Research Article

Effects of Ultrasonic-Microwave-Assisted Technology on Hordein Extraction from Barley and Optimization of Process Parameters Using Response Surface Methodology

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We investigated the process intensification of ultrasonic-microwave-assisted technology for hordein extraction from barley. Response surface methodology was utilized to optimize the extraction conditions and to analyze the interaction between four selected variables: temperature, microwave power, ultrasonic power, and extraction time. The validated extraction yield of hordein reached 8.84% at 78°C, microwave power 298 W, and ultrasonic power 690 W after 20 min as optimum conditions. Compared with traditional water-bath extraction (4.7%), the ultrasonic-microwave-assisted technology effectively increased the hordein extraction yield and shortened the extraction time. According to the obtained quadratic model ($R^2 = 0.9457$), ultrasonic power and extraction time were the first two significant factors. However, temperature limited the effects of other factors during extraction. SDS-PAGE and scanning electron microscopy were used to identify the hordein extract and to clarify the difference between the two hordein fractions extracted with new and traditional methods, respectively. Ultrasonic-microwave-assisted technology provided a new way to improve hordein extraction yield from barley and could be a good candidate for industrial application of process intensification.

1. Introduction

Hordein, as the major storage protein in barley, has attracted wide attention due to its hydrophobicity, antioxidant activity, and electrospinnability [1]. Compared with other prolamins (gliadin, secalin, and avenin), hordein is distinctive for high contents of nonpolar and hydrophobic residues and low levels of charged amino acids [2, 3]. This typical amino acid composition of hordein leads to multifunctional properties, which have been investigated in various applications. For example, continuous ultrathin fibers with nanoscale diameters were successfully prepared from hordein in acetic acid with the unfolded molecular structure and flexible conformation. In order to enhance the mechanical strength of the nanofibers, a novel assembled prolamins nanofabric which was prepared by incorporating

compact zein nanoparticles into electrospun hordein networks had been obtained [4, 5]. Hordeins are commonly subdivided by molecular weights into B-hordein (70%–80%), C-hordein (10%–20%), D-hordein, and γ -hordein (1%–5%) [6]. Numerous studies have revealed that the unique sequence partitioning of hordein may produce peptides with ferrous ion-chelating capacity or powerful antioxidant bioactivities [7]. Barley has widely been used for beer brewage, whereas considerable B-hordein and C-hordein, as the main composition of hordein protein, would remain in the spent grain after malting and brewing at high temperature [8, 9]. Therefore, it is possible to introduce the processing intensification method to improve hordein extraction yield without breaking protein molecular conformation. To our knowledge, ethanol extraction is a traditional method for extracting hordein from barley. The majority of

hordein can be extracted in the first 2 h, and the protein yield increases by 2–3% between 2 and 4 h [10]. Wang et al. have investigated several parameters, such as water to material ratio, pH, and temperature. They also found that, compared with the effects of different protein isolation methods, cold precipitation gave a higher hordein content than rotary evaporation. Up to now, highly efficient extraction method for hordein is still needed.

Microwave- and ultrasonic-assisted extractions are new process intensification technologies [11]. Microwave can effectively shorten the extraction time by intensifying heat transfer in liquid-solid system and accelerate substance dissolution from solid materials. Nevertheless, because of the heterogeneous mixture system, microwave radiation may give rise to spot heating exceedingly. Ultrasonic wave can clean the surface of solid particles in solvents by producing impact forces and lead to solid particles break or collapse as a result of instant high temperature and pressure produced by the cavity effect [12]. Barley hordein has no changes after boiling in the brewing process owing to some hordein subunits are more resistant to proteolysis and heating [13]. Therefore, heat resistance properties of hordein provide a microwave-assisted method a chance to improve protein extraction yield without irreversible degradation and denaturation.

The ultrasonic-microwave-assisted technology (UMAT) takes full advantage of the high-energy effect of microwave and ultrasonic cavitation to overcome some shortcomings of conventional extraction processes [14, 15]. Operating power and time of UMAT play significant roles in enhanced extraction process. In this study, a response surface methodology (RSM) considering ceiling temperature, microwave power, ultrasonic power, and extraction time was employed to evaluate the effects of these parameters on hordein extraction. A 5-level-4-factor central composite experimental design (CCD) was obtained. Besides, we analyzed the characteristics of two hordein fractions under different extraction conditions.

2. Materials and Methods

Barley flour (Shangdong Province, China) was purchased from a local supermarket. After defatting with hexane (10:1 v/w) at 25°C, barley flour was centrifuged at 7,000 ×g for 30 min and then air-dried. The defatted barley flour was treated with 1 M NaCl solution (10:1 v/w) for 1 h to remove albumin, globulin, and glutelin. This step was repeated twice and then washed by deionized water 3 times (1 h each time) to eliminate the disturbance of reagents and small molecule materials, and centrifuged at 7,000 ×g for 30 min at room temperature. Finally, the resulting precipitate was dried in a vacuum oven at 45°C for over 36 h, and the moisture was 13.37% of material weight. The weight losses of barley flour during defatting, salt dissolution, and water washing were 0.6%, 2.7%, and 2.2%, respectively. Anhydrous ethanol (>99%) and hexane (>97%) were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the other relevant reagents were of analytic grade.

The response surface and contour plots showed in this paper were produced by Design Expert version 8.0.5b

software without any change. The scanning electron microscope images and electrophoresis of two hordein fractions were marked by photoshop software. The diameters distribution of hordein protein was conducted by Oringing 8.0 software.

2.1. Ultrasonic-Microwave-Assisted Extraction of Hordein.

Hordein was extracted in an ultrasonic-microwave extractor. Briefly, 20 g of dried powder was transferred into a 250 ml beaker, and 200 ml of 55% (v/v) ethanol solution was then added. To decrease solvent volatilization during extraction, each beaker was sealed with parafilm. The extractor was self-assembled with a (2450 ± 50) MHz microwave oven and a 25 kHz ultrasonic processor. Microwave passed through the beaker to irradiate the pretreated sample. Once the extraction temperature exceeded the settled ceiling temperature, a platinum temperature sensor sent feedback to the temperature control system, thereby temporarily stopping microwave irradiation. Ultrasonic wave was transferred from the conical transducer assembled at the top of the oven shell via a hole. The microwave power was set from 1 to 1000 W, and the ultrasonic power was set by percentage of maximum output power (800 W) for each 10 s within 6 s pause. The experiment was duplicated twice, and the average extraction yield of hordein was taken as the response. After treatment with UMAT, resulting mixture was centrifuged (8,000 ×g for 30 min) to collect the supernatant. The yield of hordein was determined by UV-visible spectroscopy at 226 nm. Hordein fraction with 92.3% purity was used as the standard protein. The yield of barley hordein based on the dry weight of barley, defined in (1), was a valuable index for evaluating the extraction process.

$$\text{YBH}(\%) = \frac{C_H(\text{g/ml}) \times V(\text{ml})}{M_o(\text{g}) \times 1000} \times 100\%, \quad (1)$$

where C_H is the concentration of hordein in the supernatant detected by UV-visible spectroscopy at 226 nm, V is the volume of supernatant in each experiment, and M_o is the dry weight of barley flour calculated according to the weight loss.

2.2. Traditional Extraction of Hordein.

Hordein was extracted in a thermostatic water bath, 20 g of dried powder was transferred into a 250 ml beaker, and then 200 ml of 55% (v/v) ethanol solution was added into the beaker with stirring for 2 h at 65°C. To decrease solvent volatilization during extraction, each beaker was sealed with parafilm. After treatment with water-bath method, resulting mixture was centrifuged (8,000 ×g for 30 min) to collect the supernatant. The follow-up processing was same with the UMAT method mentioned before.

2.3. Experimental Design.

A 5-level-4-factor CCD was performed to optimize the hordein extraction process, and the selected variables with coded/uncoded levels are presented in Table 1. All 31 experimental conditions and results are shown in Table 2. Seven axial points were used to estimate the pure error sum of squares. The fitted polynomial

TABLE 1: Independent variables and their levels used for the CCD.

Variables	Symbols	Level				
		−2	−1	0	1	2
Temperature (°C)	X_1	45	55	65	75	85
Microwave power (W)	X_2	185	220	255	290	325
Ultrasonic power (W)	X_3	560	600	640	680	720
Extraction time (min)	X_4	12	15	18	21	24

TABLE 2: Experimental design and results of the yield of barley hordein.

Runs	X_1	X_2	X_3	X_4	Yield (%)
1	55 (−1)	290	680 (1)	21 (1)	8.15
2	55	220 (−1)	600 (−1)	15 (−1)	6.7
3	45 (−2)	255 (0)	640 (0)	18 (0)	7.12
4	75 (1)	220	680	15	8.3
5	65 (0)	325 (2)	640	18	8.6
6	75	290 (1)	680	15	8.4
7	65	255	640	24 (2)	8.45
8	75	220	680	21	8.45
9	65	255	640	18	8.5
10	65	255	720 (2)	18	8.6
11	65	255	640	18	8.31
12	55	290	680	15	7.5
13	75	220	600	15	7.16
14	65	255	640	18	8.2
15	85 (2)	255	640	18	8.45
16	75	290	600	21	8.4
17	65	255	640	18	8.45
18	55	290	600	15	6.9
19	55	220	680	15	7.3
20	75	290	600	15	7.3
21	65	255	640	18	8.29
22	65	255	640	12 (−2)	7.2
23	65	255	560 (−2)	18	7.12
24	55	220	680	21	7.8
25	75	290	680	21	8.8
26	65	185 (−2)	640	18	7.5
27	65	255	640	18	8.18
28	65	255	640	18	8.2
29	75	220	600	21	7.92
30	55	220	600	21	7.4
31	55	290	600	21	7.9

equation was expressed as surface and contour plots to visualize the relationship between the response and experimental levels of each factor and also to optimize the experimental conditions. Design Expert version 8.0.5b was used to fit the experimental data to a quadratic polynomial equation to obtain coefficients of the following equation:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i x_i + \sum_{i=1}^4 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} x_i x_j, \quad (2)$$

where Y is the response, X_i and X_j are the coded independent variables, β_0 is the constant, β_i is the linear-term coefficient, β_{ii} is the quadratic-term coefficient, and β_{ij} is the cross-term coefficient. Analysis of variance (ANOVA) was performed (Table 3), and $P < 0.05$ was considered statistically significant.

TABLE 3: Variance analysis of the regression model.

Source	Sum of squares	Degree of freedom	Mean square	F value	P value
Model	10.33	14	0.74	38.35	<0.0001
X_1	2.50	1	2.50	129.68	<0.0001
X_2	0.85	1	0.85	44.23	<0.0001
X_3	2.65	1	2.65	137.85	<0.0001
X_4	2.51	1	2.51	130.35	<0.0001
$X_1 X_2$	2.025E−003	1	2.025E−003	0.11	0.7499
$X_1 X_3$	0.11	1	0.11	5.66	0.0302
$X_1 X_4$	0.012	1	0.012	0.63	0.4395
$X_2 X_3$	6.400E−003	1	6.400E−003	0.33	0.5722
$X_2 X_4$	0.068	1	0.068	3.51	0.0793
$X_3 X_4$	0.22	1	0.22	11.23	0.0041
X_1^2	0.62	1	0.62	32.33	<0.0001
X_2^2	0.19	1	0.19	9.81	0.0064
X_3^2	0.47	1	0.47	24.64	0.0001
X_4^2	0.54	1	0.54	28.10	<0.0001
Residual	0.31	16	0.019		
Lack of fit	0.21	10	0.021	1.31	0.3869
Pure error	0.097	6	0.016		
Cor total	10.64	30			

2.4. Morphological Analysis. The ethanol supernatants obtained from UMAT extraction under optimum conditions and traditional extraction were collected, respectively. Afterwards, the hordein ethanol extracts were concentrated to one-fifth of initial volume using a rotary evaporator at 60°C under vacuum. Then, the precipitates were isolated by centrifugation (8,000 ×g for 30 min). Both of the separated hordein fractions from two methods were redissolved and dispersed equally in 55% isopropanol-water solution and freeze-dried for 18 h under the same conditions. Both hordein fractions were glued on specimen stubs by coating with gold. Each sample was observed at an accelerating potential of 20 kV under high vacuum during micrography.

2.5. Electrophoresis of Hordein Fractions. SDS-PAGE was performed to evaluate the differences between subunits in the two hordein fractions. Extracted hordein fractions were mixed with loading buffer (urea solution) and heated at 100°C for 5 min. After cooling, precipitates were removed by centrifugation, 60 µg protein sample was loaded into 12% SDS-PAGE gel, and subjected to electrophoresis at a constant voltage of 80 V. Then, the gel was stained with 0.1% (w/v) Coomassie brilliant blue-R-250 and destained with the solution containing water, methanol, and acetic acid (4:5:1, v/v).

3. Results and Discussion

3.1. Model Fitting. RSM is an effective statistical technique for process optimization, which was herein utilized to design a series of experiments to establish a predicting model for optimizing the UMAT extraction conditions for hordein.

FIGURE 1: Correlation of calculated hordein with experimentally extracted hordein.

According to CCD, 31 experimental points were run regularly. Response values in Table 2 showed that the yields of hordein ranging from 6.7% to 8.8%. The regression equation obtained for hordein extraction is presented below:

$$Y = 8.30 + 0.64X_1 - 0.38X_2 + 0.33X_3 + 0.32X_4 - 0.045X_1X_2 + 0.17X_1X_3 - 0.055X_1X_4 + 0.04X_2X_3 + 0.13X_2X_4 - 0.12X_3X_4 - 0.59X_1^2 - 0.33X_2^2 - 0.13X_3^2 - 0.14X_4^2. \quad (3)$$

The fitted equation had a determination coefficient (R^2) of 0.9457, revealing that 94.57% of variation in the response could be explained by the model [16]. The points in Figure 1 form a nearly linear pattern, indicating that normal distribution was a suitable model for this data set. The optimal extraction conditions were obtained from the fitted model as follows: 78.2°C, 298.9 W microwave power, 690.2 W ultrasonic power, and 20.4 min extraction time. According to the model, the predicted yield of hordein under the optimal conditions was 8.97%, and the mean experimental value reached 8.84% at 78°C temperature, 290 W microwave power, and 690 W ultrasonic power after 20 min.

Statistical significance of the regression model was checked by the F and P values, and the ANOVA results for response surface quadratic model are shown in Table 3. Corresponding variables became more significant with the increased absolute F value and decreased P value. The quadratic model was extremely significant ($P < 0.0001$), so it was suitable for describing the UMAT extraction process. Clearly, the four variables were extremely significant, indicating the optimization of these factors was imperative. All the quadratic terms of X_1^2 , X_4^2 , X_3^2 , and X_2^2 were significant to the extraction yield of hordein, and the first three were extremely significant. Additionally, two interaction coefficients (X_1X_3 and X_3X_4) were significant ($P < 0.05$), and the “lack of fit” of the obtained model was statistically insignificant ($P = 0.3869$).

FIGURE 2: Response surface plot and contour plot showing the effect of temperature and microwave power on the yield of barley hordein at ultrasonic power 640 W and extraction time 18 min.

3.2. Response Surface and Contour Plots. UMAT is a typical technique that intensifies bioactive extraction from raw materials [17]. However, this method is rather complicated, so it is necessary to figure out the interaction between independent variables during UMAT extraction. The three-dimensional response surface and contour plots generated from the predicting model (3) are shown in Figures 2–5. Fixing other two variables at zero level, the three-dimensional response surface showed that temperature and microwave power had quadratic effects on the yield of hordein (Figure 2). In addition, circular contour plot indicates that the interactions between corresponding variables are negligible [18]. The yield of hordein was elevated with increasing ceiling temperature and microwave power. Microwave is a rapid heating process that conducts heat from internal and external material particles simultaneously. The main disadvantage of applying microwave during bioactive extraction is inhomogeneous heating, thereby decreasing the extraction

FIGURE 3: Response surface plot and contour plot showing the effect of temperature and ultrasonic power on the yield of barley hordein at microwave power 255 W and extraction time 18 min.

yield due to partial overheating [19]. Therefore, we set ceiling temperature as a variable. According to the contour plot in Figure 2, the effect of microwave power on hordein extraction yield is limited by temperature. Once temperature was fixed, the variation of microwave power did not change the yield evidently as a result of temporary stop of irradiation.

The elliptical shape of contour plot in Figure 3 indicates that the interactions between temperature and ultrasonic power significantly affect the hordein yield. The impact of ultrasonic power at 640 W on the hordein yield was elevated from 7% to over 8% with increasing ceiling temperature. Ultrasonic wave can cause solid particles break or collapse due to the cavity effect. Subhedar and Gogate [20] studied the ultrasonic-intensified effect at a fixed microwave power and found that raising the ultrasound power hardly affected the transfer process. Table 3 shows that ultrasonic power played a more important role than microwave power, suggesting that microwave might augment the extraction

FIGURE 4: Response surface plot and contour plot showing the effect of temperature and extraction time on the yield of barley hordein microwave power 255 W and hordein at ultrasonic power 640 W.

yield mainly through rapid heating. Once the ceiling temperature was fixed, the effects of microwave were weakened. However, compared with traditional water-bath extraction, microwave shortened the heating time.

Fixing microwave power and ultrasonic power at zero level, the three-dimensional response surface in Figure 4 shows the effect of extraction time on hordein yield was nearly linear. Although the contour plot was similar to that in Figure 3, there was no significant interaction between temperature and extraction time (Table 3). Additionally, the yield of hordein from barley flour increased with rising ceiling temperature, which directly reflected the influence of microwave. To increase the extraction yield within a short time, microwave power should be elevated. A flat plain three-dimensional surface plot was showed in Figure 5, and the interaction (X_3X_4) between ultrasonic power and extraction time was the most significant one (Table 3). Probably, both of the two factors strongly stimulated the hordein yield at the same ceiling temperature.

FIGURE 5: Contour plot showing the effect of ultrasonic power and extraction time on the yield of barley hordein at temperature of 65°C and microwave power 255 W.

FIGURE 6: SEM images of (a) ultrasonic-microwave-assisted extracted hordein and (b) traditionally extracted hordein; diameter counts of (c) ultrasonic-microwave-assisted extracted hordein and (d) traditionally extracted hordein.

As shown in Figures 2–5, temperature in each experiment was the foremost limitation during the extraction of hordein from barley, and ultrasonic power interacts with suitable temperature. Both of the factors were positively correlated with the hordein yield. In addition, ultrasonic power and extraction time were interactive parameters for the extraction process, managing to augment the extraction

yield. In short, UMAT immensely shortened the extraction time and increased the extraction yield.

3.3. Morphology and Preliminary Characterization of Fractions. As shown in Figure 6, the hordein fraction from UMAT had accumulation block appearance (Figure 6(a))

FIGURE 7: SDS-PAGE of hordein fractions. UM-H: ultrasonic-microwave-assisted extracted hordein; H: traditionally extracted hordein.

surrounded by tiny globular protein. The diameter distribution of the tiny globular protein was measured around 300~2300 nm by ImageJ software (Figure 6(c)), 607.83 nm on average. In contrast, the hordein fraction from traditional ethanol solution extraction was less prone to aggregation (Figure 6(b)). The global protein diameters ranged from 400 to 1800 nm (Figure 6(d)), and the average diameter was 931 nm. As a substitute for mechanical stirring or other intensification technologies, ultrasonic waves could markedly affect the physical morphology of bioactive material and has been widely applied to some physicochemical processes for bioactive crushing [20]. In this study, the morphological difference indicated that UMAT decreased the hordein molecule size and promoted protein aggregation because of the intense vibration and mechanical function of ultrasound. SDS-PAGE (Figure 7) showed that the hordein fractions from two methods had identical subunits. The main storage subunits were B-hordein (36–43 kDa) and C-hordein (45–60 kDa).

4. Conclusion

In summary, we herein optimized the conditions of UMAT for hordein extraction from barley. The optimal extraction yield was 8.84% at 78°C temperature, 298 W microwave power, and 690 W ultrasonic power after 20 min, being consistent with the quadratic model (8.97%). The selected variables remarkably increased the hordein extraction yield from 4.7% (traditional ethanol solution extraction) to 8.84%

in the extraction process. In addition, the subunit fractions of hordein extracted from UMAT and traditional method had no obvious difference, although the particle diameters were slightly different due to the ultrasonic vibration effect.

Data Availability

All the data used to support the findings of this study are included within the article.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

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