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ANALYSIS ON EXTRACTION AND PURIFICATION OF CORN BRAN AND THE EFFECT OF ITS OXIDATION RESISTANCE ON INDUSTRIAL VALUE CREATION FROM AN INDUSTRIAL CHAIN PERSPECTIVE

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ABSTRACT

This paper studied the extraction and purification of corn bran as well as its oxidation resistance from an industrial chain perspective in order to improve the utility value of corn and increase the economic benefits of corn processing enterprises, which had great and far-reaching significance for the value creation of the corn industry. The microwave-assisted water-extraction and alcohol-precipitation method was applied for extraction of polysaccharide from corn bran. Then, the extracted polysaccharide was processed with Sevag method for deproteinization, dialysis method for removal of small molecular impurities and finally Sephadex G-75 chromatography for separation and purification. Thus, the oxidation resistance of the purified polysaccharide extracted from corn bran was studied. The results showed that corn bran polysaccharide CSP Ⅰ contained galactose, glucose, arabinose, xylose, wherein the content of glucose was the highest. Besides, no nucleic acid and small amount of protein was found in corn bran polysaccharide CSP Ⅰ. Corn bran polysaccharide CSP Ⅰ had varying degrees of scavenging effect on hydroxyl radical, DPPH • radical and superoxide anion free radical, of which the scavenging effect on hydroxyl radical was not obvious. If results of this study can be applied to the actual corn industrial chain, not only can the corn bran be turned into useful resource but also the industrial value creation can be improved through the development of high value-added products, which can bring a very broad market prospect and remarkable economic benefit.

1. Introduction

As one of the major crops in China, the production of corn reached 211 million tons in 2013. However, corn is not appropriate to be taken as staple food and about 90% of the domestic corn is used for industrial processing and consumed as animal feedstuff (Cappelli et al., 2015; England and Möller, 2015). Corn bran, containing hemicellulose, cellulose, etc., is a by-product of corn starch production process. Yet it is mainly used as feedstuff, with low economic value. With the increase of output quantity of corn non-starch components year by year, studies on the utility value of various components of corn have drawn much attention of the corn processing enterprises, which can lead the development of corn economy by extension of

corn processing chain (Weiss et al., 2010; Li et al., 2016). The corn bran contains corn bran polysaccharide, whose water-soluble and processing performance is better compared with corn bran fiber. Studies on the extraction of polysaccharide from corn bran can not only improve the value of corn, bring economic benefits to processing enterprises, but also is of great significance to the development of functional medicine and food (Nordberg, 2011; Panjkovich and Daura, 2010).

Polysaccharide, also known as N-glycans, is a kind of macromolecule substance synthesized by a great number of monosaccharide residues. Polysaccharide is the energy substance of living organisms as well as the indispensable structural material for metabolism which participates in various life activities of cells (Urai et al., 2015). In recent years, there are many experts and scholars in China and abroad who have studied the extraction and purification of corn bran and its oxidation resistance. Sadeghi et al. (2015) found in 2015 that polysaccharide was a biologically active substance which had obvious anti-tumor and anti-virus effect, without toxic and side effects. Guo et al. (2015) studied in 2015 the extraction of cottonseed meal polysaccharide by acid pickling method and obtained the optimal extraction process condition. Safa et al. (2014) in 2014 found that corn bran had lipid-lowering effect, laxative effect and anti-obesity effect, etc. Therefore, studying the extraction and purification as well as the oxidation resistance of corn bran has an important significance for industrial value creation.

2. Materials and methods

2.1. Extraction of corn bran polysaccharide

2.1.1. Materials and equipment

Materials used in this study are as follows: corn bran, phenol, concentrated sulphuric acid, ethanol, petroleum ether, glucose, thermostable α amylase.

Equipment applied in this study include: electro- thermostatic blast oven, centrifuge, rotatory evaporator, LAB-DANCER, ultraviolet and visible spectrophotometer, electric-heated thermostatic water bath, precise timing electric mixer, etc.

2.1.2. Extraction process of corn bran polysaccharide

An amount of 10 g of corn bran was weighed after it was cleaned and purified, crushed and sieved, with degreasing. Under certain microwave time, microwave power, material liquid ratio, extraction temperature and extraction time, the first extraction was carried out through centrifugation of the corn bran for 10 min with thermostable α amylase. Then, the filter liquor was collected and subsided for second extraction (Calín-Sánchez et al., 2011). Afterwards, the combined filtrate was concentrated under reduced pressure using a rotary evaporator to 10% of the original volume. Next, the concentrate was added with 3 volumes of 95% ethanol and placed for one day. Then, it was centrifuged for 10 min at 4000r/min and sediment was collected. Finally, the sediment was washed with anhydrous ethanol and acetone in turn and corn bran crude polysaccharide was obtained after processing of freeze drying (Madhurambal et al., 2015).

2.1.3. Determination of polysaccharide content

The formula for the extraction efficiency of polysaccharide content is as follow:

$$\text{Extraction efficiency},\% = \frac{\text{Extractive polysaccharide content (g)}}{\text{Gross of rawmaterial (g)}} 100 \quad (1)$$

2.1.4. Orthogonal experimental design for the extraction of corn bran polysaccharide

The orthogonal experiment was carried out based on the five factors of microwave power, microwave time, solid-liquid ratio, extraction temperature and extraction time, in which microwave power and microwave time were two main factors considered. Based on the single factor experiment, four levels were taken for each factor, taking the extraction efficiency of corn bran polysaccharide solution as the indicator, as shown in Table 1.

Table 1. Factors and levels of orthogonal experiment of corn bran polysaccharide extraction

level	A	B	C	D	E
	Microwave power (W)	**Microwave time (min)**	**solid-liquid ratio**	**Extraction temperature (°C)**	**Extraction time (h)**
1	280	2	1: 10	70	2
2	460	3	1: 20	80	3
3	600	4	1: 30	90	4
4	700	5	1: 40	100	5

2.2. Separation and purification of corn bran polysaccharide

2.2.1. Materials and equipment

Materials used in this section include: corn bran crude polysaccharide solution, phenol, concentrated sulfuric acid, phosphoric acid, chloroform, ethanol, n-butanol, sephadex G50, sephadex G75, sephadex G100 and bovine serum albumin.

Equipment applied include: electric constant temperature drying oven, centrifuge, ultraviolet and visible spectrophotometer, automatic sampling instrument, dialysis tube, etc.

2.2.2. Deproteinization with Sevag method

Firstly, the Sevag reagent was added into the corn bran water-soluble polysaccharide concentrated solution and mixed sufficiently. Then, the solution was centrifuged for 10 min at 4000 r/min. After the solution presented stratification state, the liquid supernatant was obtained. The above operation was repeated for several times. Then, V sample, V n-butyl alcohol: V chloroform, deproteinization frequency and deproteinization time were taken as four factors, with three levels in each factor. The orthogonal experiment was carried out, taking protein removal rate and polysaccharide loss rate of corn bran polysaccharide solution as indicators, as shown in Table 2.

2.2.3. Dialysis

The corn bran polysaccharide solution after deproteinization was put into a dialysis bag for 3 days of dialysis with distilled water.

2.2.4. Purification of Sephadex G-75 chromatographic column

The corn bran polysaccharide was firstly prepared into 2 mg / ml solution and then separated and purified with the Sephadex G-75 chromatographic column. Afterwards, elution was carried out on the solution with deionized water at a flow velocity of 0.26 ml/min. Then, a collector was used to collect the solution at a speed of 15 min/tube. Finally, a graph was drawn, taking number of tubes as x-coordinate and absorbance value of eluent at a wavelength of 490 nm as y-coordinate. After the eluent at peak part was combined, freeze drying process was carried out, thus corn bran polysaccharide was obtained (Akamatsu et al., 2016).

Table 2. Factor level of deproteinization with Sevag method

Level	V reagent: V sample	V n-butyl alcohol: V chloroform	Deproteinization frequency	Deproteinization time
1	1:1	1:2	2	15
2	1:2	1:3	3	20
3	1:3	1:4	4	25

2.3. Oxidation resistance of corn bran polysaccharide

2.3.1. Materials and equipment

Materials used in this section include: corn bran polysaccharide CSP I, ethanol, ferrous sulfate, salicylic acid, hydrogen peroxide, pyrogallol and DPPH.

Equipment applied include: LAB-DANCER, ultraviolet and visible spectrophotometer, thermostatic water bath pot, etc.

2.3.2. Determination of hydroxyl radical-scavenging ability

Two ml of 9 mmol/L FeS04 solution, 2 ml of mixed polysaccharide solution with polysaccharide of different concentrations, 2 ml of 9 mmol/L H2O2 solution were added into a tube successively and mixed sufficiently and then placed for 10 min. Next, 2 ml of 9 mmol/L salicylic acid solution was added into the tube and mixed sufficiently and then placed for 1 hour. The absorbancy A_i of the mixed polysaccharide solution at 510 nm was measured; distilled water was used to replace salicylic acid and then the background absorbance A_j of a certain concentration of polysaccharide was measured; the absorbancy A_0 of blank control was measured with polysaccharide solution replaced by distilled water. Thus, the calculation formula of clearance rate S is as follow:

$$S = \frac{A_0 - (A_i - Aj)}{A_0} \times 100\% \quad (2)$$

2.3.3. Determination of DPPH • radical-scavenging ability

0.2 mmol/L DPPH solution was prepared by dissolving DPPH • free radical into 95% ethanol (Noipa et al., 2011). 2 ml of mixed polysaccharide solution with polysaccharide of different concentrations and 2 ml of DPPH solution were added successively into a tube and mixed sufficiently and then placed for half an hour at dark place. Then, the absorbance A_i of the mixed polysaccharide solution at 517 nm was measured. The background absorbance A_j of a certain concentration of polysaccharide was measured by 2 ml of polysaccharide solution and 2 ml of 95% ethanol and the absorbance A_0 of blank control was measured by 2 ml of distilled water and 2 ml of DPPH solution. Thus, the calculation formula of clearance rate S is as follow:

$$S = \frac{A_0 - (A_i - Aj)}{A_0} \times 100\% \quad (3)$$

3. Results and discussions

3.1. Impact of microwave power on corn bran polysaccharide extraction rate

As can be seen from Figure 1, polysaccharide extraction rate increased with the increase of microwave power. When the microwave power reached 460 w, the polysaccharide extraction rate reached its maximum.

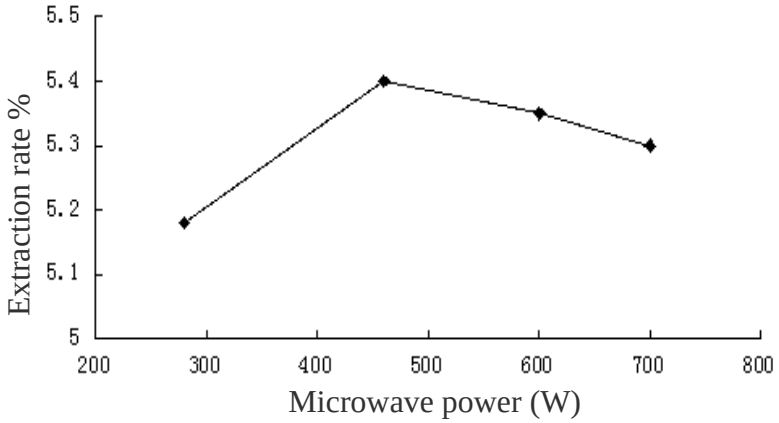

Figure 1. Impact of microwave power on corn bran polysaccharide extraction rate

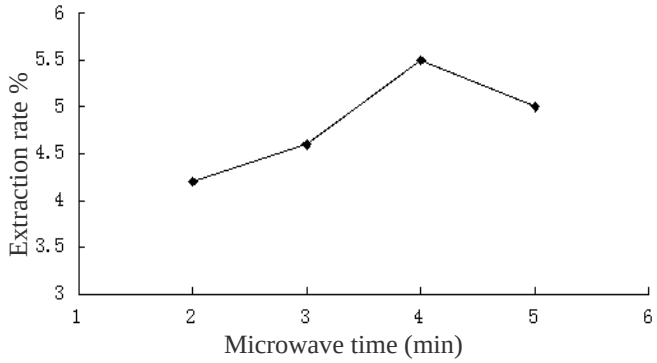

Figure 2. Impact of microwave time on corn bran polysaccharide extraction rate

However, with the continuing increase of microwave power, polysaccharide extraction rate decreased. When the microwave power was 460 w, it was in a relatively mild condition which could realize the dissolution of polysaccharide without destroying its structure. Therefore, it is considered as suitable condition when microwave power is 460 w (Cheng et al., 2015; Juanni et al., 2008).

3.2. Impact of microwave time on corn bran polysaccharide extraction rate

As can be seen from Figure 2, polysaccharide extraction rate increased with the increase of microwave time. When the microwave time reached 4 min, the polysaccharide extraction rate reached its maximum. However, with the continuing increase of microwave time, polysaccharide extraction rate decreased. When the microwave time exceeded 4 min, polysaccharide extraction rate decreased because of degradation of polysaccharide caused by saturation of solubility. Therefore, it is considered as suitable condition when microwave time is 4 min (Wlodarski et al., 2015; Bharate and Vishwakarma, 2015).

Table 3. Orthogonal experimental results of extraction of corn bran polysaccharide

Test number	Microwave power	Microwave time	liquid-to-solid ratio	Extraction temperature	Extraction time	Extraction rate %
10	3	2	4	3	1	5.1
11	3	3	1	2	4	4.7
12	3	4	2	1	3	5.2
13	4	1	4	2	3	5.4
14	4	2	3	1	4	4.9
15	4	3	2	4	1	6.0
16	4	4	1	3	2	4.5
A1	20.2	19.7	17.5	18.6	19.1	-
A2	21.6	20.2	21.1	20.1	21.3	-
A3	21.4	22.7	22.7	21.0	22.2	-
A4	20.8	21.5	22.8	24.4	21.4	-
B1	5.1	4.9	4.4	4.6	4.8	-
B2	5.4	5.0	5.3	5.0	5.3	-
B3	5.4	5.7	5.7	5.2	5.5	-
B4	5.2	5.4	5.7	6.1	5.3	-
Range (R)	0.3	0.7	1.3	1.5	0.8	-

3.3. Orthogonal experimental results of extraction of corn bran polysaccharide

As can be seen from table 3, the importance order of each factor which affects corn bran polysaccharide extraction rate is as follows: extraction temperature> liquid-to-solid ratio > extraction time > microwave time > microwave power. The best condition for corn bran polysaccharide extraction is as follows: extraction temperature: 100 °C; liquid-to-solid ratio: 1:40; extraction time: 4 hour; microwave time: 4 min; microwave power: 460w. According to the best extraction condition, corn bran polysaccharide was extracted twice and the extraction rate was 6.7% calculated by polysaccharide extraction rate formula (Müssigbrodt et al., 2015; Rahman et al., 2015).

3.4. Orthogonal experimental results of deproteinization with Sevag method

As can be seen from Table 4, the importance order of each factor which affects corn bran polysaccharide deproteinization is as follows: deproteinization frequency > V reagent: V sample > V n-butyl alcohol: V chloroform > deproteinization time. The best condition for polysaccharide deproteinization is as follows: deproteinization frequency: 4 times; V reagent: V sample: 1:2; V n-butyl alcohol: V chloroform: 1:3; deproteinization time: 25 min.

3.5. Purification results of Sephadex G-75 chromatographic column

As can be seen from figure 3, CSP Ⅰwas separated from corn bran polysaccharide through Sephadex G-75 chromatographic column. The peak pattern was close to normal distribution, suggesting its uniformity (Jie et al., 2015).

3.6. Determination of hydroxyl radical-scavenging ability

As can be seen from Figure 4, corn bran polysaccharide CSP Ⅰ had certain clearance effect on ·OH.

Table 4. Orthogonal experimental results of deproteinization with Sevag method

Test number	V reagent: V sample	V n-butyl alcohol: V chloroform	Deproteinization frequency	Deproteinization time	Protein removal rate %
1	1	1	1	1	36.4
2	1	2	2	2	41.6
3	1	3	3	3	42.7
4	2	1	2	3	44.0
5	2	2	3	1	46.7
6	2	3	1	2	38.3
7	3	1	3	2	41.5
8	3	2	1	3	35.2
9	3	3	2	1	33.9
A1	120.7	121.9	110.0	117.0	-
A2	128.9	123.5	119.5	121.5	-
A3	110.6	114.9	130.9	121.9	-
B1	40.2	40.6	36.7	39.0	-
B2	43.0	41.2	39.8	40.5	-
B3	36.9	38.3	43.6	40.6	-
Range (R)	6.1	2.9	7.0	1.6	-

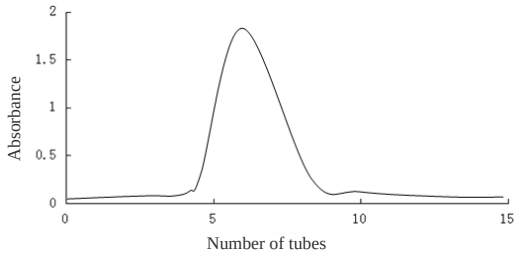

Figure 3. Sephadex G-75 elution curve of corn bran polysaccharide

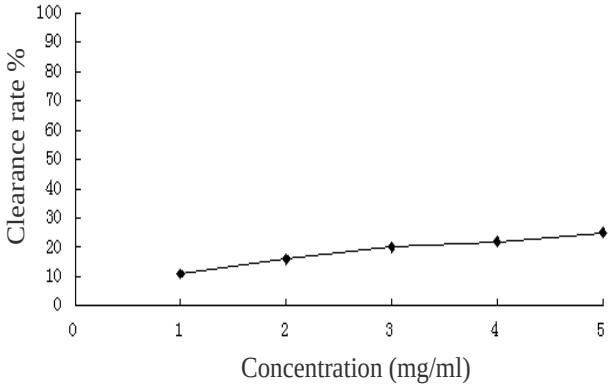

Figure 4. Hydroxyl radical-scavenging ability

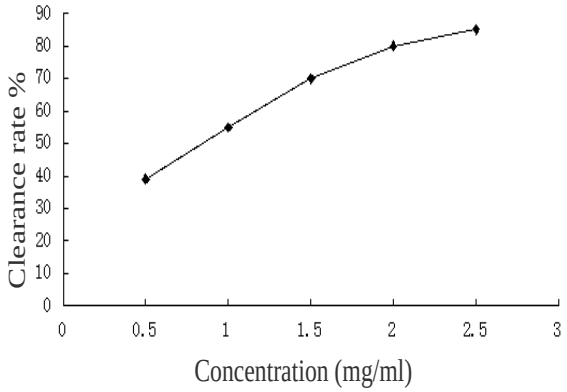

Figure 5. DPPH • free radical -scavenging ability

The clearance rate increased gradually with the increase of polysaccharide CSP concentration. However, the changes were not obvious, with the clearance rate below 50% within the range of determination (Kerry et al., 2012).

3.7. Determination of DPPH • free radical - scavenging ability

As can be seen from Figure 5, corn bran polysaccharide CSP had certain clearance effect on DPPH·. The clearance rate increased gradually with the increase of polysaccharide CSP concentration and reached 50% (Feuchtenberger et al., 2008).

4.Conclusions

Corn bran polysaccharide CSP Ⅰ had varying degrees of scavenging effect on hydroxyl radical, DPPH • radical and superoxide anion free radical, of which the scavenging effect on hydroxyl radical was not obvious. If results of this study can be applied to the actual corn industrial chain, not only can the corn bran be turned into useful resource but also the industrial value creation can be improved through the development of high value-added products, which can bring a very broad market prospect and remarkable economic benefit.

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