Combination of Ultrasound with Ammonification Pretreatment for the Fiber Composition of Corn Straw: a Predictive Model by Response Surface Methodology

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Abstract. Corn straw can be used as feed for ruminant. However, because of the fiber composition of corn straw, it is difficult for ruminant to digest and utilize corn stalk. The application of ultrasound in the fiber composition of corn straw under ammonification pretreatment was investigated in this study. Response surface methodology to the development of a predictive model for the content of various kinds of fiber in ultrasonic ammonification-assisted corn straw degradation was established. Effects of ultrasonic power, ultrasonic time, liquid-to-solid ratio and sound power density in the container were investigated. In addition, this study optimized the process parameters using response surface methodology in order to facilitate ruminant digestion and utilization of corn straw. Optimal conditions were following: ultrasonic power was 85 W; ultrasonic time was 16 min, liquid-to-solid ratio was 15:1 and sound power density in the container was 1.9 W/mL.

1. Introduction
Corn straw is a common and generous agricultural waste in China [1]. It is estimated by the National Development and Reform Commission that 0.83 billion (83 million) tons of crop straw are produced annually in China [2,3]. How to utilize effectually is one of the urgent issues of our times. Nowadays, some countries use corn straw as ruminant feed resources such as cattle and sheep. Such practice gives the serious problem that livestock competes food with people a reprieve. However, corn straw contains an abundant lignocellulosic biomass, including cellulose, hemicellulose, lignin and crude ash. For these characteristics of corn straw, it is hard for ruminant to digest and utilize [4].

In recent studies, ammonia treatment has been reported that it can destroy the morphological structure of corn straw so that the lignocellulose can be used and converted to fermentable sugars more effectively [5], the ultrasonic treatment of lignocellulose can aid in cleaving lignocellulose, decrease crystallinity of lignocellulose and increase surface area [6]. This paper, for the first time, developed a predictive model on the fiber composition of corn straw under ultrasound with ammonification pretreatment. The experimental data obtained were used for the optimization of the process parameters by means of response surface methodology (RSM) with a Central-Composite experimental design.

2. Experimental Procedure and Conditions

2.1. Corn straw preparation
The corn straw was harvested by the Northeast Agricultural University Experimental Field in Heilongjiang Province in China. The corn straw contains 0.54% lignin, 31.98% cellulose, and 20.75% hemicellulose. After being collected, corn straw was dried in the sun.

2.2. Extraction procedure and measurement
Nutrient composition of the raw and treated corn straw was analyzed at Cumberland Valley Analytical Services Inc. (MD, USA). Acid detergent fiber (#973.18), and ash (#942.05) were determined as outlined by the AOAC. Acid detergent fiber (ADF) and neutral detergent fiber were analyzed using a heat stable amylase and expressed inclusive of residual ash (NDF) as described by PJ Van Soest, JB Robertson and BA Lewis [7,8]. The calculation of hemicellulose content and lignin content is shown in formula (1) and (2).

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\text{Hemicellulose content (%) = NDF - ADF} \quad (1)
\]

\[
\text{Lignin content (%) = ADF - cellulose - ash} \quad (2)
\]

2.3. Design of experiments using response surface methodology (RSM)
Four process parameters: ultrasonic power (W), ultrasonic time (min), liquid-to-solid ratio, and sound power density in the container (W/mL), each factor selects five levels which coded as +2, +1, 0, −1, and −2 for higher, high, intermediate, low, and lower values, respectively. In this study, a random order of 31 runs was generated by Design-Expert trial version 8.0.6.

3. Results and Discussion

3.1. Statistical analysis and the model fitting
In these equations, \( X_1, X_2, X_3, \) and \( X_4 \) were the values of ultrasonic parameters, Lignin, cellulose and hemicellulose were used as valuation indicators. Response surface experimental design scheme and results are shown in table 1 below:

| Run | \( X_1 \): Ultrasonic power (W) | \( X_2 \): Ultrasonic time (min) | \( X_3 \): Liquid-to-solid ratio | \( X_4 \): Sound power density (W/mL) | Lignin content (%) | Cellulose content (%) | Hemicellulose content (%) |
|-----|---------------------------------|---------------------------------|---------------------------------|-------------------------------------|-------------------|----------------------|------------------------|
| 1   | 75 (-1)                         | 10 (-1)                         | 20 (+1)                         | 2.1 (+1)                            | 0.50              | 37.46                | 24.32                  |
| 2   | 75 (-1)                         | 20 (+1)                         | 20 (+1)                         | 2.1 (+1)                            | 0.49              | 38.11                | 26.54                  |
| 3   | 105 (+1)                        | 10 (-1)                         | 10 (+1)                         | 2.1 (+1)                            | 0.48              | 38.71                | 25.13                  |
| 4   | 75 (-1)                         | 10 (-1)                         | 10 (-1)                         | 2.1 (+1)                            | 0.50              | 36.66                | 23.24                  |
| 5   | 75 (-1)                         | 20 (+1)                         | 20 (+1)                         | 1.5 (-1)                            | 0.50              | 37.98                | 24.81                  |
| 6   | 120 (+2)                        | 15 (0)                          | 15 (0)                          | 1.8 (0)                             | 0.48              | 40.58                | 25.89                  |
| 7   | 90 (0)                          | 15 (0)                          | 15 (0)                          | 1.2 (-2)                            | 0.47              | 38.63                | 26.24                  |
| 8   | 105 (+1)                        | 10 (-1)                         | 20 (+1)                         | 1.5 (-1)                            | 0.48              | 38.83                | 25.43                  |
| 9   | 90 (0)                          | 15 (0)                          | 15 (0)                          | 1.8 (0)                             | 0.46              | 39.11                | 27.30                  |
| 10  | 60 (-2)                         | 15 (0)                          | 15 (0)                          | 1.8 (0)                             | 0.52              | 35.89                | 21.50                  |
| 11  | 90 (0)                          | 5 (-2)                          | 15 (0)                          | 1.8 (0)                             | 0.50              | 37.81                | 24.04                  |
| 12  | 105 (+1)                        | 20 (+1)                         | 20 (+1)                         | 1.5 (-1)                            | 0.47              | 39.75                | 27.29                  |
| 13  | 90 (0)                          | 25 (+2)                         | 15 (0)                          | 1.8 (0)                             | 0.46              | 39.67                | 27.16                  |
| 14  | 75 (-1)                         | 10 (-1)                         | 20 (+1)                         | 1.5 (-1)                            | 0.51              | 37.15                | 23.31                  |
| 15  | 90 (0)                          | 15 (0)                          | 15 (0)                          | 1.8 (0)                             | 0.47              | 38.52                | 27.48                  |
| 16  | 105 (+1)                        | 10 (-1)                         | 10 (-1)                         | 1.5 (-1)                            | 0.49              | 38.54                | 24.91                  |
Multiple regression analysis of the experimental data afforded the following quadratic response surface models for predicting lignin, cellulose, and hemicellulose content based on the values of the ultrasonic extraction parameters (i.e., $X_1$, $X_2$, $X_3$, and $X_4$).

Lignin content ($\%$) = $1.28839 - 0.010857X_1 - 0.010536X_2 - 6.70238 \times 10^{-4}X_3 - 0.16508X_4 + 5.56878 \times 10^{-3}X_1^2 + 3.0119 \times 10^{-4}X_2^2 + 2.0119 \times 10^{-4}X_3^2 + 0.041997X_4^2$  \hspace{1cm} (3)

Cellulose content ($\%$) = $21.9359 + 0.22133X_1 + 0.08075X_2 + 0.17446X_3 + 0.42639X_4 - 8.47062 \times 10^{-5}X_1^2 + 3.87356 \times 10^{-4}X_2^2$  \hspace{1cm} (4)

Hemicellulose content ($\%$) = $-0.9047 + 0.9397 + 0.9047 + 0.9397$  \hspace{1cm} (5)

The fitted quadratic surface models for lignin, cellulose, and hemicellulose content by ANOVA are shown in Table 2. After optimization of each model, the determination coefficients of lignin content, cellulose content and hemicellulose content were respectively 0.9047, 0.9397 and 0.958, it shows that the model can explain the change of response value well, and the predicted value has a high correlation with the actual value.

**Table 2.** Significance analysis of regression equation

| Variable | df | Sum of squares | F    | P     | Sum of squares | F    | P     | Sum of squares | F    | P     |
|----------|----|----------------|------|-------|----------------|------|-------|----------------|------|-------|
| Model    | 14 | 0.012          | 10.85| <0.0001| 33.57          | 34.38| <0.0001| 85.34          | 26.07| <0.0001|
| $X_1$    | 1  | $3.75 \times 10^{-3}$ | 48.14| <0.0001| 25.61          | 367.21| <0.0001| 21.77          | 93.12| <0.0001|
| $X_2$    | 1  | $1.35 \times 10^{-3}$ | 17.33| 0.0007| 3.91           | 56.11| <0.0001| 13.05          | 55.83| <0.0001|

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### Results of Comparison of Ultrasonic and Non-Ultrasonic Parameters

| Parameter | Value | p-value |属实 | Fitted | Pure Error | Total |
|-----------|-------|---------|------|--------|------------|-------|
| X₁ | 2.67×10⁻³ | 3.42 | 0.0828 | 2.04 | 29.2 | <0.0001 | 8.52 | 36.44 | <0.0001 |
| X₂ | 4.17×10⁻³ | 5.35 | 0.0344 | 0.39 | 5.63 | 0.0305 | 1.27 | 5.43 | 0.0332 |
| X₁X₂ | 3.47×10⁻¹⁸ | 4.45×10⁻¹⁴ | 1 | 3.91×10⁻⁰³ | 0.056 | 0.8159 | 0.016 | 0.067 | 0.7993 |
| X₁X₃ | 2.50×10⁻⁶⁶ | 0.32 | 0.5789 | 9.51×10⁻⁰³ | 0.14 | 0.7168 | 4.23×10⁻⁰³ | 0.018 | 0.8947 |
| X₁X₄ | 2.50×10⁻⁶⁶ | 0.32 | 0.5789 | 0.059 | 0.84 | 0.3721 | 0.77 | 3.27 | 0.0892 |
| X₂X₃ | 2.50×10⁻⁶⁶ | 0.32 | 0.5789 | 1.56×10⁻⁰⁴ | 2.24×10⁻⁰³ | 0.9628 | 1.17 | 4.99 | 0.0401 |
| X₂X₄ | 2.50×10⁻⁶⁶ | 0.32 | 0.5789 | 2.26×10⁻⁰³ | 0.032 | 0.8595 | 0.42 | 1.81 | 0.1976 |
| X₃X₄ | 3.47×10⁻¹⁸ | 4.45×10⁻¹⁴ | 1 | 0.016 | 0.23 | 0.6358 | 6.40×10⁻¹² | 0.027 | 0.8707 |
| X₁² | 4.49×10⁻⁰³ | 57.63 | <0.0001 | 1.2 | 17.19 | 0.0008 | 30.2 | 129.1 | 4 |
| X₂² | 1.62×10⁻⁰³ | 20.81 | 0.0003 | 0.18 | 2.52 | 0.1316 | 8.69 | 37.18 | <0.0001 |
| X₃² | 7.23×10⁻⁰⁴ | 9.29 | 0.0077 | 0.35 | 5.05 | 0.0391 | 2.22 | 9.51 | 0.0071 |
| X₄² | 4.09×10⁻⁰⁴ | 5.24 | 0.0359 | 0.13 | 1.92 | 0.1846 | 5.17 | 22.1 | 0.0002 |
| Residual | 1.25×10⁻⁰³ | | | 1.12 | | | 3.74 | |
| Lack of Fit | 10 | 6.75×10⁻⁰⁴ | 0.71 | 0.6998 | 0.9 | 2.57 | 0.1304 | 2.91 | 2.09 | 0.1906 |
| Pure Error | 6 | 5.71×10⁻⁰⁴ | | 0.21 | | | 0.84 | |
| Total | 30 | 0.013 | 34.68 | | | | 89.08 | |

### 3.2. Effects of Process Parameters on Lignin, Cellulose and Hemicellulose

As shown in Figure 1a, ultrasonic treatment with high power for long time is helpful to break the chemical bond of lignin [4-6]. Meanwhile, chemicals with formula weight rapidly volatilize resulting in pressure, promoting the production of cracks and holes on lignin fiber, and then promoting lignin degradation. However, the smaller particles of lignin degradation would not continue to decompose when the ultrasonic power exceeds the limit value [9]. Effects of process parameters on cellulose were shown in Figure 1b, when the liquid-to-solid ratio and sound power density in container were fixed, the cellulose content increased as ultrasonic power and ultrasonic time increased. It was shown in ultrasonic treatment with high power for long time causes strong cavitation, resulting in lignin broken and cellulose release from lignin. Therefore, ultrasonic parameters at high level were advantageous to cellulose extraction [10,11], the hemicellulose content increased with the increase of ultrasonic time has been shown in Figure 1c, the hemicellulose content was positively correlated with ultrasonic power when ultrasonic power was lower than 97 W, and was slightly decreased when ultrasonic power was higher than 97 W.
Figure 1. Effects of process parameters on lignin, cellulose and hemicellulose

3.3. Optimization of process parameters and validation of the model
Process optimization was conducted on the Design-Expert trial version 8.0.6 using the “response optimizer” function. Preferably ruminant feed defined as low lignin and cellulose content, and high hemicellulose. Within the tested experimental ranges, best combinations of test parameters were listed below: ultrasonic power was 85W; ultrasonic time was 16 min, liquid-to-solid ratio was 15:1, and sound power density in the container was 1.9W/mL. The verification experiments were carried out under this condition, the results show that the average on Lignin content was 0.46%, compared with the model prediction, the error range was less than 10.24%, the Cellulose content was 38.59%, the error range was less than 8.86% and Hemicellulose content was 27.23%, the error range was less than 12%.

4. Conclusion
In this study, the improvement of fiber composition of corn straw is based on ultrasonic cavitation and ammonification pretreatment. This study employed response surface methodology to develop a predictive model for fiber composition in ultrasound with ammonification pretreated corn straw, and
the results of the verification experiments was found to be in good agreement with the values predicted by the RSM model. So that, RSM can be successfully applied to modeling and optimizing the ultrasonic process with ammonification pretreatment. In addition, different ultrasonic parameters on lignin, cellulose, and hemicellulose play an important role in improving fiber composition. Results from this work would also provide useful information to utilize ultrasound in application of corn straw in ruminant feed.

Acknowledgements
This work was funded by Major Research Project of Heilongjiang provincial department of education science and technology research project (12531032).

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