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Mechanochemical-assisted extraction of polysaccharides from bamboo leaves and its optimized processing parameters

Dan SHEN[1], Tingyu JIN[1], Jianguo WANG[2], Xingyi ZHU[1,2]*

Abstract

Mechanochemical-assisted extraction (MCAE) method was applied to the extraction of polysaccharides from the bamboo leaves. The MCAE parameters were investigated via response surface methodology design experiments, the processing conditions were optimized as follows: milling time 30 min, liquid-solid ratio 2:1, extraction time 48 min. Under these conditions, the experimental value of (11.40 ± 0.12)% corresponded with the value predicted by the model and was higher than super fine-grinding extraction (SFGE) of (11.17 ± 0.15)% and heat reflux extraction (HRE) of (10.92 ± 0.17)%. The mechanism of mechanochemical-assisted extraction (MCAE) was discussed based on scanning electron microscope. Compared to HRE, the extraction time was significantly shortened, the water consumption and extraction temperature were also lowered. MCAE is a good choice for the efficient extraction of polysaccharides from bamboo leaves.

Keywords: mechanochemical-assisted extraction; polysaccharides; bamboo leaves; response surface methodology.

Practical Application: Mechanochemical-assisted extraction method is suitable for obtaining polysaccharides from bamboo leaves.

1 Introduction

Bamboo, a perennial woody grass belonging to the Gramineae family and Bambuseae subfamily, is widely distributed in tropical and subtropical areas over many countries. According to "Ben Cao Gang Mu", the leaves of some species of bamboo have the functions of antifungal activity and hemostasia, etc.. There are many effective components in bamboo leaves including flavonoids, phenolic acids, polysaccharides, anthraquinones, coumarins, special amino acids and peptides, etc. (Zhang et al., 2004, 2007; Lu et al., 2005, 2006). Many studies have shown that the polysaccharides isolated from natural plants have good biological activities (Li et al., 2020, 2021; Hu et al., 2021; Shin et al., 2021). It is found that the polysaccharides isolated from the bamboo leaves display antitumor activities, antioxidation, and immuno-modulating activities, etc. (Hu et al., 2000). Having in mind the broad spectrum of therapeutic properties and relatively low toxicity (Tzianabos, 2000; Paulsen, 2001; Wasser, 2002), it is significant to extract polysaccharides from bamboo leaves.

Conventional heat reflux extraction (HRE) method has been used to extract polysaccharides from plant. Although this method can obtain mostly active polysaccharides, heating processes consume lots of solvent and need long time and the yield is extremely low. These deficiencies highlight the necessity of alternative approaches. Mechanochemistry is a branch of chemistry used to describe the chemical and physicochemical transformation of substances during aggregation caused by mechanical energy (Guo et al., 2010). Mechanochemical-assisted extraction (MCAE) have been gaining popularity as a new means of dealing with extraction which carries out mechanochemical processing to the material with solid reagent to obtain mechanochemical composites before extraction in solvent (Zhu et al., 2011). MCAE has been frequently used in many fields of human activity, such as extractive metallurgy, crystal engineering, materials engineering, agriculture and pharmacy (Guo et al., 2010) and covers a wide range of important reactions, such as faster decomposition and synthesis (Tongamp et al., 2006; Heintz et al., 2007), polymorphic transformation (Lin, 1998) and plant materials treatment (Lomovsky, 2004). MCAE as an alternative extraction method has been used to extract triterpene acids from Siberian fir needles, (Korolev et al., 2003) to extract phytoecdysteroids from Serratula coronata L. (Lomovsky et al., 2003) to isolate lappaconitine from Aconitum septentrionale roots (Goncharov et al., 2006), chondroitin sulfate from shark cartilage (Wang & Tang, 2009), and isofraxidin from Eleutherococcus senticosus (Liu et al., 2007), etc.. However, the application of MACE in the extraction of polysaccharides from bamboo leaves has never been reported. Thus, it is of both theoretical and practical importance to conduct relevant research. In this study, the polysaccharides were extracted from bamboo leaves by MCAE method. The effects of the main operating parameters, namely, milling time, liquid-solid ratio, extraction time were optimized with response surface methodology (RSM). At the same time, the MCAE method was compared against conventional HRE and SFGE.

2 Materials and methods

2.1 Materials and reagents

The *moso* bamboo leaves were collected from Suichang country, Zhejiang province, China. They were dried in the oven and were stored in a dry and dark place until used. Standard

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[1] *Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Zhejiang University of Technology, Hangzhou, China*
[2] *College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, China*
*Corresponding author: zhuxingyi3141@163.com

glucose was purchased from Chinese Medical and Biological Products Institute (Beijing, China). Sodium bicarbonate and acetic acid (analytical grade) and other analytical-grade reagents were bought from Tianjin Yongda Chemical Reagent Development Center (Tianjin, China).

2.2 Mechanochemical-assisted extraction (MCAE)

The dried and coarse grinding bamboo leaves (20.0 g) were mixed with some sodium bicarbonate. Then, the mixture was added into AGO-2 high-intensity planetary activator (Russia). After grinding for several minutes, the powder with a particle size of about 200m was obtained. The powder was extracted with an appropriate volume of water at certain temperature for some time then isolated by filtration under reduced pressure. The filtrate was concentrated by rotary evaporator. The concentrated solution was acidified to pH 4.0 with acetic acid and precipitated by addition of a four-fold volume of anhydrous ethanol and then incubated at 4 ^{o}C for 24 h. The crude polysaccharides, namely the precipitate by anhydrous ethanol, were obtained by centrifugation, then lyophilized and analyzed by UV-Vis spectrophotometer test. The yield is expressed as follows: Yield (%) = m / M*100% (m, weight of polysaccharides analyzed by ultraviolet analysis (g); M, weight of bamboo leaves (g)).

2.3 Super-fine grinding extraction (SFGE)

The basic process is similar to mechanochemical-assisted extraction. The main difference is that the dried and coarse grinding bamboo leaves were added into AGO-2 high-intensity planetary activator (Russia) alone, without the sodium bicarbonate, but they are grinded for the same time. The equal amount of sodium bicarbonate was added when the powder was extracted with an appropriate volume of water at certain temperature. The rest step was the same as MCAE.

2.4 Heat reflux extraction (HRE)

According to the optimal conditions, the dry and coarse grinding bamboo leaves (20.0 g) were accurately weighed and extracted for two times under like conditions with a reflux apparatus. Each extracting solution collected together. The rest step is the same as above.

2.5 Scanning electron microscopy (SEM)

The changes in morphology of samples with different pretreatment was examined using a Hitachi S-4700 field scanning electron microscopy (Hitachi, San Jose, CA, USA) equipped with a scanning image device. Samples were fixed on a metal sheet, using double sides adhesive tape, then examined under high-vacuum condition with an accelerating voltage of 15.0 kV(50 µm, 1000*magnification).

2.6 Experiment design of RSM

On the base of single-factor experiment for the extraction of crude bamboo polysaccharides, proper ranges of $NaHCO_3$ content, milling time, extraction time, liquid-solid ratio, extraction temperature, acidified pH were preliminarily investigated. A three-level, three-factor Box–Behnken design (Trial Version 7.1.6, Stat-Ease Inc., Minneapolis, MN, USA) was employed to investigate the best combination of extraction variables for the extraction of bamboo leaves polysaccharides. Milling time (X_1), liquid-solid ratio (X_2), extraction time (X_3) were the independent variables selected to be optimized for the extraction of polysaccharides. The range of independent variables and their levels were presented in Table 1. The experimental design consisted of twelve factorial points and five replicates of the central point (Table 2). Yield of polysaccharides (%) was taken as the response for the combination of the independent variables given in Table 2. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the following equation: Xi= (X_i - X_0)/ΔX i = 1, 2, 3, where X_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the center point, ΔX is the step change. Based on the experimental data, regression analysis was performed and was fitted into an empirical second-order polynomial model $Y = A0 + \sum_{i=1}^{3} AiXi + \sum_{i=1}^{3} AiiXi2 + \sum_{i=1}^{2}\sum_{j=i+1}^{n} AijXij$, where Y is the dependent variable, A_0, A_i, A_{ii}, A_{ij} are the regression coefficients of variables for intercept, linear, quadratic and interaction terms respectively, and X_i, X_j are levels of the independent variables. They represent the linear, quadratic, and cross-product effects of the X_1, X_2, and X_3 factors on the response, respectively. The

Table 1. Independent variables and their levels used in the response surface design.

Independent variables	levels		
	-1	0	1
Milling time (X_1) (min)	10	20	30
Liquid-solid ratio (X_2)	15:01	20:01	25:01:00
Extraction time (X_3) (min)	20	40	60

Table 2. Box-Behnken experimental design with the independent variables.

Run	X_1	X_2	X_3	Yield of polysaccharide (%)	
				Actual values	Predicted values
1	1	0	1	10.87	11.03
2	0	1	-1	9.08	8.90
3	-1	-1	0	6.77	6.69
4	1	1	0	10.34	10.42
5	-1	0	1	9.42	9.32
6	0	0	0	11.07	11.32
7	0	-1	1	8.66	8.84
8	1	0	-1	8.89	8.99
9	0	0	0	11.45	11.32
10	-1	0	-1	7.51	7.35
11	0	0	0	11.22	11.32
12	-1	1	0	9.27	9.62
13	0	-1	-1	6.32	6.57
14	0	0	0	11.32	11.32
15	0	1	1	10.88	10.63
16	0	0	0	11.56	11.32
17	1	-1	0	9.58	9.23

effects of each independent variable were evaluated by the model via the response. Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (Trial Version 7.1.6, Stat-Ease Inc., Minneapolis, MN, USA), showed in Table 2. Three additional checking experiments were performed later on to verify the validity of the statistical experimental strategies.

2.7 Determination of polysaccharides content

The content of polysaccharide was quantified by the phenol-sulphuric acid method (Cuesta et al., 2003), glucose was used as standard, and the results were then expressed as glucose equivalents (XuJie & Wei, 2008).

3 Results and discussion

3.1 Fitting of the model

A 17-run BBD with three factors and three levels, including five replicates at the center point were used to fit a second-order response surface in order to optimize the extraction conditions. The five center point runs were carried out to measure the process stability and inherent variability, and the extraction yields of the polysaccharides from bamboo leaves were taken as the response. The design variables in coded units were given in Table 2 along with the predicted and experimental values of the response. Each run was performed in duplicate, and the extraction yields of the polysaccharides were the average of two sets of experiments in Table 2, whereas the predicted values of the responses were obtained from quadratic model fitting techniques by the mentioned software.

The predictive equation was obtained by fitting the experimental data to the BBD model, which represents an empirical relationship between the response (yield of polysaccharide) and the tested variables (in coded units): $Y=11.32+0.84X_1+1.03X_2+1.00X_3-0.43X_1X_2+0.017X_1X_3-0.13X_2X_3-0.95X_1^2-1.39X_2^2-1.20X_3^2$. F-test was used to check the statistical significance of regression model. The analysis of variance (ANOVA) for response surface quadratic polynomial model was shown in Table 3. The ANOVA of quadratic regression model demonstrated that the model was highly significant, as the Fisher's F-test had a very high model F-value (50.02) and a very low P-value ($P<0.0001$). This is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Goodness of the model can be proved by the determination coefficients (R^2) and the multiple correlation coefficients (R). Closer the values of R^2 to 1, better the correlation between experimental and predicted values (Pujari & Chandra, 2000). In this experiment, the value of R^2 (0.9848) means good agreement between the experimental and predicted values of the yield of crude bamboo leaves polysaccharides. The value of adj-R^2 (0.9653) implies that the total variation of 97% for the yield of crude bamboo leaves polysaccharides was due to the independent variables and only about 3% of the total variation cannot be explained by the model. The "Lack of Fit F-value" of 4.68 implies the Lack of Fit is not significant relative to the pure error. There is 8.51% chance that a "Lack of Fit F-value" this large could occur due to noise. Lack of fit is bad, so we want the model to fit.

The coefficient estimates of model equation and the corresponding P-values were showed in Table 3. P-values were used as a standard to test the significance of each coefficient, which also signified the mutual effect strength among each independent variable. Smaller the P-value is, more significant the corresponding coefficient is (Muralidhar et al., 2001). When the value of "probability > F" is less than 0.05, the model terms is significant. It can be seen from Table 3 that X_1, X_2, X_3, X_1X_2, X_1^2, X_2^2, X_3^2 are significant model terms.

3.2 Optimization of the procedure

The response surface curves were plotted to investigate the interactions of the variables and to determine the optimal level of each variable for the maximum response (Li et al., 2009). We judge the interaction effects of the variables on the responses through the 3D response surface plots, as presented in Figure 1.

Table 3. Analysis of variance of the experimental results of the BBD.

variables	Sum of squares	df	Mean square	F value	p-value prob>F
Model	43.02	9	4.78	50.32	<0.0001
X_1	5.63	1	5.63	59.48	0.0001
X_2	8.49	1	8.49	89.70	<0.0001
X_3	8.06	1	8.06	85.18	<0.0001
X_1X_2	0.76	1	0.76	8.00	0.0255
X_1X_3	1.225E-003	1	1.225E-003	0. 0.013	0.9126
X_2X_3	0.073	1	0.073	0.77	0.4092
X_1^2	3.79	1	3.79	40.01	0.0004
X_2^2	8.09	1	8.09	85.45	<0.0001
X_3^2	6.10	1	6.10	64.43	<0.0001
residual	0.66	7	0.095		
Lack of fit	0.52	3	0.17	4.68	0.0851
Pure error	0.15	4	0.037		
Cor total	43.68	16			

Note: df, degree of freedom. $p<0.0500$ means significance.

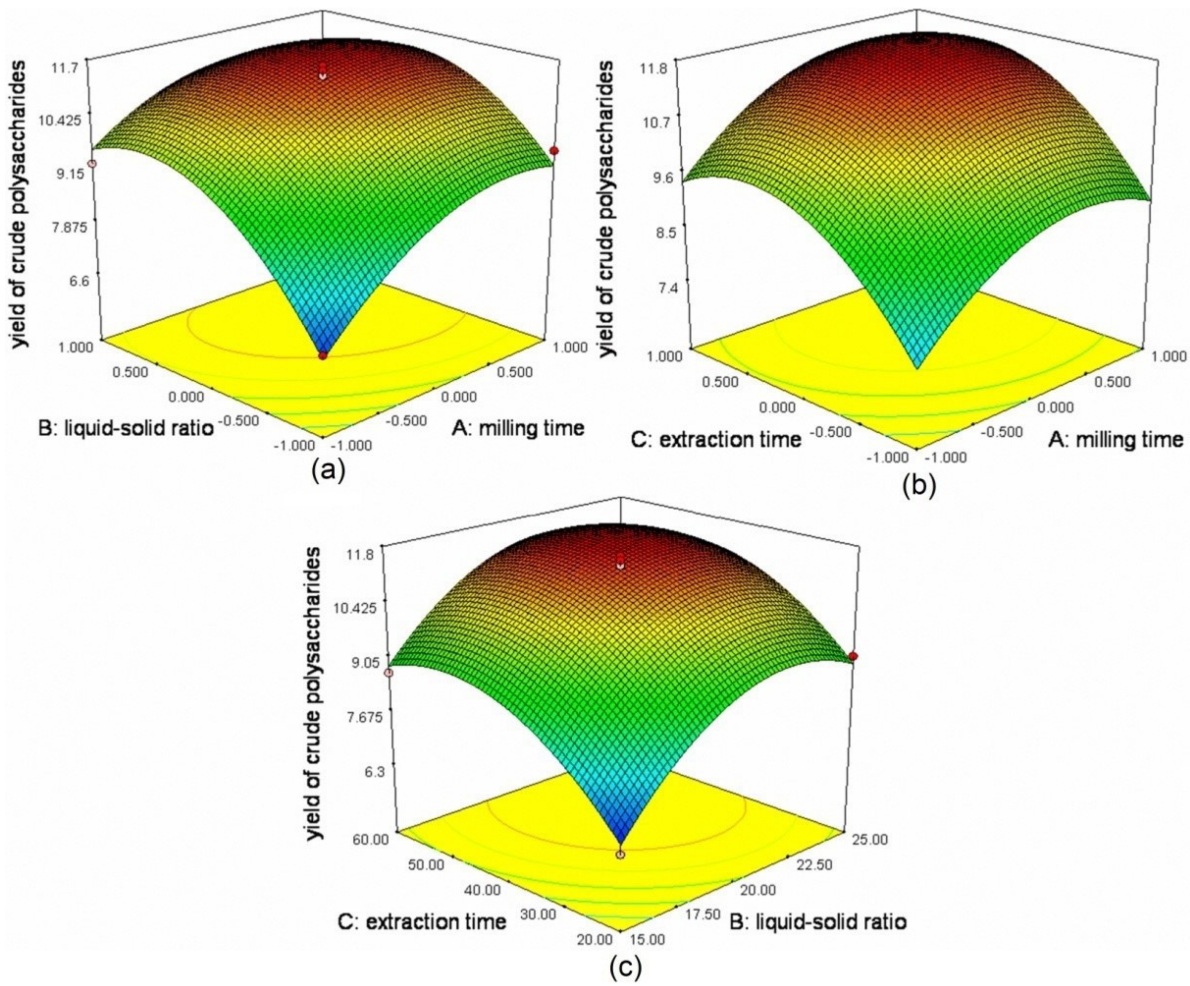

Figure 1. Response surface plots showing the effects of milling time (X_1) and liquid-solid ratio (X_2) (a), milling time (X_1) and extraction time (X_3) (b) and liquid-solid ratio (X_2) and extraction time (X_3) (c) on extraction yield of crude polysaccharides.

These types of plots revealed the effects of two variables on the response at a time. In all the presented plots, the other one variable was kept at level zero. The 3D plot in Figure 1a revealed the effect of milling time, liquid-solid ratio and their mutual interaction on extraction yield of the polysaccharides, when extraction time was fixed at 40 min. The polysaccharides yield increased when the milling time was increasing at the designed range from 10 to 30 min, indicating that temperature for the yield has a significant effect on the responses, and there was a quadratic effect on the response yield when the liquid-solid ratio was increased from 15 to 25. As shown in Figure 1b, milling time and extraction time showed a positive effect on the response yield when liquid-solid ratio was fixed at 20. There was a significant increase in the yield of polysaccharides with increase in the milling time and extraction time. Obviously, compared to the extraction time, the milling time displayed a greater influence to the yield of the polysaccharides. The yield increased slowly with the increasing of the extraction time. Likewise, Figure 1c showed that the polysaccharides yield increased with increase in the liquid-solid ratio and the extraction time.

By employing the software Design-Expert, we obtained the optimal conditions (X_1=30 min, X_2=20.97 min, X_3=48.27 min) for the extraction of the crude bamboo leaves polysaccharides. The theoretical polysaccharides extraction yield under the optimal conditions was 11.4835%. Taking account of the operated convenience, the optimal conditions were revised as follows: milling time 30 min, liquid-solid ratio 2:1, extraction time 48 min.

3.3 Validation of the model

In order to verify the prediction from the model, we do three independent replicates for the polysaccharides extraction under the revised conditions. The mean extraction yield for the polysaccharides was 11.40 ± 0.12%. Having a good agreement with the predicted value of the model equation, it was confirmed that the response model was adequate and accurate for the optimization.

3.4 Comparison of MCAE with SFGE and HRE

The yield and content of polysaccharides by different extraction methods (MCAE, SFGE, HRE) were compared under the respective optimal conditions (Table 4). The yield and content of polysaccharides using MCAE were higher than that using SFGE and HRE. The total extraction time of MCAE

Table 4. Comparison of MCAE with SFGE and HRE.

Method	MCAE	SFGE	HRE
Solvent	Water	Water	Water
Extraction time (min)	48	48	120
Extraction temperature (°C)	60	60	80
Liquid-solid ratio (mL/mg)	21	21	25
Extraction times	1	1	2
Yield (%) (g/g)	11.40 ± 0.12	11.17 ± 0.15	10.92 ± 0.17
Content of polysaccharides (%)	31.12 ± 0.19	29.87 ± 0.23	30.11 ± 0.14

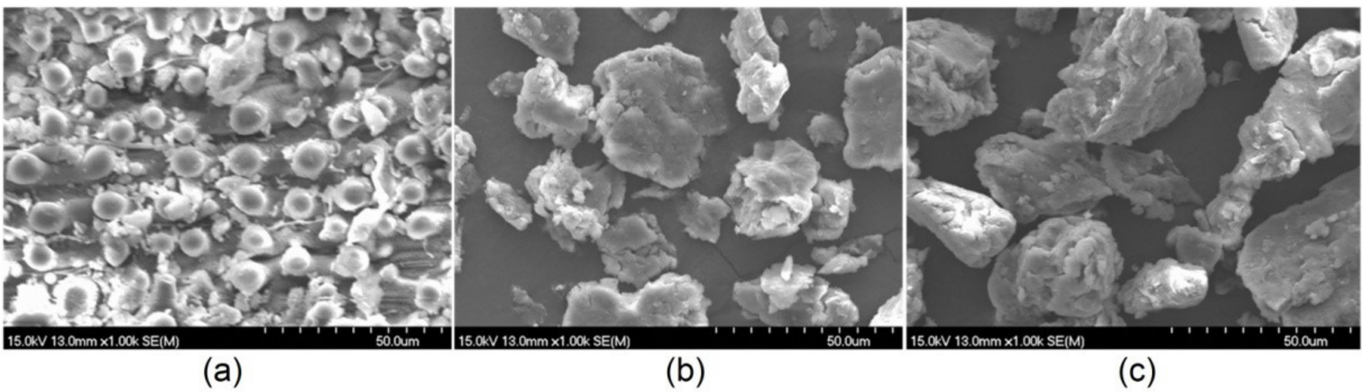

Figure 2. SEM micrographs of bamboo leaves after different treatments: (a) sample pretreated by a HX-200 pulverizer; (b) sample ground in AGO-2 planetary activator for 30 min; (c) sample co-ground with $NaHCO_3$ (5.0%, w/w) in AGO-2 planetary activator for 30 min; Each was magnified 1000 times.

was far less than HRE and the water consumption was also less than HRE. MCAE was superior to SFGE both in the yield and content, indicating that during superfine grinding extraction, mechanical activation by physically mixing sodium bicarbonate could not induce the formation of a water-soluble form of the polysaccharides immediately. Hence, it was worth noting that MCAE was a good alternative to extract the bamboo leaves polysaccharides.

3.5 Possible mechanism of the MCAE

It is well known that the extractability is affected by the sorts and amounts of the solvent, temperature, extraction time, the size of the particle and the chemical forms of the target compounds. In order to explore the mechanism of action and explain the better effect of MACE, bamboo leaves samples were probed by SEM to observe the morphological changes of samples using different extraction methods. Figure 2a showed a micrograph of coarse grinding bamboo leaves. From this, there is compelling evidence that the primary structures were not affected for a intact plant tissue and highly rough surfaces. However, from the Figure 2b and Figure 2c, the particle size was smaller obviously and no intact cell was observed, indicating a possibly complete destruction of the cell wall after the mechanical activation. There were still some differences between Figure 2b and Figure 2c. The Figure 2c showed a detailed image of bamboo leaves milled with $NaHCO_3$ for 30 min illustrated that the broken plant issue had a more coarse surface revealing that the intensity of the destruction of the cell wall was greater. The difference of the surface and the higher yield suggested mechanical activation effects on intracellular materials, presumably by increased exposure of target compounds to chemicals during destruction of the plant cell wall and enhanced potential chemical modifications happened and under the combined mechanical action of pressure and shift (Liu et al., 2007). So, because of the existence of the $NaHCO_3$, the chemical forms of the polysaccharides may change and become more water-soluble. So the cell contents pretreated by MCAE were probably released easier and faster than pretreated by SFGE and HRE and the effects will also better.

The particle size is one of the most important factors influenced the extractability. The cell wall was completely broken by the strong squeezing force and the shearing force through mechanical treatment, resulting in an increase in specific surface area. The solvent used during extraction entered the cell membrane easily to contact the target substances through various gaps and crannies, then the target compounds flowed into the extraction solvent. So the extraction rate and the yield were all enhanced. The reason for the enhancement of the yield of the polysaccharides via MCAE may be explained as the particle size reduction, cell wall breakage and chemical form transformation of the polysaccharides.

4 Conclusions

In this study, the parameters of the MCAE of the crude polysaccharides from bamboo leaves were optimized by the RSM. Under the optimized conditions, we achieve the highest yield and content of polysaccharides compared with the SFGE and HRE. The mechanism of this extraction was discussed based on SEM. According to the results, we can conclude that the main advantages of MCAE include the increased extraction yield, reduced solvent consumption, less extraction time, and lower extraction temperature. In a word, the MCAE is a good choice for the efficient extraction of polysaccharides from bamboo leaves.

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