

Water-soluble Non-starch Polysaccharides: Update on Current Extraction and Purification Techniques

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Abstract: In the past recent years, the use of plant water-soluble non-starch polysaccharides (NSPs) in food and pharmaceutical products has gained momentum and has become an interesting subject for further scientific investigations. The unique rheological behavior of water-soluble NSPs, being able to form low to high viscous solution with water, is useful in a variety of food products ranging from beverages to baked and frozen foods to improve texture and stabilize heterogeneous food mixes. Water-soluble NSPs are also widely used in the pharmaceutical industry as encapsulating matrix for drug delivery and as bioactive materials that provide preventative roles, such anticancer and antidiabetic. The prebiotic role of water-soluble NSPs to support the growth of microbiota in the gut and support the production of short-chain fatty acids is by far the most valuable scientific discovery of this material. In tune with the increasing demand for its use, advanced and novel extraction techniques has been explored and utilized in order to obtain higher yield, minimize cost, shorten extraction time and scale up the extraction processes. These include the use of ultrasound and microwave to extract water-soluble NSP that overcome some of the limitations of conventional extraction methods. Green deproteinization techniques, such as the use of freeze-thaw technique, as alternative to the commonly used chemical methods have been explored in order to minimize losses and the negative effect of deproteinization on the bioactivity of the extracted water-soluble NSP. The green purification methods to deproteinize water-soluble NSP will render the extracted material safer to be used in food and pharmaceutical products.

Key words: water-soluble non-starch polysaccharides; extraction; purification; green deproteinization techniques

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水溶性非淀粉多糖的提取纯化技术进展

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摘要: 近几年来, 植物水溶性非淀粉多糖 (NSPS) 在食品和医药产品中的应用取得了很大的发展, 已经成为能深入研究的有趣课题。水溶性 NSPS 具有独特的流变特性, 能够与水形成粘度由低到高的溶液, 可用于饮料、烘焙、冷冻等各种食品中, 以改善质地, 稳定食品混合物。水溶性 NSP 在制药工业中也被广泛用作药物输送的封装基质, 以及作为具有预防作用的生物活性材料, 如抗癌和抗糖尿病。水溶性 NSP 在促进肠道微生物群生长和支持短链脂肪酸生产方面的益生元作用是迄今为止最有价值的科学发现。由于其使用需求不断增加, 为了获得更高的得率, 降低成本, 缩短提取时间, 扩大提取工艺规模, 探索并应用了先进新颖的提取技术, 包括采用超声波和微波提取水溶性 NSP, 克服了传统提取方法的一些局限性。为了减少损失和降低脱蛋白对提取水溶性 NSP 生物活性的负面影响, 人们探索了绿色脱蛋白技术, 如使用冻融技术替代常用的化学方法。绿色的水溶性 NSP 脱蛋白纯化方法将使提取的物质更安全地用于食品和医药产品中。

关键词: 水溶性非淀粉多糖; 提取; 纯化; 绿色脱蛋白技术

Polysaccharides are among the most important and useful natural biomaterial for mankind (Gonzales, 2018). Its usefulness is evident by its presence in almost all (either food or non-food) previously used, currently in use and will be used to sustain nutrition, life and to maintain

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or promote good health (Garg, Cowman & Hales, 2011). Plant polysaccharides are heavily investigated for new and novel applications due to their wide range of functionalities to meet the increasing demand of polysaccharides for food and pharmaceutical products (Harding et al., 2017; Alén, 2018).

Starch, a major component of most plants, is

considered the most extensively studied polysaccharide and has the most industrial applications (Eliasson, 2017). The extraction of starch leads to accumulation of tons of by-products considered as “bio-agro wastes” that includes peels, stalks, roots, seeds and leaves (Hernandez et al., 2017; Eliasson, 2017). When left unutilized, these by-products will be wasted and can cause environmental concerns (Gupta & Verma, 2015). These bio-agro wastes can be a good source of the other form of polysaccharide, the non-starch polysaccharides (NSP) (Cui, 2000; Banerjee et al., 2017).

NSP is classified mainly based on its solubility in water (Choct, 2015). Therefore, it can be water-insoluble or water-soluble NSP (Pigman, 2012; Choct, 2015). Non-soluble NSPs are also known as “structural or lignocellulosic residues” which includes hemicellulose, cellulose and lignin that comprise the majority of agro-waste (Pigman, 2012; BeMiller, 2018). On the other hand, water soluble NSPs mostly includes pectic substances, gums and mucilage (Baumstark, 2017; BeMiller, 2018). Both insoluble and soluble NSPs have a variety of useful industrial applications such as in the production of composites and biomaterials (Li et al., 2016; Valdes & Garrigos, 2016), as food packaging (Jimenez et al., 2015; Cazon et al., 2017), in the development of health products as dietary fiber and prebiotic (Holscher, 2017; Dai & Chau, 2017; Amadi et al., 2017) as food additive (Stephen & Phillips, 2016), feedstock for the production of chemicals particularly in bioethanol production (Saini, Saini & Tewari, 2015; Lee & Lee, 2016; Hossain, Zaini & Mahlia, 2017), substrate biopolymer for microbial fermentation (Jakobsen et al., 2015; Conlon & Topping, 2016), and in the development of pharmaceutical products (Thakur & Thakur, 2015).

Although considered minor component than non-soluble NSPs, water-soluble NSP has gained extensive research interest due to its solubility in water that offers ease of recovery and extraction (Cui, 2005; Pigman, 2012; Pettolino et al., 2012). The number of studies conducted on the extraction and utilization of water-soluble NSPs in the past recent years is a strong evidence of its significant importance as a biomaterial with promising potential applications (Sinha et al., 2011; Dai & Chau, 2017; Amadi et al., 2017; Mahmood et al., 2017).

Despite being highly soluble in water, which makes it easier to extract than insoluble NSPs, some challenges for the extraction of water-soluble NSPs are continually being investigated (Maphosa & Jideani, 2016). Low yield and co-extraction of other unwanted components including starch, protein, lipids, and other impurities are some of these challenges (Maphosa & Jideani, 2016; Stephen & Phillips, 2016). The use of novel extraction techniques has been proposed to overcome these challenges as well as optimizing extraction conditions in order to maximize yield and minimize impurity of the extracted water-soluble NSP (Hostettmann, 2014; Castro-Alves & Nascimento, 2016).

The use of novel extraction techniques and green purification methods such as free-thaw technique have been explored to obtain and purify water-soluble NSPs in order to make it more appropriate and safer to be used in human consumed products such food and pharmaceutical products (Hostettmann, 2014; Nagy, Peng & Pohl, 2017). These novel extraction techniques and green purification methods have been proposed not only to obtain high yield and purity but also to maintain structural integrity and bioactivity of the extracted water-soluble NSP (Hostettmann, 2014).

This paper provide an update on the extraction and purification techniques utilized to extract and purify water soluble NSPs from plant sources, with emphasis on the deproteinization process that can have significant impact on the functionality of the extracted NSP. In addition, food applications and biological activities of water-soluble NSPs are summarized. This information will be useful to streamline pathway for efficient extraction of water-soluble NSP for future research on this valuable biomaterial.

1 Extraction

Water-soluble NSPs are generally extracted much easier than non-water soluble NSPs (Cui, 2005). The use of water as the extraction medium makes this material attractive for commercial and industrial use since water is readily available and relatively cheaper than other specialized biological extraction mediums such as buffered solutions (Hostettmann, 2014). While water is the most commonly used medium for the extraction of water-soluble NSPs from different materials, the

extraction method and conditions vary depending on the source and nature of the water-soluble NSP to be extracted (Cui, 2000; Cui, 2005). In all cases, the aim of the reported research was always to obtain high yield, high purity material and maintain the structural integrity

of the extracted water-soluble NSP to preserve its functionality and potential bioactivities (Cui, 2005; Goh, Kumar & Wong, 2014).

1.1 Conventional Extraction

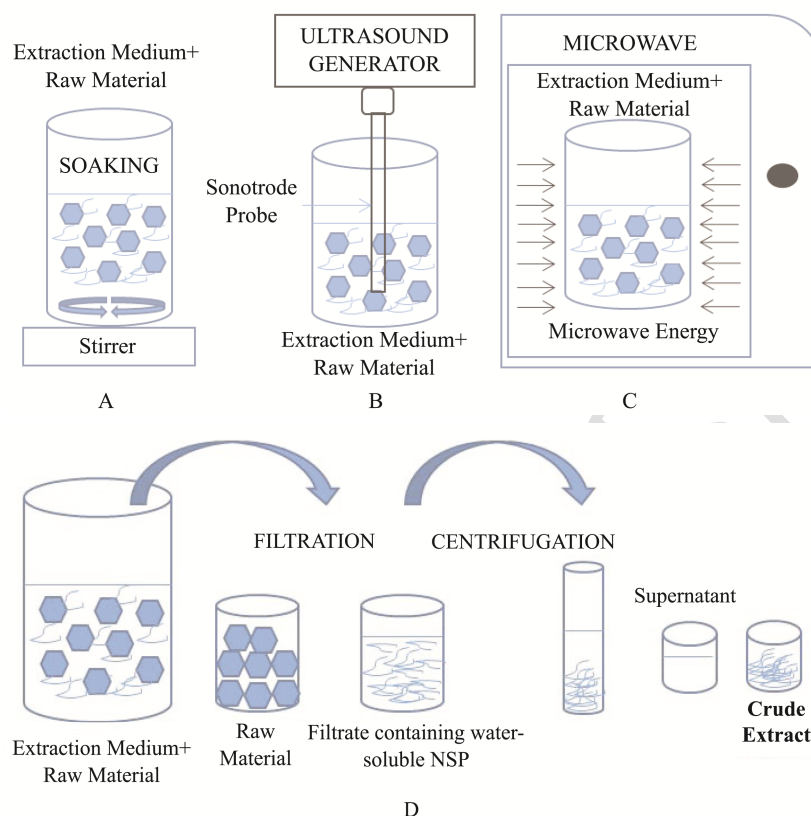


Fig.1 Schematic presentation of water-soluble NSP extraction using (A) conventional, (B) ultrasound-assisted, (C) microwave-assisted extraction, and (D) physical separation techniques to obtain crude extracts

Conventional extraction (CE) method is probably the most commonly used method to extract water-soluble NSPs (Maphosa & Jideani, 2016). This extraction method involves steeping or soaking the raw material in aqueous extraction medium where agitation can be applied to enhance the extraction process, followed by physical separation such as decantation, centrifugation or filtration to obtain the crude water-soluble NSP extract (Figure 1A) (Hostettmann, 2014; Felkai-Haddache, 2016). Although conventional extraction is efficient in extracting water-soluble NSPs, a number of limitations and drawbacks were reported on the use of this extraction method. CE is time consuming as it involves various steps (Wang, Zhang, Zhao, Wang, Wu & Yao, 2010). The physical separation steps involved in this extraction method as well contributes to polysaccharide loss (Hernandez-Carmona, Freile-Pelegrín, & Hernández-Garibay, 2013). As it is conventional, there is less control

on the different factors that may affect the yield and quality of the extracted water-soluble NSP (Bagherian, Ashtiani, Fouladitajar & Mohtashamy, 2011). CE is also not appropriate for large scale extraction process and it requires large volume of extracting solvent (Hernandez-Carmona, Freile-Pelegrín, & Hernández-Garibay, 2013). In addition, co-extraction of unwanted components is very common in CE wherein intensive purification is required for the extracted material to be of good quality (Chemat, Lagha, AitAmar, Bartels & Chemat, 2004).

1.2 Novel Extraction Techniques

Novel extraction techniques have been explored recently to be used in the extraction of water-soluble NSPs. Novel extraction techniques such as the use of ultrasound, microwave, pressurized liquid, enzymes and combination of these techniques (Vilkhu, Mawson, Simons & Bates, 2008; Chemat & Cravotto, 2012;

Farahnaky, Majzoobi & Bakhshizadeh, 2019) offers more controlled extraction conditions over CE (Hostettmann, 2014; Maphosa & Jideani, 2016; Maric et al., 2018).

1.3 Ultrasound-Assisted Extraction

Ultrasound-assisted extraction (UAE) utilizes mechanical wave to facilitate the extraction process (Hromadkova, Ebringerova & Valachovic, 2002) (Figure 1B). Its mechanism of action is attributed to the cavitation phenomena, which initiate, propagate and implode micro bubbles from dissolved gases in the extraction liquid (Fabre et al., 2015). A longitudinal displacement of molecules is generated by ultrasound waves in elastic mediums, which is caused by succession of compression and rarefaction phases of the waves (Ying, Han & Li, 2011; Benito-Roman, Alonso & Cocero, 2013). Furthermore, the explosion of cavitation bubbles produce high velocity inter-particle collisions, macro turbulence and perturbation, leading to accelerated internal and eddy diffusion that facilitates the extraction of compartmentalized compounds (Hostettmann, 2014). UAE has been reported to increase extraction yield of *Arabidopsis* adherent seed water-soluble NSP within 20 seconds ultrasound treatment (Zhao, Qiao & Wu., 2017). Similar effect was observed with water-soluble NSP extracted from *Salvia macrosiphon* seeds where the yield was increased with ultrasound time (Farahnaky et al., 2013). Although this technique is considered convenient, it has also some disadvantages such as disruption of biological cell walls of other components in the system enhancing co-extraction of unwanted material (Vilkhu, Mawson, Simons & Bates, 2008).

1.4 Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) on the other hand, involves the use of electromagnetic waves made up of two oscillating perpendicular fields (the electrical field and magnetic field) that can be used as energy vectors (Figure 1C). These waves are directly absorbed by the material and transform it into heat by molecular friction of the dipole compounds (Chemat & Cravotto, 2012). Heat generated decreases the viscosity of water-soluble NSPs and enhance its extraction (Arasi, Rao & Bagyalakshmi, 2016). MAE increased the yield of the water-soluble NSP from *Zizyphus jujube* when subjected to a microwave power of 400 W at temperature of 75 °C

(Rostami & Gharibzadeh, 2016). This was in agreement with the findings of Felkai-Haddache et al., (2016) on the mucilage of *Opuntia ficus indica* cladode where high yield was obtained with the use of MAE. Increasing microwave power enhances the solubility of the water-soluble NSPs thereby increasing the extraction efficiency (Rostami & Gharibzadeh, 2016; Felkai-Haddache et al., 2016). MAE improves the solvent penetration into the plant matrix and offers a rapid transfer of energy to the solvent and matrix allowing the dissolution of components to be extracted. However, it is important to note that successful use of MAE depends on its operation conditions that need to be optimized for different plant matrixes. Excessive microwave time and power may lead to polysaccharide degradation (Rostami & Gharibzadeh, 2016).

1.5 Pressurized Liquid Extraction

Pressurized liquid extraction (PLE) utilizes high temperature and pressure using liquid solvents that can facilitate high mass transfer kinetics (Mustafa & Turner, 2011). Under this condition, the extraction solvent is maintained in its liquid state even beyond its boiling point temperature and thus can penetrate better on the solid matrix (Pitipanapong et al., 2007; Mustafa & Turner, 2011). The high pressure can result to breakage of the polysaccharide's hydrogen bond resulting to enhanced water solubility (Lo, Tsao, Wang & Chang, 2007). This facilitates faster desorption of the analyte from the raw material enhancing the extraction process (Hostettmann, 2014). Aside from reduced extraction time, other advantage on the use of PLE is that it requires less volume of extracting solvent (Zgorka, 2009). In addition, PLE is an automated technique thus extraction condition is more controlled than CE (Mustafa & Turner, 2011). This is important for bioactive materials that losses structural integrity and bioactivity at high pressure and high temperature extraction condition (Hostettmann, 2014). Low selectivity and co-extraction of other unwanted components are the main drawback of PLE similar to UAE and MAE (Mustafa & Turner, 2011). The use of PLE (pressure: 10.1 MPa, temperature: 28 °C; extraction time: 70 min) to extract the water-soluble NSP of *Lentinula edodes* resulted to a crude polysaccharide yield of 90% that is way higher than boiling water

extraction yielding only 24.9% (Lo, Tsao, Wang & Chang, 2007).

1.6 Enzyme-Assisted Extraction

Enzyme-assisted extraction (EAE) is also considered a novel technique for extracting water-soluble NSPs (Hostettmann, 2014). EAE is considered the mildest among novel extraction techniques since it does not require intense physical treatment, high pressure or high temperature condition (Puri, Sharma & Barrow, 2012). The mechanism of action of EAE in extracting water-soluble NSPs is based on the enzyme's ability to degrade cell walls and hydrolyze bonds facilitating the release of contained polysaccharides, increase the polysaccharide's water solubility thus enhancing the extraction process (Wijesinghe & Jeon, 2012; Nadar, Pawar & Rathod 2017). The main advantage of EAE over other novel extraction techniques is its specificity. Specific enzymes are capable of reacting only to target analyte that minimizes co-extraction of other unwanted components (Puri, Sharma & Barrow, 2012; Nadar, Pawar & Rathod 2017). Another advantage of EAE is that low doses of enzymes can be efficient enough to extract water soluble NSPs making it a highly scalable extraction technique (Puri, Sharma & Barrow, 2012). However, enzyme cost, enzyme purity and the need to establish optimum enzyme activity condition are among its limitations and challenges (Hostettmann, 2014). A complex enzyme solution of 2.1% (W/V) composed of papain, pectinase and cellulase at a ratio of 1:1:1 at optimum extraction condition (extraction temperature: 61.8 °C; extraction pH: 4.14, extraction time: 3.2 h) yielded 7.53% water-soluble NSP from *Tricholoma matsutake* (Yin, You & Jiang, 2011). The use of EAE was found better than CE in extracting the water-soluble NSP of *Hericium erinaceus* fruits as evidenced by a yield increase of 67.72% from 8.03% using CE to 13.46% using EAE in extracting water-soluble NSP from *Hericium erinaceus* (Zhu et al., 2014). Some conformational changes were reported with the use of EAE along with improved extraction yield. The use of EAE resulted to structural changes characterized by larger lamellar on polysaccharide surface as seen in scanning electron microscopy (SEM) analysis. In addition, atomic force microscopy analysis revealed that EAE can reduce

large molecular aggregation of the water-soluble NSP resulting to a more extended polysaccharide chain (Zhu et al., 2014).

1.7 Combination of Extraction Techniques

Combination of different novel extraction techniques has also been explored to extract water-soluble NSPs. This is utilized to achieve synergistic effect of the different novel extraction techniques to enhance the yield even higher than the use of single extraction technique (Hostettmann, 2014). The use of combined ultrasound-enzyme assisted extraction (UEAE) technique has been reported to extract water-soluble NSP from blackcurrant fruit (Xu, Zhang, Yang, Song, & Yu, 2015). A maximum polysaccharide yield of 14.28% was obtained under the optimal condition of UEAE (ultrasonic power: 400 W; ultrasonic time: 25.6 min; enzyme ratio: (papain: pectinase) 2:1; enzyme concentration: 1.575%, pH 5.3) (Xu et al., 2015). UEAE was also used to extract water-soluble NSPs from *Epimedium* leaves using complex enzyme solution composed of papain, pectase, cellulase and α -amylase in 50, 250, 200 and 100 U/g enzyme concentrations. A 5.98% yield was obtained at ultrasound power of 311 W, ultrasound time of 42.3 min, extraction temperature of 46.8 °C coupled with the complex enzyme solution at pH 4.28 (Chen, Li, Liu, Yang & Li, 2012). In another study, the use of UEAE to extract the water-soluble NSP from corn silk was found better than CE (Chen, Chen, Tian, Wang, Wang & Xing, 2014). Under optimum UEAE condition of using cellulase, extraction yield was enhanced from 4.56% (Conventional extraction) to 7.10%. In addition to enhanced yield through UEAE, the extracted water-soluble NSP using this combined novel extraction techniques was reported to exhibit better biological activities (antioxidant and anti-tumor activities) than the conventionally extracted counterpart (Chen et al., 2014). The use of microwave-enzyme assisted extraction (MEAE) was found more efficient in extracting water-soluble NSP from the fruit of *Schisandra chinensis* Baill than the use of EAE and UAE (Cheng et al., 2015). Under MEAE optimum condition using complex enzyme solution containing papain, cellulase and pectinase (complex enzyme solution: 1.5%; pH: 4.21; extraction temperature: 47.58 °C; extraction time: 3 h; microwave time: 10 min), the polysaccharide yield is 7.38% that is higher than EAE

(6.87%) and UAE (6.44%). This observed higher yield using MEAE is attributed to the combined effect of microwave and enzyme activity in rupturing cell walls as evidence by cell wall rupture observed using scanning electron microscope. The structural change caused by MEAE was characterized by disorganization and thinning of the parenchyma. This structural change leads to better solvent penetration resulting to a more enhanced extraction (Cheng et al., 2015). A yield of 3.25% with material purity of 73.16% was obtained when optimum condition of ultrasound-microwave assisted extraction (UMAE) (microwave power: 90 W; ultrasonic power: 50 W; ultrasonic frequency: 40 kHz; solid/water ratio: 1:20 (*W/V*); extraction time: 19 min) was employed to extract the water-soluble NSP of *Inonotus obliquus* (Chen et al., 2010). Although the use of combined extraction technique offers higher yield compare to conventional extraction or with the use of single extraction method, it is important to consider that extraction using combined techniques is more complex due to the number of variables involved in the process (Hostettmann, 2014). Thus, there is always a need to establish optimum extraction conditions.

2 Isolation

Isolating the water-soluble NSP from the crude extract is achieved through chromatographic methods, ultrafiltration or by gradient non-solvent precipitation (Hu & Goff, 2018). Isolation and fractionation of water-soluble NSP are important in order to achieve higher uniformity and homogeneity needed for characterization studies (Hu & Goff, 2018). This isolation step enables high molecular weight polysaccharide to be separated to a certain degree from low molecular weight components, free sugar, salts, pigments, as well as from

glucose and peptides when starch hydrolysis and deproteinization are done prior to this step (Goh, Matia-Merino, Pinder, Saavedra, & Singh, 2011). For most water-soluble NSPs, isolation and fractionation through precipitation are commonly used as it is an inexpensive and scalable process (Hu & Goff, 2018). This is done mostly through solvent precipitation including the use of organic solvents such as ethanol, isopropanol, acetone, methanol and other precipitating agents such as dimethyl sulfoxide (Cui, 2005), ammonium sulfate ((NH_4) $_2\text{SO}_4$) (Shi et al., 2017) and polyethylene glycol (PEG) (Hu, Liu, Jin, & Tian, 2016).

The precipitation process proceeds once the precipitating agent is added into the polysaccharide solution (Figure 2). Since the precipitating agents have lower dielectric constants than water, a reduction of the dielectric constant of the water-soluble NSP solution occurs resulting in enhanced polymer-polymer interaction, conformational changes, aggregation and ultimately the precipitation of the water-soluble NSP in the solution (Jian et al., 2014). In this process, however, co-precipitation of other components other than water-soluble NSP, including low molecular weight compounds such as organic acid, minerals, sugars, peptides and free amino acids, and pigments, is possible but could be overcome by repeated fractional precipitation (Cui, 2005). Several factors control the precipitation process including the rate of the precipitant addition, the nature of the precipitant, type of precipitating agent, precipitation temperature, precipitation time, polysaccharide concentration, pH of the polysaccharide solution and presence of ions (Hu & Goff, 2018).

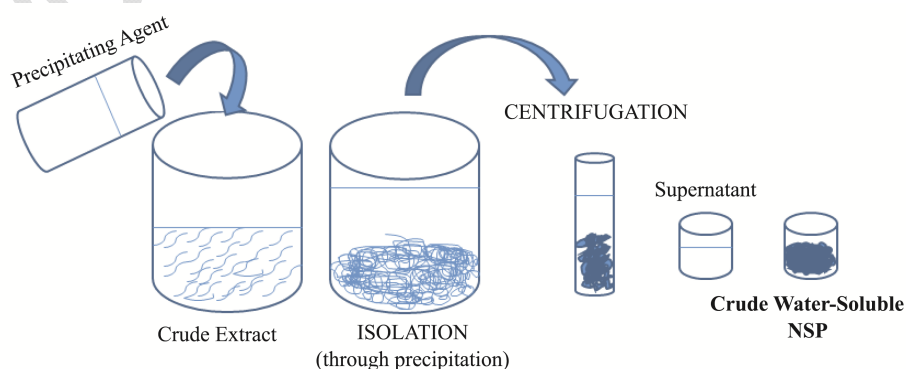


Fig.2 Isolation of water-soluble NSP from crude extract through precipitation

Ethanol ($\text{C}_2\text{H}_6\text{O}$) is the most commonly used

precipitating agent in the isolation of water-soluble NSPs

(Xu et al., 2014) although the use of acetone (C_3H_6O) and methanol (CH_3OH) has also been reported (Sarkar et al., 2014). In terms of precipitation efficiency based on dielectric constant or polarity, acetone, having the lowest dielectric constant is the most efficient precipitating agent followed by isopropanol, ethanol and lastly methanol (Hu & Goff, 2018). When used as precipitating agent to isolate the water-soluble NSP of taro (*Colocasia*

esculenta), acetone yields 9.40% while methanol only yields 1.20% (Sarkar et al., 2014). However, for safety reason especially when the material is intended for food and medical applications, the use of ethanol is preferred due to its low toxicity compared to the use of other organic solvents (Lao & Giusti, 2018).

3 Purification

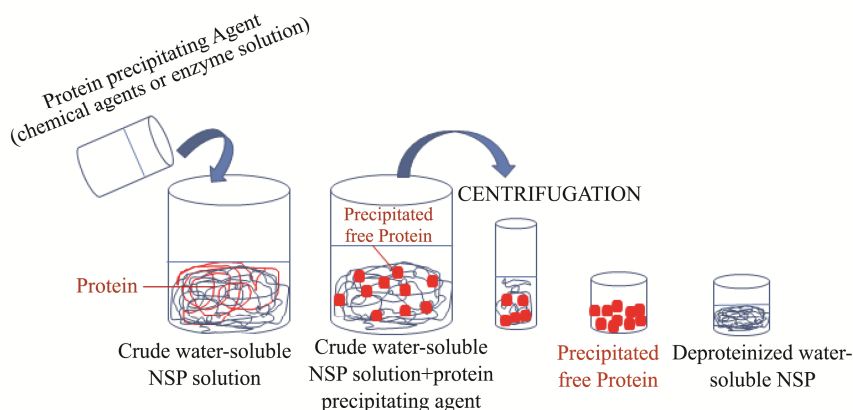


Fig.3 Schematic presentation of water-soluble NSP deproteinization using chemical agents or enzyme solutions as protein precipitating agents

Purification of the crude water-soluble NSP is vital prior to conducting characterization studies. Co-extracted components such as starch, protein, lipid, pigment, and minerals could interfere with the compositional and structural analysis that may lead to inaccurate data (Cui, 2005). In most cases, a purification step often involves starch hydrolysis and deproteinization is carried out to improve the purity of the extracted NSP. Starch removal is achieved through enzymatic hydrolysis through the use of α -amylase and amyloglucosidase where the free glucose produced from the enzymatic reaction is removed through dialysis and enzymes are deactivated through re-precipitation using an organic solvent (Bertoft, 2017; Al, 2018).

Deproteinization, on the other hand, can be achieved by various techniques including the use of chemical agents, enzymatic treatment, chromatographic techniques, use of adsorbents and greener alternative deproteinization methods such as freeze-thaw technique. Most water-soluble NSPs have tightly bound protein to its polysaccharide chain that cannot be easily removed or if treated repeatedly will result in polysaccharide loss (Wang et al., 2007). This makes the deproteinization process a crucial step in water-soluble NSP purification as intensive deproteinization treatment may result in

polysaccharide degradation affecting the yield, structural integrity, polysaccharide-protein complex functionality, and bioactivity (Chen et al., 2012). Thus, proper selection of the deproteinization method and condition is necessary in order to minimize material damage and to maintain the structural integrity and bioactivity of the extracted water-soluble NSP. Figure 3 present a schematic presentation of water-soluble NSP deproteinization process using chemical agents or enzyme solutions as protein precipitating agents.

4 Chemical Methods for Deproteinization of Water-Soluble NSPs

4.1 Sevag Method

Sevag deproteinization method is based on the precipitation of protein using chloroform and *n*-butanol (Li, Zhao, Zhou & Wu, 2012). It can be considered the most popular deproteinization method as it has been extensively used in a number of studies on water-soluble NSPs deproteinization from various sources especially the polysaccharides from *Dendrobium* (Xing et al., 2013). This method of deproteinization is widely used as it is known to be effective in reducing the amount of protein

present (Xie, Zhang, Peng & Gan, 2011). A study conducted by Chen et al., (2012), showed that use of Sevag method decreases protein concentration from 83.01 to 18.61 $\mu\text{g/mL}$ and long treatment times can increase the deproteinization efficiency from 32.50 to 84.20%. The reported deproteinization efficiency data in literature made Sevag method a common choice (Li et al., 2012; Yang et al., 2012; Zha et al., 2012).

Although Sevag method is efficient in precipitating proteins, various drawbacks have been reported for this method. These include polysaccharide loss leading to a decrease in polysaccharide recovery due to partial hydrolysis of polysaccharides as well as co-removal of glycoproteins causing inconsistency in physiological properties and decreased polysaccharide bioactivities (Wang et al., 2007; Chen et al., 2012). A decrease in polysaccharide recovery from 43.60 to 28.60% (1.77 to 0.50 g/L) was reported in the deproteinization of gellan gum (Wang et al., 2007). Similarly, studies conducted on *Stigma maydis* (Li et al., 2012) and Chinese yam (Yang et al., 2015) polysaccharides suggested a proportional relationship between number of treatments using Sevag method and loss of polysaccharides and subsequently a lower yield. For example, a four times deproteinization treatment showed an efficiency of 64.20% with increased material purity from 11.50 to 32.30% but on the expense of polysaccharide loss of 34.30% (Li et al., 2012). An increased polysaccharide loss was also reported in Chinese yam treated several times using Sevag method with an optimum deproteinization treatment of 6 times with residual protein content of 5.91% (Yang et al., 2015). The same trend was true in the deproteinization of the extracted water-soluble NSP from *Pleurotus tuber-regium* sclerotia (Wu et al., 2013). Increasing the number of treatment cycles even more as reported in the polysaccharide of *Aloe Vera Miller* up to 29 times resulted in more than 60% polysaccharide loss (Chang, Chen & Feng, 2011). This loss of polysaccharides is considered the main drawback of this method. In addition, the bioactivity of the deprotenized water-soluble NSP may also be affected since the antioxidant activity decreased by 11% after deproteinization using this method (Chen et al., 2012).

Aside from polysaccharide and bioactivity loss during repeated treatment with Sevag reagent, other

drawbacks on this deproteinization method is that it is environmentally disadvantageous due to the large amount of chloroform and *n*-butanol needed resulting in the generation of large amount of toxic organic pollutants (Wang et al., 2007). To obtain a 13.21 mg/g deproteinized water-soluble NSP from *Spirulina platensis* with residual protein of 1.07 mg/g dry sample, a 41.7 (chloroform) and 8.3 (*n*-butanol) mL/g of solvents was needed (Wu et al., 2017). The use of chloroform and *n*-butanol will carry additional economic burden and may also limit the use of the water-soluble NSP in food and pharmaceutical products (Xiong et al., 2017).

4.2 Trichloroacetic acid (TCA)

The use of trichloroacetic acid (TCA) is also one of the most utilized chemical methods to deproteinize water-soluble NSPs (Cui, 2005). TCA sequesters the protein-bound water (Chen & Huang, 2018). Its acidic nature can lower the sample pH drastically resulting in hydrophobic aggregation of protein. In addition, protein cations can bind with TCA to form insoluble salt which precipitates at $\text{pH} < \text{pI}$ (Isoelectric point) (Huang et al., 2012). Its main advantage is its high volatility thus can be easily removed after deproteinization (Xie, Zhang, Peng & Gan, 2011). As with Sevag method, its drawback also includes polysaccharide loss during deproteinization. A polysaccharide loss of 50 to 79% with deproteinization efficiency of 71 to 84% was reported for the crude polysaccharide of *Laminaria japonica* (Zha et al., 2012). TCA method has lower deproteinization efficiency (92.8%) on the water-soluble NSP of pumpkin compared to CaCl_2 and NaCl with deproteinization efficiency of 97.3% and 96.6%, respectively. Further, the polysaccharide loss was found to be higher (10.90%) with TCA than CaCl_2 (4.80%) and NaCl (5.00%) (Huang, Chen & Wang, 2011). Although TCA was found to be less effective (95.9%) in deproteinizing water-soluble NSP of garlic than HCl (98.60%), it was advantageous in terms of lower polysaccharide loss (20.4%) than using HCl (29%) (Huang et al., 2012). The advantage of using the TCA may be dependent on the material being extracted. For example, TCA was found to be better than Sevag method for the deproteinization of the water-soluble NSP of *Phellinus baumii* with deproteinization efficiency of 82% and polysaccharide

loss of 10.8% (Xie, Zhang, Peng & Gan, 2011).

4.3 Lead Acetate

Lead acetate, a heavy metallic salt, is capable of degrading and precipitating protein (Banu & Cathrine, 2015). Its use was considered the simplest approach compared to the use of Sevag method and enzyme in *Ganoderma atrum* polysaccharide deproteinization. A 0.4% (W/V) lead acetate solution showed a deproteinization efficiency of 89.4 %, which was higher than the use of Sevag method (84.2%) and papain (88.8%) (Chen et al., 2012). However, as with the use of Sevag and TCA, polysaccharide loss was also noted upon the use of lead acetate. At lead acetate concentration of 0.33%, 15.6% polysaccharide loss was observed and a further significant decrease was noted when lead acetate concentration was more than 0.52%. In addition to polysaccharide loss, excessive addition and repeated lead acetate treatment resulted in reduced polysaccharide bioactivity. A 14.5% loss in antioxidant activity was reported with the use of lead acetate concentration in the range of 0.33%~0.52% and a further loss in antioxidant activity (19.9%) was noted as lead acetate concentration increased (0.57%). This shows the negative effects of lead acetate concentration on the amount of polysaccharides and its bioactivity (antioxidant activity) (Chen et al., 2012).

4.4 Enzymatic Method

Some enzymes are capable of effective breaking of the bonds between polysaccharides and proteins and can hydrolyze free and bound proteins (Singh, Kumar, Mittal & Mehta, 2016). Thus, enzymes are useful in the deproteinization of water-soluble NSPs co-extracted with protein (Zhang, He & Simpson, 2018). The main advantage of enzymatic deproteinization is its specificity with proper enzyme selection and that low dosage is required for the deproteinization making it possible for large scale industry applications (Wang et al., 2007). The use of enzymes offers simplicity compared to other deproteinization methods. However, enzyme cost may be a considerable limitation on its use (Zhang, He & Simpson, 2018).

Papain (2 U/g) has been used effectively to decrease the protein concentration of polysaccharide extracted

from Lingzhi (*Ganoderma atrum*) to a low concentration of 12.93 µg/mL at optimum reaction time of 6 h. The deproteinization efficiency of the process was 88.8% but had a 21.3% polysaccharide loss (Chen et al., 2012). However, bioactivity (antioxidant activity) was negatively affected by increasing the papain dosage from 0.5 to 2 U/g, which reduced the antioxidant activity by 10.4 to 30.6% (Chen et al., 2012). It should be noted that the efficacy of papain for deproteinization is greatly dependent on the source of the water-soluble NSP. For example, a higher concentration of papain (2.4 U/g) than that used by Chen et al. (2012), decreased the protein content to 31.17 µg/mL of crude gellan gum with deproteinization efficiency of 86.2% (Wang et al., 2007), which is lower than that reported by Chen et al. (2012) for Lingzhi. Microbial alkaline and neutral proteases have also been reported to be useful in the deproteinization of water-soluble NSP. A deproteinization efficiency of 86.4% and 89.3% polysaccharide recovery from gellan gum was reported using alkaline protease at a dosage of 0.60 AU/g for 6 h reaction time. Lower efficiency and polysaccharide recovery (72% and 85.1%, respectively) were found with the use of neutral protease at concentration of 10.5×10^2 AU/g, which resulted in a minimum protein concentration of 62.92 mg/L. The use of trypsin (6 U/mL) in the deproteinization of the water-soluble NSP of *Laminaria japonica* resulted to a deproteinization efficiency of 72.16% with polysaccharide loss of 5.26%. In combination with Sevag method, trypsin of the same concentration resulted to a higher deproteinization efficiency (92.17%) with polysaccharide loss of 10.29% (Zha et al., 2012). Therefore, the choice of protease appears to be important and the best protease for a specific material should be optimized for various water-soluble NSP matrixes.

4.5 Aqueous Two-Phase System (ATPS)

Method

ATPS is a simple liquid-liquid fractionation technique that has been recently used to purify water-soluble NSPs (Iqbal et al., 2016). The ATPS technique has several advantages over the chemical method since it uses purification conditions without the use of highly toxic chemicals, highly controlled with high

efficiency, versatile and can be scaled up (Wang et al., 2016). For deproteinization, the use of ATPS proceeds through separating polysaccharides in the bottom phase and protein being removed in the top phase (Cheng et al., 2017). A 25% crude water-soluble NSP of *Lyceum barbarum* was purified using ATPS with the use of thermosensitive triblock copolymer (Ethylene oxide - Propylene oxide- Ethylene oxide) and an inorganic salt

(NaH_2PO_4) combined with dialysis membrane resulted in low residual protein of 5.6% with polysaccharide recovery of 96.3% (Wang et al., 2016). An ethanol ammonium-sulfate ATPS system (composed of 28.1% w/w ethanol and 20.3% W/W $(\text{NH}_4)_2\text{SO}_4$) yielded 10.71 mg/g water-soluble NSP with residual protein of 0.52 mg/g (Wu et al., 2017).

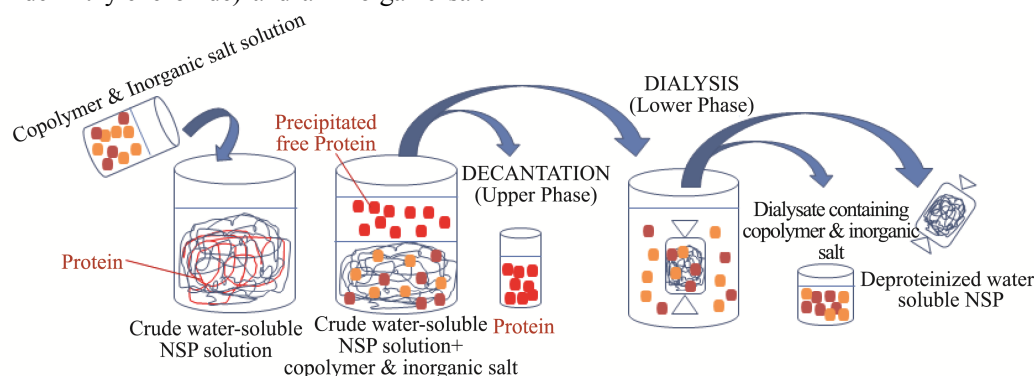


Fig.4 Schematic presentation of water-soluble NSP deproteinization using ATPS method

The use of high speed counter-current chromatography (HSCCC) with ethanol-ammonium sulfate ATPS is also a potential new deproteinization method useful for water-soluble NSPs. One-step purification of water-soluble NSP from *Spirulina platensis* using the HSCCC separation can efficiently remove protein. Water-soluble NSP purified by this method showed strong hydroxyl free radical and DPPH free radical scavenging activities. Although efficient purification can be achieved, some drawback of this method includes being time consuming at slow flow rate, while high rotation speed could cause HSCCC equipment to wear (Wu et al., 2017).

4.6 Solid-phase Extraction using Macroporous Resin

Solid-phase extraction (SPE) is a selective sample pre-concentration and purification technique that provide rapid and effective purification (Simpson, 2000; Zwir-Ferenc and Biziuk, 2006). The most common adsorption mechanisms in SPE are based on van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions) and cation-anion interactions (ionic interactions) (Huck and Bonn, 2000; Zwir-Ferenc and Biziuk, 2006). These adsorption mechanisms are dependent on the hydrophobicity and

polarity of the solute and the sorbent. The use of macroporous resins in SPE applications have been used in the purification of water-soluble NSPs to remove impurities from crude samples including deproteinization (Qin & Zhang, 2010; Liu et al., 2011; Yang et al., 2012) (Figure 5). These resins are gaining interest due to its excellent properties including great analyte retention, shorter processing times resulting in low operating costs, less use of solvents, ease of regeneration and environmentally friendly features (Li, Ren & Zou et al., 2012; Huck and Bonn, 2000; Xiaohong, Xianling, Wei, Dandan & Ying, 2017). SPE technique can also remove pigments simultaneously with deproteinization without damaging the structure of the polysaccharides and affecting its bioactivities (Yang et al., 2012). However, it is important to note that some considerations should be taken into account in selecting resins for a particular application. The physical and chemical properties including polarity, surface area, and average pore diameter are among the features that need to be considered (Huck and Bonn, 2000).

Polarity plays an important role in the absorption of proteins (Yang et al., 2012). The mechanism of action is due to reduction in the interfacial tension between the polymer surface and sample by the polar group, thereby increasing the contact between analytes and polymeric solvents (Poole, 2003). Pore diameter of the resin also

plays an important role, as exhibited by HPH480 (polar resin), having large average pore diameter showing lower adsorption ratios of protein (Yang et al., 2012). Different resins may exhibit varying adsorption (retention) and desorption (elution) rates/ profiles which is dependent on the intermolecular forces between the analyte, the active sites on the surface of the adsorbent and the liquid phase (Huck and Bonn, 2000).

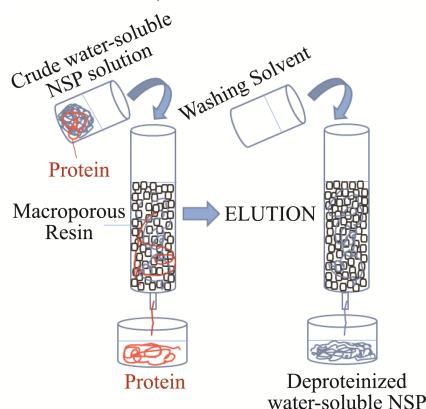


Fig.5 Schematic presentation of water-soluble NSP deproteinization following SPE using macroporous resin

The macroporous resin S-8 has been reported to be useful in the deproteinization of water-soluble NSPs. It is a hyper-cross-linked polar resin with extremely rigid networks and large internal surface areas that has more consistent structure than activated carbon (Kammerer et al., 2011). The adsorption mechanism of the resin is based on dipole-dipole forces (Zwir-Ferenc and Biziuk, 2006). Its polar group plays an important role in adsorbing protein contributing to its high deproteinization efficiency (Yang et al., 2012). S-8 resin showed higher deproteinization efficiency (75.9%) than H_2O_2 (33.9%) and active carbon (63.8%) and higher polysaccharide recovery (84.7%) than H_2O_2 (62.9%), Sevag reagent (81.7%) and active carbon (64.1%). Furthermore, nearly no degradation of polysaccharide was noted on the use of S-8 resin in the deproteinization process (Yang et al.,

2012). Aside from S-8, the use of macroporous resin ADS-7 for the removal of residuary protein resulted to a high purity (99.7%) polysaccharide from *Dendrobium denneanum* (Lou et al., 2011).

4.7 Green Alternative Deproteinization

Methods (Freeze-Thaw Technique)

Freeze-thaw treatment may cause complex changes in a food material including formation of ice crystals, phase separation, freeze-concentration of protein and solutes, and inducing pH and buffering changes (Mun et al., 2008; James, Purnell & James, 2015; Zhao et al., 2015; Jeremiah, 2019). During the freeze-thaw cycle, several changes are induced in the protein structure at the secondary, tertiary and quaternary levels that affect the protein stability (Zhang & Erbjerg, 2019). The growth of ice crystals during freezing can facilitate the opening and unfolding of protein structure (Zhao et al., 2015). Increasing the number of freezing-thawing cycles could also enhance the exposure of intermolecular disulfide bonds resulting in protein precipitation (Zhao, Sun, Li, Liu & Kong, 2017; Kolhe & Goswami, 2018). Collectively, these effects can be exploited for the deproteinization of water-soluble NSPs. This technique do not use any external processing aids (chemical or enzymatic), and thus it has been presented as a green alternative deproteinization method. Although the process requires longer time than the other previously discussed deproteinization methods, freezing-thawing technique is a simple approach and can be considered the safest among all deproteinization methods useful for water-soluble NSPs for food applications (Xiong et al., 2017). Figure 6 shows a schematic presentation of water-soluble NSP deproteinization using the freeze-thaw technique.

Table 1 Deproteinization efficiency (D_{eff}), residual protein (P_r), polysaccharide recovery (P_R) and polysaccharide loss (P_L) of different deproteinization methods

Deproteinization Method	Polysaccharide Crude extract	$D_{\text{eff}}/\%$	$P_r/\%$	$P_R/\%$	$P_L/\%$	Reference
Chemical	Sevag Reagent					
	Ganoderma (<i>Ganoderma sinense</i>) mycelium	78.90	-	-	22.90	Peng, Han, Liu & Zhou, 2016
	Brown seaweed (<i>Laminaria japonica</i>)	31.21	-	-	7.16	Zha et al., 2012
	Ganoderma (<i>Ganoderma atrum</i>)	84.20	-	-	35.60	Chen et al., 2012
	Corn Silk (<i>Stigma medic</i>)	64.20	-	-	34.30	Li, Zhao, Zhou & Wu, 2012
	Astragalus	70.80	-	-	4.44	Xia, Lu, Ge & Xu, 2007
	Gellan gum	87.90	-	28.60	-	Wang et al., 2007
	Epimedium	68.71	-	-	21.50	Zheng, Su, Tang, Liu & Sun, 2009
	Trichloroacetic acid (TCA)					
	Cucumber (<i>Cucumis sativus</i>)	95.30	-	-	18.90	Chen, Huang & Hu, 2018
	Pumpkin (<i>Cucurbita</i>)	89.30	-	-	12.00	Chen & Huang, 2018
	Cushaw (<i>Cucurbita moschata</i>)	86.90	-	-	13.50	Chen & Huang, 2018
	Ganoderma (<i>Ganoderma sinense</i>) mycelium	79.20	-	-	19.10	Peng, Han, Liu & Zhou, 2016
	Fern (<i>Asplenium australasicum</i>)	-	6.63	50.13	-	Zeng & Lai, 2016
Chemical	Brown seaweed (<i>Laminaria japonica</i>)	83.76	-	-	49.59	Zha et al., 2012
	Garlic (<i>Allium sativum</i>)	95.90	-	-	20.40	Huang, Shu, Cai, Liu & Xiao, 2012
	Mushroom (<i>Phellinus baumii</i>)	82.00	-	-	10.80	Xie, Zhang, Peng & Gan, 2011
	Hydrochloric Acid (HCl)					
	Chinese watermelon	-	1.00	96.80	-	Liu, Huang & Hu, 2018
	Garlic (<i>Allium sativum</i>)	98.60	-	-	29.00	Huang, Shu, Cai, Liu & Xiao, 2012
	Milk Thistle (<i>Silybum marianum</i>)	88.98	-	-	14.80	Dong, Zhang & Sun, 2007
	Sodium Chloride (NaCl)					
	Pumpkin (<i>Cucurbita</i>)	92.70	-	-	3.60	Chen & Huang, 2018
	Cushaw (<i>Cucurbita moschata</i>)	90.30	-	-	3.90	Chen & Huang, 2018
	Garlic (<i>Allium sativum</i>)	87.70	-	-	4.30	Huang, Shu, Cai, Liu & Xiao, 2012
	Calcium Chloride (CaCl_2)					
	Cucumber (<i>Cucumis sativus</i>)	89.60	-	-	4.30	Chen, Huang & Hu, 2018
	Pumpkin (<i>Cucurbita</i>)	94.60	-	-	4.00	Chen & Huang, 2018
Chemical	Cushaw (<i>Cucurbita moschata</i>)	93.80	-	-	4.50	Chen & Huang, 2018
	Garlic (<i>Allium sativum</i>)	90.30	-	-	5.10	Huang, Shu, Cai, Liu & Xiao, 2012
	Lead acetate					
	Ganoderma (<i>Ganoderma atrum</i>)	89.40	-	-	15.60	Chen et al., 2012
Chemical	Ammonium sulfate					
	Ganoderma (<i>Ganoderma sinense</i>) mycelium	97.00	-	-	26.90	Peng, Han, Liu & Zhou, 2016

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Enzymatic Method	Papain	Ganoderma (<i>Ganoderma atrum</i>)	88.80	-	-	21.30	Chen et al., 2012
		Gellan gum	86.20	-	78.90	-	Wang et al., 2007
	Neutral <i>Protease</i>	Gellan gum	72.00	-	85.10	-	Wang et al., 2007
	Alkaline <i>Protease</i>	Gellan gum	86.40	-	89.30	-	Wang et al., 2007
	<i>Trypsinase</i>	Brown seaweed (<i>Laminaria japonica</i>)	72.16	-	-	5.26	Zha et al., 2012
Aqueous Two-Phase System (ATPS)	HSCCC with Ethanol-ammonium sulfate	Spirulina (<i>Arthrospira platensis</i>)	-	0.52	-	-	Wu, Li, Zhao & Liu, 2017
	Thermosensitive copolymer ethylene-oxide-b-propylene-oxide-b-ethylene-oxide (EOPOEO) and NaH ₂ PO ₄	<i>Lycium barbarum</i>	-	5.60	-	-	Wang et al., 2016
Solid-Phase Extraction (SPE) using Macroporous Resin	NKA-9	<i>Cordyceps militaris</i>	62.51	-	78.08	-	He et al., 2019
	S-8	Pumpkin (<i>Cucurbita</i>)	75.90	-	84.70	-	Yang et al., 2012
Greener Alternative Methods	Freezing-Thawing	<i>Cipangopaludina chinensis</i>	87.10	-	84.91	-	Xiong et al., 2017
Combination of methods	Sevag+Papain	Mushroom (<i>Lentinus edodes</i>)	90.50	-	-	-	Zhu et al., 2018
	Proteinase+TCA	Fern (<i>Asplenium australasicum</i>)	-	2.57	59.78	-	Zeng & Lai, 2016
	Proteinase+galactanase+TCA	Fern (<i>Asplenium australasicum</i>)	-	2.32	51.99	-	Zeng & Lai, 2016
	Proteinase+mannanase+TCA	Fern (<i>Asplenium australasicum</i>)	-	2.37	52.90	-	Zeng & Lai, 2016
	Proteinase+arabinanase+TCA	Fern (<i>Asplenium australasicum</i>)	-	2.01	53.07	-	Zeng & Lai, 2016
	Proteinase+xylanase+TCA	Fern (<i>Asplenium australasicum</i>)	-	2.55	47.09	-	Zeng & Lai, 2016
	Sevag method+TCA	Brown seaweed (Fucoidan)	78.68	-	-	28.89	Zhang, Du, Zhou, Jie & Hui, 2012
	Trypsinase+Sevag Method	Brown seaweed (<i>Laminaria japonica</i>)	92.17	-	-	10.29	Zha et al., 2012

Several factors affect the deproteinization efficiency of the freezing-thawing technique. These include freezing temperature, freezing time, thawing temperature and freeze-thaw cycles (Bhatnagar, Bogner & Pikal, 2007; Xiong et al., 2017). Deproteinization efficiency increases with decreasing the freezing temperature down to -40°C where the deproteinization efficiency reach a relative steady state, with polysaccharide recovery beginning to decrease significantly as the freezing temperature is further decreased (Xiong et al., 2017). Increasing the freezing time decreases the average particle size of protein resulting in a sharp increase in surface free energy and a rapid destabilization of protein. Therefore, longer freezing time is favored for higher deproteinization efficiency. However, a higher polysaccharides loss due to

mass adsorption and embedding of protein precipitates was reported (Bhatnagar, Bogner & Pikal, 2007). Deproteinization efficiency tends to decrease with increasing thawing temperature but increases with the increase in the number of freezing-thawing cycles. Compared to Sevag method, while there was no significant difference between the two methods on the deproteinization efficiency, freezing-thawing technique had better polysaccharide recovery than the Sevag method (Xiong et al., 2017).

Table 1 presents a summary of the deproteinization efficiency, residual protein, polysaccharide recovery and polysaccharide loss of different methods utilized for the deproteinization of water-soluble NSPs.

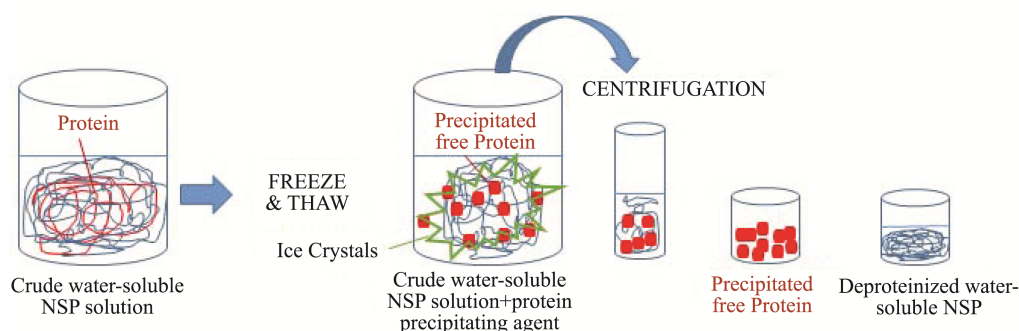


Fig.6 Schematic presentation of water-soluble NSP deproteinization following SPE using macroporous resin

5 Biological Activities

Water-soluble NSPs, particularly from botanical sources, are being sought and intensely studied for their biological activities considering their abundance and broad-spectrum therapeutic properties (Paulsen, 2001; Zong, Cao, & Wang, 2012; Liu et al., 2015). These biological activities include the water-soluble NSPs ability to enhance the immune system, exhibit anti-cancer potential, act as an antimicrobial agent, antioxidant, as prebiotic, and regulate or inhibit enzyme activities (Liu et al., 2015; Ketha & Gudipati, 2018; Yu, Shen, Song, & Xie, 2018).

Of special interest for water soluble NSPs is their ability to act as prebiotic for gut microorganisms that produces important metabolic fermentation products, the short chain fatty acids (SCFAs), mainly acetate, propionate and butyrate (Al-Sheraji et al., 2013). These short chain fatty acids play significant role in several vital organs of the body (Koh, De Vadder, Kovatcheva-

Datchary & Backhed, 2016). Acetate is the main SCFA in the blood thus is often used to monitor colonic events while propionic acid is important in liver functions and cholesterol metabolism that is capable of inhibiting hepatic cholesterol synthesis (Wong et al., 2006; Hijova & Chmelarova, 2007; Kasabuchi et al., 2015). Butyric acid functions as energy source for colonic epithelium, regulates epithelial and immune cell growth and apoptosis, provides protection against colonic cancer and colitis, exhibit anti-inflammatory properties, help modulates oxidative stress and affects composition of the mucus layer (Byrne, Chambers, Morrison & Frost, 2015). Further, butyrate promotes normal homeostasis for colonocytes and exhibit anti-tumor for tumor colonocytes (Rios-Covian et al., 2016). Other significant functions of SCFAs are its ability to act as anti-inflammatory and anti-tumor agents which are detailed in a current review by Van der Beek et al. (2017).

Overall, a major driver for research on water-soluble NSPs is their colon health potential through enhancing

intestinal transit rate, fecal moisture and lowering pH to inhibit growth of pathogenic microorganisms (Koh, De

Vadder, Kovatcheva-Datchary & Backhed, 2016; Rios-Covian et al., 2016).

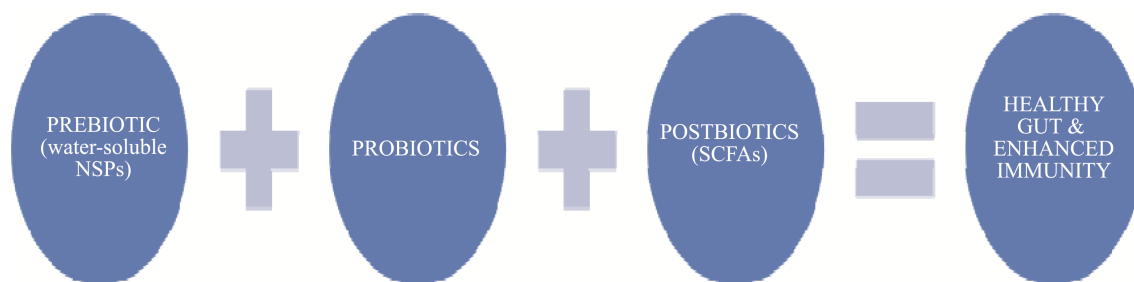


Fig.7 Schematic presentation of the symbiotic effect of prebiotics, probiotics and postbiotics on gut health and immunity

6 Food Applications of Water-Soluble NSPs

The use of natural water-soluble NSPs such as mucilage and gums encompasses a wide spectrum of both food and non-food industrial applications (Phillips & Williams, 2000; Verbeken, Dierckx, & Dewettinck, 2003). Most practical food applications of water-soluble NSPs like mucilage and gums, are based on their ability to modify texture by acting as a thickening or gelling agents and on their capacity to stabilize heterogeneous dispersions or emulsion by acting as emulsifiers (Stephen & Phillips, 2016). Depending on the food system, water-soluble NSPs contribute different functions. In dressings and sauces, its pseudoplastic behavior is ideal since it provides the suspension some stability due to high viscosity at low shear rates while the product remains pourable at higher shear rates. In addition, since dressings and sauces contains salts and are mostly acidic in nature, the rheological properties water-soluble NSPs that are not dependent or at least not greatly affected by the presence of salts and acids is appropriate to be used in order to maintain product quality (Saha & Bhattacharya, 2010; Stephen & Phillips, 2016). For the beverage industry, its low viscosity in water makes it a potential emulsifier for flavor bases and concentrates (Phillips & Williams, 2000; Milani & Maleki, 2012; Stephen & Phillips, 2016). Water-soluble NSPs improves the rheology of dough and the viscosity of mixes in baked products (Guarda, Rosell, Benedito, & Galotto, 2004; Kohajdová & Karovičová, 2009; Stephen & Phillips, 2016). Lastly, for frozen products such as ice cream, the addition of water-soluble NSPs helps in preventing product shrinkage during heat shock (temperature fluctuations) and helps control ice crystal growth (Hui, Legarretta, Lim, Murrell, & Nip, 2004; Soma, Williams,

& Lo, 2009; Stephen & Phillips, 2016).

7 Perspectives and Future Research

For future research, the following research areas are potentially worthy of investigation in order to establish the full potential use of water-soluble NSPs for industrial applications: (1) use of other novel extraction techniques such as pulse electric field (PEF) and high arc electrostatic technologies; (2) use of green (non-toxic, food and environment friendly), scalable and cost efficient isolation and purification methods. The use of ionic liquids is gaining much interest and further research is required in this field; (3) use of up-to-date and novel characterization techniques for in depth understanding of the water-soluble NSP properties and behavior including imaging technology; (4) wider utilization of water-soluble NSP in other food and non-food applications including its potential use in encapsulating bioactive materials for enhanced stability and controlled release, nano-fiber production, utilization for food product quality enhancements such as syneresis reduction, thermal stability enhancement, in the development of functional foods, waste water treatment and others; (5) water-soluble NSP modification through grafting for enhanced functionality using advance and novel techniques; (6) evaluation of other bioactivity potential including its satiety effect; and (7) investigation of the water-soluble NSPs from non-conventional and underutilized resources.

8 Conclusions

Water-soluble NSP, although considered a minor component, it is equally important as major polysaccharides like starch and water insoluble counterpart in terms of potential applications. The

extraction, isolation and purification of water-soluble NSPs can influence its recovery, composition, structure, physical properties and bioactivities. Thus, it is important to properly evaluate the appropriateness of the methods used in order to maximize recovery and maintain structural integrity and bioactivity of the extracted material. The use of novel extraction techniques and green purification methods will be useful in obtaining a higher yield of water-soluble NSP extract with high purity that is safe to use for food and pharmaceutical applications. The use of water-soluble NSP in food products create value and make it worthy to recover and utilize agro-waste leading to obvious environmental and economical benefits. Further, the various bioactivities exhibited by water-soluble NSPs make it a promising biomaterial useful for the prevention or treatment of various diseases like diabetes, cancer and for the improvement of gut health and immunity.

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References

- Ahmadi, S., Mainali, R., Nagpal, R., Sheikh-Zeinoddin, M., Soleimani-Zad, S., Wang, S., & Yadav, H. (2017). Dietary polysaccharides in the amelioration of gut microbiome dysbiosis and metabolic diseases. *Obesity and Control Therapies: Open Access*, 4(3), 1-23
- Alén, R. (2018). *Carbohydrate chemistry: Fundamentals and applications*. World Scientific Publishing Company
- Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, 5(4), 1542-1553
- Arasi, M. A. S. A. G., Rao, M. G., & Bagyalakshmi, J. (2016). Optimization of microwave-assisted extraction of polysaccharide from *Psidium guajava* L. fruits. *International Journal of Biological Macromolecules*, 91, 227-232
- Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave and ultrasound-assisted methods for extraction of pectin from grapefruit. *Chemical Engineering and Processing: Process Intensification*, 50(11-12), 1237-1243
- Banerjee, J., Singh, R., Vijayaraghavan, R., MacFarlane, D., Patti, A. F., & Arora, A. (2017). Bioactives from fruit processing wastes: Green approaches to valuable chemicals. *Food Chemistry*, 225, 10-22
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International Journal of Advanced Research in Chemical Science*, 2(4), 25-32
- Baumstark, A. L. (2017). Carbohydrate chemistry/glycoscience. *Heterocyclic Communications*, 23(3), 133-135
- BeMiller, J. N. (2018). *Carbohydrate chemistry for food scientists* (3rd Edition). United Kingdom: Woodhead Publishing and AACC International
- Benito-Román, Ó., Alonso, E., & Cocero, M. J. (2013). Ultrasound-assisted extraction of β -glucans from barley. *LWT-Food Science and Technology*, 50(1), 57-63
- Bertoft, E. (2017). Understanding starch structure: Recent progress. *Agronomy*, 7, 3, 56
- Bhatnagar, B. S., Bogner, R. H., & Pikal, M. J. (2007). Protein stability during freezing: Separation of stresses and mechanisms of protein stabilization. *Pharmaceutical Development and Technology*, 12(5), 505-523
- Byrne, C. S., Chambers, E. S., Morrison, D. J., & Frost, G. (2015). The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal of Obesity*, 39(9), 1331
- Castro-Alves, V. C., & do Nascimento, J. R. O. (2016). Speeding up the extraction of mushroom polysaccharides. *Food Analytical Methods*, 9(9), 2429-2433
- Cazón, P., Velazquez, G., Ramírez, J. A., & Vázquez, M. (2017). Polysaccharide-based films and coatings for food packaging: A review. *Food Hydrocolloids*, 68, 136-148
- Chang, X. L., Chen, B. Y., & Feng, Y. M. (2011). Water-soluble polysaccharides isolated from skin juice, gel juice and flower of *Aloe vera* Miller. *Journal of the Taiwan Institute of Chemical Engineers*, 42(2), 197-203
- Chemat, S., Lagha, A., AitAmar, H., Bartels, P. V., & Chemat, F. (2004). Comparison of conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds. *Flavour and Fragrance Journal*, 19(3), 188-195
- Chemat, F., & Cravotto, G. (2012). *Microwave-assisted extraction for bioactive compounds: Theory and practice* (Vol. 4). New York: Springer Science & Business Media
- Chen, Y., Gu, X., Huang, S. Q., Li, J., Wang, X., & Tang, J. (2010). Optimization of ultrasonic/microwave assisted extraction (UMAE) of polysaccharides from *Inonotus obliquus* and evaluation of its anti-tumor activities. *International Journal of*

- Biological Macromolecules, 46(4), 429-435
- Chen, F., & Huang, G. (2018). Extraction and antioxidant activities of cushaw polysaccharide. *International Journal of Biological Macromolecules*, 120, 1646-1649
- Chen, Y., Xie, M., Li, W., Zhang, H., Nie, S., Wang, Y., & Li, C. (2012). An effective method for deproteinization of bioactive polysaccharides extracted from lingzhi (*Ganoderma atrum*). *Food Science and Biotechnology*, 21(1), 191-198
- Chen, R., Li, S., Liu, C., Yang, S., & Li, X. (2012). Ultrasound complex enzymes assisted extraction and biochemical activities of polysaccharides from *Epimedium* leaves. *Process Biochemistry*, 47(12), 2040-2050
- Chen, S., Chen, H., Tian, J., Wang, J., Wang, Y., & Xing, L. (2014). Enzymolysis-ultrasonic assisted extraction, chemical characteristics and bioactivities of polysaccharides from corn silk. *Carbohydrate Polymers*, 101, 332-341
- Cheng, Z., Song, H., Cao, X., Shen, Q., Han, D., Zhong, F. & Yang, Y. (2017). Simultaneous extraction and purification of polysaccharides from *Gentiana scabra* Bunge by microwave-assisted ethanol-salt aqueous two-phase system. *Industrial Crops and Products*, 102, 75-87
- Cheng, Z., Song, H., Yang, Y., Liu, Y., Liu, Z., Hu, H., & Zhang, Y. (2015). Optimization of microwave-assisted enzymatic extraction of polysaccharides from the fruit of *Schisandra chinensis* Baill. *International Journal of Biological Macromolecules*, 76, 161-168
- Choct, M. (2015). Feed non-starch polysaccharides for monogastric animals: Classification and function. *Animal Production Science*, 55(12), 1360-1366
- Conlon, M. A., & Topping, D. L. (2016). Dietary polysaccharides and polyphenols can promote health by influencing gut microbiota populations. *Food & Function*, 7(4), 1730-1730
- Cui, S. W. (2000). Polysaccharide gums from agricultural products: Processing, structures and functionality. Boca Raton, Florida: Taylor & Francis
- Cui, S. W. (2005). Food carbohydrates: Chemistry, physical properties, and applications. Boca Raton, Florida: Taylor & Francis
- Dai, F. J., & Chau, C. F. (2017). Classification and regulatory perspectives of dietary fiber. *Journal of Food and Drug Analysis*, 25(1), 37-42
- Eliasson, A. C. (2017). Starch: Physicochemical and functional aspects. In A. C. Eliasson *Carbohydrates in Food* (pp. 501-600). Taylor & Francis
- Fabre, J. F., Lacroux, E., Valentin, R., & Mouloungui, Z. (2015). Ultrasonication as a highly efficient method of flaxseed mucilage extraction. *Industrial Crops and Products*, 65, 354-360
- Farahnaky, A., Bakhshizadeh-Shirazi, S., Mesbahi, G., Majzoobi, M., Rezvani, E., & Schleining, G. (2013). Ultrasound-assisted isolation of mucilaginous hydrocolloids from *Salvia macrosiphon* seeds and studying their functional properties. *Innovative Food Science & Emerging Technologies*, 20, 182-190
- Farahnaky, A., Majzoobi, M., & Bakhshizadeh-Shirazi, S. (2019). Emerging technologies for isolation of natural hydrocolloids from mucilaginous seeds. In S.M.A. Razavi *Emerging Natural Hydrocolloids: Rheology and Functions*, 451-472. John Wiley & Sons Ltd
- Felkai-Haddache, L., Remini, H., Dulong, V., Mamou-Belhabib, K., Pictou, L., Madani, K., & Rihouey, C. (2016). Conventional and microwave-assisted extraction of mucilage from *Opuntia ficus-indica* cladodes: Physico-chemical and rheological properties. *Food and Bioprocess Technology*, 9(3), 481-492
- Garg, H. G., Cowman, M. K., & Hales, C. A. (2011). *Carbohydrate chemistry, biology and medical applications* (1st ed.). Elsevier
- Goh, K. K. T., Matia-Merino, L., Pinder, D. N., Saavedra, C., & Singh, H. (2011). Molecular characteristics of a novel water-soluble polysaccharide from the New Zealand black tree fern (*Cyathea medullaris*). *Food Hydrocolloids*, 25(3), 286-292
- Gonzalez, J. T. (2018). *Carbohydrate metabolism in health and disease*. Basel, Switzerland: MDPI
- Guarda, A., Rosell, C. M., Benedito, C., & Galotto, M. J. (2004). Different hydrocolloids as bread improvers and anti-staling agents. *Food Hydrocolloids*, 18(2), 241-247
- Gupta, A., & Verma, J. P. (2015). Sustainable bio-ethanol production from agro-residues: A review. *Renewable and Sustainable Energy Reviews*, 41, 550-567
- Harding, S. E., Tombs, M. P., Adams, G. G., Paulsen, B. S., Inngjerdigen, K. T., & Barsett, H. (2017). *An introduction to polysaccharide biotechnology*
- Taylor & Francis. Hernandez-Carmona, G., Freile-Pelegrín, Y., & Hernández-Garibay, E. (2013). Conventional and alternative technologies for the extraction of algal polysaccharides. In Dominguez, H. *Functional Ingredients from Algae for Foods and Nutraceuticals*, 475-516. Woodhead Publishing
- Hernández-Carmona, F., Morales-Matos, Y., Lambis-Miranda, H., & Pasqualino, J. (2017). Starch extraction potential from plantain peel wastes. *Journal of Environmental Chemical Engineering*, 5(5), 4980-4985

- Hijova, E., & Chmellarova, A. (2007). Short chain fatty acids and colonic health. *Bratislavské Lekárske Listy*, 108(8), 354
- Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes*, 8(2), 172-184
- Hossain, N., Zaini, J. H., & Mahlia, T. M. I. (2017). A review of bioethanol production from plant-based waste biomass by yeast fermentation. *International Journal of Technology*, 8(1), 5-18
- Hostettmann, K. (2014). *Handbook of chemical and biological plant analytical methods*. John Wiley & Sons
- Hromadkova, Z., Ebringerova, A., & Valachovič, P. (2002). Ultrasound-assisted extraction of water-soluble polysaccharides from the roots of valerian (*Valeriana officinalis* L.). *Ultrasonics Sonochemistry*, 9(1), 37-44
- Hu, X., & Goff, H. D. (2018). Fractionation of polysaccharides by gradient non-solvent precipitation: A review. *Trends in Food Science & Technology*, 81, 108-115
- Hu, X., Liu C., Jin, Z., & Tian, Y. (2016). Fractionation of dextrin by gradient polyethylene glycol precipitation. *Journal of Chromatography A*, 1434, 81-90
- Huang, G., Chen, Y., & Wang, X. (2011). Extraction and deproteinization of pumpkin polysaccharide. *International Journal of Food Sciences and Nutrition*, 62(6), 568-571
- Huang, G., Shu, S., Cai, T., Liu, Y., & Xiao, F. (2012). Preparation and deproteinization of garlic polysaccharide. *International Journal of Food Sciences and Nutrition*, 63(6), 739-741
- Huck, C. W., & Bonn, G. K. (2000). Recent developments in polymer-based sorbents for solid-phase extraction. *Journal of Chromatography A*, 885(1-2), 51-72
- Hui, Y. H., Legarretta, I. G., Lim, M. H., Murrell, K. D., & Nip, W. K. (2004). *Handbook of Frozen Foods*. New York: Marcel & Dekker, Inc
- Iqbal, M., Tao, Y., Xie, S., Zhu, Y., Chen, D., Wang, X., & Hussain, H. I. (2016). Aqueous two-phase system (ATPS): an overview and advances in its applications. *Biological Procedures Online*, 18(1), 18
- Jakobsen, G. V., Jensen, B. B., Knudsen, K. B., & Canibe, N. (2015). Impact of fermentation and addition of non-starch polysaccharide-degrading enzymes on microbial population and on digestibility of dried distillers grains with solubles in pigs. *Livestock Science*, 178, 216-227
- James, C., Purnell, G., & James, S. J. (2015). A review of novel and innovative food freezing technologies. *Food and Bioprocess Technology*, 8(8), 1616-1634
- Jeremiah, L. E. (2019). *Freezing effects on food quality*. Boca Raton, Florida: Taylor & Francis
- Jian, H. L., Lin, X. J., Zhang, W. A., Zhang, W. M., Sun, D. F., & Jiang, J. X. (2014). Characterization of fractional precipitation behavior of galactomannan gums with ethanol and isopropanol. *Food Hydrocolloids*, 40, 115-121
- Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2015). Polysaccharides as valuable materials in food packaging. In G. Cirillo, U. G. Spizzirri & F. Iemma, (Volume 1) *Functional Polymers in Food Science: From Technology to Biology*, 211-251. Scrivener and John Wiley & Sons
- Kammerer, J., Carle, R., & Kammerer, D. R. (2010). Adsorption and ion exchange: Basic principles and their application in food processing. *Journal of Agricultural and Food Chemistry*, 59(1), 22-42
- Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients*, 7(4), 2839-2849
- Ketha, K., & Gudipati, M. (2018). Immunomodulatory activity of non-starch polysaccharides isolated from green gram (*Vigna radiata*). *Food Research International*, 113, 269-276
- Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*, 165(6), 1332-1345
- Kohajdová, Z., & Karovičová, J. (2009). Application of hydrocolloids as baking improvers. *Chemical Papers*, 63(1), 26-38
- Kolhe, P., & Goswami, S. (2018). Bulk protein solution: Freeze-thaw process, storage and shipping considerations. In N. W. Warne and H. C. Mahler *Challenges in Protein Product Development*, 313-336, AAPS Advances in the Pharmaceutical Sciences Series (Vol. 38). AAPS and Springer
- Lao, F., & Giusti, M. M. (2018). Extraction of purple corn (*Zea mays* L.) cob pigments and phenolic compounds using food-friendly solvents. *Journal of Cereal Science*, 80, 87-93
- Lee, O. K., & Lee, E. Y. (2016). Sustainable production of bio-ethanol from renewable brown algae biomass. *Biomass and Bioenergy*, 92, 70-75
- Li, D., Ye, Y., Li, D., Li, X., & Mu, C. (2016). Biological properties of dialdehyde carboxymethyl cellulose crosslinked gelatin-PEG composite hydrogel fibers for wound dressings. *Carbohydrate Polymers*, 137, 508-514
- Li, H. B., Ren, A. N., & Zou, Y. F. (2012). Purification technology of polysaccharide from *Chrysanthemum indicum* by macroporous adsorption resin. *Chinese Journal of Experimental Traditional Medical Formulae*, 1-7

- Li, X., Zhao, R., Zhou, H. L., & Wu, D. H. (2012). Deproteinization of polysaccharide from the *Stigma maydis* by sevag method. In H. Tan Advanced Materials Research (Volume 340), 416-420. Switzerland: Trans Tech Publications
- Liu, J., Luo, J., Sun, Y., Ye, H., Lu, Z., & Zeng, X. (2010). A simple method for the simultaneous decoloration and deproteinization of crude levan extract from *Paenibacillus polymyxa* EJS-3 by macroporous resin. *Bioresource Technology*, 101(15), 6077-6083
- Liu, J., Willför, S., & Xu, C. (2015). A review of bioactive plant polysaccharides: Biological activities, functionalization, and biomedical applications. *Bioactive Carbohydrates and Dietary Fibre*, 5(1), 31-61
- Lo, T. C. T., Tsao, H. H., Wang, A. Y., & Chang, C. A. (2007). Pressurized water extraction of polysaccharides as secondary metabolites from *Lentinula edodes*. *Journal of Agricultural and Food Chemistry*, 55(10), 4196-4201
- Luo, A., Ge, Z., Fan, Y., Luo, A., Chun, Z., & He, X. (2011). In vitro and in vivo antioxidant activity of a water-soluble polysaccharide from *Dendrobium*. *Molecules*, 16(2), 1579-1592
- Mahmood, K., Kamilah, H., Shang, P. L., Sulaiman, S., Ariffin, F., & Alias, A. K. (2017). A review: Interaction of starch/non-starch hydrocolloid blending and the recent food applications. *Food Bioscience*, 19, 110-120
- Maphosa, Y., & Jideani, V. A. (2016). Dietary fiber extraction for human nutrition: A review. *Food Reviews International*, 32(1), 98-1
- Marić, M., Grassino, A. N., Zhu, Z., Barba, F. J., Brnčić, M., & Brnčić, S. R. (2018). An overview of the traditional and innovative approaches for pectin extraction from plant food wastes and by-products: Ultrasound, microwaves, and enzyme-assisted extraction. *Trends in Food Science & Technology*, 76, 28-37
- Milani, J., & Maleki, G. (2012). Hydrocolloids in food industry. In B. Valdez Food Industrial Processes: Methods and Equipment, 17-38. IntechOpen
- Mun, S., Cho, Y., Decker, E. A., & McClements, D. J. (2008). Utilization of polysaccharide coatings to improve freeze-thaw and freeze-dry stability of protein-coated lipid droplets. *Journal of Food Engineering*, 86(4), 508-518
- Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta*, 703(1), 8-18
- Nadar, S. S., Pawar, R. G., & Rathod, V. K. (2017). Recent advances in enzyme extraction strategies: a comprehensive review. *International Journal of Biological Macromolecules*, 101, 931-957
- Nagy, G., Peng, T., & Pohl, N. L. (2017). Recent liquid chromatographic approaches and developments for the separation and purification of carbohydrates. *Analytical Methods*, 9(24), 3579-3593
- Paulsen, B. (2001). Plant polysaccharides with immunostimulatory activities. *Current Organic Chemistry*, 5(9), 939-950
- Pettolino, F. A., Walsh, C., Fincher, G. B., & Bacic, A. (2012). Determining the polysaccharide composition of plant cell walls. *Nature Protocols*, 7(9), 1590
- Phillips, G. O., & Williams, P. A. (2000). Handbook of hydrocolloids (1st Edition), 53-64
- Boca Raton, Florida: Taylor & Francis. Pigman, W. (2012). The carbohydrates: Chemistry and biochemistry (2nd Edition). London: Academic Press, Inc
- Pitipanapong, J., Chitprasert, S., Goto, M., Jiratchariyakul, W., Sasaki, M., & Shotipruk, A. (2007). New approach for extraction of charantin from *Momordica charantia* with pressurized liquid extraction. *Separation and Purification Technology*, 52(3), 416-422
- Poole, C. F. (2003). New trends in solid-phase extraction. *Trends in Analytical Chemistry*, 22(6), 362-373
- Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology*, 30(1), 37-44
- Qin, J. Z., & Zhang, H. Y. (2010). Deproteinization from *Phellinus linteus* crude polysaccharide by macroporous adsorption resin. *Science and Technology of Food Industry*, 1-12
- Rostami, H., & Gharibzadeh, S. M. T. (2016). Microwave-assisted extraction of jujube polysaccharide: Optimization, purification and functional characterization. *Carbohydrate Polymers*, 143, 100-107
- Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. *Journal of Food Science and Technology*, 47(6), 587-597
- Saini, J. K., Saini, R., & Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: Concepts and recent developments. *Biotechnology*, 5(4), 337-353
- Sarkar, G., Saha, N. R., Roy, I., Bhattacharyya, A., Bose, M., Mishra, R., & Chattopadhyay, D. (2014). Taro corms mucilage/HPMC based transdermal patch: An efficient device

- for delivery of diltiazem hydrochloride. *International Journal of Biological Macromolecules*, 66, 158-165
- Shi, X. D., Nie, S. P., Yin, J. Y., Que, Z. Q., Zhang, L. J., & Huang, X. J. (2017). Polysaccharide from leaf skin of *Aloe barbadensis* Miller: Part I Extraction, fractionation, physicochemical properties and structural characterization. *Food Hydrocolloids*, 73, 176-183
- Simpson, N. J. (2000). *Solid-phase extraction: Principles, techniques, and applications* (1st Edition). Boca Raton, Florida: Taylor & Francis
- Singh, R., Kumar, M., Mittal, A., & Mehta, P. K. (2016). Microbial enzymes: Industrial progress in 21st century. *Biotechnology*, 6(2), 174
- Sinha, A. K., Kumar, V., Makkar, H. P., De Boeck, G., & Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition: A review. *Food Chemistry*, 127(4), 1409-1426
- Soma, P. K., Williams, P. D., & Lo, Y. M. (2009). Advancements in non-starch polysaccharides research for frozen foods and microencapsulation of probiotics. *Frontiers of Chemical Engineering in China*, 3(4), 413
- Stephen, A. M., & Phillips, G. O. (2016). *Food polysaccharides and their applications*. Boca Raton, Florida: Taylor & Francis
- Sun, M., Wu, W., Liu, Z., & Cong, Y. (2017). Microbiota metabolite short-chain fatty acids, GPCR, and inflammatory bowel diseases. *Journal of Gastroenterology*, 52(1), 1-8
- Thakur, V. K., & Thakur, M. K. (2015). *Handbook of polymers for pharmaceutical technologies, Volume 3, Biodegradable Polymers*. Scrivener Publishing LLC and John Wiley & Sons, Inc
- Valdés, A., & Garrigós, M. C. (2016). Carbohydrate-based advanced biomaterials for food sustainability: A review. In *Materials Science Forum*, 842, 182-195. Switzerland: Trans Tech Publications
- Van der Beek, C. M., Dejong, C. H., Troost, F. J., Masclee, A. A., & Lenaerts, K. (2017). Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. *Nutrition Reviews*, 75(4), 286-305
- Verbeken, D., Dierckx, S., & Dewettinck, K. (2003). Exudate gums: Occurrence, production, and applications. *Applied Microbiology and Biotechnology*, 63(1), 10-21
- Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound-assisted extraction in the food industry: A review. *Innovative Food Science & Emerging Technologies*, 9(2), 161-169
- Wang, X., Yuan, Y., Wang, K., Zhang, D., Yang, Z., & Xu, P. (2007). Deproteinization of gellan gum produced by *Sphingomonas paucimobilis* ATCC 31461. *Journal of Biotechnology*, 128(2), 403-407
- Wang, J., Zhang, J., Zhao, B., Wang, X., Wu, Y., & Yao, J. (2010). A comparison study on microwave-assisted extraction of *Potentilla anserina* L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation. *Carbohydrate Polymers*, 80(1), 84-93
- Wijesinghe, W. A. J. P., & Jeon, Y. J. (2012). Enzyme-assisted extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds: a review. *Fitoterapia*, 83(1), 6-12
- Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology*, 40(3), 235-243
- Wu, G. H., Hu, T., Huang, Z. L., & Jiang, J. G. (2013). Characterization of water and alkali-soluble polysaccharides from *Pleurotus tuber-regium* sclerotia. *Carbohydrate Polymers*, 96(1), 284-290
- Wu, X., Li, R., Zhao, Y., & Liu, Y. (2017). Separation of polysaccharides from *Spirulina platensis* by HSCCC with ethanol-ammonium sulfate ATPS and their antioxidant activities. *Carbohydrate Polymers*, 173, 465-472
- Xiaohong, Y., Xianling, W., Wei, F., Dandan, S., & Ying, L. (2017). Decoloration and deproteinization of polysaccharides from American Ginseng. *Journal of Chinese Institute of Food Science and Technology*, 11, 19
- Xie, L. Y., Zhang, Y., Peng, W. H., & Gan, B. C. (2011). Study on methods of deproteinization from *Phellinus baumii* polysaccharide. *Journal of Chinese Medicinal Materials*, 34(2), 293-295
- Xing, X., Cui, S. W., Nie, S., Phillips, G. O., Goff, H. D., & Wang, Q. (2013). A review of isolation process, structural characteristics, and bioactivities of water-soluble polysaccharides from *Dendrobium* plants. *Bioactive Carbohydrates and Dietary Fibre*, 1(2), 131-147
- Xiong, Q., Huang, S., Chen, J., Wang, B., He, L., Zhang, L., & Zhang, D. (2017). A novel green method for deproteinization of polysaccharide from *Cipangopaludina chinensis* by freeze-thaw treatment. *Journal of Cleaner Production*, 142, 3409-3418
- Xu, Y., Zhang, L., Yang, Y., Song, X., & Yu, Z. (2015). Optimization of ultrasound-assisted compound enzymatic extraction and characterization of polysaccharides from blackcurrant. *Carbohydrate Polymers*, 117, 895-902
- Xu, J., Yue, R. Q., Liu, J., Ho, H. M., Yi, T., Chen, H. B., & Han, Q.

- B. (2014). Structural diversity requires individual optimization of ethanol concentration in polysaccharide precipitation. *International Journal of Biological Macromolecules*, 67, 205-209
- Yang, R., Meng, D., Song, Y., Li, J., Zhang, Y., Hu, X., ... & Li, Q. (2012). Simultaneous decoloration and deproteinization of crude polysaccharide from pumpkin residues by cross-linked polystyrene macroporous resin. *Journal of Agricultural and Food Chemistry*, 60(34), 8450-8456
- Yang, W., Wang, Y., Li, X., & Yu, P. (2015). Purification and structural characterization of chinese yam polysaccharide and its activities. *Carbohydrate Polymers*, 117, 1021-1027
- Yin, X., You, Q., & Jiang, Z. (2011). Optimization of enzyme assisted extraction of polysaccharides from *Tricholoma matsutake* by response surface methodology. *Carbohydrate Polymers*, 86(3), 1358-1364
- Ying, Z., Han, X., & Li, J. (2011). Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chemistry*, 127(3), 1273-1279
- Yu, Y., Shen, M., Song, Q., & Xie, J. (2018). Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. *Carbohydrate Polymers*, 183, 91-101
- Zgórk, G. (2009). Pressurized liquid extraction versus other extraction techniques in micropreparative isolation of pharmacologically active isoflavones from *Trifolium L.* species. *Talanta*, 79(1), 46-53
- Zha, X. Q., Xiao, J. J., Zhang, H. N., Wang, J. H., Pan, L. H., Yang, X. F., & Luo, J. P. (2012). Polysaccharides in *Laminaria japonica*: Extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of atherosclerosis. *Food Chemistry*, 134(1), 244-252
- Zhang, Y., & Erbjerg, P. (2019). On the origin of thaw loss: Relationship between freezing rate and protein denaturation. *Food Chemistry*, 299 (125104), 1-8
- Zhang, Y., He, S., & Simpson, B. K. (2018). Enzymes in food bioprocessing: Novel food enzymes, applications, and related techniques. *Current Opinion in Food Science*, 19, 30-35
- Zhao, J., Dong, F., Li, Y., Kong, B., & Liu, Q. (2015). Effect of freeze-thaw cycles on the emulsion activity and structural characteristics of soy protein isolate. *Process Biochemistry*, 50(10), 1607-1613
- Zhao, J., Sun, F., Li, Y., Liu, Q., & Kong, B. (2017). Modification of gel properties of soy protein isolate by freeze-thaw cycles are associated with changes of molecular force involved in the gelation. *Process Biochemistry*, 52, 200-208
- Zhao, X., Qiao, L., & Wu, A. M. (2017). Effective extraction of *Arabidopsis* adherent seed mucilage by ultrasonic treatment. *Scientific reports*, 7 (40672), 1-8
- Zhu, Y., Li, Q., Mao, G., Zou, Y., Feng, W., Zheng, D., & Yang, L. (2014). Optimization of enzyme-assisted extraction and characterization of polysaccharides from *Hericium erinaceus*. *Carbohydrate Polymers*, 101, 606-613
- Zong, A., Cao, H., & Wang, F. (2012). Anti-cancer polysaccharides from natural resources: A review of recent research. *Carbohydrate Polymers*, 90(4), 1395-1410
- Żwir-Ferenc, A., & Biziuk, M. (2006). Solid-phase extraction technique: Trends, opportunities and applications. *Polish Journal of Environmental Studies*, 15(5), 1-15

(上接第 29 页)

- [26] Nourmohammadi E, Sadeghi A M, Alami M, et al. Amino acid composition and antioxidative properties of hydrolysed pumpkin (*Cucurbita pepo* L.) oil cake protein [J]. *International Journal of Food Properties*, 2017, 20(12): 3244-3255
- [27] Samaranayaka A G P, Li-Chan E C Y. Food-derived peptidic antioxidants: a review of their production, assessment, and potential applications [J]. *Journal of Functional Foods*. 2011, 3(4): 229-254
- [28] ZOU Tang-bin, HE Tai-ping, LI Hua-bin, et al. The structure activity relationship of the antioxidant peptides from natural proteins [J]. *Molecules*, 2016, 21(72): 14
- [29] WANG Xueqin, YU Huahua, XING Rong, et al. Preparation and identification of antioxidative peptides from pacific herring (*Clupea pallasii*) protein [J]. *Molecules*, 2019, 24: 1946
- [30] Opie E L. On the relation of necrosis and inflammation to denaturation of proteins [J]. *Journal of Experimental Medicine*, 1962, 115: 597-608