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Research Article
Study on Technology Optimization of Lignin Removalin Cellulose Extraction from Wheat Bran by Combination of Ultrasound and Hydrogen Peroxide

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Abstract
Background: Currently, cellulose is more closely related to people's daily life and widely used in various fields, textile industry, food, medicine and health care etc. Thus the increasing research focuses on cellulose extraction. Wheat bran, by-product of flour processing, is rich in cellulose, its cellulose extraction is conducive to the rational use of wheat bran. In wheat bran, cellulose is wrapped by lignin, semi-cellulose and pectin, which prevented it from high yield, therefore the removal of lignin, semi-cellulose and pectin benefits to the extraction efficiency. **Materials and Methods:** Taking wheat bran as raw material and using the method of ultrasonic assisted, the researchers studied the factors, affecting lignin removal, to find out the optimum conditions. Effects of hydrogen peroxide concentration, liquid-solid ratio, temperature, ultrasonic pretreatment power, reaction time on removal rate of lignin were analyzed respectively. On the basis of the above experiments of single factors, design orthogonal experiment to determine the optimum conditions of lignin removal. **Results:** Experimental results showed that removal rate of lignin reached 73.87% with the ratio of liquid to solid of 15:1, the ultrasonic treatment time of 10 h at 50°C, the power of 100 W and the hydrogen peroxide concentration of 3%. **Conclusion:** Traditionally, lignin removal was carried out by chemical reagent from straw of various kinds and wood chips, along with the environmental pollution. In this study, taking wheat bran as material by the combination of ultrasound and chemical reagent process, lignin was removed to a great degree, hence cellulose was extracted with high yield and chemical reagent experienced decreased consumption, which provided enough material for food industry, medicine industry and other areas.

Key words: Ultrasonic, hydrogen peroxide, pretreatment, wheat bran, cellulose, lignin, embedded structure, sustainable

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cellulose, renewable and widespread is the most abundant organic matter[1], therefore the application of cellulose has been given ongoing focus, which is widely used in energy, medicine, food, chemistry, etc.[2-4].

Currently, materials for cellulose extraction are limited to straw of all kinds and timber for the limitation of technology. Extraction methods are only applied in laboratory, which seldom appear in industrial production. How to break through technology barrier is the key for cellulose extraction on big scale.

Bran, the outer most layer of wheat is by-product of flour processing industry. Wheat bran consists of cellulose, lignin, semi-cellulose, starch and so on, cellulose extraction from wheat bran is beneficial to its comprehensive and rational utilization.

China is an agricultural country, providing about 20 million tons wheat bran annually. For a long period, wheat bran, rich in cellulose is used as fodder, preventing it from high-value application.

Cellulose is composed of thousands of glucose molecules, which are linked by β-1,4 glycosidic bond to form a long chain, stable structure is achieved by inter-molecular force among long chain[5,6]. In wheat bran, cellulose is embedded by plenty of semi-cellulose, lignin and pectin[7], therefore, lignin removal plays an important role in cellulose extraction.

Lignin is a natural macro-molecule polymer, which plays an important role in maintaining the integrity of plant cells. Chemical methods were introduced to remove lignin, semi-cellulose and others, materials focused on straw of all kinds and wood chips, the study on cellulose extraction from wheat bran was little.

Lignin removal is feasible by the followed methods[8,9]:

- Reaction in the neutral condition by organic solvent
- Lignin dissolution in the acidic condition by organic solvent
- Separation and extraction of lignin by inorganic reagent
- Extraction of insoluble lignin by inorganic acid and other methods

Patent DE19916347A of Germany suggested a method of lignocellulose separation from timber by which material was pretreated by water or vapor, then hydrolyzed semi-cellulose was extracted in water medium, after which lignin was removed by alkanolamine and crude cellulose was obtained[10].

Nowadays, mostly widely used technologies for cellulose extraction were double enzyme degradation method, the combination of enzyme and acid hydrolysis, acidic decomposition method and alkaline hydrolysis technology, etc.[11].

Ultrasonic wave performs well in the release, diffusion and dissolution of intracellular matter via mechanic effect, heat effect and cavitation effect by which reactions were accelerated and extraction efficiency was heightened[12].

In this study, the combination of ultrasound and chemical method was introduced to remove lignin in wheat bran, which was conductive to environmental protection by the decreased amount of chemical reagent and benefited to the comprehensive utilization of large amount of wheat bran.

MATERIALS AND METHODS

Material: Wheat bran was smashed, screened and dried for use.

Main reagent: Hydrochloric acid, glacial acetic acid, α-amylase, NaOH, petroleum ether, alkaline proteinase, α-amylase, hydrogen peroxide.

Apparatus: Granding machine, KQ5200DE ultrasonic cleaner, KDC-1044 low speed centrifuge, HWS28 electric-heated thermostatic water bath, vacuum pump, electric scales, electrically heated drying oven, etc.

Methods

Pretreatment of wheat bran

Fat and starch removal: Put 1 g wheat bran into centrifuge tube, add petroleum ether and vibrate in shaking table at 34°C by 40 rpm for 4 h, there after evaporate the solvent and add α-amylase to remove starch at 55°C for 1 h, then keep its residue after centrifugation.

Pectin and semi-cellulose removal: Put distilled water into the residue of fat and starch removal by the liquid-solid ratio of 20:1 (V m^{-1}) and adjustits pH to 1.5, react for 2.5 h at 85°C, centrifuge and keep the residue, then add 6% NaOH into the residue by the liquid-solid ratio of 20:1 (V m^{-1}) at room temperature for 20 h, here after keep the residue for the followed processing.

Protein removal: Adjust pH to 9 and add alkaline proteinase to degrade protein at 45°C for 1 h, centrifuge and keep the residue, then write down the final weight.

Lignin removal: By the combination of ultrasonic and hydrogen peroxide, lignin in wheat bran was removed by per acid system, formed by glacial acetic acid and hydrogen peroxide. Ultrasonic treatment time and power, reaction temperature, liquid-solid ratio, concentration of hydrogen peroxide are single factors affecting the efficiency of lignin removal.

Effect of hydrogen peroxide concentration on lignin removal rate: Adjust pH to 4.5-5.0 with acetic acid and add hydrogen peroxide by the liquid-solid ratio of 21:1, reaction is operated at 5°C for 8 h with the power of 100 W, concentrations of hydrogen peroxide are 3, 6, 9, 12 and 15%, respectively. When the reaction is over, centrifuge the reaction solution and dry the residue. In the final, weigh the residue and analyze the effect of hydrogen peroxide concentration on lignin removal.

Effect of reaction time on lignin removal rate: Adjust pH to 4.5-5.0 with acetic acid and add 8% hydrogen peroxide by the liquid-solid ratio of 21:1, reaction is carried out at 50°C with the power of 100 W, reaction times are 2, 4, 6, 8 and 10 h. When the reaction is over, centrifuge the reaction solution and dry the residue. In the final, weigh the residue and study the effect of reaction time on lignin removal.

Effect of temperature on lignin removal rate: Adjust pH to 4.5-5.0 with acetic acid and add 8% hydrogen peroxide by the liquid-solid ratio of 21:1, reaction lasts for 8 h with the power of 100 W, reaction temperatures are 30, 40, 50, 60 and 70°C. When the reaction is over, centrifuge the reaction solution and dry the residue. In the final, weigh the residue and analyze the effect of temperature on lignin removal.

Effect of ultrasound power on lignin removal rate: Adjust pH to 4.5-5.0 with acetic acid and add 8% hydrogen peroxide by the liquid-solid ratio of 21:1, reaction is performed at 50°C for 8 h, ultrasound powers are 80, 100, 120, 140 and 160 W. When the reaction is over, centrifuge the reaction solution and dry the residue. In the final, weigh the residue and analyze the effect of ultrasound power on lignin removal.

Effect of liquid-solid ratio on lignin removal rate: Adjust pH to 4.5-5.0 with acetic acid and add 8% hydrogen peroxide, reaction is carried out at 50°C for 8 h with the power of 100 W, liquid-solid ratios are 9:1, 12:1, 15:1, 18:1 and 21:1, respectively. When the reaction is over, centrifuge the reaction solution and dry the residue. In the final, weigh the residue and analyze the effect of liquid-solid ratio on lignin removal.

Orthogonal experiment: On the basis of single factor experiments, design orthogonal experiment to optimize conditions of lignin removal:

- Data analysis software
- Orthogonal software
- Microsoft Office Excel 2003

RESULTS AND DISCUSSION

Effect of hydrogen peroxide concentration on lignin removal: Effect of hydrogen peroxide concentration on lignin removal was demonstrated in Fig. 1. Figure 1 showed that, there existed positive correlation between lignin removal rate and low hydrogen peroxide concentration. With the continuing increase in hydrogen peroxide concentration, the result tended to decrease, which reached the uttermost with 6% hydrogen peroxide.

Peroxy acid system formed by mixing hydrogen peroxide with glacial acetic acid, which performed well in lignin removal. At first, lignin removal rate went up with the increase in hydrogen peroxide concentration, until the material was saturated by the solvent with certain concentration, which led to the complete reaction, giving rise to the top removal rate. Here after higher concentration inhibited the reaction efficiency, resulting in low result.

Effect of reaction time on lignin removal: Effect of reaction time on lignin removal was shown in Fig. 2.

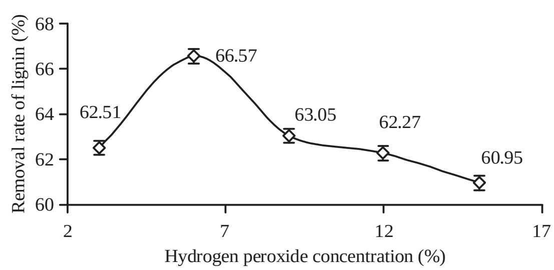

Fig. 1: Effect of hydrogen peroxide concentration on lignin removal

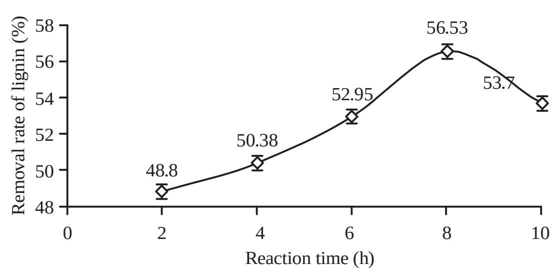

Fig. 2: Effect of reaction time on lignin removal

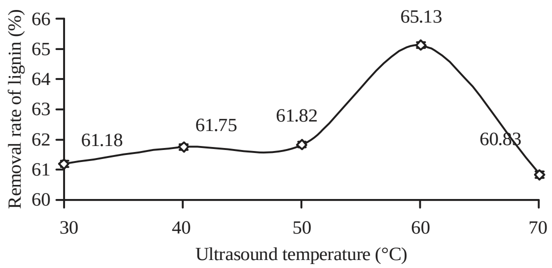

Fig. 3: Effect of reaction temperature on lignin removal

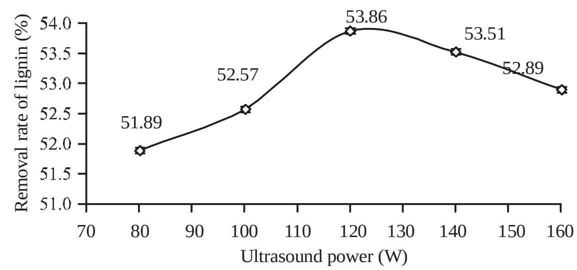

Fig. 4: Effect of ultrasound power on lignin removal

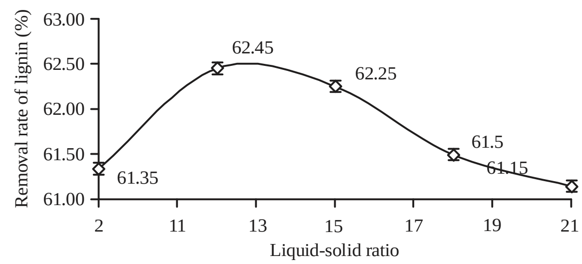

Fig. 5: Effect of liquid-solid ratio on lignin removal

Figure 2 suggested that lignin removal rate increased with the reaction time going on, which came to 56.53% by 8 h, hereafter the result decreased.

In the initial phase, lignin didn't react with peroxy acid system completely in short time. With the time continued, reaction between lignin and peroxy acid was complete, by which increasing lignin was removed. Eight hours later, by-product formed, inhibiting the reaction, therefore the result went down.

Effect of reaction temperature on lignin removal: Effect of reaction temperature on lignin removal appeared in Fig. 3. From 30-60°C with the increasing temperature, reaction rate went up, the top removal rate of 65.13% achieved at 60. When the temperature was above 60, the result dropped.

Within a certain temperature range, molecules moved fast, reaction rate was positively correlated with the increasing temperature. However high temperature led to the evaporation of solvent and the formation of by-product, which deterred the reaction rate, resulting in low lignin removal.

Effect of ultrasound power on lignin removal: Effect of ultrasound power on lignin removal was demonstrated in Fig. 4. Figure 4 showed that the lignin removal rate went up with the increasing power, which got to 53.86% when the power was 120 W, then the result went down.

In wheat bran, lignin, semi-cellulose and cellulose intertwined tightly. At the beginning, the low power didn't work well in the complete breakage of various chemical bonds, hence with the increasing power, reaction intensity strengthened, 120 W made the uttermost lignin removal rate achieved, after which the result decreased. The researchers attributed 2 aspects for the reason.

For one thing, as to the limited quantity of material, the higher ultrasound power only resulted in "Zero ultrasound effect" with energy consumption. For another, the higher power destroyed structures of semi-cellulose and cellulose, by-product appeared, preventing the continuous increase in lignin removal.

Effect of liquid-solid ratio on ligin removal: Effect of liquid-solid ratio on ligin removal was shown in Fig. 5. Figure 5 demonstrated that lignin removal rate experienced increase with the liquid-solid ratio of 9:1-12:1, after which the result decreased. Liquid-solid ratio of 12:1 made the 62.45% lignin removal rate obtained.

In the peroxy acid system, the ratio of hydrogen peroxide and acetic acid played an important role in lignin removal, high liquid-solid ratio meant too much hydrogen peroxide in the mixed solvent, its strong oxidation made side-effect accessible, weakening the reaction efficiency.

On the basis of above experiments, design orthogonal experiment to optimize reaction conditions. Arrangement and result were shown in Table 1 and 2.

As shown in Table 2, the concentration of hydrogen peroxide was the dominant factor affecting the final result, temperature ranked the second. In practice, it was essential to control the concentration of hydrogen peroxide strictly.

Intuitive analysis revealed that $A_1B_2C_2D_2E_2$ was the optimum condition for lignin removal, namely, the lignin removal rate reached 73.81% at 50°C with ultrasound power of 100 W for 6 h in the presence of 3% hydrogen peroxide, along with the liquid-solid ratio of 12:1.

Range analysis suggested that the best combination laid on $A_1B_3C_4D_2E_2$. Testified experiment represented that $A_1B_3C_4D_2E_2$ was the better, by which the result got to 73.87%, namely the combination of 3% hydrogen peroxide, liquid-solid ratio of 15:1, ultrasound power of 100 W at 50°C for 10 h gave rise to the biggest lignin removal, in contrast with that of $A_1B_2C_2D_2E_2$, 73.12%.

Table 1: Factors and levels of orthogonal experiment

Concentration of hydrogen peroxide (A)	Liquid-solid ratio (B)	Time (C)	Temperature (D)	Ultrasound power (E)
3%	9:1	4 h	40°C	80 W
6%	12:1	6 h	50°C	100 W
9%	15:1	8 h	60°C	120 W
12%	18:1	10 h	70°C	140 W

Table 2: Design and result of orthogonal experiment

	Factors					
No./Levels	A (%)	B	C (h)	D (°C)	E (W)	Lignin removal rate (%)
1	3	9:1	4	40	80	68.8
2	3	12:1	6	50	100	73.18
3	3	15:1	8	60	120	68.95
4	3	18:1	10	70	140	70.08
5	6	18:1	8	40	100	68.25
6	6	15:1	10	50	80	73.81
7	6	12:1	4	60	140	67.15
8	6	9:1	6	70	120	70.53
9	9	12:1	10	40	120	65.05
10	9	9:1	8	50	140	65.95
11	9	18:1	6	60	80	64.43
12	9	15:1	4	70	100	67.68
13	12	15:1	6	40	140	68.55
14	12	18:1	4	50	120	66.93
15	12	9:1	10	60	80	68.33
16	12	12:1	8	70	100	67.65
k1	70.25	68.40	67.64	67.66	68.84	
k2	69.93	68.26	69.17	69.97	69.19	
k3	65.78	69.75	67.70	67.22	67.87	
k4	67.87	67.42	69.32	68.99	67.93	
R	4.47	2.33	1.68	2.75	1.32	

Nowadays, research in lignin removal and extraction consists of chemical method, physical method and biological method. Chemical method depends on the combination of oxidant and catalyst or supercritical method, O_3, O_2 and H_2O_2 are widely used as oxidants.

Li *et al.*[13] studied the lignin extraction from wheat straw, it came to the conclusion that lignin degradation rate reached 72.5% under 200°C for 7 h in the presence of 0.0067 g mL^{-1} NaOH and 50% methyl alcohol. Also, lignin extraction rate was 73.7% under 120°C for 3 h by 60% 1,4-butanediol and 10% NaOH (m V^{-1})[13,14].

Wang *et al.*[15] suggested the oxidation degradation of lignin using O_2 and H_2O_2 as oxidants. Moreover, during the course, metal catalysts had to function in acid solution, alkaline solution or ionic liquid, except for metals with strong oxidation. Although, oxidants and catalysts for lignin degradation were found out gradually, low conversion efficiency and poor product selectivity prevented it from widespread utilization.

Lignin productivity of 55.7% achieved by He Wei in the presence of 80% formic acid , 15:1 (mL g^{-1}) liquid-solid ratio under 90°C for 2 h[16].

Physical method is made up of radiation, steam explosion, puffing and grinding by which lignin removal rate of 40-50% is accessible with high cost. Mixed methods of physical and chemical method are widely used to improve the extraction efficiency and reduce the cost.

Tian Yi-hong indicated the lignin yield of 35.26% from wheat straw by the incorporation of steam explosion and organic solvent at 160°C for15 min.

Research suggested that biological method for lignin removal was accessible by which various microorganisms performed well. For optimum conditions of different microorganisms varied greatly, combined actions of several microbes were essential. Whereas degradation mechanism of ligninwas explored incompletely, there existed a long way to the application of this method.

Zhang *et al.*[17] suggested that laccase, lignin peroxidase (LiP) and Mn-peroxidase (MnP) are commonly used in lignin oxidation degradation, however, heat sensitivity and instability of enzyme prevented this method from application on large scale.

Taking abundant wheat bran as material, the research studied the optimum conditions of lignin removal in cellulose extraction, in contrast with the traditional chemical method, the amount of reagent reduced, alleviating the environmental pollution. In addition, the result laid foundation for the utilization of wheat bran with high additional value, which contributed to the rapid development of cellulose industry.

CONCLUSION

China owns the output of 20 million tons wheat bran every year, using as fodder preventing it from high-value application.

In wheat bran, cellulose is embedded by lignin, semi-cellulose and pectin, which prevents it from high yield, therefore the removal of lignin, semi-cellulose and pectin benefits to the extraction efficiency.

Chemical method is introduced to remove lignin, semi-cellulose and others, materials focus on straw of all kinds and wood chips, study on cellulose extraction from wheat bran is little.

Currently, among physical methods, ultrasound is widely used in the extraction of active ingredients, which is effective in the breakage of chemical bond. Hereafter, target products release successfully, improving the production efficiency. In addition, organic solvents experience the decreased consumption in comparison with that of chemical method.

Taking wheat bran as material, by the combination of ultrasound and chemical reagent process, the researcher studied the factors affecting lignin removal to find out the optimum conditions.

Results showed that removal rate of lignin reached 73.87% with the ratio of liquid to solid of 15:1, the ultrasonic treatment time of 10 h at 50°C, the power of 100 W and the hydrogen peroxide concentration of 3%.

It was evident the technology was practicable in laboratory, which was of great importance for the further exploitation of various materials. Furthermore, in agricultural country, plenty of wastes, with little or no cost were available for cellulose extraction, which was beneficial to the industrialization of cellulose.

SIGNIFICANT STATEMENTS

China is an agricultural country, providing about 20 million tons wheat bran annually. For a long period, wheat bran rich in cellulose is used as fodder, preventing it from high-value application. Cellulose is embedded by semi-cellulose, lignin and other components, therefore, lignin removal is closely related to cellulose extraction. Chemical methods were introduced to remove lignin, semi-cellulose and others materials focused on straw of all kinds and wood chips, study on cellulose extraction from wheat bran was little. In this study, the combination of ultrasound and chemical reagent was introduced to remove lignin in wheat bran, which was conductive to environmental protection by the decreased amount of chemical reagent and benefitted to the comprehensive application of large amount of wheat bran.

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