Preparation and Physiological activities of sulphated derivative extracted from corn bran

Qing Mo¹, Linghao Dai², Jianjun Ma¹, Xiaojing Zhao¹ and Linghui Zhu²,*

¹Hangzhou Medical College, Hangzhou, China
²Zhejiang Chinese Medical University, Hangzhou, China

*Corresponding author e-mail: jhzlh1125@163.com

Abstract. In the present study, the sulphated derivative (S-CBP) with the degree of substitution (0.46) was successfully prepared from the polysaccharide extracted from corn bran. Compared with native polysaccharide, the structures of the sulphated derivative were confirmed by FT-IR and SEC-LLS and the molecular weight were changed by chemical modification. Sulfation enhanced the antioxidant activities in a dose-dependent way, which seemed to be dependent on the character of the substituted group. The results suggest that the sulphated derivative, extracted from corn bran, are potential natural antioxidant and blood fat reduce agent.

1. Introduction
Now days, an increasing number of studies have been published concerning the biological activities of polysaccharides from low-valued agricultural industry [1-3]. Researchers of corn have increased because of its uses as adhesive, thicker and stabilizer [4] and a film former and emulsifier [5-7].

To promote comprehensive utilization of corn bran, it is critical to understand the effects of chemical modification on physiological activities in detail. However, no efforts have yet been made to improve the physiological activities. In the present work, the polysaccharide, extracted from corn bran, were modified by sulfation. Instantly, their physiological activities such as antioxidant and bile acid binding capacity were investigated as a function of the effect of modified group

2. Materials and methods

2.1. Material and reagents
Corn bran was collected in Hangzhou, Zhejiang Province, China in 2015, and de-oil, de-starched according to Yadav et al. [7]. The powder of the materials was screened through a 40 mesh sieve and stored in a desiccator at room temperature. All the reagents were purchased from Aladdin Industrial Co. and used without further purification.

2.2. Extraction of polysaccharide
On the basis of previous method [7], the de-oil and de-starched material was extracted and centrifuged. The supernatant was concentrated, deproteinized using the Sevag method and then precipitated upon addition of absolute ethanol. The residue was collected by centrifuged at 5000rpm for 10min, dialyzed, lyophilized and then reserved in a dryer.
2.3. Preparation of sulphated derivative
Sulfation of the polysaccharide was carried out using the chlorosulfonic acid-pyridine method [7]. The sample obtained was coded as S-CBP.

2.4. Physicochemical characterization

2.4.1 Degree of substitution of sulphated derivative. The sulfur content of the sulphated polysaccharide was estimated using the benzidine method [1]. The DS, which is the average number of sulfate group on each sugar residue, was calculated from the sulfur content using the following equation.

\[
DS = \frac{(1.62 \times S\%)}{(32 - 1.02 \times S\%)}
\]  

Where, S% was the mass fraction of S atom.

2.4.2 FT-IR analyses. Fourier transform infrared spectra (FT-IR) of the derivatives were obtained by Nicolet FT-IR spectrometer (Magna-IR 760 E.S.P, Nicolet Instrument Corp., Madison, WI). Samples were ground with potassium bromide (KBr) at a ratio of 1:20 and pressed into a thin pellet for FT-IR analysis.

2.4.3 SEC-LLS measurement. According to our previous research, the Homogeneity and molecular mass were measured Size-exclusion chromatography combined with laser light scattering measurements were performed on the eight-angle laser photometer [9].

2.5 In vitro antioxidant activity assay

2.5.1 Hydroxyl radical scavenging assay. The hydroxyl radical scavenging activity was analyzed according to our previous researcher [9, 10].

\[
\text{Scavenging effect} (\%) = (1 - (A_1 - A_2)/A_0) \times 100\%
\]  

2.5.2 Superoxide anion scavenging assay. The assay was based on the capacity of the sample to inhibit the photochemical reduction of nitroblue tetrazolium in NADH-NBT-PMS according to our previous research [9, 10].

\[
\text{Scavenging effect} (\%) = (1 - A_0/A_1) \times 100\%
\]  

Where A_0 was the absorbance of the control (without sample) and A_1 was absorbance in the presence of the sample.

2.6 Bile acid binding capacity
Based on the method of our previous research [9], the effect of the samples on the bile acid capacity was investigated in vitro.

Statistical Analysis: All experiments were carried out in triplicate. Statistical analysis was performed using Analysis of Variance (ANOVA) and the significances of the differences between samples were determined using Duncan’s multiple range test. Statistical significance was set at a level of P < 0.05.
3. Results and discussion

3.1 FT-IR spectroscopy

The characteristic absorption peaks of polysaccharide were recorded, such as the broad intense peak at around 3400cm\(^{-1}\) related to stretching vibration of \(-\text{OH}\) and the weak peak at around 2900cm\(^{-1}\) attributed to the stretching vibration and deformation vibration of C-H. The peak at 1039cm\(^{-1}\) was due to C-O-C unsymmetrical stretching and also confirmed the presence of monopyranose component [12,13]. After sulfation, two new characteristic absorption bands at 1200cm\(^{-1}\) described an asymmetrical S=O and another at 810cm\(^{-1}\) represented a symmetrical C-O-S, confirming the existence of sulfate group. The results were similar to the observations reported in many researches [12].

3.2 Homogeneity and molecular mass

The profiles of SEC-LLS chromatograms all showed two symmetrical peaks as showed, which indicated each sample was a homogenous polysaccharide [11]. Table 1 revealed that the weight-average molecular weight (M\(_w\)) of CBP and S-CBP were 335kDa and 178kDa, respectively. Compared with the native polysaccharide, the molecular mass of the sulphated derivative degraded apparently [14].

3.3 Antioxidant activities

3.3.1 Hydroxyl radical scavenging activity. The hydroxyl radical scavenging effect is one of the most crucial indexes for the antioxidant activity [9, 10]. Fig. 2 described that the inhibitory effects on the hydroxyl radical of the polysaccharides with ascorbic acid used as the reference. The hydroxyl radical scavenging effect was in the order of S-CBP > CBP, indicating chemical modification could improve the hydroxyl radical scavenging activity. Since the native polysaccharide and the sulphated
derivatives exhibited little ferrous ions chelating activity, the mechanism of hydroxyl radical scavenging were mainly related to scavenge the hydroxyl radicals directly [9]. As a consequence, the sulfation would be an efficient way to improve the hydroxyl radical scavenging activity.

![Fig.2](image)

**Fig.2** Hydroxyl radical-scavenging activity of CBP and S-CBP. Each point is the mean ± SD of triplicates.

3.3.2 *Superoxide anion-scavenging activity*. Fig.3 showed the percentage inhibition of superoxide anion radical generation for different concentrations of the polysaccharides, compared with ascorbic acid. The S-CBP showed the higher scavenging ability (85.09 ± 1.26%) at the concentration of 6mg/mL, while the ascorbic acid scavenging ability was 65.73%, which were in accordance with Wang *et al.* [9]. The enhanced superoxide radical scavenging ability was related to the characters of the substituted groups. Consequently, the sulphated derivative would be one of the most efficient ways to improve the superoxide radical scavenging ability.

![Fig.3](image)

**Fig.3** Superoxide anion-scavenging activity of CBP and S-CBP. Each point is the mean ± SD of triplicates.
3.4 Bile acid binding capacity
The Fig.4 described the bile acid binding capacities of the samples at the concentration of 6mg/mL. And, S-CBP showed the better bile acid binding capacity (2.98μM). Compared with native polysaccharide, the bile acid binding capacity S-CBP strengthened moderately. Except for water solubility, numerous factors such as ionic interaction, hydroxyl group interaction and trapping in the overall bile acid-binding capacity of polymers [9, 15], might explain the enhanced bile acid-binding capacity of the derivatives in vitro in the present study.

![Fig. 4 Bile acid binding capacity of CBP and S-CBP.](image)

4. Conclusion
In the present study, the sulphated derivative (S-CBP) with the degree of substitution (0.46), were successfully prepared from the polysaccharide extracted from corn bran (CBP). Compared with the native polysaccharide, the molecular weights were changed by chemical modification and the structures were confirmed by FT-IR and SEC-LLS. The sulfation enhanced the antioxidant activities in a dose-dependent way, which seemed to be dependent on the character of substituted group. And the sulfation of CBP strengthened the bile acid binding capacity moderately. To promote comprehensive utilization of the corn bran, more researches on modification and the toxicological experiments need to be further focused on.

Acknowledgments
This work were financially supported by Zhejiang Chinese Medical University fund (No. 2015ZY30) and Zhejiang Medical College fund (No. 2015XZA03).

References
[1] J.T. Wang, Q. Wang, J.R. Han, Yield, polysaccharides content and antioxidant properties of the mushroom Agaricus subrubescens produced on different substrates based on selected agricultural wastes. Scientia Horticultrae, 157(2013):84-89.
[2] A. Slavov, H. Kiyohara, H. Yamada, Immunomodulatingpectic polysaccharides from waste rose petals of Rosa damascene Mill. International Journal of Biological Macromolecules, 59(2012):192-200.
[3] S.B. Liang, A.G. McDonald, E.R. Coats, Lactic acid production with underfined mixed culture fermentation of potato peel waste. Waste Management, 11(2014):2022-2027.
[4] F. Zhang, L.N. Tu, D. Kang, Q.W. Jin, H.B. Zhang, M.P. Yadav, Viscosifying properties of
corn fiber gum with various polysaccharides. Food Hydrocolloids, 43(2015):218-227.

[5] M.P. Yadav, D.B. Johnston, K.B. Hicks, Corn fiber gum: new structure/function relationships for this potential beverage flavor stabilizer. Food Hydrocolloids, 23(2009):1488-1493.

[6] M.P. Yadav, D.B. Johnston, K.B. Hick, E.A. Nothnagel, The role of lipids and protein components in the emulsification properties of gum Arabic and corn fiber gum. Foods and Food Ingredients Journal, 3(2006):245-252.

[7] M.P. Yadav, R.A. Moreau, A.T. Hotchkiss, K.B. Hicks, A new corn fiber gum polysaccharide isolation process that preserves functional components, Carbohydrate Polymers, 87(2012):1169-1175.

[8] Y. Chen, H. Zhang, Y.X. Wang, S.P. Nie, C. Li, M.Y. Xie, Sulfated modification of the polysaccharides from Ganodermaatrum and their antioxidant and immunomodulating activities. Food Chemistry, 186(2015):231-238.

[9] Y.J. Wang, Q. Mo, Z.N. Li, H.W. Lai, J. Lou, S.W. Liu, J.W. Mao, Effects of degree of carboxymethylation on physicochemical and biological properties of pachyman. International Journal of Biological Macromolecules, 51(2012):1052-1056.

[10] C.Y. Huang, S.J. Wu, W.N. Yang, A.W. Kuan, C.Y. Chen, Antioxidant activities of crude extracts of fucoidan extracted from Sargassumglaucescens by a compressional-puffing-hydrothermal extraction process. Food Chemistry, 197(2016):1121-1129.

[11] Y.B. Shen, H. Zhang, L.L. Cheng, L. Wang,. H.F. Qian, X.G. Qi, In vitro and in vivo antioxidant activity of polyphenols extracted from black highland barley, Food Chemistry, 1(2016):1003-1012.

[12] S. Bagchi, K.J. Kumar, Studies on water soluble polysaccharides from Pithecellobiumdulce (Roxb.)Benth. Seeds, Carbohydrate Polymers, 138(2016):215-221.

[13] X.Y. Li, L. Wang, Effect of extraction method on structure and antioxidant activity of Hohenbueheliaserotina polysaccharides. International Journal of Biological Macromolecules, 83(2016):270-276.

[14] Y. Liu, C. Liu, H. Tan, T. Zhao, J. Cao, F. Wang, Sulfation of a polysaccharide obtained from Phellinus ribis and potential biological activities of the sulfated derivatives. Carbohydrate Polymers, 77(2009):370-375.

[15] J.L. Wang, W. Yang, Y.Y. Tang, Q. Xu, S.L. Huang, J. Yao, J. Zhang, Z.Q. Lei, Regiselectivesulfation of Artemisia sphaerocephala polysaccharide: Solution conformation and antioxidant activities in vitro. Carbohydrate Polymers, 136(2016):527-536.