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Alkaline Extraction Method of Cottonseed Protein Isolate

Buning Zhang
School of Materials Science and Engineering
Northwestern Polytechnical University
127 Youyi Xilu, Xi'an 710072, China
E-mail:eins001@163.com

Yingde Cui (Corresponding Author)
Institute of Green Chemical Engineering
Zhongkai University of Agriculture and Engineering
24 Dongsha street, Haizhu District, Guangzhou 510225, China
E-mail: cuigdut@yahoo.com.cn

Guoqiang Yin, Xinming Li & Xiaoxia Zhou
Institute of Green Chemical Engineering
Zhongkai University of Agriculture and Engineering
24 Dongsha street, Haizhu District, Guangzhou 510225, China

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Abstract

Cottonseed protein was extracted from defatted cottonseed flour with alkaline solvent and its precipitation property in different pH value was investigated. Orthogonal experiments design was introduced and factors influencing protein extraction were studied, namely, extraction solvent type, solvent-to-flour ratio, extraction pH value, extraction temperature and time. The optimum scheme of extraction was obtained: 0.1N KOH as extraction solvent, solvent-to-flour ratio=12,pH=12.5, temperature=60℃ and time=40min.Under this condition, the cotton protein extractability is above 70%and the protein extraction can be directly applied to further chemical modification.

Keywords: Cottonseed flour, Cottonseed protein, Extraction, Precipitation

1. Introduction

Recently, protein used as a non-petroleum, safe and biodegradable and renewable resource was focused by many researchers. Applications on modification of protein, especially in nonfood industry have been increased. Carboxyl, amido, amide, hydroxy, sulfhydryl groups exist in the main chain of protein molecule. Chemical modification such as grafting, copolymerization and crosslinking could result in the desirable materials of proteins. As a herbage, cotton cultivation is traditionally for the production of fibers. Nevertheless cottonseed is also an important source of oil and protein with the content of 38%-45% protein in defatted flour (Li, 2004, PP. 34-35). Cottonseed oil is used in cooking, salads, shortenings and the flour for livestock feed. In China about 8.0 million tons of cottonseed are produced annually, which ranked the first in the world and accounted for one third production of the world(Xue, 2007, PP. 55-58). Herein, annual defatted cottonseed flour would exceed 4.0 million tons after oil extraction, which contained nearly 1.6 million tons of protein (Dai, 2001, PP. 30-32, Yang, 2000, PP. 3-4). Cottonseed flour have reasonable amino acid components and nutrition value, however, the presence of toxic gossypol in the cottonseed flour is a limiting factor for human consumption and various methods have been proposed for reduction of free gossypol content. Due to the denaturation of proteins in the thermal processing of cottonseed oil extraction, and subsequently reduction of nutrition value, this source of protein has not been intensively and extensively applied (Li, 2005, PP. 22-23). Thus, 80% defatted cottonseed have to be used as fertilizer or substrates for edible mushroom, while only 5-10% are used as feed materials, which result in a great waste of protein (Lu, 2003, PP. 48-51). Therefore, the application of protein isolated from defatted cottonseed flour in non-feed or non-food industries would have great significance in economy and society.

Isolating proteins from cottonseed was usually performed by the method of "alkali extraction and acid precipitation", which utilized the difference of protein solubility in acids and alkali, dissolved the protein in alkali solutions in specific pH and then adjusted the solution pH with acids until the isoelectric point was attained and then separated out the protein isolates. The key point of this processing was to extract protein from the cottonseed flour as much as possible. In this paper, the effect of technical conditions were investigated, and the resulting protein extraction would be taken further investigation for modification, such as grafting copolymerization with other monomers, in order to prepare different functional cottonseed protein derivates or composite materials.

2. Materials and methods

2.1 Materials

Defatted cottonseed flour was provided by China cotton-unis bioscience Co., Ltd., used for the protein extractions after screened through 80 mesh(total protein content 49.17 % by Kjeldahl Method using the conversion factor of 6.25). All other reagents were of analytical grade and used without further purification.

2.2 Extraction of cottonseed protein

Cottonseed flour (constant weight) was extracted with the extracting solutions at a solvent-to-flour ratio of 10-15 (w/v) with constant stirring after the desirable temperature and pH value were adjusted. The pH value was kept constant by extracting solutions. After extraction, solutions were filtrated immediately. The protein content was calculated after nitrogen analysis by Kjeldahl Method and UV spectrophotometry method (Guo, 1996, PP. 61-64), using the conversion factor of 6.25.The protein extraction rate was calculated according to the following formulation:

Extraction rate%=Total protein after extraction/ Total original cottonseed protein×100%

2.3 Precipitation of cottonseed protein

The cottonseed flour was extracted with 0.1 N KOH at pH 10.5 and 40℃ for 40 minutes and the content of total protein was calculated after extraction. Then at the different pH values adjusted with 0.5 N HCl or 0.5 N NaOH, the extracting solution were stirred and then kept steady at 5℃ for 24hours until precipitation, respectively. The protein content and volume of the suspension was calculated. The precipitation rate was calculated according to the following formulation:

Precipitation rate %=(Total protein after extraction-Total protein of suspension) /Total protein after extraction×100%

3. Results and discussion

3.1 Effect of medium

Generally, mediums used for extracting cottonseed protein are ammonia(Zhao, 2002, PP. 25-26), NaOH(Cui, 2003, PP. 43-45), KOH(Zhao, 2007, PP. 49-50), $Na_2S_2O_3$ (Liadakis, 1993, PP. 918-922), NaCl(El Tinay, 1988, PP. 19-27), $CaCl_2$(El Tinay, 1988,PP.19-27, 57-63), sodium hexametaphosphate (Shemer, 1973, PP. 460-462) and so on. According to the previous researches, to obtain the optimized experimental parameters, a four-factor at three-level orthogonal array experimental design $L9(3)^4$ was adopted to extract protein from cottonseed flour at a solvent-to-flour ratio of 10 (w/v). The four factors were medium, temperature, pH value and time. Based on the experimental results of the previous orthogonal design, the optimal ranges for each factor and their extraction effect could be obtained, and the optimal conditions could be speculated. The factors studied and the assignments of the corresponding levels are listed in Table 1.

The analytical results of 9 tests are listed in Table 2. The values of $|k_{max}-k_{min}|$ in Table 2 indicated that the effect of medium, temperature, pH value and time. The efficiency of these four factors were classified in the order of medium>temperature>pH value>time. Thus, medium was the major factor affecting extraction rates and 0.1 NKOH was applied for further investigation.

3.2 Precipitation characteristics of cottonseed protein

Generally, isoelectric point (*pI*) of plant oil proteins range from pH 4 to 5. Among them, *pI* of protein from soybean, peanut and rapeseed is about 4.5 while from sunflower seed 4.0. As shown from Figure 1, the highest precipitation for cottonseed protein was observed between pH 4 to 5.5. Due to different techniques of oil extraction and de-gossypol process, highest precipitation for cottonseed protein from various source usually ranged from pH 3.5 to 5.5 (King, 1977, PP. 1211-1213). Liadakis (Liadakis ,1993, PP. 918-922) found that the solubility of cottonseed protein was least at pH4.37 while Beradi found that at pH 5.0. In the present paper, the precipitation rate was highest at pH5.0, which was comparable with previous literatures and could be used for further acidic precipitation process.

3.3 Effect of solvent-to-flour ratio

Cottonseed flour (constant weight) was extracted with 0.1N KOH solution at a different solvent-to-flour ratio with constant stirring at pH 12 and 60℃ for 40 minutes and the filtrated flour was extracted again with a solvent-to-flour ratio10 (w/v) in the same condition. The results are shown in Figure 2.

When solvent-to-flour ratio was too low, cottonseed flour swelled due to water adsorption, extracting solution concentration became dense, causing the viscosity of the solution increased which hampered molecular diffusion. The velocity of the extraction decreased accordingly. Although the extracting concentrate was dense, the obtained protein amount was low. When solvent-to-flour ratio was 6, extraction was the lowest. Extraction rate increased with the increasing of solvent-to-flour ratio. When the solvent-to-flour ratio ranged from 12 to 14, extraction rates made no significant difference, due to the adverse effect of high solvent-to-flour ratio which could cause low protein content of the extraction and interfered with further chemical modification. Considering the first extraction rate and the total extraction rate after two times operation, the solvent-to-flour ratio of 12 (w/v) was acceptable and desirable.

3.4 Effect of pH value

Cottonseed flour (constant weight) was extracted with 0.1N KOH solution at a constant solvent-to-flour ratio of 12 (w/v) with constant stirring at different pH and 60℃ for 30 minutes. The results are shown in Figure 3.

Obviously, in alkaline condition, extraction rate increased with the increasing of pH value. Separation and purification of proteins for foodstuffs were generally performed under pH 10(Di, 2006, PP. 439-441, Feng, 2004, PP. 29-30, Luan, 2000, PP. 58-60). Since the further step of modification was undertaken in alkaline condition and in order to attain more protein, the pH value applied could be increased properly. But cottonseed protein was prone to degradation and denaturation and difficult to be kept in strong alkaline condition, so pH value of 12.5 was selected for further investigation.

3.5 Effect of temperature

Cottonseed flour (constant weight) was extracted at same solvent-to-flour ratio of 12 (w/v) with stirring at pH 12.5 and different temperature for 30 minutes. The results are shown in Figure 4.

With the increasing of temperature, extraction rate of protein increased. Extraction rate varied almost linearly with temperature. When temperature increased, molecules moved fast, mass transfer rate of interface between solid and liquid developed. Therefore, increasing temperature could promote mass transfer and solubility, reduce viscosity of solution and thus increase extraction rate. However, high temperature could cause the reduction of protein activity and thermal denaturation of protein. Additionally, with the increasing of temperature, color of the protein extraction became dark, so temperature of 60℃ was selected for further investigation.

3.6 Effect of time

Cottonseed flour (constant weight) was extracted at same solvent-to-flour ratio of 12 (w/v) with constant stirring at pH 12.5, temperature 60℃ for different time. The results are shown in Figure 5.

As seen from Figure 5, with the increasing of time, extraction rate increased. After 20 minutes, extraction rate amounted to 50%. While after 40 minutes the extraction curve slope decreased obviously and extraction rate increased slowly. Until 80 minutes, extraction rate increased by only 4%, so time of 40 minutes for protein extraction was suitable.

4. Conclusions

At a solvent-to-flour ratio of 10 (w/v), the protein extraction efficiency of these four factors were classified in the order of medium>temperature>pH value>time. The optimized extracting parameters of medium, solvent-to-flour ratio, temperature, pH value and time were 0.1NKOH, 12, 60℃,12.5 and 40 minutes, respectively. Under this optimum condition, the protein extraction rate was above 70% and the obtained protein extraction could be used for chemical modification directly without further process.

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Table 1. Assignments of the levels to factors in orthogonal design

Factors	Level 1	Level 2	Level 3
Medium	1%Ammonia	0.1NNaOH	0.1NKOH
pH	12	11.7	11.5
Temperature(℃)	20	40	60
Time(min)	20	40	60

Table 2. The matrix associated with the analytical results

Factors Number	pH	T/℃	t/min	Medium	Extraction rate(%)
1	12	20	20	Ammonia	14.33
2	12	40	40	NaOH	38.45
3	12	60	60	KOH	65.03
4	11.7	20	40	KOH	25.38
5	11.7	40	60	Ammonia	21.67
6	11.7	60	20	NaOH	51.36
7	11.5	20	60	NaOH	19.97
8	11.5	40	20	KOH	26.01
9	11.5	60	40	Ammonia	10.1
Average1	39.270	19.893	30.567	15.367	
Average2	32.803	28.710	24.643	36.593	
Average3	18.693	42.163	35.557	38.807	
$\lvert k_{max}-k_{min} \rvert$	20.577	22.270	10.914	23.440	

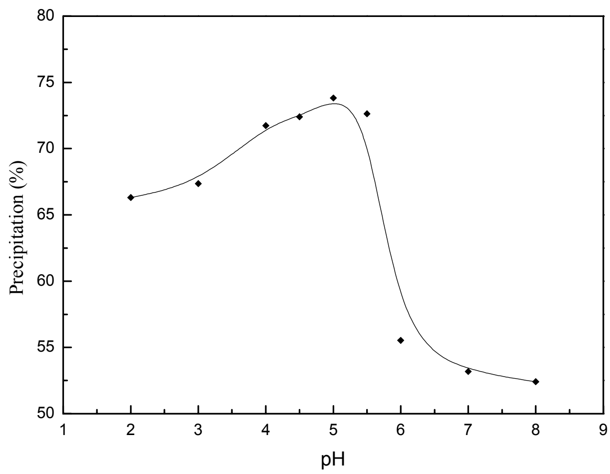

Figure 1. Effect of pH value on precipitation of cottonseed protein

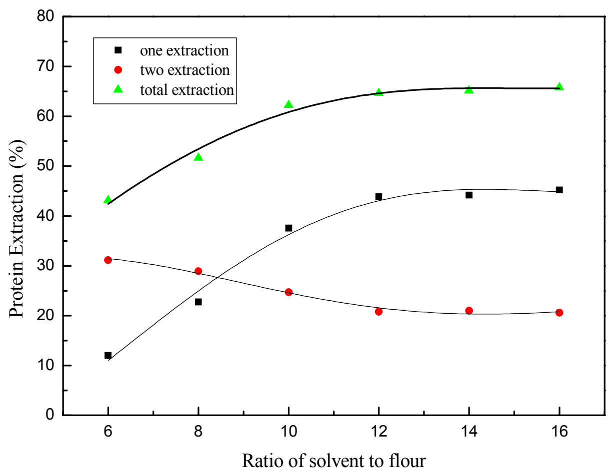

Figure 2. Effect of solvent-to-flour ratio on extraction of cottonseed protein

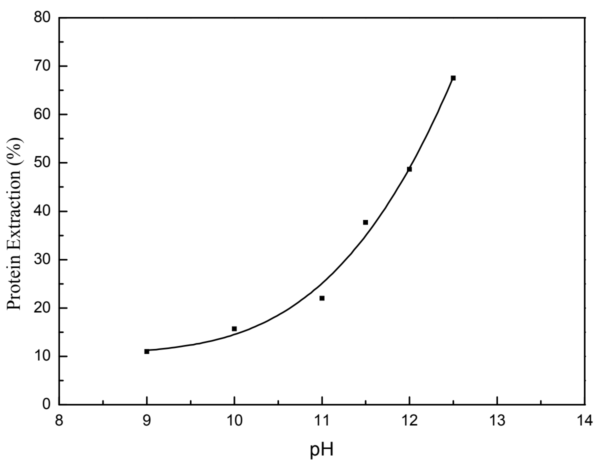

Figure 3. Effect of pH value on extraction of cottonseed protein

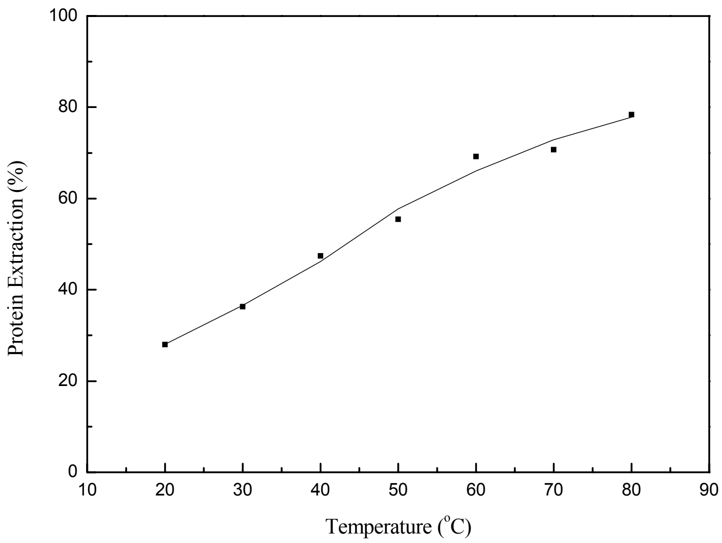

Figure 4. Effect of temperature on extraction of cottonseed protein

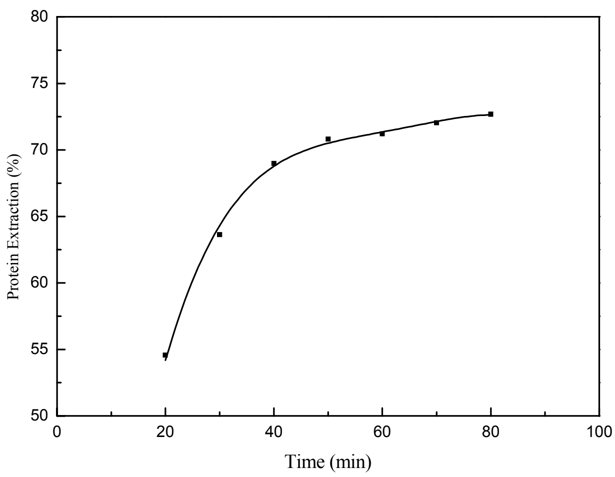

Figure 5. Effect of time on extraction of cottonseed protein