Effect of sulfation on the antioxidant properties and in vitro cell proliferation characteristics of polysaccharides isolated from corn bran

Xu Si, Zhongkai Zhou, Dandan Bu, Jing Li, Padraig Strappe and Chris Blanchard

1. Introduction

Corn bran is a by-product of deep processed corn and accounts for 15% of corn kernel. Polysaccharides are major components in corn bran, which are considered as an excellent source of edible and active plant compounds with medicinal effects on blood lipid reduction, immune function improvement and tumor suppression (Yadav, Parris, Johnston, Onwuula, & Hicks, 2010). Sulfated modification, as an approach to modify polysaccharide structure, is currently of great interest and contributes to the development of polysaccharide-related pharmaceuticals and functional foods (Franz & Alban, 1995). The sulfated glucose in a saccharide hydroxyl of sulfated polysaccharides. The formation of polyamion compounds alters the conformation and can distinctly change bioactive properties (Wang & Guan, 2000). Sulfation modified chitosan was demonstrated to exhibit a stronger antioxidant capacity compared to unmodified one, indicating a direct link between sulfation and bioactivity (Xing et al., 2005). Sulfation of polysaccharides with aminosulfonic acid is a mild and economical procedure. Aminosulfonic acid was selected as the esterifying agent to obtain sulfated barley polysaccharides with varying degrees of substitution as described by Qian, Bai, Tang, and Chen (2015), where comparisons between sulfated and non-sulfated barley polysaccharides in terms of antioxidant ability revealed that sulfated one showed stronger inhibitory effect on α-glucosidase. Meanwhile, sulfated derivatives of the P. cyrtomema polysaccharides also demonstrated a higher inhibitory activity against herpes simplex virus than that of the native material (Liu, Wan, Shi, & Lu, 2011). However, the majority of corn bran is discarded or utilized in feed production, which results in the wastage of a large amount of potential bioactive food resources. Therefore, comprehensive application of the polysaccharides in cereal is of great significance. Structure modification of polysaccharides is a favorable and necessary method to provide an opportunity to obtain new pharmacological agents with possible therapeutic utilizations (Liu et al., 2011).

ABSTRACT

Polysaccharides were extracted from corn bran (CBPs) and then modified by a sulfating reaction to obtain sulfated corn bran polysaccharides (SCBPs). Synthesized sulfated polysaccharides were located in the equatorial bonds of monosaccharide residues. A more orderly and smooth surface of SCBPs was observed. Single factor experiment results showed 2.0 g of aminosulfonic acid, 60 mL of N-dimethylformamide and 2.5 h of reaction time were the optimum conditions for SCBPs preparation. Phenolic content of SCBPs significantly decreased with increasing concentration of aminosulfonic acid, while it increased with increasing DMF concentration and reaction time. SCBPs had higher DPPH, ABTS and iron chelating capacity under the optimum conditions. SCBPs significantly inhibited the proliferation of A549 and HepG2 cell lines, which was associated with the up-regulation at the mRNA expression level of pro-apoptotic genes CASP3, CASP8, CASP9 and reduced expression of p53, gene Bcl-2 and gene iNOS.
In the present study, corn bran polysaccharides (CBPs) were extracted with hydrogen peroxide followed by a sulfated modification. The effect of aminosulfonic acid, N-dimethylformamide (DMF) and reaction conditions on the substitution degree of the sulfated corn bran polysaccharides (SCBPs) is investigated. Furthermore, total phenolic content, antioxidant activity, proliferation characteristics and the expression of apoptosis-related genes of SCBPs are evaluated using cell culture models. This study aims to investigate the characteristics and potential uses of SCBPs and their derivatives as a functional ingredient.

2. Materials and methods

2.1. Materials

CBPs were extracted from degreased corn bran by the alkaline hydrogen peroxide method, followed by removal of starch, protein and water-soluble polysaccharides. Extracted samples were freeze-dried after ethanol precipitation for further use (Kamboj & Rana, 2014). DMF and BaCl₂ were purchased from Tianjin Yongda Chemical Reagent Development Center; ethyl alcohol and ammonium acetate from Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd.; NaOH from Tianjin Northern Tianyi Chemical Reagent Factory; aminosulfonic acid, methanol, potassium peroxodisulfate and ammonium ferrous sulfate were obtained from Tianjin Fengchuan Chemical Reagent Factory; gelatin turbidity (Chaidedgumjorn et al., 2002) was obtained, gelatin; was added after shaking and cooling process using KBr.

2.2. Preparation for SCBPs

CBPs were accurately weighed and dissolved in DMF. Aminosulfonic acid was slowly added dropwise and the mixture was stirred well at 90°C and then cooled in an ice bath. 2 mol/L NaOH was used to change the pH of the solution to pH 7.0. The samples were then subjected to running water dialysis for 24 h and distilled water dialysis for another 24 h, followed by vacuum concentration of dialyzate. Precipitates obtained by ethanol precipitation method standing overnight were collected by centrifugation at 3000 rpm for 15 min and freeze-dried (Hetro Drywinner) to obtain SCBPs powder.

2.3. Identification of SCBPs

2.3.1. Molecular structure

The molecular structure of freeze-dried SCBPs was measured by the Fourier infrared spectroscopy method after a tabletting process using KBr.

2.3.2. Surface morphology

Freeze-dried SCBPs were subjected to liquid nitrogen wetting-off and metal spraying followed by analysis using a scanning electron microscope (SEM; SU-1510, Hitachi, Ltd., Japan).

2.3.3. Determination of substitution degree (DS)

The method of BaCl₂-gelatin turbidity (Chaidedgumjorn et al., 2002) was adopted to determine the degree of substitution (DS). A 0.5% gelatin solution was prepared by mixing 0.5 g of gelatin with 100 mL of distilled water. Ten grams of BaCl₂ was dissolved in 100 mL of 2% gelatin solution to obtain 1% BaCl₂-gelatin solution. K₂SO₄ (0.1814 g, accurately weighed) was dissolved in distilled water and diluted to a volume of 100 mL. The above three solutions were all stored at 4°C. Different volumes of standard solution, 1, 2, 5, 8 and 10 mL, were diluted to a volume of 10 mL and then mixed with 10 mL of 0.2 mol/L HCl and 1 mL of 2% gelatin solution, respectively. Two milliliters of BaCl₂-gelatin solution was added after shaking. Two minutes of quick jolt and 20-min standing were then performed. The absorption value was measured at 360 nm using a TU-1810 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China) with distilled water as a blank. The concentration of SO₄²⁻ was obtained, in terms of DS was calculated by Equation (1).

\[
DS = \frac{1.62 \times S\%}{32 - 1.02 \times S\%}, \quad (1)
\]

where, S% is the mass fraction of sulfur in the sample; m is the mass of SO₄²⁻; f is the conversion coefficient of the standard curve; M is the mass of sample; DS is the degree of substitution.

2.4. Factorial trial of esterification conditions

2.4.1. Aminosulfonic acid dose

The effects of variable aminosulfonic acid doses (0.5, 1.0, 1.5, 2.0 and 2.5 g) on SCBPs, under the conditions of aminosulfonic acid and DMF at a ratio of 1:30 and reaction temperature of 90°C and a reaction time of 2.5 h, were evaluated.

2.4.2. N-dimethylformamide (DMF) dose

When the aminosulfonic acid dose was fixed as 2.0 g, reaction temperature as 90°C and reaction time as 2.5 h, the effects of variable DMF volume (20, 40, 60, 80 and 100 mL) on the SCBPs were explored.

2.4.3. Reaction time

The effect of reaction time on SCBPs was determined under the variable reaction times of 1, 1.5, 2, 2.5 and 3 h and a constant aminosulfonic acid amount of 2.0 g, with an aminosulfonic acid to DMF mass ratio of 1:30 and a reaction temperature of 90°C.

2.5. Total phenolic content measurement

The Folin–Ciocalteu method, described by Kim, Tsao, Yang and Cui (2006) was used for phenolic content determination. Briefly, 0.5 mL of CBP and SCBP solution (1-10%w/v) was added to 2.5 mL of diluted Folin–Ciocalteu reagent (1:10, v: v), then 2 mL of 75 g/L of saturated sodium carbonate solution was added. The absorbance at 760 nm was mea-
2.6.2. ABTS assay
Potassium peroxodisulfate and ABTS⁺ solutions were well mixed in the dark and allowed to stand for 15 h. The freshly prepared bottle-green ABTS radical solution was diluted to an absorbance of 0.7 by methanol at 734 nm (Huang, Ou, & Prior, 2005). A darkened reaction for 10 min between 190 µL of methanol and 1 mL of ABTS radical solution was performed, and the absorbance was measured at 734 nm. Trolox solutions, set as positive control, with concentrations of 18.75, 37.5, 75, 150 and 300 mg/L, were used to construct a standard curve. SR was calculated by Equation (2). The result was expressed as mg Trolox equivalent in per gram of CBPs (mg TE/g).

\[
SR(\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100\%,
\]

where, \(A_{\text{sample}}\) is the absorbance of sample; \(A_{\text{blank}}\) is the absorbance of blank group.

2.6.3. Iron chelating capacity (ICC) assay
The method of Min, McClung and Chen (2011) was used to perform the ICC assay. A mixture of DMSO dissolved sample solution (50 µL), 1 mmol/L ammonium ferrous sulfate solution (50 µL) and 10% ammonium acetate buffer (1.3 mL) was prepared and left to stand for 5 min. A chromogenic reaction occurred after adding 50 µL of 6.1 mmol/L iron reagents and the absorbance at 562 nm was measured after 10 min. The iron chelating ability was calculated using a standard curve obtained by different concentrations of gallic acid (6.25–100 mg/L). Results were expressed as mg gallic acid equivalent per gram of CBPs (mg GAE/g).

2.7. Cell viability assay (MTT assay)
The human lung cancer cell line (A549) and the hepatoma cell line (HepG2) were resurrected and cultured in modified RPMI-1640 medium containing 10% fetal calf serum and 100 IU/mL penicillin and streptomycin and maintained at 37°C and 5% CO₂.

Cell viability was measured using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) method. Cells in logarithmic phase were collected and diluted to a concentration of 5 × 10⁴ cells/mL, 100 µL of which was added to each well of a 96 well plate (5 × 10³ cells per well). 24 h post seeding, cells were incubated with different concentrations of SCBPs ranging from 10 mg/mL to 50 mg/mL. Following a further 24 h incubation period, 20 µL of MTT solution was added and incubated for 3 h, finally 100 µL of formazan solvent was added. MTT formazan crystals were thoroughly dissolved by repeatedly pipetting and, the absorbance (OD₅₇₀ nm) was measured and cell viability was calculated as follows.

\[
\text{Cell viability} = \frac{A_{\text{sample}} - A_0}{A_{\text{control}} - A_0} \times 100\%.
\]

where, \(A\) is the absorbance associated with treated cells; \(A_0\) is the absorbance of zero setting; \(A_{\text{control}}\) is the absorbance of control.

2.8. Gene expression analysis by RT-PCR
Total cellular ribonucleic acid (RNA) was extracted using the Trizol reagent (Takara) and treated with RNase-free DNase to remove any contaminating genomic DNA. The quality and integrity of RNA was assessed by agarose gel electrophoresis and ethidium bromide staining.

Real-time PCR (RT-PCR) amplification was performed with RPL32 as reference housekeeping gene and CASP3, CASP8, CASP9, p53, Bcl-2 and INOS as target genes. single-strand cDNA was synthesized using the PrimeScript RT reagent kit with gDNA Eraser (Takara) according to the manufacturer’s instructions. PCR was performed in a reaction volume of 25 µL containing 1.0 µM of each primer (10 µM), 1.0 µL of cDNA, 12.5 µL of 2 × SYBR Primix ExTag and 9.5 µL of PC-grade water. Thermal cycling conditions included an initial denaturation step at 95°C for 5 min, and then 40 cycles of 95°C for 30 s, 58–60°C for 30 s and 72°C for 30 s. Three replicates of each reaction were carried out, and the relative expression was calculated according to the method of 2−ΔΔCT (Livak & Schmittgen, 2001; Pfaffl, 2001). Details of target genes and primer sequences used in this study are shown in Table 1.
2.9. Statistical analysis

The mean value of replicates was calculated and all data are presented as mean ± standard deviation (SD). Data were analyzed by one-way Analysis of variance (ANOVA) using SPSS software. \( p < 0.05 \) was considered statistically significant.

3. Results

3.1. Molecular structure

The changes in the FTIR spectrum before and after the sulfating reaction were recorded and demonstrated in Figure 1. It can be seen that the stretching vibration of \( \text{O}–\text{H} \) in saccharides led to the formation of an absorption peak at 3339 cm\(^{-1}\), which indicated that hydrogen bonds existed between or within molecules. The characteristic absorption peak for saccharides at 2924 cm\(^{-1}\) was caused by the stretching vibration of \( \text{C}–\text{H} \). The sulfating reaction induced \( \text{C}=\text{O} \) stretching vibration in \( \text{CHO} \), and formation of an absorption peak at 1622 cm\(^{-1}\). The stretching vibrations of two \( \text{C}–\text{O} \) bonds, including \( \text{C}–\text{O}–\text{H} \) and \( \text{C}–\text{O}–\text{C} \) in sugar rings, contributed to the absorption peak at 1027 cm\(^{-1}\).

Two new absorption peaks were found in SCBPs at 1226 cm\(^{-1}\) and 810 cm\(^{-1}\), which were an \( \text{S}=\text{O} \) asymmetric stretching vibrational absorption and a \( \text{C}–\text{O}–\text{S} \) (in \( \text{SO}_{3}^{–} \)) stretching vibrational absorption, respectively. The 810 cm\(^{-1}\) band in the FTIR spectrum showed that sulfated groups were located at the equatorial site of monosaccharide residues, demonstrating the formation of sulfated polysaccharides by the combination of sulfated groups and CBPs at the equatorial bond of monosaccharide residues.

It was also apparent in Figure 1 that the sulfating reaction broadened and strengthened the absorption, and shifted the hydroxy absorption peak wave number to 3384 cm\(^{-1}\). CBPs are equipped with a large amount of negative charge due to the substitution of sulfated group. Intermolecular hydrogen bonds were strengthened to a certain degree as many of the hydroxyl groups were exposed with a stretched and rigid coil conformation caused by the repulsive interaction between sulfated groups.

3.2. Surface morphology

SEM analysis of CBPs before and after sulfating reaction is presented in Figure 2. Sulfating reaction had a distinct effect on the surface morphology of CBPs. An interior stack and rough surface were observed in CBPs without sulfation (Figure 2(a)). In contrast, SCBPs with a distorted edge and unordered multilayer structure disappeared and were superior to CBPs in terms of orderly structure and uniform and smooth surface (Figure 2(b)). This phenomenon may be associated with non-covalent bond formation which was promoted by the distortion and modification of the saccharide ring due to the participation of the sulfated group. Also the repulsive interaction between like charge groups possibly contributed to the stretched and rigid characteristics of the curly conformation (Wang & Zhang, 2009).

3.3. Single factor experiment for sulfated modification

3.3.1. The effect of aminosulfonic acid concentration on DS and phenolic content

It was noted that the phenolic content in SCBPs was lower than that in CBPs (108 mg gallic acid/g) without exception (Figure 3(b)). DS levels rose first and then decreased later with the increase in aminosulfonic acid dose, however the opposite trend was observed with phenolic content. The highest DS (5.55) and the lowest phenolic concentration...
(39.2 mg GAE/g) were both obtained when the aminosulfonic acid amount was 2.0 g (Figure 3(a)), indicating the significant influence of aminosulfonic acid dose on the sulfating effect. Excessive acidity, caused by continuously increasing the amount of aminosulfonic acid, led to partial degradation and incomplete sulfation of CBPs potentially due to aminosulfonic acid acting on carbohydrate chains. As a result, the DS levels declined and the phenolic concentration subsequently increased. The optimum aminosulfonic acid dose was 2.0 g.

3.3.2. The effect of DMF amount on DS and phenolic content
Figure 4(b) demonstrates an increase in phenolic content of SCBPs compared with that of CBPs, except the DMF dose of 20 mL. Increases in the DMF dose resulted in increases in DS and phenolic concentration (Figure 4(a)). However, corn bran was completely soluble when the DMF dose was above 60 mL, resulting in a reduction in the DS and phenolic concentration of the SCBPs, possibly due to a lower reactant concentration reducing the probability of collisions between SCBPs, which impacted on the substitution reaction and phenolic retention. When the DMF dose was lower than 60 mL, an increased solubility and degree of esterification of CBPs combined with an increasing amount of DFM Lewis base reagent resulted in increases in the DS and phenolic concentration. The optimum DMF dose was 60 mL, which resulted in the most favorable DS (4.61) and the highest phenolic content (126.4 mg GAE/g).

3.3.3. The effect of reaction time on DS and phenolic content
Figure 5 shows that DS and phenolic content were dependent on reaction time. They both initially increased and then declined later with an extension of the reaction time at the fixed aminosulfonic acid dose and reaction temperature. These conditions resulted in the highest values for DS and phenolic content (4.65 and 119.1 mg GAE/g, respectively) at 2.5 h. This indicated that the esterification time extension contributed to an improvement in the esterification effect as well as the DS and phenolic content of the polysaccharides. However, flavonoids might be damaged due to partial degradation-induced ester bond and glycosid bond rupture, as a result of a decrease in solvent amount caused by a peracid condition and an excessive esterification time (Xu, Ye, Chen, & Liu, 2007). Thus, phenolic acid distribution was altered, leading to
decreases in the esterification degree and phenolic concentration.

### 3.4. DPPH radical scavenging ability

The DPPH radical scavenging ability of SCBPs obtained at different reaction conditions is shown in Figure 6(a–c). Higher DPPH radical scavenging values were observed in SCBPs compared with CBPs (32.7 mg TE/g), which demonstrated that DPPH radical scavenging ability were distinctly improved by sulfated modification and resulted increased DS, which is consistent with the findings of Wang et al. (2010). During the sulfating process, more active groups were involved in the free radical reaction were exposed, facilitating the capture or scavenging of which would ultimately improve bioactivity.

![Figure 5. Effect of reaction time on DS and phenolic content of SCBPs.](image)

![Figure 6. Antioxidant activities of SCBPs.](image)
The effects of three reaction factors, aminosulfonic acid dose, DMF dose and reaction time, on DPPH radical scavenging ratio can be seen in Figure 6. DPPH radical scavenging activity increased initially and then subsequently decreased with the increase in aminosulfonic acid and DMF dose as well as reaction time. The highest DPPH radical scavenging ratio was obtained at an aminosulfonic acid amount of 2.0 g (Figure 6(a)), a DMF volume of 60 mL (Figure 6(b)) and a reaction time of 2.5 h (Figure 6(c)) resulting in values of 50.2, 48.1 and 47.7 mg TE/g, respectively.

3.5. ABTS radical scavenging ability

The changing trend of ABTS radical scavenging ability with an increase of aminosulfonic acid dose, DMF dose and reaction time was similar to that seen for DPPH. A rise in value was initially observed followed by a decline as shown in Figure 6(d–f). When the aminosulfonic acid dose was 2.0 g, the strongest ABTS radical scavenging activity was obtained (3.7 mg TE/g, Figure 6(d)). With a DMF dose of 60 mL, the highest value was 4.4 mg TE/g (Figure 6(e)). In terms of reaction time, the highest ABTS radical scavenging ratio of 3.9 mg TE/g was observed at 2.5 h (Figure 6(f)). By comparison, SCBPs showed a much stronger ABTS radical scavenging ability than CBP, highlighting the contribution of sulfated polysaccharides to increased antioxidant activity.

3.6. Iron chelating capacity

The mixture of FeCl₂ and Ferrozine resulted in a high absorption value at 562 nm, which declined when the ferric ion chelator was added. The iron chelating capacity of SCBPs is shown in Figure 6(g–i).

The same characteristics of ABTS radical scavenging ability were also seen in iron chelating capacity, with increasing aminosulfonic acid amount, DMF amount and reaction time. The highest scavenging ratios were 13.3, 13.3 and 13.0 mg GAE/g, with an aminosulfonic acid amount of 2.0 g, DMF 60 mL and a reaction time of 2.5 h, respectively (Figure 6(g–i), respectively). Overall, a significant improvement was observed in iron chelating capacity values in CBPs compared to SCBPs.

3.7. Cell viability

The in vitro inhibitory effect of SCBPs on the proliferation of lung (A549) and liver (HepG2) cancer cells lines was investigated using an MTT assay and the results are summarized in Figure 7. The proliferation of both A549 cells and HepG2 cells declined sharply with increasing concentrations of CBPs or SCBPs. The maximum proliferative inhibition of SCBPs on A549 cells and HepG2 cells was 58.9% and 59.96%, respectively, which both occurred at the highest concentration of 5 mg/mL. SCBPs were associated with a lower cell viability than native CBPs at the same concentration, suggesting that the inhibition of proliferation was associated with modification of polysaccharides.

3.8. Effect of sulfation on apoptosis-related gene expression in A549 and HepG2 cells

Gene expression levels of the cysteine proteases, CASP3, CASP8 and CASP9 inducible nitric oxide synthase of iNOS (related inflammation) the tumor suppressor gene p53 and B lymphocytoma-2 gene of Bcl-2 (anti-apoptotic), were analyzed in the current experiment to explore the molecular mechanism associated with CBPs and SCBPs on proliferation of cancer cell lines.

Relative gene expression levels (Figure 8) of CASP3, CASP8, CASP9 and p53 both cell types treated with CBPs and SCBHs were all significantly up-regulated compared to the control group for both A549 and HepG2 cell lines. The degree of up-regulation in SCBP-treated cells was higher than that of CBPs group, suggesting a significantly stronger effect of SCBPs on cancer cell suppression and promotion of apoptosis. In addition, the expression of Bcl-2, with an ability to inhibit apoptosis, was down-regulated in both cell lines. A higher reduction on mRNA expression level of Bcl-2 (0.35–0.36-fold) also occurred in SCBPs treated cells. High expression of iNOS may cause uncontrolled and prolonged production of NO, resulting in liver damage, inflammation and, eventually, tumor development (Burney, Caulfield, Niles, Wishnok, & Tannenbaum, 1999; Suzuki, Menegazzi, de Prati, Mariotto, & Armato, 1995). In this study, RT-PCR results (Figure 8) showed that iNOS expression was markedly down-modulated (0.29-fold in A549 cell line and 0.35-fold

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Cell viability associated with different SCBPs concentrations.

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Viabilidad de la célula asociada con diferentes concentraciones de SCBPs.
in HepG2 cell line, respectively) following exposure to sulfated polysaccharides sulfating modification, indicating that in this scenario the potential protective effect of nitric oxide is down-regulated due to SCBP exposure.

Taken together, the gene expression levels of CASP3, CASP8, CASP9, Bcl-2 and iNOS suggest that the inhibiting effect on cancer cell line proliferation by SCBPs may be associated with the regulation at the mRNA expression level of enzymes involved in both promoting cell apoptosis and also in protecting cells from cell death. Previous reports have shown that sulfated polysaccharides obtained from the mushroom Grifola Frondosa can significantly inhibit HepG2 cell proliferation and induce apoptosis through Notch 1-NFκB pathway (Wang et al., 2013). In terms of the role of iNOS and prevention of tumor progression, Chittezhath, Deep, Singh, Agarwal, and Agarwal (2008) showed that silibinin, the major flavanolignan component of silymarin can reduce iNOS activity in A549 cells, contributing to reduced proliferation in vitro.

4. Discussion

The bioactivity of sulfated polysaccharides is dependent on the sulfur DS (Qi et al., 2005). Moderate sulfating modification improved the radical scavenging activity of CBPs significantly, but excessive modification showed an opposite effect. It is generally acknowledged that polysaccharide bioactivity is associated with its free hydroxy group on the sugar ring, and increased free hydroxy groups increases the antioxidant activity. Antioxidant activity was significantly improved after sulfation, which may be attributed to the formation of non-covalent bonds induced by distortion or transformation of the sugar ring when the hydroxy group was substituted by the sulfated group. Carbohydrate chain elongation may be due to the repulsive interaction between anionic groups and hydrogen bonds formed by a combination of sulfated groups and hydroxy groups on the sugar ring. As a result, a stretched helical structure is formed on part of the carbohydrate chain (de Souza et al., 2007), strengthening its capacity to combine with free radicals and providing polysaccharides with a higher active structure.

For example, the sulfated contrapuntal hydroxyl free radical of brown algae polysaccharides has shown strong scavenging ability (de Souza et al., 2007) and sulfated *Ganoderma lucidum* polysaccharides have also been associated with improved antitumor effects compared to native polysaccharides (Tao, Zhang, & Cheung, 2006).

The combined actions of various phenols in plants generate an antioxidant activity which is dependent on phenolic composition and concentration. A large number of reports (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Giovaneli & Buratti, 2009; Pandey & Rizvi, 2009) have found a strong association between phenolic compounds and antioxidant activity in plants. However, opposite statement that no or little correlation between phenolic compounds and antioxidant activity also exists (Nsimba, Kikuzaki, & Konishi, 2008). In this current study, the increase in aminosulfonic acid dose led to an increase in DS and a decline in phenolic concentration, which distinctly enhanced antioxidant activity. At the same time, antioxidant activity was seen to be stronger with an increased phenolic content, extended reaction time and increased DMF dose. This phenomenon demonstrated that the relationship between phenolic concentration and antioxidant capacity is not completely linear. Phenols are not the only compounds showing antioxidant activity. Other active compounds, such as flavonoids and polysaccharides, can also enhance antioxidant activity. Maillard reaction and caramelization reaction can result in antioxidant substances (Randhir, Kwon, & Shetty, 2008) and antioxidant activity can be improved by the synergistic effect of phenols and non-antioxidant compounds in extracts (Tsao, 2010).

The anticancer activity of polysaccharides and their modified derivatives have usually been attributed to the stimulation of a cell-mediated immune response. Furthermore, the introduction of sulfated groups to polysaccharides alters its physicochemical characterization and chain conformation (Wang, Li, & Chen, 2009). It has also been reported (Wang, Zhang, Li, Hou, & Zeng, 2004) that relatively expanded and stiff chain conformation of sulfated polysaccharides can improve anticancer effects.
5. Conclusion
A sulfating modification was performed on CBPs extracted by the alkaline hydrogen peroxide method. Sulfated groups were located at the equatorial bond of monosaccharide residues, and led to a more orderly structure and a smoother surface. Phenolic concentration of SCBPs was lower than that of CBPs at all-time points with increasing aminosulfonic acid amounts. However, a significant increase was noted in the phenolic content in SCBPs as the DMF dose was increased and the reaction time extended. The improvement in sulfated DS was accompanied with a decrease in phenolic content and a distinct increase in antioxidant activity. SCBPs showed superior DPPH scavenging ability, ABTS scavenging ability and iron chelating capacity, as well as inhibiting the proliferation of two cancer cell lines compared with unmodified CBPs. The inhibition of proliferation in A549 lung carcinoma and HepG2 liver carcinoma cell lines appears to be linked to activation of apoptotic pathways with enhanced mRNA expression of the pro-apoptotic caspases, CASP3, CASP8, CASP9 and reduced expression of p53, Bcl-2 and INOS genes, suggesting that SCBPs may provide unique anticancer bioactivity characteristics.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This work was financially supported by Agricultural science and Technology Achievements Transformation Fund (grant number 2014GB2A100527), National Spark Program, the NSFC (grant number 31471701), the China-European research collaboration program (grant number SQ2013ZOA100001) and ARC Functional Grains Centre, Australia.

References
Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K., & Gallagher, E. (2010). Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry, 119, 770–778. doi:10.1016/j.foodchem.2009.07.032
Burney, S., Caulfield, J.L., Niles, J.C., Wishnok, J.S., & Tannenbaum, S.R. (1999). The chemistry of DNA damage from nitric oxide and peroxynitrite. Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 424, 37–49. doi:10.1016/S0027-5107(99)00006-8
Chaidedgumjorn, A., Toyoda, H., Woo, E.R., Lee, K.B., Kim, Y.S., Toida, T., & Imanari, T. (2002). Effect of (1→3)- and (1→4)- linkages of fully sulfated polysaccharides on their antiangiogenic activity. Carbohydrate Research, 337, 925–933. doi:10.1016/S0008-6215(02)00078-2
Crittazeth, M., Dee, G., Singh, R.P., Agarwal, C., & Agarwal, R. (2008). Silibinin inhibits cytokine-induced signaling cascades and down-regulates inducible nitric oxide synthase in human lung carcinoma A549 cells. Molecular Cancer Therapeutics, 7, 1817–1826. doi:10.1158/1535-7163.MCT-08-0256
de Souza, M.C.R., Marques, C.T., Dore, C.M.G., da Silva, F.R.F., Rocha, H.A.O., & Leite, E.L. (2007). Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. Journal of Applied Phycology, 19, 153–160. doi:10.1007/s10811-006-9121-2
Franz, G., & Alban, S. (1995). Structure-activity relationship of anthrbutolic polysaccharide derivatives. International Journal of Biological Macromolecules, 17, 311–314. doi:10.1016/0141-8130(96)81187-X
Giovanelli, G., & Buratti, S. (2009). Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chemistry, 112, 903–908. doi:10.1016/j.foodchem.2008.06.066
Huang, D., Ou, B., & Prior, R.L. (2005). The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53, 1841–1856. doi:10.1021/jf030723c
Kim, K.H., Tsao, R., Yang, R., & Cui, S.W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry, 95, 466–473. doi:10.1016/j.foodchem.2005.01.032
Liu, X.X., Wan, Z.J., Shi, L., & Lu, X.X. (2011). Preparation and antitherpeitic activities of chemically modified polysaccharides from polygonatum cnytomina hu. Carbohydrate Polymers, 83(2), 737–742. doi:10.1016/j.carbpol.2010.08.044
Livak, K.J., & Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods, 25, 402–408. doi:10.1016/S0888-6277(00)00334-X
Min, B., McClung, A.M., & Chen, M.H. (2011). Phytochemicals and antioxidant capacities in rice brans of different color. Journal of Food Science, 76, C117–C126. doi:10.1111/j.1750-3841.2010.01929.x
Qi, H., Zhang, Q., Zhao, T., Chen, R., Zhang, H., Niu, X., & Li, Z. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta) in vitro. International Journal of Biological Macromolecules, 37, 195–199. doi:10.1016/j.ijbiomac.2005.10.008
Qian, J.Y., Bai, Y.Y., Tang, J., & Chen, W. (2015). Antioxidation and o-glucosidase inhibitory activities of barley polysaccharides modified with sulfation. LWT - Food Science and Technology, 64, 104–111. doi:10.1016/j.lwt.2015.05.034
Randhir, R., Kwon, Y.I., & Shetty, K. (2008). Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innovative Food Science & Emerging Technologies, 9, 355–364. doi:10.1016/j.ifset.2007.10.004
Suzuki, H., Menegazzi, M., de Prati, A.C., Mariotto, S., & Armato, U. (1995). Nitric oxide in the liver: Physiopathological roles. Advances in Neuroimmunology, 5, 379–410. doi:10.1093/0960-5428(95)00024-0
Tao, Y., Zhang, L., & Cheung, P.C.K. (2006). Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. Carbohydrate Research, 341, 2261–2269. doi:10.1016/j.carbpol.2006.05.024
Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. Nutrients, 2, 1231–1246. doi:10.3390/nu2121231
Wang, C., & Guan, H. (2000). Advances of researches on antiviral activities of polysaccharides-II. Antiviral activities of sulfated polysaccharides. Biotechnology Progress, 20(11), 3–8.
Wang, C.L., Meng, M., Liu, S.B., Wang, L.R., Hou, L.H., & Cao, X.H. (2013). A chemically sulfated polysaccharide from Grifola frondosus induces HepG2 cell apoptosis by notch1-NF-κB pathway. Carbohydrate Polymers, 95, 282–287. doi:10.1016/j.carbpol.2013.02.057
Wang, J., Guo, H., Zhang, J., Wang, X., Zhao, B., Yao, J., & Wang, Y. (2010). Sulfated modification, characterization and structure–antioxidant relationships of Artemisia sphaerocephala polysaccharides. Carbohydrate Polymers, 81, 897–905. doi:10.1016/j.carbpol.2010.04.002
Wang, L., Li, X., & Chen, Z. (2009). Sulfated modification of the polysaccharides obtained from defatted rice bran and their antitumor activities. International Journal of Biological Macromolecules, 44, 211–214. doi:10.1016/j.ijbiomac.2008.12.006
Wang, X., & Zhang, L. (2009). Physicochemical properties and antitumor activities for sulfated derivatives of lentianan. Carbohydrate Research, 344, 2209–2216. doi:10.1016/j.carres.2009.04.033
Wang, X., Zhang, L., Li, Y., Hou, X., & Zeng, F. (2004). Correlation of structure to antitumor activities of five derivatives of a β-glucan from Poria cocos sclerotium. Carbohydrate Research, 339, 2567–2574. doi:10.1016/j.carres.2004.08.003
Xing, R., Liu, S., Guo, Z., Yu, H., Wang, P., Li, C., . . . Li, P. (2005). Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. *Bioorganic & Medicinal Chemistry*, 13, 1573–1577. doi:10.1016/j.bmc.2004.12.022

Xiong, X., Li, M., Xie, J., Jin, Q., Xue, B., & Sun, T. (2013). Antioxidant activity of xanthan oligosaccharides prepared by different degradation methods. *Carbohydrate Polymers*, 92, 1166–1171. doi:10.1016/j.carbpol.2012.10.069

Xu, G., Ye, X., Chen, J., & Liu, D. (2007). Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. *Journal of Agricultural and Food Chemistry*, 55, 330–335. doi:10.1021/jf0625171

Yadav, M.P., Parris, N., Johnston, D.B., Onwulata, C.I., & Hicks, K.B. (2010). Corn fiber gum and milk protein conjugates with improved emulsion stability. *Carbohydrate Polymers*, 81, 476–483. doi:10.1016/j.carbpol.2010.03.003