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Effects of alkali treatment and subsequent acidic extraction on the properties of soybean soluble polysaccharides

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A B S T R A C T

Soybean soluble polysaccharides (SSPS) were extracted under acidic condition from the okara of producing soybean protein isolate. The methyl esterificated carboxylic group of galacturonic acid (GalA) in SSPS could be demethoxylated by alkali treatment when heated, which had effects on SSPS properties. In this study, okara was treated in six selected alkali conditions, and then SSPS were extracted under the acidic condition (115 °C, 100 min, pH 4.5). The obtained SSPS were determined to have degree of esterification (DE) of 49, 55, 60, 66, 74 and 83%, respectively. Violent alkali treatment produced SSPS with not only lower DE but also lower molecular weight (MW) compared to mild alkali treatment. MW was increased from 396 to 489 kDa with increasing DE. It was found that SSPS with lower DE possessed lower aqueous viscosity, more negative charges, better emulsifying activity in oil-in-water emulsion and stabilizing activity in acidic milk drink. When DE changed from 49 to 83%, aqueous viscosity (10% (w/w) SSPS), average particle size of fresh oil-in-water emulsion, and centrifugation precipitating rate (CPR) of acidic milk drink (pH 4.5; two-stage 30 MPa homogeneous pressure) were increased from 25.8 to 108.2 mPa s, 1.5 to 4.0 μm, and 0.20 to 0.47%, respectively; and zeta potential (pH 7) was decreased from 17 to 10 mV. These results suggested that SSPS obtained from different alkali treatment had different functional properties.

Keywords: Soybean soluble polysaccharide; Alkali treatment; Acidic extraction; Degree of esterification; Molecular weight; Functional properties

1. Introduction

Soybean soluble polysaccharides (SSPS) were constituents extracted from fibrous bean curd residue (okara) of producing soybean protein isolate, soymilk, and tofu (Cui, 2001; Maeda, 2000). Okara, the indigestible fraction of which was higher (41.6%) than seed (28.5%), was an alternative to dietary fiber (Irene and Ruperez, 2009). SSPS have been successfully added into dairy products to improve the contents of dietary fiber (Chen et al., 2010). As novel polysaccharides, SSPS successfully satisfy extensive requirements for new types of food products which consumers and food industry need.

The structure of SSPS, an acidic and peptide-linked polypeptides, has been confirmed by gradual enzymatic degradation, and clarified as main homogalacturonan and rhamnogalacturonan backbones with branched chains of galactan and arabinan (Nakamura et al., 2001, 2002, 2004). It was reported that SSPS had a pectin-like structure, but they had more branched chains and lower galacturonic acid (18% GalA) than pectin (over 75% GalA) (Schols and Voragen, 2002; Nakamura et al., 2002). One research reported that the average MW of SSPS was about 645 kDa and radius of gyration (R_g) was 23.5 ± 2.8 nm determined by size exclusion chromatography and multiangle laser light scattering (Wang et al., 2005).

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SSPS have been reported to play important roles on human health through lowering the risk of diabetes, enhancing body uptake and bioavailability, and reducing blood cholesterol (Lo et al., 1986; Tsai et al., 1987). In addition to physiological function as one kind of dietary fiber, SSPS also had some other functional properties. Some researchs reported that SSPS could be used as stabilizer in acidic milk drink, revealing that it could interact with milk protein to inhibit their precipitation in acidic condition (Nakamura et al., 2003; Liu et al., 2006). SSPS also could be used to stabilizing the oil-in-water emulsion with its peptide fraction anchoring on the oil–water interface and its carbohydrate moieties forming a thick, hydrated layer on the surface of oil droplet (Nakamura et al., 2007). Nakamura et al. (2006) obtained high molecular weight (HMF) and low molecular weight (LMF) fractions through separating crude SSPS, and it was found that HMF fraction was more effective to stabilize oil-in-water emulsion than LMF fraction. Some other researches reported that SSPS could inhibit lipid oxidation, and could be used as wall material for the microencapsulation of canthaxanthin, which was an unstable orange–red ketocarotenoid (Matsumura et al., 2003; Hojjati et al., 2011).

Generally, carboxyl group of GalA was frequently substituted with methyl ester, which could be represented by the degree of esterification (DE). It was well known that methyl esterificated carboxylic group of GalA could be demethoxylated by alkali treatment when heated. Until now, the researches about DE were mainly focused on pectin. Hotchkiss et al. (2002) and Savary et al. (2003) produced low DE pectin from the high DE pectin by a salt-independent orange pectin methylesterase, and it was found that low DE pectin showed quite different liquid behavior from the high DE pectin. Pectin with low DE had larger intrinsic viscosity (η) when dissolved in 0.005 and 0.05 M monovalent salt solutions, and lower η when dissolved in 0.2 M salt solution than pectin with high DE (Yoo et al., 2006). When pectins with different DEs were used in the acidic milk drink, high DE pectin was better as stabilizer (Liu et al., 2006), revealing that the DE greatly affected the functional properties of pectin. In addition, it was reported that pectin with high DE increased springiness, chewiness, coarseness of mass and hardness, and that the MW and DE of pectin were the most crucial factors for gel matrix (Kim et al., 2008, 2010). Although several researches have reported that pectin with different DEs possessed different properties, no researches has been conducted on the effects of DE on the properties of SSPS. It was considered that SSPS with different DEs might also show different properties.

Generally, SSPS were extracted under acidic conditions, and their rheological properties and utilizations were examined in several food systems as stated above (Chen et al., 2010; Furuta et al., 1998; Liu et al., 2013). But the effects of alkali treatment and subsequent acidic extraction on SSPS properties was not clear now. In this study, six alkali treatment conditions were selected to treat okara before acidic extraction, and the MWs, sugar compositions, protein contents, and DEs of the obtained six SSPS samples were determined, and further their viscosities, zeta potentials, emulsifying activities in oil-in-water emulsion and stabilizing activities in acidic milk drink containing milk protein were compared. It was considered that this study was very meaningful for producing SSPS with different functional properties by controlling DEs of SSPS.

Table 1 – The alkali treatment conditions of SSPS preparation.

Type of SSPS	Alkali treatment conditions		
	Temperature (°C)	Time (h)	pH
1	90	3	13
2	80	3	12
3	70	2	12
4	60	2	12
5	60	1.5	11
6	50	1.5	9

2. Materials and methods

2.1. *Materials*

The okara was obtained from Gushen Biological Technology Group Co., Ltd. (Shandong, China). The reagents were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO) or of analytical grade.

2.2. *Preparation of SSPS*

Okara was powdered by WK-800A grinder (Jingcheng Co., Ltd., Shandong, China), dispersed in distilled water at a concentration of 5% (w/w), divided into six parts, and treated in six alkali treatment conditions (Table 1) to obtain different DEs. Then the suspensions were centrifuged at 3500 × *g* for 20 min, and the supernatants were poured off. Distilled water was added into precipitates to the original weights, mixed, and treated by centrifugation (3500 × *g*, 20 min), which was repeated two more times. Distilled water was added into the final precipitates to the original weights and mixed. The suspensions, which possessed different DEs, were extracted at 115 °C using pressure cooker at pH 4.5 for 100 min. After removal of insoluble materials by centrifugation (5000 × *g*, 40 min), the extract was concentrated to approximately 10% (w/w) through rotary evaporation. Ethanol was added into the concentrated solution at a volume ratio of 3/1, and the mixtures were cut into pieces using high speed blender (10,000 rpm, 1 min, PowerGen 125, Fisher Scientific, Nepean, Canada). Each mixture was filtrated by suction filtration using vacuum pump and Buchner funnel. The residue was collected, washed three times with ethanol, and dried at 40 °C to obtain SSPS powder. The contents of protein and carbohydrate were determined using Lowry (Lowry et al., 1951) and phenol–H_2SO_4 (Dubois et al., 1956) methods, respectively.

2.3. *Determination of DE*

The DE of SSPS was determined by the titrimetric method of USP 26 NF 21 (2003) and Food Chemical Codex (FCC, 1981) with slight modifications. SSPS (500 mg) were stirred in 20 mL of ethanol–HCl mixture (5 mL of 12 M HCl in 100 mL of 70% (v/v) ethanol) for 10 min, then filtrated through filter paper and glass funnel. The collected SSPS were washed five times using the ethanol–HCl mixture as above (20 mL each time), then washed by 70% (v/v) ethanol solution until the filtrate did not have chloride ions by silver nitrate detection. Finally, SSPS were dried at 105 °C for 1 h. This treatment wiped out free sugars and salts, which transfeered the SSPS to free acid form. The dried samples (60–80 mg) were moistened with 2 mL of ethanol, dissolved into 100 mL of distilled water without carbon dioxide, and titrated by 0.1 M sodium hydroxide (NaOH)