and amino acids without any acid or alkali catalyst via a simple and environmental-friendly process. The hydrolysate of low molecular weight peptides and amino acids has excellent surface activity after acylation reaction with some content oleoyl chloride. With 4.5:1 weight ratio of 10% (wt.%) hydrolyzed low molecular weight peptides and amino acids solution/oleoyl chloride, the CMC and corresponding surface tension are 0.7 g/L and 34.0 mN/m.

Acknowledgements
The research was supported by the Program for Innovative Research Team in University (No. IRT13078).

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