Chemical Compositions of Hydrocolloids Produced from Nutlets of Salvias

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Abstract

Hydrocolloids of three species of Salvias (*S. miltiorrhiza*, *S. sclarea* and *S. viridis*) was analyzed their chemical compositions after isolation of hydrocolloids from seed coats. Isolation was conducted after expanding out completely in water. Hydrocolloids produced from *S. miltiorrhiza*, *S. sclarea* and *S. viridis* have cellulose contents about 18.6%, 25.3% and 35.4% and hemicelluloses contents about 80%, 73.4% and 62%, respectively. Native hydrocolloids produced from *S. sclarea* and *S. viridis* were rich in glucose about 48.6% and 55.4%, respectively, while the other one, *S. miltiorrhiza*, was rich in xylose, about 85.1%. Distribution of these polysaccharides in *S. miltiorrhiza*, *S. sclarea* and *S. viridis* were 86.5%, 71.0% and 63.2% (acidic polysaccharides) and 13.8%, 29.0% and 36.5% (neutral polysaccharide), respectively. Acidic polysaccharides of hydrocolloids produced from three species of Salvias contain high amount of xylose (88.8 ~ 91.9%). Neutral sugar compositions in neutral polysaccharides of hydrocolloids produced from three species of Salvias, however, were rich in glucose (25.7 ~ 37.5%) and galactose (31.3 ~ 60.4%), the ratio being changed depending on species. Acidic sugar in the acidic polysaccharides from three Salvia spp. was identified as glucuronic acid by High Performance Anion Exchange Chromatography (HPAEC). Glucuronic acid contents in the acidic fractions of *S. miltiorrhiza*, *S. sclarea*, *S. viridis* were estimated about 25%, 22% and 27%, respectively. These results elucidate that hydrocolloids have amorphous structure containing branch glucuronic acid in acidic polysaccharides structure. The present of glucuronic acid is predicted attach to xylan.

Key words: Salvia, hydrocolloids, acidic polysaccharides, neutral polysaccharides, glucuronic acid

Introduction

Cellulose is by far the basic structural component of plant cell walls as the most abundant organic substance found in nature. Cellulose makes up more than half of all living matter and extremely important to human uses as clothes (cottons, linen and rayon); paper products; cosmetic and food products (anticake agent, emulsifier, stabilizer, dispersing agent, thickener). Bacterial cellulose (*Nata de coco*) constitutes a cellulose resource gellouse-like produced by *Acetobacter xylinum*. Bacterial cellulose is consumed as a sweet dessert in Asian countries. In addition, high mechanical strength and crystallinity of the bacterial cellulose is responsible for its use as acoustic diaphragms and electronic paper (Shah and Brown 2005).

Traditionally, cellulose is harvested from plant resources as most important renewable natural resources on earth. One of the most important features of bacterial cellulose is its chemical purity which distinguishes this cellulose from plant cellulose usually embedded with hemicelluloses and lignin, removal of which is inherently difficult. Recently, cellulose is found to be present in hydrocolloids as seed mucilages of several kinds of plants, Basils (*Ocimum spp.*) and Mistletoe (*Viscum album* L.) (Azuma *et al*. 2000a; Azuma *et al*. 2000b; Azuma and Sakamoto 2003; Indrarti *et al* 2004) in addition to White mustard (*Brassica alba*), Cress (*Lepidium sativam*) and Quince (*Cydonia*) (Smith and Montgomery 1959). One of other cellulose resource is *Salvia spp*. as one of genera in Lamiaceae family found wild in South America, Southern Europe, Northern Africa and Asia which is utilized for medical, ornamental and qulinary qualities. Lamiaceae is one of a few families with various aromatic plants that provide some of essential ingredient of life in enhancing the desirable flavor, aroma of food and drink and utilizing as a folk medicine.

In a previous study, we reported pericarp structure of nutlets from more than 30 species of Salvias as well as relationship between their hydrocolloid contents and epicarp layer as origin of hydrocolloids (Yudianti *et.al*. 2005). Structural analysis of hydrocolloids produced from 11 species of Salvias was also reported including order and disorder structure by X-ray diffraction and Iα cellulose fraction of hydrocolloids by FT-IR spectra. In this report, we conducted chemical analysis of Hydrocolloids produced from three species of Salvias (*S. miltiorrhiza*, *S. sclarea and S. viridis*) about cellulosic and hemicellulosic polysaccharides of hydrocolloids and neutral sugar composition present in neutral and acidic polysaccharides analyzed by High Performance Anion Exchange Chromatography (HPAEC). Acidic polysaccharides importantly affecting hydrocolloids formation were isolated by Anion Exchange Chromatography.

Materials and Methods

Nutlets of three species of Salvias (*S. miltiorrhiza*, *S. sclarea and S. viridis*) used as hydrocolloids source were purchased from Richters Co., Ontario, Canada. Hydrocolloids were isolated from seed coat of Salvias nutlets after expanding out completely in water and freeze dried. Cellulose and hemicelluloses present in hydrocolloids were separated by extraction with 17.5% sodium hydroxide and 3% boric acid. Hemicelluloses were recovered by ethanol precipitation after neutralization of the extracted solution by acetic acid, dialysis against water and concentration to a small volume.

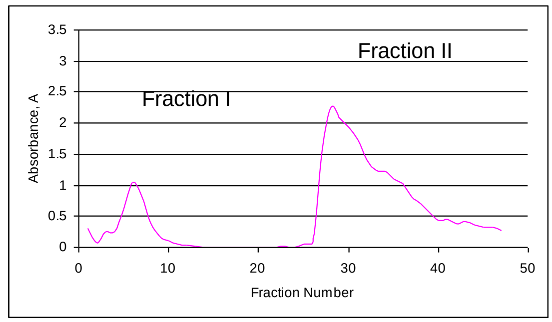
Hemicellulosic polysaccharides were separated into acidic and neutral polysaccharides by Anion Exchange Chromatography on a diethylaminoethyl (DEAE) column. Fractions were collected by fraction collector and analyzed by absorbances measurement at 280 nm for UV-absorbing materials and 490 nm for neutral sugars after developing colors. Adsorbed fractions were recovered by a linear gradient elution of NaCl to 1 M in 5 mM sodium phosphate buffer, pH 6.8. Fractionated polysaccharides were dialyzed against water and precipitated in 3 volumes of ethanol.

Neutral sugar compositions of polysaccharides of hydrocolloids produced from these Salvias were analyzed by High Performance Anion Exchange Chromatography (HPAEC) (Dionex DX-500) equipped with ED 40 electrochemical detector in a pulsed amperometric detector mode on a column of Carbopac PA 1 (4.0 mm x 25.0 cm). Polysaccharides were hydrolyzed with 1 N sulfuric acid for 6 hours at $100^{o}C$. The chromatograms were analysed by Dionex Peaknet Data Station.

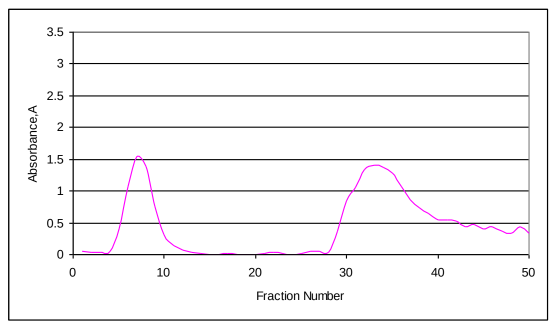
Identification of acidic sugar present in hydrocolloids from these Salvias was analyzed by HPAEC (Dionex DX-500) after methyl esterification and reduction by sodium borohydride ($NaBH_4$). The acidic sugar content present in hemicellulosic polysaccharides were analysed by measurement of UV absorbance at 525 nm after developing color by treatment with *m*-hydroxydiphenyl 0.15% (w/v) in 0.5% (w/v) NaOH.

Results and Discussion

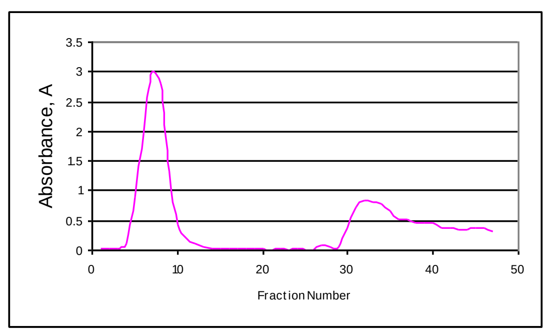
Chemical analysis was conducted to hydrocolloids produced from nutlets of three species of Salvias, *S. miltiorrhiza, S. sclarea and S. viridis*. These Salvias were used as hydrocolloids source because of high production of hydrocolloids, contents being about 10.1%, 8.7% and 16.4%, respectively. Numerical values of cellulose and hemicelluloses contents are given in Table 1. Obtained cellulose and hemicelluloses contents of hydrocolloids produced from these Salvias were about 18.6 ~ 35.4% (cellulose content) and about 62 ~ 80% (hemicelluloses content). Of the results pointed out that *S. viridis* has the lowest hemicelluloses content (62%) and the highest cellulose content (35.4%), while *S. miltiorrhiza* has the highest hemicelluloses content (80%) and the lowest cellulose content (18.6%). Meanwhile, crystallinity index of the cellulose in the hydrocolloids from nutlets of these Salvias were 8% (*S. miltiorrhiza),* 23% (*S. sclarea*) and 41% (*S. viridis)*. *S. viridis* had the highest crystallinity index (41%) and *S. miltiorrhiza* has the lowest crystallinity index (8%).



a. *S. miltiorrhiza*



b. *S. Sclarea*



c. *S. viridis*

Figure 1. Elution profiles of hemicellulosic polysaccharides from three species of Salvias (a. *S. miltiorrhiza*, b. *S. sclarea*, and c. *S. viridis*) by Anion Exchange Chromatography.

Table 1. Content of cellulose and hemicelluloses and weight fractions of polysaccharides in hydrocolloids of three species of Salvias.

No.	*Salvia spp.*	Cellulose and Hemicelluloses Contents (w/w %)		Weight Fraction of Polysaccharides (%)	
		Cellulose	Hemicelluloses	Acidic	Neutral
1.	*S. miltiorrhiza*	18.6	80.6	86.5	13.75
2.	*S. sclarea*	25.3	73.4	71.6	29.7
3.	*S. viridis*	35.4	62.3	63.2	36.5

Table 2. Neutral sugar compositions of hemicellulosic, neutral and acidic polysaccharides of three species of Salvias

No.	*Salvia spp.*	Polysaccharide	Relative Neutral Sugar Composition (%)					
			Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose
1.	*S. miltiorrhiza*	Hemicellulosic	7.5	4.0	10.9	11.1	66.4	0.0
		Acidic	3.0	4.5	1.8	1.8	88.8	0.0
		Neutral	16.7	0	31.3	33.5	16.2	2.3
2.	*S. sclarea*	Hemicellulosic	0.6	2.1	27.6	16.0	53.6	0.0
		Acidic	0.9	4.1	3.7	1.7	89.2	0.3
		Neutral	3.3	0.9	44.7	25.7	25.3	0.1
3.	*S. viridis*	Hemicellulosic	0.4	0.2	42.1	28.7	28.5	0.1
		Acidic	0.4	1.0	3.2	3.4	91.9	0.1
		Neutral	0.5	0.3	60.4	37.5	0.5	0.8

When cellulose and hemicelluloses contents and crystallinity index of hydrocolloids were compared, a conclusion is proportion of cellulose in hydrocolloids seem to be a determinat on crystalline structure of hydrocolloids. Hemicelluloses have no crystalline structure. Hemicelluloses interference might be affecting the crystalline structure of hydrocolloids.

Collection of fractions by a fraction collector gave elution profiles as shown in Figure 1. Fractions corresponding to two peaks were pooled, separately, as Fraction I (neutral polysaccharide) and Fraction II (acidic polysaccharide). Fraction I and Fraction II as neutral and acidic polysaccharides respectively were dialyzed against distillated water and precipitated by 3 vol. of ethanol. Obtained weight fractions data of acidic and neutral polysaccharides are given in Table 1. Entirely, every polysaccharide in hydrocolloids produced from three species of Salvias was greatly dominated by acidic polysaccharide. Acidic polysaccharide is predicted as an important portion which has high response to water as polar and hydrophilic part in the hydrocolloid materials. Proportion of acidic polysaccharides in *S. miltiorrhiza*, *S. sclarea* and *S. viridis* are 86.5%, 71% and 63.2%, respectively; while the values for neutral polysaccharides in *S. miltiorrhiza, S. sclarea* and *S. viridis* are 13.75%, 29% and 36.5%, respectively. The compositions of neutral sugar in acidic, neutral and hemicellulosic polysaccharides were shown in Table 2. The hemicellulosic polysaccharides of *S. miltiorrhiza* and *S. sclarea* were rich in xylose, 66.4% and 53.6% respectively, while *S. viridis* has high galactose content (42%). Acidic polysaccharides in *S. miltiorrhiza, S. sclarea* and *S. viridis* have high xylose content, 88.8%, 89.2% and 91.9%, respectively. Meanwhile, neutral polysaccharides in *S. miltiorrhiza, S. sclarea* and *S. viridis* are rich in galactose (31.3 ~ 60.4%) and glucose (25.7 ~ 37.5%). Based on these results, acidic polysaccharides of these Salvias are concluded to be rich in xylan while neutral polysaccharides are rich in galactan. Low mannose content in all polysaccharides of these Salvias constitute inhibiting factor in glucomannan formation. Therefore, the origin of glucose in the neutral polysaccharide fraction, presumed from starch because of low glucomannan content in hemicelluloses structure of Salvias.

Identification of acidic sugars present in the hydrocolloids from three species of Salvias was given in Table 3. Comparison of the data between native and reduced acidic polysaccharides indicates that glucose content is greatly increased from 3.2 ~ 6.9% to 26.9 ~ 32.6% after reduction. In addition, the values of another sugar composition (arabinose, rhamnose, galactose, xylose, and mannose) are relatively unchanged. This indicates that acidic sugar present in hemicellulosic polysaccharides of hydrocolloids from these Salvias is identified as glucuronic acid, estimated about 22 ~ 27%. Neutral and acidic sugar (glucuronic acid) contents in the acidic, neutral and hemicellulosic polysaccharides pointed out in Table 4. Acidic, neutral and hemicellulosic polysaccharides contained neutral sugar of about 34.9 ~ 49.6%, 87.2 ~ 94.2 % and 70.5 ~ 73.2 %, respectively. Meanwhile, glucuronic acid contents in acidic, neutral

Table 3. Neutral sugar compositions of native acidic and reduced acidic polysaccharides of three species of Salvias.

No.	Salvia spp.	Acidic Polysaccharide	Relative Neutral Sugar Composition (%)					
			Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose
1.	*S. miltiorrhiza*	Native	3.4	8.3	3.2	3.2	81.9	0
		Reduced	0.9	4.1	5.6	27.8	61.5	0.1
2.	*S. sclarea*	Native	2.5	9.1	10.1	5.9	72.5	0
		Reduced	0.3	3.9	6.5	26.9	62.4	0
3.	*S. viridis*	Native	0	8.9	6.8	6.9	77.4	0
		Reduced	0	1.7	3.6	32.6	62.0	0

Table 4. Neutral and acidic sugar contents in polysaccharides of Salvia spp.

No.	Salvia spp.	Polysaccharide	Neutral – Acidic Sugar Content (%)	
			Neutral	Acidic
1.	*S. miltiorrhiza*	Hemicellulosic	70.5	28.2
		Acidic	49.6	50.9
		Neutral	87.2	9.6
2.	*S. sclarea*	Hemicellulosic	75.3	29.2
		Acidic	47.6	46.7
		Neutral	92.7	3.3
3.	*S. viridis*	Hemicellulosic	73.2	34.4
		Acidic	34.9	51.9
		Neutral	94.2	5.5

and hemicellulosic polysaccharides of Salvias were about 46.7 ~ 51.9%, 3.3 ~ 9.6% and 28.2 ~ 34.4%, respectively. This indicates that hemicellulosic polysaccharides of hydrocolloids produced from these Salvias have a proportion of about 70 ~ 75% as neutral sugar and 28 ~ 35% as acidic sugar (glucuronic acid). Acidic polysaccharides have a proportion of about 35 ~ 50% as neutral sugar and 45 ~ 50% as acidic sugar, while neutral polysaccharides have a proportion of 85 ~ 90% as neutral sugar and 3 ~ 10% as acidic sugar.

Conclusions

1. High hemicelluloses contents of hydrocolloids induce low crystalline (8 ~ 41%) on hydrocolloids structure supporting structure in easily water absorption
2. Hemicellulosic polysaccharides produced from Salvias contain 70 ~ 75 % neutral polysaccharides and 28.2 ~ 34.4% acidic polysaccharides.
3. Acidic sugar of Salvias is identified as glucuronic acid.
4. Acidic Polysaccharides are rich in xylan as main chain connecting to branch glucuronic acid.

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