Preparation and physiological activities of carboxymethylated derivative purified from corn bran

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Abstract. Two water-soluble polysaccharides extracted from corn bran were chemically modified to obtain their carboxymethylated derivatives (C-CBP1, C-CBP2). The results of degree of substitution and FT-IR analysis showed the carboxymethylation of polysaccharides were successful. The average molecular weight (Mw) of C-CBP1 and C-CBP2 were 368 and 263kDa, respectively. The degree of substitution (DS) of C-CBP1 and C-CBP2 were determined to be 0.44 and 0.46. The results showed that derivatives were effective in antioxidant and bile acid binding activity in a dose dependent way. And C-CBP2 had the higher activity for hydroxyl radical, superoxide anion scavenging activities and bile acid capacity, as lower molecular weight plays a critical role in antioxidant activities and bile acid capacity. The results suggest that the carboxymethylated derivatives are potential natural antioxidant and blood fat reduce agent that can be used as drugs or functional food ingredients.

1. Introduction
Corn bran polysaccharides (CBP), derived the main component of corn bran and/or endosperm tissues, is a water-soluble polysaccharide and has significant influence on human health. In recent years, many studies have focused on finding more valuable properties of CBP including adhesive, thickening and stabilizing, and film forming and emulsifying [1-3]. However, to the best of our knowledge, there are no available references on the antioxidant activity of the carboxymethylated corn fiber polysaccharide. Our previous study reported that carboxymethylation could enhance the solubility of pachyman [4], thus resulting in the improvement of the activities. In this case, chemical modification of carboxymethylation of corn fiber polysaccharides was carried out.

2. Materials and methods
2.1. Materials and reagents
Corn bran was collected in Hangzhou, China. The materials were de-oil, de-starched in accordance with Yadav et al.[3]. All the reagents were purchased from Aladdin Industrial Co. and used without further purification.
2.2. Preparation of polysaccharide and its carboxymethylated derivatives

2.2.1. Preparation of Corn Bran polysaccharide (CBP). Corn bran polysaccharide (CBP) was extracted from the de-oiled and de-starched corn bran following the instructions provided by alkaline hydrogen peroxide procedure [3]. Then, CBP was separated by the column of DEAE Sepharose Fast Flow (2.6cm×60cm) equilibrated with distilled water [5]. CBP were isolated, then concentrated, dialyzed and lyophilized, respectively.

2.2.2. Preparation of Carboxymethylated derivatives. The carboxymethylation of CBP was carried out on the previous researches [3]. The carboxymethylated derivatives were obtained.

2.3. Characterization of carboxymethylated derivatives

2.3.1. Compositional analysis. The carbohydrate content of all the CBP samples obtained above were analyzed by the phenol-sulphuric acid method [5]. Protein was determined by Coomassie Brilliant Blue method [6]. The samples were dissolved to 1mg/mL solution and scanned with an ultraviolet spectrophotometer (U-1900UV, Hitachi high-Technologies Corp., Japan).

2.3.2. Determination of carboxymethyl content. The degree of substitution (DS) of carboxymethylated derivatives was determined by neutralization titration with a few modifications [4].

2.3.3. Homogeneity and molecular weight determination. Molecular weight (Mw) and molecular size (Mn) of the purified fractions were determined by size exclusion chromatography with laser light scattering (SEC-LLS) [4].

2.3.4. Infrared spectrum analysis. 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 then pressed into pellets for transformation infrared spectra measurement in a frequency range of 4000-500 cm⁻¹.

2.4. Antioxidant activity

2.4.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. DPPH radical scavenging activity was determined as the procedure [7]. The DPPH radical-scavenging activity (%) was calculated by the following equation:

\[
\text{Scavenging activity (\%)} = (1 - \frac{A_1}{A_0}) \times 100\% \tag{1}
\]

Where \( A_0 \) was the absorbance of the control (without sample) and \( A_1 \) was absorbance of the samples.

2.4.2. Hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity was analyzed according to our previous researcher [4].

\[
\text{Scavenging activity (\%)} = (1 - \frac{A_1 - A_2}{A_0}) \times 100\% \tag{2}
\]

Where \( A_0 \) was the absorbance of the control (without sample) and \( A_1 \) was the absorbance in the presence of the sample, \( A_2 \) was the absorbance without sodium salicylate.
2.4.3. Superoxide radical-scavenging activity. 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 [4].

\[
\text{Scavenging activity (\%)} = (1 - \frac{A_1}{A_0}) \times 100\% \tag{3}
\]

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

2.5. Bile acid binding capacity

Based on the method of our previous research [4], the effect of the samples on the bile acid capacity was investigated in vitro.

Statistical Analysis

The results were means ± standard deviation (SD) of determinations of triplicate cultures. The data were analyzed by one-way ANOVA and a level of \(P < 0.05\) was regarded as statistically significant with Duncan's new multiple ranges.

3. Result and discussion

3.1. Chemical analysis of C-CBP

|          | carbohydrate content (%) | Protein (%) | Mw/kDa | DS |
|----------|--------------------------|-------------|--------|----|
| CBP1     | 91.6                     | 0.97        | 332    | /  |
| CBP2     | 93.3                     | 1.50        | 187    | /  |
| C-CBP1   | 86.5                     | 0.82        | 368    | 0.44 |
| C-CBP2   | 84.6                     | 1.46        | 263    | 0.46 |

The DS, carbohydrate content and molecular weight of CBP and its derivatives are listed in Table 1. Compared with the native sample, not only does the carbohydrate content of CBP and C-CBP decreased significantly \((p < 0.05)\) but also the molecular weight was increased, which was in accordance with the results of Wang et al. [4]. The DS of the C-CBP1 and C-CBP2 was 0.44, 0.46 respectively. However, their carbohydrate content and molecular weight listed in a converse order, which indicated that a slight degradation might occur in the process of C-CBP preparation.

3.2. IR Spectra of CFP and its derivatives

![FT-IR spectrum of CBP1, CBP2, C-CBP1 and C-CBP2](image)
The FT-IR spectra of the crude polysaccharide (CBP1, CBP2) and its derivatives (C-CBP1, C-CBP2) are shown in Fig. 1. The intensity of bands around 3375.76 cm$^{-1}$ in the IR spectrum was due to the hydroxyl stretching vibration of the polysaccharide and as expected they were broad. The absorption bands at 2920 cm$^{-1}$ were attributed to the stretching vibration of methylene group[8]. After carboxymethylation, two new bands at 1602 cm$^{-1}$ ($\gamma_{\text{sym}}$(COO$^-$)) and 1426 cm$^{-1}$ ($\gamma_{\text{as}}$(COO$^-$)) were observed[8]. The results were similar to the observations reported in the carboxymethyl derivatives of Ganoderma lucidum [9] and Psyllium arabinoxylan [10]. Thus, the native polysaccharide was carboxymethylated successfully without change of the molecular structure in this study.

3.3. Antioxidant activities

As shown in Fig. 2, C-CBP1 and C-CBP2 exhibited higher scavenging activity than the native polysaccharide through etherifying hydroxyl groups with carboxymethyl groups. When the

![Fig. 2. DPPH radical-scavenging rates of CBP1, CBP2, C-CBP1 and C-CBP2. Each point is the mean ± SD of triplicates.](image)

![Fig. 3. Hydroxyl radical-scavenging activity of CBP1, CBP2, C-CBP1 and C-CBP2. Each point is the mean ± SD of triplicates.](image)
concentration of samples was 6.0mg/mL, DPPH free radical scavenging activities were 55.67±1.37%, 50.41±0.94%, 62.07±1.25%, and 59.97±1.29% for CBP1, CBP2, C-CBP1 and C-CBP2, respectively. This result demonstrated the importance of the carboxymethyl group in antioxidant ability, especially at a high Mw. The carboxymethylated derivatives showed excellent scavenging activity on DPPH radical, which might be attributable to its strong hydrogen-donating ability by activating the hydrogen atom of the anomeric carbon [11].

Hydroxyl radical is considered as one of the most reactive oxygen radicals, which can easily cross cell membranes, react with most biomolecules and lead to tissue damage or cell death [13]. Fig. 4 showed that the hydroxyl radical scavenging ability of C-CBP had concentration dependence although weaker than vitamin C in the same concentrations. It increased to 36.64±1.35%, 65.47±0.42% for C-CBP1 and C-CBP2 when the concentration increased to 6 mg/mL. Our study showed that scavenging hydroxyl radical activity of the carboxymethylated derivatives were improved compared native polysaccharides. This is in agreement with the results in which carboxymethylated derivatives from Auricularia auricular [14]. For hydroxyl radical, there were two types of antioxidant mechanisms [9]: One mechanism was that the effect of the metal complexes led to the suppression against hydroxyl radical generation, and the other was the direct clearance of the generated hydroxyl radical. The mechanism of C-CBPs on cleaning hydroxyl radicals needed to be further investigated.

**Fig.4.** Superoxide anion-scavenging activity of CBP1, CBP2, C-CBP1 and C-CBP2. Each point is the mean ± SD of triplicates.

Superoxide anion radical was easily transformed from molecular oxygen, and cause oxidative damage in human body [10]. Fig. 5 showed both of the native and carboxymethylated derivative had a concentration-dependent increase in the superoxide anion radical scavenging activity. In the concentration of 2.0 mg/mL, the scavenging of superoxide radicals of C-CBP1 and C-CBP2 were 32.51±1.08%, 37.67±1.08%, respectively. In the present study, comparing with the original CBP, the scavenging activity of C-CBP against superoxide anion moderately improved. The addition of electron-donating substituents probably increased radical scavenging activity as a result of increasing electron density on the heterocyclic ring of the carbons [12, 15]. After modification, carboxylic groups were introduced into the polysaccharide’s chains, which presented C-CBP with strong electron-withstanding ability and enhanced the superoxide scavenging activity.
3.4. Bile acid binding capacity

![Fig.5. Bile acid binding capacity of CBP1, CBP2, C-CBP1 and C-CBP2. Each point is the mean ± SD of triplicates.](image)

The bile acid binding capacities of the samples were presented at Fig.6. Compared with the native polysaccharide, the bile acid binding capacity became more pronounced after carboxymethylation. Particularly, the C-CBP2 showed the best capacity with 18μmol/mg, indicating the molecular weight and the substituent group played an important role in the bile acid binding capacity. Therefore, better functionality of carboxymethylated polysaccharides to bind bile acids in vitro might be partly explained by their improved molecular weight [16].

4. Conclusion

In the present study, two water-soluble polysaccharide (CBP) extracted from corn fiber were modified successfully to obtain its carboxymethylated derivatives. In general, the carboxymethylation enhanced the antioxidant and bile acid binding activities in a dose-dependent way. Higher antioxidant activities were found for the derivatives with C-CBP2 with lower molecular weight. The research will promote comprehensive utilization of the corn bran and further researchers need to be focused on. It is noteworthy that C-CBP should be explored as a novel potential antioxidant after its further research on the safety for human consumption and antioxidant activity in vivo.

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