Technical Research of Industrialized 7S and 11S Soy Protein Fractionation

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Abstract: Combining with our isolation techniques (method of Guo), this article compared different separation methods of 11S and 7S fractions with industrial methods. The principles of most separation techniques are "alkali extraction and acid precipitation" and "cold precipitation" effects. Wu's method succeeded in isolation 11S and 7S fraction for the first time, the later researches mostly focus on its improvement. The method of Guo is the only method to extract protein in a neutral condition, thus saved the usage of alkali and avoided the denaturation of protein. Meanwhile, this article pointed out the problems in the current industrial production. Although lots of improvements were made on the separation process, problem still exist, such as complicated procedures, low yields, high costs of production and so on. Therefore, the writer further proposed the directions of efforts and improvement for the realization of industrial production.

Key words: Soy protein; Separation techniques; Industrial production; 7S rich fraction; 11S rich fraction; Yield; Purity.

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工业化大豆蛋白7S和11S组分分离技术研究进展

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摘 要:该文围绕目前工业化生产11S和7S组分的分离技术成果,结合实验室的分离技术(Guo法),比较了各种分离方法的差异。结果表明:分离提取技术利用的原理几乎均建立在"碱溶酸提"和"冷沉"作用基础之上。Wu首次成功地进行了7S和11S的工业化分离,之后的研究多是对此方法的改进和修饰。而Guo法是唯一采用中性条件抽提的工业化分离方法,在节省了原料碱的同时,避免了蛋白的变性。同时,该文提出了目前工业化生产大豆组分蛋白中存在的问题,虽然不断在加工方式上进行改进,但仍存在步骤复杂、产率低、生产成本高等方面的问题,因此作者进一步对实现工业化分离大豆组分蛋白提出了需要改进和努力的方向。

关键词:大豆蛋白;分离技术;工业化生产;7S富集蛋白;11S富集蛋白;产量;纯度

1 Introduction

Soy protein, an important vegetable protein, is widely used in food industry. Two major components of soybean protein are Glycinin (11S) and β-conglycinin (7S), which account for approximately 40% and 30% of total soybean protein, respectively[1-3].

Glycinin is a heterogeneous protein having a molecular weight of 340 to 375 kDa[4-6]. Main structure of the 11S is a hexameric protein composed of several subunits, each subunit consists of an acidic (A) and basic (B) polypeptide chain connected by a disulphide linkage(An—S-S—Bn)[7]. The main body is a stable hexagonal structure of two rings which is formed by the interaction between six acidic subunits (A_1, A_2, A_3, A_4, A_5, A_6) and six basic subunits (B_1, B_2, B_3, B_4, B_5, B_6)[8-9]. Glycinin has the characteristic of "cold precipitation". About 86% glycinin can be precipitated when the defatted soybean protein extracting solution is kept in ice bath (0~2℃) overnight[10].

β-conglycinin is a glycoprotein containing 3.8% mannose and 1.2% glucosamine with a molecular weight of 126 to 171 kDa. It is composed of α′, α, and β subunits, which make up seven heterogeneity proteins including B_0(α′ββ), B_1(αββ), B_2(α′αβ), B_3(α′α′β), B_4(ααα′), B_5(ααα) and B_6(βββ) with hydrophobic and hydrogen bonding[9]. Although the isoelectric point (pI) of β-conglycinin is pH 4.8-4.9[11], each subunit has its own thermal stability[12]. By the same token, under the different pH and ionic strength,

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other characteristics, such as surface hydrophobicity, thermal stability, solubility, thermal cohesion and emulsifying capacity are substantial difference of each β-conglycinin subunit[12].

As to physical, chemical and functional properties, 7S and 11S globulin are significantly different between their amino acid compositions and structures. For instance, gel-forming ability and stability of 11S globulin are superior to 7S globulin[13]. 11S globulin presents superior elasticity, cohesion and extensibility to 7S globulin, and it can form better gel with water-holding capacity[14]. With the increasing of 11S and 7S ratio, the hardness and viscosity of protein gel increased[15-16], and emulsifying ability of soy protein showned an inverse relationship with 11/7S ratio[17]. The derivatives of 7S globulin and 11S globulin hydrolysates showed special physiological activity. It was reported that glycopeptides hydrolyzed from β-conglycinin are able to modulate the balance of gut microbiota. It inhibited not only the adhesion of pathogenic bacteria to intestinal cells but also the *Salmonella typhimurium* translocation in Caco-2 epithelial cell monolayers[18-20]. The 7S globulin hydrolysates are also presented to reduce blood cholesterol[21-22] and plasma triglyceride levels[23-25].

Therefore, no matter industrialized food ingredients and functional food productions, there is a wide market prospect of 7S and 11S protein isolation. This paper summarize the researches of 7S and 11S including separating method, product yield, product purity and industrial production, and discuss the industrialization of 7S and 11S separation technique.

2 Methods for industrialized separation of 7S and 11S fraction

The research of 7S and 11S soy globulin separation technique was started in 1960s. Wolf and others utilized the "cold precipitation" effects of 11S to get the crude 11S faction, and then prepared a relatively pure 11S globulin according to the classic method of ammonium sulfate salting-out in 1962[26]; however, this method could not be used for separation of 7S globulin at the same time[27].

The pI of 11S globulin is pH 6.4, while the pI of 7S globulin is pH 4.8 – 4.9[11,28]. With utilizing the principle of different soybean protein components could precipitate in its isoelectric point, Thanh and other[29-30] directly separated 11S globulin and 7S globulin by adjusting the pH. Soy storage protein is a kind of alkali-soluble protein. Thus, it can be extracted from the defatted soybean with alkaline solution. Meanwhile, the 7S and 11S fraction can be obtained respectively because they are precipitated in different pI. That is the so-called "alkali extraction and acid precipitation" method. It is the first time to separate 7S globulin and 11S globulin simultaneously and laid the foundation for the subsequent separation technique of the soy protein components.

Existed reports of separation technology indicated the main purpose of separation involves two aspects: one is to study the chemistry and physiological activity of 7S and 11S globulin, and the other is to use their special features for food industrial application. Therefore, in order to meet the requirements for the different use of isolates, the additives and the technical routes which used in the separation process are different. For instance, adding the reducing agent such as 2-mercaptoethanol (2-ME) or sodium bisulfite (SBS) in the extracting process to break disulfide bonds between the protein components was just for further improve the purity of isolates[31]. In order to improve the product yield, the inorganic salt is added for the "salting-out" effect breaking the electric double layer of protein and promoting the protein precipitation[32].

However, the purity requirements of 7S and 11S fraction are not so harsh if they are just applied in food industry. For the industrial production, the principles for choosing separation technique mainly contain cost savings, high yield, simple separation steps and the feasibility of industrial production as the principles for the separation. Therefore, this article elaborates the current separation methods of 7S and 11S fractions from the laboratory-scale preparation and pilot-plant-scale production.

2.1 The methods of Wu and Ricket

The methods of Thanh and Nagano are all the classical method for laboratory-scale preparing 7S and 11S components; however, these two methods are hard to reach the industrial produce for the reasons of discontinuous operation, cockamamie process and the use of non-food grade chemical reagents. Wu[33] modified

the method of Nagano[34], developed the industrial separation methods of 7S and 11S components in which the amount of raw material consumption was 20 kg.

Wu changed the pH of basic extract step to 8.5, and reextracted the flake residue for the purpose of improve the protein yield. Though the purity of protein is not high, it is still one of the important references for the technological development of industrial production. The Specific parameters and technological process were shown in Table 1.

Based on the above-mentioned method, Wu[35] developed the ultrafiltration membrane technology into the industrial separation technique in 2000. By the reason of separation 7S component, ultrafiltration and reverse-osmosis membrane technology were operated on the supernatant after extracted 11S globulin. Meanwhile, on the basis of Wu and the laboratory-scale (as already described in detail), Ricket[36] researched the pilot-plant separation technique and obtained the optimal technology framework. Meanwhile, these two modified methods have the same capacity of handing raw material (20 kg) with the Wu's first method[33]. The optimal process of each method is shown in Table 1.

Table 1　Operating Process for Several Industrial-sale Methods in Separating 7S and 11S Fracation[a]

Operating process			Wu(1)	Wu(2)	Ricket
Stage 1 Extraction		Material	DSF, H_2O	○	○
		RML(1)	1:10	○	○
		RML(2)	1:5	○	– –
		pH	8.5	>8.0	○
		Extract time(1)	1 h	○	○
		Extract time(2)	0.5 h	○	– –
		Temp.	20℃	○	45℃
		Centrifuge	5700 r · min^{-1}	○	9800 r · min^{-1}
Stage 2 Separation	Step 1: 11S fraction	Material	Sup1.	○	○
		Additive	0.98 g · L^{-1} SBS	○	○
		pH	6.4	6.0	○
		Time	Overnight	○	○
		Temp.	4℃	7℃	○
		Centrifuge	9800rpm	○	○
		Desalt	RC-30	RC-100	○
		Dry	Spary dry	○	○
	Step 2: Intermediate fraction	Material	Sup2.	– –	○
		Additive	0.25 mol · L^{-1} NaCl	– –	○
		pH	5.0	– –	○
		React time	1h	– –	○
		Temp.	5℃	– –	– –
		Centrifuge	9800r · min^{-1}	– –	○
	Step 3: 7S fraction	Material	Sup3.	○	○
		Additive	2-fold H_2O	0.02M SO2 ; water	3-fold H_2O
		pH	4.8	7.0	○
		Temp.	4℃	○	7℃
		time	Overnight	– –	○
		Centrifuge	9800r · min^{-1}	– –	○
		Desalt	RC-30	– –	– –
		Concentrate	– –	RC – 100	– –
		Dry	Spray dry	○	○

[a] Wu(1), Wu(2), and Ricket represent the different methods named by the inventers, Wu developed two methods, called Wu(1) and Wu(2), respectively; "○" represents the requirement process or condition is identical with the first method of Wu. "—" represents the process or requirement is not exist; RML(1) and RML(2) represent the ratio of material to liquid for the first time and second time to extract protein solution, respectively. Time(1) and Time(2) represent the extracting time for the first time and second time, respectively; RC-30/RC-100 represents membrane filtration system and a 30KDa/100KDa regenerated cellulose membrane; DSF, defatted soybean flake; RML.

2.2 The method of Guo

In order to fully demonstrate the functional characteristics of soybean protein components and the utilization of 7S and 11S protein components in the actual production, our laboratory studied the separation technique of soybean protein components in a pilot-plant-scale[37]. The capacity of handing raw material of this method was more than 300 kg. Meanwhile, it is the first time to extract protein in a neutral way and avoid the damage on the protein structure in an overmuch alkali. Simultaneously, it can save costs for the industrial production.

The steps are as following: defatted soybean meal was dispersed at a 1∶12 ratio of flakes to water and stirred the slurry. After the protein was extracted, the insoluble residue was separated by centrifugation with a decanter centrifuges (4000 r · min^{-1}). The ionic strength and pH values of the extraction were adjusted to 0.05 mol · L^{-1} and 5.5 mol · L^{-1}, respectively, and the food grade reducing agent was added to the extraction. With the processes of stirring and keeping the solution for a while, a decanter centrifuges (4 000 r · min^{-1}) was used for separation of components. The supernatant was just the 7S rich fraction while the precipitation was the 11S rich fraction.

The supernatant was adjusted to pH 4.8 with 2 mol · L^{-1} HCl and centrifuged (4 000 r · min^{-1}) after holding a slight time, the precipitation was just the 7S rich fraction. Meanwhile, the precipitated glycinin was redissolved in an aqueous solution acidulated to pH 6.4 and centrifuged (4 000 r · min^{-1}) after holding a slight time. The precipitation was just the 11S rich fraction. After that, the precipitated glycinin and-conglycinin fractions were redissolved in an aqueous solution. Both fractions were centrifuged again before the desalting process and neutralized and dried in a spray-dryer.

2.3 Summary

The reported pilot-plant-scale separation techniques of these four methods were mentioned above. Comparatively, the first method used by Wu[34] was more cockamamie, which adopted twice repeat extract process for the purpose of improving protein yield, however, it is relatively complicated the operate process. Only one exaction step was carried out in the method of Ricket, with simple operating procedure than Wu method, and he set the requirement for the extraction temperature. The second method of Wu[52] used was relatively simple, it was separated by 2 steps which omit the process of removal intermediate fraction, and the separation of 7S component was carried out by using ultrafiltration membrane so that the efficiency was improved. Generally, the biggest problem of ultrafiltration protein liquid was the membrane contamination, however, this method was not discussed in detail about this problem. The 7S rich fraction and 11S rich fraction were collected separately before the curd components were separated in the method of Guo, for this method, the operation is relatively simple, the requirement of equipment is not so high.

3 Comparison of separation effects on industrialized production technology methods

According to the available separation techniques, it seems that most methods were based on the principles of "alkali extraction and acid precipitation." and "cold precipitation". However, those fractionations were obviously different in some details, such as separation steps, pH value, precipitating agent, which also lead to variation of the resultant products of 7S and 11S. Subsequently, the effects of various separation techniques on the yield and purity of the product are compared.

At present, the researches for the industrial-scale separation technique are mainly four methods illustrated in Table 2. Wu studied the separation technique of protein components in pilot-plant-scale for the first time (Wu(1) in Table 2). The yields of 11S rich fraction and 7S rich fraction of this method were relatively low (9.5% and 10.7%, respectively). But the purity of 11S globulin was up to 90%.

On the basis of the previous studies, Wu simplified the separating procedure of 11S globulin and 7S globulin by the combination of ultrafiltration membrane technique and the principle of pI precipitation (Wu(2) in Table 2). The ultrafiltration membrane was used to separate and purify 7S rich fraction. In the both two methods, the yield of method 2 was almost at twice as that of the method 1, because the intermediate fraction was kept in the supernatant (after removing 11S precip-

itation fraction), while the purity of 7S rich fraction was only 62.6%, which was also caused by the existing of intermediate fraction. However, it is feasible to use method 2 in industrial production because the simple operating process and the low requirement for purity of the raw material.

Meanwhile, Rickert put forward another pilot-plant fractionation technique according to the first method of Wu. This method was relatively simple with keeping the temperature of protein extraction at 45℃ and omitting the second extracting step. The yields of 11S and 7S rich fractions were higher than that of Wu's method, due to the reason of high temperature could promote the extracting efficiency of protein. The purity of 7S rich fraction was 68%, which was lower than Wu's method, and the purity of 11S globulin was not reported.

The method of Guo which was put forward by our laboratory is very different from others on the process of separate protein components. The yield of 11S fraction was 2 times higher than Ricket's method and 3 times higher than Wu's method, while the yield of 7S rich fraction was just 5.5%. The purity of 7S component and 11S component were lower than others. It was probably caused by the low-speed centrifugation ($4{,}000\ r \cdot min^{-1}$). Comparatively, because of the high yield of 11S component, it is an effective method for the industrialized production.

4 Discussion on the industrialized production

Generally, the separation techniques vary with the different research purposes. Thus, whether the reported pilot-plant-scale production was suitable for industrial production?

Besides the feasibility of industrial production, any product which wants to realize the industrial production has to meet the following requirements as much as possible: (1) low production cost and resources consumption; (2) easy operation; (3) high output; (4) little environmental pollution

Any change could lead to a great impact on production costs. As to the operation processes of the four methods (Table 2), the first method of Wu is a 2-step extracting protein solution and 3-step separation procedure, while Ricket's method simplified the process with extracting protein solution only in one step. Although the second method of Wu carried out a 2-step extracting protein solution procedure, the 2-step fractionation was adopted to simplify the process, thereby saving the costs for the operation.

Table 2 Comparison of separation effects on industrialized production technology methods[a]

Methods	11S rich fraction			7S rich fraction		
	Yield/%	Protein content/%	Purity/%	Yield/%	Protein content/%	Purity/%
Wu(1)	9.5	91.1	90.6	10.7	97.7	70.4
Wu(2)	9.7	91.2	92.8	19.6	91.6	62.6
Ricket	11.7	99.8	--	12.25	91.3	68
Guo	28.5	94.46	86	5.5	93	65

[a]Wu(1), Wu(2), and Ricket represents the different methods named by the inventers, Wu developed two methods, called Wu(1) and Wu(2), respectively.

The material to water ratio in process markedly affects the consumption of resource. Obviously, the more water is used, the more resource is consumed. At this point, Ricket's method adopted a smallest material to water ratio (1∶10), thus saving the costs correspondingly. Water just plays a role of media in the whole separation process. The discarded waste-water would increase if the water is used too much, and then cause a relatively great environmental pollution. Nevertheless, Ricket's method put forward a demand of constant temperature for extraction (45℃) and required temperature reduction quickly after finishing extraction step. From the perspective of industrialization, the treatments of heating or cooling will increase the energy consumption and the costs and acid production. The precipitation principle of the methods of Wu and Ricket was "alkali extraction and acid precipitation". Thus, these methods require a large amount of alkaline and acid.

Feasibility is a prerequisite for the realization of industrial production. The method of using ultrafiltration membrane is a simple process with high yield. However, it is easy to cause membrane contamination. Although many researches have been done on membrane technology, it is still hard to apply it on the in-

dustrial production for the membrane contamination. On the other side, Guo's research was carried out the pilot-plant-scale experiments by produce line in Tianyuan Group in Gansu Province and High Technology Group in Heilongjiang Province, respectively. It was just dedicated to meet the needs of industrial production and the results showed it was feasible and it can be committed to the promotion of industrialization.

In summary, each method has its strengths and weaknesses, both of which should have to keep its own advantages, make up the disadvantages, minimize the production costs and improve yield as much as possible for the purpose of realize the industrial production.

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(")#)仁礼45 1&'ù-•d'趋½Ã应j ¸ 施浅见(5)4&'(H!!%%">#$!:=4"YA@7 8 Q4(6HKUWAFA@3 VAKZ 27 CBK G@@6G[62!CUK7L @L F267CKU?K@G6UKG C2/BAY@ G G2ETK@7 AYL6GCUE(5)4(2ETK@7(FAK7FK q YKFB7232IE!!%%">#$!:=4#

(&%)#娇E!谢)•4黑VE&'gù特A; JK•€(5)4&'(H!!%%"'#$=:"4"5A@2 5!mAK m54/6UUK7C G@@6G@L LKVK32H?K7C GCL@@KI E 2WG2ETK@7 HU2L6FCA27 AY PKA327IJA@7I HU2VAYFK(5)4(2ETK@7(FAK7FK q YKFB7232IE!!%%"'#$=:"4#

# #

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]/!9>4

0*/*&*4;*.

(!")#YB@7B 1 P!)i6T2 X!(BAT@@@AX40G23@@A27 @L FB@U@FCKUAJ@CA27 2WCBK?63CAHBK=(I32T63AYG 2WG2ETK@7(5)4 ^3@7C^BEGA232IE! &"=9! 9'$&"]!!4

($%)#YB@7B 1 P!)i6T2 X!(BAT@@@AX4 M@2U HU2CKAYG 2WG2ETK@7 GKKLG@GCL@AB CW2U2@L W@FCA27 @L CBKAUFB@U@FCKUAJ@CA27 BKCKU2IK7KACE 2W+KC@F27I3EFAYAY(5)4 526U7@3 2W-IUAF63G6U@3 @L `22L FBK?AGCUE! &"=!!>$&&&=]&&!&4

($&)#NK@.-! M6UHBE^-! 52B7G27 Q-4, WKAFCG 2WUKL6FAYI @K7C F27FK7CU@CA27 27 G2E HU2CKAY W@FCA27@CA27 @L W67FCA27@KACE(5)4 526U7@3 2W 22L(FAK7FK!!%%!=&"$#$/!%%]/!%O4

($!)#NK@.-! M6UHBE^-! 52B7G27 Q-4, WKAFCG 2W @3F27FK7CU@CA27 AYW6K7FK 27 ":F27I3EFAYAY EAK3L @L H6UACE L6UAYI G2E HU2CKAY W@FCA27@CA27(5)4 526U7@3 2W 22L(FAK7FK!!%%!=&">#$/!>=

($$)#<6(! M6UHBE^-! Q@2UK7FK-5! KC@34^A32CHB@7CW@FCA27@CA27 2WG2ETK@7 I3EFAYAY @L ":F27I3EFAYAY(5)4 526U7@3 2WCBK-?KUAF@7)A3/BK?AGCG(2FAKCE! &"""!='$!O9]"$4

($>)#.@@72 Y! MC2BA 2 P! PA2E6i AM! KC@34 NE7@AF VAGF2K3@GCAF GG6LE 27 CBK IK3@CA27 2W=(I32T63AY W2?G2ETK@7G(5)4 526U7@3 2W-IUAF63G6U@3 @L `22L/BK?AGCUE! &""!!>O$">&]">>4

($9)#<6(! ^@CUAFA@-M! Q@2UK7FK-5! KC@34(A?HBAAKL ^U2FKGG W2U(2ETK@7 R3EFAYAY @L ":F27I3EFAYAY AY `U@FCA27@CA27(5)4 `22L/BK?AGCUE!%%%!>O"=#$!=%]!=%O4

($')#8AFi KUCN-! 52B7G27 Q-! M6UHBE^-4 O?HU2VKL W@FCA27@CA27 2W I3EFAYAY @L ":F27I3EFAYAY @L H@CCA27AYI 2WHBEC2FBK?AF@3G(5)4 526U7@3 2W-IUAF63G6U@3 @L `22L FBK?AGCUE!%%>!9!$&=!']$>4

($=)#R62(Y! P@7*5! MK7I*! KC@34-?KCB2L W2UK!CU@FCA7I G2E TK@7 HU2CKAY(^)4/BAY@^@K7C!%%>&%%"9O'4>4