Preparation and Surface Activity of the Hydrolyzed Protein Surfactant from Chrome Shavings under the Effect of Microwave

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Abstract: The traditional production process of hydrolyzed protein surfactant, in which chrome shavings are used as the raw material, have some severe problems such as long reaction time, a lot of energy consumed and the secondary pollution to the environment, and the obtained surfactant has high ash content and low surface activity. To solve these problems, microwave heating was adopted in this study to hydrolyze the chrome shavings, and the corresponding processing parameters including microwave power, use level of calcium oxide, temperature, reaction time and liquor ratio were optimized. Results show that the microwave can shorten the processing time to twenty minutes, and the extraction yield of hydrolyzed protein is close to 75%. Then, for the chrome shavings, the pretreatment method was investigated to reduce the ash content of the final product. Results indicate that, when chrome shavings are pretreated in turn by the degreasing agent and saturated calcium hydroxide solution, the ash content of hydrolyzed protein is less than 4.5%, and the subsequent treatment of a 0.2% cation change resin for 30 minutes can further increase its ash content to 1.92%. Finally, the hydrolyzed protein surfactant was prepared by the condensation of the extracted hydrolysate to oleoyl chloride, and its surface activity was determined and compared with that of Lamepon A. Results show that the prepared surfactant has stronger ability to decrease the surface tension of solution and better foaming capacity, which imply its potential to be used in the area of high added value.

Key words: microwave; chrome shavings; hydrolyzed protein surfactant; surface activity

1 Introduction

Chrome shavings are small pieces of leather that are shaved off when the thickness of wet blue is rendered uniform by a shaving machine. During the past several decades, some rather innovative methods to treat this waste product have been developed [1]. For example, basic hydrolysis at elevated temperature and/or pressure has been used in many parts of the world for chrome recovery and for the isolation of protein fractions. As this method has high extraction yield and low cost of production, it is often used to extract protein hydrolysate as a material for the preparation of hydrolyzed protein surfactant such as Lamepon A [2]. However, the basic hydrolysis of chrome shavings have some disadvantages, such as long reaction time, a lot of energy consumed and the secondary pollution to the environment. Moreover, due to the low molecular weight and high ash content, the isolated protein hydrolysate can not be used for the manufacture of high value-added products. So, Modification for this process is necessary.

Microwave, as a kind of heating energy, has been widely used in many areas of production and living. Compared with the conventional heating mode, microwave heating has many advantages, such as high speed heating rate, energy conservation and easy control. At present, microwave heating has been successfully used in all kinds of organic and inorganic chemical reaction to improve reaction speed and increase yield [3]. In nature, the basic treatment of chrome shavings is a de-chroming reaction, where the carboxyl groups ($-COO^-$) of hide collagen coordinated to trivalent chromium are substituted by hydroxyl ion ($-OH^-$) in alkali liquor, at the same time, chromic hydroxide precipitation is generated. So, if

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microwave heating was used during the basic hydrolysis of chrome shavings, its reaction speed should be improved. The objectives of this work are three-fold: to optimize the basic treatment process of chrome shavings under the effect of microwave; to decrease ash content of the obtained protein hydrolysate by the pretreatment of chrome shavings and the adsorption action of cation change resin; and to determine the surface activity of the corresponding hydrolyzed protein surfactant.

2 Experimental

2.1 Materials

Chrome shavings used in this study were obtained from a commercial tannery in Chengdu (China). Calcium oxide (CaO), oleic acid and strong-acid styrene cation exchange resin, which are all analytic reagents, were purchased from Kelong Chemical Agent Plant (Chengdu, China). And all the other reagents used in the study were in the analytical reagent grade.

A normal pressure microwave extraction apparatus (MAS-I, Shanghai Xinyi Microwave Chemistry Co. Ltd.) was used in the study. A contact angle measuring apparatus (OCAH200, Dataphysics, Germany) was used to test the surface tension of the prepared surfactants.

2.2 Treatment of Chrome Shavings by Microwave Heating

Ten grams of chrome shavings were suspended in 50-100mL of water (500-1000% float), 0.4-0.8 grams of calcium oxide (4-8%) was added and the samples were shaken at 80-100℃ in the microwave extraction apparatus for 5-60 minutes. The power of microwave was changed from 500W to 1000W. The obtained solutions were filtered. Analyses were run on the hydrolyzed protein products.

2.3 Deashing of the Isolated Protein

To reduce ash content of the final hydrolyzed protein product, the chrome shavings were pretreated as shown in Tab. 1 and the obtained hydrolyzed protein product was treated with strong-acid styrene cation exchange resin. In the experiments of resin treatment, diluted sulfuric acid was added to fifty grams of the protein solution containing different dosage of cation exchange resin (0.1%, 0.2% and 0.3%) to adjust the pH to 4.0, and the suspension was agitated at room temperature for 10-60 minutes.

Tab. 1 Pretreatment of chrome shavings

Procedure	Chemicals and Dosage	Duration and remarks
Washing:	500% Water at 45~50℃	
	0.5% Levelling Agent O (Non-ionic surfactant)	Run 30min. Drain.
De-oiling:	500% Water at 45~50℃	
	0.5% AP-NC (Non-ionic surfactant)	
	1.5% Ammonia to adjust pH 7.0~8.0	Run 30min. Drain.
Washing:	600% Water at 45~50℃	Run 15min. Drain.
Desalting:	700% Saturated $Ca(OH)_2$ solution	Run 1h and then overnight at 25℃. Drain.
Washing:	600% Water at 25℃	Run 15min. Drain. Take out. Air dry.

2.4 Preparation of Hydrolyzed Protein Surfactant

Following a traditional three-step process [4], the hydrolyzed protein surfactants were prepared by the condensation reaction of the isolated hydrolysate and oleoyl chloride. Firstly, two kinds of protein hydrolysates were isolated from chrome shavings at the condition of optimal microwave treatment and the condition of high temperature and high pressure, respectively. Then, oleic acid reacted with phosphorus

trichloride to synthesize oleoyl chloride. Finally, the protein hydrolysates reacted with oleoyl chloride to prepare oleoyl amino acid sodium, namely, the hydrolyzed protein surfactant, and their chemical reaction is as follow:

$$C_{17}H_{33}COCl + H_2NR_1(CONHR_2)_XCOOH \xrightarrow{2NaOH} C_{17}H_{33}CONHR_1(CONHR_2)_XCOONa + NaCl + H_2O$$

2.5 Analysis

The chrome shavings were analyzed for pH, moisture, chromium, ash and protein contents as described in a previous publication [5]. The protein products were analyzed for total solids and total ash as described in a previous publication [6]. The molecular weight distribution of the hydrolysate was analyzed by polyacrylamide gel electrophoresis (SDS-PAGE). The yields of the protein products were calculated based on the dry weight of chrome shavings [5]. The prepared hydrolyzed protein surfactants were analyzed for amino substitution degree, surface tension, emulsifying and foaming properties as described in a previous publication [7].

3 Results and discussion

Previous studies demonstrated that chrome shavings in leather industry can be used to prepared hydrolyzed protein surfactants, and the typical commercial product is Lamepon A [4]. During the course of manufacture, the lime (CaO) is often used to hydrolyze chrome shavings under the condition of high temperature and pressure, and the treatment time is very long. In this present set of experiments, we wanted to determine if the microwave heating would be effective in the treatment of chrome shavings. The percent recovery of the available protein (protein in chrome shavings), the quantity of the isolated products, and the treatment speed would be the parameters by which this new microwave preparation would be evaluated. A process of pretreatment for chrome shavings and cation resin treatment for isolated protein were determined if the ash content of the final surfactant would be decreased. The prepared surfactants were compared by determining their degree of amino substitution, surface tension, emulsifying and foaming powers.

The chrome shavings used in these experiments were obtained from a commercial tannery. They were analyzed for moisture, ash, chromium, protein and pH, and the results can be seen in Tab.2. The results are typical of shavings that have been used in the past [5, 6].

Tab. 2 Chemical analysis of chrome shavings

Parameter	Moisture (%)	Ash (%)	Cr_2O_3 (%)	Protein (%)	pH
Value	33.18	21.94	5.43	72.36	3.85

3.1 Optimization of Microwave Treatment Process

3.1.1 Concentration of CaO

Five samples were run with varying Cao concentration of 4 to 8% based on the weight of the chrome shavings, and the other conditions were settled, namely, microwave power was 600W, liquor ratio is 7.0, temperature is 95℃ and irradiation time is 20 minutes. It can be seen from Fig.1 that the percent recovery of protein increases with increasing concentration of Cao. When the concentration is above 6%, there is no significant increase in the protein yield. So, 6% concentration appears to be sufficient for maximum yield of protein.

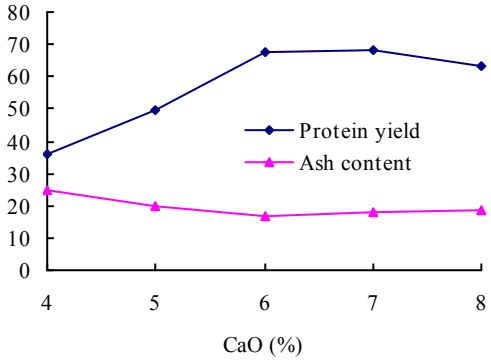

Fig. 1 Effect of CaO concentration on protein yield and ash content

3.1.2 Temperature of Treatment

The results obtained with respect to the effect of temperature on protein yield and ash content are shown in Fig.2. In this set of experiments, the temperature of treatment was varied from 80 to 100℃, with constant 6% concentration of CaO, 7.0 of liquor ratio, 600W of microwave power and 20 minutes of treatment time. It is seen that there is an gradually increase in the percent recovery of protein with increase in temperature of microwave treatment up to 95℃, and further increase in temperature results in slight decrease of the protein yield. The ash content of the isolated protein has no obvious change in the whole temperature range. Hence, 95℃ is taken as the optimized temperature of microwave treatment.

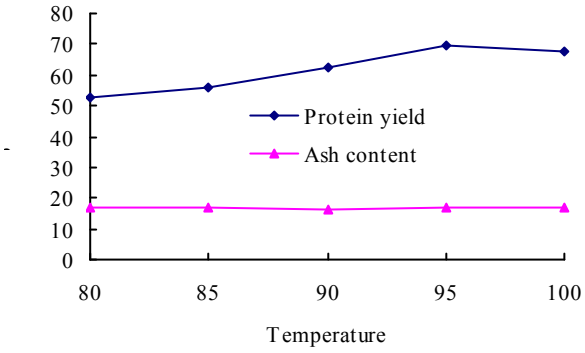

Fig. 2 Effect of temperature (℃) on protein yield and ash content

3.1.3 Power of Microwave

The protein yield and ash content at different power of microwave is shown in Fig.3. In these experiments, the concentration of CaO is 6%, liquor ratio is 7.0, temperature is 95℃ and treatment time is 20 minutes. It is evident from the figure that the protein yield increase gradually with the increase of microwave power from 500 to 700W, and further increase in power results in slight decrease of the protein yield. So, 700W is taken as the optimum microwave power, where the protein yield is 72.63%.The power of microwave has no obvious effect on the ash content of the isolated protein.

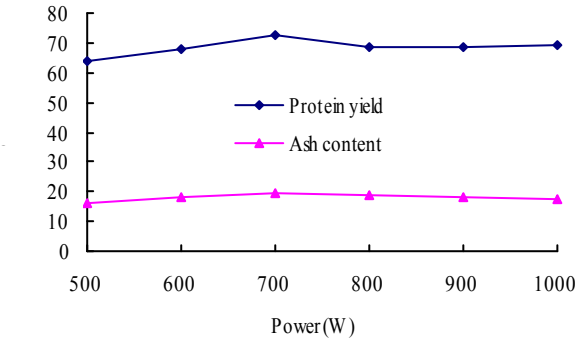

Fig. 3 Effect of microwave power on protein yield and ash content

3.1.4 Time of Treatment

The results obtained for the protein yield and ash content at different time intervals with constant of 6% concentration, 7.0 of liquor ratio, 600W of microwave power and 95℃ of treatment temperature are shown in Fig.4. From the figure, it can be seen that there is a gradually increase in the percent recovery of protein with the increase in treatment time from 5 to 20 minutes. Above 20 minutes, there is no significant increase in the protein yield. So, 20 minutes was selected as the optimal treatment time. The ash content has slight change in the different treatment time.

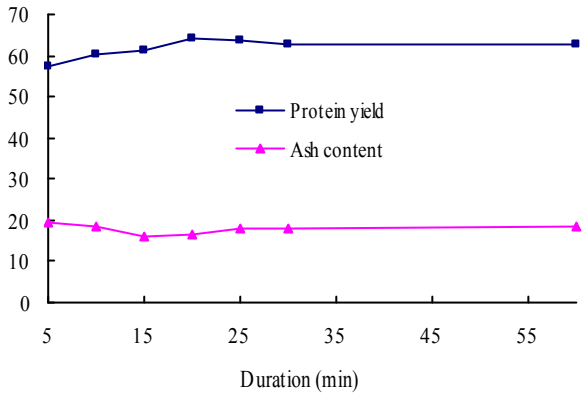

Fig. 4 Effect of treatment time on protein yield and ash content

3.1.5 Liquor Ratio

Varying the liquor ratio of 500 to 1000% based on the weight of the chrome shavings, the protein yield and ash content are given in Fig.5, where concentration of CaO is 6%, microwave power was 600W, temperature is 95℃ and irradiation time is 20 minutes. It can be seen that there is slight effect of liquor ratio on protein yield and ash content. Considering the convenience of process, the appropriate liquor ratio is selected 7.0. Hence, A 6% offer of CaO in 700% liquor ratio, at95℃ for 20 minutes and 700W has been taken as optimized condition for the hydrolysis of chrome shavings under the effect of microwave.

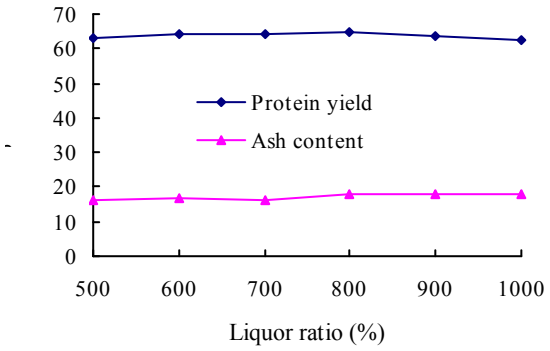

Fig. 5 Effect of liquor ratio on protein yield and ash content

3.2 Deashing of the Isolated Protein

The high ash content in the isolated protein is adverse to the surface activities of the final hydrolyzed protein surfactant. In this present set of experiments, two methods were used to decrease the ash content. Firstly, the chrome shavings were pretreated as shown in Tab.1 to remove part of soluble inorganic salts such as NaCl and Na_2SO_4. Then, the obtain protein was treated by cation exchange resin to further reduce its ash content.

3.2.1 Pretreatment of Chrome Shavings

The results for the ash content of the chrome shavings and isolated protein before and after pretreatment are shown in Fig.6. It can be seen that there are remarkable decrease in the ash content of the shavings and the protein. After pretreatment, the ash content of the chrome shavings is decreased from 21.94% to 13.79%, and for the isolated protein, its ash content is decreased from 16.92% to 4.48%.

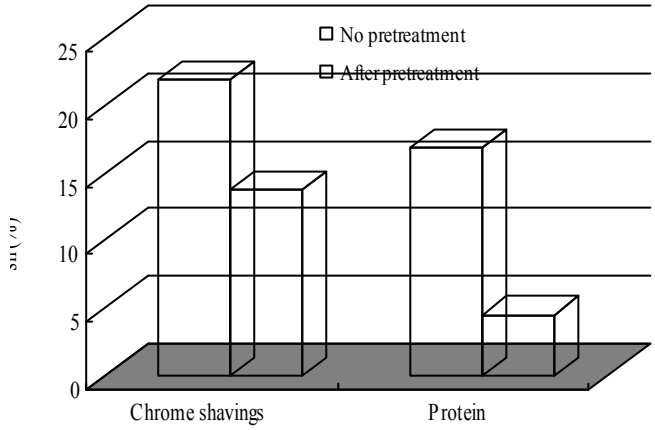

Fig. 6 Effect of pretreatment on the ash content of chrome shavings and final protein

3.2.2 Treatment of the Isolated Protein by Cation Resin

The ash content of the treated protein at different usage of cation exchange resin and different time intervals are given in Tab.3. Based on the weight of the protein solution, 0.1%, 0.2% and 0.3% offer of the cation resin were used, and the treatment time was 30 minutes. From Tab.3, it can be seen that 0.2% resin is enough to reduce the ash content below 2%, and further increased usage has no obvious effect on the decrease of ash content. At 0.2% offer of the cation exchange resin, the protein was treated for 10, 30 and

60 minutes, respectively. It is evident that from Tab.3 that the ash content decreases gradually with treatment time. 30 minutes of treatment can bring about significant decrease, and increased time interval is no necessary. Hence, a 0.2% offer of the cation exchange resin for 30 minutes has been taken as optimized conditions for much less ash content of the final protein.

Tab. 3 Effect of cation resin treatment on the ash content of protein

Resin (%)	Ash (%)	Time (min)	Ash (%)
0	4.48	0	4.48
0.1	3.00	10	3.03
0.2	1.95	30	1.92
0.3	1.80	60	1.86

3.3 Properties of the Hydrolyzed Protein Surfactant

After being pretreated, the chrome shavings were hydrolyzed at the optimum microwave condition to prepare the hydrolyzed protein. Then, a 0.2% of the strong-acid styrene cation exchange resin was used to further decrease the ash content of the isolated protein that was taken as a reactant of the subsequent condensation. For the sake of contrast, a conventional process of high temperature and high pressure was adopted to treat the chrome shavings, and the obtained protein was used to prepare the surfactant of Lamepon A. For the two kinds of hydrolyzed protein, their molecular weight distributions were determined by polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of the former is located in the range of 20.1 KDa to 97.2 KDa, whereas the latter is mainly between 14.3 KDa and 20.1 KDa. So, the protein prepared by microwave heating has higher molecular weight distribution.

Except for the treatment condition of chrome shavings, the two hydrolyzed protein surfactants was prepared by the same three-step process, and their surface tension, degree of amino substitution, Emulsifying and foaming power are shown in Fig.7 and Tab.3, respectively. It can be seen from Fig.7 that the critical micelle concentration (CMC) of the surfactant from microwave process is about 0.5 g/L, and the CMC of Lamepon A is approximate to 0.8 g/L. The results indicate that the former has stronger ability to decrease the surface tension of solution.

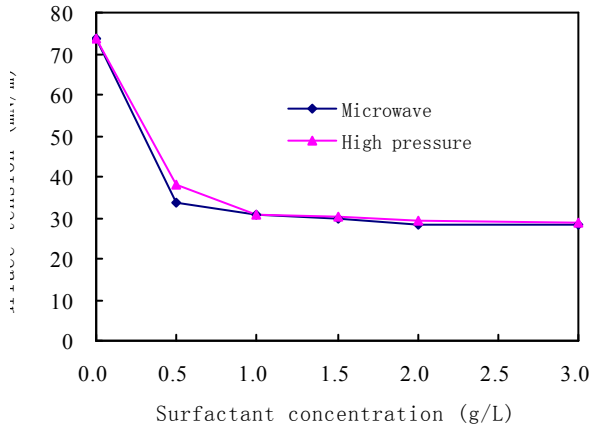

Fig. 7 Surface tension of the prepared surfactants

As shown in Tab.3, the conventional Lamepon A has much higher degree of amino substitution

because there are much more free amino groups resulted by the deeply hydrolysis of chrome shavings under the condition of high temperature and high pressure. Although the emulsifying power of the surfactant from microwave process is not as good as that of Lamepon A, it has excellent foaming power. Considering the low ash content, the hydrolyzed protein surfactant should be potential to be used in high-value products.

Tab. 3 Properties of Lamepon A and surfactant from microwave treatment

Properties		Lamepon A	Surfactant from microwave process
Degree of amino substitution (%)		78.53	56.65
Emulsifying power	Oil phase (mL)	1.0	6.0
	Emulsion phase (mL)	11.2	5.0
	Water phase (mL)	7.8	9.0
Foaming power	Initial height (mL)	28.0	50.0
	Stable height (mL)	24.0	36.0

4 Conclusions

The microwave heating is an effective approach for the extraction of hydrolyzed protein from chrome shavings, and the ash content of the isolated protein can be effectively decreased below 2% by the pretreatment of chrome shavings and the treatment of a cation change resin. Using the obtained proteins as a raw material, a hydrolyzed protein surfactant can be prepared, and the final product has excellent foaming power and can effectively decrease the surface tension of solution. It has a potential to be used in the area of high added value.

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