

Recent Advances in Soy Protein Extraction Technology

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Abstract

Protein extraction from soybeans is a vital part of the soy industry. Traditionally, the extraction of soy protein has been done by alkaline extraction and isoelectric precipitation. As technology has advanced, more extraction techniques are superior to this traditional method. In this review, the composition and classification of soy protein are summarized. Next, the current emerging technologies for soy protein extraction are highlighted. Three extraction technologies, namely reverse micellar, enzyme-assisted and membrane ultrafiltration, are reviewed in detail. Finally, the research prospects and trends of soy protein extraction technology are also summarized.

1. Introduction

Soy, an important cash crop, is used in the production of protein. Most importantly, soy protein contains a wide range of essential amino acids for humans and has a high nutritional value. In addition, soy protein is widely used in the food industry due to its high yield, low cost, and appropriate functional properties. Soy protein can be classified into four different components based on different sedimentation factors, namely: 2S, 7S, 11S, and 15S fractions. The 2S fraction includes the majority of soy protein albumins, whereas the 7S, 11S, and 15S fractions mostly contain globulins. Soybean storage proteins consist mainly of β -conglycinin and glycinin, which are 7S and 11S globulins respectively. 11S globulins make up more than 40% of total soybean protein and have better gelation properties than 7S globulins, which are often used as food additives to improve the texture and taste of foods. The 11S globulin molecule is a two-ring hexagonal structure consisting of six acidic and six basic subunits linked by disulfide bonds. Due to the tight structure of the 11S globulin molecule, most of the active groups are wrapped in the globular structure, making it difficult to express its physiological activity. Depending on the protein content, soy protein products are divided into three main categories: soy flour, soy protein concentrate (SPC), and soy protein isolate (SPI) (Wang et al., 2004). Soy flour has a protein content of 50 %. Washing the soy flour in hot water produces SPC with a much higher protein content (60-68 %). SPI has a significantly higher protein content (85-90%) than SPC and contains almost no water-insoluble carbohydrates.

Functional properties affect the use of soy protein in food, attributable to the fact that protein composition and conformation determine its physicochemical properties. The use of soy protein as a food ingredient will be more widespread when it has the appropriate functional properties (i.e. gelation, foaming, solubility, volatile compounds, emulsification, adsorption, etc.). As protein composition and functionality are influenced by processing conditions (Kinsella, 1979), it is necessary to consider preparation methods when preparing soy proteins with specific functionalities.

Currently, the widely used method for soy protein extraction is the alkali solution–acid precipitation method (ASAPM). The first disadvantage of the traditional method is the poor solubility of SPC and SPI when rehydrated (Fisher et al., 1986). This is because the extraction of proteins uses extreme conditions such as acids, bases, heat treatment, or centrifugation, resulting in protein denaturation. In addition, SPC and

SPI produced by the traditional process may contain high levels of phytic acid. Phytic acid complexes with divalent cations form phytate minerals or protein mineral phytate complexes, which affect the bioavailability of the minerals (Grynspan and Cheryan, 1989). Phytic acid may also lead to the reduced solubility of proteins. Another disadvantage of traditional methods is the pollution of the environment. Large amounts of acidic and alkaline wastewater are generated during protein extraction leading to water pollution.

To address the drawbacks of traditional extraction methods, researchers have developed emerging extraction techniques for soy protein. This review article outlines three extraction techniques, including reverse micelle extraction, enzyme-assisted extraction, and ultrafiltration membrane extraction. We first examine the principles and technical characteristics of each technique. Next, we discuss their advantages. Finally, we take an integrated overview of the novel applications of each technique in soy protein extraction.

2. Extraction technology

2.1 Reverse micelle extraction

As a promising extraction technology for protein extraction from soy, reverse micelle is a nanometer-sized aggregate that is formed by a surfactant, an organic solvent, and a small amount of aqueous solution (Bu, 2014). It takes various shapes such as spherical, oval, and rod. Surfactants dissolve in non-polar organic solvents and form reverse micelle when their concentration exceeds the critical micelle concentration. There are many different surfactants used to prepare micelles, including anionic (such as AOT and SDS), cationic (such as CTAB), zwitterionic, non-ionic (such as Spans and Tweens), and mixed surfactants (Lépori, 2016). Bis (2-Ethylhexyl) sodium sulfosuccinate (AOT), an anionic surfactant, has received a lot of attention because AOT-based reverse micelle does not require co-surfactants and can encapsulate large amounts of water in their aqueous core (Fuglestad, 2016). As shown in **Fig. 1**, in the reverse micelle, the non-polar groups of the surfactant are in contact with the organic solvent on the outside, while the polar groups are arranged on the inside, forming an aqueous core. The size, shape, and internal structure of aqueous cores, the number of aggregations, and microviscosity are critical to the solubility of the biomolecules in the micelle core. A considerable number of hydrophilic biomolecules can be dissolved in the aqueous cores of the reverse micelle, such as proteins, amino acids, enzymes, nucleic acids, short peptides, and DNA. These biomolecules are protected from denaturation by organic solvents inside the aqueous cores (Hong SC, 2015).

2.1.1 Extraction steps for the reverse micellar extraction

Protein extraction using the reverse micelle system consists of two steps: forward extraction and backward extraction. Forward extraction involves the dissolution of the protein into the polar aqueous core (**Fig. 1**). Subsequently, in the backward extraction, the protein-containing solution is released from the polar aqueous core and transferred to a fresh aqueous phase for recovery (Leser ME, 1989).

2.1.2 Factors affecting extraction rates

The reverse micelle method can effectively increase the extraction rate of proteins compared to the traditional alkali solution–acid precipitation method. As shown in **Table 1**, the percentage of soy protein extracted by the reverse micelle method can reach up to 95%. It has been shown in numerous studies that the extraction rate of proteins is influenced by many factors including molar ratio W_0 , reverse micelle diameter, aqueous phase pH and ionic strength (Rho, 2004), surfactant type and concentration (Shin, 2002), and co-surfactant (Lee, 2004). These factors affect the degree of protein solubility. Among these factors, the extraction efficiency of the proteins increases with increasing W_0 (Harikrishna, 2002). W_0 is the ratio of water to surfactant and can be altered by an aqueous buffer containing a certain amount of salt, which determines the size and molar ratio of the aqueous core (Ghazi, 2006). As W_0 increases, the core radius increases, indicating that not only small molecules of protein can enter the micelles, but also large molecules of protein. Nevertheless, when W_0 is too high, the surfactant molecules are released from the micelles into the organic phase due to hydrophobic effects, thus reducing the number of micelle aggregates and the efficiency of protein extraction (Bu, 2012). Bu et al. (2012) emphasized that the efficiency of forwarding extraction of soy protein in the AOT reverse micelle system increased with increasing reverse micelle diameter.

Interactions between reverse micelles and proteins lead to structural changes in proteins, the main driving forces of which are hydrophobic effects, hydrogen bonding and electrostatic interactions (Correa, 1998.). Forward extraction at a pH above the isoelectric point suggests a hydrophobic interaction between the soy protein and AOT. Due to reduced hydrophobic interactions, the addition of Triton-X-100 to AOT reduced the extraction efficiency compared to AOT alone. The reason for the reduced extraction efficiency of the Triton-X-100 may be the lack of a strong driving force to diffuse the soy protein into the nonionic reverse micelle core (Zhao et al., 2010a). Zhao et al. (Zhao et al., 2011b) observed that, due to the reverse micelle function, the amino groups near the surface of the 7S and 11S globulin powders were exposed through bond breakage, increasing the surface N atomic percentage in the 7S and 11S globulin powders. Small changes in powder surface composition or bulk composition have the potential to change the functional properties of 7S and 11S globulin powders. Zhao et al. (Zhao et al., 2010b) concluded that the interaction between soybean protein and surfactant was the main factor determining the extraction rate of protein from reverse micelles. Forward extraction was controlled not only by electrostatic interactions between the charged protein and the polar head of the surfactant (Luisi, 1988) but also by hydrophobic interactions between the non-polar region of the protein and the surfactant tail (Rajib, 2005). In particular, the alcohol molecule has an influence on the formation and destruction of reverse micelles and improves the efficiency of protein extraction (Hong DP, 1999).

2.1.3 Advantages of the reverse micelle extraction

Reverse micelle has a variety of advantages such as enormous interfacial area, thermodynamically stable and optically transparent, low cost due to the recovery of surfactant and nonpolar solvents, ease of scale-up and simple control of the reaction variables (Sereti V, 2014). Most importantly, due to the similarity of its aqueous cores to the physiological environment, the reverse micelles could prevent the denaturation of encapsulated biomolecules (Bu, 2014). A recent study has demonstrated that the reverse micelle method can better prevent breakage of the natural molecular structure of proteins compared to the traditional alkali solution-acid precipitation method (ASAPM) (Yao et al., 2021). Specifically, 11S globulin extracted by ASAPM had a higher β -fold content compared to 11S globulin isolated by reverse micelles containing more hydrophobic amino acids and fewer sulfur-containing amino acids. As a result, the surface hydrophobicity of 11S globulins obtained by ASAPM was increased. In addition, the 11S globulins separated using reverse micelles were more resistant to high temperatures. Similarly, Du (Du et al., 2020b) reported that more β -sheets but less turn structure was observed in the 7S globulin extracted by reverse micelle, indicating that the native folded structure of protein could be protected by the reverse micelle environment and 7S globulin formed a more compact conformation. It is well known that the functional properties of proteins are influenced by their structure. The low denaturation temperature, the poor thermal stability, and the strong hydrophobic interaction of 7S globulin prepared by the reverse micelle method affect its gelation process. Therefore, an improvement in the quality of thermally induced gelatin of 7S globulin was observed in the reverse micelle environment (Du et al., 2020b). As an advanced soybean protein extraction method, reverse micelles can not only separates and purifies soy protein but also improves the functionality, nutritional properties, and flavour of soy protein and reduces undesirable beany flavor. Zhao et al (Zhao et al., 2018b) concluded that the protein oil absorption capacity, solubility index, emulsification capacity, and stability as well as foaming capacity obtained by AOT reverse micelles were significantly higher than those obtained by alkali extraction isoelectric precipitation (AEIP). Soy is an essential source of amino acids (Zarkadas, 2007). Current research has shown that AOT reverse micelle extracted soy protein is a superior source of protein nutrition suitable for human consumption. For 11S globulins, the total amino acid content of the AOT reverse micelle extract was increased by 5.98% compared to the amino acid composition of the aqueous buffer extract, but the content of 7S globulins was similar. For both 7S and 11S globulins, the major amino acid content in the aqueous buffer solution was lower than that in the AOT reverse micelles (Zhao et al., 2011a).

2.2 Enzyme-assisted extraction

Enzyme-assisted extraction uses water and protease to extract protein from soybeans and is considered an al-

ternative extraction method to alkaline extraction which involves pollution (Campbell KA, 2011). As a mild extraction method, enzyme-assisted techniques minimize side reactions (Sari et al., 2013). Enzyme-assisted extractions are considered environmentally friendly technologies as they offer a green chemistry possibility for the food industry looking for cleaner routes. Recent studies on enzyme-assisted extraction have shown that it offers faster extraction rates, higher recoveries, less solvent use, and lower energy consumption than non-enzymatic methods and therefore represents a potential alternative to traditional solvent extraction methods (Vergara-Barber, 2015.). Compared to alkaline extraction, the addition of enzymes results in a reduction in protein size due to protein hydrolysis. As a result, proteins are more easily extracted. In addition, the use of enzymes can also be used to lower the processing pH, thus avoiding severe conditions of protein denaturation (Sari et al., 2013). Enzyme-assisted countercurrent extraction significantly increased the protein yield compared to alkaline extraction and acid precipitation. The protein had a larger molecular weight distribution, reduce flavor volatiles, higher thermal stability, and surface hydrophobicity as evidenced by the denaturation temperature and enthalpy change of the protein (Wei et al., 2017). Under alkaline pH conditions, 80% of soybean meal protein is extracted without the addition of enzymes, while the addition of enzymes increases the protein extraction yield of soybean meal to 90% (Sari et al., 2013). Many studies have shown that enzyme-assisted extraction has been used to enhance the nutritional value and alter the structural properties of proteins (Lu, 2016). The process of enzymatic hydrolysis of soy proteins has obtained many peptides in cancer prevention, anti-hypertension, and reducing blood cholesterol (Hoa N T, 2014,). Compared to natural SPI, SPI prepared by enzyme-assisted treatment has higher hydrophobic amino acid, surface hydrophobicity, and interfacial adsorption properties. This is due to the formation of small soluble aggregates accompanied by protein unfolding. In addition, the significant improvement in emulsification capacity and physical stability of the emulsions may be related to the higher surface protein loading. These results provide a viable route for the production of nutrient-enhanced soy proteins with excellent emulsification properties for application in the food industry as novel functional ingredients (Lu et al., 2016).

2.2.1 Cellulases

Cellulases are produced by molds, bacteria, or single-celled organisms and can hydrolyze cellulose and catalyze the separated links glucoside in the cellulose molecule, with the end product being glucose (Hoa N T, 2014,). The enzymes (cellulases, hemicellulases, and pectinases) seem to be effective in breaking down the structure of the cotyledon cell wall and lipid body membranes, which leads to disruption of structural integrity, thereby increasing the permeability of the cell wall, finally resulting in enhancement of the extraction yield (Puri, 2012). Several studies on enzyme-assisted extraction of soybean flour have shown promising progress in increasing protein yield (Jung, 2006.) as well as improving the nutritional and sensory properties (Wei, 2018.) of the extracted product by cellulases. A study demonstrated that treatment with cellulase, xylanase, and pectinase alone for a 2-hour alkali extraction resulted in a 13% increase in protein yield compared to a 3-hour alkali extraction. Thus, with the help of the enzymes, not only the alkaline extraction time is reduced but also the protein yield is positively affected. Furthermore, the proteins from the enzyme-assisted alkaline extraction exhibited better solubility, emulsification, and whipping properties (Perović et al., 2020).

2.2.2 Proteases

Proteases help to hydrolyze the oleosins, the lipophilic protein surrounding lipid bodies, thereby reducing the surface activity of the oleosin and removing the lipid (Rosenthal A, 1996). In terms of the enzymes evaluated, protease was the only one that led to a significant increase in protein extraction under certain circumstances, i.e. when large particles or heat-treated flour were used (Rosenthal A, 2001,). In another study, 0.5% (g enzyme per gram of biomass) of protease was combined with 0.5% of cellulase simultaneously to extract protein from soybean flakes. This combination resulted in a 75% increase in the yield of protein from soy flakes (Lamsal, 2006,). A response surface methodology was used to assess the effect of protease on protein extraction rates. The protein yield increased from 27.8% to 66.2% only when heat-treated flour or large grains of non-heat-treated flour were used in the extraction process (Rosenthal A, 2001,). The effect of two commercial endoproteinases (Protex 6L and Protex 7L) on the extraction rate of soy protein during

enzyme-assisted extraction was investigated. Protex 6L was more effective than Protex 7L in extracting free oil, protein, and total solids. The protein extraction rate was 85% using 0.5% Protex 6L (De Moura, 2008). Protease-assisted extraction methods have the potential to hydrolyze proteins. Without enzymatic treatment, the molecular weight of the soy protein ranges from 99 to 7 kDa. Protex 7L and Protex 6L hydrolyze the soybean to produce extracted proteins with molecular weights below 54.1 kDa and 30 kDa (De Moura, 2008).

2.2.3 Flavourzyme

Flavourzyme is an aminopeptidase consisting of a peptidase, a medium-sized endopeptidase, and an exopeptidase, which is a mixture. It is produced by deep fermentation of *Aspergillus oryzae* and is used to hydrolyze proteins under neutral or slightly acidic conditions. The optimum temperature for active flavor enzymes is around 50-55°C and the pH is around 5.0-7.0. The optimum pH for enzyme activity is around 7. Hoa et al. optimized the hydrolysis conditions of the soy protein using flavor enzymes to obtain the highest soluble protein recovery of 61.78% (Hoa N T, 2014,). Flavourzyme, Protamex, and Alcalase were chosen as three enzymes suited for hydrolyzing soybeans, and the three enzymes were compared for soy protein extraction at the same enzyme content. The results showed that Flavourzyme gave the highest soluble protein recovery. Finally, the enzymatic digestion was optimized with Flavourzyme and combined with heat treatment, resulting in a (62.47±0.12)% digestion rate for Flavourzyme (Anh et al., 2020).

2.3 Membrane ultrafiltration extraction

Among other new and unconventional processes, the purification of proteins using ultrafiltration membranes is an attractive alternative to the traditional isoelectric precipitation (NICHOLS D J, 1981,). Membrane ultrafiltration systems were first used in the early to mid-1970s for the separation of soy protein (Lawhon, 1978a). Lawhon et al. (Lawhon, 1978b) used a discontinuous percolation or re-ultrafiltration process to produce a soybean product with a protein content of approximately 90% (dry basis), while Olsen (S., 1978) concentrated defatted soybean extract from 5.6% to 25% of total solids by direct ultrafiltration to produce a soybean product with a protein content of 88% (dry basis). Ultrafiltration not only separates proteins from salt and sugar but also each other (M., 1992). The partial hydrolysis of SPI produces proteins with different molecular weight sizes (Zhang Y, 1996), which are separated by ultrafiltration membranes of different pore sizes. Depending on the difference in molecular size between proteins and other components, membrane ultrafiltration selectively separates and removes undesirable components, such as soy oligosaccharides (Endres, 2001), from soy. In addition, most of the protein in soy is recovered without producing a whey-like by-product.

Positively charged cations can interact with proteins (Pearson, 1983). Proteins are strongly negatively charged at this pH and therefore do not allow them to pass through the ultrafiltration membrane. In addition, at this pH, the phosphorus present in the soybean in the form of phytic acid interacts with the proteins (Garcia et al., 1997) and calcium to form a ternary complex (Grynspan and Cheryan, 1989), preventing the phytic acid and calcium from penetrating the ultrafiltration membrane together. The protein-mineral interaction reduces the amount of protein in the final product and limits its solubility after rehydration (Grynspan and Cheryan, 1989). on top of this, the permeate flux of protein decreases with time. This decrease is attributed to the accumulation of feed components in the membrane pores and on the membrane surface. When the reduction in flux is very large, membrane permeation is not attractive for protein separation.

2.3.1 Advantages of the ultrafiltration membrane method

Compared to traditional soybean processing methods, the ultrafiltration membrane method offers significant advantages as relatively new technology. The main advantages of ultrafiltration are the mild operating conditions and the high selectivity. The use of a gentle process produces less denatured protein than traditional disappointing precipitation. Intact soy proteins offer many special features such as water binding, adhesion, fat absorption, solubility, texture, emulsification, foaming, and flavor formation that are required by the processing of food products. Protein is recovered directly from the soy extract using an ultrafiltration membrane, thus avoiding the whey produced by traditional isoelectric precipitation methods. This process

not only increases the yield of the isolate (as whey protein is recovered from the isolate) but also produces a product with enhanced functionality and nitrogen solubility (Lawhon et al., 1981).

Treatment of soybean meal with commercial pectinase followed by ultrafiltration resulted in a soybean concentrate with a protein content of 78.5%, which had a very low concentration of phytic acid. Based on the calculated yield of the membrane separation technology, the protein recovery was 17% to 26% higher than the commercial process currently used for soy protein separation (Shallo et al., 2001). Membrane processing enables proteins to be maintained in their native state and therefore membrane-treated soy concentrates and soy powders are functionally similar. Although solubility and emulsion stability decreased with heating, hydration and emulsification activity were favorably correlated with denaturation. The essential amino acid profile of the membrane-treated soy concentrate was similar to that of the commercial isolate (Rao et al., 2002).

Ultrafiltration membrane extraction is a more environmentally friendly method, consuming less energy than other concentration techniques such as freeze-drying or evaporation. The lower energy consumption is because there is no change in the state of the solvent during the ultrafiltration process. Another advantage of ultrafiltration is that it can operate at low and ambient temperatures as well as high temperatures. Because the separation process does not use heat treatment or chemical reagents, ultrafiltration products offer better performance than conventionally produced SPI (Cheryan, 1983).

2.3.2 Application of ultrafiltration membrane method

Omasaiye et al. (Omosaiye et al., 1978) prepared a full-fat SPC by continuous filtration from an aqueous soybean extract using an ultrafiltration membrane method. It was found that this Diafiltration method was effective in removing oligosaccharides from the full-fat soybean extract. Shallo et al. (Shallo et al., 2001) enzymatically digested defatted soybean meal with commercial pectinase and diafiltered it using a porous stainless steel ultrafiltration membrane system. They obtained a soybean concentrate with a protein content of 78.5%, which had a reduced level of phytic acid. This process produced a soy concentrate with a protein recovery of 17% to 26% higher than current commercial processes. Kim et al. (Kim and Kim, 2015) used 100 kDa molecular weight ultrafiltration membranes to extract soybean protein concentrate from whole and defatted soybean meal. The protein content of full-fat soybean protein concentrate and defatted soybean protein concentrate was 68.6% and 80.0%, respectively. It was found that the membrane-treated SPC was lighter in weight and more yellow than the acid precipitated protein concentrate. The solubility, emulsification and stability, and foaming ability of the membrane-treated soybean protein concentrate were significantly higher than that of the acid precipitated protein concentrate. The amino acid profiles of the membrane treated and acid precipitated soy bean protein concentrates were comparable. This suggests that ultrafiltration membrane treatment can be used as a gentle and chemical-free process for soy protein extraction. It was demonstrated that the use of an 80 kDa ultrafiltration membrane resulted in an improved separation efficiency, yielding a SPI with a protein content of 90.0%, and also reduced membrane fouling. Furthermore, the total isoflavones in the soy protein product were reduced to 70.0 mg/kg. Due to its better solubility and lower content of anti-nutritional factors, the SPI product obtained by this process has potential applications in infant formulae (Yang et al., 2014). The content of anti-nutrients, phytic acid, in the soy protein was reduced by a series of ultrafiltration and percolation steps. After extraction, the soy protein was purified by sequential ultrafiltration and Diafiltration without pH adjustment or by adjusting the pH to 6.5. This purification method showed the lowest phosphorus to protein ratio (4.4 ± 0.3 mg P/g protein) and reduced membrane contamination compared to aqueous extraction conditions. This study demonstrates the feasibility of ultrafiltration membrane technology for the production of SPI with low phytic acid content. Studies have shown that phosphorus removal can be improved by combining bipolar membrane electro dialysis with ultrafiltration compared to using ultrafiltration membranes alone. This extraction method retains the whey-like proteins lost during conventional isoelectric precipitation. The ultrafiltration membrane extraction results in improved solubility of the isolate in the pH range of 2.0 to 4.5 and lower phytic acid content. Since the pH of liquid foods is around 3.5, this isolate has the potential for use in juice drinks (Ali et al., 2010). Sharapova and Moresoli (Mondor et al., 2010) compared the differences in infiltration time and

final product composition between electro-acidified (pH 6) and non-electro-acidified (pH 9) soy proteins using high shear tangential flow hollow fiber ultrafiltration membranes with a cut-off molecular weight of 100 kDa. They observed higher removal of calcium, magnesium, and phytic acid during filtration of electro-acidified proteins compared to non-electro-acidified proteins. pH adjustment from 9 to 6 not only reduced the permeate flux of the ultrafiltration membranes but also resulted in more severe membrane contamination and longer filtration times. It was found that discontinuous filtration increased the removal of carbohydrates and minerals, resulting in a higher protein content product, but did not improve the permeate flux of electroacidified proteins. Wu et al. (Wu et al., 1998) modified soybean isolates with protein hydrolases and then ultrafiltered them to separate these proteins into peptides of controlled molecular size. The hydrolysates were ultrafiltered using stirred cell and disc membranes (100, 50, and 20 kDa molecular weight cut-offs) and further fractionated into one retention (R100) and three permeates (P100, P50 and P20). The results showed that the soy protein peptides prepared from soy isolates modified by papain and ultrafiltered had a lower molecular weight, higher solubility, and emulsification. Due to these properties, they have the potential for application in the cosmetic and health food industries. Goodnight et al. have patented a process for ultrafiltration membranes that produce soy protein with improved digestibility, low phytic acid content, improved functional properties, high water solubility, and absence of soybean flavor and improved palatability. Phytic acid is removed by extracting defatted soybeans and separating insoluble material with a pH above 10.1. SPC is recovered by ultrafiltration to obtain fractions with antioxidant activity in different media. The low molecular weight fractions were the most active and were free radical scavengers. Protein hydrolysis increased the antioxidant activity of the >30 kDa fractions, although the heat treatment following protein hydrolysis may lead to protein aggregation, which has an impact on free radical scavenging capacity (Wieser, 2007).

3. Conclusion

Soy protein is widely consumed as common food. However, its extraction methods are the subject of continuous research. This is not only because emerging extraction technologies offer increasingly promising applications for soy proteins, but also because the contemporary food industry is faced with the challenge of creating protein sources with specific functional properties. It is crucial to investigate emerging extraction technologies for soy protein. The extraction techniques reviewed in this article have emerged mainly as alternatives to traditional techniques, with the main features being environmentally friendly, ease of operation, and the production of soy protein with specific functionalities. Although these techniques have been investigated extensively for soy protein extraction, they still suffer from many shortcomings for commercial application. Most of the examples outlined in this thesis and the results obtained are limited to laboratory-scale experiments. Although some authors emphasize the great scalability of the proposed processes, it is difficult to estimate whether global processes are cost-effective and economically viable based on laboratory experiments alone. However, their great potential lies in the ability to improve protein extraction conditions and to obtain proteins with appropriate functionality.

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References

- Ali F, Ippersiel D, Lamarche F, Mondor M. Characterization of low-phytate soy protein isolates produced by membrane technologies. *Innovative Food Sci Emerging Technol.* 2010;11:162-168.
- Anh TLQ, Hoa NTQ, Nguyen PDT, Thanh HV, Nguyen PB, Anh LTH, et al. Soybean Protein Extraction by Alcalase and Flavourzyme, Combining Thermal Pretreatment for Enteral Feeding Product. *Catalysts.* 2020;10, 10.3390.
- Bu G, Yang Y, Chen F, Liao Z, Gao Y, Yang H, et al. Extraction and physicochemical properties of soya bean protein and oil by a new reverse micelle system compared with other extraction methods. *Int J Food*

Sci Tech. 2014;49:1079-1089.

Bu GH, Liu HY, Chen FS, Liu KL, Yang YY, Gao YX. Effect of different factors on forward extraction of soy protein in reverse micelle systems. *Afr J Adv Biotechnol.* 2012;11, 7247–7257.

Campbell KA GC, Johnson LA, Jung S, de Moura JMN, Kapchie V, Murphy P. Advances in aqueous extraction processing of soybeans. *J Am Oil Chem Soc.* 2011;88:449–465.

Cheryan M. Application of membrane processing in the soy protein industry. *Intsoy Ser.* 1983.

Correa NM, Durantini EN, Silber JJ. Binding of nitrodiphenylamines to reverse micelles of AOT in n-hexane and carbon tetrachloride: solvent and substitute effects. *Colloid Interf Sci.* 1998;208, 296–103.

De Moura JMLN, Campbell K, Mahfuz A, Jung S, Glatz CE, Johnson L. Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification. *J Am Oil Chem Soc.* 2008;85, 985–995.

Du Y, Zhang Q, Zhao X, Chen F. Effect of reverse micelle on physicochemical properties of soybean 7S globulins. *J Food Eng.* 2020;282:110026.

Du Y, Zhang Q, Zhao X, Chen F. Effect of reverse micelle on physicochemical properties of soybean 7S globulins. *J Food Eng.* 2020;282.

Endres JG. Soy protein products: characteristics, nutritional aspects, and utilization. *J Am Oil Chem Soc.* 2001.

Fisher R, Glatz CE, Murphy P. Effects of mixing during acid addition on fractionally precipitated protein. *Biotechnol Bioeng.* 1986;28:1056-1063.

Fuglestad B, Gupta K, Wand AJ, Sharp KA. Characterization of cetyltrimethylammonium bromide/hexanol reverse micelles by experimentally benchmarked molecular dynamics simulations. *Langmuir.* 2016;32(37), 1674–1684.

Garcia M, Torre M, Marina M, Laborda F, Rodriquez AR. Composition and characterization of soybean and related products. *Crit Rev Food Sci Nutr.* 1997;37:361-391.

Ghazi K, Domimique P, Jean-Pierre K, Tahar T, Pierre L. Slow dynamics of water in reverse micelles. *Comptes Rendus Chimie.* 2006;9, 493–497.

Grynspan F, Cheryan M. Phytate-calcium interactions with soy protein. *J Am Oil Chem Soc.* 1989;66:93-97.

Guanhao B. Effects of different factors on the forward extraction of soy protein in reverse micelle systems. *Afr J Biotechnol.* 2012;11, 10.5897.

Harikrishna S, Srinivas ND, Raghavarao KSMS, Karanth NG. Reverse micellar extraction for downstream processing of proteins/enzymes. *Adv Biochem Eng Biotechnol.* 2002;75, 119–183.

Hoa N T, Minh N P. Investigation of enzymatic optimization by flavorsome and cellular for soy protein hydrolysate powder. *Int J Adv Pharm Biol Chem.* 2014;3: 563-574.

Hong DP. Evaluation of the alcohol-mediated interaction between micelles using percolation processes of reverse micellar systems. *Kuboi R Biochem Eng J.* 1999;4:23–29.

Hong SC PK, Son YH, Jung HS, Kim K, Choi SJ. AOT/isooctane reverse micelles with a microaqueous core act as protective shells for enhancing the thermal stability of *Chromobacterium viscosum* lipase. *Chang PS Food Chem.* 2015;179:263–269.

Jung S, Lamsal BP, Stepien V, Johnson LA, Murphy PA. The functionality of soy protein produced by enzyme-assisted extraction. *J Am Oil Chem Soc.* 2006;83, 71–78.

Kim HJ, Kim BK. Comparison of soy protein concentrates produced using membrane ultrafiltration and acid precipitation. *Food Sci Biotechnol.* 2015;24:67-73.

- Kinsella JE. Functional properties of soy proteins. *J Am Oil Chem Soc.* 1979;56:242-258.
- Lamsal BP, Murphy PA, Johnson LA. Flaking and extrusion as mechanical treatments for enzyme-assisted aqueous extraction of oil from soybeans. *J Am Oil Chem Soc.* 2006;83, 973–979.
- Lawhon J, Rhee K, Lusas E. Soy protein ingredients prepared by new processes—Aqueous processing and industrial membrane isolation. *J Am Oil Chem Soc.* 1981;58:377-384.
- Lawhon JTH, Mulsow D, Mattil KF. Optimization of protein isolate production from soy flour using industrial membrane systems. *J Food Sci.* 1978;43, 361-364.
- Lee BK, Hong DP, Lee SS, Kuboi R. Analysis of protein back-extraction processes in alcohol- and carboxylic acid-mediated AOT reverse micellar systems based on structural changes of proteins and reverse micelles. *Biochem Eng J.* 2004;22,71–79.
- Lepori CM, Correa NM, Silber JJ, Falcone RD. How the cation 1-butyl3-methylimidazolium impacts the interaction between the entrapped water and the reverse micelle interface created with an ionic liquid-like surfactant. *Soft Matter.* 2016;12(13), 830–844.
- Leser ME LP. The use of reverse micelles for the simultaneous extraction of oil and proteins from vegetable meal. *Biotechnol Bioeng.* 1989;34:1140–1146.
- Lu W, Chen XW, Wang JM, Yang XQ, Qi JR. Enzyme-assisted subcritical water extraction and characterization of soy protein from a heat-denatured meal. *J Food Eng.* 2016;169:250-258.
- Luisi PL, Giomini M, Pileni R. Reverse micelles as hosts for protein and small molecules. *Biochimica Biophysica Acta.* 1988;947, 209–246.
- Luo D, Zhao Q, Zhao M, Yang B, Long X, Ren J, et al. Effects of limited proteolysis and high-pressure homogenization on structural and functional characteristics of glycinin. *Food Chem.* 2010;122:25-30.
- M C. Membrane technology in food and bioprocessing. *Adv Food Eng.* 1992;165-179.
- Mondor M, Ali F, Ippersiel D, Lamarche F. Impact of ultrafiltration/diafiltration sequence on the production of soy protein isolate by membrane technologies. *Innov Food Sci Emerg Technol.* 2010;11:491-497.
- Nichols DJ. Production of soy isolates by ultrafiltration: factors affecting yield and composition. *J Food Sci.* 1981;46(42): 367-372.
- Omosaiye O, Cheryan M, Matthews M. Removal of oligosaccharides from soybean water extracts by ultrafiltration. *J Food Sci.* 1978;43:354-360.
- Pearson A. Soy proteins. *Dev Food Sci.* 1983;2:67-108.
- Perović MN, Knežević Jugović ZD, Antov MG. Improved recovery of protein from soy grit by enzyme-assisted alkaline extraction. *J Food Eng.* 2020;276, 10.1016.
- Puri M, Sharma D, Barrow, CJ. Enzyme-assisted extraction of bioactives from plants. *Trends Biotechnol.* 2012;30, 37–44.
- Rajib KM, Bidyut KB. Effect of NaCl and temperature on the water solubilization behavior of AOT/nonionic mixed reverse micellar systems stabilized in IPM oil. *Colloids Surf A Physicochem Eng Asp.* 2005;255(252), 165–180.
- Rao A, Shallo H, Ericson A, Thomas R. Characterization of soy protein concentrate produced by membrane ultrafiltration. *J Food Sci.* 2002;67:1412-1418.
- Rho SGK. Solubilization of BSA into AOT reverse micelles using the phase-transfer method: effects of pH and salts. *J Ind Eng Chem.* 2004;10, 247–251.

- Rosenthal A PD, Niranjana K. Aqueous and enzymatic process for edible oil extraction. *Enzyme Microb Technol.* 1996;19:402–420.
- Rosenthal A PDL, Niranjana K. Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. *Enzyme Microb Technol.* 2001;28(26): 499-509.
- S OH. Continuous pilot plant production of bean protein by extraction, centrifugation, ultrafiltration and spray drying. *Lebensm-Wiss U-Technol.* 1978;11: 57-64.
- Sari YW, Bruins ME, Sanders JPM. Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals. *Industrial Crops and Products.* 2013;43:78-83.
- Sereti V ZM, Papadimitriou V, Pispas S, Xenakis A. Biocolloids based on amphiphilic block copolymers as a medium for enzyme encapsulation. *J Phys Chem B.* 2014;118(132):9808–9816.
- Shallo H, Rao A, Ericson A, Thomas R. Preparation of soy protein concentrate by ultrafiltration. *J Food Sci.* 2001;66:242-246.
- Shin YO. Solubilization limit of lysozyme into DODMAC reverse micelles. *Biotech Bioeng.* 2002;80, 537–543.
- Sun X, Bandara N. Applications of reverse micelles technique in food science: A comprehensive review. *Trends Food Sci Technol.* 2019;91:106-115.
- Vergara-Barber M, Lerma-García MJ, Herrero-Martínez JM, Sim o-Alfonso EF. Use of an enzyme-assisted method to improve protein extraction from olive leaves. *Food Chem.* 2015;169, 128–133.
- Wang H, Johnson L, Wang T. Preparation of soy protein concentrate and isolate from extruded-expelled soybean meals. *J Am Oil Chem Soc.* 2004;81:713-717.
- Wei CL, Lu W, Yang J, Wang MP, Yang XQ, Wang JM. Physicochemical properties of soy protein prepared by enzyme-assisted countercurrent extraction. *Int J Food Sci Tech.* 2017;53:1389-1396.
- Wieser H. Chemistry of gluten proteins. *Food Microbiol.* 2007;24:115-119.
- Wu W, Hettiarachchy N, Qi M. Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. *J Am Oil Chem Soc.* 1998;75:845-850.
- Yang J, Guo J, Yang XQ, Wu NN, Zhang JB, Hou JJ, et al. A novel soy protein isolate prepared from soy protein concentrate using jet-cooking combined with enzyme-assisted ultra-filtration. *J Food Eng.* 2014;143:25-32.
- Yao F, Chen FS, Du Y, Zhang Q, Zhu TW. Functional and structural properties of soy 11S globulin: Influence of reverse micelle extraction. *J Food Sci.* 2021;86:3403-3412.
- Zarkadas CG, Gagnon C, Gleddie S, Khanizadeh S, Cober ER, Guillemette RJD. Assessment of the protein quality of fourteen soybean cultivars using amino acid analysis and two-dimensional electrophoresis. *Food Res Int.* 2007;40, 129–146.
- Zhang Y MK, Yamauchi F. Hydrolysis of soybean proteins by a vortex flow filtration membrane reactor with *Aspergillus oryzae* proteases. *J Food Sci.* 1996;61(65): 928-931.
- Zhao X, Chen F, Gai G, Chen J, Xue W, Lee L. Effects of extraction temperature, ionic strength and contact time on the efficiency of bis(2-ethylhexyl) sodium sulfosuccinate (AOT) reverse micellar backward extraction of soy protein and isoflavones from soy flour. *J Sci Food Agric.* 2008;88:590-596.
- Zhao X, Chen J, Lu Z, Ling X, Deng P, Zhu Q, et al. Analysis of the amino acids of soy globulins by AOT reverse micelles and aqueous buffer. *Appl Biochem Biotechnol.* 2011;165:802-813.

Zhao X, Chen J, Zhu Q, Du F, Ao Q, Liu J. Surface characterization of 7S and 11S globulin powders from soy protein examined by X-ray photoelectron spectroscopy and scanning electron microscopy. *Colloids Surf B. Biointerfaces*. 2011;86:260-266.

Zhao X, Li Y, He X, Zhong N, Xu Z, Yang L. Study of the factors affecting the extraction of soybean protein by reverse micelles. *Mol Biol Rep*. 2010;37:669-675.

Zhao X, Wei Z, Du F, Zhu J. Effects of surfactant and salt species in reverse micellar forward extraction efficiency of isoflavones with enriched protein from soy flour. *Appl Biochem Biotechnol*. 2010;162:2087-2097.

Zhao X, Zhang X, Liu H, Sun H. Optimization of AOT reversed micelle forward extraction of 7S globulin subunits from soybean proteins. *J Food Sci Technol*. 2018;55:4909-4917.

Zhao X, Zhang X, Liu H, Zhang G, Ao Q. Functional, nutritional and flavor characteristics of soybean proteins obtained through reverse micelles. *Food Hydrocoll*. 2018;74:358-366.

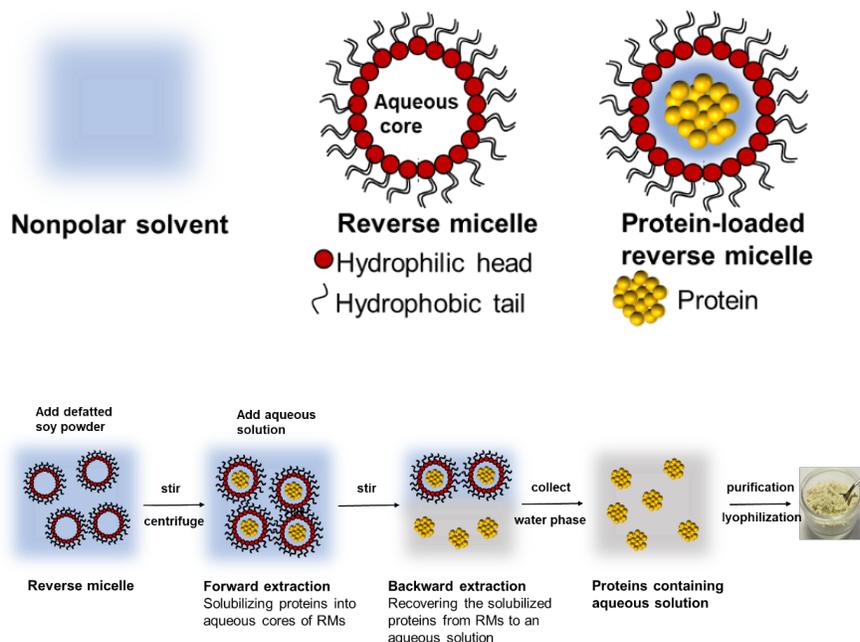


Fig. 1 Schematic diagram of soy protein extraction by reverse micelle method (Sun and Bandara, 2019).

Table 1 Extraction rates of reverse micelles with different surfactants

| Reverse micelles | Protein | Extraction rate% | References |
|------------------|-----------------|------------------|----------------------|
| AOT | 7S globulin | 78.21% | (Zhao et al., 2018a) |
| AOT | soybean protein | 80.2% | (Bu et al., 2014) |
| AOT | soybean protein | 85.5% | (Guanhao, 2012) |
| AOT | soybean protein | 95% | (Zhao et al., 2010a) |
| AOT | soybean protein | 67.6% | (Zhao et al., 2010b) |
| SDS | soybean protein | 72.9% | (Zhao et al., 2010b) |
| AOT | soybean protein | 60-70% | (Zhao et al., 2008) |