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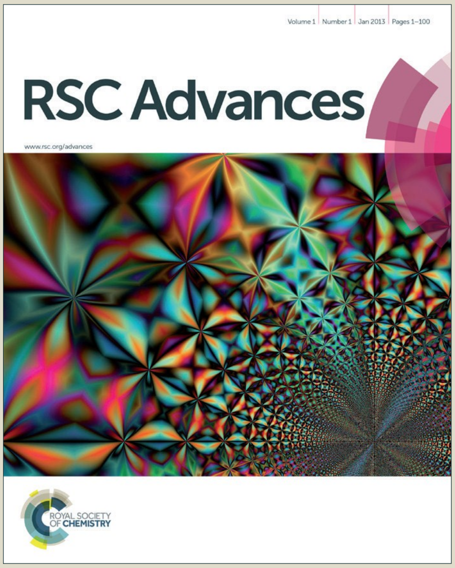

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Review on extraction, characterization and application of soybean polysaccharide

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Abstract

Soybean polysaccharide (SPS) is a class of soluble polysaccharides derived from soybean cotyledon, soybean meal or okara, and has broadly been used in food industry. In recent decades, due to its attractive physicochemical properties, SPS has been developed into versatile emulsifiers or stabilizers for beverage. Additionally, studies have emerged to reveal its potential in biomaterial and biological activities. In this review, we critically appraise the latest literature on the extraction and structural features of SPS, and perspective for the biological applications of SPS. We focus on the current strategies for extraction of this unique polysaccharide, specific structure features, and functional utilization of SPS. Notably, SPS-based food additives are demonstrated to increase the value of biological applications, such as anticancer and immunoregulation, enabling us directly use it in the area of biomedicine. Lastly, we suggest some potential directions for the development of SPS for extensive utilization in biomedicine.

Keywords

Soybean polysaccharide, assisted extraction, structural characterization, functional property, biological activity

1. Introduction

Soybean (*Glycine max*) is traditionally used to support protein or oil for human consuming. A famous food made from soybean, tofu, has attracted numerous attentions because it can provide constantly both calcium and protein for our diet. During the formation of tofu, the soybean curd residue, namely okara, is the dominating surplus material and it is always regarded as waste.[1] Okara has abundant active substances, such as protein, dietary fibre, mineral matter and oligosaccharide.[2] To reduce the cost and energy waste, okara is widely reused as the resource of polysaccharides, similarly as for soybean meal and soybean cotyledon. Intake of soybean polysaccharide (SPS) is likely to decrease human plasma cholesterol levels.[3] So far, no research has found that SPS may cause adverse biological effects to human beings. Therefore, SPS is perfectly applied in food service industry, such as enhancing stability of beverage, increasing emulsifying property of acidic solution, and utilizing as biodegradable film. Additionally, SPS is increasingly used for the purposes of anticancer or immuneregulation. However, the above mentioned properties of SPS are barely reviewed in recent literatures. In order for better development and more convenient use of this unique natural polysaccharide in broader fields of food industry and pharmaceutical chemistry, there is a pressing demand for comprehensively and efficiently analyzing the property of SPS from various angles.

Hence, in this concise review, we will firstly summarize the practical extraction methods for the preparation of SPS, followed by the introduction of the major structural characteristics which are desirable in food applications, the detailed properties of emulsification, anticancer, and immunoregulation of this special polysaccharide. Finally, we will highlight the current potential applications by employing their advantages and possible biological functions.

2. Extraction of SPS

The SPS can be extracted from various resources, including common soybean seed, soybean meal, and okara. Among them, okara, the residue after oil or protein extraction of soybean, is the most economic raw material for the extraction of SPS, particularly in East Asia. Okara contains various nutrients, including protein, dietary fiber, and some oligosaccharides.[4] Owing to its valuable nutrients, especially the components of polysaccharides, okara is increasingly used in food production. Black soybean (*Glycine max* (L.) Merr.), another species of legume commonly used in oriental diet, is also an important source of SPS.[5] Consequently, the rich resources solidly guarantee a sustainable production of SPS.

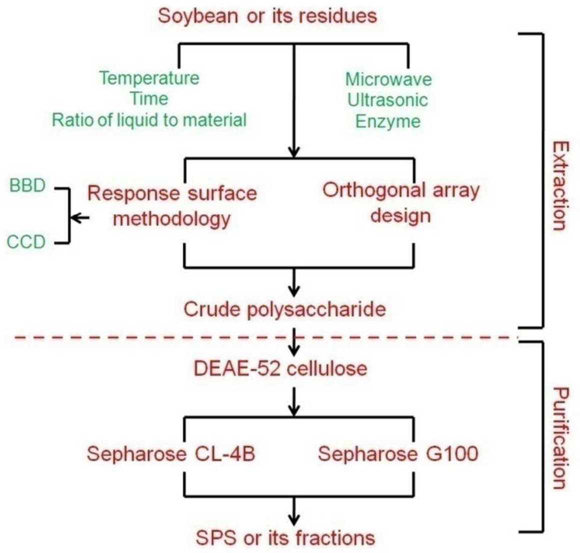

Fig. 1 Schematic technical route for effective extraction and refining of SPS from soybean. BBD, Box-Behnken design; CCD, central composite design.

Optimization of extraction solutions and procedures in versatile extracting processes is critical for the yield of SPS. The methods for extraction of SPS have been increasingly diversified over the last two decade. As shown in Fig. 1, it is the major schematic illustration of the technological process of SPS. Many options could be selected for maximizing the yield of SPS. Excepting the mentioned factors in Fig. 1, others can also profoundly affect the yield of SPS, such as pH, the origin of materials and the freshness of raw materials.

Many attempts have been made to improve the yield of SPS. Yamaguchi *et al.*[6] extracted the polysaccharide from okara using hexametaphosphate solution as extractant, and then polysaccharide was purified by DEAE cellulose column chromatography with carbonate buffer. When using alkaline water as extractant, the optimal extraction conditions are: pH 11.0, extraction temperature 120 ^{o}C, ratio of solid to liquid 1:20 (g:mL), extraction time 2 h, the polysaccharide yield is 16.24%.[7] However, for acidic water, the best parameters are: pH 4.0, extraction temperature 118 ^{o}C, ratio of material to liquid 1:30, extraction time 2.5 h, the final yield is 37.88%.[8] In order to reduce the dissolution rate of protein, organic acid solution is used to extract SPS from soybean dregs. The optimal process parameters are determined, including tartaric acid aqueous solution, pH 3.8, extraction temperature 110 ^{o}C, extraction time 1.5 h, resulting a yield of 27.65%.[9]

To maximize the yield of SPS, ultrasonic-assisted extraction method is applied. The optimum ultrasonic extraction parameters are: ultrasonic treatment time 20 min, ultrasonic power 200 W, bath temperature 90 ^{o}C, solid to liquid ratio 1:25 (g:mL), and the extracting rate is 1.87%.[10] More extraction parameters are modified, such as extraction pH 4.5, solid to liquid ratio 1:20, and ultrasonic treatment time 40 min, the yield is 8.82%.[11] Multiple approaches could be integrated in one assisted extraction. Enzymatic hydrolysis is used as well. The optimal crucial technological parameters are: ultrasonic treatment time 30 min, ultrasonic power 200 W, solid to liquid ratio 1:25 (g:mL); hydrolysis temperature 50 ^{o}C, hydrolysis duration 40 min, enzyme dosage 1.5%, and pH 5.0. Under such environment, the polysaccharide yield is 12.23%.[12]

On the other hand, soybean meal is degraded using double enzymes combination, acidic protease and flavor enzyme. The optimum conditions are: pH 6.0, enzymatic hydrolysis time 6 h, solid to liquid ratio 1:20, and amounts of 10% protease and 8% flavor enzyme, totally, yield of SPS is 9.28%.[13] Microwave-assisted extraction of SPS from soybean meal is optimized. The optimum conditions are: pH 8.0, ratio of water to raw material 1:6 (g:mL), microwave time 2.6 min, and microwave power 380 W, finally, SPS yield is 5.86%.[14] Soybean dregs are hydrolyzed with cellulase preparation under microwave assistance. The optimal procedures for extraction are: cellulase dosage 1.5%, pH 5.0, hydrolysis temperature 50 ^{o}C, hydrolysis time 40 min, material to water ratio 1:15 (g:mL), microwave power 600 W, microwave time 7 min, the maximum yield is up to 15.85%.[15]

Other extraction solution, like sub-critical water, is finely employed, its best conditions are: water temperature 150 ^{o}C, stuff mass to water ratio1:35 (g:mL), extraction time 11 min, in these conditions, the yield is 22.8%.[16] To maximize the yield of black soybean polysaccharides, Box-Behnken design is applied during the process of extraction. Liu *et al.* [17] obtained the optimal extraction conditions: ratio of water to material: 20 ml/g, extraction time: 6.4 h, extraction temperature: 92 $^{\circ}$C. Under these optimal conditions, the yields of crude SPS reach 2.56%. Taken together, various assisted extraction methods are truly benefiting both enhancement of the SPS yield and reduction of processing time.

Table 1 The fundamental characterization of SPS isolated from soybean meal, soybean cotyledon, okara, and black soybean.

Source	extraction	Fraction name	Molecular weight	Uronic acid content (%)	Monosaccharide composition (molar ratio)							References
					arabinose	rhamnose	galactose	glucose	mannose	xylose	fucose	
Soybean meal	chelating agent[a], 70 °C pH 5.2 for 1 h	ChSS	about 10^6 Da	53%	16.65	1.10	20.54	0.56	0.56	ND	ND	Huisman, 1998[18]
	0.05 mol/L NaOH 2°C for 1 h	DASS	about 10^6 Da	10%	15.99	1.10	21.09	0.56	0.56	ND	ND	
Soybean cotyledon	120 °C pH 5 for 1.5 h	SSPS	1.14×10^5 Da	23.4%	14.25	1.37	23.04	1.17	ND	3.73	2.13	Furuta, 1999[19]
	100 °C for 1 h	A1-β	2×10^6Da	ND	1.00	0.05	1.47	0.04	0.01	0.03	0.02	Hisashi, 1997[20]
Okara	120 °C pH 3 for 2 h	SSPS-L	ND	27.5%	10.19	2.36	26.81	0.89	ND	1.00	0.91	Nakamura, 2004[21] Mateos-Aparicio, 2010[22] Xiong, 2014[23]
	130 °C pH 4-5 for 3 h	SSPS-H	ND	25.6%	10.39	3.08	26.48	1.11	ND	1.47	0.79	
	120 °C pH 4-5 for 2 h	SSPS-M	ND	23.9%	13.39	2.25	26.20	0.61	ND	0.80	1.46	
	0.05 mol/L NaOH	0.05 MSF	ND	14.7%	18.00	1.30	26.30	3.40	1.50	5.50	2.30	

	1 mol/L KOH	1 MSF	ND	4.4%	16.90	ND	16.40	10.20	15.00	28.50	1.50	
	4 mol/L KOH	4 MSF	ND	5.1%	13.90	ND	18.60	12.90	5.00	36.70	2.20	
	90 °C pH 13 for 3 h	1	3.96×10^5Da	ND	18.3	3.2	53.6	6.4	ND	1.6	1.5	
	80 °C pH 12 for 3 h	2	4.11×10^5Da	ND	18.2	2.5	54.3	7.2	ND	1.9	1.1	
	70 °C pH 12 for 2 h	3	4.37×10^5Da	ND	19.4	2.7	54.9	6.8	ND	1.3	0.6	
	60 °C pH 12 for 2 h	4	4.62×10^5Da	ND	17.2	3.4	55.2	7.3	ND	0.9	1.8	
	60 °C pH 11 for 1.5 h	5	4.68×10^5Da	ND	18.1	3.3	55.7	6.5	ND	2	0.9	
	50 °C pH 9 for 1.5 h	6	4.89×10^5Da	ND	18.8	3.6	56.6	6.6	ND	1.4	1.1	
Black soybean	60~100°C for 3~7 h	BSPS-1	1.95×10^5 Da	0.14%	1.79	1.00	2.59	26.54	1.01	ND	ND	Liu, 2015[24] Liu, 2015[17]
	60~100°C for 3~7 h	BSPS-2	ND	2.98%	8.10	4.80	9.15	13.38	1.00	ND	ND	
	60~100°C for 3~7 h	BSPS-3	1.88×10^5 Da	9.13%	16.80	3.60	33.66	ND	1.00	ND	ND	

[a] chelating agent: 0.05 mol/L 1,2-diaminocyclohexane-*N*,*N*,*N*',*N*'-tetraacetic acid (CDTA) and 0.05 mol/L NH_4-oxalate in 0.05 mol/L NaAc-buffer; ND, not detected.

3. Structural characterization of SPS

SPS structure is gradually understood by researchers. Their fundamental properties are list in Table 1. SPS is extracted with water at 60 ^{o}C for 4 h, fractionated with a series of concentrations of sodium hydroxide solution, and identified six constituents of fucose, rhamnose, xylose, arabinose, galactose, and galacturonic acid, respectively.[25] Arabinogalactan, the major component of soybean seed polysaccharides, consists of arabinose and galactose residues, has an average molecular weight of 330 kDa.[26] Moreover, the arabinogalactan, derived from defatted and deproteinized soybean cotyledon meal, has the bone chain of 1→4 linked *β*-D-galactopyranose residues and a side chain containing, in general, two L-arabinofuranose residues with a 1→5 linkage.[27] Furthermore, an arabinan, found from the previous polysaccharides, is methylated and formed alditol acetates. Analysis by gas chromatography mass spectrometry (GC-MS) reveals similar structure with other arabinans.[28]

Soybean pectic polysaccharides consist of two types of regions, galacturonan and rhamno-galacturonan. The galacturonan regions are appeared both of the reducing and non-reducing ends of the chains, while the latter regions link to the side chains.[6] SPS of okara has a pectin-like structure. Its core bone contains equally L-rhamnose and D-galacturonate residues, consisting of repeating unit -4)-*α*-D-GalA-(1→2)-*α*-L-Rha-(1–and–4) -*α*-D-GalA-(1–, respectively (Fig. 2a).[29] SPS of soybean cotyledons contains acidic polysaccharides galacturonan (GN), rhamnogalacturonan (RG), and xylosyl oligosaccharides with $(\beta\text{-D-Xyl})_7$ or $(\beta\text{-D-Xyl})_4$ residues at the C-3 site.[30] The side chain of *β*-1,4-galactans is branched with fucose and arabinose residues. For GN is about 4~10 residues at the C-3 side of the galacturonates, while for RG is about 43~47 residues on the C-4 side (Figure 2b and 2c).[31]

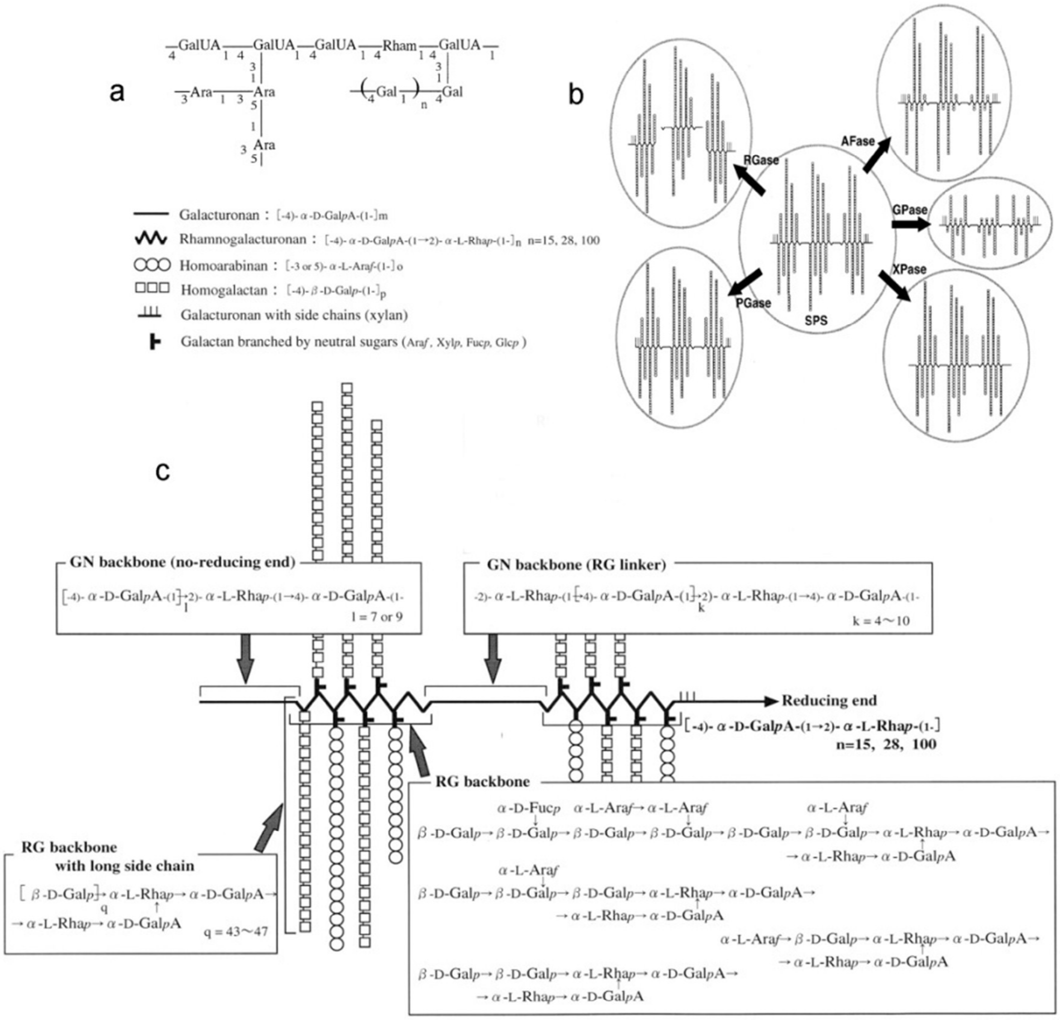

Fig. 2 The possible structure residues of SPS in alkaline condition (a) [19, 32], and the structure model of SPS possesses a globular form with arabinan and/or galactan chains that can be digested with RGase, AFase, and GPase (b and c) [33]. GalUA, galacturonic acid, Rham, rhamnose, Ara, arabinose, Gal, galactose; pectinases (polygalacturonase (PGase) and rhamnogalacturonase (RGase)) or hemicellulases (galactosidase (GPase) and arabinosidase (AFase)).

Soybean meal, the byproduct of oil extraction, is rich in proteins and polysaccharides. Two similar polysaccharides, ChSS (chelating agent soluble solids) extracted with chelating agent and DASS (dilute alkali soluble solids) extracted with dilute alkali, of soybean meal were sequentially fractionated using anion exchange chromatography.[18] To explore the detailed characterization of

ChsS, degradation of cell wall by enzymes, endo-galactanase, endo-arabinanase, rhamnogalacturonan hydrolase, rhamnogalacturonan acetylesterase and polygalacturonase-1, is performed in a rather specific way, which indicate ChSS is likely to be a highly substituted pectic structure.[34]

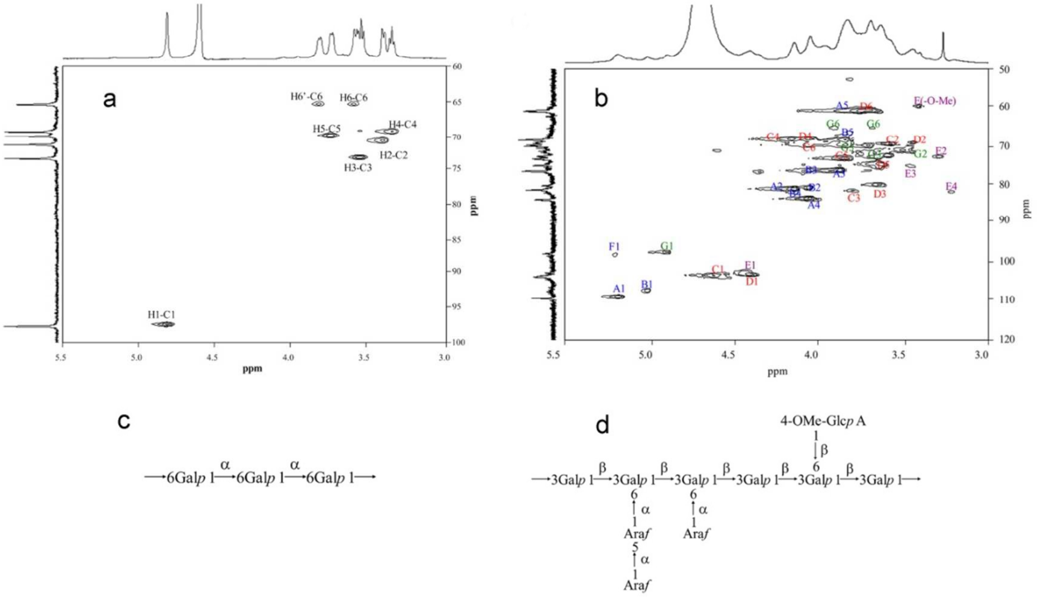

Fig. 3 HSQC (heteronuclear single quantum coherence) spectra of BSPS-1 (a) and BSPS-3 (b) in D_2O at 25 °C. H1−C1 represents the cross peak between H-1 and C-1 of →6)-*α*-D-Glc*p*-(1→ residue, etc. A1 represents the cross peak between H-1 and C-1 of residue A, etc. A, B, C, D, E, F, and G represent the residues of *α*-L-Ara*f*-(1→, →5)-*α*-L-Ara*f*-(1→, →3,6)-*β*-D-Gal*p*-(1→, →3)-*β*-D-Gal*p*-(1→, 4-O-Me-*β*-D-GlcA*p*-(1→, →2)-*α*-L- Rha*p*-(1→, and →6)-*α*-D-Glc*p*-(1→, respectively. Possible structures of BSPS-1 (c) and BSPS-3 (d) [24].

SPS from soybean cotyledons digested by five enzymes, with side chain of arabinan and galactan and backbone mainly of polygalacturonase and rhamnogalacturonase, enhances the stability of acidic beverage.[33] Comparing with those SPS treated with GPase (galactosidase), the authors indirectly confirm that native SPS is of galactan side chain and presents a branched globular conformation.[35] Combining the analysis of NMR spectra and methylation, the HSQC (heteronuclear single quantum coherence) spectra of BSPS-1 (purified fraction of black soybean polysaccharide 1) and BSPS-3 (purified fraction of black soybean polysaccharide 3) is shown in Fig. 3a and 3b as an example of compositional structural analysis. Liu *et al*.[24] identified two novel soluble polysaccharides (BSPS-1 and BSPS-3) from black soybean. BSPS-1 is a linear (1→6)-*α*-D-glucan of 195 kDa, while BSPS-3 is a type II arabinogalactan of 188 kDa (Fig. 3c and 3d). In conclusion, SPS possesses special structures that contain galacturonan and rhamnogalacturonan, suggesting its promising applications in food industry and biomedical areas.

4. Potential applications of SPS

4.1 SPS in food industry

As food additives, SPS that shows excellent stabilization and emulsifying behavior is mainly used by food researchers to improve the stability of beverage and increase emulsifying property of oil droplets in response to diverse environmental challenges. Notably, SPS-based formulations could extensively enhance their health benefits. All such promising applications of SPS may be attributable to their known physicochemical features, as summarized in Table 1 and elaborated below.

4.1.1 Emulsifying property of SPS

In aqueous environment, SPS, one of the most abundant components of the soybean byproducts, strongly endures the usual sterilization and acidic conditions.[19] SPS is a perfect candidate for interfacial film because of its high water solubility, low bulk viscosity and excellent thermostability.[21] Even in acidic and hot water conditions, within the pH range of 2~6 and the temperature range of 40~120 ^{o}C, water-soluble polysaccharides, mainly consist of rhamnogalacturonan, remain fluid.[36] Under the condition of 4 ^{o}C and 24 h, 0.5% SPS increases the rate constants of 5% starch retrogradation, and meanwhile declines the saturated dynamic storage modulus of composite system.[37] After alkali treatment and subsequently acidic extraction, SPS with lower degree of esterification exhibits highly emulsifying properties in oil-in-water and stabilizing abilities in acidic milk beverage.[23]

SPS is demonstrated to increase the emulsifying properties and then protect the unique film, which can prevent aggregation caused by steric or electrostatic repulsion among various oil droplets.[21] To understand which chains of polysaccharides are responsible for its strong emulsifying properties, SPS is digested by pectinases and hemicellulases. It is found that sugar chains, β-galactan and α-arabinan, play a notable role in emulsifying capability and stability, which provides a promising utilization of SPS for beverages.[38] The similar findings also proved that SPS could prevent the aggregation of casein micelles mutually.[39] In comparison with the concentration of sugar beet pectin (1.5%) and gum arabic (10%), SPS requires a moderate amount (4%) to surface the oil droplets and stabilize the emulsion of oil-in-water.[40] To broaden its function, SPS is phosphorylated and formed a high molecular mass complex, leading to a functional stabilization of acid particle dispersion within the pH range of 4~4.8.[41]

SPS fractions, HMF (high molecular weight) and LMF (low molecular weight), possess diverse functions. HMF is used to emulsify oil-water droplets and stabilize α-casein dispersions while LMF is better to protect emulsified lipid from oxidative aggression.[42] Compared with the stabilization of LMF of soybean cotyledons, HMF, with larger electrostatic and steric repulsive force, can clearly disperse milk proteins.[43] The presence of impure protein in LMF would increase its particle size and then change its functional performance after heating at 90 ^{o}C for 30 minutes.[44]

4.1.2 Interactions between SPS and other substance

SPS, absorbed on the droplet surface, can improve the emulsions of lactoferrin-coated oil and then prevent the lipid oxidation.[45] In addition, SPS coats, based on the electrostatic deposition layer by layer, of orange oil enhance their resistance against environmental stresses, such as versatile ions, pH, and light.[46]

Adding polysaccharide to soybean protein emulsion can decrease the initially droplet size, thereby, improve their stability.[47] Combination of SPS and dSWP (denatured soy whey protein) at a ratio of 1.5:1, forming a dSWP-SPS layer and covering oil droplets, promotes emulsion stability to prevent the coalescence and phase separation of oil-in-water.[48] Conjugating β-lactoglobulin, a whey protein of 18 kDa, with SPS in a special way would enhance the emulsifying property of this complex.[49] SPS fractions, a mixture of low and high-molecular-weight components, encapsulated with linoleic acid can increase the antioxidative capacity of this microcapsules and retard its oxidation process.[50] Cross-linked SPS with sodium hexametaphosphate via esterification reaction under acidic condition can improve the stability of soy protein isolate when stores at 4 ^{o}C.[51]

Dietary fiber in food has importantly health benefits, such as reducing blood cholesterol, decreasing the risk of diabetes, and improving bowel movement. SPS extracted and refined from okara is incorporated into thickened milkshake-style beverages. This popular beverage containing 0.015% κ–carrageenan, namely 4% SPS-fortified dairy beverage, is favored by ordinary consumers because it increases their soluble dietary fiber intake.[52] Combination of SPS and sodium carboxymethyl cellulose at a ratio of 3:1 effectively prevents the aggregation of casein and exhibits strong stabilization in acidified skimmed milk drinks.[53]

SPS can be used as an additive to improve the quality and value of food. The present of SPS from soybean cotyledons can reduce the viscosity of gelatinized starch, therefore, it is used to cook rice or noodles, which prevents them from adhering to each other.[54] Lactose, as a food additive, is widely used in infant formulas, protein powders, and candies. However, lactose can easily absorb moisture and become crystallized. Mixing with soluble soybean polysaccharide of 10 g/100 g, crystallization of spray-dried lactose powder can be remarkable delayed.[55] Anionic SPS-coated droplets and SPS-coated β-carotene droplets, are stabilized in oil-water emulsions with improved viscosity and consistency index.[56] SPS fraction of okara, glycosidoprotein with molecular weight of 14~370 kDa, is better than those of soybean hull, acidic heteropolysaccharides with molecular weight of 45~150 kDa, in terms of emulsifying performance and *in vitro* bile acid binding activity.[57]

4.2 SPS in biomedicine

Antioxidant capacity and stabilization of SPS is positively related to its concentration. SPS could scavenge hydroxyl radical and keep stable for a long time. The inhibitory rate of 0.08% SPS against oxidation is above 95% within 200 s, surprisingly, 0.2% SPS could keep this status for 20 d.[58] The soluble polysaccharide fractions of okara, namely 0.05 MSF (0.05 mol/L NaOH soluble polysaccharides), 1 MSF (1 mol/L KOH soluble polysaccharides) and 4 MSF (4mol/L KOH soluble polysaccharides), supposedly a β-glycosidic linkage, strongly scavenge ABTS (2,2'-azino-bis(3- ethylbenzothiazoline-6-sulphonic acid)) radical, and potently reduce Fe (III) to Fe (II).[22] However, crude polysaccharide of black soybean possesses higher superoxide anion and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging abilities than purified fractions (BSPS-1, BSPS-2 and BSPS-3).[17] Soybean polysaccharide degraded with hydrogen peroxide

(DPS), with a smaller molecular weight about 10.2 kDa, efficiently inhibits the formation of calcium oxalate crystallization, therefore, highly reduces the risk of kidney stone formation. In addition, DPS can distinctly weaken the external oxidative damage of renal epithelial cells of Africa green monkey, resulting in an increased cell viability.[59]

Black soybean polysaccharides, purified by column chromatography, stimulated the production of granulocyte colony-stimulating factor in peripheral blood mononuclear cells, mediated via activation of PI3K (phosphoinositide 3-kinase), ERK (extracellular signal-regulated protein kinase), PKC (protein kinase C), and NF-*κ*B (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathways.[60] PSBS, the polysaccharides of black soybean, *in vitro* accelerate myelopoiesis and increase the levels of various hematopoietic growth factors from spleen cells, and *in vivo* reconstitute bone marrow after 5-flurouracil- and irradiation-induced damage.[61] SPS as the excipient is added in *Epimedium* granules with the proportion of 1:3.5, endowing this type of granules with greater granulation, dissolubility, and applicability.[62]

Pre-treating mouse spleen lymphocytes with SPS for 2 h before X-ray radiation protects the cells from DNA damage and increases cell viability.[63] SPS shows antitumor activity via regulating immune functions of S_{180}-bearing mice, namely improving the phagocytosis and the production of NO of macrophages, and greatly increasing the number of B-lymphocytes.[64] Additionally, SPS could increase the CD_4^+ T cell numbers and the ratio of CD_4^+/CD_8^+ cells, and the level of IL-2 in serum. Obviously, SPS could notably stimulate T-lymphoid cell proliferation and IL-2 secretion.[65] Combination of cyclophosphamide and SPS shows better inhibitory effect on tumor growth and improves the thymus and spleen indices and IL-2 secretion, suggesting a synergistic anticancer effect and reduction of toxicity of cyclophosphamide.[66]

At pH 6, SPS exhibits strongly inhibitory effects on *Escherichia coli*，*Staphylococcus aures*，*Aspergillus niger* and *Penicillium chrysogenum*, and the minimal inhibitory concentration is 8, 6, 1, and 1 mg/mL, respectively.[67] Treatment with 5% SPS *in vitro* promotes the growth of *Bifidobacterium longum*, *Bifidobacterium* and *Lactobacillus*. Similar results can be seen with fructooligosaccharids.[68] Emulsifying both thyme oil and soluble soybean polysaccharide shows better antimicrobial activities against *Listeria monocytogenes* Scott A, *Salmonella enteritidis* and *Escherichia coli* O157:H7 versus thyme oil alone.[69]

4.3 SPS in biomaterials

SPS based film, a new biodegradable edible biomaterial, is a promising raw material commercially utilized for food package.[70] It has been successful gelatinized as shown in Fig. 4. Essential oils from *Zataria multiflora* Boiss or *Mentha pulegium* are incorporated with SPS to form a sandwich-like film, which promotes polysaccharide interaction, reduces water solubility, and remarkably increases elongation at break.[71] On the basis of these properties, this active edible film, additionally, inhibits the growth of gram negative and positive bacteria in a dose dependent manner, and potently scavenge free radicals, especially for *Zataria multiflora* Boiss.[72] This composite film is intensively recommended to use in food packaging.

To highly reduce nutrient cost and maximally utilize its direct value, soybean curd residue is reused as the nutrient source for solid state fermentative production of polysaccharides.[73] In comparison with normally submerged fermentation, polysaccharides fermented of okara are not only time efficiency but low cost. Li *et al*.[73] reported that polysaccharides, derived from *Wolfiporia extensa* (Peck) Ginns, fermented of okara, showed positively antioxidant abilities against DPPH and ABTS radicals.

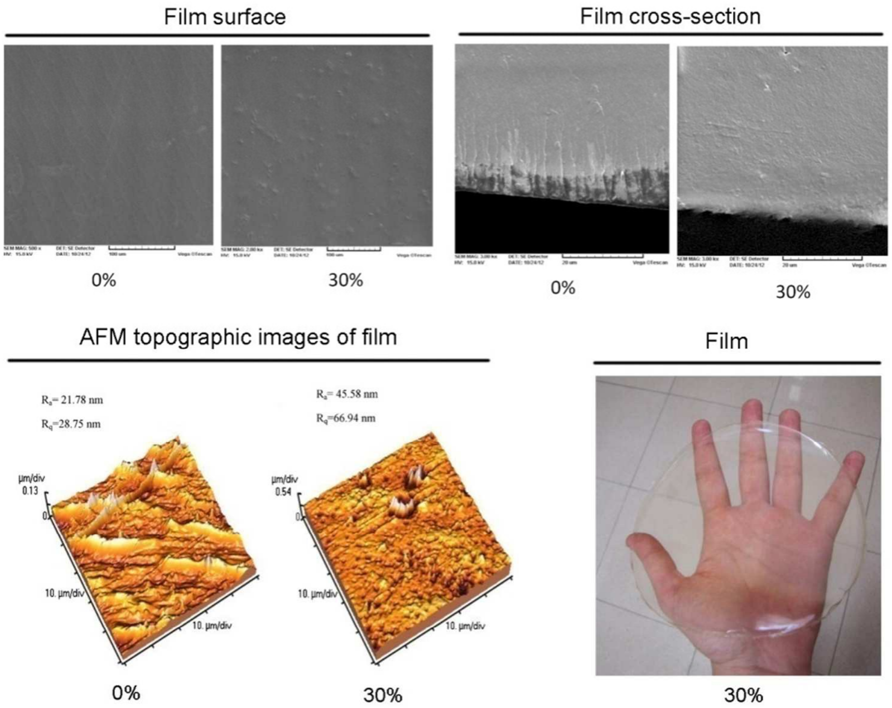

Fig. 4 Scanning electron microscope (SEM) images of surface and cross-section of SSPS films plasticized with 0% and 30% (w/w) glycerol. Atomic force microscope (AFM) topographic images of SSPS films plasticized with 0% and 30% (w/w) glycerol. The appearance of biodegradable edible films based on the formation of 0% and 30% (w/w) glycerol.[70]

5. Perspectives

Based on the physicochemical properties discussed above, SPS shows remarkable advantages in the potential applications in food additive, biomedicine and biomaterial, which motivates us to explore more possibilities of application in these fields. Future studies may be primarily focused on the following directions.

Firstly, the application of SPS in food industry could be largely broadened owing to its unique structural features and chemical properties. Actually, SPS has already been extensively applied as modifying agent. For example, it has been demonstrated that under acidic condition SPS can disperse stabilized protein solution. In this case, adding a small amount of SPS to favorable milk beverage can greatly lower its viscosity.[74] Carboxymethyl SPS, dissolving in alkali solution but not in acidic solution, inhibits the growth of *Bacillus subtilis* and *Bacillus cereus*.[75] However, little attention is paid to the adverse biological effects of modified SPS. We do not know whether these refined natural or modified SPS are harmful to the health of human being and livestock. Therefore, more comprehensive studies are highly demanded to investigate the potential influence on the health.

Secondly, it is interesting to devise novel biodegradable or edible materials based on SPS, which has been initially achieved and shown the possibilities for film. For instance, the composite films, containing 12.5% SPS, show good water soluble, incredible tensile strength and elongation rate at breaking, supposedly non-toxic and eco-friendly as well, which are extraordinary features for food packaging.[76] The preliminary findings have provided possible practical information for utilization of SPS-based biodegradable or edible films. However, factors, such as essential oils, sucrose, etc., need to be further optimized to achieve optimal gelation ability, including gel strength, gel elasticity and adhesion strength.[77]

Thirdly, application of SPS has exhibited its huge potential in the area of biomedicine, particularly for the treatment of cancer and immune regulation. However, only a few studies (as mentioned in this paper) have been involved in these interesting fields. One of the most distinct functions of polysaccharides is immunomodulation that might be closely related to its anticancer activity. At the dosage of 50~400 μg/mL, SPS exhibits great immunomodulatory activity *in vitro*, dramatically stimulating spleen lymphocyte proliferation, observably increasing the phagocytosis of macrophage, and enhancing macrophage NO production.[78] Moreover, SPS could potentially attenuate the toxicity of anticancer chemical compounds. Injecting a dose of SPS into S_{180}sarcoma mice could significantly improve parameters of immune functions, including the number of leukocytes, the level of TNF-α (Tumor necrosis factor alpha), and the ratio of CD_4^+/CD_8^+, compared to cyclophosphamide used alone . Clearly, these attempts shed light on the use of SPS for a wide range of biomedical applications. More studies are required to investigate the details of anticancer and immunoregulatory activities of SPS in animal models, and particularly in clinical trials. Whether SPS is beneficial to the prevention or treatment of oxidation- or inflammation-related diseases, such as neurodegenerative diseases, diabetes mellitus, and renal disease, is highly interesting to be explored since SPS shows strong antioxidative activities as well.

In summary, growing numbers of literatures have indicated that SPS is a promising candidate for food industry, biomedical, and biomaterial applications, in which more potentials are under exploration. However, its risk evaluation in a scientific perspective is still absent, especially for those cross-linked SPS. In this regard, substantial systemic toxicology investigations, both *in vitro* and *in vivo*, are highly demanded.

6. Conclusions

In conclusion, SPS could be effectively isolated from soybean or okara by various extracting ways, including ultrasonic assistance, microwave assistance, enzymatic treatment, and subcritical water extraction, which all show better extraction efficiency than hot water alone. Meanwhile, SPS, a linear chain of galacturonan and rhamno-galacturonan, is widely acceptable to be a food additive, showing its advantages in emulsifying and stabilizing oil-water system. Moreover, SPS has a potential in the area of biomedicine, such as antioxidant activity, antimicrobial activity, and anticancer activity. Indeed, SPS can inhibit the growth of tumor via regulating the immune function, such as increasing the level of NO and IL-2. Another promising application is to use SPS as biodegradable materials for food packaging and preservation. However, the potential risk or toxicity of SPS and its derivatives have not been reported yet. Thus, to better use of SPS and its derivatives, comprehensive toxicology studies or risk assessments, both *in vivo* and *in vitro*, within standard guidelines are highly demanded.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by the Macao Science and Technology Development Fund (074/2013/A) and the Research Fund of the University of Macau (MYRG107(Y1-L3)-ICMS13-HCW, MRG013/HCW/2014/ICMS)

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