Emirates Journal of Food and Agriculture.2017.29(10):760-769
doi: 10.9755/ejfa.2017.v29.i10.695
http://www.ejfa.me/

REGULAR ARTICLE

A cleaner production of Diosgenin from *Dioscorea zingiberensis* Wright biotransformed by *Lactobacillus casei*

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ABSTRACT

The effect of 24 probiotics on disogenin production had been conducted in our preliminary work. Consequently, *Lactobacillus casei* was selected as the fermentation strain with high potential in biotransformation and effects of main parameters on biotransformation were verified through single factor variable experiments. The fermentation conditions was optimized using response surface methodology, which would be helpful to enhance biotransformation efficiency and achieve a high-valued utilization of DZW in diosgenin production. Single factor variable experiments indicated that biotransformation was highly depend on temperature. The conversion rate of saponin reached to (22.1 ± 0.6) %, (15.5 ± 0.3) % and (17.0 ± 0.4) % under each optimal condition of 43°C, 24 hours and 4% inoculum size respectively. According to results of single factor variable experiments, fermentation conditions were optimized through response surface methodology. The 32.4% of conversion rate and 3.95 μg/mL of diosgenin concentration were predicted to be optimal value under the condition of 42°C, 27 hours, 3% inoculum size. The verification results were (32.8 ± 0.9)% of conversion rate and (4.01 ± 0.02) μg/mL of diosgenin concentration under the same conditions, which were close to the predicted values. The conversion rate increased by 41.9% compared to the initial value. Feasibility of converting saponin to diosgenin through probiotics by biotransformation was verified in this paper and the results could be the reference for a cleaner production of diosgenin.

Keywords: Biotransformation; Diosgenin; Dioscorea Zingiberensis wright; Fermentation conditions; Probiotics

INTRODUCTION

Diosorea zingiberensis C.H.Wright (DZW), also known as yellow ginger (Gao et al., 2013), was rich in bioactive components such as saponins, alkaloids, flavonoid glycosides and cardiac glycosides (Chen et al., 2003). The DZW tubers have been widely used in pharmacy, food, cosmetic and other fields. Specifically, diosgenin (2`5R-Spriost-5en-3β-OH) is commonly used as the starting material for the partial synthesis of contraceptives (Adham, et al., 2009), sex hormones and some other steroids in pharmacy (Yang, 2008). The limited raw material resource and large market requirement made the diosgenin became one of commodities in short supply at one time. With the development of the diosgenin industry and the increasing demand for DZW, farmers planted DZW on a large scale instead of the traditional crops (Li et al., 2008). Acid hydrolysis was the commonly used method to produce disogenin in large-scale. Though the method was convenient and efficient, the waste water containing high BOD and COD generated serious environmental pollution (Zhao et al., 2008). Cleaner production of diosgenin was drawing more attention to meet the demand of environment protection (Femandes et al., 2003; Mi et al., 2013).

Pretreat DZW through physical method can promote the release of saponin, which can promote its conversion to diosgenin (Wang et al., 2009). Cleaner method of producing diosgenin was established through physical separation and multi-enzymes hydrolysis (Wen et al., 2008). Proper pretreatment can reduce the energy consumption and wastewater efficiently (Qiu et al., 2011). It was also found microwave broken wall could promote the diosgenin production (Ren et al., 2015). Besides, the method of enzymatic hydrolysis also considered as a

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Received: 18 November 2016; **Revised:** 11 October 2017; **Accepted:** 15 October 2017; **Published Online:** 19 October 2017

cleaner process (Huang et al., 2013). However, the high costs of commercial enzyme coupled with feedback inhibition effect restrict its further application in large-scale production (Liu et al., 2010). As known, microorganisms can secrete enzymes rapidly to accommodate the ambient environment, meanwhile, feedback inhibition effects was reduced through metabolism process. In one word, lower costs of microorganisms instead of commercial enzyme become a more favorable method to produce the diosgenin. Several researchers explored the application of enzyme secreted from microorganisms in diosgenin production. After recovering the starch from DZW tuber, *Trichoderma reesei* exhibited a significant effect on biotransformation of saponion. Moreover, *Trichoderma harzianum* and *Aspergillus oryzae* were also found can convert saponin into diosgenin through biotransformation (Dong et al., 2009, Liu et al., 2010). Few pollutants were detected in the new method above superior to the traditional acid hydrolysis method (Zhu et al., 2010a). The potential on biotransformation were found not only in mold, so were the bacteria (Wang et al., 2009).

Inspired by this successful research, *Lactobacillus casei* had been selected as fermentation strain for biotransformation after a series of screen work (Shu et al., 2017). The aim of this paper is to explore the fundamental fermentation condition of biotransformation by probiotics. Single-factor and response surface methodology were used to find the main factors and optimize the fermentation condition of probiotics on biotransformation. The cleaner production processing could be a reference for the large-scale production in the future.

MATERIALS AND METHODS

Plant material

The *Dioscorea zingiberensis* Wright selected for this study were obtained from Shan yang in Shaanxi Province.

Microorganism and culture conditions

The *Lactobacillus* 83 was preserved in the School of Food Science & Biological Engineering, Shaanxi University of Science & Technology, which was isolated from commercial dairy product and had been identified as *L. casei* in our previous study (Shu et al., 2017). The strain was cultured in the MRS (De Man, Rogosa and Sharpe) broth three times each for 24h at 37°C to obtain the active bacterium. The inoculum size is 5% for the culture.

Reagents

petroleum ether (Tianjin Hedong Hongyan Reagent Factory), methanol (Tianjin Hedong Hongyan Reagent Factory), n-butyl alcohol (Tianjin Hedong Hongyan Reagent Factory), ammonium sulfate (Tianjin Municipality kemi'ou Chemical Reagent Co. Ltd), glucose (Tianjin Municipality kemi'ou Chemical Reagent Co. Ltd), concentrated sulfuric acid (Tianjin Hedong Hongyan Reagent Factory), vanillin (Tianjin Municipality kemi'ou Chemical Reagent Co. Ltd), glacial acetic acid (Tianjin Hedong Hongyan Reagent Factory), ethanol (95%) (Tianjin Hedong Hongyan Reagent Factory), perchloric acid (Tianjin Xinyuan Chemical Co. Ltd), sodium hydroxide (Zhengzhou Paini Chemical Reagent Factory).

Preparation of fermented DZW supernatant

Double separation was adopted to prepare the DZW fermentation supernatant. Fresh DZW were smashed mixed with water and standing for 30 minutes. Most of cellulose was removed through the multilayer gauze filter and starch was separated from the solution through centrifuging at 1000 rpm for 15 minutes. The DZW supernatant were inoculated with 5% (v/v) active strains. It's necessary to adjust the pH to 7.0 before inoculation.

Extraction of saponin and diosgenin

Saponin was extracted according to its unique solubility in water-saturated butanol. Saturated butanol was added into supernatant at a ratio of 2:1 and then extracting was conducted at room temperature for 2 hours. After removing the aqueous phase, rotary evaporating was performed at 50°C until the saponin supernatant turned into extractum.

Similarly, diosgenin can easily resolve in petroleum ether. Fermentation broth was dried in oven and Soxhlet-extraction for 2 hours. Pure diosgenin solution was obtained through the further concentration (Fig. 1).

Measurement of the content of saponin and diosgenin

Saponin was extracted from DZW by methanol and sulfuric acid. Sample solution with a volume of 0.6mL was added to the colorimetric tube and then dried in a thermostatic water bath. Then, 5ml methanol-sulfate (V/V,7:3) solution were added to the colorimetric tube and water bath heating for 1 hour at 65°C. After the colorimetric tube cooled to room temperature, then the absorbance of sample was determined at 329nm (the max absorption wavelength). The method of Vanillin-Acetic acid was employed to measure the content of diosgenin. The diosgenin extracted from the fermentation broth were dissolved in the petroleum ether. Sample solution of 1 mL was added to colorimetric tube and then dried by mean of water bath. After that, 5% vanillin-acetic acid solution of 0.2mL and perchloric acid of 0.8mL were added to the colorimetric tube one after another. Next, heat preservation was performed by thermostatic water bath at 75°C for 15 minutes and then the colorimetric tube was cooled to room temperature quickly. Acetic acid solution was added to the colorimetric tube

up to 10ml. The absorbance was measured at 450nm (the max absorption wavelength) after standing for 30 minutes.

Experimental design and statistical analysis

The effect of various parameters was evaluated by single factors experiments. Basing on the results, response surface methodology was used to optimize the fermentation conditions. Central composite design of three factors and five levels was used for exploring the optimal biotransformation condition and the mutual effects among three variables on conversion rate and diosgenin concentration. The factors level was shown in Table 1.

Dependent variable (Y_1 and Y_2) was conversion rate and diosgenin concentration.

Second-degree polynomial equation was set to evaluate fitness of data and the correlation coefficient was calculated by following equation:

$$Y=\alpha_0+\sum_{i=1}\alpha_i X_i+\sum\alpha_{ii}X_i^2+\sum_{i=1}\sum_{j=i+1}\alpha_{ij}X_iX_j \quad (1)$$

Where Y is the predicted value of the dependent variable, a is the second-order reaction constant, X is independent variables, a_i is the linear regression coefficient, a_{ii} is the quadratic regression coefficient, and a_{ij} is interaction coefficients.

The experiment design, graphs construction and results analysis was conducted by Design of Expert (DOE Version 8.0.6). The optimum value of variables was obtained by calculation of regression equation and analysis of contour plots and 3D surface plots.

Table 1: The main variables at five levels to determine the response rate

Variable	Symbol −1.682	Range of levels (coded/ actual)			
		−1	0	+1	+1.682
Temperature (°C)					
X1	36	38	40	42	44
Time (h)					
X2	19	21	24	27	29
Inoculum size (%)					
X3	2.3	3	4	5	5.7

RESULTS AND DISCUSSION

Preliminary work screened *Lactobacillus casei* 83 (L83) as the strain for further research (Shu et al., 2017). The growth stability and high conversion potential were found during the biotransformation experiment of *L. casei* 83. Based on the former research, single factor tests were conducted to explore the respective effect of temperature, time and inoculum size on biotransformation.

Effect of temperature on biotransformation

Temperature range from 35°C to 41°C were set to explore the effect of temperature on biotransformation. As shown in Fig. 2, conversion rate increased slowly when the temperature raised from 35°C to 37°C. More saponin was converting to disogenin with the temperature elevated to 39°C. After that, the conversion rate increased slowly and the highest value of (22.1 ± 0.6)% was obtained at 43°C.

The effect of temperature on fermentation could be related to the growth and metabolism of bacterial (Kumar et al., 2012). It can be seen the more saponin was converted to diosgenin at higher temperature. Probiotics tend to grow more quickly at high temperature within limits (Shu et al., 2015). Quick growth meant more nutrition demand for strains. Thus, glucosidic bond was cut off to provide more glucoside to meet the need of probiotics, thereby promote the saponin converting to diosgenin. Metabolism of bacterial was also accelerated with the increment of temperature, which was considered be another reason for enhancing the process of biotransformation when temperature upgraded. Temperature was determined to be a significant factor of biotransformation, because the conversion rate increased by more than 5 percent with temperature elevated from 35°C to 43°C.

Effect of fermentation time on biotransformation

The results of previous screen work indicated fermentation for 24 hours was more suitable to biotransformation.

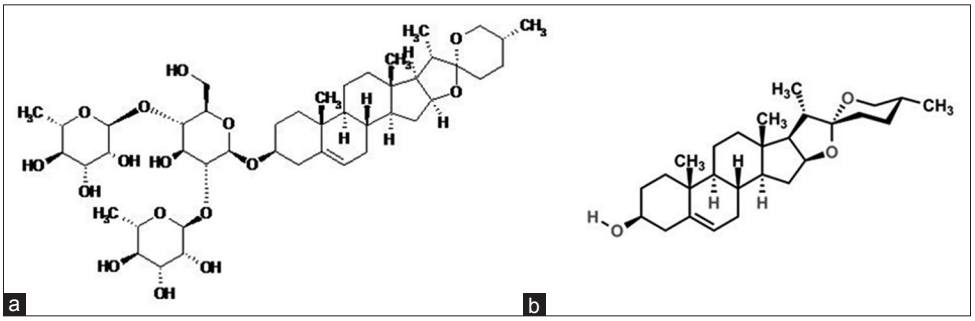

Fig 1. Structural formula of saponin (a) and diosgenin (b).

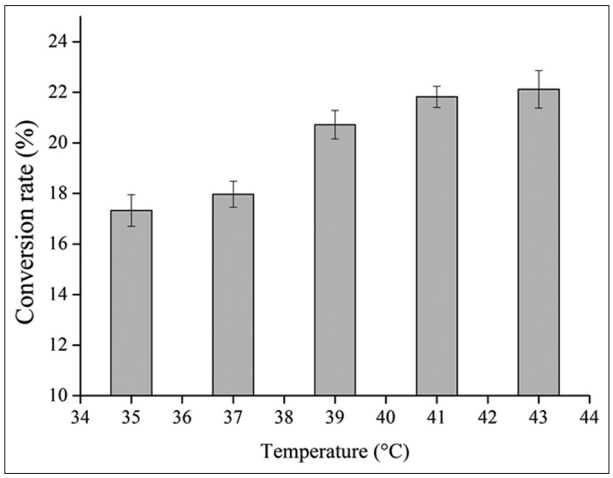

Fig 2. The biotransformation was performed in triplicate at temperature range from 35 to 43□C, and the fermented 24 hours with 5% inoculum.

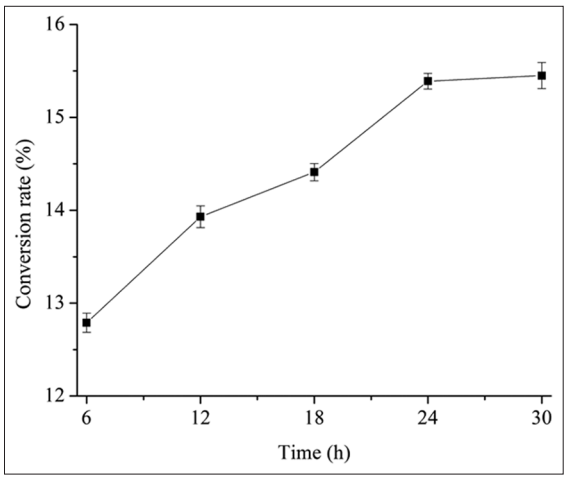

Fig 3. The biotransformation was performed in triplicate for 6 to 30 hours under 37°C with 5% inoculum.

Therefore, the strains were fermented for 6-30 hours to determine the effect of time on biotransformation. As shown in Fig. 3, the diosgenin accumulated continuously over time. The strongest increase was obvious in the range from 6 to 12 hours. During the second 6 hours, the conversion rate still boosts quickly. The quick biotransformation lasted till the 24th hours and then conversion rate slowly increased to (15.5 ± 0.3) % after 30 hours.

Theoretically, diosgenin will accumulate constantly over time. Results of experiments verified the theory predicted above. It is observed that the conversion rate increased in varying degrees at all time. Conversion rate increased rapidly owing to the quick growth in the first 6 hour. Adequate nutrition contributed to the quick growth of strains, which could be helpful to the conversion of saponin. Then, conversion of saponin became slowly due to the feedback inhibition effect by the secretions produced during the process of metabolism. Meanwhile, strains adapted themselves to the ambient change gradually, which was helpful to enhance the efficiency of conversion. However, the strains were not active as before after 24 hours' fermentation and little increment of conversion rate in the last 6 hours revealed the biotransformation came to the termination gradually. To sum up, the effect of fermentation time on biotransformation was not significant compared to temperature as less than 3% enhancement with the fermentation prolonged to 30 hours.

Effect of inoculum size on biotransformation

Bacteria counts among conversion process were largely determined by the inoculum size. More bacteria mean higher conversion rate? No, the results shown in Fig. 4 indicated the variation trend of conversion rate with the change of inoculum size. The initial conversion rate was only (14.7 ± 0.1) % with 2% probiotics inoculated. The conversion rate reached to the peak of (17.0 ± 0.4) % when the inoculum size increased to 4%. Then conversion rate began to decrease when more bacteria was inoculated into fermentation broth. The conversion rate reduced to the minimum of (14.2 ± 0.2) % with 10% bacterial inoculated.

The main influence of inoculum size on biotransformation was related to the bacterial growth (Chen et al., 2015). Probiotic strains utilized glucosides which exist in saponin as the carbon source for growth. Two percent inoculum size provided a small number of bacterial which were not enough to obtain a high conversion rate. An impressive promotion was achieved when the inoculum rose to 4%. Synergistic-competitive effect enhanced by increasing inoculum could promote the conversion of saponin. However, too much bacterial could not be survived better for the limited space and nutrition (Shu et al., 2015b). The bacterial could not be active as before and even began to die under the fierce competition. Thus, the conversion rate decreased when more bacterial was inoculated to the fermentation broth.

Optimization of the fermentation conditions through response surface method

Twenty groups of experiments had been performed to investigate the interaction among the three main factors on biotransformation. Regression equation were set to make the further analysis (Table 2):

$$R1=28.76-2.72A+1.92B-0.91C+2.28AB-2.05AC-2.13BC+0.44A^2-0.92B^2-2.67C^2 \quad (2)$$

$$R2=3.63-0.36A+0.18B-0.17C+0.18AB-0.35AC-0.37BC+0.0043A^2-0.16B^2-0.43C^2 \quad (3)$$

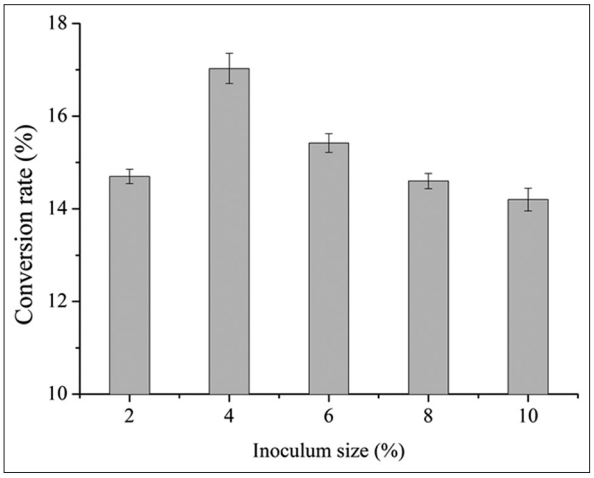

Fig 4. The biotransformation was performed in triplicate at 37°C for 24 hours with 2-10% inoculum size.

In the equation, R1 and R2 are the desirability value of conversion rate and concentration of diosgenin. A, B and C represent temperature, time and inoculum size respectively.

A: Temperature of fermentation (°C); B: Fermentation time (h); C: Inoculum size of fermentation (%); R1: Conversion rate (%); R2: Concentration of diosgenin (μg/mL)

Variance analysis was conducted to explore importance of the main factors on response rate and estimate the significance of regression equation. The results were shown in Table 3.

As shown in Table 3, *P* value lower than 0.01 indicated high significance of model. Lack of fit ($P_{\text{lack of fit}}$=0.3459) was not significant, confirming a good fitness of model established with experimental data. Model coefficients and *P* values indicated that the conversion rate of saponin was highly depend on fermentation temperature and time ($P<0.01$). Quadratic effect of the variables showed that survival rate enhanced significantly with increasing ratio of inoculum size ($P<0.01$). Moreover, all the interaction item were significant ($P<0.05$) indicated the positive mutual effects of fermentation temperature, time and inoculum size on conversion rate. Moreover, coefficient determination (R^2= 0.8984) indicated high consistence between R1 and the value predicted by model. Adjusted determination coefficient (R_{adj}^2= 0.8070) showed that over 80.70% of the response value were affected by the change of variable.

Response surface methodology was adopted to explore the interaction of various factors and obtain the optimal fermentation condition. The contour plots in Fig. 5 (a) seemed to be arc-shaped and this indicated minor mutual interaction of terms A x B for the response one (conversion rate). The 3D surface plots showed the variation trend of

Table 2: CCD experimental design matrix with experimental values of conversion rate and diosgenin concentration

Run	A (°C)	B (h)	C (%)	R1 (%)	R2 (μg/mL)
1	40	24	2.3	23.57	2.8
2	40	24	5.7	18.1	2.0
3	38	21	5.0	31.75	3.9
4	43	24	4.0	23.41	2.9
5	40	24	4.0	28.05	3.8
6	40	24	4.0	27.34	3.6
7	40	24	4.0	27.82	3.4
8	40	24	4.0	30.94	3.8
9	42	21	3.0	19.65	2.4
10	40	24	4.0	31.43	3.8
11	38	27	5.0	27.33	3.3
12	37	24	4.0	35.85	4.3
13	42	21	5.0	18.17	2.2
14	40	19	4.0	24.22	3.0
15	40	29	4.0	27.35	3.3
16	42	27	5.0	24.55	2.3
17	38	21	3.0	23.35	2.7
18	38	27	3.0	29.13	3.6
19	42	27	3.0	32.9	4.0
20	40	24	4.0	27.08	3.4

A: Temperature of fermentation (°C); B: Fermentation time (h); C: Inoculum size of fermentation (%); R1: Conversion rate (%); R2: Concentration of diosgenin (μg/mL)

R1 with the change of A and B. Maximum point didn't appeared though the R1 increased when A and B were raised to higher value respectively. Irregular contour in Fig. 5 (b) indicated that the mutual interaction of terms A x C was not significant for response one. The 3D surface plots showed that the trend of R1 was different with the increase of A and C respectively. When B was certain, R1 increased first and then decreased with sustained increase of C. On the contrary, R1 decreased and then increased with increment of A. The response tends to be stable at the saddle point. Elliptic contour plots in Fig. 5 (c) indicated a significant mutual interaction of terms B x C for response one. As the value of B and C close to the center, R1 gradually increased and maximum point still not appears.

It can be found that the model was significant as presented in Table 4 ($P<0.01$). Lack of fit ($P_{\text{lack of fit}}$=0.5811>0.05) was not significant, indicating the model established fit well with experimental data. Model coefficients and *P* values indicated that the effect of three variables were significant on diosgenin concentration ($P<0.01$). The quadratic effect showed that all the variables had significant influence on diosgenin concentration ($P<0.01$). Moreover, all the interaction item were significant ($P<0.05$), especially the mutual effects between A × C and B × C ($P<0.01$) contribute a lot to the enhancement of diosgenin concentration. Moreover, coefficient determination (R^2= 0.9573) indicated high consistence between R2 and the value predicted by model. Adjusted determination

Table 3: The ANOVA for the fitted quadratic polynomial model of the conversion rate after biotransformation

Source	Sum of squares	DF	Mean square	F value	Prob>F	Significance
Model	392.80	9	43.64	9.83	0.0007	***
A	101.39	1	101.39	22.83	0.0007	***
B	50.47	1	50.47	11.36	0.0071	**
C	11.31	1	11.31	2.55	0.1416	
AB	41.72	1	41.72	9.39	0.0119	*
AC	33.74	1	33.74	7.60	0.0203	*
BC	36.42	1	36.42	8.20	0.0168	*
A^2	2.75	1	2.75	0.62	0.4495	
B^2	12.26	1	12.26	2.76	0.1276	
C^2	102.93	1	102.93	23.18	0.0007	***
Residual	44.41	10	4.44			
Lack of fit	26.31	5	5.26	1.45	0.3459	
Pure error	18.11	5	3.62			
Cor total	437.21	19				

*Significant at 5% level; **significant at 1% level; ***significant at 0.1% level; A: Temperature of fermentation (°C); B: Fermentation time (h); C: Inoculum size of fermentation (%); R1: Conversion rate (%); R2: Concentration of diosgenin (μg/mL)

Table 4: The ANOVA for the fitted quadratic polynomial model of the diosgenin concentration after biotransformation

Source	Sum of squares	DF	Mean square	F value	Prob>F	Significance
Model	7.90	9	0.88	24.90	<0.0001	***
A	1.80	1	1.8	50.95	<0.0001	***
B	0.46	1	0.46	13.02	0.0048	**
C	0.40	1	0.40	11.42	0.0070	**
AB	0.25	1	0.25	6.94	0.0249	*
AC	0.98	1	0.98	27.78	0.0004	***
BC	1.12	1	1.12	31.89	0.0002	***
A^2	2.687E-004	1	2.687E-004	7.616E-003	0.9322	
B^2	0.38	1	0.38	10.91	0.0080	***
C^2	2.65	1	2.65	75.03	<0.0001	***
Residual	0.35	10	0.035			
Lack of fit	0.16	5	0.032	0.82	0.5811	
Pure error	0.19	5	0.039			
Cor total	8.26	19				

*Significant at 5% level; **significant at 1% level; ***significant at 0.1% level; A: Temperature of fermentation (°C); B: Fermentation time (h); C: Inoculum size of fermentation (%); R1: Conversion rate (%); R2: Concentration of diosgenin (μg/mL)

coefficient (R_{adj}^2= 0.9188) showed that over 91.88% of the response value were affected by the change of variable.

The contour plots shown in Fig. 6 (a) seemed to be arc-shaped and this indicated minor mutual interaction of terms A × B for the response two (diosgenin concentration). The 3D surface plots showed the variation trend of R2 with the change of A and B. Maximum point didn't appeared though the R2 increased when A and B were raised to higher value respectively. Arc-shaped contour in Fig. 6 (b) indicated a certain mutual interaction of terms A × C for response two. The 3D surface plots showed that the trend of R2 were different with the increase of A and C respectively. When B was certain, R2 increased first and then decreased with sustained increase of C. On the contrary, R2 decreased and then increased with increment of A. Maximum point still not appears. Elliptic contour plots in Fig. 6 (c) indicated a significant mutual interaction of terms B x C for response one. As the value of B and C close to the center, R2 gradually increased and reached to the maximum point.

The predicted optimal fermentation condition was obtained through derivation of variables in the regression. The predicted optimal conversion rate was 32.4% at 42°C, 27 hours, 3% inoculum size and 3.95 μg/mL of diosgenin concentration. The results of verification tests shown that the conversion rate was (32.8±0.9) % and diosgenin concentration was (4.01±0.02) μg/mL, which was close to the predicted values of 32.4% and 3.95 μg/mL. The conversion rate at the optimal conditions was 41.94% higher than initial value, indicating the fermentation conditions of biotransformation were successfully optimized using the response surface method.

Though pretreatment of DZW tubers could promote the extraction of diosgenin effectively (Mi et al., 2016; Pan et al., 2014; Wang et al., 2008). The disogenin

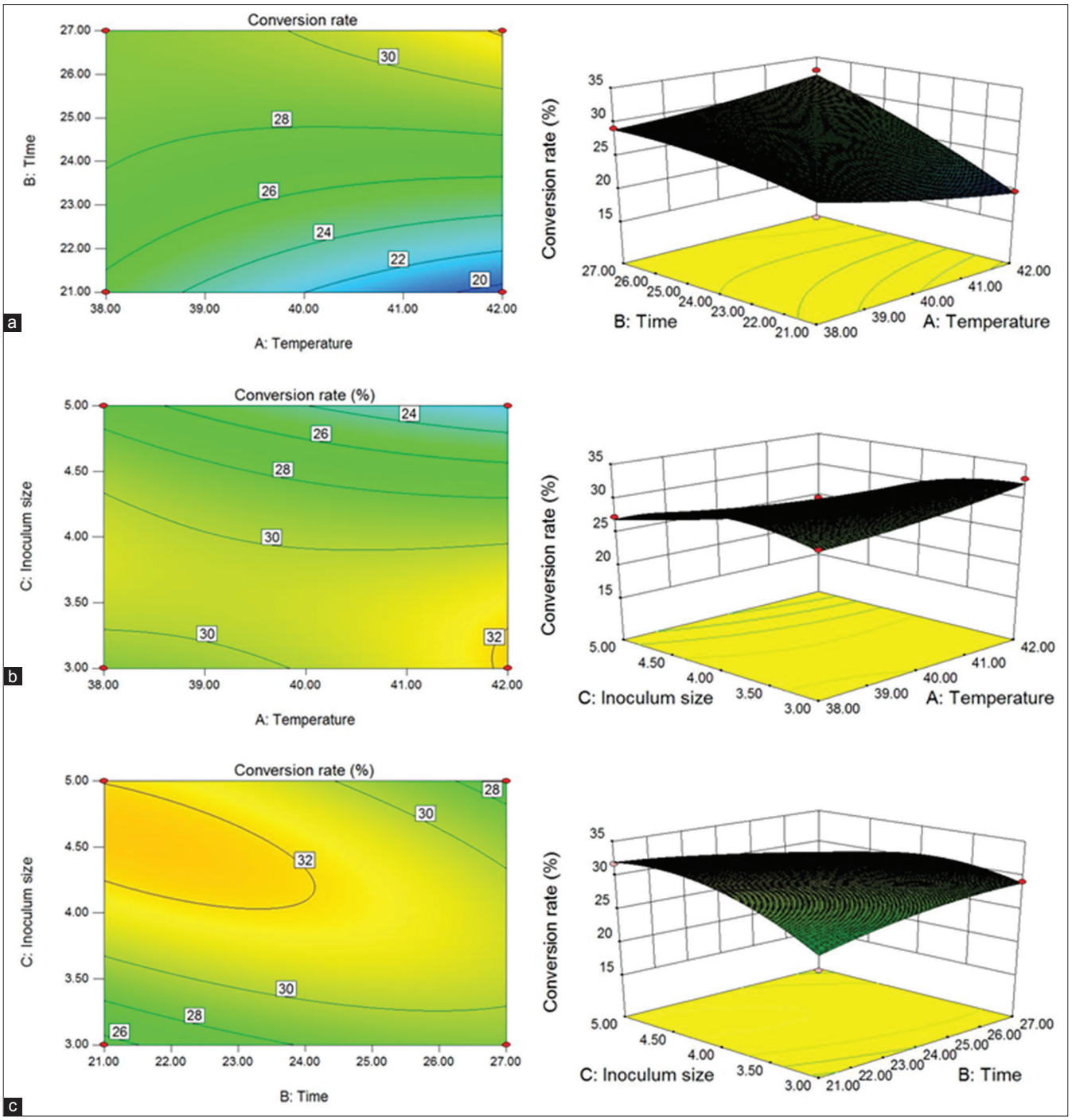

Fig 5. Response of conversion rate using the Central composite design obtained by plotting: (a) temperature versus time (inoculum size, 4%); (b) temperature versus inoculum size (24 hours); and (c) time versus inoculum size (43°C).

production still performed based on acid hydrolysis, which could not meet the demand of environmental protection. Thus, the cleaner method of producing diosgenin through biotransformation had drawn more attention recently. The potential of *β*-glucocidase derived from *A. fumigates* on producing diosgenin from various spirostanosides of DZW had been found (Lei et al., 2012). Firstly, the starch was removed, and then diosgenin was produced by microbial hydrolysis of the residue through *Trichoderma reesei* (Zhu et al., 2010b). Most of the starch (98.0%) and hemicellulose (75.4%) were separated from the material firstly. After that, large amounts of saponin (90.2%) was converted into diosgenin with the aid of *T. reesei*. Nearly all the reducing sugar, chemical oxygen demand, total organic carbo and acid were reduced in the processing wastewater, which was suitable to the environmental friendly production

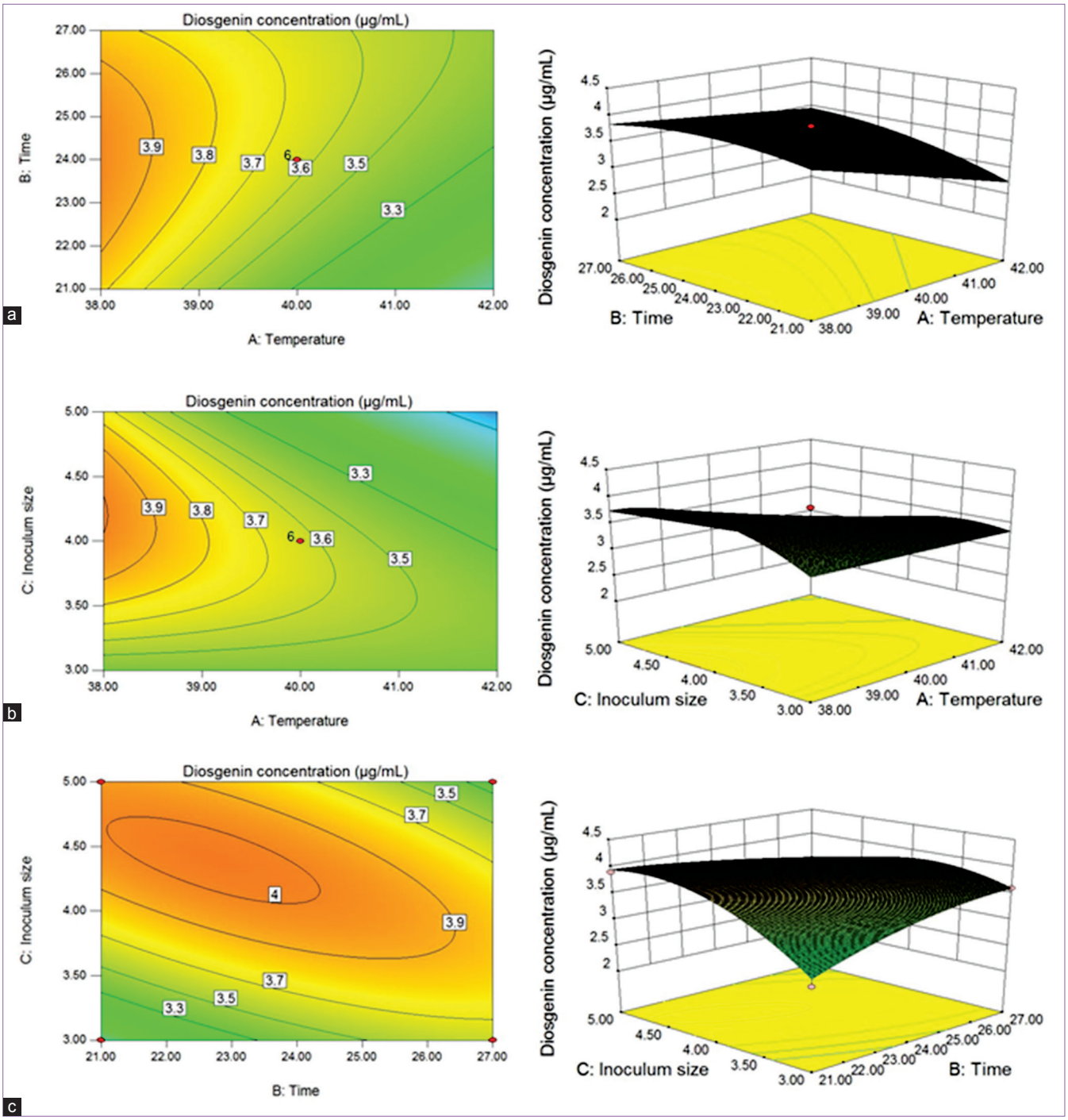

Fig 6. Response of concentration of diosgenin using the Central composite design obtained by plotting: (a) temperature versus time (inoculum size, 4%); (b) temperature versus inoculum size (24 hours); and (c) time versus inoculum size (43°C)

Overall, results of this study were valuable for following reasons. Firstly, *Aspergillus oryzae* and *Trichoderma reesei* represented considerable potentiality in diosgenin production. Compared to the results of producing diosgenin by these microorganisms, *Lactobacillus casei* in this study performed well for the highest conversion rate reach to (32.8 ± 0.9) % which was close to the value by using *Aspergillus oryzae*. Besides, less time-consuming and lower energy consumption reduced the cost effectively which make it possible to realize industrialized production. Last but not the least, once the method was successfully applied in diosgenin production; the pollution problem caused by acid-hydrolysis may no longer exist and the factor which limited further expanding of the industry will be removed (Asmuth et al., 2012). The superiority of this method in environment protection will promote the development of this industry and the local farmers who make living on planting DZW will benefit from the cleaner production.

Of course, utilizing the method for large-scale production is not easy. Though some achievements have been acquired in this paper, much more experiments need to be conducted in our later research work.

CONCLUSION

In conclusion, our results suggested that probiotics has competence of converting saponin to diosgenin through biotransformation. Especially, *Lactobacillus casei* selected in this study showed its considerable ability in diosgenin production. Temperature was found to have more significant effect on biotransformation by single factor experiments. The conversion rate of saponin after biotransformation reached to (22.1 ± 0.6) %, (15.5 ± 0.3) % and (17.0 ± 0.4) % under each optimal condition of 43°C, 24 hours and 4% inoculum size respectively. On the basis of the single factor variable experiments, optimization of the fermentation process was accomplished through response surface method. The conversion rate and diosgenin concentration were predicted to be 32.4% and 3.95 μg/mL at the optimal condition of 42°C, 27 hours, 3% inoculum size. The results of verification tests shown that the conversion rate was (32.8±0.9) % and diosgenin concentration was (4.01±0.02) μg/mL, which were close to the predicted values. The conversion rate at the optimal conditions was 41.9% higher than initial value. All in all, the study in this paper provides a cleaner method of diosgenin production. Moreover, the application of this new method in the future will benefit a lot for local farmer which can promote the agriculture development in this district.

ACKNOWLEDGEMENT

The work was partly supported by the Science and Technology Overall Planning for innovation Engineering project of Shaanxi Province, China (No. 2013KTDZ03-01-03).

Author's contributions

Z. W. conducted the experimental work and wrote the article. H. C. and G. S. designed the process of research and provided some advice for the paper.

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